NOVEL DEVELOPMENTS IN THE DIFFUSIVE GRADIENT IN THIN FILMS (DGT) TECHNIQUE FOR THE DETERMINATION OF BIOAVAILABLE MERCURY AND OTHER TRACE METALS IN AQUATIC AND TERRESTRIAL ENVIRONMENTS

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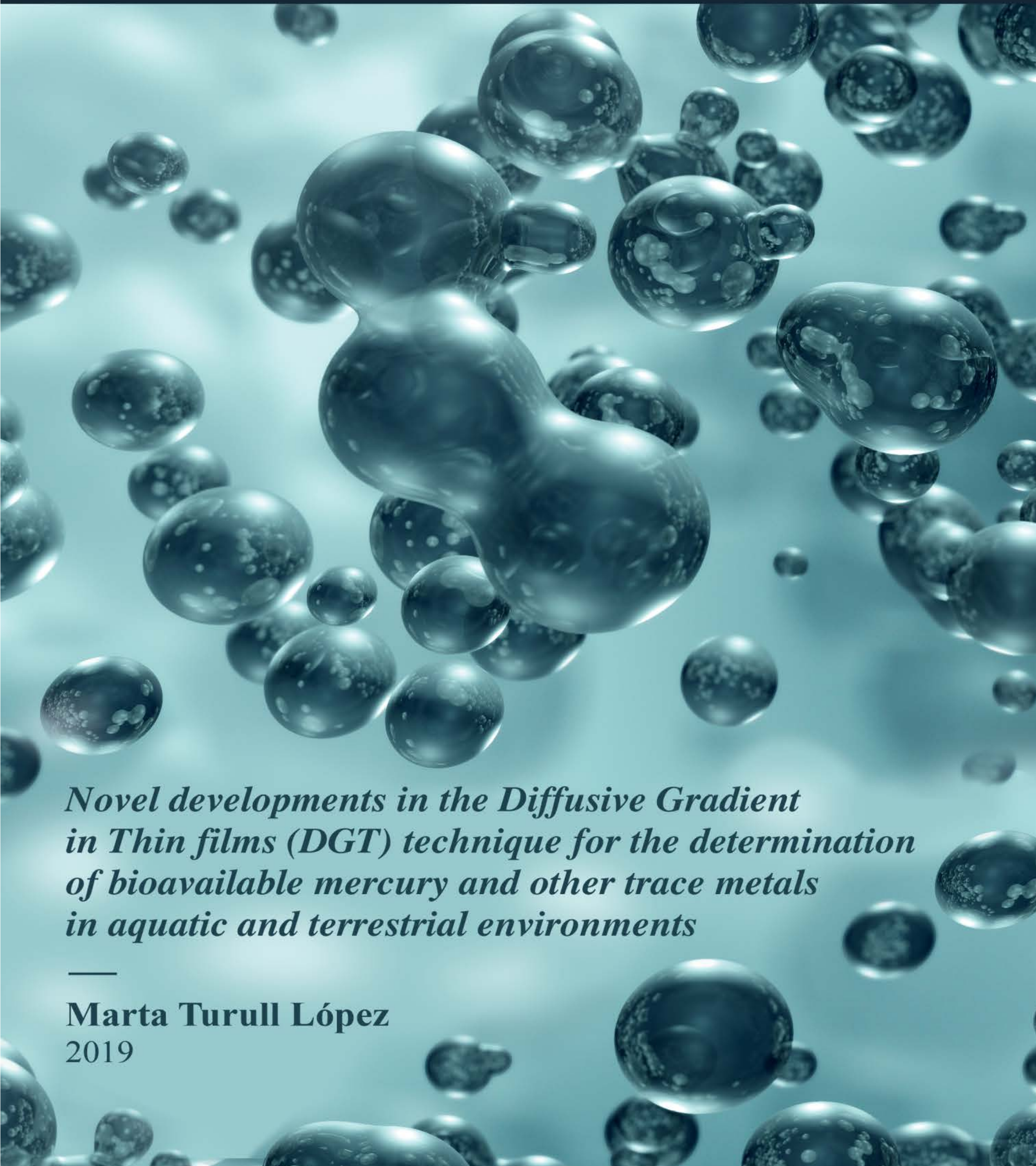

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Marta Turull López

2019

Supervised by Dr. Sergi Díez and Dr. Clàudia Fontàs
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Thesis submitted in fulfillment of the requirements for the degree of Doctor from the University of Girona

Dr. Sergi Díez, of the IDAEA-CSIC from Barcelona and Dr. Clàudia Fontàs, of the University of Girona,

WE DECLARE:

That the thesis titled "Novel developments in the Diffusive Gradient in Thin film (DGT) technique for the determination of bioavailable mercury and other trace metals in aquatic and terrestrial environments", presented by Marta Turull López to obtain a doctoral degree, has been completed under our supervision and meets the requirements to opt for an International Doctorate.

For all intents and purposes, we hereby sign this document.

Dr. Sergi Díez

Dr. Clàudia Fontàs

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Res a la vida és per ser temut,
sinó per ser comprès.
Ara és el moment de comprendre més,
per a que puguem témer menys.

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List of publications

This thesis meets all the requirements to be presented as a compendium of publications and has resulted in the publication of five scientific articles and one submitted.

Turull, M.; Elias, G.; Fontàs, C.; Díez, S. 2017. Exploring new DGT samplers containing a polymer inclusion membrane for mercury monitoring. Environ Sci Pollut Res, 24:10919-10928.
Doi: 10.1007/s11356-016-6813-z
Impact factor: 2.800
Quartile: Q2

Turull, M.; Grmanova, G.; Dago, A.; Ariño, C.; Díez, S.; Díaz-Cruz, J.M.; Esteban, M. 2017. Phytochelatin synthesis in response to Hg uptake in aquatic plants near a chlor-alkali factory. Chemosphere, 176:74-80.
Doi: 10.1016/j.chemosphere.2017.02.092
Impact factor: 4.427
Quartile: Q1

Turull, M.; Komarova, T.; Noller, B.; Fontàs, C.; Díez, S. 2018. Evaluation of Mercury in a freshwater environment impacted by an organomercury fungicide using diffusive gradiente in thin films. Sci Total Environm, 621:1475-1484.
Doi: 10.1016/j.scitotenv.2017.10.081
Impact factor: 4.610
Quartile: Q1

Turull, M.; Fontàs, C.; Díez, S. 2019. Diffusive gradiente in thin films with open and restricted gels for predicting mercury uptake by plants. Environ Chem Lett.
Doi: 10.1007/s10311-019-00864-2
Impact factor: 3.125
Quartile: Q2

Turull, M.; Fontàs, C.; Díez, S. 2019. Conventional and novel techniques for determination of Hg uptake by lettuce in amended agricultural peri-urban soils. Sci Total Environm, 668:40-46.
Doi: 10.1016/j.scitotenv.2019.02.244
Impact factor: 4.610
Quartile: Q1

Turull, M.; Fontàs, C.; Díez, S. Effect of biochar and compost on trace metal bioavailability in agricultural soils and metal uptake by lettuce. J Haz Mat. (submitted).

List of abbreviations

Δ_f	Membrane filter thickness
Δg	Thickness of the diffusion layer
φ	Soil porosity
∂	Thickness
∂^{mdl}	Thickness of the diffusion layer
2-NPOE	2-nitrophenyl octyl ether
2-NPPE	2-nitrophenyl pentyl ether
3MP	3-mercaptopropyl functionalized silica
A	Area
AAS	Atomic absorption spectrometry
Aliquat 336	Methyltrioctylammonium chloride
AMA-254	Advanced Mercury Analyzer
APA	Agarose derivative cross-linker
APS	Ammonium persulfate
AS	Ascó (in chapters 3 and 4)
AS	Agricultural soil (in chapters 7 and 8)
ATP	Adenosine triphosphate
BC	Biochar
BC3	Agricultural soil + biochar (3%)
BC6	Agricultural soil + biochar (6%)
BCF	Bioconcentration factor
BDL	Diffusive boundary layer
BH	Broken Hill
C	Control

C_a	Concentration of analytes in the eluent
CAP	Cellulose acetate propionate
C_{DGT}	Concentration measured by DGT
C_E	Effective concentration
CEC	Cation exchange capacity
C_{inorg}	Concentration of the labile inorganic species in solution
c^{ls}	Concentration of sorbed ion that can desorb within the simulation time
C_{org}	Concentration of the labile organic species in solution
CP30	Agricultural soil + compost (30%)
CRM	Certificate Reference Material
C_{soln}	Bulk pore water solute concentration
CTA	Cellulose triacetate
CTB	Cellulose tributyrate
D	Diffusion coefficient in the layer
D_0	Diffusion coefficient in water
D_{25}	Diffusion coefficient at 25ºC
D2EHPA	Di-2-(ethylhexyl) phosphoric acid
DIFS	DGT-induced fluxes in sediments
D_{gel}	Diffusion coefficient in the membrane filter
DG	Diffusive gel
DGT	Diffusive Gradient in thin Film
DMG	Diffusive milli-gel
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DOM	Dissolved organic matter

DOP	dioctyl phthalate
dp	Density of the soil particles
DT	Deltebre
dw	Dry weight
e.g.	For example
Eh	Redox potential
et al.	And others
EtHg	Ethylmercury
EU	European Union
f_e	Eluent factor
FH	Ferrihydrite
FM	Flix meander
fw	Fresh weight
GSH	Glutathione
HPCL	High performance liquid chromatography
i.e.	Id est (that is)
ICP-MS	Inductively coupled plasma mass spectrometry
IL	Ionic liquid
IRB	Iron-reducing bacteria
J	Flux
k_1 and k_{-1}	pseudo-first-order rate constants
Kd	Equilibrium constant
LC	Liquid chromatography
LDPE	Low-density polyethylene
LMS	Low molecular species

LOD	Limit of detection
LOQ	Limit of quantification
m/v	Mass by volume
M	Mass of the analyte in the resin
M_{DGT}	Mass accumulated in the DGT device
M_{inorg}	Mass of the labile inorganic species
M_{org}	Mass of the labile organic species
MeHg	Methylmercury
MEMC	Methoxy ethyl mercuric chloride
MI	Mount Isa
MT	Montmorrilonite
MWHC	Maximum water holding capacity
n	Number of replicates
NGR	Generic reference levels
NPOE	Nitrophenyloctyl ether
NRCC	National Research Council of Canada
NSW	New South Wales
nv	no value
ODL	Open diffusive layer
OM	Organic matter
Pc	Particle concentration
PC_n	Phytochelatins
PIM	Polymer inclusion membrane
PIMS	Passive integrative mercury sampler
PLM	Permeation liquid membrane

PTFE	Porous polytetrafluoroethylene
PVC	Poly(vinyl chloride)
R	Ratio between concentration measured by DGT and pore water
RB	Ribaroja
R_{diff}	Coefficient calculated by DIFS
RDL	Restricted diffusive layer
RfD	Oral reference dose
ROS	Reactive oxygen species
RS	Control site
SD	Standard deviation
SE	Sequential extraction
SLM	Supported liquid membrane
SLMD	Stabilized liquid membrane device
SPM	Suspended particulate matter
SBR	Sulphate-reducing bacteria
t	Deployment time
T	Temperature
TDS	Total suspended solids
TEMED	N,N,N',N'-tetramethylethylenediamine
THg	Total mercury
TF	Transfer factor
TFA	Trifluoroacetic acid
TOMATS	Trioctylmethylammonium thiosalicylate
UNEP	United nations environment program
UNESCO	United nations educational, scientific and cultural organization

USEPA	United States environmental protection agency
$\mathbf{V_e}$	Volume of the eluent
$\mathbf{V_g}$	Volume of the resin
vs.	Versus
w/w	Weight by weight
WA	Western Australia
WHC	Water holding capacity
WHO	World Health Organization
x	Distance
XT	Xerta

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Resum

El mercuri (Hg) és un element natural i abundant a l'escorça terrestre, però la seva presència en el medi ambient ha augmentat, en gran part, degut a les activitats antropogèniques realitzades en el passat i encara en l'actualitat. És un metall pesat amb una alta toxicitat, sobretot les espècies biodisponibles del Hg ja que es poden bioacumular i biomagnificar en les cadenes tròfiques, arribant a altes concentracions de Hg en els últims eslavons de la cadena. Tanmateix, la contaminació per part d'altres metalls és ja un problema a escala global per la seva presència (en el cas del plom o el cadmi) o pel seu excés (com el níquel, el zinc o el coure) en el medi ambient. Concretament al sòl, la incorporació de la fracció biodisponible d'aquests elements en els conreus agrícoles converteix els cultius en reservoris intermedis, podent arribar als animals i humans a través de la ingesta. Això ens porta a una imprescindible demanda per a la determinació de la fracció biodisponible dels metalls en el medi ambient per al control dels diferents focus de contaminació així com la possible remediació dels ecosistemes afectats.

Per aquest motiu, en aquesta tesi doctoral s'ha desenvolupat un sistema de mostreig passiu anomenat tècnica de "Gradient de Difusió en Capa Fina" (DGT). Aquest mostrejador passiu és capaç de mesurar la concentració làbil de l'espècie en qüestió, en un medi concret, en un temps d'exposició determinat. Mitjançant el seu mecanisme de difusió és capaç de mimetitzar les membranes biològiques i, per tant, es pot considerar una bona eina per a poder avaluar la biodisponibilitat dels metalls en el medi ambient.

Ja que aquesta tècnica es pot aplicar en diverses matrius, aquesta tesi doctoral es pot separar en dos blocs. En els capítols 3, 4 i 5 hi trobem els experiments realitzats en sistemes aquàtics, mentre que en els capítols 6, 7 i 8 hi trobem els estudis al medi terrestre.

Els estudis dels capítols 3 i 4 es van realitzar a la part baixa de la conca del riu Ebre degut a l'interès mediambiental associat a la històrica contaminació per Hg per la

planta de clor-àlcali de Flix. En el Capítol 3 s'expliquen diferents proves preliminars al laboratori aplicant membranes polimèriques d'inclusió (PIM) en el disseny de la tècnica DGT per al posterior estudi de Hg biodisponible al riu Ebre. Seguidament, el disseny de PIM-DGT més òptim es va aplicar al camp juntament amb dispositius DGT per a poder ser comparats. Els resultats obtinguts mostren un perfil d'acumulació de Hg no lineal respecte al temps d'exposició. Això limita l'aplicació de la tècnica DGT amb PIM, encara que tenint en compte l'alt rendiment d'extracció de Hg que ha mostrat el TOMATS incorporat en PIM, resultaria interessant explorar la seva utilització amb altres dissenys/tipus de mostrejador.

L'estudi del Capítol 4 es va centrar en l'avaluació del Hg aigües avall de la industria de Flix a partir de la concentració de fitoquelatines de dues espècies de plantes aquàtiques endèmiques del riu Ebre. A més a més, aquests valors es van correlacionar amb el Hg biodisponible en cada punt de mostreig mitjançant la utilització de la tècnica DGT. Encara que la major concentració de Hg va ser trobada en el material particulat, la concentració de Hg biodisponible a l'aigua va ser suficient per a activar la producció de fitoquelatines. Tanmateix, els valors trobats a les plantes i el Hg biodisponible mesurat amb els dispositius DGT, depenien de la distància en que es trobaven del focus contaminant (la industria de Flix), demostrant així que la concentració de fitoquelatines és un bon indicador del Hg biodisponible en el riu.

Finalment, l'experiment del Capítol 5 es va realitzar al riu Tully, al nord-est de Queensland (Austràlia). L'objectiu va ser determinar l'impacte produït per un fungicida que contenia una base de Hg utilitzat antigament de forma massiva en els conreus del voltant d'aquest riu per a controlar una malaltia que afectava als camps de canya de sucre. A partir d'un dels punts de mostreig de sòl -on la seva concentració era 10 vegades superior-, es va trobar un augment sobtat en la concentració de Hg biodisponible en el riu mantenint-se aigües avall, probablement degut a l'escorrentia superficial del sòl cap al riu. D'aquesta manera es va demostrar l'impacte ambiental que encara provoca l'aplicació històrica de fungicides basats en Hg en els camps de cultiu sobre el medi aquàtic.

Seguidament, els capítols 6, 7 i 8 tracten sobre estudis de DGT al medi terrestre. Concretament, els dos primers es basen en l'aplicació de dos tipus de DGTs amb diferent mida de porus en dos sòls amb característiques diferents, permetent analitzar el Hg biodisponible i, per primera vegada, el Hg inorgànic. El primer estudi es va focalitzar en l'anàlisi i comparació dels sòls esmentats, aplicant els dos tipus de DGT. Els resultats van demostrar la possibilitat de mesurar els nivells de Hg inorgànic a partir de la tècnica DGT, a l'hora que prediu l'absorció per part de l'arrel de l'enciam. El segon estudi es va centrar en la possible variació del Hg inorgànic en un mateix tipus de sòl havent aplicat dos tipus d'additius orgànics (biochar i compost), observant-se una reducció en el percentatge de Hg inorgànic a mesura que augmentava la proporció de matèria orgànica al sòl.

Seguint la mateixa metodologia anterior, en el Capítol 8 es van analitzar 6 metalls d'interès en quant a la seva toxicitat: cadmi, crom, coure, níquel, plom i zinc. Mitjançant l'extracció seqüencial i juntament amb els resultats dels enciams es va avaluar l'efecte que provoca l'aplicació del additius orgànics en quant a la biodisponibilitat de cada metall, reduint o augmentant la seva absorció a les plantes conreades en diferents sòls. Encara que els percentatges de biochar aplicats van ser baixos, es va poder observar una reducció significativa de crom i plom en planta, així com l'augment de creixement de les plantes conreades en comparació amb el sòl inicial. D'aquesta manera, es va poder determinar l'efectivitat del biochar en la immobilització de certs metalls en camps agrícoles per a la seva remediació. Tanmateix, la tècnica DGT juntament amb l'extracció seqüencial, va donar bons resultats en quant a la predicció de l'absorció de metalls en planta.

Abstract

Mercury (Hg) is a natural and abundant element in the earth's crust, but its presence in the environment has increased, mostly due to both historical and present-day anthropogenic sources. It is a heavy metal with a high toxicity, especially the bioavailable species of Hg because they can be bioaccumulated and biomagnified in the food chain, reaching high Hg concentrations in the last step of the chain. Nevertheless, contamination from other metals is a global concern because of their presence (in the case of lead or cadmium) or their excess (such as nickel, zinc or copper) in the environment. Focusing on the soil, the incorporation of the bioavailable fraction of these elements in agricultural soils turns the crops into intermediate reservoirs, reaching consumers (animals and humans) though intake. This leads to an essential claim for the determination of the bioavailable fraction of the metal in the environment for the control of different pollution sources as well as the possible remediation of affected ecosystems.

For this reason, a passive sampling technique called "Diffusive Gradient in Thin film" (DGT) was developed in this PhD. This passive sampler is able to measure the labile concentration of the species concerned in the environment, in a specific deployment time. Through its diffusive mechanism, the DGT technique is able to mimic biological membranes and, therefore, it can be considered a good tool for evaluating the bioavailability of metals in the environment.

Due to the capacity of this technique to be used in different matrixes, this thesis could be separated into two different parts. In Chapters 3, 4 and 5 the experiments in aquatic systems can be found, whereas Chapters 6, 7 and 8 contain studies conducted in terrestrial environments.

The studies described in Chapters 3 and 4 were performed in the lower part of the Ebro River basin due to the environmental interest associated with the historical Hg pollution from the chlor-alkali factory at Flix. In the study described in Chapter 3, different preliminary probes were developed in the laboratory for the application

of polymer inclusive membranes (PIM) in the DGT design for the study of Hg in the Ebro River. Subsequently, the most optimal PIM-DGT design was applied in the field together with the DGT devices so they could be compared. The results demonstrated a nonlinear accumulation profile of Hg with the deployment time. This effect limits the application of the DGT technique with PIM, although taking into account the high performance of Hg extraction that TOMATS incorporated in a PIM has shown, it is interesting to explore its use with other designs/types of samplers.

The study described in Chapter 4 was focused on the evaluation of Hg downstream of the Flix industry based on the concentration of phytochelatins in two aquatic and endemic species of the Ebro River. In addition, these values were correlated with the bioavailable Hg at each sampling point through the application of the DGT technique. Although the highest Hg concentration was found in the particulate material, the bioavailable Hg in water was sufficient to activate the production of phytochelatins. However, the values found in plants and bioavailable Hg measured by DGT, depended on the distance they were from the pollution source (the Flix factory), thus demonstrating that the concentration of phytochelatins is a good indicator of bioavailable Hg in rivers.

Finally, the experiment set out in Chapter 5 was carried out in the Tully River, in the north-east of Queensland (Australia). The aim was to evaluate the impact of the massive historical use of a Hg-based fungicide around this river to control pineapple disease in sugarcane cultivations. Based on one of the sampling points – where its concentration was 10 times higher -, a sudden increase in the concentration of bioavailable Hg was noted in the river, and maintained downstream, probably due to the superficial runoff from the soil to the water. In this way, the environmental impact that the historical application of Hg-based fungicides in agricultural fields still causes on aquatic systems has been demonstrated.

Chapters 6, 7 and 8 included studies conducted in terrestrial systems. Concretely, the first two experiments were based on the application of two different DGT devices with different pore size in two soils with different characteristics, allowing the measurement of bioavailable Hg and, for the first time, inorganic Hg. The first study was focused on the analysis and comparison of the mentioned soils, applying both types of DGT. The results showed the possibility of measuring the levels of inorganic Hg using the DGT technique, at the same time that it predicted uptake by lettuce. The second study focused on the possible variation of inorganic Hg in the same type of soil applying two types of organic amendments (biochar and compost), observing a reduction in the percentage of inorganic Hg as the proportion of the organic matter in the soil increased.

Finally, Chapter 8 describes how a similar methodology to the one described in Chapter 7 was applied for 6 different trace metals: cadmium, chromium, copper, nickel, lead and zinc. By means of sequential extraction and, together with lettuce analysis, the effect of the organic amendments to immobilize metals in soil was evaluated, reducing or increasing their uptake by plants grown in different soils. Although the percentages of biochar added to the soil were low, a significant reduction of chromium and lead could be observed, as well as an increase in lettuce growth compared to the initial soil. In this way, the effectiveness of biochar in the immobilization of certain trace metals in agricultural fields for their remediation could be determined. However, the DGT technique together with sequential extraction, gave good results in the prediction of metal uptake by lettuce.

Resumen

El mercurio (Hg) es un elemento natural y abundante en la corteza terrestre, pero su presencia en el medio ambiente ha aumentado, en gran parte, debido a las actividades antropogénicas realizadas en el pasado y aún en la actualidad. Es un metal pesado con una alta toxicidad, sobre todo las especies biodisponible del Hg ya que se pueden bioacumular y biomagnificar en las cadenas tróficas, llegando a altas concentraciones de Hg en los últimos eslabones de la cadena. Asimismo, la contaminación por parte de otros metales es ya un problema a escala global por su presencia (en el caso del plomo o el cadmio) o por su exceso (como el níquel, el zinc o el cobre) en el medio ambiente. Concretamente en el suelo, la incorporación de la fracción biodisponible de estos elementos en las cosechas agrícolas convierte a los cultivos en reservorios intermedios, pudiendo llegar a los animales y humanos a través de la ingesta. Esto nos lleva a una imprescindible demanda para la determinación de la fracción biodisponible de los metales en el medio ambiente para el control de los diferentes focos de contaminación así como la posible remediación de los ecosistemas afectados.

Por este motivo, en esta tesis doctoral se ha desarrollado un sistema de muestro pasivo llamado técnica de "Gradiente de Difusión en Capa Fina" (DGT). Este dispositivo pasivo es capaz de medir la concentración lábil de la especie en cuestión, en un medio concreto, en un tiempo de exposición determinado. Mediante su mecanismo de difusión, la técnica DGT es capaz de mimetizar las membranas biológicas y, por lo tanto, se puede considerar una buena herramienta para poder evaluar la biodisponibilidad de los metales en el medio ambiente.

Ya que esta técnica se puede aplicar en diversas matrices, esta tesis se puede separar en dos bloques. En los capítulos 3, 4 y 5 encontramos los experimentos realizados en sistemas acuáticos, mientras que en los capítulos 6, 7 y 8 encontramos los estudios en el medio terrestre.

Los estudios de los capítulos 3 y 4 se realizaron en la parte baja de la cuenca del río Ebro debido al interés medioambiental que aún supone la histórica contaminación por Hg debido a la planta de cloro-álcali de Flix. En el Capítulo 3 se explican diferentes pruebas preliminares en el laboratorio aplicando membranas poliméricas de inclusión (PIM) en el diseño de la técnica DGT para el posterior estudio de Hg biodisponible en el río Ebro. Seguidamente el diseño de PIM-DGT más óptimo se aplicó en el campo juntamente con los dispositivos DGT para poder ser comparados. Los resultados obtenidos muestran un perfil de acumulación de Hg no linear respecto al tiempo. Esto limita la aplicación de la técnica DGT con PIM, aun teniendo en cuenta el alto rendimiento de extracción de Hg que ha mostrado TOMATS incorporado en PIM, resulta interesante explorar su utilización en otros diseños/tipos de muestreadores.

El estudio del Capítulo 4 se centró en la evaluación de Hg aguas abajo de la industria de Flix a partir de la concentración de fitoquelatinas de dos especies de plantas acuáticas endémicas del río Ebro. Además, estos valores se correlacionaron con el Hg biodisponible en cada punto de muestreo mediante la aplicación de la técnica DGT. Aunque la mayor concentración de Hg se encontró en el material particulado, la concentración de Hg biodisponible en agua fue suficiente para activar la producción de fitoquelatinas. Asimismo, los valores encontrados en las plantas y el Hg biodisponible determinado por dispositivos DGT, dependían de la distancia en que se encontraba el foco de contaminación (la industria de Flix), demostrando así que la concentración de fitoquelatinas es un buen indicador de Hg biodisponible en el río.

Finalmente, el experimento del Capítulo 5 se realizó en el río Tully, al noreste de Queensland (Australia). El objetivo fue estudiar el impacto producido por un fungicida que contenía una base de Hg utilizado antiguamente de forma masiva en los cultivos de alrededor de este río para controlar una enfermedad que afectaba a los campos de caña de azúcar. A partir de uno de los puntos de muestreo del suelo –donde su concentración era 10 veces superior-, se encontró un aumento repentino en la concentración de Hg biodisponible en el río manteniéndose aguas abajo, probablemente debido a la escorrentía superficial del suelo hacia el río. De esta manera, se demostró el impacto ambiental que aún provoca la aplicación histórica de fungicidas basados en Hg en campos de cultivo sobre el medio acuático.

Seguidamente, los capítulos 6, 7 y 8 tratan sobre los estudios de DGT en el medio terrestre. Concretamente, los dos primeros se basan en la aplicación de dos tipos de DGT con diferente tamaño de poro en dos suelos con características diferentes, permitiendo analizar el Hg biodisponible y, por primera vez, el Hg inorgánico. El primer estudio se focalizó en el análisis y comparación de los suelos mencionados, aplicando los dos tipos de DGT. Los resultados demostraron la posibilidad de medir los niveles de Hg a partir de la técnica DGT, a la vez que predice la absorción por parte de la raíz de la lechuga. El segundo estudio se centró en la posible variación de Hg inorgánico en un mismo tipo de suelo habiendo aplicado dos tipos de aditivos orgánicos (biochar y compost), observándose una reducción en el porcentaje de Hg inorgánico a medida que aumentaba la proporción de materia orgánica en el suelo.

Siguiendo la misma metodología anterior, en el Capítulo 8 se analizaron 6 metales de interés en cuanto a su toxicidad: cadmio, cromo, cobre, níquel, plomo y zinc. Mediante la extracción secuencial y con los resultados de las lechugas se evaluaron los efectos que provocaba la aplicación de aditivos orgánicos en la biodisponibilidad de cada metal, reduciendo o aumentando su absorción en las plantas cultivadas. Aunque los porcentajes de biochar aplicados fueron bajos, se pudo observar una reducción significativa de cromo y plomo en planta, así como un aumento en el crecimiento de las plantas cultivadas en comparación con el suelo inicial. De esta manera, se pudo determinar la efectividad de la aplicación de biochar en la inmovilización de ciertos metales en campos agrícolas para su remediación. Asimismo, la técnica DGT juntamente con la extracción secuencial, dieron buenos resultados en la predicción de la absorción de metales en planta.

Chapter 1

Introduction

1.1 Mercury and other trace metals

Trace metals are defined as the metals present in the environment at low concentrations (mg kg^{-1} or less) (He et al., 2005). Many trace metals are essential as micronutrients for the biota and for the development of human beings. In contrast, other trace metals are toxic at very low concentrations and highly toxic for the environment. This Thesis is mainly focused on the analysis of Hg. However, other trace metals have been considered since some are micronutrients for living beings, but toxic at high concentrations (copper (Cu), nickel (Ni) and zinc (Zn)), while other elements are toxic at very low concentrations (cadmium (Cd) and lead (Pb)), being a problem just to exist in the food chain. Chromium (Cr) has also been considered, although it could be either a micronutrient (Cr^{III}), or toxic (Cr^{VI}), depending on its valence.

1.1.1 Mercury

1.1.1.1 Characteristics

Mercury is considered a unique element in the Earth because of its fascinating and singular properties. Throughout history, it has been used for different applications, such as barometers and electrical switches, but has also been considered a global pollutant due to its toxicity (Gonzalez-Raymat et al., 2017; Kabata-Pendias, 2011).

But, what are its unique properties? Besides being the 80th element of the periodic table, its silver colour and its capacity to form an amalgam with gold and other metals, Hg is the only metal which is liquid at room temperature and pressure. This is why it has historically been named “hydrargyrum”, which means “liquid silver” in Latinized Greek. Its density is 13.579 g cm^{-3} at 20ºC, and it has a boiling point of 356.58ºC and a melting point of -38.87ºC, the lowest among metals (Amde et al., 2016; Fernández Gómez, 2014; Gonzalez-Raymat et al., 2017).

Hg can exist in ecosystems in a gas or liquid state, and a large proportion is found in the atmosphere as a gas. It can also form a metal-metal bond with itself (Hg_2^{+2}), but it is not stable and rapidly breaks down into Hg^{2+} and Hg^0 (Boszke et al., 2002). Due to the fact that it has affinity to form bonds with sulfur (S^{2-}), chlorine (Cl^-) and organic compounds, Hg has different forms depending on its volatilization and mobilization in ecosystems (Kabata-Pendias, 2011):

(1) Easy volatilization: elemental mercury (Hg^0) and ethylmercury (C_5H_2Hg).

(2) Easy solubilization: mercury (II) chloride ($HgCl_2$), mercury hydroxide chloride (Hg(OH)Cl) and mercury(II) hydroxide ($Hg(OH)_2$).

(3) Low mobility: methylmercury (CH_3Hg^+) and sulfanylmethylmercury ($CH3HgS^-$).

(4) Nonreactive species: mercury sulfide or cinnabar (HgS), mercury(II) cyanide ($Hg(CN)_2$) and mercuric ion (Hg^{2+}) bound to some organic matter forming organomercury compounds, more prevalent in aquatic systems.

In this Thesis, the CH_3Hg^+ species is mentioned as MeHg and, using the same criteria, ethylmercury is referred to as EtHg.

1.1.1.2 Sources

Hg can be found in the Earth's crust (an average of 0.07 mg kg^{-1}) (IUSS Working Group WRB, 2006) as a natural component and it can be released in several ways to air, water and land. Natural sources are responsible for only 10% of the 5500-8900 Mg of total mercury emitted to the atmosphere, which includes natural mercury-containing rocks, volcanic eruptions and geothermal activity as the processes that most contribute (UNEP, 2013).

Besides, 30% of the total amount of Hg entering the atmosphere each year is from anthropogenic sources of Hg emissions. Figure 1.1 shows the two types of emissions which can be differentiated.

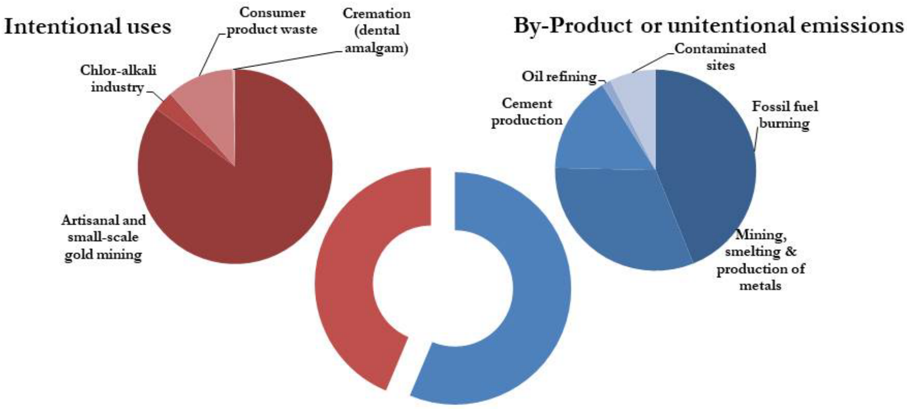


Figure 1.1: Anthropogenic Hg emissions as a percentage. Adopted by UNEP (2013).

On the one hand, Hg comes from unintentional emissions, which include processes such as oil refining, cement production, mining and smelting or fossil fuel burning. When Hg is not the primary used product, it is also emitted since it is present as an impurity in fuels and raw materials. A high degree of uncertainty exists in estimating mercury in this type of emissions because, depending on the products used, different percentages of Hg are released into the environment.

On the other hand, Hg also comes from intentional emissions, of which the major global source is artisanal and small-scale gold mining to extract gold from rocks, soils and sediments. Mercury is used by miners to create an amalgam to separate gold from other materials and, subsequently, Hg is separated from the gold by burning the amalgam. In addition to being illegal, the vapours are very toxic for people working in this industry. The main industrial use of Hg is in the chlor-alkali industry where mercury-cell technology is used in the production of chlorine and caustic soda. In this Thesis, a chlor-alcali factory (Flix factory) on the Ebro River (Spain) has been studied. In addition, several products, including batteries, fluorescent light bulbs, electrical and electronic devices, which once used Hg, still exist, and the Hg that these products contain can enter waste streams (Kabata-Pendias, 2011; UNEP, 2013).

Finally, the last 60% of Hg emissions are due to Hg re-emission and re-mobilization. These sources should not be considered as having either a natural or anthropogenic background, as the specific origin is difficult to identify by the time the Hg has been re-emitted. Re-emission is a result of natural processes converting inorganic and organic Hg forms to Hg^0, which readily returns to the air. However, estimating re-emission and re-mobilization rates is very difficult. Different processes are involved, such as forest fires, biomass burning or re-mobilization from soils or sediments caused by rain or flood events. A big concern exists due to the fact that Hg^0 can stay in the atmosphere for long periods of time and travel long distances, be deposited and later re-mobilized again. Hence, this cycle can be repeated, maintaining elevated levels in air and water, even after anthropogenic sources have been lowered (UNEP, 2013).

1.1.1.3 Biogeochemical cycle

The biogeochemical cycle of Hg is represented in Figure 1.2. Hg^0 is released into the atmosphere via natural and anthropogenic sources (see 1.1.2), oceans (re-emitted by reduction of Hg^{2+} in the water column through photochemical and biotic reactions), and from the Earth's crust due to geothermal activities (Gonzalez-Raymat et al., 2017).

When Hg^0 is in the atmosphere, it can be oxidized by many species such as O_3, OH, NO_3 or H_2O_2 (Lin et al., 2011), where Br radical is likely to be the dominant oxidant, generating different Hg^{2+} species. Hg^{2+} can be reduced to Hg^0 and the cycle can be repeated for a long time. In addition, Hg^0 can interact with different small particles (e.g. dust, sea salt aerosols) through sorption and desorption, influencing the wet (Hg^{2+} by rain and snow) and dry ($Hg^{2+}(p)$, gravitational settling and surface sorption) deposition (Obrist et al., 2018; UNEP, 2013).

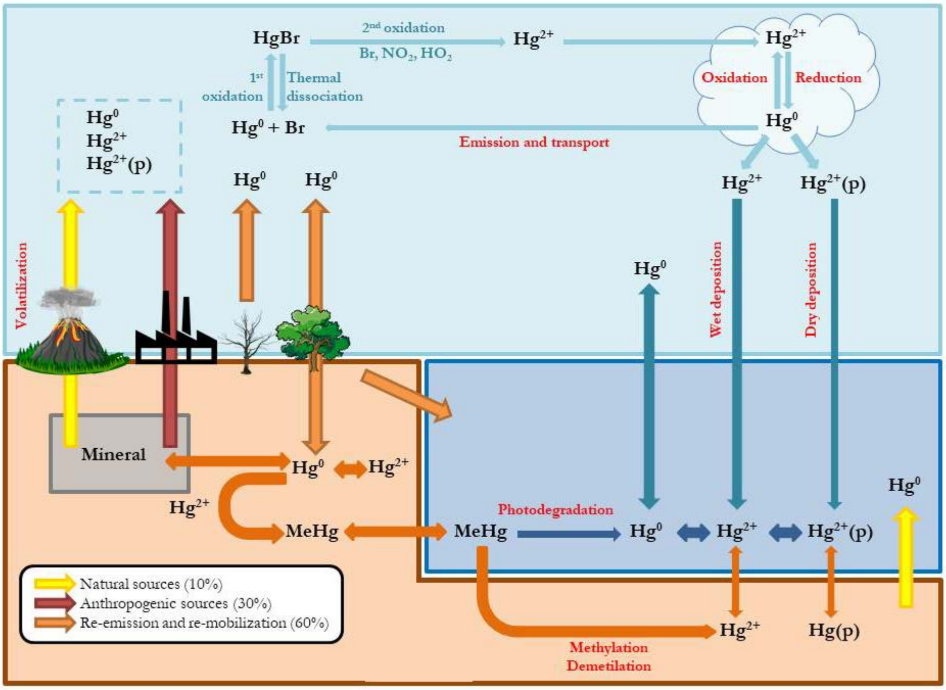


Figure 1.2: Biogeochemical cycle of Hg.

When wet and/or dry deposition is produced, Hg^{2+} can be converted into MeHg which is one of the most toxic species of Hg as it can easily be accumulated in living organisms and become concentrated through the food chain. This process is possible due to the existence of microbes which are responsible for Hg methylation/demethylation. This process depends on the specific characteristics of aquatic systems, such as stratification, depth of the anoxic hypolimnion and the organic content of sediments (Obrist et al., 2018). MeHg can be demethylated back to Hg^{2+} again or photodegraded to Hg^0, which is released into the atmosphere (Selin, 2009), making the biogeochemical cycle continue.

1.1.2 Trace metals

1.1.2.1 Cadmium

Cadmium (Cd) is a non-essential element and one of the most eco-toxic trace metals, having adverse effects on all the biological processes that are vital for living beings. Cd has only one main oxidation state (+2). Most inorganic compounds are solubles in water while cadmium sulfide (CdS) and cadmium oxide (CdO) are practically insoluble in water. It is persistent in the environment, relatively mobile in soils and freshwater systems, but bioavailability depends on its form (Kabata-Pendias, 2011; Shahid et al., 2016; UNEP, 2015). Confusion still surrounds the concept of "bioavailability" in the literature, although it may include: the physico-chemical availability of metals in the exposure medium, the demand by the biota, and the toxicological behaviour inside the organism (Landner and Reuther, 2004).

Cd is released to the environment primarily as fine suspended particulate matter from both natural and anthropogenic causes. Contributions from natural sources are volcanoes (820 Mg yr^{-1}), biogenic substances (240 Mg yr^{-1}), wind-borne dust (210 Mg yr^{-1}), terrestrial biomass burning (110 Mg yr^{-1}) and sea-salt spray (60 Mg yr^{-1}), a total of 32.5% of total Cd emissions in the mid-1990s. Contributions from anthropogenic sources include fossil fuel combustion (691 Mg yr^{-1}), non-ferrous metal production (2171 Mg yr^{-1}), iron and steel production (64 Mg yr^{-1}), cement production (17 Mg yr^{-1}) and waste disposal (40 Mg yr^{-1}), accounting for the remaining 67.5%. Therefore, the anthropogenic input is higher than the natural source, dominating the natural biogeochemical cycle of Cd (Cullen and Maldonado, 2013).

The transport of Cd in the atmosphere depends on particle size, stack height and meteorology, where wet deposition can account for 80-90% of the deposition flux of Cd in the environment (Cullen and Maldonado, 2013; UNEP, 2015). If Cd is released in the soil, it can be incorporated into minerals or bound to soil surfaces through different processes, such as absorption, co-precipitation, ion exchange and complexation reactions. It is important to say that Cd mobility in the soil decreases very slowly over time since time is needed for the complete equilibration of deposited Cd with the soil solid phase. Depending on soil properties (i.e. soil mineralogy, pH, cation exchange capacity (CEC), organic matter (OM), Fe and Mn oxides and clay particles), Cd can be free in the soil solution (Shahid et al., 2016), bioavailable for plants, and enter the food chain.

Cd may enter aquatic systems from the pollution source releases explained before, or from deposition from air or from leaching from rocks and soils to aquatic systems (National Research Council., 1997). The speciation of Cd in freshwaters depends on the pH because this influences the chemical speciation of the metal and its binding with humic substances and other ligands (Wright and Welbourn, 1994).

Cd is used in several industrial activities and nowadays the main use of Cd is in the Ni-Cd battery production sector with almost 80% of global consumption. Besides, Cd has other uses, such as protection against corrosion in the steel industry, pigments, preparation of alloys and as a stabilizer for plastics (Kabata-Pendias, 2011; Shahid et al., 2016; UNEP, 2015).

1.1.2.2 Chromium

Chromium (Cr) exists in the world in oxidation states ranging from Cr^{4-} to Cr^{6+} and the most common form in natural systems is Cr^{3+}. Cr^{6+} compounds are also found in small quantities from natural sources, but the amount in the environment has increased due to anthropogenic pollution. The particularity of this form is its higher toxicity (100-fold more toxicity than Cr^{3+}) due to its high-water solubility and mobility, as well as easy reduction (Saha et al., 2011; WHO, 2000a).

The main natural source Cr emissions are from windborne soil particles (62.4%) and volcanoes (34.7%) and, to a lesser degree, from sea salt spray, forest fires and biogenic sources (Motzer, 2004). The total residence time in the atmosphere is less than 14 days, so Cr is removed from the air by dry and wet deposition depending on the climate properties, and particles less than 5 μm in diameter remain airborne and could be transported over large distances by the wind. Anthropogenic sources of Cr also perturb the natural cycle (Motzer, 2004). Anthropogenic atmospheric releases are in aerosol form as a result of processing (FeCr, stainless steel production and fossil fuel combustion), leather tanning, electroplating, cement manufacture and wood preservation. In addition, ground and water releases exist linked to mining and milling operations, metal plating, chromite-ore processing residue and leather tanning operations. The main source of pollution is considered to be from dyestuffs and leather tanning since the wastes are discharged directly into waste streams (solid and liquid part) (Johnson et al., 2006; Kabata-Pendias, 2011).

The use of Cr is principally in the metallurgical (67%), refractory (18%) and chemical industries (15%). It was first used as a pigment because of the yellow colour of the metal. Depending on the oxidation states of Cr, it has different uses: Cr^0 is used mainly for stainless steel production, alloy production, metal and alloy manufacturing; Cr^{3+} is used for metal and alloy manufacturing, brick lining, chrome plating, leather tanning, and in textiles and copy machine toner; and, finally, Cr^{6+} is used for chrome plating, leather tanning, and in textiles and copy machine toner (Kabata-Pendias, 2011; Saha et al., 2011).

1.1.2.3 Copper

Copper (Cu) is an essential element for living beings, required in biological processes including electron transfer enzymes, even though high concentrations of Cu can be toxic. A transition metal, copper generally occurs in three different oxidation states: Cu, Cu^+ and Cu^{2+}. Cu has a wide range of applications, such as in various conductor materials, providing functions such as durable plumbing tubing and electrical transmission (Kabata-Pendias, 2011; Rauch and Graedel, 2007).

Cu is abundant in the Earth's crust, where its principal mineral is chalcopyrite ($CuFeS_2$) because it has a strong affinity for sulfur. Other minerals are bornite (Cu_5FeS_4), chalcocite (Cu_2S) and covellite (CuS) (Kabata-Pendias, 2011). Thus, the biggest mineral reserve in the Earth is copper ore.

As a natural emission source, Cu is released to the environment as the result of volcanic emissions, crustal subduction and production, sea spray, crustal rock weathering, biomass burning and the extraterrestrial flow from micrometeorites. On the other hand, some natural processes exist which can absorb part of the Cu, such as mobilization by freshwaters and plant utilization. Cu is liberated within anthropogenic emissions by fossil fuel combustion, the application of fertilizers and pesticides in agricultural crops, agricultural burning, mining, tailing and slag from metal fabrication and manufacturing and, finally, by loss from products in use (i.e. the corrosion of Cu roofs or the abrasion of Cu-containing brake linings) (Rauch and Graedel, 2007). However, the atmosphere is not a net accumulator of this metal because Cu lifetime in air is on average 10 days.

1.1.2.4 Lead

Lead (Pb) has no biological role and, indeed, is considered toxic, teratogenic and carcinogenic. It can mainly be found as Pb^{2+}, although Pb^{4+} is also found. There are two types of Pb in nature: primary Pb is of geogenic origin and was incorporated into minerals at the time of their formation; secondary Pb is of radiogenic origin from the decay of U and Th. As a result, the ratio of Pb isotopes can be used to identify pollution sources from the contamination found in a particular site (Kabata-Pendias, 2011).

The main natural sources of Pb are volcanoes, airborne soil particles, sea spray, biogenic material and biomass burning. On the other hand, the main natural source in sediments and soils is dust from rock weathering. But, the biogeochemical cycle of Pb is altered by human activities, which exceeded natural sources by an order of magnitude in 1983 (Weiss et al., 2007). Anthropogenic sources are from non-ferrous metal smelting, coal combustion, waste incineration and other industrial sources, and leaded petrol use.

Pb has a short residence time in the atmosphere (around days or weeks). So, contribution of Pb from intercontinental transport is low, and it is mainly transported local, national or regional distances. Conversely, the residence time in oceans is about 100 to 1000 years, which may indicate a greater potential for ocean transport than in the case of the atmosphere (UNEP, 2010).

Despite its toxicity, Pb has been used by humans for many centuries. One of the largest uses worldwide is in lead-acid batteries for conventional cars. Many other uses exist, such as in the manufacture of solders, alloys, pigments, cables and chemicals. It used to be added to petrol as an antiknock additive, but has been phased out in developed countries due to the high amount of Pb released into the atmosphere (Kabata-Pendias, 2011; UNEP, 2010).

1.1.2.5 Nickel

Nickel (Ni) is an essential element for healthy plant life, so it is found in most vegetables and fruits, animal species and prokaryotic organisms. Ni deficiency in plants causes a reduction in plant growth and senescence, while if it exceeds the maximum tolerable amount it is considered a potential human carcinogen (Fahad et al., 2018; Tian et al., 2012; WHO, 2000b; Wuana and Okieimen, 2011). Although Ni is an essential element for plants, it can cause toxicity at high concentrations. Ni has several oxidation states (from -1 to +4), although Ni^{2+} is the most common state in the biological systems (Fahad et al., 2018). Ni occurs naturally in the environment and is present in rocks, soils, terrestrial and aquatic species, being the fifth most common element in the Earth (Mukherjee, 1998). Most Ni is found in the inaccessible core of the Earth although it is used in over 300,000 products for consumer, industrial, military, transport, aerospace, marine and architectural applications. The main use of Ni (an estimated 68%) is used to produce stainless steel. Besides, several uses exist for Ni, such as in magnetic components, electrical equipment, tools and vessels for medicine and food technology, dyes in ceramics and also in batteries containing Ni-Cd compounds. Fourteen percent of Ni is lost to other metal markets where this metal is an unwanted constituent of carbon steel and copper alloy scrap (Kabata-Pendias, 2011; Reck et al., 2008).

Ni is released into the atmosphere from natural and anthropogenic sources. The most relevant natural sources are from windblown soil particles from eroded areas (30-50%), dust, and volcanic eruptions (40-50%). Besides, natural concentration in the air depends on local sources, specific climatic conditions and the ratio of natural to anthropogenic emissions, which is roughly one-third. On the other hand, the main anthropogenic source is oil combustion, which has been increasing in the last three decades due to the rise in the production of electricity and heat by oil combustion, accounting for almost 90% of global anthropogenic Ni emissions (Kabata-Pendias, 2011; Nieminen et al., 2007).

Depending on the type of emission, Ni can be found in the atmosphere as particulate matter (natural sources) and in the form of oxides and sulfates of small particle size (anthropogenic sources) where around 15-90% is soluble. Ni can be dispersed and deposited by both dry and wet deposition, although the dry deposition of aerosols and particulates predominates. Natural background levels in water are relatively low where divalent Ni is the predominate form (Nieminen et al., 2007; Tian et al., 2012).

1.1.2.6 Zinc

Zinc (Zn) is an essential trace metal for living beings, playing an important role in biological processes such as cell division, protein synthesis, the immune system and growth. In the environment Zn is present in the divalent free ion (Zn^{2+}), which is a source of toxicity/bioavailability. Moreover, depending on the biotic and abiotic factors of the soil and water, it can interact with various constituents and exist as many different complexes (Malle, 1992; WHO, 2001).

Natural and anthropogenic sources exist for Zn. However, the influence of natural Zn cycling processes is much more important than from human activities on a global scale. The largest natural source released into the atmosphere is sea salt followed by soil particle flux, volcanoes, fires and meteoric dust (Malle, 1992; Richardson et al., 2001). Regarding anthropogenic sources, three types of pollution can be found: controlled emissions from industrial processes; fugitive emissions from mining, handling or transport operations and diffusive emissions from the use of zinc-containing products. Controlled and fugitive emissions are released as dust and fumes to the atmosphere and as waste water to aquatic systems. Finally, diffusive emissions basically fertilizers, pesticides, sewage sludge and manure, are released to the soil (WHO, 2001).

Zn is used in many industries where its main use is mostly as a protective coating for iron and steel. It is an important component of various alloys and Zn dust is widely used as a catalyst in different chemical productions. Also, organo-Zn compounds are used as fungicides, antibiotics and lubricants. The inorganic compounds are used in batteries, and automobile equipment (1%) (Kabata-Pendias, 2011; WHO, 2001).

1.2 Concern about mercury and trace metals in the environment

1.2.1 Effect of Hg in aquatic ecosystems

Mercury can reach aquatic systems due to the deposition of Hg^0 (UNEP, 2013) where it can be transformed by the oxidation of Hg^0 to Hg^{2+}. Different processes are included in the oxidation of Hg^0: (a) a slow oxidation rate in the presence of O_2 and chloride ions; (b) photochemical reactions by OH^- and CO_3^- from the photolysis of dissolved organic matter (DOM) and NO_3^- respectively, and (c) oxidation in the dark by the interaction between Hg^0 and thiol groups present in DOM (Gonzalez-Raymat et al., 2017). In fact, Hg^{2+} is the dominant form of Hg in oceans and freshwaters, as it is more soluble, reactive and, consequently, more bioavailable for living beings.

However, Hg is lost in two ways from aquatic systems because of chemical and photochemical reactions. One way is by the reduction of Hg^{2+} to Hg^0, becoming more volatile so it can be re-emitted to the atmosphere. On the other hand, Hg^{2+} can bind to particulates in water, settling out rapidly and be buried in sediments (UNEP, 2013).

Two important processes related to the production of MeHg exist for Hg^{2+} in aquatic systems: methylation and demethylation that occur simultaneously in the environment. Both processes are important for MeHg cycling and determine MeHg levels in freshwaters and oceans:

(1) Methylation is the process by which inorganic mercury is converted into MeHg. Acidic conditions ($pH<4$) favour the formation of MeHg. There are two different pathways: biotic and abiotic. The biotic process is the dominant pathway of Hg methylation where sulphate-reducing bacteria is primarily responsible (Wuana and Okieimen, 2011). In addition, other microorganisms are involved in this process, such as sulfate and (SRB) iron-reducing bacteria (IRB) and methanogens. The abiotic pathway for MeHg formation occurs through photomethylation and other chemical pathways, but is rarely found to contribute significantly to MeHg production in aquatic systems, except in lake waters. Commonly, methylation can occur in freshwaters primarily in sediments and in oceans at intermediate depths, between 200 and 1000 metres in the water column (Hamelin et al., 2011; Li and Cai, 2013; UNEP, 2013).

(2) Demethylation is the reverse process of methylation consisting of the degradation of MeHg to inorganic Hg and CO_2, the major sink of MeHg in freshwaters. It also consists of two pathways: through biotic processes, such as SRB and methanogens, which are the primary microorganisms for this process mainly occurring in sediments and periphyton; and abiotic processes, mostly photodegradation, the dominant process in the water column (Hamelin et al., 2011; Yong et al., 2016).

When MeHg reaches aquatic systems, it can be bioaccumulated and biomagnified in living beings through the food chain thanks to its high bioavailability. Some studies have demonstrated that larger fish have higher Hg concentrations because of bioaccumulation in freshwaters (Eagles-Smith et al., 2016) and oceans (Matulik et al., 2017).

Conversely, some environmental factors exist in aquatic systems which can indirectly influence Hg concentrations in fish, such as Hg methylation rates, pH and dissolved organic carbon (DOC) concentrations (Hanna et al., 2015). In addition, studies have shown that selenium (Se), which is an essential element for functional proteins in living organisms, has a naturally protective effect against Hg toxicity because of its affinity to form stable Hg-Se compounds. Thus, Se can sequester Hg from biological processes (Arcagni et al., 2017).

1.2.2 Mobility and toxicity in soils

1.2.2.1 Mercury

The largest Hg pools in the terrestrial environment are located in soils (Obrist, 2012) and, depending on the type of rock, its abundance varies and higher concentrations are found in Histosols and Cambisols (Kabata-Pendias, 2011). Hg can be found in the soil in various inorganic and organic forms, such as Hg^0, Hg_2^{2+}, Hg^{2+} and alkylated compounds (mainly MeHg and EtHg). Besides these compounds, other Hg species found in contaminated soil-sediment include HgS (which is the most important Hg ore), $HgCl_2$, Hg_2Cl_2, HgO, CH_3HgCl and CH_3HgOH when every compound has different solubility properties (He et al., 2015). Different species of Hg are emitted to the atmosphere from some types of sources (see 1.1.1.2) and they return to the soil, which is one of the principal sources of Hg to terrestrial environments.

Different properties affect the accumulation and retention of Hg species: type of soil (i.e. moisture, pH and genesis), dissolved species (Cl^-, S^{2-}), organic matter, microbial activity, redox potentials, atmospheric deposition, sewage irrigation, fertilizers and other human activities. Besides, the distribution of Hg in soils is different, depending on the soil organic matter, latitude, annual precipitation, leaf area index and vegetation greenness (Gonzalez-Raymat et al., 2017; He et al., 2015; Obrist et al., 2018; Selin, 2009; Wang et al., 2016).

OM in the top layer of soil is more concentrated than in the other layers because of the biodegradation of dead plants by microorganisms that live in the soil. For this reason, OM plays an important role in the sorption capacity of Hg species which come from atmospheric deposition, although it also depends on the pH (Gonzalez-Raymat et al., 2017). At acid pH, OM is the most efficient Hg sorbent and at neutral to alkaline pH, mineral contents (metal oxides) are more efficient sorbents (Kabata-Pendias, 2011; Leterme and Jacques, 2013). However, Hg cannot be bound to carbons directly, but will bind to functional groups in OM, particularly S as a thiol-binding site. Moreover, in soils with low OM, Hg is more reactive and susceptible to methylation (Sipkova et al., 2016). But it is also true that it depends on the source of contamination: the bonding of Hg to sulfur is a major process in contaminated soil, while organically bound Hg is present in uncontaminated soil with soluble Hg compounds (Han et al., 2006).

1.2.2.2 Trace metals

The environmental and health effects of trace metal exposure from soil depend in particular on the mobility and availability of each metal (Borgese et al., 2013). The crustal and world-soil average for the studied trace metals are shown in Table 1.1. In fact, some parameters exist which determine these factors and, linked to these, the toxicity of the trace metals in the soil (Kabata-Pendias, 2011):

Table 1.1: Crustal and world-soil average for each element (mg kg^{-1}).

Element	Crustal average[1]	World-soil average[1]
Cd	0.1	0.41
Cr	100	59.5
Cu	55	38.9
Hg	0.07	0.07
Ni	20	29
Pb	15	27
Zn	52-80	70

[1] Kabata-Pendias (2011) pp.41-42.

(1) Soil pH directly influences the bioavailability of metal cations (Cd, Cr, Cu, Ni, Pb and Zn), whereby acidic conditions are the most favourable for their

mobility. Thus, at neutral or alkaline pH, these trace metals are not bioavailable, especially Pb and Cr, which are inherently immobile (Laghlimi et al., 2015).

(2) Redox potential (Eh) is established by oxidation-reduction from microbial activity that can convert contaminants less toxic and more stable in soil (Laghlimi et al., 2015). In addition, redox potential also controls the transformation reactivity of Fe- and Mn-oxides, which is the major sink of metals in soils (Caporale and Violante, 2016).

(3) Cation exchange capacity (CEC) varying in the order of Cd<Pb<Cu<Zn<Ni<Cr. The CEC of the soil is the total amount of exchangeable cations which the soil can absorb. This factor is directly related with OM and the clay fraction in the soil. So, decreasing CEC values depletes the capacity of the soil for retaining trace metal ions and, on the contrary, with a large CEC value the soil can retain large amounts of trace metals (Yong et al., 2016).

(4) Fine granulometric fraction (<0.02 mm) varying in the order of Cd<Pb<Cu<Zn<Ni<Cr. A relationship exists between fine particles and metal contamination because this fraction is more reactive and has a higher surface area than bigger particles (Laghlimi et al., 2015).

(5) Organic matter varying in the order of Cd<Cu<Zn<Ni<Cr<Pb. Adding OM to the soil helps to reduce the absorption of trace metals by plants. Besides increasing the CEC of soils, OM has a high affinity for trace metals because of the presence of ligands that can form chelates with them, thus reducing the bioavailability of metals due to sorption processes (Bolan et al., 2014; Laghlimi et al., 2015).

(6) Oxides and hydroxides (Fe, Mn and Al) varying in the order of Cd<Cu<Zn<Cr<Pb<Ni. Some oxides, such as Fe, Mn and Al, can easily change their oxidation state under suboxic conditions and they also have a

high surface area and a high affinity for ion absorption (Kabata-Pendias, 2011). Vodyanitskii (2006) demonstrated that, depending on the absorbed compound, Cr and Ni have more affinity to Fe-hydroxides, Cd, Cu and Ni to Mn-oxides, and Zn to phylosilicates.

(7) Microorganisms. The cell surface of the bacteria in the soil is negatively charged which can contain different types of negatively charged functional groups (i.e. carboxyl, hydroxyl or phosphoryl) that can sorb metal cations (Caporale and Violante, 2016).

Additionally, soil pH and biological processes control many chemical and biochemical processes, such as precipitation-dissolution, adsorption-desorption, complexation-dissociation and oxidation-reduction (He et al., 2005).

1.2.3 Uptake by plants

1.2.3.1 Mercury

Hg uptake depends on the ability of every plant species; even varieties of subspecies have differences. Although in some cases plants can develop a tolerance to high concentrations of Hg when they have to grow in contaminated sites (Beckers and Rinklebe, 2017; Kabata-Pendias, 2011; K. H. Kim et al., 2016). Conversely, some plants, for example *Jatropha curcas*, have the capacity to remove Hg from soils affected by gold mining. These plants are mainly used for the phytoremediation of Hg-contaminated soils (Marrugo-Negrete et al., 2016, 2015).

Translocation of Hg exists between various plant tissues such as: root to shoot, leaves to fruits, leaves to grains and even plant to seeds i.e. wheat and pea seeds treated with mercurial fungicides in the first generation seed (Kabata-Pendias, 2011). Nevertheless, when Hg is taken up by the plant from the soil, it is dominantly accumulated in roots (Chen and Yang, 2012). However, the aerial part of plants (i.e. leaf) is an important tissue for Hg accumulation. Stomata may be responsible for Hg uptake by leaves through gas exchange and can incorporate Hg^0

in tissues from the atmosphere (De Temmerman et al., 2009; Ericksen et al., 2003) and/or from soils (Millhollen et al., 2006).

The reason for the relatively easy transport of Hg within plants is its capacity to strongly bind to the amino acids of many proteins and enzymes due to its affinity for sulfhydryl groups. The most common symptoms that plants show are the stunting of seedling growth and root development, and the inhibition of photosynthesis and K^+ uptake (Kabata-Pendias, 2011; Mhatre and Chaphekar, 1984). These effects have been linked to the ultrastructural damage affecting: loss of cell shape, decrease in intercellular spaces, vascular abnormality in leaves, reduced amount of chlorophyll, breakdown of thylakoid, inhibited nicotinamide adenine dinucleotide phosphate activity. Hg produces stress in the plant, producing reactive oxygen species (ROS), which include oxygen radicals and non-radical derivatives of molecular oxygen (Chen and Yang, 2012; Ojuederie and Babalola, 2017).

To prevent ecological effects in plants due to Hg accumulated in organic soils, the recommended limits for total Hg (THg) in soil are 0.07-0.3 μg g^{-1} (Table 1.2) (K. H. Kim et al., 2016). Related to this, some studies have indicated the responsibility of Hg for the reduction of micro-biological activity vital to the terrestrial food chain in soils.

1.2.3.2 Trace metals

Depending on the properties of trace metals in soil, four categories can be found in Table 1.2, which considers their mobility and bioavalability (Kabata-Pendias, 2011).

Each plant responds in a different way depending on several factors. In fact, generalized values have been established to express the Transfer Factor (TF) by plant/soil (Table 1.2), which can be calculated as the ratio of every element concentration in plant by its total concentration in soil on a dried weight basis. When TF is less than 1 it means that the plant has the ability to transfer low levels of metals into the edible parts in the presence of high concentrations of trace metals in soil (Fan et al., 2018).

Table 1.2: Summary of the characteristics of trace metals and Hg related with plants.

Metal	Category [1]	Transfer Factor (TF)	EU legislation in vegetables [2]	Maximum allowable limit in soils [3]	Maximum allowable limit of vegetables and crops [4]
Cd	C1	10 [1]	0.050 mg kg^{-1}	3	0.2
Cr	C2	10^{-2} [5]	-	100	1
Cu	C4	10^{-1} [1]	-	100	10
Hg	C3	10^{-1} [1]	-	-	-
Ni	C4	10^{-2} [1]	-	50	0.2
Pb	C3	10^{-1} [1]	0.10 mg kg^{-1}	100	1
Zn	C1	10^{-1} [1]	-	300	50

Category 1 (C1): mobile in soil and bioaccumulated by plants; Category 2 (C2): lightly soluble in soil solution, difficult to absorb by plants; Category 3 (C3): sorbed by soil particles, not readily transported to above-ground parts of plants; Category 4 (C4): mobile and bioavailable for plants. [1]Kabata-Pendias (2011). [2]Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (European Commission, 2006). [3]World Health Organization (WHO), Food and Agricultural Organization (FAO) (Khalid et al., 2017). [4]EU European Union Standards (2006); FAO WHO/FAO 2007 (Khalid et al., 2017). [5]Liao et al. (2011).

It has to be taken into account that some trace metals such as Cr, Cu, Ni and Zn are essential, but may become toxic at higher concentrations. For example, Zn toxicity in plants inhibits metabolic functions producing retarded growth and senescence; excess of Cu produces stress and causes injury to plants, or high levels of Cr reduces germination (Nagajyoti et al., 2010). Table 1.3 shows the processes by which these essential elements are included in the plant and also the negative effects, or if the element is present in excess.

Table 1.3: Positive and negative effects on plants by trace metals.

Metal	Effect on human health	RfD (µg/kg/day)
Cd	Renal dysfunction, bone damage, lung disease and cancer, increased blood pressure, kidney disease, formation of renal stones and hypercalciuria, gastrointestinal disorder, bronchitis, premature birth and reduced birth weights [1,2]	1 [3]
Cr	Fatigue, irritability, damage to the nervous system, DNA damage, ulcers, reduction methemoglobin to hemoglobin [1,2]	1500 (Cr^{3+}) [3] 3 (Cr^{6+}) [3]
Cu	Anemia, liver and kidney damage, stomach and intestinal irritation [1,4]	40 [3]

[1](Rizwan et al., 2017) [2](Gomes et al., 2017) [3](Adrees et al., 2015) [4]Küpper and Andresen (2016)

(continue) Table 1.3: Positive and negative effects on plants by trace metals.

Metal	Effect on human health	RfD (µg/kg/day)
Ni	Heart and liver diseases, decrease in body weight and skin irritation [4]	20 [3]
Pb	Diseases of nervous systems, brain damage, liver and kidney damage, infertility, intellectual disorders and mental retardation in children [1,5]	nv [6]
Zn	Excess: Focal neural deficits, respiratory disorders and metal fume fever, gastrointestinal diseases and prostate cancer. Deficiency: Neuropsychiatric and neurosensory disorders, thymmic athrophy, skin lesions and reproductive problems. [1,7]	300 [3]

[1](Rizwan et al., 2017) [3](Adrees et al., 2015) [4]Küpper and Andresen (2016) [5](Emamverdian et al., 2015) [6]Shahid et al. (2012)

On the other hand, deficiencies of these trace metals induce different negative effects, such as abnormal pigmentation, size and shape of plant tissues, reduced leaf photosynthetic rates, and leads to various detrimental conditions.

Plants have developed different strategies for their detoxification, including chelation, immobilization, exclusion and compartmentalization. The enzymatically synthesized phytochelatins (PC_n) are one of the major groups of ligands (Küpper and Andresen, 2016). PC_n are small cysteine-rich polypeptides synthesized from glutathione (GSH) when trace metals bind to the enzyme γ-glutamyl cysteinyl dipeptidyl transpeptidase and activate it for the conversion of GSH to PC_n (Murtaza et al., 2017). Their formation is most efficiently induced by Cd and As, which are most important for detoxifying. However, they also bind Ag, Pb, Cu, Hg, Zn, Sn and Au (Andresen et al., 2018).

1.2.4 Soil remediation

Soils are contaminated worldwide by trace metals basically due to anthropogenic sources. Deterioration of the soil ecosystem, risks to soil fertility/quality and biochemical activities or the increase in metal uptake by food crops and vegetables are some examples which may entail high concentrations of metals in soil. Thus, soil remediation is necessary when these concentrations exceed the permissible limits. Different types of soil remediation can be clustered in three different groups depending on the impact on the soil: physical, biological and chemical (Khalid et al., 2017):

(1) Physical processes: this block has the advantage of being applied to highly contaminated sites, but the processes are laborious and costly. They include total or partial soil replacement by non-contaminated soil; soil isolation commonly with subsurface barriers; vitrification applying high temperature treatment at the contaminated site, and electrokinetic remediation via electrophoresis, electric seepage or electro-migration (Khalid et al., 2017).

(2) Biological processes: in this case, the cost is acceptable, but much more time is needed, and they are limited depending on the concentration of metals in the soil. Basically, they consist of phytoremediation whereby plants absorb the metal, removing the trace metals from the soil. Phytoremediation includes phytovolatilization (Padmavathiamma and Li, 2007), phytoextraction (Marrugo-Negrete et al., 2016, 2015) and phytostabilization (Castaldi et al., 2018).

(3) Chemical processes: the aim is to immobilize trace metals in soil by adding organic or inorganic amendments, such as compost (Kubna et al., 2014) or biochar (Houben et al., 2013). To use this kind of remediation the type of soil and the physicochemical nature of the soil should be taken into account. Adding the amendment to the soil can change the pH, CEC, Eh and soil microflora which are influenced directly to the mobility of the metals in the soil. The order of affinity of metal ions is: $Cu^{2+}>Hg^{2+}>Cd^{2+}>Fe^{2+}>Pb^{2+}>Ni^{2+}>Co^{2+}>Mn^{2+}>Zn^{2+}$ (Sharma and Nagpal, 2018).

1.2.5 Effects for humans

The aim of this section is to explain why these target trace metals have been selected. The negative effects on human beings, together with the protection of the environment, are significant reasons for studying the impact of metals. Nevertheless, no risk assessment was performed in this Thesis.

1.2.5.1 Mercury

Mercury has been designated as one of the ten most dangerous chemicals to public health by the World Health Organization (WHO) (Bjorklund et al., 2017). Recognized as a neurotoxicant and inmunotoxic, different outcomes can be correlated depending on the chemical form, the dose and rate of exposure of Hg in humans (M. Kim et al., 2016):

(1) Neurological effects: this is the most common and serious problem for humans. This block includes memory loss by deactivating enzymes for the production of brain cell energy and assembly of the protein tubulin into microtubules; moodiness, depression, anger and even suicidal thoughts in association with reductions in neurotransmitters dopamine, serotonin, noreprenephrine and acetyl cholinesterase (Clarkson and Magos, 2006). Effects such as neurological diseases, sleeping disorders or hearing loss have also been found in long-term exposure to low doses of Hg (Ely, 2001).

(2) Renal effects: the kidney is the primary target for mercuric ions for humans and mammals after exposure to elemental or inorganic forms of Hg (Zalups, 2000).

(3) Cardiovascular effects: Different studies have correlated exposure to Hg with increased arterial blood pressure (Azevedo et al., 2012), risk of hypertension, myocardial infarction, coronary dysfunction, atherosclerosis and risk of developing cardiovascular disease (Yoshizawa et al., 2002). It can also

influence hormones from the pituitary grand and increase the occurrence of rapid heartbeat and irregular pulse (Clarkson et al., 2007).

(4) Reproductive effects: an exposure of organic forms of Hg can be correlated with problems in the reproductive functions of males and females, such as birth defects, attention deficit disorders in children, impotency and reduction in sperm mobility leading to infertility (Arabi, 2005).

(5) Genetic-epigenetic effects: such as DNA methylation (Goodrich et al., 2013) or associated with DNA hypomethylation of brain tissue in the polar bear (Richard Pilsner et al., 2010).

1.2.5.2 Trace metals

Some trace metals, such as Cu, Zn or Fe, are necessary for humans although they are toxic at higher concentrations. On the other hand, trace metals, for example Hg or Pb, not only have no known vital effect for humans, but are even bioaccumulated in living beings and their toxicity increases (Singh et al., 2011). Table 1.4 shows the diseases and problems that trace metals can cause in human health and the oral reference dose (RfD) used to know the maximum permissible level of each metal per day.

Table 1.4: Effects on human health by trace metals and its RfD.

Metal	Effect on human health	RfD (µg/kg/day)
Cd	Renal dysfunction, bone damage, lung disease and cancer, increased blood pressure, kidney disease, formation of renal stones and hypercalciuria, gastrointestinal disorder, bronchitis, premature birth and reduced birth weights [1,2]	1 [3]
Cr	Fatigue, irritability, damage to the nervous system, DNA damage, ulcers, reduction methemoglobin to hemoglobin [1,2]	1500 (Cr^{3+}) [3] 3 (Cr^{6+}) [3]
Cu	Anemia, liver and kidney damage, stomach and intestinal irritation [1,4]	40 [3]

nv: no value. [1](Singh et al., 2011) [2](Jaishankar et al., 2014) [3](Fuentes-Gandara et al., 2018) [4](Rao, 2014) [5](Jan et al., 2015) [6]Pb has no biological function, so even at low concentration it is toxic for living beings (Fuentes-Gandara et al., 2018) [7](Plum et al., 2010).

Trace metals can disrupt metabolic functions in two ways:

1. Metals can be accumulated and damage functions in vital organs i.e. heart, brain, kidney, liver, etc.
2. They can move different vital nutritional minerals from their original place, blocking their biological functions in the body (Singh et al., 2011).

1.3 Passive sampling

During the last 20 years, many passive devices have been developed to measure a wide range of different elements in the environment. Table 1.5 shows the most common methods of passive sampling with their characteristics.

The main advantages of passive sampling are:

1. Pre-concentration of the target elements in the resin
2. Measurement of contamination during the period of the deployment
3. Reduction in matrix interferences
4. Cost effectiveness
5. Power sources are not needed for the operations (Dabrin et al., 2016; Seethapathy et al., 2008).

The key strengths which passive samplers have is that they can monitor long-term trends and measure contaminants at very low concentrations increasing measurement sensitivity (Allan et al., 2006).

Fundamentally, passive devices are based on net transport across the barrier due to molecular diffusion following Fick's first law. Passive devices consist of a barrier – a static layer of the surrounding medium or a polymer membrane - and a sorbent. The work of the barrier is to prevent the convective transport of analytes; transport is solely by molecular diffusion (Figure 1.3).

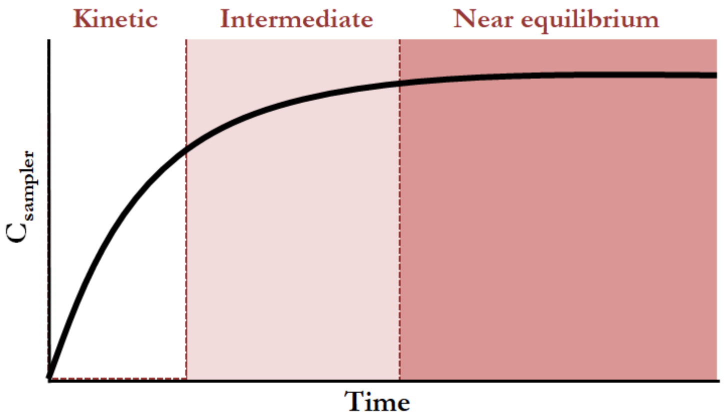


Figure 1.3: Scheme of the uptake for a passive sampling device (based on Mayer et al. (2003)).

Finally, the receiving phase can be of many different types and the membrane is responsible for the sorption of the analyte. It should be taken into account that the uptake occurs when the equilibrium between the concentration in the resin layer and in the medium has been reached.

Apart from the barrier type, the sorption material, the sampler geometry and the sampling time, environmental characteristics, such as temperature, humidity, ion strength and the analyte concentration to determine more effectively the concentration in the field, all have to be taken into consideration (Seethapathy et al., 2008).

Table 1.5: Summary of passive sampling techniques for contaminants in water. Adapted from (Miège et al., 2012; Seethapathy et al., 2008; Vrana et al., 2005)

Passive sampler	Year	Device design	Sampling aim	Target elements	Environmental matrix	Sampling deployment period	Sample preparation	Advantages	Developed by	Reference
Supported liquid membrane (SLM)	1992	Sampler involves the use of PTFE membrane, separating two aqueous solutions	Integrative field sampling, screening, mimicking biological membranes	Metal ions	Natural waters	Days	Direct analysis	Versatile	[1]	[2]
Stabilized liquid membrane device (SLMD)	1993	LDPE layflat tubing containing an acid solution	Integrative field sampling	Cd, Co, Cu, Ni, Pb and Zn (divalent metal ions)	Natural waters	Days-weeks	Acid extraction	suitable for long term, low-level monitoring of labile forms of selected metals	inorganics [3]; organics [4]	-
Diffusive gradient in thin film (DGT)	1994	membrane filter, a diffusive hydrogel and a binding layer	Integrative field sampling, screening, mimicking biological uptake, speciation	>55 elements, stable isotopes, radionuclides, phosphate and sulfide	Water, sediment and soil	Weeks	Extraction with HNO_3	Well documented (>715) in different matrixes	[5]	[6]

[1]Jönsson and Mathiasson (1992) [2]Vrana et al. (2005) [3]Brumbaugh et al.(2000) [4]Huckins et al. (1993) [5]Davison and Zhang (1994) [6]Menegário et al., (2017)
PTFE: Porous polytetrafluoroethylene; LDPE: Low-density polyethylene.

(continue) Table 1.5: Summary of passive sampling techniques for contaminants in water. Adapted from (Miège et al., 2012; Seethapathy et al., 2008; Vrana et al., 2005)

Passive sampler	Year	Device design	Sampling aim	Target elements	Environ mental matrix	Sampling deployment period	Sample preparation	Advantages	Developed by	Reference
Passive integrative mercury sampler (PIMS)	2000	LDPE lay-flat tubing	Sampling and preconcen tration	Hg	Air and freshwater	Weeks to months	Direct analysis	Membrane characteristics can be altered for control of sampling rates	[7]	-
Chemcatcher	2000	iminodiacetate functionalized chelating disk associated to a cellulose acetate membrane	Integrative in situ sampling	trace metals such as Cd, Cu, Ni, Pb	water	Days (3-28)	Acidic conditioning step	Potentially used without membrane, having more homogenus distribution in the receiving phase	[8] for organic and [9] for inorganic contaminant	[10]
Permeation liquid membrane (PLM)	2004	Microporous hydrophobic support separating test solutions from receiving solutions uptaking	Preconcen trate and screening	Cu, Pb and Ni	Natural waters	Hours	Solvent extraction		[11]	-

7Brumbaugh et al. (2002) [8](Kingston et al. (2000) [9](Persson et al. (2001) [10](Charriau et al. (2016) [11](Slaveykova et al. (2004)

(continue) Table 1.5: Summary of passive sampling techniques for contaminants in water. Adapted from (Miège et al., 2012; Seethapathy et al., 2008; Vrana et al., 2005)

Passive sampler	Year	Device design	Sampling aim	Target elements	Environmental matrix	Sampling deployment period	Sample preparation	Advantages	Developed by	Reference
Diffusive Milli-Gel (DMG)	2015	diffusive milligel beads with incorporated cation exchange resin (Chelex) particles	Integrative field sampling	Cu	Freshwater	Hours	Extraction with HNO_3	easier to implant in very various environmental media	[12,13]	[14]

[12](Perez et al. (2015) [13](Perez et al. (2016) [14](Väänänen et al. (2018)

1.3.1 Diffusive Gradient in Thin Film (DGT) technique

1.3.1.1 Theory

The Diffusive Gradient in Thin Film (DGT) technique is a passive sampling method used to determine the labile fraction of the species in the environment. The labile fraction of the target elements is considered as the potential bioavailable concentration for living beings. Nevertheless, lability has been correlated with bioavailability in many studies, in which the DGT technique is considered a useful tool for measuring the bioavailable concentration in different matrixes (Jansen et al., 2002). This method was developed by (Davison and Zhang, 1994) for *in situ* determination of kinetically labile species in aquatic systems.

The DGT technique is based on the diffusive transport of a solute across a specific concentration gradient established by a hydrogel layer and filter membrane, where it is possible to obtain values on the concentration over short time periods (Fernández-Gómez et al., 2012b). There is also the binding layer, separated from the solution by the hydrogel layer and the filter membrane, where the target element is absorbed irreversibly by diffusion. The membrane filter most commonly used for DGT is polyethersulphone with a thickness varying from 0.13 to 0.15 mm. Usually the pore size is 0.45 μm, but filters with different pore sizes could be used, depending on the species of interest (Davison and Zhang, 2016). All these components are enclosed in a plastic sampling device consisting of a piston base and a cap (Figure 1.4).

The efficiency of the DGT technique has led to provide *in situ* information on labile metal species in more matrixes in the environment, such as in seawater (Clarisse et al., 2012; M. Kim et al., 2016; Schintu et al., 2014), sediments (Divis et al., 2016; Wu et al., 2015), soils (Harper et al., 1998; Li et al., 2015), microbial mats (Davison et al., 1997), and the correlation between plant uptake and DGT resin has also been demonstrated (Qasim et al., 2016; Zhang et al., 2001).

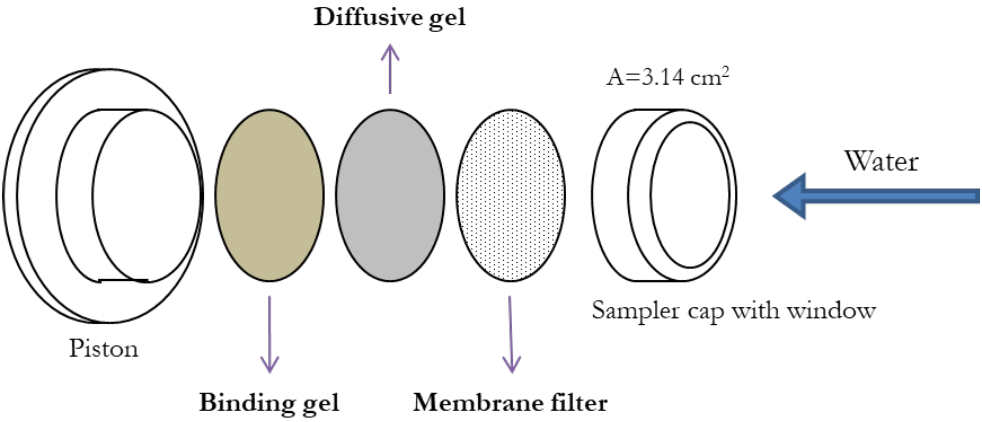


Figure 1.4: Representation of DGT components.

The first applications of DGT in soils (Zhang et al., 1998b) were to study the availability of different trace metals applying the device with the soil solution, and for the application in sediments (Zhang et al., 1995) studying trace metal concentrations in the pore water from the sediment phase.

It is important to take into account the physicochemical properties in the environment in the deployment time. The field conditions are:

(1) Temperature: the diffusive layer is dependent on the temperature and it can be corrected depending on the temperature in the deployment time. In systems where the temperature is expected to vary during the day and night, temperature loggers should be used (Österlund, 2010).

(2) pH: the pH range varies depending on the target element. For example, $pH<2$ for Cu, $pH<3.5$ for Co, Mn and Zn, and $pH<4.5$ for Cd until pH 11 (Väänänen et al., 2018).

(3) Ionic strength: to ensure that DGT measurements are accurately measured and not deteriorated, ionic strength should be higher than 1 mM (Zhang and Davison, 1999).

(4) Fouling: the suspended particles or the biofilm composed by microorganisms and algae (biofouling) can reduce the concentration of the elements in the binding layer (Díez and Giaggio, 2018; Uher et al., 2012).

1.3.1.2 Principle and calculations

1.3.1.2.1 In water

The basis of the DGT technique is Fick's first law of diffusion. A binding layer is separated from the bulk solution by a diffusive layer which has a specific thickness. A diffusive boundary layer (BDL) exists between the diffusive gel and the bulk solution where ions are transported by molecular diffusion. The binding layer is placed behind the diffusive layer in a DGT device consisting of a determined resin (depending on the target element, see Table 1.5) and typically embedded in polyacrylamide gel (Österlund, 2010; Zhang et al., 1998b). A concentration gradient is established in the diffusion gel, where the concentration is equal to the bulk concentration at the membrane/water interface, as shown in Figure 1.5.

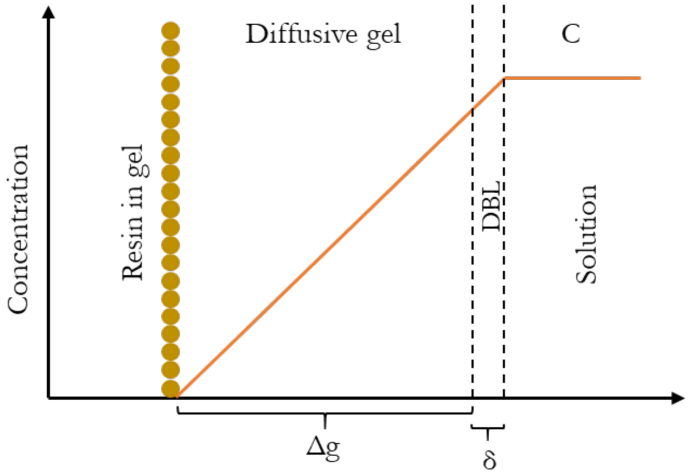


Figure 1.5: Schematic representation of the concentration of ionic species (C) through the diffusive layer in contact with aqueous solution. Adapted from Davison and Zhang (1994).

When DGT is deployed, metals are concentrated irreversibly and instantaneously in the binding layer. The measured mass accumulated in this resin provides a flux and a steady state is achieved during deployment, and a linear concentration gradient is established following equation 1.1:

$$J=D\frac{dC}{dx} \tag{1.1}$$

where J is the flux (mol cm^{-2} s^{-1}), D is the diffusion coefficient (cm^2 s^{-1}), C is the concentration (mol cm^{-3}) and x is the distance, so dC/dx (mol cm^{-4}) is the concentration gradient. This gradient induces a constant diffusion flux, whose characteristics are related to the physical properties of the gel, temperature and the concentration of labile metal in the bulk solution.

Considering Δg as the thickness of the diffusion layer (cm), the flux can be calculated as:

$$J=D\frac{C}{\Delta g} \tag{1.2}$$

After the retrieval of the DGT, the binding layer is analysed and the mass of the analyte in the resin gel (M) can be obtained using the equation 1.3:

$$M=C\frac{V_g+V_e}{f_e} \tag{1.3}$$

where V_g is the volume of the resin gel (typically 0.16 mL). V_e is the volume of the eluent and f_e is the elution factor. For trace metals, typically the recommended eluent is nitric acid and the elution factor is 0.8.

In this moment, M can be used to calculate the flux through the diffusion gel with a specific area (A, cm) during a deployment time (t):

$$J=\frac{M}{A\times t} \tag{1.4}$$

If equation 1.3 and 1.4 are combined, the concentration of the target analyte in the bulk solution can be calculated as follows:

$$C=\frac{M\times\Delta g}{A\times D\times t} \tag{1.5}$$

In practice, DBL can include the effective DBL thickness in equation 1.5 (∂) as follows:

$$C=\frac{M(\Delta g+\partial)}{A\times D\times t} \tag{1.6}$$

But this variation is only used when the thickness of the DBL is negligible if it is compared to the thickness of the diffusive gel (Gimpel et al., 2001).

When D is calculated experimentally, the temperature influences the diffusion in the gel, so D must correct the deployment temperature, T (Zhang and Davison, 1995):

$$\log D=\frac{1.37023(T\text{-}25)+8.36\cdot10^{-4}(T\text{-}25)^{2}}{109+T}+\frac{D_{25}(273+T)}{298} \tag{1.7}$$

where D_{25} is the diffusion coefficient at the deployment time at 25ºC.

Indeed, the DGT technique allows us to measure inorganic and organic species separately containing different diffusive gels with different pore sizes (Zhang and Davison, 2000). Therefore, the mass of the metal accumulated in the DGT device (M_{DGT}) is the sum of labile inorganic (M_{inorg}) and organic (M_{org}) species:

$$M_{DGT}=M_{inorg}+M_{org} \tag{1.8}$$

According to the DGT theory (equation 1.5):

$$M_{inorg}=\frac{D_{inorg}C_{inorg}At}{\Delta g} \tag{1.9}$$

$$M_{org}=\frac{D_{org}C_{org}At}{\Delta g} \tag{1.10}$$

where C_{inorg} and C_{org} are the labile inorganic and organic concentrations in solution measured by DGT, respectively.

1.4.1.2.2 In sediments and soils

The DGT technique is also capable of analysing the labile concentration of elements in soils and sediments, by pressing carefully the device in the soil paste (100% water holding capacity (WHC)) to ensure complete contact between the solid phase and the DGT device (ISO 11268-2, 2012; Zhao et al., 2006).

After analysing the amount of mass bound in the binding layer, concentration measured by DGT (C_{DGT}) can be estimated using the equation 1.11, derived from Fick's first law of diffusion (Lehto, 2017):

$$C_{DGT}=\frac{M\partial^{mdl}}{DAt} \tag{1.11}$$

where ∂^{mdl} is the thickness of the diffusion layer. Equation 1.11 is actually similar to equation 1.5, but is only used to estimate the concentration of the field for deployments which have sufficient capacity to supply solute to the DGT device interface with a flux that is the same as the diffusive flux into the DGT device.

Considering the complexities associated with interpreting the presence of solid particles and the heterogeneity in soils and sediments, a steady-state condition is rarely reached because of the lack of a convective supply of solutes and interactions between solutes and solids. In some cases, the expected dissolved ion concentration is depleted from the device interface because of the constant demand of solute by the DGT device, so the flux into the device decreases as deployment progresses. Therefore, C_{DGT} cannot be calculated with equation 1.12. If this occurs, it is useful to consider the ratio as follows:

$$R=\frac{C_{DGT}}{C_{soln}} \quad 0<R<1 \tag{1.12}$$

where C_{soln} is the bulk pore water solute concentration. Normally, C_{soln} is measured from a 0.45 μm filtered and acidified pore water sample which provides an approximate measure of the total dissolved solute concentration in the sample.

Applying equation 1.12, three models of solution supply to DGT in soils (Zhang et al., 1998b) and sediments (Zhang et al., 1995) could be represented by R and also graphically represented in Figure 1.6:

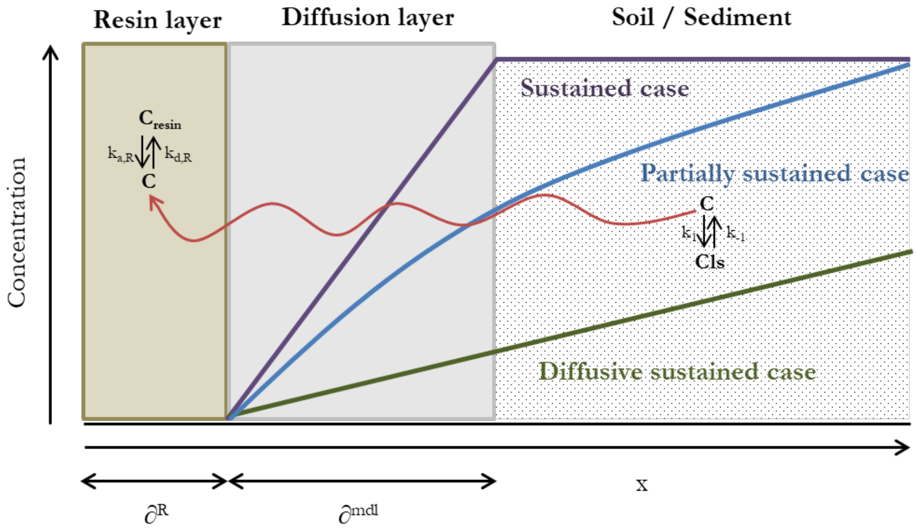


Figure 1.6: Concentrations of an ionic species in a DGT device and adjacent pore water during the deployment. Adapted from Lehto (2017) and Zhang et al. (1998b). c^{ls}: concentration of sorbed ion that can desorb within the simulation time; k_1 and k_{-1}: pseudo-first-order rate constants.

(1) Sustained case ($R > 0.8$; $C_{DGT} = C_{soln}$): continuous resupply exists from the solid phase at a rate equal to the flux into the DGT.

(2) Partially sustained case ($0.2 \leq R \leq 0.8$; $C_{DGT} < C_{soln}$): also known as the intermediate case, consists of a significant resupply of the solute from the solid phase, but it is insufficient to fully sustain pore water concentrations.

(3) Diffusive case ($R < 0.2$; $C_{DGT} << C_{soln}$): no resupply of solutes to the pore water exists. The DGT device is solely supplied by the diffusion of the solutes.

Moreover, a time-dependent model to describe the flux of solute to a DGT deployed in a soil was performed by (Harper et al., 1998). The program called "DGT-induced fluxes in sediments (DIFS)" simulates the diffusion of a dissolved ion from the pore water into a DGT device during its deployment considering soil factors and transport processes. Temporal and spatial variation can be simulated in ion concentrations to emulate the DGT-measured flux.

On the other hand, one of the most important factors in applying DGT devices in soil is to predict the bioavailability of metals to plants. Some studies have been performed comparing the total soil concentrations of trace metals with the toxic effects in plants (McLaughlin et al., 2000), but fractions unavailable to the biota exist in the total metal content in soils.

The concept of effective concentration (C_E) was introduced by Zhang et al. (2001). C_E is the concentration which would have to be present in the soil solution to supply the same mass of metal accumulated by DGT in the partially sustained case (Figure 1.6). It only takes into consideration the concentration by diffusion, which is the same process as found in the rhizosphere of plants. So, C_E can be related directly to plant uptake.

To calculate C_E, a ratio of the mean interfacial concentration with the resupply solely by diffusion to the bulk concentration should be calculated as follow:

$$C_E = \frac{C_{DGT}}{R_{diff}} \tag{1.13}$$

where R_{diff} is the coefficient determined by the geometry of the device, the deployment time and soil tortuosity. This coefficient may be calculated using a numerical solution of the diffusion equations (Harper et al., 1998) or using DIFS. Thus, C_E can be calculated dividing the concentration from the DGT device by R_{diff} (equation 1.14).

$$R_{diff}=\frac{C_{diff}}{C_{soln}} \tag{1.14}$$

In the diffusive case, the mean interfacial concentration can be calculated directly, using equation 1.15:

$$C_{diff}=\frac{M\Delta g}{DAt} \tag{1.15}$$

The application of DGT devices in soil to analyse the labile concentration and correlate the metal concentration in plants can be related with different complex methods. The Sequential Extraction (SE) method is used for the fractionation of the soil in five different blocks: water soluble and exchangeable which are the bioavailable fractions; oxide-bound, carbonate-bound and organic matter-bound which are the potentially bioavailable fractions and the residual part which is non-bioavailable (He et al., 2005). Some studies have been developed to correlate the concentration calculated in the DGT device, the first fraction of the SE and also the concentration found in plants (Ahumada et al., 2011).

1.3.1.3 Gels

1.3.1.3.1 Diffusive gel

The structure of hydrogels is intermediate between a solid and a liquid, containing up to 95% of water. Depending on their precise composition, gels can vary from viscous fluids to rigid solids. There are three types of diffusive gel commonly used in the DGT technique. In all cases, the transport of the target analytes is by diffusion (Davison and Zhang, 2016).

1.3.1.3.1.1 Agarose

Agarose is a linear polysaccharide isolated from agar (or agar agar) which consists of repeating units of agarobiose (D-galactose and 3,6-anhydro-L-galactopyranose). It may contain some impurities, such as sulphonate, ester sulphate, ketal pyruvate and carboxyl groups, but these impurities depend on the purity of the material and also the properties of the gel (Davison and Zhang, 2016).

Depending on the agarose concentration in the hydrogels, the distribution of pore sizes varies from 1 to 900 nm. The most common agarose concentration in DGT devices is 1.5% agarose which produces a gel with pore sizes ranging from 1 to 480 nm, an average value of 35-47 nm (Fatin-Rouge et al., 2003).

The thickness of the agarose hydrogel is 0.76 mm and the coefficient diffusion varies with and without DOM (Fernández Gómez, 2014).

1.3.1.3.1.2 Polyacrylamide cross-linked with an agarose derivative

Polyacrylamide cross-linked with an agarose derivative is the most commonly gel used for the diffusion layer in DGT, usually called APA (Scally et al., 2006). This hydrogel is often called an open diffusive layer (ODL) because its pore sizes are >5 nm, but not bigger than agarose pore sizes (Davison and Zhang, 2016; Shiva et al., 2015).

The most common formulation for this type of hydrogel in the DGT technique is a gel solution containing 15% monomer and 0.3% cross-linker, although the structure of the commercial product cross-linker is still unknown. The cross-linker and the acrylamide (or agarose derivative) are added to the water. Then, TEMED (N,N,N’,N’-tetramethylethylenediamine), acting as the catalyst of the reaction, and ammonium persulfate (APS), acting as the initiator for the polymerization, are added to the solution (Kovaríková et al., 2007a). Polymerization is carried out at 42-45ºC for approximately 45 min. Some studies have described the protocol (Fernández-Gómez et al., 2011).

1.3.1.3.1.3 Polyacrylamide cross-linked with bis-acrylamide

In this case, the acrylamide gels are cross-linked with bis-acrylamide (N,N’-methylene-bis-acrylamide), consisting of two acrylamide molecules linked through their aminocarbonyl groups. Using polyacrylamide gel with a bis-acrylamide cross-linker, the pore size reduces until <1 nm, usually referred to as a restricted diffusive layer (RDL) (Shiva et al., 2015). The smaller pore size affects the molecules that can diffuse through the membrane, leaving the bigger molecules outside, such as organic complexes, and allowing only free ions and small inorganic complexes to pass.

In this case, it would be better if equation 1.6 were extended to equation 1.16 because the difference is too large to ignore during calculations, while in the other cases, it does not contribute to an error (Österlund, 2010):

$$C=\frac{M}{A\times t}\left(\frac{\Delta g}{D_{gel}}+\frac{\Delta_f}{D}\right) \tag{1.16}$$

where Δg and Δ_f correspond to the diffusive gel and membrane filter thickness respectively; and D_{gel} is the diffusion coefficient in the membrane filter.

1.3.1.3.2 Binding layer

The binding layer consists of solid resins or powders which are incorporated into a gel matrix, such as polyacrylamide, to form the resin. The standard thickness of a dehydrated resin gel is 0.25 mm, which is 0.4 mm after hydration and the recommended size for the solid particles is <100 µm (Bennett et al., 2016a).

To consider a potential resin gel for an analyte/s, 3 specific points should be taken into account (Bennett et al., 2016a):

(1) The binding strength, which is correlated to a relevant equilibrium constant for the specific analyte.

(2) The intrinsic binding capacity, the sites which are able to interact with the species.

(3) The presence of competition effects, such as other ions with similar strengths.

By using different binding agents the range of species to be analysed has been extended for many trace metals (including other cations and oxyaonions) and targeted species, for example, sulfide, uranium, radium and technetium (Table 1.5). In addition to the binding agents shown in Table 1.6, mixed binding layers and multiple binding layer samples have been developed to expand the range of different analytes (e.g. Amberlite IRP69/ferrihydrite to determine K and P (Zhang et al., 2013) or Chelex-100/ZrO_2 to determine As, Fe and P (Sun et al., 2015)).

Table 1.6: Summary of the different binding agents and elements used to study some target elements with the DGT technique.

Analyte(s)	Binding agent	Eluent
THg	3-mercaptopropil functionalized silica [1]	Direct analysis
	Spheron-Thiol [2]	Direct analysis
	Duolite GT73 [3,4]	Direct analysis
	Iontosorb AV-IM [5]	Direct analysis
	Ambersep GT74 [6]	Direct analysis
MeHg	Saccharomyces cerevisiae [7]	0.6M HCl
Divalent and trivalent ions	Chelex-100[8]	2M HNO_3
Cr(VI)	N-methyl-D-glucamine [9]	1M HNO_3
Sb(III), As(III)	Mercaptopropil functionalised silica [10]	H_2O_2+NaOH
U	Manganese dioxide and Metsorb [11]	1M H_2O_2/HNO_3
Mo, Sb, V, As, W	Ferrihydrite (FH) [12]	1.4M HNO_3+0.1M HF
Cu, Cr, Cd, Mn, Ni, Pb, Zn	Montmorrilonite (MT) [13]	1M HNO_3
Cd, Co, Cu, Hg, Mn, Ni, Pb, Zn	Whatman P81 [14]	2M HNO_3
As	Amberlite IRA 910 [15]	1.2M HCl
Tc	TEVA resin [16]	4M HNO_3
As, Se	Metsorb (TiO2) [17]	1M NaOH
Antibiotics	XAD18[18]	MeOH
	XDA-1[19]	MeOH+0.1% CH_2O_2
	HLB[20]	MeOH
Sulfide	AgI[21]	12M HCl+0.25M NaOH

[1]Fernández-Gómez et al. (2011) [2]Divis et al. (2010) [3]Divis et al. (2010) [4]Pelcová et al. (2014) [5]Divis et al. (2010) [6]Pelcová et al. (2014) [7]Tafurt-Cardona et al. (2015) [8]Zhang and Davison (1995) [9]Pan et al. (2015) [10]Bennett et al. (2016b) [11]Turner et al. (2012) [12]Österlund (2010) [13]dos Anjos et al. (2017) [14]Larner and Seen (2005) [15]Rolisola et al. (2014) [16]French et al. (2005) [17]Bennett et al. (2010) [18]Chen et al. (2012) [19]Xie et al. (2018) [20]Stroski et al. (2018) [21]Teasdale et al. (1999)

(continue) Table 1.6: Summary of the different binding agents and elements used to study some target elements with the DGT technique.

Analyte(s)	Binding agent	Eluent
Phosphates	Metsorb (TiO2)[22]	1M NaOH
	Iron oxide binding layer[23]	0.25M H_2SO_4
Nitrates	Purolite A520E[24]	2M NaCl
	SIR-100-HP[25]	5% NaCl

[22]Panther et al. (2010) [23]Zhang et al. (1998a) [24]Huang et al. (2016) [25]Cai et al. (2017).

1.3.1.4 Types of DGT device

The design of the device for the deployment in solutions is a simple plastic piston shape which is shown in Figure 1.5. The window in the top of the device is 20 mm in diameter (3.14 cm^2) and the fit between the flat end of the piston and the lip of the adjacent sleeve is 1.2 mm. The most common configuration is a 0.4 mm thick binding layer with a 0.8 mm APA diffusive gel and a 0.45 μm polyethersulphone membrane filter with a 25 mm diameter of 0.014 mm thickness (Jolley et al., 2016). Therefore, the total thickness is 1.34 mm which is a good seal around the exposure window. If the total thickness is less than 1.34 mm, another diffusive gel between the binding layer and the piston surface should be placed to compensate the final thickness. It should be emphasized that the APA gel has an expansion factor of 1.6 during the hydration step, so the initial gel should be thinner (Fernández-Gómez et al., 2011).

In the case of soils, it is possible to use the sample type of device as for solutions, but the gap is slightly tapered to facilitate contact with the soil and also the diameter of the window is 18 mm for a better seal to avoid the entrance of fine soil particles.

As shown in Figure 1.7, the DGT probe is specifically for measurement in sediments. Instead of the piston form, the plastic device is a planar probe with a two-piece assembly unit in which the largest one has a molded handle on the top to facilitate probe handling as shown in Figure 1.7. The body dimensions are 18 cm x 4 cm x 0.5 cm with the 5.5 cm of the handle. The size of the cavity where the binding layer, diffusive gel and membrane filter are placed is 16.2 cm x 2.8 cm. The front piece has an open window of 1.8 cm x 15 cm with connectors to secure the base plate (Jolley et al., 2016).

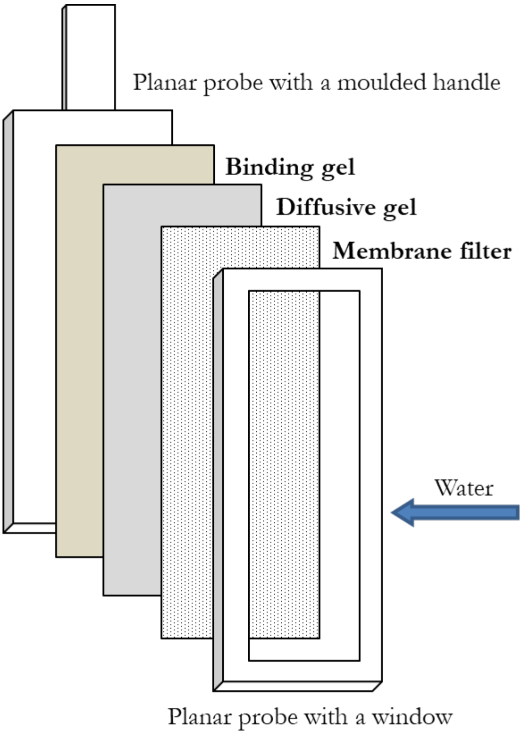


Figure 1.7: Representation of a DGT probe. Adapted from Zhang et al. (2002).

Because of their size, the resin gels in plate-type sediment probes are sliced with 1 cm resolution before elution to analyse every slice separately (Hong et al., 2014). Therefore, measurements at different sediment depths could be possible, performing the vertical distribution of the sediment pore water concentrations of DGT devices.

1.3.1.5 DGT studies

Taking into consideration the advantages that the DGT technique can provide and the ease of use of this method in the field and laboratory, more than a thousand studies using DGT devices have been performed since 1994.

Table 1.7: Summary of the published studies of DGT for the analysis of Hg in water and soil.

Water	Soil
THg [1-7]	THg [18-20]
CH_3Hg^+ [8-12]	CH_3Hg^+, Hg^{2+} [17]
Hg^{2+} [13]	
CH_3Hg^+, Hg^{2+} [14,15]	
Hg^{2+}, CH_3Hg^+, $C_2H_5Hg^+$, C_6H_5Hg [16]	
CH_3Hg^+, Hg^{2+} [17]	

[1]Docekalová and Divis (2005) [2]Rodríguez Martín et al. (2006) [3]Roig et al. (2011) [4]Gao et al. (2014) [5]Fernández-Gómez et al. (2011) [6]Fernández-Gómez et al. (2012a) [7]Pelcová et al. (2015) [8](Clarisse and Hintelmann, 2006) [9]Clarisse et al. (2009) [10]Clarisse et al. (2012) [11]Gao et al. (2014) [12]Fernández-Gómez et al. (2015) [13]Colaço et al. (2012) [14]Cattani et al. (2009) [15]Hong et al. (2011) [16]Pelcová et al. (2015) [17]Cattani et al. (2008) [18]Liu et al. (2012) [19]Senila et al. (2013) [20]Hlodák et al. (2015)

Table 1.7 shows different studies in water and in soil for Hg species. Although the binding layer is able to bind THg, depending on the treatment of the resin, it is possible to separate the different species of Hg. Pelcová et al. (2015) have studied 4 different species of Hg (Hg^{2+}, CH_3Hg^+, $C_2H_5Hg^+$ and C_6H_5Hg) in water analysing the binding layer with liquid chromatography (LC) and cold-vapour atomic fluorescence spectroscopy (CV-AFS). In addition, Cattani et al. (2008) analysed two different species of Hg (CH_3Hg^+ and Hg^{2+}) in soil using HPLC-ICP-MS.

On the other hand, Table 1.8 shows a summary of different studies of trace metals which are separated by the number of target elements analysed in every study ranging from 3 to more than 10 elements. The fact that it is possible to effectively analyse more than 3 elements at the same time with one binding layer or, in some cases, with a mix of two layers, makes a difference in terms of simplicity, effectiveness and usefulness.

Table 1.8: Summary of some published studies of DGT for the analysis of trace metals in water and soil

	3 elements	4 elements		5 elements	6 elements	7 elements	8 elements	9 elements	≥10 elements
Water	Cd, Co, Ni[1]	As, Cd, Cu, Ni[5]	Cd, Cu, Pb, Zn[14-17]	As, Cd, Cu, Mg, Mn[23]	Cd, Co, Cu, Ni, Pb, Zn[30]	Al, Cd, Co, Cu, Mn, Ni, Zn[33]	Al, Cd, Cu, Fe, Mn, Ni, Pb, Zn[40]	Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn[41]	Metals[42-50]
	Cd, Cu, Mn[2]	As, Mo, Sb, V[6]	Cu, Fe, Mn, Zn[18]	Cd, Co, Cu, Ni, Pb[24,25]	Cd, Cr, Cu, Ni, Pb, Zn[31]	Al, Cd, Cr, Cu, Fe, Ni, Pb[34]			
	Cd, Cu, Pb[3]	As, Se, V, Sb[7]	Cu, Mn, Ni, Zn[19]	Cd, Cu, Fe, Ni, Zn[26]	Cd, Cu, Mn, Ni, Pb, Zn[32]	As, Cd, Cr, Cu, Ni, Pb, Zn[35]			
	Cu, Ni, Pb[4]	Cd, Cu, Ni, Pb[8-11]	Cu, Ni, Pb, Zn[20-22]	Cd, Cu, Ni, Pb, Zn[27-29]		Cd, Co, Cr, Cu, Ni, Pb, Zn[36-38]			
		Cd, Cu, Ni, Zn[12,13]				Cd, Co, Cu, Fe, Mn, Ni, Pb[39]			

[1](Puy et al., 2014) [2](Denney et al., 1999) [3](Sui et al., 2013) [4](Cleven et al., 2005) [5](Buzier et al., 2014) [6](Zhang et al., 2017) [7](Luo et al., 2010) [8](Scally et al., 2006) [9](Unsworth et al., 2006) [10](Slaveykova et al., 2009) [11](Dakova et al., 2011) [12](Wallner-Kersanach et al., 2009) [13](Huynh et al., 2010) [14](Webb and Keough, 2002) [15](Van Leeuwen et al., 2005) [16](Gaabass et al., 2009) [17](Schintu et al., 2010) [18](Gimpel et al., 2003) [19](Forsberg et al., 2006) [20](Dunn et al., 2003) [21](Han et al., 2013) [22](Zhu and Guéguen, 2016) [23](Colaço et al., 2012) [24](Guéguen et al., 2011) [25](Levy et al., 2012) [26](Stewart et al., 2016) [27](Rodríguez Martín et al., 2006) [28](Sigg et al., 2006) [29](de Souza et al., 2014) [30](Sangi et al., 2002) [31](Soriano-Disla et al., 2010) [32](Odzak et al., 2002) [33](Yabuki et al., 2014) [34](Buzier et al., 2011) [35](Sierra et al., 2017) [36](Villanueva et al., 2013) [37](Villanueva et al., 2015) [38](Villanueva et al., 2016) [39](Warnken et al., 2008) [40](Warnken et al., 2009) [41](Warnken et al., 2007) [42](Garmo et al., 2003) [43](Lucas et al., 2015) [44](Balistrieri et al., 2007) [45](Roig et al., 2011) [46](Uribe et al., 2011) [47](Schoyen et al., 2017) [48](Wang et al., 2017) [49](Shiva et al., 2017) [50](dos Anjos et al., 2017)

(continue) Table 1.8: Summary of some published studies of DGT for the analysis of trace metals in water and soil

	3 elements	4 elements		5 elements	6 elements	7 elements	8 elements	9 elements	≥10 elements
Soil	As, Sb, Pb[51]	As, Cr, Cu, Zn[57]	Cd, Cu, Pb, Zn[64-66]	Al, Cu, Cd, Ni, Zn[68]	As, Cd, Cu, Mn, Pb, Zn[73]				Metals[77,78]
	Cd, Cu, Ni[52,53]	As, Cu, Pb, Zn[58,59]	Cr, Cu, Ni, Zn[67]	As, Cd, Pb, Sb, Zn[69]	As, Cd, Cu, Ni, Pb, Zn[74]				
	Cd, Ni, Zn[54]	Cd, Cu, Ni, Zn[59-63]		Cd, Cr, Cu, Pb, Zn[70]	Cd, Co, Cu, Ni, Pb, Zn[75]				
	Cd, Pb, Zn[55,56]			Cd, Cu, Ni, Pb, Zn[71,72]	Cd, Cu, Fe, Mn, Ni, Zn[76]				

[51](Lomaglio et al., 2017) [52](Kovaríková et al., 2007a) [53](Senila et al., 2012) [54](Ernstberger et al., 2005) [55](Fischerová et al., 2005) [56](Cornu et al., 2016) [57](Hattab et al., 2014) [58](Bade et al., 2012) [59](Hattab-Hambli et al., 2016) [60](Zhang et al., 1998b) [61](Ernstberger et al., 2002) [62](Black et al., 2011) [63](Huynh et al., 2012) [64](Nolan et al., 2005) [65](Tian et al., 2008) [66](Agbenin and Welp, 2012) [67](Ahumada et al., 2014) [68] (Li et al., 2015) [69](Qasim et al., 2016) [70](Guo et al., 2016) [71](Hamels et al., 2014) [72](Zhang et al., 2016) [73](Ciadamidaro et al., 2017) [74](Moreno-Jiménez et al., 2016) [75](Hooda et al., 1999) [76](Zhang and Davison, 1995) [77](Docekal et al., 2003) [78](Jakl et al., 2015)

Chapter 2

Objectives

The general objective of this Thesis was to improve and develop the Diffusive Gradient in Thin Film (DGT) technique as a useful tool for measuring bioavailable mercury and other trace metals in aquatic and terrestrial environments.

Thus, the specific objectives have been separated into these two different matrixes:

The objectives in aquatic systems were:

- To test the application of a specific polymer inclusion membrane (PIMS) in the DGT sampler design to analyse bioavailable mercury in the laboratory and in the field.
- To prove the correlation of the DGT technique with the amount of phytochelatins quantified in two different species of aquatic macrophytes.
- To assess the DGT technique as a control tool for pollution episodes in a river ecosystem.

And, the objectives in terrestrial systems were:

- To develop a new DGT sampler for studying inorganic mercury in terrestrial soils.
- To predict metal uptake by lettuce plants with the DGT technique.
- To determine the effectiveness of two different organic amendments (biochar and compost) for the immobilization of mercury and other trace metals in agricultural soils.

Chapter 3

Polymer Inclusion Membrane in a DGT design for mercury monitoring

This chapter is based on the article:

Turull, M., Elias, G., Fontàs, C., Díez, S., 2016. Exploring new DGT samplers containing a polymer inclusion membrane for mercury monitoring. Environ Sci Pollut Res. 24, 10919-10928. doi: 10.1007/s11356-016-6813-z

A polymer inclusion membrane (PIM) made of cellulose triacetate as a polymer and the task specific ionic liquid (IL) trioctylmethylammonium thiosalicylate (TOMATS) was assembled as a new Diffusive Gradients in Thin film (DGT) device to test its efficiency as a binding phase for mercury (Hg) monitoring. The effect of IL content was assessed, showing that higher TOMATS percentage is better for short deployment studies (up to hours), whereas for long-term exposure (up to days), a lower content can be more suitable. Different configurations of PIM-DGT samplers have been tested under controlled conditions and compared with in-house DGT conventional ones, manufactured with thiol groups as resin layer, for the determination of labile Hg. According to our results, a nonlinear accumulation profile of Hg with deployment time for the different designs of PIM-DGT was observed, limiting the range of applicability of the DGT technique. Promising results for the efficient removal of Hg from aqueous solutions and/or environmental monitoring studies were obtained with TOMATS.

3.1 Background and aims

Mercury (Hg) is considered a priority hazardous substance because of its toxicity and widespread occurrence (UNEP, 2013). This metal is released to the environment through natural (e.g., erosion and volcanism) and anthropogenic processes (e.g., coal-burning power plants, waste incinerators, and other industrial processes), and it is globally distributed. Once it enters aquatic environments, inorganic Hg can be transformed to its more toxic form, methylmercury, mainly as the result of sulfate-reducing bacteria activity (Gilmour et al. 1992; Wiener et al. 2003), that tends to bioaccumulate and biomagnify throughout aquatic trophic chain (Clayden et al. 2013; Lavoie et al. 2013). Consequently, environmental Hg monitoring and analytical procedures for the determination of the dissolved bioavailable fraction of Hg in water have attracted significant attention recently. The diffusive gradient in thin film (DGT) fulfils the requirements to be a valuable passive sampling tool for this purpose. The DGT technique was developed for the *in situ* determination of kinetically labile metal species in aquatic systems (Davison and Zhang 1994), and it has been successfully used as a means to monitor the concentration of Hg species in natural waters (Fernandez-Gomez et al. 2011, 2012, 2014, 2015). The principle of the DGT technique is based on the diffusion of the dissolved species through a membrane-diffusive layer and their accumulation in an ion-exchange resin (binding phase). These two layers are separated from the solution to be analyzed by a filter membrane (usually 0.45 µm) and are enclosed and sealed in a small plastic device, so that only the filter is exposed to the deployment solution. In solutions with no ligands, the time-averaged concentration of the metal in the solution, C, can be calculated according to the Fick's first law of diffusion as:

$$C=\frac{M\Delta g}{DAt} \tag{3.1}$$

where D is the diffusion coefficient of the metal in the diffusive layer, t is the deployment time, A is the exposure surfare area, and Δg is the thickness of the diffusive layer. The mass (M) of the analyte accumulated by the resin is experimentally measured and provides the average labile metal concentration during the exposure time.

Heavy metals are usually measured using a Chelex-100 ion exchange resin as the binding layer (resin gel). However, other kinds of even more selective resins can be employed, such as those including thiol groups specific for Hg species. In this sense, different compounds such as spheron-thiol (Docekalova and Divis 2005; Fernandez-Gomez et al. 2012) or 3-mercaptopropyl functionalized silica gel (3MP) (Clarisse and Hintelmann 2006; Fernandez-Gomez et al. 2011) have proved suitable in DGTs when incorporated into a polyacrylamide gel. It should be noted that these compounds are commonly used as a binding agents for Hg in the form of resins. In order to explore new possibilities to entangle specific ligands that can interact with Hg, we have investigated the use of a polymer inclusion membrane (PIM) containing a task-specific ionic liquid (IL) to be used as a binding agent in DGT devices.

PIMs are chemically functionalized membranes that are commonly composed of an extractant (carrier), a base polymer (usually cellulose triacetate (CTA) or polyvinylchloride (PVC)), and a plasticizer. All the components are mixed in order to be entrapped within the base polymer matrix by a combination of physicochemical interactions (Pereira et al. 2009). PIMs usually appear as flexible, thin, and stable films that are simple and cheap to prepare. Moreover, they possess good mechanical properties (e.g., strength and flexibility) and are also versatile considering the diversity of target compounds that they can extract (Almeida et al. 2012). It is important to point out that the carrier has the central role in the PIM extraction process. The carrier reactively complexes with the compound of interest, acting as the phase-transfer agent and thus extracting the compound from the aqueous phase into the membrane phase by forming a hydrophobic ion-pair or a complex. The commercial quaternary ammonium salt Aliquat 336 (trioctylmethylammonium chloride) has extensively been used as a carrier in PIMs forming suitable membranes in either CTA or PVC polymers (Vázquez et al. 2014). PIMs incorporating Aliquat 336 have been shown to effectively transport anionic species in a wide range of applications since they are suitable to transport organic compounds such as antibiotics (Garcia-Rodríguez et al. 2015), inorganic anions (Nagul et al. 2013; Cho et al. 2011), or metallic species like Cd (Pont et al. 2008) or Cr (Gherasim et al. 2011), among others. This extractant has the particularity that it is an IL at room temperature. ILs are generally defined as salts that are liquid below 100 °C and consist entirely of ions. Normally, they include an organic cation, containing nitrogen or phosphorus, and an organic or inorganic anion. They show extraordinary properties such as an extremely low vapor pressure, high thermal stability, and high viscosity, and their physicochemical properties can be tuned by modifying their chemical structure (being "task-specific" ionic liquids) (Stojanovic et al. 2010). Among other uses, IL have successfully been applied as extractants for metals and metal-containing compounds in wastewaters (Stojanovic and Keppler 2012; Fisher et al. 2011).

Since ILs also have plasticizing abilities, they form flexible and stable membranes without the need to add any other components. Hence, the formulation of the membrane composition might contain only the polymer and the IL.

Even though many separation systems based on PIMs can be found in the literature, to the best of our knowledge, there are only a few studies related to the use of PIMs in passive sampling monitoring. On one hand, Almeida et al. (2014) developed a system based on a PIM containing di-(2-ethylhexyl)phosphoric acid (D2EHPA) for the sampling of Zn^{2+} and recently, a PIM device to monitor ammonia in freshwaters (Almeida et al. 2016). On the other hand, a device with a PIM made of CTA, Aliquat 336, and the plasticizer nitrophenyloctyl ether (NPOE) was designed for the monitoring of the antibiotic sulfamethoxazole (Garcia-Rodríguez et al. 2016). However, in these works, once the analyte was extracted into the PIM, it was transported to the liquid receiving phase, placed inside the device, where it was released.

With the aim of exploring the possibility of using a PIM with a specific extractant as a binding layer in a DGT probe for mercury monitoring, we have investigated trioctylmethylammonium thiosalicylate (TOMATS) to functionalize the PIM. This reagent is also an IL, such as Aliquat 336, having both the same cationic part but bearing a thiosalicylate anion instead of the chloride. TOMATS has proven its efficiency in terms of mercury extraction when used to impregnate palm shell carbon (Ismaiel et al. 2013) or when incorporated in an electrochemical sensor for the determination of this metal (Ismaiel et al. 2014).

In this study, the preparation of PIMs with TOMATS was investigated and characterized, and membranes were included in several in-house manufactured passive sampling devices with different designs. The performance of these devices was compared with in-house DGT standard devices that were manufactured by us. Before applying the samplers in the field, a series of laboratory assays were performed. Time series experiments using a Hg^{2+} solution were carried out under controlled laboratory conditions with DGTs and PIMs, and finally, they were tested for the determination of the dissolved-bioavailable fraction of Hg in river water.

3.2 Materials and methods

3.2.1 Reagents, standards and materials

The materials and reagents employed for the preparation of the in-house manufactured DGT gels were acrylamide solution (40%), electrophoresis grade (Fisher Scientific); DGT gel cross-linker, 2% aqueous solution (DGT Research Ltd., UK); ammonium peroxydisulfate, certified A.C.S, 99% (Fisher); N,N,N',N'-tetramethylethylenediamine (TEMED) ReagentPlus, 99% (Sigma-Aldrich); 3-mercaptopropyl-functionalized silica gel (3MP) (Aldrich); and 0.25 and 0.50 mm thick skived polytetrafluoroethylene (PTFE) films (CS Hyde company, USA) as spacers. Whatman 0.45 μm pore size, 25 mm in diameter nylon membranes were used as filters to protect the diffusive gel, and plastic DGT solution deployment mouldings (3.14 cm window) (DGT Research Ltd., UK) were used to support and enclose all the layers.

TOMATS was purchased from Sigma-Aldrich and was used as received. Cellulose triacetate was purchased from Fluka (Switzerland) and chloroform was obtained from Panreac (Spain).

Mercury standards and test solutions were prepared from a 1000 mg L^{-1} mercury Standard for ICP in HNO_3 12% (Fluka Chemika). High-purity demineralized 18.2 MΩ cm Milli-Q water (Millipore, USA) was used for dilutions and test solutions.

3.2.2 DGT manufacturing

The in-house manufactured DGT devices consisted of 3MP embedded in a polyacrylamide gel as the binding agent, and 0.4 mm thick polyacrylamide gel as the diffusive layer. DGT types used a 0.1 mm thick, 0.45 μm pore size filter membrane to protect the diffusive gel (DG). The gel layers were prepared according to the procedures described below.

3.2.2.1 Diffusive gel

A procedure used by Clarisse and Hintelmann (2006) was followed for preparing polyacrylamide gels. A gel solution consisting of 15% (v/v) acrylamide and 0.3% (v/v) cross-linker was prepared first. Subsequently, polymerization was initiated by adding 7 μL of freshly prepared 10% (w/v) ammonium persulfate and 2 μL of TEMED catalyst per each milliliter of the gel solution. After mixing, the solution was immediately cast between two glass plates separated by a 0.25 mm thick Teflon spacer and kept at about 44 ± 2°C for 45 min (Figure 3.1a). The gels were hydrated in Milli-Q water for at least 24 h before use. During this hydration step, the gel expanded to its stable dimension while impurities within the gel were able to diffuse out. Given its expansion factor (1.6) (Zhang and Davison 1999), the final thickness of the polyacrylamide gel was 0.4 mm. The gels were stored in Milli-Q water until assembly.

3.2.2.2 Resin gels

The binding layer, also known as the resin gel, consisted of a thiol resin embedded in a polyacrylamide gel. It was prepared by mixing 1.2 g of 3MP with each 10 mL of gel solution (the same as used for polyacrylamide diffusive gels). Polymerization was also initiated by adding 7 μL of freshly prepared 10% (w/v) ammonium persulphate and 2 μL of TEMED per each milliliter of the gel solution. The same casting procedure as for polyacrylamide diffusive gels was used.

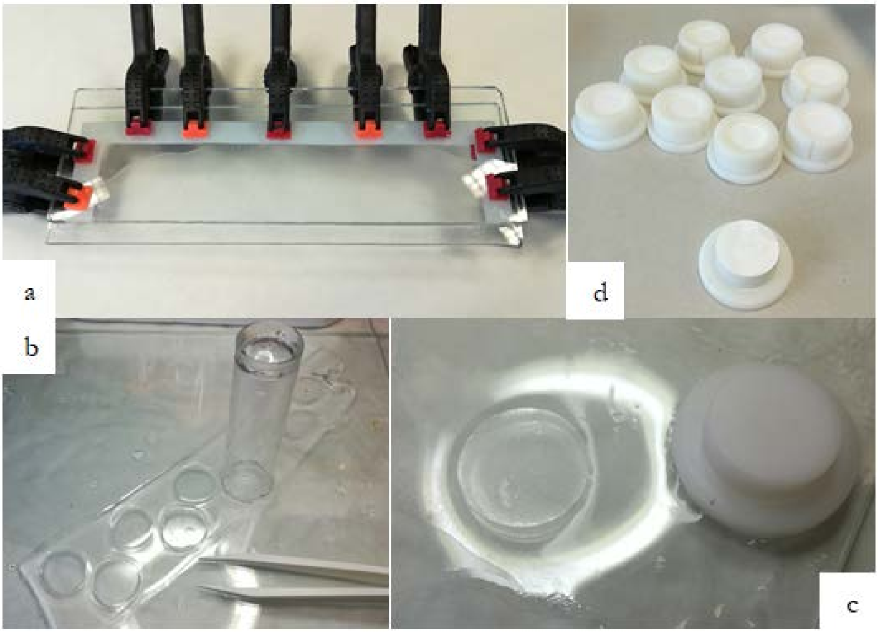


Figure 3.1: a) Preparation of gels for DGT devices between two glass plates b) Way to cut the gels c) Gel after having cut to for the assembly d) Placement of the filter before the assembly.

3.2.3 PIM preparation

PIMs were prepared by dissolving 200 mg of CTA in 20 mL of chloroform. After at least 5 h of agitation, the corresponding amount of TOMATS (see chemical structure in Table 3.2) was added: 0.2 g in the case of a PIM with a composition

50% CTA + 50% TOMATS (PIM-50% TOMATS) or 0.1 g for a 65% CTA + 35% TOMATS (PIM-35% TOMATS) (% weight). The solution was stirred by a magnetic stirrer until all membrane components had dissolved and then poured into a 9 cm diameter flat-bottom glass Petri dish, which was set horizontally and covered loosely (Figure 3.2a). The solvent was allowed to evaporate over 24 h at room temperature, and the resulting film was then carefully peeled off the bottom of the Petri dish (Figure 3.2b). Resulting membranes had an average thickness of 60 μm in the case of PIMs with 35% TOMATS and 95 μm for those containing 50% TOMATS. Circular segments were cut from the center of the membranes and placed in the DGT device.

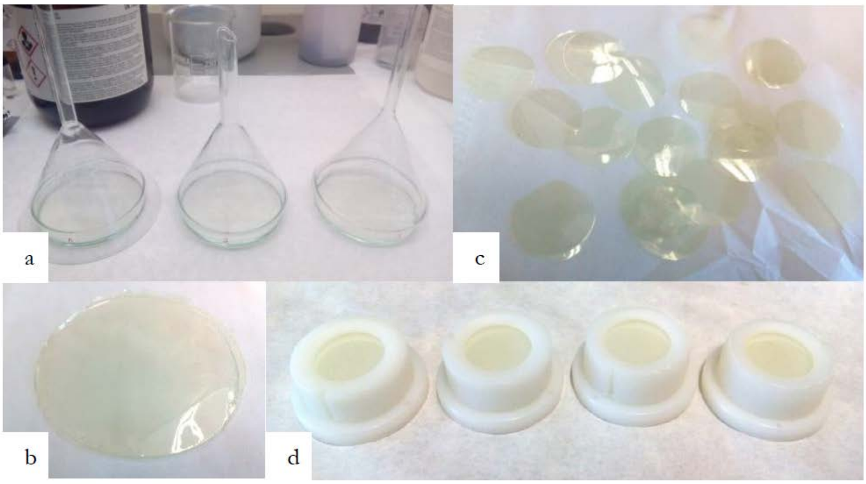


Figure 3.2: a) Preparation of PIM in the Petri dish b) Membrane of PIM before slicing c) Circular segments of PIM membrane d) Assembly of PIM in a DGT device.

3.2.3.1 PIMs characterization and stability studies

Elemental analysis of a PIM made of 70% CTA + 30% TOMATS was performed using a Perkin Elmer EA2400 instrument. Moreover, FTIR spectra for a CTA film, and PIMs containing 35% and 50% TOMATS were acquired using an Agilent Cary 630 FTIR spectrometer.

Taking into account that DGT devices must be left several days in contact with river water in the sampling points, the stability of the PIMs was investigated in terms of mass loss, which is related with the loss of the carrier (Kagaya et al. 2012). For that, PIM pieces of approximately 2 x 2 cm were dipped in 50 mL of both ultrapure water or 0.1 M NaCl solutions and were agitated in an orbital mixer for 24 h. Membranes were weighed before and after this procedure, and the mass loss was calculated.

3.2.4 Assembly of passive samplers

A plastic mold based on a simple tight-fitting piston design with a 2 cm diameter window (DGT Research Ltd., UK) was used to support the gels and to ensure that only a known surface of the DGT unit (area = 3.14 cm^2) was in contact with the solution (Figure 3.1c and Figure 3.2b). A disk of resin gel was placed on top of the cylindrical piston, with the side containing the gravity deposited resin beads facing upward (Figure 3.1b). A disk of diffusive gel was placed on top, followed by a 0.1 mm thick, 0.45 µm pore size nylon filter membrane for protection (Figure 3.1d). Care was taken to exclude air bubbles between each layer, by keeping the layers wet during assembly. The front cap was pressed down tightly, ensuring a good seal between the cap and the membrane surface.

For PIMs devices, the assembly was completed using the same plastic mold but replacing the resin gel by the PIM (e.g., PIM-50% TOMATS; PIM-35% TOMATS), which was fitted properly into the piston (Figure 3.2d). Therefore, four different designs were manufactured: PIM(A), only containing PIM; PIM(B), containing PIM and the diffusive gel (DG) plus filter; PIM(C), including PIM and filter; and PIM(D), comprising DG and PIM and DG plus filter (Table 3.1).

Table 3.1: Different designs for Diffusive Gradient in Thin film (DGT) and Polymer Inclusion Membrane (PIM) devices mounted on a simple tight-fitting piston.

Devices	Sampler Configuration
DGT	3MP + DG + 0.45 μm nylon filter
PIM(A)	PIM
PIM(B)	PIM + DG
PIM(C)	PIM + 0.45-μm nylon filter
PIM(D)	DG + PIM + DG + 0.45-μm nylon filter

The composition of PIM could be 50% CTA + 50% TOMATS or 65% CTA + 35% TOMATS and is indicated in the text as PIM-50% TOMATS or PIM-35% TOMATS, respectively
CTA cellulose triacetate, TOMATS trioctylmethylammonium thiosalicylate, 3MP 3-mercaptopropyl functionalized silica gel, DG: diffusive gel (polyacrylamide gel)

3.2.5 Hg measurement

THg in resin gels was measured using an Advanced Mercury Analyser, model AMA-254, manufactured by Altec (Prague, Czech Republic) and distributed by Leco Corp. (St. Joseph, MI, USA), which uses catalytic combustion of the sample, preconcentration by gold amalgamation, thermal desorption, and AAS. Every resin gel was put in a nickel boat, placed in the instrument and automatically introduced into the AMA. The entire analytical procedure was validated by analyzing a certified reference material consisting of dogfish (Squalus acanthias) muscle from the National Research Council of Canada (NRCC, DORM-2), with a Hg certified value of 4.64 ± 0.26 mg kg^{-1}. This reference material was analyzed in triplicate at the beginning and end of each set of (usually 10) samples, thereby ensuring that the instrument remained calibrated throughout the study. The detection and quantification limits (0.2 and 0.7 ng g^{-1} ww of Hg, respectively) were calculated based on blank measurements.

3.2.6 General procedures

All units were assembled 1 or 2 days before deployment and stored until use in a plastic container containing a 0.01 M NaCl solution in Milli-Q water. In the case of the laboratory assays, the devices deployment involved the immersion of the sampler, suspended by a nylon string, in a stirred aqueous solution. Prior to analysis, units were dismantled, resin gels or PIMs were extracted, and the amount of Hg measured directly with the AMA-254 spectrometer. Hg was determined in the spiked water solution before and after device deployment by taking a 5 mL aliquot and analyzing it immediately using the AMA-254.

3.3 Results and discussion

3.3.1 Uptake rate experiments for DGT-3MP

A time series experiment was performed with the aim of assessing the rate of Hg uptake by DGT units and obtaining the diffusion coefficient (D) of Hg ions in the respective diffusive gel layers. Two experiments were performed to identify if D is dependent of the initial concentration and/or the deployment time. Three DGT devices incorporating 3MP as the binding agent were submerged in 3 L of Hg^{2+} solution (0.01 M NaCl), stirred with a magnetic stirring bar, and held at a temperature of 20.5 ± 0.5 °C. In one experiment, sets of DGT pistons were retrieved after 2, 4, 6, 8, 24, 32, and 48 h in a solution of 12 μg L^{-1} , whereas in another experiment at a lower initial concentration (3 μg L^{-1}), pistons were retrieved after 3, 6, 9, 12, 25, 33, 49, 57, and 124 h (Figure 3.1). At each sampling interval, 5 mL of Hg solution was sampled as well to monitor the Hg concentration remaining in solution. Controls consisted of units that were not deployed at all (DGT blank) and units which were deployed for 32 h in 0.01 M NaCl (experimental blank), with values 1.09 ± 0.27 ng.

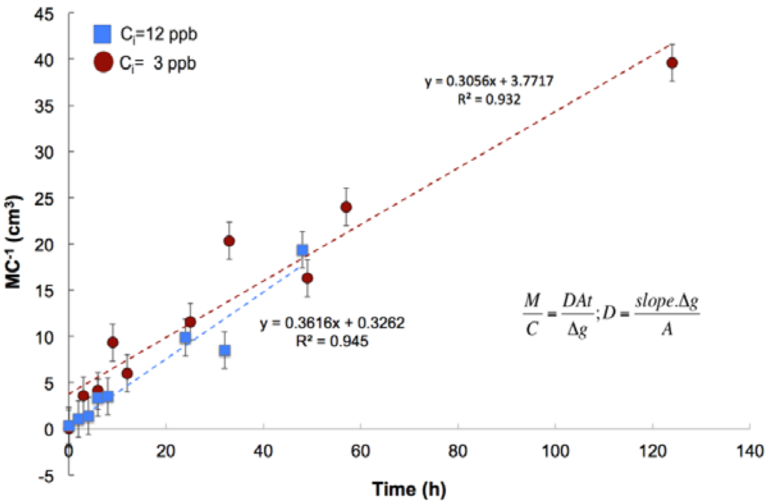


Figure 3.3: Time-series experiment for DGT-3MP. Mass of mercury accumulated in the resin (M) normalized by the Hg(II) concentration in the solution for various periods of time at two initial Hg(II) concentrations. Bars represent the mean and standard deviation of three replicates.

In accordance with the principle of DGT (equation 3.1), the mass of Hg accumulated in the resin (M) was proportional to the exposure time (t), as shown in Figure 3.3. To account for the decrease in Hg concentration in solution (C) during the experiment, caused by the DGT uptake, the mass of accumulated Hg was normalized by the solution concentration of Hg. The DGT performed equally well, as evidenced by the regression coefficients of the two linear curves ($R^2>0.93$). The two curves had similar slopes, and the diffusion coefficient (D) of Hg^{2+} in the diffusive layer was calculated from the slope (s) of the relationship between the amount of Hg accumulated by the DGT units (normalized for Hg concentration in solution) and the deployment time (equation 3.2).

$$s=\frac{DA}{\Delta g} \tag{3.2}$$

Thereby, the diffusion coefficient was calculated as follows:

$$D=\frac{s\Delta g}{A} \tag{3.3}$$

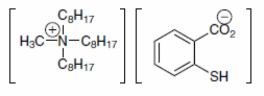
The diffusion coefficient of Hg^{2+} in the polyacrylamide DG of the DGT were calculated on the basis of Fick's law as 1.59×10^{-6} and 1.60×10^{-6} cm s^{-1}, for lower and higher Hg^{2+} concentrations, respectively, at 20°C. Diffusivities had the same order of magnitude as those obtained previously (Clarisse and Hintelmann 2006; Fernandez-Gome et al. 2011). As expected, results showed that there were no differences when DGTs were exposed to different Hg initial concentrations and longer deployment times.

3.3.2 PIM characterization and stability studies

Prior to including the PIM as a binding layer in DGT, some preliminary experiments were conducted to characterize both the membrane and extraction abilities of the IL embedded in the PIM.

Elemental analysis of a PIM made of CTA and TOMATS was performed to ensure that the IL was completely entrapped in the CTA matrix. Results presented in Table 3.2 demonstrate that the N and S (atoms only present in TOMATS molecule) contents agreed with the expected amounts, suggesting that PIMs with this IL can be well-produced. Moreover, FTIR spectra of PIMs made of pure CTA and different amount of TOMATS were performed and are presented overlapped in Figure 3.4. As can be noted from the spectra of PIMs with TOMATS, new bands appeared when compared with the one obtained with a film of 100% CTA. The bands at 2855 and 2925 cm^{-1} corresponded to stretching vibrations of CH_3 and CH_2 from TOMATS. COO- group stretching vibrations were also observed at 1598 cm^{-1}.

Table 3.2: Elemental analysis of a PIM containing TOMATS.

PIM	Element	% Calculated	% Found (n=3)
70% CTA	N	0,8	0.88 (0.03)
30% TOMATS	C	52,07	55.9 (0.2)
	H	7,83	7.26 (0.01)
	S	1,85	2.11 (0.09)

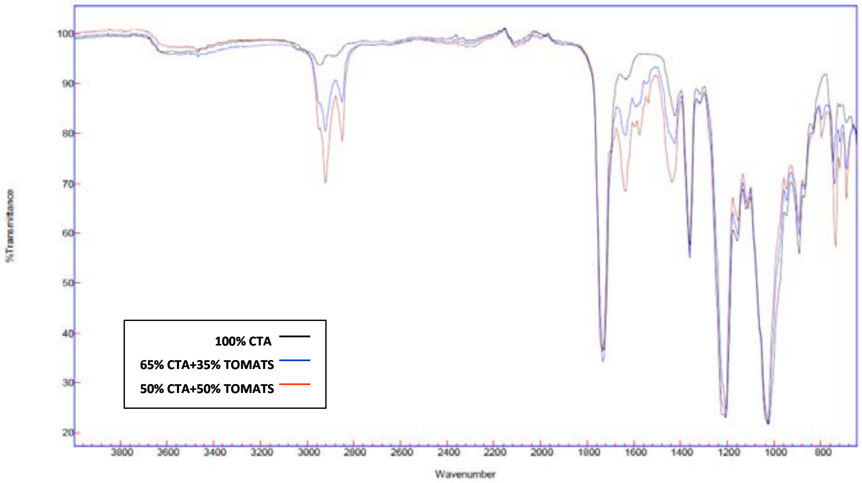


Figure 3.4: IR spectra for a pure CTA film and PIMs including 35% and 50% TOMATS.

The stability of the PIMs was investigated in both water and a saline solution. It is important to control release of the carrier from the PIM in order to ensure that the membrane maintained its extraction ability during the whole passive sampling period. Results obtained from this study are shown in Table 3.3. Both PIM compositions were very stable, with an insignificant loss of mass between 1.5 and 2.4%. These results are in great contrast with those observed by several authors when dealing with PIMs containing the IL Aliquat 336, since evidence exists that Aliquat 336 leaks from the polymeric matrix and partially solubilizes into the aqueous phases (Argiropoulos et al. 1998; Kagaya et al. 2012). Our results demonstrate that the interactions between the matrix and the IL are strong enough to form stable PIMs, and thus, this kind of membrane is a suitable option to entrap specific ligands to be incorporated in monitoring devices.

Table 3.3: Stability studies of PIMs made of CTA and TOMATS (after 24 h in contact with the corresponding solutions).

PIM composition	Solution	Mass loss (%) (n=3)
65% CTA+ 35% TOMATS	Water	1.5 (0,1)
	0.1 M NaCl	1.7 (0.7)
50% CTA+ 50% TOMATS	Water	2.3 (0.3)
	0.1 M NaCl	2.4 (0.5)

3.3.3 Hg^{2+} extraction with PIMs

Experiments were conducted to ensure the ability of the IL incorporated in a PIM to extract Hg at a very low concentration. Eight pieces of PIM-50% TOMATS (5 cm diameter, about 25 mg weight), unassembled from any piston, were placed in contact with 3 L of 1 μg L^{-1} Hg^{2+} solution (0.01 M NaCl, pH 7), and the adsorption was monitored over time. Figure 3.3a shows the percentage of Hg removal, whereas Figure 3.5b shows the amount of Hg remaining in solution. The results showed that adsorption increased rapidly during the first 2–3 h. After 3 h, the adsorption reached an equilibrium state. As is evident from Figure 3.5a, about 3 h contact time was required to attain adsorption equilibrium for the Hg solution. During this time, about 35% of Hg was adsorbed by the pieces of PIMs (i.e., between 4 and 5% of the initial Hg concentration each piece of PIM). At equilibrium, adsorption became almost constant (40–45%) up to the end of the experiment (8 h). In sum, each membrane was able to adsorb about 0.05 ng Hg g during a deployment time of 3 h. Thus, these results showed that even though TOMATS is entrapped into a polymeric matrix, this IL is a suitable extractant for Hg. It is postulated that the adsorption of Hg^{2+} can occur via the interaction of both the deprotonated carboxylic group and the S atom of the thiol group (Zhang et al. 2011).

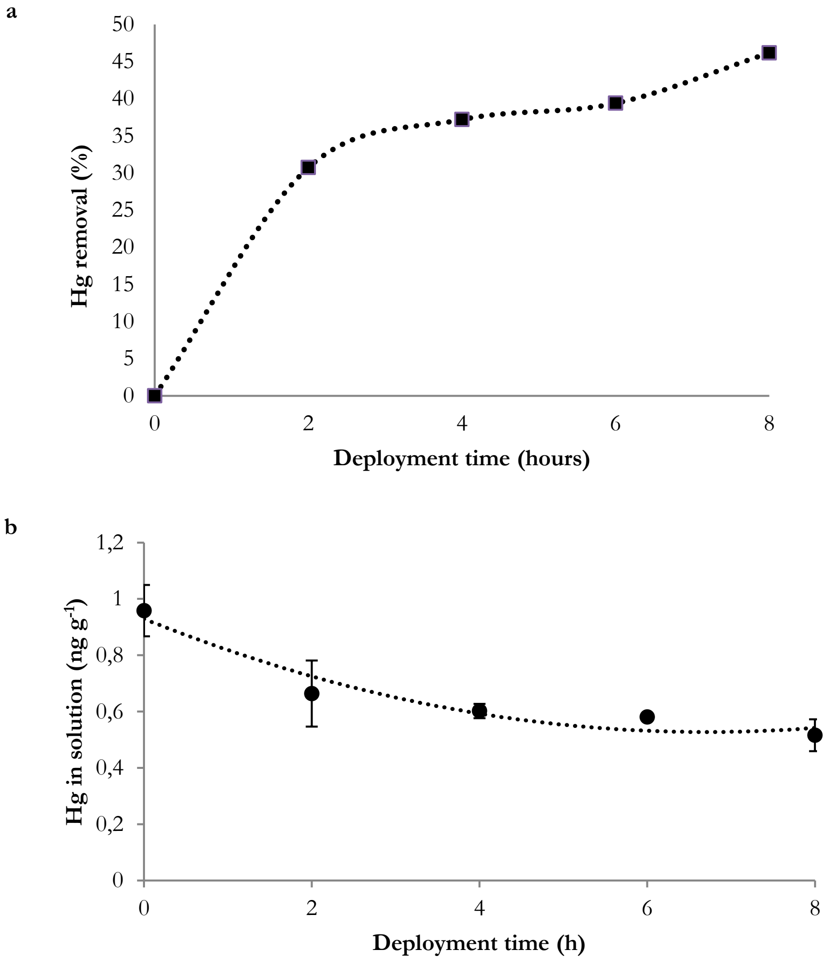


Figure 3.5: Effect of contact time on the removal of mercury by PIM-50% TOMATS (unassembled from any plastic DGT piston) submerged in 3 L of Hg(II) solution at an initial concentration of 1 µg L^{-1} (0.01 NaCl, pH 7). a) Hg(II) removal efficiency; b) amount of Hg(II) remaining in solution.

3.3.4 Mercury extraction in DGTs containing PIMs: effect of TOMATS content

The suitability of TOMATS incorporated in a PIM as a binding phase for a DGT sampler was investigated using the configuration PIM(A) at two levels of IL content: 35 and 50%. Then, different in-house manufactured PIMs samplers were prepared by including each membrane placed on top of the piston with a window on the top of the PIM. Sets of samplers were submerged in 3 L of a Hg^{2+} solution (2 µg L , 0.01 M NaCl), stirred with a magnetic stirring bar at 20 °C. Triplicates of the devices were recovered after 2, 4, 6, and 8 h (PIM-50% TOMATS) and 3, 6, and 9 h (PIM-35% TOMATS). Results presented in Figure 3.6a show higher extraction of the metal in the PIM-50% TOMATS than the one at 35%. However, taking into account that for sampling studies in the field, the DGTs should be left for several days; we also investigated the effect of Hg accumulation in DGT with a PIM content of 35% TOMATS over longer time intervals. As shown in Figure 3.6b, metal uptake vs. time follows a linear trend during the five days investigated, accumulating 780 ng g^{-1} PIM over this period. In summary, we can report that PIMs at different levels of IL are useful for Hg uptake studies, and the amount of IL can be tuned depending on the final use of the device: higher IL content is preferable for short-time studies, whereas for long-term exposure PIMs, a lower IL content is suitable.

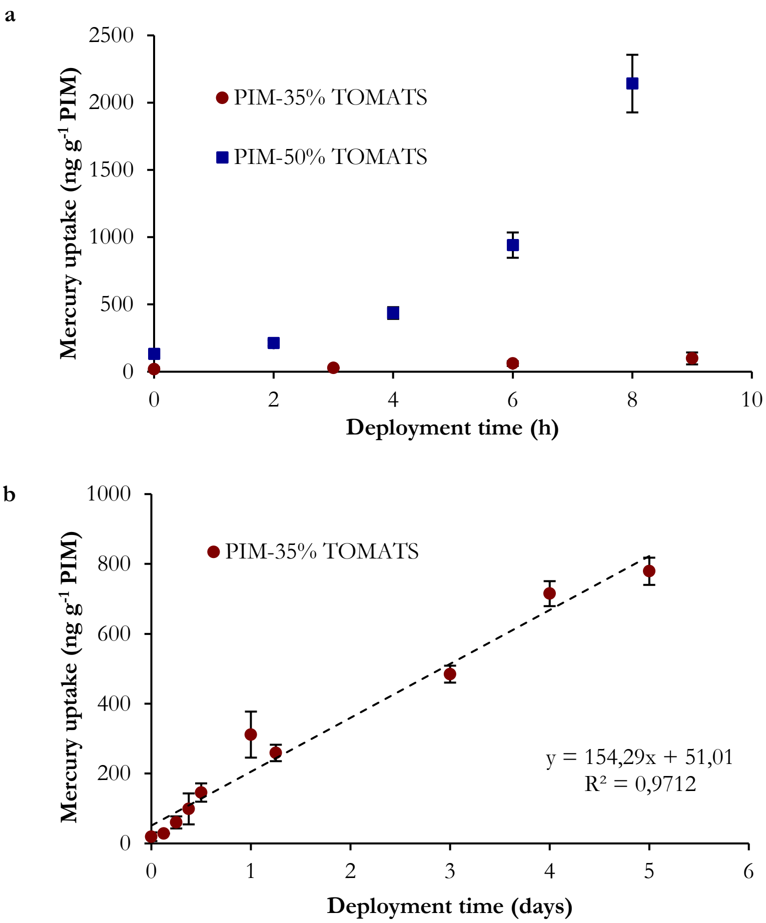


Figure 3.6: a) Uptake of mercury with two PIM compositions. PIM-50% TOMATS:50% CTA + 50% TOMATS and PIM-35% TOMATS:65% CTA + 35% TOMATS. All units were mounted on plastic DGT piston and submerged in 3 L of Hg^{2+} solution at an initial concentration of 2 µg L^{-1} (0.01 M NaCl, pH 7) at different deployment times. Bars represent the mean and standard deviation of three replicates. b) Uptake of mercury with PIM-35% TOMATS for long time deployments (5 days). All units were mounted on plastic DGT piston and submerged in 3 L of Hg^{2+} solution at an initial concentration of 0.4 µg L^{-1} (0.01 M NaCl, pH 7). Bars represent the mean and standard deviation of three replicates.

3.3.5 Uptake rate experiments for PIM-DGT

Four distinct PIM-DGT configurations to be assembled in the piston (Table 3.1) were prepared in order to elucidate their effectiveness as DGT samplers and to determine the amount of Hg uptake, depending on its unique configuration. In fact, the two models, PIM(A) and PIM(C), were designed without a diffusive gel and should not formally be regarded as DGT.

Following a similar approach as in previous experiments with DGT-3MP, a time series test was performed for each PIM-DGT design. Sets of devices were submerged in 3 L of a Hg^{2+} (2 µg L^{-1}, 0.01 M NaCl) solution, stirred with a magnetic stirring bar at 20 °C. Three devices were recovered after 2, 4, and 6 h for PIM(A), PIM(B), and PIM(C) and every hour for PIM(D). Also, at each sampling interval, 5 mL of Hg solution was analyzed to monitor the Hg concentration remaining in solution. Since experiments were performed over short-time deployments, all PIM-DGT types were prepared with PIM-50% TOMATS. Results are presented in Figure 3.7 and highlight operational differences based on PIM design, since a unique metal accumulation pattern was obtained for each device.

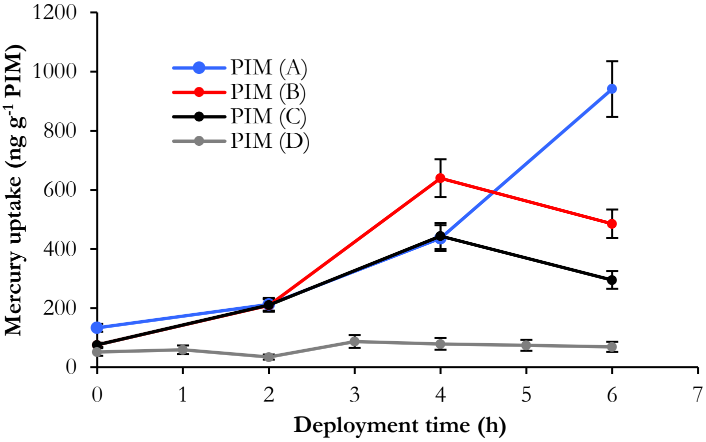


Figure 3.7: Mercury uptake with different PIM-50 % TOMATS designs (see Table 3.1 for details). Devices were submerged 3 L of Hg^{2+} solution at an initial concentration of 2 µg L^{-1} (0.01 M NaCl, pH 7). Concentrations are based on the total weight of the PIM. Bars represent the mean and standard deviation of three replicates.

In the design of DGT, a key assumption in applying equation 3.1, is that the surface of the resin layer acts as a perfect planar sink (Mongin et al. 2013). This implies that the binding to the resin should be strong, irreversible, and almost instantaneous. Because the limiting step is diffusion across the diffusive gel, the diffusion coefficient obtained in equation 3.3 is independent from the characteristics of the binding phase. So, if PIM acted as a perfect sink, we would obtain a similar diffusion coefficient for Hg, in the PIM(B) design, as with 3MP. However, for PIM(B), the linear relationship of Hg accumulation with deployment time failed ($R^2 = 0.703$), limiting the range of applicability of the DGT technique.

As shown in Figure 3.7, PIM(A), PIM(B), and PIM(C) presented a specific Hg uptake, whereas accumulation using PIM(D) was not directly proportional to the exposure time. Therefore, PIM(A) showed the highest uptake, as suggested by its value at 6 h (940 ± 140 ng g^{-1}), whereas the amounts of Hg accumulated by PIM(B) and PIM(C) were lower, 485 ± 73 and 295 ± 44 ng g^{-1}, respectively. Since the average weight of the in-house manufactured PIM units is 25 mg, then the mercury uptake by PIM(A) could be calculated as 24 ± 4 ng of Hg per binding disk at 6 h. Similarly, maximum uptake values of PIM(B) (12 ± 2 ng Hg) and PIM(C) (7 ± 1 ng Hg) were obtained per unit of disk. Our results show that the PIM(A) design, with no diffusive gels or filter membrane, was capable of uptaking the greatest amount of Hg in only 6 h relative to the other designs. In contrast, PIM(D), with two diffusive gels and a filter membrane showed the lowest sorption at top deployment time (69 ± 10 ng g^{-1}), which means 1.7 ± 0.3 ng Hg per unit of disk. Hence, the sorption of the sampler is highly affected by the presence of a double layer of DG on each side of the PIM membrane. On the other hand, it should be observed that, at 2 h deployment time, the amount of Hg taken up by all the samplers (except PIM(D)) was similar, with values around 200 ng g^{-1}. Moreover, at 4 h, PIM(A) and PIM(C) showed similar uptake (440 ng g^{-1}). These results were expected, since synthetic solutions do not form a particulate phase. However, the use of a 0.45 μm pore size filter membrane may be useful in future field studies. At higher deployment times, differences observed between all three devices were probably because PIM(B) and PIM(C) reached some equilibrium in 4 h; however, the final increase in case A is quite unexpected, and further studies with longer deployment times (up to days) are necessary to fully understand this behavior. Furthermore, more studies are needed to evaluate other membranes composition as well as other possibilities to entrap the IL in a binding phase as a perfect planar sink.

3.3.6 Field studies at the Ebro River

Because of its long and periodic history of Hg pollution (Carrasco et al. 2008, 2011), the Flix Reservoir and downstream sites were the locations within the Ebro River chosen to perform field experiments under normal environmental conditions. Over several decades, large amounts of industrial waste containing high concentrations of Hg (up to 436 μg g^{-1}) were dumped in front of a chlor-alkali plant in the Flix reservoir. So, samplers were placed downstream from the waste dumping point, at the Flix meander (FM), Ascó (AS), Xerta (XT), and Deltebre (DT) sites which were 300 m, 15 km, 65 km, and 110 km downstream from the Flix reservoir, respectively. Furthermore, a sampling point at Ribaroja (RB), 15 km upstream from the source of Hg pollution, was used as the control site. Due to the evident differences in Hg uptake capacities between DGT and PIM-DGT, samplers were deployed in triplicate for 2 months and 7 days, respectively, across all the sites at approximately 1–2 m above the sediment-water interface. Since experiments were performed over long-time deployments, PIM-DGT types were prepared with PIM-35% TOMATS. The sampling device used to hold and suspend the samplers consisted of a homemade cylindrical basket made of a plastic net. Finally, the basket was anchored to a rope with a weight on one end and a buoy on the other. Similar conductivity, approximately 1200 μS cm^{-1}, was found in the river water of the control and the target sites at the beginning and end of the experiment. A consistent pH value of 8 was measured at all sites, and the water temperature monitored throughout the experiment averaged 18°C.

The measured mass of Hg in the DGT devices deployed was used with equation 3.1 to determine the concentration of Hg in the water at the different sampling sites. Unfortunately, DGT units at RB and AS sites were lost due to flooding events in the Ebro River. DGT measurements at the FM, XT and DT sites were 10.2 ± 3.1, 1.83 ± 0.38, and 0.59 ± 1.2 ng L^{-1}, respectively. The mean value obtained at the FM site, which was closest to the source of Hg pollution, was 10 fold higher and statistically different than values obtained at XT and DT (t test, $p>0.05$). Values obtained with PIM-DGT devices (e.g., PIM(A)-35% TOMATS) at the different sites are shown in Figure 3.8. The maximum uptake was found close to the chlor-alkali plant, at FM, and gradually decreased downstream. This concentration was statistically different from the rest of the target sites (t test, $p>0.05$).

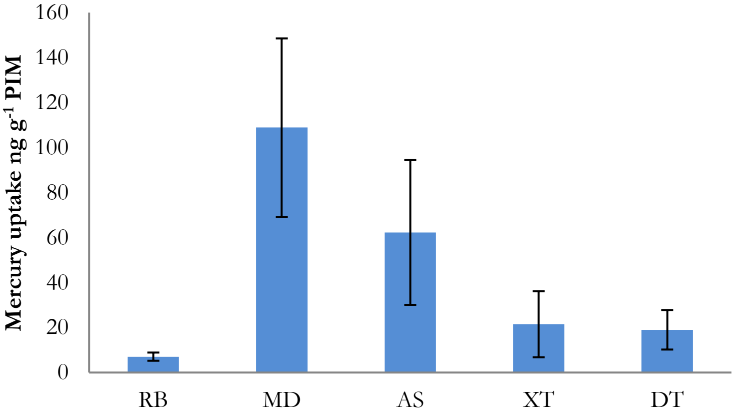


Figure 3.8: Field studies. Uptake of mercury by a PIM-device (PIM(A)-35% TOMATS) at different sites of the Ebro River (NE Spain) during 7 days. Concentrations are based on the total weight of the PIM units that were deployed mounted on pistons in triplicate. RB Ribaroja, control site, FM Meander, AS Ascó, XT Xerta, DT Deltebre. Bars represent the mean and standard deviation of three replicates.

According to tests performed in the field, DGT-3MP samplers can be used to determine the dissolved-bioavailable fraction of Hg in water, whereas Hg collected with DGTs incorporating PIMs cannot be reliably related to this fraction of metal. Further research should be done to correlate the Hg found in DGTs with PIMs as binding phases with metal species present in river water.

3.4 Conclusions

In this study, new PIMs containing the task-specific ionic liquid TOMATS were successfully prepared and characterized to be incorporated as a binding agent in

DGT samplers. Different configurations of PIMs were tested in order to explore their use as an alternative of conventional DGT-based measurements. Different PIM-DGT types were compared with an established DGT fabricated with 3MP as the resin layer, and polyacrylamide gel as the diffusive layer for Hg determination measurements in laboratory and field experiments. As expected, diffusion coefficients for DGT were equal at different initial concentrations. However, it was demonstrated that when PIM manufactured with CTA and TOMATS were assembled in the piston-like plastic molding as a binding phase and with polyacrylamide gel as the diffusive layer, it was not possible to apply the Fick's law of diffusion, limiting the range of application of the DGT technique. Nevertheless, taking into account the high performance, in terms of Hg extraction, exhibited by TOMATS incorporated in a PIM, further research will address characterizing the transport of Hg through the PIM phase, as well exploring new approaches to include TOMATS as a DGT binding phase to act as a perfect planar sink.

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Chapter 4

Phytochelatin synthesis in response to Hg uptake

This chapter is based on the article:

Turull, M., Grmanova, G., Dago, À., Ariño, C., Díez, S., Díaz-Cruz, JM., Esteban, M., (2017). Phytochelatin synthesis in response to Hg uptake in aquatic plants near a chlor-alkali factory. Chemosphere. 176, 74-80. doi: 10.1016/j.chemosphere.2017.02.092

The effects of mercury (Hg) released from a chlor-alkali factory in aquatic plants along the Ebro River basin (NE Spain) were analysed considering the phytochelatins (PC_n) and their isoforms content in these plants. These compounds were analyzed using HPLC with amperometric detection, and the macrophytes species *Ceratophyllum demersum* and *Myriopyllum spicatum* were collected in two sampling campaigns, autumn and spring, respectively. To correlate the PC_n content in macrophytes with the Hg contamination, analysis of total Hg (THg) content in plants and suspended particulate matter, as well as the dissolved bioavailable fraction of Hg in water measured by the diffusive gradient in thin film (DGT) technique were done. The results confirm the presence of PC_2-Ala in extracts of *C. demersum* and PC_2-desGly in *M. spicatum*, and the concentration of these thiol compounds depends clearly on the distance between the hot spot and the downstream sites: the higher the levels are, the closer the hot spot is. Since most of the Hg is hypothesized to be associated with SPM and transported downstream, our results of the DGT suggest that trace amounts of Hg in water can be released as free metal ions yielding a certain accumulation in plants (reaching the ppb level) that are enough for activation of induction of PCs. A few PCs species have been determined, at different seasons, indicating that they can be used as good indicators of the presence of bioavailable Hg in aquatic media throughout the year.

4.1 Background and aims

The effect that trace metal contamination has on terrestrial and aquatic ecosystems is a matter of major concern. Some trace toxic metals, like mercury (Hg), are capable of increasing in concentration upward through the food chain, reaching high levels in top predators (Morel et al., 1998). Then, an interesting point to be considered in contamination studies is the negative effect that pollutants have on living organisms. It is well known that some mammalians, plants, algae and some fungi have the capability to synthetize molecules to prevent the harmful effects produced by metals stress. In the case of plants, algae and some fungi, phytochelatin (PC_n) synthesis is considered necessary to tightly regulate the distribution of metal and to minimize damage under excess metal supply conditions. Phytochelatins are small cysteine-rich peptides, which play an essential role in heavy metal detoxification by chelating metals through thiol groups in the cythosol and transporting the complexes formed to the vacuoles. The general structure of PC_n is $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ (n = 2 to 5). Glutiathione (GSH) serves as the substrate of PC_n biosynthesis through the transpeptidation of the γ-Glu-Cys moiety of GSH onto a second GSH to form PC_2 or onto a PC_n molecule to produce an n+1 oligomer (Stillman et al., 1992; Bordin, 2000; Cobbett, 2000; Cobbett and Goldsbrough, 2002; Riordan and Vallee, 1991; Suzuki et al., 1993). By other hand, PC_n synthesis depends not only on the plant species but also of the metal stressor, being Cd the best activator followed by Ag, Bi, Pb, Zn, Cu, Hg and Au cations (Bundy et al., 2014; Dago et al., 2014b). This relationship of PC_n with trace metal contamination allows us to use PC_n content as an indicator of metal pollution. In the analysis of PC_n and their Hg complexes different methodologies have been applied. Among them the use of MS-HPLC is the most common, although detection by fluorescence or absorption spectroscopy is also considered (Serrano et al., 2015). In a previous work of our research group, a method to determine glutathione, PC_n and their Hg complexes using amperometric detection in a glassy carbon electrode has been developed (Dago et al., 2009, 2011). This method has been applied to study the effect that Hg and other metal ions have in the synthesis of PCs in *Hordeum vulgare* plants cultured in controlled conditions in the lab in the presence of these toxic substances (Dago et al., 2014a, 2014b) or in *Asparagus acutifolius* plants growing naturally in the mining district of Almadén (Dago et al., 2014c). These studies determined PC_n and their Hg-PC complexes in plants and established a correlation between the metal content in the growing media and the level of synthesized PC_n. On the other hand, some PC related compounds can also be synthesized simultaneously by plants; these compounds, also called iso-phytochelatins, are structural variants of PC_n that differ from them in one of their constituent aminoacids. Four families have been described, $(\gamma\text{-Glu-Cys})_n\text{-}\beta\text{-Ala}$, $(\gamma\text{-Glu-Cys})_n\text{-Ser}$, $(\gamma\text{-Glu-Cys})_n$ or $(\gamma\text{-Glu-Cys})_n\text{-Glu}$, that together with the phytochelatins described above form the metallothioneins class III (Grill et al., 1987; Rauser, 1995; Zenk, 1996). Another methodology, also based in HPLC with amperometric detection, has been proposed in a previous work of our research group for the determination of the isoforms of PC_2, which, in principle, are the most abundant in natural samples (Dago et al., 2015).

In the present work, a case of residue disposal from a chlor-alkali electrochemical plant sited in one bank of the Flix reservoir on the lower Ebro River (NE Spain) is considered. For more than 100 years, large amounts (*ca.* $3.5\ 10^5$ t) of hazardous industrial waste (e.g. metals and organochlorine pollutants) containing high concentrations of Hg (up to 400 mg kg^{-1}) were dumped in front of the dam riverbank causing a strong contamination (Palanques et al., 2014; Esbrí et al., 2015; Carrasco et al., 2011a; Navarro et al., 2009). In order to control the contaminant sludge effects and to remove the sludges, a retaining wall was built in 2012 around the sludge deposit. Nowadays, sludges are still being removed and transported to a controlled dumping area. A way to evaluate their impact on living organisms of the Ebro River could be through the analysis of PC_n and related compounds synthetized by macrophytes growing in the river, since they are good biomarkers for heavy metal stress. It is interesting to remark that most of the environmental studies related with this factory were devoted to the effects of Hg exposure in the aquatic environment (e.g. fish species, crayfish, molluscs) (Carrasco et al., 2008, 2011a; 2011b; Navarro et al., 2009), or focused on atmospheric Hg and its incorporation in soils and lichens (Esbrí et al., 2015), however, determination of PC_n and related compounds in aquatic plants has never been reported.

Recently, there is a growing interest in the use of aquatic macrophytes in the abatement of heavy metal pollution and as sentinel organisms of pollution in aquatic ecosystems (Rezania et al., 2016). Among them, the submerged species are particularly useful in the monitoring of heavy metals (Rai, 2009). In this sense, since *Ceratophyllum demersum* and *Myriophyllum spicatum* are invasive submerged aquatic plants and the dominant species growing in the Ebro River basin, they were selected for investigation in this work. Both have a large capacity to adsorb metal ions and by this reason both can potentially be used to remove metals from the aquatic media (Abdallah, 2012; Keskinkan et al., 2007; Milojkovic et al., 2014). However, as far as we know, studies of PC synthesis by these plants are scarce, only Mishra et al. (2006) determine the PC_n induced in *C. demersum* plants exposed to different levels of lead, and the presence of PC_2 and PC_3 was reported.

Thus, the aim of this work is to evaluate the toxic effect that upstream Hg contamination by the industrial waste and the wall built have on downstream macrophytes of this basin. In order to accomplish this, we carried out a field study in the low Ebro River basin, where two submerged macrophytes were collected and phytochelatin production was reported as a measure of the metal stress response. In order to correlate the PC_n synthetized with Hg concentration in the media, total Hg in aquatic plants, in suspended particulate matter and in sediment samples, as well as the dissolved-bioavailable fraction of Hg have also been reported.

4.2 Materials and Methods

4.2.1 Chemicals

Glutathione (GSH) was obtained from Merck (Darmstadt, Germany). Phytochelatins $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ ($n = 3–5$), as trifluoroacetate salts, were provided by DiverDrugs S.L. (Barcelona, Spain) with a purity ranging from 86.2% to 99.0%. Phytochelatin 2 (PC_2, $(\gamma$-Glu-Cys$)_2$-Gly) and its isoforms (PC_2desGly ((γ-Glu-Cys$)_2$), PC_2Ala ((γ-Glu-Cys$)_2$-Ala), PC_2Glu ((γ-Glu-Cys$)_2$-Glu) and CysPC_2 (Cys-(γ-Glu-Cys$)_2$-Gly)) were provided by Genosphere Biotechnologies (Paris, France) with a purity of 95%.

For preparing the mobile phase, acetonitrile from Panreac (Barcelona, Spain), NaCl, formic acid and KOH from Merck and trifluoroacetic acid (TFA) from Sigma-Aldrich (St. Louis, MO, USA) were used. Methanol from Merck was used for cleaning the column, and ethanol (96% purity) from Panreac was used for cleaning the glassy carbon electrode. EDTA (from Merck) was used for cleaning plants before storing at -80 ºC.

For plant extract preparation, cleaning the column and preparation of all solutions, ultrapure filtered water (18.2 MΩ cm^{-1}) obtained from a Synergy UV equipment from Merck Millipore (Darmstadt, Germany) was used.

The materials and reagents employed for the preparation of the in-house manufactured DGT gels were acrylamide solution (40%), electrophoresis grade (Fisher Scientific); DGT gel cross-linker, 2% aqueous solution (DGT Research Ltd., UK); ammonium peroxydisulfate, certified A.C.S, 99% (Fisher); N,N,N',N'-tetramethylethylenediamine (TEMED) ReagentPlus, 99% (Sigma-Aldrich); and 3MP (Aldrich). Whatman 0.45 μm pore size, 25 mm in diameter nylon membranes were used as filters to protect the diffusive gel, and plastic DGT solution deployment mouldings (3.14 cm^2 window) (DGT Research Ltd., UK) were used to support and enclose all the layers.

4.2.2 Study area

In order to cover the entire lower Ebro River (NE Spain) (Figure 4.1), from the Flix reservoir to the Ebro Delta (approximately 125 km), 5 sampling sites were selected, based on accessibility and abundance of aquatic plants. The entire monitored area has vast ecological, agricultural and recreational values. Of special interest is the Ebro Delta (320 km^2), which contains productive rice fields (210 km^2) and wetlands (80 km^2), and is rich in waterfowl and fisheries. The Ebro Delta is on the List of Wetlands of International Importance, designated under the Ramsar Convention. The strip of coastal land on the delta plain has various designations of protection: it is a Natural Park, a PEIN (Plan for Areas of Natural Interest) site, a Natura 2000 site, and it includes several nature reserves and several wetlands included on the Wetland Inventory of Catalonia. These natural values support important economic activities associated with tourism, hunting, fishing and aquaculture.

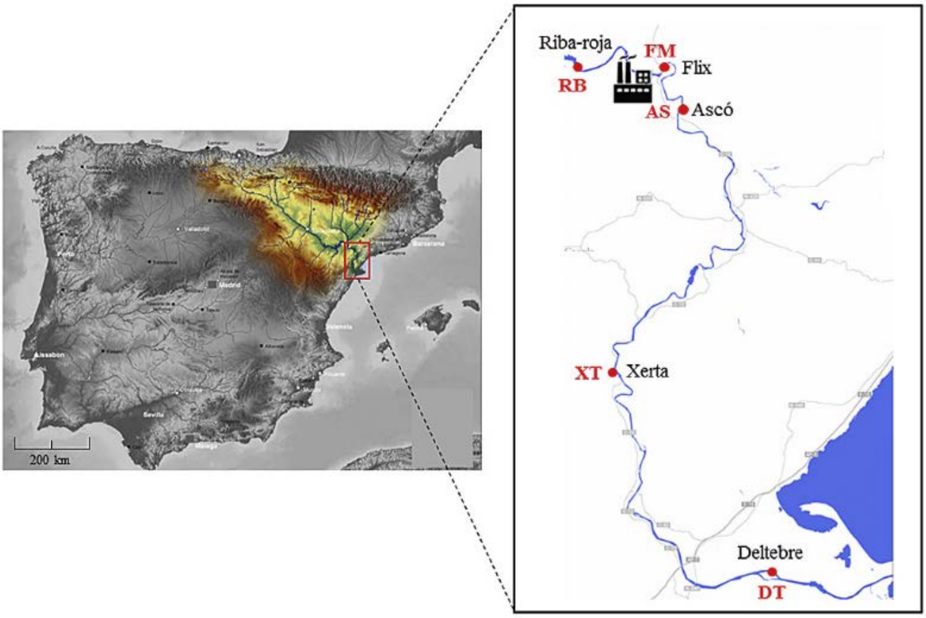


Figure 4.1: Sampling points in the Ebro River (Spain).

Although it would be interesting to sampling inside the area limited by the retaining wall, the entrance is prohibited and it was unmanageable to collect samples. The Flix meander (FM) is the sample point nearer to the contamination focus, located immediately downstream of the Flix dam; while Ascó (AS), Xerta (XT) and Deltebre (DT) sites are in consecutive sections of the river, separated by different overflow dams, located at 15, 65 and 110 km downstream of Flix, respectively. The Riba-roja dam (RB), which is used as a reference site, is located 15 km upstream of Flix, and forms a large water reservoir.

4.2.3 Plant, sediment and water sampling

Submerged aquatic plants (i.e. *C. demersum* and *M. spicatum*) are cosmopolitan species, colonising mainly eutrophic stagnant and flowing waters (Germ et al., 2006). They exhibit a similar life form, having similar "mesh-like"' architecture (i.e. branched stems and finely dissected leaves arranged in whorls around the main axis) (Rovira et al., 2016), and previous studies also showed similar adsorption capabilities for metals (Keskinkan et al., 2007). Their proliferation is different during the year, while *C. demersum* dominates in autumn and *M. spicatum* in spring. Since both macrophyte species grow in the same habitat (i.e. sampling point) and have similar plant architecture, we consider that comparisons between seasonal surveys during both the autumn and spring are feasible, because we would expect similar responses. Thus, in October 2014 (autumn sampling campaign), aquatic plants of the species *C. demersum* and water samples were sampled, and where it was possible river sediments were also collected. Plant species *M. spicatum* and water samples were collected in April 2015 (spring sampling campaign). Several physico-chemical parameters of sampling media (i.e. temperature, conductivity, total suspended solids (TDS) and pH) were measured at each sampling point with a Hydrolab DS5 multisensor probe (Hach Environmental; Loveland, CO, USA). Obtained results are shown in Table 4.1. The analysis of the variance of these values shows that in the case of temperature and pH values there is not a significant difference between values measured at the different sampling points but a significant difference between both campaigns exists. Related with conductivity and TDS a similar behaviour is observed, except for DT point where the salinity increases as a consequence of the proximity of this point to the sea. Collected plants and water samples were preserved in a fridge and transported to the laboratory along the same day.

Table 4.1: Physico-chemical parameters of river water in the different sampling points and sampling campaigns. Sites: RB Riba-roja dam; FM Flix meander; AS Ascó; XT Xerta; and DT Deltebre.

	October 2014					April 2015				
	RB	FM	AS	XT	DT	RB	FM	AS	XT	DT
Temperature (°C)	19.7	19.7	21.8	22.2	22.2	15.7	17.9	16.6	18.3	18.2
Conductivity (µS cm^{-1})	1365	1335	1367	1348	1961	783	773	796	804	1530
TDS (g L^{-1})	0.88	0.85	0.87	0.86	1.25	0.50	0.49	0.51	0.51	0.98
pH	7.74	7.83	7.88	7.92	7.96	8.22	8.47	8.26	8.22	8.58

4.2.4 Sample preparation

Once plant samples were in the laboratory, they were cleaned with ultrapure filtered water and with a solution of EDTA 0.1 mol L^{-1} to remove superficial adsorbed metals, afterward they were accurately dried with filter paper and cut into small pieces. Samples were ground with liquid nitrogen in a mortar and stored at -80 °C until analysis.

Sediments were ground and homogenized with an agate mortar and pestle and sieved through mesh to obtain a particle size lower than 200 µm.

Suspended particulate matter (SPM) was obtained passing 1 L of water through a cellulose acetate membrane filter (pore size 0.45 µm, Albet, Sant Joan Despí, Spain).

4.2.5 Analysis of phytochelatins by HPLC-ED

Prior to HPLC analysis, 100 mg of fresh plant sample were mixed with 500 µL of ultrapure filtered water at 1,500 rpm for 1 h in an Eppendorf MixMate (Hamburg, Germany) and filtered through 0.45 µm Nylon filter discs (Osmonics, Minnetonka, MN, USA).

An Agilent 1200 chromatographic system (Agilent, Santa Clara, CA, USA) equipped with a quaternary pump, an automatic injector and a vacuum degasser were used. An Ascentis C18 5 µm particle size analytical column measuring 25 cm

× 4.6 mm was provided by Supelco (Bellefonte, PA, USA). For the separation of phytochelatins (GSH, γ-Glu-Cys, and $PC_{2–5}$), a mobile phase consisted of 0.1% TFA in ultrapure filtered water pH = 2.00 and 0.1% TFA in acetonitrile was used. Separation was performed with gradient elution, as described in Dago et al., 2011. For the analysis of PC isoforms (PC_2-desGly, PC_2-Ala, PC_2-Glu and Cys-PC_2), a mobile phase consisted of 1% of formic acid with 0.1 mol L^{-1} of NaCl in ultrapure filtered water pH = 2.00 and 1% of formic acid in acetonitrile was used with isocratic elution (96:4, aqueous solution:organic solution) (Dago et al., 2015). Because larger PC_n were not present, the method to determine PC_2 isoforms was modified to include the separation of PC_3 by using gradient elution starting at 4% of organic solvent for 16 min then increasing to 20% during 6 min and keeping it constant until a total analysis time of 25 min. The injected volume was 20 μL and the flow rate was 1.2 mL min^{-1}. Amperometric detection was performed in an electrochemical flow cell from Bioanalytical Systems, Inc. (BASi, West Lafayette, IN, USA) controlled by a potentiostat μAutolab Type III (Eco Chemie, Utrecht, The Netherlands). The flow cell consists on a glassy carbon working electrode (BASi) whose surface was daily polished, a stainless steel auxiliary electrode and a Ag/AgCl (NaCl 3 mol L^{-1}) reference electrode. The optimized potential for the working electrode was 1.2 V.

4.2.6 Dissolved Hg in water

For the determination of the dissolved-bioavailable fraction of Hg in water, the diffusive gradient in thin film (DGT) technique was used as a passive sampling technique. The principle of the DGT technique is based on the diffusion of the dissolved species through a membrane-diffusive layer and their accumulation in an ion-exchange resin (binding phase). These two layers are separated from the solution to be analysed by a filter membrane (usually 0.45 μm) and are enclosed and sealed in a small plastic device, so that only the filter is exposed to the deployment solution. In solutions with no ligands, the time-averaged concentration of the metal in solution, C, can be calculated according to the Fick's first law of diffusion as:

$$C=\frac{M\Delta g}{DAt} \qquad (4.1)$$

where D (D=1.60x10^{-6} cm^{2} s^{-1}) is the diffusion coefficient of the metal in the diffusive layer, t is the deployment time, A the exposure surface area, and Δg the thickness of the diffusive layer. The mass (M) of the analyte accumulated by the resin is experimentally measured and provides the average labile metal concentration during the exposure time. The preparation of the DGT gels and assembly are described in previous works Fernández-Gómez et al., 2011,2014).

In-house manufactured polyacrylamide DGT samplers were deployed in triplicate for 7 days, in all sampling sites, approximately 1–2 m above the sediment-water interface (between 3 and 5.5 m of river depth). The sampling device used to hold and suspend the samplers consisted of a homemade cylindrical basket made of a plastic net. Finally, the basket was anchored to a rope with a weight on one end and a buoy on the other. After retrieval, DGT units were rinsed with distilled water and kept in polyethylene bags for the transport to the laboratory. Once in the laboratory, DGT units were dismantled and the resin gel was extracted and analyzed for dissolved bioavalable Hg content.

4.2.7 Analysis of total mercury concentration

Samples of plants, sediments, suspended particulate matter, and the resin gel from DGTs, were analyzed using an advanced mercury analyzer AMA-254 manufactured by Altec (Prague, Czech Republic) and distributed by Leco (St. Joseph, MI, USA), which is based on catalytic combustion of the sample, preconcentration by gold amalgamation, thermal desorption and atomic absorption spectrometry (AAS). The entire analytical procedure was validated by analyzing CRM from the National Research Council of Canada (NRCC, DORM-2: 4.64 ± 0.26 μg g^{-1} and DORM-3: 0.382 ± 0.060 μg g^{-1}) at the beginning and end of each set of samples, ensuring that the instrument remained calibrated during the course of the study. The concentrations of Hg obtained for repeated analyses (n=5) of both CRM were in good agreement to the certified values. The absolute detection limit was 10 pg, and detection limit given as treble standard deviation of Hg content in blank samples was 0.1 ng g^{-1}.

4.2.8 Statistical methods

The results for each sample were calculated as the mean ± the standard deviation from triplicate determinations. Normal distributions were obtained based on the Kolmogorov–Smirnov test for THg concentrations in aquatic plants and SPM, whereas data in DGT were not normally distributed. The student's t-test or the analysis of the variance were used to compare parametric data, and Mann-Whitney U and Kruskal-Wallis tests to compare non-parametric data. Pearson's linear correlation and regression analysis were used to establish the relationships between the variables (THg in plants, THg in SPM and THg in DGTs). Statistical significance was defined as $p \leq 0.05$. The experimental results were statistically evaluated using the IBM SPSS version 23 (Chicago, IL).

4.3 Results and discussion

4.3.1 Phytochelatin analysis

As it has been said above, a way to follow heavy metal stress response in plants is to analyse the synthesized phytochelatins. The analysis of *C. demersum* extracts corresponding to the autumn sampling campaign was done first using as mobile phase 0.1% TFA in ultrapure filtered water pH = 2.00 and 0.1% TFA in acetonitrile applying a gradient elution. Regarding the obtained chromatograms (results not shown) the presence of longer PC_n (PC_4 and PC_5) was discarded. By this reason, we decided to focus on the analysis of smaller thiols using as mobile phase formic acid with 0.1 mol L^{-1} of NaCl in ultrapure filtered water pH = 2.00 and 1% of formic acid in acetonitrile applying the elution profile described above.

Using this methodology, chromatograms indicated the highest concentration of PC_2-Ala close to the hot spot in Flix and decreasing when moving away from the contaminant focus (Figure 4.2a). To better ascertain the presence of this thiol compound, samples were spiked with different concentrations of standard and analysed. Data confirmed the presence of PC_2-Ala in the samples as the peak increases when increasing the spiked concentration of standard (Figure 4.2b). The quantification of PC_2-Ala was done using external calibration curve and analyzing three independent replicates. The obtained results were 116 ± 6 nmol g^{-1}, 77 ± 2 nmol g^{-1} and 70.1 ± 0.5 nmol g^{-1} fresh weight, for the sampling sites FM, AS and XT, respectively. In RB and in the Ebro River mouth in DT, PC_2-Ala was non-quantifiable.

Only PC_2-Ala could be detected and determined, and its concentration decreases from the source of contamination (FM, highest value) to the estuary (DT, non-quantifiable).

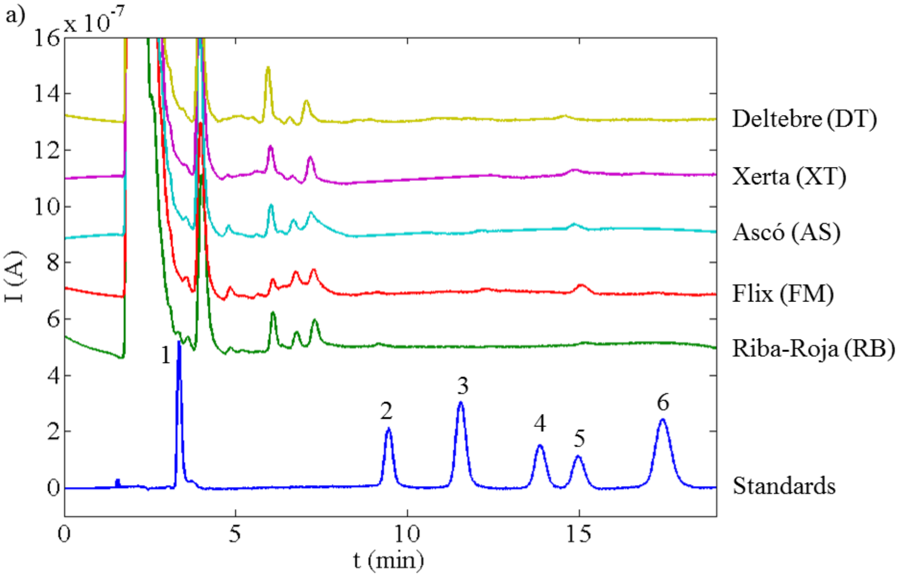
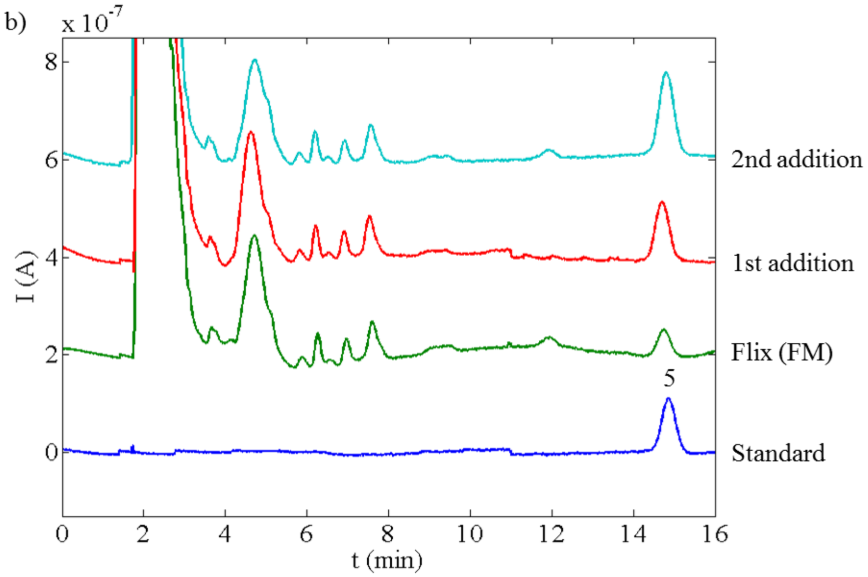


Figure 4.2: a) Chromatograms of extracts of C. *demersum* and standards of thiols (6 10-5 mol L-1); 1: GSH, 2: PC2, 3: PC2-desGly, 4: PC2-Glu, 5: PC2-Ala and 6: Cys-PC2. Mobile phase of 1% formic acid and 0.1 mol L-1 of NaCl at a pH of 2 and 1% of formic acid in acetonitrile (96:4).



(continue) Figure 4.2: b) chromatograms from Flix (FM) with two additions of 10 and 25 µL, respectively, of PC_2-Ala 10^{-4} mol L^{-1}. Mobile phase of 1% formic acid and 0.1 mol L^{-1} of NaCl at a pH of 2 and 1% of formic acid in acetonitrile (96:4).

The second sampling campaign was done in spring and the sampled aquatic plant species was *M. spicatum*. No plants were found in XT site, so only four sampling points were considered. In this case, we proceed directly to the analysis of samples using formic acid-NaCl-water (pH = 2.00) / formic acid – acetonitrile as mobile phase (Figure 4.3a). The presence of PC_2-desGly could be clearly detected and confirmed by the addition of standard (Figure 4.3b). A small peak at the retention time of PC_2-Glu seemed to appear in the chromatogram obtained from FM samples, whose presence was also confirmed by spiking the sample with the standard (Figure 4.3b). While quantifying the thiols, only PC_2-desGly could be determined with values of 63 ± 3 nmol g^{-1}, 170 ± 3 nmol g^{-1}, 154 ± 2 nmol g^{-1} and 122 ± 2 nmol g^{-1} fresh weight, for the sampling sites RB, FM, AS and DT, respectively. PC_2-Glu could only be detected in the FM samples but it was non-quantifiable.

These results show a low content in PCs in comparison with those obtained in other studies of the research group (Dago et al., 2014a, 2014c) in which plants grown in media with very high levels of Hg. Nonetheless, some PCs (PC_2 and PC_3) were found in *C. demersum*, growing in the lab and stressed with lead, at levels of the same order as those found in this work (Mishra et al., 2006). We can conclude that the available Hg uptake by the macrophytes may be low as compared to media with higher content of available metal but still suficient to promote a noticeable synthesis of PC_n.

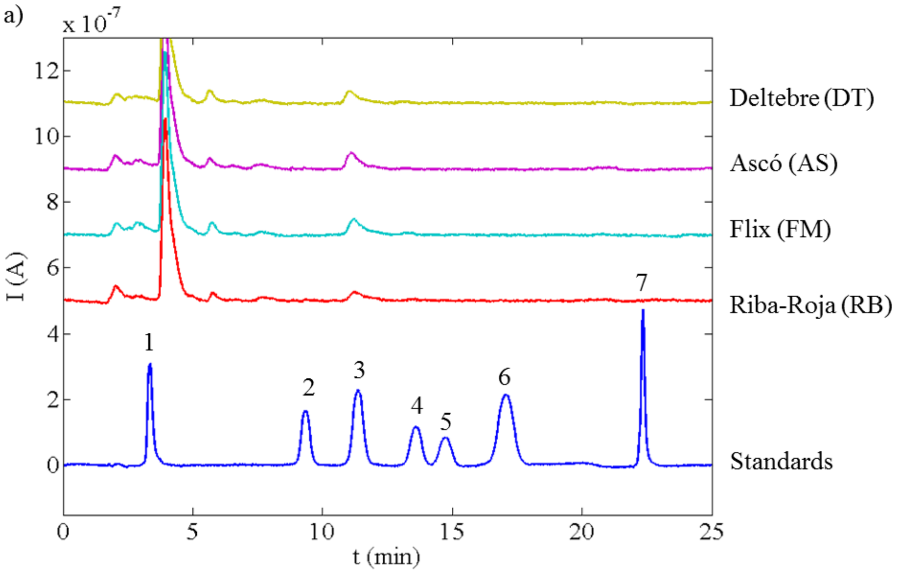
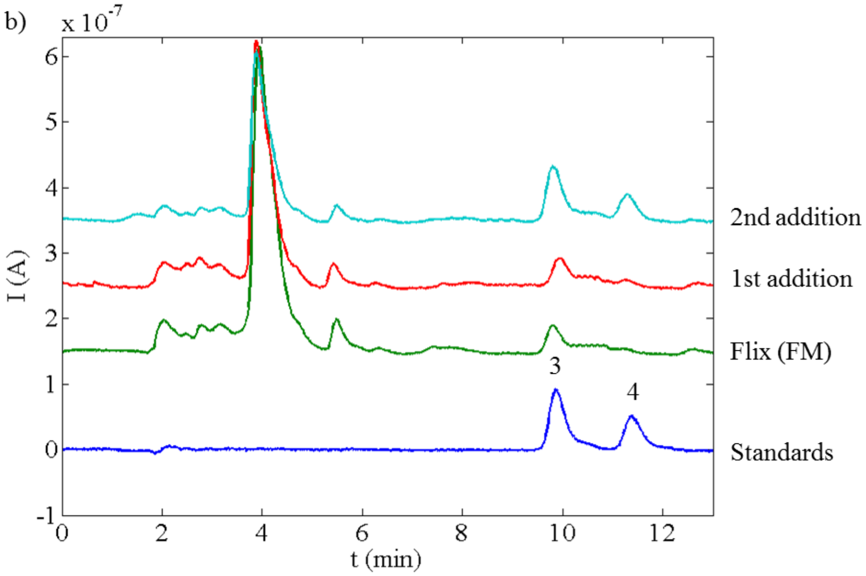


Figure 4.3: a) Chromatograms of extracts of *M. spicatum* and standards of thiols (6 10^{-5} mol L^{-1}); 1: GSH, 2: PC_2, 3: PC_2-desGly, 4: PC_2-Glu, 5: PC_2-Ala, 6: Cys-PC_2 and 7: PC_3. Mobile phase of 1% formic acid and 0.1 mol L^{-1} of NaCl at a pH of 2 and 1% of formic acid in acetonitrile with a gradient elution



(continue) Figure 4.3: b) Chromatograms of extracts of *M. spicatum* sampled in Flix (FM) with two additions of 120 and 320 µL, respectively, of a mixture of PC_2-desGly and PC_2-Glu 10^{-4} mol L^{-1}, and standards (6 10^{-5} mol L^{-1}) of 3: PC_2-desGly and 4: PC_2-Glu . Mobile phase of 1% formic acid and 0.1 mol L^{-1} of NaCl at a pH of 2 and 1% of formic acid in acetonitrile (96:4).

Regarding this aquatic plant species a relatively high concentration of PC_2-desGly could be found in all sampling stations with the highest concentration at the hot spot and decreasing downstream until the estuary. The presence of PC_2-Glu could also be detected, but not quantified because signals are below LOQ.

4.3.2 Hg concentrations in river samples

Total concentration of Hg in plants, THg in suspended particulate matter and dissolved-bioavailable Hg in water using DGTs are presented in Figure 4.4. In this figure the THg concentration in particles are expressed in contaminant mass per dry weight of particles (µg g^{-1}) and per volume of river water (ng L^{-1}).

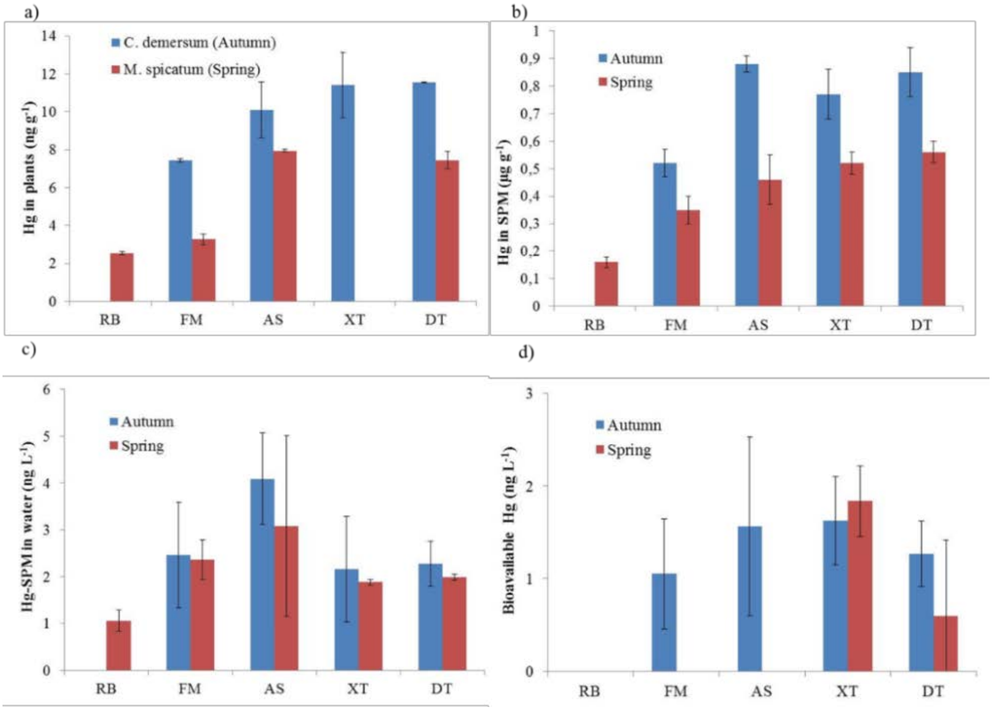


Figure 4.4: Diagrams comparing THg in plants (ng g^{-1}). a) THg in suspended particulate matter per mass weight of particles (µg g^{-1}). b) THg in suspended particulate matter per volume of water (ng L^{-1}). c) dissolved-bioavailable Hg in water (ng L^{-1}) using DGT devices (d) for the two sampling campaigns at different sampling points: Riba-roja (RB); Flix meander (FM); Ascó (AS); Xerta (XT) and Deltebre (DT).

The values of Hg in SPM are 2 and 5 orders of magnitude higher than Hg in aquatic plants and in DGTs respectively. These data show that THg is significantly higher ($p<0.05$) in plants collected in autumn (*C. demersum*) than in spring surveys (*M. spicatum*) (Figure 4.4a). These values are in good agreement with the significantly ($p<0.05$) different content of Hg also observed between seasons for SPM (Figure 4.4b). Moreover, a clear trend was observed for Hg levels found in SPM with values increasing with distance from the hot spot (FM) to downstream sites (AS, XT and DT) in both seasons. Considering THg in plants (Figure 4.4a), a similar behaviour is also observed. Indeed, statistically significant differences ($p<0.05$) in Hg concentrations were observed for *M. spicatum* between site FM and all the downstream sites, whereas no statistical differences were found for *C. demersum*. Some of these facts can be related to the two flood events occurred just before the autumn sampling campaign, when *C. demersum* was collected. Dams in the upper part of the lower Ebro River alter the downstream flow regime, since during the scouring out of deposited sediments, a downstream impact is expected. Accordingly, accumulated contaminants in sediments could be mobilized downstream during flushing operations (Kirchner et al., 2000). This occurs when an excess of rain made it necessary to open the dams of RibaRoja and Flix reservoirs. The opening of both reservoir dams dramatically increased the water flow of Ebro River downstream and consequently the amount of re-suspended particle matter as well as Hg residues associated with it. Therefore, suspended solids present in the water column varied across sampling periods and sites, decreasing during the periods of lower water flow (i.e. April 2015) and increasing during the autumn. So, observed high levels of suspended solids in autumn are related to the high flow regimen in comparison to that of the Ebro River in spring (normal/low flow). Furthermore, after the opening of dams, the concentration of THg in SPM is similar during the course of the river and toward the river downstream (Figure 4.4b, AS, XT and DT), because the Hg associated with sediments coming from upstream are flooding downstream. On the other hand, it could be observed that the amount of Hg per volume of water is decreasing (Figure 4.4c), because the amount of SPM that goes further is lower at far away sites.

These results are in the same line as those obtained previously (Carrasco et al., 2011a; Navarro et al., 2009). Maximum levels of THg in liver, kidney and muscle of feral carp (*Cyprinus carpio*), as well as the highest biological impact (i.e. increased concentration of reduced glutathione in liver and on mRNA expression of two metallothionein genes, MT1 and MT2), did not occur at the discharge sites, but several kilometres downstream.

These data are influenced by the concentration of SPM in the river water in each sampling point. As stated above, the quasi-stationary plateau reached by Hg in SPM (Figure 4.4b) is consequence of the similar concentration of THg associated with the SPM flushing down after opening the gates of the dams. Figure 4.4c shows the levels of THg in SPM per liter of water, indicating a maximum at the AS sampling point in both campaigns, and a decrease in particulate matter in the water at the XT and DT sites.

Related with sediments, they were only sampled and analysed in two sampling points (FM and AS) in the first campaign. Values of Hg in sediments (0.32 ± 0.08 $\mu g\ g^{-1}$ in FM and 0.67 ± 0.08 $\mu g\ g^{-1}$ in AS) were of similar order of magnitude than those found in SPM. On the other hand, values of the dissolved-bioavailable fraction of Hg were in the ppt range in both campaigns (Figure 4.4d). During the spring campaign, DGT devices were lost (theft or vandalized) in several stations; therefore, unfortunately, only two sampler devices (at XT and DT) were measured during this season. Although direct comparison of the dissolved-bioavailable Hg by DGT with the bioavailable Hg incorporated by the plant is not possible, the low levels of Hg measured by DGT match very well with the scarce varieties and low levels of PC_n found in plants. It must be pointed out that according to typical distribution of Hg species in river waters (Morel et al., 1998; Boszke et al., 2002), Hg mostly forms insoluble hydroxides, which are surely incorporated to SPM and sediments justifying the very low levels of bioavailable Hg found in water. The significant pH difference between both campaigns (around 0.5 pH units) does not change this distribution (Morel et al., 1998). Thus, during periods of low stream flow, sediment accumulates in the bed load of the river or the reservoir, and adsorbs Hg that becomes highly enriched in sediment. During high-flow events, this Hg enriched sediment (e.g. iron hydroxide sediment) (Rytuba, 2000) is transported downstream producing a high flux of Hg that can be a significant source of bioavailable Hg depending on site-specific conditions (availability of sulphate-reducting bacteria, electron donors, organic carbon, pH, and salinity).

Considering both sampling periods, the THg content in plants had only statistically significant correlations with THg in SPM ($r=0.828$; $p=0.008$). When exploring correlations at the different seasons, it was found, that in autumn, the THg content in plants correlates with THg in SPM (r=0.576) and THg in DGT (r=0.471), although these correlations were no significant (p>0.05). On the other hand, in spring, due to the loss of devices, the sample size disables to give reliable statistical results for DGT. Moreover, the rest of variables do not correlate at all between them.

These results suggest that the THg in plants were influenced by the content in SPM, and the bioavailable Hg measured with DGT is very similar in all cases.

4.4 Conclusions

In 2012, a retaining wall was built to contain large amounts of Hg-rich industrial waste in Ebro River. Due to the restricted access to the dumping area to obtain samples for Hg determination, the present study focused on the presence of Hg outside the wall to assess the efficiency of this barrier to prevent the release of Hg to sites downstream during removal operations. Results suggest a non-negligible Hg discharge, since relatively high levels of this metal are present in SPM (at the ppm level), which is adsorbed and transported downstream onto particulate phases.

As for the evolution of the Hg-SPM content, it increases downstream while the amount of Hg per volume of water is decreasing, because SPM can be transported away (especially during high-flow events), but tends to settle before reaching longer distances.

Meausrements of low values (at the ppt level) of Hg-DGT, indicate that trace amounts of Hg adsorbed onto SPM can be released as free metal ions yielding a certain accumulation in plants (reaching the ppb level). These trace amounts of dissolved-bioavailable Hg are enough for activation of PC_n, at different seasons, indicating that PCs can be used as a good indicators of the presence of bioavailable metal in aquatic media throughout the year.

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Chapter 5

Mercury assessment in a river impacted by an organo-Hg fungicide

This chapter is based on the article:

Turull, M., Komarova, T., Noller, B., Fontàs, C., Díez, S., (2018). Evaluation of mercury in a freshwater environment impacted by an organomercury fungicide using diffusive gradient in thin film. Sci Total Environ. 621, 1475-1484. doi: 10.1016/j.scitotenv.2017.10.081

The use of pesticides to manage pest problems for crop protection is common practice around the world, and their accumulation in soils and contamination of water bodies is a global environmental problem. In Australia, an organomercury (Hg)-based fungicide is the most popular for control of pineapple disease of sugarcane. However, the presence of Hg is of great concern because of potential adverse effects in the environment. The purpose of this work was to evaluate the residual levels of Hg in soils of sugarcane cultivation from a catchment in North Queensland (Australia). Mercury was surveyed in soils close to the Tully River at 3 different depths (100, 200 and 300 mm). Additionally, total Hg (THg) and the labile fraction of Hg in water (measured by the diffusive gradient in thin film technique) were determined in the Tully River. A pristine site, the Tully Gorge National Park upstream of sugarcane fields, was selected for background Hg concentration estimation. In soils, Hg levels ranged from 18 to 264 μg kg^{-1}, with one of the soil samples being almost 10 times higher than at other sites at the surface level (199 μg kg^{-1}). Total and labile concentrations of Hg in water increased from the Hg-elevated soil sampling sites (0.085 μg L^{-1} and 0.061 μg L^{-1}) to downstream sites (0.082 μg L^{-1} and 0.066 μg L^{-1}), which is likely due to agricultural runoff. Indeed, except for the upstream control site, the THg concentration in water is over the limit permitted by the Australian freshwater quality guideline for protection of 99% species (0.06 μg L^{-1}). These findings point to the need to perform further research to reveal the mechanisms for release of Hg from soil and whether this might be causing important adverse effects to the Great Barrier Reef located in front of this river catchment.

5.1 Background and aims

The presence of non-essential metals in agricultural soils is of increasing concern because they have the potential to be accumulated in soil and subsequently be dispersed to the surrounding aquatic environments. Furthermore, the natural concentrations in agricultural soils tend to increase due to application of metal-containing pesticides to crops. This is the case of mercury (Hg), that although it is detrimental to the environment, it has been used for many years in organomercury fungicides to protect sugarcane root seeds and other plants from fungal disease (Bhuiyan et al., 2014; Kealley, 2017; Rayment et al., 1998; Samson et al., 2005; Wickramasinghe et al., 2015).

Australia is considered the fifth export country of sugar worldwide, with an annual production of 32 million Mg of sugar (*Saccharum officinarum* L.) (ABS, 2012). Around 370 000 ha land are used for sugarcane agriculture and practically all of this area is found in the State of Queensland [a minor amount is cultivated in New South Wales (NSW) and Western Australia (WA)] (NFF, 2012). One of the most common diseases in sugarcane cultivation is called pineapple disease (sett rot) caused by the fungus *Ceratocystis paradoxa,* producing the stem rot of the plant and blocking its germination (Wickramasinghe et al., 2015). Three triazole fungicides (flutriafol, propiconazole and triadimefon), one imidazole fungicide (prochloraz), and one organomercury fungicide (methoxy ethyl mercuric chloride, MEMC) are currently registered to control pineapple sett rot in Australia (Bhuiyan et al., 2014). Therefore, besides Sinker® (500 g L^{-1} flutriafol) and Tyrant® (500 g L^{-1} propiconazole), Shirtan® 120 (Crop Care Australasia Pty Ltd, Murarrie, Queensland) is the most popular fungicide because it may stimulate germination of setts in addition to controlling pineapple sett rot. Its active ingredient is MEMC, an organo-mercury component with 68% of Hg, being the only fungicide based on Hg registered in Australia (Samson et al., 2005; Rayment et al., 1998). Since the Australian Government has signed the Minamata Convention on mercury, Hg-added products such as Shirtan® 120 are due to be phased-out by 2020 (Kealley, 2017).

The Australian Pesticides and Veterinary Medicines Authority cancelled in 1995 the registration and associated label approvals for farming products with Hg but with one exception (Shirtan® 120 Liquid Fungicide) and only if Hg concentration in the soil does not exceed background levels (Australian Government, 2017). Various studies were undertaken in the 1990s at sites around Queensland and NSW to estimate the impact from the sugarcane agriculture to the soil. A difference of specific heavy metal (mainly cadmium (Cd) from fertilizer and Hg-containing pesticide) concentrations were found between “paired” sites of similar soils types with varying in cropping history (one with a known history of sugarcane and the other with an uncropped condition) was almost threefold at depth 0-25 cm (Rayment, 2007; Rayment et al., 1997). There are no studies following 2007 on Hg concentrations in sugarcane fields, though monitoring the pollution must be done to check if there is contamination over time. This research should be performed not only for the protection of human health (due to the incorporation in the food chain) but also to re-assess Hg dispersion from sugarcane soils and to protect biota in the aquatic and terrestrial ecosystems, as well as to prevent the impact on the living Great Barrier Reef, the biggest coral reef in the world (Jardine and Bunn, 2010). In this sense, research has found that MEMC (Shirtan® 120) is extremely toxic to corals at barely detectable concentrations, affecting coral fertilisation and metamorphosis, and causing coral bleaching and host tissue death (Markey et al., 2007).

Although regional Australian surveys continue to monitor pesticides residues in food, water and sediments, studies on Hg dispersion from agricultural soils and its degree of pollution are not developed. Therefore, an intensive study was devised and conducted in the present work to profile Hg levels in soil and water within the sugarcane agricultural system area of the Tully River Basin in North Queensland. The Tully River basin was selected as an example of a catchment with intensive sugarcane cultivation on its floodplains to study the impact of the Hg from the fungicide Shirtan® 120 applied in the sugarcane farms for >40 years (Crop Care Technotes, 2017). Moreover, concentrations in the labile fraction of Hg in water measured by the diffusive gradient in thin film (DGT) technique were also performed. Such research can be viewed as an example of assessing Hg pollution status in Australia sugarcane fields, and can serve as a basis for effectively targeting policies to monitor loss soils following long-term accumulation of Hg and for ensuring food crop quality and protecting human health. Consequently, environmental Hg monitoring and analytical procedures are of great interest (Fernandez-Gomez et al., 2011; Fernandez-Gomez et al., 2012; Turull et al., 2016).

The aim of this study is to evaluate the concentration of Hg that comes from Shirtan® 120 fungicide in different environmental samples from locations in the Tully River basin, below the Tully Gorge National Park in Queensland, where >200 km^2 of sugarcane agriculture is harvested from the floodplain (Hook et al., 2017).

5.2 Material and methods

5.2.1 Materials and reagents

All reagents were of analytical grade. For the dilutions, high-purity demineralized water produced by the Millipore Milli-Q system (Labmate resin cartridge DI20NG, AQUACURE) was used.

The ready-to-use DGT-Hg units were purchased from DGT Lancaster Ltd., (Skelmorlie, Lancaster, United Kingdom). Each DGT device is composed of a piston type plastic moulding with a window 2 cm in diameter, a 0.4 mm Spheron-Thiol resin immobilized in a layer of polyacrylamide gel as the binding agent, a 0.76 mm thick agarose gel as a diffusive layer on the top of the resin layer. A 0.45 μm pore size filter membrane with a thickness of 0.13 mm is covering the last gel.

The solvent consisting of 0.5% 2-mercaptoethanol in 5% (v/v) methanol in water was used to extract the Hg amount from the DGT device. The eluent was added (1 mL) in every micro-tube with the resin and left in a roller mixer over 24 h to attain the proper extraction.

5.2.2 Study area and sampling sites

The Tully River basin (Figure 5.1) is in the wet tropical zone of North Queensland and covers an area of 1,683.5 km^2 (Brodie et al., 2007). The Tully River rises in the Great Dividing Range on the northern boundary of the Kirrama State Forest and flows north through Lake Koombooloomba and over the Tully Falls near and descends through the Tully Gorge National Park, part of the UNESCO World Heritage–listed Wet Tropics site (UNESCO, 2017). The river is joined by five minor tributaries before emptying into the Coral Sea at Tully Heads over its 133 km course. The Great Barrier Reef is in the Coral Sea 40 km east of Tully Heads. Most of this catchment remains natural (70% of total) while 30% has been modified. Sugar cane and horticulture account for 19% and the remaining 11% is grazing (7%), forestry (2%) and urban (2%) (Brodie et al., 2007).

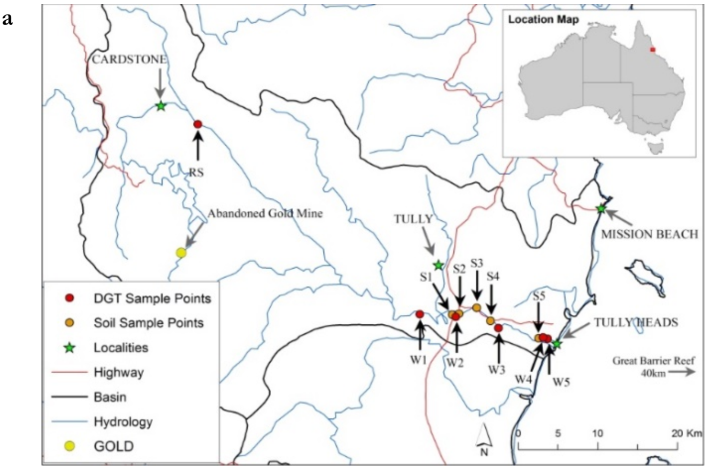
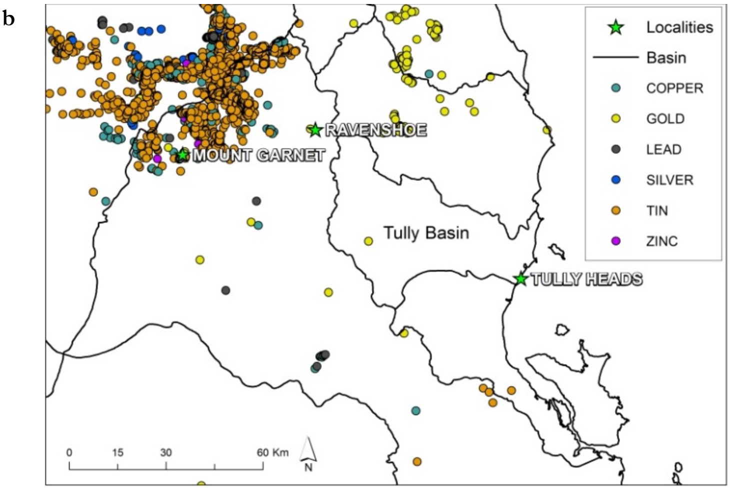


Figure 5.1. Maps of the Tully River Basin in Queensland, Australia. a) Location of sampling sites including DGT, water and soil sample sites

b



(continue) Figure 5.1. Maps of the Tully River Basin in Queensland, Australia. b) Abandoned mines in the East of Queensland including within Tully River basin (Queensland Government, 2016).

A sampling point at the undisturbed Tully George National Park, 25 km upstream from the sugarcane farms, was used as a control site (RS) (Figure 5.1a). This was the furthest upstream point available for sampling because access to the National Park was restricted. At RS soil sampling was not allowed but water could be sampled. For water, soil and DGTs, 5 sampling points downstream were chosen to cover the upper, middle and lower parts of the Tully floodplain areas where sugar cane is cultivated (Table S5.1). The water sampling sites were also selected to be above and below the soil sites. Then, 30 DGTs (5 devices at each site) were deployed for 3 days; water samples were taken at the same sampling point where DGT were deployed (one per site before and after the deployment of DGT in the field, n = 12); soil samples (n = 15) were from 5 different sites as shown in Figure 5.1a. It should be noted that sampling stations W4 and W5 are in the same cross section of the river, although the point W4 is sited in the middle of the river and the point W5 is located close to the river bank.

To help in the discussion, a map of the East of Queensland showing an abandoned gold mining area is also included (Figure 5.1b).

5.2.3 Dissolved Hg in water

For the determination of the labile fraction of Hg in water, the DGT technique was used as a passive sampling technique. This technique was developed by Davison and Zhang (1994) for the *in situ* determination of kinetically labile metal species in aquatic systems and has been successfully used as a mean to monitor the concentration of trace metals in natural waters (Clarisse and Hintelmann, 2006), metal fluxes in sediments (Harper et al., 1998), soils (Zhang et al., 1998) and to estimate the concentration of metals in pore waters (Zhang et al., 2002). The principle of this technique is based on the diffusion of the dissolved species through a membrane-diffusive layer (conventionally polyacrylamide or agarose gel) and their accumulation in an ion-exchange resin (enclosed in a polyacrylamide gel). These two layers are separated by a filter membrane with 0.45 μm of porosity and are enclosed and sealed in a small plastic device (Fernandez-Gomez et al., 2011; Destro et al., 2014). For heavy metals, the resin commonly used is Chelex-100. However, for Hg different resins are used including thiol groups specific for Hg species. In this respect, different compounds such as Spheron-thiol (Fernandez-Gomez et al., 2012) or 3-mercaptopropyl functionalized silica gel (Clarisse and Hintelmann, 2006; Fernandez-Gomez et al., 2014; Turull et al., 2016) have proved suitable in the Hg-DGT when incorporated into a polyacrylamide gel. DGT used in this work contained the resin with 0.4 mm of Spheron-Thiol gel and 0.76 mm of agarose gel. Based on the Fick's first law of diffusion the time-averaged concentration of the metal in the solution, C, can be calculated using equation 5.1:

$$C=\frac{M\Delta g}{DAt} \tag{5.1}$$

which depends on the mass of the analyte accumulated by the resin, the diffusion coefficient (D) in the diffusive gel, the deployment time in solution (t), the thickness (Δg) and the exposure surface area (A). By applying equation 5.1, the concentration of Hg in water can be calculated (Davison and Zhang, 1994; Zhang and Davison, 1995). The D used in this case was the DGT value for Hg depending on the temperature at each site (DGT Research, 2017).

5.2.4 General sampling procedures

DGT devices for sampling campaigns were stored in a refrigerator at 4 ºC until deployment at the field. The DGT devices that were deployed at each sampling site were suspended in a fabricated cylindrical basket made of a plastic net (Figure 5.2c). The basket was anchored in two points to a rope with a weight on one end and a buoy on the other (Figure 5.2b); the point without the weight was approximately 1-2 m from the surface. After retrieval, the DGT devices were rinsed with Milli-Q water and kept in the same polyethylene bags for transport to the laboratory and storage at 4 ºC.

The deployment time was 3 days and the average temperature was calculated using temperature loggers, one per site, model HOBO Pendant Temperature Data Logger, Onset, Bourne, MA 02532, USA.

Water samples were collected at the six sampling points as for the DGT devices. At each site, two water samples were taken before and after deployment of the DGTs in the field (Figure 5.2d). One of these water samples was acidified with nitric acid to preserve the metals until analyzed. The non-acidified water sample was used to measure pH, electrical conductivity and total alkalinity (Table S5.2) using an Analytical Radiometer TIM 870 combined with a sample changer SAC 80 (Radiometer, Copenhagen, Denmark).

Five sampling sites were selected for the soil sampling. At each point, 3 samples were taken at three different depths (100, 200 and 300 mm) using a stainless shovel, and were kept in a ziplock polyethylene bag (Figure 5.2a). Each soil sample was analyzed without pretreatment as described below.

Figure 5.2: a) Soil samples collected b) DGT deployment in Tully river c) DGT devices in the plastic basket d) Water samples collected.

5.2.5 Hg measurement and lead isotope analysis

Water samples from the different sampling sites were analyzed to determine total Hg (THg) concentration in two different days: when DGTs were deployed and retrieved (after 3 days). THg in the resin extract and in the water samples was measured by inductively coupled plasma-mass spectrometer (ICP-MS) using a model Agilent 8800-QQQ manufactured by Agilent Technologies (Santa Clara, CA, USA) at the Queensland Department of Health Inorganic Chemistry laboratory at Coopers Plains. The quality control of Hg analysis with this ICP-MS instrument was based on using a 5% Hg (v/v) HNO_3 solution (SRM ID 3133, SRM Lot# 060204; High Purity Standards, Charleston, SC 29423, USA) whose certified value was 10 ± 0.2 μg mL^{-1} and verified against NIST SRM 3100. Following extraction of Hg from the resin gel with the organic solvent or nitric acid, the extract was 10 times diluted. The detection limit (0.01 μg L^{-1} of Hg) was calculated based on three blanks measurement. Before the analyses, the acidified water samples were stored at 4ºC.

THg in soil was measured using an Advanced Mercury Analyzer, model AMA-254 manufactured by Altec (Prague, Czech Republic) and distributed by Leco Corp. (St. Joseph, MI, USA); this instrument uses catalytic combustion of the sample, pre-concentration by gold amalgamation, thermal desorption and AAS. Each soil sample was put in a nickel boat, placed in the instrument and automatically introduced into the AMA-254 instrument. The procedure was tested on different days with a certified material from the National Research Council of Canada (NRCC, DORM-3, Hg: 0.38 ± 0.06 mg kg^{-1}). The Hg concentration in CRM was 0.355 ± 0.02 mg kg^{-1} (n=6), in good agreement with certified values. To ensure the calibration of the instrument during the study, the reference material was analyzed in triplicate at the beginning and end of each set of 10 samples. The detection and quantification limits (0.2 and 0.7 ng g^{-1} wet weight of Hg, respectively) were calculated based on 3 blank measurements.

Lead isotope analysis was performed on Agilent 800 triple Quadrupole ICP-QQQ in ammonia reaction gas, MS/MS mode with Q1 operating as a mass filter at unit mass resolution (Farrell et al., 2016).

5.2.6 Statistical analysis

Analysis of THg in the water, DGT resin, and soil was reported as the mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to evaluate the results with IBM SPSS statistics 23 (SPSS Inc., Chicago, IL, EE.UU.). Statistical significance was declared when p value was equal to or < 0.05.

5.3 Results and discussion

5.3.1 Labile and total Hg concentration in water

The results for water samples are summarized in Table 5.1 and Figure 5.3. As it can be seen in Table 5.1, when DGTs were deployed at the river (day 1), THg in all water samples were below the detection limit (<0.01 μg L^{-1}). However, when DGTs were retrieved (day 3), THg concentration in water increased by >10 times in comparison of the first day. Such increase could be explained by the impact caused by a flood event, receiving runoff from soil between the first and last day of the deployment. On the second day of DGT deployment, the Tully River level increased dramatically up to 2.5 m after a flood event. This fact emphasizes the importance of not using spot sampling for water collection but rather time-average sampling to better detects these changes in pollutants presence.

Table 5.1: Total Hg concentration in water before and after DGTs deployment, and Australian water quality guidelines.

Sampling site	THg before DGT deployment (day 1) (μg L^{-1})	THg after DGT deployment (day 3) (μg L^{-1})
RS (Control site)	<0.01	0.106
W1	<0.01	0.141
W2	<0.01	0.155
W3	<0.01	0.170
W4	<0.01	0.163
W5	<0.01	0.152
Australian water quality guidelines for Hg (inorganic)* Trigger value (*Level of protection 99% species)*	0.06 (freshwater) 0.1 (marine)	

*ANZECC/ARMCANZ, 2000.

Labile Hg was checked in all sampling sites by determining Hg concentrations in the DGT resin. The results are shown in Figure 5.3 (Table S5.3) along with the mean value of THg in water between the first and the third day. As it can be observed, labile Hg was present at all the sampling sites evaluated. Hg concentrations found in sampling stations W1 to W5 were not statistically different using ANOVA (p>0.05). However, significant differences were found between sampling stations RS and W4 (Figure 5.3). Moreover, the total and labile Hg concentration from sampling point W3 increased to the downstream site. Also, the percentage of labile Hg in water increased downstream, after W3. The presence of labile Hg in inorganic forms is to be expected from sugarcane soil runoff rather than MEMC because MEMC is decomposed to inorganic Hg which in turn is bound to soil particles (Crop Care Technotes, 2017). However the DGT results show that retained Hg does leach slowly from soil into surface waters.

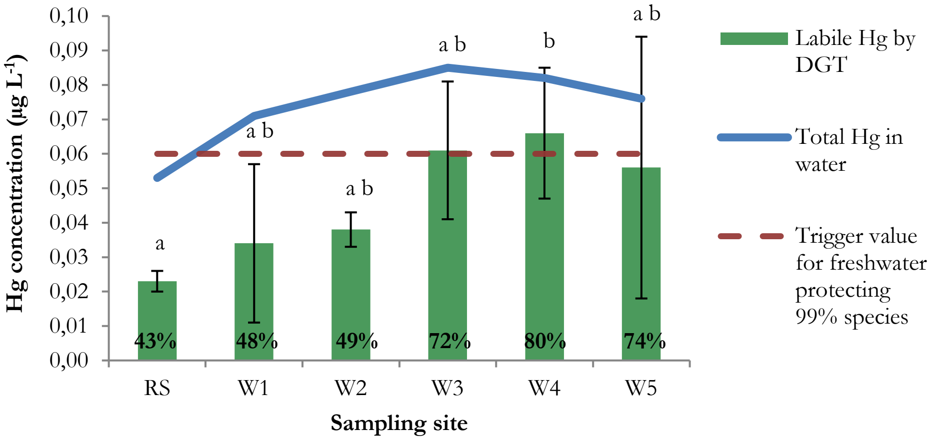


Figure 5.3: Labile Hg concentration obtained using DGT and Hg direct measurement in water by ICP-MS (average of the total Hg concentration with the first and the last deployment time). Note: Error bars represent mean ± SD (n=5). Values within the bars represent the percentage of labile Hg over THg. Different letters within the graph indicate significant differences.

Therefore, a different pattern in the Hg concentration between W4 and W5 is observed decreasing from W4 to W5. At site W4, the concentration of Hg measured using a DGT device was higher (but not significantly) than at site W5, at the riverbank. This difference is probably due to the placement of the DGT in the river, because devices deployed at site W4 were located in the middle of the river, and on the contrary, the DGT samplers at W5 were placed very close to the riverbank. A possible explanation lies in the fact that during high flow events, Hg enriched sediment and/or soil runoff from upstream sites is transported downstream and provides a high flux of Hg that can be a significant source of bioavailable Hg (Turull et al., 2017). The concentration found in the water is derived from the same dynamic process of soil runoff to the river even though the difference between sites is not remarkable. In this sense, it should be highlighted that upstream of Site RS there is an abandoned gold mining area (Figure 5.1b) where historical gold miners used Hg to amalgamate gold. Therefore, downstream export of mining sediment and associated Hg might be expected due to the legacy of historical mining activity. This result also highlights the importance of the placement of the DGT devices in the river.

Total concentration of Hg in water shows a similar pattern to labile Hg (Figure 5.3). This experiment is a good demonstration that DGT technique is a passive tool for studying labile Hg by integrating Hg accumulation. Comparison of the results in Figure 5.3 with the Australian water quality guideline (ANZECC/ARMCANZ, 2000) for THg in freshwater, shows that all the values at W1 to W5 sites exceed the limit (0.06 μg L^{-1}), excepting at upstream RS, with no impact of fungicide application. This guideline is the trigger level for 99% protection of species in the freshwater aquatic ecosystem (the Tully River in this study), while the trigger level for 99% protection of species for marine water aquatic ecosystem (0.1 μg L^{-1}) applies to the Great Barrier Reef which is 40 km east off the coastline at Tully Heads (Figure 5.1). In addition, Hg concentration in Tully River water is below the Australian drinking water guideline (1 μgL^{-1}; ADWG, 2011).

5.3.2 Total Hg concentration in soil samples

The analyses of soils from the Tully basin sugar cane cultivation areas demonstrate that there is residual THg in these soils (Figure 5.4). The mean ± SD for all the soils are 64 ± 76 μg kg^{-1}, 77 ± 104 μg kg^{-1} and 52 ± 42 μg kg^{-1} for the depths 100, 200 and 300 mm, respectively. Figure 5.4 shows the THg distribution at these three different depths for each sampling site. The THg concentrations in the sampling points S1, S2, S3, S4 and S5 are 32 ± 4 μg kg^{-1}, 194 ± 67 μg kg^{-1}, 44 ± 18 μg kg^{-1}, 34 ± 4 μg kg^{-1} and 24 ± 4 μg kg^{-1}, respectively. No statistically significant differences were found when excluding S2, with a mean value of 32 ± 12 μg kg^{-1}, so it could be posible that in S2 an unknown source of contamination exists. Due to the site having close proximity to canelands, the Hg is most likely derived from the fungicide Shirtan® 120 as the background concentration of Hg in soil is expected to be <10 μg kg^{-1} (<0.01 mg.kg^{-1}). Thus, this study demonstrates the relevance of periodic assessment of Hg dispersion from caneland soils.

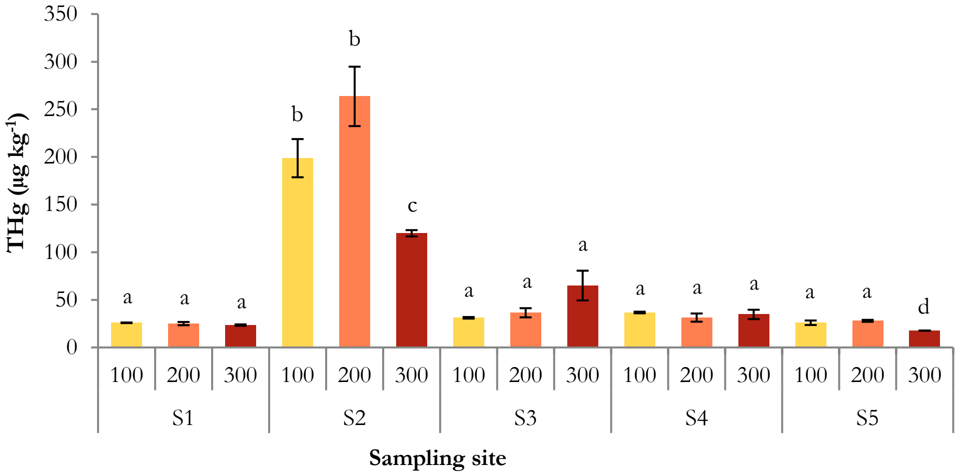


Figure 5.4: THg concentration in the soil at three different depths (100 mm, 200 mm and 300 mm). Note: Error bars represent mean ± SD (n = 3). Different letters within the graph indicate significant differences.

Table 5.2 shows a summary of studies describing THg concentration in the Queensland canelands published up to 2002 (Rayment et al., 2002) together with guideline values (NEPM, 2013), and our study.

The background value found in this study (discarding sampling point S2) is close to the range in the Earth's crust (Kabata-Pendias, 2011). The point with the highest Hg concentration exceeding the average values found in nearby areas of Queensland and New South Wales (Table 5.2), is very similar to that found during the last 20 years, compared with the range found in this study; therefore it seems that a large proportion of Hg remains in the soil.

Table 5.2: Total mercury concentration (μ kg^{-1}) in canefield soil and other comparative data.

Description	THg (μg kg^{-1})	Reference
Earth's crust	20 – 70	Kabata-Pendias, 2011
Typical soil range in Qld and NSW	10 – 500	Rayment and Barry, 1993
Qld canefield soil 0 – 250 mm	90 ± 60	Rayment et al., 1997
Qld horticultural soil		Barry, 1997
0-100 mm	42 ± 30	
100-200 mm	69 ± 33	
200-300 mm	68 ± 32	
NSW canefield soil		Rayment et al., 1998
0-100 mm	50 ± 70	
0-250 mm	80 ± 40	
250-500 mm	80 ± 20	
NSW non-canefield soil		Rayment et al., 1998
0-100 mm	80 ± 30	
0-250 mm	80± 40	
250-500 mm	60 ± 10	
Mackay, Qld		Rayment et al., 2002
0-250 mm	50	
Cairns, Qld		Rayment et al., 2002
0-250 mm	60	
Qld Tropical Coast Cane Soils:		Rayment, 2000
Tully-Mossman		
0-100 mm	120 ± 50	
0-250 mm	130 ± 50	
250-100 mm	90 ± 40	
Tully River basin		
0-100 mm	64 ± 76 (24 – 199)	This study
100-200 mm	77 ± 104 (25 – 264)	
200-300 mm	52 ± 42 (18 – 120)	
Australian Health Investigation Levels (HILs) for soil contaminants. Recreation C	Methylmercury 13000 Mercury (inorganic) 80000	NEPM, 2013

Almost all the soils show a similar pattern, except point S2, where highest THg concentrations were found in 200 mm, decreasing at lower depths (300 mm). In comparison with our study, in all cases, show the same decrease in Hg with depth.

Comparison of the THg concentrations in soil (Figure 5.4 and Table 5.2) with the Australian Health Investigation Level (Level C – recreational activity) for soil contamination with Hg (inorganic) (80 mg kg^{-1} (80,000 μg kg^{-1}); NEPM, 2013) shows that there is no identified risk to human health from these soils. Although there is no specific Hg guideline for an Ecological Investigation Level for terrestrial species in soil (NEPM, 2013), a study of Hg toxicity to the earthworm *Eisenia fetida* in three Australian soils has shown 50% lethality after 28 days in the range 152-367 mg kg^{-1} (Mahbub et al., 2017a) which is also 3 orders of magnitude above the Tully River basin soil THg's. Other studies by the same research group demonstrated that Hg inhibits soil enzyme activity (Mahbub et al., 2016) and alters the bacterial community structure and diversity (Mahbub et al., 2017b) in soil even at concentrations lower than the Australian guideline value.

5.3.3 Comparison of Hg in soil, water and DGT

To establish if there is any relationship between Hg found in water and in soils, Figure 5.5 presents a collection of all values obtained for each sampling sites for DGTs, soils and waters.

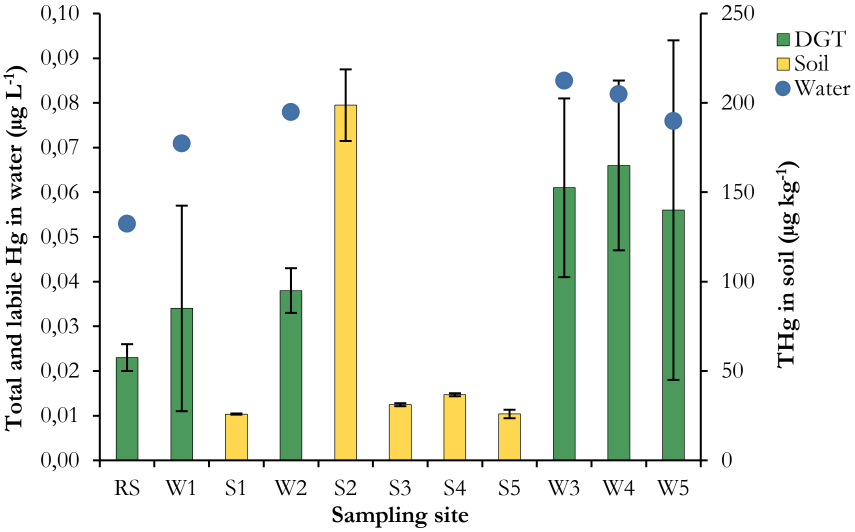


Figure 5.5: Comparison of total (in blue points) and labile Hg concentration in the water (µg L^{-1}) and soil (µg L^{-1}) in Tully River. Note: Bars represent the mean and standard deviation of three replicates. Different letters within the graph indicate significant differences.

For soils, the sampling point S2 has the highest THg concentration, which is almost 10 times higher than values at other downstream soil sites. On the other hand, for water, the THg concentration found at sampling point W3, which is downstream from soil sampling point S2, is higher in water than at sampling point

W2. Thus, Hg pollution comes from the sugarcane cultivation areas along the Tully River (e.g. S2) and consequently soil runoff could contaminate the river downstream. Finally, for the labile fraction of Hg measured by DGT, values show a similar trend at water sites, indicating that Hg amounts being removed by single rain and flood events are low.

Considering that for 40 years, >500 kg of MEMC were applied to soil in one of the Great Barrier Reef catchments and that concentrations up to 100 μg kg^{-1} in marine sediment could be found, it is mandatory to monitor the THg concentrations found in the sugar cane fields to take care of the Great Barrier Reef, one of the richest and most beautiful ecosystems in the world (GBRMPA, 2010). Comparison of Hg concentration with the Australian guideline for sediment (Interim Sediment Quality Guideline (ISQG)-Low) 0.15 mg kg^{-1} (150 μg kg^{-1}) shows that Hg accumulation in the marine ecosystem is approaching the guideline level (ANZECC/ARMCANZ, 2000). This situation contrasts with the minimal risk to terrestrial species from Hg in soil shown above, and indicates that Hg release from sugarcane field soil to Tully River waters may be a potential risk to freshwater biota and the Great Barrier Reef ecosystem.

Another highlight to discuss is the THg concentration in water found at the reference sampling site RS in the Tully Gorge National Park. At this site, fungicide Shirtan® 120 was never used and, therefore, no impact at all on the soil or any effect on water quality on Tully River due to surface runoff of this kind of Hg is expected. Nevertheless, as described above, the legacy of an abandoned gold mining area might impact site RS. After their closure, mines can still impact the environment by contaminating air, water, soil, and sediments from the scattered tailings. This is probably the case for this site, although THg level in water at site RS is 50% lower than average concentration at downstream sites W1 to W5 (Table 5.1; 0.106 μg L^{-1} vs. 0.156 μg L^{-1}).

5.3.4 Possible association of Hg with geological sources

North Queensland has extensive waste from historical gold mining which used Hg to extract the gold derived from widespread mineralization (Queensland Government, 2016; Figure 5.1b). The upper Tully River basin has occasional gold mineralization and one historical abandoned gold mine as indicated previously; some of the surrounding basins in North Queensland had extensive gold mining and use of Hg for gold recovery. In fact, Jardine et al. (2012) found Hg concentrations in water (0.1 μg L^{-1}) and soil (170 μg kg^{-1}) in different areas in the north of Queensland to assess the impact of the historical gold mines on food chains. The THg concentration in Tully River is comparatively lower than in these areas, although values are of the same order of magnitude. The environmental impacts of increasing Hg pollution caused by gold mining activities on human health and the environment can be found worldwide (Pinedo-Hernández et al, 2015; Marrugo-Negrete et al., 2015; Diringer et al., 2015; Tomiyasu et al., 2013; Balzino et al., 2015; Reichelt-Brushett et al., 2017).

Mercury in soil may be associated with other metals when derived from a geological source or mineral processing. Low concentrations of lead may be associated with Hg and be detectable via stable lead isotope ratios that are detectable by high resolution ICP-MS. Examination of stable lead isotope ratio data from a parallel study (Farrell et al., 2016) for the same soils from Sites S1 to S5 and water samples from Sites RS and W1 to W5 run in this study (Figure 5.1a) provide some insight on its sources if it is assumed that any Hg present would have a similar origin to the lead present. The lead isotope concentrations in both soil and water samples were measured using ICP-QQQ spectrometer described by Farrell et al. (2016) and in Section 5.2.5 together with four Geochemical Reference Samples from the Geological Society of Japan (JSD-1, JSD-2, JSD-3 and JLK-1) for calibration purposes (see Table 5.3). The results for the Geochemical Reference Samples from the Geological Society of Japan gave good agreement with the data from the Observatory for Science and the Universe, Grenoble, France (Jochum et al., 2005).

Table 5.3: Comparison of stable lead isotope ratios for Tully River basin soils (S) and waters (W) with known sources of lead, and reference samples from the Geological Society of Japan (JSD-1, JSD-2, JSD-3 and JLK-1).

Sample details	Pb 206/204	Pb 207/204	Pb 208/204	Hg Soil 100mm (µg.kg^{-1})	Reference
Tully S4	19.37	15.43	38.74	25.90	Farrell et al. 2016
Tully S2	19.24	15.38	38.43	199	Farrell et al. 2016
Tully S1	17.24	15.2	36.77	25.90	Farrell et al. 2016
Tully S3	18.66	15.33	37.73	31.10	Farrell et al. 2016
Tully S5	19.23	15.44	38.78	6.20	Farrell et al. 2016
Tully RS	19.02	15.62	38.59		This study
Tully W1	19.00	15.53	38.60		This study
Tully W2	18.72	15.22	38.05		This study
Tully W3	18.99	15.50	38.67		This study
Tully W4	19.10	15.56	38.91		This study
Tully W5	19.42	15.81	39.46		This study
Broken Hill galena (BH)	15.99	15.35	35.52		Townsend et al. (1998)
Brisbane petrol 1996 (BP1996)	16.81	15.51	36.57		Gulson (1996)
Mt Isa Zn concentrate (MI)	16.13	15.47	35.88		Noller et al. (2017)
Cannington Pb Ore (CAN)	17.00	17.00	36.29		Noller et al. (2017)
Kagara Pb (KGPb)	18.09	18.09	38.21		Noller et al. (2017)
Kagara Zn (KGZn)	18.05	18.05	38.13		Noller et al. (2017)
Century Pb (CENPb)	16.31	15.47	35.99		Gulson et al. (2016)
Century Zn (CENZn)	16.31	15.48	36.01		Gulson et al. (2016)
Ambient dust Karumba (AD)	18.73	15.63	38.63		Gulson et al. (2016)

(continue) Table 5.3: Comparison of stable lead isotope ratios for Tully River basin soils (S) and waters (W) with known sources of lead, and reference samples from the Geological Society of Japan (JSD-1, JSD-2, JSD-3 and JLK-1).

Sample details		Pb 206/204	Pb 207/204	Pb 208/204	Hg Soil 100 mm (µg.kg^{-1})	Reference
JSD-1[a]	Measured (Tully soil)	18.46	-	38.58		Farrell et al. (2016)
	Measured (Tully water)	18.49	15.53	38.33		This study
	Expected	18.46	15.61	38.58		Jochum et al. (2005)
JSD-2[a]	Measured (Tully soil)	18.08	-	37.72		Farrell et al. (2016)
	Measured (Tully water)	18.21	15.56	38.11		This study
	Expected	18.08	15.59	38.12		Jochum et al. (2005)
JSD-3[a]	Measured (Tully soil)	18.27	-	37.85		Farrell et al. (2016)
	Measured (Tully water)	18.45	15.55	38.57		This study
	Expected	18.37	15.65	38.88		Jochum et al. (2005)
JLK-1[a]	Measured (Tully soil)	18.48	-	38.10		Farrell et al. (2005)
	Measured (Tully water)	18.62	15.68	38.86		This study
	Expected	18.44	15.64	38.79		Jochum et al. (2005)

[a] Method used for water (This study) is the same as used for soil (Farrell et al., 2016)

Table 5.3 and Figure 5.6 give lead isotope ratios from Tully River soils, waters, known key sources of lead in Australia such as Broken Hill (BH) and Mount Isa (MI) that were used for manufacturing tetraethyl lead in petrol, local lead/zinc mine sources in Queensland and background air particulate material that would be derived from background soil. Tully basin soil and water samples differ from Broken Hill galena, Mount Isa type lead (in zinc ore) commonly used for lead in petrol and confirmed by lack of similarity with airborne lead measured in Brisbane in 1996 (BP1996) when leaded petrol was still in use (Gulson, 1996). Century zinc (CENZn) and other lead-containing ore bodies (CENPb) are similar to Mount Isa lead. Cannington lead (CAN) being different from Mount Isa shows some overlap with S1 for lower lead 206/204 and 208/204 ratios indicating that soil at S1 may be a mixture. As S1 is located adjacent the main north-south highway (Figure 5.1a), it is most likely a mixture of lead from petrol and local geological source of lead (Figure 5.6). In contrast, S2, S3, S4 and S5 and Tully River water samples are all similar to ambient dust (AD) collected 40 km from Karumba, North Queensland (Gulson et al., 2016) but different from the nearest mining of Kagara lead/zinc (KG) located at Mount Garnet (Figure 5.1b) 50 km northwest from Tully River basin. Thus, there appears to be some possible link of lead with local geology rather than from petrol. Although a soil sample in the Tully Gorge National Park would have been important to take to compare the THg in the soil and make better conclusions about the sample point in the National Park, it is demonstrated that water from the DGT Hg at Site RS has similar lead isotope ratios for all other Tully Basin samples except S1. The Hg at Site RS is not from organo-mercury applied to soil.

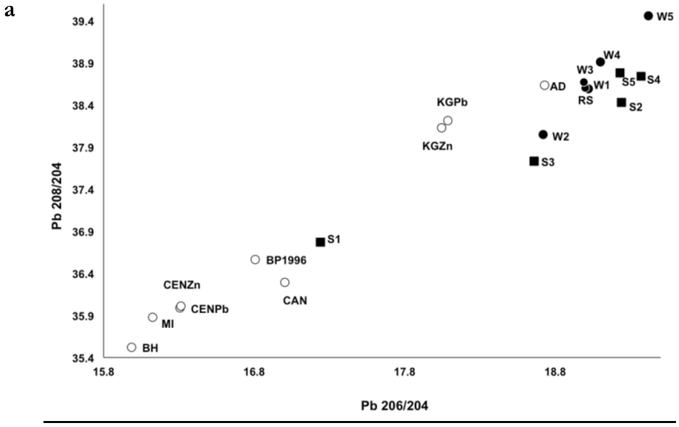
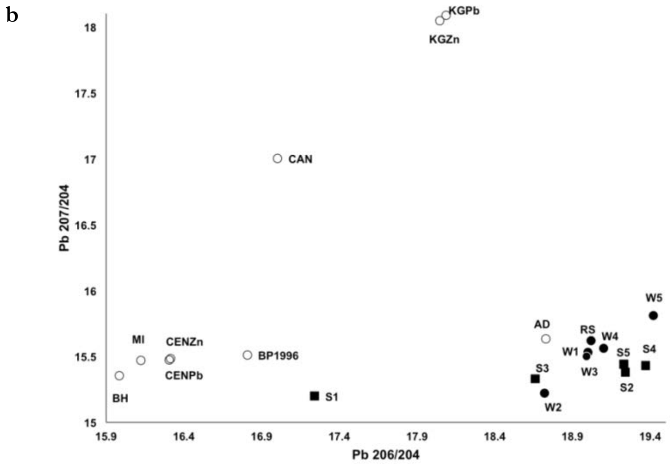


Figure 5.6: Plots of stable lead isotope ratios for Tully River basin soils with known sources of lead. a) Pb 206/204 vs 208/204.



(continue) Figure 5.6: Plots of stable lead isotope ratios for Tully River basin soils with known sources of lead. b) Pb 206/204 vs 207/204. Note: BH = Broken Hill, MI = Mount Isa, CEN = Century, BP1996 = Brisbane PM10 1996, CAN = Cannington, KG = Kagara, AD = Airborne Dust; RS and S1-S5 = Tully soils (black squares); RS and W1-W5 = Tully waters (black circles).

5.4 Conclusions

In this study, the dispersion of Hg concentration in sugarcane fields irrigated by the Tully River, where Shirtan® 120 an organomercury-based fungicide has been applied over 40 years, was assessed in water and soil. Moreover, the impact of this fungicide in sugarcane agriculture has been evaluated for the first time in 20 years. Soil samples show similar THg levels, except at one sampling site (S2) which is almost 10 times higher than the others (S1, S3, S4 and S5), where an increase of the total and labile Hg concentration is observed in water, probably due to the application of Shirtan® 120. Excluding this point, the concentration in water is higher downstream. Although this concentration is high in comparison to the Hg background in soil, the measured Hg soil concentrations do not exceed the Australian NEPM (2013) soil contamination guideline for Hg. On the other hand, the THg concentrations found in water downstream exceeded the Australian water quality guideline for 99% protection of species in freshwater except for Site RS in the Tully Gorge National Park. Nevertheless, at Site RS there is Hg concentration which could come from use of Hg in historical gold mining because there is no application of organomercury fungicide on sugarcane crops. Therefore further study is required to identify other Hg sources in the area and to evaluate their effects on the terrestrial and aquatic ecosystems.

5.5. Supplementary material

Table S5.1 GPS coordinates for DGT and soil samples.

DGT and water samples	GPS coordinates	Soil samples	GPS coordinates
RS	-17.7726, 145.65092	S1	-17.99042, 145.93797
W1	-17.99008, 145.90155	S2	-17.99204, 145.94247
W2	-17.99291, 145.94235	S3	-17.98252, 145.96583
W3	-18.00569, 145.99018	S4	-17.98252, 145.96583
W4	-18.01655, 146.04062	S5	-18.016, 146.03987
W5	-18.01602, 146.0408		

Table S5.2 Data of pH, electrical conductivity and total alkalinity for the first and the third day of deployment.

Point	Time (day)	Temperature (ºC)	pH	Conductivity (mS cm^{-1})	Alkalinity (mg L^{-1} as $CaCO_3$)
RS	First day	22.60	6.763	32.18	11.49
	Last day		6.405	32.74	10.64
W1	First day	22.45	6.877	42.6	13.13
	Last day		6.628	40.5	12.64
W2	First day	22.44	6.731	43.5	12.53
	Last day		6.596	40.1	11.73
W3	First day	22.21	6.738	43.1	12.85
	Last day		6.529	40.3	12.27
W4	First day	22.19	6.611	96.4	12.33
	Last day		6.542	81.7	11.57
W5	First day	21.02	6.684	238.6	12.8
	Last day		6.557	376.0	13.45

Table S5.3. Labile mercury from DGTs.

Point	Hg conc in DGT (ug L^{-1})	Temperature (ºC)	Diffusion coefficient (cm^2 sec^{-1})	Labile Hg in water (µg L^{-1})
RS	1.85	21.02	9.01E-06	0.023
W1	2.88	22.19	9.27E-06	0.034
W2	3.17	22.21	9.27E-06	0.038
W3	5.04	22.44	9.27E-06	0.061
W4	5.67	22.45	9.27E-06	0.066
W5	4.69	22.6	9.53E-06	0.056

5.6 References

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Chapter 6

Development of a restricted gel for the determination of inorganic mercury in soil

This chapter is based on the article:

Turull, M.; Fontàs, C. and Díez, S. 2019. Diffusive gradient in thin films with open and restricted gels for predicting mercury uptake by plants. Environ Chem Lett. doi: 10.1007/s10311-019-00864-2

The presence of mercury (Hg) in the environment is a major concern because mercury is toxic for living organisms. Estimating the Hg toxicity of environmental samples is difficult because contaminants occur in pools of various bioavailabilities, and thus, measuring the total concentration of a contaminant rarely reflects the real toxicity and risk. There is therefore a need to design advanced methods to measure the bioavailable fraction of the contaminants. Here, we used the diffusive gradient in thin films (DGT) method to determine the bioavailability of Hg in agricultural soils containing 0.02 and 0.64 mg kg^{-1} of Hg. Specifically, we used homemade manufactured open and restricted diffusive layers to distinguish between inorganic and organic bioavailable Hg species in soil. The findings demonstrate for the first time the successful use of restricted gel for the determination of inorganic Hg species in soil. Moreover, a model program has been used to calculate the effective concentration for predicting Hg concentration uptake by plants. For this purpose, lettuces were planted in the different soils tested to determine Hg soil-to-plant transfer. Concentrations of Hg obtained by DGT measurements in the soil matched the Hg concentration found in lettuce roots, confirming the effectiveness of this technique in predicting Hg uptake by plants.

6.1 Background and aims

Metal content in soils is increasing due to both natural processes and anthropogenic activities (Kabata-Pendias 2011). In the case of agricultural soils, the presence of metals is an issue of increasing concern because metal soluble forms can be transferred from soil to plants and may pose a health risk (Rodrigues et al. 2012). Different methods have been developed to assess the bioavailability of metals in soils. Chemical methods are widely used and are based on chemical extraction and further correlation of the labile metals determined by single or sequential extraction approaches (Kim and Hyun 2015). The Diffusive Gradient in Thin Film (DGT) has emerged as a good alternative for measuring metal bioavailability (Davison and Zhang 1994). Since then, a great number of studies have demonstrated that the DGT technique is a versatile *in situ* passive sampling tool for the measurement of metals and oxyanions, and also a potential tool for the determination of inorganic nutrients and organics (Li et al. 2018). The DGT technique is based on the diffusion of the dissolved species through a membrane diffusion layer and their accumulation in a resin enclosed in a hydrogel (Fernández-Gómez et al. 2014). It is well proven that this technique is highly selective for different trace elements depending on the selective resin used in the hydrogel. In the case of Hg, the specific resin includes thiol groups, such as Spheron-thiol (Fernández-Gómez et al. 2012; Turull et al. 2018) or 3-mercaptopropyl functionalized silica gel (Fernández-Gómez et al. 2011)· According to Fick's first law, the concentration of the metal in the solution (C) is:

$$C=\frac{M\Delta g}{DAt} \tag{6.1}$$

where D is the diffusion coefficient of the Hg in the diffusive layer, M is the mass of Hg accumulated by the resin, Δg is the thickness of the diffusive layer, A is the exposure surface area and t is the deployment time. Moreover, the diffusion coefficient (D) of Hg in the diffusive gel can be calculated from the slope (s) of the relationship between the amount of Hg accumulated by the DGT units (normalised for the concentration of Hg in solution) and the deployment time according to:

$$D=\frac{s\Delta g}{A} \tag{6.2}$$

The DGT technique can also be used to perform speciation studies of trace elements in which a hydrogel with a different pore size is used as the diffusive layer (Shiva et al. 2015). Generally, DGT studies use open diffusive layer, a type of polyacrylamide hydrogel with a large pore higher than 5 nm, which allows almost free diffusion of both inorganic and organic species. In comparison, very few studies employ restricted diffusive layers that uses polyacrylamide gel with a bis-acrylamide cross-linker with a pore size smaller than 1nm, which restricts the transport to inorganic species (Shiva et al. 2015). The number of studies using open and restricted diffusive layer for predicting trace metal bioavailability are scarce. Moreover, only a few studies exist on Hg soil-to-plant bioavailability (Cattani et al. 2008; Liu et al. 2012), while absolutely no studies have been conducted using restricted layer for this metal.

Our main objective was to evaluate the two types of diffusive gels with different pore size to determine the ratio of inorganic and organic Hg species in two agricultural soils. Moreover, the effectiveness of this technique was investigated by analyzing Hg uptake from lettuces growing in these soils.

6.2 Experimental design

6.2.1 Soil characteristics

An agricultural soil in the peri-urban area of Barcelona (Spain), and an ecological agricultural field used as a control were used in this study. Ten subsamples of topsoil (0–20 cm) were collected in a grid (10 m x 10 m) using a stainless-steel trowel and combined into a single sample (n=5). Soil pH and conductivity were determined with deionized water at a 1:2 solid:liquid ratio using a pH meter Crison

GLP22 and a Hach CDC401, respectively. The organic matter was analyzed as the percentage loss on ignition using a 2.0 g soil in an oven (Carbolite CWF 1300) at 550ºC for 1h (Dean 1974). Hg in all samples was determined using an AMA-254 instrument (Altec, Czech Republic) (Díez et al. 2007). The accuracy was checked with the certified material DORM-3 with values lower than 5%.

6.2.2 Pot experiments

Lettuces (*Lactuca sativa* L. cv. Batavia) were planted in 2.5 L cylindrical pots filled with 2 kg air-dried soil for every set. One lettuce was planted per pot (5 replicates per set). Plants were irrigated manually every day (50-75 mL per pot) with Tarssan nutritive solution. After 48 days of growth, leaves and roots were harvested separately and washed off with deionized water. The samples were dried in an oven at 60ºC. A mixture of soil from each set of pots was made and passed through a 0.5 mm sieve prior to analysis.

6.2.3. Diffusive Gradient in thin film (DGT)

6.2.3.1 Hydrogels and binding layers preparation

The open diffusive layers were prepared with acrylamide patented agarose-derived cross-linker (Fernández-Gómez et al. 2014). The restricted diffusive layers were prepared according to Shiva et al. (2015) with some minor modifications. Briefly, a gel solution was prepared by mixing 40% acrylamide/bis-acrylamide solution in a 1:1.66 ratio with Milli-Q water and was preserved in the fridge for at least one day. 6.67 µL of tetramethylethylenediamine and 23 µL of 10% (m/v) ammonium persulphate in Milli-Q water were added for every 3.33 mL of gel solution and then stirred vigorously. After combining these three solutions, the mixed solution was pipetted into two plates of glass separated by a 0.5 cm thick Teflon spacer and allowed to polymerize at room temperature for 45 min. After that, the polymerized gel was rinsed with Milli-Q water for at least 24 h changing the water 2-3 times.

For the binding layers, 0.33 g of 3-mercaptopropyl functionalized silica gel was used per glass, mixing with the same gel solution as for the hydrogel gels. Finally, the same casting procedure was used as for the other gels but using a 0.25 cm Teflon spacer. The characteristics of DGT gels are summarized in Table 6.1.

Table 6.1: Composition and characteristics of open and restricted diffusive layers.

Parameters	Units	ODL	RDL
Resin thickness	mm	0.4	0.25
Diffusive gel thickness	mm	0.8	0.5
Gel surface area	cm^2	3.14	3.14
Reagent		polyacrylamide cross-linked with agarose	Polyacrylamide cross-linked with bis-acrylamide
Reagent:water ratio		1:3.1	1:1.66
Setting temperature	ºC	42-45	22-25
Pore size	nm	>5	<1
Hg content: Blank (resin gel)	ng Hg	0.1	0.5

A plastic mold piston design with a 2 cm diameter window was used to assembly the gels as described elsewhere (Fernández-Gómez et al. 2014).

6.2.3.2 Determination of Hg in soils

Soil preparation consisted of adding a 40 g sample in a Petri dish and maintaining it at 100% maximum water holding capacity with Milli-Q water at 25ºC for 48 h (Figure 6.1a) to ensure the equilibrium between fractions and soil (Yao et al. 2016). Triplicates for every sample set were done. Three DGT devices per soil were pressed onto the surface of the soil slurries and kept for 24 h at 25ºC and covered by a Petri dish to avoid potential contamination and ensure that moisture was stable (Figure 6.1b). After retrieval, deionized water was used to carefully wash the filter membrane to remove soil particles from the surface. For pore water analysis, the soil slurry was centrifuged at 3000 rpm for 20 min followed by filtration (0.45 µm). Prior to analysis, each soil solution was acidified with 1M HNO_3 and stored at 4ºC.

To interpret DGT measurements, the Hg concentration from DGT (C_{DGT}) can be converted to an effective concentration (C_E) using:

$$C_E = \frac{C_{DGT}}{R_{diff}} \tag{6.3}$$

R_{diff} is the ratio of the concentration at the DGT interface to the concentration in the bulk soil solution, and it is obtained when metals reach the binding resin only through simple diffusion. It is calculated by the numerical model DIFS (DGT-induced fluxes in sediments) (Sochaczewski et al. 2007). Input parameters were used to calculate R_{diff}, soil porosity and particulate concentration (Zhang et al. 2004). C_E is the metal concentration effectively available from the soil solution and the solid phase, so, theoretically, it is the same concentration as that is found in the rhizosphere of plants. Statistical analysis was evaluated by IBM SPSS statistics 25 (SPSS Inc., Chicago, USA). Values are expressed as mean ± standard deviation. The one-way analysis of variance was used to evaluate Hg in different lines and parts of the plants. A difference of p lower than 0.05 was considered statistically significant.

Figure 6.1: a) DGT sampler deployed in the soil sample. b) DGT devices per triplicate for every soil sample.

6.3 Results and discussion

6.3.1 Soil properties

Hg concentration in each soil sample was found to be 0.016 mg kg^{-1} for the control soil and 0.641 mg kg^{-1} for the agricultural soil, below the European regulation threshold value, of 1-1.5 mg kg^{-1} (EU 1986).

Physicochemical characteristics such as texture, pH, conductivity and organic matter were sandy loam, 7.15, 1.13 mS and 2.72% for control soil, whereas for agricultural soil they were clay loam, 7.29, 1.73 mS and 6.52%, respectively. The pH and conductivity values between the studied soils were comparable, while organic matter and texture were the main parameters that could explain the different mobility of Hg. The texture of the agricultural soil had a higher sorption capacity in comparison with the control soil because of the higher clay content in soil and, depending on the organic acids, organic matter could reduce or increase Hg bioavailability by forming insoluble or soluble complexes (Hasegawa et al. 2016). In this sense, adsorbents such as clay minerals and modified clays are effective for the removal of metal ions from wastewater (Gu et al. 2018), and from soils (Kurczewska et al. 2015).

6.3.2 Studies on Hg bioavailability in soils

A time-series experiment was performed separately for both types of DGT with the aim of obtaining the diffusion coefficient of Hg in the diffusive layers. Two experiments were carried out using a glass tank (3 L) containing NaCl (0.1 M) solution and pH 7 and spiked with 4 ng mL^{-1} of Hg at 20ºC. Two DGTs were retrieved at different times to obtain the calibration curve. The value of mass (M) was used to calculate the diffusion coefficient with equation 6.1 for every time-series deployment.

Excellent linearity for both open and restricted gels was observed (Figure 6.2) over a 50 h time-series experiment, indicating the suitability of both binding layers to measure Hg. According to equation 6.2, the slope of the calibration curve was used to calculate the diffusion coefficient for both DGT types. This value was 1.79 x 10^{-6} cm^2 s^{-1} for restricted gel, whereas for open gel it was 3.08 x 10^{-6} cm^2 s^{-1}. The fact that both diffusion coefficient values were of the same order of magnitude, but the value for the restricted gel slightly lower, was also described by Shiva et al. (2015) for different metals from Hg.

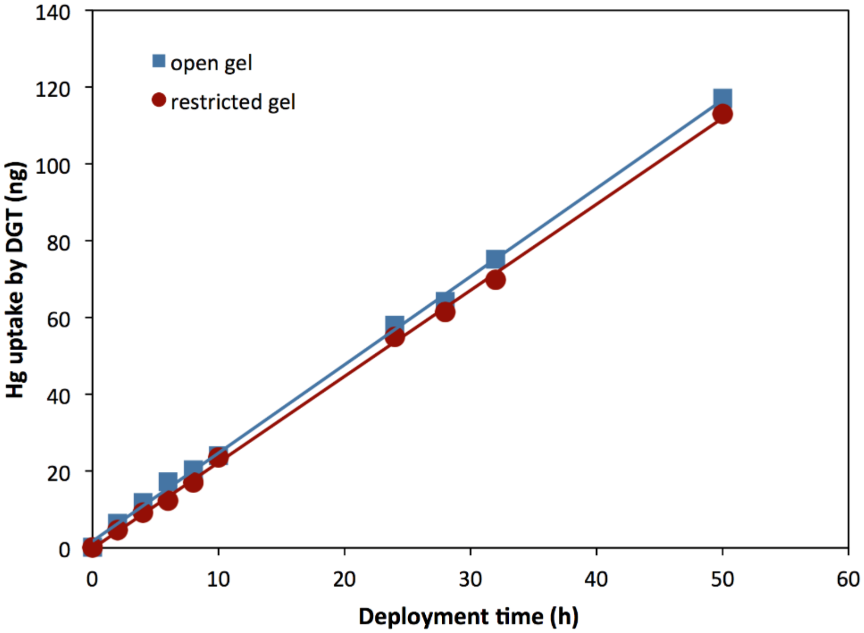

Figure 6.2: Time-series of Hg accumulation using DGT with open and restricted diffusive layer. There is a high linear relationship between the mass of Hg accumulated in the resin normalized by the Hg concentration in the solution in the period of time investigated. Linear equation for open gel: $y=2.30x+1.73$, $r^2=0.999$; and for restricted gel: $y=2.24x+0.18$, $r^2=0.999$. The diffusion coefficient (D) of Hg in the diffusive layer can be calculated from the slope of the relationship between the amount of Hg accumulated by the DGT and the deployment time according to Equation 2. The two types of DGT performed equally well, as evidenced by the regression coefficients of the two calibration curves (r^2 higher than 0.99). Note that the two curves have similar slopes, although the open gel showed the fastest rate of uptake (the greatest slope) and, therefore, a higher diffusion coefficient.

6.3.3 DGT and lettuce measurements

Applying diffusion coefficient values for restricted and open gels, Hg concentrations for both DGT were obtained (Table 6.2).

Table 6.2: Comparison of Hg concentration in soil using open (C_{DGT}-ODL) and restricted (C_{DGT}-RDL) gels, the ratio of the concentration at the DGT interface to the concentration in the bulk soil solution (R_{diff}), the calculated effective concentration (C_E) and low molecular species (LMS) for Control soil and Agricultural soil.

Line	C_{DGT}-ODL (ng mL^{-1})	C_{DGT}-RDL (ng mL^{-1})	R_{diff}	C_E (ng g^{-1})	LMS (%)*
Control soil	0.186 ± 0.040	0.130 ± 0.018	0.053	3.514	70
Agricultural soil	1.616 ± 0.170	1.567 ± 0.881	0.118	13.696	97

* LMS(%)= (C_{DGT}-RDL / C_{DGT}-ODL) x100 (indicative of inorganic Hg species in the soil)

As described above, R_{diff} is calculated by the DGT-induced fluxes in sediments model. Because the obtained R_{diff} is between 0 and 1, this value shows that only a small fraction of the Hg concentration could be determined by the DGT. C_E could be estimated by the ratio between the calculated concentration of C_{DGT}-ODL and R_{diff}. This C_E is the value to be compared with the Hg uptake by roots.

Hg concentrations found in both lettuce leaves and roots are summarized in Figure 6.3. Hg values in leaves were not significantly different between control soil and agricultural soil, despite the great difference in Hg concentration in the soils. This fact can be explained by the role of plants as either a source or a sink for atmospheric Hg through a vegetation-air mechanism, as described in Ericksen and Gustin (2004). However, in the case of roots, the uptake is only due to the presence of metal in the soil. Hg in lettuce roots grown in control was 2.88 ± 0.28 ng g^{-1}, but this value dramatically increased in the case of roots from agricultural soil, reaching a value of 14.08 ± 1.62 ng g^{-1}. It is worth mentioning that the difference between root concentration and calculated C_E was only 18% for control and 2% for agricultural soil. This good agreement demonstrates that the DGT technique can predict Hg content in plants due to its ability to mimic the process of uptake limited by diffusion, accounting for processes that traditional soil extractions are not able to determine (i.e. slow desorption) (Muhammad et al. 2012). Metal in the pore water or in soil cannot indicate the Hg available for plants because the resupply to the pore water from the solid, owing to depletion, is not taken into account (Wang et al. 2018).

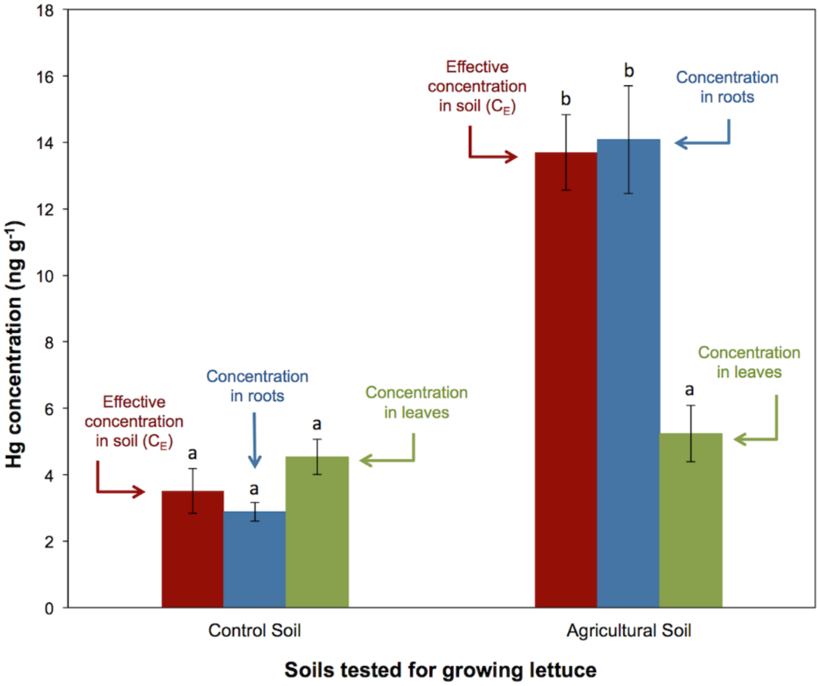

Figure 6.3: Effect of the different soils used to grow lettuces on Hg content found in lettuce roots, and leaves and effective concentration (C_E) in soil. Measurements of Hg in roots and leaves were performed by direct analysis of tissues, whereas C_E was measured by DGT in soil according to equation 6.3. Error bars are the standard deviations of quintuplicates. Different letters within the graph indicate significant differences. Note that Hg concentration measured in the soil by DGT has no significant differences with Hg in roots in both types of soil, and only with leaves in the control. This indicates that Hg concentration in soil by DGT is related to Hg in roots.

The percentage of low molecular species (LMS) of Hg, which is the fraction preferentially available by plants, is also shown in Table 6.2. A higher percentage (~10%) was found in agricultural soil versus control soil, although in both cases the predominant fraction is mainly inorganic. Higher mobility of agricultural soil can be confirmed by the coefficient R_{diff} since higher values were obtained (0.118 vs. 0.053) (Kovariková et al. 2007). Our values matched with the fraction of different metals studied in two different soils (Docekalová et al. 2007). It is known that the Hg concentration in most crops is mainly in its inorganic form (WHO 1991) which is more mobile than the organic species because of the adsorption into soil particles, reducing the direct flow into the soil water (Yu et al. 2018).

Therefore, the use of both DGT devices allows us to know which part can be rapidly uptaken by plants in the early growth stages.

6.4 Conclusion

This is the first study that successfully determines inorganic Hg species in soils using restricted diffusive layer DGT devices with a small pore size. The study results have indicated that DGT is very useful for predicting Hg uptake by lettuce roots. Moreover, combining DGT with open and restricted gels has proven to be a successful, fast and simple test for assessing bioavailable and low molecular Hg species, respectively. Hence, a great potential for determining the uptake of other toxic trace metals to vegetables can be expected. Future studies on Hg mobility and bioavailability and possibly on trace metals from different soil amendments (i.e. manure, peat, compost, or biochar), and their further incorporation in vegetable crops, are anticipated.

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Chapter 7

Prediction of mercury uptake by lettuce roots in amended agricultural soils

This chapter is based on the article:

Turull, M.; Fontàs, C.; Díez, S. 2019. Conventional and novel techniques for the determination of Hg uptake by lettuce in amended agricultural peri-urban soils. Science Total Environ. 668, 40-46. doi: 10.1016/j.scitotenv.2019.02.244

Peri-urban agriculture provides environmental benefits to the nearby urban areas. However, domestic and industrial infrastructures can be sources of pollution that can affect agricultural production. In this work, the diffusive gradient in thin film (DGT) technique was used to assess the bioavailability of mercury (Hg) in oeganic-amended agricultural soils, and uptake by lettuce. Two different amendments were studied individually in three different sets using a wood-based biochar at two rates (3% and 6% w/w), and compost at one rate (30% w/w). The effect of the amendments on Hg bioavailability, mobility and uptake was investigated by means of both DGT analyses and accumulation of Hg by lettuce. DGT manufactured in-house devices with polyacrylamide gel using both open and restricted diffusive layers (ODL and RDL, respectively) were used to determine organic and inorganic Hg labile species in soils, respectively. The Hg concentration in lettuce leaves and roots were analyzed and compared with DGT measurements to predict the uptake of Hg from the different organic-amended soils and the non-amended soils. Results show that the application of biochar reduces the bioavailability of Hg in soil and, in consequence, the Hg uptake by lettuce. Inorganic Hg species were predominant in all the different sets of the experiment (62-97%), although the addition of the different amendments reduced the free ionic species in soil.

7.1 Background and aims

Mercury (Hg) pollution is an increasing environmental problem worldwide because of its toxicity, persistence in the environment, and bioaccumulation in biota. The world-soil average of Hg is 0.07 mg kg^{-1} (Kabata-Pendias, 2011), but this concentration can be increased by anthropogenic sources, such as gold mining (Marrugo-Negrete et al., 2017), industry (Hang et al., 2017), and atmospheric deposition (de Lima et al., 2017). In addition, Hg can be introduced into agricultural sites from different amendments such as pesticides, fertilizers or poor quality urban compost (Cozzolino et al., 2016). The maximum level permissible in soils according to the EU Directive 86/278/EEC is 1-1.5 mg kg^{-1} (European Comission, 1986).

Different factors that influence the bioavailability of Hg in soil include soil pH,, interaction with other metals, cation exchange capacity (CEC), organic matter (OM) content, and sulphur compounds (Hang et al., 2017). It is well known that Hg strongly interacts with OM (humic and fulvic acids) forming stable complexes (Janowska et al., 2017). One method for remediation on soil is through amendments that are directed towards retaining the metal in the soil, and reducing availability to plants. Many studies demonstrated the efficacy of both biochar (Gilmour et al., 2018; Li et al., 2017a; O'Connor et al., 2018; Wang et al., 2018a) and compost (Janowska et al., 2017; Restrepo-Sánchez et al., 2015; Smolinska, 2015) to change the chemistry and binding properties of Hg in soil, and, hence, decreasing the amount of Hg available to different crops. It has also been demonstrated that the addition of OM for phytoremediation may increase the Hg bioavailable to plants (Smolinska, 2015).

Understanding Hg bioavailability in agricultural soils is the key for determining plant uptake and implications for food safety. Mobility, bioavailability and toxicity of Hg depend on its chemical form. Metal bioavailability in soil has been assessed by chemical extractions (Li et al., 2010), but it is well known that these methods are insufficient for determining Hg impacts on plants. To discriminate the different compounds or how in bound, sequential extractions have been done in many studies (Issaro et al., 2009). However, these techniques did not account for the depletion at the root-soil and pore-solid interfaces (Muhammad et al., 2009). Given this, a better approach to study the bioavailability of metals and to mimic the processes in the rhizosphere can be accomplished by the use of the Diffusive Gradient in Thin Film (DGT) (Davison and Zhang, 1994). This technique has been used mainly in natural waters Fernández-Gómez et al., 2014), in sediments (Wang et al., 2016) and soils (Hlodak et al., 2015) for the *in situ* determination of kinetically labile metal species (Fernández-Gómez et al., 2014). Moreover, certain studies have been demonstrated their effectiveness to predict the metal uptake by plants (Wang et al., 2018b; Zhang et al., 2004). In a recent research (Turull et al., 2019), we successfully used open (ODL) and restricted diffusive layers (RDL) to distinguish between inorganic and organic bioavailable Hg species in the soil.

In this study, an agricultural soil was selected to evaluate the effectiveness of different amendments (biochar and compost) on the mobility of different Hg compounds. To this aim, homemade manufactured DGT samplers using both open and restricted diffusive layers were used to determine different bioavailable Hg compounds. A sequential extraction prodecure was also performed to compare the results with DGT devices. Lastly, roots and leaves of lettuce were also analysed to correlate Hg concentration measured by DGT.

7.2 Materials and methods

7.2.1 Soil, biochar and compost preparation

The soil sample used was taken from an agricultural site located in the peri-urban area of Barcelona (Spain). The sample ws obtained from a mixture of 5 x 10 sub-samples taken from an area of 100 m^2 with a depth soil horizon of 0-25 cm. Air dried soil was sieved (<2 mm) to homogenize the sample and to remove large stones, roots or wastes. Afterwards, the soil sample was mixed with a wood-based biochar at two rates and compost at one rate.

The compost used in this study was manufactured and packaged by NUBA floris (Borstel, Germany), and mainly consisted of from yellow peat derived from *Sphagnum* moss and was sieved to <2 mm. Biochar was produced by Bodegas Torres (Vilafranca del Penedès, Barcelona, Spain) from vineyards by pyrolysis at 400-600ºC. The biochar was crushed and sieved to particle sizes between 0.12 and 2 mm (Hurtado Cervera, 2017). The conductivity (EC), pH and Hg concentration were 2.16 ± 0.05 mS cm^{-1}, 9.82 ± 0.04 and 29 ± 11 ng g^{-1} for biochar and 7.36 ± 0.24 mS cm^{-1}, 6.65 ± 0.35 and 39 ± 7 ng g^{-1} for the compost, respectively.

After preparing the soil and the amendments, 4 sets of different soil samples were chosen for the experiment: (1) the selected agricultural soil (AS), (2) AS with 3% w/w of biochar (BC3), (3) AS with 6% w/w of biochar (BC6), and (4) AS with 30% w/w of compost (CP30). These application rates were established on the basis of previous research with compost (Fuchs, 2002) and biochar (Hurtado et al., 2017). The time of incorporation of the amendments in soil was 72h before plant the seedlings following the protocol from Trupiano et al. (2017).

All the properties of the soil with the different amendments used for the study, as well as Hg concentration, are shown in the Table 7.1.

Table 7.1: Chemical characteristics of the different studied sets.

Parameter	Unit	AS	BC3	BC6	CP30
OM	%	6.52^{a}	9.19^{b}	11.34^{c}	12.90^{d}
Nitrate	mg kg^{-1}	9.16^{a}	9.66^{ac}	9.07^{a}	11.40^{bc}
pH	-	7.29^{a}	7.38^{b}	7.48^{c}	7.19^{d}
EC	mS cm^{-1}	1.73^{a}	1.74^{a}	1.57^{b}	5.23^{c}
Hg	mg kg^{-1}	0.640^{a}	0.628	0.619	0.578^{b}

*Different letters in the columns indicate a significant difference $p<0.05$ between treatments

7.2.2 Plant growth, sampling and analyses

Figure 7.1 shows the experimental design of this study. Briefly, lettuces (*Lactuca sativa* L. cv. Batavia) were planted in 2.5 L cylindrical pots (17 x 15.5 cm) filled with 2 kg of air-dried soil. One lettuce seedling was planted per pot, bringing a total of 5 replicates per set. During plant growth, the temperature and the amount of light were controlled (Smolinska, 2015), and lettuce plants were irrigated manually every day with Tarssan nutritive solution (50-75 mL per pot, depending on the humidity).

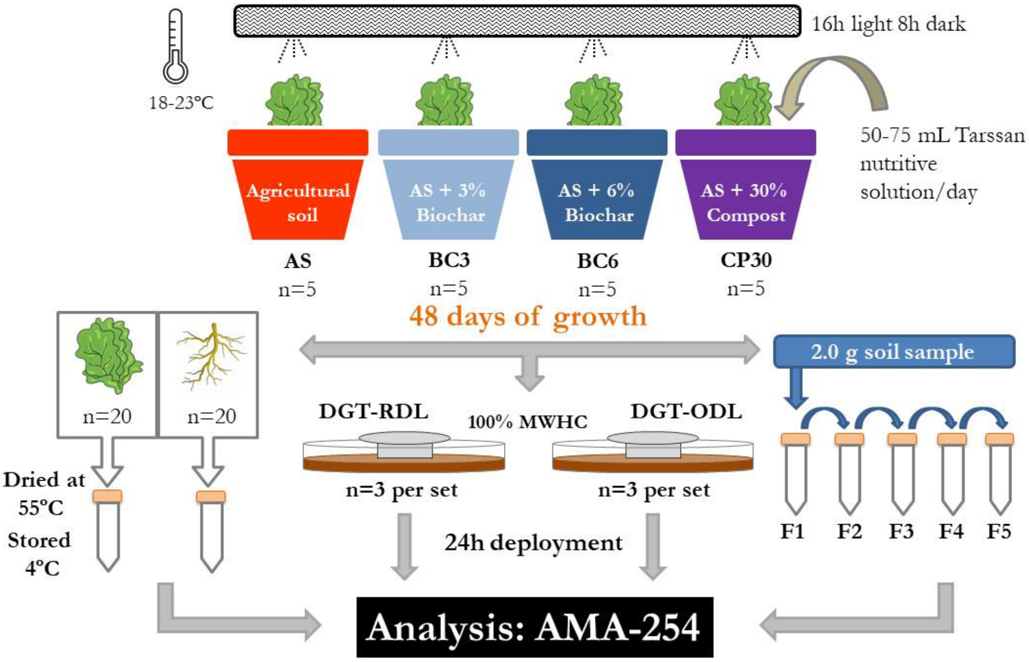

Figure 7.1: Scheme of the experiment.

After 48 days of growth, when lettuce reached commercial size, the leaves and roots were harvested separately, and the fresh weight (fw) was determined along with the length of both. Then, leaves and roots were washed off with deionized water to remove any surface contamination, and were dried in an oven at 55°C (Li et al., 2017b).

Soil pH and conductivity were determined at the end of the experiment with deionized H_2O at a 1:2 solid: liquid ratio using a previous calibrated pH meter Crison GLP22 and a previous calibrated Hach CDC401, respectively (Gramlich et al., 2018).

The OM was analysed as loss on ignition using 2.0 g of soil sample in an oven (Carbolite CWF 1300) at 550ºC for 1h with a previous drying period at 90-100ºC for 1h (Dean, 1974).

Concentration of nitrates in the soil solution was determined spectrophotometrically as nitrate nitrogen (NO_3-N) using a Hach Lange DR 1900. The extraction was carried out using 50 mL of KCl in a 5 g of soil sample and shaken for 1h (Mulvaney et al., 2016).

All the Hg analysis were performed using and Advanced Mercury Analyzer, model AMA-254 manufactured by Altec (Prague, Czech Republic) and distributed by Leco Corp. (St. Joseph, MI, USA). This instrument uses catalytic combustion of the sample, pre-concentration by gold amalgamation, thermal desorption and atomic absorption spectrometry (AAS). Every sample is put in anickel boat and automatically introduced into the apparatus. To verify the results, a certified material from the National Research Council of Canada (NRCC, DORM-3) (Hg: 0.38 ± 0.06 mg kg^{-1}) was tested before and after the analyses per triplicate. The Hg concentration in CRM is 0.386 ± 0.001 mg kg^{-1} (n=3) in good agreement with the certified values. The detection and quantification limits (0.2 and 0.7 ng g^{-1} wet weight of Hg, respectively) were calculated based on 3 blank measurements.

7.2.3 Diffusive Gradient in Thin Films (DGT)

7.2.3.1 Hydrogels and binding layers preparation

The ODL hydrogels were prepared according to Clarisse and Hintelmann (2006) and RDL hydrogels were made as stated in Shiva et al. (2015) with modifications.

For the preparation of ODL hydrogels, a gel solution consisting of 15 % (v/v) acrylamide (Sigma Aldrich) and 0.3 % (v/v) crosslinker (DGT Research Ltd., UK) was prepared before making the gels and kept at least one day at 4ºC. Then 21 μL 10 % (m/v) ammonium persulfate in Milli-Q water (Millipore, USA) and 6 μL of N,N,N',N'-Tetramethylethylenediamine (TEMED) were added to the gel solution and stir vigorously. Subsequently, the solution was cast between two glass plates separated by 0.05 cm thick Teflon spacer allowed to polymerize at 42-46ºC for 45 min. Finally, the gels were hydrated with Milli-Q water for at least 24 h and change the water 2-3 times while the gel was expanded 1.6 times to clean the impurities (Zhang and Davison, 1999).

For the preparation of RDL hydrogels, a gel solution was prepared by mixing 40% acrylamide/bis-acrylamide solution (Sigma-Aldrich) in a 1:1.66 ratio with Milli-Q water and it is preserved in the fridge for at least one day. 6.67 µl of TEMED and 23 µl of 10% (m/v) ammonium persulfate in Milli-Q water were added for every 3.33 mL of gel solution and then stir vigorously. After combine these three solutions, pipette the mixed solution in two plates of glass separated by a 0.05 cm thick Teflon spacer allowed to polymerize for 45 min at room temperature. After that, the polymerize gel was rinsed with Milli-Q water for at least 24 h changing the water 2-3 times.

For the binding layers, 0.33 g of 3-mercaptopropyl-functionalized silica (3MP) was used per glass mixing with the previous details shaking vigorously to avoid the precipitation of the resin to the bottom of the tube. Finally, the same casting procedure as for the other gels was used but in this case the separation is 0.025 cm.

A plastic mold piston design with a 2 cm diameter window (DGT Research Ltd., UK) was used to contain the gels, so it makes sure that the only part that it is in contact with the soil solution is 3.14 cm^2. A plastic tube with 2 cm diameter was used to cut the gels properly. To place the gels in the DGT device, the binding gel with the resin facing upward was added in the first place; then the diffusive gel was placed onto the resin gel and, finally, 0.45 µl pore size nylon filter membrane to protect the gels. The front cap of the DGT device was pressed down tightly ensuring a good seal to avoid any problems in the sampler.

7.2.3.2 Determination of Hg in soils by DGT

A Petri dish with 40 g of soil sample was used and maintained to 100% maximum water holding capacity (MWHC) with deionized water at 25ºC for 48 h, to ensure the equilibrium between fractions and soil (Yao et al., 2016). The water/soil mass phase ratio was 0.64 mL g^{-1}, 0.75 mL g^{-1}, 0.77 mL g^{-1} and 0.76 mL g^{-1} for AS, BC3, BC6 and CP30, respectively. Triplicates for every set and per different type of DGT (e.g. ODL and RDL) (n=24) were done.

One DGT device was pressed onto the surface of the soil slurries in one Petri dish and kept for 24 h at 25ºC. To avoid the potential contamination and to ensure stable moisture content, the top of the Petri dish was used to cover the DGT device. After the retrieval, the filter membrane was carefully washed with deionized water to remove any particles from the surface.

DGT technique follows the Fick's first law. Equation 7.1 was applied to calculate the concentration of the metal in the matrix (C) is:

$$C=\frac{M\Delta g}{DAt} \tag{7.1}$$

where D is the diffusion coefficient of the Hg in the diffusive layer, M is the mass of Hg accumulated by the resin, Δg is the thickness of the diffusive layer, A is the exposure surface area and t is the deployment time.

For the analysis of the pore water, after retrieving DGT samplers, soil slurries were centrifuged at 3000 rpm for 20 min followed by filtration through 0.45 μm filter. Prior to analyses, soil solutions were acidified with 1M HNO_3 and stored at 4ºC (Ridosková et al., 2017).

It should be noted that the DGT accumulates metal on the resin gel continuously that causes a depletion of the metal concentration in the soil solution (C_{soln}), because there is no resupply. Comparing the C_{DGT} and C_{soln} (equation 7.2) provides a ratio (R) indicating the depletion of solution concentration at the device interface depending on the adsorption-desorption kinetics within the soil (Sochaczewski et al., 2007):

$$R=\frac{C_{DGT}}{C_{soln}} \quad 0<R<1 \tag{7.2}$$

To interpret DGT measurements, C_{DGT} can be converted to an effective concentration (C_E) using equation 7.3 (Zhang et al., 2004):

$$C_E=\frac{C_{DGT}}{R_{diff}} \tag{7.3}$$

where the C_E is the metal concentration effectively available from the soil solution and the solid phase, and thus, the concentration found in the rhizosphere of the plants. R_{diff} is the ratio of the theoretical metal concentration in the diffusion only case (which means no resupply from the solid phase) and it is calculated by 2D DIFS model (DGT induced fluxes in sediments) (Sochaczewski et al., 2007). Input parameters used to calculate R_{diff} were the particle concentration (P_c), soil porosity (φ) and the diffusion coefficient in the soil (D_s) that can be calculated using equations 7.4 to 7.6 (Zhang et al., 2004):

$$P_c=\frac{m}{v} \tag{7.4}$$

$$\varphi=\frac{d_p}{(P_c+d_p)} \tag{7.5}$$

$$D_s=\frac{D_0}{(1-\ln\varphi^2)} \tag{7.6}$$

where m is the mass of the soil particles, v is the volume of the pore water, D_0 is the diffusion coefficient in water and dp is the density of the soil particles (commonly assumed 2.65 g cm^{-3} for soils).

7.2.4 Sequential extraction

In this case, a five-step sequential extraction procedure (Bloom et al., 2003; Pinedo-Hernández et al., 2015) with some modifications was used to develop an understanding of Hg compounds and binding in the soil. Accordingly, 2.0 g of dry soil sieved with a 200 µm mesh was separated in a 50 mL centrifuge tube with the corresponding extracting solution:

[F1] Water soluble fraction: 25 mL of ultra-pure water and, after that, 1 mL of 0.2 M BrCl are added to 2.0 g of every soil sample. As a rinse step, 20 mL of the same extractant are added in the tube with the soil sample.

[F2] Human stomach acid soluble fraction: residue from step 1 is added 25 mL 0.1 M CH_3COOH + 0.01 M HCl (pH 2). 1 mL of 0.2 M BrCl is added. As a rinse step, 20 mL of the same extractant are added in the tube with the soil sample.

[F3] Organo-complexed fraction: residue from step 2 is extracted with 25 mL of 1 M KOH, neutralizing the solution adding 10 mL of 0.2 M BrCl.

[F4] Elemental mercury, strongly complexed fraction: residue from step 3 is combined with 25 mL of 12 M HNO_3. Also, 1 mL of 0.2 M BrCl is added. As a rinse step, 20 mL of the same extractant are added in the tube with the soil sample.

[F5] Mercuric sulfide, residual fraction: residue from step 4 is rinsed to clean the sample with Milli-Q water, shake vigorously and centrifuge. After extract all the water, the samples are placed in the oven 50ºC overnight and analyzed directly the soil sample.

All the samples were agitated at room temperature using end-over-end shaking 18 h at 30 rpm except the last. F1, F2 and F3 fraction are filtered at the end of each step, even the rinse step. All the fractions are centrifuged at 3000 rpm for 20 min.

7.2.5 Bioconcentration factor (BCF)

The bioaccumulation factor is the index of the capacity of the plant to accumulate Hg in comparison with its concentration in the soil. BCF is calculated as Korzeniowska and Stanislawska-Glubiak, 2015:

$$\mathrm{BCF}=\frac{\text{Hg in roots}}{\text{Hg in soil}} \tag{7.7}$$

7.2.6 Statistical Analysis

Analysis of THg in the different soils, lettuce, roots and DGT devices was performed as the mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to evaluate the results for parametric values and Kolmogorov-Smirnov for no parametric values. Relationships between the parameters were performed using Pearson correlation. Statistical significance was declared when p value was equal to or <0.05. IBM SPSS statistics 25 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

7.3 Results and discussion

7.3.1 Mobility and bioavailability of Hg in soils

The concentration of Hg in biochar (29 ± 11 ng g^{-1}) and the compost (39 ± 7 ng g^{-1}) added to AS did not exceed the legislation RD 865/2010 for the application to agricultural soils (400 ng g^{-1}). The concentrations of Hg in the mobile (F1 and F2), semi-mobile (F3), and non-mobile (F4 and F5) fractions are plotted in Figure 7.2. The Hg analyzed directly from the soil sample in comparison with the Hg summed from the different fractions (90-97%) demonstrate the effectiveness of the SE procedure. The Hg concentration in the different fractions is ranged in the following order: F4 (78-83%) > F5 (14-16%) > F3 (1-5%) > F1 (1-2%) > F2 (0-1%). Our findings on the compounds of Hg in soil match with farmland soils contaminated by a chemical plant in China (Zhao et al., 2018).

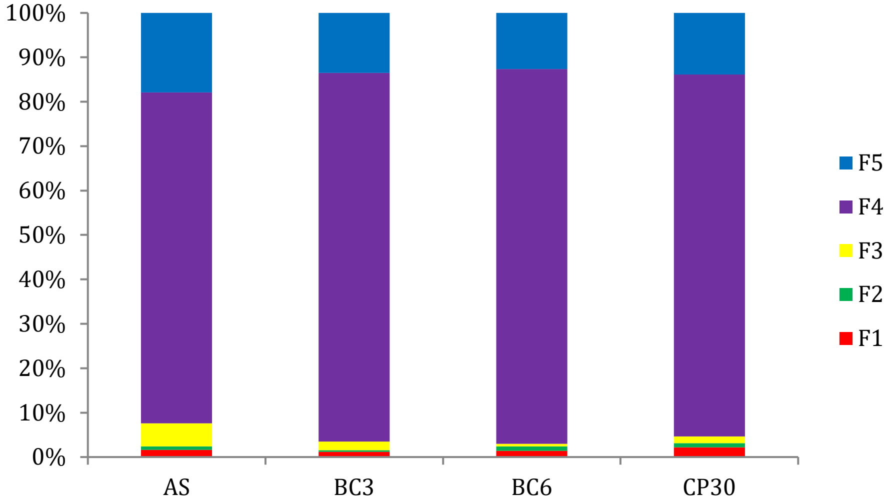

Figure 7.2: Speciation of Hg in the different soil samples.

Hg compounds present in soils depends on many factors, such as physical and chemical soil properties, seasonal temperature, downstream transport of Hg from source locations, or the time since Hg deposition, among others (Gilli et al., 2018). These factors may drastically change Hg chemistry in soil and, thereby, modify its mobility, bioavailability, and toxicity. For example, a study executed in Russia Gordeeva et al., 2017) demonstrated the difference between soils from the Hg electrolysis shop, where contamination was due to atmospheric deposition (F3 as the highest fraction), and soils from technogenic dumps (F4 and F5 as the highest part).

In this regard, the Hg aging process in soils should be considered, since a decrease of inorganic mercury and/or methylmercury extraction occurs over time (Ma et al., 2015). Soil organic content could play an important role in the Hg aging process, reducing the association rate with stronger binding sites.

Significant differences ($p<0.05$) were observed between AS and BC3, with a reduction in bioavailability of 60%. The SE showed a reduction in the mobile phases (i.e. F1, F2) and, on the contrary, the amount of Hg in F4 that is not mobile increased. In the case of BC6, the trend is similar, but the percentage in F2 increased with respect to AS (12%). However, no significant differences were obtained. In contrast, the bioavailability of Hg is significantly higher when compost is used as amendment in the soil. There was a 21% increase in F1, and a 3% reduction in F2 (16% more bioavailable than AS).

Results using biochar in the soil show that the pH slightly increased with an additional amount of biochar (Table 7.1). Moreover, previous studies have shown that biochar pH increased with increased pyrolysis temperature (Nguyen et al., 2010; Zhang et al., 2015; Yuan et al., 2011). As expected, according to the pyrolysis temperature of the used biochar (400-600 °C), we found a neutral to alkaline biochar (Li et al., 2017a; Zhang et al., 2015). On the other hand, when compost is added to the soil, pH is reduced due to its slightly acidic property. The concentration of H^+ added to the soil from the compost could explain the depletion of pH (do Carmo et al., 2016), that is related with extremely high EC in comparison with the other sets. With the addition of compost, EC increased, because of the concentration of free ions and nutrients increased. Nitrate increased from 9.2 to 11.4 mg kg^{-1} (Table 7.1). Significant inverse correlation ($R=-0.685$, $p<0.05$) between pH and F1 was observed but, on the contrary, no significant correlation exists with F2. Looking at EC, significant correlation was found with F1 but not with F2 (Table 7.2).

Table 7.2: Correlation coefficients between percentage of Hg in each fraction in the soils and the percentage of OM, pH, nitrates and EC.

	F1	F2	F3	F4	F5
OM (%)	0.390	0.470	-0.866**	0.547	0.052
pH	-0.685*	0.149	-0.331	0.710**	-0.965**
Nitrates	0.547	-0.044	-0.278	-0.109	0.678*
EC	0.748**	0.180	-0.264	-0.208	0.745**

*p<0.05 **p<0.01

Focusing on Hg bound to organic matter and sulfides (i.e. F3), significant differences from the control for all treatments were observed. Both BC amendments reduced Hg concentration in the organo-complexed fraction and for

CP30 this is the only fraction that was reduced. As can be seen in Table 7.2, the OM is highly and significantly ($p>0.05$) correlated only with F3. It is well known that the OM controls the biogeochemistry of Hg in soils, immobilizing Hg due to the high affinity of this element with the organic matter (Liu et al., 2007); however, dissolved OM in the pore water could be complexed with Hg in the soil and desorb it. The main reason is OM forms soluble metal-organic complexes, increasing Hg availability (Smolinska, 2015). Besides that, organic amendments are sometimes applied to improve the natural phytoextraction by hyperaccumulators, increasing the amount of Hg bioavailable. Moreover, Zhu et al. (2015) determined that, apart from the effect of organic fertilizers to reduce the phytoavailability of Hg in soil, when it is used in excess, the dissolvable fraction of humic acid could increase mercury desorption from the soil.

7.3.2 Accumulation of Hg from soil to lettuce

Results showed that Hg concentrations in the leaves of lettuce are not significantly different ($p<0.05$) between all the sets (Table 7.3). Several studies (Ericksen and Gustin, 2004; Frescholtz and Gustin, 2004; Leonard et al., 1998; Gustin et al., 2004) have shown that plants are a sink for atmospheric Hg. The Hg concentration in the leaves was same for all treatments ($p>0.05$). Because of this, Hg concentration in leaves are not useful for understanding the effect of Hg amendments to the soil, and Hg in roots were chosen to compare the effectiveness of the different amendments.

Table 7.3: THg in soil, and lettuce root and leaves for every set and the BCF (n=5). Different letters indicate significant differences within different columns.

	Hg concentration (ng g^{-1} in fw^{a})		
	Roots	Leaves	BCF
AS	14.08 ± 1.60^{a}	5.24 ± 0.85^{a}	0.022^{a}
BC3	10.40 ± 0.70^{b}	4.90 ± 0.56^{a}	0.017^{b}
BC6	10.24 ± 5.33^{ab}	4.86 ± 0.75^{a}	0.017^{ab}
CP30	10.96 ± 2.49^{ab}	4.87 ± 0.59^{a}	0.020^{ab}

^{a}fw: fresh weight

Roots have been shown to be an important role in the accumulation of Hg in plants, acting as a barrier to the incorporation of the metal, and affecting its translocation to aerial plant tissues (Shahid et al., 2017). In rice plants, the development of apoplastic barriers in the endodermis, the secretion of different organic acids to retain Hg in soil, and the effect of Fe to sequester the metal reduce its bioavailability (Wang et al., 2015). Therefore, the BCF for Hg in plants is low in comparison with other trace metals (Chang et al., 2014).

Results showed that Hg concentrations in the roots of lettuce are significant different ($p<0.05$) between AS and all amendments (Table 7.3). No statistical differences were found between the amendments. Moreover, a significant inverse correlation exists between the Hg concentration found in roots and the addition of OM ($R=-0.60$, $p<0.05$) (data not shown).

Using biochar amendments, the accumulation of Hg in the roots was lower than the roots of lettuces growing in AS. In fact, the percentage of uptake with respect to AS is reduced by 35% and 37% in BC3 and BC6, respectively. Although BC3 had significant differences because of the low deviation between lettuce (i.e. 0.70) in the same set, the BCF in both cases has the same value (i.e. 0.017). These results are comparable to those from Shu et al. (2016) where the amount of MeHg in rice decreased between 49 and 92% adding biochar to Hg-contaminated soils. Furthermore, biochar combined with nitrate also reduced MeHg in plant due to nitrate inhibition of the activity of sulfate reducing bacteria that are responsible of the production of MeHg (Zhang et al., 2018). Apart from its effectiveness, it is important to note that biochar is low-cost compared to other technologies (Inyang et al., 2016), and would be a good choice for large surfaces.

When compost was used as amendment, results showed that Hg uptake by roots from CP30 was 29% lower than in AS; however, it is important to note that Hg concentration in CP30 was initially lower (10%) than AS. Despite these dissimilarities, no statistical significant differences ($p>0.05$) were found in BCF between non-amended soil and the soil amended with compost (0.020 vs 0.022).

These results showed that the application of compost does not significantly reduce the concentration of Hg in the lettuce roots or reduce the BCF value of the plants. There is some controversy between studies claiming that the concentration in roots is reduced adding compost and others that confirm the ability to increase the Hg absorption in the plant. For example, Restrepo-Sánchez et al. (2015) showed the reduction of Hg transfer to plants using Hg-doped compost in the agricultural soil. While, Zhu et al. (2015) demonstrated that the addition of organic fertilizer could decrease Hg phytoavailability and Hg methylation, and when an excess of organic fertilizer was added Hg desorption from soil icreased. Cozzolino et al. (2016) investigated the combination of OM with the inoculation of arbuscular mycorrhizal fungi and showed its effectiveness to reduce Hg uptake for lettuce plants. In contrast, Muddarisna and Siahaan (2014) and Smolinska (2015) found an increase in Hg content in roots and leaves when applying compost in Hg-contaminated soils.

7.3.3 Bioavailability and mobility of Hg in soil

Concentrations of Hg by DGT were calculated using the diffusion coefficients determined by Turull et al. (2019) (3.08×10^{-6} cm^2 s^{-1} for ODL and 1.79×10^{-6} cm^2 s^{-1} for RDL) for AS, BC3 and BC6 (Table 7.4). When compost was added, the dissociation of the exchange reaction between Hg-OM and DGT was not fast enough to allow for determination of the Hg concentration available for the plant. Thus, D from a synthetic solution with compost calculated by Cattani et al. (2009) (6.70×10^{-7} cm^2 s^{-1}) was used to calculate Hg concentration in CP30. In the case of the roots, a significant inverse correlation existed between ODL values and OM ($R=-0.848$, $p<0.01$), because of the capacity of Hg to form strong ion complexes with the functional groups of OM such as sulphur or carboxylic acid groups (Smolinska and Szczodrowska, 2017).

Table 7.4: Hg content in soil solution (C_{soln}) and obtained from RDL and ODL-DGT measurements of the soil. Effective concentration (C_E) and low molecular species are calculated by R_{diff} and RDL respectively.

Line	C_{soln} (µg L^{-1})	ODL (µg L^{-1})	RDL (µg L^{-1})	R_{diff}	C_E (µg L^{-1})	LMS (%)[1]
AS	0.244 ± 0.014	1.616 ± 0.170	1.567 ± 0.649	0.118	13.696	97
BC3	0.356 ± 0.017	1.113 ± 0.171	0.905 ± 0.783	0.092	12.108	81
BC6	0.218 ± 0.059	1.122 ± 0.071	0.863 ± 0.276	0.111	10.104	77
CP30	0.076 ± 0.003	0.999 ± 0.127	0.623 ± 0.018	0.095	10.489	62

[1] LMS: Low molecular species (%): (RDL/ODL)x100

The R value is the ratio between the labile Hg concentration obtained by DGT devices and the soil solution that represents the presence of labile species in the solid phase and the speed of Hg re-supply from the solid phase to the soil solution. In Table 7.4, ratio measured from the program 2D DIFS (R_{diff}) is shown. R_{diff} value was calculated considering different parameters such as the porosity, the diffusive coefficient or the particle concentration. Also, this value was used to calculate the effective concentration (C_E) using equation 7.3. The values applied to calculate R_{diff} are summarized in Table S7.1. Therefore, C_E can be compared with the values from lettuce roots. The differences between concentration of Hg in roots and DGT values in the sets AS, BC3, BC6 and CP30 were -3, 14, -1 and -4%, respectively. These values demonstrate the effectiveness of this technique to predict metal concentration in roots. Besides that, the best significant correlation was found between DGT measurements and roots from *Lactuca sativa* (R=0.703) in comparison with the other values such as the sequential extraction (R=0.467) or C_{soln} (R=-0.314). Our results are in agreement with other studies correlating the values of Hg from DGT measurements with different crops. Ridosková et al. (2017) demonstrated the effectiveness of DGT method to predict the root uptake of Hg by C_E where its Hg correlation with *Taraxacum officinale* was 0.84. Also, Liu et al. (2012) predicted the amount of MeHg in *Oryza sativa* by DGT measurements and it was well correlated with the rice roots (R=0.85).

The amount of low molecular species is indicative of the fraction of the inorganic Hg in the soil solution (Table 7.4). Using DGT with a pore size <1 nm (i.e. RDL), the concentration of free metal species in soil could be estimated. It is important to know the fraction of soluble inorganic Hg species, because of ease of transport by natural processes, is considered a substrate for the Hg methylation (Han et al., 2003). The amount of low molecular species (as %) for AS is 97%, that was progressively reduced by the addition of organic matter in the soil. Therefore a significant inverse correlation (R=-0.586, $p<0.05$) was observed between values from RDL and the percentage of OM. In the case of biochar, adding 3% and 6%, the percentage decreased 16 and 20%, respectively. Furthermore, adding compost, the amount of inorganic species decreased by 35%.

7.4 Conclusions

In the present study, Hg concentration in an agricultural peri-urban soil from Barcelona occured in an inert form, and only a small part (1-3%) was bioavailable for lettuce. The application of biochar reduced the labile fraction of Hg. In contrast, the use of compost increased the bioavailable Hg for the plants. The concentration used to predict Hg uptake by plants, C_E, can be calculated applying the DIFS program, considering the kinetical properties of each soil. Furthermore, DGT method with a smaller pore size (RDL) was used for the determination of inorganic Hg species in the studied agricultural soils. The results showed that the soil without any amendment had the highest value of free Hg ion, whereas the amount was reduced with increased the OM content in the sample by up to 62%.

7.5 Supplementary material

Table S7.1: Values for DGT induced fluxes in sediments (DIFS).

Variables	Units	C	CS	BC3	BC6	CP30
Φs	-	0.610	0.628	0.665	0.671	0.669
Pc	g cm^{-3}	1.695	1.567	1.333	1.300	1.314
D_0	cm^2 s^{-1}	3.08E-6	3.08E-6	3.08E-6	3.08E-6	3.08E-6
C	mol cm^{-3}	3.19E-13	9.37E-13	7.93E-13	8.63E-13	2.59E-13
Cs	mol g^{-1}	1.20E-13	3.92E-12	3.42E-12	3.17E-12	3.20E-12
Kd	cm^3 g^{-1}	0.375	4.181	4.314	3.671	12.327

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Chapter 8

Measurements of trace metals bioavailability in soils using commercial Chelex-100

This chapter is based on the article:

Turull, M.; Fontàs, C.; Díez, S. Effect of biochar and compost on trace metal bioavailability in agricultural soils and metal uptake by lettuce. J Haz Mat. (submitted).

In this study, we have determined the effect of two different organic amendments on trace metal transfer from soils to crops. Agricultural soil was amended with biochar (BC) at two rates (3% and 6%, g g^{-1}), and compost (30%) to evaluate the effect of mitigation of trace metals in lettuce (*Lactuca sativa* L.). At the end of growth, Cd, Cr, Cu, Ni, Pb and Zn were determined in leaves, roots and soil, and its bioavailability and uptake was evaluated by the diffusive gradient in thin film (DGT) method. On addition of compost, the lettuce uptake of Cd, Cr, Cu and Pb was 1.7, 2.4, 1.3 and 3.7 times lower, respectively, than in lettuces with no amendments. The application of BC produced a greater weight of leaves, roots and total biomass, increased leaf length and enhanced significantly the chlorophyll content in lettuce. Moreover, BC6% mitigates Cr (33%) and Pb (36%) uptake by lettuce. In addition, the DGT-labile fractions of Cr, Cu and Pb measured in amended soil were significantly ($p<0.05$) correlated with lettuce total concentrations demonstrating that DGT is a faster and more cost-effective method than conventional ones for predicting metal uptake by plants.

SUBMITTED PAPER. EMBARGO UNTIL PUBLICATION DATE

Chapter 9

General discussion and future research

As highlighted throughout this Thesis, measuring the bioavailable fraction of trace metals is essential not only to determine their toxicity, but also to study the impact of these elements on the environment and on living beings. Accordingly, the DGT technique has been used in the present thesis due to its capacity for the *in situ* determination of kinetically labile metal species in a rigorous and easy manner.

The DGT technique is versatile, since many target trace elements can be measured in different types of matrices (e.g. water, sediments, soils, etc.). Therefore, in this thesis, we have investigated Hg and other trace metals, such as Cd, Cr, Cu, Ni, Pb and Zn, in both aquatic and terrestrial environments. These two environmental systems possess different complexities, which can affect both the way DGT devices are applied and the experimental parameters to be taken into consideration. All this has led us to study multiple ecosystems using the DGT technique and, as a result of this Thesis, new advancements and applications of DGT have emerged.

9.1 In aquatic environments

Since the DGT technique was developed in 1994 as a simple technique for measuring trace-metal concentrations *in situ* in water (Davison and Zhang, 1994), several binding agents have been tested to be applied for different target elements or compounds and in other matrices apart from water. Therefore, at the present time, components such as antibiotics, organic compounds or macronutrients can also be determined by this technique. With the aim of exploring possible new binding phases as well as new passive sampler configurations, in this Thesis we have evaluated a new membrane, with a similar design to the DGT, to measure bioavailable Hg in water. Our research group has many years of experience in polymer inclusion membranes (PIM). So, for the first time, we have investigated the incorporation of a PIM, which contained an ionic liquid (IL) able to bind Hg, specifically trioctylmethylammonium thiosalicylate (TOMATS), in the DGT design. As a result, not only have we investigated the effect of the PIM composition varying the percentage of TOMATS (35 and 50%) or device design (PIM-DGT), but also its efficacy under real conditions in the field.

The lower Ebro River (Tarragona, Spain) was selected for the field experiments due to its historic Hg pollution caused by a chlor-alkali plant located in the municipality of Flix. This chlor-alkali industry uses the traditional Hg-electrolysis method to produce chlorinated solvents. Unfortunately, due to the use of this technology, an important fraction of Hg employed during production is released through drainage into the aquatic environment and accumulated in the sediment where it can be transformed to bioavailable forms of Hg, which can be incorporated into the biota. Consequently, the river ecosystem has been affected by Hg contamination for many years (Carrasco et al., 2008).

The application of one of the customized PIM in the DGT design (based on a PIM with a 35% of TOMATS and a filter) in the Ebro River was accompanied by conventional DGT devices (with 3-mercaptopropil functionalize silica -3MP- embedded in a polyacrylamide gel as the binding agent with polyacrylamide gel as the diffusive layer) in all the different sampling points for their subsequent comparison. This river has Hg concentrations in the suspended particulate matter (SPM) 2-5 orders of magnitude higher than the free metal ions of Hg in the water. Therefore, as previous studies (Fernández Gómez, 2014) have shown, deployment times must be longer in comparison with other rivers because of the low dissolved bioavailable Hg concentrations in the Ebro River. Nevertheless, longer deployment times with high SPM concentrations in the river can cause filter clogging and devices do not work properly.

Considering the above, we hypothesize an alternative to measure the Hg bioavailable in aquatic systems with high amount of SPM. Previous studies (Serrano et al., 2015) have been demonstrated that plants, algae and some fungi have the capability to prevent the negative effects from metal stress with the production of certain compounds called phytochelatins. Accordingly, and considering the effectiveness of the DGT technique, it would be relevant to correlate this technique with the analysis of phytochelatins to monitor Hg in the aquatic systems, specifically in the Ebro River.

This is why the production of phytochelatins from two typical species of aquatic macrophytes from this river (i.e. *Ceratophyllum demersum* and *Myriopyllum spicatum*) were measured and compared with the dissolved bioavailable Hg measured by DGT devices in two different seasons. Despite the low concentrations of Hg found in the water as free metal ions, there were enough to activate the production of phytochelatins in these selected plants. Nevertheless, one of the most important limitations for the use of DGT devices in sampling campaigns was their loss during changes in weather conditions. DGT devices were only retrieved in all the sampling points in one of the three different sampling campaigns in the Ebro River. This may have occurred because the Ebro is a dam-regulated river, with many dams along the river to control the amount of water during the year. Dams store floodwater and reduce flood risks by attenuating flood peaks as well as the intensity of flooding in downstream sites. Nevertheless, when the reservoirs are full of water, it is necessary to open the floodgates, causing a sudden flood in the river. This effect might have influenced the sampling campaigns because of the increase in the force of the water reaching the river mouth. In addition, the sampling device used in these campaigns was a cylindrical basket anchored to a rope on the riverbed with a weight at one end to ensure that the device remained inside the water. The weight also prevented the baskets from being visible outside the water, so they could not be seen (even when close), and removed. Yet, if the water level increased too much, the use of the weight would make basket collection impossible due to the distance of the basket under water.

In lab experiments, the use of a PIM in a DGT design did not work properly because this configuration does not follow Fick's law, although its efficacy to absorb Hg under field conditions has been demonstrated. What it would be desirable to develop a modeling system with more PIM-DGT designs to determine its effectiveness. Conversely, the production of phytochelatins in both selected species of macrophytes was detected. Taking into consideration that both studies were performed in the same sampling points, a comparison of the results could be of interest. Figure 9.1 shows phytochelatin values together with the absorbed Hg concentration by PIMs. The order of magnitude can be seen to be the same for all the components, as well as the tendency, with PIM being significantly correlated ($R=0.744$, $p<0.05$) with *M. spicatum*.

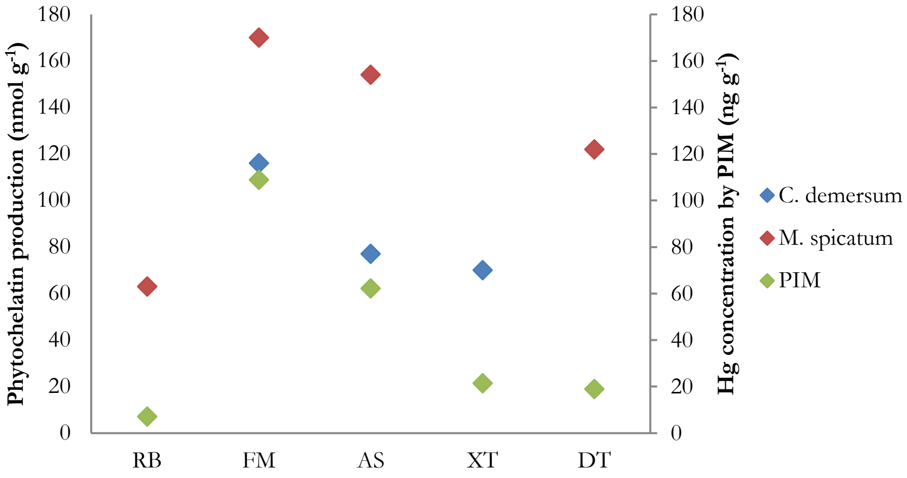

Figure 9.1: Phytochelatin production from *C. demersum* and *M. spicatum* and Hg absorption by a PIM membrane. RB: Riba-Roja, FM: Flix meander, AS: Ascó, XT: Xerta, DT: Deltebre.

Furthermore, the application of the DGT technique was assessed in a different river, specifically in the Tully River in a catchment in North Queensland (Australia). This Australian river has a lower concentration of SPM compared to the Spanish Ebro River. Therefore, the percentage of bioavailable Hg was 43-80% in relation to the total Hg found in water because of the low concentration of SPM. In this fieldwork, in contrast to the sampling technique used in the Ebro River, the placement was in the middle of the river without a weight tied to the cylindrical basket. In this way, we avoided not being able to reach the baskets due to the weight, even if the water level increased. Furthermore, this river has no reservoirs in its course. Thus, since the dynamic of this river is not naturally dam-regulated, the levels of water were not expected to rise as much in case of flood as the Ebro River.

Nevertheless, the key foundation of this work was to use DGT devices for the measurement of the average bioavailable Hg, even during a flood event, with the aim of evaluating the residual levels of Hg in soils from sugarcane cultivation that reach the aquatic environment. During this flood event, the river water levels rose about 2.5 m, but fortunately, we did not lose any baskets with DGT devices in the water. Hg concentration was successfully measured by the DGTs as well as an increase in bioavailable Hg in one sampling point was found, most probably due to the high Hg concentration found close to this soil sampling point, which should affect the river downstream.

If the measurements had been made by spot sampling, taking into account the effect of the flood event, it would probably have been difficult to identify this finding. In this case, the use of the DGT technique was fundamental for detecting that some flood episodes may increase Hg pollution in aquatic systems.

In sum, the DGT technique was successfully used in natural waters, specifically in two rivers with different hydrologic dynamics and physicochemical properties, reaching very low levels of dissolved bioavailable Hg concentrations (ng g^{-1}). Furthermore, its easy use in sampling campaigns and its quick analysis allowed effective field studies to be performed, collecting more information and avoiding potential point sources of pollution. As shown in the example plotted in Figure 9.2, the use of passive devices in field studies allowed us to obtain information closer to the real situation in comparison with spot sampling. Spot sampling depends on the number of samples and when they are collected, and is sometimes not entirely representative.

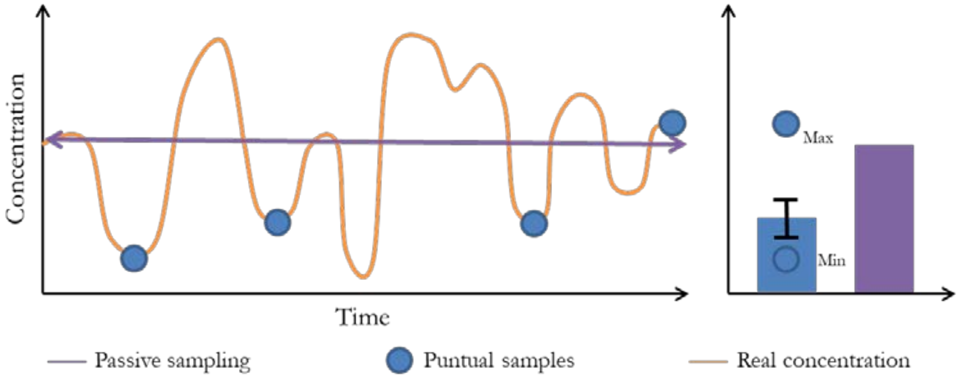

Figure 9.2: Comparison between spot sampling and passive sampling.

On the other hand, apart from the deviation in measurements due to clog procedures caused by biofouling and particulate matter, the loss of DGT devices might also cause the repetition of field work or reduced scientific accuracy. Hence, the technique used in the field is very important and the characteristics of each area to be studied must be taken into consideration in each sampling campaign to reduce the potential loss of devices.

9.2 In terrestrial ecosystems

In comparison with water, the soil matrix is more complex to study due to the interference of many factors, although soil dynamics undergo less change, and are, therefore, more stable than water. Consequently, the risks associated with trace metals in the soil are very difficult to assess. In many studies, the negative effects have been related to the total metal concentration in soil, which is not real because of the large fraction of metals not bioavailable to plants. Thus, the measurement of the bioavailable fraction is also necessary here, to perform a suitable approach for toxicological studies. Soil solution extraction or measurements of free ion activity are examples of analytical methods to obtain a better indication of the bioavailable part of trace metals, but they do not take the depletion of metal concentrations into account (Zhang and Davison, 2003). In contrast to these methods, this Thesis has demonstrated the effectiveness of using the DGT technique to determine the bioavailable fraction of Hg and trace metals in soils and their correlation to plant content.

Since the first publication in 1998 by Zhang and coworkers (Zhang et al., 1998) a large number of studies on soils (around 630 based on a Scopus database search) have been published using this technique to study the bioavailable fraction of several trace metals. Since then, many DGT compositions (different diffusive and binding layers) have been made to measure trace elements.

It is also feasible to study different metal species with the DGT technique, increasing the amount of information from a particular soil. This should be accomplished by varying the conventional pore size commonly used (>5 nm) to a pore size less than 1 nm to study the labile inorganic fraction in soil. Two studies (Docekalová et al., 2012; Kovaríková et al., 2007b) have been done for certain types of metals (Cd, Cu, Ni and Pb) in soil but not for Hg. For this reason, a laboratory study was carried out to analyze the free Hg ions in two different types of soil using two types of DGT devices WITH the same binding agent (3MP) but different cross linker (bis-acrylamide for the restricted gel and acrylamide for the open gel).

Moreover, the capacity of the DGT technique to predict metal uptake by plants has already been demonstrated (Zhang et al., 2001). The prediction of metal uptake by plants, including the kinetic properties from the studied soil, can be calculated by the use of a program, called 2D DIFS. However, most of the experiments from these studies have been carried out in different experimental arrangements, such as in the laboratory or in the field, using different types of soil, different physicochemical properties or different climate conditions. Thus, even by using the same DGT (same binding and diffusive layer), the results might be different depending on their experimental and soil characteristics. Some studies have demonstrated the effectiveness of the DGT technique to be correlated with plant uptake for a specific target element and others in which the uptake has not been able to be determined. To solve this problem, we selected two types of agricultural soils and the bioavailable fraction uptake by plants was compared with two different methods (DGT and a conventional technique). Moreover, results were correlated with the metal uptake by lettuces (*Lactuca sativa*) previously cultivated in both soils.

The possibility of predicting plant uptake without waiting for the crop to grow makes the DGT technique an important tool to be used in food security studies. Metal uptake by edible plants can be estimated in only a few days. According to EU regulations, only Cd and Pb are regulated by the legislation (European Commission, 2006) in vegetables, but the maximum trace metal concentration in soil is regulated depending on the uses allowed (industrial, urban and other uses) for 18 different trace metals including Cd, Cr, Cu, Hg, Ni, Pb and Zn (BOE, 2009). As explained before, bioaccumulation and the toxicity of elements in the environment are not related to total concentrations, but rather to the concentration of specific chemical species that may change depending on different factors. Physical and chemical soil properties, such as the pH, texture, organic matter, might vary the amount and the bioavailable fraction of trace metals. Thus, we selected an agricultural soil with a certain concentration of trace metals and we applied two types of common organic amendments used nowadays to determine how these amendments influence the bioavailable fraction of the soil and their incorporation to crops. In this case, we studied a low rate of biochar of 3% and 6% (a component which is currently being studied for its trace metal immobilizing effect), and compost at a higher rate of 30%, an amendment commonly used in the past. Furthermore, lettuces were also grown in the different experiment sets to corroborate the effectiveness of the DGTs in predicting metal uptake by the addition of these amendments.

The application of a specific binding layer to study Hg, 3MP, was suitable for predicting uptake by lettuce roots almost precisely (deviation around 5%). The lettuce leaves were discarded, since the aerial part of the plant did not allow us to distinguish if the Hg uptake came from the air or was translocated from the roots. On the other hand, roots are a better indicator to determine Hg uptake by the plant from soil since they have no other input, only the Hg concentration from the soil.

In the next study focused on food security with trace metals, such as Cd, Cr, Cu, Ni, Pb and Zn, we decided to relate DGT measurements with trace metal content in lettuce leaves since they are the part of the vegetable eaten by humans. Although this technique measures the uptake by roots and a large fraction of these trace metals were accumulated and retained in the roots, it is important to verify if DGT measurements are useful for relating the trace metals translocated/accumulated in the leaves. Therefore, a DGT with Chelex-100 was used to measure the labile fraction of trace metals, that significantly correlated with the total concentration of Cr, Cu and Pb found in lettuces. Previous studies demonstrated that, with the same binding and diffusive layer, Zn correlated well with lettuce leaves (Cornu and Denaix, 2006) and Ni with other plants (Luo et al., 2014). Consequently, we may conclude that, because Chelex-100 is not an exclusive resin and it could be possible a competition by different trace metals inside the resin, C_E could be influenced for trace metal prediction in lettuce uptake.

Therefore, not all the trace metals can be encompassed in a same resin. According to the company that commercially supplied the DGT device based on a Chelex-100 binding layer, it is able to measure up to 30 metals, but this resin was shown not to have a high degree of selectivity. Thus, depending on the metal concentration, successful measurement of the target elements would not be possible because of competition for the binding sites by the major ions (Bennett et al., 2016a). On the other hand, Chelex-100 has the ability to strongly bind transition metal ions via chelation (Bennett et al., 2016a), but Hg is not included. Accordingly, not all trace metals can be treated in the same way and be measured successfully by the same resin. Consequently, for Hg studies performed in this Thesis, a specific resin has been used for this target element.

Although the use of the Chelex-100 was not as successful as 3MP for Hg, it was possible to perform studies to determine plant uptake, even though the addition of different amendments was significantly correlated, as demonstrated in this study. Taking into account that DGT is an easy and quick technique in comparison with other techniques, it is a good tool for studying metal bioavailability, monitoring places potentially contaminated by trace metals, as well as for analyzing and controlling, over time, if different treatments for the remediation of the contaminated field are working.

In sum, this Thesis has revealed the effectiveness of the DGT technique with the use of the resin 3MP applied in soils, since it has been able to predict Hg uptake by lettuce plants in two different agricultural fields. In addition, it has demonstrated that variation by the addition of different amendments could also be monitored by DGT measurements, being a good tool (i.e. cost-effective) for studying bioavailability in field studies without needing to plant any crop and able to determine the impact by metal uptake in only a few days (i.e. fast). Furthermore, the application of a novel DGT device with a restricted diffusive layer provides additional information on the variation of the percentage of inorganic Hg when, in this Thesis, different amendments were added to the soil. As with the application of the DGT technique in aquatic systems, analyzing low concentrations could be possible.

On the other hand, the most important limitation for the uptake studies in soils using the DGT technique was in applying these devices in the field. In contrast to the studies performed in water, the variation of the climate conditions and soil parameters in a specific field should be constant during the experiment. Therefore, parameters such as temperature and water holding capacity are important and should be calculated precisely to determine uptake by plants using DIFS. Conversely, some studies have applied DGT devices in the field, although the research was conducted in paddy soils to study metal uptake by rice plants (Williams et al., 2012). This is because rice is grown in fields that are commonly flooded, so these are optimal conditions for the DGT, where the gels cannot be dried. The large majority of the studies using DGT devices in soil were performed in the laboratory and in short time periods (24-48 h) to reduce the expected variation of these conditions. Besides, other parameters, such as light or nutrients, influence metal uptake by the plant but are not captured by C_E.

9.3 Future research

Future studies and lines of research can be proposed as a consequence of the results of this Thesis.

It has been demonstrated that the application of PIMs in the DGT design was not suitable for the determination of labile Hg concentrations. However, the high capacity to accumulate Hg in water should be highlighted. This new sampler design can be used in remediation systems for the removal of Hg in aquatic ecosystems affected by Hg pollution.

Moreover, the effectiveness of a DGT design with less than 1 nm to study the inorganic Hg in two different agricultural soils has been determined. To go further, the application of this type of DGT could be applied in soils with different physicochemical characteristics.

Furthermore, the prediction of the uptake of different metals by *Lactuca sativa* was performed and successfully determined by the DGT technique. However, other vegetables should be studied to determine the effectiveness of prediction by the DGT technique, especially those that are buried in the soil (e.g. carrot, radish, etc.).

It is well known that the application of the DGT technique to predict metal uptake depends on soil characteristics. Depending on the type of soil, DGT devices may not work properly. For future studies, it would be interesting to characterize different types of soil as well as the functionality of other kinds of binding layers for different target elements to better use the DGT technique in terrestrial environments.

Chapter 10

Conclusions

The application of the DGT technique was successfully used to determine the labile concentration of different trace metals in the laboratory and in the environment. The conclusions of this Thesis, set out below, are differentiated by the matrixes in which the DGT devices were applied.

The main conclusions of the DGT technique applied in aquatic systems were:

- A polymer inclusion membrane was successfully incorporated in a DGT device to detect Hg in the Ebro River (Northeast Spain). This PIM-DGT was able to accumulate Hg in the water column of the river. However, this new device did not follow Fick's law of diffusion, so, the incorporation of a new type of binding gel in DGT design was not validated.
- Home-made DGT devices using 3-mercaptopropyl functionalized silica gel (3MP) as the binding agent and polyacrylamide as the diffusive gel were successfully used in the Ebro River basin. The concentration of labile Hg was correlated with the phytochelatins of two different species of macrophytes along the Ebro River. Therefore, the analysis of phytochelatins could be a good indicator of the bioavailable Hg in the river.
- Different commercial DGT devices based on Spheron-thiol resin and agarose as the diffusive layer were used to determine labile Hg in the Tully River (Australia). The DGT devices were successfully deployed in the river to detect Hg runoff after a flood event. The application of the DGT technique was key in detecting Hg pollution in the river from a historically polluted soil, which affected the river basin downstream.

The conclusions extracted from the studies in terrestrial environments were:

- Two home-made DGT devices with open (acrylamide) and restricted (bis-acrylamide) diffusive gel, both with 3MP as the binding agent, were used to study Hg in soils. In addition, commercial DGT devices with Chelex-100 as the binding gel were used to measure other trace metals in soils.

- DGT devices with both types of pore size were used to provide better information of the uptake of Hg from soils in a quick and effective way in comparison with other methods (i.e. sequential extraction) in terms of bioavailability.

- For the first time, inorganic Hg in soil samples was measured by the DGT technique with a pore size of less than 1 nm. As more amount of organic amendment was added, the percentage of inorganic Hg was reduced in the agricultural soil.

- Hg uptake by lettuce roots was successfully predicted by the DGT technique.

- The DGT technique was useful for predicting and comparing Hg uptake by lettuce roots through the addition of different organic amendments.

- Effective concentration (C_E) was calculated by a specific program (2D DIFS), which included the kinetic properties from the selected soil.

- Hg uptake by lettuce plants was significantly reduced by the addition of 3% of biochar.

- Significant correlations of Cr, Cu and Pb concentrations between C_E from DGT measurements with uptake by lettuces were demonstrated.

- The addition of 3% and 6% of biochar decreased significantly the uptake of Hg, Cr and Pb concentration by lettuces while the addition of 30% of compost reduced Cd, Cr, Cu and Pb in comparison with the unamended soil.

- Trace metal concentrations provided from the DGT technique and pore water were the best methods for predicting uptake by lettuces compared to sequential extraction and total metal concentration in soil.

Chapter 11

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