hydroxyphénanthrène et 17% de 1-hydroxypyrène chez *N. virens*, et 18% de 9-hydroxyphénanthrène et 20% de 1-hydroxypyrène chez *L. variegatus*. D'après ces importantes proportions en métabolites, nous présumons que les métabolites présents dans les vers sont principalement dus à la métabolisation des HAP parents plutôt qu'à la bioaccumulation à partir des sédiments où les métabolites étaient présents en faible concentration.

La réalisation de ces travaux nous aura permis d'atteindre l'ensemble des objectifs fixés. Nous avons pu mettre au point deux méthodes d'extraction sélective nous permettant de déterminer les HAP totaux biodisponibles dans les sédiments fortement contaminés par des alumineries et plus précisément les HAP avec un log K_{ow} <5,8. De plus, l'étude de la bioaccumulation nous a permis de montrer que la composition et le niveau de contamination jouaient un rôle sur la biodisponibilité des HAP. On a pu voir que les vers bioaccumulaient, en proportion, plus de HAP dans les sédiments faiblement contaminés que dans les fortement contaminés. Cette étude nous a aussi permis de déterminer que les différents paramètres toxicocinétiques (ingestion, élimination, facteurs de bioaccumulation) variaient d'un sédiment à l'autre et d'un HAP à l'autre.

ABSTRACT

Traditionally, polycyclic aromatic hydrocarbon (PAH) concentrations in soil and sediment have been determined after solid/liquid extractions using organic solvents, such as dichloromethane or hexane/acetone. However, when hydrophobic organic contaminants (HOCs), such as PAHs, enter soil or sediment, they undergo several loss or transport processes which modify their behaviour. In addition, sorption-related processes may cause these chemicals to become increasingly solvent inextractable. The results of this weathering process are a corresponding decrease in the extractability of PAHs and a decline of their availability for benthic organisms and their biodegradability by microorganisms. This phenomenon has been termed an 'aging effect'. A considerable research effort has been recently devoted to develop reliable chemical methods for the determination of labile PAHs in soils and sediments.

The aim of the present study was to establish one or more selective chemical methods allowing the characterization of bioavailable PAH concentrations in sediments with variable contamination levels and geochemical properties. Moreover, we also established accumulation temporal pattern of PAHs in those sediments in two worm species to identify toxicokinetic parameters describing uptake, elimination and bioaccumulation factors.

Thus, three series of experiments have been carried out. The first one permitted to compare three different solid/liquid extraction techniques (n-butanol (BuOH), hydroxypropyl-β-cyclodextrin (HPCD), and a solution of surfactant Brij700 (B700)) and

the second study to compare the efficiency of two passive samplers, polyoxymethylene (POM) strips and polydimethylsiloxane (PDMS) silicon tubing. Results of these two studies were compared with 28-day uptake experiments by *Nereis virens* for marine sediments and *Lumbriculus variegates* for freshwater sediments. In addition, results of the second study were compared to the previously measured B700 liquid/solid extraction. The third experiment determined the uptake temporal patterns of the different PAHs by worms (*N. virens* and *L. variegatus*) in the different studied sediments as well as the organism ability to metabolize phenathrene and pyrene by the determination of corresponding hydroxy-PAHs (9-hydroxyphenanthrene and 1-hydroxypyrene).

Results obtained during the two first studies show that strong differences were observed in quantities and proportions of PAHs obtained using different techniques for assessing the bioavailability of PAHs in sediments. Our first study shows that BuOH extracted much more PAHs than worms can bioaccumulate in their tissues, and where HPCD underestimated the bioavailability of PAHs especially for low molecular weight PAHs. On the other hand, use of the surfactant B700 revealed a good prediction capability for PAHs in highly contaminated sediments. Results of the second study show that PDMS overestimated the availability of PAHs in all studied sediments (low and highly contaminated ones), whereas the POM method provided results quite similar to the solid/liquid extraction using high molecular weight Brij®700. Indeed, bioavailability of total PAHs was correctly predicted by POM and B700 in highly contaminated aluminum smelter sediments. However, a closer examination of individual PAH results indicated that both techniques overestimated the availability of large molecules with log K_{ow} >6

suggesting the presence of a biological mechanism limiting uptake of larger PAHs which seem to be related to the molecular size of the compounds or to the steric congestion.

The results of the third study showed that *Nereis virens* and *Lumbriculus variegatus* accumulated sediment-associated PAHs rapidly and achieved apparent steady-state within seven days (168 h) for all studied sediment samples. In addition, the uptake clearance rate constants of PAHs were lower for highly than for low contaminated sediments (10^{-2}-10^{-6} and 10^{-1}-10^{-4} g dry sediment g^{-1} wet organism h^{-1}, respectively), whereas the elimination rate constants of PAHs remained in same order of magnitude for both type of sediments. Moreover, this study showed that log of the uptake clearance rate constant (log k_s) decreased with increasing hydrophobicity of PAHs, expressed by the octanol-water partition coefficient (log K_{ow}) for all studied sediment samples. The negative relationship between log k_s and log K_{ow} suggests that the desorption rate of the compounds from the sediment was an important governing factor in the accumulation by the worms. The determination of the relative proportion of phase I metabolites toward total phenanthrene and pyrene in worm tissues after a 28-day exposure indicated the presence of 24% of 9-hydroxyphenanthrene and 17% of 1-hydroxypyrene in *N. virens*, and 18% of 9-hydroxyphenanthrene and 20% of 1-hydroxypyrene in *L. variegatus*. According to these high proportions of metabolites, we assumed that the metabolites present in worms are mainly due to metabolization of the parent PAH instead of bioaccumulation from sediment where metabolites were present in low concentrations.

All the fixed objectives have been achieved by the realisation of these experiments. Indeed, we succeeded to develop two non-exhaustive extraction methods in order to

determine bioavailable PAHs in aluminium smelter highly contaminated sediments and more precisely PAHs with log K_{ow} <5,8. In addition, the bioaccumulation study showed that composition and contamination level played a key role on PAH biopavailability. We also showed that worms bioaccumulated, in proportion, more PAHs in low contaminated sediments than in highly contaminated ones. This study allowed us to establish that the toxicokinetic parameters (uptake, elimination, bioaccumulation factors) varied from a sediment to an other and from a PAH to an other.

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INTRODUCTION GÉNÉRALE

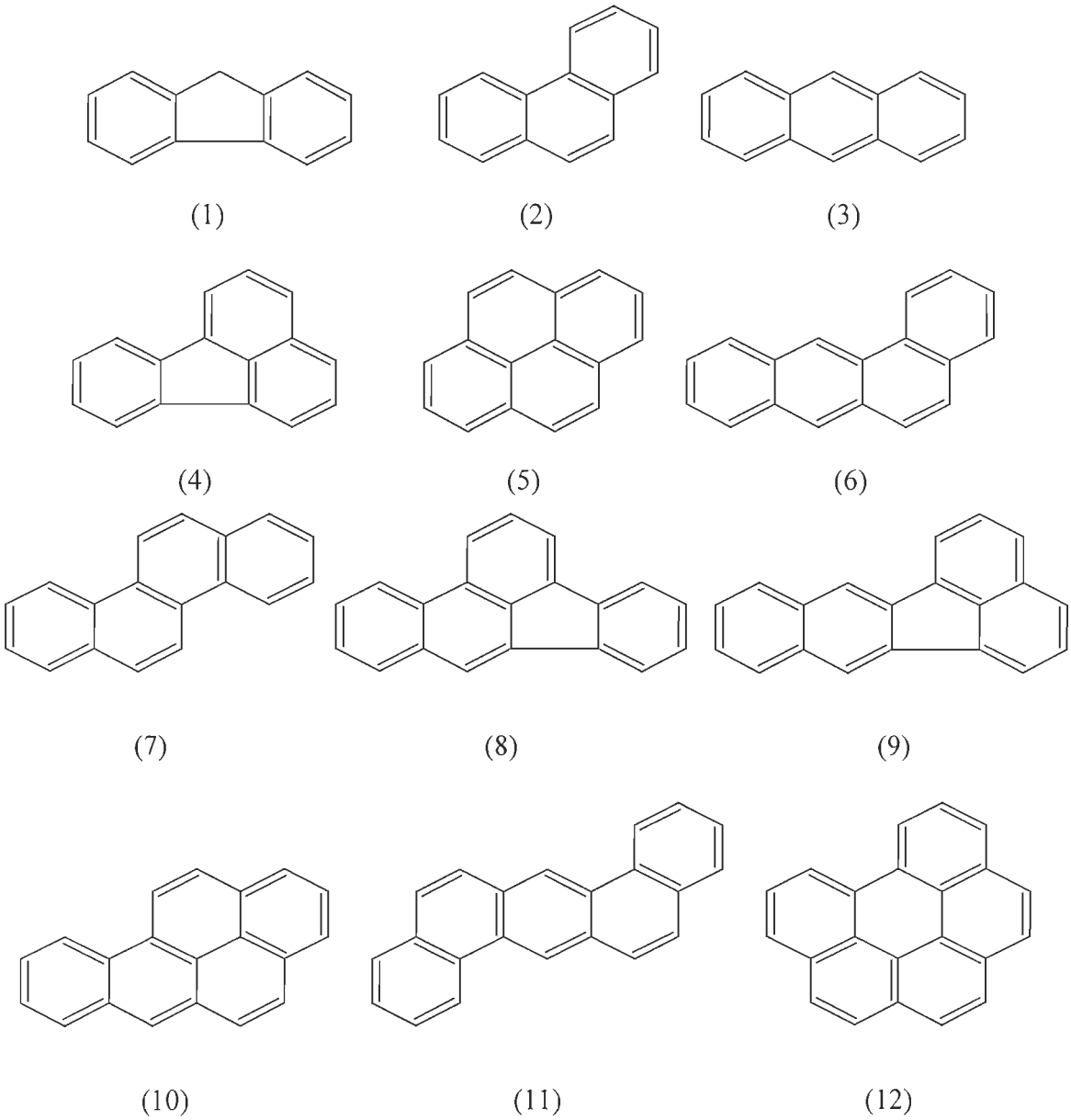
1. Les hydrocarbures aromatiques polycycliques (HAP)

Les hydrocarbures aromatiques polycycliques (HAP) sont les xénobiotiques organiques les plus présents dans les environnements estuariens et marins. Ceux-ci sont devenus extrêmement importants à cause de leurs effets cancérigènes, mutagènes et tératogènes potentiels sur les organismes aquatiques et sur l'homme (Fetzer, 2000).

1.1. Structure chimique

Les hydrocarbures aromatiques polycycliques sont un groupe de composés constitués d'atomes d'hydrogène et de carbone formant de deux à sept cycles benzéniques dans des arrangements linéaires, angulaires ou en grappes avec des groupes insaturés possiblement attachés à un ou plusieurs cycles (Fetzer, 2000). Ces composés vont du naphtalène ($C_{10}H_8$, deux cycles) au coronène ($C_{24}H_{12}$, sept cycles) (Fig. 1). En général, les HAP ont une faible solubilité dans l'eau, un haut point de fusion et d'évaporation et une faible pression de vapeur. Quand la solubilité diminue, les points de fusion et d'évaporation augmentent, et la pression de vapeur diminue avec l'augmentation du volume moléculaire qui est directement fonction du nombre de cycles (Fetzer, 2000).

Fig. 1. Structures et noms des 12 HAP étudiés: (1) Fluorène (2) Phénanthrène; (3) Anthracène; (4) Fluoranthène; (5) Pyrène; (6) Benz[a]anthracène; (7) Chrysène; (8) Benzo[b]fluoranthène; (9) Benzo[k]fluoranthène; (10) Benzo[a]pyrène; (11) Dibenz[a,h]anthracène; (12) Benzo[g,h,i]pérylène.



1.2. Les voies d'entrée des HAP dans les environnements estuariens et marins

Les routes principales d'entrée des HAP dans les environnements estuariens et marins incluent les apports atmosphériques et hydriques provenant principalement d'activités anthropiques (les effluents industriels et urbains, les déchets d'incinération, les accidents pétroliers (le pétrole brut contient de 0,2 à 7 % de HAP)) (Lima et al., 2005), la production d'asphalte et la combustion des combustibles fossiles (Dabestani et Ivanov, 1999; Oros et Ross, 2004; Yunker et al., 2002), la diagenèse de la matière organique dans les sédiments anoxiques (Lima et al., 2005) ou encore de produits et de processus naturels tels que les feux de forêts et les émissions volcaniques (Bowerman, 2004; Lu, 2003).

Presque tous les HAP provenant de l'atmosphère sont associés à la matière particulaire aérienne et aux aérosols à cause de leurs coefficients de partage octanol/eau (K_{ow}) relativement élevés, ce qui dénote un important potentiel d'adsorption sur les matières particulaires en suspension dans l'air et dans l'eau (Law et al., 2002). La pluie et la neige représentent le principal processus atmosphérique responsable du flux de HAP dans les océans mondiaux (Dickhut et al., 2000; Tsai et al., 2002) pour trouver leur destination finale dans les sédiments (Christensen et Bzdusek, 2005). Ainsi, le sédiment devient un puits pour les HAP à cause de leur relative immobilité quand ils sont non-perturbés, et de leur persistance (demi-vie de 0.3 à 58 ans pour le benzo[a]pyrène) (Ghosh et al., 2003; Gulnick, 2000).

1.3. Les HAP dans l'eau

Les eaux de surface comme les rivières et les eaux côtières peuvent être fortement polluées par les HAP en raison des industries et des ports. Les concentrations totales en HAP dans les eaux de rivières traversant des régions fortement industrialisées peuvent atteindre plus de 10 µg/L de HAP totaux. Les eaux de rivières non-polluées et l'eau de mer contiennent moins de 0,1 µg/L de HAP totaux (World Health Organization, 1998).

Les concentrations des HAP sont plus faibles dans la colonne d'eau que dans le biote et les sédiments, due en partie à la faible solubilité dans l'eau des HAP. La matière organique dissoute ou colloïdale, telle les acides humiques et fulviques, dans l'eau de mer peut agir comme un solvant pour les HAP. Plus le nombre de cycles aromatiques ou la masse moléculaire des HAP augmente, plus la solubilité diminue. Par exemple, la solubilité dans l'eau du naphtalène, un HAP à deux cycles, est d'environ 30 mg/L, alors que celle des HAP à cinq cycles varie entre 0,5 et 5,0 µg/L (Mackay et al., 1991).

1.4. Les HAP dans les sédiments

Les HAP sont facilement adsorbés à la surface des particules à cause de leur hydrophobicité, leur faible solubilité dans l'eau, leur relativement faible pression de vapeur et leur aromaticité (Pignatello et Xing, 1996). La rapidité à laquelle les HAP s'adsorbent sur la matière particulaire en suspension entraîne une concentration plus importante de ces contaminants dans les sédiments des fonds marins que dans la colonne d'eau (généralement par un facteur 1000 ou plus) (Dungavell, 2005). Les sédiments estuariens et marins

deviennent donc un réservoir majeur de HAP et une source continue de contamination pour les communautés biotiques (Ghosh et al., 2003; Gulnick, 2000).

Étant donné la proximité des sources anthropiques et de la nature hydrophobe des HAP, les dépôts marins côtiers ont des concentrations significativement élevées par rapport aux autres régions marines, tels que les sédiments des marges ou des abysses. Les zones recevant le drainage des centres industrialisés peuvent avoir des niveaux de HAP de 10 000 µg/g ou plus dans les sédiments. Dans les régions éloignées des activités anthropiques, les valeurs des HAP totaux dans les sédiments sont souvent de l'ordre du µg/g (Gulnick, 2000).

1.5. Les HAP dans le biote

Les concentrations des HAP dans les organismes aquatiques sont extrêmement variables selon les sites et les espèces aquatiques. Les valeurs rapportées sont comprises entre 0,01 et 5 000 µg/kg de poids sec (McElroy et al., 1989). Les concentrations élevées dans les organismes marins se présentent souvent dans les zones recevant des décharges chroniques d'hydrocarbures (McElroy et al., 1989).

Les organismes déposivores peuvent ingérer les contaminants organiques à partir de l'eau les entourant, de l'eau porale par respiration, filtration ou par adsorption directe, et aussi à partir des particules sédimentaires ingérées par désorption et adsorption en présence de fluides digestifs (Weston et al., 2000).

2. Biodisponibilité et toxicité

La biodisponibilité d'un composé chimique est une mesure de son accessibilité au biote dans l'environnement. Elle est définie ici comme la fraction du contaminant qui est en

fin de compte disponible pour l'ingestion, l'accumulation ou l'assimilation par un organisme. C'est un facteur important contrôlant l'ingestion de contaminants liés au sédiment dans les organismes aquatiques, et le transfert des contaminants dans la chaîne alimentaire. C'est aussi un facteur critique dans le succès des techniques biologiques de remédiation. La biodisponibilité des contaminants hydrophobes tels les HAP est déterminée par les interactions complexes de différents facteurs abiotiques et biotiques, tels que les caractéristiques du composé, les caractéristiques du sédiment, et les caractéristiques biologiques des organismes (Lu, 2003).

Les facteurs fondamentaux affectant la disponibilité des contaminants sont la matière organique des sols et sédiments (Kukkonen et al., 2003; Oleszcuk et Baran, 2004; Rust et al., 2004a; Shor et al., 2003; Thorsen et al., 2004), le pH, la nanoporosité, le pourcentage en argile (Nam et Alexander, 1998), l'hydrophobicité (Nam et Alexander, 1998), et la capacité d'échange en cations (Chung et Alexander, 2002). La matière organique inclue le matériel de décomposition des plantes et animaux aussi bien que l'humine provenant de racines. Étant donné l'affinité importante des composés organiques à la matière organique des sols et sédiments, celle-ci réduit la disponibilité de ces composés dans les sols et sédiments. Des études évaluant la biodisponibilité et la séquestration des HAP dans les sédiments ont rapporté que les concentrations en contaminants (Chung et Alexander, 1999a), le taux d'humidité (Kottler et Alexander, 2001), la remise en suspension (Talley et al., 2002; White et al., 1999a), la présence d'autres HAP (White et al., 1999a; 1999b) et la durée de contact entre le polluant et le sédiment (Macleod et Semple, 2000) sont d'autres facteurs significatifs influençant la biodisponibilité du contaminant.

Des études ont indiqué que les sols et sédiments avec un taux de matière organique élevé influence la disponibilité. Le contenu en HAP extraits avec du n-butanol a été relié au type de boue de traitement des eaux usées (Oleszczuk et Baran, 2004). Talley et al. (2002) ont rapporté que la réduction en HAP dans la fraction argile/silt se corrélait bien avec la dégradation des HAP et l'accumulation par des vers de terre. Nam et al. (1998) ont rapporté que le phénanthrène extrait du sol avec du n-butanol était proportionnel au taux de carbone organique dans le sol. La dissipation initiale des contaminants peut être attribuée au taux d'humidité du sol, à la température et à d'autres facteurs environnementaux.

Des études récentes supportent le phénomène de vieillissement, suggérant que les composés organiques persistant dans les sols et sédiments deviennent moins disponibles à l'ingestion par les organismes, deviennent moins toxiques, et sont moins disponibles pour la biodégradation par les microorganismes. Ces observations indiquent que les molécules des contaminants sont prisonnières dans des micro-sites qui ne sont pas faciles d'accès au microorganismes. Chung et Alexander (1999b) ont montré qu'il y avait une relation entre le carbone organique et des pores de diamètre 0,1-10 μm et entre le contenu en argile et des pores <102 nm. Ainsi, la fraction biodisponible des contaminants peut ne pas être déterminée précisément par des analyses chimiques déterminant les concentrations totales en polluant (Alexander, 2000).

Les premières informations sur l'effet du vieillissement et sur la biodisponibilité proviennent d'études sur les concentrations de pesticides (dichlorodiphényltrichloroéthane (DDT), aldrine, dieldrine, heptachlore et chlordane) dans les sols en culture mesurées pendant de longues périodes et de mesures de toxicité de ces pesticides pour les invertébrés

et les plantes (Decker et al., 1965; Gilbert et Lewis, 1982; Korschgen, 1971; Lichtenstein et al., 1960; Lichtenchtein et Schulz, 1965; Onsager et al., 1970; Wingo, 1966). Bien que la disparition initiale d'un pesticide soit partiellement le résultat de sa volatilisation ou de sa dégradation abiotique aussi bien que de sa biodégradation par les microorganismes, le fait que cette disparition faiblisse au fur et à mesure des années indique que ces insecticides sont devenus faiblement disponibles aux microorganismes indigènes. Les premières études toxicologiques ont aussi démontré une relation temporelle avec la diminution de la biodisponibilité. Par exemple, des dosages biologiques de la toxicité aiguë sur la mouche *Drosophila melanogaster* simultanément avec des déterminations chimiques ont donné des résultats similaires peu de temps après l'application de lindane à un sol, mais une partie importante de cet insecticide restant dans le sol après 22 mois n'affectait plus de façon détectable les mouches (Edwards et al., 1957).

Des études récentes ont montré que des composés organiques présents depuis de nombreuses années dans des sols sont moins biodisponibles que ceux fraîchement ajoutés à ces mêmes sols. Morrison et al. (2000) ont trouvé que du DDT, du dichlorodiphényldichloroéthylène (DDE) et du dichlorodiphényldichlorométhane (DDD) d'un site de déchets (contaminé 30 ans auparavant) étaient approximativement biodisponibles à 30% pour des vers. La diminution de disponibilité pour de la simazine, des HAP et du 1,2-dibromethane vieillis a aussi été démontrée (Erickson et al., 1993; Weissenfels et al., 1992).

Le vieillissement est toxicologiquement significatif parce que l'assimilation, la toxicité aiguë et chronique des composés dangereux diminuent pendant qu'ils persistent et

deviennent de plus en plus séquestrés avec le temps (Alexander, 2000). La toxicité peut être moindre que celle anticipée même quand elle est basée sur des extractions vigoureuses des sols et sédiments (Erickson et al., 1993; Meier et al., 1997; Peijnenburg et al., 2000). Bien que le vieillissement réduit l'exposition et ainsi la toxicité et le risque, il ne les élimine pas. Des composés tels que le TCDD, BPC et PBB qui ont persisté pendant de longues périodes sont toujours biodisponibles pour les mammifères. Des sols traités au DDT et au chlordane étaient toujours toxiques pour des vers même après une période de vieillissement (Alexander, 2000). Du phénanthrène et du pyrène vieillis dans un sol montrèrent une diminution de la biodisponibilité aux microbes ainsi qu'une toxicité pour des vers avec le temps (Chung et Alexander, 1999a). Il est important de noter que même si les composés vieillissent, une fraction du composé reste biodisponible et il a été montré que cette fraction s'accumule dans des insectes, plantes et bactéries.

Le gestionnaire de l'environnement est face à un dilemme majeur car l'importance de la réduction de la biodisponibilité résultant du vieillissement est différente pour le même composé dans différents sols, pour différents composés dans le même sol et pour des durées différentes d'exposition du composé dans le sol. Comment peut-on évaluer le degré d'exposition et prédire le risque d'un composé vieilli? Les biotests sont un moyen évident de faire des estimations, mais la précision de ces méthodes biologiques est parfois inadéquate pour les besoins de la réglementation. Certains prennent beaucoup de temps et sont chers. Une alternative serait un test chimique ou physique dont les résultats seraient clairement corrélés avec les résultats des biotests.

Un effort considérable a été récemment déployé pour le développement de méthodes d'extraction afin de déterminer les concentrations biodisponibles ou labiles des contaminants dans les sols et sédiments. Afin d'évaluer la fraction biodisponible des contaminants dans les sols et sédiments de nombreuses méthodes d'extraction ont été élaborées. Des méthodes d'extraction utilisant des échantillonneurs passifs ("passive sampler") tels que le Tenax, XAD, des membranes semiperméables, des disques de silice octadecyle modifié, du polyoxyméthylène (POM), des tubes de silicone (Akkanen et al., 2001; Cornelissen et al., 1998; Cornelissen et al., in press; Cuypers et al., 2002; Krauss et Wilcke, 2001; Richardson et al., 2003; Rust et al., 2004b; Tang et al., 2002; van der Wal et al., 2004; Zimmerman et al., 2004), des gaz hautement pressurisés (Hawthorne et Grabanski, 2000; Librando et al., 2004; Loibner et al., 2000; Szolar et al., 2001; Szolar et al., 2004), et l'oxydation au persulfate (Cuypers et al., 2000) ont été proposées. Récemment, des solutions aqueuses de cyclodextrines (Cuypers et al., Reid et al., 2000; 2002; Swindell et Reid, 2006) et de tensioactifs (Chang et al., 2000; Cuypers et al., 2002; Fava et al., 2004; Tang et al., 2002) ont été testées sur des sols et sédiments contaminés ainsi que des systèmes multi-colonnes (Zhao et Voice, 2000) et des extractants chimiques sélectifs (Tang et al., 2002) tel que le n-butanol (Alexander et Alexander, 2000; Liste et Alexander, 2002; Oleszczuk et Baran, 2004).

Les résultats des analyses par de telles procédures ont été corrélés avec la biodisponibilité pour différents organismes vivants. Lors de l'analyse de telles méthodes dites douces, les auteurs se sont aperçus que la diminution de la biodisponibilité en fonction du temps était accompagnée d'une chute de la quantité extraite de polluants.

Ces observations suggèrent que de telles méthodes d'extraction sélectives pourraient servir de base pour un test de substitution afin de déterminer la biodisponibilité.

3. Séquestration des contaminants organiques hydrophobes par des géosorbants

Au cours des années 1990, le professeur Luthy et collaborateurs ont proposé un modèle conceptuel pour décrire la séquestration des composés hydrophobes par les géosorbants. Les mécanismes à la base de la séquestration des contaminants organiques hydrophobes sont complexes et font appel à une succession d'étapes à partir d'une adsorption à la surface des particules suivie d'une absorption en profondeur que l'on peut schématiser ainsi (adapté de Luthy et al., 1997).

A. Absorption rapide dans la matière organique amorphe ou dans un liquide organique inter-particulaire: mécanisme plus favorable aux molécules polaires, molécules peu ou pas accessibles aux organismes, faiblement extraites.

B. Absorption lente dans la matière organique vieillie et dense: molécules peu ou pas accessibles aux organismes, séquestration complète.

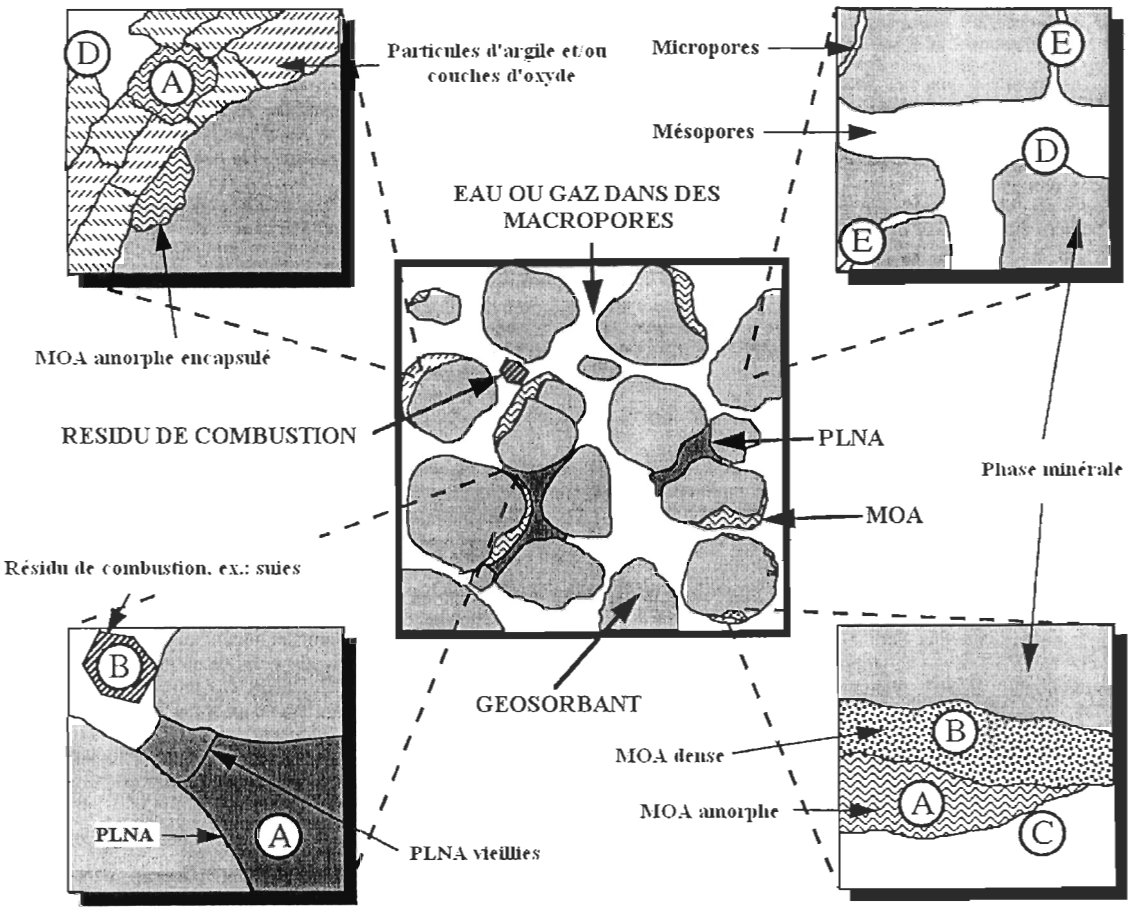
C. Adsorption rapide sur les surfaces organiques mouillées par l'eau interstitielle: interactions des régions polaires entre molécules adsorbées et l'absorbant, biodisponibles et faciles à extraire.

D. Adsorption rapide sur les surfaces minérales mouillées par l'eau interstitielle: les molécules adsorbées sont disponibles à la biodégradation et faciles à extraire.

E. Adsorption dans les micropores de la matrice minérale (argile): molécules probablement peu accessibles à la biodégradation mais demeurent extractibles avec des solvants organiques.

La Fig. 2 schématise les types de mécanisme appliqués aux divers géosorbants d'un sol.

Fig. 2. Modèle conceptuel du domaine du géosorbant. Les lettres encerclées se réfèrent aux mécanismes de sorption décrits précédemment. Le domaine du géosorbant inclus des différentes formes de matière organique adsorbante (MOA), de carbone particulaire provenant de résidus de combustion tel que les suies, et de carbone anthropique incluant les phases liquides non-aqueuses (PLNA) (traduit de Luthy et al., 1997).



4. Biodiponibilité et bioaccessibilité

De nombreuses définitions de la biodisponibilité ont été données depuis les 2 dernières décennies (Alexander, 2000; Herrchen et al., 1997; Klaasen, 1986; Kramer et Ryan, 2000; Ruby et al., 1996; Spacie et al., 1995; Van Leeuwen et Hermens, 1995). Avoir autant de définitions différentes crée une confusion chez les scientifiques environnementaux. Ainsi, Semple et al. (2004) ont proposé une définition pour la biodisponibilité et la bioaccessibilité:

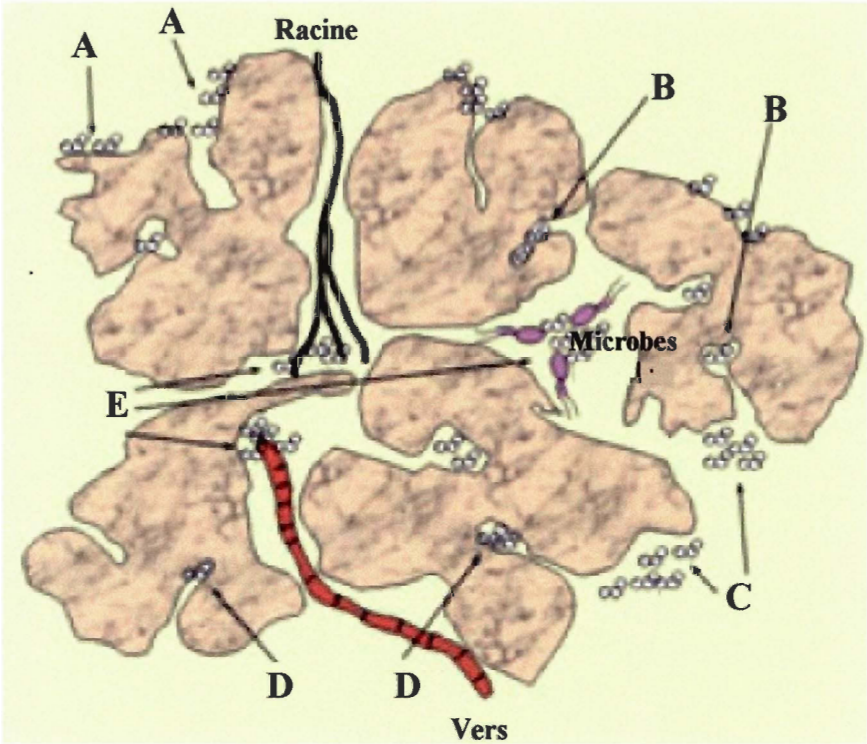
- la biodisponibilité est ce qui est librement disponible pour traverser la membrane cellulaire d'un organisme à partir du média où vit un organisme à un moment donné (Fig. 3). Une fois que le transfert à travers la membrane a eu lieu, un stockage, transformation, assimilation, ou dégradation peut se produire dans l'organisme.
- la bioaccessibilité est ce qui est disponible pour traverser la membrane cellulaire d'un organisme à partir de l'environnement, si l'organisme a accès au contaminant. Cependant, le contaminant peut être soit physiquement déplacé par l'organisme, soit devenir biodisponible après une certaine période. Dans ce contexte, physiquement déplacé peut référer à un contaminant qui est séquestré dans la matière organique et ainsi est indisponible à un moment donné ou qui occupe un espace de l'environnement différent de l'organisme (Fig. 3). Les contaminants peuvent devenir disponible suivant une libération rapide à partir d'un amalgame labile, ou l'organisme peut bouger et entrer en contact avec le

contaminant. Alternativement, une libération peut intervenir bien après (années ou décennies) et rendre le contaminant bioaccessible. En résumé, la bioaccessibilité englobe ce qui est réellement biodisponible et ce qui est potentiellement biodisponible.

Il est bien connu que la portion d'un contaminant qui est, soit biodisponible, soit bioaccessible, dans un sol ou sédiment peut différer de façon importante selon les organismes. Un avantage clair de ces définitions est leur multi-fonctionnalité, puisque ces définitions peuvent s'appliquer aux contaminants étant disponibles ou accessibles aux microorganismes, champignons, plantes, invertébrés, et animaux supérieurs par passage à travers la membrane de l'organisme en question. Ceci pourra être la membrane cellulaire d'une bactérie alors que chez les vers, par exemple, ceci englobe l'ingestion par la peau et le tractus gastrointestinal.

La distinction entre biodisponibilité et bioaccessibilité force les praticiens à considérer ce qu'ils mesurent réellement avec les méthodes biologiques et chimiques, qui sont développées afin de déterminer la fraction biodisponible.

Fig. 3. Diagramme conceptuel illustrant les fractions biodisponibles et bioaccessibles d'un contaminant dans un sol ou sédiment selon sa localisation physique (traduit de Semple et al., 2004). (A: Composé sorbé (rapidement réversible) (biodisponible ou bioaccessible: lié temporairement); B: Composé sorbé (lentement/très lentement réversible) (bioaccessible: lié temporairement); C: Composé bioaccessible (lié physiquement); D: Composé séquestré (non bioaccessible); E: Composé biodisponible) (traduit de Semple et al., 2004).



5. Travaux effectués à l'ISMER

5.1. Extraction sélective des HAP

Des techniques d'extraction sélective ont été au centre des récentes recherches dans l'espoir qu'elles puissent atteindre la fraction labile ou biodisponible. Comme on a pu le voir précédemment, plusieurs auteurs ont déjà proposé des protocoles d'extraction sélective des hydrocarbures aromatiques polycycliques (Cuypers et al., 2000; Librando et al., 2004; Oleszczuk et Baran, 2004; Reid et al., 2000; Swindell et Reid, 2006; Szolar et al., 2004). Malheureusement, la plupart de ces techniques ne miment que faiblement les processus inhérents à la biodisponibilité. De plus, celles-ci négligent le fait que les bactéries sont capables de sécréter des exoenzymes et des biopolymères, ayant des propriétés tensioactives, pour aider la dissolution partielle du matériel organique particulaire et aussi permettre le passage à travers leur paroi cellulaire. Une méthode récemment développée dans notre laboratoire (Barthe, 2002) utilise un tensioactif à haut poids moléculaire (Brij® 700) afin d'extraire la fraction biodisponible des molécules hydrophobes adsorbées sur la matrice sédimentaire. L'utilisation de tensioactifs à des concentrations proches ou supérieures à la concentration micellaire critique (CMC) permet de solubiliser des composés non-ioniques ajoutés dans un mélange d'eau et de sol (Liu et al., 1991). Notre méthode repose sur l'hypothèse que l'utilisation du tensioactif mimerait l'action des bactéries capables de sécréter des exoenzymes et des biopolymères possédant des propriétés tensioactives qui permettent une dissolution du matériel organique ainsi que la diffusion des molécules à travers leur membrane cellulaire (Barthe, 2002).

6. Objectifs

Comme on a pu le voir précédemment, les études réalisées sur les mécanismes et la cinétique de séquestration des HAP dans les sédiments ne l'ont été que sur quelques échantillons à la fois. Notre étude permettra d'avoir une idée plus précise sur les liens qu'il peut y avoir entre ceux-ci et les caractéristiques physico-chimiques des sédiments puisque nous allons étudier de nombreux sédiments lacustres et marins de provenance, de composition et de concentration totale en HAP différentes.

6.1. Objectif général

La présente étude a pour but de caractériser les mécanismes et la cinétique de séquestration des HAP dans les sédiments lacustres et marins ayant des caractéristiques géochimiques et environnementales très différentes ainsi que de déterminer la bioaccumulation des HAP afin de la corréler avec les données de séquestration obtenues.

6.2. Objectifs spécifiques

Afin d'atteindre efficacement l'objectif général présenté, la présente étude a été divisée en trois objectifs spécifiques. Les travaux d'échantillonnage, d'observation, d'analyse et d'interprétation seront ainsi répartis en fonction de chacun de ces objectifs:

1. Développer et intégrer les notions de séquestration des hydrocarbures aromatiques polycycliques (HAP) dans les sédiments marins et lacustres. Pour ce faire, nous allons étudier différentes méthodes d'extractions sélectives sur des sédiments de provenance, de composition géochimique et de concentration totale en HAP

totalement différentes afin de déterminer si la composition et la contamination de ces sédiments peuvent jouer un rôle sur la séquestration des HAP.

2. Déterminer la bioaccumulation des HAP dans les sédiments marins et lacustres en fonction de leur nature géochimique. Pour ce faire, des études de bioaccumulation seront effectuées à l'aide de polychètes et d'oligochètes afin de déterminer si la composition et le niveau de contamination des sédiments jouent un rôle sur la biodisponibilité des HAP.

3. Déterminer les patrons temporels d'accumulation des HAP pour plusieurs échantillons de niveau de contamination variable dans deux espèces de vers en déterminant les paramètres toxicocinétiques décrivant l'ingestion, l'élimination et les facteurs de bioaccumulation. De plus, l'habilité des organismes à métaboliser le phénanthrène et le pyrène sera examinée par la détermination d'hydroxy-HAP sélectionnés.

CHAPITRE I

COMPARING BULK EXTRACTION METHODS FOR CHEMICALLY AVAILABLE POLYCYCLIC AROMATIC HYDROCARBONS WITH BIOACCUMULATION IN WORMS

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Environmental context

Determining the bioavailability of organic contaminants in sediments is a critical step in assessing the ecological risks of contamination in aquatic ecosystems. Standardised sediment bioaccumulation tests using benthic organisms are often performed to determine the relative bioavailability of sediment contamination. Unfortunately biological methods are time consuming, expensive and organisms are often difficult to maintain in good health in a laboratory exposure system. Contradictory results have been reported in the last decade and factors that affect the behaviour of extractants need to be examined for a large range of sediments. A study was conducted to determine the bioavailability of polycyclic aromatic hydrocarbons (PAHs) in sediment using worms and to compare the uptake by biological samplers with mild solid/liquid extractions when exposed to unspiked low and highly contaminated marine and freshwater sediments.

Résumé

L'objet de cette étude est d'évaluer différentes techniques afin de déterminer la disponibilité des hydrocarbures aromatiques polycycliques (HAP) dans les sédiments contaminés. Ce but fut atteint en comparant les résultats d'études sur 28 jours de bioaccumulation par *Nereis virens* et *Lumbriculus variegatus* avec les HAP extraits par trois méthodes d'extraction non-exhaustives utilisant le n-Butanol (BuOH, 100%), une solution aqueuse d'hydroxypropyl-β-cyclodextrine (HPCD) et une solution de tensioactif de Brij700 (B700). Nos résultats montrent l'importance de considérer le niveau en HAP dans les sédiments ainsi que la taille moléculaire des HAP quand vient le moment de prédire leur bioaccumulation dans un échantillonneur biologique comme les vers en utilisant une méthode d'extraction solide/liquide. La solution de tensioactif B700 a eu du succès à prédire la bioaccumulation quand elle a été exposée à des sédiments fortement contaminés (25–5700 $\mu g\ g^{-1}$). Quand des sédiments faiblement contaminés (0,06–1,1 $\mu g\ g^{-1}$) ont été utilisés, le HPCD et le BuOH ont été des meilleurs agents d'extraction pour estimer la bioaccumulation alors que le B700 apparaît être trop faible pour la majorité des échantillons. Nos résultats illustrent l'intérêt et les difficultés à trouver un prédicteur chimique adéquat pour la biodisponibilité des HAP, particulièrement parce que les concentrations en HAP et les processus de séquestration jouent un rôle déterminant dans la qualité des résultats. Comme le B700 est bon marché et que les solutions d'extraction sont faciles à préparer, une procédure d'extraction utilisant ce tensioactif est proposée comme un prédicteur fiable pour les sédiments fortement contaminés.

Mots clés additionnels: biodisponibilité, Brij700, butanol, hydroxypropyl-β-cyclodextrine, hydrocarbures aromatiques polycycliques (HAP)

Abstract

The purpose of this study is to evaluate different techniques for assessing the availability of polycyclic aromatic hydrocarbons (PAHs) in contaminated sediments. This goal was achieved by comparing results from 28-day uptake experiments by *Nereis virens* and *Lumbriculus variegatus* with PAHs extracted by three non-exhaustive extraction methods using: *n*-Butanol (BuOH, 100%), an aqueous solution of hydroxypropyl-β-cyclodextrin (HPCD) and a surfactant solution of Brij700 (B700). Our results highlight the importance of considering both the PAH level in sediments and the molecular size of PAHs when attempting to predict their bioaccumulation in a biological sampler like worms using a solid/liquid extraction method. The surfactant B700 solution was quite successful to predict PAH bioaccumulation when exposed to unspiked highly contaminated sediments (25–5700 $\mu g\ g^{-1}$). When low contaminated sediments (0.06–1.1 $\mu g\ g^{-1}$) were used, HPCD and BuOH were better extractants for estimating bioaccumulation whereas B700 appeared to be too mild as extractant for most samples. Our results illustrate the interest and difficulties in finding an adequate chemical predictor for PAH bioavailabilty, particularly because PAH concentrations and sequestration processes play a determining role in the quality of results. Because B700 is not expansive and extraction solutions are easy to prepare, an extraction procedure involving this surfactant is proposed as a reliable predictor for aged highly contaminated sediments.

Additional keywords: bioavailability, Brij700, butanol, hydroxypropyl-β-cyclodextrin, polycyclic aromatic hydrocarbons (PAHs).

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are pollutants of great environmental concern because of their mutagenic and carcinogenic properties.[1,2] Traditionally, PAH concentrations in soil and sediment have been determined after solid/liquid extractions using organic solvents, such as dichloromethane or hexane/acetone. However, when hydrophobic organic contaminants (HOCs), such as PAHs, enter soil or sediment, they undergo several loss or transport processes including volatilisation and leaching, as well as chemical and biological degradation which modify their behaviour. In addition, sorption-related processes may cause these chemicals to become increasingly solvent nonextractable or irreversibly bound within the soil or sediment matrix. The result of this weathering process with time is a corresponding decrease in the extractability of PAHs. This phenomenon has been termed an 'aging effect' and is related to a decline of their availability for uptake by benthic organisms and their biodegradability by microorganisms.[3–5] A considerable research effort has been recently devoted to develop reliable chemical methods for the determination of labile PAHs. Laboratory methods based on solid-phase extraction (SPE),[6,7] persulfate oxidation,[8] solvent extraction[9,10] and supercritical fluid extraction[11] have been proposed. Recently, aqueous solutions of cyclodextrin[12,13] and surfactants[12,14] have been tested on contaminated soils and sediments.

In an attempt to compare different methods, Kelsey et al.[9] measured the extractability of phenanthrene using different mild chemical extractants to assess its bioavailability to earthworms and bacteria. Results suggested that *n*-butanol (BuOH), a small linear and polar molecule, was the most appropriate solvent for predicting bioavailability of phenanthrene to both organisms, but BuOH was unsuccessful to predict the bioavailability of atrazine. Several other studies using freshly spiked soils came in support to the BuOH-extraction procedure and showed high correlations between extraction efficiency and bioavailability of PAHs for biodegradation or bioaccumulation by worms.[10,15,16]

Cyclodextrins, a family of cyclic oligosaccharides highly soluble in water, were also considered as extractants because they have a hydrophobic organic cavity in the interior of the torus[17] that can contribute to the formation of a 1:1 inclusion complex between the cyclodextrin macromolecule and an organic moiety.[18] Aqueous solutions of cyclodextrin have been used to dissolve a large range of contaminants of low aqueous solubility. It has been shown that molecules too large to form 1:1 inclusion complexes with β-cyclodextrin can form1:2 inclusion complexes, which consist of two macrocycles and one guest.[19] The application of cyclodextrin extraction for the prediction of labile PAHs was first studied by Reid et al.[12,20] So far, only few data with PAHs in field-aged samples have been reported. Using three dissimilar spiked soils, Swindell and Reid[21] observed that cyclodextrin and BuOH showed different efficiencies especially for soils with high clay and organic contents.

The application of surfactant extraction for the prediction of chemical lability was first proposed by Volkering et al.[22] who showed that mineral oil bioavailability could be successfully predicted by an extraction with a non-ionic surfactant (Triton X-100). Triton was also used to extract PAHs from two contaminated sediments with high organic matter content (9.7 to 13.3%) and results compared with hydroxypropyl-β-cyclodextrin (HPCD) extracts.[14] Authors observed that Triton X-100 extracted more high-molecular-weight PAHs than cyclodextrin and tended to overestimate the bioavailability of PAHs. Among other surfactants that can be used in a solid/liquid extraction process, our attention was attracted by poly(oxyethylene)(100)stearyl ether (Brij700), a water soluble non-ionic high-molecular-weight surfactant, which forms micelles above its critical micelle concentration (CMC). PAHs and other hydrophobic molecules can be incorporated into these micelles, which lead to an increase of their aqueous solubility. In addition, a long poly(oxyethylene) chain (n=100) might show some similarities with biosurfactants produced by bacteria and currently found in oil contaminated soils.[23,24]

Most comparisons between extraction techniques mentioned above have been conducted with spiked soils while paying attention to aging effects by storing treated samples for weeks and months before running extraction and bioavailability experiments.[10,21] Little attention has been paid to freshwater and marine sediments with low levels of PAHs or to sediments heavily loaded with high temperature combustion residues.

In an attempt to compare the chemical lability of low and high-molecular-weight PAHs more or less sequestrated in field sediments, samples from low contaminated St.

Lawrence Estuary and from two highly contaminated industrial sites, were extracted with three non-exhaustive extraction methods using: BuOH alone,[10] an aqueous solution of HPCD[12,14] and a surfactant solution using high-molecular-weight Brij700 (B700). Sediments were not spiked with labelled PAHs to avoid possible bias introduced by the aging process recreated in the laboratory. Total extractable PAHs were determined by dichloromethane (DCM) extraction. For comparison purposes, the same sediment samples were used for bioaccumulation assessment using polychaete *Nereis virens* for marine samples and oligochaete *Lumbriculus variegatus* for freshwater samples.

MATERIALS AND METHODS

Chemicals

Calibration solutions were prepared from PAH standards (method standards for wastewater, 16 compounds 0.1 mg mL^{-1}) (Chromatographic Specialties Inc., Brockville, Canada). B700, HPCD and BuOH (99.4+%) were purchased from Sigma–Aldrich (Oakville, Canada). Acetonitrile (HPLC grade) and DCM for liquid chromatography analysis were purchased from VWR Ltd (Mississauga, Canada). The main physical and chemical properties of extractants are given in Table 1.

Table 1. Some chemical and physical properties of the extractants

	Formula	MW ($g\ mol^{-1}$)	Molecular Volume ($Å^3$)	Boiling Point (°C)
DCM	CH_2Cl_2	84.93	92	39.75
BuOH	C_4H_9OH	74.12	152	117.7
HPCD	$C_{105}H_{196}O_{56}$	1460	262	Decompose
B700 (HLB[B] = 18.8)	$C_{18}H_{37}(OCH_2CH_2)_nOH$ (n~100)	4670	Variable[A]	Decompose

[A] Surfactants aggregate into supermolecular structure in water solution forming large size micelles

[B] Hydrophilic-Lipophilic Balance

Sediment samples

Low contaminated marine sediments (total PAHs < 2μg g^{-1}) were collected in the St. Lawrence Estuary and Saguenay Fjord (Eastern Canada) in July 2004 with a Van Veen grab. Marine sediments were obtained at low tide in the Kitimat Fjord (Western Canada) in September 2003 in the vicinity of an operating aluminum smelter. Freshwater sediments were collected in the St. Louis River (SLR) (Quebec, Canada) (one low contaminated and two highly contaminated sites) in October 2003 with a hand corer also in the vicinity of an operating aluminum plant. In all cases, whole sediment samples were stored in clean and tight buckets and frozen at –20°C until analysis. A small portion (100 g) of each sample was freeze-dried for 48 h, finely ground and stored in glass desiccators. Samples were classified following their low or high content in total PAHs (ΣPAHs) and their main geochemical properties are detailed in Table 2. The organic carbon content (C_{org}) was determined by a carbon analyzer (Costech, Elemental Combustion System, CHNS-O, ECS 4010) after acidification of the sample with HCl (10 %) and digestion at 80°C for 15 h to eliminate carbonates.

Table 2. Geochemical properties of the sediment samples

Samples	% Humidity	% Clay	% Silt	% Sand	% C_{org}	Σ12PAHs ($\mu g\ g^{-1}$ w.w.)
Minette Bay	22.4	0	0	100	0.04	0.06
EGSL04-01	56.9	3.4	51.0	45.6	1.2	0.07
SLR Upstream	45.3	12.0	87.3	0.7	5.1	0.3
Hospital Beach	18.0	0	0	100	0.08	0.3
EGSL04-07	54.1	4.3	73.6	22.1	1.1	0.4
EGSL04-13	40.3	5.8	73.5	20.7	0.7	1.1
SLR Downstream	38.0	23.4	76.5	0.1	2.5	25.5
SLR Outflow	36.6	20.9	77.8	1.3	2.0	279
Scow Grid	53.8	1.9	48.1	50.0	2.41	811
Scrubber	44.5	6.5	93.5	0	41	4442
B Lagoon	53.8	5.2	89.9	4.9	29.9	5723

DCM extraction

Each dry and powdered sediment sample (1.0 g) was extracted with 10 mL of DCM by mechanical shaking for 16 h into a 35-mL Teflon tube. A surrogate solution (50 μL) of anthracene_d_{10} and fluoranthene_d_{10} at 0.4 μg mL^{-1} was added before shaking to determine the recovery yield. The tube was centrifuged for 20 min at 3000 rpm and the supernatant transferred to a conical flask. The sample was concentrated under a nitrogen flow to 0.3–05 mL, diluted in pentane (2 mL), and concentrated again to 1.0 mL. The sample was eluted through a clean-up minicolumn (Supelclean ENVI – 18 SPE 3 mL, Supelco) with 5 mL of pentane:DCM (90:10). The sample was concentrated in acetonitrile to 1.0 mL in an ice bath to minimise loss of lighter PAHs.[25] Samples were then stored in a freezer at −6°C until analysis. For comparison purposes, results were converted into wet weight (w.w.) concentrations using the known percentage humidity of each sample.

BuOH extraction

Extractions were carried out in 35-mL Teflon tubes filled with 1.0 g of wet sediment, 10 mL of BuOH and 50 μL of a surrogate solution of anthracene_d_{10} and fluoranthene_d_{10} at 0.4 μg mL^{-1}. The tubes were sealed and shaken overnight (16 h) at ambient temperature. The tubes were then centrifuged for 20 min at 3000 rpm and the supernatant transferred to a conical flask. The sample was concentrated under a nitrogen flow to 1.0 mL in an ice bath to minimise loss of lighter PAHs.[15] The samples were then stored in a freezer at -6°C until analysis.

HPCD extraction

Extractions were carried out in 35-mL Teflon tubes filled with 1.25 g of wet sediment, 25mL of HPCD (50 mM) aqueous solution and 50 μL of a surrogate solution of anthracene_d_{10} and fluoranthene_d_{10} at 0.4 μg mL^{-1}. The tubes were sealed and shaken overnight (16 h) at room temperature. The tubes were then centrifuged for 20 min at 3000 rpm. A 0.5 mL aliquot of the supernatant was then withdrawn and diluted with methanol:water (50:50) solution in a 10-mL volumetric flask. The role of the methanol was to decompose cyclodextrin complexes before analysis.[12,26] The samples were then stored in a freezer at −6°C until analysis.

Surfactant extraction

Surfactant solutions were prepared using deionised water to reach a concentration of 10 g L^{-1}. Triplicates of analysed wet sediment (1.0 g) were weighted accurately into 35-mL Teflon tubes, a surfactant aqueous solution (10 mL) and 50 μL of a surrogate solution of anthracene_d_{10} and fluoranthene_d_{10} at 0.4 μg mL^{-1} were then added to each tube. The tubes were sealed and placed on a mechanical shaker for 16 h at ambient temperature. The tubes were then centrifuged at 3000 rpm for 20 min. Each supernatant solution was transferred into a separatory funnel, and 10 mL of DCM was added to transfer the extracted PAHs into the organic phase. Water-soluble surfactants were not quantitatively extracted by DCM and did not interfere with subsequent liquid chromatography analysis. The organic phase was removed and the extraction with DCM was repeated once more. Organic

fractions were combined in a glass tube and the sample analysed as described above for the DCM extraction.[27] The samples were then stored in a freezer at −6°C until analysis.

Optimization of B700 extraction

Preliminary work with surfactants indicated that their efficiency to extract PAHs from sediments varied following their molecular structure and HLB (hydrophobic–lipophobic balance).[27] Selection criteria for the choice of the best performing surfactant were based on their efficiency to extract PAHs (results not shown) and the best reproducibility of the extraction method. High-molecular-weight B700 turns out to be the most efficient surfactant tested and the reproducibility of successive extractions of the same sample reached 99.9 ± 1.5 % (Table 3).

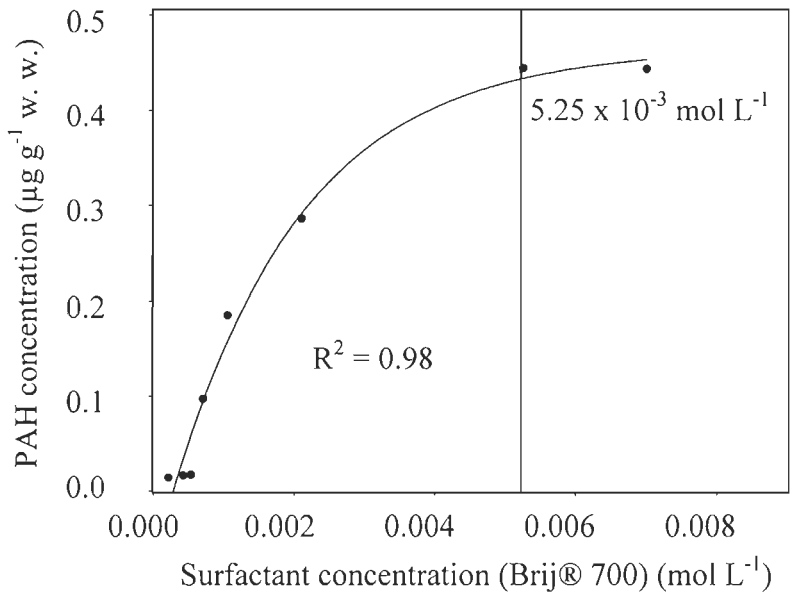
The relationship between B700 concentrations in aqueous solution and the extraction efficiency (Fig. 1) shows that the concentration of total PAHs extracted from contaminated sediment increased with the increase of the surfactant concentration up to an optimum value of $\sim 5.25\times10^{-3}$M and then reached a plateau. This phenomenon is known for surfactant extraction and corresponds to the CMC,[28] the concentration above which micelle formation becomes appreciable with a maximum hydrophobic effect and a maximum retention capacity of neutral lipophilic molecules.[29,30] Based on these results, a surfactant concentration that corresponded to 5.25×10^{-3} mol L^{-1} was chosen for the B700 extraction.

Table 3. Reproducibility of the B700 extraction repeated three times on the same marine sediment

PAHs concentration ($\mu g\ g^{-1}$ w. w.)				Mean ± SD
Fluorene	N/D[A]	N/D[A]	N/D[A]	N/D[A]
Phenanthrene	0.047	0.051	0.052	0.050 ± 0.003
Anthracene	N/D[A]	N/D[A]	N/D[A]	N/D[A]
Fluoranthene	0.216	0.218	0.213	0.216 ± 0.003
Pyrene	0.333	0.334	0.329	0.332 ± 0.003
Bz(a)Anthracene	0.049	0.046	0.042	0.046 ± 0.004
Chrysene	0.082	0.082	0.079	0.081 ± 0.002
Bz(b)Fluoranthene	0.025	0.022	0.021	0.023 ± 0.002
Bz(k)Fluoranthene	0.021	0.022	0.018	0.020 ± 0.002
Benzo(a)Pyrene	0.015	0.014	0.013	0.014 ± 0.001
Dibz(a,h)Anthracene	0.001	0.001	0.001	0.001 ± 0.000
Bz(g,h,i)Perylene	0.019	0.018	0.019	0.019 ± 0.001
Σ PAHs	0.808	0.808	0.787	0.801 ± 0.012

[A] Below detection limit

Fig. 1. Extraction efficiency of PAHs from marine sediment with a molarity gradient of commercial surfactant B700. The optimum concentration has been determined at the beginning of the plateau.



Bioaccumulation studies

Polychaetes *Nereis virens* (3 to 5 g w.w.) were collected at low tide in Parc National du Bic, near Rimouski along the St. Lawrence Estuary (Canada). Organisms were gently screened from the sediment, transported to the laboratory, and acclimated in a 15–20-cm layer of their native sediment overlaid with flow through marine water for 10 days. The worms were fed three times during these 10 days with commercial goldfish food. To estimate PAH bioavailability in marine sediments, polychaetes were placed in one-litre beakers (one polychaete per beaker) that contained 300 mL of sediment to be tested and 700 mL of seawater. Beakers were placed in an aquarium with flow-through seawater and constant aeration. At each sampling time, several worms were removed from sediment, rinsed in deionised water and allowed to purge their gut contents by standing for 8 h in circulating seawater.[31]

Freshwater oligochaetes *Lumbriculus variegatus*, ~2 cm in length, were obtained from Aquatic Research Organisms (Hampton, NH). As soon as the worms were received, they were added to the St. Louis River sediments to start the experiment without a depuration period because these worms originated from a rearing farm and were not previously exposed to contaminated soil. Exposures of worms were conducted in 500-mL glass beakers that contained 200 mL of sediment to be tested and 300mL of dechlorinated tap water in a flow-through aquarium with aeration. The number of organisms per beaker was 60, which provided a total mass of 0.4 to 0.5 g w.w. For each sampling, animals were gently sieved from the sediment, rinsed in deionised water and allowed to purge their gut for 6 h in clean dechlorinated tap water.[32] Polychaetes and oligochaetes were not fed

during the experiment. After the gut purging period, worms were put in scintillation vials, freeze-dried at −20°C for 58 h, and dry residues crushed in a fine powder for chemical extraction and analysis.

Worms (polychaetes and oligochaetes) were sampled seven times (at 0, 1, 3, 5, 7, 14 and 28 days) in triplicate (3 beakers). A total exposure period of 28 days was considered as a sufficient time to ensure a steady state tissue concentration in both polychaetes and oligochaetes.[33,34] As shown for four different samples (Table 4), the steady state plateau was reached after 3 to 14 days. General conditions of the exposure system (pH, water temperature, salinity for marine samples, dissolved oxygen and conductivity) were monitored at each sampling day and found constant for the exposure period. Only results at 28 days are reported here.

Table 4. Examples of kinetic data for bioaccumulation of total PAHs ($\mu g\ g^{-1}$)

mean ± s.d., n = 3

Time (day)	Bioaccumulation of total PAHs ($\mu g\ g^{-1}$)			
	Minette Bay	SLR Upstream	Scrubber	B Lagoon
0	0.07 ± 0.002	0.39 ± 0.01	2.11 ± 1.01	0.83 ± 0.03
1	1.19 ± 0.01	0.52 ± 0.02	104.7 ± 28.8	7.3 ± 0.3
3	1.30 ± 0.01	0.92 ± 0.04	153.5 ± 20.3	15.9 ± 0.3
5	1.32 ± 0.02	1.19 ± 0.01	163.7 ± 10.9	20.8 ± 1.1
7	1.32 ± 0.01	1.22 ± 0.01	167.1 ± 11.6	21.5 ± 0.9
14	1.32 ± 0.01	1.24 ± 0.01	167.3 ± 9.4	21.5 ± 1.4
28	1.33 ± 0.01	1.24 ± 0.02	168.7 ± 12.2	22.4 ± 0.09

PAHs in biological tissues

Surrogate solution (50 μL of anthracene_d_{10} and fluoranthene_d_{10} at 0.4 μg mL^{-1}) was added to 200 mg of dried and ground worm tissue in a 35-mL Teflon tube and stored at 4°C for 16 h. Hexane:acetone (50:50, 5 mL) was then added to the mixture. The tube was placed into an ultrasonic bath for 30 min. The tube was then mechanically shaken for 3 h and then returned again to the ultrasonic bath for 30 min. The tube was centrifuged for 15 min at 3000 rpm and the supernatant transferred to a conical flask. The extract was concentrated under a nitrogen flow at ambient temperature to a volume of 0.5 mL. The extract was eluted through a clean-up mini-column (Supelclean ENVI – 18 SPE 3 mL, Supelco) with 5 mL of hexane:acetone (90:10). The resulting clear solution was concentrated to near dryness under nitrogen flow and re-dissolved in ~1mL of acetonitrile and evaporated again to 200 μL in an ice bath to minimise loss of lighter PAHs. Analyses were conducted on triplicate samples for each organism and sampling time.

PAHs analysis

All PAH analyses were carried out by liquid chromatography (LC) with fluorescence detection. The apparatus consisted of a Rheodyne injector with a 20-μL injection loop, a Shimadzu LC-10AD pump, a Supelcosil LC-PAH column (25 cm×3 mm), and a Spectra System FL3000 fluorescence detector. All analyses were done at a constant flow rate of 0.8 mL min^{-1} with a mixture of nanopure water and acetonitrile as a mobile phase. The pump program began at 75% acetonitrile and increased to 95% in 10 min and then to 100% in the next 10 min, with a final plateau of 10 min. The cycle returned to 75% acetonitrile after a

total run time of 35 min. The excitation wavelength of the fluorescence detector was settled at 280 nm and the emission was detected at 340 nm during the first 5.5 min for lighter PAHs and then shifted to 410 nm until the end of the program for heavier PAHs. Quantification was based on peak areas using five calibration solutions (0.002–005 μgmL^{-1}). For quality control, a 0.5 μgL^{-1} PAHs Mix Standard Solution (Supelco) was analysed every 10 samples. Fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo(a,h)anthracene (DBA) and benzo(g,h,i)perylene (BPE) were quantified. Five successive injections of the same extract provided a variability of <10% for each identified and quantified peak.

Two deuterated PAHs (anthracene_d_{10} and fluoranthene_d_{10} at 0.4 $\mu g\ mL^{-1}$) were used as internal standards and were spiked in sediments and worm tissues before extraction.

Recovery of the internal standards ranged from 76.3 ± 9.5 % (mean ± standard deviation) to 98.7 ± 2.5 % (extractions of worms), from 88.9 ± 2.1 % to 91.2 ± 2.1 % (extraction by DCM), from 89.6 ± 6.9 % to 90.5 ± 3.1 % (extraction by BuOH), from 85.8 ± 2.0 % to 94.9 ± 6.4 % (extraction by B700), and from 88.9 ± 2.8 % to 94.3 ± 4.3 % (extraction by HPCD).

Data treatment

The results obtained by mild solid/liquid extractions and from worms allocated the calculation of the extraction efficiency (% XTRAC) for each sediment. % XTRAC is defined as the following ratio:

$$\%XTRAC = \frac{Q_{worms}}{Q_{DCM}} \times 100$$

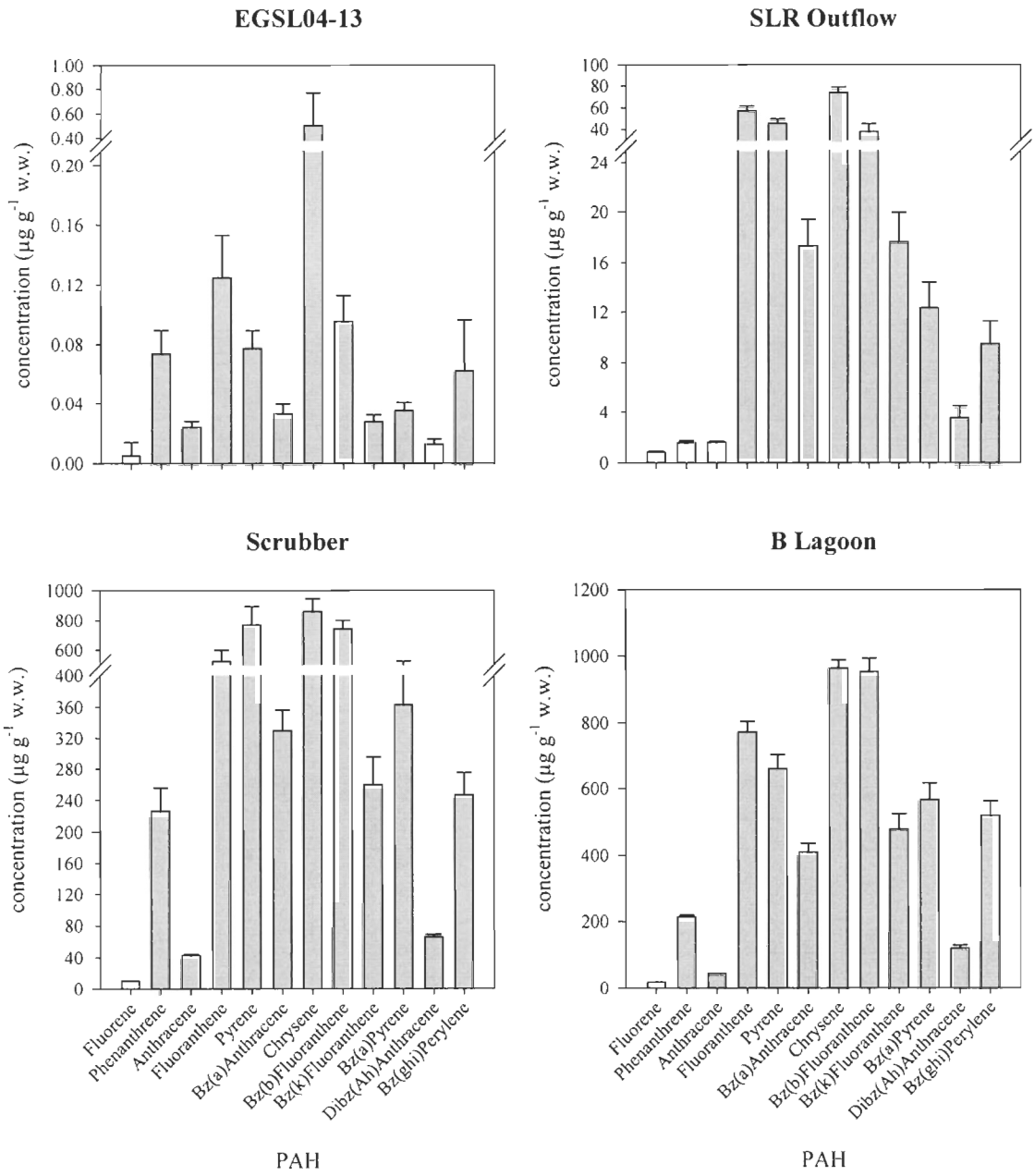
where Q_{worms} (μg g^{-1} w.w.) and Q_{DCM} (μg g^{-1} w.w.) stand for quantities extracted from worms and by DCM, respectively.

RESULTS

St. Lawrence Estuary (EGSL04-01 and -07) and Saguenay Fjord (EGSL04-13) sediments show quite similar grain-size distribution and percentage organic carbon (Table 2). Total PAHs in these deep-water marine sediments were all below 2 μg g^{-1} (w.w.) with dominance of 3–4-ring aromatics such as phenanthrene, fluoranthene, pyrene, chrysene and benzo(b)fluoranthene (Fig. 2). Particulate organic matter present in these coastal sediments is mainly derived from marine productivity and debris from terrestrial vascular plants.[35–37] Freshwater sediments from the St. Louis River (SLR samples) are also dominated by silt with a relatively high proportion of clay. Sample SLR Outflow was collected near an industrial outflow pipe from a nearby aluminum smelter and shows a relatively high level of PAHs dominated by 4–5 rings such as pyrene, chrysene, bz(b)fluoranthene, bz(k)fluoranthene and bz(a)pyrene (Fig. 2). Marine samples from Hospital Beach and Minette Bay are clean sandy sediments taken at low tide and show very low PAH levels and carbon content (Table 2). Two other samples were taken in the vicinity of an aluminum plant (B Lagoon and Scow Grid) and contained high levels of aluminum smelter residues mixed with natural muddy sediment and organic matter from a vegetated drainage basin.

Finally, one more sample was taken from the aluminum scrubber room after neutralisation. The PAH pattern of these last three industrial samples exhibits high levels of all analysed PAHs (Fig. 2).

Fig. 2. Distribution pattern of PAHs in four typical samples used to compare extraction methods and bioaccumulation using worms.



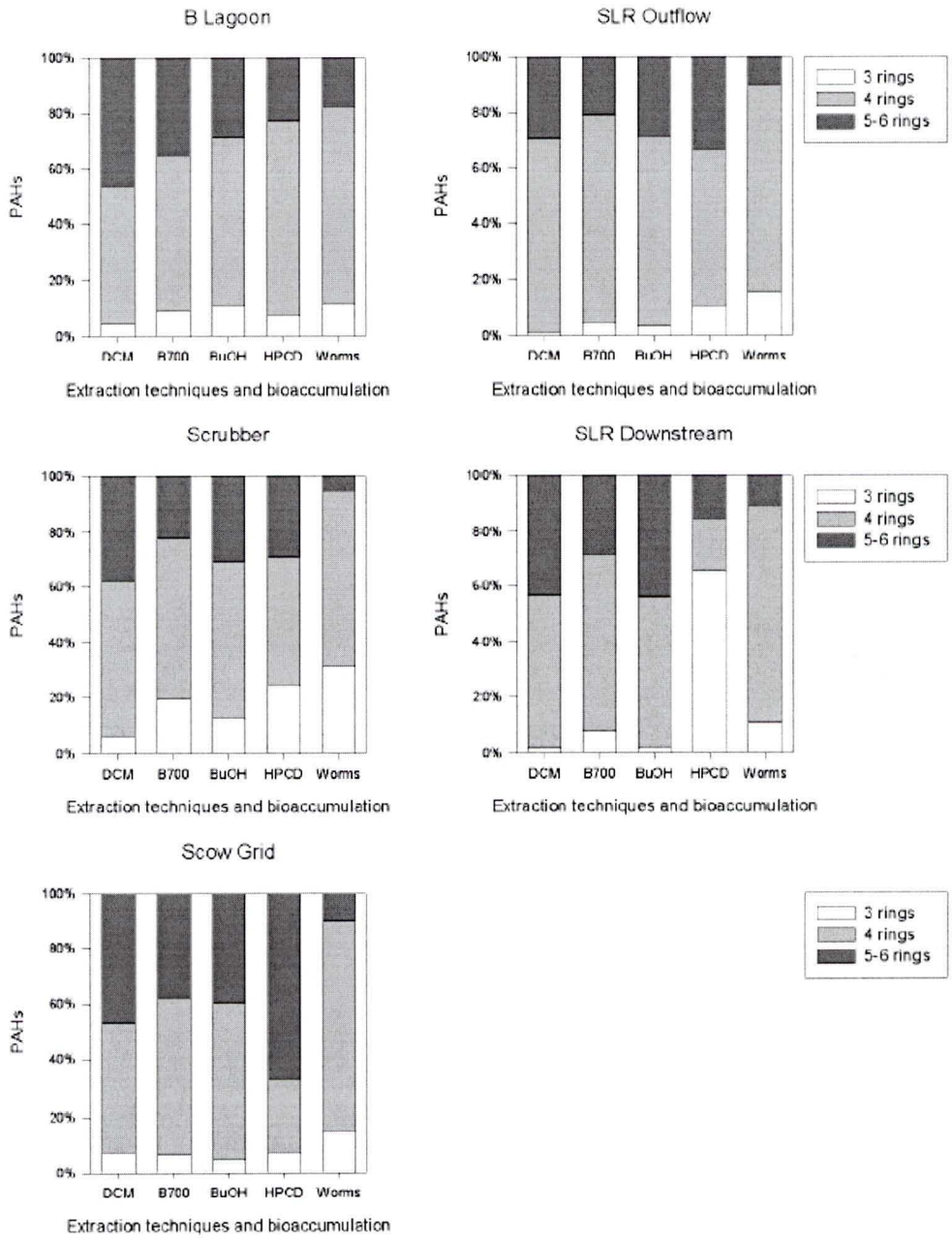
Results of extraction and bioaccumulation experiments are given in Table 5. % XTRAC of total PAHs is the yield of the chemical extraction or the worm bioaccumulation when compared with the DCM extraction, which is considered the method of extracting all PAHs present in sediment samples. In some low contaminated samples, it was often possible to extract more PAHs with BuOH and HPCD than with DCM. Similarly, worms bioaccumulated more PAHs than extracted with DCM. Only surfactant B700 was less efficient for extracting available PAHs in these samples. The efficiency of extractants was strongly reduced in highly contaminated sediments although BuOH remained quite strong with a yield that ranged from 27 to 58%. However, when comparing extractant performance with PAHs bioaccumulated in worms, it becomes clear that HPCD and B700 extracts provided a much more realistic level of PAHs than BuOH, which grossly overestimated the amount of total PAHs available to the worms in all cases.

Table 5. Results of extractions and bioaccumulation experiments with %XTRAC given in parentheses. Extractant values followed by a star are significantly different from the corresponding worm bioaccumulation value. This is an indication of the failure of the extractant to match the value of total PAHs observed in worms (P>0.01) (mean ± std. dev., n = 3)

Sediments	DCM extraction ($\mu g\ g^{-1}$ w.w.)	BuOH extraction ($\mu g\ g^{-1}$ w.w.)	HPCD extraction ($\mu g\ g^{-1}$ w.w.)	B700 extraction ($\mu g\ g^{-1}$ w.w.)	Bioaccumulation (28 days) ($\mu g\ g^{-1}$ w.w.)
Minette Bay	0.06 ± 0.02*	2.5 ± 0.5* (>100%)	0.4* (>100%)	0.02 ± 0.001* (33%)	1.3 ± 0.1 (>100%)
EGSL04-01	0.07 ± 0.03*	0.06 ± 0.01* (80%)	0.3* (>100%)	0.007 ± 0.004* (10%)	0.8 ± 0.3 (>100%)
SLR Upstream	0.3 ± 0.1*	1.2 ± 0.4 (>100%)	1.4 (>100%)	0.09 ± 0.007* (26%)	1.3 ± 0.06 (>100%)
Hospital Beach	0.3 ± 0.05*	0.07 ± 0.004* (23%)	1.0 (>100%)	0.03 ± 0.009* (10%)	1.0 ± 0.2 (>100%)
EGSL04-07	0.4 ± 0.3*	0.2 ± 0.03* (50%)	0.4* (>100%)	0.011 ± 0.002* (3%)	0.6 ± 0.1 (>100%)
EGSL04-13	1.1 ± 0.2*	0.5 ± 0.2 (45%)	0.3* (27%)	0.04 ± 0.01* (4%)	0.9 ± 0.1 (86%)
SLR Downstream	25.5 ± 2.5*	11.9 ± 0.5* (46%)	0.2* (0.9%)	0.7 ± 0.2 (2.7%)	0.8 ± 0.04 (3.3%)
SLR Outflow	279 ± 28*	163 ± 13* (58%)	4.7* (1.7%)	8.9 ± 4.1 (3.2%)	8.9 ± 0.2 (3.2%)
Scow Grid	811 ± 154*	186 ± 36* (23%)	2.5* (0.3%)	1.6 ± 0.3 (0.2%)	1.5 ± 0.2 (0.2%)
Scrubber	4442 ± 538*	1653 ± 105 (36%)	26.9* (0.6%)	86 ± 8 (1.9%)	86 ± 9 (1.9%)
B Lagoon	5723 ± 190*	1536 ± 107* (27%)	5.4* (0.1%)	30.7 ± 4.5 (0.5%)	21.3 ± 9.4 (0.4%)

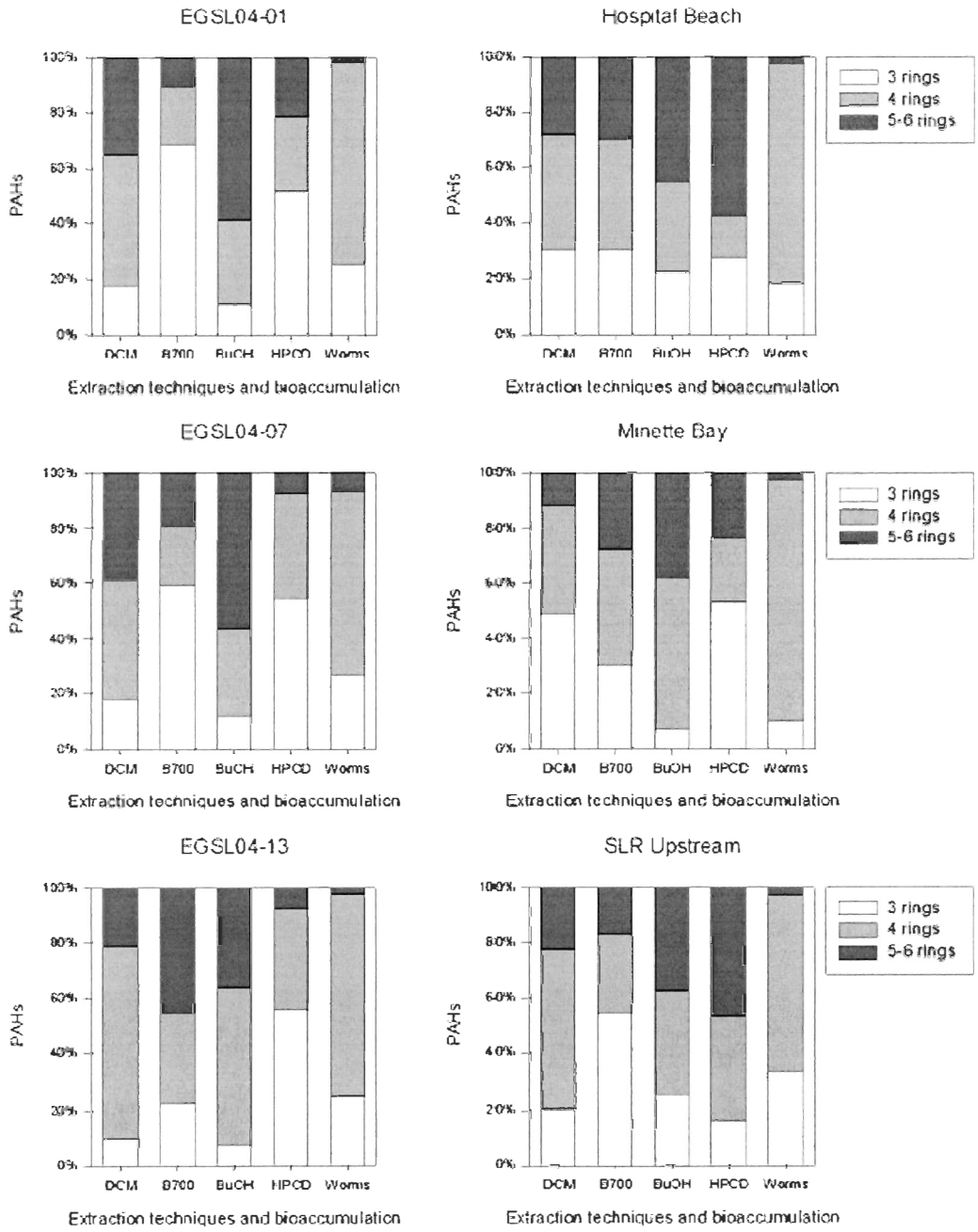
First, we examined the relative efficiency of chemical extractants to remove PAHs from sediment following their molecular size, by classifying PAHs by their number of rings. For samples highly contaminated by aluminum smelter wastes it is remarkable to see how BuOH and B700 work similarly to DCM for all samples whereas the HPCD solution seems to behave differently at least in the case of SLR Downstream and possibly with Scow Grid samples (Fig. 3). Assuming DCM extracts nearly all the PAHs present in these sediments, it becomes clear that non-exhaustive extractions with BuOH and B700 succeed to mimic, in most cases, the exhaustive extraction when considering only a relative proportion of ring numbers. The right column in each panel (Fig. 3) gives the relative proportion of 3-, 4-, and 5- to 6-ring PAHs found in worms after 28 days. In most cases the proportion of 4-ring PAHs is enhanced when compared with DCM and mild extractants, and the proportion of 5- to 6-ring PAHs is reduced. Again the HPCD solution does not perform well in the SLR Downstream and Scow Grid samples whereas B700 does very well except with ScowGrid. An early interpretation of these results would lead to the conclusion that mild extractants provide a good means to predict bioaccumulation of PAHs by their molecular size in worms, but the extraction of low contaminated sediments tells us another story.

Fig. 3. Comparison of extraction yields and worm bioaccumulation of PAH groups in highly contaminated sediments. Amounts of PAHs are normalized to the sum of PAHs extracted with each method.



We first observed that DCM extracted fairly constant proportions of 3, 4, 5 to 6 rings in similar samples (EGSL04-01, -07, and -13) and even in dissimilar samples from freshwater (SLR Upstream) and west coast samples (Hospital Beach and Minette Bay) where 3 rings account for from 20 to 40% and 5–6 rings for from 10 to 30%. HPCD and B700 solutions provided inconsistent results when compared with DCM, whereas BuOH was often quite close to DCM results except for Minette Bay (Fig. 4). Worms exposed to low contaminated sediments showed a distribution pattern of bioaccumulated PAHs quite the same in all the studied stations (4 rings>3 rings>5–6 rings with proportions of 63–87%, 11–34% and 2–7%, respectively). Very low amounts of 5–6 rings were accumulated in worms although a large proportion of these heavy PAHs were consistently extracted by solvents and extraction solutions. Neither DCM nor mild extractants correctly predicted the proportion of 4-ring PAHs biaccumulated in both freshwater and seawater worms. All results showed a similar behaviour for both worm species and were pooled together for further discussion.

Fig. 4. Comparison of extraction yields and worm bioaccumulation of PAH groups in low contaminated sediments. Amounts of PAHs are normalized to the sum of PAHs extracted with each method.



We also examined the relationship between individual PAHs bioaccumulated in worms (both species) at a steady state and their bioavailablility as predicted by each extractant for both groups of samples pooled together. Fig. 5 and Table 6 illustrate the relationship between extracted and bioaccumulated low molecular-weight (LMW: fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, and chrysene) and high-molecular-weight (HMW: benzo(b,k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i) perylene) PAHs.

PAH lability as determined by BuOH (Fig. 5a) was not in good agreement with worms data for both LMW and HMW PAH groups with high slopes and low correlation coefficients (Table 6). In the opposite direction, PAH lability as determined by HPCD (Fig. 5b) was lower, particularly with LMW compounds, than bioavailability for worms although discrepancy between both datasets is much lower than with BuOH. Finally, PAH lability, as determined by B700 (Fig. 5c) was found in good agreement with worms data particularly for LMW PAHs with a slope of 1.0 and R^2=0.83. Data points for these compounds are closely distributed around the dotted line, which represents a 1:1 relationship. Predicted PAH bioavailability was not in as good agreement for HMW PAHs (R^2=0.74) (Table 6). When LMW and HMW PAH data are included in linear regression analysis, the B700 method gave the best estimate of PAH bioavailability. Using zero-intercept fitting, linear regression resulted in a slope of 1.01 and R^2=0.82 for B700 compared with a too low slope (0.23) for HPCD and a far too high slope (18.46) for BuOH with poor R^2 in both cases.

Fig. 5. Low (LMW) and high (HMW) molecular weight PAHs extracted by BuOH (a), HPCD (b) and B700 (c) as a function of bioaccumulated PAHs in worms over 28 days. The dotted lines represent a hypothetical 1:1 correlation.

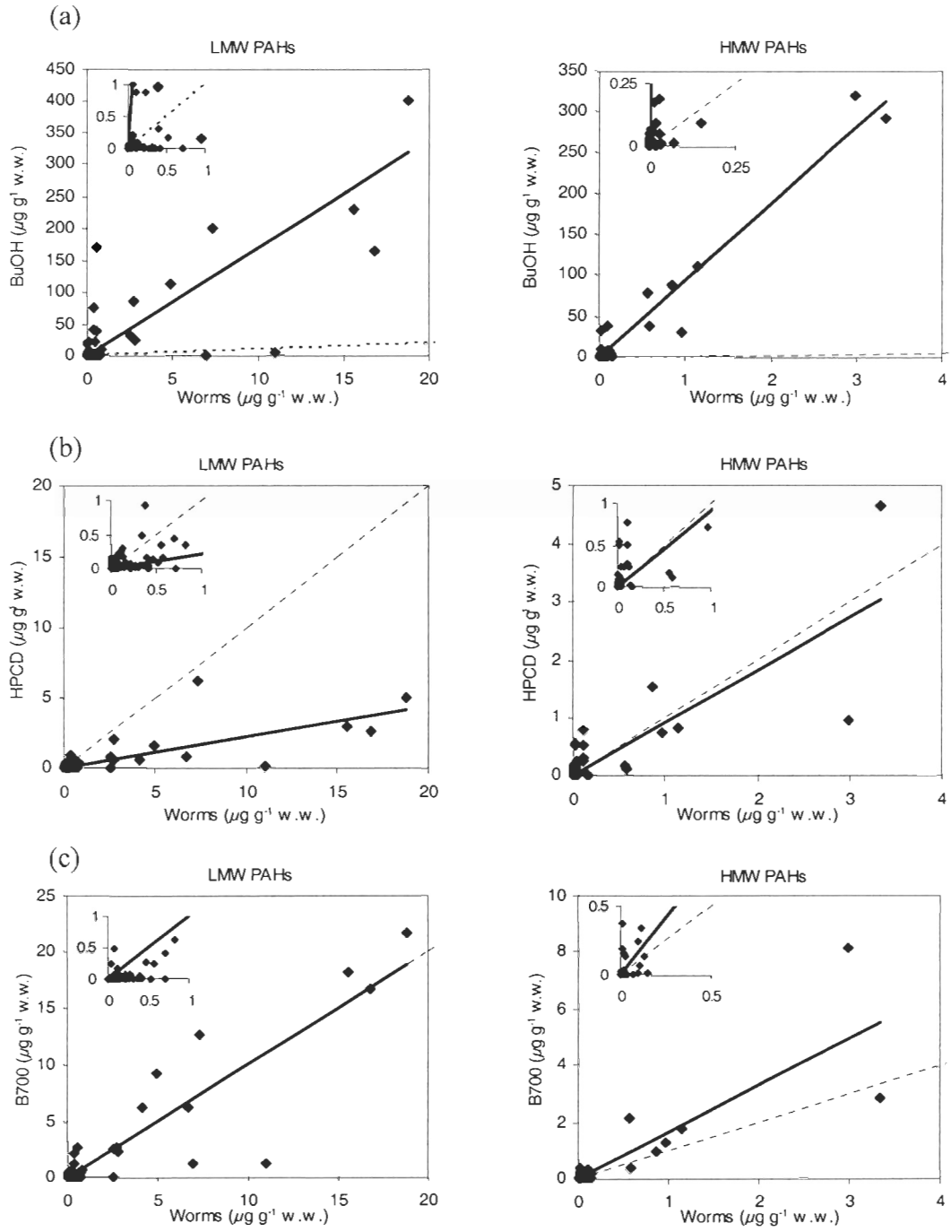


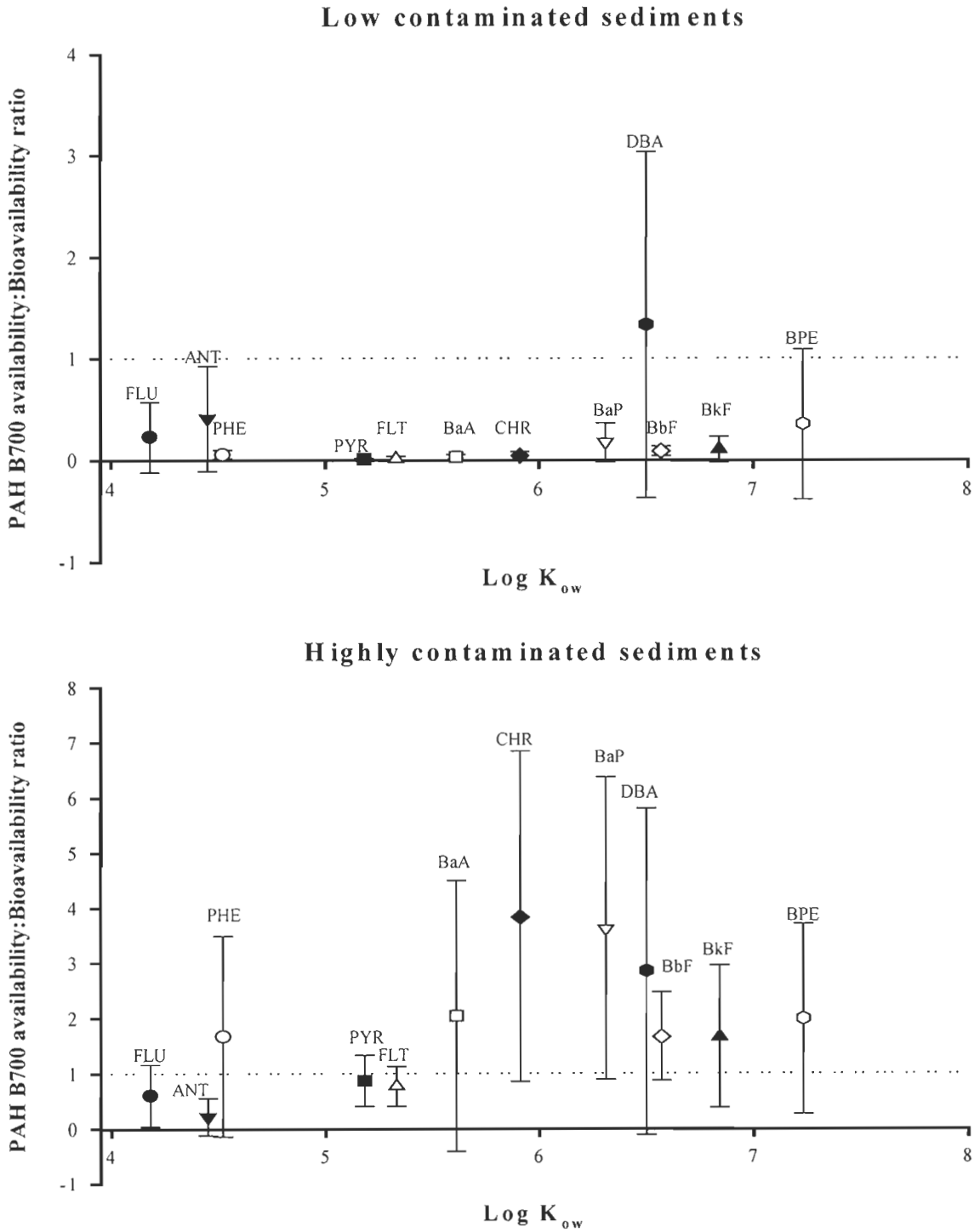
Table 6. Linear correlation parameters obtained when comparing LMW and HMW PAHs extracted with BUOH, HPCD and B700 with PAHs bioaccumulated in worms for both low and high contaminated sediments

The first line for each PAH group gives results for the intercept forced to zero and the second is for free intercept.

	BuOH extraction			HPCD extraction			B700 extraction		
PAHs	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2
LMW	16.98	0.0	0.48	0.22	0.0	0.57	1.00	0.0	0.83
	16.16	8.98	0.49	0.21	0.09	0.58	1.01	-0.06	0.83
HMW	93.71	0.0	0.95	0.91	0.0	0.66	1.65	0.0	0.74
	93.92	-0.45	0.95	0.87	0.07	0.67	1.65	0.006	0.74
All PAHs	18.46	0.0	0.42	0.23	0.0	0.48	1.01	0.0	0.82
	17.28	11.90	0.44	0.22	0.14	0.50	1.01	0.02	0.82

To gain a better understanding on the capacity of the B700 method to predict individual PAH bioaccumulation in worms, the ratio of PAH concentrations determined by B700 extraction to PAH concentrations found in worms were plotted against the PAH octanol–water partitioning coefficient (K_{ow}) (Fig. 6). A ratio approaching 1 indicates the ability to predict PAH bioavailability based on B700 extraction, whereas a ratio <1 indicates a low chemical lability, and a ratio >1 indicates an over-predicted PAH bioavailability. Low contaminated sediments show too low ratios even for most high K_{ow} PAHs. However, highly contaminated sediments present a quite different picture as all high log K_{ow} (>5.8) PAHs exhibit a ratio>1 with high variability between replicates whereas B700 extraction is a good predictor of PAH bioavailability for compounds with log K_{ow} values <5.8 (Fig. 6).

Fig. 6. Relationship between PAH octanol-water partitioning coefficient (K_{ow}) and the ability to predict PAH bioavailability using B700 availability methodology.



DISCUSSION

In this study three mild extraction methods were used with marine and freshwater sediments in an attempt to estimate the bioavailability of PAHs for invertebrates in constant contact with sediments. Our results highlight the importance of considering both the level of PAHs in sediments and the molecular size of PAHs when attempting to predict bioaccumulation in a biological sampler like worms using a solid/liquid extraction method. A surfactant B700 solution was quite successful to predict the PAH content in worms when exposed to highly contaminated sediments and smelter residues, whereas BuOH extracted 10 to 50 times too much PAHs being a far too efficient solvent for absorbed PAHs. When low contaminated sediments are used, HPCD and BuOH were revealed to be better for estimating bioaccumulation in worms whereas B700 appeared to be a too mild extractant with a very low yield for most samples.

Extractable PAHs and sediment properties

The over 100% yield (% XTRAC) often observed with low contaminated sediments using BuOH and HPCD might be in part an artifact introduced by the extraction method using DCM. As DCM is water insoluble and does not work properly with wet sediments, samples have been freeze-dried before extraction whereas wet samples were used with mild extractants. The drying process may have modified the structure of the organic matter and reduced the availability of small amounts of PAHs to DCM extraction. These anomalous results might also simply reflect analytical variability because of inhomogeneity of some

sub-samples for the same station. Extractant B700 did not give a yield of over 100% with the lowest contaminated samples which possibly indicates its low ability to disaggregate natural organic particles (pellets of invertebrates) usually present in natural sediments. Even if Hospital Beach and Minette Bay have the same sand percentage (100%) and are both low contaminated sediments, their % XTRAC by B700 was quite different (10 and 33%, respectively). This difference might be related to the size of the sand particles and the nature of the organic matter. Indeed, soils or sediments that are dominated by bigger particles generally have a greater mineralisation of PAHs.[38] A closer examination of particle size analysis (not shown) confirmed that Hospital Beach had finer sand particles than the Minette Bay sample. BuOH also extracted much more PAHs from Minette Bay than from Hospital Bay although DCM found less PAHs in Minette Bay.

The % XTRAC for two of the three St. Lawrence stations by B700 are quite similar (3% and 4% for EGSL04-07 and EGSL04-13, respectively) even though the third one is higher (10%).These results imply a possible better sequestration for stations -07 and -13 than for EGSL04-01, which has a higher sand percentage (45.6% compared with 22.1 and 20.7%) with a comparable level of organic carbon content. BuOH extraction shows the same trend. Carmichael and Pfaender[38] showed that the silt and clay fractions of the soils they studied were significantly linked to the PAH mineralised percentage. A quick examination of Fig. 4 shows the disparity of results between chemical extractants when looking at the molecular size of PAHs, particularly for St. Lawrence Estuary samples. HPCD and B700 extracted relatively more 3- and 4-ring PAHs because of their better solubility in water than 5–6-ring PAHs, which were better extracted by DCM and BuOH,

both being organic solvents able to dissolve organic lipids and reach sequestrated PAHs. Worms did not extract 5–6-ring PAHs and simply retained 3- and 4-ring PAHs.

With highly contaminated sediments, BuOH extractions never exceeded the DCM extraction efficiency, although BuOH appeared very efficient when compared with HPCD and B700. Attempts to relate % XTRAC to the geochemical properties of highly contaminated sediments were unsuccessful. The presence of huge quantities of PAHs mainly from aluminum smelter residues overwhelms other physical or chemical factors in the extraction process. The frequent observation of non-linear sorption isotherms for organic compounds introduced into soil suggests a saturation of sorption sites.[39] Such a saturation would be reflected in a declining percentage of the chemical that is sequestered as the concentration increases as shown by Chung and Alexander.[40] When examining the pattern distribution of 3-, 4- and 5–6-ring PAHs for all chemical extractions in all heavily contaminated sediments, it turns out that all patterns are quite similar (except for unexplained HPCD in the SLR Downstream sample),which confirms a similar behaviour of these extractions where solubility of the adsorbed PAHs on inert particles becomes the main factor that controls the efficiency of the extraction. As already observed for low contaminated sediments, both worm species did not extract and bioaccumulate 5–6 rings although a very large proportion of these heavy PAHs were present in these samples (Fig. 2).

In a recent paper, our laboratory examined the sorption and desorption properties of some of the samples studied here using different chemical tools.[41] We observed that PAHs in a highly contaminated B Lagoon sample were much less available to a desorption

process that PAHs in freshly released scrubber residues, although both samples showed a quite similar distribution of extractable PAHs. This reduced availability was attributed to a weathering process that resulted in a stronger sequestration of PAHs in the 20-year-old lagoon sediment.[41] This finding is supported by present results (Table 5) where % XTRAC is higher with scrubber than B Lagoon samples for all chemical soft extractions and also with worms. Worms bioaccumulated a higher proportion of 3- and 4-ring PAHs in the scrubber than in the B Lagoon sample (Fig. 3), which is in agreement with the hypothesis that LMW PAHs in the scrubber were more available than in the lagoon sediment.

Nam and Alexander[42] suggested that the bioavailability of hydrophobic compounds (such as PAHs) can be extensively reduced by particles that bear nanopores with hydrophobic surfaces. Hydrophobic compounds may become sequestered and less available to living organisms if they penetrate such porous materials.[43,44] Mayer[45] proposed that organic matter in marine sediments is protected by its location inside pores too small to allow the entrance or function of hydrolytic enzymes. Indeed, pores with a diameter of less than 100 nm have been observed in a variety of dissimilar soils.[46,47] A molecule that is entrapped in a nanopore with a diameter smaller than 100 nm is probably unavailable to any living organism since the smallest bacteria have a larger diameter.[42] Similarly, large HPCD molecules and B700 micelles most probably cannot reach these nanopores, which reduce their capacity to capture small PAHs. On the other hand, DCM and BuOH are much smaller molecules (Table 1) and can more easily access the smallest pores, which means a better extraction capacity. The situation appears different for 5–6-ring PAHs with their large volume (229Å^3 for BaP) which prevents them from penetrating nanopores and leaves

only larger pores available for large HPCD and surfactant micelles to access. This might be the reason why HPCD succeeded to predict the availability of high-molecular-weight PAHs in worms (Fig. 5).

Chemically availability v. bioaccumulation

In this study soft extraction methods were used to estimate the bioavailable fraction of PAHs from unspiked and untreated sediments. Several researchers have suggested that mild BuOH extraction may be an appropriate means for predicting PAH bioavailability.[9,10,48] However, our results indicate that BuOH-extractable PAHs do not correlate with PAHs found in worms exposed to sediments for 28 days, as BuOH overestimated by up to 540 times the PAH availability in highly contaminated samples. In a recent study by Juhasz et al. using creosote contaminated soil,[49] BuOH extraction underestimated the 3-ring and overestimated the 4-, 5- and 6-ring PAHs compared with biodegradation. Reid et al. and Macleod and Semple,[13,50] using pyrene spiked soils, also found an overestimation of pyrene bioavailability by BuOH extraction compared with bacterial mineralisation. Conversely, Kelsey et al. and Liste and Alexander[9,10] found good relationships between PAH desorption using BuOH and PAH bioavailability (estimated by microbial degradation and earthworm uptake assays) in laboratory spiked soil that contained single PAH compounds. The above studies exemplify conflicting results present in the literature on bioavailability research using spiked soils and low molecular- weight primary alcohols. Spiking and aging soil or sediments under laboratory conditions for weeks or months may not be long enough to truly reflect field conditions. In addition, contaminant bioavailability may be influenced by the presence of other organic

contaminants; a situation not taken into account in single PAH spiked soil studies. As a result, spiked soil studies (determining contaminant availability and sequestration) often fail to mimic conditions found in field contaminated soils.[51]

In the present study, the assessment of PAH bioavailability using HPCD extraction resulted in a good prediction of total PAH bioavailability in low contaminated sediment samples, but relative proportions of 3-, 4-, and 5–6-ring PAHs did not reflect the proportions found in worms. HPCD underestimated total PAHs in most highly contaminated samples. HPCD was developed as an extractant for assessing contaminant bioavailability because HPCD has a high solubility, the prevalence of hydroxy functional groups on the exterior of the torus, and a hydrophobic organic cavity, makes it possible to form an inclusion complex with PAHs.[13] HPCD extraction was in good agreement with bioavailability as determined by mineralisation in phenanthrene spiked soil.[13] Conversely, Cuypers et al. and Juhasz et al.[14,49] used petroleum-contaminated harbour sediments and creosote-contaminated soil and found HPCD extraction predicted correctly 3- and 4-ring PAH biodegradability, whereas the biodegradability of 5- and 6-ring PAHs was overestimated.

This study presents a first attempt to assess PAH bioavailability with surfactant B700. When all PAH data are included in the same linear regression analysis, the B700 method gave the best estimate of PAH bioavailability (R^2=0.82 and slope=1.01) (Table 6). However, B700 is less efficient with low contaminated sediments, most probably because of its limited capacity to penetrate lipidic particles or tissues where PAHs have been accumulated by micro- and macro-invertebrates. In both high and low contaminated

sediments, worms showed a low proportion of 3-ring PAHs and a relatively high proportion of 4-ring PAHs. This result seems to be linked to the high solubility and biodegradability of 3 rings, particularly phenanthrene. As worms were sampled after a continuous exposure of 28 days, light PAHs accumulated at the beginning of the exposure have been partly metabolised and eliminated by worms whereas heavier 4-, 5- and 6-ring PAHs were much less biodegraded. The result is an apparent low bioaccumulation of lower PAHs and an apparent excess of higher PAHs when compared with PAHs in DCM extracts.

The contrast between low and highly contaminated samples is best illustrated in Fig. 6 where the ability of B700 to predict PAH bioavailablility is assessed following its affinity for lipidic fractions (log K_{ow}). Almost all PAHs exhibit a ratio <1 in low contaminated samples, which indicates that worms cannot only adsorb surficial PAHs but also digest marine organic mater and extract PAHs already bioaccumulated by living and dead organisms present in the sediment. In contrast, highly contaminated sediments showed an interesting two-plateau pattern already observed by Juhasz et al.[49] when examining predictor capability of HPCD for PAH availability in soil highly contaminated with creosote- treated in biopile for 16 weeks. The fact that both HPCD and B700 still overestimate the availability of PAHs with log K_{ow} >5.5 might be related to their molecular size, which is smaller than enzymes and allows their accessibility to small interstices where enzymes cannot enter.

CONCLUSION

Strong differences were observed in quantities and proportions of PAHs obtained using different techniques for assessing the bioavailability of PAHs in sediments. Although several researchers have suggested that mild BuOH extractions may be an appropriate mean for predicting PAH bioavailability, our results disagree with previous studies for both low and highly contaminated sediments where BuOH was found to extract much more PAHs than worms can bioaccumulate in their tissues, and where HPCD underestimates the bioavailability of PAHs especially for LMW PAHs. On the other hand, use of the surfactant B700 revealed a good prediction for PAHs in highly contaminated sediments. Laboratory studies conducted with freshly spiked or slurried sediment may overestimate the bioavailability of contaminants compared with field situations where longer equilibration times between sediment and persistent contaminants reduce the bioavailability.[52,53] Moreover, most of these studies determined the bioavailability by measuring microbial degradation in laboratory reactors, an approach that might not be comparable with PAHs bioaccumulated by worms as worms are not passive samplers and can modify the PAH profile in their tissues.

Since risk assessments from contaminated soils are currently being overestimated by chemical analyses that rely on an initial vigorous solvent extraction, and because remediation may be suitable when none or a less vigorous treatment is required, it is essential to include bioavailability assessment in regulatory decisions. Using worm uptake and biodegradation are still the best tools to estimate PAH bioavailability, but biological

methods are time consuming and expensive. Our results illustrate difficulties in finding an adequate chemical predictor of PAH bioavailabilty, particularly because PAH concentrations and the sequestration process play a determining role in the quality of results. Because B700 is not expensive and solutions easy to prepare, an extraction procedure involving this high-molecular-weight surfactant is revealed to be useful particularly with sediments that show PAH levels >2μg g^{-1} and log K_{ow} <5.8.

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CHAPITRE II

PASSIVE SAMPLERS VERSUS SURFACTANT EXTRACTION FOR THE EVALUATION OF PAH AVAILABILITY IN SEDIMENTS WITH VARIABLE LEVELS OF CONTAMINATION

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Résumé

L'objet de cette étude était de tester l'efficacité des échantillonneurs solides passifs, des bandes de polyoxyméthylène (POM) et des tubes de silicone polydiméthylsiloxane (PDMS), à prédire le biodisponibilité des HAP présents dans les sédiments contaminés. Les résultats ont été comparés aux données de bioaccumulation et d'une extraction solide/liquide utilisant le tensioactif Brij® 700 (B700). Les deux échantillonneurs passifs ont été trouvés à agir différemment. Le PDMS surestime la disponibilité des HAP dans tous les sédiments étudiés. La méthode avec le POM produit des résultats en accord avec ceux obtenus avec l'extraction au B700. Quoi qu'il en soit, le POM et le B700 sousestiment la disponibilité des HAP dans les sédiments faiblement contaminés où les facteurs biologiques (matière organique digestible) deviennent importants. La biodisponibilité des HAP totaux a été prédite correctement par le POM et le B700 dans les sédiments contaminés par des alumineries. Un examen plus précis des résultats des HAP individuels indiquait que les deux techniques surestimaient la disponibilité des grosses molécules avec un log K_{ow} >6 suggérant un mécanisme biologique limitant l'incorporation des plus gros HAP ce qui semble être relié à la taille moléculaire des composés.

Mots clés: Échantillonneur passif, Extraction avec un tensioactif, Biodisponibilité, Bioaccumulation par des vers, HAP, Sédiment.

Abstract

The purpose of this study was to test the efficiency of passive solid samplers, polyoxymethylene (POM) strips and polydimethylsiloxane (PDMS) silicon tubing, to predict the bioavailability of native PAHs in contaminated sediments. Results were compared with worm bioaccumulation data and solid/liquid extraction using the surfactant Brij® 700 (B700). The two passive samplers were found to act differently. The PDMS sampler overestimated the availability of PAHs in all studied sediments. The POM method provided results in accordance with those obtained with the B700 extraction. However, POM and B700 methods underestimated PAH availability in low contaminated sediments where biological factors (digestible organic matter) become important. Bioavailability of total PAHs was correctly predicted by POM and B700 in highly contaminated aluminum smelter sediments. A closer examination of individual PAH results indicated that both techniques overestimated the availability of large molecules with log K_{ow} >6 suggesting a biological mechanism limiting uptake of larger PAHs which seems to be related to the molecular size of compounds.

Keywords: Passive sampler; Surfactant extraction; Bioavailability; Worm bioaccumulation; PAH; Sediment.

INTRODUCTION

Hydrophobic organic chemicals such as polycyclic aromatic hydrocarbons (PAHs) are common contaminants in industrial soils and marine sediments (Kennish, 1997). Risk assessment procedures of hydrophobic chemicals in soils and sediments are usually based on total soil/sediment concentrations or pore water concentrations estimated from field concentrations and generic organic carbon normalized partition coefficients (Doucette, 2003). These coefficients are generally based on experiments with freshly spiked standard soils and sediments and rarely consider the strong sorption to carbonaceous geosorbents (e.g., soot, coal, kerogen) that may result in increases of sorption coefficients by 1-2 orders of magnitude (Luthy et al., 1997; Cornelissen and Gustafsson, 2004).

Various chemical techniques have been developed to study bioavailability in soil and sediment. Some approaches focussed on the extraction of the weakly bound fraction. Solid sorbents such as Tenax (Yeom et al., 1996; Cornelissen et al., 1997) or XAD-2 (Carroll et al., 1994), solvents (Kelsey et al., 1997), aqueous solutions with cyclodextrin (Cuypers et al., 2002; Swindell and Reid, 2006), surfactants (Chang et al., 2000; Cuypers et al., 2002; Barthe and Pelletier, 2007), butanol (Liste and Alexander, 2002; Swindell and Reid, 2006), and highly pressurized gas (supercritical fluid extraction, (Librando et al., 2004)) have been used for this purpose. Other approaches focussed on the freely dissolved concentrations in the pore water. Freely dissolved aqueous concentrations can be determined by equilibrium dialysis (McCarthy and Jimenez, 1985), gas purging (Resendes et al., 1992), non-

equilibrium passive samplers such as semipermeable membrane devices (SPMD) (Sproule et al., 1991; Booij et al., 1998), and equilibrium passive samplers such as poly(oxymethylene) (POM) (Jonker and Koelmans, 2001), polymer-coated glass sheets or fibers (Mayer et al., 2000; Heringa and Hermens, 2003), and low-density polyethylene (LDPE) (Booij et al., 2003; Adams et al., 2007). Equilibrium passive samplers are left in contact with contaminated soil or sediment slurry, and freely dissolved aqueous concentrations can be calculated with compound-specific passive sampler-water partition coefficients.

In a previous study on chemical availability of PAHs in low and highly contaminated sediments, we compared solid/liquid bulk extraction methods with bioaccumulation in worms (Barthe and Pelletier, 2007). Results indicated strong differences in distributions of PAHs obtained using different extraction techniques. Butanol (BuOH) was found to extract much more PAHs than worms (*Nereis virens* and *Lumbriculus variegatus*) can bioaccumulate in their tissues at steady-state, and hydroxypropyl-β-cyclodextrin (HPCD) underestimated the bioavailability of PAHs, especially for low molecular weight PAHs. On the other hand, the high molecular weight surfactant Brij® 700 (B700) proved to be a good predictor for PAHs in aged highly contaminated sediments (Barthe and Pelletier, 2007).

In the present study we extended our investigation by studying the efficiency of passive solid samplers polyoxymethylene (POM) strips (Jonker and Koelmans, 2001) and polydimethylsiloxane (PDMS) silicon tubing using sediment samples already described in our previous work (Barthe and Pelletier, 2007). Results are compared with the previously measured B700 liquid/solid extraction and worm bioaccumulation. Moreover,

bioaccumulation was estimated using the biota-sediment accumulation factor (BSAF) (Boese et al., 1996) as an instantaneous measurement of normalized tissue/sediment PAH concentrations.

MATERIALS & METHODS

Chemicals

Hexanes (liquid chromatography quality) and acetone (high resolution gas chromatography) were purchased from VWR Ltd (Mississaga, Canada). n-Heptane was purchased from Merck (Darmstadt, Germany). Internal PAH standard d_{10}-phenanthrene (d_{10}-PHE) was obtained from Cambridge Laboratories (Sweden). Additive-free polymer materials were used, including medical-grade PDMS silicon tubing (core diameter 750 μm, thickness 200 μm) from A-M systems, Inc (Carlsborg WA, USA) and polyoxymethylene (POM) of 55 μm thickness from Astrup AS, Oslo, Norway (POM-55, obtained in ~1 kg cylinder-shaped blocks and sliced on a lathe equipped with a high-precision razor blade). POM-55 consisted of C-POM, which is a copolymer of $(CH_2O)_n$ produced from trioxane and other monomers.

Sediments

Marine sediments were collected during years 2003 and 2004 in Kitimat Fjord (BC, Canada), in the St-Lawrence River and Estuary and in the Saguenay Fjord (QC, Canada).

Freshwater sediments came from the St-Louis River near Montreal (Qc, Canada). Samples were stored in a freezer at –20 °C until analysis.

Table 1. Identification of samples for low and highly contaminated sediments. Total PAHs in sediment (C_{sed}) include: fluorene (FLU), phenanthrene (PHE), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DBA), and benzo[g,h,i]perylene (BPE). TOC = Total organic carbon

		Samples analyzed	C_{sed} ($\mu g\ g^{-1}$ w.w.)	TOC (%)
Low contaminated	sediments	Minette Bay (Kitimat, BC)[a]	0.06 ± 0.02	0.04
		SLR upstream (St-Louis River)[b]	0.3 ± 0.1	5.1
		Hospital Beach (Kitimat, BC)[a]	0.3 ± 0.1	0.08
		EGSL04-07 (St. Lawrence Estuary)[a]	0.4 ± 0.1	1.1
		EGSL04-13 (St. Lawrence Estuary)[a]	1.1 ± 0.2	0.7
Highly contaminated	sediments	SLR downstream (St-Louis River)[b]	25.5 ± 2.6	2.5
		SLR outflow (St-Louis River)[b]	279 ± 29	2.0
		Scow Grid (Kitimat, BC)[a]	811 ± 155	2.4
		Scrubber (Kitimat, BC)[a]	4442 ± 538	41.0
		B Lagoon (Kitimat, BC)[a]	5723 ± 190	29.9

[a] Marine sediments tested with *Nereis virens*;

[b] Freshwater sediments tested with *Lumbriculus variegatus*.

Bioaccumulation studies

Bioaccumulation tests used *Nereis virens* for marine sediments and *Lumbriculus variegatus* for freshwater sediments as previously described (Barthe and Pelletier, 2007). Tests were conducted in triplicate for 28 d, which is a sufficient time to ensure a steady-state tissue concentration in both species, at temperature ranging between 4 and 6 °C. One adult polychaete *N. virens* (3-5 g) was placed in each of the triplicate 1 L glass beaker with 300 mL of sediment to be tested and 700 mL of flow-through seawater with aeration. Sixty (0.4 to 0.5 g) adult oligochaetes *L. variegatus* (approximately 2 cm in length) were placed in each of the triplicate 500 mL glass beaker with 200 mL of freshwater sediment to be tested and 300 mL of flow-through dechlorinated tap water with aeration. Worms (polychaetes and oligochaetes) were sampled at seven times (0, 1, 3, 5, 7, 14, and 28 day) during the exposure period of 28 days, (Barthe and Pelletier, 2007). A careful examination of the results indicated no differences in uptake and elimination behavior for both worm species and bioaccumulation results were discussed together.

Analysis of worm tissues

Total lipids were determined gravimetrically following the method described by Folch et al. (1957). *Nereis virens* and *Lumbriculus variegatus* showed an average lipid content of 8.33% and 10.45%, respectively. PAH analysis in worms followed the technique previously described (Barthe and Pelletier, 2007). Briefly, samples (200 mg d.w.) were extracted with 5 ml of hexane:acetone (50:50 v/v) using an ultrasonic bath for 30 min. Each sample was then shaken for 3 h and returned to the ultrasonic bath for 30 min. The

extraction volume was reduced to 0.5 mL, solvent exchanged to acetonitrile and reduced to 0.2 mL. The extracts were analyzed by liquid chromatography (LC) with fluorescence detection.

Sediment characterization

Total PAHs, chemically available PAHs and total organic carbon (TOC) content were determined in triplicate for the sediment samples using the same procedure as previously reported (Barthe and Pelletier, 2007). Briefly, samples (1.0 g d.w.) were extracted with 10 mL of DCM. Each sample was then shaken for 16 h. The extraction volume was reduced to 0.5 mL, solvent exchanged to acetonitrile and volume reduced to 1 mL. The chemically available PAHs (C_{B700}) were extracted by B700 solution. Briefly, samples (1.0 g w.w.) were extracted with 10 mL of B700 solution. Each sample was then shaken for 16 h and then solvent exchanged to DCM. The extraction volume was reduced to 0.5 mL, solvent exchanged to acetonitrile and volume reduced to 1 mL. The extracts were analyzed on a Shimadzu LC-10AD® pump fitted with a Supelcosil™ LC-PAH column (25 cm x 3 mm) and Spectra SYSTEM FL3000 fluorescence detector (HPLC-Fl). These two methods have been shown to give good recovery (85-97%) of PAHs. TOC contents were determined by a carbon analyzer (Costech, Elemental Combustion System, CHNS-O, ECS 4010) after acidification of the dry and ground sample with HCl (10 %) and a digestion at 80 °C for 15 h to eliminate carbonates (Barthe and Pelletier, 2007). All the chemical evaluation was performed prior to the exposure of the organisms to the sediment.

Sediment characteristics for low and highly contaminated samples are given in Table 1. Low contaminated samples show PAH concentrations below 1 $\mu g\ g^{-1}$ (wet weight)

whereas highly contaminated samples contained PAHs ranging between 15.9 and 3605 μg g^{-1} (wet weight). TOC is usually higher in highly contaminated samples except for St-Louis River upstream (SLR upstream) sample located in a pristine area without industrial PAH sources.

Freely dissolved concentrations/sediment sorption experiments

All sorption studies were carried out in triplicate in 50 mL all glass flasks. Dry sediment (3-10 g), passive sampler (100-300 mg), 100 mg NaN_3 (biocide), NaCl for the marine sediments (1.25 g, i.e., 2.5%, providing a constant ionic strength comparable to the *in situ* conditions at the sampling sites), and Millipore water (alpha-Q, 50 mL) were shaken end-over-end (6 rpm; 21 d). It was recently shown that 10 d equilibration time suffices for PAHs in a sediment-water system with POM or silicon (G. Cornelissen, personnal communication). After equilibrium time, the passive samplers were removed from the system and cleaned with wet tissues.

PAH extraction from passive samplers

The clean passive sampler strips and tubing were extracted by horizontal shaking (150 rpm; 72 h) with 15 mL of heptane in the presence of an internal standard (400 ng and 40 ng d_{10}-PHE for highly and low contaminated sediments, respectively) (Cornelissen and Gustafsson, 2004). The samples were eluted through a silica micro-column with 15 mL of heptane. The extracts were analyzed by gas chromatography and mass spectrometry (GC/MS) equipped with an 30 m x 0.25 mm DB-5 fused silica column (film thickness 0.25 μm), and a ThermoFinnigan Polaris Q mass spectrometer in electron impact mode (EI^-, 70

eV). Quality control work showed recovery data ranging from 89.5 ± 5.6% (mean ± standard deviation) to 98.7 ± 2.5%. Variability of analytical results was estimated to ± 15% (n = 5) and the limit of detection was estimated at 0.001 ng μL^{-1}.

RESULTS AND DISCUSSION

BSAF calculation

BSAFs (Biota-Sediment Accumulation Factors) were calculated in worms for each analyzed PAH from the experimental data using Equation (1):

$$\mathrm{BSAF}_{\mathrm{worms}} = \frac{C_{lipid}}{C_{TOC}} \tag{1}$$

where C_{lipid} is the lipid-normalized concentration of PAHs in the organisms ($\mu g\ g^{-1}$ w.w. lipid) and C_{TOC} is the TOC-normalized concentration in the sediment ($\mu g\ g^{-1}$ w.w. organic carbon). Median $\mathrm{BSAF}_{\mathrm{worms}}$ values ranged from 0.0008 to 0.22 in nine out of ten sediments (Table 2), with the exception of SLR Upstream where the median reached 2.53. This higher $\mathrm{BSAF}_{\mathrm{worms}}$ value might be attributed to the use of *L. variegatus* for this freshwater sediment sample, but the tendency of higher bioaccumulation with *L. variegatus* was not confirmed with SLR Downstream and SLR Outflow samples. Values of BSAFs in highly contaminated samples were often two to four orders of magnitude lower than the theoretical value of 1 (DiToro et al., 1991). In low contaminated samples, ratios between measured and theoretical BSAFs (Table 2) were as high as 15, whereas in highly contaminated samples, which have been collected in the vicinity of aluminum smelters, the ratios reached 1466.

In order to include the effect of strong sorption to CGC (carbonaceous geosorbent carbon) on bioaccumulation, BSAFs can also be estimated on the basis of freely dissolved aqueous concentrations $C_{w,free}$, determined using the POM and silicon tubing passive sampler methods (Cornelissen et al., 2006) (Eq. 2).

$$C_{w,free} = \frac{C_{extract}}{m_S} \times \frac{1}{K_S} \quad (2)$$

where $C_{extract}$ is the amount in the extract (ng), m_S is the mass of the sampler (kg) and K_S is the sampler-water partition coefficient (log K; L kg^{-1}) as determined.

In this approach, the overall TOC sorption, not only amorphous organic carbon (AOC) but also CGC, is taken into account. $BSAF_{est,free}$ values (reported as $BSAF_{est,POM}$ and $BSAF_{est,silicon}$) were estimated on the basis of measured chemical parameters according to the Equation (3):

$$BSAF_{free} = \frac{K_{lipid} C_{w,free}}{C_{TOC}} \quad (3)$$

where K_{lipid} is the lipid-water partition coefficient (mL g^{-1}) and can be approximated as being equal to the octanol-water partition coefficient [K_{ow}] (Mackay et al., 1991) for the specific PAHs and $C_{w,free}$ is the freely dissolved aqueous concentration (μg mL^{-1}) determined by the POM or the silicon tubing method. Chemically estimated $BSAF_{est,free}$ values were compared with $BSAF_{worms}$ values by calculating $BSAF_{est,free}/BSAF_{worms}$ ratios (reported as $BSAF_{est,POM}/BSAF_{worms}$ and $BSAF_{est,silicon}/BSAF_{worms}$) (Table 2). Most low contaminated sediments provided relatively low ratios (all below 0.7) for POM whereas highly contaminated sediments produced ratios around 1 in most cases. Consistently,

silicon delivered lower ratios with low contaminated sediments and higher ones with sediments loaded with PAHs. However, POM (0.3-1.4) and silicon (1.4-20) predicted values are much closer to measured values than theoretical ones (0.4-1500). According to Table 2, C_w estimates by the POM are a factor of 4-20 lower than those measured by silicon tubing (data not shown). The differences between C_w estimated by the POM and the silicon could be explained by the fact that the silicon tubing-water coefficient partition (K_{sil}) for all the studied PAHs were greater than the POM-water coefficient partition (K_{POM}). PAHs could be more attracted by PDMS than POM. The difference between the sampler material is well illustrated by Rusina et al. (2007), who demonstrated that the diffusion coefficient (D) of silicon tubing was greater than POM one (log D_{sil} ranged between –9.95 and –11.35 whereas log D_{POM} were below -16).

Using previously reported data (Barthe and Pelletier, 2007) for surfactant B700, $BSAF_{est,B700}$ were estimated according to Equation (4):

$$BSAF_{B700} = \frac{K_{lipid} C_{B700}}{C_{TOC}} \quad (4)$$

where C_{B700} is the concentration of PAHs (μg mL^{-1}) determined by the B700 method. The $BSAF_{est,B700}$ values were compared to $BSAF_{worms}$ by calculating $BSAF_{est,B700}/BSAF_{worms}$ ratios (Table 2). Again, ratios with low contaminated sediments were consistently lower than those obtained with highly contaminated sediments with an average value of 1.17 (Table 2).

Table 2. Biota-sediment accumulation factors (BSAF) determined for all PAHs in all sediment samples as well as ratios between theoretical and empiric BSAFs ($BSAF_{theoretical}/BSAF_{worms}$) and between BSAFs calculated on the basis of concentrations extracted by the different methods and empirical ones ($BSAF_{est,POM}/BSAF_{worms}$, $BSAF_{est,silicon}/BSAF_{worms}$ and $BSAF_{est,B700}/BSAF_{worms}$)

	$BSAF_{worms}$	$BSAF_{theoretical}$[a]/ $BSAF_{worms}$	$BSAF_{POM}$/ $BSAF_{worms}$	$BSAF_{silicon}$/ $BSAF_{worms}$	$BSAF_{B700}$/ $BSAF_{worms}$
Minette Bay	0.16 (0.07-0.19)[b]	6.19 (5.23-30.9)	0.33 (0.15-0.62)	3.38 (1.49-34.6)	0.18 (0.16-0.28)
SLR Upstream	2.53 (1.43-4.43)	0.39 (0.24-0.69)	0.27 (0.17-3.85)	1.40 (0.79-22.9)	0.09 (0.06-0.22)
Hospital Beach	0.02 (0.02-0.06)	41.9 (17.9-54.7)	0.46 (0.34-0.71)	6.63 (4.72-21.4)	0.47 (0.19-0.60)
EGSL04-07	0.22 (0.11-0.37)	5.62 (2.75-12.7)	0.33 (0.24-2.08)	2.47 (1.43-6.28)	0.14 (0.09-0.34)
EGSL04-13	0.06 (0.03-0.19)	15.0 (5.36-35.9)	0.68 (0.49-8.38)	2.36 (1.88-25.3)	0.49 (0.36-0.52)
SLR Downstream	0.02 (0.01-0.03)	40.9 (30.0-348.2)	0.95 (0.46-2.43)	9.73 (5.02-23.4)	1.18 (0.40-1.47)
SLR Outflow	<0.01	111 (76-347)	1.01 (0.61-1.16)	20.2 (7.5-73.7)	0.99 (0.39-1.12)
Scow Grid	<0.01	1466 (1053-7005)	0.95 (0.28-2.13)	4.46 (2.02-6.05)	0.94 (0.39-1.15)
Scrubber	0.12 (0.08-0.15)	8.34 (6.59-12.04)	1.04 (0.29-2.09)	5.95 (1.79-9.92)	1.26 (0.49-1.32)
B Lagoon	0.01 (0.01-0.02)	100 (44-207)	1.37 (0.93-2.03)	7.72 (3.74-9.32)	1.50 (0.62-1.74)

[a] $BSAF_{theoretical}$ value = 1 (DiToro et al., 1991); [b] Median (interquartile ranges)

The low freely dissolved PAH concentrations determined by POM and silicon and the low concentrations extracted by the B700 method in these sediments could be explained by strong sorption to GCG (Cornelissen et al., 2006), which imply the low BSAFs observed for these sediments.

From a theoretical point of view, BSAF should approach unity for conserved organic contaminants. Bioaccumulation of hydrophobic contaminants with BSAF of approximately one has been frequently observed. For example, Ankley et al. (1992) demonstrated BSAFs of approximately one for both laboratory and field populations of oligochaetes exposed to a wide variety of polychlorinated biphenyl congeners in Fox River/Green Bay (WI, USA) sediments. Biota–sediment accumulation factors of less than unity, which appears to be the case for most sediments, and particularly those with a high PAH levels, may therefore represent direct indication of limited bioavailability (or alternatively be a function of elimination or breakdown of contaminants after uptake). So, it is possible that biodegradation of PAHs has influenced our results to a certain extent (Leppänen and Kukkonen, 2000; Jørgensen et al., 2005). Lu et al. (2003) demonstrated that differences between BSAF of desorption-resistant phenanthrene (0.59) and BSAF of reversibly sorbed (labile) phenanthrene (1.2) were not due to differences in contaminant elimination and metabolism nor in desorption kinetics nor in health of the worms but were due to a reduction in the bioavailability of the contaminants in the sediment. Recently, Kreitinger et al. (2007) used supercritical carbon dioxide extraction as a predictor of polycyclic aromatic hydrocarbon bioaccumulation and toxicity by earthworms in manufactured-gas plant site soils and also concluded that low BSAFs were related to low availability of contaminants.

In our case, we believe the low values of $BSAF_{worms}$ in highly contaminated samples can mainly be attributed to strong sorption to aluminum smelter residues well characterized by Breedveld et al (2007).

Earlier observations by Kraaij et al. (2003) suggested that freely dissolved pore-water concentrations ($C_{w,free}$) could be obtained from direct measurements using solid phase microextraction (SPME) even at low concentrations of HOCs in the pg L^{-1} range. Cornelissen et al. (2006) found that $C_{w,free}$ measured by the POM method was a good predictor for BSAFs of native PAHs in three contaminated sediments (9 to 161 mg kg^{-1}) and for two organisms (*Nereis diversicolor* and *Hinia reticulata*) which is in agreement with our results. These results are in agreement with our previous paper (Barthe and Pelletier, 2007) where it was observed that low and highly contaminated sediments presented differences in the extraction of the bioavailable PAHs by different chemical techniques. Passive samplers also illustrated a difference between low and highly contaminated sediments.

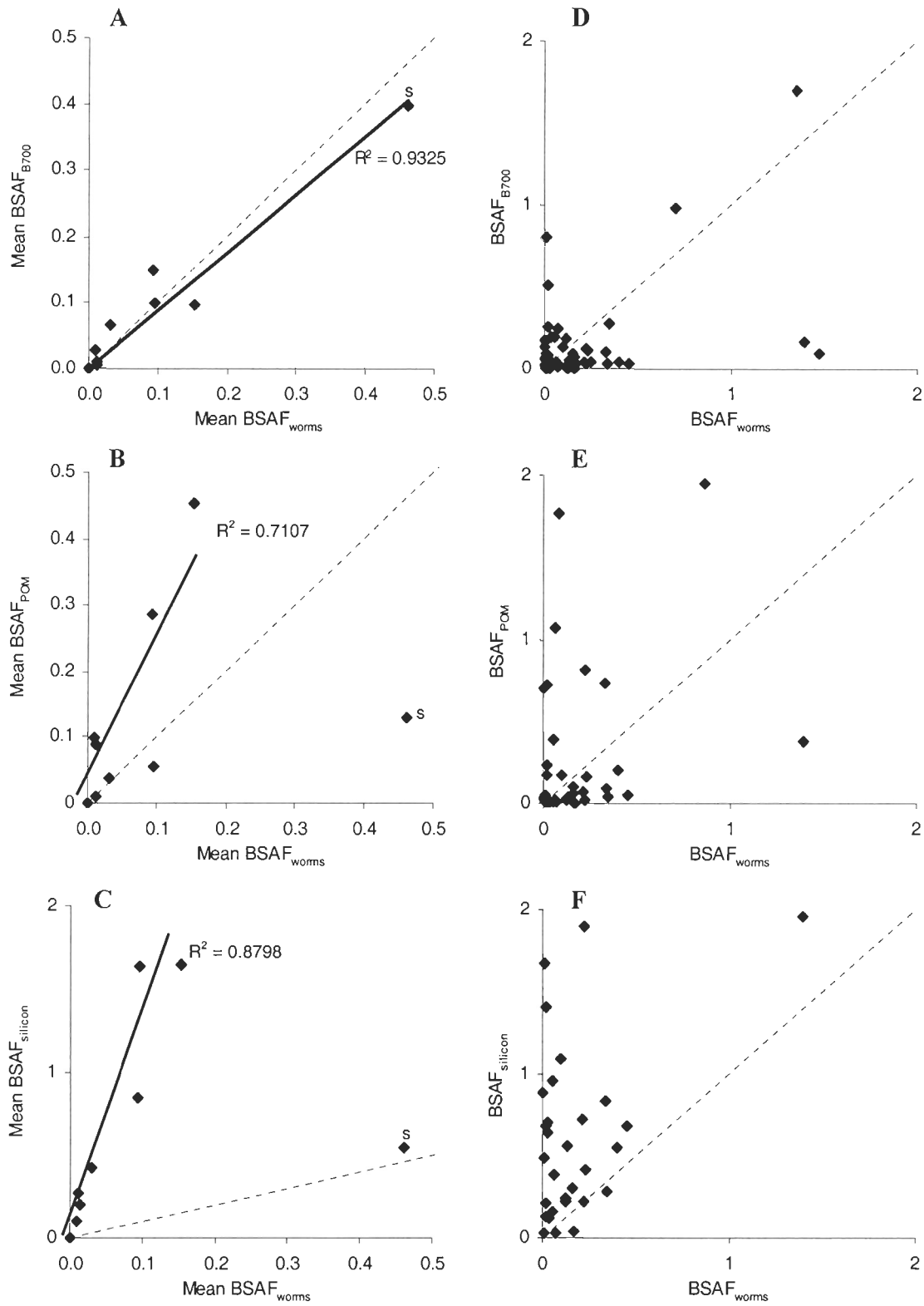
Correlation between BSAFs

In a second step, we examined the relationship between mean $BSAF_{worms}$ (for all determined PAHs) at steady-state and the mean $BSAF_{est,POM}$, $BSAF_{est,silicon}$ or $BSAF_{est,B700}$ as predicted by each method for both groups of samples pooled together (Fig. 1, left panels). The mean $BSAF_{worms}$ are reasonably well correlated with mean $BSAF_{est,B700}$ (R^2 = 0.93), $BSAF_{est,POM}$ (R^2 = 0.71) and $BSAF_{est,silicon}$ (R^2 = 0.88) when sample SLR upstream is excluded for all three samplers and when the Scrubber sample is excluded for POM and Silicon passive samplers. As already observed in Table 2, the $BSAF_{worms}$ of SLR upstream

is out of range compared with other samples because this pristine freshwater sample combines a high percentage of TOC (5.1 %) (Table 1) with a very low PAH content (0.2 μg g^{-1}) which lowers the value of C_{TOC} in equation 1 and increases $BSAF_{worms}$. The scrubber sample is a very particular case because this sample is mainly composed of aluminum smelter residues without natural sediment as described by Breedveld et al (2007). Only surfactant extraction B700 provided a consistent result for that highly contaminated sample.

The second relationship to be examined was between individual $BSAF_{worms}$ (for each analyzed PAH and both worm species) at steady-state and their predicted $BSAF_{est,POM}$, $BSAF_{est,silicon}$ or $BSAF_{est,B700}$ only for low contaminated samples (Fig. 1, right panels). These graphics illustrate the poor relationship existing between BSAF determined by chemical methods and by worms for all analyzed PAHs. Although mean BSAFs can be consistently predicted by all three samplers (left panels), individual BSAFs are not adequately predicted, particularly for low contaminated samples.

Fig. 1. Mean of $BSAF_{est,B700}$ (A), $BSAF_{est,POM}$ (B), and $BSAF_{est,silicon}$ (C) as a function of mean $BSAF_{worms}$ over 28 days for low and highly contaminated sediments (left panels). $BSAF_{est,B700}$ (D), $BSAF_{est,POM}$ (E), and $BSAF_{est,silicon}$ (F) of individual PAH as a function of $BSAF_{worms}$ over 28 days for low contaminated sediments (right panels). The dotted lines represent a hypothetical 1:1 correlation. Scrubber sample is identified with letter s.


A
Mean BSAF$_{B700}$
Mean BSAF$_{worms}$
R^2 = 0.9325
s
B
Mean BSAF$_{POM}$
Mean BSAF$_{worms}$
R^2 = 0.7107
s
C
Mean BSAF$_{silicon}$
Mean BSAF$_{worms}$
R^2 = 0.8798
s
D
BSAF$_{B700}$
BSAF$_{worms}$
E
BSAF$_{POM}$
BSAF$_{worms}$
F
BSAF$_{silicon}$
BSAF$_{worms}$

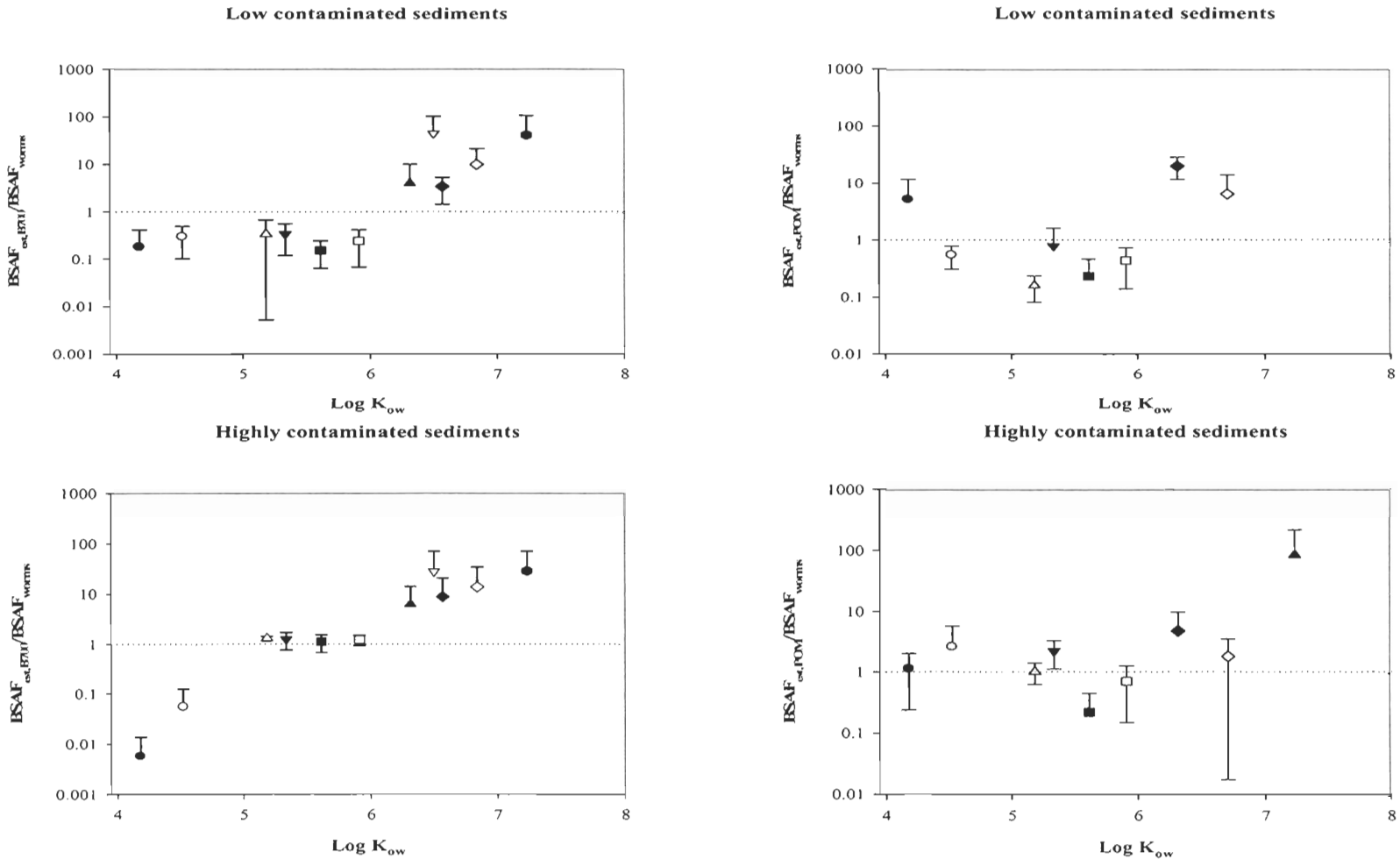
To gain a better understanding on the capacity of B700 and POM methods to predict individual PAH bioaccumulation in worms, the ratio of individual PAHs $BSAF_{est,B700}/BSAF_{worms}$ and $BSAF_{est,POM}/BSAF_{worms}$ were plotted against PAH octanol-water partitioning coefficients (log K_{ow}) for low contaminated (Fig. 2 top panels) and highly contaminated sediments (Fig. 2, bottom panels). The ratio of individual PAHs $BSAF_{est,silicon}/BSAF_{worms}$ plotted against log K_{ow} was not presented here because it has been previously shown that silicon tubing was not a good predictor of the PAH bioavailability. A ratio approaching 1 indicates the ability to predict PAH bioavailability based on B700 or POM extractions, whereas a ratio <1 indicates an underestimation by B700 or POM extraction of the biota-to-sediment accumulation factor and a ratio >1 indicates an over-prediction of the biota-to-sediment accumulation factor by the B700 or POM method. For surfactant B700, low contaminated sediments show low ratios for low log K_{ow} (<5.8) but high ratios (3.9 to 45) for high log K_{ow} (>5.8). Highly contaminated sediments presented a relatively similar picture as PAHs with log K_{ow} <5 exhibit a ratio <1, those with log K_{ow} between 5 and 5.8 exhibit a ratio almost equal to 1, and high ratios (6 to 29) are observed for PAHs with log K_{ow} >5.8 (Fig. 2).

For POM sampler, low contaminated sediments presented a pattern similar to B700 except for fluorene (FLU) which has a ratio >5 (Fig. 2). However, highly contaminated sediments show a quite different pattern from B700 with three PAHs with ratios close to one (FLU, pyrene (PYR), and chrysene (CHR)), four PAHs with ratios slightly above one (phenanthrene (PHE), fluoranthene (FLT), bz[a]pyrene (BaP), and bz[b]fluoranthene +

bz[k]fluoranthene (BbkF)), one PAH with ratios under one (bz[a]anthracene (BaA)) and one PAH with a very high ratio (bz[ghi]perylene (BPE)) (Fig. 2).

Fig. 2 illustrates contrasts and similarities between low and highly contaminated sediments for both B700 and POM methods. Almost all the low molecular-weight (LMW) PAHs (log K_{ow} <5.8) exhibit a ratio <1 in low contaminated samples indicating that worms bioaccumulated more LMW PAHs in their tissues than normally expected from samplers. As worms can digest marine organic matter, they can extract LMW PAHs already bioaccumulated by living and dead organisms present in sediment which means a better access to small PAH molecules particularly in low contaminated sediments were it is assumed that most PAHs are directly associated with organic matter freshly derived from biological activity. In contrast, both techniques are overestimating the availability of high molecular-weight (HMW) PAHs (log K_{ow} >5.8) for all sediments tested. It may indicate that both techniques do not take in account biological factors limiting bioaccumulation of HMW PAHs that seems to be related not only to log K_{ow} but also to the molecular size of the compounds. As an example, the molecular volume ($Å^3$) of fluorene is about 1.5 times smaller than the one of dibenzoanthracene.

Fig. 2. Relationship between PAH octanol-water partitioning coefficient (log K_{ow}) and the ability to predict PAH BSAF using B700 and POM methodology. The studied PAHs are: fluorene (FLU), phenanthrene (PHE), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DBA), and benzo[g,h,i]perylene (BPE).



CONCLUSION

The two passive samplers were found to act differently. The silicon sampler overestimated the availability of PAHs in all studied sediments whereas the POM method provided results quite similar to the solid/liquid extraction using high molecular weight Brij®700. However, both methods poorly predicted availability in low contaminated sediments where biological factors (digestible organic matter) become important. Bioavailability of total PAHs was correctly predicted by POM and B700 in highly contaminated aluminum smelter sediments. A closer examination of individual PAH results indicated that both techniques overestimated the availability of large molecules with log K_{ow} >6 suggesting the presence in worms of a biological mechanism limiting uptake of larger PAHs.

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CHAPITRE III

BIOACCUMULATION AND BIOTRANSFORMATION KINETICS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN SEDIMENTS WITH VARIABLE LEVELS OF CONTAMINATION

M. Barthe and É. Pelletier

Résumé

Dans cette étude, *Lumbriculus variegatus* et *Nereis virens* ont été exposés à des sédiments de composition et de concentrations variables en hydrocarbures aromatiques polycycliques (HAP). La capacité de biotransformation de *L. variegatus* et *N. virens* a été déterminée sur 28 jours en suivant la concentration des métabolites de phase I du phénanthrène et du pyrène: le 9-hydroxyphénanthrène (9-OH-PHE) et 1-hydroxypyrène (1-OH-PYR). Nous avons déterminé les coefficients de toxicocinétique, les facteurs de bioaccumulation (BAF), et les facteurs d'accumulation biote-sédiment (BSAF) qui sont les BAF normalisés par rapport au contenu lipidique de l'organisme et au contenu en carbone organique du sédiment. Les paramètres cinétiques (la constante du taux d'ingestion (k_s) et la constante du taux d'élimination (k_e)) des HAP ont été comparés avec l'hydrophobicité de ces HAP, exprimée par le coefficient de partition octanol-eau (log K_{ow}). Les résultats montrent que le log k_s diminue avec l'augmentation de log K_{ow} pour tous les sédiments étudiés. La relation négative entre log k_s et log K_{ow} suggère que le taux de désorption du composé à partir du sédiment était un facteur important dans l'accumulation par les vers. La détermination de la proportion relative des métabolites par rapport au phénanthrène total et au pyrène total dans les tissus de vers après une exposition de 28 jours indiquait la présence de 24% de 9-hydroxyphénanthrène et 17% de 1-hydroxypyrène chez *N. virens*, et de 18% de 9-hydroxyphénanthrène et 20% de 1-hydroxypyrène chez *L. variegatus*. D'après ces importantes proportions en métabolites, nous présumons que les métabolites présents dans les vers sont principalement dus à la métabolisation des HAP parents plutôt qu'à la

bioaccumulation à partir des sédiments où les métabolites étaient présents en faible concentration.

Mots clés: HAP, sédiments marin et lacustres, bioaccumulation, métabolites, cinétiques de biotransformation, hydroxy-phénanthrène, hydroxy-pyrène.

Abstract

In the present study, *Lumbriculus variegatus* and *Nereis virens* were exposed to field-collected sediments of varying composition and concentration of polycyclic aromatic hydrocarbons (PAHs). The biotransformation capability of *L. variegatus* and *N. virens* was assessed over 28 days by following the concentration of 9-hydroxyphenanthrene (9-OH-PHE) and 1-hydroxypyrene (1-OH-PYR), the phase I metabolites of phenanthrene and pyrene, respectively. Toxicokinetic coefficients, bioaccumulation factors (BAF), and biota-sediment accumulation factors ([BSAF], BAF normalized to the organism lipid content and sediment organic carbon content) were determined. The kinetic parameters (the uptake clearance rate constant (k_s) and the elimination rate constant (k_e)) of PAHs were compared to molecular hydrophobicity of these PAHs, expressed by the octanol-water partition coefficient (log K_{ow}). The results showed that log k_s decreased with increasing log K_{ow} for all studied sediment samples. The negative relationship between log k_s and log K_{ow} suggests that the desorption rate of the compound from the sediment was an important governing factor in the accumulation by the worms. The determination of the relative proportion of metabolites toward total phenanthrene and pyrene in worm tissues after a 28-day exposure indicated the presence of 24% of 9-hydroxyphenanthrene and 17% of 1-hydroxypyrene in *N. virens*, and 18% of 9-hydroxyphenanthrene and 20% of 1-hydroxypyrene in *L. variegatus*. According to these high proportions of metabolites, we assumed that metabolites present in worms are mainly due to metabolization of the parent PAH instead of bioaccumulation from sediment where metabolites were present in low concentrations.

Keywords: PAH, marine and lacustrine sediments, bioaccumulation, metabolites, biotransformation kinetics, hydroxy-phenanthrene, hydroxy-pyrene.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are common contaminants in marine environments (Kennish, 1997) that accumulate and persist in sediments and can therefore be taken up by sediment dwelling organisms (Landrum, 1989; Leppänen and Kukkonen, 1998; Loonen et al., 1997; Weston, 1990). Thus, understanding the bioavailability of these sediment-sorbed contaminants is important to determine their potential environmental risk. In a general manner, the bioavailability of sediment-sorbed contaminants decreases with the increase of the contact time (Landrum, 1989; McElroy and Means, 1988), where the contact time corresponds to the elapsed time from the introduction of the contaminant in the sediment. The contact time is also named aging time. The reduction of the bioavailable fraction can be either due to the strong binding with the sedimentary organic carbon or to the rapid metabolization of these compounds by the organisms (Pignatello, 1990). Inversely, numerous studies using spiked sediments have indicated that some chemicals, such as PAHs, did not show decrease of the bioavailability through time (Landrum, 1989; Landrum et al., 1992a). However, when comparing spiked and field polluted sediments, a lower accumulation has been shown in the polluted sediments compared to the spiked ones (Landrum et al., 1992a; Varanasi et al., 1985). These studies suggested that some compounds need more contact time (more than one year) to reach equilibrium with sediments. Due to the toxicity and the persistence of these compounds, it is important to further study their bioaccumulation potential.

Bioaccumulation studies have been widely used to evaluate the bioavailability and the ecological risk of bedded-sediments. These studies often incorporated a bioaccumulation factor (BAF), which is defined as the ratio of the contaminant in the organism at steady-state and the contaminant concentration in the environmental media (water, sediment, etc…). The disadvantage of these studies is the important time needed for the organisms to reach the steady-state for some compounds. Due to this drawback, toxicokinetic models, in particular two-compartment models using first-order kinetics are commonly used to describe accumulation and predict levels at steady-state under non-equilibrium conditions (Landrum et al., 1992b; Lydy et al., 2000; Schuler and Lydy, 2001).

The present work was undertaken as part of a larger study examining the sequestration mechanisms of PAHs in marine and lacustrine sediments with different geochemical and environmental characteristics, and to study the bioaccumulation of PAHs in order to correlate it with obtained sequestration data. The goal of the first part of this study was to evaluate three different non-exhaustive solid/liquid extraction methods (n-butanol, hydroxypropyl-β-cyclodextrin, and a surfactant solution of Brij700) for assessing the availability of PAHs in contaminated sediments. This goal was achieved by comparing results from 28-day uptake experiments by *Nereis virens* and *Lumbriculus variegatus* with PAHs extracted by these three methods. Our results showed that an extraction procedure involving the Brij700 was revealed to be useful particularly with sediments that show PAH levels > 2 $\mu g\ g^{-1}$ and log K_{ow}< 5.8 (Barthe and Pelletier 2007). The purpose of the second part of the study was to test the efficiency of passive solid samplers, polyoxymethylene (POM) strips and polydimethylsiloxane silicon tubing, to predict the bioavailability of

native PAHs in contaminated sediments. Results were compared to those of the first study. The silicon sampler overestimated the availability of PAHs in all studied sediments whereas the POM method provided results quite similar to the solid/liquid extraction using high molecular weight Brij700. However, both methods poorly predicted availability in low contaminated sediments. A closer examination of individual PAH results indicated that the POM technique overestimated the availability of large molecules with log K_{ow} >6 (Barthe et al., 2008).

The principal aim of the present study was to investigate the temporal patterns of PAH accumulation for eleven sediments with variable levels of contamination in two species of aquatic invertebrates (*Lumbriculus variegatus* and *Nereis virens*). Our specific objectives were to determine the toxicokinetic parameters describing the uptake, elimination and BAF of the eight PAHs studied in sediments exhibiting large differences in their chemical properties. Moreover, the ability of the organisms to metabolize phenanthrene and pyrene was examined by the determination of selected hydroxy PAHs (9-hydroxyphenanthrene and 1-hydroxypyrene).

MATERIALS & METHODS

Chemicals

Polycyclic aromatic hydrocarbons standards (method standards for waste water, 16 compounds 0.1 mg mL^{-1}) were purchased from Chromatographic Specialties Inc. (Brockville, Canada). 9-hydroxyphenanthrene (9-OH-PHE) (technical grade) and 1-

hydroxypyrene (1-OH-PYR) (98% purity) were purchased from Sigma-Aldrich (Oakville, Canada). Anthracene-d_{10} and fluoranthene-d_{10} were from Cambridge Isotope Laboratories (Andover, MA, USA) at 98% purity. Acetonitrile (HPLC grade) and dichloromethane (DCM) (Liquid Chromatography Analysis) were purchased from VWR (Mississauga, Canada).

Sediments

Marine sediments were collected in the St-Lawrence River and Estuary and in the Saguenay Fjord (QC, Canada) in July 2004 with a Van Veen grab and in the Kitimat Fjord (BC, Canada) in September 2003 at low tide with shovel. The whole sediment was placed into clean buckets and frozen at -20°C until analysis. Lacustrine sediments were collected in the Saint-Louis River (SLR) (QC, Canada) in October 2003 with a hand corer. The whole sediment was placed into clean buckets and frozen at -20°C until analysis. Total PAHs, and total organic carbon (TOC) content were determined in triplicate for the sediment samples using the same procedure as previously reported (Barthe and Pelletier, 2007). The sediment properties were reported in Barthe and Pelletier (2007). Low contaminated samples (EGSL04-01; -07; 13; SLR Upstream; Minette Bay; Hospital Beach) show PAH concentrations between 0.06 and 1.1 $\mu g\ g^{-1}$ (wet weight) whereas highly contaminated samples (B Lagoon; Scrubber; Scow Grid; SLR Outflow; SLR Downstream) contained PAHs ranging between 25.5 and 5723 $\mu g\ g^{-1}$ (wet weight). TOC ranged between 2.0 and 41% and 0.04 and 5.1% in highly and in low contaminated samples respectively.

Uptake experiments

Bioaccumulation tests used *Nereis virens* and *Lumbriculus variegatus* as previously described (Barthe and Pelletier, 2007). Tests were conducted in triplicate for 28 d at temperature ranging between 4 and 6°C.

Analysis of worm tissues and sediments

The PAH analysis in organisms and sediments followed the technique previously described (Barthe and Pelletier, 2007). Metabolites in biological tissues and in sediments were extracted with the same protocol used for the extraction of PAHs in organisms. Briefly, samples (200 mg d.w.) were extracted with 5 ml of hexane:acetone (50:50 v/v) using an ultrasonic bath for 30 min. Each sample was then shaken for 3 h and returned to the ultrasonic bath for 30 min. The extraction volume was reduced to 0.5 mL, solvent exchanged to acetonitrile and reduced to 0.2 mL. Concentrations of phenanthrene, 9-hydroxyphenanthrene, pyrene and 1-hydroxypyrene in sediments and in worms are presented in Table 1.

Table 1. Concentration of phenanthrene (PHE), 9-hydroxyphenanthrene (9-OH-PHE), pyrene (PYR), and 1-hydroxypyrene (1-OH-PYR) in sediments and in worms ($\mu g\ g^{-1}$ w.w.) and percentage of metabolite compared to parent PAH.

	PHE_{sed}	$9\text{-}OH\text{-}PHE_{sed}$	PHE_{worms}	$9\text{-}OH\text{-}PHE_{worms}$	%[a]	PYR_{sed}	$1\text{-}OH\text{-}PYR_{sed}$	PYR_{worms}	$1\text{-}OH\text{-}PYR_{worms}$	%[a]
B Lagoon	213	11.8	0.55	0.15	22	660	0.02	4.1	1.6	28
Scrubber	227	3.1	7.3	3.05	29	770	1.9	18.8	0.52	2.7
Scow Grid	48	0.56	0.13	0.05	28	95	0.003	0.46	0.19	29
SLR Outflow	1.6	0.58	0.71	0.13	15	46	0.002	2.77	0.69	20
SLR Downstream	0.36	0.06	0.06	0.03	32	1.9	1.0×10^{-4}	0.26	0.09	27
EGSL04-13	0.07	0.02	0.21	0.04	18	0.08	3.0×10^{-4}	0.32	0.007	2.2
EGSL04-07	0.05	0.02	0.15	0.04	24	0.06	1.0×10^{-4}	0.19	0.04	16
Hospital Beach	0.06	0.01	0.10	0.05	32	0.05	3.0×10^{-4}	0.26	0.09	27
SLR Upstream	0.06	0.05	0.42	0.02	5.3	0.06	0.004	0.62	0.08	12
EGSL04-01	0.01	0.02	0.20	0.03	11	0.02	1.0×10^{-4}	0.31	0.03	9.6
Minette Bay	0.03	0.01	0.06	0.025	29	0.014	4.0×10^{-4}	0.36	0.11	23

[a] $\% = (OH\text{-}PAH_{worms} * 100)/(PAH_{worms} + OH\text{-}PAH_{worms})$

Quantification

Metabolite analyses were carried out by liquid chromatography (LC) with fluorescence detection. The apparatus consisted of a Rheodyne® injector with a 20 µL injection loop, a Shimadzu LC-10AD® pump, a Supelcosil™ LC-PAH column (25 cm x 3 mm), and Spectra SYSTEM FL3000 fluorescence detector. All analyses were done at a constant flow rate of 0.8 mL min^{-1} with a mixture of nanopure water and acetonitrile as mobile phase. The pump program began at 75% acetonitrile and increased to 95% in 10 min and then to 100% in the next 10 min, with a final plateau of 10 min. The cycle returned to 75% acetonitrile after a total run time of 35 min. The excitation wavelength of the fluorescence detector was settled at 244 nm during the first 3.4 min and then shifted to 344 nm until the end of the program and the emission was detected at 370 nm during the first 3.4 min and then shifted to 394 nm until the end of the program. For quality control, a 0.5 µg mL^{-1} 9-OH-PHE and 1-OH-PYR mix was analyzed every 10 samples.

Fluorene (FLU), phenanthrene (PHE), fluoranthene (FLT), pyrene (PYR), bz[a]anthracene + chrysene (BaA+CHR), benzo[bk]fluoranthene (BbkF), bz[a]pyrene (BaP), bz[g.h.i]perylene (BPE), 9-OH-PHE and 1-OH-PYR were quantified.

Quality control work showed recovery data ranging from 89.5 ± 5.6% (mean ± standard deviation) to 98.7 ± 2.5%. Variability of analytical results was estimated to ± 15% (n = 5) and the limit of detection was estimated at 0.01 ng g^{-1} (d.w. sediment).

Data treatment

Contaminant accumulation data were fitted to a first-order toxicokinetic, two-compartment accumulation model to estimate both uptake and elimination for evaluation of bioavailability (Kukkonen et al., 2004; Spacie and Hamelink, 1983; Spacie et al., 1995):

$$C_{worms} = \frac{k_s C_s}{k_e}(1 - e^{-k_e t}) \quad (1)$$

where C_{worms} is the concentration of the compound in the organism (μg g^{-1} w.w.), C_s is the concentration of the compound in the sediment (μg g^{-1} d.w.), k_s is the uptake clearance rate constant of the compound from sediment (g dry sediment g^{-1} wet organism h^{-1}), k_e is the elimination rate constant of the compound (h^{-1}) in sediment, and t is time (h). The model assumes that the concentration in the sediment remains constant and there is no biotransformation of the compound. The best-fit adjustable parameter values were found using the solver function in Microsoft Excel® 7.0 (Table 2).

Table 2. Estimated wet weight-based uptake (k_s) and elimination (k_e) rate constants for the kinetics of polycyclic aromatic hydrocarbons (PAHs) in polychaetes and oligochaetes.

	Log K_{ow}[a]		B Lagoon	Scrubber	Scow Grid	SLR Outflow	SLR Downstream	EGSL04-13	EGSL04-07	Hospital Beach	SLR Upstream	EGSL04-01	Minette Bay
FLU	4.18	k_s	3.0×10^{-4}	0.01	1.6×10^{-4}	5.0×10^{-3}	2.0×10^{-3}	0.21	0.20	0.46	0.13	0.97	0.99
		k_e	0.02	0.01	0.02	0.02	0.01	0.08	0.01	0.01	0.03	0.01	0.02
PHEN	4.52	k_s	3.8×10^{-3}	5.0×10^{-4}	8.34^{e-5}	3.0×10^{-3}	2.0×10^{-3}	0.33	0.08	0.04	0.07	0.78	0.40
		k_e	0.15	0.01	0.03	0.01	0.01	0.11	0.03	0.03	0.01	0.04	0.19
FLT	5.33	k_s	1.0×10^{-4}	5.0×10^{-4}	8.41^{e-5}	5.0×10^{-4}	1.0×10^{-3}	0.07	0.07	0.88	0.56	0.53	0.89
		k_e	0.02	0.02	0.02	0.01	0.01	0.04	0.01	0.15	0.02	0.03	0.02
PYR	5.18	k_s	8.6×10^{-5}	7.0×10^{-4}	4.0×10^{-4}	5.0×10^{-4}	2.0×10^{-3}	0.21	0.05	0.50	0.14	0.82	0.86
		k_e	0.01	0.03	0.09	0.01	0.01	0.06	0.01	0.10	0.01	0.05	0.03
BaA+CHR	5.76[b]	k_s	2.1×10^{-5}	2.0×10^{-4}	2.69^{e-5}	1.0×10^{-4}	1.0×10^{-4}	2.0×10^{-3}	0.02	0.04	0.04	0.42	0.78
		k_e	0.01	0.01	0.05	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02
BbkF	6.71[c]	k_s	2.5×10^{-5}	3.8×10^{-5}	7.01^{e-6}	1.0×10^{-4}	2.0×10^{-4}	0.01	0.01	0.02	0.03	0.04	0.07
		k_e	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
BaP	6.31	k_s	1.4×10^{-5}	6.7×10^{-5}	4.7×10^{-6}	1.0×10^{-4}	8.4×10^{-5}	3.0×10^{-3}	0.01	1.0×10^{-3}	0.54	0.01	0.05
		k_e	0.02	0.03	0.03	0.02	0.01	0.01	0.01	0.01	0.04	0.01	0.01
BPE	7.23	k_s	2.3×10^{-5}	4.0×10^{-5}	4.5×10^{-6}	1.0×10^{-4}	8.5×10^{-5}	6.0×10^{-4}	4.0×10^{-4}	0.01	7.0×10^{-3}	2.0×10^{-3}	0.04
		k_e	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02

[a] From Mackay et al. (1991); [b] mean of BaA and CHR Log K_{ow}; [c] mean of BbF and BkF Log K_{ow}

Bioaccumulation factors were determined either from the measured values at the end of the exposures (BAF_{me}) as the concentration in the organisms (μg g^{-1} w. w.) divided by the concentration in the sediment (μg g^{-1} w. w.) or as calculated values from the toxicokinetics (BAF_{ca}) as the ratio of k_s/k_e to give the expected steady state BAF (Table 3).

The biota-sediment accumulation factor was calculated by normalizing BAF_{ca} by the lipid content of the organisms and the organic carbon content of the sediments (Table 3). The average lipid values for *Lumbriculus variegatus* and for *Nereis virens* were 10.45% and 8.33%, respectively. The sediment organic carbon contents were reported previously (Barthe and Pelletier, 2007).

Metabolite accumulation data were fitted to a first-order toxicokinetic, three-compartment accumulation model to estimate uptake, metabolization and elimination rate constants:

$$C_{MO} = ((C_{PS}\frac{k_1}{k_3}) \times (1 - e^{(-k_3 t)})) + ((\frac{(k_2 C_{PO_0}) - (k_1 C_{PS})}{k_3 - k_2}) \times (e^{(-k_2 t)} - e^{(-k_3 t)}) + (C_{MO_0} e^{(-k_3 t)})) \quad (2)$$

where C_{MO} is the concentration of the metabolite compound in the organism (μmol g^{-1} w.w.), C_{PS} is the concentration of the parent compound in the sediment (μmol g^{-1} d.w.), C_{PO_0} is the concentration of the parent compound in the organism at t_0 (μmol g^{-1} w.w.), C_{MO_0} is the concentration of the metabolite compound in the organism at t_0 (μmol g^{-1} w.w.), k_1 is the uptake clearance rate constant of the parent compound from sediment (μmol dry sediment g^{-1} wet organism h^{-1}), k_2 is the metabolization rate constant of the parent compound (h^{-1}), k_3 is the elimination rate constant of the metabolite compound (h^{-1}) and t is time (h). The best-fit adjustable parameter values were found using Lab Fit 7.2® (Table 4).

Table 3. Bioaccumulation (BAF) and biota-sediment accumulation (BSAF) factors for the selected polycyclic aromatic hydrocarbons.

		B Lagoon	Scrubber	Scow Grid	SLR Outflow	SLR Downstream	EGSL04-13	EGSL04-07	Hospital Beach	SLR Upstream	EGSL04-01	Minette Bay
FLU[a]	BAF_{me}	0.006	1.06	0.003	0.079	0.14	0.792	1.70	12.8	3.03	4.87	33.6
	BAF_{ca}	0.016	1.00	0.008	0.33	0.14	2.62	14.3	38.3	4.81	88.2	45.0
	BSAF	0.06	4.92	0.002	0.06	0.034	0.22	1.88	0.37	2.35	13.2	0.22
PHEN	BAF_{me}	0.003	0.03	0.003	0.45	0.17	2.80	3.00	1.70	6.90	19.9	2.10
	BAF_{ca}	0.03	0.03	0.003	0.43	0.14	3.00	2.80	1.60	7.40	19.0	2.10
	BSAF	0.09	0.16	0.0007	0.08	0.034	0.25	0.37	0.015	3.63	2.74	0.01
FLT	BAF_{me}	0.007	0.03	0.004	0.045	0.10	1.80	2.50	6.60	0.67	20.4	44.6
	BAF_{ca}	0.006	0.03	0.005	0.05	0.11	1.80	2.80	5.90	28.0	20.4	52.3
	BSAF	0.02	0.15	0.001	0.009	0.025	0.15	0.64	0.056	13.66	2.93	0.26
PYR	BAF_{me}	0.006	0.02	0.0048	0.061	0.14	4.10	3.40	5.20	11.3	16.9	25.1
	BAF_{ca}	0.006	0.02	0.0046	0.062	0.17	3.70	3.40	5.05	11.7	16.7	26.1
	BSAF	0.02	0.12	0.001	0.011	0.039	0.31	0.45	0.048	5.69	2.31	0.12
BaA+CHR	BAF_{me}	0.002	0.02	0.0005	0.013	0.011	0.15	0.88	2.35	3.55	1.98	44.2
	BAF_{ca}	0.002	0.02	0.0005	0.01	0.011	0.25	1.35	2.43	3.90	20.0	45.9
	BSAF	0.008	0.08	0.0001	0.002	0.002	0.02	0.18	0.023	1.91	2.88	0.22

		B Lagoon	Scrubber	Scow Grid	SLR Outflow	SLR Downstream	EGSL04-13	EGSL04-07	Hospital Beach	SLR Upstream	EGSL04-01	Minette Bay
BaP	BAF_{me}	0.001	0.002	0.0001	0.009	0.007	0.26	0.59	0.15	15.1	7.20	3.90
	BAF_{ca}	0.001	0.002	0.0001	0.0066	0.007	0.25	0.61	0.11	13.5	1.30	4.00
	BSAF	0.003	0.012	5.0×10^{-5}	0.001	0.002	0.02	0.08	0.001	6.58	0.19	0.02
BPE	BAF_{me}	0.001	0.005	0.0004	0.011	0.008	0.06	0.016	1.54	0.51	0.17	2.64
	BAF_{ca}	0.001	0.005	0.0004	0.009	0.009	0.07	0.022	1.62	0.58	0.15	2.75
	BSAF	0.004	0.024	0.0001	0.002	0.002	0.006	0.003	0.016	0.28	0.022	0.01
Median	BAF_{me}	0.002	0.02	0.002	0.03	0.06	1.01	1.29	2.08	3.29	6.05	15.6
	BAF_{ca}	0.004	0.02	0.002	0.03	0.06	1.58	2.08	2.18	6.13	17.9	16.3
	BSAF	0.015	0.10	0.0005	0.006	0.016	0.13	0.27	0.021	2.99	2.52	0.08

[a] FLU = fluorene, PHE = phenanthrene, FLT = fluoranthene, PYR = pyrene, BaA+CHR = bz[a]anthracene + chrysene , BbkF = benzo[bk]fluoranthene, BaP = bz[a]pyrene, BPE = bz[g.h.i]perylene.

Table 4. Estimated wet weight-based uptake (k_1), metabolization (k_2) and elimination (k_3) rate constants for the kinetics of hydroxy polycyclic aromatic hydrocarbons (OH-PAHs) in polychaetes and oligochaetes.

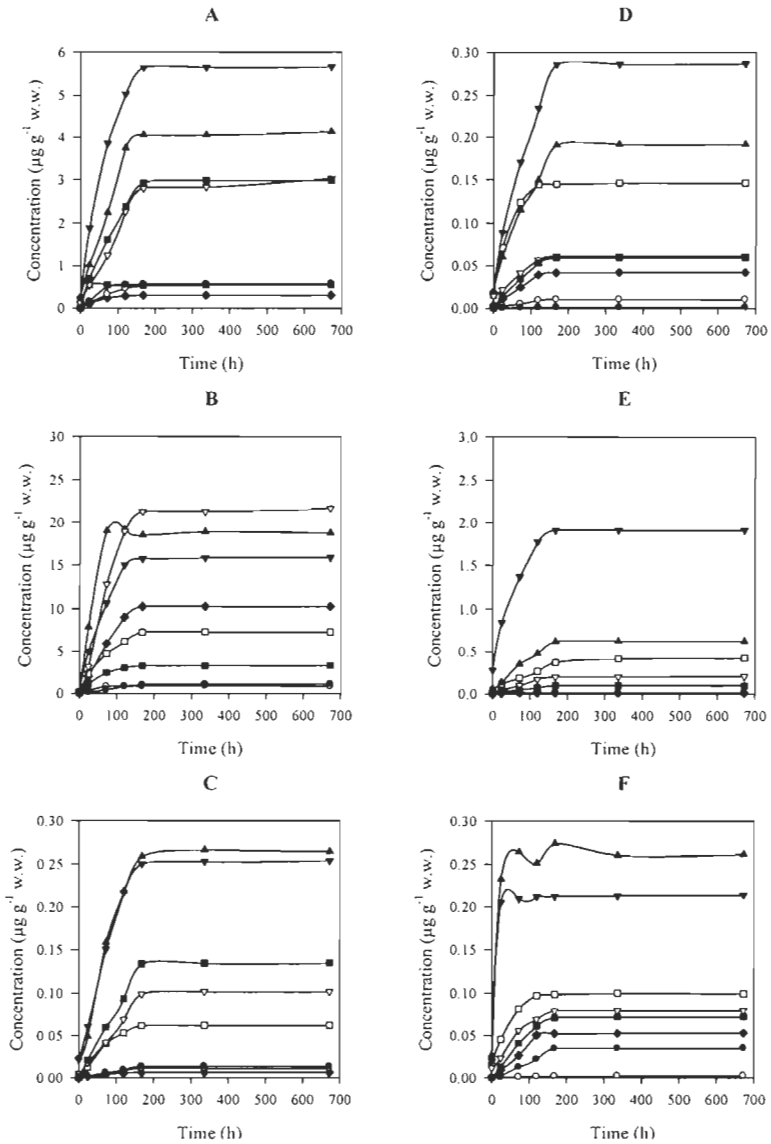
		B Lagoon	Scrubber	Scow Grid	SLR Outflow	SLR Downstream	EGSL04-13	EGSL04-07	Hospital Beach	SLR Upstream	EGSL04-01	Minette Bay
9-OH-PHEN	k_1	3.0×10^{-4}	4.0×10^{-4}	3.3×10^{-4}	0.002	0.002	0.07	0.03	0.06	0.003	0.37	0.07
	k_2	0.35	0.07	0.02	0.02	0.02	0.03	0.08	0.02	0.007	0.02	0.04
	k_3	0.43	0.03	0.31	0.02	0.04	0.11	0.031	0.09	0.008	0.14	0.08
1-OH-PYR	k_1	1.1×10^{-4}	3.1×10^{-4}	3.8×10^{-4}	3.3×10^{-4}	5.7×10^{-4}	0.004	0.01	0.28	0.003	0.02	0.77
	k_2	0.04	0.04	0.08	0.03	0.01	0.002	0.02	0.14	0.02	0.03	0.04
	k_3	0.04	0.05	0.19	0.02	0.01	0.05	0.02	0.15	0.02	0.02	0.10

RESULTS

Bioconcentration of PAH in worms

A rapid uptake of PAHs by worms was indicated by the maximum tissue levels measured within 7 days (168 h) of exposure for all target compounds in all sediment samples and both worm species. Six sediments with different characteristics and contamination levels were selected to illustrate this point (Fig. 1). For most PAHs, bioaccumulation showed a regular pattern although concentrations ranged from <0.05 $\mu g\ g^{-1}$ in low contaminated sediments to as much as 20 $\mu g\ g^{-1}$ in the highly contaminated scrubber sample. Phenanthrene revealed peak levels within 72 h and 120 h in one highly contaminated sediment (B Lagoon) (Fig. 1A) and two low contaminated sediments (EGSL04-07 and Hospital Beach), respectively (Fig. 1D and F). Pyrene also revealed peak levels within 72 h in one highly contaminated sediment (Scrubber) and in one low contaminated sediment (Hospital Beach) (Fig. 1F). Fluoranthene also showed a particular behavior in one low contaminated sediment (Hospital Beach) (Fig. 1F).

Fig. 1. Uptake kinetics of fluorene (♦), phenanthrene (□), fluoranthene (▼), pyrene (▲), bz[a]anthracene + chrysene (▽), benzo[bk]fluoranthene (■), benzo[a]pyrene (○), and benzo[g.h.i]perylene (●) in worms during the exposure period in B Lagoon (A), Scrubber (B), SLR Downstream (C), EGSL04-07 (D), SLR Upstream (E), and Hospital Beach (F) sediments. The curve corresponds to the nonlinear fit of the data using the model expressed in Eq. 1. Note different scales for PAH concentrations.

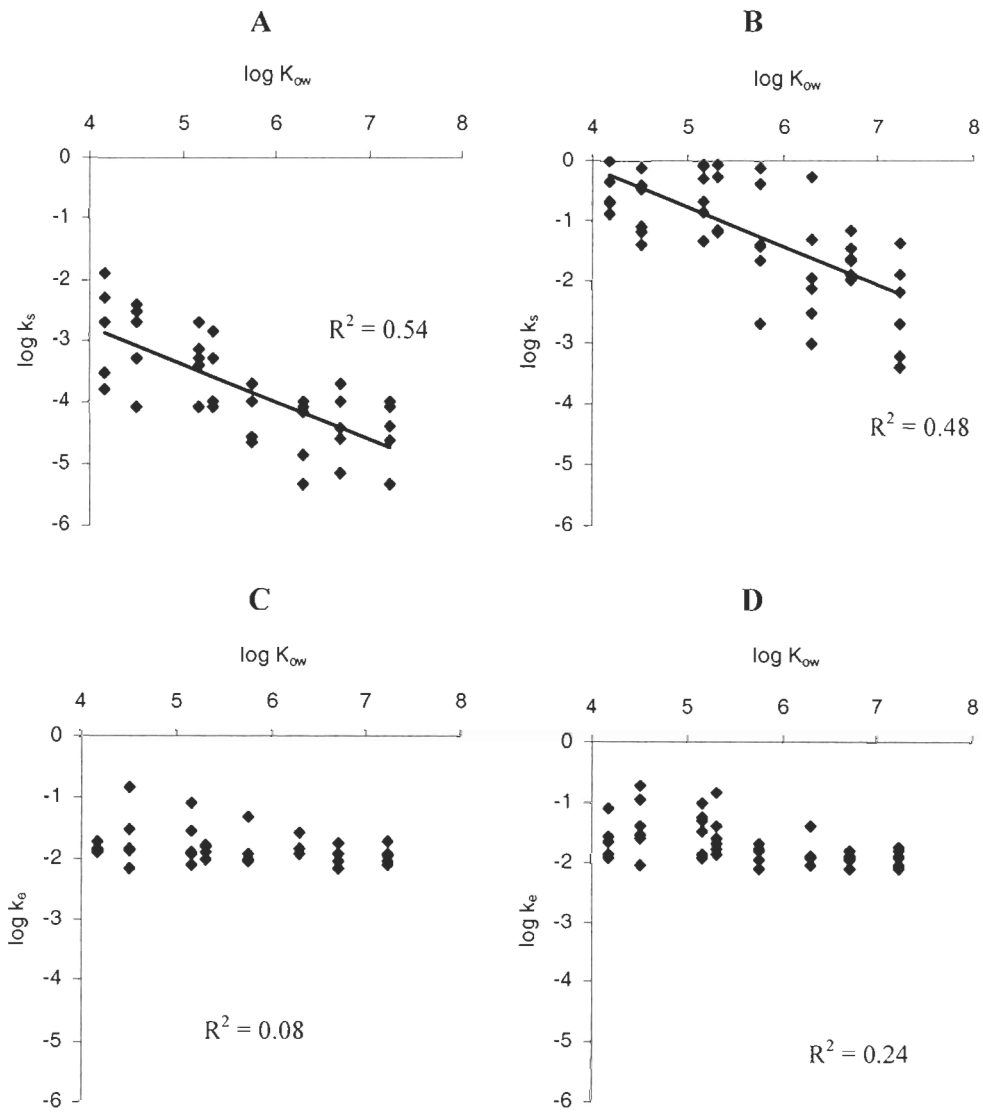


Uptake rate constants (k_s) were calculated for individual PAHs using Eq. 1 (Table 2). The relationship between uptake clearance rate constants and the molecular lipophilicity expressed by log K_{ow} is illustrated in Figure 2 (Top panel). The plots show that log k_s decreased with increasing log K_{ow} for all studied sediment samples (R^2 = 0.54 and 0.48 for highly and low contaminated sediments, respectively).

Elimination rate constants (k_e) were calculated for individual PAHs also using Eq. 1 (Table 2). The relationship between elimination rate constants and log K_{ow} has been plotted (Fig. 2 Bottom panel). The plots show that the elimination rate constant for the studied worms also tends to decrease with increasing log K_{ow} for both categories of sediment, but with low correlation coefficients (R^2 = 0.08 and 0.24 for highly and low contaminated sediments, respectively).

The bioaccumulation factors (BAFs) were estimated from $BAF_{ca} = k_s / k_e$, and also directly (BAF_{me}) from PAH concentrations in worm tissues and sediment samples, (Table 3). A decrease of BAF_{ca} was observed with increasing log K_{ow} for the low and highly contaminated sediments (data not shown). Median BSAF values ranged from 0.0005 to 0.27 in nine out of eleven sediments (Table 3), with the exception of SLR Upstream and EGSL04-01 where the medians reached 2.99 and 2.52, respectively. Values of BSAFs in highly contaminated samples were often two to four orders of magnitude lower than the theoretical value of 1 (DiToro et al., 1991).

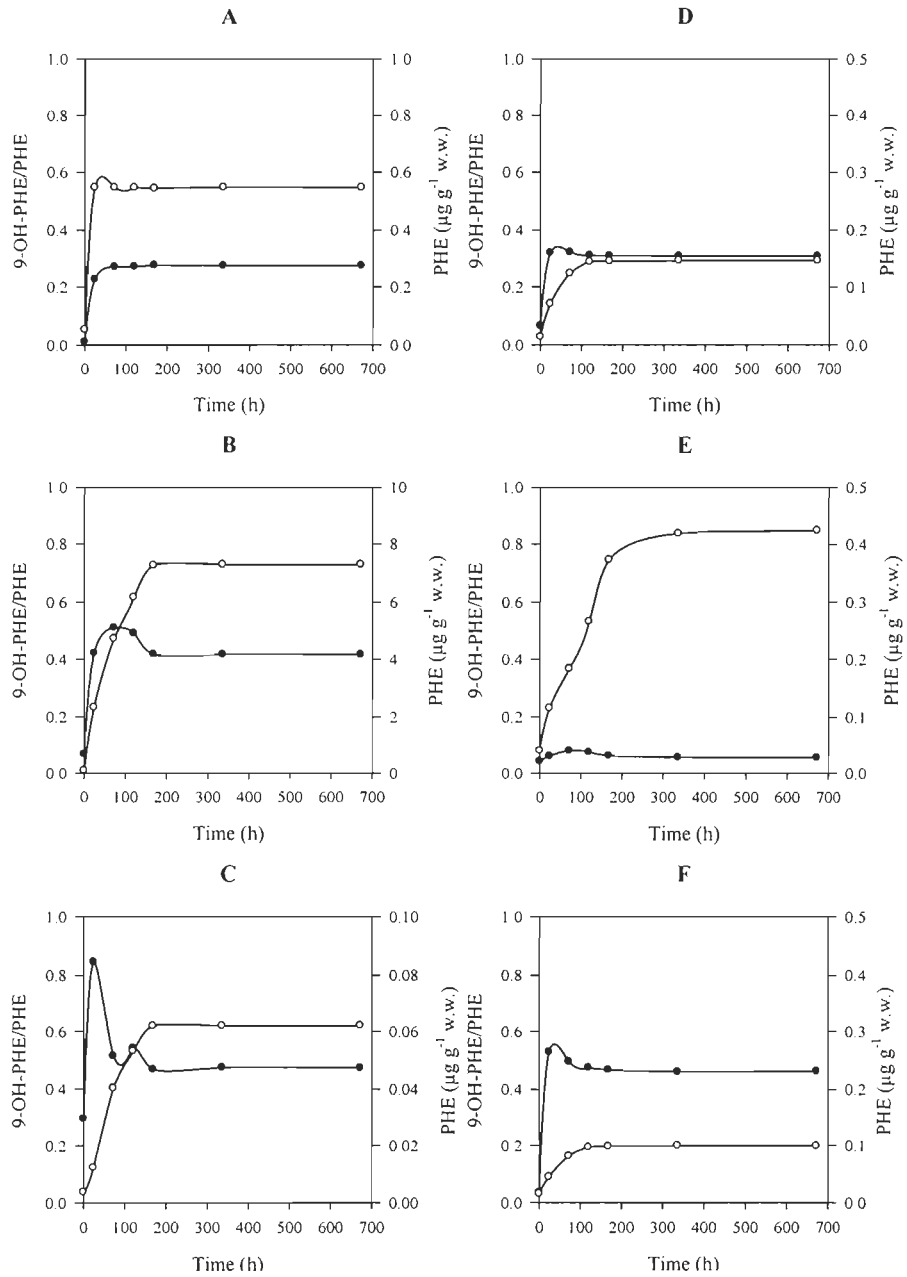
Fig. 2. **(Top)** Plot of the log of uptake clearance rate constants, log k_s, versus the log of the hydrophobicity expressed by the octanol/water partition coefficient, log K_{ow}, for highly (A) and low (B) contaminated sediments. **(Bottom)** Plot of the log of elimination rate constants, log k_e, versus the log of the hydrophobicity expressed by the octanol/water partition coefficient, log K_{ow}, for highly (C) and low (D) contaminated sediments.



Biotransformation of PAHs in worms

Two hydroxy-PAHs, 9-hydroxyphenanthrene (9-OH-PHE) and 1-hydroxypyrene (1-OH-PYR), were used as proxies to quantify the presence of hydroxy-metabolites in worm tissues and estimate metabolic activity of both worm species. After a 28-d exposure to sediments, between 5.3 and 32%, with a median value of 24%, of total phenanthrene in worm tissues was constituted by 9-OH-PHE and between 2.2 and 29%, with a median value of 20%, of total pyrene in worm tissues was constituted by 1-OH-PYR (Table 1). Results for three typical highly contaminated sediments and three low contaminated ones are illustrated in Fig. 3 and 4 for phenanthrene and pyrene, respectively. The ratio 9-OH-PHE/PHE was plotted alone with the PHE bioaccumulation curve for each sediment sample (Fig. 3). Highly contaminated B Lagoon (Fig. 3A) provides a straightforward example where ratio 9-OH-PHE/PHE increased quickly to a plateau as PHE was bioaccumulated in the worm tissues within 24h. No significant differences ($p > 0.05$) were seen in the following days and weeks. In most other cases, the 9-OH-PHE/PHE ratios increased rapidly in the first 24 to 72h, and then decreased and reached a plateau in the following days (Fig.3B, C, and D). At steady state, the 9-OH-PHE/PHE ratio ranged for about 0.3 to 0.5 with the exception of SLR Upstream (Fig.3E) where the ratio never exceeded 0.1 even with a quite high level of PHE in this low contamination sample. We found no evidence of a relationship between 9-OH-PHE/PHE and PHE concentrations in all samples studied.

Fig. 3. The ratio of 9-hydroxyphenanthrene to phenanthrene (•) and bioaccumulation kinetic of phenanthrene (○) during the exposure period in B Lagoon (A), Scrubber (B), SLR Downstream (C), EGSL04-07 (D), SLR Upstream (E), and Hospital Beach (F) sediments. Note different scales for phenanthrene concentration.



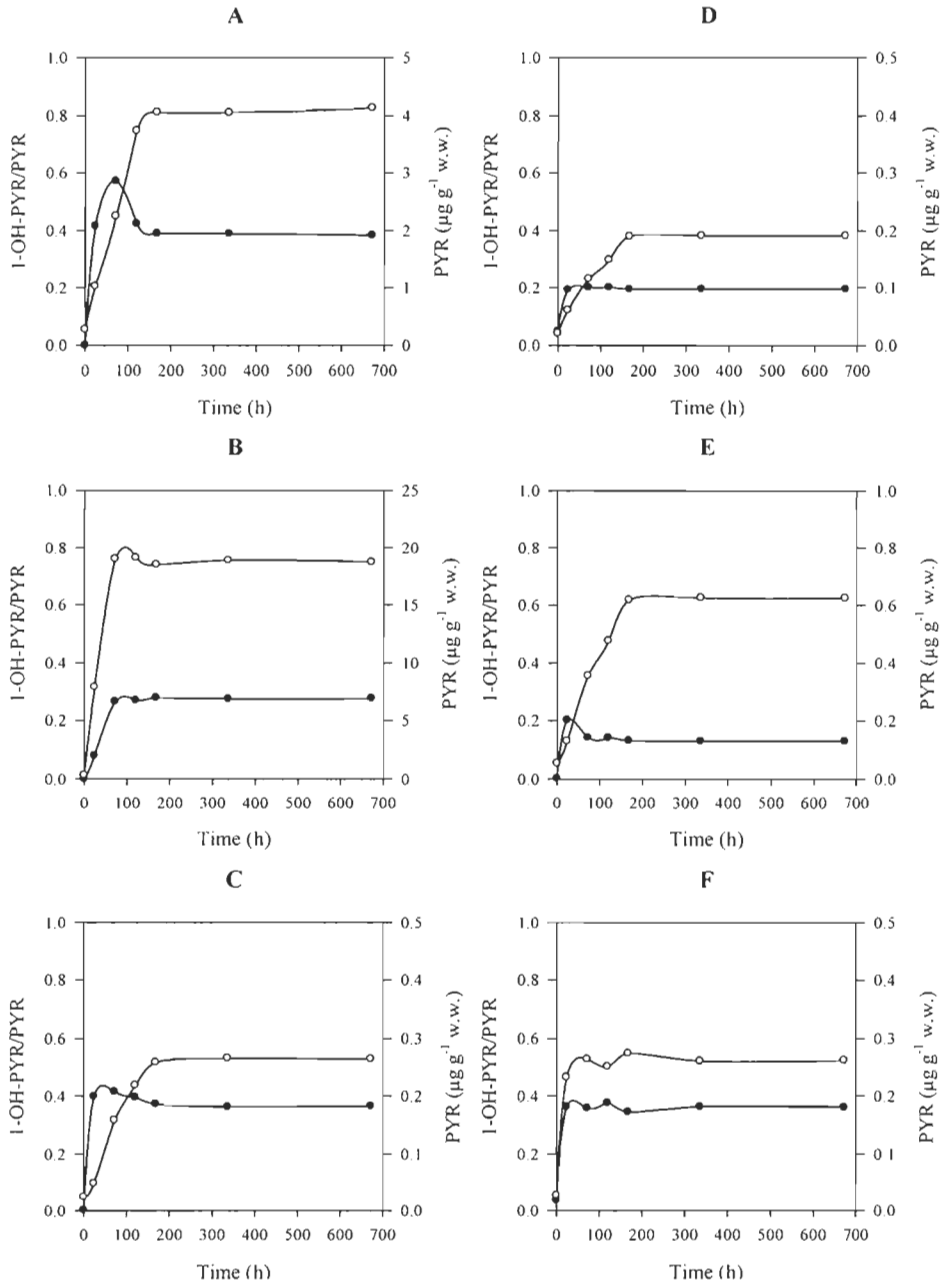
Pyrene and 1-hydroxypyrene showed a similar behavior to phenanthrene and its metabolite with a stable 1-OH-PYR/PYR ratio reaching a plateau after a few days (Fig.4). A peak is often observed early in the process (Fig. 4A and C), but seems not related to the concentration of pyrene in worms. 1-OH-PYR/PYR ratios ranged from 0.2 to 0.4 at steady state with the exception of SLR Upstream (Fig. 4E) where the ratio stabilized around 0.1 as previously observed for 9-OH-PHE/PHE.

Uptake rate constants (k_1), metabolization rate constant (k_2) and elimination rate constant (k_3) were calculated for individual OH-PAHs using Eq. 2 (Table 4). As previously observed in Table 2 for uptake rate (k_s) of the parent compound calculated with Eq.1, the uptake clearance rate constants (k_1) of phenanthrene and pyrene calculated with Eq. 2 are much lower in highly contaminated samples (averaged 9.3×10^{-4} for PHE and 3.4×10^{-4} for PYR) than in low contaminated ones (averaged 0.10 for PHE and 0.18 for PYR).

Metabolization rate constants (k_2) for phenanthrene ranged from 0.007 to 0.079 with an exceptional high value of 0.35 for B Lagoon sediment. Rate k_2 for pyrene ranged from 0.001 in EGSL04-13 to 0.14 in Hospital Beach.

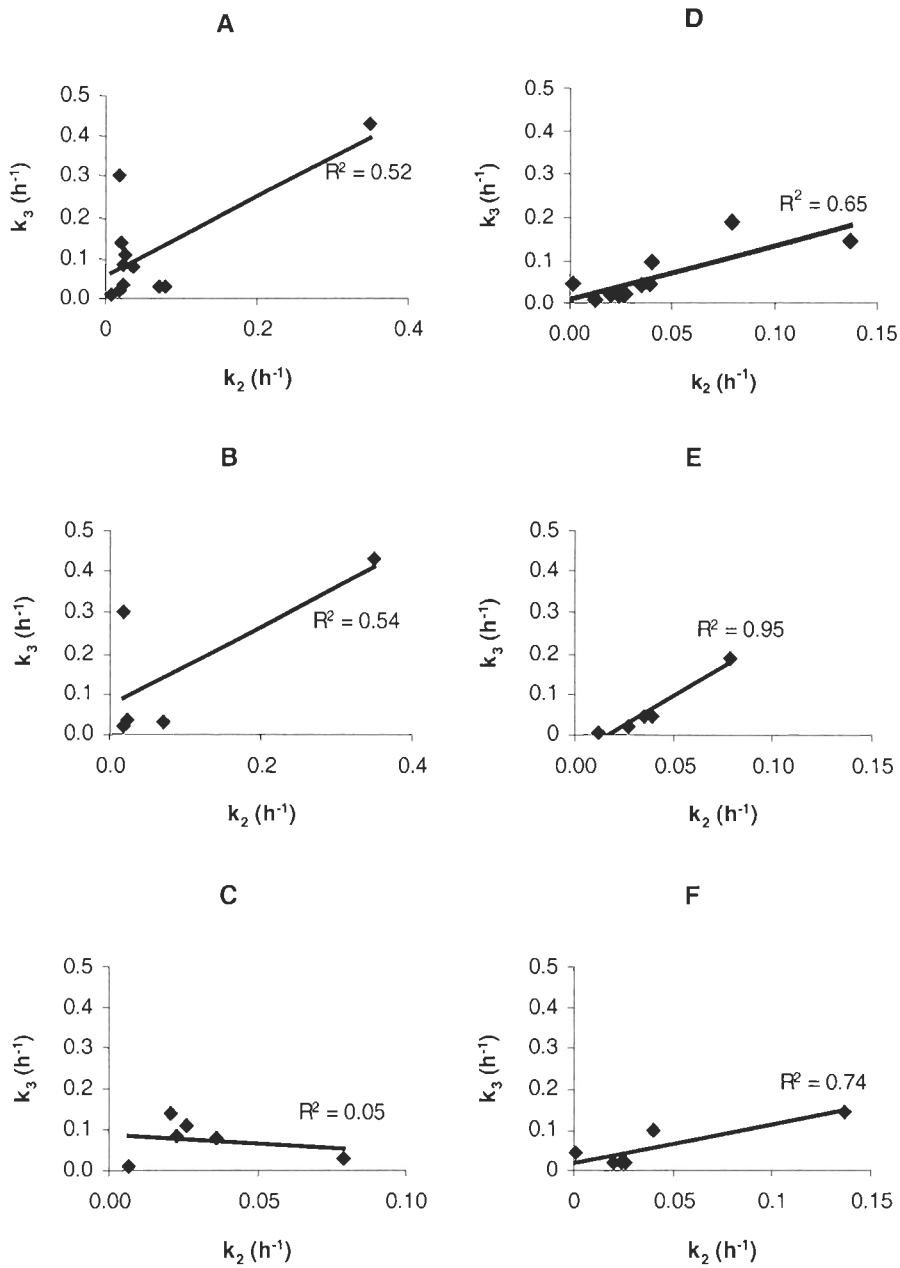
The elimination rate (k_3) of the phenanthrene metabolite ranged from 0.43 (in B Lagoon where k_2 is also high) to 0.0076 (in SLR Upstream where k_2 was particularly low). Rate k_3 for pyrene ranged from 0.01 in SLR Downstream to 0.19 in Scow Grid.

Fig. 4. The ratio of 1-hydroxypyrene to pyrene (•) and bioaccumulation kinetic of pyrene (○) during the exposure period in B Lagoon (A), Scrubber (B), SLR Downstream (C), EGSL04-07 (D), SLR Upstream (E), and Hospital Beach (F) sediments. Note different scales for pyrene concentration.



The relationship between the elimination rate constants (k_3) and the metabolization rate constants (k_2) is illustrated in Figure 5. The plots show that k_3 increased with increasing k_2 for both metabolites in all studied sediment samples with good correlation coefficients (values of R^2 between 0.52 and 0.95) with the exception of 9-OH-PHEN in low contaminated sediments ($R^2 = 0.05$). The best correlation coefficient is obtained for 1-OH-PYR in highly contaminated sediments ($R^2 = 0.95$).

Fig. 5. Plot of the elimination rate constants (k_3) versus the metabolization rate constants (k_2) for 9-hydroxyphenanthrene in all (A), highly (B), and low contaminated sediments (C) and for 1-hydroxypyrene in all (D), highly (E), and low contaminated sediments (F).



DISCUSSION

Bioconcentration of PAH in worms

The accumulation of organic compounds by *N. virens* and *L. variegatus* is complex and depends on the characteristics of the compound, its aging process in sediment, and the organism toxicokinetic characteristics. When these complex interactions are sorted out, the physicochemical properties of the compound are found to govern the accumulation of PAHs. From the relationships with log K_{ow}, we can say that the k_s values of compounds with log K_{ow} greater than 7 will be extremely small. The relationship between log k_s and log K_{ow} suggests that the desorption rate of the compound from the sediment is probably important in governing its accumulation by the organism. The change in the kinetics with increasing molecular lipophilicity expressed by log K_{ow} has been previously observed for *Pontoporeia hoyi* accumulation of PAHs from sediments (Landrum, 1989). Although the slopes were similar (-0.62 and -0.64 for highly and low contaminated sediments respectively), the displacement of the intercepts of the two lines (-0.29 and 2.4 for highly and low contaminated sediments respectively) (Fig. 2 Top panel) is thought to reflect the difference in bioavailability driven in part by differences in the total PAH content of the sediment.

Previous studies have shown that fastest elimination rate is generally for compounds with lower lipophilicity (Lotufo and Landrum, 2002). Our results are in contradiction with these results. Indeed, the present study shows now significant differences ($p > 0.05$)

between compounds with low and high lipophilicity. The difference between these two studies can mainly be due to the difference of studied species. Lotufo and Landrum (2002) studied a freshwater amphipod (*Diporeia* spp), whereas we studied a freshwater oligochaete (*L. variegatus*) and a marine polychaete (*N. virens*).

The propensity of individual PAHs to accumulate in worms can be estimated from their bioaccumulation factor. In the present study, BAFs estimated from k_s and k_e values were essentially similar to those estimated from PAH concentrations found in worm tissue and sediment samples (Table 3). At both exposure levels (highly and low contaminated sediments), BAFs decreased with increasing log K_{ow} values for different PAHs (Table 3).

As explained in Barthe et al. (2008), the low BSAF observed in highly contaminated samples can mainly be attributed to strong sorption to aluminum smelter residues well characterized by Breedveld et al. (2007).

Biotransformation of PAHs in worms

The studies of PAH metabolization capabilities of *L. variegatus* are contradictory. Indeed, some researchers (Harkey et al., 1994; Verragia Guerrero et al., 2002) found no PAH metabolites with *L. variegatus* whereas Leppänen and Kukkonen (2000), Lyytikäinen et al. (2007) and Schuler et al. (2003) found pyrene and benzo[a]pyrene metabolites in organism tissues. The differences between these two groups of studies was explained by Lyytikäinen et al. (2007) who attributed these differences in results to differences in experimental protocols. Indeed, the study by Harkey et al. (1994) was conducted at 10°C and the one by Verrengia Guerrero et al. (2002) was performed during 48 h only under an acute dosage regime, whereas studies by Leppänen and Kukkonen (2000), Lyytikäinen et

al. (2007) and Schuler et al. (2003) were performed at 20°C during 10 to 28 days. In the present study, the bioaccumulation tests were performed at 4-6°C for 28 days and PAH metabolites were observed in *L. variegatus* and in *N. virens* for all sediments tested The difference in temperature between the tests seems not to be the key factor to explain these differences between results. Even time scale seems not to be a proper explanation as we observed metabolites within 2-3 days after the beginning of the experiment. A part of the explanation may reside in metabolite detection methods used by different research teams.

We observed that total pyrene in *N. virens* tissues after a 28-day exposure was constituted by 17% 1-hydroxypyrene and 83% pyrene which is in accordance with the study of Jørgensen et al. (2005) who found 4% of 1-hydroxypyrene and 17% of pyrene toward total pyrene in *N. virens* gut tissue, which represents 23.5% of 1-hydroxypyrene compared to pyrene parent compound. According to this observation, we assumed that metabolites present in worm were mainly due to metabolization of the parent PAH instead of bioaccumulation from metabolites already present in low quantity in most sediment samples. Moreover, as worms are able to biotransform 1-hydroxypyrene and 9-hydroxyphenanthrene (phase I metabolites) to phase II metabolites (Giessing and Lund, 2002; Giessing et al., 2003; Jørgensen et al., 2005), we assume that the bioaccumulated phase I metabolites would be biotransformed to phase II metabolite quickly and would not be taken into account in the concentration measurements. The presence of 9-hydroxyphenanthrene and 1-hydroxypyrene, the phase I metabolites of phenanthrene and pyrene, respectively, in the present study supports the capability of *N. virens* and *L. variegatus* to metabolize 3- and 4-ring PAHs. This finding is in agreement with recent

Lyytikäinen et al. (2007) study in which 1-hydroxypyrene was found in *L. variegatus* tissues in sediment exposures with pyrene.

In the present study, the relative concentration of 9-hydroxyphenanthrene and 1-hydroxypyrene compared to the concentration of phenanthrene and pyrene, respectively, increased to a maximum, then decreased slightly in most case, and then reached the steady-state level after 24 to 216 h. These results are in contrast with the recent results of Lyytikäinen et al. (2007) who found that the ratio of 1-hydroxypyrene/pyrene increased linearly during the test and reached a value of ~ 1 after 15 days. Moreover, Lyytikäinen et al. (2007) observed that the concentration of 1-hydroxypyrene was the highest at the beginning of the test and decreased rapidly, whereas we found that the concentration of 9-hydroxyphenanthrene and 1-hydroxypyrene were the lowest at the beginning of the test and increased rapidly to reach a steady-state level (data not shown). Reaching a steady-state level means that elimination of the parent compound would be as efficient as biotransformation in worm tissues. This observation is confirmed by the high correlation coefficients obtained between the elimination rate constants (k_3) and the metabolization rate constants (k_2) (Fig. 5).

CONCLUSION

The results of the present study showed that *N. virens* and *L. variegatus* accumulated sediment-associated PAHs rapidly and achieved apparent steady-state within 7 days (168 h) for all studied sediment samples. In addition, the uptake clearance rate constant of PAHs

were lower for highly than for low contaminated sediments (10^{-2}-10^{-6} and 10^{-1}-10^{-4} g dry sediment g^{-1} wet organism h^{-1}, respectively), whereas the elimination rate constant of PAHs remained in same order of magnitude for both type of sediments. The relationship between log k_s and log K_{ow} suggests that the desorption rate of the compound from the sediment is probably important in governing the accumulation by the worms.

To our knowledge, the present study is the first one to report toxicokinetic data of PAH biotransformation in metabolites. Indeed, other researchers have studied the occurrence of phase I and phase II PAH metabolites in organisms during bioaccumulation and toxicokinetic studies in polychaetes and oligochaetes (Christensen et al., 2002; Jørgensen et al., 2005; Leppänen and Kukkonen, 2000; Lyytikäinen et al., 2007), but none of them has studied accumulation, or metabolization, or elimination kinetics of these metabolites. Our work opens a window in the study of the fate of PAH metabolites which deserves more scientific attention from reseachers. Additional experiments are needed to determine the possible input of microorganism-originating metabolites in worms versus the metabolic formation rate of PAH metabolites by worms. Indeed, we assumed that the PAH metabolites present in worms came mainly from the internal biotransformation of corresponding parent PAH in such a proportion that the bioaccumulated metabolites were negligible, but it would be interesting to know this proportion in order to study their bioaccumulation, biotransformation into phase II metabolites, and elimination kinetics.

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CONCLUSION GÉNÉRALE & PERSPECTIVES

Conclusions générales

Traditionnellement, les concentrations en hydrocarbures aromatiques polycycliques (HAP) dans les sols et les sédiments ont été déterminées après des extractions solide/liquide vigoureuses utilisant des solvants organiques, tels que le dichlorométhane ou l'hexane/acétone. Cependant, quand les contaminants organiques hydrophobes, tels que les HAP, entrent dans les sols ou sédiments, ils subissent un certain nombre de processus de perte ou de transport incluant la volatilisation et le lessivage, aussi bien que de la dégradation chimique et biologique qui peuvent modifier leur comportement. De plus, les processus de sorption peuvent permettre à ces composés chimiques de devenir plus résistants à l'extraction par des solvants ou d'être irréversiblement liés à la matrice interne des sols ou sédiments. Le résultat de ce processus d'altération avec le temps est une diminution de l'extractabilité des HAP. Ce phénomène a été appelé "effet de vieillissement" et est relié à un déclin de leur disponibilité pour l'absorption par les organismes benthiques et leur biodégradabilité par les microorganismes. Un effort important en recherche a été récemment consacré au développement de méthodes chimiques fiables afin de déterminer la partie biodisponible présente dans les sols et sédiments.

Les travaux de cette thèse ont donc cherché à déterminer une ou plusieurs méthodes chimiques dites douces ou sélectives permettant de caractériser les concentrations en HAP biodisponibles dans des sédiments de niveaux de contamination et de propriétés géochimiques variables mais également à établir les patrons temporels d'accumulation des

HAP dans ces échantillons dans deux espèces de vers en identifiant les paramètres toxicocinétiques décrivant l'ingestion, l'élimination et les facteurs de bioaccumulation.

Ainsi, trois séries d'expériences ont été effectuées. La première a permis de comparer trois différentes méthodes d'extraction solide/liquide (n-butanol (BuOH), hydroxypropyl-β-cyclodextrine (HPCD), et une solution de tensioactif de Brij700 (B700)) et la seconde a comparé l'efficacité de deux échantillonneurs passifs, des bandes de polyoxyméthylène (POM) et des tubes de polydimethylsiloxane (PDMS). Les résultats de ces deux études ont été comparés avec les résultats de tests de bioaccumulation sur 28 jours effectués sur les mêmes sédiments avec *Nereis virens* pour les sédiments marins et *Lumbriculus variegatus* pour les sédiments lacustres. De plus, les résultats de la seconde étude ont été comparés avec ceux obtenus lors de la première étude avec la solution aqueuse de B700. La troisième expérience a permis de déterminer les patrons temporels d'accumulation des différents HAP par les vers (*N. virens* et *L. variegatus*) dans les différents sédiments étudiés ainsi que l'habilité des organismes à métaboliser le phénanthrène et le pyrène par la détermination des hydroxy-HAP correspondants (9-hydroxyphénanthrène et 1-hydroxypyrène).

Les résultats obtenus lors de cette première étude montrent que des différences importantes ont été observées aussi bien dans la quantité que dans les proportions des HAP obtenues en utilisant différentes techniques pour déterminer la biodisponibilité des HAP dans des sédiments. Bien que plusieurs recherches ont suggéré qu'une extraction dite douce utilisant du BuOH pouvait être un moyen approprié pour la détermination des HAP biodisponibles, nos résultats sont en désaccord avec les études précédentes aussi bien pour les sédiments faiblement que fortement contaminés. Nos travaux montrent que le BuOH

extrayait plus que ce que les vers pouvaient bioaccumuler dans leurs tissus, et que le HPCD sous-estimait la biodisponibilité des HAP spécialement les HAP de faible poids moléculaire. D'autre part, l'utilisation du tensioactif B700 a montré une bonne capacité prédictive pour les HAP dans les sédiments fortement contaminés. Les études en laboratoire conduites avec du sédiment fraîchement additionné de HAP marqué peuvent surestimer la biodisponibilité des contaminants comparée aux situations de terrain où des temps d'équilibrage entre le sédiment et les contaminants persistants plus grands réduisent cette biodisponibilité (Conrad et al., 2002; Leppänen et Kukkonen 2000a). De plus, la plupart de ces études déterminaient la biodisponibilité en mesurant la dégradation microbienne, une approche qui peut ne pas être comparable avec la bioaccumulation des HAP par des vers puisque les vers ne sont pas des échantillonneurs passifs et peuvent modifier le profil du HAP dans leurs tissus.

Puisque l'évaluation du risque des sols contaminés est actuellement surestimée par l'utilisation d'analyses chimiques qui s'en remettent à une extraction vigoureuse initiale, il est essentiel d'inclure la détermination de la bioaccumulation dans les décisions de réglementation. L'utilisation de l'ingestion par les vers et la biodégradation sont encore les meilleurs outils pour estimer la biodisponibilité des HAP, mais les méthodes biologiques sont longues et coûteuses. Nos résultats illustrent la difficulté à trouver une méthode chimique adéquate pour prédire la biodisponibilité des HAP, particulièrement parce que les concentrations en HAP et le processus de séquestration jouent un rôle déterminant dans la qualité des résultats et ceci en fonction de paramètres environnementaux et historiques le plus souvent inconnus de l'utilisateur. Puisque le B700 est peu coûteux et que les solutions

sont simples à préparer, une procédure d'extraction utilisant ce tensioactif de haut poids moléculaire se révèle être une méthode utile particulièrement pour les sédiments présentant des niveaux en HAP >2 $\mu g\ g^{-1}$ et avec des log K_{ow} <5,8.

Les résultats de notre seconde étude montrent que les deux échantillonneurs passifs agissent de manière différente par rapport aux mêmes échantillons de sédiment. En effet, le PDMS surestime la disponibilité des HAP dans les sédiments étudiés (faiblement et fortement contaminés), alors que le POM produit des résultats similaires à l'extraction au B700. Quoi qu'il en soit, le POM et le B700 prédisent mal la disponibilité des HAP dans les sédiments faiblement contaminés où les facteurs biologiques (matière organique digestible) deviennent importants. Par contre, la biodisponibilité des HAP totaux a été prédite correctement par le POM et le B700 dans les sédiments fortement contaminés par des alumineries. Un examen plus détaillé des résultats obtenus pour les HAP individuels indique que les deux techniques surestiment la disponibilité des grosses molécules avec un log K_{ow} >6 suggérant un mécanisme biologique limitant l'incorporation des plus grosses molécules de HAP ce qui semble être relié à la taille moléculaire des composés ou à leur encombrement stérique; un mécanisme non directement relié à la liposolubilité de la molécule.

Les résultats de la troisième étude ont montré que *Nereis virens* et *Lumbriculus variegatus* accumulaient rapidement les HAP associés au sédiment et atteignaient un état-stable apparent en 7 jours (168 h) pour tous les sédiments étudiés. De plus, les constantes du taux d'ingestion des HAP sont plus faibles pour les sédiments fortement contaminés que pour les faiblement contaminés (10^{-2}-10^{-6} et 10^{-1}-10^{-4} g sédiment sec g^{-1} organisme humide

h^{-1}, respectivement), alors que la constante du taux d'élimination des HAP sont du même ordre de grandeur pour les deux types de sédiments. La relation négative entre log k_s et log K_{ow} suggère que le taux de désorption du composé à partir du sédiment est probablement important pour gouverner l'accumulation par les vers.

A notre connaissance, l'étude présente est la première à rapporter des données de toxicocinétiques de biotransformation de HAP en métabolite. En effet, les autres chercheurs ont étudiés l'apparition des métabolites de phase I et II dans les organismes pendant les études de bioaccumulation et de toxicocinétiques chez les polychètes et oligochètes (Christensen et al., 2002; Jørgensen et al., 2005; Leppänen et Kukkonen, 2000b; Lyytikäinen et al., 2007), mais aucun de ces chercheurs n'avaient étudié les cinétiques d'accumulation, ou de métabolisation ou d'élimination de ces métabolites. Nos travaux ouvrent une porte dans l'étude du devenir des métabolites de HAP qui nécessite une attention scientifique plus importante de la part des chercheurs. Des expériences additionnelles sont nécessaires afin de déterminer l'apport possible de métabolites provenant de microorganismes dans les vers par rapport au taux de transformation des HAP par les vers eux-mêmes. En effet, nous avons présumé que les métabolites présents dans les vers provenaient principalement d'une biotransformation interne des HAP parents correspondants dans une telle proportion que les concentrations de métabolites bioaccumulés étaient négligeables, mais il serait intéressant de connaître cette proportion afin d'étudier leurs cinétiques de bioaccumulation, de biotransformation en métabolites de phase II, et d'élimination.

Conclusion

La réalisation de ces travaux nous aura permis d'atteindre l'ensemble des objectifs fixés. Nous avons pu mettre au point deux méthodes d'extraction sélective permettant de déterminer les HAP totaux biodisponibles dans les sédiments fortement contaminés par des alumineries et plus précisément les HAP avec un log K_{ow} <5,8. De plus, l'étude de la bioaccumulation nous a montré que la composition et le niveau de contamination jouaient un rôle sur la biodisponibilité des HAP. On a pu voir que les vers bioaccumulaient, en proportion, plus de HAP dans les sédiments faiblement contaminés que dans les fortement contaminés. Cette étude nous a aussi permis de déterminer que les divers paramètres toxicocinétiques étudiés (ingestion, élimination, facteurs de bioaccumulation) variaient d'un sédiment à l'autre et d'un HAP à l'autre.

Tout au long de ce travail, nous avons parlé de biodisponibilité en général sans prendre en compte les définitions établies par Semple et al. (2004) sur la biodisponibilité et la bioaccessibilité car le but de ce travail n'était pas d'entrer dans le débat à propos des définitions pratiques de ces deux termes. Nous allons maintenant tenter de faire un lien entre ces définitions et nos travaux. Récemment ter Laak et al. (2006) ont scindé en deux les différentes méthodes d'extraction selon les critères de bioaccessibilité et de biodisponibilité. Premièrement, les méthodes qui extraient la fraction faiblement liée, dont fait partie le B700, et qui est selon ter Laak et al. (2006) la fraction bioaccessible, et deuxièmement les méthodes qui extraient les concentrations dissoutes libres présentes dans les eaux porales, ce qui inclus les échantillonneurs passifs tels que le POM et le PDMS, et

qui est appelée la fraction biodisponible. Selon les définitions de Semple et al. (2004), la partie bioaccessible est en théorie plus importante que la partie biodisponible puisque, selon ces mêmes auteurs, la bioaccessibilité englobe ce qui est réellement biodisponible et ce qui est potentiellement biodisponible. Théoriquement, si on se fie à ces définitions, les concentrations extraites par le B700 devraient être plus importantes que celles extraites par le POM ou les vers puisque ces deux dernières méthodes déterminent la partie biodisponible d'un contaminant. Or nos résultats montrent que ce n'est pas le cas. En effet, comme on a pu le voir dans le chapitre 2, les résultats obtenus avec le B700 sont similaires à ceux du POM c'est à dire une bonne estimation de la biodisponibilité des HAP de log K_{ow} <6 dans des sédiments fortement contaminés par des rejets d'alumineries. Ceci peut s'expliquer par le fait que l'extraction des concentrations biodisponibles dans les différents sédiments ne se fait pas instantanément. En effet, celle-ci dure 16 h pour le B700 et 21 jours pour le POM. En fait, les deux méthodes chimiques, ainsi que la méthode biologique (bioaccumulation par les vers), extraient la partie bioaccessible du contaminant car, selon la définition de Semple et al. (2004), la fraction biodisponible est la fraction librement disponible à un moment donné et la fraction bioaccessible est la fraction qui est disponible à ce moment donné et celle qui sera disponible plus tard.

Perspectives

De nombreux points demandent encore à être explorés et concernent en premier lieu l'aspect purement chimique. En effet, au cours de ces travaux, on a pu voir que les méthodes que nous avons proposées comme étant des méthodes adéquates pour prédire les concentrations biodisponibilites des HAP (B700 et POM) avaient des comportements

différents par rapport aux sédiments faiblement et fortement contaminés. Donc, le premier point qui pourrait être à développer est la détermination de la "limite de détection" de ces méthodes afin de définir la concentration en HAP totaux à partir de laquelle les concentrations extraites avec les méthodes chimiques deviennent en accord avec celles biodisponibles pour les organismes. Lors de nos travaux, le sédiment avec la concentration la plus élevée parmi les échantillons faiblement contaminés était la station EGSL04-07 avec une concentration totale en HAP de 1,1 $\mu g\ g^{-1}$ et celui avec la concentration la moins élevée parmi les échantillons fortement contaminés était la station SLR Downstream avec une concentration totale en HAP de 25,5 $\mu g\ g^{-1}$. On peut voir qu'il y a un facteur 23 entre les deux sédiments. Le but de cette étude serait donc de combler le trou entre ces deux échantillons en trouvant des échantillons naturels avec des concentrations en HAP totaux comprises entre 1,1 et 25,5 $\mu g\ g^{-1}$.

Deuxièmement, afin d'étendre la connaissance et les applications du POM et du B700 pour des composés organiques autres que les HAP, d'autres expériences pourraient être effectuées afin d'explorer leur performance à mesurer la fraction disponible de composés organiques tels que les BPC. Le POM a une structure rigide et une excellente résistance mécanique, ce qui fait de lui un échantillonneur potentiel pour des mesures in situ de la concentration dans l'eau porale. Quoi qu'il en soit, il y a encore quelques considérations à aborder par rapport au déploiement sur le terrain. Premièrement, il est difficile de contrôler la température sur le terrain et les variations de la température peuvent affecter l'état d'équilibre et les taux d'extraction des HAP par le POM. Ainsi il est essentiel de déterminer la déviation des performances du POM par rapport au calibrage fait en laboratoire causée

par les fluctuations de température sur le terrain. Ceci peut être accompli en effectuant des études sur les isothermes de sorption et sur la cinétique d'extraction en laboratoire à différentes températures contrôlées. Deuxièmement, la complexité de l'hydrodynamisme rencontré sur le terrain peut aussi influencer les taux d'échantillonnage du POM par rapport aux composés cibles; ainsi plusieurs tests sont suggérés afin d'examiner les effets de différents débits d'eau. De plus, d'autres paramètres importants tels que l'épaisseur de la membrane, l'aire de la surface de contact doivent aussi être évalués soigneusement afin d'optimiser les performances du POM dans ses applications sur le terrain.

Certains aspects biologiques peuvent aussi être explorés. En premier lieu, *Lumbriculus variegatus* n'est généralement pas testé à des températures aussi faibles (4 à 6°C) mais à une température d'environ 20°C. Deux possibilités s'offrent à nous, la première étant de recommencer les expériences de bioaccumulation des sédiments dulcicoles à une température de 20°C et la deuxième étant de trouver un autre organisme qui peut être utilisé à une température proche de celle que nous avons étudiée. Lors de nos tests de bioaccumulation, nous avions choisi d'avoir le moins de différence possible entre nos tests avec les sédiments marins et les sédiments lacustres. C'est pour cela que les tests avec les sédiments lacustres ont eu lieu à 4-6°C. Afin de continuer dans ce processus, il serait préférable d'utiliser des organismes qui peuvent être testés à ces faibles températures. Deux espèces d'oligochaetes d'eau douce peuvent être utilisées, *Limnodrilus hoffmeisteri* et *Tubifex tubifex* ainsi qu'une espèce d'amphipode, *Diporeia* spp.. De plus, une étude a déjà utilisé *L variegatus* à une température de 10°C (Kraaij et al., 2002).

De plus, lors des études de bioaccumulation seule une espèce marine (*Nereis virens*) et une espèce dulcicole (*Lumbriculus variegatus*) ont été utilisées. Bien que ces deux espèces soient recommandées par l'Agence de Protection de l'Environnement Américaine (US EPA) pour les tests de bioaccumulation dans les sédiments, d'autres espèces peuvent et sont utilisées pour ces tests. En effet, de nombreux chercheurs utilisent d'autres polychaetes (*Nereis diversicolor*, *Arenicola marina*) (Cornelissen et al., 2006; Kaag et al., 1997), des bivalves (*Mytilus edulis*, *Macoma balthica*) (Kaag et al., 1997), des gastéropodes (*Hinia reticulata*) (Cornelissen et al., 2006), des amphipodes (*Diporeia* spp.) (Landrum et al., 2007) ou bien la biodégradation par des bactéries (Liste et Alexander, 2002) pour déterminer la biodisponibilité des contaminants étudiés. Comme ces organismes ont déjà été utilisés, il nous serait possible d'effectuer des tests de bioaccumulation avec ces différents organismes afin de déterminer si les deux méthodes chimiques peuvent prédire la biodisponibilité des HAP seulement pour les deux sortes de vers ou alors pour d'autres sortes d'espèces.

Finalement, nous avons étudié seulement deux métabolites de HAP, le 9-hydroxyphénanthrène et le 1-hydroxypyrène. Une autre perspective serait de déterminer des métabolites d'autres HAP tels que le benzo[a]pyrène, le chrysène ou le naphtalène ou encore de déterminer d'autres métabolites du phénanthrène et du pyrène. De plus, il serait aussi bon de déterminer avec précision si les courbes de cinétique de métabolisation obtenues lors de l'étude de bioaccumulation représentent bien la métabolisation ou si elles sont des courbes de bioaccumulation de métabolites ou encore si elles représentent un mélange de bioaccumulation des métabolites et de métabolisation. Pour ce faire,

l'utilisation de métabolites marqués au C-13 serait un bon moyen pour déterminer quelle hypothèse est la bonne et si c'est la troisième, cela permettrait de déterminer en quelle proportion.

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