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Contaminants organiques (HAP, Me-HAP, PCB) en environnement: Etude de milieu naturel et de faisabilité de bioremédiation

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A dada et Maman……..

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Glossaire

POP : Polluants Organiques Persistants

HAP : Hydrocarbures Aromatiques Polycycliques

PCB : Polychlorobiphényles

MES : Matières en suspension

Me-HAPs : Methyl- Hydrocarbures Aromatiques Polycycliques

UNEP: United Nations Environment program

UNECE/CEE-ONU: United Nations Economic Comission for Europe/ Commission économique pour l'Europe des Nations unies,

EPER: European Pollutant Emission Register

US EPA: United States Environmental Protection Agency

DDT:Dichlorodiphényltrichloroéthane

Eau MilliQ : Eau ultrapure

GC-MS : Chromatographie en phase gazeuse – spectrométrie de masse

Résumé

La révolution industrielle, l'évolution technologique, la croissance démographique et l'urbanisation ont affecté non seulement l'économie ou la politique dans le monde mais également la santé environnementale. Divers types de polluants sont générés et introduits, de manière permanente, dans l'environnement via les effluents industriels, agricoles et/ou municipaux. Parmi ces polluants figurent les polluants organiques persistants ou POP qui sont susceptibles de développer une toxicité non négligeable pour la santé humaine et le bon fonctionnement de l'écosystème. En plus, ils sont détectés dans tous les composants de l'écosystème global, y compris l'atmosphère, les ressources en eaux, les sols, les sédiments et les biotes.

Depuis la découverte des insecticides (DDT) dans les tissus humains, le sort et le comportement de ces polluants dangereux ont retenu l'attention de la recherche scientifique. Dans le présent travail, on s'intéresse essentiellement aux POP du type hydrocarbures aromatiques polycycliques (HAP) et ses dérivés méthylés (Me-HAP) ainsi que les polychlorobiphényles (PCB). La première partie de l'étude est consacrée à l'évaluation de leur niveau de contamination dans les différents compartiments de l'environnement. Leur occurrence et distribution ont été évaluées dans les phases dissoutes, particulaires et sédimentaires du système d'eaux douces du bassin versant de l'Escaut dans la zone transfrontalière France-Belgique. Ceci étant dans le cadre du projet BIOFOZI, consacré à l'étude de la relation pollution-biodiversité, financé par la région Nord-Pas-de-Calais en partenariat avec la Fondation pour la Recherche sur le Biodiversité (FRB). Les mêmes investigations sont réalisées sur des sols originaires du Nord-Pas-de Calais (France) et de Madagascar. La seconde partie contribue à une étude de faisabilité de traitement biologique de sols contaminés par les HAP.

Mots clés : eau, sédiment, contamination, HAP, PCB, POP, écotoxicité, traitement

Abstract

The industrial revolution, technological change, population growth and urbanization have affected not only the economy and politics all around the world but also the environment. Various types of pollutants are generated and introduced permanently into the environment through industrial, agricultural and/or municipal discharges. These pollutants include the persistent organic pollutants or POPs which can develop dangerous effects to human health and the ecosystem. In addition, they are reported detected in all the environmental components including the atmosphere, water resources, soils, sediments and biota.

Since the discovery of the insecticides DDT in human tissue, the fate and behavior of these types of hazardous pollutants have caught the interest of researchers. In this work, the target contaminants are polycyclic aromatic hydrocarbons (PAHs) and their methylated derivatives (Me-PAHs) and polychlorinated biphenyls (PCBs). The first part of our study investigated the contamination level of theses POPs in the different compartments of the environment (water, suspended solid matters, sediment, soil). Their occurrences and distributions were evaluated in the dissolved, particulate and sedimentary phases of the freshwater system of the watershed of the Scheldt in the border area between France and Belgium. This work is included in the "BIOFOZI" program financed by the “Région Nord-Pas-de-Calais” in partnership with the “Fondation de Recherche sur la Biodiversité (FRB)”. An ecotoxicological risk assessment was also carried out. The same investigations were also conducted in soil samples originated from Northern France and from Madagascar. The second part of the present work concerns a feasibility study to eliminate PAHs in soil by bio-treatment (bioremediation).

Keywords: water, sediment, contamination, PAHs, PCBs, POPs, ecotoxicity, bioremediation

Introduction générale

La pollution environnementale joue un rôle critique sur la vie humaine et est devenue une des préoccupations majeures à l'échelle mondiale depuis quelques années. La révolution industrielle et technologique, la croissance démographique et l'urbanisation sont devenues des facteurs déterminant sur la qualité environnementale. La mauvaise gestion des déchets toxiques durant des décennies a causé la multiplication de sites pollués. Les polluants sont introduits de manière permanente dans l'environnement via les rejets industriels, municipaux et agricoles. Tous les composants de l'écosystème global, y compris l'atmosphère, les ressources en eaux, les sols, les sédiments, les biotes deviennent des récepteurs de pollution provoquant ainsi à leur détérioration progressive.

Certains composés chimiques ont leur origine naturelle mais les teneurs excessives devenues problématiques pour l'environnement sont généralement à cause des activités anthropogéniques. Dans l'environnement, on peut distinguer divers types de polluants à savoirs (i) les polluants organiques tels que les pesticides, les polychlorobiphényls, les phtalates et bisphénols issus de produits en plastiques et emballages alimentaires, les médicaments et bien d'autres, (ii) les polluants inorganiques tels que les nitrates, les sels, les métaux, (iii) les polluants organométalliques tels que les méthyl-mercure, tetraethyl de plomb, (iv) les acides, (v) les produits radioactifs comme le radium, le radon et l' uranium (vi) les polluants biologiques comme les virus, microorganismes et pollens.

Ces dernières décennies, diverses études et investigations ont été menées afin d'identifier leur potentielles sources, leur sort et transport, leur mode de dispersion dans les compartiments de l'environnement (air, eau, sédiment, sol) et également afin d'étudier leurs impacts sur leur milieux récepteurs y compris les êtres humains.

Dans ce travail de thèse, je m'intéresse plus particulièrement aux sorts et comportement de trois types de POP dont la famille des Hydrocarbures Aromatiques Polycycliques (HAP) et leurs dérivés méthylés (Me-HAP) ainsi que la famille des polychlorobiphényles (PCB) dans l'environnement aquatique et dans les sols.

Les travaux sont réalisés dans le cadre d'une thèse en cotutelle entre l'Université de Lille 1-Sciences et Technologies (Lille, France) et la Faculté des Sciences de l'Université d'Antananarivo (Madagascar), financés par le projet ERASMUS MUNDUS ACP II et le

projet « BIOFOZI » financé par la Région Nord-Pas-de-Calais en partenariat avec la Fondation pour la Recherche sur la Biodiversité (FRB) ". Ces travaux de recherches ont été menés au sein de l'équipe « Chimie Marine » du laboratoire Géosystèmes UMR 8217 désormais devenue l'« équipe physico-chimie de l'environnement » après intégration au laboratoire LASIR UMR 8516 depuis janvier 2015. Différents acteurs ont également contribué à la réalisation de ce présent travail à savoir les équipes microbiologistes du laboratoire ProBioGEM de l'Université de Lille (France) ainsi que celles au laboratoire de microbiologie de l'eau du Centre National de Recherche sur l'Environnement (CNRE, Antananarivo-Madagascar).

Ce travail considère les systèmes aquatiques d'eau douce et les sols. Les premiers objectifs étaient d'évaluer les niveaux de contamination des PCB, HAP et leurs homologues méthylés dans 15 points des rivières de l'Escaut puis d'estimer la qualité du milieu en se basant sur les normes communément établies. Cette partie s'inscrit dans le projet BIOFOZI dont son objectif était d'étudier les impacts et la corrélation entre la pollution et la biodiversité du milieu. Les niveaux de contamination de ces contaminants ont été aussi évalués dans les sols originaires de Nord Pas-de-Calais en France et d'Ambohimanambola à Madagascar. Le second objectif était d'identifier une technique de décontamination de sols pollués par les HAP par voie biologique (bioremédiation).

Ce manuscrit est divisé en trois parties. La première est dédiée à une synthèse bibliographique dans lequel je présenterai les généralités incluant le comportement et devenir des POP notamment les molécules d'intérêts (HAP, Me-HAP et PCB). Les normes en vigueurs ainsi que les différentes techniques de décontamination y sont également présentées. La seconde partie est consacrée à la présentation des matériels et méthodes présentant les étapes expérimentales adoptées pour mener à bien ces travaux. La dernière partie est réservée aux interprétations des résultats et discussions sous forme d'articles scientifiques. Une conclusion générale accompagnée de perspectives terminera ce travail.

Partie I. Synthèse bibliographique

I.1. Les Contaminants organiques dans l'environnement

I.1.1. Les polluants organiques persistants ou POP

Les POP sont des composés chimiques de synthèse et se distinguent de multitudes de substances chimiques par leur persistance, leur toxicité, leur capacité à être transportés à de très longue distance et à se bioaccumuler dans les organismes vivants (Jones and De Voogt, 1999). Ils peuvent également être transportés dans la chaine trophique et sont susceptibles de se bio-concentrer ou se bio-accumuler à partir du premier maillon de la chaîne alimentaire (à titre d'exemple : copépode, micro-algues) jusqu'aux mammifères dont les êtres humains. Cette capacité de bioaccumulation via la chaîne alimentaire engendre les effets néfastes non seulement sur le bon fonctionnement de l'environnement mais également sur la santé humaine (USEPA, 2009 ; Whylie et al., 2003). Les POPs sont des molécules hydrophobes et lipophiles d'où leur tendance préferentielle à s'adsorber dans la matrice solide que d'entrer dans la phase aqueuse de l'environnement. Dans l'organisme, ils ont une forte affinité avec les tissus lipidiques mais n'entrent pas dans la partie aqueuse des cellules de l'organisme. D'ailleurs, leur caractère persistant conduit à leur résistance aux processus de dégradation physique, chimique ou métabolique.

En effet, chaque polluant possède ses propres propriétés physico-chimiques qui contrôlent généralement leur devenir et comportement dans l'environnement. En d'autre terme, ces dites propriétés peuvent contrôler leur persistances, leurs dégradabilité dans l'environnement, la phase dans laquelle ils vont préférentiellement se concentrer (dissoute, gazeuse ou solide) ainsi que leur capacité à se bioaccumuler dans la chaîne trophique. Parmi lesquelles, ci-dessous sont citées quelques propriétés physico-chimiques importantes:

- *La solubilité aqueuse (S_W):* elle définit la capacité d'un composé à se dissoudre dans l'eau (caractère polaire). Elle est dite faible, moyenne ou importante si c'est l'ordre de µg/L, mg/L et g/L respectivement. Concernant les POP, ils sont faiblement hydrosoluble (fortement liposolubles) et peu biodégradables (très stables).

- *Le coefficient de partage carbone organique-eau (K_{OC}):* cette grandeur donne une indication sur l'aptitude d'un composé à être adsorbé ou désorbé sur la matière organique. Dans le sol (ou sédiment), cette tendance d'adsorption (ou désorption) dépend non seulement des propriétés physico-chimiques de la molécule mais également de la teneur et la nature du carbone organique dans le milieu. D'après Krauss and Wilcke (2003), certaines molécules

telles que les HAP et les PCB peuvent avoir différentes valeurs de K_{OC} selon la taille et la nature des particules. Par définition, c'est le rapport entre la quantité adsorbée du polluant par unité de poids de carbone organique du sol (ou du sédiment) et la concentration en ce même composé en solution aqueuse à l'équilibre.

- Le coefficient de partage octanol/eau (Log K_{OW}): l'utilisation de ce paramètre est un meilleur moyen, de façon indirecte, pour évaluer le potentiel d'un polluant organique à se bioaccumuler dans les tissus graisseux des organismes vivants. En effet, l'octanol est un solvant organique qui a des propriétés proches des membranes lipidiques des organismes vivants grâce à la similarité de leur polarité.

On peut prédire une bioaccumulation pour toutes substances ayant une valeur de log K_{OW} comprise entre 2 et 6. Les substances ayant un log $K_{OW}<2$ se bio-concentre[1] plus que celles ayant un log $K_{OW}>6$ (Connell, 1991 ; Kravitz et al., 2000). L'étude de Thomann (1989) a revelé qu'il y a une relation entre le K_{OW} et le potentiel de bioamplification[2] d'un composé et que l'adsorption augmente avec log K_{OW} jusqu'à un maximum de valeur de log K_{OW} entre 3 et 6 (selon la taille de l'organisme). Dans le cas où ce log $K_{OW}>6$, l'adsorption diminue. Pour les composés ayant un log K_{OW} entre 5 et 6,5, le phénomène de bioamplification peut avoir lieu via la chaîne alimentaire.

- *Le facteur de bioconcentration (BCF):* ce facteur est également très utilisé pour prédire le danger potentiel d'un produit. Il renseigne sur l'accumulation préférentielle des POP dans les organismes vivants via différentes sources d'exposition (eau, sédiment, sol) (USEPA, 2000). Généralement, on étudie la corrélation du BCF avec la S_W et log K_{OW} (Davis and Dobbs, 1984) mais on peut le calculer aussi à partir du coefficient de sorption de sol ou K_{OC} (Kenega, 1980). A titre d'exemple, dans le milieu aquatique, ceci est le ratio de la concentration du polluant dans la matière vivante ($C_{mat.vivante}$) sur celui dans l'eau (C_{eau}), d'où la formule ci-dessous:

[1] La bioconcentration désigne le phénomène qui va engendrer des concentrations dans les êtres vivants supérieures aux concentrations présentes dans le milieu

[2] La bioamplification (ou bioamplification) décrit le processus par lequel les taux de certaines substances croissent à chaque stade du réseau trophique (chaîne alimentaire).

$$BCF = \frac{C_{mati\,ère\ vivante}}{C_{eau}}$$

- *Le temps de demi-vie ($t_{1/2}$):* ce paramètre exprime la persistance ou résistance d'une molécule à la dégradation biologique (processus microbiens). Il désigne le temps nécessaire (heure, jour, mois ou même années) pour que la moitié de la concentration initiale d'une substance organique soit dégradée tout en dépendant du milieu considéré. (Rodan, 2002) Généralement, ce paramètre s'exprime par :

$$t_{1/2} = \ln 2/(kH + kB + kP)$$ (Sinkkonnen and Paasivirta, 2000)

Où k_H, k_B et k_P sont des constantes de vitesse de premier ordre pour l'hydrolyse (H), la biodegradation (B) et la photodégradation (P). Dans le cas des POP, le phénomène d'hydrolyse est extrêmement lent et k_H peut-être négligeable. Le constant k_B dépend de la composition de la population microbienne présente dans du milieu (eau, sol, sédiment, végétation) et dépend aussi de l'historique de la pollution locale.

I.1.2. Les polluants organiques persistants d'intérêt

Dans ce travail, je m'intéresse plus particulièrement aux trois grandes familles de polluants organiques dont les Hydrocarbures Aromatiques Polycycliques (HAP) et leurs dérivés méthylés ou Me-HAP ainsi que les Polychlorobiphényls (PCB). 16 molécules de HAP, celles classées comme substances prioritaires selon l'Agence de protection de l'environnement des Etats-Unis (US-EPA : United States Environmental Protection Agency); 18 molécules de Me-HAP et 28 congénères de PCB ont été étudiées. Chacune de ces famillesnde polluants est décrite comme suit :

I.1.2.1. Les HAP

Les HAP sont caractérisés par des structures chimiques complexes, constitués par l'assemblage de deux à plusieurs noyaux benzéniques (*Figure 1*). Ils sont dit légers ou de faible poids moléculaires lorsque le nombre de cycle aromatique existant est ≤ 3 tandis que ceux de cycle aromatique ≥ 4 sont catégorisés parmi les HAP lourds (ou de haut poids moléculaires). Ces composés peuvent exister naturellement suite à diverses réactions de biosynthèses par des organismes vivants. Ils peuvent être également générés par des activités anthropogéniques (industrie chimique, sidérurgie, incendies, moteurs à combustion, incinérateurs de déchets urbains, les transports et bien d'autres) (Yunker et al., 2002).

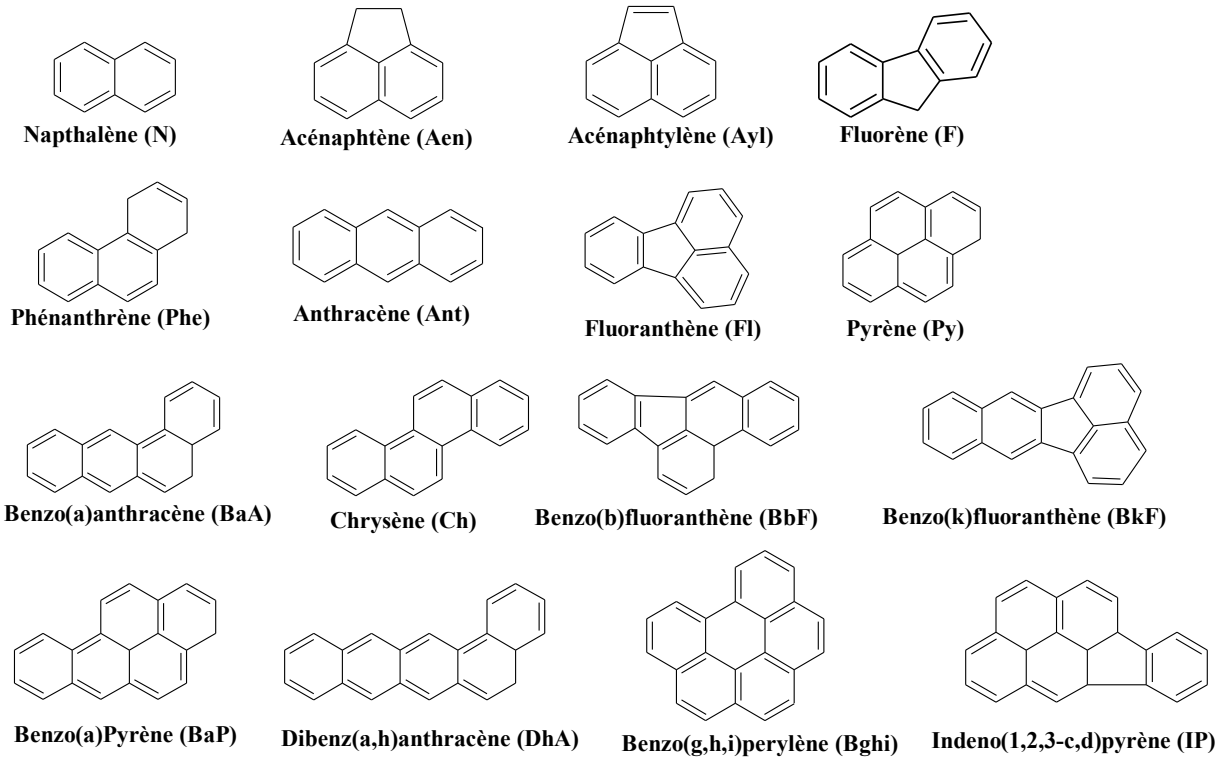


Figure 1: Structures chimiques des 16 HAP dans la liste prioritaires de l'US-EPA

I.1.2.2. Les Me-HAP

Les Me-HAP sont des composés de substitutions des HAP par le méthyl (Figure 2). Ils sont plus persistants et fréquemment plus toxiques que les HAP parents et leur toxicité augmente avec le nombre de substitutions sur le noyau benzénique. On les retrouve dans les pétroles bruts avec une forte proportion.

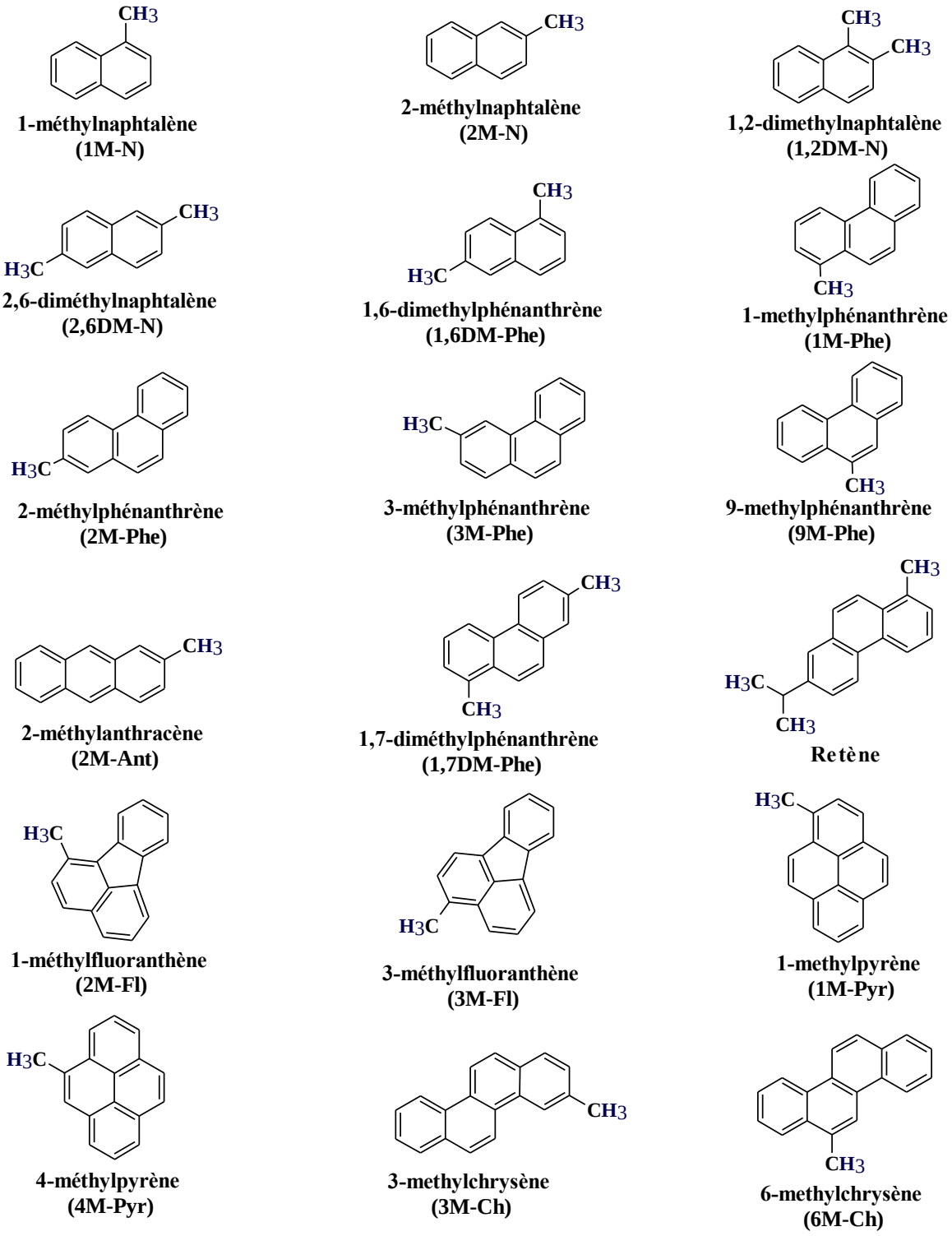


Figure 2: Structures chimiques des 18 Me-HAP étudiés

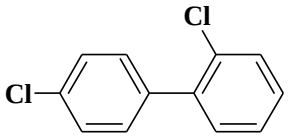
I.1.2.3. Les PCB

Les PCB sont des produits chimiques de synthèse, obtenus par chloration du biphényle et sont produits industriellement depuis 1930 sous forme de mélanges de congénères. Sur 209

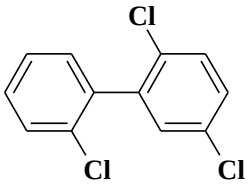
congénères enregistrés, ils se différencient entre eux par le nombre et la position des atomes de chlore sur la molécule de biphényle. Ce fait détermine ensuite leurs propriétés fondamentales et leurs activités biologiques. Ces congénères sont communément désignés par CB suivi d'un numéro correspondant à leur structure selon la nomenclature proposée par Ballschmiter et Zell (1980) tels indiqués sur la Figure 3 des 28 congenères d'intérêt.

Ils sont caractérisés par de grandes stabilités chimiques et thermiques et ont également une capacité diélectrique élevée. Ils ont été utilisés comme fluides diélectriques dans les transformateurs et les condensateurs à grandes capacité, comme liquide de refroidissement, comme additifs dans les peintures, … Ils sont incolores ou jaunâtres (selon le nombre de chlore) et ont de fortes odeurs aromatiques.

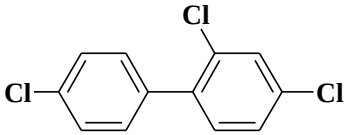
Quelques paramètres physico-chimiques (solubilité, coefficient de partage octanol/eau et coefficient de partage carbone organique/eau, …) de toutes ces molécules d'intérêt sont cités dans l'annexe I.1. D'après les données, les S_W diminuent avec le nombre de cycle aromatique pour les HAP. Pour les Me-HAP, les solubilités sont ≤0,31 mg/L à l'exception de 1-méthylnaphtalène (1M-Na ; $S_{W=}$28 mg/L) et de 2-méthylnaphtalène (2M-Na; $S_{W=}$25 mg/L) tandis que pour les PCB, les valeurs ne dépassent pas 1,2 mg/L. Ainsi, leur hydrophobicité augmente avec le nombre de cycle aromatique (dans le cas des HAP) et le nombre de chlore dans la molécule (dans le cas des PCB). Les valeurs des log K_{OW} sont presque elevées et varient de 3,3 à 6,84; de 3,87 à 6,35 et de 5,24 à 8,27 pour les molécules de HAP, Me-HAP et PCB respectivement. Quant aux valeurs de log K_{OC}, elles varient de 3,15 à 6,74 et de 4,57 à 6,4 pour les HAP (à l'exception de l'acénaphtylène) et pour les PCB selon les données disponibles dans la littérature.



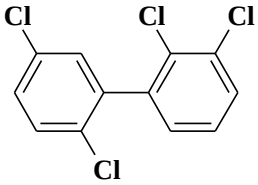
2,4'-Dichlorobiphényle (CB8)



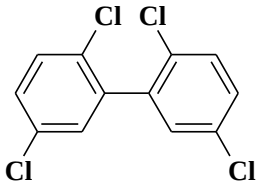
2,2',5-Trichlorobiphényle (CB18)



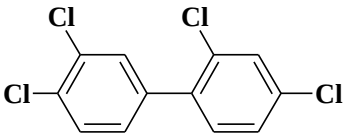
2,4,4'-Trichlorobiphényle (CB28)



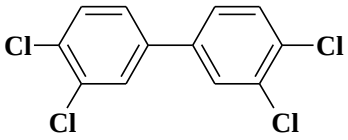
2,2',3,5'-Tetrachlorobiphényle (CB44)



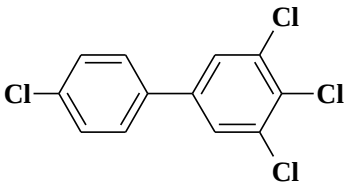
2,5,2',5'-Tetrachlorobiphényle (CB52)



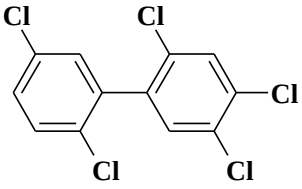
2,3',4,4'-Tetrachlorobiphényle (CB66)



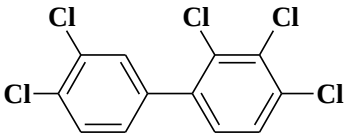
3,4,3',4'-Tetrachlorobiphényle (CB77)



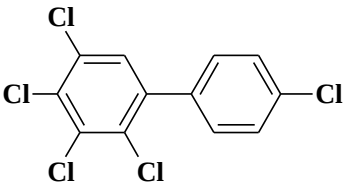
3,4,4',5-Tetrachlorobiphényle (CB81)



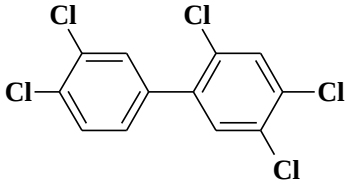
2,4,5,2',5'-Pentachlorobiphényle (CB101)



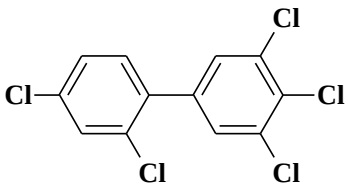
2,3,3',4,4'-Pentachlorobiphényle (CB105)



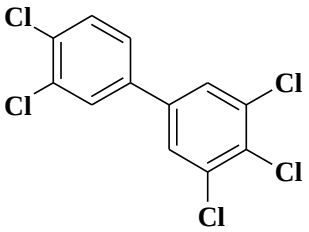
2,3,4,4'5-Pentachlorobiphényle (CB114)



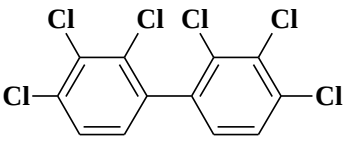
2,3',4,4',5-Pentachlorobiphényle (CB118)



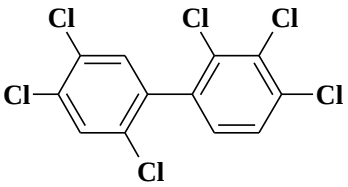
2,3',4,4',5'-Pentachlorobiphényle (CB123)



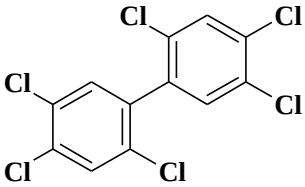
3,4,5,3',4'-Pentachlorobiphényle (CB126)



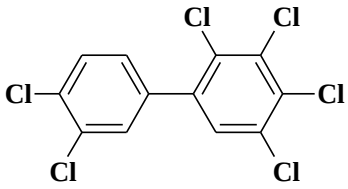
2,3,4,2',3',4'-Hexachlorobiphényle (CB128)



2,2',3',4,4',5-Hexachlorobiphényle (CB138)



2,4,5,2',4',5'-Hexachlorobiphényle (CB153)



2,3,3',4,4',5-Hexachlorobiphényle (CB156)

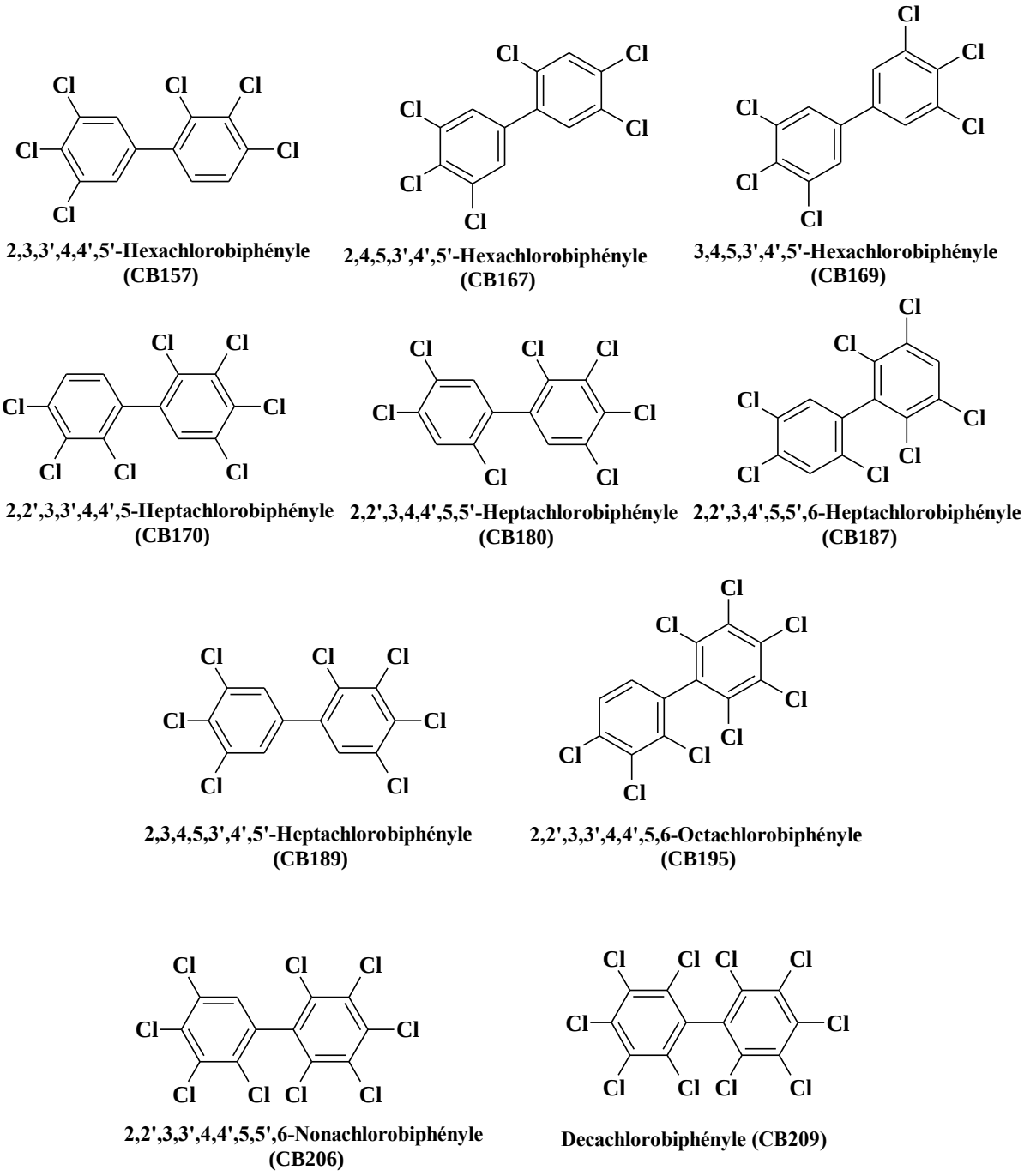


Figure 3: Structures chimiques des 28 PCB congenères étudiés

I.1.3. Sources dans l'environnement

Les HAP, Me-HAP et les PCB sont des micropolluants pouvant-être introduits dans l'environnement soit par apports diffus (lessivage des sols, retombé atmosphérique) soit par apports ponctuels (rejets industriels et les rejets municipaux). Certains produits ont des origines naturelles mais les teneurs problématiques trouvées dans l'environnement sont essentiellement liées à des origines anthropogéniques (Mehmetli & Koumanova, 2007).

Dans l'environnement aquatique (océan, eau de mer, eau douce), le dépôt atmosphérique est considéré comme principale source de contamination en plus des déversements directs (i.e., ruissellement urbain, effluents d'eaux usées, exutoires industriels) dans les rivières réceptrices (Qiao et al., 2014). Dans l'atmosphère, les micropolluants s'y introduisent via les émissions volcaniques ou les feux de brousses comme des sources naturelles et également via les émissions industrielles et les incinérateurs comme sources anthropiques. Pour les HAP et PCB produits par processus de combustion, ils entrent la phase gaseuse et particulaire. Pour les PCBs, étant banis depuis plusieurs années, leurs présences dans l'atmosphère peuvent provenir de la volatilisation à partir des surfaces sur lesquelles ils ont été utilisés, stockés ou deversés (Samara, 2007). Dans les sols et les sédiments, ils peuvent provenir des rejets municipaux, industriels ou encore agricoles.

Naturellement, les HAP sont issus de la combustion de biomasse, des éruptions volcaniques et également issus des processus diagénétiques. Leurs origines anthropiques constituent notamment la combustion des fuels, les eaux de rejets municipaux et industriels, les eaux de ruissellement ainsi que les émissions domestiques (feu de cheminé), industrielles et véhiculaires. L'origine de HAP peut être évaluée et classée soit « pyrolytique » soit « pétrogénique » selon le taux de présence des molécules HAP comparé à celui des homologues alkylés (Me-HAP) présents. En effet, l'origine est dite pyrolytique lorsque la concentration des HAP présents se trouve être élevée comparée à celle des Me-HAP; dans le cas contraire, l'origine est pétrogénique. Dans le milieu urbain, les véhicules sont cités parmi les principales sources de HAP (Zhang et al., 2008). Les effluents de raffinerie petroliers comportent des composés aromatiques polycycliques d'origine pétrogéniques (Dsikowitzky et al., 2011). Les produits diesel/biodiesel contribuent à la formation des HAP et des Me-HAP (Casal et al., 2014).

Concernant les PCB, ils ont été utilisés massivement entre 1930 et 1970 et commercialisés sous forme de mélange, principalement caractérisés par leur degré de

chloration. Parmi les gammes existants, on peut distinguer les Aroclor 1221, 1232, 1016, 1242, 1248,1254, 1260,1262. Bien que leurs utilisations et leurs fabrications aient été bannies depuis les années 70s, des teneurs en PCB restent encore detectés, actuellement, dans les différents compartiments de l'environnement (atmosphère, eau, sédiment, sol) et aussi dans les organismes vivants (Arias et al., 2015; Net et al., 2015a,b; Merhaby et al., 2015). La quantité totale de PCB non-détruits et stockés reste inconnue jusqu'à présent. Tant les PCB commerciaux que ceux extraits de l'environnement, ils contiennent un mélange complexe de congénères (Robertson and Hansen, 2015).

I.1.4. Comportement en milieu naturel

L'environnement peut être subdivisé en différents compartiments (air, eau de surface ou souterraine, sol, sédiment et biote) et qui forment des systèmes interconnectés entre eux. Des échanges chimiques, physiques ou biologiques peuvent avoir lieu entre ces compartiments. Ainsi, les déchets/rejets des contaminants dans l'un de ces compartiments peuvent migrer dans d'autres. Le bon fonctionnement de l'écosystème peuvent être alors affecté ainsi que la santé humaine (USEPA, 2009). Il est alors important de connaître le mode de transfert ainsi que la forme de distribution d'un contaminant allant d'une phase à une autre.

D'un côté, les propriétés physico-chimiques propres (S_W, leur pression de vapeur, leur constante de Henry, log K_{OW} et log K_{OC}) des polluants contrôlent leur persistance dans l'environnement, la phase dans laquelle ils vont préférentiellement se concentrer (dissoute, gazeuse, solide) ainsi que leur capacité à se bioaccumuler dans la chaîne trophique (Dsikowitzky et al., 2001). D'un autre côté, leur retenue dans les phases environnementales peut dépendre également des propriétés individuelles des phases ainsi que des facteurs variables de l'environnement tels que la température et l'humidité.

Certaines molécules de HAP, Me-HAP et PCB sont assez volatiles et peuvent subir une volatilisation pouvant être suivie d'une redéposition (cas des PCB de faible chloration ainsi que les HAP ayant deux cycles aromatiques). En effet, selon la température environnementale, les molécules s'évaporent dans l'atmosphère; se redépose par gravité ou avec les précipitations (pluie/neige) sur la surface des sols ou des eaux. Dans le milieu aquatique, ce dépôt peut aboutir dans les sédiments (Wania and Mackay, 1996). Autre que cette voie, les POP peuvent également se lier avec les particules dans les effluents industrielles ou municipaux et parcourent également tous les éventuels processus de traitement pour arriver dans les eaux de surface puis se déposent également dans les

sédiments. Ainsi, la phase sédimentaire constituent un réservoir de contaminants après accumulation, pendant de longue durée, et servent de sources de pollution dans l'écosystème avec lequel elle est en interaction (Boonyatumanod et al., 2006). A part cela, les sédiments sont aussi extrêmement importants dans la chaîne alimentaire.

Ils peuvent se volatiliser du sol, des végétations, des plans d'eau pour ensuite contaminer l'air (Jones and De Voogt, 1999) et due à leur résistance aux réactions de décomposition dans l'air, ils peuvent s'éparpiller à de très longue distance avant de se redéposer.

Comme illustration, le shéma ci-dessous (Figure 4) décrit le flux global des POPs à travers les différents compartiments de l'environnement.

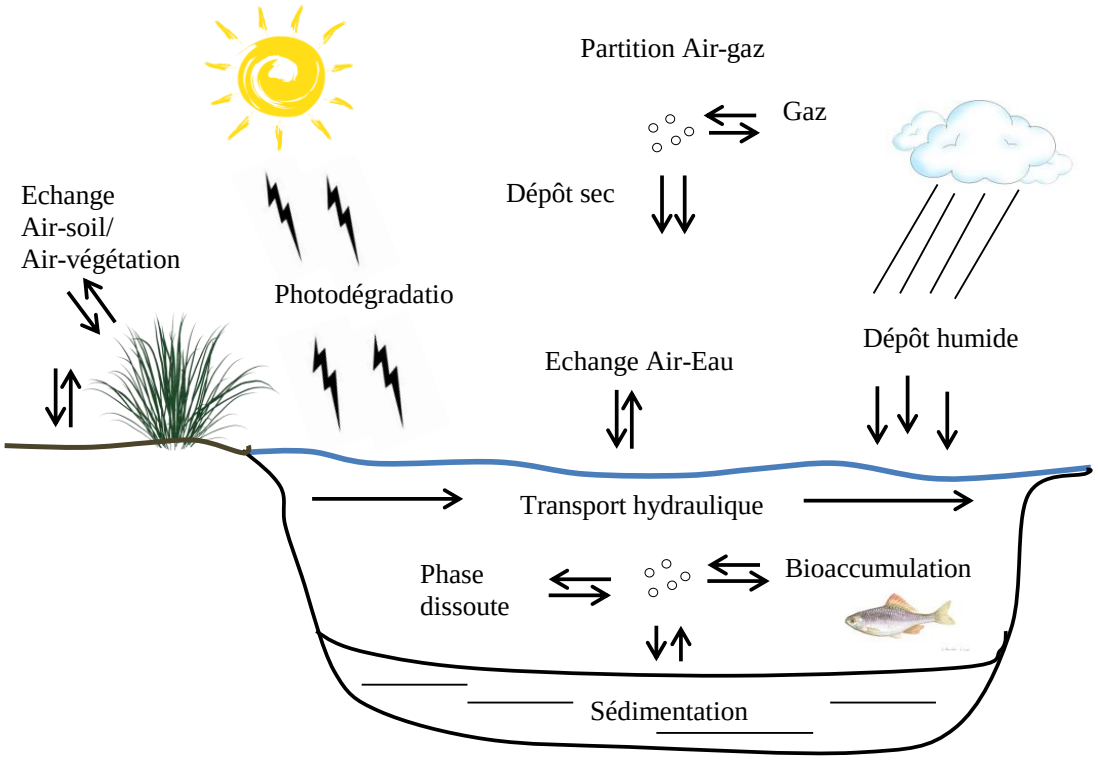


Figure 4: Flux global des POP à travers les différents compartiments environnemental (source : Jones, 2013).

I.1.4.1. Dans le système aquatique

Dans l'environnement aquatique, on peut distinguer la phase dissoute, particulaire (Skoog et al., 2003) et sédimentaire. La sorption et la partition des polluants se font selon les

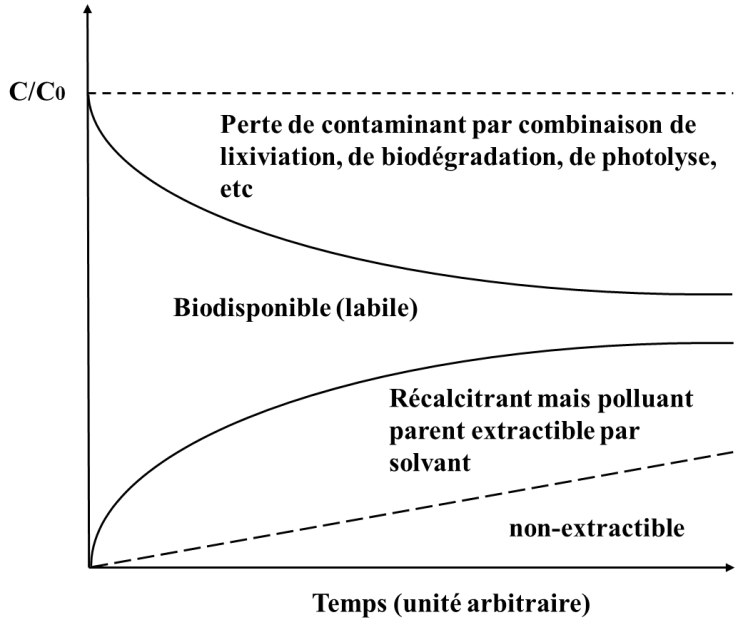
processus existant entre la colonne d'eau, l'interface du sédiment et la nature des matières organiques présentes. Dans l'eau, le transport des polluants organiques est associé à la mobilisation des particules en suspension (Douben, 2003 ; Chiou, 2003; Rugner et al., 2014). Les contaminants se fixent sur les particules organiques et inorganiques pour enfin se déposer au fond des lacs, rivières et des mers. Dans les sédiments, l'adsorption des POP dépend fortement des propriétés de chaque molécule ainsi que celles de l'adsorbant (le sédiment), de la concentration du contaminant, de la teneur en matière organique présentes, de la température et de la pression du milieu. Une fois que les contaminants se sont piégés dans les sédiments, ils deviennent moins biotransformables. Dans le cas des sédiments enciens, en plus de leurs propriétés physico-chimiques (lipophilicité et hydrophobicité), la concentration des polluants dépend également de leurs sources ainsi que de la propriété géochimique du sédiment (taille, porosité, capacité de sorption, teneur en matière organique). A titre d'exemple, les hydrocarbures sont rapidement et facilement adsorbés dans les sédiments de taille 50-500 µm. Neanmoins, des fractions de polluants piégées dans les sédiments pourraient éventuellement être libérées vers l'eau par dragages ou par d'autre processus de réhabilitation ou de remobilisation (Chiou, 2003). Quant à la désorption des polluants à partir des sédiments, ceci est généralement lente et les contaminants adsorbés y résident pour de très longues périodes (Burton et al., 2002).

Par ailleurs, les eaux interstitielles véhiculent principalement les contaminants dans les organismes aquatiques (Burton Jr, 2002). Les colloïdes facilitent aussi le transport des POP aux interfaces eau-sédiment. Plus un composé est hydrophobe plus il tend plus à se bioaccumuler dans les organismes aquatiques. Néanmoins, les microorganismes ne bioaccumulent que la fraction biodisponible des molécules.

I.1.4.2. Sorption dans le sol

Les PCB sont moins volatiles mais fortement lipophile, d'où plus de 99% de masse de PCB dans l'environnement se trouve dans le sol (Erickson, 1997). Le sol est composé de matières minérales et organiques. Dans les sols ordinaires, plus de 90% de la matière organique sèche est composée de carbone, d'hydrogène et d'oxygène avec quelques quantités mineures d'azote, de sulfure et de phosphore. Dans ce type de phase, les POP se partitionnent primairement dans les matières organiques. La volatilisation et la biodégradation sont parmi les processus influençant la concentration des POP dans le sol et plus les polluants restent piégés longtemps dans la matrice solide (effet de vieillissement de pollution) plus cela provoque la réduction de la fraction extractible voire même la non-exctractibilité de ces

polluants (Cornelissen et al., 1997). La Figure 5 ci-dessous constitue un diagramme conceptuel montrant les formes possibles des POPs dans le sol en mettant en evidence le domaine des proportions facilement, peu extractibles et non-extractibles en fonction de temps. Un minimum de changement de masse du sol pourrait certainement impacter le flux de concentration des POP dans les milieux adjacents (eau, air, etc). La propriété bio-accumulative des POP leur permet également de se transférer via la chaine alimentaire qui est un souvent élucidé dans le cas des organismes aquatiques mais concernant le sol, c'est plutôt les effets d'exposition (Jones and de Voogt, 1999).



Où **C** : concentration à t=o et **C0**: concentration à T=0

Figure 5: Diagramme conceptuel des formes possibles de POP dans le sol (Source: Jones and De Voogt, 1999)

I.2. Contamination mondiale des HAP, Me-HAP et PCB

La contamination de l'environnement par les HAP, Me-HAP et PCB est devenue une préoccupation à l'échelle mondiale. Des données sur le niveau de contamination des POPs ont été publiées suite à diverses investigations. Ces recherches sont faites dans les zones urbaines, portuaires ou dans les chantiers navals (Merhaby et al., 2015 ; Yim et al., 2014), dans des mangroves (Raza et al., 2013), des estuaires, des fleuves et rivières, des sols, voire même chez les êtres vivants (Patterson et al., 2009). Ces études ont revélé que la contamination diminue du milieu urbain au milieu rural. Dans les milieux ruraux, les principales sources de contamination sont les dépôts atmosphériques tandis qu'en milieu urbain, la contamination

provient plus des sources spécifiques locales (Krauss and Wilcke, 2003) comme les émissions industrielles et domestiques, les émissions véhiculaires, etc…

A titre informatif, selon l'Agence américaine pour la protection environnementale (US EPA), 40% des sites aux Etats-unis sont co-contaminés par les métaux et les contaminants organiques (Sandrinn & Hoffman, 2007). En 2000, selon le Registre européen des émissions de polluants (EPER), l'émission de PCB dans la région de la communauté économique européenne (CEE) s'élève à 31 016 kg.an^{-1} en Russie et 13 380 kg.an^{-1} en France (Vestreng, 2006). L'apogée de l'industrialisation a été citée comme la principale raison d'augmentation des PCB dans l'environnement dans les années 70. Néanmoins, l'utilisation et la fabrication des PCB ont fait l'objet de réglementations très restrictives dans la plupart des pays industrialisés. En Amérique, les PCB ont été interdits en 1979 et en France en 1987 (Abarnou and Blanchard, 2000). Malgré cela, les PCB restent présents dans les anciens équipements électriques encore en usage. Ces POP ont été même détectés dans de nombreux organismes vivants tels que les algues, crevettes, poissons, moules, dauphins et bien d'autres espèces (Baumard et al., 1998 ; Le Bihanic et al., 2014 ; Nakata et al., 2014 ; Net et al., 2015). Comme illustration, la figure 6 ci-dessous montre la production mondiale des PCB de 1930 à 1993dont les Etats-unis contribuent majoritairement (50%) et 10% ont été produits par la France.

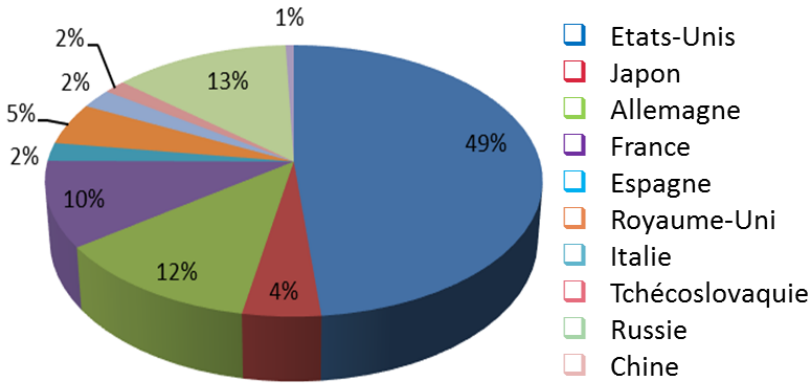


Figure 6: Répartition de la production mondiale des PCB entre 1930 à 1993 (Source : Jones and De Voogt 1999)

I.3. Toxicité et impact des HAP, Me-HAP et PCB

Pour de telles molécules hydrophobes, la bioconcentration et biomagnification sont des dangers potentiels des POP. Une fois que la concentration de contamination atteint un niveau où cela cause des effets néfastes sur les biotes, le milieu est considéré comme pollué (Burton et al., 2002).

En effet, Cette accumulation peut se faire même par interaction avec les molécules endogènes de l'organisme (ADN, métabolisme). En tout cas, l'accumulation des POP dans les organismes vivants peut se faire suivant l'un de ces trois processus: la *bioconcentration*, la *bioaccumulation* et la *bioamplification*. En effet, la bioconcentration est l'accumulation des polluants dans les organismes mais seule la matrice eau est prise en compte. Pour la bioaccumulation, il s'agit de l'accumulation des polluants dans les organismes vivants mais toute en tenant compte de toutes les voix d'accumulation (i.e l'accumulation par l'eau, la nourriture, le contact par la peau et l'inhalation par la respiration). Quant à la bioamplification (ou bioamplication), elle se réfère à la tendance des polluants de se concentrer autant qu'ils se déplacent depuis un niveau trophique vers un autre (augmentation en concentration de polluant depuis un lien dans la chaine alimentaire vers un autre) (Baumard et al., 1998). Les sédiments sont susceptibles d'accumuler une quantité excessive de contaminants qui vont affecter l'écosystème directement ou indirectement, et provoquent des contaminations significatives voire même la disparition des espèces utiles.

Pour les HAP, la propriété cancérogène est associée à la complexité de la molécule et augmente avec l'augmentation du nombre de noyaux benzénique. Le BaP est considéré comme le principal indicateur de tous les HAP cancérogènes, et le fluoranthène est considéré comme un indicateur complémentaire.

Pour les Me-HAP, les informations concernant leurs effets biologiques sont incomplètes. Néanmoins, les HAP substitués ont été montrés plus toxiques que les non substitués (Lindgren et al., 2014). L'étude menée par Vondracek et al. (2007) a démontré que le 2- et 9-methylanthracène ainsi que tous les méthylphénanthrènes se trouvent être des précurseurs de tumeurs. Dans l'environnement aquatique, les HAP sont plutôt présents sous forme de mélange, ce qui complique parfois l'évaluation des risques. Les risques potentiels engendrés par les mélanges de HAP et des alkyl-HAP ont été enregistrés sur les organismes aquatiques comme les poissons (Le Bihanic et al., 2014). Diverses anomalies morphologiques et génétiques (éclosion retardée, ADN endommagé, développement retardé, locomotion

perturbé) ont été constatées. La contamination peut aussi se transmettre via la chaîne alimentaire. Une étude faite sur les poissons zèbres, dont leur nutriment ont été exposé d'une manière chronique, depuis la phase post-fertilisation jusqu'à la phase adulte, a montré des perturbations morphologique (malformation), croissance inhibée, anomalies des activités enzymatiques intestinales et pancréatique. L'exposition à des mélanges de HAP peut provoquer des effets létaux ou sublétaux (Vignet et al., 2014a, b).

Pour les PCB, l'exposition peut survenir par inhalation, absorption ou ingestion via les chaînes alimentaires. Des organismes sont inévitablement exposés à des traces de PCB dans l'environnement. En effet, d'un côté, certains organismes peuvent accumuler ou produire des résidus chimiques sans être eux-mêmes menacés, mais leurs prédateurs souffrent de ces effets toxiques. D'un autre côté, à un certain stade de la vie d'un organisme, celui-ci peut tolérer et adsorber une certaine quantité de polluants qui pourraient lui être toxique seulement à long terme. Diverses investigations ont été menées pour comprendre le mode de bioaccumulation des POP dans les organismes aquatiques (Coudrec et al., 2015). L'ampleur de la bioaccumulation est conditionnée par l'hydrophobicité, ou la solubilité en phase aqueuses, du produit ainsi que de la faculté de l'espèce atteinte à éliminer le produit par excrétion et/ou métabolisation.

I.4. Normes en vigueur/législation

Des réglementations ont été établies par différentes entités et pays afin de fournir des bases numériques (valeurs seuils) qui servent à évaluer le niveau de contamination des POP dans l'environnement (air, eau, sol, sédiments et biote). Ces normes ne cessent d'être développées pour faire face à de nombreux problèmes environnementaux et répondre à des programmes de réglementation. Les paramètres chimiques utiles pour évaluation du danger d'un compartiment environnemental sont définis à partir des approches empiriques et statistiques. Suivant sont quelques lignes directrices adoptées dont chacune a ses propres forces et les limites.

I.4.1. La convention de Stockholm (CS)

La convention de Stockholm a été établie en mai 2001 par 152 gouvernements, dans le but de protéger la santé humaine et de l'environnement contre les POP. Cette convention est basée sur les principes de précautions nécessaires garantissant aussi bien l'élimination sûre de ces substances que la réduction de leur utilisation et de leur fabrication (Lallas, 2001; Denier

van der Gon et al. 2007). La convention a initialement définie 12 substances chimiques (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, toxaphene, PCB, hexachlorobenzene, dioxines et furanes) comme POP incluant les PCB. La convention a été entrée en force en mai 2005 et la plupart des 12 produits définis ont été bannis ou ont subi des restrictions sévères dans plusieurs pays. Malgré cela, bon nombre de ces produits sont encore en usage dans certains pays et des stocks périmés de POP existent encore (Ilyina, 2007). De plus, certains produits sont encore présents dans l'environnement dû à la forte persistance. Selon la convention de Stockholm, la persistance d'un produit chimique est basée sur 2 mois dans l'eau et 6 mois dans le sol ou sédiment.

I.4.2. La Convention sur la Pollution Atmosphérique Transfrontière à Longue Distance (CPATLD ou LTRAP, Long-Range Transboundary Air Pollution)

Cet accord a été également adopté sous l'égide de l'organisation des nations unis et de la commission économique européenne (CEE-ONU), rentré en force en Octobre 2003 dont le but est de contrôler la dispersion de certains produits chimiques dans l'environnement (Convention on Long-range Transboundary Air Pollution of the UNECE : http://www.unece.org/env/lrtap/welcome.html, visité le 21 octobre 2015). Le protocole d'Aarhus y est inclus et concerne plus particulièrment les POP dont les HAP y figurent également.

I.4.3. Les Seuils de qualité des sédiments (ou « Sediment Quality Guidelines SQGs)

Cette approche est communement appliquée pour aider à évaluer la qualité écotoxicologique d'un milieu contaminé par des polluants organiques. Ces « SQGs » ont été établis par différentes entités de différents pays en utilisant différentes approches (Long et al 1995; Long and MacDonald 1998 ; MacDonald et al 1996). Ces dernières sont de natures et structures différentes mais ont, par contre, les mêmes principaux objectifs qui consistent à évaluer la qualité de l'environnement ainsi que de veiller sur la pérennisation/entretien de bon état écologique d'un milieu. En d'autre termes, les SQGs se rapportent à:

- L'évaluation de la probabilité d'effets indésirables liés à des scénarios de gestion
- La preuve de causalité entre les effets de la pollution et la pollution dans le sédiment
- L'accroissement de la confiance (réduction de l'incertitude)

Les SQGs diffèrent dans la manière dont ils déterminent les effets de seuil, mais beaucoup sont assez similaires. Elles sont basées sur l'occurrence des effets sur les macro-invertébrés

benthiques et les concentrations totales dans les sédiments. Ces approches fixent généralement deux seuils: l'un correspond à une valeur en dessous de laquelle des effets se produisent rarement et l'autre correspond à la valeur au-dessus de laquelle des effets sont susceptibles de se produire. Ces valeurs guides sont regroupées dans le tableau 2 ci-dessous.

Tableau 1: Les valeurs guides communément utilisées

Au-dessous de lesquelles des effets se produisent rarement	Au-dessus de lesquelles des effets sont susceptibles de se produire
LEL (Lowest effect level))	SEL (severe effect level)
TEL (threshold effect level)	PEL (probable effect level)
ERL (Effect range low)	ERM (effect range median)
MET (minimal effect threshold)	TET (toxic effect threshold)
TEC (threshold effect concentration)	PEC (Probable effect concentration)

I.4.3.1. Avantages/inconvénients/limites des SQGs

Les recommandations pour la qualité des sédiments sont majoritairement basées sur les fréquences de distributions et comptent pour l'impact de tous les composés chimiques présents mais n'établissent pas les cause et effet. Ces approches ont été démontrées utile pour prédire les effets biologiques dans beaucoup (mais pas tout) de systèmes d'eau douce (Long et al 1998; Ingersoll et al 1996 ; MacDonald et al 2000). Cependant, les SQGs ont des limites d'applications. Dans certains cas, les SQGs ont abouti à des faux résultats (positifs ou négatifs) dans la prédiction de toxicité ou non-toxicité d'un sédiment. En effet, un faux positif correspond à des sédiments qui ont une contamination dépassant les valeurs recommandés définissant leurs toxicités alors que pas d'effets toxiques ont été observés. Un faux négatif est le contraire, la contamination dans les sédiments est détectée inférieure à la valeur guide de SQGs, définissant ainsi leur non-toxicité pourtant ils sont bien toxiques pour les organismes aquatiques.

I.4.3.2. Utilisation des consensus-based SQGs

Due à des similarités entre ces approches, un consensus a ensuite été développé pour les unifier d'où les Consensus-Based SQGs ou CBSQGs (Doyle et al., 2003; Macdonald et al., 2000). Le CBSQGs ont pris en compte les effets de mélanges des polluants dans un milieu (Swartz, 1999). Différentes valeurs seuils sont alors inclues à savoir :

- La TEC (Threshold Effect Concentration): la concentration seuil en dessous de laquelle les effets néfastes sur les organismes vivants dans les sédiments ne devraient pas se produire.

- Le PEC (Probable Effect Concentration): la concentration seuil au-dessus de laquelle des effets néfastes sur les organismes vivants dans les sédiments sont susceptibles d'être observés.
- Le MEC (Midpoint Effect concentration ; MEC= (TEC+PEC)/2): la concentration à effet médiane qui est recommandée pour mieux interpréter l'impact potentiel des contaminants entre le TEC et le PEC (Doyle et al., 2003).

Tableau 2: Les différentes valeurs seuils des POP selon le « CBSQGs »

	Concentrations en µg/kg p.s		
Composés	CBSQGs TEC	MEC (TEC+PEC)/2	CBSQGs PEC
Naphtalène	176	369	561
Acénaphtylène	5,9	67	128
Acénaphtène	6,7	48	89
Fluorène	77,4	307	536
Phenanthrene	204	687	1170
Anthracène	57,2	451	845
Fluoranthene	423	1327	2230
Pyrene	195	858	1520
Benz(a)Anthracène	108	579	1050
Chrysène	166	728	1290
Benzo(b)fluoranthène	240	6,820	13400
Benzo(k)fluoranthène	240	6,820	13400
Benzo(a)pyrène	150	800	1450
Indeno(1,2,3-cd)pyrène	200	1,700	3200
Dibenz(a,h)anthracène	33	84	135
Benzo(g,h,i)perylène	170	1685	3200
ΣPAH	1610	12205	22800
2-methylnaphtalène[a]	20,2	111	201
ΣPCBs	60	368	676

[a]source: CCME, 1999

Ainsi, on peut évaluer le risque écotoxicologique d'un milieu et dresser la classe de qualité d'un site selon le niveau de contamination existant. La répartition des niveaux de contamination est illustrée dans la figure 7 suivant:

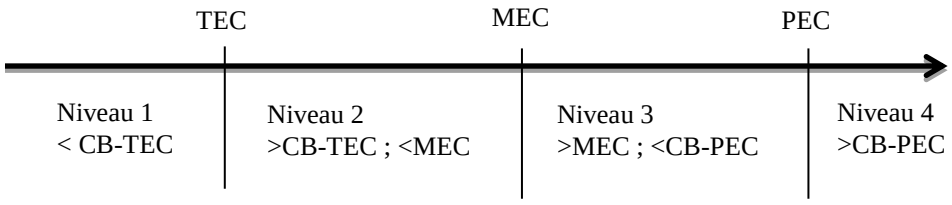


Figure 7: Les classes de niveau de contaminations des POP

I.4.4. La Directive Cadre sur l'Eau (DCE)

La Directive Cadre sur l'eau a été établi pour gérer les ressources en eau (les rivières, les lacs, les eaux souterraines et eaux littorales) sur le territoire européen. Les principaux objectifs de la DCE sont de restaurer le bon état écologique et chimique de toutes les ressources en eau dans toute la Communauté européenne pour 2015.

Ces objectifs de la DCE se repose sur :

- La prévention de détérioration supplémentaire mais plutôt sur la protection et l'amélioration de l'état des ressources en eau.
- La promotion sur l'utilisation durable des ressources en eaux
- Vise à renforcer la protection et l'amélioration de l'environnement aquatique par des mesures spécifiques pour la réduction progressive des rejets

Cette directive exige que tous les états membres doivent mettre en place une surveillance de près de la qualité des eaux et d'assurer les fréquences de contrôles en considérant les éléments de qualités listés dans la Directive n° 2000/60/CE du 23/10/00. Cet état de qualité écologique est surtout basé sur l'état biologique, hydromorpholigique et physico-chimiques des ressources. A titre d'exemple, dans les eaux côtières et estuaires, les éléments biologiques à considérer sont les phytoplanctons, les macro-algues, les benthos et les poissons. Ce bon état écologique de toutes les masses d'eau est défini si le taux d'utilisation des ressources renouvelable atteint le 40% pour satisfaire de débit écologique minimum.

Selon l'annexe V du Directive n° 2000/60/CE du 23/10/00 modifié par Directive n° 2008/32/CE du 11 mars 2008, Les états écologiques des ressources en eaux considérés (les rivières, lacs, eaux de transition et eaux côtières) peuvent-être classé par « Très bon », « bon » ou « moyen » et les eaux atteignant un état inférieur à l'état moyen sont classées« médiocres ».

Selon l'article 3, l'évaluation des risques comprend l'identification du danger, l'évaluation du rapport dose-effet et l'évaluation de l'exposition et la caractérisation du risques qui tient compte des effets toxiques potentiels non seulement sur la santé humaine mais également sur l'environnement (Annexe I, II et III).

La Directive n° 98/83/CE du 03/11/98 relative à la qualité des eaux destinées à la consommation humaine a pour objectif de protéger la santé des personnes des effets néfastes de la contaminations des eaux destinées à la consommation humaine en garantissant la

salubrité et la propreté de celle-ci. A titre d'exemple, dans le cas des HAP, il est indiqué que la teneur en HAP (Somme de benzo(b)fluoranthène, benzo(k)fluoranthène, indeno(1,2,3-c,d)pyrène et benzo(ghi)perylène) dans les eaux de consommation ne doit pas dépasser 0.10µ/L. Dans le cadre alimentaire, le composé Benzo(a)pyrène est un traceur pour tous les HAP (Wenzl et al., 2006). La Décision 2455/2001/EC désigne 33 classes de substances, qui nécessitent une surveillance de près par l'état membre de l'UE concernant les eaux de surfaces, des eaux souterraines ainsi que les eaux côtières (Roche et al 2005). Dans le cas des HAP, l'accent est mis sur 5 substances (BaP, benzo(b)fluoranthène, benzo(k)fluoranthène, indeno(1,2,3-c,d)pyrène et benzo(ghi)perylène).

I.5. Traitement

L'atténuation naturelle des polluants persistants dans les sites pollués est généralement lente voire même inhibée par le caractère récalcitrant des polluants. Différents types de techniques et technologies ont été développées afin de traiter les sites contaminés. Parmi lesquelles, il y a des approches chimiques, physico-chimiques ou thermiques mais l'approche biologique reste le plus souvent recommandée qui est une alternative de traitement faisable et rentable.

Certains micro-organismes ont été découverts comme polyvalents au catabolisme des molécules récalcitrantes et favorisent la transformation des polluants en des composés moins toxiques (dioxyde de carbone et eau) (Shuttleworth et al., 1995). La diffusion de l'oxygène est le mécanisme primaire dans le processus de biodégradation (Huesemann and Truex, 1996). Différentes espèces de bactéries ont été identifiées comme tolérantes au niveau de contamination élevé par les HAP à savoir les *Pseudomonas*, les *Bacillus* (Zafra et al., 2014), les actinomycete *Microbacterium sp*. (Salam et al., 2014). Les *Gamma-protéobactéries*, *Entérobactéries* et les genres *Pseudomonas* sont des bons bio-indicateurs pour déterminer le potentiel de dégradation des sites contaminés par les hydrocarbures (Lors et al., 2010). L'efficacité et la rapidité de la biodégradation varient notamment en fonction de la température, de l'aération du sol, de son humidité, de la biodisponibilité des nutriments, de la biodisponibilité des polluants et de la richesse microbienne (Romanstschuk et al., 2000).

Cependant, la biodégradation des micropolluants organiques peut être inhibée par la présence de métaux lourds qui sont toxiques pour les microorganismes et inhibent les activités microbiennes (activité enzymatique, transformation d'azote, génération de biomasse et bien d'autres). La complexation de ces métaux avec des ligands organiques tels que les protéines,

les acides nucléiques ainsi que les matériaux dans les parois cellulaires de microorganismes sont rarement utilisés dans le processus biochimique et conduisent à la toxicité (Sandrin and Hoffman, 2007). Certains métaux ont par contre des effets stimulateurs et que l'inhibition de la biodégradation n'apparait que sur certains populations de microorganismes particuliers (Singh and Tripathi, 2007). Les métaux tels que le cuivre, le zinc, le cadmium, le chrome (III et VI), le nickel, le mercure et le plomb sont connus comme inhibiteurs dans le processus de biodégradation.

La propriété de résister physiquement à des concentrations élevées des micropolluants comme les hydrocarbures est liée à la capacité de la bactérie à synthétiser des biosurfactants. Ces derniers protègent la bactérie du contact direct avec les hydrocarbures et lui permettent de les émulsionner en microgouttelettes plus rapidement assimilables. Généralement, les polluants sont présents dans l'environnement sous forme de mélange complexe. Certains micro-organismes ont la capacité d'utiliser un mélange de polluants suivant le phénomène appelé co-métabolisme. A titre d'exemple, pour le cas de HAP, une espèce *Mycobacterium flavenscens* est capable d'utiliser le fluoranthène en présence de pyrène alors que l'utilisation de pyrène est moindre en présence de fluoranthène qu'en son absence. De même, les espèces de *Rhodococcus* sont capables d'utiliser le fluoranthène en présence d'anthracène (Dean-Ross et al., 2002).

Dans cette étude, on va mettre plus en exergue la technique de bioremédiation dont les HAP ont été utilisé comme modèle. L'étude sur l'élimination des autres polluants d'intérêt (Me-HAP et PCB) est envisagée dans d'autres programmes de recherche à venir. Dans le cas des sols, il existe quelques de techniques de biotraitement telles que les suivantes:

I.5.1. Le compostage

Les sols contaminés par les hydrocarbures peuvent être traités par le compostage par ajouts de déchets biologiques (légumes, de fruits et de déchets de jardin) (Van Gestel et al., 2003), de coupeaux d'écorce (Jorgensen et al., 2000), ou de boue d'épuration (Namkoonga et al., 2002). Les matrices de compostage et le compost sont des sources riches en micro-organismes xénobiotiques dégradant tels que les bactéries, les actinomycètes et les champignons lignolytiques, qui peuvent dégrader les polluants et les transforment en composés inoffensifs tels que le dioxyde de carbone et l'eau (Semple et al., 2001). Selon Puglisi et al. (2007), le phénanthrène se dégrade mieux par compostage.

I.5.2. La Biostimulation et bioaugmentation

Ces techniques utilisent également des microorganismes. La biostimulation et bioaugmentation se diffèrent seulement par le fait que la biostimulation a recourt à une stimulation/acclimatation des microorganismes indigènes du site à dégrader tandis que la bioaugmentation consiste à l'introduction des microorganismes exogènes, préalablement sélectionnés, pour faire la décontamination. Pendant l'étape de biostimulation, des nutriments (source d'azote et de phosphore, pH ajusté, oligo-élements métalliques) sont fournis pour amorcer ces microorganismes indigènes. Dans la bioaugmentation, les espèces de microorganismes introduits vont agir ensemble avec la population microbienne existante dans le site à décontaminer (Kostecki, 1989).

Les conditions environnementales peuvent affecter la dégradation des micropolluants organiques dans le sol. Pour que la bioremédiation soit optimale, certains paramètres doivent être optimisés. Le tableau 3 suivant présente les conditions optimales favorisant la biodégradation .

Tableau 3: Les conditions optimales pour la biorémediation des contaminants organiques dans le sol, selon Wilson and Jones, (1993).

Paramètres	**Humidité (%)**	**Température (°C)**	**Nutriments**	**pH du sol**	**Taux en oxygène (% O_2)**
Valeurs optimales	30 - 90	20 - 30	C:N:P≈120:10:1	7 - 7,8	10 - 40

Partie II. Matériels et méthodes

Cette seconde partie est consacrée à la description des approches globales mises en œuvre au cours de la réalisation de cette thèse. Dans un premiers temps, les sites d'études sélectionnés sont présentés suivi par les méthodologies de prélèvement et d'analyse des micropolluants d'intérêt, dans l'eau en phase dissoute et, particulaire et sédimentaire, et dans le sol. Ensuite, les différentes étapes d'identification des possibles sources de POP dans les matrices environnementales étudiées sont détaillées. Enfin, je termine par la présentation de l'approche méthodologique utilisée pour l'étude de la faisabilité de bioremédiation de sol contaminé par les micropolluants organiques modèles. Quatre HAP, dont deux légers et deux lourds, sont sélectionnés comme molécules modèles de l'étude.

II.1. Présentation des sites d'études

II.1.1. Milieu aquatique : Eau, MES et Sédiment

Pour le milieu aquatique, plus de quinze sites dans les cours d'eau, rivières sont étudiés. Les niveaux de contamination des micropolluants organiques persistants (HAP, Me-HAP, PCB) sont déterminés dans trois phases à savoir la phase dissoute (eau après filtration), la phase particulaire (matières en suspension) et la phase sédimentaire. Pour le sol, plusieurs sites ont été étudiés à la fois en France et à Madagascar.

Pour l'étude dans la colonne d'eau (phase dissoute et particulaire) et de sédiments de surface, quinze sites localisés dans le bassin versant de l'Escaut, dans la région Nord-Pas-de-Calais, ont été considérés. Ces sites sont présentés sur la figure 7, l'étude s'inscrit dans le cadre du projet BIOFOZI financé par la Région Nord-Pas-de Calais en partenariat avec la Fondation de Recherche sur la Biodiversité (FRB). En effet, le Nord-Pas-de Calais est une région fortement urbanisée, densément peuplée avec une densité de 326 habitant/km^2 en 2001 (INSEE, 2014) et est historiquement une des régions les plus industrialisées en Europe (Lesven et al., 2009; Net et al., 2014a, 2015b).

Durant ma thèse, j'ai aussi contribué à l'étude d'évaluation des niveaux de contamination des HAP, Me-HAP et les PCB dans d'autres sites du Nord-Pas-de-Calais (Nord de France), en Picardie (Nord de France) et à Dakar (Sénégal, Afrique). L'accumulation des HAP, Me-HAP et les PCB dans l'eau, le sédiment et les biotes sont déterminée. Les sites d'étude et les résultats correspondant sont présentés dans les annexes. Autres que les trois polluants mentionés ci-dessus, j'ai également contribué à l'évaluation des

niveaux de contaminations d'autres contaminants organiques tels les phtalates, les pesticides et les résidus de médicaments.

Dans cette partie, l'accumulation préferentielle de polluants a été évaluée en étudiant le mode de répartition et le profil de distribution des molécules polluantes dans les trois phases du système aquatique. Ces trois phases s'agit de la phase dissoute, particulaire et sédimentaire. Ces études en parallèle sont effectuées pour l'intérêt de projet BIOFOZI. Les résultats sont présentés dans l'annexe 4.

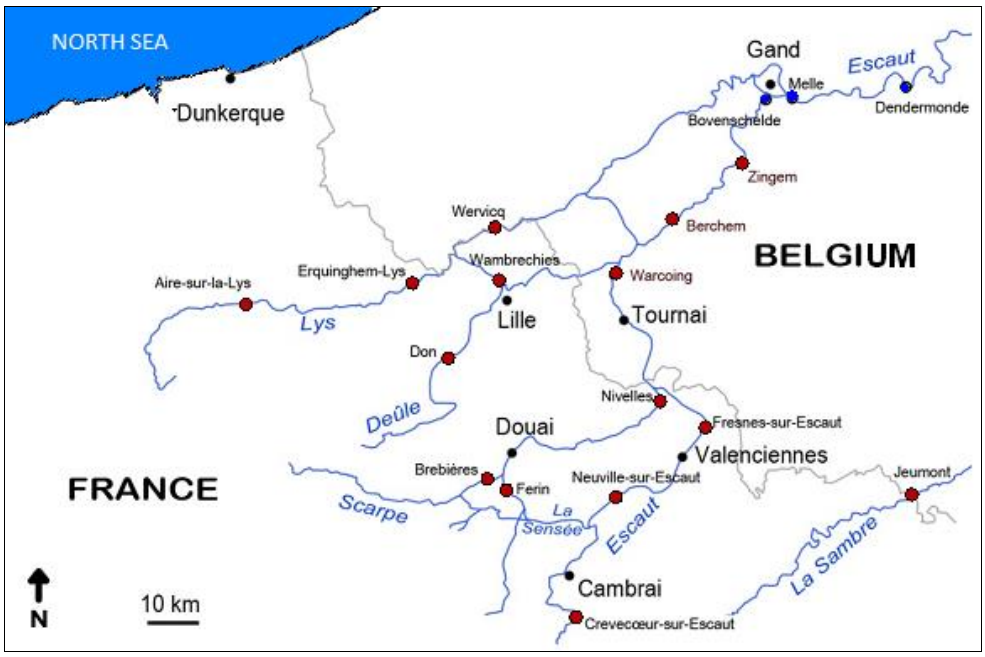


Figure 8: Les Quinze sites de prélèvement d'eau et de sédiment dans le bassin versant de l'Escaut dans le Nord-Pas-de-Calais en France et en Belgique.

II.1.2. Les sols

Pour l'évaluation des teneurs de HAP, Me-HAP et PCB sur les sols, plusieurs sites ont été sélectionnés : (*i*) à Madagascar, un site de rejet de fuel usagé d'une centrale électrique à Ambohimanambola (Figure 10), (*ii*) en France, un site situé dans une ancienne station d'essence à Flers-en Escrébieux dans le Nord-Pas-de-Calais (Figure 11), et un site situé sur le parking de campus universitaire de l'Université Lille 1-Sciences et Technologies à Villeneuve d'Ascq (Figure 12).

Figure 9: Site de prélèvement de sol, Antananarivo, Madagascar

Figure 10: Site de prélèvement, ancienne station d'essence à Flers en Escrebieux, France

Figure 11: Site de prélèvement, parking du campus de l'Université Lille1, France

II.2. Procédures d'échantillonnage

Les procédures d'échantillonnages pour chacune des matrices environnementales étudiées sont décrites séparément dans les sections ci-dessous.

II.2.1. Prélèvement d'eaux

Les échantillons d'eaux ont été prélevés en utilisant un préleveur en téflon ou en pyrex, préalablement décontaminé. L'eau prélevée est ensuite conditionnée dans des

bouteilles propres (lavées/décontaminées) en verres ambrés de 2,5 L puis transportées au frais (à 4 ± 2 °C) dans une glaciaire jusqu'à l'arrivée au laboratoire (Rabodonirina et al., 2015).

II.2.2. Prélèvement de sédiments

Des sédiments de surface (0-5 cm de profondeur) ont été prélevés des mêmes sites que précédemment. L'échantillonnage a été réalisé par carottage à l'aide d'un tube en polycarbonate de 10 cm de diamètre et 30 cm de longueur. Les carottes sédimentaires obtenues sont ensuite découpées, bien homogénéisées puis recueillies dans des barquettes en aluminium, préalablement calcinées à 550°C pendant une nuit, puis recouvertes de papier aluminium également pré-calcinés. Les échantillons sont conservés au frais dans une glaciaire jusqu'à l'arrivée au laboratoire.

II.2.3. Prélèvement de sols

L'échantillonnage de sol a été fait de manière représentative sur chaque site. A l'aide d'une benne, le sol est prélevé manuellement dans la partie rhizosphèrique[3] (10-15 cm au-dessous de la surface supérieure du sol) où se développent le plus les activités bactériennes. Les sols sont collectés dans des barquettes en aluminium, préalablement calcinés à 550°C pendant 12h, puis recouvertes de papier aluminium, également pré-calcinés à 550°C, avant d'être transportés au frais jusqu'au laboratoire. Les sols sont soient traités directement soient congelés à -20°C si les analyses prévues ne sont pas planifiées dans l'immédiat. Des équipements stériles (benne, spatules en inox, sachets) ont été utilisés pour le prélèvement des sols réservés aux analyses microbiologiques.

II.3. Préparation des échantillons

II.3.1. Cas d'échantillons liquides (phase dissoute)

Les échantillons d'eaux sont initialement filtrés sous vide au moyen d'un filtre en fibre de verre (GF/F Whatman, USA) ayant 47 mm de diamètre. Les matières en suspension (MES) retenues sur les filtres GF/F sont évaluées de manière gravimétrique qui seront destinées à

[3] La rhizosphère est la partie du sol caractérisée par sa richesse en micro-organismes (bactéries et champignons microscopiques).

l'étude dans la phase particulaire tandis que les filtrats vont servir de matrice pour l'étude dans la phase dissoute.

II.3.2. Cas d'échantillons solides (MES, sédiment et sol)

Les échantillons de sédiments et de sols sont séchés sous hotte aspirante, à l'air et température ambiants pendant quelques jours. Ils sont ensuite broyés et tamisés à 0,224 µm, puis stockés dans des contenants exempts de contamination organique, à l'abri de la lumière et au frais à -20 °C jusqu'à l'utilisation (échantillons utilisés pour la caractérisation des sites d'études et/ou comme sols pour l'étude de bioremédiation). Pour les MES, les filtres contenants les MES sont séchés puis coupé en petit morceaux avant l'extraction.

II.4. Extraction des polluants (HAP, Me-HAP et PCB)

Quelques étapes sont nécéssaires avant de pourvoir quantifier le niveau de contamination des micropolluants organiques (HAP, Me-HAP et les PCB) dans l'eau, les MES, le sédiment et le sol. La figure 13 ci-dessous présente un schéma simplifié des protocoles analytiques utilisés selon la nature de la matrice étudiée (liquide ou solide).

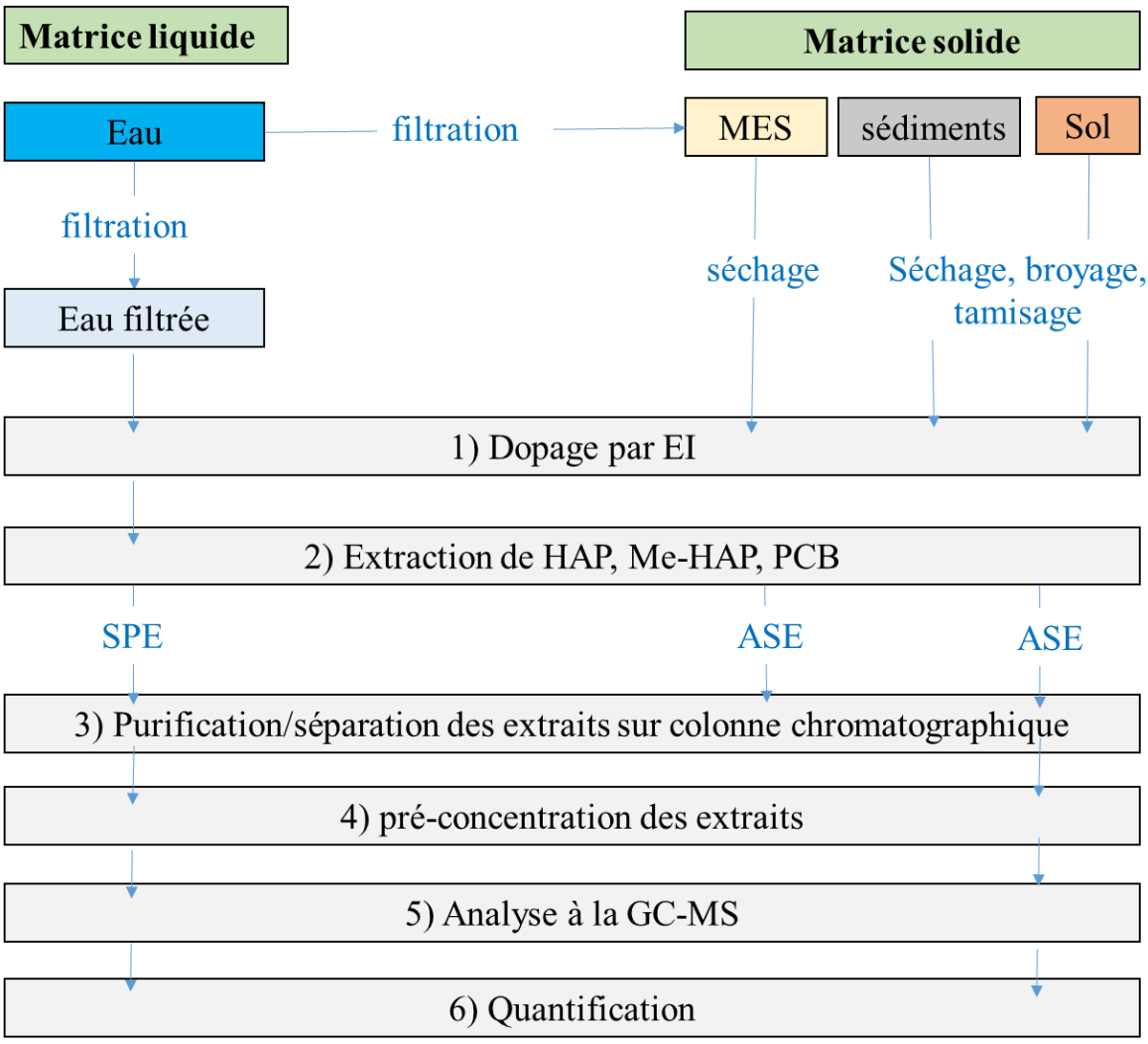


Figure 12: Les différentes étapes analytiques pour la quantification des niveaux de contamination des HAP, Me-HAP et des PCB dans les eaux, MES, sédiments et les sols.

II.4.1. Methode SPE : extraction dans une matrice liquide

Plusieurs techniques peuvent-être utilisées pour extraire les micropolluants organiques HAP, Me-HAP et PCB. Dans cette étude, on a opté pour l'extraction sur phase solide ou SPE (Solid Phase Extraction) en raison de ses nombreux avantages comparés aux autres techniques conventionnelles comme l'extraction liquide-liquide ou Soxhlet (figure 14). En effet, la SPE est une technique qui respecte de l'environnement (chimie verte), facile à mettre en œuvre, économique en solvants, rapide, semi-automatique et pluri-extraction possible. Dans cette étude, des cartouches spécifiques C-18 (StrataE, StrataM, and Supelclean Envi-18), en gel de silice greffé avec de l'octadécyle, ont été utilisées.

En premier lieu, les eaux filtrées sont dopées par des étalons internes (EI) (Tableau 4) et laissées au repos pendant une nuit. L'extraction est réalisée selon la méthode développée par Busetti et al. (2006). Brièvement, les cartouches sont initialement conditionnées séquentiellement par 9 mL d'acétonitrile, 12 mL de propan-2-ol et 12 mL de mélange eau MilliQ/propan-2-ol (85:15, v/v) acidifié à pH 2,5. Ensuite, 500 mL de l'échantillon est percolée à travers les cartouches. Puis, les cartouches sont lavés avec 30 mL de mélange eau MilliQ/propan-2-ol (85 :15, v/v) à pH 2,5 pour éliminer les interférences. Par la suite, les cartouches sont séchées sous flux d'azote pendant 15 min avant d'être éluées avec 12 mL de mélange d'hexane/acétone/propan-2-ol (95:5:5, v/v/v). Chaque extrait final est recueilli et pré-concentré pour avoir un volume final de 50-200 µL pour les analyses en GC-MS.

Tableau 4: Liste des étalons internes (EI) et leurs quantités utilisées pour le dopage des échantillons

EI	Ci	V dopé (µL)	Composés à doser
N-d8, A-d10, Phe-d10, Pyr-d10, Per-d12 (mélange)	100 ppm	35	HAP
N-d8, A-d10, Phe-d10, Pyr-d10, Per-d12 (mélange)	100 ppm	35	Me-HAP
TCN OCN CB112	236 ppm 250 ppm 100 ppm	20 20 35	PCB

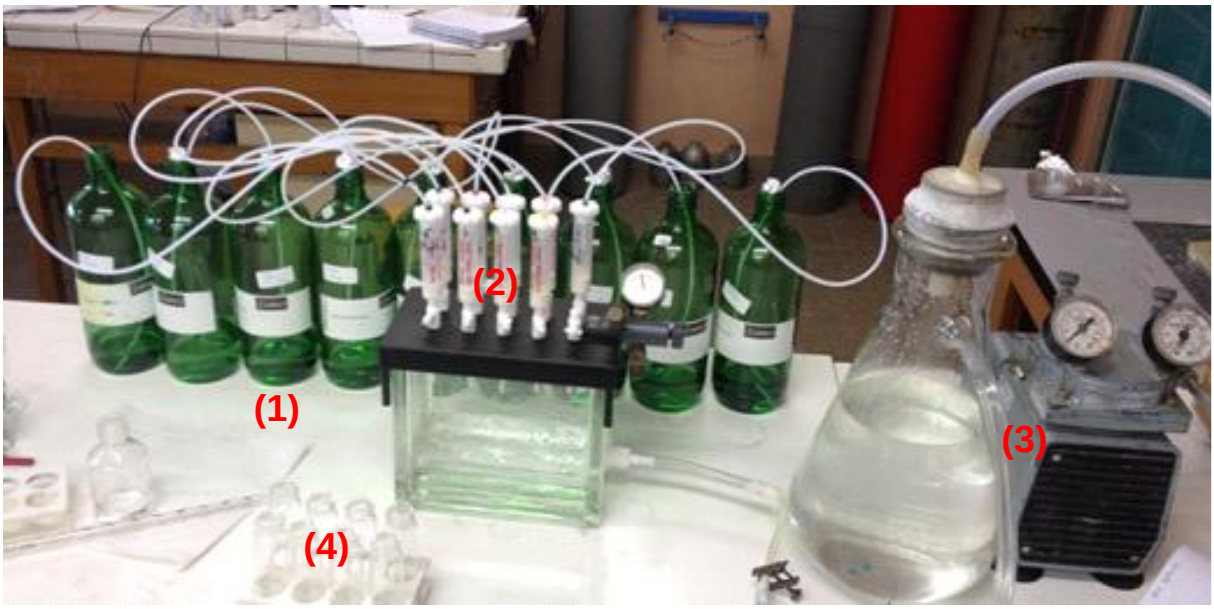


Figure 13: Montage du système d'extraction par SPE

Où (1) : bouteilles contenants les eaux filtrés dopées, (2) : les cartouches, (3) : pompe, (4) : solvant

II.4.2. Methode ASE: extraction dans des matrices solides

On a utilisé la méthode d'extraction ASE pour nos échantillons solides (MES, sédiment et sol) du fait qu'elle est fortement préconisée pour l'extraction des HAP, Me-HAP et PCB dans des matrices solides ou semi-solides. En effet, cette technique présente des avantages en comparaison aux autres techniques d'extraction du fait qu'elle est plus économique en solvants, plus rapide ce qui induit un coût d'utilisation plus faible. De plus, une bonne reproductibilité est obtenue avec l'ASE grâce à son automation.

Dans cette étude, on a utilisé le modèle ASE 200 (Dionex, USA) (figure 15). L'appareil est muni d'un four interne permettant de chauffer l'échantillon afin de faciliter l'extraction, notamment, par la diminution de la viscosité des composés présents dans les matrices solides, mais aussi par une meilleure pénétration des solvants dans ces matrices. Une pression élevée est égalemement appliquée pour que le solvant reste à l'état liquide lors de l'extraction à une température supérieure au point d'ébullition du solvant utilisé.

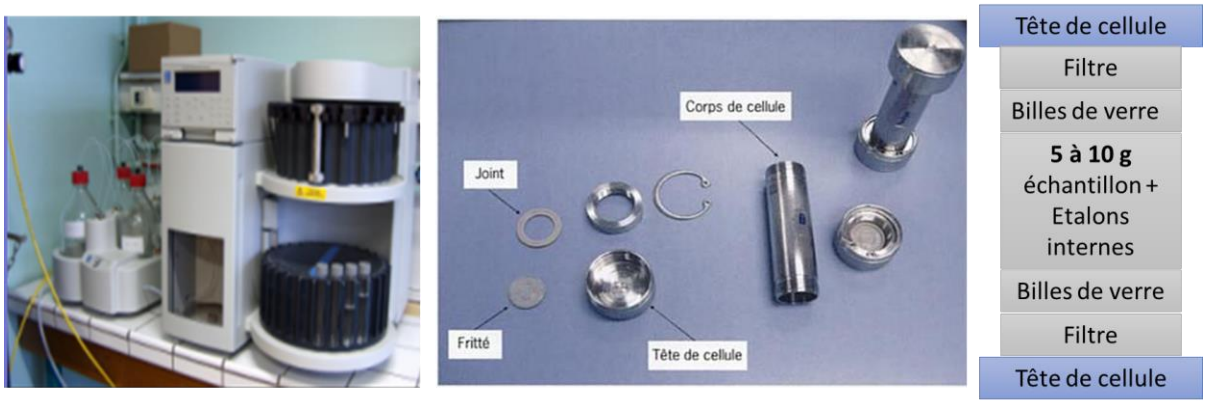


Figure 14: Préparation de la cellule d'extraction ASE

Pour les MES, les filtres sont coupés en morceaux avant d'introduire dans les cellules ASE. Pour les échantillons de sols ou de sédiments, une masse 5-15 g d'échantillons préalablement broyés et tamisés sont extraits. Des quantités connues de solutions d'étalons internes (EI), spécifiques pour la quantification des HAP, Me-HAP et PCB, ont été ajoutées. L'espace vide dans la cellule d'extraction est componsée par l'ajout de billes de verre préalablement propres et décontaminées. On laisse une nuit pour permettre une équilibration entre les EI et les matrices étudiées avant d'extraire.

Deux méthodes d'extraction ont été utilisées successivement. Les échantillons de sédiment ont été extraits selon la méthode de Tronczynski et al. (2005) tandis que les sols ont

été extraits selon la méthode développé par Net et al. (2014). Brièvement, ces conditions opératoires sont résumées dans le Tableau 5 ci-dessous:

Tableau 5: Conditions d'extraction ASE

	Tronczynski et al. (2005)	Net et al. (2014)
Solvant	Dichlorométhane	Hexane/acétone (1:1, v/v)
Temps de chauffage	5 minutes	0 minute
Température	100 °C	160 °C
Pression	138 bars	140 bars
Temps d'extraction	5 x 2 minutes / extraction	2 x 12 min
Volume du rinçage	35% pour chaque extraction	60%
Temps de rinçage	180 secondes	180 secondes

II.5. Purification des extraits

II.5.1. Désulfurisation

Les extraits contiennent un grand nombre de composés qui peuvent interférer durant le processus analytique. Dans un premier temps, des copeaux de cuivre activé sont ajoutés aux extraits afin d'oxyder le soufre moléculaire S8 sous la forme d'un précipité noir de CuS selon la réaction suivante : 8 Cu + S8 → 8 CuS (Blumer, 1957). Les extraits organiques sont ensuite transférés dans des tubes en pointe en verre puis concentrés à l'aide d'un évaporateur rotatif. Si besoin, les composés sont solubilisés à nouveau dans un peu de solvant à l'aide d'un bain à ultrasons. Le but est de concentrer chacun des extraits dans 1 mL d'hexane pour ensuite le purifier sur colonne chromatographique.

II.5.2. Séparation/purification sur colonne chromatographique

Chaque extrait issu de *II.4.* a été ensuite purifié et séparé sur colonne chromatographique réalisée à partir d'un lit de silice, d'alumine et de Na_2SO_4 mélangés avec de l'hexane. Les composés indésirables, tels que les acides humiques, acides fulviques, éventuellement co-extraits avec les molécules d'intérêt seront retenus.

Concrètement, le 1 mL d'extrait préconcentré est placé dans tête de la colonne chromatographique à l'aide d'une pipette pasteur. On procède ensuite a l'élution en ajoutant différentes proportions de solvant ou de mélange de solvant et les molécules d'intérêt sont récupérés dans des vials. Les molécules de PCB sont récupérées en éluant 20 mL d'hexane (Fraction 1). Quant les HAP et Me-HAP sont récupérés en éluant 15 mL de mélange

hexane/dichlorométhane (3:1 *v/v*) suivi de 15 mL de mélange hexane/dichlorométhane (1:1 *v/v*) (Fraction 2). Les deux fractions ont été recueillies ensembles pour assurer une quantification totale judicieuse. La figure 15 suivant montre les étapes de la séparation/purification des extraits sur colonne chromatographique.

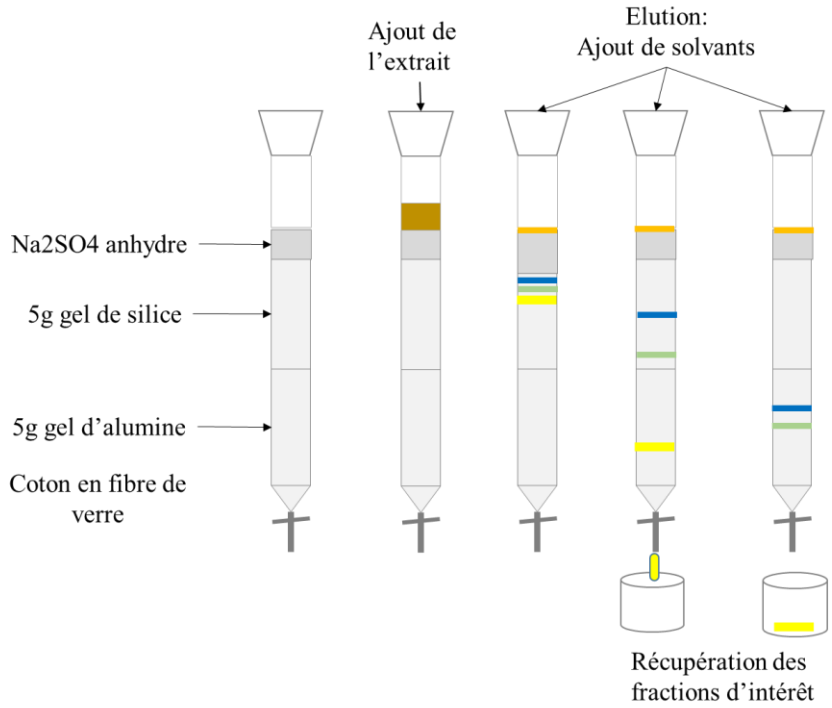


Figure 15: Les étapes de séparation/purification des extraits sur colonne chromatographique.

II.5.3. Pré-concentration des extraits

Les fractions issues de II.5.2. subissent ensuite une évaporation au moyen d'un évaporateur rotatif suivi d'un soufflé d'azote pour pré-concentrer jusqu'à 50-500 µL de volume final. Chaque extrait final est analysé à l'aide d'une chromatographie en phase gazeuse couplée à un spectromètre de masse (GC-MS). Les extraits sont conservés au frais (4 ± 2 °C) s'ils ne sont pas analysés dans l'immédiat.

II.6. Quantification des composes

II.6.1. Étalonnage interne

Dans la présente étude, le principe d'étalonnage interne a été utilisé. En effet, un étalon interne est une substance chimique de synthèse, n'existe pas dans l'environnement mais présente des propriétés physico-chimiques proches de analytes d'intérêt mais n'interfère

pas, en aucun cas, les étapes du processus analytiques. Ce principe étant utilisé dans le but de corriger les éventuelles pertes ou erreurs expérimentales de types aléatoires et/ou systématiques (skoog et al. 2003). Dans un premier temps, des gammes d'étalon de concentration croissante en HAP, Me-HAP et PCB allant de 0,1 à 5 µg/L sont préparés à partir de standards commerciaux. Ensuite, on y ajoute des quantités connues de chaque étalon interne (EI). Chaque gamme de concentration sera finalement analysée à la GC-MS. L'appareil fourni des signaux (chromatogrammes) sous forme des pics en fonction de temps de rétention et que chaque pic correspond à une molécule donnée. Chacune des molécules d'intérêts a un TR et m/z caractéristiques.

Les aires de pics de chacune des molécules sont ensuite intégrées afin de pouvoir tracer la droite d'étalonnage de la forme y=ax+b. En réalité, cette droite exprime le rapport des aires entre la molécule d'intérêt et l'étalon interne correspondant (y) en fonction du rapport de leur concentration (x) d'où l'équation suivante:

$$\frac{Ai}{Aei} = a\frac{Ci}{Cei} + b$$

Où : *Ai* et *Aei* sont les aires respectives de pic de l'analyte et celui de l'étalon interne ; *Ci* et *Cei* sont les concentrations respectives de l'analyte et celle de l'étalon interne ; *« a » est la* constante correspondant à la pente de la droite et *« b »* signifie l'ordonnée à l'origine.

Cependant, à chaque molécule correspond une équation caractéristique et la concentration finale de chacune des molécules de 16 HAP, 18 Me-HAP, 28 PCB dans un échantillon donné s'obtient de la manière suivante:

$$Ci = (\frac{Ai}{Aei} - b) * \frac{Cei}{a}$$

La figure 19 ci-dessous montre un exemple de droite d'étalonnage d'un dérivé méthylé du naphtalène (2-méthylnaphtalène).

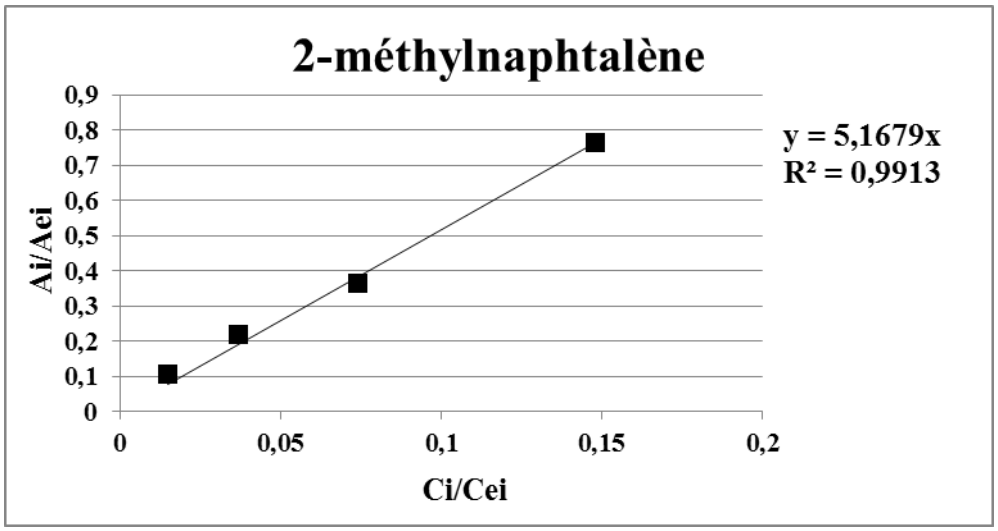


Figure 17: Exemple de droite d'étalonnage, cas du 2-méthylphénanthrène

II.6.2. Analyse en GC-MS

Chaque extrait final a été analysé au moyen d'une chromatographie en phase gazeuse (GC, Varian 3900) couplée avec un spectromètre de masse (MS, Saturn 2000) (figure 18). Les conditions opératoires utilisées lors des analyses des molécules d'intérêt sont rassemblées dans le tableau 6 ci-dessous.

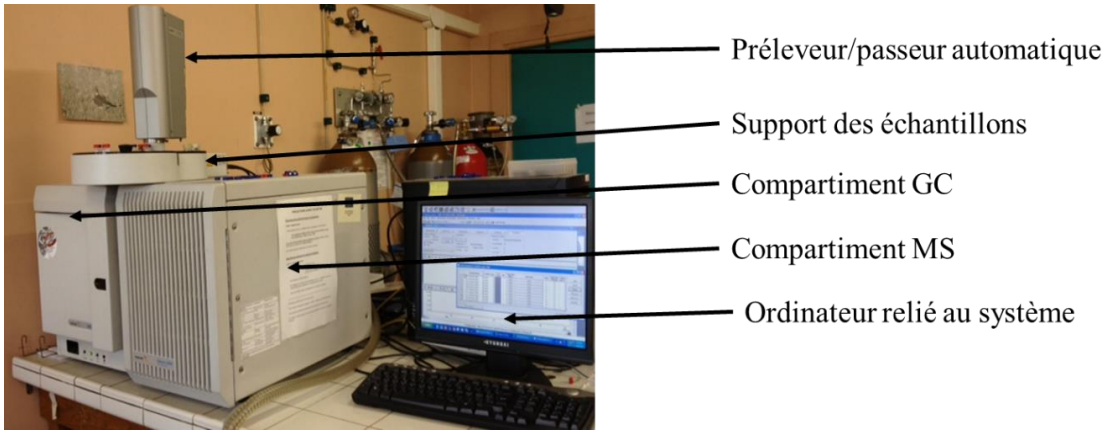


Figure 18: Présentation du système GC-MS

Afin d'identifier les molécules d'intérêt, des méthodes quantitatives précises ont été d'abord crées soient en mode SIS (Selected Ion Storage) ou MS/MS ou encore MRM (Multiple Residual Monitoring) pour des meilleures sélectivités et précisions. Cela se réalise à partir des solutions standards pures que l'on analyse à la GC en mode full scan (FS). Les

Temps de retention (TR) sur les chromatogrammes ainsi que les fragments caractéristiques (m/z) sur les spectres de masse obtenus pour chaque molécule sont ainsi repérés et qui vont servir de base de comparaisons pour les TR et m/z des échantillons.

En effet, les différents modes d'analyse sont détaillés comme suit:

- **FS** : toutes les molécules sont détectées à tout moment. Cela permet de déterminer les TR et les m/z relatifs aux composés que nous voulons étudier.
- **SIS** : une méthode SIS peut être crée seulement lorsqu'on connait préalablement les m/z et les TR de chaque molécule obtenus lors de l'acquisition en mode FS. Le mode SIS permet de stocker dans la trappe ou d'éjecter un ou plusieurs ion(s) ou une gamme d'ions spécifiques. Les ions matriciels indésirables sont ainsi éliminés, le bruit de fond est réduit et la sensibilité pour des échantillons complexes devient meilleure. Ce mode sélectif est plus adapté à l'analyse quantitative des HAP et des Me-HAP dans les extraits de sédiments.
- **MS/MS :** Il consiste à sélectionner un ion par une première spectrométrie de masse, puis à le fragmenter, et effectuer une deuxième spectrométrie de masse sur les fragments ainsi générés. Elle peut être réalisée à l'aide de nombreux appareils combinant des secteurs magnétiques, électriques, quadripolaires ou des temps de vol, mais également au sein d'un même analyseur dans le cas d'une trappe d'ions.
- **MRM :** Le principe de MRM est identique à celui du MS/MS. La seule différence est que le MRM permet de visualiser sur plusieurs canaux des pics qui ont les TR proches ou identiques. Il facilite ainsi le traitement de donnés chromatographiques.

La figure 18 montre un exemple chromatogramme du standard d'un mélange de 18 Me-HAP de concentration 0,5 µg/mL et un exemple de spectre de masse (m/z) caractéristique. Pour plus de sélectivité, la quantification a été faite en mode MS/MS ou MRM. Les temps de rétentions correspondant à chacune des molécules étudiées avec les ions quantificateur m/z sont résumés dans l'annexe I.

Tableau 6: Conditions opératoires pour l'analyse de HAP, Me-HAP et PCB sur GC-MS

GC-MS		Paramètres opératoires
Chromatographe Varian 3900		Passeur d'échantillons CP-8400
Injecteur		Type « Splitless »
Volume injecté		1 µL
Température d'injection		280 °C
Colonne capillaire silice fondue		ZB-XLB Phenomenex
Phase		si-arylene
Longueur		60 m
Diamètre interne		0,25 mm
Epaisseur du film		0,25 µm
Colonne de protection		Oui (en silice fondue 5 m, 0,25 mm diamètre interne)
Gaz vecteur		Hélium
Débit		1 mL/min
HAP et Me-HAP	Programmation du four	
	Température initiale de four	70 °C pendant 1 min
	1ère vitesse de programmation	10 °C/min
	1er palier de température	170 °C
	2ème vitesse de programmation	4 °C/min
	2ème palier de température	230 °C
	3ème vitesse de programmation	3 °C/min
	3ème palier : température finale, isotherme	300 °C pendant 13 min
PCB	Programmation du four	
	Température initiale de four	80 °C pendant 1 min
	1ère vitesse de programmation	10 °C/min
	1er palier de température	170 °C
	2ème vitesse de programmation	4 °C/min
	2ème palier de température	230 °C
	3ème vitesse de programmation	3 °C/min
	3ème palier : température finale, isotherme	300 °C pendant 19 min
Spectromètre de masse Saturn 2000		Quadripôle
Température de la trappe d'ion		220 °C
Température de la ligne de transfert		280 °C

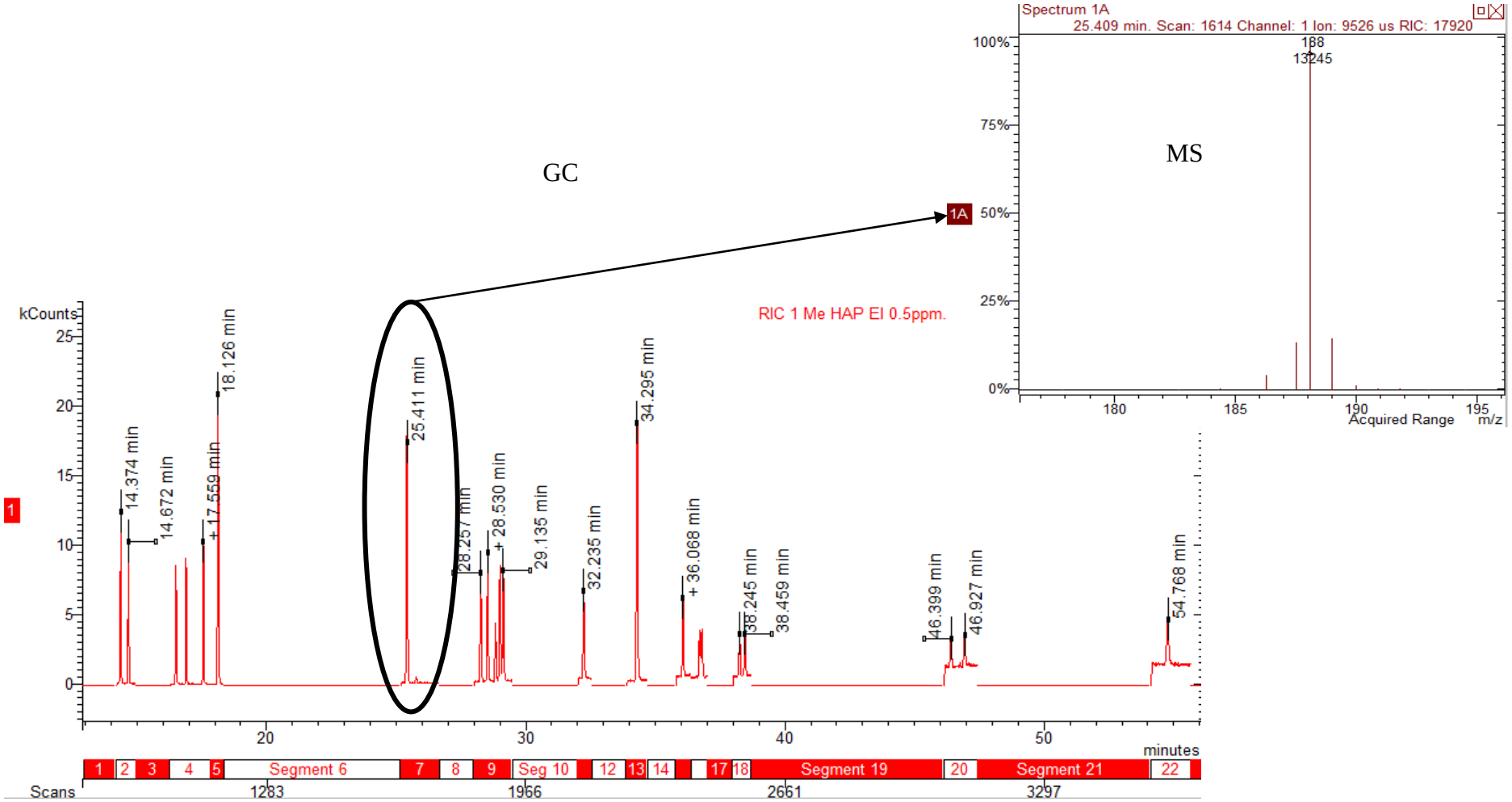


Figure 19: Exemple de chromatogramme de mélange de standard contenant 18 Me-HAP à 0.15ppm ainsi que les fragments m/z pour la molécule à TR 25.41 mn

II.7. Identification de sources de pollution

II.7.1. HAP et Me-HAP

Diverses techniques plus affinées/évolutives ne cessent d'être développées pour déterminer le niveau de contamination dans l'environnement. L'identification des possibles sources de pollution est une approche déterminante dans une démarche de gestion durable des milieux naturels. Plusieurs approches ont été adoptées pour évaluer la source des émissions des polluants et dans le cas des HAP et Me-HAP, ceci se fait généralement en examinant leur ratios moléculaires (De Luca et al., 2005; Miki et al., 2014;; Soclo et al., 2000; Yunker et al., 2002). En effet, leurs sources peuvent-être évaluées de nature pyrogénique (combustion incomplète des charbons) ou pétrogénique (pétroles ou des produits pétroliers). Le tableau 7 suivant regroupe les ratios fréquemment utilisés pour distinguer la contamination par les hydrocarbures avec des fourchettes de valeurs caractéristiques ainsi que des valeurs intermédiaires indiquant les sources mixtes.

Tableau 7: Ratios et valeurs caractéristiques des HAP d'origine pyrolytique et petrogénique

Ratio	Description	pétrogénique	combustion
FL/FL+Pyr	fluoranthène/fluoranthène+pyrene	<0,4	>0.5
Ant/Ant+Phe	anthracene/anthracene+phenanthrène	<0,1	>0,1
IP/IP+BgP	indeno(1,2,3-c,d)pyrène/indeno(1,2,3-c,d)pyrène+benzo(g,h,i)pérylène	<0,2	>0,5
BaA/BaA+Ch	benzo(a)anthracène/benzo(a)anthracène+chrysène	<0,2	>0,35
MPhe/Phe	Σ(3-methylphenanthrene+2-methylphenanthrene+9-methylphenanthrene+1-methylphenanthrene)/phenanthrène	>2	<1
LMW/HMW	Masse moléculaires légères (2-3 cycles aromatiques)/ lourdes (4-6 cycles aromatiques)	>1	<1

L'abondance des alkyl-HAP tels que les alkyl naphtalènes, alkyl phénanthrène et alkyl dibenzothiophènes marque une origine petrogénique des HAP tandis que les HAP d'origine pyrolytique sont généralement prédominés par les composés HAP non substitués (Budzinski et al., 1997; Yunker et al., 2002). L'abondance des HAP de haut poids moléculaires (HMW) démontre également une origine pyrolytique (Fernandez et al., 2000) ce qui est fréquemment observée dans les zones urbaines (Stout et al., 2004). Lors de la combustion des bois, le 1,7-DMP, le 2,6-DMP et la retène peuvent être émis (Larsen and Baker, 2003). D'autres études ont tenté de différencier les HAP d'origine véhiculaire (diesel ou non) et non véhiculaire (chauffage, combustion de bois, combustion de charbon, fumigation des grumes

de créosote, volatilisation à partir des eaux de surfaces contaminés et bien d'autres). A titre d'exemple, le pérylène a de sources biogéniques (Venkatesan, 1988), le benzo(e)perylène et le coronène sont lié à des trafics automobiles (Harrison et al., 1996), notamment lorsqu'ils sont en corrélation positive avec les monoxyde de carbone (CO) et les oxydes d'azotes (NOx). Le fluoranthène et le chrysène sont émis par le gaz d'échappement des voitures diesel et le pyrène par le gaz d'échappement des voitures à essence (Larsen & Baker, 2003).

II.7.2. PCB

Malgré le fait que la fabrication et l'utilisation des PCB ont été bannies depuis 1970, des études ont montré que la contamination par les PCB est encore d'actualité (Merhaby et al., 2015 ; Net et al., 2015a; Van Ael et al., 2013). En effet, certains produits/équipements contenant des PCB sont encore en circulation/utilisation (Lv et al., 2015). Contrairement aux HAP, l'identification de source de PCB à partir des ratios des isomères ne sont pas convenables vu que les PCB se présentent généralement sous forme de mélange. Les mélanges commerciaux existants sont caractérisés par leur degré de chloration et se présentent sous différentes gammes: Aroclor 1221, 1232, 1016, 1242, 1248, 1254, 1260, 1262. Les deux dernier chiffre traduit le pourcentage massique de chlore dans le mélange (ex : 21, 32, …) tandis que le chiffre 12 est le nombre de carbone dans les PCB, à l'exception de l'Aroclor 1016. Par conséquent, les sources possibles des PCB peuvent être évaluées en étudiant la corrélation de leurs concentrations avec d'autres paramètres polluants tels que les HAP. Dans le cas où il y a corrélation, on peut estimer que les deux types de polluants ont les mêmes sources d'émission.

II.8. Evaluation des risques éco-toxicologiques

La dégradation de l'environnement est à craindre lorsque des substances étrangères (ou xénobiotiques) sont introduits dans celui-ci. Sur ce, l'évaluation des risques écotoxicologiques, est une étape importante pour promouvoir sa protection ainsi que de prévenir la perte des potentialités des ressources naturelles. En effet, l'évaluation des risques se repose sur l'évaluation des dangers dus à l'exposition ainsi que la caractérisation des risques. Dans un premier temps, il est important de rassembler des informations sur les effets de composés polluants sur les organismes et de quantifier les risques possibles selon différentes relations dose-effet. Ensuite, il faut évaluer le degré d'exposition des organismes à une substance reconnue toxique. Sur ce, il faut tenir compte des concentrations de la

substance polluante dans le milieu et les organismes indigènes de ce milieu, ainsi que de la fréquence et la durée d'exposition des espèces les plus sensibles à ces contaminants. Généralement, l'évaluation des risques s'accompagne toujours de la gestion des risques qui est une démarche d'intervention pour éviter et réduire les dangers des activités effectuées (notamment les activités anthropogéniques).

Différentes études ne cesse d'être développées, avec une marge de sécurité, pour établir des doses ou des concentrations maximales admissibles. Sur la base des études toxicologies, on peut distinguer les outils de prévention des risques chimiques suivants :

- La notion de toxicité équivalente TEQ (Abarnou et al., 2000): qui exprime la toxicité relative des composés par rapport à une référence de base (la molécule la plus toxique connue dans la même famille, par exemple le 2,3,7,8-T4CDD pour les organochlorés). Ceci concerne uniquement les effets néfastes (cancer) découlant d'interactions avec les récepteurs. Néanmoins, ce présent système connaît une limitation notamment sur l'estimation de toxicité d'un mélange de polluant vu que de nombreuses familles chimiques ont des effets toxiques encore mal compris.
- La notion de « Predicted no effect concentration ou PNEC » : elle est basée sur la comparaison entre les concentrations d'exposition estimées (valeurs seuils) et celles trouvées pour les contaminants, la valeur au-dessous de laquelle indique qu'aucun effet néfaste n'est probable de se produire sur l'écologie.
- Les SQGs ou « Sediment quality Guidelines » : ils ont été établis afin de classer correctement les sédiments comme toxiques ou non toxiques selon les valeurs seuils ou concentrations définissant les effets probables TEL (au-dessous de laquelle des effets nocifs sur les organismes vivants ne devraient pas avoir lieu) et PEL (au-delà de laquelle les effets néfastes sur les organismes vivants sont susceptibles d'avoir lieu fréquemment).

Dans le présent travail, on a opté pour le Consensus-Based SQEs ou le CBSQGs qui a été modifié pour améliorer et unifier les différentes approches développées antérieurement (Doyle et al., 2003, Macdonald et al 2000). Les effets de mélanges des polluants dans un milieu sont pris en compte dans cette approche (Swartz, 1999). Différentes valeurs seuils sont alors à tenir compte, à savoir:

- La TEC (Threshold Effect Concentration) ou la concentration seuil en dessous de laquelle aucun effet néfaste sur les organismes vivants ne devrait pas être observé.
- Le PEC (Probable Effect Concentration) ou la concentration seuil au-dessus de laquelle des effets néfastes sur les organismes vivants dans les sédiments sont susceptibles d'être observés.
- Le MEC (Midpoint Effect concentration ; MEC=(TEC+PEC)/2 ou la concentration à effet médiane. Elle recommandé pour mieux interpréter l'impact potentiel des contaminants entre le TEC et le PEC qui ne sont pas inclus dans le CBSQGs (Doyle et al 2003).

Tableau 8: Valeurs seuils des CBSQGs correspondant aux HAP, Me-HAP et PCB.

	Concentrations en µg/kg p.s*		
Composés	CBSQGs TEC	MEC (TEC+PEC)/2	CBSQGs PEC
N	176	369	561
Ayl	5,9	67	128
Aen	6,7	48	89
F	77,4	307	536
Phen	204	687	1170
Ant	57,2	451	845
Fl	423	1327	2230
Py	195	858	1520
BaA	108	579	1050
Ch	166	728	1290
BbF	240	6,820	13400
BkF	240	6,820	13400
BaP	150	800	1450
IP	200	1,700	3200
DhA	33	84	135
Bghi	170	1685	3200
ΣPAH	1610	12205	22800
2MN[a]	20,2	111	201
ΣPCBs	60	368	676

*p.s: poids sec

II.9. Etude de Bioremédiation

II.9.1. Identification des matériels biologiques

II.9.1.1. Screening de souches de microorganismes

Des souches de bactéries ont été initialement isolées à partir des échantillons de sols pollués issus d'un site de rejet de fuels usagés d'une centrale électrique à Ambohimanambola (MADAGASCAR). Dans un premier temps, les souches bactériennes ont été isolées. Pour ce faire, 5 g de sol sec tamisé a été mis en suspension dans 45 mL d'eau distillée stérile. Cette suspension est agitée pendant 1h puis décantée pendant 30 min. Ensuite, on a procédé à une dilution successive de 10^{-1} à 10^{-5} à partir du surnageant. Deux astuces d'isolation ont été entreprises. La première s'agissait d'inoculer 1 mL du surnageant de chacune des dilutions en profondeur du milieu de culture. Tandis la deuxième astuce, seulement 100 µL de chacune des mêmes dilutions a été utilisée mais étalée à la surface de milieu de culture gélosé, préalablement disposés en boîtes de pétri. La gélose utilisée pour l'isolement étant le Trypto-caséine soja ou TSA qui présente une excellente nutritivité et permet la culture des bactéries aérobies, anaérobies voire les germes particulièrement exigeants. L'incubation a été effectuée à 30±2°C pendant un temps variant de 18 à 24 h. Un exemple des colonies bactériennes obtenues sont présentées sur la figure 20A. Ces colonies sont ensuite repiquées sur le même type de milieu jusqu'à avoir des souches pures (Figure 20B).

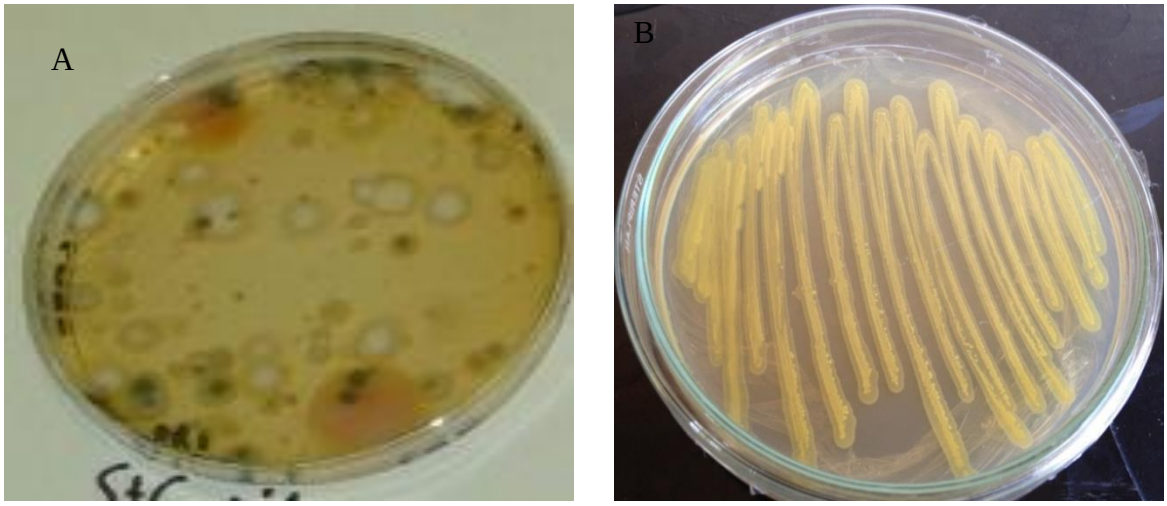


Figure 20: Milieu de culture TSA (A) avec les souches de microorganismes multiples et (B) avec une souche bactérienne pure

II.9.1.2. Acclimatation des souches

Cette étape consiste à manipuler les bactéries obtenues précédemment dans le but d'obtenir des souches qui peuvent résister et s'adapter à un milieu hautement contaminé en HAP. Cette étape permet d'étudier la capacité de ces souches à utiliser les HAP, qui sont les micropolluants d'intérêt, comme leur seule source de carbone et d'énergie pour leur métabolisme. En effet, chacune des souches a été cultivée dans des milieux liquides artificiels (bouillon nutritif), dopé par un mélange de 4 HAP. Les 4 HAP sont le fluorène, phénanthrène, pyrène et fluoranthène. Différentes doses de mélange de ces 4 HAP ont été testés : 250, 500, 1000 et 1500 $mg.L^{-1}$. Un stock de solution de mélange de HAP a été préalablement préparé en mélangeant des cristaux de fluorène, phénanthrène, fluoranthène et le pyrène dans l'acétone.

Pratiquement, une quantité précise de la solution de mélange de HAP a été distribuée dans un tube stérile. Le tube stérile contenant la solution de HAP a été gardé à la température ambiante jusqu'à ce que tout le solvant soit évaporé. Ensuite, 10 ml de bouillon nutritif stérile a été ajouté puis homogénéisé à l'aide d'un vortex. Une quantité de 100 µl de suspension microbienne, obtenues après remise en suspension des cellules pures dans du bouillon nutritif incubées à 30±2°C, a été finalement ajouté avant l'incubation à la même température. Les cellules ont été cultivées dans des conditions stationnaires (sans agitation permanente). La croissance substantielle du micro-organisme a été indiquée par la turbidité. Les critères de sélection des bactéries dégradant les HAP étaient basées sur leur capacités à croître dans le milieu hautement contaminé allant jusqu'à 1500 $mg.L^{-1}$ de mélange des 4 HAP.

II.9.1.3. Identification des bactéries

Les souches bactériennes pures sont soumises à une identification par spectrométrie de masse au moyen d'un instrument VITEK MS^{TM} (bioMérieux, Marcy-France). La technique utilise la matrice de désorption assisté/ionisation laser à temps de vol ou la MALDI-TOF (Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight). Elle est automatisée et détient un logiciel conçu pour identifier rapidement des espèces microbiennes. Actuellement, MALDI-TOF est devenu la technologie moderne, innovante et couramment utilisée dans les laboratoires de microbiologie (Nobrega de Almeida junior et al., 2014) en raison de sa précision, sa rapidité et fiabilité comparé à l'identification phénotypique classique (Bille et al, 2012; Bizzini et al, 2010). Les colonies pures sont disposées, en triplicata, sur une plaque cible de l'appareil puis laissée quelques instants à l'air ambiant avant d'insérer dans le spectromètre de masse. Un faisceau laser ionise l'échantillon, libérant ensuite un «nuage» de

protéines qui vont être accélérés par une charge électrique. Les protéines légères sont acheminées plus rapidement dans le système que les protéines lourdes. Puis, le « temps de vol » de chaque protéine est enregistré et détecté par un capteur pour créer un spectre. Ce spectre représente les empreintes de protéines de chaque échantillon. Chaque espèce d'intérêt est identifiée en comparant son spectre avec une grande base de données de spectres de bactéries bien caractérisés. Cette étape permet d'identifier les espèces, le genre et la famille des bactéries. Seules les bactéries présentant des probabilités de ressemblance élevées c'est-à-dire > 85% aux espèces répertoriées dans la base de données existantes ont été considérées (Jamal et al., 2014).

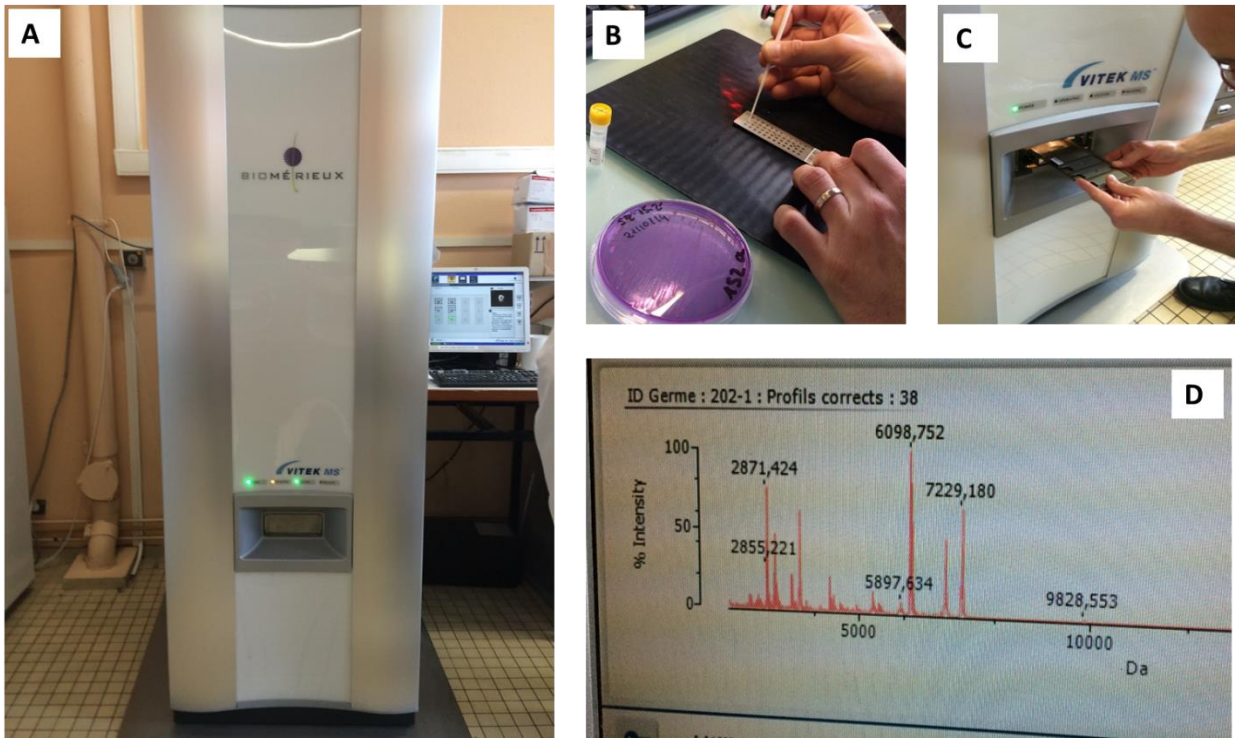


Figure 21: (A) Spectromètre de masse VITEK MS, (B) procédure d'étalement des souches bactériennes sur la plaque (cible), (C) insertion des échantillons dans l'appareil, (D) chromatogramme des protéines correspondant à la souche traitée

II.9.2. Préparation d'inoculum

L'inoculum désigne la suspension microbienne contenant les germes viables à utiliser durant l'expérimentation de biodégradation. L'inoculum a été préparé uniquement à partir de cellules résistantes observées à 1500 mg/L de mélange de 4 HAP. Ces souches constituent ainsi les souches jugées efficientes. A partir de la culture, les cellules sont d'abord repiquées de nouveau sur des boîtes de Pétri de gélose nutritive, incubées à la même température jusqu'à

l'observation des colonies bactériennes. Ces dernières ont été ensuite remises en suspension dans 10 ml de bouillon nutritif, incubé à 30±2°C jusqu'à l'apparition de trouble dans le milieu liquide. Ensuite, les cellules ont été recueillies par centrifugation (3.7x1000/mn pendant 8 min) de cette culture liquide. Les cellules sont ensuite lavées trois fois avec une solution physiologique de concentration 9 $g.L^{-1}$. Enfin, l'eau distillée stérile est ajoutée aux cellules lavées, homogénéisées. Ces dernières ont été utilisées comme inoculum.

II.9.3. Test de bioremédiation

II.9.3.1. Incubation en microcosme

Les sols utilisés sont préalablement broyés mécaniquement, séchés, tamisés à 224 µm puis stérilisés à 121°C pendant 15 min afin d'éliminer toute forme de vie pouvant interférer l'activité microbienne des bactéries sélectionnés. Chaque 5g de sols est distribué dans chaque vial ambré préalablement calcinés (à 450°C pendant 12 h) et stériles. Chaque vial contenant les 5 g de sols est placé à l'étuve à 30±2°C afin d'acclimater le milieu solide (Sabaté et al., 2004). Un volume précis de mélange de 4 HAP dans l'acétone a été ensuite ajouté pour obtenir la contamination finale de 500 $µg.g^{-1}$ de 4 HAP dans le sol sec. De l'eau distillée stérile a été ajoutée chaque semaine afin de garder le taux d'humidité compris entre 30 et 60%.

Figure 22: Incubation des sols à traiter, étude microcosme de bioremédiation

II.9.3.2. Prélèvement et extraction

Des prélèvements de sol ont été effectués à différents intervalles de temps. Le sol humide est ensuite mis dans les cellules d'extraction, mélangé avec 5 g de Na_2SO_4 par gramme de sol puis dopé avec 35 µl d'une solution d'étalon interne de concentration 100 µg/mL de Naphtalène-d8, Acenaphtene d-10, Phenanthrene d-10, Pyrène d-10, Perylène d-12 afin de corriger les éventuelles pertes et erreurs produits tout au long du protocole analytique. Chaque échantillon prélevé a été extrait, purifié/pré-séparé et analysé avec le protocole identique à celui décrit dans le paragraphe II.4.

Partie III. Résultats et discussions

Depuis plusieurs décennies, les HAP, Me-HAP et PCB dispersés dans l'environnement font partie de nombreux programmes de recherche en raison de leurs fortes toxicités et leur omniprésent dans l'environnement. En effet, ils sont détectés dans l'atmosphère, l'eau, les sédiments, le sol et même dans les biotes. Des efforts ont été portés sur l'étude de ces molécules dans l'environnement et des quantités importantes de données sur ce sujet ont été publiées. Cependant, la compréhension du comportement, du devenir et la dispersion de ces micropolluants dans l'environnement sont loin d'être entièrement parachevés. Le devenir et la dispersion de ces polluants dans l'environnement dépendent non seulement de propriétés physico-chimiques de chaque molécule mais également des caractéristiques et conditions des milieux dans lesquels ils sont présents.

Dans cette thèse, j'ai d'abord mis au point et optimisé des techniques d'analyses des HAP, Me-HAP et PCB présents à l'état de trace dans l'environnement. Ensuite, ces techniques ont été appliquées à la fois à l'étude du milieu naturel et à l'étude de faisabilité de remédiation des milieux altérés par les micropolluants organiques. En effet, mes travaux se sont focalisées sur deux axes : (i) l'étude des niveaux de contamination des milieux naturels et (ii) l'étude de faisabilités d'élimination de ces contaminants par voie biologique à l'échelle de laboratoire. Pour l'étude du milieu naturel, plusieurs matrices de l'environnement ont été étudiées; la matrice provenant du milieu aquatique (eau, MES et sédiment), les sols et aussi les biotes. Pour le milieu aquatique, les niveaux de contaminations en HAP, Me-HAP et PCB ont été déterminés dans la phase dissoute de l'eau, dans la phase particulaire (MES) et dans les sédiments dans le but de contribuer à des connaissances sur leur dispersion le long de la colonne d'eau. Les corrélations entre leurs niveaux de concentration et leur propriétés physico-chimiques tels que la solubilité, le coefficient de partage octanol-eau (K_{OW}) ont été également étudiées. Ces données sont illustrées et interprétés dans l'article 1 présentés ci-après (section III.1.1). Et afin d'avoir une vision plus large sur les contaminations et la dispersion dans l'environnement de ces micropolluants, leur concentrations ont été aussi quantifiées dans les sols et dans les organismes aquatiques. Les données sur les sols sont présentées dans le paragraphe *III.2* et les données sur les organismes aquatiques ont été valorisés sous forme d'un article à l'annexe II (article 3). Le deuxième axe de mes travaux s'est focalisé sur la faisabilité d'élimination de ces micropolluants toxiques des sols hautement contaminés par l'emploi de microorganismes (bioremédiation). Les résultats sont présentés dans la section III.2 sous forme d'un article récemment soumis dans soumission dans **Journal of Applied Microbiology.**

III.1. Evaluation du niveau de contamination

III.1.1. Cas du milieu aquatique

La qualité de l'écosystème aquatique n'est pas épargnée par l'effet de l'introduction, directe ou indirecte, de divers polluants d'origine anthropogéniques tels que les HAP, Me-HAP et PCB. Ces dernières décennies, diverses études ne cessent d'être développées dans le but d'avoir plus de compréhension sur le sort et comportement des tels polluants en fonction des différentes conditions environnementales. Malgré ces efforts, on rencontre encore des lacunes de données existent encore, tel est le cas des Me-HAP.

Dans ces travaux, 15 stations qui se trouvent dans 6 rivières qui sont des affluents de l'Escaut ont été étudiés. Ces zones sont localisées dans la Région Nord-Pas-de Calais et en Belgique. La dispersion des HAP, leurs homologues alkylés et les PCB dans la phase dissoute, particulaire et sédimentaire a été étudiés. L'objectif principale était, premièrement, d'étudié comment ces molécules de disperses et partages entre les différents phases du milieu aquatique des systèmes d'eau douce, c'est-à-dire la phase dissoute, particulaire et sédimentaire. Le deuxièmement objectif était d'étudier la corrélation entre la dispersion et les propriétés physico-chimiques de ces molécules tels que la solubilité (S_W) et le coefficient de partage octanol-eau (K_{OW}). La distribution et la répartition selon les poids moléculaires des HAP, Me-HAP ou le degré de chloration des PCB ont été également mise en exergue afin d'estimer le niveau et la forme de contamination dans chacune des rivières et dans l'ensemble du bassin versant de l'Escaut. L'article présente également des possibles sources de ces polluants dans les sites étudié en évaluant le rapport des ratios des isomères pour les HAP et Me-HAP. Dans le cas des PCB, leurs sources sont identifiées par corrélation avec les autres paramètres. Pour finir, la qualité environnementale de chaque site d'étude a été dressée sous forme de mappe après avoir effectué l'étude des risques éco toxicologiques.

Article 1

Distribution of persistent organic pollutants (PAHs, Me-PAHs, PCBs) in dissolved, particulate and sedimentary phases in freshwater systems

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Abstract

The occurrence of three groups of hazardous organic contaminants (PCBs, PAHs, Me-PAHs) in fifteen watercourses and rivers located in highly urbanized and industrialized zones was studied. The distribution of 62 organic contaminants was determined in three matrices: in the dissolved phase, associated with suspended solid matter (SSM) and in sediment. Their distributions in the aquatic environment depend strongly on their physicochemical properties. Low molecular weight PAHs were predominant in the dissolved phase while those with high molecular weight accumulated preferentially in SSM and sediments. Among the 28 PCBs congeners, only PCB153 was detected. The results showed that the contamination of these areas originated mainly from combustion processes. The three the most polluted sites identified are surrounded by big cities. Ecotoxicological assessment based on the international Sediment Quality Guidelines (SQGs) showed that the toxic effects of the sediment in these watercourses and rivers occurred due to high levels of hydrocarbons.

Keywords: POPs, sediment quality, water column, The Scheldt, ecotoxicological risk.

1. Introduction

Aquatic environments in industrialized countries are frequently exposed to numerous toxic organic pollutants generated by various discharges. Watercourses and rivers constantly receive various kind of organic contaminants from municipal, hospital and industrial wastewaters, agricultural effluents and nonpoint source pollution. To date, various watercourses, lakes, rivers, estuaries and seas have been contaminated by a wide range of organic substances including the persistent organic pollutants (POPs) (Boonyatumanond et al., 2006; Merhaby et al., 2015; Net et al., 2014a, 2014b, 2015a,b,c). Polychlorobiphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) are toxic and persistent in the environment. Due to their hydrophobicity, they tend to accumulate in solid matrices and in fatty tissues of organisms rather than entering the aqueous dissolved phase (Wenning and Martello, 2013). They have been detected in sediments and in many aquatic species such as marine algae, invertebrates, fishes, dolphins and many other aquatic organisms (Arias et al 2015a, b; Gui et al., 2014; Net et al., 2015a, b).

PAHs and Me-PAHs can originate from both natural processes and anthropogenic inputs (Lu et al., 2012). Anthropogenic origins are generally the major source of PAHs in the environment (Yunker et al., 2002). PCBs were used in industry as heat exchange fluids, in electric transformers and capacitors, as additives in paint, carbonless copy paper, and plastics (UNEP, 2008). Industries contribute a large amount of PCBs into the aquatic environment by their improper disposal (Özyürek et al., 2013). Even though their production has been banned since 1979 (US-EPA, 2013), PCBs are still present in the environment (Bigus et al., 2014; Dumoulin et al., 2013; Net et al., 2014b, 2015a, 2015b). Because of their toxic, carcinogenic and mutagenic effects (IARC, 2010; Straif et al., 2005), sixteen PAHs have been listed as priority substances by the United State-Environmental Protection Agency (US-EPA, 2013; US-Department of Health and Human Services, 1995). PCB exposure may cause endocrine disruption, abnormalities of skin and liver function, trouble in immune systems, dysfunction of neurological development and the reproductive system (Longnecker et al., 1997). PCBs have been now listed as POPs in the Stockholm Convention.

For the aquatic environment, studies have focused mainly on organic contaminants occurrence in dissolved and/or sedimentary phases. To our knowledge, there is a lack of data on the distribution of these compounds in the particulate phase of the water column. In the present work, the aims were firstly to evaluate the distribution of PCBs, PAHs and Me-PAHs in the dissolved, particulate and sedimentary phases of freshwater systems. Fifteen sampling sites located in six rivers at the cross-border area of Northern France-Belgium were studied. Secondly, the origins of the contamination and the potential environmental risks were investigated.

2. Materials and methods

2.1. Chemicals and materials

Mixed standard solutions of PAHs and Me-PAHs were purchased from Restek (Bellefonte, PA, USA) and PCB standard solutions from Accustandard (New Haven, CT, USA). Acenaphthene-d10 (A-d10), naphthalene-d8 (N-d10), perylene-d12 (Per-d12), phenanthrene-d10 (Phe-d10) and pyrene-d10 (Pyr-d10) were obtained from LGC-Promochem (Middlesex, UK) and used as internal standards for PAHs and Me-PAHs quantifications. Tetrachloronaphthalene (TCN), 2,3,3',5,6-tetrachlorobiphenyl (PCB112) and octachloronaphthalene (OCN) used for PCBs quantification were purchased from Dr Ehrenstorfer (Augsburg, Germany). HPLC-grade solvents (hexane, dichloromethane (DCM),

acetone, propan-2-ol and methanol) were purchased from Dislab (France). Ultrapure water with 18.2 MΩ/cm resistivity (Milli-Q) was produced from a Millipore apparatus. C_{18} SPE cartridges (200 mg/6 mL) were purchased from Sigma-Aldrich (Saint-Louis, USA). Merck silica gel 60 (70-230 mesh) activated at 450°C was heated at 120°C for 12 h prior to use. Glassware was systematically washed with detergent (Decon, East Sussex, UK) and acidified ultrapure water, rinsed with ultrapure water followed by acetone then dried at 120°C prior to use.

2.2. Sampling sites

The studied area is part of the watershed of the Scheldt (Fig. 1), which is surrounded by areas of high anthropogenic activity and high population density. Historically, its industrialization has been among the highest in Europe (Lesven et al., 2009; Net et al., 2014a, 2015b). Although mining and metallurgical activities in the Scheldt basins have been reduced in recent decades, the remobilization of metal trace elements buried in the sediments since the beginning of the industrial era is one of the major sources of pollution today (Lesven et al., 2009). However, only few data on organic contaminants are available in the literature (Net et al., 2014a, 2015b).

The sampling was conducted in early spring 2014 in fifteen sites located at the cross-border areas of northern France-Belgium and the watershed upstream of the Scheldt (Fig. 1). The Scheldt is a 350 km long river which crosses northern France, western Belgium and the southwestern Netherlands. It is mainly channeled and crosses five regions where it receives various wastewaters and effluents before discharging into the North Sea near Vlissingen. The present study focuses on the watercourses and rivers located at the cross-border region of northern France-Belgium. Six sites located along the Scheldt (Fresnes, Neuville, Crevecoeur, Warcoing, Berchem, Zingem), three on the Lys River (Aire sur-la-Lys, Erquinghem-Lys, Wervicq), two in the Deûle River (Don and Wambrechies), two in the Scarpe River (Brebières and Nivelle), one in the Sensée River (Férin) and one in the Sambre River (Jeumont) were studied (Fig. 1; Table 1).

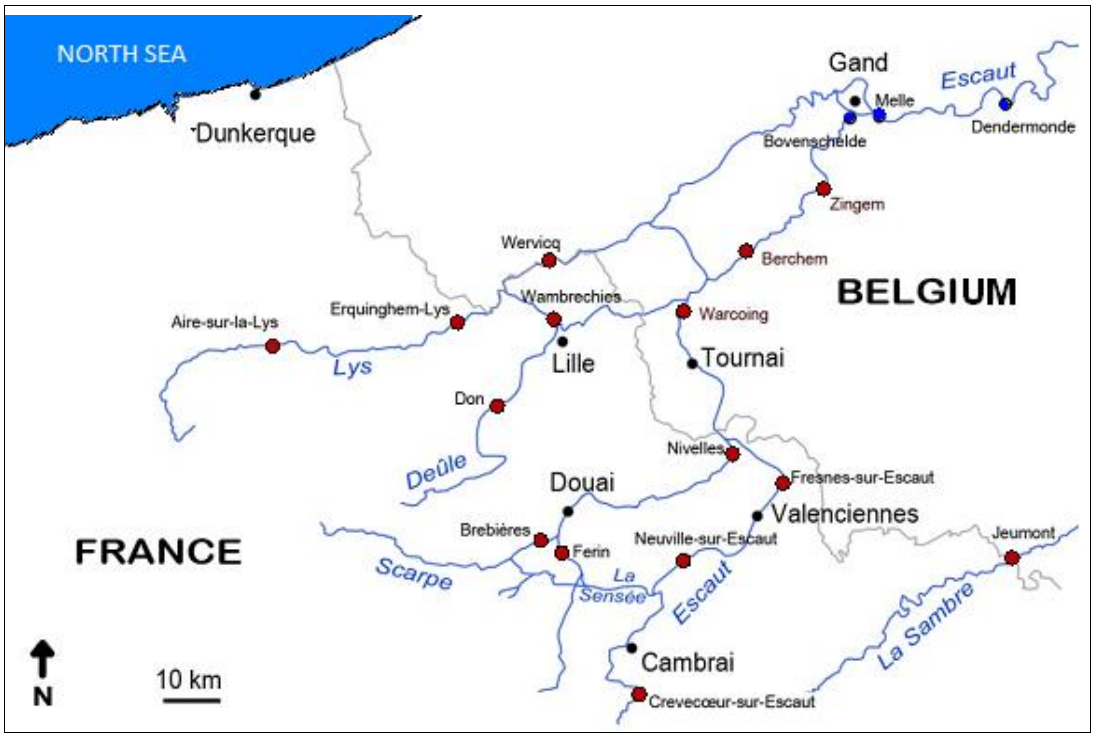

Fig. 1. Studied sites at the cross-border area Northern France-Belgium (red points).

2.3. Sampling

Water was collected using 2.5 L pre-cleaned amber glass bottles that were immediately capped with Teflon-lined lids. Sediment samples were collected from the top 0–5 cm using 10 cm diameter and 30 cm long polycarbonate tubes. The sediments were kept in pre-calcinated aluminum containers and capped with aluminum foil. The samples (water and sediment) were transported to the laboratory and directly treated without storage.

2.4. Targeted analytes

In this work, the following PCBs, PAHs and Me-PAHs were analyzed:

- *16 PAHs*: naphthalene (N), acenaphthylene (Ayl), acenaphthene (Aen), fluorene (F), anthracene (Ant), fluoranthene (Fl), benz[a]anthracene (BaA), chrysene (Ch), benz[a]pyrene (BaP), phenanthrene (Phe), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (Bghi), dibenzo[a,h]anthracene (DhA), indeno[1,2,3-cd]pyrene (IP), pyrene (Pyr).

- *18 Me-PAHs*: 1-methylnaphthalene (1M-N), 2-methylnaphthalene (2M-N), 1,2-dimethylnaphthalene (1,2DM-N), 1,6-dimethylnaphthalene (1,6DM-N), 2,6-dimethylnaphthalene (2,6DM-N), 1-methylphenanthrene (1M-Phe), 2-

methylphenanthrene (2M-Phe), 3-methylphenanthrene (3M-Phe), 9-methylphenanthrene (9M-Phe), 2-methylanthracene (2M-An), 1,7-dimethylphenanthrene (1,7-DMP), retene, 1-methylfluoranthene (1M-Fl), 3-methylfluoranthene (3M-Fl), 1-methylpyrene (1M-Pyr), 4-methylpyrene (4M-Pyr), 3-methylchrysene (3M-Ch), 6-methylchrysene (6M-Ch).

- *28 PCBs:* 8, 18, 28, 44, 52, 66, 77, 81, 101, 105, 114, 118, 123, 126, 128, 138, 153, 156, 157, 167, 169, 170, 180, 187, 189, 195, 206, 209.

The physicochemical properties of the targeted compounds are presented in Table 1S in the Supporting Information (SI). The behavior and fate of organic contaminants in the environment are controlled by their physicochemical properties such as water solubility (S_W), octanol-water partition coefficient (K_{OW}) and organic carbon partition coefficient (K_{OC}) which are generally presented as log K_{OW} and log K_{OC}. S_W controls the distribution between water, SSM and sediment. K_{OW} predicts the tendency of a contaminant to concentrate in aquatic organisms, while K_{OC} indicates the capacity to bind to organic matter in SSM or sediment. S_W values of PAHs and Me-PAHs are low and decrease with increasing numbers of aromatic rings (Table 1S). Log K_{OW} and log K_{OC} of PAHs and Me-PAHs increase with an increase in the number of cycles, which suggest that high molecular weight PAHs will be appreciably sorbed to SSM, aquatic organisms and sediment. Similarly, PCBs have low S_W and high values of log K_{OW} and log K_{OC} (Table 1S).

2.5. Sample extraction

On arrival at the laboratory, water samples were directly filtered using calcinated 0.45 µm Whatman glass microfiber filters (GF/F). Targeted compounds in dissolved phases were extracted using solid phase extraction (SPE), while compounds associated with SSM and sediments were extracted using accelerated solvent extraction or ASE (ASE200, Dionex Corp., USA).

2.5.1. SPE extraction

Filtered water samples were spiked with internal standards prior to extraction. An SPE procedure using C-18 cartridges was performed according to the method developed by Busetti et al. (2006). Briefly, the cartridges were conditioned with 9 mL of acetonitrile, 9 mL of 2-propanol and 12 mL of a mixture of Milli-Q water/2-propanol (85/15, *v/v*) acidified to pH 2.5. The samples were then passed through the cartridges (1 drop/second). Cartridges were washed with 30 mL of Milli-Q water/2-propanol mixture (85/15, *v/v*) acidified at pH 2.5 and then dried under nitrogen-flow for 10 min. Targeted compounds were eluted with 12 mL of

hexane/2-propanol/acetone mixture (90/5/5, *v/v/v*) followed by 3 mL of DCM. The extracts were then concentrated to a volume of 50–200 µL under nitrogen-flow and stored at 4°C until GC-MS analysis.

2.5.2. ASE extraction

For the targeted compounds associated with SSM, the extractions were performed on dried SSM retained on GF/F obtained from 2.5 L of water sample. Sediments were dried, finely ground and sieved at 224 µm prior to extraction. Each sample was spiked with internal standards and first extracted with DCM/acetone (1/1, *v/v*) according to the method developed by Reid et al. (2009). A second extraction was performed with DCM according to the method developed by Tronczynski et al. (2005). High purity nitrogen was employed as the purge gas. The extracts of each sample were combined before the purification step.

2.6. Purification and separation

Molecular sulfur was removed by the addition of activated copper to the extracts. The extracts were concentrated, solvent-exchanged to hexane and purified on a silica column. In the top of the column, 2 g of anhydrous sodium sulfate was added to eliminate eventual traces of water. Targeted compounds were recovered by successive elution with 20 mL of hexane, 15 mL of hexane/DCM mixture (3/1, v/v) followed by 15 mL of hexane/DCM mixture (1/1, v/v) and 15 mL DCM. Each sample was concentrated to 200 µL for SSM and 100-500 µL for sediments.

2.7. GC-MS analysis

Each compound was analyzed using a Varian 3900 gas chromatograph (GC) equipped with a deactivated fused-silica guard column (5 m, 0.25 mm i.d.) and a fused-silica capillary Phenomenex XLB (60 m length, 0.25 mm i.d., 0.25 µm film thickness) and coupled with a Varian Ion Trap Saturn 2000 Mass Spectrometer (MS). The carrier gas was helium, held at a constant flow rate of 1 mL/min. Samples were injected in the splitless mode at 280 °C and the injector was purged with helium after 1 min. Each group of organic compounds was analyzed separately. The transfer line and the ion trap were held at 280°C and 220°C, respectively. The oven temperature was programmed as follows: from 70°C (1 min) to 170°C at 10°C/min, then ramped at 4°C/min to 230°C, and then at 3°C/min (13 min) to 300°C for PAHs and Me-PAHs. For PCBs, the oven temperature was programmed from 80°C (1 min) to 170°C at 10°C/min, then to 230°C at 4°C/min, and finally to 300°C at 3°C/min (19 min).

Each targeted compound was identified based on the retention time and the mass spectrum from a chromatogram of standard solutions acquired in full scan mode. Quantification was then performed in the MS/MS or multiple residual monitoring (MRM) modes for better selectivity.

2.8. Quantitative analysis and quality control

For the quantification, a six-point internal calibration method was used. Internal standards were added for each calibration point in order to better fit to the properties of targeted compounds. To minimize the error of quantification, the procedural blank for the entire analytical procedure was performed in triplicate together with each environmental sample batch. Our results showed that the procedural blanks were free from any targeted PCBs, PAHs and Me-PAHs. This ensures that no significant contamination occurs during the procedure.

Limits of quantification (LOQs) were estimated in the range of 0.3-5, 18-38 and 0.5 µg/kg dw of sediment respectively for PAHs, Me-PAHs and PCBs. For water, LOQs were determined at 3-50, 1.5-3 and 5 ng/L respectively for PAHs, Me-PAHs and PCBs. Recoveries of PCBs, PAHs and Me-PAHs were calculated by spiking the targeted compounds into environmental matrices of interest (filtered river water, SSM retained on GF/F and sediment). The recovery rates obtained for solid matrices were >79% for PAHs, >73% for Me-PAHs and >75% for PCBs. For the dissolved phase, the recoveries of PAHs and PCBs were estimated at >76% and >68 % respectively.

3. Results and discussion

3.1. Physicochemical characteristics of water samples

During the sampling, classical parameters such as pH, temperature (T), dissolved oxygen (O_2), turbidity, and potential (E) were measured directly in the field to better understand the characteristics of the water body. In the laboratory, sulfate (SO_4^{2-}), chlorine (Cl^-) and nitrate (NO_3^-) ions were also measured using ionic chromatography. The SSM content was determined by weighing. Dissolved organic carbon (DOC) which refers to the dissolved fraction of organic carbon that passes through 0.45 µm GF/F was also determined. The values of these parameters are listed in Table 1. Generally, the water bodies were relatively neutral with pH values 6.80–7.98 and well oxygenated with dissolved oxygen ranging between 6.2 and 11.6 mg/L. The temperature ranged from 12.2 to 15.2°C. However, SSM content was highly variable ranging from 5.6 at Brebières to 88.9 mg/L at Berchem.

Nitrate concentrations were detected at high levels up to more than 25 mg/L except for Nivelles and Jeumont (Table 1).

Table 1: Location of the sampling sites and their physicochemical characteristics of the water body

Rivers	Sampling sites	GPS coordinates	pH	T (°C)	O_2 (mg/L)	Turbidity (F.N.U)	E (mV)	SSM (mg/L)	DOC (mg/L)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	NO_3^- (mg/L)
The Scheldt	Zingem	N50°53'36,30'' E3°40'50,07''	-	14.3	10.9	19.5	146	26.8	3.6	102.0	68.8	32.8
	Berchem	N50°47'36,71'' E3°30'16,74”	-	15.2	10.6	34.6	260	88.9	5.4	99.5	74.4	30.3
	Warcoing	N50°42'2,18'' E3°21'8,90''	-	15.0	10.6	28.9	288	44.7	3.5	103.8	59.8	36.9
	Crevecoeur	N50°06'16,2'' E3°14'51,1''	6.80	12.2	11.6	8.0	315	11.6	4.0	19.2	30.6	37.1
	Neuville	N50°18'05,9'' E3°21'01,1''	7.24	14.1	12.4	17.7	305	16.7	2.0	31.4	47.7	28.8
	Fresnes	N50°25'33,0'' E3°34'52,7''	7.51	13.7	11.0	14.2	283	20.1	2.2	50.7	50.6	28.9
The Lys	Wervicq	N50°46'36'' E3°02'35''	-	14.8	6.5	25.4	134	47.3	3.5	84.0	74.2	32.8
	Aire-sur-la-Lys	N50°38'45,1'' E2°24'34,8''	7.20	14.4	8.9	38	252	17.8	2.6	104.2	65.1	47.9
	Erquinghem-Lys	N50°40'37,8'' E2°50'08''	6.92	13.7	7.2	11.7	301	12.2	3.4	53.4	62.6	24.9
The Sensée	Férin	N50°32'23,8'' E3°07'24,9''	7.56	14.7	8.6	5.9	258	6.0	1.9	30.4	42.1	27.3
The Sambre	Jeumont	N50°17'52,6'' E4°06'.05,4''	7.98	13.7	10.8	11.6	262	17.6	4.4	36.6	31.9	13.8
The Deûle	Wambrechies	N50°41'10'' E3°03'10''	6.85	14.6	6.2	25.4	243	24.0	3.2	99.8	77.7	32.0
	Don	N50°32'48,8'' E2°55'14,7''	6.91	14.3	9.3	9.1	133	9.6	2.2	83.4	57.3	37.0
The Scarpe	Brébières	N50°33'47'' E3°03'23,3''	7.57	12.6	11.0	4.5	292	5.6	2.0	31.4	37.7	38.5
	Nivelles	N50°28'11,6'' E3°27'58,1''	7.65	14.4	10.3	26.2	335	34.8	6.2	175.7	68.5	17.4

Based on the values of nitrate, these surface waters were classed as quality 2. For other parameters, the measured values were quite variable and of doubtful use to judge the quality of the water body. Overall, the high levels of nitrate in these water bodies may indicate that these rivers could be significantly influenced by agricultural activities. Indeed, the principal origin of nitrates is the leaching of agricultural soils (Billen et al., 2005).

3.2. Occurrence in the three phases

The occurrence of Σ_{28}PCBs, Σ_{16}PAHs and Σ_{18}Me-PAHs both in the water column (dissolved and particulate phases) and in sediment was investigated. The concentration of each group of compounds varied significantly between stations and phases (Table 2S). High levels of Σ_{16}PAHs in SSM were identified at Zingem (3.74±0.15 µg/L), Berchem (3.58±0.20 µg/L), Warcoing (3.10±0.15 µg/L) and the highest was detected at Wervicq

(4.94±0.25 µg/L). The great majority of Σ_{16}PAHs associated with the SSM with an average of 80% versus only 20% in dissolved phase (Table 2S). This can be explained by the fact that PAHs have high affinity for solid matrices due to their low S_W and high K_{OW} and K_{OC} (Table 1S). In sediment, the Σ_{16}PAHs were detected at high levels ranging from 3.75±0.19 to 22.30±1.11 mg/kg dw with an average of 9.01±6.69 mg/kg dw (Fig. 2a, Table 2S). Above all, the highest amounts of dissolved-PAHs were detected in the Lys River. However, particulate-PAHs were detected at high levels in the Scheldt, Lys and Deûle Rivers (Fig. 2b). For sediment, the Deûle River was the most contaminated by PAHs (Fig. 2b). In contrast, the Σ_{18}Me-PAH associated with SSM was a minority (19%) compared to dissolved phase (81%) (Fig. 3a). In the water column, the highest concentration for global contamination was found at Aire-sur-la-Lys (4.40±0.22 µg/L) for the sum of dissolved and particulate phases (Σ_{D+P}) followed by Brebières (4.19±0.21 µg/L for Σ_{D+P}). In sediments, the Σ_{18}Me-PAHs were detected at high levels ranging from 0.17±0.01 to 2.62±0.13 with an average of 1.11±0.99 mg/kg dw. However, Σ_{18}Me-PAHs was nine times lower compared to Σ_{16}PAHs. A high degree of heterogeneity in each river was also observed (Fig. 3b). Highly variable concentrations of both PAHs and Me-PAHs were observed between stations for each river (Fig. 2–3). This may be due to their local sources located near to the sampling site. Above all, the Lys River seems to be the most contaminated by PAHs and Me-PAHs in the dissolved phase.

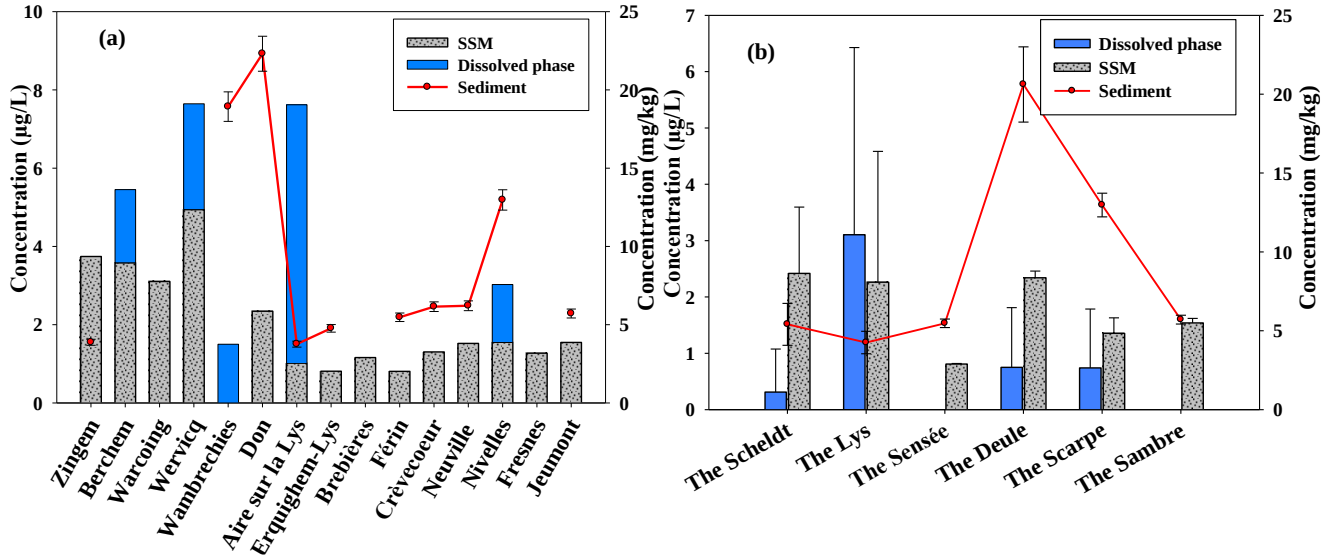

Fig. 2. Comparison of Σ_{16}PAHs contents in dissolved phase, associated with SSM and in sediment for each (a) station and (b) river.

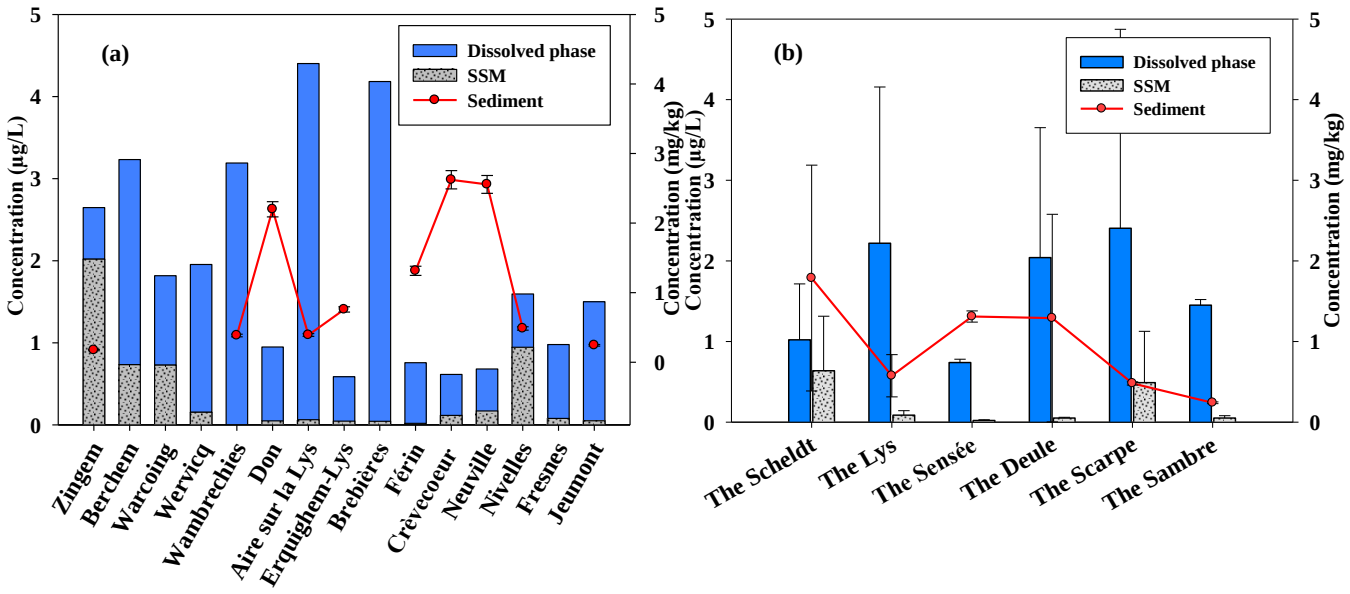

Fig. 3. Comparison of Σ_{18}Me-PAHs contents in dissolved phase, associated with SSM and in sediment for each (a) station and (b) river.

For PCBs, only PCB153 was detected among the 28 PCB congeners. A similar observation was found in a previous study for the Scheldt Estuary with dominance of PCB153 (Van Ael et al., 2012). However, our results showed that PCB153 was not detected in the dissolved phase (Table 2S, Fig. 4a, b). In SSM, PCB153 was not detected for the majority of the stations, the exceptions being Wervicq, Aire-sur-la-Lys and Nivelles (Fig. 4a, Table 2S). Although PCB153 was detected in SSM for three sampling sites, the contamination levels were relatively low. In sediments, PCB153 was detected at all sampling sites but the concentrations were relatively low in the range of 0.23-7.34 µg/kg dw (Fig. 4a, Table 2S). Indeed, PCB153 has a strong affinity to accumulate into biota, SSM and sediment due to its high K_{OW} and K_{OC}. Previous studies showed that PCBs are still present in water and sediment in freshwater in Northern France and in Belgium (Net et al., 2015a; Van Ael et al., 2012, 2013). Their presence in the aquatic environment may be due to their high persistence and their atmospheric deposition from permanent combustion process emissions (incinerators) or their release from old electronic equipment. Above all, PCB153 was detected at low levels in each river; <LOQ in dissolved phase, <13 ng/L associated with SSM and <8 µg/kg dw in sediment (Fig. 4b).

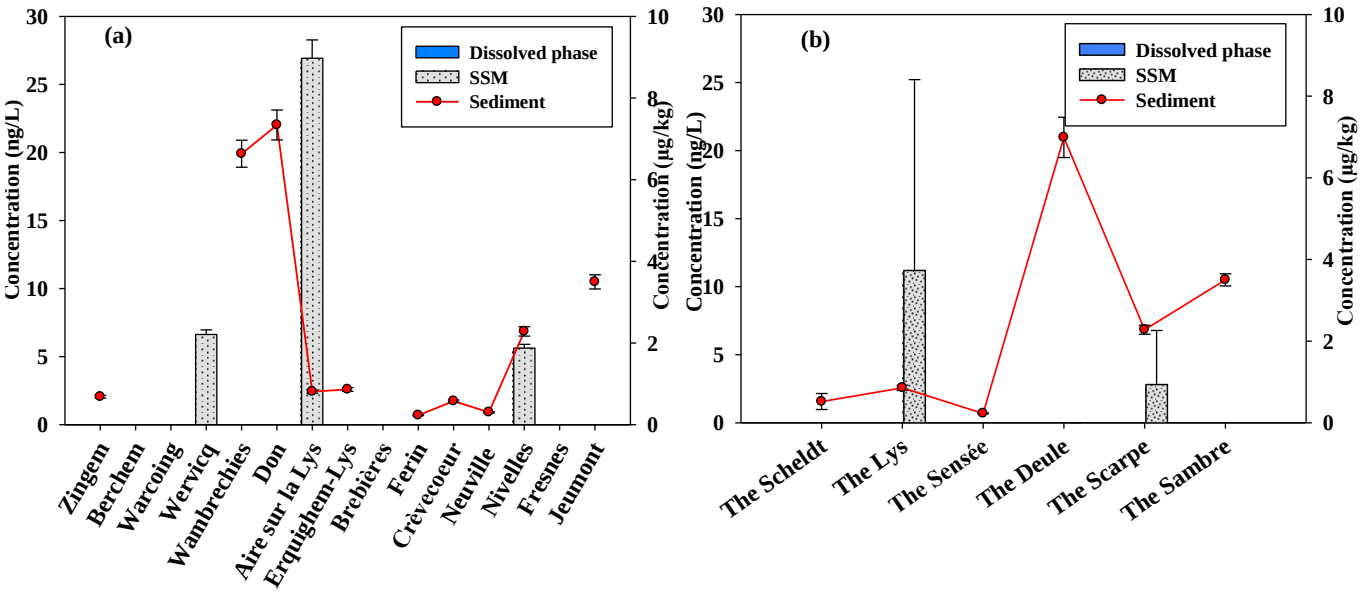

Fig. 4. Distribution of PCB153 in dissolved phase, associated with SSM and in sediment for each (a) station and (b) river.

3.3. Distribution of individual compounds in the three phases

For PAHs, the global contamination of the water columns was dominated by the low molecular weights (LMW; 2-3 aromatic rings). For all stations, LMW-PAHs represent more than 50% of the total contamination (Σ_{D+P}) (Fig. 5a). The highest level of contamination was recorded at Aire-sur-la-Lys where naphthalene was abundant (4.29±0.20 µg/L). In contrast, high molecular weight PAHs (HMW; 4-6 aromatic rings) were predominant in sediments (Fig. 5c). 87% of 4–6 aromatic ring PAHs were detected in sediments versus only 13% of 2–3 aromatic ring PAHs. This distribution was similar to that previously reported for rivers located in highly industrialized zones (Kanzari et al., 2014). Our results can be explained by the fact that HMW-PAHs are hydrophobic and more easily sorbed in a solid matrix due to their low S_W, high K_{OC} and K_{OW} (Table 1S) (Chen et al., 2004; Patrolecco et al., 2010). Fig. 6a–b shows the distributions of individual PAHs versus log K_{OW} and S_W. The proportion of PAHs in the particulate phase increases with increasing log K_{OW}. PAHs with log $K_{OW} \geq 4.45$ were detected mainly in the particulate phase (Fig. 6a). Similarly, the distribution of PAHs depends on S_W. PAHs with $S_W < 1$ mg/L were not detected in the dissolved phase (Fig. 6b) because they accumulate preferentially into SSM and sediments.

Me-PAHs with 2–3 aromatic rings represent ~100% of Σ_{D+P} for all stations (Fig. 5b). However, 4 aromatic ring Me-PAHs were predominant in sediments (Fig. 5d). In the water column, Me-PAHs are present mainly in the dissolved phase and 9-methylphenanthrene and 2-methylanthracene were predominant. Fig. 7a–b shows the correlation between the

distribution of individual Me-PAHs and their physicochemical properties. The percentage of Me-PAHs in the dissolved phase decreases with decreasing S_W (Fig. 7b). For PCBs, as reported previously, only PCB153 was detected. In the water column, PCB153 was detected only in SSM. The absence of PCB153 in the dissolved phase can be explained by the fact that this compound has very low solubility (0.001 mg/L) and high K_{OC} and K_{OW}.

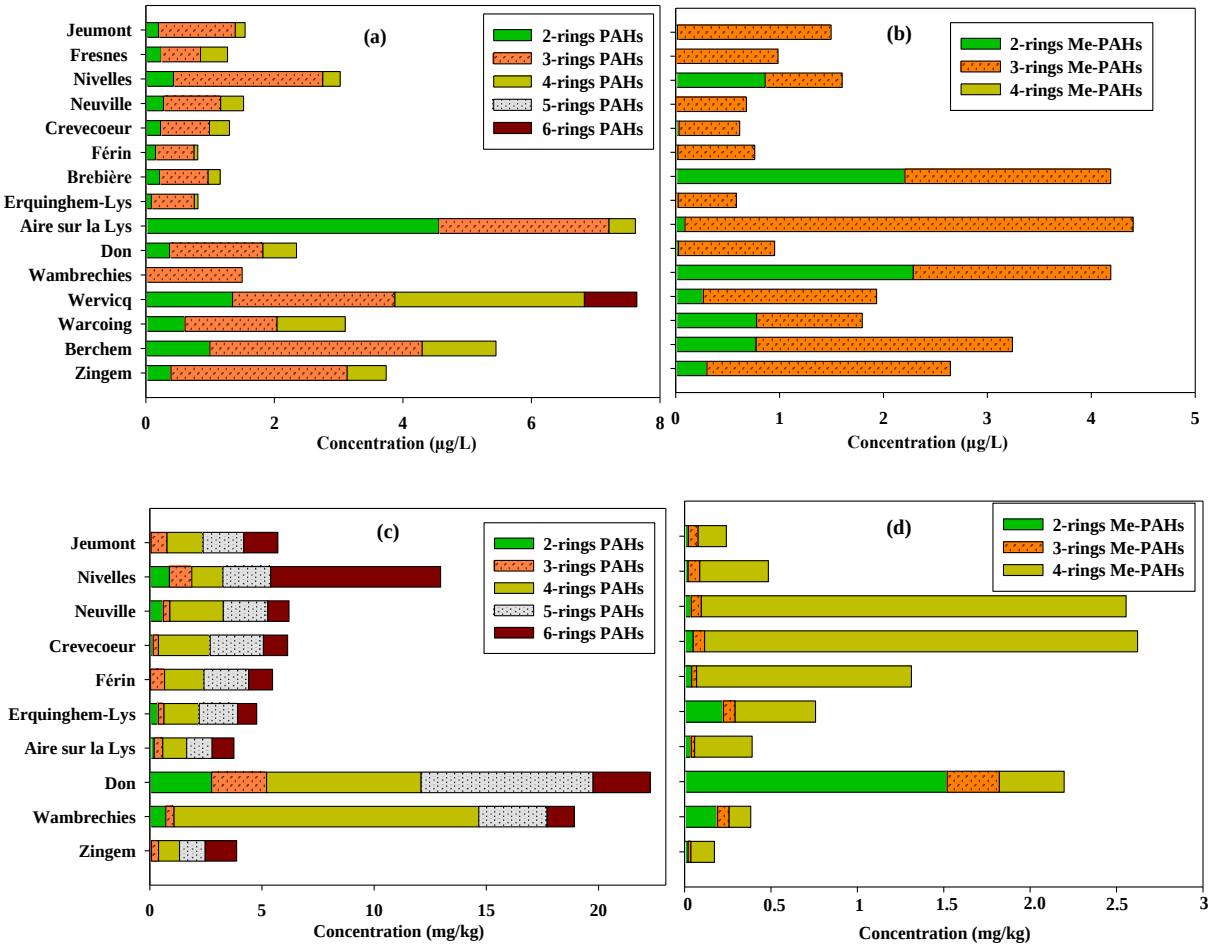

Fig. 5. Distribution of PAHs and Me-PAHs: (a) and (b) in water for the sum of dissolved and particulate phases (ΣPAH_{D+P}) and (c) and (d) in sediment.

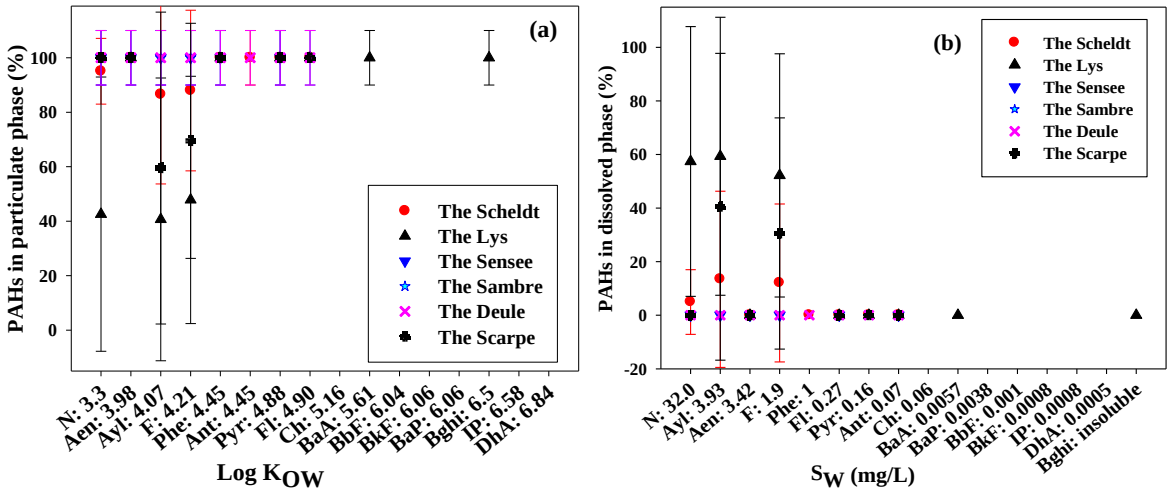

Fig. 6. Percentage of individual PAH (a) in particulate phase ($[PAH]_{in\ SSM}/([PAH]_{in\ SSM} + [PAHs]_{in\ dissolved\ phase})*100$) versus Log K_{OW} and (b) in dissolved phase ($[PAH]_{in\ dissolved\ phase}/([PAH]_{in\ SSM} + [PAHs]_{in\ dissolved\ phase})*100$) versus water solubility (S_W) for the average of each river.

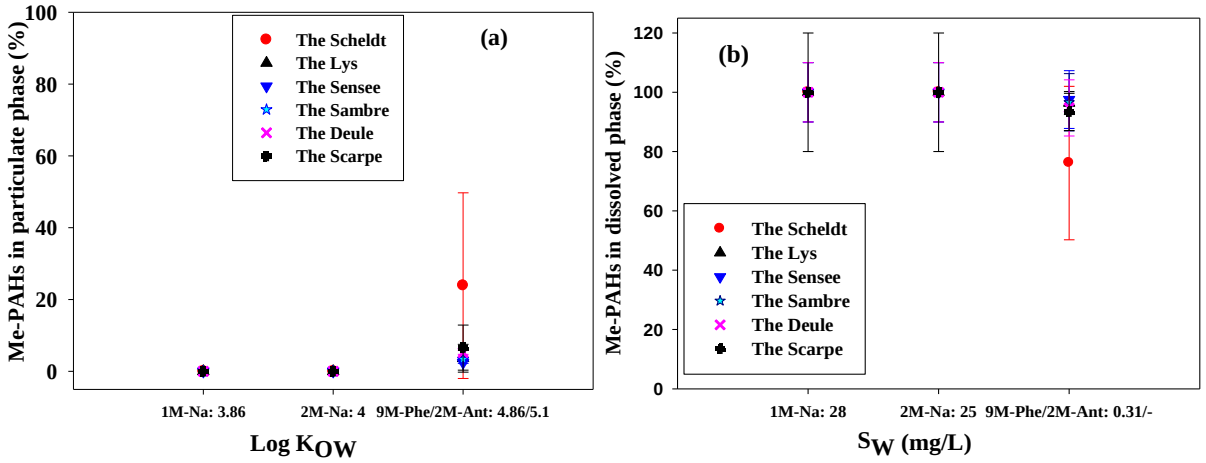

Fig. 7. Percentage of detected individual Me-PAH (a) in particulate phase ($[PAH]_{in\ SSM}/([PAH]_{in\ SSM} + [PAHs]_{in\ dissolved\ phase})*100$) versus Log K_{OW} and (b) in dissolved phase ($[PAH]_{in\ dissolved\ phase}/([PAH]_{in\ SSM} + [PAHs]_{in\ dissolved\ phase})*100$) versus S_W for the average of each river.

3.4. Source apportionment

PAHs can originate from natural or anthropogenic processes (Yunker et al., 2002), while PCBs are chemically synthesized and have no natural source (Wolska et al., 2012). Atmospheric transport/deposition, industrial and municipal effluents, domestic sewage and urban runoff can be the major point source or non-point source of PAHs and PCBs in aquatic

environments (Barakat et al., 2013; Manoli and Samara, 1999; Net et al., 2015a; Zhu et al., 2004).

The sources of parent and alkylated-PAHs can be investigated by diagnostic of different PAH isomer ratios (Miki et al., 2014; Net et al., 2014a; Soclo et al., 2000). Isomer ratios BaA/(BaA+Ch) and IP/(IP+Bghi) represent a reliable tool due to their stability (Yunker et al., 2002). BaA/(BaA+Ch) ratio <0.2 usually implies a petroleum source, 0.2–0.35 indicates a mixed source (petroleum/combustion) and >0.35 indicates a combustion origin. Similarly, IP/(IP+Bghi) ratio <0.2 indicates a petroleum origin, 0.2–0.5 indicates a mixed source and >0.5 indicates a biomass/coal combustion source. In addition, the ratio MPhe/Phe defined as the ratio of the sum of all the detected methylated phenanthrenes (3M-Phe+2M-Phe+9M-Phe+1M-Phe) to phenanthrene was also used to complement the source identification (Bakarat et al., 2011). LMW-PAHs were the contaminants at five stations (Fig. 5a) suggesting both petrogenic and combustion sources for PAHs in the water column (Zhang et al., 2004). Moreover, naphthalene, acenaphthylene and fluorene are among the predominant LMW-PAHs in the gas phase of the atmosphere, therefore hydrocarbons detected in the studied sites might originate from atmospheric fallout/deposits (Ruiz-Fernández et al., 2014).

The cross plots of IP/(IP+Bghi) and MPhe/Phe versus BaA/(BaA+Ch) in sediment (Fig. 8a-b) indicated that PAHs originated mainly from combustion sources; except for the Férin and Neuville sites where mixed sources were identified. Such a strong pyrogenic signature of PAHs was confirmed by the abundance of HMW-PAHs (Fernández et al., 2000). This fact has been reported as evident in most urban sediments (Stout et al., 2004), industrial countries and zones historically containing coal/oil tar production, processing and operations. In our case, auto/diesel/gas engine emissions represented the minor source of PAHs except in Nivelles station where the vehicle emissions tracer IP exceeded 50% of $\sum_{16}$PAHs. Other compounds such as Bghi and BkF can be also used as auto tracers (Li et al., 2012). In our case, these compounds were detected as <10% of $\sum_{16}$PAHs. Crude oil, wood and coal combustion might be the exclusive combustion origin since the markers Pyr, Fl, Phe and Ant were observed in the majority of samples (Larsen and Baker, 2003; Zhang et al., 2012).

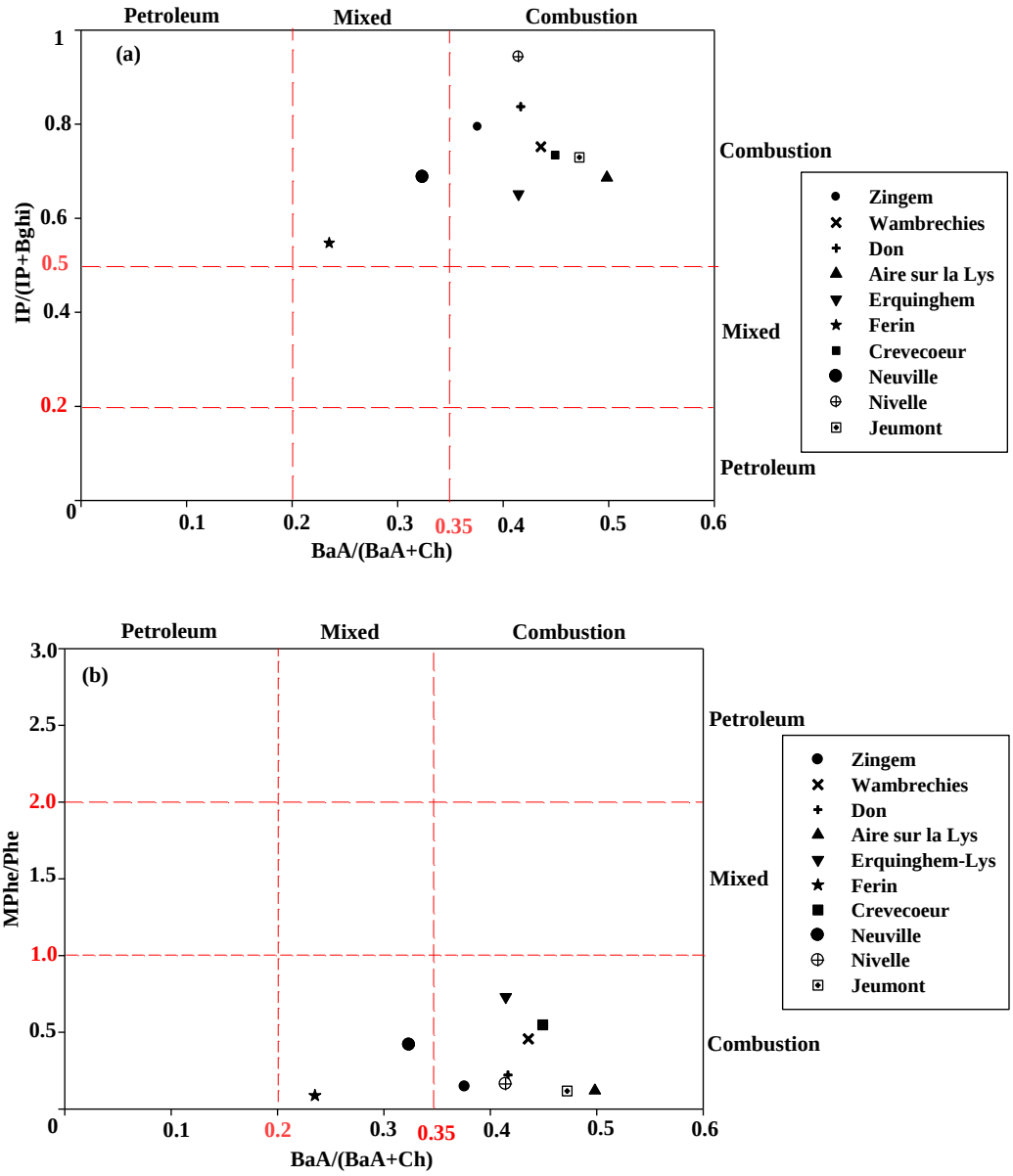

Fig. 8. Cross plots of (a) IP/(IP+Bghi) and (b) MPhe/Phe versus BaA/(BaA+Ch) for sediment samples.

The origin of PCBs has been usually identified by evaluating the similarity of the PCB patterns found in the samples compared to the principal commercial PCB mixtures (Aroclor) (Net et al., 2015a) or by examining the correlation between PCBs and other parameters (e.g. PAHs) (Merhaby et al., 2015; Wolska et al., 2014). Our results showed a linear correlation between PCB153 and Σ_{16}PAHs concentrations (R^2= 0.83, p<0.01), suggesting similar sources of emission. Therefore, PCB153 most likely also originated by pyrolysis processes such as coal, wood and peat combustion (Rose et al., 2004; Ruiz-Fernández et al., 2012).

3.5. Ecotoxicological state

Sediment Quality Guidelines (SQGs) have been established using different procedures for assessing the sediment quality and the ecotoxicological risk. Since similarities have been found between existing SQGs in many cases, "consensus" guidelines were developed to unify the guidelines and to take into account mixture effects (Macdonald et al., 2000; Swartz, 1999). Our results were comparable to the numerical consensus-based SQGs (CBSQGs) which include the Threshold Effect Concentration (TEC; the concentration below which adverse effects on sediment-dwelling organisms are not expected to occur), the Probable Effect Concentration (PEC; concentration above which adverse effects on sediment-dwelling organisms are likely to be observed) and the Midpoint Effect Concentration (MEC=TEC+PEC/2). The MEC was recommended to better interpret the potential impact of contaminants between the TEC and PEC that is not included in the CBSQGs (Doyle et al., 2003). The ecotoxicological risk of the studied sites is illustrated for four levels of sediment quality: 1(≤CBTEC), 2 (>CBTEC and ≤MEC), 3 (>MEC and ≤CBPEC) and 4 (>CBPEC) (Table 2, Fig. 9). Based on PCBs, quality 1 was assigned to all of the rivers. PCB153 was <CBTEC which predicts the absence of toxicity to benthic dwelling organisms based on this parameter. However, based on Σ_{16}PAHs, 70% of stations were identified as quality 2 and 30% identified as quality 3. Don, Wambrechies and Nivelles presented the highest ecotoxicological risk. Based on individual compounds, dibenz(a,h)anthracene was noted as the main pollutant, detected at 80% of the stations. Its concentration exceeded the generally recommended CBPEC which implies that adverse biological effects are expected to occur. On the other hand, naphthalene, phenanthrene, anthracene and fluorene were frequently detected (60–80%) but their individual concentrations were <CBTEC. 2-methylnaphthalene was detected in the majority of stations (90%) and was detected frequently >CBPEC which implies possible adverse effects on sediment-dwelling organisms. Based on 2-methylnaphthalene, the majority of the stations were identified as quality 4.

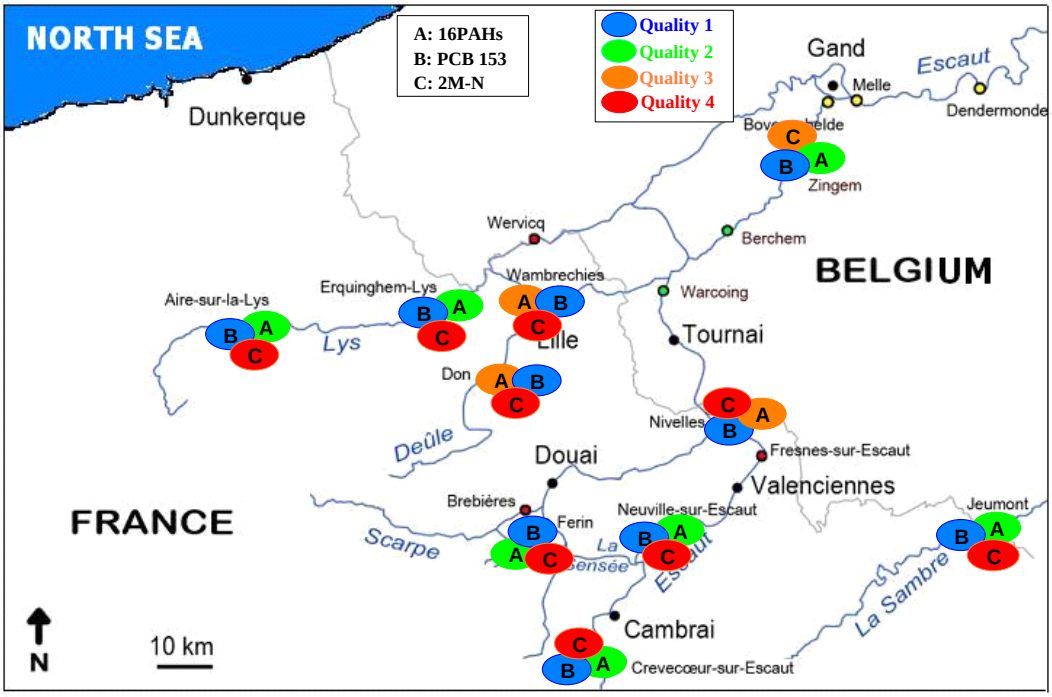

Fig. 9. Ecotoxicological risk of each site based on Σ_{16}PAHs and PCB153 and 2-methylnaphthalene.

Table 2: Consensus-based SQGs based on individual and total PAHs, 2-methylnaphthalene, total PCBs and quality of studied stations.

				Concentrations (µg/kg dw)													
				The Scheldt			The Deûle		The Lys		The Sensée	The Scarpe	The Sambre				
	µg/kg dw													% Station			
Compounds	CBSQGs TEC	MEC (TEC+PEC)/ 2	CBSQGs PEC	Zingem	Crèvecœur	Neuville	Wambrechies	Don	Aire-sur-la-Lys	Erquinghem	Ferin	Nivelles	Jeumont	Quality 1	Quality 2	Quality 3	Quality 4
Naphthalene	176	369	561	75.7	150.5	604.1	715.9	2755.2	197.8	381.3	33.8	875.5	60.8	40	10	10	40
Acenaphthylene	5.9	67	128	71.2	34.9	33.9	41.8	187.4	73.4	45.9	113.6	183.6	51.1	0	50	30	20
Acenaphthene	6.7	48	89	36.8	32.6	73.9	102.6	676.4	37.9	45.5	65.8	119.6	18.4	0	50	20	30
Fluorene	77.4	307	536	60.6	16.8	18.5	20.9	79.7	63.6	25.0	67.2	201.7	57.5	80	20	0	0
Phenanthrene	204	687	1,170	114.4	121.8	140.6	148.2	1369.9	162.0	93.6	335.7	416.0	491.4	60	30	0	10
Anthracene	57.2	451	845	32.6	28.7	24.3	54.6	136.7	41.0	44.2	40.9	73.7	90.9	70	30	0	0
Fluoranthene	423	1327	2,230	190.5	546.5	582.4	665.8	2393.6	223.1	362.9	404.8	309.1	407.1	60	30	0	10
Pyrene	195	858	1,520	421.0	1149.6	1246.1	12436.5	3468.9	387.3	788.6	897.0	722.2	748.3	0	50	40	10
Benz(a)Anthracene	108	579	1,050	120.6	275.8	180.2	208.6	427.4	228.3	173.7	107.1	149.7	214.3	10	90	0	0
Chrysene	166	728	1,290	200.4	338.1	376.7	270.1	598.6	230.0	245.5	348.4	211.5	239.5	0	100	0	0
Benzo(b)fluoranthene	240	6,820	13,400	579.2	1411.1	1148.2	1941.4	4983.6	586.9	948.9	1099.3	1276.1	1044.6	0	100	0	0
Benzo(k)fluoranthene	240	6,820	13,400	229.7	354.6	340.1	413.3	963.2	215.7	268.7	329.5	342.7	307.6	20	80	0	0
Benzo(a)pyrene	150	800	1,450	224.5	292.4	253.8	277.3	546.7	233.5	279.8	240.8	241.0	228.0	0	100	0	0
Indeno(1,2,3-cd)pyrene	200	1,700	3,200	1112.4	796.1	650.2	912.2	2129.7	668.6	555.3	582.1	7149.5	1110.3	0	80	10	10
Dibenz(a,h)anthracene	33	84	135	115.6	309.9	236.7	416.9	1173.8	98.7	207.0	324.9	236.9	227.5	0	0	20	80
Benzo(g,h,i)perylene	170	1,685	3,200	228.5	288.2	295.5	300.8	413.9	305.5	297.4	481.3	429.8	411.6	0	100	0	0
ΣPAH	1,610	12,205	22,800	3873.6	6147.4	6205.1	18926.6	22304.5	3753.2	4763.3	5472.2	12965.2	5708.9	0	70	30	0
2-methylnaphthalene	20.2	111	201	174.9	2623.8	2556.5	384.7	2199.1	392.0	759.9	1314.0	486.8	243.3	0	0	10	90
ΣPCBs	60	368	676	0.7	0.6	0.3	6.6	7.3	0.8	0.9	0.2	2.3	3.5	100	0	0	0

Conclusion

The occurrence of PAHs, Me-PAHs and PCBs was investigated in three matrices (dissolved phase, associated with SSM and sediment). Fifteen stations located in six rivers at the cross-border area of northern France and Belgium were studied. Their distribution in dissolved, particulate and sedimentary phases depended strongly on their physicochemical properties. LMW-PAHs were mainly present in the dissolved phase while HMW-PAHs accumulated preferentially in SSM and sediments. Of the PCBs, PCB153 congener remains detectable even though PCB use has been banned for many years; it was found mainly in sediment. The spatial distributions were also studied and a correlation between Σ_{16}PAHs and PCB153 concentrations was observed. Various types of combustion such as wood and coal burning and vehicular emissions could be the major sources of these contaminations for the studied sites. Industrial and domestic activities could be major contributors. The most polluted sampling stations are generally close to big cities. Moreover, high levels of nitrate indicate that agriculture may be a non-negligible source of pollution. Considering ecotoxicological assessment, based on PCB153 the sampling sites were identified as good quality. However, PAHs and 2-methylphenanthrene are likely to adversely affect aquatic wildlife.

Acknowledgment

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III.2. Niveaux de contamination des HAP, Me-HAP et PCB dans les sols

III.2.1. Occurrence et profil de distribution des HAP, Me-HAP et PCB

La distribution spatiale des HAP et Me-HAP varient selon les zones géographiques et les activités urbaines, industrielles ou agricoles entreprises localement ou dans les zones environnantes. Dans notre cas, les concentrations totales de 16 557,3±31,8 et 1790,6±195,9 µg/Kg ont été détectées dans les échantillons prélevés respectivement sur le parking du Campus de l'Université Lille 1 (Site 1) et sur une ancienne station d'essence à Villers (Site 2). Les concentrations enregistrées pour les sols originaires de Madagascar (Site 3) se trouvent inférieures aux limites de quantification (LOQ) qui varient de 0,3 à 5 µg/kg pour les HAP. En comparant avec la littérature, nos résultats se trouvent être largement supérieures à ceux obtenus dans des zones résidentielles, voire même à proximité d'une industrie chimique (Nadal et al. 2004). Les espèces majoritaires détectées sont le Benzo(a)anthracène

(106,9±2 µg/kg) et le Pyrène (672,1±144,8 µg/kg) respectivement pour le site 1 et le site 2. Quant au profil de distribution, on observe la prédominance des HAP de haut poids moléculaires (4-6 cycles benzéniques) par rapport aux HAP légers (2-3 cycles benzéniques). La somme de concentration des HAP lourds est quantifiée à 57% sur sols prélevés sur le parking de l'Université Lille 1 et 81% sur le sol de la station d'essence. Le profil de distribution des HAP et Me-HAP dans le sol sur le parking de Lille 1 est présenté sur la figurue 22A et 22B.

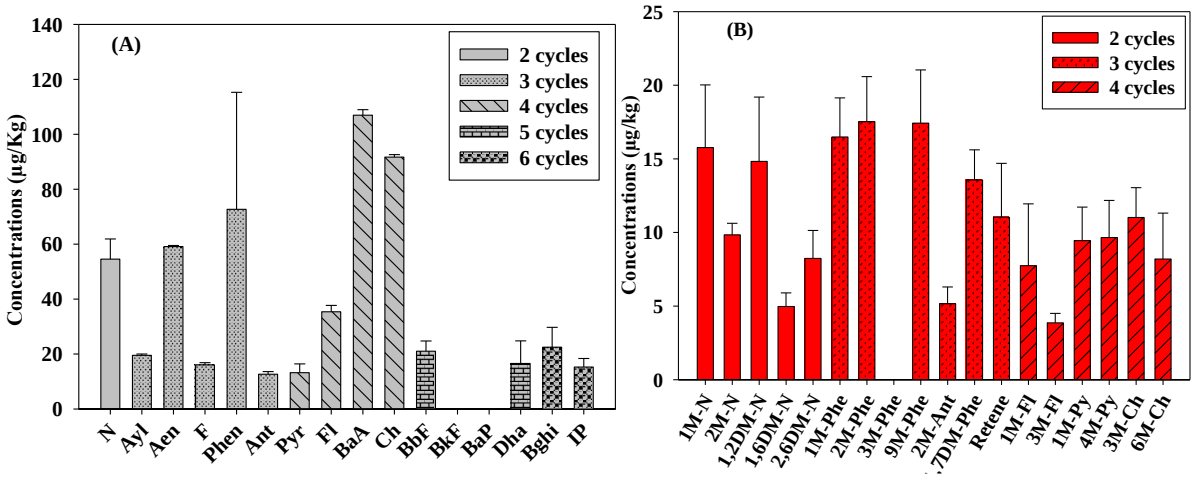

Figure 23: Profil de distribution des (A) HAP et Me-HAP(B) dans le sol sur le parking du Campus Lille 1

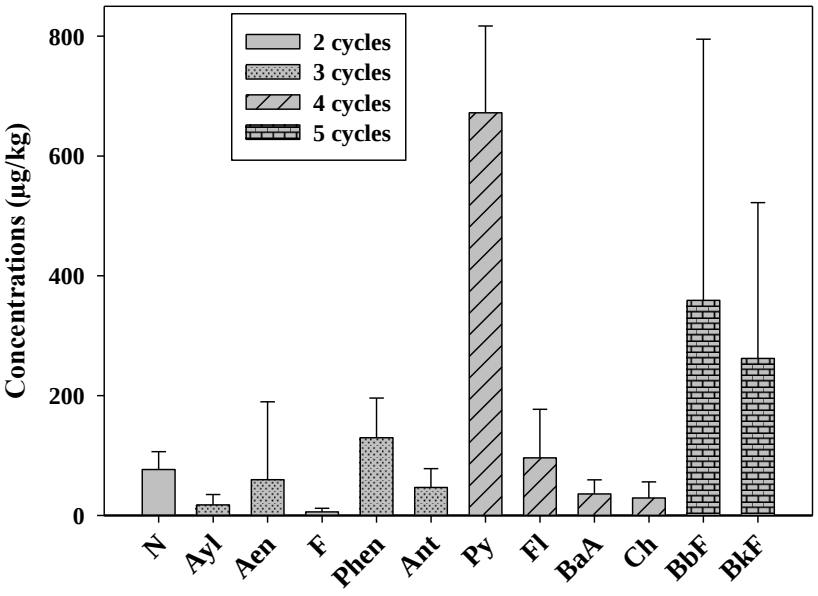

Figure 24: Profil de distribution de HAP dans le sol de Villers (Flers en Escrébieux, ancienne station d'essence)

Pour les Me-HAP, seulement le site 1 (Parking d'Université Lille 1) a fait l'objet de quantification. La somme des concentrations des 18 Me-HAP était de 184,8±4,4 µg/kg. On remarque que la teneur en HAP méthylés est généralement inférieure à celle des HAP parents (Figure 24). Les dérivés méthylés du phénanthrène participent majoritairement dans ce type de contamination avec une concentration totale Σ(1M-Phe + 2M-Phe+ 9M-Phe + 1,7DM-Phe) égale à 65 µg/kg dont 35% Σ_{18}Me-HAP.

Pour les PCB, les résultats ont montré que le parking de Campus d'Université Lille 1 était fortement contaminé. La somme des 28 congénères de PCB sélectionnés dans cette étude (Σ_{28}PCB) était de 111,8±17,4 mg/kg. Parmi les 28 congénères, 10 congénères de PCB (PCB 18, 44, 52, 44, 66, 66, 81, 118, 105, 170, 180 et 187) ont été détectés. La concentration la plus élevée est attribuée au congénère trichloré (CB18) qui représente 53% de la concentration totale. Ce congénère a été aussi détecté dans d'autres compartiments de l'environnement (sédiment) (Barakat et al., 2002, 2012 ; Hong et al., 2006 ; Net et al., 2015).

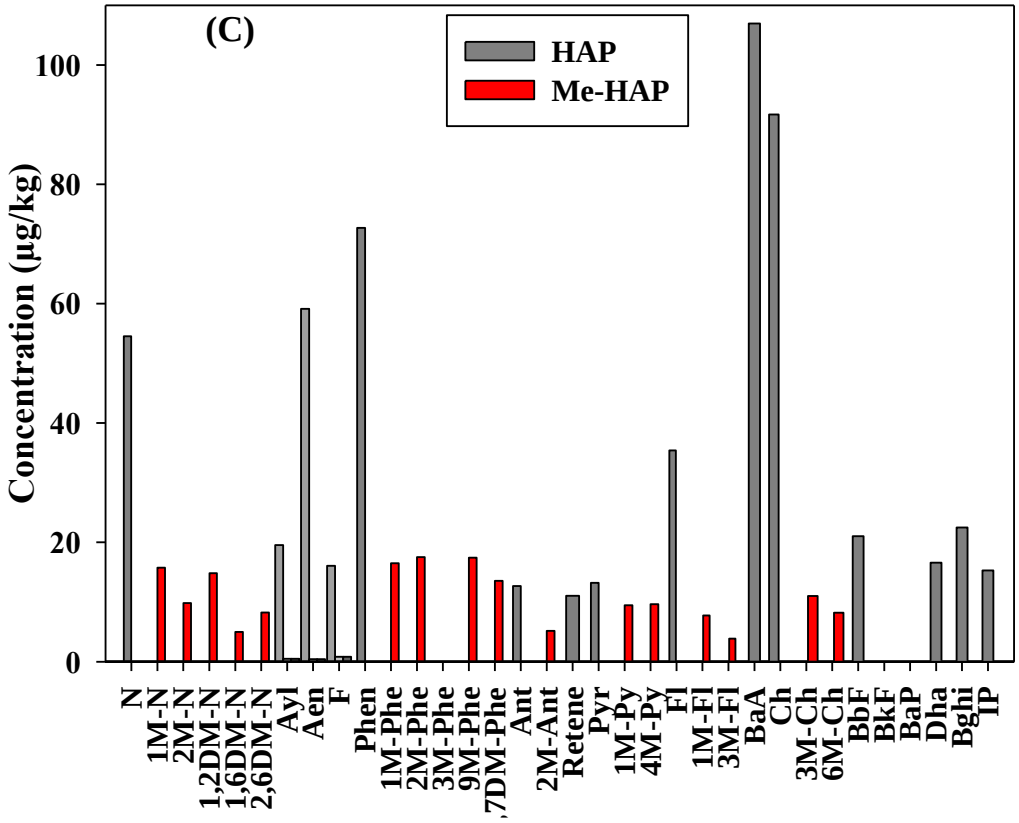

Figure 25: Répartition des Me-HAP par rapport aux HAP parents

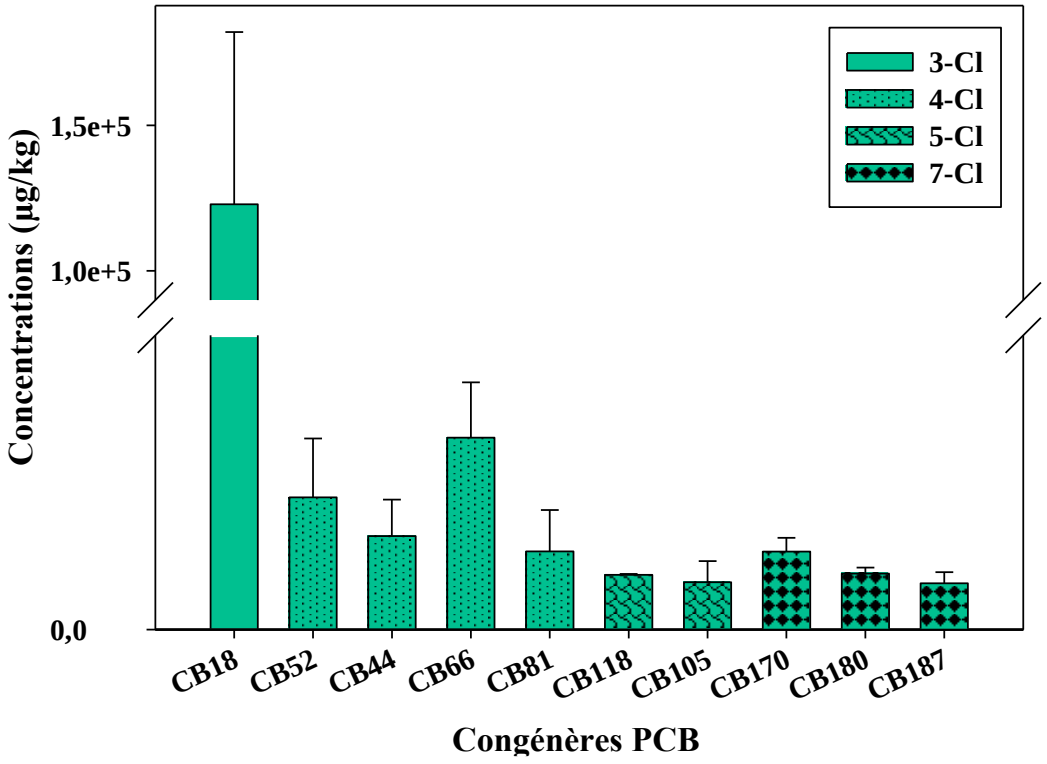

Figure 26: Profil de distribution de PCB sur le sol prélevé sur le Campus de Lille 1

Tableau 9: Les concentrations des POP dans les sols

	Moyenne ± σ en µg/kg poids sec		
Molécules	Site 1	Site 2	Site 3
N	54,6 ± 7,4	76,6 ± 29,8	<LOQ
Ayl	19,5 ± 0,5	17,7 ± 17,2	<LOQ
Aen	59,1 ± 0,4	59,7 ± 129,9	<LOQ
F	16,1 ± 0,8	5,9 ± 5,9	<LOQ
Phen	72,7 ± 42,6	129,7 ± 66,3	<LOQ
Ant	12,7 ± 0,9	46,7 ± 31,4	<LOQ
Pyr	13,2 ± 3,2	672,1 ± 144,8	< LOQ
Fl	35,4 ± 2,3	96, ± 81,1	< LOQ
BaA	106,9 ± 2	35,8 ± 23,6	< LOQ
Ch	91,7 ± 0,9	29,3 ± 26,6	< LOQ
BbF	21 ± 3,7	359 ± 435,9	< LOQ
BkF	Nd	262 ± 260,1	< LOQ
BaP	Nd	Nd	< LOQ
Dha	16,6 ± 8,2	Nd	< LOQ
Bghi	22,5 ± 7,2	Nd	< LOQ
IP	15,3 ± 3,1	Nd	< LOQ
ΣHAP	557,3 ± 31,8	1790,6 ± 195,2	< LOQ

« Nd » : non détérminé ; LD : limite de détection

III.3. Identification des sources

La prédominance des HAP de haut poids moléculaires implique généralement une origine pyrolytique des HAP. Pour mieux identifier les sources des HAP dans les sols, les valeurs de ratio des isomères des HAP individuels ont été également utilisées (Barakat et al., 2011 ; Miki et al., 2004 ; Yunker et al., 2002). Dans le présent travail, les ratio BaA/ (BaA+Ch) et Fl/(Fl+Py) sont entrecroisés ensemble et ont révélé que les HAP détectés dans sur le parking du Campus de Lille 1 (site) sont d'origine pyrogénique tandis que ceux détectés dans le site 2 ont un mélange de source (combustion/pétrogénique). Malgré le fait que le site 2 est une ancienne station d'essence, d'autres sources que pétrogénique ont contribué tels que les dépôts atmosphériques ou les effets des combustions de bois et de charbon.

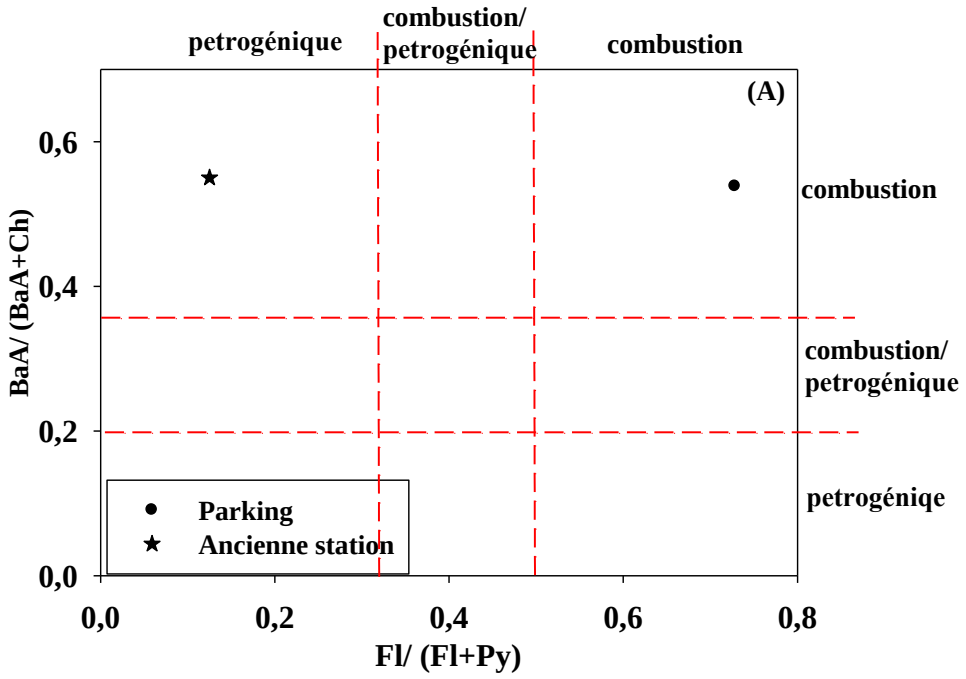

Figure 27: Ratio des isomère BaA/(BaA+Ch) vs Fl/(Fl+Py)

III.4. Etude de risque écotoxicologique

Les HAP, Me-HAP et PCB sont des substances potentiellement toxiques (US-EPA). Les directives de qualité du sol ont été établies dans le but de protéger à la fois la santé humaine et l'environnement. Les données concernant les sols sont rare mais on peut distinguer celles établies par les canadiens. Le critère de qualité des sols a été établi par le conseil canadien des ministres de l'environnement (CCME 1991).

Il est à noter que l'un des soucis majeurs dans l'évaluation des risques chimiques est l'effet de mélange (effet cocktail). Dans les cas des HAP, ils sont généralement détectés, dans l'environnement, sous forme de mélange ; à titre d'exemple, c'est rare que le naphtalène soit introduit dans l'environnement sans ses autres homologues. Ce fait nécessite une attention particulière sur les risques ainsi que les valeurs seuils acceptables pour tout l'ensemble des HAP dans le mélange mais non pas d'un seul HAP. Pourtant, il n'existe aucune norme unique destinée à pouvoir évaluer la qualité des sols contaminés et qui pourrait contribuer tant à la protection de la santé humaine que de l'environnement. Ainsi, le conseil canadien des ministres de l'environnement (CCME) propose trois étapes à suivre pour l'évaluation des risques éco toxicologiques du système terrestre (sol). En effet, pour protéger la santé humaine, il faut tenir compte des effets cancérogènes et non-cancérogènes des HAP tandis que pour

promouvoir la protection de la santé environnementale, on considère leurs effets non-cancérogènes. Le schéma suivant illustre ces trois étapes d'évaluation des risques dans les sols contaminés

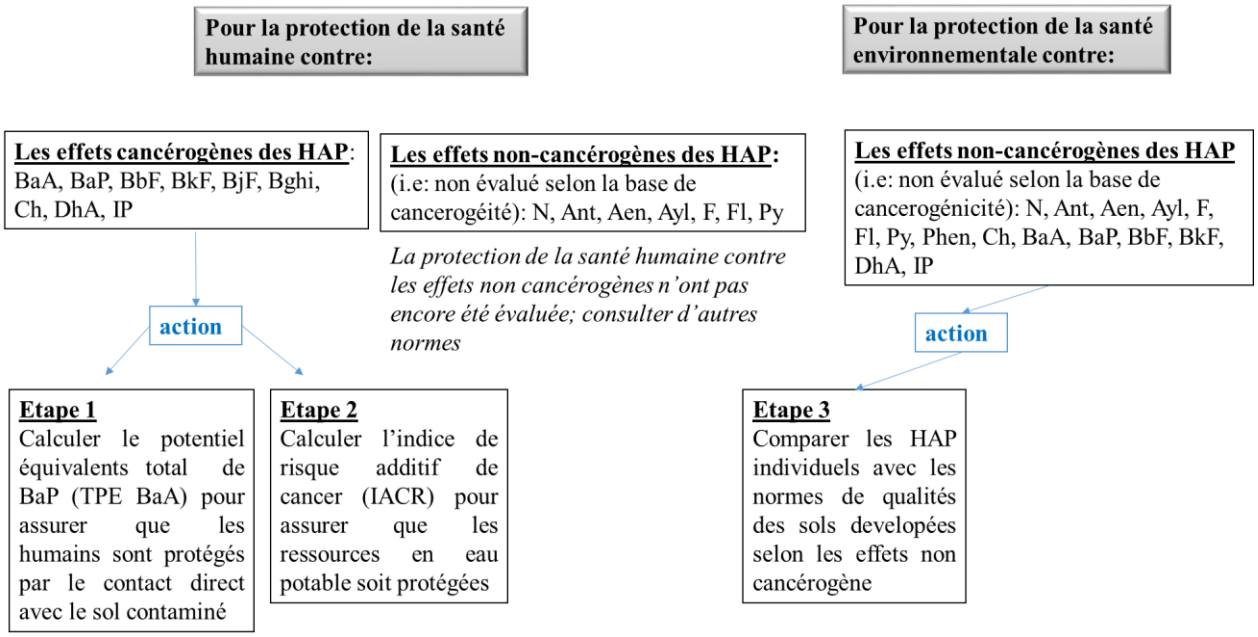

Figure 28: Les étapes d'évaluation de risque écotoxicologique lié aux sols contaminés selon le CCME

Pour les PCB, les recommandations sont catégorisées selon l'usage du sol selon le tableau ci-dessous (Tableau 10)

Tableau 10: Valeurs seuils correspondant à l'usage des sols

Sol destiné à :			
Valeur en mg/Kg			
Agriculture	Résidentiel/parc	Commerce	Industrie
0,5	5	50	50

III.5. Faisabilité de bioremédiation des HAP

La seconde publication est spécialement dédiée à l'étude de bioremédiation de quelques molécules modèles de HAP contenus dans le sol. En effet, l'article décrit toutes les étapes franchies depuis le choix des matériels biologiques nécessaires jusqu'à l'étude cinétique de dégradation des molécules HAP considérées. Il met en exergue également l'efficacité de traitement biologique en utilisant les souches bactériennes *Bacillus pumilus, Bacillus simplex et Pseudomonas stutzeri.*

Article 2

Potentiality of bacterial strains isolated from hydrocarbon-polluted soil to degrade fluorene and phenanthrene in PAHs-contaminated soil

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Abstract

Biodegradation of 3-ring and 4-ring PAHs model namely fluorene, phenanthrene, fluoranthene and pyrene was investigated. PAHs-degrading bacteria were firstly screened to select high tolerant species for insuring an efficient bioremediation. Twenty seven bacterial strains were isolated from an aged contaminated-site by oil spills before the acclimatization step. Then, each isolate was grown in four different concentrations of PAHs mixture. Among the 27 isolated strains, 8 were identified resistant to high level of PAHs (1500 mg/L) and can use PAHs as source of carbon and energy. These potent strains were then identified by mass spectrometer MALDI-TOF VITEK MS. They belonged to species *Pseudomonas stutzeri*, *Bacillus simplex* and *Bacillus pumilus* and were used for bioremediation experiment of soils contaminated by PAHs. The study was carried out under controlled conditions using soil spiked with a mixture of the target PAHs and the three microcosm strains. For 72 days of incubation, only the low molecular weight PAHs (fluorene and phenanthrene) were demonstrated efficiently removed with elimination rates between 65-86% and 86-95% for fluorene and phenanthrene, respectively. While high molecular weights PAHs (pyrene and fluoranthene) were recalcitrant to these selected microbial degradations. The biodegradation kinetics of both fluorene and phenanthrene were fitted with first order rate with R^2 values ranged from 0.88 to 0.92. The half-lives of phenanthrene (2.4 - 2.7 days) and of fluorene (3.5 - 4.6 days) were all less than 10 days implying that they were rapidly degraded with the acclimated strains.

Keywords: *Pseudomonas stutzeri, Bacillus pumilus, Bacillus simplex,* PAHs bioremediation, telluric microorganisms, bacterial identification

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are one of the major concerns in environmental pollution. Due to their toxic, carcinogenic or mutagenic effects (IARC, 2010; Wang et al., 2015), sixteen PAHs have been listed as priority substances by the United States-Environmental Protection Agency (US EPA) and six of them listed in Water Framework Directive of Europe. Their concentrations have been controlled and regulated for various environmental matrices. However, PAHs are widely disseminated in all compartment of the environment (air, fresh and sea water, sediment, soil and biota) (Merhaby et al., 2015; Rabodonirina et al., 2015). Anthropogenic activities have been reported as the major source of PAHs in the environment. High levels of PAHs were recorded more frequently in central urban than in rural area (Melnyk et al., 2015; Pozo et al., 2015).

PAHs contain two or more fused benzene rings in linear, angular or cluster arrangement and they are formed from incomplete combustion of organic substances such as coal, oil, gas, wood, garbage, and from petrochemical/oil refining industries (Yunker et al., 2002). They have a tendency to a long range transport and exhibit toxic effects toward the ecosystems and the human health. PAHs are hydrophobic and thus they preferentially sorbe into solid matrixes (organic matter, soil, sediment) or concentrate into the fatty tissues of organisms rather than entering in aqueous phase (Net et al., 2015; Wenning and Martello, 2013). Their low water solubility constitutes a limiting factor for their availability to microorganisms, which inhibit their bioremediation in the contaminated sites. Environmental factors such as (i) physical and chemical characteristics of the substrate, nutrients status, pH, temperature and (ii) biotic factors such inoculum density influence the accomplishment of any bioremediation process can also influence on their fate (Wilson and Jones, 1993).

These last decades, soil contamination by hydrocarbons has been one of the global concerns. Their elimination becomes now a necessity to reduce their impacts on ecosystem and human health and their dissemination into other compartments of the environment. Several techniques have been developed to clean-up PAHs-contaminated sites (Gonzalez et al., 2011; Hamdi et al., 2012). One of the effective techniques, the biological approach, has been widely recommended (Morillo et al., 2012) because of its cost-competitivity and environmental safe treatment. This technique exploits the ability of microorganisms to degrade and/or detoxify organic contaminants. Few studies have focused on the metabolic activities of hydrocarbon-oxidizing bacteria (Zafra et al., 2014). Mineralization,

transformation and/or immobilization of pollutant to other non- or less toxic products are the main pathway of the bacterial detoxification (Haritash and Kaushik, 2009). Some bacterial species have been reported as producer of emulsifier substances named "biosurfactants" which can effectively increase the soluble fraction and presumably the amount available for microbial uptake thus enhance the biodegradation rate of the slightly soluble contaminants (Hughes et al., 1997; Kosaric and Sukan, 1993). Some microorganisms (bacteria and fungi) species have been reported as potential species to eliminate PAHs under aerobic and/or anaerobic conditions (Seo et al., 2009).

This study aims to evaluate the ability of three bacterial strains isolated from an aged contaminated soil to remove four PAHs (fluorene, fluoranthene, phenanthrene and pyrene). Firstly, the species have been acclimatized and then laboratory-scale experiments of bioremediation have been carried out to investigate the kinetic of the degradation of the target PAHs.

2. Materials and methods

2.1. Chemicals and reagents

Powders of phenanthrene, fluorene, fluoranthene and pyrene with 98% of purity were purchased from Acros organic (France). Acenaphthene-d10 (A-d10), phenanthrene-d10 (Phe-d10) and pyrene-d10 (Pyr-d10) were obtained from LGC-Promochem (Middlesex, UK) and used as internal standards. HPLC-grade hexane, dichloromethane (DCM) and acetone were purchased from Dislab (France). Merck silica gel 60 (70-230 mesh) activated at 450°C was heated at 120°C for 12 h prior to use. Glassware was systematically washed with detergent (Decon, East Sussex, UK) and acidified ultrapure water, rinsed with ultrapure water followed by acetone then dried at 120°C prior to use. The medium were prepared aseptically.

2.1. Microorganism isolation

Bacterial strains isolation from an aged hydrocarbon-polluted soil was carried out by serial dilution method. Five grams of soil sample were taken in 45 mL of distilled water and agitated vigorously. A dilution series of the suspensions from $1x10^{-1}$ to $1x10^{-7}$ was performed and an aliquot of 0.1 mL of each dilution was spread in Tryptic soy medium (Diedrich, 2007). Petri dishes were incubated at 30±2 °C for 24-48 hours. Isolated strains were purified and preserved in 50% of glycerol at -80°C.

2.2. Microorganism acclimatization/Bio-stimulation

Isolate was tested for their tolerance of a high level of PAHs and their ability to use PAHs as their carbon and energy source. Isolate was grown on nutrient broth with ~~in~~ four different doses of PAHs mixture (250, 500, 1000 and 1500 mg/L). The PAHs mixture was prepared by mixing fluorene, phenanthrene, fluoranthene and pyrene in acetone. The mixture was put in sterile tubes and left at room temperature for 12 hours. After solvent evaporation, 10 mL of sterile nutrient broth was added and agitated. Then, 100 µL of pure bacterial culture was poured into the mixture and was incubated at 30°C for a delay between 24 h and 72 h. Cells were grown under stationary condition~~.~~ Growth strains were indicated by the troubles of the liquid medium. The criteria for selecting PAHs-degrading bacteria were based on their capacity to grow in the most contaminated media with 1500 mg/L of PAHs mixture.

2.3. Preparation of the inoculum

Inoculum was prepared from pure culture of selected isolate resistant at 1500 mg/L of PAHs mixture. Pure colonies were suspended in 10 mL of nutrient broth, incubated at 30±2°C until troubles were observed, around 24-72 h. Then, cells were collected by centrifugation (3.7x1000/mn pendant 8mn), and washed three times with a physiologic solution (9 g/L) to remove the remaining carbon source. Distilled sterile water was added, carefully agitated and 2mL per gram of soil d.w. were inoculated.

2.4. PAHs bioremediation trial

The bioremediation experiment was carried out at the laboratory during 72 days, under sterile and controlled conditions. The targeted compounds were fluorene (Fl), phenanthrene (Phen), fluoranthene (Flu) and pyrene (Pyr). 100 sterile amber vials covered with aluminum foil were prepared as microcosms. Soils samples were dried at room temperature around 10 days, sieved at 224 µm and agitated. The initial amount of PAHs in soils was quantified using Gas Chromatography coupled with a Mass Spectrometry (GC-MS). The texture of the soil was composed of sand, silt and clay with the proportion of 33.9%, 61.9% and 0.2% respectively.

Five grams of sterilized soil were put in each amber vial and spiked with the PAHs mixture, previously prepared in acetone at a final concentration of 500 mg/kg. After solvent evaporation, 2 mL of the inoculum (10^7–10^8 cfu/g) were added in the sterilized soil (Lors et al., 2012). These total bacterial populations were similar to which typically found in the

superficial layer of unpolluted soils (Taylor et al., 2002). The moisture was adjusted to 30-60% (Sabaté et al., 2004). Once a week, the microcosm was aerated, and sterile deionized water was added to maintain the moisture level. pH was maintained at pH 7; C/N ratio was 100/1.7 (Bouchez et al., 1995; Wilson and Jones, 1993). The vial content was agitated and incubated at 30±2°C during 72 days. Experiments were performed in triplicate and the whole treatment was conducted aseptically. Blank control constituted of soil spiked with PAHs without tested strains was used. The residual PAHs were determined at the beginning of incubation (t=0), 1 day then each week during 72 days and the microbial population on the onset and at the end of the experiment were enumerated.

2.5. Identification of selected isolates

Only the potent isolates were submitted for identification by VITEK MS^{TM} (bioMérieux, Marcy-l'Etoile, France). All the analysis was conducted at the clinic hospital Victor Provo in Roubaix, France. The method used the Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) which is automated with a software system designed for rapid microbial identification. MALDI-TOF has quickly become the state-of–the-art technology and has commonly used in clinical microbiology laboratory (Nobrega de Almeida Junior et al., 2014) because of its accuracy, rapidity, and cost effective compared to the conventional phenotypic identification (Bille et al., 2012; Bizzini et al., 2010).

Initially, potent isolates were cultivated in nutrient agar plates at 30°C for 48 h. Colonies were then spotted in triplicate onto the MALDI target plate, air-dried at room temperature and finally introduced to a high-vacuum environment. A precise laser burst ionized the sample releasing a “cloud” of proteins that accelerated by an electric charge. The lighter proteins travel faster than the heavier proteins and every Time of Flight of proteins was recorded and detected with a sensor to create a spectrum. The spectrum represents the protein fingerprints of each sample. Each targeted species were identified by comparing the spectrum of the sample against a large database of spectra from precisely characterized bacteria. This step allows identifying the species, their genus and family level. Only the spot returning the highest probability of identification was considered. Indeed, the identification with a score >85% was considered acceptable and reliable (Jamal et al., 2014).

2.6. Residual PAHs analysis

2.6.1. ASE extraction

Residual PAHs were extracted from 5 g of the soil samples using an Accelerated Solvent Extraction or ASE (ASE 200, Dionex Corp., USA). Firstly, soil samples were spiked with deuterated internal standards A-d10, Phe-d10 and pyr-d10. After a delay of equilibration, the soils were extracted with ASE. The extraction conditions were: heat 5 min, temperature 160°C, static solvent extraction time 12 min with 2 static cycles, pressure 14 MPa, purge 3 min and flush 60% according to the method developed by Net et al. (2014). A mixture of hexane/acetone (1/1 *v/v*) was used as extraction solvent. High purity nitrogen was employed as the purge gas.

2.6.2. Purification and pre-separation

After the extraction, the extracts were purified to eliminate the interferences. Firstly, molecular sulfur was removed by addition of activated metallic copper. The extracts were concentrated and were then purified and fractioned by liquid chromatography on a silica column to eliminate organic interferences. 2 g of anhydrous sodium sulfate was added on the top of the column to eliminate the eventual traces of water. Targeted compounds were recovered by successive elution with 20 mL of hexane, 15 mL of hexane/DCM mixture (3/1, *v/v*) followed by 15 mL of hexane/DCM mixture (1/1, *v/v*) and 15 mL DCM. Each sample was concentrated to a final volume of 1 mL and stored at 4 °C before analysis.

2.6.3. Quantification of residual PAHs

The extracts were finally analyzed with a Varian 3900 gas chromatograph (GC) equipped with a deactivated fused-silica guard column (5 m, 0.25 mm i.d.) and a fused-silica capillary Phenomenex XLB (60 m length, 0.25 mm i.d., 0.25 μm film thickness) and coupled with a Varian Ion Trap Saturn 2000 Mass Spectrometer (MS). The carrier gas was helium, held at a constant flow rate of 1 mL/min. Samples were injected in the splitless mode at 280°C and the injector was purged with helium after 1 min. The transfer line and the ion trap were held at 280°C and 220°C, respectively. The oven temperature was programmed as follows: from 70°C (1 min) to 170°C at 10°C/min, then ramped at 4°C/min to 230°C, and then at 3°C/min (13 min) to 300°C. Each targeted compound was identified based on the retention time and the mass spectrum from a chromatogram of standard solutions acquired in

full scan mode. For better selectivity and sensibility, quantification was performed in the Selected Ion Storage (SIS) mode. Response factors were determined relative to the internal standards previously chosen to better fit to the properties of each comp

2.6.4. Total PAHs-degrading microbial enumeration

Total bacterial populations on the onset and at the end of the experiment were enumerated. The viable cell count was achieved using the most probable number method (MPN). Bacterial population was expressed by colony-forming unit per g of dry soil (CFU/g soil).

3. Results and discussions

3.1. Quality assurance and quality control

Target plates were calibrated and quality controlled both by using *Escherichia coli* ATCC8739 in each run in MALDI-TOF MS system, in accordance with the manufacturer's recommendations. Uninoculated matrix was also included in each run as a negative control. For PAH quantification, procedural and method blanks were also performed by using the same pretreatment conditions as the incubated samples. Each analysis was performed in triplicate. Procedural and method blanks analysis were free from any targeted PAHs. The recoveries were 79, 103, 134 and 114% respectively for fluorene, phenanthrene, fluoranthene and pyrene with an average of 107%. The limits of quantifications (LOQs) were 0.5, 0.3, 1.0 and 0.5 µg/kg d.w. soil respectively for fluorene, phenanthrene, fluoranthene and pyrene.

3.2. Microbial isolation

A total of 27 bacterial strains were isolated from the aged-contaminated soil collected from Madagascar. Isolation of strains from a heavily contaminated site was considered because it could increase the chance of isolating high diversity of microbial population able to tolerate and degrade high concentrations of PAHs on soil (Zafra et al., 2014). Among this isolated strains, 8 were resistant to high concentration of PAHs (1500 mg/L) as carbon source. They did not show inhibitory effect during the tolerance test and exhibited a slow growth. Thus, they were selected for bioremediation experiment.

3.3. Identification of selected isolates

Preliminary morphological identification of the selected isolates showed Gram-positive and Gram-negative, bacilli bacteria. Out of the 8 isolates, 5 were identified by VITEK MS^{TM} without any extraction or purification and were achieved after a single acquisition. After elimination of duplicates, three strains were closely related to *Bacillus simplex, Bacillus pumilus* and *Pseudomonas stutzeri* with respectively 99.9%, 80.1% and 99.9% of accuracy. Unfortunately, the three other species could not be identified because their first acquisition was not contributory and even after a second acquisition, no reliable identification was obtained. This may be due to an unknown profile attributed to new species (Fernandez-Olmos et al., 2012) or an insufficient quality of the spectrum (Eigner et al., 2009). In any case, the bacteria identified in this study have already been described as hydrocarbon degraders except *Bacillus simplex* strain. In our knowledge, a few data were recorded concerning its ability in bioremediation process. According to Zafra et al. (2014), *Bacillus simplex* was moderately tolerant to high concentrations of PAHs (Phe, Pyr, BaP) and showed inhibition at 500 mg/L. Our results showed, however, that *Bacillus simplex* was 3 times more resistant; without inhibition at 1500 mg/L of PAHs mixture. *Pseudomonas* and *Bacillus* are common inhabitants of petroleum-polluted ecosystems and are well known for their capacity to degrade a range of petroleum hydrocarbons (Radwan et al., 2005; Zhang et al., 2010). They were the most reported as biological agents widely used in biodegradation process mainly for hydrocarbons attenuation (Balba et al., 1998; Cybulski et al., 2003). *Pseudomonas* species are able to degrade aromatic contaminants such as PAHs (Lors et al., 2010; Ramos et al., 1990). While *Bacilus pumilus* was reported to degrade persistent organic pollutants such as pesticides (Anwar et al., 2009). Moreover, *Bacilus pumilus* can develop at a large rage of pH and temperature, tolerant to detergents, unlike the mesophilic proteases which face these limitations (Kumar, 2002). However, few data about biodegradation of PAHs using *Bacillus pumilus* were reported.

3.4. Removal of PAHs

There was only a minimal change (0.5-0.9%) of PAHs concentrations in soil without bacterial inoculation. This indicate that abiotic loss of target PAHs was negligible in the studied conditions. The molecular weights are one of the factors which influence the biodegradability of PAHs. Our results showed that the three selected microorganisms can degrade efficiently low molecular weight PAHs (LMW-PAHs) such as fluorene and

phenanthrene. Rapid degradations of fluorene and phenanthrene were observed in early stage (within week 1) (*Figure 1*). However, no significant degradation was observed for high molecular weight PAHs (HMW-PAHs) such as fluoranthene and pyrene. Indeed, compounds with fewer aromatic rings were better removed only by bio-stimulation (Chagas-Spinelli et al., 2012) while those with 4 rings were typically recalcitrant (Alexander, 1999) and may be degraded slower (Silva et al., 2009). The effect of mixture contaminants may be the possible reason of this inhibition. Cometabolism process was also reported as a possible fate of PAHs degradation, augmentation or no effect at all (Bouchez et al., 1995). The biodegradation of complex mixture of PAHs depend not only on the presence of degraders and nutrients but also on their distribution in the different size of the soil particles (Amellal et al., 2001).

After 72 days of incubation, LMW-PAHs removal rate ranged between 65-86% and 86-95% for fluorene and phenanthrene respectively. *Figure 1A&1B* show that the degradation of fluorene and phenanthrene followed a positive exponential trend. The degradation pattern was rapid at the initial phase then the degradation slowed down relatively. More than 40% of fluorene and phenanthrene were removed within one week. In all experiment, *Bacillus simplex* degraded relatively better than *Pseudomonas stutzeri* and *Bacillus pumilus*. Fluorene was degraded slower than phenanthrene with 87% against 95% of elimination rate. Almost complete degradation of fluorene (95%) occurred within the after 72 days of treatment of incubation (*Figure 1B*). However, *Figure 1B* exhibits that no significant difference was noted between phenanthrene attenuation with the two other strains. For phenanthrene, the potential degradation of *Bacillus pumilus* and *Pseudomonas stutzeri* were similar compared to the potential degradation of *Bacillus simplex* (*Table 1*).

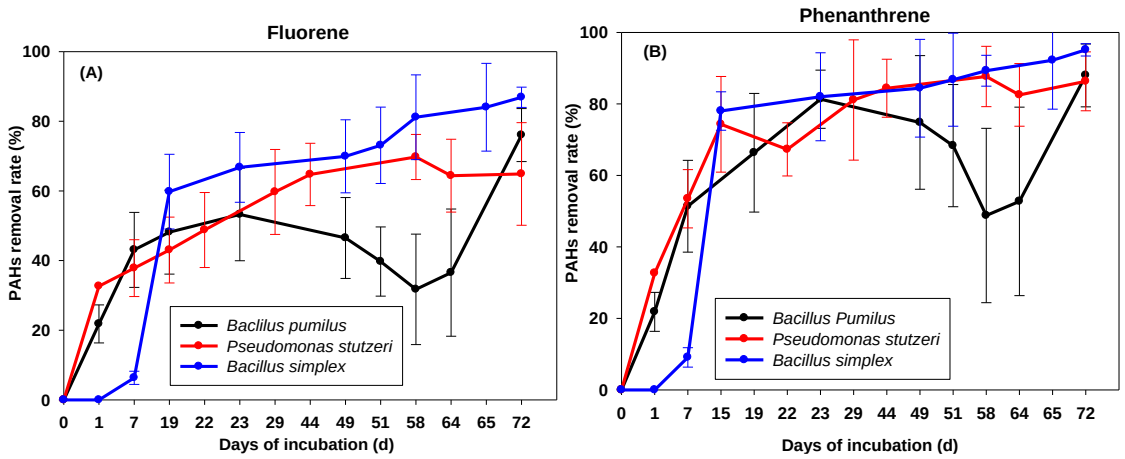

Figure 1: Degradation rate of (A) fluorene and (B) phenanthrene by Pseudomonas stutzeri, Bacillus pumilus and Bacillus simplex.

As mentioned above, no significant degradation was observed for HMW-PAHs (pyrene and fluoranthene). Ayotamuno et al. (2009) has repported that *Bacillus* and *Pseudomonas* species can degrade 3-ring and 4-ring PAHs. Our results, however, showed that there is no elimination. Environmental or nutritional conditions may be not as much favorable to the microbial growth. Microbial factors as their catabolic capacity may not be appropriate and were probably the limiting rate of the bioavailability of the HMW-PAHs to microbial attack (Zhang et al., 2006).

Table 1: summary of the pourcentage of PAHs-degradation, rate of the reaction and the half- life related to *B. simplex, B. pumillus* and *Ps. stutzeri* degrading bacteria

	Percentage of PAHs degradation rate (%)				Rate constant of the reaction/coefficient correlation			
Bacterial species	Fl	Phen	Pyr	Flu	Fl		Phen	
					k^a	R^{2b}	k^a	$R^{2\,b}$
B. simplex	87	95	nd	nd	0.19	0.90	0.29	0.89
Ps. Stutzeri	65	86	nd	nd	0.11	0.90	0.20	0.92
B. Pumilus	76	88	nd	nd	0.15	0.88	0.26	0.88

nd: not determined; [a]The degradation rate constant; [b]correlation coefficient

We suggested that consortium of these three potent strains could enhance the degradation rate as demonstrated by other studies (Chaudhary et al., 2015; Lin and Cai, 2008). Consequently, the metabolic cooperation of several microorganisms may result in enhanced PAH utilization, since metabolic intermediates produced by one group of microorganisms may serve as substrates for the growth of others (Dean-Ross et al., 2002). Supplementing additional nutrient or/and biomass may enhance also the biodegradation (Cerqueira et al., 2014). Additional of other microorganism than bacteria, may possibly enhance theses HMW-PAHs. Nevertheless, the bioremediation efficiency was likely to be more limited by the bioavailability of PAHs rather than by the total number of PAH-degraders (Hamdi et al., 2007). Nevertheless, incubation with a bacterial consortium has been suggested a promising method for bioremediation of PAH-contaminated soils (Mao et al., 2012).

3.5. Microbial enumeration

Organic solvent can be toxic to microorganisms (Ramos et al., 2002; Torres et al., 2011), the tolerance of the bacterial strains to acetone as PAH carrier solvent was assessed for the first time. According to our results, acetone used as solvent did not showed adverse effect on isolates growth. Inhibition was not observed in the control cultures without PAH and in the

treatment with acetone (Zafra et al., 2014). At the end of the treatment, the total number of bacteria population was higher in soil without spiking with PAHs.

3.6. Kinetics of PAHs

The biodegradation kinetics of organic contaminants has been described by many models (Alexander, 1999; Thiele-Bruhn and Brümmer, 2005). However, model based on the first order rate equation is more widely applied (Chen et al., 2008) and best fitted with the equation $[PAH] = [PAH_0]e^{-kt}$, where [PAH] corresponds to the PAH-concentration in the soil sample at time t (days), $[PAH_0]$ corresponds to the initial amount of PAH and *k* is the first order rate constant of the reaction. In the present study, the biodegradation kinetics of both fluorene and phenanthrene are occurred best described by the first order rate model (*Figure 2*) because the obtained R^2 value ranged from 0.88 to 0.92. The percentage of PAHs degradation, the order rate constant (*k*), the correlation coefficient (R^2) of the target PAHs are summarized in *Table 1*. The highest rate constant (k = 0.29 d^{-1}) was identified for the degradation of phenanthrene by *Bacillus simplex*. For fluorene, the rate constants were determined at 0.19 d^{-1}, 0.11 d^{-1} and 0.15 d^{-1} respectively for the degradation by *Bacillus simplex*, *Pseudomonas stutzeri* and *Bacillus pumilus*. The best fit (R^2= 0.92) was observed again with *Bacillus simplex* degrading phenanthrene.

Half-lives were also calculated for estimating the persistence of fluorene and phenanthrene. Half-live is defined as the time required of 50% of a substance initially present to be degraded. It is calculated from the first-order rate using the relationship: $t_{1/2}=\ln 2/k$. In our case, the half-lives of phenanthrene (2.4-2.7 days) were shorter than that of fluorene (3.5-4.6 days). In soil, the persistence criteria is expressed by $t_{1/2}\geq 180$ days (6 months) (Klercka et al., 2000; Webster et al., 1998) while $t_{1/2}< 10$ days indicate a rapid degradation. Accordingly, *B. simplex*, *Pseudomonas stutzeri* and *Bacillus pumilus* can be the efficient species for removal of 3-ring PAHs after acclimatization process; the LMW-PAHs half-lives were determined <10 days. Shimp et al. (1990) reported that compound with short $t_{1/2}$ showed only modest accumulation. Compared with others aerobic studies, our half-lives values are lower than those found in the literature (*Table 2*).

The difference may reflect the different environmental conditions adopted during each treatment. All experiments conducted in laboratory were run under controlled condition thus optimize the biodegradation potential but the same finding would not appear necessarily in

the field-scale bioremediation. The soil spiked with PAHs provided the lowest half-life values for most PAH compounds, suggesting a higher susceptibility of spiked PAHs to both abiotic and biological degradation (Wild and Jones, 1993). In many cases, however, degradation rates are frequently not linear so half-lives alone were demonstrated not relevant for evaluating the potential effectiveness of bioremediation of PAHs (Shuttleworth and Cerniglia, 1995).

Table 2: Half-lives values of fluorene and phenanthrene in our study and obtained from previous studies

Half-lives (in days) in our study				Half-lives (in days) values in references			
PAHs	*B. simplex*	*Ps. stutzeri*	*B. pumilus*	(Wang et al., 2010)	(Wild and Jones, 1993)	(Park et al., 1990)	(Shuttleworth and Cerniglia, 1995)
Fl	3.5	6.1	4.6	7-11.4	28	-	21 - 24
Phen	2.4	3.4	2.7	4.6	14	16-35	16 - 126

Figure 2A showed clearly a biphasic process of degradation of phenanthrene and fluorene. Firstly, there is a rapid phase of bioremediation during which the bacteria use the readily available fraction of the pollutant. Then, the second phase occurred and characterized by a slower degradation rate where microbial activity did not influence the kinetics but desorption and diffusion process may governed (Cornelissen et al., 1998; Thiele-Bruhn and Brümmer, 2005).

Our results showed that the PAHs removal rate was depend on the characteristic of the species which might be changed according to the fact that they were previously adapted to a high pollution. Besides, since the bacteria strains were isolated from a petroleum contaminated soil sample, they survived and adopted the oil contaminated soil/liquid environment easily and would be hosts for manipulated catabolic pathway (Ramos et al., 1990).

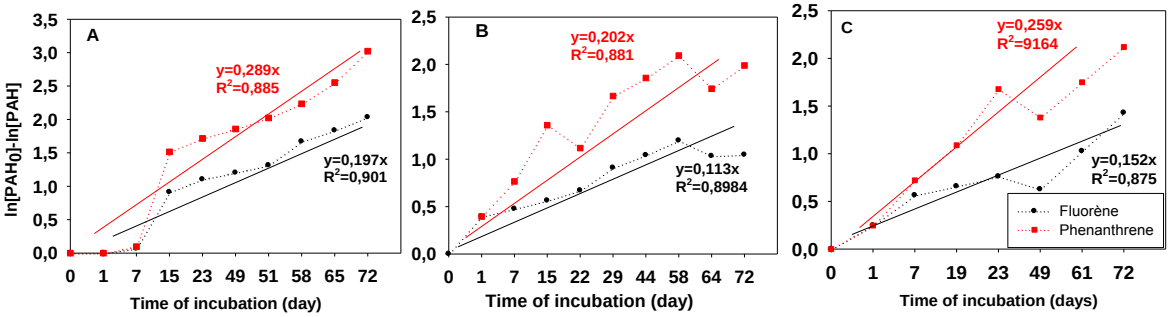

Figure 2: First order of fluorene and phenanthrene degradation by (A) *Bacillus simplex*, (B) *Pseudomonas stutzeri* and (C) *Bacillus pumilus* strains.

Conclusion

Numerous bacterial strains are able to develop and reproduce naturally in soil contaminated by hydrocarbons including the PAHs. In our case, 27 indigenous bacterial strains were isolated from an aged contaminated site and were screened individually for their resistance and ability to use PAHs as source of carbon and energy when exposed to high levels of PAHs contamination. 8 were identified potent strains and identified as *Pseudomonas stutzeri*, *Bacillus pumilus* and *Bacillus simplex* species.

In this study, they exhibit high tolerance levels to LMW and HMW-PAHs mixture exposure. However, they were observed potent only for 3-ring PAHs (fluorene and phenanthrene) clean-up; the removal of HMW-PAHs (4-rings, fluoranthene and pyrene) seems to be inhibited. Bioremediation potential using these bacterial species was occurred technically feasible, timely, thus suggested as easily to duplicate at field scale for the LMW-PAHs removal.

Further studies will be carried out to elucidate degradation pathway when using other strategical such as the use of consortium of these three strains for degrading, especially, the HMW-PAHs.

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Conclusion et perspectives

De nos jours, les activités anthropogéniques contribuent en grande partie à la détérioration de la qualité environnementale. Les mauvaises gestions des déchets toxiques ont causé la multiplication de sites pollués. Parmi les polluants introduits dans l'environnement, on distingue les polluants organiques persistants ou POP qui se caractérisent de multitudes de produits chimiques par leur persistance et leur aptitude à se mobiliser à de très longue distance dans l'environnement. L'interconnexion entre les différents compartiments de l'environnement (air, eau, sédiment, sol) favorise le transfert de contamination.

Dans cette étude, trois familles de micropolluants organiques tels que les hydrocarbures aromatiques polycycliques ou les HAP et ses dérivés méthylés (Me-HAP) ainsi que les polychlorobiphényles (PCB) ont été étudiés. Le premier objectif de l'étude était d'évaluer le mode de partition et de distribution de ces contaminants dans les différentes phases du systèmes aquatique d'eau douce (phase dissoutes, particulaires et sédimentaires) ainsi que dans des échantillons de sols issus de différents milieux en France et à Madagascar. Le second objectif se focalise sur une étude de faisabilité de bioremédiation de sols contaminés par les HAP. Pour ce faire, des espèces de bactéries ont été biostimulés puis engagés dans un traitement de dépollution en microcosme.

Les travaux de thèses ont été réalisés au sein de l'équipe chimie marine du laboratoire Géosystèmes et qui ont récemment rejoint le laboratoire LASIR UMR 8516, en collaboration avec l'IUT A de Villeneuve d'Ascq et la faculté des Sciences de l'Université d'Antananarivo (Madagascar).

Ces travaux de thèse ont permis de contribuer à certaines informations manquantes concernant le niveau de contamination et la dispersion des POP (HAP, Me-HAP, PCB) dans la zone transfrontalière du nord de la France et la Belgique. Cette étude offre également une meilleure estimation de la qualité environnementale des systèmes d'eau douce dans cette zone. L'étude de faisabilité de bioremédiation de sol pollué par les HAP a permis également de découvrir les agents biologiques (bactéries) efficientes pour une meilleurs dégradation de certaines molécules modèles de HAP rejeté dans le sol. Ainsi, cette étude offre une

information intéressante sur la pollution actuelle de l'environnement aquatique et des sols ainsi qu'un moyen exploitable de remédier un sol contaminé par les POP.

Les travaux réalisés durant ma thèse s'agissent des travaux sur terrains, sur l'optimisation des méthodes analytiques et l'étude d'évaluation des risques. Ces études contribuent à fournir d'informations essentielles pour les études environnementales, notamment sur les milieux aquatiques d'un système d'eau douce. Durant ma thèse, j'ai pu identifier, purifier des souches bactériennes dont certaines ne sont pas encore connue dans la littérature en terme d'agents de décontammination de sol pollués par les hydrocarbures. Les premiers résultats ont montré leurs capacités à éliminer les HAP légers présents dans les sols contaminés. Cependant, seulement quelques molécules modèles ont été testées et également chaque mélange des molécules modèles a été testé avec chacune de souches séparémment. Il serait alors primordial de conduire des études supplémentaires sur d'autres molécules de HAP, à la fois individuellement et en mélange afin d'avoir une idée plus large sur les capacités de chacune des espèces à dépolluer les sols contaminés par les hydrocarbures, en général sous forme de mélange complexe. De plus, il serait également intéressant d'étudier ces molécules individuelles ou en mélanges avec un consortium de microorganismes (bactéries, champignons). Les capacités de ces microorganismes méritent d'être testées aussi avec d'autres types de micro polluants indésirables tels que les PCB ou les homologues alkylés des HAP. Il est également préférable de comparer les résultats obtenus des études de microcosmes, *i.e* à l'échelle laboratoire et en milieu contrôlé, avec les données observées sur terrain. Enfin, pour les microorganismes qui ont montré de forts potentiels à bioremédier devraient être testées sur les milieux pollués.

Conclusion

Nowadays, anthropogenic activities contribute to the deterioration of the environmental quality. The mismanagement of toxic wastes has caused the multiplication of polluted in various sites. Among many types of pollutants, the released, there are persistent organic pollutants or POPs are permanently into the environment and they are characterized from multitudes chemicals by their persistence and ability to move in long range distance in the environment. The interconnection between the different compartments of the environment (air, water, sediment, soil) promoted the transfer of the contamination.
In this study, we focused particularly to the three families of organic micro-pollutants such as polycyclic aromatic hydrocarbons (PAHs) and their methylated derivatives (Me-PAHs) and polychlorinated biphenyls (PCBs). The first objective of the study was to assess the partition and distribution of these contaminants in the different phases of aquatic freshwater systems (dissolved, particulate and sedimentary phase) and in soils samples originated from different sites. The second objective focuses on a feasibility study of PAHs-contaminated soil bioremediation. To do so, bacterial species were isolated and acclimatized then the potent ones were selected and engaged in biodegradation experiment by microcosm.

My thesis was conducted in the marine chemical team of Géosystèmes laboratory that recently joined the laboratory LASIR UMR 8516, in collaboration with the IUT A of Villeneuve d'Ascq and the Faculty of Science of the University of Antananarivo (Madagascar).

This work contribute useful data that can complete the previous scientific data collected during previous studies regarding the level of POPs contamination (PAHs, Wed-PAHs, PCBs) in France and Belgium border zone. This study also provides a better estimation of the environmental quality of freshwater systems in this trans-border area between France and Belgium. The bioremediation study suggests a good way to have a better rate of biodegradation using potent biological agents (bacteria). Thus, this study provides interesting information on the current pollution of the aquatic environment and soil as well as a workable means of remedying soil contaminated by POPs.

Annexe I : données scientifiques

1. Les propriétés physico-chimiques des molécules de HAP, Me-HAP et PCB

Le tableau ci-dessous regroupe les paramètres physico-chimiques des 16 HAP, 18 Me-HAP et 28 PCB étudiés à savoir la masse molaire (MW), la solubilité (S_W), le coefficient de partage octanol/eau (Log K_{OW}) et le coefficient de partage carbone organique/eau (Log K_{OC})

HAP[j, k, l]	**N°CAS**	**Cycle**	**MW (g/mol)**	**S_W (mg/L)**	**Log K_{OW}**	**Log K_{OC}**
Naphtalène (N)	91-20-3	2	128,16	30	3,3	3,15
Acénaphtylène (Ayl)	208-96-8	3	152,20	3,93	4,07	1,4
Acénaphtène (Aen)	83-32-9		154,20	3.42	3.98	3.66
Fluorène (F)	86-73-7		166,20	1,98	4,21	6,2
Phénanthrène	120-12-7		178,20	1,2	4,45	5,15
Anthracène (Ant)	85-01-8		178,20	0,076	4,45	4,15
Fluoranthène	206-44-0	4	202,26	0,26	4,9	4,58
Pyrène (Pyr)	129-00-0		202,30	0,077	4,88	4,58
Benz(a)anthracène (BaA)	56-55-3		228,90	0,01	5,61	5,3
Chrysène (Ch)	218-01-9		224,30	$2,8 \times 10^{-3}$	5,16	5,3
Benzo(b)fluoranthène (BaF)	205-99-2	5	252,30	0,0012	6,04	5,74
Benzo(k)fluoranthène (BkF)	207-08-9		252,30	$7,6 \times 10^{-4}$	6,06	5,74
Benzo(a)pyrène (BaP)	50-32-8		252,30	$2,3 \times 10^{-3}$	6,06	6,74
Dibenzo(a,h)anthracene (DhA)	53-70-3		278,35	5×10^{-4}	6,84	6,52
Benzo(g,h,i)perylène (Bghi)	191-24-2	6	276,34	$2,6 \times 10^{-4}$	6,5	6,2
Indeno(1.2.3-c,d)pyrene (IP)	193-39-5		276,30	0,062	6,58	6,2
Methyl-HAP	N°CAS	Cycle	MW (g/mol)	S_W (mg/L)	Log K_{OW}	Log K_{OC}
1-Methylnaphtalène (1M-Na)	90-12-0	2	142	28[h]	3.87[h]	-
2-Méthylnaphtalène (2M-Na)	91-57-6	2	142	25[h]	4[h]	-
1,2-Diméthylnaphtalène (1,2DM-Na)	573-98-8	2	156	-	-	-
1,6-Diméthylnaphtalène (1,6DM-Na)	575-43-9	2	156	-	-	-
2,6-Diméthylnaphtalène (2,6DM-Na)	581-42-0	2	156	-	4.3[a]	-
1-Méthylphénanthrène (1M-Phe)	832-69-9	3	192	0.27[a]	5.08 [a]	-
2-Méthylphénanthrène (2M-Phe)	2531-84-2	3	192	0.28[a]	5.1[b]	-
3-Méthylphénanthrène (3M-Phe)	832-71-3	3	192	0.24 [a]	5.15[a]	-
9-Méthylphénanthrène (9M-Phe)	883-20-5	3	192	0.31 [a]	4.86[a]	-
2-Méthylanthracène (2M-Ant)	613-12-7	3	192	-	5.1b	-
1,7-Diméthylphénanthrène (1,7DM-Phe)	483-87-4	3	206	0.1[a]	5.44[a]	-
Retène	483-65-8	3	234	0.03[a]	6.35[a]	-
1-Méthylfluoranthène (1M-Fl)	25889-60-5	4	216	-	-	-
3-Méthylfluoranthène (3M-Fl)	1706-01-0	4	216	-	-	-
1-Méthylpyrène (1M-Pyr)	2381-21-7	4	216	-	-	-
4-Méthylpyrène (4M-Pyr)	3353-12-6	4	216	-	-	-
3-Méthylchrysène (3M-Ch)	3351-31-3	4	242	-	-	-
6-Méthylchrysène (6M-Ch)	1705-85-7	4	242	-	-	-
PCB	N°CAS	Cl	MW (g/mol)	S_W (mg/L)	Log K_{OW}	Log K_{OC}
2,4'-Dichlorobiphényle (CB 8)	34883-43-7	2	223.1	1.17[i]	5.8[c]	-
2,2',5-Trichlorobiphényle (CB18)	37680-65-2	3	257.55	0.4[i]	5.24[f]	4.57[e]
2,4,4'-Trichlorobiphényle (CB28)	7012-37-5	3	257.55	0.16[d]	5.8 [d]	5.3[d]
2,2',3,5'-Tetrachlorobiphényle (CB44)	41464-39-5	4	291.99	0.1[i]	5.75[f]	-
2,2',5,5'-Tétrachlorobiphényle (CB52)	35693-99-3	4	291.99	0.03[d]	6.1[d]	5.6[d]
2,3',4,4'-Tétrachlorobiphényle (CB66)	32598-10-0	4	291.99	0.0368[i]	6.20[c]	-

3,3',4,4'-Tétrachlorobiphényle (CB77)	32598-13-3	4	291.99	0.0027[g]	6.36[f]	-
3,4,4',5-Tétrachlorobiphényle (CB81)	70362-50-4	4	291.99	0.003[g]	6.53[g]	-
2,2',4,5,5'-Pentachlorobiphényle (CB101)	37680-73-2	5	326.44	0.01[d]	6.38[c]	5.9[d]
2,3,3',4,4'-Pentachlorobiphényle (CB105)	32598-14-4	5	326.44	0.0017[g]	6.65[c]	-
2,3,4,4',5-Pentachlorobiphényle (CB114)	74472-37-0	5	326.44	0.0026[g]	6.47[g]	-
2,3',4,4',5-Pentachlorobiphényle (CB118)	31508-00-6	5	326.44	0.002[g]	6.74[c]	-
2,3',4,4',5'-Pentachlorobiphényle (CB123)	65510-44-3	5	326.44	0.0009[g]	6.5[g]	-
3,3',4,4',5-Pentachlorobiphényle (CB126)	57465-28-8	5	326.44	0.0013[g]	6.89[f]	-
2,2',3,3',4,4'-Hexachlorobiphényle (CB128)	38380-07-3	6	360.88	0.0004[i]	6.74[c]	-
2,2',3,4,4',5'-Hexachlorobiphényle (CB138)	35065-28-2	6	360.88	0.0015[i]	6.83[f]	6.16[e]
2,2',4,4',5,5'-Hexachlorobiphényle (CB153)	35065-27-1	6	360.88	0.001[d]	6.9[d]	6.4[d]
2,3,3',4,4',5-Hexachlorobiphényle (CB156)	38380-08-4	6	360.88	0.0011[g]	6.75[g]	-
2,3,3',4,4',5'-Hexachlorobiphényle (CB157)	69782-90-7	6	360.88	0.0003[g]	6.73[g]	-
2,3',4,4',5,5'-Hexachlorobiphényle (CB167)	52663-72-6	6	360.88	0.0011[g]	6.82[g]	-
3,3',4,4',5,5'-Hexachlorobiphényle (CB169)	32774-16-6	6	360.88	0.0013[g]	7.42[f]	-
2,2',3,3',4,4',5-Heptachlorobiphényle (CB170)	35065-30-6	7	395.33	0.0035[i]	8.27[i]	-
2,2',3,4,4',5,5'-Heptachlorobiphényle (CB180)	35065-29-3	7	395.33	0.0385[i]	8.270[i]	-
2,2',3,4',5,5',6-Heptachlorobiphényle (CB187)	52663-68-0	7	395.33	0.00451[i]	8.27[i]	-
2,3,3',4,4',5,5'-Heptachlorobiphényle (CB189)	39635-31-9	7	395.33	6.3 x 10^{-5}[g]	-	-
2,2',3,3',4,4',5,6-Octachlorobiphényle (CB195)	52663-78-2	8	429.77	0.0002[i]	7.56[f]	-
2,2',3,3',4,4',5,5',6-Nonachlorobiphényle (CB206)	40186-72-9	9	464.22	0.00003[i]	7.2[g]	-
Décachlorobiphényle (CB209)	2051-24-3	10	498.66	7.43 x10^{-6}[i]	8.18[f]	-

[a]Lehndorff and Schwark 2009; [b]Boese et al., 1998 ; [c]Hawker and Connel 1988; [d]Muir and Lohmann 2013; [e]Registry 2000; [f]Fox et al., 1994; [g]Huang and Hong 2002; [h]GESTIS Substance Database ; [i]US-National Library of Medecine; [j]ATSDR, 1995; [k]EPA-TSCA ou Environmental Protection Agency-Toxic Substances Control Act ; [l]IARC: Centre International de recherche sur le cancer.

2. Temps de retention, fragment caractéristique, étalons internes

Le tableau suivant regroupe les molécules étudiées, leur temps de retention (TR) et ions quantificateurs (m/z) respectifs ainsi que les étalons internes utilisés lors des analyses sur GC-MS.

Composés	**MW (g/mol)**	**TR (min)**	**IQ ou m/z**	**EI**
HAP				
Naphtalene	128,17	12,488	102	Naphtalène d-8
Acénaphtylène	152,19	17,715	150	Acénaphtène d-10
Acénaphtène	154,21	18,130	152	Acénaphtène d-10
Fluorène	166,22	21.034	164	Acénaphtène d-10
Phénanthrène	178,23	25,677	176	Phénanthrène d-10
Anthracène	178,23	26,268	176	Phénanthrène d-10
Pyrène	202,25	32,987	200	Pyrène d-10
Fluoranthène	202,26	34,488	200	Pyrène d-10
Benzo(a)anthracène	228,29	43,727	226	Pyrène d-10
Chrysène	228,28	43,850	226	Pyrène d-10
Benzo(b)fluoranthène	252,31	51,950	250	Pérylène d-12
Benzo(k)fluoranthène	252,31	52,15	250	Pérylène d-12
Benzo(a)pyrène	252,31	55,039	250	Pérylène d-12
Dibenzo(ah)anthracène	278,35	67,880	274	Pérylène d-12
Benzo(ghi)pérylène	276,33	68,275	276	Pérylène d-12
Indeno(1,2,3-cd)pyrène	276,33	71,953	276	Pérylène d-12
Me-HAP				
1-methylnaphthalene	142,2	14,592	115	Acénaphtène d-10
2-methylnaphthalene	142,2	14,756	115	Acénaphtène d-10
1,2-dimethylnaphthalene	156,22	16,66	141	Acénaphtène d-10
1,6-dimethylnaphthalene	156,22	16,96	141	Acénaphtène d-10
2,6-dimethylnaphthalene	156,22	17,579	141	Acénaphtène d-10
1-methylphenanthrene	192,26	28,18	189	Phénanthrène d-10
2-methylphenanthrene	192,26	28,477	189	Phénanthrène d-10
3-methylphenanthrene	192,26	28,903	189	Phénanthrène d-10
9-methylphenanthrene	192,26	32,132	190	Phénanthrène d-10
2-methylanthracene	192,26		191	Phénanthrène d-10
1,7-dimethylphenanthrene	206,28		206	Pyrène d-10
Retene	234,34	35,849	234, 219	Pérylène d-12
1-methylfluoranthene	216,28	35,861	216, 215	Pyrène d-10
3-methylfluoranthene	216,28	36,613	189, 214	Pyrène d-10
1-methylpyrene	216,28	38,16	189	Pyrène d-10
4-methylpyrene	216,28	38,412	189	Pyrène d-10
3-methylchrysene	242,31	47,099	239	Pérylène d-12
6-methylchrysene	242,31		239	Pérylène d-12
PCB				
CB8	223,10	21,942	152, 222, 224	TCN
CB18	257,54	23,55	258, 256, 186	TCN
CB28	257,54	26,295	258, 256,186	TCN
CB52	291,98	27,515	292, 294, 255	TCN

CB44	291,99	28,488	292, 291, 255	TCN
CB101	326,43	31,169	292, 291, 220	CB112
CB66	291,99	31,931	326, 328, 286	TCN
CB81	291, 99	34,322	290, 292, 220	TCN
CB77	291,99	34,968	290, 292,220	TCN
CB123	326,43	35,501	326,328, 254	CB112
CB118	326,43	35,775	326,328, 254	CB112
CB114	326,44	36,398	326,328, 254	CB112
CB153	360,89	36,516	362, 361,325	CB112
CB105	326,44	37,305	326,328, 254	CB112
CB138	360,88	38,129	362, 361,325	CB112
CB170	395,33	38,656	359	CB112
CB126	326,44	39,78	325	CB112
CB128	360,88	40,13	360	CB112
CB157	360,88	41,476	288	CB112
CB156	360,88	41,709	288	CB112
CB169	360,88	41,829	359, 360, 362	CB112
CB180	395,33	43,644	359	CB112
CB187	395,33	44,414	290	CB112
CB167	360,88	46,257	324	CB112
CB189	395,33	46,424	393	CB112
CB195	429,77	46,451	360	CB112
CB206	464,22	50,848	427	CB112
CB209	498,66	53,183	428	CB112

MW : Masse molaire ; **TR** : temps de rétention ; **IQ** : ion de quantification ; **EI** : étalon interne

Annexe II : contributions scientifiques

Durant ces trois années, les fruits de mes travaux de recherche ont été valorisés sous forme de deux articles scientifiques, en tant que premier auteur, qui ont été présentés précédemment dans la partie résultats de ce présent manuscrit. Parallèlement à mes travaux de thèse, j'ai eu également l'opportunité de participer à d'autres projets gerés par et/ou en collaboration avec l'équipe scientifique de mon laboratoire d'accueil (projet IREPSE, projet BIOFOZI, …) dont les thématiques ne s'éloignent pas du cadre de mes formations. Ma participation dans ces projets comprend des travaux de terrain, des analyses, des traitements de données et des parts de rédaction dans l'élaboration de publications scientifiques. Actuellement, je suis co-auteur dans quatre autres articles scientifiques publiés dans différents journaux internationaux avec comité de lecture.

A plus des micropolluants organiques ciblés dans mes travaux de thèse (HAP, Me-HAP et PCBs), j'ai pu élargir mes compétences sur la caractérisation et la quantification des autres polluants organiques persistants dans l'environnement tels que les phtalates, les pesticides ainsi que quelques catégories de polluants émérgents comme les résidus de médicament. Dans toutes les analyses effectuées, les techniques utilisées sont:

- L'ASE pour l'extraction dans les échantillons de nature solide
- La SPE pour l'extraction dans des échantillons de nature liquide
- La quantification se fait par GC-MS

Sur ce, mes contributions scientifiques sont résumés comme suit :

Article 3 : Cet article est le fruit d'une étude d'évaluation de l'impact des rejets anthropiques sur l'écosystème écologique dans les zones côtières de Dakar. Une espèce de macro algue, deux espèces d'invertébrés et quatre espèces de poissons ont été étudiées du fait de leurs importances dans le plan économique et écologique dans les zones cotières de Dakar. Le niveau de contamination en HAP, Me-HAP, PCB et en mercure total a été évalué sur ces espèces marines. Leurs qualités ont été également évaluées suivant la norme de qualité en stipulé dans la législation européenne. Ainsi, les données obtenues completeront les données scientifiques concernant l'étude des niveaux de contamination, de leur potentiel de bioamplification et/ou processus de bioaccumulation dans la chaîne alimentaire marine.

Article 4 : cet article évalue également l'impact des activités anthropogéniques sur le système aquatique du bassin versant de l'Escaut, dans la zone transfrontalière France-Belgique qui est histroriquement industrielle et actuellement urbanisée. De ce fait, la zone est vulnérable en matière de contamination; les cours d'eau et les rivières agissent toujours comme récepteur de divers types de contaminants organiques provenant de différents types de rejet (municipal, industriels, agricoles) ainsi que d'autres sources non ponctuelles. Les phtalates, pesticides et résidus de médicaments sont les composés d'intérêt dans cet article. En effet, la particularité de ce travail se repose sur l'investigation du niveau de contamination ainsi que le mode de répartition de ces polluants organiques dans les différentes phases du système aquatique (dissoutes, particulaire et sédimentaire).

Article 5: Cette étude constitue en quelque sorte la continuité d'une étude portant sur la pollution métallique, menée par une autre équipe du laboratoire, mais se penchant notamment sur les polluants organiques du type HAP, Me-HAP et PCB. Des sédiments de rivière issus des trois rivières (la Deûle, la Sensée et la Scarpe) dans la région Nord-Pas-de-Calais ont fait l'objet d'investigation. Les principaux objectifs étant d'évaluer le niveau de concentration de 16 HAP, 18 Me-HAP et 28 PCB ainsi que leur composition et profil de distribution dans les sédiments. L'identification de sources de contamination a également été faite. En utilisant le concensus-based SQGs comme guide, une grille de qualité des sédiments a pu être dressée.

Article 6: Cette publication scientifique est le résultat de l'étude sur la rivière La Somme (Nord Pas-de-Calais, France), située à proximité d'une zone agricole et où il existe des activités de pêches. Un total de 96 composés chimiques ont été analysés incluant 28 pesticides, 16 HAP, 18 Me-HAP, 6 phthalates et 28 PCB d'ailleurs cette étude est la première concernant les phtalates dans cette rivière. Les niveaux de concentration des polluants le long de la rivière, en considérant 13 points de prélèvement, ont été évalués. La qualité de l'eau de la rivière la Somme a été évaluée en comparant les concentrations obtenues avec la directive cadre sur l'Eau (DCE) et la norme canadienne (CWQG).

Article 3

Accumulation of PAHs, Me-PAHs, PCBs and total Mercury in sediments and Marine Species in Coastal Areas of Dakar, Senegal: Contamination level and impact

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ABSTRACT

Persistent Organic Pollutants (POPs) have widely aroused public concern due to their ubiquity, environmental persistence, long-range transportability, bioaccumulation capacities and potentially adverse effects on living organisms. Dakar is located in the industrial zone of Senegal (80% of industrial activities) and inhabits 25% of Senegalese population leading to an ideal sink of these persistent organic pollutants. In the present paper, Polychlorinated Biphenyls (PCBs) and polycyclicAromatic Hydrocarbons (PAHs) were analyzed in sediments and marine organisms. The contamination level of PAHs, Me-PAHs and PCBs in surface sediment and aquatic organisms (one macroalgae, two invertebrate species, four fish species and macroalgae) were determined. The concentration levels in the sediment were detected from 2 to 636 μg/kg dw for Σ16PAHs, from 3 to 31 μg/kgdw for Σ18Me-PAHs and from 4 to 333 μg/kg dw for Σ28PCBs for the selected stations in Dakar costal area. PAHs concentrations determined in edible tissues were lower than sediment samples. Tilapia species present the highest mean levels of PAHs and Me-PAHs at 92 ± 54 and 183 ± 39 μg/kg dw respectively. For PCBs, the highest level was determined in *Perna perna* species (up to 1228 μg/kg dw) and the lowest level was found in *Penaeus kerathurus* species. At the base of the food chain, *Ulvalactula* species displayed low PCB concentrations detected at 7 ± 6 μg/kg dw. The total mercury concentration was also reported in this paper in order to complete the background of pollution degree and to study the potential processes of biomagnification and/or bioaccumulation of contaminants in marine food chain. Mercury concentration were detected in the sediment ranging from 5 to 95 μg/kg dw. For marine species, considering all organisms, the mean concentration of mercury varies between 5 and 442 μg/kg dw. Pyrogenic process was the predominant source of PAHs contamination in our sampling sites. Based on Sediments Quality Guidelines (ERM–ERL/TEL–PEL approaches) rare biological adverse effects of total mercury, PCBs and PAHs on aquatic ecosystems were expected in Dakar coastal area. Finally, in the context of human health, the edible marine species qualities obtained from three stations of Dakar coastal areas have been also evaluated. Based on the European Union legislation, the selected species present good quality for human consumption based on PCBs, PAHs and mercury.

Keywords: PAHs, PCBs, mercury, sediment and marine organisms, Dakar

1. Introduction

The environmental impact of organic pollutants and metallic trace elements in coastal environment is serious. In order to better understand the potential impact of these pollutants on the ecosystem functioning as well as human health, an increasing attention has been implemented not only on the contamination level of contaminants in water or sediment but also on their accumulation level in aquatic organism (Boon, 1985; Naes *et al.,* 1995; Neff 2002; Borgå *et al.,* 2004; Francioni, 2005; Bastami *et al.,* 2013). Senegalese coast is one of the most productive areas in the world due to the presence of coastal upwelling of deep waters rich in nutrients (Romeo *et al.,* 1999). Even if industrialization did not reach a veryhigh level compared to the developed countries, pollution of coastal aquatic habitats seems to be an inevitable problem mainly in the peninsula of Dakar which concentrates more than 80 % of the industries and inhabits more than 25 % of the population of the country (OIS 2010). Indeed, most of domestic and industrial waste waters are discharged directly into the sea. These permanent anthropogenic discharges can contribute to environmental and ecological degradation of Dakar coastal zones. Consequently, particular attentionmust be paid to the chemical qualityof edible marine organisms consumed by local human populations. Fish and invertebrates consumption remain themajor source of protein for the residents in Dakar. However, to date, only fewstudies were carried out on the trace metals and organic contamination levels in mollusk and fish species sampled from this aquatic ecosystem (Simoudou *et al.,* 2006; Ndiaye *et al.,* 2012).

Among the large variety of organic pollutants, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) are two classes of pollutants intensivelymonitored and regulated due to their toxicity, persistence and wide diffusion in the environment. PCBs werewidelyused in industries until their hazards to the environment and human health became evident. These contaminants have been reported to cause cancer in animals and humans (Bertrand *et al.,* 2010; Kramer *et al.,* 2012; Bräuner *et al.,* 2012; EPA, 2013). PCBs have primarily been used as dielectric fluids of transformers and capacitors; other applications included their use in paints, inks and pesticides; they are extremely stable compounds under environmental conditions (Bennett, 1983;WHO, 1993). Due to their toxicity, persistency and bioaccumulation capacities, these substances have been classified as persistent organic pollutants. Their production was banned by theUnited States Congress in 1979 and bythe Stockholm Convention on Persistent Organic Pollutants in 2001 (Porta and Zumeta, 2002). However, PCBs are still present in water and sediment (Turrio-Baldassarri *et al.,* 2005;

Dumoulin *et al.,* 2013; Net *et al.,* 2014a) and continue to affect aquatic organisms fromthe base of food chain (plankton, algae) to predator organisms (fish, birds, marine mammals) and consequently human health through the diet (Sun *et al.,* 2002).Another class of organic contaminants is represented by the aromatic hydrocarbons group including PAHs and Me-PAHs. These contaminants arewidely disseminated in the environment. High level of hydrocarbons represents a serious threat to the ecosystem functioning and human health via food chain and water resources. Their sources can be both natural and anthropogenic (Yunker *et al.,* 2002;Wang *et al.,* 2007; Mostert *et al.,* 2010; Net *et al.,* 2014a,b). Hydrocarbons are highly lipophilic compounds, ubiquitous in coastal, estuarine and river water column, as well as sediments in which they tend to accumulate (Chiou *et al.,* 1998; Ko and Baker, 1995; Manodori *et al.,* 2006; Cailleaud *et al.,* 2007; Gaspare *et al.,* 2009; Yunker *et al.,* 2012; Net *et al.,* 2014b). Recent studies have reported that marine organisms are prone to bioaccumulate these substances, particularly in lipidrich tissues (Neff, 2002; Francioni *et al.,* 2005; Dugan *et al.,* 2005). Due to their toxic, carcinogenic and mutagenic effects (Straif *et al.,* 2005; IARC, 2010; US Department of Health and Human Services, 2011), sixteen PAHs have been classified as priority substances by the United States Environmental ProtectionAgency(USEPA, 2002).Among toxicmetals, mercury is an element of special concern because it is known to particularly biomagnify as it moves up the aquatic food chain (Carrasco *et al.,* 2011) and thus to bioaccumulate in higher tropic level consumers (Cossa *et al.,* 1990).

The aim of the present study was to assess the impact of anthropogenic discharges on the ecological ecosystem of Dakar coastal zones. Predominant organic compound (PCBs, PAHs and Me-PAHs) were quantified in one macro algae species (Ulvalactuca), two invertebrate species (mussel: Perna perna and shrimp: Penaeus kerathurus) and four fish species (grey mullets: Mugilcephalus, tilapia Sarotherodon melanotheron, flatfish Soleasenegalensis and round sardinella Sardinella aurita). These two invertebrate and four fish species have been selected because they represent a great economic and ecological importance in these coastal zones. In addition, a toxic metal such as mercury has been also considered in this paper in order to complete the background of contamination level and to studytheir potential biomagnification and/ or bioaccumulation processes in marine food chain.

2. Materials and methods

Dakar is located in the west of Senegal, in the industrial zone (80% of industrial activities) and inhabits 25% of the Senegalese population which is an ideal sink of these

persistent organic pollutants. The sampling campaign was conducted in the south of Dakar on February 2013 during dry season in three sampling sites along the South coast of the peninsula of Dakar, noted A, B and C on the Fig. 1. Classical parameter such as pH, temperature, salinity and dissolved oxygen have been also measured simultaneously. The values of pH, temperature, salinity and dissolved oxygen were respectivelyat 7.43, 21.8°C, 36.9 PSUand 7.42mg/Lfor Soumbedioune station, 7.61, 23.4°C, 40.3 PSU and 6.19mg/L for Yarakh station and 7.64, 23.1°C, 35.8 PSU and 7.02 mg/L for Rufisque station. Surface sediment and marine species have been selected in order to understand the potential impact of micro pollutants from urban and industrial discharges on ecosystem functioning and on human health. Seven marine species were considered in this work starting frommacroalgae, bivalve, crustacean and four species of fish. The choice was based on the frequent consumption of these species by the population inhabiting the Senegalese coast. The main characteristics of marine organisms selected in this study are briefly presented as following: (i) Macroalgae: *Ulvalactuca* provide qualitative information about the contamination level and environmental quality in an ecosystem due to their lifestyle sedentary and abundance in coastal seawater (Rainbow and Phillips, 1993). (ii)Mussel: *Perna perna* is the onlymussel of this genus in theWestern coast of Africa (Sidoumou *et al.,* 2006).Mussels are sedentaryfiltering organisms, which have been widelyused as environmental sentinel for the contamination. (iii) Crustacean: *Penaeus kerathurus* is commercially one of the most important shrimp species in fishery in Senegal. It is also a target species for fishermen using trammel nets in Dakar bays. (iv) Fish species: *Sardinella aurita* is a small pelagic fish feeding on plankton. These fat fish are the more often consumed species in Senegal. In this study, *Sardinella aurita* presents from 228 to 337 cm length with mean average of 309±37 cm and from110 to 396 g with mean average of 304±91 g wet weight. *Flathead mullet Mugilcephalus* is cosmopolitan and occupies a wide variety of marine, estuarine and freshwater environment in tropical, subtropical and temperate coastal waters. This benthopelagic species is omnivorouswhich diet consistsmainly of zooplankton, benthic organisms and detritus for larger juveniles and adult stages. In this word, *Mugilcephalus* collected were from 318 to 362 cm length with mean average of 343±19 cm and from 282 to 426 g wet weight with mean melanotheron are tolerant to a broad range of environmental conditions and natural populations were found in many different habitats from freshwater to hypersaline waters (Panfili *et al.,* 2003). This species has an omnivorous diet and can change its diet in function of the environment. Tilapia is a fast-growing fish which has been an essential source of

protein food. Therefore, this fish species showan increasing demand in many developed countries. *Sarotheroron melanotheron* collectedwere from141 to 253 cmlength with mean average of 196±58 cm and from 85 to 342 g with mean average of 206 ± 129 g wet weight. *Soleasenegalensis* is one of the most abundant and representative species of theAtlantic coasts. This flat benthic fish with a practically sedentary life lives in sandy or muddy bottoms in coastal areas and feeds on benthic invertebrates such as larvae of polychaets, bivalve mollusks and small crustaceans. This species is well adapted to warm climates and have been used in field and laboratory toxicity assays because of its sensitive character to pollutants (Costa *et al.*, 2009). *Soleasenegalensis* collected were from 269 to 371 cm length with mean average of 311±40 cm and from 178 to 445 g with mean average of 279 ± 97 g wet weightThese three sampling sites were chosen due to their locations near thewaste discharge channels into the sea. Zone A (Soumbedioune) is dominated by discharges of domestic wastewater, hospital discharges and road traffics. Zones B (Yarakh) and C (Rufisque) are located in theHann Bay: Yarakh is surroundedwith industrial activities with the predominance of food industries while Rufisque is close to the Refining African society and cement factory. For each station, five superficial sediment sampleswere collected at low tide. Similarly, macroalgae and mussels were hand picked from substratum of intertidal zone.

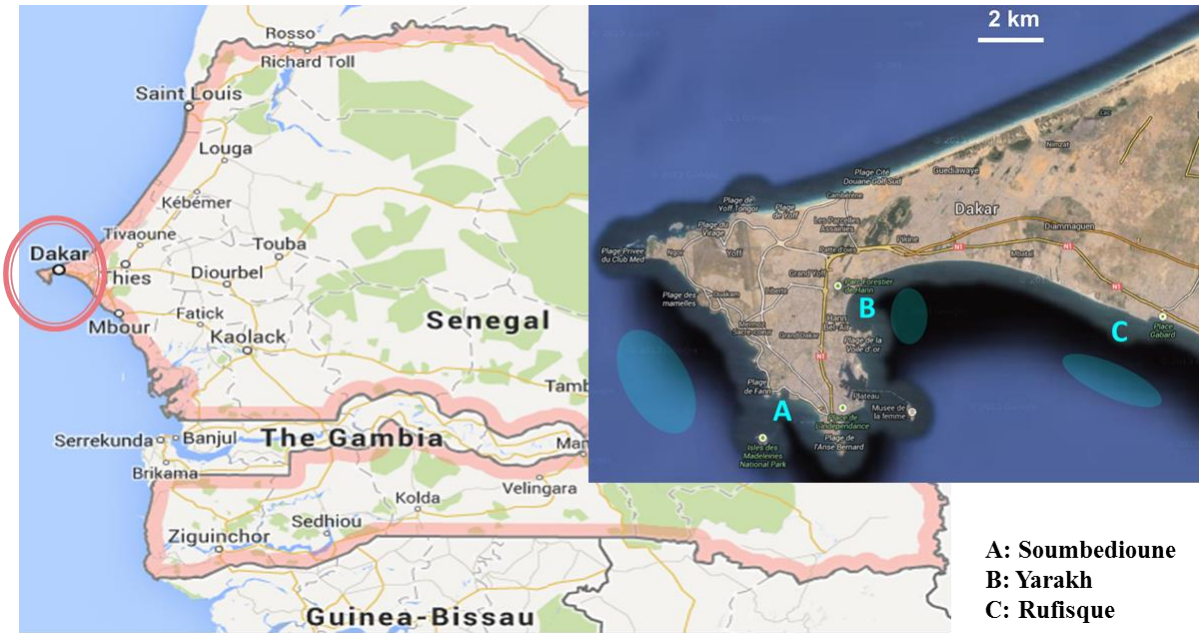

Fig. 1. Location of sampling sites from Dakar coastal zone, in Senegal

After collection, samples were transported to the laboratory in icebox and biota sampleswere rinsedwith purewater. The whole soft body of mussels was collected for chemical analysis. Samples of fish and shrimp were caught by fishermen's nets and the

fishing zones were indicated approximately in the Fig. 1. Species were purchased from the local fishermen in the same day of capture and brought to laboratory on ice immediately. Before collectingmuscle tissues, fish length andweight were measured. All samples were dried in an oven at 40°C to constant weight and were stored in individual aluminum foils at -20°C until further treatments and analysis. Dried sediment and biota samples were groundmechanicallywith an agatemortar and manually with a ceramic mortar and pestle, respectively.Mixed standard solutions of PAHs and Me-PAHs were purchased from Restek Corp (Bellefonte, PA, USA). PCBs standard solution was obtained from Accustandard, Inc. (New Haven, CT, USA). Tetrachloronaphtalene (TCN), 2,3,3',5,6- tetrachlorobiphenyl (PCB112) and octachloronaphtalene (OCN), used as internal standard for PCBs quantification, were purchased from Dr Ehrenstorfer (Augsburg, Germany). Deuterated internal standards for PAHs and Me-PAHs quantification were acenaphthene-d10 (A-d10), naphtalene-d8 (N-d8), perylene-d12 (Per-d12), phenanthrene-d10 (Phe-d10) and pyrene-d10 (Pyr-d10) and they were provided by LGC-Promochem (Middlesex, UK). HPLC-grade solvents (hexane, dichloromethane, methanol and acetone) were purchased from Dislab (France). Ultrapurewater (Milli-Q)was produced by aMillipore apparatus with 18.2 MΩ/cm resistivity. Merck silica gel 60 (70-230 mesh ASTM) activated at 450 °C was stored at 120°C for 12h prior to use. Glassware was systematically washed with detergent (Decon, East Sussex, UK), rinsed with ultrapure water and acetone and finally dried at 120 °C prior to use.

In this work, 16 PAHs, 18Me-PAHs and 28 PCBs including 12 dioxin-like PCBs (dl-PCBs) and 7 PCB indicators (PCBi) were analyzed as follow:

PAHs (16 PAHs) : naphthalene (N), acenaphtylene (Acy), acenaphtene (Acn), fluorene (F), anthracene (An), fluoranthene (Fl), benzo*[a]*anthracene (BaA), chrysene (Chr), benzo*[a]*pyrene (BaP), phenanthrene (Pn), benzo*[b]* fluoranthene (BbF), benzo*[k]*-fluoranthene (BkF), benzo*[ghi]*perylene (Bghi), dibenzo*[a,h]*anthracene (DhA), indeno*[1,2,3-cd]*pyrene (IP), pyrene (Py).

Me-PAHs (18Me-PAHs): 1-methylnaphthalene(1M-Na), 2-methylnaphthalene(2M-Na), 1,2-dimethylnaphthalene (1,2DM-Na), 1,6-dimethylnaphthalene (1,6DM-Na), 2,6-dimethylnaphtalene(2,6DM-Na), 1-methylphenanthrene (1M-Pn), 2-methylphenanthrene (2M-Pn), 3- methylphenanthrene (3M-Pn), 9-methylphenanthrene (9M-Pn), 2-methylanthracene (2M-An), 1,7- dimethylphenanthrene (1,7DM-Pn), retene, 1-methylfluoranthene (1M-Fl), 3-methylfluoranthene (3M-Fl), 1-methylpyrene (1M-Py), 4-methylpyrene (4M-Py), 3-methylchrysene (3M-Ch), 6-methylchrysene (6M-Ch).

PCBs No. (28 PCBs): 8, 18, 28, 44, 52, 66, 77, 81, 101, 105, 114, 118,123, 126, 128, 138, 153, 156,157, 167, 169, 170, 180, 187, 189, 195, 206 and 209.

Mercury: Total mercury.

Sieved powder samples were spiked with deuterated internal standardsA-d10, N-d10, Per-d12, Phe-d10 and Pyr-d10 for PAHs andMe-PAHs analyses and with TCN, PCB112 and OCN for PCBs analysis. After a delay of equilibration, samples were then extracted using an accelerated solvent extraction (ASE 200, Dionex Corp., USA). The extraction conditions were: heat 5 min, temperature 100°C, static solvent extraction time 2min with 5 static cycles, pressure 138 bars, purge 3 min and 35 % flush according to the method developed byTronczynski et al. (2005). High purity nitrogen was employed as the purge gas.

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Targeted compounds were analyzed using aVarian 3900 gas chromatograph (GC) equipped with a deactivated fused-silica guard column (5m, 0.25mm i.d.) and a fused-silica lowpolarity si-arylene ZB-XLB capillarycolumn (60mlength, 0.25mmi.d., 0.25 µmfilm thickness, Phenomenex) and coupledwith aVarian Ion Trap Saturn 2000Mass Spectrometer (MS). The carrier gas was helium held at a constant flow rate of 1 mL/min. Each group of organic compounds was analyzed separately. Temperature of the GC oven was programmed as follow: from70 °C (1min) to 170 °C at 10 °C/min, then to 230 °C at 4 °C/min, and then to 300 °C at 3 °C/min (13min) for HAPs and Me-HAPs and from80 °C(1min) to170 °Cat 10 °C/min, then to 230°C at 4 °C/min, and then to300 °Cat 3 °C/min (19min) for PCBs. Samples were injected in the splitless mode at 280 °C and the injector was purged with helium after 1 min. The transfer line and the ion trapwere held at 280 °C and 220 °C, respectively. Identification of each compound was done on the basis of the retention time and themass spectrumfromchromatograph of standard solutions acquired in full scanmode.Quantificationwas then performed in the single ion storage (SIS) mode for better selectivity. Response factors were determined relative to the internal standards previously

chosen to better fit to the properties of each compounds. No significant amount of analytes was detected in procedural blanks. Thus, the data did not need the blank corrected. The recoveryrates of the analytical procedure for extraction of PAHs and PCBs have been previously studied and validated byTronczynski *et al.* (2005). The procedure have been slightlymodified for fractionation on a silica column step. The recovery rates have been studied and validated in the laboratory by spiking the targeted compounds into the natural sediment. The recovery rates obtained weremore than 79% for PAHs, more than 73% for Me-PAHs and more than 75% for PCBs in algae and sediment samples. The limit of quantification of individual PCBs, PAHs and Me-PAHs were 0.8 µg/kg dw (except for PCB180 which was 2 µg/ kg dw), 0.1-2 µg/kg dw and 0.2-0.4µg/kg dw, respectively. Total mercuryanalysiswas carried outwith an Advanced MercuryAnalyser (Altec, AMA 254) on powder samples without treatment at least three replicates for each sample. For a known amount ofdried sample (10-40 mg), the metal was evaporated by progressive heating up to 800 °C under oxygen atmosphere and finally amalgamated on a gold-sand trap. Afterwards, the amalgamator was heated toliberatethe collected mercury, which was finally measured by atomic absorption spectrophotometry. Certified referencematerials (MESS- 3and DORM-3, National Research Council Canada) were used to assess the accuracy and precision of the analyses. Mean recovery for total mercury was more than 86 % and the limit of quantification was 5 µg/kg dried weight (dw).

3. Results and discussions

The mean contaminant levels of totalmercury, total PAHs (Σ_{16}PAHs), total Me-PAHs (Σ_{18}Me-PAHs) an total PCBs (Σ_{28}PCBs) measured in surface sedimentwer at 22±22 µg/kg dw with the range of 5-95 (n=15), 197±240 with the range of 2-636(n=15), 11±8 with the range of 3-31 (n=15) and 58±81 µg/kg dw with the range of 4-333 µg/kg dw (n=15) respectively. Mean±S.D (standard deviation) of individual concentration of targeted compounds were presented in Table 1. Individual concentration of PAH and Me-PAH detected in Soumbedioune station were one order of magnitude lower compared to previous study reported by Ndiaye *et al.* (2012) for the same station. Individual concentration of PCB were also detected at lower concentration. However, there is no data on organic contamination reported for two other sites selected in this study.Among these three sampling sites, Yarakh was the less contaminated by PAHs and PCBs. Our results showed that the distribution of PAH, Me-PAH, PCB andmercury was not homogenous. The concentration varied strongly for mone sampling point or site toanother (Table1). Despite a large range between the minimum

and maximum for contaminant levels, Σ16PAHs showed a relatively high contamination level, around 9 times higher than mercury concentration, 3 times higher than Σ_{28}PCBs concentration and 18 times higher than Σ_{18}Me-PAHs concentration.

Table 1. Σ_4PAHs, Σ_6PCBs and total mercury detected in this work (range and mean values into brackets) compared to maximum admissible content expressed in µg/kg wet weight.

Compounds	Maximum admissible content (µg/kg wet weight)			
	Mussel	Fish	Crustacean	Reference
BaP, BaA, BbF, Chr	30	30*	30*	UE N° 835/2011
	3.4-20 (8)	0.2-4.3 (1.1)[1] 8 – 34(19)[2] 3.2-18 (10.1)[3] 1.8-3.2 (2.7)[4]	8-12 (10)	This work
PCB: 28, 52, 101, 138, 153, 180	75	75	75	UE N° 1259/2011
	0.7-12 (6)	n.d-12 (5)[1] n.d2 7-10 (8.5)[3] n.d-27 (6)[4]	nd	This work
Total mercury	100-500	500-1000	100-500	UE N° 466/2001/2006
	9-17 (12)	3-101 (34)[1] 11-38 (21)[2] 9-30 (22)[3] 4-18 (7)[4]	7-12 (9)	This work

* smoked product; [1]sole; [2]sardine; [3]tilapia; [4]mullet. n.d. = not detectable (<LOQ)

The PAHs ranged between 2 and 636 µg/kg dw and the highest PAHs concentrations were obtained in sediments collected at Rufisque station and sampled from a zone close to the Refining African society and cement factory. The mean concentration of Σ_{16}PAHs was 396 ± 326µg/kgdw(Fig. 2).Our results of PAHs was at least an order of magnitude lower, compared to concentrations measured in superficial sediment collected in the Soumbedioune station (Hann Bay) during the dry season (Ndiaye *et al.*, 2012).Abdolahpur Monikh et al. (2014) have performed the study on PAHs contamination in Persian Gulf. The authors have reported the concentrations of total PAHs in sediment varied from 310.76 µg/kg dwatBoshehr province to1106 µg/kg dwat Tangestan estuary; thus two order of magnitude higher than our results obtained from Dakar coastal. Ndiaye *et al.* (2012) reported a concentration level of 19 µg/kgdw for PCBs indicator in a sediment sample fromHann Bay. The levels of PCBs indicator (Σ_7PCBs) were similar in Rufisque station and lower than literature data for the two other stations (Fig. 2). Similarly, surface sediments from two Senegalese stations (100 – 150 km South from Dakar) exhibited Σ_7PCBconcentrations ranging between 0.3 and 19.1 µg/kg dw (Bodin *et al.*,2011). Sediments from Yarakh and Soumbedioune displayed

levels of POPs lower than those from Rufisque station (Fig. 2). Yarakh station seems to be the less contaminated compared to other ones. The location of Rufisque station near the Refining African society and cement factory could explain the highest level of organic pollutants found at this station and more particularly the PAH compounds. Mercury concentrations ranged between 5 and 95 µg/kg dw and sediments from Rufisque station were the less enriched in this element. To our knowledge, no data for mercury concentration in sediments from this area or along the Senegalese coast were reported.

In general, the contamination level of pollutions are stronglyconditioned bytheir origin. Numerous methods could be used to identify the origin of PAHs contaminations (DeLucas2005; Gogou *et al.*, 1996;Simo *et al.*, 1997; Yunker *et al.*, 2002; Yunker and Macdonald, 1995; Dickhut *et al.*, 2000; Zhang *et al.*, 2005). However, to our knowledge, there is no specific method to identify the origin of PCBs and mercury. In this work, PAHs origin for the sediment samples was characterized by using the ratio of low molecular weight and high molecular weight (LMW/HMW, the sum of (2-3) / (4-6) aromatic rings). This ratios allow to distinguish the petrogenic (LMW/HMW > 1) from pyrolytic origins (LMW/HMW < 1) (De Lucas, 2005). Our results on LMW/HMW ratios revealed values less than 1, thus suggesting that combustion should be the dominant source of PAHs in the studied areas.

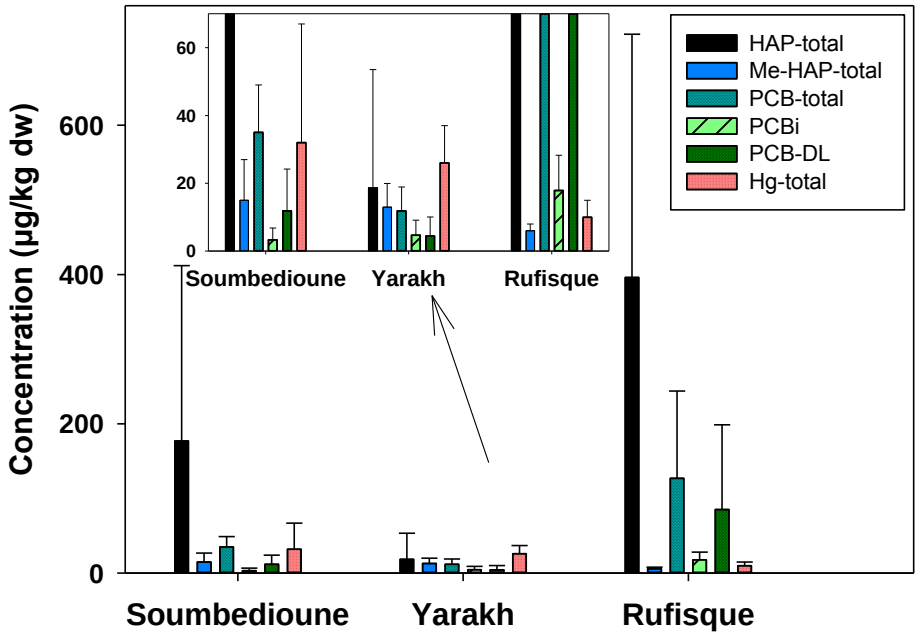

Fig. 2. Mean (±S.D.) of total PAH, Me-PAH, PCB and mercury concentrations (µg/kg dw) obtained in surface sediments collected at Soumbedioune, Yarakh and Rufisque stations.

Levels of PCB indicators (PCBi) and dioxin-like PCBs (PCB-DL) are also indicated. n=five replicates;

In addition, ratio of molecular masses 178 and 228 are commonly used t distinguish combustion from petroleum sources. For the mass 178, Ant/(Ant + Phe) <0.10 suggests pollution of petroleum origin, while a ratio >0.10 indicates a dominance of combustion (Budzinski *et al.,* 1997; Yunker *et al.,* 2002; Liu *et al.,* 2008). For the mass 228, BaA/(BaA+Ch) suggests that a ratio<0.20 indicates petroleum inputs, a ratio between 0.20 and 0.35 indicates amixed sources (either petroleum or combustion), and a ratio >0.35 indicates combustion sources (Yunker *et al.,* 2002). Our results obtained from isomers ratios of molecular masses 178 and 228 reinforce the combustion process as the major source of PAHs contamination in these three sampling sites. Moreover, such high Ant/(Ant+Phe) and BaA/(BaA+Ch) ratios involve high proportions of anthracene and benz*[a]*anthracene that are both among the most photoreactive PAHs (Gogou *et al.,* 1996). Considering a combustion/transport/deposition process, it can be admitted that no significant photolysis degradation occurred in this case study. These ratios can also imply close contamination sources that can be attributed to the nearby industrial activities or vehicle emissions of Dakar city.

However, there is no natural source of PCBs. Atmospheric depositions, runoff from the land, wood burning and food chain transport have been regarded as the major sources of PCBs in aquatic environment (Totten *et al.,* 2006). PCBs are mainly produced by pyrogenic process due to the combustion of organic matter (Pereira *et al.,* 1980; Kjeller and Rappe, 1995; Rose and Rippey, 2002; Rose *et al.,* 2004; Pandelova *et al.,* 2009). PCBs detected in Dakar coastal area could be originated fromunintentionallyatmospheric deposition, released from equipment, generators, ships, vehicles and trucks exhaust in the surrounding zone but the their origin could not be determined. Similarly, the specific origin of mercury could not identified. It is a global pollutant that is ubiquitous in the environment fromboth natural and anthropogenic sources reaching the ocean through river inputs and atmospheric deposition (Fitzgerald *et al.,* 2007). However, given their relatively low concentration of mercury detected, the domestic and industrial wastes discharged directly into Dakar Bay have not yet produced environmental mercury contamination in this coastal zone. Contrarily, twoother toxic metals such as cadmium and lead present at high concentrations level in sediment of Dakar coastal (Diop *et al.,* 2012). For the ecotoxicological risk assessment, concentrations of total PCBs or Σ28PCBs, sum of 12 PAHs or Σ12PAHs (Aen,Ayl,An, F,N, Pn, BaA, BaP,Ch,

DhA, Fl,Py) and total mercury were calculated. The mean concentrations of Σ_{12}PAHs were 279 (6 - 507), 119 (2 -298) and 18 (1.2 - 80) µg/kg dw for Rufisque, Soumbedioune and Yarakh respectively. While Σ_{28}PCBs mean concentrations detected in the sediments from Rufisque, Soumbedioune and Yarakh were 127(57 - 332), 34 (16 - 47) and 12 (4 - 22) µg/kg dw respectively. Total mercury concentrations did not exceed 95 µg/kg dw. Total mercury, Σ_{12}PAHs and Σ_{28}PCBs concentrations were compared to Sediment Quality Guidelines (SQGs) which provide a valuable tool to evaluate the potential biological adverse effect on aquatic organism caused by contaminated sediments (Long et al. 1995). The ERL/ERM (Effect range low/Effect rangemedian) developed by Long and Morgan (1990) and theTEL/PEL(threshold effect level/Probable effect level) developed by Macdonald *et al.,* (1996) are two based approaches which can be used to assess the ecological toxicity of total PAHs and PCBs concentrations (Σ_{12}PAHs and Σ_{28}PCBs) in sediments collected from Dakar coastal in Senegal. According to SQGs, the classification of biological adverse effects are rarely expected (<ERL/TEL) with minimal-effects range, occasionally (>ERL/TEL and <ERM/PEL) with possible-effects range, and frequently (>ERM/PEL) with a probable-effects range (Long *et al.*, 1995; Cardellicchio *et al.*, 2007). TEL-PEL values were 655-6676 and 22-189 µg/kg dw for the ΣPAHs and ΣPCB, respectively (Macdonald *et al.*, 1996).While ERL-ERM were 4022-44792, 23-180 and 150-710 µg/kg dw for the ΣPAHs, ΣPCBs and mercury, respectively (Long and Morgan, 1990). Our results showed that Dakar coastal sediments have rarely to occasionally biological adverse effects for mercury, PCBs and PAHs in Dakar aquatic ecosystems.

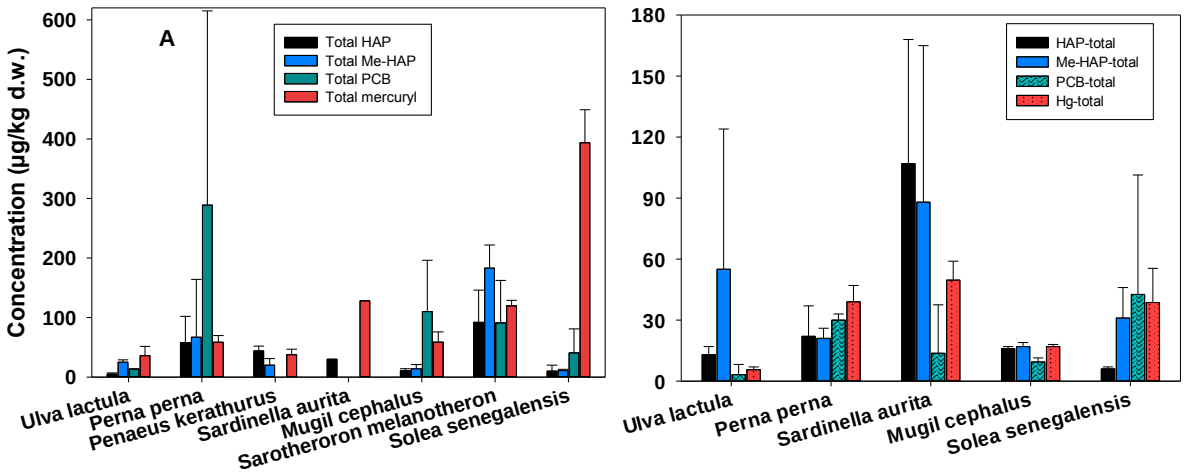

Fig. 3. Mean (±S.D.) concentrations of total PCBs, PAHs, Me-PAHs and Hg in biota samples: A) Soumbedioune station and B) Rufisque station

Overall, even if there is a strong influence of urban and industrial activities in this coastal zone, sediment contamination by PAHs, Me-PAHs, PCBs, and mercury were detected at moderate levels. This is probably due to the tidewhich is responsible to the rapid renewal of sea water, therefore sedimentation processes are not preferred. Indeed, the coastline around Dakar is called microtidal, the tidal rangewhich varies between 0.5 m (neap period) and 1.6 m (during spring tides) (Ruffman *et al.,* 1977). This suggests that urban and industrial waste discharged into Dakar coastal zones could be diluted or transported rapidly seaward. Due to their high toxicity, persistency and bioaccumulation capacities, the concentration of these contaminants have been also studied in marine organismins in order to evaluate the quality of edible species. Mean±S.D of individual concentration of PAH, Me-PAH and PCB and mercury determined in biota samples from all sampling stations of the Dakar coastal area are shown in Table 1. Comparisons of targeted compounds were significantly different between the stations and between species. Considering all organisms, the mean concentration of mercury varies between 5 and 442 µg/kg dw.Generally, muscle tissues of fishes exhibited the highest concentrations of Hg compared to macroalgae and invertebrate species. The highest levels of mercury were detected in *Solea senegalensis* from Soumbedioune station (Fig. 3). Mercury levels measured in *Sardinella aurita* were in the same order of magnitude compared to the mean concentration values reported for the same species collected along the coast of Mauritania in a previous study at 90±80 µg/kg dw (Romeo *et al.,* 1999). For PCBs concentrations, the highest level was determined in *Pernaperna* species (up to1228 µg/kg dw) and the lowest level was found in *Penaeus kerathurus* species. At the base of the food chain, *Ulvalactula* species displayed low PCB concentrations (7±6 µg/kg dw) while fish species exhibited mean values ranging between 10±20 µg/kg dw for *Sardinella aurita* and 95±32 µg/kg dw for *Sarotheroron melanotheron*. Except for *Perna perna*, *Penaeus kerathurus* and *Sardinella aurita*, PCB concentrations were in the same range between organisms and sediments while mercury concentrations were higher in biota than in sediment samples. Whereas, PAH concentrations determined in edible tissues were lower than sediment samples. This may be due to a rapid transformation of PAHs into more hydrophilic metabolites. Therefore, marine organisms exposed to these compounds indicate only trace quantities of PAHs in their tissues (Vuorinen *et al.,* 2006).The concentration level in marine organisms varied from species to species (Table 1 and Fig. 3). This may be translated by behavioral patterns of organisms such as feeding habit, the rate of movement and reproduction status (Vuorinen *et al.,* 2006). The highest mean levels of PAHs and Me-PAHs

(92±54 and 183±39 µg/kg dw respectively) were detected in the tilapia species, therefore the present study supports the idea to use this species as a sentinel in order to monitor chemical pollutants (Ndiaye *et al.*, 2012; Harrison and Whitfield, 2006). Our results showed moderate accumulations of PAHs and Me-PAHs in *Mugilcephalus* and *Soleasenegalensis*. For these two families of organic compounds, similar concentrations were determined in biota samples collected from Soumbedioune and Rufisque stations (Fig. 3). Rose *et al.* (2012) have reported the concentration of ΣPAHs in Tilapia guineensis obtained from Lagos Lagoon ofNigeria at the same order level of 62.24 µg/kg dw. This result were the same order of magnitude as the concentration of ΣPAHs in Tilapia obtained in our study. Compared to other species of fish, Mullus barbatus and Serranus cabrilla collected from Tarragona of Mediterranean accumulated ΣPAHs at the levels of 164.9 and 62.9 µg/kg dw respectively (Escartin and Porte, 1999). Amodio-Cocchieri *et al.,* (1993) reported the concentration of ΣPAHs in Engralis enchrasicholus collected from the Bay of Naples of Italy at the level of 965 µg/kg dw. The highest concentration in fish have reported by McGill et al. (1987) in Limanda limanda collected from the British North Sea at the level of 2345 µg/kg dw. This concentration was much higher than ΣPAHs concentration detected in four species selected in our study. More recently, Abdolahpur Monikh et al. (2014) have also reported the concentration of PAHs in benthic, benthopelagic and pelagic fish species from the Persian Gulf much higher than our results. Σ_{16}PAHs concentrations detected in targeted species were lower compared to concentrations in sediment. However, Σ16PAHs concentrations were detected in all marine species selected in this work. ΣMe-PAHs was present at lowlevels in the sediment samples (Fig. 2). However, their concentrations were detected at higher levels in most of marinespecies (Table 1; Fig. 3). The results clearly showed that all species does not have the same affinity to each type of contaminant. Generally, we observed the bioaccumulation of these contaminants inmarinespecies. However, the bioaccumulation level varies stronglyfrom one species to another (Table 1; Fig. 3). Each species accumulates at least two types of pollutants. Among targeted species, both *Sarothororon melanotheron* and *Perna perna* accumulated all type of contaminants (PAHs, Me-PAHs, PCBs andmercury). The contamination level of PCBs in *Sarinella aurita* and *Penaeus Kerathurus* were under the detection limits (Fig. 3). For Yarakh station, all targeted compounds were quantified in the sediments. However, only mercury was quantified in marine species. For marine species collected inYarakh site, high levels of mercury were detected in *Sardinella aurita* (71.6±23.3 µg/kg dw) and *Sarothororon melanotheron* (71.3±28.0µg/kg dw). *Ulvalactula,*

Mugilcephalus, Perna perna, Penaeus Kerathurus and *Solea senegalensis* species accumulated levels of total mercury at 40.7±5.6, 21.7±6.0, 49.0, 31.0, 15.7±3.0 µg/kg dw respectively. To estimate the potential public health risks, the European Union legislation has established maximum allowed levels in aquatic products for different toxic compounds including some PAHs, PCBs and mercury (Table 2). PAHs, PCBs and mercury concentrations obtained in dry weight (µg/kg dw) were converted into µg/kg wet weight (w.w.) in order to compare with the guideline values. According to the weighed mass before and after drying, the following factors were applied to obtain the concentrations in wet weight: 0.23 for muscle of *Mugilcephalus, Sarotherodon melanotheron, Solea senegalensis and* edible tissue of *Perna perna, 0.30* for muscle of *Sardinella aurita and 0.26* for *Penaeus kerathurus*. Two limit values are listed for mercury depending on the type of mussel and fish (Table 2). For all species, mercury and PCBs levels do not show concentrations exceeding limits fixed by the European Union. In addition, the levels of these compounds in the investigated area were low compared with these threshold values.

Concerning PAHs, the proposed limit values are for smoked products of fish and crustacea species because recentlytheEuropean legislation (Officia Journal oftheEuropeanUnion, 2011) abrogated the limit ofPAHs in these fresh seafood because it has been shown that PAHs are quickly metabolized in these species (Storelli *et al.*, 2013). In our study, limit level (30 µg/kg wet weight) was exceeded only in a *Sardinella aurita* sample with a muscle tissue concentration of 34µg/kg wet weight.Based on European legislation, the selected species present good quality for food based on PCBs, PAHs and mercury.

Table 2. Means ± S.D of individual concentration of targeted compounds (16PAHs, 18Me-PAHs, 28PCBs and total mercury) analyzed in sediment and marine organisms collected from three sampling stations in the Dakar coastal area: invertebrate species (soft body) and fish species (muscle). nd = level not detected (<LOQ) and na = not analysis. (n >3 replicate

Compound	Sediment	Ulvalactula (green algae)	Perna perna (mussel, mollusks)	Penaeus kerathurus (shrimp, crustaceans)	Sardinella aurita (sardine, pelagicfish)	Mugilcephalus (mullet, bentho-pelagic fish)	Sarotheroron melanotheron (tilapia, bentho-pelagic fish)	Soleasenegalensis (sole, benthic fish)
Soumbedioune								
PAHs (µg/kg dw)								
N	3.4±6.3	nd	nd	nd	nd	nd	nd	nd
Acy	6.7±8.9	0.5±0.0	1.9±1.4	1.9±0.3	1.3	0.5±0.3	6.5±0.9	0.8±0.1
Acn	nd		nd	nd	nd	nd	nd	nd
F	4.2±5.7	0.4±0.2	1.5±2.0	0.3±0.2	0.9	nd	1.1±0.3	nd
An	25.7±35.1		1.4±2.8	0.2±0.4	nd	nd	1.5±1.8	nd
Fl	3.2±7.1	0.5±0.7	3.7±5.7	nd	nd	nd	8.9±8.4	nd
BaA	63.3±86.8	0.3±0.0	22.7±9.2	35.2±6.1	24.2	9.6±3.5	4.7±2.2	6.3±9.6
Chr	6.4±12.9	0.3±0.0	5.4±4.7	4.3±0.7	2.9	1.2±0.4	6.9±3.1	1.3±0.9
BaP	nd	nd	nd	nd	nd	nd	nd	nd
Pn	1.6±3.6	1.8±0.6	3.1±1.7	0.2±0.2	nd	nd	5.4±5.1	nd
BbF	33.7±35.5	0.3±0.0	11.6±16.7	nd	nd	nd	32.2±28.1	0.3±0.2
BkF	24.5±33.4	0.3±0.0	1.5±3.3	nd	nd	nd	11.8±15.0	0.3±0.2
Bghi	nd	nd	nd	nd	nd	nd	nd	nd
DhA	nd	nd	nd	nd	nd	nd	nd	nd
IP	nd	nd	nd	nd	nd	nd	nd	nd
Py	4.5±5.5	0.9±0.6	5.6±7.4	1.6±0.2	0.7	0.3±0.1	13.1±4.2	0.6±0.3
Me-PAHs (µg/kg dw)								
1M-Na	2.9±2.6	3.9±3.6	6.9±6.3	17.6±9.1	nd	4.0±5.7	28.2±31.9	nd
2M-Na	1.0±1.1	2.8±3.0	3.1±5.2	4.1±7.7	nd	0.1±0.1	38.4±30.7	nd
1,2-DM-Na	2.4±1.7	4.4±4.0	9.6±3.5	17.6±8.7	nd	6.2±1.9	27.5±31.0	9.5±1.2
1,6-DM-Na	2.7±2.0	5.8±6.8	7.1±11.4	8.4±17.3	nd	0.7±0.3	84.7±69.4	0.6±0.3
2,6DM-Na	0.9±1.0	1.2±0.0	1.6±1.1	0.6±1.0	nd	0.4±0.5	4.7±2.2	0.7±0.4
1M-Pn	nd	0.7±0.2	nd	nd	nd	nd	nd	nd
2M-Pn	1.2±2.7	1.8±0.5	nd	nd	nd	nd	nd	nd
3M-Pn	nd	2.1±0.4	nd	nd	nd	nd	nd	nd
9M-Pn+2Me-An	1.4±3.1	1.3±0.6	nd	nd	nd	nd	nd	nd
1,7DM-Pn	1.7±3.8	0.9±1.3	nd	nd	nd	nd	nd	nd
Retene	0.8±1.8	nd	11.9±22.7	1.6±1.0	nd	nd	nd	nd
1M-Fl	nd	nd	1.2±2.7	nd	nd	nd	nd	nd
3M-Fl	nd	nd	5.5±12.3	nd	nd	nd	nd	nd
1M-Py	nd	nd	6.6±14.7	nd	nd	nd	nd	nd
4M-Py	nd	nd	3.6±8.2	nd	nd	nd	nd	nd
3M-Ch	nd	nd	3.9±8.6	nd	nd	nd	nd	nd
6M-Ch	nd	nd	6.1±13.5	nd	nd	nd	nd	nd
PCBs n° (µg/kg dw)								
8	8.1±5.3	4.1±1.2	nd	nd	nd	nd	nd	nd
18	3.4±5.7	nd	nd	nd	nd	nd	nd	nd
28	0.8±1.2	0.9±0.2	nd	nd	nd	nd	5.5±5.5	nd
44	nd	nd	0.9±1.1	nd	nd	nd	1.6±2.7	0.9±1.0
52	nd	nd	7.4±7.6	nd	nd	nd	2.0±3.5	nd
66	6.4±14.1	nd	4.1±6.1	nd	nd	nd	nd	2.3±4.0
77	nd	nd	42.4±94.8	nd	nd	nd	nd	15.0±18.2
81	nd	nd	nd	nd	nd	nd	nd	nd
101	nd	nd	14.4±21.9	nd	nd	4.7±6.7	12.5±12.4	0.8±1.4
105	2.7±3.5	nd	nd	nd	nd	nd	nd	nd
114	nd	nd	88.3±197.4	nd	nd	13.3±5.4	44.3±76.7	nd
118	1.2±2.4	nd	5.6±8.0	nd	nd	nd	nd	nd
123	2.5±5.3	nd	nd	nd	nd	nd	nd	nd
126	nd	nd	nd	nd	nd	nd	nd	nd
128	nd	nd	nd	nd	nd	nd	nd	nd
138	0.8±1.5	4.0±1.1	54.4±116.8	nd	nd	35.3±42.8	nd	8.8±7.6
153	1.4±0.6	nd	42.1±61.1	nd	nd	27.0±19.3	7.7±13.4	1.0±8.5
156	nd	nd	nd	nd	nd	nd	nd	nd
157	nd	nd	nd	nd	nd	nd	nd	nd
167	nd	nd	nd	nd	nd	nd	nd	nd
169	nd	nd	nd	nd	nd	nd	nd	nd
170	nd	nd	23.7±37.5	nd	nd	2.4±3.3	nd	4.5±4.1

180	nd	nd	5.7±5.5	nd	nd	24.3±19.3	nd	nd
187	nd	nd	nd	nd	nd	nd	nd	nd
189	2.7±6.0	nd	nd	nd	nd	nd	nd	nd
195	2.3±4.2	4.1±0.7	nd	nd	nd	2.9±4.1	17.0±29.5	3.2±5.4
206	nd	nd	nd	nd	nd	nd	nd	nd
209	nd	nd	nd	nd	nd	nd	nd	nd
Total mercury (µg/kg dw)								
Mecury	32.0±35.0	36.0±15.0	58.8±11.3	37.6±9.2	128.0±0.0	58.7±17.1	119.7±9.3	393.7±55.5

Compound	Sediment	Ulvalactula (green algae)	Perna perna (mussel, mollusks)	Penaeus kerathurus (shrimp, crustaceans)	Sardinella aurita (sardine, pelagicfish)	Mugilcephalus (mullet, bentho-pelagic fish)	Sarotheroron melanotheron (tilapia, bentho-pelagic fish)	Soleasenegalensis (sole, benthic fish)
Rufisque								
PAHs (µg/kg dw)								
N	2.8±1.7	nd	nd	na	nd	nd	na	nd
Acy	10.9±8.8	0.4±0.1	0.7±0.1	na	3.7±1.6	0.4±0.4	na	1.3±0.0
Acn	nd	nd	nd	na	nd	nd	na	nd
F	7.6±6.1	0.3±0.1	0.3±0.2	na	1.9±2.2	0.3±0.1	na	0.1±0.0
An	76.2±76.3	0.1±0.2	2.1±0.1	na	9.6±6.2	0.5±0.7	na	0.7±1.1
Fl	1.4±1.9	0.4±0.7	0.9±0.6	na	3.1±5.3	nd	na	nd
BaA	117.0±95.0	7.2±2.2	13.3±1.3	na	68.0±30.2	11.3±1.2	na	0.8±0.2
Chr	0.1±0.2	0.9±0.3	1.6±0.4	na	8.3±3.7	1.4±0.1	na	0.8±0.2
BaP	nd	nd	nd	na	nd	nd	na	nd
Pn	83.3±186.3	2.2±0.6	2.9±1.5	na	7.6±7.6	1.5±1.5	na	nd
BbF	68.9±46.3	nd	nd	na	nd	nd	na	0.7±0.0
BkF	47.9±32.2	1.0±1.8	nd	na	nd	nd	na	0.7±0.0
Bghi	nd	nd	nd	na	nd	nd	na	nd
DhA	nd	nd	nd	na	nd	nd	na	nd
IP	nd	nd	nd	na	nd	nd	na	nd
Py	3.6±3.1	1.0±0.7	0.4±0.1	na	5.1±5.7	0.6±0.2	na	1.1±0.2
Me-PAHs (µg/kg dw)								
1M-Na	2.2±1.3	7.2±7.5	8.6±0.8	na	35.9±43.3	7.5±0.4	na	10.8±6.9
2M-Na	0.1±0.1	2.9±2.5	0.4±0.2	na	12.9±12.7	0.4±0.1	na	4.3±6.6
1,2-DM-Na	2.4±1.4	35.4±5.5	8.0±0.9	na	33.8±40.3	7.2±0.5	na	5.8±7.0
1,6-DM-Na	0.2±0.2	6.8±5.6	2.1±0.5	na	26.6±32.5	0.5±0.1	na	9.2±15.3
2,6DM-Na	nd	2.5±2.2	1.4±0.3	na	5.4±0.3	0.8±0.3	na	0.6±0.7
1M-Pn	nd	nd	nd	na	3.6±5.0	nd	na	nd
2M-Pn	nd	nd	nd	na	5.0±7.1	nd	na	nd
3M-Pn	nd	nd	nd	na	6.8±9.6	nd	na	nd
9M-Pn+2Me-An	nd	nd	nd	na	nd	nd	na	nd
Retene	nd	nd	nd	na	nd	nd	na	nd
1,7DM-Pn	0.6±1.4	0.2±0.4	nd	na	nd	nd	na	nd
1M-Fl	0.1±0.1	nd	nd	na	2.4±3.4	nd	na	nd
3M-Fl	nd	nd	nd	na	nd	nd	na	nd
1M-Py	nd	nd	nd	na	nd	nd	na	nd
4M-Py	0.1±0.1	nd	nd	na	nd	nd	na	nd
3M-Ch	nd	nd	nd	na	nd	0.7±1.2	na	nd
6M-Ch	nd	nd	nd	na	nd	0.4±0.6	na	nd
PCBs N° (µg/kg dw)								
8	15.8±9.3	nd	nd	na	nd	nd	na	nd
18	1.5±2.9	nd	nd	na	nd	nd	na	nd
28	2.0±2.3	nd	nd	na	nd	nd	na	0.8±1.1
44	nd	nd	nd	na	nd	nd	na	nd
52	nd	nd	nd	na	nd	0.9±0.9	na	nd
66	nd	nd	nd	na	nd	nd	na	nd
77	nd	4.4±6.2	nd	na	nd	nd	na	nd
81	nd	nd	nd	na	nd	nd	na	nd
101	5.3±6.0	nd	nd	na	nd	1.0±0.9	na	1.5±1.9
105	5.5±9.2	nd	nd	na	nd	1.1±1.9	na	nd
114	3.4±5.5	0.8±0.6	6.8±9.6	na	nd	nd	na	nd
118	3.2±3.0	nd	nd	na	nd	nd	na	nd
123	2.0±1.3	nd	nd	na	nd	nd	na	nd
126	4.9±8.2	nd	6.7±9.5	na	nd	nd	na	nd
128	1.0±2.1	nd	nd	na	nd	nd	na	nd
138	1.0±2.2	nd	nd	na	nd	3.4±0.7	na	10.8±16.3
153	0.2±0.3	nd	1.5±2.1	na	nd	3.4±1.2	na	3.8±5.4
156	nd	nd	nd	na	nd	nd	na	nd
157	2.4±5.4	nd	nd	na	nd	nd	na	nd
167	31.0±69.3	nd	nd	na	nd	nd	na	nd
169	nd	nd	nd	na	nd	nd	na	nd
170	1.0±1.4	nd	nd	na	nd	nd	na	1.7±2.6

180	6.3±11.6	nd	nd	na	nd	0.9±0.8	na	12.1±24.1
187	nd	nd	nd	na	nd	nd	na	nd
189	32.8±42.4	nd	nd	na	nd	nd	na	nd
195	7.6±11.0	nd	nd	na	13.7±23.8	nd	na	0.8±1.6
206	nd	nd	nd	na	nd	nd	na	nd
209	nd	nd	nd	na	nd	nd	na	nd
Total mercury (µg/kg dw)								
Mercury	10.0±5.0	5.6±1.4	39.0±8.0	na	49.7±9.3	17.0±1.0	na	38.7±16.8

Compound	**Sediment**	**Ulvalactula (green algae)**	**Perna perna (mussel, mollusks)**	**Penaeus kerathurus (shrimp, crustaceans)**	**Sardinella aurita (sardine, pelagicfish)**	**Mugilcephalus (mullet, bentho-pelagic fish)**	**Sarotheroron melanotheron (tilapia, bentho-pelagic fish)**	**Soleasenegalensis (sole, benthic fish)**
Yarakh								
PAHs (µg/kg dw)								
N	nd	na	na	na	na	na	na	na
Acy	0.2±0.0	na	na	na	na	na	na	na
Acn	1.2±1.8	na	na	na	na	na	na	na
F	nd	na	na	na	na	na	na	na
An	nd	na	na	na	na	na	na	na
Fl	5.38±11.7	na	na	na	na	na	na	na
BaA	1.3±2.6	na	na	na	na	na	na	na
Chr	1.7±2.5	na	na	na	na	na	na	na
BaP	nd	na	na	na	na	na	na	na
Pn	0.9±0.8	na	na	na	na	na	na	na
BbF	0.7±1.0	na	na	na	na	na	na	na
BkF	nd	na	na	na	na	na	na	na
Bghi	nd	na	na	na	na	na	na	na
DhA	nd	na	na	na	na	na	na	na
IP	nd	na	na	na	na	na	na	na
Py	6.8±14.2	na	na	na	na	na	na	na
Me-PAHs (µg/kg dw)								
1M-Na	1.7±1.4	na	na	na	na	na	na	na
2M-Na	0.9±1.4	na	na	na	na	na	na	na
1,2-DM-Na	1.7±1.1	na	na	na	na	na	na	na
1,6-DM-Na	3.1±2.7	na	na	na	na	na	na	na
2,6DM-Na	0.7±0.9	na	na	na	na	na	na	na
1M-Pn	0.6±1.2	na	na	na	na	na	na	na
2M-Pn	0.2±0.2	na	na	na	na	na	na	na
3M-Pn	0.4±0.6	na	na	na	na	na	na	na
9M-Pn+2Me-An	1.7±1.6	na	na	na	na	na	na	na
Retene	nd	na	na	na	na	na	na	na
1,7DM-Pn	nd	na	na	na	na	na	na	na
1M-Fl	nd	na	na	na	na	na	na	na
3M-Fl	nd	na	na	na	na	na	na	na
1M-Py	1.4±3.1	na	na	na	na	na	na	na
4M-Py	0.3±1.0	na	na	na	na	na	na	na
3M-Ch	nd	na	na	na	na	na	na	na
6M-Ch	nd	na	na	na	na	na	na	na
PCBs n° (µg/kg dw)								
8	2.4±5.4	na	na	na	na	na	na	na
18	0.8±0.5	na	na	na	na	na	na	na
28	nd	na	na	na	na	na	na	na
44	nd	na	na	na	na	na	na	na
52	nd	na	na	na	na	na	na	na
66	nd	na	na	na	na	na	na	na
77	3.2±3.6	na	na	na	na	na	na	na
81	nd	na	na	na	na	na	na	na
101	1.6±1.8	na	na	na	na	na	na	na
105	nd	na	na	na	na	na	na	na
114	nd	na	na	na	na	na	na	na
118	1.0±2.2	na	na	na	na	na	na	na
123	nd	na	na	na	na	na	na	na
126	nd	na	na	na	na	na	na	na
128	nd	na	na	na	na	na	na	na
138	0.9±0.9	na	na	na	na	na	na	na
153	1.5±1.3	na	na	na	na	na	na	na
156	nd	na	na	na	na	na	na	na
157	nd	na	na	na	na	na	na	na
167	nd	na	na	na	na	na	na	na
169	nd	na	na	na	na	na	na	na
170	0.8±0.7	na	na	na	na	na	na	na

180	nd	na	na	na	na	na	na	na
187	nd	na	na	na	na	na	na	na
189	nd	na	na	na	na	na	na	na
195	nd	na	na	na	na	na	na	na
206	nd	na	na	na	na	na	na	na
209	nd	na	na	na	na	na	na	na
Total mercury (µg/kg dw)								
Mercury	26.0±11.0	40.7±5.6	49.0±0.0	31.0±0.0	71.6±23.3	21.7±6.0	71.3±28.0	15.7±3.0

4. Conclusion

Our study provide a data base about the organic contamination levels of PCBs, PAHs, Me-PAHs and total mercury in sediments and marine organisms obtained fro Dakar coastal zone. The distributions of PAHs and Me-PAHs suggested that their sources were mainly originated from pyrolytic origin of nearby activities. The evaluation of ecotoxicological risk based on SQGs suggested that the biological adverse effects of PAHs, PCBs and total mercury are expected from rarely to occasionally for marine organisms. However, even if the impact of this type of pollution is moderate, it is now urgent to adopt adequate pollution control strategies into this coastal area before the problem become irreversible given the high density of population in this zone and continuous discharges of domestic and industrial effluents for most case without any treatment. Indeed, most of domestic and industrial waste waters are rejected directly into the sea and these permanent anthropogenic discharges can produce environmental and ecological degradation in the coastal zones. Moreover, the tide is quiet important in this coastal zone leading to a significant transport process of pollutants out to the sea. Particular attention must be paid to the chemical levels in edible marine organisms consumed by local population. Fish and invertebrates consumption remain the major source of protein for the residents in Dakar area. The result on the contamination level ofPCBs, PAHs, Me-PAHs and total mercury in marine comestible species showed rarely to occasionally impact on aquatic organisms and no significant impact on human health by consuming targeted species namely mussel, crustacean, sole, sardine, tilapia, and mullet sampled from these coastal areas of Dakar. However, given the only few data available on the degree of contamination levels in these marine species and before making any final conclusion, it is strongly recommended to carry out complementary studies on these comestible species and others.

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Article 4

Distribution of phthalates, pesticides and drug residues in the dissolved, particulate and sedimentary phases from transboundary rivers (France–Belgium)

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Abstract

Various drug residues, pesticides and phthalates are ubiquitous in the environment. Their presence in the environment has attracted considerable attention due to their potential impacts on ecosystem functioning and on public health. In this work, 14 drug residues, 24 pesticides and 6 phthalates have been quantified in three matrices (in the dissolved phase, associated to suspended solid matter (SSM), and in sediment) collected from fifteen watercourses and rivers located in a highly industrialized zone at the cross-border area of Northern France and Belgium. The extractions have been carried out using accelerated solvent extraction (ASE) for solid matrices (SSM and sediment) and using solid phase extraction (SPE) for liquid matrix. The final extract was analyzed using GC–MS technique. Among the three classes of compounds, phthalates have been found at highest level compared to pesticides and drug residues. The Σ6PAE concentrations were ranging from 17.2±2.58 to 179.1±26.9 µg/L in dissolved phase, from 2.9±0.4 to 21.1±3.2 µg/L in SSM and from 1.1±0.2 to 11.9±1.8 µg/g dw in sediment. The Σ_{14}drug residue concentrations were lower than 1.3 $\mu g.L^{-1}$ in the dissolved phases, lower than 30 ng/L associated to SSM and from nondetectable levels to 60.7±9.1 ng/g dw in sediment. For pesticides, all compounds were below the LOQ values in dissolved phase and in sediment, and only EPTC could be quantified in SSM.

***Keywords**: Phthalates, Pesticides, Drug residues, Water, Sediment, SSM, GC–MS*

1. Introduction

Drug residues, pesticides and phthalates or phthalic acid esters (PAEs) are widespread used in worldwide scale. (i) PAEs are widely used in the manufacturing and processing of plastic products as plasticizers. Global production of plastics has reached a level of 150 million tons. 6 million tons of PAEs is also consumed each year, of which European consumption accounts for approximately 1 million tons (Mackintosh et al., 2006). PAEs are used in a very broad range of application (Serôdio and Nogueira, 2006) and their content can reach up to 10–60% w/w (Earls et al., 2003; Van Wezel et al., 2000). Not chemically bound in the polymeric matrix, PAEs can be easily released directly and/or indirectly into the environment, during manufacture, use, and disposal (Cadogan et al., 1993). To date, PAEs are ubiquitous in the environment both in municipal solid waste compost and sludge (Dargnat et al., 2009; Reid et al., 2009), surface and ground water (Gao et al., 2014; Net et al., 2014a), river and marine sediments (Chang et al., 2005), landfill leachate (Buszka et al., 2009), and

biota and air (Staples et al., 1997; Wang et al., 2014). Some PAEs such as BBzP, DnBP, DEHP and DiNP are endocrine disrupting chemicals and their environmental behavior has attracted considerable attention due to their potential impacts on the ecosystem and on public health (Net et al., 2015b,c; Oehlmann et al., 2009; Van Wezel et al., 2000; Ye et al., 2014; Chen et al., 2014). (ii) While pesticides are commonly used in agriculture to protect crop production from harmful species, they can lead to contamination of surface and ground waters (El-Osmani et al., 2014a; Net et al., 2014a). The presence of pesticides in aquatic environments, as well as their ecotoxicological effects on flora and human health have been well documented (Jurewicz and Hanke, 2008; Huen et al., 2012). Urban effluents, agricultural runoff and leaching are the main sources of pesticides in surface and groundwater. Pesticides can cause neurological disorders, affect growth, malfunction of the immune and reproductive systems, cancer and endocrine disruption (Kettles et al., 1997; Petrelli and Mantovani, 2002; Meyer et al., 2003). (iii) Another family of organic contaminants is characterized by drug residues. These compounds can be introduced into the aquatic environment via urban, agricultural and livestock effluents. The fate and behavior of drug residues as well as their metabolites in wastewaters and surface waters have been the subject of numerous studies (Pérez and Barceló, 2007; Barber, 2014). Indeed, wastewater treatment plants or lagoons are not able to completely eliminate drug residues and lead to their presence in natural water. Previous studies reported that surface water, groundwater and drinking water contain hormones from contraceptive treatments, anticancer drugs, opioids, anti-inflammatories and antibiotics (Tauxe-Wuersch et al., 2005; Togola and Budzinski, 2008). Thus, drug residues may present non-negligible environmental risk given the fact that these compounds are initially produced to be biologically active. Drug residues could cause endocrine disruption, and changes in behavior and genetic responses (Amiard and Amiard-Triquet, 2008).

Estuaries and their tributaries in industrialized countries are among themost polluted and degraded streams. Indeed,watercourses and rivers in urbanized and industrial zones always act as receptor for various kinds of organic contaminants frommunicipal, hospital, industrial, agricultural effluents, as well as organic chemicals in use and non-point source pollutions. Biodiversity of an ecosystem is intrinsically linked to water quality (Yüksek et al., 2006; Cardinale, 2011). In the present work, we aim to assess the impact of anthropogenic activities and to evaluate the distribution of PAEs, drug residues and pesticides in dissolved, particulate and sedimentary phases of fresh water system in the transboundary area (Northern France–Belgium) and the watershed upstream of the Scheldt. Fifteen sampling sites located in

a highly anthropized areawith a high population density and historical industrialization among the highest in Europe have been selected. We aim to contribute some key data on the contamination state of these vulnerable areas, and to determine the phases in which these organic compounds tend to preferentially accumulate. It also constitutes some important data for the study on the processes how these organic contaminants can affect the aquatic wildlife (i.e., via water or contaminated food).

2. Materials and methods

2.1. Chemicals and materials

PAE, drug residue and pesticide standards were purchased from Sigma-Aldrich (Saint-Louis, USA) and Restek (Bellefonte, USA). Diphenyl isophthalate and pentachloronitrobenzene with a respective purity of 99% and 94% were used as internal standards for PAEs and pesticides. Paracetamol-d4, estradiol-d5 and caffeine-13C were used as internal standards for drug residues. Supel-Select HLB SPE cartridges (200 mg/6 mL) and C18 cartridges (200 mg/6 mL) were purchased from Sigma-Aldrich (Saint-Louis, USA). HPLC grade ethyl acetate, dichloromethane (DCM), methanol, acetonitrile and hexane were purchased from Dislab (Lens, France). Ultrapure water with 18.2 MΩ cm resistivity (Milli-Q) was produced by a Millipore apparatus. Sodium chloride (NaCl, 99.5%) and analytical grade hydrochloric acid (HCl, 37%) were purchased from Merck (Darmstadt Germany). Potassium hydroxide (KOH, 0.5 mol/L in methanol) was purchased from Panreac Quimica (Barcelona Spain). Prior to use, sodium sulfate, silica and diatomaceous earth were calcinated at 500°C overnight to remove organic materials. GC–MS calibration was performed using eight calibration solutions ranging from 5 ng/mL to 5 μg/mL directly prepared from stock solutions.

2.2. Study sites

The studied area is part of the watershed of the Scheldt (Fig. 1), which presents high anthropogenic activities and a high population density. Moreover, it presents historical industrialization among the highest in Europe (Net et al., 2014a,b, 2015a). Although the mining and metallurgical activities in the Scheldt basin have been reduced in recent decades, remobilization of many metallic contaminants buried in sediments since the beginning of the industrial area constitutes one of the major sources of pollution (Lesven et al., 2009; Louriño-Cabana et al., 2011). However, only very few data concerning organic pollutions are available for these areas (Net et al., 2014b, 2015a). And lack of data concerning PAEs, pesticides and drug residues are available in the literature for the selected sampling sites.

The sampling campaign was conducted in early spring 2014, from 07/04 to 10/04/2014 in fifteen rivers and streams located at the crossborder area Northern France–Belgium and the watershed upstream of the Scheldt (Fig. 1). Six stationswere located along the Scheldt (Fresnes, Neuville, Crevecoeur, Warcoing, Berchem, Zingem), three on the Lys River (Aire sur-la-Lys, Erquinghem-Lys, Wervicq), two on the Deûle River (Don, Wambrechies), two in the Scarpe River (Brebière and Nivelles), one in the Sensée River (Férin) and one in the Sambre River (Jeumont).

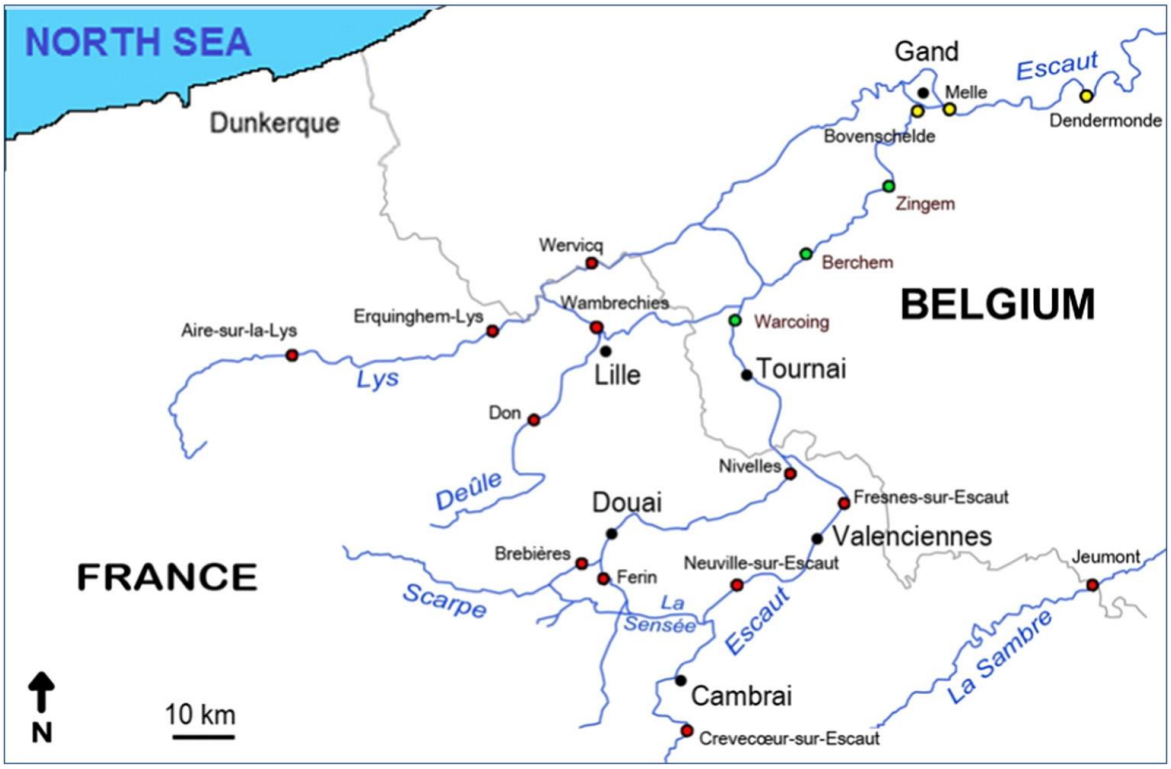

Fig. 1. Studied sites at the cross-border area of Northern France–Belgiumand the watershed upstream of the Scheldt. Sampling stations are indicated by red and green cycles respectively for the studied areas located in France and in Belgium.

2.3. Sampling

Water samplings were performed using pre-cleaned amber glass 2.5 L bottles that were immediately capped with Teflon-lined lid. Surface sediment samples (0–10 cm approximately) were collected using an Ekman bottom grab samplers. Previously calcinated diatomaceous earth, used as blank of the procedure, was brought to the sampling field and was subjected to the same pretreatment conditions as the sediment samples. Each sediment sample was homogenized before being transferred into pre-calcinated aluminum containers

that are capped with pre-calcinated aluminum foils. Sediment samples were transported to the laboratory and were dried at room temperature under a laminar hood without any storage step.

2.4. Targeted analytes

In this work, the following PAEs, drug residues and pesticides were analyzed.

2.4.1. Phthalates (6 PAEs)

Dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butylphthalate (DnBP), butyl benzyl phthalate (BBP), di-2-ethylhexyl phthalate ester (DEHP) and di-n-octyl phthalate (DnOP).

2.4.2. Drug residues (14 compounds)

Caffeine, imipramine, doxepin, aspirin, paracetamol, gemfibrozil, metoprolol, naproxen, atenolol, ketoprofen, carbamazepine, diclofenac, alpha-estradiol and estriol.

2.4 .3. Pesticides (24 compounds)

Alachlor, atraton, atrazine, butachlor, butylate, cyanazine, cycloate, diphenamide, (S)-ethyl-N,N-dipropylthiocarbamate (EPTC), N-(2-ethylhexyl)-5-norbornene-2,3-dicarboximide (MGK264), metolachlor,molinate, napropamide, pebulate, prometon, prometryn, pronamide,propazine, propachlor, terbutryn, trifluralin, triadimefon, vernolate,and hexazinone.

Physicochemical properties of each PAE, pesticide and drug residuefor a total of 44 targeted compounds selected in this study are presented in Table 1S in Supporting information. Limits of quantification (LOQ) and extraction yield with interest matrices are presented in Table 2S.

2.5. Sample preparation

Back to the laboratory, water samples were immediately filtered using previously calcinated 0.7 µm Whatman glass microfiber filters. Targeted organic contaminants in dissolved phase were extracted using solid phase extraction (SPE) technique. Organic contaminants associated to SSMand sedimentswere extracted using accelerated solvent extraction (ASE). For the quantification of targeted compounds associated to SSM, 2.5 L of water was filtered and total SSMretained on filters was used for ASE extraction. Procedural blanks were performed in triplicate with each set of samples.

2.5.1. SPE extraction

Filtered water samples were separated in three aliquots of 500 mL, which were spiked with the appropriate internal standards prior to SPE extraction. The SPE cartridges, containing hydrophilic–lipophilicbalanced (HLB) copolymer, were chosen because of their ability to extract a broad range of compounds. For the extraction of pesticides, the aliquot was adjusted to pH 6 and extracted according to the procedure developed by El-Osmani et al. (2014b). For PAEs, the extraction was carried out using C18 SPE cartridges according to the procedure described by He et al. (2013). Drug residues were extracted according to the method developed by Togola and Budzinski (2008). For the three classes of compounds, after the elution, 5 g of pre-calcinated anhydrous sodium sulfate was added to the extracts to eliminate eventual trace of water. Each sample was then concentrated and solvent exchanged to 200 µL in hexane for PAEs, 50 µL in acetonitrile for drug residues and 50 µL in AcOEt for pesticides prior to GC–MS analysis.

2.5.2. ASE extraction

PAEs, drug residues and pesticides associated to SSM and sediments were extracted using ASE (ASE 200, Dionex Corp., USA). For SSM, the extractions were performed on dried filter containing SSM previously cut into small pieces. For sediment, the extractions were performed on finely ground sediment previously sieved at 224 µm. Each sample was spiked with internal standards. After a delay of equilibration, each sample was extracted with two methods successively. Firstly, the sample was extractedwith DCM/acetone (1/1 v/v) according to the method developed by Reid et al. (2009) for PAE extraction. Secondly the extraction was performedwith DCMas extracting solvent according to themethod developed by Tronczynski et al. (2005). High purity nitrogen was employed as the purge gas. Extracts were then combined and subjected to a purification step described hereafter with the aim to remove the interferences.

2.6. Purification and pre-treatment of the extract from sediments

Molecular sulfur in the sediment extracts was removed by addition of activated metallic copper to the extracts. The sediment extracts were concentrated, solvent-exchanged to hexane, and were purified and fractioned on a silica column to eliminate organic interferences. Targeted compounds were recovered by successive elution with 20 mL of hexane, 15 mL of hexane/DCM mixtures (3/1 v/v) followed by 15 mL of hexane/DCM mixture (1/1 v/v), 15 mL DCM and then 15 mL of acetonitrile. Each sediment sample was concentrated to 500 µL in hexane for sediment sample. For SSM sample, the extract was

concentrated to 200 μL in hexane without purification step. PAEs, pesticides and some drug residues (caffeine, imipramine and doxepin) were analyzed directly with GC–MS. For other drug residues (aspirin, paracetamol, gemfibrozil, metoprolol, naproxen, atenolol, ketoprofen, carbamazepine, diclofenac, alpha-estradiol and estriol) analyses, the extracts were evaporated to dryness with slight flow nitrogen. 40 μL of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) was added into the dried sample and heated at 75°C during 45 min then kept a fewminutes at room temperature prior to GC–MS analysis.

2.7. GC–MS analysis

The extracts were analyzed using a Varian 3900 gas chromatograph (GC) equipped with a deactivated fused-silica guard column (5 m, 0.25mm i.d.) and a fused-silica capillary Phenomenex XLB (60mlength, 0.25 mm i.d., 0.25 μmfilmthickness) and coupledwith a Varian Ion Trap Saturn 2000 Mass Spectrometer (MS). The carrier gas was helium, held at a constant flow rate of 1 mL min−1. Samples were injected in the splitless mode at 280 °C and the injector was purged with helium after 1 min. Each group of organic compounds was analyzed separately. The temperature of the GC was programmed as follows: initial temperature 70°C, held for 1 min, 10 °C/min ramped to 170°C then 4°C/min ramped to 220°C, and finally 2.5°C/min to 280°C and held for 12.50 min for pesticide analysis. For PAEs, the temperature of the GC was programmed as follows: initial temperature 80 °C, held for 1 min, 10°C/min ramped to 170°C then 4°C/min ramped to 230°C, and finally 3°C/min to 280°C and held for 7 min. For drug residues, the temperature of the GC was programmed as follows: initial temperature 90°C, held for 2 min, 5°C/min ramped to 250°C then 3°C/min ramped to 300°C and held for 2.33 min. The transfer line and the ion trap were respectively held at 280°C and 220°C. Each targeted compound was identified based on its retention time and its mass spectrum obtained from chromatogram of standard solutions acquired in full scan mode. Quantification was then performed in the single ion storage (SIS), MS/MS or multiple reaction monitoring (MRM) modes for better selectivity. Response factors were determined relative to the internal standard response and to standard mixtures. The retention times, detection mode, quantifier ions and LOQ of each targeted compound are presented in Table 2S in Supporting information.

3. Results and discussion

The primary issue for the quantification of organic contaminant and particularly PAEs is the sample contamination that can occur during sample treatment and analysis (Fankhauser-Noti and Grob, 2007; Marega et al., 2013; Net et al., 2015c). Indeed, PAEs are ubiquitous in plastics, in the laboratory environment, products, reagents and solvents, analysis of real samples with a low PAE background can be difficult (Prokůpková et al., 2002; INERIS, 2009; Net et al., 2015c). In this work, procedural blanks for pesticides and drug residues analysis were free from any targeted pesticides and drug residues. However, significant levels (higher than LOQ) of PAEswere sometimes detected in procedural blanks, especially for DnBP and DEHP for which values measured in the blanks could reach up to 8% of the concentrations of the environmental samples. For this reason, results of DnBP and DEHP were systematically corrected by subtracting PAE values found in procedural blanks. Individual concentrations of the PAE, pesticide and drug residue are presented in Table 3S in the Supporting information.

3.1. Occurrence of PAEs, drug residues and pesticides in dissolved, particulate and sedimentary phases

The Σ_6PAE concentrations were found to vary significantly for the fifteen studied stations either in dissolved phase, associated to SSM and sediment (Fig. 2A). By considering the six studied rivers, Fig. 2B represents the average concentrations of the Σ_6PAEs in the three phases.

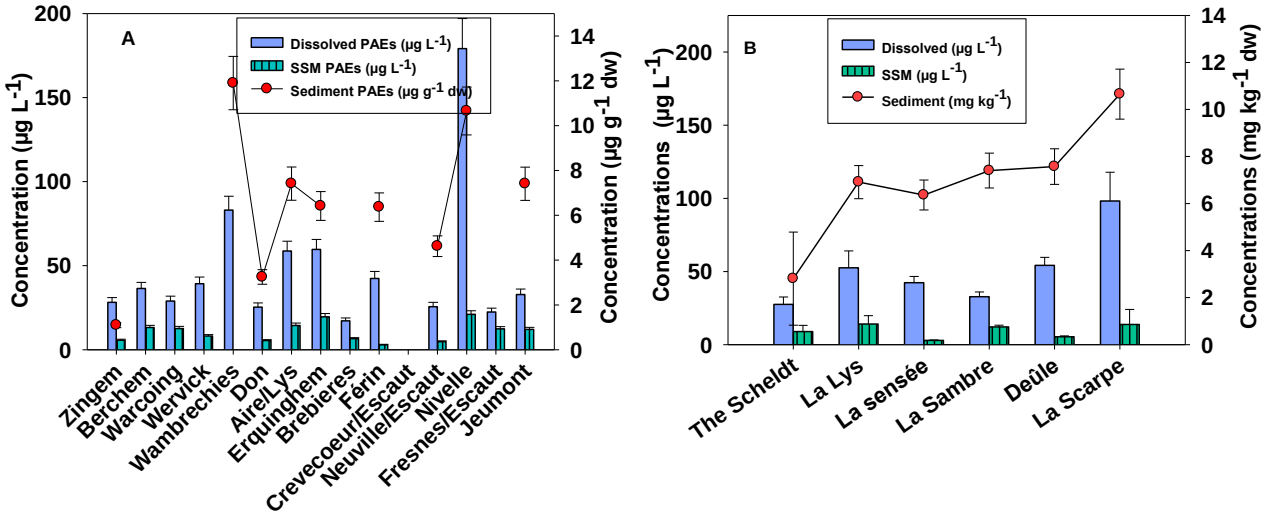

Fig.2. Comparison of $\sum_6$PAEs contents in water, associated to SSM and in sediment A) for each station and B) for each river.

The Σ_6PAE concentrations in dissolved phase of water samples were ranging from 17.2±2.58 to 179.1±26.9 µg/L with an average value of 46.8±3.9 µg/L . In SSM, the Σ_6PAE concentrations were found to range from 2.9±0.4 to 21.1±3.2 µg/L with an average value of

10.3±1.5 µg/L , that corresponds in terms of mass concentration to 148.7±2.3 - 1603.0±240.4 µg/g dw with an average value of 589±88.3 µg/g dwof SSM. These values are similar to those reported by Zheng et al. (2014) for anthropized areas. The highest level of Σ_6PAEs was detected at station Nivelles with 179.1±26.9 µg/L in dissolved phase and 21.1±3.2 µg/L associated to SSM (i.e., 605.7±90.8 µg/g of SSM dw) (Fig. 2). In sediment, the Σ_6PAEs ranged from 1.09±0.2 to 11.89±1.8 µg/g dw for an average of 6.22±0.9 µg/g dw with the maximum concentrations recorded at Wambrechies and Nivelles (Fig. 2). The Scheldt was found to be the less contaminated with an average Σ_6PAE level of 27.5±5.1 µg/L in dissolved phase and 2.8±1.9 µg/kg in sediment. Similar contamination levels were detected for the Lys, the Sensée, the Sambre and Deûle Rivers for both water and sediment compartments. The Scarpe River was identified as the most contaminated river for both the dissolved (98.2±19.6 µg/L) and sedimentary phases (10.7±1.1 µg/g) (Fig. 2B). A previous study showed that the sediments of the Scarpe River were also highly contaminated by PAHs and PCBs compared to the Deûle and the Sensée Rivers (Net et al., 2015a).

Fig. 3 presents the concentration of the Σ_{14}drug residues in the three phases A) for each sampling site and B) for each river. The concentrationof drug residues was found to vary stronglywhatever the sampling site. In the dissolved phase, concentrations of Σ_{14}drug residues were lower than 1 µg/L except for the Don station in the Deûle River. The highest concentration was recorded at Don (1290±193.5 ng/L), followed by Férin (800±120 ng/L) and Erquighem-Lys (654±98 ng/L) (Fig. 3A). The total concentrations of drug residues associated to SSM were always lower than 30 ng/L, much lower than in the dissolved phase. Although low levels of drug residues were detected in the water column of the Scarpe River, their concentrations were detected at high level in the sediment (Fig. 3B). The highest concentration of drug residues was found in Nivelles (station located in the Scarpe River) with 60.7±9.1 ng/g dw followed by Zingem located along the Scheldt (27.8±4.2 ng/g dw) and Don along the Deûle River (26.9±4.0 ng/g dw).

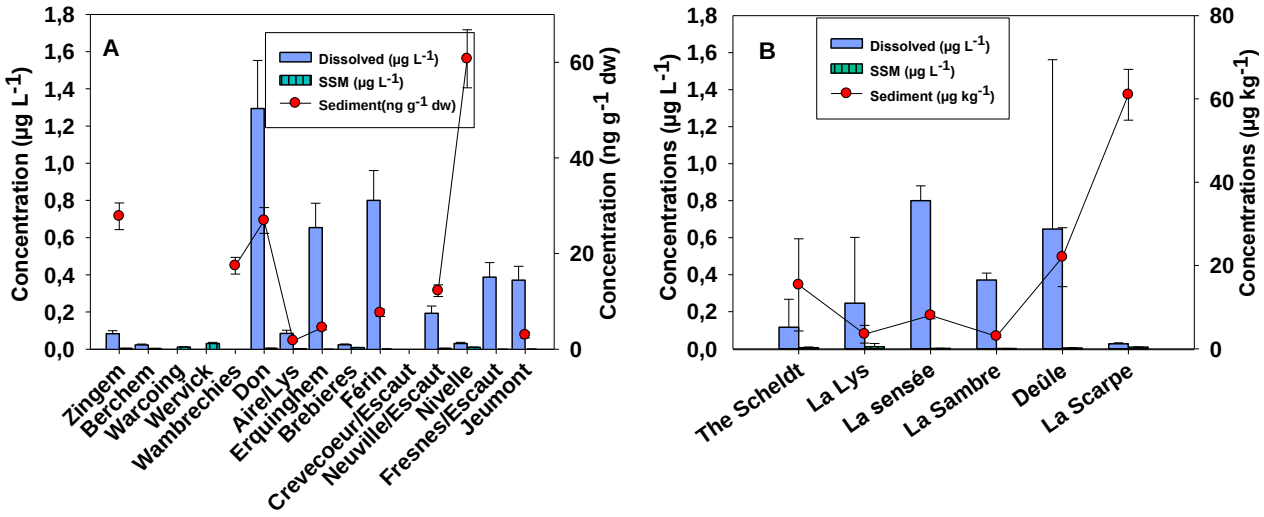

Fig. 3. Comparison of Σ_{14}drug residue contents in water, associated to SSM and in sediment A) for each sampling station and B) for each river.

3.1.1. SSM pesticide concentration (ng/g)

Among the 24 studied pesticides, only EPTC could be quantified when associated to SSM (Fig. 4A and B). In dissolved phase and in sediment, the pesticide concentrations were under the LOQ values. Highest levels were recorded in SSM at Zingem, Nivelle and Jeumont with concentrations that can reach 113±11, 152±15 and 1288±138 ng/L respectively. Overall, based on the targeted PAEs, pesticides and drug residues selected in this study, Nivelles station located in the Scarpe River was found to be the most contaminated. Thismay be due to the fact that Nivelles is closely surrounded by the big cities (Lille, Douai, Cambrai and Valenciennes) that concentrate many urban and industrial activities.

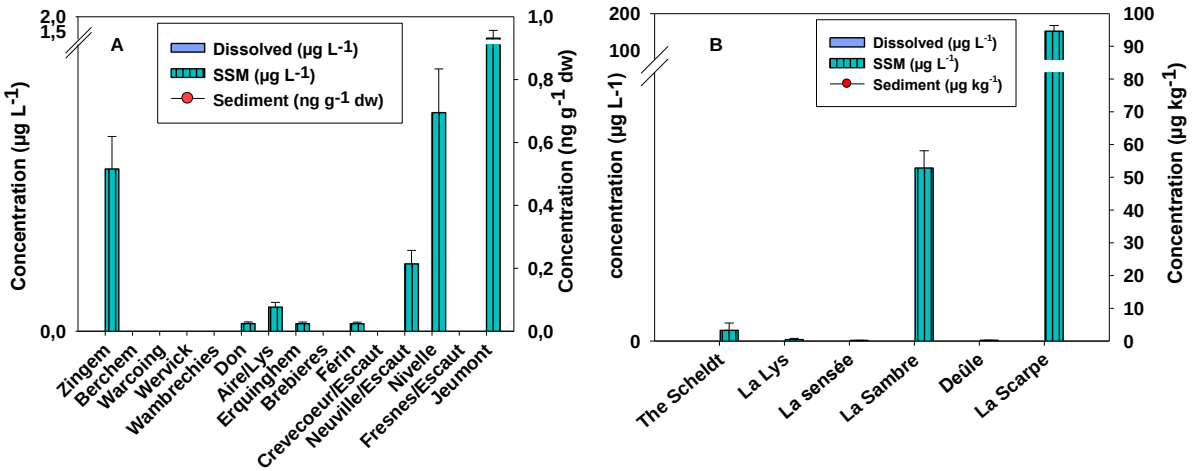

Fig.4. Comparison of EPTC contents in water, associated to SSM and in sediment A) for each station and B) for each river.

3.2. Distribution of PAEs, drug residues and pesticides in the three phases

Fig. 5 shows the distribution of the six PAEs (DMP, DEP, DnBP, BBP, DEHP, DnOP) in the fifteen watercourses and rivers located in crossborder of Northern France–Belgium. These six PAEs are listed as priority substances and are known to be the most toxic and the most predominant PAEs in the environment. Present results show that DEHP was the predominant species among the six selected PAEs. It accounted for 54%, 64% and 57% of the Σ_6PAEs in dissolved phase, SSM and sediment respectively (Fig. 5).

3.2.1. SSM PAEs concentration

DnBP was also found in abundance in both the dissolved phase and SSM with 22% and 14% of the Σ6PAEs respectively. The concentrations of individual PAE varied strongly depending on the location of sampling sites. Worldwide contamination levels of individual PAE in fresh water can exceed 500 mg/L (Fatoki and Ogunfowokan, 1993). However, individual PAEs are generally detected at concentration varying from the order of tens ng/L to few hundred μg/L (Baig et al., 2009; Dargnat et al., 2009; Gao et al., 2014; He et al., 2013; Net et al., 2014a, 2015b; Zheng et al., 2014). In the present study, DMP and DEPwere found predominant in dissolved phase and account for 84.4 and 83.7% of the Σ6PAEs respectively. Much lower proportion of DMP (16%) and DEP (16%) was found associated to SSM probably because of their high SW and relatively low log KOW (Table 1S). DnBP and BBP with moderate SW and higher log K_{OW} were associated to SSM in low proportion of 21.8% and 19.5% respectively. DnBP and BBPwere indeed mainly detected in dissolved phase (87% for DnBP and 92% for BBP) (Fig. 5). Similar observation has been reported for the St. Lawrence River (Canada), where only 14% of DnBP was sorbed to SSM while the rest was present in the dissolved phase (Germain and Langlois, 1988). DEHP and DnOP, with longer alkyl chains, have lower S_W and high log K_{OW} (Table 1S) compared to DMP, DEP, DnBP and BBP. Consequently, they were found at higher level associated to SSM, i.e., 37 and 56% for DEHP and DnOP respectively (Fig. 5). Furtmann (1993) has reported quite similar observations where only 15–17% of the low molecular weight PAEs (DMP, DEP, DnBP and BBP) were sorbed, while DEHP and DnOP bound to particulate phase accounted for 53–74%. Low molecular weight PAEs in water column present mainly in dissolved phase while higher molecular weight PAEs (DEHP, DnOP) accumulate more easily in solid matrices. The distribution of each PAE between dissolved and particulate phase may be controlled by the physicochemical properties. Fig. 6 shows the correlation between the physicochemical properties (S_W and log K_{OW}) and the percentage of distribution of each PAE in the dissolved

phase. The results show the slight tendency in decreasing the proportion of PAE in dissolved phase when log K_{OW} increases. Similarly, the proportion of PAE concentration in dissolved phase increases with the increasing of SW (Fig. 1S in Supporting information).

Highest levels of PAEs were detected at Nivelles and Wambrechies stations (Fig. 5). This can be attributed to the presence of urban and industrial activities nearby. Metropolitan areas, that concentrate urban and industrial activities, are known to be a major source of PAEs in aquatic system surrounding. Sánchez-Avila et al. (2009) reported for instance high concentrations of PAEs (0.12−7.05 µg/L for DMP; 22.2– 192 µg/L for DEP; 0.03–10 µg/L for BBP; 3.24–33.6 µg/L for DnBP and 4.47–278 µg/L for DEHP) in Spanish surfacewater in an area that concentrates industrial and urban activities. Similar levels were recorded in South Africa (i.e., 103 µg/L for DEP; 10 µg/L for BBP; 2131 µg/L for DnBP and 280 µg/L for DEHP detected in Veldwachters River) (Olujimi et al., 2012). Focusing in France, PAEs levels recorded for the present fifteen watercourses and rivers were higher than those that can be found in the Seine River estuary (France), the Marne River (France) and the Somme River (France) reported respectively by Baig et al. (2009), Dargnat et al. (2009) and Net et al. (2014a). However, present value remains much lower than the concentrations reported by Sánchez-Avila et al. (2009) and Olujimi et al. (2012).

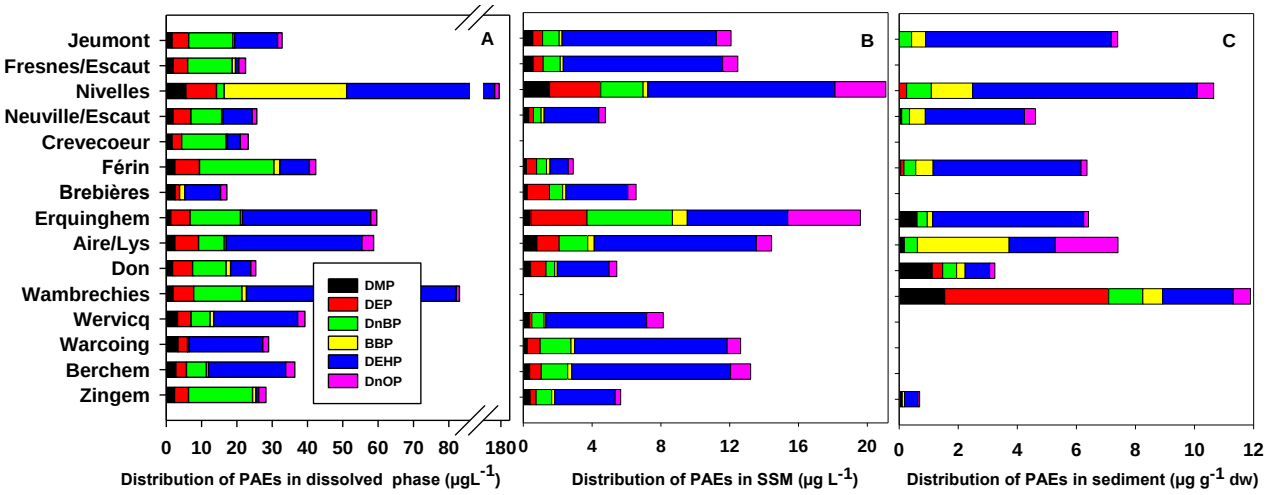

Fig. 5. Distribution of individual PAE in dissolved, particulate and sedimentary phases

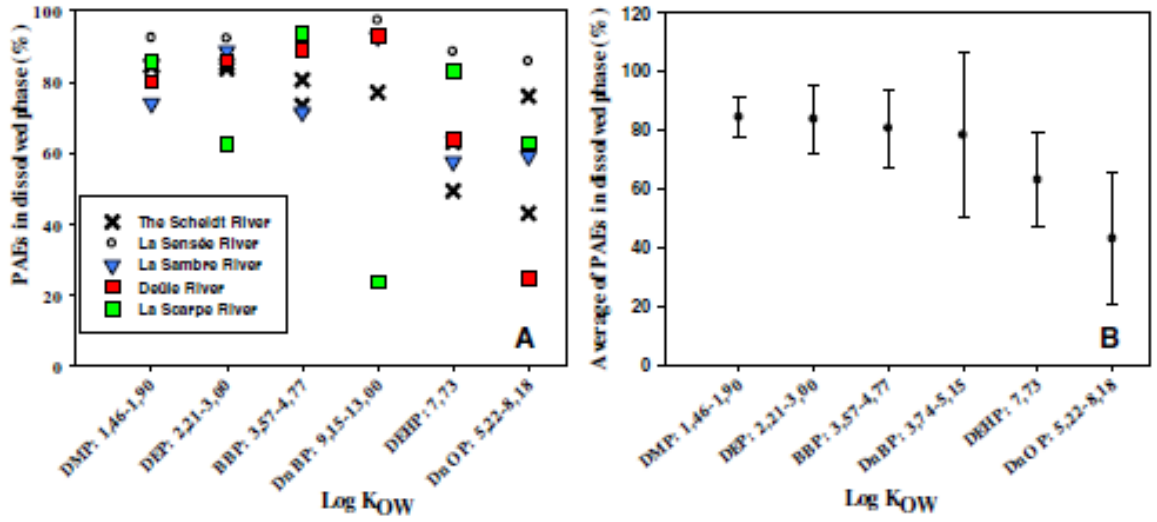

Fig. 6. Percentage of individual PAE concentration in dissolved phase ([PAE]in dissolved phase / ([PAE]in dissolved phase+[PAE]in SSM) ∗ 100) versus log KOW for the average of (A) each river and (B) all rivers.

3.2.2. SSM pesticide concentration

For pesticides, as reported previously, only EPTC was detected among the 24 pesticides. Itwas lower than 2.5 ng/kg dw of sediment. In water column, EPTC was detected only when associated to SSM. This may be due to the fact that EPTC has log K_{OW} of 3.21 which indicates sorptive capacity. Moreover, its S_W varies from 0 to 375 mg/L (Table 1S) which indicates that it can be moderately soluble or insoluble depending on the conditions of the environment (i.e., temperature).

Among the 14 studied drug residues, only one (caffeine) was detected in dissolved phase, seven (aspirin, atenolol, estriol, gemfibrozil, metoprolol, naproxen, paracetamol) in particulate phase and two (imipramine, paracetamol) in sediment. Fig. 7 shows the distribution of individual drug residue in dissolved, particulate and sedimentary phases.

3.2.3. SSM drugs concentration

In the dissolved phase, only caffeine was detected with concentrations that can be up to 1.3 μg/L (Fig. 7). The presence of caffeine in environmental matrices mainly originates from coffee consumption. Even if caffeine can be eliminated from wastewater via biological process (Joss et al., 2005), its extensive use and permanent release have resulted in its presence in the urbanized watercourse, river and estuarine systems (Togola and Budzinski, 2008). In SSM, several drug residues were detected at lowconcentration. Besides, even if caffeine was detected at considerable levels in dissolved phase, no trace of caffeine could be

detected in SSM and sediment. This can be explained by the fact that caffeine has a high solubility in water (21,600 mg/L at 25°C) and a low capacity of sorption (Log K_{OW}= −0.07).

Seven drug residues were detected in SSM, but only estriol and metoprolol were quantified. Metroprolol has a high solubility in water and a moderate capacity of sorption (Table 1S). For its part, estriol has moderate solubility and sorption capacity (Table 1S). Even if they were detected in SSM, their concentrationswere relatively lowwith an average of 6 ng/L (236 ng/g) and 0.6 ng/L (53 ng/g) respectively for metoprolol and estriol (Fig. 7). Fig. 2S in Supporting information shows the average concentration of drug residues associated to SSM versus (A) water solubility, (B) versus log K_{OW}. However, there is no correlation between the physicochemical properties (SWand log K_{OW}) and the distribution of each drug residues in the dissolved and particulate phases. Indeed, it is difficult to find out a clear correlation of the distribution of the majority of interest drug residues due to the fact that they present at low concentration in our selected sampling sites. Most of the drug residues were not quantified in dissolved and sedimentary phases. This may be due to their high LOQ values of some compounds (i.e., 50 ng/L for alpha-estradiol, estriol and diclofenac) (Table 2S in Supporting information). Indeed, only 500 mL of water sample was used for quantification of drug residue in dissolved phase. For particulate phase, SSM obtained form 2.5 L of water sample was used for each extraction.

In sediment, imipramine was detected but its concentration was lower than LOQ (Table 2S). Only paracetamol was present at quantifiable level. Its concentration in sediment was ranging from lower than LOQ values to 61 ng/kg dw. Paracetamol was quantified only in three sampling sites at Zingem, Don and Nivelles at 28±4, 27±4 and 61±9 ng/kg dw respectively (Fig. 7).

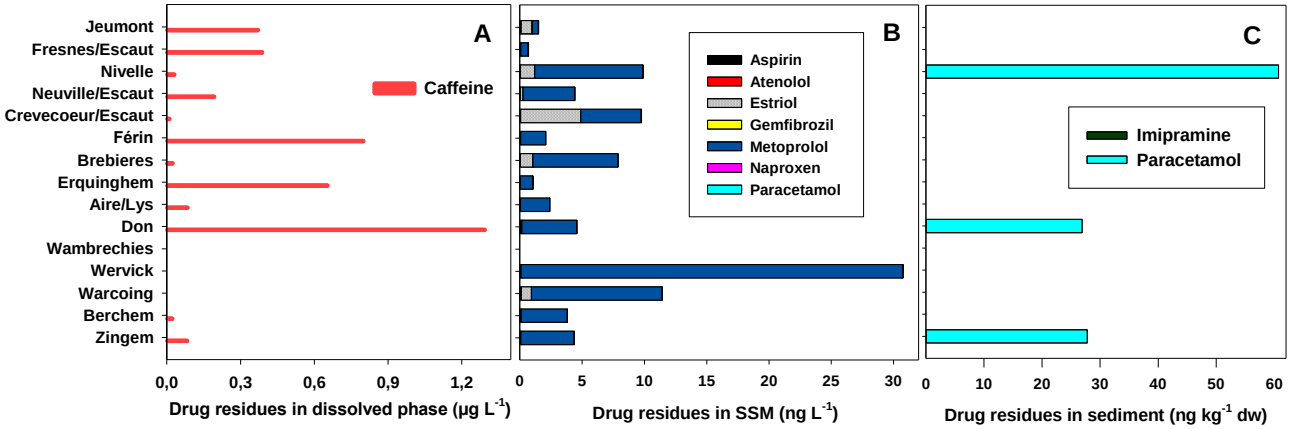

Fig. 7. Distribution of individual drug residue in dissolved, particulate and sedimentary phases.

4. Conclusion

Contamination of fifteen watercourses and rivers located at the cross-border area Northern France and Belgium have been evaluated. Three groups of organic pollutants (drug residues, pesticides and phthalates) for a total of 44 organic contaminants have been studied for three matrices (in dissolved phase, associated to SSM and in sediment) obtained from fifteen stations. These fifteen stations are located in six rivers namely the Scheldt, the Lys, the Deûle, the Scarpe, the Sensée and the Sambre Rivers. Nivelles and Wambrechies stations were the most contaminated by PAEs. High concentrations of paracetamol were also detected in the sediment obtained from Nivelles. Globally, the Scarpe River was the most contaminated and the lowest contamination levels were obtained for the Scheldt. The distributions of targeted compounds strongly depend on their physicochemical property (S_W, log K_{OW}). Low molecular weight PAEs (DMP, DEP, DnBP and BBP), with high SW and lower log KOW compared to those with higher molecular weight (DEHP, DnOP), were associated in low proportion (8–22%) to SSM while DEHP and DnOP accounted for 37–56% bound to particulate phase. The sorption capacities of PAEs lead to their accumulation in sediment. Higher levels of PAEs were indeed found in sediment. For drug residues, caffeine, which has high SW and very low log K_{OW}, was only quantified in the dissolved phase. For other drug residues, their distribution in the different phases could not be well defined due to their low concentrations in the interest studied sites. For pesticides, EPTC was the only pesticide detected in this work and it accounted for 100% in particulate phase.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.03.087.

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Article 5

Overview of persistent organic pollution (PAHs, Me-PAHs and PCBs) in freshwater sediments from Northern France

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Abstract

PCBs, parent and alkyl-PAHs have been quantified in sediments collected from three canalized rivers (Deûle, Sensée and Scarpe) all located in a highly industrialized zone of the Nord Pas-de-Calais region, Northern France. Quantification using GC–MS allowed the determination of the dispersion trend, the origin as well as the relative potency of the studied sediments. Contamination depth profiles of PCBs, parents and alkyl PAHs have been studied for the three sediment cores. Total concentrations of PCBs (Σ28PCBs) have revealed a higher contamination level for the Scarpe River (ranging from 126.8 to 194.4 μg/kg dw) by comparison with the Sensée River (from 15.1 to 34.0 μg/kg dw) and the Deûle River (from n.d. to 15.6 μg/kg dw). Sedimentary depth profiles of total PAHs (Σ16PAHs) and alkyl-PAHs (Σ18Me-PAHs) suggest a significant recent contamination of these three studied sites according to the high concentrations recorded in the surface of sediment cores (up to 33.7 mg/kg dw for the Scarpe River). The possible sources of PCBs have been identified through a principal component analysis, while the pyrolytic origins of PAHs have been determined using the molecular indexes. The Scarpe River reveals to be the most polluted river according to the consensus-based sediment quality guidelines.

***Keywords**: PAHs, Me-PAHs, PCBs, GC–MS, Sediment quality guidelines, Source apportionment*

1. Introduction

Organic compounds discharged into aquatic environment can bring negative impacts on aquatic ecosystem by direct and indirect toxic effects on organisms (Fleeger et al., 2003). Actually, organic contaminants are a major environmental cause for concern due to their persistence, long-range transportability and their potentially adverse effects on living organisms. Moreover, most of organic contaminants are fat-soluble and can lead to bioaccumulation, thus affecting not only aquatic ecosystems but also human health via drinking water resources and food chain. River water acts as receiving water for various kinds of organic contaminants from municipal and industrial wastewaters (Malve et al., 2003; Singh et al., 2004; Zhang et al., 2004). For the ecosystem protection and to keep water resources clean, it is important to identify the nature of contaminants, their contamination levels aswell as their sources.

Organic contaminants are a group of chemicals that have been intentionally or inadvertently produced and introduced into the environment. Polychlorobiphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs and Me-PAHs) belong to the class of persistent organic pollutants (POPs). PCBs have been commonly used as dielectric fluids or transformers and capacitors, and also in paints, inks and pesticides until the hazard posed to both the environment and human health by their use became evident. They are extremely stable compounds under environmental conditions (WHO, 1993). Due to their toxicity, chronic persistence and bioaccumulation, they have been banned or restricted, and some of them have been included in the list of priority pollutants in many countries. However, PCBs remain present in water and sediment (Dumoulin et al., 2013; Smith et al., 2009) and continue to affect aquatic organisms all along the food chain and consequently human health through the diet (Sun et al., 2002). Dissolved PCBs in water only represent a small fraction of total PCBs due to their hydrophobic character, which causes their rapid association to organic entities such as sediments, algae and protozoa (Booij and van den Berg, 1994; Brannon et al., 1993; Eganhouse and Gossett, 1991; Hargrave et al., 1992). PAHs and Me-PAHs can be originated from the incomplete combustion of wood, coal, industries and vehicle emissions (Wang et al., 2007; Yunker et al., 2002). They can also come from seepage of crude oil and coal or oil spills. Hydrocarbons are highly lipophilic compounds, ubiquitous in the water column of coastal, estuarine and river, as well as in sediments in which they tend to accumulate (Cailleaud et al., 2007; Chiou et al., 1998; Gaspare et al., 2009; Ko and Baker, 1995; Manodori et al., 2006; Net et al., 2014; Yunker et al., 2012). Recent studies showed thatmarine organisms are prone to bioaccumulation of these substances, especially in lipid-rich tissues (Dugan et al., 2005; Francioni et al., 2005; Neff, 2002). Because of their toxic, carcinogenic and mutagenic effects (IARC, 2010; Straif et al., 2005) sixteen PAHs have been listed as priority pollutants by the U.S. EPA. The aim of the present study was to investigate the concentration levels and the sources of these organic contaminants in order to assess the quality of river sediment from the three sites in the Nord Pas-de-Calais region, Northern France. Samples were analyzed for 28 PCBs, 16 PAHs and 18 Me-PAHs using gas chromatography/mass spectrometry (GC/MS).

2. Materials andmethods

2.1. Reagents

Sediments samples were analyzed for 16 PAHs, 18 alkylated polycyclic aromatic hydrocarbons (Me-PAHs) and 28 PCBs including 12 dioxin-like PCBs (PCB-DL) and the 7

indicators PCB (PCBi). Mixed standard solutions of PAHs andMe-PAHs were purchased fromRestek Corp (Bellefonte, PA, USA). PCB standard solution was obtained from Accustandard Inc. (New Haven, CT, USA). Tetrachloronaphthalene (TCN), 2,3,3′,5,6-tetrachlorobiphenyl (PCB112) and octachloronaphthalene (OCN), used for PCB quantification, were purchased from Dr Ehrenstorfer (Augsburg, Germany). Deuterated internal standards for PAHs and Me-PAHs (acenaphthene-d10 (A-d10), naphtalene-d8 (N-d10), perylene-d12 (Per-d12), phenanthrene-d10 (Phe-d10) and pyrene-d10 (Pyr-d10)) were provided by LGC-Promochem (Middlesex, UK). HPLC-grade solvents (hexane, dichloromethane, methanol and acetone) were purchased from Dislab (France). No significant amount of targeted analytes was showed in procedural blanks. Ultrapure water (Milli-Q) was produced by a Millipore apparatus with 18.2 MΩ/cm resistivity. Merck silica gel 60 (70–230 mesh ASTM) activated at 450°C was heated at 120°C for 12 h prior to use. Glassware was systematically washed with detergent (Decon, East Sussex, UK), rinsed with ultrapure water and acetone and finally dried at 120°C prior to use.

2.2. Sampling sites

The three sampling sites selected for this work are located in a 15 km zone in the "Nord Pas-de-Calais region" (France) near Douai city (Fig. 1). This heavily industrialized area is already studied by our group due to its severe metallic pollution (Boughriet et al., 2007; Kadlecová et al., 2012; Lesven et al., 2009; Prygiel, 2013; Superville et al., 2014). However, besides data provided by the French Water agencies in connection with the Ministry for Sustainable Development, bibliographic data concerning organic contamination in this area are still lacking.

2.2.1. Deûle River at Courcelles-lès-Lens

The Deûle, a tributary of the Lys River, is a 60-km long river beginning at Souchez (Pas-de-Calais, France). As a wide-gauge canal, the Deûle River is highly frequented by commercial barges. The sampling site was chosen at Courcelles-lès-Lens near a former smelter (Metaleurop), which was the third largest nonferrous smelter in the world during the first half of the 20th century. Nowadays, this industrial site has been replaced by a recycling and waste treatment center. Nevertheless, smelting activities are still continuing in this area according to the presence of twometallurgical factories (Nyrstar and Umicore) located at about 4 km downstream. Although contamination of the Deûle River by trace metals is well

documented, bibliographic data concerning organic contamination in this area remain scanty (Net et al., 2014).

2.2.2. Scarpe River at Râches

The Scarpe is a 102-km long river flowing from Berles-Monchel (Pas-de-Calais, France) to Mortagne -du-Nord, (Nord, France) where it flows into the Scheldt. Mostly canalized and characterized by a low flow, this river is under the influence of many effluents and various industrial and urban emissions, which widely affect sediment chemistry (Alary and Demougeot-Renard, 2010; Alary et al., 2011; Isaure et al., 2002). This medium-gauge canal is no longer navigated.

2.2.3. Sensée canal at Goeulzin

Sensée canal has been created to link the canal du Nord to the Deûle and the Scheldt. This area presents similar navigation traffic as the Deûle River according to the data provided by Voies Navigables de France, a public institution in charge of inland waterways. Contrary to the two previous sites, the Sensée canal is less affected by metallurgical activities since this area is dedicated to agriculture (Prygiel, 2013).

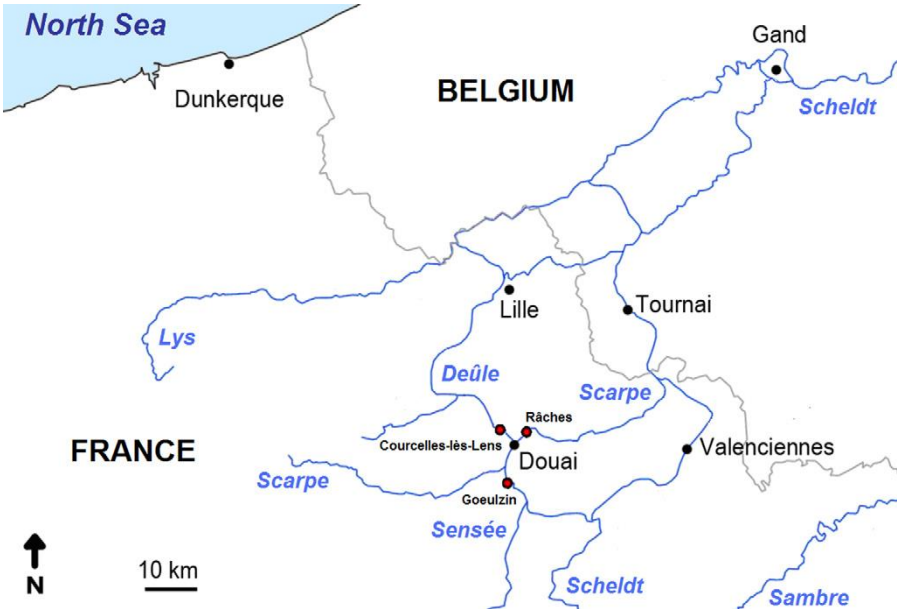

Fig. 1. Location of the three study sites.

2.3. Sampling

The sampling campaign was conducted in early spring 2012, on 22/03/2012, 06/04/2012 and 12/04/2012 respectively for the Scarpe, Deûle and Sensée rivers. Sediments

cores of approximately 10 cm length and 10 cm diameter were collected using 35-cm long polycarbonate tubes. Sediment cores were sliced in centimeter-sized slices immediately after sampling. Each slice was homogenized before being transferred into pre-calcinated aluminum containers capped with aluminum foils. Sediment samples were transported in the laboratory and were dried at room temperature in a laminar hood without storage step.

2.4. Targeted analytes

In thiswork, the following PCBs, PAHs and Me-PAHswere analyzed:

PCBs No. (28 PCBs): 8, 18, 28, 44, 52, 66, 77, 81, 101, 105, 114, 118, 123, 126, 128, 138, 153, 156, 157, 167, 169, 170, 180, 187, 189, 195, 206 and 209.

PAHs (16 PAHs): naphthalene (N), acenaphthylene (Ayl), acenaphthene (A), fluorene (F), anthracene (Ant), fluoranthene (Fl), benz[a]anthracene (BaA), chrysene (Ch), benz[a]pyrene (BaP), phenanthrene (Phe), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (Bghi), dibenzo[a,h]anthracene (DhA), indeno[1,2,3-cd]pyrene (IP), pyrene (Pyr).

Me-PAHs (18 Me-PAHs): 1-methylnaphthalene (1M-N), 2-methylnaphthalene (2M-N), 1,2-dimethylnaphthalene (1,2DM-N), 1,6-dimethylnaphthalene (1,6DM-N), 2,6-dimethylnaphthalene (2,6DM-N), 1-methylphenanthrene (1M-Phe), 2-methylphenanthrene (2M-Phe), 3-methylphenanthrene (3MPhe), 9-methylphenanthrene (9M-Phe), 2-methylanthracene (2M-An), 1,7-dimethylphenanthrene (1,7-DMP), retene, 1-methylfluoranthene (1M-Fl), 3-methylfluoranthene (3M-Fl), 1-methylpyrene (1M-Pyr), 4-methylpyrene (4M-Pyr), 3-methylchrysene (3M-Ch), 6-methylchrysene (6M-Ch).

2.5. ASE extraction

Extraction steps were performed on finely ground sediment previously sieved at 224 µm. Sieved sediment sampleswere spikedwith deuterated internal standards A-d10, N-d10, Per-d12, Phe-d10 and Pyr-d10 for PAH and Me-PAH analysis and with TCN, PCB112 and OCN for PCB analysis. After a delay of equilibration, sediments were then extracted using an accelerated solvent extraction (ASE 200, Dionex Corp., USA). The extraction conditions were heat 5 min, temperature 100°C, static solvent extraction time 2 min with 5 static cycles, pressure 138 bars, purge 3 min and 35% flush according to the method developed by Tronczynski et al. (2005). High purity nitrogen was employed as the purge gas.

2.6. Purification and pre-separation

Molecular sulfurwas removed by addition of activated metallic copper (Blumer, 1997) to the extracts. The extracts were concentrated, solvent-exchanged to hexane, and were then purified and fractioned by liquid chromatography on a silica column to eliminate organic interferences (Jeanneau, 2007). PCBs were recovered by elution with 20 mL of hexane (Fraction 1), and aromatic hydrocarbons (PAHs and Me-PAHs) were recovered by 15 mL of hexane/dichloromethane mixtures (3/1 v/v) followed by 15 mL of hexane/dichloromethane mixture (1/1 v/v) (Fraction 2). Each fraction was concentrated using a rotary evaporator followed by a slight stream of nitrogen before analysis.

2.7. Gas chromatography analyses

Targeted compounds were analyzed using a Varian 3900 gas chromatograph (GC) equippedwith a deactivated fused-silica guard column (5 m, 0.25 mm i.d.) and a fused-silica low polarity si-arylene ZB-XLB capillary column (60 m length, 0.25 mm i.d., 0.25 mm film thickness, Phenomenex) and coupled with a Varian Ion Trap Saturn 2000 Mass Spectrometer (MS). The carrier gas was helium held at a constant flow rate of 1 mL/min. Each group of organic compounds was analyzed separately. Temperature of the GC oven was programmed as follows: from 70°C (1 min) to 170°C at 10°C/min, then to 230°C at 4°C/min, and then to 300°C at 3°C/min (13 min) for HAPs and Me-HAPs and from 80°C (1 min) to 170°C at 10°C/min, then to 230°C at 4°C/min, and then to 300°C at 3°C/min (19 min) for PCBs. Samples were injected in the splitless mode at 280°C and the injector was purged with helium after 1 min. The transfer line and the ion trap were respectively held at 280°C and 220°C. Identification of each compound was done on the basis of the retention time and the mass spectrum from chromatograph of standardsolutions acquired in full scan mode. Quantificationwas then performed in the single ion storage (SIS) mode for better selectivity. Response factors were determined relative to the internal standards previously chosen to better fit to the properties of each compound.

3. Results and discussions

3.1. PCB distribution and composition profiles

Among the 28 studied PCBs, nine were detected and quantified at least one time (PCBs 8, 18, 28, 52, 44, 66, 101, 118 and 123). The highest concentration was found in surface sediment of the Scarpe River (Σ_{28}PCBs = 194.4 µg/kg dw). The Sensée and the Deûle River were found to be less contaminated in PCBs with concentration maxima (Σ_{28}PCBs) reaching 34.0 and 15.6 µg/kg dw respectively. In this study, special attention was accorded to

the seven PCB indicators (Σ7PCBi _ i.e. PCB 28, 52, 101, 118, 138, 153 and 180), that are among the most frequently detected congeners in the environment, and also to the twelve dioxin-like PCBs (Σ12PCB-DL _ i.e. PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) known to be highly toxic to humans and to persist in the environment (Kimbrough et al., 2010; Tanabe and Minh, 2010). Depth contamination profiles for Σ_{28}PCBs, Σ_{7}PCBi and Σ_{12}PCB-DL, for the three sediment cores, are presented in Fig. 2.

As above-mentioned, the highest concentrations were measured in the sediment from the Scarpe River with Σ28PCBs varying from 126.8 to 194.4 μg/kg dw. The maximal concentration was recorded in the surface of the sediment core (0–1 cm depth). Sensée and Deûle sampling sites were found to be less contaminated with Σ28PCBs respectively ranging from 15.1 to 34.0 μg/kg dw and from n.d. to 15.6 μg/kg dw. In the case of the Sensée River, the highest concentration was also encountered in surface sediment. Indicators PCBs were found to represent 100, 68.9 and 60.5% of the total detected PCBs for the Deûle, the Sensée and the Scarpe River respectively. As expected, a good correlation could be found between distributions of "total" PCBs (Σ28PCBs) and indicators PCBs (Σ7PCBi) for all the three sites (Rsquared = 0.93, p b 0.01). No significant amount of dioxin-like PCB (Σ_{12}PCB-DL) could be found in the Deûle River sediment core. The highest levels of PCB-DL were detected for the Scarpe samples, ranging from 6.9 to 60.3 μg/kg dw with an average concentration of 39.4 μg/kg dw. The Σ12PCB-DL depth profile of the Scarpe sediment core was also characterized by a low-contaminated zone at 4–6 cm depth. The Sensée samples showed much lower concentrations with an average value of 5.3 μg/kg dw. As noticed elsewhere (Babut et al., 2009), Σ12PCB-DL values were found to be correlated with Σ_{28}PCBs (R-squared = 0.67, p b 0.01) for all the three sites. The homologue composition of PCBs in most samples was characterized by mixtures with a low chlorination level (tri-, tetra- and penta-PCBs). Hexa-, hepta- and octa-PCBs were absent, whereas di-PCB could only be detected in the Sensée canal with a mean proportion of 7.2% of total PCB concentration.

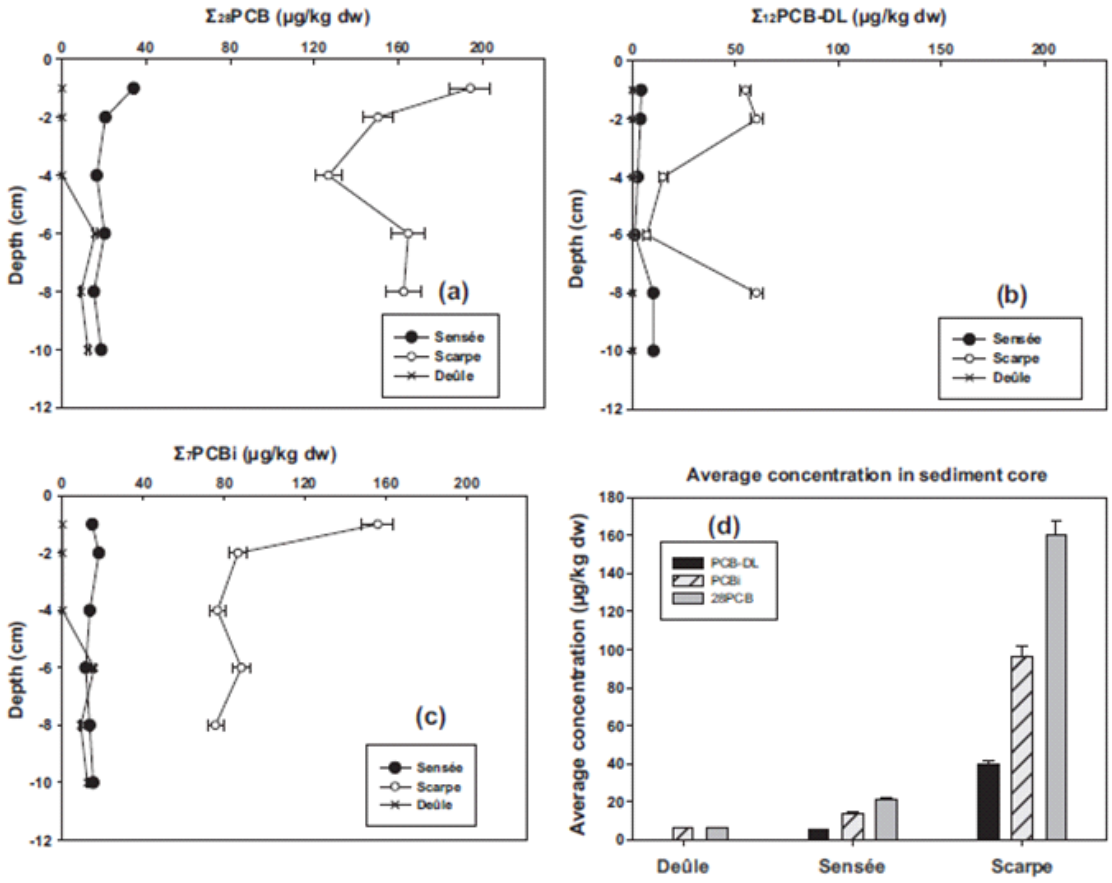

Fig. 2. PCB depth profiles in the three sediment cores of (a) Σ28PCBs, (b) Σ12PCB-DL, and (c) Σ7PCBi and (d) the average concentrations of Σ28PCBs, Σ12PCB-DL and Σ7PCBi in each sediment core.

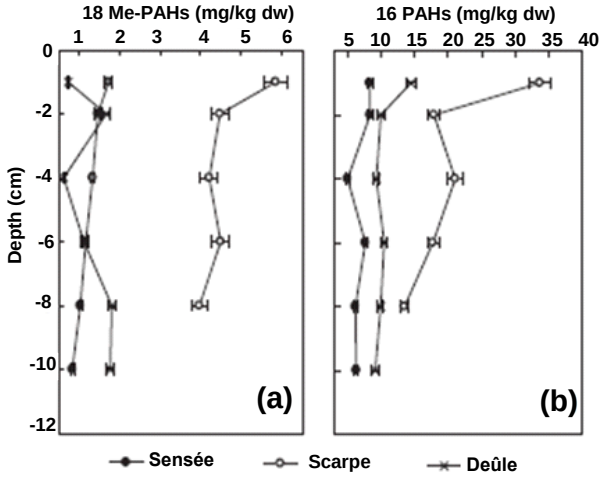

Fig. 3. Sedimentary depth profiles in the three sediment cores of (a) Σ18Me-PAHs and (b) Σ_{16}PAHs.

3.2. PAH and Me-PAH distribution and composition profiles

The Σ_{16}PAH and Σ_{18}Me-PAH concentrations were also plotted against sediment depth for the three sampling sites (Fig. 3). Concerning Σ_{16}PAHs, sediments of the Scarpe River showed the highest contamination with concentrations varying from13.4 to 33.7 mg/kg dw with an average of 20.7 mg/kg dw. Lower concentrations ranging from 9.1 to 14.4 mg/kg dw (average value of 9.9 mg/kg dw) and from 6.1 to 8.2 mg/kg dw (average value of 6.4 mg/kg dw) were found respectively for the Deûle and Sensée samples. Surface sediment (0–1 cm) was systematically found to be more contaminated than deeper sediment. This observation was particularly prominent for the Scarpe River, which, in our case, is the only one to be not navigated and thus inclined to a higher sedimentation rate than the Deûle and the Sensée River. Such depth profiles tend to indicate a recent contamination and are in agreement with a previous study carried out in the region on sediments under urban influence (Charriau, 2009). Concerning methylated homologues (Me-PAHs), all concentrations of individual species were found to be lower than 1 mg/kg dw, ranging from non-detectable levels to few hundreds $\mu g.kg^{-1}$ dw. The average Σ_{18}Me-PAH concentrations in sediment cores of Sensée, Deûle and Scarpe were 1.3, 1.3 and 4.6 mg/kg dw respectively. For all the three sites, depth profiles of Σ_{18}Me-PAHs were found to be correlated with Σ_{16}PAHs (Rsquared = 0.74, $p<0.01$).

In terms of composition profiles, PAH species were dominated by four and five ring structures whatever the depth with average proportions of 20, 36 and 41% of four rings and 48, 37 and 45% of five rings respectively for the Scarpe, Sensée and Deûle. Lower molecular weight PAHs (i.e. two ring structures) were predominantly found in the Scarpe River sediment, but did not exceed 12% of total PAH (Σ16PAHs) concentration. Such low levels of two ring PAHs can be explained both by their higher water solubility and their lower stability towards (bio)-degradation (Quantin et al., 2005). On the other hand, methylated PAHs were found to be dominated by two- and three-aromatic ring structures with average proportions respectively ranging from 37 to 49% and from 47 to 53%.

3.3. Source apportionment

A relationship could be found between Σ_{28}PCB and Σ_{16}PAH distribution profiles (R-squared = 0.65, $p<0.01$). This observation suggests that their sources are mainly located at similar regions. Atmospheric depositions, runoff from the land, and food chain transport have been regarded as the major sources of PCBs in aquatic environments (Totten et al., 2006). The

predominance of lower chlorinated congeners in our samples (tri-, tetra- and penta-PCBs) would favor an atmospheric transport-deposition process reinforced by the strong metallurgic activity nearby (Yang et al., 2009). However, PCBs result fromindustrial production without any known natural source, and it is also possible to identify the possible sources by evaluating the similarity of the PCB patterns found in the river sediment samples with that of the principal commercial PCB mixtures. A principal component analysis (PCA) was also performed toobtain further informationon PCB sources by comparing sample composition and commercial Aroclor mixtures (1221, 1232, 1242, 1248, 1254, 1260 and 1262) (Škribić and Durišić-Mladenović, 2007; Zhou et al., 2012). The compositions of Aroclors 1221, 1232, 1242, 1248, 1254 and 1260 (Frame et al., 1996) were normalized with respect to 28 congeners concerned in this study. The first two principal components (PCs) were extracted by PCA, explaining 42.4% and 22% of the total variance, respectively. PC1 was basically defined by the contributions of highly chlorinated congeners, whereas PC2 was influenced mainly by di-, tri-, tetra- and penta-PCBs. The results of the PCA are presented in Fig. 4.

The score plot suggests that Aroclor 1248 was the most common mixture used both in the Scarpe and Sensée river areas. However, it cannot be excluded that the contamination profile can also be influenced by othermixtures such as Aroclors 1232, 1242 and, to a lesser extent, 1254. Concerning the Deûle River, the PCB contamination score plot tends to show a complex influence of Aroclors 1221, 1232 and 1242, all characterized by the prevalence of low molecular weight PCBs. All these Aroclor mixtures were mostly used in electrical capacitors and electrical transformers, and can be released into the environment from landfills containing PCB waste materials and products of municipal refuse and sewage sludge incineration, and improper disposal of PCB materials. Only Aroclors 1260 and 1262 can be dismissed as potential sources in the study areas since their composition is dominated by highly chlorinated PCB congeners (hexa- to hepta-PCBs).

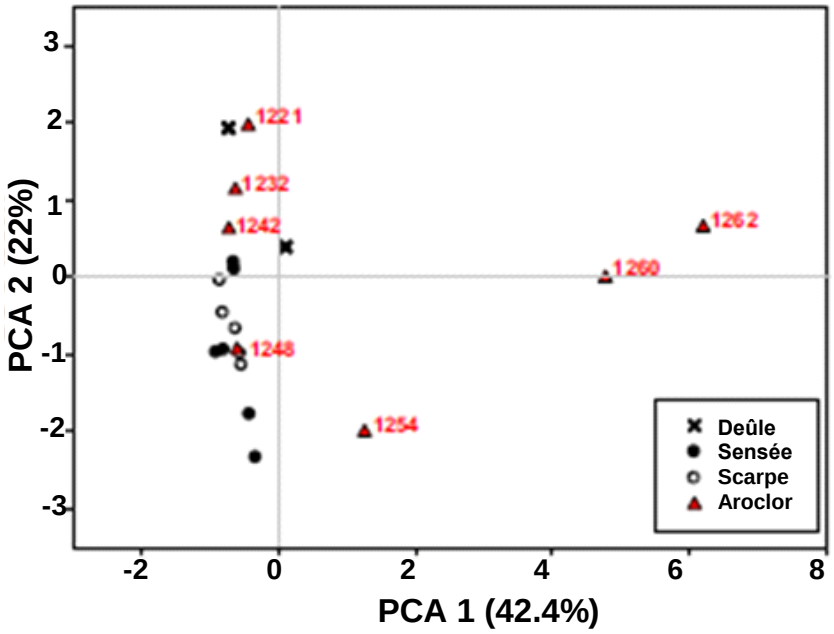

Fig. 4. Principal component analysis of PCB compositions and comparison with standard mixtures.

Concerning polycyclic aromatic hydrocarbons, anthropogenic releases can be attributed to petrogenic and pyrolytic origins. PAHs of petrogenic origins are usually characterized by the predominance of low number of aromatic rings (i.e. 2 and 3 rings), while high proportion of above 4 aromatic ring PAHs characterizes PAHs originated frompyrolytic origins. The ratio of low molecular weight and high molecular weight (LMW/HMW, the sum of (2–3)/(4–6) aromatic rings) is commonly used to distinguish the petrogenic (LMW/HMWN 1) from pyrolytic origins (LMW/HMW b 1) (De Lucas et al., 2005). As depicted in Fig. 5a, LMW/HMW ratios revealed values ranging from 0.09 to 0.99, thus suggesting that combustion should be the dominant source of PAHs in the studied areas even if petrogenic inputs can be suspected for the sediment core of the Scarpe River at 4–6 cm depth. In addition, all the sampling sites showed high proportions of parent PAHs (Σ_{16}PAHs/Σ_{16}PAHs + Σ_{18}Me-PAHs average = 0.84, 0.89 and 0.81 for Sensée, Deûle and Scarpe rivers respectively), which support combustion as the primary PAH source. Furthermore, discrimination between petrogenic and combustion sources can be ensured using specific ratios involving alkyl PAHs (Gogou et al., 1996; Simo et al., 1997; Yunker et al., 2002). For this purpose, cross plots of C0/C0 + C1 ratios in both the phenanthrene/anthracene (Phe/Ant) and fluoranthene/pyrene (Fl/Pyr) series are presented in Fig. 5b. With petroleum/combustion transitions set at 0.4 and 0.5 for the Phe/Ant and the Fl/Pyr series respectively, C0/C0+C1

ratios corroborate the combustion source. Further isomeric molecular ratios can also be used to differentiate potential sources of PAHs in sediment (Budzinski et al., 1997; Dickhut et al., 2000; Yunker, 2002; Yunker and Macdonald, 1995; Zhang et al., 2005). Nevertheless, such ratios should be treatedwith caution as biogeochemical and physical processes may alter PAH signatures due to variations in PAH reactivity, volatility, water solubility and sorption rates (Dickhut et al., 2000; Wagener et al., 2010; Yunker et al., 2002). PAHs of molecular masses 178 and 228 are commonly used to distinguish combustion from petroleum sources according to the values of the anthracene to anthracene plus phenanthrene ratio (Ant/(Ant + Phe)) and the benz[a]anthracene to benz[a]anthracene plus chrysene ratio (BaA/(BaA+ Ch)).

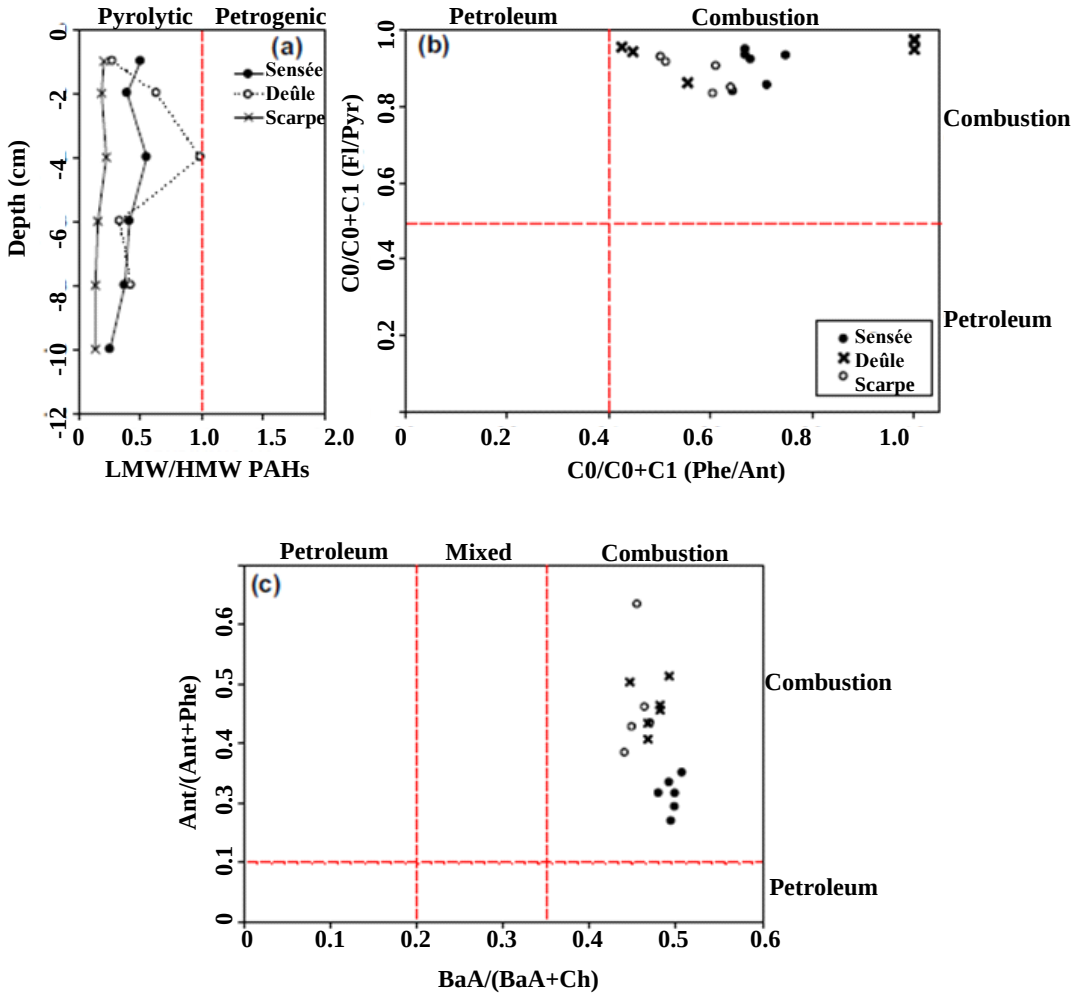

Fig. 5. (a) Depth profiles of low molecular and high molecular weight PAH ratios (LMW/HMW) of PAHs in the sediment cores. (b) Cross plots of C0/C0+C1 in the Phe/Ant vs. the Fl/Pyr series. (c) Cross plot isomeric ratios of: Ant/(Ant + Phe) vs. BaA/(BaA +Ch).

In our case, as depicted in Fig. 5c, cross plots for the ratios Ant/(Ant+ Phe) vs. BaA/(BaA +Ch) reinforce the combustion process as the major source of contamination in the studied area. Moreover, such high Ant/(Ant+Phe) and BaA/(BaA+Ch) ratios involve high proportions of anthracene and benz[a]anthracene that are both among the most photoreactive PAHs (Behymer and Hites, 1988; Gogou et al.,1996). Considering a combustion/transport/deposition process, it can be admitted that no significant photolysis degradation occurred in this case study. These ratios can also imply close contamination sources that can be attributed to the nearby industrial activities.

3.4. Sediment quality evaluation

Numerical sediment quality guidelines (SQGs) for river sediment have been developed using a variety of approaches. SQGs include both a threshold effect concentration (TEC), which identifies contaminant concentrations below which adverse effects are not expected to occur, and a probable effect concentration (PEC)which identifies contaminant concentrations above which harmful effects on sediment-dwelling organisms were expected to occur frequently (MacDonald et al., 2000a). SQGs have first been used to identify contaminants of concern in aquatic ecosystems and to rank areas of concern on a regional or national basis (US EPA, 1997). Then, consensus-based SQGs (CBSQGs) have then developed for PCBs and PAHs with the aim to provide a unified synthesis of the existing guidelines, reflecting causal rather than correlative effects, and taking into account the effects of contaminant mixtures in sediment (MacDonald et al., 2000b; Swartz, 1999). The certainty in predicting the absence or presence of sediment toxicity occurs at sediment concentration that are bTEC or NPEC values, respectively. For the values between consensus-based TEC (CBTEC) and consensus-based PEC (CBPEC), toxicities and effects to benthic macroinvertebrate species related to reductions in survival, reproduction, and growth, lead to bioaccumulation, and benthic community alterations that correspondingly increase with the increasing of the concentration of contaminants. Consequently, with the aim to better interpret the potential impacts between the TEC and PEC values of the CBSQGs, it has been recommended to consider an additional midpoint effect concentration (MEC = (TEC + PEC/2)). Four possible ranges of concentration can thus be applied for describing the level of sediment quality: Levels 1, 2, 3 and 4 respectively refer to ≤CBTEC; NCBTEC ≤ CBMEC; NCBMEC ≤ CBPEC; and NCBPEC. Sediment quality evaluations for the three sampling sites are presented in Fig. 6. Sediment quality evaluation based on Σ_{28}PCBs showed a quality level of 1 whatever the depth for sediment obtained from the Sensée and the Deûle River, whereas the Scarpe River sediment

quality was found to be at level 2. Based on Σ16PAHs, studied sediments showed poorer quality with average levels reaching 2 and 3. Level 4 was even achieved for top surface sediment (0–1 cm) of the Scarpe River.

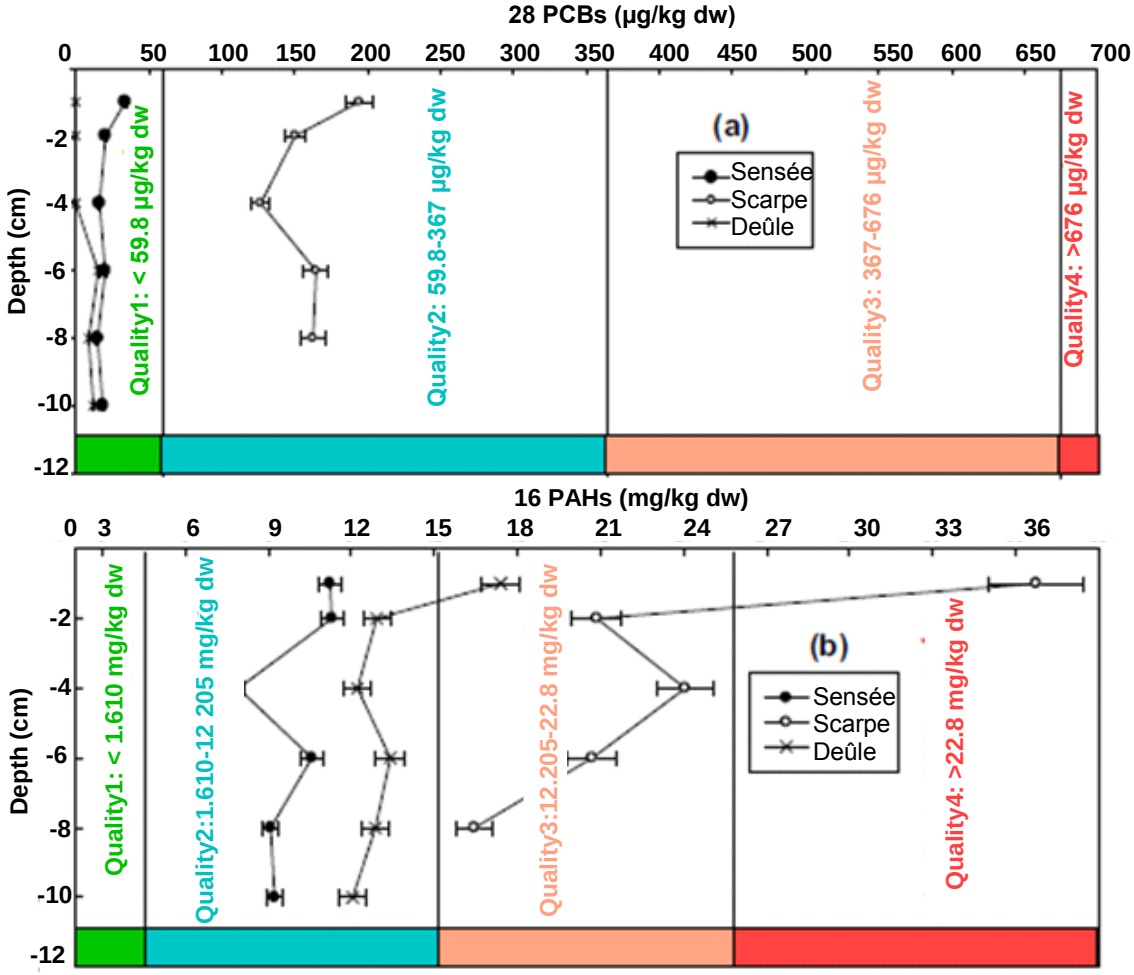

Fig. 6. Levels of sediment quality compared to recommended sediment quality guidelines.

4. Conclusion

Contamination of riverine sediments by persistent organic pollutants (PCBs, PAHs and Me-PAHs) has been studied for the first time of the three sites located in the Nord Pas-de-Calais region, Northern France (Scarpe, Deûle and Sensée rivers). The distributions of PCBs and PAHs established to be correlated, thus suggesting that their sources are mainly located at similar regions with highly metallurgical and residential activities. Total concentrations indicate a greater contamination level for the Scarpe River sediment. Recent contamination trend is suspected for all the sites according to the higher concentrations recorded in surface sediments. According to the predominance of low

chlorinated PCB congeners, atmospheric deposition can be regarded as the major source of contamination. The possible sources of PCBs have further been studied through a principal component analysis based on PCB composition, while the pyrolytic origin of aromatic hydrocarbons has been determined through the use ofmolecular indexes. Sediment quality evaluation based on consensus-based SQGs revealed that the Scarpe River is more contaminated with persistent organic pollutants than the Sensée and the Deûle River. Complementary studies are already ongoing to go further into the organic contamination in this region heretofore suffering from a severe historical metallic pollution.

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Article 6

Case study of PAHs, Me-PAHs, PCBs, Phthalates and Pesticides contamination in the Somme River water, France

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Abstract

Surface waters, especially natural rivers always act as receiving waters for various kinds of organic contaminants from municipal and industrial wastewaters, agricultural activities, organic chemicals in use, non-point source pollutions. Due to their toxicity, persistency and wide diffusion, polychlorinated biphenyls (PCBs), pesticides, phthalates, polycyclic aromatic hydrocarbons (PAH) and their alkylated homologues (Me-PAHs) are among the organic contaminants the most often monitored in the environment. Determination of the contamination level is the crucial first step of environmental research. Field investigations have clearly demonstrated their importance on various studies on the contamination levels, the origin, and impact of contaminants in the aquatic ecosystems. The present paper is an effort on the field studies focusing on persistent organic pollutants: PCBs, PAHs and Me-PAHs, phthalates and pesticides in Somme River water located on Northern France. The sampling zone is characterized by fields of agriculture surrounding urbanized and industrialized areas and constitutes a place embedded with strong fishing activities. However, the river section of interest is also known for the high levels of PCB in sediments. The water were analyzed for 28 PCBs, 16 PAHs and 18 Me-PAHs, 6 phthalates and 28 pesticides with the aim to determine the dispersion trend and the water quality of the sampled water.

Key words: *PAHs, Me-PAHs, Phthalates, PCBs, Pesticides, LLE, GC-MS, Somme River*

1. Introduction

Actually, organic contaminants are major environmental concern due to their ubiquitous, their persistence, long-range transportability and potentially adverse effects on living organisms. River water acts as receiving water for various kinds of organic contaminants from municipal and industrial wastewaters (Malve *et al.*, 2003; Singh *et al.*, 2007; Zhang *et al.*, 2004). For the ecosystem protection and to keep water resources clean, it is important to identify the nature of contaminants, their contamination levels as well as their sources. Polycyclic aromatic hydrocarbons (PAHs) and their alkylated homologues (Me-PAHs), polychlorobiphenyls (PCBs), phthalates and pesticides are the principal classes of organic contaminants in aquatic ecosystem. In this context, twenty-three priority substances and other priority pollutants (PPs) were established by European Commission in the Water Framework Directive 2008/105/EC (European Commission, 2008).

(*i*) Aromatic hydrocarbons including PAHs and Me-PAHs are widely disseminated in the environment with sources that can be both natural and anthropogenic (Yunker, 2002; Wang, 2007; Mostert, 2010). They are highly lipophilic compounds, ubiquitous in coastal, estuarine and river water column, as well as sediments in which they tend to accumulate (Cailleaud, 2007; Chiou, 1998; Manodori, 2006, Gaspare, 2009; Ko, 1995; Yunker, 2012; Net *et al.,* 2014). High level of aromatic hydrocarbons represents a serious threat to the ecosystem functioning and human health via food chain and water resources. Recent studies have indeed reported that marine organisms are prone to bioaccumulate these substances, particularly in lipidrich tissues (Neff, 2002; Francioni, 2005; Dugan, 2005). Due to their toxic, carcinogenic and mutagenic effects (Straif, 2005; IARC, 2010; U.S. Department of Health and Human Services, 2011), sixteen PAHs have been recommended as priority pollutants by the United States Environmental Protection Agency (US EPA, 2002). (*ii*) Another class of contaminant is represented by the pesticides. It is known that pesticides used for agriculture activities may lead to contamination of surface and ground waters (Kaushik *et al.,* 2010, Navarro *et al.,* 2010; Hancok *et al.,* 2008). Pesticide contamination of the aquatic environment as well as their ecotoxicological effects for aquatic flora, and human health has also been well documented (Wania and Mackay 1999; Sanborn *et al.,* 2007; Jurewicz and Hanke, 2008; Huen *et al.,* 2012). Runoff from urban areas, return flow waters from agricultural fields, and leaching are considered important inputs to pesticide contamination of surface and groundwater. (*iii*) One other group is characterized by polychlorobiphenyles (PCBs). These compounds have been commonly used as dielectric fluids or transformers and capacitors, in paints, inks and pesticides until the hazard posed to both the environment and human health by their use became evident. They are extremely stable compounds under environmental conditions (WHO, 1993). Due to their toxicity, chronic persistence and bioaccumulation, they have been banned or restricted, and some of them have been included in the list of priority pollutants in many countries. However, PCBs are still present in water and sediment and continue to affect aquatic organisms from the top of food chain (plankton, algae) to predator organisms (fish, birds, marine mammals) and consequently human health through the diet (Sun *et al.,* 2002). (*iv*) Nowadays, phthalates or phthalic acid esters have become also a group of contaminants of environmental concern. Large scales of phthalates have been produced due to the wide variety of uses. They are used in common household products, cosmetics, detergents, flame retardants, plastics, inks, adhesives and medical devices. Several million tons of phthalates have been produced each year. Some phthalates are suspected to act as

endocrine disruptors (Gomez-Hens and Aguilar-Caballos, 2003; Ghisari and Bonefeld-Jorgensen, 2009; Lau *et al.*, 2005). Phthalates are currently listed as priority pollutants in many countries due to their link to several human cancer diseases (Kaneco *et al.*, 2006). According to US EPA, diethyl phthalate (DEP), dimethyl phthalate (DMP), di(2-ethylhexyl) phthalate (DEHP), butyl benzyl phthalate (BBP), di-n-butyl phthalate (DBP) and di-n-octyl phthalate (DOP) should be considered Priority Toxic Pollutants (US EPA, 1999). Their entry into the surface water occurs directly from the production of plastic materials and indirectly *via* volatile emissions and leaching from their parent polymeric material (Stanley *et al.*, 2003, Petrovic *et al.*, 2001; Gomez-Hens and Aguilar-Caballos, 2003 and Kavlock *et al.*, 2002).

The aim of the present study was to investigate the concentration levels of these four classes of organic contaminants in order to assess quality of river water from fourteen sampling sites of the Somme River in Northern France. Samples were analyzed for 16 PAHs and 18 Me-PAHs, 6 phthalates, 28 pesticides and 28 PCBs, using gas chromatography/mass spectrometry (GC/MS). Results obtained from this multiresidue study aim to fill the lack of data concerning water contamination in this region.

2. Material and methods

Mixed standard solutions of PAHs and Me-PAHs were purchased from Restek Corp (Bellefonte, PA, USA). PCBs standard solution was obtained from Accustandard, Inc. (New Haven, CT, USA). Phthalates and pesticides standards were purchased from Sigma- Aldrich (Saint-Louis, USA) and Restek (Bellefonte, USA). Tetrachloronaphtalene (TCN), 2,3,3',5,6-tetrachlorobiphenyl (PCB112) and octachloronaphtalene (OCN), used for PCB quantification, were purchased from Dr Ehrenstorfer (Augsburg, Germany) Deuterated internal standards for PAHs and Me-PAHs (acenaphthene-*d*10 (A-*d*10), naphtalene-*d*8 (N-*d*10), perylene-*d*12 (Per-*d*12), phenanthrene-*d*10 (Phe-*d*10) and pyrene-*d*10 (Pyr*d*10)) were provided by LGC-Promochem (Middlesex, UK). Benzyl benzoate and pentachloronitrobenzene with a purity of 99% and 94% respectively were used as internal standard for phthalates and pesticides, and they were purchased from Sigma-Aldrich (Saint-Louis, USA). HPLC-grade solvents (hexane, dichloromethane, methanol and acetone) were purchased from Dislab (France). No significant amount of targeted analytes was showed in procedural blanks. Ultrapure water (Milli-Q) was produced by a Millipore apparatus with 18.2 MΩ/cm resistivity. Merck silica gel 60 (70-230 mesh ASTM) activated at 450°C was heated

at 120°C for 12h prior to use. Glassware was systematically washed with detergent (Decon, East Sussex, UK), rinsed with ultrapure water and acetone and finally dried at 120°C prior to use.

The sampling campaign was conducted on October 2012 in Somme River (Picardie region in northern France). The sampling was done from downstream at Béthencourt-sur-Somme to Saint-Quentin (Fig. 1). Water samples were collected from 11 sites of the Somme River from the first station at Béthencourt-sur-Somme (Station 001103) to Gauchy (station 116500). Two additional samplings were performed in two ponds around Béthencourt-sur-Somme with the aim to evaluate a potential accumulation of contaminants. The sampling zone is characterized by fields of agriculture surrounding urbanized and industrialized areas (e.g. Saint-Quentin, Ham) and constitutes a place embedded with strong fishing activities. However, the river section of interest is also known for the high levels of PCBs in sediments (Dumoulin *et al.*, 2013).

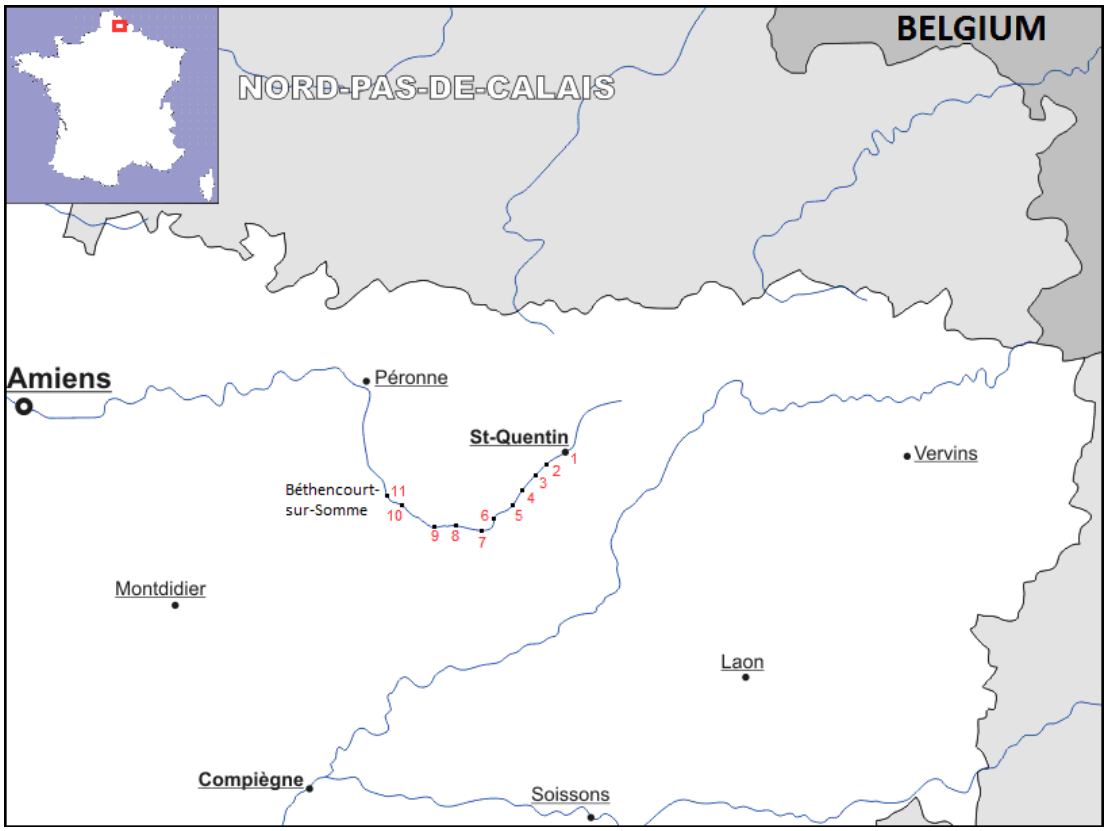

Fig. 1. Location of sampling sites in the Somme River in Picardie region, Northern France

In this work, samples were analyzed for 16 PAHs, 18 Me-PAHs, 6 phthalates, 28 pesticides and 28 PCBs including 12 dioxin-like PCBs (dl-PCBs) and 7 PCBs indicators (PCBi) as following:

PAHs (16 PAHs) : naphthalene (Na), acenaphtylene (Ayl), acenaphtene (Aen), fluorene (F), anthracene (An), fluoranthene (Fl), benz*[a]*anthracene (BaA), chrysene (Ch), benz*[a]*pyrene (BaP), phenanthrene (Phe), benzo*[b]*fluoranthene (BbF), benzo*[k]*fluoranthene (BkF), benzo*[ghi]*perylene (Bghi), dibenzo*[a,h]*anthracene (DhA), indeno*[1,2,3-cd]*pyrene (IP), pyrene (Py).

Me-PAHs (18 Me-PAHs) : 1-methylnaphthalene (1MNa), 2-methylnaphthalene (2M-Na), 1,2- dimethylnaphthalene (1,2DM-Na), 1,6-dimethylnaphthalene (1,6DM-Na), 2,6-dimethylnaphtalene (2,6DM-Na), 1-methylphenanthrene (1M-Phe), 2-methylphenanthrene (2M-Phe), 3-methylphenanthrene (3M-Phe), 9-methylphenanthrene (9M-Phe), 2-methylanthracene (2M-An), 1,7-dimethylphenanthrene (1,7DM-Phe), retene, 1-methylfluoranthene (1M-Fl), 3-methylfluoranthene (3M-Fl), 1-methylpyrene (1M-Py), 4-methylpyrene (4M-Py), 3-methylchrysene (3M-Ch), 6-methylchrysene (6M-Ch).

Phthalates (6 PAE): dimethyl phthalate (DMP), diethylphthalate (DEP), di-n-butyl phthalate (DBP), butylbenzyl phthalate (BBP), di-2-ethylhexyl phthalate ester (DEHP) and di-*n*-octyl phthalate (DNOP).

Pesticides: alpha-lindane, gamma-lindane, betalindane, delta-lindane, heptachlor, aldrin, transchlordane, cis-chlordane, 4,4'-DDE, endosulfan I, dieldrin, endrin, 4,4'-DDD, endosulfan II, 4,4'-DDT, endrin aldehyde, methoxychlor, endosulfan sulfate, endrin ketone, chloroneb, chlorothalonil, DCPA methyl ester, heptachlor epoxide (isomer A and B), transnonachlor, chlorobenzilate, trans-permethrin and cispermetrin.

PCBs No.(28 PCBs) : 8, 18, 28, 44, 52, 66, 77, 81, 101, 105, 114, 118, 123, 126, 128, 138, 153, 156, 157, 167, 169, 170, 180, 187, 189, 195, 206 and 209.

Water sampling was performed using pre-cleaned amber glass 2.5 L bottles that were immediately capped with Teflon-lined lid. Samples were maintained at 4°C before analysis. Back to the laboratory, samples were rapidly filtered using 0.7 µm Whatman glass microfiber filters and extracted using liquid–liquid extraction (LLE) technique. The applications of LLE in water and other liquid matrixes have been widely accepted in standard methods for various classes of organic contaminants such as PAHs, pesticides, and PCBs analysis (USEPA, 1996; JISC, 2005; Boussahel *et al.*, 2000; Barcéló, 1993; T90-120, AFNOR 1990; USEPA, 2008). Various solvent can be used depending on the nature of interest compounds. Dichloromethane (DCM) has been widely adopted for the extraction of POPs whereas *n*-hexane has often been used for PCB and PAHs extraction (Turrio-Baldassarri *et al.*, 2005; DIN EN ISO 17993). In

this study, in order to increase the extraction efficiency, each water sample (1L) was spiked with internal standards and then extracted four times with 60 mL of *n*-hexane followed by four times with 60 mL of DCM. The extracts were then pooled and dried using Na2SO4. Finally, the extract was concentrated using a rotary evaporator followed by a slight stream of nitrogen before analysis. The recoveries of PAHs, Me-PAHs, phthalates and PCBs using this protocol were estimated at 71.3-106.2%, 68.2-115%, 72.1-107.6%, 72.5-112.0% respectively.

The extracts were analyzed using a Varian 3900 gas chromatograph (GC) equipped with a deactivated fused-silica guard column (5 m, 0.53 mm i.d.) and a fused-silica capillary Phenomenex XLB (60 m length, 0.25 mm i.d., 0.25 μm film thickness) and coupled with a Varian Ion Trap Saturn 2000 Mass Spectrometer (MS). The carrier gas was helium, held at a constant flow rate of 1 mL/min. Samples were injected in the splitless mode at 280°C and the injector was purged with helium after 1 min. Each group of organic compounds was analyzed separately. The transfer line and the ion trap were respectively held at 280°C and 220°C. Each contaminant was identified based on the retention time and the mass spectrum from chromatogram of standard solutions acquired in full scan mode. Quantification was then performed in the single ion storage (SIS) mode for better selectivity. Response factors were determined relative to the deuterated internal standards response and to standard mixtures. Deuterated standards were chosen in order to better fit to the properties of each group of contaminants.

3. Results and discussions

As depicted on fig. 2, the total concentration of the 16 PAHs (Σ_{16}PAH) varies significantly along the river section with a mean value of 284 ng/L. Two subsurface maxima of concentration were recorded at Fontaine-les-Clercs (station 3 - Σ16PAHs = 513 ng/L) and Artemps (station 5 - Σ_{16}PAHs = 831 ng/L). On the other hand, the total concentration of 18 Me-PAHs (Σ_{18}Me-PAH) was also found to vary significantly from 75 ng/L at Dury (station 7) to 440 ng/L at Artemps (station 5) with an average concentration of 185 ng/L . A strong relationship can be found between the total concentration evolution of Σ16PAHs and Σ18Me-PAHs (R-squared = 0.78, P <0.01). In terms of the compositional profiles (Fig. 3), the concentrations of low molecular weight PAHs (2-3 rings) were significantly higher than high molecular weight PAHs. Three-rings PAHs accounted for the most abundant species with an average of 60% of the total concentration of PAHs (Σ_{16}PAHs). No significant traces of five and six ring-membered PAHs were detected in the water samples. Concerning Me-PAHs, two-ring species were predominant with an average proportion of 79% of Σ18Me-PAHs.

At this stage, some PAHs diagnostic ratios were used as a tool for attempting to identify pollution emission sources (Yunker *et al.*, 2002). For example, the anthracene/(anthracene+phenanthrene) ratio (Ant/(Ant+Phe)) is commonly used to distinguish petrogenic (<0.1) from pyrogenic (>0.1) origins, whereas the fluoranthene/(fluoranthene+pyrene) ratio (Fl/(Fl+Py)) allows distinction between petrogenic (<0.1), fossil fuel combustion (0.4-0.5) and grass/wood/coal combustion (>0.5). Both these ratio have been widely used to assess contamination sources in water samples (Wang *et al.*, 2009; Opuene *et al.*, 2009; Tobiszewski *et al.*, 2010). In the case of the Somme river water, Ant/(Ant+Phe) ratios support a pyrogenic origin with a mean value of 0.46. The Fl/(Fl+Py) ratios corroborate this hypothesis with a mean value of 0.55 attributable to grass/wood/coal combustion. However, sites 1, 2 as well as the two ponds were not taken into account for the determination of the Fl/(Fl+Py) ratios since no significant trace of fluoranthene could be detected in the corresponding water samples. This might be explained by the fact that PAHs in water samples easily undergo photolysis, which may alter values of diagnostic ratios (Jacobs *et al.*, 2008; Tobiszewski and Namiesnik, 2012). Nevertheless, high proportions of parent PAHs (Σ_{16}PAHs/Σ_{16}PAHs+Σ_{18}Me-PAHs average value = 0.6) support combustion as the primary PAHs source. Moreover, discrimination between petrogenic and combustion sources was ensured using specific ratios involving alkyl PAHs in the phenanthrene/anthracene (Phe/Ant) series. With petroleum/combustion transitions set at 0.4, the C0/C0+C1 (Phe/Ant) ratios determined in our samples corroborate the combustion source with a mean value of 0.77.

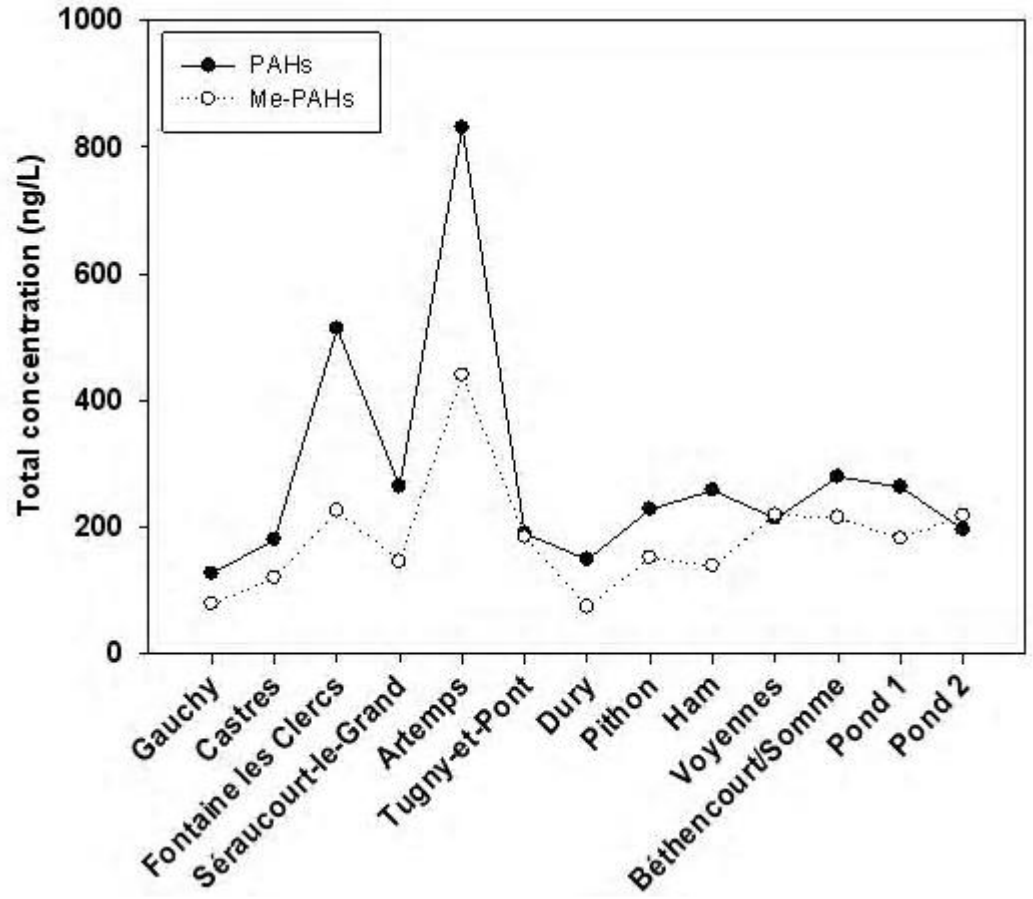

Fig. 2. PAHs and Me-PAHs repartition in surface water samples of the Somme River

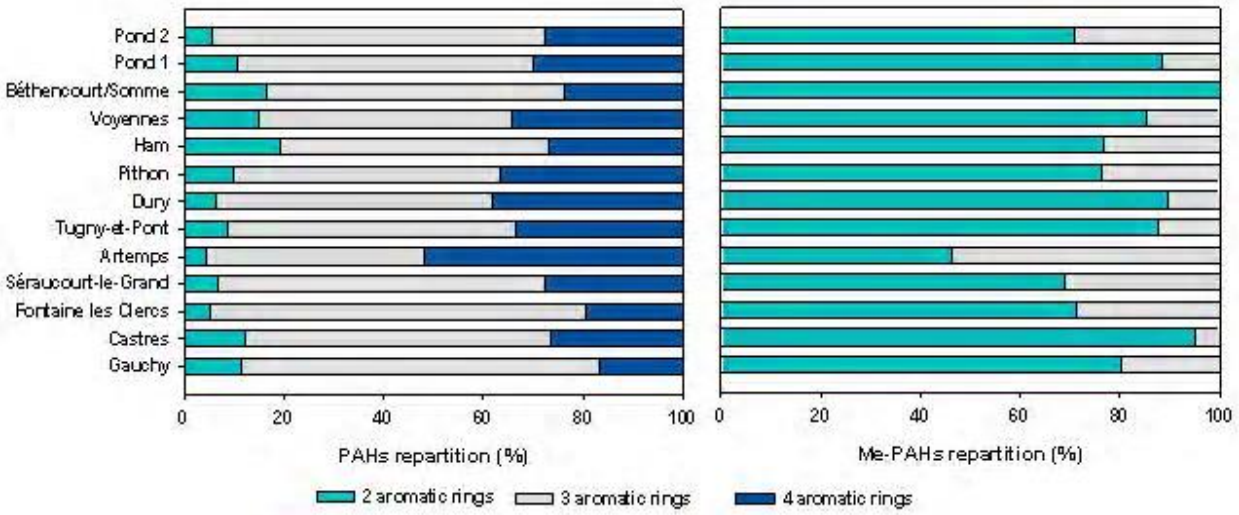

Fig. 3. PAHs and Me-PAHs composition profiles in the Somme River water samples

Large variations of phthalates concentrations (Σ_6PAEs**)** were observed, ranging from 6.93 ng/L at Artemps (station 5) to 23.34 ng/L at Pond 2 (station 13). DEHP was found to be

the most abundant specie with concentration ranging from 5.16 to 20.76 ng/L for a mean value of 10.23 ng/L (accounting for 68 % of mean Σ6PAEs). No trace of benzyl butyl phthtalate and di-octyl phthalate could be detected in the samples, whereas low levels of hydrophilic phthalate (DMP) were recorded. No significant correlation could be found between phthalate species distribution. Detailed concentrations are presented in Table 1. The relative high concentrations recorded for phthalates can be explained by the fact that these compounds have now become ubiquitous in water. DEHP and DBP are the most frequently detected and in surface water, wastewater and tap water with concentrations that can easily reach a few dozen µg/L (Fromme *et al.,* 2002; Aparicio *et al.,* 2007; Meng *et al.*, 2011). In our case, the low recorded levels of DMP can be linked to the higher degradation rates of short chain phthalates (Staples *et al.,* 1997).

Among the 28 studied pesticides, only three were detected and quantified at least one time in four samples. Other compounds of interest were below the limit of detection. Gamma-lindane and chloroneb were detected in the four above-mentioned samples corresponding to sites 2, 3, 5 and 11, with concentrations respectively ranging from 177 to 281 ng/L and from 90 to 131 ng/L. The alpha isomer of lindane was only detected in sites 2 (89 ng/L) and 3 (355 ng/L) (Table 2). The sources can be attributed to the nearby agricultural activities of sampling stations. Somme River sediments are known to be highly contaminated by PCBs. The Agence de l'Eau Artois-Picardie (AEAP), which is a public institution of the Ministry for Sustainable Development, has carried out several studies on contamination of sediment of the Somme River by PCBs since 1997. These investigations have led to highlight three sites in the Somme River par ticularly affected by PCBs contamination of sediment: Fontaine-les-Clercs (station 3), Séraucourt-le-Grand (station 4), and Artemps (station 5), with concentrations of PCBi higher than 200 µg/kg dw of sediment. No accumulation of PCBs could be noticed downstream watershed (AEAP, 2009; Dumoulin *et al.,* 2013). Besides, no data concerning contamination levels of PCBs in the water column were indicated for these study sites. The present study also aims to provide additional information for these sampling sites with a focus on the water column. Fig. 4 showed the total concentrations (Σ28PCBs), dl-PCB and PCBi in surface water of Somme River. Large variations of PCBs concentrations were observed for selected sampling sites. High concentrations of Σ_{28}PCB were detected at Artemps (201 ng/L), Pithon (246 ng/L) and Voyennes (179 ng/L) (Fig. 4), whereas PCBs were present at nondetectable levels for 6 stations (Castres, Séraucourtle-Grand, Tugny-et-Pont, Dury, Pond 1 and Pond 2). No specific correlation could be found between Σ_{28}PCB and

both indicator and dioxin-like PCBs for the seven concerned stations. PCBi were detected in only four stations and accounted for a mean value of 61% (ranging from 26 to 100%). On the other hand, dl-PCB, which are usually measured in biota, were detected at non-negligible levels in six water samples with concentrations ranging from 23 ng/L at Ham (station 9) to 116 ng/L at Voyennes (station 10) with a mean value of 60 ng/L. PCB77 was the dominant dioxin-like congener with an average proportion of 78%. These concentrations in the water column were not correlated with value previously recorded in sediment for the same sampling sites (Dumoulin *et al.,* 2013). This can be explained by low solubility of PCBs in water by the non-significant remobilization of PCBs from sediment to water column in the studied river section. Moreover, dissolved PCBs in water represent a small fraction of total PCB in the water column due to their rapid association to organic entities such as sediment, algae and protozoa (Brannon, 1993, Hargrave *et al.,* 1992, Eganhouse and Gossett, 1991 and Booij and van den Berg, 1994). However, it is interesting to note that PCBs are bioaccumulable compounds, which even at low concentration in water can affect strongly the aquatic organisms. Moreover, PCBs are extremely stable compounds under environmental conditions (WHO, 1993). Even, their concentrations in surface water were relatively low, it can contaminate underground water which could be source of drinking water and can impact consequently human health.

Numerous Water Quality Guidelines (i.e. European, Canadian, USA, Australian…) for Marine and Fresh Water Quality have been developed. The specific guidances are corresponding to the type of effluent (surface water, groundwater, freshwater) and to the intended use of the water. Nevertheless, for certain compounds, regulation of hazardous substances (organic micro pollutants) was incomplete or even not available in the literature in the past operation of worldwide water policy (Kallis *et al.,* 2001). Progressively, revisions have been developed to provide a tool for simplifying the reporting quality data. The assessment of the water quality and the contamination level evaluation of each studied site were performed by referring to the environmental quality standards (EQS) indicated through threshold values (European Commission, 2008). For River water quality, the threshold values are currently given for PAHs, phthalates and pesticides. No threshold values available for Me-PAHs. Concerning the PCB contamination, actually, insufficient data allow to set the threshold value. However, according to the circular EU-WFD 2005/12 of 28 July 2005, the interim EQS of PCBs for water inland surface, transitional and territorial marine interior is set

to 0.001 μg.L^{-1} (INERIS, 2011). Threshold values of EQS of individual PAH, phthalate and pesticides, and contamination levels detected in Somme River are presented in Table 3.

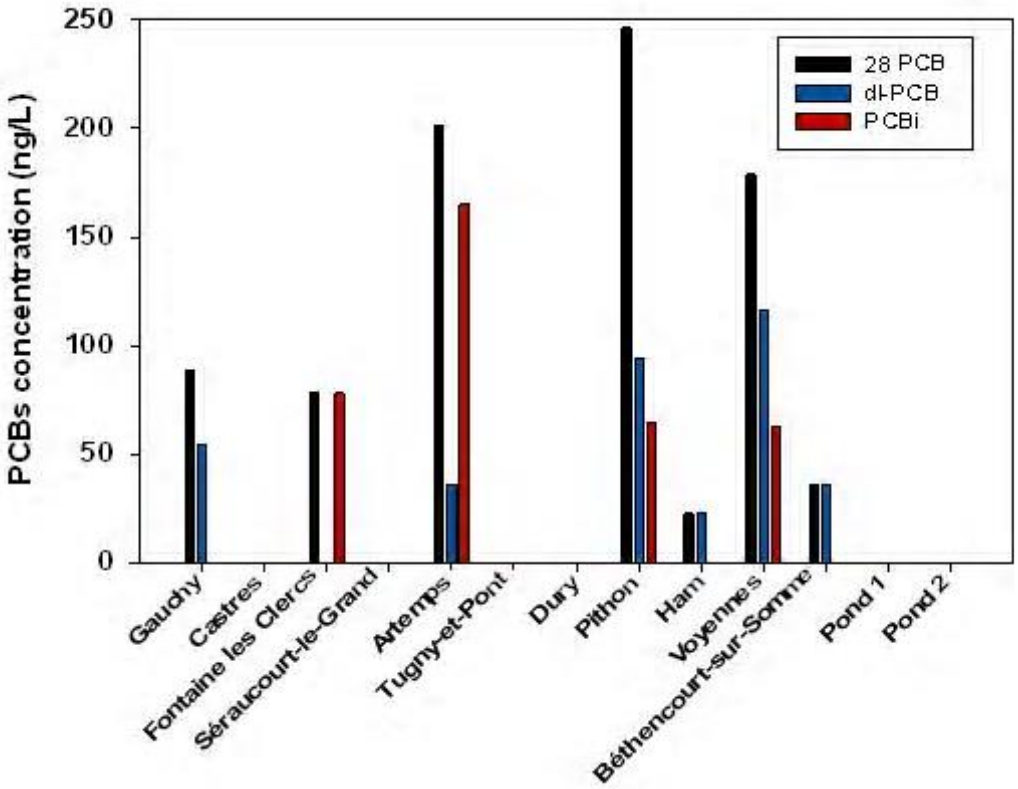

Fig. 4. Concentration of Σ28PCB, dl-PCB and PCBi in Somme River

The results in Table 3 show the contamination level of phthalates and pesticides under the maximum allowance concentration (MAC) of EU-WFD. However, the concentration of alpha-lindane and betalindane exceed CWQG value (> 0.01 μg/L) for some sampling points (at Fontaine-les-Clercs and Castres for alpha-lindane, and at Fontaine-les-Clercs, Artemps, Castres and Berthencourt-sur-Somme for beta-lindane). For PAH, the concentration detected at Somme River water do not exceed the MAC of EU-WFD. Nevertheless, some measured points showed the value higher than interim guideline set by CWQG for An, Fl, BaA and Py.

Table 1. Individual phthalate concentrations in the 13 sampling sites of the Somme River

Phthalate concentrations (µg/L)								
N° Station	Corresponding City	DMP	DEP	DBP	BBP	DEHP	DOP	Σ6phthalates
1	Gauchy	0.08	5.35	2.92	-	13.30	-	21.65
2	Castres	-	0.46	0.24	-	20.76	-	21.46
3	Fontaine les Clercs	0.25	2.12	3.28	-	8.88	-	14.53
4	Séraucourt-le-Grand	0.20	-	3.77	-	7.46	-	11.43
5	Artemps	0.02	0.26	0.43	-	6.22	-	6.93
6	Tugny-et-Pont	0.14	1.28	2.01	-	6.57	-	10.00
7	Dury	0.03	0.48	0.54	-	17.93	-	18.89
8	Pithon	0.13	4.92	2.97	-	5.16	-	13.18
9	Ham	0.10	3.01	3.86	-	5.86	-	12.83
10	Voyennes	0.15	3.62	1.98	-	6.84	-	12.59
11	Béthencourt/Somme	0.03	0.52	0.22	-	9.39	-	10.16
12	Pond 1	0.06	6.83	0.77	-	11.23	-	18.89
13	Pond 2	0.22	6.98	2.78	-	13.36	-	23.34

* « - » : not detected « + » : value below the limit of quantification (< 0.01 µg/L)

Table 2. Pesticides repartition in the surface water in Somme River

Pesticide concentrations (ng/L)													
N° Station	1	2	3	4	5	6	7	8	9	10	11	12	13
Alpha-lindane	-	89	355	-	-	-	-	-	-	-	-	-	-
Gamma-lindane	-	271	177	-	281	-	-	-	-	-	231	-	-
Chloroneb	-	90	131	-	117	-	-	-	-	-	103	-	-
Σ28pesticides	-	450	663	-	398	-	-	-	-	-	334	-	-

* « - » : not detected

Table 3. Threshold values of EQS of individual PAH, phthalate and pesticide (Canadian Environmental Quality Guidelines, 2007; Directive n° 2013/39/UE of 12/08/13 modifying the directives 2000/60/CE and 2008/105/CE), and contamination levels detected in Somme River

Compounds	**EU-WFD**	**CWQG**	**Somme River (Average)**
PAHs (µg/L)			
Na	130**	1.1*	0.011-0.048 (0.026)
Ayl			0.040-0.081 (0.057)
Aen		5.8*	n.d. – 0.066 (0.017)
F	0.12**	3*	n.d. – 0.060 (0.011)
An	0.1**	0.012*	n.d. – 0.044 (0.027)
Fl	0.12**	0.04*	n.d. – 0.101 (0.032)
Ch		Insufficient data	n.d. – 0.138 (0.011)
BaP	0.27**	0.015*	n.d.
Pn		0.4*	0.008-0.194 (0.047)
BbF	0.017**		n.d.
BkF	0.017**		n.d.
Bghi	0.0082**		n.d.
DhA			
IP	Insufficient data		n.d.

BaA		0.018*	n.d. – 0.068 (0.006)
Py		0.025*	0.021-0.123 (0.041)
Pesticides (µg/L)			
Alpha-lindane	0.04	0.0	n.d.-0.355 (0.029)
Gamma-lindane	0.04	0.01	n.d. – 0.281 (0.064)
Beta-lindane	0.04	0.01	n.d.
Delta-lindane	0.04	0.01.	n.d
Heptachlor	0.0003	Insufficient data	n.d.
Aldrin	Insufficient data	Insufficient data	n.d.
Trans-chlordane		Insufficient data.	n.d
Cis-chlordane		Insufficient data	n.d.
Endosulfan I	0.01**	0.02	n.d.
Dieldrin	Insufficient data	Insufficient data	n.d.
Endrin	Insufficient data	Insufficient data	n.d.
Endosulfan II	0.01**	0.02	n.d.
4,4'-DDT	Insufficient data	Insufficient data	n.d.
Endrin aldehyde		Insufficient data	n.d.
Endosulfan sulfate		0.02	n.d.
Endrin ketone		Insufficient data	n.d.
Heptachlor epoxide (isomer A and B)	0.0004**	No objet	n.d.
Chloroneb			n.d. – 0.094 (0.046)
Isodrine	Insufficient data		n.d.
P,p-DDT	Insufficient data		n.d.
Phthalates (µg/L)			
DMP			n.d. – 0.25 (0.110)
DEP		16	n.d. – 6.98 (3.001)
DBP		19	0.22 – 7.58 (2.404)
BBP			n.d.
DNOP		Insufficient data	n.d.
DEHP	Insufficient data		0.34– 20.76 (9.591)

Eu-WFD: European Union Water Framework Directive
CWGQ: Canadian Water Quality Guidelines for the protection of Aquatic life
*Interim Guideline **Maximum Allowance Concentration

4. Conclusion

The extract water samples was analyzed for 16 PAHs and 18 Me-PAHs, 6 phthalates, 28 pesticides, 28 PCBs, using gas chromatography/mass spectrometry (GC/MS) for the total of 96 targeted compounds. Each contaminant was quantified to assess their contamination levels in 13 sites of Somme River in northern France. Recorded concentrations showed significant contaminations of Somme River. Large variation of concentration was observed from one sampling site to others for the concentration level of hydrocarbons. Hydrocarbons contaminations were dominated by two rings and three rings respectively for Me-PAHs with average of 72% and PAHs with average of 75%. This work reported for the first time the

contamination level of phthalates in Somme River. The same order of magnitude of phthalate concentration was observed with fifteen sampling stations in Somme River; the Σ6phthalates varies from 7 to 23 µg/L. DEHP and DEP are phthalates the most abundance in this River; their average represents respectively around 63% for DEHP and 20% for DEP. Among 28 targeted pesticides, only chloroneb, alphalindane and beta-lindane were detected and quantified. Moreover, their concentrations were relatively low close to limited guidelines for drinking water which limited the sum of pesticides at 500 ng/L. According to the guideline values set by EU-WFD, Somme River present the good quality respect to these five families selected (PAHs, Me-PAHs, PCBs, phthalates and pesticides). However, these contamination levels could affect the aquatic life because they are frequently detected at the values exceeded the guideline values set by CWQG. It is interesting to note that Somme River is a place where there is a breeding fish and local fishing activities. The generally gap information could be a major source uncertainty in evaluation of water quality and so on the decision that the authorities could take. Nevertheless, there is growing evidence that these kinds of study are potentially important sources of information which contribute to the quality evaluation of the aquatic ecosystems and some decision of local authority (i.e. fishing activities, breeding fish).

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