



Nutrient-assisted phytoremediation of wood preservative-contaminated technosols with co-planting of *Salix interior* and *Festuca arundinacea*

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Abstract

The remediation of wood preservative-contaminated sites is an important issue due to the carcinogenic nature of some contaminants derived from wood preservatives (e.g., Cr⁺⁶, arsenate, and pentachlorophenol). This study evaluated the effects of fertilizer application on remediation potential of co-plantings of *Salix interior* Rowlee. (*Salix*) and *Festuca arundinacea* Schreb. (*Festuca*) in a wood preservative-spiked technosol while considering the potential contaminant and nutrient leaching. Two levels of nitrogen (N) and phosphorus (P) fertilizers, NaNO₃ and NaH₂PO₄ (25 and 75 mg L⁻¹), were applied to achieve three N:P ratios, i.e., 3:1 (75:25), 1:3 (25:75), and 1:1 (25:25), that were compared with a control treatment (0:0 N:P) in a mesocosm experiment. Roots of the plant supplied with 1:1 and 1:3 N:P had more than double arsenic (As) and copper (Cu) amounts (i.e., biomass × concentration) compared to the control ones. Highest As and Cu amounts in shoots were found for *Salix* stems and *Festuca* leaves in the 1:3 and 1:1 N:P treatments, respectively. Arsenic and P leaching was high in mesocosms supplied with 1:3 N:P. Contamination and nutrient leaching in the 1:1 N:P treatment did not differ from the control, except for Cu. In conclusion, 1:1 N:P treatment yielded the best results in terms of metal(loid) uptake and contaminant and nutrient leaching. In 1:1 N:P treatment, the maximum values of percent As, Cr, and Cu in *Salix* and *Festuca* aboveground were 0.18%, 0.024%, and 1.20% and 0.89%, 0.08%, and 1.78%, respectively.

Keywords Phytoremediation · Fertilization · Chromated copper arsenate · Pentachlorophenol · Leaching · Willow · Fescues

Introduction

Pentachlorophenol (PCP) and chromated copper arsenate (CCA) are two common chemicals that have been widely used

in North America since 1941 and 1933, respectively, to protect wood products against fungi, bacteria, and insects (Balasoïu et al. 2001; Coudert et al. 2013). Pentachlorophenol is an organic oil-borne compound that contains chlorophenols and some persistent hydrocarbon impurities such as polychlorinated dibenzo-dioxins/furans (PCDD/Fs). Chromated copper arsenate is a waterborne inorganic preservative made of hexavalent chromium, cupric oxide, and arsenic pentoxide (Wood preservation Canada 2012). Pentachlorophenol and CCA can be toxic and carcinogenic depending on their dosages. Based on the US Environmental Protection Agency, maximum acceptable level (MCL) for total arsenic (As), chromium (Cr), copper (Cu), and PCP in drinking water is 0.01, 0.1, 1.3, and 0.001 mg L⁻¹, respectively (EPA 2018). In Canada and the USA, the application of PCP and CCA has been restricted to industrial use, such as treatment of utility poles, railroad ties, and outdoor construction timber. However, on utility or storage sites where PCP- and CCA-treated wood are outdoor exposed to rain, these chemicals can leach from treated wood into the soil and

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groundwater, toxifying the ecosystem and wildlife habitats (Thomasson et al. 2015). Due to their potential danger to human health and ecological toxicity, they are considered priority pollutants whose remediation is essential (EPA 2004).

As a set of remediation phytotechnologies, phytoremediation has the potential to facilitate degradation and biodegradation of organic compounds and promote root uptake of metal(loid)s, followed by either root sequestration or partial transfer to the shoots depending on elements, plant species, and their populations (Mills et al. 2006; Truu et al. 2015). However, phytoremediation of sites contaminated with mixed pollutants is complex. The interaction between organic and inorganic pollutants can lead to unexpected chemical reactions that affect their bioavailability and toxicity in soil (Wang et al. 2012). The resulting conditions can in turn influence plant growth and remediation efficiency (Guemiza et al. 2017). Depending on dose and chemical speciation, metal(loid)s and their methylated chemical forms may have toxic effects on microbial activities in the root zone that can inhibit microbial biodegradation of organic pollutants in soils (Sandrin and Maier 2003; Lin et al. 2006; Tchounwou et al. 2012). Furthermore, organic contaminants may reduce the phytoavailability of metals (e.g., Cu) by combining with them to form sparingly water soluble organo-metallic compounds (Chen et al. 2004; Kobyłeczka and Skiba 2008).

Therefore, PCP- and CCA-contaminated soils require specific strategies in order for phytoremediation to be successful. In previous studies, we evaluated two ecological approaches for phytoremediation of wood preservatives (Lachapelle et al. 2020; Heine et al. 2021), the use of native plants and co-planting of selected plant species. Using native plant species may be beneficial, since they are likely to be adapted to the local environment and more tolerant to harsh soil conditions (Heckenroth et al. 2016). Their use also helps ensure local biodiversity and avoids the possible introduction of invasive plant species (Dagenais et al. 2018). Co-planting species may improve performance through complementarity and increase productivity as the result of interspecific interactions that build up resistance against stress in a highly stressed environment (Wang et al. 2014; Craven et al. 2016).

Soils in industrial areas contain little or no nutrients and organic matter, which reduces productivity and hampers establishment and growth of phytoremediation plants (Barrutia et al. 2009; Babu et al. 2013). Providing phytoremediation plants with essential nutrients may enhance their ability to remediate PCP- and CCA-contaminated sites. Adding nutrients to the system can stimulate root production that, in turn, induces root exudate secretion and improves microbial activity. The combined effect of plant exudates and microbial activity can increase mobility of and root exposure to As, Cr, and Cu and favor degradation of organic compounds (Marschner et al. 2001; Fitz and Wenzel 2002; Zayed and Terry 2003; Greger 2005). However, chemical reactions occurring

between contaminants and nutrient ions may affect the bioavailability, toxicity, and/or leaching of elements. For example, competitive effects have been observed between arsenate and phosphate in soil. Phosphate may indeed suppress the sorption of arsenate by soil and poses an increased risk of groundwater contamination (Livesey and Huang 1981). In contrast, arsenate in high concentration can decrease the soil sorption of phosphate (Gao and Mucci 2001). Competition for soil binding sites has also been reported between sodium (a common accompanying cation in fertilizers) and copper, which affects the bioavailability and toxicity of the latter in soil (Lock et al. 2007). Therefore, the application of plant nutrients to mixed-contaminated soils to improve phytoremediation is not a trivial procedure and requires thorough evaluation.

The objective of this research was to evaluate the effect of nitrogen (N) and phosphorous (P) addition, as inorganic fertilizers, on biodegradation of PCP- and phytoextraction of CCA-contaminated technosols. To achieve this objective, two levels of N and P fertilizers at three N:P ratios were applied to a co-planting phytoremediation system growing on technosols spiked with wood preservatives under controlled greenhouse conditions. Plant phytoremediation performance and occurrence of contaminants and nutrient leaching in the fertilized treatments were compared to an identical system that received no fertilizers.

Materials and methods

Experimental set-up and treatments

A mesocosm experiment was set up in a controlled greenhouse at the *Institut de recherche en biologie végétale* located at the Montreal Botanical Garden, Canada. The mesocosms consisted of polyethylene containers 50 cm in height and 35 cm in diameter (48 L). No drainage holes were drilled at the bottom of the mesocosms. Before filling them with soil, the bottom 5 cm of each mesocosm was filled with coarse gravel (3/4 inch, Bomix®). A PVC tube (diameter 2.5 cm and height 70 cm) was installed vertically down to the bottom of each mesocosm to collect drainage water.

In this experiment, a technosol (hereafter “soil”) was artificially formulated and spiked with PCP and CCA to have identical condition for all treatments and replicates. In fact, we reduced the high variability in soil properties and contamination levels by not using a real contaminated soil from a wood preservation site. Subsequently, the artificial constructed soil was stored in closed containers for 2 years to allow the soil to react extensively with the added contaminants (Christodoulatos and Mohiuddin 1996) and then thoroughly mixed with a mechanical soil mixer to maximize soil homogeneity prior to the experiment.

The soil had a loamy sandy texture and consisted of a mixture of 50% volume of calcareous stone dust (< 5 mm), 12.5% sand (2 mm), 12.5% construction limestone gravel (6.35 mm), and 25% topsoil. All soil components were purchased from a landscaping supplier, Matériaux Paysagers Savaria Ltée. Crystals of PCP (97% grade purity, Stella Jones Inc.) and liquid CCA-C (60% purity, Stella Jones Inc., containing 47.5% CrO₃, 18.5% CuO, and 34% As₂O₅) were used to spike the soil. Pentachlorophenol was introduced into the soil with the use of a surfactant, cocamidopropyl betaine, to enhance solubilization (Reynier et al. 2014). The concentrations of the PCP and CCA added were determined to reproduce a contaminant level similar to field conditions at wood preservative-contaminated areas. At the start of the experiment, the soil contamination levels of As, Cr, and Cu exceeded recommended environmental criteria C, A, and B, respectively, of the province of Quebec, where the experiment was conducted (Table 1) (Beaulieu 2019). The concentration of PCP and PCDD/F exceeded criterion B (Beaulieu 2019).

A co-plantation of sandbar willow (*Salix interior* Rowlee.) (hereafter “*Salix*”) and tall fescue (*Festuca arundinacea* Schreb.) (hereafter “*Festuca*”) was selected due to their documented performance for the phytoremediation of PCP- and CCA-contaminated soil (Desjardins et al. 2018; Frédette et al. 2019). Robust cuttings of *Salix* and seeds of *Festuca* were purchased from the Aiglon-Indigo nursery. The cutting of *Salix*, approximately 20 cm in length, was planted in sandy rooting medium on May 14, 2019. After 1 month, one rooted *Salix* stem was transferred to each mesocosm. Indigenous *Festuca* seeds (3.13 g pot⁻¹) were sown in the mesocosms after the early establishment of the *Salix* plant on June 18, 2019. Earlier trials showed that this sequence allowed for optimal establishment of both species together.

The fertilizer treatments were applied on July 9, 2019, when the plants were fully established (Lin and Mendelssohn 1998; Landmeyer 2011). Two levels of N and P (25 and 75 mg kg⁻¹) were used as fertilizer treatments for a total of three N:P ratios: 3:1 (75:25), 1:3 (25:75), and 1:1 (25:25). Nitrogen and P were added as sodium nitrate (NaNO₃) and monosodium phosphate (NaH₂PO₄). The application of NaNO₃ was preferred in this study rather than fertilizers with acidifying potential (e.g., ammonium sulfate) to limit the mobility and leaching of the metal(loid)s in soil (Schmidt 2003). The same accompanying cation (Na⁺) was chosen for N and P to prevent introduction of confounding variables. A control treatment with no added fertilizer (0:0 N:P) was included. Other essential nutrients (K, Mg, S, and micro-nutrients except Cu) were supplied to all fertilized mesocosms in constant amounts as a 1/2 Hoagland solution (Hoagland and Arnon 1950). Additive levels were determined following chemical analysis of the initial soil material

Table 1 Soil properties at the beginning of the experiment (mean ± SE, n = 6)

Parameters	Values
Field capacity (%)	15 ± 0.2
pH	8.9 ± 0.03
Electrical conductivity (μS cm ⁻¹)	234 ± 11
Total organic carbon (%)	6 ± 0.3
Pentachlorophenol (mg kg ⁻¹)	1.6 ± 0.1
Polychlorinated dibenzo-dioxins/furans (pg g ⁻¹)	132 ± 11
Water soluble (mg L ⁻¹)	
As	2 ± 0.1
Cr	0.03 ± 0.001
Cu	0.01 ± 0.0003
Fe	0.2 ± 0.1
Mn	0.002 ± 0.0002
Mo	0.002 ± 6E-05
Zn	0.02 ± 0.003
Acid extractable (mg kg ⁻¹)	
As	220 ± 5
Cr	142 ± 5
Cu	114 ± 3
Fe	8467 ± 162
Mn	479 ± 15
Mo	<1.0
Zn	78 ± 32
TKN	306 ± 19
NO ₃ ⁻ -N	95 ± 3
NH ₄ ⁺ -N	<5.0
K	861 ± 26
P	<20
Ca	21,1250 ± 5258
Mg	26,167 ± 976

(Table 1). Calcium (Ca) was not added because the soil material already contained a high Ca concentration. The pH of fertilizer solutions was set at 6.5 ± 0.5 with dilute HCl or NaOH.

Each treatment (three fertilizer levels + one control) had six replicates, for a total of 24 mesocosm units which were positioned in a randomized complete block design in greenhouse. The plants were allowed to grow for 14 weeks, until October 9, 2019. The temperature inside the greenhouse was adjusted to mimic the temporal variation in outside air temperature, ranging from 15 to 30°C.

The plants were watered with tap water (Table 2). To minimize leaching at the base of the mesocosms, the volume of water added was determined based on the soil field capacity (FC) using a hygrometer (TDR 150, Spectrum® Technologies, Inc.). The soil moisture content was maintained

Table 2 Chemical composition of tap water (mean \pm SE; $n=3$)

Parameters	NO ₃ -N	NH ₄ -N	PO ₄ ⁻³	Ca ²⁺	Mg ²⁺	Cl ⁻	TOC
mg L ⁻¹	0.34 \pm 0.01	< 0.020	0.002 \pm 3E-05	32 \pm 0.3	8 \pm 0.1	26 \pm 0.3	2 \pm 0.03

around the FC level via a daily watering session and following the hydrometer readings.

Data collection

Soil chemistry One random soil sample was collected from each mesocosm at the beginning of the experiment to determine the initial soil properties. Soil pH and EC were measured at the IRBV's laboratories. Concentration of water-soluble (As, Cr, Cu, Fe, Mn, Mo, and Zn) and acid-extractable elements (As, Cr, Cu, Fe, Mn, Mo, Zn, Ca, Mg, and K), concentration of nutrients (total nitrogen Kjeldahl (TKN), NH₄-N, NO₃-N, PO₄⁻³), PCP, PCDD/F, and total organic carbon (TOC) were determined by an accredited analytical laboratory (Veritas Laboratories, Montreal). Results are reported in Table 1.

The procedure for measuring pH, EC, and water-soluble elements in soil solution followed Séguin et al. (2004). For water-soluble elements, a mass of 3.5 g soil was shaken with 35 mL of ultra-pure water (1:10 soil:water) in a 50-mL centrifuge tube for 2 h. The suspension was then centrifuged at 1400g for 15 min. A 10-mL sub-sample of this solution was used for pH and EC analyses using a pH/electrical conductivity meter (Orion Star A215). The remaining solution was filtered using nylon syringe filters (0.45 μ m) and stored at 4°C in plastic bottles containing 2% HNO₃ before analysis of As, Cr, Cu, Fe, Mn, Mo, and Zn by inductively coupled plasma mass spectrometry (ICP-MS, Agilent).

To determine acid extractable As, Cr, Cu, Fe, Mn, Mo, Zn, Ca, Mg, and K in soil, 1 g of oven-dried soil samples (105°C) was digested with 4 mL of 50% HNO₃ (V/V) and 10 mL of 20% HCl (V/V), heated for 30 min (MA.200-Mét. 1.2, Rev. 5, CEAEQ 2020), and analyzed by Agilent ICP-MS.

Levels of TKN and NH₄-N were determined using acid digestion– and sodium salicylate–automated colorimetric methods, respectively (MA.300-NTPT 2.0, Rev. 2, CEAEQ 2020; MA.300-N 2.0, Rev. 2, CEAEQ 2020) and detected at 660 nm by an automated colorimetric analyzer (Thermo Fisher Gallery Plus). NO₃-N was evaluated by ion chromatography (MA. 300-Ions 1.3, Rev. 3, CEAEQ 2020) and analyzed with an ion chromatography apparatus (Thermo Fisher ICS-1600). PO₄⁻³ was determined by colorimetry (MA.303-P 1.1, Rev. 2, CEAEQ 2020) and measured at 880 nm using Agilent ICP-MS. TOC was detected using infrared detection (MA. 300-C 1.0, Rev. 6, CEAEQ 2020). For analysis of TOC, the soil sample was introduced into a tube heated to 680°C.

The combustion and degradation compounds in the form of CO₂ were analyzed with a TOC analyzer (Leco SC-632).

PCP was determined using gas chromatography assay coupled to a mass spectrometer (GC-MS, Agilent) (MA. 400-Phé 1.0, Rev. 3, CEAEQ 2020). PCDD/F was determined by high-resolution gas chromatography coupled to a high-resolution mass spectrometer (HRGC/HRMS, Thermo Fisher) (MA. 400-D.F. 1.1, Rev. 1, CEAEQ 2020).

Soil water content FC was measured using both mass determination (Salter and Haworth 1961) and hygrometer readings. Six pots (3 L) were filled with a known mass of oven-dried soil samples and saturated with water. Excess water was allowed to drain out from the bottom holes of the pots. Immediately after drainage ceased, the pots were covered with polyethylene sheets to prevent water evaporation from the soil surface. The mass of the pots (container, soil, and water) was measured until a constant mass to obtain FC as the mass of water divided by the mass of dry soil. Soil water content at FC was measured by a hygrometer vertically inserted from the soil surface to a depth of 12 cm. Soil FC was determined to be around 15% of the soil mass and the hygrometer reading corresponding to this moisture content was 30%.

Soil solution The leachates reaching the bottom of the soil containers were collected weekly with a peristaltic pump using the PVC tube inserted in the soil, and then disposed of safely in accordance with Canadian Biosafety Standard (CBS) regulations by the University of Montreal Health and Safety Division.

A sample of the collected leachate from each mesocosm was retained once a month for analysis according to Veritas Laboratories protocol (CEAEQ 2020) and analyzed by their laboratory. The concentrations of total dissolved NH₄-N, NO₃-N, Cl, Na, Cr, Cu, As, PO₄⁻³, PCP, and PCDD/F in leachate samples were determined using the methods described previously for soil samples. Dissolved organic carbon (DOC) was determined in leachate samples following the method described for TOC measurement using a Shimadzu TOC-VCPN apparatus. The pH and EC of leachate samples were measured soon after each sampling, as above.

All analyses were performed according to standard protocols and following quality control–quality assurance (QC/QA) procedures. The QC/QA procedure for As, Cr, Cu, and PCP involved laboratory reagent blank (LRB), laboratory fortified blank (LFB), and duplicate analysis (CEAEQ 2020). The recovery values for all analyses were in a control limit

range of 90–110%. The limit of detection (LoD) for As, Cr, Cu, and PCP in leachate samples was 0.001, 0.005, 0.001, and 0.001 mg L⁻¹, respectively.

Plant chemistry During the second and third months of the experiment, total chlorophyll content was measured by the dimethyl sulfoxide (DMSO) extraction method (Garg 2012). Accordingly, the youngest developed leaf was removed from each plant species per pot. A 100 mg mass of leaf tissue was placed in 15-mL centrifuge vials containing 7 mL DMSO. The vials were then incubated at 65°C for 4 h. Afterwards, the liquid extract was transferred to another 15-mL centrifuge vials and the volume was reduced to 10 mL with DMSO. Absorbance was recorded using a spectrophotometer (DR2800, HACH).

At the end of the experiment, the shoots were cut from the roots at the soil-atmosphere interface. The roots were excavated and the soil particles were manually separated from the roots. We did not distinguish between *Salix* and *Festuca* fine roots, so that the root tissues of both plant species were combined as one sample. *Salix* shoots were divided into stem and leaves. The collected shoots and roots were rinsed with tap water and oven dried at 65°C until constant mass and weighed separately to determine biomass dry weight. They were then analyzed at Veritas Laboratory to determine the concentration of As, Cr, Cu, PCP, and PCDD/F according to the methods described previously for soil samples. Pentachlorophenol and PCDD/F concentrations were only detected in root tissues. The LoD for As, Cr, Cu, and PCP in plant samples was 0.5, 0.5, 1.0, and 0.1 mg kg⁻¹, respectively. The contaminant amount (mg) in the plant tissues was calculated by multiplying dry biomass (kg) by the concentration of contaminants (mg kg⁻¹) for each plant part in a pot.

Statistical analysis

Treatment effects were tested by two-way analysis of variance (ANOVA) using JMP statistical software version 15 (SAS Institute Inc., Cary, NC). Multiple comparisons of means were used to determine statistically significant differences between treatments by Tukey's test ($\alpha = 0.05$).

The values presented in this study are mostly the means of six replicates ($n=6$) of each treatment except the data for tap water (Table 2), *Salix* chlorophyll content (Fig. 2), and PCP and PCDD/F (Table 1; Figs. 3 and 6). The ANOVA for chlorophyll content of *Salix* was performed with four replications of each treatment due to the scarcity of leaves in some mesocosms. PCP and PCDD/F were analyzed in soil, drainage water, and plant roots on the first two replications of each treatment throughout the experiment ($n=2$) to limit analytical costs. Standard errors (SE) were calculated from the replicates and presented as \pm or error bars.

Results

Plant growth responses

Belowground biomass production increased in all fertilized pots (Fig. 1). The increase was almost double in 1:1 and 1:3 N:P treatments compared to control. Fertilizer did not influence *Salix* aboveground biomass (data not presented), while *Festuca* aboveground biomass in 1:1 and 1:3 N:P treatments was significantly higher than control.

There were no significant differences in total leaf chlorophyll content of *Salix* and *Festuca* on the first sampling date (data not presented). On the second sampling date, there was no significant difference between treatments for *Salix* chlorophyll content, but the addition of 1:1 N:P fertilizer had a positive effect on total chlorophyll content of *Festuca* leaves compared to control (Fig. 2).

Metal(loid), PCP, and PCDD/F concentrations and amounts in plant tissues

The metal(loid) concentrations in root tissues, *Salix* leaves, and *Salix* stems were not influenced by the fertilizer applied (Fig. 3). The concentration of As and Cr in *Festuca* leaves was higher in the control treatment, followed by 1:3 and 1:1 N:P treatments, respectively.

To assess the impact of N and P addition on the metal(loid) alleviation, the values of As, Cr, and Cu of plants tissues in the pair treatments 25:25 vs. 75:25 N:P and 25:25 vs. 25:75 N:P were compared (*T* test, $\alpha = 0.05$). No significant difference between each pair was found except for the effect of N on Cr alleviation in *Festuca* aboveground (data not presented).

Unlike PCDD/F, PCP concentration in root tissues was affected by the fertilizer treatments and was significantly higher in 1:1 N:P treatment compared to control.

Arsenic, Cr, and Cu amounts were much higher in root tissues than in aboveground tissues of *Salix* and *Festuca* (Fig. 3). The highest root As and Cu amounts were in 1:1 N:P treatment, which were significantly greater than control. In aboveground tissue of *Salix*, only stem As amount was affected by fertilizer treatments and was significantly higher in 1:3 and 3:1 N:P treatments compared to control. *Festuca* leaves had a significantly higher Cu amount in 1:1 N:P treatment. Fertilizer treatments had no significant effect on Cr amount in root tissues. Although the translocation of Cr to shoots was detected, the Cr amount translocated to shoots was low. In *Salix* stems and leaves, Cr amount was insignificant among treatments. However, in *Festuca* leaves, Cr amount decreased when the plant received fertilizers, significantly in 3:1 and 1:3 N:P treatments.

Addition of fertilizers significantly increased PCP amount in plant tissues, particularly in 1:1 N:P treatment (Fig. 3). No effect of fertilizer on PCDD/F amount was observed.

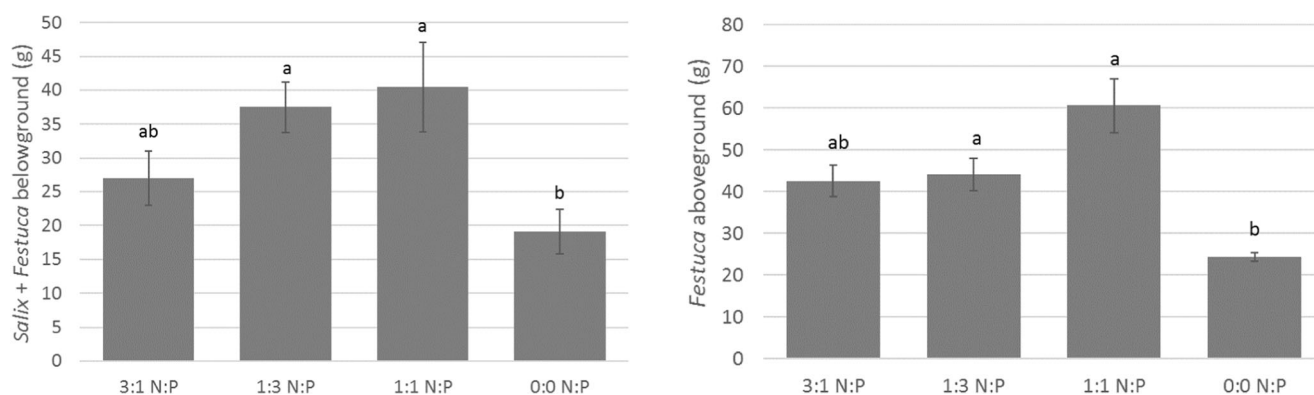


Fig. 1 Mean (with standard error) belowground dry biomass of *Salix* and *Festuca* and aboveground dry biomass of *Festuca*. Belowground biomass is the combined root biomass of the two plant species. Bars with identical letters are not significantly different according to Tukey HSD test, $\alpha < 0.05$

Phosphorus and nitrogen concentrations in plant tissues

Addition of fertilizers had no effect on N concentration in plant tissues (Supplementary Materials-Table I). The added fertilizers significantly affected P concentration in root tissues and *Festuca* leaves (Fig. 4). The significantly lowest P concentrations in roots and *Festuca* leaves were detected in the control treatment. Concentration of P in *Salix* leaves and stems was not influenced by the added fertilizers.

Leachate volume and analysis

The plant species treated with fertilizers required significantly more water compared to those in the control treatment (Fig. 5). However, the amount of leachate was significantly higher in the control treatment, with an average of 13.45 L during 14 weeks, approximately half of the water volume they received.

The concentrations of trace elements, DOC, PCP, and PCDD/F, in the drainage water are shown in Fig. 6. Regarding the metal(loid)s, As concentration in drainage

water was quite high in 1:3 N:P treatment at the first sampling time. Thereafter, no significant difference in As concentration was observed between treatments. The Cr concentration in drainage water did not differ between treatments at the first and third sampling times. But its concentration was lower in all fertilized treatments than control at the second sampling time. At the last sampling, Cr concentration was significantly lower in drainage water of the 3:1 N:P treatment. The concentration of Cu in drainage water was consistently higher in all fertilized treatments throughout the experiment. There was no significant difference between treatments in DOC concentration in drainage water at the first sampling time. A higher concentration of DOC was found in drainage water of all fertilized treatments at the second sampling time. Thereafter, the 3:1 N:P treatment had a significantly higher concentration of DOC drainage compared to control. The PCP concentration in drainage water did not significantly differ between treatments on all sampling dates, but PCDD/F concentration was significantly higher in the drainage water of the control on the first sampling date.

The concentrations of nutrients, Cl and Na, in the drainage water are shown in Fig. 7. The drainage water from 3:1 N:P

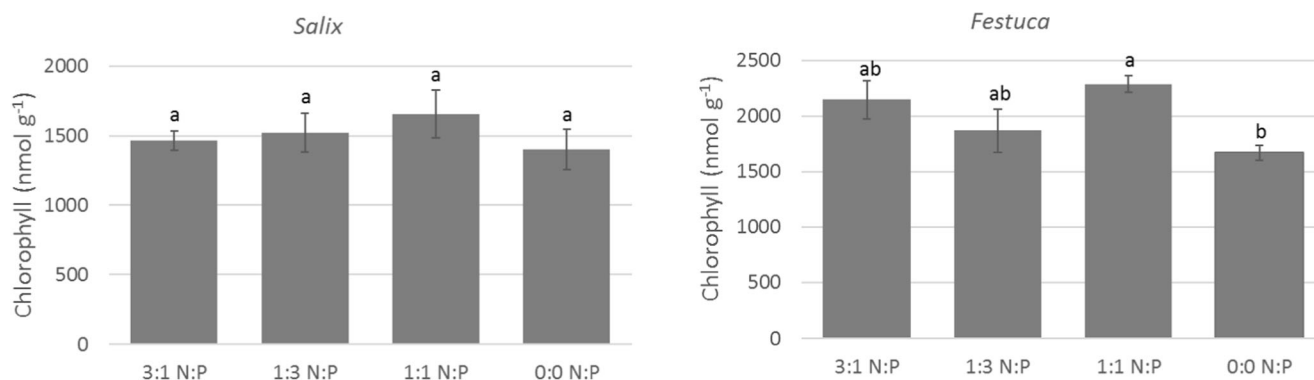


Fig. 2 Mean (with standard error) chlorophyll content in *Salix* and *Festuca* leaves on September 23, 2019. Bars with identical letters are not significantly different according to Tukey HSD test, $\alpha < 0.05$

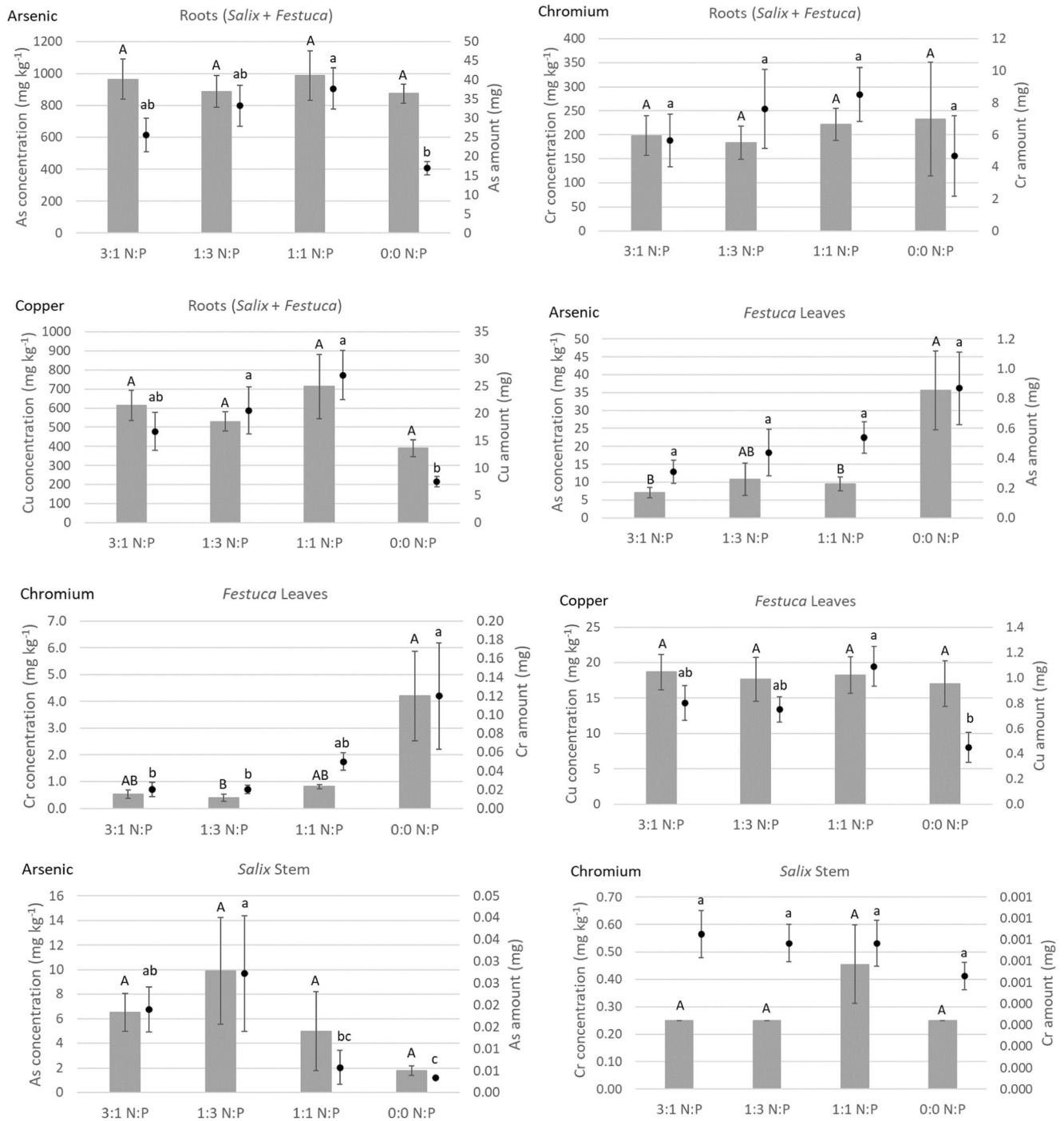


Fig. 3 Mean (with standard error) contaminant concentration (bars) and total amount (dots) in *Salix* and *Festuca* tissues, 14 weeks after fertilizer application. Identical uppercase and lowercase letters are not significantly

different for concentration and total amount, respectively, according to Tukey HSD test, $\alpha < 0.05$

treatment contained the highest concentration of nitrate, while the drainage water from 1:3 N:P treatment had the greatest concentration of orthophosphate on all four sampling dates. High concentrations of Na and Cl (the cations and anions bound to the added fertilizers) were also observed in drainage water of the fertilized treatments. The drainage water of 1:3

and 3:1 N:P treatments contained a significantly higher Na concentration than control on all sampling dates. Chlorine concentration in drainage water of all fertilized treatments was significantly higher throughout the experiment. However, its concentration decreased at the end of the experiment, and no significant difference in Cl concentration was

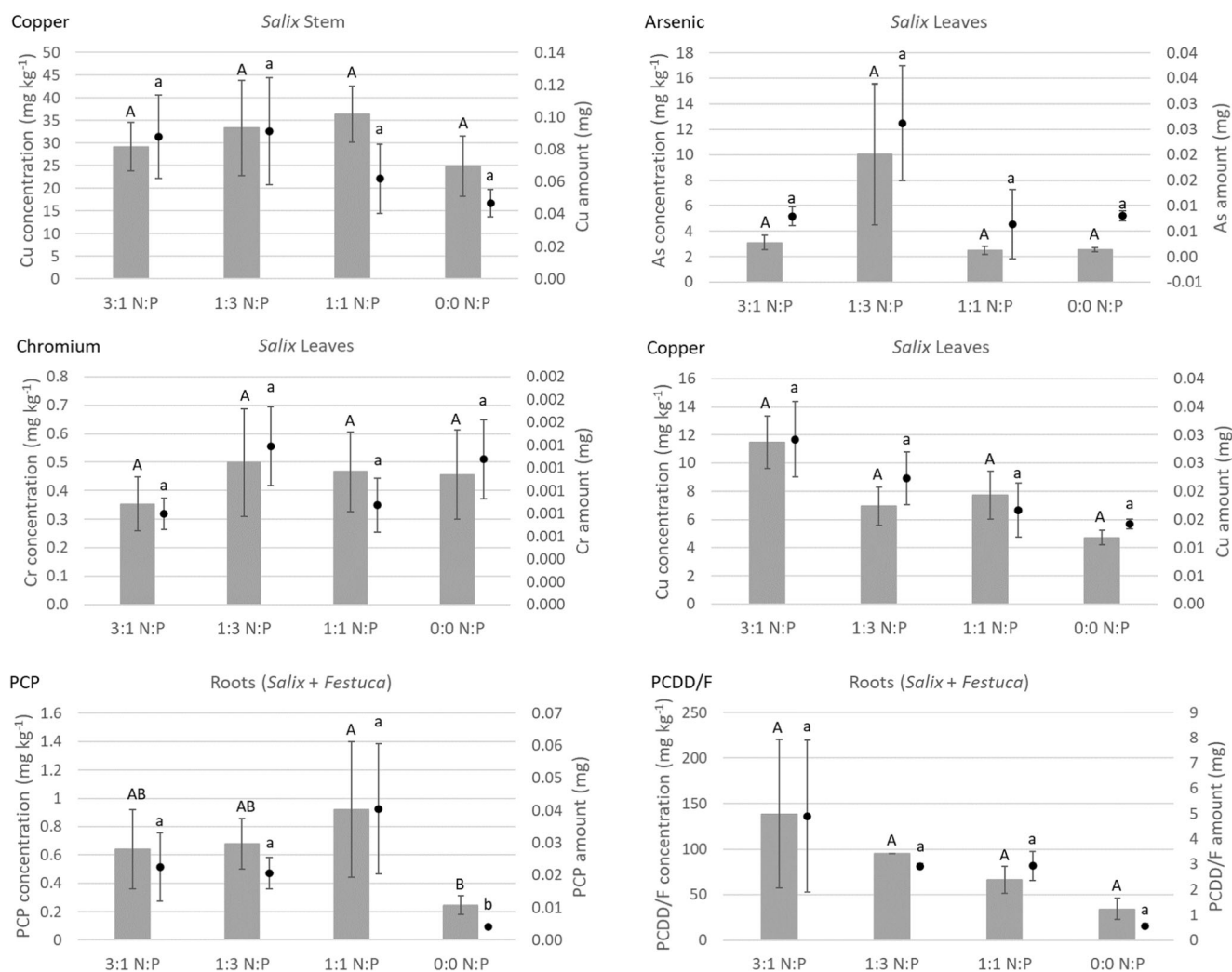


Fig. 3 continued.

found between control and the fertilized treatments, except for 3:1 N:P treatment.

Discussion

Although *Salix* spp. and *Festuca* spp. are known to be tolerant to a certain range of trace elements (Pulford et al. 2002; Brye and Pirani 2006; Marmioli et al. 2011), they demonstrated signs of phytotoxicity at the contaminant concentration levels applied here. In fact, it is well established that the bioavailability and toxicity of metal(loid)s are higher in spiked technosols than aged contaminated fields, where the combined effects of aging and leaching decrease the ionic strength of metal(loid)s (Smolders et al. 2009). Also, the presence of different metal(loid)s (As, Cr, and Cu) and/or other aspects of environmental stresses (e.g., PCP, pH, and P deficiency) may cause strong synergistic effects that lead to significant overexpression of

metal(loid) effects in plants (Mahmood et al. 2014; Timmerer et al. 2020).

The soil conditions in control treatment

In this study, the soil properties in the control treatment produced a stressful environment for *Salix* and *Festuca* since both plant species were negatively affected. *Salix* and *Festuca* growth ceased in the control treatment in the last month of the study and leaves showed chlorosis symptoms, being a result of insufficient amounts of chlorophyll in plants (Busato et al. 2010). Insufficient production of photosynthetic pigments and reduction of CO₂ assimilation can consequently cause decline in plant dry biomass (Singh et al. 2013).

Inhibition of plant growth and chlorosis could be the result of phytotoxicity induced by the presence of excess As, Cr, and Cu in soil (Mengle and Kirkby 1982; Panda and Choudhury 2005; Hasanuzzaman et al. 2015). *Salix purpurea* cv. 'Fish

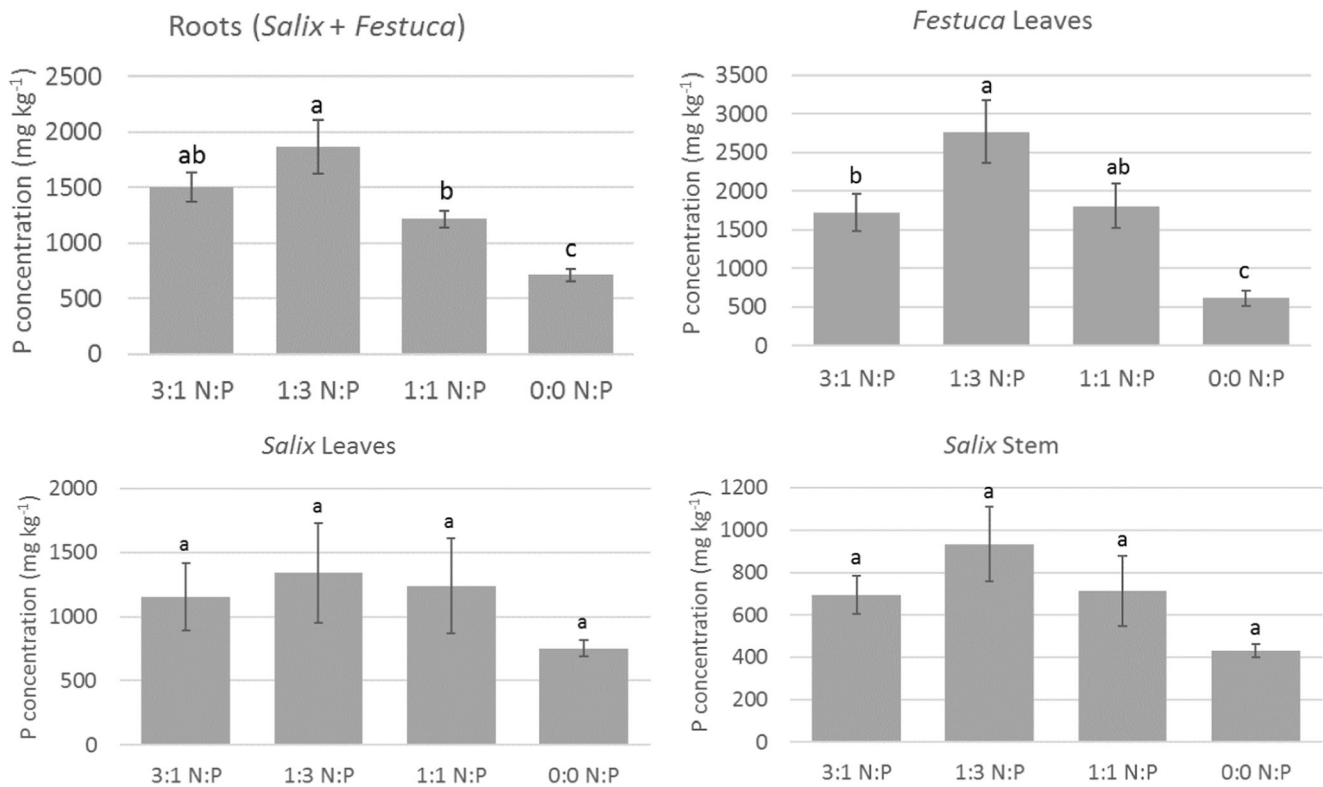


Fig. 4 Mean (with standard error) phosphorus concentration in *Salix* and *Festuca* tissues 14 weeks after fertilizer application. Bars with identical letters are not significantly different according to Tukey HSD test, $\alpha < 0.05$

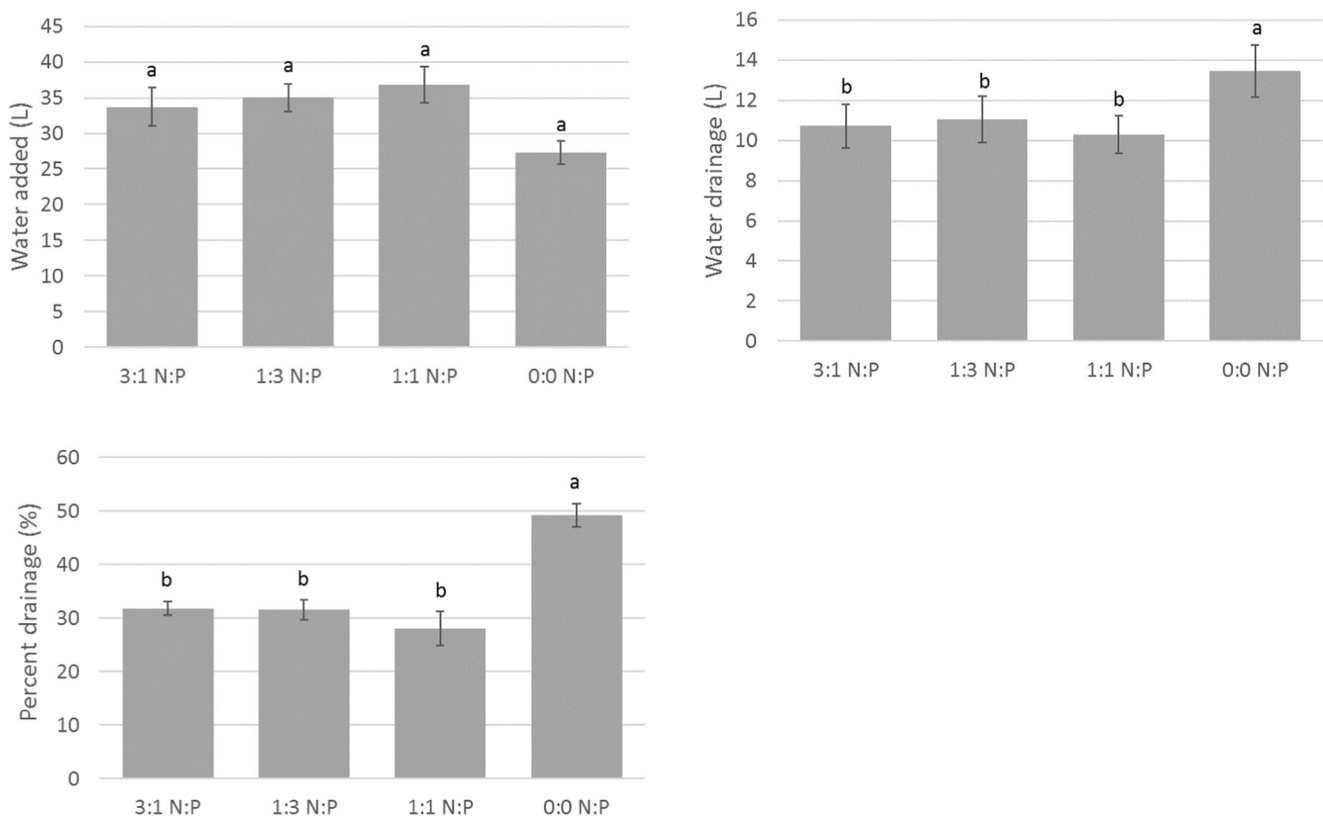


Fig. 5 Mean (with standard error) volume of water added, water drainage at the bottom of the mesocosm, and percent drainage for each treatment during the 14-week experiment. Bars with identical letters are not significantly different according to Tukey HSD test, $\alpha < 0.05$

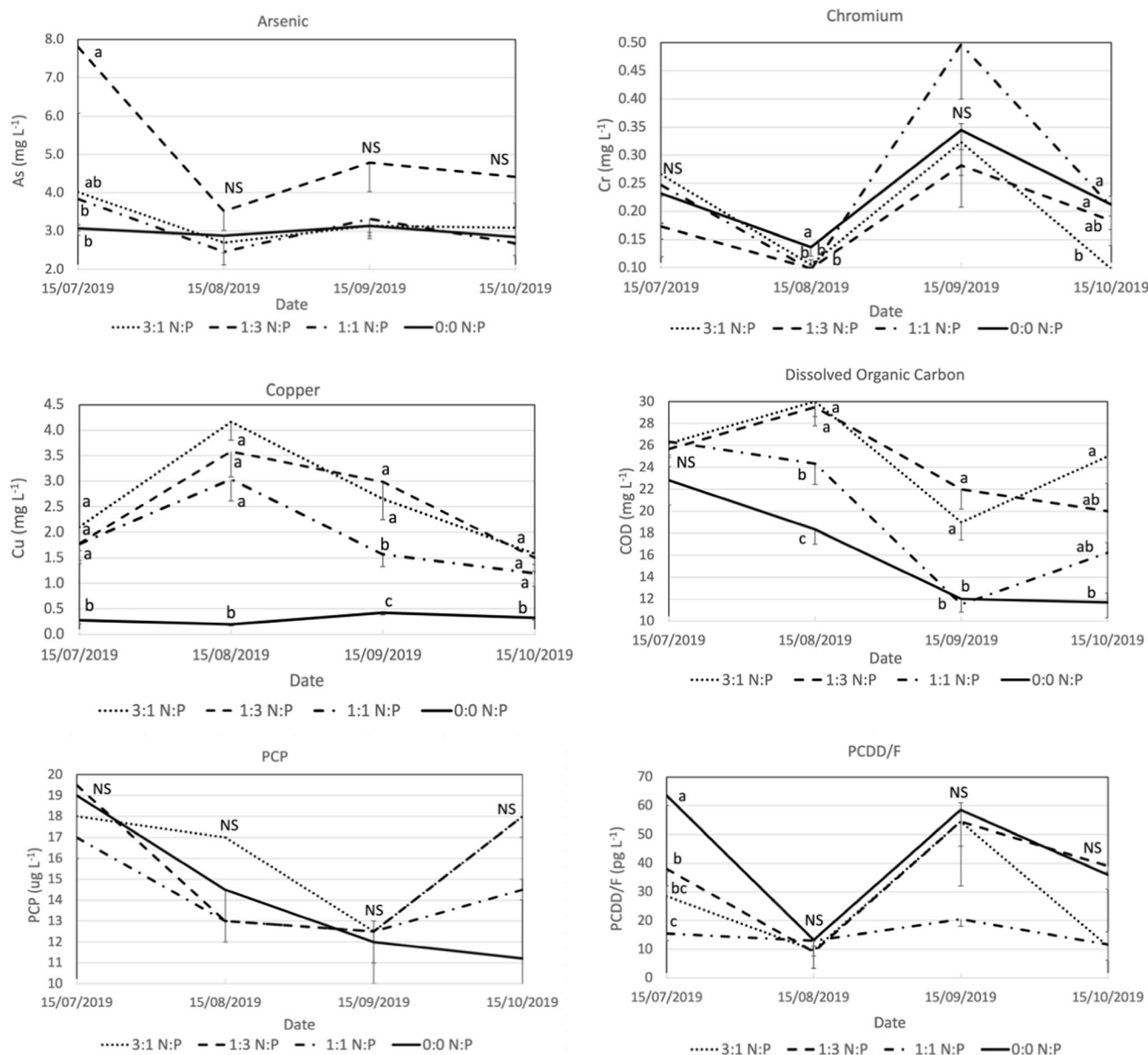


Fig. 6 Average (with standard error) monthly concentrations of contaminants and dissolved organic carbon in drainage water collected from mesocosms for each treatment. For a given sampling date, identical

letters are not significantly different according to Tukey HSD test, $\alpha < 0.05$. NS, no significant difference between means of different treatments for a given sampling date

Creek' has been shown to experience a substantial reduction in dry biomass (49.1%) compared to control plants after 4 weeks of hydroponic exposure up to 5 mg As L⁻¹. The plant accumulated up to 183 mg As kg⁻¹ in roots, while the value for shoots was below the detection limit (< 5 mg kg⁻¹) (Yanitch et al. 2017). In a hydroponic culture, application of 5 mg Cr⁺⁶ L⁻¹ decreased tall fescue biomass up to 17% and chlorophyll content up to 10% after 12 days. The plant accumulated 191.7 and 12.8 mg Cr kg⁻¹ in root and shoot tissues, respectively (Huang et al. 2018). *Populus × euramericana* clone Adda showed a strong decrease of 50% in plant biomass at 6.3 mg Cu L⁻¹ in Hoagland's solution after 34 days. Root

Cu concentration was found to be 3000 mg kg⁻¹, while the values for leaves and stem tissues were 7 and 5.5 mg kg⁻¹, respectively (Borghi et al. 2007), below the foliar Cu toxicity threshold levels (20–100 mg kg⁻¹) reported by Kabata-Pendias (2010).

Excess As, Cr, and Cu can affect amounts of chlorophyll content in different approaches. Arsenic can cause injuries in chloroplast membrane and negatively affect fundamental photosynthetic process (Abbas et al. 2018). Excess Cr⁺⁶ decreases chlorophyll content by impairing activities of various enzymes involved in chlorophyll biosynthesis (Singh et al. 2013). High Cu levels can induce a decrease in chlorophyll

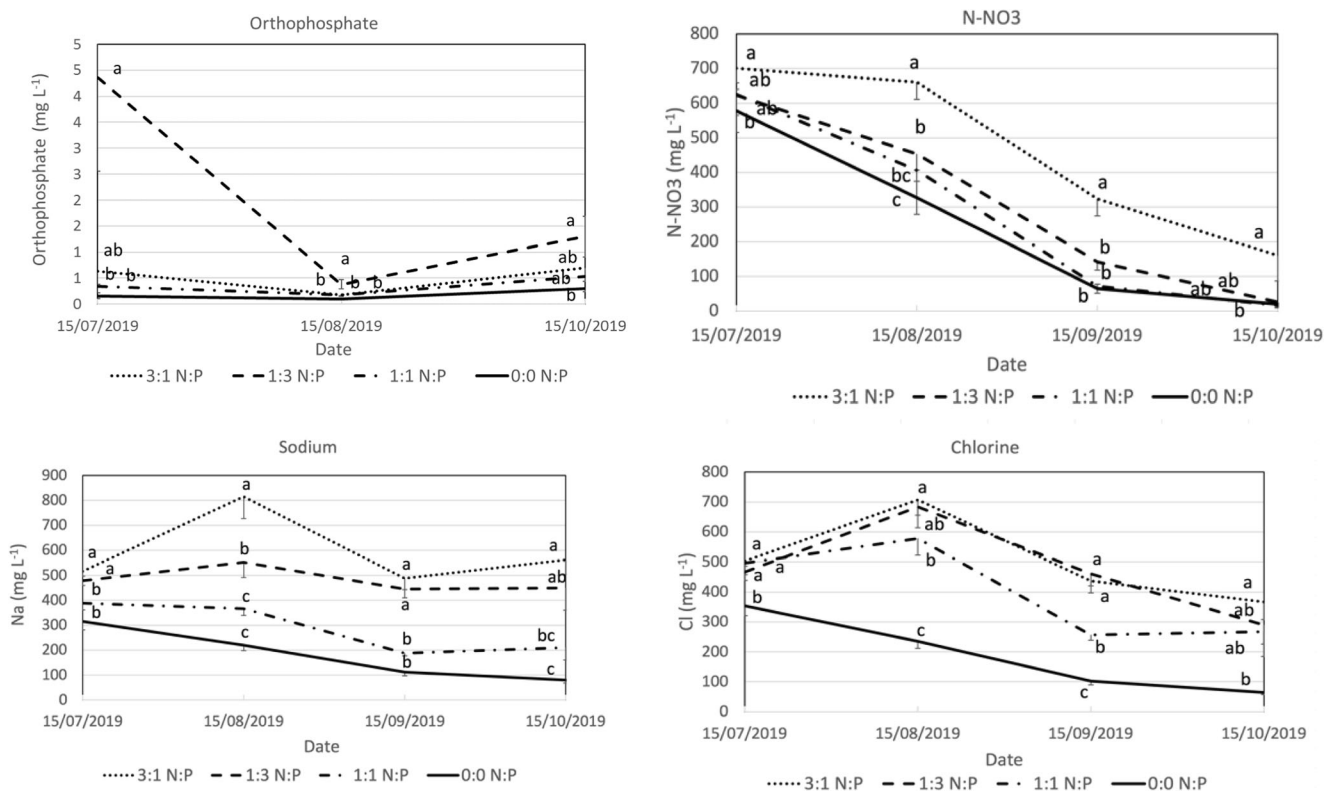


Fig. 7 Average (with standard error) monthly elemental concentrations in drainage water collected from mesocosms for each treatment. For a given sampling date, identical letters are not significantly different according to Tukey HSD test, $\alpha < 0.05$

content by destruction of inner structure of chloroplasts and alternation of thylakoid membranes (Pätsikkä et al. 2002).

In addition to the phytotoxicity of trace elements, the stunted growth and chlorosis of *Salix* and *Festuca* in the control treatment could also be related to N, P, and Fe deficiencies in plant tissues (Taiz and Zeiger 2006; Zhang et al. 2019). Nitrogen concentrations in *Festuca* leaves were lower than the critical deficiency value of 15,000 mg kg⁻¹ reported by Errecart et al. (2012). *Salix* showed foliar N concentration of 14,800 mg kg⁻¹, which was lower than the critical level for optimal growth of *Salix* species (21,000 mg kg⁻¹; Fillion et al. 2009). Phosphorus concentration in roots and *Festuca* leaves in the control treatment was lower than the adequate P concentration in plant tissues (2000 mg kg⁻¹; Paredes et al. 2011). Iron status in *Salix* and *Festuca* leaves (Supplementary Material-Table II) was very close to the critical level of 50 mg kg⁻¹ required for sufficient plant growth according to Hagemeyer (2004).

At the soil pH level in this study, 8.9, the solubility of Fe³⁺ could be extremely low. *Salix* and *Festuca* may employ different strategies to acquire highly insoluble Fe³⁺ in soil. *Salix* may reduce Fe³⁺ to Fe²⁺ by releasing protons and subsequent acidifying the rhizosphere. *Festuca* can complex Fe³⁺ by exuding the high-affinity Fe³⁺ phytosiderophores (Colombo et al. 2014; Walter et al. 2017). However, it is likely that Cu competes with Fe in both cases. It has been shown that Fe reductases are

capable of reducing Cu²⁺ and phytosiderophores are able to cause Cu complexation that can affect both Cu speciation and mobilization in soil (Ryan et al. 2013).

Copper interferes with Fe uptake by plants. Excess Cu in a hydroponic medium induces Fe deficiency in bean (*Phaseolus vulgaris*) plants which leads to a reduction in chlorophyll content. In fact, Cu²⁺ and Fe²⁺ compete for ion uptake and leaf metabolic processes (Pätsikkä et al. 2002).

In addition to Cu, a high Cr concentration has been shown to induce Fe chlorosis. Hexavalent chromium (Cr⁶⁺) can interfere with the absorption of Fe and decrease Fe accumulation, which is required for the biosynthesis of chlorophyll and heme enzymes (catalase and peroxidase) (Gopal et al. 2009). Exposure of cabbage (*Brassica oleracea*) to 26 mg L⁻¹ Cr³⁺ in a sand culture reduced the Fe concentration in leaves by half over 6 weeks compared to control plants. The chlorophyll concentration (60%) and the activities of the heme enzymes (CAT (80%) and POX (35%)) were also significantly decreased (Pandey and Sharma 2003).

Chromium has been shown to interfere with N metabolism in plants by altering activity of various enzymes related to N assimilation, e.g., nitrate reductase (NR) and nitrite reductase (NiR) (Ertani et al. 2017). Joshi et al. (2003) showed decreased activity of NR and NiR in leaves of guar (*Cyamopsis tetragonoloba*) in the presence of 2–6 mg Cr kg⁻¹. The reduced NR and NiR activities upon Cr exposure

may be related to either decreased nitrate uptake by roots or decreased CO₂ fixation, as NR activity is dependent on the photosynthetic rate (Vijayaraghavan et al. 1982; Kleinhofs and Warner 1990). A decline in the activity of N assimilatory enzymes in plants has also been observed under high As and Cu concentrations (Jha and Dubey 2004; Xiong et al. 2006).

Arsenate (AsO₄³⁻) can interfere with uptake of phosphate (PO₄³⁻) in plant roots due to their similar electron structures and chemical properties (Tu and Ma 2003). Arsenate can replace phosphate in its metabolism and various phosphorylation reactions such as ATP synthesis, which is toxic to the plant (Dixon 1997). Excess Cr has also been observed to strongly reduce the proteins involved in mitochondrial oxidative phosphorylation and induce a decline in ATP levels in plants (Santos et al. 2012).

Impact of fertilizer addition on plant growth and the metal(loid) concentrations and amounts

The application of N and P fertilizers to the soil resulted in the increase in dry biomass of the root system and leaves of *Festuca* and in the enhancement of chlorophyll content in *Festuca* leaves. Addition of the N and P fertilizers alleviated the metal(loid) phytotoxicity at the tested concentrations for this technosol. The mitigating effect of N and P fertilizers on the metal(loid) phytotoxicity and the stimulation of plant growth have been addressed in the scientific literature (Sayantan 2013; Zhang et al. 2014; Baldi et al. 2018). Nitrogen and P have been shown to reverse oxidative stress induced by over-accumulation of As, Cr, and Cu through regulation of nitric oxide (NO) formation, which acts as a signaling molecule to increase antioxidant enzyme activities and protect against injury caused by excess As, Cr, and Cu (Jin et al. 2010; Zhang et al. 2014).

Alleviation of the metal(loid) phytotoxicity through adding N and P fertilizers can also be related to the increase in plant dry biomass and consequently the increase in cells and available vacuolar compartmentalization of metal(loid)s which leads to increase in the plants' tolerance and accumulation of metal(loid)s (Guo et al. 2012).

In this study, the growth of *Salix* was stunted in the control treatment. However, application of N and P fertilizers did not significantly increase the growth of *Salix*. In the fertilized treatments, *Festuca* responded immediately to fertilization, and its enhanced growth may have increased its competitive advantage over *Salix* in all the fertilized treatments.

The plant analysis in our study found lower As concentrations in the *Festuca* leaves in the presence of N and P fertilizers. Our results concur with studies that showed a reduction of As uptake as a result of adding N and P fertilizers. Phosphorus is preferentially absorbed by phosphate transporters in competition with As, which leads to a reduction in As uptake (Singh and Ma 2006; Purdy and Smart 2008).

In our study, addition of N and P significantly decreased Cr concentration in the *Festuca* leaves in 1:3 N:P treatment. The lower uptake of Cr with added P fertilizer was also reported by López-Bucio et al. (2014). They suggested that there is probably competition between chromates and phosphates for cell entry (López-Bucio et al. 2014).

Application of N and P fertilizers did not affect Cu concentrations in *Salix* and *Festuca* tissues. These results are compatible with the previous findings showing that NO₃-N and P fertilizers increase Cu uptake by roots (Tills and Alloway 1981; Huang et al. 2018).

Application of the fertilizers increased the As and Cu amounts in the combined root tissues of *Salix* and *Festuca* and in *Salix* stem and *Festuca* leaves, respectively. In fertilized plants, the higher root As and Cu amounts were related to the effect of fertilizers on root biomass as well as root As and Cu concentrations. Although root As and Cu concentrations did not differ between treatments, the higher root biomass in the fertilized treatments resulted in a significant difference in As and Cu amounts. In *Salix* stems, the increase in As amount was entirely due to an increase in As concentration, since the fertilizers did not affect stem biomass. Conversely, Cu amount in *Festuca* leaves was more related to biomass yield than foliar Cu concentration.

Adsorption and absorption of PCP by roots

The highest PCP concentration was found in the root system of the fertilized plants. The presence of PCP in the root tissues can be the result of PCP adsorption on root surfaces as well as its absorption by plants. Pentachlorophenol is a lipophilic compound which has a strong affinity to root lipophilic molecules (He et al. 2009). The higher total root mass in the fertilized treatments might increase the root surface area, for greater PCP adsorption. Adsorption of organic compounds onto the root surface is reported to be a critical step in phytoremediation of organic compounds as either plant uptake or degradation by rhizosphere microorganisms (Schwab et al. 1998).

Few studies have addressed the absorption of PCP in plant tissues (Reischl et al. 1989; Huelster et al. 1994; Nunes et al. 2014; Frédette et al. 2019). Factors such as number of chlorine atoms in molecules and hydrophobicity have been demonstrated to influence PCP uptake by roots (Nunes et al. 2014). The effect of fertilizers on absorption of PCP by plants in this study can be explained through the effect of fertilizers on the binding compounds for PCP that are released with root exudates. These compounds are responsible for maintaining chlorophenol in the soil solution and forming a hydrophilic complex appropriate for absorption by the plants (Campanella et al. 2002).

Here, we did not measure PCP volatilization, although it has been shown that a small proportion of PCP may volatilize

in the presence of fertilizers (Mueller et al. 1991; Bacci et al. 1992).

Contaminant leaching in the soil

Leachate was collected monthly from each mesocosm and analyzed to monitor contaminants and nutrients. The purpose was to determine if the application of fertilizer to the phytoremediation system increases the risk of ground water contamination. In general, the volume of leachate generated was less in all fertilized treatments, which was probably due to the increasing vegetation growth achieved by the addition of the fertilizers, resulting in a higher evapotranspiration rate. The amount of dry mass produced from assimilated carbon depends on the inward diffusion of CO₂ from leaf stomata, which is quantitatively correlated with diffusion of water vapors out of the leaf (Sermons et al. 2017). This result is consistent with those of other studies that demonstrated that unvegetated control produced more leachate than fertilized vegetated treatments with tall fescue and Bermuda grass (Hutchinson et al. 2001).

Higher As loss from 1:3 N:P treatment on the first sampling date demonstrated competitive interference between As and P in soil. Phosphate increases arsenate concentrations in the soil solution by displacing it from soil particle sorption sites (Fitz and Wenzel 2002; Smith et al. 2002). The Cr concentration leached from the fertilized treatment may have been lower than control due to Cr immobilization in the root zone. Root exudates can decrease Cr mobility in the soil by reducing Cr⁺⁶ to Cr⁺³ (Banks et al. 2006). Unlike Cr, the leachate of Cu increased in the presence of fertilizers. The application of cations such as K⁺ in fertilizer and Na⁺ bonded to N and P fertilizers increased the cationic competition with Cu for retention on negatively charged soil exchange sites, causing leaching of Cu (Morton et al. 2004). In addition, the mobility of Cu can be related to DOC concentration, since Cu has a high affinity for organic matter, thus tending to form organo-metallic compounds (Lockwood et al. 2015). Addition of fertilizers increased DOC concentrations in drainage water. This result confirms those of a study conducted by Henry et al. (2005). These authors reported that the application of N fertilizer increased the release of root soluble carbon to soil, which might be related to increased root activity associated with the uptake and reduction of N. However, microbial mineralization or respiration of solubilized carbon is reported to lower DOC concentrations in drainage water over time (Clay et al. 1995).

Pentachlorophenol leaching occurred in all mesocosms regardless of the presence of the fertilizers. According to Guemiza et al. (2017), leaching tends to increase with high PCP input, alkaline pH, high level of soil humidity, and low organic matter content in the soil. Here, soil moisture and low organic matter present in the soil could be the cause of PCP

leaching. However, the PCP concentration in drainage water was below the Canadian drinking water standard level of 60 µg L⁻¹ (Health Canada 2019). Unlike PCP, the solubility of PCDD/F in water is very low and may be highly persistent in the soil (Kitunen et al. 1987; Bhattacharya et al. 2002; Gumezia et al. 2017). However, it can be transported by water and mobilized in soil by adsorbing to suspended soil particles and dissolved organic matter due to its density and hydrophobic properties (Kim et al. 2009). In this study, PCDD/F leaching decreased significantly with the application of fertilizer on the first sampling date. This may be due to dechlorination of PCDD/F in redox conditions that occurred in the contaminated soils as a result of fertilizer addition as well as microorganism actions such as the reduction of nitrates or Fe (Yu et al. 2014). In addition to dechlorination, our results showed that PCDD/F was more subject to plant uptake in the presence of fertilizers, as discussed previously.

A large quantity of N was found in the leachate of all treatments. This may be due to the initial N content of the soil, as indicated by the high TKN values. Total nitrogen Kjeldahl was mostly in the form of organic N and not readily bioavailable at the beginning of the experiment. Mineralization of organic N throughout the experiment could have increased the concentration of inorganic N in the soil and in the leachate, as reported by Burgos et al. (2006). Moreover, addition of N fertilizer at the higher concentration (3:1 N:P) increased the amount of N leachate. Much like for N, application of a fertilizer higher in P (1:3 N:P) led to higher drainage of P from the soil. Elevated N and P leaching following application of large doses of those fertilizers indicates that excess amounts of applied N and P could not be retained in the soil or taken up by plants (Sevel et al. 2014).

The application of Cl⁻ and Na⁺ as accompanying ions in the fertilizers also elevated leaching of Cl and Na in drainage water. Cl⁻ and Na⁺ are readily mobile and subject to leaching, especially in sandy and sandy loam soils (Freeman et al. 2006; Chen et al. 2010).

Overall, our results suggest that the application of 1:1 N:P fertilizer is the optimal treatment for co-planting *Salix* and *Festuca* to overcome the stress conditions in a spiked technosol mimicking wood preservative-contaminated soils. The addition of 1:1 N:P fertilizer considerably elevated plant biomass production and the amount of metal(-loid)s and PCP in the plants during the 14 weeks of the experiment. In 1:1 N:P treatment, the percentages of the metal(-loid)s in the plants as determined by the amounts of As, Cr, and Cu in the aboveground biomass of *Salix* and *Festuca* were 0.18%, 0.024%, and 1.20%, and 0.89%, 0.08%, and 1.78%, respectively. A monthly leaching analysis demonstrated that 1:1 N:P fertilizer potentially posed a lower pollution linkage to water resources, since it decreased both the volume of drainage water and leaching of most contaminants and nutrients.

Conclusion

Our study demonstrated that the application of N and P fertilizers (NaNO_3 and NaH_2PO_4) mitigated stress conditions for *Salix* and *Festuca* in a wood preservative-spiked technosol. Addition of the fertilizers increased chlorophyll content and aboveground biomass of *Festuca* as well as belowground biomass of *Salix* and *Festuca*. In the presence of 1:1 N:P fertilizer, As and Cu amounts in belowground of *Salix* and *Festuca* doubled and quadrupled, respectively, and Cu amounts increased twofold in *Festuca* leaves. The application of a higher dosage of fertilizers (1:3 and 3:1 N:P) increased the leaching of metal(loid)s and nutrients into drainage water. The optimum effect of the fertilizers was observed in mesocosms supplied with 25 mg kg^{-1} of each of N and P (1:1 N:P), which enhanced the phytoremediation potential of the plants with minimal impact on the drainage content. However, the success of this strategy relies on the presence of appropriate nutrient types and dosage, as a function of the initial soil conditions. The impact of nutrients accompanying anions and cations, and their potential interactions with metal(loid)s and organic compounds, all require further investigation. Future study should be conducted on real brownfield soils to answer questions regarding the actual processes involved.

Abbreviations ANOVA, Analysis of variance; CCA, Chromated copper arsenate; DMSO, Dimethyl sulfoxide; DOC, Dissolved organic carbon; EC, Electrical conductivity; FC, Field capacity; PCDD/Fs, Polychlorinated dibenzo-dioxins/furans; PCP, Pentachlorophenol; SE, Standard errors; TOC, Total organic carbon

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contribution Conceptualization: JB, FC, and SY; methodology: JB, FC, and SY; formal analysis and investigation: SY; writing - original draft preparation: SY; writing - review and editing: JB and FC; funding acquisition: JB; resources: JB; supervision: JB and FC. All authors have read and agreed to the published version of the manuscript.

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Declarations

Ethical approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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