Ecological engineering for the bioremediation of aquatic systems : effects of the combined bioturbation and phytoremediation on cadmium and atrazine removal.

Trung Kien Hoang

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THÈSE

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Trung Kien HOANG

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Ingénierie écologique pour la biorémédiation des systèmes aquatiques: effets du couplage de la bioturbation avec la phytoremediation sur le cadmium et l'atrazine

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"It always seems impossible until it's done" – Nelson Mandela

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RESUME FRANÇAIS

Ce sujet de recherche vise mieux comprendre les processus de bio remédiation qui participent à la réduction des charges en polluants dans les écosystèmes aquatiques du type zones humides, en tant que question d'actualité en ingénierie écologique. L'efficacité des processus de phytoremédiation a été largement démontrée par des applications individuelles sur le sol et les sédiments. Cette thèse a pour objectif de démontrer la participation d'une population d'invertébrés dans l'efficacité de la réduction de polluants des sédiments aquatiques en combinant le processus de bioturbation avec la phytoremédiation. Les hypotheses de recherche ont été testée expérimentalement en conditions de laboratoire à l'aide d'une série de microcosmes reproduisant chacun une portion d'interface eau/ sédiments similaire aux conditions en zones humides. Dans nos expériences, la bioturbation est réalisée par une population d'oligochètes Tubificidae bien connue comme un ingénieur écologique. La phytoremédiation associée est effectuée par une plante aquatique *Typha latifolia* connue pour sa capacité à extraire les polluants organiques et inorganiques des sédiments par l'accumulation dans leur biomasse. L'influence de cette biodiversité sur les flux et bilan de masse de polluants modèles, a été démontré à l'aide de 2 expériences de laboratoire mettant en oeuvre des séries de microcosmes contaminées avec du Cadmium en tant que métal trace avec une concentration initiale de 20 $\mu g.L^{-1}$, dans l'eau surnageante, et de l'atrazine marquée avec une concentration de 5 $\mu g.g^{-1}$ de sédiment frais en tant que micropolluant organique persistant et herbicide.

Les résultats de ces expériences démontrent que le bio-transport créé par la population de tubificidae ainsi que la bioremédiation sont toujours actifs en présence de contamination ce qui confirme le potentiel de dévelopement de ces organismes en ingénierie écologique. La bioadvection du sédiment et des contaminants par les tubificidae est quantifiée grâce à l'utilisation de luminophores (traceurs particulaires). Le processus de bioconvoyage engendre l'ensevelissement et le renouvellement de la

quantité de polluant autour du système racinaires des *Typha latifolia*. Les coefficients d'enrichissement des racines (EC_R) pour le Cadmium sont plus élevés en présence de bioturbation. L'interaction entre l'activité des tubificidés et la contamination par le Cd depusi la colonne d'eau influence le bilan de masse du cadmium. Les flux de métaux du sédiment vers les racines sont estimés de l'ordre de 0.02+/-0.00 et 0.07+/-0.03 μg Cd par jour sans et avec bioturbation, respectivement. Dans le cas de l'atrazine, l'influence de la bioturbation sur les propriétés chimiques du sédiment (pH, porosité, matière organique, etc.) explique les modifications observées au niveau du processus d'adsorption-désorption de l'atrazine. Il est démontré que la bioturbation par les tubificidae accélère la mobilité de l'atrazine et favorise sa biodisponibilité en passant de la forme adsorbée sur les particules de sédiment vers la forme libre dans l'eau interstitielle. Les coefficients d'enrichissement de l'atrazine dans les racines de typha sont également plus élevés en présence de bioturbation (7.16 ± 0.66) que sans bioturbation (5.99 ± 0.64). La biodégradation de l'atrazine est significativement plus avancée en présence de bioturbation comme le démontre un nombre plus élevé de métabolites, et une quantité de métabolites plus importante mesurés dans les racines *Typha latifolia*.

Notre étude démontre le potentiel apporté par la bioturbation comme facteur d'amélioration des performances de la phytoremédiation, pour un métal lourd comme pour un micro-polluant organique. Les recherches futures sur la base de ces résultats devraient s'intéresser à mettre en évidence l'influence de la bioturbation sur les communautés de micro-organismes en charge de la dégradation des composés organiques persistants.

Mots clés: Ingénierie écologique; bioturbation; phytoremediation; pollution aquatique; zone humide; cadmium; atrazine; ^{14}C.

SUMMARY

The development of efficient bioremediation techniques to reduce pollutant loads in aquatic ecosystems is a challenging research question for ecological engineering. The accuracy of phytoremediation processes has been primarily demonstrated by individual applications on soils or water sediments. The present Ph.D. aims to demonstrate the interest of additional bioturbation combined to phytoremediation processes for the improvement bioremediation efficiency of aquatic sediments. This strategy benefits are tested experimentally in controlled laboratory conditions with a serie of microcosms reproducing each a portion of water/sediment interface such as in wetland areas. In our experiments, bioturbation was carried out by a conveyor-belt invertebrate population, the tubificidae oligochaetes *Tubifex tubifex*, well known as an active ecological engineer. The phytoremediation was conducted by the riparian plant *Typha latifolia* known for its ability to remove organic and inorganic pollutants from sediments by accumulation into its biomass. The experiments were managed to demonstrate the effects of this biological influence (plant and inveterbrate) on the mass balances and fluxes of one metal and one pesticide as models of pollutants. Cadmium as a heavy, inorganic and conservative metal pollutant was introduced as a pulse input in the overlying water of the contaminated microcosms, with a cadmium concentration of 20 $\mu g.L^{-1}$ in at the initial time of the experiment that lasted one month. In a second experiment, atrazine was mixed in the whole sediment column at the initial time in order to reach a concentration of 5 $\mu g.g^{-1}$ of fresh sediment as a source of organic micropollutant and herbicide in the microcosms. The pesticide was radiolabeled with ^{14}C. Fluxes from water to sediment, and from sediment to plants were assessed in experimental conditions with several treatments (+/- plants, +/- bioturbation, +/- pollutants) allowing to demonstrate the effects of the biological influence.

Our results indicated that the tubificids and the related bioremediation influences are still efficient under cadmium and atrazine contaminations in aquatic

systems. Biotransport due to tubificids changed the distribution of cadmium across the sediment column and enhanced the pumping of cadmium from the water to surface sediment and then to the anoxic underlying sediment surrounding the plant roots. Thereby this biotransport increased the bioaccumulation of cadmium in the root system of *Typha latifolia* as demonstrated by the roots enrichment coefficients (EC_R). Combining Cd contamination and bioturbation optimized the transport of the metal (flux) from sediment to plants with fluxes estimated to 0.02+/-0.00 and 0.07+/-0.03 µg Cd per day without and with bioturbation respectively. In the case of atrazine contamination, bioturbation influence on the chemical properties of sediment (pH, porosity, Organic matter) is explaining the adsorption-desorption behavior of atrazine in sediment, resulting in the acceleration of atrazine mobility and bioavailability of the pesticide. The transfert of pollutant that passed from attached forms onto sediment particles into a free fraction in pore water in the sediment column was enhanced under bioturbation. Consequently higher values of atrazine were measured in the roots system with ECs of 7.16 ± 0.66 with bioturbation compared to 5.99 ± 0.64 in the treatment without bioturbation. The metabolization of the pesticides was also significantly increased under the effects of bioturbation on the sediment microbiome as demonstrated by enhanced number and quantity of metabolites in *Typha latifolia* roots in this treatments.

Our study, therefore, highlights the potential of bioturbation addition of the phytoextraction for integrated bioremediation strategies of metallic and organic polluted sediments of aquatic ecosystems. Further researches need to take into account the bioturbation influence on the microorganism communities in the relationship with organic compounds degradation.

Keywords: Ecological engineering; bioturbation; phytoremediation; water quality; wetland; atrazine, cadmium, ^{14}C.

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Abbreviations list

ATR	Atrazine
DEA	Deethylatrazine
DEHA	Desethylhydroxyatrazine
DIA	Deisopropylatrazine
DIDA	Didealkylatrazine
EC	Enrichment coefficient
ESA	Ecological Society of America
GLWD	Global Lake and Wetland Database
HA	Hydroxylatrazine
HO-A	Hydroxyatrazine
ITIS	Integrated Taxonomic Information System
K_d	Sorption coefficient
Koc	Partition coefficient
K_{ow}	Octanol–water partition coefficient
NRC	National Research Council
PHEs	Potentially harmful elements
POPs	Persistent organic pollutants

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INTRODUCTION

L'accumulation de polluants dans les sédiments des écosystèmes aquatiques, tels que les métaux et les polluants organiques persistants, est à l'origine de disfonctionnement des milieux naturels. Non seulement, ces polluants présentent un risque pour les communautés aquatiques due à leur exposition à des concentrations anormales, mais ces polluants peuvent également entrer dans les organismes *via* le phénomène de bioaccumulation le long des réseaux trophiques. Le défit associé à la qualité de la ressource en eau d'une part, la mise en évidence du rôle prépondérant des zones humides dans le service de regulation de la qualité de l'eau des hydrosystèmes et et la mise en évidence de la toxicité des polluants mesurés dans les sédiments de ces zones humides d'autre part, font que la contamination des sédiment attire une attention grandissante. Bien que la limitation de l'arrivée de polluants dans ces milieux soit la priorité, la recherche de solutions pour restaurer la qualité de l'eau et des sédiments sousjacents est une question qui relève de l'ingénierie écologique. La bioremédiation est la partie de l'ingénierie écologique qui vise à réduire la quantité de polluants dans un milieu naturel en mettant en œuvre, une population ou une communauté d'organismes vivants. Ce type d'ingénierie est aujourd'hui la seule solution durable pour améliorer le fonctionnement des zones humides, en apportant des méthodes lentes mais pérennes pour diminuer la charge en polluants en se basant sur les connaissances issues de l'écologie fonctionnelle des écosystèmes aquatiques.

L'avancée des recherches sur la compréhension des processus biologiques participant aux flux de matière et d'énergie à l'interface eau-sédiment apportent une source d'information intéressante pour l'identification des techniques de bioremediation faisant participer ces processus. La démonstration de l'influence de la biodiversité dans les flux de matière et à l'origine de ces processus de biodegradation est toujours une question de recherche active. La mise en évidence des bénéfices et services naturels retirés du fonctionnement naturel des écosystèmes a récemment relancé l'attention sur ces processus. Plus précisément, le rôle de la biodiversité dans le service naturel de régulation de la qualité de l'eau est toujours une question de

rechercheà résoudre. L'application des enseignements issus de ces recherches pour améliorer la qualité de l'eau des écosystèmes naturels par bioremédiation réponds à une véritable demande pour le maintien des services naturels. Les solutions fondées sur la nature sont l'illustration d'une telle démarche qui s'inspire aussi bien des processus d'origines biologiques et physiques pour répondre à une question de société: "Comment dépolluer de manière durable les milieux aquatiques". D'une manière générale, les solutions fondées sur la nature participe à la résilience des écosystèmes dans lesquels elles sont appliquées et quelques une de ces solutions contribuent à la bioremédiation. Parmi les méthodes d'ingénierie écologique déjà à l'œuvre et pouvant s'inscrire parmi les solutions inspirées de la nature, la phytoremédiation est maintenant largement reconnue pour son potentiel à réduire les quantités de polluants d'un sol ou d'un sédiment. De nombreuses recherches ont eut pour objectif de démontrer les capacités d'espèces de plantes variées à extraire certains polluants des sols et sédiments, tels que les métaux (Ali et al., 2013; Barceló & Poschenrieder, 2003; Guo et al., 2012; Klink et al., 2013; Lyubenova et al., 2013; Pandey et al., 2014; Wani et al., 2017; Weis & Weis, 2004), et les POPs (Frazar, 2000; Ibrahim et al., 2013; Marcacci, 2004; Merini et al., 2009; Moore et al., 2013; Pascal-Lorber et al., 2010; Tournebize et al., 2013; Wang et al., 2015), et beaucoup reste encore à faire pour développer les applications de ces connaissances en milieu naturel. Cependant, les plantes ne sont parfois pas suffisante pour faire face au large spectre de polluants présents dans les sédiments aquatiques, et il devient alors intéressant de chercher des solutions pour améliorer les capacités épuratives de la phyto-extraction. Parmi les voies de recherche vers une bioremédiation optimale, l'association avec d'autres processus naturels est une possibilité encore peu explorée. Cette thèse vise à mettre en évidence s'il existe un véritable intérêt à associer le phénomène de bioturbation avec la phytoremediation « classiquement » mis en œuvre. La bioturbation est un processus naturel qui a été étudié depuis longtemps dans les sédiments marins, d'eau douce et les sols terrestres. Plus précisément, la bioturbation sous l'impulsion des multiples activités des

invertébrés benthiques à l'interface eau-sédiment, présente la capacité d'accélérer tout les flux de matière à travers cette interface, de même que les transformations chimiques et biologiques de la matière naturelle et anthropique dans le sédiment (Anschutz et al., 2012; Baranov et al., 2016a; Baranov et al., 2016b; Bundschuh et al., 2016; Ciutat et al., 2007; Ciutat et al., 2006; Delmotte et al., 2007; Devault et al., 2009; Gerino et al., 1994, 1998, 2003; Li et al., 2016; Mermillod-Blondin et al., 2001, 2002, 2003, 2004, 2005; Pigneret et al., 2016). Bien que son influence sur les propriétés physiques, chimiques et biologiques du sédiment soit internationalement reconnue, son intégration dans les méthodes de bioremédiation reste encore timide à ce jour (Leveque et al., 2014; Megharaj et al., 2011; Yu et al., 2005).

Cette thèse est basée sur la combinaison de 2 processus connus pour leur capacité à générer des flux de matière dans les sédiments, la phytoextraction et la bioturbation. L'hypothèse principale sous-jacente à ce travail est que ces 2 processus peuvent être complémentaires pour faciliter la mobilisation et la réduction des polluants présents à l'interface eau-sédiment. Avec son influence sur les flux de polluants à travers l'interface eau-sédiment, il est suggéré que la bioturbation puisse améliorer l'arrivée des polluants en profondeur, et ainsi renouveler la quantité de polluant à proximité du réseau racinaire des plantes. Si cette hypothèse est vérifiée alors les capacités de phytoextraction pourraient être augmentées en présence d'invertébrés bioturbateurs dans le même sédiment. Dans le milieu naturel dans lesquels la phytoextraction est mise en œuvre, les communautés d'invertébrés sont souvent développées de manière spontanée dans le sédiment, et il est possible que leurs activités participent aux résultats de phytoremediation mais leur rôle dans les performances mesurées ne sont jamais pris en compte. De même si ces organismes tendent naturellement à coloniser les sédiments des zones humides construites de type filtres plantés il serait alors intéressant de démontrer s'il serait utile de favoriser leur colonisation, voire de la provoquer.

La thèse présentée ici a pour but de tester cette hypothèse de travail en utilisant 2 types de polluants: le cadmium comme modèle de métal lourd présent dans les sédiments de nos région, et l'atrazine, un polluant de type micro-organique persistant interdit en France depuis plusieurs années mais toujours présents dans de nombreux sédiments. Les effets de la bioturbation sur les flux et bilans de masse de ces polluants sont mis en évidence à l'aide d'une approche expérimentale de laboratoire utilisant une série de microcosmes reproduisant l'interface eau-sédiment de zones humides. L'influence de la biodiversité est mise en évidence, mesurée et discutée à partie de 2 expériences visant à suivre une stratégie de complexité croissante au niveau de la composition des microcosmes et de leur contamination:

- une première expérience a été mise en oeuvre avec une contamination initiale et ponctuelle dans la colonne d'eau avec une concentration de 20µg de Cd/L (Cadmium Nitrate – $Cd(NO_3)_2.4H_2O$), le polluant étant le cadmium, représentatif d'un type de polluant relativement conservatif. L'expérience a durée 30 jours et l'ensemble des mesures ont été réalisées au temps initial et à la fin de l'expérience.

- une deuxième expérience a été mise en oeuvre avec une contamination initiale dans la colonne de sédiment, le polluant étant l'atrazine, toujours présent en excès pendant toute la durée de l'expérience dans ce compartiment. Ce type de molécules pouvant être biotransformée durant le temps de l'expérience (26 jours), la molécule utilisée a été marqué au ^{14}C. La concentration d'atrazine égale à 2 $\mu g.g^{-1}$ de sédiment frais a été estimée 15 jours avant l'introduction de la biodiversité, la durée de l'expérience en présence de biodiversité a été de 26 jours.

- dans ces 2 expériences, les luminophores ont été introduits à la surface du sédiment en tant que traceurs purement conservatifs pour mesurer le transport de sédiment et les polluants associés durant la durée de l'expérience.

Cette approche expérimentale a été menée avec le même type de biodiversité pour représenter la source de phytoextraction et de bioturbation. Une population

d'oligochètes tubificidés *Tubifex tufifex* a été utilisée comme bioturbateurs connus pour être à l'origine du processus de convoyage à l'interface eau-sédiment. Pour la phytoextraction, c'est l'espèce de plante riparienne *Typha latipholia* qui a été sélectionnée pour ces capacités de résistance à la contamination par les herbicides et sa croissance rapide. Plusieurs conditions expérimentales ont été mises en place pour permettre de tester isolément ou en combinaison l'effet de ces organismes sur les flux de polluants. L'introduction de ce manuscrit présente la revue bibliographique de l'état de l'art sur les processus mis en jeu et les caractéristiques de polluants ciblés, La partie suivante correspond à la méthodologie commune au 2 experiences mises en oeuvre. La partie III regroupe l'ensemble des results permettant de démonstrer les conclusions de ce manuscript. Plus précisément, la partie 3 se decompose en 3 sous parties correspondant chacune a une experience 3A et 3B et enfin à la comparaison des resultats de ces 2 experiences dans la partie 3C. L'ensemble de ces recherches a été réalisé pour répondre aux objectifs spécifiques enoncés ci dessous :

(*i*) l'estimation de l'influence de la population d'invertébrés sur le processus de phytoremédiation à partir du suivi temporel des concentrations des 2 types de polluants différents (Parties 3A et 3B). La mise en évidence des effets de ce couplage sur l'évolution des concentrations de Cd a été l'objet d'un papier publié en *2018* dans la revue internationnale: *Science of the Total Environment - 618 : 1284–1297; https://doi.org/10.1016/j.scitotenv.2017.09.237):* sous le titre "**Bioturbation effects on bioaccumulation of cadmium in the wetland plant *Typha latifolia*: A nature-based experiment**" et avec les auteurs « Trung Kien Hoang, Anne Probst, Didier Organe, Franck Gilbert, Arnaud Elger, Jean Kallerhoff, Francois Laurent, Sabina Bassil, Thi Thuy Duong, and Magali Gerino ».

(*ii*) évaluer l'efficacité de détoxication sous l'influence de cette biodiversité en utilisant 2 types de méthodes complémentaires :

- les mesures directes des concentrations de polluants dans l'eau, le sédiment et les plantes (résultats et discussions des parties III.A and III.B) ;

- les mesures indirectes de toxicité de l'eau surnageante dans les microcosmes et l'eau interstitielle des sédiments à la fin de l'expérience à l'aide de tests écotoxicologiques. Ces tests ont été mis en œuvre à l'aide d'une algue connue pour sa sensibilité à la contamination de l'eau par les POPs: *Chlorella vulgaris* (résultats et discussion de la partie III.B).

(iii) démontrer l'effet de la bioturbation sur la biodisponibilité des polluants vis a vis des plantes, en développant la mise en évidence des modifications physico-chimiques du sédiment (pH, potentiel redox, porosité, matière organique, etc…) sous l'influence de cette biodiversité, (Résultat et discussion de la partie III.B).

(iv) évaluer l'efficacité de mitigation de la pollution par le couplage entre la bioturbation et la phytoremediation, sur les 2 types de polluants (métal and herbicide) par l'estimation de leurs bilans de masse et les flux entre compartiments (eau surnageante, sédiment, et plante) (Parties résultat et discussion de III.B et III.C) et en comparant les effets de la méthode de bioremédiation testée sur ces 2 types de polluants (Partie III.C du manuscrit).

INTRODUCTION (English version)

Despite not being able to induce lethal effects rapidly, the accumulation of the contaminants, such as nutrients, metals, or organic pollutants in the water-sediment interface, causes long-term potentiation to the biota by direct uptake or through the food web. By the potential risk associated with their toxicity, the arrival of these contaminants in aquatic environments, as well as their becoming, requires increasing attention. Although the limitation of the appearance of these molecules in the natural environment is the priority, all the natural processes allowing the elimination of these molecules in the aquatic ecosystems are welcome.

With the rise of sustainable management, the development of useful tools for rehabilitation of aquatic sites with contaminated water and sediment, and the purification of water as a vital resource for urban areas has become a considerable concern. Sustainable bioremediation techniques are a part of ecological engineering that involves biodiversity (the variety of plant and animal life in the world or in a particular habitat) in management strategies focusing on the reduction of pollutant loads of ecosystems. The demonstration of these processes participating in the essential service of self-purification of the soils, rivers, and wetlands and the associated biodiversity is still in progress in the international community. This demonstration of benefits belonging to increased biodiversity in aquatic systems relies not only on the capacity to help at the restoration of polluted sites but also as ecological engineering strategy to facilitate the resilience of the environments. Ecological engineering is based on the knowledge gained from research on the functioning of ecosystems, the role of biodiversity in these environments, and the associated flows of materials. The integration of biodiversity into know-how in bioengineering appears to be a guarantee of the sustainability of the functioning of the target environments. Ecological engineering methods, such as engineered wetland phytoremediation used in the purification of wastes, or bioturbation as ecosystem engineering, have been studied independently in an aquatic environment so far. Different types of natural processes, on

the other hand, can be combined to enhance the treatment efficiency from the advantages of individual systems, such as the integration of vertical and horizontal filter stages in most hybrid constructed wetlands. Current bioremediation relies on a multidisciplinary approach that crosses hydrological, chemical, physical, biogeochemical, and ecological processes. The application of a combination of several ecological processes could be therefore considered as a potential, and prospective trend to amplify the possibility of bioremediation when compared with single-process application performed so far.

The present Ph.D. works are, therefore, based on the combination of two ecological processes, which are bioturbation and phytoremediation, known in isolation for their ability to generate pollutant flows and integrated into wetland restoration approaches. The efficiency of phytoremediation has been demonstrated as an applied bioremediation process for persistent pollutants, such as metals in soils and sediments (Ali et al., 2013; Barceló & Poschenrieder, 2003; Guo et al., 2012; Klink et al., 2013; Lyubenova et al., 2013; Pandey et al., 2014; Wani et al., 2017; Weis & Weis, 2004), and some works for TOCs and POPs (Frazar, 2000; Ibrahim et al., 2013; Marcacci, 2004; Merini et al., 2009; Moore et al., 2013; Pascal-Lorber et al., 2010; Tournebize et al., 2013; Wang et al., 2015). Bioturbation in aquatic sediments is the source of material flow and stimulates the activity of microbial communities (Anschutz et al., 2012; Baranov et al., 2016a; Baranov et al., 2016b; Bundschuh et al., 2016; Ciutat et al., 2007; Ciutat et al., 2006; Delmotte et al., 2007; Devault et al., 2009; Gerino et al., 1994, 1998, 2003; Li et al., 2016; Mermillod-Blondin et al., 2001, 2002, 2003, 2004, 2005; Pigneret et al., 2016), has significant implications for the structure and functioning of ecosystems (Schiffers et al., 2011), with potential to be exploited in ecological engineering. On the other hand, the cooperation of bioturbation as a stimulator of the phytoremediation's efficiency has almost never been explored in a context of bio-elimination of inorganic and organic pollutants, except for previous studies on metal phytoremediation under the effect of earthworms (Leveque et al., 2014; Megharaj et al., 2011; Yu et al., 2005). By controlling

pollutant fluxes through the water-sediment interface and into the sediment, the present study starts from the suggestion that bioturbation should increase the pollutants availability for phytoremediation by the plant root system. The bioturbation and phytoremediation association should enhance the capacity of environmental restoration of polluted aquatic sites.

The proposed Ph.D. work aims to demonstrate the influence of the combination of bioturbation on the efficiency of the phytoremediation of metal (cadmium) and pesticide (atrazine). This coupling is suggested as an innovative strategy based on the combination of these two ecological processes: bioturbation and phytoremediation. These two processes being known for their influence on pollutant behaviors in the aquatic sediments, the current demonstration in this Ph-D will not include the demonstration of the isolated processes efficiency.

The study tackles experimentally the coupling of these two methods to enhance biotransport of a pollutant from overlying water into the sediment column by bioturbation, then from sediment to plant by the combination of both bioturbation and phytoremediation. The biotransport created by the benthic community activities is well known as a process that enhances the mixing of pollutants into the bioturbated layer. The bioturbation may increase not only the burial of pollutant coming from the overlying water but also renew the sediment and the related contaminant in the vicinity of the plant root system.

The working hypotheses of the influence of the bioturbation on the efficiency of the phytoremediation have been tested with two types of pollutants and an inert tracer that are more precisely:

- the luminophores particles, as luminescent sediment particles, used as conservative tracers to give evidence and quantity the biotransport under the biological effect of two populations of plant and invertebrate;

- a trace metal, the cadmium, as a conservative pollutant;

- a pesticide, the atrazine, as a more reactive pollutant although it makes part of the persistent micro-organic pollutants (POP). This herbicide was radiolabeled with ^{14}C for the need of the experiment, making it possible to follow the mother molecule and its metabolites into the different compartments under plant and invertebrate effects: overlying water, sediment, and plant organs.

Using a controlled-environment experiment made of microcosms mimicking a wetland water-sediment interface, the detailed objectives are:

(*i*) to estimate the influence of an invertebrate population in the combination of a species of riparian plant in the bioremediation process by using different types of pollutants, Cd as a model of heavy metal and atrazine as a model of herbicide. The results of this study were published to the Journal of *Science of the Total Environment - 618 (2018) 1284–1297; https://doi.org/10.1016/j.scitotenv.2017.09.237):* "**Bioturbation effects on bioaccumulation of cadmium in the wetland plant *Typha latifolia*: A nature-based experiment**". Trung Kien Hoang, Anne Probst, Didier Organe, Franck Gilbert, Arnaud Elger, Jean Kallerhoff, Francois Laurent, Sabina Bassil, Thi Thuy Duong, and Magali Gerino.

(*ii*) To assess the detoxication efficiency under biological influence of two populations of plants and invertebrates using two types of complementary method:

- direct measure of pollutant concentrations in water, sediment, and plan (*results and discussion of Parts III.A and III.B*);

- an indirect measure of toxicity using ecotoxicology tests (*results and discussion of Part III.B*).

(iii) to demonstrate the impact of bioturbation on pollutants' bioavailability for the plant root system in the sediment using its influences on physicochemical properties of the sediment, such as pH, sediment porosity, sediment organic matter, etc. affecting their mobility and transformation within the sediment matrix (*results and discussion of Part III.B).*

(iv) to compare the mitigation efficiency of this coupled bioremediation strategy (bioturbation and phytoremediation) on the two types of pollutants (metal and herbicide) via their mass balance calculation (fluxes and quantities) in the different experimental compartments (overlying water, sediment, and plant) (*results and discussion of Parts III.A and III.B*) and comparing the effects of the bioremediation method tested on these two types of pollutants (Part 3C of the manuscript).

This bioremediation strategy has been tested with two laboratory experiments to set different treatments with and without plant and invertebrates effects. In our experiments, bioturbation is carried out by a conveyor-belt species, a *Tubificidae Oligochaetes* invertebrate species, well known as an active ecological engineer. Also, the associate phytoremediation is conducted by a riparian plant *Typha latifolia* whose ability to remove inorganic and organic pollutants from sediments by accumulation into their biomass has been previously demonstrated. At last, a deterministic model was applied to estimate the intensity of the sediment bio-transports to further highlight the oligochaetes influence on pollutant fluxes in the sediment column.

The first experiment was run using cadmium, one heavy metal initially introduced in the overlying water (20µgCd/L of Cadmium Nitrate – $Cd(NO_3)_2.4H_2O$) as a source of conservative pollution of urban hazard that bioaccumulates in plants as a function of time (30 days of the experiment). The second experiment was started with homogeneously contaminated sediment with radiolabeled-^{14}C atrazine with an initial concentration of 2 $\mu g.g^{-1}$ wet sediment, as a non-conservative and organic pollutant to be followed in the aquatic environment during 26 days of the experiment. The influence of macro-organisms on radiolabeled-^{14}C atrazine fluxes in the multi-compartments setup (water, sediment, and plant) is explored to achieve a precise mass balance of this pollutant that may undergo biodegradation during the time of the study. At the same time, the effectiveness of this bioengineering was tested by ecological bioassay using fresh blue-green algae – *Chlorella vulgaris*, which is well known as a useful bioindicator

for water pollution, to assess the evolution of the toxicity of the overlying and interstitial waters due to plants and invertebrates influences.

PART I. BIBLIOGRAPHY SYNTHESIS

I.A. AQUATIC CONTAMINATION BY METALS AND PESTICIDES

I.A.1 AQUATIC ECOSYSTEM

Natural ecosystems are highly complex systems sheltering several communities of living organisms as called the biocenosis (plants, vertebrate, macrofauna, meiofauna, microorganisms) in a physical area of the environment with similarity enough that may be called "habitats" or biotopes, composed with the non-living components (water, sunlight, soils) that they interact with. They are structured environmental complexes in which matter and energy exchanges arise due to the interactions between living and non-living organisms. Aquatic ecosystems encompass a wide variety of environments, all characterized by the omnipresence of water (sweet, salty, lively or slow). Communities of organisms have evolved and adapted to water habitats over millions of years, while aquatic habitats supply the food, water, shelter, and space essential for the survival of aquatic animals and plants.

Table I.1.
Comparison of the Earth surface areas and known species for freshwater, terrestrial, and oceanic ecosystems (McAllister et al., 1997). This table does not contain symbiotic species.

Ecosystems	Freshwater	Terrestrial	Oceanic
The Earth surface area	0.8 %	28.4%	10.8%
Percentage of known species	2.4%	77.5%	14.7%
Relative diversity in species	3	2.7	0.2

Freshwater ecosystems cover only 0.8% of the Earth's surface (Table I.1), yet they are the hotspots and livelihoods not only for 2.4% of all known species but also for human activities with billions of people especially in developing countries (Strayer & Dudgeon, 2010).

Most aquatic ecosystems have been developed, modified, and profitably used by humans, for various needs or environmental improvement purpose. The term

"anthropogenic hydro system" is sometimes used to refer to an aquatic ecosystem integrating the human component. Freshwaters including lakes, streams, ponds, and wetland ecosystems, the major types of aquatic ecosystems, are essential due to their incredible diversity and productivity, providing vital ecosystem services for humans (Hitt et al., 2015; Poff et al., 2002). According to Global Lake and Wetland Database - GLWD (Lehner & Döll, 2004), lakes and reservoirs cover a total of about 2.0% of the Earth's surface, while wetlands are estimated to cover less than 9 %. They are, however, very vulnerable to changes (Prowse et al., 2006; Vincent et al., 2011) due to human impacts, mainly changes of biodiversity, such as the extinction and invasion of species. This biodiversity erosion leads to ecosystem services limitation, especially natural services of regulation, such as water flow and quality regulation (Martín-López et al., 2018). Other disturbances can be global climate change and the growing radiating activity of ultraviolet rays (Dodds et al., 2013; Poff et al., 2002). Conspicuously, even though a small area of the Earth (6.2 – 7.6%, according to Lehner and Döll (2004)) is covered by wetlands, they represent an extremely valuable resource of ecosystems due to their critical role in climate regulation, biodiversity habitats, hydrology control, and human health. Wetlands also provide more direct benefits to people (ecosystem services): food provisioning, water quality regulation as natural services by filtering out sediment and contaminants from the surrounding environment, flood abatement, and carbon management (Zedler & Kercher, 2005). However, wetland losses and degradation, that results in these last ecosystem services depletion (Zedler & Kercher, 2005), are continuing worldwide and becoming one of the most threat with their global declined extent between 64 – 71% in the 20th century (Gardner et al., 2015). The main direct drivers of wetland degradation and associated biodiversity loss are the expansion of crop and grazing lands into native vegetation, unsustainable agricultural and forestry practices, climate change, and, in specific areas, urban expansion, infrastructure development, and extractive industry. The wetlands degradation contributes to the decline and eventual extinction of species and the loss of ecosystem services to

humanity, making avoidance, reduction, and reversal of land degradation essential for human well-being. Studies from Asia and Africa indicate that the cost of inaction in the face of land degradation is at least three times higher than the cost of action. On average, the benefits of restoration are ten times higher than the costs, estimated across nine different biomes. While challenging, the benefits of restoration include, but are not limited toregional biodiversity, physical, and chemical function s, such as water quality improvement but also increased employment, increased business spending, improved gender equity, increased local investment in education and improved livelihood of a wetland (IPBES 2018).

Ecosystem functioning

"Ecosystem functioning reflects the fluxes of matter an energy that are resulting from the collective life activities of plants, animals, and microbes, such as production, consumption, and excretion, and that are influencing thephysical and chemical conditions of the environment." (the statement of Ecological Society of America – ESA on biodiversity and ecosystem functioning, ESA 1999, p. 3) (Virginia & Wall, 2013). These activities are influencing the properties of the environment via biotic factors such as functions displayed by the species communities, and interactions between species (Humbert & Dorigo, 2005). Some of the tasks rendered by the ecosystem can be useful to humans. For examples, plant production of food and fuel. Alternatively, the cycling and mineralization of organic matter by active organisms in the ecosystem make it possible to play a purifying role and to improve the physicochemical quality of the water. Human has exploited freshwaters and their surroundings natural resources for other needs, such as drinking and irrigation water, agricultural use for plants, fish, and minerals harvesting or gathering, transportation, electricity generation, waste management (Strayer & Dudgeon, 2010). Besides, freshwater benefits evaluated as ecosystem services are worth estimation of USD 6.5 trillion/y, one-fifth of the global ecosystems' value (Costanza et al. 1997).

Wetlands and riparian ecosystems are the most productive ecosystems as "biological supermarkets" (Thomas C. O'Keefe, 2000) providing critical ecosystem services, particularly in restoring the viability of cities and rural areas. Common multi-beneficial ecosystem services from wetlands include carbon retention (Bridgham et al., 2006; Mitsch et al., 2013), water quality improvement in watersheds (Mitsch et al., 2001; Verhoeven, 2006; Zedler & Kercher, 2005), coastal protection (Gedan et al., 2011; Temmerman et al., 2013), groundwater level and soil moisture regulation (Hefting et al., 2004; Xiong et al., 2003), flood regulation (Acreman & Holden, 2013; Groot et al., 2002) and biodiversity support (Gibbs, 2000), nutrient cycling, water storage and flood abatement, and habitat provision (Mitsch and Gosselink, 2000).

I.A.2. CONTAMINATION IN AQUATIC ECOSYSTEMS

A healthy aquatic ecosystem is defined in such a way that human activities do not disturb the natural functioning (e.g., nutrient cycling) nor appreciably modify the system structural design (e.g., species composition). An unhealthy aquatic ecosystem, on the other hand, is defined with imbalanced occurred to the natural state due to anthropogenic impacts, either *physical* (e.g., streaming in abnormal hot water), *chemical* (e.g., applying toxic wastes at a concentrations where species suffer from harms or damages), or *biological* (e.g., supporting non-native species invasion).

The aquatic environment as a whole is a very complex system which is the seat of some chemicals, physical and biological reactions, and this system is closely related to all the other systems or compartments (Westrich & Förstner, 2007). Because of the water cycle, aquatic ecosystems are likely to be contaminated by accidental or chronic pollutions. Excess of natural products and many human-made molecules are therefore expected to pollute aquatic ecosystems. Nutrients and contaminants transport via inland waters in polluted aquatic systems have been receiving increasing attention because these inland waters are the central support, the backbone of continuum linkage

between land and sea waters. In this hydraulic system of watersheds, the wetlands are playing an essential role as buffer zones for wastewater mitigation (Billen & Garnier, 2007; Le et al., 2010).

Long regarded as a passive conduit between land and ocean, inland water systems are significantly more complex and influential than previously believed (Böhlke et al., 2009). They do not only transport materials from land to sea, but they internally store sediments and liquid influents, support the food web of the aquatic environment and bring the water cleaning regulation for human needs.

Wilson and Carpenter (1999) claimed that continental aquatic systems are in the middle of a tightly linked network of interests, regarding economy, politics, and environment, which can be proved by the numerous uses that human civilization harness from these systems. In contrast, those relying on the water source suffer from various contaminants (mineral, inorganic, and organic) during these uses.

Sources of pollution of the aquatic environment

As a sink for pollutants in the aquatic environment, the water-sediment interface, known to be one of the most vulnerable (Devault et al., 2009) receiving a hefty source of pollution from agricultural practices and metropolis areas. The accumulation of contaminants, such as nutrients, metals, or a persistent toxic organic compound (PCBs) in the water-sediment interface at levels that are not rapidly lethal may result in long-term, subtle effects to the biota by direct uptake or through the food web.

Metallic pollution

Trace metals, or potentially harmful elements (PHEs), among the most effective environmental contaminants (Bini and Bech, 2014) at the water-sediment interface, are raising concerns (Guo et al., 2012; Horowitz et al., 1999; Schäfer et al., 2009). They are considered as micro-pollutants since they are present in the various compartments of the environment in the trace state. Nevertheless, they are compounds that are

dangerous for living beings, both because of their toxicity and their persistence (Dirilegen, 2000; Chouteau, 2004) that drive to bioaccumulation processes.

The metallic pollution can be due to various metals sources, such as aluminum, arsenic, chromium, cobalt, copper, manganese, molybdenum, nickel, zinc, cadmium, mercury, or lead. They are not degradable and accumulate in aquatic sediments or soils as very highly persistent compounds (Kermani et al., 2010). Some heavy metal ions (Pb, Hg, Cr, Cd, As, Mn...) are commonly found in industrial wastewater. Most metals are highly toxic to humans and animals. Since they are not biodegradable, they concentrate on the living bodies throughout the food chains. They can so reach very high rates in certain species consumed by the man, as the fishes. This "bioaccumulation" explains their extreme toxicity. The primary sources for aquatic environments are:

- Industrial wastes (cadmium, chromium) of paper factories (mercury), factories of chlorine (mercury) and iron, etc... (currents and orpheline sites)
- Agricultural fertilizers or residual muds of water-treatment plants,
- Fungicides (mercury),
- The fallout from atmospheric dust emitted during the incineration of waste (mercury) or combustion of gasoline (lead),
- With the streaming of rainwater on roofs and roads (zinc, copper, lead)
- Silver exploration in the rivers (mercury)

As very high resistant compounds, these metals cannot be reduced by chemical or microbial processes, so that they accumulate in the aquatic sediment or soil as a natural receptacle of all suspended matters that my transit by free running water. Being the most common toxic chemicals, PHEs such as arsenic, mercury, lead, or cadmium, etc. these metals are polluting aquatic systems and resulted in severe health problem to humans due to bioaccumulation along the trophic webs.

Cadmium, as an example

Cadmium is one of the common toxic metals found in freshwater ecosystem sediments that act as a final sink (N'guessan et al., 2009). Furthermore, cadmium, listed as a general and widespread metal pollutant that is toxic at a high level to humans and most plants (Barceló & Poschenrieder, 2003). The tendency of using Cd is on the rise in electroplating, coating, plastic manufacturing, and battery manufacturing industry. This metal has recently been responsible for the high risk of contracting osteoporosis and kidney diseases among several thousands of Tak residents (Thailand) due to the mining industry, which causes high levels of Cadmium reaching from 0.1 to 44 mg/kg in the area. Besides, the amount of Cd in garlic and soy is also much higher than its standard from 12 to 126 times (Liu et al., 2005). Cadmium pollution was also recognized in some reservoirs that provide a primary freshwater source for daily human life. In South of France, Cd transfer to the downstream site of food farms is raising worries for the toxicity in many aquatic areas, as in the case of the Lot–Garonne-Gironde fluvial system. Cd concentrations in oysters from industrial zone of the Gironde estuary were found at 100 times higher than those oysters from non-industrial areas. The daily fluxes of total Cd range from 0.26 to 966 kg/day in the Lot River and from 0.31 to 1360 kg/day in the Garonne River (Audry et al., 2004). In Vietnam, Cd accumulation in seafood of estuarine site of the Cai River at Nha Trang is one of the many examples as well in this country.

Micro-organic pollution

Most of the organic pollutants are composed of primary herbicides (Dorigo et al., 2007), which are used not only in agriculture but also for many other purposes (from household gardening to railway weed treatment). These herbicides can enter aquatic ecosystems as a result of terrestrial runoff, stormwater and to a lesser extent, of direct application and aerial spraying (Carter, 2000). The distribution of pesticides within the freshwater ecosystem depends upon the formulations in which they are carried and

upon the chemistry and physics of the environment where they settle down. Many pesticides are soluble in water. When pesticides enter the water in these forms, quick dispersion usually takes place. The emulsions spread throughout the water, but the dispersion can be affected by water currents, surface winds, and water temperature. Once in the water, the fate and transport of organic contaminants in aquatic systems are highly dependent on physical and chemical properties of the molecule (Imfeld et al., 2009; Suthersan et al., 2017) moreover, defined by:

- the type and kind of external sources;
- the transport of the substances via various elements of the hydrological cycle;
- the chemical, biological, or biochemical transformation of these substances.

Besides their severe risks to humans, these persistent contaminants are slowly reduced by chemical or microbial processes so that accumulation of mother molecules and metabolites may occur in the aquatic sediment, as a final receptacle, with long residence times (N'guessan et al., 2009). The POPs, such as pesticides, are exceptionally hazardous due to their toxicity to human beings by nature, their impact on non-target organisms, their bioaccumulation capability in the tissues of animals and humans via the food chain, and their long-term persistence in the environment.

Atrazine, as an example

Atrazine (2-chloro-4-(aminoethyl)-6-(aminoisopropyl)-s-1,3,5-triazine) is a photosynthesis inhibitors herbicide. It was used in pre- and post-emergence control of annual broad-leaved weeds and yearly grasses mainly in maize and sorghum but also for sugar cane, vines, lemon and banana among other crops. It is also used in non-food crops and at industrial sites such as roads and railways (Douglass et al., 2015; Smith et al., 2005). It was applied intensively during 40 years, and several tens of thousands of metric tonnes of atrazine are used every year in the word. Roughly 33 to 36 tones of atrazine are sold to the United States each year (Tillitt et al., 2010), where its concentrations (Henderson et al., 2006; Schreiner et al., 2016; Thelin & Stone, 2010) are

exceeding the European Union standard of 0.1 μgL^{-1} in surface fresh water. A monthly import of atrazine to Vietnam is about 250,000 USD in 2017 (http://www.exportgenius.in/vietnam-imports-data/atrazine-import-data.php).

Atrazine is a highly effective and moderately persistent organic pollutant. The average half-life of atrazine in soil (DT50) is 40 days (Kontchou & Gschwind, 1995; Sun et al., 2016) but depending on the various environments may be as long as 166 weeks, for example in sandy loam soils (Bowmer, 1991). The substance was found to be a major contaminant of water, polluting both surface water (Garmouna et al., 1998) and underground water, and finally resulting in its banning in European Union in 2004. Atrazine is suspected to be an endocrine disruptor, particularly in male frogs (Hayes et al., 2003) and to synergize the amphibian-sensitivity to virus infections, causing the decline of the amphibian population in the world (Forson and Storfer, 2006).

As a moderately hydrophilic molecule (Graymore et al., 2001) used as a pre-emergent herbicide, atrazine is moderately to highly mobile in soils, especially in soils having low clay and organic matter contents. Due to its low adsorption in soils or sediments (Koc = 128 $mL.g^{-1}$) (Sun et al., 2010) and moderate aqueous solubility of 22 $mg\ L^{-1}$, residual atrazine, and its metabolites, show a strong potential risk of dispersion in aquatic environments (Gustafson, 1989).This behavious is confirmed by its persistent and robust occurence in aquatic environments with a weak affinity for soil organic matter (Koc - the partition coefficient of the compound in organic matter over water ~ 100 $mL\ g^{-1}$) Even though it has been banned since several years in European Union and spite of its rapid disappearance from the areas sprayed, the repeated use of atrazine has resulted in atrazine and its metabolites accumulation in aquatic sediments. Atrazine is a highly efficient and moderately persistent organic herbicide (a lengthy soil half-life of 60 to > 100 days) widely used in agriculture to control broadleaf and grassy weeds (Douglass et al., 2015; Smith et al., 2005), especially in the United States (Henderson et al., 2006; Thelin and Stone, 2010, Schreiner et al., 2016). Atrazine concentrations can exceed 0.1 $\mu g\ L^{-1}$ in natural surface water (the European Union standard in surface fresh

water). In the Garonne rivers, sediment of the Malause reservoir downstream Toulouse Devault et al (2009) measured an average (n=19 samples) concentration of Atrazine 0.128 (μg/g) in sediment fraction finer than 2 mm, with highest measured concentrations of 0.83 μg/g.

Atrazine (ATR) is considered to break down with some difficulty in the soil (Kaufmann and Kearney, 1970). Microorganisms break down atrazine into deethylatrazine (DEA), deisopropylatrazine (DIA) and didealkylatrazine (DIDA) or hydroxyatrazine (HO-A), hydroxylatrazine (HA) and desethylhydroxyatrazine (DEHA) (Graymore et al., 2001) (Fig.I.1).

Due to its low adsorption in soils or sediments (Koc =128 mL g^{-1}) (Sun et al., 2010), residual atrazine and its metabolites, such as DEA or DIA have a high potential to contaminate tap water, surface waters, groundwaters and adjacent soils (Kolpin et al., 1996; Lewis et al., 2009; Zaya et al., 2011) from agricultural soils via leaching and surface runoff (Douglass et al., 2017; Pascal- Lorber et al., 2011).

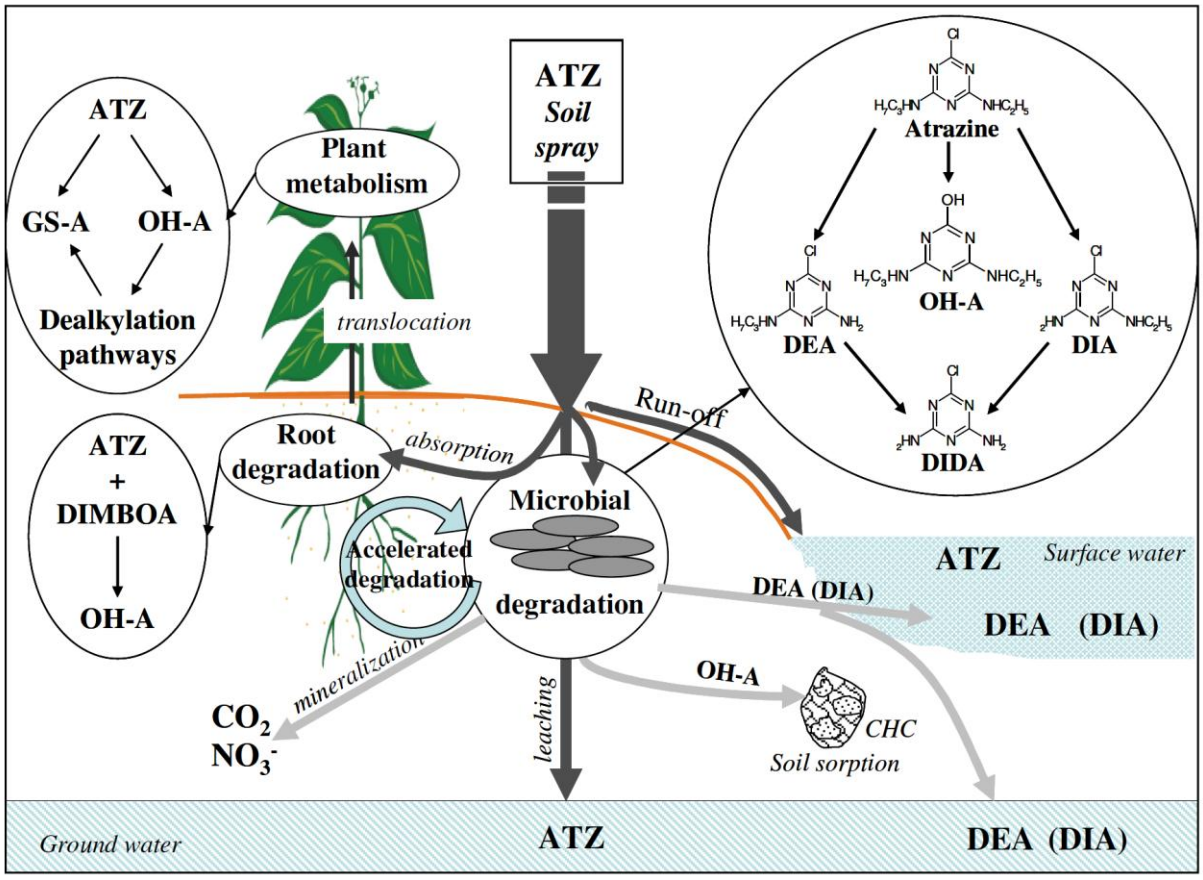


Figure I.1. Scheme of atrazine fate in the environment from fields to aquatic networks that shows the impact of microbial atrazine-degradation on the atrazine-residues transfer towards aquatic biotopes from INRA communication (Pascal-lorber & Laurent, in prep.)

Atrazine has a molecular weight of 215.70 g/mol and a melting point between 175-177 °C (Solomon et al., 1996). The water solubility of atrazine is 33µg/mL at 22 °C; the Log Kow is 2.68 at 25 °C (Solomon et al., 1996). This herbicide was stable to hydrolysis for 30 days at pH 5-9 and 25 °C. The aqueous photolysis half-life (T ½) for atrazine with a natural light measured was 17.5 hours at pH 7, 12 days under a Mercury lamp, and 45 days under a Xenon lamp (Solomon et al., 1996). The soil (Kd) and organic carbon partition, (Koc), coefficients for atrazine and its primary metabolites in a Maryland clay were reported as 2.46 and 87.0, respectively (Solomon et al., 1996). The presence of these biocides or their metabolites in soil, water, plants, and even the atmosphere, together with their potential pharmacodynamic properties, have harmful effects on the environment (fauna and flora) or human health. In countries belonging to

the European Union, regulations aim to reduce risks at the lowest level, but it is not the case everywhere. Some problems should now be overcome.

Soil adsorption Coefficients

Other indexes for atrazine adsorption and mobility in the sediment, including sorption coefficient (Kd) and soil organic carbon-water partitioning coefficient (Koc) were determined. The coefficient (Koc) has been so far used as a quantitative measure of the magnitude of the binding affinity of organic matter for atrazine. Also, this constant allows the calculation of the proportion of atrazine bound to organic matter, which is essential for predicting the fate of atrazine in soils and water bodies (Kulikova and Perminova, 2002). **Soil adsorption coefficient (Kd)** measures the amount of chemical substance adsorbed onto soil per amount of water (Linde, 1994). Since the adsorption occurs predominantly by a partition into the soil organic matter, it is more useful to determine a soil's ability to adsorb in Koc (Linde, 1994).

$$\textbf{Kd (mL.g}^{-1}\textbf{)} = \frac{Concentration\ of\ chemical \in soil\left(\frac{\mu g}{g}\right)}{Concentration\ of\ chemical \in water\left(\frac{\mu g}{mL}\right)}$$

$$\textbf{Koc (mL.g}^{-1}\textbf{)} = \frac{Kdx100}{Organic\ carbon})$$

Octanol/Water Partition Coefficient – K_{ow}

Lipophilicity of a pesticide is measured by the octanol : water partition coefficient (log Kow: the ratio of chemical that is soluble in octanol (organic or non-polar solvent) divided by its concentration in water (a polar solvent)) (Linde, 1994). This value is a good indicator of a herbicide's lipophilic or hydrophilic nature.

I.B. ECOLOGICAL ENGINEERING AS SOURCE OF NATURAL ATTENUATION PROCESSES FOR AQUATIC SYSTEMS

I.B.1. ECOLOGICAL ENGINEERING AND ECOSYSTEM RESTORATION

I.B.1.1. Ecological engineering

Ecological engineering is an emerging part of the environmental sciences integrating ecology and engineering knowledge and concerned with the design, monitoring, and construction of new sustainable ecosystems, which benefit of both human and nature well-being (Mitsch & Jørgensen, 2004). It is also the practice of fitting environmental technology with ecosystems self-design for maximum performance (Odum & Odum, 2003), with the aim at (1) conserving and (2) restoring ecological systems, (3) modifying ecological systems to increase the quantity, quality, and sustainability of particular services they provide, or (4) building new ecological systems that would provide natural services. Those task without ecological engineering would otherwise be provided through more conventional engineering based on non-renewable resources with high energy and economic costs (Barot et al., 2012). Thus, the objective of ecological engineering is to better understand the natural functioning of the ecosystem, to better favor their resiliences, and to seek solutions of bioremediation. In other words, ecological engineering plays a significant role in a sustainable society by providing benefits for humankind without destroying the ecological balance (Mitsch & Jørgensen, 2004). These approaches are seeking for solving environmental questions that are not only useful for our human well-being but also for aquatic systems conservation.

Finding new solutions based on natural functions of ecosystems for climate change mitigation, pollution removal of ecosystems and others majors issues of environment has been recently generalized as an innovative concept of Nature-based

solutions (NBS) (Eggermont et al., 2015; Maes & Jacobs, 2017). Even though environmental management tools are now more and more numerous and complex, they include the simultaneous application of environmental technology, cleaner technology, environmental legislation, ecological engineering, and ecosystem restoration (Fig.I.2). Likewise, realizing nature-based solutions requires political, economic, and scientific challenges to be tackled (Maes & Jacobs, 2017). Ecological innovation such as ecological engineering is a key to designing nature-based solutions which effectively contribute to sustainable economic growth (Maes & Jacobs, 2017).

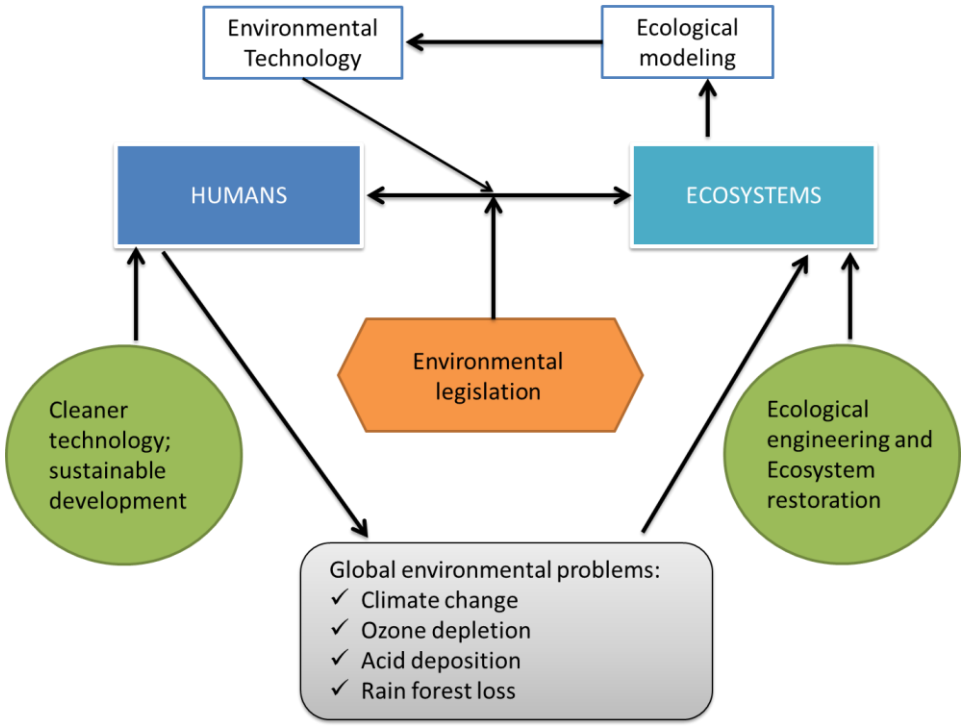


Figure I.2. Environmental management strategy in the 21st century with the specified place for ecological engineering (Jørgensen & Bendoricchio, 2001)

I.B.1.2. Restoration and rehabilitation of ecosystems

Restoration

The term ecological restoration has come into everyday language to describe operations carried out on the environment to repair damage, malfunctions, or improving existing ones. The standardized definition provided by the Society for Ecological Restoration (SER, 2004) is as follows: "Ecological restoration is the process of

assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed". It also provides clear concepts, models, methodologies, and tools to assist practitioners in their practices to identify appropriate indicators of restoration success. Restoring natural processes is an intentional act, and the most effective ways accelerate ecosystem recovery about its specific composition, community structure, ecological functioning and hence resilience (Angeler et al., 2013; Spears et al., 2015). Restoration might relate directly to the provision of ecosystem services and securing biodiversity (Hooper et al., 2005) and therefore "returns an ecosystem to a -as close as possible- approximation of its condition before disturbance" (the National Research Council – NRC, 1992).

The benefits of restoration regarding conservation are apparent. The restoration of ecosystems is valuable because of its inherent capacity to offer people an opportunity to repair ecological damage and thus to bring economic, environmental, health, and food benefits to improve the human well-being (Meli et al., 2014). The focus of ecosystem restoration has recently shifted from pure rehabilitation objectives to both improving ecological functioning and the delivery of ecosystem services (Friberg et al., 2017).

Rehabilitation

Rehabilitation emphasizes the repair and recovery of processes and therefore, the products and services of the ecosystem, while restoration also aims to restore pre-existing biotic integrity regarding the specific composition and structure of the communities. Rehabilitation involves ecosystems that have undergone more significant degradation and for which the thresholds of biotic and even abiotic irreversibility have been crossed. The objective is to improve or restore specific processes or functions with as a model the state of the ecosystem preceding degradation. Interventions that influence the physical environment, such as erosion control, improved soil resources or terrain remodeling, are then implemented to repair degraded sites. These interventions

sometimes make it possible to return to the original ecosystem, but most often lead to an alternative state.

I.B.2. WETLAND AS FOCUSING SITES FOR ECOLOGICAL ENGINEERING

Wetlands are known for their provisioning of ecosystem services and thus have great potential to be used as nature-based solutions to address a variety of environmental, social and economic challenges (Thorslund et al., 2017).

Ecological engineering strategies may be based on one or more of the following classes described by Mitsch & Jørgensen (Table I.2, 2004). Those strategies that are particularly suitable for wetland restoration are classes (1) and (2) below:

(1) Ecosystems services are used to reduce or solve a pollution problem that otherwise would be (more) harmful to other ecosystems. A typical example is the use of natural wetlands for wastewater treatment.

Natural wetlands are well known for their ability to remove sediments, nutrients, and other contaminants from water. They have been used as convenient wastewater discharge sites treatments (Ballantine et al., 2017; Hu et al., 2017; Kadlec & Wallace, 2009; Zedler & Kercher, 2005).

(2) Ecosystems are imitated or copied to reduce or solve a pollution problem, leading to constructed ecosystems. Examples are artificial fishponds and constructed wetlands for treating wastewater or diffuse pollution sources.

Wetlands construction to treat various wastewaters has been accelerating around the world since 1985 (Kadlec & Wallace, 2009) and successfully applied for decades as a sustainable wastewater management option worldwide (Wang et al., 2017). Constructed wetlands for water treatment are complex, integrated systems of water, plants, animals, microorganisms, and the environment (EPA, 2016). The use of natural wetlands for wastewater treatment has recently became limited due to being protected by Federal

law, while constructed wetlands provide a relatively simple and inexpensive solution for controlling many water pollution problems without detrimentally affecting natural wetlands resources (Kadlec & Wallace, 2009). Creating and restoring constructed wetlands is a typical example of imitating or copying an existing ecosystem by creating or restoring one that mimics field patterns of the ecosystem to solve a pollution problem (Mitsch & Gosselink et al., 2000).

(3) The recovery of ecosystems after significant disturbances. Examples are coal mine reclamation and restoration of lakes, estuaries, and rivers.

(4) The use of ecosystems for the benefit of humanity without destroying the ecological balance (i.e., the utilization of ecosystems on an ecologically sound basis). Typical examples are the use of integrated agriculture and development of organic agriculture; this type of ecological engineering finds wide application in the ecological management of renewable resources.

Table I.2.
Examples of ecological engineering approach for terrestrial and aquatic systems according to types of applications (Mitsch & Gosselink et al., 2000).

Ecological Engineering Approaches	Terrestrial Examples	Aquatic Examples
1. Using ecosystems to solve a pollution problem	Phytoremediation	Wastewater wetland
2. Imitating or copying ecosystems to reduce or solve a problem	Forest restoration	Replacement wetland
3. Recovering an ecosystem after significant disturbance	Mine land restoration	Lake restoration
4. Existing ecosystems are modified in an ecologically sound way	Selective timber harvest	Biomanipulation
5. Using ecosystems for benefit without destroying the ecological balance	Sustainable agro-ecosystems	Multi-species aquaculture

I.B.3. PHYTOREMEDIATION

The use of plants for bioremediation purposes notably relies on their ability to degrade or extract some contaminants (Moore et al., 2013; Murphy & Coats, 2011; Qu et al., 2017; Vangronsveld et al., 2009). Phytoremediation is defined as the use of plants to contain, degrade, remove, or transform pollutants into less toxic compounds (Fulekar, 2012). Although plants have long been used for soil decontamination, significant scientific discoveries over the last decade have contributed to improve the process and extend its scope. It can be used to clean up metals, pesticides, solvents, explosives, crude oil and other contaminants from soils, water (surface and underground water), and gaseous pollutants. When the plants have absorbed and accumulated contaminants, they can be harvested and discarded.

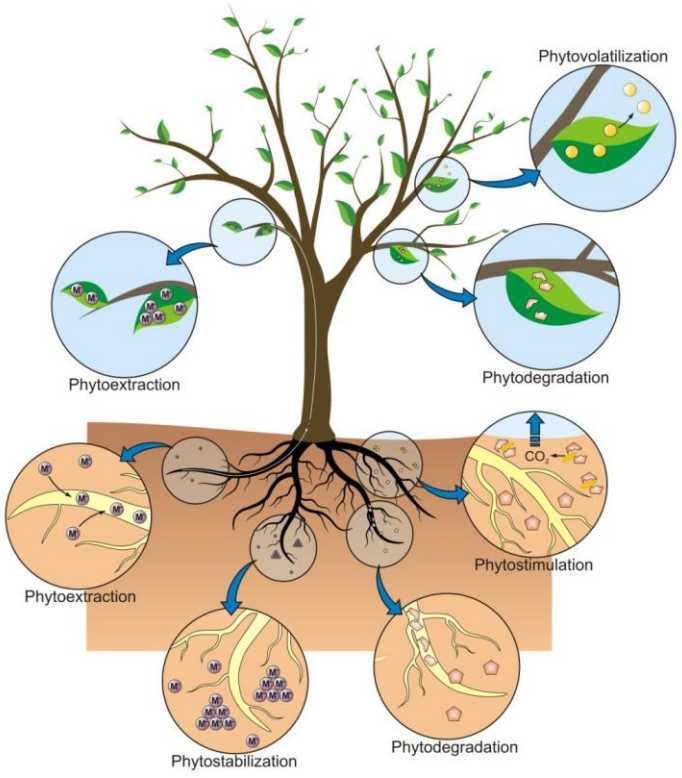


Figure I.3. Different types of phytoremediation process

Phytoremediation of inorganic and organic pollutants may involve several processes (Paz-Ferreiro et al., 2014):

(I) phytoextraction: use of plants to extract contaminants from the soil and concentrate them in harvestable plant's tissues. Phytoextraction is the absorption and translocation of contaminants by plant roots into the portions of the plants (shoots) that can be harvested and burned energy and recycling the metal from the ash.

(II) rhizofiltration: use of roots to absorb and sequester pollutants from contaminated water,

(III) phytostabilization: use of plants to prevent erosion and to immobilize pollutants by limiting their mobility and availability in the surface layers, in particular avoiding their migration to the surface and underground water,

(IV) phytovolatilization: use of plants to extract pollutants from the soil and convert them into volatile compounds

(V) phytodegradation: use of plants to degrade soil organic pollutants by plant tissues.

(VI) rhizodegradation (phytostimulation): use of plants to degrade organic contaminants in the rizhosphere thanks to microbial activity surrounding the roots.

(VII) phytodesalination: use of plants to remove excess salts from saline soils by halophytes.

In these ways, plants may be viewed as hyperaccumulators, with several species known to accumulate >100 $mg.kg^{-1}$ Cd, Cr, Co or Pb; or >1000 $mg.kg^{-1}$ Ni, Cu, Se, As or Al; or >10 000 $mg.kg^{-}$ Zn or Mn in their above-ground biomass (dry weight) (Gifford et al., 2007). Phytoremediation was demonstrated to be efficient in removing both inorganic and organic compounds contaminated waters (Favas et al., 2014; Lyubenova & Schröder, 2011), sediments (Klink et al., 2013; Pandey et al., 2014; Sasmaz et al., 2008; Weis & Weis, 2004) or soils (Leveque et al., 2013).

Biomechanism of heavy metal removal by plants

During plants' lifespan, both macronutrients like N, P, K, S, Ca, and Mg, and micronutrients such as Fe, Zn, Mn, Ni, Cu, and Mo are considered essential. Plants have undergone evolution to develop mechanisms in which they absorb, distribute, and store the nutrients at various levels. An example is a protein, which serves as transportation for metal through plants' membranes. Another characteristic of these mechanisms is selectiveness. Metal ions are kept concentrated at the cell level, and in plants process of ions uptake, some ions types are more preferred than others.

Roots make up 20 - 50% of plant biomass; it absorbs the elements in plant tissues (except carbon) from the soil and transports them to the above-ground biomass. The mobility of metallic elements in the soil is following different pathways:

- Molecules which complex with metals may exist in the form of complexes in the root and soluble metals in the soil. The process of extracting metals by plants is primarily performed by complexion (Dang, 2009).

- The roots can reduce the concentration of metal ions in the soil as a response to a special need for the plant metabolism, and the agent may increase the beneficial properties of the metal. Deficiency of Fe or Cu elements in Pea plants, for example, increased the ability to remove Cu^{2+} and Fe^{3+} ions from soil (Dang, 2009).

- By soil acidification, the roots can dissolve heavy metals in the soil and push the metal ions from the complex (Dang, 2009).

- The roots can coordinate with rhizosphere microorganisms (mycorrhizal fungi or bacteria living on the roots) to enhance the absorption ability of metals in the soil. Microbial populations are higher in the rhizosphere than in the root-free soil. This results from a symbiotic relationship between soil microorganisms and plants. This symbiotic relationship can enhance some bioremediation processes. Plant roots also may provide surfaces for sorption or precipitation of metal contaminants (Sas-Nowosielska et al., 2008).

Typha latifolia – a suitable wetland plant for metal and pesticides bioaccumulation

Among aquatic macrophytes, *Typha latifolia* is a common wetland plant that grows broadly in tropical and warm regions with a high capacity for uptaking heavy metals into its body. Sasmaz et al., (2008) indicated that the *Typha latifolia L.* root system was suitable for metal bioaccumulation. His results also showed that *T. latifolia L.* in contaminated water and sediments or soil could also be used as bio-monitoring for Zn, Ni, Cu, Pb, Co, Mn and Cd (Sasmaz et al., 2008). *T. latifolia* also has capable of significantly remove atrazine molecules generating flows between the soil and the root system (Moore et al., 2013), or convert atrazine to other metabolites, such as hydroxyatrazine, DEA, and DIA (Mezzari & Schnoor, 2006).

I.B.4. BIOTURBATION

Bioturbation is driven by the activities of invertebrates in the sediment. It is a natural process defined as "all transport processes carried out by animals that directly or indirectly affect sediment matrices" (Kristensen et al., 2012). Bioturbation is the source of significant changes in biological and physicochemical properties of soils and sediments (Baranov et al., 2016; Kristensen et al., 2012; Peterson et al., 1996), as well as several types of biotransports (Gerino et al., 2003).

I.B.4.1. Different types of bioturbation

The bioturbation processes include particle reworking and burrow ventilation (Delmotte et al., 2007; Kristensen et al., 2012) depending on the species or functional group of bioturbators. Four major types of particle reworking conducted by benthic fauna in aquatic environments are being identified that are common in marine and freshwater sediment (François et al., 2002; Gerino et al., 2003) (Fig. I.4):

(A)Biodiffusors: Organisms with activities that usually result in a constant and random local sediment biomixing over short distances resulting in transport of particles analogous to molecular diffusion.

(B)Upward conveyors: Vertically oriented head-down species at depth in the sediment. They transport particles from deep horizons to the sediment surface. The particles are returned to bottom by gravity under fecal pellets accumulation at the sediment surface

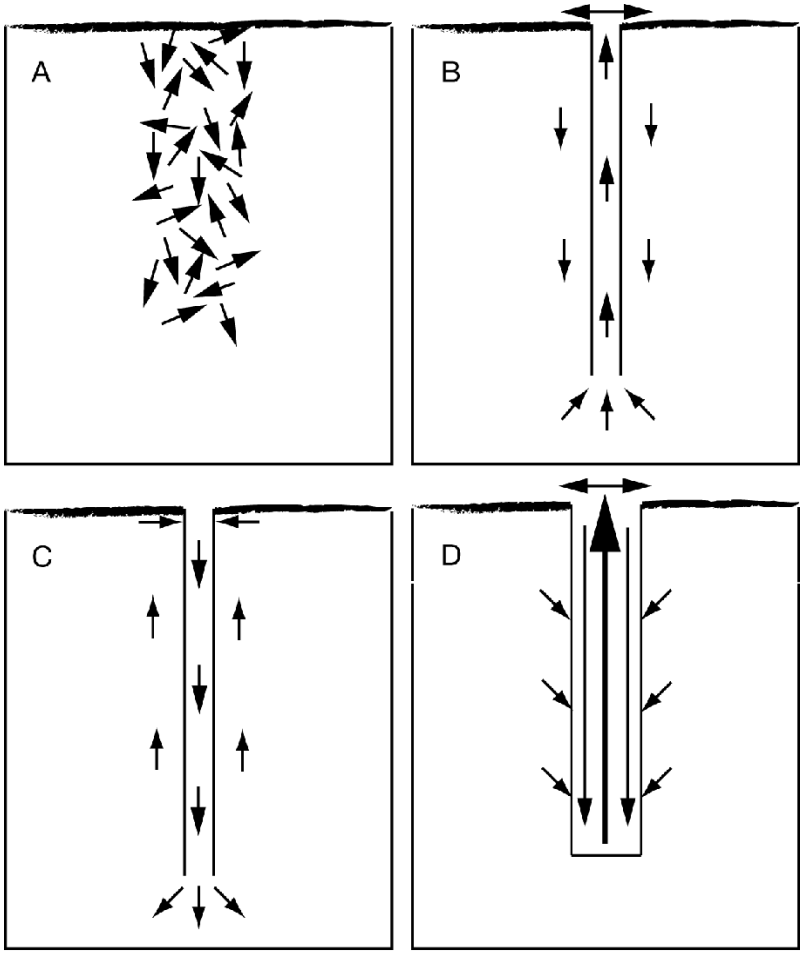


Figure I.4. Four main categories of particle reworking by bioturbation (François et al., 2002).

(C)Downward conveyors: Vertically oriented head-up feeders actively select and ingest particles at the surface and egest this non-locally as feces in deeper sediment strata

(D)Regenerators: excavators that dig and continuously maintain burrows in the sediment and by doing so transfer sediment from depth to the surface

I.B.4.2 Tubificid worms – a typical example of bioturbation in aquatic systems

Among the benthic invertebrate community that inhabits the freshwater wetland, the oligochaetes are the organism among the most resistant to chemical perturbations. It is widely known that Tubificid Oligochaeta (ITIS - Integrated Taxonomic Information System), is at the source of bioturbation processes that are related to conveying biotransport of sediment in the majority of the freshwater deposit sediment with fine granulometric (McCall & Fisher, 1980). Tubificids are described as small (vertically) burrowing worms (which are often about 2-5 cm long and roughly 1 mm in diameter), living in the sediment-water environments. They have located vertically in the surface muddy sediments with a head-down orientation so that they are feeding at depth in the sediment and are continuously egesting its fecal pellets at the surface of the sediment. With large densities of tubificids in aquatic sediment, they are generating the conveyor belt phenomena. During their activities, they consume and dispose sediment particles from the bottom layers to the superficial sediment layers (Cunningham et al., 1999) in a typical conveying process with related bioadvection of the surrounding sediment. Bioadvection is a process with the similarity of the natural sedimentation but with higher rates of sediment burial due to the tubificids that defecate at the sediment surface so that the surface sediment in buried under deposition of large quantities of fecal pellets. This bioadvection is a downward advection of the water-sediment interface under the influence of large densities of the tubificid population, as head-down deposit feeders. This biotransport modifies the distribution of fine particles that increases in the surface layer where fecal pellets accumulate (Ciutat et al., 2006).

I.B.4.3 Influence of bioturbation on the fate of aquatic pollutants

Bioturbation and related biotransports may significantly influence the physicochemical properties of sediment (Mermillod-Blondin et al., 2001, 2002, 2003, 2004; Pigneret et al., 2006), and the contaminant incorporation into the sediment (Delmotte et al., 2007; Devault et al., 2009). In most cases, bioturbation effects on the physical, chemical, and biological properties of sediment-water result in increased mineralization rate of organic matters in marine sediment (Gerino et al., 1998). By changing the ratio between sediment and water, bioturbation also possibly modify the vertical distribution of pollutants in the sediment column (Anschutz et al., 2012; Ciutat et al., 2007, 2006; Delmotte et al., 2007; Gerino et al., 2014; Hoang et al., 2018; Hölker et al., 2015; Kristensen et al., 2012; Teal, Parker, & Solan, 2013). Bioturbation, generated by invertebrates' benthic activities, therefore is controlling the fluxes of organic matter and nutrients through water-sediment interface, and the contaminant incorporation into the sediment.

The redistribution of the sediment particles due to bioturbation could lead to a change in its grain size in different sediment layers. The bioturbation activity can modify the vertical granulometric distribution of the sediment. Other conveyors organisms are responsible for a granulo-reclassification of particles: they ingest the finest particles at depth and reject the fecal pellets on the surface, resulting in a depletion of fine particles in depth and enrichment in the surface layers. This is the case of the polychaetes *Arenicola marina* (Rasmussen et al., 2000) and *Naineris laevigata* in the marine environment; tubificids in freshwater (McCall & Fisher, 1980; Ciutat et al., 2006); and earthworms in the terrestrial environment. Fecal pellets produced by conveyor organisms are often agglomerated by mucus, generating larger particles that increase the porosity of this layer of fecal pellets and, together, the fluxes through the interface by simple diffusion. It is important to emphasize that these fecal pellets contain a relatively high proportion of low-density organic matter. Also, the balls are not bonded together, thus reducing the compactness and cohesively compared to the non-

bioturbated sediment, depending on the type of bottom (sandy or muddy) considered. This layer of fecal pellets is systematically more abundant in water, which will facilitate its resuspension by currents or other organisms (McCall & Fisher, 1980; Rhoads & Young, 1970). Downward transport of the surface sediment results from the accumulation of fecal pellets at the sediment surface, simultaneously with sediment depression in deeper layers due to sediment ingestion by the worm feeding (Anschutz et al., 2012; Ciutat et al., 2006). Consequently, bioturbation creates two distinct layers in the bioturbated sediment: a top layer corresponding to the fecal pellets accumulation from ingested anoxic sediment, and a bottom layer of with increasing particle size (Anschutz et al., 2012).

Some burrowing species can generate resuspension of sediment particles during the construction of their burrows: this is the case of *Hexagenia rigida* larvae in freshwater (Saouter, 1990) or the amphipod *Corophium volutator* in an estuarine environment (Ciarelli et al. 1999). The presence of galleries and burrows leads to an increase in the contact surface between the sediment and supernatant water (Charbonneau & Hare, 1998) and therefore a potential increase in the exchange of matter between these two compartments. The digging of the galleries increases the porosity and the water content of the sediment. Compaction, the roughness of the bottom surface, ease of erosion, or permeability is also modified (McCall, 1979; McCall & Fisher, 1980; Sandnes et al. 2000). The biological activity will, therefore, involve both transport of particles and molecules associated with them and the transport of interstitial water and solutes. Bioirrigation of burrows will increase the potential for exchange of material between interstitial water and the water column (Figure I.5.), for example, by increasing the depth of the oxic zone. This will profoundly alter the chemistry of bioturbated sediment layers, especially when mixing between areas with different redox potentials, as well as interface chemistry, which is the critical area for flow control between the column of water and sediments.

Bioadvection, on the other hand, will bring into contact two geochemically very different zones: a deep reduced zone and the oxidized surface zone (Fisher & Matisoff, 1981). Bioturbation increases the area of the oxic-anoxic interface leading to the alteration of the redox zonation geometry and potential increase of the number of sites suitable for coupled nitrification-denitrification reactions (Fig.I.5).

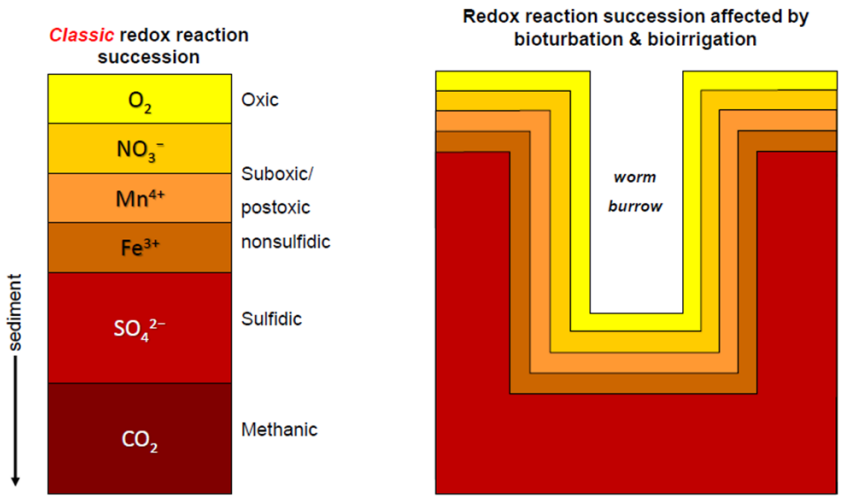


Figure I.5. Influence of bioturbation and bioirrigation on redox reaction in the sediment matrix (stimulated by Aller, 1994)

The bioturbation process, carried out by the invertebrate species, *Oligochaetes Tubificidae Tubifex tubifex*, generates vertical material flow by biotransport and stimulating the activity of microbial communities (Ciutat et al., 2007; Devault et al., 2009; Anschutz et al., 2012). A previous study showed that tubificids increased cadmium scavenging into the sediment because of the renewal of the adsorption site at the sediment surface (Ciutat et al. 2005). Anschutz et al., (2012) showed that bioadvection by tubificid was a pump of dissolved sulfate from the surface to the anoxic sediment. Influence of bioturbation and bioirrigation on redox reaction in the sediment matrix was reported by Aller et al. (1994). Bioturbation also can control the fate of organic matter and nutrients as well as fluxes of nutrients between sediments and water (Hölker et al., 2015) and therefore facilitate the transformation of pollutants (Gerino et al., 2014; Hölker et al., 2015; Monard et al., 2008).

I.B.4.4 Resistance and tolerance of invertebrate animals towards pollutants

Some invertebrate oligochaetes are very widely distributed and frequently dominant in freshwater benthic communities; they have a high level of resistance to unfavorable treatments, especially organic pollution associated with severe hypoxic treatments (McCall & Fisher, 1980). An indoor microcosm carried out by Ciutat et al., (2005) tested the bioaccumulation kinetics of Cd in tubificids worms during a 56-day exposure period. As a consequence, high bioaccumulation levels could be found in the worms (50 $mg.g^{-1}$ dry *wt.*) concerning with their detoxification or sequestration processes under polluted environment. Whitley (1967) reported that tubificids had high tolerance limits for solution contaminated by Pb and Zn. The median tolerance limit for Pb and Zn was 49 and 46 mg/1, respectively. Several authors have described resistance to high metal accumulation levels for other Oligochaeta species, based on efficient detoxification mechanisms, such as intracellular compartmentalization, involving lysosomes, sphero crystals, and/or metal-containing granules (Brown, 1982); metal inactivation by binding to metalloproteins (Dallinger, 1994) or metallothionein-like proteins (Gillis et al., 2002).

For organic compounds, both acute and chronic toxicity has been investigated in some invertebrate worms. Dad et al., (1982) reported that tubificids worms (a mixture of *T. tubifex* and *L. hoffmeisteri*) were able to tolerate high concentrations of insecticides with Presumable Harmless concentration of Furadan 3G found to be at 4.3742 and 3.2167mg/l, respectively.

Earthworms in general (especially *E. fetida*) are highly resistant to many chemical contaminants including heavy metals and organic pollutants in soil and have been reported to bio-accumulate them in their tissues. Earthworms which ingested TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) contaminated soils were shown to bio-accumulate dioxin in their tissues and concentrate it on average 14.5 fold (Satchell, 1983).

I.B.5. COMBINED EFFECTS OF BIOTURBATION AND PHYTOREMEDIATION AS A BIOLOGICAL PROCESS FOR POLLUTANTS REMOVAL IN AQUATIC SYSTEMS

With the rise of sustainable management, the development of useful tools for rehabilitation of aquatic sites with contaminated water and /or sediment, and the purification of water as a vital resource for urban areas has become a significant concern. Investigation of innovative solutions based on natural functions of ecosystems to develop safer and cleaner technologies is one of the current challenges of ecological engineering. Sustainable bioremediation techniques are a part of ecological engineering that involves biodiversity in management strategies focusing on the reduction of pollutant loads of ecosystems. Although current bioremediation relies on a multidisciplinary approach that crosses hydrological, chemical, physical, biogeochemical and ecological processes (Gifford et al., 2007), the integration of different ecological engineering methods, such as bioturbation and phytoremediation was seldom explored so far.

Bioturbation has been proved to promote pollutant fluxes in aquatic sediments, while phytoremediation was demonstrated to be efficient in removing both inorganic and organic compounds contaminated waters, sediments or soils. However, investigation of innovative bioremediation strategies for polluted aquatic sites based on the combination of these two processes to enhance the efficiency of contaminant removal has not been performed thoroughly. Up to now, few combined solutions are recognized within the international scientific community. Few ecological researches, however, has focused on structure and relative abundance of invertebrate communities, as a source of bioturbation, in associations with phytoremediation by riparian plants (Hann, 1995) or influences of these both ecological tools on biogeochemical processes (Mermillod-Blondin et al., 2008; Thomaz et al., 2008)) rather than their interactions in aquatic pollutants removal. Mermillod-Blondin et al. (2008), who studied on relative influences of *Tubifex tubifex* and *Myriophyllum spicatum* (a submersed riparian plant),

suggested that the bioirrigation process by the invertebrate species and radial oxygen loss due to the macrophyte plant's activity are major functional traits affecting biogeochemical functioning at the water-sediment interface of wetlands.

Recently, positive and complementary influences of aquatic bioturbation combined with phytoremediation process for enhancing cadmium fluxes from overlying water to sediment and then into plant's root system have been demonstrated (Hoang et al., 2018). The efficiency of phytoremediation has been largely demonstrated as a bioremediation engineer on heavy metal such as lead, cadmium, copper, arsenic (Leveque et al., 2013; Lyubenova & Schröder, 2011; Pandey et al., 2014), but with more seldom demonstrations on the organic compounds, such as atrazine (Ibrahim et al., 2013). Phytoremediation of persistent organic pollutants (POP) notably relies on their ability to take up, accumulate, or detoxify organic compounds or their indirect role in stimulating the soil microbial or fungal activities in the breakdown of organic compounds (Pascal-Lorber et al., 2011). Stated otherwise, although invertebrates and riparian plants often are both key players in biogeochemical processes occurring at the water-sediment interface of aquatic ecosystems, the impacts of the invertebrate bioturbation on the transfer and transformation of the pollutants, such as inorganic and/or organic compounds by riparian plants for bioremediation purpose are still remains understudied.

I.B.5.1 The potential to inorganic pollutants remediation

Most of the toxic metals belong to an insoluble form and are difficult to move freely in the vascular system of plants. Most of them exist in the form of complexes, such as sulfate, carbonate or phosphate precipitates. Toxicity of metals in soil depends not only on the environment and soil pH, flooding and organic matter content, but it is also affected by the initial chemicals already existing in the sediment and other forms of existence of fauna and flora in the soil system.

Note that biogeochemical processes, including the exchange of materials between the water-sediment interface as well as their transport within the sediment (Liu et al., 2006), which are considerably impacting on contaminants behavior in aquatic environment, are playing an essential role in designing and developing remediation systems (Piwoni & Keeley, 1990; Suthersan et al., 2017). In the other hand, sediment-to-plant mobility of pollutants, such as potentially harmful elements, or plant's phytoremediation potential, which can be assessed by using enrichment coefficients (EC) (Ravanbakhsh et al., 2016), transfer coefficient (Coumar et al., 2016), or transfer factor (Cheng et al., 2015), etc., depends not only on characteristics of plant species or plant parts, but also on physicochemical properties of sediment matrix (Antoniadis et al., 2017). Within the sediment matrix, the pore water is the most active component where the transfer and transformations of contaminants are taken place (Li et al., 2016b). Plants only uptake/accumulate metals in soluble (bioavailable) forms as free metal ions, soluble metal complexes, or adsorbed form to inorganic soil constituents (Sheoran et al., 2016). Bioavailability refers to the ability of a chemical to be absorbed by an organism. It can exist in three different forms: (1) dissolved, (2) sorbed to biotic or abiotic components and suspended in the water column or deposited on the bottom, and (3) incorporated (accumulated) into the organisms (EPA, 2000). According to Sposito (1989), a chemical element is bioavailable if it is present as, or can be transformed readily to, the free-ion species, if it can move to plant roots on a time scale that is relevant to plant growth and development, and if once absorbed by the root, it affects the life cycle of the plant. Bioavailability of metals to plant roots is considered as the critical factor limiting the efficiency of phytoextraction (Felix, 1997). Metal(loid) bioavailability is influenced by biokinetics, organism behavior and physiology, and sediment chemistry, particularly factors that impact the partitioning between aqueous and solid phases and metal speciation (Simpson & Batley, 2007).

Previous studies have demonstrated that bioturbation enhances the bioavailability of inorganic pollutants by affecting physicochemical properties of the

water-sediment interface as well as sediment matrix, habitat for aquatic invertebrate animals. Indeed, bioturbation modifies sediment particle size, porosity, water content, nutrient content, turbidity, TOC, etc., of the overlying water and sediment (Anschutz et al., 2012; Ciutat et al., 2006; Mermillod-Blondin et al., 2008). On the other hand, the bioturbation of sediments by large benthic invertebrates alters sediment redox chemistry by mixing pre-stratified zones in the sediment, and increasing the penetration of electron acceptors, such as dissolved O_2, NO_3^- and SO_4^{2-} into anoxic sediment (Aller et al., 2001). The geochemical changes in bioturbated sediment can affect the behavior of metals (Gilbert et al., 2007). Redox changes can alter metal binding affinities between the solid and dissolved phases, significantly modifying the speciation and bioavailability of most metals in sediments (De Jonge et al., 2012). The changes in redox potential, pH, organic matter, and porosity under bioturbation effects can influence the sorption capacity of the pollutant on the sediment particles and thus change the solid/liquid partition equilibrium of metals, e.g. Zn and Cd (Cheng & Wong, 2002; Shaheen et al., 2016; Shaheen et al., 2014; Yu et al., 2005). Via its influence on physicochemical properties of sediment, bioturbation shifts the metal binding affinities between the solid and dissolved phases and can also significantly modify the speciation and bioavailability of most metals in sediments (De Jonge et al., 2012; Remaili et al., 2015). Combining the bioadvection effect with the Cd source in the overlying water results in doubling the pollutant loading in sediment due to the bioturbation effect (Ciutat et al., 2005, 2006, 2007). Li et al., (2016b) demonstrated that Pb distribution and migration in the contaminated sediment is due to the changing of sediment structure and sediment particles caused by tubificid bioturbation. Delmotte et al., (2007) reported that cadmium was found within the surface layers of sediments (few millimeters from the surface) under bioturbation conditions, and then via bioadvection was transferred into the deeper layer. Consequently, bioturbation generates material fluxes via biotransportation of sediment and associated contaminants through the water-sediment interface and inside the inhabited layer that are estimated to double the incorporation of

these contaminants into the sediment (Anschutz et al., 2012; Ciutat et al., 2006; Ciutat et al., 2007; Delmotte et al., 2007; Gerino et al, 2014; Hölker et al., 2015; Kristensen et al., 2012; Teal et al., 2013).

I.B.5.2 The potential to organic pollutants remediation

Translocation of organic compounds in sediment is governed by lipophilic properties such as the octanol-water partition coefficient, K_{ow}. Herbicide with a low log K_{ow} value such as 0.6 is more water-soluble (hydrophilic or polar), while a herbicide with a high log K_{ow} value, such as 2.61 (atrazine) is more lipid soluble (lipophilic). Pesticides absorption by plant roots is typically characterized by rapid initial entry to the tissue. Root absorption capacity is often expressed regarding the root concentration factor (EC_R). EC_R value < 1.0 indicates incomplete permeation of the tissue, whereas a value > 1.0 indicates accumulation in the tissue.

Atrazine, for example, has a log K_{ow} of 2.56 at 25 °C, and Topp et al. (1986) indicated a positive correlation between initial absorption rates, enrichment coefficient of pesticides for plant root (EC_R) and lipophilicity (K_{ow} coefficient). Polar compounds enter the root cells less rapidly and are initially restricted to the free space, resulting in EC_R of 0.6 – 1.0. Lipophilicity compounds, on the other hand, enter the root cells rapidly and can accumulate in lipid-rich domains in the tissue, resulting in a greater value of EC_R (Topp et al., 1986). Sorption of organic compounds to organic matter lowers its bioavailability for a plant (Binet et al., 2006). Partition coefficient (K_{oc}) has been so far used as a quantitative measure of the magnitude of the binding affinity of organic matter for pesticides. This constant allows measuring the mobility of a substance in soil or sediment (from sediment particles to sediment pore water) when calculating the proportion of the substance bounding to organic matter thus predicting the fate of organic compounds in soils and water bodies (Kulikova & Perminova, 2002). A very high value of Koc means it is strongly adsorbed onto soil and organic matter and does not move throughout the soil. A very low value means it is highly mobile in soil. It has

been demonstrated that bioturbation can control the fate of organic matter and nutrients via digestion (Hölker et al., 2015; McCall & Fisher, 1980; Mermillod-Blondin et al., 2001) and therefore facilitate the mobility (Farenhorst et al., 2000a, 2000b) and transformation of pollutants (Gerino et al., 2014).

On the other hand, plants provide a substrate (roots, stems, and leaves) as a habitat for microorganisms that they can grow, break down organic materials, and uptake heavy metals. Degradation of pesticides can include photolysis, chemical transformations and biological transformation (Stangroom et al., 2000), of which microbial processes usually dominate (Vink & Van der Zee, 1997). The interactions between the plants and microorganisms that live in the soil can also contribute to phytoremediation, often called rhizosphere bioremediation. The importance of the rhizosphere microbial communities for the breakdown of organic contaminants was described by Anderson et al, (1994).

The degradation of the organic compounds such as herbicides mostly occurs in wetland areas (Mudhoo & Garg, 2011) or in topsoils (Douglass et al., 2015), where a physical (photo-oxidation), chemical and biological degradation processes may occur. Atrazine-degrading microbial communities (Krutz et al., 2012), as well as other important chemical factors (soil pH, organic material, and moisture) exerting controls on the plant up taking, (Wehtje et al., 1983), have been considered as the primary mode of the attenuation. Soil microbial communities play a crucial role in biodegradation of the *s*-triazine herbicides such as atrazine into metabolites (Udiković-Kolić et al., 2012;). As a growth substrate (C and/or N source), atrazine is used by microorganisms via catabolic pathways of xenobiotic due to *atz/trz* genes coding for the enzymes responsible for the mineralization (Udiković-Kolić et al., 2012). Atrazine-degrading bacteria typically include members of genera *Pseudomonas, Acinetobacter, Agrobacterium, Arthrobacter, Rastonia and Norcardioides* (Strong et al., 2012), as well as some fungi such as *Aspergillus, Rhizopus, Fusarium, Penicillium, Phanerochaete* (Mougin et al., 1994; Islas-Pelcastre et al., 2013).

Typha latifolia, with rapid growth and high resistance in polluted environments, shows the capacity to significantly remove atrazine molecules by generating flows between the soil and the root system (Moore et al., 2013). This plant, as other species also converts atrazine to other metabolites, such as hydroxyatrazine, DEA, and DIA (Mezzari & Schnoor, 2006). The bioturbation type, carried out by the conveyor species (Gerino et al. 2003), such as Oligochaetes Tubificidae, generate vertical material flow by biotransport and stimulate the activity of natural heterotrophic microbial communities (Gerino et al., 2007, Ciutat et al., 2007; Devault et al., 2009; Anschutz et al., 2012), and the processing of organic matter in aquatic sediments (McCall & Fisher, 1980; Mermillod-Blondin et al., 2001). The flux dispersion by the oligochaetes also enhanced both aerobic and anaerobic microbial activities (Mermillod-Blondin et al., 2001), in which heterotrophic bacteria in the sediment has a significant correlation with the presence of tubificid worms (McMurtry et al., 2011). Likewise, the organic matter modification by earthworms during the soil bioturbation also positively impacted biodegradation by increasing atrazine adsorption on their microsites (Farenhorst et al., 2000; Kersanté et al., 2005). Starting from this evidence, previous studies with contaminated aquatic sediment began to show how bioturbation could also facilitate the transformation of micro-organic pollutants (Gerino et al., 2014). Previous studies also reported the modification of size, structure, and activity of the indigenous atrazine-degrading bacteria group (Monard et al., 2008) or acceleration of atrazine mineralization in bioaugmented soil in a relationship with earthworms bioturbation (Kersanté et al., 2005).

These both microbial and chemical factors that directly influence the biodegradation of the pesticide can be positively altered by the presence of independently natural processes, such as bioturbation (Hölker et al., 2015; Monard et al., 2008), phytoremediation (Moore et al., 2013; Murphy & Coats, 2011; Qu et al., 2017). The bioturbation process, carried out by the invertebrate species, *Oligochaetes Tubificidae*, has a capacity of not only generating vertical material flow by biotransport, but also

stimulating the activity of microbial communities (Anschutz et al., 2012; Ciutat et al., 2007; Devault, Delmotte, et al., 2009). Previous studies also reported the modification of size, structure, and activity of indigenous atrazine-degrading bacteria (Monard et al., 2008) or acceleration of atrazine mineralization in bioagmented soil in a relationship with earthworms bioturbation (Kersanté et al., 2005).

I.B.5.3 What's about the terrestrial systems?

Similar to aquatic invertebrate, soil bioturbator animals (earthworms) can also modify the soil characteristics through their bioturbation activities such as burrow creation, production of cast thus the mixing of litter and soil (Nahmani et al., 2007). Besides the accumulation in their tissues and their survivability in metals (Cd, Cu, Zn and Pb), pesticides, and lipophilic organic micropollutants like the polycyclic aromatic hydrocarbons (PAH) from contaminated soils, some earthworms could show the ability to biodegrade the chemicals contaminants by stimulating soil microbial activity (Morgan et al., 1989; Sinha et al., 2009). Earthworms have thus been recognized as typical ecosystem engineers (Farenhorst et al., 2000a, 2000b; Jones et al., 2008; Monard et al., 2008), and a potential partner for humans in managing ecosystem services (Byers et al., 2006). They are also potential providers of hot spots (in their gut) for microbial communities, specifically the bacterial degrading function (Sinha et al., 2009) and indirect biodegrade organic contaminants through enhancing microbial activities (Ma et al., 1995). Bacterial species associated with the intestine and vermicasts of the earthworms, such as *Pseudomonas*, *Paenibacillus*, *Azoarcus*, *Burkholderia*, *Spiroplasm*, *Acaligenes,* and *Acidobacterium* were reported by Singleton et al. (2003). The digestion by earthworms during the soil bioturbation also positively impacted biodegradation by increasing atrazine adsorption on their microsites concerning with organic matter modification (Farenhorst et al., 2000b; Kersanté et al., 2005).

It is also well-known that soil to plant transfer of metals depends strongly on pollutant compartmentalization and speciation that are directly concerning soil

properties such as soil organic matters or pH (Shahid et al., 2014). Significant improvement in the soil quality was observed where the worms inhabited. This change was explained by worms effects on leading the mobility of metal and thus increase its bioavailability (Capowiez et al., 2011; Sizmur & Hodson, 2009) in the soil through their burrowing activity, thereby improving the efficiency of phytoremediation (Cheng & Wong, 2002).

Recently, Leveque et al. (2014) documented the investigation of the earthworm activity's influence on the phytoavailability of metals from soil. The study indicated that earthworms resulted in significant increases metal phytoavailability by creating soil macroporosity and producing casts near the plant roots, in which physical impact of the earthworm's bioturbation appears to be the principal mechanism (Fig.I.6).

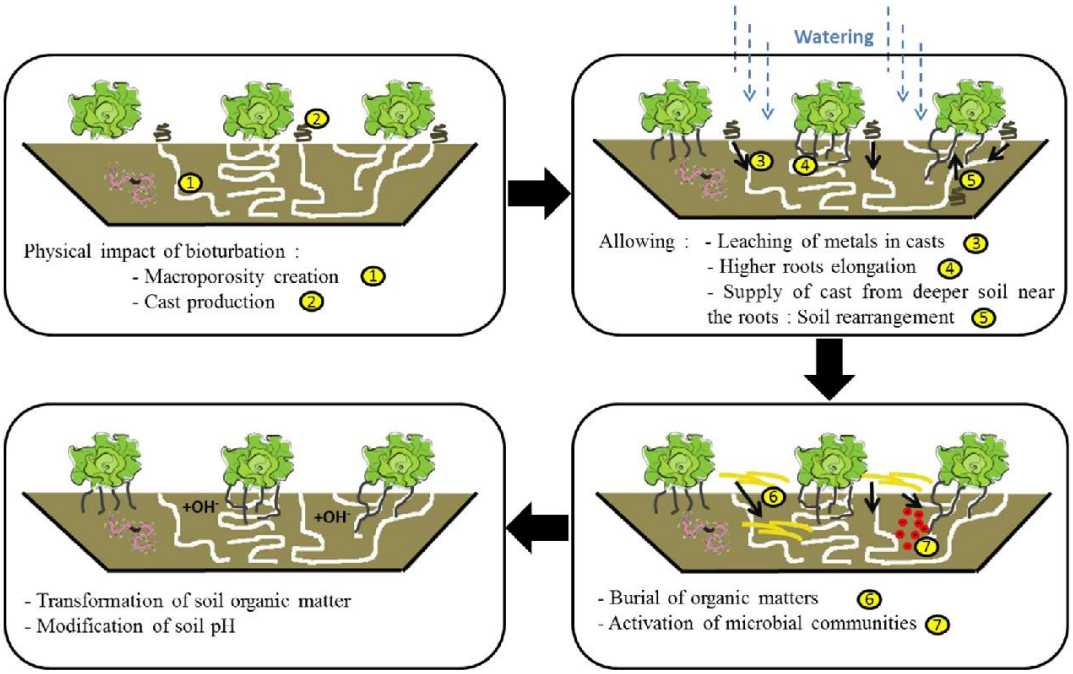


Figure I.6. Proposed mechanisms to explain the increase of metals phytoavailability in relation to earthworms' activities (Leveque et al., 2014)

An application of a novel combination between bioturbation and phytoremediation processes could be therefore considered as a potential and prospective study with the aim of amplifying the possibility of bioremediation if compared with single-process application performed so far.

PART II. METHODOLOGY

II. A. MICROCOSM STRUCTURE AND EXPERIMENTAL DESIGN

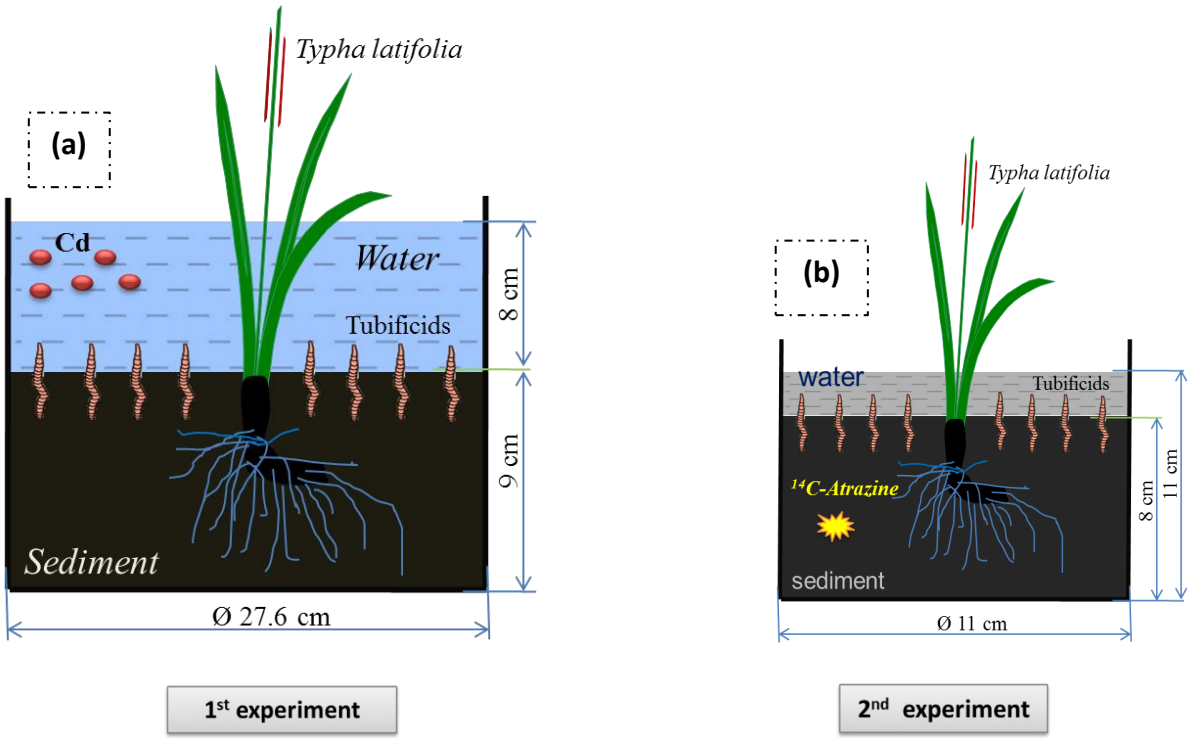


Figure II.1 Microcosm design of two experiments mimicking wetland patterns

(a) 1st experiment using cadmium as a conservative pollutant; (b) 2nd experiment using atrazine as a non-conservative pollutant

Our bioremediation strategy benefits were tested with 02 controlled laboratory experiments mimicking natural-based patterns; these experiments were using a series of microcosms with different treatments with and without biological effects of plant and invertebrate. Each microcosm consists of a mixed biotope "water column/sediment" interface as in wetland areas (*Fig. II.1*). This pattern was used for the two different experiments (*Fig. II.1*).

The 1st experiment (*Fig. II.1a*) was run using Cadmium, one heavy metal initially introduced in the overlying water as a source of *conservative pollution* and representative of urban hazard. The experimental condition was set to allow the metal pollutant

bioaccumulating in plants as a function of time (30 days of the experiment). The 2nd experiment was started with homogeneously contaminated sediment with radiolabeled-^{14}C atrazine as a *non-conservative and organic pollutant* to be followed in the aquatic environment during 26 days of the experiment.

Depending on the size of each experiment, the microcosms were set up in experimental pails made by plastic or glass material, and designed as follows for each microcosm:

- 1st experiment (*Fig II.1a*): sediment surface area = 0.6 dm^2; sediment volume = 5.4 dm^3; water volume = 4.8 dm^3; experimental pail made by plastic material
- 2nd experiment (*Fig II.1b*): sediment surface area = 0.1 dm^2; sediment volume = 0.76 dm3; water volume = 0.28 dm^3; experimental pail made by glass material

II. A.1 SAMPLING SITE AND PROCESSING OF SEDIMENT AND PLANTS

The Aussonnelle River catchment - the studied site, where water, sediment, and plant samples were collected, is a first rank tributary and a small watershed of just 0.28 km^2 of Garonne River (Selery area, Colomier, France). The part of this watershed upstream Selery area is essentially covered by residential and green areas. This first rank little river is an affluent of the Aussonnelle River that is itself an affluent of the Garonne (Fig. II.2).

The sediment was defaunated firstly by incubating sediment for 40 days in a room at 40°C into closed tanks to perform anaerobic condition in the sediment. Secondly by a mechanical mixing process of the sediment, using a motor-mixer, which eliminated the last macro fauna and homogenized the sediment at the same time. Then, the defaunated sediment was introduced into each experimental bucket (pail, 13 liters),

and then de-chlorinated tap water was carefully added to the upper part of the pails to avoid disturbances at the sediment surface.

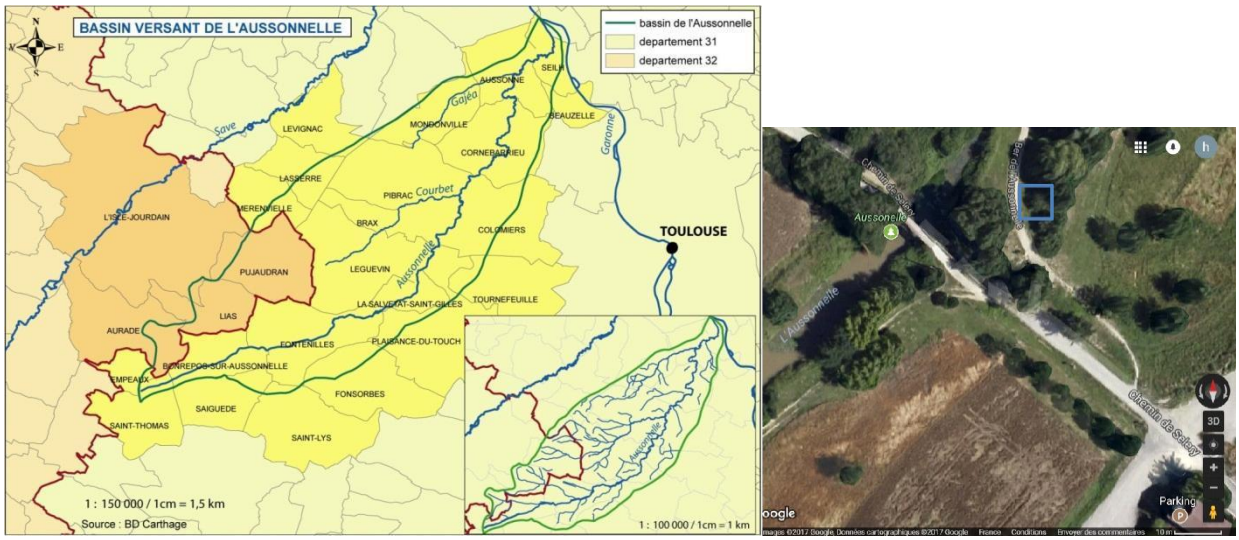


Figure II.2. Aerial view of the Aussonnelle basin, the watershed study, and location of sampling point (Blue Square) for water and sediment located on the site of Sélery. *Source: adapted from Google map*

II. A.2 EXPERIMENTAL DESIGNS

II. A.2.1 Cadmium experiment – a conservative pollutant case study (1st experiment)

Plants manipulation

Figure II.3. Acclimating the *Typha latifolia* in the experimental room (1st experiment). The individual biomass was homogenised before starting the experiment by cutting some rhizome excess.

In the 1st experiment, a set of 32 microcosms was displayed in one thermostatic experimental room. Each microcosm was filled with sediment and water and additional

biological factors (plant and invertebrate) and cadmium depending on the treatment to be imposed.

The collected *Typha latifolia* individual plants (*Fig II.3)* were stored a thermostatic experimental room for acclimatisation for 60 days until the start of the experiment. Healthy plants showing no signs of rotting and having approximately the same height were selected for experiments. One individual was taken and prepared for analyses to have the reference information of metal content. Just before starting the experiment, the roots and rhizomes of the other *T. latifolia* plants were carefully cleaned up with tap water to completely remove field non-manipulated sediments and then precisely cut to achieve, as far as possible, homogeneous biomass among the plants. From these plants, three were selected randomly (at t=0) for initial metal analysis. The remaining were planted into microcosms (one by the bucket) and collected on the last day of the experiment (t=30).

Experimental design

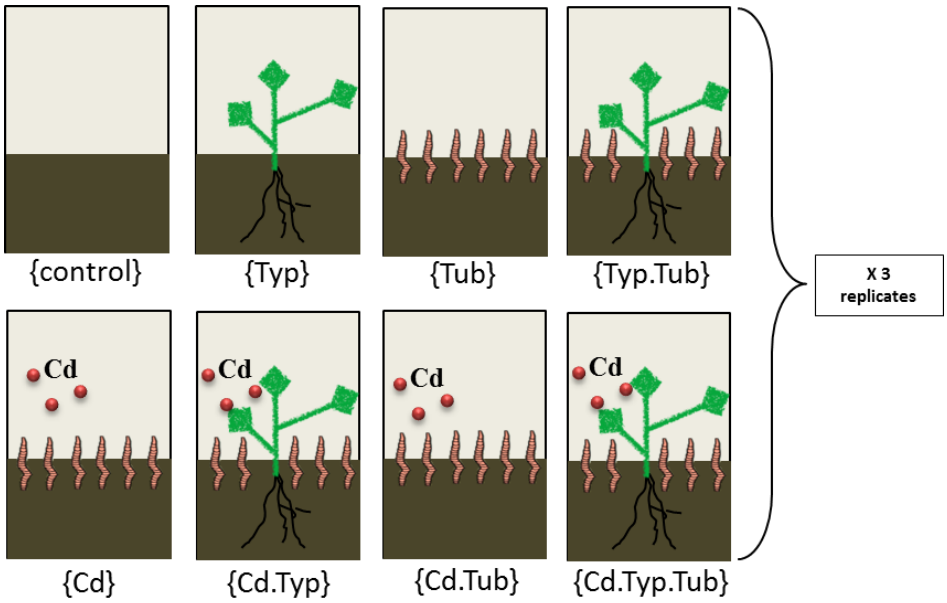


Figure II.4. 1st experimental treatments

A full-factorial design was used, crossing three tested factors: Cd concentration (Cd-enriched or not), plant influence (presence/absence of *T. latifolia*) and macrofauna influence (presence/absence of tubificid worms) (see Table II.1).

All possible combinations of the different levels of each factor were thus tested and distributed into the different microcosms (*Fig II.1a and II.4*). Three replicates per combination were performed. The experimental units were distributed in four blocks, each including one replicate of each combination of the three tested factors. The position of the microcosms within each block was randomly determined to avoid any location effects. Tap water was supplemented in each microcosm every few days (approximately 3% of the total water volume per day) to compensate for evaporation and to keep the water level constant. Microcosms were set-up in a thermostatic experimental room with homogeneous physicochemical conditions (T°C, light, sediment, water).

Table II.1
Main acronyms for different experimental treatments used in the cadmium experiment

Treatments noted	Time (day)	Description
Field	t = - 60	Samples collected from the field (before starting the experiment)
Initial	t = 0	Initial samples (at the beginning of the experiment)
{Control}	t = 30	Control treatment (no plant added, no metal added, no invertebrate added)
{Typ}	t = 30	No metal added, with plant added, no invertebrate added
{Tub}	t = 30	No metal added, no plant added, with invertebrates added
{Typ.Tub}	t = 30	No metal added, with plant added, with invertebrates added
{Cd}	t = 30	With metal added, no plant added, no invertebrate added
{Cd.Typ}	t = 30	With metal added, with plant added, no invertebrate added
{Cd.Tub}	t = 30	With metal added, no plant added, with

		invertebrates added
{Cd.Typ.Tub}	t = 30	With metal added, with plant added, with invertebrates added

Typ: *T.latifolia* plant; Cd: cadmium; Tub*:* tubificids invertebrates

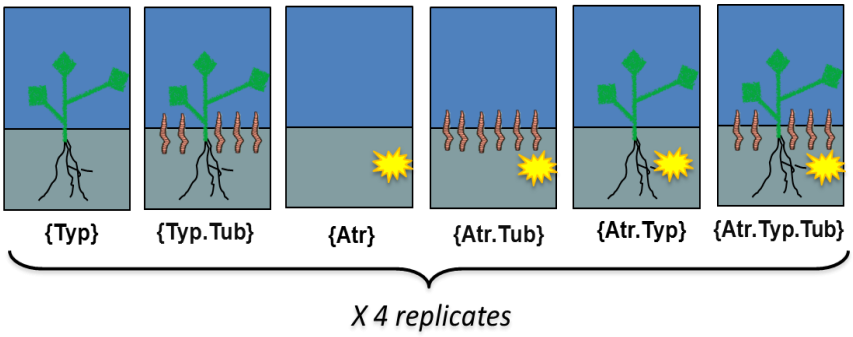
Light provided by three electric bulbs (400 Watts each), 12h of light each day, room temperature at 18°C. Photon fluence rate (the unit concerning the light in the plant production) in the experimental room was 18.1 ± 2.4 $\mu mol.m^{-2}.s^{-1}$ at the surface of the overlying water and 28.0 ± 2.2 $\mu mol.m^{-2}.s^{-1}$ at the top of *T. latifolia* plants.

Two weeks after worm introduction, cadmium provided as cadmium nitrate ($Cd(NO_3)_2.4H_2O$), with an initial contamination level of 20µg Cd/L was gently added to the overlying water. This concentration was selected from the previous experiment with cadmium and tubificid worms (Ciutat et al., 2005b) considering that this concentration is frequent in contaminated waters by mining wastes (Salvarredy-Aranguren et al., 2008) or industrial sites (Andres et al., 1999). In order to avoid any bias resulting from nitrate addition in the metal-treated pails, a neutral salt in the form of calcium nitrate $Ca(NO_3)_2.4H_2O$, with the same nitrate concentration (20µg Ca/L), was added in microcosms not contaminated by cadmium.

The experiment lasted 30 days after the introduction of the contaminant in the overlying water on the first day (t=0). This is the time which was considered needed to allow: (i) plant growth, (ii) bioturbation, and (iii) metal transfer within each compartment as well as their bioaccumulation in the plant system, without having extra-environmental disturbances. Water, plants, and sediment were sampled on the last day (t=30).

II. A.2.2. Atrazine experiment – a non-conservative pollutant case study (2nd experiment)

The 2nd experiment was also performed in controlled laboratory conditions with a series of 24 microcosms, each copying a portion of the water/sediment interface as in wetland areas. Each microcosm was enriched or not with additional worms and contaminant depending on the selected treatment, including 3 tested factors, which are atrazine concentration (atrazine or not), plant influence (presence/absence of *T. latifolia*), and macrofauna influence (presence/absence of tubificids worms) (*Fig II.5* and Table II.2). They were set up through four blocks, each including one replicate of each combination of the three tested factors. These blocks were placed in a climatic chamber, primarily devoted to radiolabeled experiment (UMR Toxalim, INRA, Toulouse, France), under controlled conditions 16 h day photoperiod (light provided by a mix of low-pressure and high-pressure sodium lamps of 400 watt each, at 24 °C during the day and 20 °C during the night under a 16 h photoperiod.



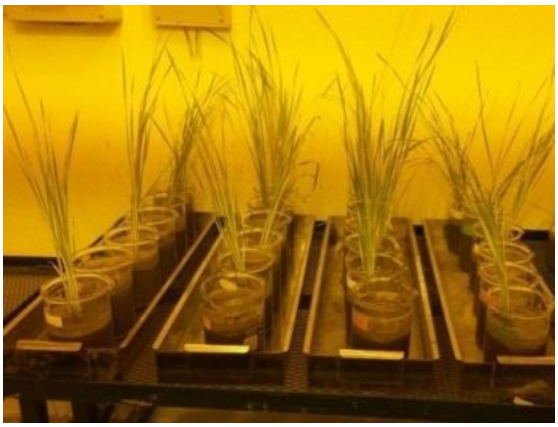


Figure II.5. 2nd experimental room with block design

Table II.2
Main acronyms for different experimental treatments used in the atrazine experiment

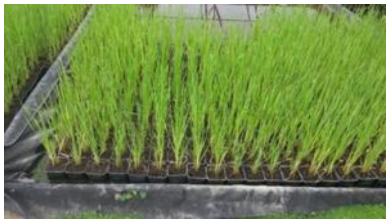
Treatments noted	Time (day)	Description
{Atr}	t = 26	No plant added, no invertebrate added, with atrazine added
{Atr.Tub}	t = 26	No plant added, with invertebrate added, with atrazine added
{Typ}	t = 26	With plant added, no invertebrate added, no atrazine added
{Atr.Typ}	t = 26	With plant added, no invertebrate added, with atrazine added
{Typ.Tub}	t = 26	With plant added, with invertebrate added, no atrazine added
{Atr.Typ.Tub}	t = 26	With plant added, with invertebrate added, with atrazine added

Atr: atrazine; Typ: *T.latifolia* plant; Tub: tubificids invertebrates

Two weeks (t= -15 days) before starting the experiment, water from the Ausonelle River was spiked with radiolabeled [^{14}C]-atrazine and then homogeneously introduce in the sediment with an initial concentration of 2 µg.g^{-1} wet sediment, as a non-conservative and organic pollutant. The experiment lasted for 26 days at the laboratory of UMR TOXALIM, INRA, Toulouse.

Plants manipulation

They were watered every second day with running tap water.



Typha latifolia collected from ALISHMA

Acclimating the Typha at laboratory (INRA2, Toxalim, France)

Selecting the best Typha for experiment at ***T=0***

Figure II.6. Collecting and acclimating the *Typha latifolia in the* experimental room (2nd experiment)

In this 2nd experiment, *T. latifolia* plants were provided by ALISMA commercial farm (France) and then stored about one month in the climatic chamber in order to keep it growing after the collecting. Before putting them into the experimental pails (at t= 0), their root and rhizome systems were cleaned with tap water to remove un-defaunated sediments completely.

II. A.3 ECOLOGICAL ENGINERING TOOLS USED

II. A.3.1 Tubificids worms

The biological model used as bioturbation generating organism is *Tubifex tubifex* (Oligochaeta Tubificidae), a species of tubificid worms that inhabits the sediments of lakes and rivers on several continents. The batches of *tubifex* worms were bought to GREBIL company and the delivered organisms consisted of a mixture of different tubificid species.

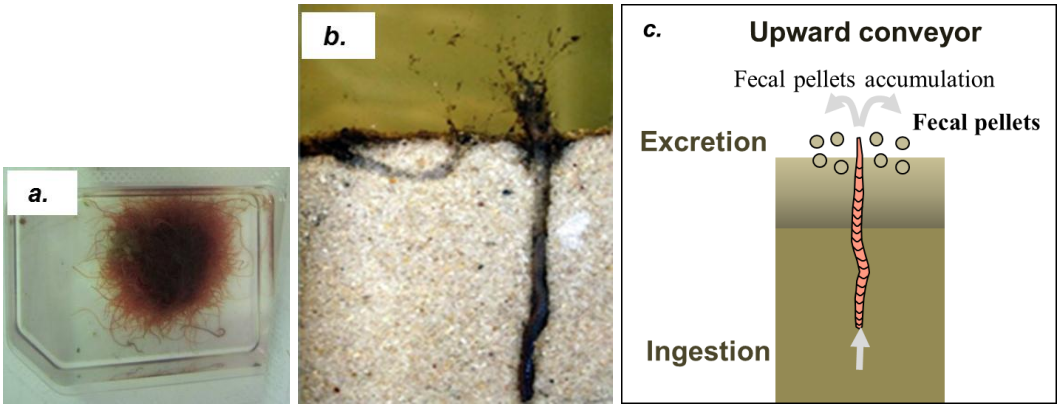


Figure II.7. *Oligochaetes tubificidae* worms and their mode of bioturbation.
a. a tangled ball of tubificid worms in an aquatic medium without sediment for their burrowing; *b*. an individual worm burrowing in sediment; *c.* particle reworking model of tubificid worms as a conveyor-belt species.

These worms, usually in red (their blood contains a pigment close to hemoglobin), are described as small burrowing worms which are often about 2-5 cm long and roughly 1 mm in diameter when fully mature (Aston, 1973; Cunningham et al., 1999). They do not have eyes.

They can constitute a large part of the benthic fauna of continental aquatic ecosystems and represent one of the main bioturbating agents of these systems (McCall & Fisher, 1980). Their natural maximal densities in the sediment can reach up 10^4 to 10^5 individuals.m^{-2}, or even to 60,000 individuals.m^{-2} (Ciutat et al., 2005; McCall & Fisher, 1980). They are resistant to high concentrations of organic matter, anoxia (transient anaerobic metabolism possible), heat and dehydration; they constitute a dominant group within benthic invertebrate communities in disturbed environments (chemical pollution). Their lifespan is a few years.

The tubificids worms usually inhabit muddy substrates with their anterior ends (prostomium) buried and tails (pygidium) protruding and undulating in the water above (Aston, 1973). During their activity, they ingest sediment particles at depth and deposit undigested materials at the sediment-water interface in the form of fecal balls,

after having extracted a part of the assimilable organic matter. This process leads to downward sediment migration and thus induces a recovery of the oxidized sediment of the surface by reduced sediment coming from lower horizons. Through the feeding zone, such sediment could be recycled many times before ultimate burial below the deepest feeding zone (Matisoff et al., 1999). By its metabolism on prevention of the establishment of the anoxic layer at the top of the sediment, tubificids have a significant effect on the exchange of redox-sensitive species across the sediment-water interface (Anschutz et al., 2012).

Fecal pellets contain material with high oxygen demand (FeS for example), whereas the oxidized sediment of the interface is progressively reduced during its burial (McCall & Fisher, 1980). This type of bioturbation is bioadvection; tubificids are conveyor-belt organisms (Rhoads, 1974). Oligochaetes do not irrigate their burrows (McCall & Fisher, 1980). Their resistance to low concentrations of dissolved oxygen is an adaptation related to this ecological niche.

The tubificid worms used in this study were provided by the GREBIL Company (Arry, France). In each microcosm with tubificids, the worms were introduced at the surface of the sediment with the density varying a bit between both experiments (Table II.3)

Table II.3. Tubificids worms' density and fresh biomass

Experiment	surface of an experimental pail (dm^2)	Number of worms in a pail	Density (ind.dm^{-2})	Fresh biomass (g per microcosm)
Cd experiment	6	800	133	17.80 ± 3.1
ATR experiment	1	100	100	4.45 ± 0.53

II. A.3.2 Riparian plant - Typha latifolia

The Broadleaf Cattail (*Typha latifolia*), also known as the cane reed, rauche, or cattail in Canada, is a plant of the family Typhaceae. It is the most common species of

the genus Typha, a tall monocot that forms dense and highly productive monospecific stands in wetlands (Rocha & Goulden, 2009).

T. latifolia contains rhizomes that are buried in sediment, submerged under water, and often shaded by a dense layer of litter and existing plants. Initial leaf growth is supported by carbohydrates that are either mobilized from rhizomes or translocated from older leaves. Depending on sediment thickness and water depth, and the density of the litter layer and existing canopy, the lower (younger) 50–100 cm of a *Typha* leaf may experience almost total darkness (Rocha & Goulden, 2009).

This riparian plant has a large distribution under various hydrological conditions (Aulio, 2015), with populations found in temperate, tropical, humid coastal, and dry continental climates, or in both flooded and non-flooded conditions (Pandey et al., 2014). On the other hand, the cattail riparian plant reveals a rapid growth and high resistant characteristics in polluted sites with a high capacity for uptaking metals into its body, particular their root systems. In spite of non-essential element for the growth of plants (Williams et al., 2000), Cd is well uptaken by roots and translocated into leaves in many plants species (Manousaki et al., 2008). It's also reported that the *Typha latifolia*'s root system can be used as bio-indicator or bio-accumulator for water and sediments polluted by metals, such as Zn, Ni, Cu, Pb, Co, Mn and Cd (Sasmaz et al., 2008; Klink et al., 2013). Also, the capable of significantly remove organic molecules generating flows between the soil and the root system (Matthew T. Moore, Tyler, & Locke, 2013), or convert pesticide, such as atrazine to other metabolites, such as hydroxyatrazine, DEA, and DIA (Mezzari & Schnoor, 2006) has been documented. Clearly, T.latifolia is well known as a key species for the restoration of some essential wetland functions (Wild et al., 2001) and also a valuable tool to mitigate nutrient enrichment in impacted wetlands (Chua et al., 2012; Zhao et al., 2012) or pollutants for phytoremediation purpose (Aulio, 2015; Ciotir et al., 2013; Favas et al., 2014; Klink et al., 2013; Moore et al., 1999; Moore et al., 2013; Pandey et al., 2014; Sasmaz et al., 2008; Xu et al., 2011).

II.B. METHODOLOGICAL TOOLS

The method of sampling and luminophore addition were almost the same in the two experiments. The few difference of procedure is pointed out below.

II.B.1 CORING SEDIMENT AND SAMPLING OF INTERSTITIAL WATERS AND SEDIMENT PARTICLE

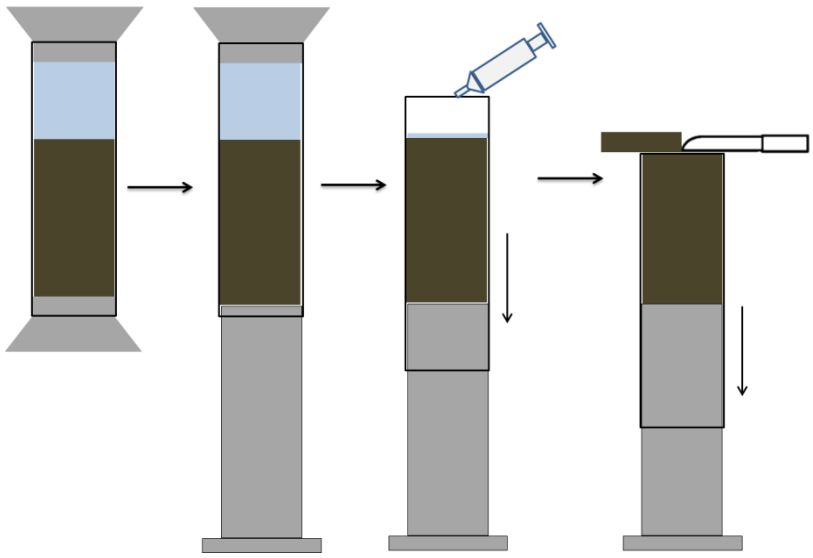


Figure II.8. Procedure for overlying water collection and slicing sediment cores by pushing from underneath of microcoms with a sediment extractor device

At the end of the first experiment, sediment samples were collected by coring and then cut into different layers depending on the purpose of each experiment. The water column was first gently sucked with a pipe (siphoning) and then with a large syringe (Figure II.8). A plexiglass cylinder 5.3 cm in diameter was embedded in the sediment and then removed with the sediment core inside. The thickness of the sediment slices was defined by a system of stops spaced 0.5 cm apart. The sediment slices were cut with disposable plastic knives, which were changed after each slice to prevent contamination from one slice of sediment to another.

II.B.2 BIOTURBATION ACTIVITY MEASUREMENTS

The bioturbation activity of tubificids in the two experiments was quantified using the fluorescent inert tracer technique (Gerino et al., 1994; Lagauzère et al. 2011). Luminophores are natural sand particles coated with pink fluorescent paint with size

ranging from 63 – 125 µm (Partrac Ltd, UK). Twenty-four hours after worm introduction (the time needed for the worms to restore from handling), luminophores were homogeneously deposited at the sediment surface in the form of a 3-mm-thick frozen mud cake (a mixture of 30g of the same sediment with 4g of the tracers). At the end of the experiment (t=30 days), the sediment was cut into twelve layers (0.5-cm thick layers from the surface down to 3 cm depth, then 1-cm thick layers down to 9 cm depth) to assess the vertical distribution of luminophores in the sediment column. Each layer of fresh sediment was homogenized before sub-sampling. Detection of luminophores was performed with a Synergy Microplate reader (Biotek, USA) according to a protocol adapted from Lagauzère et al. 2011. Fluorescence reading was used with 565 nm as excitation and 602 nm as emission wavelength. After estimation of the luminophore concentrations along with depth in the sediment column, the bioadvection–biodiffusion model (Gerino et al., 1994; Officer and Lynch, 1982) was applied to fit the measured tracer profiles:

$$\frac{\delta C}{\delta t} = Db\frac{\delta^2 C}{\delta z^2} - V\frac{\delta C}{\delta z} \frac{\delta C}{\delta t} = Db\frac{\delta^2 C}{\delta z^2} - V\frac{\delta C}{\delta z} \qquad \textit{(eq. 1)}$$

With *t* as the time, *z* as the depth, and *C* as the tracer concentration.

This model allows to estimate the theoretical concentration of tracers under the effects of the two biotransport parameters: V ($cm.y^{-1}$) as the bioadvective velocity or bio-sedimentation rate that represents the downward transfer of the tracers and Db ($cm^2.y^{-1}$) as the biodiffusion coefficient that reflects the dispersion rate of the tracers in omnidirectional directions. This model was first developed by Officer and Lynch (1982) and then used for describing marine (Gerino et al., 1994) and freshwater bioturbation using luminophores (Delmotte et al., 2007). Estimation of bioadvection and biodiffusion rates in the experiment was performed by fitting the theoretical concentrations obtained

with the bioturbation model to the measured luminophore concentrations with a least-squares procedure for each microcosm.

II.B.3 CADMIUM MEASUREMENT

II.B.3.1. Water, sediment, and plant sampling and pre-treatment for Cd analysis

The overlying water was sampled at the beginning (t=0) and the end (t=30) of the experiment. In order to determine dissolved cadmium concentration in water, 40mL from each sample was filtered with a mesh of standard pore diameter of 0.22 µm. Solutions were acidified using a 65% ultra pure HNO_3 solution to maintain metal in solution and then kept in cold and dark conditions (5°C) before the measurements. All materials used (filtration devices, pipettes, box filters, etc.) were plastic made to avoid any metal contamination of samples.

The sediment was sampled three times during the experiment, after the defaunation process: initial samples (t=-60), at the beginning (t=0) before adding contaminant into the overlying water, and on the last day (t=30). For the samples at t=30, the sediment column was carefully removed from the microcosm and then cut into two layers (L1 and L2) connected to the bioturbation process by worms: L1 (0 – 1cm) corresponds to a surface zone composed entirely of fecal pellets ejected and L2 (1 – 5 cm) corresponds to an ingested area of particles (Ciutat et al., 2006). All sediment samples were kept in plastic bottles and stored frozen.

The sediments from the bottles were then spread into plastic Petri dishes and dried in an oven at 40°C, with smooth manipulation (without crushing minerals, sand, gravel, etc...) during drying to avoid aggregation. Once the sediment was dry, and the main organic debris removed with clean plastic tweezers, they were sieved through a plastic mesh sieve to get the fraction < 63µm (accounting for 26.3 ± 1.7% of the total

fraction), subsequently used for Cd analysis. This fraction is supposed to be the most metal enriched and available for plants (Förstner & Solomons, 1980; Probst et al., 2003).

The *Typha latifolia*, collected before the experiment (field sample, at t= -60) at the initial (t=0) and the end of the experiment (t=30), were cut into the following parts: the above part (stem and leaves), the rhizome, and the roots. Before analysis, plant materials were carefully cleaned with distilled water and cut to a constant weight between samples. To ensure the efficiency of the dissolution procedure of the different plant compartments before analysis, they were carefully cut into pieces using a ceramic knife and then ground in liquid nitrogen using a pestle and mortar.

II.B.3.2. Solid sample dissolution procedure and Cd analysis

The sediment fraction (< 63 μm) used for ICP-MS analysis was dissolved by a well-calibrated procedure in the EcoLab cleanroom (ISO 7) (N'guessan et al., 2009). Approximately 100 mg of homogenized plant or sediment material was inserted into a Teflon reactor (Savillex) with a clean plastic spatula and subsequently digested with a mixture of concentrated supra pure HNO_3 and HF at 90 °C overnight. The solution was then evaporated and hydrogen peroxide solution H_2O_2 was added to destroy the organic matter. The procedure was defined with different steps repeated until the dissolution is complete. Finally, after evaporation, 11 ml miliQ water was added.

All samples were transferred to tubes and were diluted so that the element concentrations were lower than 0.8 g/l in order to avoid ICP-MS saturation during the analysis. The samples were then acidified with nitric acid (HNO_3 69%) to obtain a 2% HNO_3 solution. Finally, Indium/Rhenium (In/Re, conc. 0.4037ppm) was added to samples in order to control the machine deviation during the analytical process.

The analytical data quality was checked by including simultaneously in the procedure the blank samples and two standards (SRM-1515 apple leaves, from National Institute of Standards and Technology, USA, and STSD-1 Stream Sediment Reference materials, from Canada Certified Reference Materials Project – CCRMP, Canada) to

verify accuracy and reproducibility of the dissolution process for plant and sediment samples.

Cd concentration was analyzed in water, plants (root and above parts) and sediment samples by spectrometry using a Q-ICP-MS (Perkin-Elmer ELAN 6000) at the OMP-GET (Geoscience Environment Toulouse, Observatoire Midi-Pyrénées, Toulouse, France). Detection limits for Cd were between 10^{-3} to 10^{-2} $\mu g.L^{-1}$. The concentrations of some elements measured in the two standards were used for the analytical recovery (QA/QC) calculation by comparison with their reference values. For most elements, the recovery of the reference material SRM-1515 ranged between 112% and 166%, in which Cd element was at 124%. Because the standard STSD-1 is not certified for Cd element, we used the sediment samples collected in the Aussonnelle River, (initial sample of the study) for comparison with the sediment samples, which were collected in the same station (Aussonnelle, Garonne river bank) by Proffit & Probst (2007) and with the Gascogne river studied by N'guessan et al., (2009). Our results showed that the initial sediment Cd concentrations in the present study were in the same range as those measured by these authors. The mean concentrations of cadmium in the Gascogne stream sediments (fraction <63 μm) and in the Gascogne bedrock (Molasse) samples were 0.30 and 0.20 $\mu g.g^{-1}$, respectively (N'guessan et al., 2009), while the Cd concentration in sediment from this study averaged 0.21 ± 0.02 $\mu g.g^{-1}$.

II.B.4. PHYSICOCHEMICAL VARIABLES OF SEDIMENT AT THE END OF THE EXPERIMENTS

1st experiment with cadmium

Grain-size of the sediment particles was measured with a Laser Scattering Particle Size Distribution Analyser – LA – 950V2 (HORIBA Japan), for sizes ranging from 0.01 to 3000 μm. The Wentworth's classification (Wentworth, 1922) was used to define the various grain-size fractions of four experimental treatments {control}, {Tub},

{Cd}, and {Cd.Tub}: fine silts and clays (< 15 μm), medium silts (15– 30 μm), coarse silts (30–63 μm), very fine sands (63– 125 μm), fine sands (125 –250 μm), medium sands (250 – 500 μm) and coarse sands (> 500 μm). This analysis also provided the mean size of sediment particles, which is an integrative parameter allowing to compare the grain size of a given sediment layer in different treatments.

To study the influence of different treatments on the physicochemical sediment properties, pH value, and pore water content were measured in fresh sediment samples from 4 distinguishable layers collected. These layers thickness were defined according to tubificid worms activity, trying to selectively sample the fecal pellets layer, then the bioturbated layer and finally the non-bioturbated sediment (see §2.4.1). The pore water content and porosity were assessed by drying sediment samples overnight at 105°C, while pH was measured in a water suspension (1.5; v/v) according to the norm ISO 10390.

2nd experiment (atrazine treatment)

Some physical-chemical parameters that affect the degradation of atrazine in sediment including organic matter, organic carbon, and pH were measured vertically in three distinguishable sediment layers (1st layer – L1: 0-1cm; 2nd layer – L2: 1-3cm; 3rd layer – L3: 3-8cm) from two experimental treatments without radioactivity at t=26days: {Typ} and {Typ.Tub}. While sediment porosity was determined in fresh sediment samples from the ten distinguishable layers as in the bioturbation activity measurement. Organic matter content in the sediment has been measured through a semi-quantitative method based upon the indiscriminate removal of all organic matter followed by gravimetric determination of sample weight loss after sample burning(loss-on-ignition method) (ASTM, 2000).

II.B.5. ATRAZINE AND ITS METABOLITES MEASUREMENTS

[^{14}C]-(Atrazine, metabolites) concentrations in all treatments in the 2nd experiment having the contaminant added at initial time were measured in vertical

profile with ten different layers: 0-0.5; 0.5-1; 1-1.5; 1.5-2; 2-2.5; 2.5-3; 3-4; 4-5; 5-6; and 6-8 cm.

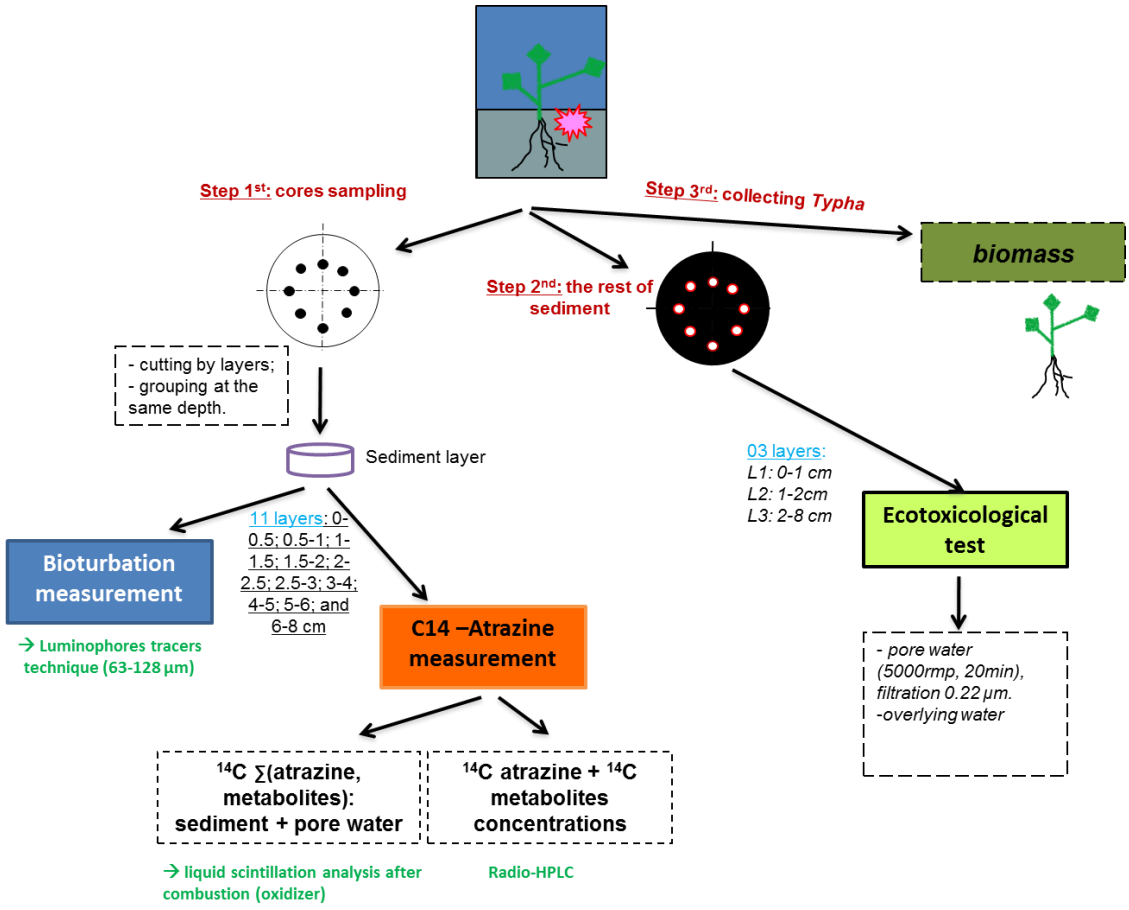


Figure II.9. Sampling procedure to collect total sediment, pore water, and sediment particles fractions for samples of the atrazine experiment

II.B.5.1. Reagents and chemicals

[U-*ring*-^{14}C]Atrazine was purchased from Institute of Isotopes Co Ltd, Hungary (specific activity of 545.45 pg/DPM, radiochemical purity of > 98%). The analytical standard for atrazine was obtained from Sigma-Aldrich (Saint-Quentin Fallavier, France). Solvents used for extractions and radio-RP-HPLC analyses were provided by Cluseau Info lab (Ste Foy La Grande). Unless otherwise specified, all other chemicals were of analytical grade.

II.B.5.2. Atrazine and its metabolites concentrations determination

Total concentrations of atrazine residues [^{14}C]-(atrazine + metabolites) in each constitutive compartment (water, sediment, and plant) were determined in the four experimental treatments having atrazine contamination: {Atr.Typ.Tub}, {Atr.Typ}, {Atr.Tub}, and {Atr} at initial time (t = 0 day) and at the end of the experiment (t= 26 days). The total concentration of [^{14}C]-atrazine was determined in overlying water and the ten distinguishable sediment layers. The activity measurement of the layers was estimated after partitioning liquid and solid phases by centrifugation: the pore waters and the sediment particles.

II.B.5.3. Determination of Radioactivity

For quantification of [^{14}C] radioactivity in overlying water and pore water, 1 mL of each prepared sample was mixed with 10 mL of liquid scintillation cocktail (Ultima Gold™) before measuring with a liquid scintillation counter (LSC) (Packard Tri-Carb^{R} 2910TR, PerkinElmer Life and Analytical Sciences, Courtaboeuf, France). For the sediment particles and plant part samples, approximately 300 mg of homogenized subsamples were used for oxidative combustion to convert [^{14}C] radioactivity to [^{14}C]-CO_2 using a Sample Oxidizer Packard 307 (PerkinElmer Life and Analytical Sciences). The resulting [^{14}C]-CO_2 was trapped in a scintillation cocktail (Permafluor/Carbosorb, 10:7, v/v), and then the detection of radioactivity was performed by LSC. The efficiency of the oxidizer was measured by combustion of ^{14}C-labeled standard, and the recoveries of radioactivity from samples were adjusted to allow for the efficiency of the oxidizer. Quantity of ΣATR was calculated from the specific activity of [^{14}C]-atrazine.

Liquid scintillation principles

Figure II.10 provides a graphic illustration of the way the emitted radiation interacts with the cocktail (a mixture of a solvent and a solute) leading to a count being recorded by the system.

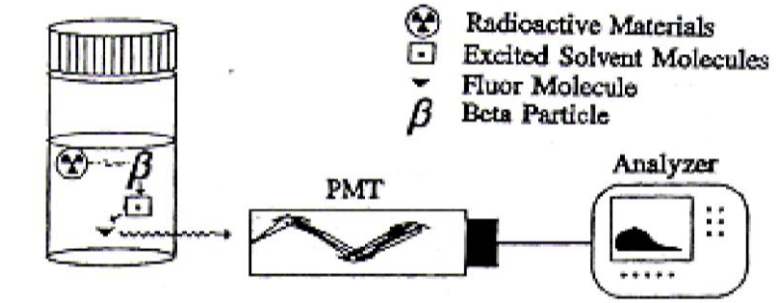


Figure II.10. Liquid Scintillation Counting

The 6 steps for the radioactivity measurements are detailed below:

Step 1. Beta particle (e^-) is emitted in radioactive decay.

Step 2. For ^{14}C, the kinetic energy of beta particles is relatively low, from 0 to 156 Kev. In the relatively dense liquid, the ^{14}C beta particle travels only short distances before all of its kinetic energy is dissipated. The energy is absorbed by the medium in 3 forms: heat, ionization, and excitation. Some of the beta energy is absorbed by solvent molecules making them excited (not ionized).

Step 3. The energy of the excited solvent is emitted as UV light and the solvent molecule returns to ground state. The excited solvent molecules can transfer energy to each other and to the solute. The solute is a fluor. An excited solvent molecule which passes its energy to a solute molecule disturbs the orbital electron cloud of the solute raising it to a state of excitation. As the excited orbital electrons of the solute molecule return to the ground state, a radiation results, in this case, a photon of UV light. The UV light is absorbed by fluor molecules which emit blue light flashes upon return to ground state. Nuclear decay events produce approximately 10 photons per keV of energy. The energy is dissipated in a period of time on the order of 5 nanoseconds. The total number of photons from the excited fluor molecules constitutes the scintillation. The intensity of the light is proportional to the beta particle's initial energy.

Step 4. Blue light flashes hit the photocathode of the photomultiplier tube (PMT). Electrons (proportional in number the blue light pulses) are ejected producing an electrical pulse that is proportional to the number of blue light photons. An LSC normally has two PMT's. The amplitude of the PMT pulse depends on the location of the event within the vial. An event producing 100 photons will be represented by a larger pulse if the event is closer to the PMT than if the event is more remote. The signal from each PMT is fed into a circuit which produces an output only if the two signals occur together, that is within the resolving time of the circuit, approximately 20 nanoseconds (coincidence circuit). By summing the amplitude of the pulses from each PMT, an output is obtained, which is proportional to the total intensity of the scintillation. This analog pulse rises to its maximum amplitude and falls to zero.

Step 5. The amplitude of the electrical pulse is converted into a digital value and the digital value, which represents the beta particle energy, passes into the analyzer where it is compared to digital values for each of the LSC's channels. Each channel is the address of a memory slot in a multi-channel analyzer which consists of many storage slots or channels converting the energy range from 0-156 keV.

Step 6. The number of pulses in each channel is printed out or displayed on a CRT. In this manner, the sample is analyzed and the spectrum can be plotted to provide information about the energy of the radiation or the amount of radioactive material dissolved in the cocktail.

II.B.5.4. Extractable and Un-extractable Residues

After harvested, *T. latifolia* was cut in two parts, roots and shoots (aerial part). Each part was weighed and dried for 48 h at 60°C in the oven. Then, samples were ground in ball grounder.

Ground samples were transferred in centrifuge tubes of 25 mL (screw cap Corex) containing a methanol/dichloromethane/water mixture (2:1:0.8, v/v/v) (8 mL g−1 DW). The sample was homogenized every 15 min for four h in a vortex, stored overnight at 4 °C then it was sonicated with an ultrasonic cell disrupter (Branson sonifier 450, Fischer Bioblock, Illkirch, France) for 5 min. Homogenate was centrifuged at 10,000g for 10 min. The pellet was washed twice with the solvent mixture. The three supernatants, which contained the soluble (extractable) residues, were combined and evaporated under vacuum to remove organic solvents. Samples were then evaporated under nitrogen to 100−200 µL (to avoid any metabolite loss) and brought to the volume of the injection with solvent A before HPLC analysis. The pellets, which consisted of the unextractable residues, were allowed to air-dry for 24 h at room temperature to remove organic solvents and then freeze-dried for 60 h to remove the water before weighed and oxidative combustion.

II.B.5.5. Radio-HPLC analysis of atrazine and its metabolites in T. latifolia

Atrazine and its metabolites are mostly formed through biodegradation (Graymore et al., 2001). A reversed-phase high-performance liquid chromatography (RP-HPLC) method was used to determine [^{14}C]-atrazine and its [^{14}C]-metabolites in two different parts (roots and shoots) of the plant using radioisotope detection and liquid scintillation counting quantitation. Liquid chromatography was performed on a Spectra-Physics (Les Ulis, France) P4000 apparatus equipped with a Rheodyne model 7125 injector (Rheodyne, Cotati, CA). The flow rate was 1 mL.min^{-1} at 40°C. The column consisted of a C18 Bischoff reverse-phase column (Prontosil Eurobond, Interchim, Montluçon, France) (250 x 4.6 mm, 5 µm) coupled to a C18 guard precolumn (10 x 4.6 mm, 5 µm, Interchim). The column was equilibrated with 100% mobile phase A (water/acetonitrile/acetic acid, 95/5/0.001). Elution conditions were performed as follows: gradient from 100% at T= 0 to 100% mobile phase B (acetonitrile/acetic acid,

100/0.001) followed by isocratic phase of 10 min at 100% phase B. The HPLC system was connected for on-line radioactivity detection to a Packard Flo-One β A500 instrument (cell volume, 0.5 mL; scintillation cocktail ratio, 2 mL of scintillation liquid/ mL of HPLC effluent) (PerkinElmer Life and Analytical Sciences) using Flo-Scint II as the scintillation cocktail (PerkinElmer Life and Analytical Sciences) to establish the atrazine profile or to a Gilson model FC-204 fraction collector set at 2 fractions per minute (Gilson Medical Electronics, Middleton, WI) for atrazine and its metabolites identification. An aliquot of each fraction was counted after mixing with 2 ml in a scintillation counter to monitor radioactivity. In this condition, a run time of standard atrazine was 20.5 min.

II.B.6. CALCULATIONS AND DATA TREATMENTS

II.B.6.1 Enrichment coefficient and Translocation factor

The effectiveness of atrazine bioaccumulation in *T. latifolia* was evaluated by using ECs (enrichment coefficients) and TF (transfer factor) values as follows:

EC_R = ratio of the concentration of metal or pesticide in roots over the concentration of metal or pesticide in sediment (fraction< 63 μm only for the Cd experiment)

EC_L = ratio of the concentration of metal or pesticide in leaf over the concentration of metal or pesticide in sediment (fraction< 63 μm only for the Cd experiment)

TF = ratio of the concentration of metal or pesticide in leaf over the concentration of metal or pesticide in roots

II.B.6.2 Statistical analysis

Data collected from the 1st experiment

To evaluate statistically any significant difference between mean values among all experimental treatments at the beginning (at t=0) and at the end of the experiment (t=30), Cd concentrations in plant samples, enrichment factors (EC), transfer factors (TF), bioadvective (V) and biodifusive rates (Db), percentage of relative recovery, and cadmium fluxes between experimental compartments were firstly compared using one-way ANOVA. Prior to ANOVA tests, homoscedasticity and normality of the residues were checked with Bartlett and Shapiro-Wilk tests, respectively. Following ANOVAs, the mean values of each treatment were compared with each other using Tukey HSD's method. Dunnett test was also used for comparing data at t=30 days with initial values (t=0 day) used as references.

Secondly, the possible interactive effects of cadmium enrichment and tubificids on the bioaccumulation of *T. latifolia* and cadmium fluxes – F3 (from sediment to plant) were studied by using two-way ANOVA. Two-way ANOVAs were also performed on the average size of sediment particles separately for each sediment layer, to assess the impact of bioturbation and cadmium enrichment on sediment texture.

Three-way ANOVA was applied on the bioturbation bioadvective rates - V and the cadmium concentrations, and the cadmium quantities in the sediment samples to investigate for possible interactive influences of worm addition, cadmium enrichment, and plant presence/absence on bioturbation activities, cadmium behavior in sediment, and cadmium mass balance.

Data collected from the 2nd experiment

To evaluate statistically any significant difference among all experimental treatments (t = 26), the mean values of bioadvective rate (V); biodiffusive rate (Db), ^{14}C-∑(atrazine, metabolites) concentrations and quantities in water, sediment, plant samples; water content in the sediment; Kd, Koc, percentage of relative recovery, and percentage inhibitions of *C.vulgaris* at 72h were first compared using one-way ANOVA. Following ANOVAs, the mean values of the various treatments were compared with

each other using Tukey HSD test. Dunnett test was also used for comparing the total quantity of [^{14}C]-(atrazine, metabolites) in experimental compartments: water, fresh sediment, plant, and whole test-system at t=226 days with that at t= -15 days used as references.

To investigate the single and interactive effects of atrazine and tubificids on the bioadvective rates, the mean values in the treatments including plants (*i.e.* {Typ}, {Typ.Tub}, {Atr.Typ}, and {Atr.Typ.Tub}) were compared using two-way ANOVA. Another two-way ANOVA was conducted on all the atrazine-contaminated treatments (*i.e.* {Atr}, {Atr.Tub}, {Atr.Typ}, and {Atr.Typ.Tub}) to evaluate the single and interactive effects of plants and tubificids on bioadvective rates. Likewise, two-way ANOVA was also used to assess the influences of *T. latifolia* plants and worms on the changes of the quantity in water (ΔQ_1), sediment (ΔQ_2), microcosm (ΔQ), and percentage of relative recovery, respectively.

Student *t*-test was applied to the mean values of organic matter, organic carbon, C:N ratio, pH in the sedimentary layers, enrichment factors (EC), transfer factors (TF) in *T. latifolia* and [^{14}C]-(atrazine, metabolites) quantities in root and leaf parts of *T. latifolia* at t = 26 days to evaluate statistically significant differences between experiment treatments with and without worms: {+Tub} and {-Tub}.

Significance of the observed effects was assessed at the $p \leq 0.05$, 0.01, 0.001, and 0.0001 levels. All analyses were performed using the statistical software R, version 3.0.2 (2013-09-25), and statistical GraphPad Prism software, version 6.01.

II. C. ASSESSMENT THE EFFICIENCY OF THE COMBINED BIOREMEDIATION TOOLS BY USING TOXICOLOGICAL BIOASSAY

Phytoplankton communities have been considered to be indicators of the bioactivity of industrial wastes, and they vary in their response to a variety of toxicants

(Ma et al., 2002) as well as herbicide contaminations among the living aquatic microorganisms (Leboulanger et al., 2001). *Chlorella vulgaris* is green unicellular alga and a significant primary producer of the aquatic environment and has been widely used to evaluate the toxicity of chemicals present in water as a bioindicator of water pollution (Naessens et al., 2000; Shitanda et al., 2005). Herbicides can affect the structure and function of aquatic communities by altering the species composition of an algal community (Ma et al., 2002).

To assess the toxicity of the water in our experiments, the ecotoxicological method was applied by using phyotoplankton bioassay test under experimental conditions. Overlying water and sediment interstitial waters samples contaminated with atrazine, collected from the experimental microcosm, were undertaken using single species algal microplate bioassay. The tests were based on the use of exponentially growing cells of *Chlorella vulgaris* that were exposed for 72h in a 96-well microplate filled up with the of liquid solutions. The test procedure consists of three main steps as below (Fig. II.11):

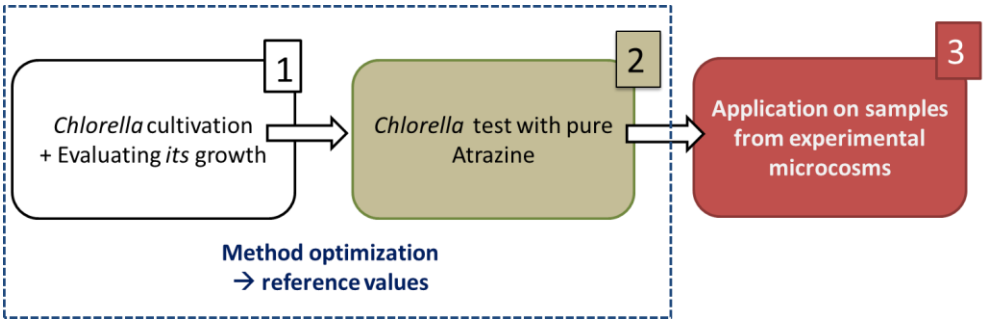


Figure II.11. Summary of the three steps procedure followd to make the ecotoxicity test using *Chlorella vulgaris*

II.C.1. MICROALGAE STRAIN AND CULTIVATION.

The green algae *Chlorella vulgaris* culture was used run in the thermostatic room ECOLAB in the University Paul Sabatier - 4R1 building). The microalgae were cultivated using CB nutrient medium and incubated under controlled experimental

conditions of temperature and light using the incubation chamber. Several previous tests were undergone in order to identify the best conditions to run the ecotoxicological tests and to allow their reproducibility.

II.C.2. MEDIUM

CB medium has the following composition (in milligrams per liter of deionized distilled water): $Ca(NO_3)_2.4H_2O$, 150; KNO_3, 100; $MgSO_4.7H_20$, 40; β - sodium glycerophosphate, 50; bicine, 500; biotin, 0.0001; vitamin B12, 0.0001; and thiamine hydrochloride, 0.01, with 3 ml of PIV metals. PIV metals consisted of the following (in milligrams per 100 ml of deionized distilled water): $FeCl_3$ $6H_20$, 19.6; $MnCl_2.4H_20$, 3.6; $ZnSO_4.7H_20$, 2.2; $CoCI_2.6H_20$, 0.4; $Na_2MoO_4.2H_20$, 0.25; and disodium EDTA $2H_20$, 100. The pH of the CB medium was adjusted to 9.0.

II.C.3. DETERMINATION OF CHLORELLA GROWTH CURVE FROM DIFFERENT GENERATIONS

To determine the best-cultivated age for the microalgae to be used for toxicity tests, *Chlorella vulgaris* solutions made with different culture generations (Table II.3) were tested in 96-well microplate at 5 different times during 96h (Fig. II.12). In order to determine the best cultivated old age the test microalgae for toxicity testing, the *Chlorella vulgaris* solutions at different generations (table II.4) were collected from the incubation chamber for their growth evaluation. These algae solutions were put into plastic 96-wells microplate under the same condition as in the incubation progress with controlled temperature, lighting, and nutrient regimes. The growth curve of the selected *Chlorella* solutions was determined via its density using Spectrophotometric method at 682nm (optimal wavelength for *Chlorella vulgaris*). The produce test includes: test format: 96-wells microplate, five replicates per each dilution; well volume content: 220µL (same volume as in a toxicity test afterward); growth determination: OD_{682nm} at

24h and 0, 24, 48, 72, and 96h. Due to high variability of initial OD_{682} values caused by different generations between *Chlorella* solutions, the dilution step of these 04 solutions by CB medium was performed to set a similar initial optical density for the growth evaluation starting from the beginning (t = 0h) (Table II.4).

Table II.4
Different generations of the tested *Chlorella vulgaris*

	Microalgae	Cultivated old age (days)	Code sample	Initial OD_{682}	OD_{682} at 0h after dilution
1	*Chlorella vulgaris*	55	Ch7	1.43 ± 0.01	0.18 ± 0.00
2	*Chlorella vulgaris*	41	Ch9	1.31 ± 0.03	0.17 ± 0.00
3	*Chlorella vulgaris*	25	Ch10	0.91 ± 0.04	0.15 ± 0.00
4	*Chlorella vulgaris*	6	Ch11	0.16 ± 0.00	0.00

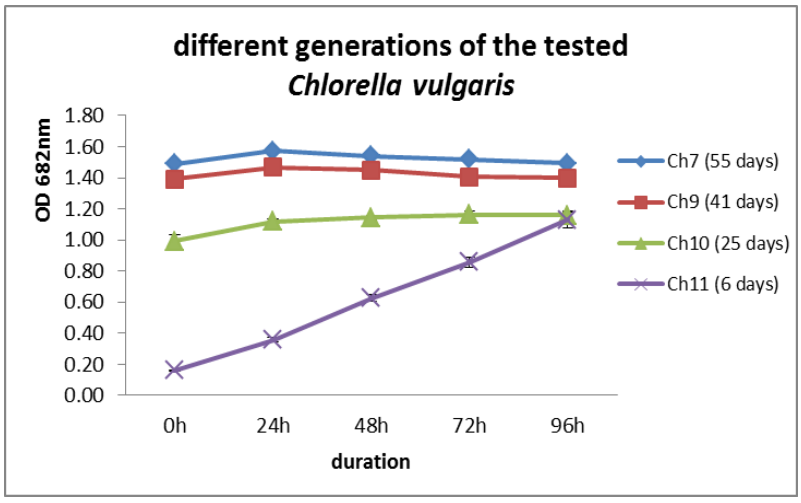


Figure II.12. Growth curves of *Chlorella vulgaris* at different generations

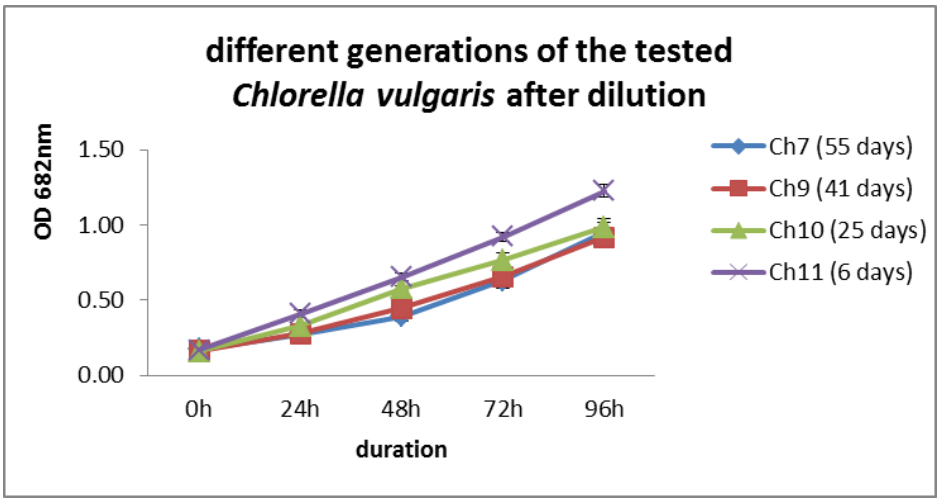


Figure II.13. Growth curves of *Chlorella vulgaris* at different generations after the dilution

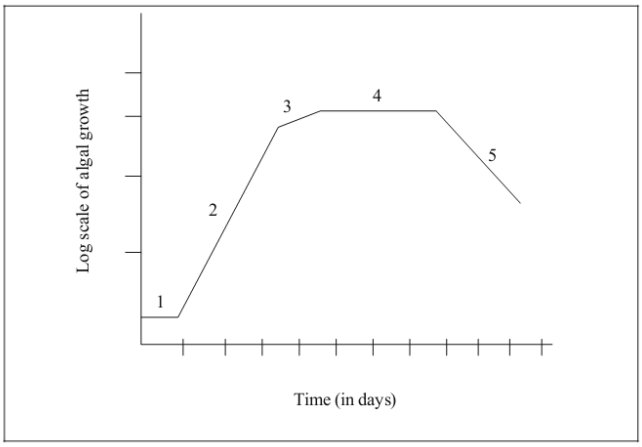


Figure II.14. Growth phases of algal cultures: Lag *(1)*, Exponential *(2)*, Declining growth rate *(3)*, Stationary *(4)*, Death *(5)*, according to Fogg (1965).

Fig.II.14 showed the theorethical growth of algae culture in different phases, under controlled conditions of light, temperature, and nutrients. After inoculation into growth medium with a fixed number of physiologically-active cells, population growth patterns normally include: *(1)* lag or introduction phase: little increase in cell density occurs; *(2)* exponential phase: cell density increase as a function of time; *(3)* phase of declining relative growth: Cell division slows down when nutrients, light, pH, carbon dioxide or other physical and chemical factors begin to limit growth; (4) stationary phase: balance of growth rate resulting in a constant cell density due to limit factors; *(5)*

death phase: depletion of nutrient and water quality leads to a rapid decrease in cell density.

Data in Fig.II.12 indicated that the youngest *Chlorella vulgaris* strain Ch11, 6 days of old age, continuously and steadily increased during 96h of the incubation. While the older *C. vulgaris* cultures, Ch10, Ch9, and Ch7 with their old ages of 25, 41, and 55 days, respectively, displayed a constant the balance or even decline of OD growth rate due to their state out of exponential growth phase (Fig.II.14).

As can be gleaned from Fig.II.13, different tested *Chlorella* straints were diluted to set up similar OD at the initial time (0h). Then the comparison of growth speed between these *Chlorella* strains during 96h of incubation provided more evidence that help in selecting the best one with the highest growth speed. The obtained result indicated that the youngest algal taken from 6 days old growth culture (Ch11), belonging to the exponential phase with the increase of optical density as a function time, also indicated the highest growth speed in comparison with the other older *C. vulgaris* cultures.

From our previous test, it was concluded that for the successful conduct of phytotoxicity tests, it was imperative to use as inoculum cells from algal stocks which are in exponential phase (4-8 days old cells). This ensures a shortened lag phase and optimal growth rates leading to cell densities that will allow adequate comparisons between control and sample-exposed growth at the end of the exposure period (Walsh, 1988).

II.C.4. *CHLORELLA* TEST WITH PURE ATRAZINE SOLUTION.

This series of test aims to optimize the protocol for the toxicity tests to find the best dilution with optimal sensibility range of testing, based on endpoint determination, for the real samples contaminated by atrazine.

Under ideal conditions, a phytotoxicity test should include 1) a concentration that will have no effect on algal growth (0% growth inhibition), 2) a concentration that

will induce an intense or total algistatic effect (90-100% growth inhibition), 3) two concentrations below a 50% growth effect and 4) two concentrations above a 50% growth effect (Blaise and Vasseur, 2005). However, since the toxicity of the (overlying and pore water) samples tested is unknown, selecting test concentrations for samples should start with the high concentration that could be found from the experiment. The highest concentration of the pure atrazine solution was therefore selected at 2500µg/l (the atrazine concentrations in overlying water and pore water samples at the initial time of the experiment were determined at 3600 and 1950 µg/l, respectively).

Preparing pure atrazine solution for testing

The testing stock atrazine solution was prepared by dissolving 2500 µg pure atrazine power with a little acetone (5ml) and then filled up by distilled water in 200ml. In the next step, 10ml of the stock solution was taken and filled up by DW in order to get a final concentration of 2500µg/l. The media of *C. vulgaris* were treated with various atrazine concentrations from 2500µg/l (T1) and then diluted to 1250 (T2), 500 (T3), 100 (T4), 75 (T5), 50 (T6), 25 (T7), 10(T8), 5 (T9) and 1 (T10) µg/l (*see protocol #1*). For running the test, 200 µL of each specific test solution concentration was introduced into a pre-defined well to which are also added 10 µL of nutrient spike and 10 µL of algal inoculum.

***Protocol #1**: Experimental configuration of a 96-well microplate for toxicological testing with pure atrazine solution*

ROW \ COLUMN	1	2	3	4	5	6	7	8	9	10
A	IC	IC	IC	IC	IC	NC1	NC1	NC1	NC1	NC1
B	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
C	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
D	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
E	NC2	NC2	NC2	NC2	NC2	PC	PC	PC	PC	PC
F	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
G	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
H	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10

Note:

- The assay was run with six replicates for each of the 10 tests concentrations with initial *Chlorella* and control wells located in rows A and E, respectively.
- IC - Initial *Chlorella*: 220 µL *Chlorella* (4-6 days cultivated old ages, before centrifugation)
- NC1 - Negative control 1: 200µL DW + 10µL CB 11X nutrient spike + 10µL *Chlorella* inoculum
- NC2- Negative control 2: 200µL DW having Aceton as used for atrazine preparation + 10µL CB 11X nutrient spike + 10µL *Chlorella* inoculum
- PC - Positive control: 200µL formaldehyde + 10µL CB 11X nutrient spike + 10µL *Chlorella* inoculum
- T1, T2, T3,...,T10 - Test solutions: 200µL atrazine solution with diffrent concentrations + 10µL CB 11X nutrient spike + 10µL *Chlorella* inoculum.

Preparation of the algal inoculum solution

The *Chlorella,* cultivated at 4-8 day old ages, was collected from growth culture for making the algal inoculum (withdraw 30 mL of the stock culture algae with a 10 mL pipette and dispense into 15 mL plastic centrifuge tubes). At the initial OD_{682nm} determined to be 0.197 ± 0.006, these stock solutions then were centrifuged at 2000 RMP, rotor 8-well for the tube of 15 mL in 20 min at 20°C, after which the supernatant is poured off and replaced with a few mL (5mL) of buffered water. Buffered water solution or water-bicarbonate solution (15mg/L $NaHCO_3$) is essentially used as a reagent during the centrifugation of algal cells in order to concentrate their numbers and to wash them to prepare an algal inoculum for testing. The capped tubes were

shaken vigorously by hand (or vortex) to resuspend the algae and determine the density of the solution after centrifugation. The most suitable wavelength to use for monitoring culture growth of *C. vulgaris* was fixed at 682 nm after scanning the absorption of wavelength ranging from 540 to 750 nm. The selected wavelength was close to the Kasai et al., (1993) that reported on the strong correlation between cell numbers and OD_{680nm}. Growth of algal cells was calculated indirectly using spectrophotometric data.

Note that the algal inoculum is prepared no more than 2-3 h prior to testing.

Endpoint determination

In the algal toxicity tests, the determination of the estimated sample concentration, at which a specified percent reduction in growth occurs compared to control algae, is a recommended endpoint. The inhibitor concentration - IC_{50}, represents the concentration of atrazine, at which *Chlorella*'s growth rate is reduced twofold – 50%. It was used as an endpoint (Blaise and Vasseur, 2005). In this study, percentage inhibition values, relative to growth or cell density of *C. vulgaris* in control systems, were calculated using spectrophotometric data (Fig. II.15).

Growth-inhibiting toxicity values as representative of 10 atrazine concentrations were used to plot a dose-response curve based on percent growth or cell density inhibition of *Chlorella* tested (y-axis) versus test atrazine concentration (x-axis). The GraphPad Prism software, version 6.01, was used to determine the endpoint (IC_{50}).

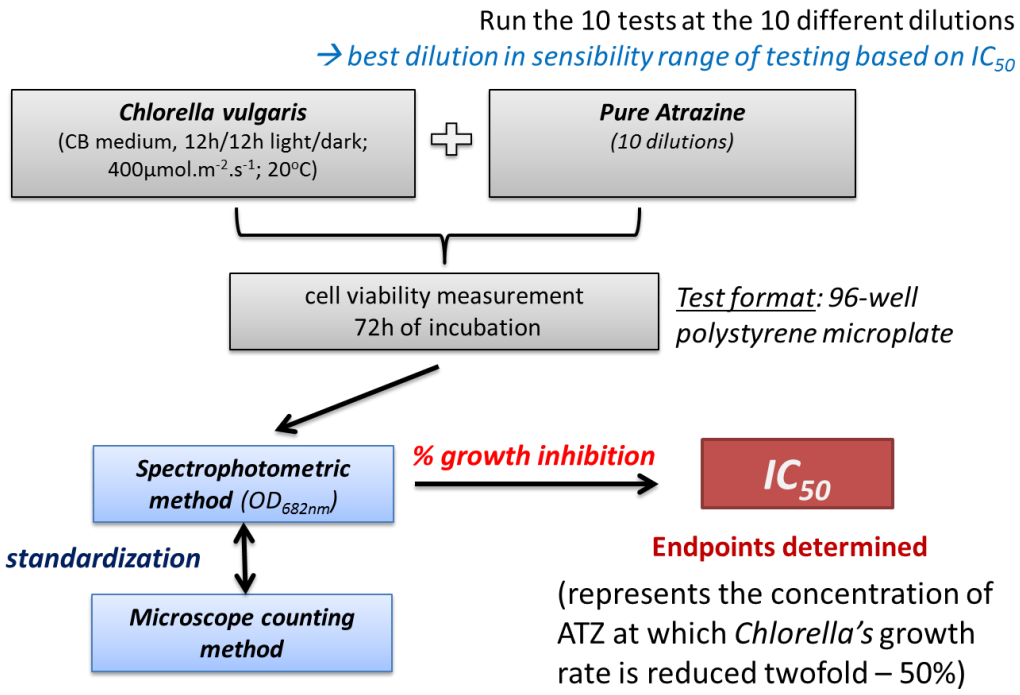


Figure II.15. The test procedure of *Chlorella* with pure atrazine solution

The test system makes use of exponentially growing cells the *Chlorella* that is exposed for 72 h in a 96-well microplate to varying concentrations of atrazine solutions under the same condition as in the incubation progress with controlled experimental conditions of temperature and light. The experimental configuration included control wells with different variants; 10 serial dilutions of the atrazine test solution, each with five replicates (*see Protocol #1*). *Chlorella*'s growth was measured after 72h by spectrophotometric measurement at 682nm (optimal wavelength of *Chlorella vulgaris*). The reference toxicant (positive control) was a formaldehyde solution.

Figure II.17 showed the effect of atrazine (pure solutions) in different concentrations starting from the lowest concentration (1µg/L) that has no effect on algal growth. A similar value of Delta OD_{682nm} (0.266) was recognized at the lowest concentration of atrazine (1µg/L) when compared to the control samples having DW or DW and acetone (OD_{682nm} = 0.273 and 0.267, respectively). These concentrations have not given any effect on the algal growth, with a mean value of 0.38% of growth inhibition (Fig.II.17). While, the highest concentration of atrazine at 2500 µg/L induced an intense

or total algistatic effect (the property of inhibition algal growth) to *C. vulgaris*, with Delta OD_{682nm} of 0.036 (Fig. II.16) and the growth inhibition of 74.47% (Fig.II.17). The concentrations of 500, 100, 75, and 50 µg/L induced effects that below 50% with the growth inhibition values of 43.48, 43.7, 36.23, and 32.20, respectively.

Percent growth inhibition (Blaise and Vasseur, 2005)

$$\mathbf{I = [(OD_c - OD) \div OD_c] \times 100}$$

where:

I = the percent inhibition in algal growth

OD_c = the OD_{682nm} for control algae after 72h

OD = the OD_{682nm} for each testing sample after 72h

Toxicological test of Chlorella vulgaris on pure atrazine solutions

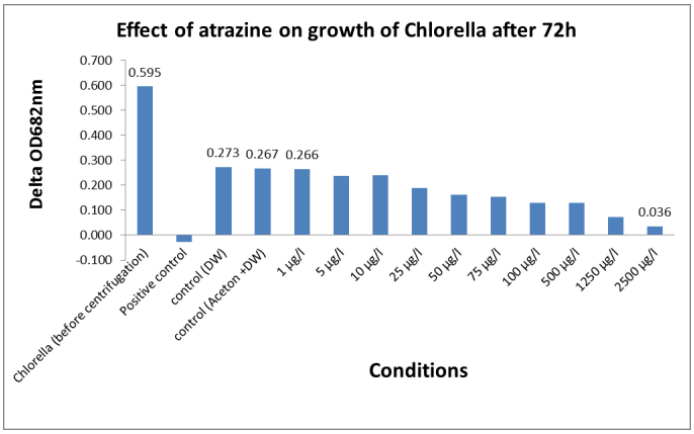


Figure II.16. Growth curves estimated by Delta OD 682nm of *Chlorella vulgaris* treated by atrazine solutions. Delta OD682 is the difference of DO between initial time and after 72 h.

The dose-response curve obtained for the 72-h duration of the inhibitor atrazine is presented in Fig.II.17. For *C.vulgaris,* the atrazine dose-response curve provided the IC_{50} of 60.41 µg/L with 95% confidence intervals ranging from 59.27 to 70.68 µg/L, according to the Graphpad' calculation. The EC_{50} of *C.vulgaris* was found to be at 42 µg/L (Leboulanger et al., 2001) when the algae were exposed to atrazine, which is consistent with our finding. The concepts of IC_{50} and EC_{50} are fundamental to pharmacology. IC_{50} is used in case there is an essay in which the inhibitor generates a

decline by half in activity of the response. The EC_{50} is the effective concentration of a drug that gives half-maximal response, and it is used for dose-response curves that go up hill. In some cases, it is also used for a curve that goes downhill. While IC: means inhibitory concentration, so is used for dose-response curves that go downhill, because the drug inhibits a response.

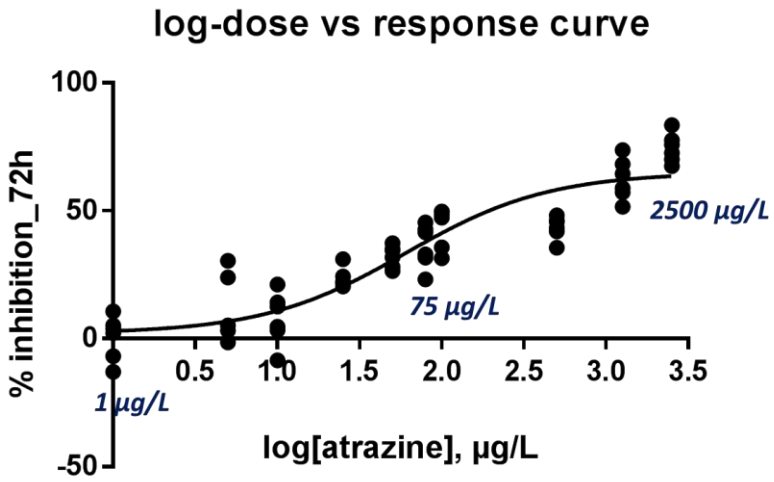


Figure II.17. Dose–response curve for tested *C.vulgaris* and atrazine as an inhibitor after 72h (mean of % inhibition ± SD, n=6). The values for this curve were obtained from the GraphPad Prism software (version 6.01) with the endpoint (IC_{50})

The obtained results were consistent with the previous studies on the toxicity of Atrazine toward *Chlorella vulgaris* as well as useful for validating the toxicological method for further tests with the real water samples or pore water (collected from sediment) contaminated by atrazine

II.C.5. APPLICATION ON SAMPLES FROM EXPERIMENTAL MICROCOSMS.

After collecting sediment samples by coring for bioturbation and pesticide concentration measurements, the rest of the sediment in each experimental pail (Fig.II.9) were used for ecotoxicology test with *Chlorella vulgaris* species using the spectrophotometric method. Overlying water and sediment samples were collected at

the end of the experiment. The collected sediment samples were centrifuged at 5000 rmp, 10 minutes for collecting pore water. The filtration step (by filter paper - 0.22 µm) was carried out to completely remove the sediment particles in both the overlying and pore water samples. These prepared samples were used for the ecotoxicology test using the same test procedure as in the test of pure atrazine on 96-wells microplates (Fig. II.18).

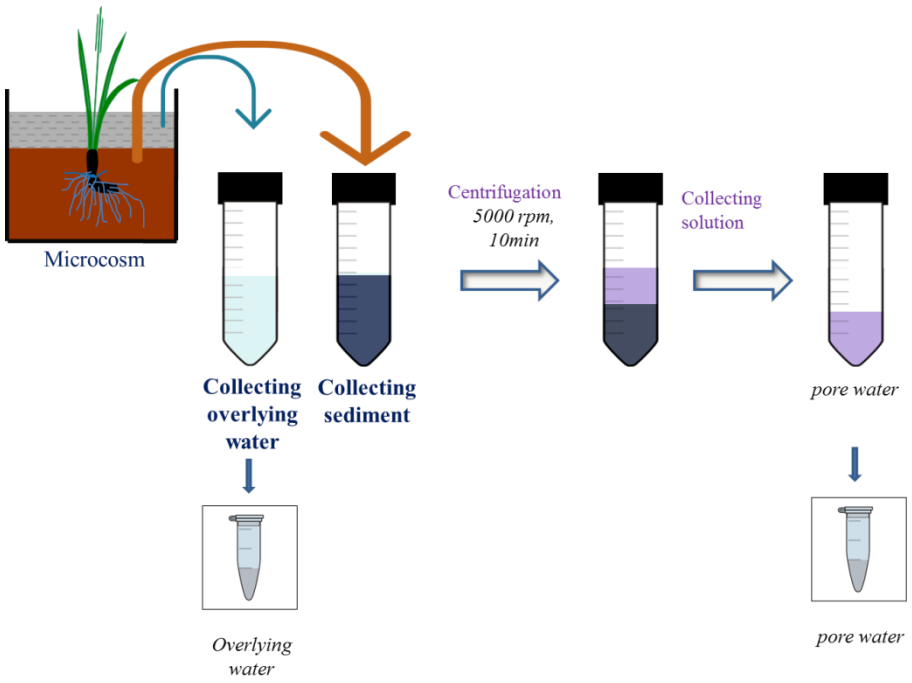


Figure II.18. Sampling and processing of overlying water and sediment interstitial waters samples from the 2nd experiment. The number of replicate per each sample was 04 and controlled sampled were run simultaneously.

For running the test, 200 µL of each test solution (overlying water or sediment interstitial water) was introduced into a pre-defined well to which are also added 10 µL of nutrient spike and 10 µL of algal inoculum (*see protocol #2*).

***Protocol# 2**: Chlorella testing with the real samples*

ROW \ COLUMN	1	2	3	4	5	6	7	8	9	10	11	12
A	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
B	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
C	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
D	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
E	NC	NC	NC	NC	NC	NC	PC	PC	PC	PC	PC	PC
F	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
G	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
H	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS

- Algal inoculum (by centrifugation): 10µL *Chlorella vulgaris*
- NC - Negative control : 200µL DW + 10µL CB medium 11X + 10µL *Chlorella*
- PC - Positive control: 200µL formaldehyde + 10µL CB medium 11X + 10µL *Chlorella*
- TS - Test sample: 200µL testing sample + 10µL CB medium 11X + 10µL *Chlorella*

PART III. RESULTS AND DISCUSSION

This part presents and discusses the results obtained from the two experiments mimicking *in situ* wetlands ecosystems. By focusing on this type of ecosystems, the objective was to use the biodiversity of this habitat to question the efficiency of the innovative strategy based on the combination of bioturbation and phytoremediation process. This strategy efficiency was tested with two different types of pollutants: one type of heavy metal (cadmium, 1st experiment), also used in these researches as a model of conservative pollutant to be able to quantify the fluxes in our experimental systems, and secondly, one micro-organic pollutant (atrazine, 2nd experiment) as a non-conservative contaminants. Fluxes of two types of pollutant were followed between the different compartments of the experimental system under the biological and physical processes. In addition, it was expected that the micro-organic pollutant might undergo biodegradation process during the period of the experiment. In order to precisely measure the contaminant fluxes of atrazine and its metabolites in this framework, a radiolabelled atrazine was used in the second experiment.

The first chapter III.A is devoted to the study of bioturbation and phytoremediation combined effects on the removal of cadmium from the water and sediment, with pollutant initially introduced in the overlying water of our experiment microcosm. This metal is representative of urban hazard that bioaccumulates in *T. latifolia* as a function of time (during 30 days of the 1st experiment).

The second chapter III.B shows the results and discusses the influence of the bioturbation on the efficiency of the phytoremediation of the radiolabeled-[^{14}C] atrazine initially introduced in the sediment compartment. This contamination was homogenized in the whole sediment column previous to the starting of the experiment.

The third chapter III.C, is devoted to the comparison of the mitigation efficiencies measured in the second experiment with these two types of pollutants (metal - conservative and herbicide – non conservative) via their mass balance calculation

(based on quantities and fluxes) of contaminants in the four different experimental compartments (overlying water, sediment, plant, and worms).

CHAPTER III.A. INFLUENCE OF COMBINED BIOTURBATION AND PHYTOREMEDIATION ON CONSERVATIVE POLLUTANT (CADMIUM AS AN EXAMPLE) IN AQUATIC SEDIMENT

The conveyor belt species of invertebrates *Oligochaeta tubificidae*, are known as an active ecological engineer to be able to generate continuous vertical flows of matter between water-sediment interfaces. This bioturbation is viewed as a source for contaminants burial, via biotransport. Bioturbation mechanisms on metal bioavailability in the water-sediment condition are well described previously. Cadmium is expected to be transferred into the depth of sediment via bioadvection by tubificid worms. Our study starts from the hypothesis that bioturbation favors the burial and bioaccessibility of cadmium in aquatic sediment for the plant, thus optimizes their bioaccumulation in the plant.

This chapter, therefore, examines the influence of combined bioturbation and phytoremediation on cadmium removal in aquatic sediment. A population of the tubificid worms was used as a bioturbation source. Phytoremediation was carried out by a riparian plant species, *Typha latifolia* is known for its ability to accumulate the metal in their roots system from sediment. Cadmium, a conservative pollutant of an urban hazard was initially introduced in overlying water of microcosms reproducing a portion of water/sediment as in wetland condition. Principal results presented in this chapter highlighted the role of plant and invertebrate of the observed fluxes in the microcosms.

By comparison Cd concentration bioaccumulated in *T. latifolia* as a function of time (30 days of the 1st experiment) with and without bioturbation, the first part (III.A.1) purposes to estimate the putative increase of phytoremediation potential under sediment bioturbation. In the second part (III.A.2), calculation of cadmium quantity in each experimental treatment makes it possible to estimate mass balance and fluxes

through both major interfaces water/sediment and sediment/plant. From the estimation, the change in flows under the effect of the plant and invertebrate will be highlighted. Toxic effect of cadmium on a population of tubificid worms as well as the possibility to apply the bioturbation to remediate other metals will be discussed in the last part of this chapter (III.A.3).

III.A.1. BIOTURBATION EFFECT ON *T. LATIFOLA*'S BIOACCUMULATION RATES

The results of this study were published to the Journal of *Science of the Total Environment - 618 (2018) 1284–1297; https://doi.org/10.1016/j.scitotenv.2017.09.237):* "**Bioturbation effects on bioaccumulation of cadmium in the wetland plant *Typha latifolia*: A nature-based experiment**". Trung Kien Hoang, Anne Probst, Didier Organe, Franck Gilbert, Arnaud Elger, Jean Kallerhoff, Francois Laurent, Sabina Bassil, Thi Thuy Duong, and Magali Gerino.

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Bioturbation effects on bioaccumulation of cadmium in the wetland plant *Typha latifolia:* A nature-based experiment

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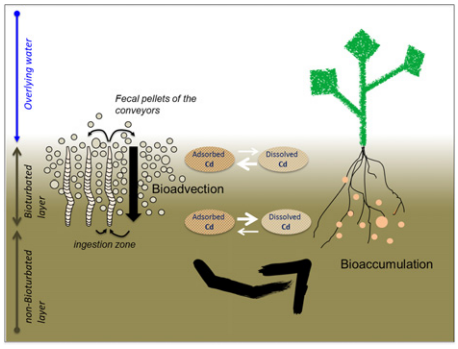
HIGHLIGHTS

- A nature-based solution for metal bioremediation in sediments is proposed.
- Ecological engineering relies on combined bioturbation and phytoremediation.
- Tubificids are transporting Cd downward in sediments surrounding the plant roots.
- Cd bioaccumulation increases in *T.latifolia* roots with bioturbation.
- Combining Cd contamination and worm bioturbation optimizes Roots Enrichment (EC_R).

GRAPHICAL ABSTRACT



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ABSTRACT

The development of efficient bioremediation techniques to reduce aquatic pollutant load in natural sediment is one of the current challenges in ecological engineering. A nature-based solution for metal bioremediation is proposed through a combination of bioturbation and phytoremediation processes in experimental indoor microcosms. The invertebrates *Tubifex tubifex* (Oligochaeta Tubificidae) was used as an active ecological engineer for bioturbation enhancement. The riparian plant species *Typha latifolia* was selected for its efficiency in phyto-accumulating pollutants from sediment.

Phytoremediation efficiency was estimated by using cadmium as a conservative pollutant known to bio-accumulate in plants, and initially introduced in the overlying water (20 μg Cd/L of cadmium nitrate – $Cd(NO_3)_2 \cdot 4H_2O$). Biological sediment reworking by invertebrates' activity was quantified using luminophores (inert particulates).

Our results showed that bioturbation caused by tubificid worms' activity followed the bio-conveying transport model with a downward vertical velocity (V) of luminophores ranging from 16.7 ± 4.5 to 18.5 ± 3.9 cm·year^{-1}. The biotransport changed the granulometric properties of the surface sediments, and this natural process was still efficient under cadmium contamination. The highest value of Cd enrichment coefficient for plant roots was observed in subsurface sediment layer (below 1 cm to 5 cm depth) with tubificids addition.

We demonstrated that biotransport changed the distribution of cadmium across the sediment column as well as it enhanced the pumping of this metal from the surface to the anoxic sediment layers, thereby increasing the

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bioaccumulation of cadmium in the root system of *Typha latifolia*. This therefore highlights the potential of bioturbation as a tool to be considered in future as integrated bioremediation strategies of metallic polluted sediment in aquatic ecosystems.

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1. Introduction

The accumulation of contaminants at the water-sediment interface, in particular for potentially harmful elements (PHEs), is raising concerns (Guo et al., 2012; Horowitz et al., 1999; Schäfer et al., 2009). Metals and metalloids such as arsenic, mercury, lead, or cadmium are the most common toxic chemicals involved in soil and water contamination and result in serious health problem to humans (Simmons et al., 2005; Teeyakasem et al., 2007). These metals are not degradable and accumulate in aquatic sediment or soils as very highly persistent compounds (Kermani et al., 2010). Among those, cadmium is a widespread trace element that is extremely toxic to living organisms, such as aquatic animals (Bur et al., 2010; Nair et al., 2013) and most of the plant species (Barceló and Poschenrieder, 2003; Liu et al., 2005). Human populations are exposed to Cd via drinking water and contaminated food (Byrne et al., 2009; EFSA, 2012), resulting in adverse health effects, such as neurologic, metabolic, carcinogenic and immunological disorders (Breton et al., 2013; Go et al., 2014). A European citizen absorbs cadmium orally between 0.29 and 1.17 $\mu g \cdot kg^{-1} \cdot day^{-1}$ (EFSA, 2012).

The natural abundance of Cd in the terrestrial crust is 0.11 mg/kg (Kabata-Pendias, 2011). Indeed, Cd is frequently found in river bed sediment, which acts as a final sink of natural and anthopogenic sources (N'guessan et al., 2009). The tendency of using Cd is on the rise in electroplating, coating, plastic and battery manufacturing industry. Cadmium contamination was also evidenced in some reservoirs that provide a major fresh water source for domestic use, e.g. in Milluni Grande lake, the main reservoir of water supply for La Paz, where Cd content reaches >30 times the WMO guideline value (Salvarredy-Aranguren et al., 2008). In France, such contamination occurs as a consequence of leaching from mining exploration: daily fluxes of total Cd range from 0.26 to 966 kg/day in the Lot River (a tributary of the Garonne River) and from 0.31 to 1360 kg/day in the downstream Garonne River itself (Audry et al., 2004).

Faced to such metal accumulations and toxicity risks, several methods are already being used to clean up the environment from such environmental contaminants. But most of them are costly and far away from their optimal performance. In parallel, the nature-based solutions (Eggermont et al., 2015) inspired strategies for pollution bioremediation and water resource sustainability. The ecological processes of phytoremediation and bioturbation were studied independently up-to-now. They are both known to be efficient within ecological engineering to promote pollutant fluxes in aquatic sediment. Although the influence of bioturbating mechanisms on metal bioavailability in the water-sediment conditions is now relatively well understood, phytoremediation applications coupled with bioturbation have not yet been investigated for pollutant removal strategies.

The application of knowledge of nature-based solutions might inspire future options for pollution attenuation (Eggermont et al., 2015). Up to now, few solutions are recognized within the international scientific community. Among them, phytoremediation is highlighted as an efficient and sustainable way to stabilize and/or remove commonly encountered pollutants occurring in the environment (EPA, 2012). Phytoremediation is a method using plants to remove contaminants, such as metals, from soils and sediment without destroying soil structure (Ghosh and Singh, 2005; Zhang et al., 2010). Phytoremediation was demonstrated to be efficient in removing metals such as lead, cadmium, copper, arsenic, uranium from contaminated waters (Favas et al., 2014; Lyubenova and Schröder, 2011), sediment (Klink et al., 2013; Pandey et al., 2014; Sasmaz et al., 2008; Weis and Weis, 2004) or soils (Leveque et al., 2013) by accumulation in plant organs. It is a cost-effective, efficient, friendly-environmentally, and in-situ applicable remediation strategy for environmental restoration (Barceló and Poschenrieder, 2003; LeDuc and Terry, 2005; Sinha et al., 2009). The mitigation of metal contamination by plants through phytoremediation process involves the mechanisms of phytoextraction, phytostabilisation, rhizofiltration, and phytovolatilization (Prasad, and Freitas, H. M. de O., 2003; Vangronsveld et al., 2009). Up to now, >450 existing species of "metal hyperaccumulator" plants have been discovered in the world (Ghosh and Singh, 2005; Prasad, and Freitas, H. M. de O., 2003). Their efficiency depends on many factors, in which availability and mobility of PHEs are very important factors contributing to the success of the phytoremediation technology (Antoniadis et al., 2017; Felix, 1997). Broadleaf cattail, or *Typha latifolia,* is one of the more common wetland plant species for phytoremediation application, with a large distribution under various hydrological conditions, both flooded and non-flooded (Aulio, 2015) and under various climates in temperate, tropical, humid coastal and dry continental ones (Pandey et al., 2014). *Typha latifolia* reveals a rapid growth and resistant characteristics in polluted sites with a high capacity for metal uptake into its organs, particular in the root system. The root system can be used as a bio-indicator or bio-accumulator for waters and sediment polluted by metals, such as Zn, Ni, Cu, Pb, Co, Mn and Cd (Klink et al., 2013; Sasmaz et al., 2008). However, although non-essential for the growth of plants (Williams et al., 2000), Cd is well uptaken by roots and translocated into leaves of many plant hyperaccumulators, without displaying harmful effects to growth and development (Manousaki et al., 2008).

Bioturbation is driven by the activities of invertebrates in the sediment. It is a natural process defined as: "all transport processes carried out by animals that directly or indirectly affect sediment matrices" (Kristensen et al., 2012). These processes include particle reworking and burrow ventilation, so that bioturbation is the source of significant changes in biological and physico-chemical properties of soils and sediment (Baranov et al., 2016; Kristensen et al., 2012; Peterson et al., 1996), as well as several types of biotransports (Gerino et al., 2003). Consequently, bioturbation generates material fluxes via biotransportation of sediment and associated contaminants through the water sediment interface and inside the inhabited layer that are estimated to double the incorporation of these contaminants into the sediment (Anschutz et al., 2012; Ciutat et al., 2007; Ciutat et al., 2006; Delmotte et al., 2007; Gerino et al., 2014; Hölker et al., 2015; Kristensen et al., 2012; Teal et al., 2013). One specific type of bioturbation called bioadvection is produced by conveyors-belt species like tubificids that feed at sediment depth and defecate at the sediment surface. This bioadvection is a downward advection of the water sediment interface when the tubificids population, as head-down deposit feeders, feed at depth in the sediment and egest faecal pellets at the sediment surface. This biotranport modifies the distribution of fine particles into the sediment column that increases in the surface layer where feacal pellets accumulate (Ciutat et al., 2006). The dimension of the worms' prostomium limits the sediment particle size that can be ingested and this will thereafter be transported to the surface by the turbificid guts (Anschutz et al., 2012; Ciutat et al., 2006). Besides their survival in metal contaminated soils, some invertebrate animals (earthworms) can accumulate metals, such as Cd, Cu, Zn, and Pb, in their tissues

(Morgan et al., 1989). The geochemical changes in bioturbated sediment are able to affect the behaviour of metals (Gilbert et al., 2007). For instance, the changes in redox potential, pH, OM, and porosity under bioturbation effects can influence the sorbtion capacity of the pollutant on the sediment particles and thus change the solid/liquid partition equilibrium of metals, e.g. Zn and Cd (Cheng and Wong, 2002; Rinklebe et al., 2016; Shaheen et al., 2016; Yu et al., 2005). Via its influence on phisicochemical properties of sediment, bioturbation shifts the metal binding affinities between the solid and dissolved phases and can also significantly modify the speciation and bioavailability of most metals in sediment (Remaili et al., 2015). Therefore combining the bioadvection effect with the Cd source in the overlying water, results in doubling the pollutant loading in sediment due to the bioturbation effect (Ciutat et al., 2005a, 2006, 2007). This should alows metal transfer and availability into the sediment surrounded by the plant's root system (Yu et al., 2005), and therefore bioturbation, by tubificids in the presence of cadmium, might simultaneously favor metal uptake by plants. The experiment described in this paper was perfomed in order to test whether bioturbation could be a source of improvement of the efficiency of phytoextraction.

Our research aimed to give evidence of the potential of combined bioturbation and phytoremediation for the remediation of cadmium-contaminated sediment by mimicking field patterns of aquatic systems contamination from the water during a flood event. Our experimental study involves these two methods to enhance cadmium transfer from water into sediment and from sediment into plant organs. Using a controlled-environment experiment, the objectives were: (i) to evaluate the putative increase of phytoremediation potential under sediment bioturbation with tubificid's conveyors, (ii) to estimate the metal bioaccumulation dynamic by a riparian plant coupled to tubificid worms' activity.

2. Materials and methods

2.1. Sampling and processing of sediment and plants

Sediment samples and *Typha latifolia* species were collected from the Aussonnelle River catchment, a first rank tributary of the Garonne River (Sélery area, Colomiers, France) in January 2013, 60 days before starting the experiment. The sediment was firstly defaunated by incubating for 40 days at 40 °C into closed tanks in order to generate anaerobic conditions in the sediment, and secondly by a mechanical mixing process of the sediment using a motor-mixer to both mechanically kill the last alive macrofauna and homogenize the sediment. The defaunated sediment was then introduced into each microcosm (a pail of 13 L-volume, see Fig. 1). Each microcosm roughly consisted of a sediment layer of 9 cm depth (approximately 7.3 kg of sediment) and of a water column of 8 cm height of de-chlorinated tap water (approximately 4.8 L of overlying water) (Fig. 1).

After collecting *T. latifolia* from the field, individual plants were stored in a thermostatic experimental room for acclimatation during 60 days until the start of the experiment. Healthy plants showing no signs of rotting and having approximately the same height were selected for experiments. One individual was taken and prepared for analyses in order to have the reference information of metal content. Just before starting the experiment, the roots and rhizomes of the other *T. latifolia* plants were carefully cleaned up with tap water to completely remove field non-manipulated sediment and then carefully cut to achieve, as far as possible, a homogeneous biomass among the plants. From these plants, three were selected randomly (at $t = 0$) for an initial metal analysis. The remaining were planted into microcosms (one by bucket) and collected on the last day of the experiment ($t = 30$).

2.2. Experimental design and microcosm setup

A full-factorial design was used, crossing 3 tested factors: Cd concentration (Cd-enriched or not), plant influence (presence/absence of *T. latifolia*), and macrofauna influence (presence/absence of tubificid worms) (see Table 1). All possible combinations of the different levels of each factor were thus tested and distributed into the different microcosms (Fig. 1). Three replicates per combination were performed. The experimental units were distributed in four blocks each including one replicate of each combination of the 3 tested factors. The position of the microcosms within each block was randomly determined. Tap water was supplemented in each microcosm every few days (approximately 3% of the total water volume per day) to compensate for evaporation and to keep the water level constant. Microcosms were set-up in a thermostatic experimental room with homogeneous physico-chemical conditions (T°C, light, sediment, water): light provided by 3 electric bulbs (400 W each), 12 h of light each day, room temperature at 18 °C, photon fluence rate (the unit concerning the light in the plant production) in the experimental room was 18.1 ± 2.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the surface of the overlying water and 28.0 ± 2.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the top of *T. latifolia* plants.

The tubificid worms used were provided by the GREBIL Company (Arry, France). In each microcosm with tubificids, approximately 800 worms were introduced at the surface of the sediment corresponding to approximately 135 worms $\cdot dm^{-2}$, with a fresh biomass of 17.8 ± 3.1 g per microcosm. Two weeks after worms introduction, cadmium provided as cadmium nitrate ($Cd(NO_3)_2 \cdot 4H_2O$), with an initial contamination level of 20 μg Cd/L was gently added to the overlying water. This concentration was selected from the previous experiment with

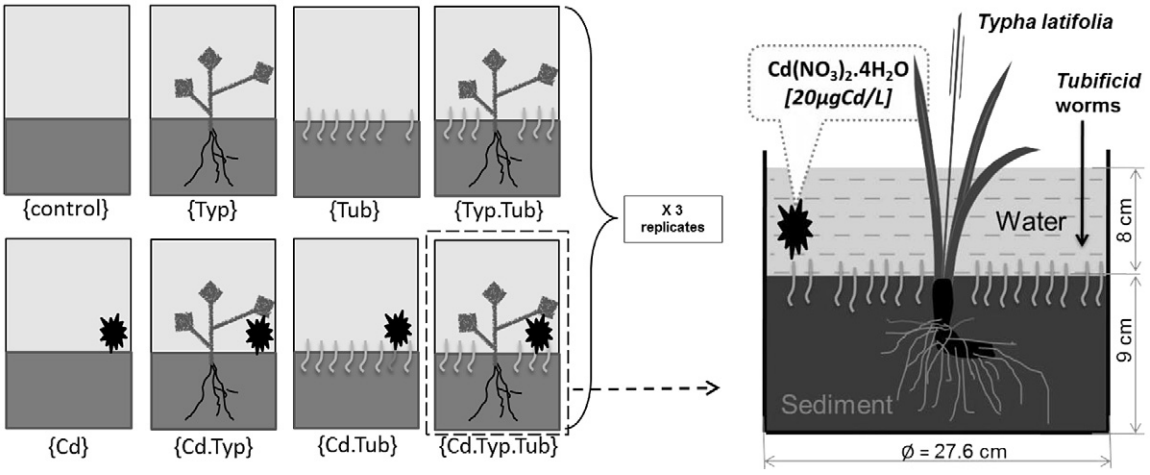

Fig. 1. Microcosm experimental design (see Table 1 for acronym definitions corresponding to the different treatments).

Table 1
Main acronyms for different experimental treatments used in the experiment.

Treatments noted	Time (day)	Description
Field	t = −60	Samples collected from the field (before starting experiment)
Initial	t = 0	Initial samples (at the beginning of experiment)
{Control}	t = 30	Control treatment (no plant, no metal, no invertebrates)
{Typ}	t = 30	No metal, with plant, no invertebrates
{Tub}	t = 30	No metal, no plant, with invertebrates
{Typ.Tub}	t = 30	No metal, with plant, with invertebrates
{Cd}	t = 30	With metal, no plant, no invertebrates
{Cd.Typ}	t = 30	With metal, with plant, no invertebrates
{Cd.Tub}	t = 30	With metal, no plant, with invertebrates
{Cd.Typ.Tub}	t = 30	With metal, with plant, with invertebrates

Typ: *T. latifolia* plant; Cd: cadmium; Tub: tubificid invertebrates.

cadmium and tubificid worms (Ciutat et al., 2005b) considering that this concentration is frequent in contaminated waters by mining wastes (Salvarredy-Aranguren et al., 2008) or industrial sites (Andres et al., 1999). In order to avoid any bias resulting from nitrate addition in the metal-treated pails, a neutral salt in form of calcium nitrate $Ca(NO_3)_2 \cdot 4H_2O$, with the same nitrate concentration (20 μg Ca/L), was added in microcosms not contaminated by cadmium.

The experiment lasted 30 days after the introduction of the contaminant in the overlying water on the first day (t = 0). This is the time which was considered needed to allow: (i) plant growth, (ii) bioturbation, and (iii) metal transfer within each compartment as well as their bioaccumulation in the plant system, without having extra-environmental disturbances. Water, plants, and sediment were sampled on the last day (t = 30).

2.3. Bioturbation activity measurements

The bioturbation activity of tubificids was quantified using the fluorescent inert tracers technique (Gerino et al., 1994; Lagauzère et al., 2011). Luminophores are natural sand particles coated with pink fluorescent paint with size ranging from 63 to 125 μm (Partrac Ltd., UK). Twenty-four hours after worms introduction (the time needed for the worms to restaure from handling), luminophores were homogeneously deposited at the sediment surface in the form of a 3-mm-thick frozen mud cake (a mixture of 30 g of the same sediment with 4 g of the tracers). At the end of the experiment (t = 30 days), the sediment was cut into twelve layers (0.5-cm thick layers from the surface down to 3 cm depth, then 1-cm thick layers down to 9 cm depth) in order to assess the vertical distribution of luminophores in the sediment column. Each layer of fresh sediment was homogenized before sub-sampling. Detection of luminophores was performed with a Synergy Microplate reader (Biotek, USA) according to a protocol adapted from Lagauzère et al., 2011. Fluorescence reading was used with 565 nm as excitation and 602 nm as emission wavelength. After estimation of the luminophore concentrations along depth in the sediment column, the bioadvection–biodiffusion model (Gerino et al., 1994; Officer and Lynch, 1982) were applied to fit the measured tracer profiles:

$$\frac{\delta C}{\delta t} = Db\frac{\delta^2 C}{\delta z^2} - V\frac{\delta C}{\delta z} \quad (1)$$

with t as the time, *z* as the depth, and *C* as the tracer concentration.

This model allows to estimate the theoretical concentration of tracers under the effects of the two biotransports parameters: V ($cm \cdot y^{-1}$) as the bioadvective velocity or bio-sedimentation rate that represents the downward transfert of the tracers and Db ($cm^2 \cdot y^{-1}$) as the biodiffusion coefficient that reflects the dispersion rate of the tracers in omnidirectional directions. This model was first developped by Officer and Lynch (1982) and then used for describing marine (Gerino et al., 1994) and freshwater bioturbation (Delmotte et al., 2007). Estimation of bioadvection and biodiffusion rates in the experiment was performed by fitting the theoretical concentrations obtained with the bioturbation model to the measured luminophore concentrations with a least-squares procedure for each microcosm.

2.4. Cadmium measurements

2.4.1. Water, sediment, and plant sampling and pre-treatment for Cd analysis

The overlying water was sampled at the beginning (t = 0) and the end (t = 30) of the experiment. In order to determine dissolved cadmium concentration in water, 40 mL from each sample was filtered with a mesh of standard pore diameter of 0.22 μm. Solutions were acidified using a 65% ultra pure HNO_3 solution to maintain metal in solution and then kept in cool and dark conditions (5 °C) before the measurements. All materials used (filtration devices, pipettes, box filters, etc.) were plastic made to avoid any metal contamination of samples.

The sediment was sampled three times during the experiment, after the defaunation process: initial samples (t = −60), at the beginning (t = 0) before adding contaminant into the overlying water, and on the last day (t = 30). For the samples at t = 30, the sediment column was carefully removed from the microcosm and then cut into two layers (L1 and L2) connected to the bioturbation process by worms: L1 (0–1 cm) corresponds to a surface zone composed entirely of feacal pellets ejected and L2 (1–5 cm) corresponds to an ingested area of particles (Ciutat et al., 2006). All sediment samples were kept in plastic bottles and stored frozen.

The sediment from the bottles were then spread into plastic Petri dishes and dried in an oven at 40 °C, with smooth manipulation (without crushing minerals, sand, gravel, etc.) during drying to avoid aggregation. Once the sediment was dry, and the main organic debris removed with clean plastic tweezers, they were sieved through a plastic mesh sieve to get the fraction <63 μm (accounting for 26.3 ± 1.7% of the total fraction), subsequently used for Cd analysis. This fraction is supposed to be the most metal enriched and available for plants (Förstner and Solomons, 1980; Probst et al., 2003).

The *Typha latifolia*, collected before the experiment (field sample, at t = −60) at the initial (t = 0) and at the end of the experiment (t = 30), were cut into the following parts: the above part (stem and leaves), the rhizome, and the roots. Prior to analysis, plant materials were carefully cleaned with distilled water and cut to a constant weight between samples. To ensure the efficiency of the dissolution procedure of the different plant compartments before analysis, they were carefully cut into pieces using a ceramic knife and then ground in liquid nitrogen using a pestle and mortar.

2.4.2. Solid sample dissolution procedure and Cd analysis

The sediment fraction (< 63 μm) used for ICP-MS analysis was dissolved by a well-calibrated procedure in the EcoLab cleanroom (ISO 7) (N'guessan et al., 2009). Approximately 100 mg of homogenized plant or sediment material was inserted into a Teflon reactor (Savillex) with a clean plastic spatula and subsequently digested with a mixture of concentrated supra pure HNO_3 and HF at 90 °C overnight. The solution was then evaporated, and hydrogen peroxide solution H_2O_2 was added to destroy the organic matter. The procedure was defined with different steps repeated until the dissolution is complete. Finally, after evaporation, 11 mL miliQ water was added.

All samples were transferred to tubes and were diluted so that the element concentrations were lower than 0.8 g/l in order to avoid ICP-MS saturation during the analysis. The samples were then acidified with nitric acid (HNO_3 69%) to obtain a 2% HNO_3 solution. Finally, Indium/Rhenium (In/Re, conc. 0.4037 ppm) was added to samples in order to control the machine deviation during the analytical process.

The analytical data quality was checked by including simultaneously in the procedure the blank samples and two standards (SRM-1515 apple leaves, from National Institute of Standards and Technology,

USA, and STSD-1 Stream Sediment Reference materials, from Canada Certified Reference Materials Project – CCRMP, Canada) to verify accuracy and reproducibility of the dissolution process for plant and sediment samples.

Cd concentration was analyzed in water, plants (root and above parts) and sediment samples by spectrometry using a Q-ICP-MS (Perkin-Elmer ELAN 6000) at the OMP-GET (Geoscience Environment Toulouse, Observatoire Midi-Pyrénées, Toulouse, France). Detection limits for Cd were between 10^{-3} to 10^{-2} $\mu \cdot L^{-1}$. The concentrations of some elements measured in the two standards were used for the analytical recovery (QA/QC) calculation by comparison with their reference values. For most elements, the recovery of the reference material SRM-1515 ranged between 112% and 166%, in which Cd element was at 124%. Because the standard STSD-1 is not certified for Cd element, we used the sediment samples collected in the Aussonnelle River, (initial sample of the study) for comparison with the sediment samples, which were collected in the same station (Aussonnelle, Garonne river bank) by Proffit and Probst (2007) and with the Gascogne river studied by N'guessan et al. (2009). Our results showed that the initial sediment Cd concentrations in the present study were in the same range as those measured by these authors. The mean concentrations of cadmium in the Gascogne stream sediment (fraction <63 μm) and in the Gascogne bedrock (Molasse) samples were 0.30 and 0.20 $\mu g \cdot g^{-1}$, respectively (N'guessan et al., 2009), while the Cd concentration in sediment from this study averaged 0.21 ± 0.02 $\mu g \cdot g^{-1}$.

2.5. Texture, pH, and pore water content analyses of sediment at the end of the experiment

Grain-size of the sediment particles was measured with a Laser Scattering Particle Size Distribution Analyser – LA – 950 V2 (HORIBA Japan), for sizes ranging from 0.01 to 3000 μm. The Wentworth's classification (Wentworth, 1922) was used to define the various grain-size fractions of four experimental treatments {Control}, {Tub}, {Cd}, and {Cd.Tub}: fine silts and clays (<15 μm), medium silts (15–30 μm), coarse silts (30–63 μm), very fine sands (63–125 μm), fine sands (125–250 μm), medium sands (250–500 μm) and coarse sands (>500 μm). This analysis also provided the mean size of sediment particles, which is an integrative parameter allowing to compare the grain size of a given sediment layer in different treatments.

To study the influence of different treatments on the physico-chemical sediment properties, pH value, and pore water content were measured in fresh sediment samples from 4 distinguishable layers. These layers were defined according to tubificid worms' activity (see Section 2.4.1). The pore water content and porosity were assessed by drying sediment samples overnight at 105 °C, while pH was measured in a water suspension (1:5; v/v) according to the norm ISO 10390.

2.6. Calculations and data treatment

2.6.1. Enrichment coefficient and translocation factor

The enrichment coefficient for roots (EC_R), enrichment coefficient for leaf parts (EC_L) and transfer factor (TF) were calculated as follows:

EC_R = concentration of metal in roots / concentration of metal in sediment (fraction < 63 μm)

EC_L = concentration of metal in leaf / concentration of metal in sediment (fraction < 63 μm)

TF = concentration of metal in leaf / concentration of metal in roots

2.6.2. Statistical analysis

To evaluate statistically any significant difference between mean values among all experimental treatments at the beginning (at t = 0) and at the end of the experiment (t = 30), Cd concentrations in plant samples, enrichment factors (EC), transfer factors (TF), bioadvective (V) and biodifusive rates (Db) were firstly compared using one-way ANOVA. Prior to ANOVA tests, homoscedasticvity and normality of the residues were checked with Bartlett and Shapiro-Wilk tests, respectively. Following ANOVAs, the mean values of each treatment were compared with each other using Tukey HSD's method. Dunnett test was also used for comparing data at t = 30 days with initial values (t = 0 day) used as references.

Secondly, the possible interactive effects of cadmium enrichment and tubificids on the bioaccumulation of *T. latifolia* were studied by using two-way ANOVA. Two-way ANOVAs were also performed on the average size of sediment particles separately for each sediment layer, to assess the impact of bioturbation and cadmium enrichment on sediment texture.

Three-way ANOVA was applied on the bioturbation bioadvective rates - V and the cadmium concentration in the sediment samples to investigate for possible interactive influences of worms addition, cadmium enrichment, and plant presence/absence on bioturbation activities and cadmium behaviour in sediment.

Statistical analyses were performed using the statistical GraphPad Prism (version 6.01) and R software (version 3.2.5).

3. Results

3.1. Sediment particle bioturbation

In sediment without tubificids (Fig. 2, black dots), about 70% of luminophores remained in the surface layer (0–0.5 cm) after 30 days. The remaining luminophore particles were located below the sediment surface (about 25% and 5% of the tracers were found at the layers from 0.5 to 1.0 cm and from 1.0 to 1.5 cm, respectively). The luminophore concentration profiles recorded under the influence of the tubificids provided evidence of sediment biotransport (Fig. 2, white dots): 30 to 40% of the tracers, initially deposited at the sediment surface, were spread down to rather than 2 cm depth with a concentration peak of luminophore particles at 2 cm depth after 30 days. The experiment indicates a significant increase of the bioadvective rates (V) in presence of worms (three-way ANOVA, $F_{1,16} = 160.05$, $P < 0.0001$) (Table 2).

The bioadvective rates were significantly and consistently different between treatments with or without tubificids addition (Tukey HSD test; Fig. 3). The mean values, for sediment with tubificids, ranged from 16.7 ± 4.5 to 18.5 ± 3.93 $cm \cdot year^{-1}$, while it varied only from 0.6 ± 0.5 to 2.5 ± 3.1 $cm \cdot year^{-1}$ when they were absent (Fig. 3). No other factor had any significant influence on V, either alone or in interaction with the presence of tubificids (Table 2). The corresponding biodiffusion coefficients (Db) did not significantly vary among treatments but tended to be higher in presence of worms (from 1.0 ± 0.6 to 1.6 ± 0.3 $cm^2 \cdot year^{-1}$) compared to abiotic controls (from 0.8 ± 0.1 to 1.4 ± 0.5 $cm^2 \cdot year^{-1}$) (Fig. 3). It is particularly remarkable that no significant difference for either V or Db was observed when comparing sediment with or without added cadmium.

3.2. Physico-chemical sediment properties with and without bioturbation

The mean grain-size and the percentage of each sediment fraction in the two sediment layers (L1 (0–1 cm) and L2 (1–5 cm)) at the end of the experiment are given in Table 3 for the four experimental conditions: {Control}, {Tub}, {Cd}, and {Cd.Tub}. After 30 days, when no tubificids, the smallest particles (fine silt, clay, medium silt, and coarse silt with grain-size fraction <63 μm) accounted for up to 54.7 and 49.6% of the sediment volume in L1 and L2, respectively in {Control} unit. The grain size distributions were almost similar in both treatments ({Control} and {Cd} treatments). When worms were present, the relative proportion of the sediment fractions <63 μm increased significantly at the end of the experiment to 84.5 and 87.1% in the top layer (L1) of the two treatments ({Tub} and {Cd.Tub}), respectively. At the same time, in a consistent way, the relative proportion of the sediment fractions <63 μm decreased in the second layer (L2) to 33.6 and 38.9% for these treatments. In contrast, the largest particles (notably coarse, medium

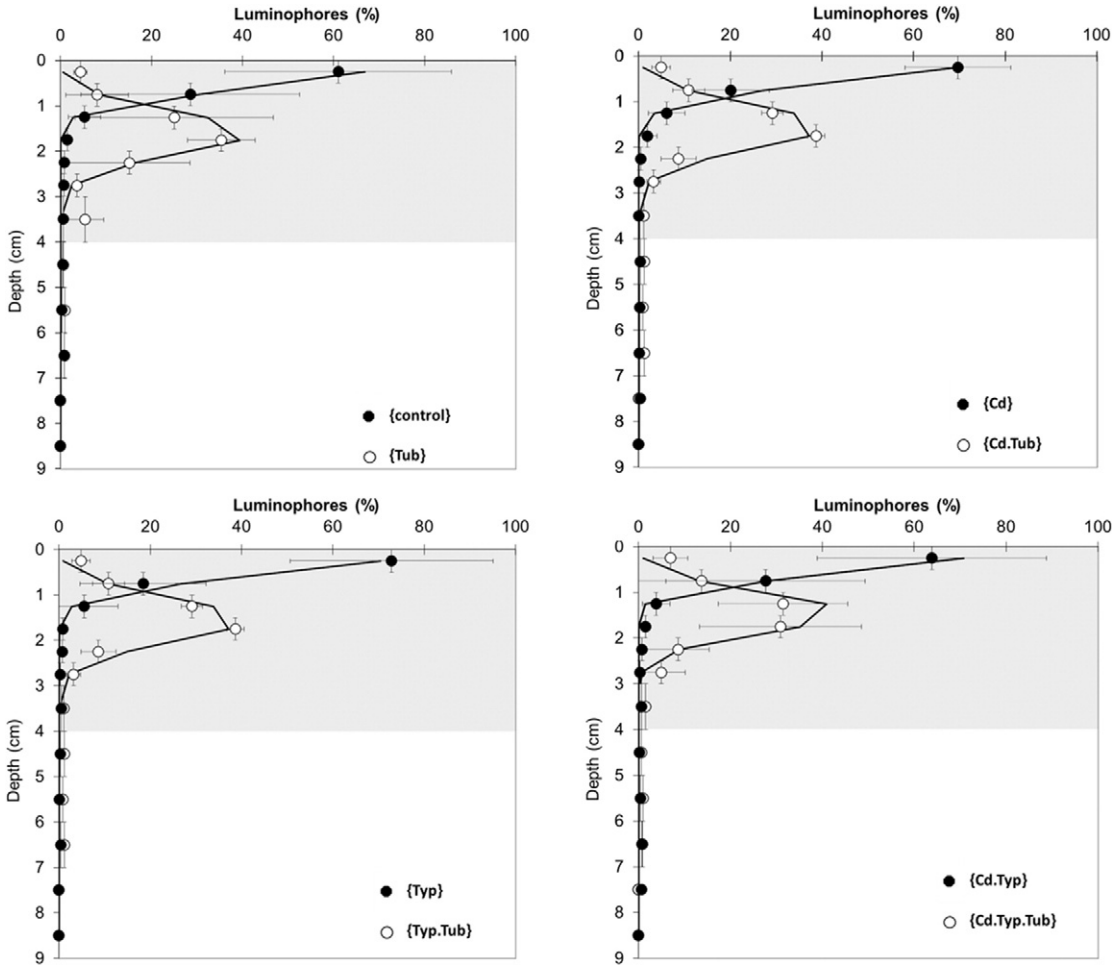

Fig. 2. Vertical profiles of luminophores in the sediment after 30 days for treatments with and without tubificids bioturbation. White and black dots are mean values of the measured luminophore concentrations (%); Solid lines are theoretical profiles of the tracer concentrations obtained by using bioadvective (V) and biodiffusive (D_b) rates calculated independently for each microcosm; The part in grey color is the thickness of the bioturbated layer in the treatments with tubificids (see Table 1 for the acronym definitions corresponding to the different treatments).

and fine sands) recorded in L1 for these two treatments were significantly less abundant compared with those without worms (Table 3).

A significant effect of tubificids addition was recorded in the first layer (L1) with a lowering of the mean grain size of particles, and the reverse for the second layer (L2), as evidenced by using two-way ANOVA ($F_{1,32} = 179.4$ and 26.97, respectively, $P < 0.0001$) (Table 4). The effect of cadmium enrichment was significant, although to a lesser extent only on the mean grain size in layer 2 (two-way ANOVA, $F_{1,32} = 4.951$, $P = 0.0333$). No significant interaction between the two factors tested (Cd enrichment and tubificids addition) was found for any of the two layers (Table 4).

Table 2
Three-way ANOVA performed on the bioadvective rates (V). ***: significant difference for V at $P \leq 0.001$; ns: non significance according to three-way ANOVA.

Factors	$F_{1,16}$	P
Cd enrichment	0.0668	0.7994^{ns}
T. latifolia treatment	0.1427	0.7106^{ns}
Tubificids addition	160.0475	$<\mathbf{0.0001}^{***}$
Cd:typ interaction	0.4304	0.5211^{ns}
Cd:tub interaction	0.0232	0.8808^{ns}
Typ:tub interaction	0.4842	0.4965^{ns}
Cd:typ:Tub interaction	0.3173	0.5810^{ns}

The bold in the table is to highlight the significant difference of V (bioadvective rate), compared to the other factors.

For the treatment {Cd.Typ}, the porosity of sediment in the column varied from 33.5% to 25.7%, respectively for L1 and L2. With addition of worms, the porosity has only increased in the first layer L1 with a mean of 47.4%. Fig. 4 shows the pH variation according to the depth in the microcosms for {Cd.Typ} and {Cd.Typ.Tub} treatments. The pH of

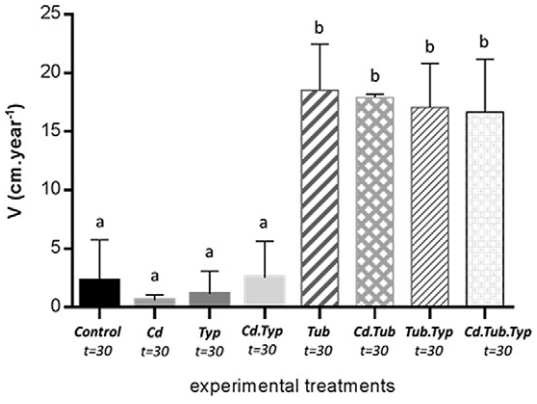

Fig. 3. Bioadvective rates (V) estimated after 30 days for the different experimental treatments; different letters indicate the significant differences ($P < 0.05$) of V between treatments as analyzed by TUKEY HSD multiple comparison tests. Values are mean ± SD, $n = 3$ per treatment group (see Table 1 for the acronym definitions used for the different treatments).

Table 3
Percentages of the various size-fractions of particles and mean grain-size in each sediment layers (mean ± SD, $n = 9$) at t = 30 days.

Grain-size fraction (%)		1st layer (L1)				2nd layer (L2)			
	Grain size (μm)	Control.t30	Tub.t30	Cd.t30	Cd.Tub.t30	Control.t30	Tub.t30	Cd.t30	Cd.Tub.t30
Fine silt and clay	<15	33.2 ± 3.2	55.9 ± 2.9	29.7 ± 2.5	58.7 ± 4.0	28.2 ± 2.2	15.4 ± 2.1	30.5 ± 1.7	18.8 ± 2.3
Medium silt	15–30	11.1 ± 0.8	15.4 ± 0.6	10.4 ± 1.2	16.2 ± 1.6	10.3 ± 0.6	6.5 ± 0.5	10.8 ± 0.7	7.6 ± 1.2
Coarse silt	30–63	10.4 ± 0.6	13.2 ± 0.8	10.7 ± 1.6	12.1 ± 1.1	11.2 ± 0.6	11.7 ± 1.7	10.7 ± 1.3	12.5 ± 1.7
Fine sediment (particles < 63)		54.7 ± 4.0	**84.5 ± 2.2**	50.8 ± 4.2	**87.1 ± 1.8**	49.6 ± 3.1	**33.6 ± 2.8**	51.9 ± 2.3	**38.9 ± 4.4**
Very fine sand	63–125	13.5 ± 0.9	11.0 ± 1.4	14.5 ± 1.6	9.2 ± 0.9	14.8 ± 0.9	12.5 ± 0.9	14.5 ± 1.0	12.3 ± 0.9
Fine sand	125–250	12.9 ± 0.9	3.8 ± 0.9	14.6 ± 0.6	3.0 ± 0.5	14.7 ± 0.5	18.1 ± 2.4	15.1 ± 0.6	17.7 ± 1.7
Medium sand	250–500	14.2 ± 1.8	0.7 ± 1.0	14.7 ± 2.2	0.6 ± 0.7	15.9 ± 1.7	25.5 ± 3.3	15.1 ± 1.6	25.3 ± 3.1
Coarse sand	>500	4.7 ± 3.1	0.0 ± 0.0	5.4 ± 3.9	0.1 ± 0.4	5.0 ± 2.1	10.3 ± 6.2	3.3 ± 1.3	5.9 ± 2.5
Mean grain size (μm)		107.82 ± 21.26	28.64 ± 4.18	126.14 ± 33.35	26.15 ± 5.41	125.03 ± 17.33	207.34 ± 70.62	112.44 ± 11.96	162.99 ± 21.48

The bold in the table is to make the highlights of the worms' effects on fine sediment fraction.

the overlying water were found to be at 8.03 and 8.20 in conditions with and without bioturbation, respectively. The bioturbation effect was only detectable in the surface sediment layers (from 0 to 4 cm) where the pH decreased from 8.03 ± 0.05 to 7.73 ± 0.02 under tubificids' influence. Without bioturbation effect, the pH is even lower and reaches 7.47 ± 0.06 at 2 cm depth (Fig. 4).

3.3. Cadmium concentrations in overlying water

Dissolved cadmium concentration in the overlying water of the microcosms contaminated by Cd was initially introduced at 20 $\mu g \cdot L^{-1}$ (corresponding to a total amount of 95.6 μg Cd per microcosm). Few hours after the contaminant introduction (t = 0), the average dissolved cadmium concentrations were found to be 1.01 ± 0.31 $\mu g \cdot L^{-1}$ and 0.01 ± 0.00 $\mu g \cdot L^{-1}$ (means ± SD, n = 3) in the treatments with {+Cd} and without cadmium enrichment {−Cd} respectively. Thirty days after the beginning of the experiment, the Cd concentration in water was very low, ranging from 0.01 ± 0.00 to 0.03 ± 0.01 $\mu g \cdot L^{-1}$ in all experimental conditions, with no significant difference between all contaminated treatments.

3.4. Cadmium concentrations in sediment

Before adding the contaminant into the overlying water, Cd concentrations in sediment ranged from 0.195 ± 0.014 (layer L2) to 0.202 ± 0.003 $\mu g.g^{-1}$ dry wt. (layer L1) (Fig. 5A). At t = 30 days, Cd concentration in the sediment of {Control} microcosms and the microcosms with *T. latifolia* only {Typ} ranged from 0.180 to 0.205 $\mu g \cdot g^{-1}$ dry wt. and were similar to the initial conditions, in both sediment layers. For all these treatments, no significant difference in Cd concentration was found between top (L1) and deeper (L2) layers.

In the absence of worms (Fig. 5A), but with Cd addition, i.e. {Cd} and {Cd.Typ}, the average Cd concentrations significantly increased in the first layer (0.331 and 0.339 $\mu g \cdot g^{-1}$ dry wt., respectively) compared to control units, while in the second layer the values remained the same as in the control microcosms {Control} (0.180 and 0.196 $\mu g \cdot g^{-1}$ dry wt., respectively). The Cd concentrations did not differ significantly depending on the presence or absence of *T. latifolia.*

Table 4
Effect of bioturbation and cadmium enrichment and their interactions performed on the mean grain size of sediment particles, using two-way ANOVA.

Factors	1st layer (L1)		2nd layer (L2)	
	$F_{1,32}$	P	$F_{1,32}$	P
Cadmium enrichment	1.40	0.2456^{ns}	4.95	0.0333^{*}
Tubificids addition	179.40	$<0.0001^{***}$	26.97	$<0.0001^{***}$
Interaction	2.42	0.1295^{ns}	1.54	0.2235^{ns}

(*) and (***) indicate significance at p ≤ 0.05 and 0.001, respectively; ns: non significance according to two-way ANOVA.

When worms were present (Fig. 5B) without Cd enrichment ({Tub}, {Typ.Tub}), compared with the control {Initial} unit at t = 0, and {Control} and {Typ} units at t = 30 days, Cd concentrations increased in L1 (to 0.325 and 0.336 $\mu g.g^{-1}$ dry wt.), and decreased in L2 (to 0.138 and 0.145 $\mu g.g^{-1}$ dry wt.) in {Tub}, {Typ.Tub}, respectively.

When both worms and Cd were present, the highest concentrations of Cd were found in the layer L1 (0.498 and 0.459 $\mu g \cdot g^{-1}$ dry wt., in {Cd.Tub} and{Cd.Typ.Tub} respectively), and there was no significant difference in the presence or absence of *T. latifolia*. Cd concentrations in the layer L2 of these two treatments were found to be 0.168 and 0.179 $\mu g \cdot g^{-1}$ dry wt., respectively (Fig. 5), again independently of *T. latifolia* presence.

The different levels of Cd concentrations between the first and the second layer, expressed as specific ratio *R* observed in all experimental treatments, ranged from 1.0 to 1.8 in the absence of worms' activities (Fig. 5A) and from 2.3 to 3.0 when worms are present in the sediment (Fig. 5B).

The effects of both cadmium enrichment and tubificids addition on Cd concentrations in the top layer were analyzed using the three-way ANOVA ($F_{1,11} = 56.12$, $P < 0.0001$ and, $F_{1,11} = 39.97$, $P < 0.0001$, respectively) as well as the effect of tubificids only in the second layer (three-way ANOVA, $F_{1,11} = 12.56$, $P = 0.0046$) (Table 5). There were no significant interactions between these factors (three-way ANOVA, $P > 0.05$).

3.5. Cadmium concentrations in T. latifolia organs

The mean Cd concentration in the roots and leaves of *Typha latifolia* in field sample collected 60 days before the beginning of the experiment and in the different treatments at initial set up and and end of experiment is reported in Fig. 6. The concentrations in the leaf parts (Fig. 6B) averaged 0.158 ± 0.063 $\mu g \cdot g^{-1}$ dry wt. in {Typ} to 0.248 ± 0.222 $\mu g \cdot g^{-1}$ dry wt. in {Cd.Typ.Tub} treatments and was much lower by a factor 10 than in the roots for all treatments. No significant differences ($P > 0.05$) in Cd concentrations were observed in the leaf parts of any treatment at the beginning and at the end of the experiment, despite a slight increase observed in the presence of worms ({Cd.Typ.Tub}). In the roots (Fig. 6A), there were significant differences in average Cd concentrations among treatments at the beginning (t = 0) and at the end of the experiment (t = 30) when measured values were analyzed all together (one-way ANOVA, $F_{4,10} = 4.67$, $P = 0.0219$). The mean value of all the treatments at the end of the experiment (t = 30) significantly differed from the average initial Cd concentration (t = 0) (from the Dunnett multiple comparisons, $P > 0.05$ in all cases) (Fig. 6A). However, a significant effect of cadmium enrichment on Cd bioaccumulation by *T. latifolia* roots ($F_{1,8} = 17.37$, $P = 0.0030$) and to a lesser extent of the interaction between the two tested factors (Cd and tubificids) in interaction ($F_{1,8} = 6.569$, $P = 0.0335$), were evidenced by a two-way ANOVA (Table 6). Cadmium concentration increased significantly in the roots during the

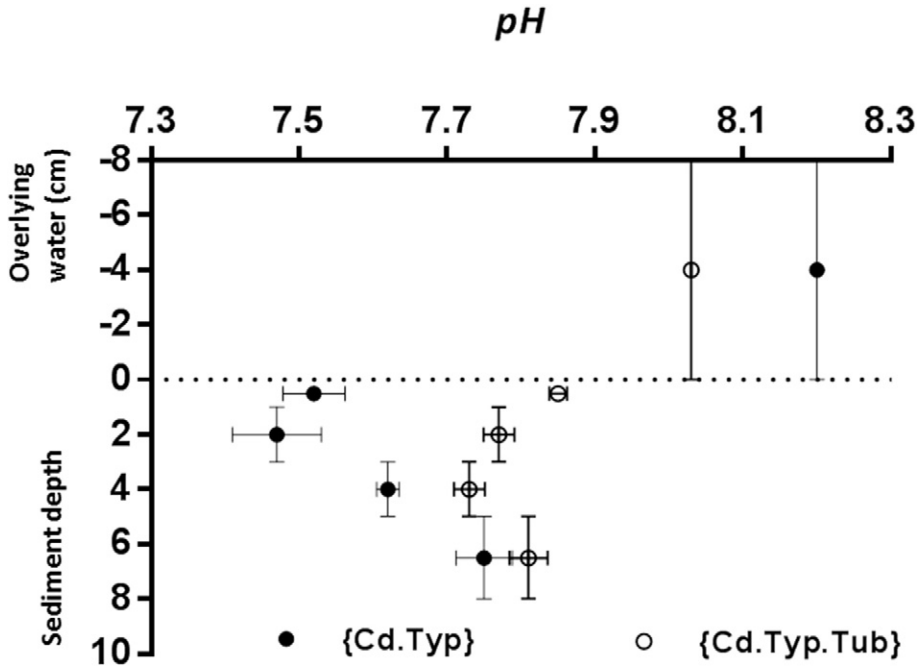

Fig. 4. Comparision of pH profiles in the overlying water and sediment column for treatments with (white dots) and without tubificids (black dots) bioturbation. Values are mean ± SD, $n = 3$ per treatment group (see Table 1 for the acronym definitions used for the different treatments).

experiment, when both cadmium and worms were together ({Cd.Typ.Tub}), when compared with the treatment having worms only {Typ.Tub} (Tukey HSD, $P = 0.0060$) (Fig. 6A). A significant increase was also observed with bioturbation {Cd.Typ.Tub} (mean = 2.90 $\mu g \cdot g^{-1}$ dry wt.) compared to {Typ} (mean = 1.41 $\mu g.g^{-1}$ dry wt.) treatments at t = 30 (Tukey HSD, $P = 0.0307$). No significant difference was observed between the two conditions {Cd.Typ} and {Cd.Typ.Tub} ($P = 0.1575$) neither between the two conditions {Cd.Typ} and {Typ} ($P = 0.6630$) according to Tukey post-hoc test (Fig. 6A).

3.6. Indicators of phytoremediation potential

Plant phytoremediation potential can be assessed by using the enrichment coefficient (EC) and translocation factor (TF). In Table 7 and Fig. 7, two EC values, that do not consider the reference conditions (field sample (t = −60) and initial samle (t = 0)), were calculated for the two experimental sediment layers (L1 and L2) for roots (EC_R) and for leaves (EC_L). As indicated in Table 7, mean values of EC_L ranged from 0.54 to 0.86 in top layer L1 and from 0.85 to 1.39 in layer L2, whereas the EC_R showed higher values, with average ranging from 2.65 to 8.27 (Fig. 7A) and from 6.15 to 16.24 (Fig. 7B) in L1 and L2, respectively. The TF was smaller than 1 and varied between 0.02 and 0.12.

The enrichment coefficient for the leaf parts (EC_L-L1 and EC_L-L2) and the transfer factor (TF) (Table 7) did not show any significant difference among experimental treatments at the beginning (t = 0) and at the end (t = 30) of the experiment (one-way ANOVA, $F_{4,10} = 0.7450$, $P = 0.5830$; $F_{4,10} = 0.3955$, $P = 0.8075$; $F_{4,10} = 1.417$, $P = 0.2976$, respectively). In contrast, the enrichment coefficient for the roots (Fig. 7) showed significant differences among compared treatments at the beginning and at the end of the experiment (EC_R-L2, ANOVA test, $F_{4,10} = 4.354$, $P = 0.0270$) and among treatments at t = 30 (EC_R-L1, EC_R-L2, one-way ANOVA test, $F_{3,8} = 6.4$, $P = 0.0161$, $F_{3,8} = 7.075$, $P = 0.0122$, respectively).

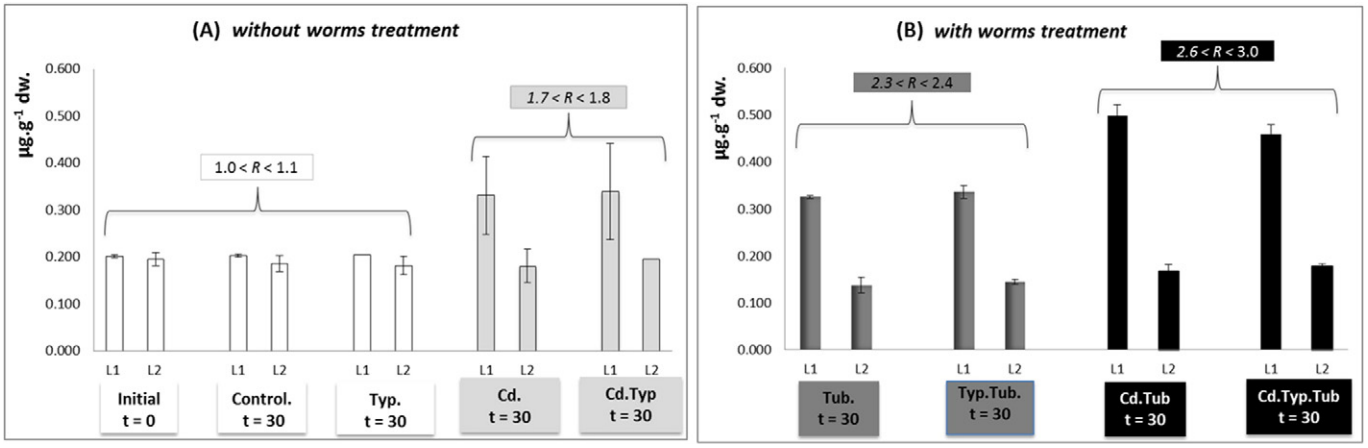

Fig. 5. Mean concentrations of cadmium ($\mu g \cdot g^{-1}$ dry wt.) in the sediment layers (fraction <63 μm) at the initial: t = 0, mean ± SD ($n = 3$), and at the end: t = 30, mean ± SD ($n = 2$); (L1 = surface layer of sediment: 0–1 cm; L2 = deeper layer of sediment: 1–5 cm); R = ratio between concentration of Cd in L1 over concentration of Cd in L2. (A) - treatment group without tubificids, (B) - treatment group having tubificids (see Table 1 for the acronym definitions corresponding to the different treatments).

Table 5
Effects of Cd enrichment and tubificids on cadmium concentrations in the top sediment layer (L1) and in the deeper layer (L2) to investigate the interactions between experimental treatments.

Experimental treatments	Cd conc. in layer 1 (L1)		Cd conc. in layer 2 (L2)	
	$F_{1,11}$	P	$F_{1,11}$	P
Cadmium enrichment	56.12	1.21×10^{-5} ***	1.08	0.319763
T. latifolia treatment	0.32	0.5834	0.02	0.878950
Tubificids addition	39.97	5.66×10^{-5} ***	12.56	0.004593**
Cd:Typ interaction	0.22	0.6455	0.95	0.348630
Cd:Tub interaction	0.14	0.7169	2.83	0.120637
Typ:Tub interaction	0.16	0.6966	0.16	0.693625
Cd:Typ:Tub interaction	0.40	0.5421	0.29	0.598204

(**) and (***) indicate significance at $p \leq 0.01$ and 0.001, respectively; (ns)-non significance according to three-way ANOVA.

Table 6
Two-way ANOVA performed on cadmium concentration in *T. latifolia* to investigate the interaction between experimental treatments.

Microcosms	Cd conc. in root		Cd conc. in leaf	
	$F_{1,8}$	P	$F_{1,8}$	P
Cadmium enrichment	17.73	0.0030**	0.1979	0.6682ns
Tubificids addition	0.6568	0.4411ns	0.4956	0.5014ns
Interaction	6.569	0.0335*	0.1240	0.7339ns

(*) and (**) indicate significance at $P \leq 0.05$ and 0.01, respectively; (ns)-non significance according to two-way ANOVA.

Significant interactions between Cd enrichment and tubificids addition were observed in the enrichment coefficients for roots in both the layer L1 and layer L2 (two-way ANOVA, $F_{1,8} = 11.10$, $P = 0.0104$; $F_{1,8} = 5.985$, $P = 0.0402$, respectively) (Table 7). However, the EC_R values were differently influenced by the various factors in the two layers: there was a significant effect of tubificids addition in the layer L1 (two-way ANOVA, $F_{1,8} = 5.564$, $P = 0.0460$) and of cadmium enrichment in the layer L2 (two-way ANOVA, $F_{1,8} = 13.07$, $P = 0.0068$) (Table 7). Although the enrichment factors for the roots (EC_R) did not reveal any significant difference between the two compared treatments {Cd.Typ} and {Typ}, nor between {Cd.Typ} and {Cd.Typ.Tub} for the L1 (Tukey HSD post hoc test, P values ranging from 0.0918 to 0.8990) (Fig. 7A), the bioaccumulation capacity of *T. latifolia* in the roots was significantly increased for the L2 in the presence of both cadmium and worms (Fig. 7B). The Tukey's multiple comparisons tests indicated the significant differences between {Cd.Typ.Tub} and {Typ.Tub} treatments in EC_R-L1 and EC_R-L2 ($P = 0.038$, 0.0114, respectively), and between {Cd.Typ.Tub} and {Typ}treatments in EC_R-L2 ($P = 0.0288$).

4. Discussion

4.1. Influence of bioturbation on particle mixing

In control plots without *Tubifex* worms (Fig. 2), no particle mixing occurred since 70% of luminophore tracers remained at the surface of the sediment. Some luminophore particles were found in the deeper layers probably due to inherent tracer movments during experimentation or because of the activity of some smaller invertebrates that survived to the initial sediment defaunating process. A significant bioadvection of sediment occured during the experiment only under the worms' activities as attested by the stastitistical analysis (Table 2).

In the presence of tubificid worms with a density of 135 worms·dm^{-2} (equivalent to a fresh biomass of 17.8 ± 3.1 g per microcosm), a subsurface peak of tracers in the 2 cm layer indicated that a conveying transport was created by the tubificids with average rates of 16.7 ± 4.5 to 18.5 ± 3.93 cm·$year^{-1}$. This downward transport of the surface sediment resulted from the accumulation of faecal pellets at the sediment surface, simultaneously with sediment depression in deeper layers due to sediment ingestion by the worms feeding activities (Anschutz et al., 2012; Ciutat et al., 2006). Since coarse particles as luminophores and sand particles are too large to be ingested by tubificid worms, at depth, the same feeding behaviour induces a decrease of the silt-clay fraction, since worms are avoiding larger sand particles (Rodriguez et al., 2001). Consequently, bioturbation creates 2 distinct layers in the bioturbated sediment (Fig. 2): a top layer corresponding to the faecal pellets accumulation from ingested anoxic sediment and a bottom layer of with increasing particle size (Anschutz et al., 2012). The maximal ingestion depth was located below 3 cm and the bioturbated layer was estimated to be from 0 to >3 cm since luminophores occurred down to this level (Fig. 2) after 30 days. The obtained rates of bioadvection in presence of tubificids (Fig. 3) were in the same order of magnitude of those estimated by other previous authors with tubificids population of various densities (Ciutat et al., 2005b; McCall and Fisher, 1980; Matisoff et al., 1999). Indeed, Ciutat et al. (2005a, 2005b) who studied bioadvection under a tubificids population of 600 worms.dm^{-2} reported an ingestion zone in the 3–5-cm layer and an advection rate of 42 cm^2·$year^{-1}$. The higher density in the previous studies explains the differences in the bioadvection rates obtained in our experiment (16.7 ± 4.5 to 18.5 ± 3.93 cm·$year^{-1}$). Compared to control experimental units, the recycled material at the end occurs as several centimetre-thick (>3 cm) surface layers enriched in water content (as observerd by Anschutz et al., 2012 with higher value of porosity found in surface that was close to 85%). This layer was characterized by a significant concentration of dissolved nitrate and sulphate, depleted in oxygen

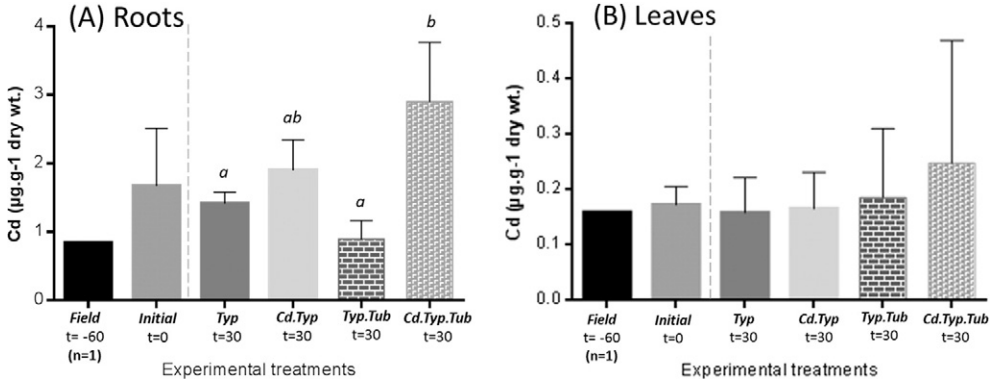

Fig. 6. Mean concentrations of cadmium in *Typha latifolia* roots (A) and leaves (B) for each experimental condition, before the experiment (field sample on t = −60), at the initial time (t = 0), and at the end of the experiment (t = 30). Values are means ± S.D., replicate (n) = 3 per treatment group, except for the field sample (n = 1). Different superscript letters indicate the significant differences for Cd concentrations in the root parts between conditions at $p < 0.05$ level as analyzed by Tukey HSD test. No significant difference was observed among the leaf parts of the various experimental treatments. The vertical scales used in root and leaf's Cd contents are different. (See Table 1 for the meaning of acronyms corresponding to the different treatments.)

Table 7
Cadmium transfer factors and enrichment coefficients for root (EC_R) and leaf parts (EC_L) of *Typha latifolia* for the field sample (t = −60), and in the different conditions at the beginning (t = 0), and at the end (t = 30) of the experiment.

Treatments	EC_R-L1	EC_R-L2	EC_L-L1	EC_L-L2	TF
Field sample (t = −60) (n = 1)	4.15	4.29	0.79	0.82	0.19
t = 0 (n = 3)	8.27 ± 4.20	8.55 ± 4.34	0.86 ± 0.17	0.89 ± 0.17	0.12 ± 0.07
{Typ} t = 30 (n = 3)	6.89 ± 0.84^{a}	7.77 ± 0.94^{ab}	0.79 ± 0.00^{a}	0.87 ± 0.35^{a}	0.11 ± 0.04^{a}
{Typ.Tub} t = 30 (n = 3)	2.65 ± 0.81^{b}	6.15 ± 1.88^{b}	0.55 ± 0.37^{a}	1.28 ± 0.86^{a}	0.02 ± 0.07^{a}
{Cd.Typ} t = 30 (n = 3)	5.59 ± 1.32^{ab}	9.72 ± 2.30^{abc}	0.49 ± 0.19^{a}	0.85 ± 0.33^{a}	0.09 ± 0.02^{a}
{Cd.Typ.Tub} t = 30 (n = 3)	6.32 ± 1.89^{ac}	16.24 ± 4.85^{c}	0.54 ± 0.48^{a}	1.39 ± 1.24^{a}	0.09 ± 0.09^{a}
P value two-way ANOVA					
Cadmium enrichment	0.1499^{ns}	0.0068**	0.4875^{ns}	0.9326^{ns}	0.0976^{ns}
Tubificids addition	0.0460*	0.1788^{ns}	0.6921^{ns}	0.3291^{ns}	0.2519^{ns}
Interaction	0.0104*	0.0402*	0.5211^{ns}	0.8907^{ns}	0.3649^{ns}

Values are means ± S.D., *n* = 3 per treatment group.; different superscript letters indicate significant differences for EC_R between treatments at t = 30, assessed separately at the two different sediment layers (L1 and L2) (Tukey HSD tests); * and ** indicate the significance of *F* values at $P \le 0.05$ and 0.01, respectively, according to two-way ANOVA.

and slightly increased in pH according to Anschutz et al. (2012) for similar worms population conditions.

4.2. Influence of bioturbation processes on cadmium behaviour in sediment

During the experiment, there were no significant physical processes occuring, such as sediment compaction, that might affect the contaminant distribution with time. This is supported by (i) the similar Cd concentration profiles between the three control treatments, at t = 0 (before adding the contaminant into the overlying water), the control treatment {Control} (t = 30), and the experimental unit having *T. latifolia* treatment only {Typ} at t = 30, with an average Cd concentration ranging from 0.180 to 0.205 $\mu g \cdot g^{-1}$ dry wt. (Fig. 5A); (ii) the similar specific ratio of Cd concentrations (*R*) close to 1 between L1 and L2 of sediment among these three conditions. This highlights also the relevance of the reference conditions as it is consistent with previous concentrations measured on the same sediment (Probst and Guilloux, in prep.) and in the rivers from the area (N'guessan et al., 2009).

After 30 days, the rapid adsorption of Cd on surface sediment particles following Cd addition in the overlying water at t = 0 was attested by the decreased of the initial concentration (20 $\mu g \cdot L^{-1}$) in the overlying water to an average of 1.01 $\mu g \cdot L^{-1}$ for the contaminated treatments {+Cd}. The strong and rapid adsorption process of Cd was explained by the high pH conditions (>7.5) of carbonate sediment in the sampling area (N'guessan et al., 2009; Pokrovsky et al., 2012). Cd adsorption was demonstrated to be a fast process (>95% of the adsorption took place within the first 10 min) (Santillan-Medrano and Jurinak, 1975).

In the presence of only cadmium (Fig. 5A), physical processes of Cd adsorption on the surface sediment occurred, as indicated by the recorded profiles obtained after 30 days. In the aquatic environment, cadmium was found dissolved in the pore water or adsorbed on to particles (Mustafa et al., 2004). Molecular diffusion of Cd^{2+} was estimated between 189 and 220 $cm^2 \cdot year^{-1}$ for temperature ranging between 18 and 25 °C (Shackelford and Daniel, 1991; Delmotte et al., 2007). The mobility and fate of metals in the sediment environment are directly related to their partitioning between free and solid fractions (Sakultantimetha et al., 2009). The presence of trace metals on to the solid phase is a result of precipitation (with sulfides), co-precipitation (with Fe hydroxides), adsorption, complexation to sediment components (clay minerals, organic matter), those processes being highly pH dependent (Lukman et al., 2013) and indirectly affected by redox potential (E_H) (Du Laing et al., 2009). The estimated partition coefficients Kd for Cd ranges from 8 to 4000 mL/g and depends on the pH, here ranging from 5 to 8 (EPA, 1999). Because it is sensitive to pH, the Cd adsorption and desorption in the sediment of our microcosm depends on pH and redox condition, that are shown to vary under bioturbation effects in the sediment (Bradl, 2004; Fu and Allen, 1992). Indeed, the hydrogen ions affect the surface charge of the adsorbent, the degree of ionization, and the speciation of the adsorbate (Lee et al., 1996), which explains the high dependence of Cd (II) adsorption on pH. Moreover, the metal adsoption is also dependant on the active surface of sediment particles (Gerth et al., 1993; Naidu et al., 1997), the higher adsoption capacity occuring with larger specific surface area (Ljung et al., 2006) of sediment with fine particle size (Gong et al., 2014; Sutherland, 2003). The adsorption of cadmium onto riverbed sediment was reported to range from 30% at pH 2.0 to 80% at pH 7.0 for the coarser sediment fraction (210 ± 250 mm) (Palheiros et al., 1989). These processes lead to higher values of R in the {Cd} and {Cd.Typ} plots (1.8 and 1.7, respectively). The

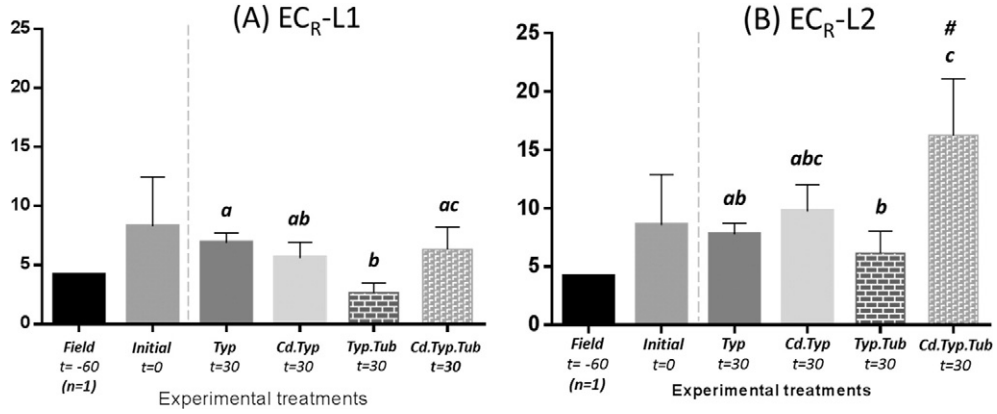

Fig. 7. Enrichment coefficients for the roots (EC_R) of *Typha latifolia* at two different sediment layers ((A): L1; (B): L2). Values are means ± S.D., *n* = 3 per treatment group, except for the field sample (*n* = 1). $^{(\#)}$ indicates significant differences between a given treatment at t = 30 and the initial EC_R value (Dunnett test); Different superscript letters indicate significant differences for EC_R between treatments at t = 30, assessed separately at the two sediment layers (Tukey HSD test). See Table 1 for the meaning of acronyms corresponding to the different treatments.

deposition of Cd from the overlying water was thus possible in the surface sediment where about 80% of Cd was trapped due to higher pH conditions (Bradl, 2004) with the value of 7.52 ± 0.04 in the surface sediment (Fig. 4).

In the treatment with tubificids and without Cd addition ({Tub} and {Typ.Tub} units, Fig. 5B), a significant increase of the cadmium concentration in L1 was closely related to bioturbation phenomenon and linked to the change of texture (84.5 to 87.1% of fine particle in the top layer, Table 3), and physico-chemistry of the environment by worms effects. A similar effect was previously mentionned by Ciutat et al. (2007) who observed the remobilization and bioavailability of cadmium from historically contaminated sediment. That the Cd enrichment in this layer is the result of the accumulation of fine fraction made of rejected feacal pellets which have a free surface area larger that the coarse particles. This was evidenced by the increase of Cd concentration in this layer and of *R* values (2.4 and 2.3 in {Tub} and {Typ.Tub} units, respectively). These results were in agreement with previous studies that showed how tubificids bioturbation changes the distribution of metals in the sediment column, as well as, the chemical speciation in pore waters (Ciutat et al., 2007, 2006; Peterson et al., 1996).

In sediment inhabited by tubificids, Ciutat et al. (2003 and 2006) also observed cadmium associated with the faecal pellets in the surface layer whose mean grain size was 20 μm in diameter and including 78% of particles <63 μm in diameter. Thus, redistribution of the sediment particles by bioturbation leads to a vertical change in its grain size and the redox conditions in the sediment layers where the tubificid worms act (Table 3) (McCall and Fisher, 1980). Moreover, tubificids through their activity continuously renew anoxic sediment particles coming from the depth to the sediment surface (Ciutat et al., 2005a). We demonstrated here that tubificids influenced significantly the mean grain size of sediment particles in the bioturbation layer, and how this might affect Cd enrichment (Table 4). In the case of cadmium enrichment, grain size also encreased significantly in the first layer, indicating that ecological engineering by bioturbation is active even under such level of the metal contamination.

The same worms' influence in addition to the physico-chemical processes of adsoption and molecular diffusion was observed in all treatments with worms and Cd addition ({Cd.Tub} and {Cd.Typ.Tub}) as indicated the highest values of *R* (3 and 2.6, respectively). This phenomenon mainly results from ingestion of anoxic sediment at depth by tubificids. When this sediment reaches the surface, the redox condition of the newly arrived sediment is favoring the adsoption with consequences on the solid/liquid partitionning of Cd. Thus, the adsoption that was observed in the surface layer of abiotic plots is increased by the bioturbation effect that continuously supplies the sediment surface with new anaerobic sediment with low Cd concentrations.

Likely, the effect of tubificids on Cd partitionning could favor the trapping of Cd from the overlying water into the top layer made of feacal pellets. The bioadvection, evidenced by luminophores, could transport surface sediment with associated Cd at depth until the bottom of the ingestion zone estimated to be 3 or 4 cm in average from previous studies (Ciutat et al., 2006; Delmotte et al., 2007). The metal accumulation in the new layer of faecal pellets may, therefore, be extended deeper than L1, but the choice of having the second layer thickness from 1 to 5 cm has limited this observation. This burial of contaminants is supplying the vicinity of the plant root system with Cd.

At depth, when worms are present, a lower pH (Fig. 4), anoxic conditions, and higher redox potential may also favor the desorption of Cd into the pore water. The release dynamics and mobilization of Cd in a water saturated sediment appeared to be positively related to redox potential (E_H) as governed by the behaviour of dissolved organic carbon and SO_4^{2-} concentrations under oxidizing conditions (Rinklebe et al., 2016; Shaheen et al., 2016), while pH commonly shows a negative relation with dissolved Cd (Rinklebe et al., 2016). Generally, metal cations are released from organic matter and other sorbents such as clay mineral surfaces when pH decrease (Du Laing et al., 2009; Frohne et al., 2014). This contributed to increasing the Cd mobility and thus bioavailability (Lee et al., 2009; Wang et al., 2016) in the deeper layers. By changing the water-sediment ratio, redox conditions in the sediment (Anschutz et al., 2012), and the physico-chemical conditions of the sediment (surface area, oxidation, organic matter digestion, etc..) (McCall and Fisher, 1980; Kristensen et al., 2012; Simpson and Batley, 2003), the presence of tubificids increased ammonia concentrations in interstitial water (Kikuchi and Kurihara, 1977) leading to a decrease of pH gradient in the sediment (Fisher and Matisoff, 1981; Fu and Allen, 1992). Consequently, metal solubility (Probst et al., 2009) and speciation (Ciutat et al., 2007, 2006; Weis and Weis, 2004) increased at depth as a consequence of shifting redox conditions (Du Laing et al., 2009). Finally, even if not considered in this study, it can also be supposed that Cd might compete with other metals present in this "natural" field sediment in term of adsorption/desorption and complexation processes, which might influence Cd behaviour and other physico-chemical parameters (Liao et al., 2007).

The absence of interaction between cadmium enrichment and tubificids addition (three-way ANOVA, Table 5) indicated that the effects of worms bioturbation on Cd concentration in sediment not depend on Cd-enriched or not. In the absence of Cd-enriched, bioturbation influence, linked to the change of sediment texture, on Cd partitionning is still existing in {Tub} and {Typ.Tub} plots (Fig. 5B) due to the amount of Cd that was available in the sediment before starting the experiment (the Cd concentrations ranged from 0.195 ± 0.014 to 0.202 ± 0.003 $\mu g.g^{-1}$ dry wt.). The effect of worms was similar when combined with Cd-enriched (the treatments {Cd.Tub} and {Cd.Typ.Tub}) is interpreted by the assumption of tubificids' tolerance to metal toxicity (Ciutat et al., 2005b).

4.3. Phytoremediation potential with bioturbation

The values of Cd concentration in *Typha latifolia* plants (Fig. 6) were in agreement with previous studies, as well as the overall trend of cadmium and almost other metals in the different organs (roots > leafs) (Klink et al., 2013; Pandey et al., 2014; Sasmaz et al., 2008; Singh et al., 2010). Our study showed higher Cd concentrations in roots of the contaminated treatments ({Cd.Typ} and {Cd.Typ.Tub}) than those of plants growing in the uncontaminated microcosms - {Typ} and {Typ.Tub} (although the difference in both cases was only significant for one of them – {Cd.Typ.Tub} unit) (Fig. 6A). Such a pattern was also reported by Singh et al. (2010) for Cd as well as other metals. It is well known that *Typha latifolia* has a dense root mat leading to a high effective rhizosphere surface area, which favors metal uptake (Pandey et al., 2014). For many plants growing in metal contaminated soils, roots are the specific metal storage compartment, which acts as a barrier to prevent metal transfer to the upper parts (Probst et al., 2009; Liu et al., 2005).

The enrichment for the roots was enhanced when both cadmium and tubificids were occurring together, in comparison with the other treatments without tubificids. The significant effects of cadmium in interaction with tubificids for Cd concentrations in the roots (Fig. 6A and Table 6) indicated that bioturbation generated by worms is favoring the phytoremediation. This phenomenon is only efficient if tubificids transfer the cadmium to deeper layers in the sediment column with a flux that is important enough to change the concentration in the roots of *T. latifolia.* This also confirmed that the depth of the bioturbated layer was deep enough to correspond to the depth of the root system that was estimated to range from 0 to 6 cm. The bioingestion zone should get deeper in the sediment column with time when the tubificids population increases in biomass and organisms size. This extention of the bioingestion zone should allow the burial of contaminants coming from the overlying water in an extended bioturbated sediment down to 5 cm, 7 cm, and even 13 cm after 24, 38, and 93 days, respectively (Ciutat et al., 2006). This vertical extention of the

bioturbation zone should also favor the access to bioavailable Cd in the environment of an extending root system in the sediment column.

Enrichment coefficients for plant roots and vegetative parts (EC_R and EC_L, respectively) (Table 7) indicated the sediment-to-plant mobility of the metal element as well as plant accumulation capacity for phytoremediation purpose. These indexes depend not only on both characteristics of plant species or plant parts and physico-chemical properties of sediment matrix, but also the nature of metal elements (Antoniadis et al., 2017). Metals with high p*K* values tend to have the higher mobility in the sediment-to-plant system (i.e., Cd (10.1), Ni (9.9), Co (9.7), Zn (9.0)) (Antoniadis et al., 2017). Availability of PHEs to plant roots is considered as the key factor limiting the efficiency of phytoextraction (Felix, 1997). Plants only uptake/accumulate metals in solube (bioavailabe) forms as free metal ions, solube metal complexes, or adsorbed form to inorganic soil constituents (Sheoran et al., 2016). In our study, significant highest EC_R values in layer L2 in the condition {Typ.Cd.Tub} at t = 30 (Table 7) provided evidence of an effective improvement of cadmium bioavailability and thus bioaccumulation in the plant's root under the influence of tubificids bioturbation. Therefore, this result demonstrated that bioturbation plays a significant role on the modification of physico-chemical properties of the sediment matrix which influences on cadmium bioaccumulation efficiency. Cd concentration in the deeper layer of the bioturbated zone, L2, is not so important since more Cd effectively arrived into this layer due to tubificids biotransport, but the Cd is stimultaneously removed by plant roots in this horizon.

These results also demonstrated that bioturbation is able to create changes in sediment properties that enhance metal bioaccumulation in *T. latifolia* roots when cadmium addition occurs from the overlying water. Worms were indeed able to resist to Cd contamination and to maintain a significant bioturbation activity in contaminated sediment. Ecological engineers should be able to handle toxicity of the environment where they are supposed to participate at the bioremediation process.

Our results on the bioturbation effect on Cd incorporation and partitioning in the sediment according to depth are in agreement with previous research studies with tubificids populations (Anschutz et al., 2012; Ciutat et al., 2007, 2006; Gerino et al., 2014). Those studies demonstrated complementary effects of bioturbation by this invertebrate species on the physico-chemical properties of sediment. The originality of the results from the present study relies on the positive influence of the bioturbation on phytoremediation performance. We demonstrated here that tubificids create a biological burial of sediment and incorporated contaminant such Cd that renews the pollutant loads in the immediate environment of plants' root system. Consequently, at depth, metal passing from bound to soluble forms, from sediment to pore water (Ciutat et al., 2005a), occurs and thus promotes the absorption by plants (Weis and Weis, 2004), as well as enhances the efficiency of phytoremediation.

TF is an important asset to recognize the phytostabilization potential (preventing the lateral or vertical migration of toxic metals by leachating) of the desired plant (Ma et al., 2001). TF value < 1.0 means that a plant has poor translocation efficiency of metals from root to rhizome, or from the rhizome to leaf, and can be used preferentially for phytostabilization purposes (Garba et al., 2013; Mendez and Maier, 2008). The low values of TF observed for all Cd-treated samples at t = 30 ({Cd.Typ} and {Cd.Typ.Tub}) (Table 7), indicated that *T. latifolia* has a poor translocation efficiency of Cd from its roots to its upper parts. It may also happen a non-proportional translocation into the upper parts, with this Cd behaviour, being previously observed when plants are grown on contaminated substrates by comparing with natural background substrate conditions (Probst et al., 2009). These results are in agreement with previous studies that aldready showed the low value of TF given by *T. latifolia*. Sasmaz et al. (2008) reported the EC_R and TF factors of *T. latifolia* collected from polluted sites by Water Treatmen (Kehli Stream, Elazig, Turkey) ranging from 0.78 to 3.95 and from 0.21 to 0.7, respectively. According to Probst & Guilloux (in prep), *T. latifolia* plants collected from natural background sediment, preferentially store Cd, Cr, Pb, As and U in roots than in their upper parts (leaves, stems; TF factors ≪ 1). This is also confirmed by Pandey et al. (2014) study where TF values of *T. latifolia* growing in flooded and non-flooded areas, were lower than 1.0 for all metals except Mn.

Our study indicated that *T. latifolia* has an important capacity to accumulate cadmium in roots, and its ability to concentrate the metal is enhanced under bioturbation process. Although the transportation of cadmium to the leaves is not so high, in some cases, the absolute quantity of metal stored in leaf parts might be higher than in the root system, even if relative concentrations are not significantly different, due to the main advantage of this riparian plant of having a high biomass of above parts (leaves, steams) compared to the root system (Probst & Guilloux, in prep.).

5. Conclusion

Our preliminary experiment reproduces field conditions where bioturbation caused by tubificid worms' activities in the presence of plants follows the bio-conveying transport model with significant bioadvective rates. This natural process is still efficient under cadmium contamination of 20 μ/L^{-1} in the overlying water. The present study suggests that biotransport enhanced cadmium pumping from the surface to deeper anoxic sediment layers, modified the distribution and speciation of cadmium in the sediment column thereby increasing the bioaccumulation possibility by *Typha latifolia*. It was demonstrated here that bioturbation improves the phytoremediation by the plant's root system. Indeed, more investigations should be done such as metal partitioning among the phases and more precise depth definition in the bioturbation effect and root system. Besides, the efficiency of bioturbation on phytoremediation processes could be tested with different cadmium contamination levels to find the limit of the worms' efficiency as ecological engineers can tolerate. The comparison of Cd bioaccumulation in *T. latifolia* with and without tubificids influence permitted to estimate the positive and complementary influences of benthic faunal bioturbation combined with phytoremediation. This first preliminary and multidisciplinary experiment was positive enough on the advantages of the coupling strategy between bioturbation and phytoremediation to open the door for further estimation of biodiversity influence on cadmium fluxes from overlying water to sediment and from sediment to plants. This study should be carried out via a whole metal budget in the different experimental compartments: overlying water, pore water and sediment, worms, roots and leaves as parts of the plants. Additionally, further research is required to verify the observed mobilization kinetics of Cd with natural water and sediment contaminated with Cd under field conditions. In particular, the role of bioturbation on E_H, pH, and other governing factors changes (i.e., DOC, Mn, Fe, SO_4^{2-}), thus affecting Cd solubility and uptake in the flooded systems should be elucidated more precisely.

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III.A.2. EFFECTS OF TUBIFICID WORMS AND *T. LATIFOLIA* PLANT ON CADMIUM MASS BALANCE AND FLUXES

The water-sediment interface, known to be one of the most vulnerable (Devault et al., 2009), is often assumed to act as a sink receiving a hefty source of pollution from agricultural practices and metropolis areas that may cause long-term effects to the biota by direct uptake or through the food web. Metal contaminants are differentially associated with the three compartments of the water-sediment interface that are the particulate and pore waters fractions of the sediment and overlying waters. The amount of metal in each of this compartment is depending on the partition coefficient Kd of the metal - the ratio of sorbed metal concentration to a dissolved metal concentration that is dependent upon various geochemical characteristics of the soil and its porewater (Reible et al., 1996). By mimicking in-field source ofcontamination from water, our closed experimental system was set up to test the efficiency of the bioremediation on cadmium fluxes between water, sediment, and plant. The previously obtained results (section III.A.1) demonstrated that the phytoremediation potential is enhanced under sediment bioturbation by tubificid's conveyors as well as the metal bioaccumulation temporal dynamic of *T. latifolia* when it is coupled with bioturbation. Tubificid activity was proved to generate a downward bioadvection of Cd into the sediment surrounding the *T. latifolia*'s roots and thus favoring the Cd bioaccumulation into the roots system. However, after the demonstration of bioturbation influence on cadmium concentrations in the different compartments, it may be then relevant to study the variations of the metal quantities between these compartments in the same experimental conditions. The comparison of Cd quantities in water, sediment, with and without the worms and the plant influences will permit to quantify the influence of the biodiversity on the cadmium fluxes through the compartment interfaces. These fluxes are making part of the Cadmium mass balance in the different treatment conditions.

The mass balance concept has been important to the development of science in general, and it has played an especially significant role in the environmental sciences

(Groffman et al., 2004). A mass balance study essentially accounts for all sources, pathways, transformation, and sinks for a particular pollutant in a given area. The mass balance approach traces the flow of a pollutant through an area, in which a chemical, physical, or ecological interaction and a mass flow rate are quantified.

The mass balance for any material in an ecosystem in one compartment can be represented by a general equation (Groffman et al., 2004):

Input + generation – Output – consumption = Change of stored or accumulated quantity

It should be noticed that *generation* and *consumption* terms refer only to the generation of products and consumption of reactants as a result of a chemical reaction. In case of no chemical reaction as for the Cadmium these terms are zero.

Mass balance studies were thus applied to estimate metal fluxes that flow through each of the interfaces of water and sediment and sediment to plant within the closed experimental aquatic system under effects of tubificids worm's bioturbation and *T. latifolia* plant.

III.A.2.1. Calculation of mass balance and percentage relative recovery of cadmium in experimental compartments.

In overlying water compartment

Mass of cadmium in overlying water was calculated based on the following formula:

Mass of cadmium in overlying water (µg) = total concentrations (dissolved and particulate fractions) of cadmium in overlying water (µg/L) x volume of overlying water column (L).

Since cadmium concentration was determined in the dissolved fraction only, the concentrations of the particulate fraction was estimated based on the research of Ciutat et al., (2005), who performed the same type of experiment, with the same source of Cd

as experimental contaminant with initial concentration of 20 µg.L^{-1}, in the overlying water. Ciutat et al. (2005) recorded the proportion of dissolved and total concentration of cadmium in the overlying water of microscosms with and without tubificid addition. The author indicated that mean dissolved Cd concentration represented 86% and 98.4% of total Cd concentrations of this compartment with and without tubificidsrespectively.

In sediment compartment

Mass of cadmium in sediment was calculated based on the following formula:

The mass of cadmium in bulk sediment (µg) = total cadmium concentrations of all the different fraction sizes in the bulk sediment (µg/kg dry wt.) x weight of the bulk dried sediment (kg dry wt.).

The weight of bulk sediment (kg dry wt.) = volume of sediment (cm^3) x sediment density (g dry wt.cm^{-3})/1000. Densities of sediment were determined in different sedimentary layers with and without bioturbation (see *Annex 2*).

Since the cadmium concentration was determined only in fine sediment (fraction < 63µm), the cadmium concentration in the bulk sediment was calculated based on the Distribution Factor (DFx) given by Acosta et al., (2009) following the formula:

$$DFx = Xfraction/X\ bulk$$

in which: Xfraction and Xbulk are contents (mg.kg^{-1}) of heavy metal in a given fraction and into the bulk sample, respectively. This factor was used to estimate in which size fraction the heavy metals are preferentially enriched. According to Gong et al. (2014), the smallest fractions (<53µm) occupied only 5.08 to 9.57 %, and they had the highest distribution factor (DF) of 3.5 for cadmium.

The total cadmium concentrations in bulk sediment were therefore calculated following the formula:

The total cadmium concentration in bulk sediment (µg/kg) = total cadmium concentration in fine sediment (fraction < 63 µm) (µg/kg) / Distribution Factor of 3.5 (DF).

In plant compartment

Mass of cadmium in the plant was calculated based on the following formula:

Mass of cadmium in the plant (µg) = sum of the mass of cadmium in each plant's part (leaves + rhizome + root) (µg/kg dw.).

Mass of cadmium in each plant's part = cadmium concentration in plant's part (µg/kg dw.) x biomass of plant's part (kg dw.)

Since the cadmium concentration in *T. latifolia* was determined only in leave and root parts, the cadmium concentrations in the rhizome part were calculated based on the ratio of Cd accumulation in the different part of the *Typha latifolia* under the non-flooded condition given by (Pandey et al., 2014). In this study Cd concentration in the rhizome was estimated about 57% of total cadmium concentration in root systems.

In the worms:

In the experimental treatments having Cd-enriched from overlying water ({Cd.Tub} and {Cd.Typ.Tub}), Cd quantity bioaccumulated in the tubificid worms was estimated based on the bioaccumulation rate of about 50 µgCd.g^{-1} dry wt of worms during 56 days as previously estimated by Ciutat et al., (2005), who performed the same type of experiment, with an initial concentration of 20 µg.L^{-1} introduced from overlying water. This estimation took into account the experimental duration of 30 days as well as the tubificid population of 135 worms.dm^{-2}, compared to 56 days and 600 worms.dm^{-2} carried out in the study of Ciutat et al. (2005).

In the absence of Cd-enriched ({Tub} and {Typ.Tub} treatments), the amount of Cd lost after the experiment was mainly allocated to the worms accumulation and to the attachment by plastic material of the experimental pail wall. In this case, Cd

quantities incorporated to plastic material (ΔQ_5, Fig. III.1) were calculated based on the difference in total Cd quantities in each microcosm between before (t=0day) and after (t=30 days) the experiment, after having taken into account the Cd amount lost due to quantities accumulated in the worms.

In microcosm

The mass of cadmium in one microcosm is estimated as the sum of cadmium mass in all compartments : water, sediment, plant, and worms. The percentage of relative recovery of cadmium is the total mass of cadmium in each microcosm at the end of the experiment (t = 30) divided by the total cadmium mass that was added at the initial time (t = 0). These data are provided in Table III.1.

III.A.2.2. Estimated mass balance and percentage relative recovery of cadmium

Table III.1 shows the total quantity of Cd in the whole microcosm before (t=0) and after (t=30) the experiment. A marginal loss of the metal estimated to 5.8 %, with about 94.20% of relative recovery was observed in the {control} treatment, with the absence of any abiotic factor, i.e. worms and plant. This implied that this loss was due to the Cd attachment to the experimental containers' walls which are made of plastic material. Similar levels of relative recovery were also observed with no significant differences of the values ($P > 0.05$, Dunnett's multiple tests, Table III.1) between the other treatments without tubificid addition ({Typ} and {Cd.Typ} treatments) and the {Control} treatment, except for the {Cd} treatment ($P < 0.05$ Dunnett's multiple test, Table III.1).

A significant difference in the relative recovery was observed in the {Cd} treatment with the percentage of 79.13%, compared to the value of the {control} treatment that could be explained by the physical sorption of Cd to the containers made of plastic. In this case, presumably, Cd remained a longer period of time in the overlying water after the enrichment to the overlying water at the initial time. The experiment lasted for 30 days under steady-state conditions without any magnitude

mixture or transport from the water into the sediment or another compartment due to the absence of bioturbation or plant. The only transport from water to sediment was due to the physical process of the molecular diffusion into the interstitial water of the surface sediment. Plastic-metal interactions have been described previously as a consequence of the adsorption of chemical contaminants, such as organic compounds (Rochman et al., 2013), or metals (Holmes et al., 2012), by plastic debris from surrounding water. The loss of trace metals on container walls during experimental manipulations (sampling, handling, storing) of aqueous solutions has been reported by (Massee, et al., 1981; Struempler, 1973). According to these authors, the amount of trace metals, including Silver, Arsenic, Lead, Zinc, or Cadmium was lost on an experimental container made of plastic material has a close correlation with aqueous pH medium. These studies also confirmed that no sorption of Cd on polyethylene container occurs below pH 2 (Struempler, 1973) and pH 4 (Massee et al., 1981). Shendirkar et al., (1976) did not observe significant sorption of Cd at pH below the value of 7, while (Gardiner, (1974) reported a 10% loss of cadmium from the natural water on the same material at pH 7.5-8.0. Massee et al., (1981) indicated that not only pH but also the ratio – *R* between the inner container surface and the volume of the solution (unit of cm^{-1}) are the dominant factors controlling the sorption of cadmium. Accordingly, his experiment set up during 28 days at pH 8.5 resulted at 12% and 56 % of Cd lost with the ratio - *R* of 1.4 and 3.4, respectively. Our experiment was carried beyond the 30-day period, the amount of Cd lost from the plastic container in the {Cd} treatment was estimated to 16.11% at pH in overlying water of 8.2 (Fig. 4, section III.A.1) with the ratio - *R* of 0.37 cm^{-1} (inner surface of container = 1789.94 cm^2; volume of water = 4783.85 cm^3). The calculated Cd lost was about an order of magnitude higher than that reported by Massee et al., (1981) with at pH 8.5 and *R* 1.4. This data calculation was taken into account the amount of Cd physically accumulated to sediment particles at the surface (layer L1: 0 – 1cm, Fig III.1) from the overlying water.

Table III.1
Cadmium mass balance and percentage of relative recovery of cadmium after the experiment in microcosms.

Treatments	*time*	total quantity of Cd in microcosm (µg) (mean ± SD, n =3)	Relative recovery (%) (mean ± SD, n =3)	P value
{Control}	*0 day*	417.37 ± 20.82	-	-
{Tub}	*0 day*	417.37 ± 20.82	-	-
{Typ}	*0 day*	419.26 ± 21.84	-	-
{Typ.Tub}	*0 day*	419.26 ± 21.84	-	-
{Cd}	*0 day*	513.34 ± 20.81	-	-
{Cd.Tub}	*0 day*	513.34 ± 20.81	-	-
{Cd.Typ}	*0 day*	515.23 ± 21.83	-	-
{Cd.Typ.Tub}	*0 day*	515.23 ± 21.83	-	-
{Control}	*30 days*	393.59 ± 32.65	94.20 ± 3.13	-
{Tub}	*30 days*	304.64 ± 9.55	73.04 ± 1.36***	0.0007
{Typ}	*30 days*	388.93 ± 38.14	92.62 ± 4.28ns	0.9994
{Typ.Tub}	*30 days*	320.66 ± 29.65	76.37 ± 3.10**	0.0036
{Cd}	*30 days*	407.22 ± 54.26	79.13 ± 7.37*	0.0136
{Cd.Tub}	*30 days*	384.85 ± 10.48	75.00 ± 1.00**	0.0019
{Cd.Typ}	*30 days*	439.32 ± 19.09	85.26 ± 0.26ns	0.2134
{Cd.Typ.Tub}	*30 days*	404.16 ± 72.93	78.14 ± 10.85**	0.0084

*ns indicates nonsignificant differences and *, **, *** indicate significant differences at P < 0.05, 0.01, and 0.001, respectively of relative recovery between a given treatment and the {Control} treatment at t=30 (Dunnett's multiple comparison test). See Table II.1 for the definitions of acronym corresponding to the different treatments*

III.A.2.3. Cd quantity in sediment and transport of the contaminant under bioturbation

Environmental Cd quantity in sediment was found at 417 ± 20 µg before adding the contaminant into the overlying water. At t = 30 days, the amounts in the sediment of microcosms without Cd-enriched were lower than the initial condition, mainly in deeper layers (from 1 to 9 cm, layers L2 + L3), probably due to the loss of Cd to the experimental containers as denoted by the increase of ΔQ_{5b} values in the Fig. III.1.

In the absence of Cd-enriched, a negligible amount of Cd from the surface sediment (layer L1) was released into the overlying water by physical diffusion of the contaminant from a region of high concentration (sediment) to a region of low concentration (overlying water), with the small values of ΔQ_1 increased in the overlying

water in {Control}, {Tub}, {Typ}, and {Typ.Tub} treatments. Small levels of contaminant fluxes – F2 from sediment to the overlying water in these microcosms were, therefore, calculated that indicated a negligible mobilization from sediment to water for Cd.

About treatments having worms' addition ({Tub} and {Typ.Tub}), previous studies revealed a significant release of cadmium-induced by bioturbation from the sediment to the overlying water, mostly in the particulate form (Ciutat et al., 2007; Rasmussen et al., 2000). Schaller (2014) indicated that bioturbation caused by invertebrate *Chironomus plumosus* strongly affects remobilization from sediment into the water column for some metal elements, such as Mg, Ca, Sr, Mo, and U. For some other elements, such as Mn, Ni, As, or Cd, the remobilization was mentioned to release them to water when the invertebrates were burrowing (Schaller, 2014). His experiment reported a significant remobilization rise of Cd concentrations in the overlying water during the first 5h under bioturbation at 5 times orders of magnitude higher than that in the control treatment and then followed by a decrease after one day. In the absence of bioturbation, Cd was observed to be released from the sediment in dissolved form, by molecular diffusion (Ciutat et al., 2007). Accordingly, the remobilization from sediment to the overlying water for Cd could, therefore in our experiment, be explained by either the molecular diffusion or bioturbation. A higher quantity of Cd was observed in the enriched L1 in the treatment with tubificid but without Cd addition ({Tub} and {Typ.Tub}) that was explained previously (*see §4. Discussion, section III.A.1*) with the same pattern being observed about the concentrations in this surface layer. Bioturbation process modifies sediment texture to finer particles (84.5 to 87.1 % of fine particle in the top layer, Table 4, section III.A.1) in the top layer, thus results in an increase of Cd quantity due to higher adsorption surface of finer sediment particles for Cd.

When Cd was present, after 30 days, the rapid adsorption of Cd on surface sediment particles followed Cd-introduction in overlying water at t=0. This rise was again confirmed by the amount of Cd in the top layer as indicated by the positive value

of ΔQ_{2a} in the enriched layer - L1 for the microcosms having Cd in the treatments ({Cd}, {Cd.Tub}, {Cd.Typ}, and {Cd.Typ.Tub} units).

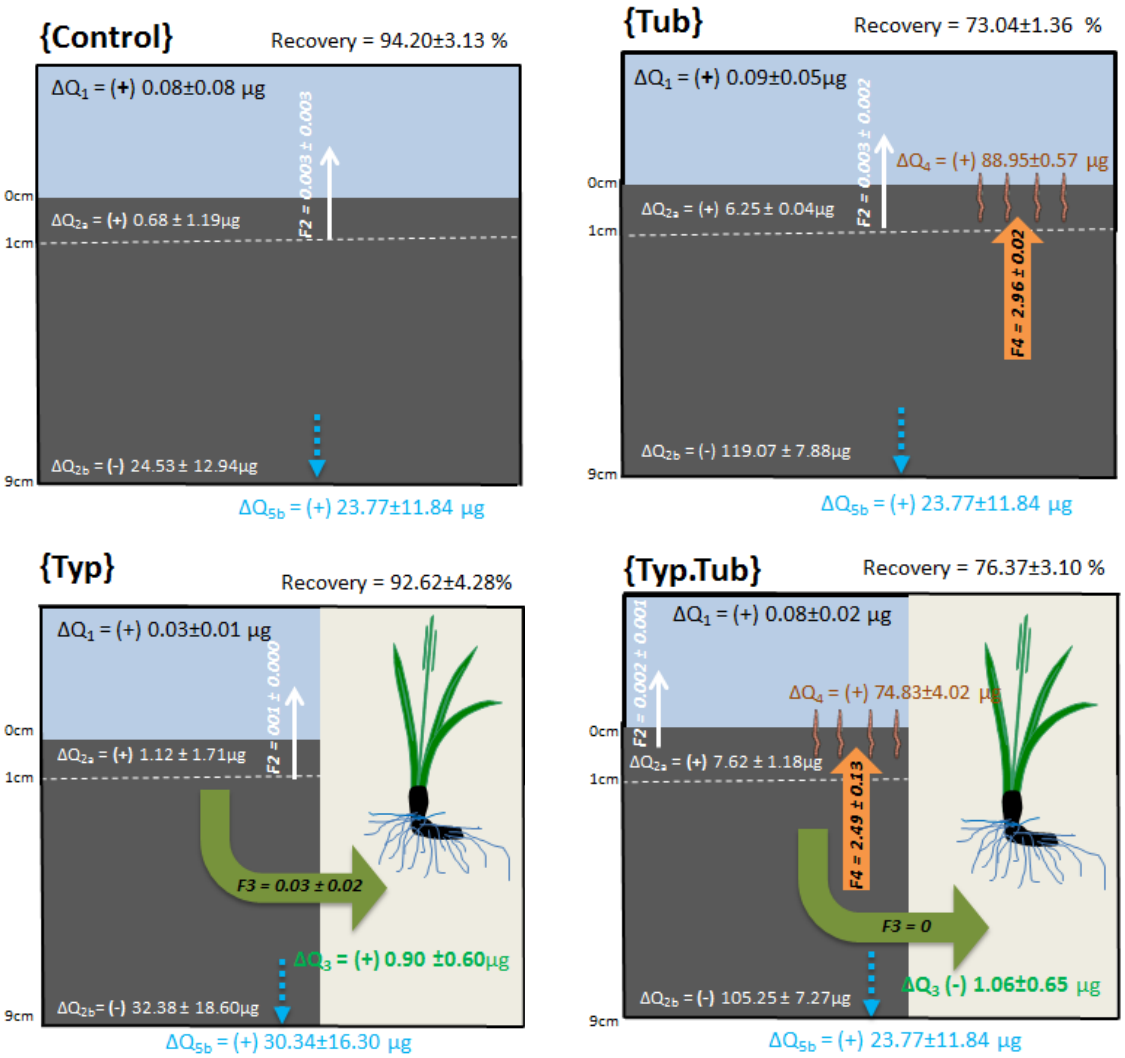
At the same time, significant decreases of the initial Cd quantity (96 µg) in the overlying water with the negative values of ΔQ_1 being equal to 95.88, 95.86, 95.84, and 95.67 µg were observed in microcosms having Cd treatments ({Cd}, {Cd.Tub}, {Cd.Typ}, and {Cd.Typ.Tub} units, respectively). The rapid adsorption process of Cd on the surface sediment was previously discussed (*see §4. Discussion, section III.A.1*) as influenced by the high pH conditions (> 7.5) (N'guessan et al., 2009; Pokrovsky et al., 2012; Santillan-Medrano and Jurinak, 1975).

Budgets of Cd in the microcosm are the descriptions of Cd flux from one compartment to another (Fig.III.1). Without bioturbation, the flux F1 of 1.79 ±1.48 µg/day from water to sediment was observed in the treatment having both Cd and plant addition ({Cd.Typ}). This flux was estimated higher than the one of 0.78 ±0.43 µg/day estimated in the {Cd} treatment where only physicochemical processes of adsorption and molecular diffusion of Cd were occurring. The fluxes difference between {Cd.} and {Cd.Typ} is allocated to the effects of the plant that can only uptake Cd in pore water. So that it is suspected that plant uptake in pore water, leads to an increase the concentration gradient between sediment and water and therefore increase the fluxes from overlying water to sediment.

The effect of tubificids on the fluxes of Cd from overlying water to sediment is demonstrated in treatments having both worms and Cd addition ({Cd.Tub} and {Cd.Typ.Tub}). The highest values of fluxes – F1 from overlying water to sediment of 2.83+/-0.00 µg/day were observed in both cases (two-way ANOVA) with bioturbation. These higher fluxes values with bioturbation lead to increases in metal quantity in the sediment when bioturbation is happening. This phenomenon is explained by the accumulation of fecal pellets at the sediment surface that results from ingestion of anoxic sediment at depth by tubificids. When this sediment reaches the surface, the

redox condition of the newly arrived sediment is favoring the adsorption with consequences on the solid/liquid partitioning of Cd. Thus, the adsorption that was observed in the surface layer of abiotic plots is increased by the bioturbation effect that continuously supplies the sediment surface with new anaerobic sediment with low Cd concentrations.

ΔQ_{2b} are representative of the amounts of Cd lost from the sediment during the experimental duration. With negative values of ΔQ_{2b} estimated to 60.69 ± 11.10 and 40.53 ± 59.62 µg Cd in both {Cd.Tub} and {Cd.Typ.Tub} treatments, respectively. These losses are explained as mainly due to worms bioaccumulation and physical attachment to the plastic containers in {Cd.Tub}(Fig.III.1), and with *T. latifolia* uptake in addition in case of {Cd.Typ.Tub} treatments.



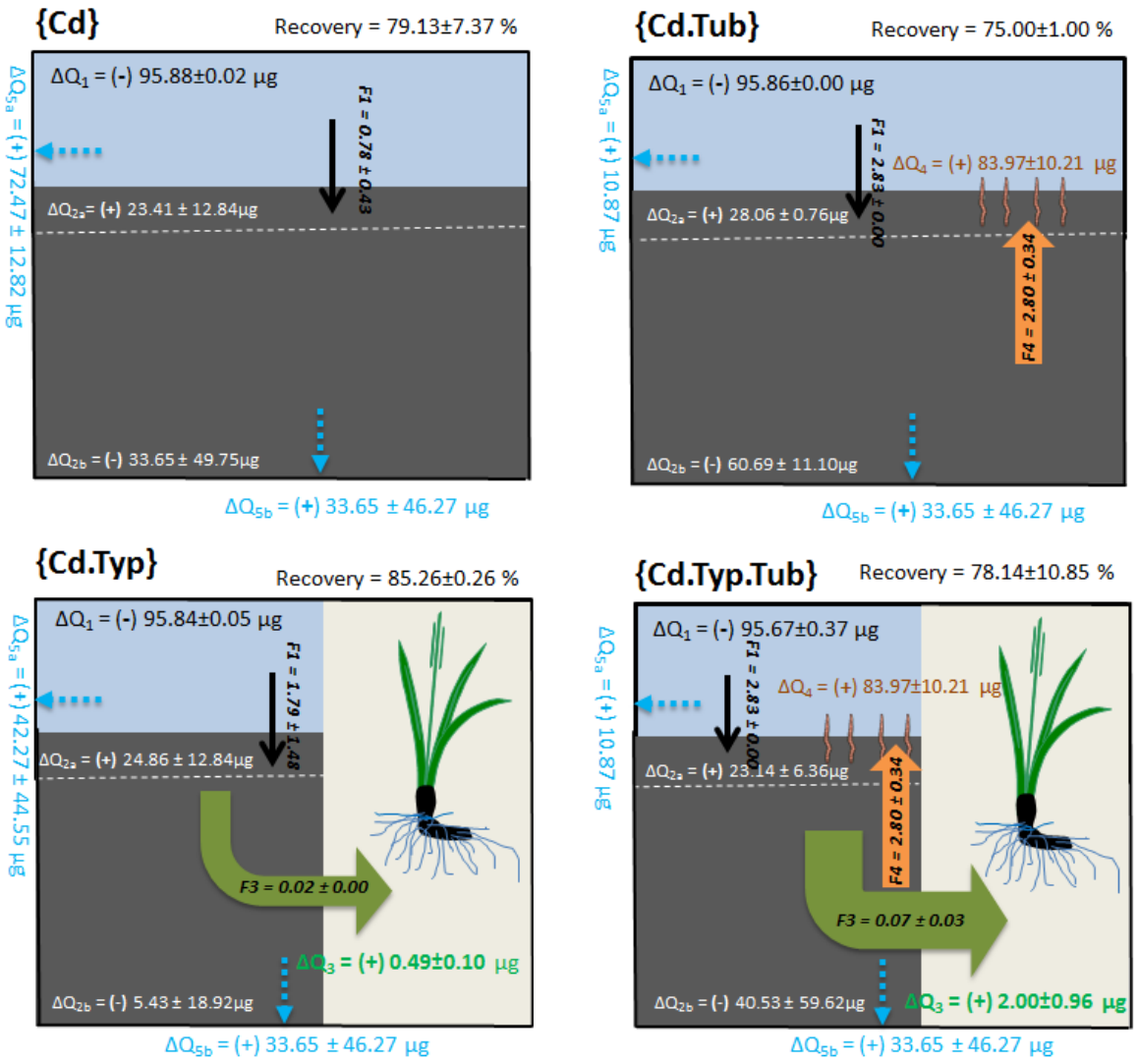


Figure. III.1. Change (ΔQ) in Cd quantity before (t=0 day) and after the experiment (t=30 days) and estimated fluxes of total Cd between experimental compartments.

F1, F2, F3, F4 are fluxes from water (blue block) to sediment (black block), from sediment to water, from sediment to plant (tan block), from sediment to worms body, respectively.

White and black solid arrows represent transport of Cd from sediment to water and water to sediment compartments, respectively. Green arrows represent the fluxes of Cd from sediment to plant compartment. Blue and red broken line arrows represent lost of the contaminant out of the microcosm due to: (1) attachment to pail's wall, (2) worms bioaccumulation.

$\Delta Q_{1,2a,2b,3,4,5a,5b}$ values (mean ± SD) in each compartment (water, n=3; sediment, n=2; plant, n=3) imply the estimated change in Cd quantity between t = 30 and at t = 0 day in water, sediment, plant, worms, experimental containers from water and from sediment, respectively.

Table III.2.
Effects of Cd enrichment, tubificids, and plant occurrence on Cadmium quantities in the top layer (0 to 1 cm) and in the deeper layer (1-9 cm) to investigate the interactions between experimental treatments.

Experimental treatments	Cd quantity in layer 1 (0-1cm)		Cd quantity in layer 2 (1-5cm), and layer 3 (5-9cm)	
	$F_{1,8}$	P	$F_{1,8}$	P
Cadmium enrichment	22.552	**0.001447****	2.7856	0.13366
T. latifolia treatment	0.0087	0.927811	0.4144	0.53775
Tubificid addition	0.7234	0.419769	7.3939	**0.02628***
Cd:Typ interaction	0.0898	0.772012	0.2523	0.62897
Cd:Tub interaction	0.2688	0.618166	1.5541	0.24779
Typ:Tub interaction	0.0947	0.766185	0.0260	0.87593
Cd:Typ:Tub interaction	0.1716	0.689608	0.1240	0.73383

(*) and (**) indicate significance at p ≤ 0.51 and 0.01, respectively; (ns)-non significance according to three-way ANOVA

The effects of cadmium enrichment ($F_{1,8}$ = 22.552, P < 0.01), tubificid and *T. latifolia* addition on Cd quantities in the top layer – L1 and the deeper layers L2 and L3 were analyzed using the three-way ANOVA (Table III.2). There were no significant interactions between these factors (three-way ANOVA, P > 0.05). Although tubificids can enhance Cd incorporation into the sediment surface layer ($F_{1,11}$ = 7.39, P < 0.05) where worms fecal pellets accumulate at the sediment surface and then creating changes of the sediment properties in this area (Table 4 and 5, section III.A.1). The increased amount of Cd (ΔQ_{2a} in layer L1 in all treatments) at the end of the experiment (t=30) are dependent on not only the new arrival of Cd from the overlying water but also on the transport from surface to deeper sediment layers generated by bioturbation and the loss by worms' bioaccumulation on their tissue. The bioadvection, evidenced by luminophores, transported surface sediment with associated Cd at depths until the bottom of the ingestion zone estimated to be 3 or 4 cm in average from previous studies (Ciutat et al., 2006; Delmotte et al., 2007). The metal accumulation in the new layer of fecal pellets may, therefore, be extended deeper than layer L1, but the choice of having the second layer thickness from 1 to 5 cm has limited this observation. This metal

accumulation is known from treatment with without Cd-enrichment, where the significant increase (ΔQ_{2a} in layer L1 of {Tub} and {Typ.Tub} units), mainly due to initially existing cadmium in natural sediment was associated with the fecal pellets accumulation created by bioturbation, although to a lesser extent. This obtained result, all together, explained why bioturbation effects on the Cd quantity are only observed in the deeper zone, but not in the top layer, according to the three-way ANOVA (table III.2).

Table III.3.
Two-way ANOVA performed on cadmium fluxes-F3 (from sediment to *T. latifolia* plant) to investigate the interaction between experimental treatments having plant.

Experimental treatments	Fluxes - F3	
	$F_{1,8}$	*P*
Cadmium enrichment	8.308	**0.0204***
Tubificid addition	0.9231	0.3648
Cd:Tub interaction	14.77	**0.0049****

(*) and (**) indicate significance at p ≤ 0.05 and 0.01, respectively; (ns)-non significance according to two-way ANOVA

Table III.4.
Tukey's multiple tests performed on cadmium fluxes-F3 (from sediment to *T. latifolia* plant) to compare the experimental treatments having plants.

Experimental treatments	Fluxes - F3	
	P	Significance
{Typ} *vs.* {Cd.Typ}	0.9021	ns
{Typ} *vs.* {Typ.Tub}	0.2513	ns
{Typ} *vs.* {Cd.Typ.Tub}	0.0991	ns
{Cd.Typ} *vs.* {Typ.Tub}	0.5553	ns
{Cd.Typ} *vs.* {Cd.Typ.Tub}	**0.0381**	*
{Typ.Tub} *vs.* {Cd.Typ.Tub}	**0.0063**	**

(*) and (**) indicate significance of P ≤ 0.05 and 0.01, respectively; (ns)-non significance according to two-way ANOVA

The transport of Cd from sediment to the plant was noted as the fluxes - F3 ($\mu g.day^{-1}$) calculated in all treatments having *T. latifolia* ({Typ}, {Typ.Tub}, {Cd.Typ}, and {Cd.Typ.Tub}, Fig.III.1). Significant effect of cadmium enrichment on the Cd fluxes – F3

from sediment to *T. latifolia* ($F_{1,8}$ =8.308, P<0.05) as well as a greater extent of the interaction between the two tested factors (Cd and tubificid) ($F_{1,8}$ =14.77, P<0.01), were evidenced by a two-way ANOVA (Table III.3). The Cd fluxes increase significantly in the treatment having both cadmium and worms ({Cd.Typ.Tub}), compared with the treatment having tubificid only ({Typ.Tub}) (Tukey HSD, P < 0.01, Table III.4). When Cd was enriched, a significant increase of the plant uptake was also observed with bioturbation ({Cd.Typ.Tub} treatment), when compared with treatment having no bioturbation ({Cd.Typ}) (Tukey HSD, P <0.05, Table III.4). No significant difference was observed between the two treatments {Cd.Typ} and {Typ} (P >0.05) according to Tukey posthoc test (Table III.4). Although, the amount of Cd transferred from sediment to plant in the presence of tubificid worms ({Cd.Typ.Tub} treatment) was estimated much lower than the loss due to the worms' bioaccumulation (Fig. III.1), our results, at least, demonstrated the influence of bioturbation on changing sediment properties that enhance the Cd bioaccumulation in *T. latifolia* when cadmium addition occurs from the overlying water. In addition to the significant role on the physic-chemical properties modification of the sediment matrix (see §3. Results - *Cd concentration in sediment*, section III.A.1) which influences on cadmium bioaccumulation efficiency and sediment-to-plant mobility (see §3. Results - *Indicators of phytoremediation potential*, section III.A.1), tubificid worms were proved to be able to accumulate Cd as a rapid and efficient process to resist to Cd contamination in aquatic sediment (will be discussed in the next part III.A.3). These results also demonstrated that the transport of Cd from sediment to the plant is only enhanced if both cadmium and tubificid were added together (Fig. III.1). The worms bioaccumulation is influencing cadmium mass balance also to the plant uptake so that the two processes occur in parallel.

III.A.3. TOXICITY OF CADMIUM AND THE POSSIBILITY TO APPLY THE BIOTURBATION AS A BIOREMEDIATION STRATEGY

All aquatic invertebrates are known to accumulate trace metals in their tissue, whether essential or not (Eisler, 1981), either from the aquatic medium or from food with the vast range of accumulated trace metal concentrations (Rainbow, 2002). Several benthic invertebrates such as freshwater oligochaetes worms, widely distributed in contaminated surficial sediments, have been proved to be tolerant to pollution. Due to an affinity for sulfur and nitrogen molecules (Nieboer and Richardson, 1980) containing in amino acids, trace metals the have potential to bind to proteins within living cells (Rainbow, 2002). Toxicity of a trace metal to aquatic invertebrates will depend on their accumulation pathways as either a metabolically available or detoxified types which are induced by either essential or non-essential meals (Rainbow, 2002). Cadmium, a non-essential metal for aquatic invertebrates, which is defined as in the detoxified category, is suspected to represents no danger to the metabolism of the animal to a certain threshold of concentrations in the surrounding environment Rainbow (2002) furthermore mentioned that he trace metals may undergo detoxified forms of cadmium, lead, or mercury with no requirement of minimum concentration and need to be detoxified. They might be accumulated by invertebrates with no threshold of total metal concentration as well as no relationship between total body metal concentration and toxicity (Rainbow, 2002). Toxic effects as well as bioaccumulation of Cd in oligochaetes organisms such as *Tubifex* worms (Bouche et al., 2000; Bervoets et al., 1997; Ciutat et al., 2005), *Lumbriculus variegatus* (Ankley et al., 1994), have been previously studied.

III.A.3.1. Toxic effects and bioaccumulation of cadmium in oligochaete worms

Toxicity of Cd in oligochaetes worms have been reported in various cases. Bouche et al. (2000), studying the acute toxicity of Cd in *Tubifex tubifex,* indicated that sublethal toxicity with morphology impacts, starting mortality, and complete mortality (after 3 days) were observed at 10, 20, and 100 $\mu g.L^{-1}$ Cd, respectively. Previous studies also indicated that difference in test conditions, such as water or sediment environment,

pH, O2 saturation, or water hardness, etc. may significantly influence the test results. Acute toxicity tests studied by Brkovic-Popovic and Popovic (1977), Bouche et al., (2000), and Chapman et al., (1982) under different conditions using distilled water or hardness-water as a dilution medium for Cd exposure presented various Cd concentrations as LC50 after 96h for Tubifex worms, of 0.003 and 0.03 $mg.L^{-1}$, 0.32 $mg.L^{-1}$ (hardness of 5.3$mg.L^{-1}CaCO_3$). Some other authors presented higher LC50 values for Tubifex after 96h, at 1.032 mg/L (Fargasova, 1994), or 47.5 mg/L (Khangarot, 1991).

In our experiment, the tubificid worms consisted of a mixture of three species: *Tubifex tubifex, Limnodrilus hoffmeisteri, and Limnodrilus claparedeianus*. Sediment reworking intensity by these invertebrate species was quantified using luminophores as conservative tracers. After the introduction of the worm at the surface of the sediment, cadmium was added into the overlying water with the initial concentration of 20µgCd/L (*see §2.2. Experimental design and microcosm setup,* section III.A.1). No significant difference for the bioadvective rates - *V* was observed when comparing experimental treatments with or without Cd-enriched (Fig. 3, section III.A.1). The absence of interaction between cadmium enrichment and tubificid addition factors (three-way ANOVA, Table III.3) also confirmed that the effects of worms bioturbation on the biological reworking in sediment via the bioadvective rates do not depend on Cd-enrichment or not. The obtained rates of bioadvection in presence of tubificids (Fig. 3, section III.A.1) were also in the same order of magnitude of those estimated by other previous authors with tubificids population of various densities (Ciutat et al., 2005b; Mc Call and Fisher, 1980; Matissof et al., 1999).

In a special case, with Oligochaeta *Linnodrilus hoffmeisteri* worms inhibiting the Foundry Cove site (Hudson River, New York, USA), one of the most severely Cd contaminated site in the world, this species was observed to survive a 28-day exposure to extremely high level of Cd in sediment ranged from 500 to more than 200,000 $\mu g.g^{-1}$ dry wt. (Klerks and Bartholomew, 1991; Knutson et al., 1987; Wallace et al., 1998). These

authors also reported the great ability of the worms to accumulate Cd until 1800 $\mu g.g^{-1}$ dry wt. (Klerks and Bartholomew, 1991; Wallace et al., 1998). Regulating, storing, and detoxifying trace metals were found related to higher metallothionein-like (MT) protein and metal-rich granules (MRG) possessed in the worms that make them resistant to trace metals, such as cadmium, mercury, zinc, copper, gold, and silver (Klerks and Bartholomew, 1991; Roesijadi, 1992). A genetic study by Martinez and Levinton (1996) indicated that the resistance of the Oligochaete *Linnodrilus hoffmeisteri* worms evolved within 4 to 36 generations.

Likewise, Cd bioaccumulation by aquatic invertebrates depends on the dose, duration of exposure, and environmental concentrations (sediment or water-only conditions) (Rainbow, 2002). Cd bioaccumulation in Tubifex worms was found significantly correlated to the metal concentrations in both sediment and overlying water (Łuszczek-Trojnar et al., 2014). Cd accumulated by the worms is a rapid and efficient process with bioaccumulation rates of 80.4 and 189.5 $\mu g.g^{-1}$ dry wt. depending on the exposure concentration of 5 and 10 $\mu g.L^{-1}$ Cd from water, respectively (Bouche et al., 2000). In sediments, the highest Cd concentrations (1.1 ± 0.002 mg/kg) were observed in Tubifex worms after 7 days of exposure to 2.5 mg Cd/kg (Łuszczek-Trojnar et al., 2014). In agreement with the present study (with the same Cd concentration contaminated and similar type of tubificid worms used), results by Ciutat et al., (2005) also noted the high level of Cd bioaccumulation in tubificids worms at 47.1 ± 8.9 $\mu gCd.g^{-1}$ dry wt. after 56 days of exposure to a constant Cd concentration in the overlying water of 20 μg/L. The study also suggested the relation of detoxification/ sequestration processes to the resistance of the tubificid worms. Based on the bioaccumulation rate of about 50 $\mu gCd.g^{-1}$ dry wt. estimated by Ciutat et al., (2005), we calculated the Cd quantity accumulated in the experimental worms might reach about 83.97 ± 10.21 μg Cd (Figure III.1). This amount was, therefore, applied to our microcosm having Cd-enrichment as denoted by the positive value of ΔQ_4 presented in the {Cd.Tub} and {Cd.Typ.Tub} treatments (Figure III.1). This calculation took into account

the experimental duration of 30 days as well as the tubificid population of 135 worms.dm^{-2}, compared to 56 days and 600 worms.dm^{-2} carried out in the study of Ciutat et al., (2005). In the absence of Cd-enrichment ({Tub} and {Typ.Tub} treatments, the calculated Cd quantities accumulated in the worms was estimated to 88.95 ± 0.57 and 74.83 ± 4.02 µg Cd, respectively, and were found in the agreement with the estimated value from Ciutat et al., (2005).

III.A.3.2. Possibility to apply tubificid worms as a bioremediation strategy in metal polluted aquatic systems.

Tubificid worms are typically found in heavily polluted rivers and lakes with high densities of over a million individuals per square meter (Appleby and Brinkhurst, 1970). Some species are not only able to regulate respiration at low oxygen concentrations, but also to carry out other vital activities under these conditions (Aston, 1973).

Although toxicity test was not conducted in the present study to give evidence on the epersistence of the tubificid worms to cadmium in different levels, at least, our study implied that bioturbation still remains active in natural aquatic sediments that do not exceed the used concentration at 20µg/L which is frequently found in contaminated waters from mining wastes (Salvarredy-Aranguren et al., 2008) or industrial sites (Andres et al., 1999). The involvement of efficient detoxification mechanisms explained why tubificids worms can accumulate more significant amounts of Cd without exhibiting any toxic symptoms.

To handle pollution in aquatic systems contaminated by multiple metals, bioremediation strategy relied on the bioturbation process should take into account the accumulation patterns for different trace metals. Non-essential metals (for invertebrates metabolism) in detoxified forms like Cd, such as Pb, Hg would have no required minimum concentration and need to be detoxified (Rainbow, 2002). For essential metals, such as Zn or Cu, a certain amount is required to play essential roles in

metabolisms, such as an enzyme or respiratory protein operation. An extra amount of such metals accumulated has the potential to be toxic to invertebrates, initially sublethal, but eventually lethal (Rainbow, 2002).

The study of Back (1990) on Cd and Zn accumulation in *Tubifex* and *Limnodrilus* worms indicated that Cd (non-essential metal) was more enriched than Zn (essential metal) in both worms biomass. On the other and, an increase in bioavailability of a trace metal will cause an increase in the uptake rate of that metal into the aquatic invertebrates body (Rainbow, 2002). The median tolerance limit (i.e. the concentration at which 50% of the worms survived for 24 h) for lead (Pb) was 49 ppm at a pH of 6.5 and 27.5 ppm at a pH of 8.5. For zinc, the median tolerance limit was 46.0 ppm at a pH of 7.5. The combination of copper and lead ions has been examined by (Jones, 1938) with the decrease of toxicity to *Tubifex tubifex* as an antagonism.

On the other hand, the possibility to apply bioturbation as a bioremediation strategy for trace metals also needs to take into consideration their mobility within a solid and liquid matrix. Solid/liquid partition coefficient - Kd for the metal in sediment indicates mobility of trace metals between water and sediment as well as its bioavailability for the bioremediation purpose. The index depends not only on physicochemical properties of a material (suspended matter, sediment, or soil, etc.) but also the nature of metal elements. Accordingly, the patterns of decreasing Kd for metals in sediment was reported by Allison et al. (2005) in the order: Pb > Hg > Cr^{III} > Cu > Ni > Zn > Cd > Ag > Co > As. Metals with higher mean Kd values, such as Cu (Kd = 4.2), Cd (Kd = 3.6) (Table 1, EPA, 2005) tend to have the higher capacity of the mobility and thus higher capacity to be transported (pump) due to tubificids between water and sediment, compared to As (lower Kd = 2.5) (Allison and Allison, 2005). Moreover, progress of decreasing affinity for sorption material has also been exhibited in the following order: suspended matter > sediment > soil by Allison and Allison (2005).

CHAPTER III.B. INFLUENCE OF COMBINED BIOTURBATION AND PHYTOREMEDIATION ON A MICRO-ORGANIC POLLUTANT: ATRAZINE

III.B.1. INTRODUCTION

The actual study mainly tackles the pivotal role of faunal biodiversity of aquatic habitat via bioturbation processes for bioremediation and how it influences the atrazine fluxes and biodegradation at the water-sediment interface. This study aims to demonstrate the influence of bioturbation (caused by invertebrate worms Oligochaeta *Tubifex tubifex*) alone and its combination with phytoremediation (conducted by a riparian plant *Typha latifolia*) to enhance water and sediment quality. The natural environment was reproduced in a series of 24 microcosms that mimics the wetland bottom conditions with sediment, plants, and invertebrates. Six different treatments crossing three tested factors were applied to allow the test of different research hypotheses (Table II.2): presence/absence of atrazine as a source of organic compound contamination; presence/absence of plant influence (*T. latifolia*); and presence/absence of invertebrate influence (tubificid worms). The experiment was started with homogeneously contaminated sediment with radiolabeled [^{14}C]-atrazine at an initial time with an initial concentration of 2µg/g wet sediment 15 days before starting the experiment. The experiment with plant and invertebrate in the microcosms lasted during 26 days (*see §II.A.2.2*). The radiolabeled pesticide allowed tracking the distribution of the total quantity of pesticide (mother molecule and metabolites) within all experimental compartments: water, sediment, and plant. Concentrations of [^{14}C]-atrazine were explored in each physical compartment (free water, interstitial, water and particles of sediment, and plant) to estimate the changes of concentrations and fluxes under the effects of bioturbation by the species of invertebrate tubificidae. The success of the bioremediation was made evident in two complementary ways:

- The direct measurement of ^{14}C-[atrazine, metabolites] concentrations in the different compartments at an initial time and the end of the experiment to demonstrate

the dynamics of the pesticide during the experiment lag of time. By following the concentrations changes of atrazine and its metabolites in the different compartments, it is possible to emphasize the pathways and behavior of the molecule and its metabolites in the aquatic model;

- Indirect measurements of the water quality (free and interstitial water) by using ecotoxicological bioassay to assess the toxicity of the water. This toxicity evolution with time in our microcosms should permit to assess the efficiency of the bioremediation. *Chlorella vulgaris*, the green algal, was used as a model organism, well known for its sensitivity to micro-organic pollutants (Naessens et al., 2000; Shitanda et al., 2005). The methodology of these tests is given in detail in Chapter II. Methodology.

This study tackles experimentally the coupling of bioturbation and phytoremediation to enhance atrazine biodegradation and transfer from sediment compartments into the plant organs. The objectives of the study are:

(i) to estimate the influence of the bioturbation on a labeled [^{14}C]-organic pollutant behavior within the sediment compartment using a controlled-environmental experiment. The effects of bioturbation is given in evidence through the exploration of the biotransport intensity and the related effects on the physicochemical properties of the sediment (porosity, OM, OC, C:N, pH, Koc, and Kd). The influence of oligochaetes on the atrazine partitioning in the sediment column (pore water and solid fractions) in these conditions is demonstrated by comparison of the vertical distributions of [^{14}C]-atrazine concentrations in water and sediment with and without bioturbation. The biotransport intensity and type is estimated under these conditions that also allow studying the influence of pesticide contamination and plant occurrence on the bioturbation process; This part (i) is making the contents of one future paper whose title is “Bioturbation effects on atrazine behavior in aquatic sediments” and included in the *Annex 13.*

This paper is planned to be submitted in the Journal of *Ecotoxicology and Environmental Safety*.

(ii) to observe the influence of invertebrate (tubificid worms) (relationships with microbial consortium) on the biotransformation of atrazine into metabolites. Since metabolites were only searched into the plant biomass during the duration of this Ph.D., the influence of the bioturbation on the transformation of atrazine in the sediment is estimated by comparing the metabolites composition in the plants in treatment with and without bioturbation. Further measurements of metabolites in sediment samples are now running at INRA so that this information will be included in the next papers extracted from this study.

(iii) to discuss the bioavailability of atrazine and its metabolites for plant from the sediment compartment by comparison of atrazine and metabolites concentrations in porous water and particles fractions

(iv) to estimate the plant effect (phytoremediation) in the pesticides attenuation in the contaminated sediment by comparison of the labeled atrazine (concentration, metabolites, quantity, mass balance) in the treatments with and without plant ({Atr.Typ} and {Atr} treatments, Fig. II.5);

(v) to estimate the influence of plant and bioturbation combination on atrazine and metabolites (concentration, quantity, fluxes, and, mass balance) by comparison with and without the combination of plant and worms in the bioremediation treatments. The first working hypothesis is that combination effect is higher than bioturbation effect alone or phytoremediation alone; the second (even better) hypothesis is that combination effect is higher than the sum of bioturbation and phytoremediation effects ({Atr} and {Atr.Typ.Tub} treatments, Fig. II.5);

(vi) to assess atrazine toxicity mitigation by bioremediation engineering via toxicity tests of the interstitial and overlying water in different treatments. The comparison of the toxicity at the beginning and the end of the experiment allows

elucidating the evolution of this toxicity with time under plant and invertebrate influence.

III.B.2. RESULTS

III.B.2.1. Sediment reworking measurement

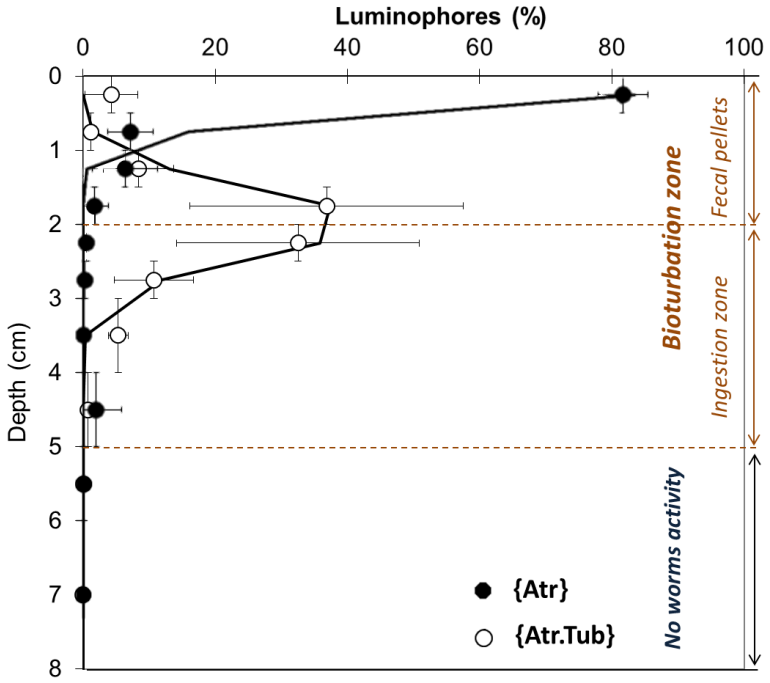


Figure. III.2. Vertical profiles of luminophores in the sediment after 26 days for treatments with and without tubificids introduction. *Dots are mean values of the relative luminophore concentrations (%) measured in treatments with (white) and without (black) tubificid introduction;* (*see* ***Table II.2*** *for the definitions of acronym corresponding to the different treatments*). *The bioturbation zone is defined by the maximum depth where luminophores were found at the end of the experiment.*

In the sediment without Tubifex worms (Fig. III.2), no particle mixing occurred since most of the luminophore tracers (80%) remained at the surface of the sediment (0-0.5cm), and about 10% were located below the surface (0.5 - 2 cm, respectively) after the experiment (black dots). Some luminophore particles (about 2%) were found in the deeper layers probably due to inherent tracer movements during experimentation or

because of the activity of some smaller invertebrates that survived to the initial sediment after the defaunating process.

Table III.5

Two-way ANOVA assessing the influences of atrazine and worms on bioadvective rates (V) performed with all treatments. ****: *significant difference for V at P ≤ 0.0001; ns: non significance.*

Factors	$F_{1,17}$	*P*
Atrazine contamination	2.536	0.6631^{ns}
Tubificid addition	*392.4*	*<0.0001***
Atrazine x tubificid interaction	0.005214	0.9433^{ns}

Table III.6

Two-way ANOVA assessing the influences of *T. latifolia* plants and worms on bioadvective rates (V) performed with all treatments. ****: *significant difference for V at P ≤ 0.0001; ns: non significance according to two-way ANOVA.*

Factors	$F_{1,17}$	*P*
T.latifolia treatment	2.333	*0.11450* ^{ns}
Tubificid addition	*434.5*	*<0.0001****
T.latifolia : tubificid interaction	0.5536	0.4670 ^{ns}

Luminophore profiles (Fig. III.2) showed an increase in the depth location of the maximal luminophore concentration with bioturbation. In the presence of worms, the tracers (about 10 to ~ 60%) were mostly grouped in the sediment layer between 1 and 3 cm. This profile attests of the biotransport of tracers downward in the sediment under fecal pellets accumulation at the sediment surface, and it is typical of bioadvective processes under tubificids bioturbation (white dots) with the conveyor-belt process. These deposit feeder invertebrates ingest sediment at depth in the sediment and egest fecal pellets at the sediment surface. The accumulation of fecal pellets at the sediment surface induces the burial of the tracers that migrate downward in the sediment with a vertical velocity depending on the worms density. Once luminophores reached the ingestion layer, they are transferred upward again for the smallest particles. Some

others remain at depth since they are too large particle to be ingested by oligochaetes. The deepest occurrence of luminophores in the sediment (5 cm) at the end of the experiment indicated the depth of the sediment ingestion layer.

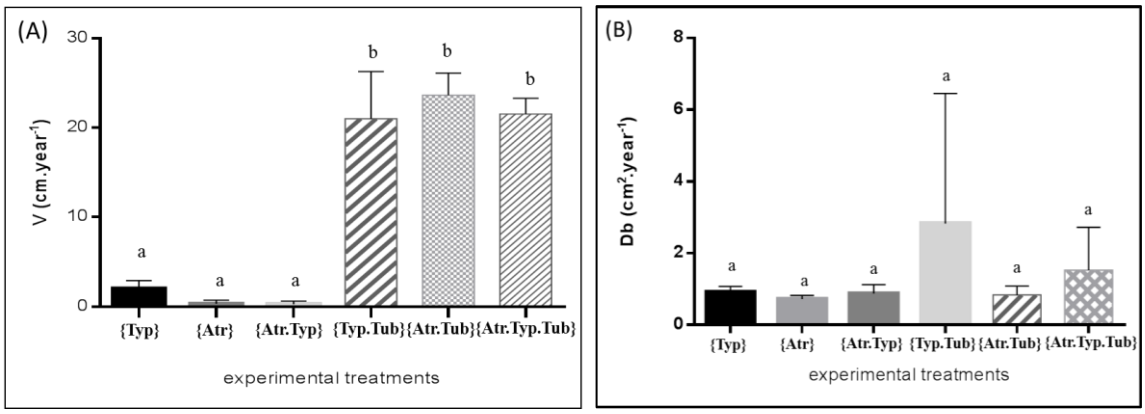


Figure. III.3. *(A):* bioadvective rates -V, and (*B*): biodiffusive rates - Db, estimated after 26 days for the different experimental treatments. *For each parameter, the same letters at the top of the bars indicate treatments that were not significantly different (P < 0.05) as analyzed by TUKEY HDS multiple comparison test. Values are means ± SD, n = 4, except for {Typ.Tub}, {Atr.Tub}, {Atr.Typ.Tub} with n =3. (see* ***Table II.2*** *for the definitions of acronym corresponding to the different treatments).*

A significant effect of tubificids addition on bioadvective rates was evidenced by using two-way ANOVA, either when crossing this factor with atrazine ($F_{1,17}$ = 392.4, P < 0.0001, Table III.5) or with the presence of *T. latifolia* plants ($F_{1,17}$ = 434.5, P < 0.0001, Table III.6). None of the other factors tested (atrazine or *T. latifolia* plant) had any significant influence on V, either alone or in interaction with the presence of tubificid (Table III.5 and III.6).

In the treatment having worm addition, the bioadvective rates (V) obtained by using the bioadvection–biodiffusion model (Gerino et al. 1994) corresponded to a rate ranging from 15.00 to 26.50 $cm.y^{-1}$ for a tubificid density of 10,000 $individuals.m^{-2}$, while it varied only from 0.00 to 3.00 $cm.year^{-1}$ when the worms were absent (Fig. III.3A). Only the bioadvective rates showed a significant difference between treatments with or without tubificid addition, while biodiffusion remained constant in the same conditions (Tukey HSD test; Fig. III.3A). It is particularly remarkable that no significant difference for either V (Fig. III.3A) or Db (Fig. III.3B) was observed when comparing

corresponding experimental treatments with or without added atrazine (Table III.5), and with or without plant (Table III.14).

III.B.2.2. [^{14}C] ∑(atrazine, metabolites) concentration in compartments

Overlying-water

Two weeks before starting the experiment (at t = - 15 days), [^{14}C]-(atrazine, metabolites) concentration in the overlying water was found to be at 3681.55 ± 71.30 µg.L^{-1} (mean ± SD, n = 2). Twenty-six days after the beginning of the experiment with plant and invertebrate in the microcosms (t = 26 days), the [^{14}C]-(atrazine, metabolites) concentration was deficient, ranging from 66.54 ± 17.90 to 211.09 ± 7.13 µg.L^{-1} (mean ± SD, n=4). Tubificid addition had a significant effect on the concentrations in the overlying water ($F_{1,11}$ = 16.57, P < 0.01, see *Annex 3*) and the interaction between the tubificids and *T. latifolia* ($F_{1,11}$ = 28.99, P < 0.001, see *Annex 3*) was also highly significant as evidenced by two-way ANOVA performed on the concentrations of all pesticide contaminated treatments: {Atr}; {Atr.Tub}; {Atr.Typ}; and {Atr.Typ.Tub} (Fig. III.4).

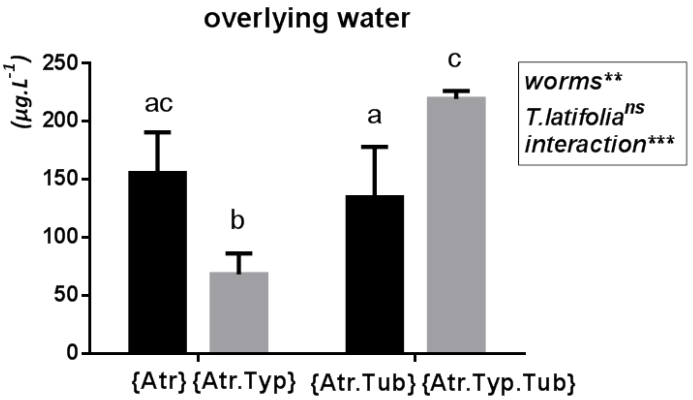


Figure. III.4. [^{14}C]-(atrazine + metabolites) concentrations in the overlying water of all contaminated treatments at the end of the experiment (t = 26 days). *Values are means ± S.D., replicate (n) = 4 per treatment group (except for {Atr.Typ.Tub} with n=3). (**) and (***) indicate significant (p<0.01) and (p<0.001) effect of worms addition and interaction with plants according to two-way ANOVA (performed on the replicate concentrations of the four treatments). Different superscript letters indicate the significant differences for the mean values of the concentrations between the experimental treatments as analyzed by post-hoc Tukey HSD test. (see* ***Table II.2*** *for the definitions of acronym corresponding to the different treatments*).

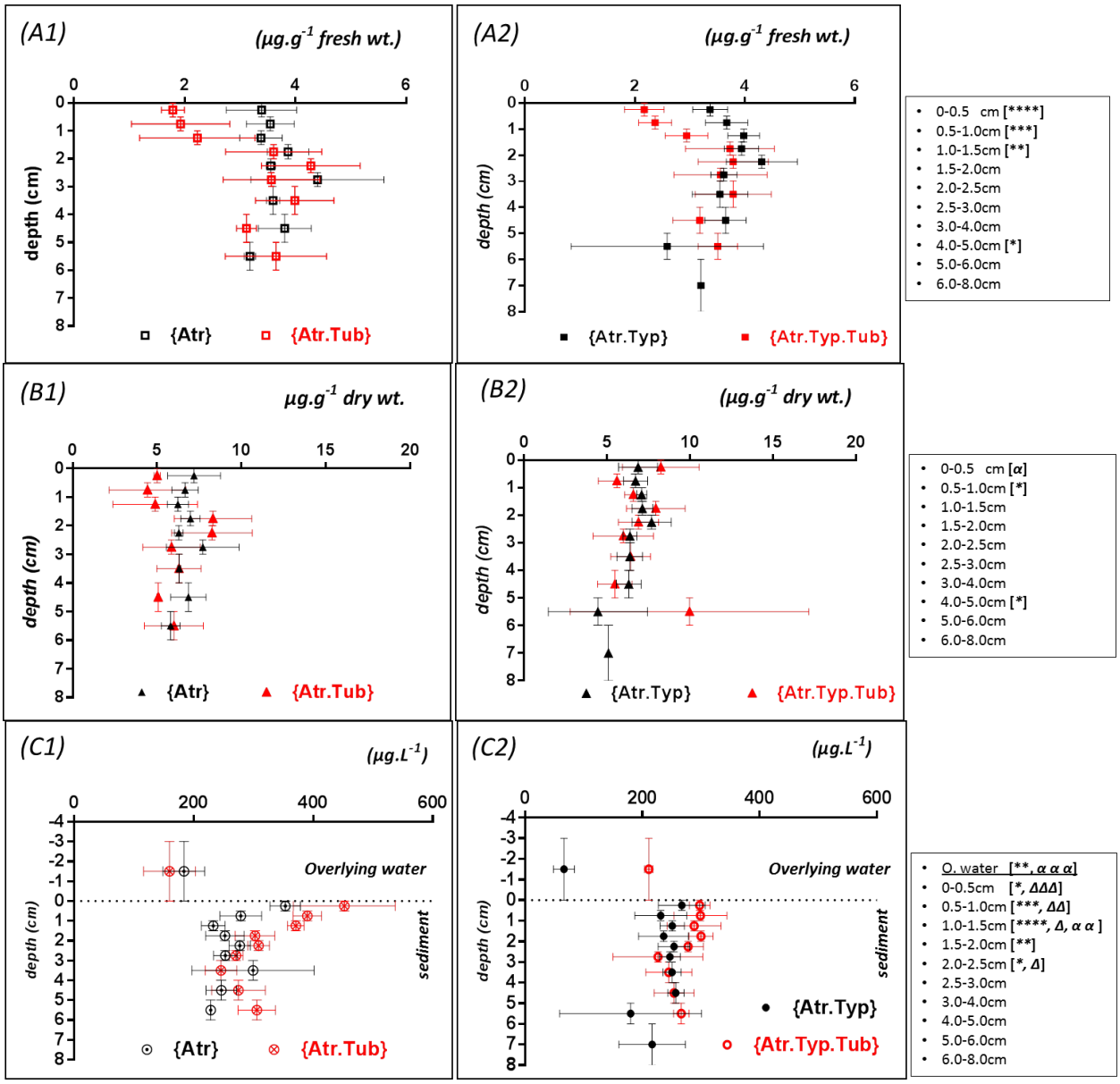


Figure. III.5. [^{14}C]-(atrazine + metabolites) concentrations according to depth and experimental treatments for different sediment fractions *(A1 and A2: fresh sediment (or bulk sediment: being the sum of sediment particles and interstitial water); B1 and B2: sediment particles; C1 and C2: overlying water and pore water) at the end of the experiment (t=26). Values are means ± S.D., replicate (n) = 4 per treatment group. (*), (**), (***), and (****) indicate significance effects of worms addition at P ≤ 0.05, 0.01, 0.001, and 0.0001 respectively according to the two-way ANOVA (The test were performed on the concentrations of the four treatments: {Atr}, {Atr.Typ}, {Atr.Tub}, and {Atr.Typ.Tub}). Similarity, the number of Δ and α indicate the level of significance of T. latifolia effect the level of the two factors interactions, respectively at different levels of P values according to the two-way ANOVA (see* ***Table II.2*** *for the definitions of acronym corresponding to the different treatments).*

During the experiment, [^{14}C]-(atrazine, metabolites) concentrations increased significantly in the overlying water when tubificids were occurring ({Atr.Tub}) and when both worms and *T. latifolia* were together {Atr.Typ.Tub}, when compared with the

treatment having *T. latifolia* only {Atr.Typ} (Tukey HSD, P = 0.0461 and 0.0002, respectively) (Fig. III.4). A significant increase was also observed with bioturbation only ({Atr.Tub}) compare to treatment having *T. latifolia* only ({Atr.Typ}) at t = 26 days (Tukey HSD, P = 0.0183). In the other hand, [^{14}C]-(atrazine + metabolites) concentrations decreased significantly in the treatment having *T. latifolia* ({Atr.Typ}) compared to treatment without *T. latifolia* ({Atr.}) (Tukey HSD, P = 0.0092) (Fig. III.4).

Bulk sediment (total fraction)

Two weeks before starting the experiment, mean value of [^{14}C]-(atrazine, metabolites) concentration in the fresh sediment (total fraction or bulk fraction) was found to be 1.95 ± 0.02 µg.g^{-1} fresh *wt (weight of fresh sediment)*. At the end of the experiment, the concentrations in the sedimentary layers in the absence of worms ranged from 3.18 ± 0.10 to 4.40 ± 1.20 µg.g^{-1} fresh *wt.* and from 2.59 ± 1.75 to 4.31 ± 0.65 µg.g^{-1} fresh *wt.* (total fraction), respectively in {Atr} and {Atr.Typ} treatments (Fig.III.5A). Significant effect of tubificids were recorded in the upper layers (0-0.5, 0.5-1.0, 1.0-1.5 cm, with P < 0.0001, 0.001, and 0.01, respectively) where lower concentrations were measured with bioturbation, and higher concentration in the deeper layer (4-5 cm, P < 0.05) as evidenced by the performed two-way ANOVA (Fig. III.5A) (see *Annex 4* for the P values).

Impact of the interactions between bioturbation and plant on [^{14}C]-(atrazine, metabolites) quantities in the bulk sediment were estimated by using Tukey HSD test focusing on the three sedimentary layers as defined in § III.B.4.1 according to the bioturbation depth: the fecal pellets zone (0-2 cm), the ingestion zone (2-5 cm), and the "no worm activity" zone (5-8 cm). These layers thickness were defined based on the luminophores distribution under bioturbation (Fig. III.2). The post-hoc test was used to compare, between treatments, the pesticide quantities mean values in these layers at the end of the experiment (Fig. III.6A). In the fecal pellets zone, a significant depletion of [^{14}C]-(atrazine, metabolites) quantity was observed in the presence of worms, when

comparing between {Atr} and {Atr.Tub}, between {Atr} and {Atr.Typ.Tub}, between {Atr.Typ} and {Atr.Tub}, and between {Atr.Typ} and {Atr.Typ.Tub} treatments (Tukey HSD, P=0.0149, 0.0013, 0.0087, and 0.0008, respectively, see *Annex 8*). The quantity did not differ independently on the presence or absence of *T. latifolia* in the top layer with any significant difference between treatments (Tukey HSD, P > 0.05, Fig. III.6A). In the ingestion zone (2-5 cm), the values remained the same in all treatments (Tukey HSD, P > 0.05, Fig. III.6B). The significant lower quantity was only found in the deepest layer (5-8 cm) (Tukey HSD, P = 0.0239, Fig. III.6C) of the treatment {Atr.Typ} when compared with {Atr.Typ.Tub}.

Sediment particles

A significant effect of tubificids on [^{14}C]-(atrazine, metabolites) concentrations of sediment particles was observed only in the two layers from 0.5 to 1.0 and from 4.0 to 5.0 cm (two-way ANOVA, P = 0.032 and 0.0138, respectively) (Fig. III.5B) where lower concentrations were observed with bioturbation. Any significant effects of *T. latifolia* on the concentrations were observed in all layers of sediment particles as evidenced by the two-way ANOVA test (P > 0.05, Fig. III.5B) (see *Annex 5* for the P values).

A similar consequence of tubificid addition on the quantity of [^{14}C]-(atrazine, metabolites) in the fecal pellets layers (0-2 cm) of fresh sediment was also recorded in the sediment particle fraction with significant depletion of the quantity in the presence of worms. By comparing the quantities, the post-hoc Tukey HSD tests also showed significant lower quantities in treatments having the worms {Atr.Tub}; {Atr.Typ.Tub}; and {Atr.Tub} compared to the treatments without the worms {Atr}, {Atr.Typ} (Fig.III.6B). The quantities again showed independence on the presence or absence of *T. latifolia* in the top layer with any significant difference between treatments (Tukey HSD, P > 0.05, Fig. III.6B). No significant difference was found between treatments in the deeper layers (from 2 to 8 cm) (Tukey HSD, p > 0.05, Fig. III.6B) (see *Annex 9* for the P values).

Pore water

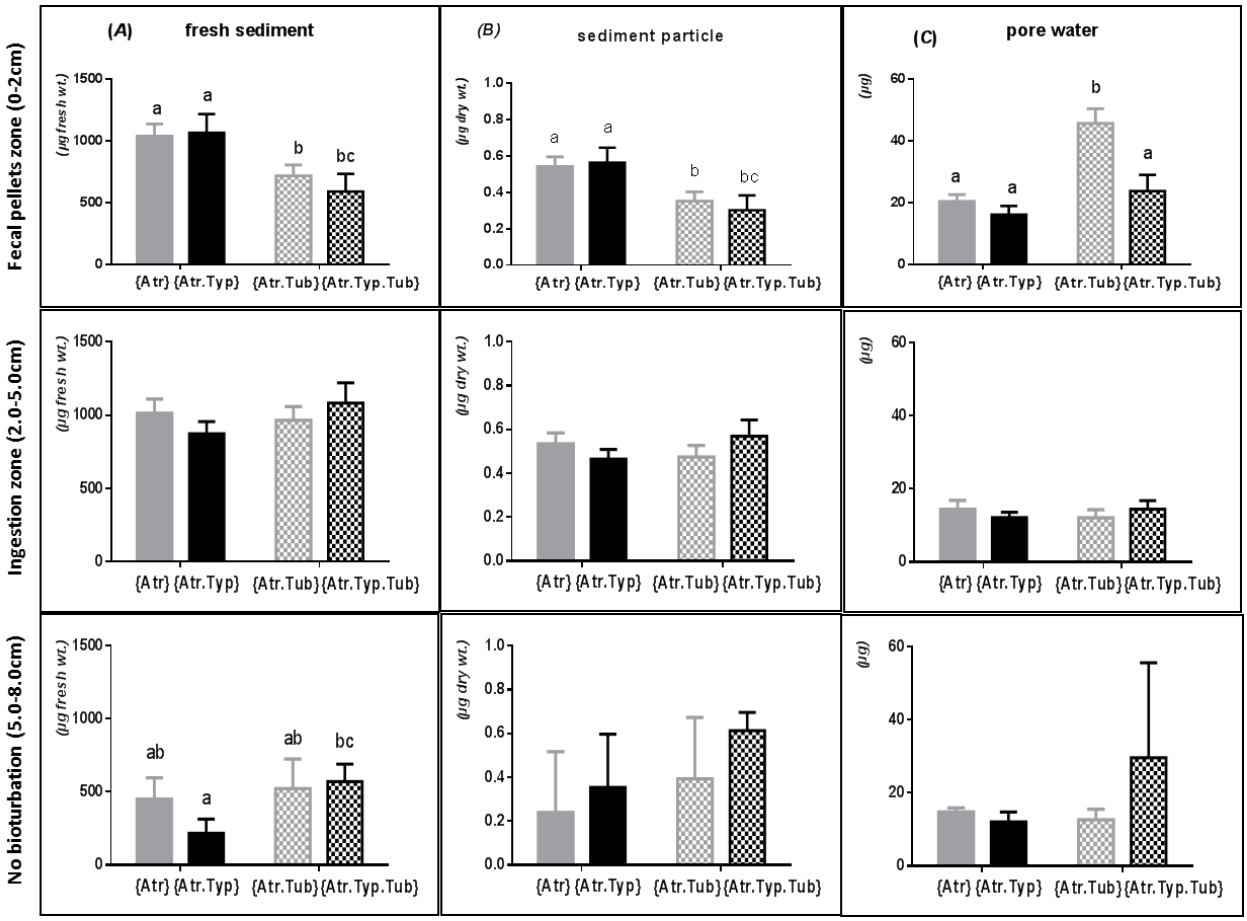


Figure. III.6. HSD Tukey test performed on ^{14}[C]-(atrazine + metabolites) quantities in the sedimentary layers defined by bioturbation depth in different sediment fractions. *Values are means ± S.D., replicate (n) = 4 per treatment group. Different superscript letters indicate the significant differences for the mean values of the concentrations between the experimental treatments as analyzed by post-hoc Tukey HSD test. (see* ***Table II.2*** *for the definitions of acronym corresponding to the different treatments).*

At the end of the experiment, the effects of tubificids addition were significant from the surface sediment until 2.5 cm depth with higher [^{14}C]-(atrazine, metabolites) concentrations in pore water with bioturbation. The effect of the plants was also significant in the same layers, but with lower concentrations when *T. latifolia* was occurring, except for the layer from 1.5 to 2.0 cm (two-way ANOVA, Fig.III.5C, see *Annex 6*). A significant interaction between the two tested factors was only found for layer from 1.0 to 1.5 cm (Fig.III.5C) (see *Annex 6* for the P values).

In the presence of tubificid worms only ({Atr.Tub} treatment), the quantity of [^{14}C]-(atrazine, metabolites) in the fecal pellets layer (0-2 cm) increased significantly

compared to treatments without the worms addition ({Atr} and {Atr.Typ}, respectively) (Tukey HSD tests, P < 0.0001, Fig.III.6C). No significant difference of the quantities between treatments was found for either the ingestion or the no-bioturbation zones (Fig.III.6C) (see *Annex 10* for the P values).

III.B.2.3. Porosity of experimental sediment

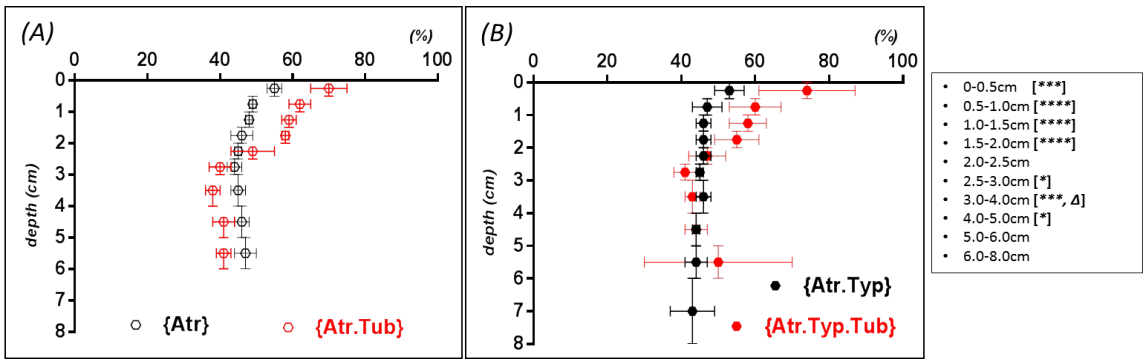


Figure. III.7. Sediment porosity profiles of (A): {Atr} and {Atr.Tub}; (B): {Atr.Typ} and {Atr.Typ.Tub} treatments versus sediment depth at the end of the experiment. *Values are means ± S.D., replicate (n) = 4 per treatment group. (*), (***), and (****) indicate significant effects of worms addition at p ≤ 0.05, 0.001, and 0.0001 respectively according to two-way ANOVA (effects of worms and T. latifolia treatment performed on the percentage of water content in these four treatments); Δ indicate significant effect of T. latifolia at p ≤ 0.05 according to the two-way ANOVA test. No significant interaction between these two tested factors was observed among the treatments of all the layers (see* ***Table II.2*** *for the acronym definitions corresponding to the different treatments).*

At the end of the experiment, the effect of bioturbation on the water content in sediment column was significantly (P < 0.05, 0.001, 0.001) at all depth from surface until 2.5 cm, excepted for the layers from 2.0 to 2.5 cm, as evidenced by the two-way ANOVA (Fig. III.7). Significant effect of *T. latifolia* on the change was observed only in the layer from 3.0 to 4.0 cm (P > 0.05, two-way ANOVA, Fig. III.7**).** However porosity increased under worms effects in the surface layers from 0 to 2 cm, and inversely, the porosity was lower in the deeper layers, as analyzed by Tukey HSD multiple comparison test (Fig. III.7 and III.8, see *Annex 7* for the P values)

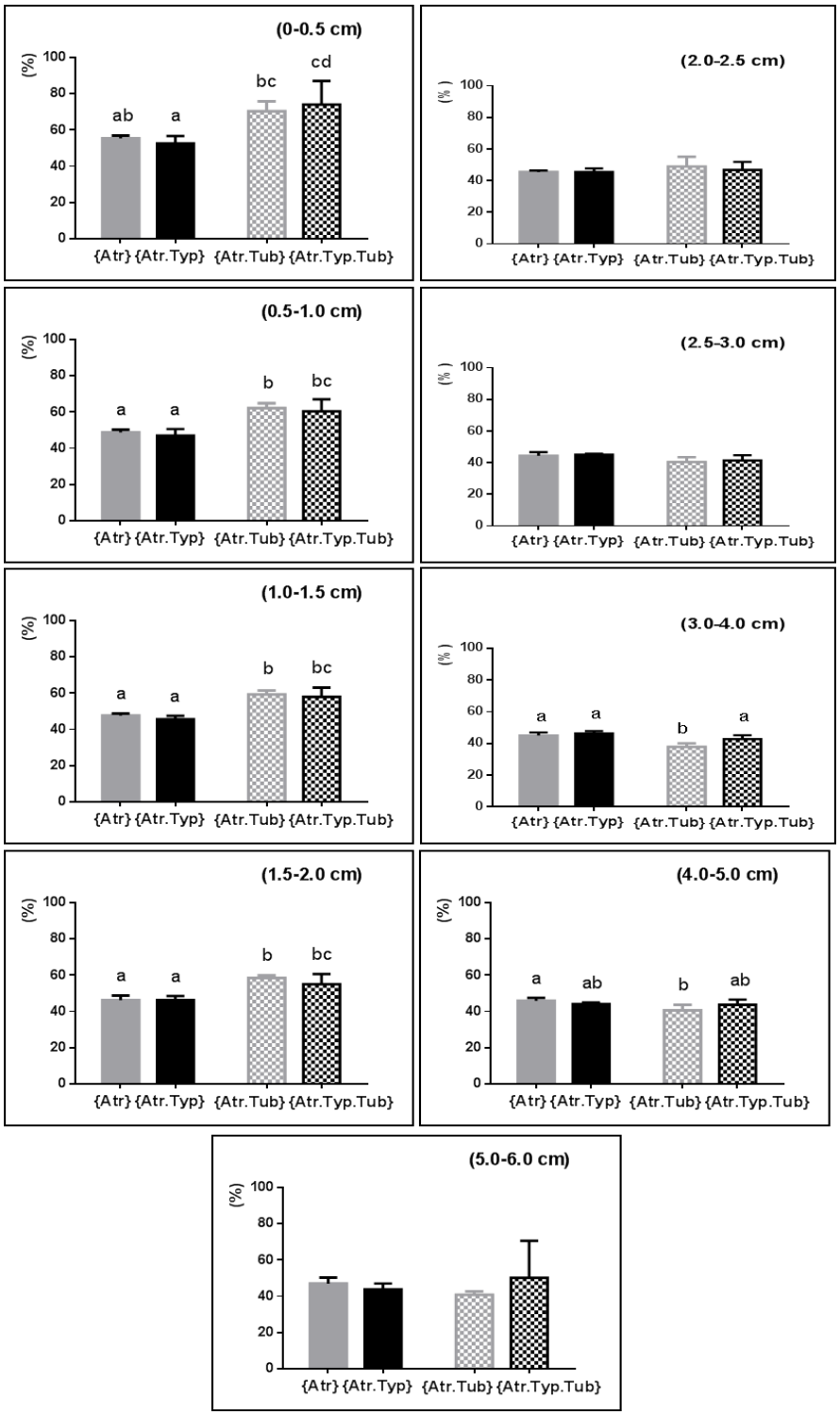


Figure. III.8. HSD Tukey test performed on sediment porosity in each sedimentary layer of all experimental treatments. *Values are means ± S.D., replicate (n) = 4 per treatment group. Different superscript letters indicate the significant differences for the mean values of the concentrations between the experimental treatments as analyzed by post-hoc Tukey HSD test. (see* ***Table II.2*** *for the definitions of acronym corresponding to the different treatments).*

In the absence of worms ({Atr} and {Atr.Typ} treatments), the porosity of sediment varied from 43 ± 6% to 53 ± 4% in the sediment column (Fig. III.7A and III.7B), with no significant difference between the two treatments (P > 0.05, Tukey HSD test, Fig. III.8) (see *Annex 11* for the P values).

III.B.2.4. Organic matter, carbone, C/N, pH, and *partition coefficients*

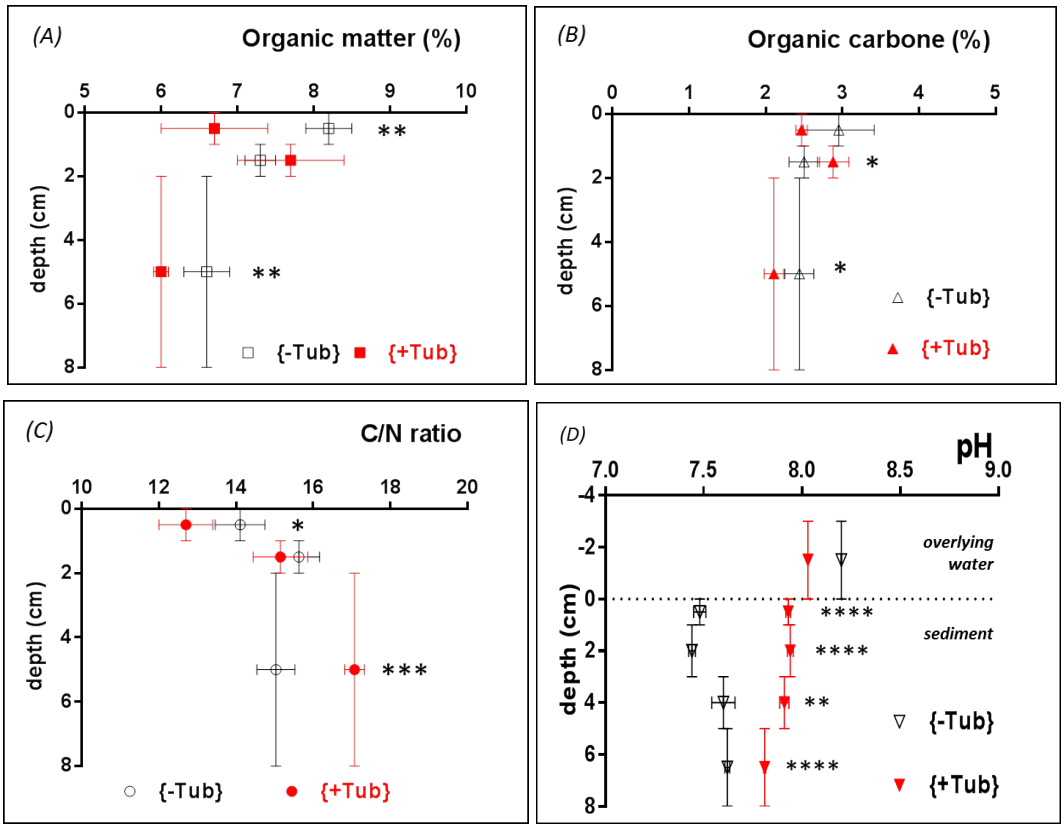


Figure. III.9. Physico-chemical variables at t=26 days with *(A)*: organic matter content; *(B)*: organic carbon; *(C)*: C/N ratio; *(D)*: pH (*see Table II.2 for the definitions of acronym corresponding to the different treatments*). (*), (**), and (***) indicate significance at p ≤ 0.05, 0.01 and 0.001, respectively according to unpaired Student *t-test*.

In the presence of the worms, organic matter contents (Fig III.9A) significantly decreased after 26 days in the upper sediment layers (0-1 cm) and in bottom layers (2 – 8 cm) (p < 0.01, Student *t*-test) in the treatment {+Tub}, compared to the treatment without the worms. Similarity, organic carbon content showed a significant decrease in the layer from 2 to 8 cm with worms ({+Tub} treatment (p < 0.05, Student *t-test*), but the higher C contents were found mostly in the upper layer (1-2 cm) (Fig. III.9B). This leads to a significant decrease of organic carbone C:N ratio in the presence of the worms

({+Tub}) in the top layer (0-1 cm) at the end of the experiment, but a significant increased in the bottom layer (2-8 cm) compared to treatment without worms addition ({-Tub}, Fig. III.9C). The pH level became significantly higher in whole sediment column when the worms were occurring, by comparison with the treatment without bioturbation ($P < 0.0001$ and 0.05, Student *t*-test, Fig. III.9D).

III.B.2.5. Partition coefficients

Worms and *T. latifolia* significantly affected soil adsorption coefficient Kd in bioturbated layers from 0 to 5 cm, as evidenced by two-way ANOVA test performed on the values of all treatments contaminated with atrazine ($p < 0.001$ and 0.0001, respectively, Fig. III.10). A significant effect of worms for water partitioning coefficient was observed only in the fecal pellets zone (0-2.0cm) ($p < 0.001$, two-way ANOVA, Fig. III.10), while *T. latifolia* significantly affected the whole bioturbated layers from 0 to 5 cm ($p < 0.0001$, two-way ANOVA, Fig. III.10).

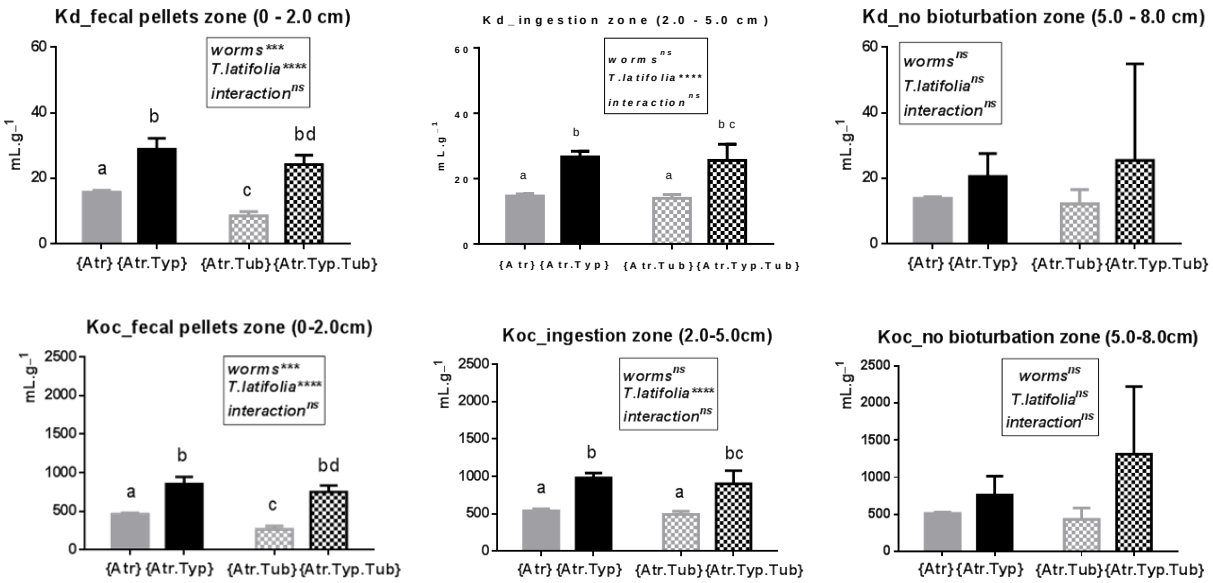


Figure. III.10. Soil adsorption coefficients – Kd and organic carbon-water partitioning coefficients – Koc affecting the mobility of atrazine and its metabolites in sediment. *Values are means ± S.D., replicate (n) = 4 per treatment group. (*), (***), and (****) indicate significant effects of worms addition and T.latifolia treatment as well as a significant interaction between the two factors tested at $p \leq 0.05$, 0.001, and 0.0001 respectively according to two-way ANOVA; ns – no significant difference.*

Lower values of this sorption coefficient (Kd) and water partitioning coefficient (Koc) were also observed in the presence of worms ({Atr.Tub} treatment) compared to treatments without worms addition ({Atr} and {Atr.Typ}, respectively) with significant differences in the bioturbated layers (0 – 5.0 cm) for Kd (Tukey HSD test, Fig. III.10) and in the fecal pellets layer for Koc (Tukey HSD test, Fig.III.10).

III.B.2.6. Atrazine and its metabolites in T. latifolia root systems

The typical radio-reverse-HPLC chromatogram in Fig. III.11A displayed the presence of atrazine authentic standard peak (***Atr***) with the retention time - t_R - equal to 24.00 minutes. Identification of atrazine compounds in the plant samples of treatments {Atr.Typ} and {Atr.Typ.Tub} was achieved by comparison with the obtained retention time in the radio-reverse-HPLC chromatogram of the authentic standard. This atrazine peak (***Atr***) was tentatively assigned to atrazine by coelution in HPLC with an authentic standard.

The chromatogram of atrazine and its metabolites in the *T. latifolia* root system (Fig.III.11B and Fig.III.11C) showed the presence of peaks of several metabolites in addition to the atrazine authentic standard. After the experiment, besides the atrazine peak, some metabolites appeared on the radio-HPLC profiles of both treatments with and without bioturbation ({Atr.Typ} and {Atr.Typ.Tub} treatments), such as the peaks with t_R of 15.70 and 16.70 minutes. Identification of the metabolites of [^{14}C]-atrazine was, however, not performed. Several new metabolites, which could not be detected in the condition without worms treatment {Atr.Typ}, were found only in the treatment with worm addition ({Atr.Typ.Tub}) (peaks **1**, **2**, **3**, **4** with t_R = 17.60, 17.90, 18.60, and 20.90 min, respectively) by comparison of their retention times in HPLC with the atrazine authentic standard (Fig. III.11B).

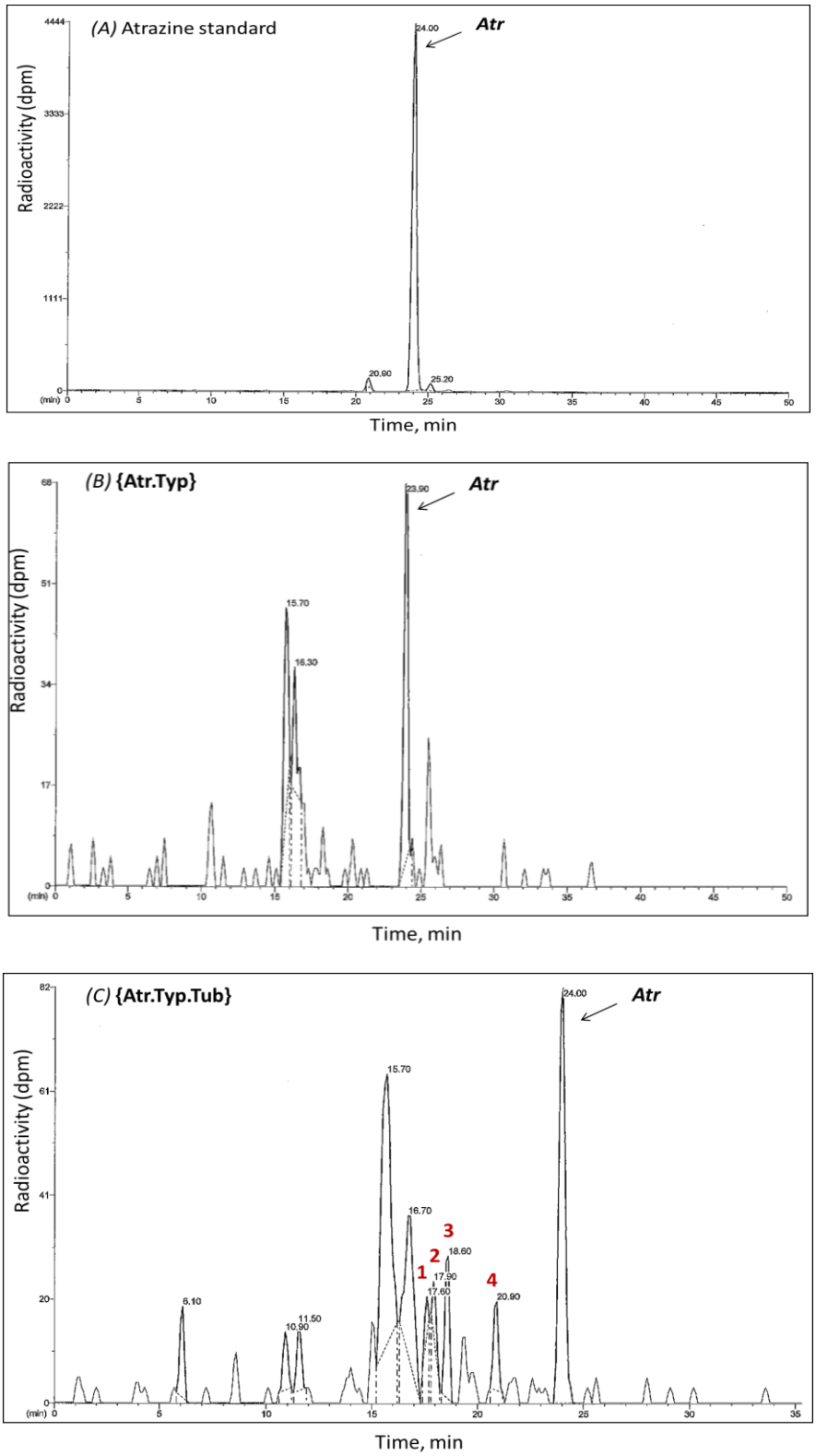


Figure. III.11. Chromatograms showing retention time of atrazine standard (*A*), atrazine and its metabolites in *T. latifolia* root part of two experimental treatments: *(B)* without worms {Atr.Typ} treatment and *(C)* with worms {Atr.Typ.Tub} treatment. ***Atr*** is the peak of atrazine; **1**, **2**, **3**, and **4** are metabolites peaks among new ones formed in the {Atr.Typ.Tub} treatment after the experiment.

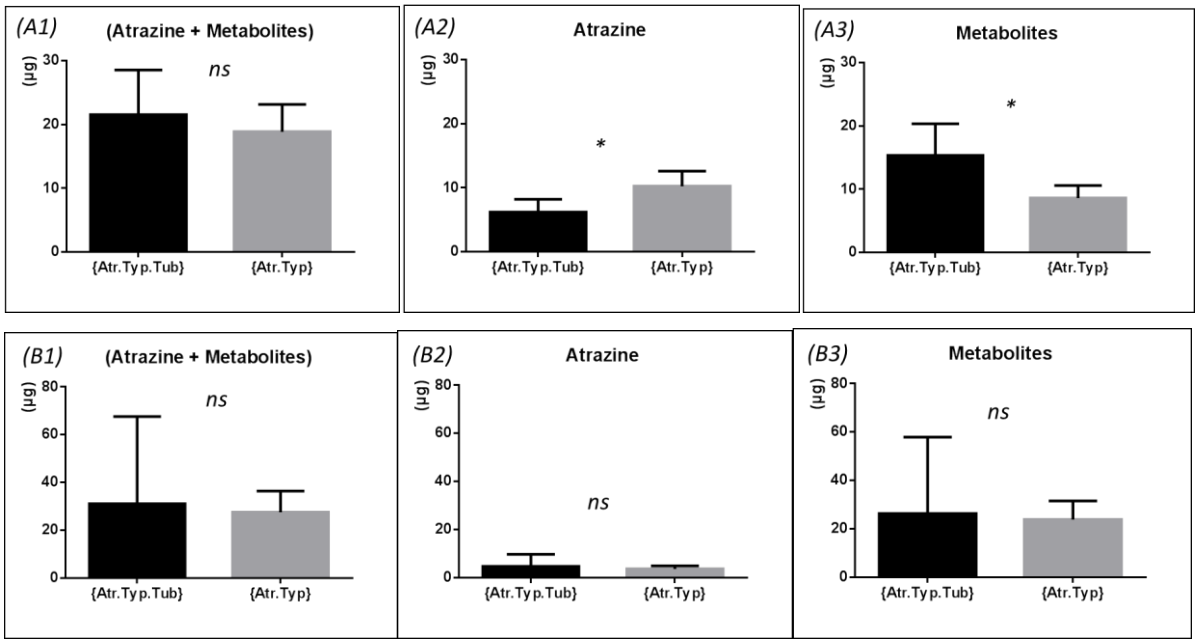


Figure. III.12. [^{14}C]-(atrazine, metabolites) quantities in root and leaf parts of *T. latifolia* at t = 26 days with and without bioturbation in contaminated treatments. *(A1), (A2), (A3) are the quantities determined in the root part; (B1), (B2), (B3) are the quantities determined in the leaf parts. (*) indicates significance at p ≤ 0.05 and ns indicates no significant difference (P > 0.05) according to unpaired Student t-test.*

Quantity of atrazine and its metabolites were calculated based on peak areas obtained from the radio-HPLC profiles (Fig.III.12). No significant difference in the total quantity of [^{14}C]-(atrazine, metabolites) were found either in *T. latifolia*'s root system and the leaves of {Atr.Typ.Tub} and {Atr.Typ} microcosms (Fig.III.12A1). Twenty-six days after the plant and invertebrate addition in the microcosms, the quantities found in the leaves were lower than in the root systems. The quantity of pure atrazine in the roots, as representative of the parent compound, was significantly lower in the treatment having worms when compared with conditions without bioturbation ($P < 0.05$, Student t-test) (Fig.III.12A2). The percentages of atrazine in the roots systems, related to the total quantities found in each part of the plants, accounted for 28.74 and 54.34% with and without worms, respectively. In the leaf parts, the atrazine accounted for 16.30 and 13.22% of the total pesticides in the presence or absence of worms, respectively, and these pesticide expressed in quantities did not significantly differ ($P > 0.05$, Student *t*-test) (Fig.III.12B). Biodegradation of atrazine into metabolites appeared

on both treatments with and without worms {Atr.Typ.Tub} and {Atr.Typ}. Derivatized products of atrazine accounted for high amounts, with 71.26% and 45.66% in roots, respectively, and 83.70 and 86.78% in leaves of both treatments, respectively. The quantities of the metabolites showed a significant increase under the worm treatment ($P < 0.05$, Student *t-test*) in the root parts only, with no noticeable changes observed in the leaves parts (Fig.III.12A3).

III.B.2.7. Phytoremediation potential indicators

Plant phytoremediation potential for pesticide can be assessed by using the enrichment coefficient (EC) and transfer factor (TF). Fig. III.13 indicated the EC values for root (EC_R) and leaves (EC_L) and TF estimated for the two experimental treatments {Atr.Typ} and {Atr.Typ.Tub}. The enrichment coefficient for the leaves part (EC_L) did not show any significant difference between the two treatments after 26 days of the experiment ($p > 0.05$, Student *t*-test), with averages ranging from 5.32 ± 4.57 {Atr.Typ} to 7.55 ± 1.59 {Atr.Typ.Tub}.

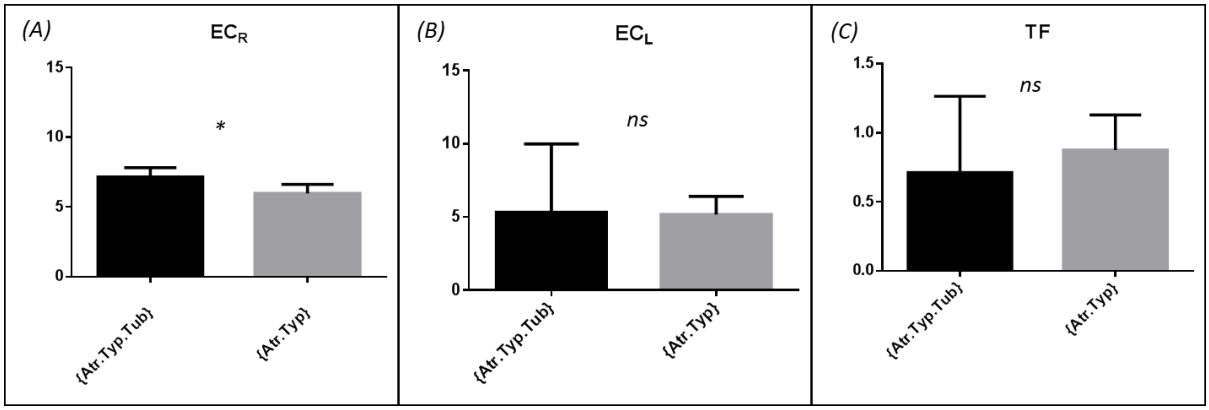


Figure. III.13. *(A)* Enrichment coefficients for the roots (EC_R); *(B)* Enrichment coefficients for the leaves (EC_L); *(C)* transfer factor (TF) of *Typha latifolia* for the two different experimental treatments at the end of the experiment. *(*) indicates significance at $p \leq 0.05$; ns: non significance according to unpaired Student t.test*

In contrast, the enrichment EC_R of the roots (Fig. III.13A) showed a significant difference between the two treatments (Fig. III.13B), with higher values of 7.16 ± 0.66 found with bioturbation ({Atr.Typ.Tub} treatment) compared to lower values of 5.99 ± 0.64 in the {Atr.Typ} treatment. Means of the transfer factor were found at the low level

(< 1) in both conditions, with values being equal to 0.71 ± 0.55 in the {Atr.Typ.Tub} treatment and 0.88 ± 0.25 in the {Atr.Typ} treatment, and no significant differences between the two treatments (P > 0.05, Student *t*-test, Fig.III13C).

III.B.2.8. Atrazine and its metabolites mass balance

Quantity of [^{14}C]-(atrazine, metabolites) was estimated at 3,310.02 ± 279.95 µg after adding the contaminant into the microcosm at t = -15 days (table III.7). Table III.7 showed the total quantity of [^{14}C]-(atrazine, metabolites) in the water, sediment, plant compartments, and in the whole test-system at the initial time (t = -15days) and the end of the experiment (t = 26 days). These first estimations of the total quantities in the microcosm as used for the relative recovery calculation were only based on measurements in the water, sediment and plant, without including the quantities in worm biomass. No significant differences (P > 0.05, Dunnett test, table III.7) in quantity of [^{14}C]-(atrazine, metabolites) in the whole microcosm of experimental treatments without worms ({Atr} and {Atr.Typ}) after the experiment compared to that in initial condition (t = -15 days) were observed. At the end of the experiment (t=26 days), marginal losses of the atrazine and its metabolites in these treatments, followed the relative recoveries, Re.(A), and Re.(C), equal to 93.52 ± 5.82 and 93.99 ± 3.26 %, respectively, presented in the Fig. III.14.

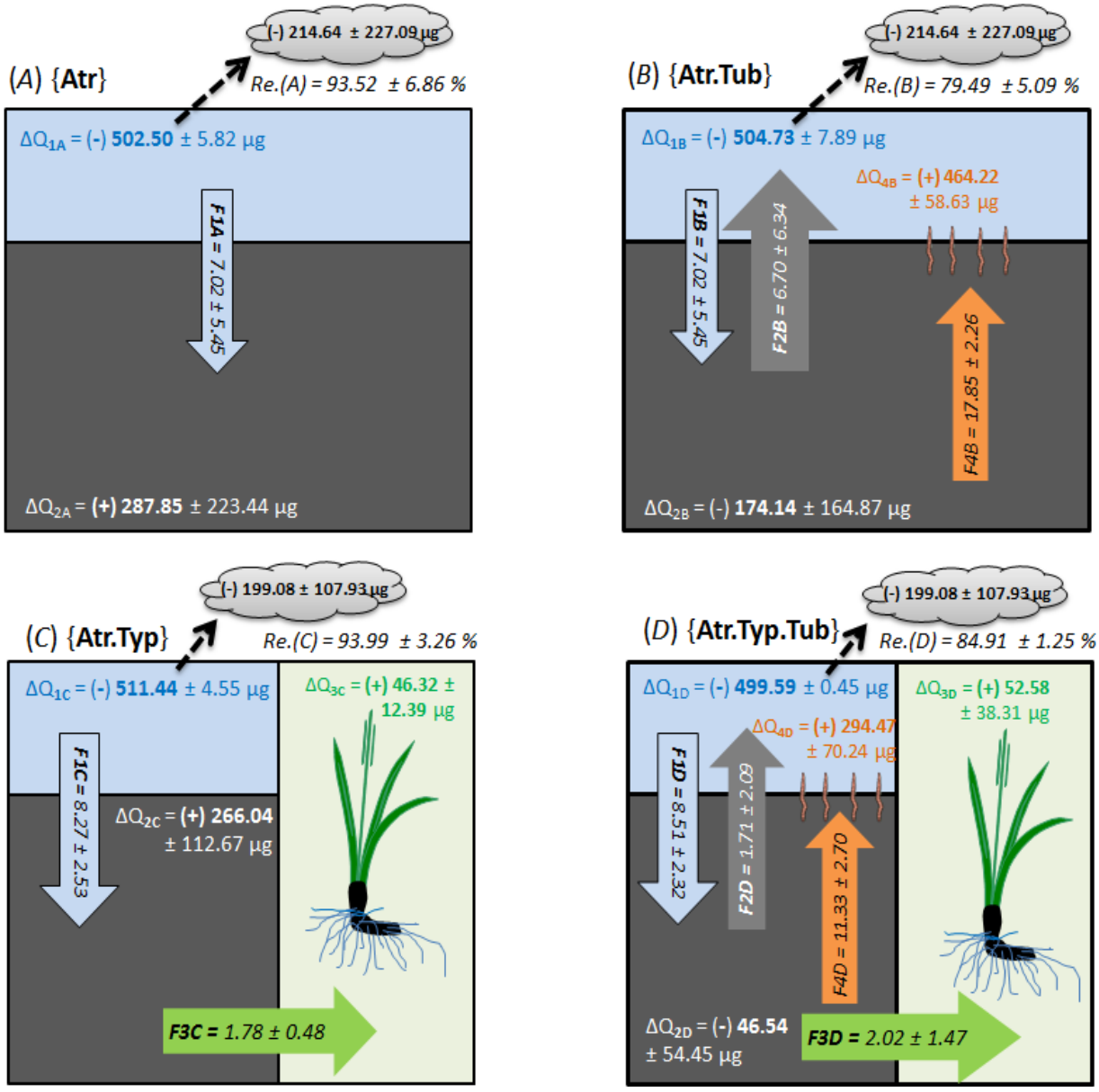


Fig. III.14. Mass balance of [^{14}C]-(atrazine, metabolites) at t = 26 days after the plant and invertebrate introduction in experimental microcosms: **(*A*)** {Atr}, **(*B*)** {Atr.Tub}, **(*C*)** {Atr.Typ}, and **(*D*)** {Atr.Typ.Tub} treatments. All fluxes (F1 to F4) are expressed as µg per day.

$\Delta Q_{1A,B,C,D}$, $\Delta Q_{2A,B,C,D}$ represent differences of quantities of total [^{14}C]-(atrazine, metabolites) in the water (Q1) and sediment (Q2) compartments of the 04 microcosms, between t = -15days and the end of the experiment (at t=26 days); $\Delta Q_{3,C,D}$ represents changes in the quantity in plant for the two microcosms having *T. latifolia*, respectively. $\Delta Q_{4,C,D}$ represents changes in the quantity in worms biomass for the two microcosms having tubificid, respectively. (-) and (+) before ΔQ indicate decrease and increase of the quantity in the compartment during the 26 days of the experiment duration. Arrows represent transport of the pesticides between the compartments. F1A,B,C,D are fluxes of total [^{14}C]-(atrazine, metabolites) in the 04 treatments, respectively from water (blue block) to sediment compartments (black block). F2B,D are fluxes from sediment contaminant into overlying-water in the 02 treatments having worms, these fluxes in the other

treatment being considered as neglectable. F3C,D are fluxes in the 02 treatments having *T. latifolia* added, from sediment to plant compartment (light green block). F4B,D are fluxes of the contaminants from sediment into the worms biomass. Percentages of relative recovery (Re.(A),(B),(C),(D) of the 04 treatments, respectively) is the total amount of [^{14}C]-(atrazine, metabolites) in each microcosm (without taking into account the worms biomass) at t=26 days divided by the quantity that was added at initial time (t = -15 days). Black and broken line arrows are figuring contaminant fluxes of from overlying water out of the microcosms due to volatilization.

Table III.7

Total quantity of [^{14}C]-(atrazine, metabolites) in experimental compartments: water, fresh sediment, plant, and whole test-system (mean ± SD, n = 4, except for initial sample (n=2)). (*) and (**) indicate significant difference at P < 0.05, 0.01, respectively according to the Dunnett's multiple comparison test between each treatment with {initial} (at t= -15 days) treatment.

Treatments	***time***	**Water (µg)**	**Sediment (µg)**	**Plant, (µg)**	**Microcosm**	
					(µg)	P value
{initial} (n=2)	*-15 days*	523.29 ± 10.13	2787 ± 290	-	3,310.02 ± 279.95	
{Atr}	*26 days*	26.20 ± 5.82	3075 ± 223	-	3,095.38 ± 227.09	0.4077
{Atr.Tub}	*26 days*	26.10 ± 7.89	2613 ± 165	-	2,631.16 ± 168.36**	***0.0014***
{Atr.Typ}	*26 days*	11.00 ± 4.55	3053 ± 113	46.30 ± 12.40	3,110.94 ± 107.93	0.4697
{Atr.Typ.Tub}	*26 days*	24.10 ± 0.45	2740 ± 540	52.60 ± 38.30	2,816.47 ± 37.69*	***0.0147***

Table III.8

Two-way ANOVA assessing the influences of *T. latifolia* plants and worms on the changes of the quantity in water (ΔQ_1), Sediment (ΔQ_2), the microcosm (ΔQ), and Relative recovery, respectively. Relative recovery does not include the quantities estimated in the worms biomasses. ****: significant difference for the values at P ≤ 0.001; ns: non significance according to two-way ANOVA.*

	ΔQ_1 in Water		**ΔQ_2 in Sediment**		**ΔQ in Microcosm**		**Relative recovery**	
Factors	$F_{1,11}$	*P*	$F_{1,12}$	*P*	$F_{1,12}$	*P*	$F_{1,12}$	*P*
T.latifolia	0.4201	0.5302	0.4828	0.5004	1.736	0.2123	1.735	0.2123
Tubificid	2.671	0.1305	***25.87***	***0.0003******	***24.76***	***0.0003******	***24.78***	***0.0003******
Interaction	5.730	0.0356	0.9629	0.3458	1.240	0.2874	1.237	0.2879

At t = 26 days, the total amounts in the whole test-system having bioturbation ({Atr.Tub} and {Atr.Typ.Tub} treatments) were significantly lower than the initial condition ($P < 0.01$ and 0.05, Dunnett test, Table III.7), mainly due to the loss of atrazine and its metabolites from the water and sediment compartment as denoted by the negative values of ΔQ_1 and ΔQ_2 Fig. III.15). However, the increases of the pesticide quantity in the sediment compartment were observed in the treatments without worms ({Atr} and {Atr.Typ}) with the positive values of ΔQ_2 (Fig. III.14). Fluxes of the contaminant from water to sediment (F1, Fig. III.15) occurred in all treatments having atrazine addition and indicated the occurrence of physical transport of the contaminant from water to sediment. These fluxes from overlying water into the sediment were understood as a consequence of adsorption processes of the organic compound from water to sediment particle during the whole experiment duration (15 days of contamination plus 26 days having plant and worms treatments). The transport of the pesticide from sediment to water could be found in the treatments having worms only ({Atr.Tub} and {Atr.Typ.Tub}), as denoted by the similar values of F1b in both cases (Fig. III.15).

A significant difference in the total quantity of [^{14}C]-(atrazine, metabolites) before and after the experiment was, however, observed in the experimental treatments having worms addition ({Atr.Tub} and {Atr.Typ.Tub}, $P < 0.01$ and 0.05, respectively, Dunnett test, Table III.7). Significant effect of tubificid worms on changes of the sediment quantity in the sediment (ΔQ_2), in microcosms quantities (ΔQ), and relative recovery (Re.) were evidenced by two-way ANOVA ($P < 0.001$, Table III.8). Relative recovery in the treatment having atrazine and worms only ({Atr.Tub}) (Re(B) = 79.49 ± 5.09 %) was significant lower than that in the {Atr} treatment (Re(A) = 93.52 ± 6.86 %) ($P < 0.01$, Tukey HSD test, see Annex 12) and in the {Atr.Typ} treatment (Re(C) = 93.99 ± 3.26 %) ($P < 0.01$, Tukey HSD test, see Anne 12), and this point indicated along with a loss of atrazine and its metabolites from the previously investigated compartments (water, sediment, and plants) in the presence of bioturbation.

In the presence of plants ({Atr.Typ} and {Atr.Typ.Tub} treatments), after 26 days, atrazine and its metabolites were transported into plants biomass as resulted in the positive values of ΔQ_3 as well as the fluxes F2 from sediment to plant (Fig.III.14). No significant difference of the atrazine quantities (ΔQ_3), as well as the fluxes (F2) between these two treatments with and without bioturbation, were observed (P = 0.7668, unpaired Student *t*-test, *data not shown*) during the time duration of our experiment.

III.B.3. DISCUSSION

III.B.3.1. Influence of bioturbation on particle mixing and tubificid resistance to atrazine

In the presence of tubificid worms with a density of 100 worms.dm^{-2} (equivalent to fresh biomass of 4.45 ± 0.53 g per microcosm), a subsurface peak of tracers in the 2 cm layer indicated that conveying transport were created by tubificids. This downward transport of the surface sediment resulted from the accumulation of faecal pellets at the sediment surface, simultaneously with sediment depression in deeper layers due to sediment ingestion by the worm feeding activities (McCall & Fisher, 1980; Matisoff et al. 1999; Anschutz et al., 2012; Ciutat et al., 2006). Selective feeding behavior by tubificid worms based on particle size, avoiding coarse particles, such as luminophores and sand particles, induces a decrease of the silt-clay fraction at depth (Rodriguez et al., 2001). Consequently, bioturbation creates two distinct layers in the bioturbated sediment: a surface layer corresponding to the fecal pellets accumulation from ingested anoxic sediment with an enhanced fine fraction, and a bottom layer with increasing particle size (Anschutz et al., 2012). Our results (Fig.III.2) indicated that bioturbation caused by worm activities followed the bio-conveying transport model and allowed us to identify three distinguishable layers: *(i) a surface bioturbated zone* (0 - 2.0 cm) composed entirely of fecal pellets resulting from the conveying process, with about 75% of the luminophore tracers initially deposited at the sediment surface buried down to 2.0 cm depth at the end of the experiment (Fig.III.2); *(ii) an ingestion zone* (2.0 – 5.0 cm) which

indicates the maximal ingestion depth by the worms; and *(iii) a deep zone* (5.0 – 8.0 cm) where no worm activity has been detected. The maximal ingestion depth found in this study was located below 2.0 cm, and the bioturbated layer was thus estimated to be from 0 to more than 5 cm since luminophores occurred down to this level (Fig. III.2) after 26 days. Depending on the bioturbating time, the thickness of the surface zone could be extended to about more than 4 cm after 93 days having the same species treatment at a higher density of 620 ± 40 worms.dm^{-2} (Ciutat et al., 2006). The obtained rates of bioadvection in the presence of tubificids (Fig. III.2) were in the same order of magnitude of those estimated by other previous authors with tubificid population of various densities (Ciutat et al., 2005; Hoang et al., 2018).

The estimation of the interactive effect between atrazine and tubificid addition on bioadvective rates (two-way ANOVA, Table III.13) indicated that bioturbation was not altered by atrazine-contamination. There was no significant difference of V among the experimental treatments having worms with or without added atrazine {Typ.Tub}, {Atr.Tub}, {Atr.Typ.Tub} (Tukey HSD test, $P > 0.05$, Fig. III.3A). This result is in agreement with previous studies that found out, using Cd-spiked aquatic sediment, that bioadvective rates were not affected by Cd enrichment (Anschutz et al., 2012; Ciutat et al., 2005; Hoang et al., 2018). Although, few studies previously tested the bioadvection rates under contamination with pesticides such as atrazine, oligochaete worms could be therefore considered as ecological engineers with high resistance to both inorganic and organic contaminants from the aquatic environment. Indeed, some invertebrate oligochaetes, which are very widely distributed and frequently dominant in freshwater benthic communities, show a high level of resistance to unfavorable treatments, especially organic pollution associated with severe hypoxic treatments (Brinkhurst and Cook, 1974). An indoor microcosm was carried out by Ciutat et al., (2005) to test the bioaccumulation kinetics of Cd, added to the water column at 20 µg/L, in tubificid worms during a 56-day exposure period with bioaccumulation levels as high as 50 mg.g^{-1} dry *wt.* found in the worms. The resistance of these organisms was

related to their detoxification or pollutant sequestration abilities under polluted environments. Whitley (1967) reported that tubificids showed high tolerance limits for water contaminated by Pb and Zn, with the median tolerance limit at 49 and 46 mg/L, respectively. Both acute and chronic toxicity of organic compounds has been previously investigated in some invertebrates. Dad et al., (1982) reported that tubificid worms (a mixture of *T. tubifex* and *L.hoffmeisteri*) were able to tolerate high insecticides concentrations of with presumed harmless concentrations of Furadan 3G (carbofuran) and Malataf 50E found to be at 4.37 and 3.22 mg/L, respectively. Although atrazine is considered as highly toxic for freshwater aquatic invertebrates (US Environmental Protection Agency, 2003), the hydrophobic chemical was bioaccumulated across the body wall of benthic Oligochaeta *Lumbriculus variegatus* with a wide range of bioaccumulation factors (BAFs), from 0.19 to 19.56 after 240 h of exposure (Jantunen et al., 2008). Although this study does not give an evidence on the persistence of the worms to the high level of the pesticide with concentration as high as 6.4 µg/g (compared with 17 µg/L) which is normally found out in natural sediment in rural pond in Canada (Frank et al., 1990), our study implied that bioturbation should remain active in natural sediments with relatively high concentration, the used concentration of 2µg/g in the present experiment did not exceeding the concentration of 5 µg/L in the previous studies with the same invertebrate group.

III.B.3.2. Influence of biotransport by tubificid on behavior and mobility of atrazine within the sediment

Tubificids affect physical and chemical characteristics, including sediment particle size, porosity, water content, nutrient content, turbidity, TOC, etc., of the sediment and overlying water near the water-sediment interface (Anschutz et al., 2012; Ciutat et al., 2006; Cunningham et al., 1999; Hoang et al., 2018; Mermillod-Blondin et al., 2013; Mermillod-Blondin et al., 2008). The significant increase of water content observed in the top sediment layers (0-2 cm) when the worms were occurring in the experimental treatment {Atr.Tub} (Fig. III.2 and Fig. III.7) is explained by the physical effect of

tubificid worms. Through the conveying activity, worms accumulate their fecal pellets made with smaller particles (fine fraction extracted at depth) at the surface of the sediment. This phenomenon due to bioturbation led to a vertical change in the distribution of sediment grain sizes in the sediment layers where the tubificid worms occurred. As a consequence, the mean grain size of sediment particles became smaller in the upper layer (0-1 cm) and greater in the deeper layers (Hoang et al., 2018). Additionally, fecal pellets getting a larger diameter than sediment particles, this fact is leading to increased sediment porosity in the surface layer. This porosity promoted water and solute exchanges thought the water-sediment interface by simple molecular diffusion. That also explained the significant decrease of water content in the deepest layers when worms were present ({Atr.Tub} treatment, Fig. III.7) along with the least abundance of small particles (fine silt, clay, medium silt, and coarse silt) and more higher abundance of larger particles (coarse, medium and fine sands) in agreement with previous observations of Ciutat et al., (2006) and Hoang et al. (2018). Other types of bioturbation may leas to increased water fluxes through the water-sediment interface by bio-irrigation such as Wood et al., (1975) reported that the tubificid *Limnodrillus hoffmeisteri* generates a burrow flushing rate of 9.5–15 L water per worm per hour (20°C). At typical densities of 10^4 –10^5 ind. m^{-2}, this would result in the exchange of 25 L m^{-2} d^{-1}.

The increase of water content in the top sediment also explained the significant depletion of the total [^{14}C]-(atrazine, metabolites) concentrations and quantity in upper layers with worms ({Atr.Tub}) (Fig. III.5 and III.6). The enhanced porosity caused a dilution of the total atrazine and metabolites concentrations in the upper bioturbated sediment layers. When the surface zone is composed entirely of worms' fecal pellets, it also extends the thickness of the layer (Ciutat et al., 2006), the difference of volume is explained by the enhanced water content.

Aquatic sediments are one of the largest reservoirs of organic matter (Breithaupt et al., 2012). Organic matter in sediment consists of a heterogeneous mixture of carbon

and nutrients, notably in the form of carbohydrates, proteins, fats, and nucleic acids (Zonneveld et al., 2009). Bacteria, as heterotrophic microorganisms living in carbon-rich sediments, play an essential role in the transformation and mineralization of organic matter in aquatic sediments (Boudreau, 1992). Tubificid worms play a significant role in the processing of organic matter in aquatic sediments by direct ingestion of by microbial gardening (McCall & Fisher, 1980; Mermillod-Blondin et al., 2001). During feeding activities of Tubifex worms, the fecal pellets accumulated at the sediment surface are covered by worm's digestive mucus that increases the colonizable area for bacteria. In this study, tubificid worms consequently modified the organic matter distribution in the sedimentary layers (Fig. III.5A and III.5B), with a significant increase in the surface layer due to fecal pellets arrival with mucus made of exopolysaccharides, and a depletion of the organic matter content in deeper layers (2-8 cm) to ingestion. The depletion of C and OM observed in the top sediment is explained by the new fecal pellets arrival, where the microbial community didn't get the time to develop yet. The burial over time of these pellets will be followed by the development of microbial biofilm that explains the OM matter inversion at the bottom of the surface layer. A similar profile of organic carbon content, hence, could be found in the presence of tubificid worms (Fig. III.9).

In addition to these physicochemical parameters, pH is also considered as an important factor controlling the adsorption of atrazine in the soil/sediment. Gradually, positive mobilization of herbicides, such as atrazine and metribuzin in soil has been reported to increase as the pH increased (James et al., 1976). When pH decrease, the molecules became protonated that led to complex the triazine molecules with H^+ on the clay surface (Weber, 1970). In our experiment, the relatively high pH (8.03) in overlying water along was observed together with the significant increase of water content in the surface sediment layers (Fig. III.7) due to the intrusion of overlying water in the interstitial space of the fecal pellet layers (Fig. III.9D). This leads to the increase of pH in this boundary layer as observed in the {Atr.Tub} treatment. Under the conveying

process, the new pH conditions are spread down into the whole bioturbated sediment. Previous studies also reported sediment pH increase under bioturbation, which was explained by an accelerated removal of acid metabolites from sediment (Yingst and Rhoads, 1980).

Soil organic carbon-water partitioning coefficient (Koc) has been so far used as a quantitative measure of the magnitude of the binding affinity of organic matter for atrazine. Also, soil adsorption coefficients (Kd) are interesting indices for predicting pesticide mobility in the aquatic environment (Kulikova and Perminova, 2002). Study of sorption affinities of 101 soil samples from New Zealand was reported by Ahmad and Rahman (2009) with sorption coefficients (Kd) ranging from 0.7 to 52.1 $mL.g^{-1}$, and by Kookana et al. (2008) with the values in 31 soils ranging from 0.51 to 5.48 $mL.g^{-1}$.

Bioavailability and mobility of pesticides in natural environments under bioturbation are strongly affected by their solubility variations (sorption to soil particles and organic matter, dissolution in pore water, or uptake by plants). Pesticides with low Koc or low distribution coefficient have the highest potential for movement; they are highly water soluble, relatively persistent, and not readily sorbed to soil particles. In contrast, the higher the Koc value, the more strongly the pesticide is sorbed onto soil and organic matter, which lowers its bioavailability (Binet et al., 2006). It has been shown that atrazine adsorption is associated with fine particle fraction consisting of clay minerals and organic matter. Koc, however, is a more important factor affecting the sorption than particle size. Atrazine binding to soil organic matter is not extremely strong (**Koc~128 $mL.g^{-1}$**, the moderate water solubility of **33 $mg.L^{-1}$**, average field half-life 60 days) (Lesan and Bhandari, 2000) leading to the high potential for movement from particles to dissolved state. This becomes obvious when compared with benomyl, a systemic agricultural fungicide having low movement rating with the solubility of 2 $mg.L^{-1}$ and Koc of 1,900 $mL.g^{-1}$. A wide variation of partitioning coefficient (Koc), from 30 to 680 $mL.g^{-1}$ (Kookana et al., 2008), with a mean of 126.9 $mL.g^{-1}$, was also found by Ahmad and Rahman (2009). The Koc coefficients in our study (Fig. III.10) were in the

same order of magnitude as the data provided by the authors cited above, with the means varying from 265 ± 131.76 to 537 ± 114.50 mL.g^{-1}. The two coefficients generally increased with soil organic carbon content (Fig. III.10).

It is suggested that the significant depletion of organic matter in the surface and deeper sediment layers under the influence of tubificid worms ({Atr.Tub} treatment) contributed to the higher desorption/movement of atrazine and its metabolites from organic matter to the pore water, compared to the treatments without worms ({Atr} treatment, Fig. III.5C and Fig. III.9A). Organic matter degradation due to worm activity consequently increased [^{14}C]-(atrazine, metabolites) concentration in pore water and thus released more free fraction of atrazine and its metabolites into the interstitial environment (Fig.III.16B). Therefore, in the sediment column, bioturbation accelerates the mobility of atrazine (and presumably its metabolites) from attached forms (onto sediment particles) into a free fraction (in pore water) with lower values of Kd, Koc.

III.B.3.3. Influence of tubificids oligochetes on the biotransformation of atrazine and the bioavailability for plant

Due to its low adsorption in soils with Koc of 128 mL g^{-1} (Sun et al., 2010), residual atrazine and its metabolites, such as deethylatrazine (DEA) or deisopropylatrazine (DIA) have a high potential to contaminate surface waters, groundwaters and adjacent soils (Kolpin et al., 1996; Lewis et al., 2009; Zaya et al., 2011) from agricultural soils via leaching and surface runoff (Douglass et al., 2017; Pascal-Lorber et al., 2011). The degradation of the herbicide mostly occurs in wetland areas (Mudhoo and Garg, 2011) or in topsoils (Douglass et al., 2015), where a physical (photo-oxidation), chemical and biological degradation processes may occur. Atrazine-degrading microbial communities (Krutz et al., 2012), as well as other important chemical factors (soil pH, organic material, and moisture) exerting controls on the plant up taking, (Wehtje et al., 1984), have been considered as the primary mode of the attenuation. Soil microbial communities play a crucial role in biodegradation of the *s*-

triazine herbicides such as atrazine into metabolites (Udiković-Kolić et al., 2012). As a growth substrate (C and/or N source), atrazine is used by microorganisms via catabolic pathways of xenobiotic due to *atz/trz* genes coding for the enzymes responsible for the mineralization (Noor et al., 2014; Udiković-Kolić et al., 2012). Atrazine-degrading fungi normally include members of genera *Pseudomonas, Acinetobacter, Agrobacterium, Arthrobacter, Rastonia and Norcardioides* (Strong et al., 2002) as well as some mycorrhizae such as *Aspergillus, Rhizopus, Fusarium, Penicillium, Phanerochaete* (Mougin et al., 1994; Islas-Pelcastre et al., 2013).

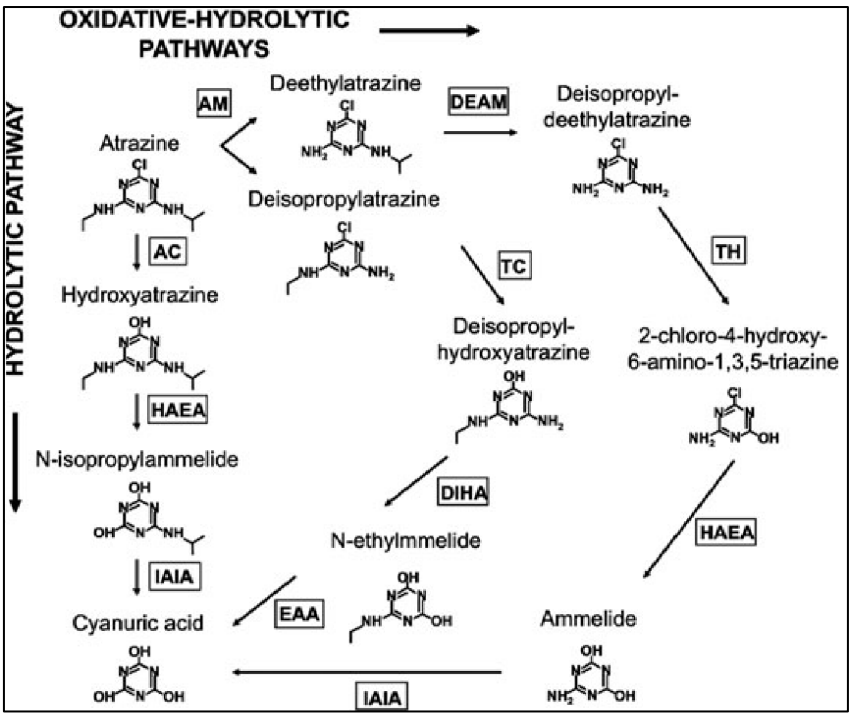


Figure III.15. Atrazine degradative pathways (Govantes et al., 2009). *Catalyzing atrazine to the common intermediate cyanuric acid by enzymatic steps. AC, atrazine chlorohydrolase; HAEA, hidroxyatrazine ethylaminohydrolase; IAIA, N-isopropylammelide isopropylamidohydrolase; TC, s-triazine chlorohydrolase; AM, atrazine monooxygenase; DEAM, deethylatrazine monooxygenase; DIHA, deisopropyhidroxylatrazine amidohydrolase; EAA, N-ethylammelide amidohydrolase, TH, s-triazine hydrolase.*

Since this transformation occurs at depth in the sediment, the microbial community involvement is suspected as a significant effect compared to chemical or physical influences (Kerle et al., 2007) and this biotransformation should be led by the microbial consortium in the sediment surrounding the plant roots. Also, the water and

organic matter contain in the bioturbated layer (5 cm) was modified that was suspected to be at the source of an acceleration of the atrazine and its metabolites mobility from sediment particles to pore water (lower the values of Kd, Koc) under bioturbation, thus promoting the uptake by the plant. Bioturbation is creating fluxes of sediment and related OM that influences the vertical distribution of organic matter. Since organic matter is controlling the atrazine adsorption capacity in the solid fraction, bioturbation is suspected to influence the adsorption capacity depending on the depth in the sediment column. Besides, C/N ratio is increasing with depth in the sediment under bioturbation of the sediment column; C/N ratio and organic matter variation together attest of enhancement of the organic matter-mineralizing by heterotrophic microbial degradation pathways in bioturbated sediment.

Octanol/Water partition coefficient (K_{ow}) is an indicator of the environmental fate of a pesticide. Due to a very high polarity of water, the more polar a molecule is, the more soluble it will be in water (Linde, 1994). A pesticide with a low log K_{ow} value such as 0.6 is more water-soluble (hydrophilic or polar), while pesticide with a high log K_{ow} value, such as 2.61 (atrazine) is more lipid soluble (lipophilic). Our results on the mobilization of atrazine and its metabolites in the sediment fractions under the impact of bioturbation were illustrated in Fig. III.5. By stimulating organic content degradation, tubificids favored the mobility of atrazine and its metabolites from sediment particles (organic matter) to pore water in the sedimentary layers of {Atr.Tub} microcosms. This release of the atrazine and related compounds in the free fractions, that were then taken up by the plant, was demonstrated by the significant increase of ^{14}C-(atrazine, metabolites) concentrations in the pore water of the fecal pellets zone between the treatments having a plant with and without bioturbation {Atr.Typ} and {Atr.Typ.Tub} (Fig.III.6C). Effects of worms and plants were significant on Kd and Koc coefficients in the fecal pellets zones of the two experimental treatments (Fig. III.10). Inversely the high values of Kd and Koc in the presence of the plant only in the lower layers indicated the

strong adsorption or immobilization of residual pesticides molecules onto sediment particles and organic matter in these conditions.

Uptake of lipophilic compounds like atrazine depends mainly on their ability to partition into cell membranes. Atrazine penetrates the plant by a simple phenomenon of passive diffusion (McCloskey et al., 1997). When tissues or cells of plant are exposed to atrazine (nonpolar or lipophilic, high log K_{ow}) and its metabolites such as DEA, DEHA, DIA, DIHA (less polar), the molecules quickly permeates the tissue via the xylem vascular system (apoplastic movement) and remains into cytoplasm of cells, resulting in the passive absorption and accumulation in the cells. Equilibrium between the cytoplasm and chloroplast thus will be established (Shimabukuro and Swanson, 1969). In contrast, polar molecules slowly flow across lipophilic cell membranes, even not pass into the cells. Although rapid to cross the cell membranes, the lipophilic organic compounds are still adsorbed by organic matter in the sediment. It causes the competition between organic compounds complexes formation with atrazine and organic matter and their phyto bioavailability. The more organic matter content in the sediment, the less organic compounds are available for plant uptake. However, the depletion of organic matter according to the depth of the sediment ({Typ.Tub} in the microcosm, Fig. III.10) and due to bioturbation affected this competition in favor of the bioavailability for the plants. This process together with the move of atrazine and its metabolites from sediment particle to pore water (lower the values of Kd, Koc), thus promoted the taken up by plants.

On the other hand, the metabolism of atrazine is very complex in the sediment and the plant and may result in the involvement of 15 to 20 different compounds (Fig. III.15). The atrazine metabolization under the impact of tubificids worms was illustrated in Fig. III.11 with the increased number of less polar metabolites in the root system of plants in treatment with bioturbation {Atr.Typ.Tub} compared to without bioturbation {Atr.Typ}. Although total quantity of [^{14}C]-(atrazine, metabolites) were not different when the worms were occurring, the quantity of [^{14}C]-metabolites significantly

increased ($P < 0.01$, t.test) along with significant decrease of [^{14}C] atrazine quantity ($P < 0.001$, t.test) in the plant root system of the treatment having worms ({Atr.Typ.Tub}, when compared to that in the treatment without bioturbation ({Atr.Typ}) (Fig. III.12). Sensitive plants, such as oats, cucumber, and alfalfa, are unable to detoxify atrazine resulting in chlorosis and death (Hull 1967), while resistant plant species, such as *T. latifolia* accumulate and metabolize atrazine to hydroxyatrazine and amino acid conjugates (Ronald, 1989). Beside the ability of *T. latifolia* to accumulate atrazine and convert them into the derivatized product by themselves, the present study thus demonstrated that bioturbation ({Atr.Typ.Tub} treatment) considerably enhanced the accumulation and metabolization process of atrazine that started into the sediment under microbial processes and thus resulted in increases metabolites in the plant roots system.

One more consequence of bioturbation works in the direction of pesticides biodegradation in the sediment: atrazine metabolization requires additional nutrients and carbon to feed the micro-organisms. By increasing flux dispersion, the oligochaetes also enhanced the nutrient availability in all microenvironments of the columns, stimulating both aerobic and anaerobic microbial activities. Mermillod-Blondin et al., (2001) and McMurtry et al., (2011) also reported a significant correlation between the abundance of heterotrophic bacteria in sediments and tubificid preference.

III.B.3.4. Plant influence on bioturbation intensity and pesticide attenuation

Several studies have revealed the plant influences on aquatic bioturbation intensity in aquatic sediment notably for large bioturbators such as ghost shrimps (Berkenbusch et al., 2007) and the polychaete *Nereis diversicolor* (Hughes et al., 2000). Interaction between belowground biomass of the seagrass *Zostera* and burrowing bioturbators activities have been investigated previously with negative effects of root density on the intensity and mode of sediment particle mixing by benthic infaunal

activities in the coastal environment (Bernard et al., 2014). These findings, however, are not consistent with the present study that revealed the independence of bioturbation intensity with the occurrence of the riparian *T. latifolia* plant. Neither significant difference in bioadvective rate (V) (Fig. III.3A) and biodiffusive rate (Db) (Fig. III.9B) nor the interaction between plant occurrence and worm treatment (Table III.14) were observed.

It should be noticed that the contradictory results from that obtained in the present study might be due to the differences in bioturbation modes, in types of aquatic environment studied, or in species/ functional type of aquatic plant selected. This suggested that the bioturbation type of biotransport produced by tubificid worms still remains active and is not affected by the plant treatment, when other types of bioturbation as biodiffusion of regeneration made by large crustacean may be affected with the proximity of plants.

Moreover, the fact of using individual species of bioturbators rather than a whole community, as well as longer time duration for carrying out this experiment (26 days) could lead to different observations of the interactions between plants and invertebrates bioturbators.

III.B.3.5 Mass balance and contaminant fluxes between experimental compartments

Due to the low adsorption of atrazine to solid (Koc =128 mL g^{-1}) (Sun et al., 2010) compared to a rapid uptake by plant root (in the soluble form only), the sediment column was homogeneously contaminated at initial time of the experiment with a water solution containing [^{14}C]-atrazine and that was 15 days before the introduction of the worms and plant to the microcosm. The choice to work in the present experiment with contaminating sediment, instead of only contaminated overlying water as in the previous experiment with cadmium (see Chapter III.A), was made in order to get a completed adsorption of atrazine first to the sediment, and before it might be impacted by other processes, including bioturbation and phytoremediation. On the other hand,

the effects of tubificids on the biotransportion of pollutant is considered as already done in the previous study. Furthermore, the present study is more focusing on biogeochemical properties modifications in the sediment compartment where the changes of pesticide concentration and fluxes might be observed. Atrazine and its metabolites were however explored in all physical compartments, including free water, interstitial water, sediment particles, and plants, except in the worm's biomass.

In the absence of worms in {Atr} and {Atr.Typ} treatments, the decrease of [^{14}C]-(atrazine, metabolites) quantities in the overlying water (negative values of ΔQ_{1A} and ΔQ_{1B} at t = 26 days) along with the increase of the quantity in the sediment compartment (positive values of ΔQ_{2A} and ΔQ_{2C}, Fig.III.14A) were observed as a consequence of completed adsorption of atrazine from free water to sediment particles during 31 days of contamination (including the 15 days before biodiversity introduction). Atrazine, with a Koc > 100 ml/g and capable of donating and accepting hydrogen bonds (Welhouse et al., 1993), can interact and sorbt to sediment organic matter by multiple mechanisms depending on its organic content (Guo et al., 2016). In the presence of worm ({Atr.Tub} and {Atr.Typ.Tub} treatments), the significant depletion of [^{14}C]-(atrazine + metabolites) concentrations in the first 2 cm of sediment (Fig.III.9A) as well as the negative values of ΔQ_{2B} and ΔQ_{2D} (Fig.III.14A) are explained by the sediment porosity increase and the occurrence of fluxes (F1B and F1D, Fig.III.14) from sediment to water due to the bioturbation.

The calculated fluxes of atrazine and its degradation products from water to sediment in {Atr.Tub}, {Atr.Typ}, {Atr.Typ.Tub} treatments (F1B,C,D, Fig.III.14) showed no significant differences compared to that in the control ({Atr} treatment) ($P > 0.05$, Dunnett multiple test, data not shown). Tubificid activity impacted the sorption and desorption of the moderate hydrophobic atrazine between attached-particles and free fractioning forms within sediment compartment with an increase of the concentrations of the molecules in the pore water that is partly explained by the organic matter reduction under tubificids feeding. The influences of bioturbation by tubificids on these

chemical properties of sediment have consequences on the adsorption-desorption behavior of the herbicide in the sediment compartments, without affecting the total fluxes from water to sediment.

The relative recovery, (*i.e.*, the total amount of [^{14}C]-(atrazine, metabolites) in the whole test-system (overlying water, sediment, and plant) at the end of the experiment divided by that added at the beginning of the experiment), was calculated in each treatment with and without bioturbation ({Atr.Tub} and {Atr}; {Atr.Typ.Tub} and {Atr.Typ}). This allows the assessment of the faunal biodiversity influence in the bioremediation process. The obtained data from the treatment having tubificid worms ({Atr.Tub} and {Atr.Typ.Tub} treatments), indicated relative recovery approximately equal to 79.5 % and 84.9%, respectively. This values suggested additional pesticide loss or uptake. This additional process might be worms' bioaccumulation or enhanced pesticides mineralization under higher bioturbation. While, a marginal loss of the pesticide, approximately 93.5% and 94% of relative recovery observed in the absence of the worms ({Atr} and {Atr.Typ} treatments, respectively) (Fig.III.14), implied *(1)* a volatilization of atrazine to the air phase via the mineralization into CO_2 or *(2)* an adsorption to experimental pail walls. This finding was consistent with the previous study of Bundschuh et al., (2016) on the role of pesticide properties on remobilization from sediment to the overlying water phase.

III.B.3.6. Phytoremediation potential on the pesticide attenuation under bioturbation process

The remediation of atrazine by plants has been proved in previous studies (Ibrahim et al., 2013; Murphy and Coats, 2011; Susarla et al., 2002). Herbicide absorption by plant roots is typically characterized by rapid initial entry into the tissues. Root absorption capacity is often expressed in terms of the root concentration factor (EC_R). Initial absorption rates and EC_R are positively correlated with lipophilicity (octanol/water partition coefficient, K_{ow}) (Top et al., 1986). Polar compounds enter the

root cells less rapidly and are initially restricted to the free space, resulting in EC_R ranging from 0.6 to 1.0 (uncompleted permeation). Lipophilicity compounds, on the other hand, enter rapidly the root cells and can accumulate in lipid-rich domains of the tissues, resulting in a greater value of EC_R, higher than 1.0 (Top et al., 1986). Significant increase of EC_R with a mean value of 7.55 ± 1.59 (Fig. III.14) when the worms were added in {Atr.Typ.Tub} microcosms indicated that bioaccumulation of *T. latifolia* was increased in the roots in the presence of bioturbation.

The water content was consistently lower at depth than in the upper layers in profiles of the treatments having both worms and *T. latifolia* (Fig. III.7B). The tubificids effects were obvious along the whole bioturbation zones, and with any noticeable interactions effects between worms and Typha. However the significant reduction of the water contains was only observed in the layer 3-4 cm that indicated the role of a plant during their solution uptake at the depth where root is located.

III.B.3.7. Toxicological test using Chlorella vulgaris

Toxicological test of Chlorella vulgaris on experimental pore water samples

It was indicated in Fig. III.5C that there were more atrazine and its metabolites in the first 2cm of sediment pore water when the worms are occurring, in both conditions with and without plants. Under the activities of worms, bioturbation may increase the bioavailability of the atrazine and its metabolites via the depletion of organic matter by feeding activity and microbial degradation. These changes the equilibrium between the complexes of pesticides and organic matter and thus promote the release of less polar molecules of herbicides and metabolites into the pore water. As a result, higher concentrations and quantities of atrazine and metabolites in the pore water are observed, particularly in the condition {Atr.Tub}. However, lower concentration of the herbicides could be found in the upper layers in the presence of the plant {Atr.Typ.Tub} that are explained by the *Typha*'s root system uptake.

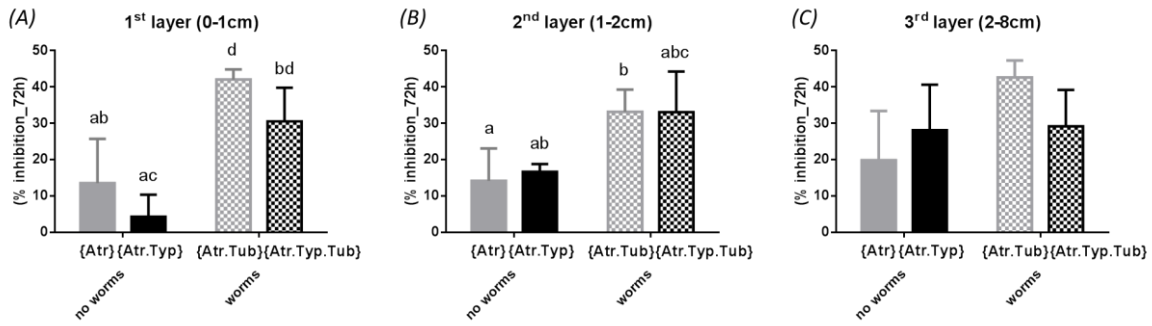


Figure III.16**.** Percentage of inhibitions of *C. vulgaris* in experimental pore water samples collected in different layers of microcosms sediment at t = 26 days **[A]:** *1st layer from 0 to 1cm*; **[B]:** 2nd *layer from 1 to 2 cm*; **[C]**: 3rd *layer from 2 to 8cm* at 72h. Different superscript letters indicate the significant differences for the percentage of inhibition between experimental treatments at P≤ 0.05 level as analyzed by Tukey HSD test. Values are means ± SD (n = 4, except for {Atr}, {Atr.Typ}, {Atr.Typ.Tub} at the 2nd layer, n = 3 and for {Atr.Typ.Tub} at the 3rd layer, n =3). No significant difference was observed among the treatments of the 3rd layer. (*see* ***Table II.2*** *for the definitions of acronym corresponding to the different treatments*).

Table III.9.

Effects of *T. latifolia* and tubificids on the percentages of inhibition of *C. vulgaris* in experimental pore water of different sedimentary layers, using two-way ANOVA

Factors	**1st layer (0-1cm)**		**2nd layer (1-2cm)**		**3rd layer (2-8cm)**	
	F (1,12)	**P**	**F (1,9)**	**P**	**F (1,11)**	**P**
T.latifolia treatment	6.143	0.029*	0.08252	0.7804ns	0.2155	0.6515ns
Tubificid addition	42.96	<0.0001****	17.04	0.0026**	4.485	0.0578ns
T.latifolia : tubificid interaction	0.0781	0.7846ns	0.08889	0.7724ns	3.709	0.0804ns

(*), (**), and (****) indicate significance at P≤ 0.05, 0.01, and 0.0001 respectively; (ns)-non significance according to the two-way ANOVA tests. (*see* ***Table II.2*** *for the definitions of acronym corresponding to the different treatments*).

At the end of the experiment, atrazine concentrations in overlying water and pore water of our microcosm were found to be at about 200 and 400µg/L, respectively (Fig. III.9). The concentrations were not consistent with actual environmental concentrations in Europe because of the herbicide's prohibition set in European Union since 2004 and actual measured level in natural waters not exceeding to 28 µg/L (Munz et al., 2013). However, the ecotoxicological results using *Chlorella vulgaris* with the IC_{50} found to be at 60.41 µg/L (Fig.III.16) showed % of inhibition lower than 50% obtained

and these results with to be consistent with the estimated herbicides levels in our microcosm.

As can be gleaned from Fig. III.16A, by comparing with and without worms treatments ({Atr} and {Atr.Tub}, Tukey HSD test), the pore water is significantly more toxic in the sediment layers when the worms are occurring. This pattern was always observed in all the 3 investigated layers from 0 to 8 cm, where the bioturbation is acting. For the conditions having the plant treatments ({Atr.Typ} and {Atr.Typ.Tub}, Tukey HSD test, Fig. III.16B), the pore water was also significantly more toxic in the top and bottom layers when the worms were occurring in the experimental conditions. While the decrease of toxicity in the layer from 1.0 to 2.0 cm having worm treatment is likely due to the plant root.

This higher toxicity of the porous water when bioturbation is acting is explained by 1. the enhanced pesticides concentrations in the interstitial water in treatment with tubificids and the higher toxicity of the metabolites for the algae species. Although the toxicity was not significantly lower when *Typha latifolia was acting*. the plant effects were still significant when tested by the ANOVA. This last point underlined the double and opposite effects of the Tubificids on the toxicity of the porous water : i. the worms are increasing the water toxicity, probably due to the influence on the microbial degradation into more harmful metabolites, and ii) the plant effects that slightly lower the toxicity by uptaking the harmful compounds into its root system. These results about the plant capacity of atrazine removal are consistent with previous observations from other authors, like Moore et al. (2013) and (Mezzari and Schnoor, 2006) who observed that high resistant aquatic plant in a polluted environment, are capable of significantly atrazine molecules removal. This removal is generating flows between the soil and the root system or transfer of atrazine into other metabolites, such as hydroxyatrazine, DEA, and DIA.

On the other hand, the bioturbation is proved to be a positive ecological tool which significantly impacts on the contaminant incorporation into the sediment (Ciutat et al., 2007 Delmotte et al., 2007; Devault et al., 2009;). Bioturbation also can control the fate of organic matter and nutrients as well as fluxes of nutrients between sediments and water (Anschutz et al., 2012; Hölker et al., 2015) and therefore facilitate the transformation of pollutants by the microorganisms communities (Gerino et al., 2014). The pore water toxicity was increased in the bioturbated conditions that might be explained by the increase of the metabolites' bioavailability (more atrazine and its metabolites concentrations in pore water). The decrease of toxicity in the layer from 1.0 to 2.0cm (Fig. III.16B and Fig.III.16D) having worms treatment and in the deepest layer (2.0 – 8.0cm) in the conditions without worm treatment ({Atr.Typ} and {(Atr}) might be due to the plant root' activity in the middle layer of the sediment column.

On the one hand, bioturbation is known to promote microbial activity that may support the degradation of such compounds, but on the other hand, the release of this compound under bioturbation effect may increase its toxicity to these organisms. Thus, in the same time, the bioturbation is facilitating the biodegradation of persistent pollunant by way of interaction with sedimentary micro-organisms, but also simultaneously enhance the toxicity in the surface layers where the plants are not extracting the pesticides yet. The superimposition of these processes in the bioturbated layer may lead to controversy if the details of the mechanism behind the toxicity evolution are not explained with the help of the other results.

III.B.4. CONCLUSION

In aquatic systems, atrazine is among the most largely occurrent herbicides in the continental sediments within deposition areas such as wetlands and ponds. Its moderate hydrophobic character makes it a sensitive candidate to investigate its behavior at the water-sediment interface as a response to biodiversity-related processes such as bioturbation. Our experimental study using radiolabeled [^{14}C]-atrazine showed that bioturbation is still maintained in contaminated sediment with or without plants. Under worm activities, the overall depletion of atrazine and its metabolite concentrations in the surface sedimentary layers was observed together with the redistribution of organic matter and C:N ratio in the sediment column. The influences of bioturbation by tubificids on these chemical properties of sediment have consequences on the adsorption-desorption behavior of the herbicide. Therefore bioturbation accelerates the mobility of atrazine (and presumably its metabolites) from attached forms (onto sediment particles) into a free fraction (in pore water) with lower values of Kd, Koc. The change of the partition balance gets consequences on the toxicity of the sediment pore water that is globally enhanced under bioturbation and with a more advanced bioavailability for microbial communities and plant uptake. This indirect bioturbation influence on the partitioning of atrazine in sediment suggests that atrazine should be more available for the other organisms that live in the sediment.

The bioaccumulation (ECs) and metabolization of the pesticides were significantly enhanced when the worms were present as observed in *T. latifolia* roots. This mobility should also increase its toxicity towards some other organisms (microbes, and invertebrates) that live in the sediment layer, but may also promote its uptake by other plants. Bioturbation has effects on biogeochemical cycle inside the sediment compartment by changing the organic matter content where the worms' feeding and gardening are happening. Phytoremediation then played an important role in the process by up taking the released available toxic molecules in the pore water. In this sequence of the process, the phytoextraction efficiency looks favored by the

bioturbation because a more accessible quantity of pesticide is made available in the pore water in the surrounding sediment of the roots systems. The production of more toxic metabolites under bioturbation may be counteracted by the uptake of the whole compounds by the plants.

The role of microorganism communities in the degradation of atrazine in bioturbated sediment should be addressed in the next studies. Further analytical works, *i.e.* identification of atrazine metabolites in sedimentary layers and in the plant system as well as polarity determination of new metabolites (via Kow values) which is formed in the plant are also needed to test the hypotheses of metabolization process under the bioturbation.

CHAPTER III.C. COMPARISON OF BIOREMEDIATION EFFECTS ON TWO DIFFERENT TYPES OF POLLUTANTS

The impact of human activity on the aquatic ecosystems has produced a global limitation of water resource availability and quality in many places in the word. Water resources scarcity and risks to ecological health such as water contamination call for new solutions that help to sustain the wetlands ecosystem as all other types of aquatic ecosystems. For the restoration of wetlands, environmental engineering goals identify contaminant behaviors, prevent its bioavailability and try to reduce pollution loads in human-made and natural systems, and, above all, attempt to minimize the negative impact, or ecological footprint, of contaminants accumulation on all aquatic systems (Dresp-Langley, 2008). Several examples of constructed wetlands utilize ecologically engineered systems to treat, recycle, and permit the reuse of wastewater (Todd et al., 2003). Prior methods development applied to treat most frequent pollution such as nutrients excess, coliforms and excess of sediment build-up, with lots of emphasizes on the wetland capacity to reduce nitrate and ammonia (Prior and Johnes 2002). The plants are a critical element in most of these technologies, as attested the actual large display of planted filters over the world to treat waste water before getting in the natural environment. Their roots provide surface areas and nutrient support for microbial communities, some nutrient uptake, and they shade/inhibit suspended algae in the wetlands. Two major processes involved in the phytoremediation are depending on whether the pesticide processing takes place outside or within the plant. These processes are rhizodegradation and phytoextraction. In the first case, the root system acts as a support for the soil microflora. The root exudates, consisting of sugars, amino acids, and organic acids, enhance the development of a cortege of bacteria and fungi forming the rhizosphere that leads to an increase of the microbial biomass versus a not planted soil (Bowen and Rovira, 1999; Weyens et al., 2009).

The effectiveness of bacteria and fungi in the degradation of organic compounds

has long been used in remediation processes (Pothuluri and Cerniglia, 1994). In phytoremediation, the use of soil microflora in the rhizodegradation process, therefore, consists in enhancing bacterial or fungal development to increase the capacity of the soil to break down pesticides (Anderson et al., 1993). The ability to phytoextract metals from sediment was largely demonstrated (Leveque et al., 2013; Lyubenova and Schröder, 2011; Pandey et al., 2014). More recently, micro-organic pollutants in the wetland water raised more concerns and difficulties to reduce this type of pollution due to the resistance of the compounds to microbial degradation. Few methods were tested to address bioremediation strategy towards micropollutants for removing persistent organic pollutants (POPs) such as pesticides, PCBs, PAHs and PPCPs from wastewater with plants or other strategies. Most of the tested methods are related to various microorganisms' capacity to breakdown the complex compounds through biodegradation, biostimulation, or bioaugmentation process (Gaur et al., 2018).

Facing the refractory character of these anthropic molecules and their harmful effects on human and natural health, ecological engineering is providing a set ofpossibilities that is worth it to explore. Furthermore, bioremediation that involves the capabilities of microorganisms or plants in the removal of pollutants is the most promising, relatively efficient, cost-effective and sustainable methods for natural polluted aquatic systems. However, the current bioremediation approaches suffer from a number of limitations which include the poor capabilities of microbial communities in the field, lesser bioavailability of contaminants on spatial and temporal scales, and absence of benchmark values for efficiently testing the bioremediation for its widespread application in the field (Megharaj et al., 2011). Furthermore, the combination of different methods, or natural processes, to influence the fate of contaminants, are still poorly investigated and remained question-unknown. This Ph.D.'s objective is, therefore to question an innovative strategy based on the combination of bioturbation and phytoremediation processes to increase the removing efficiency of pollution in aquatic sites. These strategy benefits were tested with two

controlled laboratory experiments with the two types of pollutants (metal - conservative and herbicide – non-conservative).

Our results indicate that bioturbation process has been undergoing with regular intensities in the presence of cadmium in the overlying water and atrazine in the sediment, with pollutants concentration representative of the aquatic system under high chemical pressures. With cadmium contamination from the water, bioturbation has been active at conveying sediment from the surface to deeper layers under worms feeding activity. This activity results in two significant processes that were influencing the metal behavior: (*1*) at the sediment surface, the metal was continuously adsorbed on new sediment particles coming from deeper layers; (*2*) the metal was then transported downward by bioadvection from the surface into the anoxic sediment at depth. These two processes together, thereby significantly increased or renewed the concentration of metal in the sediment surrounding the root system of *T. latifolia* (see Chapter III.A). With atrazine contamination starting from the sediment, the bioturbation was occurring with a regular intensity, but the fact that initial contamination was homogenously mixed in the sediment column didn't allow to get noticeable effects of the biotransport on the atrazine vertical distribution in the sediment column. It is however suspected that the concentrations in the vicinity of the roots were certainly renewed in microcosms with bioturbation. In this case of micro-organic pollution in the sediment, the bioturbation effects are more likely to be given in evidence on the process of biodegradation by the microorganisms. Bioturbation phenomena are well known, not only to create biotransport of particles, but also to influence the microbial composition and activities by changing the sediment properties patterns and the supply of solutes in the pore water (Aller et al., 1994; Anschultz et al. 2012). If the microbial capacities to breakdown persistent micro-organics are demonstrated in many studies, on the other hand, bioturbation is able to indirectly influence the microbial patterns, community composition, richness, abundance, and turn-over in the sediment column (Yao et al., 2017). The results of this invertebrates and microbial relationships in the contaminated

sediment is a metabolization of atrazine into derivatives (Monard et al., 2008; Hölker et al., 2015) products via microbial biodegradation, including atrazine-degrading bacteria and fungies (Krutz et al., 2012). In the present study, one of our main questions is related to how the bioturbation influence on the microbial consortium may contribute to bioremediation towards a refractory herbicide.

The influence of bioturbation on microbial community breakdown of pesticide was suspected in this study since the number of metabolites found in the sediment was increased in the plants roots of microcosms with bioturbation. Some authors also mentioned the capacity of worms to increase the microbial community evolution, in the way that the richness turnover of the bacteria community composition was increased. The renewal of the community consortium of the sediment is explained by the filtering of microbial species produced during the transit through the worms' guts. By increasing the turnover of the microbial community composition, the invertebrate grazing of the biofilm lying on the sediment particle is enhancing the capacity of the microbial community to evolve with species adapted to the chemical composition of the environment. Some authors further mentioned that worm internal microbial consortium is playing as inoculum in the natural communities (Kauppi et al., 2018).

In these microcosms, the bioturbation displayed a second type of effects with consequences on bioremediation efficiency due to the influence of worms on the physical properties of the sediment. The bioavailability of the atrazine's metabolites may be increased due to a bioturbation effect on sediment porosity that may enhance atrazine and metabolites molecules bioavailability. This process gets incidences on the disponibility of the pollutant for uptake by the plant roots that was certainly also occurring into the microcosms sediment with bioturbation.

Finally, the bioturbation involvement in the bioremediation efficiency results from its ability to change the physical properties, but also the chemical and biological composition of the sediment and to generate biotransports of sediment. For non-

conservative pollutants such as POPs, all these processes are possibly occurring simultaneously with relative incidence depending on the location of the source of contamination. For conservative pollutant, all except biodegradation will be playing (see Chapter III.B).

The third chapter – III.C, complementary to the first and second one, is devoted to comparing the mitigation efficiency of this bioremediation strategy towards the two types of pollutants (metal - conservative and herbicide – non-conservative) by studying their mass balance fluxes between overlying water, sediment, and plant compartments.

III.C.1. TRANSPORT AND BIOAVAILABILITY OF POLLUTANTS EXAMPLES OF CADMIUM AND ATRAZINE

III.C.1.1. Mobility of the pollutants within the sediment

Our bioremediation studies were mimicking natural-based patterns of wetland at the interfaces between the overlying water and the sediment. In this habitat, the oligochaetes community may reach high species richness and densities, and tubificids are a ubiquitous group that usually inhabits wetland sediment in the word. Bioturbation caused by tubificid worms' activities (with or without plants) follows the regular bio-conveying transport model with significant bioadvective rates.

The present study demonstrated that biotransport affected mobility and fate of the pollutants within the sediment and thus created enhanced pollutant fluxes between water, sediment, and plant compartments. Bioturbating mechanisms influencing the mobilization depend upon not only on the type of pollutants (conservative or non-conservative) but also on the design of the applied experiments. Tubificids affect physical and chemical characteristics of sediment and overlying water near the water-sediment interface, including sediment particle size, porosity, water content, pH, nutrient content, turbidity, TOC, etc., (Anschutz et al., 2012; Ciutat et al., 2006; Cunningham et al., 1999; Hoang et al., 2018; Mermillod-Blondin et al., 2008; 2013). The mobilization and bioavailability of PHEs - Potential Harmful Elements (Rinklebe et al.,

2016; Shaheen et al., 2016) as well as adsorption and desorption of pesticide in a water-saturated sediment strongly correlated to these physicochemical parameters, in which pH and OM content are considered as important factors (Bradl, 2004; Fu and Allen, 1992).

Worm activity transferred reduced particles and pore water at the sediment surface during the feeding process of the tubificids population. This recycled material results in a several centimeter-thick layers enriched in water content, dissolved nitrate and sulphate, and depleted in oxygen, ammonium and dissolved Mn(II) (Ciutat et al., 2006; Anschutz et al., 2012). Oxygen penetration depth is usually shallower in this type of bioturbated sediments, as mentioned by the same authors, because, tubificids do not irrigate their burrows (McCall & Fisher, 1980). The presence of tubificids increases ammonia concentrations in interstitial pore water that leads to a decrease of pH with depth in the sediment when compared to the water-sediment interface, where cadmium accumulated in the sediment of our microcosms from the beginning of the 1st experiment. The pH value decreased from 7.9 ± 0.01 in the top layer of 0.5 cm and 7.73 ± 0.02 in the layer of 4 cm under tubificids influence (Fig. 4, section III.A.1). The influence of tubificids bioturbation on pH distribution therefore directly drives the mobility of the heavy metals. The pH influence on the solid/liquid partition coefficient - Kd for the metal in the sediment is driving the mobility of tracer metals between water and sediment as well as its bioavailability for the bioremediation purpose. The estimated partition coefficients Kd for Cd ranges from 8 to 4000 mL/g as a function of pH from 5 to 8 (EPA 1999). When pH increases, the partition will be in favor of the particulate fraction so that the dissolved Cd precipitated in the surface oxic layer. When fecal pellets arrive at the sediment surface, the transfer of anoxic sediment, with low pH, to contact with the overlying water, with higher pH, creates a change of the sediment physicochemical properties. This change certainly favors the adsorption of Cd from the overlying water onto the particles of sediment. When this contaminated sediment is buried downward, bioturbation process thus causes new and inverse shifting of the

redox conditions (Du Laing et al., 2009) along with the release of protons H^+ (Fisher and Matisoff, 1981; Fu and Allen, 1992) in the sediment. In non-bioturbated sediment, the pH gets even lower at depth, so that adsorbed metals may also release into the pore water. However the surface process of Cd trapping being absent without bioturbation, the final transfer of Cd at depth in the sediment remains minimal without worms conveying, so that the release in these microcosms sediment remained also poor. Accordingly, PHEs cations are released from organic matter and other sorbents, such as clay mineral surfaces when pH decrease (Du Laing et al., 2009; Frohne et al., 2014) and consequently increase the Cd mobility and bioavailability for the plants (Lee et al., 2009; Wang et al., 2016) in the deeper layers. With the conveyor-belt process generated by the tubificids, this upward and downward biotransport is occurring continuously in the boundary layer of the bioturbated sediment in the wetlands. It is thus suggested here that bioturbation in natural wetland may continuously act as a pump of metal from the water into the sediment, for those metals and other pollutants that gets similar Kd coefficients to Cd. However, partition coefficients of trace metals are all different so that other metals behavior facing pH change under bioturbation is very specific to each metal and difficult to anticipate. Nevertheless, Alison & Alison (2005) ranked the metal in the sediment according to their specific Kd with the order:

Pb>Hg>Cr>Cu>Ni>Zn>Cd>Ag>Co>As

Mobilization of herbicides in the soil, by changing the free/adsorbed balance in favor of the free fraction, has been reported to increase as the pH increased (James et al., 1976). When pH decreases, the molecules became protonated that could have led to complex the triazine molecules with H^+ on the clay surface (Weber, 1970). In the 2nd experiment, the physical impact of tubificid led, similarly to the first experiment, to the significant increase of pH in sediment (section III.B), when compared to non-bioturbated sediment (Fig. III.9). Thus bioturbation may favor the bioavailability of the herbicide as well even if atrazine was initially introduced and adsorbed in the contaminated sediment since the beginning of the beginning. In our two different

experiments (with Cd and atrazine), the pH profiles displayed the same patterns with a lower value in the sediment than in the overlying water, but the pH remaining higher in the sediment with tubificids than without (Fig.4 - section III.A.1 and Fig.III.9 - section III.B). Besides, it is also suggested that the significant depletion of organic matter content in the surface (0-1 cm) and deep sediment layers (2-8 cm) under the biofilm and detritus grazing by tubificid worms enhanced desorption/movement of atrazine and its metabolites from organic matter to the pore water (Fig. III.5C and Fig. III.9A). Depletion of organic matter due to worm feeding probably acted in the complement of the pH pattern changes in bioturbated sediment and both processes resulted in the acceleration of the mobility of atrazine (and its metabolites) from attached forms into a free fraction in pore water (Fig. III.17).

It is concluded that in both cases of conservative metal or non-conservative persistent herbicide, the influence of the tubificids bioturbation on the biochemistry of the sediment is acting in favor of a contaminant flux from the overlying water into the sediment, and from adsorbed on sediment particles to free form in the pore water. Also, the conveying of sediment in the bioturbated layer continuously transports downward sediment and pollutants from the surface layer until depth where the plants' roots system is occurring. This conveying is poorly influencing the atrazine distribution because of the homogeneous contamination of the sediment column but probably worked at renewing the sediment and its related contaminant loads in the vicinity of the roots during the experiment lapse of time.

III.C.1.2. Bioavailability for plant

Mechanisms of bioturbation on PHEs bioavailability in the water-sediment conditions are now relatively well investigated in the literature. Likewise, the bioavailability of pesticides, correlated with its solubility, is strongly affected by the bioturbation effects on sediment physicochemical properties. As mentioned above (III.C.1.1), pH changing and organic matter depletion due to tubificid activity are main

factors controlling mobilization and thus bioavailability of atrazine in the sediment for the plant. Via its influence on these sediment properties, bioturbation shifts metal binding affinities between the solid and dissolved phases and can thus modify the speciation and bioavailability of most metals in sediments (Remaili et al., 2015). That promotes the absorption of PHEs by plant with the increase of bioaccumulation possibility by *T. latifolia* as demonstrated in our study (section III.A.1). Due to an important role of PHEs' mobility and bioavailability on the phytoremediation technology, efficiency of applications coupling phytoremediation and bioturbation for the PHEs removal should be therefore enhanced.

Unlike cadmium, atrazine, however, as a non-conservative pollutant can be degraded into many different metabolites (about 15 to 20 compounds). The polarity of atrazine and its metabolites affect their penetration capacity into the plants. By enhancing the metabolization of atrazine, our study with the 2nd experiment indicated that bioturbation enhanced bioaccumulation of the pesticides metabolites with lower polarity molecules in the plant roots (see III.B.4.2).

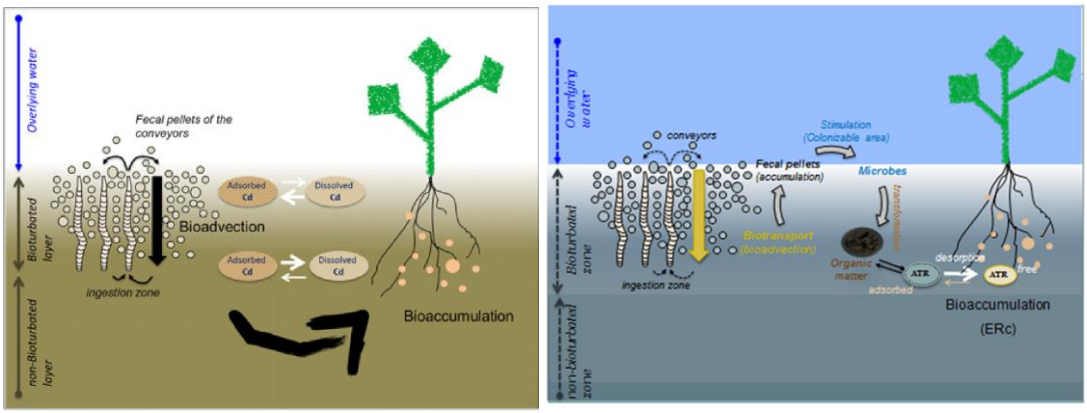


Figure III.17. Scheme of bioturbation processes affecting behavior and mobility of (*A*) - cadmium and (*B*) atrazine within sediment compartment

In many cases, the impacts of bioturbation on the bioavailability of these pollutants follow different pathways depending on the possibility of biodegradation of these compounds. For the conservative pollutant, the efficiency of phytoremediation

also depends on metal concentrations in plant and the sediment. In the plant, the capacity to phytoextract is dependent on the quantity that is previously bioaccumulated (Sumiahadi & Acar, 2018). In the sediment, the plant root system is potentially efficient at removing the metal in the direct environment of the roots. Hence phytoextraction should slow down proportionally to the decontamination of the immediate surrounding sediment when this sediment is immobile. Bioturbation thereby provides an additive impact on the phytoremediation process by changing the distribution of the pollutant across the sediment column when creating the pumping of this pollutant from the surface to the anoxic sediment layers. In case of pesticides, phytoremediation relies on the ability of plants to take up (phytoextraction), adsorb to their roots (rhizofiltration), degrade directly (phytotransformation, contaminant inside plant tissues can be transformed by plant enzymes), or degrade indirectly (rhizoremediation by microbes) the contaminants (Van Aken, 2008). Bioturbation is known to promote microbial activity that may also support the degradation of the pesticides (Yao et al., 2017) (Krutz et al., 2012).

On the other hand, the accumulation in a plant strongly depends on the hydrophobicity of the micro-organic compounds with an optimum log K_{ow} around 2 (Briggs et al., 1982). This coefficient largely varies among mother compounds and its derivative products that may occur in natural sediments. In the present study, bioturbation significantly enhanced the metabolization of atrazine in a sediment into the pore water and then uptaken by plants.

III.C.1.3. Pollutant transport between compartments

Transport and dispersion of the pollutants in experimental compartments (water, sediment, plant) in our two experiments followed different pathways. The two experiments setup was different on the contaminant source compartments: cadmium initially in the overlying water, while atrazine was homogeneously contaminated with water and sediment. Indeed, transport of cadmium from water to sediment was due to

the physical process of the molecular diffusion and adsorption in the absence of bioturbation. With bioturbation, physical processes still occurred, but biotransport due to tubificid worms enhanced adsorption and diffusion rates and thus results into cadmium pumping from the surface to deeper sediment layers as attested by significantly higher fluxes of cadmium from overlying water to sediment (F_1) in the treatments having worms (Fig.III.1). Worms almost double the fluxes of Cd from overlying-water into the sediment as estimated by the change of Cd Quantities in the surface sediment, in agreement with results by Ciutat et al. (2005).

The physical adsorption and diffusion were not directly measured in our study, but the estimated Cd fluxes from water to sediment permitted to suggest that physical processes involved in the Cd integration into the sediment were certainly enhanced under bioturbation. Inversely, remobilizations of cadmium from sediment to water (F_2) under bioturbation were found to be negligible after 30 days of the 1st experiment in treatment with Cd (Fig.III.1). Schaller (2014) indicated that metal remobilization from sediment due to bioturbation/bioirrigation depend on chemical characteristics of the element. Cd remobilization is highly influenced by bioturbation, especially at the start of the experiment when worms dig into the sediment and afterward sharply decrease (Schaller, 2014). In our experiment, the effects of the tubificids on Cd remobilization was measured in the treatment without Cd contamination.

In these conditions, the metal gradient was low enough in the overlying water so that remobilization from natural sediment was detectable, in agreement with the previous observation of Ciutat et al. 2007. In the presence of worms, the transport of cadmium between experimental compartments was mainly following a downward flow following the sequence:

(i) Overlying water → sediment → plant (Fig.III.1).

In the 2nd experiment, transport of atrazine under bioturbation followed the 3 different pathways:

(1) Overlying water → sediment particle (*adsorption*) → pore water (*desorption*) → plant (*phytoremediation*)

(2) Sediment → worm (*bioaccumulation*)

(3) Sediment → overlying water (*remobilization*) → atmosphere (*volatilization*)

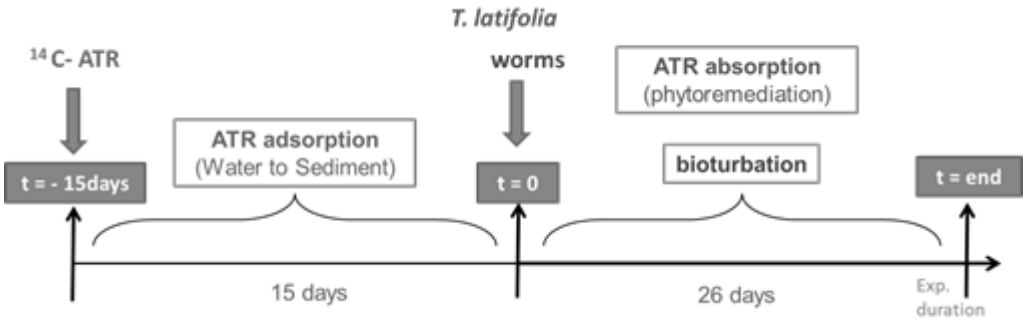


Figure III.18. Different processes of atrazine experiment duration

Before starting the experiment, (t = -15days), atrazine was mixed with the humid sediment before the introduction into the microcosm. Sedimentation process afterward in the microcosm created two distinguished compartments: the overlying water with a concentration estimated to 3.68 ± 0.07 $\mu g.L^{-1}$ and sediment column with a concentration estimated to 1.95 ± 0.02 $\mu g.g^{-1}$ before introducing the plant and invertebrate. However, due to low absorption rates of atrazine in soil and sediment (Koc =128 mL g-1) (Sun et al., 2010), adsorption of atrazine from overlying water to sediment particles was processed slowly after the sedimentation that resulted in the transport of the contaminant from water to sediment (flux - F1C, {Atr.Typ} treatment, Fig. III.14). In the presence of worms, bioturbation process began at t = 0 day and last during 26 days of the experiment (Fig. III.18).

Without bioturbation, the plant uptake rate (*phytoremediation*) of atrazine and metabolite (F3C on Fig. III.14.) was estimated to be equal to 1.78 ± 0.48 $\mu g.day^{-1}$ in {Atr.Typ}. Under the effect of the worms, the plant uptake following pathway (*1*), resulting in the fluxes F3D on figure Fig. III.14., was estimated to 2.02 ± 1.47 $\mu g.day^{-1}$ in

{Atr.Typ.Tub} treatments. Besides, another type of transports of atrazine was occurring in the microcosm environment in addition to the plant uptake.

Atrazine fluxes that transferred by the pathway (3) from sediment to water could be estimated to 6.70 ± 6.34 µg.day-1 in {Atr.Tub} (F2B) and 1.71 ± 2.09 µg.day^{-1} in {Atr.Typ. Tub} in (F2D Fig. III.14). This finding was consistent with the previous study of Bundschuh et al., (2016) on the role of pesticide properties on remobilization from sediment to the overlying water phase.

After making the sum of ^{14}C labeled pesticides in all compartments, there remained a marginal loss of atrazine at the end of the experiment as estimated by calculation of the relative recovery (Fig.III.14). This ^{14}C-atrazine and metabolites loss in the treatments without bioturbation {Atr} and {Atr.Typ}, approximately 93.5%, and 94%, respectively was explained by the volatilization of atrazine into the air phase via the mineralization into CO_2. The relative recovery estimated with tubificid worms in {Atr.Tub} and {Atr.Typ.Tub}, approximately 79.5%, and 84.9% respectively, suggested pesticide losses due to not only mineralization [pathway (3)], but also the bioaccumulation into the worm's' body - [pathway (2)]. The transport of the contaminant via the pathway (2) is resulting in the positive value of ΔQ4B and ΔQ4D estimated in the treatments having tubificids added {Atr.Tub} and {Atr.Typ.Tub}, respectively. It is concluded that the tubificids also directly interfered with the atrazine and metabolites distribution by bioaccumulation in the worm biomass. This bioaccumulation was estimated to be with a rate of 17.85 ± 2.26 µg.day^{-1} (F4B in {Atr. Tub}) and 11.33 ± 2.70 µg.day^{-1} (F4D in {Atr.Typ.Tub} of figure III.14) as estimated in the conditions having worms and atrazine contamination, respectively.

III.C.2. COMPARISON OF BIOREMEDIATION OF WATER AND SEDIMENT TOXICITY BETWEEN NON-CONSERVATIVE AND CONSERVATIVE POLLUTANTS

III.C.2.1. Effects of the pollutants on tubificid resistance

Our results from the cadmium and atrazine experiments demonstrated that the biological reworking of sediment by tubificid worms as estimated via the bioadvective rates do not depend on pollutant-enrichment or not. The bioadvective rates obtained in all treatments having tubificids (Fig. 3, section III.A.1, and Table III.10) were in the same order of magnitude of those estimated by other previous authors with tubificids population of various densities (Anschutz et al., 2012; Ciutat et al., 2005b; Mc Call and Fisher, 1980). In both case, these natural processes are still efficient under cadmium contamination of 20µg.L^{-1} in the overlying water or atrazine 5µg.g^{-1} in the sediment. Oligochaete worms could be therefore considered as ecological engineers with high resistance to both inorganic and organic contaminants from the aquatic environment and therefore good candidates for being displayed into bioremediation of polluted sediments.

Table III.10.
Biomass of tubificid worms in our two experiments, and estimated bioaccumulated quantity during the experimental duration, 30 days for Cd and 26 days for atrazine

Exp.	surface of microcosm (dm^{-2})	Number of worms per microcosm	Fresh biomass (g) per microcosm	Density (worms.dm^{-2})	Bioadvection rate (cm.$year^{-1}$)	Estimated accumulated quantity (µg)
Cd	6.00	800	17.8 ± 3.1	133	16.7 to 18.5	83.97 ± 10.21
Atrazine	1.00	100	4.45 ± 0.53	100	15.0 to 26.5	from 294.47 ± 70.24 to 464.22 ± 58.63

NA: not available

III.C.2.2. Comparison of pollutant removal rates by the plants

The efficiency of phytoremediation has been primarily demonstrated as a bioremediation engineering on heavy metal such as lead, cadmium, copper, arsenic (Leveque et al., 2013; Lyubenova & Schröder, 2011; Pandey et al., 2014), but with more seldom demonstrations on the organic compounds, such as atrazine (Ibrahim et al., 2013). For metal contaminants, plants show the potential for uptake and recovery of contaminants into above-ground biomass (phytoextraction), filtering metals from water onto root systems (rhizofiltration), or stabilizing waste sites by erosion control and evapotranspiration of large quantities of water (phytostabilization) (Williams, 2002). Phytoremediation of organic chemical notably relies on plant ability to take up, accumulate, or detoxify organic compounds to less toxic metabolites or their indirect role in stimulating the soil microbial or fungal activities in the breakdown of organic compounds (Pascal-Lorber et al., 2010). Impact of bioturbation, as mentioned above, on behavior and transport of the cadmium and of atrazine follows different mechanisms, and it consequently enhanced phytoremediation efficiency in different pathways. The phytoextraction rates measured in our experiment was estimated to 7.30 ± 0.00 $\mu g.year^{-1}$ for Cd without bioturbation ({Cd.Typ} treatment) that corresponds to 5.5% of relative uptake of cadmium from sediment at the end of the experiment (Table III.11). In the presence of tubificids, these uptakes rates were estimated to be 730 ± 350.4 $\mu g.year^{-1}$ in agreement with the flux of 0.07 ± 0.03 $\mu g.day^{-1}$ of Cd from sediment to plant (F3 in Fig.III.1) and the relative uptake of 9.7 % estimated in the treatment {Cd.Typ.Tub} (Table III.11). Bioturbation process significantly increased Cd uptake in our experimental conditions. However, these rates remain much lower than the previous uptake rates estimated in the literature by other experiments with *Typha latifolia* or other riparian plant species (Table III.12). This difference is explained by the specific experimental conditions of each author that remains differences in temperature, light, plant biomass, or experimental duration, etc. with consequences on the obtained removal rates. Our removal rates were indeed not optimal due to some lack of expertise

on the phytoextraction that did lead to optimal rates. Our plant growth rate may have suffered from some limiting factors such as nutrients or light during the duration of the experiment. However, the most important point is that the experimental conditions were similar for all the treatments, with randomization of the microcosm location, so that the observed difference between treatments was possibly allocated to the biodiversity composition (plant and invertebrate) that made the difference. In other terms, although the absolute uptake rates were not optimal, the main goal of this research was to provide evidence of the bioturbation effects on these rates that were different according to the treatments.

The enrichment coefficient for the root (EC_R) with Cd when compared the two treatments with and without added tubificid, is also useful to give evidence of the changes of efficiency under bioturbation (Table III.11). Enrichment coefficients were significantly higher for both type of pollutants when investigated in the root when the enrichment coefficient remained unchanged in the leaves. These results may be explained by the duration of the experiment that did not last long enough to get the pollutant to migrate until the leaves. This point as the whole set of fluxes estimated in this experiment need not be validated with longer experimental times.

In the case of atrazine, uptake rates of 1.78 ± 0.48 $\mu g.day^{-1}$ were estimated in {Atr.Typ}, with removal efficiency of 1.40 ± 0.37 %. Under the effect of the worms, the plant uptake was estimated to 2.02 ± 1.47 $\mu g.day^{-1}$ in {Atr.Typ.Tub} treatments, with a removal efficiency of 1.59 ± 1.16 %, that remain quite similar from the uptake potential estimated without bioturbation. The lack of difference with and without bioturbation is explained by the fact that the sediment column was homogeneously contaminated with pesticide during the experiment duration so that the effects of the biotransport by the tubificids were not efficient in that special experimental design. In the second experiment, the bioturbation effects were more evident in the process of biodegradation by the microorganisms, but this fact is only a consequence of the experimental design. Besides the influence of worms on the physicochemical properties of sediment that

caused higher mobility of atrazine within the sediment, the major impact of bioturbation in this second experiment was demonstrated on the microbial community breakdown capacity for pesticide. This fact was suspected in this study with the increase of a number of metabolites in microcosms with bioturbation. Similarly to Cadmium, the removal efficiency measured with the atrazine experiment is much lower than previous measurements found in the literature with *T. latifolia* and other aquatic plants. This fact suggests limitation of the plant growth during the time of the experiment the again, but also raise a question on the experimental duration that was much lower in our study that in the literature experiment. Without changing our positive conclusions on the efficiency of the plant and invertebrate on the tested bioremediation, this comparison rise motivation to run new experiments with more attention paid at the experimental conditions, in order to test the influence of bioturbation in optimal conditions.

Table III.11
Removal efficiency, enrichment coefficients, and transfer factor calculated from two experiments

Treatments	cadmium			atrazine		
Plant removal rates						
	µg.day^{-1}	µg.year^{-1}	Relative uptake, ‰	µg.day^{-1}	µg.year^{-1}	removal efficiency, %
Without bioturbation	0.02 ± 0.00	7.30 ± 0.00	5.5	1.78 ± 0.05	649.7 ± 18.25	1.40 ± 0.37
With bioturbation	2.00 ± 0.96	730 ± 350.4	9.7	2.02 ± 1.47	737.3 ± 536.55	1.59 ± 1.16
Enrichment coefficients						
	ECR	ECL	TF	ECR	ECL	TF
Without bioturbation	8.98 ± 2.13	0.78 ± 0.31	0.09 ± 0.02	5.99 ± 0.64	5.17 ± 1.24	0.88 ± 0.25

With bioturbation	13.83 ± 4.13	1.18 ± 1.06	0.09 ± 0.09	7.16 ± 0.66	5.32 ± 4.66	0.76 ± 0.92

Relative uptake: ratio of total Cd content of plant over sediment after treatment;
Removal efficiency: ratio of total atrazine and its metabolites content taken up by plant over sediment at initial time;
ECR: enrichment coefficient for root= root/sediment;
ECL: enrichment coefficient for leaf= leaf/sediment;
TLF: transfer factor = leaf/root

Table III.12
Removal efficiency, enrichment coefficients, and transfer factor of cadmium from literatures

Initial conc. (mg.kg^{-1} dry wt.)	Plants species	Removal rates (µg.day−1)	Relative uptake ‰	ECR	ECL	TF	In lab/ in field	duration	References
50	*E. japonicus*	10.87 ± 2.75	9.13				lab	84 days	Guo et al. 2012
50	*P. tobira*	5.07 ± 3.69	12.74				lab	84 days	
50	*C. Blue Ice*	8.54 ± 2.13	7.17				lab	84 days	
0.23	*T. latifolia*			2.47	1.16	0.47	field		Sasmaz. et al 2008
0.77 ± 0.84	*T. latifolia*			6.60	0.05	0.01	field		Klink et al. 2013
0.47 ± 1.41	*navel orange*			7.75	0.73	0.09			Cheng et al., 2015

Table III.13
Removal efficiency, enrichment coefficients, and transfer factor of atrazine from literature

initial concentration (mg.kg^{-1} dry wt. or µg.L^{-1}	**Plants species**	**removal efficiency %**	**In lab/ in field**	**duration**	References
100 µg/L	*Azolla caroliniana*	6.56	lab (greenhouse)	6 days	GUIMARÃES, F.P. et al. 2011
100 µg/L	*L. gibba*	5.07	lab (greenhouse)	6 days	GUIMARÃES, F.P. et al. 2011
100 µg/L	*S. minima*	5.60	lab (greenhouse)	6 days	GUIMARÃES, F.P. et al. 2011
1000 µg/L	*Azolla caroliniana*	0.43	lab (greenhouse)	6 days	GUIMARÃES, F.P. et al. 2011
1000 µg/L	*L. gibba*	0.38	lab (greenhouse)	6 days	GUIMARÃES, F.P. et al. 2011
1000 µg/L	*S. minima*	0.45	lab (greenhouse)	6 days	GUIMARÃES, F.P. et al. 2011
0.5 mg/kg	*P. crispus*	91.0 ± 3.2	lab	45 days	
20 µg/L	*T. latifolia*	35 ± 8	lab	6h	
1 mg/kg	*Lolium multiflorum*	50.00	lab	21 days	Merini et al., 2009
3.5 mg/L	*Acorus calamus*	57.00		6 days	Marecik 2012
4 mg/L	*Iris pseudacorus*	75.60	hydroponic system	20 days	Wang et al., 2012
4 mg/L	*Lythrum salicaria*	65.50	hydroponic system	20 days	Wang et al., 2012
4 mg/L	*Acorus calamus*	61.80	hydroponic system	20 days	Wang et al., 2012

73 and 147 µg/L		52 (transformed)	constructed wetland	35 days	Moore et al., 2000
0.005 µg/L	*Phragmites australis, Eleocharis sphacelata, Schoenoplectus validus, Baumea articulate, Typha orientalis*	60.00	constructed wetland (0.11km2)	28 days	Page et al., 2011
9.2 ± 0.8 µg/L	*Typha latifolia*	53 ± 1.9 decrease	storm runoff	4 hours retention time	Moore et al., 2017
12 ± 0.4 µg/L	S. americanum	25% ± 16	storm runoff (4 hours)		Moore et al., 2017
3.1 ± 0.2 µg/L	*L. oryzoides*	51 ± 6.1	storm runoff (4 hours)		Moore et al., 2017

PART IV. CONCLUSIONS AND PERSPECTIVES

Although the influence of bioturbating mechanisms on pollutant bioavailability at the water-sediment boundary layer in now relatively well understood, applications of phytoremediation combined with bioturbation for pollutants removal strategies in aquatic sites were not investigated yet previously to this Ph.D. The mission of the present work is thus to precisely separate the individual roles of worms and plant as well as their combined actions in order to understand the advantage of their combination on pollutant fate. Our experiments reproducing field conditions aimed to test the purification capacity associated with two populations representative of the wetland biodiversity (plant and invertebrate) and concerning two different types of pollutants in order to begin to face the many varieties of contaminants stored in sediment through nature-based solutions. The main conclusions that may be drawn after the completion of these two experiments are listed below:

- The tubificids group, as represented by the *T. tubifex* population seems to be a good candidate as a population of ecological engineers able to develop a significant conveying of sediment and associated pollutants downward into the contaminated sediment. The intensity of the bioadvection was still efficient (and similar to biotransport by this species in non-bioturbated medium), under cadmium and atrazine contaminations in aquatic systems of 20 $\mu g.L^{-1}$ in overlying water and $5\mu g.g^{-1}$ in sediment, respectively.
- The tubificids population maybe also involved in the pollutant removal process by bioaccumulation. This uptake is well known for the metal, but few have been demonstrated about this process for the pesticide as atrazine so far.
- The plant species that were selected to drive these experiments seem to be resistant enough to the same contaminations to be an interesting source of phytoextraction. The uptake rates of this plant for trace metals and cadmium was previously emphasized in the literature, but few researches tested the

capacity of this plant species to maintain its phytoextraction towards an herbicide.

- The interaction between plants and invertebrates were given in evidence using the *Typha latifolia* and *Tubifex t.* species, chosen as model species, so that it is suspected that other species of plant and invertebrate might contribute to these types of interaction as well.
- The recorded uptake rates are in the lower part of the uptake rates previously measured in the literature for trace metals. This fact is explained by the experimental conditions that may not have been optimal during the first experiment. More particularly, a lack of light or nutrients may have acted as a limiting factor for better growth and metabolism of the plants during the experimental period. However, this point does not change the main conclusion about the efficiency of the coupling strategy since the experimental conditions were similar for all the treatments. The conclusion about combination efficiency rose up from the main comparison between treatments with and without faunal biodiversity.
- The bioturbation influence displayed by the conveyor belt species is favoring the burial of the surface sediment and the newly incorporated pollutant coming from the overlying water so that the bioturbation is increasing the arrival of pollutants in the vicinity of the plant root systems. This conclusion is right for the metal, and the pesticides tested so that it is possible to write that bioturbation improved phytoremediation of cadmium and atrazine by *T. latifolia's* root systems.
- Bioturbation by tubificids continuously influenced physicochemical properties of the 5 first centimeters of sediment and thus adsorption-desorption behavior of atrazine in sediment, resulting in acceleration of atrazine delivery in the interstitial water by mobility from attached forms

(onto sediment particles) into a free fraction (in pore water) under pH variations.

- The bioavailability, the bioaccumulation in the roots as evidenced by the enrichment factors (ECs) and the metabolization of the tested pesticide were significantly enhanced in *T. latifolia* roots when the worms were occuring.
- The ecotoxicological tests did show that the toxicity after the biological influence of plant and invertebrate was not lower, but even high than toxicity level at the initial time. This fact is explained by the toxicity of the produced metabolites that is higher than the mother compound. This fact does not modify the conclusion about the bioremediation strategy efficiency for removing pollutants. It is, however, raising new questions on the toxicity related to metabolites that are continuously produced in the fields under the effects of natural biodiversity.

The present work by reproducing field conditions is innovative for the scientific field by providing more useful scientific knowledge and evidence of biodiversity involvement in the biodegradation, bioremediation, and attenuation of pollutants in aquatic systems. These first preliminary and multidisciplinary experiments were positive enough on the advantages of the coupling strategy between bioturbation and phytoremediation to open the door for further estimation of plant and invertebrate influence on aquatic pollutants. Additionally, these research results might be relevant to society with the expectation of applying technology and scientific knowledge on ecological engineering in real wetland bioremediation. By increasing the biodiversity (plants, invertebrates, and microbial consortium), the application of combined natural processes (bioturbation and phytoremediation) also contributes to the improvement of bioremediation strategy in nature-based solutions or constructed wetlands facing inorganic and organic pollutants contaminations. Enhanced biodiversity designs of ecological engineering methods for the rehabilitation of natural or constructed wetland

should lead to improve the water quality at the outlet of the sites. Cases of sewage water to be treated in this type of natural or semi natural systems are world wide spreaded and contamination is mots of the time a cocktail of heavy metals and/ or pesticides due to urban, industrial, or agricultural activities. Enhanced biodiversity treatment system should be developed in order to contribute to the establishment of sustainable water resources management.

The variety of contaminated biotopes, as the number of pesticides to depurate, is huge. This means that many researches still need to be developed that may improve the ecological engineering based on the coupling of invertebrate and plants (1), or by coupling with other ecological engineering tools (2):

- ✓ Many other plants are good candidates, and the plant choice must be done according to pollutant properties and with roots depth in agreement with bioturbation depth. High variability of plant tolerances does make this choice even more difficult. Three types of plants are particularly useful: graminae in buffer zones, trees such as poplar or willow in riparian zones or phytoremediation processes due to large evapotranspiration capacities, and aquatic plants for waste depuration processes. The difficulties to find a polyvalent wild plant, lead to the search for new methods to select plants more efficiently.
- ✓ Many bioremediation tools may be combined to address the broad spectrum of harmful molecules and sites to be restored. We tried to combine the bioturbation with the phytoextraction, with one first species of invertebrate as a model of bioturbation. Other bioremediation strategies are searching to develop new genetic engineering technologies. Bioremediation via genetic engineering relies on the enhancement of bacterial growth to improve the degradation of the contaminants. The complementarity of

these technologies (bioturbation and genetic engineering) should supply possible opportunities to broaden the scope of ecological engineering even more. Two strategies are usually listed as useful, the biostimulation that supplies limiting nutrients like nitrogen or carbon to enhance the development of indigenous microorganisms, and bioaugmentation, which provides non-indigenous strains able to metabolize the contaminants.

- ✓ It is clear from the present studies that the easiest way to combine bioturbation with genetic engineering should be via biostimulation to favor the development of indigenous micro-organisms species adapted to the breakdown of the harmful molecules. This is undoubtedly the process that we already observed in our experiment with atrazine and a higher number of metabolites attesting of more advanced biodegradation in bioturbated sediment. The combination of previously tested biostimulation techniques with bioturbation should certainly bring interesting results in the future.
- ✓ Many other invertebrate species are good candidates for bioturbation. Natural wetlands are sheltering benthic communities with a large biodiversity richness. Those benthic communities are indeed the source of many other bioturbators as ecological engineers to be combine with the phytoextraction. Benthic organisms with similar sediment mixing modes in sediment have been classified according to their bioturbation functions (Gerino et al., 2003). T invertebrate community of wetlands is composed of functional groups that drive other types of biotransports such as biodiffusion, bioregeneration or non-local biotransports. These other biotransports may generate other effects on the pollutants behaviors at the water-sediment interface, with some favoring the resuspension of pollutants, other more inclined to increase the burial of pollutants into the sediment column.

- ✓ Since functional bioturbators have different modes of sediment reworking and irrigation, we hypothesize that they also have different influences on the dynamics of pollutants and then their potential phytoextraction. Biodiffusers, for example, induce a diffusive transport of sediment by randomly moving particles over short distances, while gallery-diffusers move particles from the surface sediment directly to the bottom of the burrow. Anyway the super-imposition of several types of bioturbation in the wetland boundary layers should contribute to maintain the sediment surface biologically active, with nutrients, organic matter vertical fluxes, . The bioturbation not only influence s the microbiological composition, but the different biotransport of solutes and particles also contribute overall positively on the biological succession of the invertebrate community with time. The bioturbaytion is supposed to be one of the main driver for the benthic boundary layer resilience towards more natural colonisation by fauna and plants. We demonstrated that bioturbation stimulates the phytoextraction by the plants and together with bioturbation involvement in the ecological succession, it is suggested that exploration of this phenomenon should bring useful insights for the restoration strategies of contaminated sites.
- ✓ Some researches are also required to find out the role of microorganism communities in the biodegradation of organic pollutants under bioturbated conditions. Further analytical works concerning the identification of atrazine metabolites in sedimentary layers and the plant system, as well as the polarity of new metabolites (via Kow values) formed in the plant, thus also need to be carried to evidence metabolization process under the bioturbation.
- ✓ Bioturbation is known to promote microbial activity and its turn over for more adapted species to the pollutant cocktail they are facing. That may

support the degradation of such compounds. The release of these compounds under bioturbation, however, may increase the toxicity of the interstitial water for invertebrates. Her is a feedback loop that slow down the bioturbation effects until a certain shreshold where the water quality does not allow any inbertebrates colonization any more.

There was a risk in the selection of the atrazine for this Ph.D. demonstration since the exposure of macrophytes to atrazine in the amounts observed in watercourses causes a significant reduction in their biomass.

Authors mentioned that this decline might follow acute exposure after a period of substantial leaching, for example, or at smaller chronic doses (Cunningham et al., 1984; Kettle et al., 1987). We demonstrated that with relative moderate atrazine concentration the phytoextration with Typha was still possible. One possible research development would be to explore the maximum exposure concentrations that this plant may handle to sustain bioremediation. A threshold certainly exists where the strategy may not be any more efficient. The same concern have been already addressed towards the invertebrates as a source of bioturbation. It is suggested here as a conclusion of this work that bioturbation combination with phytoextraction may get its limits of application where both types of organisms are getting the threshold of exposures.

Our project aimed to demonstrate the efficiency of bioturbation and phytoremediation combination to remove metal and a persistent micro-organic pollutant from out of aquatic sediments. The next experiments will be run in Vietnam to complete the demonstration of the efficiency of bioremediation by the Oligochaeta *Tubificidae*, *Typha latifolia,* and other aquatic species. The next experiments will be run at the Institute of Environmental Technology IET from the VAST-Vietnam Academy of Sciences and Technology, in the cooperation with UMR ECOLAB and Toxalim from Toulouse (France), UMR INRA Agroecology at Dijon, USTH and IBT-Vast (Vietnam). These next researches on ecological engineering will be funded with the help of

financial support obtained for two years from IFS (International Foundation for Sciences). A part of this fund is allocated to the analytical measurements of atrazine and the microbial communities in the cooperation with UMR from INRA Toxalim from Toulouse and Agroécologie from France, and IBT-Vast (Vietnam).

The application of these researches is already taking place in the project and concept of Smart Clean Garden, that aims to develop these ecological engineering in water treatment technology of planted filters, especially in tropical countries. This project is completed in cooperation with UMR ECO&SOLS (IRD, CIRAD, INRA et SupAgro Montpellier), UMMISCO (IRD), the USTH, the VAST, the IRD, and a toulousain firm EPURTECK. The technology of water reuse is answering a rising request from society, and this project is using the university campus as demonstration sites. In the Smart Clean Garden, the water treatment technology is secured with the association of innovating technology sensors for water quality and properties survey (Internet Of Things) in the filters. The first filter is now being implemented (2018/19) to treat the waste water coming on the VAST campus of Hanoi. With three replicates filters working in en parallel, this device is supplying an ideal experimental platform for the continuity of these researches in bioremediation, especially with the opportunity to test other biodiversity combinations.

CONCLUSIONS en français:

Bien que l'influence de la bioturbation sur les proprieties des sediments soit rlativement biencomprise maintenant, son application pour améliorer les performances de phytoextraction n'avait pas encore été explore jusque là. L'objectif des rechercehs menées dans cette thése a été de s'apuyer sur les connaissances déjà disponibles sur les processus individuel de bioturbation et de phytoextraction pour mettre en evidence les interaction possible et leur influence su rle devenir des pollluants après leur arrive à l'interface eau-sédiment. Nos experiences , en reproduisant une portion de zones humide avec une biodiversité simplifiée en microcosms ont permi de commencer a tester l'efficacité d'une combinaison d'espèces plante-invertebré pour 2 differents type de polluants particulièrement rémanent. Nos premieres resultat offre une premiere demonstration de l'intêret de developer ces approches fnctionnelles en croisant des groups fonctionels distincts pour faire face à la multiplicité des pollutants presents dans les zones humides naturelles ou construites. L'amplification de la biodiversité invertébrés au memetre titre que l'augmentation des communauté microbiennes dans les zones humides pour améliorer les processus de bioremediation fait partie des solutions inspirées de la nature qui peuvent venir améliorer l'efficacité des procédés d'épuration des eaux usées en vue de leur recyclage.

Les principales conclusions dressées à l'issue de cette thèse sont :

- Les oligochètes tubificidae mis en oeuvre dans nos expériences avec une contamination par le cadmium et l'atrazine de 20 $\mu g.L^{-1}$ dans l'eau surnageante et de 5$\mu g.g^{-1}$ dans le sédiment, respectivement, ont maintenu une vitesse de biotransport par bioadvection similaire à celle sans contamination. Ce résultat indique que ce groupe d'invertébrés d'eau douce est un bon candidat comme source de bioturbation pour accompagner la bioremédiation dans les sédiments contaminés.

- la bioturbation par les tubificidae convoyeurs influence continuellement les propriétés physico-chimiques dans les 5 premiers centimètres de sédiment. Ce processus a des

conséquences sur les capacités d'adsorption et désorption des polluants testés, notamment en déplaçant l'équilibre en faveur des formes libres dans l'eau interstitielle en profondeur dans la colonne de sédiment.

La bioturbation mesurée met en évidence un ensevelissement des polluants dans la couche de sédiment dans laquelle se trouve également le système racinaire des plantes. Ce phénomène accompagné du déplacement de l'équilibre vers les formes libres à pour conséquence de favoriser la biodisponibilité des polluants pour les plantes.

- La plante ryparienne *Typha latipholia* est restée active et a démontrer une capacité de bioaccumulation significative au niveau du système racinaire pendant la durée expérimentale, pour les 2 types de polluants utilisés. Si les performances de cette plante vis à vis de la phytoextraction du cadmium, comme d'autres métaux lourds était déjà reconnue, sa résistance et ses capacités en présence d'une contamination par un herbicide n'était pas démontrée jusque là.

- La biodisponibilité, la bioextraction et la métabolisation de l'atrazine par les racines de *T. latifolia* est plus importante dans les sédiment bioturbés, ces résultats sont démontrés par un nombre de métabolites plus grands et une quantité de métabolites de l'atrazine significativement plus élevée dans le système racinaire en présence de bioturbation.

- Les taux de phytoextraction de métaux mesurés expérimentalement restent faible comparés aux taux mis en évidence dans la littérature par ailleurs, mais la comparaison avec les témoins sans contamination nous permet de conclure qu'il s'agit probablement d'un alea des conditions expérimentales (ex manque de lumière et de nutriment pendant la durée de l'expérience) qui a entrainé un taux de croissance et/ou un niveau de santé des plantes non optimaux. Cette faiblesse au niveau des plantes utilisées ne change pas les résultats sur l'influence de la biodiversité testée, car la démonstration est basée sur la comparaison de traitements expérimentaux avec et sans biodiversité, tous exposées à des conditions expérimentales uniformes.

- Les tests d'écotoxicologues n'ont pas permis de mettre en évidence une baisse de la toxicité après l'application de la stratégie de bio remédiation testée, mais au contraire une augmentation de la toxicité de l'eau interstitielle de manière généralisée en présence de biodiversité. Ces résultats sont expliqués par le niveau de toxicité des métabolites formés qui pourrait vraisemblablement être plus élevé que celui de la molécule mère.

- L'évaluation de la toxicité des molécules métabolisées en présence de bioturbation et l'évolution de la composition de la communauté microbienne responsable de ces réactions de biodégradation en présence de bioturbation est l'étape suivante pour la continuité de ces recherches.

- Si les interactions invertébrés benthiques et plantes se sont trouvées favorables envers l'amélioration des possibilités des capacités de phytoextraction avec les 2 premières espèces testées *Tipha l.* et *Tubifex t.,* il est possible qu'il existe d'autres espèces animales et végétales qui puissent être associées pour optimiser l'efficacité de bio remédiation. Non seulement d'autres espèces, par exemple avec d'autres modes de bioturbation qui peuvent générer d'autres biotransports plus favorable à la phytoextraction, ou pour d'autres polluants, mais plus encore l'association de plusieurs espèces de bioturbateurs peut être un véritable atout pour faire face à la multitude de formes de pollution qui arrive dans les milieux naturels. Il est également probable que d'autres type d'interactions existent avec d'autres combinaisons d'espèces vivant à l'interface eau-sediment et leurs interventions en ingenierie écologique restent à être testées.

- Enfin, l'association des 2 outils de bioremédiation testés s'est avérée fructueuse ce qui laisse présager d'autres associations bénéfiques au rendement de bioremédiation. Par exemple, la bioamplification pour le développement de communautés de micro-organismes adaptées à la dégradation de certaines polluants pourrait certainement participer à l'amélioration des performances de bioremédiation par phytoextraction et bioturbation.

Cette thèse avait pour objectif de réaliser les premiers tests pour examiner la pertinence d'intégrer la bioturbation dans les outils de bioremédiation par phytoextraction, la démonstration étant concluante, d'autres expériences vont suivre en particulier au Vietnam pour compléter cette démonstration avec les oligochaetes *Tubificidae*, et les plantes *Typha latifolia* mais aussi avec d'autres plantes aquatiques. Les prochaines expériences se tiendront en conditions de laboratoire à l'IET - Institute of Environmental Technology IET à Hanoi, un institut de la VAST (Vietnamese academic of Sciences and Technologie), en coopération avec les UMR ECOLAB, les UMR de l'INRA Toxalim à Toulouse, et l'UMR Agroécologie à Dijon, et au Vietnam à l'IBT-Vast et à l'USTH (Université des Sciences et Technologie de Hanoi). Les prochaines recherches seront financées en partie par l'ISF (International Sciences Foundation) pendant 2019 et 2020. Une partie de ces financements seront notamment alloués à l'analyse des communautés microbiennes impliquées dans la dégradation de l'atrazine en coopération avec l'UMR Agroécologie, de Dijon et l'Institut de Biotechnologie de la VAST (IBT-Vast -Vietnam).

L'application de ces recherches prend déjà place dans le concept et projet de Smart Clean Garden dont l'objectif est de déployer ces innovations d'ingénierie écologiques dans l'adaptation des technologies d'assainissement par filtres plantés dans les pays tropicaux. Ce projet est réalisé en coopération avec les UMR ECO&SOLS (IRD, CIRAD, INRA et SupAgro Montpellier), l'UMR UMMISCO (IRD), l'USTH, la VAST, l'IRD et l'entreprise toulousaine EPURTECK. La technologie de réutilisation des eaux usées traitées réponds à une demande sociale grandissante, et ce projet vise à utiliser les campus universitaires comme sites de démonstration prioritaires. Dans le Smart Clean Garden, la technologie d'assainissement des eaux est sécurisée par l'association de technologie innovante au niveau des capteurs de surveillance de la qualité de l'eau, niveau d'eau, pH, etc.. en développant la puissance des IOT (Internet des Objets) dans les filtres plantés. Un premier filtre a été inauguré en 2019 à Hanoi sur le campus de la VAST dans le cadre de ce projet pour traiter une partie des eaux usées

domestiques du campus de la VAST. Avec ces 3 dispositifs/répliquas en parallèle, ces filtres apportent une plateforme expérimentale idéale pour la continuité des recherches en bio remédiation, notamment avec la possibilité de tester d'autres combinaison de biodiversité sur les eaux usées d'un bâtiment de recherche.

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ANNEXS

ANNEX 1. Liste des publications et communications

Hoang Trung Kien, Probst Anne, Orange Didier, Gilbert Franck., Elger Arnauld, Kallerhoff Jean, Laurent François, Sabina Bassil, Duong Thi Thuy, Gerino Magali. Bioturbation effects on bioaccumulation of Cadmium in the wetland plant *Typha latifolia*: a mesocosm experiment. *Science of the Total Environment - 618 (2018) 1284–1297; https://doi.org/10.1016/j.scitotenv.2017.09.237)*

Hoang Trung Kien, Laurent François, Orange Didier, Sophie Lorber, Arnaud Elger, Frederic Julien, Sabina Bassil, Duong Thi Thuy, Gerino Magali. Bioturbation effects on atrazine behavior in aquatic sediments (will be submitted in *Journal of Ecotoxicology and Environmental Safety* on July 2019)

Hoang Trung Kien, Laurent François, Orange Didier, Duong Thi Thuy, Bassil Sabina, Gerino Magali. Ecological engineering for humid polluted systems: coupled bioturbation and phytoremediation. *ICEEA 2017 8th International Conference on Environmental Engineering and Applications July 2017.*

- *Awards: Best presenter in the section of Environmental Adaptation and Ecosystem Management*

Nereis Park 2014. Bioturbation poster / Poster Sự xáo trộn sinh học. Vietnamese version, Edition by ECOLAB.
http://www.nereispark.org/divers/NPretsop/sretsop/Vietnamese45f23/A0%20Bioturbation%20VI.pdf

ANNEX 2. Density measurement of sediment samples used in the 1st (cadmium) experiment.

Experimental treatment	**g sediment dry wt./cm³**		
	Layer 1 (0-1cm)	Layer 2 (1-5cm)	Layer 2 (5-10cm)
Without bioturbation	1.03	1.41	1.41
With bioturbation	0.74	1.40	1.40

ANNEX 3. Two-way ANOVA assessing the influences of *T. latifolia* plants and worms on [^{14}C]-(atrazine + metabolites) concentrations in the overlying water

	SS	DF	MS	F (DFn, DFd)	P value
Interaction	27337	1	27337	F (1, 11) = 28.99	0.0002
Worms	15622	1	15622	F (1, 11) = 16.57	0.0019
Typha latifolia	6.880	1	6.880	F (1, 11) = 0.007297	0.9335
Residual	10372	11	942.9		

ANNEX 4. Two-way ANOVA assessing the influences of *T. latifolia* plants and worms on [^{14}C]-(atrazine + metabolites) concentrations in bulk sediment

Layer 1 (0-0.5cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.1561	1	0.1561	F (1, 12) = 0.9232	P = 0.3556
Worms	7.800	1	7.800	F (1, 12) = 46.12	P < 0.0001
Typ	0.1272	1	0.1272	F (1, 12) = 0.7519	P = 0.4029
Residual	2.029	12	0.1691		

Layer 2 (0.5-1.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.1038	1	0.1038	F (1, 12) = 0.3417	P = 0.5697
Worms	8.545	1	8.545	F (1, 12) = 28.14	P = 0.0002
Typha	0.3121	1	0.3121	F (1, 12) = 1.028	P = 0.3307
Residual	3.644	12	0.3037		

Layer 3 (1.0-1.5cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.01217	1	0.01217	F (1, 12) = 0.03312	P = 0.8586
Worms	4.817	1	4.817	F (1, 12) = 13.11	P = 0.0035
Typha	1.739	1	1.739	F (1, 12) = 4.732	P = 0.0503
Residual	4.411	12	0.3676		

Layer 4 (1.5-2.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.002355	1	0.002355	F (1, 12) = 0.005696	P = 0.9411
Worms	0.2201	1	0.2201	F (1, 12) = 0.5325	P = 0.4796
Typha	0.03697	1	0.03697	F (1, 12) = 0.08943	P = 0.7700
Residual	4.961	12	0.4134		

Layer 5 (2.0-2.5cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1.556	1	1.556	F (1, 12) = 3.805	P = 0.0749
Worms	0.04083	1	0.04083	F (1, 12) = 0.09984	P = 0.7575
Typha	0.06805	1	0.06805	F (1, 12) = 0.1664	P = 0.6905
Residual	4.908	12	0.4090		

Layer 6 (2.5-3.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.6042	1	0.6042	F (1, 12) = 0.8094	P = 0.3860
Worms	0.7996	1	0.7996	F (1, 12) = 1.071	P = 0.3211
Typha	0.6126	1	0.6126	F (1, 12) = 0.8206	P = 0.3828
Residual	8.957	12	0.7465		

Layer 7 (3.0-4.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.02375	1	0.02375	F (1, 12) = 0.07623	P = 0.7872
Worms	0.3931	1	0.3931	F (1, 12) = 1.262	P = 0.2832
Typha	0.06813	1	0.06813	F (1, 12) = 0.2187	P = 0.6484
Residual	3.738	12	0.3115		

Layer 8 (4.0-5.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.04586	1	0.04586	F (1, 11) = 0.2678	P = 0.6151
Worms	1.241	1	1.241	F (1, 11) = 7.248	P = 0.0209
Typha	0.01070	1	0.01070	F (1, 11) = 0.06251	P = 0.8072
Residual	1.884	11	0.1713		

Layer 9 (5.0-6.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.1173	1	0.1173	F (1, 8) = 0.4058	P = 0.5419
Worms	0.2006	1	0.2006	F (1, 8) = 0.6938	P = 0.4290
Typha	0.01125	1	0.01125	F (1, 8) = 0.03892	P = 0.8485
Residual	2.313	8	0.2891		

ANNEX 5. Two-way ANOVA assessing the influences of *T. latifolia* plants and worms on [^{14}C]-(atrazine + metabolites) concentrations in sediment particle

Layer 1 (0-0.5cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	12.59	1	12.59	F (1, 12) = 5.469	P = 0.0375
Worms	0.6640	1	0.6640	F (1, 12) = 0.2884	P = 0.6011
Typha	8.571	1	8.571	F (1, 12) = 3.723	P = 0.0777
Residual	27.63	12	2.302		

Layer 2 (0.5-1.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1.219	1	1.219	F (1, 12) = 0.6416	P = 0.4387
Worms	11.18	1	11.18	F (1, 12) = 5.886	P = 0.0320
Typha	1.531	1	1.531	F (1, 12) = 0.8059	P = 0.3870
Residual	22.80	12	1.900		

Layer 3 (1.0-1.5cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.6912	1	0.6912	F (1, 12) = 0.3918	P = 0.5431
Worms	3.479	1	3.479	F (1, 12) = 1.972	P = 0.1856
Typha	6.605	1	6.605	F (1, 12) = 3.744	P = 0.0769
Residual	21.17	12	1.764		

Layer 4 (1.5-2.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.2733	1	0.2733	F (1, 12) = 0.1204	P = 0.7346
Worms	4.664	1	4.664	F (1, 12) = 2.055	P = 0.1772
Typha	0.04653	1	0.04653	F (1, 12) = 0.02050	P = 0.8885
Residual	27.23	12	2.269		

Layer 5 (2.0-2.5cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	7.627	1	7.627	F (1, 12) = 3.524	P = 0.0850
Worms	1.379	1	1.379	F (1, 12) = 0.6372	P = 0.4402
Typha	0.002380	1	0.002380	F (1, 12) = 0.001100	P = 0.9741
Residual	25.97	12	2.164		

Layer 6 (2.5-3.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	2.133	1	2.133	F (1, 12) = 0.7735	P = 0.3964
Worms	5.093	1	5.093	F (1, 12) = 1.847	P = 0.1991
Typha	1.423	1	1.423	F (1, 12) = 0.5160	P = 0.4863
Residual	33.09	12	2.757		

Layer 7 (3.0-4.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.003428	1	0.003428	F (1, 12) = 0.003666	P = 0.9527
Worms	0.0008478	1	0.0008478	F (1, 12) = 0.0009068	P = 0.9765
Typha	0.03809	1	0.03809	F (1, 12) = 0.04074	P = 0.8434
Residual	11.22	12	0.9349		

Layer 8 (4.0-5.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.8433	1	0.8433	F (1, 11) = 1.126	P = 0.3113
Worms	6.407	1	6.407	F (1, 11) = 8.557	P = 0.0138
Typha	0.01453	1	0.01453	F (1, 11) = 0.01940	P = 0.8917
Residual	8.236	11	0.7488		

Layer 9 (5.0-6.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	9.846	1	9.846	F (1, 7) = 0.6258	P = 0.4549
Worms	11.84	1	11.84	F (1, 7) = 0.7525	P = 0.4144
Typha	11.32	1	11.32	F (1, 7) = 0.7194	P = 0.4244
Residual	110.1	7	15.73		

ANNEX 6. Two-way ANOVA assessing the influences of *T. latifolia* plants and worms on [^{14}C]-(atrazine + metabolites) concentrations in pore water

Layer 1 (0-0.5cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	4667	1	4667	F (1, 12) = 1.908	P = 0.1923
Worms	16675	1	16675	F (1, 12) = 6.819	P = 0.0227
Typ	57532	1	57532	F (1, 12) = 23.53	P = 0.0004
Residual	29344	12	2445		

Layer 2 (0.5-1.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1904	1	1904	F (1, 12) = 1.314	P = 0.2739
Worms	32024	1	32024	F (1, 12) = 22.11	P = 0.0005
Typha	19098	1	19098	F (1, 12) = 13.19	P = 0.0034
Residual	17380	12	1448		

Layer 3 (1.0-1.5cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	10145	1	10145	F (1, 12) = 12.97	P = 0.0036
Worms	30782	1	30782	F (1, 12) = 39.36	P < 0.0001
Typha	4118	1	4118	F (1, 12) = 5.266	P = 0.0406
Residual	9384	12	782.0		

Layer 4 (1.5-2.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	176.1	1	176.1	F (1, 12) = 0.1611	P = 0.6952
Worms	13017	1	13017	F (1, 12) = 11.91	P = 0.0048
Typha	347.9	1	347.9	F (1, 12) = 0.3183	P = 0.5830
Residual	13116	12	1093		

Layer 5 (2.0-2.5cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	60.93	1	60.93	F (1, 12) = 0.1185	P = 0.7367
Worms	3100	1	3100	F (1, 12) = 6.027	P = 0.0303
Typha	2895	1	2895	F (1, 12) = 5.629	P = 0.0352
Residual	6172	12	514.3		

Layer 6 (2.5-3.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1470	1	1470	F (1, 12) = 0.8798	P = 0.3667
Worms	5.291	1	5.291	F (1, 12) = 0.003166	P = 0.9561
Typha	2521	1	2521	F (1, 12) = 1.509	P = 0.2429
Residual	20052	12	1671		

Layer 7 (3.0-4.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	2276	1	2276	F (1, 12) = 0.7064	P = 0.4171
Worms	3496	1	3496	F (1, 12) = 1.085	P = 0.3181
Typha	2420	1	2420	F (1, 12) = 0.7513	P = 0.4031
Residual	38655	12	3221		

Layer 8 (4.0-5.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	919.9	1	919.9	F (1, 11) = 1.011	P = 0.3364
Worms	596.7	1	596.7	F (1, 11) = 0.6555	P = 0.4353
Typha	99.70	1	99.70	F (1, 11) = 0.1095	P = 0.7469
Residual	10012	11	910.2		

Layer 9 (5.0-6.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	59.90	1	59.90	F (1, 8) = 0.01037	P = 0.9214
Worms	18880	1	18880	F (1, 8) = 3.270	P = 0.1082
Typha	5384	1	5384	F (1, 8) = 0.9324	P = 0.3625
Residual	46195	8	5774		

ANNEX 7. Two-way ANOVA assessing the influences of *T. latifolia* plants and worms on [^{14}C]-(atrazine + metabolites) concentrations in sediment porosity

Layer 1 (0-0.5cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	41.44	1	41.44	F (1, 12) = 0.7666	P = 0.3985
Worms	1324	1	1324	F (1, 12) = 24.50	P = 0.0003
Typha	0.8154	1	0.8154	F (1, 12) = 0.01508	P = 0.9043
Residual	648.7	12	54.06		

Layer 2 (0.5-1.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.01550	1	0.01550	F (1, 12) = 0.0009365	P = 0.9761
Worms	712.4	1	712.4	F (1, 12) = 43.04	P < 0.0001
Typha	12.85	1	12.85	F (1, 12) = 0.7761	P = 0.3956
Residual	198.6	12	16.55		

Layer 3 (1.0-1.5cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.5289	1	0.5289	F (1, 12) = 0.05942	P = 0.8115
Worms	577.9	1	577.9	F (1, 12) = 64.93	P < 0.0001
Typha	11.36	1	11.36	F (1, 12) = 1.277	P = 0.2806
Residual	106.8	12	8.901		

Layer 4 (1.5-2.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	11.61	1	11.61	F (1, 12) = 1.027	P = 0.3310
Worms	445.4	1	445.4	F (1, 12) = 39.38	P < 0.0001
Typha	11.78	1	11.78	F (1, 12) = 1.041	P = 0.3276
Residual	135.7	12	11.31		

Layer 5 (2.0-2.5cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	4.444	1	4.444	F (1, 12) = 0.2535	P = 0.6237
Worms	22.05	1	22.05	F (1, 12) = 1.258	P = 0.2839
Typha	4.045	1	4.045	F (1, 12) = 0.2308	P = 0.6396
Residual	210.3	12	17.53		

Layer 6 (2.5-3.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.1607	1	0.1607	F (1, 12) = 0.02515	P = 0.8766
Worms	59.40	1	59.40	F (1, 12) = 9.300	P = 0.0101
Typha	2.821	1	2.821	F (1, 12) = 0.4416	P = 0.5189
Residual	76.65	12	6.387		

Layer 7 (3.0-4.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	12.94	1	12.94	F (1, 12) = 3.154	P = 0.1011
Worms	118.9	1	118.9	F (1, 12) = 28.98	P = 0.0002
Typha	37.04	1	37.04	F (1, 12) = 9.028	P = 0.0110
Residual	49.24	12	4.103		

Layer 8 (4.0-5.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	23.12	1	23.12	F (1, 11) = 4.762	P = 0.0517
Worms	28.59	1	28.59	F (1, 11) = 5.889	P = 0.0336
Typha	1.311	1	1.311	F (1, 11) = 0.2700	P = 0.6136
Residual	53.40	11	4.855		

Layer 9 (5.0-6.0cm)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	109.1	1	109.1	F (1, 8) = 0.6759	P = 0.4348
Worms	0.03780	1	0.03780	F (1, 8) = 0.0002342	P = 0.9882
Typha	28.47	1	28.47	F (1, 8) = 0.1764	P = 0.6856
Residual	1291	8	161.4		

ANNEX 8. Tukey's multiple comparisons test performed on [^{14}C]-(atrazine + metabolites) quantity in bulk sediment

Treatments	**Adjusted P Value**		
	Fecal pellets zone (0-2cm)	*Ingestion zone (2-5cm)*	*No bioturbation zone (5-8cm)*
{Atr} vs. {Atr.Typ}	0.9897	0.2770	0.1655
{Atr} vs. {Atr.Tub}	0.0149	0.9066	0.8993
{Atr} vs. {Atr.Typ.Tub}	0.0013	0.7892	0.6705
{Atr.Typ} vs. {Atr.Tub}	0.0087	0.6184	0.0528
{Atr.Typ} vs. {Atr.Typ.Tub}	0.0008	0.0630	0.0239
{Atr.Tub} vs. {Atr.Typ.Tub}	0.4979	0.4143	0.9672

ANNEX 9. Tukey's multiple comparisons test performed on [^{14}C]-(atrazine + metabolites) quantity in sediment particle

Treatments	**Adjusted P Value**		
	Fecal pellets zone (0-2cm)	*Ingestion zone (2-5cm)*	*No bioturbation zone (5-8cm)*
{Atr} vs. {Atr.Typ}	0.9659	0.3348	0.9076
{Atr} vs. {Atr.Tub}	0.0098	0.4597	0.8135
{Atr} vs. {Atr.Typ.Tub}	0.0017	0.8136	0.2447
{Atr.Typ} vs. {Atr.Tub}	0.0044	0.9941	0.9962
{Atr.Typ} vs. {Atr.Typ.Tub}	0.0008	0.0862	0.5328
{Atr.Tub} vs. {Atr.Typ.Tub}	0.7376	0.1306	0.6484

ANNEX 10. Tukey's multiple comparisons test performed on [^{14}C]-(atrazine + metabolites) quantity in pore water

Treatments	Adjusted P Value		
	Fecal pellets zone (0-2cm)	*Ingestion zone (2-5cm)*	*No bioturbation zone (5-8cm)*
{Atr} vs. {Atr.Typ}	0.4521	0.4292	0.9860
{Atr} vs. {Atr.Tub}	< 0.0001	0.4123	0.9929
{Atr} vs. {Atr.Typ.Tub}	0.6272	> 0.9999	0.3615
{Atr.Typ} vs. {Atr.Tub}	< 0.0001	> 0.9999	0.9999
{Atr.Typ} vs. {Atr.Typ.Tub}	0.0731	0.4474	0.2358
{Atr.Tub} vs. {Atr.Typ.Tub}	< 0.0001	0.4301	0.2588

ANNEX 11. Tukey's multiple comparisons test performed on sediment porosity

Treatments	Adjusted P values								
	Layer 1 (0-0.5cm)	*Layer 2 (0.5-1.0cm)*	*Layer 3 (1.0-1.5cm)*	*Layer 4 (1.5-2.0cm)*	*Layer 5 (2.0-2.5cm)*	*Layer 6 (2.5-3.0cm)*	*Layer 7 (3.0-4.0cm)*	*Layer 8 (4.0-5.0cm)*	*Layer 9 (5.0-6.0cm)*
{Atr} vs. {Atr.Typ}	0.9495	0.9297	0.7678	> 0.9999	> 0.9999	0.9836	0.8206	0.6256	0.9932
{Atr} vs. {Atr.Tub}	0.0582	0.0027	0.0007	0.0012	0.6679	0.1603	0.0014	0.0402	0.9504
{Atr} vs. {Atr.Typ.Tub}	0.0170	0.0080	0.0018	0.0136	0.9677	0.3717	0.3738	0.5225	0.9900
{Atr.Typ} vs. {Atr.Tub}	0.0231	0.0010	0.0002	0.0012	0.6773	0.0896	0.0003	0.2428	0.9904
{Atr.Typ} vs. {Atr.Typ.Tub}	0.0067	0.0029	0.0004	0.0134	0.9709	0.2259	0.1015	0.9978	0.9118
{Atr.Tub} vs. {Atr.Typ.Tub}	0.8928	0.9154	0.9215	0.5012	0.8968	0.9356	0.0244	0.3063	0.7708

ANNEX 12. Tukey's multiple comparisons test performed on percentage of relative recovery calculated from atrazine experiment

Treatments	Adjusted P Value
{Atr} vs. {Atr.Typ}	> 0.9999
{Atr} vs. {Atr.Tub}	0.0061
{Atr} vs. {Atr.Typ.Tub}	0.1342
{Atr.Typ} vs. {Atr.Tub}	0.0047
{Atr.Typ} vs. {Atr.Typ.Tub}	0.1041
{Atr.Tub} vs. {Atr.Typ.Tub}	0.5080

ANNEX 13. Bioturbation effects on atrazine behavior in aquatic sediments

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(i) that the work as submitted has not been published or accepted for any publication;

(ii) that the work conforms to the legal requirements of the country in which it was carried out, including those relating to conservation welfare, and to the journal's policy on these matters;

(iii) that all authors and relevant institutions have read the submitted version of the manuscript and approve its submission;

(iv) that all persons entitled to authorship have been so included.

Abstract:

Bioturbation driven by the activities of invertebrates is a natural process with a potentially large implication in ecological engineering. Our work aims to test the effect of

bioturbation, carried out by a sediment-dwelling *Tubificidae Oligochaeta* invertebrate species, on atrazine behavior in the sediment under controlled laboratory conditions. A series of microcosms reproducing each a portion of water/sediment interface such as in wetland areas was displayed with homogeneously spiked sediment with [^{14}C]-atrazine at an initial concentration of 2µg/g wet sediment, to be monitored during 26 days. Concentrations of [^{14}C]-atrazine were explored in each sedimentary fraction to estimate the physicochemical influences of the aquatic invertebrates. Bio-conveying transport by tubificid worms was independent on the atrazine contamination (bioadvection rate ranged from 15.00 to 26.50 cm.year^{-1} for 100 *Tubifex tubifex* worms.dm^{-2}). Three distinguishable layers were identified: *(a)* (0-2.5 cm) feacal pellets accumulation zone; *(b)* (2.5-5 cm) sediment ingestion layer; and *(c)* (5-8 cm) no activity of worms. The significant depletion of [^{14}C]-(atrazine + metabolites) concentration in the first 2 cm of sediment is explained by the sediment porosity increase due to the bioturbation. Tubificid activity impacted the sorption and desorption of the moderate hydrophobic atrazine between attached-particles and free fractioning fomrs within sediment compartment with an increase of the molecules concentration in the pore water, that is partly explained by the organic matter reduction under tubificids feeding.

Keywords: bioturbation, atrazine, ecological engineering, aquatic sediment.

1. Introduction:

Bioturbation, caused by the activities of invertebrates, is proved to create significant impacts on the physicochemical properties of sediment (Baranov, Lewandowski, Krause, et al., 2016; Kristensen et al., 2012). Sediment particles reworking and biotransports by bioturbators in aquatic sediment can be categorized as different types, including biodiffusor, conveyor, and regenerator (François et al., 1997, Gerino et al. 2003). These modes cause different effects on vertical distribution of organic matter, particle sorting, and grain size distribution in sediment column (Kristensen et al., 2012) and consequently may generates contaminant fluxes throught the water sediment interface during or their deposition in the sediment compartment (Ciutat et al., 2007). These fluxes should be at the source of a remobilization or resuspension of pollutants from the sediment into the overlying water (Bundschuh et al 2016) (Schaller, 2014, Sommerfreund et al. 2010) or in an opposite direction, bioturbation could also increase pollutant incorporation and burial into the sediment (Hoang et al. 2018). The remobilization or the burial of the contaminant largely depends on their hydrophobicity, and the type of bioturbation that is playing in the sediment. The bioturbation process carried out by conveyor invertebrate species, *Oligochaeta Tubificidae,* can generate continuous vertical material flow through upward and downward biotransports and stimulate the activity of microbial communities (Ciutat et al., 2007; Devault et al., 2009; Anschutz et al., 2012). Previous studies showed that bioturbation can also control the fate of organic matter and nutrients as well as fluxes of nutrient pollutant transports between sediments and water (McCall and Fisher, 1980; Hölker et al., 2015; Florian Mermillod-Blondin et al., 2001), and therefore facilitate the transformation of pollutants (Gerino et al., 2014). Likewise, the digestion by earthworms during

the soil bioturbation positively impacted biodegradation by increasing atrazine adsorption on their microsites concerning with organic matter modification (Farenhorst et al., 2000; Kersanté et al., 2005). Bioturbation activity by tubificid worms could also stimulate organic matter mineralization and the release of pollutants in storm water sediment (Mermillod-Blondin et al., 2005). Previous studies also reported the modification of size, structure and activity of indigenous atrazine-degrading bacteria (Monard et al., 2008) or acceleration of atrazine mineralization in bioaugmented soil in a relationship with earthworms bioturbation (Kersanté et al., 2005). Recently, positive and complementary influences of bioturbation combined with phytoremediation process for enhancing cadmium fluxes from overlying water to sediment and then into the plant root system have been demonstrated (Hoang et al., 2018).

Atrazine is a highly efficient and moderately persistent organic herbicide (a lenghthy soil half-life of 60 to > 100 days) widely used in agriculture to control broadleaf and grassy weeds (Douglass et al., 2015; Smith et al., 2005), especially in the United States (Henderson et al., 2006; Thelin and Stone, 2010, (Schreiner et al., 2016). As a moderately hydrophilic molecule (Graymore et al., 2001) used as a premergent herbicide, atrazine is moderately to highly mobile in soils, especially in soils having low clay and organic matter contents. Due to its low adsorption in soils or sediments (Koc =128 mL g^{-1}) (K. Sun et al., 2010), residual atrazine and its metabolites, such as deethylatrazine (DEA) or deisopropylatrazine (DIA) have a high potential to contaminate tap water, surface waters, groundwaters and adjacent soils (Kolpin et al., 1996; Lewis et al., 2009; Zaya et al., 2011) from agricultural soils via leaching and surface runoff (Douglass et al., 2017; Pascal- Lorber et al., 2011). Atrazine concentrations can exceed 0.1 μg L^{-1} in natural surface water (the European Union standard in surface fresh water).

The water-sediment interface, known to be one of the most vulnerable (Devault et al., 2009) receiving a hefty source of such hydrophophic chemicals (Jantunen et al., 2008) from agricultural practices and urban areas that may cause long-term effects to the biota by direct uptake or through the food web. Several physical, chemical and biological methods, involving activated carbon adsorption, air stripping, catalytic degradation, membrane filtration, and biological treatments could recently provide capacity to remove these molecules from aquatic systems. However, they were still limited due to the cost with high capital expenditure and manpower (Susarla et al., 2002; Guo et al., 2007). Bioengineering strategies advantage in providing sustainable methods with low cost of functioning for natural waters. Pesticides are degraded by microbial activity, chemical activity, or sunlight. The degradation of the herbicide, mostly occur in top soils (Douglass et al., 2015) or in wetland areas (Mudhoo & Garg, 2011) where atrazine-degrading microbial communities (Krutz et al., 2012) and other critical chemical factors for plant uptake, such as soil pH, organic matter and moisture (Wehtje et al., 1983), have been considered as the primary mode of attenuation. These microbial and chemical factors that directly influence the biodegradation of the pesticide can be positively influenced by the presence of bioturbation (Monard et al., 2008; Hölker et al., 2015) and an active use of phytoremediation (Murphy and Coats, 2011; Moore et al., 2013; Qu et al., 2017).

Since plants are part of the natural environment of the wetland, it makes part of natural component that may influence the bioturbation intensity. The plant root system is viewed as a part of the sediment compartment that may modulate or inhibit the bioturbation intensity and type of sediment particle mixing (Bernard et al., 2014). As a natural component of the biodiversity of the wetlands, a plant species was introduced in the experimental microcosm to

simulate the natural conditions. Broadleaf cattail (*Typha latifolia*) is one of the commonest wetland plant species with a large distribution under various hydrological conditions (Aulio, 2015). *T. latifolia* reveals rapid growth and high resistance in polluted environments with the ability to bioaccumulate both inorganic (Lyubenova et al., 2013) and organic pollutants (Langan & Hoagland, 1996).

The objective of this paper is to estimate the bioturbation intensity and physicochemical effects (porosity, OM, OC, C:N, pH, Koc, and Kd) on a pesticide behaviour within sediment contaminated by a labeled [^{14}C]-organic pollutant using a controlled-environmental experiment. The natural environment was reproduced in a serie of microcosm that mimics the wetland buttom conditions with sediment, plants and invertebrate. The bioturbation intensity and type (biotransport by bioadvection) was estimated under these conditions to study the influence of pesticide contamination and plant occurrence on the bioturbation process. The influence of oligocheates on the atrazine partitioning in the sediment (pore water and solid fractions) in these condition is also demonstrated by comparison of the labeled-[^{14}C]-atrazine concentrations in water and sediment with and without bioturbation. In particular, this paper discusses the pivotal role of the tubificids on water and sediment quality using atrazine as a model of POPs in realistic aquatic habitat.

2. Materials and methods:

2.1. Experimental design and microcosm setup

The experiment was carried out in controlled laboratory conditions with a series of 24 microcosms, each mimicking a portion of water/sediment interface as in wetland areas. Three factors were considered in the experimental setup: organic compound contamination (presence/absence of atrazine) plant influence (presence/absence of *T. latifolia*), and invertebrate influence (presence/absence of tubificid worms) (Table 1). Due to space limitation, the choice was made to not use a full-factorial design, but to focus on the treatments necessary to assess (1) the influence of atrazine and worms on the bioturbation process and (2) the influence of worms and *T. latifolia* plants on the fate of atrazine in water and sediment layers. The microcosms were set-up in a thermostatic experimental room with similar physico-chemical conditions (light provided by six electric bulbs of 400 Watt each, 12h of light each day, temperature at 18°C, light intensity in the experimental room of 6000 ÷ 8000 lux (120 ÷160 µmol.m^{-2}.s^{-1}) at the top of *T. latifolia* plants). They were arranged in four blocks, each including one replicate of the different combinations of factors tested..

Table 1

Main acronyms for different experimental treatments used in the experiment

Treatments noted	Time (day)	Description
{Typ}	t = 26	With plant, no invertebrate, no atrazine
{Typ.Tub}	t = 26	With plant, with invertebrate, no atrazine

{Atr}	t = 26	No plant, no invertebrate, with atrazine
{Atr.Tub}	t = 26	No plant, with invertebrate, with atrazine
{Atr.Typ}	t = 26	With plant, no invertebrate, with atrazine
{Atr.Typ.Tub}	t = 26	With plant, with invertebrate, with atrazine

Typ: *T. latifolia* plant; W*:* tubificids worms; Atr: Atrazine

The sandy-muddy sediment samples were collected from the Aussonnelle River catchment, a first rank tributary of the Garonne River (Sélery area, Colomiers, France). A mechanical mixing process of the sediment was performed by using a motor-mixer to eliminate the antecedent macrofauna and to homogenise the sediment. Then, the defaunated sediment was introduced into each microcosm, which roughly consisted of a sediment layer of 8 cm depth (approximately 0.6 kg of fresh sediment) and of a water column of 3 cm height of dechlorinated tap water (approximately 0.2 L of overlying water).

Tap water was supplemented in each microcosm every few days (approximately 3% of the total water volume per day) to to keep a constant level of water despite evaporation and transpiration by the plant. *T. latifolia* plants were provided by ALISMA commercial farm (Plantes aquatiques, Taurignan Castet, France) in June 2015 and then stored about one month at the thermostatic experimental room in order to keep it growing after the delivery. Before putting them into the experimental pails (at t=0), their root and rhizome systems were cleaned with tap water to completely remove un-defaunated sediments.

Two weeks (t= -15 days) before starting the experiment, radiolabeled [^{14}C]-atrazine solution was mixed within water from the Garonne River and then homogeneously contaminated sediment with an initial concentration of 2 µg.g^{-1} wet sediment, as a non-conservative and organic pollutant to be followed in the aquatic environment during 26 days of the experiment.

Invertebrate Oligochaeta *Tubifex tubifex* (tubificid worms), well known as active ecological engineers, used as a source of bioturbation, were provided by the GREBIL Company (ARRY, France). The tubificid worms were introduced into the microcosms at the surface of the sediment at the initial time of the experiment (t=0) with a density of 10,000 individuals.m^{-2}, corresponding to a fresh biomass of 2.13 ± 0.06 g per microcosm.

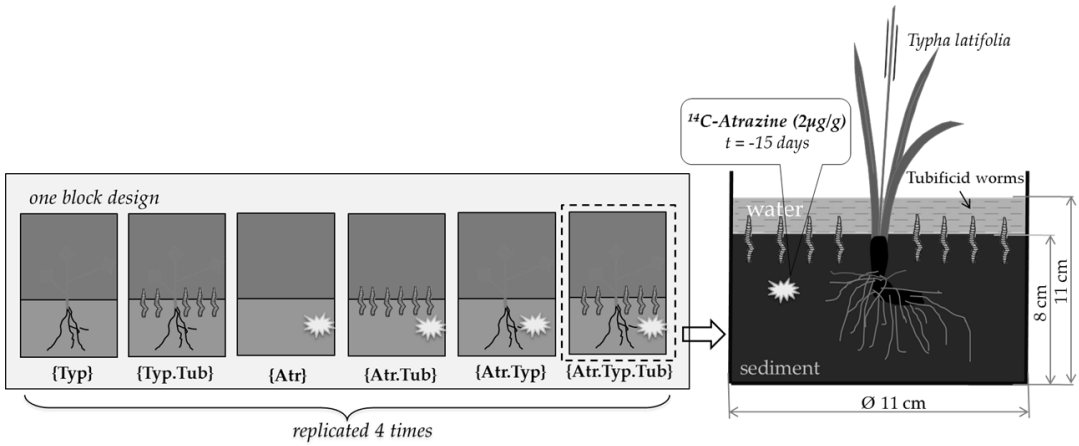


Fig. 1. Microcosm experimental design (see **Table 1** for the acronym definitions corresponding to the different treatments). The four replicates for each treatment were arranged in four separate blocks to avoid any confounding effect due to possible variations of environmental parameters within the thermostatic room.

2.2. Bioturbation activity measurements

The bioturbation activity of tubificids was quantified using fluorescent inert tracer technique (Gerino et al., 1994; T.K. Hoang et al., 2018). About 2g of luminophores tracers (natural sand and particles coated with pink fluorescent paint with size ranging from 63 to 125µm (Partrac Ltd., UK) were deposited at the sediment surface by spraying method one day after tubificid introduction. At the end of the experiment (t=26), the sampling of sediment along with the deposited tracer was carried out by sub-coring in the microcosms. Then sediment core was cut into ten layers with high definition: 0.5-cm thick layers from the surface down to 3 cm depth, then 1-cm thick layers down to 6 cm depth, and 2-cm thick layers down to 8cm depth.

Detection of luminophores was performed with a Synergy Microplate Reader (Biotek, USA) according to a protocol adapted from Majdi et al. (2014). After estimation of the luminophore concentrations along depth in the sediment column, the bioadvection–biodiffusion model (Officer & Lynch, 1982; Gerino et al., 1994, Ciutat et al 2005, Delmotte et al . 2007) was applied to fit the measured tracer profiles:

$$\frac{\delta C}{\delta t} = Db\frac{\delta^2 C}{\delta z^2} - V\frac{\delta C}{\delta z} \qquad (eq.\ 1)$$

With *t* as the time, *z* as the depth, and *C* as the tracer concentration. This model allows to estimate the theoretical concentration of tracers under the effects of the two biotransports parameters: V ($cm.y^{-1}$) as the bioadvective velocity or bio-sedimentation rate that represents the downward transfert of the tracers under conveying bioturbation and Db ($cm^2.y^{-1}$) as the biodiffusion coefficient that reflects the mixing rate of the tracers in omnidirectional directions in the sediment.

2.3. Physico-chemical analyses of sediments

Two experimental treatments with and without bioturbation (no contamination, no plant addition): {-W} and {+W} were set up using similar experimental design in order to determine physico-chemical factors affecting the degradation of atrazine in sediment. Organic matter, organic carbon, C/N ratio, and pH were measured vertically in three distinguishable sediment layers (0-1; 1-3; 3-8cm), excepted for pH with four layers (0-1; 1-3; 3-5; 5-8 cm). Sediment porosity was determined in fresh sediment samples from the 10 distinguishable layers with high definition as in the bioturbation measurement (*section 2.2*). Organic matter content in the sediment was measured through a semi-quantitative method based upon the indiscriminant removal of all organic matter followed by gravimetric determination of sample weight loss using muffle furnace at 500°C during 4h (loss-on-ignition method) (ASTM, 2000). Organic carbon and C/N ration were determined using Elemental Analyzers - FLASH 2000 (Thermo Scientific). Sediment pH was measured using a pH probe Multi 3420 WTW.

2.3. Atrazine and its metabolites concentration determination

Total concentration of [^{14}C]-(atrazine + metabolites) in the two experimental treatments having atrazine contamination: {Atr} and {W,Atr} was determined in overlying water and in the ten distinguishable sediment layers at the beginning (t=0) and at the end of the experiment (t = 26days). These layers were defined as in the bioturbation activity measurement partitioning liquid and solid phases: the pore waters and the sediment particles.

For quantification of [^{14}C] radioactivity in overlying water and pore water, 1 mL of each prepared sample was mixed with 2 mL of liquid scintillation cocktail (Ultima Gold™) before measuring with a liquid scintillation counter (LSC) (Packard Tri-Carb^{R} 2910TR, PerkinElmer Life and Analytical Sciences, Courtaboeuf, France). For the sediment particles and plant part samples, approximately 300 mg of homogenized subsamples were used for oxidative combustion to convert [^{14}C] radioactivity to $^{14}CO_2$ using a Sample Oxidizer Packard 307 (PerkinElmer Life and Analytical Sciences). The resulting $^{14}CO_2$ was trapped in a scintillation cocktail (Permafluor/Carbosorb, 10:7, v/v), and then the detection of radioactivity was performed by LSC. The efficiency of the oxidizer was measured by combustion of ^{14}C-labeled standards, and the recoveries of radioactivity from samples were adjusted to optimize the efficiency of the instrument. Atrazine and its metabolites were considered together through a global quantification, and their quantity was calculated from the specific activity of [^{14}C]-atrazine.

Didier's comment: And nothing about the metabolites concentration ? Do you determine the metabolite such as DEA and DIA ?

2.4. Calculations and data analysis

Sediment adsorption Coefficients

Sorption coefficient (Kd) and soil organic carbon-water partitioning coefficient (Koc), as indexes for respectively atrazine adsorption and its mobility into the sediment were calculated. The Koc coefficient has been so far used as a quantitative measure of the magnitude of the binding affinity of atrazine for organic matter. This constant allows the calculation of the proportion of atrazine bound to organic matter which is essential for predicting atrazine fate in

sediments and water bodies (Kulikova and Perminova, 2002). **The sorption coefficient (Kd)** measures the amount of chemical substance adsorbed onto sediment per amount of water (Linde, 1994). Since the adsorption occurs predominantly by a partition into the soil organic matter, it is more useful to determine a soil's ability to adsorb through Koc (Linde, 1994).

$$\textbf{Kd } (\text{mL.g}^{-1}) = \frac{Concentration\ of\ chemical\ in\ soil\ (\frac{\mu g}{g})}{Concentration\ of chemical\ in\ water\ (\frac{\mu g}{mL})}$$

$$\textbf{Koc } (\text{mL.g}^{-1}) = \frac{Kd\ x100}{\%\ Organic\ carbon}$$

Statistical analysis

To evaluate statistically any significant difference among all experimental treatments (t = 26), the mean value of bioadvective rate (V) and biodiffusive rate (Db) obtained from the *eq.1* were first compared using one-way ANOVA. Following ANOVAs, the mean values of the various treatments were compared with each other using Tukey HSD test. To investigate the single and interactive effects of atrazine and tubificids on the bioadvective rates, the mean values in the treatments including plants (*i.e.* {Typ}, {Typ,W}, {Typ,Atr}, and {Typ,W,Atr}) were compared using two-way ANOVA. Likewise, another two-way ANOVA was conducted on all the atrazine-contaminated treatments (*i.e.* {Atr}, {W,Atr}, {Typ,Atr}, and {Typ,W,Atr}) to evaluate the single and interactive effects of plants and tubificids on bioadvective rates. .

Student *t*-test was applied on the mean values of ^{14}C-∑(atrazine, metabolites) concentrations in overlying water and sediment, as well as the mean values of water content, organic mater, organic carbon, and C:N ratio, K_d, K_{oc} in the sedimentary layers to evaluate statistically significant differences between experiment treatments with and without worms: {W,Atr} and {Atr}.

Significance of the observed effects were assessed at the $p \leq 0.05$, 0.01, 0.001, and 0.0001 levels. All analyses were performed using the statistical software R, version 3.0.2 (2013-09-25) and statistical GraphPad Prism software, version 6.01.

3. Results:

3.1. Sediment reworking measurement

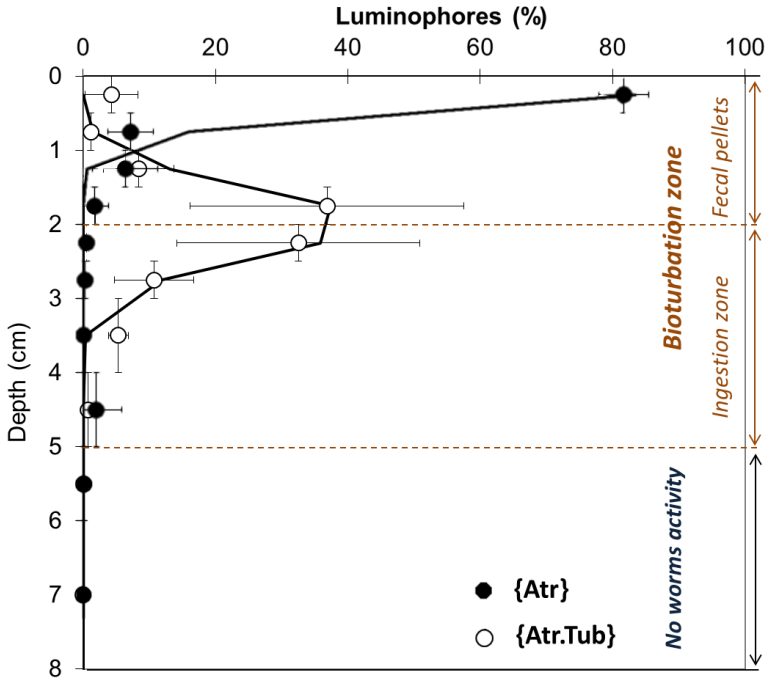


Fig. 2. *Mean values and their standart error (horizontal bars) of the luminophore concentrations* in the sediment after 26 days for treatments with and without tubificids (*black and grey dots, respectively); (see* ***Table 1*** *for the definition of acronyms corresponding to the different treatments*).

In the sediment without *Tubifex* worms (Fig. 2), no particle mixing occurred since most of luminophore tracers (70-80%) remained at the surface of the sediment (0-0.5cm), and about 20%, and 5% were located below the surface (0.5-1.5 cm, 1.5-2 cm, respectively) after the experiment (grey profile). Some luminophore particles were found in the deeper layers probably due to inherent tracer movements during experimentation or because of the activity of some smaller invertebrates that survived to the initial sediment defaunating process.

Luminophore profiles (Fig. 2) showed an increase in the depth location of the maximal luminophore concentration under the conditions with and without bioturbation. In the presence of worms, the tracers (about 20 to 45%) mostly distributed at the 2-3 cm sediment layer with bioturbation (black profile) evidenced sediment bioadvective processes induced by tubificids. Since luminophores were too large to be ingested by oligochetes, their transfert upward was not possible once they reached the ingestion layer. The deepest occurrence of luminophores in the sediment (5 cm) at the end of the experiment indicated the depth of the sediment ingestion layer.

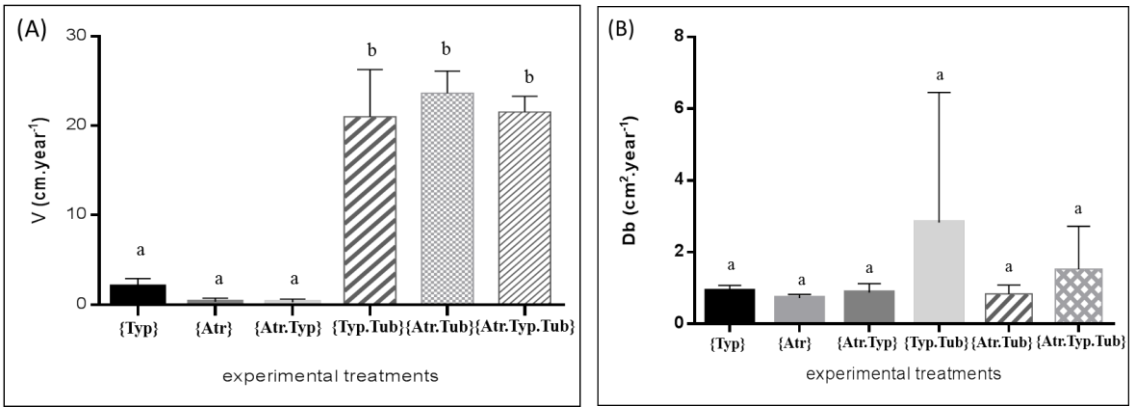


Fig. 3. *(A):* bioadvective rates -(V,) and (*B*): biodiffusive rates - (Db,) estimated after 26 days for the different experimental treatments. For each parameters, thevariable same letters at the top of the bars indicate treatments that were not significantly different ($P < 0.05$) as analyzed by TUKEY HDS multiple comparison test. Values are means ± SD, n = 4, excepted for {Typ.Tub}, {Atr.Tub}, {Atr.Typ.Tub} with (n =3) . (*see* ***Table 1*** *for the definitions of acronym corresponding to the different treatments*).

In the treatment having worm addition, the bioadvective rates (V) obtained by using the bioadvection–biodiffusion model corresponded to a rate of 21.53 ± 1.74 to 23.63 ± 2.49 cm.year^{-1} for a tubificid density of 10,000 individuals.m^{-2}, while it varied only from 0.26 ± 0.36 to 2.03 ± 0.88 cm.year^{-1} when the worms were absent (Fig. 3). Only the bioadvective rates showed significant difference between treatments with or without tubificid addition (Tukey HSD test; Fig. 3A).

It is particularly remarkable that no significant difference for either V (Fig. 3A) or Db (Fig. 3B) was observed when comparing corresponding experimental treatments with or without added atrazine, and with or without plant.

Table 2

Two-way ANOVA assessing the influences of atrazine and worms on bioadvective rates (V) (performed on the treatments with plants, *i.e.* {Typ}, {Typ.Tub}, {Atr.Typ} and {Atr.Typ.Tub}). ****: *significant difference for V at P ≤ 0.0001; ns: non significance.*

Factors	$F_{1,10}$	*P*
Atrazine contamination	0.2014	0.6631^{ns}
Tubificid addition	**214.3**	*<0.0001*****
Atrazine x tubificid interaction	0.7001	0.6631^{ns}

Table 3

Two-way ANOVA assessing the influences of *T. latifolia* plants and worms on bioadvective rates (V) (performed on the treatments with atrazine, *i.e.* {Atr}, {At.Tubr}, {Atr.Typ},

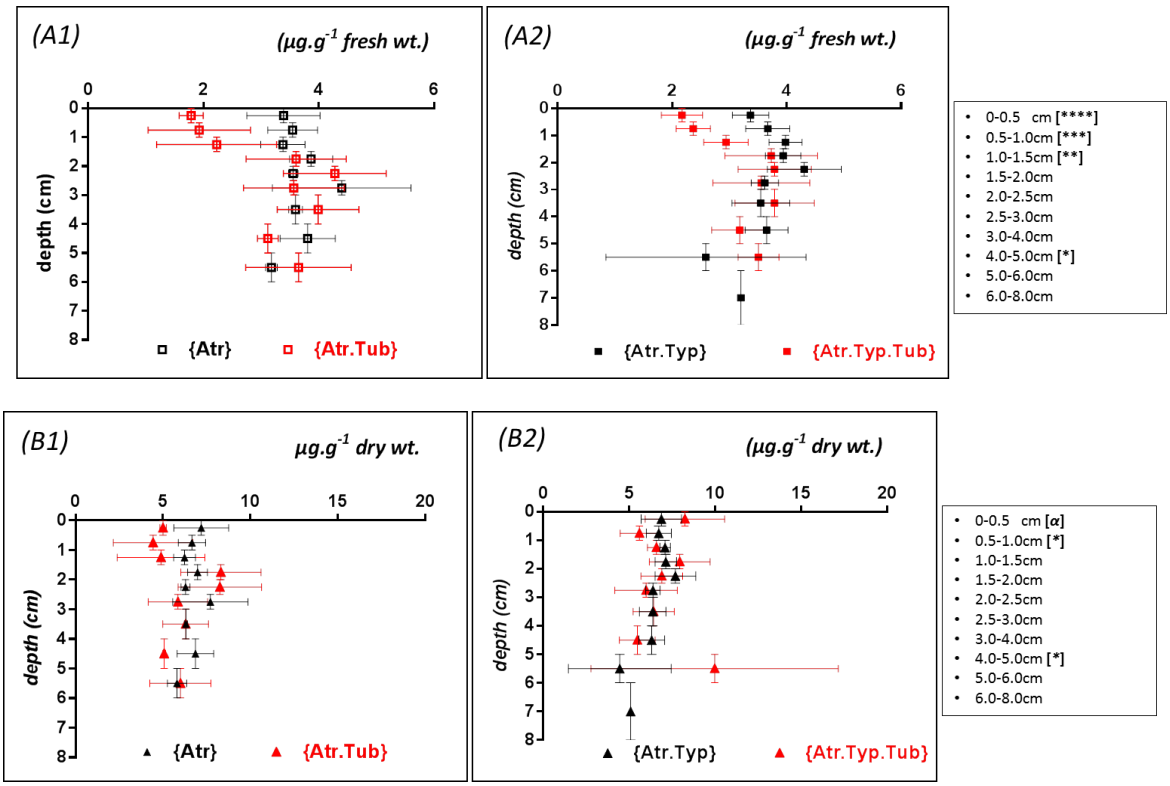
{Atr.Typ.Tub}). ****: *significant difference for V at P ≤ 0.0001; ns: non significance according to two-way ANOVA.*

Factors	$F_{1,10}$	P
T.latifolia treatment	2.078	*0.1800* ns
Tubificid addition	**879.3**	*<0.0001***
T.latifolia : tubificid interaction	1.827	0.2062 ns

A significant effect of tubificid addition on bioadvective rates was evidenced by using two-way ANOVA , either when crossing this factor with atrazine ($F_{1,10} = 214.3$, $P < 0.0001$, Table 2) or with the presence of *T. latifolia* plants ($F_{1,10} = 879.3$, $P < 0.0001$, Table 3). None of the other factors tested (atrazine or *T.latifolia* plant) had any significant influence on V, either alone or in interaction with the presence of tubificids (Tables 2 and 3).

3.2.[^{14}C]-(atrazine, metabolites) concentration in overlying-water and sediment

At the initial time (two weeks before starting the experiment), [^{14}C]-(atrazine, metabolites) concentration in the overlying water was found to be at 3.68 ± 0.07 µg.mL^{-1} (mean ± SD, n=2). Twenty-six days after the beginning of the experiment, the total concentrations were very low, ranging from 0.16 ± 0.04 to 0.18 ± 0.03 µg.mL^{-1} (mean ± SD, n=4), with no significant difference between the pesticide-contaminated treatments {Atr} and {Atr.Tub} (data not shown).



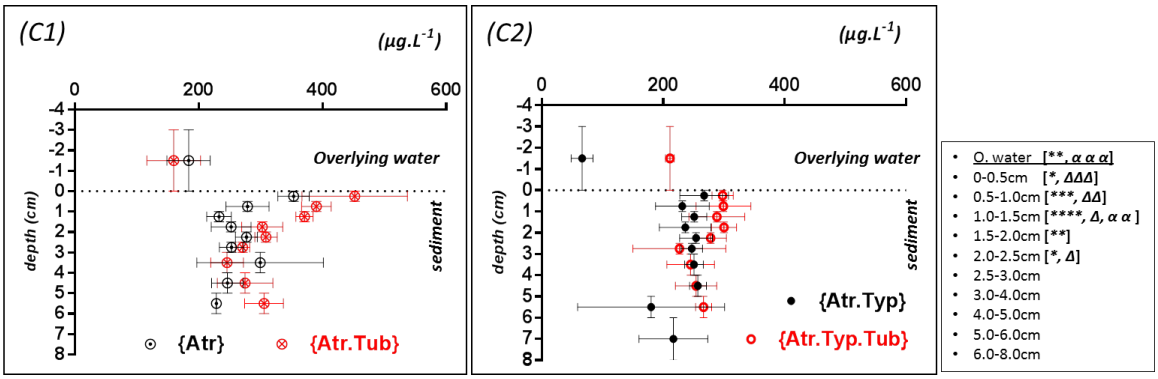


Fig. 4. [^{14}C]-(atrazine + metabolites) concentrations according to depth and experimental treatments for different sediment fractions (A1 and A2: *fresh sediment (or bulk sediment: being the sum of sediment particles and interstitial water); B1 and B2: sediment particles; C1 and C2: overlying water and pore water*) at the end of the experiment (t=26). *Values are means ± S.D., replicate (n) = 4 per treatment group.* (*), (**), (***), and (****) indicate significance effects of worms addition at P ≤ 0.05, 0.01, 0.001, and 0.0001 respectively according to the two-way ANOVA (The test were performed on the concentrations of the four treatments: {Atr}, {Atr.Typ}, {Atr.Tub}, and {Atr.Typ.Tub}). Similarity, the number of Δ indicates the level of significance of *T. latifolia* effect and α the level of the two factors interactions, respectively at different levels of pP values according to the two-way ANOVA (*see* ***Table 1*** *for the definitions of acronym corresponding to the different treatments*).

Bulk sediment (total fraction)

Two weeks before starting the experiment, mean values of [^{14}C]-(atrazine, metabolites) concentration in the fresh sediment (total fraction or bulk fraction) was found to be 1.95 ± 0.02 µg.g^{-1} fresh *wt (weight of fresh sediment)*.. At the end of the experiment, the concentrations in the sedimentary layers in absence of worms ranged from 3.18 ± 0.10 to 4.40 ± 1.20 µg.g^{-1} fresh *wt.* and from 2.59 ± 1.75 to 4.31 ± 0.65 µg.g^{-1} fresh *wt.* (total fraction), respectively in {Atr} and {Atr.Typ} treatments (Fig.4A). Significant effect of tubificids were recorded in the upper layers (0-0.5, 0.5-1.0, 1.0-1.5 cm, with p < 0.0001, 0.001, and 0.01, respectively) where lower concentrations were measured with bioturbation, and higher concentration in the deeper layer (4-5 cm, pP < 0.05) as evidenced by the performed two-way ANOVA (Fig. 4A).

Impact of the interactions between bioturbation and plant on [^{14}C]-(atrazine, metabolites) quantities was estimated by using Tukey HSD test focusing on the three sedimentary layers as defined in § III.B.4.1 according to the bioturbation depth : the fecal pellets zone (0-2 cm), the ingestion zone (2-5 cm), and the "no worm activity" zone (5-8 cm). These layers thickness were defined based on the luminophores distribution under bioturbation (Fig. 2).

Sediment particles

A significant effect of tubificids on [^{14}C]-(atrazine, metabolites) concentrations of sediment particles was observed only in the two layers: from 0.5 to 1.0 and from 4.0 to 5.0 cm (two-way ANOVA, P =0.032 and 0.0138, respectively) (Fig. 4B) where lower concentrations were observed with bioturbation. Any significant effects of *T. latifolia* on the concentrations was

observed in all layers of sediment particles as evidenced by the two-way ANOVA test ($P > 0.05$, Fig. 4B).

A similar consequence of tubificid addition on quantity of [^{14}C]-(atrazine, metabolites) in the fecal pellets layers (0-2 cm) of fresh sediment was also recorded in the sediment particle fraction with significant depletion of the quantity in the presence of worms.

Pore water

At the end of the experiment, effects of tubidicids addition were significant from the surface sediment until 2.5 cm depth with higher [^{14}C]-(atrazine, metabolites) concentrations in pore water with bioturbation. The effect of the plants was also significant in the same layers, but with lower concentrations when Typha was occuring, except for the layer from 1.5 to 2.0 cm (two-way ANOVA, Fig.4C). Significant interaction between the two tested factors was only found for layer from 1.0 to 1.5 cm (Fig.4C).

3.3. Porosity of experimental sediments

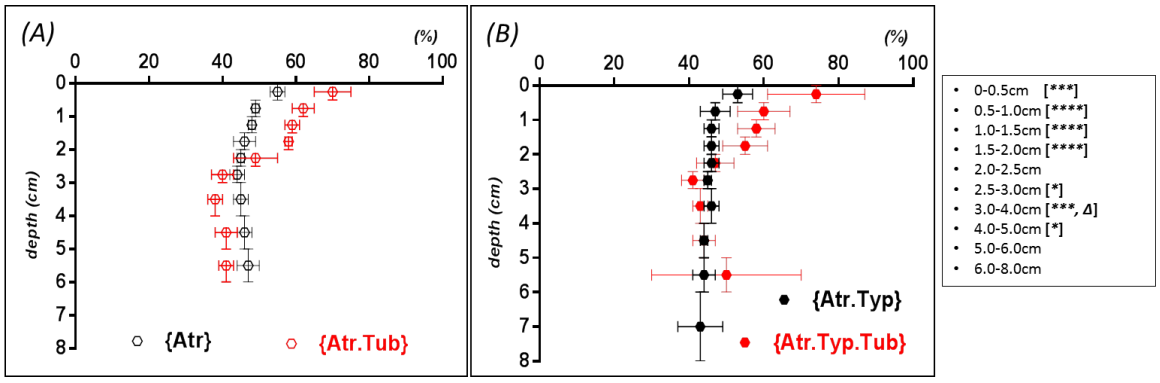


Fig. 5. Sediment porosity profiles of (A): {Atr} and {Atr.Tub}; (B): {Atr.Typ} and {Atr.Typ.Tub} treatments versus sediment depth at the end of the experiment. *Values are means ± S.D., replicate (n) = 4 per treatment group.* (*), (***), and (****) indicate significance effects of worms addition at $p \leq 0.05$, 0.001, and 0.0001 respectively according to two-way ANOVA (effects of worms and *T. latilolia* treatment performed on the percentage of water content in these four treatments); Δ indicate significant effect of *T. latifolia* at $p \leq 0.05$ according to the two-way ANOVA test. No significant interaction between these two tested factors was observed among the treatments of all the layers (*see* ***Table II.2*** *for the acronym definitions corresponding to the different treatments*).

At the end of the experiment, effect of bioturbation on water content in sediment column was significantly ($P < 0.05$, 0.001, 0.001) at all depth from surface until 2.5 cm, excepted for the layers from 2.0 to 2.5 cm , as evidenced by the two-way ANOVA (Fig. 5). Significant effect of *T. latifolia* on the change was observed only in the layer from 3.0 to 4.0 cm ($P > 0.05$, two-way ANOVA, Fig. 5**).** However porosity increased under worms effects in the surface layers from 0 to 2 cm, and inversely, the porosity was lower in the deeper layers, as analyzed by Tukey HSD multiple comparison test (see Annex)

In the absence of worms ({Atr} and {Atr.Typ} treatments), the porosity of sediment varied from 43 ± 6% to 53 ± 4% in the sediment column (see Annex), with no significant difference between the two treatments (P > 0.05, Tukey HSD test, see Annex).

3.4. Physicochemical factors affecting the mobility of atrazine and its metabolites

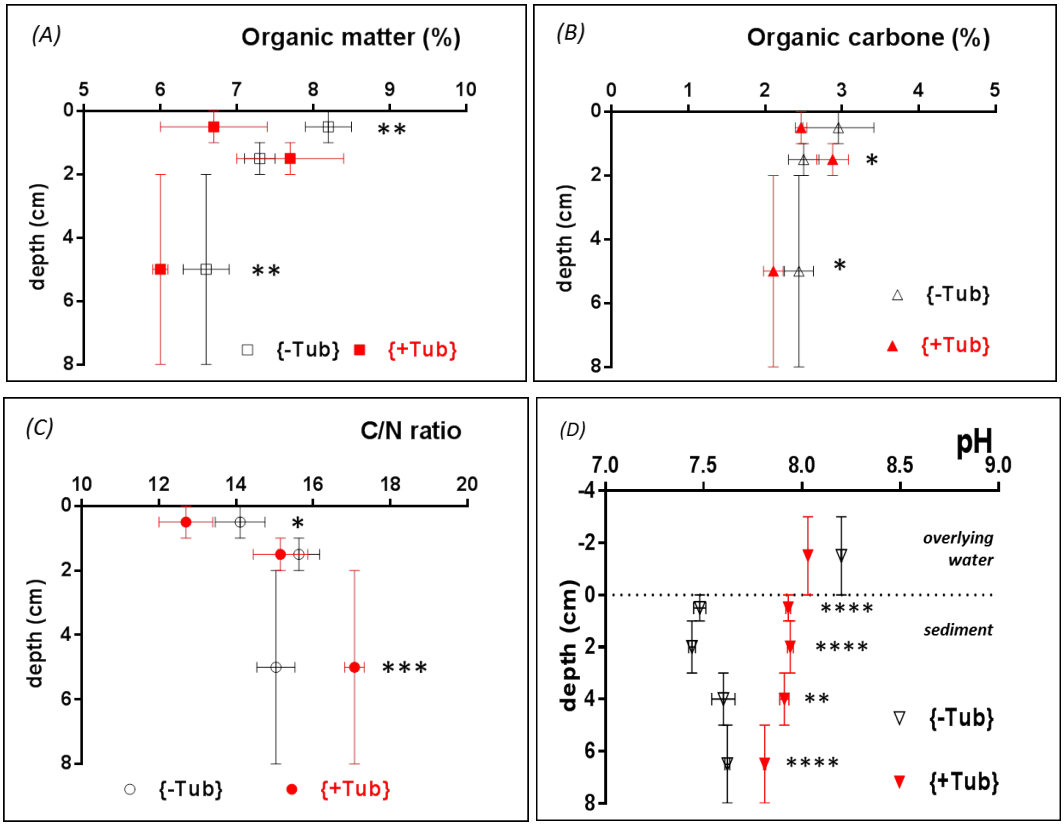


Fig. 6. Physico-chemical variabless at t=26 days with *(A)*: organic matter content; *(B)*: organic carbone; *(C)*: C/N ratio; *(D)*: pH (*see **Table 1** for the definitions of acronym corresponding to the different treatments*). (*), (**), and (***) indicate significance at p ≤ 0.05, 0.01 and 0.001, respectively according to unpaired Student *t-test.*

In the presence of the worms, organic matter contents (Fig III.6A) significantly decreased after 26 days in the upper sediment layers (0-1 cm) and in bottom layers (2 – 8 cm) (p < 0.01, Student *t*-test) in the treatment {+Tub}, compared to the treatment without the worms. Similarity, organic carbon content showed a significant decrease in the layer from 2 to 8 cm with worms ({+Tub} treatment (p < 0.05, Student *t-test*), but the higher C concents were found mostly in the upper layer (1-2 cm) (Fig. III.6B). This leads to a significant decrease of organic carbone C:N ratio in the presence of the worms ({+Tub}) in the top layer (0-1 cm) at the end of the experiment, but a significant increased in the bottom layer (2-8 cm) compared to treatment without worms addition ({-Tub}, Fig. III.6C). The pH level became significantly higher in whole sediment column when the worms were occurring, by comparison with the treatment without bioturbation (P < 0.0001 and 0.05, Student *t*-test, Fig. III.6D).

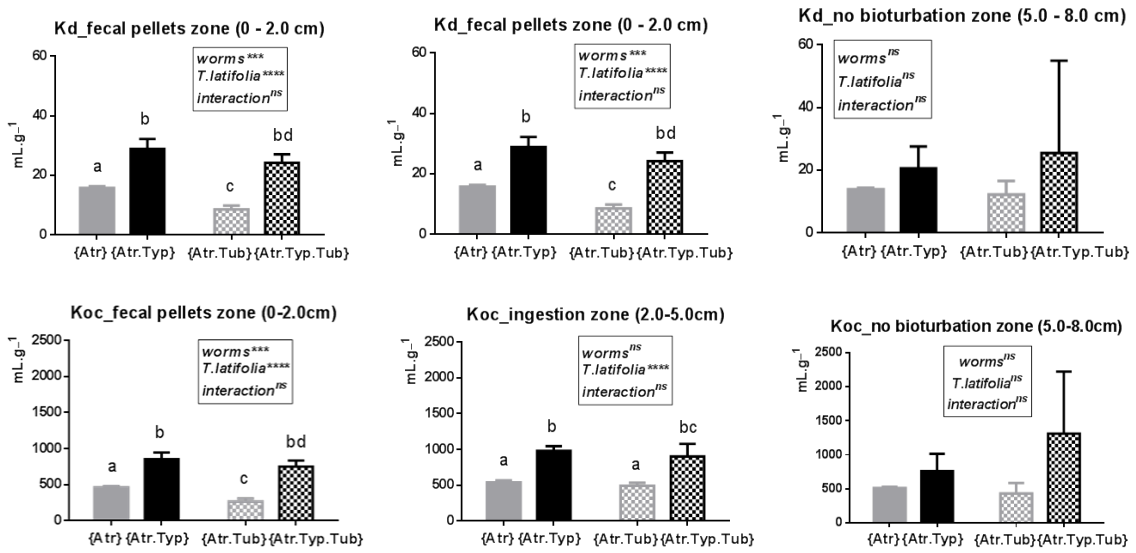


Fig. 7. soil adsorption coefficient – Kd and organic carbon – water partitioning coefficient – Koc affecting mobility of atrazine and its metabolites in sediment. *Values are means ± S.D., replicate (n) = 4 per treatment group.* (*), (***), and (****) indicate significance effects of worms addition and T.latifolia treatment as well as significant interaction between the two factors tested at $p \leq 0.05$, 0.001, and 0.0001 respectively according to two-way ANOVA; ns – no significant difference

Worms and *T. latifolia* significantly affected soil adsorption coefficient Kd in bioturbated layers from 0 to 5 cm, as evidenced by two-way ANOVA test performed on the values of all treatments contaminated with atrazine ($p < 0.001$ and 0.0001, respectively, Fig.7). A significant effect of worms for water partitioning coefficient was observed only in the fecal pellets zone (0-2.0cm) ($p < 0.001$, two-way ANOVA, Fig.7), while *T. latifolia* significantly affected the whole bioturbated layers from 0 to 5 cm ($p < 0.0001$, two-way ANOVA, Fig.7).

Lower values of this sorption coefficient (Kd) and water partitioning coefficient (Koc) were also observed in the presence of worms ({Atr.Tub} treatment) compared to treatments without worms addition ({Atr} and {Atr.Typ}, respectively) with significant differences in the bioturbated layers (0 – 5.0 cm) for Kd (Tukey HSD test, Fig.7) and in the fecal pellets layer for Koc (Tukey HSD test, Fig.7).

4. Discussion:

4.1. Influence of bioturbation on particle mixing and tubificid resistance to atrazine

In the presence of tubificid worms with a density of 10,000 worms.m^{-2} (equivalent to a fresh biomass of 4.45 ± 0.53 g per microcosm), a subsurface peak of tracers in the 2 cm layer indicated that a conveying transport was created by tubificids. This downward transport of the surface sediment resulted from the accumulation of faecal pellets at the sediment surface,

simultaneously with sediment depression in deeper layers due to sediment ingestion by the worm feeding activities (Anschutz et al., 2012; Ciutat et al., 2006). Selective feeding behaviour by tubificid worms based on particle size, avoiding larger coarse particles, such as luminophores and sand particles induces a decrease of the silt-clay fraction at depth(Rodriguez et al., 2001). Consequently, bioturbation creates two distinct layers in the bioturbated sediment: a surface layer corresponding to the faecal pellets accumulation from ingested anoxic sediment, and a bottom layer with increasing particle size (Anschutz et al., 2012). Our results (Fig. 2) indicated that bioturbation caused by worm activities followed the bio-conveying transport model and allowed us to identify three distinguishable layers: *(i) a surface zone* (0 - 2.0 cm) composed entirely of fecal pellets resulting from the bioadvective transport process with about 75% of the luminophore tracers, initially deposited at the sediment surface, were spread down to 2.0 cm depth at the end of the experiment (Fig. 2); *(ii) an ingestion zone* (2.0 – 5.0 cm) which indicates the maximal ingestion depth by the worms; and *(iii) a deep zone* (5.0 – 8.0 cm) where no worm activity has been detected. The maximal ingestion depth found in this study was located below 2.0cm and the bioturbated layer was thus estimated to be from 0 to more than 5 cm since luminophores occurred down to this level (Fig. 2) after 26 days. Depending on the bioturbating time, thickness of the surface zone could be extended to about more than 4 cm after 93 days having the same species treatment at higher density of 62,000 ± 4,000 worms.m^{-2} (Ciutat et al., 2006). The obtained rates of bioadvection in presence of tubificids (Fig. 3) were in the same order of magnitude as those estimated by other authors with tubificid populations at various densities (Ciutat et al., 2005; Hoang et al., 2018).

The absence of interactive effect of atrazine and tubificid addition on bioadvective rates (two-way ANOVA, Table 2) indicated that bioturbation was not altered by atrazine-contamination. Indeed, there was no significant difference in V among the experimental treatments having worms but with or without atrazine added {Typ.Tub}, {Atr.Tub}, {Atr.Typ.Tub} (Tukey HSD test, $P > 0.05$, Fig. 3A). This result is in line with previous studies that found out, using Cd-spiked aquatic sediment, that bioadvective rates were not affected by Cd enrichment (Anschutz et al., 2012; Ciutat et al., 2005; Hoang et al., 2018). Oligochaete worms could be therefore considered as ecological engineers with a high resistance to both inorganic and organic contaminants from aquatic enviroment. Indeed, some invertebrate oligocheates, which are very widely distributed and frequently dominant in freshwater benthic communities, show a high level of resistance to unfavorable treatments, especially organic pollution associated with severe hypoxic treatments (Brinkhurst and Cook, 1974). An indoor microcosm carried out by Ciutat et al., (2005) to test the bioaccumulation kinetic of Cd, added to the water column at 20 µg/L, in tubificid worms during a 56-day exposure period with bioaccumulation levels as high as 50 mg.g^{-1} dry wt found in the worms. The resistance of these organisms was related to their detoxification or sequestration abilities under polluted environment. Whitley (1967) reported that tubificids showed high tolerance limits for water contaminated by Pb and Zn, with the median tolerance limits at 49 and 46 mg/L, respectively. Both acute and chronic toxicity of organic compounds has been previously investigated in some invertebrate worms. Dad et al., (1982) reported that tubificid worms (a mixture of *T. tubifex* and *L.hoffmeisteri*) were able to tolerate high insecticide concentrations with presumed harmless concentrations of Furadan 3G (carbofuran) and Malataf 50E found to be at 4.37 and 3.22 mg/L, respectively. Although atrazine is considered highly toxic to freshwater aquatic invertebrates (US Environmental Protection Agency, 2003), the hydrophobic chemical was bioaccumulated across

the body wall of benthic oligochaeta *Lumbriculus variegatus* with a wide range of bioacumulation factors (BAFs) from 0.19 to 19.56 after 240 h of exposure (Jantunen et al., 2008). Although this study does not give an evidence of worm persistence at high pesticide levels, with concentration as high as 6.4 μg/g (compared with 17 μg/L) which is normally found in natural sediment in rural ponds in Canada (Frank et al., 1990), our study implied that bioturbation should remain active in natural sediments that does not exceed the used concentration (at 2μg/g, compared with 5 μg/L).

4.2. Plant influence on bioturbation intensity

Several studies have revealed plant influences on aquatic bioturbation intensity in aquatic sediment. Interactions between nbelowground biomass of the seagrass *Zostera* and burrowing bioturbator's activities have been investigated previously, with negative effects of root density on the intensity and mode of sediment particle mixing by benthic infaunal activities in coastal environment (Bernard et al., 2014), notably for large bioturbators such as ghost shrimps (Berkenbusch et al., 2007) and the polyochaete *Nereis diversicolor* (Hughes et al., 2000). These findings, however, are not consistent with the present study that revealed the independence of bioturbation intensity to the occurence of the riparian *T.latifolia* plant. Neither significant difference in bioadvective rate (V) (Fig. 3A) and biodiffusive rate (Db) (Fig. 3B) nor interaction between plant occurrence and worm treatment (Table 3) were observed when comparing experimental treatments with or without worms and plant added. This suggested that burrowing bioturbation by tubificid worms still remains active and is not affected by the plant treatment.

It should be noticed that the contradictory results from that obtained in the present study might be due to the differences in biotubation modes, in types of aquatic environment studied, or in species / functional type of aquatic plant selected. Moreover, the fact of using individual species of bioturbators rather than a whole community, as well as a short time duration for carrying out this experiment (26 days) could have led to incomplete observations to evidence these expecting influences.

4.3. Influence of biotransport on behavior and mobility of atrazine in sediment

Tubificids affect physical and chemical characteristics, including sediment particle size, porosity, water content, nutrient content, turbidity, TOC, etc., of the sediment and overlying water near the water-sediment interface (Anschutz et al., 2012; Ciutat et al., 2006; Cunningham et al., 1999; Hoang et al., 2018; Mermillod-Blondin et al., 2013; Mermillod-Blondin et al., 2008). The significant increase of water content observed in the top sediment layers (0-2 cm) when the worms were occurring in the experimental treatement {Atr.Tub} (Fig. 5) is explained by the physical effect of tubificid worms. Through the burrowing activity, worms create the accumulation of feacal pellets in the surface layer. The redistribution of sediment particles due to feacal pellets and undigested sediment particle accumulation by bioturbation led to a vertical change in grain size in the sediment layers where tubificid worms occured. Faecal pellet getting a larger diameter than sediment particles, this increased sediment porosity. This porosity change promoted water and solute exchanges thought the water sediment interface by simple molecular diffusion. As a consequence, mean grain size of sediment particles became smaller in

the upper layer (0-1 cm) and greater in the deeper layers (Hoang et al., 2018). That explained the significant decrease of water content in the deepest layers (3-8 cm) when worms were present ({Atr.Tub} treatment, Fig. 5) along with the least abundance of small particles (fine silt, clay, medium silt, and coarse silt) and higher abundance of larger particles (coarse, medium and fine sands) according to Ciutat et al., (2006) and Hoang et al. (2018). Wood et al., (1975) reported that the tubificid *Limnodrillus hoffmeisteri* generates a burrow flushing rate of 9.5–15 L water per worm per hour (20°C). At typical densities of 10^4 –10^5 ind m^{-2}, this would result in exchange of 25 L m^{-2} d^{-1}.

The increase of water content in the top sediment also explained the significant depletion of total [^{14}C]-(atrazine, metabolites) concentration in upper layers in case of worm addition ({Atr.Tub}) (Fig. 4A) due to bioturbation effect. The physical impact of worm activity caused a dilution of this total concentration at the interface between overlying water and sediment. When the surface zone is composed entirely of faecal pellets ejected by worms, it also extends the thickness of the layer (Ciutat et al., 2006), the difference of volume being explained by the enhanced water content.

Aquatic sediments are one of the largest reservoirs of organic matter (Breithaupt et al., 2012). Organic matter in sediment consists of heterogeneous mixture of carbon and nutrients, notably in the form of carbohydrates, proteins, fats and nucleic acids (Zonneveld et al., 2009). Bacteria, as heterotrophic microorganisms living in carbon-rich sediments, play an important role in the transformation and mineralization of organic matter in aquatic sediments (Boudreau, 1992). Tubificid worms play a significant role in the processing of organic matter in aquatic sediments (McCall and Fisher, 1980; Florian Mermillod-Blondin et al., 2001). During feeding activities of *Tubifex* worms, the downward transport of the surface sediment resulted from the accumulation of faecal pellets at the sediment surface (0-2.5 cm, Fig. 2) simulatneously to sediment ingestion at depth. The faecal pellets are covered by worm's digestive mucus that increase the colonizable area for bacteria. By increasing flux dispersion, oligochaetes also enhanced the nutrient availability in all microenvironments of the columns, stimulating both aerobic and anaerobic microbial activities (Florian Mermillod-Blondin et al., 2001, McMurtry et al., (2011) also reported a significant correlation between the abundance of heterotrophic aerobic bacteria in sediments and tubificid preference. In this study, tubificid worms consequently modified the organic matter distribution in the sedimentary layers (Fig. 6A and 6B), with a significant increase of feacal pellet contribution at the bottom of the surface layer and a depletion of the organic matter content in deeper layers (2-8 cm). The depletion of C and OM observed in the top sediment is explained by the new faecal pellet arrival, where the microbial community didn't get the time to develop yet. The burial over time of these pellets will be followed by the developpment of microbial biofilm that explains the OM matter inversion at the bottom of the surface layer. Similar profile of organic carbon content, hence, could be found in the presence of tubificid worms (Fig. 6B). In addition to these physicochemical parameters, pH is also considered as an important factor controlling adsorption of atrazine in soil/sediment. Gradually, positive mobilization of herbicides, such as atrazine and metribuzin in soil has been reported to increase as the pH increased (James et al., 1976). The adsorption of triazine molecules on soil matters is influenced by the pH at which the molecules became protonated at pH decrease that attributed to complexing of the triazine molecules with H^+ on the clay surface (Weber, 1970). In our experiment, the high pH (8.03) in overlying water, along with the

significant increase of water content (Fig. 5), leads to the increase of pH in surface sediment layers as observed in the {W,Atr} treatment. This is due to the intrusion of overlying water in the interstitial space of the faecal pellet layers (Fig. 6D). Under conveying process the new pH conditions are spread into the whole bioturbated sediment. Previous studies also reported sediment pH increase under bioturbation, which was explained by an accelerated removal of acid metabolites from sediment (David, 1974; Yingst & Rhoads, 1980).

Soil organic carbon-water partitioning coefficient (Koc) has been so far used as a quantitative measure of the magnitude of the binding affinity of organic matter for atrazine. In addition, soil adsorption coefficients (Kd) are indices for pesticide mobility in the aquatic environment, which are important for predicting the fate of atrazine in soils and waterbodies (Kulikova and Perminova, 2002). Study of sorption affinities of 101 soil samples from New Zealand were reported by Ahmad and Rahman (2009) with sorption coefficients (Kd) ranging from 0.7 to 52.1 $mL.g^{-1}$, and by Kookana et al. (2008) with the values in 31 soils ranging from 0.51 to 5.48 $mL.g^{-1}$.

Bioavailability and mobility of pesticides, determined via their fate in the environment under natural processes like bioturbation are strongly affected by the solubility (sorption to soil particles and organic matter, dissolution in pore water, or uptake by plants). Pesticides that are highly water soluble, relatively persistent, and not readily sorbed to soil particles (low Koc or low distribution coefficient) have the greatest potential for movement. In contrast, the higher the Koc value, the more strongly the pesticide is sorbed onto soil and organic matter which lowers its bioavailability (Binet et al., 2006). It has been shown that atrazine adsorption is associated with fine particle fraction consisting of clay minerals and organic matter (Paya-Perez et al., 1992). Koc, however, is a more important factor affecting the sorption than particle size (Rao 1983). Atrazine binding to soil organic matter is not extremely strong (**Koc~128 $mL.g^{-1}$**, moderate water solubility of **33 $mg.L^{-1}$**, average field half-life 60 days) (Lesan and Bhandari, 2000; Burnett et al., 2000) leading to high potential for movement from particle to dissolved state. This becomes obvious when compared with benomyl, a systemic agricultural fungicide having low movement rating with solubility of 2 $mg.L^{-1}$ and Koc of 1,900 $mL.g^{-1}$. A wide variation of partitioning coefficient (Koc), from 30 to 680 $mL.g^{-1}$ (Kookana et al., 2008), with a mean of 126.9 $mL.g^{-1}$, was also found by Ahmad and Rahman (2009). The Koc coefficients in our study (Fig.7) were in the same order of magnitude as the data provided by the authors cited above, with the means varying from 265.0 ± 131.8 to 537.0 ± 114.5 $mL.g^{-1}$. The two coefficients generally increased with soil organic carbon content (Fig. 7A and 7B).

It is suggested that the significant depletion of organic matter in the surface and deep sediment layers under the influence of tubificid worms ({Atr.Tub} treatment) contributed to the higher desorption/movement of atrazine and its metabolites from organic matter to pore water, compared to the treatments without worms ({Atr} treatment, Fig. 5C and Fig. 6A). Organic matter degradation due to worm activity consequently increased [^{14}C]-(atrazine, metabolites) concentration in pore water and thus released more free fraction of atrazine and its metabolites into the interstitial environment.

The relative recovery, *i.e.* the total amount of [^{14}C]-(atrazine, metabolites) in the whole test-system (overlying water and sediment) at the end of the experiment divided by that added at the beginning of the experiment, was calculated in each treatment with and without

bioturbation ({Atr.Tub} and {Atr}). This will allow to estimate the influence of faunal biodiversity in the bioremediation process. The obtained data from the treatment having tubificid worms ({Atr.Tub}), approximately 79.7 %, suggested a pesticide loss a a consequence of bioaccumulation to the worms' body. While, a marginal loss of the pesticide, approximately 93.7% of relative recovery, observed in the asbsence of worms {Atr} (data not shown), implied *(1)* a volatilization of atrazine to the air phase via the mineralization into CO_2 or *(2)* an adsorption to experimental pail walls. These finding was consistent with previous study of Bundschuh et al., (2016) on the role of pesticide properties on remobilization from sediment to overlying water phase.

5. Conclusion:

In aquatic systems, atrazine is among the most largely occurent herbicides in the continental sediments within deposition areas such as wetlands and ponds. Its moderate hydrophobic character makes it a sensitive candidate to investigate its behavior at the water sediment interface as a response to biodiversity-related processes such as bioturbation. Our experimental study using radiolabeled [^{14}C]-atrazine showed that bioturbation is still maintained in contaminated sediment with or without plants. Under worm activities, a biological mixture between water-sediment interfaces was created, causing the depletion of atrazine and its metabolite concentrations in the surface sedimentary layers and the redistribution of organic matter and C:N ratio in the sediment column. The influences of bioturbation by tubificids on these chemical properties of sediment have consequences on the adsorption-desorption behavior of the herbicide. Therefore bioturbation accelerates the mobility of atrazine (and presumably its metabolites) from attached forms (onto sediment particles) into a free fraction (in pore water) with lower values of Kd, Koc. This indirect bioturbation influence on the partitioning of atrazine in sediment suggests that atrazine should be more available for the other organisms that live in the sediment. This mobility should increase its toxicity towards some other organisms (microbes and invertebrates) that live in the sediment layer, but may also promote its uptake by plants.

The role of microorganism communities in the degradation of atrazine in bioturbated sediments should be addressed in next studies. On the one hand bioturbation is known to promote microbial activity that may support the degradation of such coupounds, but on the other hand, the release of this coumpound under biotubation may increase its toxicity to these organisms. Further analytical works concerning the identification of atrazine metabolites in sedimentary layers and the plant system, as well as the polarity of new metabolites (via Kow values) formed in the plant, also need to be carried to evidence metabolization process under the bioturbation.

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