Rôles comparés des métaux, des espèces végétales et des traitements des sédiments sur la diversité microbienne, la croissance végétale et la réduction des métaux dans les effluents au sein de microcosmes représentant des solutions fondées sur la nature pour le traitement des eaux pluviales

Comparative roles of metals, plant species and sediment treatments on microbial diversity, plant growth and metal reduction in effluents within microcosms representing nature-based solutions for stormwater treatment

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RÉSUMÉ

L'imperméabilisation des sols renforcent la nécessité de disposer de systèmes efficaces de traitement des eaux pluviales entre autres pour éliminer les éléments traces métalliques (ETMs) qui s'accumulent dans les eaux de ruissellement urbaines. Les solutions fondées sur la nature (SFN) s'appuient sur les interactions entre les plantes et les microorganismes pour retenir les ETMs, cependant les rôles respectifs et l'interaction de la végétation, des communautés microbiennes et des apports métalliques restent mal compris. Cette étude a examiné comment *Phragmites australis* et *Plantago lanceolata*, combinés à des communautés microbiennes naturelles ou réduites, influencent l'élimination du cuivre, du zinc, du nickel et du cadmium dans des microcosmes contrôlés. Les plantes ont été cultivées dans des sédiments autoclavés (Mi-) ou non autoclavés (Mi+), avec ou sans enrichissement en ETMs. La croissance des plantes et les concentrations en ETMs dans les effluents ont été déterminés pendant 12 semaines. La structure des communautés microbiennes a été étudié à 3 et 12 semaines. La croissance des plantes était plus élevée dans les conditions Mi-. Les analyses des effluents ont révélé des concentrations plus élevées de Cu et de Zn dans les conditions Mi-. Après trois semaines, les communautés bactérienne associées aux racines sont influencées par la stérilisation des sédiments. Dans l'ensemble, les résultats préliminaires suggèrent que la stérilisation des sédiments a un impact plus important que les apports de métaux sur les performances des plantes, la dynamique des métaux et les communautés microbiennes.

ABSTRACT

Soil sealing reinforces the need for effective stormwater treatment systems, among others for removing metal trace elements (MTEs) that accumulate in urban runoff. Nature-based solutions (NBS) rely on interactions between plants and microorganisms to retain MTEs, but the respective roles and interactions of vegetation, microbial communities and metal inputs have rarely been investigated. This study examined how *Phragmites australis* and *Plantago lanceolata*, combined with natural or reduced microbial communities, influence the removal of copper, zinc, nickel and cadmium in controlled microcosms. Plants were grown in autoclaved (Mi-) or non-autoclaved (Mi+) sediments, and with or without MTE enrichment. Plant growth, MTE concentrations in effluents, and microbial community structure were determined over 12 weeks. The structure of microbial communities was studied at 3 and 12 weeks. Plant growth was higher under Mi- conditions. Effluent analyses revealed no differences between plant species, but higher concentrations of Cu and Zn were observed under Mi-conditions. After three weeks, root-associated bacterial communities were influenced by the autoclaving process of sediments. Overall, preliminary results suggest that autoclaved sediment has a greater impact than metal inputs on plant performance, metal dynamics and microbial communities.

MOTS CLÉS

eaux pluviales, espèces fongiques, éléments traces métalliques, interactions plante-microorganismes, solutions fondées sur la nature

1 INTRODUCTION

Urban runoff mobilizes (among others) metal contamination, which often is discharged without treatment into receiving waters, posing significant environmental risks (Becouze et al., 2009; Brudler et al., 2019). Nature-based solutions (NBS) such as bioswales and infiltration basins are increasingly used to treat stormwater by mimicking natural soil filtration processes (Biswal et al., 2022; Wu et al., 2023). These systems rely on layered substrates and vegetation to retain contaminants through filtration, adsorption, sedimentation, and precipitation.

Vegetation contributes to pollutant removal by stabilizing the substrate, maintaining hydraulic conductivity, and participating in phytoremediation processes (Bonanno & Lo Giudice, 2010; Khan et al., 2015). Furthermore, plant–microorganism interactions influence NBS efficiency. Root exudates enrich the rhizosphere, which stimulate microbial growth and activity (Ma et al., 2019). In turn, microorganisms support plant growth under metal stress by solubilizing nutrients, producing siderophores and phytohormones, and forming biofilms that immobilize metals (Khatoon et al., 2024). Microorganisms contribute to MTE removal through biosorption, precipitation, redox reactions and bioaccumulation (Chepsergon & Moleleki, 2023; He et al., 2025).

Despite growing evidence, few studies evaluate plant–microbe interactions specifically in stormwater-treatment NBS. Better understanding these interactions is essential for optimizing the long-term performance of NBS in removing metals from urban runoff. Thus, the aims of this study were to determine i) which combination of two plants*, Phragmites australis* and *Plantago lanceolata*, with microorganisms, allows for better removal of Copper, Zink, Nickel and Cadmium, ii) if there is a removal of metals from the effluent, iii) in which compartment MTEs are immobilized (rhizosphere soil, roots, shoots), and, iv) to describe the effect of MTEs cocktail loading and microbial communities from sediments on *P. australis* and *P. lanceolata* performance and traits. To answer these questions, individuals of *Plantago lanceolat*a and *Phragmites australis* were cultivated in microcosms under different conditions of MTEs feeding (presence/absence), and in abundance reduction of microorganisms vs. natural abundance.

2 MATERIALS AND METHODS

Microcosms were set up to mimic infiltration basin conditions. The top organic layer of bioswales and infiltration basins near Lyon (Villeurbanne and Chassieu) was used as a substrate, referred to as sediment for simplicity, which were already contaminated with metals. Half of these sediments were autoclaved twice (20 min at 120 °C for both cycles) to reduce the microbial abundance and diversity (Mi- conditions), while the rest represented the condition with no modification of microorganism abundance (Mi+).

Two plant species*, Phragmites australis* (Poaceae) (Pa), which is often planted during establishment of vertical-flow NBS for stormwater treatment, and *Plantago lanceolata* (Plantaginaceae) (Pl), which secondarily colonizes the systems, were used. Seeds were grown in pots with ¾ sterilized sand and ¼ autoclaved sediments (from above-described infrastructures). When *P. australis* sized between 1-3 cm and *P. lanceolata* between 6-8 cm, they were transplanted into microcosms.

The microcosms were built from 83 PVC-U columns (diameter 12.5 cm, length 39 cm). A soft drainage tube was attached at the bottom of each column to allow water to drain away 22 hours after watering. They were filled (from the bottom) with 5 cm of sterilized gravel, 24 cm of sterilized sand, and 8 cm of sediments from the above-described stormwater NBS. Half of the columns received the autoclaved sediment, and the other half received the untreated sediment with the microorganisms. In each microcosm, two individual plants of the same species were transplanted.

Every week, half of the microcosms were watered with 800 mL of one quarter-strength Hoagland solution (Me-condition). For the other half, MTEs were added to the nutritive solution (Me+ conditions) to achieve the target concentrations of 212 µg/L of Zinc (Zn), 50 µg/L of Copper (Cu), 2.2 µg/L of Cadmium (Cd) and 2.9 µg/L of Nickel (Ni) consistent with concentrations commonly reported in stormwater (Becouze et al., 2009; Tondera et al., 2018). Every three weeks, 30 mL of the effluents were collected to measure MTE concentration and determine their conductivity and pH. The whole experiment was conducted for 12 weeks. Every week, measurements were taken on the plants (maximum height, number of leaves, leaf chlorophyll content).

Plant and soil samples were collected at two different times, 3 and 12 weeks after starting the experiment.

Measurements were performed on the aboveground parts of plants to assess their health and accumulation of MTEs (leaf traits: Leaf Dry Matter Content (LDMC), Surface Leaf Area (SLA), individual weight, C and N content, MTE concentrations) and on the roots (C and N content, MTE concentrations). The soil samples were analyzed to assess possible changes induced by conditions: C and N content, concentration of total and bioavailable MTEs, pH, conductivity, % of organic matter, % of carbonate. Subsamples of the rhizosphere soil and roots were stored at -80 °C for microbial analyses. PCR were carried out on both compartments targeting the fungal ITS2 region (gITS7/ITS4) and the bacterial 16S V3-V4 region (341F/805R). Seven or eight replicates per condition were send to ProfileXpert for paired-end 2*250 pb sequencing on MiSeq / Illumina.

3 SUMMARY OF RESULTS AND FUTURE DIRECTIONS

From 21 days onwards, for both plant species, the average sizes were significantly higher for the Mi- conditions compared to the Mi+ conditions (ANOVA and Kruskal-Wallis: p-value <0.05). The chlorophyll content in the leaves was higher in Mi- compared to Mi+ conditions at 7, 21, 64 and 90 days for *P. australis* and at every measurement for *P. lanceolata* (ANOVA with post-hoc Tukey test and Kruskal-Wallis: p-value<0.05). In conclusion, the plant growth is higher in autoclaved sediments compared to untreated sediments. One possibility is that the autoclaving modified the nutrient accessibility of the sediment and released some carbon sources. However, this has not yet been confirmed by analyses.

Effluent MTE concentrations have been analysed every third week. For each time point, differences among the eight conditions were evaluated. Under the same conditions of metals and sediment treatment, there were no difference between plant species during the entire experiment (Kruskal-Wallis: p-value>0.05) (Figure 1).

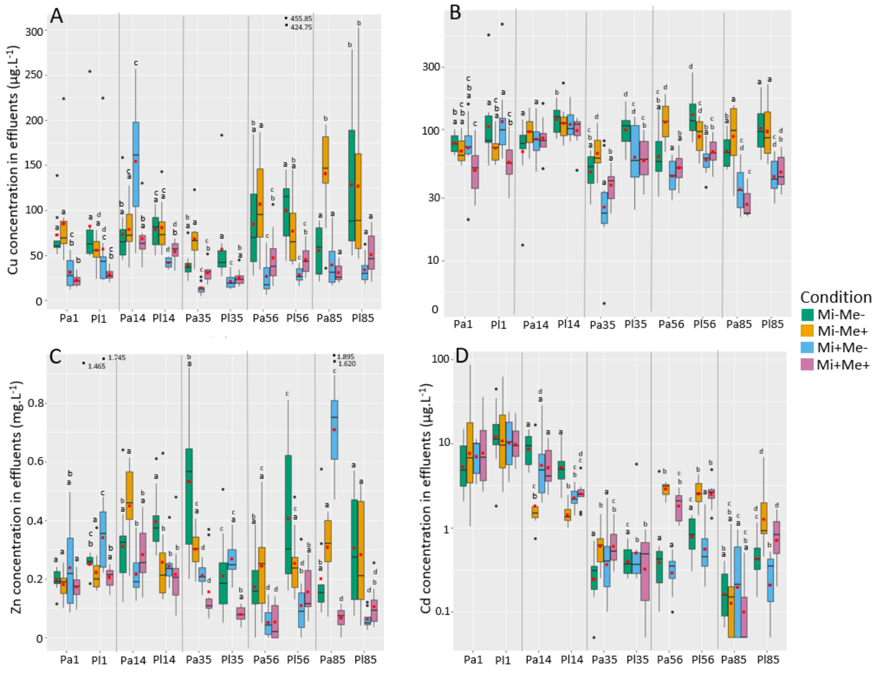

Figure 1: Concentration of metals in the effluents of microcosms experiment containing Phragmites australis (Pa) and Plantago lanceolata (Pl) every three weeks in each condition (Mi-: autoclaved sediments, Mi+: untreated sediments, Me-: watering without metals, Me+: watering with metals), for Copper (A), Nickel (B), Zinc (C) and Cadmium (D). The lower-case letters indicate significant differences between all conditions at a given time (Kruskal-Wallis, Dunn's test, p-value < 0.05).

The analyses of bacterial communities associated with roots at the third week has already been completed. The bacterial communities associated with roots show disparities in the taxa present between conditions. Eight percent of the total variations are induced by the plant species and 6% by autoclaving of sediments (Permanova on Bray-Curtis dissimilarity index: p-value<0.05). Although the orders vary, no differences in alpha diversity have been observed between all the conditions (Kruskal Wallis test: p-value>0.05). The communities have high taxa variability, distributed evenly. The addition of MTEs had no effect on the bacterial root-associated communities after 3 weeks. This could be explained by initial concentrations of the MTEs in the sediments; the addition of

MTE was probably not high enough to induce changes in communities. The remainder of the sequencing is currently underway.

At present, the results show no effect of metal addition to the feed water. Differences that are mainly induced by the autoclaving process of the sediments. For the analysis of samples after 12 weeks, we expect an effect of metal addition on microbial communities. This study will provide an initial understanding of the fate of metals in NBS and identify certain microorganisms that are tolerant to the presence of metals or promote plant growth.

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