Mise en œuvre de la technique des membranes semi-perméables pour la caractérisation des teneurs en polluants organiques hydrophobes

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1. Les SPMD comme réponse au faible niveau de concentration en phase liquide

L'évaluation du niveau de contamination du bassin par les molécules organiques est particulièrement difficile à obtenir pour au moins deux raisons majeures : (i) les molécules en questions sont en grand nombre, d'origines très variées), et ont des devenirs forts différents (durée de vie dans le milieu, comportement physico-chimique etc…), et (ii) certaines d'entre elles sont dangereuses et/ou présentes à de très faibles concentrations. Ainsi, des méthodes lourdes et coûteuses doivent être déployées pour les rechercher dans le milieu (eau, sédiments, organismes…), comprenant les étapes de collecte, de pré-traitement puis d'analyse des échantillons. Les tendances actuelles de la législation nationale et européenne avancent aujourd'hui sur deux points qui concernent particulièrement les contaminants organiques : (i) la notion de bon état écologique qui inclut le niveau de contamination des organismes et les conséquences sur leur physiologie (ii) et la production de listes de substances à risque qui incluent quelques métaux mais surtout beaucoup de contaminants organiques.

Les questions de la représentativité des échantillons et des limites de détection se posent de manière moins sensible pour les sédiments et les organismes car dans les deux cas il y tendance à l'accumulation pour beaucoup de molécules[1] (bioaccumulation ou sorption) en liaison avec leur caractère plus ou moins hydrophobe, et parce que organismes et sédiments ont généralement des temps de séjour suffisants dans le milieu pour permettre une évaluation intégrée de la contamination

[1] Mais certainement pas de manière équivalente pour la totalité des produits cependant.

du milieu, au moins qualitativement, sous réserve que l'échantillonnage soit réalisé avec discernement. C'est dans la partie liquide du système que les problèmes d'échantillonnage se posent car on ne dispose pas de matrice accumulatrice et que les contaminants sont très dilués, ce qui induit de nouvelles difficultés à l'analyse : seuils analytiques trop hauts, bruit de fond trop fort pour identifier et quantifier les contaminants.

Les systèmes SPMD (Semi-Permeable Membrane Devices) ont été créés pour répondre à ce problème. Il s'agit d'un système d'échantillonnage intégratif, composé d'une membrane en polyéthylène contenant une lipide (trioleine) et qui fonctionne en dialysant le milieu externe (Huckins et al., 1993). La trioleine ne peut pas franchir la membrane, et les HAP, comme la plupart des autres contaminants hydrophobes, diffusent vers la trioleine où ils peuvent se concentrer en fonction de leur hydrophobie. La trioleine est un lipide naturellement présent dans de nombreux organismes, ce qui a valu aux systèmes SPMD le surnom de "poisson artificiel". De nombreuses études en laboratoire ont permis de mettre en évidence des corrélations entre les facteurs de concentration à l'équilibre dans les SPMDs et ceux dans divers organismes biologiques et ceci pour bon nombre de contaminants comme par exemple pour des pesticides avec *Anodonta piscinalis* [Sabaliunas et al, 1998], pour des chlorophénols avec *Carassius auratus* [Wang et al, 1998]. In situ, ces corrélations sont moins évidentes à cause notamment des temps d'exposition des SPMDs nécessairement plus longs pour atteindre l'équilibre. Même s'il n'est pas possible d'établir simplement des relations systématiques entre les concentrations mesurées in situ dans les SPMDs et les organismes ou le sédiment, du fait des processus chimiques et biologiques différents entre ces systèmes (durée d'équilibration, métabolisation, dégradation, contamination par voie particulaire possible chez les organismes…), il est néanmoins envisageable de caractériser le danger toxique par identification des substances présentes dans les SPMDs exposées in situ. La SPMD a déjà été utilisée avec succès dans les milieux aquatiques pour échantillonner une grande panoplie de familles de molécules: des pesticides organochlorés, des polychlorobiphényles (PCB), des hydrocarbures aromatiques polycycliques (HAP) [Petty et al, 1995 et 1998], des dioxines et assimilés (polychlorés dibenzodioxines : PCDD, et dibenzofuranes : PCDF) [Lebo et al, 1995, Rantalainen et al, 1998], des alkylphénols dont le nonylphénol [Bennett, E. R. et al, 2000], des insecticides organophosphorés ou pyrèthroïdes, des nitroanilines [Sabaliunas, D. et al 1997], des diphényléthers polybromés [Booij K. et al, 2002] et même des organométalliques [Folsvik et al, 2000 et 2002].

Il y a cependant une différence significative entre l'évaluation de la biodisponibilité, ou de son ersatz, la SPMD-disponibilité, et celle de la concentration dissoute. En effet, comme pour les métaux (voir Tusseau-Vuillemin et al., ce volume), seule une fraction des contaminants dissous sera capable de traverser les membranes biologiques ou la membrane des SPMD. Pour bien comprendre les relations qui existent entre concentrations dissoutes et concentrations mesurables par SPMD, il est nécessaire d'évaluer également le rôle des matières organiques dissoutes qui sont probablement capables de retenir une partie des contaminants organiques. Cet effet de rétention en phase dissoute devrait être sensible de la même façon pour les SPMD et pour des organismes modèles.

2. Rôle potentiel des matières organiques dissoutes sur la disponibilité des HAP.

Ces travaux ont été réalisés en utilisant des daphnies comme molécules modèles. Un article, synthésisant les résultats obtenus a été récemment soumis. Il est donné en annexe, et nous en rappelons ici les principaux résultats (Gourlay et al, soumis, donné en annexe).

Des essais au laboratoire ont été entrepris pour mesurer la capacité de la matière organique dissoute à limiter la biodisponibilité des HAP. Le HAP modèle utilisé est de benzo(a)pyrène (BaP), alors que la daphnie est l'organisme modèle. Tous les essais sont réalisés dans des conditions standards, avec de daphnies d'une même lot (en reproduction asexuée) et de même âge. Elles sont exposées durant 4 heures alors que le BaP était mis en solution une heure avant les daphnies. Les concentrations utilisées sont relativement élevées, l'équivalent de 1 $\mu g.L^{-1}$ étant mis en solution, et dont nous avons prouvé que seulement 60% restaient en solution dans les conditions expérimentales utilisées.

Il est connu depuis longtemps que les acides humiques, aux concentrations natuelles sont capables de limiter fortement la biodisponibilité des HAP. Mais nous avons montré récemment que d'autres molécules organiques naturellement présentes dans les milieux naturels ont également cette propriété (Gourlay et al., 2003). Il était alors important de chercher à mieux caractériser le rôle potentiel de ces molécules. Pour disposer d'un ensemble de molécules susceptibles d'être présentes en Seine, nous avons procédé à des essais de biodégradation de deux types de matières organiques : du Vandox®, essentiellement composée de protéines et glucides d'origine animale et des extraits d'algues réalisés après culture au laboratoire. Les expériences de biodégradation duraient 15 à 20 jours.

Les résultats sont exprimés sous la forme de K_{DOC}, qui caractérisent l'affinité des matières organiques dissoutes pour le BaP. Ils sont estimés à partir de l'expression suivante qui donne la fraction biodisponible, que nous mesurons par accumulation dans les daphnies, et la concentration de matière organique présente (notée [DOC]) :

$$\frac{F}{F_0} = \frac{1}{1 + K_{DOC}[DOC]}.$$

Quel que soit le substrat initial (Viandox ou extrait d'algues), on note une forte augmentation du K_{DOC} avec le temps, c'est à dire avec le niveau de dégradation. Les K_{DOC} en fin d'expérience atteignent des valeurs de 2 à 3 10^4 qui deviennent comparables avec celles des acides humiques.

Parallèlement, nous avons mesuré l'absorbance UV des matières organiques. En effet l'absorption des la lumière dans l'UV est principalement le fait des cycles aromatiques, et ces cycles constituent des sites très hydrophobes au sein des macromolécules organiques, en particulier dans les acides humiques. Le SUVA (Specific UV absorbance) est ainsi un indicateur d'hydrophobie facile à mettre en œuvre.

Alors que les K_{DOC} augmentaient significativement pour les deux matières organiques dégradables modèles, il n'en a pas été des même des SUVA, qui sont restés faibles dans l'essai réalisé à partir d'exsudats algaux. Il est probable que ce phénomène soit du à la présence d'alginates. Ces composés d'origine algale sont exempts de cycles ont de longues chaînes alkyles, Ils sont susceptibles d'interactions hydrophobes sans toutefois absorber la lumière UV.

Ces résultats confirment l'importance des composés non humiques, présents dans les systèmes anthropisés, sur la disponibilité des HAP, qu'ils soient apportés au milieu sous forme de rejet ou produits *in situ*. On a montré que ces composé étaient susceptibles de faire baisser la fraction biodisponible à moins de 50% de la fraction dissoute totale pour des concentrations en COD inférieures à 5 $mg.L^{-1}$, soit tout à fait compatibles avec celles qu'on rencontre dans le bassin de la Seine. Ces composés actifs du point de vue de la séquestration des composés hydrophobes se forment après quelques jours de dégradation des substances fraîches, ils pourraient être en partie constitués de sous-produit de la dégradation microbienne (SMP : Soluble Microbial Products).

3. Evaluation du niveau de contamination par des SPMD *in situ*

L'utilisation standard des SPMD consiste à les immerger pour un certain temps (quelques jours ou semaines) dans un milieu et à observer l'accumulation des composés hydrophobes d'intérêt (tous les composés hydrophobes sont accumulés). Tant qu'on est suffisamment éloigné de l'équilibre final entre le SPMD et le milieu extérieur, l'accumulation est sensiblement linéaire et réputée proportionnelle à la concentration disponible dans le milieu. Les concepteurs des SPMD (Huckins et al., 1993) ont proposé des valeurs de R_S pour faire la relation entre la concentration disponible externe et la vitesse d'accumulation. Le R_S (en $L.j^{-1}$) est en quelques sorte le volume d'eau de Seine dans lequel un composés hydrophobes est capté tous les jours. En fait, la mécanique du procédé n'est pas simplement la captation de tous les composés dans un volume d'eau donné, mais la diffusion dans l'eau puis à travers la membrane. Les valeurs de RS sont donc dépendantes de plusieurs facteurs, dont le composé lui-même, la température et dans une certaine mesure de la vitesse de l'eau.

Les SPMD ont été déployées dans le bassin de la Seine et aussi dans des stations d'épuration lyonnaise à titre de comparison. Le déploiement dans des stations d'épuration du bassin de la Seine est

prévu en 2004. Les résultats obtenus font l'objet d'une publication acceptée dans Polycyclic Aromatic Hydrocarbons (Miège et al.), donnée en annexe, et dont nous rapportons ici les traits essentiels.

Les SPMD ont été placées en trois stations de mesure en Seine, aux Quatre Cents, un petit ruisseau forestier dans le bassin de l'Orgeval, à Saint Maurice en Marne et à Andrésy en Seine. Les campagnes de mesure sont été réalisées en février et en avril. En mai, des SPMD ont été placées à l'exutoire de stations d'épuration.

L'accumulation observée est raisonnablement linéaire en fonction du temps, ce qui suugère que le modèle proposé par Huckins peut être utilisé. L'utilisation de R_S mesurés au laboratoire (Huckins et al., 1999) permet d'estimer des concentrations disponibles, et réputées dissoutes et non complexées. Comme on pouvait s'y attendre, les concentrations obtenues augmentent d'amont en aval, mais elles sont relativement élevées (plus de 100 $ng.L^{-1}$ en HAP totaux à Andrésy). La comparaison avec les concentrations totales montre des facteurs de disponibilité (availability indicator : le rapport entre la concentration estimée par les SPMD et la concentration totale) souvent supérieurs à 1, alors qu'on s'attendrait au contraire à ce que la fraction disponible ne soit qu'une partie de la fraction dissoute, soit une petite partie de la fraction totale (les valeurs supérieures à 1 sont forcément irréalistes). Pour des raisons de limite de détection, nous n'avons hélas que peu de données de HAP dissous pour comparer aux accumulations dans les SPMD. D'une certaine façon, ceci confirme l'intérêt des SPMD qui permettent de s'affranchir de ces problèmes.

Malgré le biais apparent dans la réponse absolue de la SPMD, un certain nombre d'éléments concordants ressortent néanmoins de l'analyse des différents facteurs susceptibles d'influencer l'accumulation par les SPMD. Par exemple, les plus faibles aromaticités de la matière organique dissoute donnent des accumulations plus fortes, de même que les plus faibles biodégradabilité, ce qui est cohérent avec les résultats obtenus avec les daphnies et le BaP. De même de plus fortes teneurs en suspensions, également susceptibles de piéger des HAP sous une forme non disponible tendent à diminuer les accumulations.

Il ressort de ces premières applications *in situ* des SPMD que les facteurs attendus répondent dans le bon sens, mais que d'autres facteurs mal contrôlés nuisent à la qualité de l'ensemble et en tous cas occasionnent des vitesses d'accumulation plus fortes que celles qui ont été mesurées au laboratoire. Pour parvenir à des résultats mieux quantifiés, il est nécessaire de mieux quantifier le comportement des SPMD in situ, en particulier en fonction des conditions d'agitation du milieu. Les Permeability Reference Compounds (PRC) injectés au sein de la SPMD avant son immersion, et dont on peut mesure la fuite hors des SPMD, fournissent une solution potentiellement intéressante.

4. Utilisation des PRC (Permeability Reference Compounds)

4.1. Fonctionnement des SPMD

4.1.1 Modèle de diffusion simple

Théoriquement, en prenant un modèle d'accumulation simple, on peut simuler le comportement d'un SPMD par les équations suivantes en reprenant les notations proposées par Huckins et al. (1999) :

$$\frac{dC_{SPMD}}{dt} = k_u C_W - k_e C_{SPMD}.$$

Toutes les concentrations, dans l'eau comme dans la SPMD sont données en $ng.L^{-1}$.

A l'équilibre, on a :

$$\frac{C_{SPMD}}{C_W} = K_{SMPD} = \frac{k_u}{k_e}$$

En phase d'accumulation linéaire, soit lorsque $k_u C_W >> k_e C_{SPMD}$, soit encore lorsqu'on est encore loin de l'équilibre on a :

$$\frac{dM_{SPMD}}{dt} = V_{SPMD}\frac{dC_{SPMD}}{dt} = V_{SPMD}k_u C_W = V_{SPMD}k_e K_{SPMD} C_W = R_S C_W ,$$

ce qui définit R_S, qu'on peut considérer comme équivalent au volume d'eau échantillonné par le SPMD chaque jour. Attention, en réalité le processus physique n''st pas l'épuisement d'un volume d'eau chaque jour, mais un phénomène diffusif, et R_S dépend de nombreux facteurs, dont le composé hydrophobe accumulé. R_S dépend aussi de la température et de l'agitation.

En phase de désorption, en supposant les concentrations dans l'eau toujours nulles, ce qui est le cas des PRC, on a :

$$\frac{dC_{SPMD}}{dt} = -k_e C_{SPMD} \cdot$$

La valeur de k_e peut être déduite de l'évolution du PRC dans le SPMD (qui doit être une exponentielle décroissante), et on peut en déduire R_S à condition que K_{SPMD} soit connu. Deux hypothèses sont proposées par Huckins et al. (2002). Le première consiste à écrire que le processus limitant est le même pour tous les composés au delà d'un log(K_{OW}) de 4 environ[2], on fait l'hypothèse (validé au laboratoire) que c'est la diffusion externe, dans l'eau ou dans le biofilm, qui est limitante. Sous cette hypothèse, à partir de la mesure in situ de k_e grâce au PRC, on peut recalculer le k_e de l'espèce d'intérêt, qui dépend en réalité de K_{SMPD}, voir ci-dessous, les k_u de tous les composés si leurs coefficients de partage à l'équilibre K_{SPMD} sont connus. Une autre hypothèse moins forte consiste à injecter initialement plusieurs PRC de K_{OW} variables et de calculer par interpolation en fonction de K_{OW} le k_e à appliquer à chaque molécule d'intérêt, puis le k_u de la même façon en tenant compte du K_{SPMD}. Un problème est que les k_e ne sont plus guère mesurables en pratique pour les K_{OW} élevés.

4.1.2 Relation entre le coefficient d'échange et K_{OW}.

Plusieurs modèles plus complexes à 2 ou 3 couches limitantes ont été proposés pour mieux simuler le comportement des contaminants hydrophobes dans les SPMD. On va prendre un modèle assez simple à deux couches (l'eau et la membrane) similaire à celuiproposé par Vrana et Schüürmann (2002).

En état permanent le flux à travers la couche limite aqueuse est égal au flux à travers la membrane. Le premier peut s'écrire :

$$F = A\alpha_W (C_W^\infty - C_W) ,$$

où A est la surface du SPMD, α_W est le coefficient de diffusion dans la couche limite externe, C_W^∞ est la concentration dans l'eau loin de la membrane, et C_W la concentration dans l'eau au voisinage immédiat de la membrane.

$$F = A\alpha_M (C_W^M - C_L^M) ,$$

où α_M est le coefficient de diffusion dans la membrane, et C_W^M et C_L^M les concentrations dans la membrane au voisinage de l'eau et du lipide (trioleine) respectivement. A partir de l'hypothèse que les deux flux sont égaux fonctionnement permanent dans chacun des interfaces, on montre que :

$$F = A\frac{\alpha_W \alpha_M K_{MW}}{\alpha_W + \alpha_M K_{MW}}\left(C_W^\infty - \frac{C_L}{K_{LW}}\right) = A\alpha_G\left(C_W^\infty - \frac{C_L}{K_{LW}}\right),$$

où K_{MW} et K_{LW} sont les coefficients de partage membrane-eau et lipide-eau respectivement. α_G est un coefficient de diffusion global égal à α_W ou $\alpha_M K_{MW}$ selon que le processus limitant est la diffusion au travers de la couche externe (couche limite aqueuse éventuellement colmatée par du

[2] Le K_{OW} est le coefficient de partage Octanol-Eau, c'est une propriété caractérisant globalement l'hydrophobie d' une molécule.

biofilm) ou la membrane. L'idée générale est que lorsque K_{OW} croit, le terme $\alpha_M K_{MW}$ l'emporte sur le terme α_W et que la diffusion dans la couche limite externe devient limitante.

En identifiant au formalisme du paragraphe précédent, on obtient :

$$k_e = \frac{A \times \alpha_G}{V_{SPMD} \times K_{LW}} \text{ et } k_u = \frac{A \times \alpha_G}{V_{SPMD}} .$$

K_{LW}, comme K_{SPMD} augmentent avec K_{OW}. On montre ainsi que k_e diminue avec le degré d'hydrophobie, ce qui explique pourquoi il est conseillé de choisir des PRC pas trop hydrophobes pour que la diffusion dans l'eau soit le processus diffusif limitant effectivement, mais pour que le k_e ne soit pas trop faible et que la perte soit mesurable en cours d'exposition.

On doit noter également que selon ce modèle, la vitesse d'accumulation devrait être la même pour tous les composés suffisamment hydrophobes (i.e. diffusion externe limitante) ce qui ne correspond pas exactement à l'expérience (voir Figure 1). Mentionnons tout de même que, alors que les K_{LW} comme les K_{OW} et comme les ke varient selon plusieurs ordres de grandeur, les valeurs expérimentales de R_S (proportionnel à k_u) ne varient que 2 à 3,. On peut exprimer ceci en écrivant que R_S est remarquablement constant alors sur une plage de variation de K_{OW} de plusieurs ordres de grandeur, c'est qu'une question de point de vue.

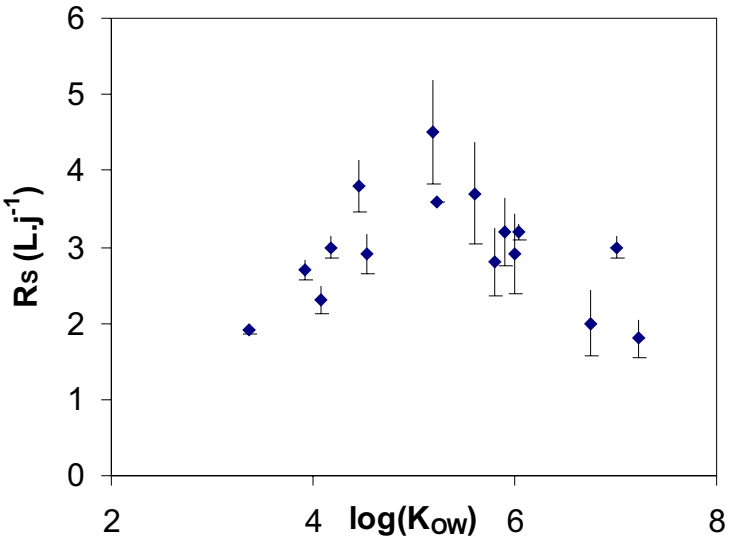


Figure 1 : Les R_S à 10°C au laboratoire selon Huckins et al. (1999)

Cependant, les variations quoique limitées de R_S devraient être mieux comprises pour affiner l'évaluation des concentrations disponibles à partir des vitesses d'accumulation dans les SPMD. Deux pistes en particulier devraient être suivies. La première concerne le comportement du film plastique qui peut être considéré comme un gel hydrophobe (modèle implicite ici avec α_M est à peu près constant) ou une milieu poreux ou α_M serait inversement relié à l'affinité du composé pour la phase "solide". La deuxième concerne le comportement de composés très hydrophobes, réputés dissous, mais qui pourraient ne pas l'être complètement (formation de micelles par exemple ou associations colloïdales), dans la couche limite au voisinage du film de plastique.

Ces considérations demandent des développements qui dépassent le cadre de ce projet. En attendant les développements ultérieurs nécessaires, nous utiliserons la méthode proposée par Huckins et al. (2002) en considérant les variations de R_S comme des variations de second ordre. Le PRC donne une estimation de k_e, ce qui permet d'estimer son k_u grâce à une référence de K_{SPMD}, et donc son R_S. Bien que les R_S devraient être tous constants, on tiendra compte des observations

expérimentales de Huckins pour corriger le R_S du HAP considéré par un facteur R_S(HAP)/R_S(PRC) où les valeurs de R_S sont les références proposées par Huckins et al. (Figure 1).

4.2. Technique mise en œuvre et plan d'échantillonnage

4.2.1 Plan et sites d'échantillonnage

La campagne menée en novembre 2003 préfigure les séries de mesures que nous envisageaons pour l'année 2004. Cinq stations ont été échantillons : l'Orgeval au Theil à l'aval du bassin versant expérimental du même nom, à Guérard dans le Grand Morin, à l'aval de Coulommiers, mais à l'amont des prémisses de l'agglomération de Marne-la-Vallée, à Meaux dans la Marne, à l'amont également de l'agglomération parisienne, à St Maurice, à l'amont de la Confluence entre la Marne et la Seine, et enfin à Andrésy, à l'aval de la confluence avec l'Oise mais au centre du bras gauche de l'île Denouval.

Les SPMD étaient placées dans des cages de vingt centimètres de coté, avec des mailles centimétriques, disposées en spitrale autour de deux tiges placées côte à côte en évitant tout recouvrement du SPMD sur lui-même. Deux SPMD (ou plus) étaient systématiquement placées dans la même cage pour valider la reproductibilité des mesures.

Les cages étaient immergées à un mètre de profondeur à Meaux, Saint Maurice et Andrésy, à une profondeur moindre à Guérard ou au Theil faute d'une hauteur d'eau suffisante. Les cages étaient systématiquement recouvertes d'un plastique noir pour limiter la photo-dégradation des HAP.

Les SPMD ont été relevées au bout de 13 jours, mais nettoyées (sédiments enlevées des SPMD) après une semaine d'exposition. A Saint Maurice une expérience cinétique a été faites sur 21 jours, la moitié des SPMD étant nettoyées deux fois par semaine plutôt qu'une fois par semaine.

Trois échantillons d'eau ont été collectés sur chaque site au cours de la période d'exposition. Ils sont destinés à des mesures de HAP dissous et particulaires, des analyses de COP, COP et SUVA. Les analyses de HAP particulaires ne sont pas encore complétées, elles seront discutées ultérieurement . Ce rapport est principalement destiné à exposer la manière dont les données d'accumulation dans les SPMD peuvent être exploitées.

4.2.2 Techniques de prélèvement et analyses (HAP)

Les SPMD sont utilisées dans la configuration recommandée par l'USGS, il s'agit de sacs en polyéthylène de 96 cm de long et 2,5 cm de large, dont la membrane a une épaisseur de 70 µm, emplis de 1 mL de trioleine. (Exposmeter Tavelsjo, Suède).

4 PRC deutérés ont été ajoutés dans les PRC en début d'expérience (anthracène-D10, fluorantène -D10, pyrène-D10 et benzo(ghi)pérylène-D12) par injection directe dans les SPMD, puis le trou a été scellé de nouveau. Après exposition, les SPMD sont dialysées deux fois dans de l'heptane. Une série d'étalons internes deutérés également sont ajoutés à cette étape pour contrôler le bon déroulement des étapes d'évaporation et éventuellement purification sur colonne de silice.

Les analyses sont réalisées ensuite par chromatographie gazeuse couplée à la spectrométrie de masse. Les 16 HAP de la liste EPA sont analysés. Des solutions certifiées ont été utilisées pour établir toutes les gammes.

Dans la série des HAP analysés, le naphtalène et l'acénaphtylène (les deux plus légers) ne peuvent pas être pris en considération car ils sont relativement volatils alors que le protocole utilisé ne permet pas de garantir que les pertes par volatilisation seront négligeables.

4.3. Résultats et discussion

4.3.1 Comportement dynamique des SPMD

Echappement des PRC

L'évolution des PRC est présenté sur la Figure 2. On y observe les évolutions attendues, malgré une variabilité analytique qui semble élevée pour le fluoranthène-D10. Pour ce dernier, dont le K_{OW} est plus élevé, on ne parvient plus à mesurer d'évolution, le k_e est effectivement beaucoup plus

faible. Vrana et Schüürmann (2002) avaient aussi utilisé l'anthracène-D10 pour leurs essais. Nos résultats sont bien dans l'ordre de grandeur de ce qu'ils avaient observé (de l'ordre de 50% de perte en 300 heures), bien que les vitesses utilisées au cours de leurs essais soient très inférieures aux vitesses observées en rivière aux différents points de mesure, mais probablement proches des vitesses à l'intérieur des cages. Pour les molécules les plus hydrophobes, il est donc probable que la limitation soit contrôlée par la vitesse de l'eau et puisse donc être évaluée à partir des mesures de R_S effectuées au laboratoire, comme l'ont suggéré Huckins et al. (2002).

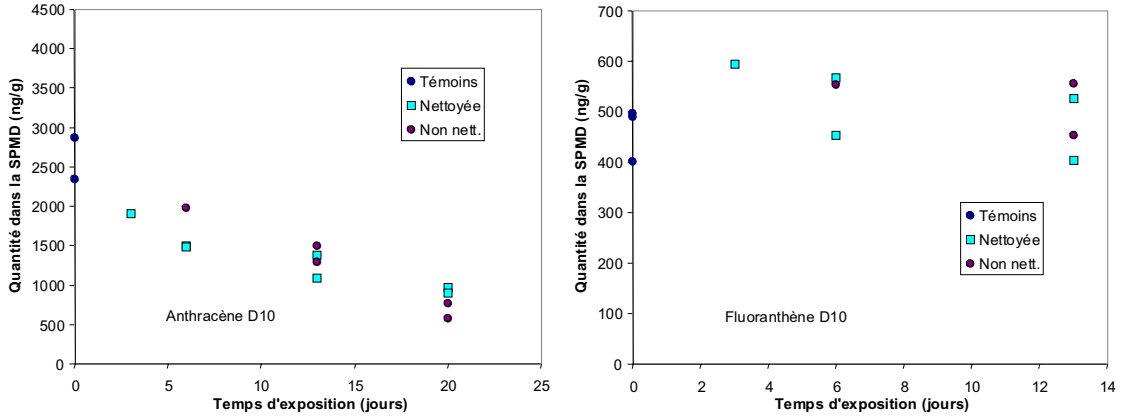


Figure 2: Evolution de deux PRC en cours d'exposition à la station de Saint Maurice

On notera (Figure 3) que les teneurs en Ant-D10 restant dans les SPMD après 13 jours diminuent progressivement d'amont en aval. Ceci est probablement lié aux vitesses de l'eau au voisinage des cages ou à l'agitation qu'elles ont subi. L'augmentation de k_e d'amont en aval est probablement un hasard, mais elle pourrait introduire un biais dans l'interprétation des résultats d'accumulation si on ne la prend pas en compte. Le rapport des corrections aux concentrations estimées est d'un facteur 3 entre Le Theil et Andrésy.

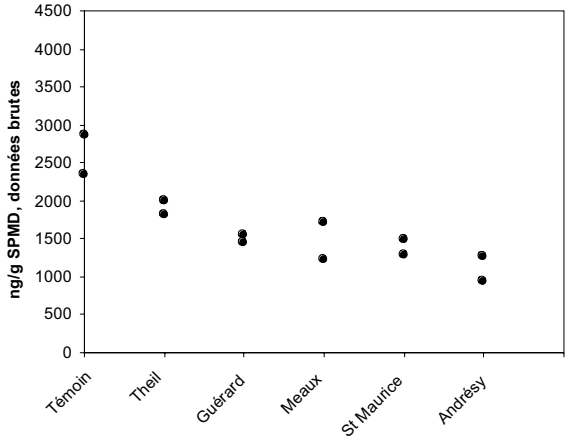


Figure 3: Evolution de l'échappement de l'anthracène-D10 après 13 jours d'exposition d'amont en aval.

Evolution en cours d'exposition

Plusieurs évaluation de la dynamique de contamination in situ ont été réalisés en Marne et en Seine à St Maurice et Andrésy, d'autres devront être encore réalisées dans des stations situées plus à l'amont dans le bassin ou les conditions d'exposition sont sensiblement différentes. En février et mars 2003, des cinétiques d'accumulation linéaires ont pu être observées.

A St Maurice, en Novembre 2003, ce comportement attendu a été perturbé par une pollution localisée, tout à fait exceptionnelle aux dires des agents du SNS en charge de l'exploitation du site, due à un problème mécanique sur un bateau, ayant occasionné une forte émission d'un mélange de fuel et d'huile. L'incident est survenu très peu de temps avant notre expérimentation (ni le fautif ni le

moment précis de l'incident n'ont été identifiés), et les traces (irisations) sont restées visibles quelques jours après le début de l'exposition. Une telle durée de l'impact ne peut être expliquée que par la fixation d'une partie des contaminants les différentes parties des ouvrages de navigation et la biomasse (biofilm, filaments…) qui les recouvre.

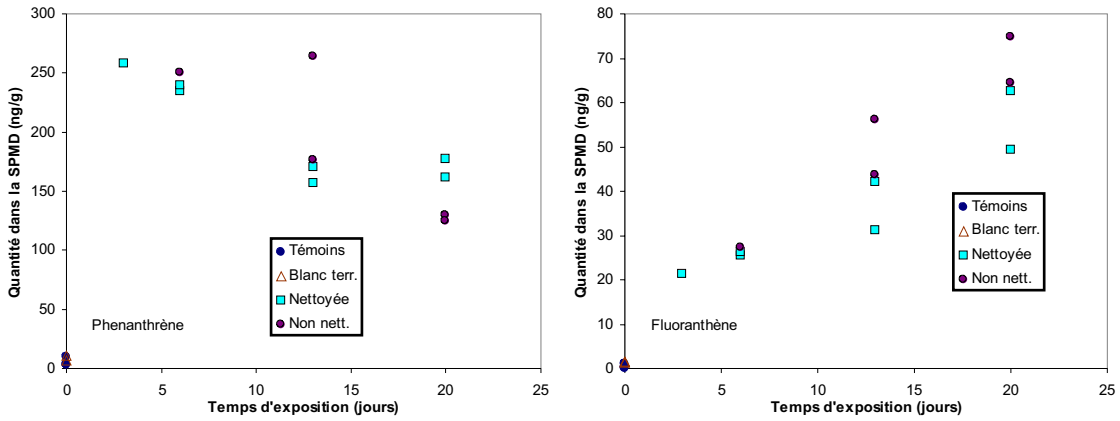
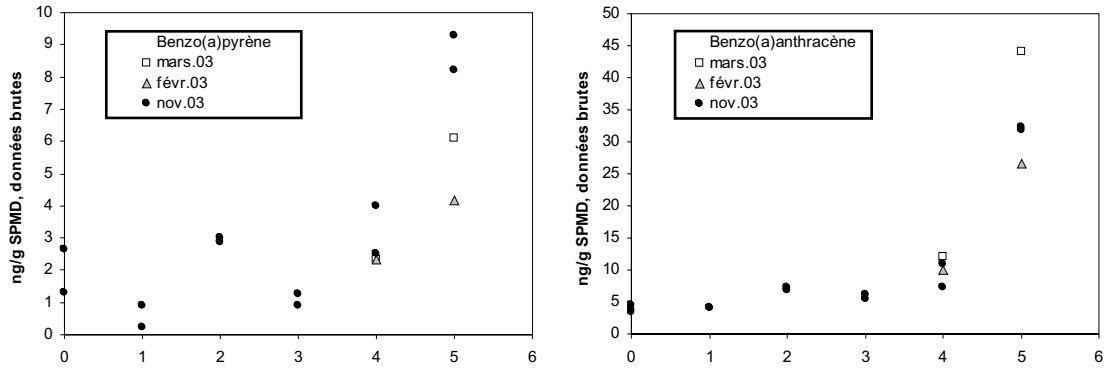


Figure 4: Evolution de la teneur an HAP dans le SPMD pour le phénanthrène et le fluoranthène à Saint Maurice en novembre 2003.

On note que les conséquences de l'incident ne sont pas complètement comparables pour tous les HAP. En particulier, certains HAP parmi les plus légers donnent des valeurs très fortes au cours de la première période d'exposition. Nous ne connaissons pas précisément la composition du rejet accidentel, mais effectivement, de très fortes teneurs en fluorène et phénanthrène (24 et 82 ng.L^{-1}) ont été mesurées au moment de la pose des SPMD à St Maurice, alors que les autres HAP ont des concentrations de l'ordre ou inférieures au ng.L^{-1}. C'est bien le fluorène et le phénanthrène qui donnent des réponses très élevées au jour 3 d'exposition des SPMD. Le fait qu'une valeur pic puisse être obtenue à un moment donné avec les SPMD signifie très clairement qu'elles ne fonctionnent pas uniquement en accumulation, au moins pour ces HAP légers, et donc que les cinétiques d'échange sont assez rapides pour ces composés

4.3.2 HAP dans les SPMD

L'évolution amont-aval (Theil, Guérard, Meaux, St-Maurice, Andrésy) des teneurs en différents HAP dans les SPMD est donnée sur les graphes de la Figure 5. Il s'agit de teneurs après 13 jours d'incubation effectivement mesurées pour la campagne de novembre 2003. Pour février et mars 2003, des teneurs à 21 jours et 14 jours respectivement ont été ramenées à 13 jours en faisant une hypothèse d'accumulation linéaire.



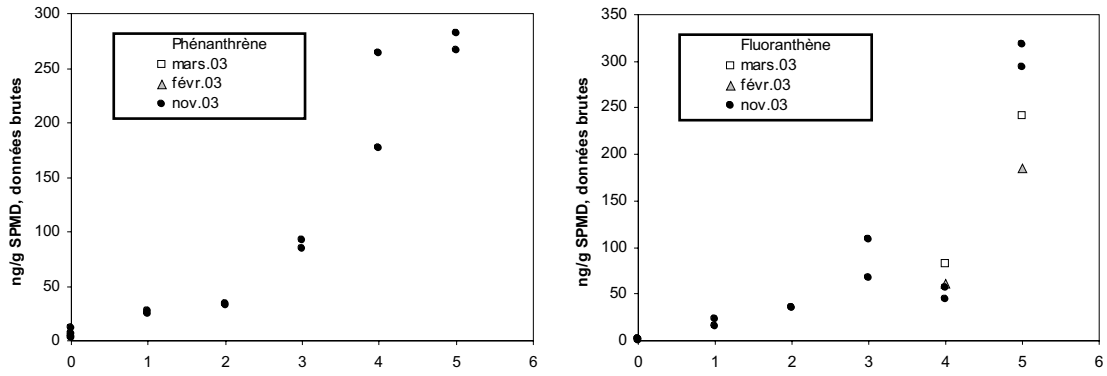


Figure 5 : Evolution amont-aval des teneurs en quelques HAP dans le SPMD.

On observe des évolutions amont aval différentes pour les différents HAPs. La tendance générale est à une forte augmentation, mais pour les 3 points Guérard, Meaux et St-Maurice certains groupes de HAP donnent des teneurs plus élevées à Guérard (B(a)P, B(k)F et B(b)F notamment). On notera cependant l'excellente reproductibilité des résultats obtenus, pour deux SPMD différentes immergées au même point. Les résultats sont donc bien significatifs, même les variations observées dans le secteur Meaux-Guérard demeurent inexpliquées et sont probalement le fait d'origines différentes.

Comme indiqué plus haut dans la partie méthodologie, on applique à ces données d'accumulation les coefficients R_S établis par Huckins et al. (1999), que l'on corrige des valeurs de k_e mesurées par la fuite de l'Anthracène-D10 hors du SPMD. Les résultats obtenus sont résumés sur les Figure 6. Malgré les corrections appliquées en fonction des quantités de Ant-D10 restantes, les tendances mises en évidence à partir des teneurs dans les SPMD demeurent observables.

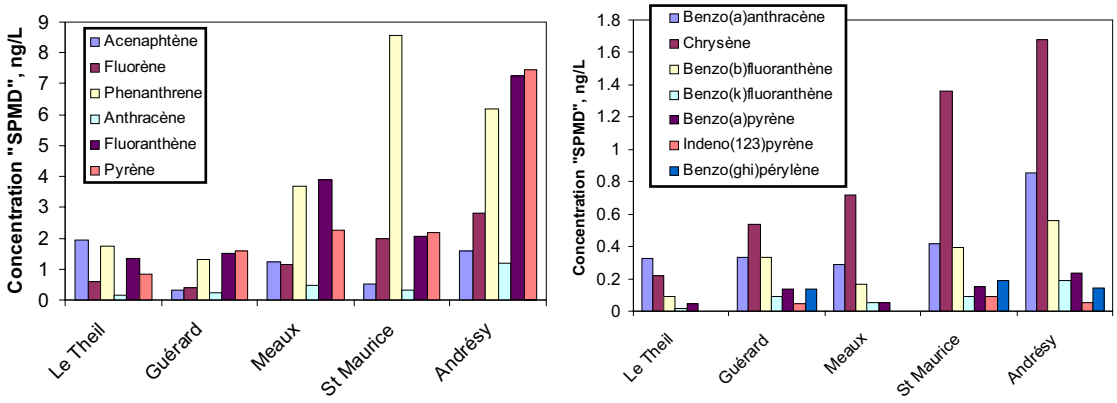


Figure 6 : Teneurs disponibles en HAP en novembre 2003 en Marne et en Seine selon une hypothèse d'accumulation.

Comme les valeurs de k_e que nous avons obtenues sont supérieures à celles de Huckins et al. (1999) au laboratoire, les facteurs d'accumulation R_S sont beaucoup plus importants (d'un facteur 3 à 5). L'accumulation étant plus rapide, bien que les résultats acquis en laboratoire montrent que l'accumulations est linéaire sur les 15 premiers jours -voir Luellen et Shea (2002) par exemple-, il est important de vérifier si cette hypothèse reste valide pour ces essais in situ. Pour ce faire, on peut calculer les concentrations dans l'eau à partir d'un modèle d'équilibre. Peu de données valeurs de K_{SPMD} sont disponibles dans la littérature, nous prendrons celles que propose Hoffmans (1999).

A partir des teneurs mesurées dans les SPMD, on peut calculer des concentrations dissoutes sous une hypothèse d'équilibre (C_{eq}), qu'on comparera aux concentrations dissoutes obtenues par le modèle d'accumulation linéaire (C_{acc}). Pour une concentration C donnée dans le milieu, la teneur en HAP dans le SPMD en phase d'accumulation est nécessairement inférieure à le teneur à l'équilibre. Donc réciproquement, pour une teneur mesurée dans le SPMD, la concentration estimée dans le

milieu C_{acc} doit être nettement supérieure à C_{eq}, sinon l'hypothèse "accumulation" ne tient plus On note que pour tous les HAP plus légers que l'anthracène (anthracène compris), ne sont pas très éloignés de 1 (à un facteur 2 près), et que l'hypothèse de quasi-équilibre devrait être examinée également. L'expérience de St Maurice en novembre, avec une contamination forte en début de période d'intégration confirme bien que pour les HAP les plus légers, très présents de surcroît dans la contamination accidentelle, on a bien un effet d'équilibre et non d'accumulation qui se traduit par une diminution des teneurs dans les SPMD après disparition de la contamination.

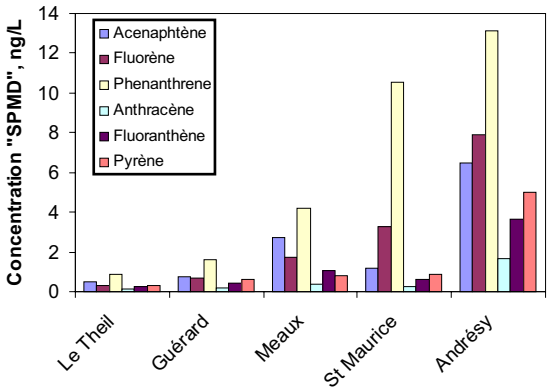


Figure 7: Concentrations disponibles en HAP (pour les plus légers), selon une hypothèse d'équilibre

La comparaison des valeurs obtenues avec des données de HAP dissous n'est pas évident, car ces données sont rares, surtout pour les HAP les plus lourds dont les concentrations sont les plus faibles. A Achères, en novembre 2003, les concentrations de phénanthrène dissous étaient en moyenne de 17 ng..L^{-1} (12 à 22 ng..L^{-1} pour les 3 échantillons collectés au cours de la période d'immersion des SPMD), suffisamment élevés pour être mesurées avec une bonne précision, à comparer aux 14 ng.L^{-1} estimés par les SPMD. Le fluoranthène est mesuré avec moins de précision à 9 ng.L^{-1} en moyenne, à comparer aux 7 ng.L^{-1} estimés par les SPMD. Le pyrène est mesuré à 10 ng.L^{-1}, contre 7.5 estimés par les SPMD. Compte tenu des valeurs de blanc de quelques ng.L-1 pour les mesures dissoutes directes, la comparaison est assez bonne. Pour les autres stations, nous ne disposons hélas pas de données de concentrations dissoutes de qualité suffisante pour faire des comparaisons.

5. Conclusions

Ces premiers résultats, qui devront être encore confrontés aux autres données collectées au cours de ces campagnes (encore en traitement) montrent que les SPMD sont un outil intéressant pour estimer les concentrations en HAP. L'utilisation de PRC indispensable pour bien évaluer le comportement in situ des SPMD et corriger les R_S de la littérature. La poursuite de ces campagnes en 2004 nous permettra de compléter l'image de l'évolution amont-aval des HAP et nous l'espérons de mettre en évident in situ le rôle des matières organiques dissoutes sur la disponibilité.

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The ability of dissolved organic matter (DOM) to influence benzo[a]pyrene bioavailability increases along DOM mineralization

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Abstract

The biodegradation of two substrates was monitored in reactors along with the ability of dissolved organic matter (DOM) to influence benzo[a]pyrene bioavailability, as DOM mineralization is progressing. Substrates were composed of algae extracts and an artificial substrate miming raw wastewater. They were considered as autochthonous and allochthonous anthropogenic DOM models respectively. Soluble microbial products formed during the biomass activity were also studied. The aromaticity of DOM was followed with specific ultra-violet absorbance. Partitioning coefficients between DOM and benzo[a]pyrene, $K_{DOC}(biol)$, were biologically-determined by means of 4-hour bioaccumulation experiments on *Daphnia magna*.

Parent as well as degraded substrates always significantly reduced the bioaccumulation of benzo[a]pyrene at environmental DOM concentrations. Soluble microbial products also significantly affected the benzo[a]pyrene bioaccumulation. $K_{DOC}(biol)$ ranged between $2x10^4$ and $4x10^5$ L/kg. While the artificial wastewater was biodegrading, DOM aromaticity increased, as well as $K_{DOC}(biol)$. During the biodegradation of algae extract DOM, $K_{DOC}(biol)$ increased, whereas their aromaticity slightly decreased.

Key-words :

Dissolved Organic Matter, biodegradation, bioavailability, partition coefficient, Kdoc, aromaticity, benzo[a]pyrene, *Daphnia magna*, bioconcentration, Soluble Microbial Products.

INTRODUCTION

Organic matter plays a dominant role on many aspects of the aquatic environment. Its availability controls the bacterial loop in aquatic ecosystems and consequently affects most living organisms. It also affects the fate and bioavailability of xenobiotics (Stumm and Morgan, 1981). In the water column, a large fraction of hydrophobic persistent organic pollutants (POPs) may bind to dissolved organic matter (DOM), which modifies their bioavailability to living organisms (see review in Haitzer et al, 1998). Most studies report that the presence of DOM in the exposure media reduces the bioaccumulation of POPs in pelagic crustaceans (Leversee et al, 1983, Kukkonen and Oikari, 1991, Haitzer et al, 2001), in fishes (McCarthy and Jimenez, 1985a, Freidig et al, 1998) or in benthic organisms (Landrum et al, 1985, Haitzer et al, 1999a). The generally accepted assumption is that only the unbound fraction of contaminants (also referred to as the "free fraction") is bioavailable to these organisms (Landrum et al, 1985).

The strength of the interactions depends on the characteristics of DOM. Because of their high hydrophobicity, humic substances have a great ability to bind POPs (Kukkonen et al, 1990). Most published studies focus on the effect of humic substances or humic-rich natural waters on the bioavailability of POPs (Haitzer et al, 1998). However, recent studies showed that non-humic natural riverine DOM (with low aromaticity and low carboxylic content) could also affect the bioavailability of POPs (Akkanen et al, 2001) and also that highly biodegradable DOM was able to reduce the bioavailability of some polycyclic aromatic hydrocarbons (Gourlay et al, 2003).

Waters bodies under anthropogenic influence contain high levels of non-humic allochthonous organic matter of urban origin from industrial or domestic effluents (Namour and Muller, 1998). Moreover, it may be subject to further biodegradation in the medium. Tusseau-Vuillemin and Le Reveille (2001) found that 25 to 61% of DOM from wastewater treatment

plant effluents was biodegradable. In the highly anthropized river Seine (France) area, 80% of DOM in combined sewer overflows and 40% of DOM in river water are biodegradable (Seidl et al, 1998). Since anthropogenic nutrient discharges may also enhance eutrophication, anthropized waters may also contain high levels of autochthonous biodegradable DOM from algal origin. Mean and Kirchmann (2001) measured that 68% of DOM produced during a phytoplanktonic bloom was mineralized within 18 days. Not only DOM would impact on the bioavailability of POPs, but the progressive mineralization of degradable DOM is likely to modify again the bioavailability of POPs. However, no comprehensive study on the effect of the DOM present in anthropized waters on the bioavailability of POPs is available, although they carry the highest concentrations of micro-contaminants.

This study aims at evaluating the ability of two types of DOM to influence the bioavailability of benzo[a]pyrene (BaP) and the evolution of this influence as DOM is mineralized and becomes more refractory. Two models for anthropogenic water DOM are studied. Soluble microbial products (SMP), i.e. DOM formed by biomass production process, are also analysed for their relative importance in refractory DOM composition and their influence on the bioavailability of BaP.

MATERIALS AND METHODS

Water, organic matter, chemicals and solvents.

A solution of 1 mg/L benzo[a]pyrene (BaP) in methanol was prepared in the laboratory from solid PAH powder (purity: 98%, Aldrich, Steinhein, France). A mixture of high purity dichloromethane and methanol (4:1 v/v) (LiChroSolv, Merk Eurolab, Fontenay-sous-Bois, France) was used for the extraction of BaP in organisms.

Mineral water (Evian® Evian, France; pH: 7.2, dissolved solids: 309 mg/L, conductivity: 567 µS/cm).was used both for experiments and daphnid culture. Its composition is stable, with a neutral pH and a moderate mineralization.

Raw wastewater and activated sludge were sampled at the Noisy-Le-Grand (France) combined sewer treatment plant. Wastewater was filtered on a pre-combusted 0.7 μm glass-fiber filter (GF/F Whatmann®, Kent, UK) and used within four hours after sampling. Activated sludge was kept under constant aeration until use.

Wastewater and river waters may have highly variable compositions and contain some toxic compounds. In order to get reproducible results, model substrates were used to evaluate the influence of DOM on BaP bioavailability. A commercially available mixture composed of meat and vegetal extracts and sugars (Viandox®, Rueil Malmaison, France) was chosen for that purpose as a model for allochthonous anthropogenic DOM. It contains 42 g/L of carbohydrates, 173 g/L of proteins and 0.77 mg/L of fats (Pernelle et al, 2001). So-called "artificial wastewater" was prepared by diluting Viandox to 1% in mineral water. As a model for autochthonous DOM, a solution of algae extracts was prepared as follows : *Selenastrum capricornutum* were cultured, concentrated by decanting and autoclaved for 90 min at 120 °C to accelerate cell lysis. The solution was filtered on a pre-combusted GF/F filter in order to preserve only the dissolved fraction. Both substrates were prepared the day of experiment.

Biodegradation experiments

The biodegradation of each model substrate was monitored in a 6L cylindrical open reactor equipped with a mechanical stirrer. The temperature was controlled at 15°C. 4.5 litres of the initial DOM stock solution was put in the reactor, with a 4.5 ml activated sludge inoculum. The solution was regularly aerated in order to keep oxic conditions. The biodegradation process was monitored for about 15 days. We particularly focused on the first days, when the biodegradation was the most rapid. Degrading solutions were regularly sampled and filtered on pre-combusted GF/F Whatmann filters. For each sample, dissolved organic carbon concentration (DOC) and particulate organic carbon concentration (POC) were measured using a Carbon Analyzer (O.I. Analytical, College Station, TX, USA). The absorbance at 254

nm (A_{254}) of the filtrate was measured using a spectrophotometer (Lambda 11 Perkin-Elmer, Courtabeuf, France) and the Specific Ultra-Violet Absorbance (SUVA = 1000 x A_{254}/DOC in $cm^{-1}.g^{-1}.L$) was calculated. Other aliquots were periodically sampled, filtered and used to test the influence of DOM on the bioaccumulation of BaP by daphnids as described below.

In order to validate the use of Viandox® as a model for an artificial wastewater, the biodegradation of the real wastewater sample was also monitored under the same conditions. DOC, POC and SUVA were regularly measured for 22 days.

Soluble Microbial Products experiments

Soluble microbial products (SMP) released into the solution from biomass activity and decay were produced as suggested by Hejzlar and Chudoba (1986): 4 liters of a glucose solution (685 mg/L DOC) was prepared in mineral water with additional nutrients and 10 ml of activated sludge; Its biodegradation at 15°C was monitored under oxic conditions. Biomass activity in the batch (data not presented) showed that the glucose biodegradation was very fast and completed during the first three days. Since glucose is totally degradable, the dissolved fraction of DOM after 4 days was only composed of newly-formed SMP, as observed by Hezjlar and Chudoba (1986). The fourth day, the solution was filtered and analysed for its DOC, SUVA and tested for its influence on the bioaccumulation of BaP.

Bioaccumulation experiments

The influence of the DOM on the bioavailability of BaP was measured following the method detailed in Gourlay et al (2003). Bioaccumulation experiments were performed using 5-7 day-old daphnids (*D. magna*). One hour before exposure, the organisms were moved into clean mineral water in order to clear their gut. DOC was measured in the stock solution just before the experiment. Five or six exposure solutions were prepared by diluting the DOM solution in mineral water to a final volume of 300 ml. DOM concentrations in exposure media ranged from 0 to 30 mg/L DOC, which is within the range of DOM concentrations in urban effluents

or polluted rivers (Tusseau-Vuillemin and Le Réveillé, 2001). An additional solution was prepared with no DOM. The solutions were next spiked with 300 µl of the BaP solution, to a final nominal concentration of 1 µg/L. Exposure solutions were let to settle for one hour before introducing the organisms. This equilibrium duration was shown to be sufficient to allow interactions between BaP and DOM (McCarthy and Jimenez, 1985b, Schlautman and Morgan, 1993, Krop et al, 2001). 30 daphnids were exposed to each of the solution for 4 hours at room temperature in the dark.

After exposure, organisms were removed and gently rinsed with milli-Q water. Two samples of fifteen organisms were put in two glass tubes with 4 ml of organic solvent. Samples were sonicated for 1 minute. The tubes were then stored in the dark at 4°C until analysis. The samples were filtered on a GF/F filter and immediately put in 1-cm quartz cuvette for fluorescence analysis of the daphnid extract.

BaP content in daphnid extract was measured by spectrofluorimetry (Gourlay et al, 2002). The emission fluorescence spectrum of the extract was recorded between 350 and 500 nm. Excitation wavelengths were set to 265 nm. Fluorescence measurements were performed at room temperature. The intensities of the measured peaks were corrected for baseline by subtracting the minimum fluorescence intensity at the left of the peak. Gourlay et al (2002) showed that the BaP concentration in daphnids could be linearly related to the fluorescence intensity of daphnid extract at 407 nm (F_{407}) and 417 nm (F_{417}).

One control blank solution was prepared with DOM and daphnids only. DOC measured at the beginning and at the end of the exposure did not significantly evolve during the 5 hour-experiment. No fluorescence was measured on the extracts of daphnids exposed in the control blank solution

For two experiments, the BaP concentrations in the beakers were measured after exposure using liquid-liquid extraction in dichloromethane. We verified that, although lower than the

nominal initial concentration, BaP concentration was constant among the beakers, whatever the presence and the concentration of DOM ($[BaP]_{water}$ = 0.61 ± 0.07 µg/L, n =6 and $[BaP]_{water}$ = 0.59 ± 0.06 µg/L, n =6).

Data processing

The processing of biodegradation was scaled with reference to a biodegradation index f_{ref} ranging from 0 to 1. f_{ref} was computed as the ratio between the final and the current DOC concentration (*DOC(f)* and *DOC(t)* respectively): $f_{ref} = DOC(f)/DOC(t)$. Practically, f_{ref} gives an estimation of the refractory DOM fraction in the total DOM and increases up to 1 at the end of the mineralization experiment..

From bioaccumulation experiments, partitioning coefficients between DOM and BaP, K_{DOC} *(biol)* were determined. We verified that the ratio F_{417}/F_{407} was constant within each series of experiments (Gourlay et al, 2003). The relative effect of DOM on PAH bioaccumulation was estimated with the ratio F/F_0, F_0 being the average F_{407} in the two pseudo-replicates of daphnid extracts exposed to BaP only and F being F_{407} in daphnid extract exposed to BaP and DOM.

The reduction of bioaccumulation in the presence of DOM can be explained by the formation of DOM-BaP complexes that are too large and too polar to cross biological membranes (Landrum et al, 1985). Therefore, the bioaccumulation in the presence of DOM can be related to the bioavailable fraction of PAHs that is referred to as the freely dissolved fraction of PAHs in the soution. A carbon-normalised partition coefficient K_{DOC} is commonly used to estimate the free PAH concentration, which leads to the following expression relating the bioavailable fraction to DOC (Gourlay et al, 2003):

$$\frac{F}{F_0} = \frac{1}{1 + K_{DOC}[DOC]} \qquad (1)$$

From Equation (1), a biologically-determined partitioning coefficient $K_{DOC(biol)}$ could be estimated from non linear regression of F/F_0 against DOC data points. The Levenberg-Marquardt method was applied with the XLStat software. $K_{DOC(biol}$) measures the ability for DOM to influence and reduce the bioavailability of BaP to daphnids.

RESULTS

Evolution of organic matter concentrations in the reactors

The evolutions of particulate and dissolved organic carbon in the batch during the mineralization of the model substrates and the real wastewater are displayed on Figure 1. All substrates were highly degradable : after 15 days, 76% of the start algal extracts and 90% of the artificial wastewater were mineralised. After 22 days, 83% of the real wastewater was mineralised. More than 60 % of initial DOM disappeared in less than 2.2 days in the three experiments. Following Billen and Servais (1989), this fast biodegradation corresponds to the digestion of easily degradable molecules in the substrate. Concurrently, we observed an increase of particulate organic carbon corresponding to newly-formed biomass. Then the biodegradation process of DOM got slower and particulate organic matter concentrations decreased, because of the biomass mortality and degradation. The formation and then the degradation of the microbial biomass during the mineralization of the substrates indicates that Soluble Microbial Products are very likely to be present in the remaining DOM.

SMP formed by the total biodegradation of glucose accounted for 36.3 mg/L DOC, which represents 5% of the initial DOC content. This result is in agreement with Shin and Kang (2003) who observed that SMP from activated sludge fed with glucose were 4% of the initial DOC content. Since the production of SMP follows a complex kinetic that depends on the substrate and the kind of microorganisms (Laspidou and Rittman, 2002), it is difficult to precisely assess the fraction of SMP along the other substrate degradation experiments. However, a crude estimation can be obtained assuming that the concentration of SMP

($DOC_{SMP}(t)$) amounts 5% of DOC decrease, as found at the end of the glucose degradation experiment :

$$DOC_{SMP}(t) = 0.05\ (DOC(0) - DOC(t)) \quad (2)$$

Consequently, SMP would represent 50% and 25 % of DOM at the end of the artificial and real wastewater degradation respectively, and only 16% when starting with DOM of algal origin.

Evolution of DOM aromaticity

The Figure 2 shows the 254 nm-specific absorbance (SUVA) of degrading DOM plotted against the biodegradation index. Following Traina et al. (1990), the specific absorbance at 240-280 nm can be considered as an indicator of the aromaticity of DOM. Initially, the three substrates displayed very low SUVA, compared to natural humic substances (for example SUVA = 27-62 $cm^{-1}.g^{-1}.L$ for various humic substances, Haitzer et al, 1999c). The aromaticity of the artificial wastewater-DOM increased along the mineralization, whereas the aromaticity of algae extract-DOM exhibited a slight decrease. The evolution of the real wastewater aromaticity is comparable to the one of the artificial wastewater, the real wastewater having somehow higher SUVA. The similarity of POC, DOC and SUVA evolutions with the mineralization of the artificial and the real wastewater allows us to validate the use of the artificial substrate as a model for domestic wastewater.

The SUVA-value measured on the SMP sample is reported on the Figure 2 ($SUVA_{SMP}$ = 22.5 $cm^{-1}.g^{-1}.L$). It compares well with data obtained by Shin and Kang (2003) who measured SUVA of SMP obtained from glucose degradation between 17 and 19 $cm^{-1}.g^{-1}.L$.

Evolution of DOM influence on BaP bioavailability

All the tested DOM significantly reduced the bioaccumulation of BaP in daphnids at the tested concentrations (0-30 mg/L DOC). The effect of DOM from the artificial wastewater and from algal origins on the bioaccumulation of BaP at four different biodegradation times is

displayed on Figure 3A and 3B. For each DOM solution, the relative bioaccumulations of BaP by daphnids (F/F_0) in the presence of increasing amounts of DOM are plotted, as well as the regression curve following the Equation 1. The specific effect of DOM increases as the biodegradation is progressing. As an example, 32 mg/L DOC from fresh algae extracts are necessary to get a 50 % bioaccumulation reduction whereas the same effect is obtained with only 3.5 mg/L DOC of 15 day-degraded algae extracts. Similarly, 16mg/L DOC of 0.8 day-degraded artificial substrate lead to the same 50%-effect on BaP bioaccumulation than 2.8 mg/L DOC only of 16 day-degraded substrate. The bioaccumulation of BaP was reduced by 50% in the presence of 4.3 mg/L DOC from Soluble Microbial Products.

A biologically-determined partitioning coefficient $K_{DOC}(biol)$ could be estimated for all the bioaccumulation experiments. The Figure 4 shows the evolution of $K_{DOC}(biol)$ along the mineralization of allochthonous or autochthonous DOM, as the remaining DOM becomes more and more refractory. For both substrates, the partitioning coefficient increases as DOM becomes more refractory. $K_{DOC}(biol)$ ranges between $2x10^4$ and $30x10^4$ L/kg for DOM from algae origin and between $6x10^4$ and $36x10^4$ L/kg for DOM from the degrading artificial wastewater. The $K_{DOC}(biol)$ estimated for SMP ($24.8x10^4 \pm 3.5x10^4$ L/kg) is also reported on Figure 4.

DISCUSSION

Validity of K_{DOC}(biol) estimation and comparison with natural DOM

A very similar technique was used by Haitzer et al (1999a, 1999b) to estimate K_{DOC}, the bioaccumulation measurement being performed after 48 hours of exposure, when equilibrium between the organisms and the media was achieved. Since the organic matters studied here are biodegradable, they may evolve during the exposure although most heterotrophic bacteria have been eliminated by filtration (nominal GF-F cut-off : 0.7µm). Consequently, our experimental protocol must be as short as possible. Moreover, short-term and steady-state

exposure experiments have been shown by Gourlay et al. (2003) to allow the same estimation of K_{DOC}, under a generally accepted first-order toxico-kinetic hypothesis. Even if K_{DOC} values are strongly dependant on the estimation methods (Krop et al, 2001), Akkanen and Kukkonen (2003) showed that the equilibrium dialysis method and the biological methods were in good agreement for K_{DOC} estimation. Consequently, K_{DOC} values from this study were compared to those obtained with the equilibrium dialysis technique, or with the biological method.

The partitioning coefficients obtained in this study with artificial non-refractory DOM are in the same order of magnitude as the ones of DOM from European rivers estimated with the equilibrium dialysis technique (0.7- 24.10^4 L/kg, Akkanen et al, 2001). Although somehow lower, they are also in the same order of magnitude as the K_{DOC} estimated with the biological technique for natural stable humic substances (20 - 49.10^4 L/kg, Haitzer et al, 1999b). Our data show that biodegradable or recently stabilised DOM may have an effect comparable to long-term stable DOM.

How does DOM mineralization affect BaP bioavailability?

The SUVA of the artificial wastewater DOM increased during the biodegradation process. The molecular composition of the artificial wastewater is complex with mainly non aromatic, hydrosoluble sugars and proteins, leading to a low global SUVA. It may also contain some aromatic compounds. Along the mineralization process, the smallest and easily hydrolysable molecules are quickly assimilated by bacteria, whereas complex macromolecules, and particularly aromatic molecules, are less easily degraded (Henze, 1992). Thus, the proportion of aromatic compounds is increasing while other compounds are degraded, which leads to a global increase of the aromaticity of DOM. The same phenomenon is very likely to occur along the biodegradation process of the real wastewater effluent, as SUVA also increased. Indeed, the increase of the aromaticity of DOM along the mineralization process of wastewater was previously observed: Dignac (1998) found that the SUVA of raw and treated

wastewater DOM were respectively 9 and 23 $cm^{-1}.g^{-1}.L$ and that they contained 7% and 32% of aromatic compounds respectively. Imai et al (2001, 2002) measured that the specific absorbance at 260 nm of a raw domestic wastewater DOM was lower (about 6 $cm^{-1}.g^{-1}.L$) than the ones measured in treated wastewaters from several sewer treatment plants (11-19 $cm^{-1}.g^{-1}.L$). In the experiment with the artificial wastewater, we found a positive correlation between the binding capacity of DOM with BaP, $K_{DOC(biol)}$, and the aromaticity (correlation coefficient : 0.85), as previously observed in most studies on natural DOM (McCarthy et al, 1989, Kukkonen and Oikari, 1991, Akkanen et al, 2001) and humic substances (Haitzer et al, 1999c). Interactions between DOM and hydrophobic organic compounds are primarily hydrophobic interactions (Scharzenbach et al, 1993).Therefore, the increasing presence of aromatic structures that form hydrophobic sites in DOM may explain the increase of the affinity of DOM for hydrophobic compounds.

However, an anti-correlation was obtained (poor correlation coefficient : -0.55) between SUVA and $K_{DOC(biol)}$ in experiments with algal extracts : the more degraded algae extracts, the more they bind BaP and the less aromatic they appear. The very low SUVA of autochthonous fresh or degraded DOM was previously observed. Fukushima et al (1996) measured that the 260 nm- specific absorbance of DOM from aquatic origin produced in a lab pond was 12 ± 4 cm-1.g-1.L and did not significantly evolve during DOM mineralization on a sand filter. Zumstein and Buffle (1989) reported that the 285 nm-specific absorbance was ten times lower for aquagenic refractory DOM than for pedogenic refractory DOM. Despite their low aromaticity, algae DOM strongly influence the bioaccumulation of BaP, which means that algal extracts DOM contains some hydrophobic sites for BaP binding. Some refractory substances, called algaenans, have already been isolated from algae residues (Allard et al, 1998). Algaenans are component of microalgae cell walls and are defined as non-hydrolysable, poorly soluble biopolymers. They are formed by long aliphatic chains (Derenne

et al, 1992), which should form hydrophobic sites and bind hydrophobic contaminants. Since algaenans are also highly refractory to biodegradation (Allard et al, 1998), their proportion in the degraded algal solution may increase along the mineralization process, leading to a global increase of the binding capacity of DOM with BaP.

Although chemical bonds between benzenic cycles may favour the binding of POPs onto organic matter, the data presented here show that SUVA is not a good indicator of the binding potential of model organic matter mixture for POPs. However, the proportion of residual hydrophobic compounds appears to be a common process explaining the increased affinity for BaP during biodegradation.

Along the degradation, the relative proportion of SMP increases. Therefore, the increase of $K_{DOC}(biol)$ along the biodegradation process may also be due to the increasing presence of SMP in the solution. SMP were proved to significantly influence the bioavailability of BaP at low concentrations ($K_{DOC}(biol) = 24.8 \times 10^4 \pm 3.5 \times 10^4$ L/kg) They contain some aromatic structures, as revealed by their high SUVA. They are also composed of large macromolecules, 73% of SMP have molecular weight over 10 kD (Shin and Kang (2003)). These characteristics may explain the high propensity of SMP to bind BaP (Chin et al, 1997).

Fractionation of DOM into biodegradability classes : relevance of aromaticity and binding capacity

In the reactor, the DOM solution ($DOC(t)$) can be is theoretically fractionated into biodegradable compounds ($DOC_{biodeg}(t)$), initial present refractory compounds (DOC_{ref}) and SMP produced during the biodegradation process ($DOC_{SMP}(t)$). In the following, we make the hypothesis that each fraction can be characterised by a specific SUVA ($SUVA_{biodeg}$, $SUVA_{ref}$ *and* $SUVA_{SMP}$ respectively), and a specific binding capacity ($K_{DOC,\ biodeg}$, $K_{DOC,\ ref}$ *and* $K_{DOC,\ SMP}$ respectively). Consequently, the 254 nm-absorption of the global solution will be given by:

$$SUVA(t).DOC(t) = SUVA_{biodeg}\, DOC_{biodeg}(t) + SUVA_{ref}\, DOC_{ini\ ref} + SUVA_{SMP}\, DOC_{SMP}(t) \qquad (3)$$

Equation 3 can be re-written as a linear function of $SUVA(t)$ against f_{ref} (see details in Appendix) :

$$SUVA(t) = SUVA_{biodeg}\ 1.05[1 - f_{ref}] + SUVA_{ref}\ [1.05 - 0.05DOC(0)/DOC(f)]\ f_{ref} + SUVA_{SMP}\ 0.05[DOC(0)/DOC(f)\ f_{ref} - 1] \quad (4)$$

For each DOM origin, $SUVA_{biodeg}$ and $SUVA_{ref}$ were estimated from linear regressions of $SUVA(t)$ against f_{ref} . Regression curves are shown on Figure 2. The measured values for $SUVA_{SMP}$ was an input of the regression. Results are displayed in Table 1. Results show the relatively low aromaticity of biodegradable compounds, whatever their origin ($SUVA_{biodeg}$ <13 $cm^{-1}.g^{-1}.L$). Refractory initial molecules of the artificial and the real wastewater effluent are more aromatic than SMP. On the contrary, the refractory initial molecules from algae extracts have a very low SUVA. This finding supports the importance of aliphatic molecules in the refractory algae extracts.

It should be underlined that this estimation only gives an order of magnitude of the aromatic characteristics of the substrates. It is based on the assumption of a unique SUVA for each DOM, although they may be composed of very various molecules. Satisfying determination coefficients (r^2>0.85) and significant parameters (T-test, p<0.01) were obtained for the three regressions, which tends to corroborate this assumption. We also assumed that the $SUVA_{SMP}$ value measured from glucose degradation could represent the SUVA of SMP formed during any other substrate degradation.

The same linear equation theoretically relies $K_{DOC}(t).DOC(t)$ and $DOC(t)$, since the partitioning coefficient K_{DOC} between BaP and DOM follows the relationship:

$$K_{DOC}(t)\ .DOC(t) = K_{DOC\ biodeg}\ .\ DOC_{biodeg}(t) + K_{DOC\ ref}\ .\ DOC_{ref} + K_{DOC\ SMP}.DOC_{SMP}(t) \quad (5)$$

Consequently, $K_{DOC\ biodeg}$ and $K_{DOC\ ref}$ were estimated in the same way. Results are displayed in Table 2 and regression curves are reported on Figure 4.

A significant estimation was obtained from the regression with data from the artificial substrate experiment (T-test, p<0.01). Biodegradable as well as refractory compounds from

the artificial substrate have a significant influence on the binding of BaP. $K_{DOC\ ref}$ values obtained for refractory compounds is similar to the ones of natural humic substances (Kukkonen et al, 1990, Haitzer et al, 1999) and is higher than measured $K_{DOC\ SMP}$. For DOM from algal origin, we were not able to get any significant K_{DOC} values for DOM fractions. This shows that the assumption of a unique binding capacity for each DOM biodegradability class may not be always a relevant model for the estimation of the global binding capacity of a degrading DOM. However, more studies are needed in order to precise the relevance of biogeochemical fractionation of DOM to estimate its binding capacity for organic micropollutants.

CONCLUSION

We monitored the biodegradation of two different substrates and evaluated the changes in aromaticity and influence on the bioavailability of benzo[a]pyrene. Both substrates were chosen as model mixture for allochthonous and autochthonous DOM in anthropized ecosystems. Both substrates were highly biodegradable and significantly affected BaP bioavailability. The more degraded they were, the higher their K_{DOC} were. We observed that refractory algae component as well as degraded artificial wastewater DOM decreased the bioavailability of BaP by 50% at DOM concentrations lower than 4 mg/L DOC, which is in the range of DOM concentrations in river waters. Conversely, initial non degraded products, had a much lower influence on the bioavailability of BaP. This study confirms the need to consider such types of DOM in addition to more widely studied humic substances when estimating the bioavailability organic pollutants in surface freshwaters.

Urban discharges increase DOM concentrations rivers, either of autochthonous (algal blooms triggered by nutrient discharges) or allochthonous origin (organic matter from sewer treatment plant, stormwater or sewer overflows). While persistent organic pollutants are little degraded by the aquatic micro-organisms, the transformation of DOM inside microbial food web will

likely greatly modify the bioavailability of POPs. This study points out the need to consider the dynamics of biodegradable DOM when evaluating the bioavailability of organic pollutants.

APPENDIX

Mathematical expressions of DOM biodegradability fractions

In the reactor, the DOM content can be expressed as the sum of biodegradable and refractory compounds and some SMP :

$$DOC(t) = DOC_{biodeg}(t) + DOC_{ref} + DOC_{SMP}(t) \qquad (a)$$

At the end of the experiment, only refractory compounds and SMP remain in the solution.

$$DOC_{ref} = DOC(f) - DOC_{SMP}(f) \qquad (b)$$

Since $DOC_{SMP}(t) = 0.05\ (DOC(0) - DOC(t))$ (Equation 2), DOC_{ref} can be written as:

$$DOC_{ref} = 1.05\ DOC(f) - 0.05\ [DOC(0) - DOC(f)] \qquad (c)$$

$$DOC_{ref} = 1.05(DOC(f) - 0.05DOC(0)$$

The biodegradable compounds can be expressed as a function of $DOC(t)$ (Equation a). :

$$DOC_{biodeg}(t) = DOC(t) - DOC_{ref} - DOC_{SMP}(t) \qquad (d)$$

Using the estimation of $DOC_{SMP}(t)$ (Equation 2) and DOC_{ref} (Equation c)

$$DOC_{biodeg}(t) = DOC(t) - 1.05[DOC(f) - 0.05DOC(0)] - 0.05[DOC(0) - DOC(t)] \qquad (e)$$

$$DOC_{biodeg}(t) = 1.05(DOC(t) - DOC(f))$$

Finally, the Equation 3 can be re-written as :

$$SUVA(t).DOC(t) = SUVA_{biodeg}\ 1.05[DOC(t) - DOC(f)] \qquad (f)$$
$$+ SUVA_{ref}\ [1.05\ DOC(f) - 0.05DOC(0)]$$
$$+ SUVA_{SMP}\ 0.05[DOC(0) - DOC(t)]$$

Equation g is obtained when dividing Equation f by $DOC(t)$ and using the definition of f_{ref} :

$$SUVA(t) = SUVA_{biodeg}\ 1.05[1 - f_{ref}] \quad (g)$$
$$+\ SUVA_{ref}\ [1.05\ f_{ref} - 0.05DOC(0)/DOC(f)\ f_{ref}]$$
$$+\ SUVA_{SMP}\ 0.05[DOC(0)/DOC(f)\ f_{ref} - 1]$$

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FIGURES CAPTIONS

Figure 1 : particulate (dotted line, POC) and dissolved (continuous line, DOC) organic carbon concentration evolution during the biodegradation of the three substrates.

Figure 2 : Evolution of the specific UV- absorbance at 254 nm (*SUVA*, in cm-1.g-1.L) during the biodegradation of the artificial domestic wastewater (crosses), the real wastewater (circles) algae extracts (triangles). he *SUVA* value of Soluble Microbial Products is also reported.

Figure 3: relative bioaccumulation of benzo[a]pyrene in daphnids (F/F_0) in the presence of organic matter from artificial wastewater and algae extracts, at different biodegradation times, from the initial substrate to a 16 day-biodegraded solution

Figure 4 : Evolution of the biologically-determined partition coefficients between dissolved organic matter and benzo[a]pyrene ($K_{DOC}(biol)$) along the biodegradation of the substrates. The $K_{DOC}(biol)$ value of Soluble Microbial Products is also reported.

Figure 1

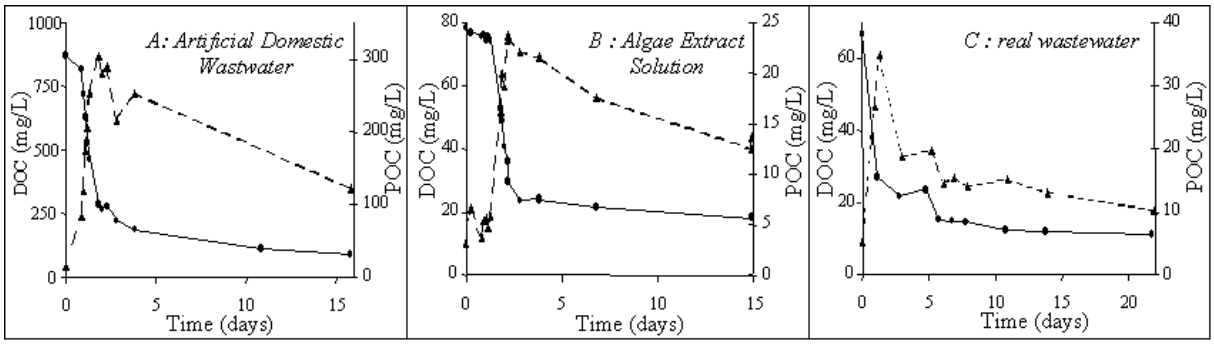

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Figure 2

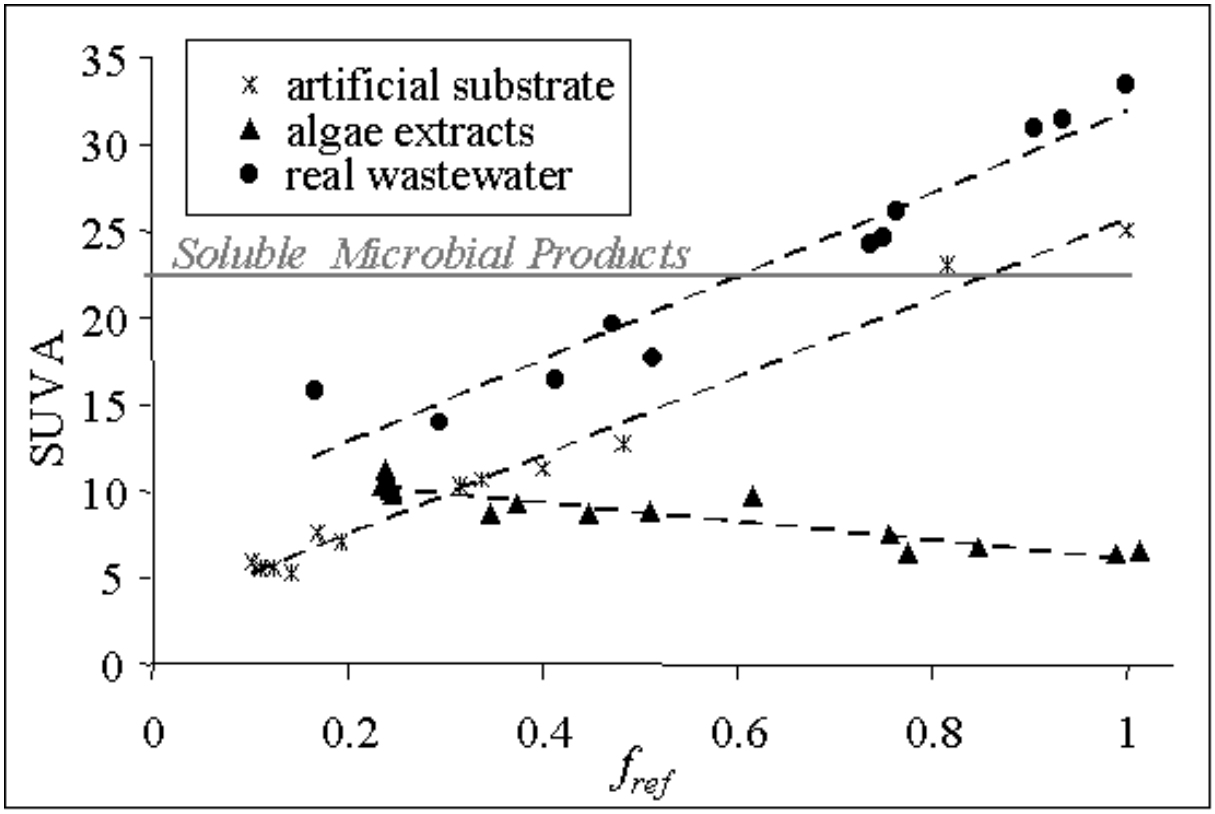

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Figure 3

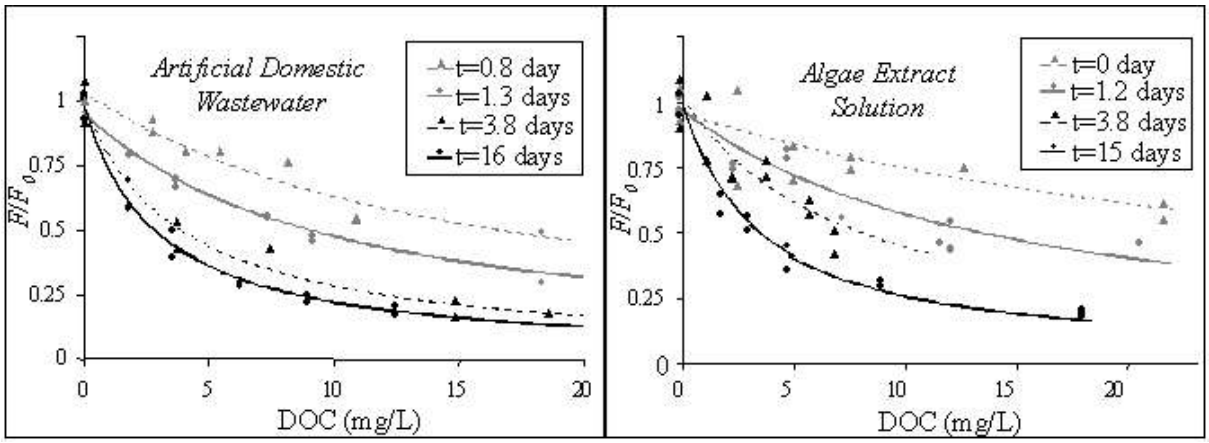

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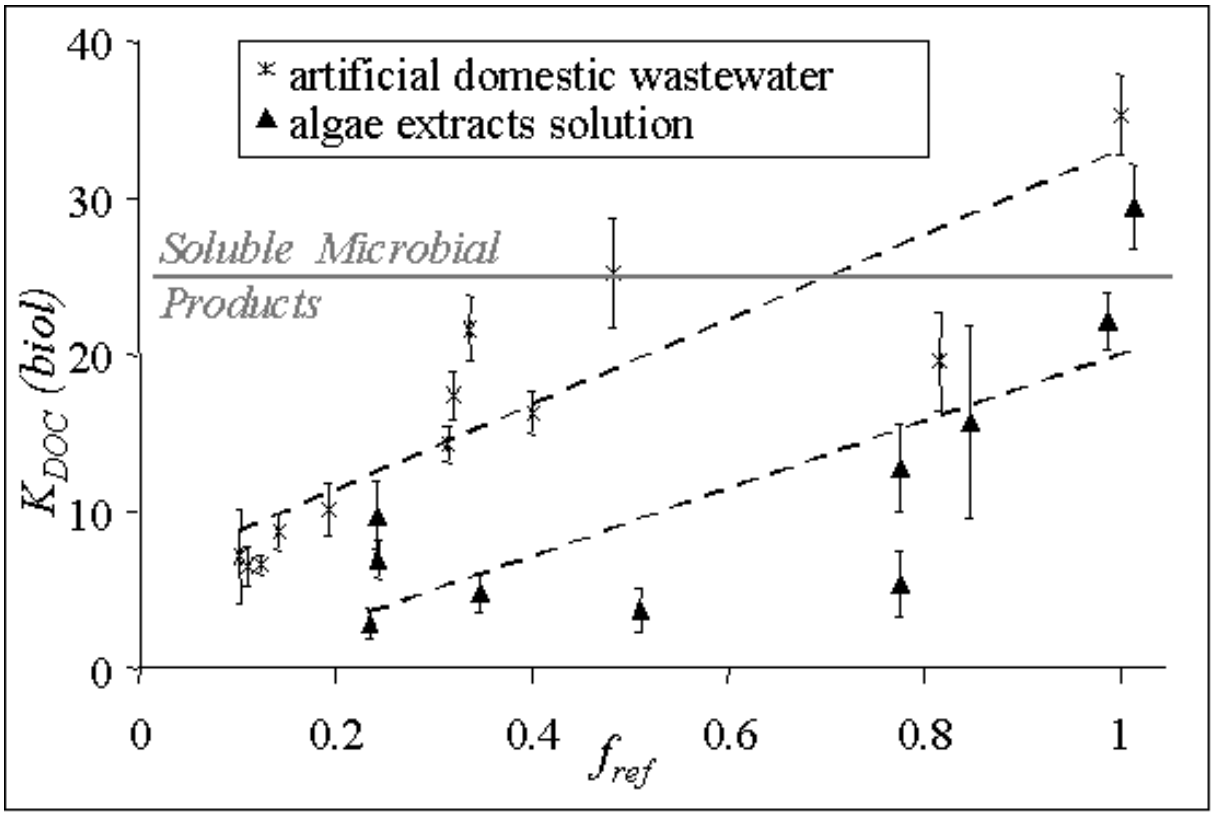
Figure 4



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Variability of SPMD (Semi-Permeable Membrane Device)-availability of polycyclic aromatic hydrocarbons (PAHs) in river waters and wastewater treatment plant effluents

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ABSTRACT

River waters and wastewater treatment plant effluents contain hydrophobic organic compounds (HOCs) such as polycyclic aromatic hydrocarbons (PAHs) which are persistent, bioaccumulative and dangerous for the environment. The biological risk assessment of HOCs requires the estimation of their bioavailable fraction in addition to the total contamination of the media. The bioavailability of HOCs is dependent on the aquatic environment characteristics. In particular, the presence of organic matter (OM) usually reduces the bioavailability of HOCs by trapping them and preventing them from crossing biological membranes.

The SPMD (Semi-Permeable Membrane Device) technique is used to evaluate bioavailable fractions of HOCs. We tested the influence of aquatic and more particularly OM characteristics upon the PAHs SPMD-availability, in several river waters (up and downstream a big town) and in wastewater treatment plant effluents. 13 priority PAHs were analyzed in total water and in SPMDs. Aquatic environments were characterised for their pH, ionic strength, temperature, chlorophyll A and suspended solids contents. Total and dissolved OM was characterised for its aromaticity, biodegradability, molecular weight and hydrophobicity. The total PAHs contamination increased downstream the river ; wastewater effluents were proved to be a source of SPMD-available PAHs. Relationships between the characteristics of organic matter and the SPMD-availability were attempted to be established in order to initiate an aquatic environment typology : the SPMD-availability was negatively related to dissolved OM molecular weight and aromaticity and positively related to biodegradability.

Keywords : Semi-Permeable Membrane Device, Polycyclic Aromatic Hydrocarbons; Aquatic environment typology, River waters, Wastewater treatment plant effluents.

1 – INTRODUCTION

River waters and wastewater treatment plant effluents contain hydrophobic organic compounds (HOCs) such as polycyclic aromatic hydrocarbons (PAHs) which are persistent, bioaccumulative and dangerous for the environment. The biological risk assessment of HOCs requires the estimation of their bioavailable fraction in addition to the total contamination of the media. The bioavailability of HOCs is dependent on the aquatic environment characteristics. In particular, HOCs may be bounded by suspended or dissolved organic matter (OM) making them more mobile and less biodegradable by biological/chemical processes. The presence of OM also usually reduces the bioavailability of HOCs [1-4]. The generally accepted assumption, which can be referred to as the "free pollutant model" is that pollutants that are bound to OM are too large to cross biological membranes [5]. The intensity of interactions between OM and HOCs, and consequently the bioavailability of HOCs, depend on OM characteristics. However, the influence of environmental conditions on the bioavailability of HOCs need to be better understood.

The evaluation of bioavailable contaminants in situ remains very difficult because of (i) the influence of environmental conditions and (ii) the low concentrations found in real media. As a tool to sample and evaluate in situ bioavailable HOCs, Semi-Permeable Membrane Devices (SPMDs) have been developed by J.N. Huckins , J.D. Petty et al from the CERC (Columbia Environmental Research Center, Columbia, MO) in the beginning of the 90's [6,7]. SPMDs are passive sampling devices consisting of a tubular layflat low density polyethylene (LDPE) membrane with a 1000 daltons (about 10 Å) nominal molecular weight cutoff, and containing a thin film of a high-molecular weight lipid (triolein) which is a major non polar lipid found in aquatic organisms. The passive sampling is driven by membrane-lipid-water partitioning and can be applied to non ionic compounds with log Kow values > 1. Because nearly all environmental contaminants are only slightly smaller than 10 A° (1000 d), mainly truly dissolved organic contaminants diffuse through the membrane and are concentrated by the triolein [7]. Moreover, the cross-sectional diameters of biological membranes range from 4 to 45 A° [8]. Therefore the SPMD-available fraction of contaminants in the media is considered to be similar to the readily bioavailable fraction [7].

The most common applications of SPMDs are the determination of the presence, source and time-weight-average concentrations of HOCs in various aquatic environments (natural waters [9-11] or wastewater effluents [12,13]) and in the atmosphere [14,15]. They can also been

used as surrogates for, or in addition to, biomonitoring studies to improve estimates of HOCs exposure [16,19] and to predict concentrations of specific contaminants in the tissues of organisms [11]. Recently, Leppänen et al [20] have tested the influence of sediments on HOCs bioavailability using SPMDs. Ravelet et al [21] used SPMDs in laboratory to study the influence of various aquatic dissolved organic matters (DOM) on PAHs bioavailability.

In this study, we tested the efficiency of SPMD to concentrate PAHs in several contaminated environments, and we more particularly focused on the role of organic matter (OM) upon SPMD-availability of PAHs. SPMDs were deployed in river water (up and downstream a large town) and in wastewater treatment plant (WWTP) effluents. The EPA priority PAHs were analyzed in whole samples and in SPMDs. Aquatic environments were characterised for their pH, ionic strength, temperature, chlorophyll A and suspended solids contents. Total and dissolved organic carbon were measured, and in order to initiate a typology of OM and assess its influence on PAH bioavailability, OM was characterised by its aromaticity assessed by its specific UV adsorption , biodegradability, molecular weight and hydrophobicity.

2 - MATERIALS AND METHODS

2.1. Chemicals

Acetonitrile, acetone, dimethyl-formamide, toluene, heptane of "pestipur" grade were obtained from SDS (Peypin, France). Heptane, methanol, acetonitrile, toluene, of LichroSolv grade were obtained from VWR (Fontenay-sous-Bois, France). Ultrapure water was obtained from a Milli-Q water system (Millipore, Molsheim, France). Standard nitrogen grade was obtained from Air Liquide (Paris, France). Filtrations were conducted on pre-combusted GF-F Whatmann glass fiber filter. Chromabond C18 6 ml / 2g cartridges (Macherey-Nagel, Hoerdt, France) were used for river water extraction. Silica cartridges Chromabond 6 mL / 1 g (Macherey-Nagel, Hoerdt, France) were used for SPMD purification. Priority PAHs at 10 $mg.L^{-1}$ in acetonitrile were obtained from Cil-Cluzeau (Ste Foy la Grande, France).

2.2. Aquatic environments characteristics

We have sampled 3 stations inside the Seine watershed : (i) Orgeval : a small forested river which is a tributary of the Grand Morin and Marne rivers, (ii) Saint-Maurice locks:

immediately upstream of Paris, on the Marne river, (iii) Andrésy locks, on the Seine river, about 10 km downstream of the main wastewater treatment plant of Paris.
We proceeded with 2 measurement campaigns : in February and in April 2003.

In May 2003, we have sampled effluents of 4 WWTP near Lyon (France), having various capacities, influent origins and treatment processes. Their characteristics are as follows (theoretical daily) :
AS1 : capacity : 3500 p.e., daily load : 900 m^3/j, 230 BOD_5 kg/j, activated sludge with continuous aeration, effluent of urban origin (domestic wastewaters and urban run-off), on the Saône river.
AS2 : capacity : 8000 p.e., daily load : 1600 m^3/j, 430 BOD_5 kg/j, activated sludge, domestic wastewaters and urban run-off plus dand-craft and industrial effluents (food processing industries, chemistry), on the Rhône river.
BF1 : capacity : 45000 p.e., daily load : 9670 m^3/j, 1800 BOD_5 kg/j, biofilters, with plated settling tanks, domestic wastewaters and urban run-off plus Hand-craft and industrial effluents (food processing industries, chemistry), on the Saône river.
BF2 : capacity : 35000 p.e., daily load : 6000 m^3/j, 2000 BOD_5 kg/j, biofilters, with plated settling tanks, domestic wastewaters and urban run-off plus Hand-craft and industrial effluents (food processing industries, chemistry), on the Rhône river.

Aquatic environments were characterized by their pH, ionic strength, temperature, chlorophyll A and suspended solids contents. Total and dissolved organic carbon was measured, and OM was characterised by its aromaticity assessed by its specific UV adsorption, biodegradability (according to [22]), molecular weight and hydrophobicity according to [21].

2.3. SPMD sampling

We used standard SPMDs (0.96 cm long, 2.5 cm wide, 75-90 µm wall thickness, filled with 1 mL of triolein) obtained from Exposmeter (Tavelsjo, Sweden).
In order to avoid or limit biofouling, SPMD were dipped twice a week during 5 min in a copper sulfate solution (1 g/L), and gently wiped.

SPMDs were exposed in river water for 3, 7 and 21 or 14 days (first and second campaign respectively), with duplicate devices for each time of exposure, except for the first campaign in Andrésy (only 3 SPMD analyzed, no duplicate).
SPMDs were exposed during 3, 7 and, when possible, 14 days in treated wastewater, depending on the quality of the effluents. When biofouling was too important, devices were removed. We set 3 SPMDs in each site.
After exposure and before extraction, SPMDs were washed with a tissue impregnated with ultrapure water in order to remove sorbed particles and associated PAHs.
We proceeded with blanks with unexposed SPMD, and with SPMD exposed on site to the atmosphere (just during the setting and the removal of the SPMDs) in order to evaluate possible contamination while setting, removing or washing the devices.

2.4. PAH analytical procedure

2.4.1. Sample pretreatment

Extraction of water samples : As for rivers, two liters of raw water were filtered on pre-combusted GF-F Whatmann glass fiber filter (0.7 µm nominal pore diameter). PAHs adsorbed on suspended solids were retained in the filter which was extracted by Soxhlet (with 80 mL of heptane during 7 h). PAHs in filtered water were solid-phase-extracted using C-18 silica cartridges. Cartridges were conditioned with 6 ml of methanol and then 6 ml of milli-Q water. One liter of the filtered water was percolated. Elution was achieved with 6 ml of acetonitrile and 6 ml toluene.
As for effluents, they were liquid-liquid extracted (3 successive fold during 3 min checking with 50 mL of heptane / L of sample).
After addition of 250 µL of dimethyl-formamide to the extract, they were concentrated with a rotary evaporator (at 40 °C) and under nitrogen flow to 250 µL. The final volume was adjusted to 1 mL in graduated tubes with acetonitrile for HPLC injection.

Extraction by dialysis for SPMD samples and purification : SPMDs were extracted by dialysis in heptane for 48 h. The extract was evaporated with a rotary evaporator (at 40 °C) until 1 mL. It was then purified by percolation on silica cartridge (preconditioned at 105 °C during 4 h and conditioned with percolation of 5 mL of a mixture of heptane/toluene (2/1))

and eluted with 10 mL of a mixture of heptane/toluene (2/1). After addition of 250 µL of dimethyl-formamide to the extract, heptane and toluene were evaporated with a rotary evaporator (at 40 °C) and under nitrogen flow. The final volume was adjusted in graduated tubes with acetonitrile for HPLC injection.

2.4.2. HPLC analysis

The HPLC system consisted of a Kontron 422 S pump coupled with a Jasco FP 920 fluorescence detector (UVK-LAB Service, Trappes, France). A 250 x 3 mm Bakerbond PAH-16 Plus column including guard column was used (Machery Nagel, Hoerdt, France). An Igloo-Cil oven (Interchim, Montluçon, France) was used for setting up the column temperature.
Acetonitrile and water were used as elution solvents at a flow rate of 1 $mL.min^{-1}$. The gradient elution program was : 60 % acetonitrile and 40 % water during the first 3 minutes, followed by a linear gradient up to 70 % acetonitrile after 17 min, followed by another linear gradient up to 100 % acetonitrile after 30 min, and isocratic elution with 100 % acetonitrile for 5 min. The column temperature was maintained at 30 °C. The fluorescence excitation and emission wavelengths were changed during the chromatographic separation in order to obtain better sensitivity. The excitation/emission wavelengths were set as follows : 280/340 nm from the acenaphtene to the anthracene elution, 280/430 nm from the fluoranthene to the chrysene elution and 285/460 nm from the benzo[b]fluoranthene to the indeno[123cd]pyrene elution.
The 16 priority PAHs were analyzed except naphtalene (because of its volatity and poor recovery after extraction), acenaphtylene (because it does not fluoresce) and phenanthrene (because of interfering compounds).

The whole protocol including in situ sampling and laboratory analysis is summarized in Table 1.

3 - RESULTS AND DISCUSSION

3.1. PAH concentrations in the total water compartment

Figure 1 shows the total high weight and low weight PAH concentrations for each site. We define PAHs with 3 aromatic cycles and log K_{ow} lower than 5 as low weight PAHs (Ace, Flu, Ant), and PAHs with more than 4 aromatic cycles and log Kow higher than 5 as high weight PAHs (10 PAHs, from Fla to IndP).

In WWTP effluents, high weight PAHs were mainly represented by Fla and Pyr (with similar concentrations). The 3 most hydrophobic PAHs (IndP, BghiP and DaA) are systematically absent. This can be explained by their absence or low concentration above the wastewater treatment plant and by lost by adsorption during the successive steps of the wastewater treatment.

Concentrations increase from Orgeval (the small forest river) to St Maurice and Andrésy (upstream and downstream of Paris), which reveals the influence of urban contamination.

For river waters, we have found a higher level contamination during the first campaign, which can be explained by the household heating in winter that induces higher PAHs atmospheric emissions and then higher contamination of aquatic environments by precipitation or deposition.

The level of contamination of treated wastewaters and river waters are similar. Among the WWTP, the AS1 effluent is significantly less contaminated, whereas BF2 displays the highest PAHs concentrations. However, due to its important treatment capacity (35000 p.e.), the PAH discharge to the river is by far the highest at BF2 (6000 $m^3.d^{-1}$).

3.2. SPMD measurements

3.2.1. In situ SPMD sampling efficiency

The accumulation of HOCs in the SPMD can be simply modelled by considering that the system is reduced to two compartments with a diffusion process in between : the contaminated water and the whole SPMD (membrane and triolein) [7]. The accumulation rate into the SPMD is supposed proportional to the SPMD-available concentration in water

(external) and the elimination rate from the SPMD is supposed proportional to the concentration in the SPMD (internal) :

$$\frac{dC_{PAH}^{SPMD}}{dt} = kuC_{PAH}^{water} - keC_{PAH}^{SPMD} \quad (1)$$

with : C_{PAH}^{SPMD} : the concentration of PAH in the SPMD (ng/g),

C_{PAH}^{water} : the SPMD-available concentration of PAH in water (ng/L),

ku : the accumulation rate constant ($L.d^{-1}.g^{-1}$),

ke : the elimination rate constant (d^{-1}).

During a first accumulation phase, the PAH elimination from SPMD is negligible and the accumulation linearly raises with time. After a second intermediary accumulation phase, the equilibrium is finally reached (phase 3).

SPMDs were exposed as described in the experimental part 2. Biofouling was limited in river waters and SPMDs were not damaged during the exposure, while previous experiments without copper treatment nor wiping showed a serious deterioration of the SPMDs. In sewer treatment plant outlets, we were forced to remove some SPMDs before the end of the 14 days exposure because of the hard exposure conditions (high water velocity, high suspended solids) and strong biofouling. Consequently SPMDs were exposed for only 7 days in BF2 and AS2 effluents.

During the first campaign in river, SPMDs were set during 21 days. But devices in Orgeval that were removed at 21 days contained systematically and significantly much lower quantities of PAHs than devices that were removed at 3 or 7 days. This can be explained by a possible PAHs photodegradation during exposure. Indeed, SPMDs were deployed right under the water surface because of the very low water level in Orgeval (about 30 cm deep). For the other sites, SPMDs were deployed at more that 1 meter deep, where light is very limited, which avoids most photodegradation processes. Therefore, the data obtained after 21 days exposure at Orgeval were not used in this study.

The mean relative standard deviation (RSD) among all PAHs replicates measurements is 18 %, and 75 % of RSD among replicates measurements are below 24 %, which is quite

satisfying. This deviation is comparable to RSD of PAHs in replicate SPMDs reported in other studies (6-31 % from [23] and 14-56 % from [19]). As shown in Figure 2, for the second campaign in St Maurice, accumulation was linearly increasing during the exposure period, with correlation coefficients (R^2) higher than 0.9. We observed that accumulation in SPMDs was still linear after 14 days for all tested PAHs and all stations.

The linear accumulation of PAHs can be described by the following equation :

$$C_{PAH}^{SPMD} \times M_{SPMD} = C_{PAH}^{water} \times R_S \times t \quad (2)$$

With : R_S : the uptake rate constant ($L.d^{-1}$), i.e. the volume of water cleared per unit time, as defined by Huckins et al.

M_{SPMD} : the SPMD mass (g),

t : the exposure time (d).

Accordingly, we calculated PAH SPMD-available concentration in water as :

$$C_{PAH}^{water} = \frac{M_{SPMD} . C_{PAH}^{SPMD}}{R_S . t} \quad (3)$$

Rs values depends on the contaminant, the SPMD size as well as environmental conditions. They are estimated for each compound from laboratory calibration. In this study, we used the R_S values for PAHs determined for standard SPMDs by Huckins at 10 °C for river waters and 18 °C for effluents [24].

SPMD-available concentrations of PAHs should be lower than the total concentrations, but this was not systematically verified with our results (as seen in Figure 3) : the SPMD-available concentration of PAH represented 50 to 450 % of the total concentration in the water. SPMDs are unexpectedly able to concentrate much more PAHs than what was expected from reported R_S coefficients. This phenomenon was already observed elsewhere. Stuer-Lauridsen et al [23] found that the SPMD-available concentration of PAHs in water represented 30 to 437 % of the total concentrations as measured by liquid-liquid extraction in

wastewater. Axelman [19] also observed that the SPMD-water concentration may represent up to 9 to 10 fold the concentration of PAH in the water.

These unexpected results could be explained by the time averaged concentration obtained from SPMDs that are compared with an average concentration obtained from several instantaneous samplings. Indeed, during the exposure time, we observed a high RSD among PAHs concentration from successive instantaneous samplings (up to 34 % for the sum of the 13 PAHs in Andrésy, second campaign). Another possible explanation is that the R_S values proposed by Huckins et al. might be inapplicable for the exposure conditions encountered in these studies. We also found higher R_S values in well controlled laboratory experiments a previous study devoted to 5 selected PAHs [25].

The reason for the observed discrepancies is however unclear. Temperatures and water velocity may influence on the kinetic of PAH accumulation inside the device, but available experimental data do not allow to derive R_S values for any temperature or agitation conditions. Temperatures in river water were in the range from 5 to 11 °C and 14 to 20 °C for effluents, which is generally lower than the reference temperature studied by Huckins (10 °C and 18 °C respectively used for river waters and effluents). Since R_S coefficients are expected to increase with temperature, this factor cannot be retained as a possible explanation. Previous work [26] indicated that a 50 times increased shear flow along the SPMD surface by (from 0.01 to 0.50 $cm.s^{-1}$) induces only a slightly higher sampling rates Rs for the higher Kow PAHs (from 8 % for pyrene to 40 % for benzo[b]fluoranthene), and no significant Rs increase for the lower Kow PAHs.

As linearity and repetability are satisfying, and since biofouling was avoided, we conclude that Huckins values cannot be directly transposed in our study to obtain quantitative results, but that SPMD nevertheless behaved as expected with a linear accumulation.

Nevertheless, in order to provide standardized results, SPMD-available concentrations will be computed using the R_S values proposed by Huckins et al.

3.2.2. SPMD-availability in river waters and WWTP effluents

In this paragraph, results are presented as a unit-less ratio (SPMD-available concentration / total PAH concentration) in order to keep track of the level of contamination in total water samples. Because possibly higher than 1, this ratio is not representative of a real fraction of SPMD-available PAHs inside the total water compartment, we shall simply call it availability

indicator of PAHs. The higher the indicator, the more the PAHs are SPMD-available and the more the PAHs are possibly available to aquatic organisms.
Figure 3 shows the indicator values for the sum of the 13 PAHs and for each site. The average availability indicator in treated wastewater is much higher than what was obtained for the river water, while the total PAH concentration was approximately the same for both types of aquatic environment, as previously observed (part 3.1). The different factors which could explain such a behavior need to be explored.

3.3. Influence of the exposure characteristics upon the SPMD-availability of PAHs.

In order to initiate a typology of the exposure characteristics according to their influence on the PAH availability to SPMD concentration, correlations between these characteristics and the availability indicators were tested. Exposure characterization was based on water pH, temperature, conductivity, water velocity and suspended matter content. Dissolved and particulate organic matter was measured and OM was characterized for its aromaticity (by SUVA254, ie UV254/ DOC), and biodegradability. We also made experiments in the laboratory to study the influence of the size (molecular weight) and hydrophobicity of OM.

The mean values for each of the descriptors for river waters and for wastewater effluents are reported in Table 2. Temperature, chlorophyll a content and SUVA were not measured in WWTP effluents. But some authors reported SUVA values (measured at 260 nm) ranging from 11 to 19 $cm^{-1}.g^{-1}.L$ according to the treatment [27,28]. M.F. Dignac et al [29] measured a SUVA value (at 254 nm) of 23 in the effluents of the WWTP of Compiègne (near Paris, France). These values are similar to ours in river waters.
We observe that conductivity, suspended matter, total, dissolved and biodegradable organic carbon are significantly higher in effluents.
According to the free pollutant model, bioavailability should decrease with the increase of suspended matter, total and dissolved organic carbon, which is not verified. At this step, we can wonder if these 3 quantitative descriptors are determining. Perhaps, they have combined effects ? The well-known solubilization effect of PAHs with the increase of OM content in water may induce an increasing SPMD-availability if these are small size OM that can pass through the SPMD membrane. Qualitative descriptors (aromaticity, biodegradability, hydrophobicity, size) are perhaps more representative of matrices influence upon

bioavailability : with regard to the fraction of biodegradable organic carbon, it is higher in effluents, and this can explain the higher SPMD-availability in effluents. Indeed, this descriptor is often correlated with a small size of OM (then less steric obstruction when crossing the biological membrane) or a poor aromaticity of OM (then weaker interactions with PAHs). It is just like there are some intra-matrix descriptors (i.e. applicable inside one kind of matrix, as for the quantitative ones) and inter-matrices descriptors (applicable whatever the kind of matrix, as for the qualitative ones). Anyway, for the next, we have chosen to study these 2 kinds of matrices separately.

3.3.1. Influence of physical and chemical- descriptors

We did not observe any correlation between the SPMD availability indicator and pH, conductivity or temperature, whereas some correlations are brought to the fore with the suspended solids concentration.

As shown in Figure 4, the SPMD-availability indicator decreases when the suspended solids concentration increases in effluents and river waters. This can be explained by the free pollutant model since PAHs trapped by suspended matter become less available. This trend must be confirmed with more values. Since this descriptor seems to be applicable inside one kind of matrix (intra-matrix descriptor), it must be considered as a secondary descriptor in the typology.

3.3.2. Influence of organic matter

On the one hand, we did not find any correlation between SPMD availability and TOC, DOC or chlorophyll A content (not enough data in this case). On the other hand, we observe in river waters a decreasing SPMD-availability while OM aromaticity (measured by SUVA) increases (Figure 5). We verified in situ that aromatic organic matter reduces the availability of contaminants, since aromatic structures in OM may form hydrophobic sites for PAH binding. This phenomenon was already observed in various studies in the laboratory [30,31,32]. In river and effluents, the availability indicator also increases when biodegradable DOC decreases (Figure 6). From [33], we know that SUVA and biodegradable carbon content are

correlated : a high value of biodegradable carbon content induces a low SUVA (a low aromatic molecules content).

We also made some experiments in laboratory with various DOM characterized by a large range of average molecular weight (Mw), from 610 to 4100 daltons [21]. They are listed on the abscissa of Figure 7. On the ordinate is reported the availability indicator for BaP, in tap water spiked with the DOM at 10 mg/L. We first note that in well-controlled laboratory conditions, the availability indicators are always lower than 1. We observe that the availability indicator increases with the decrease of the average molecular weight. This is in agreement with the free pollutant model : small structures, when they interact with PAHs, induce less steric obstructions when crossing the SPMD membrane than large OM. We also know that lower Mw is correlated with higher biodegradable content and lower aromaticity.
Until now, we did not find
any interesting results with the OM hydrophobicity.

3.4. Conclusion

SPMDs were deployed in various river and sewer treatment plant waters in order to sample bioavailable PAHs in addition to PAHs in the total water. Although SPMD may overestimate water concentration in the media, they were used as a new tool to understand the influence of aquatic environments on organic pollutants bioavailability.
From the in situ experiments, wastewater effluents seems to be a source of SPMD-available PAHs. Whatever the kind of aquatic environment, the SPMD-availability tends to be positively related to the biodegradability and negatively related to the aromaticity of DOM. When considering river waters or WWTP effluents separately, suspended matter content increases while the SPMD-availability decreases. All these tendencies are in good concordance with the free pollutant model but they must be confirmed with more points in others rivers and effluents. The influence of the average OM molecular weight must be confirmed in situ and the OM hydrophobicity must be still studied (in laboratory and in situ).
Although interesting results regarding factors affecting availability have been obtained, it should be kept in mind that the behaviour of SPMD in situ is still poorly understood since numerous higher than one availability indicators have been obtained. A significant effort should be made in the future to better assess this behavior. Reference compounds placed

inside the SPMD before exposure may be a valuable way to evaluate in situ R_S values, according to the simple two-compartment model commonly used. However, more work should also be devoted to the in situ behavior of the membrane, and to its possible long-term interactions with dissolved or adsorbed organic matter.

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Table 1 : Sampling and analytical protocols.

Site	Type	Sampling Period	Duration, (number of replicate)	Water analysis (technique), (replicate number)
Orgeval	Small forest river	Feb. 03	3d (2), 7d (2), 21d (2)	Filtered water (SPE), particles (Soxhlet) (3)
St Maurice	Anthropic river upstream Paris	Feb 03 April 03	3d (2), 7d (2), 21d (2) 3d (2), 7d (2), 14d (2)	Filtered water (SPE), particles (Soxhlet) (3) Filtered water (SPE), particles (Soxhlet) (3)
Andrésy	Anthropic river downstream Paris STP	Feb 03 April 03	3d (1), 7d (1), 21d (1) 3d (2), 7d (2), 14d (2)	Filtered water (SPE), particles (Soxhlet) (3) Filtered water (SPE), particles (Soxhlet) (3)
AS2	Small WWTP (domestic effluents)	May 03	3d (1), 7d (1), 14d (1)	Raw and filtered water (LLE, 1)
BF2	Large WWTP (domestic /industrial effluents)	May 03	3d (2), 7d (1)	Raw and filtered water (LLE, 1)
BF1	Large WWTP (domestic /industrial effluents)	May 03	3d (2), 7d (1)	Raw and filtered water (LLE, 1)
AS1	Small WWTP (domestic /industrial effluents)	May 03	3d (1), 7d (1), 14d (1)	Raw and filtered water (LLE, 1)

d : day ; SPE : solid phase extraction ; LLE : liquid-liquid extraction

Table 2 : Mean values and standard deviations for some descriptors of river waters and wastewater effluents.

	pH (is)	Conductivity (µS/cm à 25°C)	T° (°C)	Susp. matter (mg/L)	TOC (mg/L)	DOC (mg/L)	Suva $_{254}$ ($cm^{-1}.g^{-1}.L$)	biodeg. DOC (% DOC)
Mean values for river waters	**7,7**	**547**	**6,8**	**14,71**	**4,93**	**3,35**	**21,67**	**21 %**
Standard deviations	0,7	177	2,1	4,66	1,38	0,98	5,30	
Mean values for effluents	**7,8**	**1173**	**nm**	**41,56**	**22,10**	**16,39**	**nm**	**49 %**
Standard deviations	0,2	199	nm	29,00	11,03	9,71	nm	
	=	#	/	#	#	#	/	#

nm : not measured, SUVA : ABS 254 nm/DOC

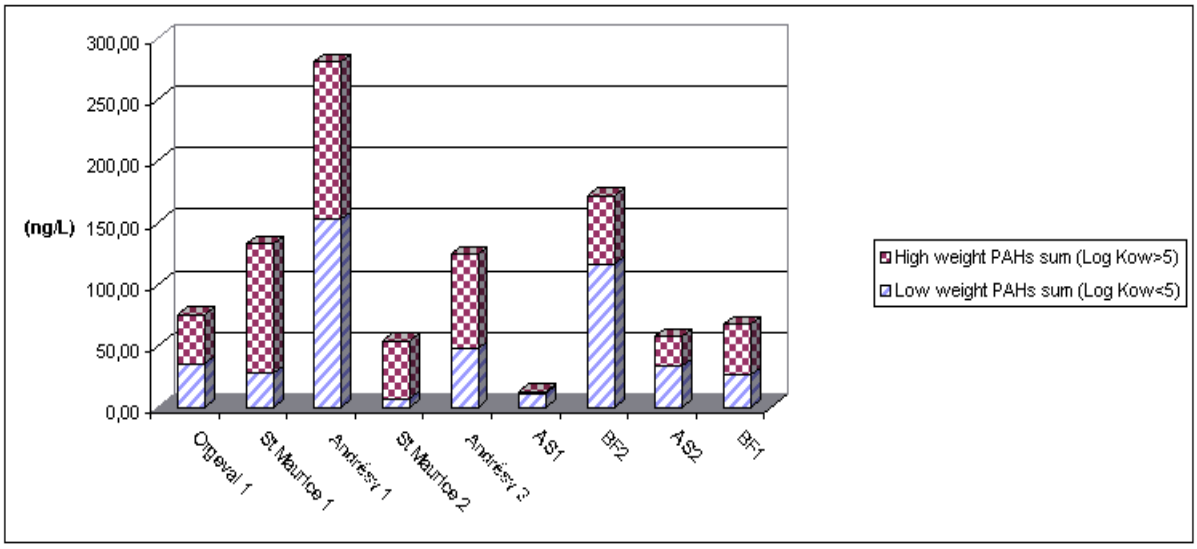
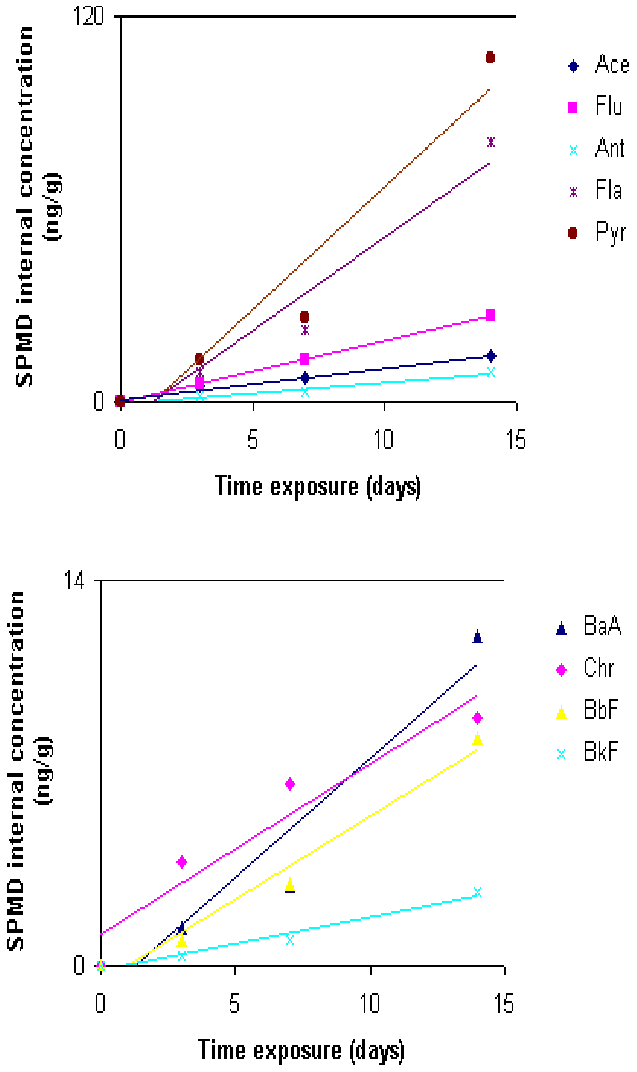


Figure 1 : Total PAH concentrations, 1 : first campaign in February, 2 : second campaign in April (with Orgeval dried up).



	R^2
Ace	0,986
Flu	1,000
Ant	0,975
Fla	0,945
Pyr	0,928
BaA	0,926
Chr	0,917
BbF	0,974
BkF	0,970

Figure 2 : Accumulation curves in St Maurice (second campaign) and correlation coefficients (R^2).

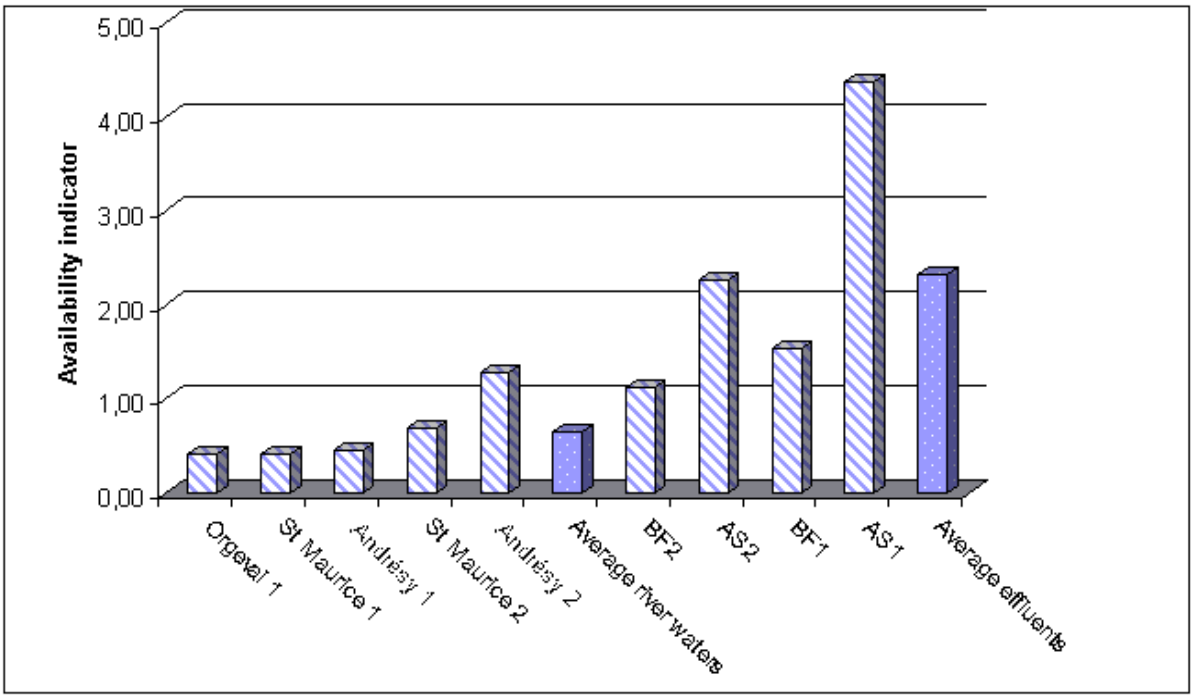


Figure 3 : Availability indicator (ratio SPMD-available concentration / total PAH concentration) and average values in river waters and effluents.

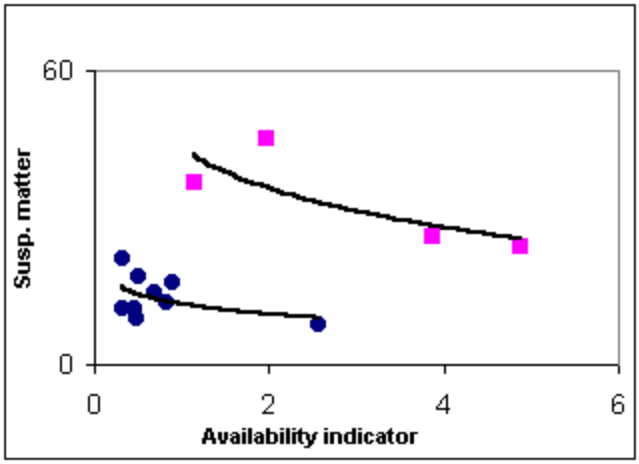


Figure 4 : Influence of the suspended matter content (mg/L) on the availability indicator, in river water (o) and WWTP effluents ( ).

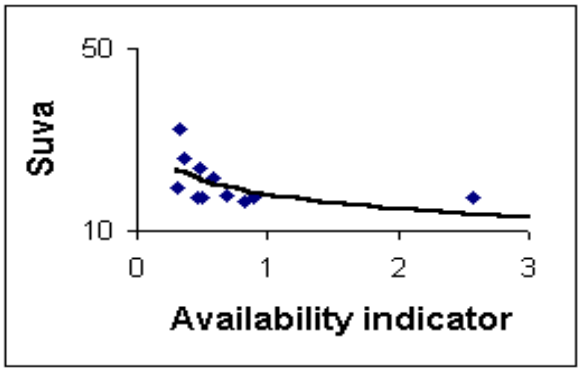


Figure 5 : Influence of SUVA ($cm^{-1}.g^{-1}.L$) on the availability indicator, in river water (SUVA was not measured in effluents).

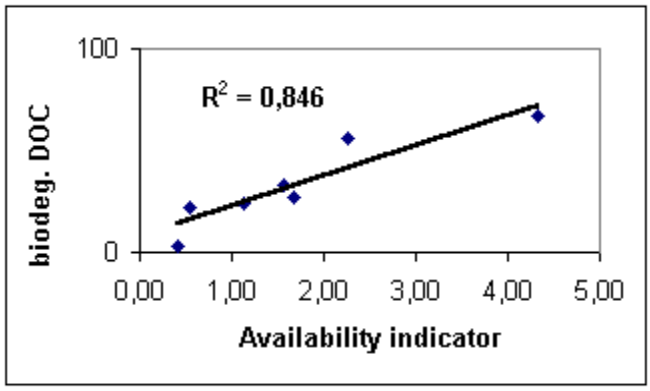


Figure 6 : Influence of biodegradable DOC (% DOC) on the availability indicator, measurements were conducted in river and effluents.

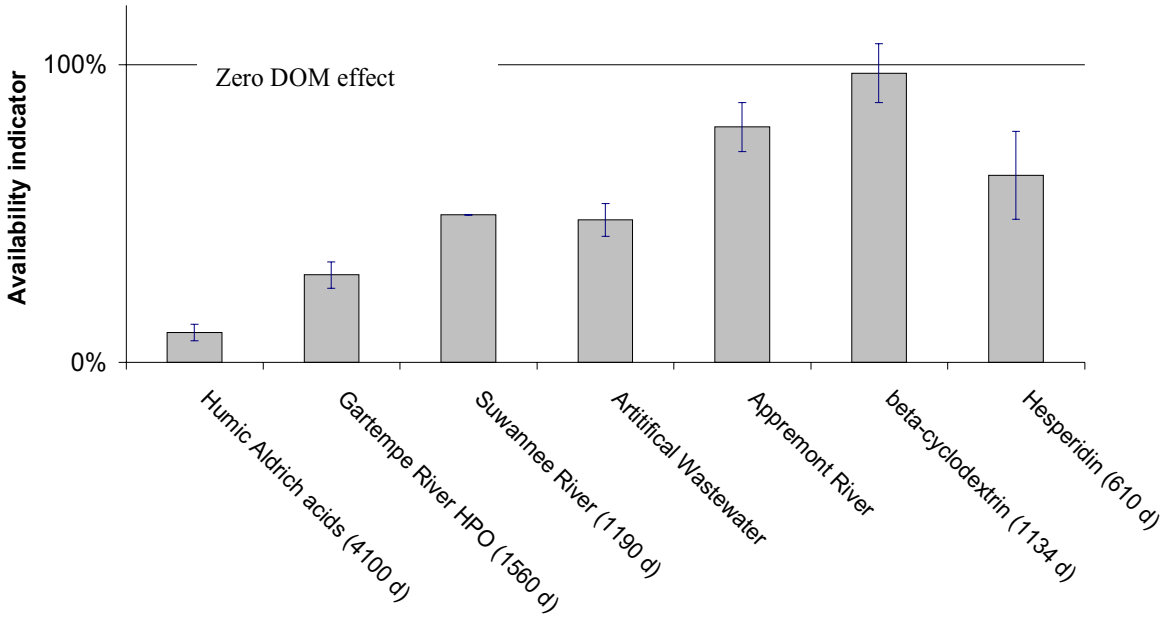


Figure 7 : Influence of average molecular weight Mw (dalton) on the BaP availability indicator, measurement in tap water spiked with 10 mg/L DOM, from [21].