Université de Montréal

Impacts d’amendements organiques sur les capacités phytoremédiatrices de saules et de peupliers

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Mémoire présenté dans le but de l’obtention du grade de

M.sc. en sciences biologiques

Août 2023

Université de Montréal

Département des sciences biologiques, Faculté des arts et sciences

Ce mémoire intitulé

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RÉSUMÉ

Les sites industriels abandonnés présentent souvent des sols ayant des propriétés physico-chimiques atypiques et des concentrations élevées en métaux traces, rendant leur dépollution coûteuse de surcroît quand les superficies sont grandes. Les phytotechnologies, comme la phytoremédiation, émergent alors comme des options économiques et socialement acceptables. Cette étude, conduite en conditions semi-contrôlées, a examiné la capacité de saules (*Salix miyabeana* 'SX67') et de peupliers (*Populus* DNxM '915508') à phytoremédier des sols contaminés, notamment par le cuivre, et a comparé leurs performances suivant l'application de frass, un résidu de l'élevage d'insectes, et de fumier de poule, tous deux produits dans une démarche d'économie circulaire. Dans l'ensemble, aucune tendance claire n'a pu être identifiée entre les traitements et les espèces concernant la production de biomasse, les paramètres physiologiques, ou l'assimilation des éléments traces. Cependant, les saules amendés avec du frass ont atteint une hauteur moyenne de 210 cm, significativement supérieure à celle des saules non fertilisés. Les concentrations de cuivre extrait par les plantes n'ont pas été affectées par les deux types d'amendements organiques. Comme pour le cuivre, qui a montré des concentrations de 407 mg kg^{-1} dans les racines et de 14 mg kg^{-1} dans les parties aériennes, la majorité des dix éléments étudiés a été principalement retrouvée dans les racines. Seuls le zinc et le cadmium ont montré un facteur de translocation supérieur à un. En ce qui concerne les différentes fractions de cuivre dans le sol, aucune différence significative n'a été observée entre les traitements pour les fractions labiles et phytodisponibles, bien qu'une tendance puisse être notée pour les peupliers amendés avec du fumier. Les macronutriments tels que l'azote et le phosphore ont montré une interaction complexe impliquant les espèces, les amendements, et la contamination en cuivre. Malgré des résultats ambigus et une complexité inattendue, cette étude semble indiquer que le frass et le fumier de poule sont des matériaux efficaces en comparaison des engrais inorganiques communément utilisés. Néanmoins, ils soulignent également la nécessité d'une recherche plus approfondie sur l'utilisation de ces amendements organiques issus de l'économie circulaire afin d'optimiser leur utilisation. Une telle exploration, en alignement avec les principes de durabilité et de recyclage, pourrait éventuellement orienter nos stratégies vers des solutions plus efficaces, viables et économiques, contribuant ainsi à l'avenir de la restauration écologique des terrains industriels abandonnés.

Mots-clés : *Salix*, *Populus*, phytoremédiation, cuivre, fertilisant organique, frass, fumier de poule

ABSTRACT

Brownfield sites, characterized by soils with atypical physicochemical properties and high trace element (TE) concentrations, are generally expensive to decontaminate especially when large areas are involved. Economical and socially approved phytotechnologies, such as phytoremediation, offer environmentally conscious solutions that require an approach distinct from those using chemical fertilizers in industrial or agricultural contexts. This study, conducted under semi-controlled conditions, examined the ability of willows (*Salix miyabeana* 'SX67') and poplars (*Populus* DNxM '915508') to phytoremediate contaminated soils, especially those contaminated with copper, and compared their performance following the application of frass, a residue from insect breeding, and chicken manure, both produced in a circular economy approach. The study examined biomass production, specific physiological properties, and the assimilation of copper and other trace elements into plant tissues. Results showed no discernible pattern between the treatments and the species. However, willows treated with frass achieved significantly greater heights, averaging 210 cm. Neither organic amendment influenced the level of copper phytoextracted with, in most cases, higher concentrations of trace elements in roots rather than in the aerial parts, exemplified by copper levels of 407 mg kg^{-1} in roots and only 14 mg kg^{-1} in leaves. Notably, only zinc and cadmium had translocation factors greater than one. Concerning soil copper fractions, there were no statistical differences in both labile and phytoavailable copper across treatments despite a visible trend for frass-amended poplars. Macronutrients such as nitrogen and phosphorus demonstrated complex interactions dependent on the species, amendments, and Cu contamination. Despite ambiguous results and unexpected complexity, this study seems to indicate that frass and chicken manure are effective materials compared to commonly used inorganic fertilizers. However, they also emphasize the need for more in-depth research on the use of these organic amendments from the circular economy to optimize their utilization. Such exploration, in alignment with principles of sustainability and recycling, could potentially guide our strategies towards more effective, viable, and economical solutions, thereby contributing to the future of ecological restoration of abandoned industrial sites.

Keywords: *Salix*, *Populus*, phytoremediation, copper, organic fertilizer, frass, hen manure

REMERCIEMENTS

Mon parcours à l’Institut de recherche en biologie végétale fut enrichissant et fort agréable. Il m’a permis de me familiariser avec les phytotechnologies et de côtoyer plusieurs scientifiques de renommée ou encore de rencontrer ceux de demain. Il me faut tout d’abord mentionner que ce parcours n’aurait surement pas vu le jour sans la rencontre de mon directeur, Michel Labrecque, et d’Amandine Bonet, tout cela orchestré par, Vanessa Grenier

À travers différents projets expérimentaux, le mien comme ceux de mes collègues, j’ai eu la chance de rencontrer Patrick Benoist, Joan Laur, Louise Hénault-Ethier et Adrian Paul. Ces gens m’ont conseillé et m’ont permis d’orienter mes réflexions. Je voudrais également remercier TriCycle et l’usine de biométhanisation de Saint-Hyacinthe pour leur contribution au développement scientifique.

J’aimerais également remercier ceux avec qui je partageais mon quotidien : Noémie Legault, Eszter Sas, Adrien Frémont, Nicolas Perreault, Laurianne Bédard et Henry Beral. Certains se sont également impliqués à des moments cruciaux comme le lancement de l’expérience, Noémie Legault et l’équipe de terrain tandis que d’autres étaient présents à la récolte finale tel que Noémie Hotte et Ezster Sas.

Finalement, bien que tous ces gens portent une place particulière dans mon esprit, j’aimerais donner des remerciements particuliers à Adrian Paul et Ezster Sas pour leur aide et leur soutien en plus de ma famille qui m’a supporté tout au long de mon retour aux études.

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English

ANOVA: Analysis of variance

BCF: Bioconcentration factor

COPT: Copper transporter

DTPA: Diethylenetriaminepentaacetic acid

GHG: Greenhouse gas

LOD: Limit of detection

$N\text{-}NH_4$: Ammonium

$P\text{-}PO_4$: Phosphate

TE : Trace element

TF: Translocation factor

TON: Total oxidized nitrogen

Français

CEC : Capacité d'échange cationique

CICR : Culture intensive sur courte rotation

CO : Carbone organique

COD : Carbone organique dissout

ETM : Élément trace métallique

GES : Gaz à effet de serre

HAP : Hydrocarbure aromatique polycyclique

MO : Matière organique

PEH: Peuplier hybride

QC : Québec

1. INTRODUCTION

1.1. MISE EN CONTEXTE

Les espaces historiquement exploités à des fins industrielles, mais aujourd'hui à l'abandon, sont communément appelés « friches industrielles » et représentent, inexorablement, une perte de revenus pour les municipalités (Beaulieu, 2021). Ces friches génèrent également une pollution visuelle, des îlots de chaleur et favorisent des activités de déversement sauvage et peuvent, à terme, contribuer à l'établissement de plantes envahissantes (Labrecque, 2022). En raison de leur héritage industriel, les sols issus de ces friches peuvent être pollués et nécessitent, selon leur vocation future, d'être décontaminés, ce qui peut freiner la revitalisation de quartiers qui voisinent ces terrains (Beauchamp et al., 2018). Il s'agit donc d'un enjeu grandissant à travers le Canada et en particulier au Québec où l'on dénombre plus de huit mille terrains contaminés (Gouvernement du Québec, 2023).

Plusieurs types de contaminants peuvent être présents dans les sols de friches industrielles. Parmi ceux-ci figurent (1) les composés organiques notamment les hydrocarbures aromatiques polycycliques, les hydrocarbures pétroliers C_{10}-C_{50} ou les biphényles polychlorés et (2) les éléments inorganiques tels que les éléments traces métalliques (e.g., Cd, Cu, Ni, Pb, Zn) et métalloïdes (e.g., As, Sb, Se, Te). Certains de ces éléments, appelés microéléments, sont indispensables au monde vivant, mais demeurent toxiques en quantité trop importante (e.g., Cu, Zn, Fe, Mo). D'autres, en revanche, ne sont pas essentiels et sont nocifs même à faible dose (e.g., Cd, Pb, Hg, Sn—Guerra et al., 2011; Schützendübel & Polle, 2002).

1.2. LE CUIVRE

Retrouvé naturellement dans les sols, la concentration en Cu varie de 3 à 140 mg kg^{-1} entre les différentes composantes de la croûte terrestre, avec une moyenne de 36 mg kg^{-1} (Shabbir et al., 2020). Cependant, divers facteurs, qu'ils soient naturels ou anthropiques, peuvent contribuer à la hausse de sa concentration dans les sols (e.g., volcans, exploitation minière ou divers processus de transformation industriels—Rehman et al., 2019). Ainsi, cet élément trace métallique (ETM) peut être retrouvé à des concentrations atteignant plusieurs centaines de mg kg^{-1} dans certains substrats (González et al., 2010; Guidi et al., 2012; Karczewska, 1996). En zones agricoles, le Cu est généralement issu de l'application de fertilisants phosphatés et de biocides (Husak, 2015; Li et al., 2020). La majeure partie du Cu se trouve généralement dans les premiers horizons, à proximité de la surface en raison de sa faible mobilité et de son affinité pour la matière organique (MO) (Bravin et al., 2009; Ginocchio et al., 2004; Ju et al., 2019). La décomposition de cette dernière peut, néanmoins, acidifier le sol et accentuer sa mobilité en formant des liaisons Cu-COD (carbone organique dissout) (Araújo et al., 2019), Cu-acide humique et Cu-acide fulvique (Hsu & Lo, 2000). À l'inverse, certains composés de nature alcaline (e.g., les cendres et boues rouges), les carbonates, les phosphates et l'argile peuvent limiter son lessivage (Ciccu et al., 2003; Kabata-Pendias, 2010; Kumpiene et al., 2008). Ainsi, le pH, les teneurs en Ca et en MO sont connues pour influencer sa forme et indirectement sa phytodisponibilité (Brümmer & Herms, 1983; Elbana & Selim, 2011; Sauvé et al., 1997; Yruela, 2009).

Dans les sols, le Cu se retrouve principalement sous deux états d'oxydation (Cu (I) et Cu (II)—Kabata-Pendias, 2010). Sous la forme Cu (II), il forme des liaisons avec la MO et des matières inorganiques telles que les oxydes (i.e., Fe, Al et Mn) (Cui et al., 2017; Kabata-Pendias, 2010; F. Wang et al., 2009). Lorsqu'il est présent dans des sols alcalins (i.e., pH > 7), il se retrouve majoritairement sous sa forme réduite (Cu (I)), et sa mobilité diminue en raison d'une affinité accrue avec certains complexes organiques (Carrillo-González et al., 2006; Ponizovsky et al., 2006; Rehman et al., 2019; Strobel et al., 2005). Ces deux formes demeurent assimilables par les plantes : 1) le Cu (II) est réduit à surface de la membrane des cellules des tissus racinaires et assimilé par les protéines de la famille COPT/Ctr (Carrió-Seguí et al., 2019; Ryan et al., 2013) ou 2) Cu (II) pénètre *via* les *Zinc Iron transporters proteins* ou *Heavy metal ATPase*, et est subséquemment réduit par des ATPases de type P 1-B (Kumar et al., 2021; Ogunkunle et al., 2019; Yruela, 2009).

1.2.1. Le cuivre : micronutriment

Le Cu est un micronutriment essentiel pour les plantes et de nombreux autres organismes (Festa & Thiele, 2011). Il est notamment impliqué dans le fonctionnement de certains systèmes enzymatiques (Rehman et al., 2019; Shabbir et al., 2020). Le Cu peut contribuer à :

1. La photosynthèse (plastocyanine) et la respiration (cytochrome oxydase) (Baron et al., 1995). Une carence en Cu peut entraîner la protéolyse de la plastocyanine (Li & Merchant, 1995), ainsi que la chlorose et la nécrose des jeunes feuilles (Kabata-Pendias, 2010);

2. La synthèse et l'activité de la cytochrome c oxydase impliquée dans la chaîne respiratoire des mitochondries (Garcia et al., 2014);
3. L'absorption et le transport de l'eau en jouant un rôle dans la synthèse des parois cellulaires et des tissus du xylème (Burkhead et al., 2009);
4. La production de graines (Burkhead et al., 2009; Kabata-Pendias, 2010);
5. La défense des plantes face aux lésions physiques, oomycètes, champignons et bactéries. (Cona et al., 2006; La Torre et al., 2018; Marusek et al., 2006; Rodríguez et al., 1999).

Les facteurs physico-chimiques du sol ne sont pas les seuls à influencer la biodisponibilité du Cu. En effet, les racines sécrètent plusieurs composés (e.g., acides organiques) dans la rhizosphère, qui peuvent augmenter la concentration de COD et accroître sa biodisponibilité (Badri & Vivanco, 2009; Narasimhan et al., 2003; Nguyen et al., 2017). À l'inverse, la biodisponibilité du Cu peut être négativement influencée par certains éléments (e.g., N, P, Zn, Mn, Mo) et engendrer des disfonctionnements physiologiques (i.e., déficience) au même titre qu'une concentration élevée, qui peut devenir phytotoxique (Hodges, 2010; Kabata-Pendias, 2010; Yruela, 2009).

1.2.2. Phytotoxicité du cuivre

Pour la majorité des espèces végétales, on rapporte une phytotoxicité potentielle à des concentrations se trouvant entre 150 et 300 mg kg^{-1} de Cu dans les sols (Hodges, 2010). Cependant, la concentration totale d'un métal dans le sol n'est pas considérée comme un critère suffisant pour juger de sa toxicité (Sauvé et al., 1998). Le type de sol et sa composition dictent la biodisponibilité du Cu et, par conséquent, sa toxicité (La Torre et al., 2018).

Dans les tissus des plantes, la concentration en Cu varie entre 5 à 25 mg kg^{-1} dépassant rarement les 20 mg kg^{-1} dans les parties aériennes (Hodges, 2010; Kabata-Pendias, 2010; Reeves, 2006; Shabbir et al., 2020). Cependant, lorsqu'exposé à des concentrations élevées, les plantes qui ne disposent pas de mécanismes de régulation adaptés, vont absorber et stocker des concentrations phytotoxiques de Cu, ce qui génère, entre autres:

1. Des taux élevés d'espèces réactives de l'oxygène (ERO), provoquant une multitude de stress physiologiques (e.g., déséquilibre hydrique) (Gong et al., 2019);
2. Un changement dans la composition lipidique, réduisant la fluidité membranaire des thylakoïdes (i.e., membranes photosynthétiquement actives retrouvées dans les chloroplastes) (Quartacci et al., 2000);

3. Une diminution de la teneur en glucides, expliquée par une baisse de la production photosynthétique et une hausse de la consommation de sucres solubles pour des besoins physiologiques de bases (Xing et al., 2010).
4. Une lignification accrue des tissus racinaires, causant leur épaississement et décoloration provoqués par la production excessive de précurseurs de la lignine (e.g., monolignols) *via* la voie des phénylpropanoïdes (Tugbaeva et al., 2022; Wairich et al., 2022).

Cependant, certaines plantes appelées hyperaccumulatrices ont développé des mécanismes, pour des raisons encore mal connues, leur permettant de concentrer des teneurs exceptionnelles (> 300 mg kg $^{-1}$) de Cu dans leurs tissus (Reeves, 2006; van der Ent et al., 2013). Aujourd'hui, on recense plus de 50 espèces végétales hyperaccumulatrices de Cu reparties à travers le monde, dont la majorité sont endémiques de la ceinture de Cu de Katanga en Afrique centrale (i.e., République démocratique du Congo et Zambie)(Reeves et al., 2018; van der Ent et al., 2019).

Parmi ces plantes qui ont le potentiel d'accumuler des concentrations de Cu plus élevées, aucune ne se retrouve naturellement au Québec en raison de la glaciation récente qui n'a pas laissé place à l'apparition de ce trait (Rajakaruna et al., 2009). De plus, ces espèces étant majoritairement endémiques aux zones tropicales, le climat québécois n'offre pas les conditions propices à leur établissement, ce qui complique grandement leur utilisation localement pour la phytoremédiation (Baker & Brooks, 1989; Schmöger et al., 2000).

1.3. LES PHYTOTECHNOLOGIES POUR RÉSOUDRE UNE PROBLÉMATIQUE ENVIRONNEMENTALE URBAINE

1.3.1. LA PHYTOREMÉDIATION

La phytoremédiation offre une solution aux enjeux environnementaux, économiques et sociaux auxquels nous confrontent les friches industrielles. Cette technique profite d'espèces végétales, préférablement indigènes, disposant de caractéristiques physiologiques particulières leur permettant de prospérer dans des substrats aux propriétés chimiques (e.g., contaminants, pH) et physiques (e.g., texture, compacité) hostiles (Guidi Nissim & Labrecque, 2021). Outre leur résilience face à des conditions inhospitalières pour la majorité des plantes, ces espèces sont aussi en mesure d'immobiliser des contaminants ou même de les absorber et de les transférer dans leur biomasse aérienne qui peut ensuite être récupérée et traiter selon les besoins et normes en vigueur. La phytoremédiation permet ainsi la valorisation des sols contaminés, une approche favorisée par le *Guide d'intervention – Protection des sols et réhabilitation des terrains contaminés* (Beaulieu, 2021). Par ailleurs, leur utilisation en milieu urbain engendre des effets bénéfiques sur l'environnement (réhabilitation des sols, séquestration du carbone, réduction des ilots de chaleurs, biodiversité et amélioration des paysages) et sociaux (Guidi Nissim & Labrecque, 2021). En sorte, la phytoremédiation permet la réappropriation des friches industrielles au profit d'un équilibre social pour certains quartiers ou les politiques d'équité environnementale sont *quasi* absentes (Cruz-Sandoval et al., 2020).

1.3.2. Le culture intensive en courte rotation

La culture intensive en courte rotation (CICR), un mode de production appelé « short rotation coppice » en anglais et parfois traduit par taillis sur rotation courte, a été appliquée comme une approche efficace pour la phytoremédiation en raison de (1) la grande densité de plantes utilisées et (2) les recepages fréquents (Desjardins et al., 2018; Labrecque & Lajeunesse, 2017; Padoan et al., 2020). Cette dernière caractéristique prive la tige primaire de sa dominance apicale, stimule le développement de bourgeons secondaires (Paukkonen et al., 1992) et crée plusieurs ramifications et conséquemment la production de nombreuses tiges à partir de la souche (Guidi et al., 2013; Verlinden et al., 2015). Ainsi, la CICR appliquée à la phytoremédiation facilite l'extraction des contaminants du sol par les plantes, en vue de leur enfouissement après production de bioénergie (Marmiroli et al., 2011), réduisant ainsi considérablement les volumes à enfouir, et limite les risques d'érosion et de lessivage en particulier lorsque des espèces à système racinaire dense sont sélectionnées.

1.3.3. Les genres *Salix* et *Populus* en phytoremédiation

L'hétérogénéité (i.e., édaphique, climatique, chimique) qui caractérise les sites contaminés contraint les intervenants à porter une attention particulière lorsqu'il s'agit de déterminer les espèces végétales les plus adaptées (Laghlimi et al., 2015). Bien que la recherche en phytoremédiation ait initialement ciblé les espèces hyperaccumulatrices, leur production de biomasse est souvent faible (Lunáčková et al., 2003). De même, leur réseau racinaire est généralement peu étendu tandis que leur faible taux de transpiration restreint l'absorption de l'eau et, *ipso facto*, des contaminants par la plante sur une courte saison de croissance (Lunáčková et al., 2003). Ainsi, les caractéristiques à retenir pour la sélection d'une espèce, en particulier au Québec, doivent dépasser le seul aspect de l'accumulation et prendre en compte la dimension du système racinaire, la vitesse de développement et la production de biomasse (Marques et al., 2009). L'utilisation d'espèces ligneuses à croissance rapide comme les saules (*Salix* spp.*)* et les peupliers (*Populus* spp.*)* constitue donc un choix bénéfique en phytoremédiation pour le Québec, car elles démontrent :

1. Des capacités d'adaptation à diverses conditions pédologiques et climatiques (Pulford & Watson, 2003);
2. Des croissances rapides et des productions importantes de biomasse (Chen et al., 2014) ;
3. Des facilités de propagation à partir de boutures (Guidi Nissim & Labrecque, 2016; Zalesny, Jr. et al., 2005);
4. Des systèmes racinaires denses et ramifiés (Isebrands et al., 2014; Kuzovkina & Volk, 2009);
5. Des aptitudes à bien répondre à la taille (Paukkonen et al., 1992);
6. Des facultés à absorber et transpirer de grands volumes d'eau (Frédette et al., 2019) ;

Parmi les espèces, *Salix miyabeana, S. alba* et *S. viminalis* ainsi que plusieurs cultivars de peupliers ont démontré de bonnes capacités à croître dans des sols contaminés (Desjardins et al., 2018; Hu et al., 2013; Keller et al., 2003; Wani et al., 2020). Plusieurs études ont d'ailleurs démontré que des espèces du genre *Salix* possèdent la capacité d'accumuler des concentrations élevées de Cd et Zn et modérées d'As, Mn et Se (Courchesne et al., 2017; Dickinson & Pulford, 2005; Krishna et al., 2012; Labrecque et al., 1995; Moreno-Jiménez et al., 2009; Vamerali et al., 2009). Toutefois, les Salicacées ne parviennent généralement pas à stocker suffisamment certains contaminants (e.g., Cu, Cr, Ni, Pb) dans leurs tissus aériens (Bissonnette et al., 2010; Labrecque et al., 1995). Par exemple, *S. nigra* '5005' dans une expérience en pot utilisant des sols dopés à 800 mg Cu kg^{-1} n'a accumulé que 125 et 112 mg Cu kg^{-1} dans les parties aériennes et racinaires respectivement (Massenet et al., 2021).

De manière générale, les peupliers montrent des caractéristiques analogues aux saules (i.e., niveaux de tolérance et des physiologies distinctes selon les espèces). Ils permettent donc également le développement d'approches prometteuses de phytoextraction et de stabilisation des contaminants toxiques dans le sol (Di Baccio et al., 2003; Frenette-Dussault et al., 2019; Labrecque et al., 2020; McCutcheon & Schnoor, 2003; Sebastiani et al., 2004; Vigl & Rewald, 2014).

1.3.4. *Salix miyabeana* 'SX67'

Au Québec, plusieurs études se sont intéressées à l'établissement (Guidi et al., 2012) et au rendement (Fontanaet al., 2017; Fontana et al., 2020) de *S. miyabeana* 'SX67'. Ce cultivar peut être planté en « macro-boutures » (i.e., >50 cm), en boutures « traditionnelles » (i.e., 20 - 25 cm) ou en « micro-boutures » (i.e., 5 cm), ces derniers pouvant réduire les coûts de plantation (Frenette-Dussault et al., 2019; Guidi Nissim & Labrecque, 2016). Il montre des rendements supérieurs à d'autres cultivars (*S. gmelinii* 'India', *S. purpurea* 'Fish Creek') avec une production de biomasse entre 10 et 13 t ha^{-1} a^{-1} (Frenette-Dussault et al., 2019) en sol modérément contaminé de type G2[1]. Néanmoins, il peut souffrir d'une fluctuation importante de rendement qui dépend principalement des propriétés du sol, mais aussi des conditions climatiques (Fontana, et al., 2017). Par exemple, les teneurs en limon et en MO favoriseraient la croissance des saules alors que le Ca échangeable serait, au contraire, un facteur limitant (Fontanaet al., 2017).

En phytoremédiation, ce cultivar a montré une tendance intéressante à tolérer des contaminations de toutes sortes, comme l'arséniate de Cu chromaté et de pentachlorophénol, deux composés utilisés historiquement pour la préservation du bois (Frédette et al., 2019). Autre exemple, *S. miyabeana* (i.e., 'SX61' et 'SX67') aurait obtenu, dans une étude comparant onze cultivars de saules, les meilleurs rendements en sol contaminé aux hydrocarbures pétroliers (Grenier et al., 2015).

1.3.5. *Populus* DNxM – 915 508

Utilisés en sylviculture comme en CICR, les peupliers hybrides (PEH) démontrent des productions élevées (accroissement annuel moyen de 11,6 m^3 ha^{-1} a^{-1} – Ménétrier, 2008). Parmi les PEHs recommandés au Québec, on retrouve l'hybride femelle DNxM-915508 issu du croisement entre *P. deltoïdes x nigra* (DN) et *P. maximowiczii* (M) (Truax et al., 2012, 2014, 2018). Néanmoins, seulement quelques études ont été menées, à l'heure actuelle, sur sa capacité à croître dans un substrat contaminé et phytoextraire des éléments traces (ETs). L'unique investigation complète à ce jour a montré que ce clone, en une saison de croissance, pouvait produire 6,4 T ha^{-1} et accumulé jusqu'à environ 15 mg Cu kg^{-1} dans ses parties aériennes (Guidi Nissim & Labrecque, 2023).

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[1] Moyen et de classe texturale L-S-A (loam sablo-argileux)

De même, seulement quelques études ont été menées à l'heure actuelle sur les rendements de phytoextraction et l'accumulation des éléments traces (ETs) par le DNxM. Pour la première année de croissance du DNxM-915508 en sol de friche industrielle présentant une concentration de 78,5 mg Cu kg $^{-1}$, des concentrations moyennes de 0,01 mg As kg^{-1}, 9,10 mg Cu kg^{-1}, 0,72 mg Pb kg^{-1} et 89,9 mg Zn kg^{-1} ont été rapportées (Guidi Nissim & Labrecque, 2023) .

1.4. LES FERTILISANTS ORGANIQUES

La « bioéconomie » fait référence à l'utilisation durable des ressources organiques dans une approche renouvelable pour générer de nouveaux produits ou procédés comme les fertilisants et la bioénergie (McCormick & Kautto, 2013). Une telle économie est dite circulaire puisqu'elle incorpore systématiquement des sous-produits dans son cycle de production et concerne diverses activités telle l'agriculture, tout en atténuant les impacts biogéochimiques sur l'environnement grâce au retour des nutriments à la source (Murray et al., 2017).

L'agriculture est l'une des plus grandes sources d'émission de gaz à effet de serre dont 30 % proviennent de l'énergie nécessaire à la production de fertilisants inorganiques (Stocker et al., 2014). En comparaison, l'usage d'engrais organiques comme le lisier de porc permettent de générer des rendements en biomasse végétale similaires (McLaughlin et al., 2000). Dans la majorité des cas, l'incorporation d'engrais organiques réduit considérablement les gaz à effet de serre (GES) engendrés par la production de fertilisant. Cependant, l'activité microbienne et la décomposition pourraient entraîner une augmentation des émissions de GES une fois incorporés aux champs (Walling & Vaneeckhaute, 2020).

Bien que la production de fertilisants inorganiques soit coûteuse et énergivore, l'utilisation des fertilisants organiques, qui reste niche malgré un récent gain de popularité, offre des avantages économiques et des impacts environnementaux mineurs, augmentant ainsi l'attractivité de nombreuses activités telles que la phytoremédiation (Chabbi et al., 2017; Paes et al., 2019; Rumpel et al., 2020; Sharma et al., 2019; Solinas et al., 2021).

La principale différence entre fertilisants inorganiques et fertilisants organiques réside dans la teneur plus importante en composés carbonés de ces derniers qui, dès lors, contribuent davantage à l'augmentation du taux de carbone et d'azote dans le sol. Ainsi, d'une année à l'autre, la quantité de fertilisant organique nécessaire pourrait contribuer à la diminution de l'apport en fertilisants inorganiques (Hepperly et al., 2009; Lazcano et al., 2013; Yang et al., 2016).

Le carbone organique (CO) et la matière organique (MO) affectent plusieurs propriétés fonctionnelles et physiques du sol :

1. La MO (≥ 2 %) influence la structure des sols la rendant moins érosive et limitant le lessivage des nutriments en augmentant la capacité d'échange cationique (CEC) (Murphy, 2015; Oades et al., 1989; Ramos et al., 2018; Rosewell & Loch, 2002);
2. Le CO contribue à la stabilité des agrégats, augmentant la cohésion des particules minérales et ralentissant la pénétration de l'eau dans le sol (Annabi et al., 2007; Kong et al., 2005);
3. Ils stimulent la densité de microarthropodes et l'activité des microorganismes (González et al., 2010; Meidute et al., 2008; Petersen, 2003);
4. Le CO agit comme réservoir et source de nutriments pour la production agricole (Murphy, 2015).
5. Le CO tamponne l'acidification des sols provoquée par les fertilisants inorganiques (Dai et al., 2021).

En phytoremédiation, la fertilisation peut également avoir des effets significatifs sur l'extraction des contaminants (Brown et al., 2003; Puschenreiter et al., 2005). Par exemple, la décomposition de la MO peut générer du carbone organique dissous qui influence à la hausse la solubilité et la biodisponibilité de certains métaux (Antoniadis & Alloway, 2002). Néanmoins, en leur présence, les végétaux, dont les saules, ont tendance à développer un réseau racinaire limité, réduisant sensiblement leur volume d'action (Fortier et al., 2019; Heinsoo et al., 2009; Jerbi et al., 2015). De plus, certains fertilisants organiques, selon leur origine et nature, peuvent entraîner des coûts de transport et de manutention importants et contenir des agents pathogènes et des éléments traces (e.g., le chrome, le cadmium, le plomb, l'arsenic) comme le fumier (Khan et al., 2018; Petersen, 2003; Rashmi et al., 2020).

1.4.1. Le fumier de poule

Au Québec, l'élevage de volaille génère une quantité importante de fumier, qui est déjà valorisé comme engrais organique et montre des rendements similaires ou supérieurs en fonction des conditions édaphiques et des espèces. Une telle augmentation a été observée chez le tournesol et le maïs pour lequel un épandage de 2 t ha^{-1} de fumier de poule permettait de doubler la biomasse produite et de rehausser significativement la CEC du sol (Boateng et al., 2006). Au regard des saules, une hausse de 38 % des parcelles amendées en fumier de poule (34,8 T ha^{-1} en 3e année) a été notée après trois années de croissance dans un loam limoneux graveleux (Adegbidi & Briggs, 2003). En somme, les sols amendés en fumier de poule composté montrent généralement des concentrations en K, Mg et P et des pH plus élevés ce qui contribuerait, selon toute vraisemblance, à une réduction de la biodisponibilité des ETM (Basta et al., 2005).

1.4.2. Le frass de ténébrion meunier

En quête de protéines ayant un impact environnemental moindre que celui engendré par l'industrie de la viande, l'élevage d'insectes comestibles connaît une popularité grandissante partout à travers le monde (Dicke, 2018). L'élevage de ténébrions meuniers (Tenebrionidae: *Tenebrio molitor* Linnæus, 1758), particulièrement dominant au Québec, génère, non seulement moins de GES que les élevages porcins et bovins, mais également un sous-produit composé de résidus (i.e., fèces, exuvies et substrats alimentaires non consommés), à fort potentiel baptisé frass (Fowles & Nansen, 2020; Hénault-Ethier et al., 2017; Oonincx et al., 2010). Ces résidus ont récemment démontré des impacts agronomiques positifs (e.g., augmentation de la biomasse, meilleure absorption des nutriments), lorsqu'amendés sur des parcelles d'orge (*Hordeum vulgare* L.) ou de tomate (*Solanum lycopersicum* var. Moneymaker) (Houben et al., 2020). Par sa nature, il stimulerait l'activité microbienne (e.g., favoriserait la synthèse d'hormones de croissance) du sol dont certains utilisés en ingénierie agricole et enrichirait le sol en carbone et azote par la dégradation de la chitine (Barragán-Fonseca et al., 2022; Francesca et al., 2015; Houben et al., 2020; Jacquiod et al., 2013; Poveda et al., 2019). Le frass serait aussi capable d'aider les plantes à résister aux stress abiotiques (e.g., sécheresse, salinité), biotiques (e.g., ravageurs entomologiques ou fongiques), et pourrait potentiellement diminuer le pH du sol (Dulaurent et al., 2020; Hénault-Ethier et al., 2023; Poveda et al., 2019). Néanmoins, sa teneur en nutriments est généralement dépendante de la diète donnée aux insectes, ce qui rend ses propriétés fertilisantes variables contrairement aux fertilisants inorganiques (Poveda et al., 2019).

1.5. Objectifs et hypothèses de recherche

1.5.1. Objectifs

L'objectif principal de ce projet de maîtrise est de comparer l'impact de deux formules fertilisantes sur les performances phytoremédiatrices d'un cultivar de saule (*Salix miyabeana* 'SX67') et d'un peuplier hybride (clone 915508), tous deux cultivés sur un sol contaminé au Cu. En effet, en comblant les besoins nutritionnels de ces deux Salicacées, il serait possible d'accroître leur performance de croissance et, par conséquent, d'augmenter leur production de biomasse corrélée au potentiel de phytoextraction du Cu et d'autres contaminants inorganiques. L'effet potentiel de ces amendements organiques sur la biodisponibilité du Cu, le rendent propice à l'absorption, sera suivi. Le cas échéant, la MO pourrait former des complexes avec les métaux pouvant contribuer au ralentissement du lessivage vertical de ceux-ci.

1.5.2. Hypothèses

Hypothèse générale : Les amendements permettront d'optimiser le rendement de phytoextraction du cuivre chez les saules et/ou les peupliers.

Hypothèse 1 : Les différentes formules auront des effets distincts sur la biodisponibilité du Cu, entraînant des accumulations variables dans les tissus des plantes.

Sous-hypothèse 1.1 : Les amendements provoqueront une diminution du pH du sol au cours de l'expérience.

Hypothèse 2 : Les saules et/ou les peupliers présenteront des réponses différentes aux divers traitements.

Sous-hypothèse 2.1 : Une variation intraspécifique de la production de biomasse sera observée en fonction des différents traitements.

Sous-hypothèse 2.2 : Le taux de phytoextraction du Cu variera selon les différents traitements appliqués.

CONTRIBUTION À L'ARTICLE SCIENTIFIQUE

Je déclare être le premier auteur de cet article présenté dans le cadre de ma candidature à la maîtrise en sciences biologiques.

Cet article est la présentation des résultats obtenus dans le cadre de mon projet de maîtrise se déroulant entre janvier 2021 et décembre 2022. Ce projet s'est réalisé sous la direction de mon directeur Michel Labrecque et avec l'assistance de Louise Hénault-Ethier, Amandine Bonet, Adrian Paul.

Marc Olivier Brunette : Conceptualisation, Méthodologie, Monitoring, Prise des mesures, Analyse des résultats, Recherche, Écriture – Version préliminaire, Écriture - Révisions & Corrections.

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Louise Hénault-Ethier: Conceptualisation, Méthodologie, Recherche, Supervision, - Révisions & Corrections, Financement.

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2. Impacts of Organic Amendments on the Phytoremediation Capacities of Willows and Poplars

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2.1. Introduction

Industrial and, by extension, anthropogenic activities have historically and profoundly contaminated natural ecosystems in direct proximity to populations worldwide (Alker et al., 2000; De Sousa, 2006; Song et al., 2019). Some of these sites, commonly defined as brownfields, are now abandoned in urban or peri-urban sectors after all economic operations—whether industrial or commercial—ceased and cities expanded (Drenning et al., 2020). Within the broad spectrum of contaminants encountered in brownfield soils previously occupied by the petrochemical industry, organic compounds, including polycyclic aromatic hydrocarbons (PAHs), and inorganic elements such as trace elements (TE — e.g., As, Cd, Cu, Pb) are predominant (Roy et al., 2005; Wang et al., 2016). If their concentrations exceed legal criteria in soils, these contaminants cause substantial income losses for municipalities, as the land remains unoccupied (Beaulieu, 2021; Granda & Théorêt, 2016; Jorat et al., 2020). For example, copper is an essential micronutrient for plants and many other organisms but is biotoxic in excessive quantities (Kumar et al., 2021). Consequently, it is one of the primary contaminants worldwide that prevent the reallocation of large areas in socioeconomic-pressured neighborhoods (Festa & Thiele, 2011; Rehman et al., 2019; Shabbir et al., 2020). Hence, TE removal is believed to provide long-term economic benefits and immediate environmental and social services (Guidi Nissim & Labrecque, 2021).

Phytoremediation, a technique that takes advantage of certain plant species capable of thriving in hostile conditions (e.g., contaminants, pH, texture), provides a solution to the challenges faced by stakeholders managing postindustrial sites (Todd et al., 2016). Although the potential for copper phytotoxicity in most plants has been reported at concentrations between 150 mg kg^{-1} and 300 mg kg^{-1}, some species demonstrate resilience in adverse conditions, allowing them to immobilize contaminants and, in rare instances, uptake and translocate contaminants to their aerial biomass, which may then be recovered after the harvest of aerial parts (Hodges, 2010; Kabata-Pendias, 2010) . Willows and poplars, in particular, when grown as short-rotation coppice, a mode of production characterized by high planting density and repeated coppicing (Desjardins et al., 2018; Labrecque & Lajeunesse, 2017; Padoan et al., 2020), evidenced significant extractions of TE (Marmiroli et al., 2011) as well as a substantial capacity to prevent erosion and leaching (Dos Santos Utmazian & Wenzel, 2007; Lebrun et al., 2018; Wieshammer et al., 2007).

At the forefront of green management for contaminated sites, stakeholders developing phytoremediation projects must constantly reassess their environmental impacts and incorporate sustainable and renewable approaches to generate new commodities (e.g., fertilizers) or processes (e.g., bioenergy, phytochemical) (McCormick & Kautto, 2013; Sas et al., 2021). In return, the import and export of by-products, according to the needs, can positively affect local practices and mitigate biogeochemical effects (Murray et al., 2017; Walling & Vaneeckhaute, 2020). Agriculture is one of the largest sources of greenhouse gas (GHG) emissions, with 30% of its carbon footprint resulting from the energy required to produce inorganic fertilizer (Stocker & Qin, 2014). In comparison, although microbial activity and decomposition may produce more GHG emissions once incorporated into fields, relatively inexpensive organic fertilizers have the potential to significantly reduce upstream GHG emissions (Sharma et al., 2019; Walling & Vaneeckhaute, 2020). Some industries (e.g., forestry) have already adopted organic by-products as fertilizers, which generally do not contain nutrient forms contributing to soil deterioration (e.g., $NaNO_3$, NH_4NO_3, KCl, K_2SO_4, NH_4Cl) (Savci, 2012). Overall, organic fertilizers have a higher C content, especially C-H linkage, thus participating substantially more in soil enrichment than inorganic fertilizers and limiting nutrient leaching (Shaji et al., 2021). If generalized, their use may eventually decrease fertilizer demand in the long term (Hepperly et al., 2009; Lazcano et al., 2013; Yang et al., 2016). Organic fertilization can also significantly affect the bioavailability of contaminants through, among other things, the decomposition of organic matter into dissolved organic carbon (Antoniadis & Alloway, 2002; Puschenreiter et al., 2005). In parallel, some organic fertilizers, depending on their nature, require significant transport and handling costs and may contain pathogens and TEs (Khan et al., 2018; Petersen, 2003; Rashmi et al., 2020). Local availability is a determining factor for using organic fertilizers (Dorais, 2007; Larney & Angers, 2012). Poultry farming induces a considerable amount of manure, which may, depending on soil conditions and species, generate higher yields and improve soil cation exchange capacity (CEC) (Boateng et al., 2006). For example, in a study focusing on willows amended with 69.3 Mg ha^{-1} of hen manure, annual stem biomass production soared by 30-38%, and soil pH increased, probably leading to a decrease in TE bioavailability (Adegbidi & Briggs, 2003; Basta et al., 2005).

The pursuit of sustainable management practices aimed at upcycling food waste into the food system to mitigate environmental impacts has led to a growing global interest in the recovery of by-products derived from the breeding of edible insects. (Dicke, 2018). The breeding of mealworms (Tenebrionidae: *Tenebrio molitor* Linnæus, 1758) not only generates fewer GHG emissions than pig and cattle breeding but also yields a value-added by-product called frass (i.e., feces, exuviae, and uneaten food substrates) (Fowles & Nansen, 2020; Hénault-Ethier et al., 2017; Oonincx et al., 2010). Frass fertilizing properties highly depend on the insect diet, but they generally enrich soils with carbon and nitrogen and may stimulate soil microbial activity (e.g., chitin degradation into chitosan, plant growth hormone biosynthesis) (Barragán-Fonseca et al., 2022; Francesca et al., 2015; Houben et al., 2020; Jacquiod et al., 2013; Poveda et al., 2019). It has recently demonstrated positive agronomic effects (e.g., increased biomass, better nutrient uptake) when amended on barley (*Hordeum vulgare* L.) and other vegetables, herbs, and flowers (Hénault-Ethier et al., 2023; Houben et al., 2020; Przemieniecki et al., 2021). Additionally, frass may help plants resist abiotic and biotic stresses, such as drought, salinity, and fungal pests (Dulaurent et al., 2020; Hénault-Ethier et al., 2023).

To substitute synthetic fertilizers with regionally available organic fertilizers could substantially reduce production costs and environmental impacts across various sectors, particularly if local recycling loops are established (Chabbi et al., 2017; Paes et al., 2019; Rumpel et al., 2020; Solinas et al., 2021). Following the enactment of Article 31.68 of the Environment Quality Act in Quebec, Canada, and the identification of eight thousand contaminated sites, municipalities in the province became incentivized to develop, optimize, and apply phytoremediation (Guidi Nissim & Labrecque, 2021).

This study aimed to evaluate better the potential of incorporating specific organic fertilizers from local industries in phytoremediation projects conducted in a pot trial under semi-controlled conditions. The primary objectives were to compare the impact of chicken manure and frass on the phytoremediation performance of two Salicaceae taxa (willow and poplar) in copper-contaminated soil. Secondary goals included investigating the effect of these organic fertilizers on soil TEs, nitrogen (N), and phosphorus (P) and exploring the overall response of the two plant species to these fertilizers over a 14-week growing period.

2.2. Method

2.2.1. Location and selected species

The study was conducted at the Montréal Botanical Garden (45° 33′ N, 73° 57′ W), Quebec, Canada. The trial was undertaken over three months, starting May 2021, in an open-sided growth tunnel. In this region, the growing season lasts for about four months (mid-May to mid-September) with an average daily high temperature above 20 °C (https://www.ouranos.ca).

Cuttings from *Salix miyabeana* 'SX67 'and *Populus deltoïdes x nigra* × *P. maximowiczii* (clone DN × M-915508) were collected from plants growing on a contaminated brownfield in the Rivière-des-Prairies/Pointe-aux-Trembles neighborhood of Montréal (QC, Canada —45° 63 ′ N, 73° 50′ W) during the autumn of 2020 and kept below 0° C in cold chamber until the setup of this experiment.

2.2.2. Soil preparation

The soil originated from the top 30 cm of the contaminated brownfield (80 cm of a sandy clay loam backfill over an undisturbed silty clay soil). After excavation, the soil was mechanically homogenized *in situ*, air-dried, and larger debris were removed by hand. Following the drying, samples were sifted (850 µm screen) and analyzed following Mehlich 3 (Mehlich, 1984) to determine the initial soil chemical characteristics (Table 1).

The soil was then amended with copper chloride $CuCl_2 \cdot 2H_2O$ in solution to 30 kg soil batches placed in large plastic containers. All treatment groups were amended to reach a Cu concentration of 1,000 mg kg^{-1}, mimicking the concentrations observed at the site from which the soil was collected. After the amendment, the soil was watered to achieve field capacity and vigorously mixed once a week for three months before initiating the experiment to ensure equilibration. Soil copper concentration values determined according to various methods to evaluate its bioavailability, are presented in the Results section in Table 2

TABLE 1: INITIAL TOTAL AND EXTRACTABLE SOIL ELEMENT CONCENTRATIONS AND GENERAL PROPERTIES OF THE SANDY CLAY LOAM COLLECTED FROM A BROWNFIELD IN THE RIVIÈRE-DES-PRAIRIES/POINTE-AUX-TREMBLES NEIGHBORHOOD OF MONTRÉAL, QC, FOR THE STUDY.

Properties	Units		Soil
OM	%		4.40
Density	$t.m^{-3}$		1.11
C/N			24.3
pH	pH		7.20
CEC	$meq.100g^{-1}$		42.8
Elements	**Unit**	**Extraction**	**Soil**
$N\text{-}NO_3$		-	5.00
$N\text{-}NH_4$		-	8.00
Total N		-	3,000
P_2O_5		-	2.70
K_2O		-	84.1
Ca		Pseudo-Total	5,100
Mg		Pseudo-Total	160
		Pseudo-Total	88.9
Cu		Phytoavailable	43.9
	$mg\ kg^{-1}$	$CaCl_2$	NA
		Pseudo-Total	342
Mn		Phytoavailable	22.6
		$CaCl_2$	NA
		Pseudo-Total	90
Zn		Phytoavailable	13.0
		$CaCl_2$	NA
Fe		Total	160
Na		Total	24
Al		Total	264

2.2.3. Treatments and experimental design

In May 2021, two willows (20 cm each) or three poplar cuttings (15 cm each) were placed horizontally in 12 containers each (50 cm x 37 cm x 34 cm) containing Cu-spiked soil and allocated one of the three fertilizing treatments (hen manure, frass, and the unfertilized control). A randomized multifactorial block design was used for the experiment with four replications for each species: treatment combination. The first fertilizer (i.e., chicken manure), provided by

Actisol™, was primarily composed of hen manure and bone meal (Table S1). The mealworm frass (Tenebrionidae: *Tenebrio molitor* Linnæus, 1758) was offered by TriCycle Inc, a Quebec company specializing in entotechnologies (Table 2). Treatments were applied twice, at a two-month interval based on total N (at the start and again seven weeks later, in July—125 kg N, 125 kg P and 82 kg K ha^{-1} for frass and 125 kg N, 312 kg P and 62 kg K ha^{-1} for hen manure), with the initial application covered with a layer of compost. Watering with tap water was performed every other day to maintain water-holding capacity until harvest.

2.2.4. Growth and physiological parameters

Shoots were measured at harvest (14 weeks, T_f). Leaf samples were collected after 7 weeks ($T_{1/2}$) prior to the second fertilizer application and at the end (14 weeks, T_f) of the experiment for photosynthetic pigment quantification of chlorophyll α, β, and carotenoids, following the protocol proposed by Garg (2012). To this end, leaf samples (100 mg) were placed in 15 mL falcon tubes, submerged in 10 mL of dimethyl sulfoxide, and heated at 65 °C using a water bath. After two hours, samples were vortexed and analyzed (Multiskan SkyHigh Microplate Spectrophotometer — ThermoFisher Scientific). The concentrations were determined using the equations developed by Wellburn (1994). At the end of the experiment, after three months of cultivation (September 2021), the aerial biomass was harvested and separated into two fractions: leaves and stem, in addition to roots. Subsequently, the plant samples were oven-dried at 65 °C, ground, and weighed. In parallel, soil samples (50 grams) were air-dried at room temperature for two weeks, sieved to 850 µm, and freed from all visible roots.

2.2.5. Chemical analysis of soil and plant material samples

To determine Cu fractions in soils at harvest, three extraction methods were used. The $CaCl_2$ extraction (0.01 *M*), evaluating the soil solution concentrations was carried out according to Houba (1986). Phytoavailable concentrations in soils were assessed using the diethylenetriaminepentaacetic acid (DTPA) extraction developed by Lindsay and Norvell (1978). To quantify pseudo-total concentrations, plant and soil samples were digested following Wilson (2005) Briefly, subsamples (~200 mg) were weighed, digested with 2 mL 70% HNO_3 in a block heating system for 6 h at 115 °C (Kjeldaltherm-Gerhardt), and diluted with distilled water to 50 mL.

Total P and Kjeldahl N concentrations in plants and available N, P, and K were determined using methods described by Carter & Gregorich (2007). For the total P and KN, subsamples (~200 mg) were weighed, digested in 10 mL of concentrated H_2SO_4 for 3 hours to 220 °C, then 360°C for 3 more hours, and diluted with 25 mL deionized water before analysis. For the available N, P, and K in soils, subsamples (approx. 5 g) were shaken with 50 mL of extraction solution (2.0*M* KCl for N and modified Kelowna for K and P (Ashworth & Mrazek, 1995) for 30 minutes and filtered.

All digests and available K and P were analyzed *via* inductively coupled plasma atomic emission spectroscopy (NexION 300—Perkin Elmer at the University of Montréal or Thermo iCAP6300 Duo—Thermo Fisher at the University of Alberta). Total P, KN, and available N concentrations were analyzed *via* a colorimetric method on a Thermo Gallery Plus Beermaster (Thermo-Fisher) at the University of Alberta. Certified reference materials from the National Institute of Standards and Technology were added to each sample batch to evaluate the quality of extraction or digestion. In parallel, internal controls were included to test the quality of the analytical measurements.

2.2.6. Statistical analysis and indexes

All statistical investigations were made using the R software, version 4.2.1. Means +/- standard deviation were determined by applying descriptive analysis. Significant differences were assessed employing a two-way analysis of variance (ANOVA) with a 95% confidence level (α=0.05), followed by Tukey's honestly significant difference (HSD). If any assumptions (e.g., normality, homoscedasticity, independency of the data) were violated, data were transformed to meet the necessary prerequisites *via* log transformations.

Nitrogen uptake in each pot was calculated by multiplying plant yield by nitrogen concentration in the aerial biomass at harvest. Nitrogen use efficiency was calculated using the difference between the mean nitrogen uptake of both treatments per species and the mean nitrogen uptake when no nitrogen was added per species, divided by the applied rate of nitrogen.

2.3. Results

2.3.1. Biomass yield

After the 14-week growth period, both plant species' mortality rates were marginal, with only two willow cuttings in the control group experiencing mortality. Despite unequal survival rates, no statistical difference ($p > 0.05$) was detected in shoot number, aboveground, or root biomass, with aboveground and root average biomass reaching approximately 75 g and 23 g for both species, respectively (Fig.1). Nevertheless, tendencies for each species were observed for aerial biomass when amended with frass or chicken manure versus unfertilized control (70 g vs. >75 g for poplars and >80 g for Salix). In comparison, trends in root biomass could only be observed in fertilized treatments for willows (25 g vs. 20 g). In the same way, no significant difference was observed in aerial biomass repartition, with the ratio of leaves to stem remaining similar in all treatments. In contrast, tendencies were noted in the final heights of species, as willows exhibited greater heights than poplars ($p = 0.07$). We also noted that willows treated with frass displayed a significantly greater height than unfertilized control plants ($p < 0.01$) while remaining statistically similar to plants amended with chicken manure.

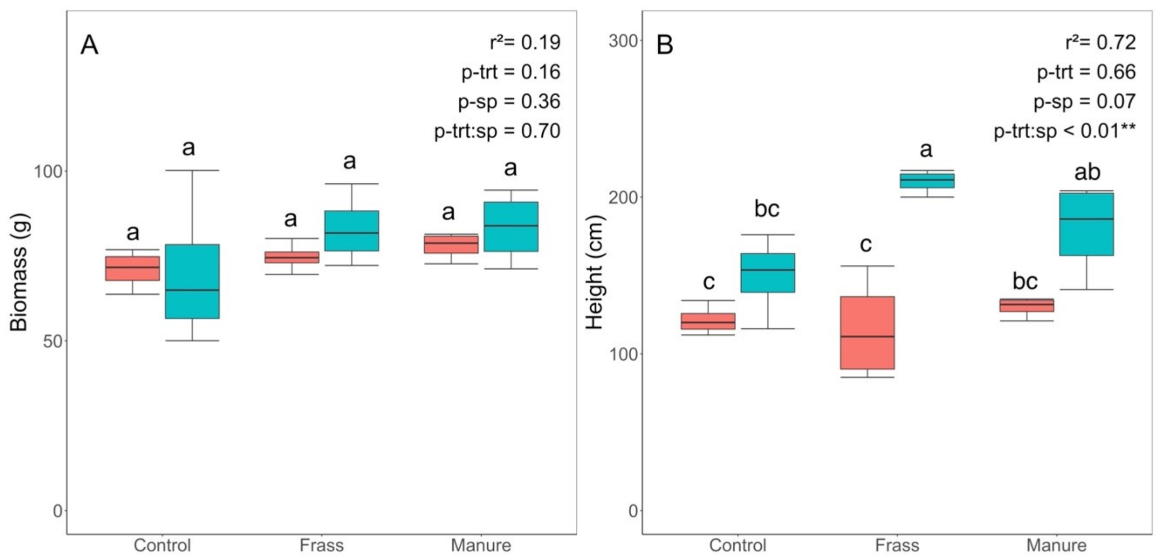

FIGURE 1: GROWTH OF TESTED SPECIES ON CU-CONTAMINATED SOIL ACCORDING TO THE AMENDED FERTILIZER USED. A: ABOVEGROUND YIELD IN GRAMS. B: TREE HEIGHTS IN CENTIMETERS. FOR BOTH FIGURES, RED: POPLAR TREES, AND BLUE: WILLOW TREES. ACCORDING TO TUKEY'S HSD TEST, BOX-AND-WHISKER TOPPED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT THE 5% LEVEL.

2.3.2. LEAF CHLOROPHYLL AND CAROTENOID CONCENTRATIONS

No difference between species and treatments were observed after six weeks (Fig. 2) in leaf pigments. The average values at $T_{1/2}$ for chlorophyll a, chlorophyll b, and total carotenoids were approximately equal to 1.00 µg mg^{-1}, 0.15 µg mg^{-1}, and 0.20 µg mg^{-1}, respectively. After 14 weeks, clear differences were observed between species for chlorophyll-a indicating a significant interaction between time and species ($p<0.01$) as values for willows increased and values for poplar decreased with time. Furthermore, an interaction between treatment:time:species was noticeable for chlorophyll-a and chlorophyll-b as significant difference between $T_{1/2}$ and T_f were only observed for willows in controls while a significant decrease was observed in the level of carotenoids in the frass treatment of both studied species.

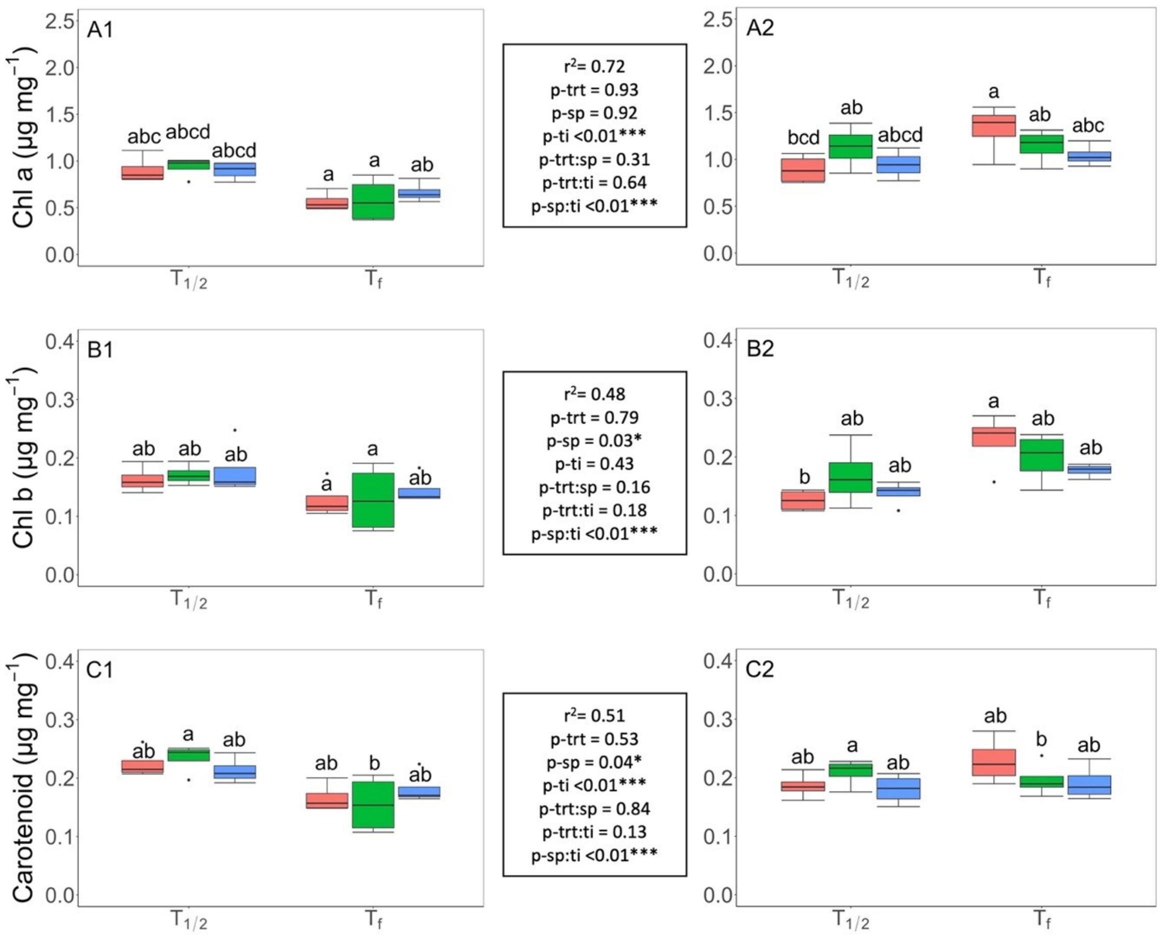

FIGURE 2: PHOTOSYNTHETIC PARAMETERS OF TESTED SPECIES ON CU-CONTAMINATED SOIL ACCORDING TO THE AMENDED FERTILIZER USED. A1, B1 AND C1 CORRESPOND TO POPLAR MEASUREMENTS FOR CHLOROPHYLL A, CHLOROPHYLL B, AND TOTAL CAROTENOIDS, RESPECTIVELY. A2, B2, AND C2 CORRESPOND TO WILLOWS MEASUREMENTS FOR CHLOROPHYLL A, CHLOROPHYLL B, AND TOTAL CAROTENOIDS, RESPECTIVELY. FOR ALL FIGURES, RED: CONTROL, GREEN: FRASS AND BLUE: MANURE.

CONCENTRATIONS ARE IN MG MG^{-1}. ACCORDING TO TUKEY'S HSD TEST, BOX-AND-WHISKER TOPPED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT THE 5% LEVEL.

2.3.3. CONCENTRATIONS OF COPPER AND OTHER METALLIC TRACE ELEMENTS

PLANT MATERIAL

Overall, concentrations at harvest averaged around 14 mg Cu kg^{-1} in aerial biomass while, in comparison, approximately 400 mg Cu kg^{-1} was found in roots, resulting in a mean bioconcentration factor (BCF) of 0.01 and a translocation factor of 0.07 for this element (Fig 3). Other TE of interest naturally present in the studied soil, including Mn, Pb, and Se, had similarly limited root and aerial mean concentrations, with the latter reaching 26.8 mg kg^{-1}, 0.59 mg kg^{-1}, and 0.20 mg kg^{-1}, respectively (results not shown). With average concentrations greater than 236 mg kg^{-1} and 3.27 mg kg^{-1}, only Zn and Cd displayed an average BCF greater than one, with a maximum of 5.02 and 5.45 respectively observed in poplar specimens amended with chicken manure (based on results from Tables S1-2-3). Although no treatment or species differences were observed for most TE concentrations in either roots or aerial biomass, root Pb concentrations were significantly greater ($p = 0.04$) in trees treated with chicken manure while Cd root concentration ($p = 0.01$) were found greater in poplars (Tables S2-3).

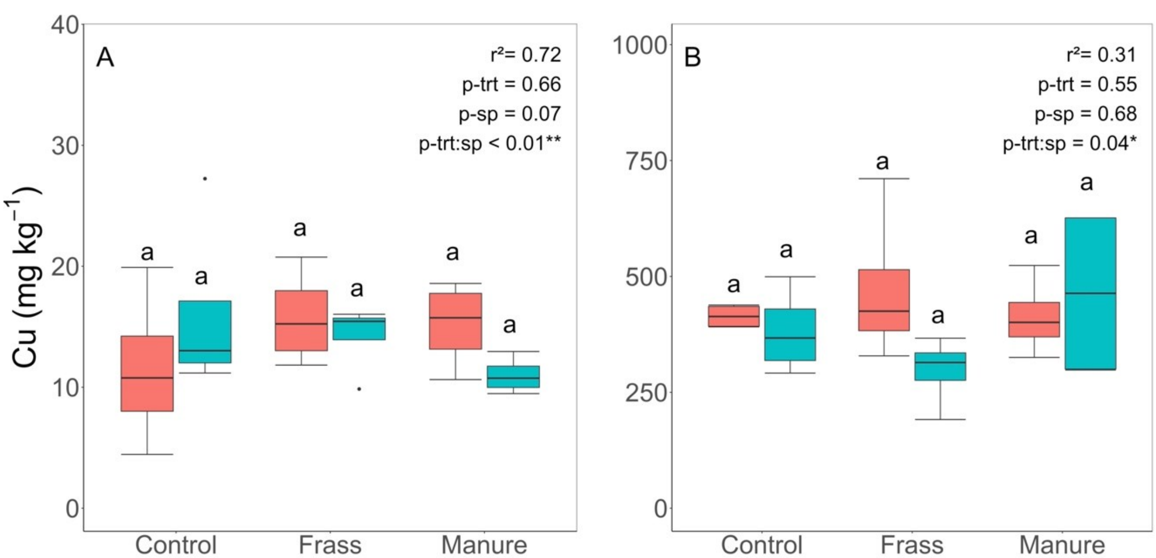

FIGURE 3: CU CONCENTRATIONS IN PLANTS AT HARVEST OF TESTED SPECIES ON CU-CONTAMINATED SOIL ACCORDING TO THE AMENDED FERTILIZER USED. A: COPPER CONCENTRATIONS IN AERIAL BIOMASS IN MG KG^{-1}, B: COPPER CONCENTRATIONS IN ROOT BIOMASS IN MG KG^{-1}. FOR ALL FIGURES, RED: POPLAR TREES AND BLUE: WILLOW TREES. CONCENTRATIONS ARE IN MG KG^{-1}.

ACCORDING TO TUKEY'S HSD TEST, BOX-AND-WHISKER TOPPED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT THE 5% LEVEL.

IN SOIL

Although some trends were observed for the phytoavailable fraction, no significant differences were statistically determined for Cu concentration in the labile, phytoavailable, or pseudo-total fractions (Table 2). Regarding the labile fraction (extracted using $CaCl_2$), Cu concentrations at the end of the experiment were approximately 1.00 mg kg^{-1}, regardless of the treatment or species (Table 2). Apart from Cu, Zn was the only other element detected above the limit of detection (LOD), but no significant differences were observed between treatments while concentrations averaged 0.1 mg kg^{-1}.

In contrast, phytoavailable Cu concentrations exhibited a noticeable trend ($p = 0.06$) in Populus, where Cu in pots amended with fertilizers was more available, particularly in the frass treatment. On the contrary, no difference was identified in the pots containing Salix trees (Table 2). Phytoavailable concentrations of Pb and Zn but did not show any significant differences, with both having mean values averaging 0.25 mg kg^{-1} (results not shown).

Lastly, pseudo-total Cu concentrations observed in all pots were similar, ranging between 863 mg kg^{-1} and 1110 mg kg^{-1}, and comparable to concentrations observed in the bulk soil. Similarly, concentrations of other measured elements, including Mn, Pb, and Zn, did not change significantly compared to the bulk soil.

TABLE 2 : LABILE, PHYTOAVAILABLE, AND TOTAL CU CONCENTRATIONS IN SOILS AT HARVEST ACCORDING TO THE AMENDED FERTILIZER AND SPECIES. WITHIN A COLUMN, VALUES WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT THE 5% LEVEL, ACCORDING TO TUKEY'S HSD TEST. FORMAT: MEAN (STANDARD DEVIATION)

Species	Treatment	Labile ($CaCl_2$)	Phytoavailable (DTPA)	Pseudo-total (HNO_3)
Populus	Control	0.91[a] (0.09)	409[a] (40.7)	872[a] (114)
	Frass	0.91[a] (0.17)	570[a] (72.0)	1030[a] (138)
	Chicken manure	1.05[a] (0.12)	539[a] (114)	1110[a] (268)
Salix	Control	1.00[a] (0.06)	447[a] (82.0)	944[a] (93.8)
	Frass	0.96[a] (0.12)	408[a] (115)	871[a] (239)
	Chicken manure	0.90[a] (0.17)	421[a] (111)	863[a] (216)

2.3.4. Concentrations of N and P and other Macronutrient

Plant material

Aerial parts harvested at the conclusion of the experiment revealed significant interspecific differences in total nitrogen (TN) and total phosphorus (TP) concentrations (p=0.01) (Fig.4). Populus exhibited notably higher values of TP in comparison to control (1.95 g kg^{-1} vs. 1.65 g kg^{-1}), while Salix demonstrated significantly greater values of TN (16.1 g kg^{-1} vs 12.6 g kg^{-1}—Fig 4). Conversely, no intraspecific differences were detected for either TN or TP. Nevertheless, the concentration of TN displayed a discernible trend (Chicken manure > Frass > Control) for both species, whereas no such trend was apparent for TP.

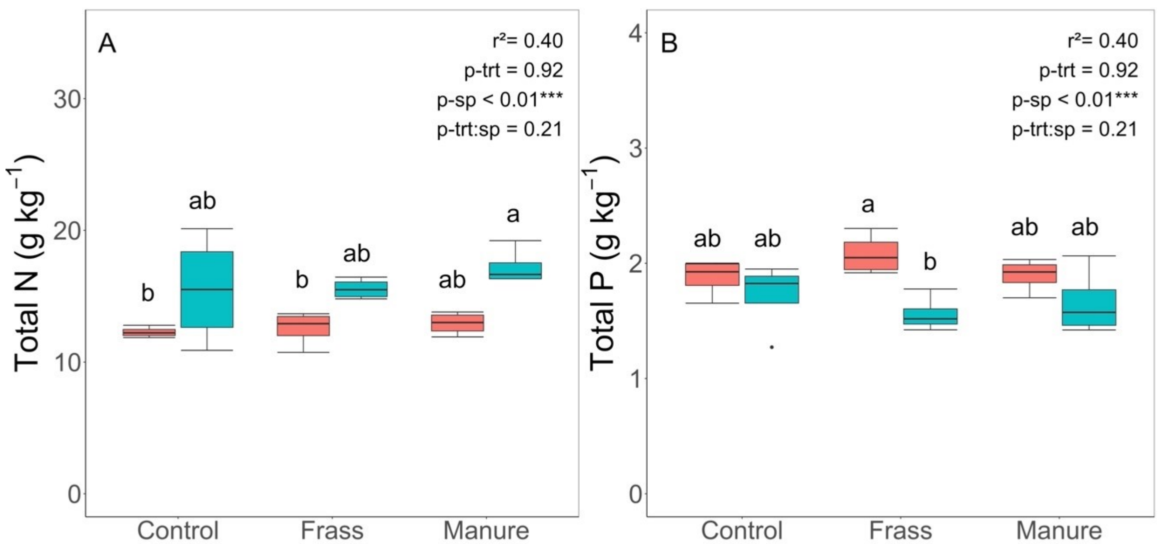

Figure 4: Macronutrient concentrations in plants at harvest of tested species on Cu-contaminated soil according to the amended fertilizer used. A: Total N concentrations in aerial biomass in mg kg^{-1}, B: Total P concentrations in aerial biomass in mg kg^{-1}. For all figures, red: poplar trees and blue: willow trees. Concentrations are in g kg^{-1}. According to Tukey's HSD test, box-and-whisker topped by the same letter are not significantly different at the 5% level.

Soil

The average mean concentrations of ammonium (NH_4-N) and total oxidized nitrogen (TON-N) ranged from 3.66 to 4.60 mg kg^{-1} and 6.91 to 16.1 mg kg^{-1} in soils at the end of the experiment, respectively, regardless of species and treatments. While no significant difference was found between treatments nor species the concentrations of TON and NH_4 in Salix containers amended with frass (9.24 and 3.66 mg kg^{-1}) appeared visibly lower than those without amendment (Fig. 5).

On the other hand, a significant interspecific difference ($p < 0.01$) was observed for TON concentrations, with soils hosting Salix exhibiting higher TON levels (13.2 mg kg^{-1}) compared to those hosting Populus (7.95 mg kg^{-1}). In the case of phosphate (P-PO4), no significant differences nor trends were identified, with concentrations averaging 40.7 mg kg-1.

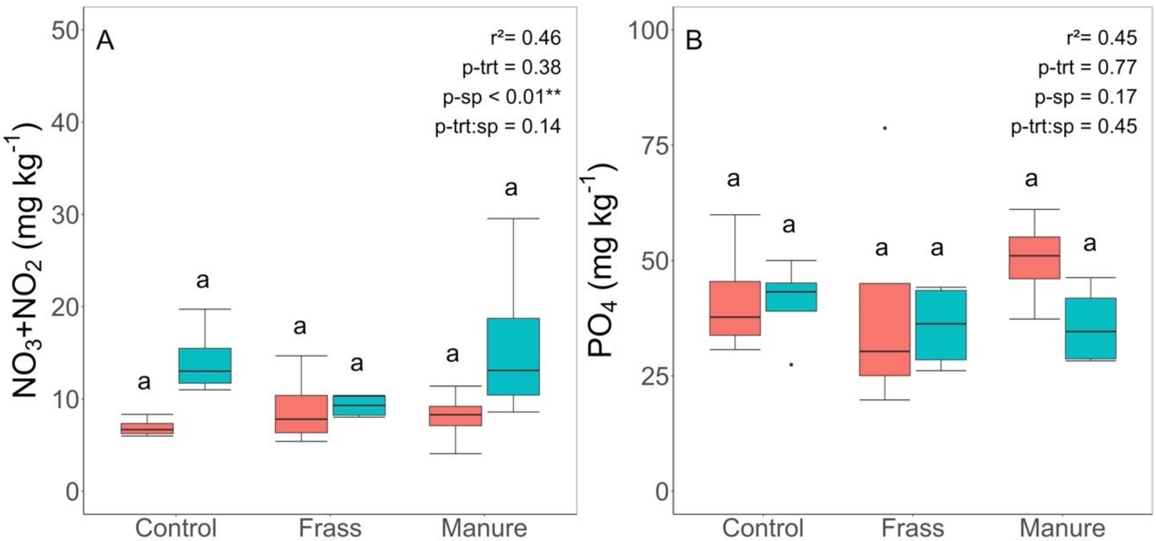

FIGURE 5 : MACRONUTRIENT CONCENTRATIONS IN SOILS AT HARVEST OF TESTED SPECIES ON CU-CONTAMINATED SOIL. ACCORDING TO THE AMENDED FERTILIZER USED. A: SOIL NO_3+NO_2 CONCENTRATIONS IN AERIAL BIOMASS IN MG KG^{-1}, B: SOIL PO_4 CONCENTRATIONS IN AERIAL BIOMASS IN MG KG^{-1}. FOR ALL FIGURES, RED: POPLAR TREES AND BLUE: WILLOW TREES. CONCENTRATIONS ARE IN G KG^{-1}. ACCORDING TO TUKEY'S HSD TEST, BOX-AND-WHISKER TOPPED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT THE 5% LEVEL.

2.3.5. UPTAKE AND USE EFFICIENCY OF N

Fertilization significantly affected N uptake in Salix only (p-value <0.01), while no influence was observed in Populus trees (p-value >0.05). Overall, the mean N uptake of Populus and the control group of Salix ranged from 41.6 kg ha^{-1} to 59.4 kg ha^{-1} (Table 3). In comparison, fertilized Populus values averaged 73.4 kg ha^{-1} and reached 83.3 kg ha^{-1}, with no statistically significant difference observed based on the type of fertilizer used. However, a higher mean was obtained for chicken manure (77.1 kg ha^{-1}) compared to the alternative (69.7 kg ha^{-1}). Results for N use efficiency demonstrated similar trends to N uptake while exhibiting lower statistical differences in the treatment and species interactions, but N use efficiency of frass in Salix was not statistically different from frass in Populus (Table 3). Nonetheless, the species effect persisted (p-value <0.01).

TABLE 3: NITROGEN UPTAKE AND USE EFFICIENCY OF TESTED SPECIES ON CU-CONTAMINATED SOIL ACCORDING TO THE AMENDED FERTILIZER USED. WITHIN A COLUMN, VALUES WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT THE 5% LEVEL, ACCORDING TO TUKEY'S HSD TEST. FORMAT: MEAN (STANDARD DEVIATION)

Species	Treatment	N Uptake (kg ha^{-1})	N use efficiency (%)
Populus	Control	47.1^{b} (4.77)	-
	Frass	50.8^{b} (7.06)	$1{,}46^{b}$ (2,82)
	Chicken manure	54.5^{b} (4.72)	$2{,}94^{b}$ (1,88)
Salix	Control	55.2^{b} (3.47)	-
	Frass	69.7^{a} (9.12)	$5{,}82^{ab}$ (3,64)
	Chicken manure	77.1^{a} (6.88)	$8{,}77^{a}$ (2,75)

2.4. DISCUSSION

The adoption of more sustainable methods for promoting plant species growth is vital for the preservation of our ecosystems and is considered one of the most urgent implementations to be generalized, especially in green technologies like phytoremediation (Megharaj & Naidu, 2017; Mench et al., 2010). The latter often targets soils with suboptimal physicochemical conditions that exhibit characteristics hindering both the short-term establishment and long-term productivity of most plant species without the implementation of proper agronomic practices despite favoring a wider species richness (Iverson et al., 2012; Schadek et al., 2009).

Fertilizers are critical in creating optimal growing conditions in challenging environments, such as brownfields (Megharaj & Naidu, 2017), while over-fertilizing can cause adverse effects, including TEs and nutrient leaching (Yavari et al., 2021). Although equal quantities of N were added to the soil in both treatments (i.e., frass and chicken manure), the treatments offered differing amounts of macro (P, K, Ca, Mg, etc.) and micronutrients (Mn, Zn, etc.) due to their unique compositions (see Table S1). Frass, which is less nutrient-dense overall, tend to supply lower concentrations of these elements to the soil, especially P and Ca which may create challenges in agricultural or forestry areas lacking these nutrients. In the contrary, it may present advantages by not over-enriching areas already high in Ca or P, or exacerbating eutrophication in vulnerable catchment basins. Additionally, it may help reduce metal accumulation, a phenomenon detrimental to biotopes and surrounding ecosystems often observed in production systems where manure by-products, enriched in metals such as As, Cu and Zn, are used as nutrient sources without well-established guidelines (Bolan et al., 2004; Sharpley et al., 1998). A metal enrichment partially attributed to the increasing use of these metals as feed additives in animal production industries (Bolan et al., 2004; Nicholson et al., 1999). In contrast, mealworm farming typically employs vegetable wastes, promoting economic circularity that optimizes agronomic resources and generates insect proteins and by-products with high fertilizing potential, competing with conventional fertilizers but containing reduced TE (Derler et al., 2021; Hénault-Ethier et al., 2023; Moruzzo et al., 2021).

Although the lack of competition for sunlight does not apply universally to the vast array of species that thrive in abandoned lands, it offers plants with pioneering characteristics, including invasive species, an optimal environment for their establishment and spread (Clemants & Moore, 2003; Duguay et al., 2007; Schadek et al., 2009). Hence, the faster growth of frass-amended willows could be advantageous in resource competition, particularly in remote or inaccessible areas where weeding or monitoring proves challenging (Sher et al., 2002). While rapid N mineralization is commonly associated with chicken manure, it can also be hypothesized that the labile C concentration in frass promotes rapid N mineralization making N readily available in the rhizosphere, likely at a faster rate than chicken manure (within just a few days) due to its smaller particle sizes, as emphasized by the N use efficiency trends observed between treatments (chicken manure > frass) (Houben et al., 2020; Rothé et al., 2019). In contrast, the intraspecific differences detected in the last index highlight disparities in intrinsic needs between willows and poplars (Labrecque & Lajeunesse, 2017; Richardson & Isebrands, 2013). Nevertheless, the nutritional requirements of the studied species were vastly exceeded, resulting in no significant yield or nutrient level differences. Other parameters, including water, were supplied to attain optimal conditions, limiting the potential benefits of applying frass. However, under suboptimal conditions, a few studies have demonstrated frass's ability to mitigate damage from drought or soil water saturation, factors that significantly affect yield in silviculture and agriculture making frass a potential solution in some North American regions, where Salix production via short rotation intensive coppices is often hindered by water availability during summer (Fontana et al., 2017; Labrecque & Teodorescu, 2003; Toillon et al., 2013; Zhivotovsky & Kuzovkina, 2010).

Although soil alkalinity was not exceptionally high, the pH coupled with the high concentrations of Ca—an element commonly found in high concentrations in brownfields (Jorat et al., 2020)—exceeding 5,000 mg kg^{-1} and Cu concentrations, were likely sufficient to limit the phytoavailability of essential elements such as B or S, especially when naturally present in low concentrations in the rhizosphere (Guidi Nissim & Labrecque, 2021; Guidi et al., 2012). Nevertheless, both Salicaceae species demonstrated a significant capacity to phytostabilize most of the TEs, including Cr, Mn, Ni, Cu, As, Se, Ba, and Pb despite the inherent limitations associated with pot experiments indicating their effectiveness in mitigating leaching. Furthermore, both Cd and Zn exhibited BCF greater than 1, suggesting moderate extractive capacities that appeared to be enhanced in poplars amended with frass, possibly due to an increase in root exudate production that stimulated the microbial community in the rhizosphere. The effects of high Cu concentrations on poplars and willows remain uncertain, as no observable qualitative phytotoxicity signs were detected including the preventing of mycorrhizal symbiosis, a phenomenon known to significantly reduce N and P uptake (Fortin Faubert et al., 2022; Selle et al., 2005) that may occur under adverse conditions (Chhabra & Dowling, 2017; Mackie et al., 2013; Quoreshi & Khasa, 2008), potentially impacting the overall health and growth of plants.

Interspecific and intraspecific differences were noted in the leaves' chlorophyll a and b concentrations at harvest. The observed interspecific differences can logically be attributed to the distinct traits inherent to each species. In contrast, counterintuitively, unfertilized plants displayed superior chlorophyll concentrations, despite established research suggesting low nitrogen availability negatively impacts chloroplast development and photosynthetic pigment concentrations (Dhami & Cazzonelli, 2020). This unexpected outcome could potentially be explained by a rise in evapotranspiration to complete nutrient needs, a theory consistent with the known relationships between transpiration, plant turgor, photosynthesis, and nutrient assimilation (Hsiao & Xu, 2000; Rouphael et al., 2012).

An alternative hypothesis suggests that the increase in NH_{4+} concentrations by approximately 5%, particularly in the surface soil where the fertilizer was applied, could have significantly impacted chlorophyll content, potentially due to the reduced uptake of essential cations, primarily Ca and K, among other factors (Guo et al., 2019; Heuer, 1991). Despite these differences, the chlorophyll concentrations in amended pots were within the range typically observed for willows grown in the brownfield where the soil originates (Palm et al., 2022). In contrast, the carotenoid concentrations obtained in all pots were slightly below the values found in the field, which may be attributed to reduced luminosity or high soil concentrations of TEs (Eismann et al., 2020; Esteban et al., 2014; Giannakoula et al., 2021; Palm et al., 2022).

2.5. Conclusion

This experiment aimed to assess the effects of two organic fertilizing amendments on *Salix miyabeana* 'SX67' and *Populus* (DNxM) '915508' grown in artificially Cu-contaminated brownfield soil. Interspecific differences revealed inherent traits, but no clear pattern emerged regarding the fertilization of both species, as the unfertilized treatment did not differ significantly for most of the studied parameters besides the greater heights of willows when fertilized with frass. In terms of TE in plant tissues, only Zn and Cd showed a BCF and translocation factor greater than 1 while the other TEs, including Cu, remained in the soil or roots. Globally, treatments had minimal impacts on soil-available nutrients and TEs. Although frass showed a trend of increasing phytoavailable Cu in poplar pots, similar Cu concentrations were observed in aerial parts of both species, suggesting that treatments had limited effects on soil available TEs and their uptake by plants.

3. Discussion générale

L'objectif principal de cette recherche était d'analyser l'influence des amendements organiques sur la capacité de phytoremédiation de deux espèces, *Salix miyabeana* ‘SX67' et *Populus* (DNxM) ‘915508', lorsque cultivées sur un sol enrichi en cuivre et amendé avec des engrais organiques issus de l'économie circulaire. Globalement, aucune tendance n'a pu être établie quant à l'effet des fertilisants comparativement au groupe témoin, cependant, des différences physiologiques notables ont été observées entre les deux espèces. En particulier, le saule, lorsqu'amendé avec du frass de ténébrions meuniers, a montré une croissance significativement plus élevée, probablement attribuable à l'aptitude de cet engrais à préserver l'équilibre hydrique et à fournir un approvisionnement en eau adéquat en permettant une meilleure rétention.

Malgré des besoins nutritionnels satisfaits par tous les traitements à l'exception du groupe témoin, une absence de différences intraspécifiques a été obtenue. Celle-ci pourrait probablement être attribuée aux caractéristiques du sol et à des facteurs abiotiques particuliers (par exemple, le pH, la texture du sol) qui auraient limité la disponibilité des nutriments fournis par les amendements. Cependant, il est important de noter que seul l'apport en azote était contrôlé (250 kg N ha^{-1}) dans cette étude. Par conséquent, les apports en macro et micronutriments étaient susceptibles de varier en fonction de l'amendement utilisé : le frass fournissait des quantités de phosphore nettement inférieures à celles du fumier de poule, qui était, en revanche, enrichie en calcium et en sodium, le rendant moins approprié pour une utilisation dans des bassins versants vulnérables ou soumis à l'eutrophisation.

Concernant les éléments traces métalliques (ETM) assimilés et transférés vers les tissus aériens des plantes, seuls le zinc et le cadmium ont démontré à la fois un facteur de bioconcentration et un facteur de translocation supérieurs à 1. Les autres ETM, y compris le cuivre, ont soit été retenus dans le sol, soit se sont accumulés dans les racines. De plus, aucun effet des amendements sur la biodisponibilité des ETM dans le sol n'a été observé, à l'exception du cuivre dans la rhizosphère des peupliers amendés avec du frass. Toutefois, ces derniers présentaient des teneurs en cuivre comparables à celles des autres combinaisons traitement-espèce dans les parties aériennes, ce qui suggère que les capacités d'absorption intrinsèques des plantes étaient atteintes, remettant ainsi en question l'hypothèse générale de cette étude. Les variations des concentrations d’ETM comme le nickel, le sélénium et le plomb dans les racines, qui dépendent de l'amendement utilisé, ne peuvent être expliquées par le protocole expérimental de cette étude, mais pourraient être attribuées à l'influence des amendements sur les propriétés physico-chimiques du sol ou à leur capacité à induire la formation de microbiomes distincts. Ces questions pourraient être élucidées par des recherches plus approfondies, incluant l'étude de différents horizons du sol et une analyse métagénomique.

Cette recherche, menée dans un environnement semi-contrôlé, a permis d'étudier la capacité du saule et du peuplier à se développer et à interagir dans un milieu enrichi en cuivre, qui peut être phytotoxique pour certaines espèces végétales. Toutefois, comme tout environnement contrôlé, cette étude a créé un contexte artificiel sensiblement différent de celui auquel les arbres sont normalement exposés en friche. Par exemple, en zone industrielle, le vent peut entraîner le dépôt de contaminants aérosols, issus des activités environnantes, sur la végétation, alors que les précipitations et les températures tout au long de la saison estivale peuvent également avoir un impact significatif, positif ou négatif, sur la production de biomasse. Également, un tel milieu de culture doit normalement résister au stress du gel hivernal. Il n'a donc pas été possible d'observer l'hibernation, la dormance et le deuxième bourgeonnement des arbres au printemps. De plus, en champ, les racines ont la possibilité d’éviter les zones riches en contaminants alors qu’elles y sont contraintes en pot. En fin de compte, la durée allouée à l'expérience a fait en sorte que les arbres ont eu une période de croissance limitée, également réduite par le temps nécessaire au bourgeonnement des boutures. Pour finir, l'espace restreint pour l'installation des pots de l'étude a également limité le nombre total d'arbres, ce qui a réduit la puissance statistique qui aurait pu être obtenue *in situ* sur un terrain plus vaste.

Les connaissances acquises au cours de cette expérience pourraient permettre d'ajuster certains paramètres et de changer d'échelle, malgré la nécessité d'effectuer des études en milieu contrôlé. Une éventuelle étude *in situ* pourrait être réalisée sur un terrain contaminé qui n'aurait pas besoin d'être préalablement dopé. D'autres espèces végétales pourraient être intégrées (compagnon), alors que les conditions météorologiques et la présence de mauvaises herbes seraient contrôlées par des désherbages et arrosages méthodiques pour une production de biomasse optimale. En outre, des études supplémentaires pourraient être menées afin d'optimiser la densité de plantation, le type d'implantation et de prolonger la période de croissance - trois paramètres clés influençant la production de biomasse. Ces recherches pourraient aider à résoudre ces questions, optimiser les stratégies d'épandage et, à long terme, favoriser l'adoption de ces amendements dans le cadre de la phytoremédiation.

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TABLE S1: TOTAL TE CONCENTRATIONS (MG KG^{-1}) IN SOILS AT HARVEST OF TESTED SPECIES ACCORDING TO THE AMENDED FERTILIZER USED. WITHIN A COLUMN, VALUES WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT THE 5% LEVEL, ACCORDING TO TUKEY'S HSD TEST. FORMAT: MEAN (STANDARD DEVIATION)

Species	Treatment	Cr	Mn	Ni	Cu	Zn	As	Se	Cd	Ba	Pb
Populus	*Control*	30.5[a] (4.07)	367[a] (17.0)	32.7[a] (0.87)	872[a] (114)	154[a] (13.9)	5.02[a] (0.18)	2.88[a] (0.46)	0.48[a] (0.06)	168[a] (9.85)	80.8[a] (11.9)
	Frass	28.9[a] (3.02)	399[a] (24.4)	33.5[a] (2.12)	1030[a] (138)	167[a] (22.6)	5.27[a] (0.18)	4.69[a] (4.22)	0.47[a] (0.06)	167[a] (19.9)	75.2[a] (12.4)
	Actisol	28.5[a] (0.99)	378[a] (22.4)	34.1[a] (3.40)	1110[a] (268)	156[a] (5.96)	5.12[a] (0.26)	2.71[a] (0.36)	0.45[a] (0.04)	162[a] (12.8)	75.0[a] (5.64)
Salix	*Control*	29.2[a] (1.42)	397[a] (28.3)	33.3[a] (1.80)	944[a] (93.8)	172[a] (29.7)	5.03[a] (0.53)	2.62[a] (0.14)	0.46[a] (0.04)	154[a] (6.02)	78.9[a] (4.57)
	Frass	29.1[a] (2.05)	356[a] (24.1)	33.9[a] (0.71)	871[a] (239)	160[a] (17.4)	5.22[a] (0.10)	2.89[a] (0.50)	0.47[a] (0.05)	169[a] (7.86)	75.3[a] (11.0)
	Actisol	34.4[a] (8.78)	365[a] (25.3)	33.3[a] (1.53)	863[a] (216)	168[a] (9.83)	5.40[a] (1.16)	2.90[a] (0.32)	0.51[a] (0.05)	166[a] (28.4)	107[a] (48.5)

TABLE S2: TRACE ELEMENT CONCENTRATIONS (MG KG^{-1}) IN PLANT AERIAL BIOMASS AT HARVEST OF TESTED SPECIES ACCORDING TO THE AMENDED FERTILIZER USED. WITHIN A COLUMN, VALUES WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT THE 5% LEVEL, ACCORDING TO TUKEY'S HSD TEST. FORMAT: MEAN (STANDARD DEVIATION)

Species	Treatment	Cr	Mn	Ni	Cu	Zn	As	Se	Cd	Ba	Pb
Populus	*Control*	0.48[a] (0.20)	22.9[a] (2.48)	2.90[a] (0.50)	11.5[a] (6.49)	224[a] (30.3)	0.33[a] (0.18)	0.18[a] (0.07)	2.29[a] (1.82)	11.6[a] (6.51)	0.75[a] (0.43)
	Frass	0.59[a] (0.13)	28.0[a] (9.83)	3.76[a] (1.28)	15.8[a] (3.98)	261[a] (20.0)	0.37[a] (0.18)	0.23[a] (0.04)	3.60[a] (1.67)	17.7[a] (3.86)	0.74[a] (0.44)
	Actisol	0.49[a] (0.06)	36.5[a] (11.9)	4.45[a] (1.10)	15.2[a] (3.61)	262[a] (81.9)	0.30[a] (0.15)	0.22[a] (0.09)	4.31[a] (1.88)	19.7[a] (9.28)	0.56[a] (0.43)
Salix	*Control*	0.43[a] (0.14)	25.6[a] (10.8)	3.65[a] (1.03)	16.1[a] (7.49)	214[a] (5.82)	0.36[a] (0.23)	0.17[a] (0.02)	2.94[a] (0.92)	12.9[a] (3.13)	0.45[a] (0.37)
	Frass	0.45[a] (0.15)	25.6[a] (10.9)	3.42[a] (1.16)	14.2[a] (2.91)	239[a] (49.2)	0.36[a] (0.22)	0.20[a] (0.03)	3.41[a] (1.85)	12.9[a] (6.48)	0.54[a] (0.35)
	Actisol	0.34[a] (0.14)	22.3[a] (8.53)	3.30[a] (1.36)	11.0[a] (1.53)	216[a] (32.7)	0.32[a] (0.17)	0.18[a] (0.04)	3.08[a] (1.49)	15.2[a] (8.81)	0.50[a] (0.11)

TABLE S4: TRACE ELEMENT CONCENTRATIONS (MG KG^{-1}) IN PLANT ROOT BIOMASS AT HARVEST OF TESTED SPECIES ACCORDING TO THE AMENDED FERTILIZER USED. WITHIN A COLUMN, VALUES WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT THE 5% LEVEL, ACCORDING TO TUKEY'S HSD TEST. FORMAT: MEAN (STANDARD DEVIATION)

Species	Treatment	Cr	Mn	Ni	Cu	Zn	As	Se	Cd	Ba	Pb
Populus	*Control*	7.97[a] (1.39)	97.2[a] (22.1)	8.58[ab] (1.16)	414[a] (25.7)	63.9[bc] (5.22)	1.92[a] (0.26)	0.87[ab] (0.16)	0.83[bc] (0.21)	44.3[a] (8.73)	14.8[ab] (3.92)
	Frass	6.87[a] (1.38)	86.3[a] (24.2)	7.55[b] (2.15)	472[a] (166)	59.6[bc] (10.3)	1.61[a] (0.24)	0.78[b] (0.20)	0.76[c] (0.09)	37.9[a] (4.14)	12.0[ab] (2.61)
	Actisol	6.87[a] (3.79)	78.4[a] (44.3)	7.17[b] (3.16)	413[a] (83.2)	54.7[c] (16.4)	1.78[a] (0.79)	0.64[b] (0.22)	0.80[c] (0.14)	37.0[a] (17.1)	11.0[ab] (5.13)
Salix	*Control*	8.15[a] (3.71)	127[a] (57.5)	10.8[ab] (2.49)	381[a] (92.2)	135[a] (7.32)	2.03[a] (0.64)	0.99[ab] (0.14)	1.49[a] (0.43)	42.0[a] (9.65)	12.9[ab] (5.36)
	Frass	8.19[a] (2.08)	97.4[a] (11.8)	10.4[ab] (1.42)	297 [a] (74.9)	125[a] (45.2)	1.68[a] (0.13)	0.91[ab] (0.33)	1.27[abc] (0.35)	39.0[a] (3.05)	10.7[b] (1.08)
	Actisol	10.8[a] (4.06)	126[a] (46.2)	12.7[a] (2.51)	463 [a] (189)	115[ab] (34.5)	2.15[a] (0.51)	1.29[a] (0.07)	1.42[ab] (0.23)	53.3[a] (17.2)	21.1[a] (6.95)