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MERCURY CONTAMINATION OF RIVERS SEDIMENTS IN THE NORTHERN FRANCE AND CZECH REPUBLIC

CONTAMINATION MERCURIQUE DES SÉDIMENTS ET COURS D'EAU DU NORD DE LA FRANCE ET DE LA RÉPUBLIQUE TCHEQUE

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Spécialité : Optique, Lasers, Physico-Chimie, Atmosphère

par

Ing. Milada Kadlecova

CONTAMINATION MERCURIQUE DES SÉDIMENTS ET COURS D'EAU DU NORD DE LA FRANCE ET DE LA RÉPUBLIQUE TCHEQUE (Mercury Contamination of Rivers Sediments in the Northern France and Czech Republic)

Thèse dirigée par : *Baghdad OUDDANE (Directeur) et Hana DOCEKALOVA (Co-directeur)*

Soutenue le 15 décembre 2011

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Zadání dizertační práce

Číslo dizertační práce:	**FCH-DIZ0051/2011**	Akademický rok:	**2011/2012**
Ústav:	Ústav chemie a technologie ochrany životního prostředí		
Student(ka):	**Ing. Milada Kadlecová**		
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Vedoucí práce	**prof. RNDr. Hana Dočekalová, CSc.**		
Konzultanti:			

Název dizertační práce:

Role sedimentů jako zdroje nebo úložiště znečištění rtutí, geochemická studie

Zadání dizertační práce:

Dizertační práce se zabývá aspekty znečištění rtutí přírodního prostředí a možností využití in-situ vzorkovací techniky s použitím nových sorpčích gelů v technice DGT.
Primární cíle dizertační práce jsou:
i) Optimalizace metody stanovení methylrtuti pomocí extrakce a ethylace spécií rtuit s použitím headspace a automové fluorescenční detekce a zavedení této techniky do běžné laboratorní praxe.
ii) Terénní studie distribuce rtuti v sedimentech říčních systémů Jižní Moravy (v České republice) a regionu Nord Pas de Calais (ve Francii).
iii) Studie metylačního a demethylačního potenciálů velmi znečištěných sedimentů
iv) A využití různých sorpčních gelů v technice DGT pro stanovení rtuti v sedimentech.

Termín odevzdání dizertační práce: 29.2.2012

Dizertační práce se odevzdává ve třech exemplářích na sekretariát ústavu a v elektronické formě vedoucímu dizertační práce. Toto zadání je přílohou dizertační práce.

Ing. Milada Kadlecová Student(ka)	prof. RNDr. Hana Dočekalová, CSc. Vedoucí práce	doc. Ing. Josef Čáslavský, CSc. Ředitel ústavu

V Brně, dne 1.9.2007

prof. Ing. Jaromír Havlica, DrSc.
Děkan fakulty

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In Brno, December 2011 *Milada Kadlecová*

ABSTRAKT

Rtuť je v přírodě přirozeně se vyskytujícím toxickým prvkem, jehož globální emise jsou ovlivňovány zejména antropogenními zdroji znečištění. Obrovský globální nárůst v usazování rtuti, zejména ve vodných ekosystémech, byl zaznamenán současně s počátkem průmyslové revoluce.

Sedimenty jsou posledním místem úložiště nejrůznějších komplexů rtuti. Rtuť však zde může být přeměněna na toxičtější organickou formu, methylrtuť, pomocí transformačních procesů kontrolovaných různými fyzikálními, chemickými, ale i biologickými faktory. Navíc mohou být specie rtuti remobilizovány ze sedimentů pomocí difuze a resuspenzace a tak se sedimenty mohou stát i potenciálním zdrojem rtuti. Proces bioakumulace a bioobohacování tak pokračuje v potravním řetězci, ve kterém se člověk, i další zvířata, stává konzumentem methylrtuti.

Stanovení celkové koncentrace rtuti není dostačující k porozumění osudu rtuti v přírodním prostředí a tak stanovení MeHg poskytuje nezbytnou doplňující informaci. Dostatečně citlivá a přesná analytická metoda pro stanovení specií rtuti je nezbytným nástrojem environmentální chemie. Metody vhodné pro stanovení specií rtuti v sedimentech jsou popsány v části metodologie disertační práce. Metoda stanovení methylrtuti v sedimentech pomocí automatické Headspace vybavené pastí („trap“) a spojené s plynovou chromatografií a fluorescenční detekcí je zde také popsána. Zvláštní pozornost je také věnována potřebám zásad čistého vzorkování, skladování vzorků a přípravě vzorků před samotou analýzou, jakož i samostatné části věnující se terénní studii rtuti a methylrtuti v sedimentech vytipovaných lokalit. Sedimenty jižní Moravy a severní Francie jsou srovnány z hlediska znečištění rtutí. Specie rtuti a další ukazatele (Fe, Mn, S) byly analyzovány v sedimentech, pórové vodě a povrchové vodě řek Deûle a Lys (Francie) a Jihlava a Morava (Česká republika).

Z hlediska posouzení vodních ekosystémů a jejich znečištění rtutí, je vhodné znát koncentraci rtuti v pórové vodě a posoudit dostupnost rtuti ze sedimentů. Technika difuzního gradientu v tenkém filmu je vhodným způsobem jak stanovit koncentraci rtuti v pórové vodě sedimentů. Do roku 2005 bylo použití této techniky pro měření rtuti značně limitováno. Ale nedávný pokrok především v dostupnosti možných sorpčních gelů

vhodných pro stanovení rtuti umožnilo využití této techniky i pro stanovení rtuti. Byly použity různé sorpční gely: Spheron.Thiol, Duolite GT-73 a TiO_2.

Řeka Deûle představuje past enormního množství antropogenní rtuti pocházející z průmyslových zdrojů a je považována za potenciální významný zdroj methylrtuti pro okolní prostředí a živé organismy především. Poslední část dizertační práce se zabývá aplikací dobře zavedeného experimentu využívajícím stabilní isotopy ke studiu metylačních a demethylačních procesů v sedimentech řeky Deûle. Obohacené stabilní značkovače rtuti v anorganické formě (^{199}Hg) and methylované formě ($^{201}MeHg$) byly přidány do sedimentů. Tyto označené specie rtuti tak pomohly sledovat osud specií rtuti a vypočítat rozsah jejich přeměny v průběhu experimentu.

ABSTRACT

Mercury is naturally occurring toxic element; however global mercury emissions are dominated by anthropogenic sources. The global cycle of mercury has seen an increase in mercury deposition, especially in aquatic ecosystems, since the beginning of the industrial revolution. The sediment in aquatic systems may acts as the ultimate sink, where mercury in its various complexes is deposited. The mercury in sediments can then be converted to its more toxic organic form, methylmercury (MeHg), by the transformation processes controlled by various physical, chemical and biological factors. More over remobilization of mercury species from sediments is possible due to diffusion and resuspension and so sediments may act as potential source of mercury for aquatic biota. Bioaccumulation and biomagnifications can then continue up the food chain where humans, among other animals, consume the organic mercury.

It is clear that determination of total mercury is not sufficient to understand its fate in the environment; determination of MeHg provides very useful additional information. The sensitive and precise analytical method for MeHg determination is necessary. The methodological part of the thesis deal with the methods for determination of mercury species in sediments. The method for methylmercury determination in sediments using automated Headspace sampler equipped with Trap and coupled with Gas Chromatography and Atomic Fluorescence Detector was developed and is define. The special attention is also given to the necessity of clean sampling procedures and the proper storage and pre-treatment of the samples and the field study of Hg distribution in sediments. The mercury contamination of sediments from the South Moravia and Northern France are compared. The mercury species and other elements (Fe, Mn, S) were analysed in sediments and/or pore water and/or surface water collected from the sampling sites in the Deûle and Lys River (France) and Jihlava and Morava River (Czech Republic).

In order to better assess the mercury contamination of aquatic ecosystem, the pore water concentration could be evaluated to understand the availability of mercury from sediment. The use of diffusive gradient in thin film (DGT) technique is applied to measure pore water mercury concentration in river sediments. Till 2005 the development of DGT for measuring mercury has been limited. But the recent progress of the availability of ion exchange resins capable of adsorbing mercury enables the use of DGT technique for mercury

measurement. Different resins gels for mercury determination are used: Spheron-Thiol, Duolite GT-73 and TiO_2.

River Deûle act as a sink for enormous anthropogenic Hg from the industrial activities and is considered as a potential significant source of methylmercury to the surrounding environment. The last part of thesis deal with the application of well-established isotope experiments to study methylation/demethylation processes in sediments of Deûle River. For this purpose, species-specific isotopically enriched tracers in the form of inorganic mercury IHg (^{199}Hg) and MeHg (^{201}MeHg) have been added to the sediment slurries. Mercury labelled species were used as the tracers to follow their chemical fate and calculate the extent of the transformation reaction yield occurring during the 24 hours experiment.

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TITRE DE LA THESE :

CONTAMINATION MERCURIQUE DES SÉDIMENTS ET COURS D'EAU DU NORD DE LA FRANCE ET DE LA RÉPUBLIQUE TCHEQUE

RESUME :

Depuis de nombreuses années, les métaux traces ont fait l'objet de recherches importantes dans les domaines de l'environnement et de l'écotoxicologie. Le mercure (Hg), élément trace, a été étudié depuis l'époque où sa responsabilité dans la contamination de la baie de Minamata au sud-ouest du japon a été mise en évidence. Il est le seul élément chimique dont l'introduction dans le milieu marin par l'activité humaine ait entraîné mort d'homme. Les sédiments jouent un rôle important dans le cycle biogéochimique du mercure en milieu aquatique, qui sont considérés comme piège de la contamination par le mercure, les sulfures jouent un rôle important dans le contrôle de sa spéciation et les risques environnementaux générés dans les milieux aquatiques. Le mercure peut être transformé sous ses formes methylées et être remobilisé ou encore transféré dans la chaine trophique. Dans certaines conditions les sédiments peuvent être aussi une source de contamination par le mercure. La première partie de cette étude est axée sur la mise au point d'une nouvelle méthode de mesure et de spéciation du mercure dans les sédiments en utilisant, le couplage éthylation en solution du mercure et du methylmercure avec la méthode Headspace et Headspace avec Trap (piège Tenax), séparation par Chromatographie Gaz et détection par la technique de spectroscopie de fluorescente atomique à vapeur froide (CV-AFS : Cold Vapor Atomic Fluorescence Spectroscopy). L'étude de terrain été réalisée dans les canaux « Deûle et Lys » de la région Nord-Pas de Calais, côté français et dans deux fleuves côté République Tchèque « le Morava et le Jihlava ». Les techniques d´échantillonnages classiques des sédiments par carottage et à l´aide des méthodes de diffusion sur gel DGT (Diffusion Gradient Thin film) pour la mesure du mercure *in situ* ont été utilisées. La méthode DGT permet de déterminer le métal dissous labile (ion libre, complexes minéraux et complexes organiques peu stables) et dépend du type de capteur DGT utilisé. Différents gels ont été utilisés et comparés pour la détermination du mercure: Spheron-Thiol, Duolit GT-73 et TiO_2.
Les résultats obtenus montrent que le Canal de la Deûle est un site caractérisé par une contamination très forte et ancienne. Les transformations et la partition des espèces du mercure ont été évaluées grâce à l´utilisation de traceurs isotopiques (^{199}IHg et ^{201}MeHg) en même temps que la réactivité biogéochimique du sédiment. Les comportements respectifs des espèces naturelles (endogènes) et ajoutées (exogènes) du mercure ont été comparés pour chaque couche du sédiment.

Soutenance le 15/12/2011 à 9h 30
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CHAPITER V.

LIST OF ABBREVIATION

AVS	Acide volatile sulfides
CRS	Chromium reducible sulfides
DGT	Diffusive gradient in thin film technice
DET	Diffusive equilibrium in thin film
MeHg	methylmercury
HgT	total mercury
MerA	mercuric reductase
CH_3^-	alkyl anion group
SRB	Sulphate-reducing bacteria
PE	polyethylen
PP	polypropylen
Milli-Q	Millipore water

GENERAL INTRODUCTION

Mercury (Hg) is naturally occurring toxic element; however global mercury emissions are dominated by anthropogenic sources. The global cycle of mercury has seen an increase in mercury deposition, especially in aquatic ecosystems, since the beginning of the industrial revolution. Theoretical part of the thesis summarizes the source of mercury, its properties and toxicity from the general point of view, but also the current state of knowledge on the biogeochemical cycle of mercury and in particular behaviour of mercury inside the sediments of the aquatic environment and factors influencing its transformation into methylated form. The sediment in aquatic systems may acts as the ultimate sink, where mercury in its various complexes is deposited. The mercury in sediments can then be converted to its more toxic organic form, methylmercury (MeHg), by the transformation processes controlled by various physical, chemical and biological factors. More over remobilization of mercury species from sediments is possible due to diffusion and resuspension and so sediments may act as potential source of mercury for aquatic biota. Bioaccumulation and biomagnifications can then continue up the food chain where humans, among other animals, consume the organic mercury.

It is clear that determination of total mercury is not sufficient to understand its fate in the environment; determination of MeHg provides very useful additional information. The sensitive and precise analytical method for MeHg determination is necessary. The methodological part of the thesis deal with the methods for determination of mercury species in sediments. The method for methylmercury determination in sediments using automated Headspace sampler equipped with Trap and coupled with Gas Chromatography and Atomic Fluorescence Detector was developed and is define. The special attention is also given to the necessity of clean sampling procedures and the proper storage and pre-treatment of the samples and the field study of Hg distribution in sediments. The mercury

contamination of sediments from the South Moravia and Northern France are compared. The mercury species and other elements (Fe, Mn, S) were analysed in sediments and/or pore water and/or surface water collected from the sampling sites in the Deûle and Lys River (France) and Jihlava and Morava River (Czech Republic).

In order to better assess the mercury contamination of aquatic ecosystem, the pore water concentration could be evaluated to understand the availability of mercury from sediment. The use of diffusive gradient in thin film (DGT) technique is applied to measure pore water mercury concentration in river sediments. Till 2005 the development of DGT for measuring mercury has been limited. But the recent progress of the availability of ion exchange resins capable of adsorbing mercury enables the use of DGT technique for mercury measurement. Different resins gels for mercury determination are used: Spheron-Thiol, Duolite GT-73 and TiO_2.

River Deûle act as a sink for enormous anthropogenic Hg from the industrial activities and is considered as a potential significant source of methylmercury to the surrounding environment. The last part of thesis deal with the application of well-established isotope experiments to study methylation/demethylation processes in sediments of Deûle River. For this purpose, species-specific isotopically enriched tracers in the form of inorganic mercury IHg (^{199}Hg) and MeHg (^{201}MeHg) have been added to the sediment slurries. Mercury labelled species were used as the tracers to follow their chemical fate and calculate the extent of the transformation reaction yield occurring during the 24 hours experiment. This experiment methodology is refined by applying advanced matrix algebra to resolve the contributions of several different enriched stable isotope species specific tracers to the isotope pattern found, making the calculation of methylation/demethylation rates possible.

ÚVOD

Rtuť (Hg) je v přírodě přirozeně se vyskytujícím toxickým prvkem, jehož globální emise jsou ovlivňovány zejména antropogenními zdroji znečištění. Obrovský globální nárůst v usazování rtuti, zejména ve vodných ekosystémech, byl zaznamenán současně s počátkem průmyslové revoluce. Teoretická část dizertační práce shrnuje poznatky o rtuti obecně, o jejich vlastnostech a toxicitě, ale také shrnuje současné poznatky o biogeochemickém cyklu rtuti a zejména o chování rtuti v sedimentech vodních systémů a faktorech ovlivňujících transformaci rtuti do methylované formy.

Sedimenty jsou posledním přírodním úložištěm nejrůznějších komplexů rtuti. Anorganické sloučeniny rtuti zde mohou být přeměněny na toxičtější organické formy, methylrtuť (MeHg), pomocí transformačních procesů kontrolovaných různými fyzikálními, chemickými, ale i biologickými faktory. Navíc mohou být specie rtuti remobilizovány ze sedimentů pomocí difuze a resuspenzace a tak se sedimenty mohou stát i potenciálním zdrojem rtuti. Proces bioakumulace a bioobohacování tak pokračuje v potravním řetězci, ve kterém se člověk, stává konzumentem toxických sloučenin rtuti.

Stanovení celkové koncentrace rtuti není dostačující k porozumění osudu rtuti v přírodním prostředí a tak stanovení MeHg poskytuje nezbytnou doplňující informaci. Dostatečně citlivá a přesná analytická metoda pro stanovení specií rtuti je nezbytným nástrojem environmentální chemie. Metody vhodné pro stanovení specií rtuti v sedimentech jsou popsány v části metodologie disertační práce. Detailněji je popsána metoda stanovení methylrtuti v sedimentech pomocí automatické Headspace se sorpční kolonkou (trap) pro předkoncentraci sloučenin, spojené s plynovou chromatografií a fluorescenční detekcí, která je v práci používána. Zvláštní pozornost je také věnována potřebám zásad čistého vzorkování, skladování vzorků a přípravě vzorků před samotou analýzou, jakož i samostatné části věnující se terénní studii rtuti a methylrtuti v sedimentech vybraných lokalit. Sedimenty jižní Moravy a severní Francie jsou srovnány

z hlediska znečištění rtutí. Specie rtuti a další ukazatele (Fe, Mn, S) byly analyzovány v sedimentech, pórové vodě a povrchové vodě řek Deûle a Lys (Francie) a Jihlava a Morava (Česká republika).

Z hlediska posouzení vodních ekosystémů a jejich znečištění rtutí, je vhodné znát koncentraci rtuti v pórové vodě sedimentů a posoudit dostupnost rtuti ze sedimentů. Technika difuzního gradientu v tenkém filmu je vhodným nástrojem pro stanovení koncentrace rtuti v pórové vodě sedimentů. Do roku 2005 bylo použití této techniky pro měření rtuti značně limitováno. Ale nedávný pokrok především v dostupnosti možných sorpčních gelů vhodných pro stanovení rtuti umožnilo využití této techniky i pro stanovení rtuti. Byly použity různé sorpční gely: Spheron.Thiol, Duolite GT-73 a TiO_2.

Řeka Deûle je lokalitou enormního množství antropogenní rtuti pocházející z průmyslových zdrojů a je považována za potenciální významný zdroj methylrtuti pro okolní prostředí a zvláště pro živé organismy. Poslední část dizertační práce se zabývá aplikací stabilních isotopů ke studiu metylačních a demethylačních procesů v sedimentech řeky Deûle. Isotopově obohacené značkovače rtuti ve formě ^{199}Hg a $^{201}MeHg$ byly přidány do sedimentů. Tyto označené specie rtuti tak pomohly sledovat osud specií rtuti a vypočítat rozsah jejich přeměny v průběhu experimentu. Experiment umožnil určit izotopové poměry přírodních i isotopově přidaných specii rtuti a kalkulaci možné míry metylace a demethylace v tomto značně rtutí kontaminovaném sedimentu.

INTRODUCTION GENERALE

Depuis de nombreuses années, les métaux traces ont fait le sujet de recherches importantes dans les domaines de l'environnement et de l'écotoxicologie. Le mercure (Hg) est un élément trace qui a été étudié depuis l'époque où sa responsabilité dans la contamination de la baie de Minamata au sud-ouest du Japon a été mise en évidence. Il est le seul élément chimique dont l'introduction dans le milieu marin par l'activité humaine ait entraîné mort d'homme. La partie étude bibliographique résume ses propriétés physico-chimiques, sa circulation dans l'ensemble des milieux naturels (air, eau, sol, sédiment) et les transformations des formes inorganiques en milieu aquatique (sous l'action des bactéries) à sa forme méthylée la plus toxique, le monométhylmercure (CH_3Hg^+). La toxicité du mercure est abordée d'une façon générale.

Les sédiments sont considérés comme piège de la contamination par mercure, les sulfures jouent un rôle important dans le contrôle de sa spéciation et les risques environnementaux générés dans les milieux aquatiques. Le mercure peut être transformé à sous ses formes methylées et être remobilisé ou encore transféré dans la chaine trophique (i.e., les transferts). Dans certaines conditions les sédiments peuvent être aussi une source du mercure. Alors le processus bioaccumulations et biomagnification continue et le consommateur peut ainsi être exposé à des doses qui peuvent, dans des cas extrêmes, occasionner à long terme une neurotoxicité grave puis la mortalité

La partie analytique de l'étude est axée sur l'analyse du mercure total et la mise au point d'une nouvelle méthode spéciation dans les sédiments en utilisant, le couplage éthylation en solution du mercure et du methylmercure avec la méthode Headspace et Headspace avec trap (piège Tenax), séparation par Chromatographie gaz et détection par la technique de spectroscopie de fluorescente atomique à vapeur froide (CV-AFS : Cold Vapor Atomic Fluorescence Spectroscopy). L'étude de terrain été réalisée dans les canaux

« Deûle et Lys » de la région Nord-Pas de Calais, côté français et dans deux fleuves côté République Tchèque « le Morava et le Jihlava ». Le mercure et d´autres paramètres (Fe, Mn, S) ont été analysés dans les sédiments, dans l´eau interstitielle et dans l´eau de surface. Les techniques d´échantillonnage classique des sédiments par carottage et à l´aide des méthodes de diffusion sur gel DGT (Gradient de Diffusion Thin film) pour les mesures de mercure *in situ* sont présentées. La technique DGT permet de déterminer les métaux dissous labiles (l´ion libre, les complexes minéraux et les complexes organiques peu stables) dépend du type de capteur DGT utilisé. Pour la détermination du mercure, les différents gels ont été utilisés: Spheron-Thiol, Duolit GT-73 et TiO_2.

Le Canal de la Deûle est un site caractérisé par une contamination très forte et ancienne dû à une pollution industrielle. Les transformations et la partition des espèces du mercure ont été évaluées grâce à l´utilisation de traceurs isotopiques (^{199}IHg et ^{201}MeHg) en même temps que la réactivité biogéochimique du sédiment. Les comportements respectifs des espèces naturelles (endogènes) et ajoutées (exogènes) du mercure ont été comparés pour chaque couche du sédiment.

Chapter I: BACKGROUND

I.I. Mercury and its compounds

I.I.1. Physical and chemical properties

Mercury (Hg) is considered as a very dangerous trace element because of its accumulative and persistent character in the environment and biota.

In the periodic table mercury can be found among the group of elements known as the transition metals, group IIB nevertheless it has unusual physical and chemical properties that distinguish it from other transition metals. It is only metal (shiny, silver-white) liquid at the temperature ambient with relatively high vapour pressure and is dense. The general properties of mercury are listed in the ***Table I.1*****.**

Table I.1: *General properties of mercury.*

Atomic weight	200.6	Melting point	-38.9°C
Atomic number	80	Boiling point	356.6°C
Density	13.6	Oxidation states	+1, +2

Mercury occurs as elemental mercury and as inorganic and organic compounds. Elemental, metallic mercury (vapour, liquid) is rather volatile and mercury vapour is regarded as insoluble in water, nevertheless its solubility at room temperature is approximately 60 μg Hg/L. In the presence of oxygen, metallic mercury is rapidly oxidized to ionic form.

In compounds mercury occurs as monovalent mercurous mercury and divalent mercuric mercury. Mercuric salts (like halides, sulphates and nitrates) are water soluble contrary to mercusous salts which are unless some exception water insoluble (e.g. mercurous nitrate). Mercuric ion Hg^{2+} is able to form many stable complexes with biologically important molecules, such as sulfhydryl groups contrary to mercurous mercury which is rather unstable in presence of biological molecules.

In nature mercury also exists as organometallic compounds (mercurials) in which mercury forms chemi*cally stable carbon-mercury covalently bound, because of low affinity of mercury for oxygen (the type of compounds RHg^{+} and RHgR´, where R, R´represent the organic moiety). The short-chain alkyl mercury compounds form salts with the halogens, hydroxides and nitrates but halogens salts are highly volatile already at room temperature so also more hazardous. Among the mercurials compounds belongs also alkoxyalkylmercury compounds, phenyl mercury compounds and the salts of methylmercury and phenylmercury but in spite of potentially large number of organic mercury compounds, by far the most common organic mercury compound in the environment is methylmercury (MeHg).

Hg has seven stable isotopes (^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg and ^{204}Hg) with a relative mass difference of 4%, and it undergoes redox transformations involving compounds with a high degree of covalent character, measurable stable isotope fraction of Hg could occur during its transformations in the environment.

I.I.2. Natural and anthropogenic sources

Mercury is naturally present in the earth, however; it is estimated that large quantities of the Hg currently in the atmosphere was derived from anthropogenic activity (*Table 2.*). Average abundance in the earth´s crust is approximately 0.05 mg/kg. In recent times, mercury has been used for many different purpose such as burning coal, chloralkali industry, certain products manufacture etc. and the amount of mercury released to nature (water, air, soils) have enormously increased.

Natural sources of mercury include volcanoes, forest fires, evaporation from soil and water surface and degradation of minerals. The main deposit of naturally occurring mercury is cinnabar, the red sulphide (HgS). This is also the main component of mercury-rich ores, that are mined and they may contain up to 70% mercury (Berlin, 2007). In 1985 global natural mercury emissions were estimated at 2 500 - 30 000 tonnes per year

(Lindqvist and Rodhe, 1985), but the lower end of the given range is most reliable, since then natural emissions were estimated at approximately 3 000 ton per year (Nriagu, 1989). However emission estimation for natural sources are difficult to assess. But a large source of mercury into the atmosphere is coming from human activities, anthropogenic sources, primarily from coal-burning power plant, fuel combustion, gold refining production, metals smelting, solid waste incineration, cement production, steel and mercury production (Pacyna et al., 2006). Another 15% of anthropogenic mercury emissions are coming to the earth from direct application of fertilisers and fungicides and municipal solid waste and another 5% from direct discharge of commercial effluent to water objects (Stein et al., 1996).

Estimates of total anthropogenic emissions of mercury from the major sources in 1995 given by (Pacyna et al., 2006) are given in ***Table I.2.***

Table I.2: *Global anthropogenic emissions of Hg in 2000 (in ton) (Pacyna, et al., 2006).*

Continent	Stationary combustion	Cement production	Metals smelting	Steel production	Gold production	Mercury production	Caustic soda production	Waste	Total
Africa	205.2	5.3	7.9	0.4	177.8	0.1	0.3	-	398.4
Asia	878.7	89.9	87.6	11.6	47.2	0.1	30.7	32.6	1179.3
Australia	112.6	0.8	4.4	0.3	7.7		0.7	0.1	126.6
Europe	88.8	26.5	10.0	10.6	-		12.4	11.5	175.1
Russia	26.5	3.7	6.9	2.7	3.1		8.0	3.5	72.6
S.America	31.0	6.5	25.4	1.4	-	22.8	5.0	-	92.1
N.America	79.6	7.7	6.4	4.3	12.2	0.1	8.0	18.7	145.8
Total	1422.4	140.4	148.6	31.3	248.0	23.1	65.1	66.4	2189.9

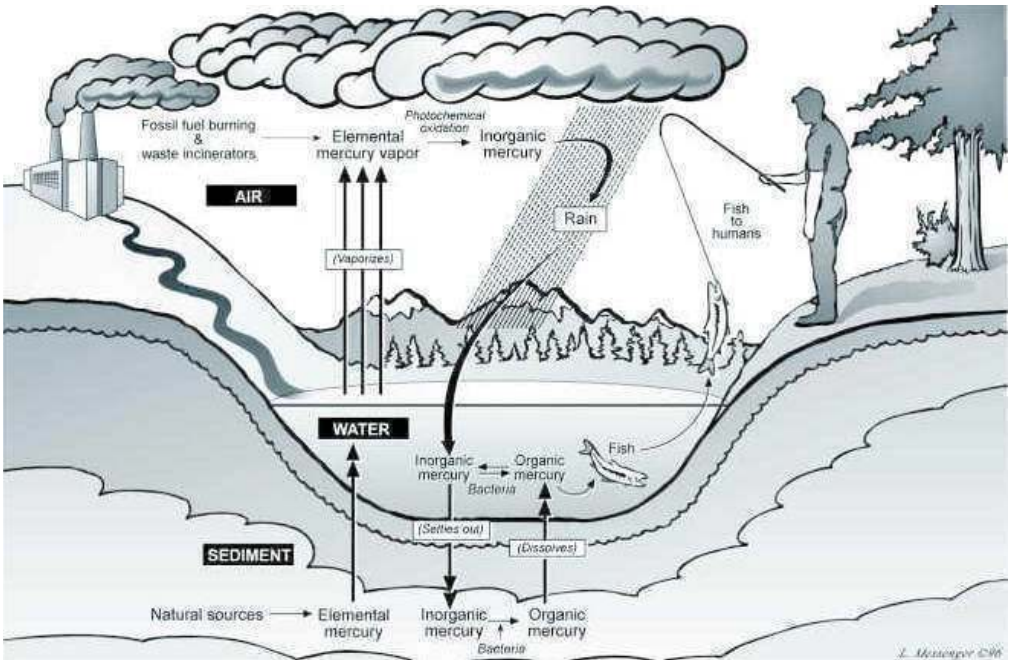
I.I.3. The transformation and mercury cycling in the environment

In the biogeochemical cycle, mercury may be transformed between elemental Hg (Hg^0), divalent mercury (Hg^{2+}) and methylmercury (monomethylmercury MeHg, CH_3Hg^+; dimethylmercury DMeHg, CH_3HgCH_3). All these species may interchange in atmospheric, aquatic and terrestrial environments (Ullrich et al., 2001; Leermakers et al., 2005). In natural aquatic systems transformation processes and species distribution are controlled by physical, chemical and biological factors. So the mercury levels found in different aquatic environmental matrices and in biota are dependent on a variety of physical changes,

geochemical reactions and biochemical interactions which are specific for the given location.

Once released from natural or anthropogenic source as a volatile Hg^0, mercury can circulate for up to a year and be widely dispersed. The ultimate source of mercury to aquatic ecosystems is deposition from atmosphere in the inorganic form (after photochemical oxidation) along with a rainfall. Exceptions are point sources of mercury contamination into the aquatic ecosystems. In the water mercury enters a complex cycle in which one form can be converted to another; can be brought to the sediments by particle settling and released by diffusion or resuspension later; can settled into the sediment as insoluble mercury sulphides can entered the food chain; and can be released back to the atmosphere by volatilization. The main reactions in the environment are reduction and methylation. The first one is related with subsequent volatilization into the atmosphere, the second one with subsequent bioaccumulation in a tropic level.

Figure I.1: *Mercury cycle.*



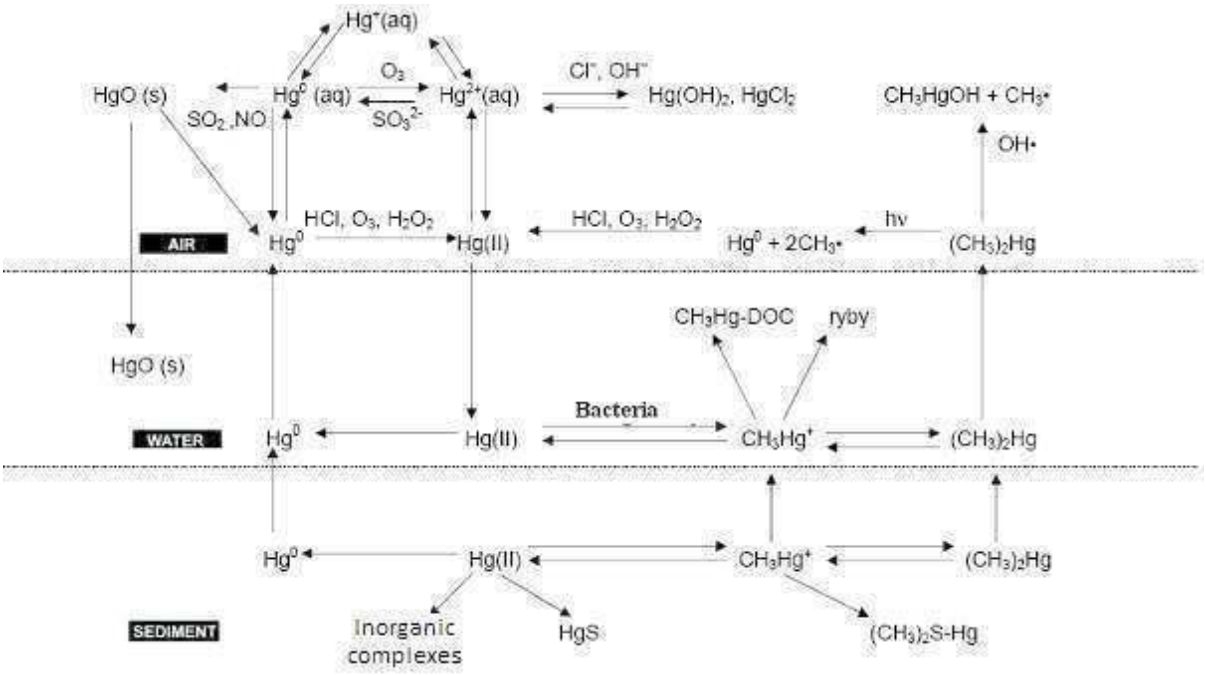
More than 50 years of research of the possible biotic and abiotic sources, sinks and transformations of Hg have greatly enhanced the understanding of Hg´s global and watershed cycling. Biological processes (microbial metabolism) play an important role in A) the formation and degradation of MeHg (Fitzgerald et al., 2007) and B) redox

transformation of inorganic Hg potentially controlling the concentration of the substrate for methylation (Barkay et al., 2003) and evasion of Hg(0) from open waters (Mason et al., 1995).

However, we do not have a high degree of confidence regarding the absolute and relative contributions of these processes to the formation of MeHg. Issues such as the clear identification of source and sinks for Hg in the environment, the in situ pathways leading to toxicity, and the nature and evolution of redox reactions are key to the understanding of Hg biogeochemistry and also vitally pertinent to the development of management policies for the control of environmental Hg contamination (Lindberg et al., 2007).

Depending on local condition, mainly sulphate reducing bacteria may in the suboxic milieu convert some of the deposited Hg to MeHg. This conversion is important for the reason of: higher toxicity of MeHg, bioaccumulation in biota, bio-amplification along the food chain and long elimination time from organisms. ***Figure I.1.*** shows the overall cycle of mercury in the environment and ***Figure I.2.*** the main transformations phenomenons of Hg in the aquatic ecosystems.

Figure I.2: *Main transformation processes.*



I.I.4. Transformation of Hg in the aquatic environment

Microbes play vital roles in the biogeochemical cycling of Hg (Barkay et al., 2003) by mediating redox transformations of Hg and formation and degradation of MeHg in aquatic environments. The main mechanisms of Hg transformation are displayed on ***Figure I.3***.

Hg (II) reduction (HgII –> Hg0)

Reduction of HgII results in the partitioning of Hg into the air due to the product´s low aqueous solubility (60 μg/L water at 25°C) and high volatility. The best documented mechanism of Hg **biological reduction** is mediated by the inducible bacterial enzyme mercuric reductase (MerA). Microbes disposing MerA remove Hg(II) from contaminated aquatic environments leading to a decrease in the availability of the substrate of methylation. Concentrations of total mercury (HgT) in most natural environments are at very low levels, which might not be sufficient for induction of MerA. In such an environments Hg(II) may be reduced by photosynthetic organisms or by Fe(II)-dependent reduction process among acidophilic thiobacilli (Barkay et al., 2003).

Abiotically Hg(II) is reduced as a result of photochemical transformation due to the formation of reducing organic free radicals that are produced by photolysis from dissolved organic carbon (Nriagu, 1994), dissolved oxygen and organic carbon complexes and Fe(III) organic acids coordination compounds (Zhang and Lindberg, 2001).

Hg (II) methylation (HgII –> MeHg)

The methylation of inorganic mercury in waters and sediments constitutes a key step in the cycling of mercury in aquatic ecosystems and takes place in both remote and impacted environments. Methylation occurs predominantly in sediments, less in the water column. Since both methylation and demethylation processes occur, environmental MeHg concentrations reflect net methylation rather than actual rates of MeHg synthesis. It appears that the combined effect of MeHg production and degradation leads to a state of equilibrium with a near constant level of MeHg in sediments, that rarely exceeds 1,5% of HgT concentration, whereas the proportion of MeHg in fish and other aquatic biota may be

much higher (Ullrich et al., 2001). Maximum methylation rates in sediment usually occur at the redox boundary layer which may vary seasonally.

Methylation of the metal (transformation of a methyl group from an organic compound to the metal ion) is not facile chemical reaction, at least in aqueous solution. The methylation of Hg requires the presence of a suitable methyl donor molecule. It ultimately requires the transfer of an alkyl anion group (such CH_3^-), a strong base highly unstable in water. Thus, methylation reaction either is the result of photochemical processes or need to be catalyzed by microorganisms (Morel et al., 1998). In the natural aquatic environment, **biotic and abiotic mechanisms** are possible as a large variety of potential methylating compound, all of them being biologically synthesised (Ullrich, et al., 2001).

Biomethylation

Organisms capable of the Hg methylation in sediment have been found both anaerobic and aerobic but the potential for microbial methylation is generally through to be higher under anaerobic conditions. Sulphate-reducing bacteria (SRB) have been identified as the principal biotic methylators of inorganic Hg (Compeau and Bartha, 1985; Gilmour et al., 1998; King et al., 2000). Later work has shown also methylation by iron reducting bacteria that belong to the Deltaproteobacteria (Fleming et al., 2006; Kerin et al., 2006).

Mercury methylation by organisms may be enzymatic or nonenzymatic. Enzymatic methylation requires the presence of actively metabolising organisms, while nonenzymatic methylation requires only methylated products of active metabolism.

There exist several chemical agents able to methylate inorganic mercury. Methylcobalamin (CH_3CoB_{12}), the derivate of vitamin B_{12}, produced by many organisms, is one of the most studied in the environment (Craig and Moreton, 1986). Methylcobalamin is involved in microbial Hg methylation. The process involved nonenzymatic transfer of the methyl group, methylanion (CH_3^-) to the mercuric ion (Hg^+) according to the next equation:

$$CH_3CoB_{12} + Hg^{2+} + H_2O \rightarrow CH_3Hg^+ + H_2OCoB_{12}^+$$

It can also lead to dimethylmercury, but this step is slower than the first one.

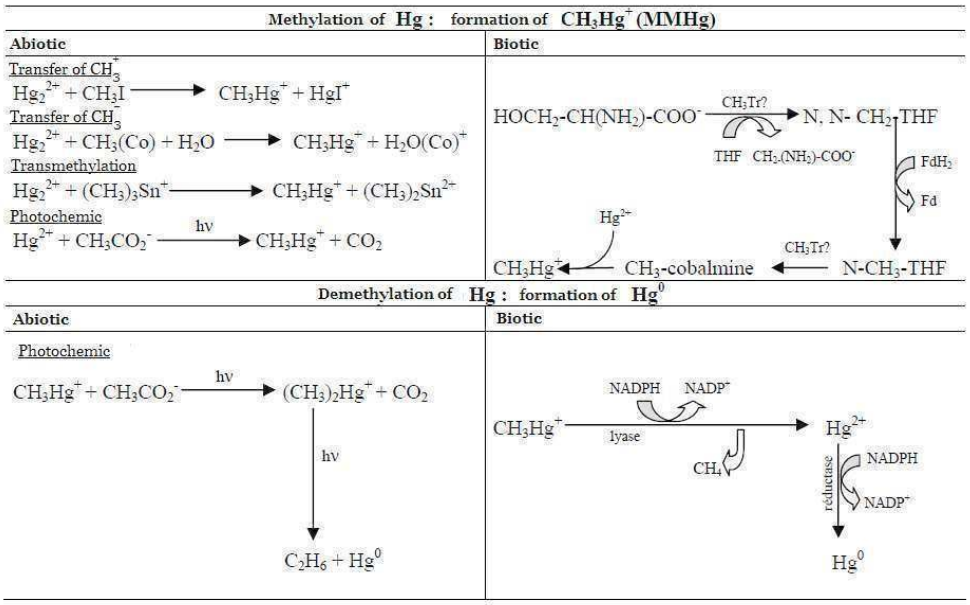
Abiotic Methylation

Chemical methylation of Hg is also possible if suitable methyl donors are present. Chemical agents able to photochemical methylation of Hg^{2+} like methanol, ethanol, acetic

acid and propionic acid can be effective (Miller et al, 1979), Sewage effluent and industrial wastewater have also been reported as methyl sources in the photochemical methylation of Hg (Ullrich et al., 2001). At last, Hg methylation can also occur as a result of transalkylation reaction between Hg and lead and tin alkyls occurring in the environment (Stochiev, 2002). Finaly, humic matter may be another significant environmental methylation agent (Nagase et al., 1982; Nagase et al., 1986)

The relative importance of abiotic versus biotic methylation mechanisms in the nature aquatic environment has not yet been established, but it is generally believed that Hg methylation is predominant a microbial mediated process. (Ullrich et al., 2001)

Figure I.3: *The main mechanisms of methylation/demethylation.*



MeHg Demethylation (MeHg –> Hg(0) + CH_4/CO_2

The biological and abiological decomposition of methylated Hg species is an important process regulation the organic Hg content of sediments and waters. Methylmercury degradation is through to be predominantly **microbially mediated**. Numerous bacterial strains capable of demethylating MeHg are known; including both aerobic and anaerobic species, but demethylation appears to be predominantly accomplished by aerobic organisms (Compeau and Bartha, 1984). The reductive pathway of microbial degradation of MeHg produce CH_4 and Hg(0) and oxidative pathway of demethylation produce CO_2 and small quantity of CH_4. Oxidative demethylation is

significant in both contaminated and uncontaminated river sediments and is most pronounced at sediment surface (Ullrich et al., 2001).

In the sediments rich in sulphides, MeHg can be chemically decomposed according to the equation:

$$2\ CH_3Hg^+ + S^{2-} \rightarrow (CH_3Hg)_2S \rightarrow (CH3)_2Hg + HgS$$

Photolytic decomposition appears to be only significant abiotic decomposition mechanism Abiotically MeHg is degraded by sunlight at a wavelength range of 280-400 mm (Suda et al., 1993). In light exposed environment, such as wetlands and lakes, photo-degradation may be the major mechanism for MeHg degradation, but in the sediments and bottom waters, where MeHg accumulates following methylation, this process may have little impact on demethylation and microbial processes most likely dominate.

I.I.5. Toxicity

Speciation of mercury affects its bioavailability and toxicity. The extent of the adverse effects depends not only on the form of Hg at the time of exposure, but also on the duration of exposure, and the route of exposure. In general all forms of Hg induce toxic effects in mammalian species, including humans. From a toxicological point of view, mercury compounds should be divided into inorganic and organic compounds. Among the inorganic compounds, elementary mercury and the divalent mercury salt and among the organic compounds, many times more toxic, methylmercury are the compounds of toxicological interest (Berlin, 2007). The main rout of mercury exposure for the general population is inhalation of mercury vapour – elemental form and intake of food containing MeHg and the target for mercury toxicity in humans where toxic effects of Hg have been demonstrated are cardiovascular system, liver, kidneys, immune system and nervous system of people all ages (Berlin, 2007).

I.I.6. Impacts of mercury on the environment - Ecotoxicity

A very important factor in the impacts of mercury to the environment is its ability to build up in the organisms and up along the food chain. Methylmercury is, from the other mercury forms, mostly absorbed and accumulated. Inorganic mercury can also be absorbed, but is generally taken up at a slower rate and with lower efficiency than is methylmercury (US EPA, 1997). The biomagnifications of methylmercury has a most

significant influence on the impact on animals and humans. Methylmercury is very bioavailable and accumulates in fish through the aquatic food web, nearly 100 percent of mercury that bioaccumulates in predator fish is methylmercury. Most of the methylmercury in fish tissue is covalently bound to protein sulfhydryl groups. This binding caused in a long half-life for elimination (about two years) (Wiener, 1996). As a consequence, there is a selective enrichment of methylmercury (relative to inorganic mercury), because in a higher trophic level the methylmercury content is higher than in previous trophic level.

The HgT and MeHg concentration and percentage of MeHg in different fish are presented in ***Table I.3*** (Stein et al., 1996). The limit value for total mercury, fixed by European Commission Decision is 0.5 $mg.kg^{-1}$ wet weights, except for some mercury species.

Table I.3: *Total Hg and MeHg contents and percentage of MeHg with respect to total Hg in different edible fish species from the Mediterranean Sea.*

Species	HgT ($mg.kg^{-1}$ wet weight)	MeHg ($mg.kg^{-1}$ wet weight)	MeHg (%)	Reference
Hake (Merluccius mecluccius) Ionian Sea	ND – 0,30	ND – 0,30	73-100	(Storelli et al., 2005)
Hake (Merluccius mecluccius) Adriatic Sea	0,04 – 0,48	0,04-0,48	60 – 100	(Storelli et al., 2005)
Striped mullet (Mullus barbatus) Adriatic Sea	0,08 – 1,74	0,08 – 1,74	68 - 100	(Storelli et al., 2005)
Ghostshark (Chimaera onstrosa)	1,30 – 5,16	1,14 – 4,56	74 - 97	(Storelli et al., 2002a)
Electric ray (Torpedo nobilliana)	1,65 – 3,59	1,15 – 2,76	51 - 97	(Storelli et al., 2002a)
Eagle ray (Mytilus galloprovincialis)	0,67 – 1,01	0,40 – 0,84	61 - 83	(Storelli et al., 2002a)
Mussel (Mytilus galloprovincialis)	0,04 – 0,83	0,02 – 0,12	14 - 98	(Ipolyi et al., 2004)
Albacore (Thunnus alalunga)	0,84 – 1,45			(Storelli et al., 2002b)
Bluefin tuna (Thunnus thynnus)	0,16 – 2,59			(Storelli et al., 2002b)
Bluefin tuna (Thunnus thynnus)	1,02			(Storelli and Marcotrigiano, 2001)
Swordfish (Xiphias gladius)	0,49			(Storelli and Marcotrigiano, 2001)
Sardine (Sardinella aurita) Tunisia	0,19 – 0,32		85 - 97	(Joiris et al., 1999)
Sardine (Sardinella pilchardus) Tunisia	0,26 – 0,42		85 - 97	(Joiris et al., 1999)

Chapter II: METHODS

II. I. Determination of mercury species in sediments

The determination of the HgT is not sufficient to understand its fate in the environment. In addition to the monitoring of HgT concentrations in the environment, speciation analysis provides very useful additional information (Leopold et al., 2010). The International Union of Pure and Applied Chemistry (IUPAC) states that the speciation analysis is analytical activity for identification and/or measuring one or several individual chemical forms of an element (Templeton et al., 2000). Speciation analysis implies the determination of very low concentration of minor species and in the case of mercury speciation in sediments it means that sometimes concentrations nearly ng g^{-1} have to be handled. Usually, MeHg in sediments does not exceed 1,5% of HgT present (Horvat et al., 1993; Benoit et al., 1998; Heyes et al., 2004, Ouddane et al., 2008; Wu et al,. 2011). Highly sensitive detection techniques are available for mercury, but speciation analysis also requires effective and sensitive pre-concentration and separation. The development of sensitive, reliable, simple and cost-effective procedure for speciation analysis is still one of the principal research axes in analytical chemistry. In addition to the demand for high sensitivity, the preservation and integrity of the sample and the Hg species of interest during sampling, storage and pre-treatment are crucial.

The main steps to consider for Hg speciation in sediment samples are (Stoichev et al., 2006):

- Sample collection with special attention to clean procedures
- Sample preservation, storage and pre-treatment to preserve mercury species concentration and distribution
- Extraction from the sediment sample securing the integrity of the species
- Preconcentration to achieve a final concentration matching the LODs accessible with detection technique selected

- Separation of mercury species of interest
- Detection and quantification

Numerous standards and certified reference materials are available for verifying the reliability of new or modified methods. The regular use of certified reference material and quality control materials is indispensable in procedure development, validation, verification and internal quality control (QC).

II. I. 1.Sampling and storage

While applying the method for trace mercury determination, one of the greatest difficulties could be precluding sample contamination during collection, transport and analysis.

Rigorous cleaning procedures must be used for all laboratory ware and other equipment that comes into contact with samples (Stoichev et al., 2006). Usually cleaning for several days in acid bath is included (Jackson, 1988).

The contamination risk for sediments is less important than for waters but the technical problems can be very complicated. The sampling strategy depends on the objective of the study. For the investigation of temporal variation it is necessary to collect the samples from the same site. Using Global Positioning System is more than recommended. For the stratigraphic studies it is obligatory not to mix sedimentary layers and for anoxic sediments, the sample oxidation has to be minimal (Stoichev et al., 2006).

Bottom sediments contain terrigenous particles and precipitated materials from chemical and biological processes in river lake or ocean waters. Sediments consist of particles of different size, shape and composition, so it constitute delicate and complex milieu for studying. Natural processes responsible for the formation of bottom sediments are altered by human activities. Many man-made compounds with complex chemical composition and physico-chemical properties have entered streams through atmospheric deposition, runoff from the drainage basin or direct discharge into the water. Most organic contaminants, metal compounds and nutrients entering water bodies become associated with particulate matter which is carried by currents into quiet areas in the rivers, lakes and oceans where it settles and accumulates in bottom sediments. Under certain conditions the contaminants in bottom sediments may become released into water or accumulate in the

food chain. Consequently, bottom sediments are a sink as well as a source of contaminants in an aquatic environment. Particular distribution equilibrium of contaminants is established among suspended and bottom sediments, sediment pore water, overlying water column and biota. This equilibrium is affected by the physico-chemical regime which controls the kinetics of different reactions taking place within the system.

The surface sediments situated in the first 10 cm depth from the sediment/water interface are usually taken with sediment grabs (Stoichev et al., 2004a). For sampling the sediment cores (profiles), different plastic tubes are used (Baeyens, 1992). After sampling, the cores are sliced in the inert atmosphere. The sediments can be sieved with Nylon sieves to eliminate stones and other rough particles. They are transferred in acid-cleaned vials and immediately frozen to increase the stability of $MeHg^+$. In the laboratory the sediments can be dried either with clean air flux or freeze-dried (Varekamp et al., 2000).

Attention must be paid also to sample preservation to avoid perturbing the distribution of mercury species in the sample. The preservation of aqueous samples is often accomplished using acidification. But the suspended matter must be removed prior to acidification and dimethylmercury and Hg(0) have to be removed or else conversion of these species into methylmerucry and mercury (II) can occur (Liang et al., 1996). In sediments, immediate freezing of sample after collection is recommended in order to retard microbial activities and to increase the stability of $MeHg^+$. Another possibility is drying the sample in the laboratory, but for mercury determination, especially MeHg either with clean air fluxes upon laboratory temperature, because under higher temperature there is a risk of losses from volatilization or freeze-dried (Varekamp et al., 2000).

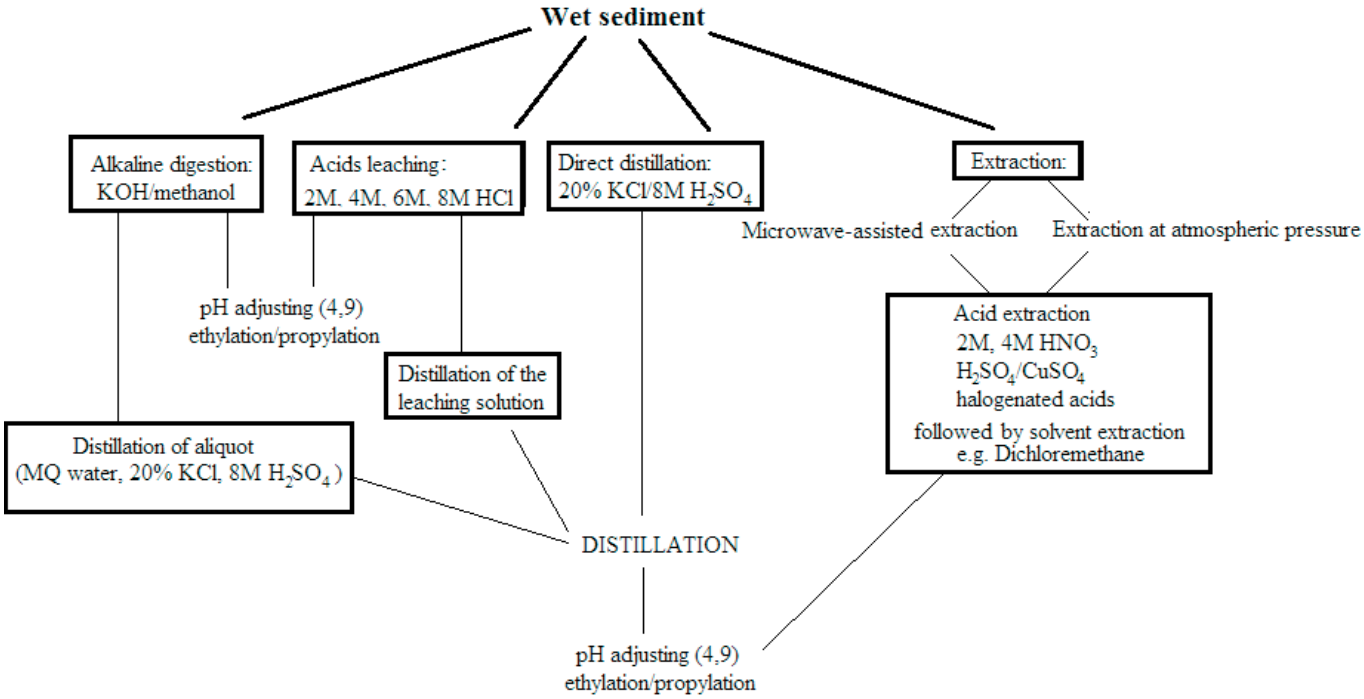
II. I. 2.Isolation and pre-concentration

Mercury species „extraction" from the environmental sample (e.g. from soil, sediment or biota) securing the integrity of the sought species as they are in the sample. To start with the extraction, the environmental sample should be made homogenous and perhaps preservative agents could be needed in order to prevent species degradation before final analysis. It is well known that organometallics can be degraded in many ways including micro-organisms action, oxidation, or even UV irradiation. The matrix of sample can play an important role in the stability of the sought compound.

HgT can be extract from sediments with concentrated HNO_3 (Pereira et al., 1998; Mikacet al., 1999) or acid mixtures (Baeyens et al., 1997; Gilmour et al., 1998) under efficient reflux. Since 1986, the special single-purpose atomic absorption spectrometer called Trace Mercury Analyzer has been used in majority of Czech and Slovak analytical laboratories. In 1994, the second generation of this instrument, called Advanced Mercury Analyzer AMA-254, was introduced (Operator Manual, 1994). Instrument enable direct analysis of dry solid samples or liquid samples without any pre-treatment. Principe of the instrument is described in Chapter 3. In natural waters, the concentration of mercury and the water sample volume possible for introduction into the AMA-254 is too low for ***dissolved HgT*** determination. The most common technique used to monitor HgT in waters is cold vapour atomic fluorescence spectrometry (CV-AFS). Before the analysis, natural water samples are digested to transform all Hg species to free inorganic Hg^{II} ions by addition of a strong oxidant (e.g. bromine chloride) and than reduced to elemental Hg^0 by tin (II) chloride. Elemental mercury is then purged out of the reaction solution by an inert carrier gas (e.g. argon) and cold vapours of Hg^0 is then pre-concentrated on a gold trap by formation of an amalgam. Subsequent heating of the gold trap releases the mercury, which is transported to the detection cell where mercury resonance fluorescence is measured at a wavelength of 253,7 nm. The detection limit is dependent on the purity of the reagents, the inert gas used and the cleanliness of the working procedures (Labatzke et al., 2004).

Extracting the sought species of **MeHg** from the solid environmental sample is a delicate process depending on both the nature of the species and of the sample matrix. In sediments the extraction is probably the most crucial step of the whole Hg speciation strategy. The mostly commonly used procedures for the isolation of MeHg from environmental samples *are acid/solvent extraction, distillation* (vacuum, stream or gas-stream distillation) and *alkaline digestion* (Lorenzo et al., 1999; Bowles and Apte, 2000). Separation of mercury species from matrix is one of the most critical steps. Adequate recovery and preventing losses are the two conflicting issues need to be addressed. Isolation of MeHg is a very delicate step, because the whole species content may not be liberated and artifacts can occur so MeHg can be destroyed or formed. The various approaches for MeHg isolation from sediment are schematically shown in ***Figure II.1.***

Figure II.1: *Brief scheme of various approaches for MeHg isolation from sediment*



Distillation is a simple and specific isolation procedure for MeHg. The advantage of the distillation method over alkaline digestion is avoidance of matrix effects during the ethylation process. Distillation gives consistent and high recoveries and therefore provides more accurate results.. In order to extract the mercury species intact, several types of milder or diluted agents are used, e.g. KBr/$CUSO_4$/H_2SO_4 (Lambertsson et al., 2001); urea/HCl/$CuSO_4$ (Gilmour et al., 1992); dilute HCl/HNO_3 mixtures (Dietz et al., 2001), or HNO_3 (Tseng et al., 1997).

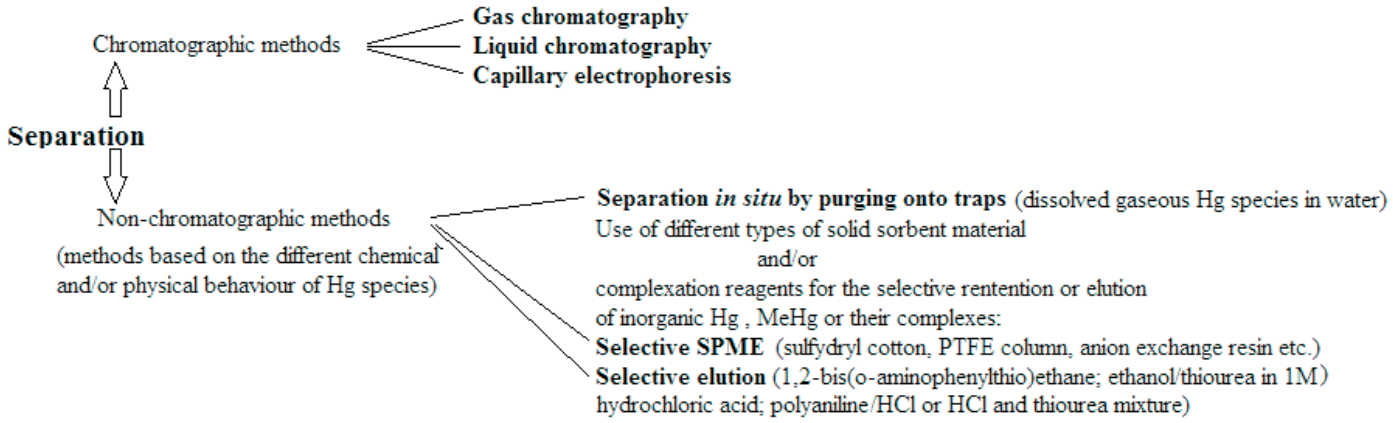
Alkaline digestion based on extractions in KOH-methanol have been proposed to release MeHg from biological samples and sediments, nevertheless for sediments, high levels of organic matter, sulfides or ferric ions pose the problems due to its co-extraction with MeHg species.

Extraction is efficient procedures for isolation of various organic and organometallic compounds from environmental matrices. For MeHg acid extraction at the room temperature, microwave-assisted acid extraction (Tseng et al., 1997) and microwave-assisted organic solvent extraction (Vazquez et al., 1997) can be used and has been evaluated in various environmental applications. MW extraction is extremely fast method (Lorenzo et al., 1999) and both open vessel (working at atmospheric pressure) and closed vessel (working under controlled pressure) microwave ovens can be used for alkaline or acid extractions of mercury species from the sediments. MW extraction requires smaller volumes of organic solvents than the conventional techniques and simultaneous extraction of several samples is possible. On the other hand, the MW extraction of sediments should, however, be used with caution since it may also suffer from artifact formation of $MeHg^+$. (Sanz Landaluze et al., 2004). Avoiding artificial methylmercury production is important during the isolation, because natural sediments often contain very low amounts of MeHg (not more than 1,5% of HgT) so even a small proportion (0,02 – 0,03% of inorganic Hg) artificial Hg methylation occurs, it can result in 30-80% overestimation of MeHg concentrations in sediment. Acid leaching with H_2SO_4/KBr/$CuSO_4$ at room temperature followed by CH_2Cl_2 extraction and back extraction in water did not give rise to methylation artifacts (Bloom et al., 1997). The isolation procedure is followed by a separation step, such as ion exchange. Separation is generally performed by gas chromatography often with comvination with a derivatization step (ethylation, hydrid generation).

II. I. 3.Derivatisation and separation of mercury species

Separation methods for Hg speciation can be classified into two general approaches: chromatographic methods (GC, LC, CE) and non-chromatographic methods based on the chemical and physical properties of different Hg species. The scheme on ***Figure II.2***. shows the various approaches for Hg species separation.

Figure II.2: *Scheme of various approaches for MeHg separation*



Chromatography is usually a more powerful separation technique than non-chromatographic approaches. However, simple non-chromatographic approaches can be successful to separate adequately one or two species in a given sample (Sánchez Uria and Sanz-Medel, 1998).

Non-chromatographic methods for Hg speciation mostly determine only one species or one fraction rather than the simultaneous determination of all Hg species present. From non-chromatographic methods, selective SPME has several advantages over other separation techniques, especially with regard to the preservation of the sample. In the last decade SPME methods have been shown to be a good approach for the storage of Hg species. The analyte species are adsorbed on a solid phase in the field rather than stabilized in a liquid sample for transport and storage. However, practical benefits are only achieved if the applied method has few procedural steps and uses easily cleanable reagents with low reagent consumption.

In fact, speciation of Hg in environmental samples is mainly carried out by chromatographic separation techniques and particularly gas chromatography (GC) (Bouyssiere et al. 2002). In contrast to other speciation approaches chromatographic methods are able to separate all Hg species in a single step. GC is the preferred method because Hg is occurring as volatile species or compounds able to form easily volatile species without uncontrolled changes of speciation during separation processes (Sánchez Uria and Sanz-Medel, 1998). There is a trend today to use open capillary columns, of the non-polar type. It appears that this type of columns provide more efficient separations and better resolution, as compared with Chromosorb-type GC columns. Moreover the more common GC detectors might be lacking the required selectivity to be used in speciation of Hg in environmental samples.

Prior to gas chromatographic separation, Hg species are usually derivatised in order to form non-polar or volatile species, respectively. These can be separated on non-polar packed (OV3 on Chromosorb W) and/or capillary columns

Derivatisation reactions are a key step in the analytical procedure for Hg speciation following separation and are likely to have the most significant impact on accuracy (Leopold et al. 2010). Different derivatisation techniques for Hg speciation have been reported. The most common are hydride generation with sodium borohydride, ethylation or propylation with sodium tetraethylborate or tetrapropylborate, respectively and derivatization with a Grignard agent. The ethylation reaction is usually achieved by derivatisation in the solution of Hg^{2+} and MeHg at pH 4,9 with sodium tetraethylborate to form volatile diethylmercury and methylethylmercury, respectively. The principal chemical reactions are:

$$Hg^{2+} + 2NaB(C_2H_5)_4 \rightarrow Hg(C_2H_5)_2 + 2Na^{+} + 2B(C_2H_5)_3$$

$$CH_3Hg^{+} + NaB(C_2H_5)_4 \rightarrow CH_3HgC_2H_5 + Na^{+}\ B(C_2H_5)_3$$

The optimum pH necessary for derivatisation reaction is 4,9 and is reached addition of acetate buffer.

The ethylated species are volatile and can be purged from solution at room temperature and collected on sorbents, such as Carbotrap or Tenax. After thermal release, the Hg compounds are transferred to a GC system, where individual Hg compounds are separated by cryogenic, isothermal or temperature-programmed GC. The Hg species eluted from the column are thermally decomposed in a pyrolitic step before being measured by a Hg-specific detector (e.g.CV-AFS, CV-AAS, QF-AAS, MIP-AES or ICP-MS).

The critical part of this procedure is sample preparation prior to ethylation. MeHg must be removed from its binding sites to facilitate the ethylation and inferring compounds (e.g. chlorides and sulfides) must be also removed. Ethylation cannot be used for the determination of other organomercurials and is not specific in cases where ethylmercury compound are present in the original sample. That's the reason why other derivatisation agents have been investigated (Leermakers et al., 2005).

II. I. 4.Detection of mercury species

The analytical sensitivity and selectivity requirements for reliable Hg-speciation analysis can be achieved by using hyphenated techniques, coupling chromatographic separation methods on-line to Hg-specific detectors (Leermakers et al., 2005).

The most common detector used for mercury species are Electron Capture Detector (ECD), element-selective detectors as the Atomic Absorption Detector (AAS), Atomic Fluorescence Detector (AFS), Microwave Induced Plasma Atomic Emission Detector (MIP-AES), Inductively Coupled Plasma Atomic Emission Detector (ICP-AES) and Inductively Coupled Plasma Mass Spectrometry Detector (ICP-MS).

Detectors used with HPLC to determine organomercury species in sediments are AFS (Hintelmann and Wilken, 1993) and ICP-MS. (Wilken and Falter, 1998) The detectors used with GC for analysis of environmental sediments samples are Cold Vapour Atomic Absorption Detector (CV-AAS)(Rodriguez Pereiro et al., 1998), AFS (Bowles and Apte, 2000; Tseng et al., 2004), MIP-AES (Dietz et al., 2001; Sanz Landaluze et al., 2004) and ICP-MS.(Jitaru and Adams, 2004; Rodriguez Martin-Doimeadios et al., 2004). ***Table II.1*** resumes different detectors used for Hg speciation with respective detection limits (Sánchez Uria and Sanz-Medel, 1998; Leermakers et al., 2005) and ***Table II.2*** resumes the detailed information on the selected methods for determining Hg in sediment samples.

MeHg in environmental samples could be determinate using a rapid and automated method: Headspace gas chromatography with atomic fluorescence detection in combination with aquous phase ethylation (**Chapter III**).

In the analytical techniques applied in stable isotope tracer study, precise and accurate determinations of isotopes are required. Since a mass spectrometer separates and detects ions of slightly different masses, it easily distinguishes different isotopes of mercury. From this point of view ICP-MS technique allows the measurement of individual isotopes with sufficient sensitivity (**Chapter IV**).

Table II.1: *The separation techniques with specific detection for Hg speciation (Sánchez Uria and Sanz-Medel, 1998).*

Separation	Detectors	Detection limits
GC	CV-AAS	5 – 167 pg
HPLC	CV-AAS	4 - 16 $\mu g.l^{-1}$
GC	ETAAS	0,04 ng
GC	CV-AFS	0,6 – 1,3 pg; 0,01 – 6 $ng.l^{-1}$
HPLC	CV-AFS	0,015 – 0,1 μg
GC	MIP-AES	0,04 – 10 $ng.l^{-1}$
HPLC	CV-MIP-AES	0,35 $ng.ml^{-1}$
GC	ICP-AES	3 pg; 0,6 $ng.l^{-1}$
HPLC	ICP-AES	0,1 $ng.ml^{-1}$
GC	ICP-MS	0,12 – 1 pg
HPLC	ICP-MS	16 – 400 $ng.l^{-1}$

Table II.2: *Methods for determination of mercury species (Stoichev et al., 2006;* ***U.S. Department of health and human services****, March 1999).*

Sample	Preparation method	Analytical method	Sample detection limit	Reference
Soil, sediments, sludge	Digestion of sample with aqua regia and permanganate in stream bath or with HNO_3/H_2SO_4 and permanganate in autoclave, reduction with hydroxylamine, purging to detector	CV- AAS	0,1 mg/L	(Beckert et al., 1990)
Soil, sediment (methyl Hg, phenyl Hg)	SFE of spiked sample using CO_2 methanol containing diethlammonium diethyditiocarbamate, dilution with octane, addition of pentylMgBr to form pentyl derivatives, addition of H2SO4, extraction of organic phase with water, treatment with anhydrous magnesium sulphate	GC/AED	2,5 ng/mL in extract	(Liu et al., 1994)
Sediment (HgT)	Digestion of sample with concentrated acid; evaporation, redissolution in HNO_3 and dilution with water ; reduction of sample with $SnCl_2$ in HNO_3; purging to detector	CVAAS	0,1 ng/L	(Soo et al., 1989)
Sediment (HgT)	Digestion of sample with HCl/HNO_3 and heat in Teflon bomb; oxidation with potassium permanganate solution; reduction with sodium borohydride; purging to plasma	ICP/MS	2 ng/g	(Haraldsson et al., 1989)
Solid samples (HgT)	Introduction of a slurry of sample in nitric acid into FIA system using on-line microwave digestion, mix with tin (II) chloride to form elemental mercury	CV-AFS	0,09 ng/g	(Morales-Rubio et al., 1995)
1g sediment sample (Hg^{2+}, $MeHg^{+}$)	MW extraction, HNO_3	Eth-CT-GC-AAS	0,5 ng/g	(Tseng et al., 1998)
5g sediment sample ($MeHg^{+}$, $EtHg^{+}$)	Citrate buffer extraction/back extraction	HPLC-VGAFS	0,1 ng/g	(Hintelmann and Wilken, 1993)
0,5g sample, ($MeHg^{+}$)	$KBr/Cu^{2+}/H_2SO_4$ extraction/back extraction	Eth-PTI-CGC-ICP-IDMS	0,3 ng/g	(Lambertsson et al., 2001)
0,4g sample	MW extraction, diluted aqua regia	Eth-CCT-CGC-MIP-AES	0,08 ng/g	(Dietz et al., 2001)
1g wet sample ($MeHg^{+}$)	$KCl/ Cu^{2+}/H_2SO_4$ (stream distilation)	Eth-PTI-GC-AFS	0,01 ng/g	(Bowles and Apte, 2000)
0,5g samle ($MeHg^{+}$)	$NaCl/ Cu^{2+}/H_2SO_4$ (vacuum distilation)	HG-CT-GC-AFS	0,2 ng/g	(Morrison and Weber, 1997)
1g sample (Hg^{2+}, $MeHg^{+}$)	MW extraction HNO_3	Eth-CT-GC-AFS	0,02 ng/g (MeHg)	(Stoichev et al., 2004b)
1g sample (Hg^{2+}, $MeHg^{+}$)	MW extraction HNO_3	Eth-Extraction-MCGC-MIP-AES	0,2 ng/g	(Rodriguez Pereiro, et al., 1998)

II. II. Conditions for ultra-trace Hg analysis

While applying the method for trace mercury determination, one of the greatest difficulties could be precluding sample contamination during collection, transport and analysis. Therefore, it is imperative that extreme care must be taken to avoid contamination when collecting and analyzing the samples for trace mercury. The choice of suitable material for sampling is indispensible. For manipulation with water samples while mercury should be determined, it is recommended to use fluoropolymer or glass vessel. Polypropylen (PP) or polyethylene (PE) sampling materials pose the problems caused by Hg sorption on the walls of vessels.

A lot of analytical results could be incorrect due to the contamination of samples during sampling, transport, storage and manipulations. The critical point of Hg speciation analysis is the cleaning of the material and the vessels which are used for the sampling, the storage and samples preparation. Hence these steps have to be carefully controlled because of possible source of error.

II. II. 1. Cleaning

The bottles for water sampling were cleaned by heating to 65 – 75°C in 4M HCl for 48 hours. After cooling down, the bottles were rinsed three times with reagent water, and filled with reagent water containing 1% HCl. The bottles were capped and placed in a clean oven at 60°C for 24 hours. After cooling they were rinsed with Milli-Q water and filled with reagent water containing 0,4% HCl. The bottles were tightly capped and placed into the double zip-bags.

The equipment for sediment sampling were cleaned by washing using Milli-Q (18.2 MΩ cm, Millipore) water after 24 hours soaking in the diluted nitric acid (HNO_3:H_2O = 1:10, V/V for glass vessel, HNO_3:H_2O = 1:100, V/V for plastic and Teflon vessels), 24 hours soaking in the diluted chloric acid (HCl:H_2O = 1:10, V/V for glass vessel, HCl:H_2O = 1:100, V/V for plastic and Teflon vessels) and then dried under streamline flow.

All **laboratory glass, plastic and Teflon vessels** which are in contact with the sample must be also well decontaminated before using in order to avoid the contamination. The following protocol of decontamination was applied:

1. Hot RBS (detergent) and water bath, overnight
2. Hot water washing,
3. Milli-Q water washing, 1 hour in ultrasonic bath
4. First bath of 10% HNO_3 (for glass vessels) or 1% HNO_3 (for Teflon materials), 1 hour in ultrasonic bath
5. Milli-Q water washing and hot Milli-Q water bath for 1 hour in ultrasonic bath
6. Second bath of 10% HNO_3 (for glass vessels) or 1% HNO_3 (for Teflon materials), 1 hour in ultrasonic bath
7. Milli-Q water washing and hot Milli-Q water bath for 1 hour in ultrasonic bath
8. Bath of 10% HCl (for glass vessels) or 1% HCl (for Teflon materials), 1 hour in ultrasonic bath
9. Milli-Q water washing and hot Milli-Q water bath for 1 hour in ultrasonic bath
10. Drying under laminar flow hood

All cleaned materials were stored in double zip-bags until using.

Trace analysis **cleaning** and handling techniques ware also used throughout the study of using **DGT technique** for Hg determination. All plastic ware and glass plates (for gel casting) were acid-washed in nitric acid (HNO_3:H_2O = 1:10, V/V) followed by thorough rinsing with Milli-Q water.

All cleaning as well as solution preparation and sample handling were undertaken wearing disposable powder free gloves.

II. II. 2. Certified reference materials

For check the quality and traceability of product and to control the analytical protocol the certified reference materials (CRM) were used. Estuarine sediment IAEA 405/IAEA 433/IAEA 158 and CRM 580 purchased from the International Atomic Energy Agency (IAEA, Vienna, Austria) and from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium), respectively were used to validate the analytical method for total Hg and MeHg analysis. Each analysis of sample and validation of

analytical protocol followed the analysis of CRM. Certified mercury and methylmerucry contents are listed in ***Table II.3.***

Table II.3: *Certified values for HgT and MeHg.*

CRM Sediment	HgT (mg kg^{-1})	MeHg (mg kg^{-1})
IAEA - 405	0,810 ± 0,030	5,9 ± 0,57
IAEA - 433	0,168 ± 0,004	0,17 ± 0,05
IAEA - 158	0,132 ± 0,014	-
CRM - 580	132,0 ± 3,0	75,5 ± 3,7

II. III. Determination of HgT

II. III. 1. Determination of HgT by AMA 254

Advance Mercury analyzer (AMA-254, Altec, s.r.o. Praha, CZ) is device based on the dry decomposition of liquid or solid sample in a stream of oxygen and passage of the combustion gases through the catalytic column, followed by trapping Hg^0 on the gold amalgamator. Heating the amalgamator rapidly evaporates preconcentrated mercury which is then transported into the system of measuring cells, and atomic absorption of free mercury atoms is measured. The whole analysis is performed automatically without the need of preliminary dissolution and/or decomposition of the sample. Instrument has a good sensitivity (detection limit 0,01 ng Hg), reproductibility (< 1,5%) and reliability of operation. Working range is 0,05 to 500 ng Hg (AMA, 1994). The results of mercury determination by AMA-254 suggested good agreement with certified values of reference materials (Duran and Adams, 2002). During the years several instruments based on similar principle developed including some commercially available devices (Brandvold et al., 1993; Bin et al., 2001; Cizdziel et al., 2002; Operator Manual, 2003).

II. III. 2. Determination of HgT by AFS

For determination of mercury in water the method of cold-vapor fluorescence spectrometry (CVAFS) was used (EPA 245.7). This method may be used to determine Hg up to 200 $ng.L^{-1}$. The method was used for determination of total dissolved mercury (all $KBrO_3$/KBr-oxidizable mercury forms and species found in the filtrate aqueous solution. The principle of determination is reduction of Hg(II) to Hg^0. The Hg^0 is then liberated from the sample solution and pre-concentrated on a gold surface before being thermally desorbed prior to analysis.

II. IV. Article: Improvement in determination of methylmercury in sediments by Headspace Trap Gas Chromatography and Atomic Fluorescence Spectrometry after aquous phase ethylation

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Abstract:

An automated method for determination of methylmercury in sediments was developed using Headspace sampler equipped with Trap coupled to Gas Chromatography (GC) with Atomic Fluorescence Detection (AFS) in combination with aqueous phase ethylation. The appropriate volume of ethylating agent was found. The method avoids the problems associated with the purge and trap technique. The Headspace only (HS) or Headspace Trap (HS Trap) mode can be used. The results shown that combination of HS with Trap offered better performances for methylmercury determination than the HS only, regarding several aspects. The organic extraction of methylmercury from sediment samples was used (the procedure include the use of $CuSO_4/KBr/H_2SO_4$ solution). The method was tested using the sediment reference materials (IAEA-405; IAEA-433) and the results were in good agreement with certified values. Due to the low detection limit the method can be used for analysis of contaminated as well as uncontaminated sediment samples.

Keywords: methylmercury, Headspace, Gas Chromatography, Atomic Fluorescence Spectrometry

Introduction

Organomercury compounds especially methylmerucury (MeHg) belong to the most toxic and dangerous contaminants in the environment. Due to its high bioaccumulation and biomagnification potencial along the food chain MeHg can be found concentrated at the

end of the food web – in the top predators even through the low concentration in the environment. The lack of knowledge on the toxic impact of MeHg in sediment and the need to understand better the environmental pathways justify its monitoring in the different matrices - water, sediment, biota (Quevauviller et al., 1998). Considerable effort in the development of reliable, sensitive and precise analytical methods for MeHg determination was made (Berzas Nevado *et al*., 2008; Yang *et al*., 2009; Davis *et al*., 2004; Yair, 1975; Horvat *et al*., 1990; Bloom, 1989). The determination of methylmercury is a special challenge due to its low concentration in environmental samples and difficulties in samples handling, preparation technique before analysis and problems with possible methylation artefacts during analysis run.

The automated method for determination of MeHg after extraction and aqueous phase ethylation using headspace (HS) with atomic fluorescence detection (AFS) was firstly reported by (Leermakers *et al*., 2003) for analysis of MeHg in sediments, waters and biological samples. A new in this paper is investigation the potential of Headspace method for the analysis of MeHg in sediments samples. The Headspace is coupled to a gas chromatograph and enable direct analyte introduction to the system (HS only) or precontration on the Tenax Trap (HS Trap). The different operation conditions for Headspace and in addition also the different ethylation condition were compared.

Methods and materials

Reagents

For the sample extraction sulphuric acid (96% H_2SO_4 p.a.) and potassium bromide (KBr p.a.) were purchased from Merck (Darmstadt, Germany) whereas copper sulphate pentahydrate ($CuSO_4.5H_2O$ p. a.) was purchased from Acros Organics (New Jersey, U.S.A). 18% (w/v) KBr in 5% (v/v) H_2SO_4 and 1M $CuSO_4$ solutions were prepared in glass bottles by dissolving in Milli-Q water.

1 g Sodium tetraethylborate (min 98%, Stream Chemicals, Newburyport, U.S.A.) was used to prepare a 1% solution in 100 mL Milli-Q water containing 2% KOH (p.a., Merck, Darmstadt, Germany). The solution was divided into 10 mL Teflon bottle and stored deep-frozen until use. The acetate buffer (2M) was prepared by dissolving 82 g sodium acetate (CH_3COONa p.a., Merck, Darmstadt, Germany) and 59 mL glacial acetic acid (CH_3COOH, Scharlau Extrapur) in 500 mL Milli-Q water.

Dichloromethane, the organic solvent, used were of HPLC grade. All other chemicals and analytical agents used were of highest available purity. Deionised water obtained from Milli-Q system was used for preparation of all dilutions. Argon C-50 (Air liquid, France) was used as a carrier gas at the transfer line and AFS detector.

Standard solutions and Certified Reference Sediment

Stock standard solutions of 1 mg L^{-1} of Hg^{2+} and MeHg were prepared in volumetric flask by diluting 100 µL a commercially available mercury standard solution (1 000 ppm Hg in 2 mol L^{-1} HNO_3, Merck, Darmstadt, Germany) in deionised Milli-Q water (18.2 MΩ cm, Millipore, France) with 0.5 ml HNO_3 (65%, Merck, Darmstadt, Germany) to a final volume of 100 mL and 50 mL monomethylmercury chloride

(CH_3HgCl, Stream Chemicals, Newburyport, U.S.A) in Milli-Q water with 2-3 mL methanol to a final volume of 50 mL, respectively. These solutions are stable few months and were used for preparation standard solutions of 1 μg L^{-1}. All stock solutions were stored in dark in brown glass bottle at a temperature 4°C. Working standard solutions used for calibration were freshly prepared daily by proper dilution with Milli-Q water. Certified Reference Sediment for methylmercury (IAEA-405 and IAEA-433) from the International Atomic Energy Agency (IAEA) was used for the optimisation, the recommended value of methylmercury concentration are: 5.49 ± 0.53 ng Hg $.g^{-1}$ for IAEA-405 and 0.17 ± 0.07 ng Hg $.g^{-1}$ for IAEA-433.

Cleaning procedure

All glassware and Teflon vessel used in the analysis were soaked in 5% nitric acid overnight, then rinsed with Milli-Q water, soaked in Milli-Q water overnight and rinsed again. Teflon and glassware used in the extractions were soaked in diluted acids (5% HNO_3 + 1% HCl) and rinsed with Milli-Q water. All was dried under laminar flow.

Sample preparation

Organic solvent extraction

For quantification of MeHg concentration the method firstly porposed by (Bloom *et al.*, 1997) is used. 0.1 – 0.2 g sediment is weight and placed into a 25-mL glass vial. 1 mL of 1 $Mol.L^{-1}$ $CuSO_4$ solution and 5 mL of 18% (w/v) KBr in 5% H_2SO_4 (v/v) solution are

added. The mixture is shaken in vertical position on 400 rpm for 50 min and then after adding 10 mL CH_2Cl_2 it is shaken for additional 50 min, followed by centrifugation for 15 min at 3000 rpm to separate organic, aqueous and solid layer. 5 mL of organic layer is transferred to a 50 mL conic glass bottle with 20 mL Milli-Q water.

Leermakers *et al* (2003) included in the procedure a CH_2Cl_2 extraction step, in order to eliminate the matrix effects. The results shown that inorganic Hg is not quantitatively extracted with CH_2Cl_2, however for MeHg analysis, it has the advantage that no very large inorganic Hg peaks were observed when analysing contaminated sediments.

CH_2Cl_2 layer was back-extracted into the Milli-Q water by solvent evaporation at 46°C (water bath - Compatible Control CC3, Offenburg/Germany) and under constant N_2 flow, when there were no more visible drops of CH_2Cl_2. CH_2Cl_2 extraction and back-extraction in water showed no methylation artefact in the samples analysed (Leermaker *et al.*, 2003).

After extraction, the samples are analysed same day. For analysis, 5 mL aqueous extract was transformed and diluted in the 22 mL headspace vials with 5 mL Milli-Q water.

Ethylation

For analysis, 5 mL aqueous extract is transformed and diluted in the 22 mL headspace vials with 5 mL Milli-Q water. 50 µL of acetate buffer for optimal pH 4.8 – 5.2 (Lansens *et al*., 1991; Tseng *et al*., 1997) and sodium tetraethylborate solution for

ethylation are added. The vials were sealed with Teflon-coated butyl rubber septa and Al crimp caps. Before analysis, the solution was shaken and allowed to react in the dark (Leermakers *et al*., 2003).

Optimization was carried out on MeHg standard solutions (5 – 50 ng L^{-1}). Procedure was firstly performed and validated on standard IAEA 405, estuarine sediment.

Analytical instrument analysis:

The analytical system consists of HS - TurboMatrix 40 automated Headspace sampler and Gas chromatograph Clarus 500 from Perkin Elmer. Transfer line (heated fused silica) is directly connected to the packed GC non-polar capillary column consists of a 80 cm long Teflon tube - PTFE O.D. ¼", I.D. ⅛", 15 x 0.25 mm x 0.25 μm (Altech) packed with 10% OV3 on Chromosorb WAW DMCS (60/80 mesh). The outlet of gas chromatography is coupled to a cold-vapor atomic-fluorescence detector, Model 2600 CVAFS Mercury Analysis System from Tekran via a pyrolysis unit from Varian. The pyrolysis oven - ETC-60 was provided with an Electrothermal temperature Controler panel. All data were acquired by TotalChrom Navigator System, Version 6.3.0.0445 (Perkin Elmer).

Operating conditions for HS-GC-AFS and pyrolysis are based on parameters previously optimized (Bloom, 1989; Liang *et al*., 1996; Leermaker *et al.*, 2003; Berzas Nevado *et al.*, 2008) and modified by varying during optimization procedure.

Headspace only (HS) and Headspace Tenax trap (HS Trap)

The sample vials (22 mL) with 10 mL of sample are thermostated for 5 min at 70°C. The pressurization time is 30 s and injection time is 15 s. A modification of the technique is used Tenax trap for precontcentration. Thermostating time is 5 min, presurization time 30 sec, injection time 15 s and withdrawal time is 20 s. The needle temperature and transfer line temperature is 100°C and 105°C, respectively, to reduce condensing and injection of water vapour.

Gas Chromatograph (GC) - Atomic-Fluorescence Detector (AFS)

Optimal Ar gas flow rate of 60 mL/min, maintained through analysis, allow to injection of volatile species into the GS system and passing through the gas chromatographic column. The temperature program of chromatograph oven is chosen in order to improve separation between mercury species thus the initial temperature is 50°C, final temperature is 100°C and ramp temperature is 25 $mL.min^{-1}$.

The mercury compounds are then transferred through the pyrolysis oven, where the temperature is held on 800°C, to the atomic fluorescence detector. Signal processing is performed using TotalChrom Navigation system.

Results and discussion

Ethylation

The analytes react with $NaBEt_4$ in the following way to give the corresponding volatile mercury species (Rapsomanikis et al., 1991):

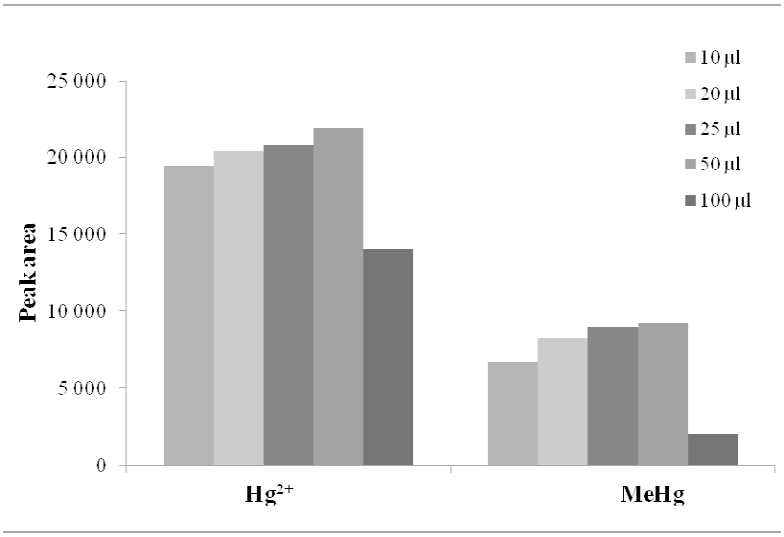
$$Hg^{II} + 2\ NaBEt_4 \rightarrow HgEt_2 + 2Na^+ + 2BEt_3$$

$$MeHg^+ + NaBEt_4 \rightarrow MeHgEt + Na^+ + BEt_3$$

BEt_3 represents an unstable compound, which reacts with air and water.

Concentration (1%) of ethylating agent and reaction time was based on the experiences with headspace analysis of MeHg (Lansens and Baeyens, 1990). For optimization of conditions the standard solutions contain MeHg (1 – 50 $ng.L^{-1}$) only or mixture of MeHg and HgT was used. 50 µL aliquot of acetate buffer added to the sample was found to be optimal to stabilise the pH at 4,7 – 5,0. The volume of ethylation agent ($NaBEt_4$) added has the main effect on formation of ethylated compounds. The results on fig. 1 show that the optimal volume is 50 µL.

Fig. 1 Optimization of volume $NaBEt_4$

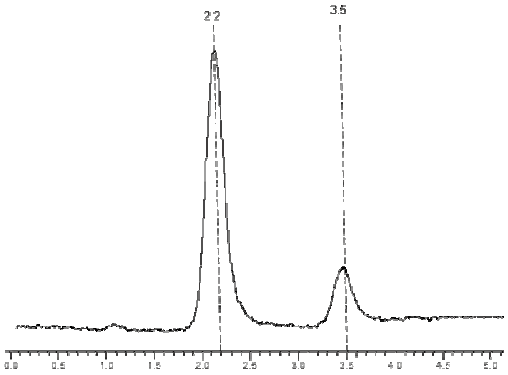


Significance is also a minimum reaction time of ethylation, at 20°C a minimum of 45 min was found to be necessary for ethylation reaction to obtain a stable signal.

Headspace only (HS) and Headspace Tenax trap (HS Trap)

The volatiles ethylation derivates in samples were directly introduced to the GC column by HS or injected after pre-concentration (trapping) on Tenax column (HS Trap). Mercury species (Hg^0: MeHg determined as methylethyl mercury - MeHgEt and Hg^{2+} determined as diethyl mercury – $HgEt_2$) are than separated by isothermic chromatography in gaseous phase and the compounds outgoing from the chromatographic column are thermal decomposed under the elementary form of mercury and measured by AFS. The species are identified on their retention time, peak at 2.25 min corresponds to MeHg while peak at 3.5 corresponds to Hg^{2+} (see fig. 2).

Fig. 2 Chromatogram of 20 ng.L^{-1}MeHg standard after ethylation (50µL NeBEt$_4$) and HS Trap GC-CV-AFS analysis peak area as function of retention time in min (MeHg peak at 2.2 min retention time)



Operating conditions for HS-GC-AFS and pyrolysis were based on parameters previously optimized (Leermakers *et al*. 2003; Bloom, 1989; Liang *et al.*, 1996; Berzas Nevado *et al*., 2008) and modified by varying during optimization procedure. Parameters are summarized in Table 1.

Table 1.: HS-GC operation conditions:

Headspace parameters	
Thermostatic heating time	5 min
Pressurization time	30 s
Injection time	15 s
Withdrawal time	20 s
Needle temperature	100°C
Transfer line temperature	105°C
Sample volume	10 ml
Gas-Chromatography parameters	
Column	PTFE O.D. ¼", I.D. ⅛",15 x 0.25 mm x 0.25 µm, Altech
Initial temperature	50°C
Initial time	1 min
Ramp temperature	25°C/min
Final temperature	100°C
Final time	2 min
Carrier gas flow rate	60 ml/min
Pyrolysis	
Pyrolysis temperature	800°C

Headspace temperature and dry purge time are the main factors affect the species separation and determination. Dry purge time adjustments influence the injection of water vapours to the GC. When the dry purge time is too short the *'false peak'* appears at the time of 0.5 s, while longer dry purge time cause the elimination of water vapours introduction into the GC and its detection (see fig. 3). Water vapours introduced to the GC may cause quenching during CV-AFS detection and false peak appears due to scattering of the excitation radiation. The false peak may influence the interpretation of the MeHg and Hg^{2+} content in the sample.

Different dry purge times were tested and the results are shown on the fig. 4. Increase of dry time purge decrease the recovery of Hg^{2+} till the 3 min and so the better survey of MeHg peak area is achieved. But the dry purge time longer than 3 min affected the increase the recovery of Hg^{2+} and the survey of MeHg peak area is negatively influenced. The dry time purge of 3 min is optimal for the best sensitivity for MeHg recovery is achieved.

Fig.3 Comparison of chromatograms (20 ng.L^{-1}MeHg standard after ethylation with 50µL NeBEt$_4$ and HS Trap GC-CV-AFS analysis, peak area as function of retention time in min) with different dry purge time (0.1 min and 5 min)

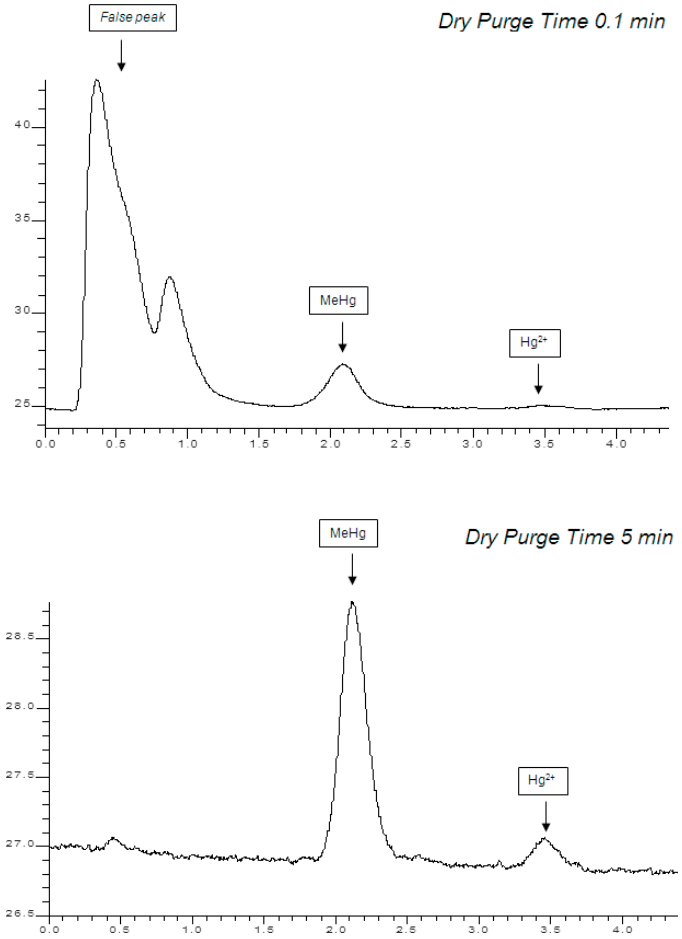
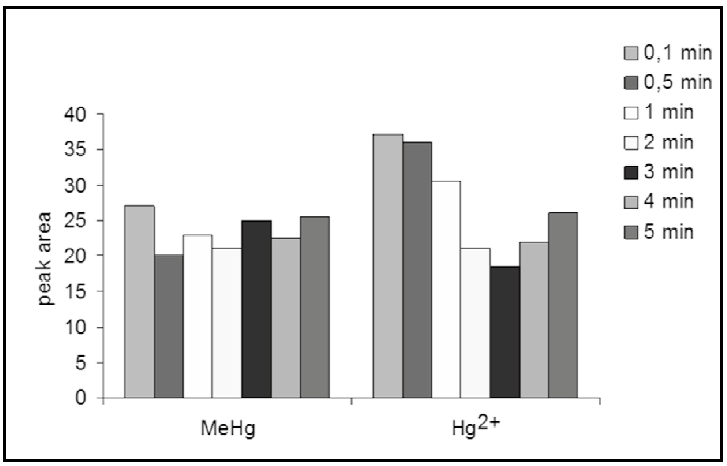


Fig.4 Peak area recovery after analysis of 20 ng.L^{-1}MeHg standard (ethylation: 50µL NeBEt$_4$) by HS Trap GC-CV-AFS



The species are quantified on the peak area. The automated Headspace enables not only the HS Trap selection, but also the selection of HS Trap conditions. For headspace, the temperature of equilibration is important. The use of HS Trap enabled use of higher thermo-stating temperature (up to 94 °C, comparing to HS where temperature was limited on 70 °C), due to possibility of elimination of water vapours from the gas phase during the drying step of the trap. Peak heights increased with increasing temperature. The optimal temperature is 74°C, because further increase of temperature leads to undesired water vapour injection and peak tailing of the Hg^0 – false peak and so the survey of MeHg is influenced (figure. 5 and figure. 7).

Fig.5 Calibration of 5, 10, 15, 20 ng.L^{-1}MeHg standard; ethylation: 50µL NeBEt$_4$ and analysis by HS GC-CV-AFS while using HS only, HS Trap with thermo-stating temperature 74°C and 90°C

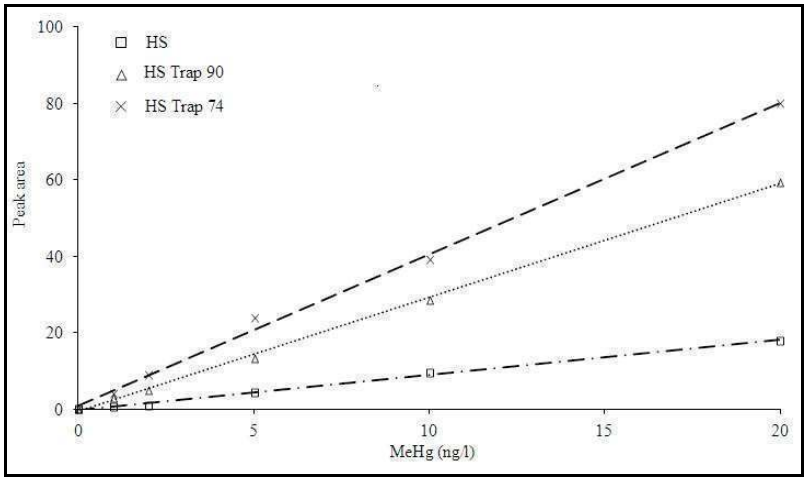
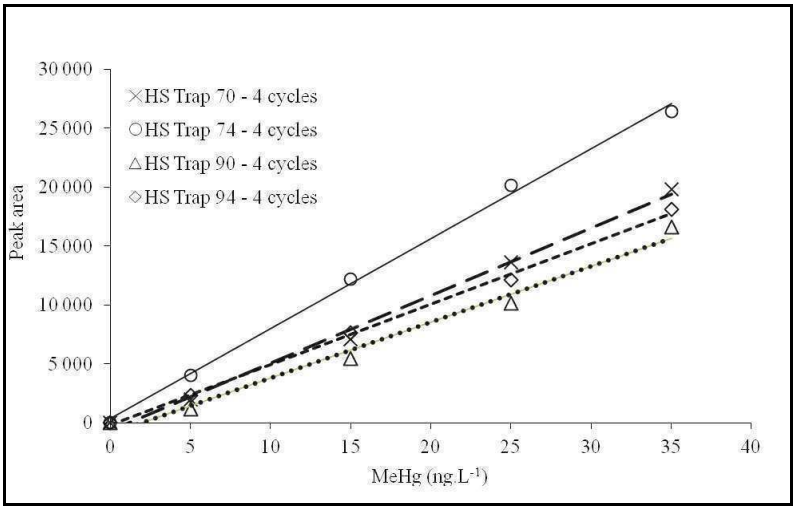
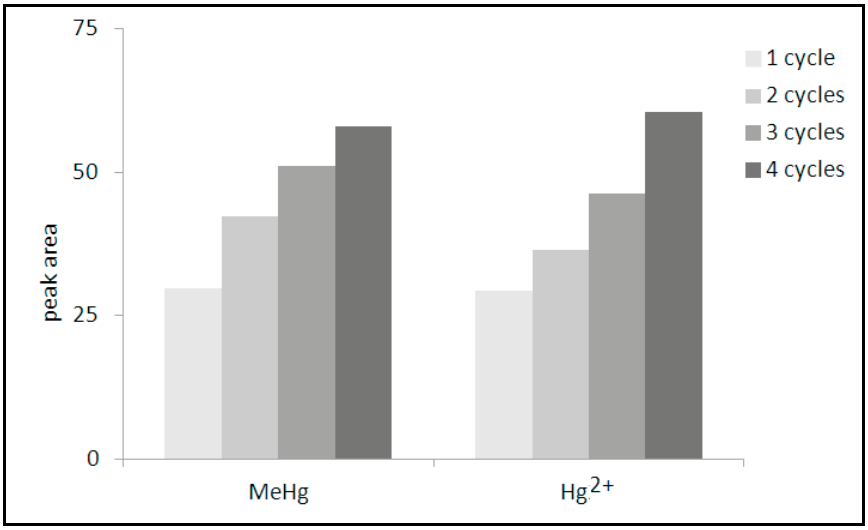


Fig.6 Calibration of 5, 15, 25, 35 ng.L^{-1}MeHg standard; ethylation: 50µL NeBEt$_4$ and analysis by HS GC-CV-AFS while using HS Trap with 4 cycles and thermo-stating temperature 70°C/74°C/90°C/94°C



HS Trap system offered also better recovery of methylmercury and also better detection limit due to possibility of pre-concentration in several cycles, so a greater part of the analyt is introduced into GC (figure. 6, figure 7).

Fig.7 Recovery (%) after pre-concentration of 10 ng.L^{-1}MeHg standard; ethylation: 50µL NeBEt$_4$; analysis by HS GC-CV-AFS while using HS Trap with thermo-stating temperature 70°C



Calibration and evaluation of detection limit, limit of quantification and precision

A validation procedure (organic solvent extraction and ethylation) was carried out for MeHg. A five-point calibration was used during the measurements to obtain high confidence for the calibration line. Statistical evaluation of method for MeHg determination was based on ISO-84661. Ten triplicate analyses of a 10 ng/L MeHg and 20 ng/L shoved a RSD of 3.7 % and 3.1% respectively (method HS Trap 74°C). The detection limit calculated as three times the standard deviation of the noise (blank determination) was 1.12 $ng.L^{-1}$ as Hg (HS Trap 74°C). The accuracy of the extraction procedures with cleanup step included was tested by the method HS Trap 74°C and analyses of reference material (IAEA-405, IAEA-433) and analysis of real river sediments (taken from the river Deule – the results presented in Kadlecova et al., 2011). The results obtain by testing the certified reference materials (IAEA-405: 5.85 ± 0.45 ng Hg $.g^{-1}$, IAEA-433: 0.18 ± 0.09 ng Hg $.g^{-1}$) were in good agreement with certified value (IAEA-405: 5.49 ± 0.53 ng $Hg.g^{-1}$, IAEA-433: 0.17 ± 0.07 ng $Hg.g^{-1}$). Repeatability (RSD; n=5) calculated for the analysis of certified material was 4.86% and a detection limit of 0.27 $\mu g.kg^{-1}$ for 0.2 g sediment and back-extraction of 5 mL of organic layer into 20 mL Mili-Q water was obtained.

Conclusion

The benefit of this paper is use of automated Headspace as a sample introduction system for MeHg analysis by GC – CV – AFS instrument. A Perkin Elmer TurboMatrix HS-40 Trap (filed with Tenax sorbent for MeHg pre-concentration) coupled with GC (Clarus 500, Perkin Elmer) and CV-AFS detector (Tekran 2600) was tested for analysis of

methylmercury in sediments and methods HS only and HS Trap are compared. Optimal volume 50 μL of ethylation agent was found to be appropriate. The results shown that combination of HS with Trap offered better performances for methylmercury determination than the HS only, regarding several aspects. The use of trap enabled use of higher thermostating temperature (the use of trap enable elimination of water vapors from the gas phase during the drying step of the trap) and offer better detection limit due to possibility of pre-concentration in several cycles, so a greater part of the analyt is introduced into GC. The method avoids the problems associated with the purge and trap technique and sufficient sensitivity can be achieved to analyze MeHg in sediments samples, this is confirmed by sediments analyses of certified reference materials IAEA 405 and IAEA 433.

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Chapter III: DETERMINATION OF MERCURY SPECIES IN AQUATIC ECOSYSTEMS

Article: Speciation of mercury in the strongly polluted sediments of the Deûle River (France).

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PAPER

1

Speciation of mercury in the strongly polluted sediments of the Deûle River (France)

Milada Kadlecová, Baghdad Ouddane* and Hana Dočekalová

We demonstrate that the concentrations of dieldrin, DDT, DDE, and DDD in surficial sediments from Long Island Sound have not decreased significantly over the past twenty years.

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PAPER

Speciation of mercury in the strongly polluted sediments of the Deûle River (France)

Milada Kadlecová,[ab] Baghdad Ouddane*[a] and Hana Dočekalová[c]

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The Deûle River in Northern France experienced serious contamination from the metallurgical industry, especially from the smelter Metaleurop prior to 2003. In 2002 the surface sediments were collected from the bed of the river around 10 km above and 10 km below the smelter. Total mercury (HgT) and methylmercury (MeHg) concentrations exceeded the background value of 0.1 mg kg^{-1}. The average concentrations were 19.67 ± 1.02 mg kg^{-1} and 10.88 ± 1.08 μg kg^{-1}, respectively. In 2003 the sediment core samples were collected at two different sites near the factory for survey depth profiles of Hg contamination. The concentrations of HgT and MeHg in sediment cores varied from 10.47 to 259.44 mg kg^{-1} and from 3.24 to 82.61 μg kg^{-1}, respectively. The concentration of total mercury was significantly correlated with the methylmercury concentration in the sediment below a depth of 23.5 cm ($R^2 = 0.81$, $p < 0.01$). This may suggest that the production of MeHg is directly related to the HgT concentration. Nevertheless the MeHg/HgT ratio in the upper part of the sediment core was higher than that in the lower part. This suggests that HgT and MeHg may have been co-deposited together. However, the methylmercury production takes place in the surface sediment by microorganisms. The strong correlation observed between MeHg and acid volatile sulfides (AVS) suggests that MeHg variability is associated with the bacterial activity (presence of AVS).

Introduction

Mercury (Hg) is a toxic element, widely distributed in the environment and is also present in the aquatic systems. It exists in different chemical and physical species with a wide range of properties and its toxicological and ecotoxicological effects are strongly dependent on the chemical form present.[1,2] Inorganic mercury Hg(II) is the main form of Hg in waters and sediment samples which can be naturally converted to a much more toxic form methylmercury (MeHg). MeHg is accumulated by aquatic organisms and is known to be bio-amplified along the food chain, and it poses a threat to humans consuming fish.[1] Knowledge of the concentration, transport and speciation of mercury compounds in aquatic ecosystems is needed to predict the potential impact on human and aquatic life.[3]

Extensive waste inputs from industrial and/or agricultural and/or urban sources have contributed to the significant increase of pollution in rivers. This is also the case for Northern France, where the increase in population as well as development of industry contributed to a contamination of once clean aquatic ecosystems. This is also the case for the highly populated and greatly industrialized region of Northern France. This region is known for its extensive metallurgical industry. Near Douai city, the lead and zinc smelter Metaleurop contributed to one-third of the French production of zinc (*i.e.* 100 000 t). Metaleurop was in

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Environmental impact

In this work, we have investigated some of the main geochemical factors affecting the speciation and distribution of mercury in highly contaminated sediments from the Deûle River. The behaviour and fate of this metal contaminant in sediments are examined using new analytical methods. Under anoxic conditions, transformation of inorganic mercury to methylmercury occurs *via* sulfate-reducing bacteria even though the sediment is highly contaminated by other trace metals. The importance of biogeochemical processes in this anoxic sediment was verified.

active for more than a century and was closed in January 2003; however the waste is still located in the vicinity and can spread into all fields of the environment, massively accumulating and endangering the people living in the proximity of such a contaminated place.[4,5] We focused our study on total mercury (HgT) and methylmercury (MeHg) in the river sediments taken from the strongly polluted point of the Deûle River near Metaleurop.

In this study, HgT and MeHg concentrations in depth profile of sediments were determined. For MeHg determination after ethylation, the Headspace injection technique, followed by gas chromatography separation and atomic fluorescence spectrometry detection, is used.

Materials and methods

Sampling and sample treatment

Samples were collected within a part of the Stardust project (INTEREG III) realized along the Deûle canal. The sampling sites are shown in Fig. 1. In July 2002 the surface sediments were taken using a Van Veen grab system (just 5 cm of the depth). This first series was realized around 10 km distance at 17 sites downstream and upstream of Metaleurop smelter. Subsequently undisturbed core samples were collected together with their overlying water in November 2003. Two sampling sites located in a part of the Deûle River that was considered as its most polluted zone had been chosen. The first site was upstream (Fig. 1, core A, site 7) and the second one downstream (Fig. 1, core B, site 8) of the factory. The core samples were collected using a hand-driven gouge sampler and polyethylene core. The length of the sampler tube was 80 cm and the inner diameter was 7 cm. The upper part of the sediment core tube was cut at a thickness of 2 cm and the lower part at 3 cm (under 10 centimetres of the sediment depth). Cutting of the sediment was performed under N_2 atmosphere (in a N_2-filled glove bag) to prevent any oxidation reactions in the sedimentary material. Sliced sediment samples as well as surface sediment samples were placed and preserved in hermetically closed plastic bags and vessels, transported to the lab and stored in the fridge (4 °C). An additional sediment core was collected for measurement of potential.

Collection, storage and preservation followed US EPA Method 1631 (Revision E, 2002). All glass and Teflon vessels were cleaned by washing using Milli-Q (18.2 MΩ cm, Millipore) water after 24 hour soaking in the diluted nitric acid ($HNO_3 : H_2O = 1 : 10$, v/v) and then dried under streamline.

Determination of HgT concentrations

Before HgT and MeHg analysis in the laboratory, the samples were dried at the room temperature and then sieved through 63 μm sieves to remove rough particles.

For the total mercury analysis in dry sediment samples without any pre-treatment, a one-purpose atomic absorption spectrometer Advanced Mercury Analyser, model AMA 254 (Altec Ltd., Czech Republic), was used. This analyzer is based on thermal combustion of a known amount of dried sediment in oxygen atmosphere, a selective trap of mercury on an amalgamator and subsequently, after heating Hg–Au amalgam, on atomic absorption spectroscopy detection of the released Hg^0. The amalgamator and the block of measuring cuvettes are kept at a temperature of 120 °C to prevent water condensation. After selective trapping, mercury is released from the amalgamator by a short heating-up of the Hg–Au amalgam and is transferred by a carrier gas through the long measuring cuvette, gathered in a delay vessel and transferred through a short cuvette out of the instrument. Consequently the same quantity of mercury is measured twice with different sensitivities, resulting in a dynamic range of 0.05 ng Hg of the analyser in a single measurement.[6] The detection limit for the analysis of 100 mg of the sample is 0.1 μg kg^{-1}.

Analysis of the samples followed the analysis of the certified reference material (CRM) from the International Atomic Energy Agency (*e.g.*, IAEA 405, 433 and 158). Certified mercury contents are listed in Table 1.

Determination of MeHg concentrations

For the determination of MeHg in sediments, the aqueous phase ethylation followed by Headspace (HS) injection, Gas Chromatography separation and Atomic Fluorescence Spectroscopy (GC-AFS), proposed by Leermakers *et al.*,[7] was used. The precision and accuracy of the technique have been verified by calibration exercises and the analysis of the reference material (*e.g.*, IAEA 405). An overview of the analytical procedure is

Table 1 Measured and certified values of HgT (mg kg^{-1})

CRM sediment	Measured value ($n = 6$)	Certified value
IAEA-405	0.750 ± 0.060	0.810 ± 0.030
IAEA-433	0.155 ± 0.010	0.168 ± 0.017
IAEA-158	0.121 ± 0.013	0.132 ± 0.014

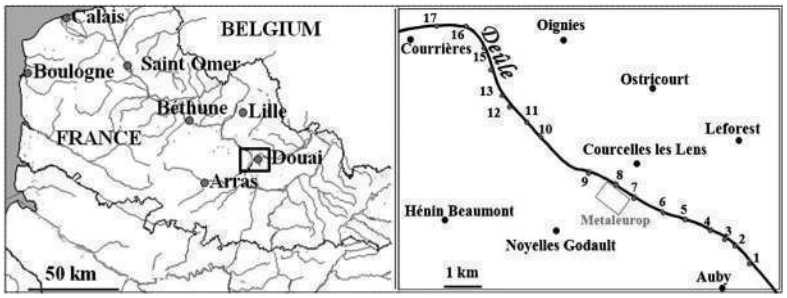

Fig. 1 Position of sampling sites.

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given here. For quantification of the MeHg concentration, a known amount of the sediment (100.0 mg for more contaminated or 200.0 mg for less contaminated sediments) was placed into a 25 mL glass vial, to which 1 mL of 1 M $CuSO_4$ (Acros Organics, New Jersey, USA) solution and 5 mL of 18% KBr (w/v) (Merck, Darmstadt, Germany) in 5% H_2SO_4 (v/v) (Merck, Darmstadt, Germany) solution were added. The mixture in vertical position was shaken at 400 rpm for 45 min and then after adding 10 mL CH_2Cl_2 it was shaken for additional 45 min, followed by centrifugation for 15 min at 3000 rpm to separate the organic, aqueous and solid layers. 20 mL Milli-Q water was added to a 50 mL conical glass bottle and then 5 mL of CH_2Cl_2 was transferred. The organic layer was back-extracted in Milli-Q water by solvent evaporation at 46 °C (water bath—Compatible Control CC3, Offenburg, Germany) under constant N_2 flow until there were no more visible drops of CH_2Cl_2.[7] All sample extracts were analysed on the same day. For analysis, 5 mL aqueous extract was diluted with 5 mL Milli-Q water in the 22 mL Headspace vials. 50 μL of acetate buffer (82 g CH_3COONa, p.a., Merck, Darmstadt, Germany and 59 mL CH_3COOH, Scharlau Extrapur in 500 mL Milli-Q water) were added to get the optimal pH (between 4.8 and 5.2).[8,9] Finally 50 μL of sodium tetraethylborate (min 98%, Stream Chemicals, Newburyport, USA) solution was added for the ethylation reaction. The vials were sealed with Teflon-coated butyl rubber septa and Al crimp caps. Before analysis, the solution was shaken and allowed to react for 1 hour in the dark.[10]

Optimization was carried out on MeHg standard solutions (1–50 ng L^{-1}). The procedure was firstly performed and validated on a certified reference material IAEA 405.

The analytical system for methylmercury determination, present in the sediment, consists of a Headspace sampler (PerkinElmer HS TurboMatrix 40) coupled to a Gas Chromatograph (Clarus 500, PerkinElmer, USA) through a heated fused silica transfer line. The transfer line is directly connected to the packed GC non-polar capillary column which consists of an 80 cm long Teflon tube (PTFE O.D. ¼″, I.D. 1/8″, 15 × 0.25 mm × 0.25 μm, Altech) filled with 10% OV3 on a Chromosorb WAW DMCS (60/80 mesh). The outlet of the GC is coupled to a Cold-Vapour Atomic-Fluorescence detector (Tekran, Model 2600 CVAFS Mercury Analysis System, USA) *via* a pyrolysis unit. The pyrolysis oven (ETC-60 Electrothermal Temperature Controller, Varian, Australia) was provided with a temperature control set. All data were acquired by a TotalChrom Navigator System, Version 6.3.0.0445 (PerkinElmer). The operating conditions for HS-GC-AFS and pyrolysis were based on the parameters previously optimized[10–13] and modified by varying them during the optimization procedure. The parameters are summarized in Table 2.

Table 2 HC-GC-AFS parameters used for MeHg determination

Headspace parameters	
HS temperature	70 °C
Thermostatic heating time	5 min
Pressurization time	30 s
Injection time	15 s
Withdrawal time	20 s
Needle temperature	100 °C
Transfer line temperature	105 °C
Sample volume	10 mL
Gas-chromatography parameters	
Column	PTFE O.D. ¼″, I.D. 1/8″, 15 × 0.25 mm × 0.25 μm, Altech
Initial temperature	50 °C
Initial time	1 min
Ramp temperature	25 °C min^{-1}
Final temperature	100 °C
Final time	2 min
Carrier gas flow rate	60 mL min^{-1}
Pyrolyser	
Pyrolysis temperature	800 °C

Determination of reduced sulfur species

The majority of mercury can be associated with organic matter, where it binds strongly to reduced sulfur groups. Reduced sulfur (S) species were determined in the sediment cores, which were sectioned under N_2 atmosphere, after their conversion into H_2S gas following sequential extraction procedures described previously by Cornwell *et al.*[14] and Canfield *et al.*[15] and optimized by Billon *et al.*[16] Briefly, about 1 g of frozen and wet sediment was introduced into a flask. This manipulation was carried out inside a glove box. The extraction of acid volatile sulfides (AVS), which are mainly composed of amorphous FeS, poorly crystallized gregite and/or mackinawite, was performed by the addition of 6 M HCl solution to the anoxic sediment sample at ambient temperature over one hour. This procedure led to the generation of H_2S gas, which was drawn off with N_2 and trapped into a 2 M NaOH solution, containing further ascorbic acid and ethylenediaminetetraacetic acid, disodium salt dihydrate to give $2Na^+$ and S^{2-}. The content of trapped sulfide ions was titrated by a 10^{-3} M $AgNO_3$ solution by means of a specific sulfide membrane electrode (Orion). The accuracy of these two methods was estimated to be <8% and the lower limit of determination of this method (1 g of sediment) was about 20 mg kg^{-1} of S in the sediment. The optimisation of this method and the artefact related to sampling and determination were discussed by Billon *et al.*[16] and by Lesven *et al.*[17]

Calibration and evaluation of detection limit, limit of quantitation and precision

A validation procedure was carried out for the MeHg calibration line. A five-point calibration was used during the measurements to obtain high confidence for the calibration line. Statistical evaluation of the method for MeHg determination was based on ISO 8466-1.[18] The detection limit (LOD) and limit of quantitation (LOQ) calculated as three times and ten times the standard deviation of blank determination were 1.12 ng L^{-1} and 3.74 ng L^{-1} as Hg respectively. The precision of the method, indicated as relative standard deviation (RSD) for ten replicate analyses of 10 ng L^{-1} MeHg, was 3.7%.

The accuracy of the MeHg extraction procedure with CH_2Cl_2 and subsequent back extraction into Milli-Q water was evaluated in the participation of the certified material IAEA-405 and the obtained result (5.85 ± 0.45 ng Hg g^{-1}) was in good agreement with the recommended value (5.49 ± 0.53 ng Hg g^{-1}). Repeatability (RSD; $n = 5$) calculated for the analysis of certified material IAEA-405 was 4.86% and a detection limit of 0.27 μg kg^{-1} for 0.2 g sediment and back-extraction of 5 mL of CH_2Cl_2 layer into 20 mL Milli-Q water was obtained.

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Results and discussion

Surface sediments—horizontal distribution

The horizontal distribution of HgT and MeHg in surface sediment samples is shown in Fig. 2. HgT and MeHg concentrations ranged from 2.1 to 78.4 mg kg^{-1} (mean 19.67 ± 19.97 mg kg^{-1}) and from 1.1 to 46.4 μg kg^{-1} (mean 10.89 ± 11.80 μg kg^{-1}), respectively. The background concentration of uncontaminated sediments of Hg in this region (Nord-Pad de Calais) is 0.1 mg kg^{-1} HgT.[19] We observed approximately from 10 to 800 times higher Hg concentration in the surface sediments than the background value at all the sampling points of the Deûle River.

Indeed, the highest Hg concentrations in the surface sediments were observed at site 8 (see Fig. 2), which is located near the former industrial zone (see Fig. 1) while they decrease with distance from the pollution source. Surface sediments are strongly contaminated in the place where the dust from Metaleurop was spread and the effluent discharged. In Table 3 concentrations of both HgT and MeHg found in the surface sediment in the Deûle River are compared with those observed in other rivers and environmental causality affected by mining or other industrial activities. Higher concentrations of HgT and MeHg have been detected in river sediments where industrial sources of Hg (*e.g.* mining activities) are found, like in the Slovenia (Idrijca River) and Spain (Valdeazogues River).

Vertical distribution of HgT and MeHg

The results show (Fig. 3) that the HgT concentration ranged from 10.47 to 13.28 mg kg^{-1} (mean 10.80 ± 3.11 mg kg^{-1}) in core A and from 10.89 to 259.44 mg kg^{-1} (mean 64.51 ± 78.90 mg kg^{-1}) in core B. The MeHg concentration in sediment samples was in the range from 3.24 to 18.91 μg kg^{-1} (mean 10.36 ± 5.55 μg kg^{-1}) in core A and from 2.31 to 82.61 μg kg^{-1} (mean 23.4 ± 26.14 μg kg^{-1}) in core B. The repeatability was checked by making triplicate measurements of each sample. The variability ranged from 0.3 to 8.8% (mean 3.3%). A large difference in the sediments depth and a difference in the mercury concentration between these sampling places (7 and 8) close to each other are evident. The sediment layer is larger in core B, most probably on account of industrial activity, because deeper sediments can be found near the field where the dust from lead and zinc metallurgical production was spread.

The vertical distribution of Hg concentrations showed variability and no consistent gradient with increasing depth in shallow sediments (core A and till 23.5 cm in core B) (Fig. 3a). This is not uncommon and has been also found *e.g.* by ref. 20 and 21 in the cores taken from northern Kazakhstan and Texas. This variability in Hg concentrations in sediment cores may be partly explained by bioturbulation, redistribution of sediments during seasonal changes or by boat traffic which tends to mix sediment levels.

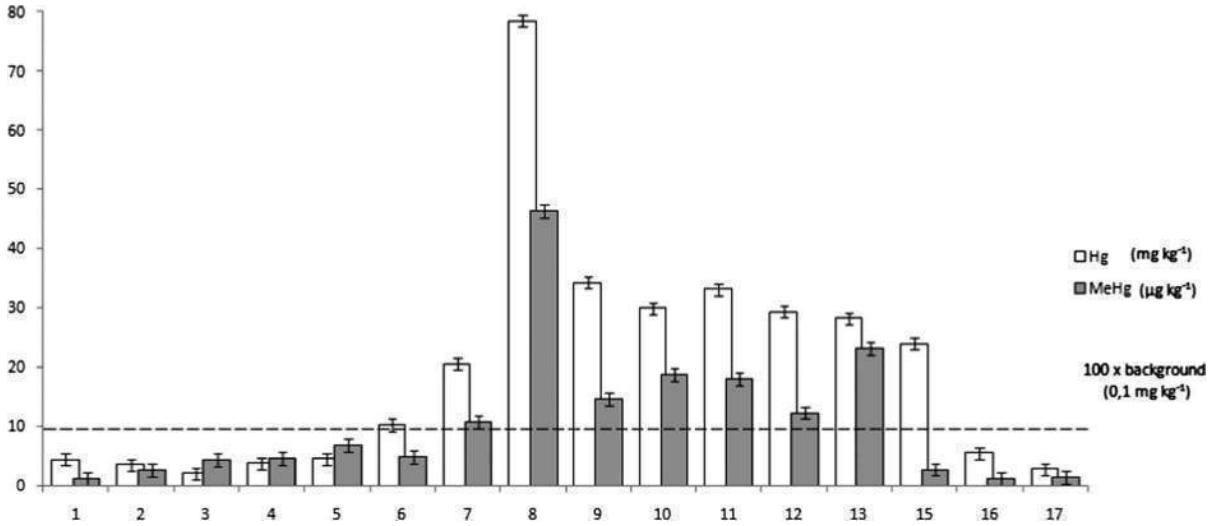

Fig. 2 Horizontal distribution of HgT and MeHg in surface sediments at 17 sampling points.

Table 3 Comparison of HgT and MeHg concentrations in river sediments

River	HgT/mg kg^{-1}	MeHg/μg kg^{-1}	Source	Reference
Idrijca (Slovenia)	5–727	3–10	Mercury mine (stopped in 1994)	24
Soča (Slovenia)	1.3	0.8–3	Mercury mine (stopped in 1994)	25
Valdeazogues River (Spain)	7.2–74	8.6–880	Mining activities	26
Wuli River (China)	0.8–48	0.18–35	Zn smelter and chlor-alkali facility	27
Haihe River (China)	0.55–8.8	0.7–21.7	Industrial and domestic effluents	28
Steamboat Creek (Nevada, USA)	0.03–8.53	0.03–2.98	Gold and silver extraction	29
Deûle River (France)	2.1–78.4	1.1–46.4	Zn–Pb smelting factory	This study

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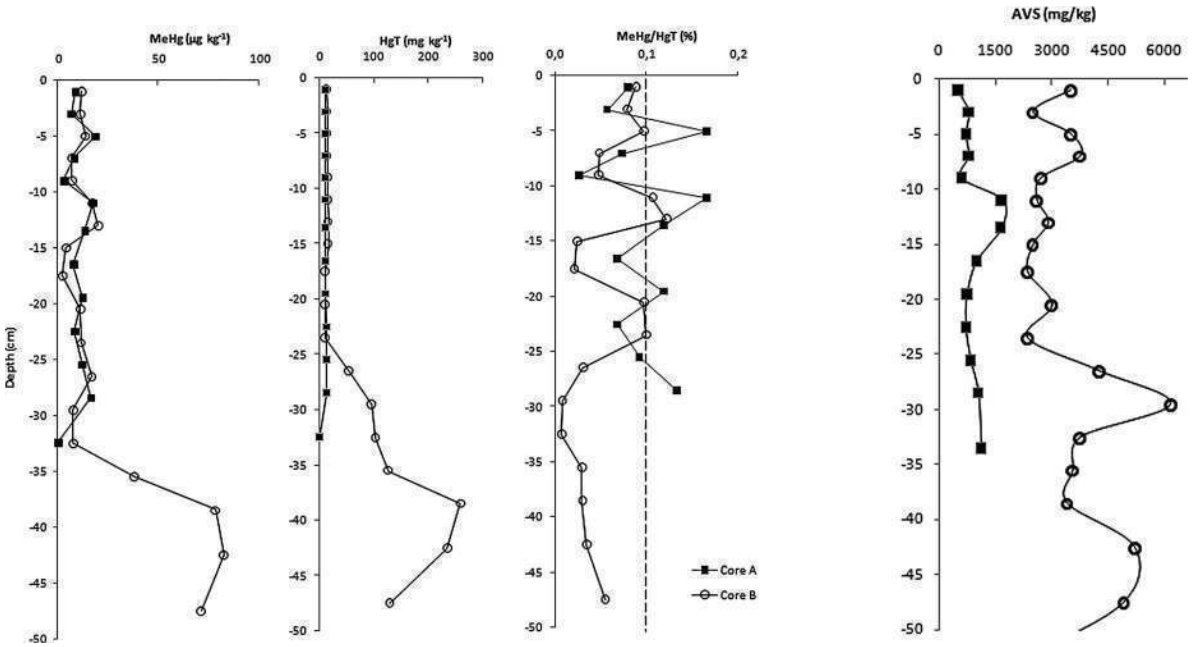

Fig. 3 (a) Concentration of HgT and MeHg in a sediment core. (b) AVS depth profiles.

As shown in Fig. 4, when the HgT concentration in the sediment was plotted against the MeHg concentration, no correlation was found in the surface sediments. Actually some studies also reported that there can be a weak correlation between HgT and MeHg concentrations in sediments.[22] As reported[23] the Hg methylation is active in surface sediments where the production of MeHg is not consequently directly bound to the total Hg concentration, but depends also on a lot of environmental variables, such as pH, temperature, complexing agent, and biological activities. Bloom and Lasorsa[30] observed that in the stable aquatic systems no significant changes in HgT and MeHg were seen. But when the organic-rich sediments are redistributed (*e.g.* boat traffic) MeHg in the water column increases (by factor 30, mostly in the suspended matter[30]), while the increase of HgT is not so noticeable (factor 2, ref. 30). Thus MeHg can be eluted from the sediment and is less fixed in sediment matter than inorganic mercury.

A positive and noticeable correlation was found ($r = 0.81$, $p < 0.01$) between MeHg and HgT in the deeper sediment (under 25 cm). We observed growth concentrations of MeHg and HgT in the deeper sediment, which increased up to ten times compared to surface sediments. This suggests the persistence of MeHg in older, buried sediment, and the positive and prominent correlation between HgT and MeHg concentrations suggests that they have been co-deposited together or that MeHg production is a function of HgT concentration as observed also by Benoit *et al.* (1998).[31]

In the upper part of sediment cores without big differences in the concentration of HgT as well as MeHg, the proportion of MeHg (%MeHg) given by the amount of inorganic Hg present (%MeHg = (MeHg/Hg) × 100) is near 0.1 ± 0.02%. These low % MeHg are typical at high sulfate concentrations.[31,32] Sulfate reducing bacteria (SRB) are important methylators of mercury.[33] At low sulfate concentrations, sulfate stimulates sulfate-reduction

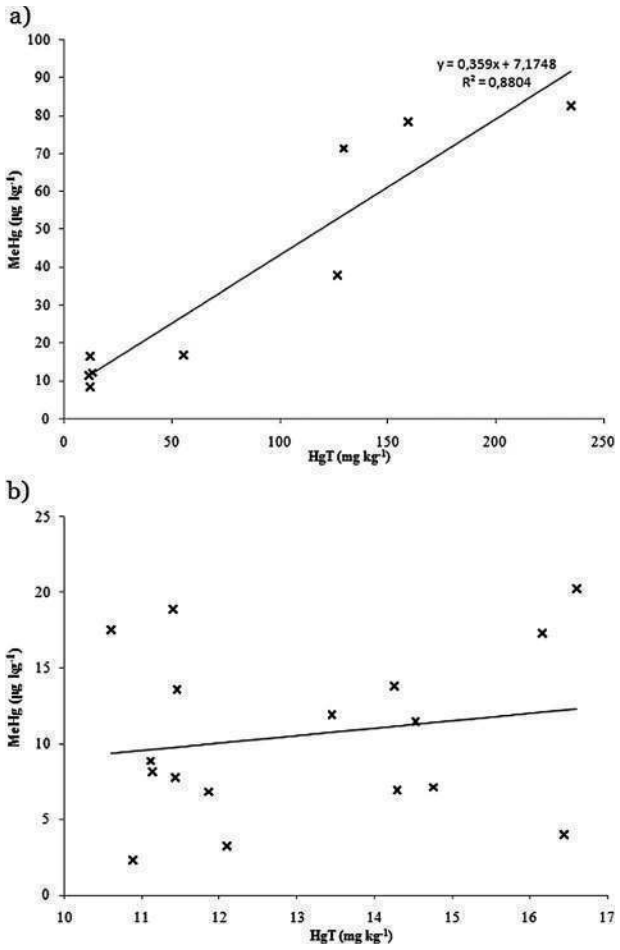

Fig. 4 Correlation between HgT and MeHg: (a) deeper sediments (>23.5 cm; cores A and B); (b) surface sediments (cores A and B).

Table 4 HgT and MeHg in ores previously treated by Metaleurop

Sample	MeHg/μg kg^{-1}	HgT/mg kg^{-1}	MeHg/HgT (%)
Tara	<0.1	12.21	—
MWZ	<0.1	3.93	—
Ammechag	7.79	12.10	0.06
Los Frailes	24.8	0.51	0.05
Oxyde Turc	<0.1	6.55	—

as well as Hg methylation; however at high sulfate concentration inorganic mercury is less available for methylation due to its complexation with sulfidic compounds.[34] This is also the case for Deûle River. The AVS and MeHg concentration profiles show a similar trend. In the work of Mikac *et al.* in some of the investigated sediments, very good correlations were established between MeHg and AVS, indicating an efficient adsorption of MeHg onto iron monosulfides or something common in the mechanisms of their formation in sediments.[35–37] Mikac *et al.*[37] also observed a good agreement between MeHg profiles in the sediment and the depth profile of AVS. This relation suggests that conditions which are favorable for formation of AVS are also favorable conditions for Hg methylation (MeHg increases as AVS increases). Conditions could be favorable for Hg methylation not only due to high activity of SRB (recognized by efficient formation of AVS), but also due to particular speciation of Hg in pore water at the relevant depth and the presence of Hg–sulfur species which are readily available for methylation.[35,37] In contrast, total mercury concentrations were observed to be inversely correlated with AVS; this suggests that the accumulation of total mercury is negatively related to sulfur levels. In core B, an increase of AVS concentration is observed in a deeper part of the core (25 cm, Fig. 3b). It suggests that sediment SRB activity is not limited only to the surface layers.

Additional analyses of ores previously treated by Metaleurop confirmed the notion that methylation is caused by microbial activity and other environmental conditions as MeHg present in these ores is not eminent. In these natural ores, MeHg was not found and if it was, the ratio MeHg/HgT was not significant (Table 4). Also when the MeHg concentration in the minerals was plotted against the HgT concentration, the significant correlation factor was not found ($r = 0.19$) between their concentrations. Anyway additional work is necessary for the survey of sulfur behaviour and of potential MeHg production (methylation rate)/MeHg degradation (demethylation rate) determinations as well as for investigation of mercury speciation in water, suspended solids and sediments pore water.

Conclusions

We can consider that the HgT concentration is one of the factors influencing the presence of MeHg and its distribution in the sediments. The higher ratio of MeHg/HgT in the surface (less than 25 cm) *versus* that in deeper sediments and the value near 0.1% (Fig. 3) suggest that the production of MeHg is mainly limited to the surface sediment where there is high microbial activity as reported earlier.[35] The positive correlation trend suggests that there is a competition between methylation and demethylation. In deeper sediments the microbial activity and its potential for methylation are not sufficient or because of the high concentration of total mercury, the microbial association becomes inactive and hence the production of MeHg is limited. But the conditions in deeper sediments are stabilized, so the production of MeHg is mainly caused by HgT bulk. In deeper sediments, the high Hg concentration indicates its persistence in the long term.

The total Hg concentration is constant in the upper 25 cm of core B. This is probably a consequence of sediment deposition in the recent time period. The increase of the Hg concentration with depth in core B and also the greater sediment layer in core B in comparison with core A may be the consequence of a higher anthropogenic load of Hg and matter in the past—during the industrial times.

The factory Metaleurop is considered a certain agent responsible for mercury pollution of the Deûle River ecosystem. Hg speciation in surface sediments was examined at 17 sites around 10 km distance and deep sediment profiles at two sites in proximity of the old lead and zinc Metaleurop factory, considered as the most polluted zone (highest contamination by Hg and MeHg).

MeHg and HgT concentrations in deeper sediments were significantly correlated and the MeHg/HgT ratio here and so the methylmercury production are influenced by the HgT pool, but on the other hand in surface sediments, the other factors controlling MeHg productions are also important. We noticed that HgT and MeHg were deposited together into the buried sediments from where, we consider, they may diffuse back to the water column during dredging to preserve canal navigability. For considering resuspension (mercury quantity eluted from sediment) additional work is needed for estimating the HgT and MeHg concentrations in pore and surface water. Thus we could roughly evaluate what concentration of MeHg the aquatic organisms can be exposed to. The need of additional work and continuation of monitoring this area is evident.

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III.I. Comparisons of mercury contamination sediments from contaminated and uncontaminated sites

This work was supported by the Egide/Barrand project between Czech Republic (*Vývoj a použití nových metod pro stanovení biodostupných forem kovových polutantů a organokovových sloučenin v sedimentech řek, vodních toků a nádrží ke studii biogeochemických cyků*; MEB 020918) and France (*Évolution et usage de nouvelles techniques DGT pour l´étude de la bio-accessibilité des métaux et organométaux toxique en milieu aquatique et sédimentaires*; 19494QF).

III.I.1. Study area

River of Nord Pas de Calais

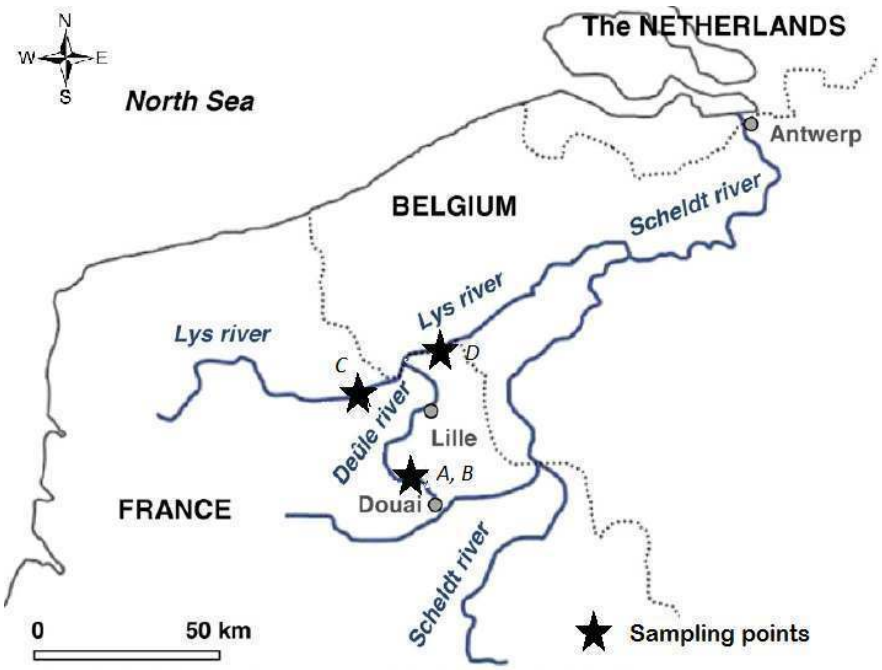
The region Nord Pas de Calais is industrial region in northern France. Since the middle of the 19th century, industrial activities, including coal mining, metallurgical plants, textile and chemical industries, have led to very high inputs of contaminants into the nearby Rivers and its tributaries.

River Deûle is a major drinking-water source for the population living in this region, however, with severe metallic pollution, particularly, in a 3 km zone from nearby two smelters (Metaleurop and Umicore) near city Douai. For more than century the factory Metaleurop was in activity and was closed in 2003. Umicore factory has still been in activity since 19 century. The waste is still located in the vicinity and has been spread into the all fields of environment, massively accumulated and endangered the people living in the proximity of such contaminated place. The lots of effort have been done for significant improvements of the water quality in this area. However, numerous sediments are still highly polluted and may now act as a potential “time bomb”, particularly in case of them being disturbed (Charriau, 2009).

The Lys River flows through France and Belgium, in one of the most industrialized and urbanized area of Western Europe. The Lys River is the tributary of Scheldt River and Deûle River is the tributary of the Lys River. The Lys River with total length 202 kilometers makes the frontier between the France and Belgium approximately 25 km. In general it is polluted river due to the high density of population and industry. The quality of water is good in the upper, non canalized part, because of less anthropogenic source of contaminants. Near the Lille agglomeration and after the tributary of Deûle River the water quality is very bad (AEAP, 2005).

Sediments samples were taken from Deûle River (A, B), from the two sites. One site, which was considered as most polluted zone (very close to the factory Metaleurop) and the second upstream the factory. Samples were taken also from the Lys River, on the one site considered as the good water quality (C) and on the second site in the lower part of River in Warneton (D), after the flow of Deûle River, on the frontier between the France and Belgium (***figure III. 1***).

Figure III. 1: *Sampling sites in France*



Rivers of South Moravia (Czech Republic)

The study area in Czech Republic was located in the lowlands of the Lower Morava Valley, in the eastern part of the Czech Republic. The Morava River basin is part of the Danube River basin and the Black Sea drainage area. Fluvial sedimentation of Morava River, a left-hand tributary to the Danube River, started with deposition of aggrading braided-channel gravels and sands. The river was embanked in the first half of the twentieth century when the main channel was straightened and shortened by about 40%. The Morava River is the largest river of the Morava basin, with a total length of 284 km and a catchment area of 9533 km2. The average annual discharge of the Morava is 52,6 m^3/s as recorded at the gauge station Kroměříž. The majority of the river basin is constituted by agricultural land. Surface-water resources within the Morava River basin fulfill a number of uses and functions which can be split into the water supply for public, industry and agriculture, recreation and fishing, nature conservation, flood management and disposal of effluent. The biggest users of surface-water abstractions are power stations, which use large amounts of water for cooling purposes. Municipalities represent the largest source of surface water pollution, contributing approximately 90% of organic pollution and nearly 50% of the nutrient load. Almost all municipalities with more than 5,000 person equivalents in the Czech part of the basin have waste-water treatment plants, the majority of them are presently undergoing some upgrading or extension aimed mostly at introducing of new technology for nutrient reduction.

The most significant industries from the pollution point of view are food processing, textile, rubber, tannery, paper and chemical manufacturing. While the textile industry is located in the upper part of the basin, the food industry and chemistry is concentrated mainly in its middle and lower part

Aquatic systems are not isolated, but interact with other compartments like sediments, which are often polluted due to industrial, domestic and agricultural discharges. The inorganic pollutants like metals and organic persistent pollutants that are accumulated in the sediments may, however, be solubilized and diffuse back to the water column. Despite that lot of efforts was made for setting environmental quality standard by reducing

point discharges of toxic substances in surface waters, considerable amounts of contaminants could be still release from sediments.

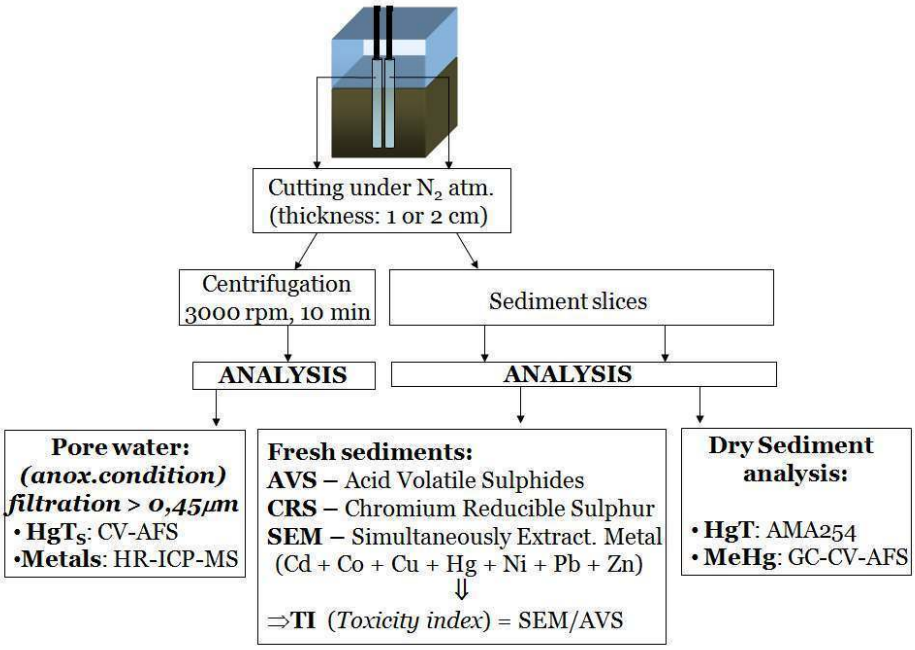
III.I.2. Sampling strategy:

Sampling in France

The samples were taken in March and April 2009 and February and March 2010 at two sampling sites located on Deûle River and Lys River. Sampling was performed from the bank river, only samples from Deûle River was facilitate using the boat (L´Agence de l´Eau Artois Picardie in France). From each sampling site the four sediment cores were taken for survey of profiles contamination. Undisturbed sediments cores were collected together with their overlying water using hand-driven gouge sampler and polyethylene tube. The length of the sampler tube was 80 cm and inner diameter 7 cm.

- One of the sediment core was dedicated to **pH and redox potential measurements**,
- Second and third sediment cores were dedicated to **DGT and DET** deployment and determination of **mercury depth profiles** and **(bio)available part of metals**.
- Fourth sediment core was transported to the laboratory in the polyethylene tube and was employed for **analysis of: trace metal total concentration in pore waters, acid volatile sulfides (AVS), simultaneously extracted metals concentrations, HgT and MeHg concentrations in sediment** ***(figure III. 2)***.

Figure III. 2: *Sampling strategy.*



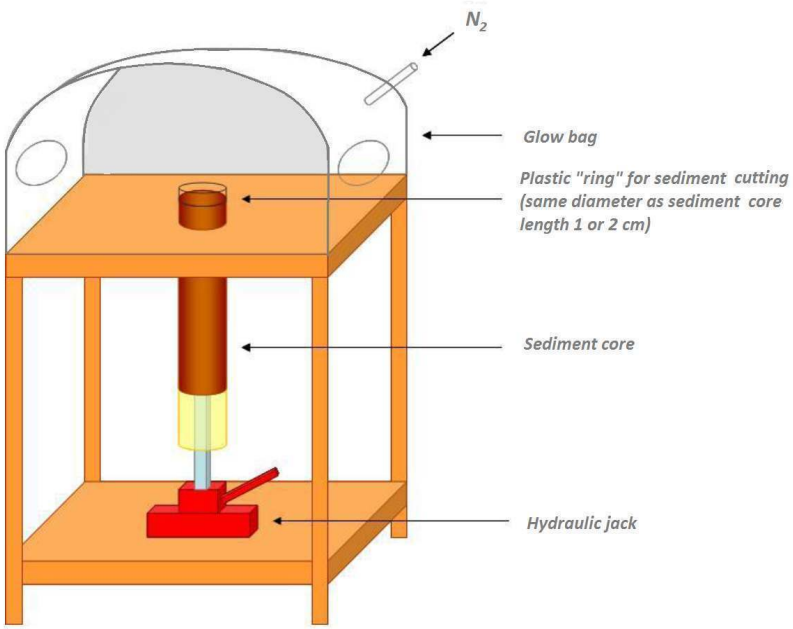
Redox potential and pH measurement was performed directly in the field. The tubes with had pre-drilled holes (1 cm interval), covered with tape during the collection of sediments were used. Electrodes were inserted in the holes to measure pH and redox potential. Measuremts of redox potential were realized with a combination platinum electrode and measurements of pH were realized with combination glass electrode. For both electrodes, the reference electrode is Ag/AgCL, [KCl] = 3 M (***figure III. 3***).

Figure III. 3: *Collection of sediment cores and measurement of redox potential.*

Into the two sediment cores, DET and DGT probes were inserted back to back in the sediment cores for 48 hours (in Deûle River) and 72 hours (in Lys River).

The last sediment core was introduced in a glove box, previously flushed with nitrogen and after removal of the overlying water cut every 1 or 2 cm under N_2 to prevent any oxidation of the reduced species present in the anoxic sediments (***figure III. 4***).

Figure III. 4*: The table with glove box adapted for work under nitrogen atmospherel:*



Each sediment slice was split into three parts in the glove box: One part was stored untreated in a plastic bag at -18°C under nitrogen prior to sulphur and metal analysis, second part was dried in laboratory under laminar flow and the third part was introduced in a centrifugation tube in order to extract interstitial water. Sediment slices were centrifuged (3000 rpm, 10 min) and the extracted pore water were immediately filtrated (Whatman or Supor®-450; 0,45 µm) under N_2, mainly to avoid the oxidation of Fe(II) and Mn(II). The pore water was acidified with HNO_3 (Merck, suprapur) and stored in the fridge until analysis of metals and total dissolved mercury.

The dry sediment samples were homogenized and total mercury and methylmercury was measured.

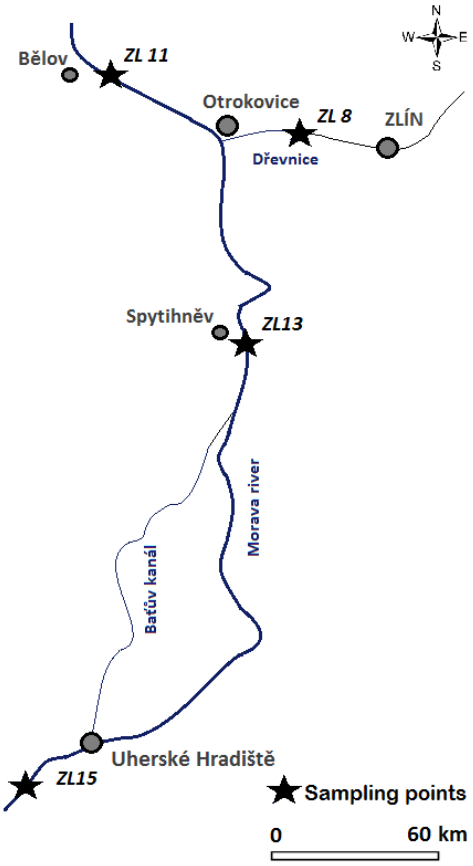
Sampling in Czech Republic

Samples were taken in March and April in 2008 at four sampling sites (***figure III. 7***) located on the Morava River (Otrokovice: ZL8; Bělov ***figure III. 5;*** ZL11; Spytihněv: ZL13; Uherské Hradišťě: ZL15 – ***figure III. 6***) within the work supported by the GA ČR (project No 525/09/P583).

Figure III. 5: *The photo from sampling site near the Bělov.*

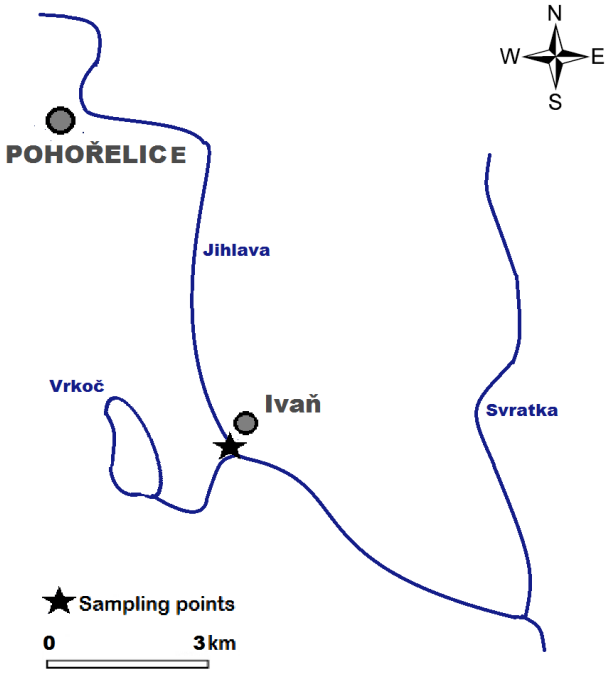
Figure III. 6: *The photo from sampling site near the Uherské Hradiště.*

Figure III. 7: *Sampling sites in Czech Republic – Morava River.*



The aim of the work was monitoring of selected metals and characterization of aquatic systems of selected areas in the Region Zlín. In addition to the project set up, the determination of HgT and MeHg was carrying out with the view of comparisons of mercury sediments contamination in Czech Republic ("uncontaminated sites") and France ("contaminated sites"). Just surface sediments were taken from Morava River manually because the conditions of bed stream disable- the sediment cores sampling. Generally, the rivers in the Czech Republic are different as in France. The sediments are shallow so it is difficult to find the river where the sediment cores could be taken and physical parameters and concentration of pollutants in sediment profiles can be observed. The river sediments in Czech Republic are much sandier (it is not suitable for DGT and/or DET deployment) and fast vertical lithology changes are common. The sediment character on different sampling place in South Moravia were explore within the preparation of the new research project on the Brno University of Technology. We manage to take the sediment cores from only one site - River Jihlava (***figure III. 8***) near the village Ivaň at April 2010.

Figure III. 8: *Sampling sites in Czech Republic – Jihlava River.*



Surface sediments were collected manually. From Ivaň two sediment cores were taken for survey of profiles contamination. Undisturbed sediments cores were collected together with their overlying water using 40 cm long polyethylene tube (inner diameter 6 cm). One core was cut every 2 cm. Sediments were dried in the laboratory under laminar flow.

DGT probes were inserted back to back in the sediment cores for 48 hours. Before the deployment, the DGT probes were de-oxygenated by immersing them for 24 hours in a container with metal free NaCl (0,01 M) solution. After deployment, probes were rinsed with Mili-Q water and the resin gel was cut into 5 mm interval using a Plexiglas gel cutter.

The water samples were taken into cleaned Teflon bottles and preserved by adding of HNO_3 concentrated (Suprapur). All water samples were stored in the dark cold place (-4°C) until analysis. The water temperature, pH and conductivity were measured in the river.

III.I.3. Analysis

Determination of HgT

For the total mercury analysis in dry sediment samples without any pre-treatment, one-purpose atomic absorption spectrometer Advanced Mercury Analyser, model AMA 254 (Altec Ltd., Czech Republic) was used. The principle is described in detail in the **part II of this chapter**: *Speciation of mercury in the strongly polluted sediments of the Deûle River (France)*.

Determination of MeHg

An automated method for the determination of MeHg in sediment samples using Headspace Gas Chromatography with atomic fluorescence detection in combination with aqueous phase ethylation was used. The principle is described in detail in the **Chapter II. – part III**: *Improvement in determination of methylmercury in sediments by Headspace Trap Gas Chromatography and Atomic Fluorescence Spectrometry after aqueous phase ethylation.*

Determination of dissolved HgT

Determination of mercury in pore water (as well as surface water) the method of cold-vapor fluorescence spectrometry (CVAFS) was used (EPA 245.7). The limit of detection is 1,8 ng.L-1 and minimum level of quantification 5,0 ng.L-1. Before the analysis all samples were filtered (0,45 μm) and preserved by adding of concentrated acid (HCl). Before the introduction into the instrument, all Hg species are oxidized by a potassium bromated/potassium bromide reagent. After oxidation the sample is sequentially pre-reduced with $NH_2OH.HCl$ to destroy the excess bromine. The ionic Hg is then reduced with $SnCl_2$ (1% solution in 5% HCl) to convert Hg(II) to volatile Hg0. The volatile species of Hg are separated from the solution by purging with high purity argon gas through a semi permeable dryer tube. The Hg is carry by the gas stream into the gold cell of cold-vapor

atomic fluorescence spectrometer and after the thermal desorption the concentration of Hg is determined by atomic fluorescence spectrometry at 253,7 nm.

Sulfur (S) analysis

Two major sinks of reduced S in aquatic sediments are: acid-volatile sulfur (AVS – reactive sulphide phase), mostly FeS_x ($0.9 < x < 1,5$) and highly resistant to recycling and terminal sink - pyrite (chromium reducible sulfur - CRS). AVS pool is often found as a minor constituent of reduced S and is believed to be a precursor which converts to the pyrite during early diagenesis. AVS measure sulfides liberated from mineral phases that are soluble in 6M HCl. From the sediment, sulfide is first volatilized (addition of acid) and gaseous hydrogen sulfide (H_2S) at ambient temperature is purged from the sample by nitrogen flow and trapped inside an antioxidant solution (1 M EDTA in 1M NaOH). The CRS sequentially follow the AVS extraction and mixture of HCl and Cr(II) is used. The concentrations of AVS and CRS were determined with potentiometric titration of the H_2S with a 100 $mg.L^{-1}$ Cd solution using a Metrohm 736 GP Titrino system. The reproducibility of the entire procedure was estimated by Billon et al. (2001) to be better than 8%.

III.I.4. Results

Mercury speciation in sediments and surface water

The surface water, pore water and sediments were analyzed. Total dissolved mercury in the surface water (HgT_{SW}) and in the pore water (HgT_{PW}) was measured using CV-AFS; total "insoluble" mercury in the surface water (HgT_{ins}) was measured after filtration of surface water in sediments by subsequent analyses of the filters using AMA254. Mercury was also measured by technique of diffusive gradient in thin film with different resin gels (Chelex-100, Dulit GT-73, TiO_2, Spheron-Thiol, ***Chapter V***). Total

mercury in dry sediments (HgT_{sed}) was analyzed using AMA254, methylmercury (MeHg) using HS-GC-AFS.

Surface sediments and surface water

Concentrations of HgT and MeHg in surface sediment samples are listed in the ***table III.2***. For the comparisons of sites the values for the surface sediments (0-5 cm), surface water and pore water (0-5 cm) were average. The surmise was, that the sites in Northern France are highly contaminated compared the sites in South Moravia due to extensive industrial activities. This suggestion is confirm just partially. The concentration of dissolved Hg in surface water is lower while the concentration of insoluble HgT in surface water and HgT in pore water and MeHg in sediments in Morava River as well as Jihlava River is more or less comparable or just slightly lower than the concentration in Deûle and Lys River. Contrary, the average HgT concentration in sediments of Deûle-Lys Rivers is more than 20 times higher compared Morava River. This can prove the lower anthropogenic contamination of sediments. The exception is the Jihlava River, where the concentrations of all Hg species are comparable with the sites at the Lys River (***figure III. 9***).

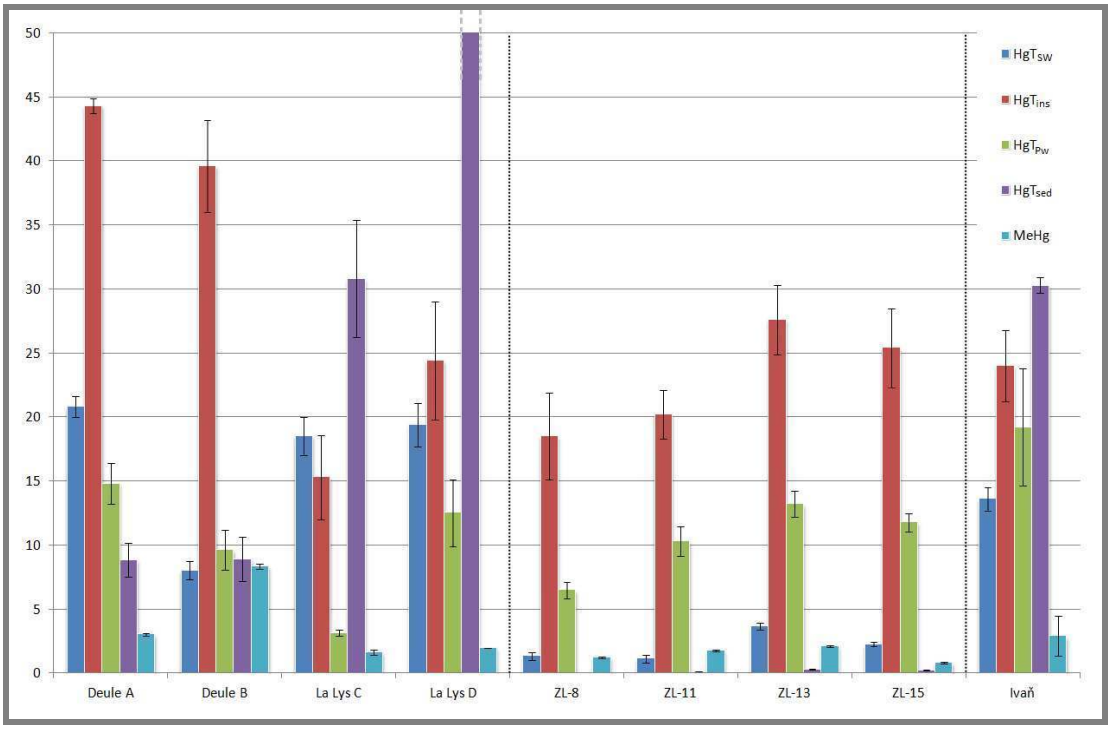
This can be explained by the slowly flowing Jihlava River and more over the sediments were taken in the cove where the contaminants could be setting. The sediments taken from the Jihlava River (muddy sediment with the sand) has also different composition compared the Morava River (primarily the sand and gravel with a lot of shell and small animal being).

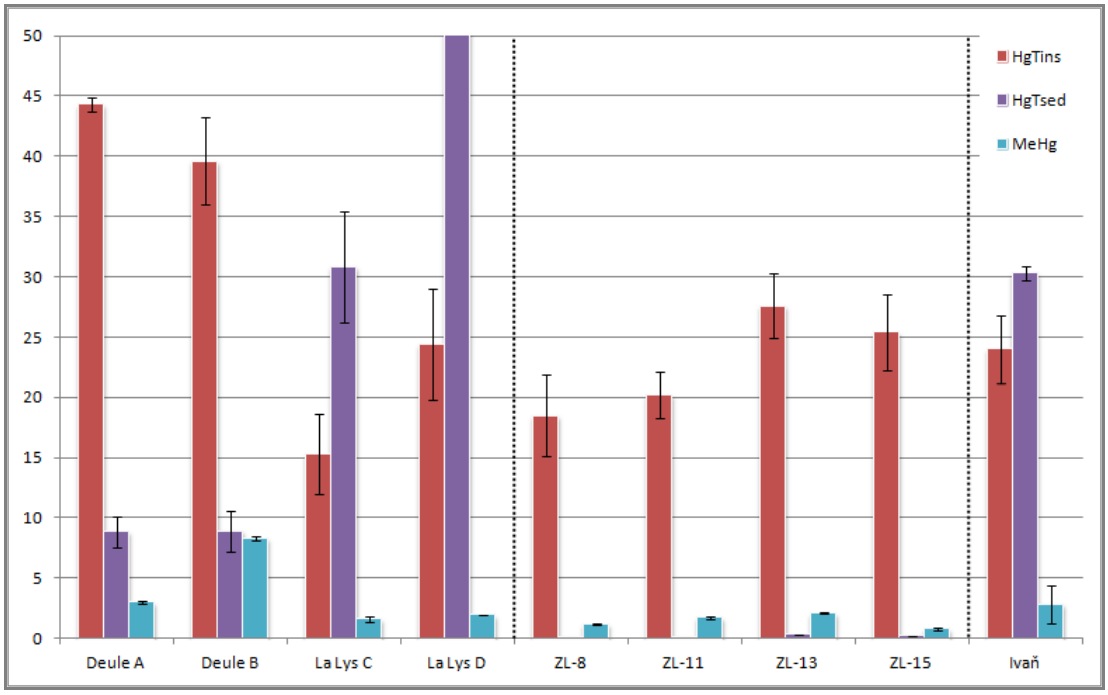
***Table III.2**: Hg species in surface samples (red color of the cell – highest concentration of given Hg species among the sampling sites, green color of the cell – lowest concentration of given Hg species among the sampling sites.*

Sampling points	Surface water		Pore water	Surface sediment	
	HgT_{SW} $ng.L^{-1}$	HgT_{ins} $ng.L^{-1}$	HgT_{PW} $ng.L^{-1}$	HgT_{sed} $mg.kg^{-1}$	MeHg $\mu g\ kg^{-1}$
Deûle A	20,8 ± 0,8	44,3 ± 0,6	14,8 ± 1,62	8,85 ± 1,31	3,03 ± 0,10
Deûle B	8,0 ± 0,71	39,6 ± 3,6	9,61 ± 1,55	8,91 ± 1,71	8,32 ± 0,22
La Lys C	18,5 ± 1,5	15,3 ± 3,3	3,13 ± 0,24	30,8 ± 4,6	1,62 ± 0,20
La Lys D	19,4 ± 1,7	24,4 ± 4,6	12,5 ± 2,6	461 ± 117	1,95 ± 0,03
ZL-8	1,32 ± 0,29	18,5 ± 3,4	6,47 ± 0,63	0,049 ± 0,013	1,21 ± 0,04
ZL-11	1,11 ± 0,33	20,2 ± 1,9	10,3 ± 1,15	0,102 ± 0,015	1,74 ± 0,09
ZL-13	3,68 ± 0,27	27,6 ± 2,7	13,2 ± 1,02	0,307 ± 0,045	2,10 ± 0,07
ZL-15	2,25 ± 0,18	25,4 ± 3,1	12,8 ± 0,71	0,227 ± 0,030	0,80 ± 0,10
Ivaň	13,6 ± 0,9	24,0 ± 2,8	19,2 ± 4,6	30,3 ± 0,6	2,87 ± 1,56

The background values in Czech Republic for uncontaminated water (according with the Environmental Quality Standard – NV *NEK-RP* NV č. 61/2003 Sb. and NV č.23/2011 Sb) for Hg is 0,05 $\mu g.L^{-1}$ and for sediments 0,1 $mg.kg^{-1}$. The Global background concentration from Turekian and Wedepohl (1959) is 0,4 $mg.kg^{-1}$ for Hg. The values of natural background concentrations, necessary for evaluation and classification of the recent river sediment contamination are not known for sites in South Moravia. The possible way to obtain the background concentrations are sampling and analysis of deeper deposited, anthropogenic uncontaminated, sediment layers in the fluvial alluvium areas. While the background values are not known the results were compared with the Environmental Quality Standard and Global background concentration. The values were below this Standard or Global background, with exception of place ZL-13 (Morava River), where the value was exceeded 3 times and in the Jihlava River the concentration of HgT in sediments were largely exceeded (more than 300 times).

***Figure III. 9**: Dissolved total mercury concentration in the surface water (HgT_{SW}), total mercury concentration in the surface water (HgT_{ins}), total mercury contamination in the pore water (HgT_{PW}), total mercury (HgT_{sed}) and methylmercury (MeHg) in sediments – the values only for surface sediments layer (0-5 cm), all values are average for comparison of Hg contamination of sampling places.*





The background concentration for Hg in Northern France (region Nord-Pad de Calais) is 0,1 $mg.kg^{-1}$ (Agence de l´Eau, 1997). This concentration was from 80 to 4000 times higher in surface sediments than background value at all sampling points of Deûle and Lys River (maximum Lys River – D). This can be attributed to the metallic industrial impact (e.g. Metaleurop factory).

Surface contamination in Northern France:

In Northern France the higher concentration was expected at the place Deûle B, located in proximity of the closed factory Metaleurop. But the higher values of almost all Hg species were found at the place Deûle A. This can be explained by navigation of the boat, the churn up the water and sediments and settling the particles in the cove, which is the one of the sampling place (***figure III. 1***, Deûle A).

Sampling site La Lys C was considered as the place with the best water and sediments quality (Etat qualitative des cours d´eau – La Lys canalisée a Erquinghem/Lys). The Deûle River has been impacted by the waste discharges and so is characterized as high-polluted water system. Site La Lys D is situated after the confluence of River Deûle and Lys. This fact supposes that the River Deûle influences the water and sediment quality. From the ***figure III.9*** is evident that the concentration is lowest at the site La Lys C (except the HgT_{SW} and HgT_{sed}). While concentration of HgT in particles (HgT_{ins}) is lower at the uncontaminated site La Lys C and this concentration is again higher after the confluence of two Rivers, the concentrations of deposited HgT in sediments (HgT_{sed}) is lower in the Deûle River and 3-50 times higher in the Lys River. The boat traffic at this place is not so intense, so the Hg in particles in lower while Hg is higher in the settle sediments. Mercury has a high tendency to be sorbed on surfaces. And in natural waters Hg is mostly bounded to sediments and a large proportion of Hg in water phase is attached to suspended particles (Ullrich, 2001). Hg bound to inorganic particles can be transported by the suspended matted from the place with a high boat traffic (Deûle – A, B) to the moderate place (La Lys – D), where the particles can settle. This is why the higher HgT concentration is found at the less contaminated place.

Surface contamination in South Morava:

The sampling place Belov ZL-11 was supposed to be the site relatively clear, without the industry and pollution discharges. The only source of pollution was the traffic. The sampling plase Zl-8 is situated on the river Dřevnice with the extensive industrial factories in proximity and traffic as well. The concentrations of Hg species in the studied environmental compartment (water, sediment) at these two places are comparable. The traffic seems to be the main factor responsible for the Hg contamination of aquatic system in this locality. Concentrations of the Hg species in studied environmental compartments are highest at the Zl-13 after the confluence of the Morava and Dřevnice River. The concentration is supposed to be influenced by the both stream, but also by the increasing industry in the proximity of this place. The concentrations decrease at the place Zl-11. This could be influenced by the fact, that no industry is located between the site Zl-13 and Zl-15, the pollution is result of previous contamination and the concentrations are lower.

Depth profiles sediments

The sediment cores for survey of depth profiles were taken at all sampling place in France (Deûle - A B, La Lys - C D) and just at the one sampling sites in the South Moravia - Ivaň, because of sediments compositions (in general the composition of the sediments on Moravia are too sandy) and missing suitable equipment (sediment sampler). The missing suitable equipment (anoxic condition for the sediment cutting) was the reason why only the HgT and MeHg in dry sediment were measured.

The average concentration of HgT and MeHg at Ivan is 30,3 ± 1,35 $mg.kg^{-1}$ and 2,87 ± 1,56 $\mu g.kg^{-1}$, respectively (***figure III. 10***). The concentration of Hg species in Deûle and Lys River are in the ***table III. 3*** and ***table III. 4***. The values were average for comparison of sampling places.

Figure III. 10: *HgT and MeHg depth profiles at Ivan.*

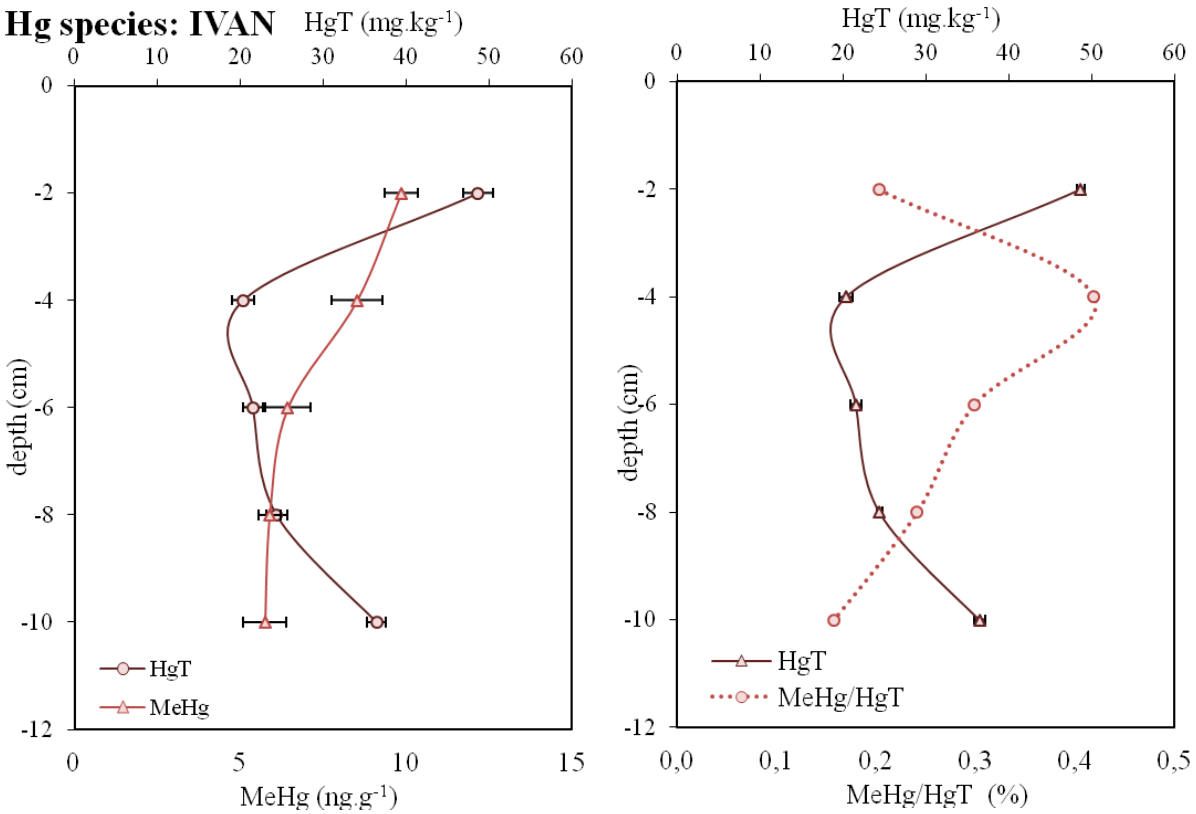


Table III. 3: *Average concentration of Hg species in the Deûle and Lys River.*

Sampling points	Surface water		Pore water	Sediment		MeHg / HgT_{sed}
	HgT_{SW} ng.L^{-1}	HgT_{ins} ng.L^{-1}	HgT_{PW} mg.kg^{-1}	HgT_{sed} mg.kg^{-1}	MeHg µg kg^{-1}	%
Deûle A	20,8 ± 0,8	44,3 ± 0,6	16,0 ± 3,2	9,92 ± 2,22	3,56 ± 1,30	0,21
Deûle B	8,0 ± 0,71	39,6 ± 3,6	9,83 ± 2,03	9,11 ± 1,85	7,61 ± 3,67	0,87
La Lys C	18,5 ± 1,5	15,3 ± 3,3	5,88 ± 0,35	42,0 ± 2,3	1,63 ± 0,09	0,42
La Lys D	19,4 ± 1,7	24,4 ± 4,6	11,4 ± 3,5	527 ± 13	1,86 ± 0,23	0,04

***Table III. 4:**. The range of Hg species concentration in the Deûle and Lys River.*

Sampling points	Pore water	Sediment		MeHg / HgT_{sed}
	HgT_{PW} $mg.kg^{-1}$	HgT_{sed} $mg.kg^{-1}$	MeHg $\mu g.kg^{-1}$	%
Deûle A	*11,9 – 24,1*	*6,89 – 16,0*	*1,01 – 5,06*	*0,12 – 0,73*
Deûle B	*7,17 – 11,8*	*6,36 – 11,76*	*2,81 – 12,2*	*0,26 – 1,92*
La Lys C	*1,02 – 8,53*	*25,9 – 61,0*	*1,35 – 2,31*	0,25 – 0,65
La Lys D	*0,98 – 28,9*	*180 – 944*	*0,49 – 3,16*	*0,01 – 0,09*

The content of Hg decreasing: **La Lys D:** HgT_{sed} > HgT_{PW} > MeHg > HgT_{ins} > HgT_{SW} ; **La Lys C:** HgT_{sed} > HgT_{PW} > MeHg > HgT_{SW} > HgT_{ins}; **Deûle B:** HgT_{PW} > HgT_{sed} > MeHg > HgT_{ins} > $HgT_{SW;}$ **Deûle A:** HgT_{PW} > HgT_{sed} > MeHg > HgT_{ins} > HgT_{SW}. HgT concentrations are significantly higher in the Lys River especially in the site La Lys – D (527 $mg.kg^{-1}$), while the Deûle sediment ranged from 6,36 to 16 $mg.kg^{-1}$. Contrary the MeHg concentration are lower in the Lys River (0,49 – 3,16 $\mu g.kg^{-1}$) and higher at the Deûle River (1,01 – 12,2 $\mu g.kg^{-1}$). The proportion of the MeHg/HgT is also higher at the Deûle River (0,12 – 1,92%) this may suggest also higher methylation in this area. When the MeHg concentration was plotted against HgT concentration in the sediments the noticeable negative correlation was observed only at the place Deûle-B (***figure III. 11***). This suggest no significant relationship between MeHg and HgT concentration except the place Deûle-B (r^2 = 0,79; n = 7), where the negative correlation is observed. At the Ivan, just weak positive correlation was observed (r^2 = 0,22; n = 5). HgT is not assumed as a predictor of MeHg concentration. Especially at the place La Lys – D where is a HgT concentration highest (180 – 944 $mg.kg^{-1}$), the % MeHg doesn´t exceed 0,01 %. Low MeHg concentrations are may be caused by the inhibition of microbial activity at high HgT levels (Chen, 1996). At the place Deûle - B, %MeHg is highest among the other place, ranged from 0,26 to 1,92%. This suggests that methylation is stronger, but is not only related to HgT concentration. All graphs of depth profiles of Hg species are presented at the figures in the **Appendix (C.I – C.IV).**

Total Hg concentrations vary with the depth at all sampling sites and no clear gradient is found (see ***figure III. 12***). The concentration is higher in the deeper sediment layer compared to the bottom sediment layer.

Figure III. 11*: Correlation HgT and MeHg.*

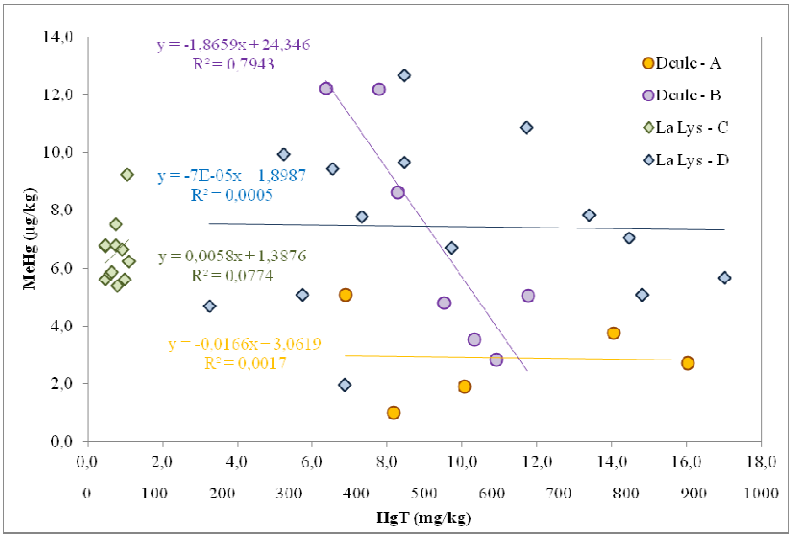
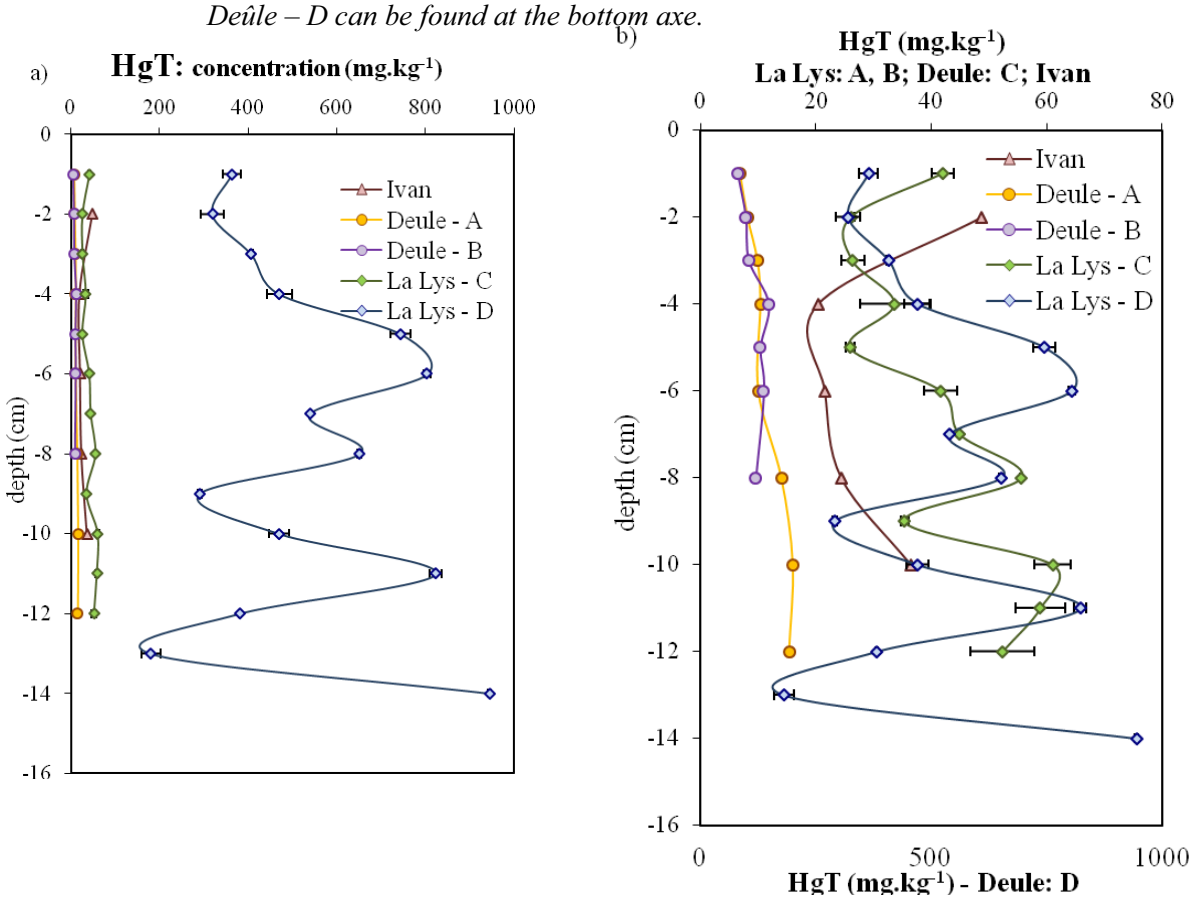
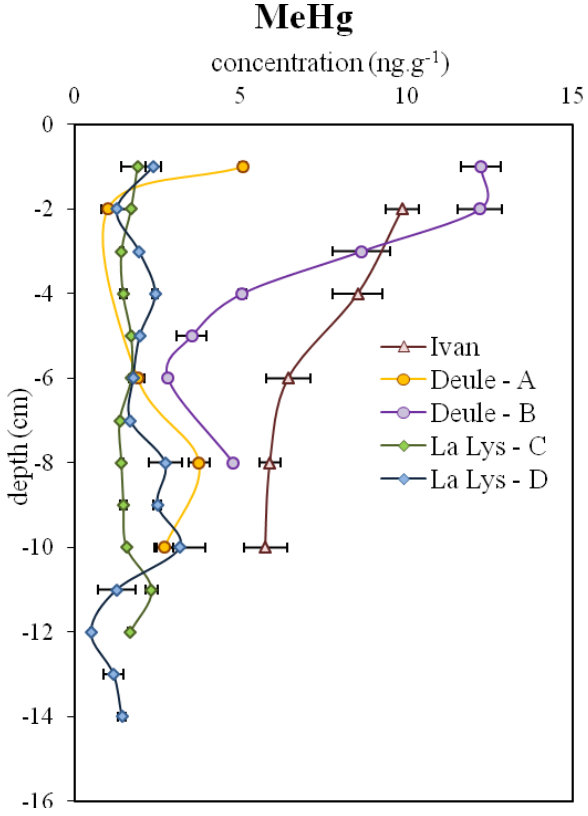


Figure III. 12*: Concentration of HgT: a) Concentration of HgT in the depth profiles at the all sampling place; b) Values for concentration of HgT in depth profile at the place Deûle – D can be found at the bottom axe.*



MeHg at the place La Lys – C and La Lys – D varied little among the top, middle and bottom sediment layers while at the sampling place of River Deûle and River Jihlava (Ivan) a clear gradient is found, MeHg concentration being higher in the top than in the bottom layer (*figure III. 13*). Higher MeHg concentrations are commonly found in the top layer of river sediments - in the oxic zone, which is attributed to factors such as higher organic matter and bacterial activity. While in the anoxic sediments the Hg is controlled especially by sulfides (Ulrich et al., 2001). The redox potential (Eh) was measured in the sediments dept profiles. In the Deûle River the Eh values were about 0 mV in the surface layer and values rapidly decrease already in the first three centimeters of sediments depth (values from -190 to – 250 mV). This corresponds with a higher MeHg concentration in surface layer. While in the Lys River redox potential was lower already in the surface sediments (values range from -100 to -120 mV) and decrease only gradually (lowest values range from – 150 mV to -167 mV).

Figure III. 13: *Concentration of MeHg in the depth profiles at the all-sampling places.*



Sulfur speciation

The range of AVS and CRS in depth profiles of investigated sediment cores are listed in the ***table III.5*** The graphs of depth profiles are appended **(D.I – D.IV).**

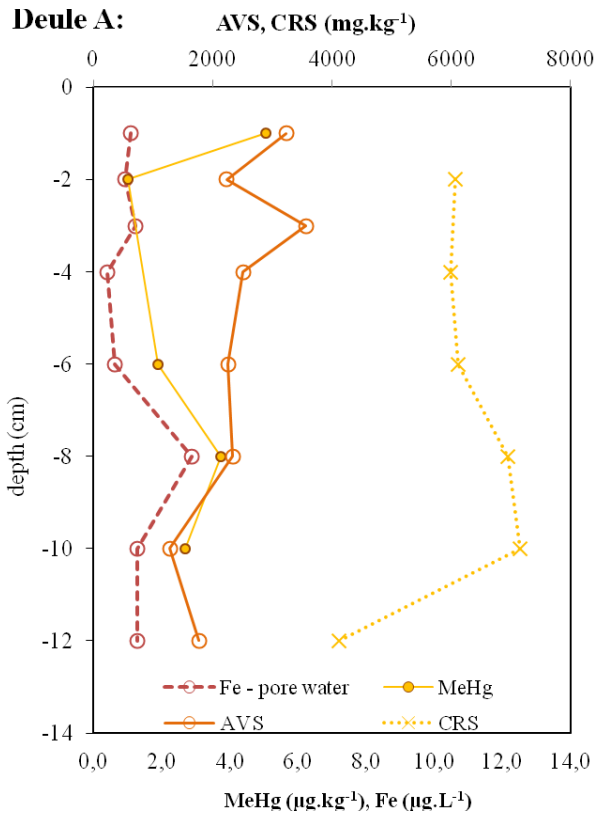
Table III. 5: *Range of AVS – CRS concentration (mg.kg^{-1}).*

Sampling points	AVS	CRS	AVS+CRS	AVS/CRS
Deûle A	1285 – 3565	4114 – 7154	3565 – 9284	0,18 – 0,43
Deûle B	404 – 3035	5399 – 9931	8109 - 12966	0,19 – 0,50
La Lys C	1106 – 2194	1208 – 3461	2248 – 5370	0,55 – 1,09
La Lys D	389 - 2495	1815 - 7280	1820 - 8955	0,17 – 0,43

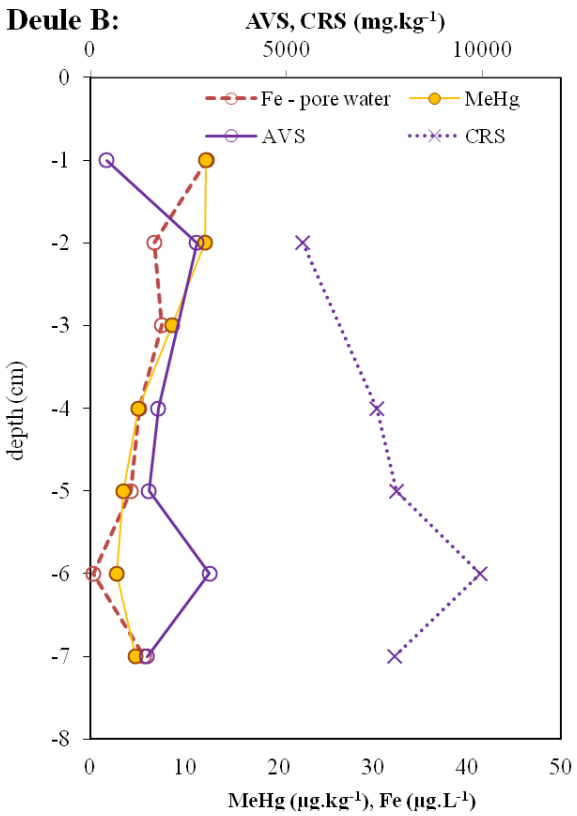
Relatively high concentrations of AVS and CRS are observed in sediment cores at all sampling places indicating significant activity of sulfate reducing bacteria in these sediments. These bacteria are responsible for the formation of solid sulfides, but they are also the principal methylators of mercury in sediments. The highest concentration of AVS was observed at the place Deûle-A (ave. 2397 ± 89 mg.kg^{-1}) and it is 1,3 – 2 times higher than at the other places. Anyway such a high values indicate the high anoxic conditions throughout the sediment cores. The AVS and CRS depth profiles do not show any increasing or decreasing tendency. Depth profiles of AVS and CRS and their ratio (AVS/CRS) indicate the degree of stability of anoxic conditions in the sediment column. Because the difference between upper and lower sediment layers is less evident, the stable redox conditions already in the upper sediments layers are pattern. High concentration of AVS and CRS in sediment cores corresponds to the low levels of dissolved Fe and Mn in porewater (***figure III. 14 a) – d)***; Fe profiles: more in **Chapiter V**). In the sediment profile Deûle D, the concentration AVS around - 9 cm depth is lower than average contrasting to higher concentration of dissolved Fe and MeHg found at this depth. The concentration of Fe and Mn are eliminated from porewater by precipitation of FeS and stable anoxic conditions are developed at these sediment. These anoxic conditions are not favorable for Hg methylation.

***Figure III. 14**:a) – d) Profiles of AVS, CRS , dissolved Fe in pore water and MeHg in sediments.*

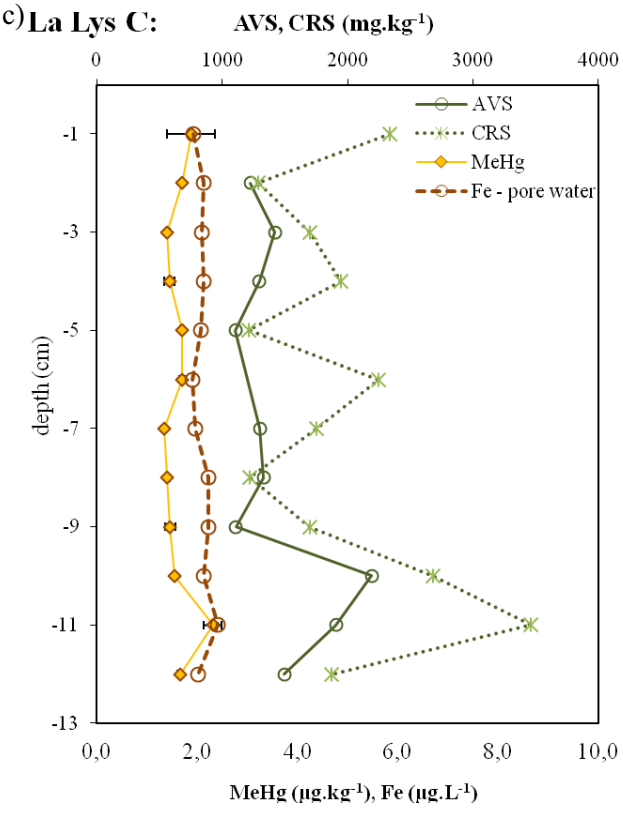
a)



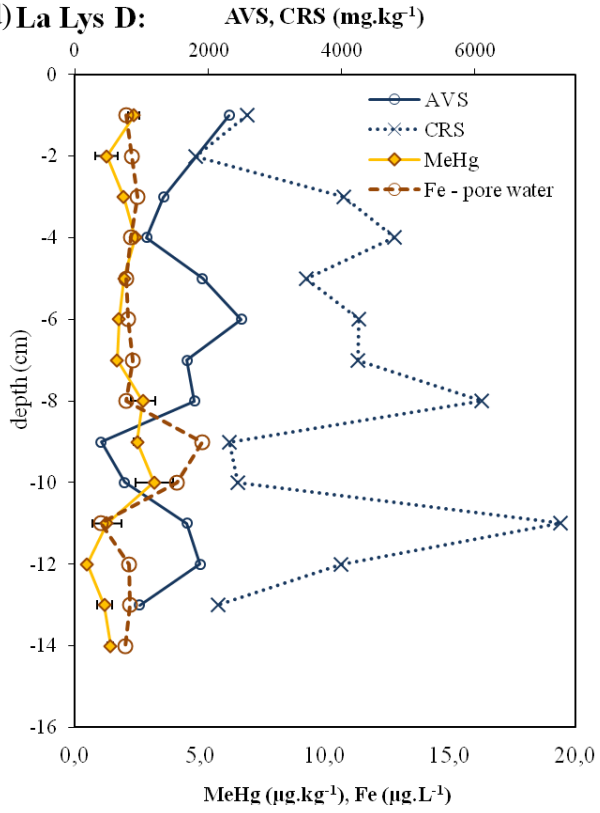
b)



c)



d)



III.I.5. Conclusion

Horizontal mercury distribution: The Hg species (dissolved, insoluble) in the surface water and pore water are more or less comparable in the Czech Republic and France. But the HgT concentration in sediments of Deûle and Lys River are higher than in Morava River. This can prove the lower anthropogenic contamination of aquatic systems at the South Moravia.

Sediments composition: The sediments composition is different. The sediments taken in Czech Republic were much sandier while the sediments in Northern France were muddy with the anoxic conditions (redox potential was evaluated) already in the first centimeters of its surface. The exception is sediments of Jihlava River, where the sediments composition is comparable with the sites of Lys River. This corresponds also with the approximately similar concentrations of all studied Hg species at these sites.

Mercury and environmental standards: The values of Hg species in the Morava and Jihlava River were below the value of Standard or Global background, with exception of site ZL-13 (Morava River), where the value was little bit exceeded and site of Jihlava River where the concentration of HgT in sediments were largely exceeded. In Northern France the background value of Hg is 0,1 $mg.kg^{-1}$. This values of Hg concentration was exceeded at all sampling points of Deûle and Lys River.

Vertical mercury distribution: HgT concentrations are significantly higher in the Lys River especially in the site La Lys – D (527 $mg.kg^{-1}$), than in the Deûle River. Contrary the MeHg concentrations are lower in the Lys River (0,49 – 3,16 $\mu g.kg^{-1}$) and higher at the Deûle River (1,01 – 12,2 $\mu g.kg^{-1}$). Not a significant correlation was observed between the HgT and MeHg. This suggests HgT is not a predictor of MeHg concentration. The sediments may be assessed as a dominant sink for mercury. While compared the redox potential and depth profiles of mercury and sulfides we can suggest not favorable conditions for Hg methylation. The methylation and demethylation yield of these sediments were investigated within the experiment using species specific stable isotopes tracers (***Chapiter IV***).

Mercury and sulfides: In sediments mercury can be bound to sulfides and other sulfur compounds. The sampled depth sediments are characterized by the anoxic conditions. Of the many sediment components that influence mercury behavior, the primary controller of much mercury in anoxic sediments of Deûle and Lys River may be sulfides.

The inorganic mercury in sediments bound to sulfides and other compounds generally represents less of an immediate biological hazard than organic – methylated form. However it should be bring to the surface layer or up into the water column (e.g. by flossing disturbances, bioturbation, release with sulfide gases, dragging etc.) and relatively harmless Hg forms can be transformed into more hazardous and more bioavailable forms.

Chapter IV: METHYLATION AND DEMETHYLATION YIELD CALCULATIONS USING SPECIES SPECIFIC STABLE ISOTOPES TRACERS

SCIENTIFIC BACKGROUND

IV. I. Stable isotopic tracers

Stable isotopes have the same atomic number and are placed at the same spot in the periodic table and participate in the same chemical reactions. But because they have different atomic masses (because of variable numbers of neutrons in the nucleus), the molecules made up of different stable isotopes have different vibration frequencies which in turn lead to differences in heat capacities, entropies, diffusivities, velocities, free energies and rates of reaction of the molecules (Bigeleisen, 2005).

Out of 108 naturally occurring elements, 87 have more than one stable isotope. Mercury has seven stable isotopes (^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg and ^{204}Hg) with minimum relative mass difference of 4% between ^{196}Hg and ^{204}Hg. Relative abundance and spins of mercury isotopes are mentioned in table IV.1.

Table IV.1: *Natural abundance of stable isotopes of mercury.*

Isotope	Abundance (%)	Nuclear Spin
^{196}Hg	0,15	0
^{198}Hg	9,97	0
^{199}Hg	16,87	1/2
^{200}Hg	23,10	0
^{201}Hg	13,18	3/2
^{202}Hg	29,86	0
^{204}Hg	6,87	0

In thesis I applied the isotopically enriched species of mercury, solutions of IHg: ^{199}Hg and MeHg: ^{201}Hg.

IV.I. 1. Applications of stable isotopic enriched species

The combination of the use of stable isotopes and precise detection technique (typically based on mass spectrometry) offers a suitable technique for speciation analysis and permit the follow the transformation of the different species in the environment.There exist two types of experiments used the stable isotopes species:

- Isotope dilution experiments
- Isotopic tracers experiments

The **technique of isotopic dilution** (ID, offend inductively coupled plasma-isotope dilution mass spectrometry: ICP-IDMS) is a quantification technique with high potential for analysis of trace elements (Heumann, 2004). This technique has low limits of detection which restrict the concentration range and disable the experiments focused on the tracking of the reactivity of species. Previously used radioactive isotopic tracers (Ramlal et al., 1986; Gilmour and Riedel, 1995) augment the load of mercury to the environment and impacted the achieve results. Before 1990 determination of trace elements and less frequently, element species by isotope-dilution mass spectrometry (IDMS) was almost exclusively performed by use of thermal ionization (TI-IDMS)(Heumann, 1990). Since 1990 an increasing number of ICP-IDMS analyses have been published (Lu et al., 1993; Enzweiler et al., 1995) and in 1994 the first papers about element speciation coupling HPLC with ICP-IDMS appeared in the literature (Brown et al., 1994; Heumann et al., 1994).

In an other hand, the use of the **stable isotopic tracers** enables the suitable tool for study of the environmental reactions and interactions (transfers and transformations). Nowadays stable isotopic tracers are used in various scientific areas to investigate pathways of minerals, trace elements and biomolecules in many different biological systems (Stürup et al., 2008), ranging from estimating human absorption of iron (Roe et al., 2005), to studies into the bioaccumulation of mercury in aquatic food web (Pickhardt et al., 2005), to tracking parasitoid wasps in farmland (Wanner et al., 2007).

The choice of isotope experiment depends on the biological process under investigation. The various isotope experiments have been further developed and refined within the individual research areas to fit a particular type of investigation (Stürup et al., 2008).

In thesis I applied well-established isotope experiments to study methylation/demethylation processes in aquatic ecosystems especially sediments. This experiment methodology is refined by applying advanced matrix algebra to resolve the contributions of several different enriched stable isotope species specific tracers to the isotope pattern found, making the calculation of methylation/demethylation potentials possible.

IV.I. 2. Analytical methods for Hg speciation analysis

In all analytical techniques applied in enriched stable isotope tracer studies, precise and accurate determination of isotopes are required in samples that already contain a large amount of the same element, but with natural isotopic composition. The shift in isotopic composition induced by the enriched stable isotope tracer needs to be quantified (Stürup et al., 2008). This involves determining the isotope ratio of the tracer isotope over a reference isotope. Generally, a minimal measurement precision of 1% is required for isotope ratio determinations. The most applied techniques are inductively coupled plasma mass spectrometry (ICP-MS) and thermal ionization mass spectrometry (TIMS). TIMS offers good sensitivity and high precision and accuracy of measurements. This technique has several disadvantages, like previous fully separation of the analyte from the sample matrix (in order to ensure efficient ionization), which evoke a time-consuming sample preparation and a low sample output. Contrary of the TIMS, ICP-MS offers a simple sample preparation and higher output.

Since a mass spectrometer separates and detects ions of slightly different masses, it easily distinguishes different isotopes of mercury. The precise isotopic composition must be determined. The ICP-MS technique allows the measurement of individual isotopes with sufficient sensitivity, which is essential if we want to apply enriched mercury isotopes in stable isotope tracking experiments (Hintelmann and Evans, 1997).

It seems nowadays, that ICP-MS, with its recent technological developments (high resolution, multicollector systems reaction/collision cell) has become the preferred technique. However, it is important to evaluate the accuracy and precision in the sample matrix, because several factors (mass bias, detector dead time, interferences) do may affected both.

Mass bias is factor causing deviations of measured isotopic rations from the expected ratios. Mass bias derives from the different transmission of ions of different mass from the point at which they enter the sampling device until they are finally detected by the electron multiplier, however the true cause of these effects in not completely understood. (Monperrus et al., 2004) Mass bias can amount to 5% per mass unit depending on the element measured and instrument setup.

Detector dead time is the time during which the detector does not record and when some ion during this time interferes with the surface of the detector it will not be detected. This tends to underestimate the real rate. The dead time arise from the electronics devices of the detector system (amplifier, discrimitator and counting system) and is changing with the age of the detector. Reevaluation of detector dead time periodically is recommended.

Interferences are dependent on the sample matrix, but recent improvements offer unique capabilities for interference removal (reaction/collision cell) without any significant loss of sensitivity and good isotope ratio precisions. Moreover, in the case of Hg, atomic and polyatomic interferences are limited.

IV.I. 3. Isotopic tracer experiments

In the aquatic systems all Hg species undergo to the antagonist transformation reactions (methylation and demethylation) and so it is necessary to resolve the contribution of each transformation process in the aquatic system and to resolve if the new inputs of mercury into the system are driving the biogeochemical transformation and bioaccumulation processes or if the old mercury present in the system is responsible for the overall effect (Hintelmann and Harris, 2004). The use of isotopic tracers enables the tracking of dynamics of the substance of interest (e.g. mercury), the subject of the antagonist reactions like adsorption / desorption (Hintelmann and Harris, 2004), methylation / demethylation (Rodriguez Martin-Doimeadios et al., 2004), oxydation / reduction (Whalin and Mason, 2006), assimilation / excretion (Rodriguez-Gonzalez et al., 2005), etc.

The technique enables not only the determination of the species transformation parameters (i.e. ratio – net and rough of conversion) and kinetic constant, but also enables the follow the transformation process of the natural present species (endogenous) and the isotopic labeled added species (exogenous species) simultaneously. The majority of the previous studies show the higher reactivity of the added isotopic labeled species (Hintelmann et al., 2000; Benoit et al., 2002),

particularly regarding the methylation of the inorganic isotopic tracers of Hg. So the determined transformation yields represent the potential of transformations rather than exact yield. However this means that the reactivity of the added isotopic labeled species (even in the minor quantity) is subjected to its own reactivity which is different from the reactivity of endogenous species.

Nowadays the use of isotopic tracers has not the clearly established procedures. The different forms of the tracers may be added to the environments in equilibrium with investigated environmental compartment or just approximately. The quantity of added isotopic tracers is one of the main subject of investigation for the transformation processes: very low concentrations can be used if we want to respect the conditions of non contaminated place or higher concentrations can be used in the cases where we want to evaluate the response of the environment to the environmental changes.

IV.I. 4. Example of the stable isotopic tracers use

Some representative examples of the use of stable isotopic in experimentation to evaluate Hg species reactivity are listed in the ***table IV.2***.

The most investigated environmental compartment is sediment. The separated incubations of sediment slurries (the layers form the different sediments depth) or directly incubations of the sediment cores and the water columns are possible. The main advantage of the slurry incubations is the possibility of the kinetic studies (Rodriguez Martin-Doimeadios et al., 2004). The great advantages are also the homogeneity of the incubations conditions and the easily measured physico-chemical parameters which permits simulate the Hg speciation (Drott et al., 2008a; Drott et al., 2008b). And finally the significant advantage is also the possibility of easily distinguished control above the different phases (solid, water, gas).

The **incubation realized directly in the sediments cores** offer the incubation condition closed to the real systems, but also not so well controlled. The isotopes are injected by the needle at the different intervals. At the end of the incubation, the core is sliced and the concentration is determined in each slice. The determined transformation yields are generally calculated from the *in situ* concentrations of the Hg species. However the isotopically enriched species may be redistributed within the core and the spiked species could not be perfectly recovered along the core compared to the theoretically spiked amount at each depth section (Monperrus et al. 2007). The result may be affected by the heterogeneities of the sediment (e.g. due to occurrence of shell).

Table IV.2*:The use of enriched Hg stable isotopes in aquatic ecosystems.*

Isotopes	Labeling technique and aim of study	Analytical techniques	Methylation	Demethylation	Reference
^{200}Hg, ^{201}Hg	$^{201}Hg^{2+}$ and $CH_3\ ^{200}Hg^{+}$ were added to experimental tanks in order to investigate the influence of algal biomass on Hg accumulation through the food web.	Cold vapor generation HR-ICP-MS. Isotope ratio, precision 0.1% RSD.	-	-	(Pickhardtet al., 2002)
^{200}Hg, ^{201}Hg	Mesocosm experiments with ^{201}Hg2+ and $CH_3\ ^{200}Hg^{+}$ to evaluate the influence of zooplankton composition, algal abundance and Hg speciation on the ability of zooplankton to accumulate Hg from phytoplankton and transfer Hg to planktivors.	Cold vapor generation HR-ICP-MS. Isotope ratio precision 0.1% RSD.	-	-	(Pickhardt, et al., 2005)
^{202}Hg	^{202}Hg2+ was added to mesocosms in a lake to study dissolved gaseous mercury production and evasion.	Dissolved gaseous mercury determined by gold trap HR-ICP-MS.	-	-	(Poulainet al., 2006)
^{199}Hg ^{201}MeHg	Experiment designed to unravel mercury species reactivity in superficial coastal sediments oscillation between oxic and anoxic condition.	GC-ICPMS-CT-GC-AFS	Estuarine sediment: 1,2 % Lagoon sediment: 0,5%	Estuarine sediment: 92% (49 – 98%) Lagoon sediment: 47% (4 - 69%)	(Bouchetet al., 2011)
^{200}Hg ^{202}Hg	$^{200}Hg^{2+}$ and $^{202}Hg^{2+}$ isotopes were added to mesocosms in a lake in order to study biogeochemical cycling of Hg in food web and to estimate the contribution of added Hg to MeHg accumulation in fish and other biota.	Total mercury in water, fish and particles by isotope dilution ICP-MS, MeHg by GC-ICP-MS	-	-	(Paterson, 2006)
^{199}Hg ^{201}Hg	Study of mercury isotope fractionantion during photoreduction in natural water	MC-ICP/MS for isotope ratio measurements	-	-	(Zheng and Hintelmann, 2009)
^{199}Hg, ^{199}Hg ^{201}MeHg	199Hg was addend to sediments 199Hg and 201 MeHg was addend to the water samples. Methylation rates of Hg in surface sediments and the water column were determined. The experiment was conducted in order to evidence and evaluate the fate of MeHg influenced by benthic and pelagic dynamics.	GC-ICPMS	Rates range: $0 – 6,3\%.day^{-1}$	Rates range: $6,4 – 24,5\% .day^{-1}$	(Monperruset al., 2007)
^{199}Hg ^{201}MeHg	Study of mercury transformations (methylation, demethylation and volatilization) in estuarine sediments under biotic and	GC-ICPMS	Anox-abiot: 3,34% Anox-biot: 0,178%	Anox-abiot: 9,10% Anox-biot: 32,45%	(Rodríques Martín-Doimeadios et al.,

Isotopes	Labeling technique and aim of study	Analytical techniques	Methylation	Demethylation	Reference
	abiotic, oxic and anoxic conditions)		Ox-biot: 0,196% Ox-abiot: 0,148%	Ox-biot: 2,16% Ox-abiot: 7,86%	2004)
^{199}Hg	Estimation of Hg methylation in river sediments by injection of $^{199}Hg^{2+}$ into sediment cores. (MeHg/HgT: 0,07 – 0,3%)	ICP-MS for 199Hg isotope determinations.	Chesapeake Bay1%(Mason et al. 1999) Lavaca Bay:0,6%(Bloom et al. 1999) Fundy: 0,6% (Sunderland et al. 2004)	Chesapeake Bay: 12 – 48% $.h^{-1}$ Fundy: 10 – 30%$.h^{-1}$	(Heyes et al., 2004)
^{199}Hg ^{201}Hg	Rates of Hg methylation and MeHg demethylation in sediment of the Hudson River, Chesapeake Bay and Fundy.	HgT: CV-AFS MeHg: Purge and trap GC-CVAFS Detection ICP-MS	Hudson River: 0,2% Patuxent River: 0,23% Bay of Fundy: 0,7%	Hudson River: 6,4 – 9 - 11%.MeHg loss hr^{-1} Bay of Fundy: 25 - 29 %.MeHg loss hr^{-1}	(Heyes, Mason et al 2006)
^{203}Hg ^{14}C-MeHg	Transformation of Hg species in sediment from the second largest Hg mine – Idrija Mercury Mine, Slovenia (Gulf Trieste). Determined using radio-techniques	GC-CVAFS	0,1 – 1,9%	0,004 – 1%$.h^{-1}$	Hlines, E., Faganeli, J. et al 2006
^{199}Hg	Diagenetic behavior of mercury species in surface sediment of the Gironde Estuary (south-west France)	GC-ICP-MS	0,28%	11% h^{-1}	Schäfer et al 2010

IV.I. 5. Measurements of mercury species transformation

It is nowadays established that observed methylmercury concentrations in aquatic sediments is the net product between mercury methylating and methylmercury demethylating processes affected by micro organisms. It is also necessary to gain knowledge about the rates at which these reactions occur. That information is valuable in risk assessment of Hg polluted industrial areas and development of preventive measures to minimize Hg methylation at such sites. There have been several methods and strategies by which Hg species transformation rates have been determined, but none of them was ideal. Jensen and Jenelov (Jensen and Jernelov, 1969) studied mercury methylation by spiking sediments with inorganic mercury of natural isotopic composition. This method required very high concentrations of inorganic Hg to differentiate any significant methylation of the added inorganic Hg spike from MeHg already present in sediment and moreover large amount of added inorganic mercury could affect the incipient Hg species equilibria and microbial fauna drastically. Methods utilizing the radiochemically labeled tracers (Rudd et al., 1980; Ramlal et al., 1986) are relatively simple and methods can easily be adopted for field study. But the Hg radioactive tracer ($^{203}Hg^{2+}$) are usually of low specific activity and the samples have to be spiked to unrealistically high concentrations to detect the methylated tracer. And even if the tracer has very high specific activity (Gilmour and Riedel, 1995), the selectivity of the methylmercury extractions used in radiotracer methods is questionable and mercury species transformation - methylation rates may be overestimated. Significant improvement was made by Hintelmann and co-workers about 15 years ago, when they presented a method that made the use of enriched stable inorganic Hg as a tracer to monitor mercury methylation in combination with gas chromatography inductively coupled plasma mass spectrometry instrumentation (Hintelmann et al., 1995). They spiked the sediment slurries with $^{199}Hg^{2+}$ very low incipient concentrations and determined the changes in incipient methylmercury levels over a period of thee week. They further work enabled measurement of demethylation rates simultaneously in the same sample as methylation rates due to development of methylmercury tracer (Hintelmann and Evans, 1997; Hintelmann et al., 2000).

Calculation

Nowadays there exist four mathematical approaches for multiple spiking species-specific isotope dilution analysis: Calculation of Stable Isotope Concentrations, Speciated Isotope Dilution Analysis, Species-Specific Isotope Dilution Analysis and Isotope Pattern Deconvolution. In 1997

Hintelmann and Evans presented the first speciation analysis based on multiple spike and its application on MeHg and IHg quantification (Calculation of Stable Isotope Concentrations) taking in a account the methylation and demethylation (the opposites processes). In 1998 Kingston et al presented the model called Speciated Isotope Dilution Analysis. The methodology developed by Ruiz Encinar et al (2002) and improved by Rodriguez-Gonzales et al. (2004, Multiple Species-specific isotope dilution analysis) with three enriched species was used for quantification of butyltin. Finally, the quantification of transformation rate (Isotope Pattern Deconvolution) was presented by Meija et al. in 2006.

The methods Calculation of Stable Isotope Concentrations and Isotope Pattern Deconvolution provide advantages such as the qualitative information on any non-spiked species present in the samples, In addition, method Isotope Pattern Deconvolution can be used as a internal procedure for mass bias correction without the additional measurement of reference isotope ratios. (Rodriguez-Gonzalezm P., 2007)

IV.I. 6. Methylation and demethylation potential measurement

If an isotope enriched inorganic mercury tracer is added to a sediment (e.g. $^{199}Hg^{2+}$), the isotopic abundance ratio between ^{199}Hg and a reference isotope (^{202}Hg) for that species will be shifted from natural ratio to a higher value, that depend on the amount of added tracer. Methylmercury produced from the inorganic mercury present in the sample will display as 199/202 ratio that is higher than the natural 199/202 ratio. The amount of methylmercury formed ($^{199}Hg^{+}$) is proportional to the degree that the measured methylmercury 199/202 ratio exceeds the natural 199/202 ratio and can usually be quantified with high accuracy and precision especially if the tracer experiment is combined with SSID calibration during sample treatment. The limit of detection for Hg methylation using stable isotope methodology will be dependent on the incipient concentration of methylmercury. To detect a significant methylation of the added tracer ($^{199}Hg^{2+}$), the resulting increase in the 199/202-methylmercury ratio has to exceed the natural 199/202 ratio by an extent equivalent to three times the standard deviation interval at which the 199/202 ratio can be measured (Lambertsson, 2005). The incipient methylmercury concentration determines the methylation limit of detection in the sample, as a higher incipient amount of methylmercury means that a higher amount of the tracer has to be methylated to produce a significant increase in the 199/202 methylmercury ratio and conversely. The strategy for methylmercury demethylation potential measurements using stable isotopes is based on the decrease in concentration of the added

methylmercury tracer during the incubation period. It is necessary mentioned the fact that methylmercury is generally present at 1-5% of inorganic mercury concentrations. This would render very high demethylation detection limits if measurements were based on an increase of the inorganic tracer/reference isotope ratio due to methylmercury tracer demethylation.

To determine the amount of methylated and demethylated Hg, at least three isotopes of Hg must be monitored: one representing the newly produced MeHg from inorganig mercury (IHg) trace addition (isotope 199), one representing the demethylation of the MeHg tracer addition (isotope 201) and last one representing the changes in MeHg concentrations derived from the Hg, originally present in the sample (isotope 202**; *figure IV.1***).

The total concentration of methylated Hg produced in the sediment from the spike ($^{1}MeHg_{sp}$) can be calculated by using the equation: (Hintelmann, et al., 1995; Rodriguez Martin-Doimeadios, et al., 2004)

$$^{1}MeHg_{sp} = \frac{\sum {}^{2}MeHg - R_n \sum {}^{1}MeHg}{(R_{sp} - R_n)\,{}^{1}A_{sp}} \qquad [IV.1]$$

The superscript 1 refers to the isotope 199, superscript 2 refers to the isotope 202. The superscript *n* refers to the natural methylmercury that was originally present in the sample before trace addition. The subscript *sp* distinguish the newly produced methylmercury from the spike, R_{sp} is calculated from the total mercury isotope ratio measurement in the tracer solution used for spiking. R_n is the isotope ratio corrected for mass bias of the unspiked sediment sample.

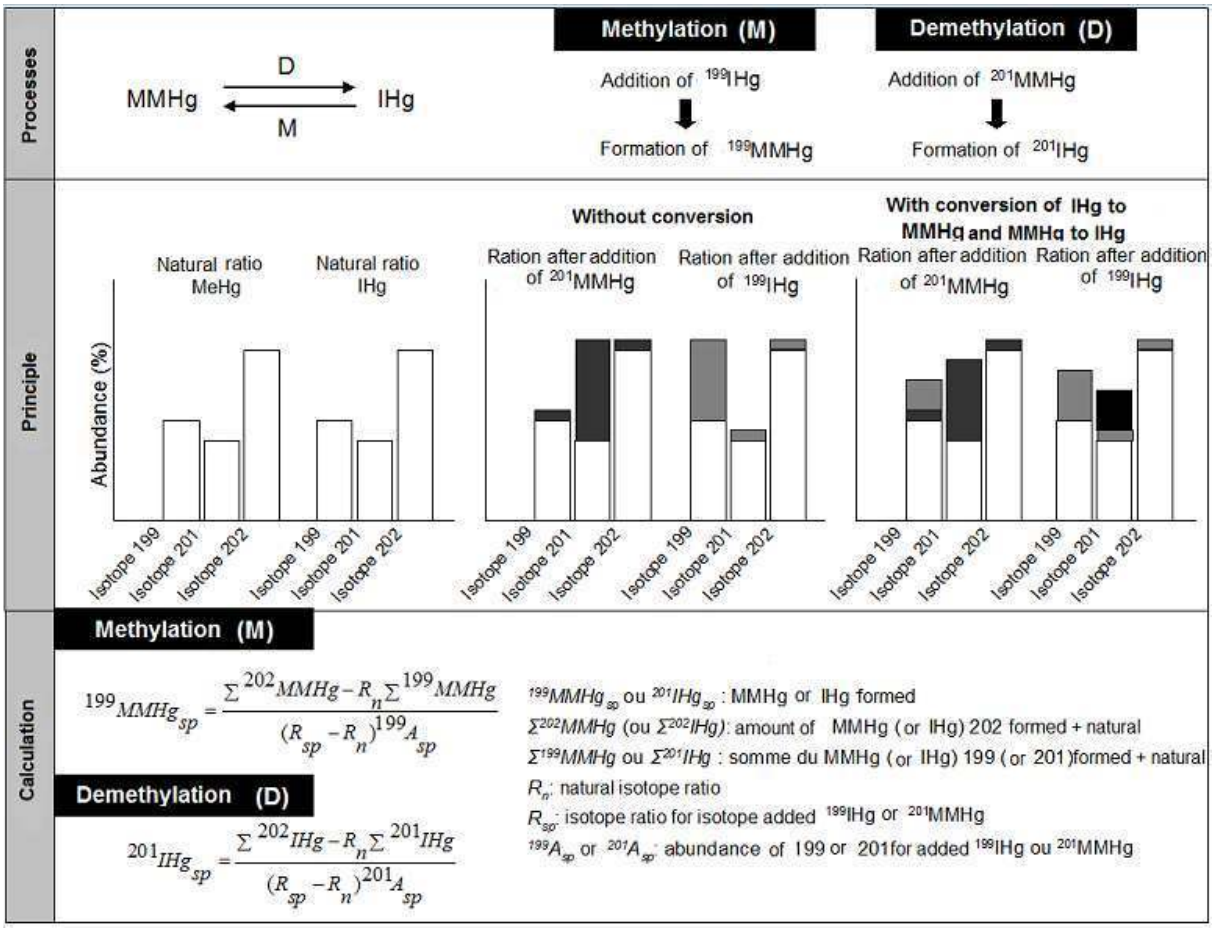
$^{1}MeHg$ represents the total concentration of enriched isotope tracer coming from the sum of the newly produced and already present MeHg. $^{2}MeHg$ correspond to the corrected concentration of natural MeHg for produced MeHg impurities in the tracer solution. And $^{1}A_{sp}$ correspond to the abundance of isotope 1 in tracer solution.

The amount of demethylated MeHg from enriched spike is calculated in the same way following the isotope 201 instead of isotope 199. The demethylation is calculated by substraction of the initial ^{201}MeHg amount added to the final ^{201}MeHg amount.

The principles of mercury transformation (methylation/demethylation) as well as calculation are resumed on the ***Figure IV.1.*** Measured yields of methylation and demethylation are assumed to define methylation and demethylation potentials in the considered system. The methylation yield is calculated by dividing the amount of ^{199}MeHg formed by the amount of ^{199}IHg

recovered after the incubation period. The demethylation yield is calculated by dividing the quantity of ^{201}IHg formed by the quantity of ^{201}MeHg added. If it is assumed that the spiked mercury species are equally or more available than the natural Hg species, the calculated yield represent the maximum yield that can be obtained under natural conditions (Monperrus et al., 2007).

***Figure IV.1**: Principle of the isotopic tracers use for methylation and demethylation potential.*



The use of stable tracers in environmental studies offers a lot of advantages. ICP-MS instrumentation offers exceptionally low detection limits, however the response is calculated from changes in the isotope ratios of the Hg and reflect the behavior of the stable tracers. Isotope ratio measurements allow the use of very low spike concentrations which does not exceed natural levels. The great benefit gained in using the stable isotope tracer methodologies is the possibility to follow more than one process. So reversible methylation/demethylation transformation potentials, can be determined simultaneously in one sample with high specificity and with very low tracer additions, which enables the establishment of net methylation rates for the study system.

Study of methylation and demethylation potentials of highly contaminated sediments

IV. II. Materials and methods

IV.II.1. Cleaning procedures

All equipment used for field sampling, sample incubation, storage and laboratory analysis were cleaned using specific protocols adapted from trace metal analyses. Briefly all material was cleaned with specific detergent, rinsed with MQ water then cleaned in 10% HNO_3 (twice) and 10% HCl bath, rinsed with Mili-Q water, dried under laminar flow hood and stored in double sealed polyethylene bags until use.

IV.II.2. Reagents and standards

Hg standard solution enriched in ^{199}Hg (^{199}Hg - 91,71%; 1 mL solution stabilized with nitric acid) was purchased from ISC Science (Oviedo, Spain) and was of isotopic composition:

Isotope	Hg-196	Hg-198	Hg-199	Hg-200	Hg-201	Hg-202	Hg-204
Content (%)	0,092	1,489	91,974	4,859	0,680	0,791	0,115

Enriched MeHg standard solution in ^{201}Hg (^{201}Hg - 96,5%; 1 mL solution in acetic acid/methanol 3:1) was purchased from ISC Science (Oviedo, Spain) and was of isotopic composition:

Isotope	^{196}Hg	^{198}Hg	^{199}Hg	^{200}Hg	^{201}Hg	^{202}Hg	^{204}Hg
Content (%)	< 0,01	0,043	0,109	0,890	96,495	2,372	0,091

All chemicals were at least of analytical reagent grade (list of reagent and producers are mentioned in ***Appendix B***). Ultrapure water was obtained from a Mili-Q system (Quantum EX, Milipore, USA). The acid solution of HNO_3 (6M) was prepared by diluting with water the appropriate volume of concentrated acid. Glacial acetic acid and sodium acetate were used to

prepare 0,1 M acetate buffer solution. Solution (1%) of tetraethylborate was prepared daily and stored in a refrigerator (4°C) until use. All stock solutions and standards were stored at 4°C and protected from the light. Stock standard solutions (1000 $\mu g.mL^{-1}$) of IHg and MeHg of natural isotopic composition were prepared by dissolving mercury (II) chloride in 1% HNO_3 and methylmercury chloride in methanol, respectively. Working standard solutions were prepared daily by appropriate dilution of the stock standard solutions with 1% HCl and stored in the dark at 4°C

IV.II.3. Preparation of the isotopically enriched tracer solutions

Spike solutions of ^{199}Hg and $^{201}MeHg$ were prepared by diluting the stock solutions with the natural water collected in the study sites. Hg species were then equilibrated with the water for 30 minutes before their addition into the samples. The spiked solutions were stored acidified till the analysis to determine precisely the Hg species concentrations and the relative isotopic abundances.

IV. III. Study area – sampling

The Deûle River area which is affected by severe metallic pollution, was studied. Samples were taken from the polluted site in proximity of the smelter Metaleurop, near the Douai city. Metaleurop was in activity for more than a century and was closed in January 2003. The sediment cores were taken in March 2010 in order to investigate the methylation and demethylation potential at two locations (***figure IV.2*** site I – Metaleurop;site II – Deûle Amont). Site I was directly impacted by the extensive mercury pollution (I.) and the second was located before the contamination source (II.) (***figure IV.3***). Cores were taken carefully in order to maintain the integrity of the sediment-water interface. Sediments were collected using hand-driven gouge sampler and Plexiglas tubes (80 cm length, diameter 7 cm) and sealed underwater with rubber caps. The pictures of sediment cores are present the figure IV.4. Two replicates were taken at each site.

***Figure IV.2**: Sampling sites – I. Metaleurop site impacted directly by extensive mercury pollution; II. Deûle Amont site located befor the contamination source.*

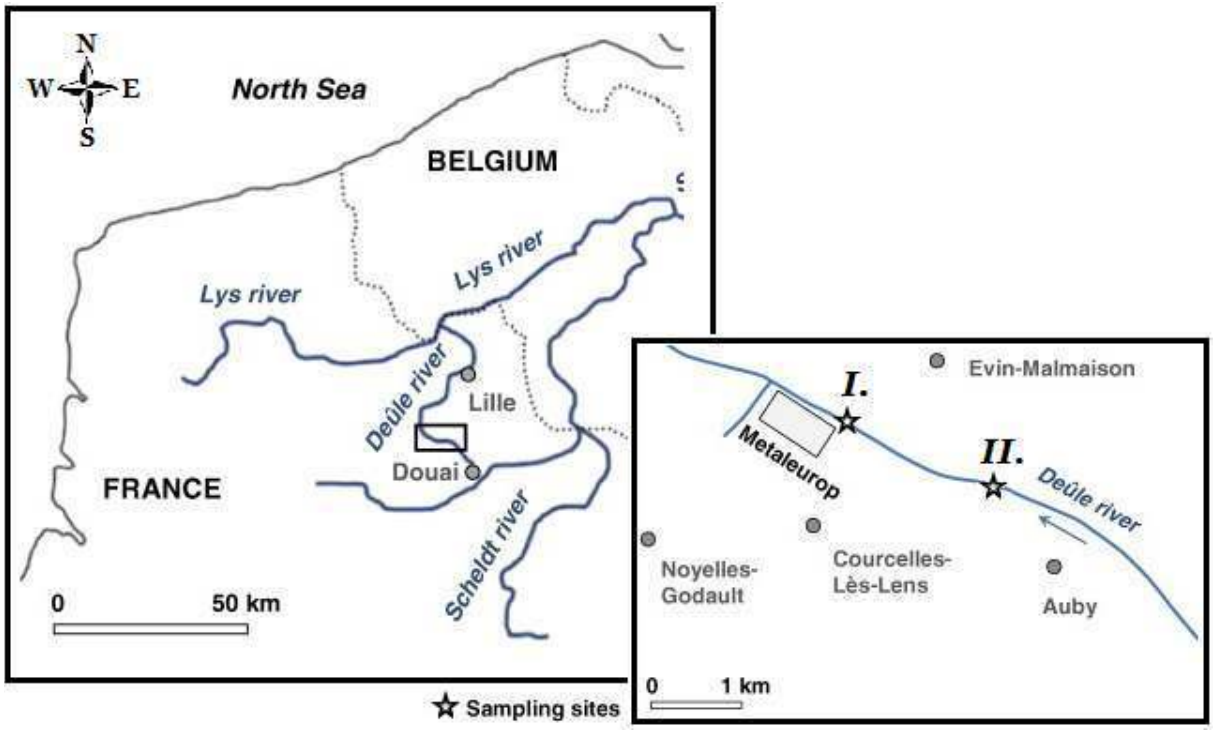
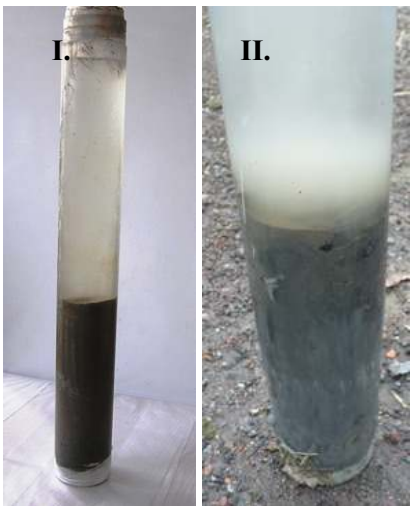


***Figure IV.3**: Picture of Sampling sites*

***Figure IV.4**: Picture of sediment cores*



IV. IV. Preparation of sediment – spiking, incubation and storage

Under a nitrogen atmosphere inside a glove box, the cores were sectioned by depth (in 3 cm layers). A part of these sliced sediments was kept in plastic bags previously purged with N_2 for analysis of acid volatile sulphides (AVS) and Chromium Reducible Sulfur (CRS). Approximately 5 g of wet sediment from each slice (mass was precisely balanced) was transferred into the 22 mL headspace vials and 5 mL of overlying water was added. The slurry was spiked with a known amount of isotopically labelled Hg species. Because the same samples were used for methylation and demethylation yield determination, two isotope enriched mercury species (^{199}IHg, ^{201}MeHg) were added to the samples at concentration levels of 2 µg/g IHg and 20 ng/g MeHg approximately. Sediment samples were spiked with an aqueous solution of ^{201}MeHg (0,2 µg/g) to 0,1% of HgT (10 µg/g) concentration and ^{199}IHg to (10 - 40%) of the HgT concentration. Duplicates sample of each sediment layer for this experiment was prepared. Sediment slurries were sealed gas-tight and incubated for 24 hours in the dark and at bottom water temperature (12 – 14°C). After the incubation period, slurries were stored in -20°C until next handling. For accurate determination of IHg and MeHg concentrations in the sediment, two slurries controls (t_0) from each core were spiked and directly stored without incubation period: the top sediment layer ($t_{0, -3}$ = -3 cm depth) and the deepest sediment layer ($t_{0, -30}$ = -30 cm) were frozen immediately after spiking.

Additionally, control assays were performed on water used for preparation of slurries. Six water samples (5 mL) were spiked with the same amount of isotopically labeled Hg species (^{199}IHg, ^{201}MeHg). The process of incubations in three water samples was stopped directly after adding enriched stable isotope Hg species (t_0) by adding high purity HCl (1% v/v). Remaining three samples were incubated and after 24 h, the incubations were stopped by adding high purity HCl (1% v/v) and stored at 4°C in the dark until analysis.

IV. V. Analysis:

The Concentrations of Hg species in sediments were determined by different analytical methods:

- MeHg concentrations by HS-GC-CVAFS
- HgT concventraions by AAS
- MeHg and Hg(II) for each isotpe by GC-ICPMS

Measurements of MeHg in dried sediment samples were performed using ethylation HS-GC-CVAFS described in the **Chapter III**. Total Hg concentration was determined directly on ~10-50 mg of dried sediment by atomic absorption spectrometry after incineration (O_2 stream) and amalgamation, using AMA 254. The sample preparation prior to analysis and method of HgT and MeHg determination is described in the **Chapter III**. The results were expressed in mg.kg^{-1} (dry weight) and detection limits (3 SD of the blank values, determined daily) were < 0,004 mg.kg^{-1}. The analytical results were quality checked analyzing international certified reference material CRM (IAEA 405). Precision was better than 4% (r.s.d.) and results were consistently within the certified ranges.

Determination of the concentration of each Hg species for each isotope was carried out to evaluate methylation and demethylation yields. The sample preparation for determination of stable isotopes of MeHg and Hg(II) is described below (part ***Pre-treatment – lyophilisation and extraction, Derivatisation and Hg, MeHg determination***). The whole procedure of the experiment is schematically present on the ***figure IV.5.***

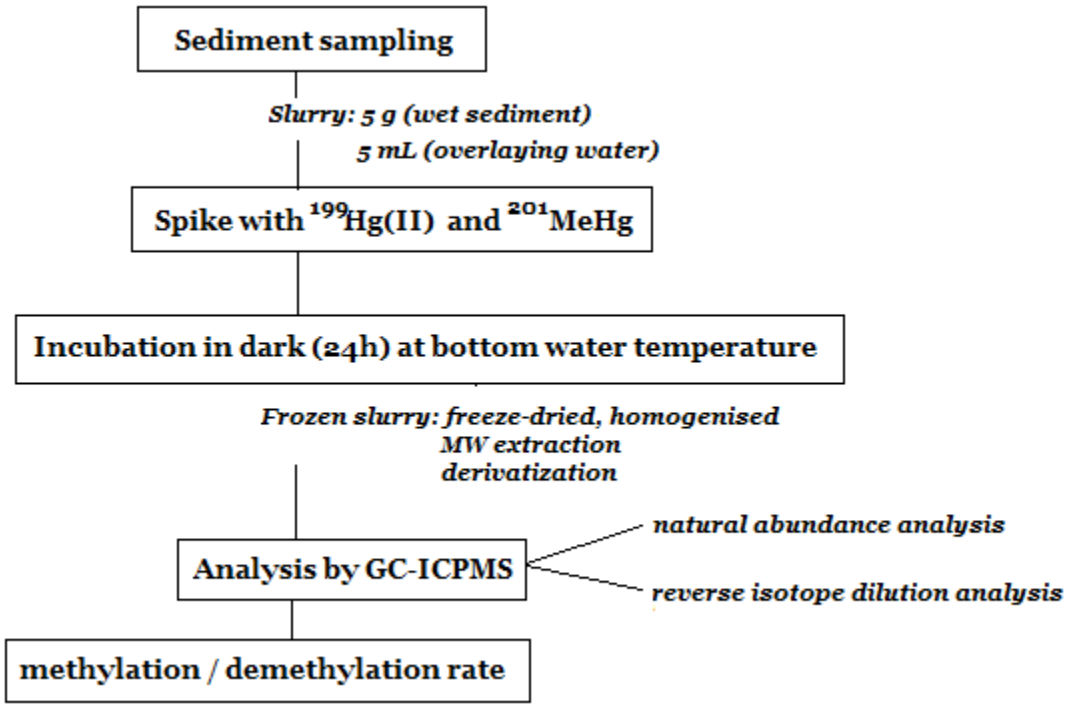
The detection limits, accuracy and precision obtained using the CRM analysis (e.g. IAEA-405, IAEA-158, BCR-580) are listed in the ***table IV.3.***

Instrument detection limit is given by the detector used for analyses of mercury species. Detection limits were calculated as three times the standard deviation of blank determination. Precision of analytical procedure (RSD) and method detection limit has been verified by the calibration exercises and analysis of certified reference material (*MeHg by HS-GC-CVAFS*: 0,2 g sediment IAEA-405 and back-extracted of 5 ml of CH_2Cl_2 layer into 20 ml Mili-Q water; *HgT by AAS*: 0,02 g sediment IAEA-405 and IAEA-158; *MeHg, Hg(II) by GC-ICPMS*: 250 mg taken for the MW extraction, derivatisation: 10µl of extract). RSD is given for five repletion of CRM measurement.

Table IV.3: *The accuracy and precision of the analytical methods used for Hg speciation analysis.*

Type	HS-GC-CVAFS (MeHg)	AAS (HgT)	GC-ICPMS (MeHg; Hg(II))
Instrument Detection Limit	< 0,1 pg Hg (AFS)	0,01 ng Hg	0,01pg; 0,02 pg
Detection limit (3xSD blank)	1,12 ng/kg	0,1 ng/g	0,02 ng/g; 0,05 ng/g
Method Detection Limits	0,27 µg/kg	-	0,05 ng/g; 0,1 ng/g
Precision (RSD)	4,86 %	1,2%	2,1 %; 1,8%

Figure IV.5*: Procedure of experiment*



Additional analysis: AVS and CRS were evaluated on wet sediments and determined after their conversion into H_2S gas by the sequential extraction prcedures described by Billon et al. (2001). Briefly, AVS compounds have been extracted with 1 M HCl solution during 4 hour. Consecutively, a hot digestion of the sediment residue during 2h after addition of a Cr(II) solution leads to a significant recovery of sedimentary pyrite and elemental sulphur. The accuracy od the AVS and CRS results has been estimated at 8% (Billon, 2001).

IV. VI. Determination of the concentration of each Hg species for each isotope

IV.VI.1. Pre-treatment – lyophilisation and extraction

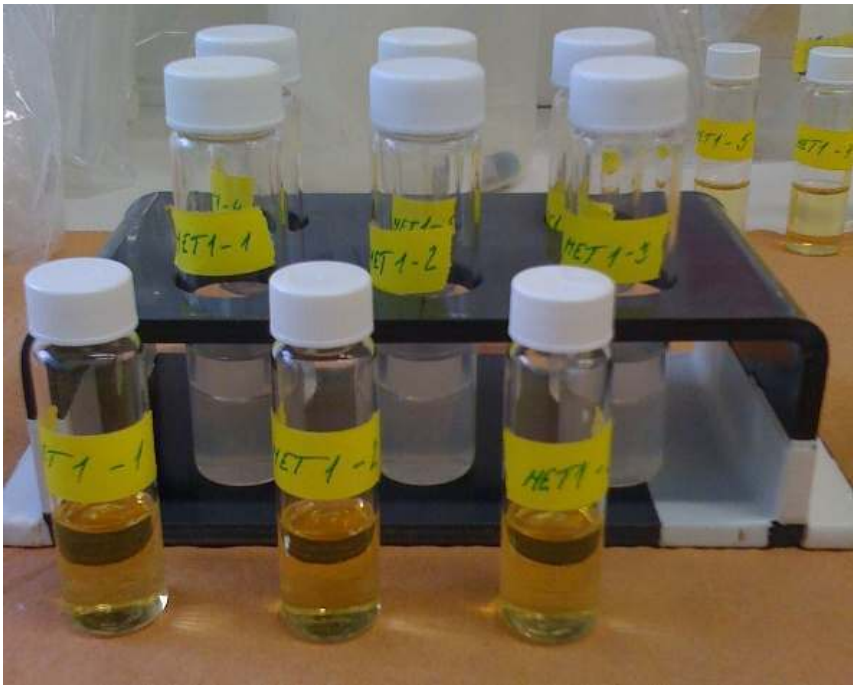
The frozen slides of sediment have been freeze-dried (LABCONCO, USA) under vacuum (-70°C) for 48 hours. Freeze-dried samples of powder form were homogenized and stored in a cold and dry place until analysis.

A commercial open atmospheric-pressure focused microwave system (model PROLABO A301, France) was used for extraction of Hg species from sediment samples. A sample of 250 µg of dried and homogenated sediment was suspended in 5 mL of HNO_3 (6M) and exposed to microwave irradiation (40 W, 3 min). The supernatant solution was separated after centrifugation at 2500 rpm for 5 min, poured into 22 mL Pyrex vials with Teflon caps and stored in a fridge until analysis. Extraction blank determinations were also performed to control and to detect any contamination during the extraction, sample preparation and sample analysis procedures. Previous work of (Rodriguez Martin-Doimeadios et al 2003; Monperrus et al, 2008; Ogric et al. 2007) demonstrated that using a nitric acid extraction, all Hg(II) is totally extracted from the sediment matrix.

IV.VI.2. Derivatisation and mercury species determination

10 µL of sediment extract was buffered with 5mL of 0,1 M sodium acetate buffer (pH4) and the pH was adjusted to 4 by addition of concentrated NH_3. 1ml of sodium tetraethylborate ($NaBEt_4$) was added as derivatisation agent and 3mL of isooctane was added to extract to the ethylated compound formed. The flask was immediately capped and vigorously manually shaken for 5 min (***figure IV.6*** – extracts of the sediments in HNO_3 and preparation of derivatisation).

Figure IV.6*: Extracts of the sediments in HNO_3 and preparation of derivatisation.*



Then organic layer was transferred to a 2mL glass vial and injected in duplicate into the GC-ICPMS using an autosampler (AS3000m Thermo scientific) or stored at -18°C until analysis. The samples were analyzed by Gas Chromatography (Focus GC, Thermo Element) coupled to Inductively Coupled Plasma Mass Spectrometry (ICPMS X7, Thermo Element), GC was equipped with a capillary column MXT-1 (Crossbond 100% dimethylplysiloxane 30m, id 0,53 mm and 1 mm coating).

The instrument configuration allows working in wet plasma conditions. The silcosteel capillary was inserted into the torch injector, and the connection to the torch was realized by means of glass T-piece. A Scott cooled (2°C) spray chamber and a conventional Babington nebulizer were

connected to this T-piece and enabled continuous aspiration of standard solution (Tl, 10 µg.L^{-1}). This configuration allowed optimization of instrument performance and simultaneous measurement of ^{203}Tl and ^{205}Tl for mass correction during the chromatographic run. Previously established GC-ICPMS conditions were used (Rodriguez anal chemistry 2003). GC separation parameters were previously optimized in order to obtain symmetrical peak, thus minimizing peak integration errors.

External calibration was performed daily to check the instrumental sensitivity. The mixed calibration standards of MeHg (0,1; 0,2; 0,5 ng.mg^{-1}) and IHg (1; 2; 5 ng.mg^{-1}) were prepared daily from working solution of natural composition of 10 ng.mL^{-1} and 100 ng.mL^{-1} respectively. Ethylation blanks were prepared each day and allowed the detection of possible contamination during the ethylation and the organic solvent extraction. The calibration standards and ethylation blanks were derivatized, extracted in isooctane and analyzed by GC-ICPMS as described previously. Each sample was injected two times and blanks were checked to control for contamination.

Speciation analysis for non spiked sediment sample and CRM was performed using **isotope dilution** analysis by using isotopic enriched Hg species as analytical spikes (Monperuss et al. 2005). The working enriched solution of MeHg and IHg was prepared by dissolving the stock enriched solutions of 0,1 µg.L^{-1} and 10 µg.L^{-1}m respectively in 1% HCl. Isotope dilution (***Appendix E***) is based on the addition to the sample of a precise amount of an isotopically labeled form of the analyte. The concentration of the analyte in the sample can be calculated from the observed isotope ratios when the natural and enriched isotope ratios and the masses of sample and spike are known (Monperrus et al. 2005).

Speciation analysis for spiked sediment samples was carried out by using natural abundance standards as analytical spikes (**reverse isotope dilution analysis**). The determination of the species concentrations of each isotope requires two analyses: first the determination of the relative isotopic abundance of the Hg species in the sample calculated by **isotopic deconvolution** analysis and second, the determination of the total concentrations of each species calculated by **reverse isotopic dilution analysis** using natural abundance species as analytical spikes.

The analytical method was validated with two different certified reference sediments (IAER 405 and CRM 580) certified for total Hg and MeHg. The comparison between the certified values and the experimental values from several independent replicates is given in ***table IV.4.***

Analysis of the certified sediment (IAEA 405 and BCR 580) show that the values found by speciation analysis (MeHg concentrations: 26,9 ± 1,7 pmol.g^{-1} and 348 ± 18 pmol.g^{-1} respectively;

IHg concentration: 3,99 ± 0,7 nmol.g^{-1} and 653 ± 15 nmol.g^{-1} respectively) are in a good agreement with the certified values (MeHg concentrations: 25,5 ± 2,5 pmol.g^{-1} and 347 ± 17 pmol.g^{-1} respectively; IHg concentration: 4,04 ± 0,2 nmol.g^{-1} and 658 ± 15 nmol.g^{-1} respectively). Moreover no transformations of the enriched ^{199}IHg and ^{201}MeHg analytical spikes have been detected during the analyses of the CRM.

Table IV.4: *Certified and measured values of Hg species concentrations found for CRMs.*

Reference Material	Species		Certified values	Measured values
IAEA 405	HgT	mg.kg^{-1}	0,81 ± 0,04	0,80 ± 0,01
	MeHg	µg.kg^{-1}	5,49 ± 0,53	5,39 ± 0,33
CRM 580	HgT	mg.kg^{-1}	132 ± 3	131 ± 3
	MeHg	µg.kg^{-1}	75,0 ± 3,7	70,0 ± 3,6

IV.VI.3. Data processing

The data obtained from the GC-ICPMS analysis were transferred from Plasmalab program (ICP-MS) to TST program (Traitement de Signaux Transitoires, LCABIE, France) as *.csv files. Chromatographic peaks were integrated manually using valley to valley technique. Obtained peak areas were transferred to the Excel and processed.

Calculation of stable isotope Hg species concentrations:

- Isotope patern deconvolution: used for the determination of relative isotopic abundances and the molar fractions of each Hg isotope contributing to the isotope pattern for each Hg species
- Reverse isotope dilution: used for the calculation of the total concentration of each species

Isotope pattern deconvolution:

Mathematical technique for distinct isotope signatures from mixture of natural abundance and enriched tracers (Isotope pattern deconvolution) was used. Isotope pattern Deconvolution can be used to achieve quantifications with the same metrological quality than Stable Isotope Dilution Analysis but without the requirement of a methodological calibration. The total amount of a given elemental species in the system is the sum of all its analogues with a different isotopic composition (Rodriguez-Gonzalez et al., 2011). Briefly, the approach is based on the determination of the **molar fractions** for each pure isotope pattern (^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{204}Hg) contributing to the isotope pattern observed in the mixture of natural abundance and labeled molecules by multivariate linear regression.

The molar fractions are defined by the equations:

$$ {}^{a}x_{nat} = \frac{{}^{a}N_{nat}}{\left({}^{a}N_{nat} + {}^{a}N_{tracer,1} + \ldots {}^{a}N_{tracer,n}\right)} = \frac{{}^{a}N_{nat}}{{}^{a}N_{T}} \qquad [IV.3] $$

$$ {}^{a}x_{spike,n} = \frac{{}^{a}N_{spikeN}}{\left({}^{a}N_{nat} + {}^{a}N_{tracer,1} + \ldots {}^{a}N_{tracer,n}\right)} = \frac{{}^{a}N_{tracer,n}}{{}^{a}N_{T}} \qquad [IV.4] $$

For: species *a* (IHg; MeHg) present in the sample as a mixture of the species with natural isotope abundances

N number of moles

x refers to the molar fraction

$^{a}N_{T}$ total number of moles

The subscript: *nat* refers to the species of natural isotopic composition

tracer refers to the different isotopically enriched added tracers

n number of spikes (isotopic tracers)

Using gas chromatography coupled to a mass spectrometer, the chromatographic peak of each isotopes is monitored. The species-specific isotope abundance can be easily calculated from the species-specific isotope intensities. The abundance in the sample is a linear combination of the individual isotopic sources present in the system. General linear regression for the isotope *1* of the species *a* is:

$$ {}^{a}A^{1}{}_{sample} = {}^{a}A^{1}{}_{nat} \cdot {}^{a}x_{nat} + {}^{a}A^{1}{}_{tracer,1} \cdot {}^{a}x_{tracer,1} + \ldots {}^{a}A^{1}{}_{tracer,n} \cdot {}^{a}x_{tracer,n} \qquad [IV.5] $$

Considering all the available isotopes of the Hg, the general linear regression can be rewritten as:

$$y = A \cdot x + e \qquad \text{[IV.6]}$$

where: y – measured variable (isotope abundance);

A – independent variable;

x – regression coefficient;

e – noise

A matrix notation for the system in this work experiment where the samples containing IHg and MeHg the enriched spikes of ^{199}Hg and ^{201}MeHg are added the following equations are applied for IHg and MeHg, respectively:

$$\begin{pmatrix} {}^{IHg}A^{196}_{sample} \\ {}^{IHg}A^{198}_{sample} \\ {}^{IHg}A^{199}_{sample} \\ {}^{IHg}A^{200}_{sample} \\ {}^{IHg}A^{201}_{sample} \\ {}^{IHg}A^{202}_{sample} \\ {}^{IHg}A^{204}_{sample} \end{pmatrix} = \begin{pmatrix} A^{196}_{natural} & A^{196}_{tracerMeHg201} & A^{196}_{tracerIHg199} \\ A^{198}_{natural} & A^{198}_{tracerMeHg201} & A^{198}_{tracerIHg199} \\ A^{199}_{natural} & A^{199}_{tracerMeHg201} & A^{199}_{tracerIHg199} \\ A^{200}_{natural} & A^{200}_{tracerMeHg201} & A^{200}_{tracerIHg199} \\ A^{201}_{natural} & A^{201}_{tracerMeHg201} & A^{201}_{tracerIHg199} \\ A^{202}_{natural} & A^{202}_{tracerMeHg201} & A^{202}_{tracerIHg199} \\ A^{204}_{natural} & A^{204}_{tracerMeHg201} & A^{204}_{tracerIHg199} \end{pmatrix} \cdot \begin{pmatrix} {}^{IHg}x_{natural} \\ {}^{IHg}x_{tracerMeHg201} \\ {}^{IHg}x_{tracerIHg199} \end{pmatrix} + \begin{pmatrix} {}^{MeHg}e^{196} \\ {}^{MeHg}e^{198} \\ {}^{MeHg}e^{199} \\ {}^{MeHg}e^{200} \\ {}^{MeHg}e^{201} \\ {}^{MeHg}e^{202} \\ {}^{MeHg}e^{204} \end{pmatrix} \qquad \text{[IV.7]}$$

$$\begin{pmatrix} {}^{MeHg}A^{196}_{sample} \\ {}^{MeHg}A^{198}_{sample} \\ {}^{MeHg}A^{199}_{sample} \\ {}^{MeHg}A^{200}_{sample} \\ {}^{MeHg}A^{201}_{sample} \\ {}^{MeHg}A^{202}_{sample} \\ {}^{MeHg}A^{204}_{sample} \end{pmatrix} = \begin{pmatrix} A^{196}_{natural} & A^{196}_{tracerMeHg201} & A^{196}_{tracerIHg199} \\ A^{198}_{natural} & A^{198}_{tracerMeHg201} & A^{198}_{tracerIHg199} \\ A^{199}_{natural} & A^{199}_{tracerMeHg201} & A^{199}_{tracerIHg199} \\ A^{200}_{natural} & A^{200}_{tracerMeHg201} & A^{200}_{tracerIHg199} \\ A^{201}_{natural} & A^{201}_{tracerMeHg201} & A^{201}_{tracerIHg199} \\ A^{202}_{natural} & A^{202}_{tracerMeHg201} & A^{202}_{tracerIHg199} \\ A^{204}_{natural} & A^{204}_{tracerMeHg201} & A^{204}_{tracerIHg199} \end{pmatrix} \cdot \begin{pmatrix} {}^{MeHg}x_{natural} \\ {}^{MeHg}x_{tracerMeHg201} \\ {}^{MeHg}x_{tracerIHg199} \end{pmatrix} + \begin{pmatrix} {}^{MeHg}e^{196} \\ {}^{MeHg}e^{198} \\ {}^{MeHg}e^{199} \\ {}^{MeHg}e^{200} \\ {}^{MeHg}e^{201} \\ {}^{MeHg}e^{202} \\ {}^{MeHg}e^{204} \end{pmatrix} \qquad \text{[IV.8]}$$

A simple equation is employed for the determination of the concentration of the analyte as the ratio of molar fractions is equal to the ratio of molar concentrations in the mixture. The molar fractions can be calculated from natural abundance species. Once the molar fractions of different isotopic sources are known, the concentration from the **total number of moles** of the sample has to be calculated.

Reverse isotope dilution:

Number of moles in the samples is calculated conventionally by isotope dilution analysis using natural abundance standards as analytical spikes (reverse isotope dilution). Once the total number of moles ${}^{a}N_T$ of the sample is calculated the individual concentrations of ambient and isotopically added species can be deconvoluted after the environmental process has taken place by rearranging equations [IV.3] and [IV.4]:

$$\begin{aligned} {}^{IHg}N_{nat} &= {}^{IHg}x_{nat}.\,{}^{IHg}N_T & {}^{MeHg}N_{nat} &= {}^{MeHg}x_{nat}.\,{}^{MeHg}N_T \\ {}^{IHg}N_{spikeIHg199} &= {}^{IHg}x_{spikeIHg199}.\,{}^{IHg}N_T & {}^{MeHg}N_{spikeIHg199} &= {}^{MeHg}x_{spikeIHg199}.\,{}^{MeHg}N_T \\ {}^{IHg}N_{spikeMeHg201} &= {}^{IHg}x_{spikeMeHg201}.\,{}^{IHg}N_T & {}^{MeHg}N_{spikeMeHg201} &= {}^{MeHg}x_{spikeMeHg201}.\,{}^{MeHg}N_T \end{aligned} \quad \text{[IV.9]}$$

Calculation of methylation / demethylation yield:

The methylation / demethylation yields were calculated for 24 hours experiment in two different ways:

- Measuring the increase of the newly formed species concentrations
- Measuring the decrease of the initial added species concentration

The simplest case of two endogenous natural abundance species of the same element (a_{nat} and b_{nat}) contained in a system in which two enriched analogues enriched in a different isotope are added ($a_{tracer,1}$ and $b_{tracer,2}$) was considered. After the process under study has taken place, the final concentration of the enriched analogues will provide the potential methylation and demethylation yields. The potential formation of a from b (F_a(%)) was calculated from the amount of the newly formed a_{spike2} found after the incubation and the newly formed b_{spike1} whereas the potential formation of b from a (F_b*(%)*) was calculated from the amount of b_{spike1} found after the incubation and the newly formed a_{spike2}. Such calculations are given in following equations Rodriguez-Gonzalez et al., 2011 :

$$F_a(\%) = \frac{a_{tracer,2}}{b_{tracer,2} + a_{tracer,2}} \times 100 \quad \text{[IV.10]}$$

$$F_b(\%) = \frac{b_{tracer,1}}{a_{tracer,1} + b_{tracer,1}} \times 100 \quad \text{[IV.11]}$$

In the similar way, degradation yields can be determined from the degradation of spiked species (D_a(%) and D_b(%)) were calculated using the equations:

$$D_a(\%) = \frac{(a_{tracer,1})_0 - a_{tracer,1}}{(a_{tracer,1})_0} \times 100 \quad \text{[IV.12]}$$

$$D_b(\%) = \frac{(b_{tracer,2})_0 - b_{tracere,2}}{(b_{tracer.2})_0} \times 100 \quad \text{[IV.13]}$$

Where the subscription 0 corresponds to the amount of species determined for a non incubated sample.

In the purpose of my work *tracer,1* refers to ***201*** and *tracer,2* refers to ***199***. In this case the most accurate calculation of methylation corresponds to the amount of the newly formed ^{201}MeHg (F_a). Demethylation corresponds to degradation of the ^{201}MeHg (D_a) rather than regarding the formation of ^{201}IHg, because of important differences between the two concentration (small quantity of ^{201}MeHg is not easily detectable on a great peak of IHg).

IV. VII. Results

IV.VII.1. Geochemical characteristics

The speciation of the sulfides species may influence the bioavailability of Hg and production of MeHg (e.g. high sulfide concentrations can stop the MeHg production; Benoit et al, 2002; Glimour and Riedel, 1995). Two reduced sulfur fractions: acid-volatile sulfur (AVS), predominantly monosulfides and chromium reducible sulfur (CRS), predominantly pyrite were determined. AVS is a measure of sulfide liberated from mineral phases that are soluble in 6M HCl. The CRS sequentially follow the AVS extraction and mixture of HCl and Cr(II) is used. The average concentrations with standard deviations of AVS and CRS in the sediment cores for each site are summarized in ***the table IV.5***. Generally the concentrations of AVS as well as CRS are lower in the sediment cores taken at the site II. Deûle – Amont compared to the site I. Metaleurop.

The concentrations of AVS and CRS in the I. Metaleurop sediment increase after -9 cm of sediment depth and maximum is 3412 ± 86 $mg.kg^{-1}$ and 8167 ± 83 $mg.kg^{-1}$ respectively at -15 cm (***figure IV.7 a), b)***). Deeper the concentrations decreasing and oscillate around the 1500 $mg.kg^{-1}$ (AVS) and 2600 $mg.kg^{-1}$ (CRS) with the second maximum for AVS concentration at -24 cm (value 2786 ± 59 $mg.kg^{-1}$).

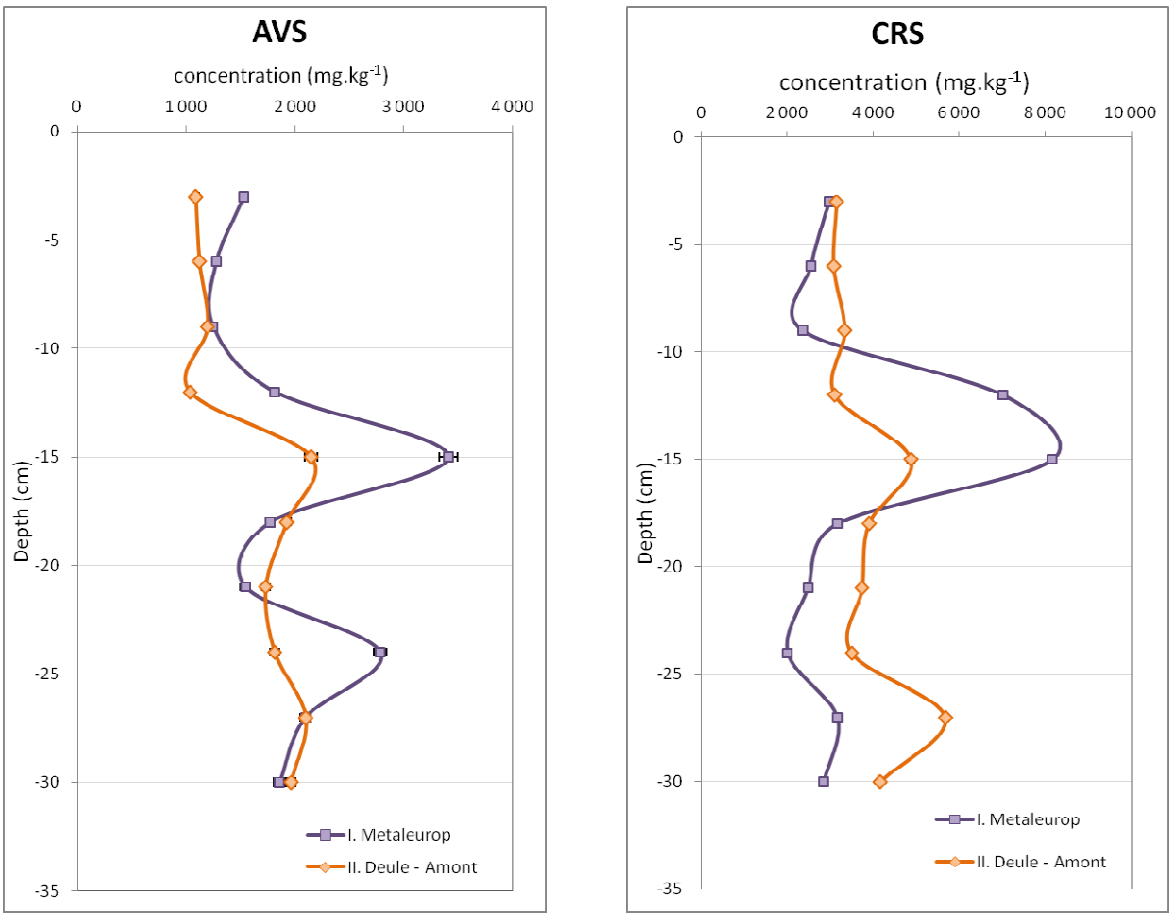
The concentration profiles of AVS and CRS in the sediments taken at the site II. Deûle – Amont, in first sediment centimeters (to -12 cm depth) oscillate between 1150 $mg.kg^{-1}$ and 3150 $mg.kg^{-1}$, respectively. The maximum AVS concentration 2151 ± 59 $mg.kg^{-1}$ and CRS concentrations 4882 ± 86 $mg.kg^{-1}$ can be found at -15 cm (***figure IV.7***). The concentrations AVS and CRS in deeper sediment fluctuate around 1900 $mg.kg^{-1}$ and 4050 $mg.kg^{-1}$m respectively.

Sulfur chemistry is a particulary important factor controlling methylation. The high concentration of both AVS and CRS observed at the sampling place indicate the significant activity of sulfate reducing bacteria (SRB) responsible for formation of sulfides. SRB are important methylators of mercury in anaerobic sediments and sulfate simulates microbial Hg methylation at the low sulfate concentrations. However, at high levels in reducing conditions methylation is inhibited due to sulfide formation which may be one of the

reasons why MeHg levels in sediments rarely exceed 1% of HgT (MeHg/HgT % see below, ***table IV.8***).

The AVS/CRS ratio is used by the geochemists for estimation of AVS to CRS conversion. The obtained values are relatively low 0,3 – 1,4 Metaleurop site and 0,3 – 0,5 Deûle – Amont site. This ratio range shows the high degree of AVS conversion to pyrite (Billon G., 2001).

Figure IV.7*: The average concentration of a) AVS and b) CRS at both sampling sites.*



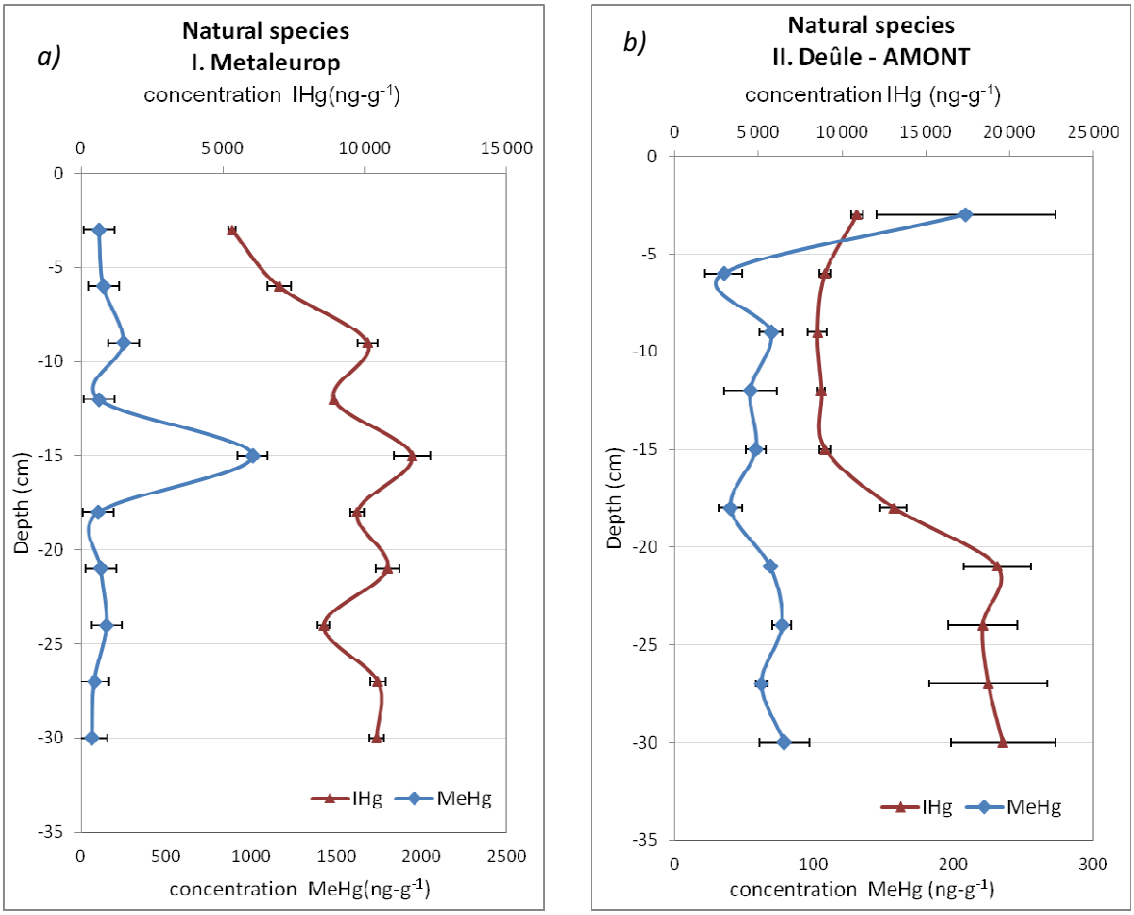
IV.VII.2. Hg species concentrations

Endogenous (natural) mercury species concentrations:

The average natural Hg species (IHg and MeHg) concentrations in sediments of both sites are displayed in ***figure IV.8 a) - b)*** and summarized in ***table IV.5***. The sediments sampled in the Metaleurop site show considerably higher natural IHg (5 323 – 11 658 $ng.g^{-1}$) and natural MeHg (64 - 1007 $ng.g^{-1}$) contents compared to the sediments sampled at the Deûle – Amont (***figure IV.9 a) – b))***

When the MeHg was plotted to IHg no significant relationship was observed (I. Metaleurop: $R^2 = 0,042$; II. Deûle – Amont: $R^2 = 0,0013$; ***appendix F.I. and F.II.***).

Figure IV.8*: The depth profiles of natural IHg and MeHg concentrations at the sampling site a) I. Metaleurop and b) II. Deûle – Amont.*

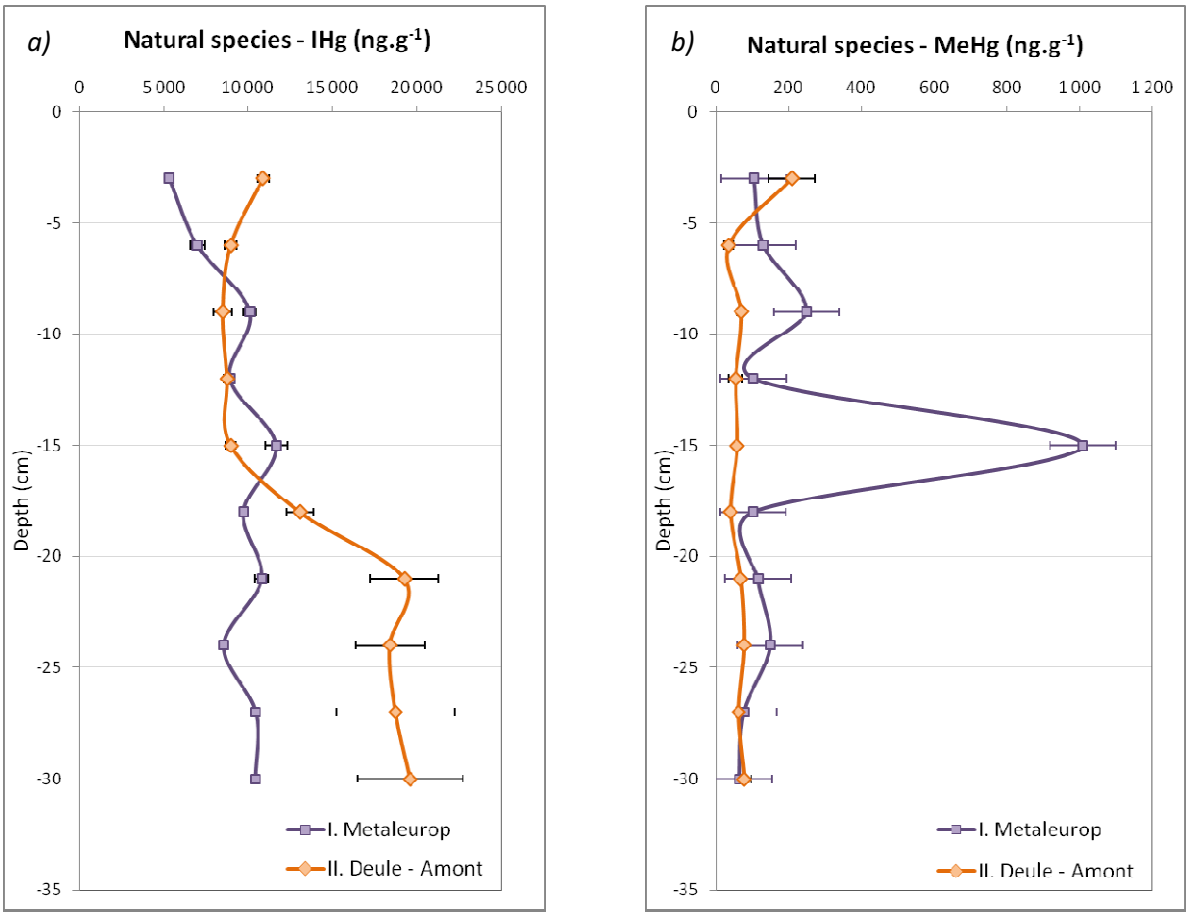


The pool of HgT in the sediment is substatial with ranging from 5,33 to 11,66 $mg.kg^{-1}$ at the site I.Metaleurop and even higher values ranging from 8,51 to 19,60 $mg.kg^{-1}$ at the site II. Deûle – Amont were observed.Concentrations were variable. Similar, there was difference in MeHg concentration between sites, ranging from 64 to 1 007 $\mu g.kg^{-1}$ at the I. Metaleurop site and 35 – 208 $\mu g.kg^{-1}$ at the II. Deûle – Amont site. Maximum of MeHg concentrations can be found at the 15 cm sediment depth for the I. Metaleurop site, while the maximum of MeHg content for II. Deûle-Amont site can be found at the surface layer. The percent of HgT that occured as MeHg range from 0,62 to 11,31 % (I. Metaleurop) and 0,25 to 1,92 % (II. Deûle – Amont). The values around 1% were found at Chesapeake Bay (Mason et al., 1999), around 0,6% at Lavaca Bay (Bloom et al., 1999) and Baltimore Harbor (Mason and Lawrence, 1999).

Table IV.5*: The average concentration and concentration range of two sites.*

Sampling ste	**I. Metaleurop**		**II. Deûle - Amont**	
	ave	range	ave	range
^{201}MeHg ($ng.g^{-1}$) recovered	17,1 ± 3,0	*9,43 – 32,8*	14,9	*9,88 – 21,6*
^{199}MeHg($ng.g^{-1}$) formed	4,20 ± 0,83	*0 – 17,1*	0,916 ± 0,514	*0 – 4,69*
^{201}IHg($ng.g^{-1}$) formed	11,7 ± 0,4	*0 – 32,1*	7,99 ± 1,85	*0 – 54,1*
^{199}IHg ($ng.g^{-1}$) recovered	2110 ± 67,3	*1663 – 3253*	1824 ± 164	*1246 - 2437*
MeHg natural ($ng.g^{-1}$)	210 ± 49	*64 – 1 007*	75,1 ± 15,1	*35,1 - 208*
IHg natural ($ng.g^{-1}$)	9 292 ± 296	*5 323 – 11 658*	13 517	*8 507 – 19 604*
MeHg/IHg (%)	2,13	*0,62 – 8,64*	0,62	*0,31 – 1,92*
AVS ($\mu g\ S.g^{-1}$)	1 932 ± 45	*1 248 - 3412*	1 613 ± 37	*1 036 - 2151*
CRS ($\mu g\ S.g^{-1}$)	3 673 ± 48	*2 015 – 8167*	3 855 ± 40	*3 082 - 5675*

***Figure IV.9**: The depth profiles of natural a) IHg and b) MeHg concentrations at the both sampling sites.*



Exogenous (added) mercury species concentrations:

IHg

Depth profiles of ^{199}IHg contents recovered after incubation as well as ^{199}IHg contents theoretically spiked for I. Metaleurop site are presented in ***figure IV.10 a)*** and the same parameters for samples of II. Deûle – Amont are presented in ***figure IV.10 b)***. When compared the theoretically spiked ^{199}IHg and experimentally measured contents (***table IV.6***) the values are slightly different. The values higher in theoretically spiked ^{199}IHg contents than measured suggest the worse recovery of ^{199}IHg (the recovery range of ^{199}IHg: 29-102%; average 77%; with two exceptions at I. Metaleurop surface sediment layer and II. Deûle – Amont deepest sediment layer the value of recovery of ^{199}IHg slightly exceeded 100%). The values lower in theoretically spiked ^{199}IHg contents than measured may suggest the underestimation of IHg content in sediments and can also be explained by losses during the experimental procedures certainly mainly by adsorption

to the vessel walls. Compared two sites, the recoveries are lower for site Deûle – Amont. At this site also lower sulfide concentrations can be found. This may suggest that IHg has a lower potential of complexation with sediment sulfide and so the higher losses of IHg spiked by adsorption on the vessels walls.

***Figure IV.10**: The depth profiles of theoretically spiked and recovered ^{199}IHg for the a) I. Metaleurop and b) II. Deûle – Amont site.*

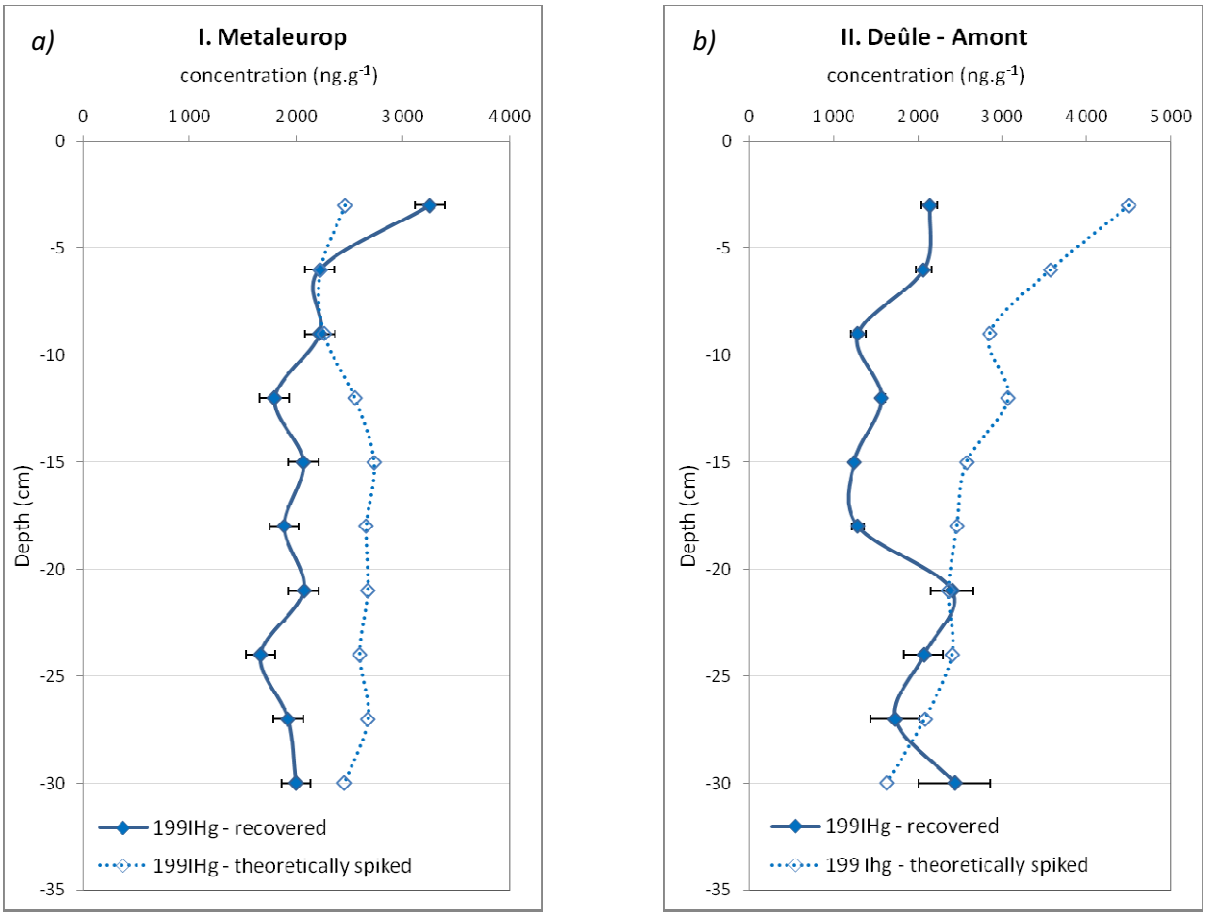


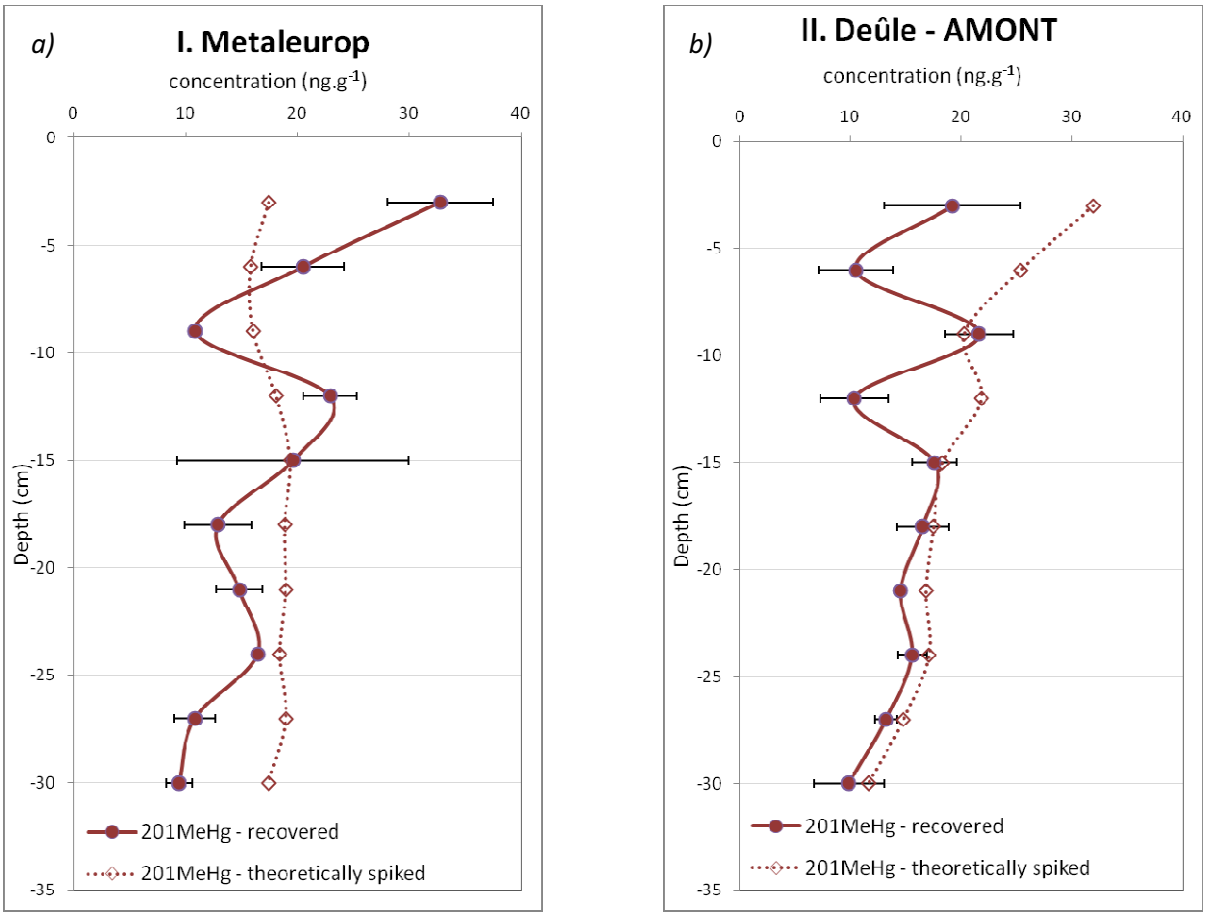
***Table IV.6**: The average concentrations of the recovered spike of ^{199}IHg compared to theoretical spike and the range of the concentrations for both sites.*

Sampling site	**I. Metaleurop**		**II. Deûle - Amont**	
	ave	range	ave	range
^{199}IHg (ng.g^{-1}) measured	2110 ± 67	*1663 – 3253*	1824 ± 164	*1246 - 2437*
^{199}IHg (ng.g^{-1}) spiked	2527 ± 176	*2226 - 2729*	2753 ± 216	*1637 - 4499*
^{199}MeHg (ng.g^{-1}) formed	4,20 ± 0,9	*0,46 – 17,1*	0,92 ± 0,23	*0,50 – 4,69*

MeHg

Depth profiles of ^{201}MeHg contents recovered after incubation as well as ^{201}MeHg contents theoretically spiked for the sediment samples of the Metaleurop are presented in ***figure IV.11 a)*** and the same parameters for samples of Deûle – Amont are presented in ***figure IV.11 b)***.

Figure IV.11*: The depth profiles of theoretically spiked and recovered ^{201}MeHg for the a) I. Metaleurop and b) II. Deûle – Amont site.*



In the both cases the mean contents of the theoretically spiked ^{201}MeHg (about 17,5 ng.g^{-1}) are almost totally recovered when compared with experimentally measured contents (see ***table IV.7***). The maximum of ^{201}MeHg measured contents is found at the surface layer in the site I. Metaleurop and in the site II. Deûle – Amont at -9 cm (21,6 ng.g^{-1}) and the second maximum at the surface sediment (19,2 ng.g^{-1}).

Table IV.7*: The average concentrations of the recovered spike of ^{201}MeHg compared to theoretically spiked and the range of the concentrations for both sites.*

Sampling site	**I. Metaleurop**		**II. Deûle - Amont**	
	ave	range	ave	range
^{201}MeHg (ng.g^{-1}) measured	17,1 ± 3,0	*9,43 – 32,8*	14,9 ± 2,6	*9,88 – 21,6*
^{201}MeHg (ng.g^{-1}) spiked	17,9 ± 1,2	*15,8 – 31,9*	17,3 ± 3,1	*11,6 – 19,4*
Recovery (%)	96	*54- 126*	80	*41 - 106*
^{201}IHg (ng.g^{-1}) formed	11,7 ± 0,6	*7,87 – 32,1*	7,99 ± 1,26	*4,52 – 24,1*

The depth profiles of endogenous (natural) Hg and exogenous (added) Hg are present on the ***figure IV.12 a) – b)*** for IHg specie and ***figure IV.13 a) – b)*** for MeHg specie.
The ^{201}MeHg is almost entirely recovered as ^{201}IHg, this may suggest the involved of oxidative demethylation.

IV.VII.1. Hg species transformation

Methylation and demethylation can be calculated in two different ways: by measuring the increase of the newly formed species concentration (values *M*) and by measuring the decrease of the initial added species concentrations (values *D*). The methylation / demethylation yields are experesed in %. The methylation transformation yield is calculated as the ratio of the sub-products of the reaction by the initial reactant:

$$M^{199}(\%) = \frac{^{199}MeHg}{^{199}IHg + ^{199}MeHg} \times 100$$

The demethylation yield is calculated as the difference between the ^{201}MeHg concentration and ^{201}MeHg initial concentation, divede by the initial ^{201}MeHg concentration:

$$D^{201}(\%) = \frac{(^{201}MeHg)_{t0} - ^{201}MeHg}{(^{201}MeHg)_{t0}} \times 100$$

***Figure IV.12**: The depth profiles of endogenous and exogenous IHg for the a) I. Metaleurop and b) II. Deûle – Amont site.*

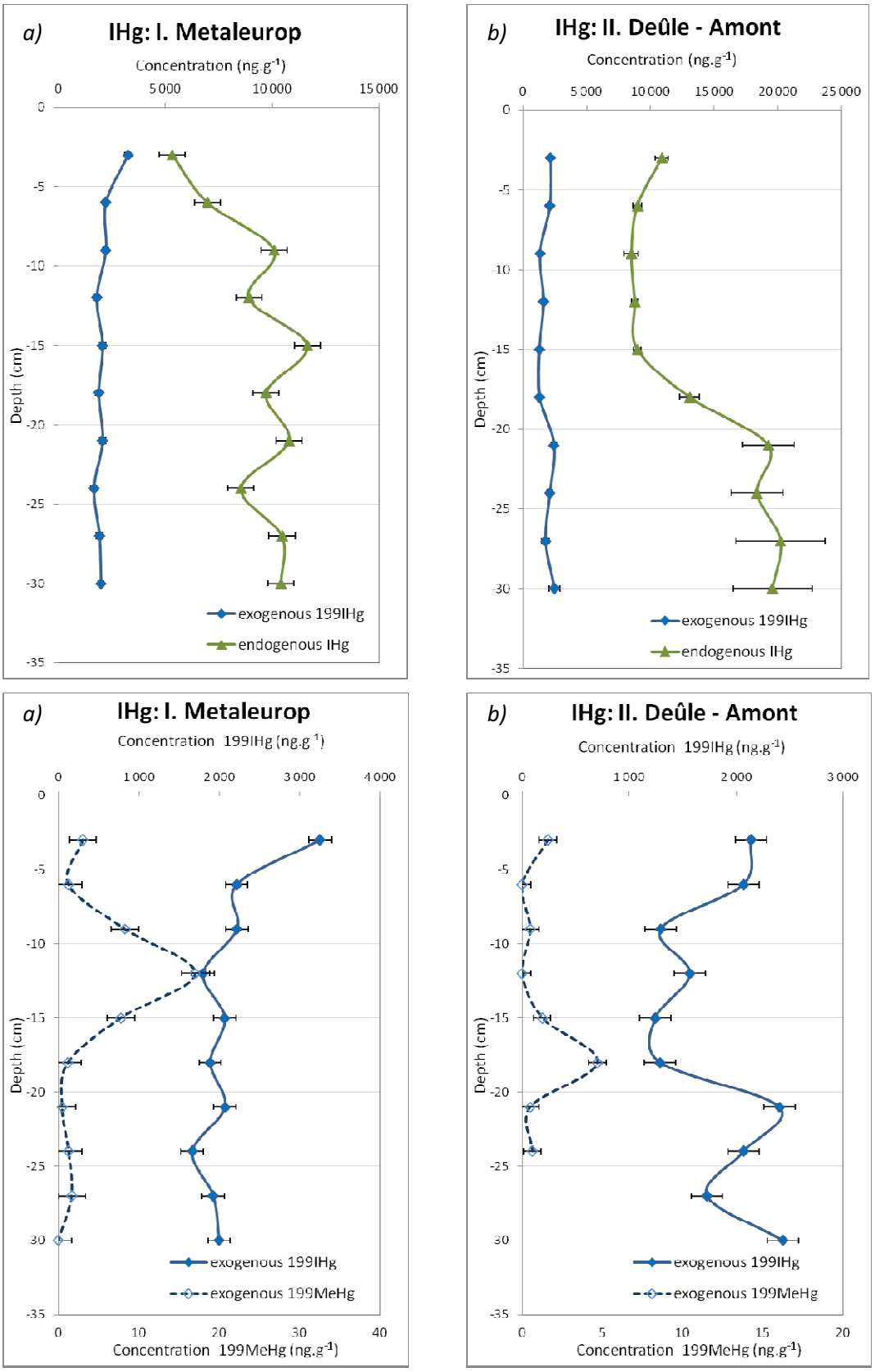
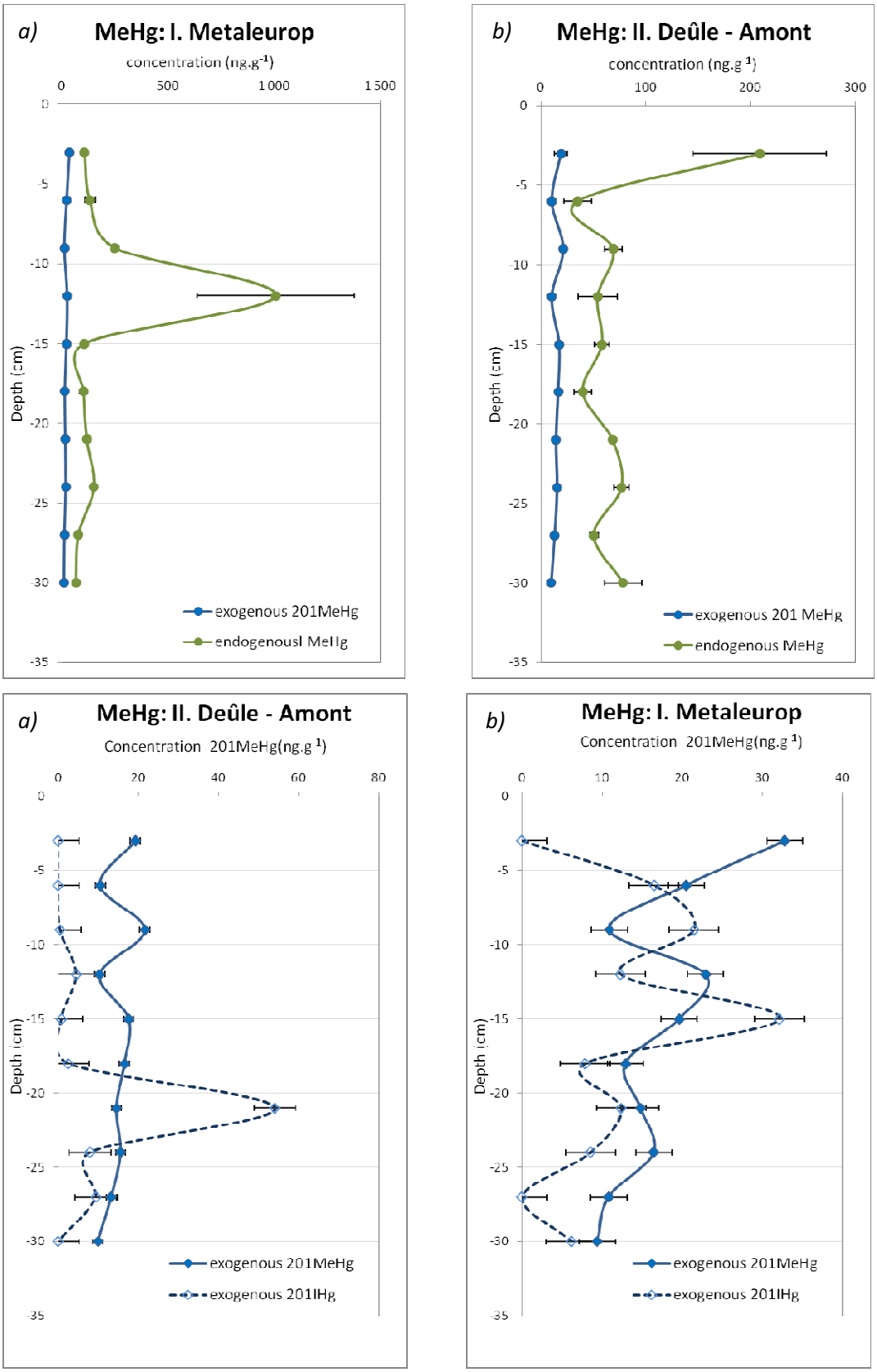


***Figure IV.13**: The depth profiles of endogenous and exogenous MeHg for the a) I. Metaleurop and b) II. Deûle – Amont site.*



The average values, range and maximum of methylation and demethylation yields for the both sites are presented in the ***table IV.8.*** The maximum of methylation yield of the both place was found in deeper sediment rather than surface. Maximum of methylation for I. Metaleurop was found at the 12 cm of sediment depth while for II. Deule – Amont at the 21 cm of sediment depth where is also reach the maximum of exogenous ^{199}MeHg formation (***figure IV.13***).

Methylation yields are found higher for I. Metaleurop compared to II. Deule – Amont with average values of 0,24 and 0,08%, respectively. For demethylation, similar yields are found for both sites with average values of 27,3 and 28,8, respectively. These values are in agreement with field observation made for methylation potentials previously reported for sediment (Schäfer et al. 2010, Heyes J.M. et al. 2006, Hlines. E. 2006, table IV.2). Values close to 0,2 % were observed also by Rodrigez Martin-Doimeadios et al.(2004) when study mercury transformations in estuarine sediments under anoxi-biotic conditions. Similar demethylation yields to those observed by Rodrigez Martin-Doimeadios et al.(2004) for anox.-biot. condition (32,45 %).were also found.

Moreover the I. Metaleurop sediment presented a mean endogenous MeHg proportion (calculated as the ration of MeHg and THg concentration) 2,1% (***table IV.8***) higher than the II. Deûle – Amont sediment (0,6%), confirming the higher net MeHg production potential at the Metaleurop site.

In situ MeHg concentration and the amount of ^{199}MeHg were correlated (R^2 = 0,73 the positive and noticeable correlation at the site I. Metaleurop ***Appendix F.III***). The production of MeHg is independent of the amount of ^{199}Hg added, suggesting that small variation in Hg concentration are not important to MeHg production in systems with high HgT concentration (no correlation was found when HgT was plotted against the MeHg) (***Appendix F.I, F.II***). This is confirmed by the lack of relationship between HgT and MeHg concentration. As Hg concentration and Hg speciation do not appear to affect Hg methylation, the overriding control in this system is likely microbiological activity (Heyes, A., 2004)

The maximum of demethylation yield of the I. Metaleurop site was found high around the 10 cm sediment depth and higher values also under 18 cm of sediment depth. In the II. Deule-Amont site demethylation yield was highest almost at surface (6 cm depth), next maximum around the 12 cm depth and from the 15 cm of sediment depth values of demethylation yield increasing with depth (***figure IV. 14***). From the M/D (%) ratio (***figure IV. 14)*** is pattern the methylation zone at the point where the demethylation is low, the M/D ratio is high. Also around the -15 cm where

the highest value of M/D ratio can be found the highest concentration of CRS can be found. It seems that the methylation is controlled by the CRS concentrations.

The methylation yield calculated at the I. Metaleurop is higher (0,24%) than the II. Deûle – Amont sediment (0,08%). These values are in agreement with field observation made by the (Schäfer et al. 2010, Heyes J.M. et al. 2006, Hlines. E. 2006, ***table IV.2***). The values close to the 0,2 % were observed also by Rodrigez Martin-Doimeadios et al.(2004) when study mercury transformations in estuarine sediments under anoxi-biotic conditions.

Table IV.8*: Methylation and demethylation yield.*

Sampling site	**I. Metaleurop**			**II. Deûle - Amont**		
	ave	range	max.	ave	range	max.
M - endogenous MeHg proportion	2,13 ± 0,28	*0,62 – 8,64*	8,64 ± 1,07	0,62 ± 0,07	*0,2 – 1,9*	1,92 ± 0,13
M^{199} (%)	0,24 ± 0,05	*0,02 – 0,94*	0,94 ± 0,09	0,08 ± 0,02	*0,02 – 0,19*	0,19 ± 0,03
D^{201} (%)	27,3 ± 9,92	*2,21 – 59,9*	59,9 ± 7,95	28,8 ± 6,44	*0 – 52,9*	52,9 ± 7,5

The I. Metaleurop sediment presented a mean demethylation potential 27,3 ± 9,92% similar as the II. Deûle – Amont sediment: 28,8 ± 6,44 % (***table IV.8***). The demethylation yield observed by Rodrigez Martin-Doimeadios et al.(2004) for anox.-biot. conditions was 32,45 %.

When the methylation yield was plotted to AVS, no significant correlation was observed. But when the methylation was plotted to CRS concentration, significant correlation was observed, especially at the I. Metaleurop site (R^2= 0,96; II. Deûle – Amont R^2 = 0,62; ***figure IV.15***). CRS seems to control the MeHg production. The high concentration of CRS (predominantly pyrite) the higher methylation is observed. The study of Benoit et al. (1999) have shown that the inhibitory effect of sulfide on Hg methylation is not due to HgS precipitation, but the sulfides lowers the availability of Hg for bacterial methylation by formation of less bioavailable charged Hg-S complexes.

No correlation was observed when demethylation yield was plotted to AVS or CRS concentrations.

***Figure IV.14**: The depth profiles methylation yield and demethylation yield at the a) I. Metaleurop and b) II. Deûle – Amont site.*

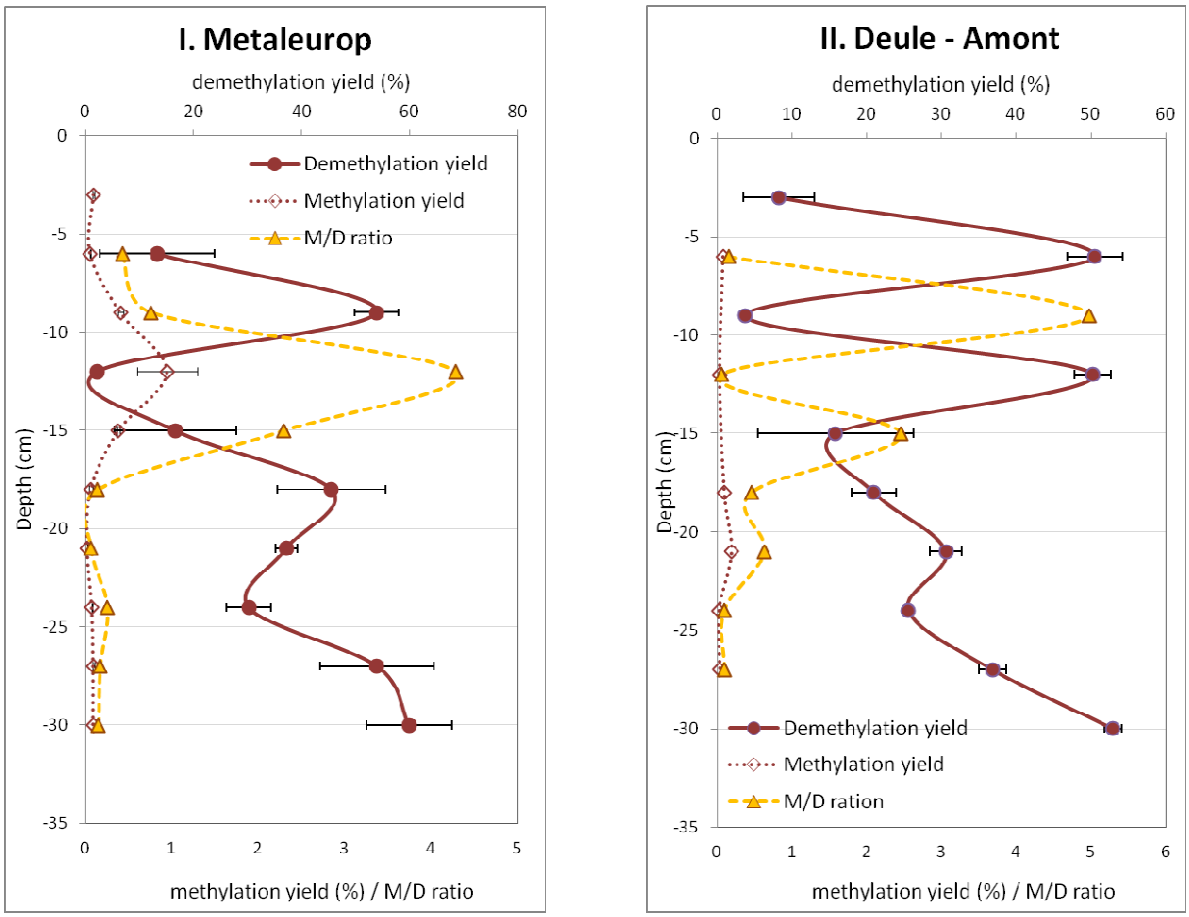
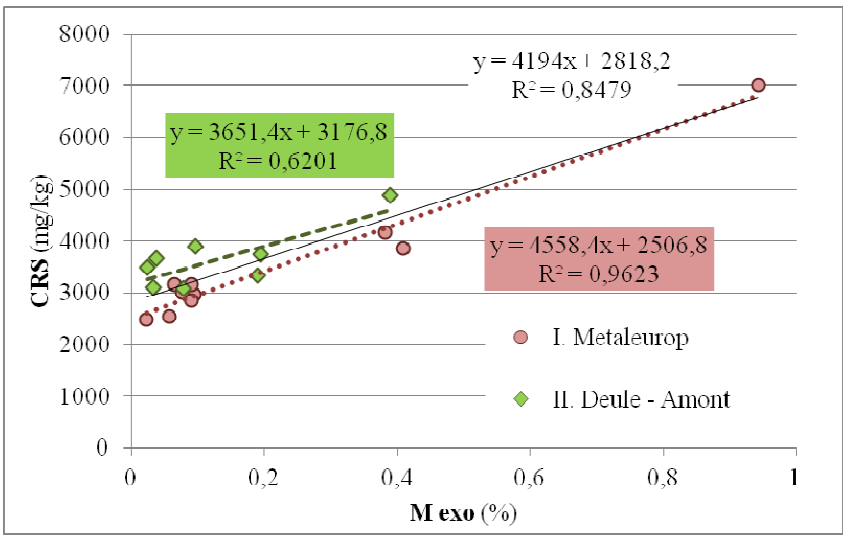


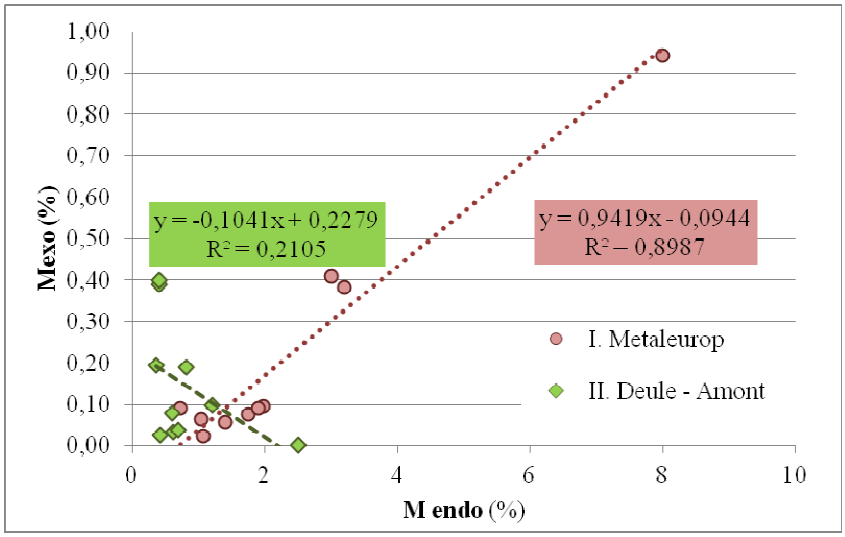
***Figure IV.15**: Correlation between the methylation yield and CRS concentration.*



Previously studies have already demonstrated a large methylation of added species (Benoit et al.,2002; Hintelmann et al., 2002; Hintelmann et al. 2000) suggesting a higher availability. Net methylation can be assumed when the endogenous and exogenous Hg methylation are compared. The endogenous and exogenous pools exhibited a similar behavior (student test, p-values < 0,01) at the I. Metaleurop site. When the exogenous methylation yield was plotted against the MeHg/IHg proportion the I. Metaleurop sediment exhibited a slightly higher ^{199}IHg methylation (slope 0,9) than the endogenous one. The correlation was significant this site (R^2 = 0,89). While in II. Deûle – Amont sediment the correlation was negative (slope -0,1) and insignificant (R^2 = 0,21) (***figure IV.16)***. Nevertheless the relative reactivity of the exogenous Hg in these sediments demonstrated that the importance of exogenous methylation to the MeHg production would depend on their intrinsic properties.

Net methylation is dependent not only on the methylation yield, but also on the demethylation yield. When the proportion of methylation yield and demethylation yield was calculated the only the negative values were observed at the II. Deûle – Amont site. No production zone of MeHg is observed and thus the demethylation is predominant. At the I. Metaleurop site the maximum of MeHg is located not at the surface or subsurface sediment, but deeper around the 12 cm of sediment depth.

Figure IV.16*: Correlation between the endogenous methylation yield and exogenous methylation yield.*



IV. VIII. Conclusions

There are many factors that can hinder the measurement of true methylation and demethylation potential. The incubations were perfored with regard to simulate the in situ conditions. The isotopes were equilibrated with overlaying water (not with the surface water) to injection to minimize speciation changes during the assay (MeHg is probably efficiently stabilized by dissolved organic matter and Hg species are pre-equilibrated with natural ligands). This lead to more representative transformation yield (Rodriguez-Gonzalez et al., 2011). The spike concentrations was kept close to the natural concentrations (MeHg the 85-95% of the in situ concentration IHg just 15-25%) to minimize the potential increase in Hg bioavailability that may have occured with excess Hg addition. The conditions of experimetn was chosen with a view to the ability to detect changes in concentration and the maintenance of realistic microbial and geochemical conditions. The comparison of the different ractivity of the exogenous and endogenous species permit the qualification of reactivity of natural Hg with regard to the labile Hg added.

The previous study made by the Rodrigez Martin-Doimeadios et al.(2004) showed the strong dependence of ^{199}Hg methylation on redox conditions and biological activity. The different variables tested the combination of anaerobic and biotic condition was the most conducive to methylmercury production and the values of methylation / demethylation yield was in agreement as the values observed for this yield study.

The concentration of Hg in river sediment has not a significant influence on the MeHg cocnentration and the production of MeHg is independent of the amount of ^{199}Hg added. The factors other than total Hg concentration are important. This is well known and the primary factors influencing methylation have been previously outlined (Benoit et al., 2003), e.g. Organic metter, temperatur (winter/summer season), presence of sulfate reducing bacteria etc.

The factors that affect Hg methylation can be separated into those that affect the biovailability of Hg to the methylating organisms and those that affect the activity of the Hg methylating bacteria (Heyes A., Mason, P.R., 2006). The study made by Compeau and Bartha (1985) showing that sulfate reducing bacteria appear to be primary mercury methylators in anoxic sediment. And must be point that the availability of sulfate is suggested to be a substrate limiting

factor for sulfate reducing bacteria. Among the many factors that may influenced the transformation processes, just the concentration of sulfide was studied in this work. The significant correlation between CRS and methylation yield was observed.. MeHg concentration tend to decrease with sulfide concentration increase. When the sulfate can stimulate methylation (due to higher sulfate reducing bacteria activity), the accumulation of sulfide can inhibit methylation (decrease of bioavailability) (Glimour et al., 1998). The methylation is not inhibited by the HgS precipitation, but by less (bio)availbable charged Hg-S for bacteria methylation. Thus it seem that the higher CRS concentration increased the (bio)available Hg-S and influenced the methylation yield.

The values comparable with the study made by Rodrigez Martin-Doimadios (2004) for methylation / demethylation yield proove the anaerobic biotic conditions in the sediment. Also the other authors e.g. The Fagestorm and Jernelov (1972), Olson and Cooper (1976) and Compeau and Bartha (1984) found higher methylation activity and higher persistance of MeHg under anaerobic conditions. However in the sediments taken from the Deûle river especially the demthylation is extensive (~28% of spiked methylmercury had been demethylated).

Calculation of the methylation / demethalation (M/D) ratios enable the indication the extent to which environmental disturbances may affect the net rate of mercury methylation and hence the methylmercury concentrations in aquatic environments. The M/D ratio determines the balance of methylating and demethylating activity and thus the methylation or demethylation zone can be determined. When ratio is > 1 the conditions had higher potential to the methylmercury production (case I. Metaleurop, around 12 cm of sediment depth), while ratio < 1 favour the demethylation. This is the case of sediment II. Deûle – Amont where MeHg production zone was not found (values < 1). This site wasn´t affected in the past by the mercury pollution. MeHg production was low and the MeHg degradation was comparable with the site I. Metaleurop. The maximum production of MeHg is generally observed in the surface or sub-surface sediment. This is not case of I.Metaleurop site, either the II. Deûle – Amont. Thus in terms of possible remobilization of the MeHg to the water column and the other compartments the MeHg is highly cached.

Further investigation of other parameters responsible for Hg transformation, e.g. organic carbonm at this place should be studied as well as composition of the microbiological comunity of these sediment. Also the study of dredging and its impact related to MeHg release to the water colum and difference between the methylation / demethylation yield in the winter and summer season should bring the information about increase or decrease contribution of demethylation or methylation.

Chapter V: THE USE OF DIFFERENT RESIN GEL FOR DGT TO MEASURE HG IN SEDIMENTS

V. I. Diffusive gradients in thin films (DGT)

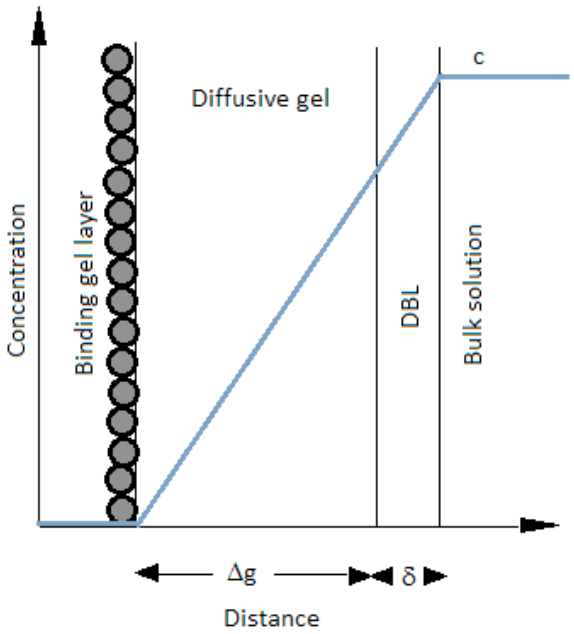
The DGT technique is an in-situ analytical method that is designed to accumulate labile species in environmental systems (Davison and Zhang, 1994; Zhang and Davison, 2000; Zhang, 2004). Generally, in a typical experiment, DGT devices are deployed in an aquatic environmental system (e.g. sediment, water) for a time period ranging from days to months. The accumulation of chemical species occurs *in-situ*, and accumulated species are measured conventional by a suitable analytical technique. Since the first publications describing the use of DGT, by Davison and Zhang in 1994 and 1995 (Davison and Zhang, 1994; Zhang and Davison, 1995), it has been applied to the accumulation of a many trace metals and semi-metals in waters, soils, and sediments, both under controlled laboratory conditions, and in field studies (Zhang et al., 1995; Zhang et al., 1998a; Zhang et al., 1998b; Cattani et al., 1999; Denney et al., 1999; Teasdale et al., 1999; Naylor et al., 2004; Diviš et al., 2005; Diviš, 2005; Dočekalová and Diviš, 2005; Ernstberger et al., 2005; Merritt and Amirbahman, 2006; Naylor et al., 2006; Dunn et al., 2007; Cattani et al., 2009).

V. I. 1. DGT theory

The DGT technique uses an adsorbent, usually immobilized in a polyacrylamide hydrogel (binding gel layer), to adsorb solutes from solution. The binding gel layer is separated from the bulk solution by a permeable gel (i.e. diffusive gel) that is well defined in terms of its thickness (Δg) and porosity (***Figure V.1***).

While DGT is applied **in water solutions**, a diffusive boundary layer (DBL) of thickness δ exists between the diffusive gel and the bulk solution. The transport of solutes from the bulk solution to the binding gel layer is by molecular diffusion through the DBL and the diffusive gel. The diffusive gel generally controls the overall rate of mass transport to the binding gel layer irrespective of the hydrodynamics of the bulk solution. Rapid and irreversible binding of solute to the adsorbent ensures that a concentration gradient is quickly established between the bulk solution and the binding gel layer.

Figure V.1: *Schematic draft of the concentration gradient of a species through a DGT assembly as represented by the bold line. (Zhang, et al., 1998a)*



The DGT technique is based on Fick's first law of diffusion (Zhang and Davison, 1995). The flux, F, of a species to the binding gel layer is given by equation 5.1, where D is the species diffusion coefficient in the diffusive gel, C is the species concentration in the bulk solution, C' is the species concentration at the interface between the binding gel layer and diffusion gel layer, and Δg and δ are the thicknesses of the diffusive gel and DBL, respectively.

$$F = \frac{D(C - C')}{\Delta g + \delta} \qquad (5.1)$$

If the solute binds rapidly to the adsorbent, C' is effectively zero (provided the adsorbent is not saturated), and assuming δ is negligibly small compared to Δg (which is generally assumed for well-mixed solutions), then equation 5.1 simplifies to equation 5.2.

$$F = \frac{DC}{\Delta g} \tag{5.2}$$

The flux can also be determined from the mass, *M*, diffused through an area, *A*, after a given time, *t* (equation 5.3).

$$F = \frac{M}{At} \tag{5.3}$$

Combining equations 5.2 and 5.3, and rearranging, gives equation 5.4. This equation is known as the DGT equation and is used to calculate the concentration of solute in the bulk solution from the known values of Δg, *D, A*, the deployment time, *t*, and the mass of solute accumulated on the binding gel layer, *M*.

$$C_{DGT} = \frac{M\Delta g}{DtA} \tag{5.4}$$

At the completion of a DGT deployment, the binding gel and the diffusive gel are separated and the accumulated solute is eluted from the binding gel layer. For metals this is usually achieved by using 1 to 2 mol.L^{-1} HNO_3 (Davison and Zhang, 1994; Zhang and Davison, 1995; Warnkenet al., 2006). The concentration *Ce*, of solute in the eluent is then determined by an appropriate analytical technique. The accumulated mass *M*, of solute on the binding gel layer can then be calculated using equation 5.5, where *Ve* and *Vg* are the volumes of the eluent and binding gel layer, respectively, and E_f is the elution factor.

$$M = \frac{Ce(Ve - Vg)}{E_f} \tag{5.5}$$

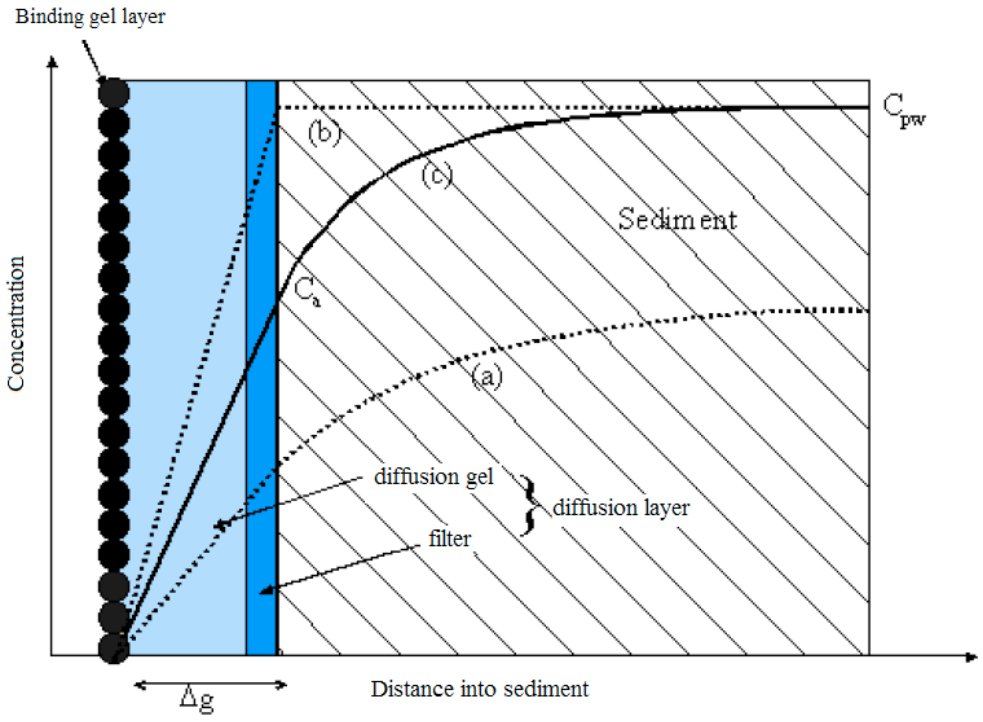
E_f is the ratio of the eluted to bound metal and values of E_f of 0.8 have been reported for Zn, Cd, Cu, Ni an Mn when using 1 or 2 M HNO_3 to elute from Chelex resin (DGT Research, 2011).

The different adsorbents have been used with the DGT technique to bind the solute of interest from solutions. The gels are 95 % water (DGT Research, 2011) and generally have from 2 to 5 nm pore size (Davison and Zhang, 1994; Zhang and Davison, 1995).

However by varying the amounts of reagent used to prepare the diffusive gels, pore sizes in the range of 1 to 20 nm can be prepared (Zhang and Davison, 2000). The diffusion coefficients of metal ions in the diffusive gel are normally between 85 to 100 % of the diffusion coefficients in water (Zhang and Davison, 1995; 1999; Scally et al., 2006; Warnken et al., 2006). These values depend on the solute accumulated. The diffusion coefficients of hydrated metals in the diffusive gel are commonly in the range 4.5 to 8.0 x 10^{-6} cm^2 s^{-1} at 25°C. (Garmo et al., 2003; Scally et al., 2006). The diffusion coefficients of species in the diffusive gel are dependent on temperature due to changes in water viscosity (Zhang and Davison, 1995).

Application of the **DGT probe into the sediment** (or soil) may be use to assess labile metal concentration in pore waters. The interpretation of DGT measurement in sediment is not so easy like in solutions which are well mixed. The diffusive boundary layer between the diffusive gel and the bulk solution pore water (sediments, soils) is omitted. In the sediment pore water there is no mixing processes and the transport of ions is possible only due to diffusion of ions or its releasing from the solid phase. The ions, has to be simultaneously added into the sediment pore water, if not the metals concentration adjacent to the DGT device significantly decreasing with the deployment time.

***Figure V.2**: Schematic representation of a cross section through a DGT device in contact with sediment – three cases: (a) unsustained, (b) sustained, (c) the general or partially sustained case. Modified form DGT Research Ltd., UK.*



To interpret DGT measurements in sediment it is instructive to consider two significant differences from the use of DGT in water:

1. Due to the lack of mixing it must be assumed that, in general, pore water concentrations adjacent to the DGT device become depleted (cases (a) and (c) ***figure V.2***) and if there is no supply of solutes the zone of depletion adjacent to the DGT device becomes progressively larger with deployment time. (DGT Research Ltd., UK).
2. Due to a constant resupply of solutes from solid phase, interfacial pore water concentration between the sediment and DGT device (C_a) is relatively constant during deployment and the theory for solution can be adapted to deployments in sediments:

$$C_{DGT} = C_a = \frac{M\Delta g}{DtA} \qquad (5.7.)$$

C_{DGT} correspond to the pore water concentration adjacent to the DGT device C_a. The DGT concentration will be C_{DGT} less than or equal to the concentration of labile species in the pore water C (measured by independent analytical method). So the degree of ions refilled from the solid phase into the pore water can be found due to the ratio R:

$$R = \frac{C_{DGT}}{C},\ 0 < R < 1 \qquad (5.8.)$$

If the standardized methodologies and samplers (DGT research Ltd., UK) are used value R depend on kinetics of solutes resupply from solid phase and capacity of the solid phase to solutes resupply. R may be obtained experimentally and use to characterize the solute removal to the DGT device as one of the three cases illustrated **in *figure V.2*.**, which show a cross section through a DGT device during deployment (DGT Research Ltd., UK):

figure V.2*: (a):* Unsustainde Case: The value R is close to 0, there is no resupply of solutes to the pore water. The device is supplied only by the diffusion and the concentration of solutes adjacent to the DGT device decreasing with the deployment time.

(b): Sustained Case: The value R is close to 1, mainly the labile forms of metal are present in the sediment and the capacity of the solid phase to resupply the pore water is large and rate of resupply from solid phase is fast compared to the rate of solutes removal to the DGT device. The concentration measured by DGT device is interpreted like actual

concentration of labile metal species on the interface of DGT device and sediment:

$$C_{DGT} = C_{sed} = C_a = \frac{M\Delta g}{DtA} \qquad (5.9.)$$

(c): Partially Sustained Case: The resupply of the solute from solid phase is significant, but it is insufficient to sustain fully pore water concentration. The concentration measured by DGT device is average concentration of solute on the surface of diffusive gel during the deployment time:

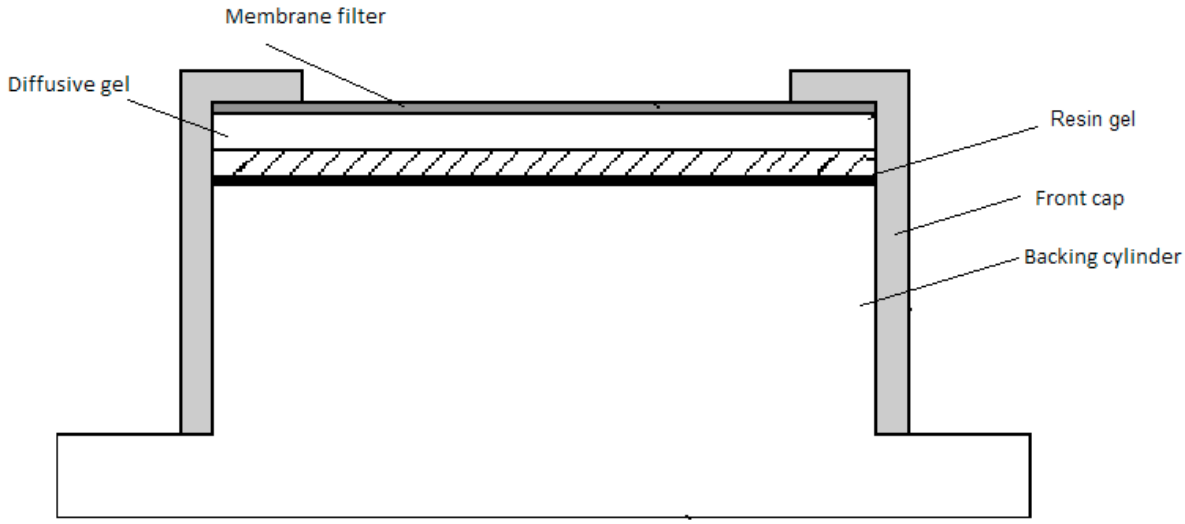
$$C_{DGT} = \frac{1}{t}\int_{t_i=0}^{t} C_a(t_i)dt \qquad (5.10.)$$

Where $C_a(t_i)$ is concentration C_a of time function and t is deployment time.

V. I. 2. DGT assembly

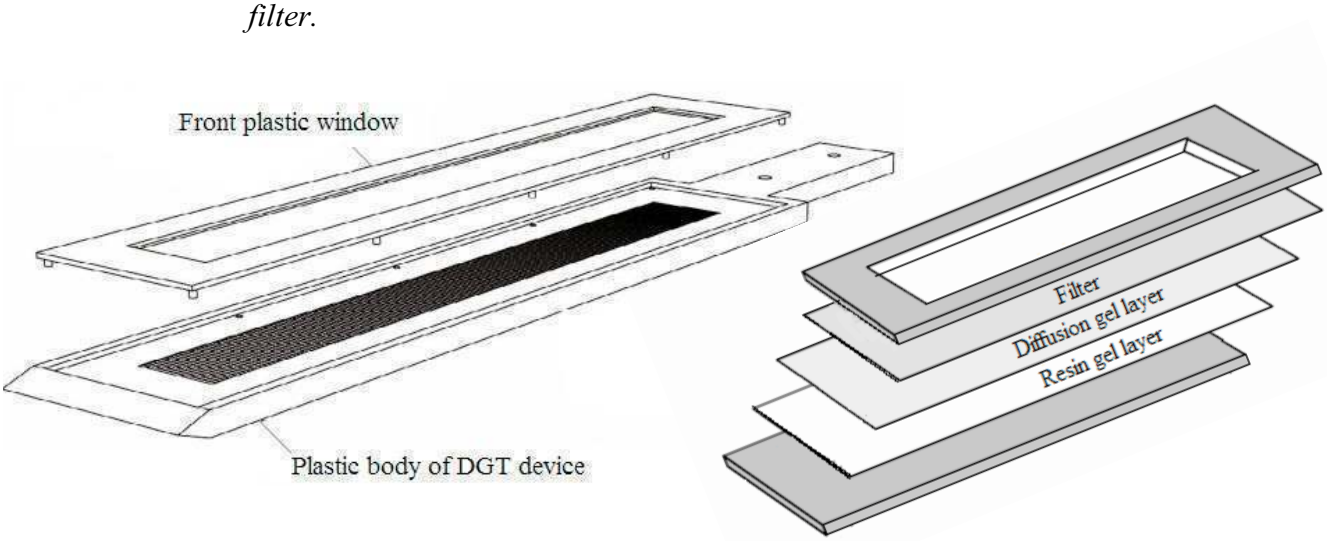
For deployment in waters, the binding and diffusive gels are accommodated within a DGT device which is based on a simple piston design consisting of a backing cylinder and a front cap (***figure V.3***). The binding and diffusive gels are placed sequentially on top of the backing cylinder and a membrane filter is usually placed on top of the diffusive gel to protect it and prevent the adhesion of particles. (Davison and Zhang, 1994) The membrane filter is generally treated as an extension of the diffusive layer (Davison and Zhang, 1994). The front cap, which has a window that allows species to diffuse from the bulk solution to the binding gel layer, is pushed down tightly onto the backing cylinder to hold the three layers firmly in place.

Figure V.3: *Schematic of a DGT device showing the placement of the binding gel layer (resin gel), diffusive gel, and the membrane filter.*



For deployment in sediments (measurement of metals concentration in pore water), DGT devices are 240 x 40 x 5 mm in size, with a window of 150 x 18 mm open to the sediment – sediment probe (***figure V.4***). By slicing the resin gel (used in the DGT device deployed in sediments – ***figure V.8***) on the 5 mm slices prior to analysis it is easy to use DGT to make measurements of solutes at high vertical resolution.

Figure V.4: *Schematic of DGT sediment probe used for deployment in sediments and placement of the binding gel layer (resin gel), diffusive gel, and the membrane filter.*



V. I. 3. Speciation measurements using DGT

The DGT technique measures the free metal ion and metal in labile complexes that can diffuse through the pores of the diffusive gel, and dissociate while diffuse through this layer (Zhang and Davison, 1995; 2000). The solute must then form a stable complex with the adsorbent in the binding gel layer (Davison and Zhang, 1994). Hence, the measurement of species by the DGT technique is governed by the adsorbent, the diffusive layer thickness, and the pore size of the gel (Zhang and Davison, 1995).

Effect of diffusion layer thickness

The contribution of metal from metal complex dissociation will depend on the thickness of the diffusive gel as this determines the time-scale in which dissociation of the metal-complex can occur (Zhang and Davison, 1995; 2000). When the complex dissociates, the concentration gradient towards the adsorbent ensures that the free metal ion diffuses towards the binding gel layer and hence diminishes reformation of the complex. The removal time of a species in the diffusive layer can be approximated by equation 5.11. (Zhang and Davison, 2000; Scally et al., 2003; Scally et al., 2006)

$$t = \frac{\Delta g^2}{2D} \qquad (5.11)$$

For a simple metal ion, the residence time in a 0.8 mm diffusive layer is ~ 2 min; for a metalfulvate complex this time would be considerably larger (~ 15 min).

Effect of adsorbent binding strength

The binding strength of the adsorbent has been shown to influence the fraction of metal measured by the DGT technique (Diviš, et al., 2005; Liet al., 2005). When using adsorbents that may not be sufficiently selective for the metal fraction of interest, the metal may not be quantitatively sampled by DGT technique when is present in the complex form (e.g. with humic acids) and in the presence of competing major ions. For example, Dočekalová and Diviš (Diviš, et al., 2005) used DGT (some devices contained Chelex-100 and some contained Spheron-Thiol as the adsorbent) to measure Hg concentrations in synthetic solutions and a stream (in-situ). For simple synthetic solutions good agreement was obtained for the calculated Hg concentrations when using the Chelex-100 and

Spheron-Thiol adsorbents. However, when DGT was applied to a natural water, the Hg concentration calculated from the mass accumulated in the Spheron-Thiol DGT device was ~ 3 times higher than the Hg concentration calculated from the mass accumulated in the Chelex-100 device. Dočekalova and Diviš (Diviš, et al., 2005) explain this difference by the higher affinity of thiol groups than iminodiacetate group of Chelex-100 to Hg. This affinity affects results in the Spheron-Thiol adsorbent 'inducing' dissociation of Hg from its natural complexes.

Effect of pore size

The DGT device can provide information about the actual labile Hg species present in the environment by varying the pore size of the diffusion gel layer (Gimpel et al. 2003). DGT generally measures a greater amount of dynamic species; however DGT devices with very small gel pore size have been claimed to largely exclude metal humic complexes (Zhang, Davison, 2000; Zhang, Davison, 2001(a), Zhang, Davison, 1999). Metal bound to large colloids and particulate material are excluded from the diffusive layer due to the pore size of the gel.

V. I. 4. Advantages of DGT

The *in-situ* capabilities of the DGT technique is one of the major advantages of the method; it provides a **labile species measuring** in natural systems **without most of the problems associated with collection and storage of samples**. DGT measures the kinetically labile fraction of a metal. The DGT technique **preconcentrates the solute of interest** and therefore allows the measurement of species at very low concentrations. When deploying a DGT device for 24 h with a 0.8 mm thick diffusive gel and 3.14 cm^2 diffusion area, and assuming a diffusion coefficient of 6 x 10-6 cm^2 and an eluent volume of 1 mL, the concentration of solute in the eluent will be ~ 100 times greater than the concentration in the bulk solution. The amount of solute preconcentrated when deploying DGT device into the water can be higher by increasing the deployment time. Furthermore, DGT **allows the solutes to be separated from complex matrices** such as seawater; this is advantageous as it is well known that matrix species can interfere with many analytical measurements.

The DGT measurement is time-integrated, that is, it measures the average concentration over the deployment period. The DGT technique ensures that the

contribution of short-term changes in concentration is included in the measurement. In comparison, grab samples provide a measurement of solutes concentration at discrete times; changes in concentration that occur outside of the sampling period may not contribute to the measurement.

The measurement of **solutes concentrations** by the DGT technique can be carried out over a **wide pH and ionic strength range**. The actual ranges depend on the adsorbent used. The pH range for use of DGT with the most common adsorbent, Chelex-100, is between ~ 5 and ~ 9. (Buffle, 2000) At low pH the uptake of metal by the Chelex-100 resin is reduced, and swelling effects of the diffusive gel at pH > 9 may affect DGT measurements (Buffle, 2000). It has been reported by some authors that at low ionic strengths (0.0001 up to 0.001 mol L^{-1} $NaNO_3$) the concentration measured by DGT does not agree with the concentration in the bulk solution (Alfaro-De la Torre et al., 2000; Sangi et al., 2002; Peters et al., 2003; Warnken et al., 2005). It has been shown that if the diffusive gels are conditioned and deployed in 0.001 mol L^{-1} $NaNO_3$ prior to used, good agreement between the concentration measured by DGT and the concentration in the bulk solution is obtained (Warnken et al., 2005) even at the low ionic strength. The preparation procedure and recommendation proposed by DGT research must be kept, thus the prepared diffusive gel has to be conditioned, stored in 0.01 – 0.1 M $NaNO_3$ (DGT Research Ltd, 2011).

In higher ionic strength solutions (e.g. seawater) the diffusion coefficients of species are ~ 10 % lower than that at low ionic strength; this is due to a difference in viscosity of the solution (Buffle, 2000). In addition, adsorbents such as cation-exchange resins can quickly become exhausted at high ionic strength. (Chang et al., 1998)

V. I. 5. DGT and mercury determination

The diffusive gradient in thin films technique, developed by Davison and Zhang (Davison and Zhang, 1994) for in situ determination of kinetically labile metal species in aquatic systems has been successfully used as a means to follow the concentration of trace metals in natural waters (Denney et al., 1999; Torre et al., 2000; Dahlqvist et al., 2002; Dunn et al., 2003), metal fluxes in sediments (Zhang et al., 1995; Fones et al., 2001) and soils (Zhang, et al., 1998b; Zhang et al., 2001; Dočekal, 2003) and also to estimate the concentration of metals in pore waters (Zhang et al., 2002; Diviš, 2003). Till 2005 the DGT technique gives useful information about wide range of metal species but not for mercury. As Dočekalová and Diviš (Dočekalová and Diviš, 2005) published mercury binding on the amide groups within polyacrylamide gel rather than free diffusion does not

allow use of this diffusive gel and DGT technique for mercury determination. DGT with polyacrylamide gel commonly used is unsuitable (Dočekalová, Diviš, 2005) as diffusive medium for Hg determination, because during diffusion to the resin layer, mercury ions are covalently bound to amide groups of plyacrylamide diffusive gel. (Dočekalová and Diviš, 2005) Agarose gel, has different structure from polyacrylamide gel and was found to be suitable as the diffusive gel for mercury measurements.

Two different resin Chelex-100 and Spheron-Thiol with –SH groups prepared by Smrž and Hradil (Smrž M., 1978), intensively studied by Dočekal and Slovák (Slovák et al., 1979) were firstly used for Hg DGT measurement. The value of diffusion coefficient of mercury in agarose gel calculated on the base of Fick´s law was found same for both Chelex-100 and Spheron-Thiol resins, in mercury solution 8,97 x 10^{-6}. This value is very similar to that in water 9,13 x 10^{-6} (Dočekalová and Diviš, 2005). During the field study using both resin gels was found that concentration of Hg in river water measured by DGT with Spheron-Thiol resin layer was higher than that measured by Chelex-100. This is because higher affinity of thiol groups to Hg(II) bound in non-labile complexes. Moreover in real water sample, there are also strong complexes with natural ligands as fulvic acids and humic acides which are measured by DGT with Spheron-Thiol resin and not measured by Chelex-100. (Dočekalová and Diviš, 2005) Iminodiacetic groups of Chelex-100 enable to assess only ionic mercury and weak complexes of mercury.

Upon this, Diviš and Leermakers used the DGT to measure depth profiles of mercury in river and marine sediments. The DGT with agarose diffusive gel was found suitable for measuring Hg concentrations and fluxes in sediment pore water and the pre-concentration capability of the DGT makes it possible to measure very low concentrations of Hg in sediment pore waters with high vertical resolution. (Diviš et al., 2005) The results obtained using the DGT technique with Spheron-Thiol resin are in good agreement with the results obtained after centrifugation, so the Spheron-Thiol DGT is a representative of total dissolved mercury in the sediments.

Clarisse and Hintellman deal with determination of MeHg by DGT. In their work (Clarisse and Hintelmann, 2006), the extraction of MeHg from the thiol resin and the operational pH range, where the thiol resin efficiently accumulates MeHg was defined. They developed the new mercaptopropyl functionalized resin (Clarisse and Hintelmann, 2006). The DGT technique has been successively developed to monitor MeHg concentration in natural waters, but one of the most important applications of DGT could be the measurement of MeHg to assess the bio-available pool of MeHg in sediments. In

this environment the DGT flux measurement reflects the concentration in the pore waters. (Clarisse and Hintelmann, 2006) Any other work considering measurement of MeHg by DGT was published till present.

Another paper of Diviš et al. follow the previous work and study the possible alternatives of Spheron-Thiol resins, because Spheron-Thiol prepared by Smrž and Hradil (Smrž M., 1978) is not available in the market nowdays (Diviš et al., 2009). Commercially available Duolite GT73 resin, used for the pre-concentration of noble metals and in industrial processes for removing heavy metals from wastewater and the application of new Intosorb AV-MP (6-mercaptopurine functionalized sorbent) resin synthesized in the laboratory was studied. Diviš et al proved that both resins can be used in the DGT technique as mercury specific resins after a pretreatment in the laboratory (Duolite GT-73: graining, sieving and acid washing) (Diviš et al., 2009)

Within my thesis I deal with preparing optimization and consequently testing the resin gels with Titanium dioxide (TiO_2). (*Szkandera, Dočekalová, Kadlecová, Trávníčková, Diviš: Sorpční gel s oxidem titaničitým pro stanovení rtuti technikou DGT: Chemické listy, peer review*).

V. II. Article: Sorption gel with titanium dioxide for determination of mercury using the diffusive gradient in thin film technique (DGT)

Submitted article in Czech language is in the Appendix

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Article: Sorption gel with titanium dioxide for determination of mercury using the diffusive gradient in thin film technique (DGT)

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Key words: DGT; titanium dioxide; mercury; sorption gel

Introduction

Mercury (Hg) is considered by the Environmental Protection Agency (EPA) as a highly dangerous element, because of its accumulative and persistent character in the environment and biota. The fast development of analytical methods during the last decade enable also the development of analytical methods for mercury and mercury compounds determination in the different environment compartments, which were consequently used in many environmental sudies[1-4]. However Hg determination in the different environmental matrices is still complicated for reason of many difficulties during the sampling and analysis (e.g. low concentration of Hg in the samples – not more than $ng.kg^{-1}$ or $ng.dm^{-3}$. Determination such a low concentrations required the special instrumentation[5-6] and use of the non-contaminating[7-8] protocols.

Clean sample collection and storage are important due to the low concentration in the environment. Even minor contamination of the reagents used, storage containers or other tools

have a deleterious effect[9-15]. Also inter-conversion between Hg species has to be avoided during sample collection, pre-treatment and storage. In situ techniques overcome serious problems related to system changes due to sampling and additional sample treatments. Even through more than decade's works in the field of in situ measurement, today we can still speak about the beginning of the practical used in situ methods, because theirs development and validation are usually really difficult and time consuming.

During the nineties, DGT technique[16, 17] has been investigated for measuring more than 50 elements in situ. The pre-concentration capability of the DGT makes it possible to measure very low concentrations of Hg in sediment pore waters with high vertical resolution.

Nowadays the DGT is commonly used for in situ determination of kinetically labile metal species in aquatic systems, for determination of trace metals in natural waters[17, 18], metal fluxes in sediments[21-23] and soils[19,20] and also to estimate the concentration of metals in pore water.

A major advantage of DGT technique is simplicity, relatively fast response times, determination of quite a lot of elements and the ability to perform measurements at high spatial resolution and already mentioned the pre-concentration capability.

The DGT technique employs two layers of hydrogel: a dissuasive layer and a binding gel layer. The both gels are placed in the DGT unit and covered with membrane filter and sealed. Dissolved metal species which are smaller than membrane pore size, diffuse through the diffusion gel layer, of thickens Δg and area A (window of DGT unit; cm^2) and are accumulated by an solute-selective adsorbent in binding phase of gel. The mass of metal M (ng) on the resin layer accumulated during the deposition time t (s) is usually measured after elution with acid by, for example, AAS of ICP-MS. The mercury in resin gels is advantageously measured using one-purpose atomic absorption spectrometer Advanced Mercury Analyser, model AMA 254 directly in the gel discs without previous elution.

Polyacrylamide gel is commonly used as diffusive gel and Chelex-100 is normally used as a binding gel layer. Polyacrilamide gel is unsuitable as dissuasive medium for mercury determination, because during diffusion to the resin embedded in the resin layer, mercury ions are covalently bound to amide groups of polyacrilamide diffusive gel and affect the diffusion of mercury. Agarose gel was found to be suitable as a diffusive layer[23]. Moreover, the use of another resin, with – SH groups, instead of the frequently used Chelex-100 resin, is recomended[23]. Thiol groups of Spheron-Thiol are capable of reacting with mercury bonded even in very strong complexes[23].

During the study of Hg behavior in sediment was prove, that the Spheron-Thiol concentration is very similar to that obtained by direct sampling and analysis of pore water using centrifugation. Concentration of Hg measured by Chelex-100 is lower. This is because, the DGT with Chelex-100 does not have such a high affinity to Hg and it measures only the labile Hg species, such as inorganic ions and weak inorganic complexes of Hg[23,24].

Because Spheron-Thiol is not available in the market at present, therefore possible alternatives were seeking, synthesized, validated and during the analyses of real samples tested the resins contain the thiol groups, Duolit and Iontosorb[21, 25].

This study of Hg measurement in natural waters using the DGT technique, deal with optimization and testing the resin gel, containing the particles of titanium dioxide (TiO_2), as a sorption layer. TiO_2 is known as a very good adsorption medium for metal ions. It is commonly used for pre-concentration of a huge scale of metals with a method of solid-phase extraction (SPE) and its determination using the spectral methods[28-30], for removing the heavy metals from waste water[31-32] and removing of the Hg residue of combustion during the coal combustion[33-34]. The use of TiO_2 as a sorption layer for DGT technique was recently presented by the Bennet et al.[35]. They use Metsorb adsorbent for determination of inorganic form of arsenic and selenium in the natural waters. Panther et al.[36] report the use of resin gel with TiO_2 for determination of radioactive phosphorus in natural waters.

Experimental

Reagents and chemicals

A sorption hydrogel was prepared using the acrylamide (Boehringer, Germany), patented agarose-based cross-linker (DGT Research Ltd., Lancaster, United Kingdom), ammonium persulfate (Lachema, Czech Rpublic), N,N,N′,N′-tetramethylenethylendiamin TEMED (Sigma-Aldrich, Germany) and titanium dioxide – anatase, particle size < 44 μm (Sigma-Aldrich, Germany). All of the reagents were of analytical-reagent grade and water used in the study was high-purity demineralized water provided by Milli-Q (Millipore, USA). For preparation of agarose diffusive gel a 1,5% solution of agarose was used (Merck, Germany). Testing mercury solutions were prepared from a 1 $g.dm^{-3}$ stock standard solution (Astasol®, Analytica Ltd. Prague, Czech Republic). For ionic strength adjustment the sodium nitrate (Lachema, Czech Republic) was used. The pH of testing solutions was modified with sodium hydroxide (Suprapur® 30%, Merck, Germany) and nitric acid (Suprapur® 65%, Merck,

Germany). For study of effect of natural ligands on Hg sorption, the sodium chloride (Lachema, Czech Republic) and mixture of humic acids (Prod. N° 53680, Fluka, Switzerland). DGT probes (piston type with an exposed area of 3,14 cm^2) were obtained from DGT Research Ltd. (Lancaster, United Kingdom).

Apparatus

The determination of total mercury in testing solutions and in sorption gels a one-purpose atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser, Altec, Praha, Czech Republic) was used. For dispersion of TiO_2 particles dispersion the ultrasonic batch (Powersonic PSO 3000A, Ultrashalltechnik AG, Straubehhart, Germany) was used. Testing Hg solutions were stirred using a magnetic stirrer (Hei-Standard, Schwabach, Germany) and pH of these solutions were measured with multimeter (WTW-320, Weilheim, Germany) calibrated by buffer solutions ph 4 and 7 (Analytika, Praha, Czech Republic)

Procedures

Preparation of resin and diffusion gels followed the procedure recommended by Zhang and Davison[17] with a few modifications. A diffusive agarose gel (1,5%) was prepared by dissolving the agarose in an appropriate volume of 80°C warm demineralized water and hot dissolved agarose solution was pipetted between two preheated glass plates separated by plastic spacers of 0,5 mm thickness. After cooling down to the gelling temperature (36°C or below) the solution made the agarose hydrogel. Preparation of resin hydrogel sheets with particles of TiO_2 follow the experiences gained in during the preparation of ion exchanging resin Spheron-Thiol[18] and Chelex-100[23].

To 0,4 g of dried TiO_2, which guarantee the sufficient sorption capacity of the DGT disc units for long-term use in real aquatic systems, 2 mL of gel solution was added and mixture was 5 minutes stirred. For homogenical distribution of TiO_2 in hydrogel, the gel solution was placed into the ultrasonic bath after TiO_2 addition. This facilitates the pipetting of solution into the glass form and formation of better quality hydrogel. From the prepared gels plates the round discs with diameter of 25 mm, were cut using a plastic knife. The discs of agarose gel and resin gel with TiO_2 were hydrated in demineralized water for at least 24 hour. After hydration, the discs were stored in demineralized water

DGT assemblies were set just before the use. Gels were placed on the top of the piston, the resin gel was covered by diffusive gel and by the polyethylenesulfone membrane filter (Supor®-450, Pall Corporation, USA) with a 0,45 μm pore size to protection of gels against the damaged. The unit was closed with front cap with exposure 2cm diameter window.

The prepared DGT units were placed into a 5 L of stirred testing Hg solutions of selected conditions without presence of other substances. The concentration of Hg in solution was 20 $\mu g.dm^{-3}$. The pH in the bulk solution was 6. pH of solutions for effect of different pH testing was in the range from 2 to 10. For testing of different ionic strength testing the Hg solutions of ionic strength from 0,001 to 0,5 $mol.dm^{-3}$ were prepared. Concentration of chlorides in testing solution was 0,001 – 0,5 $mol.dm^{-3}$ and concentration of humic acids was 0,01 – 10 $mg.dm^{-3}$.

After exposure, DGT units were taken from the solution, the units were taken into parts and the gel layers were separated. During all of the tests, the concentration of Hg in tested solution was controlled by taking the aliquot volume of solution before DGT units insertion into the solution and after their removing by filtration through membrane filter with a 0,45µm pore size and acidification using nitric acid. The determination of Hg in solutions C_{SOL} and in the gel discs was performed using AMA-254. Average Hg concentration measured by DGT was calculated using the following equation:

$$C_{DGT} = M.\Delta g / D\ t\ A, \qquad (1)$$

where M (ng) is mass of Hg accumulated in the resin gel layer during the deployment time t (s). A is exposure area and Δg is the thickness of the diffusive gel, D is the diffusion coefficient of Hg in agarose gel.

Results and discussion

Characterization of sorption gel

In prepared sorption gels with TiO_2 Hg content was determined by subtraction of Hg from Hg content found after DGT exposition in solution. The average value of Hg found in the unexposed gel disc was 0,13 ± 0,05 ng (n=10), this correspond with minimal measurable

concentration of Hg 3,4 ng.dm^{-3} (for DGT deployment time of 24 hours). The lower Hg concentrations can be measured by increasing of deployment time of DGT units.

While multiple DGT discs Hg ions overcharging, the capacity of the discs was found to be 2,5 μmol/disc (fig.1), which is sufficient high for several weeks also month deployment of DGT unit with this sorption gels in a natural water systems.

Validation of DGT technique with TiO_2 sorption gel

Concentration of Hg (C_{DGT}; calculated from content of Hg bound into sorption gel) within 4h exposition calculated using equation (1) are compared with the immersion solution concentration measured during the experiment. The differences are within 5% and so it respects the DGT Research recommendation[37].

The mass of Hg accumulated in the resin gel increased linearly with the time (fig.2) and the measured mass of accumulated Hg in DGT sampling units agrees with a theoretical prediction using equation (1). These results prove that TiO_2 resin gel in DGT technique in order to measure mercury guarantee the reliable data for Hg determination.

The diffusion coefficient in agarose gel used with TiO_2 resin gel was calculated from slope (α) of the dependence bounded Hg content in resin gel (M, ng) during the time (t, s), thickness of the diffusive gel (Δg, cm), exposure area (A, cm^2) and Hg concentration in solution.

$$D = \alpha \Delta g / AC \qquad (2)$$

The calculated diffusion coefficient D of Hg in agarose gel for TiO_2 is (8,90 ± 0,13)×10^{-6} cm^{2}s^{-1} and correspond to the values found for Chelex-100 and Spheron-Thiol and to the tabulated value of Hg in water 9,13.10^{-6}.cm^2.s^{-1}.

Influence: pH, ionic strength and selected natural ligands

The pH value plays an important role with respect to the adsorption of different ions on oxide surface. Influence of different pH on Hg sorption was study within the range 2-10. The study proves that resin gel bounds Hg well in the range 4-8 (fig.3). The decrease of pH leads to the neutralization of surface charge, and overlay of active sites on the surface of TiO_2,

so the adsorption of cation onto TiO_2 decrease quickly.[39] pH of natural waters is in the rage 6,5 – 9. The DGT technique with TiO_2 resin gel might be used without the trouble in most of the natural aquatic systems.

Influence of ionic strength on the Hg sorption in the range from 0,001 to 0,01 mol.dm^{-3} was neglectable. These values correspond to a common range of ionic strength in surface water (0,002 – 0,02 mol.dm^{-3}). With an ionic strength 0,1 mol.dm^{-3} diminution of Hg sorption was observed (fig.4). Crucial effect on Hg determination using DGT technique has concentration of natural ligands.

In this study, the influence of chlorides and mixture of humic acids was investigated. The concentration of chlorides less than 0,03 mg.dm^{-3} in the tested solution already influenced the Hg sorption (25% of diminution). The concentration of chlorides over 3 mg.dm^{-3} implicates stable chlorcomplex formation, which cannot be measured by DGT technique (fig.5).

Presence of humic acids in the tested solution also influence the Hg content captured in resin gels by stable complex formation (fig.6). Already the concentration 1 mg.dm^{-3} of humic acids cause the diminution of Hg capture about 40%, the concentration 10 mg.dm^{-3} about 80%.

The concentration of chlorides in clean surface water never exceeds 0,05 mg.dm^{-3} and the concentration of humic acids vary in units mg.dm^{-3} in natural waters[40]. The result of basic tests prove, that DGT technique with TiO_2 resin gel cannot be used for Hg determination in marine waters, because Hg is due to high chlorides concentration (as far as 22g.dm^{-3}) present in the stable chlorcomplexes.

Also in natural waters with a high concentration of humic acids, the Hg bound into the strong complexes, which cannot be capture by DGT technique with TiO_2 resin gel. In the natural waters with content of humic acids no more than 1 mg.dm^{-3} DGT technique with TiO_2 resin gel can be successfully used for determination of labile Hg species. The combination of DGT units with TiO_2 resin gel and resin gel containing the thiol groups, as a Spheron-Thiol or Duolit GT73 enable the prediction of different Hg forms in natural aquatic systems.

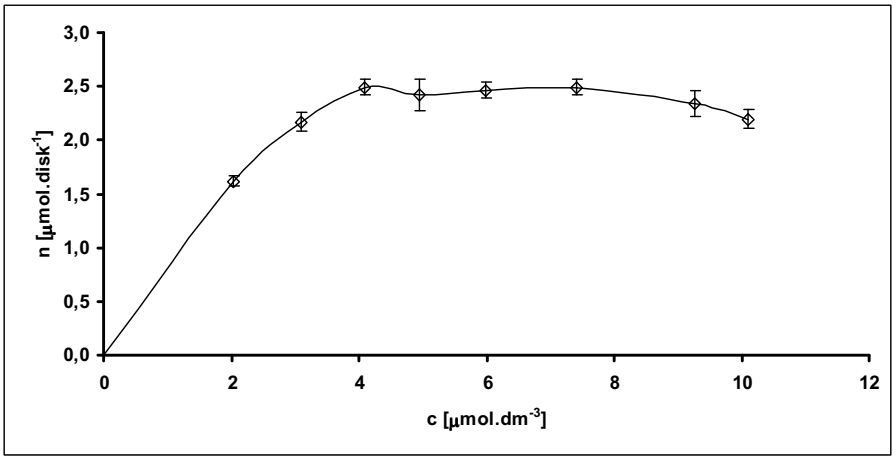

Fig.1 Dependence of accumulated Hg content in discs (n, µmol) on its concentration in solution (c, µmol.dm^{-3})

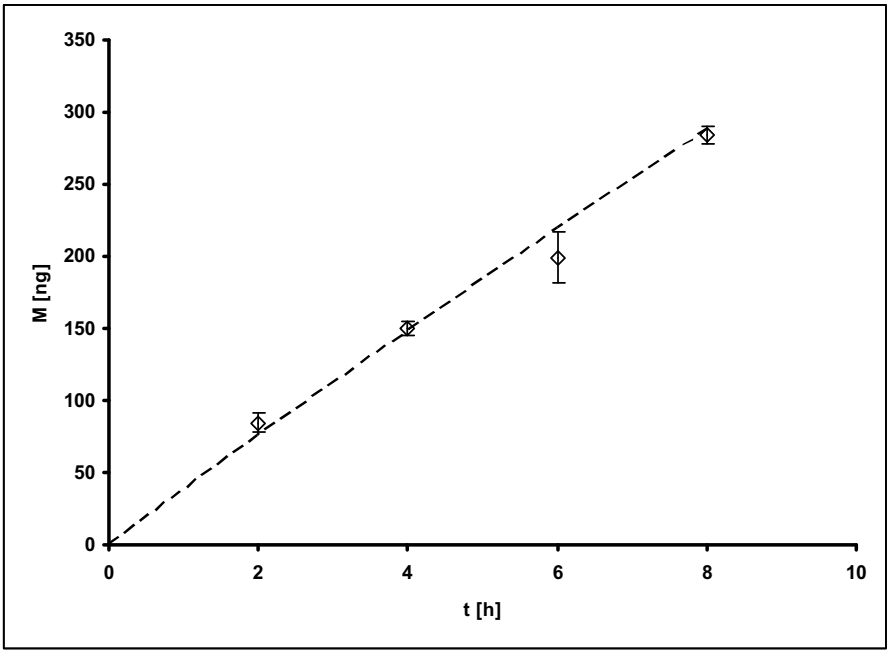

Fig.2 Dependence of accumulated Hg content (M_{Hg}, ng) in single sorption gel on a time (t, h) for Hg concentration in solution 20 µg.dm^{-3}, hatched line represent the theoretic dependence from the equation (1)

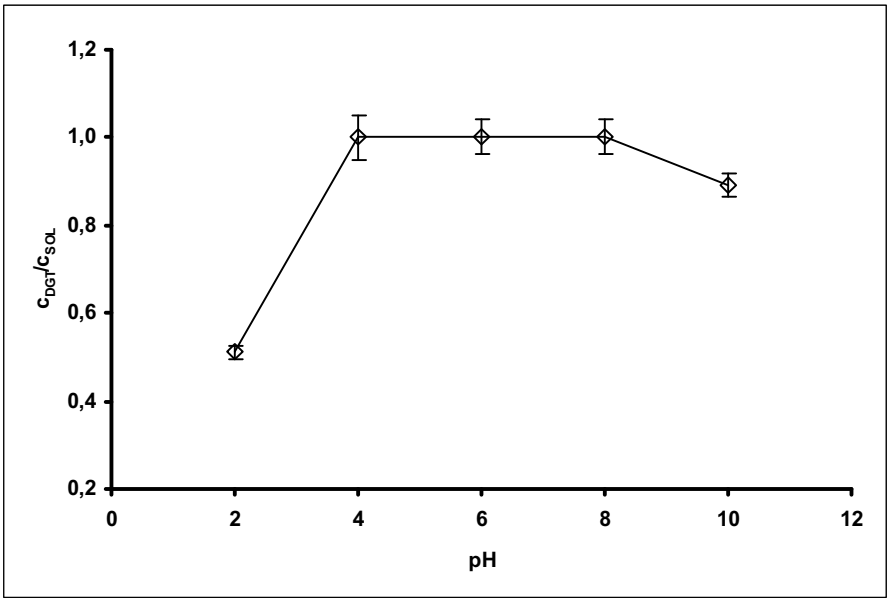

Fig.3 Dependence of Hg sorption on a pH of solution

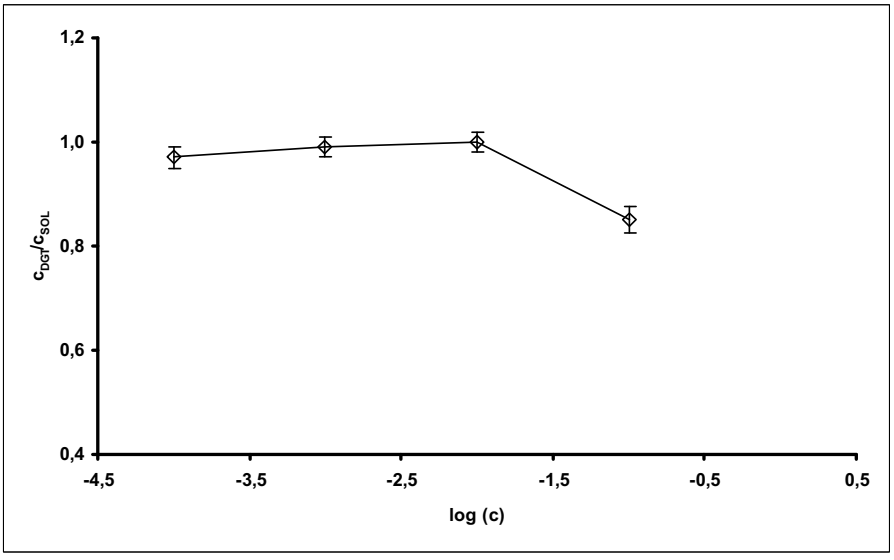

Fig.4 Dependence of Hg sorption on an ionic strength of solution (c, mol.dm^{-3})

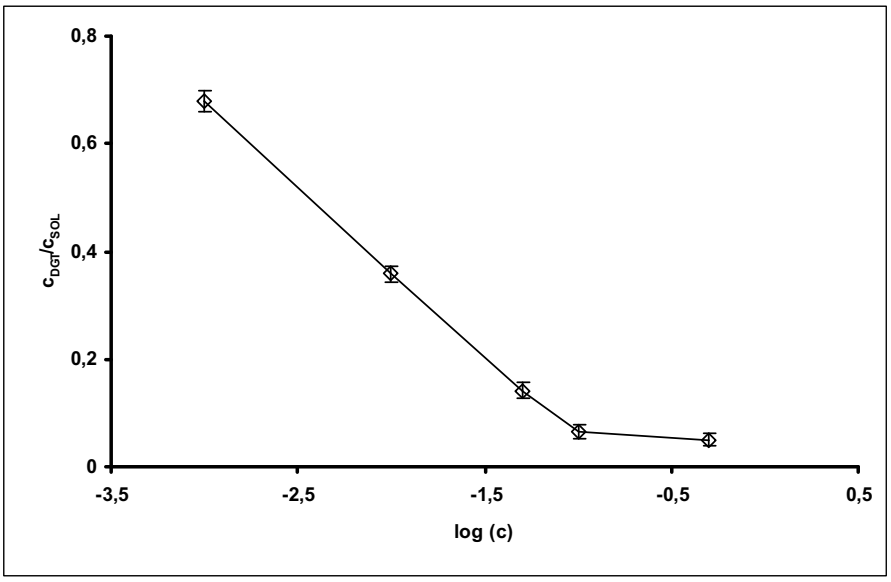

Fig.5 Dependence of Hg sorption on a chlorides concentration (c, mol.dm^{-3})

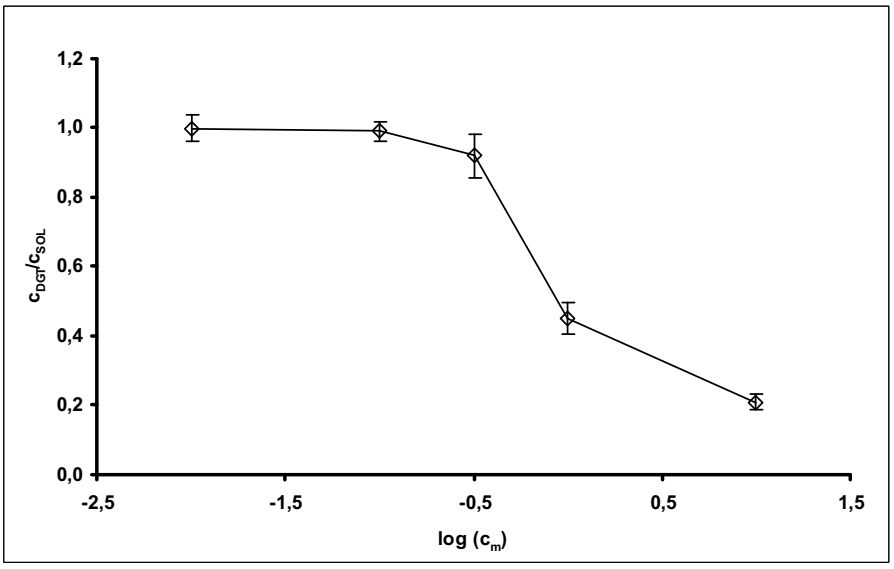

Fig.6 Dependence of Hg sorption on a concentration of humic acids (c_m ,mg.dm^{-3})

Conclusion

TiO_2 resin gels in DGT technique can be used for determination of labile Hg species in most of natural freshwater. Method is not suitable for Hg determination in marine water with a high chlorides concentration, because of stabile Hg complex formation. On the other hand DGT technique with TiO_2 resin gel can be applied in the water containing the humic acids with concentration lower than 1 $mg.dm^{-3}$, similary like resin gels containing the thiol groups (Duolit GT73 and Spheron-Thiol)[41]. Determination with a commonly used Chelex-100 is influenced by the ordinary lower humic acids concentration.

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V. III. The use of different resin gel for DGT in the field study

V. III. 1. Materials and methods

Preparation of resin and diffusion gels

The procedures of preparation resin gels followed the procedure used by Davison and Zhang (1995). Briefly, the polyacrylamide gel solution was prepared by mixing aqueous solutions of acrylamide (40% solution, Sigma-Aldrich) and cross-linker (DGT Research Ltd., UK). The given amount of resin (Chelex-100: Bio-Rad Laboratories, USA; Duolite GT73: Sigma-Aldrich, Germany; Spheron-Thiol: Lachema, Czech Republic) were added to the 10 mL of the polyacrylamide gel solution and the polymerization was initiated by adding freshly prepared ammonium persulfate and catalyzed with TEMED (N, N, N′, N′ - tetra-methylethylenediamine). Space between two glass plates separated by plastic side spacers was filled with the well mixed resin gel solution mixture. The procedures using Chelex-100 and Spheron-Thiol is in detail describe in Dočekalová and Diviš 1995 and using Duolite GT73 in Diviš et al. 2009 and also in Szkandera 2011 (doctoral thesis).

The procedures of preparation TiO_2 gel and agarose gel is describe in the **Chapter V. II.**

As published Dočekalová (Dočekalova and Diviš, 2005) commonly used polyacrylamide gel in DGT technique is unsuitable as diffusive gel for mercury determination. The agarose gel is suitable gel and was used as diffusive gels in combination with any sorption gel for mercury measurements.

Preparation of DGT end DET assembly

For the laboratory testing the gels were cut to circular disks of 2,4 cm diameter before used. The DGT units were assembled by inserting the Chelex-100 or Spheron-Thiol or Duolit or TiO_2 gel disk on the piston base, overlying it with first agarose gel, then 0,45µm pore size filter membrane (Milipore, USA) and closed by the plastic molding which holds the layers inside the DGT unit and prevent leakage.

For the field study, the sheets of 16 x 2,7 cm in size were cut from the prepared gels using a plastic knife. The DGT probes were assembled by inserting the Chelex-100 or Spheron-Thiol or Duolit or TiO_2 gel sheets inside the rear part of the DGT probe (DGT Research Ltd. UK). The resin gel was then covered with agarose diffusive gel sheet and with 0,45µm pore size membrane filter (Milipore, USA) and closed with the front part DGT probe equipped with a window.

DET probes preparation procedure was similar to that of Zhang et al. (1995). A gel containing 1,5% agarose was prepared by its dissolving in an appropriate volume of warm Mili-Q water. The mixture was placed in a boiling water bath, covered and gently stirred until all the agarose was dissolved and the solution was immediately pipette into a preheated gel-casting probe and left to cool down to its gelling temperature. The gels were covered with a 0,45 µm cellulose acetate filter (Milipore) and finally the window plate was put on top of the probe and all the elements gently pressed together.

Basic DGT performance test

In order to test the validity of DGT measurement for mercury analysis, the recommended basic test (time-dependence test) was carried out using 20 - 50 $\mu g.l^{-1}$ mercury solution pH 4-6 and ionic strength 0,01 $mol.L^{-1}$ (using $NaNO_3$), during the 8 hours.

The prepared DGT units were placed into a 5 L of stirred testing Hg solutions of selected conditions without presence of other substances. The concentration of Hg and pH in solution was monitored during the whole experiment by taking the aliquot volume of Hg solution. The concentration was measured after filtration through membrane filter with a 0,45µm pore size

and acidification using nitric acid. Filtration is necessary, for obtain the comparable values of Hg content in DGT resin gel and bulk solution, because the previous experiments prove the sorption of Hg in membrane filter (Feldmannová 2002; Klímová 2003).

After exposure, DGT units were taken from the solution, the units were taken into parts and the gel layers were separated. The determination of Hg in solutions C_{SOL} and in the gel discs was performed using AMA-254. Average Hg concentration measured by DGT was calculated using the following equation:

$$C_{DGT} = M.\Delta g / D\ t\ A, \qquad (5.7)$$

where M (ng) is mass of Hg accumulated in the resin gel layer during the deployment time t (s). A is exposure area and Δg is the thickness of the diffusive gel, D is the diffusion coefficient of Hg in agarose gel.

The diffusion coefficient in agarose gel used with Duolite resin gel was calculated the same way as for TiO_2 resin gel (**Chapter V.II**) and is $(8{,}90 \pm 0{,}13)\times10^{-6}$ cm^2s^{-1} and correspond as well to the values found for Chelex-100 and Spheron- Thiol and to the tabulated value of Hg in water $9{,}13.10^{-6}.cm^2.s^{-1}$.

V. III. 2. Study areas and sampling

The samples were taken from the River Deûle in 03/2009 and 04/2009 and from Lys River in 02/2010 and 03/2010 (***figure V.5, figure V.6***). The sampling sites are generally same with those periodically monitored by water authority (Agence de l´Eau) for controlling the water quality. The sampling strategy is described in the **Chapter III**. Briefly the two sediment cores (polyethylene tube; length 80 cm, diameter 7 cm) were taken in order to deployment of DGT and DET units. Another sediment core was employed for pH and redox measurements in the sampling place at the 1 cm interval. The last core was transported to the laboratory introduced into a glove box previously flushed with nitrogen and after removal of the overlying water sectioned. Each slice of sediment was split into two parts in a glow box. One part was stored in the dark cold place under nitrogen till the analysis of sulphur and metals

(analytical method, results and discussion in **Chapter III. part II.**); other part was centrifuged in the tube in order to extract the interstitial water. An aliquot of overlying water was filtered under nitrogen and acidified for metal analysis, while other aliquots were stored for analysis of dissolved THg (water preserved by adding 5 ml/l BrCl solution to the sample according the EPA method 1631, revision E). Finally the surface water samples were collected by hand, in acid-washed bottles.

To provide reliable and accurate trace metal determination, clean techniques were used for handling and analyzing samples. All material coming in contact with samples were cleaned and stored according to the conditions for ultra-trace Hg analysis (**Chapter II.)**

Figure V.5: *Sampling sites*

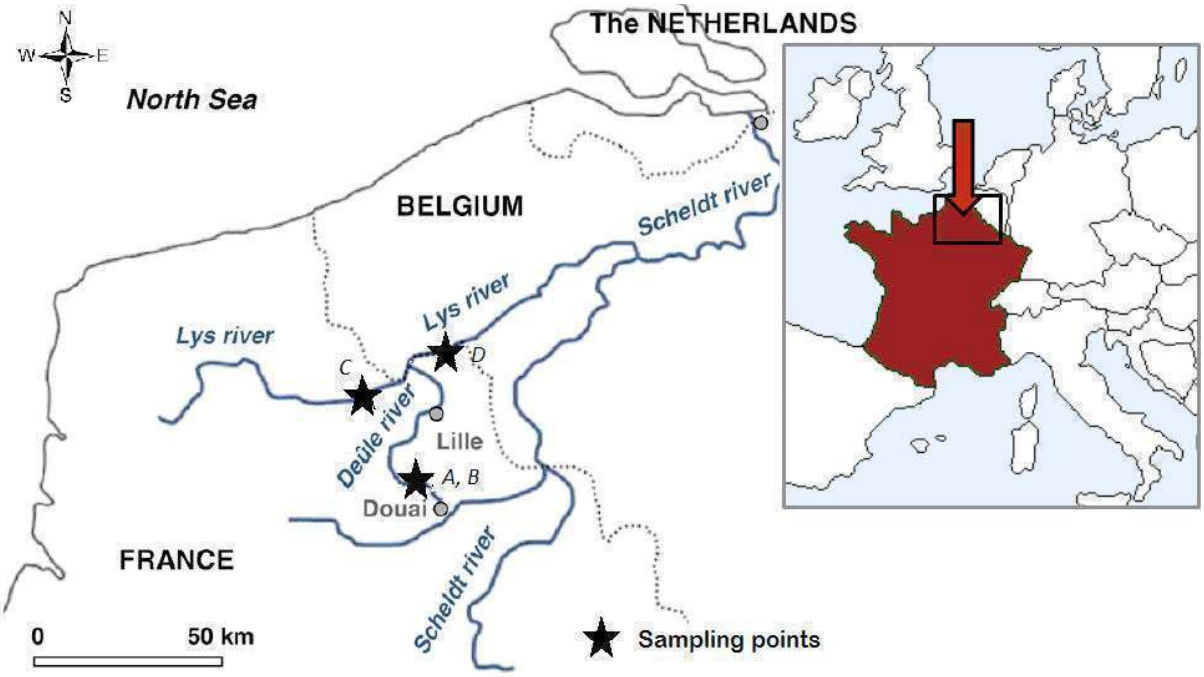
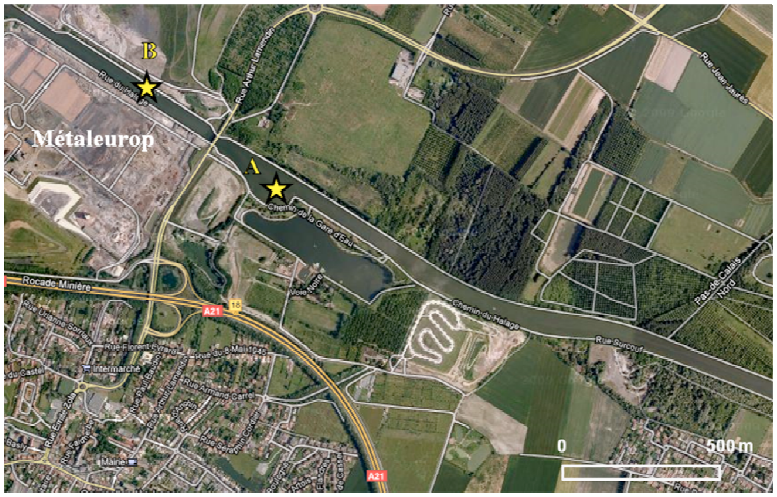


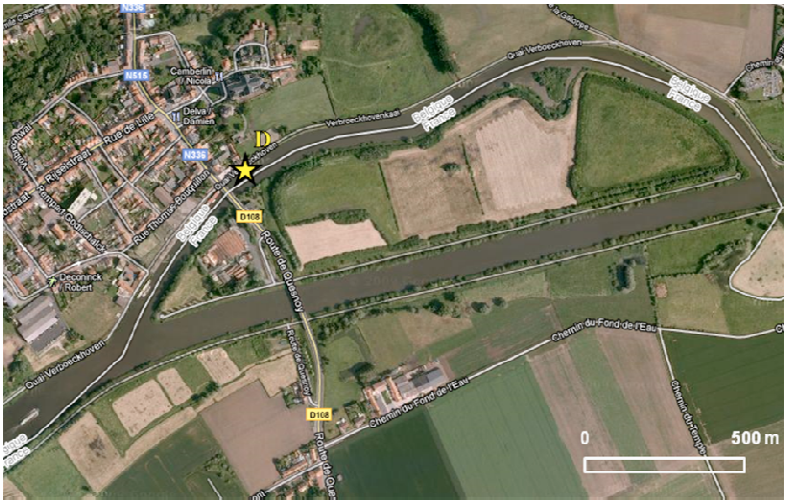
Figure V.6: *The map of sampling site (a) at Deûle River A and B near the Metaleurop; (b) at Lys River C; (c) at Lys River D*

(a)



(b)

(c)



The sampling strategy demonstrates the ***figure V.7*** DET and DGT probes were inserted back to back in the sediment cores for 48 hours (in Deûle River) and 96 hours (in Lys River). Before the deployment, for remove metals, the DET and DGT probes were de-oxygenated by bubbling with oxygen free nitrogen gas for 24 hours while were immersed in a container with $NaNO_3$ (0,01 M) solution. After deployment, probes were rinsed quickly with Mili-Q water. DET gels were extracted from the small slits of the probe, while the resin gel was cut into 5 mm interval using a Plexiglas gel cutter. Each gel slice was then eluted in a 1 M HNO_3 solution and diluted for analysis by ICP-MS. The procedure of preparation DGT and DET probes is described in the section V.III.1.

Figure V. 7: *Sampling strategy*

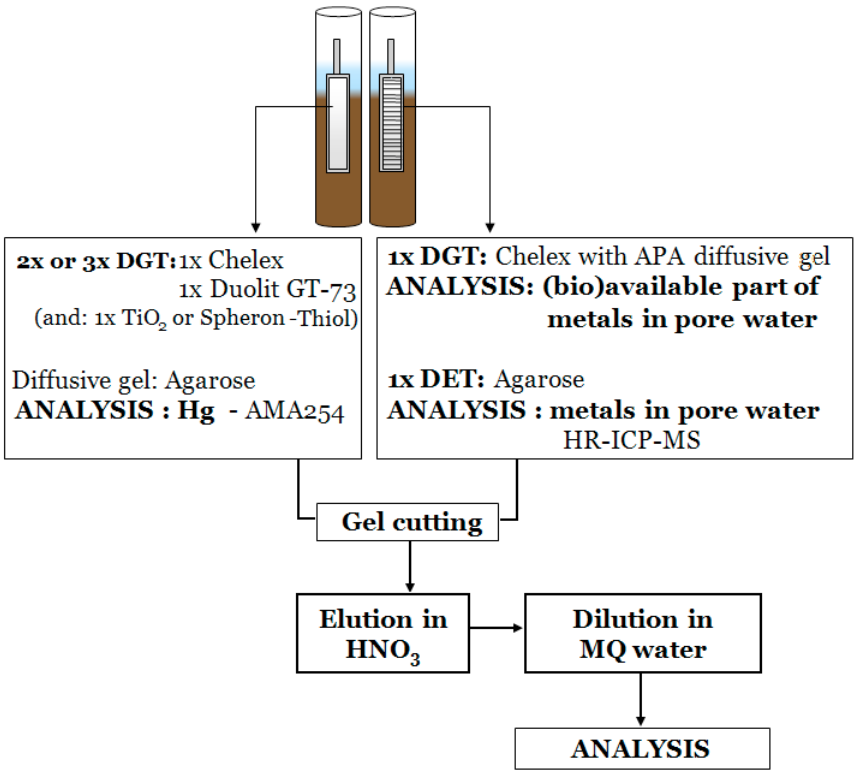


Figure V. 8: *Deployment of DGT probes into the sediment*

V. III. 3. Analysis

Basic DGT performance test

To verify the application of DGT for mercury measurements the experiment concerned the time-dependence test was performed. Results of previous work prove (Dočekalová, 2005), that DGT with Chelex-100 resin measure only cations and small inorganic complexes while Spheron-Thiol resin measure even more stable Hg species (e.g. complexes with natural ligands and fluvic acids and humic acids. Thus resin with thiol-groups present the best choice for total mercury determination.

Unfortunately nowadays Spheron-Thiol is not available in the market and therefore possible alternatives were studied by Diviš et al. (2009). Because there are no mercury specific resins applicable for direct use in DGT technique on the market, the resin gels has to be pretreated in the laboratory. This is also the case of Duolite GT73, which has to be grained,

sieved, washed in acid and incorporated into the polyacrylamid (Diviš et al. 2009). The basic test with Duolit GT73 was performed also in my work.

Another possibility is use of resin gel, containing the particles of titanium dioxide (TiO_2), as a sorption layer. This gel was characterized and validated. The results are presented in the **Chapter V.II.**

Determination of insoluble part of HgT in water, Hg species in sediments and pore water – DGT technique

For the total mercury analysis in dry sediment samples without any pre-treatment, in dry filters (used for filtration of water and determination the THg in particles) and in DGT resin gels, one-purpose atomic absorption spectrometer Advanced Mercury Analyser, model AMA 254 (Altec Ltd., Czech Republic) was used. The principle is described in the **Chapter II. – part II.**

Determination of Hg species in the pore water – Centrifugation

Determination of mercury in pore water (as well as surface water) the method of cold-vapor fluorescence spectrometry (CVAFS) was used (EPA 245.7). The principle is described in detail in the **Chapter III. – part IV.**

Determination of metals - HR-ICP-MS

The Inductively Coupled Plasma sector type mass spectrometer (ICP-MS) used to analyze all samples was a X Series, Thermo Elemental (University of Lille) or Thermo Finnigan Element II, Thermo Scientific, USA (Vrije Universiteir in Brusels). Its combination of sensitivity and very low background noise makes it particularly suitable for elemental trace analysis. The content of metals (Fe, Mn) in the pore water of sediments and in resin gels extracts (1% HNO_3) and in 6M HCl extracts of sediment was determined.

V. III. 4. Results

Basic DGT performance test

Firstly the concentration of Hg in prepared gels was measured using AMA254. The 10 discs of each prepared gel were used for blank measurements. The values are listed in the *table V. 2* and were used as a blank concentration for correction of accumulated Hg concentration in the gels after exposition.

Table V.2: *The blank concentration in the prepared gels.*

Resin	Concentration of Hg in ng/disc
Chelex - 100	0,125 ± 0,015
Spheron-Thiol	0,540 ± 0,099
Duolite GT-73	0,141 ± 0,014
TiO_2	0,122 ± 0,018

All the Hg blank concentrations are negligible when compared the concentration Hg determined. The highest value of blank concentration was found in the Spheron-Thiol resin gel.

The conditions and results of basic DGT test for the Duolit GT-73 resin gel test are listed in the ***table V. 3***. The difference between the average concentrations measured in the bulk solution (after filtration) compared average concentration in the DGT resin gel is lower than 10% and is possible to use DGT resin gel in the aquatic system.

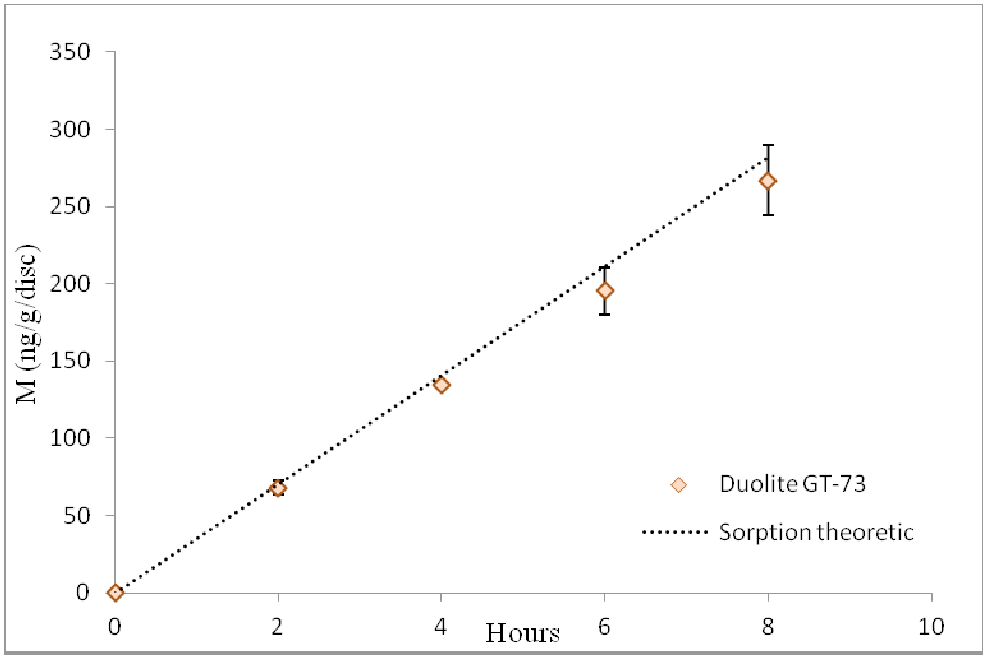
Table V.3: *Test conditions and the concentration of Hg in the solution (filtrated and non-filtrated) and in the (testing Duolit GT-73).*

hours	pH	temp. (°C)	C_{sol} (µg.L^{-1})		C_{DGT} (µg.L^{-1})	Δc (%)
			Non-filtrated	Filtrated		
2 (start)	4,51	24,8	24,81	19,45	21,17	3,7
4	4,51	24,8	24,28	19,31	20,89	3,0
6	4,50	25,0	22,14	17,69	19,22	3,0
8 (end)	4,50	25,1	21,02	16,97	18,73	4,5
Ave.	4,5	24,9	24,06 ± 1,79	18,36 ± 1,22	19,01 ± 1,21	3,5

The concentration measured by DGT has to be compared with concentration filtered in bulk solution concentration while the resin and diffusive gels are covered by the membrane filter. The 0,45 µm pore size filter was used.

The mass of Hg accumulated in the Duolite GT-73 resin gel (figure V. 9.) increased linearly with the time and the measured mass of accumulated Hg in DGT sampling units agrees with a theoretical prediction using First Fick´s low (equation 5.7).

Figure V. 9*: The mass of Hg accumulated in the Duolite GT-73 resin gel within the time*



These results prove that Duolite GT-73 resin gel in DGT technique in order to measure mercury guarantee the reliable data for Hg determination.

The conditions and results of basic DGT test for the TiO_2 resin gel test are mentioned in the **Chapter V.II**, and the results of basic DGT are listed in Dočekalová and Diviš 1995, Diviš et al. 2009, Szkandera 2011 (doctoral thesis).

Mercury speciation in sediments, pore water and surface water

The surface water, pore water and sediments were analyzed. Total dissolved mercury in the surface water (HgT_{SW}) and in the pore water (HgT_{PW}) was measured using CV-AFS. Total "insoluble" mercury in the surface water (HgT_{ins}) was measured after filtration of surface water in sediments by subsequent analyses of the filters using AMA254. Mercury was also measured by technique of diffusive gradient in thin film with different resin gels (Chelex-100, Dulit GT-73, TiO_2, Spheron-Thiol). Total mercury in dry sediments (HgT_{sed}) was analyzed using AMA254, methylmercury (MeHg) using HS-GC-AFS **(Chapter III).**

The average concentration of mercury species are in the ***table V.4***. The values were average for comparison of sampling places.

Table V.4: *The average concentration of Hg.*

Sampling points			**Deûle A**	**Deûle B**	**La Lys C**	**La Lys D**
Surface water	HgT_{SW}	ng/l	20,8 ± 0,8	8,0 ± 0,71	18,5 ± 1,5	19,4 ± 1,7
	HgT_{ins}		44,3 ± 0,6	39,6 ± 3,6	15,3 ± 3,3	24,4 ± 4,6
Pore water	HgT_{PW}		16,0 ± 3,2	9,83 ± 2,03	5,88 ± 0,35	11,4 ± 3,5
	Chelex-100		9,98 ± 7,15	2,03 ± 1,68	0,676 ± 0,37	2,18 ± 1,12
	Duolit GT-73		19,1 ± 4,7	11,3 ± 3,0	4,94 ± 0,8	5,35 ± 1,40
	Spheron-Thiol		17,7 ± 4,6	11,7 ± 2,9	-	-
	TiO_2		-	-	0,768 ± 0,29	3,21 ± 1,41
Sediment	HgT_{sed}	mg/kg	9,92 ± 2,22	9,11 ± 1,85	42,0 ± 2,3	527 ± 13
	MeHg	µg/kg	3,56 ± 1,30	7,61 ± 3,67	1,63 ± 0,09	1,86 ± 0,23
$MeHg / HgT_{sed}$		%	0,21	0,87	0,42	0,04

For the better characterization of the sediments depth profiles the concentration of Fe and Mn measured by DGT, DET and conventionally by centrifugation was observed.

Comparison of resin gels

A commonly used Chelex-100 resin was used for comparison with Spheron-Thiol, Duolit GT-73 resin and TiO_2 adsorbent. Chelex-100 is not suitable ion-exchange resin for total dissolved mercury measurements. The iminodiacetic function groups of Chelex-100 resin are only able to react with weak complexes of mercury. The concentration of mercury in pore water as measured by Chelex 100 DGT probe was quite low than that of Spheron-Thiol

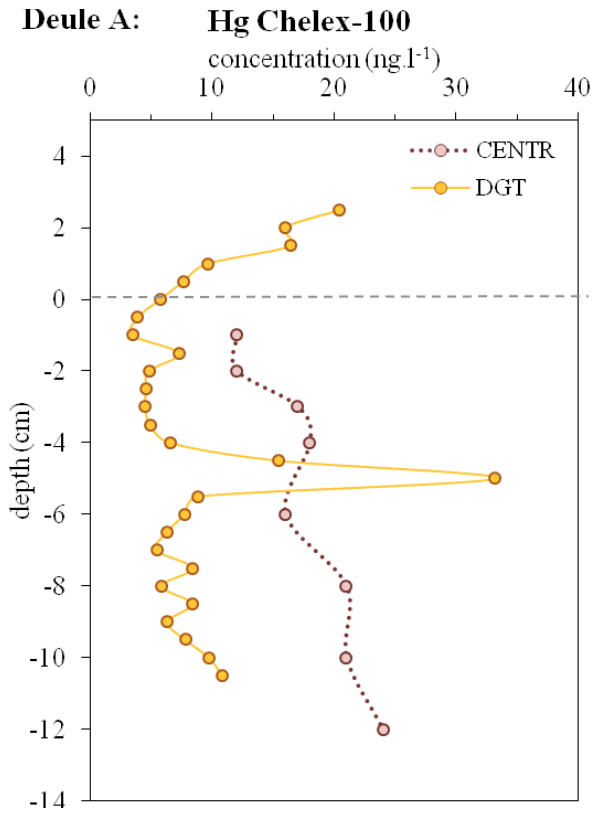
or Duolit GT-73 probe (average concentration are listed in the ***table V.3***.; depth profile are shown on the ***figure V.10 a)–d)***). The specific resin with -SH function groups are able to react with a mercury bounded in the strong complexes with natural ligands and organomercurial compounds.

Concentration of Hg measured by Spheron-Thiol resin or Duolit GT-73 approaching the concentration of dissolved mercury in pore water (HgT_{PW}) with exception Duolite GT-73 at the point La Lys D, where seems to be more labile species rather than at the point La Lys C. Another explanation is that in the case of the site La Lys D, there is no supply of mercury to the pore water (e.g. from the sediment) and all mercury species are strongly bounded in the solid part of sediments.

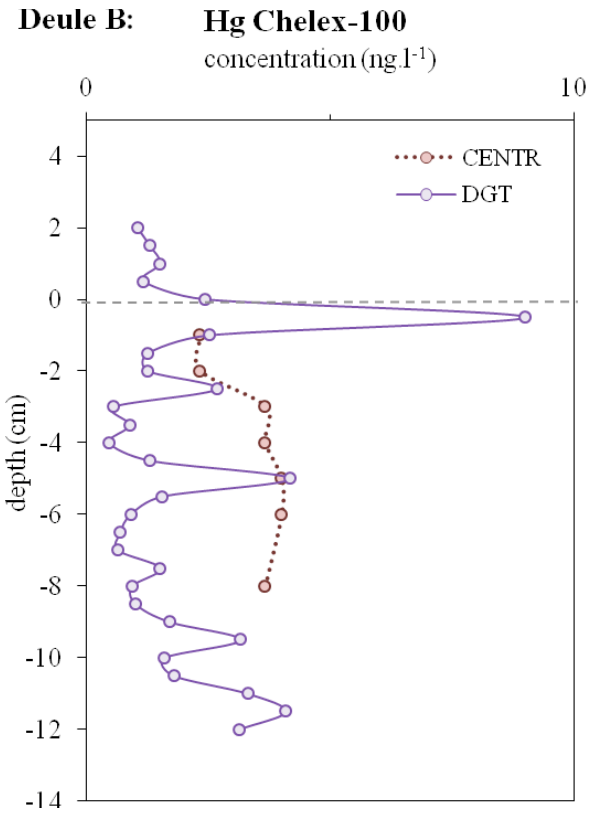
The results in this yield study prove that the sorbent with titanium dioxide cannot be used in the measurement in the real aquatic environment. The sorption is different due to the content of an inorganic as well as organic substances in the aquatic systems compared to the model solution prepared in the laboratory. These substances can be bound to the active place of titanium dioxide strongly than the Hg. Because of different binding efficiencies of the TiO_2 resins compared e.g. Spheron-Thiol or Duolit GT-73, the concentration of Hg in pore water as measured by the TiO_2 is lower and comparable with Hg in pore water measured by Chelex-100. TiO_2 resin gels is only able to react with weak complexes of mercury, as hydrated mercury ions and mercury bonded in labile inorganic and organic complexes.

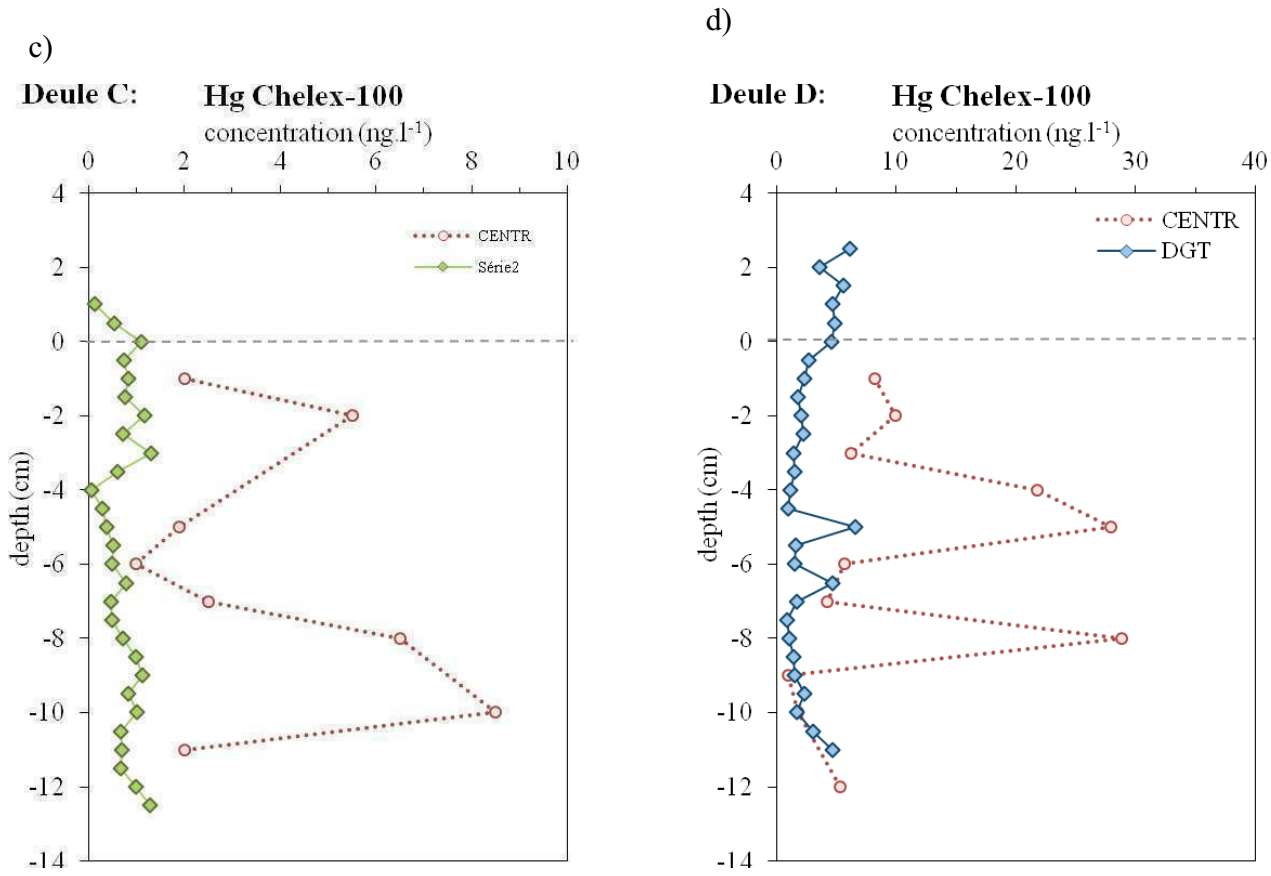
Figure V.10: *The depth profiles of mercury in pore water measured by the Chelex-100 DGT probe and obtained using centrifugation at the sampling place a) Deûle A, b) Deûle B, c) La Lys C, d) La Lys D.*

a)



b)





Blanks for DGT gels (Hg in Duolit GT-73, Spheron-Thiol, TiO_2, and Chelex-100, Fe, Mn in Chelex-100) as well as blanks for DET gels (Fe, Mn) were determined. Five strips of prepared DGT gels (5 mm) and ten strips of prepared DET agarose gel (2 mm) without exposition of metals were used for blanks determination. Comparing blanks concentrations of Fe and Mn with metals (Fe, Mn) concentration ranges observed in the sediment cores, the concentrations are almost always higher in the sediment than the blanks. Blanks for DGT and DET gels and concentration of Fe and Mn are shown in ***table V.5***. Blanks were calculated using deployment time of 24 h.

Table V.5.: *The average DGT and DET metals blanks concentrations compared to range concentration in the sediment pore water.*

	DGT ($\mu g.L^{-1}$)		DET ($\mu g.L^{-1}$)	
	blank	Sedim.Range	blank	Sedim.Range
Fe	1,78 ± 0,24	30 – 12 220	237 ± 46	< dl - 269 210
Mn	0,053 ± 0,011	20 – 780	3,21 ± 0,32	31 – 13 570

The observed concentration of Hg in the prepared resin gels - in the discs are summarized in ***table V.2***. The Hg blank concentrations in the DGT are quite small when compared the concentration Hg in the sediments. The blank concentrations of Hg in strips measured by Duolit GT-73 DGT was 0,045 ng.cm^{-1}, by Chelex-100 was 0,040 ng. cm^{-1}, by Spheron-Thiol was 0,172 ng. cm^{-1} and by TiO_2 was 0,039 ng. cm^{-1}

Sediment depth profiles: Deûle A - Deuel B:

The content of Hg at **Deûle A** (*table V.3*) decreasing:

$HgT_{sed} > HgT_{ins} > HgT_{SW} >$ *Hg DGT (Duolit GT-73)* $> HgT_{PW} >$ *HgT DGT (Spheron-Thiol) > HgT DGT (Chelex)*

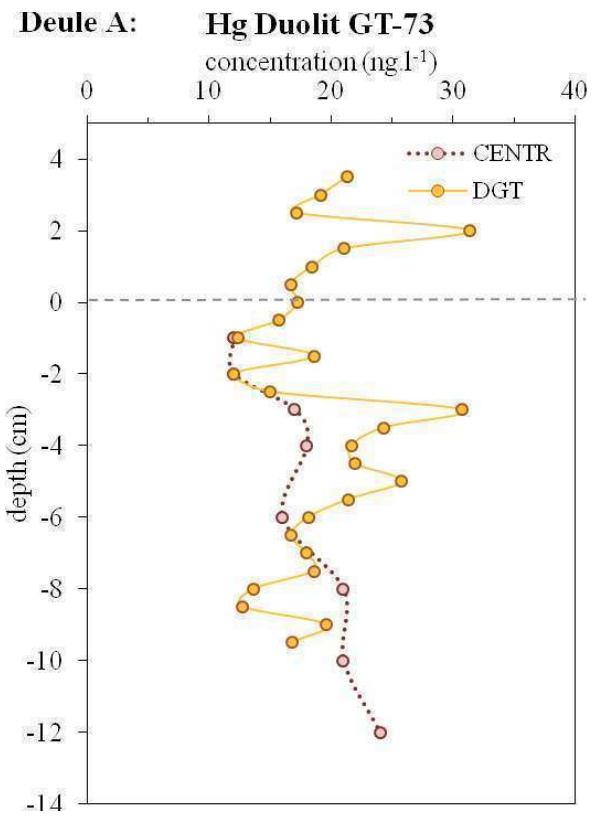
Because of expected high mercury contamination (**Chapter III**.), the chosen deployment time of 48 hours was chosen, with a view on the obtained results.

Th

e deployment of the six different DGT probes, containing either Spheron-Thiol or Duolit GT-73 or Chelex-100 resin resulted in the concentration depth profiles of mercury with similar trends but concentration scales vary (***figure V.11 a) – b) figure V.10 a)***) The concentration of mercury in pore water as measured by the Chelex-100 (range from 3,54 to 33,2 ng.L^{-1}, average 9,98 ± 7,15 ng-L^{-1}) is half of concentration measured by the Duolit GT-73 (range from 12,0 to 31,3 ng.L^{-1}; average 19,12 ± 4,74 ng-L^{-1}) and Spheron-Thiol (range from 11,9 to 32,0 ng.L^{-1}; average 17,69 ± 4,64 ng-L^{-1}).

Figure V.11: *The depth profiles of mercury in pore water measured by the Duolit GT-73 (a) and Spheron-Thiol (b) DGT probe and obtained using centrifugation at the sampling place Deûle A.*

a)



b)

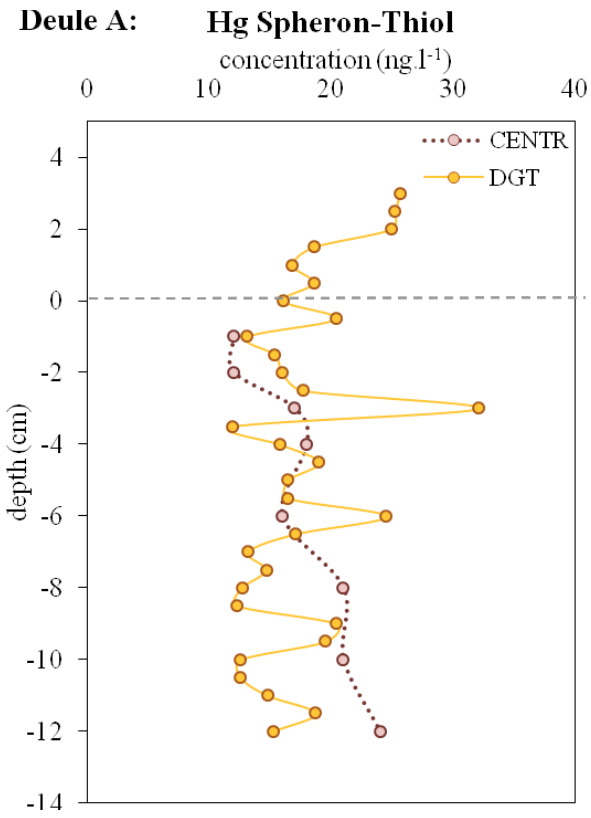
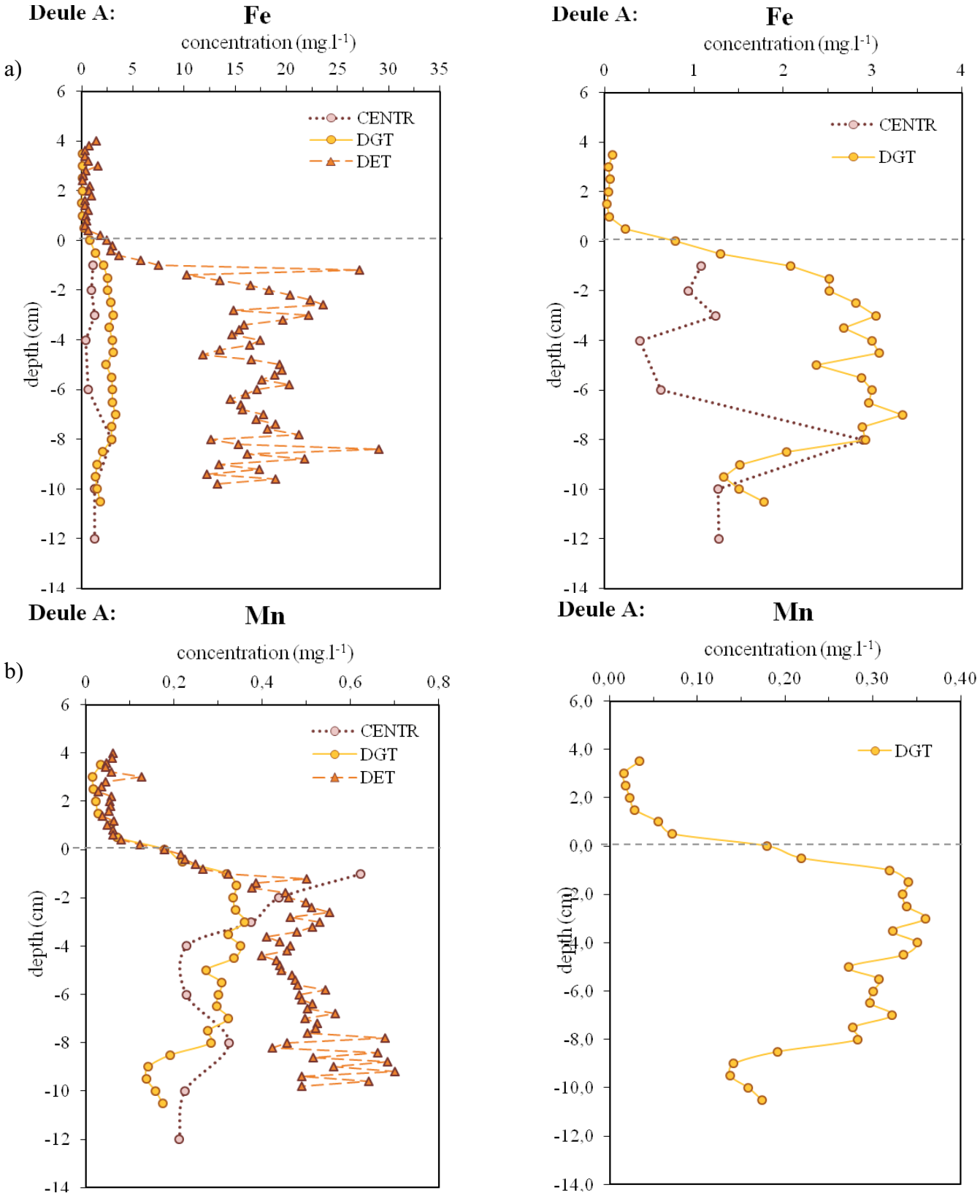


Figure V.12: *The depth profiles of a)Fe and b) Mn at the Deûle A*



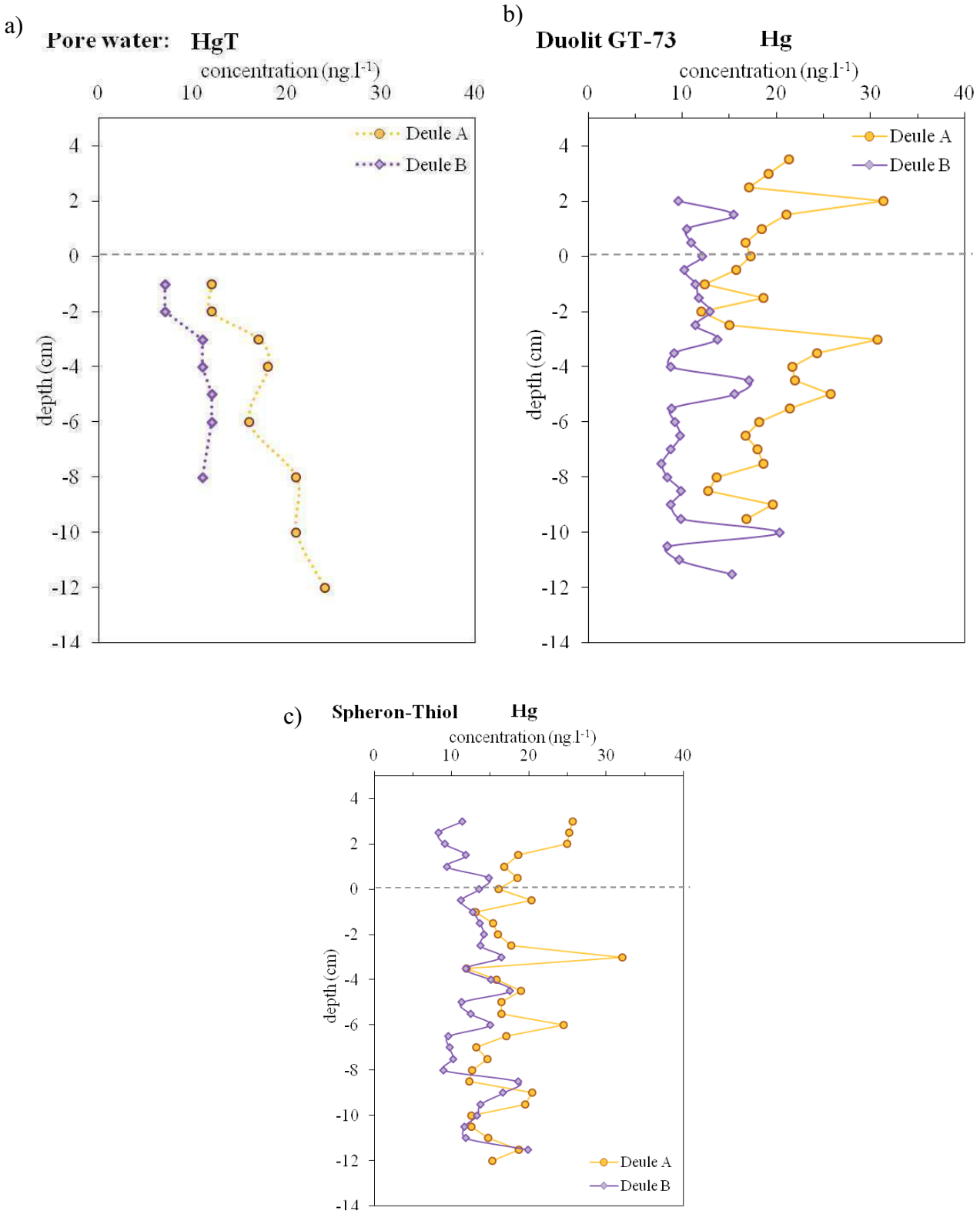
The depth profiles of Fe and Mn follow the same shape profile. Both increased from a low value at the sediment-water interface to reach the high concentration of DGT- and DET-measured Fe and Mn in the first centimeters of the sediment depth. DGT measured concentrations were lower compared DET measured concentration. The concentration of Fe obtained by the DGT technique is slightly higher than classic centrifugation technique; the concentration of Mn is comparable.

The content of Hg at **Deûle B** (***table V.4***) decreasing:

$$HgT_{sed} > HgT_{ins} > \text{Hg DGT (Duolit GT-73)} > \text{HgT DGT (Spheron-Thiol)} > HgT_{PW} > HgT_{SW} > \text{HgT DGT (Chelex)}$$

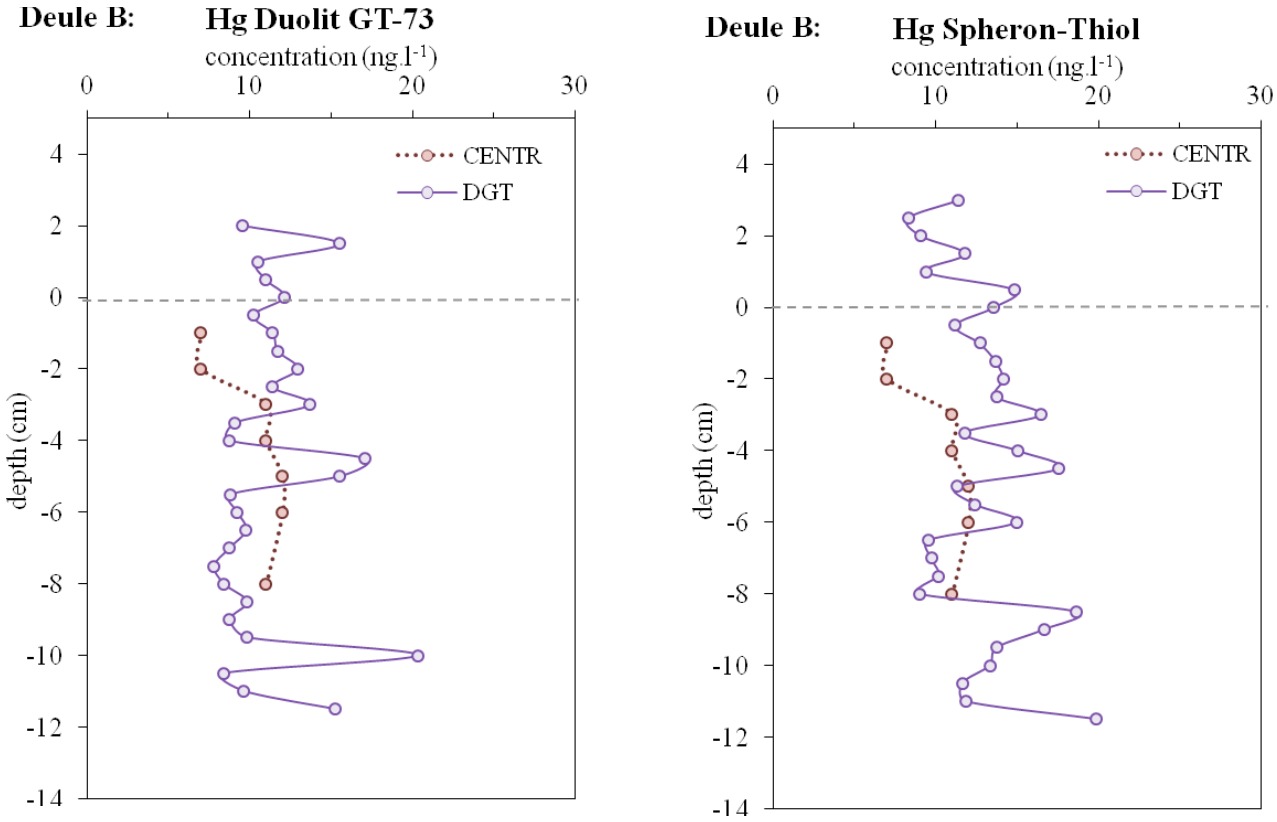
The deployment time was similar as at sampling site Deûle A (48 hours), but the sampling was carry out differently because of inaccessibility of the river bank. The samples were taken from the middle of the stream using the boat during the sampling. Nevertheless the depth profiles of mercury concentration in pore water obtained using centrifugation as well as measured by the Duolit GT-73 or Spheron-Thiol DGT probes at the sampling place A and B are more or less similar at the first 6 cm (***figure V.13 a) b)c)***).

Figure V.13: *Hg concentration measured in the a) pore water, b) byDuolit GT-73 and c) Spheron-Thiol*



The six DGT probes containing either Spheron-Thiol or Duolit GT-73 or Chelex-100 resin were deployed into the sediments. The concentration of mercury in pore water as measured by the Chelex-100 (range from 0,47 to 9,02 ng.L^{-1}, average 2,03 ± 1,68 ng-L^{-1}) is five times lower than concentration measured by the Duolit GT-73 (range from 7,80 to 20,31 ng.L^{-1}; average 11,26 ± 3,01 ng-L^{-1}) and Spheron-Thiol (range from 8,98 to 19,88 ng.L^{-1}; average 11,73 ± 2,90 ng-L^{-1}).

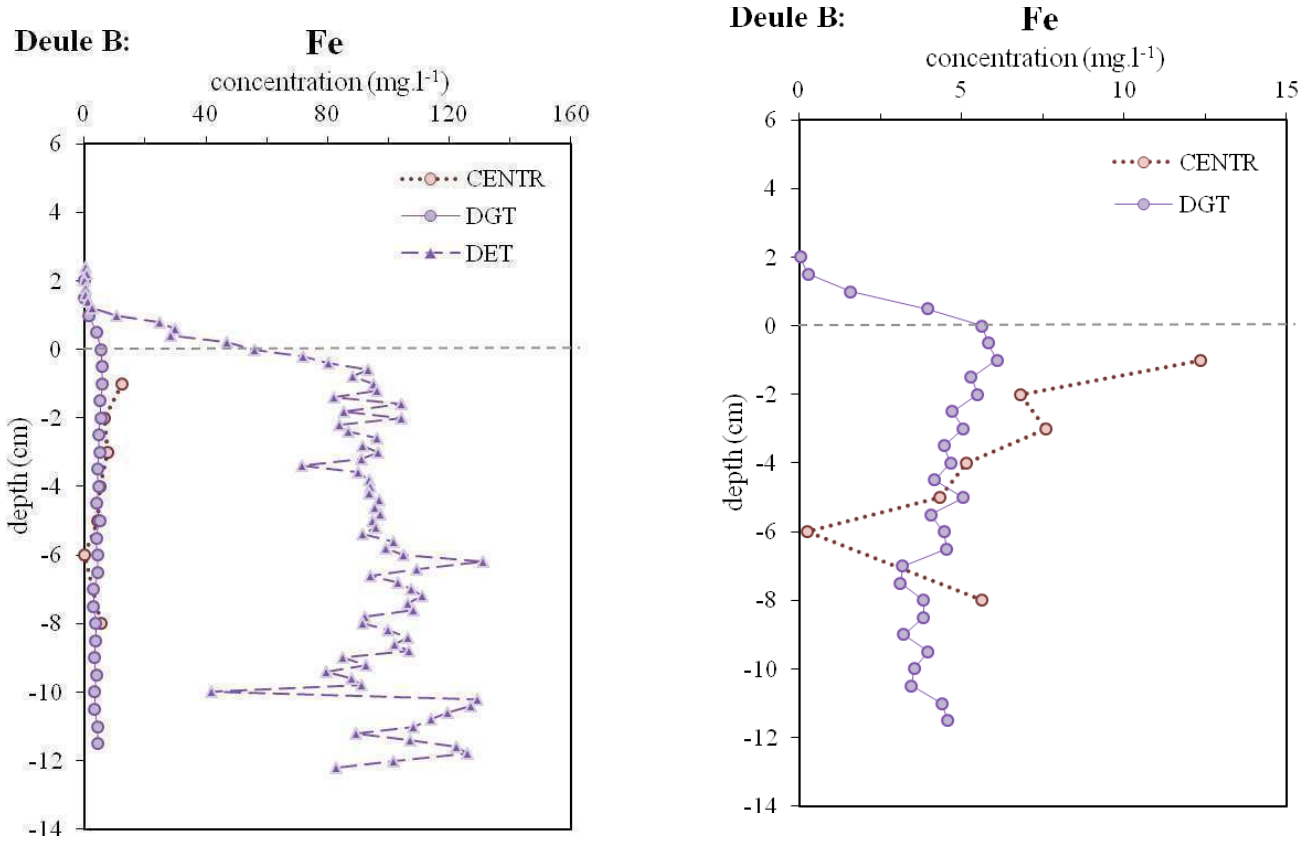
Figure V.14: *The depth profiles of mercury in pore water measured by the Duolit GT-73 (a) and Spheron-Thiol (b) DGT probe and obtained using centrifugation at the sampling place Deûle A.*

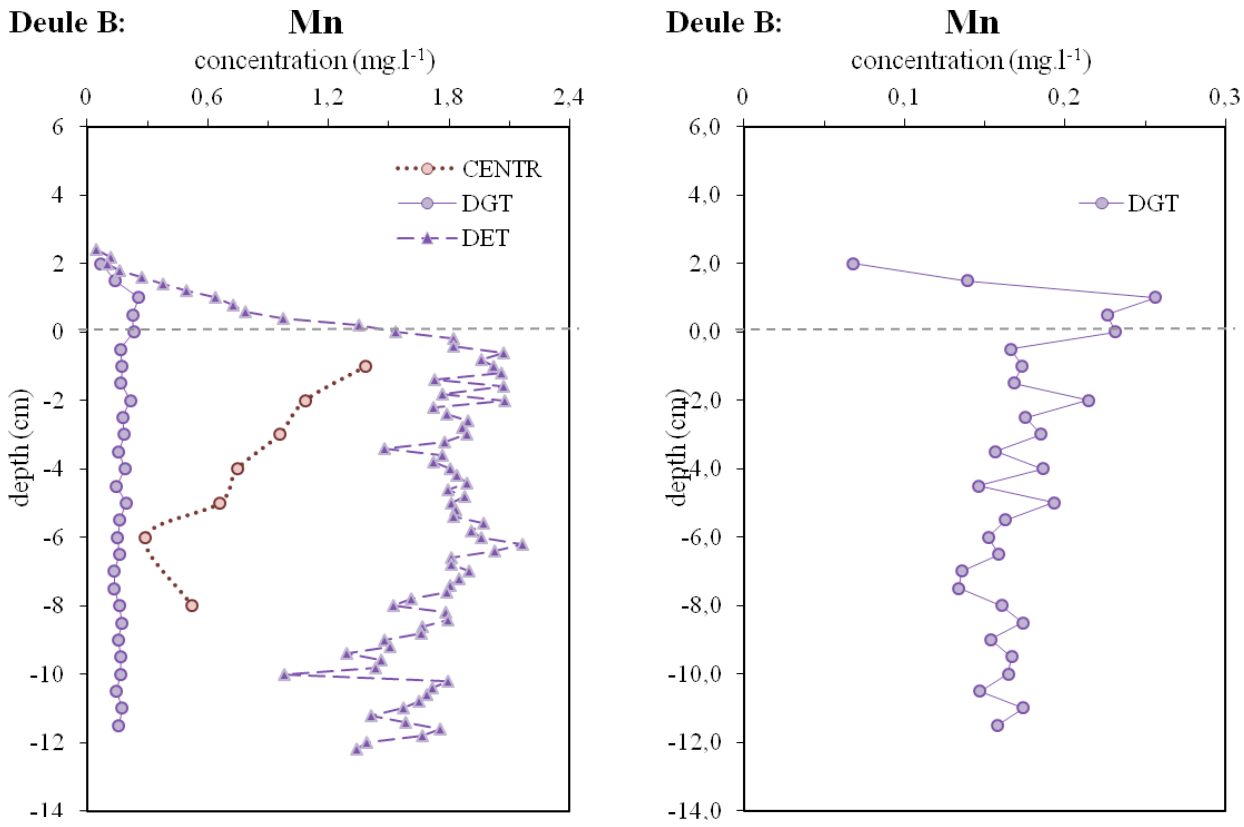


In the both sampling sites (Deûle A and B), Mn and Fe measured by DET and DGT increased sharply very close to the sediment-water interface. The depth profiles of Fe and Mn follow the same shape profile. Both increased from a low value at the sediment-water interface to reach the high concentration of DGT- and DET-measured Fe and Mn in the first centimeters of the sediment depth. While the concentration measured after centrifugation was

highest at the surface sediment layers. Mn concentration measured after centrifugation was highest at the first sediment layer and decrease with the sediment depth. Concentrations of Fe measured by DET were higher than mean pore water concentration measured after centrifugation and DGT measured concentrations, by a factor approaching 13 and 20 respectively. The similarity was observed for Mn where concentration measured by DET were higher than mean pore water concentration measured after centrifugation and DGT measured concentrations, by a factor approaching 2 and 10 respectively (***figure V.15. b)***).

Figure V.15: *The depth profiles of a)Fe and b) Mn at the Deûle B*





Sediment depth profiles: La Lys C - La Lys D:

The content of Hg at **La Lys C** (***table V.4***) decreasing:

$$HgT_{sed} > HgT_{SW} > HgT_{ins} > HgT_{PW} > Hg\ DGT\ (Duolit\ GT\text{-}73) > HgT\ DGT\ (TiO_2) > HgT\ DGT\ (Chelex)$$

Because of expected lowest concentration of the mercury contamination, the deployment time of 96 hours was chosen, with a view on the obtained results.

The deployment of the six different DGT probes, containing either TiO_2 or Duolit GT-73 or Chelex-100 resin resulted in the concentration depth profiles of mercury with similar trends but concentration scales vary (***figure V.16 a) – b) figure V.10 c)***) The concentration of mercury in pore water as measured by the Chelex-100 (range from 0,057 to 1,31 $ng.L^{-1}$,

average 0,71 ± 0,30 ng-L^{-1}) is more or less equal the concentration measured by TiO_2 (range from 0,41 to 2,13 ng.L^{-1}; average 0,863 ± 0,31 ng-L^{-1}), but the four times lower than concentration measured by the Duolit GT-73 (range from 1,10 to 4,44 ng.L^{-1}; average 2,93 ± 0,84 ng-L^{-1}).

The depth profiles of Fe increased from a low value at the sediment-water interface to reach the high concentration of DGT- and DET-measured Fe concentration in the deeper sediment layer. The Mn concentration is highest at the sediment-water interface. DGT measured concentrations were also lower compared DET measured concentration. The concentration of Fe and Mn obtained by the DGT technique are comparable to classic centrifugation technique.

Figure V.16: *The depth profiles of mercury in pore water measured by the Duolit GT-73 (a) and TiO_2 (b) DGT probe and obtained using centrifugation at the sampling place Deûle A.*

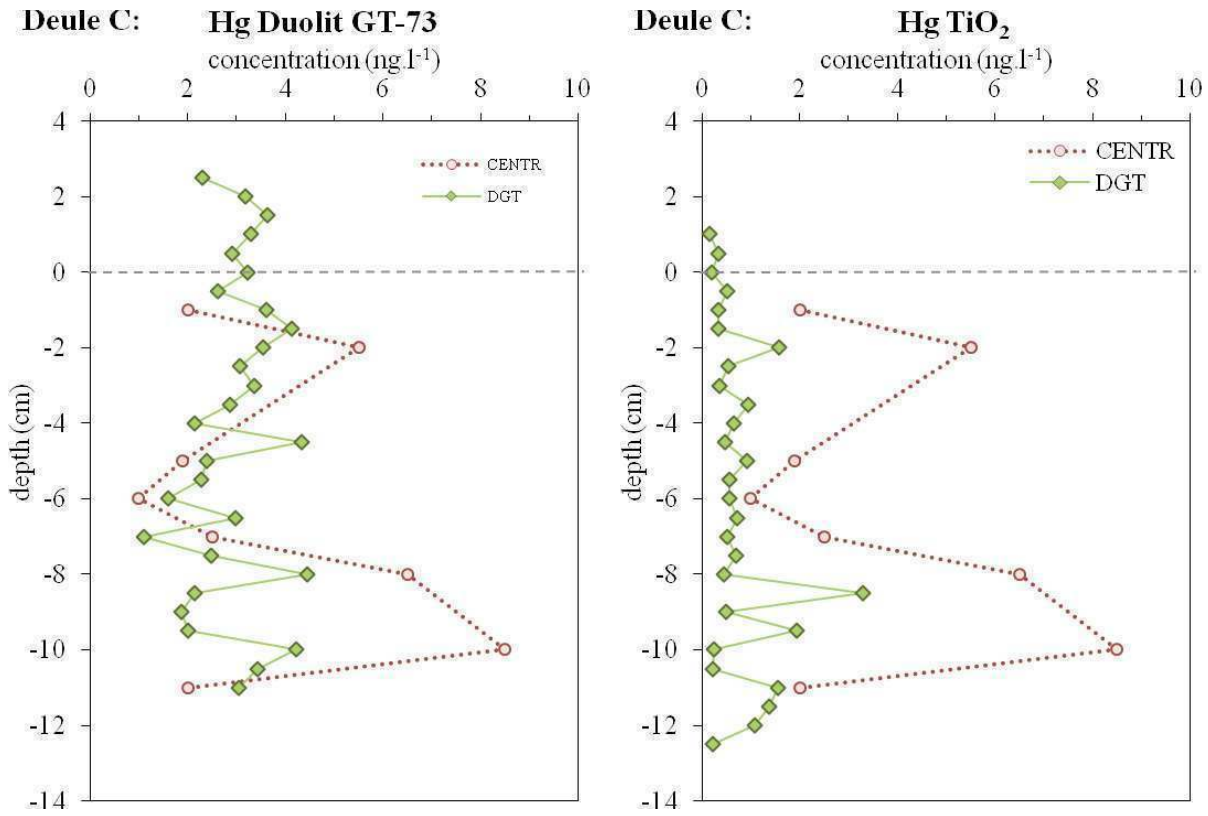
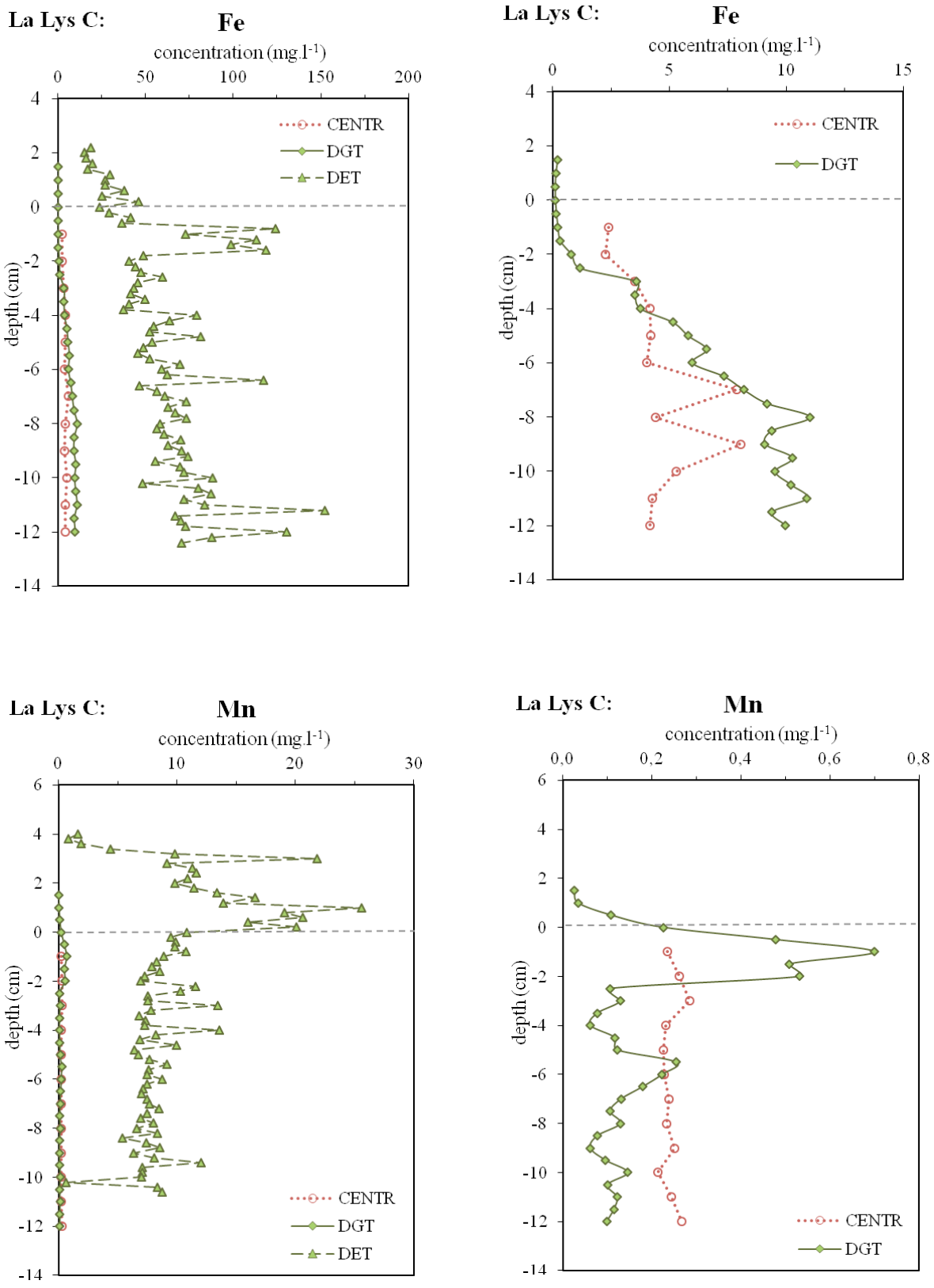


Figure V.17: *The depth profiles of a)Fe and b) Mn at the La Lys C*

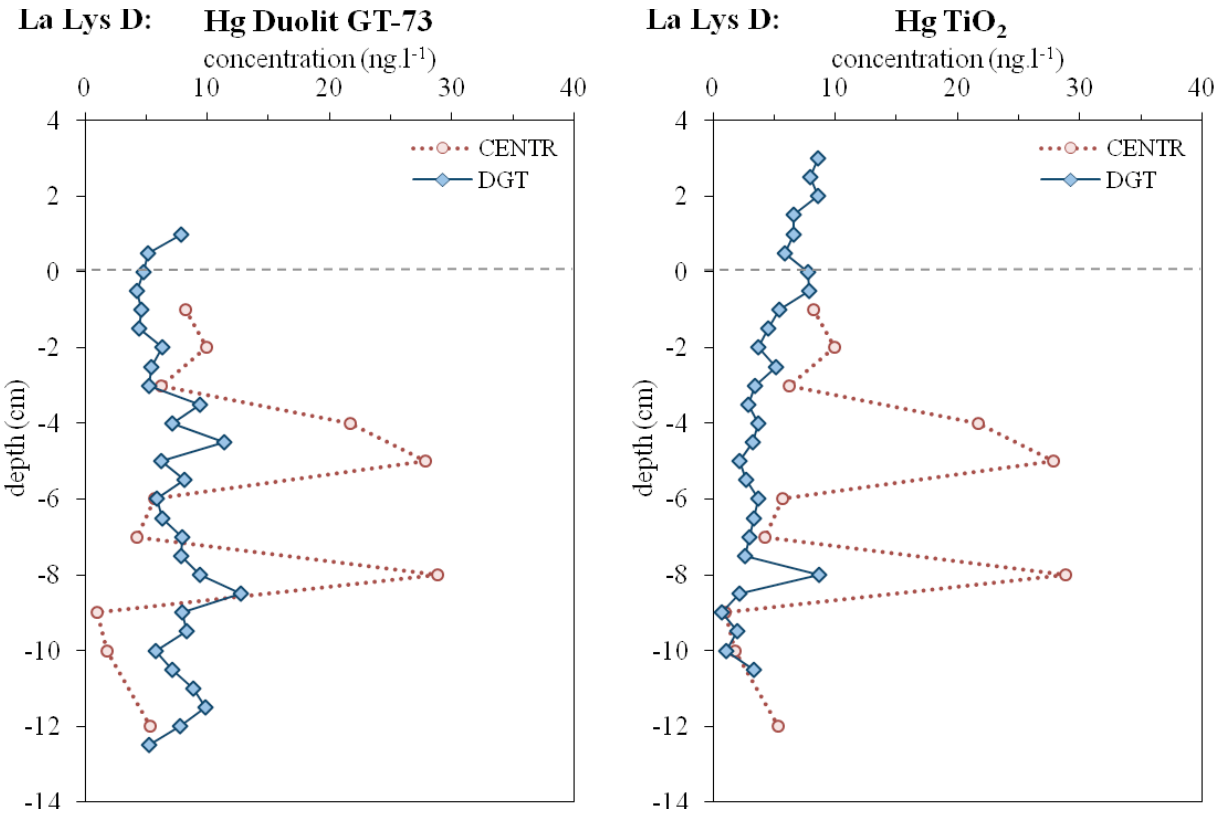


The content of Hg at **La Lys D** (***table V.4***) decreasing:

HgT_{sed} > HgT_{ins} > HgT_{SW} > HgT_{PW} > Hg DGT (Duolit GT-73) > HgT DGT (TiO_2) > HgT DGT (Chelex)

The deployment time of 96 hours was chosen. The concentration depth profiles of mercury are shown on the ***figure V.18 a) – b)***

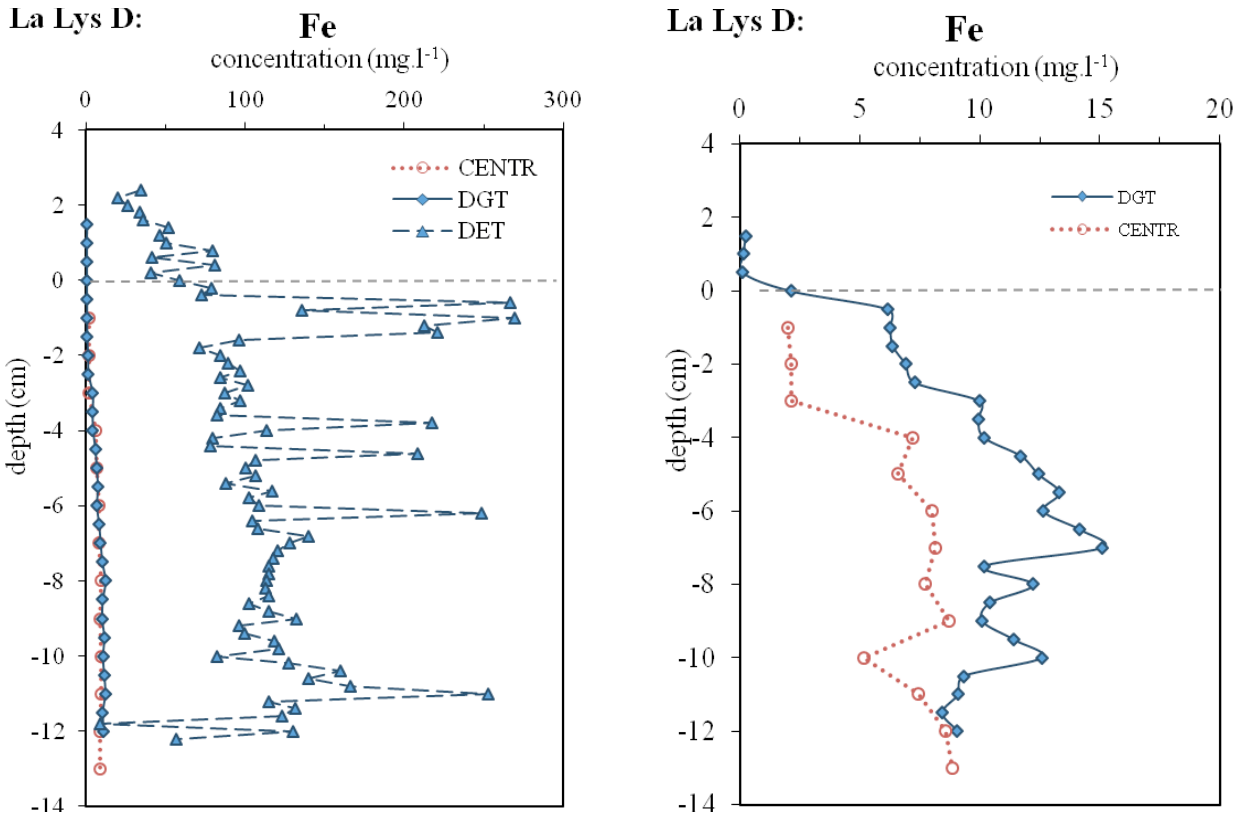
Figure V.18: *The depth profiles of mercury in pore water measured by the Duolit GT-73 (a) and TiO_2 (b) DGT probe and obtained using centrifugation at the sampling place La Lys D.*



The average concentration of mercury in pore water as measured by the Chelex-100 is 2,74 ± 1,67 ng-L^{-1} (range from 0,89 to 6,59 ng.L^{-1}) and by TiO_2 is 4,56 ± 2,51 ng-L^{-1} (range from 0,73 to 8,63 ng.L^{-1}). The concentration measured by the Duolit GT-73 is around two times higher (range from 4,24 to 12,72 ng.L^{-1}; average 7,27 ± 2,09 ng-L^{-1}).

The concentration depth profiles of Fe and Mn are shown on the *figure xxx a) – d).* The average Fe concentration measured by DGT technique, DET technique or classic centrifugation technique was highest compared the other sampling place (table xxx).

Figure V.19: *The depth profiles of a)Fe and b) Mn at the La Lys D*



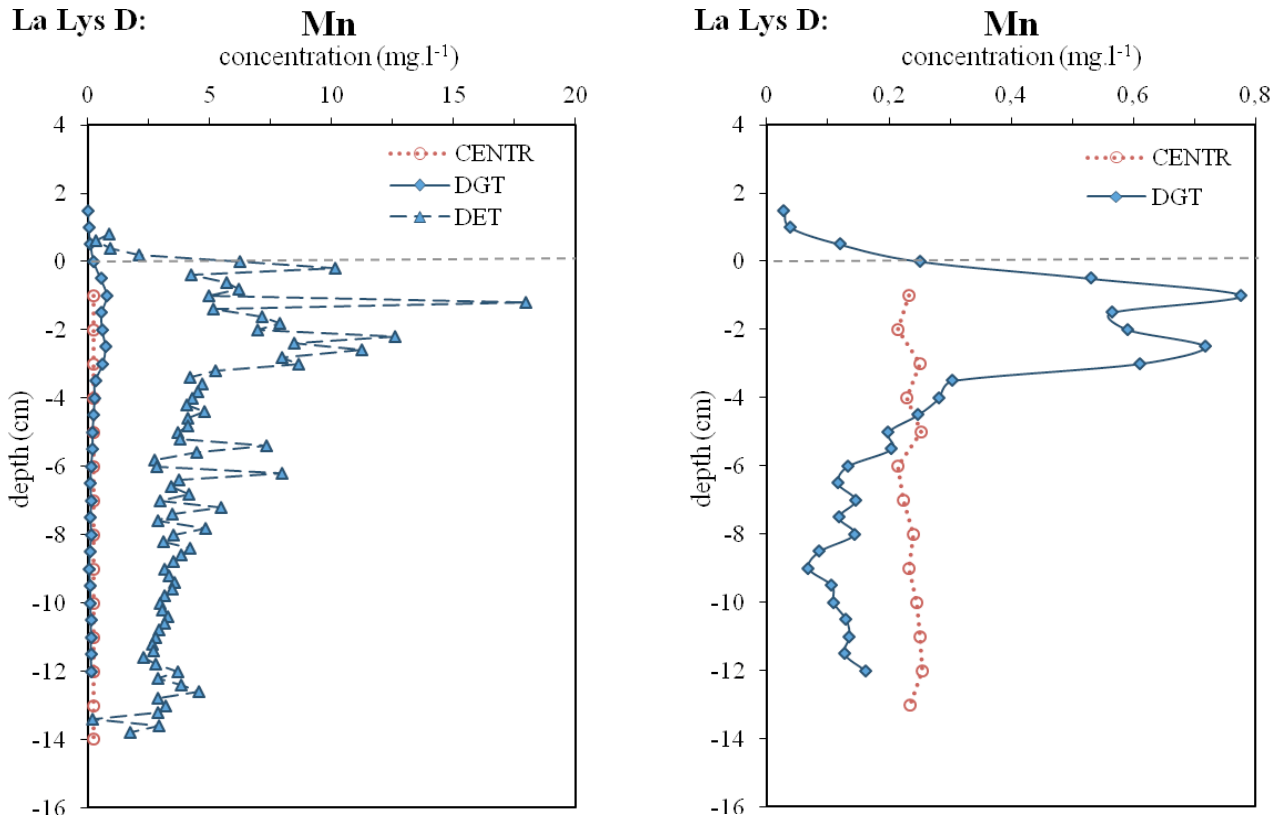


***Table V.6**: Average concentration of Fe and Mn in the pore water of each sampling sites:*

Sampling site	Fe			Mn		
	Centr.	DET	DGT	Centr.	DET	DGT
Deule A	1,22 ± 0,70	11,5 ± 8,36	1,82 ± 1,16	0,33 ± 0,13	0,35 ± 0,21	0,21 ± 0,12
Deule B	6,01 ± 3,38	83,0 ± 34,8	4,01 ± 1,42	0,81 ± 0,34	1,54 ± 0,52	0,17 ± 0,03
La Lys C	4,55 ± 1,07	66,6 ± 24,8	5,43 ± 4,04	0,24 ± 0,02	8,13 ± 1,97	0,18 ± 0,16
La Lys D	6,52 ± 2,51	120 ± 52	6,03 ± 4,49	0,23 ± 0,01	3,62 ± 1,17	0,25 ± 0,21

DGT measured Fe depth profile show the similarity with the centrifugation depth profiles. The low values were observed at the sediment water interface and highest concentration around 8 – 9 cm of sediment depth. DET measured Fe depth profile is around

20 times higher. DGT and DET measured Mn depth profiles show the highest values at the first centimeters of surface sediment while the centrifugation depth profile is fairly uniform within the sediment depth.

Disscusion

The redox potential (**Chapter III**) shown a completely anoxic environment for the sediment of Lys River. The values of Eh in the cores of Deule River at the surface sediments were about 0 mV and rapidly decrease. The anoxic environment is evident. Such conditions point out the increase or production of iron(II) and manganese(II) – redox sensitive elements. The higher concentrations of Fe and Mn for both DET and DGT samplers can be found in the sediment cores compared the concentration in the overlying water column (***figure V.12, V.15, V.17 and V.19***). However high concentration of AVS and CRS in sediment cores corresponds to the lower levels of dissolved Fe and Mn in pore water (***figure III. 14 a)-d)***) of such anoxic sediment compared to the aerobic sediment. Also the low values of the AVS/CRS ratio shows the high degree of sulfides conversion to pyrite.

The difference DET measurements of Fe and Mn compared to measurements by the classic centrifugation technique may simply reflect heterogeneity, the possibility that DET overestimates Fe and Mn at depth, or that there was some loss of Fe from the pore waters due to oxidation of dissolved Fe(II) into solid Fe(III), cannot be excluded. Fe could possibly bind to the agarose gel or there could be some in situ oxidation. Although the probes were deployed in an oxygen-free environment prior to deployment, traces of oxygen would be taken up as they were quickly transferred to and inserted in the sediment cores.

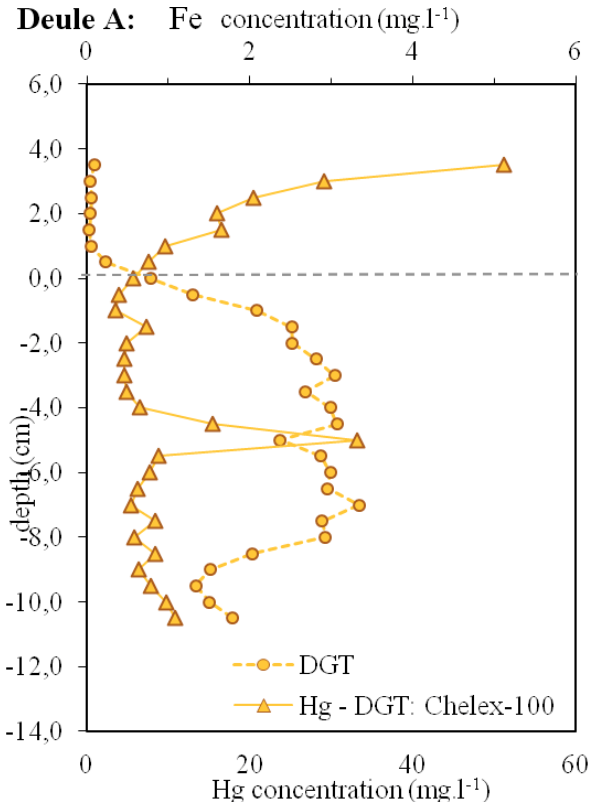
Comparison between the centrifugation technique and DGT technique should be carried out with caution. DGT measures the local concentration in a small volume of sediment close to the DGT probe, whereas classical centrifugation techniques measure bulk water concentration average from whole bulk pore water in the sediment layer.

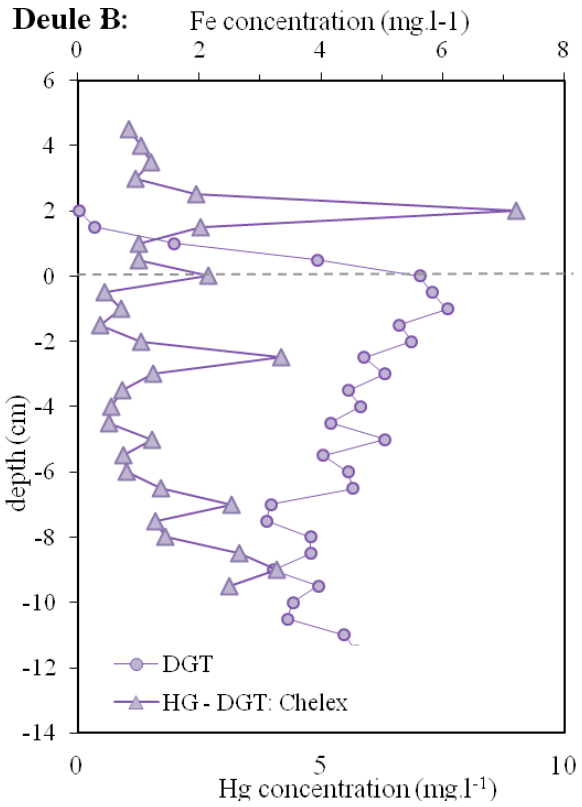
The differences between DET and DGT can be due to the pore size of the gels (20 nm for DET and 5 nm for DGT) or due to competition between the DGT resin and complexing ligands when metal complexes are abundant in the pore water. DGT technique measured only the labile part of metals (simple metal ions, hydrated ions and labile complexes) while DET

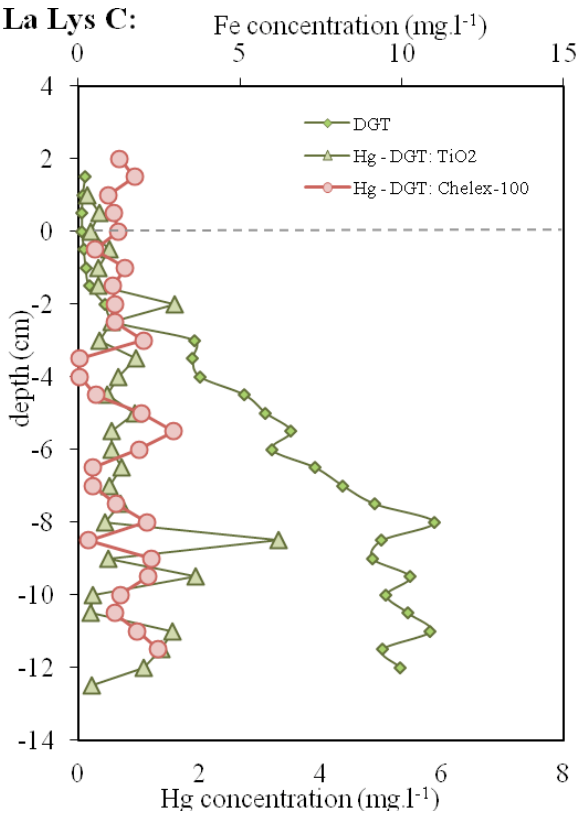
measured the whole dissolved concentration of the metal (free ions as well as small anorganic and organic complexes). The average DGT/DET ratios observed are following: at Deule A 1/2 for Mn and for 1/6 Fe; at Deule B 1/10 for Mn and 1/31 for Fe, at La Lys C 1/45 for Mn and 1/12 for Fe; at La Lys D 1/14 for Mn and 1/20 for Fe. At Deule A, DET and DGT profiles are thus most close to each other. The highest Fe and Mn labile fraction is observed in the Deule A sediments.

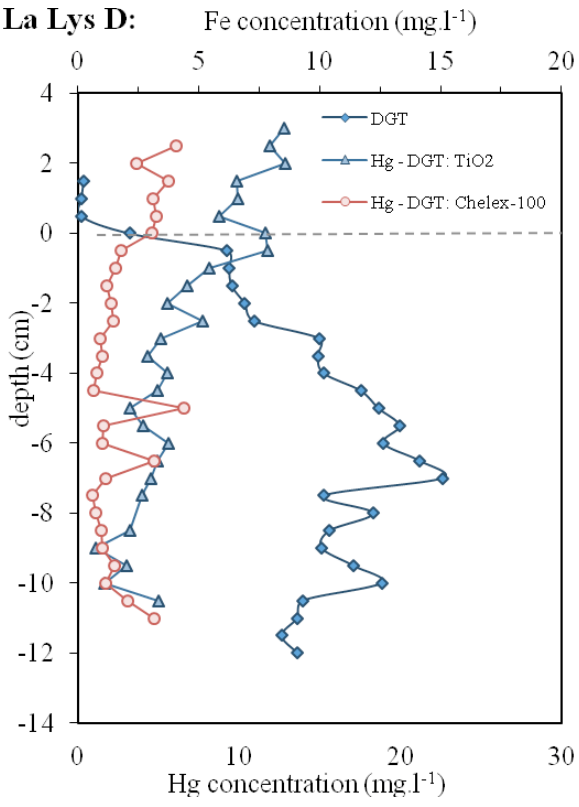
Dissolved Fe and Mn (measured by DET) in pore waters exhibited a classic profile of sharp increase in upper layers of the sediment cores. Mn and Fe DET profiles are very similar with exception of the site La Lys C where the maximum of Mn is located in the surface water in the 0,8 cm up to the sediment layer, while the maximum of Fe is located in the 1 cm of sediment depth. At the Deûle A and La Lys D sites Mn and Fe DET profiles are characterized by a very large gradient at the surface sediment while at the Deûle B the gradient starts already in the surface water (around 1 cm up to the sediment layer). Fe and Mn oxides in sediment influence the concentration of trace metals including also Hg in sediments. The amount of oxidants in the surface sediments is decreasing with sediment depth. The oxic zone becomes anoxic in the first centimeters of sediment depth. The reduction of iron and manganese oxides liberate dissolved Fe(II) and Mn(II), which diffuse through the gel. Distribution of dissolved Fe appears to be restricted by sulfide, possible due to the precipitation of FeS. The FeS formation may be responsible for the decrease in dissolved Hg. The study with anoxic sediment slurries made by Han (2008) investigate that at low Fe(II), dissolved Hg increased, but at high Fe(II), dissolved Hg decreased markedly, which could be caused by sulfide removal. The depth profile of the Fe measured by the DGT with Chelex-100 (and TiO_2 at the sites La Lys C and La Lys D) are shown on the figure ***V.20 a) – d)***.

Figure V.20: *The depth profiles of labile part of Fe measured by the DGT and labile part of Hg species measured by the Chelex-100 resin at the site a) Deule A and b) Deule B and measured by the Chelex-100 resin and TiO_2 adsorbent at the site c) La Lys C and d) La Lys D*









V. III. 5. Conclusions

A resin with thiol groups presents the best choice for total mercury determination while the TiO_2 measured only the labile species of Hg (like Chelex-100). The good agreement was observed between the concentration of Hg in pore water and concentration measured by the Duolit GT-73 or Spheron-Thiol. Because the Spheron-Thiol is no longer on the market and Duolit GT-73 required the laboratory pretreatment before the gel preparation, the alternatives should be sought.

When compared the sampling sites not Deule B site (located in the vicinity of the pollution) but the site La Lys D seems to be the most contaminated. The concentration found at this place was 120 $mg.l^{-1}$. Quite fast change from the oxic zone to the anoxic zone was observed in the sediment profile of the all sampling places (the gradual was in the sediment profile of La Lys C). While oxic zone change to the anoxic zone the decrease of oxygen is characteristic till the whole depletion. In the anoxic sediment hydrated oxid of Fe and Mn are reduced to the Fe(II), Mn(II) and dissolved. Meanwhile the batteries "consuming" the sulfates and produce the sulfides. The high concentration of AVS and CRS was found in the investigated sediments. Sulfides control the dissolved Fe, as a consequence of binding to the FeS. The production of FeS may influenced the reduction of the dissolved mercury. Thus these high concentration of sulfides decrease the amount of bio(available) mercury, which can be measured by the DGT with TiO_2 and Chelex-100. At the site Deule A and La Lys D the higher concentration of the labile species mercury was observed compared to the La Lys C and Deule B.

It is not evident which site is the most contaminated, nevertheless the Hg concentrations exceed the background values given for this sites. We can assume that the elevated concentration of Hg in sediment of the studied places may occur due to the past release of effluents from the smelter factory Metaleurop. Nowadays the sediment act both as a

sinks and secondary source of Hg. The concentrations found in the surface water and in the water particles are higher than the pore water concentration. However the lower pore waters Hg contents may be continuously release by the redistribution of the sediment due to the extensive boat traffic on the Deule River. The high concentrations of Hg found at the place La Lys D suggest the transport of polluted sediment from the source of contamination and redistribution of the sediments.

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CONCLUSIONS AND PROSPECTS

This work on the *Mercury Contamination of Rivers Sediments in the Northern France and Czech Republic* reports on the field study of some chosen sites in northern France, which are considered to be the contaminated and the sites in the Czech Republic, considered as uncontaminated. The aim of the study is development of DGT technique for Hg measurements and its application in the aquatic environment (sediment). The one of the goal is also the evaluated of the endogenous and exogenous Hg species transformation of the highly contaminated sediments.

The theoretical part of the thesis briefly reviewed the properties of mercury and summarized information about mercury cycle, transport and speciation processes in the environment.

The first achievement of the work was established the automated Headspace method coupled with the Gas Chromatography and Atomic Fluorescence Detector for determination of MeHg in the sediment samples to the common laboratory routine. The optimal conditions of several parameters were found during the optimization procedure by varying one parameter while holding the other constant.

The second part of work consists from the comparison of sampling sites in France (heavy industrial region) and site in Czech Republic, more precisely at the Moravia region (rather agriculatural land, without extensive industry compared the Northern France). Sediments in France have different compositions, are deeper and are extensively contaminated by the mercury. Because the conditions of bed stream disable the sediment cores sampling in the Czech Republic just the surface sediment were investigated. The

concentration of Hg in sediments extensively exceeded the background values given by the Northern France by Agence de l´Eau (0,1 $mg.kg^{-1}$). While the background values are not known for the Moravia Region, the contamination were also compared with the Global background concentration (0,4 $mg.kg^{-1}$). The concentration was from 20 to 1000 times higher in the surface sediments of Deûle and Lys River in Northern France and from 3 to 300 times higher in the surface sediments of Morava and Jihlava River in the Moravia Region.

The third part of study reports on behaviors of natural (endogenous) and added (exogenous) Hg species (inorganic mercury and methylmercury) in the highly contaminated sediments of Deûle River. The results indicate similar behaviors towards demethylation. The geochemical characteristics suggest that the sediments are mostly anoxic. The methylation seems to be controlled by the CRS concentrations (mostly pyrite). On the other hand no correlation was observed between the AVS concentration and methylation yield and AVS or CRS concentration and demethylation yield. Net methylation was assumed (the endogenous and exogenous Hg methylation was compared) and the ratio M/D was calculated. From the ratio M/D, the methylation zone was pattern at the point where the demethylation yield was lower. Methylation wasn´t located at the surface or subsurface sediment but deeper (around the -12 cm sediment depth at the site I. Metaleurop; around -10 cm and -15 cm sediment depth at the site II. Deûle – Amont). Nevertheless the net methylation is dependent not only on the methylation yield, but also on the demethylation yield. And demethylation yield is in the sediments predominant.

Because the maximum production of MeHg is not located to the surface sediment layer, in terms of possible remobilization of the MeHg to the water column and the other compartments the MeHg is highly cached.

The Metaleurop factory should be considered like a certain agent responsible for the mercury contamination and its environmental implications of the Hg fate for this contaminated site. Nowadays the sediments act as a sink for Hg pollution but if the river sediment will be dredge up (to preserve the navigability of the Deûle River) the Hg may be remobilized and thus sediment may act as a source of the pollution. Moreover despite the fact that the Metaleurop has been closed since 2003, Hg is still located at the environmental surrounding and could be transported over the hundred kilometers to the sea.

Finally this work contributed to the new findings on the field of Hg measurement using DGT technique.The recently developed Diffusion Gradient in Thin-films (DGT) technique is based on a simple device that accumulates metal in situ, over time in a binding gel. The different gels used in DGT technique enables the determination of different mercury species. DGT devices equipped with Chelex-100 or TiO_2 were able to measure labile part of Hg species in the sediment pore waters while when the resin with –SH function group is used, the total mercury in the pore waters of sediment can be determined. The application of DGT technique using the specific ion-exchange resin, Chelex-100, Spheron-Thiol, Duolite and adsorbent TiO_2, provided the assessment of mercury in sediment pore water. DGT and DET techniques together with centrifugation provided also high resolution depth profiles of Fe and Mn in pore water. It is not possible to exactly establish which sampling site was more contaminated. Nevertheless the Hg concentration exceeds the background value at all these sites. Thus the Deûle River as well as La Lys River can be classified as contaminated rivers. The contamination of Hg in La Lys River can by caused by transport of Hg with particles from the sites located near the place affected by the contamination. Correlation between mercury species and sulfides content confirm that total mercury and sulfides content count among the factors influencing the mercury release to pore water and controlling the processes of methylation and demethylation.

The DGT technique emerged the tool capable of measuring the (bio)available part of Hg in the pore water of river sediment. Potentially, one of the most important applications of DGT will be the measurement of methylmercury to assess the (bio)available part of MeHg in sediments. For this purpose the new resin gel as well as the elution protocol for methylmercury has to be developed, optimized and validated.

ZÁVĚRY

Předkládáná dizertační práce na téma *Mercury Contamination of Rivers Sediments in the Northern France and Czech Republic* pojednává o terénní studii vybraných míst severní Francie, pro která je charakteristická dávná kontaminace rtutí, a míst v České republice, která jsou považována za nekontaminovaná. Jedním z cílů byl vývoj techniky difuzního gradientu v tenkém filmu (DGT) pro měření rtuti a aplikaci ve vodním prostředí (sedimentu). Dalším z cílů bylo také zhodnocení transformace endogenních a exogenních spécií rtuti ve vysoce rtutí kontaminovaném sedimentu.

Teoretická část teze pojednává o vlastnostech rtuti a shrnuje informace o cyklu rtuti v přírodě.

První část experimentální práce byla zaměřena na stanovení jednotlivých forem v sedimentech za užití etylace a zavedením automatické Headspace techniky (past Tenax), separací plynovou chromatografií s detekcí pomocí atomové fluorescenční spektroskopie s generováním studených par (CV-AFS: Cold Vapor Atomic Fluorescence Spectroscopy) do běžné laboratorní rutiny. Optimální podmínky pro analýzu byly nalezeny měněním jednoho z parametrů, zatímco ostatní parametry byly ponechány konstantní.

Druhá část experimentální práce se skládala z porovnání vzorkovacích míst ve Francii (regionu zasaženého těžkým průmyslem) a míst v České republice, přesněji na Moravě (kde převažuje spíše zemědělství a lehký průmysl ve srovnání s regionem v severní Francií). Sedimenty odebrané ve Francii mají zcela jiné složení, jsou hlubší a mnohem více znečištěné rtutí. Protože podmínky dna říčních toků v České republice neumožnili odebrání hloubkových sedimentů, byly odebrány a analyzovány pouze vzorky povrchového sedimentu. Koncentrace

rtuti v sedimentech extrémně překročili tzv. pozaďové hodnoty prostředí, které jsou pro Francii dané Agenturou na ochranu přírodních vod (0,1 $mg.kg^{-1}$). Protože pozaďové hodnoty pro Moravu nejsou známy, byly hodnoty porovnány také s Globálními pozaďovými koncentracemi (0,4 $mg.kg^{-1}$). Koncentrace v sedimentech byla 20 až 1000 krát vyšší v povrchovém sedimentu řek Deûle a Lys v severní Francii a 3 až 300 krát vyšší v řekách Jihlava a Morava v České republice.

Třetí část experimentální části se zabývá chování přírodních (endogenní) a přidaných (exogenních) spécií rtuti (anorganické rtuti a metylrtuti) v značně kontaminovaných sedimentech řeky Deûle. Výsledky poukazují na stejný chování spécií směrem k demetylačnímu procesu. Geochemická charakteristika ukazuje na anoxické prostředí v sedimentech. Metylace je spojena s převážně s pyritovou formou síry (koncentrace CRS). Žádná korelace nebyla pozorována mezi koncentrací AVS a metylací a AVS a/nebo CRS a demetylací. Na základě poměru M/D byla vyhodnocena tzv. čistá metylace. Produkční zóna metylrtuti byla pozorována v místech kde byla demetylace nejnižší. Zóna metylace nebyla nalezena v povrchovém či podpovrchovém sedimentu, ale hlouběji (kolem -12 cm hloubky v sedimentu místa I. Metaleurop; a kolem -10 cm a -15 cm hloubky v sedimentu místa II. Deûle – Amont). Nicméně čistá metylace je závislá nejenom na metylaci, ale i na demetylaci. A demetylace je v tomto sedimentu převažující.

Protože maximum produkce metylrtuti není na povrchu sedimentu, z hlediska možné remobilizace metylrtuti do vodního sloupce a dále do přírodního prostředí můžeme říct, že metylrtuť je v sedimentu přeážně zadržována.

Továrna Metaleurop je považována za původce znečištění rtutí v jejím okolí. V současnosti sedimenty řeky Deûle, která je v její bezprostřední blízkosti, toto znečištění převázně zadržují, ale v případě, že sedimenty budou vybagrovány (z důvodu zachování splavnosti řeky), rtuti může být remobilizována a sedimenty se mohou stát zdrojem znečištění pro okolní prostředí. Navíc přestože továrna Metaleurop byla zavřena v roce 2003, antropogenní rtuť pocházející z této továrny je v přírodním prostředí stále přítomna může být rozšířena i stovky kilometrů daleko id tohoto zdroje.

V neposlední řadě dizertační práce přispěla k novým závěrům v rámci použití techniky DGT pro měření rtuti. V nedávné době vyvinutá techniky DGT je založena na jednoduchém zařízení, které je uzpůsobeno tak, že vazebná vrstva gelu v čase akumuluje kovy in situ. Různé gely umožňují stanovení různých forem rtuti. DGT, které je naplněné gelem Chelex-100 nebo TiO_2 umožňovalo stanovit labilní část spécií rtuti v pórové vodě sedimentů zatímco gely s funkčními skupinami –SH umožňovali stanovit celkovou rozpuštěnou rtuti. Není možné přesně říci, které z míst je nejvíce znečištěno rtutí. Nicméně pozaďové koncentrace byly překročeny na všech místech. Proto je řeka Deûle i Lys zařazena mezi znečištěné řeky. Kontaminace řeky Lys může být ovlivněna transportem částic vázajícím rtuť z míst, která byla dříve zasažena kontaminací rtutí (řeka Deûle – Metaleurop). Korelace mezi rtutí a sírou potvrzuje, že celková rtuť a obsah síry ovlivňují uvolňování rtuti do pórové vody a podílí se na procesech metylace a demetylace.

Technika DGT představuje nástroje vhodný k měření bio-dostupné části rtuti v pórové vodě sedimentl. A potenciálně, jednou z nejdůležitějších aplikací techniky DGT by mohlo být in situ měření metylrtuti ke stanovení její bio-dostupné části v sedimentech. Pro tento účel je nutné vyvinout nový gel a eluční protokol pro metylrtuti a proces optimalizovat a validovat.

CONCLUSION GENERALE

Cette thèse sur la *Contamination mercurique des sédiments et cours d´eau du nord de la France et de la République tchèque* s´est focalisée sur l´étude des sites au Nord de la France (les canaux de La Lys et la Deûle), caractérisé par une contamination ancienne en métaux et particulièrement en mercure et sur les site non-contaminés au Sud de la Moravie (les fleuves: Jihlava et Morava). Ce travail s'est également focalisé sur la dévélopement du technique de diffusion sur gel, capteur DGT pour la détermination du mercure dans les sédiments et dans la colonne d'eau. Enfin dans le but d´évaluer la dynamique des espèces mercurielles (IHg, MeHg) dans des sédiments très contaminés, les espèces du mercure isotopiquement enrichies ont été utilisées.

Dans la première partie de la thèse, nous avons résumé la littérature récente publiée sur la pollution par le mercure et les nouvelles avancées sur l'étude du cycle biogéochimique du mercure, son transport et sa spéciation dans l´environnement.

Sur le plan analytique, une nouvelle méthode d'analyse du mercure dans les sédiments en utilisant, le coupage éthylation en solution du mercure et du méthylmercure avec la méthode Headspace avec piège (Trap Tenax), séparation par Chromatographie gaz et détection par la technique de spectroscopie de fluorescente atomique a vapeur froide a été mise en place. Plusieurs paramètres de la méthode ont été optimisés pour améliorer la sensibilité et la précision des mesures, des standards de références ont été utilisés pour la validation de la méthode avec une participation à un exercice d'intercalibration international organisé par l'AIEA.

La partie second de la thèse est consacrée à une étude de terrain réalisée dans les canaux de la Deûle et de La Lys (région Nord-Pas de Calais) et dans les fleuves Jihlava et Morava (region Moravie). Les sédiments cotés Français ont sont très contaminés en mercure comparés aux sédiments de surface prélevés dans les fleuves Jihlava et Morava en république Tchèque. Les concentrations en mercure total dans les sédiments de Nord de la France dépassent considérablement la valeur limite en Hg du fond géologique proposé par L´Agence de l´Eau Artois Picardie (0,1 $mg.kg^{-1}$), les valeurs limites ne sont pas connus pour la Sud de la Moravie, le choix c'est donc porté sur la valeur Shales (0,4 $mg.kg^{-1}$) qui correspond aux sites non contaminés pour faire les comparaison. Les concentrations en mercure total pour les sédiments surfaces sont 20 à 1000 fois plus élevé dans le Nord de la France et 3 a 300 fois plus élevés en Sud de Moravie par rapport aux limites.

Les comportements des espèces du mercure (mercure inorganique et methylmercure) naturelles (endogène) et ajoutés (exogène) en utilisant des traceurs isotopiques stables des formes chimiques du mercure sont présentés dans la troisième partie. L'évaluation en simultané des processus net de méthylation et déméthylation sont possibles par cette technique. Les caractéristiques physicochimiques confirment le caractère anoxique des sédiments de la Deûle. La méthylation est corrélé avec les sulfures pyritiques CRS, néanmoins aucune corrélation n'a été observée entre les sulfures fraichement précipité AVS et le potentiel de méthylation ou de déméthylation. La méthylation a été estimée par les calculs des rapports et abondances isotopiques de chaque espèce considérée. La zone de méthylation n´est pas situé en surface mais plus en profondeur (à -12 cm). Le maximum de production du MeHg n´est pas localisé sur les sédiments de surface ce qui limite les possibilités de rémobilisation du MeHg dans la colonne d'eau.

Finalement, ce travail contribue aux développements de nouveaux outils de mesure du mercure dans l'eau et les sédiments au moyen des techniques de diffusion sur gel (DGT). Cette technique permet de concentrer et de déterminer différentes espèces en fonction de la nature et les composés intégrés au gel. Le TiO_2 ou la Chelex-100 permette la détermination de la fraction labile des espèces du mercures, tandis que les gels avec des résines comportant des groupes fonctionnels -SH permettent la détermination du mercure total dans l´eau interstitielle d'ou l'intérêt d'utiliser plusieurs type de gel ayant des capacités de piégeage et spécifiques de différentes formes du mercure dans les sédiments.

La technique DGT apparaît comme un bon outil de détermination de la part (bio) accessible du mercure dans l´eau interstitielle. Une de plus important application de la téchnique DGT sera dans le futur la détermination du methylmercure pour évaluer avec précision aussi la partie (bio) accessible, dans ce but, de nouvelles résines et gels doivent être développés.

APPENDIX A

List of Apparatus:

AMA 254	**Altec, Czech Republic**
Analytical Balance Genius	**Sartorius, USA**
Analytical Balance Mettler AT 250	**Mettler Toledo, Swiss**
Centrifuge X340	**Prolabo, France**
Gas Chromatography (GC) Clarus 500	**Perkin Elmer, USA**
TurboMatrix Headspace Sampler	**Perkin Elmer, USA**
CV-AFS (model 2600)	**Tekran, USA**
Pyrolytic oven	
Drying Oven	**Binder GmbH, Germany**
Shaker KS 250 basic	**IKA Labortechnik, Germany**
Water bath and circulator Polystat	**Fisher Scientific, USA**
HR-ICP-MS Thermo Finnigan Element	**Thermo Finnigan Element II, USA**
ICP-MS	**X Series Thermo elemental**
Titrator	**Metrohm, Titrino 736 GP**
Freeze Dryer	**Christ Alpha 1-2 LD; Labconco freeZone 4.5**
Microwave	**Microwave system Explorer (CEM)**
Autosampler	**AS 3000 Thermo scientific**
GC	**Focuse GC, Thermo Element**
ICPMS	**X2 Thermo Fisher or X7 Thermo Element**

APPENDIX B

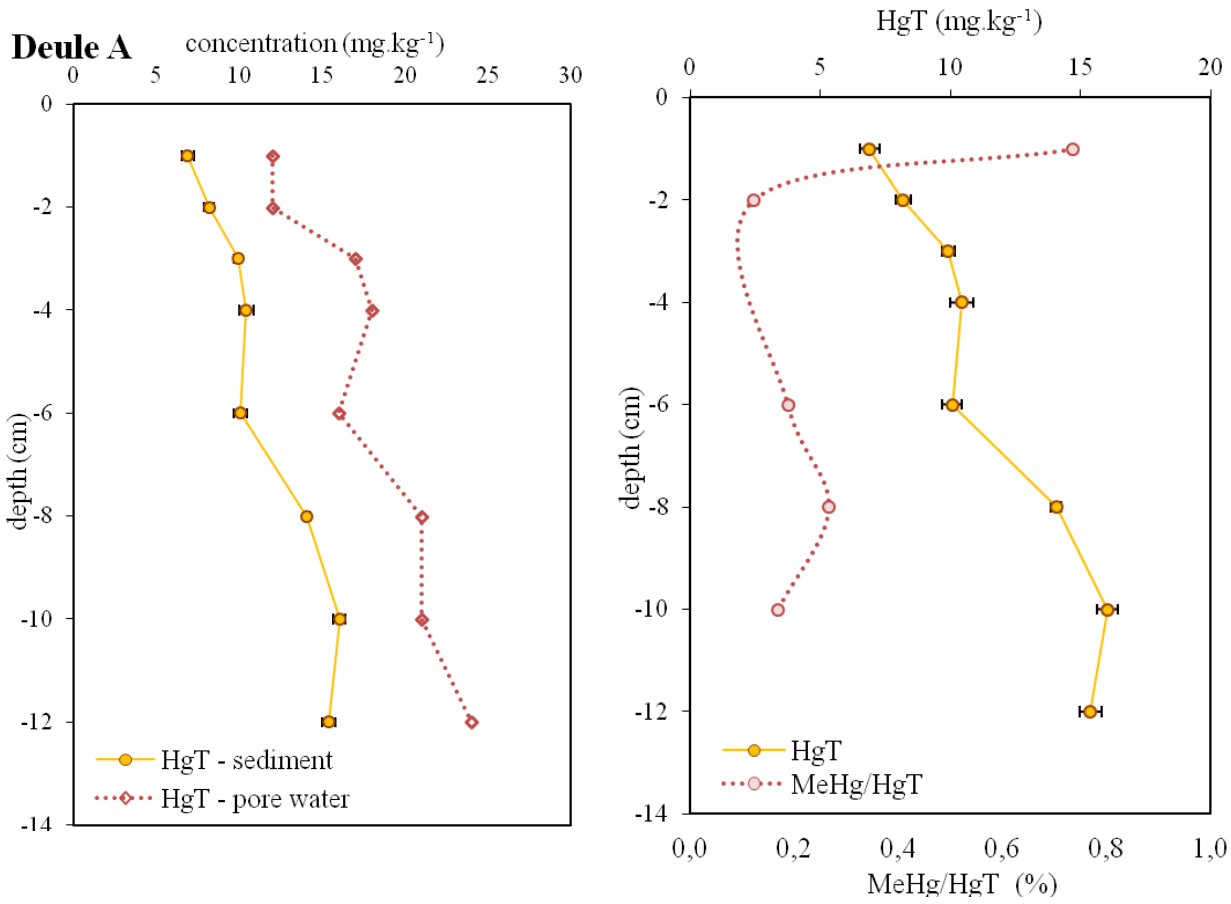
List of reagent and standards:

Reagent	
Nitric acid	65 % Fluka
Nitric acid	1% Merck
Glacial acetic acid	Merck, p.a.
Sodium acetate	Sigma
$NaBEt_4$	98%, Strem Chemicals
Ammonium hydroxide	Fluka
Mercury (II) chloride	Sterm Chemicals
Methylmercury chloride	Sterm Chemicals
Methanol	Merck
Chloric acid	Ultrex, JT Baker

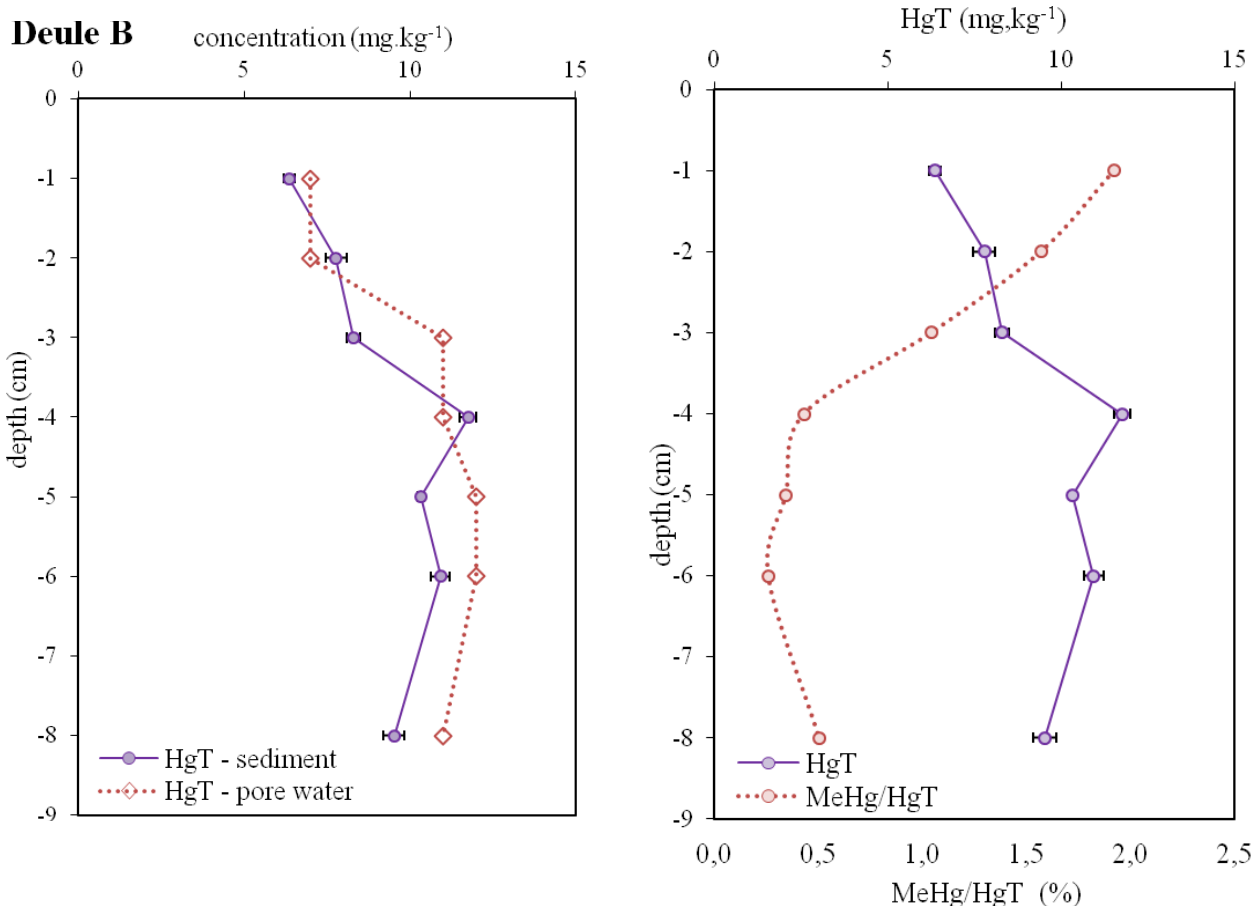
APPENDIX C

Depth profiles of mercury species and mercury proportion found at the sampling place Deûle A (C.I), Deûle B (C.II), La Lys C (C.IIII) and La Lys D (C.IV):

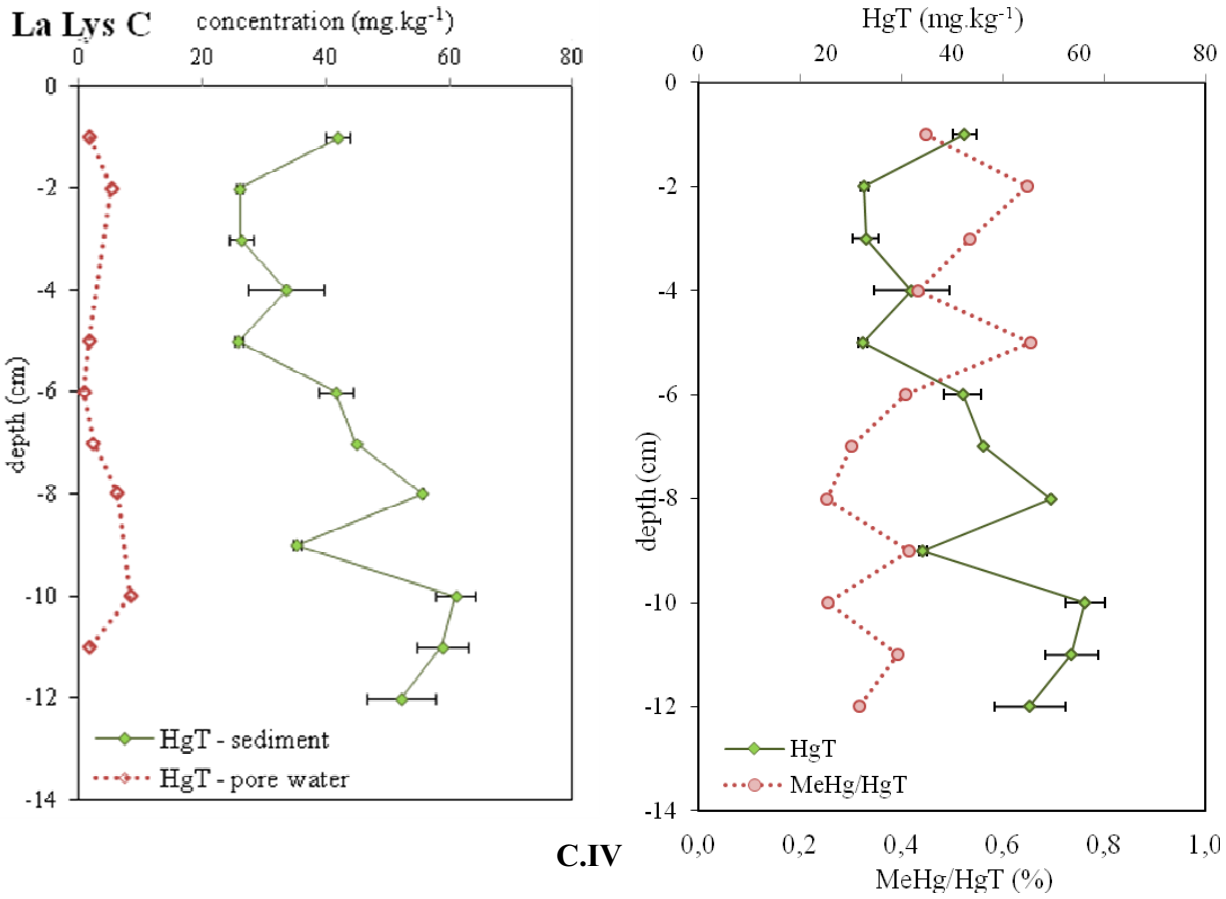
C.I.



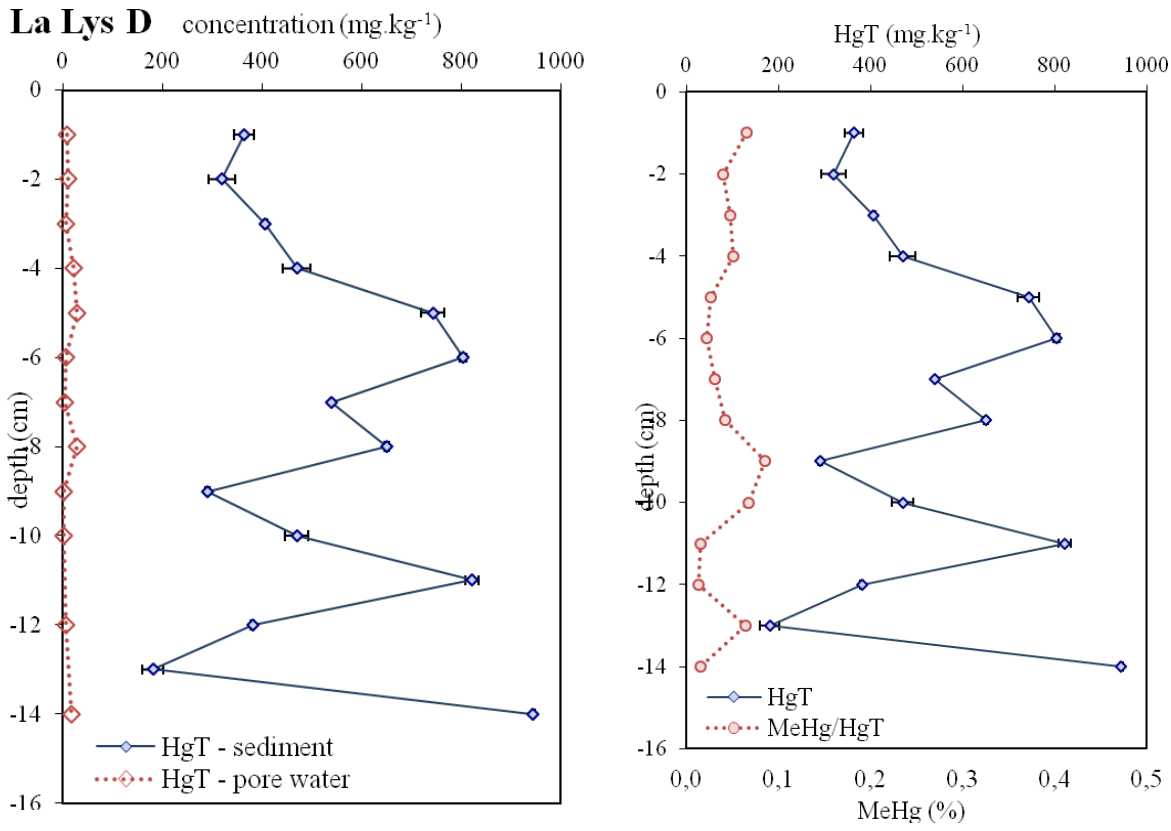
C.II



C.III



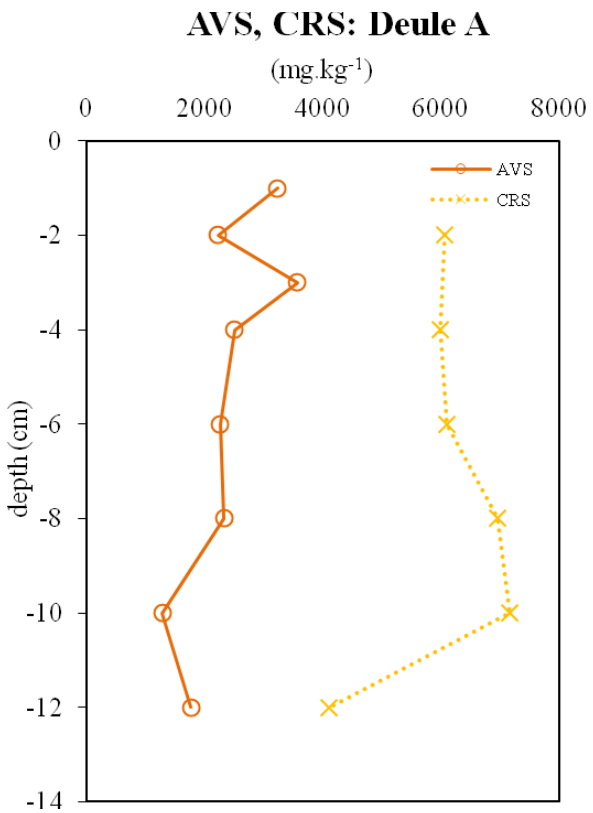
C.IV



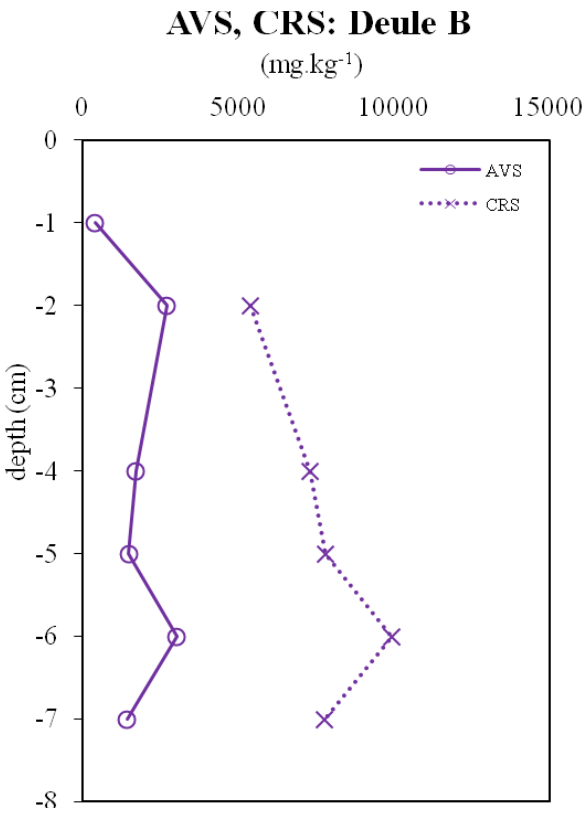
APPENDIX D

Depth profiles of AVS and CRS found at the sampling place Deûle A (D.I), Deûle B (D.II), La Lys C (D.IIII) and La Lys D (D.IV):

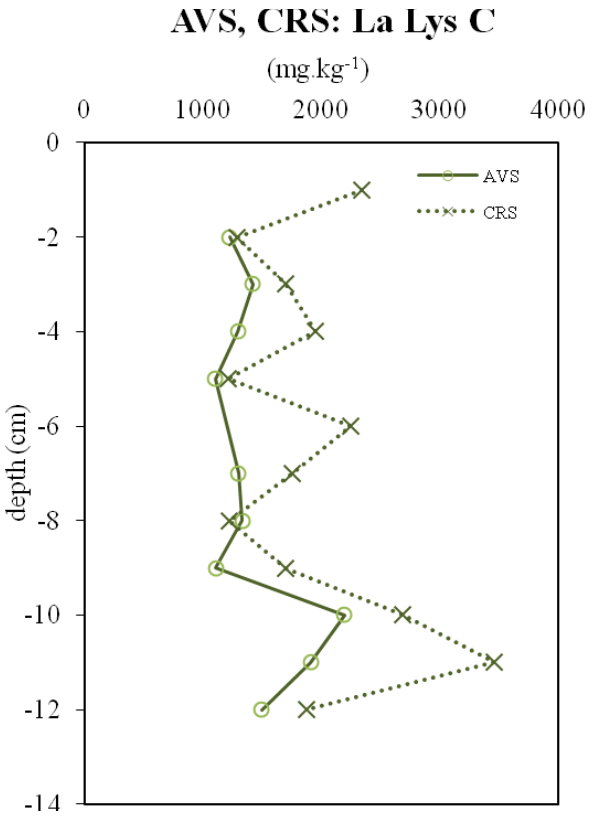
D.I



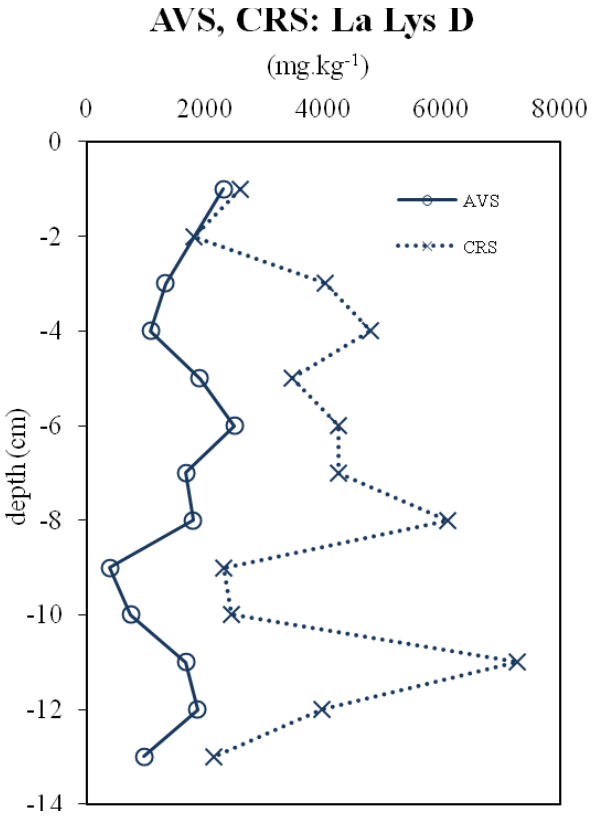
D.II



D.III



D.IV



APPENDIX E

Isotope dilution is based on addition to the sample of a precise amount of an isotopically labeled form of the analyte. The concentration of the analyte in the sample can be calculated from the observed isotope ratios when the natural and enriched isotope ratios and the masses of sample and spike are known. The spiking procedure is a critical stage that requires full equilibrium and the same behavior for both the analyte and the analogue during the analytical procedure. To minimize errors in the isotope ratio in the final determination, the amount of enriched standard added to the sample is adjusted in order to obtain a spike to analyte isotopic ratio close to unity.

The amount of methylated Hg deriving from the enriched isotope 199 during the incubation can be calculated by using the isotope dilution equation (Hintelmann et al, 1997):

$$c_{CH_3Hg^+} = \frac{\frac{201}{200.59} \times \left[CH_3^{201}Hg^+\right] \times \left(R_{sample} - R_{tracer}\right)}{\left(R_{natural} - R_{sample}\right) \times A_{201} \times sample\ weight} \qquad [IV.2]$$

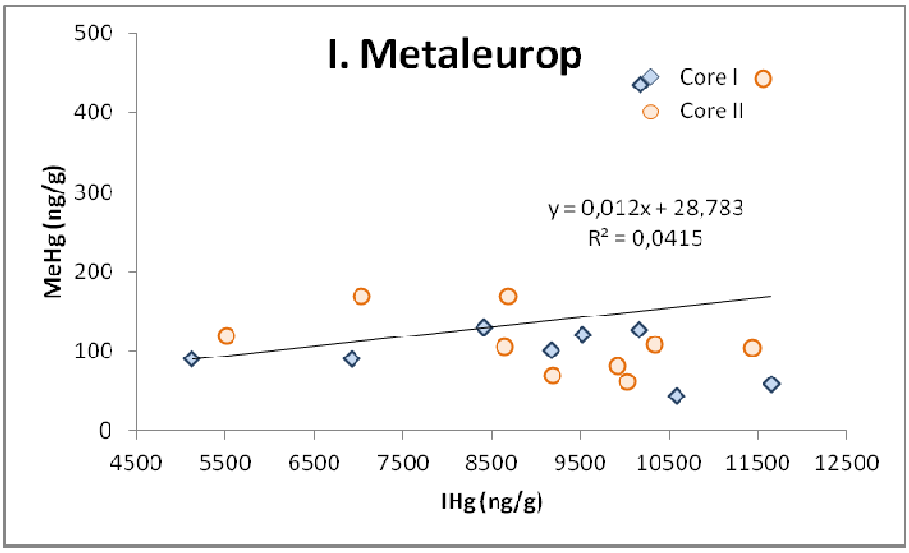
$c_{CH_3Hg^+}$	is concentration of CH_3Hg^+ in sample;
$[CH_3^{201}Hg^+]$	is amount of added $CH_3^{201}HgCl$ in ng
R_{sample}	is measured ration of $CH_3^{202}Hg$/ $CH_3^{201}Hg$ in sample after tracer addition
R_{tracer}	is measured ration of $CH_3^{202}Hg$/ $CH_3^{201}Hg$ in tracer solution
$R_{natural}$	is measured natural ration of $CH_3^{202}Hg$/ $CH_3^{201}Hg$
A_{201}	relative isotopic abundance of $CH_3^{201}Hg$

The amount of demethylated methylmercury from the enriched spike is calculated in a similar way following the 201 isotope.

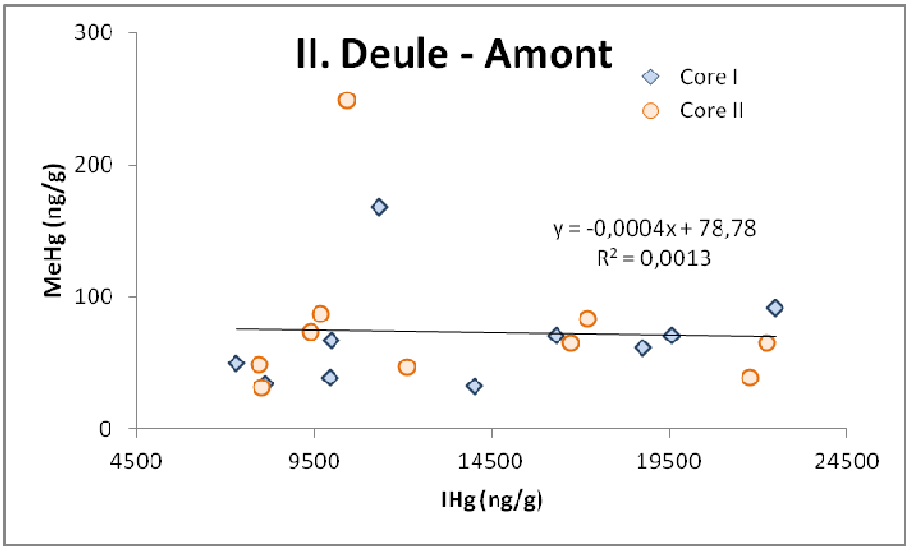
APPENDIX F

Relationship between in situ MeHg concentration and HgT cocnentation in the F.I sediments of I.Metaleurop; F.II. II.Deûle – Amont and c) percentage isotope methylated and in situ MeHg concentration.

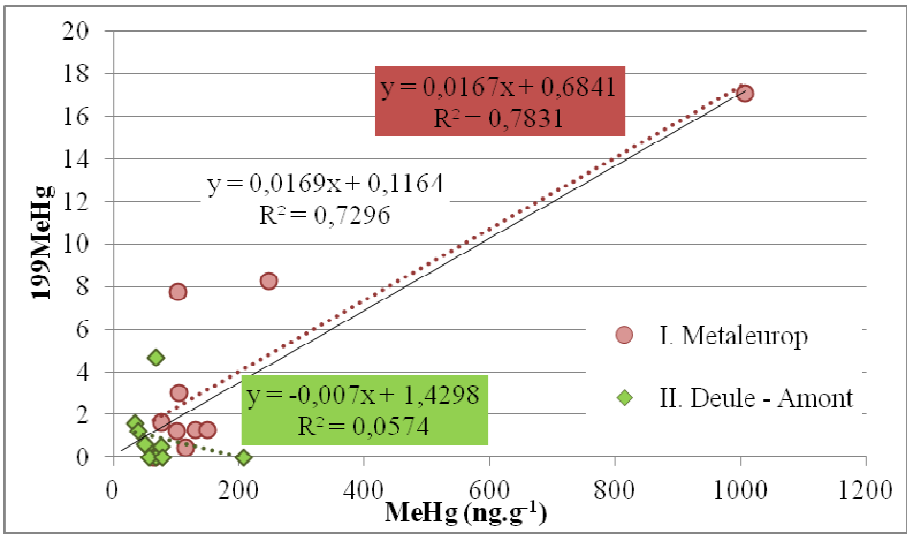
F.I.



F.II



F. III. Correlation between the MeHg concentration and amount of ^{199}MeHg.



APPENDIX G

Article: Sorption gel with titanium dioxide for determination of mercury using the diffusive gradient in thin film technique (DGT) in Czech language

Sorpční gel s oxidem titaničitým pro stanovení rtuti technikou difuzního gradientu v tenkém filmu

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Klíčová slova: DGT; TiO_2; rtuť; sorpční gel

Úvod

Rtuť patří pro své toxické vlastnosti a vysokou schopnost bioakumulace k prvkům, kterým je věnována mimořádná pozornost. Rychlý rozvoj analytických metod v posledních desetiletích vedl k vývoji nových analytických technik umožňujících stanovení rtuti a jejich sloučenin v různých

složkách životního prostředí, jež byly následně použity v mnoha environmentálních studiích[1-4]. Stanovení rtuti v jednotlivých matricích životního prostředí se však i přes tento pokračující vývoj stále potýká s řadou problémů. Ty převážně souvisí s nízkou koncentrací rtuti a jejich specií v měřených vzorcích, často na úrovni $ng.kg^{-1}$ respektive $ng.dm^{-3}$. Stanovení tak nízkých koncentrací vyžaduje použití speciálních zařízení[5-6] a bezkontaminačních postupů[7-8]. S takto nízkou koncentrací souvisí výskyt možných ztrát, na druhé straně možnost kontaminace vzorku během odběru, transportu a úpravy k analýze[9-15]. Se změnou fyzikálně-chemických parametrů může docházet rovněž ke změnám speciace. Minimalizaci manipulace se vzorkem a zamezení či výraznému snížení kontaminace řeší použití *in situ* technik. I přes více jak desetiletí práce v oblasti *in situ* měření je však v současnosti možno mluvit pouze o počátcích praktického používání *in situ* metod, neboť jejich vývoj a ověření jsou často velmi obtížné a zdlouhavé.

Pokrokem v *in situ* měření solutů ve vodných systémech je v 90. letech minulého století nově vyvinutá vzorkovací gelová technika, technika difuzního gradientu v tenkém filmu (Diffusive Gradients in Thin films technique, DGT)[16, 17], jejíž další nespornou výhodou pro měření velmi nízkých koncentrací, je kromě použití *in situ* prekoncentrační schopnost.

Technika DGT je dnes již běžně používána pro stanovení koncentrací široké škály labilních kovových specií stopových, ale i minoritních a majoritních kovů v přírodních vodách, jezerech, řekách a v mořích[17, 18]. Je využívána i pro měření koncentračních gradientů a toků látek v půdách[19,20] a sedimentech[21-23].

Předností techniky DGT je její jednoduchost, finanční nenáročnost, možnost stanovení celé řady prvků a již zmiňovaná prekoncentrační schopnost.

Technika DGT využívá dva typy hydrogelů: difuzní a sorpční. Oba typy gelů jsou společně s membránovým filtrem utěsněny ve vzorkovací jednotce ve tvaru pístu. Ionty kovů, jejich mobilní a

labilní formy, difundují kruhovým okénkem vzorkovací jednotky DGT o ploše A (cm^2) difuzní vrstvou známé tloušťky Δg (cm) a vážou se na vhodné sorpční médium zakotvené v sorpčním gelu. Množství kovu M (ng) vázaného během doby expozice t (s) v sorpčním gelu se obvykle stanovuje po eluci sorpčního gelu kyselinou dusičnou metodami atomové spektrometrie, v případě rtuti lze s výhodou použít stanovení rtuti bez eluce přímo v disku sorpčního gelu pomocí jednoúčelového atomového absorpčního spektrometru AMA 254 . Běžně používaným hydrogelem je polyakrylamidový hydrogel, který slouží jako difuzní gel. Polyakrylamidový sorpční gel má v sobě zabudováno sorpční médium, pro stanovení kovů a jejich specií to bývá iontoměnič Chelex-100. Tento typ DGT s polyakrylamidovým difuzním gelem nelze použít pro stanovení rtuti, protože rtuť se váže na volné aminové skupiny hydrogelu a omezuje difuzi rtuti k iontoměniči. Proto byl polyakrylamidový difuzní gel nahrazen agarosovým gelem[23]. Pro záchyt většího počtu specií rtuti bylo místo iontoměniče Chelex-100 navrženo použití iontoměniče Spheron-Thiolu s –SH skupinami které silně vážou ionty rtuti a jsou schopny konkurovat i pevnějším komplexům rtuti s přírodními ligandy[23]. Při studiu chování rtuti v sedimentech bylo prokázáno, že koncentrace rtuti nalezené DGT se Spheron-Thiolem odpovídají celkové rozpuštěné rtuti změřené klasickým postupem po centrifugaci. Násobně nižší obsahy nalezené DGT s Chelexem-100 pak odpovídají rtuti v iontové formě a rtuti ve slabých anorganických a malých organických komplexech[23,24].

Vzhledem k současné komerční nedostupnosti Spheron-Thiolu byly hledány, syntetizovány, validovány a při analýze reálných vzorků použity iontoměniče obsahující thiolové skupiny, Duolit a Iontosorb[21,25]. Byly připraveny a použity iontoměniče umožňující stanovení methylrtuti[26,27].

Tato práce se zabývá optimalizací přípravy a testováním sorpčního gelu s částicemi oxidu titaničitého jako sorpčního média pro stanovení rtuti v přírodních vodách technikou DGT. Oxid titaničitý je znám jako velmi dobré adsorpční médium pro kovové ionty. Je úspěšně používán pro zakoncentrování široké škály kovů metodou extrakce tuhou fází

(SPE) pro jejich stanovení pomocí spektrálních metod[28-30], k odstraňování těžkých kovů z odpadních vod[31,32] a k odstraňování rtuti ze spalin při spalování uhlí[33,34]. Použití oxidu titaničitého jako sorpčního média pro techniku DGT popsali v nedávné době Bennet a kol.[35], kteří použili adsorbent Metsorb založený na částicích TiO_2 k přípravě sorpčního gelu pro stanovení anorganických forem arzenu a selenu v přírodních vodách pomocí techniky DGT. Panther a kol.[36] referovali o použití sorpčního gelu s oxidem titaničitým na stanovení reaktivního fosforu v přírodních vodách.

Experimentální část

Použité chemikálie

Pro přípravu sorpčního gelu byl použit akrylamid (Boehringer, Německo), patentované agarosové síťovadlo (DGT Research, Lancaster, UK), peroxosíran amonný (Lachema, ČR), N,N,N‘,N‘ – tetramethylethylendiamin TEMED (Sigma-Aldrich, SRN) a oxid titaničitý (Sigma-Aldrich, SRN) - anatáza, velikost částic menší než 44 μm.

Ve všech experimentech byly použity chemikálie čistoty p.a. a deionizovaná voda připravená přístrojem Milli-Q Academic (Millipore, USA). Pro přípravu agarosového difuzního gelu byla použita agarosa (Merck, SRN). Pro přípravu modelových roztoků rtuti byl použit standardní roztok Hg o koncentraci 1 $mg.cm^{-3}$ (Astasol®, Analytika Praha, ČR). Pro úpravu iontové síly v modelových roztocích rtuti byl použit dusičnan sodný (Lachema). Hodnota pH modelových roztoků byla upravována pomocí hydroxidu sodného Suprapur® 30% (Merck) a kyseliny dusičné Suprapur® 65% (Merck). Pro studium vlivu přírodních ligandů na sorpci rtuti v sorpčním gelu byly použity chlorid sodný (Lachema) a směs huminových kyselin (Prod. Num. 53680, Fluka, Švýcarsko). Vzorkovací

jednotky DGT s expoziční plochou 3,14 cm^2 byly zakoupeny u firmy DGT Research Ltd. (Lancaster, UK).

Použité přístroje

Ke stanovení celkového množství rtuti v modelových roztocích a sorpčních gelech byl použit jednoúčelový atomový absorpční spektrometr AMA-254 (Advanced Mercury Analyser, Altec, Praha, ČR). Pro dispergaci oxidu titaničitého v gelovém roztoku byla použita ultrazvuková lázeň Powersonic PSO 3000 A (Ultrashalltechnik AG, Straubehhardt, SRN). Modelové roztoky rtuti byly míchány laboratorní míchačkou Hei-Standard (Schwabach, SRN) a pH těchto roztoků bylo sledováno pomocí pH metru WTW 320 (Weilheim, SRN) kalibrovaného pufračními roztoky o pH 4,0 a 7,0 (Analytika Praha, ČR).

Pracovní postupy

Difuzní a sorpční gely byly připravovány dle postupu doporučovaného Zhang a Davisonem[17] s mírnými modifikacemi. Difuzní agarosový gel (1,5%) byl připraven rozpuštěním příslušného množství agarosy v horké deionizované vodě a vzniklý roztok byl nalit mezi dvě skla oddělená distanční fólií o tloušťce 0,5 mm. Ochlazením na teplotu místnosti roztok ztuhl a vytvořil agarosový hydrogel. Při přípravě sorpčního gelu s TiO_2 se vycházelo ze zkušeností s přípravou gelů s iontoměniči Spheron-Thiol[18] a Chelex-100[23].

K navážce 0,4 g suchého TiO_2, která zaručovala dostatečnou sorpční kapacitu disku DGT jednotky pro dlouhodobé použití v reálných systémech přírodních vod, byly přidány 2 ml gelového roztoku a směs byla míchána na míchačce 5 min. Pro homogennější rozložení částic TiO_2 ve vzniklém gelu byl gelový roztok s TiO_2 vložen do ultrazvukové lázně, což usnadnilo dávkování do skleněné formy a vznik kvalitnějšího gelu. Z plátků vyrobených gelů byly plastovým nožem

vykrajovány kruhové disky o průměru 25 mm. Disky difuzního agarosového a sorpčního gelu s oxidem titaničitým byly před použitím uchovávány v deionizované vodě.

Vzorkovací jednotky DGT byly sestaveny těsně před použitím tak, že na vnitřní stranu jednotky byl uložen disk sorpčního gelu, překryt diskem difuzního gelu a nakonec polyethersulfonovým membránovým filtrem (Supor®-450, Pall Corporation USA) s póry o velikosti 0,45 μm pro ochranu proti poškození. Jednotka byla uzavřena prstencovým krytem s expozičním okénkem o průměru 2 cm.

Připravené jednotky DGT byly vloženy do míchaných modelových roztoků rtuti o objemu pěti litrů za vybraných podmínek bez a za přítomnosti dalších látek. Koncentrace rtuti v modelových roztocích byla 20 $\mu g.dm^{-3}$. Hodnota pH v základním modelovém roztoku byla upravena na hodnotu 6. pH roztoků k testování vlivu kyselosti se pohybovalo v rozmezí hodnot 2-10. Pro testování vlivu iontové síly byly připraveny modelové roztoky rtuti o iontové síle 0,001 až 0,5 $mol.dm^{-3}$. Koncentrace chloridů v testovaných roztocích byla 0,001 – 0,5 $mol.dm^{-3}$ a koncentrace huminových kyselin 0,01 – 10 $mg.dm^{-3}$.

Po uplynutí expoziční doby byly jednotky z roztoku vyjmuty, rozebrány a jednotlivé vrstvy gelů odděleny. Pro srovnávací měření byly z modelových roztoků odebírány alikvotní vzorky roztoku a to před vložením a po vytažení jednotek a zfiltrovány přes membránový filtr o velikosti pórů 0,45 μm a okyseleny kyselinou dusičnou. Koncentrace rtuti v odebraných vzorcích roztoků C_{SOL} a obsah rtuti v sorpčních gelech byl stanoven na přístroji AMA 254. DGT časově průměrná koncentrace C_{DGT} byla vypočítána dle rovnice:

$$C_{DGT} = M \Delta g / D\ t\ A, \qquad (1)$$

kde M (ng) je množství rtuti navázané na sorpční gel během doby expozice t (s). A (cm^2) je plocha expozičního okénka a Δg (cm) je tloušťka difuzního gelu, D je difuzní koeficient rtuti v agarosovém gelu.

Výsledky a diskuse

Charakteristiky sorpčního gelu

V připravených sorpčních gelech s TiO_2 byl stanoven obsah rtuti, který byl odečítán od obsahu rtuti nalezeného po expozici jednotek DGT v roztocích. Průměrná hodnota množství rtuti nalezená v neexponovaných gelech byla 0,13 ± 0,05 ng (n=10), což při expoziční době DGT vzorkovacích jednotek 24 hodin odpovídá minimální měřitelné koncentraci rtuti 3,4 $ng.dm^{-3}$. Nižší koncentraci rtuti lze měřit zvýšením expoziční doby jednotek DGT.

Při několikanásobném přetížení disku ionty rtuti byla nalezena kapacita disků 2,5 μmol/disk (obr. 1.), která je dostatečně vysoká pro několikatýdenní i několikaměsíční expozici jednotky DGT s tímto sorpčním gelem v přírodních vodných systémech.

Validace techniky DGT se sorpčním gelem s TiO_2

Koncentrace rtuti vypočtená z množství rtuti navázané na sorpční gel za čtyři hodiny pomocí rovnice (1) odpovídala koncentraci rtuti změřené přímo v roztoku. Odchylky mezi koncentracemi nepřesahovaly 5 % a splnily tak kritéria doporučovaná DGT Research[37].

Množství rtuti vázané v sorpčním gelu rostlo lineárně s časem expozice (obr. 2) a odpovídalo teoretickému množství vypočítanému z rovnice (1). Tyto výsledky ukazují, že technika DGT se sorpčním gelem s TiO_2 poskytuje spolehlivá data pro stanovení rtuti.

Ze směrnice závislosti (α) navázaného množství rtuti (M, ng) na sorpční gel za čas (t, s), tloušťky difuzního gelu (Δg, cm), expoziční plochy (A, cm^2) a koncentrace rtuti v roztoku (C_{SOL}, ng mL^{-1}) byl vypočítán difuzní koeficient pro rtuť v agarosovém gelu s sorpčním gelem TiO_2

$$D = \alpha \Delta g / AC \qquad (2)$$

Jeho hodnota $(8{,}90 \pm 0{,}13).10^{-6}.cm^2.s^{-1}$ odpovídala hodnotám nalezeným pro Chelex 100 a Spheron Thiol[23] a hodnotě difuzního koeficientu rtuti ve vodě $9{,}13.10^{-6}.cm^2.s^{-1}$ z tabulek[38].

Vliv pH, iontové síly a vybraných přírodních ligandů na stanovení rtuti DGT s TiO_2

Vliv kyselosti roztoku na sorpci rtuti byl sledován v rozmezí pH 2-10. Bylo prokázáno, že sorpční gel váže rtuť plně v rozmezí pH 4-8 (obr. 3). Při nižším pH dochází k neutralizaci povrchového náboje a k překrytí aktivních míst na povrchu TiO_2 což vede k celkovému snížení adsorpce[39]. Hodnota pH přírodních povrchových vod se pohybuje v rozmezí 6,5-9. Techniku DGT se sorpčním gelem obsahující TiO_2 tedy lze bez problémů ve většině přírodních vod použít.

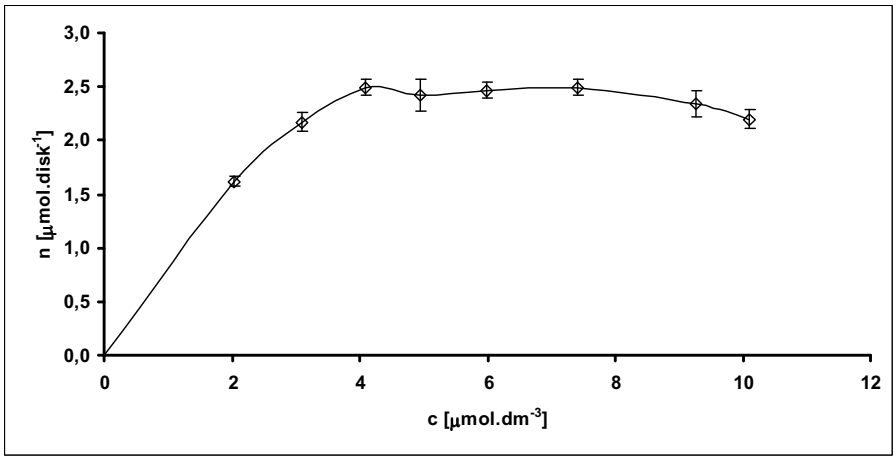
Vliv iontové síly na sorpci rtuti v sorpčním gelu s TiO_2 byl v rozmezí hodnot 0,001 až 0,01 $mol.dm^{-3}$ zanedbatelný. Tyto hodnoty odpovídají běžnému rozsahu iontové síly v povrchových vodách (0,002 - 0,02 $mol.dm^{-3}$). K 20% poklesu sorpce rtuti v sorpčním gelu docházelo až při iontové síle roztoku 0,1 $mol.dm^{-3}$ (obr. 4).

Zásadní vliv na stanovení rtuti technikou DGT má koncentrace přírodních ligandů. V této práci byl studován vliv chloridů a směsi huminových kyselin. Již při koncentraci chloridů v modelovém

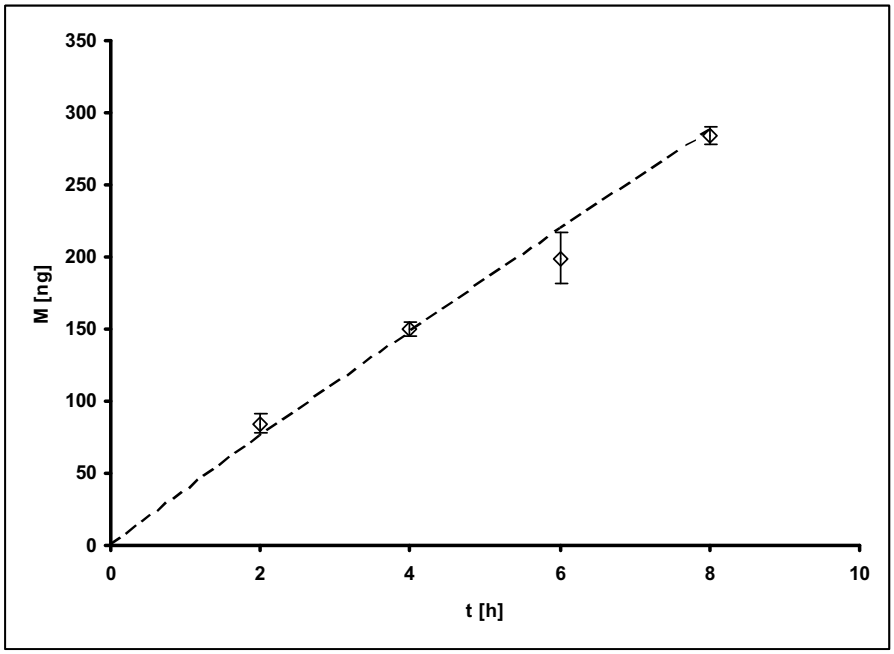
roztoku 0,03 mg.dm^{-3} docházelo k 25% poklesu sorpce rtuti. Zvyšování obsahu chloridů v roztoku na hodnotu 3 mg.dm^{-3} vedlo k tvorbě stabilních chlorokomplexů, které nebyly technikou DGT zachyceny (obr. 5).

Přítomnost huminových kyselin v roztoku rovněž ovlivňovala množství rtuti zachycené v sorpčním gelu tvorbou pevných komplexů (obr. 6). Již koncentrace huminových kyselin 1 mg.dm^{-3} způsobila snížení záchytu rtuti o 40%, koncentrace 10 mg.dm^{-3} potom o 80%.

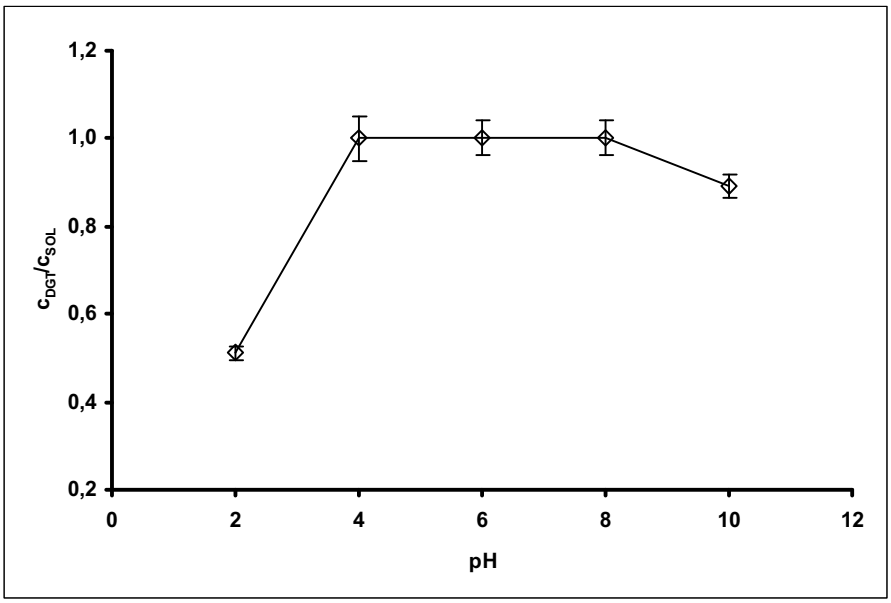
Koncentrace chloridů v čistých povrchových vodách nepřesahuje 0,05 mg.dm^{-3} a koncentrace huminových kyselin se pohybuje v jednotkách mg.dm^{-3} v přírodních vodách[40]. Výsledky prováděných testů prokázaly, že techniku DGT se sorpčním gelem s TiO_2 nelze použít pro měření koncentrací rtuti v mořských vodách, neboť zde je rtuť díky vysoké koncentraci chloridů (až 22 g.dm^{-3}) přítomna ve stabilních chlorokomplexech. Rovněž v přírodních vodách s velkým obsahem huminových látek je rtuť vázána v pevných komplexech, které nejsou jednotkou DGT se sorpčním gelem s TiO_2 zachyceny. V přírodních povrchových vodách s obsahem huminových látek do 1 mg.dm^{-3} je možné techniku DGT s TiO_2 sorpčním gelem s úspěchem použít pro stanovení labilních specií rtuti. Kombinace DGT jednotek s TiO_2 s jednotkami se sorpčním gelem obsahujícím thiolové skupiny, jako je Spheron-Thiol nebo Duolit GT73, umožňuje odhad různých forem rtuti v přírodních vodných systémech.



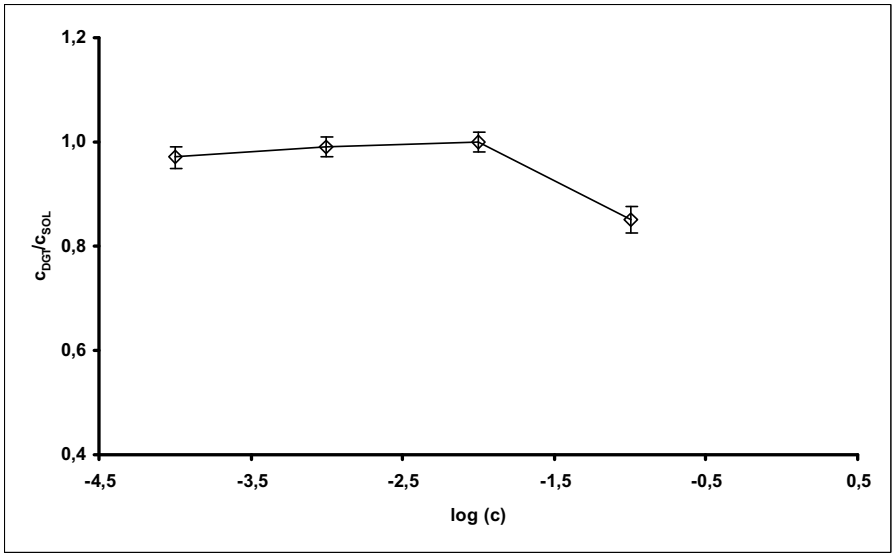
Obr. 1. Závislost akumulovaného množství rtuti n (μmol) v disku na její koncentraci v roztoku c (μmol.dm^{-3})



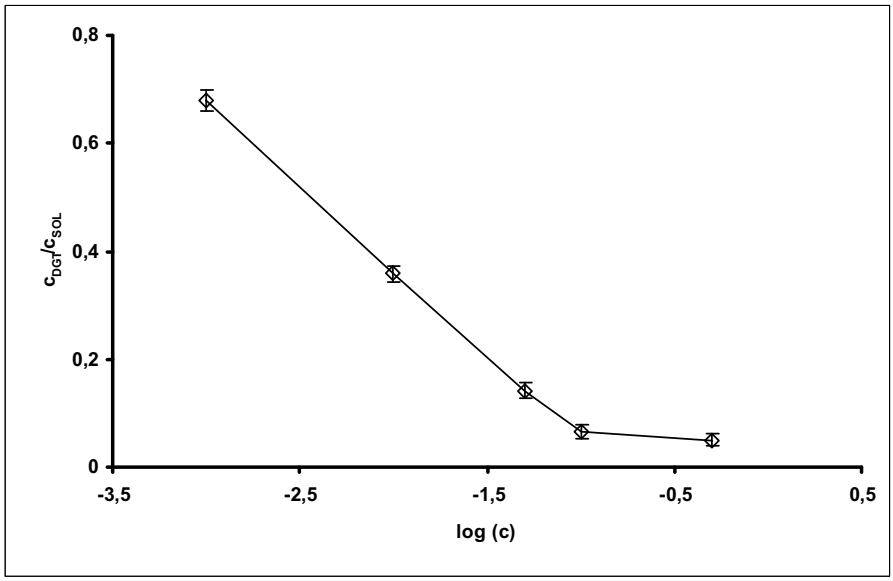
Obr. 2. Závislost množství rtuti M_{Hg} (ng) sorbované v jednom sorpčním disku na čase t (h) pro koncentraci rtuti v roztoku 20 μg.dm^{-3}, čárkovaná čára znázorňuje teoretickou závislost z rovnice (1)



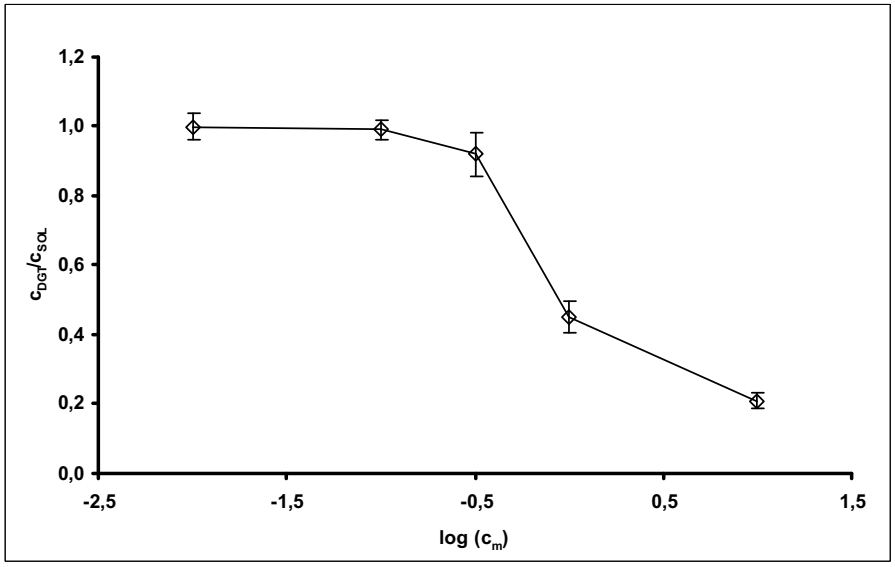
Obr. 3. Závislost sorpce rtuti na hodnotě pH vnějšího roztoku



Obr. 4. Závislost sorpce rtuti na iontové síle vnějšího roztoku c ($mol.dm^{-3}$)



Obr. 5. Závislost sorpce rtuti na koncentraci chloridů c (mol.dm^{-3})



Obr. 6. Závislost sorpce rtuti na koncentraci huminových kyselin c_m (mg.dm^{-3})

Závěr

Techniku DGT se sorpčním gelem obsahujícím TiO_2 lze použít pro stanovení labilních specií rtuti ve většině sladkovodních přírodních vod. Není vhodná pro stanovení rtuti v mořské vodě, která obsahuje vysokou koncentraci chloridů, s nimiž rtuť tvoří stabilní komplexy. Naopak lze DGT s TiO_2 použít ve vodách obsahujících huminové kyseliny do 1 $mg.dm^{-3}$ obdobně jako s iontoměniči s thiolovými skupinami Duolite GT73 a Spheron-Thiol[41]. Stanovení s běžně používaným iontoměničem Chelex-100 je ovlivňováno řádově nižšími koncentracemi huminových kyselin.

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