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Diversité végétale en phytoremédiation

La complémentarité fonctionnelle pour gérer efficacement la contamination multiple des sols

par

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Résumé

La phytoremédiation est une technologie de dépollution des sols basée sur les habiletés naturelles des végétaux à épurer leur substrat des contaminants qui s'y trouvent. Cette approche comporte plusieurs avantages en comparaison aux méthodes conventionnelles utilisées en gestion des terrains contaminés. Contrairement à ces dernières, elle n'implique que rarement des travaux d'excavation et de transport de sol, ce qui contribue grandement à son faible impact environnemental et son coût abordable. Ces avantages économiques et environnementaux s'ajoutent au caractère esthétique et écologique des systèmes végétaux employés en phytoremédiation.

Le type de contamination du sol déterminera l'approche de phytoremédiation à adopter. Toutefois, les composés chimiques exogènes présents dans le sol d'un site dégradé sont souvent de nature variée, en plus d'être distribués de manière hétérogène. La phytoremédiation doit pouvoir tenir compte de cette situation complexe. La littérature scientifique fait état de nombreuses espèces végétales présentant des habiletés de remédiation des sols. Les principales habiletés sont la capacité d'absorber et de métaboliser les contaminants, ou encore celle de dégrader des composés organiques directement dans le sol, grâce à la production d'exsudats racinaires et avec l'aide de la communauté microbienne de la rhizosphère.. Les espèces végétales présentant certains de ces traits sont nombreuses, mais rares sont celles qui possèdent des habilités de remédiation pour plusieurs composés chimiques. Dans un contexte de contamination multiple, il apparait alors judicieux d'assembler différentes espèces de manière à obtenir un ensemble complémentaire d'habiletés de remédiation qui sera adapté au site visé. En plus de cet argument technique, une telle approche promouvant la diversité s'inscrit dans la lignée des nombreuses études mettant en évidence les bienfaits de la biodiversité pour la productivité et le fonctionnement des agro-écosystèmes.

De manière à explorer la pertinence d'une approche de diversification végétale pour la phytoremédiation, trois études ont été menées. En premier lieu, une investigation fut conduite sur le site d'un ancien bassin de décantation. Celle-ci visait à mettre en lumière l'influence que pouvait avoir l'hétérogénéité de la contamination sur la répartition en espèces d'une communauté végétale. Une analyse multivariée a pu démontrer qu'une grande partie de la distribution spatiale de la végétation sur le site pouvait être expliquée par celle des contaminants. Ce résultat suggère l'existence de niches écologiques de résistance aux contaminants et appuie la pertinence d'utiliser différentes espèces végétales en phytoremédiation en situation de contamination multiple.

Une deuxième étude, réalisée en pots, avait pour objectif d'explorer le potentiel de quatre espèces commercialement disponibles, c.-à-d. le saule arbustif (*Salix miyabeana* `SX67`), la moutarde indienne (*Brassica juncea*), la luzerne (*Medicago sativa*) et la fétuque érigée (*Festuca arundinacea*), pour la phytoremédiation d'un sol contaminé par trois éléments traces, soit l'argent (Ag), le cuivre (Cu) et le zinc (Zn). Chacune des espèces a su démontrer des aptitudes concrètes de phytoextraction, mais pour seulement un des éléments traces. Les résultats issus de cette deuxième étude suggèrent à nouveau qu'un système de phytoremédiation à plusieurs espèces serait approprié pour la phytoremédiation des sols à contamination multiples.

Enfin, une troisième étude en mésocosmes a porté sur la richesse spécifique en phytoremédiation, en comparant des systèmes végétaux à une, deux et trois espèces, pour la remédiation d'un sol contaminé par huit éléments traces. En monoculture, *S. miyabeana* 'SX67', *M. sativa* et *F. arundinacea* ont présenté des patrons d'allocation de biomasse complémentaires, ainsi que des habiletés de remédiation distinctes. Bien que pour un élément trace donné, assembler les espèces ne semblait pas représenter une solution plus performante, en considérant la remédiation d'un ensemble d'éléments, une approche de combinaisons d'espèces semblait répondre plus efficacement à une problématique de contamination complexe. Des interactions facilitatrices d'accessibilité en azote ont aussi pu être observées dans certains assemblages, ajoutant à l'intérêt d'adopter une approche multispécifique.

Cette thèse de doctorat a été en mesure d'amasser une quantité substantielle d'évidences scientifiques originales suggérant que les assemblages d'espèces sont appropriés pour la phytoremédiation des sols à contaminations multiple.
Mots-clés : Phytoremédiation, contamination multiple, sol, écologie, complémentarité, saule, luzerne, fétuque

Abstract

Phytoremediation is a plant-based technology using plant's natural abilities to remove contaminants from their substrate. This technique comes with numerous advantages compared to conventional contaminated soil management. Since it is usually implemented *in situ*, it rarely involves soil excavation and transport, which contributes to its negligible environmental impact and affordable cost. These economic and environmental advantages add to the valuable esthetic and ecological properties of the plant systems involved in phytoremediation.

By its nature, soil contamination can represent a challenge for phytoremediation. Chemical compounds inside the soil of degraded areas are often miscellaneous as well as heterogeneously distributed. This level of complexity in soil contamination must be taken into account when designing phytoremediation initiatives. The scientific literature reports several plant species presenting characteristics of interest for phytoremediation such as inorganic element absorption or organic compounds degradation by the internal metabolism or the rhizospheric community that is supported by the plants. However, although these plant species are numerous, only a small number are known to possess remediation abilities towards multiple compounds. In a context of complex soil contamination, it therefore appears wise to assemble plant species in order to obtain a set of remediation abilities that will be adapted for the target site. In addition to this technical argument, such an approach promoting diversity goes along with studies highlighting benefits of biodiversity for productivity and general functioning of agro-ecosystems.

In order to explore the relevance of adopting of a diversified approach in phytoremediation, three studies were conducted. A first investigation was led on the area of a former decantation basin. The objective was to investigate the influence that the spatial distribution of the contaminants could have on the species repartition of a ruderal plant community. A multivariate analysis was used to demonstrate that most of the species spatial pattern could be explained by the contaminant distribution on this site. This evidence suggests the existence of ecological niches of resistance to pollutants and therefore supports the use of different plant species for phytoremediation of complex contaminated soil.

A second study conducted as a pot trial aimed at exploring the potential of four commercially available species i.e. willow (*Salix miyabeana* `SX67`), alfalfa (*Medicago sativa*), indian mustard (*Brassica juncea*) and tall fescue (*Festuca arundinacea*), for phytoremediation of a soil contaminated by three trace elements, namely silver (Ag), copper (Cu, and zinc (Zn). Each of the species demonstrated phytoextraction abilities but only for one of the trace elements. The results gathered from this experiment point towards the existence of species-specific remediation abilities and suggest that plant combination could be appropriate for remediation of soil contaminated by multiple elements.

A third experiment in mesocosm directly investigated the effect of plant diversity in phytoremediation by comparing one, two and three species systems for remediation of a soil contaminated by eight trace elements. In monoculture, *S. miyabeana*, *M. sativa* and *F. arundinacea* demonstrated complementary biomass allocation patterns as well as distinct remediation abilities. While for the remediation of a specific trace element assembling species did not seem to enhance phytoremediation efficiency, when considering a set of trace elements simultaneously, an approach combining species seems to address most efficiently a complex soil contamination issue. Facilitative interactions towards nitrogen accessibility were also identified in certain assemblages, adding to the interest of combining species in phytoremediation.

This thesis gathered important scientific evidences suggesting that assemblages of species should be employed for phytoremediation of soil contaminated by multiple compounds.

Keywords : Phytoremediation, multiple contamination, soil, ecology, complementarity, trace elements, willow, alfalfa, tall fescue

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Liste des abréviations

BPC :	Biphényles Polychlorés
D&F :	Dioxines et Furanes
ET :	Élément trace
GTC :	Système de Gestion des Terrains Contaminés, du ministère québécois du Développement durable, de l'Environnement et de la Lutte contre les Changements Climatiques (MDDELCC), anciennement du Développement durable, de l'Environnement, de la Faune et des Parcs (MDDEFP).
HAM :	Hydrocarbure Aromatiques Monocycliques
HAP :	Hydrocarbure Aromatiques Polycycliques
HPT :	Hydrocarbure Pétroliers Totaux
MTEA	Accumulation de Multiples Éléments Traces
MTEE :	Extraction de Multiples Éléments Traces
RDA :	Analyse de redondance canonique
TCE :	Trichloréthylène
TNT :	Trinitrotoluène

Je dédie cette thèse à mon père Viateur, ainsi qu'à mon grand-père Anatole, qui ont su faire naitre en moi ma plus grande passion, celle de la nature.

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Chapitre 1 | INTRODUCTION GÉNÉRALE

Mon projet de doctorat traite de la phytoremédiation des sols contaminés par plusieurs composés, un contexte qui apparait propice à l'utilisation de différentes espèces végétales. Dans ce chapitre d'introduction, je décrirai la problématique propre à la contamination multiple, ainsi que les arguments scientifiques suggérant que la diversité végétale soit une approche adaptée à ce contexte particulier. Sur la base de cette information, trois objectifs de recherche ont été établis. Les chapitres deux, trois et quatre présentent le travail qui a été entrepris pour atteindre ces trois objectifs.

1.1. La contamination des sols

La pollution[1] des sols est un type de dégradation causé par la présence dans le sol de composés chimiques en concentrations suffisantes pour causer des effets écotoxicologiques sur les environnements terrestres et aquatiques (Fent, 2004). La plupart du temps, les terrains pollués sont la conséquence de l'abandon d'installations industrielles, militaires ou minières, du déversement accidentel de polluants, parfois suite au transport sur de longues distances, ou de la disposition inadéquate de déchets industriels, miniers ou municipaux (EEA-UNEP, 2000). Le phénomène est d'une ampleur préoccupante; la pollution du sol se rencontre dans toutes les régions du globe et les frais associés à la gestion de sites contaminés sont très élevés, parfois

[1] La pollution et la contamination sont deux concepts similaires qui sont parfois utilisés de manière interchangeable. Toutefois, des subtilités les distinguent. De manière générale, la contamination peut être considérée comme la présence d'une substance naturellement absente du milieu ou en concentration supérieure aux teneurs de fond naturelles, alors que la pollution est un type de contamination qui cause ou peut causer des effets biologiques négatifs (Chapman, 2007). Les différents acteurs du domaine de la remédiation des sols dégradés ne discriminent pas toujours les deux termes. Il en a été fait de même dans ce texte.

au-delà de la capacité des instances locales (Grimski and Ferber, 2001). Dans l'Union européenne (UE), le nombre de sites contaminés est estimé à 2.5 millions (Panagos et al., 2013) et on évalue à 6.5 milliards d'Euros les sommes dépensées annuellement pour la gestion de ces sites. Ces fonds proviennent en bonne partie du secteur privé, mais un coût élevé reste tout de même à payer par les contribuables (EEA, 2014). Du côté des États-Unis, le Département de l'Énergie gère un inventaire incluant 6.5 trillion des litres d'eau souterraine, ainsi que 40 millions de mètres cubes de sol, contaminé aux radionucléides, métaux et polluants organiques (U.S. Departement of Energy, 2017). Le bureau de gestion environnemental de ce même département détenait en 2016 un budget de plus de 6 milliards de dollars pour mener à bien ses activités (U.S. Department of Energy, 2017).

Au Canada, la remédiation des sols constitue un marché de 30$ milliards (Gouvernement du Canada, 2011). Depuis le début des années 2000, ce secteur prend chaque année de l'ampleur et le nombre de sites contaminés rapportés a presque doublé, atteignant à l'heure actuelle plus de 22 000 (Environnement et changement climatique Canada, 2015). Au cours de l'exercice 2014-2015, 263$ millions de dollars ont été investis dans le cadre du *Plan d'Action pour les Sites Contaminés Fédéraux* (PASCF) pour des activités d'assainissement et de gestion des risques (Environnement et changement climatique Canada, 2015). Le Ministère québécois du Développement durable, de l'Environnement, de la Faune et des Parcs (MDDEFP) avait quant à lui enregistré, au 31 décembre 2010 (bilan le plus récent disponible en date du 30 juin 2017), 8334 inscriptions au système de gestion des terrains contaminés (GTC). Ce chiffre est loin de représenter un inventaire exhaustif des terrains contaminés au Québec; il exprime seulement une compilation du nombre de cas portés à l'attention du ministère qui dépassaient le critère B de contamination établi par ce même ministère. Le critère B exprime la limite acceptable pour des terrains à vocation résidentielle, récréative et institutionnelle (Beaulieu, 2016). Les seuils à partir desquels un sol est considéré comme contaminé dépendent du composé chimique considéré, des niveaux naturellement présents dans le sol, mais aussi d'autres considérations tels les impacts potentiels sur la santé et l'environnement, qui sont spécifiques à chaque région (Jennings and Petersen, 2006). Au Québec, les sites contaminés sont répartis dans les 17 régions administratives, la région de Montréal et la Montérégie rassemblant la majorité des cas. Le secteur privé est propriétaire de 78% de ces terrains (Hébert and Bernard, 2013).

Les sommes investies pour réhabiliter les terrains contaminés sont justifiées par le fait que ces terrains peuvent représenter des risques importants pour la santé humaine et celle des écosystèmes (Li et al., 2014). On reconnait maintenant une multitude d'impacts négatifs des sites pollués sur nos sociétés et notre environnement.

1.2. Impacts de la contamination

L'agence de protection environnementale des États-Unis (USEPA) reconnait à la présence de friches industrielles un large spectre de conséquences négatives. L'organisme regroupe ces effets en trois catégories. D'abord, un premier groupe d'effets est relié à la sécurité, puisque des sites pollués laissés à l'abandon se trouvent parfois à proximité de zones habitées. Ensuite, il existe des impacts socio-économiques, telle la réduction de la valeur des terrains avoisinants, qui se traduit par une entrée inférieure de taxes foncières à l'échelle locale et ultimement, une potentielle réduction des services sociaux. Finalement, la troisième catégorie englobe la problématique environnementale, parmi laquelle on peut mentionner les divers effets négatifs sur la santé des écosystèmes naturels et humains causés par la dispersion des polluants dans l'environnement (USEPA, 2006). Mes travaux de doctorat sont motivés plus particulièrement par cette troisième catégorie d'effets négatifs, bien que les solutions proposées aient aussi un impact bénéfique sur les deux premières catégories.

Différents contaminants auront, en fonction de leurs propriétés, des effets différents sur la santé humaine et sur l'environnement. Dans les sites sévèrement contaminés, des effets aigus de la contamination peuvent survenir, mais l'essentiel du problème provient des effets chroniques qui surgissent sur le long terme (Fent, 2004). Les effets écotoxicologiques peuvent se manifester à tous les niveaux d'organisation biologiques, de la molécule à l'écosystème. À titre d'exemple, il est reconnu que les éléments métalliques peuvent interférer avec les processus métaboliques (Jaishankar et al., 2014). Ces effets dépendront de la dose absorbée, de la voie d'absorption, ainsi que de la durée d'exposition. Une contamination métallique des cellules provoquera généralement l'apparition de radicaux libres, ainsi que la production d'antioxydants en guise de défense, ce qui induira un stress oxydatif potentiellement dommageable (Patlolla et al., 2009). Certains polluants organiques sont quant à eux reconnus comme ayant des effets carcinogènes, mutagènes et tératogènes (Boström et al., 2002).

1.3. Types de contamination

De manière générale, on distingue les contaminants du sol en deux grandes catégories : les composés organiques et inorganiques. À titre indicatif, les pourcentages d'inscription au système québécois de Gestion des terrains contaminés (GTC) étaient en 2010 de 73%, 12% et 15% pour des contaminations de nature organique, inorganique et mixte (organique et inorganique), respectivement (Hébert and Bernard, 2013).

1.3.1. Composés organiques

Les contaminants organiques, parfois regroupés sous le terme xénobiotique, sont un vaste groupe de molécules qui ont en commun une structure de base faite de chaine de carbone. À tire d'exemple, les hydrocarbures (aliphatiques, aromatiques monocycliques (HAM) et polycycliques (HAP)), les biphényles polychlorés (BPC), les dioxines et furanes (D&F), le trichloréthylène (TCE) et le trinitrotoluène (TNT) sont parmi les plus fréquemment rencontrés (Figure 1.1).

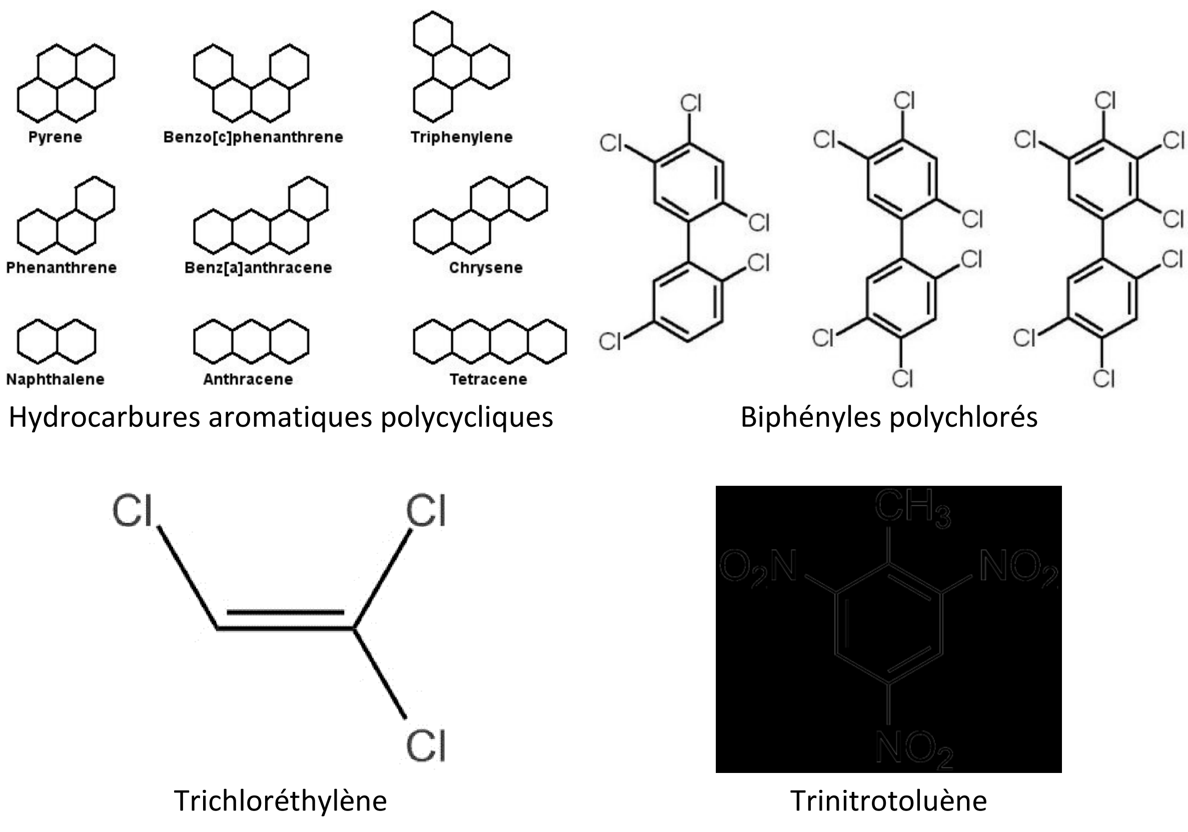

Figure 1.1 Composés organiques fréquemment rencontrés dans les sols contaminés.

La présence de contaminants de nature organique dans les sols est essentiellement d'origine anthropogénique. Les sources possibles sont multiples (déversements, activités militaires et industrielles, agriculture, traitement du bois, etc.). Ces polluants étant pour la plupart des composés de synthèses, leur diversité n'a d'égal que les fonctions pour lesquelles ils ont été créés. Par exemple, on parle souvent des congénères de BPC, puisque le terme BPC regroupe en fait plus de 200 composés.

1.3.2. Composés inorganiques

Les contaminants inorganiques dans l'environnement peuvent provenir de processus géologiques naturels, mais aussi d'activités humaines. Cette catégorie de contaminants inclue des macronutriments, tels les nitrates et phosphates (Horne et al., 2000), des éléments traces métalliques tels l'argent (Ag), le chrome (Cr), le cuivre (Cu), le fer (Fe), le manganèse (Mn) et le zinc (Zn) (McIntyre, 2003), des éléments non essentiels comme l'aluminium (Al), le césium (Cs), le cadmium (Cd), le cobalt (Co), le fluor (F), le mercure (Hg), le sélénium (Se) et le plomb (Pb) (Horne et al., 2000; Pilon-Smits, 2005), d'autres éléments qualifiés de métalloïdes tel le bore (B), le silicium (Si), l'antimoine (Sb) et l'arsenic (As) (Alloway, 2013), ainsi que des isotopes radioactifs tels le césium 137 (^{137}Cs), le strontium 90 (^{90}Sr) et l'uranium 235 at 238 ($^{235,238}U$) (Dushenkov, 2003). Parmi les sources anthropogéniques les plus communes, notons la disposition des effluents industriels, l'épandage des boues d'épuration, les opérations militaires, d'enfouissement domestique et de minage, ainsi que l'utilisation de produits chimiques pour l'agriculture et la production de carburant et d'énergie (Lasat, 2014; Seaward and Richardson, 1989). Peu importe la source de ces contaminants, des concentrations excessives de certains éléments métalliques posent des risques significatifs pour la santé de la faune, des humains et des écosystèmes (Blaylock and Huang, 2000).

1.4. Remédiation conventionnelle et phytoremédiation

Il est possible de traiter les sols contaminés par des procédés de types chimiques, physiques et biologiques (McEldowney et al., 1993). Les méthodes physico-chimiques impliquent la plupart du temps le transport des sols dans des lieux de confinement où sont effectués par exemple, un lavage ou une désorption thermique (Smith et al., 2001) pour séparer les fractions contaminées, ou une inactivation chimique des composés (Pilon-Smits, 2005). Ces volumes excavés doivent évidemment être remplacés par de nouveaux sols venus d'ailleurs. Cette façon de gérer les sols contaminés est extrêmement énergivore, puisqu'elle implique le transport de grandes quantités de matière minérale souvent dangereuse, en plus d'être associée à une utilisation importante de machinerie lourde d'excavation (Pilon-Smits, 2005). De plus, le danger n'est pas complètement écarté, puisqu'il est possible qu'un lixiviat contaminé provenant de ces sites d'entreposage s'échappe dans l'environnement. Ce dernier problème est tel que l'agence de protection environnementale des États-Unis a mis sur pied un programme spécialement dédié à la problématique du lessivage des installations de traitement (USEPA, 2013).

C'est dans ce contexte que se créa un besoin pour la recherche d'alternatives plus durables et respectueuses de l'environnement en gestion de sites contaminés. Dans cette lignée, quelques techniques firent leur apparition. Parmi celles-ci, les biopiles sont maintenant utilisées pour le traitement des polluants de nature organique. Cette technique consiste à stimuler la flore microbienne naturelle ou inoculée du sol en prodiguant aération et nutrition aux microorganismes qui peuvent dégrader les composés à base de carbone par des processus métaboliques naturels (Jørgensen et al., 2000). C'est une technique relativement accessible let efficace lorsque le temps nécessaire est disponible et que le type de contamination s'y prête, puisqu'elle ne permet que de s'attaquer qu'aux polluants de nature organique.

Dans la lignée des alternatives possibles à la gestion conventionnelle des sites contaminés, la phytoremédiation fit aussi son apparition. Le terme phytoremédiation fait référence à la remédiation biologique de contaminants environnementaux par l'utilisation de végétaux et des microorganismes qui leur sont associés (Salt et al., 1995). Cette méthode met à profit les processus naturels par lesquels les plantes et leurs microorganismes rhizosphériques dégradent ou séquestrent des polluants de nature organique ou inorganique (Pilon-Smits, 2005). La technique est applicable à des substrats solides, liquides et gazeux. La phytoremédiation a acquis une popularité grandissante auprès des agences gouvernementales et industries depuis les années 90. Cette popularité est expliquée en partie par le coût relativement faible de la méthode, combiné aux budgets limités pour le nettoyage environnemental qui lui, nécessite des sommes impressionnantes. On estime à environ un dixième le coût de la phytoremédiation comparé aux techniques conventionnelles (Glass, 1999). Le fait que la phytoremédiation est habituellement appliquée *in situ* contribue à son bon rapport coût/efficacité et aussi à la diminution des risques potentiels d'exposition des substrats contaminés aux humains, à la faune et à l'environnement (Pilon-Smits, 2005). Elle gagne aussi en popularité auprès du grand public, qui requiert maintenant des alternatives plus vertes et respectueuses de l'environnement pour aborder les problématiques environnementales (Hou and Al-Tabbaa, 2014).

Il existe plusieurs modes d'action possibles pour un système de phytoremédiation. Les principaux sont décrits dans le Table 1.1.

Table 1.1 Modes d'action possibles pour un système de phytoremédiation.

Mode d'action	Description
Phytoextraction	Prélèvement des composés du sol et concentration de ceux-ci dans les tissus récoltables de la plante
Phytostabilisation	Diminution de la mobilité des contaminants par l'absorption ou l'adsorption aux tissus souterrains de la plante
Phytodégradation	Importation et dégradation des contaminants par le métabolisme interne de la plante ou par exsudats racinaires
Rhizodégradation / Phytostimulation	Biodégradation des polluants organiques par les microorganismes rhizosphériques stimulés par les exsudats racinaires
Phytovolatilisation	Volatilisation des contaminants depuis le sol vers l'atmosphère par le processus d'évapotranspiration
Rhizofiltration	Absorption ou adsorption de contaminants présents dans l'eau

Ces modes d'action peuvent aussi être représentés graphiquement en fonction du devenir que subit le contaminant lorsqu'il est intégré par le système de phytoremédiation (Figure 1.2).

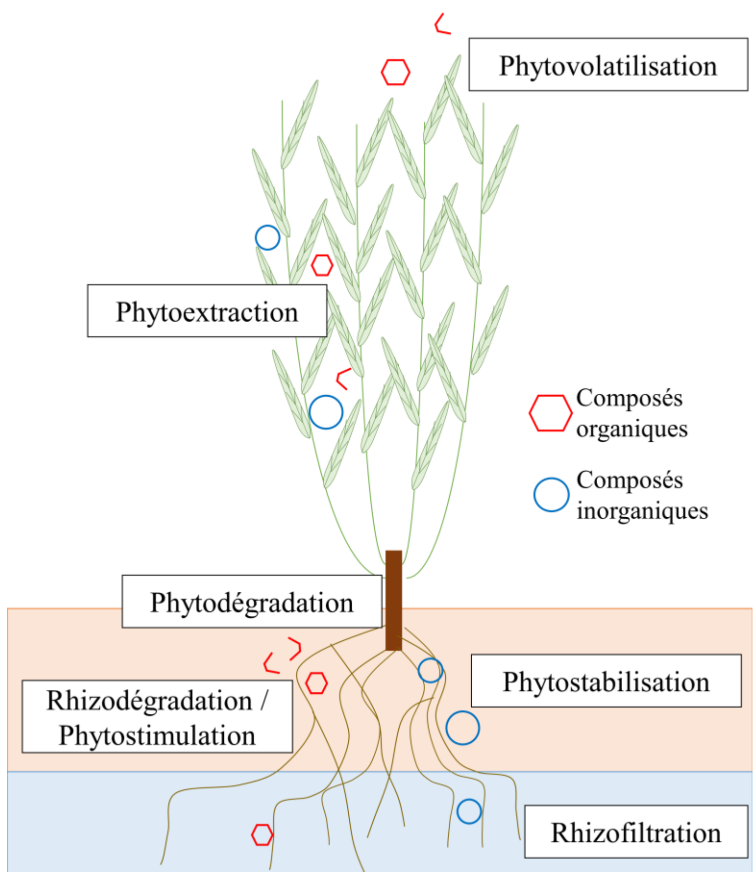

Figure 1.2 Schéma représentant différents mécanismes de phytoremédiation. Par D. Desjardins (2017). Reprise de Townie (Arulnangai & Xavier Dengra).

Ceux-ci ont été décrits à maintes reprises, parfois avec quelques variantes (Blaylock and Huang, 2000; Chaney et al., 2000; Chaudhry et al., 1998; McCutcheon and Schnoor, 2003; Salt et al., 1995).

1.4.1. Disponibilité des contaminants

La phytoremédiation est une technique qui comporte plusieurs avantages économiques et environnementaux, ainsi que certaines limites. Pour agir sur les polluants, les végétaux doivent être en mesure de s'établir directement là où ils se trouvent. La profondeur d'enracinement et celle de la contamination sont donc déterminantes; les végétaux ne peuvent traiter des substrats leur étant inaccessibles, sauf peut-être dans le cas où les eaux de ruissellement contaminées seraient récupérées pour l'irrigation. Les caractéristiques physiques du sol et le climat, qui influencent la profondeur d'enracinement, sont donc à considérer.

Une fois le système racinaire bien implanté dans la zone contaminée, la biodisponibilité des contaminants pour la plante devient décisive. Celle-ci est influencée par une combinaison de facteurs incluant les propriétés du sol (pH, capacité d'échange cationique (CEC), matière organique, texture), la forme chimique des contaminants (spéciation) et l'activité biologique du système plante-sol-contaminant (Pilon-Smits, 2005).

Le pH a un effet bien connu sur la disponibilité des éléments inorganique pour les végétaux. En situation acide, les ions H^+ se font plus abondants et compétitionnent avec les cations pour les sites de liaisons présents à la surface des particules d'argile ou de matière organique, ce qui laisse plus de cations en solution qui sont facilement disponibles (Allen et al., 2001). La CEC exprime quant à elle la quantité de cations adsorbés par unité de poids de sol (McBride, 1994).

Mesurer la CEC permet d'obtenir une indication de la quantité de nutriments échangeables et donc potentiellement disponibles pour la plante. Elle est donc influencée par le pH du sol, mais également par la présence d'humus et d'argile. L'humus est composé en grande partie d'acides humiques et fulviques, des macromolécules organiques qui offrent beaucoup de sites de liaisons potentiels pour les contaminants. Finalement, les particules d'argiles étant de faible diamètre, la surface spécifique des sols argileux est plus importante que dans un sol sableux (Pennell, 2002), ce qui offre aussi plus de sites de liaisons potentiels pour les ions, en particulier les cations (ex. Ag^{2+}, Cd^{2+}) (Taiz and Zeiger, 2002).

Les contaminants du sol peuvent se distribuer sous différentes formes chimiques dans les phases gazeuse, solide et liquide. Les végétaux retirant leurs nutriments principalement de la solution du sol, il est généralement convenu que les éléments traces dissouts sont les plus facilement biodisponibles pour les plantes (Barber, 1995). Les éléments métalliques dans la phase aqueuse du sol peuvent ensuite se distribuer sous plusieurs formes selon la nature de leurs liaisons (ions libres, complexes ioniques, ions complexés aux anions organiques, ions complexés aux macromolécules organiques, ions complexés aux colloïdes inorganiques) (Hinsinger and Courchesne, 2008) ayant des niveaux de biodisponibilité différents, la forme libre étant considérée comme la plus étroitement reliée à la bioaccumulation (Ge et al., 2000).

L'activité biologique de plusieurs organismes (plantes, bactéries, champignons) influence également la biodisponibilité des éléments traces. Par leur métabolisme, ces organismes catalysent des réactions d'oxydo-réduction et agissent sur l'acidité du sol (Sposito, 2008). La réduction des éléments peut en retour limiter leur mobilité ou entrainer leur précipitation (Violante et al., 2010).

La biodisponibilité peut être influencée dans une certaine mesure par des amendements, la concentration en matière organique dans le sol étant corrélée positivement avec la CEC, ainsi qu'avec la capacité de lier des polluants organiques hydrophobes. Toutefois, selon le type d'amendement et le contaminant considéré, des effets antagonistes peuvent survenir (Beesley et al., 2010). Quoi qu'il en soit, augmenter la biodisponibilité implique une augmentation de la mobilité des contaminants et donc aussi du risque de lessivage de ceux-ci vers d'autres compartiments environnementaux. Ce dernier processus doit donc être considéré avec vigilance.

En raison des limites précédemment mentionnées, le temps nécessaire à la phytoremédiation pour diminuer les concentrations du sol à des niveaux jugés acceptables peut être plus important que pour une intervention conventionnelle. Néanmoins, les deux approches ne sont pas mutuellement exclusives. Souvent, le type de pollution du sol pourra être très varié et la distribution de celle-ci sera hétérogène sur un site (Desjardins et al., 2014; French et al., 2006). Une combinaison de techniques conventionnelles et biologiques peut donc s'avérer la meilleure solution, compte tenu du rapport coût/efficacité (Pilon-Smits, 2005). Ceci illustre la complexité qui peut exister dans la contamination d'un site. Les interventions en phytoremédiation des sols doivent tenir compte de cette complexité. Ceci peut être fait en sélectionnant adéquatement les espèces végétales à employer.

1.5. Spécificité des végétaux et complémentarité

La répartition des végétaux dans les nombreux écosystèmes terrestres témoigne bien de leur extraordinaire capacité d'adaptation. Des espèces ont développé au cours de l'évolution des aptitudes à tolérer des stress environnementaux qui peuvent être aussi divers que sévères. Par exemple, selon le degré de disponibilité de l'eau dans leur environnement, les végétaux auront des adaptations morphologiques complètement différentes. Chez les espèces de milieu xérique, le faible nombre de stomates et leur position enfoncée dans le mésophylle a pour fonction de limiter la perte d'eau, alors que chez les espèces de milieu humide, les stomates sont au contraire abondants, de manière à maximiser la transpiration.

La recherche en phytoremédiation a permis de bien observer les différences de tolérance à la pollution entre les espèces et ainsi d'associer un taxon à un ou plusieurs contaminants. Les adaptations des plantes pour tolérer les polluants sont surtout d'ordre physiologique. Les transporteurs moléculaires dans la membrane de la vacuole (Clemens, 2001), ainsi que la production de molécules de type métallothionéines et phytochélatines jouent un rôle dans les mécanismes de détoxification mis en place par les végétaux pour tolérer la présence de contaminants dans leur organisme (Yang and Chu, 2011). Ces adaptations sont variables d'une espèce à l'autre, ce qui implique des niveaux de tolérance et des capacité de remédiations différentes entre les espèces. À titre d'exemple, la base de données PhytoRem du gouvernement du Canada (McIntyre, 2001) a recensé plusieurs espèces végétales dont le potentiel à remédier certains polluants en particulier a été observé. Parmi les exemples bien connus, notons la capacité des espèces arbustives du genre *Salix* a accumuler des concentrations parfois impressionnantes de cadmium et de zinc (Vandecasteele et al., 2002), alors que leur habileté à extraire le chrome et le nickel est plus limitée (Meers et al., 2007).

Tel que mentionné précédemment, la contamination des sites est rarement de nature simple et la présence de plusieurs contaminants différents complexifie les risques de toxicité et limite les options de remédiation. C'est dans ce contexte qu'il apparait pertinent d'explorer de quelle façon la phytoremédiation peut répondre efficacement aux cas de contamination complexes. Dans cette thèse, les expressions « contamination complexe » et « contamination multiple » seront toutes les deux utilisées pour décrire la présence de plusieurs (au moins trois) contaminants dans le sol. Ce contexte concerne les contaminants tant organiques qu'inorganiques, bien que la plupart des travaux présentés subséquemment concernent des éléments inorganiques (ou éléments traces). Dans ces conditions particulières, les végétaux se retrouvent exposés à plusieurs stress, ce qui perturbe leur métabolisme et ultimement leur croissance (Yadav, 2010). La productivité des végétaux est toutefois un élément clé de la réussite en phytoremédiation (Vamerali et al., 2010). Assurer un rendement élevé doit être une priorité pour que l'on puisse observer de manière rapide et importante l'activité biologique qui est à la source des processus de phytoremédiation.

De nombreux travaux scientifiques ont cherché à comprendre le rôle que la diversité pouvait jouer dans la productivité des écosystèmes. C'est un domaine de recherche de grande ampleur, en partie motivé par la prise de conscience d'une perte importante de biodiversité à l'échelle mondiale. Dans des revues de littérature sur ce sujet, Hooper et al. (2005), ainsi que Spehn et al. (2005), ont mentionné une tendance vers un effet positif de la diversité sur la productivité de certains assemblages d'espèces. Il semble de plus que cette tendance positive puisse être conservée au travers du temps (Van Ruijven and Berendse, 2009), des lieux et des scénarios de changements environnementaux (Isbell et al., 2011), en raison de l'existence d'une complémentarité entre les espèces (Cardinale et al., 2007). Il est maintenant aussi suggéré que les effets positifs de la biodiversité dans les écosystèmes soit encore plus important lorsque l'on considère plus d'une fonction écosystémiques à la fois (multifonctionnalité des systèmes) (Lefcheck et al., 2015) et non seulement la productivité. Ceci pourrait s'avérer particulièrement pertinent dans une situation de contamination multiple, où de nombreuses habiletés de remédiation sont nécessaires.

Parmi les mécanismes en cause dans la relation positive entre biodiversité et productivité, la complémentarité des niches fonctionnelles est déterminante (Loreau, 2000; Van Ruijven and Berendse, 2009). On entend, par complémentarité des niches fonctionnelles, que différentes espèces remplissent des fonctions différentes dans leur écosystème. Le résultat de cette répartition des tâches étant une utilisation plus optimale des ressources et de l'espace. Toutefois, un autre mécanisme, auquel ont attribut souvent le terme effet de sélection, peut aussi être en cause dans une relation diversité-productivité positive. On entend par effet de sélection la probabilité grandissante d'introduire dans l'assemblage une espèce très productive si l'on augmente le nombre d'espèces présentes (Loreau, 2000). Le gain effectué sur le plan de la productivité serait donc causé par la présence de cette espèce productive plutôt que par une synergie entre les espèces. Il est donc important de faire la distinction entre complémentarité fonctionnelle et effet de sélection dans les expériences sur la biodiversité. Néanmoins, les démonstrations empiriques de l'effet positif de la biodiversité sur la productivité abondent. Parmi les preuves qui ont contribué à convaincre la communauté scientifique des effets positifs de la biodiversité, notons l'article de Tilman et al. paru en 2001 présentant les résultats de plusieurs années d'expérimentation dans les prairies américaines (Figure 1.3).

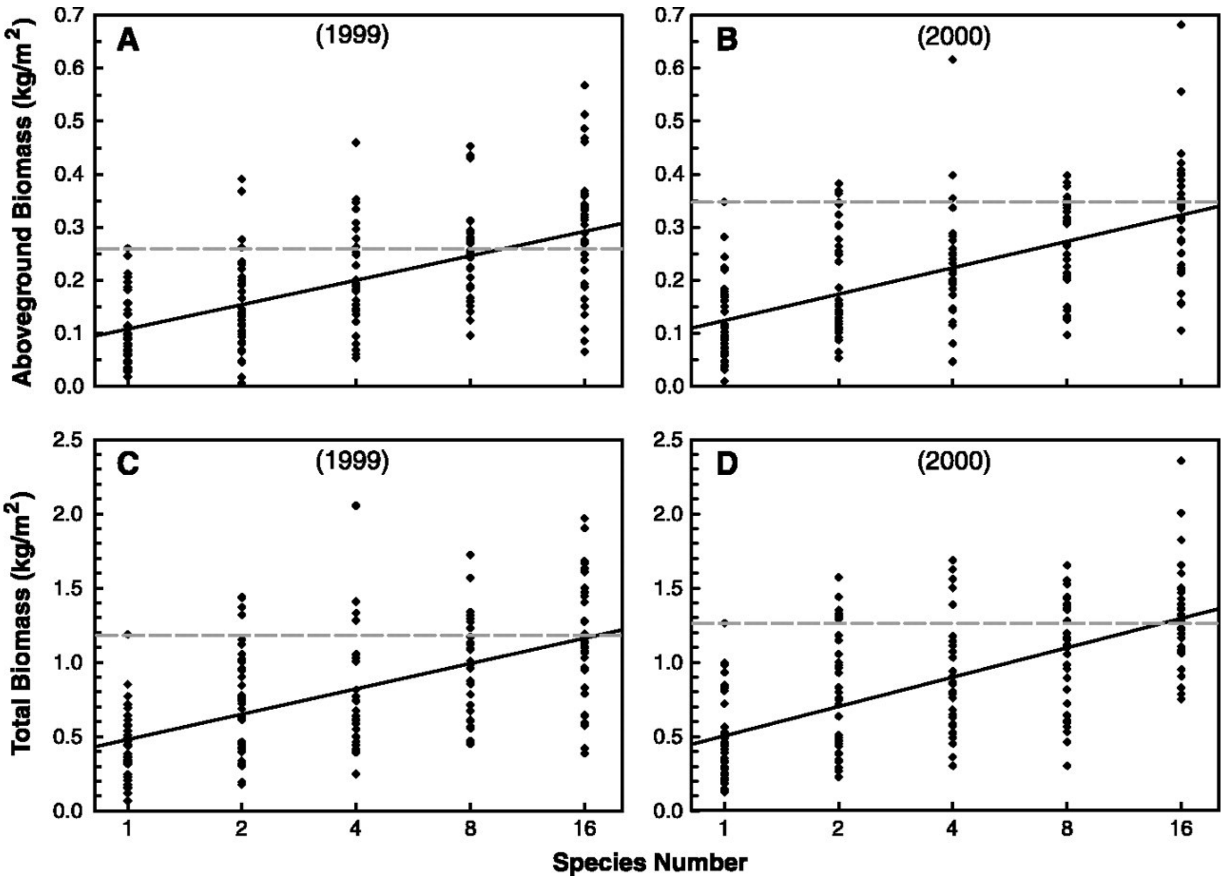

Figure 1.3 Influence de la biodiversité sur la productivité des prairies américaines. Tiré de Tilman et al. (2001). Effet du nombre d'espèces plantées sur la biomasse aérienne (**A** et **B**) et totale (**C** et **D**) en 1999 et 2000. La ligne brisée montre le rendement de la meilleure monoculture pour une année donnée. La ligne droite montre la régression de la biomasse sur le logarithme du nombre d'espèces. Le logarithme a été utilisé en raison de la relation légèrement meilleure qui en résultait.

1.6. Biodiversité et phytoremédiation

La théorie des niches fonctionnelles et le principe de complémentarité qui caractérisent les écosystèmes naturels apparaissent comme étant directement applicables à la phytoremédiation. Ce lien peut être fait si l'on considère les systèmes de phytoremédiation comme fournissant un service écologique de remédiation du sol, ce service résultant de l'expression par les végétaux d'un ensemble de fonctions de remédiation (par exemple, phytoextraction du cadmium et phytodégradation de HAPs). En reconnaissant que chaque espèce possède un ensemble limité d'habiletés de remédiation (Sarma, 2011), il apparait judicieux d'employer des assemblages d'espèces possédant des habiletés complémentaires de remédiation lorsque l'on fait face à des sols à contamination complexe (Batty and Dolan, 2011; Courchesne et al., 2017; Kidd et al., 2015).

Il devient ainsi évident que la diversité fonctionnelle, bien que parfois difficile à identifier et mesurer, devrait être considérée dans les processus de décision en restauration (Cadotte et al., 2011). Mettre en place des dispositifs de phytoremédiation fonctionnellement diversifiés pourrait permettre de maintenir un ensemble de fonctions nécessaires à la remédiation d'un sol contaminé par plusieurs composés et ce, au travers du temps et des perturbations environnementales. Cette notion de conservation des fonctions sur le long terme est appropriée pour la phytoremédiation, puisque celle-ci est susceptible de s'opérer sur de longues périodes. D'autres facteurs permettent aussi de croire que la phytoremédiation pourrait bénéficier de l'adoption d'une approche tenant compte de la biodiversité.

Premièrement, il peut exister un bénéfice direct à jumeler des espèces, suite à la mise en place d'un mécanisme de facilitation. C'est le cas en agriculture lorsque l'on mélange graminées et légumineuses pour améliorer la valeur nutritive du fourrage (Klabi et al., 2017). Par exemple, Moukoumi et al. (2012) ont observé dans les prairies canadiennes un bénéfice sur le rendement par hectare de *Salix miyabeana* lorsque cultivé en compagnie de *Caragana arborescens* (Fabaceae), mais seulement dans les sites où les sols étaient les plus pauvres. En situation de ressources abondantes, la présence de *C. arborescens* diminuait le rendement de *Salix* en raison de la compétition interspécifique pour les ressources. Dans les sites les plus pauvres, jusqu'à

60% de l'azote foliaire de *S. miyabeana* était d'origine atmosphérique, donc préalablement fixée par *C. arborescens*. On pourrait croire en l'établissement possible d'un processus semblable dans les systèmes de phytoremédiation, ceux-ci étant généralement implantés sur un substrat de très faible qualité. De plus, *S. miyabeana* est une espèce fréquemment utilisée dans les essais de phytoremédiation (Courchesne et al., 2017; Desjardins et al., 2016; Guidi et al., 2011). Dans une autre étude sur l'impact de la diversité phylogénétique, fonctionnelle et des traits sur la productivité des communautés, Cadotte et al. (2009) ont quant à eux observé que la présence d'un fixateur d'azote était un des meilleurs prédicteurs de la productivité des communautés dans une prairie américaine.

Nous savons aussi que les plantes promeuvent la présence de microorganismes dans le sol, principalement par le biais de la production d'exsudats racinaires (Megharaj et al., 2011) et que certains de ces microorganismes peuvent dégrader des polluants organiques (Chaudhry et al., 2005). Un assemblage diversifié de végétaux pouvant supporter une communauté plus diversifiée de microorganismes rhizosphériques (Eisenhauer et al., 2010), il est possible de supposer que l'utilisation de plusieurs espèces de plantes en phytoremédiation pourrait permettre de supporter une communauté plus diversifiée de microorganismes, travaillant de façon complémentaire, pour s'attaquer à une gamme plus grande de polluants organiques ou du moins, une communauté ayant plus de probabilités de contenir au moins une souche pouvant dégrader efficacement les polluants organiques. Dans une étude comparant différentes compositions végétales sur la dégradation des hydrocarbures pétroliers totaux (HPT), Phillips et al. (2009) n'ont pas observé d'avantages à utiliser plusieurs espèces simultanément, mais suggèrent toutefois que des facteurs atténuants, telle l'augmentation de la désorption d'hydrocarbures préalablement non disponibles, pourraient causer une sous-estimation de la dégradation réelle.

D’autres études ont par contre identifié qu’une augmentation dans la diversité végétale améliorait la dégradation du pyrène, phénanthrène et autres HAPs (Maila et al., 2005; Wei and Pan, 2010; Xu et al., 2006). Les recherches allant en ce sens sont toutefois limitées et le lien entre les diversités végétales et microbiennes n’a pas été étudié pour sa pertinence dans l’augmentation de la dégradation de la pollution (Batty and Dolan, 2011).

Concernant les polluants inorganiques, Wang et al. (2014) ont identifié un effet positif de la richesse en espèce sur l’extraction de métaux depuis un substrat de résidus miniers. Yang et al. (2012) ont quant à eux démontré un effet positif de la diversité de plantes compagnes sur la réduction des concentrations en métaux chez une espèce de grande culture. Le domaine des marais filtrant s’intéresse lui aussi à des approches multispécifiques (Button et al., 2016; Rodriguez and Brisson, 2016). Kearney et Zhu (2012) suggèrent qu’en présence de polluants multiples, la fonction optimale d’un système de filtration végétal serait plus susceptible d’apparaître dans un système contenant un assemblage de plusieurs espèces.

À la lumière de l’information présentée en introduction, il semble pertinent que la recherche en phytoremédiation s’intéresse aux situations de contamination complexes. Certaines études récentes considèrent une gamme plus large de contaminants dans leurs travaux (Chigbo and Batty, 2015; Lu et al., 2014; Marchand et al., 2015), mais celles-ci restent néanmoins relativement rares.

En faisant un amalgame de connaissances issues de l’étude des écosystèmes et de la phytoremédiation, il semble que la diversité végétale puisse jouer un rôle d’importance en phytoremédiation des sols à contamination multiple. Toutefois, pour établir ce lien, les arguments allant en ce sens doivent être rassemblés et des démonstrations empiriques faites. C’est à cette tâche que s’est attelée la thèse présenté dans ce document.

1.7. Objectifs de la thèse

L'objectif général de cette thèse est d'évaluer dans quelle mesure les propriétés spécifiques des végétaux peuvent être exploitées seules ou en combinaisons pour remédier des sols à contamination multiple. Pour ce faire, les activités de recherche se sont articulées autour de trois sous-objectifs, constituant autant de chapitres, pour lesquels une hypothèse de recherche a été associée. Chaque objectif correspond aussi à un article scientifique.

Chapitre 2 (premier chapitre de résultats):

Objectif : Identifier des indices supportant l'existence de niches de tolérance aux contaminants sur un site pollué laissé à l'abandon

Hypothèse : La distribution spatiale de la contamination du sol influence la répartition des espèces végétales sur un tel site.

Chapitre 3 (deuxième chapitre de résultats):

Objectif : Tester quatre espèces connues en phytoremédiation et offertes commercialement pour la remédiation d'un sol contaminé par l'argent, le cuivre et le zinc.

Hypothèse : Certaines des espèces testées peuvent simultanément accumuler dans leurs parties aériennes l'argent, le cuivre et le zinc en concentrations similaires à celles du sols.

Chapitre 4 (troisième chapitre de résultats):

Objectif : Tester des solutions à plusieurs espèces en situation de contamination multiple.

Hypothèse : Un assemblage d'espèces sera en mesure d'extraire du sol une quantité plus importante d'éléments traces que l'une ou l'autre des monocultures.

Chapitre 2 | ASSOCIATIONS PLANTES-CONTAMINANTS AYANT COURS SUR LE SITE D'UN ANCIEN BASSIN DE DÉCANTATION

Dans le chapitre d'introduction, une série de résultats scientifiques mettant en lumière les relations entre la diversité d'une communauté végétale et les services rendus par cette communauté a été présentée. Ces démonstrations appuient la théorie des niches fonctionnelles. Il est proposé dans cette thèse que cette théorie soit applicable en contexte de sol contaminé : les espèces végétales occuperaient des niches différentes selon leurs niveaux de tolérance aux contaminants. Si la théorie s'avère applicable à ce type de système, on devrait pouvoir observer des associations préférentielles entre les contaminants du sol et les espèces se trouvant au même endroit. Chacune de ces associations démontrerait la capacité d'une espèce de tolérer un polluant et donc potentiellement de remédier le sol qui le contient. C'est dans cette lignée que fut menée, sur le site d'un ancien bassin de décantation, une étude consistant à déterminer la distribution des contaminants dans le sol en plus d'effectuer un inventaire floristique sur ce même site, situé à Varennes, dans la province de Québec au Canada. Les deux types de données ont ensuite servi à explorer les liens potentiels entre contamination du sol et composition floristique.

Objectif : Identifier des indices supportant l'existence de niches de tolérance en terrain contaminé par plusieurs composés.

Hypothèse : La distribution spatiale de la contamination du sol influence la répartition des espèces végétales sur un site contaminé par plusieurs composés.

DISTRIBUTION PATTERNS OF SPONTANEOUS VEGETATION AND POLLUTION AT A FORMER DECANTATION BASIN IN SOUTHERN QUÉBEC, CANADA

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2.1. Abstract

After industrial activities shut down, the brownfields remaining on abandoned sites are often left to revegetate naturally, a process that reflects the site's biotic and abiotic characteristics, including spatial pollutant distribution. The soil of a former decantation basin in Varennes (southern Québec, Canada) was systematically sampled and described in terms of concentration of PAHs (polycyclic aromatic hydrocarbons), PHs (petroleum hydrocarbons C_{10}-C_{50}), various trace metals as well as ruderal plants abundance and diversity. Partial redundancy analysis was used to investigate the effect of heterogeneous pollution on the plant community's spatial distribution. Up to 61% of variance in spontaneous plant distribution was explained by the pollutant dispersion pattern on the study site. These findings provide guidelines for the design of site-specific and within-site remediation or rehabilitation promoting natural processes that are already in progress. They also suggest using local vegetation and a greater diversity of plant species when conditions are conducive as this may have many associated benefits. The resulting design, which promotes development of the local plant community, can be a more cost effective and environmentally sustainable alternative to traditional plant-based remediation approaches.

Key words: pollution, spontaneous vegetation, distribution patterns, partial redundancy analysis, site-specific, remediation

2.2. Introduction

Industrial activities are a vital economic sector, valuable to local populations and governments around the globe. Industrial development is often associated with negative impacts such as the discharge of pollutants into the environment, partly due to inadequate sustainable development policies. Long after industrial activities on a site have ceased, a variety of heterogeneously distributed pollutants can remain (French et al., 2006). Commonly-found contaminants include complex organic compounds such as polycyclic aromatic hydrocarbons (PAHs) and petroleum hydrocarbons (PHs), as well as inorganics (trace elements). PAHs and PHs, characterized by fused benzene rings and long carbon chains, are generally highly lipophilic, although to varying degrees, and consequently have the potential to bioaccumulate in the food chain (Jones and de Voogt, 1999), resulting in human health issues even at low concentrations (Qing Li et al., 2006) to the point that some are classified as carcinogenic (Weil et al., 2016). Trace elements, unlike organic compounds, do not decay and may persist in the environment for an extended period (Järup, 2003).

When industrial activities are shut down, brownfields on the site are often left to revegetate spontaneously. Patterns of plant distribution resulting from this process reflect plant interactions with site characteristics, as plants seek out vacant and suitable niches (Treshow, 1980). Ruderal vegetation will therefore appear distributed in a spatial pattern corresponding to variation of the site's specific attributes, including water conditions, pH, soil structure and associated biotic and abiotic stresses (Osmond et al., 1987). It has also been observed that vegetation assemblages can reflect anthropogenic metalliferous soil contamination (Gallagher et al., 2008). Eventually, vegetation will spread to cover most of the area, contributing to soil rehabilitation (Gao and Zhu, 2003).

Observations regarding natural revegetation patterns on former industrial sites as well as precise information on pollutant distribution should be taken into account when determining an ecologically responsible and site-specific rehabilitation approach (Danh et al., 2009). Several, possibly native, plant species should be favored, according to the contamination of the site. Addressing degraded land issues in this way would maintain greater local biodiversity, in contrast to more common phytoremediation practices (which have traditionally used high-maintenance, short-term intensive agricultural techniques including planting selected species) and hence provide ecological services adapted to site specificity. The use of a greater number of plant species would also lead to a greater diversity of associated soil microorganisms (Eisenhauer et al., 2010), resulting in possible more effective degradation of organic pollutants. The literature suggests that the interaction between plant and microbial communities within the rhizosphere is critical to remediation success and the use of diverse communities may further enhance this potential, but a specific understanding of function within the community is required before this can be achieved (Batty and Dolan, 2011).

Unstable heterogeneous environments (e.g. polluted sites) can be remediated in two main ways: imposed or self-organized (Mitsch and Jørgensen, 2003). The first, typical of many "conventional" phytoremediation approaches, may result in rigid plant assemblages with little potential for adaptation to changing conditions. Self-organization, which can be achieved by keeping the system open and allowing self-introduction of locally-present species, provides higher potential for adaptation (and resilience) to new situations (Mitsch, 1998). In addition, this approach fosters maintenance of native biodiversity and hence the ecological functions naturally performed on the site.

The aims of this study were to describe a former decantation basin in southern Québec (Canada) in terms of spontaneous vegetation distribution as well as organic compounds and trace elements in soil, and to evaluate whether pollutant distribution could explain variation in the distribution of spontaneous plant communities. Since plant tolerance to specific pollutants can vary by species, we hypothesized that distribution patterns of spontaneous vegetation in a former decantation basin could be partially explained by the distribution of contaminants in the soil. Information on conjoint variation in pollution and vegetation distributions could subsequently be used to develop a rehabilitation plan for a site such as the one we studied. Promoting presence of ruderal species within on-site zones where they appear to have found a suitable niche would enhance natural remediation processes already in progress toward an ecological and sustainable site rehabilitation. The originality of our research lies in the use of a statistical tool to produce a spatial distribution model of the site's pollution and vegetation. Such a model provides relevant information that can be used to not only enhance the cost-effectiveness and ecological character of traditional phytoremediation approaches, but also render them infinitely adaptable to varying site conditions and requirements.

2.3. Methods

2.3.1. Study area

The study area was located in the industrial zone of Varennes, few kilometres south of Montréal, Québec, Canada (45°40'N; 73°25'W). The site lies on the shores of the St-Lawrence

River, the primary drainage conveyor of the Great Lakes Basin. It is flat land with a temperate climate (annual average temperature: 6.2 °C; annual precipitation: 978.9 mm (www.stat.gouv.qc.ca). While the chemistry and ecology of the site have been previously described in detail (Guidi et al., 2011), it is important to note that it once hosted primarily petrochemical activities, but was also used for ethanol and titanium dioxide pigment production. Currently, the industrial zone is surrounded mainly by agricultural activities.

Subsequent to industrial use, parts of the zone served as a sedimentation basins for various industrial wastes that had been generated on-site. Industrial activities ceased in 2008. One sedimentation basin was emptied and left opened to spontaneous revegetation (Figure 2.1).

Figure 2.1 Decantation basin area in Varennes, Canada. Quadrats for ruderal vegetation and soil sampling (0-30 cm depth) are located inside a former decantation basin (zone marked in red, 20m X 22m). Basins still in operation are visible. The surrounding area is characterized mostly by industrial activities. Image was taken before the beginning of the study while colonization of the former basin by ruderal plants was already ongoing.

2.3.2. Data sampling

In the summer of 2012, the basin's vascular plants were sampled systematically. Twenty (20) quadrats, each 1 m^2, were staked out on the site in order to represent the range of conditions observed. For each quadrat, the number of species and percentage of canopy coverage per species were recorded. A *Braun-Blanquet* (Braun-Blanquet and Springer-Verlag, 1951) canopy cover index was calculated for each species. Rare species (< 5% cover) were excluded from data analysis. Each individual of the 23 species found on the site was identified to the species level, except those either missing reproductive parts essential to clear identification due to the season or poorly developed, probably as a result of poor soil quality (Table 2.1).

Table 2.1 List of plant species found on the study site in Varennes, Canada. Botanical information from the Database of Vascular Plants of Canada (VASCAN) (Brouillet et al., 2010+)

Scientific name	English name	Botanical family	Canadian status
Alisma triviale Pursh	northern water plantain	Alismataceae	Native
Bidens vulgata Greene	big devils beggartick	Asteraceae	Native
Butomus umbellatus L.	flowering rush	Butomaceae	Introduced
Chenopodium glaucum L.	oakleaf goosefoot	Amaranthaceae	Native
Cirsium arvense (L.) Scop.	Canada thistle	Asteraceae	Introduced
Cirsium vulgare (Savi) Ten.	bull thistle	Asteraceae	Introduced
Eleocharis obtusa (Willd.) Schult.	blunt spikerush	Cyperaceae	Native
Eupatorium L.	thoroughwort	Asteraceae	Native
Hordeum jubatum L.	foxtail barley	Poaceae	Native
Licaria Aubl.	licaria	Lauraceae	Native
Linaria Mill.	toadflax	Plantaginaceae	Introduced
Lycopus europaeus L.	gypsywort	Lamiaceae	Introduced
Oenothera biennis L.	common evening primrose	Onagraceae	Native
Panicum capillare L.	common panicgrass	Poaceae	Native
Polygonum lapathifolium L	curlytop knotweed	Polygonaceae	Introduced
Polygonum persicaria L.	spotted ladysthumb	Polygonaceae	Introduced
Populus balsamifera L.	balsam poplar	Salicaceae	Native
Sagittaria latifolia Willd.	broadleaf arrowhead	Alismataceae	Native
Solidago canadensis L.	Canada goldenrod	Asteraceae	Native
Sonchus arvensis L.	field sow-thistle	Asteraceae	Introduced
Sporobolus vaginiflorus Alph. Wood	sheathed dropseed	Poaceae	Native
Tussilago L.	coltsfoot	Asteraceae	Introduced
Typha angustifolia L.	narrow-leaved cattail	Typhaceae	Native

The soil of each quadrat was sampled (0-30cm depth) and analyzed by GC-MS for organics and ICP-MS for inorganic elements. We determined that a depth of 0-30cm was appropriate, based on our investigation of root length of individuals outside the sampling quadrats. Data regarding organic compounds in soil was regrouped into petroleum hydrocarbons C_{10}-C_{50} (PHs) and polycyclic aromatic hydrocarbons (PAHs). PAHs were considered as a group rather than individually, for reasons explained below. The concentrations of 10 elements (Al, B, Cu, Cr, Fe, Mg, Mn, Ni, Pb, Zn) and basic soil properties was also recorded (Table 2.2). C.E.C. was estimated by NH_4^+ saturation of soil at pH 7.0 followed by NaCl washing (CRAAQ, 2010). pH was obtained by electrometric method (CEAEQ, 2014). Organic matter (%O.M.) content was obtained by loss on ignition (Rabenhorst, 1988).

Table 2.2 Chemical characteristics of sampling quadrats

Quadrat	C.E.C	pH	%O. M.	PHs	PAHs	Al	B	Cu	Cr	Fe	Mg	Mn	Ni	Pb	Zn
1	24.1	6.9	7.4	890	10.8	899	0.88	46	141	437	1553	47.8	64	15	136
2	24.5	7.3	4.3	530	8.4	869	0.84	44	197	413	1397	27.8	71	15	224
3	23.1	7.1	4.1	290	2.4	900	0.9	43	140	367	1455	24.6	69	15	138
4	29.8	7.7	5.3	590	6.8	762	2.39	142	175	306	1851	44.5	72	15	170
5	28.1	7.3	6.1	1470	9.1	717	1.21	138	128	396	1425	56.3	66	15	124
6	22.6	7.5	3.1	3340	320.4	894	0.92	48	114	469	2175	51.4	67	15	105
7	35.3	7.6	3.4	1420	30.0	753	0.7	20	91	362	928	110.8	53	40	50
8	21.5	7.3	6.1	20700	2652.4	618	1.02	45	113	350	1965	76.8	65	15	100
9	28.9	7.5	5.9	360	14.8	761	1.61	20	102	457	1696	55.8	58	15	101
10	31.9	7.7	3.3	17500	595.6	806	0.85	45	108	438	1745	83.8	65	15	50
11	30.2	7.6	9.4	26300	4133.8	620	1.52	48	125	644	1223	85.7	64	15	124
12	38.2	7.4	7.6	9480	277.9	750	0.096	56	188	569	1372	75.5	69	15	196
13	38.2	7.6	4.1	5350	504.7	777	0.89	48	115	597	1547	109	65	15	109
14	36.1	7.6	3.8	8200	1444.8	765	1.28	45	113	713	1613	153.9	67	15	104
15	24.7	7.5	4.5	9470	955.7	671	1.09	46	110	587	1467	100.5	67	15	104
16	25.9	7.5	2.8	5000	451.1	851	0.95	52	112	485	2019	99.1	67	15	104
17	30.3	7.4	11.5	870	8.6	450	1.34	20	120	342	1093	77.1	68	15	110
18	45.6	7.4	5.9	1020	2.3	612	0.72	20	117	303	1545	33.1	71	15	110
19	25.7	7.2	7.7	990	13.8	306	0.78	72	237	346	946	27.3	62	15	274
20	30.6	7.4	6.7	2070	11.1	869	1.75	64	161	513	2093	23.8	66	76	245

All compounds and elements concentrations are expressed in mg kg^{-1} of soil. C.E.C. is expressed in $cmol_{(+)}$ kg^{-1}.

2.3.3. Statistical analysis

We divided vegetation data into two sets in order to perform two analyses. First, to obtain an estimate of general plant distribution on the site, three parameters were considered for each quadrat i.e. species richness, percent canopy cover and α-diversity using Shannon's (*H*) index. The latter takes into account species richness and equitability (Peet, 1974). According to a previous study (Mouillot and Leprêtre, 1999), Shannon's index has been shown to have a lower root-mean-squared error (RMSE) for assemblages containing 10 or 25 species, which makes it an appropriate measurement tool for our study. We obtained the Shannon index with the *diversity* function of the *Vegan* package (Oksanen et al., 2007) in R software. A second data set contained abundance of each species separately.

Partial redundancy analysis (pRDA) was used to analyse data. Redundancy analysis is the direct extension of multiple regression to multivariate response data (Legendre and Legendre, 2012), where redundancy is synonymous with explained variance (Gittins, 1985). This allowed us to determine whether and how much of the variation in vegetation distribution is explained by variation in pollutant dispersion. We first considered variation in vegetation distribution in terms of diversity (Shannon's index), absolute number of species and canopy cover percentage and secondly by abundance of each species.

The effect of environmental covariables i.e. pH, organic matter percentage and cation exchange capacity (C.E.C) as a source of variation was taken into account (partialized) by the model, to better relate variance in vegetation distribution to variance in pollutant dispersion. Scaling of type 2 when plotting the ordination diagrams allowed us to interpret angles between plant responses and pollutant distribution as their correlation, as well as between plant responses and pollutant concentrations individually (Borcard et al., 2011). Species abundances were Hellinger-transformed to avoid double-zero problems (Borcard et al., 2011).

The interpolation of the concentrations presented on the maps of Figure 2.3 was produced by ordinary kriging of the soil sampling data. Kriging methods have shown to provide a high prediction accuracy of the mean concentration of soil heavy metals (Xie et al., 2011) and are appropriate to estimate the pollutants distribution on the study site.

2.4. Results and discussion

2.4.1. Diversity index, number of species and canopy cover

Influence of soil parameters distribution on Shannon's diversity index, canopy cover and number of species is presented in Figure 2.2a.

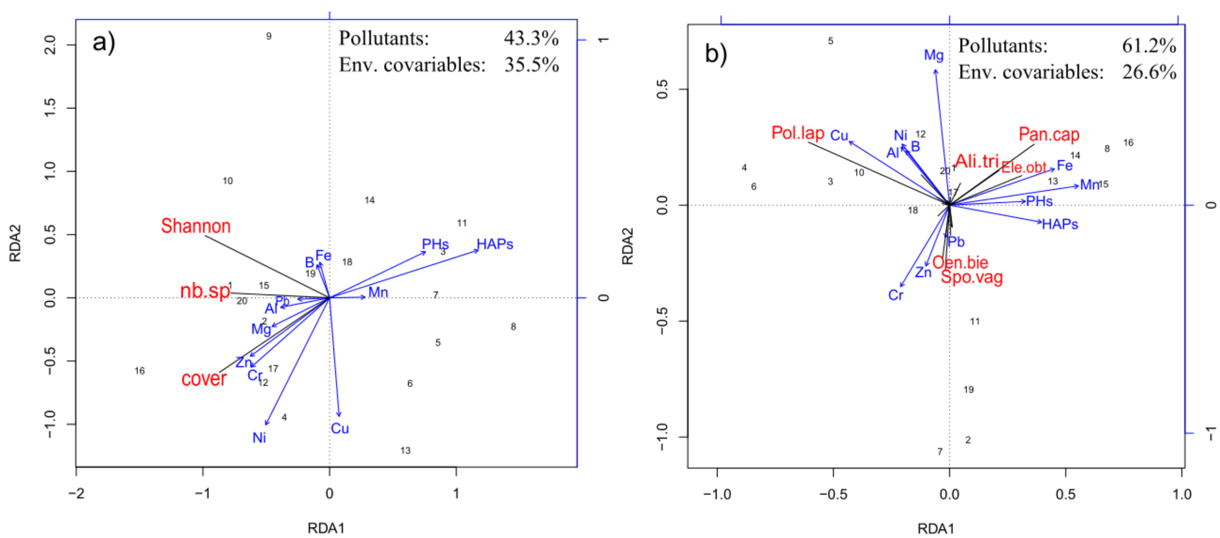

Figure 2.2 Partial RDAs modelling vegetation distribution explained by pollutant dispersion. Models are adjusted for effect of pH, organic matter percentage and cation exchange capacity (C.E.C.) (environmental covariables). In both ordinations, numbers represent sampling quadrats. Percent of variation in plant distribution explained by soil parameters are given. a) Soil parameters dispersion explaining 43.3% of variance in vegetation distribution in terms of

canopy cover (cover), number of species (nb.sp) and Shannon's diversity index Shannon; b) Soil parameters dispersion explaining 61.2% of variance in the distribution of *Sporobolus vaginiflorus, Eleocharis obtusa, Alisma triviale, Polygonum lapathifolium., Panicum capillare* and *Oenothera biennis* on the site.

As much as 43.3% of spatial variation in the three vegetation responses is explained by spatial variation of pollutants . Shannon's index has been used in ecological studies to assess the response of plant communities' diversity to soil contamination. For instance, this index was found to be lower under hydrocarbon-contaminated as compared to uncontaminated soil in semi-arid grasslands of western Canada (Robson et al., 2004). Similar results on influence of hydrocarbons on plant diversity are described below.In our analysis, PAHs is the sum of all PAH compounds identified through chemical analysis. This parameter was established based on previous analyses conducted with each PAH compound separately that showed they all occupied the same small area on ordination diagrams and hence all provide the same explanation of vegetation distribution. We found that the different pollutant compounds were scattered heterogeneously on the site, as illustrated by Figure 2.3.

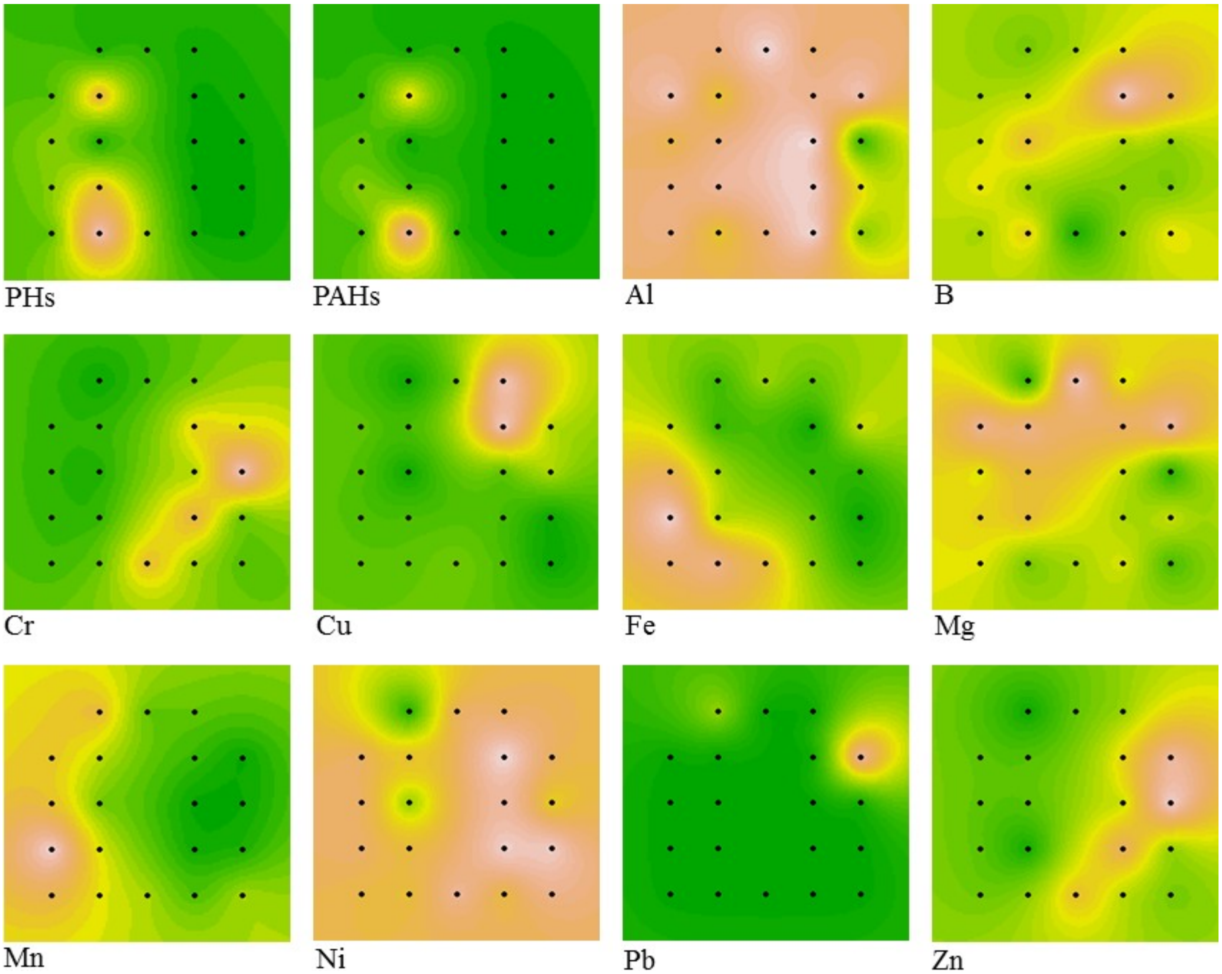

Figure 2.3 Spatial heterogeneity of various compounds in Varennes's basin. Petroleum hydrocarbons (PHs), petroleum aromatic hydrocarbons (PAHs) and trace elements (Al, B, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn) concentrations at 0-30cm depth. Sampling quadrates are indicated with points. Mapping was produced by kriging sampling data (Table 2.2) with R software. Warmer colors indicate higher concentration of target compound, with no relations between maps. Spatial allocation of sampling quadrats reflects exclusion of the basin's center area, where weeding had been performed for a previous study. Concentration ranges across sampling quadrats of 290-26300 mg PHs kg^{-1}, 2.3-4133.8 mg PAHs kg^{-1}, 306-900 mg Al kg^{-1}, 0.096-2.39 mg B kg^{-1}, 20-142 mg Cu kg^{-1}, 91-237 mg Cr kg^{-1}, 303-713 mg Fe kg^{-1}, 928-2175 mg Mg kg^{-1}, 23.8-153.9 mg Mn kg^{-1}, 53-72 mg Ni kg^{-1}, 15-76 mg Pb kg^{-1} and 50-274 mg Zn kg^{-1}.

Spatial allocation of sampling quadrats reflects exclusion of the basin's center area, where weeding had been performed for a previous study.

All three vegetation responses were positively correlated ($0.58 < r < 0.76$) with each other, and negatively correlated with organic compounds ($-0.51 < r < -0.38$) (Table 2.3).

Table 2.3 Pearson correlations among pollutant concentrations in soil and number of species, canopy coverage, Shannon's diversity index and individual species abundance.

	PHs	PAHs	Al	B	Cu	Cr	Fe	Mg	Mn	Ni	Pb	Zn
Number of species	-0.44	-0.51	0.24	-0.33	-0.14	0.33	-0.12	-0.01	-0.31	-0.01	0.10	0.33
Canopy cover	-0.42	-0.5	-0.13	-0.19	0.06	0.47	-0.16	-0.12	-0.31	0.44	0.07	0.48
Shannon's diversity	-0.38	-0.47	0.07	-0.13	-0.2	0.27	-0.10	0.01	-0.35	0.13	0.04	0.30
Sporobulus vaginiflorus	-0.14	-0.12	0.03	-0.19	-0.24	0.28	-0.19	-0.42	-0.28	-0.68	0.34	-0.33
Oenothera biennis	-0.26	-0.21	-0.54	-0.27	-0.09	0.48	-0.04	-0.32	-0.44	0.13	-0.12	0.43
Polygonum lapathifolium	-0.25	-0.22	0.16	0.58	0.75	0.18	-0.38	0.17	-0.29	0.34	-0.13	0.08
Panicum capillare	0.03	-0.02	0.13	-0.04	-0.01	-0.02	0.13	0.29	0.27	0.05	-0.11	-0.18
Alisma triviale	0.16	0.18	0.02	0.08	-0.12	-0.3	0.58	0.06	0.61	0.4	-0.13	-0.26
Eleocharis obtusa	0.01	0.05	0.14	-0.08	-0.06	-0.02	0.47	0.12	0.53	0.01	-0.11	-0.17

Ni, Cr and Zn all had a positive effect on canopy cover ($0.44 > r > 0.48$). These results imply that we could consider PHs and PAHs as having a wider range of phytotoxicity than other pollutants, at least on this site, and hence a greater impact on diversity. Polycyclic aromatic hydrocarbons have been shown to be highly toxic to many plants, primarily affecting germination and seedling growth, although the extent of plant sensitivity varies according to species (Hong et al., 2009) and chemical compound (Sverdrup et al., 2003).

We can summarize the previous results from a rehabilitation point of view. It suggests that a more specialized approach would be more appropriate for areas within-site that have high levels of organic compounds in soil, based on a smaller number of species tolerant of the specific pollutants present on the site. On the other hand, the presence in other areas of metallic elements like Zn and Cr, which are positively correlated to diversity on our site, would not be an obstacle to the use of a higher number of local herbaceous species for site rehabilitation.

2.4.2. Species abundance

The six plant species (Sporobolus vaginiflorus (Nash) Scribn.., Eleocharis obtusa (Willd.) Schult., Alisma triviale Pursh., Polygonum lapathifolium L., Panicum capillare L., and Oenothera biennis L.) identified by the model as those that are most responsive to variations in site characteristics (highest RDA scores) are presented in the second ordination diagram. Figure 2.2b (61.2% of explained variation) shows the distribution pattern of these species according to the various types of pollutants.

The positions of these species on the ordination diagram fall into three well-defined groups based on different distribution patterns, suggesting species-specific tolerance towards pollutants.

Based on Figure 2.2b, *Eleocharis obtusa*, *Panicum capillare* and *Alisma triviale* may be tolerant to PAHs and PHs, but univariate correlations show that their position is also positively attributable to the distribution of Fe and Mn (Table 2.3) on the study site. This information is partially confirmed by findings in previous studies, which showed that *Panicum spp.*, like other members of the grass family (*Poaceae*), is highly tolerant to PAH contaminated soil (Hong et al., 2009). On the other hand, species of the *Eleocharis* genus have been shown to be very effective in phytoremediating mine tailings and drainage rich in heavy metals, including Cr, Cu, Zn, Ni, and Mn (Ha et al., 2011). Members of the same family (*Cyperaceae*) have been known to be highly resistant to petroleum pollution (Merkl et al., 2005). These results suggest that in areas where petroleum and Mn contamination is predominant and hence a high plant diversity strategy inappropriate (negative correlations with Shannon index, see Table 2.3), species like *P. capillare* and *E. obtusa* could be considered for use in rehabilitation.

In our study, *Polygonum lapathifolium* was positively correlated with variations in B, and Cu distribution (r = 0.58 and 0.75 respectively), which concurs with previous studies that showed that several members of the *Polygonum* genus have a high affinity for trace metals (Kim et al., 2003) and boron (Ozturk et al., 2010). This species may therefore be a particularly good candidate for rehabilitating sites with high levels of copper.

Based on our findings, we could also assume that *Oenothera biennis* has a high tolerance for Zn and Cr (r = 0.43 and 0.48 respectively). The fact that these two elements did not negatively affect diversity (Figure 2.2a) would hence not necessarily point towards a specialist species approach, as mentioned in the case of organic compounds and Mn contamination, to foster greater species diversity. The species could therefore be used with other Zn and Cr tolerant species in rehabilitation. *O. biennis* was also negatively affected by the presence of Al (r = -0.54). The position of *Sporobulus vaginiflorus* on the ordination diagram reflects a variety of interactions with Mg, Zn, Ni (r = -0.42, -0.33 and -0.68 respectively) and Pb (r = 0.34). Among the six species presented in Figure 2.2b, only *S. vaginiflorus* is positively correlated with the presence of Pb. The genus S*porobolus* (*sp. pungens*) has been previously described as highly tolerant of moderate Pb accumulation (García et al., 2003), which highlights this species as promising for rehabilitation of Pb contaminated areas within-site.

2.5. Conclusion

Our study showed that pollutant and elemental dispersion can explain most of the distribution pattern of spontaneous vegetation on a former industrial site in southern Québec. Knowing that contamination tends to be highly site-specific and plant response to pollution contaminant-species dependent, knowledge about contaminant distribution and soil characteristics on a degraded site is an important prerequisite for designing an efficient and ecological rehabilitation approach using local species already present on site. Not only is this information relevant for an over-all site-specific design, it also suggests area-specific phytoremediation strategies within a given site. Furthermore, it makes it possible to propose the use of a greater number of species when conditions are conducive. Another important advantage of our method lies in the possibility of identifying local species that may be new candidates for further remediation or rehabilitation research. Notwithstanding the relatively limited data, our approach can be extended and refined to adapt to any scale required for the design of remediation strategies. While nurturing the revegetation process occurring naturally on a site may appear to be a slow-paced approach to rehabilitation, it may ultimately be more cost-effective and environmentally sustainable. Encouraging species to spread in a niche where they have already established and begun to thrive could reduce weeding, watering and soil amendment costs. Less soil disturbance would also foster action performed by soil microorganisms, further stabilizing the rehabilitation process.

The integration of data on spatial distribution in the design of plant-based rehabilitation methods hence offers numerous advantages. It allows conceiving a site-variable community-based phytoremediation approach. Conventional polluted land management and bioremediation techniques not being mutually exclusives, integration of natural remediation as proposed in this paper into polluted site management plans could ultimately have wide-ranging ecological benefits and also enhanced the cost-effectiveness of a remediation strategy.

2.6. Acknowledgments

This study was made possible in part thanks to financial support from Genome Québec and Genome Canada. Our thanks also to Petromont for providing access to the site, as well as to Stéphane Daigle for help in interpretation of results, Stuart Hay for species identification and Karen Grislis for English revision. At the time of the study, Alexandre Naud was part of the team of the Institut de recherche en biologie végétale.

2.7. Synthèse du chapitre 2

Parmi les conclusions du chapitre 2, il a été possible de démontrer le lien qui existe entre la distribution d'une végétation et celle de la contamination du sol sur un même site. Il apparait ainsi possible d'utiliser les associations observées entre espèces locales et contaminants pour inspirer le design d'une approche de phytoremédiation adapté à ce même site. Toutefois, il est difficilement concevable de mettre sur pied une approche de phytoremédiation qui associera une espèce locale en particulier à chacun des contaminants présents en raison de deux principaux obstacles. Premièrement, il n'est pas toujours possible d'utiliser un relevé floristique comme point de départ pour le design d'une approche de phytoremédiation, puisque bien souvent, au moment de décider du type d'intervention, les sites sont dénudés de végétation. Dans le cas où une végétation rudérale est présente, les espèces retrouvées peuvent aussi ne pas être disponibles commercialement, ce qui engendre des défis d'approvisionnement peu intéressants à surmonter pour un gestionnaire de site. Ensuite, comme les sites contaminés sont souvent caractérisés par une contamination complexe en termes de diversité de composés, mais aussi de distribution spatiale, un échantillonnage extensif du site est nécessaire pour déterminer où implanter chacune des espèces. Ce type de travaux devient rapidement onéreux et donc difficilement envisageable.

Une solution à ces deux problèmes serait d'employer une espèce disponible commercialement, qui serait aussi tolérante à plusieurs contaminants à la fois, permettant alors d'implanter cette espèce sur l'ensemble du site. C'est dans cette optique que fut menée l'expérience décrite dans le chapitre 3.

Chapitre 3 | HABILETÉS DE REMÉDIATION MULTIPLES

Comme vu précédemment, des associations préférentielles entre espèces végétales et contaminants peuvent se mettre en place de manière naturelle. Utiliser cette information pour le design d'une approche de phytoremédiation sur le terrain d'origine serait avantageux sur le plan de biodiversité, tout en impliquant quelques défis opérationnels .

Alternativement, y aurait-il des espèces commercialement disponibles qui pourraient posséder des habiletés de phytoremédiation envers plusieurs composés? Quatre espèces connues dans le domaine de recherche en phytoremédiation ont servi à explorer cette question.

Objectif : Tester quatre espèces couramment utilisées en phytoremédiation pour la remédiation d'un sol contaminé par l'argent (Ag), le cuivre (Cu) et le zinc (Zn).

Hypothèse : Certaines des espèces testées peuvent simultanément transloquer dans leurs parties aériennes l'argent, le cuivre et le zinc en concentrations similaires à celles présentes dans le sol.

DIFFERENTIAL UPTAKE OF SILVER, COPPER, AND ZINC SUGGESTS COMPLEMENTARY SPECIES-SPECIFIC PHYTOEXTRACTION POTENTIAL

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3.1. Abstract

The aim of our study, conducted as a pot experiment, was to assess the potential of willow (*Salix miyabeana*), alfalfa, (*Medicago sativa*) tall fescue (*Festuca arundinacea*) and Indian mustard (*Brassica juncea*) to remediate two brownfield soils differentially contaminated with Ag, Cu and Zn (up to 113.60, 47.50 and 117.00 mg kg^{-1} respectively). While aboveground Ag accumulation was highest in *B. juncea* (4.60±2.58 mg kg^{-1}), lower levels were also measured in *M. sativa* and *F. arundinacea*. Cu accumulation was observed in all species, but only in underground parts, and was highest in *F. arundinacea* (269.20±74.75 mg kg^{-1}), with a bioconcentration factor of 13.85. *Salix miyabeana* was found to have the highest Zn aerial tissue concentration (119.96±20.04 mg kg^{-1}). Because of its high Ag uptake, the remediation potential of *B. juncea* should be evaluated more extensively on the site from which we excavated the soil for this study. Given the multiple forms of contamination on the site and the differential specie-related uptake evident in our findings, we submit that an optimal plantation allowing expression of complementary remediation functions would include *B. juncea* for extraction of Ag, in combination with *F. arundinacea* for stabilization of Cu and *S. miyabeana* for extraction of Zn.

3.2. Introduction

Trace elements (TE), which include heavy metals and metalloids (Alloway, 2013), can be found in high concentrations in soils, especially on sites with extensive industrial activities. TE at some concentrations can pose a serious threat to ecosystem and human health due to the immutable nature of TE and possible bioaccumulation through the food chain (Alkorta et al., 2004). Phytoremediation has been proposed as a sustainable alternative to conventional approaches to management of metal polluted soil (Garbisu and Alkorta, 2001), but the technique's efficiency requires further enhancement to improve both its acceptability and application (Padmavathiamma and Li, 2007). The efficiency of a TE phytoremediation system is in large part a function of the level of concentration of one or several TE that can be measured in plant tissues as a result of uptake. Sequestration of TE in underground parts is referred as phytostabilization, whereas translocation of TE to aerial and hence harvestable biomass is called phytoextraction (Sarma, 2011). The latter constitutes an ideal strategy for removing TE from soil. Both TE and plant identity have a strong influence on the plant tissues in which TE accumulate (Padmavathiamma and Li, 2007).

Numerous studies have investigated the biogeochemical properties of TE, and research underway on this topic includes testing the potential of several TE to bioaccumulate in plant tissues. Sheoran *et al*. (2010), Sarma (2011) and Krämer (2010) provide useful reviews of the literature on TE bioaccumulation mechanisms in plants, and especially on hyperaccumulation (uptake of TE from the soil at high rates, translocation and accumulation of the same in shoot organs, stem and leaves) (Maestri et al., 2010). Silver (Ag) is among the TE that have been identified in brownfields and can represent a threat to the environment and health (Ratte, 1999).

Bioaccumulation and toxicity of silver in different organisms have also been investigated in recent years, but far less extensively than many other TE and often only in aqueous environments such as specially designed hydroponic systems (Banach et al., 2012; Guidi Nissim et al., 2014; Ha et al., 2011; Harris and Bali, 2008; Odjegba and Fasidi, 2004; Ratte, 1999; Sasmaz and Obek, 2012; Xu et al., 2010). Regarding bioaccumulation in higher plants, Gardea-Torresdey *et al*. (2003) have reported on formation of silver nanoparticles in alfalfa (*Medicago sativa)* grown in an artificially contaminated solid substrate. Borovička et al. (2007) have also described silver accumulation in two ectomycorrhizal macrofungal *Amanita* species, where concentration in fruit bodies was commonly 800–2500 times higher than in underlying soils.

Multiple TE can be present conjointly and in variable concentrations, and this is commonly the case in brownfields. Exposing plants to variable TE mixtures in phytoremediation research is therefore relevant for deriving fundamental knowledge about how plants respond to this complex type of stress. However, such research requires considerable investment of financial resources, time and expertise in order to capture the complexity of the processes involved. Pilot pot studies that expose plant species to soil excavated from a site contaminated with various TE are nonetheless useful to effectively control for environmental sources of variation, likely to be encountered in an *in situ* phytoremediation initiative, as well as to identify which plant species are most effective for soil remediation. Such pot studies thus lay the foundation for designing phytoremediation approaches that can be adopted on actual sites, and hence increase their potential efficiency. At the same time, they produce relevant knowledge on metal-plant interactions.

This study was conducted on soils from the site of a former petrochemical refinery that were contaminated with Ag, Cu and Zn, of which two zones were targeted for phytoremediation. The aim was to identify one or more species that could accumulate silver as well as copper and zinc, all of which were present in the soils studied. The scarcity of information in the literature regarding the fate of silver in soil-plant systems as well as the need for designing mixed contamination phytoremediation approaches both justify such experimental investigation.

3.3. Methods

3.3.1. Experimental design

A pot experiment was conducted outdoors on the site of the Montreal Botanical Garden (**latitude:** 45.560002 | l**ongitude:** -73.563009) in the summer of 2012. The experimental units (fifteen-liter pots) were placed on a layer of gravel on the ground. To prevent water and thereby pollutants from leaching onto the experimental site, each pot was lined with a 0.5 millimeter plastic bag. A double-bottom layer of inert volcanic stone topped with a fitted geotextile membrane was added to retain soil in the upper part while allowing water to drain normally. To intercept rainwater and prevent the pots from flooding, a polyethylene roof was installed on a tunnel shaped structure over the pots. Plant water needs were fulfilled by an automated drip-irrigation system.

Two substrates (S1 and S2) with some differing physicochemical characteristics (Table 3.1) were used in the experiment.

Table 3.1 Total concentrations of Ag, Cu and Zn and other physicochemical parameters in S1 and S2 soils.

Parameter	Soil	Value	
Ag	S1	34.6±8.7b	mg kg^{-1}
	S2	113.6±4.4a	
Cu	S1	47.5±5.5a	
	S2	20.0±0.1b	
Zn	S1	162.2±19.0a	
	S2	177.0±3.3a	
P[1]	S1	35.0±3.6a	kg ha^{-1}
	S2	28.0±5.0a	
K[1]	S1	456.3±79.7a	
	S2	340.3±45.7a	
pH (H_2O)	S1	8.3±1.0a	
	S2	7.5±0.2a	
O.M.	S1	2.3±0.2b	%
	S2	3.9±0.3a	
C.E.C.	S1	49.8±11.8a	$cmol_{(+)}$ kg^{-1}
	S2	36.9±3.0a	

[1]Extraction method according to Mehlich (1984). O.M. = organic matter percentage; C.E.C. = cation exchange capacity. Different lowercase letters indicate significant differences between the two soils for a given metal (n=5) or other parameter (n=3).

They were excavated in two different zones of the same brownfield located in an industrial zone in the City of Montreal, and were both mechanically homogenized to reduce heterogeneity of pollutant distribution. This handling produced a comparable physico-chemical substrate in each experimental unit of the same substrate. The fact that the experiment was conducted outdoors made it possible to maintain environmental conditions that resembled those on the site of origin.

For each of the two types of substrate, four plant species, i.e. three herbaceous; *Brassica juncea* (Indian mustard), *Medicago sativa* (alfalfa), *Festuca arundinacea* (tall fescue) and one woody species, *Salix miyabeana* (willow, cultivar SX67), were sown or planted (20 cm cuttings in the case of willow) in late May 2012. Each experimental pot received either one cutting of willow or the equivalent of 15 kg ha^{-1} of seeds for the herbaceous species. These species were selected because of their remediation capacities for TE other than silver, demonstrated in numerous earlier studies (Lu et al., 2014; Maxted et al., 2007; Singh et al., 2013; Vamerali et al., 2011), and also for their availability on the market and suitability for growing at northern latitudes. Even though these species are not necessarily considered hyperaccumulators, the greater growth potential and high yields of field crop species allow them to uptake an appreciable total amount of TE, which compensates for the lower concentration they accumulate in tissues. Moreover, they can rapidly establish a dense green canopy, hence improving the landscape and, most importantly, reducing the mobility of pollutant trough water and wind erosion, as well as trough water percolation (Vamerali et al., 2010).

An unplanted treatment pot acting as a control was included in the experimental design. N-P-K Plant Prod® fertilizer (20-20-20) was applied twice during the growing season, in the early stages of growth and at mid-season. In summary, four species and one unplanted control, 2 types of substrate (S1 and S2) and two replications for each of the treatments formed a design of 20 pots. This was reproduced over five randomly distributed experimental blocks for a total of 100 pots.

3.3.2. Soil and biomass analysis

The experimental conditions were maintained for a total of four months. At the end of September, or July in the case of *B. juncea* since it had completed its growing season then, aboveground biomass of plants was cut, dried to constant mass and weighed. The samples of soil containing roots were transported to the laboratory, and all soil was removed from roots manually using pliers, tap water and 1mm sieves. To avoid quantification of TE potentially adsorbed to the surface of the roots and thus not accumulated within underground tissues, the latter were soaked in a 0.5mM $CaCl_2$ solution (Rauser, 1987) and rinsed again with demineralized water. Roots were dried to constant weight and weighed.

Soil samples were homogenized, dried, grinded and sieved (<0.5mm) prior to digestion with HNO_3 and HCl (1:3 ratio). Plant tissues were dried to constant weight, homogenized and digested with HNO_3. Trace element contents of both soil and tissue samples were quantified by ICP-OES. Values for P and K were obtained by the Mehlich III method (Mehlich, 1984). pH was obtained by electrometric method (CEAEQ, 2014). Soil organic matter was obtained by loss on ignition (Rabenhorst, 1988). C.E.C. was estimated by NH_4^+ saturation of soil at pH 7.0 followed by NaCl washing (CRAAQ, 2010). Significant differences in TE concentrations between the two soils were observed for Ag and Cu, the former being higher in S2 soil and the latter higher in S1 (Table 3.1). The percentage of organic matter (O.M. %) was higher in S2 soil. Other parameters measured (Zn, P, K, pH and cation exchange capacity (C.E.C.)) were constant between the two soils. Ag, Cu and organic matter content are therefore considered the main drivers of the differences in plants responses between S1 and S2.

3.3.3. Calculation and presentation of results

Bioconcentration factor (BCF); dry weight concentration ratio of a plant part per soil concentration.

$$BCF = [metal]_{plant} / [metal]_{soil} \quad (1)$$

BCF is used here to calculate root uptake relative to soil concentration only. Aerial part uptake is described with the translocation factor. Translocation factor (TF) is the concentration ratio of aerial parts per underground parts.

$$TF = [metal]_{shoot} / [metal]_{root} \qquad (2)$$

3.3.4. Statistical analysis

Statistical analyses to compare means (ANOVA) were performed with SAS JMP (v. 8.0) software and R (v. 2.15.2) open source software. Significant differences were determined using Tukey's HSD test with a threshold of $\alpha = 0.05$. Data transformations (log 10) were carried out where data did not fit the assumptions of the test.

3.4. Results and discussion

3.4.1. Plant establishment and biomass production

The willows and three herbaceous species successfully colonized the pots. After one week, germination and growth were observed for all species. Biomass allocation into (a) roots and (b) shoots is shown in Figure 3.1.

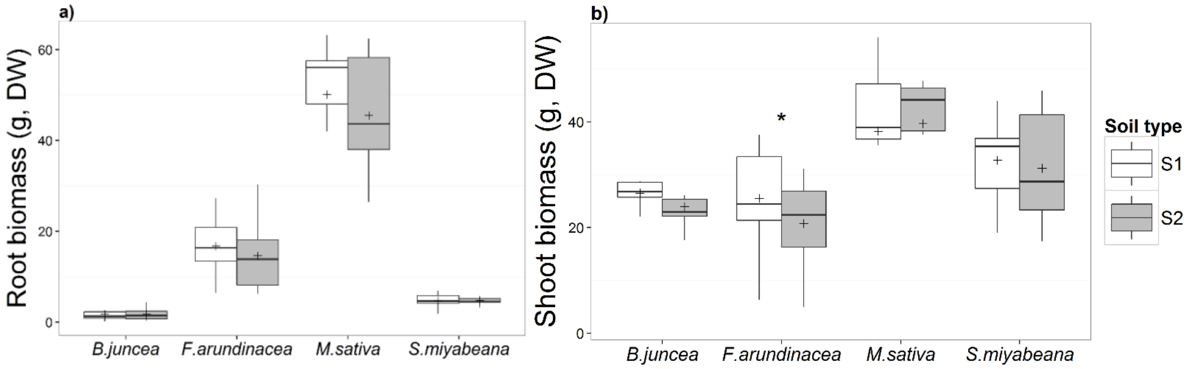

Figure 3.1 Biomass of species grown in S1 and S2 soils. (a) root and (b) shoot biomass (g, dry weight material) for all species, per pot (15 liters). Significant difference between soils is identified by an asterisk. Cross marks represent the mean (n=10).

Our investigation aimed to identify significant differences solely between substrates, since interspecific variations in dry biomass yield among species were attributed to genetic and physiological factors and were thus irrelevant for this analysis. The parameters significantly differing between S1 and S2 (Ag, Cu and % organic matter) appear to have no global significant influence on vegetation yield, with the sole exception of slightly decreased aerial biomass production in *F. arundinacea* grown in S2 soil compared to S1. Minimal differences in plant yield could be attributed to the low individual influence of the parameters studied on biomass production or to an antagonist effect. As the influence of individual parameters cannot be investigated with this experimental design, it is impossible to confirm either hypothesis. However, higher organic matter content is usually associated with greater crop yield (Bauer and Black, 1994), and raising Cu and other TE concentration in soil has been identified as a possible pressure that can reduce biomass production (Reichman, 2002).

3.4.2. Trace element uptake

Chemical analysis for bioaccumulation of TE was conducted on below- and aboveground tissues of all plants. Figure 3.2 shows the concentrations (mg kg^{-1}) of Ag, Cu and Zn in the four species grown in S1 and S2 soil.

The three TE are present in belowground parts of all species. Significant differences in root concentrations between S1 and S2 soil are only observed for Ag, while Cu and Zn are present at equivalent concentrations in the roots of a species regardless of substrate type. Therefore, the values for Cu and Zn concentrations mentioned in the following paragraphs will be the mean of the concentrations found in plants grown in S1 and S2.

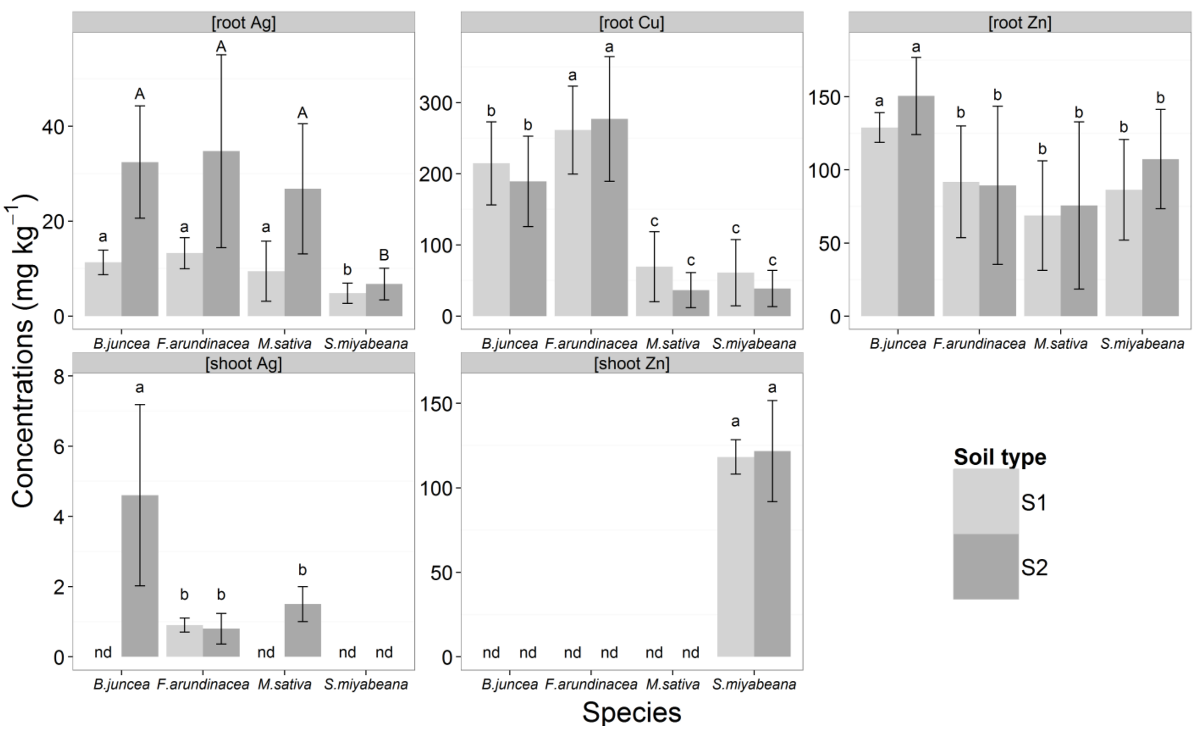

Figure 3.2 Root and shoot concentrations (mg kg^{-1}) for all species in S1 and S2 soils. Uppercase letters are used when significant differences were observed between soils (n=5). Different letters identify differences between species. The abbreviation 'nd' is used when concentrations were below detection limits (0.5, 40 and 100 mg kg^{-1} for Ag, Cu and Zn respectively)..

Root concentrations of all trace elements varied according to species in both S1 and S2 soils. Ag root concentration was equivalent in the three herbaceous species (S1 herbaceous mean = 11.33±4.08 mg kg^{-1}; S2 herbaceous mean = 34.68±15.29 mg kg^{-1}) and lower in *S. miyabeana* (4.80±2.16 mg kg^{-1} in S1; 6.74±3.33 mg kg^{-1} in S2). Cu root concentration varied between 60.50 mg kg^{-1} and 277.00 mg kg^{-1}, and was found in species in the following decreasing order: *F. arundinacea* > *B. juncea* > *M. sativa* = *S. miyabeana*. *B. Juncea* had the highest Zn root concentration of all species (139.80±18.31 mg kg^{-1}), the three others having equivalent lower root concentrations (mean = 83.93±42.58 mg kg^{-1}).

In S1 soil, aboveground concentrations of Ag were found in *F. arundinacea* (0.90±0.20 mg kg^{-1}) only. Regarding S2 soil, *B. juncea* (4.60±2.58 mg kg^{-1}), *F. arundinacea* (0.80±0.44 mg kg^{-1}) and *M. sativa* (1.50±0.50 mg kg^{-1}) had accumulated Ag in their shoots. Cu in aerial tissues of the species included in the experiment was below the detection limit (100 mg kg^{-1}). Zn concentration in shoots was only observed in *S. miyabeana* and at equivalent levels in both substrates (119.96±20.04 mg kg^{-1}). Previous studies have reported a bioaccumulation of copper and zinc in higher plants (Evlard et al., 2014; Purakayastha et al., 2008; Puschenreiter et al., 2013; Wu et al., 2011; Żurek et al., 2013), but to our knowledge, this is the first evidence of silver translocation in non-transgenic vascular plant aboveground biomass from a brownfield soil matrix.

3.4.3. Bioconcentration and translocation factors

Bioconcentration factor (BCF) is defined here as the metal concentration ratio of a given plant part per soil, and the translocation factor (TF) as the concentration ratio of plant aerial parts per underground parts. BCF was used to describe root uptake, and TF for shoot uptake. Table 3.2 shows the BCF of the four species in regard to Ag, Cu, and Zn in soils S1 and S2, with lowercase letters making comparison possible among species and substrate simultaneously. A value of 1 indicates that the same concentration was found in roots and in the corresponding soil.

Table 3.2 Bioconcentration factor (BCF) of silver (Ag), copper (Cu) and zinc (Zn) in roots of *B. juncea*, *F. arundinacea*, *M. sativa* and *S. miyabeana* grown in S1 and S2 soil.

	Ag		Cu		Zn	
	S1	S2	S1	S2	S1	S2
B. juncea	0.33±0.07ab	0.29±0.10ab	5.11±1.39abc	9.46±3.18a	0.80±0.06a	0.85±0.15a
F. arundinacea	0.38±0.09a	0.31±0.18ab	6.22±1.47ab	13.85±4.39a	0.74±0.02a	0.84±0.05a
M. sativa	0.28±0.19ab	0.24±0.12ab	2.04±1.08cd	3.03±1.10bcd	0.77±0.00a	1.01±0.00a
S. miyabeana	0.14±0.06bc	0.06±0.03c	2.10±0.93d	3.33±0.25bcd	0.68±0.08a	0.69±0.07a

Lowercase letters allow significant comparison of all species and substrates for a given TE (n=5).

3.4.3.1. Bioconcentration factor

When comparing Ag BCF for each substrate, we see interspecific differences isolating *S. miyabeana*, with a lower value, from *F. arundinacea* in S1 soil, and from the three other species in S2 soil. *F. arundinacea* had a significantly higher value for Cu root BCF than *M. sativa* and *S. miyabeana* in S1 soil; *B. juncea* and *F. arundinacea* had higher values in S2 soils. No significant differences appear in Cu BCF between substrates for any of the species. Marchiol et al. (2004) recorded much lower Cu root BCF (1.17) and slightly higher Zn root BCF (1.01) in *B. juncea*. Sekara et al., (2005) have reported *M. sativa* root concentrations equivalent to a similar BCF for Cu (2.86) and a lower BCF for Zn (0.19). Lu et al., (2014) reported lower Cu BCF for *F. arundinacea* (between 1.6 and 2.5, calculated from concentrations). *S. miyabeana* was also observed to have accumulated Cu and Zn from soil in underground tissues at lower rates (in concentrations leading to BCF of 0.31 and 0.27 respectively) (Pitre et al., 2010)

3.4.3.2. Translocation factor

The translocation factors of Ag and Zn were calculated. As mentioned earlier, Cu was not recorded in the aerial biomass of any species grown in our experiment, although it has been in previous studies in *B. juncea* (Marchiol et al., 2004), *F. arundinacea* (Zhao et al., 2013), *M. sativa* (Peralta et al., 2001) and other *Salix* species (Kuzovkina et al., 2004). Translocation of TE to aerial and harvestable plant tissues is among the most important feature in phytoremediation of TE-rich substrate because it makes it possible to remove the TE from the soil system. *B. juncea* translocated Ag at the highest rate (TF of 0.21, n=3) in S2 soil. *F. arundinacea* had an Ag TF of 0.1 in S1. *S. miyabeana* had a TF of 1.06 and 0.96 for Zn in S1 and S2 substrates respectively (n=3). Zn TF in *Salix* species has previously been observed to be between 2 and 4, with substrate concentrations between 490 and 955 mg kg^{-1} (Wieshammer *et al*. 2007), at 1.54 with a substrate concentration of 184 mg kg^{-1} (Bissonnette et al., 2010) and at about 1.3 when grown on substrate with concentrations ranging from 470 and 2044 mg kg^{-1} (Vandecasteele et al., 2002).

3.4.4. Influence of soil concentrations on root uptake

To help visualize the influence of initial total soil concentration of TE on root uptake, Figure 3.3 presents log-transformed soil and root concentrations for both substrates as well as tendency lines between their means.

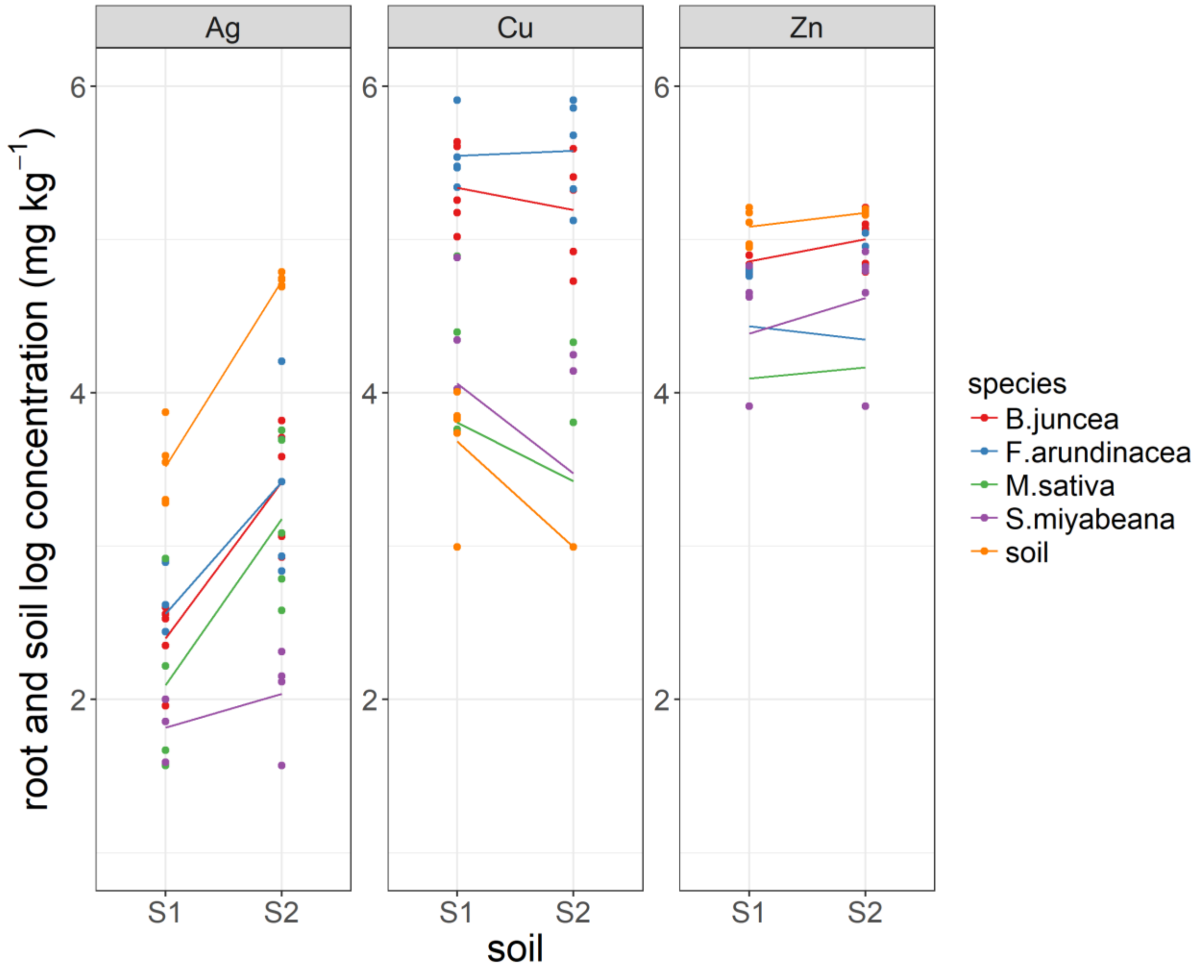

Figure 3.3 Variation of Ag, Cu and Zn soil and root concentrations between S1 and S2 soils. *B. juncea, F. arundinacea, M. sativa* and *S. miyabeana* are represented with their log-transformed concentrations (n=5).

In the previous sections, we showed that Ag concentration was significantly higher in S2 than in S1 (Table 3.1), which led to significantly higher root uptake for all species (Figure 3.2), and equivalent root BCF (Table 3.2). This implies that the variation of Ag concentrations between the two substrates (S2 having 3.3 times S1 mean Ag concentration) led to a proportional fluctuation in root concentration for each species.

A different pattern emerges from results regarding Cu. The concentration of this TE was significantly lower (2.4 times lower) in S2 soil, but led to equivalent root concentrations (Figure

3.2), as well as equivalent root BCF (Table 3.2). This may appear inconsistent; the same level of root accumulation in a soil with a lower concentration of this TE should have led to higher BCF values. This phenomenon could be attributed to a relatively high variation (high standard deviation) in our results, which may have prevented emergence of statistically significant differences in the BCF. However, Figure 3.3 still illustrates the tendency, particularly in *B. juncea* and *F. arundinacea,* to produce higher BCF values, due to equivalent Cu root concentration in both soils.

Since the two substrates were excavated from the same site and had been polluted for the same length of time by the same industrial activities (source), it seems unlikely that the distinct pattern associated to Cu phytoextraction is due to the presence in these soils of different species of Cu thus having different mobility and potential phytoavailability. Moreover, pH was constant in S1 and S2, and this parameter is known to be of primordial importance for speciation of Cu (Alloway, 2013) and most TE (Kabata-Pendias, 2010). We hypothesize that the higher organic matter content of S2 soil is the driving factor of the tendency toward higher root BCF for Cu, highlighting, at least for this particular TE, the importance of considering this variable in phytoremediation studies. Interaction between organic matter and metal is known to have a significant impact on the biogeochemical properties of the latter (Rule, 1999). McBride et al. (1997) suggested that solid organic matter limits Cu activity, whilst dissolved organic matter has the opposite effect in aged soils.

Regarding Zn uptake, Figure 3.3 shows equivalent soil and root concentration between S1 and S2 soil. This pattern is consistent with the statistically equivalent BCF results obtained between substrates (Table 3.2).

3.4.5. Estimation of extraction yield

A possible exercise to estimate *in situ* remediation potential is to extend the concentration found in plant tissues to the total crop yield (Kuzovkina et al., 2004; Nehnevajova et al., 2008; Ruttens et al., 2011). Results from such a calculation should be interpreted with caution, but small scale extension, by square meter (m^2) for instance, is useful for estimating potential extraction yield while maintaining a reasonably low level of uncertainty. We calculated extraction yield based on aerial biomass produced during one growing season in order to evaluate potential annual harvest yield. Among the estimation scenarios, *B. juncea* shows the highest potential Ag phytoextraction yield (0.39 g m^{-2}) in S2 soil. *S. miyabeana* would show a similar Zn extraction yield in both substrate types: 15.8 g m^{-2} in S1 soil and 14.1 g m^{-2} in S2 soil. No calculations were made for Cu, since none was recorded in aerial biomass of any species grown in either substrate.

3.5. Conclusion

Based on our results, *B. juncea* must be considered for use in remediation of the original site, particularly given its capacity to translocate Ag into its shoots at a relatively high rate. However, other species have also demonstrated good remediation capacities. *F. arundinacea* is the only species that had Ag in aerial parts when grown in the two substrates. The same species also showed the highest root BCF recorded for Cu (6.22±1.47 in S1 and 13.85±4.39 in S2 soil). *S. miyabeana* is also a good candidate for Zn extraction on the original site. Given this information and relating it to the site characteristics, we hypothesize that the best field results would be obtained by combining these species in a multi-species function-complementary phytosystem. A combination of *B. juncea* and *F. arundinacea* for extraction of Ag, the latter species also for immobilization of Cu, and *S. miyabeana* for extraction of Zn appears to be ideal based on our results. Further investigation of the ecological interactions between species of such a system is underway in our laboratory to verify this hypothesis.

3.6. Acknowledgement

This experiment was conducted with financial support from Mitacs. Dominic Desjardins is supported by a FRQNT BMP doctoral scholarship. The research team would like to thank all the individuals who assisted in sample collection, processing and data analysis, as well as Karen Grislis for English revision.

3.7. Synthèse du chapitre 3

L'étude présentée au chapitre 3 a révélé les aptitudes de remédiation différentes de quatre espèces de plantes pour trois contaminants inorganiques soit l'argent, le cuivre et le zinc. L'hypothèse préalablement émise stipulait qu'au moins une des quatre espèces pouvait accumuler les trois éléments, ce qui fut le cas pour *B. juncea*, mais seulement dans les tissus racinaires. Tous tissus confondus, les plus hautes concentrations des trois métaux ont été obtenues par *B. juncea* pour la phytoextraction (tiges) de l'Ag, *F. arundinacea* pour la phytostabilisation (racines) du Cu et *S. miyabeana* pour la phytoextraction (tiges) du Zn. L'étude conclut que la meilleure approche pour la phytoremédiation des sols ayant servi à l'étude consisterait à implanter un système végétal combinant ces trois espèces, de manière à rassembler des habiletés de remédiation envers chacun des éléments problématiques présents dans le sol.

C'est pour faire suite à cette étude que l'expérience présentée au chapitre 4 a été menée.

Chapitre 4 | COMPLÉMENTARITÉ FONCTIONNELLE EN PHYTOREMÉDIATION

Les habiletés de remédiation spécifiques à l'espèce mises en lumière dans le chapitre précédent constituent autant d'indices empiriques suggérant que combiner des espèces serait une approche appropriée pour la phytoremédiation des sols à contamination complexe. Dans cette optique, un essai de phytoremédiation en mésocosmes comparant le potentiel de trois espèces végétales en monoculture et en combinaisons a été mené. Les capacités d'absorption des éléments traces présents dans le sol ont servi de point de comparaison entre les différentes approches.

Objectif : Comparer les monocultures et les combinaisons d'espèces végétales pour leurs capacités de remédiation d'un sol contaminé par plusieurs éléments traces.

Hypothèse : Les assemblages d'espèces seront en mesure d'extraire du sol une quantité plus importante d'éléments traces que chacune des monocultures respectives.

COMPLEMENTARITY OF THREE DISTINCTIVE PHYTOREMEDIATION CROPS FOR MULTIPLE-TRACE ELEMENT CONTAMINATED SOIL

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4.1. Abstract

Trace element (TE) contaminated land represents an important risk to the environment and to human health worldwide. These soils usually contain a variety of TEs which can be a challenge for plant-based remediation options. As individual plant species often possess a limited range of TE remediation abilities, functional complementarity principles could be of value for remediation of soil contaminated by multiple TEs using assemblages of species. Monocultures and polycultures of *Festuca arundinacea*, *Medicago sativa* and *Salix miyabeana* were grown for 4 months in aged-polluted soil contaminated by Ag, As, Cd, Cr, Cu, Pb, Se and Zn. Above and belowground biomass yields, root surface area (RSA) and TE tissue concentrations were recorded. In monoculture, the greatest aboveground biomass was produced by *S. miyabeana* (S), the greatest belowground biomass was from *M. sativa* (M) and *F. arundinacea* (F) produced the highest RSA. The polycultures of F+M, F+S and F+M+S produced among the highest values across all three traits. *F. arundinacea* monoculture and its combination with *S. miyabeana* (F+S) accumulated the highest amounts of total TEs in belowground tissues, whereas the most effective combination (or monoculture) for aboveground extraction yields varied depending on the TE considered. The crops demonstrated complementarity in their biomass allocation patterns as well as facilitative interactions. When considering contamination with a particular TE, the best phytomanagement approach could include a specific monoculture option; however, when biomass allocation patterns, TE-remediation abilities as well as nitrogen accessibility are considered, co-cropping all three species (F+M+S) was the most robust scenario for remediation of multiple-TE contaminated land. By more effectively addressing a diversity of TE, species assemblage approaches could

represent an important advancement towards enabling the use of plants to address contaminated-land issues worldwide.

Keywords:

Phytoremediation, trace elements, soil remediation, polycultures, ecology, functional complementarity

Highlights:

- Three phytoremediation crops are grown in mono- and polyculture
- Common trace element (TE) contamination above Canadian regulation thresholds
- Polycultures matched the highest monoculture biomass and TE extraction yields
- Distinct biology of *F. arundinacea, M. sativa* and *S. miyabeana* can be complementary
- Polycultures are a promising option to improve phytoremediation system flexibility

4.1.1. Graphical abstract:

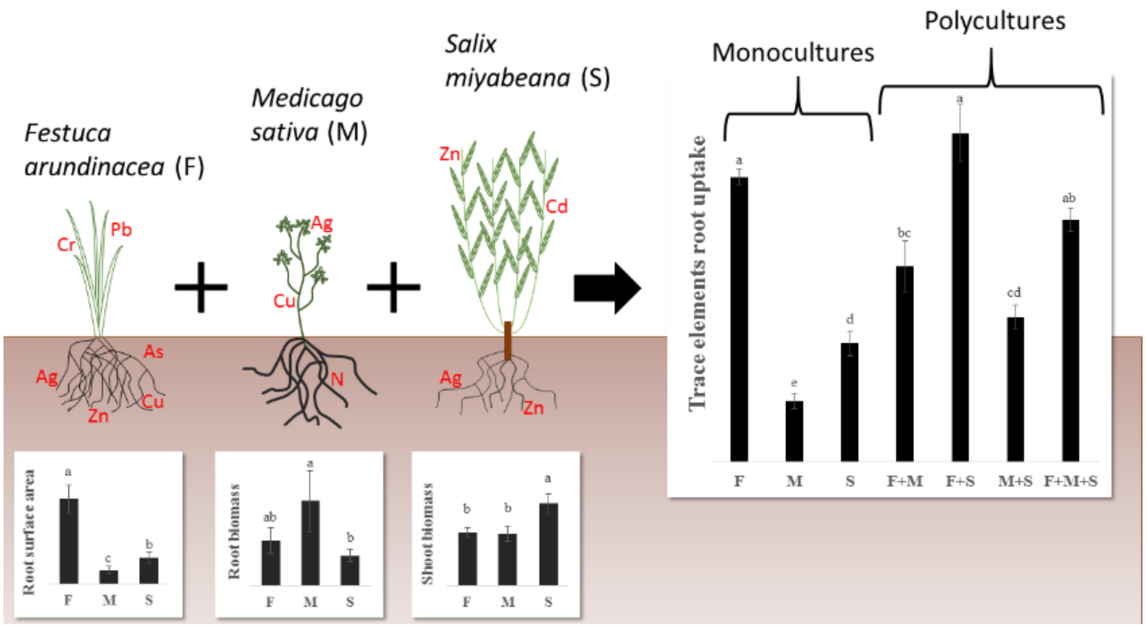

Figure 4.1 Graphical abstract representing the different biomass allocation patterns and remediation abilities of *Festuca arundinacea*, *Medicago sativa* and *Salix miyabeana*.

4.2. Introduction

Trace elements (TEs) are metals and metalloids that can be toxic to living organisms if present in their environment in sufficient bioavailable concentrations (Adriano, 2001). They are ubiquitous anthropogenic contaminants in agricultural and metropolitan soils and can represent important human and environmental risks around the world (Purves, 2012). In Europe, 342, 000 contaminated sites have been identified as in need of remediation measures although the real number is estimated at around 2.5 million (Panagos et al., 2013). TE contamination represent approximately 35% of these sites with €6.5 billion per year devoted managing such land (EEA, 2014). In China, the TE concentrations of a wide range of TEs exceed soil background values in a large number of urban areas (Luo et al., 2012), but also across agricultural regions (Wei and Yang, 2010). North America also suffers from extensive TE land contamination, 30,000 in Canada and 384,400 in the US (Simons, 1998; Sousa, 2001), compelling large scale remediation initiatives, such as United States Superfund Redevelopment Initiative (EPA, 2017) and Canada's Contaminated Site Inventory (Treasury Board of Canada Secretariat, 2017).

While conventional remediation operations for TE contaminated land is often impracticable due to the associated high costs (Grimski and Ferber, 2001), when remediation activities do occur, they can have direct detrimental impact on environmental and human health (Harbottle et al., 2008). The thresholds of TEs at which soil is considered "contaminated" vary depending on natural background levels and with the consideration of environmental and health impact often specific to countries or regions (Jennings and Petersen, 2006). There are a wide number of regulatory frameworks for defining such levels, such as the Quebec province criteria (QPC) in Canada. A less conventional approach for remediation of contaminated land currently being explored to reduce high costs and both environmental and health impacts is phytoremediation, the use of contamination tolerant plants to decontaminate and rejuvenate soils (Pulford and Watson, 2003; Vara Prasad and de Oliveira Freitas, 2003). Setting phytoremediation objectives based on local legislation, such as QPC, may help in establishing communication with landowners and promote phytomanagement alternatives (Cundy et al., 2013). Phytomanagement is the combination of a phytoremediation processes, such as phytoextraction (uptake and translocation of TEs aboveground) and phytostabilisation (reduced TE mobility to migration pathways), with the production of valuable biomass on contaminated land (Kidd et al., 2009; Robinson et al., 2009).

Increased diversity in cultivated plant species, such as using polyculture over monoculture, can have positive effects which increase biomass yield per area of land (Smith et al., 2008). These beneficial effects can be attributed to interactions between plant species, such as stress resistance caused by TE contamination (Craven et al., 2016; L. Li et al., 2014; Michalet et al., 2006; Wang et al., 2014). Functional diversity and complementarity could be selected to help positive species interactions to occur, providing ecological services (Faucon et al., 2017) such as soil remediation, as individual plant species may possess only a limited set of remediation abilities (and therefore fulfil a limited set of remediation functions) (Sarma, 2011). As TE soil contamination is often complex in both contaminant variety (Van der Perk, 2012; Zhao et al., 2015) and distribution (Desjardins et al., 2014; French et al., 2006), multiple-TEs could be phytomanaged by using assemblages of species grown in polyculture if phytoremediation abilities were complementary.

To explore this potential for complementarity, a polyculture trial was conducted, involving: *Festuca arundinacea* Scherb (tall fescue), a herbaceous species of the *Poaceae* family with an extensive fine-root system (Picon-Cochard et al., 2012), *Medicago sativa* L. (alfalfa), a herbaceous species of the *Fabaceae* family capable of association with *rhizobium* for nitrogen fixation (Tate, 1995) and *Salix miyabeana* \`SX67\`, a fast growing woody crop (Guidi Nissim et al., 2013). These three species have been studied as potential phytoremediation crops (Albornoz et al., 2016; Desjardins et al., 2016; Marchand et al., 2016) and can produce high yields in temperate climates with low maintenance requirement. All species are compared in monoculture and polyculture using aged multiple-TE contaminated soil from a former industrial site in the region of Montréal, Canada.of Montréal, Canada.

4.3. Methods

4.3.1. Site and treatment

The experiment was conducted outdoors on the site of the Montréal Botanical Garden (MBG; 45.563788, -73.562837, altitude = 26m) from mid-May to mid-September (122 days). Annual average climatic characteristics are as follow: temperature = 6.8ºC, liquid precipitations = 784mm, solid precipitations = 210cm, growing degree-days < 1800 (Environment Canada, between 1981 and 2010). 100 dm^3 plastic containers were used: 37 cm height, 45 cm width and 60 cm length. Soil (Table 4.1) was excavated from the site of a former petroleum refinery in Varennes, southern Quebec, Canada. The soil was mixed thoroughly to reduce pollution heterogeneity. Three plant species: *Festuca arundinacea* Scherb. (F), *Medicago sativa* L. (M) and *Salix miyabeana* \`SX67\` (S) were cultivated in monoculture (F, M and S alone) and polyculture (F+M, F+S, M+S and F+M+S) according to a replacement series design where initial density is constant among treatments (Ewel et al., 2015; Jolliffe, 2000). The equivalent of 15, 7.5 and 5 kg ha^{-1} of seeds or 6, 3 and 2 willow cuttings were introduced in the 1, 2 and 3 species treatments, respectively. Cuttings were planted in one or two rows (where six cuttings were present), mimicking small intercropping cultures. The randomized block experimental design consisted of 32 mesocosms ((7 treatments + 1 control) x 4 replicates). A double layer bottom in each experimental unit allowed water drainage without leaching to the environment.

4.3.2. Soil and plant tissue analyses

Soil samples were collected at the beginning (May 15th) and end (September 13th) of the experiment, and frozen (-20ºC) until analyzed. Samples destined for TE measurements were dried until constant weight and sieved to <500µm. For determination of the water extractable fractions of TE, 4.0g of soil was mixed with 40ml of MilliQ water and shaken for 2 hours. Samples were then centrifuged for 15m at 1400 G. This was followed by filtration through a 0.45µM nylon membrane. A 15ml aliquot was kept for analysis, for which 0.04ml HNO_3 50% (v/v) was added as a preservative (Hendershot et al., 2008). For total recoverable TE determination, 200mg of soil and plant material was incorporated to 2ml of HNO_3 70% (V/V) (trace metal grade) for 16 hours before digestion at 120ºC for 5 hours. The resulting digested material was mixed with MilliQ water to a total volume of 50ml. After overnight resting, samples were filtered through 0.45µM nylon in-line filters (Wilson et al., 2005). Soil samples were diluted 10X and plant tissues 2X before being analyzed by ICP-MS at the Chemistry Department of the Université de Montréal for concentrations of eight TE, namely Ag, As, Cd, Cr, Cu, Pb, Se and Zn (Table 4.1). These TE were present above detection limits (Ag=0.003, As=0.089, Cd=0.004, Cr=0.075, Cu= 0.005, Pb=0.001, Se=0.227, Zn=0.034 mg kg^{-1}) in all soil samples (n=32).

Table 4.1 Properties of the soil used in the diversity experiment

Parameter	mean	QPC	unit	samples
Al	453.3 (55.2)		mg kg^{-1}	n=4
Ca	8660.9 (401.7)			
Cl	5.1 (1.4)			
Fe	309.2 (23.8)			
K	196.1 (6.9)			
Mg	381.2 (22.8)			
Mn	37.7 (4.3)			
Na	81.8 (6.3)			
P	25.1 (1.0)			
C.E.C.	47.0 (2.2)		$cmol_{(+)}$ kg^{-1} $100g^{-1}$	
pH-H_2O	7.9 (0.2)		log	
N	0.18 (0.02)		%	
Org. mat.	7.8 (3.2)			
clay	20.6 (3.0)			
sand	52.3 (3.8)			
silt	27.1 (2.0)			
C/N	30.5 (0.5)		ratio	
Recoverable (HNO_3) concentrations of trace elements				
Ag	**85.7** (53.4)	2.0	mg kg^{-1}	n=32
As	3.4 (0.4)	6.0		
Cd	0.9 (0.1)	1.5		
Cr	67.0 (6.1)	100.0		
Cu	**79.3** (54.8)	50.0		
Pb	**66.3** (77.9)	50.0		
Se	0.5 (0.1)	1.0		
Zn	**230.1** (26.6)	110.0		
Water-soluble (H_2O extraction) concentrations of trace elements				
Ag-H_2O	0.51 (0.48)		µg kg^{-1}	n=32
As-H_2O	21.05 (0.53)			
Cd-H_2O	0.32 (0.07)			
Cr-H_2O	15.7 (2.49)			
Cu-H_2O	165.7 (10.8)			
Pb-H_2O	0.85 (0.17)			
Se-H_2O	5.44 (0.69)			
Zn-H_2O	48.01 (19.67)			

Mean values of various soil properties. Values in bold exceed Quebec province criteria (QPC) of trace element soil concentration for residential land use. C.E.C. = cation exchange capacity.

Biomass dry weight (DW) yield (g m^{-2}) of shoots and roots were measured separately, as well as root surface area (RSA, cm^2 of root per dm^3 of soil). RSA was obtained by optical scanning of clean root systems using a flat-bed scanner (400 DPI, Perfection V700 Photo, Epson) before image analysis with *WinRhizo Reg* 2012a software (Regent Instruments Inc., 2012). TE tissue concentrations are expressed as mg per kg of biomass whereas TE accumulation (belowground) and extraction (aboveground) are expressed in mg per square meter of land (mg TE m^{-2}). Nitrogen proportion (%) of plant tissue was measured using 2.5 mg of ground sample material by a *Fisons* C, H, N, S/O analyzer (CHNS-O EA1108) at the Biological Science Department of the Université de Montréal. Al, Ca, Cl, Fe, K, Mg, Mn, Na and P values were determined according to Mehlich (1984). Organic matter fraction was calculated following loss on ignition at 575°C for 15min (Rabenhorst, 1988). Soil texture was obtained by Bouyoucos method (Bouyoucos, 1962). pH was obtained by electrometric method (CEAEQ, 2014). C.E.C. was obtained by NH_4^+ saturation of soil at pH 7.0 followed by NaCl washing (CRAAQ, 2010)

4.3.3. Measurement indices and statistical analysis

Multiple-TE Accumulation (MTEA) belowground and Multiple-TE Extraction (MTEE) aboveground were used to compare uptake of several TE simultaneously. A geometric mean, the *n*th root of the product of *n* numbers, is employed for MTEE and MTEA as more appropriate for allowing comparison of multiple TEs (Ebert and Welsch, 2004) (as opposed to arithmetic mean). The selection of TEs included in the calculation of the MTEA and MTEE was based on local legislated environmental limits (Québec Province Criteria, QPC), which are set at 2.0 mg kg^{-1} for Ag, 6.0 mg kg^{-1} for As, 1.5 mg kg^{-1} for Cd, 100.0 mg kg^{-1} for Cr, 50.0 mg kg^{-1} for Cu, 50.0 mg kg^{-1} for Pb, 1.0 mg kg^{-1} for Se and 110.0 mg kg^{-1} for Zn (Gouvernement du Québec, 1998) (Table 4.1). MTEA and MTEE were calculated as follow:

$$MTEA = \sqrt[4]{[rAg][rCu][rPb][rZn]}$$

$$MTEE = \sqrt[4]{[sAg][sCu][sPb][sZn]}$$

where *[rTE]* is the root concentration of a TE and *[sTE]* is the shoot concentration of a TE.

Statistical analyses were performed using R software (R Development Core Team, 2008) and SAS JMP v.10.0.0 (SAS Institute Inc, 2012). A mixed-model ANOVA (with the bloc factor as a random variable) along with Tukey's HSD post hoc test were performed to compare biomass, root surface area (RSA), plant tissues TE concentrations, plant TE uptake and nitrogen content. Student's t-test was used for comparisons of observed and expected yields. Log transformation, or box-box transformation when log was insufficient, were used when necessary to meet normality and homoscedasticity.

4.4. Results

4.4.1. Biomass

4.4.1.1. Aboveground biomass

Salix miyabeana produced the most aboveground biomass (Tukey, $p < 0.05$) of the three species grown in monoculture, with an aboveground biomass yield of 546 (± 67) dry weight (DW) g m^{-2} of TE contaminated soil, whereas *Medicago sativa* produced 347 (± 51) DW g m^{-2} and *Festuca arundinacea* produced 352 (± 31) DW m^{-2} (Figure 4.2A).

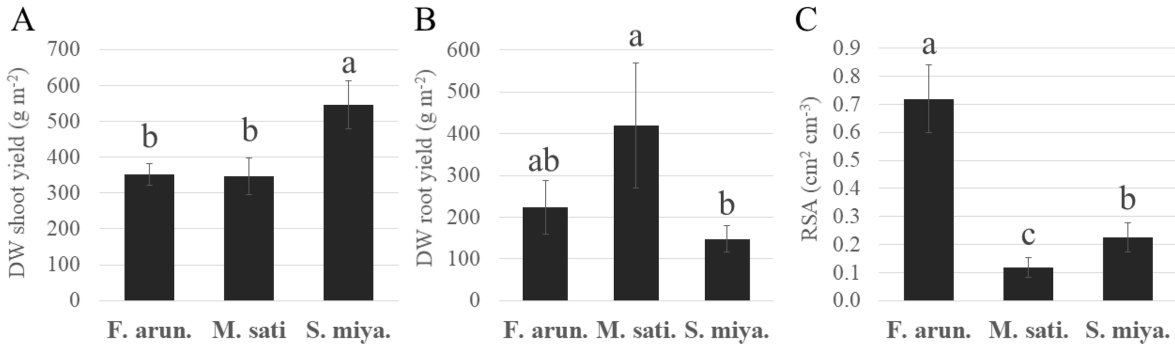

Figure 4.2 Biomass allocation of *F. arundinacea*, *M. sativa* and *S. miyabeana* grown in monoculture. Mean values of A) shoot biomass, B) root biomass and C) root surface area (RSA) of *F. arundinacea*, *M. sativa* and *S. miyabeana* grown in monoculture. Different letters identify significant differences (Tukey, $p < 0.05$). Error bars represent standard error ($n = 4$ mesocosms plots).

The total aboveground yield of the four polycultures were not significantly different from *S. miyabeana* monoculture (Figure 4.3A).

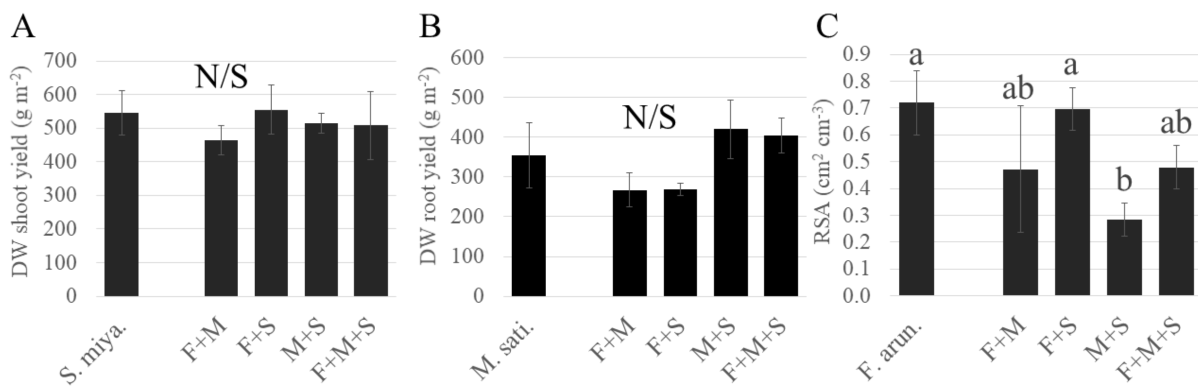

Figure 4.3 Biomass allocation of *F. arundinacea*, *M. sativa* and *S. miyabeana* grown in polycultures. Mean values of A) shoot biomass, B) root biomass and C) root surface area (RSA) of *F. arundinacea* (F), *M. sativa* (M) and *S. miyabeana* (S) grown in polycultures compared to the most productive monoculture for each trait. Different letters identify significant difference (Tukey, $p < 0.05$). Error bars represent standard error ($n = 4$ mesocosms plots).

When polyculture assemblages were compared with their respective monocultures, all polycultures which included *S. miyabeana* produced an equivalent amount of biomass to *S. miyabeana* monoculture. In contrast to this, the polyculture F+M produced a significantly higher amount of aboveground biomass than both respective monocultures (Tukey, $p < 0.05$) (Figure

S4.1A). When comparing the observed aboveground yields of each species in polyculture to those expected (based on monoculture yields), observed yields of *F. arundinacea* were significantly greater (t-test, $p < 0.05$) by 84 % when co-cropped with *M. sativa* (F+M), by 48% when co-cropped with *S. miyabeana* (F+S) and by 65% when co-cropped with the two other species (F+M+S) (Figure 4.4A).

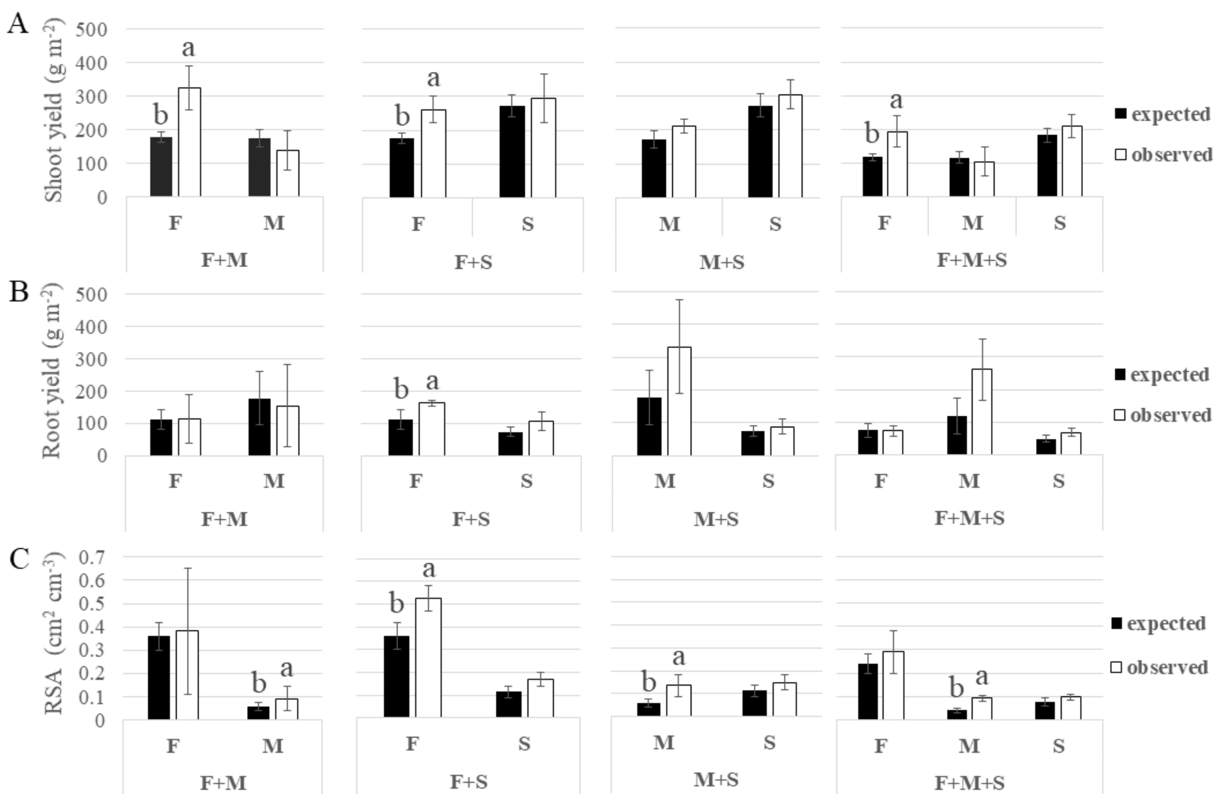

Figure 4.4 Observed and expected biomass of *F. arundinacea*, *M. sativa* and *S. miyabeana*. Mean values of observed and expected (based on monoculture results) A) shoot biomass, B) root biomass and C) root surface area (RSA) of polycultures components. F= *F. arundinacea*, M= *M. sativa*, S= *S. miyabeana*. Different letters identify significant differences (Tukey, $p < 0.05$). Error bars represent standard error (n = 4 mesocosms plots).

4.4.1.2. Belowground biomass

Root biomass production of monocultures was greatest in *M. sativa* and was significantly more (Tukey, $p < 0.05$) than *S. miyabeana* (Figure 4.2B). Root productivity of *F. arundinacea* was intermediary, in being not significantly different from the other species. Total root yields of plant polycultures were all not significantly different from M. sativa monoculture (Figure 4.3B).

Polycultures which included *M. sativa* all produced a total belowground biomass comparable to *M. sativa* in monoculture (B). When comparing the belowground yields of each species in polyculture to those expected from relative monoculture yields, *F. arundinacea*, when mixed with *S. miyabeana* (F+S), had an observed yield significantly (t-test $p < 0.05$) higher, by 45%, than the expected yield (Figure 4.4B).

4.4.1.3. Root surface area (RSA)

The three species had significantly different root surface areas (RSA) when grown in monoculture (Figure 4.2). The total RSA of the plant polycultures was not different from *F. arundinacea*, at the exception of the significantly smaller M+S, with 0.28 (± 0.06) cm^2 cm^{-3} (Tukey, $p < 0.05$) (Figure 4.3C).

Polycultures of F+M and F+S produced RSA equivalent to *F. arundinacea* monoculture (Figure S4.1). The polyculture of M+S had RSA equivalent to *S. miyabeana* in monoculture. Polyculture of the three species (F+M+S) produced a total RSA significantly lower than *F. arundinacea* in monoculture, but greater than *M. sativa* and *S. miyabeana* monoculture (Tukey, $p < 0.05$). When comparing the observed RSA of each species in polyculture to those expected from monocultures results, in F+S, *F. arundinacea* had an observed RSA significantly higher than expected by 46% (t-test, $p < 0.05$). In F+M, M+S and F+M+S, *M. sativa* also had an observed RSA significantly higher than expected (t-test, $p < 0.05$) by 54%, 128% and 134% respectively (Figure 4.4C).

4.4.1.4. Nitrogen tissue content

The nitrogen percentage in the aboveground and belowground tissues of the three species grown in monoculture was compared to their nitrogen percentage in polyculture. There were no significant differences between any of the species in shoot nitrogen percentage in polyculture, with *F. arundinacea* shoot nitrogen percentage ranging from 1.10 % (± 0.03) to 1.29 % (± 0.11), *M. sativa* shoot nitrogen percent ranging from 2.85 % (± 0.29) to 3.09 % (± 0.37) and *S. miyabeana* shoot nitrogen percentage ranging from 0.95 % (± 0.19) to 1.22 % (± 0.14) (Table S4.1).

The nitrogen percentage in roots of *F. arundinacea* was the lowest in monoculture (0.75 % ± 0.08) and when in presence of *S. miyabeana* (0.89 % ± 0.03), whereas it was significantly higher than in monoculture when co-cropped with both *M. sativa* and *S. miyabeana,* with 1.11 % (± 0.12), and with *M. sativa*, with 1.14 % (± 0.12) (Tukey, $p < 0.05$) (Figure 4.5).

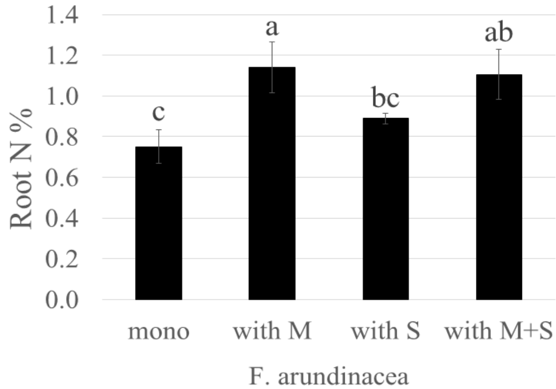

Figure 4.5 Nitrogen (N) percentage in roots of *F. arundinacea* (F) in monoculture or in polyculture with *M. sativa* (M) and *S. miyabeana* (S). Values are mean of 4 mesocosms plots. Different letters identify significant differences (Tukey, $p < 0.05$).

The nitrogen percentage in roots of *M. sativa* was not significantly different between polycultures, ranging from 0.96 % (± 0.11) to 1.28 % (± 0.42), or in roots of *S. miyabeana* between different polycultures, ranging from 0.78 % (± 0.11) to 0.89 % (± 0.19).

4.4.2. Trace element concentrations

4.4.2.1. Aboveground concentrations

The three monocultures were compared for the aboveground and belowground concentrations of eight TEs present in the soil samples: Ag, As, Cr, Cd, Cu, Pb, Se and Zn. A number of shoots had highly variable levels of Ag, Cu and Pb however; substantial and significant variation was identified between the shoot concentrations of the monocultures for Ag, Cr, Cu, Pb and Zn (Table 4.2).

Table 4.2 Shoots trace element concentrations

	Ag (mg kg^{-1})		As (mg kg^{-1})		Cd (mg kg^{-1})		Cr (mg kg^{-1})	
F. arundinacea	5.34(1.13)	a	0.68(0.13)		1.32(0.17)	b	12.23(1.83)	a
M. sativa	10.75(5.91)	a	1.13(0.47)	ns	0.48(0.06)	c	21.80(10.38)	a
S. miyabeana	0.38(0.04)	b	0.86(0.05)		7.92(0.36)	a	4.77(0.13)	b
	Cu (mg kg^{-1})		**Pb** (mg kg^{-1})		**Se** (mg kg^{-1})		**Zn** (mg kg^{-1})	
F. arundinacea	80.98(53.60)	ab	200.63(192.54)		0.22(0.06)		153.95(17.23)	b
M. sativa	104.47(32.10)	a	76.86(43.39)	ns	0.30(0.06)	ns	200.30(9.83)	b
S. miyabeana	25.72(0.83)	b	9.23(1.91)		0.27(0.05)		637.36(24.59)	a

Monoculture mean values (n=4 mesocosms plots) ± standard error (brackets) of trace element (TE) concentrations (mg kg^{-1}) in shoots of *F. arundinacea*, *M. sativa* and *S. miyabeana*. Different letters identify significant differences (Tukey, $p < 0.05$). ns = no significant differences.

The shoot concentrations of TE being statistically not different ranged for As from 0.68 (± 0.13) to 1.13 (± 0.47) mg As kg^{-1}, for Se from 0.22 (± 0.06) to 0.30 (± 0.06) mg Se kg^{-1} and for Pb from 9.2 (± 1.9) to 200.6 (± 192.5) mg Pb kg^{-1}. *F. arundinacea* and *M. sativa* Ag shoot concentrations were not significantly different, but were both significantly higher than that of *S. miyabeana*. Similarly, *F. arundinacea* and *M. sativa* Cr shoot concentrations were not significantly different, but were again both significantly higher than in *S. miyabeana*. *M. sativa* had higher Cu shoot concentrations than *S. miyabeana*, while *F. arundinacea* was not significantly different from the two others. *S. miyabeana* had the highest concentrations of Cd and Zn of the three species

In polycultures, the TE concentration in the shoots of some species were significantly different from their concentration in monocultures. For *S. miyabeana* in the presence of *M. sativa* (M+S), As significantly decreased from 0.86 ± 0.05 to 0.60 ± 0.02 mg As kg^{-1} and Zn significantly decreased from 637.4 (± 24.6) to 495.6 (± 7.1) mg Zn kg^{-1} (Tukey, $p < 0.05$) (Figure S4.2A and

B). In *M. sativa* in the presence of *F. arundinacea* (F+M), Cd significantly decreased from 0.50 (± 0.06) to 0.31 ± (0.03) mg Cd kg^{-1} (Figure S4.2C).

4.4.2.2. Belowground concentrations

F. arundinacea had significantly greater root concentration of Cu (1354.3 ± 210.2 mg kg^{-1}) than *S. miyabeana* (Tukey, $p < 0.05$) (Table 4.3).

Table 4.3 Roots trace element concentrations

	Ag (mg kg^{-1})		**As** (mg kg^{-1})		**Cd** (mg kg^{-1})		**Cr** (mg kg^{-1})	
F. arundinacea	178.84 (30.35)	a	5.97 (0.92)	a	3.12 (0.39)	a	165.55 (29.86)	a
M. sativa	22.33 (4.37)	b	0.86 (0.16)	b	0.77 (0.11)	b	27.09 (5.26)	b
S. miyabeana	101.69 (8.48)	a	3.81 (0.39)	a	2.93 (0.19)	a	92.37 (16.97)	a
	Cu (mg kg^{-1})		**Pb** (mg kg^{-1})		**Se** (mg kg^{-1})		**Zn** (mg kg^{-1})	
F. arundinacea	1354.32 (210.22)	a	94.34 (15.00)	a	1.80 (0.09)	a	747.76 (86.53)	a
M. sativa	101.69 (12.26)	c	19.60 (3.65)	b	0.28 (0.08)	b	153.19 (18.18)	b
S. miyabeana	687.35 (53.77)	b	59.19 (9.12)	a	1.38 (0.11)	a	540.28 (33.35)	a

Monoculture mean values (n=4 mesocosms plots) ± standard error (brackets) of root trace element (TE) concentrations in roots of *F. arundinacea* (F), *M. sativa* (M) and *S. miyabeana* (S). Different letters identify significant differences (Tukey, $p < 0.05$). ns = no significant differences.

Ag, As, Cd, Cr, Pb, Se and Zn root concentrations were not significantly different between *F. arundinacea* and *S. miyabeana,* and were both significantly higher than in *M. sativa*. There were no significant differences in root TE concentrations between monoculture and polyculture, for any of the species. TE tissues concentrations of species grown in polyculture is available in supplementary material (Table S4.2).

4.4.3. Trace element uptake

Each species and species assemblage was compared to investigate which could accumulate the highest amount of TEs.

4.4.3.1. Trace element root accumulation

Comparisons of all monoculture and polycultures identified significant differences of accumulation yields for each TE (Tukey, $p < 0.05$) (Table 4.4).

Table 4.4 Trace element accumulation (root) yields

Species	Ag ($mg\ m^{-2}$)		As ($mg\ m^{-2}$)		Cd ($mg\ m^{-2}$)		Cr ($mg\ m^{-2}$)	
F	37.84 (4.05)	a	1.25 (0.08)	a	0.66 (0.04)	ab	34.35 (2.72)	a
M	6.86 (0.59)	c	0.27 (0.03)	c	0.26 (0.05)	c	8.37 (1.20)	b
S	14.85 (1.65)	bc	0.56 (0.07)	bc	0.42 (0.03)	bc	13.37 (2.51)	b
F+M	16.67 (4.22)	bc	0.46 (0.10)	bc	0.52 (0.10)	bc	12.73 (2.76)	b
F+S	50.44 (6.27)	a	1.00 (0.12)	a	0.89 (0.07)	a	27.25 (2.47)	a
M+S	16.01 (1.95)	bc	0.43 (0.04)	bc	0.45 (0.05)	bc	8.96 (0.86)	b
F+MS	21.91 (1.12)	b	0.61 (0.03)	b	0.51 (0.02)	bc	15.02 (0.77)	b
	Cu ($mg\ m^{-2}$)		**Pb** ($mg\ m^{-2}$)		**Se** ($mg\ m^{-2}$)		**Zn** ($mg\ m^{-2}$)	
F	283.94 (10.51)	a	19.75 (1.34)	ab	0.40 (0.05)	ab	160.01 (7.75)	abc
M	33.30 (5.50)	c	6.25 (1.15)	b	0.09 (0.03)	d	49.86 (7.15)	d
S	99.06 (5.60)	c	8.59 (1.39)	ab	0.20 (0.02)	cd	79.77 (10.18)	d
F+M	244.85 (55.50)	ab	21.57 (3.93)	a	0.18 (0.05)	cd	104.11 (23.57)	bcd
F+S	349.32 (17.36)	a	19.11 (3.59)	ab	0.43 (0.02)	a	182.84 (15.78)	a
M+S	128.88 (8.76)	bc	13.62 (2.83)	ab	0.18 (0.03)	cd	83.97 (6.37)	cd
F+M+S	249.95 (42.04)	a	20.63 (4.69)	a	0.26 (0.01)	bc	174.69 (28.92)	ab

Mean values (n=4 mesocosms plots) ± standard error (brackets) of the root accumulation yield ($mg\ m^{-2}$) of *F. arundinacea* (F), *M. sativa* (M) and *S. miyabeana* (S) in monocultures and polycultures for eight trace elements. Different letters identify significant differences between treatments (Tukey, $p < 0.05$).

F. arundinacea and F+S accumulated similar amounts of Ag that were both significantly higher from the other species or species assemblages. Similarly, the root accumulation yields of As were significantly higher for *F. arundinacea* and F+S than all other species or species assemblages. The root accumulation yields of Cd were not significantly different under F+S and *F. arundinacea*, and only F+S was significantly higher than other species or species assemblages. Cr accumulation yields were not significantly different in *F. arundinacea* and F+S, but both were significantly higher than the other species or species assemblages. Cu accumulation yields were not significantly different for *F. arundinacea*, F+M, F+S and F+M+S and all but F+M were significantly higher than Cu accumulated by *M. sativa*, *S. miyabeana* and M+S. The Pb accumulation yields of F+M and F+M+S were significantly higher than *M. sativa*, while the other species and species assemblages were not significantly different from these. Se root accumulation yields were similar and not significantly different between F+S and *F. arundinacea,* while only the F+S was significantly higher than F+M+S. All other species and species assemblages accumulated amounts of Se ranging from 0.09 to 0.20 mg Se m^{-2}). Zn root accumulation yields of F+S, F+M+S and *F. arundinacea* were similar and not significantly different, with only F+S being significantly higher than the other species and species assemblages.

4.4.3.2. Trace element extraction aboveground

Each species and species assemblage was compared altogether to investigate which cropping approach could extract the highest amount of TEs. Significant differences between TE extraction yields were identified for five elements, namely Ag, Cd, Cr, Cu and Zn (Table 4.5).

Table 4.5 Trace element extraction (shoot) yields

Species	Ag (mg m^{-2})		As (mg m^{-2})		Cd (mg m^{-2})		Cr (mg m^{-2})	
F	1.84 (0.30)	a	0.24 (0.03)		0.46 (0.05)	c	4.22 (0.42)	ab
M	4.11 (2.56)	a	0.42 (0.21)		0.18 (0.03)	d	8.28 (4.58)	ab
S	0.21 (0.03)	b	0.46 (0.02)		4.34 (0.41)	a	2.60 (0.13)	b
F+M	3.10 (0.67)	a	0.35 (0.05)	ns	0.49 (0.05)	c	6.73 (1.17)	a
F+S	1.29 (0.26)	a	0.35 (0.01)		2.69 (0.15)	b	4.36 (0.47)	ab
M+S	2.72 (0.25)	a	0.52 (0.07)		2.29 (0.14)	b	7.52 (1.49)	a
F+M+S	1.86 (0.64)	a	0.36 (0.07)		1.85 (0.19)	b	5.77 (1.65)	a

	Cu (mg m^{-2})		Pb (mg m^{-2})		Se (mg m^{-2})		Zn (mg m^{-2})	
F	26.18 (16.12)	ab	62.20 (59.22)		0.08 (0.01)		53.53 (4.18)	d
M	37.71 (12.75)	a	29.81 (18.22)		0.11 (0.03)		69.99 (8.64)	cd
S	14.01 (0.79)	b	5.02 (0.98)		0.15 (0.03)		349.24 (31.4)	a
F+M	32.61 (11.14)	ab	10.68 (2.05)	ns	0.12 (0.02)	ns	79.19 (4.08)	c
F+S	16.11 (1.53)	ab	4.69 (0.501)		0.11 (0.02)		225.23 (7.80)	b
M+S	24.16 (1.39)	ab	8.31 (2.45)		0.20 (0.02)		193.79 (7.37)	b
F+M+S	31.67 (9.69)	ab	48.92 (38.08)		0.12 (0.02)		178.02 (3.34)	b

Mean values (n=4 mesocosms plots) ± std. err. (brackets) of the shoot extraction yield (mg m^{-2}) of *F. arundinacea* (F), *M. sativa* (M) and *S. miyabeana* (S) in mono and polycultures for eight trace elements. Different letters = significant differences between treatments ($p < 0.05$). ns = no significant differences.

Ag extractions yields were not significantly different between species or species assemblages, ranging from 1.3 to 4.1 mg Ag m^{-2}, with the exception of *S. miyabeana* monoculture, which extracted a significantly lower (Tukey, $p < 0.05$) amount of Ag of 0.21 (± 0.03) mg Ag m^{-2}. Cd extraction yields varied and formed four significantly different groups: *S. miyabeana* had the highest Cd extraction yield, followed by a second group of F+S, M+S and F+M+S, while *F. arundinacea* and F+M extracted equivalent amounts of Cd and finally, *M. sativa* extracted the least. Cr extraction yields were significantly different between *S. miyabeana* and a group of F+M, M+S and F+M+S, while the other treatments were not significantly different from the previous. Cu extraction yields were significantly different between *S. miyabeana* and *M. sativa*, while *F. arundinacea* and all of the species assemblages were not significantly different. The Zn extraction yield of *S. miyabeana* was significantly higher than a group formed of F+S, M+S and F+M+S, while F+M, *M. sativa* and *F. arundinacea* extracted significantly lower amounts than the other species or species assemblage. The extraction yields of As, Pb and Se did not vary significantly between species or species assemblages.

4.4.3.3. Multiple-TE Accumulation belowground and Multiple-TE Extraction aboveground

Multiple-TE accumulation (MTEA) into roots was calculated using the geometric mean of the accumulation yields from TEs exceeding the Quebec province criteria (QPC) for residential land use, namely Ag, Cu, Pb and Zn (Table 4.1). The comparison of the root MTEA yields identified that *F. arundinacea* (75.9 ± 2.1 mg m^{-2}), F+S (87.5 ± 7.6 mg m^{-2}) and F+M+S (64.4 ± 3.1 mg m^{-2}) were not significantly different, but that only *F. arundinacea* and F+S were significantly higher (Tukey, $p < 0.05$) than root MTEA values of F+M (52.1 ± 6.7 mg m^{-2}) (Figure 4.6A).

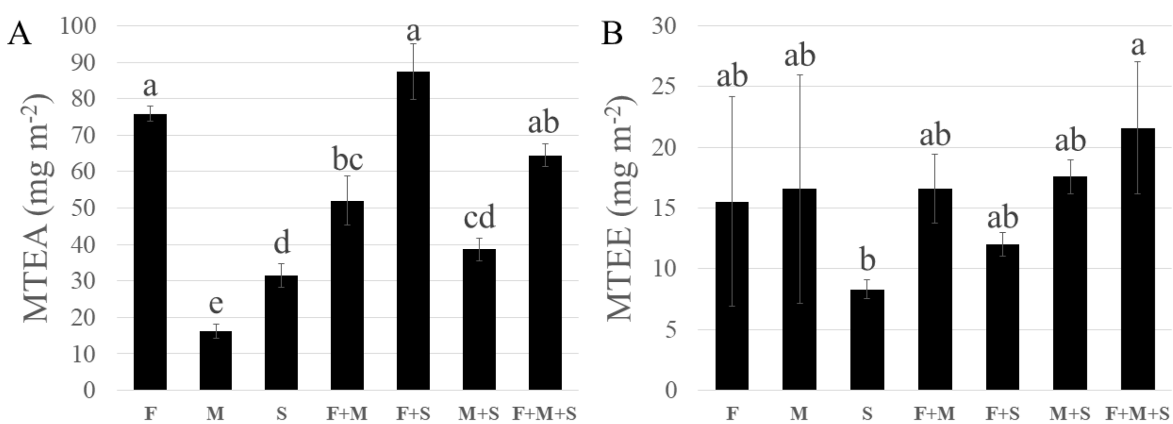

Figure 4.6 Multiple-TE Accumulation (MTEA) and Multiple-TE Extraction (MTEE) yield of *F. arundinacea* (F), *M. sativa* (M) and *S. miyabeana* (S) in single and mixed-cropping system.Bars are mean values (n=4 mesocosms plots) ± standard error. Different letters identify significant differences (Tukey < 0.05). MTEE and MTEA were obtained by calculating the geometric mean of the uptake yields of the trace elements.

Multiple-TE Extraction (MTEE) aboveground was calculated using the geometric mean of the extraction yields, again using the TEs exceeding QPC (Ag, Cu, Pb and Zn) (Figure 4.6B). F+M+S extracted significantly more (Tukey, $p < 0.05$) of these TEs (21.6 ± 5.5 mg m^{-2}) than *S. miyabeana* (8.3 ± 0.8 mg m^{-2}), while the other species and species assemblages were not significantly different from one another, ranging from 12.0 to 17.6 mg m^{-2}.

4.5. Discussion

4.5.1. Aboveground biomass

Salix miyabeana was the most productive monoculture aboveground. This is perhaps unsurprising as it is the only fast-growing tree species used and due the use of cuttings, which is the conventional planting practice, provides an increased resource source at establishment compared to seed. *Festuca arundinacea*, *Medicago sativa* and the genus *Salix* usually have overlapping yield ranges (Laurent et al., 2015) when grown under less challenging conditions (non-contaminated soil), so the difference observed here may also reflect a particular tolerance of *S. miyabeana* to this contamination or to contaminated soils in general.

All the species assemblages yielded similar amounts of aboveground biomass. Every combination of species which included *S. miyabeana* yielded as much biomass as *S. miyabeana* in monoculture. This observation could be attributed to a mechanism of dominance (Aarssen, 1997; Huston, 1997) (also referred to as a selection effect), where productivity of a plant species assemblage is determined by its most productive species in monoculture. While there seems to be no gain in productivity by using assemblages of species including *S. miyabeana* over *S. miyabeana* in monoculture, these findings do suggest that by using a polyculture including *S.*

miyabeana it is possible to introduce other species, that possess different and potentially complementary ecological functions and TE-remediation abilities, without any loss in biomass productivity.

Interestingly, the species assemblage F+M produced more than each of its respective species grown in monoculture. This evidence of a positive effect of co-cropping in these two species could arise from a facilitation mechanism (L. Li et al., 2014) occurring in a stressful environment such as TE-contaminated soil (Wang et al., 2014). Depending on environmental conditions, interactions between plant species have the potential to shift from competition to facilitation under a certain level of environmental stress (Michalet et al., 2006). One potential mechanistic explanation could result from the higher percentage of nitrogen in roots of *F. arundinacea* (from 0.75% to 1.14%) when co-cropped with the leguminous *M. sativa* (Figure 4.5). This highlights the positive impact a nitrogen fixator can have in species assemblages, by introducing additional atmospheric nitrogen into the system (Moukoumi et al., 2012) and potentially reducing the long term fertilization requirement of a co-cropped plantation (Strecker et al., 2015). While the findings presented here are from study of one growth season alone, these observations do allow for positive expectations longer term.

The yield of each species component within polycultures were compared with their *expected yield* (De Wit, 1960), predicted from monoculture yields. Expected yield has been widely used to compare plant performance in monocultures and mixtures (Weigelt and Jolliffe, 2003). *F. arundinacea* produced more aboveground biomass than expected in every polyculture combinations where it was present i.e. F+M, F+S and F+M+S by 84%, 48% and 65% respectively. This could be due to a reduction of the relative intraspecific competition of *F.*

arundinacea in polyculture (Iverson et al., 2014). If true, this would suggest that the collective inter + intraspecific competition in polyculture was lower than the intraspecific competition in monoculture. Relatively reduced intraspecific competition can be the consequence of the two species occupying different spatial niches in a complementarity manner, therefore reducing resource competition (Hooper et al., 2005). However, doubts concerning the attribution of niche differentiation and complementarity of resource use as a mechanistic explanation for similar results have been raised (Jolliffe, 2000), as single-density replacement series (such as used here) make it difficult to meaningfully assess the levels of competition present in the association. An experimental design extended to test other initial plant densities could have allowed to precise the mechanisms behind the observation made.

4.5.2. Belowground biomass

M. sativa was identified as more productive belowground than *S. miyabeana*, while *F. arundinacea* produced an intermediary amount of root biomass. This distinct partitioning of belowground biomass by *M. sativa* provides this species with the greatest potential sink of the three species for root TE accumulation and therefore could induce the most phytostabilisation of TEs. Phytostabilisation refers to the use of plants to reduce the mobility and prevent TEs in the soil from reaching potential migration pathways (Kidd et al., 2009), and can be of significant importance to avoid groundwater contamination (Salt et al., 1995). Similar to aboveground yields, where *S. miyabeana* dominated the polycultures, *M. sativa* was the most dominant species belowground in the polycultures i.e. polycultures including *M. sativa* produced as much root biomass as *M. sativa* in monoculture. This suggests that polycultures containing *M. sativa* can offer a similar global sink size for TE root accumulation, while extending the potential range of TEs that can be accumulated in the roots of the system by introducing other species possessing different TE uptake abilities. These other species can in turn benefit from the greater nitrogen accessibility. Although there may be compromises necessary regarding the accumulated amount of certain TEs, extending the range of TEs that could be sequestered and/or tolerated by at least one other constituent species within a polyculture can represents an interesting advantage for phytoremediation of multiple-TE contaminated soil.

The comparison of the observed and expected yields of each component of all polycultures revealed that *F. arundinacea* produced more root biomass than expected (45%), when co-cropped with *S. miyabeana* (F+S), as it also did for the aboveground biomass. Again, the reduction of the relative effect of intraspecific competition in *F. arundinacea* could explain this result, potentially reflecting complementarity in belowground space between a tree and a grass species (Van Noordwijk et al., 2015).

4.5.3. Root surface area

While above and belowground biomass productivity of plants are commonly measured parameters in plant TE uptake studies, root surface area (RSA) can also be of interest in representing the area of interface between the soil matrix and the plant (the rhizospheric interface), where critical exchanges of nutrients and exudate as well as TE uptake occurs (Gobran et al., 2000). In monoculture, while *M. sativa* might represent the highest belowground biomass sink for TE accumulation, *F. arundinacea* had the highest RSA of the three species, perhaps unsurprising as members of *Poaceae* are known to have high root density among the various plant species used for phytoremediation (Gawronski and Gawronska, 2007). *F.*

arundinacea possesses a rare ability to absorb significant amounts of several TEs such as Ag (Desjardins et al., 2016), Cu, Pb (Nissim et al., 2015), Ni (Park et al., 2013) and Zn (Rizzi et al., 2004) in its root system. A dominance effect was again observable; when *F. arundinacea* was present in any polyculture, the total RSA (including partner species) was generally equivalent to *F. arundinacea* in monoculture. Such impact to polyculture root structure could have important ramifications to phytoremediation efficiencies. In terms of the relative contribution of RSA in polyculture, the observed *F. arundinacea* RSA was significantly higher (46%) than expected from monoculture when co-cropped with *S. miyabeana* (F+S), as was the observed *M. sativa* RSA in all combinations: +54% (F+M), +128% (M+S) and +134% (F+M+S). This maintenance of total root biomass equivalent to monoculture but with higher RSA should provide a greater total area for TE uptake and so could potentially be a morphological alteration advantageous for *M. sativa* phytoremediation. While the impact of increased *M. sativa* RSA requires specific experimental inquiry to establish either the benefit or detriment to a particular ecological (or remediation) function, what is clear here is that a distinct and consistent phenotypic alteration has occurred from the co-cropping this species.

4.5.4. Biomass allocation

The monocultures possess distinct morphologies, each with the highest value for a different biomass-related trait (aboveground biomass, belowground biomass and RSA). Three of the assemblages (F+M, F+S, F+M+S) produced values as high as the most productive monoculture for all of these three traits, a feature that no monoculture could replicate. When considered broadly, if the different phenotypic characteristics from these species can be maintained in polyculture, then there may be benefits from combining these species when compared to their monoculture through improved flexibility to space use, while also potentially improving other ecological attributes such as ground cover (Altieri, 1999), resistance to weed colonization (Steinauer et al., 2016), habitat creation (Mathey et al., 2015) and soil microbial properties (Strecker et al., 2015). To explore the impact of such different developmental responses of mono and polyculturing on TE uptake, the mobility of TEs from soil to plant tissues was investigated.

4.5.5. Trace element concentrations

4.5.5.1. Belowground concentrations

F. arundinacea and *S. miyabeana* both accumulated similar root concentrations of all TEs, with the exception of Cu, which was present in higher concentration in *F. arundinacea*`s roots. The presence of one or two other species did not affect root TE concentrations. However, although the TEs concentrations were relatively constant within species assemblages, suggesting potentially strict regulation of internal TE concentration, the changes in total belowground biomass should influence the absolute amounts of TEs accumulated in roots.

4.5.5.2. Aboveground trace element concentrations

Three species assemblages led to reduced shoot TE concentrations compared to respective monocultures: *S. miyabeana* shoot As concentration fell by 30% when mixed with *M. sativa* and its shoot Zn concentrations decreased by 22% when mixed with both other species. Similarly, *M. sativa* saw its shoot Cd concentrations decreased by 22% when mixed with *F. arundinacea*. These reductions might lead to lower TE extraction yields and particular attention must be paid to such effects if these metals are identified as a remediation priority, or if sites are contaminated only by these individual metals. Apart from these exceptions, in most of the cases (69 out of 72), the concentrations of TEs found in shoots of a species was constant whether grown alone or in species mixture. As with belowground TE tissue concentrations, there seems to have been little "competition" for TE uptake when mixing these species. From a phytoextraction perspective, the consistency in TE shoot concentrations suggests that the amount of TE that can be extracted from the soil and translocated to shoots of the mixed-crops systems versus single crop systems should be determined by the biomass yields. To explore the combined impact of biomass yield and TE tissue concentration on TE soil remediation, above and belowground amounts of TE uptaken by the plants was investigated.

4.5.6. Trace element uptake

4.5.6.1. Individual trace element accumulated belowground

F. arundinacea and F+S accumulated the highest amounts of all TEs in their root systems. While it was previously hypothesized that *M. sativa* could accumulate the highest amount of TEs of the monocultures because of its greater root sink, it appears that, with regard to root morphology, the higher root surface area (RSA) of *F. arundinacea* may be a more desired root trait. It is also noted that *M. sativa* had a higher than expected RSA in species assemblages, however, this was not followed by a corresponding increase in root TE concentration. Therefore, the increase of the RSA of *M. sativa* might alone be insufficient to induce more TE uptake but also, the TE remediation abilities of *F. arundinacea* could be driven by properties other than RSA, such as more efficient root metal import machinery, a solubilizing effect of specific root exudates (Malinowski et al., 2004) or associations with arbuscular mycorrhizal (Shabani et al., 2016). Fine roots, such as presented by *F. arundinacea*, are recognized as an important factor for soil aggregate stabilization (Gyssels et al., 2005), therefore enhancing substrate quality and plant growth (Passioura, 2002). Although, based on these findings, the impact of root morphology on TE uptake is unclear; TE accumulation yields do suggest that both *F. arundinacea* and F+S assemblage could be used effectively in diverse TE-contamination contexts for phytostabilisation.

4.5.6.2. Individual trace element extracted aboveground

Ultimately, management of TE-contaminated land is contingent on how much TE can actually be extracted from the soil by a phytomanagement approach. High extraction could be realized by either using hyperaccumulator plants (Krämer, 2010) or high yielding biomass crops (Evangelou et al., 2015). *S. miyabeana* in monoculture extracted the highest amount of Cd and Zn to harvestable aboveground tissues. This implies that for land only contaminated with TEs for which a species has a particularly high TE-extraction capability, such as *S. miyabeana* for Cd and Zn, an approach involving this single species could be preferable. While TE-contaminated lands rarely suffer from single TE contamination (Van der Perk, 2012), there are a few examples, such as with Cd in Canadian agricultural soils (Roberts, 2014), where these findings could be of a particular interest. Regarding the other TEs, there was no consistent pattern suggesting that either a monoculture or a polyculture approach would be superior for remediation when considered as contaminating land in isolation.

4.5.6.3. Multiple-TE accumulation in roots

As contaminated soils are rarely contaminated by single TEs, phytomanagement approaches need to be able to address real-world complexity in land contamination. The soil used in this experiment exceeded legislated environmental limits, Quebec province criteria (QPC), of soil TE-contamination levels for residential land use (Gouvernement du Québec, 1998) for four TEs, namely Ag, Cu, Pb and Zn (Table 4.1). These four elements therefore represent a remediation priority and considering them collectively, here calculated as Multiple-TE accumulation (MTEA) and Multiple-TE extraction (MTEE) indices, is likely to enhance the attractiveness of these approaches to local remediation practitioners. Calculating indices such as MTEA and MTEE also corresponds to the *averaging approach*, as described by Byrnes et al. (2014), useful for evaluating the multifunctionality of a system.

F. arundinacea monoculture, F+S and F+M+S assemblages all produced the highest values of root MTEA of Ag, Cu, Pb and Zn. These findings confirm that *F. arundinacea* possesses a wide range of belowground remediation abilities, and that this species could be considered as flexible towards multiple-TE-contamination. Also, from these findings, it could be recommended that *F. arundinacea* could be co-cropped with *S. miyabeana* (F+S) or both *M. sativa* and *S. miyabeana* (F+M+S) without any losses in terms of accumulation of multiple TEs compared to *F. arundinacea* monoculture (Figure 4.6A).

4.5.6.4. Multiple-TE extraction aboveground

For the subset of the four TEs exceeding the QPC regulation for residential land use, the highest Multiple-TE extraction (MTEE) value was obtained by the polyculture of F+M+S, which is the only co-cropping system that simultaneously translocated significantly more of these problematic TEs to harvestable aboveground biomass than *S. miyabeana* monoculture. This suggests that TE-contaminated land management practitioners opting for a green and sustainable technology, such as phytomanagement, should strongly consider multiple-species approaches, as they might benefit from the expression of multiple-TE remediation functions simultaneously.

Ultimately, aboveground and belowground systems are inseparable and must be considered in conjunction in order to select the best remediation option possible. The F+M+S assemblage produced amongst the highest values for the three biomass-related traits. MTEA and MTEE findings also showed that F+M+S had, jointly, amongst the highest multiple-TE accumulation yields belowground and level of simultaneous extraction of Ag, Cu, Pb, and Zn per area of land. It has also been demonstrated that the presence of *M. sativa* had a positive effect on *F. arundinacea* nitrogen accessibility. The combination of all these observations point towards the preferential use of the three species together (F+M+S) to obtain the most flexible and effective overall remediation efficacy from the different species and species assemblages investigated, particularly when contaminants were present at levels considered as potentially detrimental to human health (QPC).

Whilst these benefits are clear in the short term, longer term studies would be needed to establish additional benefits from co-cropping. However, in light of the positive impact of *M. sativa* on *F. arundinacea* nitrogen accessibility, the long term prospects are promising.

4.6. Conclusions

Distinct morphological strategies were reflected in the development of monocultures on TE contaminated soil with *S. miyabeana* producing the most aboveground biomass, *M. sativa* producing the most belowground biomass and *F. arundinacea* producing the highest root surface area (RSA). These strategies lend themselves well to polyculture as species assemblages of F+M, F+S and F+M+S produced among the highest yields of aboveground and belowground biomass as well as root surface area (RSA).

In polycultures, TE tissue concentrations were generally maintained from monoculture. However, the *amounts* of TE accumulated in root tissues differed, as did the amount of TEs extracted from soil and translocated to aboveground tissue, between species mixtures due predominantly to biomass yield variation. When combining uptake of the TEs which exceeded legislated residential health limits in Canada, the highest amounts accumulated in roots (Multiple-TE Accumulation; MTEA) and translocated to harvestable biomass aboveground (Multiple-TE Extraction; MTEE) were achieved by three cropping approaches: *F. arundinacea* monoculture, F+S and F+M+S assemblages. However, it was also demonstrated that *F. arundinacea* benefits from the presence of *M. sativa*, potentially by having an improved access to nitrogen. These results suggest the three species cultivated together provide the most flexible and efficient phytoremediation option tested. The use of multiple species may well be a preferable generalized phytomanagement approach for complex (real-world) contaminated land.

4.7. Acknowledgments

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Research Council of Canada (NSERC), Green Municipal Fund (FCM) and the Montréal Botanical Garden.

4.8. Supplementary material

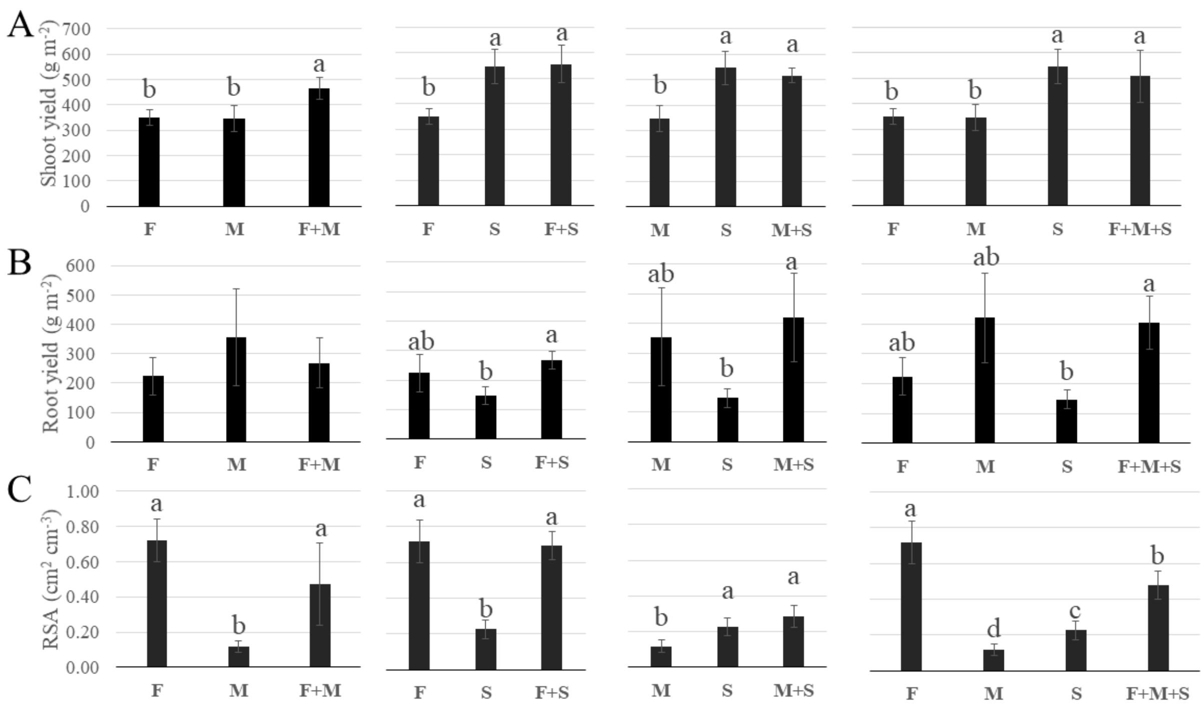

Figure S4.1 Comparison of biomass allocation of *F. arundinacea*, *M. sativa* and *S. miyabeana* polycultures with their respective monocultures. A) shoot biomass, B) root biomass and C) root surface area (RSA). Different letters identify significant differences (Tukey, $p < 0.05$). Error bars represent standard error ($n = 4$)

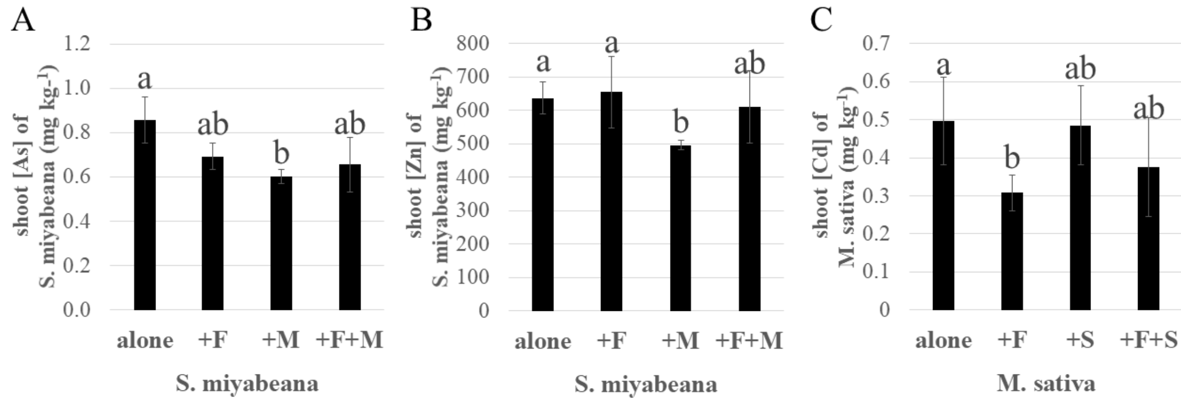

Figure S4.2 TE shoot concentrations for which differences were recorded between a species cultivated alone and the same species grown with one or two others. Mean values of A) As, B) Zn and C) Cd shoot concentrations (mg of TE per kg of dry weight plant material) for which differences were recorded between a species cultivated alone and with one or two others. *F. arundinacea* = F, *M. sativa* = M and *S. miyabeana* = S. Different letters identify significant differences (Tukey < 0.05). Error bars represent standard error (n = 4).

Table S4.1 Nitrogen % in plant tissues of *F. arundinacea*, *M. sativa* and *S. miyabeana* grown in mono- and polycultures

Species	partner	Nitrogen shoot %
F. arundinacea	monoculture	1.23 (0.13)
	with M	1.28 (0.31)
	with S	1.10 (0.03)
	with M+S	1.29 (0.11)
M. sativa	monoculture	2.85 (0.29)
	with F	2.95 (0.25)
	with S	3.09 (0.37)
	with F+S	3.02 (0.10)
S. miyabeana	monoculture	1.15 (0.22)
	with F	0.95 (0.19)
	with M	1.22 (0.14)
	with F+M	1.11 (0.21)

Species	partner	Nitrogen root %	
F. arundinacea	monoculture	0.75 (0.08)	c
	with M	1.14 (0.12)	a
	with S	0.89 (0.03)	c
	with M+S	1.11 (0.12)	b
M. sativa	monoculture	1.05 (0.12)	
	with F	1.28 (0.42)	
	with S	1.03 (0.27)	
	with F+S	0.96 (0.11)	
S. miyabeana	monoculture	0.78 (0.11)	
	with F	0.81 (0.06)	
	with M	0.86 (0.08)	
	with F+M	0.89 (0.19)	

Mean values of nitrogen (N) percentage (%) in shoot (upper table) and roots (lower table) of *F. arundinacea* (F), *M. sativa* (M) and *S. miyabeana* (S) in monoculture and polycultures. Different letters identify significant differences (Tukey < 0.05). Value in brackets are standard errors (n=4).

Table S4.2 TE concentrations in species grown in polycultures

bloc	species	partner	organ	Ag	As	Cd	Cr	Cu	Pb	Se	Zn
1	*F. arundinacea*	M	shoot	12.42	1.10	1.40	27.10	228.06	48.05	0.41	190.99
2	*F. arundinacea*	M	shoot	10.18	0.90	1.37	17.71	37.34	12.56	0.31	186.33
3	*F. arundinacea*	M	shoot	3.29	0.45	1.23	9.71	48.69	12.15	0.12	154.09
4	*F. arundinacea*	M	shoot	5.58	0.76	1.47	12.22	44.98	17.56	0.12	172.65
1	*F. arundinacea*	S	shoot	6.45	0.68	1.25	14.74	43.10	13.24	0.12	164.43
2	*F. arundinacea*	S	shoot	3.13	0.42	1.18	8.55	24.71	7.26	0.12	130.31
3	*F. arundinacea*	S	shoot	5.47	0.56	1.21	12.72	23.41	7.94	0.12	148.42
4	*F. arundinacea*	S	shoot	3.07	0.53	1.32	7.93	29.29	7.31	0.12	136.15
1	*F. arundinacea*	M+S	shoot	6.86	0.80	1.10	20.80	423.85	1252.95	0.33	200.30
2	*F. arundinacea*	M+S	shoot	6.56	0.67	1.46	14.00	36.54	20.95	0.35	204.03
3	*F. arundinacea*	M+S	shoot	4.81	0.64	1.48	11.50	32.28	11.33	0.12	149.62
4	*F. arundinacea*	M+S	shoot	2.91	0.44	1.55	7.38	54.06	17.42	0.12	141.97
1	*M. sativa*	F	shoot	5.77	0.90	0.34	15.28	58.87	23.61	0.42	166.01
2	*M. sativa*	F	shoot	9.25	0.78	0.34	17.07	66.68	63.45	0.30	182.81
3	*M. sativa*	F	shoot	1.95	0.48	0.24	6.36	44.43	19.88	0.31	145.73
4	*M. sativa*	F	shoot	4.40	0.71	0.31	8.92	56.52	29.35	0.33	167.71
1	*M. sativa*	S	shoot	12.28	1.35	0.42	24.08	64.42	21.08	0.59	207.59
2	*M. sativa*	S	shoot	11.60	1.29	0.45	21.55	57.00	19.96	0.35	201.47
3	*M. sativa*	S	shoot	15.09	2.85	0.64	50.24	67.09	59.05	0.57	223.92
4	*M. sativa*	S	shoot	10.14	0.97	0.43	16.81	83.39	14.19	0.41	187.31
1	*M. sativa*	F+S	shoot	2.34	0.48	0.31	10.51	51.91	46.23	0.39	164.91
2	*M. sativa*	F+S	shoot	3.78	0.43	0.30	8.48	45.39	7.53	0.25	132.78
3	*M. sativa*	F+S	shoot	16.88	2.01	0.57	46.72	70.31	110.62	0.39	228.35
4	*M. sativa*	F+S	shoot	1.52	0.40	0.32	5.69	8.40	8.16	0.40	139.51
1	*S. miyabeana*	F	shoot	0.43	0.68	9.49	5.74	31.36	9.02	0.28	689.91
2	*S. miyabeana*	F	shoot	0.33	0.67	6.61	5.11	26.27	7.75	0.28	522.21
3	*S. miyabeana*	F	shoot	0.36	0.78	8.33	5.76	28.77	10.85	0.12	775.03
4	*S. miyabeana*	F	shoot	0.27	0.64	8.61	3.90	24.54	4.62	0.34	630.79
1	*S. miyabeana*	M	shoot	0.39	0.62	7.00	6.10	39.87	10.66	0.37	507.73
2	*S. miyabeana*	M	shoot	0.31	0.63	7.57	5.03	26.61	4.87	0.24	507.69
3	*S. miyabeana*	M	shoot	0.37	0.56	7.89	5.23	32.68	11.63	0.43	486.55
4	*S. miyabeana*	M	shoot	0.43	0.60	6.42	4.63	32.74	4.61	0.26	480.21
1	*S. miyabeana*	F+M	shoot	0.25	0.84	7.25	4.10	25.97	17.75	0.12	774.03
2	*S. miyabeana*	F+M	shoot	0.29	0.59	7.25	5.56	28.72	6.60	0.35	557.23
3	*S. miyabeana*	F+M	shoot	0.32	0.59	6.69	5.05	25.70	4.53	0.12	563.59
4	*S. miyabeana*	F+M	shoot	0.23	0.60	7.73	4.31	29.44	4.78	0.12	547.55
1	*F. arundinacea*	M	root	125.38	4.58	4.07	124.47	4121.81	118.03	1.67	871.94
2	*F. arundinacea*	M	root	122.78	3.25	3.40	84.85	1999.88	61.45	1.52	725.79
3	*F. arundinacea*	M	root	133.58	2.42	4.84	61.17	2250.59	52.66	0.95	806.32
4	*F. arundinacea*	M	root	130.53	3.23	4.00	96.18	1395.32	85.74	1.38	816.75
1	*F. arundinacea*	S	root	162.33	2.85	2.77	91.50	1918.27	50.79	1.53	597.41
2	*F. arundinacea*	S	root	298.33	5.24	3.29	145.78	1424.92	123.46	1.84	895.79
3	*F. arundinacea*	S	root	272.25	4.27	4.37	149.42	1530.47	79.26	1.81	817.38
4	*F. arundinacea*	S	root	155.35	4.73	2.88	119.85	1520.14	82.78	1.53	734.23
1	*F. arundinacea*	M+S	root	115.64	3.11	2.04	76.15	1415.46	54.42	1.52	857.18
2	*F. arundinacea*	M+S	root	163.14	4.26	2.83	109.98	2948.38	78.05	1.91	825.61

3	*F. arundinacea*	M+S	root	154.58	5.24	2.99	125.71	2285.40	97.08	2.02	1502.96
4	*F. arundinacea*	M+S	root	150.52	5.23	3.37	99.45	1698.54	91.95	1.64	3061.93
1	*M. sativa*	F	root	15.71	0.56	0.56	14.33	138.70	138.62	0.33	142.32
2	*M. sativa*	F	root	21.66	0.78	0.60	16.51	144.51	36.98	0.06	148.50
3	*M. sativa*	F	root	12.35	0.45	0.55	14.14	66.03	82.79	0.06	75.35
4	*M. sativa*	F	root	20.97	1.07	0.69	29.38	66.50	23.37	0.28	95.54
1	*M. sativa*	S	root	25.05	0.78	0.70	17.41	149.86	28.15	0.43	156.92
2	*M. sativa*	S	root	19.76	0.59	0.48	15.65	173.24	52.76	0.06	123.42
3	*M. sativa*	S	root	8.38	0.30	0.45	6.16	52.67	12.22	0.06	74.72
4	*M. sativa*	S	root	6.47	0.27	0.37	8.96	83.75	34.34	0.06	84.05
1	*M. sativa*	F+S	root	24.30	0.77	0.81	30.62	132.52	187.08	0.39	216.58
2	*M. sativa*	F+S	root	14.85	0.43	0.39	11.61	120.03	41.70	0.06	134.05
3	*M. sativa*	F+S	root	6.00	0.20	0.18	6.80	34.41	10.44	0.06	39.57
4	*M. sativa*	F+S	root	7.64	0.32	0.35	8.13	34.75	14.31	0.06	63.99
1	*S. miyabeana*	F	root	162.29	2.65	3.75	74.04	1180.49	36.90	1.54	586.99
2	*S. miyabeana*	F	root	90.88	2.74	2.49	51.32	806.10	58.63	1.30	506.50
3	*S. miyabeana*	F	root	206.59	3.24	4.34	81.94	848.64	51.86	1.68	685.97
4	*S. miyabeana*	F	root	129.69	2.84	3.45	58.37	688.74	53.36	1.47	518.51
1	*S. miyabeana*	M	root	219.84	4.58	3.67	91.49	1510.08	61.97	2.39	615.23
2	*S. miyabeana*	M	root	126.73	3.35	3.06	71.54	1249.37	44.18	1.58	566.33
3	*S. miyabeana*	M	root	133.05	3.33	3.69	54.35	920.85	44.31	1.77	625.84
4	*S. miyabeana*	M	root	77.99	2.22	2.90	40.60	815.20	31.65	1.11	485.84
1	*S. miyabeana*	F+M	root	114.80	2.05	2.55	68.02	802.62	68.46	1.10	576.67
2	*S. miyabeana*	F+M	root	145.80	3.03	3.11	74.40	2098.61	38.49	1.90	594.58
3	*S. miyabeana*	F+M	root	132.72	3.32	3.43	78.51	1069.86	66.36	1.48	614.55
4	*S. miyabeana*	F+M	root	97.64	2.85	2.81	48.64	861.89	36.11	1.39	637.70

Mean values (n=4) of trace element (TE) concentrations (mg kg^{-1}) in shoot and roots of *Festuca arundinacea* (F), *Medicago sativa* (M) and *Salix miyabeana* (S) grown in different polycultures.

4.9. Synthèse du chapitre 4

Dans le quatrième chapitre, la comparaison des monocultures de trois espèces avec les polycultures de ces mêmes espèces a permis de faire plusieurs observations intéressantes. *F. arundinacea*, *M. sativa* et *S. miyabeana* ’SX67’ont présenté des patrons d’allocation de biomasse très complémentaires. Ceci a mené à des productivités élevées chez les assemblages de ces espèces. Pour la remédiation du sol, les trois espèces ont démontré des aptitudes différentes envers les éléments traces. Lorsqu’un seul élément était considéré, un assemblage d’espèces ne parvenait pas à surpasser le rendement de remédiation de la meilleure des trois monocultures. Toutefois, en considérant l’accumulation et l’extraction de plusieurs éléments traces, l’assemblage des trois espèces semblait se démarquer de manière positive. De plus, la présence de *M. sativa* a su favoriser l’accessibilité à l’azote chez *F. arundinacea*, ce qui ajoute à l’intérêt de jumeler ces espèces pour la conservation des fonctions de remédiation sur le long terme.

Chapitre 5 | DISCUSSION GÉNÉRALE

5.1. Rappel de la problématique et du contexte d'étude

La contamination des sols et la gestion de ceux-ci représentent une problématique d'une telle ampleur que des alternatives ou des compléments durables à la gestion conventionnelle de ces sites se font nécessaires (Caliman et al., 2011). La phytoremédiation constitue une solution prometteuse, puisqu'elle présente plusieurs avantages économiques et environnementaux (Wuana and Okieimen, 2011). Toutefois, cette technique fait face à un défi particulier lorsque le sol à traiter est contaminé par plusieurs composés chimiques différents, une situation relativement répandue.

De nombreuses espèces végétales ont été identifiées comme possédant des aptitudes de tolérance et de remédiation envers certains composés chimiques susceptibles de contaminer les sols (Kabata-Pendias, 2010). Toutefois, ces aptitudes sont limitées à seulement quelques contaminants et celles-ci peuvent varier d'une espèce à l'autre. Dans un contexte de contamination multiple des sols par une vaste gamme de composés chimiques, il semble pertinent de rassembler les aptitudes de remédiation nécessaires à la décontamination en jumelant les espèces possédant ces dernières (Batty and Dolan, 2011).

De plus, la littérature scientifique fait état de nombreux arguments suggérant que la diversité végétale aurait un effet bénéfique sur la productivité et le fonctionnement des agro-écosystèmes (Isbell et al., 2017). Il était considéré en prémisse de cette thèse que les mêmes arguments étaient aussi valables pour les systèmes végétaux implantés en terrain contaminé.

5.2. Rappel de l'objectif principal

C'est dans le contexte précédemment décrit que s'est inscrit ce projet de recherche doctorale. L'objectif principal des travaux était d'évaluer dans quelle mesure les propriétés spécifiques des végétaux peuvent être exploitées seules ou en combinaisons pour remédier des sols à contamination multiple. Pour ce faire, trois études ont été menées; une première étude *in situ*, une deuxième en pots et une troisième en mésocosme.

5.3. Retour sur les hypothèses et faits saillants

5.3.1. Chapitre 2 | Associations plantes - contaminants

Le premier objectif était d'identifier des indices supportant l'existence de niches de tolérance aux contaminants sur un site pollué laissé à l'abandon. L'hypothèse associée à cette première étude soutenait qu'un site pollué de manière hétérogène constitue un ensemble de niches spatialement distincte qui seront occupées par des espèces végétales possédant les aptitudes de tolérances spécifiques à ces niches. Suite à un relevé de la végétation et un échantillonnage systématique du sol sur le site d'un ancien bassin de décantation, une analyse multivariée a permis de révéler que la majorité (61%) de la répartition spatiale des six espèces les plus abondantes sur le site pouvait être expliquée par la concentration dans le sol de différents contaminants. Ce résultat suggère que la répartition des espèces à l'intérieur d'une communauté végétale établie sur un site contaminé est influencée par la distribution spatiale des contaminants du sol. Cette répartition des espèces en fonction de leurs tolérances à certains contaminants appuie l'importance qui doit être accordée à la spécificité des aptitudes de remédiation que possèdent les différentes espèces végétales. Il serait donc cohérent d'adopter un patron d'implantation conséquent de la contamination en situation de contamination hétérogène. Bien que ceci puisse s'avérer difficilement réalisable sur de grande surface, les observations rapportées suggèrent néanmoins qu'il serait adéquat d'employer plusieurs espèces possédant des aptitudes de remédiation différentes en phytoremédiation des sites à contamination multiples.

5.3.2. Chapitre 3 | Habiletés de remédiation multiples

Le second objectif était d'explorer le potentiel de quatre espèces disponibles commercialement pour la remédiation de deux sols contaminés par plusieurs contaminants inorganiques en l'occurrence, l'argent, le cuivre et le zinc. Considérer des espèces couramment utilisées en agriculture pour la phytoremédiation a l'avantage de pouvoir assurer la disponibilité des semences et boutures en grande quantité et au moment voulu, en plus de permettre de baser leur sélection sur certaines caractéristiques agronomiques et de remédiation déjà rapportées dans la littérature. L'hypothèse que parmi les quatre espèces testées, certaines pourraient bioaccumuler les trois éléments traces dans leurs parties aériennes dans une concentrations équivalente à celle du sol avait été émise. Une deuxième étude, effectuée en pots, a révélé cette hypothèse comme étant fausse. La translocation des éléments traces dans les parties aériennes s'est avérée inégale; la moutarde indienne (*Brassica juncea*), la luzerne (*Medicago* sativa) et la fétuque érigée (*Festuca arundinacea*) ont été en mesure de transloquer de l'argent, mais aucune espèce n'a pu le faire avec le cuivre et seulement le saule (*Salix miyabeana* 'SX67') a extrait et transporté du zinc vers ses parties aériennes. Du point de vue de la décontamination, employer une seule espèce pour la phytoremédiation du site d'origine des sols à l'étude semble peu envisageable. Un assemblage conséquent de certaines des espèces est suggéré comme une approche potentiellement plus appropriée.

5.3.3. Chapitre 4 | Complémentarité fonctionnelle en phytoremédiation

Le troisième objectif visait à explorer directement l'effet de la richesse spécifique (une, deux et trois espèces) sur la remédiation d'un sol contaminé par plusieurs éléments inorganiques. L'hypothèse de départ était qu'à densité égale, un système à plusieurs espèces parviendrait à extraire du sol une quantité plus importante d'éléments traces que les monocultures correspondantes. Cette troisième étude, effectuée en mésocosme, a révélé qu'en considérant l'extraction d'un seul élément trace à la fois, les assemblages d'espèces ne sont pas parvenus à surpasser le rendement d'extraction de la meilleure des monocultures testées. En adoptant une approche différente, qui consistait à considérer l'extraction d'un ensemble d'éléments simultanément, bien que les différences entre traitements n'aient pas toutes été statistiquement significatives, la valeur la plus élevée a été produite par la polyculture de la fétuque érigée (*Festuca arundinacea*), de la luzerne (*Medicago* sativa) et du saule (*Salix miyabeana* 'SX67'). À l'intérieur de cet assemblage, il a de plus été possible de montrer chez la fétuque une accessibilité à l'azote facilitée par la présence de la luzerne. Une fois de plus, les observations faites lors de cette expérience permettent de suggérer qu'une approche multi-spécifique serait plus appropriée qu'une monoculture pour la phytoremédiation des sols à contamination multiple.

5.4. Synthèse et comparaison des résultats

La rareté des études similaires à celles présentées par cette thèse témoigne de l'originalité des questions qui ont été explorées. Quelques exemples peuvent toutefois servir de point de comparaison. Dans le domaine des marais filtrants, l'effet de la richesse spécifique sur l'enlèvement des polluants a aussi été exploré. Li et al. (2012), ainsi que Rodriguez et Brisson (2016) ont observé, bien que dans des contextes expérimentaux différents (microcosmes avec algues et mésocosmes avec plantes vasculaires), peu de bénéfices à combiner des espèces pour la dépollution de l'eau. En sol minier, Wang et al. (2014) ont rapporté, à l'instar de ce qui a été présenté au chapitre 4 de cette thèse, des interactions facilitatrices ayant cours dans les assemblages d'espèces, qui ont eu un effet positif sur la minéralomasse (quantité d'un élément trace donné) des végétaux.

Au moment de la rédaction de ces lignes, l'auteur n'avait pris connaissance d'aucune étude ayant évalué la performance de systèmes végétaux multispécifiques pour un ensemble de fonctions de remédiation, tel que présenté à la section 4.4.3.3 (MTEA et MTEE). Il semble toutefois surprenant que ce type d'approche ne soit pas plus répandu, puisque le principe de complémentarité fonctionnelle, qui est relativement bien connu, sous-entend que les bénéfices de la biodiversité sont plus susceptibles de se manifester sur un ensemble de fonctions plutôt que sur une seule (Meyer et al., 2018). Toutefois, l'idée d'appliquer à d'autres domaines de recherche les concepts écologiques qui ont été élaborés en étudiant les systèmes naturels est en expension (Mariotte et al., 2017).

Pour atteindre les objectifs de cette thèse, des preuves scientifiques ont été amassées de manières diversifiées, par une série d'études s'étant déroulées sur le terrain, ainsi qu'en milieu semi-contrôlé. Explorer une problématique avec différentes méthodes permet d'approfondir la compréhension de celle-ci. La phytoremédiation, en raison de sa nature complexe, implique effectivement une recherche qui transcende les disciplines (Ali et al., 2013). Cette thèse a tenté d'adopter une telle approche en s'intéressant à la biologie de plusieurs espèces de plantes, à leurs interactions dans un contexte particulier qu'est celui des sols contaminés, en considérant finalement ces informations dans un objectif de génie environnemental. Les observations rapportées dans cette thèse ont indiqué qu'en situation de contamination multiple du sol, la communauté végétale à l'étude a semblé réagir comme en milieu naturel et s'organiser en niches de tolérances à son milieu (chapitre 2) et que la spécificité des aptitudes de remédiation que possèdent les espèces rend difficile la remédiation d'un tel sol par une seule espèce (chapitre 3). Ces observations suggéraient qu'une approche prônant la biodiversité serait plus appropriée pour la phytoremédiation du type de sol à l'étude, ce qu'il a été possible de démontrer en considérant plusieurs fonctions simultanément (chapitre 4) (Figure 5.1).

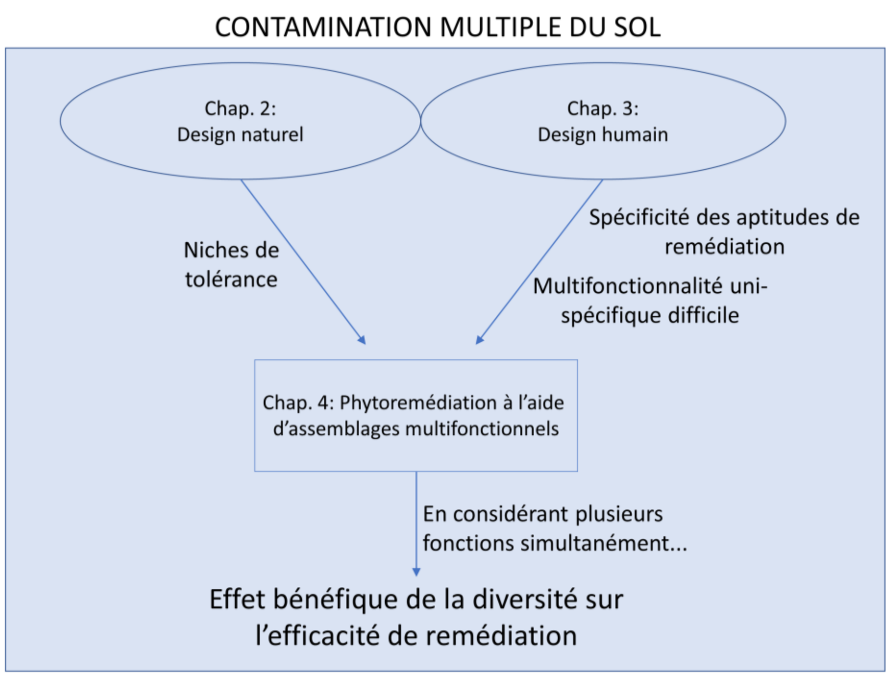

Figure 5.1 Schéma synthèse des résultats de la thèse

Les démonstrations scientifiques ayant mené à ce constat ont été faites en adoptant des protocoles expérimentaux rigoureux. Ce document devrait constituer un outil supplémentaire pour convaincre les acteurs du domaine de la phytoremédiation d'accorder plus d'importance à l'écologie et la biodiversité.

5.5. Limites des études et questionnements soulevés

Les conclusions de cette thèse constituent un apport important pour le domaine de la recherche en phytoremédiation. Quelques limites des études menées peuvent toutefois être mentionnées. Les différents essais décrits dans ce document ont été menés sur une période relativement courte, soit lors d'une seule saison de croissance (mai à septembre). Bien que les résultats tirés des deux expériences en milieu semi-contrôlé soient généralisables principalement à l'année d'implantation des dispositifs de phytoremédiation, cette première étape reste déterminante dans le succès d'une initiative d'implantation végétale.

Les deux études susmentionnées ont été réalisées *ex-situ*, un contexte qui pourrait paraitre éloigné d'une réalité de terrain. Toutefois, conduire des expériences en mésocosmes permet de mieux contrôler les variables environnementales confondantes qui abondent sur le terrain et permet ainsi la réplication et la vérification statistique des réponses (Crossland and La Point, 1992). Contrôler l'effet de cette variabilité sur le terrain nécessiterait une réplication d'une ampleur la plupart du temps difficilement envisageable. De plus, il est pertinent de mentionner que du sol excavé à même un site contaminé a servi aux expériences. Dans les deux cas, celui-ci était donc caractérisé par des propriétés physico-chimiques identiques à celles retrouvées sur le site d'origine, à l'exception d'une porosité potentiellement plus importante causée par les activités d'excavation, ce qui a pu faciliter l'implantation des végétaux.

Il est considéré que les contaminants présents dans les sols utilisés pour les expériences se sont présentés sous leurs différentes formes chimiques dans les mêmes proportions que sur le site d'origine. Ceci représente un élément clé de la transférabilité des résultats des expériences aux sites similaires. En effet, les conséquences du vieillissement (*aging*) sur les propriétés des contaminants sont déterminantes pour la spéciation de ceux-ci et donc pour leur disponibilité envers les végétaux (Smolders et al., 2009).

Suite à la présentation des constats et limites de cette thèse, des questions de recherches s'ouvrent. Un travail d'optimisation des pratiques culturales adoptées (densité, travail du sol, amendements) pourrait-il améliorer la performance des systèmes? La complémentarité observée chez les espèces étudiées se conserverait-elle sur de nombreuses années? Les résultats obtenues seraient-ils maintenus dans des cas de contamination plus sévère? Des considérations d'ordre agronomiques, temporels et de sévérité de la contamination soulèvent, selon l'avis de l'auteur, les questionnements découlant les plus directement des conclusions de cette thèse.

5.6. Perspectives

La recherche en phytoremédiation a été grandement stimulée par les nombreux problèmes associés aux terrains contaminés. L'objectif principal a toujours été d'offrir des solutions alternatives à la gestion conventionnelle des sites contaminés. Bien qu'ayant beaucoup évoluée depuis son apparition il y a quelques décennies, le constat se doit d'être fait que cette technologie peine à prendre une place importante parmi les options choisies par les gestionnaires de sites. Le développement de la capacité locale à comprendre et appliquer la phytoremédiation, ainsi que l'établissement d'un cadre réglementaire efficace font partie des obstacles à surmonter (UNEP, 2017). La vitesse à laquelle s'opère la phytoremédiation constitue aussi un frein à son application. Les activités de remédiation des sols étant souvent enclenchées seulement lorsqu'un promoteur met en branle un projet de développement pour le site, la phytoremédiation se retrouve écartée des possibilités d'intervention.

Des indices suggèrent que procéder par associations d'espèces, tel que proposé dans ce document, peut améliorer la performance et le caractère écologique de la phytoremédiation et ainsi aider à en faire la promotion. Le choix des espèces employées est évidemment crucial. La complémentarité dans l'utilisation de l'espace aérien et souterrain, l'absence d'interactions alléllopathiques négatives, ainsi que le maintien des performances individuelles lorsque les espèces sont jumelées sont des critères de sélection à considérer. Ce type d'information n'étant pas nécessairement disponible, davantage d'études doivent être conduites afin d'acquérir ces connaissances.

Du côté sociétal, implanter la phytoremédiation dès qu'un terrain contaminé est laissé à l'abandon serait idéal. Les années qui passent avant que le site ne soit destiné à d'autres fins pourraient bien avoir permis à la phytoremédiation de réduire les niveaux de plusieurs contaminants et du même coup les frais associés à une remédiation conventionnelle. De plus, des revenus alternatifs pourraient être tirés de la biomasse produite durant cette période (Evangelou et al., 2015). Cette dernière possibilité pourrait s'avérer un élément important pour la suite du développement et de la commercialisation de la phytoremédiation, en autant que les infrastructures nécessaires à la transformation de la biomasse soit disponibles.

La promotion de la phytoremédiation en général pourrait bien entendu bénéficier d'un soutien gouvernemental plus important. Implanter la phytoremédiation sur les nombreux sites dégradés dont le gouvernement possède et hérite périodiquement serait un gage de confiance envers cette technologie et aurait assurément un effet stimulant pour l'adoption à plus grande échelle de la phytoremédiation. De plus, les normes actuellement en vigueur pour caractériser un sol contaminé sont basées sur les concentrations totales des contaminants, alors que des nuances pourrait être apportées. Comme ces normes visent ultimement à gérer le risque associé à la présence des contaminants, une approche plus près des réalités de toxicité, comme celle de considérer la fraction soluble des contaminants, serait appropriée pour atteindre cet objectif. Par le fait même, ceci aurait pour conséquence de rendre la phytoremédiation encore plus intéressante, puisque c'est en premier lieu les fractions les plus facilement disponibles des contaminants qu'elle est en mesure de (Barber, 1995).

Malgré les obstacles à surmonter avant de voir la phytoremédiation appliquée à grande échelle, celle-ci continue de se développer et de démontrer son efficacité. Dans un avenir rapproché, les preneurs de décisions feront face à une pression de plus en plus grande pour adopter des pratiques de gestion respectueuses du développement durable. La phytoremédiation s'inscrit indéniablement dans cette lignée.

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