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Influence du phytomanagement sur un écosystème contaminé par des métaux : Application à *Miscanthus* × *giganteus*

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Résumé

Influence du phytomanagement sur un écosystème contaminé par des métaux : Application à *Miscanthus* × *giganteus*

La phytoremédiation est présentée comme une option pour gérer des sols dégradés. Les potentialités de *Miscanthus* × *giganteus* ont été évaluées, dans des conditions *ex situ* et *in situ*, avec pour objectif de produire une biomasse sur des sols agricoles fortement contaminés par Cd, Pb et Zn. Il a été dressé un bilan sur le comportement de la plante face à un gradient de contamination des sols, en intégrant les variations saisonnières et différentes pratiques agronomiques (choix du cultivar, densité de plantation, amendement biologique, fertilisation azotée). Il a aussi été étudié l'influence du miscanthus sur la mobilité des métaux dans les sols. Les résultats ont montré que la contamination des sols ne perturbe pas la croissance de *M.* × *giganteus* et le rendement de la biomasse récoltée. Le cadmium, Pb et Zn sont principalement accumulés dans les racines et d'une façon générale, *M.* × *giganteus* réduit leur transfert vers les organes aériens. Cette plante présente un bon potentiel pour la phytostabilisation des métaux et d'une façon plus globale, pour le phytomanagement. Prises individuellement, les pratiques agronomiques n'ont pas d'effet sur le comportement de la plante. En revanche, la fertilisation azotée d'une part, et l'interaction entre le cultivar et l'ajout de l'inoculum endomycorhizien d'autre part, favorise légèrement l'accumulation de Cd et Zn dans les organes aériens des cultivars étudiés. Compte tenu du caractère pérenne de la plante, il conviendrait de valider ces résultats sur le long terme, d'étudier les effets du stress métallique sur la santé de la plante et d'évaluer le devenir des polluants en lien avec l'accumulation des matières organiques dans le sol.

Mots clés : Miscanthus, culture énergétique, phytostabilisation, phytomanagement, métaux, sol, contamination, mobilité des métaux.

Abstract

Influence of phytomanagement on a metal-contaminated ecosystem : Application to *Miscanthus* × *giganteus*

Phytoremediation is considered as an option for management of degraded soils. The potential of *Miscanthus* × *giganteus* were assessed in *ex situ* and *in situ* conditions, with the aim of producing biomass on agricultural soils heavily contaminated by Cd, Pb and Zn. The study mainly focused on the behavior of *M.* × *giganteus* growing on soils presenting a contamination gradient, and included seasonal variations and different agronomic practices, i.e., choice of the cultivar, planting density, biological soil amendment and nitrogen fertilization. The influence of the plant on metal mobility in soils was also assessed. The results show that soil contamination does not affect *M.* × *giganteus* growth and shoot yields. Cadmium, Pb and Zn are mainly accumulated in roots and in general *M.* × *giganteus* reduces their transfer to the aboveground organs. This plant presents therefore a great potential for metal phytostabilisation, and in a more comprehensive way, for phytomanagement. Taken individually, agronomic practices did not affect the plant behavior. However, nitrogen fertilization on one hand, and the interaction between the cultivar and the endomycorrhizal inoculum on the other hand, increase the Cd and Zn accumulation in miscanthus organs. Given the perennial nature of the plant, long-term studies are needed to validate the present results, to assess the effects of metal-induced stress on the plant health as well as the fate of contaminants in relation to the accumulation of soil organic matter in miscanthus plantations.

Key words: Miscanthus, energy crop, phytostabilisation, phytomanagement, metal, soil, contamination, metal mobility.

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Avant-propos

La pollution des sols par les éléments traces métalliques (ETM) représente des dangers environnementaux et sanitaires. C'est notamment le cas en région Nord-Pas de Calais et plus particulièrement, dans l'ancien Bassin Minier caractérisé par un important passé industriel et une forte densité de population. Dans cette région, l'une des préoccupations majeures concerne la gestion durable des sols contaminés par des ETM dans des écosystèmes fortement anthropisés. Cette problématique s'inscrit dans les thématiques développées par l'Equipe Sols et Environnement (ESE) du Laboratoire Génie Civil et géoEnvironnement (LGCgE). Les travaux de cette thèse s'intègrent dans un programme pluridisciplinaire intitulé « PHYTENER » coordonné par l'ESE-LGCgE et soutenu par l'ADEME (2009-2015). Ce programme a pour objectif de développer le phytomanagement sur les sols fortement contaminés par les ETM en y intégrant la production de cultures énergétiques. Ce faisant, il porte sur la gestion durable de parcelles agricoles affectées par les émissions atmosphériques passées de l'ancienne fonderie de plomb Metaleurop Nord installée à Noyelles Godault sur une durée de plus d'un siècle jusqu'à sa fermeture en 2003. Il s'agit de contribuer à la reconversion d'une agriculture fortement pénalisée par la contamination des sols en utilisant des techniques respectueuses de l'environnement pour réduire les dangers associés à la production sur ces terres de denrées destinées à l'alimentation. Ainsi, les aspects développés dans ce programme intègrent l'évaluation de la viabilité écologique et de l'intérêt socio-économique de modes de gestion basés sur l'utilisation de végétaux pour réduire la mobilité des contaminants dans les sols et dans la biosphère en général, et sur la valorisation de la biomasse à des fins énergétiques essentiellement. Deux modes de gestion reposant sur la phytostabilisation assistée ou pas ont été évalués; deux filières de production de biomasses non alimentaires ont été étudiées : le bois (*Robinia pseudoacacia*, *Alnus glutinosa*, *Quercus robur*, *Acer pseudoplatanus)* et une végétation herbacée (*Miscanthus* × *giganteus*).

Le dispositif relatif à la filière boisée a été mis en place en 2000. Les espèces choisies pour l'expérimentation sont connues pour leur tolérance à une contamination en métaux des sols, leur capacité à limiter d'une part, la mobilité de ces éléments notamment le plomb (Pb), le zinc (Zn) et le cadmium (Cd) dans le sol et d'autre part, leur transfert vers les organes aériens (thèse de Bidar, 2007). L'apport d'amendements minéraux (cendres silicatées et sulfo-calciques), en vue d'une phytostabilisation aidée, a été effectué pour accroître

l'immobilisation des ETM et favoriser le développement de la biomasse en améliorant les paramètres physico-chimiques des sols (thèse de Lopareva-Pohu, 2011).

Les travaux de la présente thèse portent sur l'étude des potentialités de *Miscanthus* × *giganteus*, à des fins de production d'une biomasse et sur les effets de cette plante et des pratiques agronomiques sur les paramètres physico-chimiques des sols lesquels influencent le fonctionnement de ceux-ci. Dans une perspective de gestion durable, l'objectif spécifique de la thèse est d'étudier le transfert de Cd, Pb et Zn du sol à la plante modèle et les facteurs susceptibles d'influer sur le comportement des métaux et le fonctionnement des sols dans une plantation de *M.* × *giganteus*. Les facteurs retenus incluent notamment l'origine du cultivar, des pratiques agronomiques (mode et densité de plantation) et des amendements biologique (utilisation d'un inoculum endomycorhizien) et chimique (fertilisation azotée). La recherche s'appuie principalement sur un dispositif expérimental *in situ* localisé aux alentours de l'ancienne fonderie qui a été mis en place en 2007 et 2010 dans le cadre de PHYTENER. Ce dispositif comprend également une parcelle considérée comme peu affectée par les activités humaines. En parallèle, un dispositif *ex situ* a été mis en place dans des conditions semi-contrôlées et ceci, dans l'objectif de confronter les résultats des deux dispositifs et de faciliter une meilleure interprétation des acquis de notre approche expérimentale.

Introduction générale

1. Contexte

La contamination des sols par les éléments organiques et inorganiques constitue un risque sanitaire et environnemental dans le monde (Kabir et al., 2012; Su et al., 2014). L'Agence Européenne pour l'Environnement (AEE) estime à environ 3 millions le nombre de sites potentiellement contaminés dans l'Union Européenne des 27 et à 250 000 le nombre de sites qui nécessitent des mesures urgentes de réhabilitation (EC, 2013; Panagos et al., 2013). Les éléments trace métalliques (ETM) sont les contaminants majoritairement présents dans les sols et les eaux souterraines (37 %). Parmi ces contaminants majeurs, le plomb (Pb), le chrome (Cr), le cuivre (Cu), le mercure (Hg), le nickel (Ni) et le zinc (Zn) sont les plus fréquents dans les écosystèmes terrestres (EC, 2013). Il convient de noter que dans les conditions naturelles, leurs teneurs dans la composition du substrat géologique ne dépassent pas 0,1 % ou 1 g kg^{-1} d'où l'appellation « éléments traces métalliques » (Baize, 2009). En Chine et en Afrique, la superficie des terres contaminées par ces éléments est estimée à 8 100 000 et 12 000 000 ha respectivement (Evangelou et al., 2012).

Dans la plupart des pays industrialisés, notamment sur le continent européen, la contamination des sols est associée à un passé industriel (EC, 2013; Panagos et al., 2013). Partout dans le monde, les activités industrielles, minières, l'agriculture et le transport ont été et sont les sources majeures de contamination en ETM de divers compartiments des écosystèmes terrestres et aquatiques (**Fig. 0.1**). La contamination des sols est en général liée aux dépôts atmosphériques résultant de différentes sources dont principalement les activités industrielles. Elle est due aussi à certaines pratiques culturales et au recyclage inadéquat de déchets et d'eaux usées (Belluck et al., 2006). A long terme, l'utilisation intensive d'intrants (engrais phosphatés, lisiers, produits phytosanitaires, déchets domestiques, boues de stations d'épuration) contenant des éléments traces conduit à l'enrichissement et à la contamination des sols en ces éléments (He et al., 2005; Luo et al., 2009).

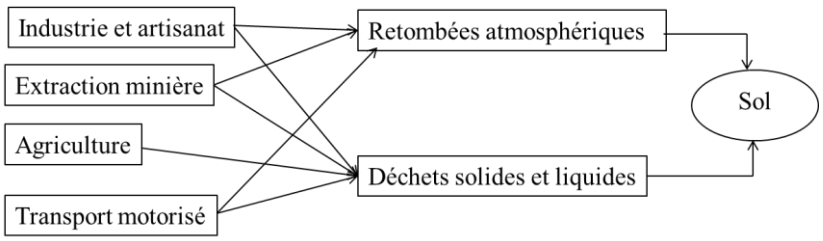

Fig. 0.1. Les principales sources de contaminants métalliques du sol.

Les sols contaminés et/ou pollués présentent un risque réel ou potentiel pour l'environnement et pour la santé humaine en fonction des usages qui en sont faits (Maldeveanu, 2014). Selon l'exposition et la topographie du site, les polluants ou contaminants peuvent se disperser sous l'effet du vent (envols de poussières) ou sous l'effet des eaux (percolation dans le sol, ruissellement à la surface du sol) (He et al., 2005; Carrillo-Gonzalez et al., 2006).

L'exposition excessive aux ETM via les différentes voies (solution du sol, inhalation, ingestion, transfert dans la chaîne alimentaire) engendre des toxicités sur les récepteurs biologiques (organismes vivants) terrestres et aquatiques (Tang et al., 2009; EC, 2013; Su et al., 2014). Certains éléments tels que le Ni, Cu et Zn ont des fonctions biologiques connues, et sont toxiques à une forte concentration (Duruibe et al., 2007). D'autres, tels que le Cd, Hg et Pb n'ont aucune fonction biologique connue et sont toxiques à des concentrations beaucoup plus faibles. Chez l'homme, l'intoxication par les ETM peut causer des maladies neurologiques et des cancers (Bernard, 2008; Mudgal et al., 2010). Les cas emblématiques des maladies de Minamata (due au Hg) et Itai-Itai (due au Cd) au Japon démontrent bien les dangers sanitaires que peuvent engendrer les flux des contaminants non-contrôlés (Kaji, 2012; EHSD, 2013).

1.1. Options de gestion des sols contaminés

La majorité des sols très contaminés par des ETM correspondent à des friches industrielles et aux espaces localisés aux alentours de sites industriels (EC, 2013). Il peut s'agir de sols boisés, de sols résidentiels, de sols récréatifs (aires de jeux, pelouses…), de sols utilisés à des fins de productions alimentaires (potagers, parcelles agricoles labourées, prairies). Sans aucune mesure de remédiation ou de requalification, ces sols peuvent représenter des dangers sanitaires et environnementaux majeurs. En effet, les ETM présentent la particularité de ne pas se dégrader et donc de s'accumuler dans les sols. Par conséquent, un manque de gestion spécifique peut conduire non seulement au dysfonctionnement du sol et à la dispersion spatiale des polluants, mais aussi constituer des dangers pour la santé humaine. Dans la plupart des pays, il appartient aux services publics de mener les actions et/ou d'impliquer les acteurs dans la gestion des pollutions historiques liées aux activités industrielles et de prévenir les pollutions futures (Chen, 1998; Freier and Grimski, 1998; Belluck, et al., 2006; Pavel and Gavrilescu, 2008).

Les techniques de dépollution varient selon les polluants, la nature et l'étendue du milieu pollué, et l'objectif visé en lien avec les usages des sols (Pavel and Gavrilescu, 2008;

CGDD, 2013). Pour les fortes contaminations en ETM dans le sol, l'excavation, le confinement et leur remplacement par des sols «sains» sont les techniques les plus souvent utilisées pour de petites superficies ou quand les mesures de dépollution s'avèrent très urgentes (Yao et al., 2012). Cependant, si ces techniques ont l'avantage d'être rapides et efficaces en termes de traitement de la pollution, elles ont aussi l'inconvénient d'être relativement coûteuses. Elles posent aussi la question de l'approvisionnement en terres non-contaminées et de la gestion des terres contaminées. D'autres techniques, notamment celles reposant sur l'usage de produits chimiques, peuvent conduire à une dégradation des paramètres physico-chimiques du sol. A l'opposé, les techniques douces de remédiation, reposant sur l'utilisation de plantes et les microorganismes associés, sont connues pour améliorer le fonctionnement des sols. Au cours de ces vingt dernières années, cette technique communément appelée « phyoremédiation » est préférée aux techniques physico-chimiques non respectueuses de l'environnement. Les termes « phytotechnologies » et « techniques douces de remédiation-*Gentle remediation options* » sont couramment utilisés pour désigner l'application des sciences et de l'ingénierie pour offrir des solutions basées sur la phytoremédiation (Conesa et al., 2012; Henry et al., 2013; Kumpiene et al., 2014). Celle-ci prend donc diverses formes comme indiquées ci-dessous (**Fig. 0.2;** Favas et al., 2014).

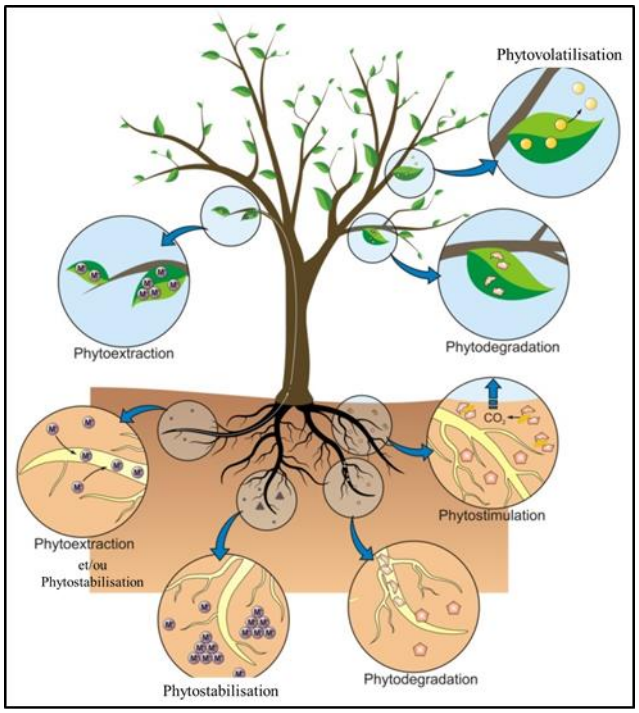

Fig. 0.2. Différentes formes de phytoremédiation du sol (Favas et al., 2014).

La phytodégradation est la dégradation et la transformation de substances toxiques sous les formes moins ou non-toxiques par voies métaboliques dans les tissus des plantes et/ou par les microorganismes de la rhizosphere associés à la plante. Dans la rhizosphère, les plantes peuvent stimuler les activités des microorganismes favorables à la dégradation de certains polluants, d'où l'utilisation du terme de phytostimulation. Les produits issus de la dégradation peuvent être incorporés dans des processus métaboliques ou stockés dans les tissus végétaux. Ils peuvent aussi être volatilisés au niveau des organes aériens. Dans ce cas, on parlera de la phytovolatilisation. La phytodégradation convient aux contaminants organiques qui sont, de par leur nature, potentiellement dégradables par voies enzymatiques et physico-chimiques, contrairement aux ETM qui sont non-dégradables. Mis à part le Hg et les métalloïdes tels que le sélénium (Se) et l'arsenic (As) qui peuvent être phytovolatilisés, aucun de ces trois processus ne permet la remédiation de la contamination et/ou pollution due aux ETM. Par conséquent, la phytostabilisation et la phytoextraction peuvent être utilisées.

La phytoextraction est l'extraction des contaminants par la culture des espèces/variétés végétales. Les plantes utilisées doivent être capables de transférer et de concentrer une grande partie des contaminants dans les tissus des organes aériens récoltables. Cependant, la forte contamination peut induire un stress, réduire la croissance et par conséquent, la production de la biomasse aérienne et/ou récoltable (Baker, 1987; Mendez and Maier, 2008). La plupart des plantes accumulatrices produisent peu de biomasse aérienne, ce qui induit un faible rendement et allonge énormément le processus de phytoextraction (Mendez and Maier, 2008). De plus, l'accumulation est spécifique, d'où la faible applicabilité de la phytoextraction sur les sols/matrices multi-contaminés. La phytoextraction reste donc une technique de décontamination et de dépollution partielle visant à réduire la concentration des contaminants dans le sol. Il s'agit plutôt de réduire, sur le long terme et via le prélèvement par la plante, la fraction disponible des contaminants dans les sols; l'élimination complète des contaminants est souvent impossible.

La phytostabilisation consiste en l'utilisation de plantes pour réduire la mobilité des contaminants dans le sol et leur transfert dans les différents compartiments de l'écosystème dont la biosphère. Les ETM sont immobilisés dans les organes souterrains ou tout simplement dans la rhizosphere. L'immobilisation est basée sur la sorption des contaminants sur les surfaces minérales ou organiques présentes, sur l'adsorption sur les surfaces racinaires, et l'absorption dans les racines et le stockage dans les structures permettant l'isolation des contaminants. De plus, un bon développement du système racinaire permet de stabiliser le sol

et de réduire les transferts des contaminants qu'ils soient horizontaux en limitant leur ruissellement en surface du sol et verticaux en diminuant la percolation des eaux. Il convient de souligner que cette option de gestion n'est pas une technique de dépollution. Elle permet toutefois de maîtriser les dangers présentés par le sol pollué via la stabilisation des contaminants dans le sol.

Quelle que soit la forme de la phytoremédiation, l'installation d'un couvert végétal est connue pour limiter la dispersion des ETM dans l'environnement en protégeant les sols contaminés contre l'érosion hydrique et éolienne, ainsi que pour réduire le transfert des contaminants vers les eaux souterraines (Mendez and Maier, 2008; Henry et al., 2013). Compte tenu des objectifs visés, il convient de noter que des amendements biologiques (bactériens et fongiques) et physico-chimiques apportés au système sol-plante peuvent améliorer l'efficacité des processus. Dans ce cas, on parle de la phytoremédiation aidée ou assistée. Par exemple, pour la phytostabilisation, l'effet « immobilisant » des végétaux sur les ETM du sol peut être renforcé en utilisant des amendements minéraux ou organo-minéraux capables de réduire la mobilité et la phytodisponibilité de ces contaminants. L'association des deux techniques est connue sous le terme de phytostabilisation aidée (Vangronsveld et al., 2009; Mench et al., 2010; Pourrut et al., 2011). Les amendements chimiques comprenant les matériaux alcalins, les matières organiques, les matériaux apportant des oxydes métalliques et des alumino-silicates améliorent l'efficacité de la phytostabilisation (Mench et al., 2010).

1.2. Choix des plantes pour la phytoremédiation

Selon sa nature et sa concentration, la contamination en ETM peut induire un stress et une toxicité chez les plantes (Hossain et al., 2012; DalCorso et al., 2013). Ainsi, les plantes sont choisies selon leur capacité à se développer sur les sols contaminés. Ceci requiert une tolérance non seulement à la pollution mais aussi, aux conditions pédoclimatiques du site (Mench et al., 2010). La tolérance peut être inhérente à la plante ou peut être acquise grâce à l'association entre la plante et les microorganismes bactériens ou fongiques, eux-mêmes tolérants à la toxicité induite par les ETM (Göhre and Paszkowski, 2006; He et al., 2013; Sessitsch et al., 2013). En effet, ces deux types de microorganismes, surtout les champignons mycorhiziens arbusculaires, peuvent diminuer la toxicité via l'accumulation d'une partie des ETM dans leurs hyphes (Joner et al., 2000; Pal et al., 2010).

Selon les espèces, les variétés et les processus impliqués dans la rhizosphère (mobilisation ou immobilisation des ETM dans les sols), différents mécanismes de tolérance aux contaminants

métalliques peuvent être observés chez les plantes. Trois grands types de plantes sont distingués (**Fig. 0.3**; Baker, 1981; Tlustoš et al., 2006) :

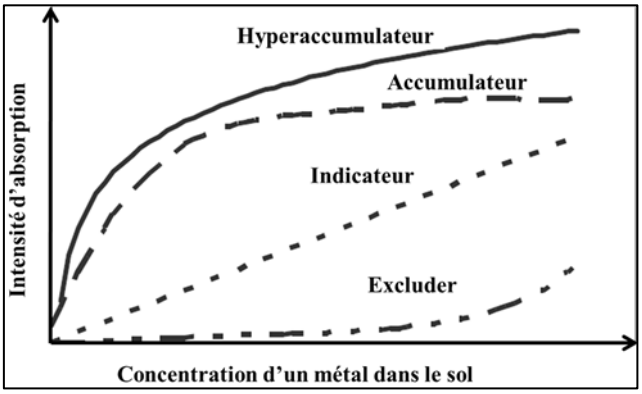

Fig. 0.3. Comportement des plantes face à l'exposition aux ETM dans le sol (Tlustoš et al., 2006).

- les *excluders* qui réduisent l'absorption et l'accumulation des ETM en limitant leur transport et leur translocation des racines vers les organes aériens. Ces plantes aboutissent à immobiliser les ETM dans les racines et à diminuer ainsi le transfert vers les parties aériennes;
- les *accumulateurs* qui sont capables d'absorber et de concentrer les ETM dans leurs organes aériens. Ces plantes ont développé des mécanismes complexes pour tolérer, absorber, transporter et stocker des quantités significatives de contaminants dans ces organes. Sur des sols contaminés, ces plantes sont généralement caractérisées par un facteur de bioconcentration (ratio entre les concentrations d'un ETM dans l'organe de la plante sur sa concentration dans le sol) et un facteur de translocation (ratio entre les concentrations d'un ETM dans les organes aériens de la plante sur sa concentration dans les organes souterrains) qui sont supérieurs à 1 (Mench et al., 2010). En référence aux concentrations fréquemment rencontrées dans les feuilles de plante « standard » ou non accumulatrice (0,05, 0,2, 1, 1,5, 10, 50 mg kg^{-1} MS pour Cd, Co, Pb, Cr/Ni, Cu et Zn respectivement), certaines plantes nommées « hyperaccumulateurs » présentent des concentrations pouvant être 100 à 1000 fois supérieures. L'hyperaccumulation des ETM se trouve chez un petit nombre de plantes (environ 450 à 500 espèces) dont la majorité est inféodée aux sols métallifères ou ultramafiques (Reeves, 2006; Rascio and Navari-Izzo, 2011; van der Ent et al., 2013);
- les *indicateurs* dont l'accumulation des ETM dans les organes est fonction du degré de contamination de leur milieu de croissance. Le stade de développement de la plante ou

ses organes, l'intensité de l'exposition et la disponibilité en eau sont autant de facteurs clés qui modulent le transfert et les concentrations des polluants chez les indicateurs.

Le choix d'un de ces types de plantes dépend de la technique de phytoremédiation visée. Ainsi, on choisira les excluders pour la phytostabilisation et les accumulateurs pour la phytoextraction. De plus, à part la tolérance aux contaminants présents dans le sol, les espèces ou variétés végétales choisies doivent présenter des caractéristiques leur permettant de se développer et de produire une biomasse non négligeable dans les conditions pédoclimatiques du site concerné (Mench et al., 2010). Ainsi, est-il important d'étudier le comportement de la plante choisie face à l'exposition de contaminants. En effet, selon les stratégies des plantes, il existe un compromis entre la croissance ou allocation de la biomasse et la tolérance aux ETM (Audet and Charest, 2008; Maestri et al., 2010). La plupart des plantes (hyper)-accumulatrices produisent peu de biomasse aérienne, ce qui induit un faible rendement et allonge énormément le processus de phytoextraction (Mendez and Maier, 2008). De même, les plantes excluders investissent plus dans la croissance des organes souterrains afin de limiter le transfert des ETM dans les parties aériennes (Audet and Charest, 2008). Cependant, certaines plantes, notamment celles à croissance pérenne, produisent une biomasse aérienne très importante malgré la forte contamination du sol et/ou des concentrations relativement faibles dans les parties aériennes. Chez ces plantes pérennes, un bon développement des organes souterrains permet, à long terme, d'accroitre la capacité d'accumulation et de séquestration des ETM dans la rhizosphère. Dans cette zone, la faible mobilité et solubilité des contaminants est due à des modifications importantes affectant des paramètres physico-chimiques du sol. Les modifications sont essentiellement dues à l'accumulation de la matière organique dans le sol et au développement des microorganismes (bactériens et fongiques) tolérants aux ETM et qui interviennent énormément dans la nutrition minérale de la plante (Göhre and Paszkowski, 2006; Kidd et al., 2009).

Une étude de la distribution de contaminants dans les différentes parties de la plante permet d'évaluer l'option de phytoremédiation. Cependant, même si la mise en œuvre de chacune des techniques de phytoremédiation a ses avantages et ses inconvénients (Mendez and Maier, 2008; Mench et al., 2010), l'optimisation des avantages découle de l'intérêt économique et écologique des espèces choisies. La considération de ce point dans le choix de l'option retenue s'inscrit dans la gestion durable des sites et sols contaminés, ce qui fait l'objet du phytomanagement (Conesa et al., 2012).

1.3. Objectif du phytomanagement

Dans le cadre du phytomanagement, les phytotechnologies sont choisies et évaluées en tenant compte non seulement de leur efficacité de remédiation, mais aussi en y intégrant un volet socio-économique (**Fig. 0.4**). Il ne suffit pas seulement de réhabiliter le sol, mais aussi de lui trouver des usages qui répondent aux attentes sociétales, voire économiques. Les objectifs primordiaux sont 1) d'assainir les matrices contaminées dont les eaux, les effluents, les sols, les sédiments, les déchets miniers et l'air (Nsanganwimana et al., 2014), 2) d'améliorer la sécurité des aliments (Singh et al., 2011), 3) de rétablir les services écosystémiques et 4) de répondre à des besoins économiques avec la production de matières premières renouvelables à usages industriels et à faible impact sur les émissions de gaz à effet de serre (Conesa et al., 2012; Evangelou et al., 2012). Un des objectifs du phytomanagement peut être la production de biomasses pour des applications industrielles liées au développement durable avec un approvisionnement de matières premières. En effet, la biomasse issue des terres marginales et/ou contaminées peut être valorisée dans de nombreux domaines dont la production des énergies renouvelables (les biocarburants) et divers éco-matériaux. En produisant la biomasse sur ces sols contaminés et marginaux, dans le sens où ces terres ne conviennent pas à la production alimentaire, le phytomanagement permet d'une part, d'éviter la controverse liée à la production de bioenergies sur des terres à vocation alimentaire et d'autre part, d'accroître la superficie des terres dédiées à la production de biomasse (Nsanganwimana et al., 2014). Ceci est d'autant plus vrai dans les régions à forte démographie et connaissant une forte pression foncière. Ce faisant, le phytomanagement est respectueux de l'environnement; il est bien perçu par les populations car il tient compte à la fois des attentes environnementales, sociétales, économiques et peut constituer un mode de gestion durable intégrant une viabilité écologique et économique.

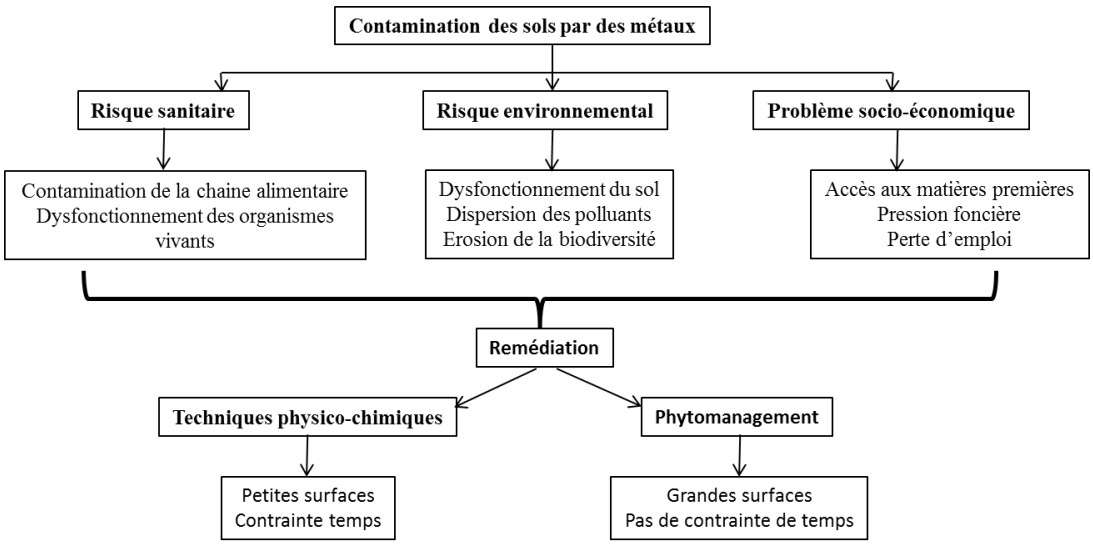

Fig. 0.4. Cadre conceptuel du phytomanagement.

1.4. Contexte national de la contamination des sols

En France, le nombre actuel de sites contaminés recensés s'élève à 4 772 (CGDD, 2013). Les anciennes régions industrielles, minières et les zones fortement urbanisées concentrent la majorité des sites et sols pollués **(Fig. 0.5)**. C'est notamment le cas en régions Rhône-Alpes (601 sites, 14,5 %), Nord-Pas de Calais (571 sites, 13,8 %) et en Ile de France (429 sites, 10,4 %) qui comptent à elles-seules 40 % des sites et sols polluées, soient 1601 sites. Parmi les ETM les plus fréquemment détectés, Pb est présent dans 15 % des sites contaminés, suivi par Cu (12 %), Cr (12 %) et As (11 %).

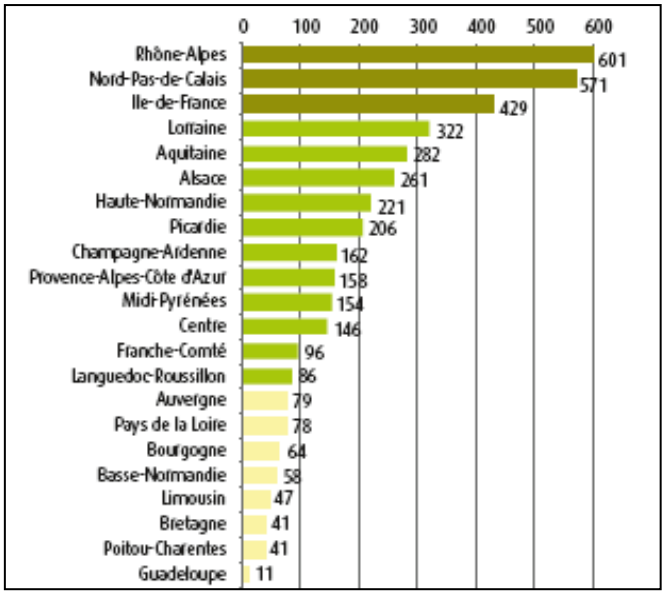

Fig. 0.5. Nombre de sites et sols pollués recensés en France métropole jusqu'à la fin de 2012 (CGDD, 2013).

En Région Nord-Pas de Calais, qui est la deuxième région ayant le plus grand nombre de sites contaminés en France, les sols ont été affectés par le passé par les activités minières, les industries chimiques ainsi que par les anciennes fonderies de métaux, notamment de plomb et de zinc. La plupart des sites contaminés se trouve dans l'ancien Bassin Minier et/ou aux abords des installations industrielles. C'est le cas du site atelier « Metaleurop » avec une superficie qui est estimée à 120 km^2 (**Fig. 0.6;** http://www.safir-network.com/site_metaleurop.html).

Les activités de la fonderie de plomb Metaleurop Nord, installée à Noyelles Godault durant plus d'un siècle, et de l'usine métallurgique Nyrstar, qui est toujours en fonctionnement à ce jour, ont rejeté dans le passé des quantités considérables de poussières contaminées dans l'atmosphère. Ceci a entrainé une forte contamination des sols en Cd, Pb et Zn et dans un moindre degré, en d'autres ETM (Hg, Se, As…) (Frangi and Richard, 1997; Sterckeman et al., 2000; Sterckeman et al., 2002).

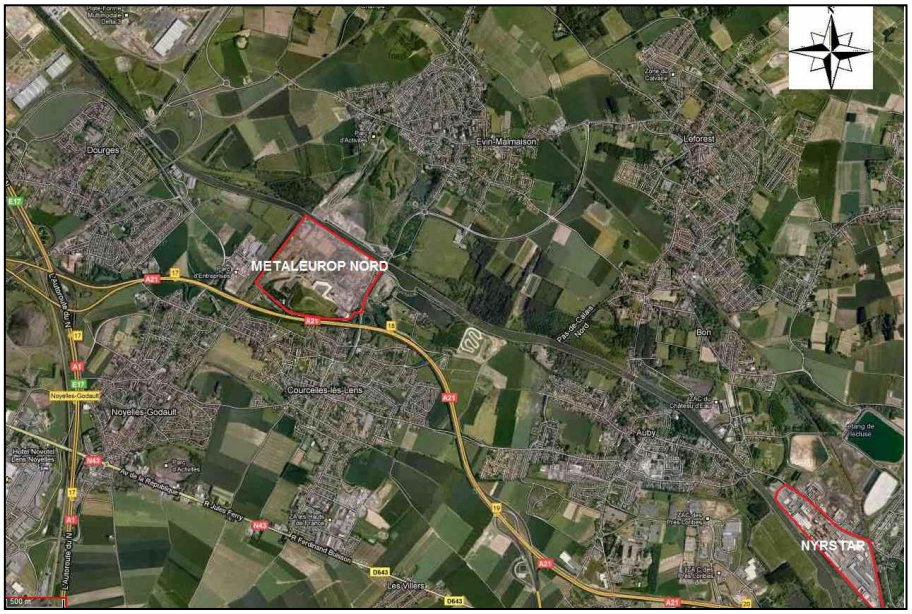

Fig. 0.6. Vue aérienne du site atelier « Metaleurop ».

Les sols sont contaminés à des degrés divers selon leur distance aux sources de contamination (**Fig. 0.7**). Les horizons labourés (0 - 25 cm) des sols agricoles les plus proches de l'ancienne fonderie présentent des concentrations en Cd, Pb et Zn respectivement 20 à 50 fois supérieures aux teneurs agricoles habituelles (TAH) régionales (Sterckeman et al., 2002).

Les productions agricoles végétales obtenues sur ces sols présentent très souvent des concentrations en Pb et Cd qui dépassent les valeurs réglementaires en vigueur pour

l'alimentation humaine et animale (Pruvot et al., 2006; Douay et al., 2013). La population vivant sur ce vaste territoire est exposée à de multiples voies de contamination. Ainsi, à la contamination directe liée à l'ingestion et à l'inhalation de poussières contaminées, s'ajoute une exposition en lien avec un transfert des ETM dans la chaîne alimentaire. Ces constats découlent de nombreux rapports et études menés sur les voies d'expositions, la mobilité et bioaccessibilité des métaux (Cd, Pb et Zn) dans les sols ainsi que sur la qualité des productions végétales issues des exploitations agricoles et des très nombreux potagers (Mazzuca et al., 2005; Douay et al., 2013; Pelfrêne et al., 2013).

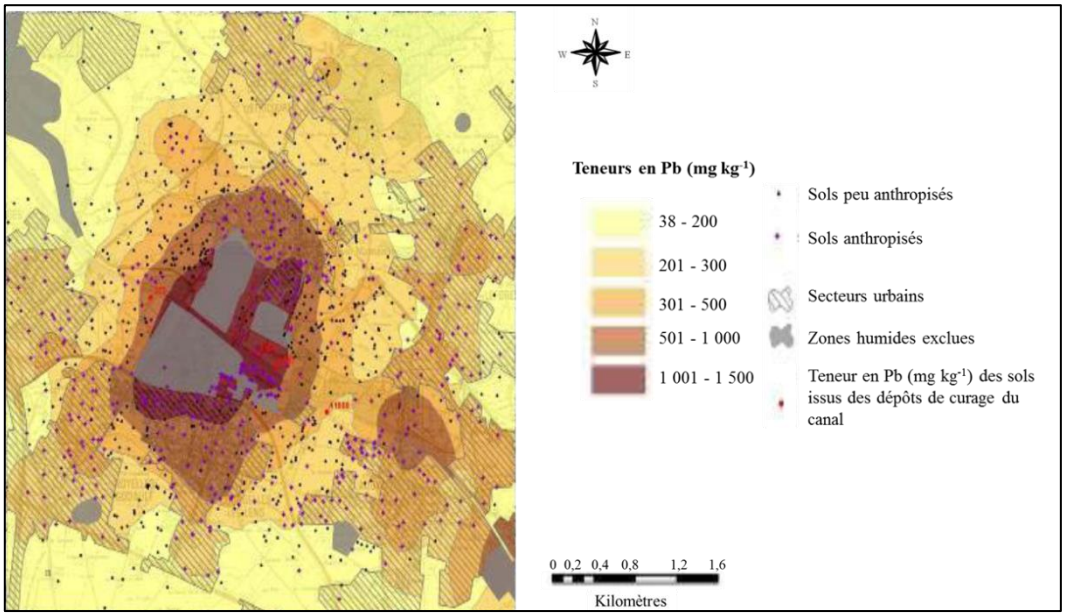

Fig. 0.7. Carte d'isoconcentration en Pb des sols (0 - 25 cm) du site atelier « Metaleurop ».

1.5. Gestion des sols agricoles du site atelier « Metaleurop »

Sur le site atelier « Metaleurop », la surface des terres agricoles contaminées à plus de 200 mg de Pb kg^{-1} dans l'horizon labouré est estimée à environ 750 hectares. Ceux-ci concernent à divers degrés 36 exploitations (Douay, 2012). Les techniques classiques de remédiation (décapage et remplacement des terres contaminées) ne conviennent pas; elles seraient inappropriées, coûteuses et difficiles à mettre en œuvre (Douay et al., 2008). En revanche, les techniques privilégiant l'utilisation des végétaux sont envisageables. En 1996, l'usine Metaleurop Nord a entrepris la création d'une ceinture verte constituée par des plantations arborées sur les sols les plus contaminés (> 500 mg de Pb kg^{-1}). La démarche prolongée par l'ADEME quelques années après la liquidation de l'usine en 2003 avait pour objectif d'exclure de la production agricole les terres les plus contaminées et de créer un écran naturel

entre le site industriel et les communes voisines. Parallèlement, un dispositif expérimental a été mis en place en 2000, sur une ancienne parcelle agricole située à Evin-Malmaison, dans le cadre d'un partenariat industriel (Metaleurop Nord, EDF, Surschiste, Apinor), institutionnel (Etablissement Foncier Nord-Pas de Calais) et universitaire (ISA Lille, Lille 1) afin d'évaluer les effets de ce mode de gestion sur le comportement des ETM et les paramètres physico-chimiques des sols. Ce fut le cadre de deux thèses d'Université. L'une, a porté sur l'étude du transfert des métaux (Cd, Pb et Zn) du sol vers la végétation herbacée (*Lolium perenne* et *Trifolium repens*) et vers les feuilles et les brindilles des cinq essences présentes (le robinier faux-acacia-*Robinia pseudoacacia*, l'aulne-*Alnus glutinosa*, le chêne pédonculé-*Quercus robur*, l'érable-*Acer pseudoplatanus* et le saule blanc-*Salix alba*) sur le dispositif expérimental (Bidar, 2007). Pour l'aulne, le robinier faux-acacia et l'érable, il a été montré leur capacité à tolérer la forte contamination métallique des sols et le faible transfert de Pb, Cd et Zn du sol vers les organes aériens. La seconde thèse a étudié les effets de deux amendements minéraux (cendres sulfo-calciques et silico-alumineuses) sur le comportement des métaux, les paramètres physico-chimiques des sols et les plantes (Lopareva-Pohu, 2011). Les deux thèses ont démontré la capacité de phytostabilisation de Cd, Pb et Zn dans les sols du dispositif expérimental et concluent qu'une production de biomasse non-alimentaire peut être développée sur les sols agricoles fortement contaminés du site atelier « Metaleurop ».

La biomasse produite peut être destinée à la production énergétique ou utilisée comme biomatériaux. Ce mode de gestion permet de développer une activité économique et d'assurer un revenu aux agriculteurs. Cependant, si les essences arborées retenues répondent aux critères de la phytostabilisation, l'absence de revenu saisonnier ou annuel peut constituer un frein pour les gestionnaires et notamment, les agriculteurs. En effet, selon les essences, la récolte de biomasse demande au moins 3 à 6 ans pour les taillis à très courte rotation, et 6 à 12 ans pour les taillis à courte rotation. Pour pallier à ce défaut, les recherches menées au sein du laboratoire ont été orientées vers une graminée pérenne, miscanthus (*Miscanthus* × *giganteus*).

1.6. Caractéristiques du miscanthus et ses atouts pour le phytomanagement.

D'une hauteur moyenne de 2 à 3 mètres, le miscanthus ressemble visuellement beaucoup à la canne à sucre (**Fig. 0.8A**). Il possède des rhizomes qui sont les organes de réserve d'éléments nutritifs (**Fig. 0.8B).**

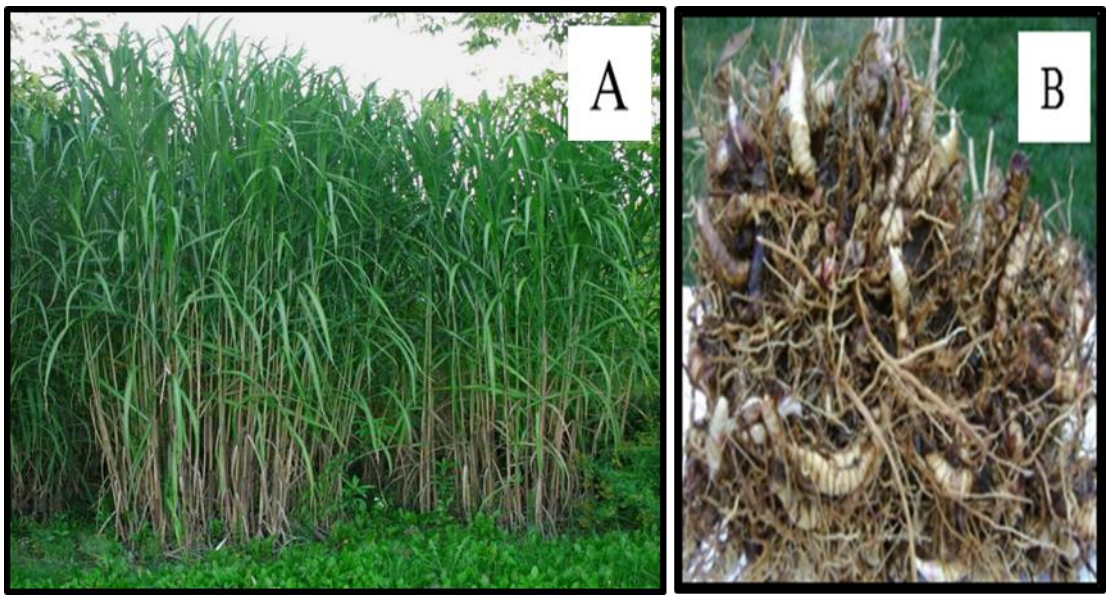

Fig. 0.8. Miscanthus. **A**) physionomie aérienne, **B**) rhizomes.

Cette plante, d'origine tropicale, est un hybride résultant du croisement entre *Miscanthus sinensis* et *Miscanthus sacchariflorus* (**Fig. 0.9**). Elle est cultivée à des fins commerciales en Europe depuis les années 1990, avec un rendement moyen de 15 à 30 t MS ha^{-1} an^{-1} pour des plantations agées de plus de 3 à 5 ans (Heaton et al., 2008).

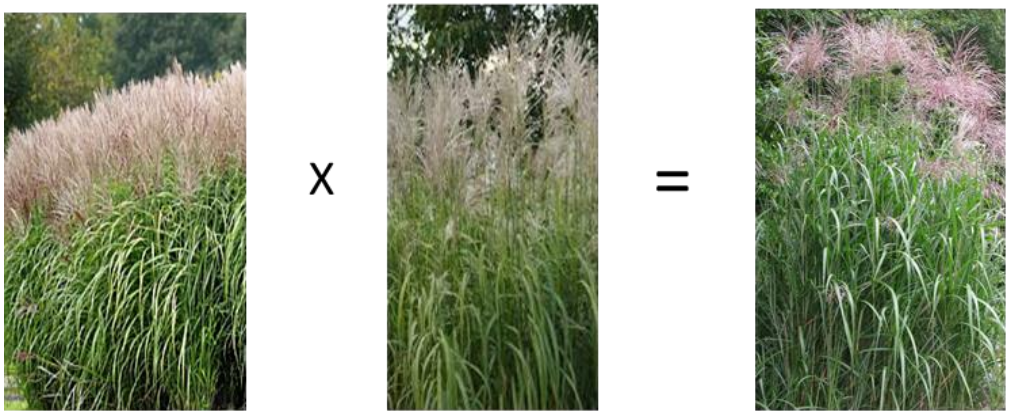

Fig. 0.9. Origine parentale de *Miscanthus* × *giganteus.*

Comme ses parents, *M.* × *giganteus* est une plante pérenne et rhizomateuse dont les parties aériennes poussent à partir des rhizomes. Dans les régions tempérées, le cycle de croissance ou de developpment de cette plante suit la succession des saisons (**Fig. 0.10**). Des pousses commencent à émerger du sol dès le debut du printemps. C'est à cette période qu'est faite la mise en place des plantations au moyen de rhizomes ou de plantules (plants pré-démarrés). La date d'implantation optimale se situe dans la deuxième moitié d'avril afin d'éviter les gélées

tardives et améliorer le taux de levée. La densité de plantation conseillée est de 2 plants m^{-2}. Miscanthus talle après la levée, notamment à partir de la deuxième année, ce qui permet de produire de multiples tiges par plante (ou pied) à partir de la plantule initiale, formant ainsi des touffes denses. En géneral, le nombre de pousses augmente rapidement au cours des mois de mai, juin et juillet. Les mois d'août, septembre et voire même octobre, correspondent à la croissance et donc à la production progressive de la biomasse aérienne.

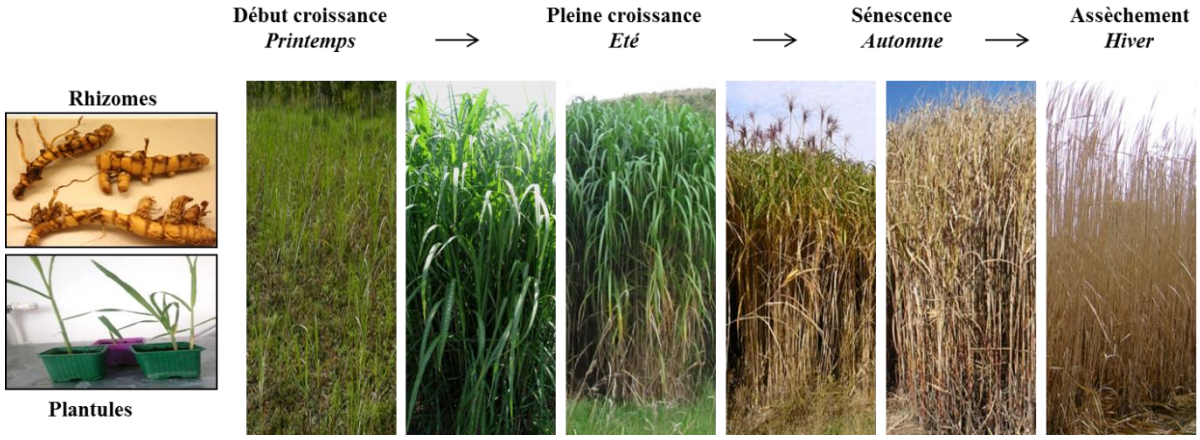

Fig. 0.10. Cycle de croissance et développement de miscanthus.

La fin de la croissance coïncide avec une baisse de température. La sénescence des feuilles est progressive commençant par des feuilles les plus agées. Pendant la senescence, certains nutriments sont remobilisés à partir des parties aériennes vers les rhizomes. La sénescence complète des parties aériennes se produit à partir des premières gélées. Les feuilles sèchent très vite et se détachent des tiges, constituant ainsi un mulch qui couvre entièrement le sol. Les tiges séchent progressivement pendant l'hiver.

Au regard de ce cycle de croissance annuel, la récolte de la biomasse aérienne de miscanthus peut se faire à deux periodes. En effet, la récolte peut se réaliser soit dès la fin de la croissance (mi-octobre à mi-décembre) ou pendant l'hiver. La première periode de récolte, encore appelée « récolte en vert » ou « récolte précoce » permet d'obtenir le maximum de biomasse car la plante contient encore toutes les tiges et une biomasse importante des feuilles. En revanche, cette biomasse contient beaucoup d'humidité (> 50 %), ce qui augmente le coût de séchage avant l'utilisation à des fins énergétiques. Avec la récolte en hiver, encore appelée « récolte en sec » ou « récolte tardive », on obtient un rendement moindre dû à la chute des feuilles. Cependant, cette récolte présente l'avantage d'obtenir une biomasse moins humide. Les feuilles tombées au sol constituent une litière (mulch) qui pourra enrichir le sol en matière organique et en élements nutritifs. Aussi, ce mulch inhibe la pousse des advanetices et protège le sol contre l'érosion hydrique et éolienne.

A la différence de ses parents, *Miscanthus* × *giganteus* se caractérise par un faible potentiel invasif car la multiplication des individus se fait uniquement par reproduction asexuée au moyen de rhizomes. En effet, à ce jour, il n'existe pas de données qui montrent sa dispersion à partir des plantations, même dans les pays européens tels que le Royaume Uni, l'Allemagne et l'Italie où des plantations sont établies depuis 20 ans.

Le miscanthus est bien perçu par la population locale grâce à des opportunités ou impacts économiques et environnementaux avérés et attendus, lesquels sont associés à une biomasse à usage multiple : chauffage, compostage, raffinerie (production de biofuel pour bioéthanol et autres produits dérivés), litière pour animaux (cheval, volaille), paillage, biochar pour amendements de sols (ValBiom, 2009). De plus, sa forte productivité et son caractère pérenne (15 à 20 ans sans rotation) permettent une séquestration du carbone, ce qui correspond aux attentes environnementales en ce qui concerne l'abattement de CO_2 atmosphérique.

Parmi les cultures énergétiques en France, miscanthus représente 56 % de la surface dédiée, suivi par les Taillis Courte Rotation (TCR) de peupliers (*Populus sp.*, 14 %), de robiniers faux-acacias (*Robinia pseudoacacia*, 12 %), de saules (*Salix sp.*, 8 %), d'eucalyptus (*Eucalyptus sp.*, 5 %) et enfin, par la culture de panic érigé (*Panicum virgatum*, 5 %) (FranceAgriMer, 2012). Le rendement moyen d'une plantation âgée de 3 ans et plus est de 15 à 25 t MS ha^{-1} an^{-1} (Zub et al., 2011) et dépasse de loin celui d'autres graminées à vocation énergétique telles que le panic érigé (*P. virgatum*), le triticale (*Triticale sp*) et le sorgho (*Sorghum bicolor*) (Cadoux et al., 2014).

Parmi ces cultures énergétiques, les peupliers et les saules sont les plus utilisés ou étudiés pour le phytomanagement des sites contaminés par des ETM dans des régions à climat tempéré. La biomasse produite par ces arbres est utilisée en combustion (Witters et al., 2012; Delplanque et al., 2013). La forte productivité, un rendement annuel régulier et de nombreuses utilisations de la biomasse font du miscanthus un candidat plus avantageant que les essences arborées pour la gestion de ces sols. Cependant, à nos jours, il existe très peu d'études sur le comportement de cette plante face aux contaminants métalliques dans le sol. Ainsi, dans le cadre de cette thèse, une analyse bibliographique a été réalisée sur les espèces du genre *Miscanthus* les plus cultivées en vue de leur utilisation pour le phytomanagement d'anciens sites et friches industrielles contaminés (**chapitre I**). Ceci a permis d'orienter les travaux de recherche qui sont présentés dans ce mémoire.

2. Structure du mémoire

Pour faciliter la lecture et garder la cohérence entre les différents points développés, le contenu de ce travail est structuré en huit chapitres.

Le chapitre I dresse un aperçu des potentialités du genre *Miscanthus* pour le phytomanagement de sites et sols contaminés et dégradés, ainsi que la restauration des services écosystémiques sur ces milieux. C'est à partir de cette revue bibliographique que le choix de l'espèce *Miscanthus* × *giganteus* a été fait pour le phytomanagement du site et des sols qui font l'objet de notre étude.

Le chapitre II décrit les dispositifs expérimentaux mis en place pour apporter des réponses aux différentes questions soulevées par la problématique de la gestion des sols du site atelier « Metaleurop ».

Les chapitres III et IV concernent l'étude de l'accumulation des métaux (Cd, Pb et Zn) dans les organes de *M.* × *giganteus* et des effets de cette culture sur les paramètres physico-chimiques des sols et la mobilité des métaux dans les conditions *ex situ* (chapitre III) et *in situ* (chapitre IV). Malgré la similarité des objectifs, le choix a été fait de séparer ces deux chapitres car les données sont acquises dans les conditions différentes et à des pas de temps différents.

Les chapitres V, VI et VII mettent l'accent sur les facteurs influençant le transfert et/ou l'accumulation des métaux chez *M.* × *giganteus*, dont notamment les variations saisonnières (chapitre V), le stade de développement et/ou la dynamique de croissance (chapitre VI) et les pratiques culturales axées sur l'origine des cultivars, la densité de plantation et les amendements apportés au sol (chapitre VII).

Enfin, le chapitre VIII dresse une synthèse des principaux résultats obtenus et place ces derniers dans le contexte général et spécifique de ce travail. En outre, il propose les pistes de recherche pour les études ultérieures.

Chapitre I :

Potentialités du *Miscanthus* pour gérer des sites dégradés et/ou contaminés

Préambule

La forte contamination des sols par des substances inorganiques et/ou organiques constitue un danger majeur dans les pays industrialisés. Le phytomanagement est un mode de gestion utilisant des plantes tolérantes aux contaminants en vue d'une part, de réduire les risques environnementaux et sanitaires et d'autre part, de produire une biomasse répondant à des besoins sociétaux et/ou économiques. De par sa forte productivité et sa tolérance aux stress abiotique et biotique, *Miscanthus* pourrait répondre aux exigences du phytomanagement. Parmi les espèces de *Miscanthus*, *M.* × *giganteus*, un hybride issu du croisement entre *M. sinensis* et *M. sacchariflorus*, se distingue par une plus forte biomasse. Il est cultivé depuis longtemps dans les régions tempérées en Europe et en Amérique du Nord. Contrairement aux autres espèces dotées d'une reproduction sexuée, *M.* × *giganteus* présente un faible potentiel invasif car sa multiplication se fait essentiellement par les rhizomes. Sa biomasse riche en lignocellulose se prête à de nombreuses voies de valorisation, notamment en bioénergies et en production de matériaux biosourcés. Il existe toutefois peu de travaux sur l'utilisation du miscanthus pour le phytomanagement. Pourtant, de nombreux sites ayant eu une activité passée industrielle ou minière nécessitent d'être requalifiés et de retrouver une activité économique selon les attentes des gestionnaires et des populations. Il en est de même pour les sols agricoles sur lesquels ne peut être maintenue une production alimentaire au regard des valeurs réglementaires en vigueur. *Miscanthus* nécessite peu d'intrants et sa croissance pérenne permet non seulement un recyclage des nutriments dans le sol, mais aussi protège ce dernier contre l'érosion hydrique et éolienne. Ce chapitre dresse un état de l'art sur les potentialités du miscanthus pour le phytomanagement de sites dégradés et/ou contaminés, et propose des pistes de recherche afin d'optimiser le rendement.

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Suitability of *Miscanthus* species for managing inorganic and organic contaminated land and restoring ecosystem services. A review

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Abstract

The mitigation of potential health hazards and land scarcity due to land use change can be addressed by restoring functional and ecosystem services of contaminated land. Physico-chemical remediation options are criticized as being costly and not providing environment-friendly solutions. The use of plants and associated microorganisms could be a sustainable, cost-effective option to reduce pollutant exposure. Phytomanagement aims at using valuable non-food crops to alleviate environmental and health risks induced by pollutants, and at restoring ecosystem services. Suitable plant species must be tolerant to contaminants, reduce their transfer into the food chain, and efficiently produce marketable biomass. Based on *Miscanthus*' capacity to sequestrate inorganic contaminants into the root system and to induce dissipation of persistent organic contaminants in soil, these plant species are favorable for phytostabilization and phytodegradation. Among *Miscanthus* species, the non-invasive hybrid *Miscanthus* × *giganteus*, with a high lignocellulosic content, is a promising biomass crop for the bioeconomy, notably the biorefinery and bioenergy industries. Planting this species on contaminated and marginal land is a promising option to avoid changes in arable land use to mitigate the food vs. biofuel controversy. Key issues in promoting sustainable management of *Miscanthus* sp. on contaminated land are: a) crop suitability, integration, and sustainability in a region with a potential local market; b) site suitability in relation to the species' requirements and potential (c) biotic interactions in the landscape diversity; d) increase in shoot yields in line with various stressors (e.g., pollutants, drought, cold temperatures), and with minimal inputs.

Key words: biomass, *Miscanthus*, phytostabilization, phytodegradation, soil contamination

1.1. Introduction

Industrial, mining, urban, and agricultural activities have resulted in worldwide soil and water contamination by inorganic contaminants (Mench et al., 2010; Panagos et al., 2013) such as metals (e.g., Cd, Cu, Cr, Pb, Zn, Ni, and Hg) and metalloids (e.g., As, Se, and Sb), subsequently referred to as trace elements (TE). Frequently, TE contamination co-occurs with organic xenobiotics such as polycyclic aromatic hydrocarbons (PAH), dioxins/furans, petroleum hydrocarbons, and volatile chlorinated solvents (Panagos et al., 2013). Such

contaminants affect the functioning of natural and cropped ecosystems. Some TE (e.g., Cd, Zn, Se, Mo, As) can be transferred into the food chain from primary producers to animals and humans constituting both environmental and health hazards (Chapman et al., 2003; Reichenauer and Germida, 2008; Rajaganapathy et al., 2011).

Worldwide, some 22 million ha of land have been estimated to be contaminated by TE (GACGC, 1994). Updated figures on contaminated areas (in ha) are more readily available for the European Union (EU-18: 4,099,220-4,797,260), the USA (2,600,000), Australia (60,000), and China (8,100,000) than for emerging countries (Africa: 12,000,000) (Evangelou et al., 2012). There are up to 1 million potentially contaminated sites in the EU-33 (Panagos et al., 2013), and some of them are unsuitable and/or prohibited for food agriculture (Lebeau et al., 2008; Wang et al., 2012). The remediation of such sites is a challenge because of persistence and/or multiple contaminant occurrences in the environment. Conventional soil remediation commonly involves physical methods such as excavation and removal of soil to landfills (Dermont et al., 2008). However, such methods are unfit for large areas, notably those with diffuse pollution, and are criticized as being expensive and environmentally unfriendly (Conesa et al., 2012).

Phytoremediation is a set of alternative remediation technologies based on the concomitant use of tolerant plants and associated microorganisms to alleviate pollutant linkages and risks due to excessive contaminants in soils, water, and sediments. The types and processes of plant-based technologies have been extensively reviewed (Reichenauer and Germida, 2008; Mench et al., 2010; Conesa et al., 2012). Phytoremediation of TE-contaminated land mainly includes: (1) phytoextraction, i.e., the uptake and accumulation of TE from the soil to harvestable plant parts; and (2) phytostabilization, i.e., the use of plants and associated microorganisms to enhance TE immobilization in the rhizosphere. Organic contaminants can be dissipated by either phytodegradation via plant enzymes and secondary metabolites or rhizoremediation via exoenzymes and rhizosphere microorganisms (Reichenauer and Germida, 2008). For matrices (co)-contaminated with organic pollutants, phytodegradation and rhizodegradation can occur simultaneously with either phytoextraction or phytostabilization. All phytoremediation options can be aided with chemical (Shi et al., 2009; Mench et al., 2010; Komárek et al., 2013) and biological (Göhre and Paszkowski, 2006; Glick, 2010) amendments depending on the site and soil characteristics.

Sustainable management of contaminated sites based on phytoremediation options, hereafter referred to as phytomanagement, requires the use of economically valuable non-food crops

(Conesa et al., 2012; Evangelou et al., 2012). Their biomass production should replace non-renewable carbon materials and promote C sequestration and other ecosystem services such as improvement of soil and water quality, reduced erosion and increased niches for biodiversity (Evangelou et al., 2012). The lack of plants that can naturally produce high biomass and accumulate more than one type of contaminant is a major drawback for using phytoextraction (Maestri et al., 2010). Moreover, handling contaminated biomass and avoiding further environmental and food chain contamination remain controversial (Dickinson et al., 2009). Consequently, phytostabilization and phyto-/rhizodegradation are suggested as more relevant options for managing such contaminated sites. These techniques require a perennial plant activity to (1) actively minimize pollutant linkages and labile contaminant pools in soils, (2) optimize the maintenance costs and fertilizer inputs, and 3) improve site/soil ecological characteristics (Dickinson et al., 2009; Mench et al., 2010). Therefore, choosing appropriate plants entails the consideration of their ability to produce high biomass, contain the soil contamination, avoid health risks, and provide ecosystem services. Low contamination of biomass produced by these remediation techniques is crucial for socioeconomic development of marginal land, including brownfields and contaminated sites, and lands with a low agricultural value partly due to overfarming, erosion, and flooding (Cai et al., 2011; Gopalakrishnan et al., 2011).

Miscanthus species are high-yielding, non-food perennial grasses, considered a promising biomass crop for energy, bio-based products and raw materials for various industrial activities (Valbiom, 2009; Acikel, 2010). They are capable of growing in various climates from tropical to temperate regions and can adapt to a wide range of soil conditions including marginal lands. This review focuses on the suitability of *Miscanthus* species in phytomanagement projects aiming at reducing human and environmental risks, restoring ecosystem services, and promoting the bio-economy. This work does not examine *Miscanthus* physiology and agronomic practices because both topics have already been reviewed (Caslin et al., 2010; Heaton et al., 2010; Zub and Brancourt-Hulmel, 2010).

1.2. Identity, distribution and economic importance of miscanthus

1.2.1. Description and taxonomy

Miscanthus genus comprises C_4 perennial, woody, rhizomatous, bamboo-like grasses native from tropical and subtropical regions of Asia and Southeast Africa (Lewandowski et al., 2000, Chung and Kim, 2012). The genus forms a monophyletic group consisting of 11-20 species

(Deuter, 2000; Anderson et al., 2011) with the highest basic chromosome number (n = 19) within the Poaceae (Deuter, 2000). Cultivated species with an economic use have been thoroughly identified. They include *Miscanthus sinensis*, *M. sacchariflorus*, *M. floridulus*, and *M.* × *giganteus*, an interspecific hybrid between the tetraploid *M. sacchariflorus* and the diploid *M. sinensis* (Deuter, 2000).

All *Miscanthus* species develop tuffs with high shoot density. They are generally 1.5-4 m high with 1-to 2-cm stem diameter, but some species such as *M. floridulus* and *M. lutarioriparius* can reach 6-7 m high (Yan et al., 2012). Their leaves have a prominent white midvein, with the size varying from 20 to 100 cm long and 1 to 3 cm wide depending on the species (Sun et al., 2010). Flowers are generally formed between July and September (Sun et al., 2010). Inflorescence consists of a fan-shaped plume made up of long branches attached to a central axis.

1.2.2. Ecology of *Miscanthus* genus

Due to their tolerance to various ecological conditions, *Miscanthus* species grow on grasslands, abandoned milling sites, forest edges, streamsides, foothills, mountain slopes, wastelands, and coastal areas, often favoring damp habitats (Chou, 2009; Stewart et al., 2009; Sun et al., 2010). The species occurrence and distribution map in their native area, namely East Africa and Asia, are reviewed in Chung and Kim (2012). Most of the species occur at altitudes below 2,400 m, but some Asian endemic species including *M. transmorisonensis* strictly occur between 2,600 and 3,500 m (Chou, 2009). Some *Miscanthus* species such as *M. sinensis* grow on neutral or acid-sulfate soils (pH = 4-6) that often have relatively high Al levels (Stewart et al., 2009).

Miscanthus species are commonly naturalized in the temperate regions of Europe, North America, (Heaton et al., 2010, Anderson et al., 2011). Details on *Miscanthus* production map in these regions are given in Heaton et al. (2010).Well-drained silt and light clay, sandy or loamy soils with pH ranging from 5.5 to 7.5 support their optimal growth (DEFRA, 2007; Picco, 2010). However, growth and distribution of *Miscanthus* may be limited by harsh winter conditions, and productivity is low in dry environments (Kering et al., 2012). Negative soil temperatures are generally lethal (up to 50%) to *M.* × *giganteus*, and this species is more cold sensitive than *M. sinensis* (Clifton-Brown and Lewandowski, 2000; Farrell et al., 2006). In such conditions, a low survival rate of first-year *Miscanthus* plantations is observed, but plants that survive the first winter grow well (100% of survival rate) in the next season

(Maughan et al., 2012). In Europe, *M. sinensis*, *M. sacchariflorus*, and *M.* × *giganteus* grow well in Mediterranean, warm, temperate regions that meet optimal soil and water conditions. They also occur in high latitudes up to 57°N (Lewandowski et al., 2000), but optimal productivity is limited by the short growing season.

1.2.3. Biomass production of cultivated species

Field trials conducted in temperate climates of Europe and the USA resulted in mean aboveground biomass of 15-30 t DW ha^{-1} for 2- to 3-year-old plantations (Picco, 2010).In Europe, shoot DW yield is higher in southern countries with both a longer growing season and higher temperatures (**Table 1.1**).

The biomass production depends on species and genotypes (Jørgensen, 1997; Zub et al., 2011; Gauder et al., 2012), management practices (Angelini et al., 2009), soil types (Richter et al., 2008), and other environmental factors such as the length of the vegetation growing season (Zub and Brancourt-Hulmel, 2010). Water stress and frost negatively affect shoot DW yield, while irrigation and high temperatures improve the production in dry temperate regions (Lewandowski et al., 2003; Zub and Brancourt-Hulmel, 2010).

Within the growth life cycle, biomass production occurs from the second year because the first year ensures crop establishment (Zub et al., 2012). Out of common cultivated species, *M.* × *giganteus* has a great potential for biomass production in various European and North American environments and is the most productive species of the genus. In a 3-year study comparing the shoot DW yield potential of *M.* × *giganteus*, *M. sinensis* (hybrid), and *M. sacchariflorus*, higher winter yields (19 t ha^{-1}) were achieved by *M.* × *giganteus* (Zub et al., 2011).

1.2.4. Potential economic uses of *Miscanthus* biomass

Miscanthus biomass offers a wide range of end-products and utilizations depending on the transformation processes (**annex 1**). Nowadays, the growing need for renewable energies and plant-based chemicals has made *Miscanthus* a genus of interest for industries (Brosse et al., 2012).

Table 1.1. *Miscanthus* shoot yields (t DW ha^{-1}) in various countries.

Location	Autumn harvest	Winter harvest	Species	References
Austria	17-30	22	*M. × giganteus*	1
Belgium		16		1
China		17	*M. lutarioriparius*	2
Denmark	17	10	*M. × giganteus*	1
		11	*M. sinensis*	3
France	42-49	30	*M. × giganteus*	1
		15.8	*M. sinensis*	4
		12	*M. sacchariflorus*	4
Germany	17-30	10-20	*M. × giganteus*	1
Greece		20-26		1
USA (Illinois)				1
Ireland	18	14		1
Italy		15-27		1
Japan		6-22	*M. sinensis*	5
Latvia			*M. × giganteus*	1
Lithuania	18-28			1
Poland		16.5		6
		6.2	*M. sacchariflorus*	6
Portugal	39	26-30	*M. × giganteus*	1
Slovakia		21	*M. sinensis*	7
Spain		14	*M. × giganteus*	1
Switzerland		10-20	*M. sinensis*	8
Taiwan		16-27	*M. floridulus*	9
The Netherlands	25	16-17	*M. × giganteus*	1
UK		11-17	*M. × giganteus*	1

1. Anderson et al. (2011); 2. Liu et al. (2012); 3. Jørgensen (1997); 4. Zub et al. (2011); 5. Stewart et al. (2009); 6. Borkowska and Molas (2013); 7. Porvaz et al. (2012); 8. Corbière-Nicollier et al. (2001); 9. Huang et al. (2011).

1.2.4.1. Energy and biofuel production

Innovative technologies allow the transformation of lignocellulosic biomass into heat, biofuels, electricity (Brosse et al., 2012; Dickerson and Soria, 2013), and valuable by-products such as activated carbon, biochar, and fermentable hydrocarbon compounds, which can be used to produce bio-based chemicals (Villaverde et al., 2010). A high lignocellulosic content (**Table 1.2**) makes *Miscanthus* a promising crop to produce second-generation bioethanol (Han et al., 2011). *Miscanthus* biomass has a high heating value (17.7 MJ kg^{-1}; Collura et al., 2006), which is suitable for heat production. It can be also used in co-combustion with wood and coal in heating plants (Wagenaar and van den Heuvel, 1997).

Table 1.2. Lignocellulosic content (% DW) of some biomass crops (Byrt et al., 2011).

Plant biomass	Lignin	Hemicellulose	Cellulose	Location
Miscanthus	9-11	16-34	43-58	USA
Corn stover	10	28	35	USA
Sugarcane	7	8	24	Brazil
Switchgrass	6	36	32	USA
Sweet sorghum	7	20	26	USA
Poplar	20	23	40	Canada

1.2.4.2. Pulp and fiber

Successful trials have demonstrated *Miscanthus* potential to produce pulp and fibers (Kirwan et al., 2007; Marin et al., 2009; Acikel, 2011). A high pulp yield (70-80% DW) is due to the high holocellulose content, which forms 66-76% of the lignocellulosic content (Marín et al., 2009; **Table 1.2**). The pulp can also be harnessed into methylcellulose used as food additives and in many industrial applications (Ye et al., 2005). *Miscanthus* fiber provides raw material for reinforcement of biocomposite or synthetic materials (Kirwan et al., 2007; Valbiom, 2009; Acikel, 2011).

1.2.4.3. Building and construction materials

Miscanthus has been used as a thatching material for centuries in Japan (Stewart et al., 2009). In Denmark, Ireland, and the UK, thatching projects have been initiated since the 1990s to build ecological houses (Fowler et al., 2003). Building materials produced from *Miscanthus* include pure fiberboards (Velasquez et al., 2003), composite particleboards in combination with wood (Park et al., 2012), and building concrete and blocks (ValBiom, 2009). Building blocks made of *Miscanthus* biomass offer properties suitable for noise isolation. The use of *Miscanthus* as the core material for a light natural sandwich has been emphasized in the UK and Denmark (Bullard and Nixon, 1999) and demonstrated in Germany (Fowler et al., 2003). These light building materials are used for plane and mold structural parts with high form stability and low weight.

1.2.4.4. Agricultural uses

In agriculture, *Miscanthus* canes support ornamental potted plants and its straw is used in soil mulching to retain soil moisture, inhibit weed growth, and prevent superficial erosion (Fowler et al., 2003). *Miscanthus* straw is also used as bedding for poultry, cattle, pigs, horses, and companion animals (Caslin et al., 2010). In Switzerland, *Miscanthus* for horse bedding is

combined with production of organic fertilizer (ValBiom, 2009). The biochar produced from *Miscanthus* pyrolysis could be used as amendment to improve the soil physico-chemical properties associated with fertility (Melligan et al., 2012) and increase the yields of some crops such as maize (Kwapinski et al., 2010).

1.3. Phytomanagement of contaminated sites

1.3.1. Potentials for phytostabilization of TE-contaminated soils

1.3.1.1. Accumulation of TE in *Miscanthus* organs

The uptake and accumulation of TE by plants are among the characteristics investigated prior to plant selection for phytomanagement. Generally, TE accumulate more in belowground (BG) than in aboveground (AG) *Miscanthus* organs (**Table 1.3)**. Trace element concentrations in organs depend on a given element, but they generally occur in the decreasing order: roots > rhizomes > stems and leaves for all elements reviewed in this study. Arsenic, Cr, Pb, and Zn are more accumulated in leaves than stems, while the opposite is true for Cd. Arsenic and Pb concentrations in the AG part of *M. floridulus* can exceed 300 and 200 mg kg^{-1} DW, respectively. This species and *M.* × *giganteus* may highly accumulate Zn (> 400 mg kg^{-1}) in harvestable AG parts, which could be due to higher concentrations in the growing medium (**Table 1.3**). The bioconcentration factors (BCF, the ratio of TE concentrations in AG parts to soil) and the translocation factor (TF, the ratio of TE concentrations in AG to BG or roots), which are generally less than 1, suggest a low TE transfer to aerial organs. Due to insufficient and more heterogeneous data, it is not easy to compare the species with reference to BCF and TF. Nonetheless, considering long-term TE exposure (**Table 1.3**), the TF is higher for As, Cr, and Pb in *M. floridulus* than *M.* × *giganteus* and *M. sinensis*.

The season and the plantation age influence TE accumulation in *Miscanthus* species. Cadmium, Cu, Pb, and Zn concentrations decrease with plantation age in *M.* × *giganteus* AG organs (**Table 1.4**).

Table 1.3. Overview of TE concentrations and distribution in *Miscanthus* organs from contaminated soils.

Growing medium				**Concentrations in *Miscanthus* organs (mg kg^{-1} DW)**										
TE	**Concentration (mg kg^{-1} DW)**	**Type**	**pH**	**Roots**	**Rhizome**	**Stem**	**Leaves**	**BG [a]**	**AG [b]**	**BCF [c]**	**TF [d]**	**Species**	**Exposure duration**	**References**
As	3.7-1,605	Soil	5.1-5.5					5.6-659	1.4-333	0.21-0.38	0.25-0.51	*M. floridulus*	nd	Leung et al. (2007)
	78		8.5						0.4-0.7	< 0.01		*M.* × *giganteus*	8 months	Hartley et al. (2009)
	83,000	Technosol	3.4	1,284	49.5	5.4	17.1						3 months	Wanat et al. (2013)
	1727		6.1	602	48.3	3.6	4.3							
	418.7		6	34.4					0.45	< 0.01	0.01	*M. sinensis*	nd	Zhu et al. (2010)
Cd	4.7-51.7	Soil	5.1-5.8					1.1-56	0.1-3.8	0.02-0.07	0.07-0.09	*M. floridulus*	nd	Leung et al. (2007)
	13.7	Soil	5.2			3.6	2.3-5.3					*M.* × *giganteus*	2 years	Barbu et al. (2010)
	20.8		6.5						4.3	0.21			3 years	Pogrzeba et al. (2011)
Cu	7.7-95.9	Soil	5.1-5.8					13.6-179	1.8-7.5	0.08-0.23	0.04-0.13	*M. floridulus*	nd	Leung et al. (2007)
	42			16.2					7.1	0.17	0.44		nd	Wang et al. (2012)
	134.3	Sewage sludge	6.8						1.8	0.1		*M.* × *giganteus*	3 years	Fernando et al. (2004)
	130	Soil							2.3	0.02			4 years	Lord et al. (2008)
Cr	100	Mine tailing		31					51	0.31	1.65	*M. floridulus*	nd	Wang et al. (2012)
	55	Sewage sludge	6.8						0.32	< 0.01		*M.* × *giganteus*	3 years	Fernando et al. (2004)
	95.2	soil	6	14.6					9.9	0.10	0.68	*M. sinensis*	nd	Zhu et al. (2010)
Pb	88-2,582	Soil	5.1-5.8					172-519	36-254	0.1-0.41	0.21-0.50	*M. floridulus*	nd	Leung et al. (2007)
	344		5.5-5.9	10.4		5.4	12.3						nd	Li et al. (2011)
	2500	Mine tailing		217					324	0.13	1.5		nd	Wang et al. (2012)

Table 1.3. Overview of TE concentrations and distribution in *Miscanthus* organs from contaminated soils (Continued).

Growing medium				Concentrations in *Miscanthus* organs (mg kg^{-1} DW)										
TE	**Concentration (mg kg^{-1} DW)**	**Type**	**pH**	**Roots**	**Rhizome**	**Stem**	**Leaves**	**BG [a]**	**AG [b]**	**BCF [c]**	**TF [d]**	**Species**	**Exposure duration**	**References**
Pb	94	Sewage sludge	6.8						0.09	< 0.01		*M.* × *giganteus*	3 years	Fernando et al. (2004)
	271	Soil							0.36	< 0.01			4 years	Lord et al. (2008)
	547		6.5						158	0.29			3 years	Pogrzeba et al. (2011)
	682.5		5.2			3-6	4.1-22						2 years	Barbu et al. (2010)
	400-630		6.3						10-30	0.03-0.05			3 years	Kocoń and Matyka (2012)
	15,200	Technosol	3.4	327	30.6	29.5	43						3 months	Wanat et al. (2013)
	200		6.1	38	3.8	0.6	1							
Zn	49.6-1,927	Soil	5.1-5.8					61-801	13-605	0.26-0.31	0.21-0.76	*M. floridulus*	nd	Leung et al. (2007)
	9375	Mine tailing		1,231					1,163	0.12	0.95		nd	Wang et al. (2012)
	940	Sewage sludge	6.8						17	0.02		*M.* × *giganteus*	3 years	Fernando et al. (2004)
	365	Soil							48	0.13			4 years	Lord et al. (2008)
	2,174.5		6.5						450	0.21			3 years	Pogrzeba et al. (2011)
	635-1,000								200-600	0.31-0.60			3 years	Kocoń and Matyka (2012)

[a] BG: Belowground organs (roots and rhizomes); [b] AG: Aboveground organs (stems and leaves).

[c] BCF: Bioconcentration factor, i.e, the ratio of TE concentrations in aboveground organs to the growing medium.

[d] TF: Translocation factor, i.e, the ratio of TE concentrations in aboveground to belowground organs or roots.

[e] nd: Not determined for monitoring studies.

Many factors including growth kinetics, root-microorganism symbiotic associations, and plant detoxification mechanisms could be involved. The dilution of TE concentrations in AG organs may occur as less biomass is produced during the first year vs. the following years. The increase in root volume and surface results in enhanced TE sequestration, which may decrease TE transfer to AG parts. Also, a well-developed root system is associated with increased rhizodeposition, which supplies nutrients and an energy source for some TE-tolerant microorganisms, thereby increasing the TE-binding surface within the rhizosphere (Neagoe et al., 2013).

Table 1.4. Seasonal and annual variations in trace elements (TE) concentrations (mg kg^{-1} DW) in *Miscanthus* × *giganteus* grown on contaminated soils.

Reference [a]	Country	Sampling date [b]	TE in soils		Concentration in organs		
			TE	Concentration	Stems	Leaves	AG [c]
Barbu et al. (2010)	Romania	March 2009	Cd	13.7	3.6	5.3	
		August 2009			2.9	3.6	
		March 2010			2.1	2.3	
Pogrzeba et al. (2013)	Poland	Season 2		20.8			4
		Season 6					2
Fernando et al. (2004)	Portugal	Season 1	Cu	134			6.2
		Season 3					1.8
Fernando et al. (2004)	Portugal	Season 1	Pb	94			1
		Season 3					0.1
Barbu et al. (2010)	Romania	March 2009		682.5	5.5	22	
		August 2009			4.9	6.4	
		March 2010			3.7	4.1	
Pogrzeba et al. (2013)	Poland	Season 2		547			160
		Season 6					122
Kocoń and Matyka (2012)	Poland	Season 1		400-630			10-30
		Season 2					< 3
		Season 3					< 3
Fernando et al. (2004)	Portugal	Season 1	Zn	940			35.9
		Season 3					17
Kocoń and Matyka (2012)	Poland	Season 1		635-1,000			200-600
		Season 2					200-300
		Season 3					80-220
Pogrzeba et al. (2013)	Poland	Season 2		2,174.5			630
		Season 3					210

[a] Full details are given in the reference list provided in the main document.

[b] For season 1, 2, 3 and 6, sampling was done at the end of growing season in late autumn.

[c] AG: Aboveground organs.

1.3.1.2. Response of *Miscanthus* to potential TE-induced toxicity

The plant exposure to TE may result in accumulation of these elements in their tissues, which could induce stresses or affect the metabolic pathways such as photosynthesis, water status, and mineral nutrition, consequently reducing growth (Pourrut et al., 2011; Hossain et al., 2012). The literature of TE-induced toxicity in *Miscanthus* is scarce. However, short-term hydroponic experiments (≤ one growing season) demonstrate that in *M.* × *giganteus*, *M. sinensis*, and *M. sacchariflorus*, shoot and root growth and essential mineral (K, Mg, N, and P) uptake are affected by acute exposure to high Al and TE (Cd, Cu, Cr, Pb, and Zn) concentrations (**Table 1.5**).

The response to TE exposure and induced toxicity differ among *Miscanthus* species. Inhibitory effects on the growth (radicle and plumule) of *M. floridulus* and *M. transmorrisonensis* seedlings exposed to very high concentrations of Cd, Cu, Hg, and Pb in culture solutions were observed (Hsu and Chou, 1992). At 10 mg L^{-1} (1 µM Cd, 0.6 µM Cu, 2 µM Hg, and 2 µM Pb), the seedling growth of *M. floridulus* was 70%, 66-85%, 11-69%, 11-25%, and 70% inhibited by Cd, Cu, Hg and Pb, respectively. Conversely, 2 µM Pb did not reduce *M. transmorrisonensis* growth, and the growth inhibition rate due to Cd, Cu, and Hg in this species was less than in *M. floridulus*. Similarly, in hydroponics, seedlings of *M. sinensis* and *M. sacchariflorus* exposed to the 0-5,930 µM Al for 42 days displayed different behaviors. *Miscanthus sinensis* accumulated more Al in roots than did *M. sacchariflorus*. The biomass reduction occurred at 185 µM Al for *M. sinensis* and at 74 µM Al for *M. sacchariflorus*. The reduction of mineral uptake (K, P, and N) due to Al toxicity was higher in the latter species, and concentrations higher than 1,480 µM Al caused a progressive growth collapse up to death in both species. Overall, TE-induced toxicity as well as the upper critical threshold values depend on the species. *Miscanthus* species protect their photosynthetic system by regulating and/or limiting the TE transfer to their shoots (**Table 1.3**). On highly TE-contaminated soils with acid pH, the reduction in shoot growth or yields is first due to high TE availability and uptake (Pavel et al., 2014) or TE negative effects on the root growth in combination with other damages, notably on the photosynthetic apparatus (Wanat et al., 2013). In field experiments (≥ two growing seasons), *M.* × *giganteus* does, however, grow well on multiple TE contaminations such as soils contaminated by industrial activities (Lord et al., 2008; Pogrzeba et al., 2013) or by amendment with metal-contaminated sewage sludge (Fernando et al., 2004). Moreover, higher tolerance to Al in *M. sinensis* (usually dominating

poor nutrient acid soils in Japan) than in *M. sacchariflorus* (dominant in coastal and flooding plains) was observed by Kayama (2001).

Similarly, populations of *M. transmorrisonensis* and *M. floridulus* collected from contaminated sites are more tolerant to Pb, Zn, Cd, and Hg than those collected from uncontaminated sites (Hsu and Chou, 1992). This suggests that plant species interact with their growing medium in various ways, and resistance levels to stresses depend on growing conditions and can differ among species and populations.

Avoidance of local metal accumulation and distribution may constitute a homeostasis mechanism to avoid acute toxicity. In *M. sinensis*, excessive metal ions (Al, Cr, and Zn) are removed from the growing root tip tissues and are stored in mature ones or distributed to shoots (Ezaki et al., 2008). A high Al ion transport ratio (23-30%) from roots to aerial parts in *M. sinensis* suggests avoidance of local accumulation and distributing excess Al to aerial parts for vacuolar storage (Ezaki et al., 2008). These mechanisms, which alleviate internal metal ion toxicity, possibly involve the formation of inert metal-organic acid (citrate, malate and oxalate) complexes in the apoplast and vacuoles (Ma, 2000; Lyubenova et al., 2013).

The phytotoxic symptoms induced by Cd (Scebba et al., 2006) and Cr (Sharmin et al., 2012) include oxidative stress generation revealed the activation of antioxidant enzymes, and lipid peroxidation (**Table 1.5**). In plants, the production of a large amount of reactive oxygen species and the resulting generation of oxidative stress are among the best-known and earliest aspects of TE toxicity (Clemens, 2006). An activation of antioxidant enzymes (catalase, ascorbate peroxidase and superoxide dismutase) is observed more rapidly in roots than in leaves of Cd-treated (0-6.6 µM in hydroponic culture) *M.* × *giganteus* (Scebba et al., 2006). Similarly, by exposing *M. sinensis* to increasing Cr (VI) concentrations (0-1,000 µM), the antioxidant enzymes are readily activated in roots, and lipid peroxidation likely occurs at concentrations higher than 300 µM Cr (VI) in the growing medium (Sharmin et al., 2012). However, molecular mechanisms involved in TE tolerance and detoxification (Pourrut et al., 2013) are still unknown in *Miscanthus* species grown in field conditions.

Table 1.5. Aluminum (Al) and trace elements (TE) toxic effects on *Miscanthus* species and upper critical threshold concentrations in hydroponic conditions.

References	Pollutant	Growing conditions	Experimental concentrations	Exposure duration	*Miscanthus* species	Phytotoxic effects*	Upper critical threshold concentrations
Kayama (2001)	Al	3-week old seedlings grown in hydroponics, under natural illumination, 18-25°C, pH = 4	0-3,334 µM	42 days	*M. sacchariflorus*	Shoot (-72%) and root (-83%) DW; and shoot Mg (-40%)	≥ 74 µM
						Shoot P (-50%), root K (-58%)	≥ 185 µM
						Shoot K (-36%) and root P (-33%)	≥ 370 µM
						Root Mg (-50%)	≥ 1,480 µM
			0-5,930 µM		*M. sinensis*	Shoot (-30%) and root (-40%) DW	≥ 185 µM
						Shoot K (-75%), Mg (-47%), P (-89%), and root K (-91%), Mg (-73%), P (-70%)	≥ 1,480 µM
Arduini et al. (2004)	Cd	20-cm-tall plants pre-grown for 4 weeks in hydroponics (pH = 7.5, conductivity = 3.8 mS cm^{-1}) under open-air conditions.	0-6.6 µM	93 days	*M.* × *giganteus*	Root length (-82%), volume (-73%), DW (-38%) and rhizome DW (-38%) Stem height (-18%), number (-33%), and shoot DW (-37%)	6.6 µM
Arduini et al. (2006a)	Cd	20-cm-tall plants pre-grown for 81 days in hydroponics (pH = 7.5, conductivity = 3.8 mS cm^{-1}) under open-air conditions.	0-26.7 µM	36 days	*M.* × *giganteus*	Stem number (-22%), and height (-36%) Shoot N (-60%) Green leaf number (-42%) and leaf N (-58%) Root N (-58%) and rhizome N (-51%)	≥ 6.6 µM
Arduini et al. (2006b)	Cr	20-cm-tall plants pre-grown for 81 days in hydroponics (pH = 7.5, conductivity = 3.8 mS cm^{-1}) under open-air conditions.	0-3,846 µM	36 days	*M.* × *giganteus*	Stem height (-14%), number -18%) and shoot DW (-27%) Green leaf number (-26%) Rhizome DW (-14%)	≥ 960 µM
						Root DW (-43%)	≥ 1,334 µM

* % toxic effect was calculated as follows: (Plant performance with treatment – Control performance)/Control performance.

Table 1.5. Aluminum (Al) and trace elements (TE) toxic effects on *Miscanthus* species and upper critical threshold concentrations in hydroponic conditions (continued)

References	Pollutant	Growing conditions	Experimental concentrations	Exposure duration	*Miscanthus* species	Phytotoxic effects*	Upper critical threshold concentrations
Scebba et al. (2006)		20-cm-tall plants pre-grown for 1 month in hydroponics (pH = 7.5, conductivity = 3.8 mS cm^{-1}) under open-air conditions.	0-6.6 µM	3 months	*M.* × *giganteus*	Increased shoot and root DW: hormesis effect Increased antioxidant enzyme activity	2.2- 4.4 µM
						Decreased shoot, rhizome and root DW, Decreased antioxidant enzyme activity	6.6 µM
Ezaki et al. (2008)	Al	10-day-old seedlings grown in hydroponics at pH = 4.2, 16 h illumination, 24°C	0-900 µM	10-14 days	*M. sinensis*	Decreased root growth rate (40%)	≥ 300 µM
						Increased SOD and CAT activity, oxidative damage to root	900 µM
	Cd	10-day-old seedlings grown in hydroponics at pH = 5.7, 16 h illumination, 24°C	0-150 µM			Decreased root growth rate (-90%)	≥ 0.50 µM
	Cr		0-15 µM			Decreased root growth rate (-25%)	≥ 10 µM
	Cu		0-15 µM			Decreased root growth rate (-85%)	≥ 15 µM
	Zn		0-200 µM			Decreased root growth rate (-25%)	≥ 100 µM
Sharmin et al.(2012)	Cr	4-week-old seedlings grown in hydroponics, pH = 5.8, under controlled illumination (16/8 light/dark period) at 25°C.	0-1,000 µM	3 days	*M. sinensis*	Decreased root length (-1%), and DW (-20%) Inhibition of new root formation Lipid peroxidation due to oxidative stress	≥ 300 µM

* % toxic effect was calculated as follows: (Plant performance with treatment – Control performance)/Control performance.

1.3.1.3. Effects of *Miscanthus* on TE mobility

The plant cover changes the physico-chemical soil parameters and determines TE speciation in contaminated soils (Yang et al., 2010). Vegetation may influence soil TE bioavailability by modifying the distribution of these elements between labile and less labile fractions. In field conditions, *M. floridulus* reduced TE availability by increasing their fraction associated with organic matter content as follows: 20%, 40-50%, and 60%, respectively, for Cd or Zn, Pb, and Cu (Zhang et al., 2009). Similarly, a reduction of the TE available fraction was observed in acid technosols highly contaminated by As and Pb and planted with *M.* × *giganteus* (Ollivier et al., 2012). After 3 months, the available fractions obtained with 0.01 M $CaCl_2$ extraction decreased by 52% for Pb mg kg^{-1} and 19% for As.

The capacity of *Miscanthus* to reduce TE availability in the root zone could be higher than that of annual rotation crops. The TE availability and speciation was investigated in slightly alkaline silt loam soils containing Cd, Cu, Pb, and Zn and cultivated with either a *M.* × *giganteus* or annual (wheat, broad bean, and sugar beet) cropping system (Iqbal et al., 2013). EDTA-extractable Cu and Pb fractions in soils sampled in the 3-year-old *M.* × *giganteus* plantation were lower (i.e., 43.7% and 93.7% for Cu and Pb, respectively) than in annual-cropped soils (Cu: 46.6% and Pb: 98.2%). Conversely, EDTA-extractable Cd and Zn fractions did not differ between the two cropping systems. The negative correlation between soil (20-50 µm) organic carbon and EDTA-extractable Cu and Pb fractions suggest that both elements could form organometallic complexes, hence less mobile in soils.

The reduction of TE mobility should not be considered as a general rule but rather specific to TE and/or their interactions with soil physico-chemical properties and prevailing local weather conditions. Indeed, long-term cropping of *Miscanthus* on agricultural soils increases humus content, cation exchange, and water retention capacities (Kahle et al., 1999; Lewandowski et al., 2000). The decay of *Miscanthus* belowground organs and litter contribute to nutrient recycling and soil carbon/organic matter (Kahle et al., 2001; Dondini et al., 2009). The estimated contribution of underground parts and litter inputs to soil total carbon under *M.* × *giganteus* plantation is 10 t ha^{-1} and 1.5 t ha^{-1} $year^{-1}$, respectively (Amougou et al., 2011). Both Cu and Pb strongly sorb to soil organic matter (Guo et al., 2006). Therefore, their mobility is likely lower than that of Cd or Zn under miscanthus plantations. However, *Miscanthus* will poorly influence the mobility of TE such as As whose solubility increases with increasing pH and carbon content (Hartley et al., 2009).

Rhizosphere processes and conditions under *Miscanthus* plantations could influence TE mobility. In TE-contaminated soils, the plant cover improves soil moisture retention, while

root exudates support microorganisms including bacteria by providing nutrients (Zhan and Sun, 2011). The quality and quantity of root exudates of *M.* × *giganteus* was studied by Formánek et al. (2009), Kaňová et al. (2010) and Hromádko et al. (2010). Dominant molecules in root exudates include various amino acids and organic acids such as proprionic, succinic, and citric acids. These molecules, notably organic acids, influence rhizosphere pH, desorption/dissolution of mineral nutrients, and formation of metal complexes in soil (Jones, 1998; Ryan et al., 2001). Kayama (2001) hypothesized that higher tolerance to Al in *M. sinensis* than in *M. sacchariflorus* was due to its capacity to release much more citric and malic acids to form Al chelates. Indeed, the anionic chemical forms of organic acids such as oxalate, citrate, and malate form relatively stable complexes with metals (e.g., Al, Cd, Cu, Zn, Pb) in soil solutions and increase TE bioavailability and root uptake and accumulation (Jones, 1998). Within the plant cells and tissues, mainly in roots, organic acids mediate ionic TE chelation, vacuolar compartmentation, and detoxification (Lyubenova et al., 2013). Moreover, root exudates are a food resource for bacterial and fungal microorganisms that either accumulate TE or stimulate root growth, thereby increasing the TE binding surface in the rhizosphere (Neagoe et al., 2013). Thus, rhizosphere processes could influence *Miscanthus* TE exclusion and sequestration capacity, but the driving mechanisms have not been elucidated yet.

1.3.2. Phyto- and rhizodegradation of organic contaminants

Unlike TE, organic contaminants can be degraded by living organisms through the intervention of various enzymes (Gerhardt et al., 2009). Plant species influence the establishment of specific degrader microorganism communities and increase both nutrients and oxygen supply in the soil-root interface, which stimulate microorganism growth and diversity, thereby increasing their degradation capacity (Reichnauer and Germida 2008; Megharaj et al., 2011). Both phytodegradation and rhizodegradation processes rely on synergistic relationships between plants and their rhizosphere microorganism communities.

Miscanthus could present great potentials for biodegradation of organic contaminants. Some studies conducted *in vitro* and in microcosm conditions, using root exudates collected from *M.* × *giganteus*, demonstrated the capacity of this species to promote bacterial diversity and activity capable of degrading PAH, namely pyrene and phenanthrene (Técher et al., 2011, 2012a, 2012b). More than 10 bacterial isolates belonging to the Proteobacteria group were identified as the main degraders and their overall activity almost doubled in presence of *M.* × *giganteus* root exudates (Técher et al., 2011). Polyphenolic (gallic, chlorogenic, and caffeic acids) and flavonoid (quercetin, rutin, and catechin) compounds present in the *M.* × *giganteus*

rhizosphere are the major contributors to the biostimulation of PAH-utilizing bacteria. It is hypothesized that on contaminated soils, the lower hydrophobicity of quercetin compared with that of PAH may also enhance its absorption by bacteria and activate enzymes involved in PAH detoxification (Técher et al., 2012a). Moreover, some plant enzymes such as dehalogenase, laccase, monooxygenase, and peroxidases are involved in degradation of organic xenobiotics (Gerhardt et al., 2009), but the extent of their influence in the *Miscanthus* rhizosphere and tissues is not known yet.

1.3.3. Reduction of human and environmental risks

Phytomanagement aims at remediating the environment and protecting local inhabitants from the harmful effects of exposure to hazardous substances. The selected plant species/varieties should reduce contaminant dispersion, exposure to airborne contaminants, and their transfer into the food chain (Henry et al., 2013). *Miscanthus* grown on contaminated soils can contain higher shoot TE concentrations, but the TF, which is for the most part less than 1, indicates that root-to-shoot TE transfer is minimized (**Table 1.3**). The combination of this trait with low BCF and higher TE concentrations in roots than in shoots demonstrate the capacity to retain TE in soils. Owing to the perennial growth and its ability to stabilize TE and degrade some organic pollutants, *Miscanthus* could potentially limit pollutant transfer into different environmental compartments by reducing (1) pollutant leaching from the root zone and groundwater contamination, (2) pollutant run-off (water erosion) and surface water contamination, (3) dust emission into the atmosphere due to wind erosion and seasonal soil tillage, and (4) pollutant transfer into plant AG parts and thus transfer into food chains. Therefore, as non-food crops, *Miscanthus* form a potential resource for phytomanagement of contaminated areas, with the option of TE phytostabilization and/or organic pollutant degradation, hence the opportunity to reduce both human and environmental risks.

1.3.4. Biomass production on contaminated sites: challenges and opportunities

For sustainable biomass production on contaminated lands, three challenges have to be addressed: (1) maintaining *Miscanthus* growth and productivity on contaminated soils, (2) monitoring the quality of the biomass in line with the specific requirements of local conversion chains, and (3) mitigating the production cost and market availability.

High TE concentrations in the growing medium negatively affect growth due to root damage and reduced mineral nutrition, particularly N and P in *M.* × *giganteus* and K, P, and Mg in *M. sinensis* and *M. sacchariflorus* (**Table 1.5**). To avoid and anticipate such undesirable effects, which reduce potential shoot DW yield, identifying, assessing, and modeling the response of

Miscanthus species or cultivars to contaminated matrices should be a prerequisite for large-scale field plantation. Moreover, depending on soil parameters and contaminant linkages, the use of chemical or biological amendments could be another option to reduce TE bioavailability, and increase plant productivity (Lee et al., 2014; Pavel et al. 2014).

Thermo-chemical technologies including combustion, gasification, and pyrolysis are the main conversion routes of miscanthus biomass (**Annex 1**; Brosse et al., 2012). These technologies operate at high temperatures to optimize output. This suggests a high risk for metal volatilization or their recovery in the end-products. The mean TE concentrations in *Miscanthus* biomass from uncontaminated land are: 0.2 (As), 0.1 (Cd), 2 (Cu), 0.03 (Hg), 2 (Ni), 2 (Pb), and 10 (Zn) mg kg^{-1} and such concentrations do not cause any environmental risks during and after biomass combustion (Obernberger et al., 2006). However, biomass from highly contaminated sites can contain higher TE concentrations (**Table 1.3**), and the TE behavior biomass transformation needs to be known for subsequent handling of the end-products. For instance, TE such as Cd, Pb, and Zn are highly volatile and accumulate in fly ashes during willow biomass gasification (Vervaeke et al., 2006). Similarly, the gasification of *Miscanthus* biomass containing Pb and Zn resulted in high Zn levels in flue gas at 900-1000°C (Porbatzki et al., 2010). Therefore, assessment of the biomass quality in standing crop or at harvest is needed so as to mitigate potential risks that can be associated with biomass conversion. Moreover, depending on the temperature, the degradation or the formation of organic pollutants should be also monitored (Han and Kim, 2008). Appropriate transformation pathways and equipment to reduce environmental risks are needed when dealing with contaminated plant biomass.

Establishing *Miscanthus* plantations requires a financial investment. In European countries, the rhizome plantation cost is within the range of 1,500-3,000 € per hectare and this cost increases depending on other required farm-to-gate operational costs, notably soil ploughing, machinery hire, land rental, fertilization, and irrigation (Bocquého and Jacquet, 2010; Caslin et al., 2010; Lychnaras and Schneider, 2011). However, this establishment cost is relatively low for a perennial crop that does not require seasonal plantation for about 20 years (Lewandowski et al., 2000). The annualized production cost including plantation management, harvest, transport, and storage ranges from 430 to 828 € ha^{-1} with a gross revenue of 326-628 € ha^{-1} $year^{-1}$ (Styles et al., 2007; Krasuska and Haka, 2011; Lychnaras and Schneider, 2011). The price for *Miscanthus* chips varies according to countries and regions: e.g. 60 € t^{-1} in Ireland (Caslin et al., 2010) and 70.3 € t^{-1} in Poland (Krasuska and Haka, 2011). This price may be competitive with wood (120 € t^{-1} DW) used in heat generation by large consumer industries (Caslin et al., 2010). The selling price for *Miscanthus* can fluctuate

depending on its biomass form and uses: e.g., the price may reach approximately £ 240 (280 €) t^{-1} for *Miscanthus* pellets in the UK (Biogreentech, 2013), which is higher than wood pellets (200 € t^{-1}) (Caslin et al., 2010). However, *Miscanthus* remains an advantageous biomass crop for local consumers, given that its low bulk density (0.09 t m^{-3}) compared to wood density (0.65 t m^{-3}) probably increases the long-distance transport cost (Caslin et al., 2010). Thus, mitigating the production cost and establishing *Miscanthus* biomass markets are of prime concern.

Moreover, there should be a difference in the way economic considerations for *Miscanthus* planted on uncontaminated and contaminated land are addressed. Currently, on contaminated land, the prime concern is to limit environmental and human risks by improving ecosystem services of the sites concerned. Therefore, assessment of economic profitability should consider and price their re-establishment that meets both local population expectations and environmental policies. Other opportunities could be explored, including co-firing with coal or wood (Wagenaar and van den Heuvel, 1997; Caslin et al., 2010) or the integration of *Miscanthus* biomass produced from phytostabilization of patchy and small land plots into the existing production agrosystem (Christou et al., 2005).

1.4. Ecosystem services provided by miscanthus

1.4.1. Low production inputs and reduced greenhouse gas emissions

Miscanthus presents the advantage of reduced cost due to a single planting season and high productivity that is maintained for approximately 20 years (Lewandowski et al., 2000). Moreover, the translocation and storage of nutrients into rhizomes at the end of the growth cycle allow nutrient recycling and availability for the following cycles. For comparison purposes, both quantitative and qualitative assessments of *Miscanthus* agronomic and ecological advantages vs. other biomass crops are summarized in **Table 1.6**.

To date, few pests and pathogens affect *Miscanthus* growth in Western countries suggesting a limited use of pesticides (Anderson et al., 2011). In USA, few grass crop pests such as the yellow sugarcane aphid - *Sipha flava*, corn leaf aphid - *Rhopalosiphum maidis* (Bradshaw et al, 2010), nematodes - *Xiphinema* sp., and fungi (*Fusarium* sp., *Leptosphaeria* sp.) (Anderson et al., 2011) were observed in field conditions. Artificial infestations under greenhouse conditions demonstrated that the maize pest western corn rootworm - *Diabrotica virgifera virigifera* (Spencer and Raghu, 2009; Gloyna et al., 2011), and fall armyworm - *Spodoptera frugiperda* (Prasifka et al., 2009) can successfully grow in young *Miscanthus* plants.

The occurrence of such pests raises the debate and uncertainty on natural resistance to pests and their effects on *Miscanthus* productivity in field conditions. However, *Miscanthus* does not constitute a preferential food source for insects such as *Aphis fabae*, *Myzus persicae*, and *Rhopalosiphum padi*, and do not support their reproduction, which reduces their survival rate (Coulette et al., 2013).

In field conditions, the southwestern corn borer, *Diatraea grandiosella*, reduces the shoot biomass of young *M.* × *giganteus* up to 12-30%, but this effect decreases with stem size and crop age (Pasifka et al., 2012). Conversely, in greenhouse culture, artificial soil and rhizome inoculation with pathogenic fungal species including *Fusarium avanaceum*, *F. oxysporium*, and *Mucor hiemalis* resulted in reduction of the rhizome sprouting rate as well as shoot and root growth of *M.* × *giganteus* (Covarelli et al., 2012). The occurrence of such rhizome-infesting *Fusarium* sp. and growth reduction in field conditions was confirmed in Belgium (Sclauflaire et al., 2013).

Miscanthus cultivation requires a limited use of herbicides, which could only be applied during the establishment phase (1-2 years). After the second year, the closed canopy formed by mature miscanthus stands reduces weed growth. Moreover, with winter harvest (after leaf fall), the mulch formed by dead leaves rapidly covers the soil and prevents weed growth (Amougou et al., 2012).

The perennial growth and small production inputs suggest a reduction of greenhouse gas emissions along the biomass production chain, especially on-site NO_2 and CO_2 emissions (Davis et al., 2010). In a life cycle assessment, *M.* × *giganteus* grown in Europe shows higher CO_2 savings and biomass production than other energy crops such as *Panicum virgatum*, *Cynara cardunculus*, *Sorghum bicolor*, *Z. mays*, *Triticum* sp., and *Brassica napus* (Fazio and Monti, 2011; Cadoux et al., 2014; Felten et al., 2013). The reduction of CO_2 and other greenhouse gas emissions across the production process is mainly due to the best trade-off between low nutrient requirements and high biomass productivity per land surface unit. Despite lower water requirements than tree species such as *Salix* sp. and *Eucalyptus* sp. (**Table 1.6**), *M.* × *giganteus* has a higher evapotranspiration rate than *P. virgatum* and *Z. mays* (Zhuang et al., 2013) and requires sustained water availability in soil to maintain high productivity (Richter et al., 2008). Drought conditions can cause up to 40% of its potential biomass production (Richter et al., 2008). Lower establishment rate and shoot productivity than perennial grass such as *Arundo donax* and *P. virgatum* were observed in semi-arid environments of Southern Oklahoma in the US (Kering et al., 2012).

Table 1.6. Agronomic characteristics of the most frequently used bioenergy crops in Europe. Modified from Nsanganwimana et al. (2014).

Characteristics [a]	***Miscanthus* sp.**	***Sorghum bicolor***	***Panicum virgatum***	***Arundo donax***	***Populus* sp.**	***Salix* sp.**	***Eucalyptus* sp.**	***Cannabis sativa***	***Brassica carinata***
Biomass yield (t DW ha^{-1})	15-30	5-30	10-25	14-45	7-28	10-30	10-25	12-23	2.6-4
N requirement (kg ha^{-1})	0-100	56-224	0-70	40-60	110-450	80-150	60-125	100-220	80-170
Nutrient use efficiency	+++	++	+++	+++	++	+++	+++	+++	++
Nutrient recycling	+++	+	+++	+++	+++	+++	+++	+	+
Water needs (P_{mm}) [b]	700-800	300-700	450-750	380-650	> 350	1000	870-1100	400-600	
Water use efficiency	+++	++	+++	++	+	++	++	++	++
Pest resistance	+++	+++	+++	+++	++	++	++	+++	++
Noninvasiveness	++ [c]	++ [c]	+++	++ [d]	++	++	++	++	++
Ecological benefits [e]	+++	+++	+++	+++	+++	+++	+++	+++	+

[a] Favorable characteristics and effects are indicated by +, with +++ being the most favorable.

[b] Ranges of annual rainfall needed for optimal productivity.

[c] Nonhybrid *Miscanthus* species such as *M. sinensis* can become invasive outside their native distribution ranges.

[d] *Arundo donax* is an aggressive riparian invasive in nonagricultural ecosystems.

[e] The ecological benefits mainly refer to the plant's potential to improve soil and water quality, to grow on and add value to marginal land, and to reduce greenhouse gas emissions during its life cycle.

1.4.2. Improvement of soil quality

Under poor and/or TE-contaminated soil conditions, *Miscanthus* species such as *M. floridulus* (Zhang et al., 2009) and *M.* × *giganteus* (Técher et al., 2012c) increase carbon inputs and promote microorganism diversity and activity, which are important in soil particle aggregation and rehabilitation processes. *Miscanthus* × *giganteus* was successfully established on former fly ash deposit sites presenting alkaline pH, nutrient deficiency, and little water-holding capacity (Técher et al., 2012c). Despite a low total chlorophyll content and small shoot DW yield on such conditions, roots and rhizomes grew quite well and were able to support and enhance nitrification processes. Similarly, in the monitoring study of a Chinese Cu mining and tailing site, plant communities including *M. floridulus* and *M. sinensis* were able to restore several parameters (increased pH, structure, and microbial diversity including nitrogen-fixing proteobacteria, organic matter, and water contents) of TE-contaminated soils (Zhan and Sun, 2011).

Miscanthus × *giganteus* has a dense and superficial root system with 90% of the root biomass exploring the first 0.35 m of the soil profile (Monti and Zatta, 2009). This provides resistance to water erosion (Tsuyuzaki, 2002) and an effective rhizosphere for trapping nutrients and preventing nitrate leaching into water drainage (Ng et al., 2010; Wu and Liu, 2012).

Miscanthus ability to improve soil quality could be used in combination with organic amendments on soils with low agronomic value. Fernando et al. (2004) and Kalembasa and Malinowska (2009) report the great potential to increase *M.* × *giganteus* and *M. sacchariflorus* biomass production by fertilizing with TE- and nutrient-contaminated sewage sludge. Therefore, fertilizing *Miscanthus* plantations on poor nutrient soils with sewage sludge and/or irrigation by TE- and nutrient-contaminated wastewater on sites with limited nutrient and water availability can offer a three-fold advantage: improving soil productivity, increasing biomass yields, and reducing costs for treatment and disposal of sewage sludge in line with the specific legislation in each country. Planting *Miscanthus* on contaminated and marginal land and its use to recycle nutrients from sewage sludge and to clean-up wastewater, notably in dry regions, are phytomanagement scenarios that avoid changes in arable land use to mitigate the food vs. biofuel controversy.

1.4.3. Effects on biodiversity and biotic interactions

Large-scale monoculture plantations have been associated with reduction of ecological niches for wildlife and other plant species (Biemans et al., 2008). *Miscanthus* is not a preferential food source for most of animals and insects, but its closed canopy and residues left after

harvest provide nesting, shelter, and brood places for many invertebrates including pollinators, wild mammals, and birds, especially during winter (Semere and Slater, 2007a, 2007b; Sage et al., 2010; Stanley and Stout, 2013). These animals presumably provide ecosystem services, notably mediation of pollination and control of crop pests in agroecosystems In comparison to annual crops, *M.* × *giganteus* increases biodiversity due to a lack of seasonal tillage and reduced pesticide levels (Felten and Emmerling, 2011; Jørgensen, 2011). In TE-contaminated soils, increased density and diversity of soil invertebrates and stable communities of soil-dwelling invertebrates result from provision of food sources (for saprophages and rhizophages), reduced soil toxicity (for geophages), and improved soil structure and rhizosphere quality (Hedde et al., 2013a, 2013b). However, these positive effects on biodiversity may diminish with crop age and canopy closure (Bellamy et al., 2009), probably due to species specialization.

The most cultivated *Miscanthus* species, *M.* × *giganteus*, has a lower probability of becoming invasive (Gordon et al., 2012) than its partner species including *M. sinensis* (Quinn et al., 2010; Barney et al., 2012). *Miscanthus sinensis* and *M. sacchariflorus* produce viable seeds, suggesting that intra- and interspecific variability exists, and that their invasion potential is unpredictable. On the other hand, the *M.* × *giganteus* hybrid is sterile without fruitful sexual reproduction, which reduces its invasion risk. To date, there has been no report on the threat of invasion due to rhizome growth extension from long-term commercial plantations to neighboring arable land.

As previously mentioned, pathogens and parasites in *Miscanthus* plantations can affect its productivity but may also present potential ecological risks. Indeed, pests associated with *M.* × *giganteus* may transmit parasites (viruses, bacteria, and fungi) or *Miscanthus* plantations may be a pest reservoir for susceptible conventional cereal crops such as maize (Spencer and Raghu, 2009; Gloyna et al., 2012). However, until now, the occurrence of this risk is only suspected and has not been measured elsewhere in field conditions.

1.5. General conclusion and research perspectives

Management of marginal land, which include brownfields, contaminated sites, and land with a low agricultural value, has become a crucial concern in developed and densely populated countries. The management of this marginal land, which covers large surfaces, requires innovative and sustainable solutions to reduce human and environmental risks. Phytomanagement integrating phytotechnologies as well as societal and socioeconomic aspects is a potential option that must be site-specifically appraised.

In the last decade, a growing interest in *Miscanthus* as a promising biomass crop has been shown worldwide. Thanks to its capacity to accumulate more TE in roots, limit TE transfer to shoots, promote degradation of organic xenobiotics, and improve soil quality at contaminated sites, *Miscanthus* is suitable crop for combining biomass production and ecological restoration of contaminated and marginal land. Among *Miscanthus* species, due to its non-invasive character and higher biomass production, *M.* × *giganteus* appears to be a relevant species to reduce human and environmental risks and consequently a suitable option for the phytomanagement of contaminated sites.

Most research investigating *Miscanthus* has focused on uncontaminated agricultural lands. Therefore, there is a gap in our knowledge of the factors that can best explain the species resistance and growth on contaminated and degraded soils. A holistic appraisal of phytomanagement should consider (1) *M.* × *giganteus* ability to grow on marginal land, (2) the effects of *M.* × *giganteus* on pollutant behavior, (3) pollutant transfer into the biosphere, (4) human and environmental risk assessment, (5) influence of *M.* × *giganteus* crops on biodiversity, (6) trade-offs between economic and social benefits and costs, and (7) crop life cycle assessment considering environmental aspects and sustainability in a region with a potential local market.

These considerations and other studies reviewed herein can form the basis for breeding more productive and stress-tolerant genotypes suitable for sustainable management of contaminated and degraded lands.

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Objectifs de la thèse

L'analyse bibliographique montre un manque d'information sur les potentialités de *M.* × *giganteus* pour le phytomanagement et son comportement face aux contaminants métalliques dans le sol. En effet, il existe très peu de travaux sur l'accumulation des ETM dans les différents organes de miscanthus. Les expérimentations *ex situ* menées par Arduini et al. (2004, 2006) en hydroponie et ceux de Wanat et al. (2013) sur des Technosols, ne représentent pas les conditions de terrain, et encore moins le transfert des ETM dans le système sol-plante. Quant aux expérimentations *in situ*, l'intérêt est plus porté sur la biomasse aérienne et sénescente au moment de la récolte (Lord et al., 2008; Barbu et al., 2010; Pogrzeba et al., 2013). De tels travaux permettent d'évaluer le rendement et la qualité de la biomasse sans pour autant renseigner sur la distribution des métaux dans les organes souterrains et les effets de la culture sur le comportement des ETM dans le sol. De même, il a été constaté que les concentrations dans la biomasse récoltée diminuent avec l'âge de la plantation mais aucune étude n'a démontré la relation entre les teneurs en ETM dans les organes de miscanthus et la cinétique de croissance.

Pour que le phytomanagement soit un mode de gestion durable, il s'avère nécessaire de bien maitriser les flux de transferts d'ETM dans l'écosystème sol-plante et de favoriser la productivité végétale. Une meilleure évaluation de l'efficacité de ce mode de gestion requiert d'associer l'étude du degré de contamination et des paramètres physico-chimiques du sol au comportement de l'espèce végétale choisie comme modèle. En effet, l'influence des paramètres physico-chimiques tels que le pH, la capacité d'échange cationique, les teneurs en phosphates, en matières organiques et en oxydes/hydroxydes sur la mobilité et la disponibilité environnementale des métaux est mieux connue (Carrillo-Gonzalez et al., 2006; Dube et al., 2001; Sparks, 2005; Pinto et al., 2014). L'acquisition des données sur ces paramètres permet d'interpréter au mieux le comportement de la plante face aux métaux. De plus, au sein d'une espèce végétale, le comportement face aux métaux diffère selon les cultivars (Guo et al., 2011; Song et al., 2011; Ding et al., 2014; Long et al., 2013). Cette information est à prendre en compte afin d'identifier les cultivars les plus efficaces selon l'option de phytomanagement.

L'objectif principal de ce travail est d'évaluer les potentialités de *Miscanthus* × *giganteus* pour le phytomanagement de sols agricoles fortement contaminés par des ETM. L'approche utilisée dans notre démarche vise à dresser un bilan sur le comportement de *M.* × *giganteus* face à la contamination métallique des sols. Ceci repose sur l'évaluation des effets de cette plante sur les paramètres physico-chimiques des sols étudiés, la mobilité de Cd, Pb et Zn ainsi que le transfert de ces éléments dans le système sol-plante.

Notre travail a pour objectifs spécifiques d'étudier l'influence **1)** du degré de contamination et des paramètres physico-chimiques des sols, **2)** du stade de croissance de la plante et de la saison et **3)** de pratiques culturales (cultivar, densité de plantation et amendement biologique) sur le transfert et l'accumulation des métaux du sol dans les organes aériens et souterrains de *M.* × *giganteus*. La prise en compte des variations saisonnières et du stade de croissance sur l'accumulation des métaux et des nutriments dans *M.* × *giganteus* vise à évaluer à la fois le degré de transfert des métaux au cours du cycle végétatif et la qualité de la biomasse à la récolte. De même, la productivité de *M.* × *giganteus*, mise en évidence par la détermination de la biomasse aérienne, est évaluée sur un gradient de contamination des sols et selon les pratiques culturales considérées dans le cadre de ce travail.

Chapitre II :

Description des dispositifs expérimentaux

2.1. Justification du choix de la démarche expérimentale

Sur le site atelier « Metaleurop », les sols agricoles sont contaminés à des degrés divers selon la distance aux sources de contamination (Sterckeman et al., 2002). Ainsi, la contamination en ETM diminue avec la distance à l'ancienne fonderie Metaleurop Nord. Les parcelles intégrées au programme PHYTENER ont été choisies en considérant cette composante. Ainsi, les parcelles représentent le gradient de contamination qui caractérise le site.

Les travaux de la thèse s'appuient sur deux dispositifs expérimentaux complémentaires en conditions *in situ* et *ex situ*. Le dispositif expérimental *in situ* a été mis en place dès 2007 dans l'objectif d'une part, de tenir compte de la réalité de terrain avec des plantations ayant acquis une certaine maturité et d'autre part, de démontrer la faisabilité du phytomanagement. Toutefois, ce type d'expérimentation *in situ* est souvent sujet aux contraintes environnementales imprévisibles. Il a donc été complété en 2012 par la mise en place d'un dispositif *ex situ*, en milieu semi-contrôlé, pour étudier le comportement du miscanthus face aux ETM en limitant les variabilités environnementales rencontrées dans les conditions *in situ*.

2.2. Expérimentation *in situ*

2.2.1. Localisation des parcelles expérimentales

Le dispositif expérimental *in situ* comprend quatre parcelles agricoles (nommées M200, M500, M500-B et M700) localisées en zone péri-urbaine, autour de l'ancienne fonderie de plomb (**Fig. 2.1**).

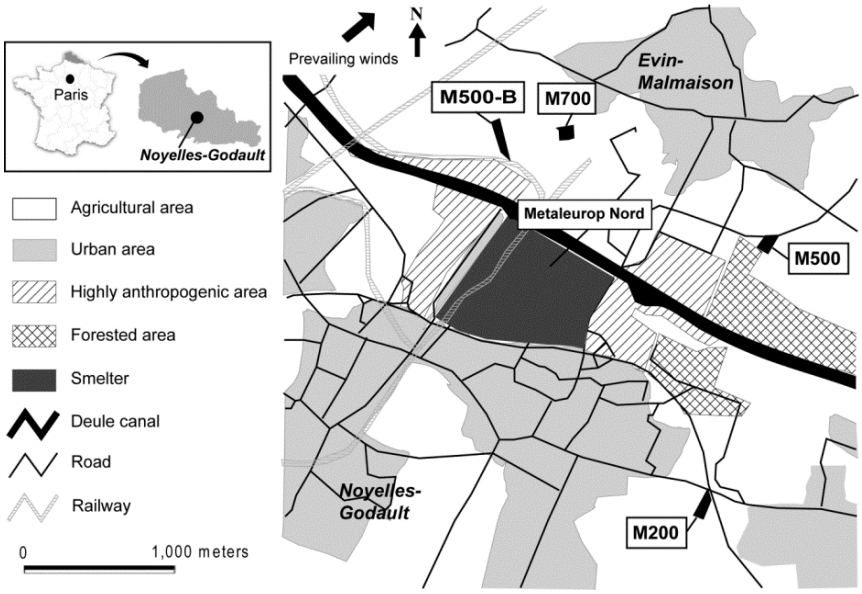

Fig. 2.1. Localisation des parcelles agricoles/des sols du dispositif expérimental *in situ*.

La parcelle M200 (1,4 ha) est sur le site atelier la plus éloignée, à environ 1,8 km de l'ancienne fonderie, et aussi la moins contaminée. Elle est située sur la commune de Courcelles-lès-Lens. La parcelle M500 (0,8 ha) est à une distance d'environ 1,4 km de l'ancienne usine, sur la commune d'Evin-Malmaison. Elle se situe dans la vallée alluviale de la Deûle. Les parcelles M500-B (1,5 ha) et M700 (0,8 ha) se situent dans le périmètre le plus proche de l'ancienne usine métallurgique, à environ 1 km au Nord de l'ancien site industriel.

Ce dispositif comprend également une parcelle témoin (MC) considérée comme non-massivement contaminée dans la démarche. D'une surface de 1,3 ha, elle est localisée en zone rurale, à Linzeux (50°20′46″N 2°12′15″E), à environ 75 km du site atelier.

2.2.2. Caractéristiques des sols des parcelles étudiées

Sur chacune des cinq parcelles étudiées, trois échantillons composites de sol ont été constitués dans l'horizon labouré (0 - 25 cm). Ils ont été analysés par le Laboratoire d'Analyses des Sols de l'INRA d'Arras selon des protocoles normés : granulométrie (NF X31-107), pH eau (NF ISO 10390), carbonates totales (NF ISO 10693), carbone organique (CO), azote total (NF ISO 13878), phosphore assimilable (P_2O_5, NF X31-161, méthode Joret-Hébert), capacité d'échange cationique (CEC, NF X31-130), cations échangeables (NF X31- 108), fer libre et manganèse (NF X31-120, méthode Mehra-Jackson), concentrations totales en Cd, Pb et Zn (NF X31-147). Le **Tableau 2.1** rassemble les données analytiques obtenues.

Les teneurs en limons fins et grossiers de MC (690 g kg^{-1}) sont plus élevées que celles des sols des autres parcelles étudiées (498, 522, 569 et 530 g kg^{-1} pour M200, M500, M500-B et M700 respectivement). La fraction sableuse est plus élevée dans les sols des parcelles M200 (289 g kg^{-1}), M500-B (257 g kg^{-1}) et M700 (274 g kg^{-1}). Le sol de la parcelle M500 est plus argileux (311 g kg^{-1}) que ceux des autres parcelles dont la teneur en argile avoisine 200 g kg^{-1}.

Au sein d'une parcelle, la texture des sols a été considérée comme relativement homogène. Ceci n'est pas le cas pour la parcelle M200 où trois types de sols, avec différents niveaux d'engorgement en eaux (g) pendant la période pluvieuse, ont été clairement identifiés (**Fig. 2.2**). En effet, la topographie de cette parcelle correspond à une pente régulière inclinée vers le nord-est. Sur la partie haute, les sols sont développés sur des limons argileux (M200-1) et des limons argilo-sableux (M200-3).

Tableau 2.1. Paramètres physico-chimiques des sols (0 - 25 cm) des parcelles de miscanthus étudiées (n = 3).

	Unité	M200	M500	M500-B	M700	MC
Paramètres agro-pédologiques						
Argile	g kg^{-1}	213 ± 47^{b}	311 ± 19^{a}	175 ± 12^{b}	195 ± 20^{b}	200 ± 32^{b}
Limons fins	g kg^{-1}	177 ± 19^{b}	183 ± 21^{b}	191 ± 12^{b}	180 ± 3^{b}	265 ± 0^{a}
Limons grossiers	g kg^{-1}	332 ± 71^{b}	339 ± 18^{b}	378 ± 26^{b}	350 ± 22^{b}	425 ± 13^{a}
Sables fins	g kg^{-1}	259 ± 35^{a}	147 ± 46^{b}	227 ± 25^{b}	234 ± 10^{a}	105 ± 18^{c}
Sables grossiers	g kg^{-1}	30 ± 8^{ab}	21 ± 6^{b}	30 ± 3^{b}	40 ± 3^{a}	6 ± 2^{c}
pH eau		$7,8 \pm 0,5^{a}$	$8,0 \pm 0,0^{a}$	$8,1 \pm 0,1^{a}$	$8,2 \pm 0,1^{a}$	$6,1 \pm 0,6^{b}$
Carbonates totales	g kg^{-1}	$7,73 \pm 7,91^{bc}$	$22,73 \pm 2,80^{a}$	$6,63 \pm 1,10^{b}$	$10,15 \pm 3,32^{b}$	$\leq 1,10^{c}$
Carbone organique	g kg^{-1}	$16,4 \pm 1,8^{b}$	$35,0 \pm 1,6^{a}$	$16,5 \pm 0,7^{b}$	$18,2 \pm 0,4^{b}$	$8,9 \pm 5,6^{c}$
N total	g kg^{-1}	$1,22 \pm 0,08^{b}$	$2,8 \pm 0,15^{a}$	$1.22 \pm 0,06^{b}$	$1,20 \pm 0,03^{b}$	$0,90 \pm 0,53^{b}$
C/N		$13,4 \pm 0,7^{b}$	$12,8 \pm 0,1^{b}$	$13,6 \pm 0,4^{b}$	$15,2 \pm 0,4^{a}$	$9,3 \pm 0,9^{c}$
P_2O_5 Joret-Hébert	g kg^{-1}	$0,16 \pm 0,02^{a}$	$0,11 \pm 0,03^{b}$	$0,12 \pm 0,01^{b}$	$0,16 \pm 0,01^{a}$	$0,13 \pm 0,05^{ab}$
CEC	$cmol^{+}$ kg^{-1}	$16,33 \pm 4,0^{b}$	$32,83 \pm 1,3^{a}$	$14,8 \pm 0,7^{b}$	$14,90 \pm 1,6^{b}$	$10,00 \pm 2,3^{c}$
Ca^{2+}	$cmol^{+}$ kg^{-1}	$17,16 \pm 3,50^{b}$	$33,93 \pm 1,15^{a}$	$16,4 \pm 0,5^{b}$	$16,63 \pm 1,50^{b}$	$9,97 \pm 2,60^{c}$
Mg^{2+}	$cmol^{+}$ kg^{-1}	$0,72 \pm 0,27^{ab}$	$1,00 \pm 0,22^{a}$	$0,65 \pm 0,05^{b}$	$0,61 \pm 0,07^{b}$	$0,50 \pm 0,12^{c}$
Na^{+}	$cmol^{+}$ kg^{-1}	$0,04 \pm 0,01^{a}$	$0,07 \pm 0,02^{a}$	$0,04 \pm 0,03^{a}$	$0,08 \pm 0,02^{a}$	$0,06 \pm 0,02^{a}$
K^{+}	$cmol^{+}$ kg^{-1}	$0,75 \pm 0,10^{a}$	$0,63 \pm 0,17^{ab}$	$0,43 \pm 0,03^{c}$	$0,60 \pm 0,04^{b}$	$0,26 \pm 0,04^{d}$
Fe libre	g kg^{-1}	$4,50 \pm 0,37^{c}$	$7,62 \pm 0,66^{b}$	$3,05 \pm 0,09^{d}$	$4,90 \pm 0,25^{c}$	$9,73 \pm 0,55^{a}$
Mn^{2+}	g kg^{-1}	$0,34 \pm 0,02^{b}$	$0,13 \pm 0,01^{d}$	$0,23 \pm 0,01^{c}$	$0,24 \pm 0,01^{c}$	$0,39 \pm 0,02^{a}$
Concentrations totales en métaux						
Pb	mg kg^{-1}	215 ± 24^{c}	532 ± 46^{b}	547 ± 32^{b}	731 ± 67^{a}	20 ± 3^{d}
Cd	mg kg^{-1}	$3,8 \pm 0,6^{c}$	$9,7 \pm 0,7^{b}$	$11,0 \pm 1,0^{b}$	$14,1 \pm 1,4^{a}$	$0,2 \pm 0,1^{d}$
Zn	mg kg^{-1}	330 ± 43^{c}	584 ± 49^{b}	813 ± 74	1000 ± 88^{a}	62 ± 2^{d}

Le sol M200-1 est développé sur des argiles profondes et homogènes présentant un drainage modéré à imparfait (g : 30 - 50 cm). Le sol M200-3 est issu des limons argilo-sableux profonds, bien drainés reposant vers 1,10 cm sur une craie (g : 30 - 50 cm). Dans la partie basse, le recouvrement limoneux s'épaissit pour excéder 1,20 m (M200-2).Ce dernier sol est issu des limons très profonds, homogènes et bien drainés (g > 1,20 cm).

Les sols de la parcelle M500 sont issus d'alluvions limono-argileuses calcaires (g : 30 cm). Les sols des parcelles M500-B et M700 sont constitués de limons et de limons argileux reposant sur des matériaux argilo-limoneux à argilo-sableux (g : 30 - 50 cm). Enfin, les sols de la parcelle MC sont développés sur des limons loessiques de plus de 1,20 m d'épaisseur (g > 1,20 cm).

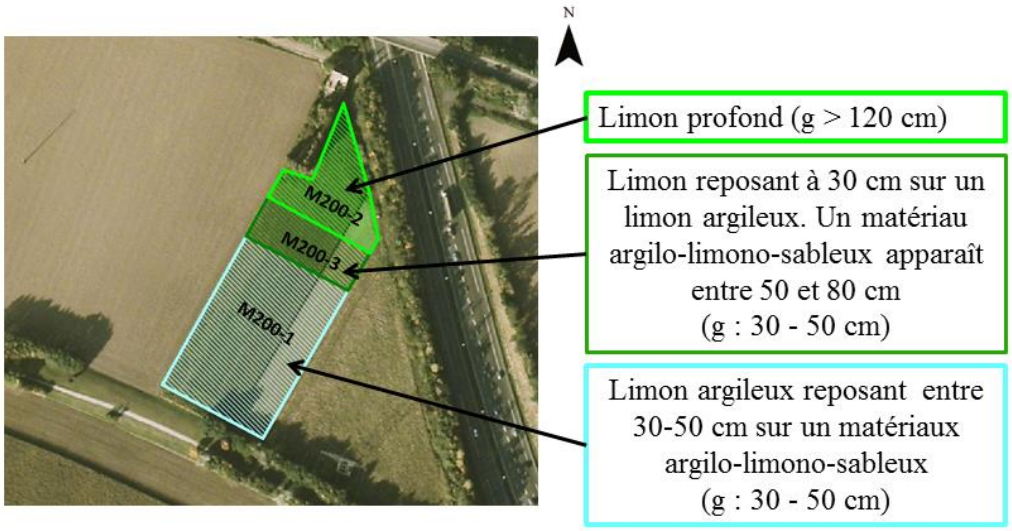

Fig. 2.2. Types de sols sur la parcelle M200.

Le pH des sols des parcelles contaminées est légèrement alcalin (7,8, 8 et 8,2 respectivement pour M200, M500 et M700). En revanche, le sol de la parcelle MC a un pH légèrement acide (pH = 6,1).

Les teneurs en carbonates totales, en azote total, en carbone organique, CEC, Ca^{2+} et Mg^{2+} sont les plus élevées sur la parcelle M500 et sont significativement différentes des teneurs en ces éléments mesurées sur les autres parcelles. Il est à noter qu'en comparaison des autres parcelles, le sol de la parcelle MC se différencie par des teneurs en carbonates totales, carbone organique, une CEC et un ratio C/N significativement plus faibles. Ce ratio C/N est plus élevé sur le sol de la parcelle M700 (15,2) que sur les autres parcelles.

La teneur en P_2O_5 est plus élevée sur les parcelles M200 (0,16 g kg^{-1}) et M700 (0,16 g kg^{-1}) que sur M500 et M500-B. Les teneurs Fe libre et en Mn^{2+} sont plus élevées sur la parcelle MC.

Outre des différences significatives en lien avec l'origine des matériaux parentaux et les paramètres agro-pédologiques, les sols des parcelles étudiées diffèrent par leur degré de contamination (**Tableau 2.1**). Les concentrations en Cd, Pb, et Zn mesurées dans les sols permettent d'établir un classement des quatre parcelles selon l'ordre décroissant suivant : M700 > M500-B > M500 > M200 > MC. Pour les parcelles contaminées, un gradient de contamination est constaté selon la distance à l'ancienne fonderie (**Fig. 2.1**). Cependant, les concentrations en Cd et Pb ne sont pas différentes entre M500 et M500-B. Les concentrations en ETM du sol de la parcelle MC sont légèrement inférieures aux Teneurs Agricoles Habituelles (TAH) des sols limoneux de la région (Sterckeman et al., 2002).

L'analyse géostatistique (par krigeage ordinaire) de la distribution des teneurs en Cd, Pb et Zn dans les sols a mis en evidence une variabilité spatiale de la contamination (**Fig. 2.3 et 2.4;** Douay et al., 2014). Cette composante, ainsi que les différences au niveau des paramètres agro-pédologiques, a été intégrée dans la mise en place des protocoles d'échantillonnage et d'analyse des données obtenues. Ainsi, pour chaque campagne d'échantillonnage, le sol était prelevé au droit de chaque pied de miscanthus échantillonné afin de constituer un couple sol/plante. Afin d'étudier la relation entre le degré de contamination des sols et l'accumulation des ETM dans les organes de miscanthus, les facteurs de biococentration (BCF) de Cd, Pb et Zn ont été calculés pour chacun des quatre organes (feuilles, tiges, rhizomes et racines). Il s'agit du ratio entre la concentration d'un ETM dans l'organe végétal sur celle mésurée dans le sol.

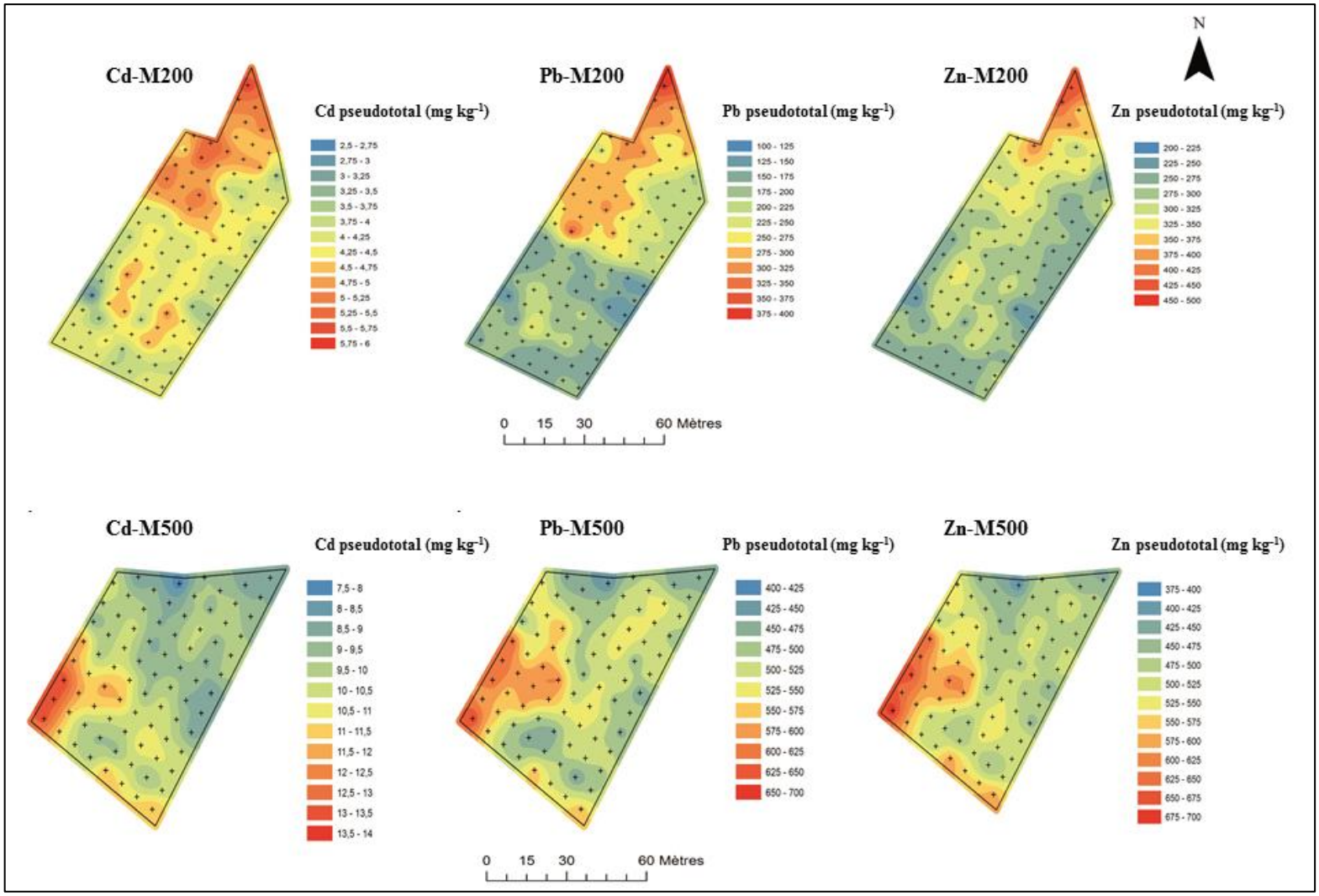

Fig. 2.3. Cartes des concentrations pseudototales en Cd, Pb et Zn de l'horizon labouré des parcelles M200 et M500 (Douay et al., 2014).

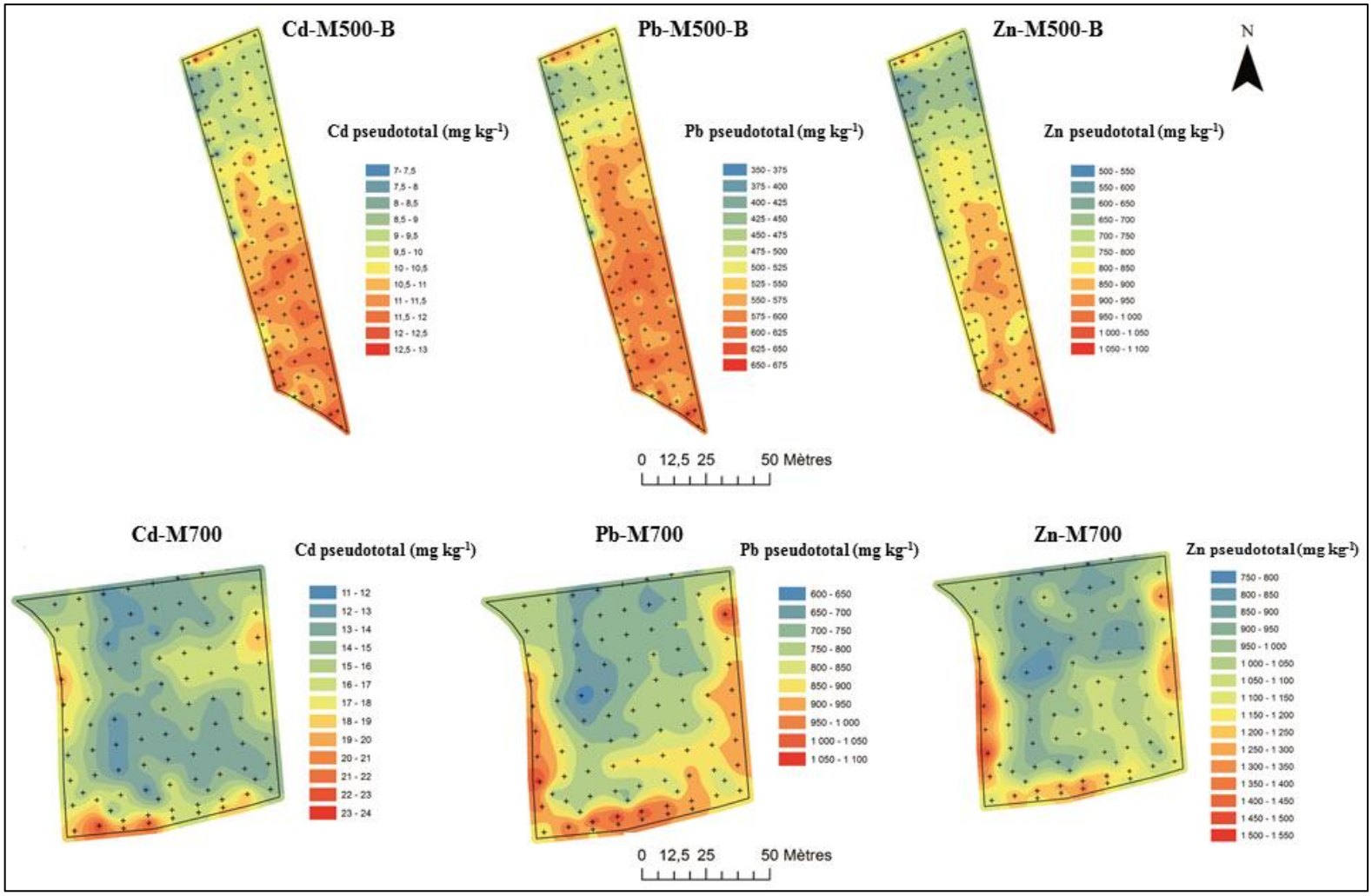

Fig. 2.4. Cartes des concentrations pseudototales en Cd, Pb et Zn de l'horizon labouré des parcelles M500-B et M700 (Douay et al., 2014).

2.2.3. Mise en place des plantations de miscanthus

Les plantations de miscanthus ont été établies sur les parcelles avec un objectif double : 1) étudier le comportement de *Miscanthus x giganteus* face au gradient de contamination des sols et 2) étudier l'influence des pratiques culturales sur le transfert de Cd, Pb et Zn du sol vers les organes du miscanthus ainsi que sur la capacité de cette plante à produire une biomasse (**Table 2.2**).

Tableau 2.2. Synthèse des paramètres étudiés selon les parcelles de miscanthus.

Parcelles		Année de plantation	Pratiques agronomiques				
			Origine du miscanthus	Nature des plantations	Densité[1]	Ajout ou pas d'un inoculum mycorhizien[2]	Fertilisation azotée[3]
Contaminées	M700	2010	Bical	Plants prédémarrés	LD/HD	M	F
			Rhizosfer	Plants prédémarrés	LD/HD	M	F
			Exploitation agricole	Plants prédémarrés	LD/HD	M	F
	M500-B	2010	Bical	Plants prédémarés	LD		
		2010	Rhizosfer	Rhizomes	HD		
	M500	2008	Bical	Rhizomes	HD		
	M200	2008	Bical	Rhizomes	HD		
Témoin	MC	2007	Bical	Rhizomes	HD		

[1]LD : densité équivalente à 15 000 plants à l'hectare.

[1]HD : densité équivalente à 20 000 plants à l'hectare.

[2]M : ajout de 10 g d'un inoculum endomycorhizien lors de la plantation.

[3]F : fertilisation à partir de la 3ème année.

Pour répondre au premier objectif, les rhizomes de miscanthus (Mis-B) provenant de la société Bical France (actuellement NovaBiom) ont été plantés sur les parcelles MC, M200 et M500 au moyen d'une planteuse à pommes de terre avec une densité de 20 000 plants ha^{-1}. L'ensemble de ces parcelles n'a fait l'objet d'aucune fertilisation minérale. Ce mode de gestion reflète les pratiques agricoles mises en œuvre en région.

Sur la parcelle M700, le dispositif mis en place correspond au deuxième objectif. Les paramètres correspondant aux pratiques culturales choisies comprennent :

- l'origine des plants de miscanthus,
- la densité de plantation : 15 000 et 20 000 plants ha^{-1},

- l'ajout d'un inoculum mycorhizien commercial,
- la fertilisation azotée (dès la 3ème année).

Les rhizomes de miscanthus utilisés ont trois origines distinctes. Les rhizomes Mis-A ont été fournis par la société Rhizosfer. Les rhizomes Mis-I ont été fournis par une exploitation agricole située en région parisienne. Les rhizomes Mis-B ont été prélevés sur la parcelle MC plantée à l'origine au moyen de matériel fourni par la société Bical.

Contrairement aux autres parcelles, il a été planté des plants de miscanthus pré-démarrés sur M700. Pour la production de ces plants, réalisée sous serre par l'Institut de Genech, des fragments de rhizomes portant 2 à 3 bourgeons ont été placés dans des pots en polyéthylène contenant du terreau. Ceux-ci ont été arrosés régulièrement. Les plants pré-démarrés ont été installés manuellement en mai - juin 2010 dans des sillons (**Fig. 2.5)**.

Fig. 2.5. Mode de plantation des plants pré-démarrés de miscanthus sur la parcelle M700.

Afin de déterminer l'influence de l'origine des plants, de la densité de plantation, de l'ajout d'un inoculum endomycorhizien et de la fertilisation azotée sur le miscanthus, 24 modalités, avec 3 réplicats pour chacune, soit 72 placettes, ont été installées sur la parcelle M700 (**Fig. 2.6**). La répartition des modalités et des répétitions dans les blocs inoculés ou non a été réalisée de façon aléatoire par tirage au sort. Elles ont été réparties sur six blocs séparés par une bande de 5 m de large.

Chacun de ces blocs est divisé en placettes (4 x 10 m) plantées de six rangs de miscanthus espacés de 0.80 m. La densité de plantation a été obtenue en faisant varier la distance entre les plants sur les rangs. La densité de plantation a été de 15 000 ou de 20 000 pieds ha^{-1} pour la faible et forte densité respectivement.

Un inoculum endomycorhizien commercial a été utilisé. Il s'agit de SolRize® (Agrauxine, Saint Evarzec, France), une souche de champignons endomycorhiziens *Glomus LPA*. Lors de la plantation, l'inoculum a été apporté à raison de 10 g par plant. Compte tenu de la topographie de la parcelle, les placettes comportant l'ajout de l'inoculum endomycorhizien ont été regroupées dans la partie basse de la parcelle de façon à limiter une éventuelle

propagation, par ruissellement superficiel des eaux pluviales, des mycorhizes vers les plantations n'ayant pas été inoculées.

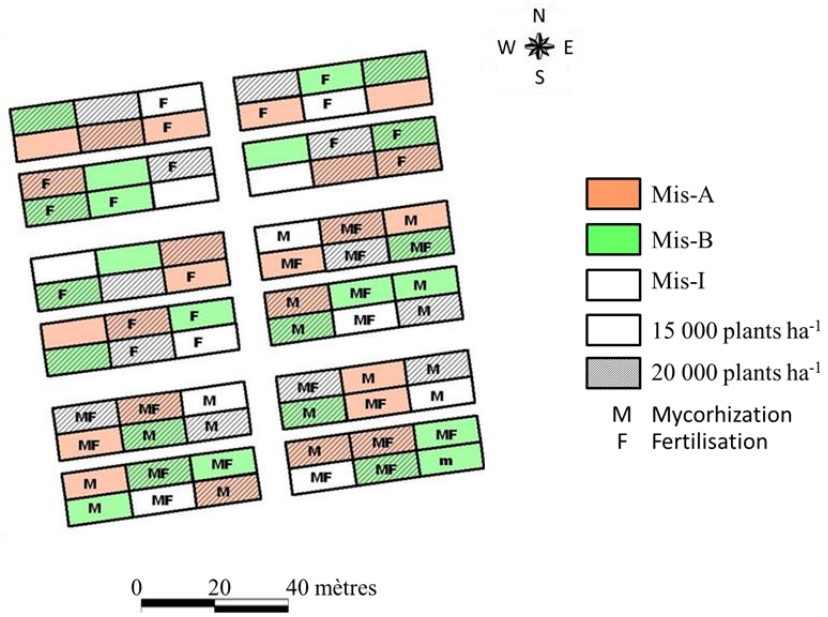

Fig. 2.6. Schéma de disposition des modalités mises en œuvre sur la parcelle M700.

Un à deux litres d'eau a été apporté par plant lors de la mise en place des plants pré-démarrés. Compte tenu d'un printemps particulièrement sec, l'irrigation des plants a été renouvelée à plusieurs reprises.

Après la plantation des plants de miscanthus, un grillage a été installé autour de la parcelle pour limiter les dégâts engendrés par les lapins.

En juin 2012, 50 unités d'azote (ammonitrate, 27 %), ont été apportées manuellement sur la moitié du dispositif (soit 36 placettes). Cette fertilisation a pour but de compenser les exportations éventuelles.

2.3. Expérimentation *ex situ*

En février 2012, des rhizomes ont été prélevés sur un seul pied de miscanthus de la parcelle MC et ceci, pour constituer des plants du même génotype. Comme pour l'expérimentation *in situ*, des fragments de rhizomes portant 2 à 3 bourgeons ont été placés dans des pots en polyéthylène contenant du terreau. Ils ont été disposés en serre et arrosés régulièrement. Des plants ayant atteint un même stade de croissance (tiges atteignant environ 25 cm de hauteur) ont été sélectionnés et repiqués individuellement dans un pot contenant 2,5 kg de terre provenant des parcelles du dispositif *in situ*.

Les six sols utilisés (MC, M200-1 et M200-3, M500, M500-B et M700) présentent un gradient de contamination tel que décrit plus haut (**Tableau 2.1**). Pour chacun des sols, 6 réplicats ont été constitués, soient 36 pots plantés de plants de miscanthus.

La culture du miscanthus en pots a été effectuée du 12 avril au 23 juillet 2012, soit pendant 93 jours.

Chapitre III :

Potentialités de *Miscanthus* × *giganteus* pour la phytostabilisation des sols contaminés par des métaux: expérimentation *ex situ*

Préambule

Le phytomanagement est l'une des solutions proposées et adaptées à la gestion de grandes surfaces comme sur les parcelles agricoles du site atelier Metaleurop. Les plantes choisies doivent permettre la réduction des risques environnementaux et sanitaires qu'engendre cette contamination. Elles doivent aussi permettre une reconversion de l'agriculture soucieuse des enjeux économiques en lien avec la production de matières renouvelables. Miscanthus (*Miscanthus* × *giganteus*) se développe bien dans les conditions pédoclimatiques de la région et pourrait être un bon candidat pour le phytomanagement des sols fortement contaminés en Cd, Pb et Zn du site Metaleurop.

Afin de mieux discerner le comportement du miscanthus face à la contamination métallique des sols et de s'affranchir des contraintes liées aux expérimentations *in situ*, cette plante a été cultivée en serre sur les sols issus des parcelles agricoles expérimentales situées aux alentours de l'ancienne fonderie (**cf. Chapitre II**). Le gradient de contamination et la variabilité des paramètres physico-chimiques des sols ont été pris en compte dans l'étude du comportement du miscanthus.

Les objectifs spécifiques de cette étude sont :

- étudier les effets à court terme du miscanthus sur la distribution et la mobilité de Cd, Pb et Zn dans les sols étudiés,
- mesurer les paramètres de croissance en lien avec la production de biomasse,
- déterminer l'accumulation et la distribution des métaux dans les organes du miscanthus (racines, rhizomes, tiges, feuilles).

La culture de miscanthus en pots sur les sols contaminés (M200-1, M200-3, M500, M500-B et M700) et le sol non contaminé (MC) a été effectuée du 12 avril au 23 juillet 2012, soit pendant 93 jours.

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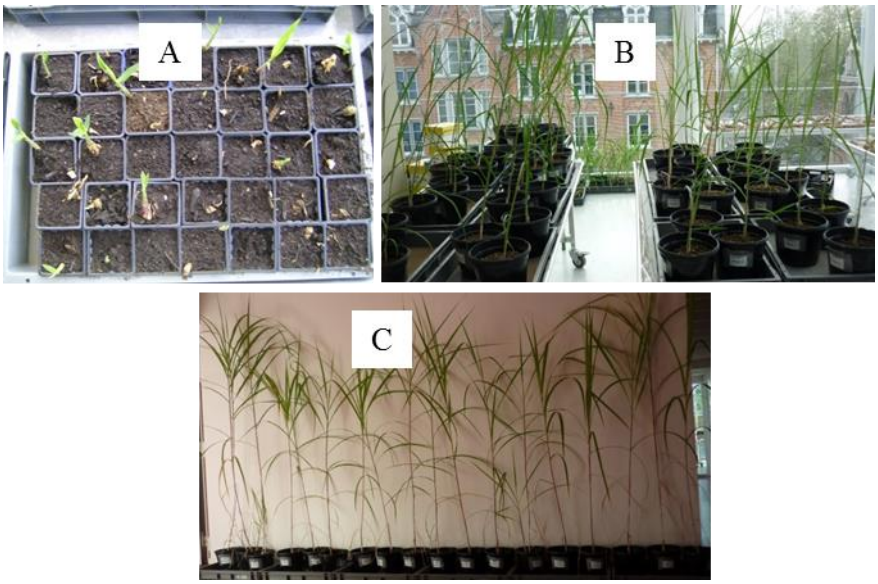

Fig. 3.1. Differentes étapes de la culture du miscanthus lors de l'experimentation : **A**) prédémarrage des plants (février 2012), **B**) culture et croissance sur les sols contaminés (mai 2012), **C**) fin de l'experimentation (juillet 2012).

Ex situ growth of *Miscanthus* × *giganteus* reveals its potentials for phytostabilization of trace element-contaminated soils

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Abstract

Soil contamination by potentially toxic trace elements (TE) is of great concern worldwide. Phytomanagement is proposed as a cost-effective, environmentally-friendly option for sustainable use of large contaminated areas. Preliminary studies of TE mobility in soils and their accumulation in candidate plants grown in microcosms help in the choice and validation of the management options to apply so as to contain or decrease pollutant linkages. For the present study, the energy crop miscanthus (*Miscanthus* × *giganteus*) was grown in *ex situ* conditions on agricultural soils presenting a Cd, Pb and Zn contamination gradient. After 93 days of culture, shoot and root growth parameters were measured. Also, soils and plants were sampled to study TE accumulation in miscanthus, the effects of this plant on TE mobility in soils. The results show that miscanthus growth performance depended more on the silt content in the soils rather than on TE-contamination level. The overall TE fractionation did not significantly differ between unplanted and planted soils. Some observed changes in pH and in TE mobility could be due to complex rhizosphere processes driving plant mineral uptake, and organic carbon inputs in soils. In contaminated soils, miscanthus accumulated Cd, Pb and Zn mainly in roots, and strongly reduced transfer of these elements from soil to all organs and from roots to rhizomes, stems and leaves. Therefore, miscanthus could be considered as a TE-excluder, hence a potential candidate crop for coupling phytostabilization and biomass production on studied TE-contaminated soils.

Key words: Miscanthus, Excluder, Rhizosphere, Trace Element Mobility, Phytomanagement

3.1. Introduction

Soil contamination by potentially toxic elements is of great concern worldwide (Kabir et al., 2012; Panagos et al., 2013). For instance, most of trace element (TE) enrichment in soil is due to deposit from industrial or associated anthropogenic activities (Panagos, et al., 2013). Crop production for food or fodder on contaminated soils may result in the accumulation of toxic TE such as cadmium (Cd) or lead (Pb) in edible crop parts, hence increased health risks (Douay et al., 2013). Physico-chemical methods can be used for soil remediation but they are

expensive, environmentally-unfriendly, and can only be efficient for small surfaces (Yao et al., 2012). As an alternative, phytoremediation is a cost-effective and environmentally-friendly technology using plants and associated microorganisms (bacteria and arbuscular mycorrhizal fungi) for remediation of contaminated soils, water and sediments (Mench et al., 2010). Phytoremediation of TE-contaminated land is mainly based on (1) phytoextraction, i.e. the uptake and accumulation of TE from the soil into the harvestable plant parts; and (2) phytostabilization, i.e. the use of plants and associated microbes to enhance TE immobilization in the rhizosphere and the roots.

To optimize the phytoremediation processes, suitable plant species and/or their cultivars should be tolerant to targeted contaminants and to on-site abiotic and biotic stresses (Mench, et al., 2010). Preliminary studies of TE accumulation in candidate plants grown in microcosms help in the choice and/or validation of the phytoremediation options and appropriate agronomic practices to apply so as to contain or decrease pollutant linkages. In *ex situ* conditions, such studies allow overcoming confounding effects due to additive stress from *in situ* environmental variations, e.g. water availability, wind exposure, nutrient gradient, pathogens, and herbivores (Vangronsveld et al., 2009). Moreover, field plantations are expensive to put in place, hence microcosms studies can help to optimize large scale field plantations (Alkorta et al., 2004).

A sustainable phytoremediation hereafter referred to as phytomanagement, couples mitigation of environmental and health risks, restoration of ecosystem services with the production of valuable plant-based feedstock (Conesa et al., 2012). The energy crop *Miscanthus* × *giganteus*, hereafter referred to as miscanthus, was proposed as a good candidate for phytomanagement of contaminated sites/soils (Nsanganwimana et al., 2014; Pidlisnyuk et al., 2014). Indeed, this plant has a high pest resistance, can grow on a wide range of climatic conditions, and has a high productivity with minimal nutrient requirements (Lewandowski et al., 2000; Nsanganwimana et al., 2014). Moreover, long-term cultivation of this crop on agricultural soils increases soil carbon, cation exchange and water retention capacities (Kahle et al., 1999).

Despite some field experiments demonstrating the capacity of this species to produce energy-dedicated biomass on TE-contaminated soils (Barbu et al., 2013; Pogrzeba et al., 2013), little is known on TE accumulation patterns in its organs. To date, the only studies on TE distribution patterns were realized in hydroponic conditions spiked with Cd (Arduini et al., 2006) and in mining wastes (Technosols) contaminated by As and Pb (Wanat et al., 2013). Though these studies provide insights into the matter, it is worth noting that they do not

represent real soil conditions. Therefore, there is still much knowledge lacking with regard to TE accumulation in miscanthus grown on TE contaminated soils so as to determine the phytoremediation option stemming from its cultivation on these soils. Also, there is a need to assess effects on soil contamination level and physico-chemical parameters on TE accumulation in miscanthus organs.

For the present study, miscanthus was grown in greenhouse on agricultural soils presenting a Cd, Pb and Zn contamination gradient and different physico-chemical parameters. The work here reported aimed at a) studying short-term effects of miscanthus on TE distribution and mobility in soils, b) assessing its potential for biomass production and c) TE accumulation and distribution patterns in its organs in contaminated soils.

3.2. Materials and methods

3.2.1. Plant material

The plant material used during this experiment came from a 4-year old miscanthus plantation established in the Northern France at Linzeux (50°20'46''N, 2°12'15''E) in uncontaminated agricultural soil (MC). This plantation consists of the commercial hybrid *Miscanthus* × *giganteus,* supplied by NovaBiom France. Rhizomes were sampled in winter (January 2012) from one miscanthus clump. The samples were directly put into a polyethylene bag, and preserved at 4°C prior to the experiment.

3.2.2. Soil origin

Six soils, hereafter named MC, M200-1, M200-3, M500, M500-B and M700 were used to grow miscanthus under greenhouse conditions. Soils are composite samples which were homogenized, dried and sieved to pass through a 5 mm mesh. The soil MC was collected from uncontaminated agricultural plot, whereas the other five soils were collected from the contaminated agricultural plots located around the closed-down Pb smelter, Metaleurop Nord (**Fig. 2.1**). In this area, agricultural soils are contaminated by Cd, Pb and Zn at levels of 20 to 50-fold higher than the frequent regional concentrations in arable soils (Sterckeman et al., 2002). As the contamination level decreases with the distance to the location of the former Pb smelter (**Fig. 2.1**), the five soils present a Cd, Pb and Zn contamination gradient with M200-1 or M200-3 and M700 being the least and the most contaminated respectively.

3.2.3. Plant growth

3.2.3.1. Rhizome sprouting

Clean polyethylene pots (7 x 7 x 8 cm, Desch PlantPak) were filled with plant compost in which small pieces of miscanthus rhizomes (5-7 cm long, 2-3 buds) were introduced horizontally at 4-5 cm deep and completely covered. Tap water was sprayed on the newly planted compost to keep it moist. Planted pots were provided with a retention tray, and rested on a table under greenhouse conditions.

3.2.3.2. Transplantation of miscanthus plantlets

Prior to the plantation, studied soils were first air-dried, milled and sieved to 10 mm. For miscanthus culture, pots (4 liter-21 cm, Desch PlantPak, Germany) were filled with 2.5 kg of soil. In total, 36 pots were prepared comprising 6 pots for each soil. Plantlets with 25 cm high were considered for transplantation. During this operation, plantlets were carefully removed from the pre-plantation pots and directly deposited in the transplantation pots at a depth of 5 cm. Growth occurred in ambient air and light conditions of the greenhouse, and day temperature variations remained in the range of 16-22°C. Permutation of pot positions was carried out every two weeks to avoid point and borderline effects. When necessary, a capillary irrigation was applied using composite water (pH = 7.2) made from osmotic water and tap water in 4:1 ratio, v/v. Soil humidity was maintained above 70% across the experiment for 93 days (T_{93}). Neither fertilizers nor pesticides were used. Weeds were manually removed and left on the soil surface. There was no fungal and algal growth on soil surface or on pot walls during the experiment.

3.2.4. Sampling and preparation for analysis

3.2.4.1. Plant samples

Prior to harvest, growth parameters including the leaf number, stem height and diameter were taken. The diameter was taken at the mid-point between the two basal nodes on each stem. The shoots were cut with clippers at 1 cm above soil. They were separated into stems and leaves, which were directly weighed for fresh weight (FW) determination. The belowground parts were manually separated and sorted from soils. They were firstly washed with tap water and then thoroughly rinsed in osmotic water. Roots were separated from rhizomes and both parts were cut into very small pieces. All samples were oven-dried at 40°C up to constant weight for chemical analysis, and a small fraction of each sample was dried at 105°C overnight for determination of dry weight (DW) according to ISO 11465.

Dry samples were ground into a fine powder with a cutting mill (GM200, Retsch, Germany) for leaves and roots, and with an ultracentrifuge mill (ZM200, Retsch, Germany) for stems and rhizomes. A sample (300 mg) was directly weighed into a digestion tube. Concentrated nitric acid (HNO_3, 70%, 5 mL) was added and then the aliquot heated at 95°C for 75 min using a digestion plate (HotBlock Environmental Express, USA). After cooling, hydrogen peroxide (H_2O_2, 30%, 5 mL) was added and the mixture heated again at 95°C for 3 hours. Quality control for chemical extractions and sample digestion was performed by including internal reference sample. The digested aliquot was adjusted to 25 mL volume with osmotic water and filtered (0.45 µm, cellulose acetate membrane, Minisart).

3.2.4.2. Soil samples

At T93, soils were emptied from the pots and spread on plastic trays to remove all plant debris. They were oven-dried at 40°C, and then crushed (ZM 200 Mill, Retsch, Germany) and sieved to 2 mm for pH measurement and 250 µm for other analyses described herein.

Soil physico-chemical parameters were analysed according to standardized protocols. The particle size distribution was determined by sedimentation and sieving after destruction of organic matter by H_2O_2 according to the French standard NFX 31-107. The pH (H_2O) was measured after stirring a mixture of soil and deionized water (1:5, v/v) according to the standard ISO 10390. The total carbonate content was determined by measuring the CO_2 formed after adding HCl (4 M) in the aliquot according to NF ISO 10693. The organic carbon (OC) content was extracted and measured according to the standard ISO 14235. The total N content was determined by dry combustion method according to ISO 13878. The phosphorus (P_2O_5) was extracted in ammonium oxalate solution ($(NH_4)_2C_2O_4$, 0.1M, pH = 7) and measured according to the French standard NFX 31-161 and Joret and Hébert (1955). The cationic exchange capacity(CEC) was determined after percolation of CH_3COONH_4 (1M, pH = 7) solution into soil samples followed by an extraction of ammonium ions (NH_4^+) with sodium chloride (NaCl, 1 M) according to the French standard NF X31-130. Iron and Mn oxides were extracted at high temperature (80°C) in the presence of sodium citrate ($C_6H_5Na_3O_7$, 0.3 M) as a complexing agent, sodium bicarbonate ($NaHCO_3$, 0.11 M) as a pH buffer and sodium dithionite ($Na_2S_2O_4$, 1 M) as a reducing agent according to Mehra and Jackson (1960). The determination of Fe and Mn contents soils extracts was performed by flame atomic absorption spectrometry (AA-6800).

For determination of Cd, Pb, Zn pseudototal concentrations, soil digestion was performed in aqua regia using a digestion plate. A soil sample (300 mg) sieved at 250 µm was directly

measured in digestion tube in which a mixture (6 mL) of concentrated acids HCl and HNO_3 was added in 3:1 ratio. Quality control for chemical extractions and sample digestion was performed by including internal reference sample. The aliquots were heated on a digestion at 120°C during 75 min. After cooling, the volume was brought to 25 mL with osmotic water and filtered (0.45 µm, Cellulose acetate membrane).

For the determination of the easily soluble Cd, Pb and Zn concentrations, the soil was mixed with 0.01 M calcium chloride ($CaCl_2$) solution in a ratio of 1:10, m/v according to (Waterlot and Douay, 2012).

For Cd, Pb and Zn fractionation, a four-step extraction procedure was used according to (Waterlot et al., 2012). The extractions allowed to obtain fractions named A, B, D, and R, and defined respectively as: (i) exchangeable, water- and acid-soluble (0.11 mol L^{-1} acetic acid), (ii) reducible (0.5 mol L^{-1} hydroxylammonium chloride), (iii) oxidizable (8.8 mol L^{-1} H_2O_2, followed by 1.0 mol L^{-1} ammonium acetate at pH = 2), and (iv) residual (aqua regia). The quality of analytical data was verified by including the certified reference material CRM BCR®-701.

3.2.4.3. Determination of TE concentrations in plant and soil extracts

The atomic absorption spectrophotometer (AA-6800, Shimadzu) was used to determine Cd, Pb and Zn concentrations in plant and soil extracts. The results were expressed on DW basis.

3.2.6. Data analysis

Analysis of variance was realized on the parameters analyzed and measured in soil and plant samples. The normal distribution of data (Shapiro-Wilk test), and equality of variances (Bartlett test) were checked. Fisher statistics was considered for significance ($p \leq 0.05$), and Tukey HSD test was immediately used for pair-wise comparisons of statistical groups. Principal component analysis was performed to study the relationship between measured miscanthus growth parameters, TE concentrations in miscanthus organs, soil pseudototal and $CaCl_2$-extractable concentrations. All statistical tests were performed using the XLSTAT (Addinsoft™ software 2012).

3.3. Results

3.3.1. Soil physico-chemical parameters

Before the greenhouse experiment, soil physico-chemical parameters were determined (**Table 3.1**). Since soils were thoroughly homogenized, only one sample was analyzed for each soil.

Table 3.1. Soil physico-chemical parameters before (T0) miscanthus greenhouse culture.

Parameters	MC	M200-1	M200-3	M500	M500-B	M700
Clay (%)	21.3	25.4	19.1	29	16.8	15.8
Silt (%)	69.2	44.4	54.7	51.1	61.4	58.8
Sand (%)	9.5	30.2	26.2	19.9	21.8	25.4
pH (H_2O)	5.9	7.6	7.8	8.1	7.7	8.1
Carbonates (g kg^{-1})	< 1	2.1	1.4	22.2	< 1	4.2
Organic C (g kg^{-1})	18.1	15.1	17.3	28.2	15.8	14.2
Total N (g kg^{-1})	1.73	1.22	1.23	2.46	1.18	1.12
C/N	11.2	13.2	13.5	12.7	13.8	14.1
P_2O_5 (g kg^{-1})	0.09	0.15	0.14	0.09	0.09	0.16
CEC ($cmol^+$ kg^{-1})	12.1	13.4	17.3	23	12	11.2
Fe (g kg^{-1})	9.70	4.49	4.50	7.62	3.05	4.9
Mn (g kg^{-1})	0.39	0.29	0.22	0.13	0.23	0.24

Soils M200-1 and M200-3 were collected on the plot M200. Apart from differences in TE concentrations (**Table 3.2**), the six soils differ according to their physico-chemical parameters. Briefly, the MC soil has a lower (acid) pH and higher Fe and silt contents than other soils. There is more clay and lower silt contents in M200-1 than in M200-3. The cation exchange capacity (CEC), total carbonates, organic carbon (OC) and total nitrogen contents are the highest in M500. The M500-B presents the same Cd and Pb contamination level as in M500 but it has lower pH, CEC, carbonates, Fe, organic carbon and total nitrogen contents than in M500. The M700 differs from the M500-B by higher carbonate and P_2O_5 contents.

The pH and the organic carbon were considered as physico-chemical parameters which inform on fast and short-time changes in a planted system; both parameters were therefore measured at T_0 and at T_{93} **(Table 3.2)**. At T_{93}, the mean pH (6.4) in MC was lower than in contaminated soils. In these soils, the pH in M500 (7.6) and M700 (7.5) are higher than in M200-1, M200-3, and M500-B. The pH increased by 10.2% in MC whereas it decreased by 3.7 to 6.7% in contaminated soils. The organic carbon content was lowest in M200-1 (19.4 g kg^{-1}) and highest in M500 (35.4 g kg^{-1}) (**Table 3.2**). Whatever the soil, the organic carbon contents at T_{93} were significantly higher than at T_0, and increased by 25.5-45.3%.

3.3.2. Soil TE pseudototal concentrations

At T_{93}, the TE contamination gradient was maintained among the studied soils (**Table 3.2**). There were some changes in pseudototal concentrations depending on the soil and the element. In MC, Cd concentrations significantly decreased by 63.3%. Pb concentrations did not generally differ from T_0 to T_{93}. Apart from M700 which was characterized by a slight decrease, Zn concentrations generally increased by 3.6-5.5% in the studied soils.

Table 3.2. The pH, the organic carbon (OC), pseudototal and $CaCl_2$-extractable concentrations of Cd, Pb and Zn in soils used for greenhouse miscanthus culture. Different letters refer to significant differences between soils at T_{93} (Tukey HSD test $p \leq 0.05$, $n = 6$).

		MC	M200-1	M200-3	M500	M500-B	M700
pH	T_0	5.9	7.6	7.8	8.1	7.7	8.1
	T_{93}	6.4 ± 0.1^{d}	7.3 ± 0.0^{c}	7.4 ± 0.0^{bc}	7.6 ± 0.0^{a}	7.4 ± 0.1^{b}	7.5 ± 0.0^{ab}
OC (g kg^{-1})	T_0	18.1	15.1	17.3	28.2	15.8	14.2
	T_{93}	24.2 ± 1.3^{b}	19.4 ± 0.8^{d}	18.9 ± 0.8^{d}	35.4 ± 2.7^{a}	22.5 ± 0.6^{bc}	20.6 ± 1.3^{cd}
		Pseudototal concentrations (mg kg^{-1})					
Cd	T_0	0.57	5.0	5.1	10.6	10.9	15.9
	T_{93}	0.2 ± 0.0^{d}	5.0 ± 0.1^{c}	4.8 ± 0.0^{c}	10.4 ± 0.3^{b}	10.1 ± 0.5^{b}	15.2 ± 0.9^{a}
Pb	T_0	11.1	213.5	215.2	476.7	481.7	726.5
	T_{93}	9.5 ± 2.3^{d}	206.9 ± 5.1^{c}	208.8 ± 8.5^{c}	492.3 ± 9.0^{b}	481.4 ± 23.3^{b}	728.3 ± 25.2^{a}
Zn	T_0	51.7	324.0	335.7	546.9	679.2	1012.1
	T_{93}	54.5 ± 1.6^{e}	348.3 ± 11.0^{d}	340.6 ± 17.6^{d}	566.8 ± 9.0^{c}	709.2 ± 26.7^{b}	974.5 ± 49.6^{a}
		$CaCl_2$-extractable concentrations (mg kg^{-1})					
Cd	T_0	0.14	0.21	0.23	0.20	0.42	0.43
	T_{93}	0.12 ± 0.01d	0.19 ± 0.02^{b}	0.18 ± 0.02^{b}	0.16 ± 0.02^{b}	0.30 ± 0.01^{a}	0.31 ± 0.01^{a}
Pb	T_0	0.02	0.03	0.04	0.02	0.04	0.04
	T_{93}	0.02 ± 0.01^{cd}	0.01 ± 0.01^{d}	0.02 ± 0.01^{cd}	0.03 ± 0.00^{bc}	0.05 ± 0.01^{a}	0.04 ± 0.02^{ab}
Zn	T_0	0.50	0.58	1.01	0.51	1.82	1.34
	T_{93}	0.38 ± 0.12^{d}	0.49 ± 0.03^{d}	0.79 ± 0.03^{c}	0.45 ± 0.07^{d}	1.58 ± 0.07^{a}	1.27 ± 0.05^{b}

3.3.3. Extractable Cd, Pb and Zn fractions by 0.01 M $CaCl_2$

The results of 0.01 M $CaCl_2$-extraction are presented in **Table 3.2**. Overall, the extractable TE concentrations increased with soil contamination level and depended on soil physico-chemical parameters. Whatever the soil, Cd and Zn extractable concentrations generally decreased from T_0 to T_{93}. In contaminated soils, Pb concentrations decreased by 52-56% in M200-1 and M200-3.

3.3.4. Cadmium, Pb and Zn fractionation

Sequential extraction allowed obtaining four fractions **(Fig. 3.2**). Each TE fraction is expressed as the mean percentage (%) of the total sum of the four fractions.

Though there were some little differences between T_0 and T_{93}, the overall TE distribution did not change whatever the soil, and TEs are mainly available in fraction A and B. Here, for the purpose of brevity, we will only comment the results on important changes in TE fractions in order to compare the effects of miscanthus culture on TE distribution in the soils (**Table 3.3**). Overall, changes in TE distribution depended on soil and on a given TE. Apart from very few exceptions, the cultivation of miscanthus resulted in a slight reduction of Cd and Pb in

fraction A and a slight increase in the fraction B for both TEs. Unlike changes in Cd and Pb fractionation, the Zn fraction D slightly increased in five out of six studied soils.

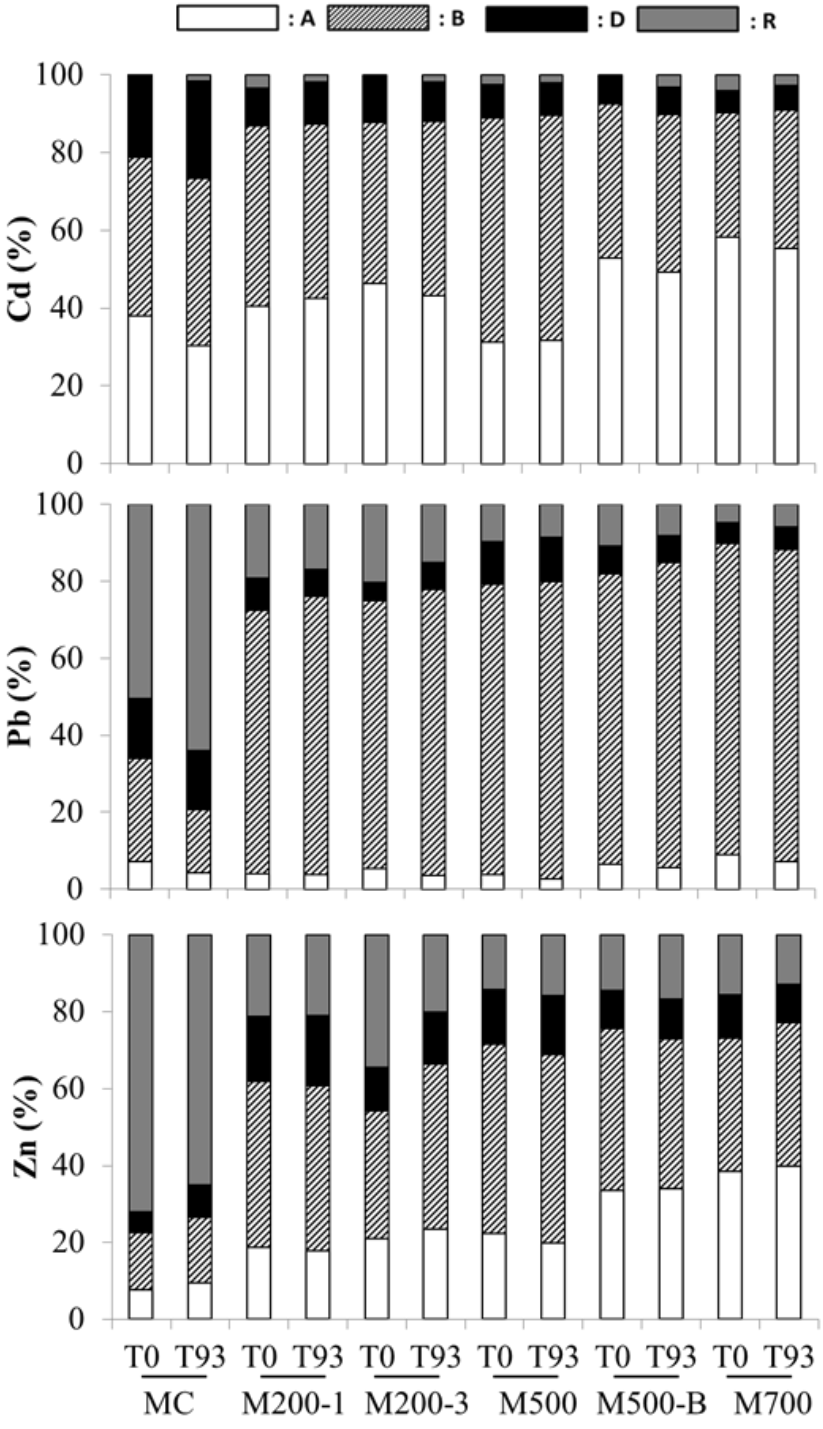

Fig. 3.2. Fractionation (%) of Cd, Pb and Zn in studied soils. The piled bars represent means of each fraction in soils before (T_0) and after (T_{93}) miscanthus plantation. **A**: Exchangeable, water- and acid-soluble; **B**: reducible, mainly occluded in soil oxides; **D**: oxidizable, mainly associated with soil organic matter; **R**: residual, relating to the soil crystalline matrix.

Table 3.3. Changes in Cd, Pb and Zn fractionations in the studied soils. Values represent means of percentage differences (T_{93}-T_0) of each fraction. A: Exchangeable, water- and acid-soluble; B: reducible, mainly occluded in soil oxides; D: oxidizable, mainly associated with soil organic matter; R: residual, relating to the soil crystalline matrix.

Trace element	Fraction	Soils					
		MC	M200-1	M200-3	M500	M500-B	M700
Cd	A	-7.7	2.0	-3.1	0.4	-3.6	-2.9
	B	2.3	-1.4	3.1	0.3	0.8	3.6
	D	3.9	1.0	-1.6	-0.2	0.2	-2.8
	R	1.5	-1.6	1.5	-0.5	2.6	-1.2
Pb	A	-3.0	-0.3	-1.8	-1.2	-0.7	-1.8
	B	-10.3	4.0	4.8	2.0	3.7	0.3
	D	-0.3	-1.3	2.2	3.2	-0.1	0.4
	R	13.6	-2.4	-5.1	-1.3	-2.9	1.1
Zn	A	1.9	-0.8	2.4	-2.6	0.4	1.4
	B	2.1	-0.4	9.8	-0.1	-3.1	2.7
	D	3.0	1.5	2.1	1.0	0.5	-1.2
	R	-7.0	-0.3	-14.3	1.6	2.2	-2.8

3.3.5. Trace element accumulation and distribution in miscanthus

3.3.5.1. Trace element concentrations in miscanthus organs

In general, the Cd concentrations in the four miscanthus organs decreased in the following order: roots >> rhizomes ≥ stems ≥ leaves (**Fig. 3.3**).

The mean concentrations in leaves varied from 0.4 to 2.9 mg kg^{-1} DW. They were significantly higher in M700 and in M500-B than in other soils. The stem concentrations in MC, M200-1, M200-3, and M500 did not significantly differ from leaf concentrations, and are higher in M500-B (3.2 mg kg^{-1}) and M700 (2.3 mg kg^{-1}). Similarly, in M700 and M500-B, the rhizome concentrations (2.2-2.7 mg kg^{-1}) and root concentrations (96.5-118.8 mg kg^{-1}) are significantly higher than in other soils. Cadmium concentrations in rhizomes and roots are significantly lower in MC (0.3 and 2.2 mg kg^{-1} for rhizomes and roots respectively).

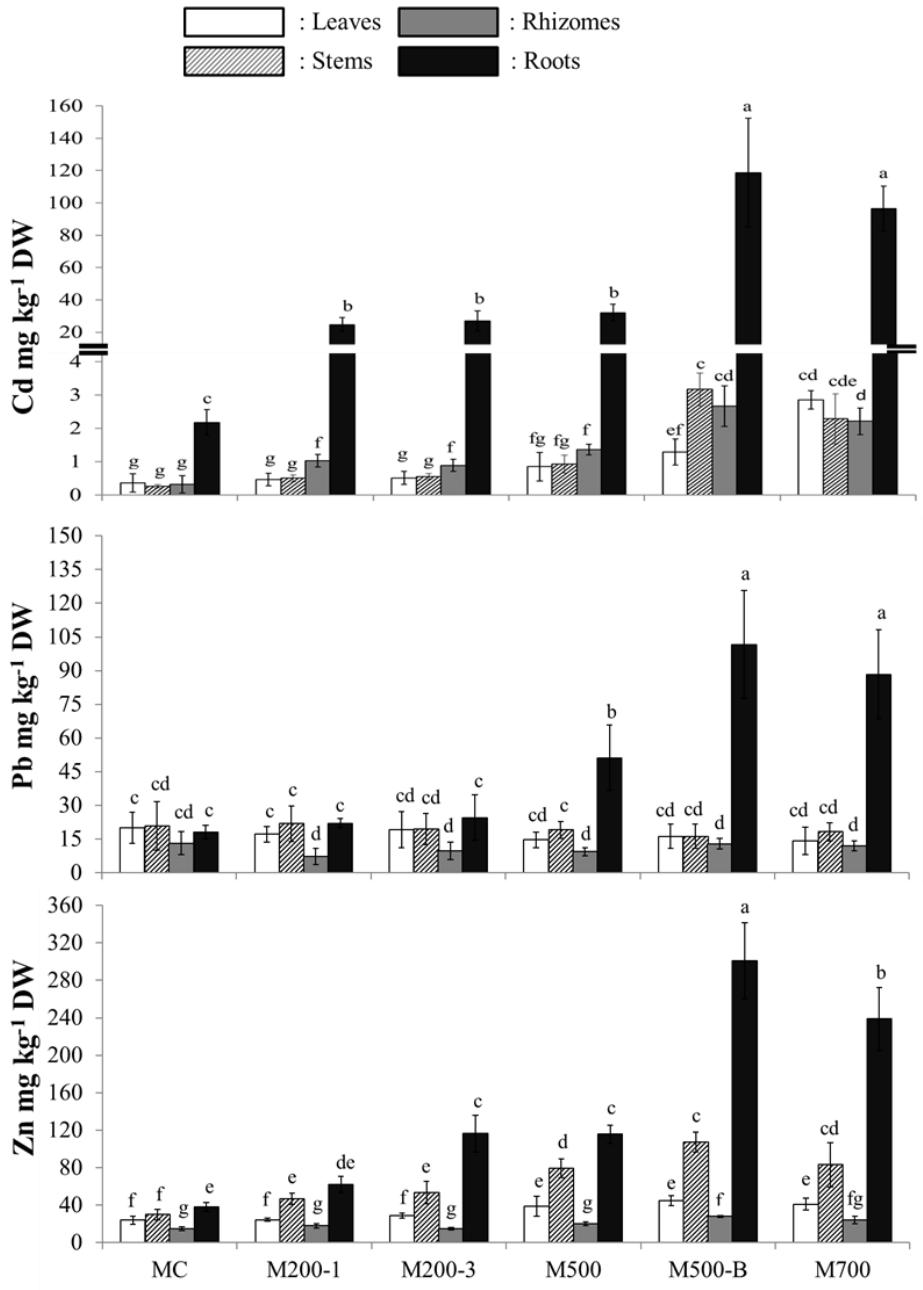

Fig. 3.3. Concentrations (mg kg^{-1} DW) of Cd, Pb and Zn in miscanthus organs. The histograms represent means and associated standard deviations. The different letters refer to significant differences (Tukey HSD test, $p \leq 0.05$) between organs and depending on soil contamination levels.

The Pb concentrations in the four miscanthus organs can be ranked as follows: roots >> stems = leaves ≥ rhizomes (**Fig. 3.3**). The concentrations in leaves and in stems varied from 14.7 to 21.9 mg kg^{-1} and did not significantly differ whatever the soils. Similarly, there was no significant difference in rhizome concentrations (7.3-13.2 mg kg^{-1}) among the soils. The root Pb concentration in MC is 18.0 mg kg^{-1}, which is 1.2, 1.4, 2.8, 5.6, and 4.9-fold lower than in M200-1, M200-3, M500, M500-B and M700 respectively. These concentrations were not significantly different between MC and M200-1 and M200-3 on one hand, and between M500-B and M700 on the other hand.

Whatever the soil, Zn concentrations in miscanthus organs can be ranked as follows: roots > stems > leaves > rhizomes (**Fig. 3.3**). In MC, M200-1 and M200-3, the concentrations in leaves (23.8 to 28.6 mg kg^{-1}) were not significantly different and were lower than in M500, M500-B and M700 (**Fig. 3.3**). The stem concentrations were the lowest in MC (29.9 mg kg^{-1}), and highest in M500-B (107.3 mg kg^{-1}) and in M700 (83.0 mg kg^{-1}). Similarly, the rhizome concentrations in M500-B (27.8 mg kg^{-1}) are higher than in other soils whose concentrations varied from 14.8 to 21.1 mg kg^{-1}. The root concentrations increased with the soil contamination level though they were the highest in M500-B (300.7 mg kg^{-1}).

Overall, whatever the TE, concentrations were higher in roots than in rhizomes and both aboveground organs. Cadmium and Zn in the four organs positively correlated to their pseudototal and $CaCl_2$-extractable concentrations in soils (**Fig. 3.4**). Conversely, apart from roots, and to a lesser extent from the rhizomes, Pb in stems and leaves was poorly and negatively correlated to its pseudototal and $CaCl_2$-extractable concentrations in soils (**Fig. 3.4**).

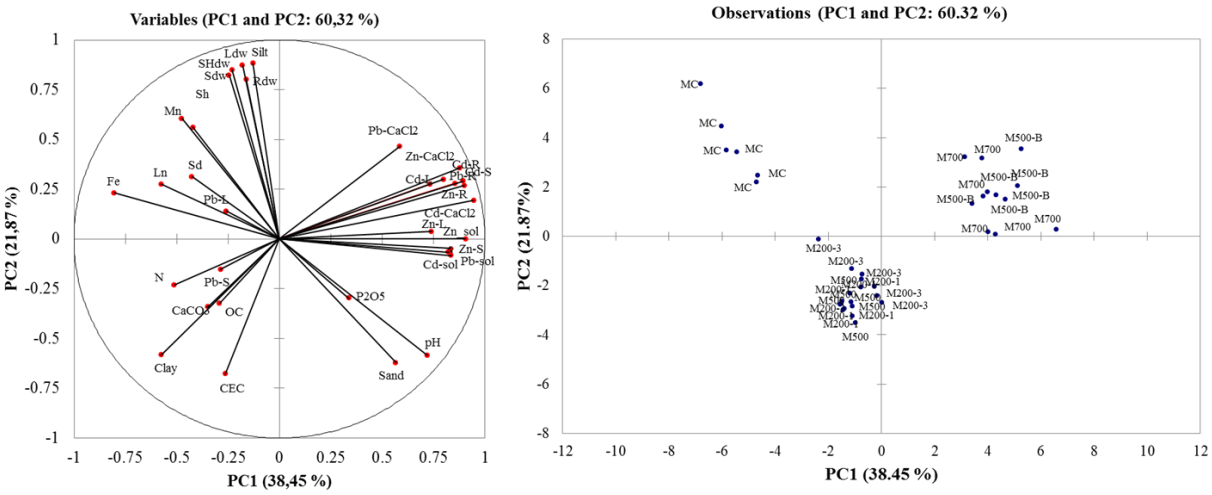

Fig. 3.4. The relationship between miscanthus grow parameters, TE concentrations in miscanthus organs, soil physico-chemical parameters, 0.01 M $CaCl_2$ and pseudototal TE concentrations. Variable correlation circle (left) and soil positions on the first two principal components PC1 and PC2 (right).

Meaning of the parameters

Growth parameters

- Ln: leaf number
- Ldw: Leaf dry weight
- Sd: stem diameter
- Sh: stem height
- Sdw: stem dry weight
- SHdw: shoot dry weight
- Rdw: root dry weight

TE concentrations in miscanthus

- Cd-L: leaf Cd concentrations
- Cd-S: stem Cd concentrations
- Cd-R: root Cd concentrations
- Pb-L: leaf Pb concentrations
- Pb-S: stem Pb concentrations
- Pb-R: root Pb concentrations
- Zn-L: leaf Zn concentrations
- Zn-S: stem Zn concentrations
- Zn-R: root Zn concentrations

Soil TE concentrations and physico-chemical parameters

- Cd-sol: soil Cd pseudototal concentrations
- Pb-sol: soil Pb pseudototal concentrations
- Zn-sol: soil Zn pseudototal concentrations
- Cd-CaCl2: soil Cd concentrations extractable with $CaCl_2$ (0.01 M)
- Pb-CaCl2: soil Pb concentrations extractable with $CaCl_2$ (0.01 M)
- Zn-CaCl2: soil Zn concentrations extractable with $CaCl_2$ (0.01 M)
- Fe: iron content (Mehra -Jackson method)
- Mn: manganese content (Mehra -Jackson method)
- N: soil total nitrogen
- OC: Organic carbon content
- CaCO3: total Carbonate content
- CEC: Cation exchange capacity
- P2O5: soluble phsphorus (Joret-Hébert Method)

3.3.5.2. Trace element transfer from soil to miscanthus organs

The TE transfer ratio from the soil to miscanthus was assessed using the bioconcentration factor-BCF, i.e., the ratio of TE concentrations in plant organs to TE concentrations in soils (**Table 3.4**).

Table 3.4. Comparison of BCFs and TFs of miscanthus organs. Values represent means ± standard deviations. Different letters refer to significant differences (Tukey HSD test, $p \leq 0.05$, $n = 6$).

TE	Soil	BCF				TF		
		Leaves	Stems	Rhizomes	Roots	Leaves	Stems	Rhizomes
Cd	MC	1.76 ± 1.35^{c}	1.26 ± 0.26^{c}	1.59 ± 1.26^{c}	10.45 ± 1.81^{a}	0.17 ± 0.12^{ab}	0.12 ± 0.02^{a}	0.17 ± 0.14^{ab}
	M200-1	0.09 ± 0.04^{f}	0.10 ± 0.02^{f}	0.21 ± 0.04^{de}	3.75 ± 0.90^{bc}	0.03 ± 0.01^{bc}	$0.03 \pm 0.01b^{c}$	0.06 ± 0.01^{b}
	M200-3	0.11 ± 0.04^{f}	0.12 ± 0.02^{f}	0.19 ± 0.04^{e}	5.39 ± 1.27^{b}	0.02 ± 0.01^{bc}	0.02 ± 0.00^{bc}	0.04 ± 0.01^{b}
	M500	0.08 ± 0.04^{f}	0.09 ± 0.02^{f}	0.13 ± 0.01^{f}	3.08 ± 0.53^{c}	0.03 ± 0.02^{bc}	0.03 ± 0.01^{bc}	0.04 ± 0.01^{b}
	M500-B	0.13 ± 0.04^{f}	0.31 ± 0.06^{d}	0.26 ± 0.07^{de}	11.71 ± 3.16^{a}	0.01 ± 0.00^{c}	0.03 ± 0.01^{bc}	0.02 ± 0.01^{bc}
	M700	0.19 ± 0.02^{e}	0.15 ± 0.06^{ef}	0.15 ± 0.03^{ef}	5.68 ± 0.94^{b}	0.03 ± 0.01^{bc}	0.03 ± 0.01^{bc}	0.03 ± 0.01^{bc}
Pb	MC	1.93 ± 1.21^{a}	2.26 ± 1.33^{a}	0.37 ± 0.62^{bc}	1.97 ± 0.49^{a}	0.99 ± 0.49^{ab}	1.15 ± 0.55^{a}	0.22 ± 0.37^{c}
	M200-1	0.08 ± 0.05^{bc}	0.11 ± 0.04^{b}	0.04 ± 0.03^{c}	0.11 ± 0.01^{b}	0.75 ± 0.58^{abc}	1.00 ± 0.36^{ab}	0.38 ± 0.24^{bc}
	M200-3	0.07 ± 0.04^{bc}	0.09 ± 0.03^{b}	0.01 ± 0.02^{c}	0.12 ± 0.05^{b}	0.74 ± 0.52^{abc}	0.99 ± 0.78^{abc}	0.27 ± 0.14^{c}
	M500	0.02 ± 0.01^{c}	0.04 ± 0.01^{c}	0.01 ± 0.01^{c}	0.10 ± 0.03^{b}	0.27 ± 0.16^{bc}	0.39 ± 0.08^{bc}	0.11 ± 0.08^{c}
	M500-B	0.02 ± 0.02^{c}	0.03 ± 0.01^{c}	0.02 ± 0.01^{c}	0.21 ± 0.09^{b}	0.10 ± 0.08^{c}	0.19 ± 0.11^{c}	0.12 ± 0.10^{c}
	M700	0.02 ± 0.01^{c}	0.02 ± 0.01^{c}	0.01 ± 0.01^{c}	0.12 ± 0.03^{b}	0.15 ± 0.11^{c}	0.18 ± 0.12^{c}	0.12 ± 0.07^{c}
Zn	MC	0.44 ± 0.07^{bc}	0.55 ± 0.10^{ab}	0.27 ± 0.04^{d}	0.70 ± 0.09^{a}	0.64 ± 0.14^{a}	0.79 ± 0.17^{a}	0.40 ± 0.06^{bc}
	M200-1	0.07 ± 0.00^{g}	0.13 ± 0.02^{f}	0.04 ± 0.00^{h}	0.18 ± 0.03^{ef}	0.40 ± 0.06^{bc}	0.76 ± 0.14^{a}	0.24 ± 0.04^{de}
	M200-3	0.08 ± 0.01^{g}	0.16 ± 0.03^{f}	0.05 ± 0.01^{h}	0.34 ± 0.06^{cd}	0.25 ± 0.03^{d}	0.46 ± 0.08^{bc}	0.16 ± 0.03^{f}
	M500	0.07 ± 0.02^{gh}	0.14 ± 0.02^{f}	0.04 ± 0.00^{h}	0.20 ± 0.02^{ef}	0.33 ± 0.08^{cd}	0.69 ± 0.09^{a}	0.18 ± 0.03^{ef}
	M500-B	0.06 ± 0.01^{gh}	0.15 ± 0.02^{f}	0.04 ± 0.00^{h}	0.43 ± 0.10^{bc}	0.15 ± 0.03^{f}	0.37 ± 0.10^{bc}	0.09 ± 0.02^{g}
	M700	0.04 ± 0.01^{h}	0.09 ± 0.02^{g}	0.02 ± 0.00^{i}	0.24 ± 0.04^{de}	0.17 ± 0.03^{ef}	0.35 ± 0.08^{cd}	0.10 ± 0.02^{g}

Due to low concentration in MC, cadmium BCFs are higher than 1 whatever the organ. In contaminated soils, the BCFs for the leaves, stems and rhizomes varied from 0.09 to 0.31 and are significantly lower than root BCFs (3.08 to 11.71). The BCFs allowed ranking organs as follows: roots >> rhizomes > stems = leaves ≥ in M200-1 and M200-3; roots >> rhizomes = stems= leaves in M500 and M700; and roots >> rhizomes = stems > leaves in M500-B. The leaf BCFs in M700 and stem BCFs in M500-B are higher than in other soils. The rhizome BCFs vary greatly but were lower in M500. There is no significant difference between the aboveground organs and rhizomes apart from in M500-B where stem and rhizome BCFs are higher than in other soils. The root BCFs in M500-B (11.7) are 3.1, 2.2, 3.8 and 2.1-fold higher than in M200-1, M200-3, M500 and M700 respectively.

Apart from rhizomes, the lead BCFs are higher than 1 in MC whereas they were lower than 1 in contaminated soils. In these soils, the BCFs vary from 0.02 to 0.21, and they allow ranking organs as follows: roots = stems ≥ leaves ≥ rhizomes for M200-1 and M200-3, and roots > stems = leaves = rhizomes in other soils. The stem BCFs in M500, M500-B and M700 are significantly lower than in M200-1 and M200-3.

Unlike Cd and Pb, the zinc BCFs are less than 1 whatever the organ and the soil. The zinc BCFs allow classifying the organs as follows: roots ≥ stems > leaves > rhizomes. The BCFs are higher in MC than in other soils. In contaminated soils, stem (0.09) and rhizome (0.02) BCFs are the lowest in M700, whereas root BCFs are the highest (0.43) in M500-B.

Overall, in contaminated soils, the BCFs allow ranking the accumulation of the studied TEs in miscanthus organs as follows: Cd >> Zn > Pb (roots), Cd > Zn > Pb (rhizomes), Zn ≥ Cd > Pb (stems) and Cd > Zn > Pb (leaves). Cadmium is the most accumulated in the belowground organs. Lead is the least accumulated in all organs. Apart from Pb in stems and in leaves, TE concentrations in miscanthus organs are positively correlated with soil pseudototal and 0.01M $CaCl_2$-extractable concentrations but negatively correlate with soil CEC, clay, Fe and Mn contents (**Fig. 3.4**).

3.3.5.3. Trace element transfer ratio from roots to rhizomes, stems and leaves

The TE transfer ratio from roots to other miscanthus organs was assessed using the translocation factor- TF, i.e., the ratio of TE concentrations in aboveground organs or in rhizomes to TE concentrations in roots (**Table 3.4**).

The cadmium, lead and zinc TFs are higher in MC than in contaminated soils. In these soils, cadmium TF range between 0.01 and 0.06 and are not significantly different whatever the organ. The lead TFs vary from 0.11 to 1.15, with leaf and stem TFs being generally higher in

MC than in contaminated soils. The zinc TFs vary from 0.09 to 0.79, and they allow ranking the organs as follows: stems > leaves > rhizomes.

Overall, whatever the soil, the TFs allow ranking of TE transfer as follows: Pb = Zn >> Cd (rhizomes); Pb = Zn >> Cd (stems) and Pb = Zn >> Cd (leaves). Obviously, the Cd is the least transferred from roots to other organs whereas it is the most transferred from soil to roots.

3.3.6. Growth and biomass production

The ability of miscanthus to grow on TE contaminated soils was assessed with reference to the uncontaminated soil. In general, the measured parameters are consistently higher in MC **(Table 3.5)**.

Table 3.5. Miscanthus growth parameters related to biomass productivity on studied soils after 93 days under greenhouse culture. Different letters in the same row refer to significant differences (Tukey HSD, $p \leq 0.05$, $n = 6$).

Parameters	MC	M200-1	M200-3	M500	M500-B	M700
Leaf number	10.6 ± 0.97^{a}	8.67 ± 0.52^{c}	9.33 ± 0.52^{bc}	10.17 ± 0.75^{ab}	9.17 ± 0.41^{bc}	9 ± 0.63^{bc}
Stem height (cm)	181 ± 19^{a}	144 ± 13^{b}	158 ± 16^{ab}	159 ± 8^{ab}	155 ± 10^{ab}	156 ± 34^{ab}
Stem diameter (cm)	0.72 ± 0.06^{a}	0.64 ± 0.02^{ab}	0.62 ± 0.03^{b}	0.67 ± 0.04^{ab}	0.63 ± 0.06^{b}	0.65 ± 0.09^{ab}
Leaf DW (g)	5.97 ± 1.06^{a}	3.53 ± 0.51^{bc}	3.40 ± 0.66^{c}	4.00 ± 0.37^{bc}	4.6 ± 0.53^{bc}	4.83 ± 0.94^{ab}
Stem DW (g)	12.76 ± 3.58^{a}	6.80 ± 1.64^{b}	7.41 ± 2.08^{b}	7.37 ± 1.21^{b}	8.79 ± 1.23^{b}	9.88 ± 3.19^{ab}
Root DW (g)	8.91 ± 1.55^{a}	3.93 ± 0.93^{c}	4.24 ± 1.20^{c}	5.41 ± 1.22^{bc}	7.02 ± 1.50^{ab}	5.79 ± 0.99^{bc}
Shoot DW (g)	18.72 ± 4.57^{a}	10.33 ± 2.11^{b}	10.81 ± 2.72^{b}	11.37 ± 1.46^{b}	13.39 ± 1.60^{b}	14.71 ± 4.02^{ab}
Shoot/root DW ratio	2.13 ± 0.48^{a}	2.74 ± 0.76^{a}	2.58 ± 0.41^{a}	2.19 ± 0.58^{a}	1.99 ± 0.48^{a}	2.52 ± 0.40^{a}

Despite the soil contamination gradient, stem height, stem diameter and shoot DW did not significantly differ in the contaminated soils. In these soils, root DW in M200-1 and M200-3 is lower than in M500-B. There is no significant correlation between measured growth parameters and soil contamination level (**Fig. 3.4**). Conversely, these parameters positively correlate with soil silt and Fe contents whereas they negatively correlate with soil pH and sand content (**Fig. 3.4**).

3.4. Discussion

The potential of miscanthus as a candidate crop for phytomanagement of TE-contaminated soils was assessed in semi-controlled greenhouse conditions. The plants were grown on soils presenting different physico-chemical parameters and contamination levels. At the end of the experiment, soil pH, organic carbon content and TE mobility were assessed with reference to T_0. The determination of TE accumulation and distribution in the four miscanthus organs

allowed highlighting the management option stemming from miscanthus cultivation on TE-contaminated soils.

3.4.1. Impact of miscanthus growth on soil physico-chemical parameters

In soil-plant systems, pH is mainly controlled by complex rhizosphere processes including the release of protons (H^+) or hydroxyls (OH^-) at the root surface and in the rhizosphere during root nutrient uptake (Hinsinger et al., 2003; Blossfeld et al., 2010). Rhizosphere pH also depends on root exudate composition and ionic forms, which may lead to acidification of alkaline soils or to alkalization of acidic soils (Hinsinger et al., 2003). However, the plant effects on soil pH depend on the species and their interactions with the growing medium. In a growth medium with pH ranging from 5.6 to 7.4, *Lolium perenne* alkalized the rhizosphere whereas *Zea mays* acidified it (Blossfeld et al., 2010). Conversely, Faget et al. (2013) found that rhizosphere alkalization by *Zea mays* may occur with time. With regard to our results, while working with one genotype of miscanthus, we observed alkalization of the rhizosphere at slight acid pH and acidification in slight alkaline pH. The same results were found in barley and soybean grown on clay loam soils, for which the alkalization occurred in the rhizosphere at an initially low pH (4.8), and acidification at high initial pH values (7.1) (Youssef and Chino, 1989). The soil alkalization occurs as part of plant mechanism to alleviate toxicity due to acidic pH, (Hinsinger et al., 2003). According to these authors, uptake of ions such as Fe, K and P that are necessary for plant growth may cause acidification over time and space. Therefore, the observed pH changes in studied soils could be explained by nutrient uptake and avoidance of toxicity and nutrient depletion in the miscanthus rhizosphere.

In the studied soils, the organic carbon content significantly increased from T_0 to T_{93}. In soil-plant systems, plant decaying residues and rhizodeposits are the main carbon sources (Kuzyakov and Schneckenberger, 2004). In our experiment, the increase in carbon could mainly be due to miscanthus root exudates as there were no decaying plant residues. Indeed, the composition of miscanthus root exudates mainly comprises carbohydrates, proteins and amino acids which are source of organic carbon (Hromádko et al., 2010). Also, rhizosphere biota including free living fungi, bacteria and soil microfauna could contribute to this increase. Indeed, miscanthus rhizosphere hosts a large microbial diversity due to high quantity and diversified composition of its root exudates (Técher et al., 2012). In field conditions, Stępień et al. (2014) found that in the miscanthus rhizosphere, the number of heterotrophic bacteria was more than three, and that of fungi more than two orders greater than the size of the groups in the non-rhizosphere.

3.4.2. Impact of miscanthus growth on TE distribution in soils

After 93 days of miscanthus culture, the pseudototal concentrations decreased for Cd, and increased for Zn whereas no differences could be observed for Pb. These changes could be due to the plant effects on soil physico-chemical parameters and nutrient uptake. Indeed, Cd and Zn are naturally more available in soil liquid phase than Pb (Kabata Pendias, 2004; Carrillo-Gonzalez et al., 2006). The decrease of Cd in studied soils could be due to plant uptake, and this is supported by the fact that this element was the most accumulated in miscanthus roots. As it has been observed in rhizosphere of *Lolium multiflorum* and *Agrostis capillaris* (Houben and Sonnet, 2012; Houben et al., 2014), the increased Zn concentrations in all soils could be due to soil acidification and this element mobilization for plant uptake.

The overall fractionation of the studied TEs did not change as they remained mainly present in the fractions A and B at the end of the experiment. However, Cd and Pb fractions A decreased whereas their fractions B increased in contaminated soils. For Zn, the general trend was an increase of the fraction D. This suggests the cultivation of miscanthus could decrease TE soluble and exchangeable fractions. Indeed, the $CaCl_2$ (0.01 M)-extractable Cd and Zn concentrations decreased whatever the soil and this is different from what would be expected in studied soils as pH decrease should have noticeable effects on the increased solubility of TE in the rhizosphere. Thus, the reduced extractability of these two elements could be due to their association with more soil stable components, especially the organic matter or oxides/hydroxides in oxidized rhizosphere.

3.4.3. Trace element concentrations in miscanthus organs

In contaminated soils, the Cd, Pb and Zn concentrations in roots were higher than in rhizomes, stems and leaves of miscanthus. Moreover, apart from Pb in stems and in leaves, the concentrations of these elements in the organs were positively correlated with their soil pseudototal concentrations. With comparison to short-term studies in controlled conditions, our results are consistent with those of Arduini et al. (2006) who exposed miscanthus seedlings to increasing Cd concentrations under hydroponic conditions; and with those of Wanat et al. (2013) obtained in acidic Technosols contaminated by low to high Pb concentrations.

Whatever the TE, the BCFs for roots were higher than for rhizomes, stems and leaves. Also, the BCFs for all organs, apart from Cd in roots, were lower than 1 in contaminated soils suggesting that miscanthus limits TE root uptake and reduces their transfer to aboveground organs (Pavel et al., 2014). Similarly, the TFs which are lower than 1 whatever the organs

corroborate the facts that the studied TEs are mainly accumulated in miscanthus roots. However, the BCFs and TFs differed depending on TEs. While Cd is the most transferred TE from soil to all organs, the TFs show that it is the least transferred from root to other organs. The Pb is the least transferred to miscanthus organs. The different patterns in the transfer of the studied elements from soil-to miscanthus could depend on potential TE mobility in soil. Indeed, Cd and Zn are more mobile in soil than Pb (Kabata Pendias, 2004; Carrillo-Gonzalez et al., 2006), and this pattern was more demonstrated by the calculated root BCFs for each TE.

The low BCFs and TFs on contaminated soils demonstrated miscanthus TE-exclusion or avoidance strategy. This is one of the tolerance strategies which allow plants, so-called excluders, to grow in a wide range of phytotoxic TE concentrations and significantly prevent them from entering roots or restricting their transfer to the shoots (Baker, 1981; DalCorso et al., 2013). The plant TE-exclusion may depends on complex extra and intracellular mechanisms that either restrict TE entry into the roots or reduce the root-to shoot TE transport. These include precipitation of TE into root iron plaques (DalCorso et al., 2013), development of exodermis and endordermis to limit TE apoplastic movement (Lux et al., 2011), immobilization of TE in root cell apoplast by complexation with pectin carboxylic groups in the cell wall (Pourrut et al., 2011) or TE chelation by phytochelatins and storage in root cell vacuoles (Pourrut et al., 2011; DalCorso et al., 2013). Although not studied yet, these mechanisms could be key players in enhancing the root sequestration capacity of miscanthus.

Although Cd and Zn concentrations in miscanthus in all organs, and Pb concentrations in leaves increased with soil contamination level, their uptake could be influenced by soil physico-chemical parameters. For instance, the TE contamination level in M500 is higher than in M200-1 and M200-3, but TE concentrations in miscanthus organs in these soils are not significantly different. Also, despite the same contamination level in Cd and Pb in M500 and M500-B, these two TE concentrations were higher in M500-B than in M500. Certainly, the reduced metal uptake in M500 could be explained by a lower metal phytoavailability in relation with CEC, carbonate, Fe oxide, and organic carbon contents which are higher in this soil than in others. Indeed, soils rich in carbonates, organic matter and Fe/Mn oxy(hydro)oxides have a higher buffering capacity capable of delaying metal dissolution and maintaining them in the soil solid phase (Carrillo-Gonzalez et al., 2006; Buekers et al., 2007). Accordingly, both high TE concentrations and BCFs in miscanthus organs in M500-B could be due to a higher TE solubility as even evidenced by higher TE-extractable concentrations.

Despite a positive correlation between TE concentrations in soil and their concentrations in miscanthus organs, the aboveground biomass did not generally differ among contaminated soils. Moreover, though all measured growth parameters were consistently higher in uncontaminated soil, there was no correlation between these parameters and TE concentrations in soils. Conversely, growth parameters were positively correlated with soil silt content. This suggests that observed difference between uncontaminated and contaminated soils could not only be due to soil TE contamination, but also to soil physico-chemical parameters. Accordingly, the higher silt content and the favourable structure in uncontaminated soil could allow better water accessibility, hence better miscanthus growth.

Overall, our results demonstrate that, as biomass crop, miscanthus could be a good candidate to stabilize Cd, Pb and Zn contamination in the studied soils. The observed increase in organic carbon content suggests that long-term cultivation of this species could increase TE sequestration in the rhizosphere. Indeed, in a 3-year-old miscanthus plantation, Iqbal et al. (2013) found that TE (e.g. Cu and Pb) mobility could be decreased probably due to an increase in organic carbon content. However, we cannot up to now, predict the effects of progressive accumulation of organic matter on TE speciation in the studied soils planted with miscanthus. Moreover, long-term study in Cd, Pb and Zn contaminated sandy loam soils showed that miscanthus productivity decreased with soil contamination level (Pavel et al., 2014), which is different from what we observed in the present study. Therefore, we would recommend long-term field studies to focus on miscanthus productivity and its effects on TE contaminated soils.

3.5. Conclusion

After 93 days, miscanthus grown in greenhouse conditions demonstrated its ability to grow on TE (Cd, Pb and Zn)-contaminated soils, and to reduce the accumulation of these TEs into the aboveground organs. Moreover, the observed changes in TE distribution and mobility could be due to complex rhizospheric processes driving pH, root exudation and/or inputs of organic matter in planted system. Therefore, considering the capacity of miscanthus to reduce TE potential mobility, to mainly accumulate TE in roots and limit their transfer to aerial organs, this species could be considered as a TE-excluder, hence a potential candidate crop for coupling phytostabilization and biomass production on studied contaminated agricultural soils.

Further studies should focus on key areas to evaluate phytostabilization efficiency and sustainability, notably miscanthus physiological response and/or tolerance mechanisms to

various stress inducers in both *ex situ* and *in situ* conditions, and the long-term effects of this species cultivation on TE mobility in the studied soils. As this last point greatly depends on rhizosphere composition and processes, it would be more interesting to characterize miscanthus root exudates and how these molecules influence soil biological activities. Last but not the least, different miscanthus genotypes should be screened in order to get more insights into the suitability of miscanthus genetic resources for phytomanagement of TE-contaminated soils/sites.

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Chapitre IV :

Potentialités de *Miscanthus* × *giganteus* pour la phytostabilisation des sols contaminés par les métaux : expérimentation *in situ*

Préambule

Il a été montré précédemment que dans des conditions *ex situ*, miscanthus accumule davantage Cd, Pb et Zn dans les racines et limite l'accumulation de ces éléments dans les parties aériennes (tiges et feuilles) quel que soit le degré de contamination des sols étudiés. La mobilité des métaux dépend des paramètres physico-chimiques des sols (CEC, teneurs en carbone organique, carbonates et fer). Ainsi, bien que la contamination des sols de la parcelle M500 excède largement celle de M200, aucune différence n'a été observée dans les concentrations en métaux pour un organe donné du miscanthus.

Dans le présent chapitre il s'agit d'étudier le comportement du miscanthus cultivé dans des conditions *in situ*. Le dispositif expérimental correspond à celui mis en place dans le cadre du projet PHYTENER. Les objectifs spécifiques de la présente démarche sont :

- d'étudier la disponibilité environnementale de Cd, Pb et Zn au moyen d'extractions simples (0.01 M $CaCl_2$) et séquentielles;
- de déterminer le profil d'accumulation et de distribution des métaux dans les organes de miscanthus selon un gradient de contamination des sols;
- d'établir les relations potentielles entre les paramètres physico-chimiques, le degré de contamination des sols et l'accumulation des métaux dans les organes du miscanthus.

La démarche repose sur les parcelles contaminées M200 et M500, ainsi que sur la parcelle considérée comme non massivement contaminée (MC). Trois à quatre années après la plantation de miscanthus, il a été réalisé en septembre 2011 un échantillonnage des plantes (racines, rhizomes, tiges et feuilles) et des sols qui ont porté ces cultures.

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Miscanthus × *giganteus*: A trace element-excluder perennial grass suitable for phytostabilization of contaminated agricultural soils

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Abstract

Soil contamination by trace elements (TEs) is of major concern worldwide. Phytostabilization is proposed as an environmentally-friendly option for phytomanagement of large surface of TE-contaminated lands. The present study aimed to assess the capacity of the energy crop *Miscanthus* × *giganteus* for phytostabilization in combination with a biomass production on agricultural lands located in the vicinity of the former lead smelter (Metaleurop nord, France). In this area, Cd, Pb and Zn concentrations in soils are 20 to 50-fold higher than the regional background values. *Miscanthus* × *giganteus* experimental plantations comprised two agricultural plots established around the former smelter, and one agricultural plot considered as a control. Four year after the crop establishment, topsoil (0-25 cm) and plant samples were collected in order to study the soil physico-chemical parameters and TE (Cd, Pb and Zn) mobility, and to establish the relationship between TE accumulation in *M.* × *giganteus* organs and the soil physico-chemical parameters. Soil pH, cation exchange capacity, total carbonates and soluble phosphorus contents negatively correlated with TE accumulation in *M.* × *giganteus* organs, suggesting the soil buffering capacity could reduce TE solubility and transfer to the plant organs. Cadmium, Pb and Zn concentrations in *M.* × *giganteus* organs, and their bioconcentration or translocation factors demonstrate that this species accumulates the studied TEs mainly in roots and strongly reduces their transfer to aboveground parts. Moreover, TE concentrations in leaves and stems did not significantly differ between contaminated and uncontaminated plots. Overall, these results show that *M.* × *giganteus* is TE-excluder plant and could be a potential candidate crop for coupling phytostabilization and biomass production on TE-contaminated agricultural soils.

Key words: *Miscanthus*, biomass crop, phytomanagement, trace element-exclusion

4.1. Introduction

Soil metal-contamination by industrial activities, mining wastes, and agricultural fertilizers, is of great concern worldwide (Su et al., 2014). In the Northern France, coal mining and many other industrial activities have contributed to the contamination of terrestrial ecosystems. Among the industrial activities, the former lead (Pb) smelter (Metaleurop Nord, Noyelles Godault), which had operated for up to 100 years until its closure in 2003, has contaminated its surrounding environment via atmospheric fallouts and trace element (TE)-enriched dust

emissions (Frangi and Richard, 1997; Sterckeman et al., 2002). The affected area, referred to as Metaleurop site (http://www.safir-network.com/), covers about 120 km^2.

The study conducted within the site demonstrates that cropland topsoils are contaminated mainly by Cd, Pb and Zn, at levels equivalent to 20 to 50-fold higher than regional agricultural topsoil concentrations (Sterckeman et al., 2002). In the latter study, it is indicated that near the former Pb smelter, Cd, Pb and Zn concentrations in agricultural topsoils can reach 21, 1132 and 1378 mg kg^{-1}, respectively. Moreover, the contamination is mainly limited to the ploughed horizons (0-30 cm) and it decreases with distance from the contamination sources.

The contaminated agricultural soils within this site poses both environmental and health risks. Some food crops including wheat, barley, forage maize and potatoes contain Cd and Pb concentrations that exceed legislation thresholds for food or feedstuff (Douay et al., 2013). Moreover, during dry periods, the agricultural practices on contaminated soils result in emission of contaminated particles (Douay et al., 2005), which potentially increases the exposure of the local inhabitants to contaminants. The human bioaccessibility tests showed that for soils contaminated at 6 ± 2.5 mg Cd kg^{-1}, 279 ± 153 mg Pb kg^{-1} and 486 ± 238 mg Zn kg^{-1}, the % average of bioaccessible gastric fractions can be 82, 55 and 33 for Cd, Pb and Zn respectively (Perfrêne et al., 2011). This study also highlighted that a high availability of these TEs in the potentially mobile fractions, especially for Cd, could increase their environmental availability, phytoavailability and human bioaccessibility.

Protecting inhabitants, especially by reducing their exposure to contaminated dusts and ingestion of contaminated foodstuff, are of prime concern. In a new policy awaiting approval, the local authorities have set out that agricultural lands with topsoils presenting Cd and/or Pb concentrations higher than 10 and/or 500 mg kg^{-1}, respectively, should not be used for crop production. Moreover, topsoils with concentrations from 4 to 10 mg Cd kg^{-1} and/or from 200 to 500 mg Pb kg^{-1} should not be used for commercial food and feed production. Overall, 750 ha of cropped lands are concerned, which cannot be economically managed by conventional physico-chemical methods. Therefore, a sustainable management of these agricultural soils is crucial and should meet both local population's needs and environmental quality in a producing agro-ecosystem.

Phytoremediation, i.e. the use of plants and associated microorganisms to alleviate pollutant linkages and risks due to excessive exposure to TEs and/or organic contaminants in soils, water and sediments (Mench et al., 2010) can be a cost-effective and an environmentally

friendly option to manage such a big area. Phytoremediation of TE-contaminated land mainly consists of (1) phytoextraction, i.e. the uptake and accumulation of TE from the soil into the aboveground plant parts, and (2) phytostabilization, i.e. the TE immobilization in the rhizosphere and/or in the plant roots.

A sustainable management of contaminated sites, hereafter referred to as phytomanagement, suggests the use of phytoremediation options for the production of economically valuable non-food crops (Robinson et al., 2009). The biomass products (biofuels, biochemicals, animal feed, soil improvers, etc.) create income-generating activities for the local inhabitants (Evangelou et al., 2012; Witters et al., 2012). Moreover, such crops should promote carbon sequestration and other ecosystem services such as improvement of soil and water quality, reduced erosion and increased niches for biodiversity (Evangelou et al., 2012). Given many drawbacks associated with phytoextraction (Dickinson et al., 2009), phytostabilization is suggested as a more relevant option for managing large and highly contaminated sites. This technique requires a perennial plant activity to a) decrease TE availability in soils, b) reduce emissions of contaminated particles released by seasonal soil tillage and c) improve soil and site ecological characteristics (Dickinson et al., 2009; Mench et al., 2010).

On the Metaleurop site, a study is currently being conducted on the ability of the non-food perennial grass, *Miscanthus* × *giganteus* (subsequently referred to as miscanthus) for phytomanagement that couples phytostabilisation and energy-dedicated biomass production. Miscanthus grows well under regional climatic and edaphic conditions (Zub et al., 2011), and can provide raw materials for house building, chemical and energy production, and paper production (Nsanganwimana et al., 2014). *In situ* growth of this species on TE (Cd, Pb and Zn)-contaminated acid (pH = 5.3-6.9) sandy clay soils shows that it retains TEs in its belowground organs (Pavel et al., 2014). However, shoot TE concentrations may increase in case of high TE-contaminated acid sandy or loamy soils as it has been found for Pb and Zn (Kocoń and Matyka, 2012; Pogrzeba et al., 2011). Therefore, it is necessary to assess soil TE mobility and TE concentrations in miscanthus organs in relation with soil characteristics before establishing large-scale plantations on TE-contaminated soils. Such a study can help in the choice regarding farming practices, mitigation of environmental risks and further biomass uses.

This paper reports the results obtained from a 4-year field growth of miscanthus on former agricultural plots with soils presenting different TE (Cd, Pb and Zn) contamination levels as well as physico-chemical characteristics. Topsoil and plant samples were collected for studying (1) soil physico-chemical parameters, (2) TE availability and mobility by single and

sequential extractions, (3) TE distribution in miscanthus organs along the soil contamination gradient and (4) the relationship between soil characteristics and TE transfer and accumulation in miscanthus organs.

4.2. Material and methods

4.2.1. Characterization of experimental plots

Three agricultural plots were used. The two contaminated plots, named M200 (50°24′52″N, 3°01′51″E, 1.1 ha, Courcelles-les-Lens) and M500 (50°25′49″N, 3°02′13″E, 0.8 ha, Evin-Malmaison), are located within the Metaleurop site (**Fig. 0.6; Fig. 2.1**). Here, the landscape presents a high degree of anthropization with residential suburbs, agricultural and woodlands, and transport networks. The plots are 1.8 km Southeast (for M200) and 1.4 km Northeast (for M500) of the former Pb smelter (50°25′42″N, 3°00′55″E, Noyelles-Godault). The M500 is the closest to the contamination source and more exposed to dominant winds. The third plot, hereafter called MC (50°20′46″N, 2°12′15″E, 1.3 ha, Linzeux), is not contaminated and is at about 75 km from the Metaleurop site. It is located in a rural area, within an agrosystem landscape.

The soils of these three plots differ according to their descriptive characteristics. The MC soil is a thick (> 1.20 m deep), well-drained loessic loam lying on a plateau. The M200 plot is located in a slightly northeastward-sloping topography. During wet periods, soil waterlogging conditions in the plot occur at a depth of 30-50 cm. Due to the depth of the geological substrate, two sub-plots (M200-1 and M200-3) are differentiated. The M200-1 sub-plot is located in the upper slope with a sandy clay loam soil lying over a clayed substrate at 30-50 cm deep. The M200-3 sub-plot is located at the lower slope. Its soil is composed of a loam layer (up to 30 cm) under which a clay loam layer lies on a clayed substrate at 50-80 cm deep. The M500 plot is located in the alluvial plain with clay loam developed from the calcareous alluvial deposits. During wet periods, the waterlogging conditions in this soil occur at 30 cm deep.

4.2.2. Miscanthus plantations

Miscanthus plantations were established in 2007 for MC and in 2008 for M200 and M500. The soils were ploughed in late winter and prepared for plantation in early spring. Rhizomes of *Miscanthus* × *giganteus* Greef & Deuter ex Hodkinson & Renvoize (Hodkinson and Renvoize, 2001) were supplied by NovaBiom, formerly Bical Biomass France. Rhizomes with at least 2 buds were planted at 10-20 cm of soil depth and at a density of 20,000 plants ha^{-1}. A potato planter assisted in the plantation.

Weed growth was suppressed by herbicide (Roundup® Original) spraying during the first 2 years of miscanthus establishment. Irrigation was never necessary because the region benefits from the oceanic temperate climate characterized by sufficient rain throughout the year. Annual precipitations and average temperatures are 738.7 mm and 10.8°C for both M200 and M500, and 996.5 mm and 11°C for the MC. Fertilization was not applied, and pesticides were not necessary because no diseases were detected during the experimental period.

4.2.3. Plant and soil sampling

Sampling was carried out in September 2011 when miscanthus plantations were in the 3rd- to 4th-year growth season. The first 2 years corresponded to the plant establishment phase, which was inappropriate for assessment of TE accumulation in miscanthus. Five miscanthus clumps were randomly chosen within each plot. Care was taken to avoid borderline effects. First, plant parts were sampled. The shoots were cut with clippers and separated into stems and leaves. The belowground parts composed of roots and rhizomes were sampled and were not separated directly. Second, the soil under the root influence of each sampled plant was finally collected using an auger (composite sample from topsoils, 0-25 cm). A total of five couples of plant-soil samples were collected. All samples were put into polyethylene bags for transportation to the laboratory.

4.2.4. Sample preparation and analysis

4.2.4.1. Plant material

Leaf and stem samples were washed in three successive baths of osmotic water. The belowground organs were washed thoroughly with tap water to remove the soil particles. Rhizomes were separated from roots with scissors. Both organs were rinsed in three successive osmotic water baths. All plant samples were cut into small pieces, oven-dried at 40°C and then ground into fine powder using a knife mill (GM200, Retsch) for leaves and roots, and an ultracentrifuge mill (ZM200, Retsch) for stems and rhizomes. The samples were digested by adding 5 mL of nitric acid (HNO_3, 70%, Intra-Baker Analyzed Reagent) in a tube (50 mL Digestion Cup, Environmental Express) containing 300 mg of plant powder. The tube was heated to 80°C on a digestion block (HotBlock™, Environmental Express) for 1 h under a hood box. After cooling, 5 mL of hydrogen peroxide (H_2O_2, 30%, Baker Analyzed Reagent) were added to the digest and the mixture was again heated at 80°C for 3h. After cooling, the volume was adjusted to 25 mL with double-distilled water and filtered (0.45 µm acetate membrane filters, Minisart). Filtrates were stored at 4^{o}C before Cd, Pb and Zn determination by atomic absorption spectrophotometry (AA-6800, Shimadzu).

Quality control for chemical extraction and digestion was performed by including blanks, internal and certified (Polish Virginia tobacco leaves, INCT-PVTL-6, Poland) reference materials. The mean recovery rates in the reference material are 106.5% (Cd), 93.6% (Pb) and 104.5% (Zn). The residual moisture was determined according to ISO 11465 and was used to apply the moisture correction factor so as to express the results on a dry weight (DW) basis.

4.2.4.2. Soils

The samples were dried at 40°C, ground and passed through a 2-mm sieve and 250 µm using a mill (ZM200). The physico-chemical analyses were performed according to French standardized protocols (AFNOR, 1996). The soil particle size distribution, the pH, the cationic exchange capacity (CEC), exchangeable cations and phosphorus (P_2O_5) contents were determined on soil samples sieved to 2 mm. The total carbonates , organic carbon (OC), Fe and Mn oxides, and TE concentrations in samples were measured from soils sieved to 250 µm. As for plant samples, TEs (Cd, Pb and Zn) were determined by atomic absorption spectrophotometry (AA-6800, Shimadzu).

a) Physico-chemical parameters

The particle size distribution was determined by sedimentation and sieving after destruction of organic matter by H_2O_2 according to the French standard NFX 31-107. The pH (H_2O) was measured after stirring a mixture of soil and deionized water (1:5, v/v) according to the standard ISO 10390. The phosphorus (P_2O_5) was extracted in ammonium oxalate solution ($(NH_4)_2\ C_2O_4$, 0.1M, pH = 7) and measured according to the French standard NFX 31-161 and Joret and Hébert (1955). Iron and Mn oxides were extracted at high temperature (80°C) in the presence of sodium citrate ($C_6H_5Na_3O_7$, 0.3 M) as a complexing agent, sodium bicarbonate ($NaHCO_3$, 0.11 M) as a pH buffer and sodium dithionite ($Na_2S_2O_4$, 1 M) as a reducing agent according to Mehra and Jackson (1960). The determination was performed by flame atomic absorption spectrometry (AA-6800). The total carbonate content was determined by measuring the CO_2 formed after adding HCl (4 M) in the aliquot according to NF ISO 10693. The organic carbon (OC) content was extracted and measured according to the standard ISO 14235. The CEC was determined after percolation of CH_3COONH_4 (1M, pH = 7) solution into soil samples followed by an extraction of ammonium ions (NH_4^+) with sodium chloride (NaCl, 1 M) according to the French standard NF X31-130. Exchangeable cations (Ca^{2+}, K^+, Mg^{2+}, and Na^+) were extracted with a CH_3COONH_4 (0.1 M, pH = 7) solution, and measured according to the French standard NF X 31-108.

b) Pseudototal trace element concentrations

The pseudo-total Cd, Pb and Zn concentrations were determined after acid digestion in aqua regia ($HCl:HNO_3$, 3:1 v/v, 6 mL) of 300 mg of soil using the digestion block at 95°C for 75 min. After cooling, the volume was adjusted to 25 mL with double-distilled water and the solution was filtered (0.45 µm cellulose acetate filters). The quality control of the extraction and analysis was provided by the introduction of two internal reference samples and a certified soil reference (CRM 141, IRMM, Belgium). The mean recovery rates in the reference soil material are 92.2%, 101.7% and 101.7% for Cd, Pb and Zn, respectively. As for the plant sample analysis, the moisture correction factor was applied so as to express the results on DW basis.

c) Single and sequential extractions

The single extraction was performed using a calcium chloride ($CaCl_2$, 0.01 M,) solution according to the protocol that is fully described in Waterlot et al. (2011). For each TE, the results are expressed as the percentage (%) of the pseudo-total concentrations.

The sequential extractions were done to study the fractionation of Cd, Pb and Zn. A four-step extraction procedure was used according to Waterlot et al.(2012). The extractions allowed to obtain fractions noted A, B, D and R, and defined respectively as: exchangeable, water- and acid-soluble (0.11 mol L^{-1} acetic acid), reducible (0.5 mol L^{-1} hydroxylammonium chloride), oxidizable (8.8 mol L^{-1} H_2O_2, followed by 1.0 mol L^{-1} ammonium acetate at pH = 2) and residual (aqua regia). The quality of analytical data was verified by including the certified reference material BCR®-701. The results of each TE fraction are expressed as the percentage of the total sum of the four fractions. In the reference material, the recovery rates in different fractions are: 98.5 (A), 95.6 (B), 99.5 (D) and 102.9 (R) for Cd; 101.8 (A), 94.5 (B), 98.2 (D) and 100.6 (R) for Pb; and 100.4 (A), 98.9 (B), 106.5 (D) and 106.6 (R) for Zn. In the studied soils, the recovery rate of the pseudo-total concentrations ranges from 92.9 to 114%, 87.6 to 113.7% and 92.3 to 115.7% for Cd, Pb and Zn, respectively.

4.2.5. Data analysis

Statistical tests performed using the XLSTAT (Addinsoft™ software, 2012) included each of the parameters analyzed and measured in soil and plant samples. For plant samples, data also included the BCF (bioconcentration factor) (the ratio of TE concentrations in plant organs to TE concentrations in soils) and the TF (translocation factor) (i.e., the ratio of TE concentrations aboveground organs or rhizomes to TE concentrations in roots) so as to compare TE accumulation and distribution among samples from different plots and sub-plots.

Analysis of variance was performed. The normal distribution of data (Shapiro-Wilk test) and equality of variances (Bartlett test) were checked. When both tests proved conformity, Fisher statistics was considered for significance ($p \leq 0.05$) and the Tukey (HSD) test was used for pair-wise comparisons of statistical groups. The Kruskal-Wallis test was performed for data that were not distributed normally. If this test was significant ($p \leq 0.05$), the groups were compared with the Mann-Whitney test. Principal component analysis was performed to study the relationship between the soil physico-chemical parameters and TE mobility. Pearson's correlation coefficients were calculated to relate the soil physico-chemical parameters and TE accumulation in miscanthus organs.

4.3. Results

4.3.1. Soil physico-chemical parameters

The physico-chemical parameters of topsoils (0-25 cm) differ significantly depending on the plots (**Table 4.1**).

Silt dominates in the particle size of studied soils. The sand fraction in MC soil is lower than in M200 and M500 soils whereas the clay fraction is higher in M500 soil than in MC and M200 soils. In M200 soils, there are lower clay and higher silt fractions in M200-3 than in M200-1 soils.

The pH in MC soil (6.5) is slightly acidic, whereas it is slightly alkaline in the other soils (pH = 7.4-7.6). The high carbonate content (22.6 g kg^{-1}) in M500 soil confirms its calcareous origin (**Section 2.1**). The MC soil has the lowest carbonate content (0.82 g kg^{-1}), while significant differences were observed between the M200-1 (2.5 g kg^{-1}) and M200-3 (9.1 g kg^{-1}) soils. The OC content (27.6 g kg^{-1}) in the M500 soil is nearly 2-fold higher than in the other soils.

The highest P_2O_5 levels were measured in the M200-1 (0.43 g kg^{-1}) and M200-3 (0.41 g kg^{-1}) soils, which are 2- and 4-fold higher than in M500 and MC soils respectively.

Table 4.1. Physico-chemical parameters of the soils (0-25 cm). Soil particle sizes are expressed in %, n = 1. For other parameters, values are means ± standard deviations, n = 5. The different letters represent significant differences (Tukey HSD test, $p \leq 0.05$) between soils.

Parameters	MC	M200-1	M200-3	M500
Clay (%)	21.3	25.4	19.1	29.0
Fine silt (%)	27.8	16.7	18.8	16.4
Coarse silt (%)	41.4	27.5	35.9	34.7
Fine sand (%)	8.5	27.4	23.5	18.2
Coarse sand (%)	1.0	3	2.7	1.7
pH (H_2O)	6.5 ± 0.6^{b}	7.5 ± 0.1^{a}	7.4 ± 0.2^{a}	7.6 ± 0.1^{a}
Carbonates (g kg^{-1})	0.82 ± 0.46^{d}	2.51 ± 1.25^{c}	9.14 ± 3.98^{b}	22.55 ± 3.43^{a}
OC (g kg^{-1})*	16.84 ± 1.74^{b}	14.75 ± 0.69^{b}	15.09 ± 1.29^{b}	27.60 ± 1.76^{a}
P_2O_5 (g kg^{-1})	0.12 ± 0.05^{c}	0.43 ± 0.04^{a}	0.41 ± 0.06^{a}	0.22 ± 0.02^{b}
Fe (g kg^{-1})	8.62 ± 3.36^{a}	4.78 ± 0.12^{b}	4.65 ± 0.52^{b}	7.78 ± 0.71^{a}
Mn (g kg^{-1})	0.35 ± 0.06^{a}	0.21 ± 0.03^{b}	0.27 ± 0.03^{b}	0.13 ± 0.01^{c}
CEC ($cmol^{+}$ kg^{-1})	15.6 ± 1.0^{c}	16.3 ± 0.5^{c}	19.9 ± 0.4^{b}	25.4 ± 0.9^{a}
Ca^{2+} ($cmol^{+}$ kg^{-1})	9.55 ± 1.80^{c}	19.57 ± 0.67^{b}	30.61 ± 11.02^{ab}	40.80 ± 11.29^{a}
K^{+} ($cmol^{+}$ kg^{-1})	0.38 ± 0.11^{b}	0.61 ± 0.08^{a}	0.61 ± 0.03^{a}	0.35 ± 0.02^{b}
Mg^{2+} ($cmol^{+}$ kg^{-1})	0.76 ± 0.07^{bc}	0.88 ± 0.14^{ab}	0.72 ± 0.12^{c}	1.06 ± 0.04^{a}
Na^{+} ($cmol^{+}$ kg^{-1})	0.07 ± 0.02^{a}	0.03 ± 0.00^{b}	0.02 ± 0.01^{b}	0.05 ± 0.01^{a}

*OC: organic carbon

There is no significant difference in Fe content between the M200-1 and M200-3 soils whose concentrations are 1.6-1.8-fold lower than in the MC (8.62 g kg^{-1}) and M500 (7.78 g kg^{-1}) soils. The concentration of Mn^{2+} is highest in the MC soil (0.35 g kg^{-1}), and it is 1.5- to 2.6-fold higher than in the M200 and M500 soils. The CEC is the lowest in the MC soil (15.6 $cmol^{+}$ kg^{-1}) and the highest in the M500 soil (25.4 $cmol^{+}$ kg^{-1}). The CEC is lower in the M200-1 soil (16.3 $cmol^{+}$ kg^{-1}) than in the M200-3 soil (19.9 $cmol^{+}$ kg^{-1}). All soils have a saturated exchange complex largely dominated by Ca^{2+} ($Ca^{2+} >> Mg^{2+} \geq K^{+} > Na^{+}$) with higher Ca^{2+} content in the M500 soil (40.8 $cmol^{+}$ kg^{-1}) than in the other soils. Higher Ca^{2+} contents also suggests that $CaCO_3$ is dominant among the carboantes in the studied soils.

4.3.2. Trace elements in topsoils

4.3.2.1. Cadmium, Pb and Zn pseudototal concentrations

For all the TEs, the soils present a contamination gradient as follows: M500 > M200-1 = M200-3 >> MC (**Table 4.2**). The mean concentrations of Cd, Pb and Zn in the MC soil are, 0.18, 14 and 53 mg kg^{-1} respectively. These concentrations are nearly equal to or lower than frequent regional TE concentrations in cultivated loamy soils (Sterckeman et al., 2002). The M500 soil has the highest Cd (8.3 mg kg^{-1}), Pb (461 mg kg^{-1}) and Zn (523 mg kg^{-1})

concentrations. These concentrations are, respectively, 46, 33 and 10 times higher than those measured in the MC soil, and 2-, 2.5- and 1.5-fold higher than in the M200-1 and M200-3 soils.

Table 4.2. Pseudo-total concentrations (mg kg^{-1} DW) and 0.01M $CaCl_2$-extractable fractions (%) of Cd, Pb and Zn in the studied soils. The different letters refer to significant differences between soils (Tukey HSD test for pseudo-total concentrations, and Mann-Whitney test for extractable fractions. $p \leq 0.05$).

Soils	Pseudo-total concentrations (mg kg^{-1} DW)			$CaCl_2$-extractable fractions (%)		
	Cd	Pb	Zn	Cd (%)	Pb (%)	Zn (%)
MC	0.18 ± 0.13^{c}	13.67 ± 7.90^{c}	52.27 ± 6.32^{c}	0.609 ± 0.411^{a}	< DL	0.311 ± 0.128^{a}
M200-1	4.02 ± 0.22^{b}	204.91 ± 30.60^{b}	351.26 ± 24.45^{b}	0.219 ± 0.074^{b}	0.006 ± 0.001^{a}	0.067 ± 0.064^{ab}
M200-3	4.24 ± 0.67^{b}	201.88 ± 15.19^{b}	343.69 ± 26.73^{b}	0.252 ± 0.133^{b}	0.005 ± 0.001^{a}	0.114 ± 0.047^{b}
M500	8.26 ± 0.52^{a}	460.93 ± 44.86^{a}	522.95 ± 68.47^{a}	0.400 ± 0.277^{ab}	0.003 ± 0.000^{b}	0.044 ± 0.008^{c}

< DL: below detection limit

4.3.2.2. Extractable Cd, Pb and Zn fractions by 0.01 M $CaCl_2$

The $CaCl_2$-extractable Cd, Pb and Zn fractions are presented in (**Table 4.2**).

The fraction of each TE is substantially less than 1% in all soils. In the MC soil, Cd and Zn fractions are 0.61 and 0.31%, respectively, and both TEs present a higher extractability than Pb. The extractable fraction of each of the three TEs does not differ between the two M200 soils. The Pb (0.003%) and Zn (0.044%) extractable fractions are the lowest in the M500 soil.

4.3.2.3. Cadmium, Pb and Zn fractionation and relationship with physico-chemical parameters

The Cd, Pb and Zn distribution in different fractions in the studied soils is presented in **Fig. 4.1**. Principal component analysis was performed to study the relationship between TE fractions and soil physico-chemical parameters **(Fig. 4.2).**

a) Cadmium

Cadmium is highly present in fractions A and B. The fraction A in the M200-1 and M200-3 soils (51 and 46%, respectively) is higher than in the M500 (35%) and MC (33%) soils. Conversely, the fraction B is higher in the M500 soil (49%) than the in the MC soil (40%). In the M200, this fraction is lower in the M200-1 (30%) than the M200-3 (35%) soils. Fraction D is higher in the MC soil (16%) than in the other soils whose fractions are about 5%. The soils do not differ with regard to fraction R (11-14%).

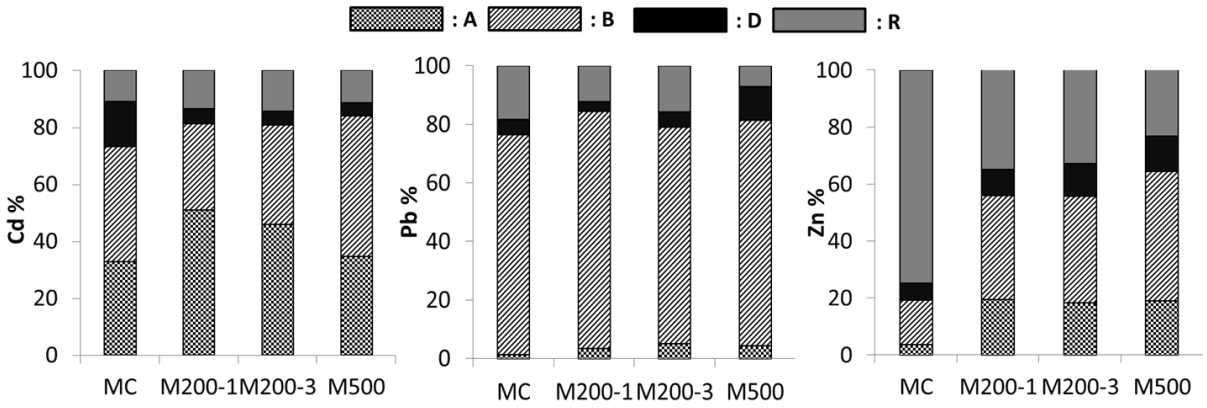

Fig. 4.1. Fractionation (%) of Cd, Pb and Zn in the studied soils. The piled bars represent means of each fraction in soils. **A**: Exchangeable, water and acid-soluble; **B**: reducible, mainly occluded in soil oxides; **D**: oxidizable, mainly associated with soil organic matter; **R**: residual, relating to the soil crystalline matrix.

b) Lead

Lead is highly present in fraction B with 75, 74, 81 and 77% in the MC, M200-1, M200-3 and M500 soils, respectively. Fraction A (3.6-5.4%) does not differ between the contaminated soils and is the lowest in the MC soil (1.5%). The M500 soil presents a higher fraction D (11.5%) than the other soils, whose fractions vary between 3.4 and 5.1%. Conversely, fraction R decreases with soil contamination level; it is lower in the M500 (7%) and higher in MC (18%) soils than in M200 soils.

c) Zinc

Zinc is highly present in fractions B and R. Fraction B is the highest in the M500 soil (45%), which is 1.2 and 3-fold higher than in the M200 and MC soils, respectively. Fraction R is the highest in the MC soil (74.8%), and it is 2 and 3-fold higher than in the M200 and M500 soils, respectively. Conversely, fraction A is the lowest in the MC soil (3.6%), which is more than 5-fold lower than in the other soils. Similarly, fraction D was lower in the MC (5.9%) and higher in the M500 (12.4%) soils than in the M200 soils.

Overall, the correlation between soil physico-chemical parameters and TE mobility differentiates the soils of the three plots (**Fig. 4.2**). The MC soil is characterized by low pH but with high Fe and Mn oxide contents as well as high fraction R and $CaCl_2$-extractable fraction. The M200-1 and M200-3 soils constitute one group characterized by high Cd in fraction A, P_2O_5 and K^+ contents, but low Fe oxide and Na^+ contents. The M500 soil is mainly characterized by a high Pb in fraction D and high CEC, OC, carboantes ($CaCO_3$), Ca^{2+} and Mg^{2+}contents.

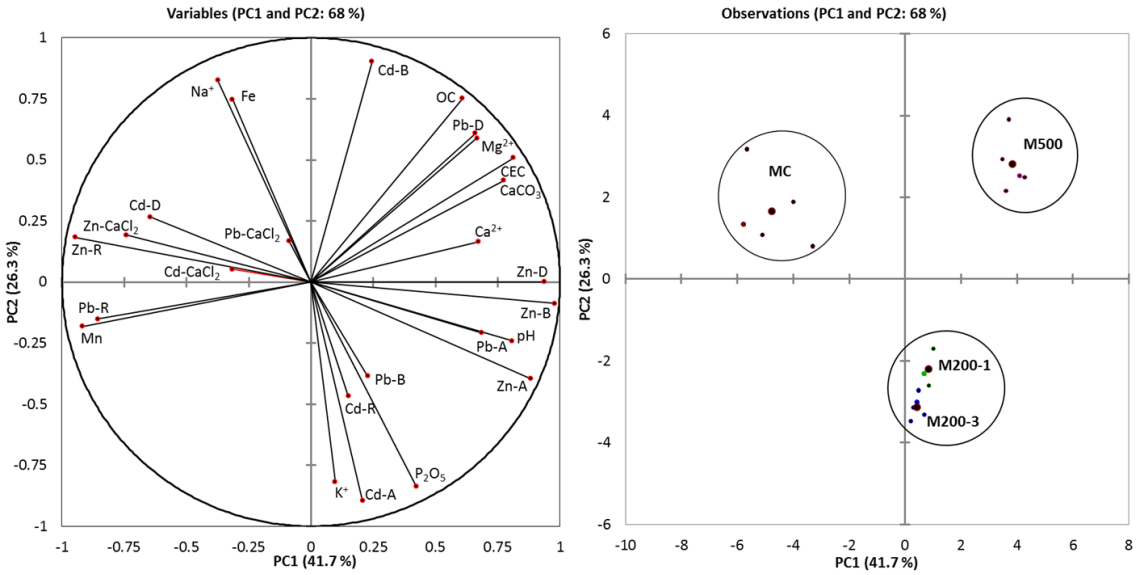

Fig. 4.2. Correlation of soil physico-chemical parameters, 0.01M $CaCl_2$ and sequential TE-extractable fractions. Variable correlation circle (left) and soil positions on the first two principal components PC1 and PC2 (right).

4.3.3. Trace element distribution in miscanthus organs

4.3.3.1. Cadmium, Pb, and Zn concentrations

a) Cadmium

The mean concentrations of Cd in organs are in the following descending order: root >> rhizomes > stems = leaves (**Fig. 4.3**).

On the MC soil, the mean concentrations in the four organs vary between 0.4 and 0.7 mg kg^{-1} and are not significantly different. The root concentrations on the M200-1 (11.6 mg kg^{-1}), M200-3 (10.1 mg kg^{-1}) and M500 (20.9 mg kg^{-1}) soils are, respectively, 16.6, 14.5 and 29.8 times higher than on the MC soil. The rhizome concentrations on the M200-1, M200-3 and M500 soils do not differ and are 10 to 12-fold lower than in the roots.

b) Lead

The mean Pb concentrations in organs can be ranked as follows: roots > rhizomes = stems = leaves on contaminated soils and leaves = stems >> roots > rhizomes on the MC soil (**Fig. 4.3**). The rhizome concentrations (< 0.05 mg kg^{-1}) on the MC soil are the lowest of all organs, while their concentrations on contaminated soils are nearly identical. On the M200-1, M200-3 and M500 soils, the roots present mean concentrations of 31.6, 26.6 and 34.0 mg kg^{-1} respectively, and these concentrations do not significantly differ. The mean leaf and stem concentrations vary between 8.0 and 12.5 mg kg^{-1}, and do not differ whatever the soil.

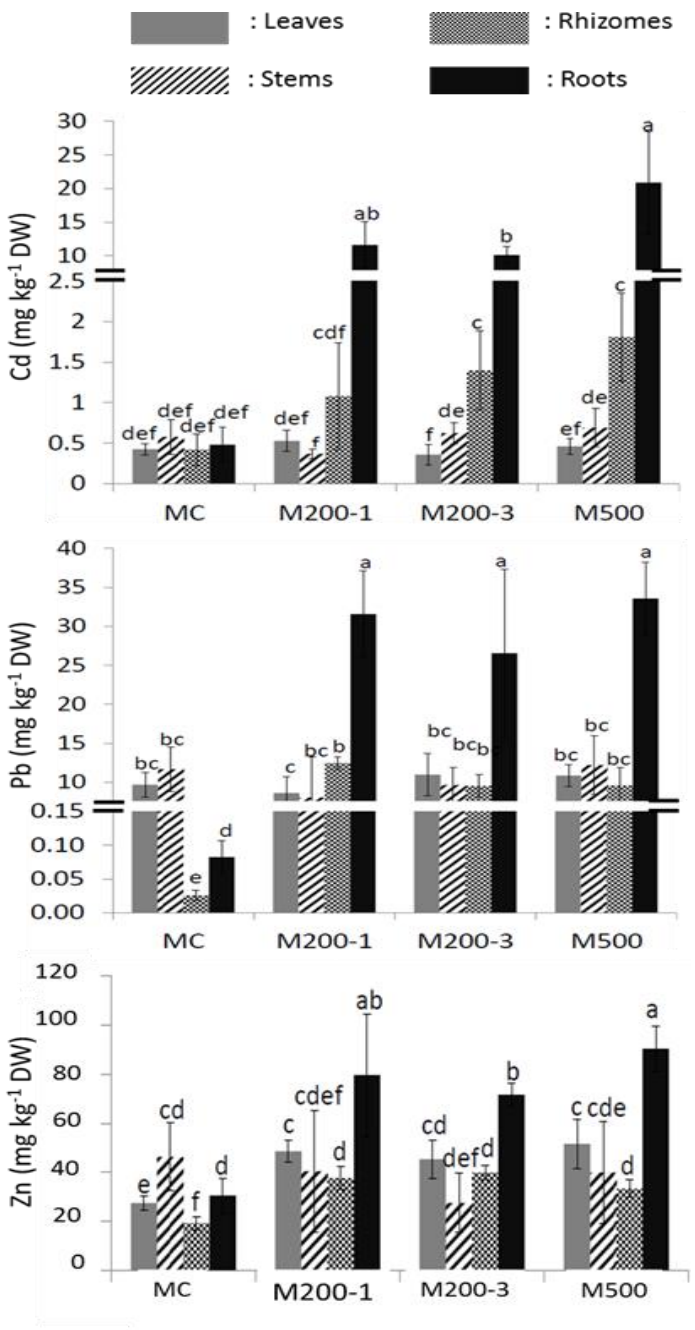

Fig. 4.3. Concentrations (mg kg^{-1} DW) of Cd, Pb and Zn in miscanthus organs. The histograms represent means and associated standard deviations. The different letters refer to significant differences (Tukey HSD test, $p \leq 0.05$) between organs and depending on soil contamination levels.

c) Zinc

The mean Zn concentrations in organs can be ranked as follows: roots > rhizomes = stems = leaves on contaminated soil, and roots = stems > leaves > rhizomes on the MC soil (**Fig. 4.3**). The root concentrations (30.5 mg kg^{-1}) on the MC soil are, respectively, 2.6, 2.4 and 3 times lower than those on the M200-1, M200-3 and M500 soils. Also, stem concentrations (27.6-46.4 mg kg^{-1}) do not significantly differ whatever the soil and are higher than in leaves and rhizomes on the MC soil. The rhizome (33.4-40 mg kg^{-1}) and leaf (45.4-51.6 mg kg^{-1})

concentrations on contaminated soils are higher than on the MC soil (27.5 and 19.3 mg kg^{-1} for rhizomes and leaves, respectively).

4.3.3.2. Trace element transfer from soil to miscanthus organs

The TE transfer ratio from the soil to miscanthus was assessed using the BCFs (**Table 4.3**).

Table 4.3. Comparison of BCFs and TFs of miscanthus organs. Values represent means ± standard deviations. Different letters refer for significant differences depending on organs and soils (Mann-Whitney test, $p \leq 0.05$, n = 5).

TE	Soils	BCF				TF		
		Leaves	Stems	Rhizomes	Roots	Leaves	Stems	Rhizomes
Cd	MC	3.28 ± 1.97^{a}	4.36 ± 1.68^{a}	3.48 ± 2.17^{a}	3.63 ± 2.02^{a}	0.80 ± 0.43^{c}	1.14 ± 0.90^{c}	0.78 ± 0.60^{c}
	M200-1	0.13 ± 0.04^{cd}	0.09 ± 0.04^{d}	0.28 ± 0.19^{bcd}	2.99 ± 1.05^{a}	0.05 ± 0.01^{ab}	0.03 ± 0.01^{ab}	0.09 ± 0.03^{abc}
	M200-3	0.09 ± 0.03^{d}	0.15 ± 0.02^{c}	0.33 ± 0.11^{b}	2.43 ± 0.45^{a}	0.04 ± 0.04^{ab}	0.06 ± 0.01^{ab}	0.14 ± 0.04^{bc}
	M500	0.06 ± 0.01^{e}	0.08 ± 0.03^{de}	0.22 ± 0.06^{b}	2.51 ± 0.87^{a}	0.02 ± 0.01^{a}	0.04 ± 0.02^{ab}	0.09 ± 0.03^{bc}
Pb	MC	1.02 ± 0.72^{a}	1.31 ± 1.02^{a}	nd	0.01 ± 0.00^{e}	nd	1.31 ± 1.02^{c}	0.27 ± 0.16^{bc}
	M200-1	0.04 ± 0.02^{cde}	0.04 ± 0.02^{cde}	0.06 ± 0.02^{cd}	0.16 ± 0.00^{b}	0.4 ± 0.10^{c}	0.16 ± 0.05^{b}	0.001 ± 0.001^{a}
	M200-3	0.06 ± 0.02^{cd}	0.05 ± 0.01^{d}	0.05 ± 0.01^{d}	0.13 ± 0.06^{b}	0.43 ± 0.2^{c}	0.42 ± 0.1^{c}	0.14 ± 0.19^{abc}
	M500	0.02 ± 0.00^{e}	0.03 ± 0.01^{e}	0.02 ± 0.00^{e}	0.07 ± 0.02^{c}	0.24 ± 0.11^{bc}	0.31 ± 0.14^{bc}	0.04 ± 0.06^{a}
Zn	MC	0.53 ± 0.09^{b}	0.89 ± 0.27^{a}	0.39 ± 0.05^{c}	0.58 ± 0.09^{b}	0.93 ± 0.19^{cd}	1.63 ± 0.79^{d}	0.66 ± 0.19^{b}
	M200-1	0.14 ± 0.02^{e}	0.12 ± 0.07^{ef}	0.11 ± 0.01^{e}	0.24 ± 0.09^{d}	0.63 ± 0.12^{b}	0.34 ± 0.14^{a}	0.51 ± 0.22^{ab}
	M200-3	0.13 ± 0.02^{e}	0.08 ± 0.04^{ef}	0.12 ± 0.01^{e}	0.21 ± 0.01^{d}	0.64 ± 0.14^{b}	0.39 ± 0.19^{ab}	0.56 ± 0.05^{b}
	M500	0.10 ± 0.02^{e}	0.08 ± 0.03^{ef}	0.06 ± 0.01^{f}	0.18 ± 0.04^{d}	0.58 ± 0.15^{b}	0.45 ± 0.25^{ab}	0.38 ± 0.08^{a}

nd: not determined

On the MC soil, the mean BCFs calculated for Cd vary from 3.28 (for leaves) to 4.36 (for stems), and are not significantly different. For Pb, the mean BCFs are 1.02 (leaves) and 1.31 (stems) and are higher than those of roots and rhizomes, which are for the most part less than 0.1. Whatever the organ, zinc BCFs are less than 1 and can be ranked as follows: stems > leaves = roots > rhizomes. However, given the low soil TE concentrations in this soil (**Table 4.2**), the BCFs are less relevant for the assessment of miscanthus TE accumulation behaviour with regard to toxic TE concentrations.

On contaminated soils, whatever the TE, BCFs are higher for roots than for rhizomes, stems and leaves. Also, apart from Cd roots' BCFs (2.99, 2.43 and 2.51, on the M200-1, M200-3 and M500 soils respectively), the BCFs for all organs are much lower than 1.

Overall, in contaminated soils, the BCFs decrease with soil contamination and allow ranking the three TE accumulations in miscanthus as follows: Cd >> Zn > Pb (roots), Cd > Zn > Pb (rhizomes), Cd = Zn > Pb (stems) and Cd ≥ Zn > Pb (leaves). Cadmium is the most accumulated in the underground organs with BCFs ≤ 0.33 (rhizomes) and ≤ 2.99 (roots). Lead is the least accumulated in all organs with BCF ≤ 0.06 for aerial parts and rhizomes, and ≤ 0.16 for roots. Zinc accumulation does not generally differ between aerial parts and rhizomes.

The relationships among TE accumulation in miscanthus organs, and between TE accumulation in miscanthus organs and the soil physico-chemical parameters are presented in **Table 4.4.**

Table 4.4. Pearson's correlation coefficients between BCFs and soil physico-chemical parameters. Bold values are statistically significant at $p \leq 0.05$ with Bonferroni correction (L: leaves; S: stems; Rhi: rhizomes; R: roots).

		Cd				**Pb**				**Zn**			
		L	S	Rhi	R	L	S	Rhi	R	L	S	Rhi	R
Cd	L	**1.00**											
	S	**0.97**	**1.00**										
	Rhi	**0.94**	**0.98**	**1.00**									
	R	0.64	0.66	0.62	**1.00**								
Pb	L	0.60	0.63	0.61	**0.84**	**1.00**							
	S	0.67	0.69	0.66	**0.82**	**0.98**	**1.00**						
	Rhi	-0.40	-0.38	-0.36	-0.15	-0.32	-0.35	**1.00**					
	R	-0.05	0.06	0.17	0.43	0.64	0.54	0.06	**1.00**				
Zn	L	**0.80**	**0.77**	**0.80**	0.60	**0.79**	**0.82**	-0.41	0.38	**1.00**			
	S	**0.87**	**0.83**	**0.81**	**0.76**	**0.87**	**0.91**	-0.44	0.28	**0.93**	**1.00**		
	Rhi	**0.86**	**0.80**	**0.80**	0.68	**0.81**	**0.83**	-0.37	0.30	**0.96**	**0.96**	**1.00**	
	R	0.70	0.61	0.65	0.45	0.62	0.64	-0.37	0.25	**0.94**	**0.83**	**0.91**	**1.00**
Soil parameters	pH	**-0.89**	**-0.92**	**-0.93**	-0.65	**-0.76**	**-0.83**	0.39	-0.26	**-0.88**	**-0.90**	**-0.86**	-0.72
	OC	-0.28	-0.28	-0.29	-0.24	-0.26	-0.28	-0.43	-0.21	-0.36	-0.30	-0.42	-0.33
	$CaCO_3$	-0.49	-0.46	-0.54	-0.34	-0.44	-0.48	-0.20	-0.28	-0.62	-0.56	-0.73	-0.68
	P_2O_5	-0.61	-0.59	-0.60	-0.43	-0.56	-0.60	0.72	-0.18	-0.68	-0.68	-0.60	-0.64
	Fe oxides	0.25	0.15	0.08	0.43	0.61	0.60	-0.54	0.21	0.45	0.56	0.44	0.43
	Mn oxides	0.56	0.48	0.45	0.45	0.63	0.65	-0.11	0.20	**0.75**	**0.73**	**0.81**	**0.73**
	CEC	-0.51	-0.49	-0.50	-0.37	-0.45	-0.47	-0.13	-0.23	-0.59	-0.54	-0.66	-0.54
	Ca^{2+}	-0.53	-0.56	-0.60	-0.46	-0.49	-0.55	0.05	-0.20	-0.61	-0.58	-0.60	-0.53
	K^+	-0.47	-0.44	-0.36	-0.22	-0.29	-0.38	0.70	0.23	-0.33	-0.43	-0.27	-0.24
	Mg^{2+}	-0.36	-0.31	-0.29	-0.15	-0.19	-0.24	-0.27	-0.01	-0.38	-0.31	-0.44	-0.38
	Na^+	0.46	0.49	0.54	0.52	0.70	0.68	-0.56	0.54	0.66	0.63	0.56	0.56
	Cd-total	-0.72	-0.65	-0.67	-0.51	-0.60	-0.63	0.06	-0.23	**-0.83**	**-0.78**	**-0.89**	**-0.85**
	Pb-total	-0.66	-0.60	-0.63	-0.47	-0.57	-0.60	0.00	-0.24	**-0.79**	**-0.74**	**-0.85**	**-0.81**
	Zn-total	**-0.78**	-0.71	-0.73	-0.51	-0.65	-0.69	0.17	-0.24	**-0.90**	**-0.85**	**-0.94**	**-0.91**
	Cd-$CaCl_2$	0.69	**0.76**	**0.80**	0.11	0.06	0.12	-0.19	-0.18	0.44	0.35	0.39	0.34
	Pb-$CaCl_2$	0.49	0.56	0.61	-0.09	-0.10	-0.05	-0.23	-0.16	0.26	0.15	0.19	0.24
	Zn-$CaCl_2$	0.46	0.44	0.44	0.71	**0.88**	**0.87**	-0.39	0.55	0.69	**0.78**	**0.77**	0.61

Leaf and stem BCFs are positively correlated whatever the TE. Moreover, Zn accumulation is positively correlated with Cd accumulation in all organs. However, Zn accumulation in the leaves, stems and rhizomes positively correlates with Pb in the leaves and stems, but it negatively correlates with Pb in the rhizomes. The TE accumulation in miscanthus organs is negatively correlated with TE pseudototal concentrations and physico-chemical parameters of the studied soils except for Fe/Mn oxides and Na^+ contents. Cadmium and Zn accumulations

are positively correlated with their $CaCl_2$-extractable fractions in soils, although the coefficients are very low for Cd in roots (r = 0.11) or not significant for root and leaves. Conversely, Pb accumulation in all organs is poorly and negatively correlated with its $CaCl_2$-extractable fraction.

4.3.3.3. Trace element transfer from roots to rhizomes, stems and leaves

The TE transfer ratio from the roots to other miscanthus organs was assessed using the TF (**Table 3).** On the MC soil, the mean TFs for all organs are higher than on the M200-1, M200-3 and M500 soils, and they are higher than 1 only for stems (1.14, 1.31 and 1.63 for Cd, Pb and Zn, respectively). On contaminated soils, the TFs are lower than 1 for all organs. Overall, the TFs allow ranking TE transfer as follows: Zn >> Cd > Pb (rhizomes); Zn > Pb > Cd (stems) and Zn > Pb > Cd (leaves). Cadmium is the least transferred to stems and leaves. Both high Cd root concentrations and BCFs are not followed by high TFs, suggesting the capacity of miscanthus to reduce Cd accumulation in shoots.

4.4. Discussion

The present work aimed at assessing the use of miscanthus for phytostabilization of highly TE-contaminated agricultural soils. The experimental set-up allows the study of soil physico-chemical parameters and TE behavior under a perennial crop-managed agrosystem. The phytostabilisation option is appraised, with reference to TE mobility in soils and their accumulation patterns in non-senescent miscanthus organs.

4.4.1. Soil TE mobility and physico-chemical parameters

Single and sequential extraction schemes are commonly used to assess soil TE mobility (Violante et al., 2010). The 0.01-M $CaCl_2$ extraction is indicated as a suitable method for determination of easily soluble Cd, Cu, Pb and Zn fractions in neutral to slightly alkaline soils without inducing pH changes in the process (Pueyo et al., 2004). Sequential extractions aim at simulating conditions under which TEs associated with different soil components in the solid phase can be released into the soil solution, hence allowing to determine their potential mobility and (phyto)availability (Kabata Pendias, 2004; Violante et al., 2010).

In the studied topsoils, single extractions show that after 3 to 4 years of miscanthus growth, TEs are potentially slightly mobile and their potential solubility differs depending on a given element. Cadmium and Zn are more extractable than Pb for all the studied soils. These results corroborate with those reported by Pogrzeba et al. (2013), who used the same extractant in silt-clay loam soils polluted by smelting activities and planted with miscanthus. Indeed, Cd and Zn are loosely adsorbed in cation exchange positions and are easily complexed with Cl^-

anions in the extraction solution (van der Sloot et al., 1996), whereas Pb is naturally less mobile because it is strongly sorbed in Fe/Mn oxy(hydro)oxides and organic matter (Cerqueira et al., 2011; Violante et al., 2010).

In sequential extractions, TEs are highly available in the exchangeable, water- and acid-soluble (A), and reducible (B) fractions, which indicates their anthropogenic origin (Kaasalainen and Yli-Halla, 2003). Most particularly, Cd and Zn are more available in fraction A than Pb. Also, Cd, Pb and Zn are mainly available in fractions A, B and R, respectively. These results show that TE distribution in soils does not differ from their usual distribution in topsoils from the Metaleurop site (Douay et al., 2009; Pelfrêne et al., 2011; Waterlot et al., 2012).

A relatively greater Cd and Zn availability in fraction A than Pb could be due to their usual higher solubility (Kabata Pendias, 2004) in acid conditions. Both elements are usually more adsorbed in cation exchange positions of the soil solid phase (van der Sloot et al., 1996), suggesting that their availability could likely increase in oxidizing conditions and in soils presenting a relatively higher CEC. This is more valid for Zn in fraction A, which is highly positively correlated with pH and CEC. Also, notwithstanding the ability of phosphates to immobilize TEs (Waterlot et al., 2011), the correlation of Cd in fraction A with soluble phosphorus (P_2O_5) content in the studied soils, especially in the M200 soils, suggests that high phosphate contents could influence TE availability and/or solubility in agricultural soils. Lambert et al. (2007) demonstrated that long-term soil fertilization with granular commercial monammonium phosphate leads to enhanced Cd solubility, possibly due to the formation of soluble phosphate complexes such as $CdHPO_4$.

The higher presence of TEs in the reducible fraction, especially Pb, suggests their association with Fe and Mn oxy(hydro)oxides in soils (Kaasalainen and Yli-Halla, 2003). Indeed, the studied soils present higher Fe oxide contents, suggesting more sites for TE sorption and long-term retention in the soil matrix (Komárek et al., 2013). The negative correlations between Fe/Mn oxide contents and fraction A of all the three studied TE emphasize the role played by Fe/Mn oxides in TE retention in the studied soils.

Among the physico-chemical parameters of studied soils that can influence TE mobility, pH, clay, organic carbon, calcium carbonates and Fe/Mn oxide contents could be the most important (Pinto et al., 2014). Generally, some TEs (Pb, Cd and Zn) are more soluble in an acidic pH (< 6.5), whereas they are less available at a pH that is close to neutral or slightly alkaline (6.6-7.5) (Remon et al., 2005). At alkaline pH, the negative charges of clay minerals and oxides, and humic compounds are higher (Sparks, 2005), thus increasing the possibility of

formation of precipitates and organometallic complexes. Moreover, in soils with alkaline pH, relatively high contents of carbonates, organic carbon and Fe/Mn oxy(hydro)oxides often confers a high buffering capacity capable of delaying the TE dissolution and maintaining them in the soil solid phase (Carrillo-Gonzalez et al., 2006; Buekers et al., 2007). With reference to the studied soils, the physicochemical parameters in the MC soil (slightly acidic pH, low carbonate content and low CEC) could potentially increase TE mobility. Conversely, the M500 soil markedly differs from the MC and M200 soils in its high CEC, clay, carbonate and organic carbon contents, which could reduce TE mobility and phytoavailability.

4.4.2. Trace element accumulation in miscanthus organs

The roots constitute the main TE entry in terrestrial plants and high TE concentrations in cells and tissues potentially induce toxicity (DalCorso et al., 2013). To overcome TE toxicity, plants have developed two main tolerance strategies (Baker, 1981). The exclusion or avoidance strategy allows plants (excluders) to grow within a wide range of phytotoxic TE concentrations and significantly prevent them from entering root or restricting their transfer to the shoots. The accumulation strategy permits plants (accumulators) to uptake TEs by roots and transfer a significant fraction to leaves. The accumulators have more active root and leaf detoxification mechanisms that include TE homeostasis and sequestration (DalCorso et al., 2013).

The present results show that on contaminated soils, miscanthus mainly accumulates Cd, Pb and Zn in roots and strongly reduces their transfer to the rhizomes and shoots. These results are consistent with those of Arduini et al. (2006), who exposed miscanthus seedlings to increasing Cd concentrations (0.75-3 mg L^{-1}) under hydroponic conditions, and those of Wanat et al. (2013) obtained in acidic Technosols contaminated by low to very high Pb concentrations (23,325 and 15,200 mg kg^{-1}). In roots and rhizomes, TE concentrations were significantly lower in uncontaminated soil than in contaminated ones whereas TE concentrations in stems and leaves did not significantly differ in all soils. Moreover, the TE concentrations in the four miscanthus organs are not significantly different on the studied contaminated soils. This suggests that in studied soil conditions, TE uptake and accumulation in miscanthus organs are controlled and do not increase with the soil contamination gradient. These findings are in disagreement with those of Arduini et al. (2006) for Cd and Wanat et al. (2013) for Pb, which show that in all miscanthus organs, the concentrations of these elements increase with the contamination gradient. They are also not consistent with the results obtained by Pavel et al. (2014), which show that Cd, Pb and Zn accumulation in miscanthus stems and leaves increases with increasing soil contamination level (4.4-10.3, 146-419 and

315-673 mg kg for Cd, Pb and Zn, respectively). However, it should be noted that these three studies were conducted in different conditions from ours: spiked-hydroponic culture (Arduini et al., 2006), highly acidic Technosols (Wanat et al., 2013), and contaminated acid (pH = 5.3-6.9) sandy clay soils (Pavel et al., 2014).

In contaminated conditions, both BCFs and TFs are commonly used to assess plant TE uptake efficiency and accumulation mechanisms, and ultimately to select suitable plants for phytoremediation of TE-contaminated matrices (Mench et al., 2010). A plant can be considered as accumulator when the BCF and TF are higher than 1, whereas it is considered as a non-accumulator or excluder when the BCF and TF are lower than 1 (Mench et al., 2010). Our results show that apart from Cd roots' BCFs, both BCFs and TFs for all organs or TEs are less than 1 on contaminated soils. Moreover, TE concentrations in each organ and their TFs do not generally differ on these soils. This demonstrates that TE root uptake from the soil and their transfer to aerial organs is tightly controlled. The low soil-to-miscanthus TE transfer could be associated with the natural soil buffering capacity due to neutral or alkaline pH, higher carboantes (especially $CaCO_3$), Fe and Mn oxide contents, low content of sandy fraction in these soils. Indeed, the BCFs were generally negatively correlated with physico-chemical parameters, $CaCl_2$-extractable TE fractions as well as pseudototal TE concentrations in the studied soils. The significant correlation of the BCFs with pH suggests that soil pH is the driving factor of soil-to-miscanthus TE transfer. Accordingly, apart from MC soil with a slightly acid pH, M200 and M500 soils have a neutral to slightly alkaline pH, which potentially reduces TE solubility in soil solution, hence root absorption (Kabata Pendias, 2004; Violante et al., 2010). It is worth noting that leaf, stem and rhizome BCFs for the three TEs are significantly lower on the M500 soil than on the M200-1 and M200-3 soils, which suggests that the soil-to-miscanthus TE transfer is restricted with increasing soil contamination. Also, this difference could be due to a low TE phytoavailability, especially Pb and Zn in the M500 soil. Lower phytoavailability and/or a higher TE retention capacity in this soil could be due to the clay, carbonate and organic carbon contents, which are significantly higher in the M500 soil than in the M200 soils.

The positive correlation between TE accumulation in miscanthus leaves and stems suggests common mechanisms of TE transfer and accumulation within miscanthus tissues. Cadmium and Pb have no known beneficial functions in plant physiology (DalCorso et al., 2013), hence their transfer and accumulation within the plant should mainly be due to the TE concentration gradient in the soil solution or water transport from the soil to the plant (Violante et al., 2010). Moreover, TE sequestration in cell vacuoles and their sorption on cell walls are common

mechanisms involved in alleviating excess TE ion toxicity, which can partly explain the positive correlation between TE accumulation in a given plant organ (DalCorso et al., 2013; Pourrut et al., 2013). Conversely, Zn is an essential micronutrient and its supply at cell level implies active and passive mechanisms (Sadeghzadeh and Rengel, 2011, Pinto et al., 2014), and this possibly explains the fact that it is the most accumulated and transferred to miscanthus aboveground organs.

Overall, high Cd, Pb and Zn accumulation in roots and both BCFs and TFs lower than 1 in contaminated soils indicate that *M.* × *giganteus* is an excluder species suitable for phytostabilization of the studied TEs. In plants, high root TE concentrations or TE retention capacity suggest the presence of active TE sequestration mechanisms. These include TE adsorption onto root cell wall pectins, synthesis and secretion of TE ligands or chelators such as phytochelatins and low molecular carboxylic organic acids (DalCorso et al., 2013; Pinto et al., 2014). In Poacea, the formation of TE complexes in the apoplast such as Zn silicates in *Oryza sativa* (Gu et al., 2012), or the Si influence on the development of casparian bands and suberin lamallae below the endodermic layer in *Sorghum bicolor* (Lux et al., 2003) and *Zea mays* (Vaculík et al., 2012), are among the dominant mechanisms that limit diffusive TE (e.g., Cd) fluxes into the symplast and increase their sequestration in the apoplast (Vaculík et al., 2012). Altogether, despite the lack of information, these mechanisms could be key players in regulating root-to-shoot TE transfer and could enhance the root sequestration capacity of miscanthus.

The use of *M.* × *giganteus* for phytostabilization could not only offer the opportunity to produce the biomass on contaminated soils, but could also improve soil parameters and or site ecological conditions (Nsanganwimana et al., 2014). Our results suggest that planting this perennial grass on TE-contaminated lands could be a good phytomanagement option to reduce environmental and health risks due to contaminant exposures. Moreover, this plant grows quite well on the studied contaminated plots and the average biomass yield during the third year was 14 t DW ha^{-1} (personal data). Therefore, *M.* × *giganteus* could increase arable surface area for biomass crop production, hence stimulation of bioeconomy and mitigation of the current food vs. biofuel controversy.

4.5. Conclusion

Despite different physico-chemical parameters and the contamination gradient of the studied soils, TE accumulation patterns in miscanthus organs are more or less similar on contaminated soils. On these soils, miscanthus accumulates TE mainly in roots and reduces their transfer to the aerial organs. The studied TE concentrations in leaves and stems did not significantly differ between contaminated and uncontaminated plots. Both low BCFs and TFs on contaminated plots prove that *M.* × *giganteus* is a TE-excluder species. Therefore, this species could be a potential candidate crop for phytomanagement that couples phytostabilization and production of a valuable biomass on contaminated soils.

Our study shows that the cultivation of miscanthus did not induce significant changes in TE distribution and mobility in soils. However, a sustainable TE-contaminated soil management using miscanthus needs regular monitoring of soil physico-chemical parameters, biomass productivity and TE accumulation in miscanthus organs throughout the growth cycle and at harvest time. As phytomanagement aims at promoting ecosystem services on contaminated sites, miscanthus effects on TE behaviour, toxicity alleviation, soil biological functions and biodiversity as a whole should also be assessed. Information from such studies could constitute a key tool for optimization of soil management as well as the allocation of biomass uses with minimal environmental risks.

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Chapitre V :

Influence des variations saisonnières sur l'accumulation des métaux chez *Miscanthus* × *giganteus*

Préambule

Dans les chapitres précédents, l'étude de l'accumulation des métaux dans les organes de miscanthus a été réalisée sur des échantillons prélevés après 93 jours de culture pour l'expérimentation *ex situ* et au début de l'automne pour celle menée *in situ*. Or, il est connu que l'accumulation des éléments minéraux dans les plantes varie selon la saison et leur stade de développement. Miscanthus étant une plante à croissance pérenne, la considération des variations saisonnières s'avère nécessaire dans l'étude de l'accumulation des métaux dans ses organes.

La démarche a pour objectif d'une part, de confirmer le caractère excluder du miscanthus selon les saisons et d'autre part, d'évaluer la qualité de la biomasse aérienne obtenue lors de la récolte. Cinq échantillons de végétaux et de sols ont été prélevés sur chacune des parcelles M200, M500 et MC. L'échantillonnage a été effectué durant une même année culturale à trois périodes différentes : pleine croissance (juillet), fin de croissance (septembre) et senescence (févier).

Sur les échantillons de sol, ont été déterminés les paramètres physico-chimiques et les concentrations pseudototales en Cd, Pb et Zn. Dans les végétaux (racines, rhizomes, tiges et feuilles), les concentrations en métaux ont été mesurées. Comme dans la démarche précédente, les facteurs de bioconcentration et de translocation ont été calculés.

Valorisation : Ce chapitre a été soumis pour publication à GCB Bioenergy sous le titre « **Seasonal variations in metal accumulation in energy crop *Miscanthus* × *giganteus* growing in contaminated agricultural soils** ».

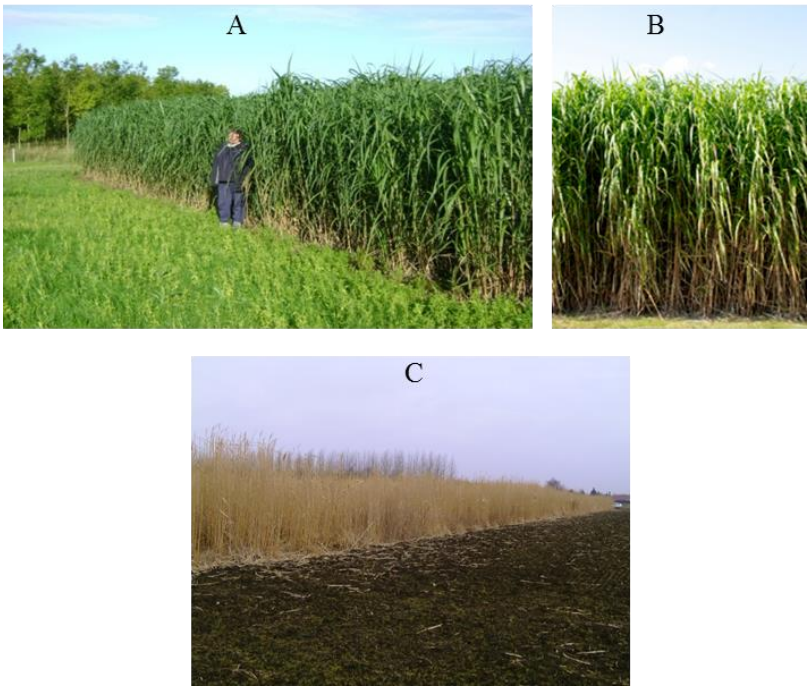

Fig. 5.1. Physionomie du miscanthus lors de l'échantillonnage. A) Juillet 2010, B) Septembre 2010, C) Février 2011.

Seasonal variations in metal accumulation in energy crop *Miscanthus* × *giganteus* growing in contaminated agricultural soils

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Abstract

Plant biomass is a promising renewable energy resource that provides fuel and high-grade bioproducts. Marginal lands that include metal-contaminated sites offer the opportunity to increase energy crop-dedicated land surface and to mitigate environmental and health risks due to exposure to contaminants. The present study demonstrates the possibility of using miscanthus (*Miscanthus* × *giganteus*) for phytostabilization of metal-contaminated agricultural soils in a smelter-impacted agrosystem in Northern France. Beginning in spring 2007, miscanthus experimental plantations were established on two agricultural plots (M200 and M500) presenting a metal contamination gradient as well as on an uncontaminated plot (MC). After 3-4 years, plant and soil samplings were scheduled in mid and late summer and in the winter in order to assess seasonal variations in Cd, Pb and Zn concentrations in miscanthus organs. The results show that Cd, Pb and Zn were mainly accumulated in roots for all sampling periods. Despite different soil physico-chemical parameters and contamination levels, metal concentrations, bioconcentration (BCF) and translocation (TF) factors in miscanthus organs did not significantly differ between the two contaminated plots. In these soils, the BCFs and TFs were generally less than 1, suggesting that miscanthus reduces uptake and accumulation of studied metals. In roots, metal concentrations did not significantly differ throughout the study period, whereas rhizomes and stems reflected fluctuations depending on the metal. In leaves, metal concentrations were not significantly different in the three soils during the growing seasons. Conversely, during winter, leaf metal concentrations were higher in contaminated soils than in the uncontaminated soil, whereas the stem metal concentrations did not differ in these soils. The low metal concentrations in aerial parts, notably stems, which constitute the late harvestable biomass, make miscanthus a promising candidate energy crop for phytostabilization and valuable biomass production on metal-contaminated soils/sites.

Key words: Energy crop, Marginal land, Miscanthus, Phytostabilization, Soil contamination.

5.1. Introduction

Fossil fuels account for more than 80% of the global energy supply, but their fluctuating prices and current global warming have boosted the ambition of many countries to include plant biomass among the inexpensive renewable energy sources to simultaneously meet energy demand and reduce greenhouse gas emissions (Beringer et al., 2011; Bonanno et al., 2013). Besides, plant biomass is a polyvalent resource that can be used to produce energy and bioproducts for different industrial activities, thereby diversifying current agricultural

feedstock markets and promoting sustainable agriculture development (Christou et al., 2010). The sustainability of bioenergy production depends on both biomass supply and land availability (Beringer et al., 2011). However, the cultivation of energy crops on food-dedicated agricultural land may not only lead to changes in landscapes and/or loss of biodiversity (Beringer et al., 2011; Rahman et al., 2014), but it can also potentially increase the "food vs. fuel" controversy (Valentine et al., 2012). To mitigate these challenges, the use of marginal lands, i.e. lands that are not suitable for food crop production because of poor soil quality, nutrient depletion or metal/organic contamination, is proposed as an alternative option (Bonanno et al., 2013; Cai et al., 2011).

In Northern France, the former Pb smelter Metaleurop Nord (Noyelles Godault) operated for up to 100 years until its closure in 2003. The large area impacted by its activities, referred to as the Metaleurop site (http://www.safir-network.com/), presents land with a contamination gradient from the source. In this area, cropland (Sterckeman et al., 2002) and woodland (Douay et al., 2009) are contaminated by Cd, Pb and Zn at levels equivalent to 20- to 50-fold higher than regional agricultural topsoil concentrations (Sterckeman et al., 2002). The great concern is that some food crops, including wheat, barley, forage maize and potato, from the cultivated soils contain Cd and Pb concentrations that are higher than the legislation limit for food or fodder (Douay et al., 2013). Moreover, the agricultural practices on these soils can lead to emission of contaminated particles, which potentially increases environmental dispersion of the contaminants and their exposure to local inhabitants (Douay et al., 2005). Therefore, sustainable management of this area is crucial to mitigate environmental and health risks while maintaining a viable economy on contaminated agricultural lands (Bonanno et al., 2013).

On metal-contaminated soils or sites, the successful implementation of energy schemes requires the selection of suitable phytotechnologies to mitigate both environmental and health risks due to contaminant exposure (Henry et al., 2013). Among these technologies, phytostabilization, i.e. the use of plants and associated microorganisms to enhance metal immobilization in the roots and the rhizosphere appears to be cost-effective for large surface areas and allows producing a biomass with low metal contents. Phytostabilization requires a perennial plant activity to (1) reduce metal mobility in soils, (2) optimize maintenance costs and fertilizer inputs and 3) improve site/soil ecological characteristics (Dickinson et al., 2009; Mench et al., 2010). The plants used should preferably present characteristics such as tolerance to both abiotic and biotic stresses and high aboveground biomass (Mench et al., 2010).

Among perennial energy crops, the hybrid grass *Miscanthus* × *giganteus* (subsequently referred to as miscanthus) is currently greatly appreciated for its high productivity, adaptation to a wide range of climate conditions, tolerance to metal contamination and low metal accumulation in its shoots (Nsanganwimana et al., 2014). Miscanthus presents considerable potential for a high biomass production in regions with temperate climates such as the North of France (Cadoux et al., 2014) and can provide raw materials for house building, chemical and energy production, and paper production (Nsanganwimana et al., 2014). The establishment of miscanthus on contaminated soils of the Metaleurop site was proposed as a cost-effective solution to maintain viable agriculture and reduce environmental and health risks, thereby meeting both local population needs and environmental quality in a producing agro-ecosystem. To this end, it is crucial to assess miscanthus potential for metal stabilization in soils and metal concentrations in harvestable plant parts.

In situ growth of this species on cadmium- (Cd), lead- (Pb) and zinc (Zn)-contaminated acid (pH = 5.3-6.9) sandy clay soils showed that it retains metals in the belowground organs and that metal concentrations in leaves and stems increase as the soil contamination level rises (Pavel et al., 2014). Similarly, shoot metal concentrations can increase in case of highly metal-contaminated acid sandy or loamy soils, as it has been found for Pb and Zn (Kocoń & Matyka, 2012; Pogrzeba et al., 2013; Pogrzeba et al., 2011). This suggests that metal accumulation in miscanthus should be appraised in a soil- or site-specific fashion before establishing large-scale plantations on metal-contaminated soils. Also, the above studies showed that metal concentrations in harvested shoots vary greatly depending on the harvest period and plantation age. However, information is lacking on the variation in metal concentrations in individual miscanthus organs. Consequently, the plant's potential for phytostabilization throughout the growing cycle cannot be detailed, nor can the biomass quality be determined at autumn or winter harvest time.

The present study addresses the following questions: Is miscanthus a potential candidate species for metal (Cd, Pb and Zn) phytostabilization in contaminated agricultural soils such as those found on the Metaleurop site? Do metal concentrations in the aboveground miscanthus organs differ according to the soil contamination level? Do metal accumulation and distribution in miscanthus organs change with the season or growing phase?

5.2. Materials and methods

5.2.1. Characterization of experimental plots

Two metal-contaminated agricultural plots (M200 and M500) and one uncontaminated plot (MC) were used. The M200 (50°24′52″N, 3°01′51″E, 1.1 ha, Courcelles-les-Lens) and M500 (50°25′49″N, 3°02′13″E, 0.8 ha, Evin-Malmaison) are located within the Metaleurop site (**Fig. 2.1**). The site landscape presents a high degree of anthropization with residential suburbs, agricultural and wood lands, and transport networks. The plots are 1.8 km Southeast (for M200) and 1.4 km Northeast (for M500) of the former Pb smelter (50°25′42″N 3°00′55″E, Noyelles-Godault). The MC (50°20′46″N 2°12′15″E, 1.3 ha, Linzeux) is at about 75 km from the former Pb smelter. It is located in a rural area, within an agrosystem landscape.

The soils of these three plots differ according to their particle size distribution (**Table 1**) and their descriptive characteristics. The MC soil is a thick (> 1.20 m deep), well-drained loessic loam lying on a plateau. The M200 is located in a slightly northeastward-sloping topography. Only the lower part of this plot was considered for the present study (**Fig. 2.1**). In this part, the soil is composed of a medium to thick loessic loam layer (50-120 cm) lying over a clay substrate. The M500 has a calcareous clay loam soil that developed from alluvial deposits. During wet periods, the waterlogging conditions in M200 and M500 soils occur at a depth of 30-50 cm and 30 cm, respectively.

5.2.2. Establishment of miscanthus plantations

Miscanthus plantations were established in 2007 for MC and in 2008 for M200 and M500. The soils were ploughed in late winter and prepared for plantation in early spring. Miscanthus rhizomes were supplied by NovaBiom France. Rhizomes with at least one pair of buds were planted at10-20 cm deep, and at a density of 20,000 plants ha^{-1}. A potato planter was used for planting.

Weeds were suppressed by glyphosate spraying during the first 2 years of miscanthus establishment. Irrigation was never necessary because the region benefits from an oceanic temperate climate characterized by sufficient rain throughout the year. Annual precipitation and average temperatures are 738.7 mm and 10.8°C for both M200 and M500 plots, and 996.5 mm and 11°C for MC. Fertilization was not applied, and pesticides were not necessary as no disease symptoms were detected during the experimental period.

5.2.3. Plant and soil sampling

Samples were collected from each of the experimental plots at three periods. The first sampling (S1) was conducted in July 2010 during the maximum growing period. The second one (S2) in September 2010 corresponded to the final growth phase. The third sampling (S3) was done in February 2011 at the end of the senescence before harvest. Care was taken to avoid borderline effects in selecting sampling points in each plot. Five miscanthus clumps were randomly chosen within each plot and at each sampling period. First, plant parts were sampled. The shoots were cut with clippers and separated into stems and leaves. The belowground parts composed of roots and rhizomes were sampled and were not separated directly. Second, the soil (0-25 cm) corresponding to the rhizosphere was sampled to constitute couples of plant-soil sample. In total, there were 5 soil and 5 plant samples per plot for each sampling period. All samples were put into polyethylene bags for transport to the laboratory.

5.2.4. Sample preparation and chemical analysis

5.2.4.1. Soil

After removing plant debris, soils were transferred into trays, oven-dried at 40°C, then ground (ZM 200 Mill, Retsch, Germany) and sieved to constitute 2-mm and 250-µm fractions. The pH, cationic exchange capacity (CEC) and soluble phosphorus (P_2O_5) content were determined on soil subsamples sieved to 2 mm. Total carbonate, total carbon (total C), Fe and Mn oxide, total nitrogen (total N) contents, and metal (Cd, Pb and Zn) concentrations were measured on soil subsamples sieved to 250 µm.

Soil pH was measured after 1 h shaking in deionized water (1:5, v/v) at 20°C and a 2-h resting period (ISO 10390). The carbonate contents were determined by measuring the CO_2 released after HCl (4 M) treatment according to ISO 10693. The P_2O_5 was extracted with ammonium oxalate solution ($(NH_4)_2C_2O_4$, 0.1 M, pH = 7) and determined according to Joret & Hébert (1955) and the French standard NF X31-161. Iron and Mn oxides were extracted by a solution containing sodium citrate (0.27 M), sodium bicarbonates (0.11 M) and sodium dithionite (200 g L^{-1}) according to Mehra & Jackson (1960) and determined using a flame atomic absorption spectrometer (AA-6800, Shimadzu).

The CEC was obtained by percolation of ammonium acetate solution (CH_3COONH_4, 1 M, pH = 7) on soil samples followed by an extraction of ammonium with sodium chloride (1 M) according to the French standard NF X31-130. Ammonium was determined by a spectrometric measurement at 655 nm (UV-1800, Shimadzu).

The total C (ISO 10694) and total N (ISO 13878) contents were determined by dry combustion at the INRA Soil Analysis Laboratory (Arras, France), which holds the accreditation for soil analytical measurements in France.

For determination of Cd, Pb and Zn concentrations, acid digestion of soil samples was performed using a digestion plate (HotBlock™, Environmental Express, USA). A soil sample (300 mg) was measured in a digestion tube. Aqua regia solution (HCl + HNO_3, 3:1, 6 mL) was added and the aliquot heated at 95°C for 75 min. After cooling, the digest was adjusted to 25 mL with double-distilled water and filtered (0.45 µm cellulose acetate membrane). Concentrations of Cd, Pb and Zn were measured using atomic absorption spectrophotometry (AA-6800). The accuracy and precision of the chemical analysis were verified using a certified reference material (CRM-141R, Belgium) at all steps of extraction and measurements. The residual moisture was determined according to ISO 11465 and was used to apply the moisture correction factor so as to express all results on a dry weight (DW) basis.

5.2.4.2. Plant

The leaf and stem samples were washed in osmotic water. The belowground organs were washed thoroughly with tap water to remove the soil particles, partitioned into roots and rhizomes, and then rinsed in deionized water baths. All samples were cut into small pieces, oven-dried at 40°C, and then ground into fine powder using a knife mill (GM200, Retsch) for leaves and roots, and an ultracentrifuge mill (ZM200, Retsch) for stems and rhizomes.

Digestion was done by adding 5 mL of nitric acid (HNO_3, 70%) in a tube containing 300 mg of plant powder. The tube was covered with a watch glass and heated at 80°C on a digestion block for 1 h. After cooling, 5 mL of hydrogen peroxide (H_2O_2, 30%) were added, and the mixture was again heated at 80°C for 3 h. After cooling, the volume was adjusted to 25 mL with double-distilled water and filtered (0.45 µm). Metals (Cd, Pb and Zn) in digests were measured using atomic absorption spectrometry (AA-6800). The accuracy and precision of the analytical determinations were verified using a certified reference material (Polish Virginia Tobacco Leaves, INCT-PVTL-6, Poland). As for soils, the moisture content was determined to express the results on DW basis.

5.2.5. Data analysis

Physico-chemical soil parameters and metal concentrations in miscanthus organs are presented as the means and standard deviations of five replicates for each sampling period. Also, the BCFs-bioconcentration factors (ratio of metal concentrations in plant organs to metal concentrations in soils) and the TFs-translocation factors (ratio of metal concentrations aboveground organs and rhizomes to metal concentrations in roots) were computed so as to

compare metal accumulation and distribution in miscanthus organs depending on soil contamination levels and sampling periods. Analysis of variance was performed. The normal distribution of data (Shapiro-Wilk test) and equality of variances (Bartlett test) were checked. The Fisher statistics was considered for significance ($p \leq 0.05$), and Tukey HSD test was used for pair-wise comparisons of statistical groups. All statistical tests were performed in XLSTAT software (Addinsoft^{TM} software 2012).

5.3. Results

5.3.1. Soil physico-chemical parameters and metal contamination levels

The physico-chemical parameters and metal concentrations in the studied soils are presented in **Table 5.1**. Generally, there were no significant within-plot differences across the sampling periods (**Annex 2**). Consequently, all the data were grouped together so as to focus on between-plot differences.

Table 5.1. Physico-chemical parameters and metal concentrations in soils of the studied plots (0-25 cm). Values represent means ± standard deviations. The different letters represent significant differences between plots (Tukey HSD test, $p \leq 0.05$).

Parameters	**Plots**		
	MC	**M200**	**M500**
Physico-chemical parameters			
Clay (%)	20.0	18.8	31.1
Fine silt (%)	26.5	18.7	18.3
Coarse silt (%)	42.5	35.9	33.9
Fine sand (%)	10.5	24.2	14.7
Coarse sand (%)	0.6	2.6	2.1
pH	6.6 ± 0.4^{c}	7.3 ± 0.2^{b}	7.6 ± 0.1^{a}
Carbonates (g kg^{-1})	0.5 ± 0.2^{c}	1.5 ± 1.0^{b}	20.6 ± 5.0^{a}
P_2O_5 (g kg^{-1})	0.1 ± 0.0^{c}	0.3 ± 0.2^{a}	0.3 ± 0.2^{a}
Fe (g kg^{-1})	8.1 ± 1.1^{a}	4.1 ± 0.7^{b}	8.9 ± 1.2^{a}
Mn (g kg^{-1})	0.5 ± 0.1^{a}	0.3 ± 0.0^{b}	0.2 ± 0.0^{c}
Total C (g kg^{-1})	18.5 ± 1.9^{b}	19.0 ± 1.5^{b}	34.9 ± 4.1^{a}
Total N (g kg^{-1})	1.9 ± 0.2^{b}	1.4 ± 0.1^{c}	2.8 ± 0.3^{a}
C/N	10.0 ± 0.3^{c}	13.3 ± 0.7^{a}	12.6 ± 0.4^{b}
CEC ($cmol^{+}$ kg^{-1})	11.7 ± 0.8^{b}	12.4 ± 0.8^{b}	25.2 ± 4.2^{a}
Metal concentrations			
Cd (mg kg^{-1})	0.6 ± 0.1^{c}	5.4 ± 0.5^{b}	11.9 ± 0.8^{a}
Pb (mg kg^{-1})	23.7 ± 9.8^{c}	227.0 ± 16.4^{b}	523.2 ± 35.8^{a}
Zn (mg kg^{-1})	47.5 ± 4.4^{c}	358.9 ± 19.0^{b}	572.6 ± 46.2^{b}

The MC soil is slightly acidic (pH = 6.6) and there was a difference of 0.6-1 pH units between this soil and the M200 and M500 soils. The CEC, carbonate and total C contents were higher in M500 soil than in MC and M200 soils. The P_2O_5 contents were higher in M200 and M500 than in MC soil. Fe contents are about twice as high in MC and M500 than in M200 soil. The Mn content in M500 soil was the lowest, whereas it was the highest in MC soil. Total N content in M200 soil (1.4 g kg^{-1}) is about 1.4- and 2-fold lower than in MC and M500 soils, respectively. However, the C/N ratio in MC soil was about 1.3-fold lower than in M200 and M500 soils.

Whatever the metal and the sampling period, the studied soils present a contamination gradient as follows: M500 > M200 >> MC. In MC soil, metal concentrations were 9 and 21 (Cd), 9.6 and 22 (Pb) and 7.6 and 12 (Zn)-fold lower than in M200 and M500 soils, respectively. Also, in MC soil, metal concentrations are slightly equal or lower than frequent regional metal concentrations in cultivated loamy soils (Sterckeman et al., 2002).

5.3.2. Metal accumulation and distribution in miscanthus

5.3.2.1. Metal concentrations in miscanthus organs

The mean concentrations of Cd, Pb and Zn in aboveground (leaves, stems) and belowground (rhizomes, roots) miscanthus organs are presented in **Fig. 5.2, 5.3 and 5.4.** Overall, concentrations significantly differed according to the organ and the plot **(Table 5.2).**

Table 5.2. Level of significance (p-values) of the effect of the organ, sampling period and plot on metal (Cd, Pb and Zn) concentrations, the bioconcentration factors (BCFs) and translocation factors (TFs). Values were obtained from ANOVA (n = 5, $p \leq 0.05$).

Source of variation	Metal concentrations				BCFs				TFs			
	df	Cd	Pb	Zn	df	Cd	Pb	Zn	df	Cd	Pb	Zn
Organ	2	< 0.0001	< 0.0001	< 0.0001	2	0.0001	< 0.0001	< 0.0001	2	< 0.0001	**0.186**	< 0.0001
Plot	3	< 0.0001	< 0.0001	< 0.0001	3	< 0.0001	< 0.0001	< 0.0001	2	< 0.0001	**0.250**	< 0.0001
Period	2	**0.758**	**0.868**	< 0.0001	2	0.043	**0.581**	< 0.0001	2	**0.269**	0.001	< 0.0001
Organ*Plot	6	< 0.0001	< 0.0001	< 0.0001	6	< 0.0001	< 0.0001	< 0.0001	4	< 0.0001	0.012	0.021
Organ*Period	4	**0.791**	**0.436**	0.003	4	0.003	**0.582**	0.034	4	**0.671**	**0.177**	< 0.0001
Plot*Period	6	**0.580**	0.011	< 0.0001	6	**0.566**	< 0.0001	0.001	4	**0.166**	**0.173**	0.003
Organ*Plot*Period	12	**0.931**	**0.182**	< 0.0001	12	**0.562**	< 0.0001	**0.065**	8	**0.508**	0.029	0.001

a) Cadmium

The Cd distribution in miscanthus organs can be classified as follows: roots > rhizomes = stems > leaves in MC, and roots > rhizomes > stems > leaves in both M200 and M500 (**Fig. 5.2**).

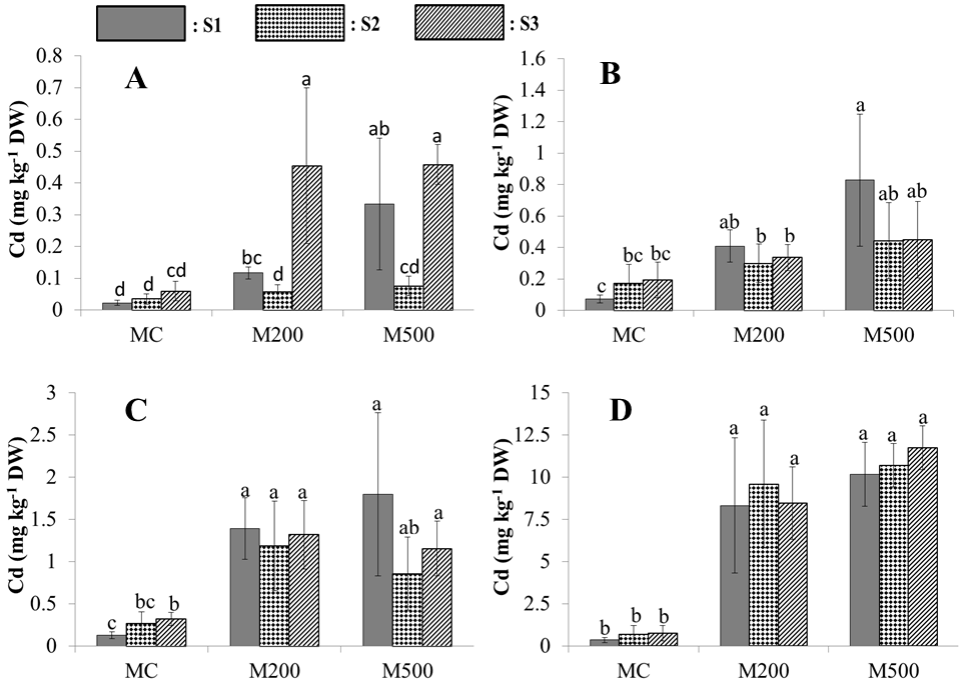

Fig. 5.2. Cadmium concentrations (mg kg^{-1} DW) in miscanthus organs (A: leaves, B: Stems, C: rhizomes, D: roots). The histograms represent means and associated standard deviations. The different letters refer to significant differences (Tukey HSD test, $p \leq 0.05$, n = 5) between sampling periods and depending on plots.

The leaf concentrations did not significantly differ in MC over all the sampling periods. However, concentrations at S1 (0.02 mg kg^{-1}) and S2 (0.03 mg kg^{-1}) in this plot were 6- and 16.5-fold lower than in M200 and M500, respectively. At S3, concentrations in leaves in MC were 7.5-fold lower than in both M200 and M500. In these two plots, concentrations decreased from S1 to S2 and then increased (5- to 6-fold) from S2 to S3.

The stem concentrations in MC (0.07 mg kg^{-1}) at S1 were 6- and 12-fold lower than in M200 and M500, respectively. At S2 and S3, the concentrations (0.17-0.45 mg kg^{-1}) did not significantly differ between MC and the contaminated plots.

Considering the three sampling periods, the rhizome concentrations in MC (0.13-0.32 mg kg^{-1}) were 4.1- to 10.7- and 3.2- to 13.8-fold lower than in M200 and M500, respectively. In MC, these concentrations did not differ at S1 and S2, but they were significantly higher at S3 than at S1. In M200 and M500, the rhizome concentrations did not significantly differ across the seasons.

The root concentrations in MC (0.35-0.75 mg kg^{-1}) were 11.3- to 25.2- and 15.1- to 29.2-fold lower than in M200 and M500, respectively. There was no within-plot difference in root concentrations and no significant difference between M200 and M500.

b) Lead

Whatever the sampling period and the plot, the Pb concentrations in miscanthus organs can be classified as follows: roots > leaves > stems = rhizomes (**Fig. 5.3**).

Considering the three sampling periods, the leaf concentrations in MC (0.09-0.44 mg kg^{-1}) were 3.2- to 4.4- and 5.6- to 8.8-fold lower than in M200 and M500, respectively. In all three plots, the concentrations increased from S1 or S2 to S3.

The stem concentrations in MC varied from 0.29 mg kg^{-1} (S1) to 0.76 mg kg^{-1} (S3) and were significantly higher at S3 than at S1. Similarly, the concentrations in M200 (0.24-0.75 mg kg^{-1}) and M500 (0.27-0.51 mg kg^{-1}) did not significantly differ between the two plots. However, they were 3.1-fold lower at S1 than at S3 in M200 soil, whereas there was no significant seasonal difference in M500.

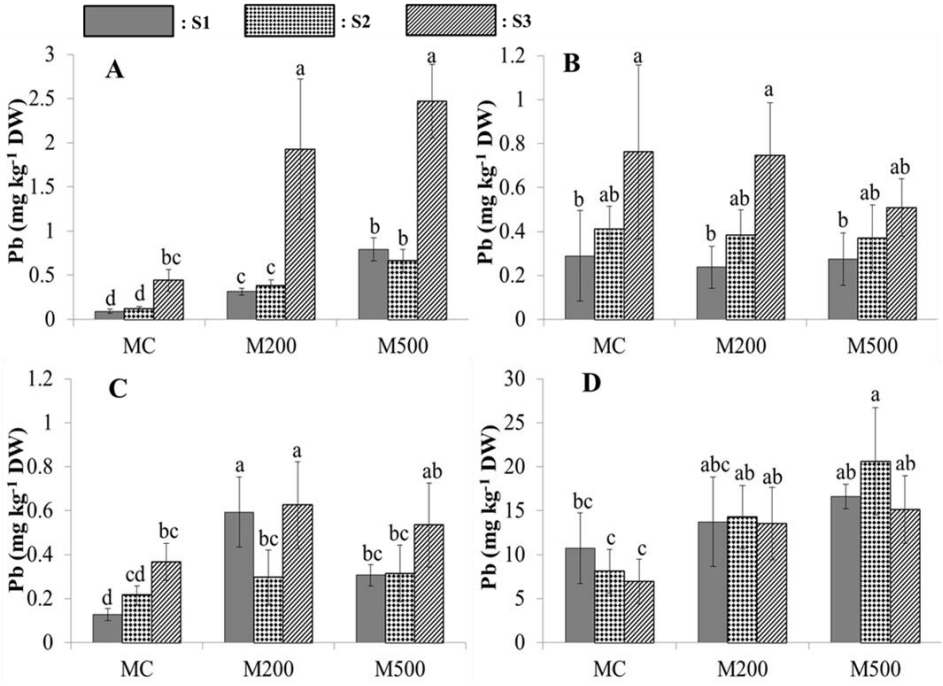

Fig. 5.3. Lead concentrations (mg kg^{-1} DW) in miscanthus organs (A: leaves, B: Stems, C: rhizomes, D: roots). The histograms represent means and associated standard deviations. The different letters refer to significant differences (Tukey HSD test, $p \leq 0.05$, n = 5) between sampling periods and depending plots.

The rhizome concentrations in MC at S1 (0.13 mg kg^{-1}) and S2 (0.22 mg kg^{-1}) did not differ significantly. At S3, the concentrations (0.37 mg kg^{-1}) were significantly higher than at S1. At each sampling season, there was no significant difference between MC and contaminated plots. In M200, the concentrations at S1 (0.59 mg kg^{-1}) and S3 (0.63 mg kg^{-1}) were higher than at S2 (0.30 mg kg^{-1}). In M500, the rhizome concentrations varied from 0.31 to 0.53 mg

kg^{-1} and did not differ at the different sampling periods. The rhizome concentrations were significantly higher in M200 than in MC at both S1 and S3, whereas they were only higher than in M500 at S1.

Depending on the plot, the root concentrations did not significantly differ across the seasons. They varied from 6.96 to 10.92 mg kg^{-1}, 13.54 to 14.27 mg kg^{-1}, and 15.14 to 20.61 mg kg^{-1} in MC, M200 and M500, respectively, and they were significantly lower in MC than in the two contaminated plots at S2 and S3.

c) Zinc

At all sampling periods, root concentrations in MC were generally lower than in both M200 and M500 (**Fig. 5.4**). Moreover, in M200 and M500, Zn concentrations in miscanthus organs can be classified as follows: roots > leaves ≥ stems > rhizomes.

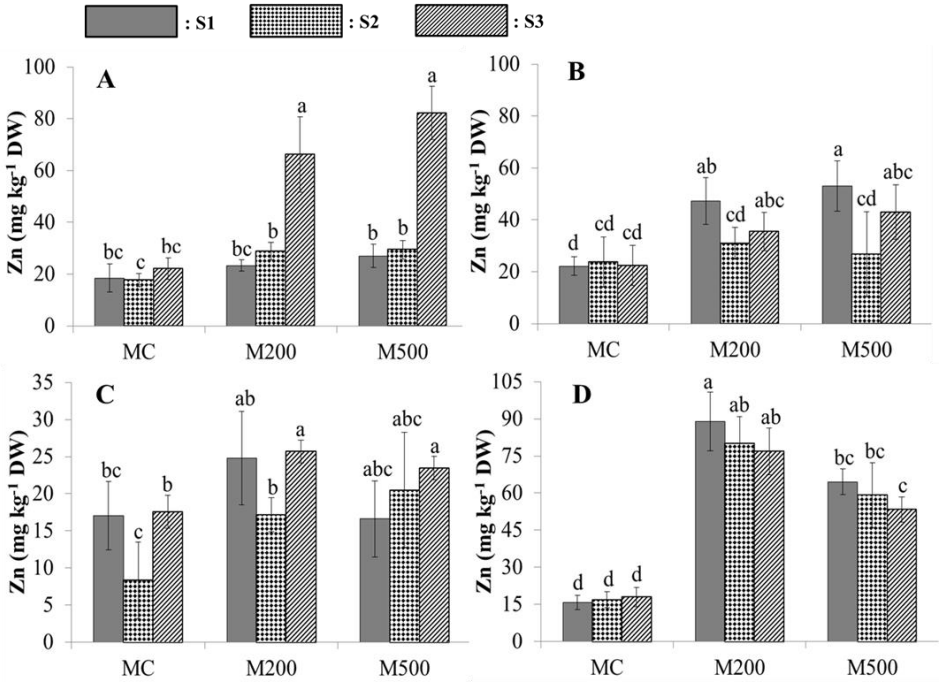

Fig. 5.4. Zinc concentrations (mg kg^{-1} DW) in miscanthus organs (A: leaves, B: Stems, C: rhizomes, D: roots). The histograms represent means and associated standard deviations. The different letters refer to significant differences (Tukey HSD test, $p \leq 0.05$, $n = 5$) between sampling periods and depending on plots.

Leaf concentrations in MC (17.68-22.07 mg kg^{-1}) did not significantly differ according to season. At S3, these concentrations were 3- and 3.7-fold lower than in M200 and M500, respectively. In these plots, leaf concentrations at S1 and S2 (23.32-27.02 mg kg^{-1}) did not significantly differ and were lower than at S3 (66.19-82.18 mg kg^{-1}).

Stem concentrations in MC (22.17-23.88 mg kg^{-1}) were 1.3 to 2.1 and from 1.1 to 2.4-fold lower than in M200 and M500 soils, respectively. However, the concentrations at S1 (47.23 mg kg^{-1}) in M200, and at both S1 (53.01 mg kg^{-1}) and S3 (42.97 mg kg^{-1}) in M500 were significantly higher than in MC. Depending on the sampling period, the stem concentrations in both M200 and M500 did not significantly differ, and they decreased from S1 and S2, and then increased from S2 to S3.

The rhizome concentrations in MC at S1 (17.05 mg kg^{-1}) and S3 (17.56 mg kg^{-1}) did not significantly differ. At S2, the concentrations (8.32 mg kg^{-1}) were significantly lower than at S3. In M200 and M500, the concentrations did not significantly differ at each sampling season. However, in M200, concentrations at S2 (17.5 mg kg^{-1}) were significantly lower than at S3 (25.70 mg kg^{-1}). Also, the concentrations in both M200 and M500 were higher than in MC at S3.

The root concentrations did not differ across the sampling periods. In MC, they were from 4.3- to 5.6-fold and from 3- to 4.1-fold lower than in M200 and M500, respectively. The concentrations in M200 at S1 (88.91 mg kg^{-1}) and at S3 (76.68 mg kg^{-1}) were significantly higher than in M500.

5.3.2.2. Metal transfer from soil to miscanthus organs

The metal transfer ratio from the soil to miscanthus organs was assessed using the BCFs (**Table 5.3).** Cadmium and zinc BCFs depended on the organ, the plot and the sampling period, whereas there was no significant effect of the sampling period on lead BCFs **(Table 5.2).**

a) Cadmium

Overall, for all plots and sampling periods, the BCFs were higher in roots and lower in leaves and stems. In MC, the leaf BCFs varied from 0.03 (S1) to 0.12 (S3) and did not significantly differ according to the season. The same patterns were observed for stems with BCFs ranging from 0.11 to 0.39. For rhizomes and roots, the BCFs were lower at S1 (0.19 and 0.54 for rhizomes and roots, respectively, and significantly higher at S3 (0.64 and 1.53 for rhizomes and roots, respectively).

In M200 and M500, the leaf BCFs varied from 0.02 to 0.08, and they were significantly higher at S3 than at S1 and S2. In these plots, the stem BCFs (0.04-0.08) did not significantly differ across the seasons. Moreover, there was no significant difference between leaf and stem BCFs at S3. The rhizome BCFs (0.21-0.28 and 0.07-0.15 for M200 and M500, respectively) did not differ across the sampling seasons in each of these plots. However, they were

significantly higher in M200 than in M500 at S2. Root BCFs (1.46-1.69) in M200 did not significantly differ across the sampling periods and were significantly higher than in M500 during all seasons. The root BCFs did not significantly differ between MC and the contaminated plots except at S1 where BCFs were lower in MC than M200.

Table 5.3. Comparison of BCFs of miscanthus organs. Values represent means ± standard deviations. Different letters refer to significant differences depending on metals and plots (Tukey HSD test, $p \leq 0.05$).

Metals	Soils	Sampling period	BCFs			
			Leaves	Stems	Rhizomes	Roots
Cd	MC	S1	0.03 ± 0.01^{fg}	0.11 ± 0.04^{de}	0.19 ± 0.06^{d}	0.54 ± 0.29^{c}
	M200		0.02 ± 0.00^{g}	0.08 ± 0.02^{ef}	0.28 ± 0.07^{d}	1.69 ± 0.79^{a}
	M500		0.03 ± 0.02^{fg}	0.07 ± 0.03^{ef}	0.15 ± 0.07^{de}	0.85 ± 0.15^{bc}
	MC	S2	0.07 ± 0.07^{efg}	0.35 ± 0.25^{cd}	0.55 ± 0.29^{cd}	1.46 ± 1.11^{ab}
	M200		0.01 ± 0.00^{g}	0.05 ± 0.02^{ef}	0.21 ± 0.09^{d}	1.64 ± 0.59^{a}
	M500		0.01 ± 0.00^{g}	0.04 ± 0.02^{f}	0.07 ± 0.03^{ef}	0.88 ± 0.13^{bc}
	MC	S3	0.12 ± 0.07^{def}	0.39 ± 0.24^{cd}	0.64 ± 0.18^{c}	1.53 ± 1.00^{ab}
	M200		0.08 ± 0.04^{ef}	0.06 ± 0.01^{ef}	0.24 ± 0.09^{d}	1.53 ± 0.37^{ab}
	M500		0.04 ± 0.00^{f}	0.04 ± 0.02^{f}	0.10 ± 0.03^{de}	1.03 ± 0.08^{b}
Pb	MC	S1	0.005 ± 0.003^{ef}	0.004 ± 0.001^{f}	0.007 ± 0.003^{ef}	0.519 ± 0.136^{a}
	M200		0.001 ± 0.000^{g}	0.001 ± 0.000^{g}	0.003 ± 0.001^{g}	0.062 ± 0.020^{c}
	M500		0.002 ± 0.000^{g}	0.001 ± 0.000^{g}	0.001 ± 0.000^{g}	0.033 ± 0.003^{d}
	MC	S2	0.007 ± 0.003^{ef}	0.021 ± 0.007^{e}	0.012 ± 0.005^{e}	0.430 ± 0.152^{ab}
	M200		0.002 ± 0.000^{g}	0.002 ± 0.001^{g}	0.001 ± 0.000^{g}	0.060 ± 0.017^{c}
	M500		0.001 ± 0.000^{g}	0.001 ± 0.000^{g}	0.001 ± 0.000^{g}	0.038 ± 0.011^{cd}
	MC	S3	0.019 ± 0.014^{ef}	0.059 ± 0.056^{cde}	0.016 ± 0.011^{ef}	0.256 ± 0.087^{b}
	M200		0.009 ± 0.003^{ef}	0.003 ± 0.001^{g}	0.003 ± 0.001^{g}	0.062 ± 0.019^{c}
	M500		0.005 ± 0.001^{ef}	0.001 ± 0.000^{g}	0.001 ± 0.000^{g}	0.030 ± 0.009^{d}
Zn	MC	S1	0.39 ± 0.14^{abc}	0.47 ± 0.10^{ab}	0.35 ± 0.07^{abc}	0.33 ± 0.05^{bc}
	M200		0.07 ± 0.01^{i}	0.13 ± 0.02^{gh}	0.07 ± 0.02^{i}	0.25 ± 0.03^{cde}
	M500		0.05 ± 0.01^{i}	0.09 ± 0.02^{hi}	0.09 ± 0.02^{hi}	0.11 ± 0.01^{gh}
	MC	S2	0.35 ± 0.04^{abc}	0.48 ± 0.18^{ab}	0.16 ± 0.10^{fghi}	0.34 ± 0.08^{bcd}
	M200		0.08 ± 0.01^{hi}	0.08 ± 0.02^{hi}	0.05 ± 0.01^{i}	0.22 ± 0.03^{def}
	M500		0.05 ± 0.01^{i}	0.05 ± 0.03^{i}	0.05 ± 0.03^{i}	0.10 ± 0.02^{hi}
	MC	S3	0.50 ± 0.10^{a}	0.49 ± 0.14^{a}	0.40 ± 0.07^{ab}	0.40 ± 0.07^{ab}
	M200		0.19 ± 0.04^{efg}	0.10 ± 0.02^{hi}	0.07 ± 0.00^{i}	0.22 ± 0.03^{def}
	M500		0.15 ± 0.01^{g}	0.08 ± 0.02^{hi}	0.05 ± 0.03^{i}	0.10 ± 0.01^{h}

b) Lead

For all plots and sampling periods, the organs' BCFs can be classified as follows: roots >> rhizomes = stems = leaves at both S1 and S2, and roots >> leaves = rhizomes = stems at S3. Apart from a few exceptions, the BCFs for all organs (0.005-0.019 for leaves, 0.015-0.137 for

stems, 0.007-0.016 for rhizomes and 0.256-0.519 for roots) in MC were higher than in M200 and M500. Depending on organs, the BCFs do not significantly differ across the seasons in the contaminated plots apart from the root BCFs at S1 (0.033) and S3 (0.062) in M200, which were significantly higher than in M500. Moreover, the leaf BCFs in these two plots were significantly higher at S3 than at S1 and S2.

c) Zinc

In the contaminated plots, the organs' BCFs can be classified as follows: roots > rhizomes = stems = leaves at S1 and S2, and roots > leaves > stems = rhizomes at S3. In MC, the BCFs for leaves (0.35-0.50) and stems (0.47-0.49) did not differ according to sampling period and they were significantly higher than in M200 (0.07-0.19 and 0.08-0.13 for leaves and stems, respectively) and in M500 soil (0.05-0.15 and 0.05-0.09 for leaves and stems, respectively). In M200 and M500, leaf BCFs at S3 were significantly higher than at S1 and S2. The rhizomes' BCFs in MC at S1 (0.35) and at S3 (0.40) were significantly higher than at S2 (0.16). Whatever the sampling period, there was no significant difference between rhizome BCFs in M200 (0.05-0.07) and M500 (0.05-0.09). Moreover, these BCFs were significantly lower than those in MC at S1 and S3.

Root BCFs in MC varied from 0.33 (S1) to 0.40 (S3) and they were not significantly different across the seasons. In M200, the root BCFs (0.22-0.25) did not significantly differ across the seasons and were significantly higher than those in M500 (0.10-0.11). The root BCFs in MC were significantly higher than in M500 for all seasons, whereas they were only significantly higher than in M200 at S3.

5.3.2.3. Metal transfer from miscanthus roots to rhizomes, stems and leaves

The metal transfer ratio from the roots to other miscanthus organs was assessed using the TFs (**Table 5.4)**. There was no significant effect of the organ and the plot on lead TFs (**Table 5.2**).

a) Cadmium

The cadmium TFs in MC (0.07-0.08 for leaves, 0.23-0.27 for stems and 0.39-0.62 for rhizomes) did not significantly differ depending on organ and sampling period. In this plot, the leaf TFs were significantly lower than stem and rhizome TFs. Moreover, the TFs in MC were generally higher than in M200 and M500. In these plots, the TFs did not differ at each sampling period and depending on the organs, but they were higher for rhizomes (0.13-0.19 and 0.08-0.18 in M200 and M500, respectively) than for leaves and stems.

b) Lead

In general, at S1 and S2, the TFs for all organs (0.01-0.06) did not significantly differ for any plot or sampling period. However, at S3, the leaf TFs in M200 (0.15) and M500 (0.18) were significantly higher than at S1 and S2. There was no significant difference between S1, S2 and S3 for stem and rhizome TFs for all plots.

Table 5.4. Comparison of TFs of miscanthus organs. Values represent means ± standard deviations. Different letters refer to significant differences depending on metals and plots (Tukey HSD test, $p \leq 0.05$).

Metals	Soils	Sampling period	TFs		
			Leaves	Stems	Rhizomes
Cd	MC	S1	0.07 ± 0.02^{de}	0.23 ± 0.08^{bc}	0.39 ± 0.12^{abc}
	M200		0.02 ± 0.01^{f}	0.05 ± 0.01^{de}	0.19 ± 0.07^{c}
	M500		0.03 ± 0.02^{ef}	0.08 ± 0.03^{de}	0.18 ± 0.09^{c}
	MC	S2	0.06 ± 0.03^{de}	0.27 ± 0.09^{bc}	0.44 ± 0.20^{ab}
	M200		0.01 ± 0.00^{f}	0.04 ± 0.03^{def}	0.13 ± 0.04^{cd}
	M500		0.01 ± 0.00^{f}	0.04 ± 0.03^{def}	0.08 ± 0.04^{d}
	MC	S3	0.08 ± 0.02^{de}	0.26 ± 0.07^{bc}	0.62 ± 0.44^{a}
	M200		0.05 ± 0.02^{de}	0.04 ± 0.01^{e}	0.17 ± 0.06^{c}
	M500		0.04 ± 0.01^{e}	0.04 ± 0.02^{ef}	0.10 ± 0.03^{cd}
Pb	MC	S1	0.01 ± 0.01^{c}	0.03 ± 0.03^{c}	0.01 ± 0.01^{c}
	M200		0.03 ± 0.01^{c}	0.02 ± 0.01^{c}	0.06 ± 0.03^{c}
	M500		0.05 ± 0.01^{c}	0.02 ± 0.01^{c}	0.02 ± 0.00^{c}
	MC	S2	0.02 ± 0.00^{c}	0.05 ± 0.01^{c}	0.03 ± 0.01^{c}
	M200		0.03 ± 0.01^{c}	0.03 ± 0.03^{c}	0.02 ± 0.01^{c}
	M500		0.03 ± 0.01^{c}	0.02 ± 0.01^{c}	0.02 ± 0.01^{c}
	MC	S3	0.07 ± 0.03^{bc}	0.22 ± 0.23^{abc}	0.06 ± 0.02^{c}
	M200		0.15 ± 0.05^{ab}	0.06 ± 0.04^{c}	0.05 ± 0.01^{c}
	M500		0.18 ± 0.07^{a}	0.04 ± 0.01^{c}	0.04 ± 0.02^{c}
Zn	MC	S1	1.22 ± 0.50^{abc}	1.43 ± 0.31^{ab}	1.07 ± 0.13^{abc}
	M200		0.27 ± 0.06^{hi}	0.53 ± 0.07^{defg}	0.28 ± 0.06^{hi}
	M500		0.42 ± 0.09^{gh}	0.83 ± 0.19^{bcde}	0.26 ± 0.09^{hi}
	MC	S2	1.08 ± 0.20^{abc}	1.44 ± 0.52^{ab}	0.50 ± 0.32^{efghi}
	M200		0.37 ± 0.06^{gh}	0.39 ± 0.07^{gh}	0.22 ± 0.02^{i}
	M500		0.52 ± 0.14^{defg}	0.44 ± 0.25^{ghi}	0.37 ± 0.18^{ghi}
	MC	S3	1.27 ± 0.36^{abc}	1.26 ± 0.36^{abc}	1.03 ± 0.35^{abcde}
	M200		0.88 ± 0.28^{bcde}	0.46 ± 0.09^{fghi}	0.34 ± 0.05^{gh}
	M500		1.56 ± 0.28^{a}	0.81 ± 0.19^{bcde}	0.44 ± 0.07^{gh}

c) Zinc

For all organs and sampling periods, the zinc TFs in MC (1.08-1.27 for leaves, 1.26-1.44 for stems and 0.50-1.07 for rhizomes) were higher than in M200 and M500. Moreover, in MC,

the zinc TFs did not significantly differ across the sampling period, except for rhizome TFs at S2 (0.50), which were significantly lower than at S1 (1.07). In M200, rhizome TFs at S2 (0.22) were significantly lower than at S3 (0.34), whereas there was no significant seasonal difference for stems. In M500, the rhizome TFs varied from 0.26 to 0.44 and did not significantly differ from those in M200 for all seasons. Conversely, the leaf TFs in these two plots increased across the seasons, and they were significantly higher at S3 than at S1 and S2.

5.4. Discussion

The present study aimed to assess the potential of miscanthus for phytostabilization of metal contamination in agricultural soils. The soil contamination gradient allowed assessing the effects of the metal contamination level on metal accumulation and distribution in miscanthus belowground and aboveground organs. The sampling scheduled at three periods allowed us to consider seasonal variations among the factors that influence the metal transfer rate from soil to miscanthus organs. The suitability of miscanthus biomass to thermochemical conversion technologies was assessed with reference to metal concentrations in the aboveground organs.

The results show that in contaminated soils, the Cd, Pb and Zn concentrations in miscanthus roots are higher than in rhizomes, stems and leaves. Apart from root BCFs for Cd, the BCFs and TFs for all organs are less than 1, suggesting that miscanthus limits root uptake of metals and their transfer to aboveground organs. Indeed, in contaminated conditions, a plant is considered as a non-accumulator or metal excluder when the BCFs and TFs are lower than 1 (Mench et al., 2010). Therefore, these results show that miscanthus is an excluder plant. In plants, metal-exclusion depends on the functional role of roots, which consists of precipitation and sequestration of metals in the rhizosphere, metal immobilization in root cell apoplast by complexation with pectin carboxylic groups in the cell wall or metal chelation by phytochelatins, and storage in root cell vacuoles (Hossain et al., 2012). These results also show that metal concentrations in the stems do not differ between uncontaminated and contaminated plots during winter (S3), a period which is indicated as the most suitable for miscanthus harvest in temperate climates (Lewandowski & Kicherer, 1997). Given a high metal accumulation in roots and reduced transfer to aboveground organs, miscanthus could allow metal stabilization in contaminated soils. Therefore, as suggested by Nsanganwimana et al. (2014) and Pidlisnyuk et al. (2014), miscanthus is a suitable candidate crop for phytomanagement of studied metal-contaminated soils with low metal concentrations in harvestable biomass.

Despite different physico-chemical parameters and contamination levels, metal concentrations, BCFs and TFs in miscanthus organs do not generally differ between M200 and M500. This is not consistent with the results obtained by Pavel et al. (2014), which showed that Cd, Pb and Zn accumulation in miscanthus stems and leaves increased as the soil contamination level increased. However, that study was conducted on contaminated acid (pH = 5.3-6.9) sandy clay soils. With regard to the present study, root Cd, Pb and Zn concentrations in contaminated plots were higher than in MC, suggesting that metal entry in roots does somehow depend on their concentrations in soils. The lack of direct proportionality between the soil contamination level and metal accumulation in miscanthus organs shows that the metal transfer from soil to miscanthus is restricted with increasing soil contamination. This could mainly result from miscanthus metal-exclusion as suggested above. Moreover, reduced metal uptake could be explained by lower metal phytoavailability, in relation with CEC, carbonate, Fe oxide and carbon contents, which are higher in M500 than in M200 soil. Indeed, soils rich in carbonates, organic matter and Fe/Mn oxy(hydro)oxides have a higher buffering capacity capable of delaying metal dissolution and maintaining them in the soil solid phase (Carrillo-Gonzalez et al., 2006; Buekers et al., 2007).

Metal concentrations in roots did not significantly differ across the sampling periods. Conversely, their accumulation patterns in rhizomes and stems showed many fluctuations and depended on the element studied. Cadmium, Pb and Zn concentrations and transfer rates (BCFs and TFs) were generally lower in the final growth phase (S2) than in winter (S3). Interestingly, the leaf concentrations and transfer rates of these metals were lower during the growth period and significantly increased during the senescence period. These results are consistent with the literature on seasonal variations of shoot or leaf metal concentrations in perennial grasses such as *Phragmites australis* (Baldantoni et al., 2009; Bragato et al., 2009; Kastratović et al., 2013), *Arrhenatherum elatius* (Deram et al., 2008), *Lolium perenne* (Bidar et al., 2009) and *Bromus carinatus* (Silk et al., 2006). Moreover, higher metal concentrations in senescent/old leaves than in young/growing leaves appear to be a common trend in perennial plants. This is also true for other non-grass perennial shrubs including *Camellia sinensis* (Han et al., 2007), *Vaccinium myrtillus* (Kandziora-Ciupa et al., 2013), *Fagopyrum esculentum* (Tani and Barrington, 2005), and trees such as *Populus* sp. (Laureysens *et al.*, 2004). The low concentrations in aboveground miscanthus organs during the growing seasons could be explained by the dilution effect due to the dry matter increment. This corroborates with results obtained on *Ruppia maritima* (Malea et al., 2008) and *Bromus carinatus* (Silk et al., 2006), which proved that during growth, Fe and Cd in *Ruppia maritima* and Cu in *Bromus carinatus* concentrations were low and negatively correlated with plant biomass. With regard

to our study, and as suggested by Bragato et al. (2009) in *P. australis*, it can also be hypothesized that the root-to-leaf translocation could be activated at the end of the growing season so as to allocate toxic elements in the inactive senescent tissues and to protect both dormant belowground organs and young tissues at the start of the growing season from metal toxicity. Indeed, in the present study, the Cd, Pb and Zn concentrations in leaves were not significantly different in the three soils during the growing seasons (S1 and S2). Conversely, during winter (S3), leaf Cd, Pb and Zn concentrations were significantly higher in contaminated soils than in the uncontaminated soil, whereas the stem metal concentrations did not differ between these soils.

Notwithstanding the plant tolerance strategy that consists of sequestrating potentially toxic metal in inactive organs, the high metal concentrations in senescent or old leaves could be due to long-term metal uptake, exposure and accumulation (Weis et al., 2003; Windham et al., 2001). Although we did not consider leaf age during sampling, we can suggest that high metal concentrations in leaves at S3 are due to long duration to metal exposure. This corroborates with the results obtained in miscanthus during a short-term hydroponic culture in Cd-spiked growth media (Arduini et al., 2004). These authors found that at the 29th day, Cd concentrations in miscanthus stems and leaves were lower than at the 93rd day. Moreover, there could be a close relationship between metal accumulation and the plant's water status. Here, we refer to senescence, which is associated with the drying phenomenon, low water uptake and increased transpiration rate (Sarath et al., 2014). In *Populus* sp. (Laureysens et al., 2004) and *Fagopyrum esculentum* (Tani and Barrington, 2005), high leaf metal uptake and concentrations were related to a high transpiration rate, suggesting that metal transfer from roots to shoots or leaves in miscanthus could occur during senescence.

The increased metal concentrations in harvestable plant organs, especially in leaves, seem questionable with regard to biomass quality. Here, the question is "how suitable is miscanthus biomass produced from metal-contaminated soils to thermochemical conversion?" Indeed, combustion, gasification and pyrolysis are the main thermochemical conversion pathways for miscanthus biomass (Brosse et al., 2012). These technologies operate at high temperatures (e.g. > 800°C for combustion, 700 - 1500°C for gasification, 200 - 1000°C for pyrolysis), and metal oxides are likely released in the processing. Porbatzki et al. (2011) found that the gasification of commercial miscanthus biomass containing concentrations of 150 mg Zn kg^{-1} resulted in a high detection level of this element in flue gas at 900 - 1000°C. Our results show that Zn concentrations in leaves and stems vary from 35 to 82 mg kg^{-1}, which is much lower than the above value reported to be problematic. Moreover, in the studied contaminated plots, the stem concentrations for Cd, Pb and Zn did not significantly differ from those measured on

the uncontaminated plot. This suggests that as an excluder plant, miscanthus biomass produced on contaminated and uncontaminated soils may have the same metal composition. However, attention should be paid to the metal composition of leaves given that metal concentrations in these organs tend to increase at the time of harvest. Scheduling the harvest later in the winter may make it possible to obtain a biomass with low contamination because a large quantity of the leaves is lost (Lewandowski and Kicherer, 1997).

5.5. Conclusion

Overall, whatever the sampling period, metals mainly accumulated in miscanthus roots and their transfer to rhizomes and shoots was reduced. Metal concentrations in roots did not significantly change over time, whereas on contaminated soils, the leaves' metal concentrations increased during winter period. A high accumulation of metal in roots, as well as low BCFs and TFs in contaminated soils, highlight a metal-exclusion strategy of *Miscanthus* × *giganteus*. During winter, Cd, Pb and Zn concentrations in leaves were significantly higher in contaminated soils than in uncontaminated one whereas the stems' metal concentrations did not differ between these soils. The low metal concentrations in aerial parts, notably stems which constitute the harvestable biomass, make *Miscanthus* × *giganteus* is a promising candidate energy crop for phytostabilization and valuable biomass production on studied metal-contaminated agricultural plots.

For sustainable management of miscanthus plantations established on contaminated land, further studies need to focus on the quantification of metal and mineral contents (mineralomass) throughout the growing cycle and in the yields. Information from such a study could allow the comprehensive assessment of the biofuel quality, the schedule of harvest time and the reduction of ultimate environmental risks. The increase of metal concentrations in senescent leaves suggests that leaf mulch or litter could be a source of metals in soils planted with miscanthus. Thus, metal concentrations in the mulch should be quantified so as to predict their inputs in soils as well as the ecological impacts of their decomposition on soil biota.

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Chapitre VI :

La cinétique d'accumulation de métaux et des nutriments dans les organes aériens de *Miscanthus* × *giganteus*

Préambule

La croissance du miscanthus suit un rythme saisonnier spécifique du fait de son caractère rhizomateux. Ainsi, le début du printemps correspond à la sortie des pousses qui marque le démarrage de la croissance de la plante avec une utilisation de ses réserves nutritives stockées dans les rhizomes. Jusqu'à la fin de l'été, la plante est en pleine croissance, période caractérisée par une activité photosynthétique maximale. L'automne correspond à la fin de la croissance, au début de la sénescence et à la translocation des nutriments des parties aériennes vers les rhizomes. La sénescence se prolonge en hiver jusqu'à l'asséchement de la biomasse aérienne.

Le chapitre précédent présente les résultats issus d'un échantillonnage des végétaux et des sols à trois périodes (juillet, septembre et février). Il a été constaté une augmentation des concentrations en métaux dans les feuilles pendant la sénescence alors que les concentrations dans les racines ne varient pas significativement quelle que soit la saison. Si la démarche mise en œuvre constitue une approche saisonnière globale, elle ne permet pas d'établir une cinétique précise de l'accumulation des métaux dans les différents organes du miscanthus. Se pose aussi la question de l'évolution temporelle des concentrations en éléments nutritifs dans les parties aériennes du miscanthus cultivé sur des sols contaminés.

Pour apporter des éléments de réponse à ces questionnements il a été procédé à un échantillonnage mensuel des organes, de mai à décembre 2012. Comme précédemment, l'étude porte sur les parcelles M200, M500 et MC et comprend la détermination de la composition minérale des organes aériens de miscanthus. Les concentrations en métaux (Cd, Pb et Zn) et en éléments minéraux (N, P, K, Ca, Mg, et Na) des échantillons issus des parcelles contaminées sont comparées à celles des échantillons issus de MC.

Valorisation : Ce chapitre a été soumis pour publication à BioEnergy Research sous le titre « **Metal and nutrient accumulation during the growing cycle of the energy crop *Miscanthus* × *giganteus* established on metal-contaminated agricultural soils** ».

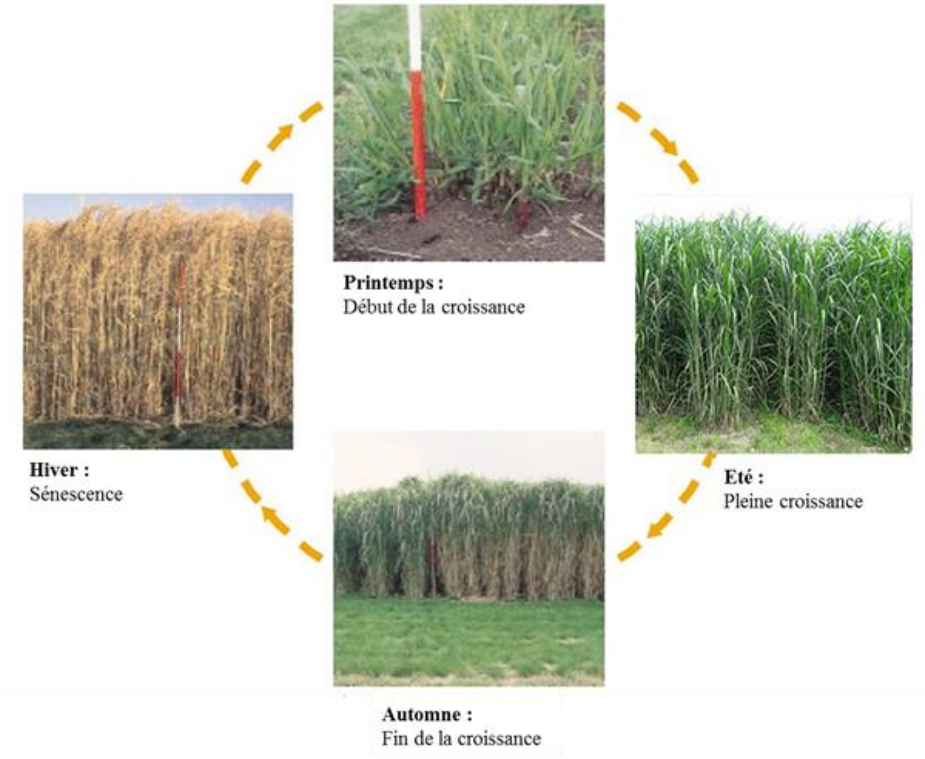

Fig. 6.1. Cycle de croissance du miscanthus dans les régions tempérées.

Metal and nutrient accumulation during the growing cycle of the energy crop *Miscanthus* × *giganteus* established on metal-contaminated agricultural soils.

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Abstract

The perennial grass, *Miscanthus* × *giganteus*, is considered as potential candidate crop for phytomanagement of metal-contaminated soils. The biomass quality of this plant has been extensively characterized in uncontaminated agricultural soils while very little is known in contamination conditions. As part of biomass quality appraisal, the main objective of this study was to assess the metal and nutrient composition of the standing aerial biomass along the growing period in 5-to-6 year-old *M* × *giganteus* experimental plantations established on former agricultural plots (M200 and M500) contaminated by Cd, Pb and Zn, and from uncontaminated plot (MC). Monthly samplings of the aboveground parts were realized from mid-spring to late autumn in 2012. Metals (Cd, Pb and Zn) and nutrients (N, P, K, Ca, Mg and Na) were determined in leaves and stems separately. Overall, metal concentrations depended on the plot, the organ and the sampling period. Our results show that on M200 and M500, metals, mainly Zn and Cd, were higher than in MC. During summer and autumn, Zn and Pb concentrations were higher in leaves than in stems whereas Cd concentrations did not significantly differ between the two organs. In these seasons, Cd and Zn concentrations in both leaves and stems did not significantly change whereas Pb concentrations in leaves increased. The nutrient concentrations can be ranked as follows: K > N > P ≥ Ca > Mg > Na. Apart from Mg and Na, there was no plot effect on nutrient concentrations. Also, before senescence onset, N, P, K and Mg decreased across the study period whereas Ca and Na increased. Nevertheless, the same nutrient concentrations, especially N, P, K, and Ca, between the contaminated and uncontaminated plots suggest that metal contamination may not influence nutrient uptake in miscanthus. Further studies should focus on quantification of metal and nutrient concentrations in harvestable biomass across the possible harvest windows, and on the potential metal and nutrient inputs via litter decomposition.

Key words: Miscanthus, biomass, phytomanagement, growing season, senescence

6.1. Introduction

Worldwide, large surface of land are reported to be contaminated by metals (Evangelou et al., 2012; Panagos et al., 2013). Soils on such lands require appropriate management and remediation options to avoid loss of arable surface areas, to produce economically valuable biomass while alleviating metal exposure and associated environmental/health risks (Evangelou et al., 2012; Witters et al., 2012). Due to its high productivity, its tolerance to

abiotic and biotic stresses, and the multiple uses of its biomass, the perennial grass miscanthus (*Miscanthus × giganteus)* is considered as a suitable candidate plant to couple management of metal-contaminated lands and biomass production (Nsanganwimana et al., 2014). Moreover, miscanthus is characterized by a high nutrient use efficiency and a high nutrient turnover in soils as a result of nutrient translocation and mulch decomposition, hence little requirements for mineral fertilization (Nassi et al., 2011; Cadoux et al., 2014).

To maintain local farmer's activities, the cultivation of miscanthus was proposed as sustainable management option of metal-contaminated agricultural soils within Metaleurop site located in Northern France (http://www.safir-network.com/site_metaleurop.html). This site covers a large surface (about 120 km^2), mianly contaminated by atmospheric emission of the former lead smelter called Metaleurop Nord. This smelter had released large quantities of metal (mainly Cd, Pb and Zn)-contaminated dust for more than a century until its closure in 2003 (Douay et al., 2008). This has resulted in a high soil contamination. After its closure, most of the crops grown on these soils still present Cd, Pb and Zn concentrations which are higher than the maximum threshold allowed for food and feed (Pruvot et al., 2006; Douay et al., 2008; Douay et al., 2013). Experimental miscanthus plantations were established since 2007 to evaluate the potential of this plant for the production of energy-dedicated biomass on contaminated soils and to limit environmental and human risks around the former Metaleurop smelter. Assessing the metal and nutrient composition of aboveground parts is of crucial importance in order to adapt best agronomic practices for management of the plantations, and for the optimization of biomass quantity and quality.

Studies of metal concentrations in miscanthus biomass from contaminated soils have only focused on late harvest but failed to characterize the metal composition in the standing biomass across the growth cycle (Barbu et al., 2013; Pogrzeba et al., 2013). Yet, in perennial plants, the leaf fall across the growth cycle forms the litter and their decomposition may result in metal enrichment in soils (Van Nevel et al., 2014). Thus, there is a need to get information on the metal concentrations in leaves. In our previous study on the influence of seasonal variations on Cd, Pb and Zn concentrations in miscanthus, these elements were higher in senescent leaves than in middle and late summer green leaves (**Chapter 5**). In other studies, high concentrations in shoots are usually found for Zn regardless of the degree of soil contamination (Nsanganwimana et al., 2014), and for Cd and Pb in case of highly contaminated soils (Kocoń and Matyka, 2012; Pogrzeba et al., 2013). However, none of the abovementioned studies covered the entire miscanthus growing cycle which spans from

spring to mid- autumn for the biomass building or photosynthetic active period and from late autumn throughout winter for senescent or drying period (Heaton et al., 2010).

Up to now, there is no data on nutrient concentrations in aerial miscanthus biomass produced on metal-contaminated soils. On uncontaminated agricultural soils, the nutrient concentrations (N, P, K, S, Ca, Mg and Na) in aerial miscanthus organs gradually decrease from the senescence onset due to their translocation to underground organs, notably in rhizomes, at the end of the growing season (Beale and Long, 1997; Himken et al., 1997; Iqbal and Lewandowski, 2014). Also, nutrient decrease in senescent shoots can result from bleaching due to rainwater in field conditions (Iqbal and Lewandowski, 2014). Here, two questions should be addressed: 1) does nutrient composition in miscanthus leaves and stems differ between uncontaminated and contaminated conditions? 2) Does soil metal contamination affect mineral uptake and seasonal variations across the growth cycle?

As part of standing biomass quality appraisal, the main objective of the present study was to determine metal and nutrient concentrations in leaves and stems of miscanthus growing on uncontaminated and on metal (Cd, Pb and Zn)-contaminated soils. Samplings were scheduled across the entire growth cycle from May (mid-spring) to December (late autumn) so as to include all growth phases characterizing miscanthus.

6.2. Materials and methods

6.2.1. Soil and plant sampling

Soil and plant samples were collected from 5 to 6-year old miscanthus (*M.* × *giganteus*) plantations established in contaminated agricultural plots named M200 and M500, and in uncontaminated one named MC. Details about the study area and soil physico-chemical parameters were provided previously (**chapter IV and V**). Monthly changes in rainfall and air temperature were recorded from the nearby weather station (**Table 6.1**).

As indicated in **Table 6.1**, monthly plant samplings were conducted from the beginning of the growing season in May 2012 (S1) to the end of the growing season (senescent phase) in December 2012 (S8).

Table 6.1. Monthly rainfalls and air temperatures during the sampling period. Meteorological data were recorded from the nearby station (Lille-Lesquin, France). Values were computed cumulative sum of daily rainfalls and average temperature) to fit the interval time between two sampling dates.

Sampling period	Rainfall (mm)	Temperature max (°C)	Temperature min (°C)
22/05/2012 (S1)	79.7	15.5	8.0
19/06/2012 (S2)	77.7	20.4	11.6
19/07/2012 (S3)	113.0	21.3	13.3
22/08/2012 (S4)	58.5	24.9	14.4
24/09/2012 (S5)	30.3	21.5	11.0
23/10/2012 (S6)	127.2	16.4	9.2
22/11/2012 (S7)	67.4	11.0	5.1
22/12/2012 (S8)	56.4	7.2	2.1

At S1, 5 miscanthus plants (clumps) were first identified in each of the three studied miscanthus plots. Plants were randomly selected and care was taken to avoid borderline effects. Then, in each clump, shoots which reached the same developmental stage were tagged to constitute a sampling pool. At this date, 5 plant samples (two shoots from each clump) were collected from each studied plot. The soil (0-25 cm) corresponding to the clump rhizosphere was sampled to constitute a couple of plant-soil sample at S1, and to determine the soil metal (Cd, Pb and Zn) contamination level, the pH, the cationic exchange capacity (CEC) and the organic carbon content. For the next sampling dates (S2 to S8), only plant parts were collected from fixed miscanthus clumps and tagged shoots at S1. The shoots were harvested at approximately 5 cm above ground level. All samples were put into polyethylene bags for transportation to the laboratory.

6.2.2. Sample preparation and physico-chemical analyses

6.2.2.1. Soils

After removing organic debris, soils were transferred into plastic trays; dried at 40°C, and then ground (ZM 200 Mill, Retsch, Germany) to pass through 2mm and 250 µm sieves.

The pH (H_2O) was measured after stirring a mixture of soil and deionized water (1:5, v/v) for 1 h at 20°C, followed by a two-hour resting time before measurement (ISO 10390). The organic carbon (OC) content was determined according to the standard ISO 14235. The cation exchange capacity (CEC) was obtained by percolation of CH_3COONH_4 (1 M, pH = 7) solution into soil samples according to the French standard NF X31-130.

For determination of metal (Cd, Pb, and Zn) pseudototal concentrations, acid digestion of soil samples was performed using a digestion plate (HotBlock$^{(TM)}$ Environmental Express, USA).

The aqua regia solution (HCl + HNO_3, 3:1, 6 mL) was added and the aliquot heated at 95°C for 75 min. The accuracy and precision of the metal determination were verified by using a certified reference material (CRM-141R, Belgium).

The exchangeable, water- and acid-soluble metal concentrations were extracted by using a 0.11 mol L^{-1} of concentrated acetic acid according to (Waterlotet al., 2012). The quality of analytical data was verified by including the certified reference material BCR®-701.

Soil metal concentrations were measured using atomic absorption spectrophotometry (AA-6800, Shimadzu).

6.2.2.2. Plants

The shoots were first separated into stems and leaves, and thoroughly washed in osmotic water. They were then oven-dried at 40°C until constant weight, and finally ground into fine powder using a knife mill (GM200, Retsch) for leaves, and an ultracentrifuge mill (ZM 200, Retsch) for stems. As described earlier (**chapter III and IV)**, sample digestion was realized by using nitric acid (HNO_3, 70%) and hydrogen peroxide (H_2O_2, 30%).

The concentrations of metals and nutrients (Ca, K, Mg, and Na) in digests were measured using spectrophotometry (AA-6800). For Ca and Mg, sample solutions were diluted into lanthanum chloride solution ($LaCl_3$, 1%) to meet requirements for atomic absorption spectrophotometry. The accuracy and precision of the analytical determinations for these four nutrient elements and three metals was verified using internal and certified reference materials (Polish Virginia Tobacco Leaves, INCT-PVTL-6, Poland).

The N (NH_4-N) and P (P_2O_5) were analyzed in sample digests according to the Kjeldahl digestion procedure as modified by Saha et al. (2012). 150 mg of plant sample were measured in a digestion tube. After adding 3 g of a catalyst composed by the mixture potassium sulfate (K_2SO_4), copper sulfate ($CuSO_4.5H_2O$), and titanium oxide (TiO_2) at proportion of 33:1:1 for K_2SO_4, $CuSO_4.5H_2O$ and TiO_2 respectively, the sample was thoroughly vortexed. Ten mL of concentrated (96.8%) sulfuric acid (H_2SO_4) and 10 mL of 30% hydrogen peroxide (H_2O_2) were successively added slowly to each tube and then mixed by swirling. The digestion tubes were thereafter placed on a digestion block (DK Heating Digester, Velp® Scientifica), heated at 200°C for 20 min and at 390°C for 45 min. The samples were digested until the solution was green and clear. Digests were cooled for 15 min at room temperature, and diluted by adding distilled water to constitute a solution of 100 mL volume.

Determination of N and P in extracts was carried out by manual colorimetric method (UV/VIS spectrophotometer, Multiskan® GO) according to Saha et al. (2012) for N and

according to the ascorbic/molybdate method as described in French standard NFX 31-161 and in Joret and Hébert (1955) for P. The accuracy and precision of the analytical N and P determinations were verified using internal material and a certified reference sample BCR®-129 (IRMM, Belgium).

The residual moisture was determined according to ISO 11465 and was used to apply the moisture correction factor so as to express all results on a dry weight (DW) basis.

6.2.3. Data analysis

Metal and nutrient concentrations in miscanthus leaves and stems are expressed and presented as the means and standard deviations of five replicates for each plot and sampling period. Analysis of variance (ANOVA) was realized to evaluate within- and between-plot differences of element concentrations in leaves and stems across the sampling periods. The normal distribution of data (Shapiro-Wilk test), and equality of variances (Bartlett test) were checked. Fisher statistics was considered for significance ($p \leq 0.05$), and Tukey HSD test was used for pair-wise comparisons of statistical groups. All statistic tests were performed in XLSTAT software (Addinsoft^{TM} software 2012).

6.3. Results

6.3.1. Soil physico-chemical parameters and metal concentrations

The soil physico-chemical and metal concentrations are presented in **Table 6.2**. The pH is lower in MC soil than in M200 and M500 soils. The organic carbon content and the CEC are significantly higher in M500 soil than in the MC and M200 soils. There is a Cd, Pb and Zn contamination gradient, with the M500 soil being the most contaminated whatever the metal. In MC soil, the studied metal concentrations are in the same order as those measured in uncontaminated agricultural loamy soils in the region (Sterckeman et al., 2002). The acetic acid extraction shows that the exchangeable, acid and water-soluble metal concentrations increase with Cd and Zn contamination whereas there is no significant difference between M200 and M500 soils for Pb. However, considering the percentage of extractability, Cd and Zn are more extractable in M200 soil (62% and 30 % for Cd and Zn respectively) than in MC and M500 soils. Pb was more extractable in MC soil (10%) than in M200 (6%) and M500 (3%) soils.

Table 6.2. Physico-chemical parameters and metal concentrations in the rhizosphere (0-25 cm) of each sampled plant at S1. Values represent means ± standard deviations. Different letters in columns refer to significant differences between plots (Tukey HSD test, n = 5, $p \leq 0.05$).

	MC	M200	M500
Physicochemical parameters			
pH $_{(H2O)}$	6.5 ± 0.3^{b}	7.3 ± 0.2^{a}	7.5 ± 0.0^{a}
Organic C (g kg^{-1})	18.6 ± 1.1^{b}	17.3 ± 2.7^{b}	31.9 ± 3.0^{a}
CEC (cmol^{+} kg^{-1})	16.4 ± 1.8^{b}	17.0 ± 2.8^{b}	28.9 ± 5.2^{a}
Pseudototal metal concentrations (mg kg^{-1})			
Cd	0.3 ± 0.0^{c}	3.6 ± 0.3^{b}	8.8 ± 0.6^{a}
Pb	19.1 ± 1.1^{c}	226.4 ± 19.2^{b}	486.2 ± 15.8^{a}
Zn	44.3 ± 1.7^{c}	301.2 ± 28.8^{b}	511.8 ± 26.6^{a}
Exchangeable, acid- and water-soluble metal concentrations (mg kg^{-1})			
Cd*	0.1 ± 0.0^{c} (40^{b})	2.0 ± 0.4^{b} (10^{a})	2.3 ± 0.9^{c} (5^{c})
Pb*	2.3 ± 0.3^{b} (62^{a})	12.4 ± 1.1^{a} (6^{b})	90.3 ± 13.1^{b} (30^{a})
Zn*	3.1 ± 0.3^{a} (35^{c})	13.9 ± 2.87^{a} (3^{c})	118.8 ± 5.6^{a} (23^{b})

*****Values in brackets represent % of pseudototal concentrations

6.3.2. Metal concentrations in leaves and stems

Metal concentrations in miscanthus leaves and stems are presented in **Fig. 6.2 & 6.3** (for within- and between-plot comparison). Overall, the variations in concentrations depended on the plot, the sampling period and the organ (**Table 6.3**).

6.3.2.1. Cadmium

Along the sampling period, leaf Cd concentrations varied from 0.04 to 0.27 mg kg^{-1}, 0.22 to 0.53 mg kg^{-1}, and 0.18 to 0.81 mg kg^{-1} on MC, M200 and M500 respectively (**Fig. 6.2**). Stem Cd concentrations varied from 0.05 to 0.44 mg kg^{-1}, 0.26 to 3.19 mg kg^{-1}, and 0.31 to 3.99 mg kg^{-1} on MC, M200 and M500 respectively (**Fig. 6.2**). Whatever the plot, leaf and stem concentrations were higher at S1 and S2 than at the rest of the sampling period. From S1 to S3, the concentrations were significantly higher in stems than in leaves, but did not generally differ at the end of growth and during senescence (from S4 in MC and M200, and from S5 in M500). Leaf and stem concentrations in MC are significantly lower than in M200 and M500 (**Fig. 6.3**). Leaf concentrations in M200 and M500 did not significantly differ apart from at S2 where concentrations are higher in M500. The stem concentrations in M500 are higher than in M200 during the growing period from S2 to S5 but there were not significantly different at the end of the growing season.

Table 6.3. Level of significance (p-values) of the effect of the organ, sampling period and plot on metal and nutrient concentrations (ANOVA, n = 5, $p \leq 0.05$).

Source of variation	Metal concentrations				Nutrient concentrations						
	Df*	Cd	Pb	Zn	Df*	N	P	K	Ca	Mg	Na
Plot	2	< 0.0001	< 0.0001	< 0.0001	2	0.061	0.143	0.059	0.496	< 0.0001	< 0.0001
Period	7	< 0.0001	< 0.0001	< 0.0001	4	< 0.0001	< 0.0001	< 0.0001	0.011	< 0.0001	< 0.0001
Organ	1	< 0.0001	< 0.0001	< 0.0001	1	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Plot x Period	14	< 0.0001	< 0.0001	< 0.0001	8	0.014	0.013	0.21	0	< 0.0001	< 0.0001
Plot x Organ	2	< 0.0001	< 0.0001	0.144	2	0.556	0.002	0.66	0.168	0.787	0.003
Period x Organ	7	< 0.0001	< 0.0001	< 0.0001	4	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.001
Plot x Period x Organ	14	< 0.0001	< 0.0001	< 0.0001	8	0.133	0.001	0.613	0.035	0.015	0.193

df: degrees of freedom

6.3.2.2. Lead

Along the sampling period, leaf Pb concentrations varied from 0.01 to 0.21 mg kg^{-1}, 0.30 to 0.84 mg kg^{-1}, and 0.13 to 0.94 mg kg^{-1} on MC, M200 and M500 respectively (**Fig. 6.2**). Stem Pb concentrations varied from 0.01 to 0.67 mg kg^{-1}, 0.01 to 0.19 mg kg^{-1}, and 0.02 to 0.15 mg kg^{-1} on MC, M200 and M500 respectively (**Fig. 6.2**). On the three plots, stem concentrations were higher at S1 and S2 than other periods, and they did not significantly differ from S3 to S8. On MC, the leaf concentrations were relatively lower and stable from S1 to S3, increased at S4 and remained stable up to S8. Conversely, leaf concentrations in M200 and M500 increased along the study period. Pb concentrations in stems were significantly lower than in leaves apart from some exceptions, namely at S3 in MC, S2 and S3 in M200, and S1 in M500 (**Fig 6.3**). Leaf concentrations were generally lower in MC than in M200 and M500 (**Fig. 6.3**). Stem Pb concentrations did not significantly differ whatever the plot, apart from at S1 with higher concentrations in MC (**Fig. 6.3**).

6.3.2.3. Zinc

Leaf Zn concentrations varied from 21.1 to 40.7 mg kg^{-1}, 31.1 to 51.4 mg kg^{-1}, and 35.6 to 60.6 mg kg^{-1} on MC, M200 and M500 respectively (**Fig. 6.2**). The stem Zn concentrations varied from 12.5 to 47.5 mg kg^{-1}, 29.3 to 78.2 mg kg^{-1}, and 27.4 to 90.7 mg kg^{-1} on MC, M200 and M500 respectively (**Fig. 6.2**). In MC, leaf concentrations significantly decreased from S1 to S2, increased and remained relatively stable from S3 to S6, and then decreased up to S8. On this plot, stem concentrations generally decreased from S1 to S5, and then remained stable from S5 to S8. In contaminated plots, leaf decreased from S1 to S2, and then increased and remained relatively stable from S3 to S8. Conversely, stem concentrations increased from S1 to S2, and then decreased and remained relatively stable from S4 to S8.

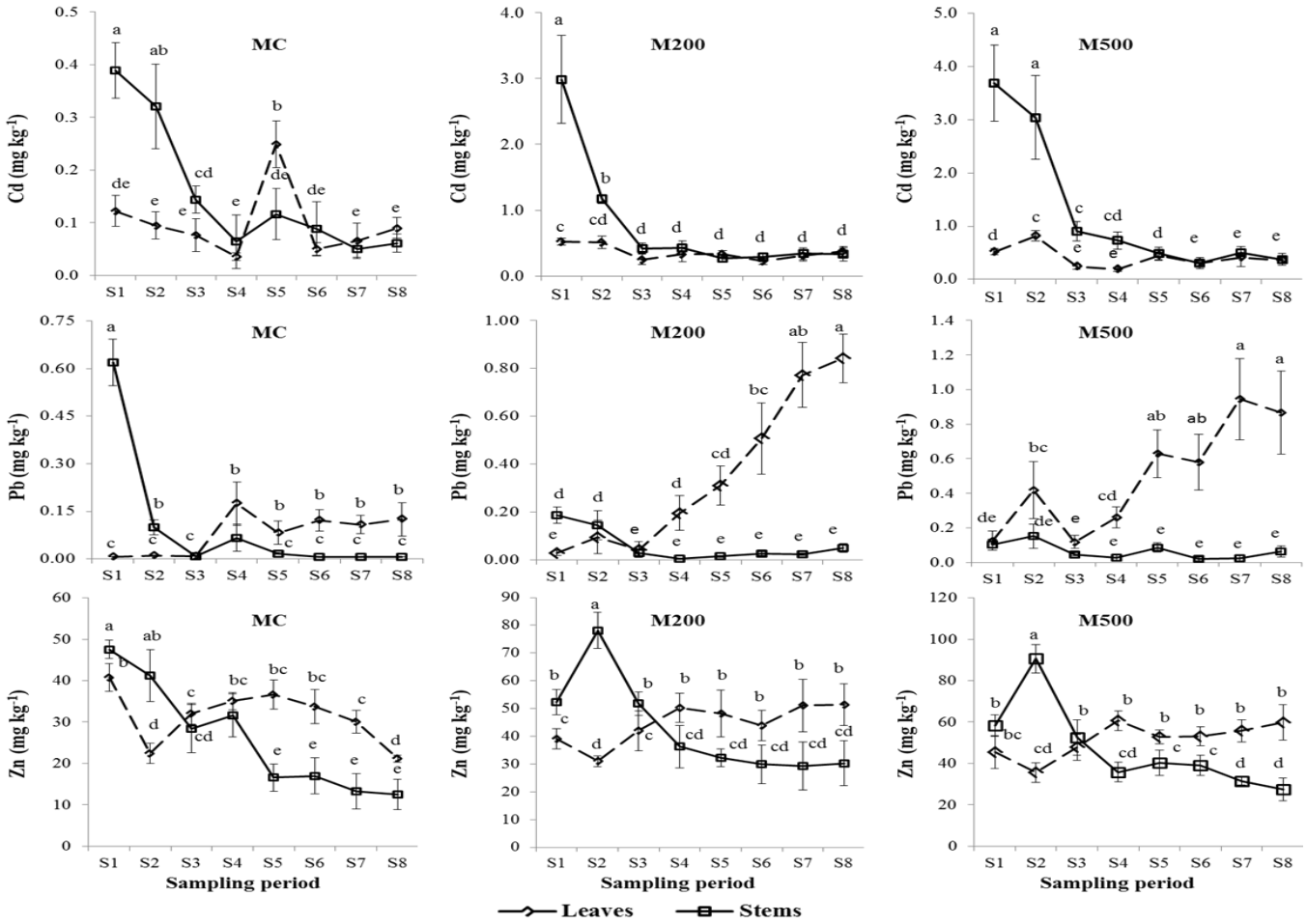

Fig. 6.2. Within-plot differences of metal (Cd, Pb and Zn) concentrations in leaves and stems of miscanthus growing in uncontaminated (MC) and contaminated (M200 and M500) agricultural plots. Analyzed samples were collected from S1 (May) to S8 (December) in 2012. Metal concentrations are presented as means ± SD of five samples for each sampling period. Different letters represent significant difference between the two organs (Tukey HSD, n = 5, $p \leq 0.05$).

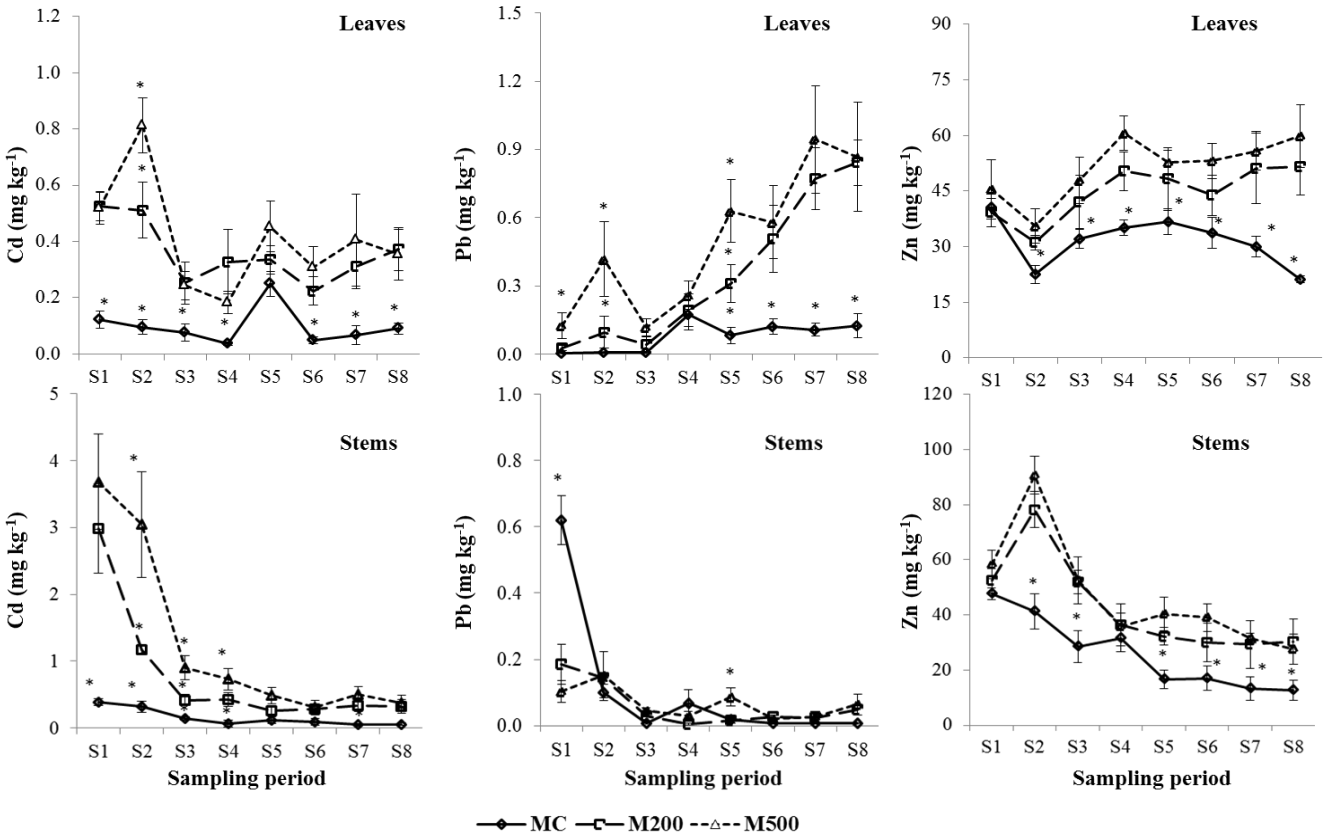

Fig. 6.3. Between-plot comparison of metal (Cd, Pb and Zn) concentrations in leaves and stems of miscanthus growing in uncontaminated (MC) and contaminated (M200 and M500) agricultural plots. Analyzed samples were collected from S1 (May 2012) to S8 (December 2012). Metal concentrations are presented as means ± SD of five samples for each sampling period and plot. The stars (*) represent any significant difference between plots (Tukey HSD, n = 5, $p \leq 0.05$).

In all plots, apart from at S3 and S4 in MC, and S3 in M200 and M500, Zn concentrations in stems were significantly lower than in leaves (**Fig. 6.3**). Apart from S1 for leaves, and S1 and S4 for stems, Zn concentrations were significantly higher in M200 and M500 than in MC (**Fig 6.3**). Moreover, there was no significant difference between M200 and M500 whatever the organ and sampling period.

6.3.3. Nutrient concentrations in leaves and stems

The nutrient concentrations are presented in **Fig. 6.3** and **6.4** (for between- and within-plot comparison). Unlike metal, only variations in Mg and Na concentrations depended on the plot (**Table 6.3**).

6.3.3.1. Nitrogen (N)

The mean leaf and stem N concentrations decreased along the study period and did not significantly differ among the plots apart from stem N concentrations in MC and M500 were higher than in M200 at S1 (**Fig. 6.4**). They varied from 4.8 to 41.6 g kg^{-1} for leaves, and from 1.7 to 40.2 g kg^{-1} for stems. The leaf concentrations at S1 decreased by 86% at S7 or S8 whereas stem decreased by 95.6% from S1 to S5, S7 and S8. Whatever the plot, N concentrations in leaves were generally higher than in stems (**Fig. 6.5**).

6.3.3.2. Phosphorus (P)

The mean leaf and stem P concentrations decreased along the study period varying from 2.6 to 10.1 g kg^{-1} and 1.3 to 9.9 g kg^{-1} in leaves and stems respectively (**Fig. 6.4**). The leaf P concentrations at S1 decreased by 74.3% at S7 or S8 whereas stem P concentrations decreased by 87.3% from S1 to S8. Apart Pconcentrations in leaves which were higher at S5 in MC, there was no significant difference between the three plots. Also, P concentrations at S1 did not significantly differ between leaves and stems whereas they were significantly higher in leaves than in stems at S8 (**Fig 6.5**).

6.3.3.3. Potassium (K)

Leaf and stem K concentrations decreased along the study period and did not significantly differ between plots (**Fig. 6.4**). They varied from 2.1 to 31.6 g kg^{-1} for leaves, and from 6.4 to 71.1 g kg^{-1} for stems. The leaf concentrations at S1 decreased by 93.3% at S8 whereas stem decreased by 90% from S1 to S5, S7 and S8. Whatever the plot, K concentrations were higher in stems than in leaves from at S1 and S3, whereas there was no significant difference between the two organs from S5 to S8 (**Fig. 6.5**).

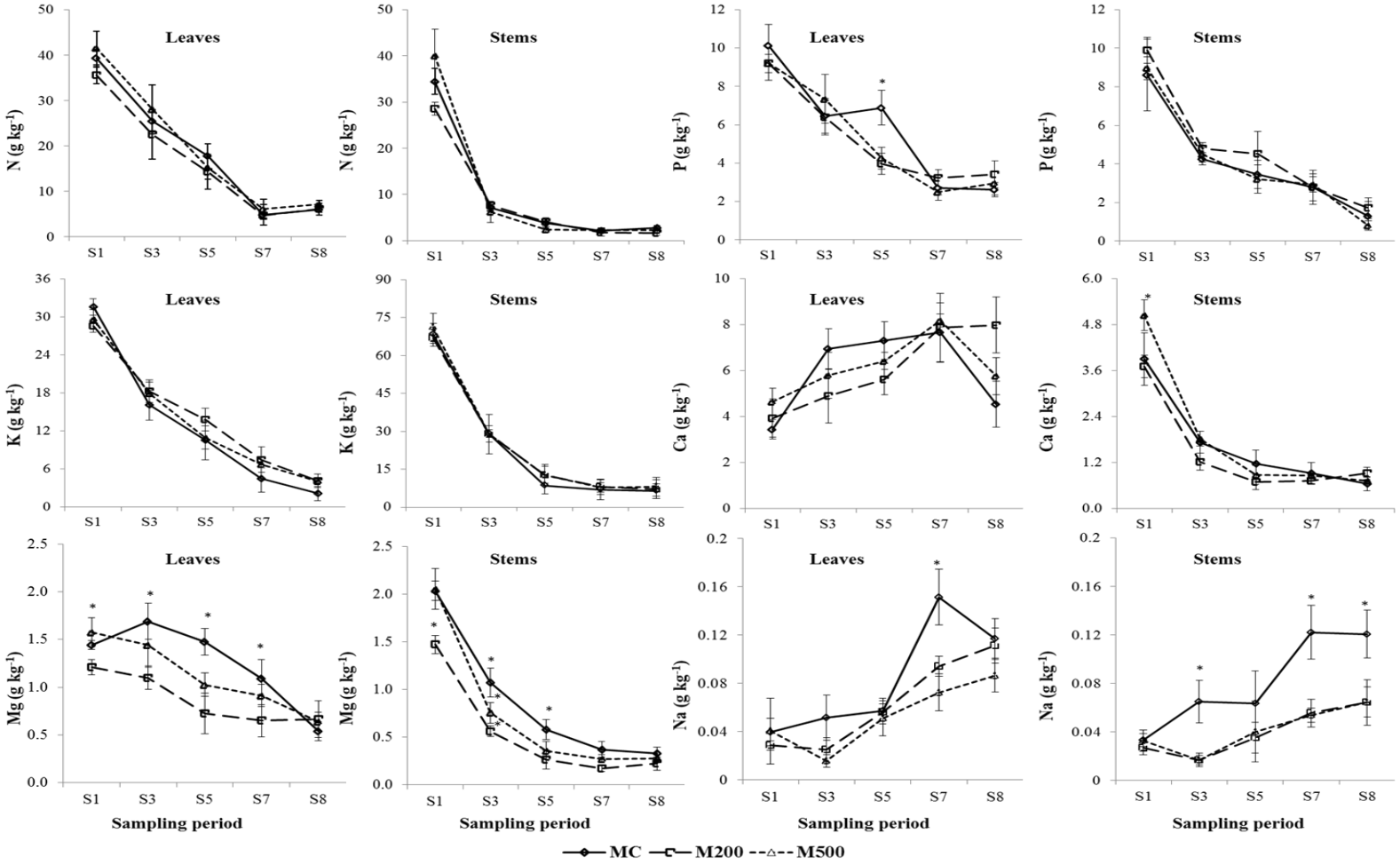

Fig. 6.4. Between-plot comparison of nutrient (N, P, K, Ca, Mg, and Na) concentrations in leaves and stems of miscanthus growing in uncontaminated (MC) and contaminated (M200 and M500) agricultural plots. Analyzed samples were collected from S1 (may 2012) to S8 (December 2012). Metal concentrations are presented as means ± SD of five samples for each sampling period and plot. The stars (*) represent any significant difference between plots (Tukey HSD, $n = 5$, $p \leq 0.05$).

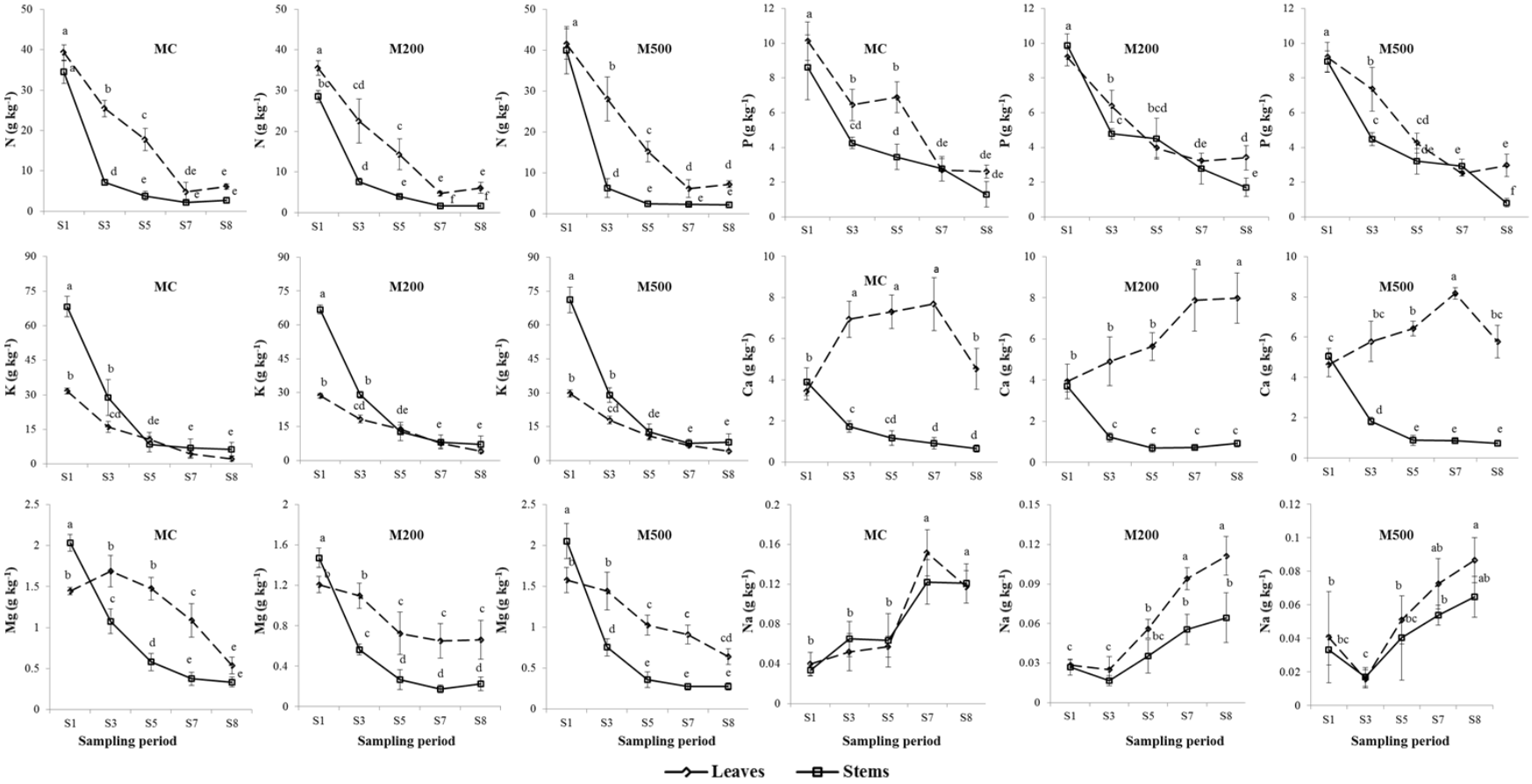

Fig. 6.5. Within-plot differences of nutrient (N, K, Ca, Mg, and Na) concentrations in leaves and stems of miscanthus growing in uncontaminated (MC) and contaminated (M200 and M500) agricultural plots. Analyzed samples were collected from S1 (May) to S8 (December) in 2012. Nutrient concentrations are presented as means ± SD of five samples for each plot and sampling period. Different letters represent significant difference between the two organs (Tukey HSD, $n = 5$, $p \leq 0.05$).

6.3.3.4. Calcium (Ca)

Leaf Ca concentrations varied from 3.4 to 8.2 g kg^{-1} (**Fig. 6.4**). Although not significantly different, these concentrations increased by 58.5% from S1 to S7, and then decreased (especially in MC and M500) up to S8 (**Fig. 6.5**). The stem Ca concentrations varied from 0.7 to 5.1 g kg^{-1}, and they decreased by 86.3% from S1 to S5, S7 and S8. At S1, stem Ca concentrations in M500 were higher than in MC and M200 (**Fig. 6.4**). Whatever the plot, leaf and stem Ca concentrations did not significantly differ at S1 whereas they were higher in leaves than in stems from S3 to S8 (**Fig. 6.5**).

6.3.3.5. Magnesium (Mg)

Whatever the plot, leaf and stem Mg concentrations decreased across the sampling periods (**Fig. 6.4**). The concentrations varied from 0.5 to 1.7 g kg^{-1} and 0.2 to 2.1 g kg^{-1} for leaves and stems respectively. The leaf concentrations at S1 decreased by 70.6% at S8 whereas stem decreased by 90.5% from S1 to S7 and S8. At S1, the concentrations in MC and M500 were not significantly different and were higher than in M200, whereas there was no significant difference between the three plots at S8. Also, whatever the organ, the concentrations in M200 were significantly lower than in MC from S1 to S7. Whatever the plot, the concentrations were higher in stems than in leaves at S1 whereas they were higher in leaves than in stems from S3 to S8 (**Fig. 6.5**).

6.3.3.6. Sodium (Na)

Whatever the plot, leaf and stem Na concentrations increased across the study period with concentrations varying from 0.02 to 0.15 g kg^{-1} for leaves, and from 0.02 to 0.14 g kg^{-1} for stems (**Fig. 6.4**). From S1 to S8, the leaf and stem increased by 86.6 % and 85.7% respectively. Whatever the sampling period, stem Na concentrations did not differ between M200 and M500 and they were lower on these two plots than in MC plot from S3 to S8. In MC and M500, leaf and stem Na concentrations did not differ whatever the sampling period whereas concentrations in leaves were significantly higher than in stems in M200 from S3 to S8 (**Fig. 6.5**).

6.4. Discussion

The present study aimed at determining metal and nutrient concentrations in aboveground miscanthus organs along the growing cycle. The leaves and stems were analyzed separately so as to compare their element composition in contaminated and uncontaminated field conditions. The study was also framed between mid-spring and late autumn so as to focus on element composition pertaining to the physiologically active phase of these two organs.

Metal concentrations in organs, apart from Pb in stems, were higher on M200 and M500 than on MC. This suggests that concentrations increased with the soil contamination level. However, despite high metal contamination in M500 soil, there was little or no significant difference between M200 and M500 for Cd, Pb and Zn concentrations in miscanthus along the studied period. The lack of proportionality between metal concentrations in miscanthus organs and metal contamination in M500 could be attributed to the potential low mobility of metals in the soils as demonstrated by the lower percentage of exchangeable, water and acid-soluble fraction than in M200 (**Table 6.2**). Moreover, in our previous study (**chapter IV**), there was a negative correlation between metal accumulation and soil physico-chemical parameters such as pH, CEC, phosphorus, carbonate, and organic carbon contents. Therefore, on M500, the higher CEC, carbonate and organic carbon contents could play a key role in reducing metal availability in soil solution, hence low plant uptake of these elements (Kabata Pendias, 2004).

In miscanthus leaves and stems, Zn concentrations were the highest whereas Pb concentrations were the lowest. However, the analysis of the bioconcentration factors (BCFs-ratios of metalconcentrations in leaves or stems to metal concentrations in soils) shows that the soil-to-shoot transfer of Cd is about the same level as Zn, and Pb is the least transferred from soil to miscanthus organs (**Annex 3 & 4**). This suggests that metal concentrations in miscanthus depended on potential element mobility in soils. Indeed, the percentage of available Pb in exchangeable and water soluble fraction is lower than that of Cd and Zn in the soils of the studied plots. Moreover, as shown by Kabata Pendias (2004), Pb is usually less mobile in soil than Cd and Zn which can partly explain its low concentrations in miscanthus organs. The higher Zn concentrations in the leaves can be explained by its functions as an enzyme cofactor in various metabolic pathways (Cakmak, 2000). Generally, the low Cd and Pb concentrations in the leaves show that miscanthus limits the accumulation of these toxic elements in the photosynthetic organs.

Apart from Pb concentrations in leaves, metal concentrations in miscanthus organs were generally higher at S1 and S2 than at other sampling periods. Also, apart from some few exceptions, metal concentrations were higher in stems than in the leaves during the spring (S1 and S2). Conversely, during summer and autumn (S3/S4 to S8), Zn and Pb concentrations in leaves were higher than in the stems whereas Cd concentrations in the two organs did not significantly differ at the end of growth (S5), and this whatever the plot. This suggests that seasonal variations in metal concentrations depended on the element, organ, and growth phase. With regard to the latter point, and apart from leaf Pb concentrations, the lower metal concentrations in summer, a season which corresponded to a steady increase of biomass

(**Annex 5**), could be resulted from dilution effect. However, on contaminated soils, leaf Pb concentrations were not affected by dilution. The steady increase of leaf Pb concentrations across the study period, as it was observed in other perennial grasses such as *Phragmites australis* (Kastratovic et al., 2013), could be due to natural low mobility of this element (Pb) in plant tissues (Pourrut et al., 2011), leading to progressive accumulation along the plant growth cycle.

The present study shows that the nutrient concentrations in miscanthus aerial organs can be ranked as follows: K > N > P ≥ Ca > Mg > Na. To our knowledge, there is no data in the literature on mineral distribution in miscanthus grown in contaminated soils. However, our results are closely related with those obtained in miscanthus shoots from uncontaminated soils (Beale and Long, 1997; Himken, et al., 1997). Moreover, apart from Na, nutrient concentrations were higher in leaves than in stems, which are consistent with the results obtained by Monti et al. (2008).

The nutrient concentrations in plant organs depend on element specific functions, the plant nutrition needs, and the complex interactions between the metabolic rate, the uptake or transport processes and environmental factors (Conn and Gilliham, 2010). There were some common variation patterns between nutrient concentrations in the stems and leaves across the study period. In these organs, N, P, K and Mg concentrations were higher early in the growing period and decreased progressively until senescence. Indeed, these four elements are abundant in active aerial plant organs in the beginning of the growing season (Himken et al., 1997; Iqbal and Lewandowski, 2014). The high concentrations can be attributed to the abundance of the active tissues since these elements, especially K and Mg, are usually present as free ions in plants, and they are among key enzyme activators and osmotic regulators. More particularly, Mg forms the ring structure of the chlorophyll molecule. The strong decrease in nutrient concentrations during high growth period can be explained by dilution effect (Nassi et al., 2011) which is most probably due to the increase of biomass production during spring and summer (**Annex 5**). At the end of growth, and/or during senescence, their low concentrations could be due to their translocation to underground organs, notably into rhizomes (Himken et al., 1997). During this period, the increased proportion of older tissues and reduced plant nutrition demands may explain the low nutrient concentrations in the aboveground organs (Kering et al., 2012). Also, unlike Ca, K and Mg elements are easily leachable by rainfall following the alteration of plant aerial tissues during senescence (Iqbal and Lewandowski, 2014).

Calcium and Na leaf concentrations increased along the study period. Conversely, stem concentrations decreased for Ca, and increased for Na across the sampling period. Indeed, there is no strong evidence supporting active transport and redistribution of Na via phloem vessels. As its transfer from roots to aboveground organs is mainly due to transpiration (Conn and Gilliham, 2010), Na is not readily redistributed once transported into transpiring organs. Thus, apart from transpiration, the organ age could explain its gradual accumulation across the sampling period and in senescent miscanthus leaves. Given the higher Ca concentrations in leaves than in stems, this element is, like Na, less mobile in leaf tissues. Moreover, in transpiring plant organs, the excess of Ca is assimilated into cell wall pectin complexes, which explain its low mobility and relatively stable concentrations with time (Conn and Gilliham, 2010).

Cadmium, Pb and Zn concentrations in miscanthus organs were generally higher in contaminated soils than in uncontaminated soils. However, the same nutrient concentrations in organs on the three plots suggest that soil metal contamination does not influence nutrient uptake in miscanthus. The high nutrient concentrations in the aboveground miscanthus organs during the period of high growth (late spring and summer) suggest a high uptake of the essential nutrients, notably N, P, and K. These elements should be supplied in sufficient amounts to maintain a high growth and productivity, and to eventually compensate for their removal upon harvest (Cadoux et al., 2012). Also, our study shows that nutrient concentrations are higher in leaves than in stems. Yet, the fallen leaves form the litter which can release back nutrients into the soil during its decomposition. Thus, the quantification of potential amount of nutrient inputs from the litter should be included or considered in the calculation of nutrient requirements for futher fertilization. Moreover, the decaying litter can increase metal availability and enrichment in the topsoils (Van Nevel et al., 2014). The higher Pb and Zn concentrations in leaves than in stems of miscanthus suggest that the quantification of metals in the litter should be considered in the management of risks associated with metal release into the soil.

It is admitted that lower metal and nutrient concentrations in plant biomass increase their quality for thermochemical conversion such as combustion, gasification and pyrolysis (Obernberger et al., 2006). Indeed, high nutrient concentrations increase the ash and noxious oxide contents during this conversion process. Moreover, the elements such as K decrease the melting point, which consequently results in fouling, slagging and increased ash and corrosion of the boilers (Obernberger et al., 2006). Our results suggest that good biomass quality will be obtained by avoiding the harvest during the growing phase. Delaying the harvest later in the

winter and allowing the biomass bleaching in the field are both strategies to decrease the leaf proportion in the harvested biomass (Lewandowski et al., 2003). However, as late harvest decreases biomass (Lewandowski et al., 2003), scheduling the harvest time requires considering the trade-off between biomass quality vs quantity. Our study demonstrates that nutrient concentrations decrease across the growth cycle and that low concentrations from the end of growth and/or during early senescence suggest a better quality of the harvestable parts for thermochemical conversion. Thus, for a good schedule of the harvest time, the analysis of metal and nutrient concentrations in the aboveground biomass along the possible harvest windows should be realized to complete the present study.

6.5. Conclusion

As part of biomass quality appraisal, this study focused on the metal and nutrient composition of the leaves and stems of *M.* × *giganteus* growing on uncontaminated and metal-contaminated agricultural plots.

Metal concentrations in miscanthus depended on the plot, the sampling period and the organ. Zinc and Cd concentrations in the two organs, and Pb concentrations in leaves, were higher on contaminated plot than on uncontaminated plot. In summer and autumn, Zn and Pb concentrations were higher in leaves than in stems whereas Cd concentrations did not significantly differ between the two organs. In these seasons, Cd and Zn concentrations in both leaves and stems did not significantly change whereas Pb concentrations in leaves increased.

The nutrient concentrations were ranked as follows: K > N > P ≥ Ca > Mg > Na. The N, P, K and Mg concentrations were higher in the green parts during spring and decreased progressively in summer whereas Ca and Na increased. The observed variations of these element concentrations during the study period could be attributed to the organ growth and development stage on one hand, and on element functions and mobility inside the plant tissues, on the other hand.

The same nutrient concentrations, especially N, P, K, and Ca, between the contaminated and uncontaminated plots suggest that metal contamination may not influence nutrient uptake in miscanthus. Given higher concentrations of metals and nutrients in the leaves, there is a need to quantify metal and nutrient concentrations in these organs and in the litter across the possible harvest windows. Such studies will inform not only on the quality of harvested biomass but also on the potential metal and nutrient inputs in soil via litter decomposition.

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Chapitre VII :

Influence des pratiques agronomiques sur l'accumulation des métaux chez *Miscanthus* × *giganteus*

Préambule

Dans les quatre chapitres précédents, l'accumulation de Cd, Pb et Zn dans les organes du miscanthus est étudiée pour un même génotype. Or, l'accumulation des métaux chez les plantes peut varier selon les espèces et les variétés. De plus, le rendement d'une culture et la phytodisponibilité des métaux dans le sol peuvent être modulés par les amendements apportés au sol et la densité de plantation.

En considération de ces facteurs, un dispositif expérimental (**cf. Fig. 2.6, chapitre II**) a été mis en place *in situ* afin d'évaluer l'influence de pratiques agronomiques sur le rendement et l'accumulation en Cd, Pb et Zn dans les organes (racines, rhizomes, tiges, feuilles) et l'ensemble des parties aériennes récoltées du miscanthus. Les pratiques agronomiques étudiées comprennent :

- trois cultivars,
- deux densités de plantation : faible (15 000 plants ha^{-1}) et élevée (20 000 plants ha^{-1}),
- un amendement biologique : inoculum endomycorhizien commercial,
- une fertilization azotée pendant la $3^{ème}$ année de culture.

L'échantillonnage des sols et des plantes a été fait à des périodes différentes selon l'objectif visé :

- en septembre 2011, pour étudier l'accumulation de Cd, Pb et Zn dans les quatre organes du miscanthus,
- en février 2012 et janvier 2013, pour évaluer le rendement et les concentrations en métaux dans la biomasse aérienne.

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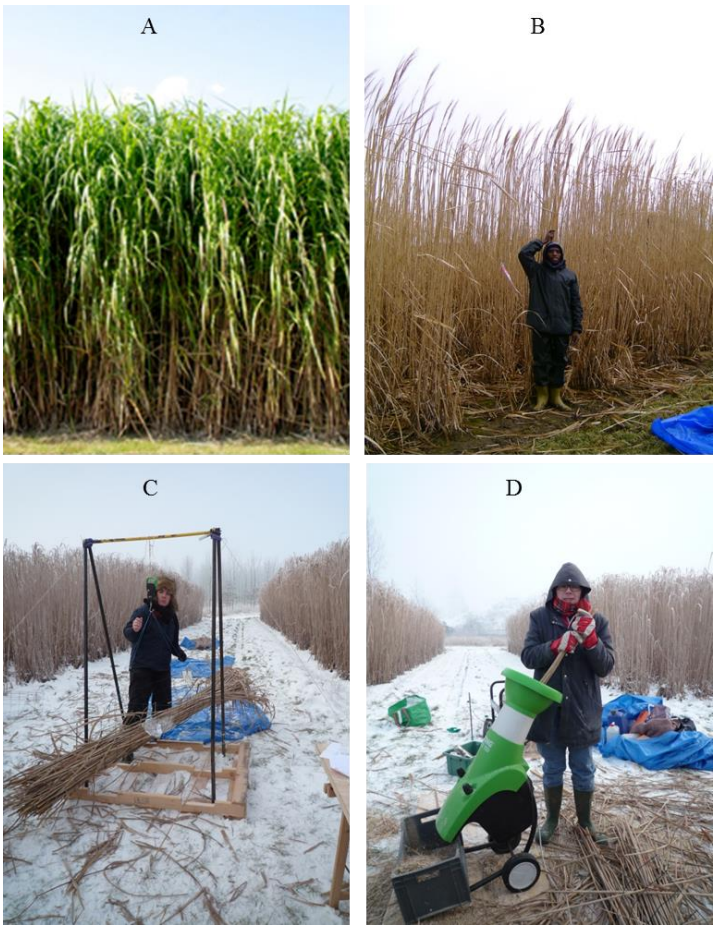

Fig. 7.1. Echantillonnage du miscanthus. A. en septembre 2011, B) en janvier 2012, C) mesure de la biomasse aérienne, D) broyage des parties aériennes récoltées.

Metal accumulation and shoot yield of *Miscanthus* × *giganteus* growing in contaminated agricultural soils: Insights into agronomic practices

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Abstract

The choice of agronomic practices for phytomanagement of metal-contaminated soils is of crucial importance to optimize plant biomass yields and to mitigate both environmental and health risks due to metal exposure. The present study aimed to assess the effects of agronomic practices on metal (Cd, Pb and Zn) accumulation in the organs and shoot yields of the energy crop miscanthus (*Miscanthus* × *giganteus*) grown in metal-contaminated agricultural soils. Three miscanthus cultivars, hereafter named Mis-A, Mis-B and Mis-I, were planted at low and high density. An endomycorrhizal inoculum (*Glomus LPA* Val 1) was added during plantation, and nitrogen fertilization was applied during the third growing season. Metal accumulation in miscanthus organs was determined during the second growing season, whereas shoot yields and their metal concentrations were determined during both the second and the third growing seasons. Based on metal concentrations and bioconcentration factors (BCFs), the three cultivars mainly accumulate metals in their roots. The shoot yields increased from 3.7-10.3 t DW ha^{-1} in the second growing season to 15.8-23.3 t DW ha^{-1} in third growing season. There were no or very few significant differences in metal concentrations and shoot yields within treatments comprising the same cultivar. The addition of endomycorrhizal inoculum increased metal (mainly Cd and Zn) accumulation in miscanthus organs and in the shoot yields and this was more observed in the cultivars Mis-B and Mis-I than in Mis-A. Shoot yields in treatments comprising different cultivars depended not on fertilization but on the interactions between cultivar and planting density, and between cultivar, planting density and endomycorrhizal inoculum. Whatever the treatment and the sampling period, Pb concentrations did not significantly differ in shoot yields. The interaction between cultivar and planting density resulted in higher Cd concentrations in the yields of Mis-B planted at low density during the third growing season. Zn concentrations increased with fertilization in all treatments, and with the addition of the endomycorrhizal inoculum in Mis-B and in Mis-I. Overall, the results demonstrate that the three cultivars could be potential candidates for coupling phytostabilization and biomass production on metal-contaminated soils.

Key words: Miscanthus, phytomanagement, phytostabilization, bioconcentration factor, endomycorrhizal inoculum, metal accumulation.

7.1. Introduction

Soil metal-contamination by industrial activities, mining wastes, and agricultural fertilizers, is of great concern worldwide (Su et al., 2014). Protecting inhabitants, especially by reducing their exposure to contaminated dusts and ingestion of contaminated foodstuff, is of prime concern. To this end, phytomanagement, which uses phytoremediation techniques, is a very promising option (Conesa et al., 2012). Phytoremediation is a cost-effective, environmentally-friendly remediation using plants and associated microorganisms (bacteria and fungi) for either (1) phytoextraction, i.e. the uptake and accumulation of metals from the soil to aboveground plant parts; or (2) phytostabilization, i.e. metal immobilization in the rhizosphere and in the roots (Mench et al., 2010). The biomass from contaminated/marginal lands is expected to provide industrial feedstock for bioenergy and green chemicals, hence avoiding the nexus of food *vs* fuel controversy (Gopalakrishnan et al., 2011; Witters et al., 2012). The sustainability is well ensured when selected plants are able to tolerate on-site contaminants and other local environmental stresses whilst providing a high and economically valuable biomass production (Conesa et al., 2012).

Around the former lead smelter Metaleurop Nord (http://www.safir-network.com/site_metaleurop.html), agricultural soils are contaminated at various levels and their contamination is mainly limited to the ploughed horizon (Sterckeman et al., 2002). Some food crops including wheat, barley, forage maize, and potato produced on such soils present Cd and Pb concentrations that often exceed legislation thresholds for food or feedstuff (Douay et al., 2013). Due to its high tolerance to abiotic stresses and low metal accumulation in aboveground biomass, *Miscanthus* species are among potential candidate crops for phytostabilization (Nsanganwimana et al., 2014). Consequently, the cultivation of *M.* × *giganteus* was proposed as a sustainable management option to maintain local farmers' activities on Metaleurop site contaminated agricultural soils. In our previous work, we demonstrated the capacity of one cultivar of *Miscanthus* × *giganteus* for phytostabilization of Cd, Pb and Zn contaminated agricultural soils (**chapter III, IV and V**). In both *ex situ* and *in situ* conditions, miscanthus did not increase mobility of Cd, Pb and Zn in studied soils, accumulated these elements mainly in roots and strongly reduced their transfer into aboveground parts.

In the last 10 years, many works have been done on these species with a special emphasis on water and nutrient use efficiencies, and on their ability to tolerate abiotic stresses such drought and chilling temperature (Zub and Brancourt Hulmel, 2010). In North America, Europe, and Asia, some intra- and inter-specific commercial cultivars have been identified for

biomass production (Clifton-Brown and Lewandowski, 2002; Yan et al., 2012; Sacks et al., 2013). Much knowledge and progress have been gained in these fields in order to breed suitable genotypes depending on expected biomass uses and local environmental conditions (Gauder et al., 2012; Robson et al., 2013; Slavov et al., 2013). However, up to date, there are still very few works on the ability of *Miscanthus* species to grow on metal-contaminated soils. Moreover, there is a need to fully appraise the potential influence of different miscanthus cultivars and agronomic practices on metal mobility into the soils and accumulation into plant organs to maximize biomass production and to decrease pollutant linkages in soils (Tang et al., 2012).

Among the agronomic practices, plantation density and soil amendments allow mitigating negative effects of multiple stresses on plant performance, hence increasing the establishment rate and productivity (Singh et al., 2011). For trees and seeded crops, plantation density and fertilization increase the yields during the establishment phase in contaminated soils (Kidd et al., 2014). The mycorrhizal fungi play a key role in mineral uptake by plants, especially in nutrient (e.g. P-deficient conditions) (Leung et al., 2013). Moreover, in metal-contaminated soils, these fungi can influence metal uptake but the extent to which this happens depends on plant species, cultivars and on fungal strains (Göhre and Paszkowski, 2006).

The present study addresses two main questions: 1) can metal accumulation and productivity in *M* × *giganteus* differ among cultivars? 2) do planting density, endomycorrhizal fungi and nitrogen fertilization have effects on biomass production and metal (Cd, Pb and Zn) accumulation in *M* × *giganteus* grown in highly contaminated field conditions?

7.2. Materials and Methods

7.2.1. Description of the experimental plot

The studied experimental agricultural plot, earlier named M700, is located at Evin-Malmaison (50°26'15.0"N 3°01'05.7"E), at approximately 1 km from the former lead smelter, Metaleurop Nord (**Fig. 2.1, chapter II**). The site landscape presents a high degree of anthropization with residential suburbs, agricultural and woodlands, and transport networks. The soil physico-chemical parameters and metal concentrations are presented in **Table 7.1**. The soil is a clay sandy loam dominated by silt (53%), and with a slightly alkaline pH. The carbonate, organic carbon, total nitrogen, and P_2O_5 contents are higher in top horizon than in deep horizons. The soil metal contamination is restricted to ploughed horizon (0-30 cm). Obviously, the soil is mainly contaminated by Cd, Pb, and Zn at concentrations of 14.1 ± 1.4 mg kg^{-1}, 731 ± 67 mg kg^{-1} and 1,000 ± 88 mg kg^{-1} respectively. These concentrations are 33, 23 and 15-fold (for Cd,

Pb and Zn respectively) higher than their concentrations in regional uncontaminated agricultural soils (Sterckeman et al., 2002).

Table 7.1. Mean values of soil physico-chemical parameters and metal concentrations on the plot before miscanthus plantation. Values are means ± standard deviations of the analyzed composite samples, n = 3. The different letters represent significant differences (Tukey HSD test, $p \leq 0.05$).

	0-30 cm	30-60 cm	60-90 cm
Agronomic parameters			
Clay (%)	19.5	28.2	26.2
Silt (%)	53	39.1	29.4
Sand (%)	27.5	32.7	44.4
pH (H_2O)	8.2 ± 0.1	8.0 ± 0.1	8.1 ± 0.1
Carbonates (g kg^{-1})	10.2 ± 3.3	< 1	1.6 ± 0.4
C total (g kg^{-1})	18.2 ± 0.4	3.6 ± 0.7	2.5 ± 0.6
C/N	15.2 ± 0.4	8.3 ± 0.3	7.7 ± 0.6
P_2O_5 (g kg^{-1})	0.16 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
CEC ($cmol^+$ kg^{-1})	14.9 ± 1.6	18.9 ± 1.9	18.0 ± 0.8
Metal concentrations (mg kg^{-1})			
Cd	14.1 ± 1.4^a	0.44 ± 0.06^{ab}	0.36 ± 0.08^b
Pb	731 ± 67^a	25.1 ± 2.4^b	24.6 ± 2.0^b
Zn	1,000 ± 88^a	70.8 ± 9.2^b	65.4 ± 6.0^b
Co	9.3 ± 0.7^a	11.4 ± 0.3^c	11.1 ± 0.1^b
Cr	61.1 ± 3.1^a	63.6 ± 1.9^a	72.9 ± 16.9^a
Cu	36.8 ± 1.1^b	11.3 ± 1.8^{ab}	10.2 ± 2.0^a
Mo	0.54 ± 0.01^a	0.48 ± 0.07^a	0.38 ± 0.06^a
Ni	20.0 ± 1.9^a	26.5 ± 4.5^a	25.9 ± 5.8^a
Ti	0.72 ± 0.05^b	0.42 ± 0.08^{ab}	0.39 ± 0.07^a

7.2.2. Miscanthus pre-growth in greenhouse

Three different miscanthus (*M* × *giganteus*) cultivars, named Mis-A, Mis-B, and Mis-I were used in this experiment. Mis-B and Mis-A were respectively supplied by Bical France (currently NovaBiom, France) and Rhizosfer (Brienne sur Aisne, France). The Mis-I plants were supplied by a private farmer. Prior to field planting, the rhizomes of miscanthus cultivars were propagated in greenhouse. The rhizome fragments with 2-3 buds were placed in polyethylene pots containing potting soil. They were watered regularly.

7.2.3. Experimental design and miscanthus field growth

Soil tillage was conducted early in spring 2010. Thereafter, the plot was designed into a randomized split-plot comprising 6 blocks (**Fig. 2.6, chapitre II**). Each block was divided into 12 subplots. Depending on treatments, a subplot (4 m x 10 m) incorporated four variables, namely the cultivar, the plantation density, the endomycorrhizal inoculation and the nitrogen fertilization. The treatments applied to each of the three miscanthus cultivars are described in **Table 7.2**. There are three replicates for each treatment **(Fig. 2.6, chapitre II**).

Table 7.2. Description of the treatments applied to each of the three miscanthus cultivars.

Abbreviation	Description
LD	Low density (15,000 plant ha^{-1})
HD	High density (20,000 plant ha^{-1})
LD-M	Low density with addition of mycorrhizal inoculum
HD-M	High density with addition of mycorrhizal inoculum
LD-F	Low density with fertilization (50 units of ammonium nitrate)
HD-F	High density with fertilization (50 units of ammonium nitrate)
LD-MF	Low density with addition of mycorrhizal inoculum and fertilization
HD-MF	High density with addition of mycorrhizal inoculum and fertilization

The plantlets with approximately 20-30 cm high were hand-planted into trenches (10-15 cm deep) in late spring 2010. For each subplot, six rows separated by 0.8 m were planted. The planting density was obtained by varying the distance between the plants in the rows so as to reach 15,000 and 20,000 plants ha^{-1} for low and high density respectively. During the plantation, 10 g of a commercial endomycorrhizal inoculum (SolRize®, Agrauxine, Saint Evarzec, France), were added per plant. According to the furnisher, *Glomus* LPA Val 1 is the active mycorrhizal fungal strain in inoculum. Considering the ground topography, the subplots with the addition of mycorrhizal inoculum were grouped in the lower part of the plot in order to limit further mycorrhizal spread by runoff into non-inoculated subplots **(Fig. 2.6, chapitre II**).

Irrigation (2 liters per plant) was regularly applied during the first two weeks after the plantation. No pesticides were applied. The plantation was kept weed free by applying herbicides (Roundup®) in the first growth season, and were not necessary thereafter. The spaces between blocks were planted with ryegrass so as to cover the soil, to limit dust emission and weed development. Fertilization was applied during the second growing season in June 2012. Fertilized subplots/treatments received 50 units of nitrogen (ammonium nitrate, 27%).

7.2.4. Plant and soil sampling

Samples were collected at three different periods depending on the objective.

The first sampling was realized during the second year of growth in September 2011 so as to study metal distribution and accumulation in miscanthus organs. For each treatment, three plants (clumps) were randomly selected for sampling. Shoots were cut at 5 cm above the ground level. The belowground organs were then sampled. Plant samples were separated into

leaves, stems, rhizomes and roots so as to study metal accumulation in each organ. About 1 kg of soil was collected from the rhizosphere (0-25 cm) of each sampled plant. For the cultivar Mis-I, only treatments with low density were sampled because the treatments with high density for this cultivar were established one year later.

The second and the third samplings were respectively realized on February 2012 and on January 2013, in order to determine shoot yields and quality with reference to their metal concentrations. Eight plants were selected from each subplot, and their shoots were cut manually at 10 cm above the ground level. They were then weighed for fresh matter. Subsamples were immediately chopped (GE150, Viking®) and put into polyethylene bags for transportation. At the laboratory, the chopped miscanthus was divided into two lots: one lot was dried at 40°C for the determination of metal concentrations in the biomass whereas the other lot was dried at 105°C for the determination of moisture content and dry weight (DW). The mean DW for the sampled shoots (DW $plant^{-1}$) was estimated from the harvested fresh weight by multiplying the dry matter content of each subsample by its proportion of the fresh weight. The shoot yield (DW ha^{-1}) per treatment was then calculated by multiplying the DW $plant^{-1}$ by the corresponding plantation density.

7.2.5. Plant and soil physico-chemical analysis

7.2.5.1. Plants

For metal accumulation in the four miscanthus organs (leaf and stem, rhizome, and root) sampled in 2011, samples were thoroughly washed in osmotic water. Shoots collected in 2012 and 2013 were not washed so as to represent the usual treatment conditions of the harvested biomass. All samples were oven-dried at 40°C and then ground into a powder using a knife mill (GM200, Retsch) for leaves and roots, and an ultracentrifuge mill (ZM200, Retsch) for stems, rhizomes, and shoots.

The samples were digested by adding 5 mL of nitric acid (HNO_3, 70%) and 5 mL of hydrogen peroxide (H_2O_2, 30%) as describe previously in **chapter III and IV**. Filtrates were stored at 4°C before Cd, Pb, and Zn determination by atomic absorption spectrophotometry (AA-6800, Shimadzu). Quality control for chemical extraction and digestion was performed by including blanks, internal and certified (Polish Virginia tobacco leaves, INCT-PVTL-6, Poland) reference materials. The residual moisture was determined according to ISO 11465 and was used to apply the moisture correction factor so as to express the results on a dry weight (DW) basis.

7.2.5.2. Soils

The samples were dried at 40°C and ground (ZM200) to pass through 2mm and 250 µm sieves.

The pH (H_2O) was measured after stirring a mixture of soil and deionized water (1:5, v/v) for 1 h (ISO 10390). The organic carbon (OC) content was determined according to the standard ISO 14235. The Cationic Exchange Capacity (CEC) was obtained by percolation of CH_3COONH_4 (1 M, pH = 7) solution into soil samples according to the French standard NF X31-130. The carbonate content was determined by measuring the CO_2 formed after adding HCl (4 M) in the aliquot according to ISO 10693. The phosphorus (P_2O_5) was extracted in ammonium oxalate solution ($(NH_4)_2C_2O_4$, 0.1 M, pH = 7) and measured according to the French standard NFX 31-161, and Joret and Hébert (1955).

The pseudo-total Cd, Pb, and Zn concentrations were determined after acid digestion in aqua regia ($HCl:HNO_3$, 3:1 v/v, 6 mL) of 300 mg of soil using the digestion block at 95°C for 75 min. After cooling, the volume was adjusted to 25 mL with double-distilled water and the solution was filtered (0.45 µm cellulose acetate filters). The quality control of the extraction and analysis was provided by the introduction of two internal reference samples and a certified soil reference (CRM 141, IRMM, Belgium).

The $CaCl_2$-extractable Cd, Pb and Zn concentrations were determined according to (Waterlot et al., 2011). The dry soil was mixed with 0.01 M calcium chloride ($CaCl_2$) solution in a ratio of 1:10, m/v.

Sample extracts were stored at 4°C prior to Cd, Pb, and Zn determination by spectrophotometry (AA-6800). As for the plant sample analysis, the moisture correction factor was applied so as to express the results on DW basis.

7.2.6. Data analysis

Data are presented as the means and standard deviations. The analysis of variance (ANOVA) was realized. When necessary, data were log-transformed to meet normality conditions. Fisher statistics was considered for significance ($p \leq 0.05$). The Tukey HSD test was performed for pair-wise comparison of treatments. All statistic tests were performed in XLSTAT software (Addinsoft^{TM} software 2013).

7.3. Results

7.3.1. Soil physico-chemical parameters and metal concentrations

The determination of metal concentrations and physico-chemical parameters was realized in sampled soils (**Table 7.3**). The pH, CEC, carbonate and P_2O_5 contents did not significantly differ whatever the treatment. The organic carbon contents in B-HD-M (12.7 g kg^{-1}) and in I-LD-M (14.6 g kg^{-1}) were significantly lower than in I-LD-NM (16.5 g kg^{-1}).

The pseudototal concentrations varied from 11.6 to 14.7 mg kg^{-1}, 674.7 to 814.5 mg kg^{-1}, and 819.0 to 1,080.9 mg kg^{-1} for Cd, Pb and Zn respectively. The Pb concentrations did not significantly differ whatever the treatment whereas Cd concentration in B-LD-M was lower than in A-HD-M and Zn concentration in B-LD-M lower than in the other treatment plots. Whatever the metal, the $CaCl_2$-extractable concentrations were less than 1 mg kg^{-1} and did not significantly differ between treatments. Lead is the least extractable with mean concentrations ranging between 0.02 and 0.05 mg kg^{-1}.

Overall, the standard deviations show that there was little within-treatment variation of pH and P_2O_5 content and relatively high variation of CEC, organic carbon, carbonate contents and metal concentrations. These observations suggest that the sampled plants were not exactly exposed to the same conditions. Therefore, to limit potential bias, the bioconcentration factor (BCF, i.e. the ratio of metal concentrations in plant organs to metal concentrations in soils) was also calculated by considering the plant-soil sample to compare metal accumulation in the three studied cultivars.

Table 7.3. Soil physico-chemical parameters and metal concentrations in sampled treatments or subplots. Values represent means ± standard deviations. Different letters in columns refer to significant differences between subplots (Tukey HSD test, n = 3, $p \leq 0.05$).

Treatments	Physico-chemical parameters					Pseudototal metal concentrations			$CaCl_2$-extractable metal concentrations		
	pH	CEC	Carbonates	Organic C	P_2O_5	Cd	Pb	Zn	Cd	Pb	Zn
		$cmol^+ kg^{-1}$	$g\ kg^{-1}$	$g\ kg^{-1}$	$g\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$
A-LD-M	7.7 ± 0.1^a	16.6 ± 1.5^a	7.4 ± 2.8^a	14.8 ± 1.6^{ab}	0.4 ± 0.1^a	14.3 ± 0.8^{ab}	788.6 ± 44.1^a	1,045.9 ± 68.2^a	0.30 ± 0.06^a	0.05 ± 0.04^a	0.40 ± 0.12^a
A-HD-M	7.8 ± 0.1^a	14.8 ± 0.4^a	6.3 ± 1.2^a	13.6 ± 0.9^{ab}	0.4 ± 0.0^a	14.7 ± 1.1^a	814.5 ± 42.4^a	1,080.9 ± 105.1^a	0.38 ± 0.08^a	0.03 ± 0.03^a	0.54 ± 0.16^a
A-LD-NM	7.6 ± 0.0^a	16.1 ± 1.0^a	5.1 ± 1.5^a	13.1 ± 1.5^{ab}	0.4 ± 0.1^a	13.3 ± 0.5^{ab}	765.4 ± 30.1^a	969.5 ± 54.7^a	0.39 ± 0.08^a	0.04 ± 0.02^a	0.56 ± 0.11^a
A-HD-NM	7.8 ± 0.1^a	16.4 ± 0.5^a	6.8 ± 3.9^a	13.7 ± 1.1^{ab}	0.4 ± 0.1^a	14.0 ± 1.5^{ab}	777.7 ± 88.4^a	1,022.2 ± 120.1^a	0.22 ± 0.04^a	0.02 ± 0.01^a	0.41 ± 0.13^a
B-LD-M	7.7 ± 0.0^a	16.9 ± 2.0^a	5.7 ± 1.6^a	13.8 ± 0.8^{ab}	0.4 ± 0.0^a	11.6 ± 0.3^b	674.7 ± 21.2^a	819.0 ± 17.9^b	0.26 ± 0.11^a	0.03 ± 0.05^a	0.66 ± 0.33^a
B-HD-M	7.7 ± 0.1^a	15.8 ± 0.6^a	7.2 ± 5.1^a	12.7 ± 0.8^b	0.4 ± 0.0^a	12.3 ± 1.1^{ab}	727.1 ± 77.4^a	899.8 ± 114.4^a	0.31 ± 0.14^a	0.03 ± 0.03^a	0.72 ± 0.38^a
B-LD-NM	7.7 ± 0.0^a	16.0 ± 1.1^a	5.4 ± 1.0^a	13.6 ± 0.4^{ab}	0.3 ± 0.1^a	12.7 ± 0.7^{ab}	707.8 ± 8.5^a	886.8 ± 33.8^a	0.22 ± 0.07^a	0.06 ± 0.03^a	0.55 ± 0.35^a
B-HD-NM	7.7 ± 0.1^a	17.6 ± 1.9^a	6.8 ± 0.7^a	13.8 ± 1.1^{ab}	0.4 ± 0.0^a	13.2 ± 1.5^{ab}	750.4 ± 103.5^a	957.8 ± 148.1^a	0.21 ± 0.03^a	0.03 ± 0.03^a	0.50 ± 0.14^a
I-LD-M	7.7 ± 0.1^a	14.8 ± 0.9^a	5.2 ± 1.3^a	14.6 ± 0.1^b	0.3 ± 0.0^a	12.8 ± 0.6^{ab}	734.6 ± 53.8^a	905.1 ± 72.5^a	0.29 ± 0.10^a	0.03 ± 0.01^a	0.72 ± 0.12^a
I-LD-NM	7.7 ± 0.1^a	16.9 ± 1.7^a	5.9 ± 1.1^a	16.5 ± 1.1^a	0.4 ± 0.0^a	13.2 ± 0.8^{ab}	723.9 ± 47.5^a	938.2 ± 48.9^a	0.22 ± 0.03^a	0.03 ± 0.01^a	0.50 ± 0.12^a

7.3.2. Metal concentrations in the organs of the studied miscanthus cultivars

Metal concentrations in the studied miscanthus cultivars are presented in **Fig. 7.2**. Given lack of significant difference between treatments, samples were grouped together to represent metal concentrations and distribution in the four organs of the studied cultivars. We note also that for a given metal and organ, there was no significant differences among the cultivars. Cadmium concentrations in leaves varied from 0.7 to 1.0 mg kg^{-1} and were lower than in other organs. Stem and rhizome concentrations did not differ and varied from 1.6 to 2.4 mg kg^{-1}. Leaf, stem and rhizome concentrations were lower than the root concentrations (32.8 to 41.0 mg kg^{-1}).

Whatever the cultivar, Pb concentrations in leaves, stems and rhizomes did not significantly differ and varied from 8.2 to 14.3 mg kg^{-1}. These concentrations were significantly lower than root concentrations (35.9 to 41.2 mg kg^{-1}).

Zinc concentrations in the leaves and rhizomes varied from 53.1 to 68.2 mg kg^{-1} and did not significantly differ. Zinc concentrations in stems varied from 84.1 to 120.3 mg kg^{-1}. These concentrations were not significantly different from root Zn concentrations for the cultivars Mis-A and Mis-I; they were also not significantly different from leaf and rhizome Zn concentrations for the cultivar Mis-B.

Overall, metal concentrations in the roots were higher than in rhizomes, stems and leaves. Whatever the organ, Zn concentrations were higher than Cd and Pb concentrations. The concentrations of latter two metals in the roots are about the same whereas Cd concentrations in rhizomes, stems and leaves are lower than Pb concentrations in these three organs.

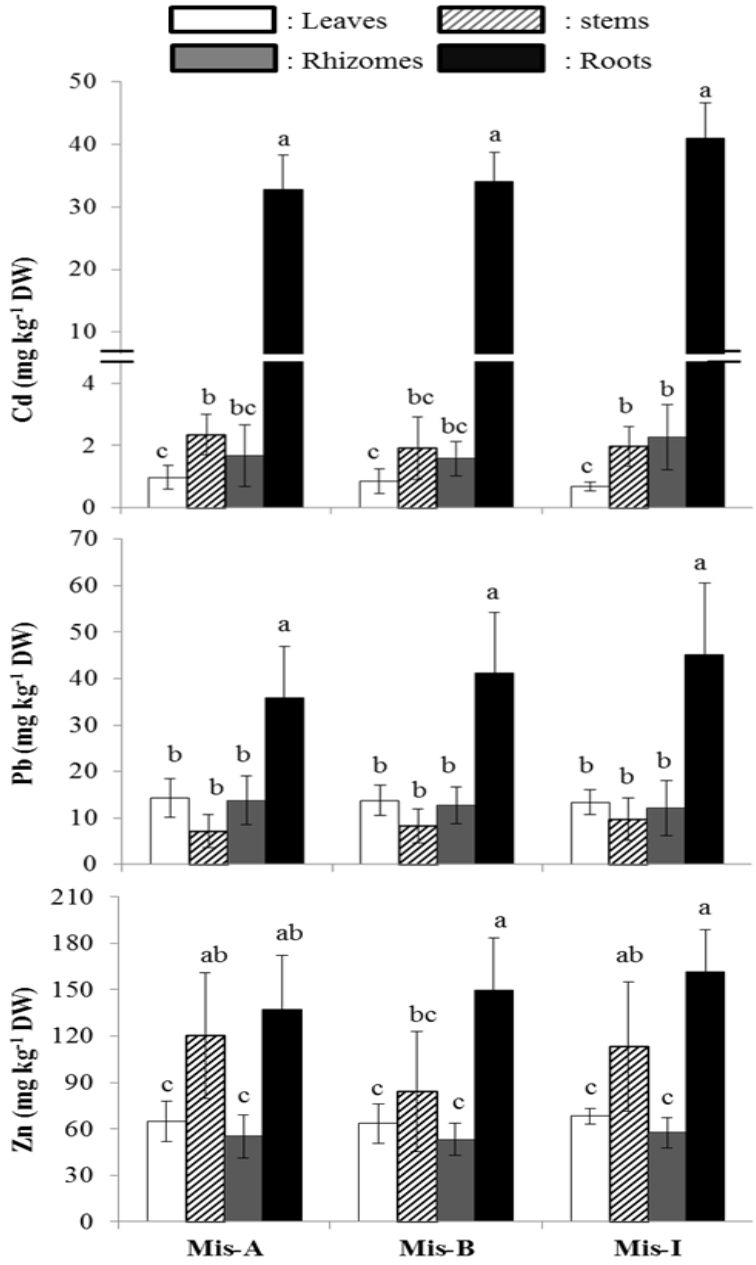

Fig. 7.2. Concentrations (mg kg^{-1} DW) of Cd, Pb, and Zn in leaves, stems, rhizomes and roots of the studied miscanthus cultivars during the 2nd growing season (September 2011). The histograms represent means and associated standard deviations. The different letters refer to significant differences between organs (Tukey HSD test, $p \leq 0.05$).

7.3.3. Treatment influence on metal accumulation rate in the organs of the studied miscanthus cultivars

The BCFs were used to study the accumulation rate of metals in the organs of the studied miscanthus cultivars. Depending on the organ, the BCFs did not significantly differ between the three cultivars (**Fig. 7.3**).

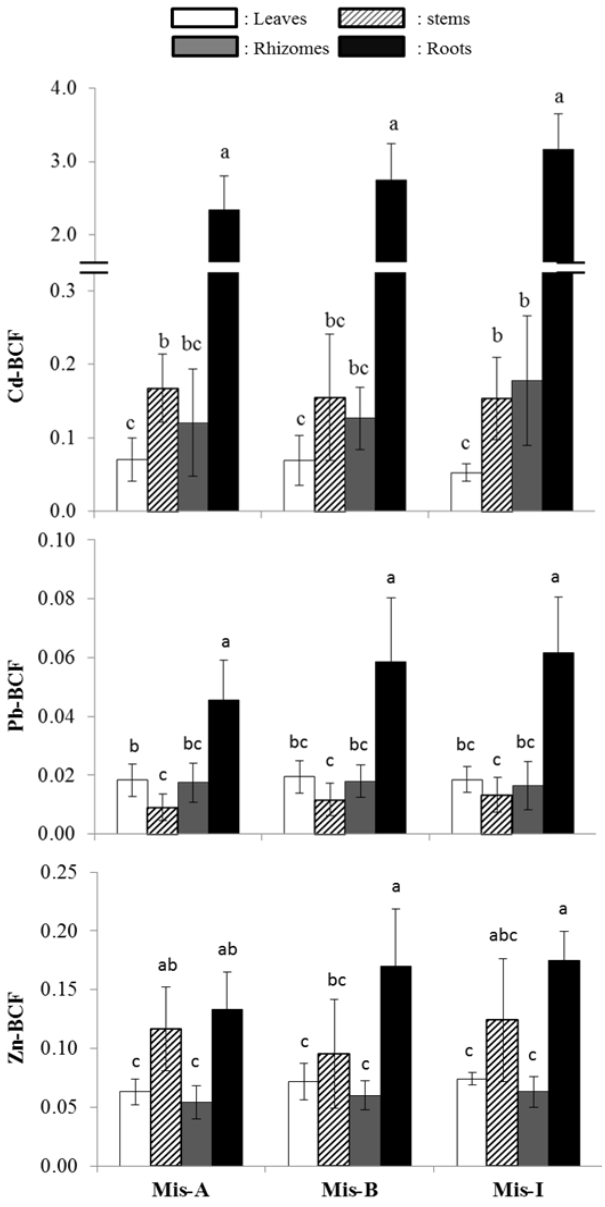

Fig. 7.3. Bioconcentration factors (BCF) of Cd, Pb, and Zn in the organs of the studied miscanthus cultivars during the 2nd growing season (September 2011). The histograms represent means and associated standard deviations. The different letters refer to significant differences between organs (Tukey HSD test, $p \leq 0.05$).

The cadmium BCFs for leaves (0.05-0.07), stems and rhizomes (0.12-0.18) were highly lower than for roots (2.4-3.2). The lead BCFs for leaves, stems, and rhizomes (0.01-0.02) are significantly lower than for roots (0.05-0.06). The zinc BCFs for leaves and rhizomes (0.05-0.07) were lower than for stems and roots (0.10-0.18). Overall, the BCFs allowed ranking the accumulation of the metals as follows: Cd >> Zn > Pb (roots), Cd > Zn > Pb (rhizomes), Cd ≥ Zn > Pb (stems) and Cd = Zn > Pb (leaves).

The analysis of variance and pair-wise comparison of treatments revealed some significant effects on metal accumulation in organs, mostly due to the addition of the endomycorrhizal inoculum (**Fig. 7.4**).

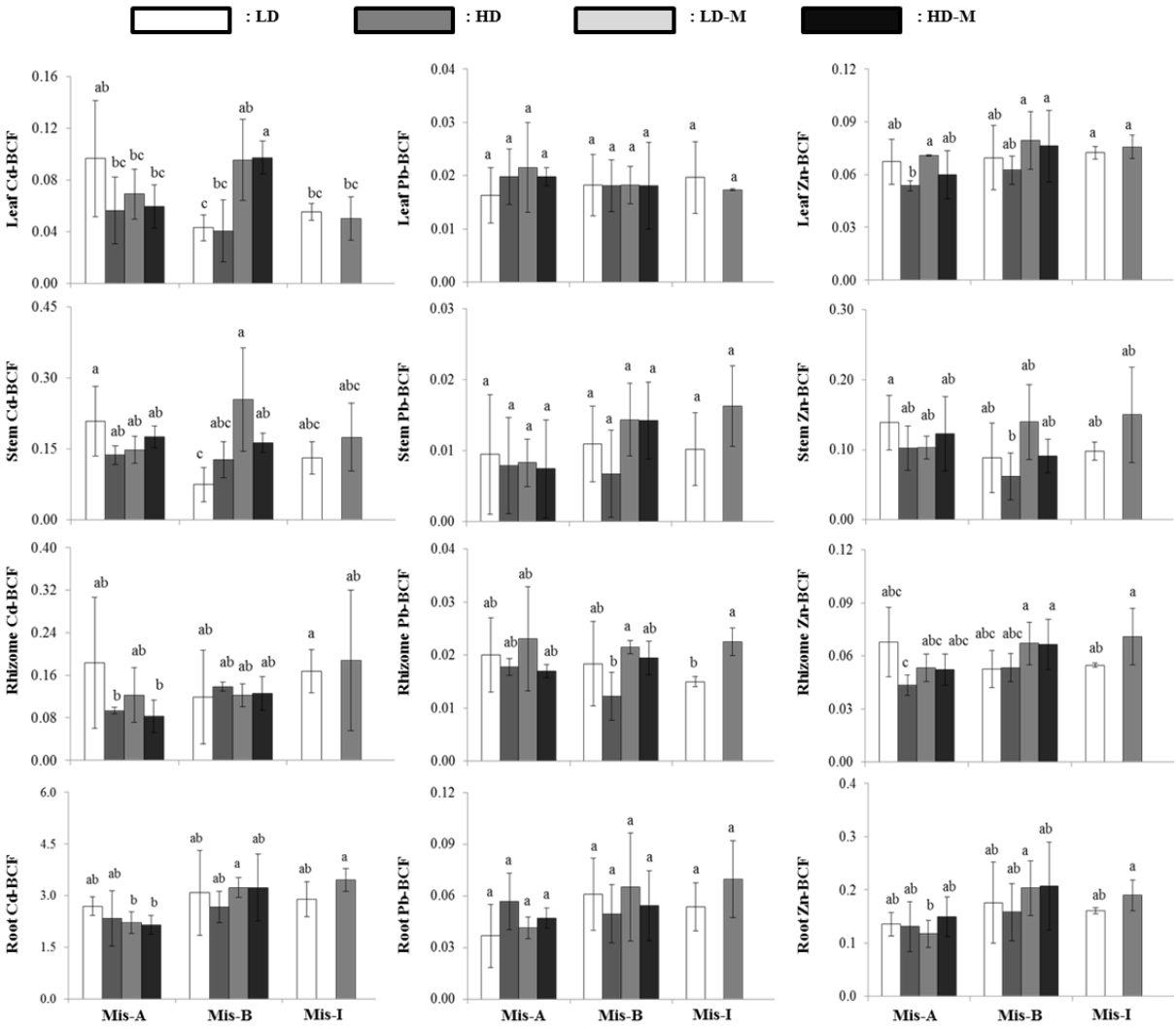

Fig. 7.4. Between-treatment comparison of bioconcentration factors (BCFs) of Cd, Pb, and Zn in the organs of the studied miscanthus cultivars during the 2nd growing season (September 2011). Values represent means ± standard deviations. Different letters refer for significant differences depending on organs and treatments (Tukey HSD, $p \leq 0.05$)

Briefly, there was a significant effect of the interaction between cultivar and endomycorrhizal inoculum on the accumulation of Cd in the leaves (F = 6.61, p = 0.006) and in the roots (3.75, p = 0.04), and on the accumulation of Zn in the roots (F = 4.48, p = 0.03). Indeed, whatever the density, the addition of the inoculum to the cultivar Mis-B increased leaf BCFs. The Cd and Zn root BCFs were higher in the inoculated cultivars Mis-B and Mis-I than in Mis-A. There was also the effect of the interaction between cultivar, planting density, and endomycorrhizal inoculum on the Cd accumulation in stems (F = 4.01, p = 0.03). At low

density, the stem BCFs for Mis-B were higher in the inoculated than in non-inoculated treatments. The Pb accumulation in the rhizomes was influenced by the addition of the inoculum ($F = 8.02$, $p = 0.04$). The rhizome BCFs for Mis-I were higher in the inoculated than in non-inoculated treatments.

7.3.4. Shoot yield and metal concentrations

After the second growing season, the shoot yield ranged from 3.7 to 10.3 ton DW ha^{-1} (**Fig. 7.5**).

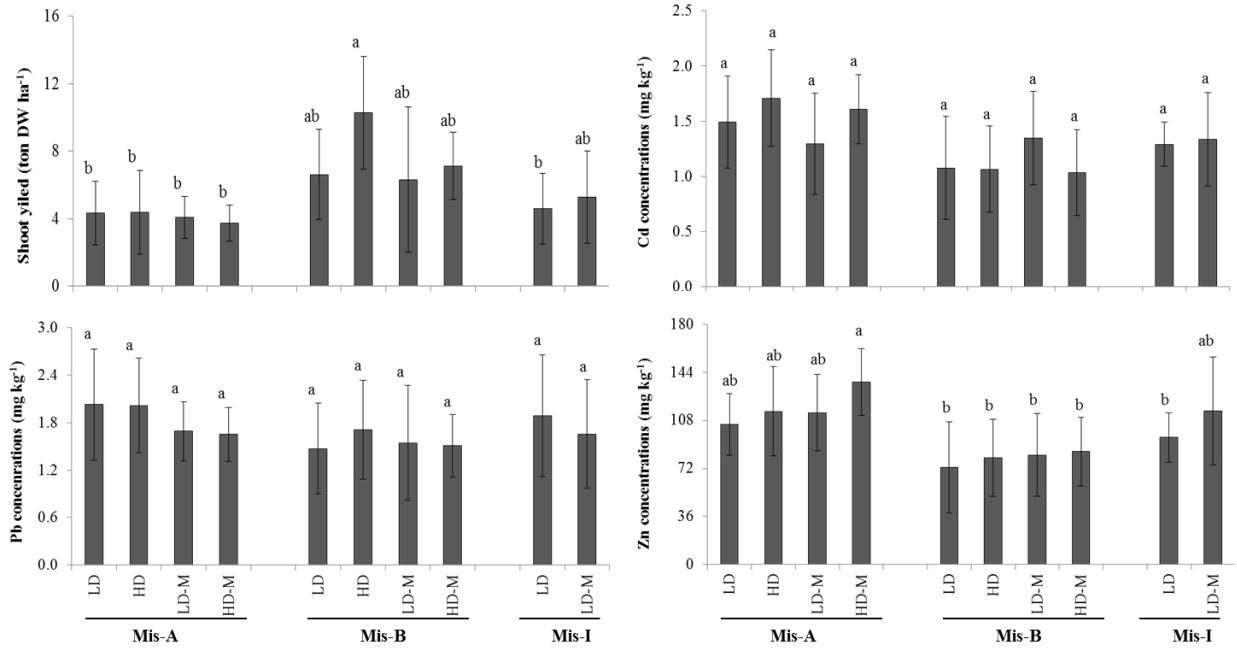

Fig. 7.5. Shoot yields (t DW ha^{-1}) and metal concentrations in the studied cultivars after the 2nd growing season. Different letters refer to significant differences between treatments (Tukey HSD test, $p \leq 0.05$).

The maximum and the minimum yields were recorded in Mis-B and Mis-A respectively. The yield depended on the interaction between cultivar and density ($F = 5.64$, $p = 0.002$). The cultivar Mis-B planted at high density produced more biomass than all the treatments comprising Mis-A, and Mis-I planted at low density. Within the cultivar, the yields did not significantly differ whatever the treatment.

Whatever the treatment, the shoot yields increased significantly during the third growing season **(Fig. 7.5)**. In average, the yields varied from 15.8 to 23.3 t DW ha^{-1}. The yield depended on the interaction between cultivar, density, and endomycorrhizal inoculum ($F = 5.77$, $p = 0.020$). Indeed, the pair-wise comparison of treatments showed that there was no significant difference among treatments comprising Mis-B and Mis-I during the third growing

season. Conversely, among the treatments comprising Mis-A, the yields were generally higher in treatments with high planting density and addition of inoculum.

Metal concentrations in shoot yields are presented in **Fig. 7.5** and 7.**6**. After the second growing season, Cd, Pb and Zn concentrations in the shoots varied from 1 to 1.7 mg kg^{-1}, 1.5 to 2 mg kg^{-1}, and 80 to 137 mg kg^{-1} respectively (**Fig. 7.5**). Whatever the treatment, Cd and Pb concentrations did not significantly differ. Conversely, the differences in Zn concentrations between the treatments depended on the cultivar ($F = 4.2$, $p = 0.02$) and on the addition of the inoculum ($F = 4.6$, $p = 0.04$). Indeed, lower Zn concentrations were measured in treatments comprising the cultivar Mis-B whereas the addition of the inoculum increased Zn concentrations in treatments comprising Mis-A and Mis-I.

After the third growing season, Cd, Pb, and Zn concentrations in the yields varied from 0.8 to 1.5 mg kg^{-1}, 1 to 1.7 mg kg^{-1}, and 28 to 74 mg kg^{-1} respectively (**Fig. 7.6**). Shoot zinc concentrations were two-fold lower than those measured during the second growing season. Pb concentrations did not differ whatever the treatment. Conversely, Cd concentrations depended on the interaction between cultivar and planting density ($F = 2.80$, $p = 0.048$). Overall, Cd concentrations were higher in Mis-B planted at low density. The differences of Zn concentrations among treatments could explained by fertilization effect ($F = 3.83$, $p = 0.05$) and the interaction between cultivar, planting density and endomycorrhizal inoculum ($F = 3.69$, $p = 0.001$). Indeed, Zn concentrations increased with fertilization in all treatments, and with the addition of the endomycorrhizal inoculum in Mis-B and in Mis-I.

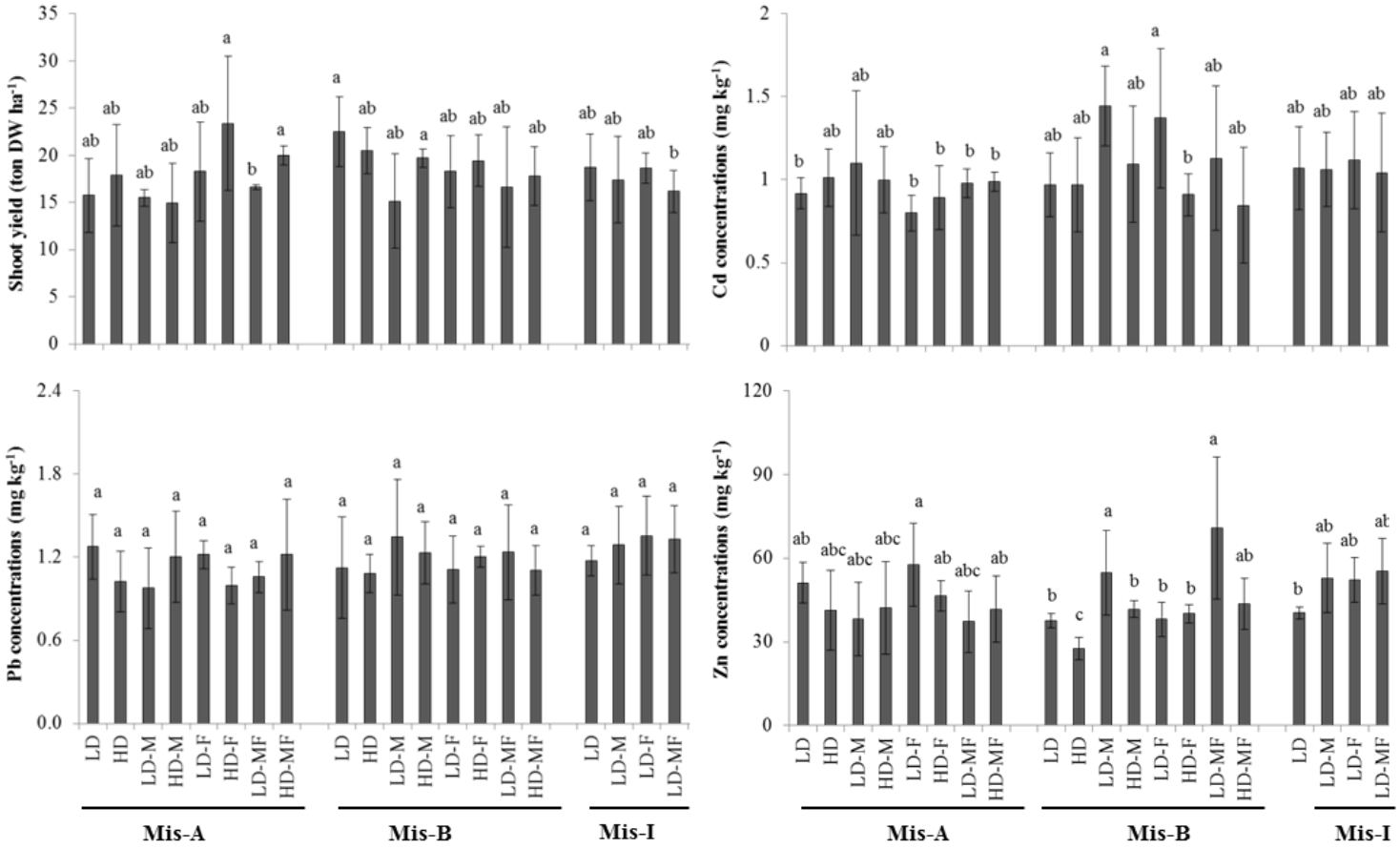

Fig. 7.6. Shoot yields (t DW ha^{-1}) and metal concentrations (mg kg^{-1}) in the studied cultivars. The harvest was carried out in January 2013 after the 3rd growing season. Different letters refer to significant differences between treatments (Tukey HSD test, $p \leq 0.05$).

7.4. Discussion

This study focused on the distribution and accumulation of metal (Cd, Pb, and Zn) in the organs of the three miscanthus cultivars grown on metal-contaminated agricultural soils. The influence of the planting density, endomycorrhizal inoculum and nitrogen fertilization on metal accumulation and on shoot biomass production were assessed.

7.4.1. Metal distribution and accumulation in the organs and shoot yields

Whatever the cultivar, metals were mainly accumulated in the roots. Apart from Cd in roots, the BCFs were less than 1, suggesting that metal transfer from the soil to miscanthus is limited. Also, the BCFs were higher in roots than in rhizomes, stems and leaves, suggesting that roots play a key role in reducing the metal transfer to the rhizomes and the aboveground organs. These observations corroborate with our previous results (**chapter III, IV and V**). Indeed, *M.* × *giganteus* is an excluder species suitable for phytostabilization (Nsanganwimana et al., 2014; Pidlisnyuk et al., 2014). The lack of significant difference in accumulation of metals among the cultivars could be due to the same behavior with regard to soil metal contamination. Moreover, as a hybrid species reproducing solely by rhizome propagation, there could be little room for genetic variability among the studied cultivars. Indeed, lack or very low genetic variability and diversity among European and North American cultivated *M.* × *giganteus* was evidenced (Cichorz et al., 2014; Yook et al., 2014).

Whatever the organ and the cultivar, Zn concentrations were higher than Cd and Pb concentrations. However, the BCFs show that Cd and Pb respectively are the most and the least transferred from the soil to miscanthus organs. This information suggests that metal accumulation in the three cultivars depend on their function and potential mobility in the soil. Zinc is an essential element in plant nutrition as it is involved in many metabolic processes as an enzyme cofactor (Cakmak, 2000). Cadmium and Pb are toxic elements without known biological functions (DalCorso et al., 2013). Moreover, Pb is naturally less mobile in soil, hence less available for plant uptake (Kabata Pendias, 2004). High Cd concentrations in roots are not followed by high Cd accumulation in the aboveground organs. This could be due to reduced symplastic transport after absorption from the soil solution. Indeed, Cd usually accumulates more in roots than in shoots (Lux et al., 2011). The high transfer from soil to roots is due to the fact that Cd is more available in the rhizosphere and uses various pathways of entry including no-specific transporter, Ca^{2+} channels, ZIP and IRP transporters (Lux et al., 2011; DalCorso et al., 2013). Inside root tissues, Cd accumulates in the endodermis and in

xylem cell wall, or is chelated by phytochelatins that mediate vacuolar storage (Lux et al., 2011; Akhter et al., 2014).

There was no significant planting density effect on metal concentrations in miscanthus. However, there were significant effects of the interactions between cultivar and planting density for Cd concentrations in shoots during the third growing season, and between cultivar, planting density, and endomycorrhizal inoculum for stem cadmium BCFs and for Zn concentrations in the shoots. These effects were more observed in Mis-B and Mis-I than in Mis-A. Moreover, regardless of the planting density, the addition of the inoculum often increased the Cd and Zn accumulation in Mis-B and Mis-I. All these observations suggest that planting density could have little impact on metal accumulation in the organs of miscanthus cultivars. This could be due to the young age of the studied miscanthus plantation with little root competition for nutrient uptake. The situation could change in the future when the rhizomes will completely colonize the available space.

Our results also show that the inoculum could increase Cd and Zn concentrations in the shoots. This differs from what is commonly known from *Glomus* species which is considered as the active endomycorrhizal fungus in the used inoculum. For instance, in maize (*Zea mays*), *Glomus* species increased metal (Cd and Pb) accumulation in the root and decreased its accumulation in the shoots (Sudová and Vosátka, 2007; de Andrade and da Silveira, 2008; Zhang et al., 2010). However, the mycorrhizal symbiosis is a complex living mechanism; arbuscular mycorrhiza can either reduce metal content of the plants or increase metal absorption from polluted soils, depending on growth conditions, fungal strain and given metals (Hall, 2002; Göhre and Paszkowski, 2006; Hu et al., 2013). To date, there is no work in the literature about mycorrhizal symbiosis in *M.* × *giganteus* growing either in contaminated or uncontaminated soils. Given lack or very few differences within treatments comprising the same cultivar, and the young age of the plantation during the study period, further research should be conducted on mature plantations to confirm the present results.

Zinc concentrations in shoots significantly decreased from the second to the third growing season. However, pair-wise comparison of treatments showed that fertilization resulted in their increase during the third growing season. Indeed, in plants, ammonium nutrition results in rhizosphere acidification due to protons release by plant roots (Hinsinger et al., 2003), which potentially increase metal mobility in soils. In our experiment, though we did not study metal mobility and soil physico-chemical parameters after fertilization, we can suggest that the root uptake of the used ammonium nitrate could have increased metal mobility. In

Triticum sp, high Zn translocation to shoot and in grain were observed following N supply in the growing media (Erenoglu et al., 2011; Barunawati et al., 2013). Therefore, though Zn is usually more abundant in miscanthus tissues than Cd or Pb, the observed increase in its concentrations in shoots could be due to ammonium-based N fertilization.

7.4.2. Potential for biomass production

Whatever the treatment, shoot yields in the third growing season were significantly higher than in the second growing season. Indeed, the first three years correspond to establishment phase in miscanthus species, which explains a steady increase between the two seasons (Clifton-Brown and Lewandowski, 2002; Miguez et al., 2008; Zub et al., 2011). Moreover, the shoot yields are as the same as those reported on uncontaminated agricultural soils (Zub et al., 2011). This suggests that the studied cultivars are able to maintain their potential productivity despite soil metal-contamination.

During the establishment phase, *M.* × *giganteus* shoot biomass increases with the planting density (Danalatos et al., 2007; Maughan et al., 2012). This is different from our results which show that in treatments comprising the same cultivar, plant density did not have a significant effect on shoot yields. Conversely, the observed differences in shoot yields were due to effects of interactions between cultivar and planting density during the second growing season, and between cultivar, planting density and endomycorrhizal inoculum during the third growing season. Indeed, during the second growing season, the cultivar Mis-B planted at high density produced higher biomass than any treatment comprising Mis-A, and non-inoculated Mis-A. However, the shoot yield obtained after the third growing season show that the biomass increased whatever the treatment and that there was no significant difference between Mis-B and Mis-A planted at high density. Moreover, yield of Mis-A planted at high density significantly increased during this season. This suggests that results obtained during the establishment phase are not sufficient for long-term prediction of the cultivars' potential for biomass production and their interaction with the agronomic practices. We recommend a regular follow-up of studied parameters until the yield stabilization is reached so as to avoid biaised conclusion. Nevertheless, the lack of or very few significant differences between treatments comprising the same cultivar suggests that there is no need to plant at high density to optimize the yield. Moreover a low planting density allows reducing the crop establishment cost.

The endomycorrhizal inoculum which is classified as a fertilizer was added during the plantation so as to stimulate rooting and promote the plant establishment. Indeed, mycorrhizal

fungi assist plants in mineral and water nutrition (Leung et al., 2013). Maize plants inoculated with *Glomus* species produced more shoot biomass and have higher nutrient contents (P, N, and S) in their tissues than non-inoculated plants (Sudová and Vosátka, 2007; de Andrade and da Silveira, 2008; Zhang et al., 2010). Our results did not show significant effects of the application of the inoculum on shoot yields. Nevertheless, there was a significant effect of the interaction between cultivar, planting density, and endomycorrhizal inoculum. Yet, this effect could mainly be due to planting density because the pair-wise comparison of treatment showed that this effect was only significant in Mis-A planted at higher density. Here, the lack of inoculum effect on plant growth, along with it effect on metal transfer to miscanthus organs, raises the issue of mycorrhizal efficiency for phytostabilization. The determination of the root mycorrhization degree of the studied cultivars will inform on the establishment rate of the used endomycorrhizal inoculum in studied metal-contaminated soils.

Whatever the treatment, there was no nitrogen fertilization effect on shoot yields. Our results corroborate with those obtained by Larsen et al. (2014). Moreover, on agricultural soils, miscanthus biomass production depends on the crop age and the soil agronomic parameters, and nitrogen fertilization becomes usually more efficient after 2-3 years (Miguez et al., 2008). As mentioned earlier above, the first three years correspond to the plant establishment, mainly development of underground organs. During our experiment, fertilization was applied in the third growing season. At that time, only one harvest was realized, suggesting that soil N was not yet depleted by biomass exportations. It will be interesting to monitor the evolution of the productivity in a multi-year cropping rotation.

7.5. Conclusion

The results show that whatever the cultivar, Cd, Pb, and Zn are mainly accumulated in the roots. The BCFs which are higher in roots than in rhizomes, stems and leaves, suggest that roots play a key role in reducing metal transfer to the rhizomes and the aboveground organs. The addition of endomycorrhizal inoculum increased metal (mainly Cd and Zn) accumulation in miscanthus organs and in the shoots; this was more observed in the cultivars Mis-B and Mis-I than in Mis-A. Whatever the treatment and the sampling period, there was no significant effect of the studied factors on Pb concentrations in shoots whereas Zn concentrations increased with fertilization. Shoot yields depended not on fertilization but on the interaction between cultivar and planting density or between cultivar, planting density, and endomycorrhizal inoculum. During the second growing season, Mis-B planted at high density produced higher shoot yield than Mis-A and Mis-I. In the third growing season, the yields generally increased with interaction between plant density and addition of inoculum,

and this was more observed for Mis-A than Mis-B. Overall, low metal accumulation into the aboveground parts and high biomass production suggest that the studied cultivars are potential candidates for phytostabilization of metal-contaminated soils.

This study was conducted during the plant establishment. Further studies should assess the effects of the agronomic practices on the shoot yield and metal accumulation of these cultivars in mature plantations. The determination of the root mycorrhization degree will inform on the success of the used endomycorrhizal inoculum in studied metal-contaminated soils. As the aim of phytostabilization is to decrease soil metal mobility and to improve soil functions, the effects of the agronomic practices on metal mobility and on soil physico-chemical parameters should be monitored at different time intervals so as to assess the phytostabilization efficiency.

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Chapitre VIII :

Synthèse générale des résultats et Perspectives

8.1. Synthèse et discussion

Les résultats obtenus concernent la capacité de miscanthus (*Miscanthus* × *giganteus*) à produire une biomasse sur des sols contaminés en Cd, Pb et Zn, l'accumulation de ces éléments dans les organes de cette plante et l'influence de celle-ci sur la mobilité des métaux dans les sols étudiés. L'accumulation des polluants a été étudiée en considérant différentes pratiques agronomiques et phases du cycle végétatif de miscanthus. Ceci a permis d'une part, de déterminer l'aptitude de miscanthus à stabiliser les métaux dans le sol et d'autre part, d'évaluer la qualité de la biomasse aérienne en lien avec sa composition en métaux et en éléments essentiels.

8.1.1. Croissance et rendement du miscanthus sur les sols contaminés

L'un des objectifs du phytomanagement est de produire une biomasse végétale sur des sols contaminés (Robinson et al., 2009; Conesa et al., 2012). La capacité du miscanthus à se développer sur les sols contaminés en ETM a été évaluée dans les conditions *ex situ* et *in situ*.

Dans les conditions *ex situ*, les paramètres de croissance qui déterminent le rendement ont été mesurés après 93 jours de culture en pots en juillet, période qui correspond à la phase de pleine croissance chez miscanthus. Sur un gradient de contamination des sols en Cd, Pb et Zn, les résultats montrent que la croissance du miscanthus est beaucoup plus dépendante des paramètres physico-chimiques que du degré de contamination des sols (**chapitre III**). De plus, la biomasse aérienne ne diffère pas significativement quel que soit le degré de contamination des sols. La croissance élevée observée sur la terre non massivement contaminée (issue de la parcelle MC) s'explique davantage par sa texture limoneuse. En effet, même si la granulométrie des terres étudiées est dominée par les limons, la particularité de la terre non contaminée réside dans une meilleure structure, facilitant ainsi la croissance racinaire et l'accès par capillarité à l'eau apportée pendant l'expérimentation.

Après la troisième année de culture dans les conditions *in situ*, le rendement moyen (14 tonnes MS ha^{-1}) sur les parcelles MC, M200 et M500 n'était pas significativement différent et ceci, malgré des paramètres physico-chimiques et des degrés de contamination des sols différents. Pour étudier l'influence des pratiques agronomiques (choix entre trois cultivars, deux densités de plantation, amendement par ajout d'un inoculum mycorhizien lors de la plantation des plants, fertilisation azotée durant la troisième année), le rendement a été déterminé après les deuxième et troisième années de culture sur M700, qui est la parcelle la plus contaminée du dispositif expérimental.

Quelle que soit la modalité étudiée, l'augmentation du rendement entre la deuxième et la troisième année montre que la contamination ne perturbe pas la croissance de miscanthus. En effet, chez miscanthus, la biomasse aérienne augmente progressivement durant les premières années qui correspondent à la phase d'installation de la culture (Clifton-Brown and Lewandowski, 2002; Miguez et al., 2008; Zub et al., 2011). De plus, le rendement obtenu à l'issue de ces deux saisons culturales est comparable à celui obtenu sur des sols non contaminés (Zub et al., 2011).

Audun des facteurs étudiés n'a d'effets significatifs sur le rendement. De plus, au sein d'un même cultivar, il y a peu ou pas de différence significative entre les différentes modalités. En général, pendant la phase d'installation du miscanthus (2 à 3 ans), le rendement augmente avec la densité de plantation (Danalatos et al., 2007; Maughan et al., 2012). Or, ceci n'a pas été le cas dans notre étude : au sein d'un même cultivar, il n'y a pas de différence significative en lien avec la densité de plantation. En revanche, il a été mis en évidence un effet de l'interaction entre le cultivar et la densité de plantation après la deuxième année. De plus, il y a un effet de l'interaction entre le cultivar, la densité de plantation et l'apport d'un inoculum mycorhizien après la troisième année. Cet effet se traduit par une augmentation du rendement sur les modalités correspondant aux plantations à densité élevée (**chapitre VII**). Tous ces constats montrent que la plante s'est installée très vite et que la combinaison des pratiques agronomiques étudiées peut influer sur les rendements.

Dans les sols contaminés, le développement des hyphes de mycorhizes arbusculaires augmente classiquement la surface de sorption des métaux à l'intérieur des racines et dans la rhizosphere, protégeant ainsi la plante contre la toxicité induite par l'excès d'ions métalliques (Göhre and Paszkowski, 2006; Leung et al., 2013). Les mycorhizes jouent aussi un rôle capital dans la nutrition minérale, favorisant ainsi la croissance végétale (Leung, et al., 2013). Par exemple, cultivés sur des sols contaminés en Cd et Pb, des plants de maïs (*Zea mays*) inoculés avec des champignons du genre *Glomus* ont produit plus de biomasse que des plants non inoculés (Sudová and Vosátka, 2007; Zhang et al., 2010). De ces faits, peut résulter une certaine ambivalence dans le rôle des mycorhizes dans les sols contaminés par les métaux. Dans le cadre de notre travail, l'inoculum contenant la souche de *Glomus LPA* Val 1 a été ajouté lors de la plantation afin de favoriser la reprise des plants et surtout de stimuler leur croissance. Pour les trois cultivars étudiés, l'ajout de l'inoculum n'a pas eu d'effet significatif sur le rendement. Ceci pourrait s'expliquer par la bonne fertilité du sol de la parcelle M700 qui pourrait masquer l'effet des mycorhizes. Celui-ci est en effet connu sur des sols à faible fertilité en lien notamment avec une carence en éléments nutritifs comme le phosphore

(Karandashov and Bucher, 2006). Ceci n'est pas le cas pour le sol de la parcelle M700 cultivé de façon intensive jusqu'à la mise en place des plantations de miscanthus. Cette caractéristique expliquerait aussi en partie l'absence d'effet de la fertilisation azotée sur le rendement. Par ailleurs, d'autres facteurs peuvent expliquer ce constat. Il s'agit du jeune âge de la plantation ou tout simplement du fait que le miscanthus répond généralement très peu ou pas à la fertilisation azotée. Bien souvent, la fertilisation n'a pas d'effet significatif sur le rendement du miscanthus avant la troisième, voire même la quatrième année de culture (Miguez, et al., 2008; Davis et al., 2014). Ceci est expliqué d'une part, par le système racinaire qui n'est pas encore très développé pour optimiser l'absorption des nutriments et d'autre part, par la faiblesse des exportations via les récoltes à ce stade de développement de la culture. Dans notre expérimentation, on peut penser que l'azote était toujours en quantité optimale pour assurer la croissance de miscanthus durant les trois premières années.

8.1.2. Accumulation de Cd, Pb et Zn dans les organes du miscanthus

L'accumulation des ETM dans les organes du miscanthus a été étudiée dans les conditions *ex situ* et *in situ*. Nos résultats démontrent que, dans les deux conditions, Cd, Pb et Zn sont davantage accumulés dans les racines que dans les rhizomes et les organes aériens. En effet, mises à part les plantes hyperaccumulatrices, les ETM sont d'une façon classique plus accumulés dans les racines que dans les organes aériens. Ce constat tient au fait que les racines sont les organes qui sont en contact direct et étroit avec les contaminants du sol (Nagajyoti et al., 2010). Elles constituent une barrière, notamment via l'endoderme, pour contrôler les transferts d'éléments potentiellement toxiques aux organes assurant la photosynthèse (DalCorso et al., 2013).

Les facteurs de bioconcentrations (BCFs) et de translocation (TFs) sont utilisés pour évaluer le degré d'accumulation des ETM chez les plantes (Menchet al., 2010). Pour ces deux facteurs, les valeurs supérieures à 1 caractérisent les plantes accumulatrices, tandis que les valeurs inférieures à 1 caractérisent les excluders. Nos résultats montrent que les BCFs et les TFs sont largement inférieurs à 1. Ceci argumente donc en faveur d'une stratégie du miscanthus qui restreint le transfert des métaux étudiés du sol à ses organes et plus particulièrement des racines aux rhizomes, tiges et feuilles. *Miscanthus x giganteus* est un excluder. Chez les excluders, la capacité à limiter le transfert et à stocker les métaux dans les tissus racinaires suppose la présence au sein de ces organes souterrains de structures spécifiques capables de limiter l'entrée massive des métaux. Chez les graminées, il a été constaté le renforcement de la couche endodermique, laquelle limite le transfert des ETM dans le xylème (Lux et al., 2003). De plus, chez les plantes, la paroi cellulaire joue un rôle

important dans la séquestration des ETM (DalCorso et al., 2013). Au sein des cellules racinaires, la présence de ligands tels que les phytochélatines et les acides organiques forment des complexes organométalliques qui sont transférés dans les vacuoles pour limiter la toxicité (Hossain et al., 2012). A l'extérieur de la racine, la séquestration des ETM dans la rhizosphère se fait grâce à des modifications des paramètres physico-chimiques qui limitent la mobilité et la solubilité des ETM, et donc les rendent moins phytodisponibles. Dans le système sol-plante, l'augmentation de la teneur en matières organiques permet aussi d'accroitre la capacité de la rhizosphère à séquestrer les ETM (Guo et al., 2006). Pendant la croissance, les racines libèrent des exsudats qui sont riches en éléments organiques tels que les acides organiques et les phytosidérophores (Dakora and Phillips, 2002). Le rôle de ces exsudats racinaires dans la phytoexclusion est très controversé car ils peuvent augmenter ou diminuer le transfert des ETM du sol à la plante (Nigam et al., 2001; Hill et al., 2002; Han et al., 2006). Néanmoins, sous leurs formes anioniques, les acides organiques tels que les acides citriques et maliques constituent des complexes organométalliques plus ou moins stables (Francis et al., 1992), et peuvent diminuer les transferts des ETM vers les parties aériennes (Nigam et al., 2001). Chez les graminées telles que *Zea mays*, *Hordeum vulgare*, il a été montré que les phytosidérophores, qui sont libérés pour solubiliser et chélater le fer, forment des complexes métalliques dans la rhizosphere et diminuent le transfert des ETM vers les tissus végétaux, notamment les racines (Hill et al., 2002; Kudo et al., 2007). Ainsi, même si nous n'avons pas mis en évidence leur présence et leur rôle, ces mécanismes pourraient conférer à miscanthus son caractère excluder.

Quelles que soient les conditions de culture du miscanthus ou la période d'échantillonnage, nous avons montré que les concentrations en Zn des organes de la plante sont plus élevées que celles en Cd et Pb. De plus, le transfert de cet élément des racines aux feuilles est souvent plus élevé. Ce comportement du Zn s'explique par sa fonction biologique dans différents processus métaboliques (Cakmak, 2000) contrairement à Cd et Pb qui n'ont pas de fonctions biologiques connues chez les êtres vivants (DalCorso et al., 2013). Pour Pb, les faibles BCFs peuvent aussi être expliqués par sa faible mobilité dans le sol (Kabata Pendias, 2004). Il est toutefois à noter que, chez le miscanthus, et de façon générale chez les plantes, Cd s'accumule principalement dans les racines. Sa séquestration dans les tissus racinaires réduit son transfert vers les organes aériens (Lux et al., 2011).

Dans les conditions *ex situ* et *in situ*, les profils d'accumulation des métaux sont identiques : ils sont plus accumulés dans les racines que dans les rhizomes et les organes aériens. Cependant, leurs concentrations dans les organes sont plus élevées dans les conditions *ex situ*

qu'*in situ*. Dans les conditions *ex situ*, les pots utilisés pour la culture de miscanthus constituent un milieu très restreint, ce qui fait que tout le volume du pot est colonisé par les racines. Ceci augmente le contact des racines avec la terre, et donc l'absorption des métaux par les racines. De plus, sur une courte durée, la biomasse produite reste plus faible dans les conditions *ex situ*, ce qui fait que les concentrations mesurées dans les organes sont moins diluées dans la biomasse aérienne. Ainsi, le volume de la rhizosphere et la biomasse végétale qui sont très importants dans les conditions *in situ* pourraient conduire à une dilution des concentrations en métaux. Il est à penser qu'après 4 années de culture en conditions *in situ*, le système racinaire et les rhizomes sont très développés, et colonisent probablement des horizons peu ou pas contaminés (Monti et Zatta, 2009). Rappelons que la contamination des sols agricoles aux alentours de l'ancienne fonderie est limitée à l'horizon labouré (Sterckeman et al., 2000). De plus, l'élévation des teneurs en matières organiques et le développement des communautés microbiennes sont aussi des facteurs qui peuvent contribuer à limiter les flux de métaux du sol à la plante.

8.1.3. Effet du gradient de contamination sur l'accumulation de Cd, Pb et Zn dans les organes de miscanthus

Les sols étudiés présentent un gradient de contamination en Cd, Pb et Zn. Dans les conditions expérimentales *ex situ*, les concentrations dans les organes du miscanthus permettent de répartir les sols étudiés en deux groupes : les sols M200-1, M200-3 et M500 et les sols M500-B et M700 (**chapitre III**). Dans chacun de ces groupes, les concentrations en métaux dans les organes du miscanthus ne sont pas, dans la plupart des cas, significativement différentes. De même, dans les conditions *in situ*, il a été montré que les concentrations en métaux dans les organes du miscanthus ne sont pas significativement différentes entre les sols M200-1, M200-3 et M500. Ces constats montrent que les concentrations pseudototales ne peuvent à elles seules expliquer le transfert des métaux dans le système sol-plante. En revanche, ce transfert peut être expliqué par les paramètres physico-chimiques des sols, lesquels influencent la mobilité et la phytodisponibilité des ETM dans le sol. L'analyse des paramètres physico-chimiques des sols étudiés a montré que les sols contaminés présentent un pH légèrement alcalin et une texture à dominante limoneuse. Néanmoins, quelques différences ont été observées en ce qui concerne la CEC, les teneurs en $CaCO_3$, en carbone organique et en oxydes de Fe. En effet, les sols riches en $CaCO_3$, en matières organiques et en oxydes de Fe / Mn ont souvent des pH alcalins, ce qui leur donne un pouvoir tampon plus élevé, capable de retarder la dissolution des métaux et de les maintenir dans la phase solide du sol (Dube et al., 2001). Ainsi, la faible extractabilité des métaux au $CaCl_2$ (0,01 M), la CEC et les teneurs en

carbonates, carbone organique, oxydes de Fe qui sont plus élevées dans le sol M500, contribuent à expliquer les faibles concentrations en métaux des organes du miscanthus obtenu sur ce sol par rapport au sol M500-B. Aussi, bien que le degré de contamination en métaux du sol M500-B soit inférieur à celui du sol M700, l'extractabilité au $CaCl_2$ (0,01 M) plus élevée, les faibles teneurs en carbonates et en oxydes de Fe dans le sol M500-B expliquent la similitude des concentrations en métaux dans les organes du miscanthus obtenus sur ces deux sols.

8.1.4. Influence des variations saisonnières et de la phase de croissance sur l'accumulation d Cd, Pb, Zn et des éléments nutritifs chez miscanthus

Chez les végétaux, la teneur en éléments minéraux dépend du stade de croissance de la plante ou des organes de cette dernière. Les éléments nutritifs sont généralement plus concentrés dans les organes jeunes et actifs que dans les organes sénescents et inactifs. Pendant la croissance, ces éléments sont transférés des organes vieillissants aux organes actifs. Miscanthus étant une plante pérenne, les concentrations en éléments nutritifs, mais aussi en ETM, peuvent évoluer durant le cycle végétatif et selon le stade de développement des organes.

Les concentrations en Cd, Pb, Zn et en éléments nutritifs ont été mesurées durant le cycle végétatif de miscanthus (**chapitres V et VI**) : en phase de démarrage de la croissance (printemps), de pleine croissance (été), de sénescence (automne et début d'hiver), asséchement (hiver).

Concernant les métaux, leurs concentrations sont plus élevées dans les tiges que dans les feuilles au printemps. Ensuite, l'évolution des concentrations dans ces organes aériens dépend de l'élément. Il a été constaté que pendant la phase de croissance, les concentrations en Zn et Pb sont plus élevées dans les feuilles et dans les tiges alors que les concentrations en Cd dans ces deux organes ne diffèrent pas significativement. Aussi, dans cette phase de croissance, les concentrations en Cd et Zn ne changent pas significativement quel que soit l'organe alors que les concentrations en Pb dans les feuilles augmentent progressivement, voire même pendant la senescence. Malgré cette augmentation, les concentrations en Pb restent très faibles et moins élevées que celles en Cd et Zn. L'augmentation progressive en Pb dans les feuilles pourrait s'expliquer d'une part, par le mouvement et le transport de cet élément qui est beaucoup plus dépendant des mécanismes de transpiration de la plante et d'autre part, la faible mobilité de Pb dans les tissus végétaux.

Sur les sols contaminés, les concentrations en Cd, Pb et Zn dans les racines ne changent pas significativement quelle que soit la saison ou la phase de croissance étudiée. Dans les feuilles, les concentrations en ces trois métaux sont plus élevées pendant la phase d'asséchement que pendant la phase de croissance. Ceci a été observé chez la plupart des plantes à croissance pérenne comme *Phragmites australis* (Kastratovic et al., 2013), *Arrhenatherum elatius* (Deram et al., 2008), *Lolium perenne* (Bidar et al., 2009) et *Bromus carinatus* (Silk et al., 2006). Chez miscanthus, ceci pourrait être en lien avec l'âge de l'organe : les concentrations les plus élevées sont observées dans les organes ayant été exposés plus longuement aux ETM tels que Cd (Arduini et al., 2004). De plus, comme les métaux ne sont pas généralement mobiles dans les tissus végétaux, la transpiration et l'asséchement accéléré peuvent conduire à l'accumulation des ETM dans les feuilles qui sont des récepteurs terminaux. Enfin, les concentrations élevées dans les feuilles sont à mettre en parallèle avec une translocation des éléments minéraux vers les organes souterrains.

Concernant les éléments nutritifs, les teneurs en azote, phosphore, potassium et magnésium sont plus élevées au printemps et diminuent pendant la phase de croissance (chapitre 6). Dans les feuilles, cette diminution est progressive le long du cycle végétatif alors que les teneurs dans les tiges sont plus ou moins stables dès la fin de la croissance et pendant la senescence. La diminution des teneurs pendant la phase de croissance peut être expliquée par la dilution due à l'augmentation de la biomasse (Nassi et al., 2011). Pendant la senescence, cela pourrait être en lien avec la translocation de ces éléments vers les organes souterrains, notamment les rhizomes (Beale and Long, 1997; Himken et al., 1997). Les teneurs en éléments nutritifs identiques quel que soit le degré de contamination des sols suggèrent que la contamination des sols en Cd, Pb et Zn n'a pas d'effet significatif sur la composition minérale des organes étudiés.

8.1.5. Influence des pratiques agronomiques sur l'accumulation de Cd, Pb et Zn dans les organes de miscanthus

Les pratiques agronomiques étudiées comprennent trois cultivars, deux densités de plantation, l'ajout d'un inoculum mycorhizien contenant la souche *Glomus LPA* Val 1 ainsi que la fertilisation azotée (**chapitre VII**).

La distribution et les concentrations en métaux dans les organes des cultivars étudiés ne diffèrent pas significativement. En effet, miscanthus cultivé actuellement en Europe et en Amérique du Nord est un hybride sans reproduction sexuée, ce qui suppose une très faible variabilité entre les génotypes (Cichorz et al., 2014; Yook et al., 2014).

La densité de plantation et l'ajout de l'inoculum n'ont pas d'effet significatif sur l'accumulation des métaux dans les organes des génotypes de miscanthus. Cependant, l'interaction entre le cultivar et l'inoculum mycorhizien a montré que l'inoculation des cultivars augmente plus le transfert de Cd et Zn dans les organes de Mis-B et Mi-I que ceux de Mis-A. Ceci suggère que l'inoculation induit des comportements différents chez les cultivars. Aussi, quel que soit le cultivar, la fertilisation augmente les concentrations en Zn dans les parties aériennes. Le même constat a été observé chez *Triticum sp*, où les concentrations en Zn dans les parties aériennes et dans les grains augmentent suite à l'ajout de nitrate d'ammonium dans le substrat de culture (Erenoglu et al., 2011; Barunawati et al., 2013). En effet, la nutrition d'ammonium conduit à l'acidification de la rhizosphère en raison de protons libérés par les racines des plantes (Hinsinger et al., 2003). Ceci augmente la mobilité des métaux dans les sols. Ainsi, l'augmentation des concentrations en Zn dans les parties aériennes traduit une augmentation de la phytodisponibilité de cet élément dans la rhizosphere, laquelle pourrait s'expliquer par l'acidification due à la fertilisation azotée.

8.1.6. Effet de miscanthus sur la mobilité de Cd, Pb, Zn et les paramètres physico-chimiques des sols.

Les plantes peuvent affecter la spéciation des ETM dans le sol via des changements du pH, du degré d'aération et d'hydratation du sol, ou tout simplement via un changement de l'environnement chimique dans la rhizosphère (Kabata Pendias, 2004). A nos jours, il existe très peu de travaux portant sur les effets de *M.* × *giganteus* sur la mobilité des ETM dans les sols. Nos résultats obtenus dans les conditions *ex situ* et sur un court délai (93 jours) et dans les conditions *in situ* après 2 à 4 années de culture montrent que le pH a augmenté dans le sol non contaminé alors qu'il a diminué dans les sols contaminés. En effet, le pH est légèrement acide dans le sol non contaminé et légèrement alcalin dans les sols contaminés. Or, la plupart des minéraux nutritifs sont solubles à pH = 6 - 7.5 (Fernandez and Hoeft, 2009). L'acidification du sol est l'une des stratégies de la plante pour accéder aux nutriments. Ceci se fait par libération directe des ions H^+ et acides organiques dans le sol (Hinsinger et al., 2003). Malgré cette acidification du sol, il a été constaté, dans les conditions *ex situ*, une légère diminution des concentrations extractibles au $CaCl_2$ (0,01 M) et des fractions échangeables et solubles de Cd et Pb. Aussi, la fraction réductible pour Cd et Pb, et la fraction associée à la matière organique pour Zn, ont augmenté. Ces constats montrent que la culture de miscanthus peut réduire le pool labile des métaux étudiés dans le sol. Les travaux d'Iqbal et al. (2013) ont montré que, dans des plantations de miscanthus, l'augmentation des teneurs en carbone organique réduit la mobilité des ETM dans le sol.

Les résultats sur la mobilité des métaux dans les conditions *in situ* représentent un bilan fait trois ans après la culture de miscanthus. En général, la fraction extractible au $CaCl_2$ (0,01 M) pour chacun des trois métaux est largement inférieure à 1 %, ce qui montre leur faible mobilité. Cependant, les extractions séquentielles montrent que ces éléments sont plus présents dans les fractions échangeables, solubles et réductibles que dans les fractions oxydables et résiduelles. En comparaison avec les autres résultats portant sur la mobilité des métaux, il apparait que la culture de miscanthus n'a pas globalement changé la distribution des métaux dans les sols agricoles telle qu'on la connaît sur le site atelier Metaleurop (Pelfrêne et al., 2011)}.

8.2. Conclusion et perspectives

8.2.1. Conclusion

L'objectif principal de la thèse était d'évaluer les potentialités de *Miscanthus* × *giganteus* pour le phytomanagement de sols agricoles fortement contaminés par les émissions atmosphériques passées de la fonderie de plomb Metaleurop Nord. Ce travail s'inscrit dans un programme plus global, nommé PHYTENER et soutenu par l'ADEME, et qui vise à évaluer l'intérêt de ce mode de gestion en vue de contribuer à la reconversion de l'agriculture sur un vaste territoire fortement pénalisé par un siècle d'activités industrielles. Afin d'évaluer l'aptitude de cette plante à immobiliser les métaux (Cd, Pb et Zn) dans la rhizosphère, à produire une biomasse, les travaux ont mis l'accent sur trois points principaux : la capacité de cette plante à se développer sur des sols fortement contaminés, l'accumulation dans les différents organes des métaux et des éléments minéraux essentiels à la nutrition végétale, et l'influence de miscanthus sur la mobilité des trois métaux et les paramètres physico-chimiques des sols étudiés.

Quelles que soient les conditions de culture, la phase de croissance et la période d'échantillonnage, l'analyse des concentrations en métaux dans les organes de la plante ainsi que les facteurs de bioconcentration (BCFs) et de translocation (TFs) montrent que *M.* × *giganteus* est un excluder. Cette plante est donc une bonne candidate pour la phytostabilisation de sols contaminés par Cd, Pb et Zn. Les concentrations en ces éléments dans les organes de cette plante augmentent avec le degré de contamination des sols, mais ceci dépend plutôt de leurs paramètres physico-chimiques. Dans les sols présentant une CEC et des teneurs en $CaCO_3$, P_2O_5 et en carbone organique élevées, le transfert des trois métaux étudiés du sol aux organes de *M.* × *giganteus* est très limité.

Dans les conditions *ex situ et in situ,* l'étude de la mobilité de Cd, Pb et Zn au moyen d'extractions simples et séquentielles montre que la culture de miscanthus ne conduit pas à une augmentation de la mobilité des polluants dans le sol. Dans les conditions *ex situ,* elle peut même diminuer la mobilité de ces derniers, mais ceci dépend de l'élément. Pour Cd et Pb, les fractions échangeable et soluble diminuent au détriment de l'augmentation de ces deux éléments dans la fraction réductible. Pour Zn, il a été constaté une augmentation de la fraction oxydable.

L'étude de la cinétique d'accumulation de Cd, Pb, Zn et des éléments nutritifs dans les organes aériens du miscanthus a montré que la contamination métallique des sols n'affecte pas leur composition en éléments nutritifs. Ce constat suggère que les métaux interfèrent peu ou pas sur la nutrition minérale chez miscanthus.

Vu le jeune âge de la plantation, prévue pour durer une vingtaine d'années au regard des expériences acquises sur des sols non contaminés, il serait prématuré de tirer une conclusion finale sur les effets observés des pratiques agronomiques étudiées sur l'accumulation et la croissance de miscanthus. Après trois ans, il a toutefois été montré que l'origine du cultivar, la densité de plantation et l'ajout de l'inoculum mycorhizien n'ont pas d'effet significatif sur les concentrations et la distribution des trois métaux dans les organes de miscanthus. Cependant, les interactions entre le cultivar et l'ajout de l'inoculum pourraient légèrement augmenter le transfert de Cd et Zn du sol aux organes des cultivars étudiés. De même, la fertilisation azotée pourrait augmenter la mobilité des métaux dans le sol et leur transfert dans les organes aériens.

8.2.2. Perspectives

Sur les trois premières années de culture, nous avons montré que le miscanthus tolère bien la contamination en Cd, Pb et Zn des sols étudiés et fournit une biomasse comparable à celle obtenue sur un sol réputé non contaminé. Son comportement vis-à-vis des trois métaux étudiés est celui d'un excluder. Sur la période étudiée, nous avons constaté, dans des conditions *ex situ* et *in situ,* une légère diminution du pH et une augmentation de la teneur en carbone organique des sols. Compte tenu de la durée du cycle de culture (20 ans en moyenne), se pose la question de l'influence à long terme de la plante sur les paramètres physico-chimiques des sols et la mobilité des métaux. Un suivi temporel s'avère nécessaire pour compléter les résultats. Il en est de même avec la démarche engagée dans le cadre de PHYTENER en ce qui concerne la réponse de la plante au regard de la toxicité des métaux. L'étude de biomarqueurs de stress a mis en évidence l'existence d'un stress métallique chez

miscanthus sans toutefois affecter le rendement (Douay et al., 2014). Cependant, les résultats d'une étude utilisant une palette beaucoup plus importante de biomarqueurs et portant sur les échantillons de miscanthus de l'expérimentation *ex situ* (données non publiées) sont en désaccord avec ceux obtenus *in situ*. Ces résultats, possédant une robustesse statistique très supérieure (n = 7 vs n = 3), démontrent un léger stress oxydatif mais pas d'impact génotoxique sur les sols contaminés. De plus, aucune différence significative entre les sols contaminés n'a été observée, ce qui est cohérent avec les résultats portant sur la biomasse. Cependant, un suivi complet d'une palette de biomarqueurs pertinents sur des plantations matures permettrait de préciser d'une part, la réponse de la plante vis-à-vis d'un stress engendré par la contamination et d'autre part sa capacité à maintenir une productivité élevée sur le long terme dans ces conditions.

Les variations temporelles ou saisonnières observées dans l'accumulation de Cd, Pb et Zn et des éléments nutritifs dans les organes de miscanthus ont été expliquées par des phénomènes de dilution pendant la période de forte croissance (printemps et été) et de translocation à la fin de la croissance (automne). Plusieurs pistes peuvent être envisagées pour vérifier ceci. A titre d'exemple, l'effet de dilution pourrait être évalué en couplant la détermination de la biomasse avec les concentrations en métaux et en nutriments. De même, la translocation des nutriments pourrait être mise en évidence par l'analyse temporelle des teneurs en ces éléments dans les rhizomes et les organes aériens notamment, les feuilles. Enfin, l'étude des concentrations en ETM et en éléments nutritifs dans les sèves xylémiques et phloémiques pourrait apporter une réponse précise quant à la translocation de ces éléments.

Il a été suggéré que la durée d'exposition à Cd, Pb et Zn ainsi que la transpiration accélérée expliqueraient l'augmentation des concentrations en ces métaux dans les feuilles pendant la sénescence. Cependant, il est à noter que les feuilles ont été échantillonnées sans tenir compte de leur stade de développement. Un échantillonnage sélectif des feuilles établi selon leur stade de développement mettrait en évidence l'effet de la durée d'exposition et de la sénescence sur les teneurs en métaux de ces organes.

Les concentrations en métaux, surtout en Zn et Pb, sont plus élevées dans les feuilles que dans les tiges de miscanthus. Ceci suggère que la décomposition de la litière pourrait être une source d'enrichissement en métaux de l'horizon labouré, ce qui pourrait aussi affecter la mobilité et la biodisponibilité des polluants. Par ailleurs, se pose la question du devenir des métaux dans la litière mais aussi, dans le sol après la destruction de la culture, soit dans un délai d'une vingtaine d'année. Le retour à une rotation culturale traditionnelle pourrait affecter le cycle des éléments mais aussi celui des métaux. Une étude du vieillissement

artificiel de litières contaminées en milieu contrôlé complétée par un suivi des paramètres physico-chimiques et biologiques des sols après une destruction anticipée de cultures de miscanthus pourrait apporter des éléments de réponse à ces questionnements.

Les résultats de l'influence des pratiques agronomiques sur le rendement et l'accumulation des métaux ne permettent pas de conclure fermement sur le long terme. Ainsi, l'évaluation du rendement et de la capacité d'accumulation des métaux chez les cultivars étudiés devrait être faite sur des plantations suffisamment matures. Par ailleurs, l'évaluation du degré de mycorhization des racines devrait être effectuée pour déterminer le taux de succès de l'inoculum endomycorhizien utilisé dans les conditions étudiées. Pour évaluer l'efficacité de la phytostabilisation au moyen du miscanthus, les effets des pratiques agronomiques sur la mobilité des métaux et les paramètres physico-chimiques des sols devraient être suivis à différents intervalles de temps. Il pourrait être aussi intéressant d'évaluer les potentialités d'autres génotypes de miscanthus afin d'identifier des ressources disponibles et appropriées selon les conditions des sites.

Notre étude montre l'aptitude de miscanthus à gérer des sols agricoles qui présentent une bonne fertilité agronomique bien qu'ils soient massivement contaminés par Cd, Pb et Zn. Ceci est rarement le cas pour des sols dits marginaux qui présentent souvent de plus une contamination mixte associant des polluants inorganiques et organiques de nature et dans des proportions très variables. C'est notamment le cas des friches industrielles qui présentent un intérêt moindre pour l'agriculture traditionnelle mais de réels dangers environnementaux et sanitaires. Les potentialités de miscanthus pour le phytomanagement sur ces sols pourraient ainsi être évaluées.

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Valorisation scientifique

A. Articles publiés dans des revues à comité de lecture

A.1. **Nsanganwimana, F.,** Pourrut, B., Mench, M., Douay, F., 2014. Suitability of *Miscanthus* species for managing inorganic and organic contaminated lands and restoring ecosystem services. A review**.** Journal of Environmental Management 143, 123-134.

A.2. Nsanganwimana, F., Marchand, L., Douay, F., Mench, M., 2014. *Arundo donax* L., a candidate for phytomanaging water and soils contaminated by potentially toxic trace elements and producing plant-based feedstock*:* A review**.** International Journal of Phytoremediation 16, 982-1017.

A.3. Marchand, L., **Nsanganwimana, F.**, Lamy, J.B, Quintela-Sabaris, C., Gonnelli, C., Colzi, I., Fletcher, T., Oustrière, N., Kolbas, A., Kidd, P., Bordas, F., Newell, P., Alvarenga, P., Deletic, A., Mench, M., 2014. Root biomass production in populations of six rooted macrophytes in response to Cu exposure: Intra-specific variability versus constitutive-like tolerance. Environmental Pollution, 193, 205-215.

A.4. Marchand, L., **Nsanganwimana, F**., Vystavna, Y., Huneau, F., Lecoustumer, P., Lamy, J.B., Cook, B., Mench, M., 2014. Trace element transfer from soil to leaves of macrophytes along the Jalle d'Eysines River, France and their potential use as contamination biomonitors. Ecological Indicators, 46, 425-437.

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A.6. Pelfrêne, A., Kleckerová A., Pourrut B., **Nsanganwimana F.,** Douay F., Waterlot C., 2014. Effect of miscanthus cultivation on metal fractionation and bioaccessibility in smelter-impacted agricultural soils: comparison between greenhouse and field experiments**.** Environmental Science and Pollution Research. DOI: 10.1007/s11356-014-3585-1

A.7. Kubná, D., Elbl, J., Plošek, L., **Nsanganwimana, F.**, 2014. Effect of compost amendment on arbuscular mycorrhiza in relation to bioavailability of heavy metals in contaminated soils**.** *In*: Polák, O., Cerkal, R., Škarpa, P. (*Ed*), ***Proceedings of International PhD students Conference*-MendelNet 2014**, pp. 278-283, Mendel University, Brno (Czech Republic). ISBN: 978-80-7509-174-1.

A.8. Plošek, L., **Nsanganwimana, F**., Pourrut, B., Elbl, J., Hynšt, J., Kintl, A., Kubná, D., Záhora, J., 2013. The effect of compost addition on chemical and nitrogen characteristics, respiration activity and biomass production in prepared reclamation substrates. International Journal of Environmental Science and Engineering 7 (11), 364-369.

B. Articles soumis dans des revues à comité de lecture

B.1. Nsanganwimana, F, Pourrut, B., Waterlot, C., Douay, F., 2014. *Miscanthus* × *giganteus*: A trace element-excluder perennial grass suitable for phytostabilization of contaminated agricultural soils. Soumis à Journal of Environmental Management.

B.2. Nsanganwimana, F., Bidar, G., Waterlot, C., Pourrut, B., Douay, F., 2014. Seasonal variations in metal accumulation in energy crop *Miscanthus* × *giganteus* growing in contaminated agricultural soils. Soumis à GCB Bioenergy.

B.3. Nsanganwimana, F., Pourrut, B., Pelfrêne, A., Waterlot, C., Douay, F., 2014. *Ex-situ* growth of *Miscanthus* × *giganteus* reveals its potentials for phytostabilization of trace element-contaminated soils. Soumis à Environmental and Experimental Botany.

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C. Communications orales

C.1. Kubná, D., Elbl, J., Plošek, L., **Nsanganwimana, F.**, 2014. Effect of compost amendment on arbuscular mycorrhiza in relation to bioavailability of heavy metals in contaminated soils***. 21st Proceedings of International PhD students Conference***, 19-20 November 2014, Brno (Czech Republic).

C.2. Nsanganwimana, F., Pourrut, B., Waterlot, C., Bidar, G., Douay, F., 2014. Intérêt de *Miscanthus* × *giganteus* pour le phytomanagement de sols agricoles contaminés par des éléments traces métalliques dans le Nord de la France. ***3ème Rencontres nationales de la recherche sur les Sites et Sols pollués, ADEME,*** 18 - 19 novembre 2014, Paris (France).

C.3. Nsanganwimana, F., Pourrut, B., Waterlot, C., Bidar, G., Douay, F., 2014. Comportement de *Miscanthus* × *giganteus* cultivé sur les sols contaminés par les métaux. ***Gestion et requalification durable des Sites et Sols dégradés: Expériences en Nord-Pas de Calais***, 25 - 26 Septembre 2014, Lille (France).

C.4. Pourrut, B., **Nsanganwimana, F.**, Waterlot, C., Douay, F., 2014. *Miscanthus* × *giganteus:* a promising perennial grass for a sustainable phytomanagement of contaminated

sites in Northern France. ***The 3rd International Conference on Sustainable remediation,*** 17-19 September 2014, Ferrara (Italy).

C.5. Nsanganwimana, F., Pourrut, B., Waterlot, C., Douay, F., 2014. *Miscanthus* × *giganteus* : une graminée pérenne pour la gestion de sols agricoles contaminés par les éléments traces métalliques dans le nord de la France. ***Journée de l'Environnement,*** 27 mars 2014, Beauvais (France).

C.6. Plošek, L., **Nsanganwimana, F**., Pourrut, B., Elbl, J., Hynšt, J., Kintl, A., Kubná, D., Záhora, J., 2013. The effect of compost addition on chemical and nitrogen characteristics, respiration activity and biomass production in prepared reclamation substrates. ***34th International Conference on Agriculture, Bioengineering, Biological and Biosystems Engineering-ICABBBE***, 14-15 November 2013, Venice (Italy).

C.7. Nsanganwimana, F., Douay, F., Waterlot, C., Pourrut, B., 2013. *Miscanthus* × *giganteus*: a perennial grass suitable for coupling biomass production and management of metal-contaminated sites in Northern France. ***Sustainable Approaches to Remediation of Contaminated Land in Europe*** **(SARCLE-2013/** ***Contaminated site Management in Europe*** **(CSME-2013),** 21-24 October 2013, Amsterdam (The Netherlands)**.**

C.8. Marchand, L., Mench, M., **Nsanganwimana F.**, Oustrière, N., 2013. Intra-specific variability of Cu-tolerance in populations of six rooted macrophytes. ***12th International Conference on the Biogeochemistry of Trace Elements,*** 16-20 June 2013, Athens, GA, (USA).

C.9. Marchand, L., Mench, M., **Nsanganwimana, F**., Oustrière, N., Fletcher, T., 2012. Copper tolerance in macrophyte populations: Innate tolerance and/or phenotypic plasticity? ***Panamerican Conference on Wetland Systems for water quality improvement, management and treatment,*** 26 February - 1 March 2012, Technological University of Pereira, Pereira (Colombia).

D. Communications par affiches (Posters)

D.1. Waterlot, C., Pelfrêne, A., **Nsanganwimana, F.**, Pourrut, B., Douay, F., 2014. Evaluation des effets de *Miscanthus* × *giganteus* sur la distribution de Cd, Pb et Zn dans les sols contaminés d'une parcelle agricole. ***3ème Rencontres nationales de la recherche sur les Sites et Sols pollués, ADEME,*** 18 - 19 novembre 2014, Paris (France).

D.2. Galende, M.A., Kolbas, A., Marchand, L., Oustrière, N., **Nsanganwimana, F**., Douay, F., Mench, M., 2014. *Miscanthus* × *giganteus* as a candidate for phytomanaging Cu-

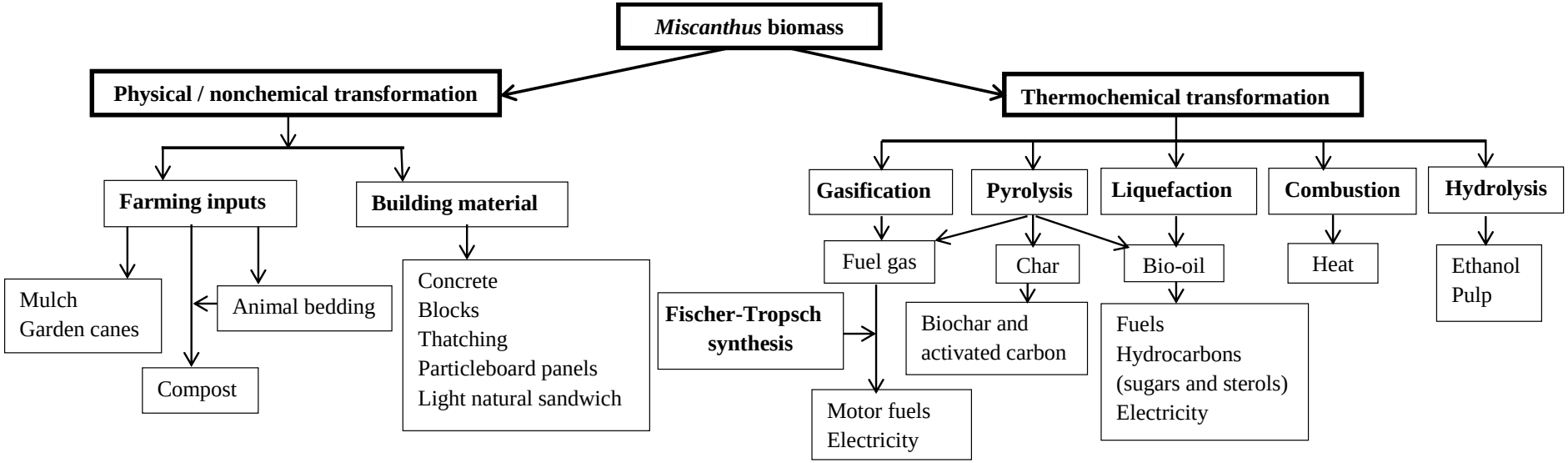
contaminated soils. ***International Congress of Phytoremediation of Polluted Soils (PPS)***, 29-30 July 2014, Vigo (Spain)**.**

D.3. Nsanganwimana, F., Pourrut, B., Douay, F., 2013. Influence du degré de contamination du sol en Cd, Pb et Zn sur l'accumulation de ces éléments dans les organes de *Miscanthus* × *giganteus* après 4 ans de plantation. ***Journée de recherche à l'Université Catholique de Lille,*** 31 mai 2013, Lille (France).

D.4. Marchand, L., Mench, M., **Nsanganwimana, F**., 2012. Copper tolerance in macrophyte populations: Innate tolerance and/or phenotypic plasticity? **9th** ***International Conference on Phytotechnologies, "Plant-based strategies to clean water, soil, air and provide ecosystem services"***, 11-14 September 2012. University of Hasselt, Diepenbeek (Belgium).

D.5. Nsanganwimana, F., 2012. *Miscanthus* × *giganteus*, a perennial grass suitable for phytostabilization of trace elements and production of biomass on contaminated sites in Northern France. ***Journée de recherche à l'Université Catholique de Lille,*** 30 mai 2012, Lille (France).

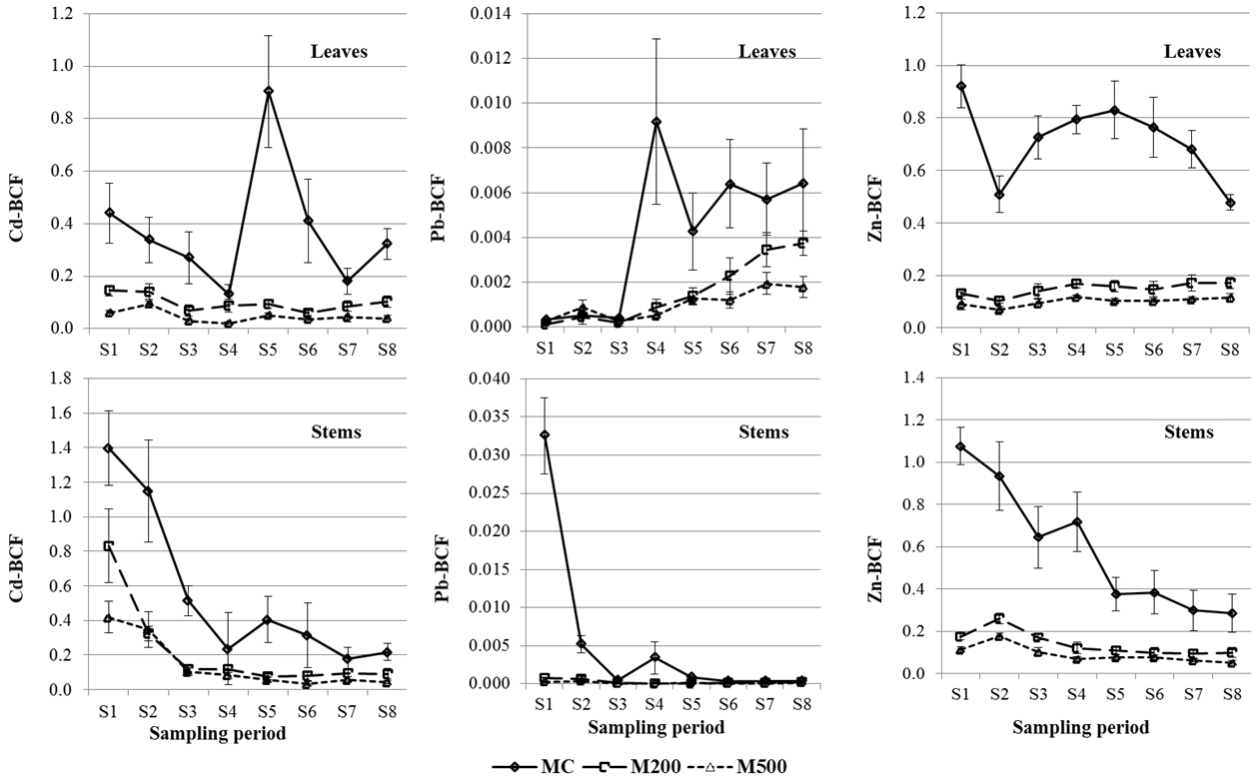
Annexes



Annex 1. *Miscanthus* biomass transformation pathways and products

Annex 2. Physico-chemical parameters and metal concentrations in topsoils (0-25 cm) during the study period. Values represent means ± standard deviations. The different letters represent significant differences between plots and sampling periods (Tukey HSD test, $p \leq 0.05$, $n = 5$).

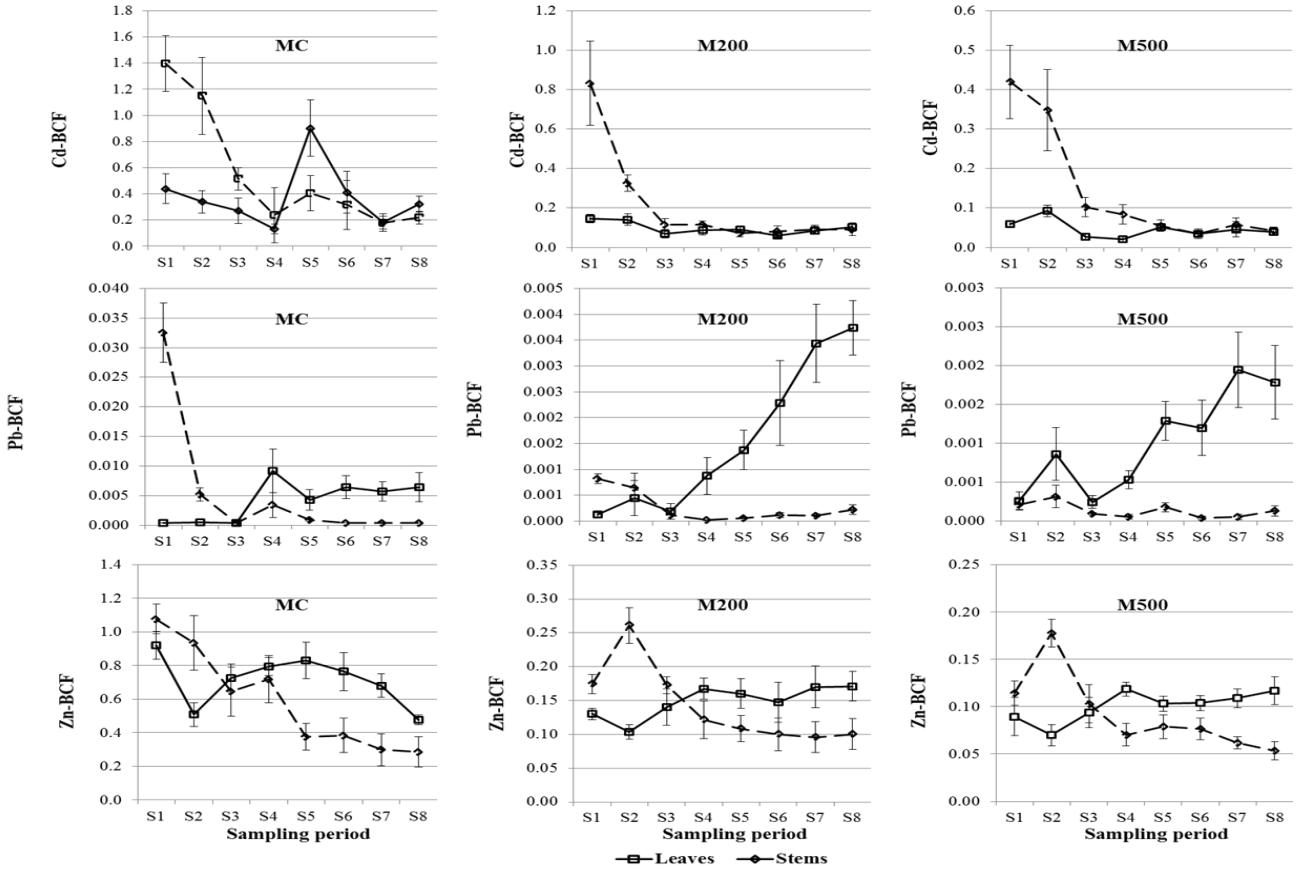
Plots	**MC**			**M200**			**M500**		
Sampling period	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
pH	6.7 ± 0.4^{b}	6.5 ± 0.3^{b}	$6.5 \pm 0.5)^{b}$	7.3 ± 0.2^{a}	7.2 ± 0.2^{a}	7.4 ± 0.2^{a}	7.5 ± 0.1^{a}	7.5 ± 0.1^{a}	7.6 ± 0.1^{a}
$CaCO_3$ (g kg^{-1})	0.4 ± 0.1^{c}	0.4 ± 0.1^{c}	0.6 ± 0.3^{c}	0.8 ± 0.4^{bc}	1.0 ± 0.8^{bc}	2.7 ± 1.8^{b}	21.4 ± 5.9^{a}	20.7 ± 5.1^{a}	19.7 ± 5.0^{a}
P_2O_5 (g kg^{-1})	0.1 ± 0.0^{b}	0.1 ± 0.1^{b}	0.1 ± 0.1^{b}	0.4 ± 0.3^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}	0.5 ± 0.4^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}
Fe (g kg^{-1})	7.4 ± 1.1^{a}	8.5 ± 1.0^{a}	8.5 ± 1.0^{a}	3.5 ± 0.2^{c}	4.0 ± 0.3^{b}	4.9 ± 0.5^{b}	8.6 ± 1.0^{a}	8.4 ± 1.3^{a}	9.7 ± 1.1^{a}
Mn (g kg^{-1})	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	0.5 ± 0.1^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.2 ± 0.0^{c}	0.2 ± 0.0^{c}	0.2 ± 0.0^{c}
OC (g kg^{-1})	18.3 ± 2.3^{b}	19.1 ± 1.3^{b}	18.0 ± 2.1^{b}	18.9 ± 1.6^{b}	19.6 ± 1.9^{b}	18.6 ± 0.9^{b}	33.7 ± 3.8^{a}	36.1 ± 5.4^{a}	34.9 ± 3.3^{a}
Total N (g kg^{-1})	1.9 ± 0.2^{b}	1.9 ± 0.2^{b}	1.8 ± 0.2^{b}	1.4 ± 0.1^{c}	1.5 ± 0.1^{c}	1.4 ± 0.1^{c}	2.7 ± 0.3^{a}	2.8 ± 0.3^{a}	2.8 ± 0.3^{a}
C/N	9.9 ± 0.3^{b}	10.1 ± 0.2^{b}	9.9 ± 0.4^{b}	13.5 ± 0.6^{a}	13.2 ± 1.2^{a}	13.2 ± 0.4^{a}	12.4 ± 0.5^{a}	12.8 ± 0.4^{a}	12.7 ± 0.2^{a}
CEC ($cmol^{+}$ kg^{-1})	12.0 ± 0.9^{c}	11.7 ± 0.9^{c}	11.5 ± 0.8^{c}	12.0 ± 0.9^{c}	12.6 ± 0.7^{c}	12.5 ± 0.7^{c}	22.6 ± 2.2^{b}	22.6 ± 2.2^{b}	30.4 ± 1.9^{a}
Cd (mg kg^{-1})	0.7 ± 0.1^{d}	0.5 ± 0.0^{e}	0.5 ± 0.0^{e}	4.9 ± 0.2^{c}	5.8 ± 0.4^{b}	5.5 ± 0.3^{b}	11.9 ± 0.7^{a}	12.3 ± 1.0^{a}	11.4 ± 0.7^{a}
Pb (mg kg^{-1})	21.0 ± 6.8^{c}	20.1 ± 6.1^{c}	30.0 ± 13.4^{c}	220.7 ± 10.0^{b}	240.4 ± 13.5^{b}	220.0 ± 17.9^{b}	511.9 ± 26.1^{a}	546.2 ± 39.1^{a}	514.0 ± 38.1^{a}
Zn (mg kg^{-1})	47.8 ± 5.0^{c}	49.9 ± 3.6^{c}	44.7 ± 3.6^{c}	358.2 ± 4.7^{b}	371.3 ± 25.2^{b}	347.7 ± 16.5^{b}	571.1 ± 33.6^{a}	588.1 ± 57.8^{a}	560.9 ± 51.6^{a}



Annex 3. Comparison of bioconcentration factors (BCFs) of metals (Cd, Pb and Zn) for leaves and stems of miscanthus growing in uncontaminated (MC) and contaminated (M200 and M500) agricultural plots. Analyzed samples were collected from S1 (May) to S8 (December) in 2012. The BCFs are presented as means ± SD of five samples for each sampling period.

Note: The bioconcentration factors (BCFs) were calculated using the following formula:

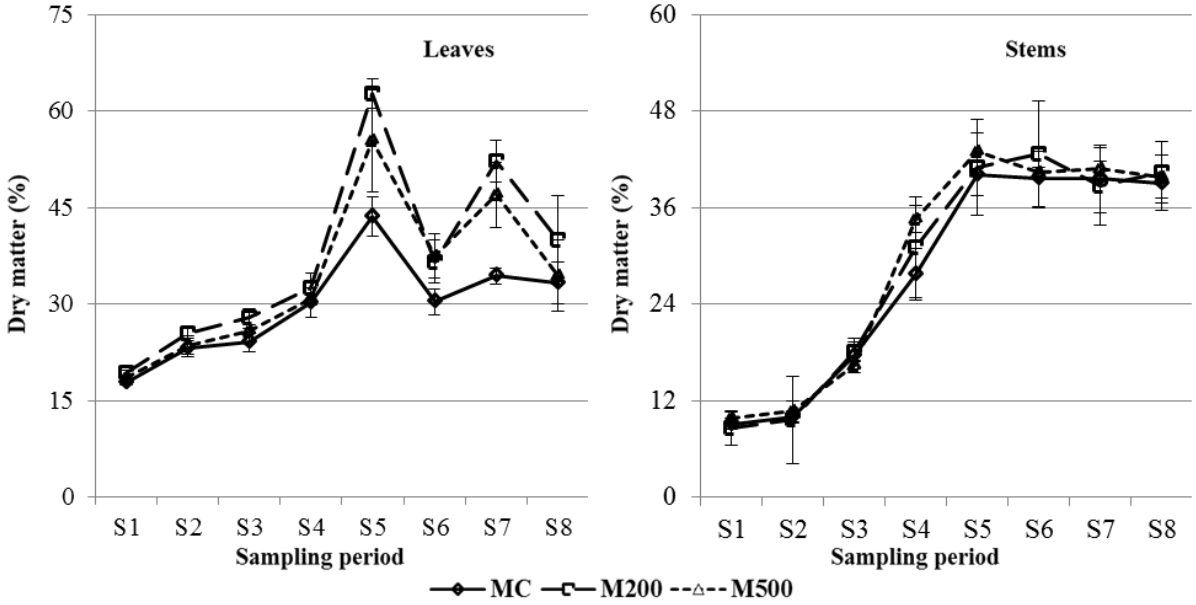
BCF= Metal concentrations in leaves or stems/ pseudototal metal concentrations in soils



Annex 4. Within-plot comparison of the bioconcentration factors (BCFs) of metals (Cd, Pb and Zn) for leaves and stems of miscanthus growing in uncontaminated (MC) and contaminated (M200 and M500) agricultural plots. Analyzed samples were collected from S1 (May) to S8 (December) in 2012. The BCFs are presented as means ± SD of five samples for each sampling period.

Note: The bioconcentration factors (BCFs) were calculated using the following formula:

BCF= Metal concentrations in leaves or stems/ pseudototal metal concentrations in soils



Annex 5. Biomass (expressed as dry matter %) in leaves and stems of miscanthus growing in uncontaminated (MC) and metal-contaminated (M200 and M500) agricultural plots. Analyzed samples were collected from S1 (May) to S8 (December) in 2012. Metal concentrations are presented as means ± SD of five samples for each sampling period.

Note: The dry matter content was determined by oven-drying of fresh samples at 105°C until constant weight according to ISO 11465.