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Research article

Growth, physiology, and phytoextraction potential of poplar and willow established in soils amended with heavy-metal contaminated, dredged river sediments

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ABSTRACT

Phytotechnologies have been used worldwide to remediate and restore damaged ecosystems, especially those caused by industrial byproducts leaching into rivers and other waterways. The objective of this study was to test the growth, physiology, and phytoextraction potential of poplar and willow established in soils amended with heavy-metal contaminated, dredged river sediments from the Great Bačka Canal near Vrbas City, Serbia. The sediments were applied to greenhouse-grown trees of *Populus deltoides* Bartr. ex Marsh. clone 'Bora' and *Salix viminalis* L. clone 'SV068'. Individual pots with trees previously grown for two months were amended with 0, 0.5 and 1.0 kg of sediment containing 400 mg Cr kg^{-1}, 295 mg Cu kg^{-1}, 465 mg Zn kg^{-1}, 124 mg Ni kg^{-1}, 1.87 mg Cd kg^{-1}, and 61 mg Pb kg^{-1}. Following amendment, trees were grown for two seasons (i.e., 2014, 2015), with coppicing after the first season. In addition to growth parameters, physiological traits related to the photosynthesis and nitrogen metabolism were assessed during both growing seasons. At the end of the study, trees were harvested for biomass analysis and accumulation of heavy metals in tree tissues and soils. Application of sediment decreased aboveground biomass by 37.3% in 2014, but increased height (16.4%) and leaf area (19.2%) in 2015. Sediment application negatively impacted the content of pigments and nitrate reductase activity, causing them to decrease over time. Generally, the effect of treatments on growth was more pronounced in poplars, while willows had more pronounced physiological activity. Accumulation patterns were similar to previously-published results. In particular, Zn and Cd were mostly accumulated in leaves of both poplar and willow, which indicated successful phytoextraction. In contrast, other metals (e.g., Cr, Ni, Pb, Cu) were mostly phytostabilized in the roots. Differences in metal allocation between poplar and willow were recorded only for Cu, while other metals followed similar distribution patterns in both genera. Results of this study indicated that the composition of heavy metals in the sediments determined the mechanisms of the applied phytoremediation technique.

1. Introduction

Rapid economic growth and industrialization have led to degradation of water bodies worldwide (Dipak et al., 2017; Jeelani et al., 2017; Wu et al., 2016; Xu et al., 2017). Of concern is the impact of dredging river systems to minimize flooding, maintain navigation, and receive discharge wastewater from industrial and municipal sources (Gurnell et al., 2007). Domestic sewage and wastewater pollution has caused the presence of large amounts of inorganic and/or organic contaminants in water, which are rapidly deposited and strongly attached to the sediments (dos Santos et al., 2018; Thanh Vu et al., 2017; Yu et al., 2017). For example, heavy metals present in rivers accumulate in such sediments, as well as bacteria, tubicids, fish, and humans (Mulligan et al., 2001). River sediments can act as main sinks for trace metals and become potential secondary sources of pollutants in aquatic environments when river conditions change. In particular, sediments can accumulate and deposit heavy metals with fine particles causing gradual or immediate deterioration of the aquatic systems (Chen et al., 2016; dos

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Santos et al., 2018). In lieu of mechanical treatment of the sediments, phytoremediation offers the use of a publicly-accepted green technology.

Phytoremediation consists of the use of green plants, including grasses, forbs, and woody species, to remove, contain, or render harmless environmental contaminants such as heavy metals, trace elements, and organic compounds (Hinchman et al., 1995). This technology utilizes naturally occurring processes by which plants and their rhizosphere microorganisms degrade and sequester inorganic and organic pollutants (Pillon-Smits, 2005). Among potential plant species, trees have been used for phytoremediation of heavy metals because they provide a number of beneficial attributes such as: 1) large biomass, 2) genetic variability, 3) established management practices, 4) economic value, 5) public acceptance, and 6) site stability (Pulford and Dickinson, 2006). More specifically, short rotation woody crops (SRWC) have been used as a solution for cleanup of contaminated soils and waters. Short rotation coppice biomass plantations rely on the ability to resprout vigorously to achieve system sustainability and phytoextraction (Kuzovkina and Volk, 2009). Due to decades of research and experience in poplar and willow biomass and biofuel cultivation (Vance et al., 2010; Zalesny et al., 2016a, 2016b), scientific understanding of their biology and ecology makes them ideal candidates for phytotechnologies such as phytoremediation.

Poplars and willows are the most commonly-used trees for phytoremediation in the northern hemisphere (Kuzovkina and Volk, 2009; Nikolić et al., 2008; Zalesny et al., 2016b). Rapid growth grate, high biomass production, coppicing ability, propagability, low nutrient demands, efficient photosynthesis, and tolerance of high plantation densities are among the primary characteristics supporting their use for such phytotechnologies (Kuzovkina and Volk, 2009; Licht and Isebrands, 2005). Numerous researchers have reported on the successful remediation of inorganic and organic contaminants of soil and water (Dickinson and Pulford, 2005; Hasselgren, 1999; Pajević et al., 2009; Pilipović et al., 2005; Sebastiani et al., 2004; Vervaeke et al., 2003; Zalesny et al., 2011), yet there is limited information about phytotechnologies involving dredged river sediments. As a result, novel studies addressing the potential use of nutrient-rich, dredged river sediments as fertilizer for SRWC are needed, especially as they relate to biological treatment of the contaminants.

This is especially important because the presence of heavy metal contamination causes disturbance of metabolic and physiological processes in plants that often results in losses of vitality and decreases in growth. Disturbance is expressed through basic plant processes such as nitrogen metabolism and nitrate reductase activity (Matraszek, 2008; Pilipović et al., 2012), as well as biochemical processes such as glutathione metabolism (Di Baccio et al., 2005; Nikolić et al., 2008). Disturbed metabolic and biochemical processes in plants are further expressed through impacted photosynthetic parameters (Borghi et al., 2008; Borišev et al., 2016; Tognetti et al., 2004) that ultimately affect morphology (Di Baccio et al., 2009; Nikolić et al., 2017) and growth (Watson et al., 2003; Župunski et al., 2016). Heavy metals can enter root tissue by altering pH through efflux of hydrogen (H^+) ions, resulting in an electrochemical gradient that facilitates transport of cations and anions (proton pump) and requires cellular energy in the form of adenosine triphosphate (ATP), where mostly divalent cations are absorbed (Arthur et al., 2005). These ATP-ases are also involved in pumping of metal ions from pericycle cells to xylem vessels (Zhao and McGrath, 2009). Depending upon the accumulation in different plant tissues, individual species or genotypes can be characterized as plants for phytostabilization or phytoextraction of heavy-metal contaminated soils (Giachetti and Sebastiani, 2006a).

Roots, stems, and leaves are functionally interdependent and these three systems maintain a dynamic balance in biomass which reflects relative abundance of aboveground resources (e.g., light and CO_2) compared with belowground resources (e.g., water and nutrients) (Poorter et al., 2012). Unfavorable conditions such as heavy-metal contamination affect living processes in plants resulting in eventual growth decreases. Therefore, morphological traits, such growth parameters, are generally used to study plant responses to the influence of stressors (Borghi et al., 2008; Borišev et al., 2016; Katanić et al., 2015). Although the growth of plants is exhibited by diameter, height, and biomass, which are quantitatively inherited and a reflection of numerous anatomical properties, as well as physiological and biochemical processes (Orlović et al., 1998), the effect of unfavorable environmental conditions (e.g., contamination, low fertility, drought) can often diminish the genetic background of the plants (Pilipović et al., 2012). The effect of different factors on plants can be obtained through yield assessment, which is a composite trait that can be tested directly or indirectly via the individual physiological traits that affect plant performance (Marron and Ceulemans, 2006).

Effective phytoremediation of contaminated soils and sediments can have many obstacles which are not evident during the design and establishment of specific phytotechnologies. For example, uneven distribution of contamination has caused the avoidance of hotspots by plants roots (Pulford and Dickinson, 2006), resulting in significant decreases of phytoremediation effectiveness. Avoiding such problems can be achieved by application of contaminated sediment to trees that are already developed. The objective of this study was to test the growth, physiology, and phytoextraction potential of poplar and willow established in soils amended with heavy-metal contaminated, dredged river sediments from the Great Bačka Canal near Vrbas City, Serbia. While the sediments and clonal material are specific to Serbia, the results and applications of the current study are useful for researchers, managers, and academicians developing systems throughout the world in regions experiencing similar environmental concerns, such as the Great Lakes Basin, USA.

2. Materials and methods

2.1. Site description and sediment collection

The Great Bačka Canal in the vicinity of Vrbas City, Serbia is highly contaminated with heavy metals. As a consequence of decades of disposal of industrial and agricultural waste, more than 400,000 m^3 of sediments are present nowadays. As a result, based on heavy metals content and associated regulations on the limit of pollutant concentrations in surface water, ground water, and soils, these sediments have been classified as highly polluted (Official Gazette RS, 50/2012). Thus, abatement methods are mandatory, including 1) disposal of the dredged materials under controlled conditions and with special protection measures to prevent further distribution of hazardous materials in the environment, 2) deployment of biological systems such as phytoremediation, or 3) a combination of such methods. Physico-chemical parameters and limits for heavy metals of the river sediments are listed in Table S1.

In the current study, during spring 2014, contaminated river sediments were dredged and collected from a 6-km long section of the Great Bačka Canal (45.58,889°N, 19.61,639°E). The sediments were transported to the Faculty of Sciences – Department of Chemistry, Biochemistry and Environmental Protection laboratories at the University of Novi Sad (Novi Sad, Serbia) and stored in closed barrels (without air) in the dark for two months. Just before the start of treatment, sediment was dried at room temperature in the dark, subsampled and diluted with a predetermined amount of distilled water. Sediment prepared in this way was than incorporated with common alluvial soil (Table S2) at the greenhouse of the Faculty of Sciences – Department of Biology and Ecology to create two sediment amendment treatments: 1) 0.5 kg of sediment diluted with 1 l of deionized water and added to the alluvial soil of each 10-L pot, and 2) 1.0 kg of sediment diluted and added to the alluvial soil of each pot. A control treatment consisting of pure alluvial soil (i.e., without sediment amendments) also was tested.

2.2. Genotype selection and experimental design

During winter 2014, dormant whips from poplar clone 'Bora' (*Populus deltoides* Bartr. ex Marsh) and willow clone 'SV068' (*Salix viminalis* L.) were collected from the gene bank located at the Experimental Estate of the Institute of Lowland Forestry and Environment (ILFE), University of Novi Sad (former Poplar Research Institute) (45.29,444 °N, 19.88,556 °E). The female poplar clone was obtained from controlled pollinations and subsequent selections at the ILFE. The female willow clone was selected from half-sib, open-pollinated seeds, obtained from international exchanges in the 1960s. Whips were processed into 20-cm long cuttings containing 3 to 5 healthy buds, with the first bud located not more than 1 cm from the top of each cutting. During early May 2014, cuttings were soaked in water for 20–24 h to initiate rooting and treated with a 0.5% solution of copper oxychloride (to reduce potential fungal growth that could impact the growth of the trees) before being planted into 10-L pots containing the alluvial soil described above.

After two months of growth in the alluvial soil, the aforementioned sediment amendment treatments were applied to the alluvial soil in July 2014, and trees were grown and tested in a split-plot design consisting of three random block effects (with four trees per treatment × genus interaction per block), three fixed sediment treatment whole plots (i.e., 0.0, 0.5, and 1.0 kg of sediment per pot), and two fixed genus sub-plots (i.e., poplar and willow), for a total of 72 trees being tested. Treatments and genera were arranged in randomized complete blocks to minimize potential greenhouse environmental gradients. Following application of the sediment treatments, trees were kept watered to field capacity and regularly monitored for health impacts.

2.3. Growth parameters

Following cessation of growth during September 2014 (i.e., after two months of growth in the alluvial soil followed by another two months in the amended soil), diameter (mm) was measured from 1 cm above the point of attachment between the shoot and original cutting, and height (cm) was measured from the point of attachment to the tip of the terminal bud. Total leaf area per tree was measured using an ADC AM300 Leaf Area Meter (ADC Bioscientific, Ltd., United Kingdom). In addition, aboveground biomass was harvested and immediately weighed, whereby shoots were dissected at the point of attachment and leaves were separated from shoots. Following dissection, shoots and leaves were oven-dried at 50 °C until constant mass, and dry mass (g) of each tree tissue was recorded.

The trees re-sprouted and grew during the 2015 growing season, and similar growth parameters as 2014 were measured following growth cessation during September 2015. In particular, height, aboveground biomass, and leaf area were determined, as well as harvesting, washing, and weighing of root systems. For the latter, soil was washed from the belowground biomass, and roots were separated from the original cutting. As in 2014, all tree tissues were oven-dried at 50 °C until constant mass, and dry mass (g) of each tree tissue was recorded.

From biomass measurements and leaf area, specific leaf area (SLA) was calculated for 2014 and 2015 as the ratio between leaf area and leaf dry mass ($m^2\ kg^{-1}$). Additionally, the following parameters were calculated in 2015: 1) leaf mass ratio (LMR) as the ratio between leaf dry mass and total (leaf + root + shoot) dry mass ($kg_{leaf}\ kg_{tree}^{-1}$), 2) leaf area ratio (LAR) as the ratio between leaf area and total dry mass [calculated as LAR = SLA × LMR ($m^2\ kg_{tree}^{-1}$)], and 3) root mass ratio (RMR) as the ratio between root dry mass and total (leaf + root + shoot) dry mass ($kg_{root}\ kg_{tree}^{-1}$).

2.4. Physiological parameters

Physiological parameters were measured during August of both years. In 2014, the content of photosynthetic pigments (mg $g_{dry\ mass}^{-1}$) [i.e., chlorophyll A, chlorophyll B, total chlorophyll (A + B) and carotenoids] was determined spectrophotometrically in acetone extracts according to Wettstein (1957). Gas exchange measurements were made with an ADC LCPro + Portable Photosynthesis System (ADC Bioscientific, Ltd., United Kingdom) under controlled, constant light conditions of 1000 μmol $m^{-2}\ s^{-1}$, constant ambient air supply of 100 μmol s^{-1}, and ambient levels of air humidity and temperature. Investigated gas exchange parameters included photosynthetic rate (A) (μmol$m^{-2}\ s^{-1}$ O_2) and transpiration rate (E) (mmol$m^{-2}s^{-2}$ H_2O). Measurements were performed on the first set of fully developed leaves. Instantaneous water use efficiency (WUE) (μmolO_2mmolH_2O^{-1}) was computed as the ratio of net photosynthesis to transpiration (A/E) (Farquhar et al., 1989). In addition, SPAD index values were determined from the same leaves that were used for gas exchange measurements using a Minolta SPAD 502 Portable Chlorophyll Meter (Konica Minolta, Inc., Osaka, Japan). The *in vivo* nitrate reductase activity (NRA) (μmol NO^{2-} $g_{fresh\ mass}^{-1}h^{-1}$) in tree leaves was assayed using the method of Hageman and Reed (1980).

In 2015, photosynthetic rate (A), transpiration rate (E), water use efficiency (WUE), and nitrate reductase activity (NRA) were measured.

2.5. Heavy metals accumulation

Concentrations of targeted heavy metals (Cd, Cr, Cu, Ni, Pb, Zn) (mg kg^{-1}) were analyzed in soils that were collected during the harvest and processing of tree tissues in both years (i.e., 2014 = leaves, shoots; 2015 = leaves, roots, shoots). Samples were prepared using a Milestone Start E Microwave Extraction System (Milestone, Inc., Shelton, Connecticut, USA) according to manufacturer recommendations. Specifically, 0.5 g of soil and/or plant sample was measured and added to a Teflon beaker along with 7 ml of HNO_3 and 2 ml of 32% H_2O_2. The sample mixture was heated to 85 °C for 4 min, 145 °C for 9 min, 200 °C for 4 min, and then kept at a constant temperature of 200 °C for an additional 14 min. The Teflon beaker with the mixture was then left to cool and was transferred to a 50-ml volumetric flask and filled with deionized water. Sample analyses were conducted using a Perkin Elmer Analyst 700 Atomic Absorption Spectrometer (Perkin Elmer, Waltham, Massachusetts, USA). The analyses were conducted using a graphite furnace and flame techniques according to US EPA methods (EPA7010, EPA7000B). Adequate quality assurance/quality control (QA/QC) analyses were followed through laboratory analyses. The accuracy of the analyses were controlled by using reagent blanks, triplicate samples, and certified reference samples (Trace Element on Fresh Water Sediment) from Fluka. The recoveries for investigated metals of the standard reference samples ranged from 92 to 107%. The analytical precision for replicate samples for all metals was within 5–10%.

Using the results from the heavy metals accumulation, bioconcentration factors (BCF) were used to express the effectiveness of heavy metal phytoextraction, calculated as the ratio of the concentration of individual metals in tree tissues with associated concentrations in the soil (Dickinson and Pulford, 2005). To test the phytoextraction potential of the trees, BCF was calculated separately for each heavy metal and tree tissue (i.e., leaves, roots and shoots).

2.6. Data analysis

All growth, physiology, and phytoextraction data were subjected to analyses of variance (ANOVA) and analyses of means (ANOM) according to SAS® (PROC GLM; PROC ANOM; SAS Institute, Inc., Cary, North Carolina, USA) assuming the aforementioned split-plot design including the main effects of block (random), treatment (fixed whole-plots), and genus (fixed sub-plots), and their interactions. Block × genus interactions with $P > 0.25$ were pooled with the error term, which was then used to test genus main effects.

Thus, the following two linear additive models were used, based on pooling:

$Y_{ijk} = \mu + B_i + T_j + BT_{ij} + G_k + BG_{ik} + TG_{jk} + \text{Error (without pooling)}$

$Y_{ijk} = \mu + B_i + T_j + BT_{ij} + G_k + TG_{jk} + \text{Pooled Error (with pooling)}$

where: Y_{ijk} = response variable to be analyzed, μ = overall mean, B_i = main effect of ith block, T_j = main effect of jth treatment, BT_{ij} = effect of interaction between ith block and jth treatment, G_k = main effect of kth genus, BG_{ik} = effect of interaction between ith block and kth genus, TG_{jk} = effect of interaction between jth treatment and kth genus, and pooled error = error term resulting from pooling of BG_{ik} and BTG_{ijk} terms, defined as: effect of interaction among ith block and kth genus, and effect of interaction among ith block, jth treatment, and kth genus, respectively.

Additionally, Pearson (i.e., phenotypic) correlation coefficients were generated using PROC CORR of SAS® in order to test for relationships among growth and physiological parameters during both years. Fisher's protected least significant difference (LSD) was used to separate means of main effects at a probability level of $P < 0.05$.

3. Results

3.1. Growth and physiology

3.1.1. First growing season (2014)

The treatment main effect was significant for aboveground dry mass, chlorophyll A content, total chlorophyll content, carotenoid content, and nitrate reductase activity (NRA), while genera differed for diameter, aboveground dry mass, chlorophyll A content, chlorophyll B content, total chlorophyll content, carotenoid content, photosynthetic rate (A), water use efficiency (WUE), NRA, and SPAD (Table S3). Despite these significant treatment and genus main effects, the interaction between treatment and genus governed chlorophyll A content, total chlorophyll content, carotenoid content, and NRA (Table S3).

Aboveground dry mass for the control treatment was 33% greater than the overall mean and 60% greater than for either of the sediment amendment treatments, which were not significantly different from each other nor the overall mean (Fig. 1).

Furthermore, differences between genera showed that poplars exhibited greater growth while willows had higher levels of physiological activity. Specifically, diameter and aboveground dry mass were 18% and 41% greater for poplars than willows, respectively. Physiologically, willows exhibited 1.2 times as much chlorophyll B content, as well as higher rates of A (2.7×), WUE (1.7×), and SPAD (1.2×) (Table 1). With the exception of NRA, the significant treatment × genus interactions corroborated the advantage of willows versus poplars for content of chlorohyll A, total chlorophyll, and carotenoids (Fig. 2). The genera exhibited consistent yet different trends across treatments. For poplar, chlorophyll and carotenoid content was generally greatest for the control treatment, with the 1.0-kg treatment having similar chlorophyll A and total chlorophyll content as the control yet significantly less carotenoid content. For all three traits, the 0.5-kg treatment was significantly less than both the control and 1.0-kg treatments, in addition to being 26–38% lower than the overall mean. For willow, chlorophyll and carotenoid content was greatest for the control, followed by 0.5- and 1.0-kg treatments. For all traits, the control was 11–16% greater than the overall mean. Additionally, poplar exhibited significantly greater NRA than willow, with the poplar control treatment having 1.5 times more NRA than the 1.0-kg treatment that was 1.9 times better than the 0.5-kg treatment. The treatments did not differ for willow and were 50–80% less than the overall mean (Fig. 2).

Phenotypic correlations among growth and physiological parameters across genera ranged from −0.84 (poplar shoot dry mass-NRA) to 0.97 (willow aboveground dry mass-shoot dry mass), and there were

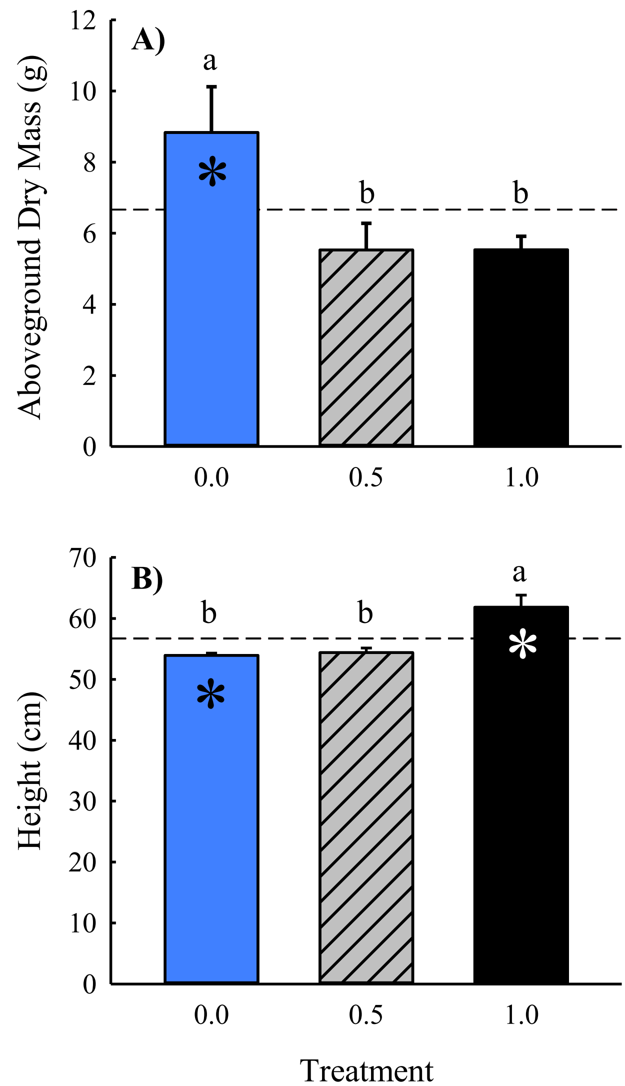

Fig. 1. Aboveground dry mass (A) and height (B) for the soil treatment main effect (n = 24) in a study testing the capability of poplar clone 'Bora' (*Populus deltoides* Bartr. ex Marsh) and willow clone 'SV068' (*Salix viminalis* L.) for phytoextraction of heavy metals from contaminated river sediments after being grown for the 2014 (A) and 2015 (B) growing seasons. The three soil treatments included a control of alluvial soil without sediment amendments (i.e., 0 kg) and two amendments consisting of the addition of 0.5 and 1 kg of sediment per pot. The dashed line represents the overall mean, while bars with asterisks indicate means that differ from the overall mean at $P < 0.05$. Bars with the same letters were not different according to Fisher's protected LSD at $P < 0.05$.

distinct trends within and between genera (Table S4). Poplars exhibited fewer significant correlations among growth traits, with diameter-height and leaf dry mass-aboveground dry mass being the only significant relationships. In contrast, for willows, the only non-significant relationships were for leaf dry mass-diameter, leaf dry mass-height, and aboveground dry mass-height (Table S4). While both genera exhibited highly positive correlations among chlorophyll and carotenoid content, relationships among WUE and NRA were generally non-existent for willow yet chlorophyll A, total chlorophyll, and carotenoid content were correlated with WUE and NRA for poplar. Similarly, A and transpiration rate (E) were not correlated for willow but were for poplar. In contrast, leaf area was positively correlated to both A and E for willow, with a lack of relationships for poplar (Table S4). All significant growth-growth and physiology-physiology correlations were positive (Table S4). Furthermore, growth-physiology correlations showed similar results. Despite significant leaf area-leaf dry mass and leaf area-

Table 1
Mean value (± standard error; n = 36) for growth and physiological parameters of poplar clone 'Bora' (*Populus deltoides* Bartr. ex Marsh) and willow clone 'SV068' (*Salix viminalis* L.) grown for the **2014 and 2015 growing seasons** in a study testing the capability for phytoextraction of heavy metals from contaminated river sediments. All means within a parameter were different at $P < 0.05$.

Parameter	Poplar	Willow
2014		
Diameter (mm)	5.01 ± 0.49 a	4.23 ± 0.67 b
Aboveground dry mass (g)	7.77 ± 1.04 z	5.50 ± 0.43 y
Chlorophyll B content (mg g^{-1})	2.03 ± 0.11 b	2.48 ± 0.12 a
Photosynthetic rate (A) (μmol O_2 $m^{-2}s^{-1}$)	7.68 ± 0.36 y	20.72 ± 1.76 z
Water use efficiency (WUE) (μmol O_2mmol H_2O^{-1})	7.27 ± 0.20 b	12.24 ± 0.84 a
SPAD (index value)	29.63 ± 0.37 y	34.35 ± 1.19 z
2015		
Leaf dry mass (g)	2.30 ± 0.21 a	0.93 ± 0.05 b
Shoot dry mass (g)	3.30 ± 0.29 z	1.14 ± 0.03 y
Aboveground dry mass (g)	5.60 ± 0.44 a	2.07 ± 0.07 b
Total dry mass (g)	6.84 ± 0.56 z	3.15 ± 0.14 y
Root mass ratio (RMR) (g g^{-1})	1.58 ± 0.02 b	3.03 ± 0.03 a

aboveground dry mass correlations for both genera, poplar height increased with increasing chlorophyll A, total chlorophyll, and carotenoid content, while these traits were unrelated for willow (Table S4).

3.1.2. Second growing season (2015)

The treatment main effect was significant for height, leaf area, E, and NRA, while genera differed for leaf dry mass, shoot dry mass, aboveground dry mass, total dry mass, leaf area, root mass ratio, E, WUE, and NRA (Table S5). Despite these significant treatment and genus main effects, the interaction between treatment and genus governed leaf area, E, WUE, and NRA (Table S5). Height for the 1.0-kg treatment was 9% greater than the overall mean and 14% greater than for both of the remaining treatments, which were not significantly different from each other despite the control being 5% shorter than the overall mean (Fig. 1).

Furthermore, differences between genera showed that poplars exhibited greater growth while willows allocated a greater proportion of their total biomass to roots relative to leaves and shoots. Specifically, leaf, shoot, aboveground, and total dry mass were 2.5, 2.9, 2.7, and 2.2 times greater for poplars than willows, respectively. In contrast, poplar root mass ratio was 17% less than that of willow (Table 1).

According to the treatment × genus interaction for leaf area, poplars exhibited 2.5 times greater leaf area than willow, with poplar leaf area across treatments ranging from 30 to 57% greater than the overall mean and willow leaf area ranging from −46 to −37% less than the overall mean. For poplar, the greatest leaf area was for trees grown in

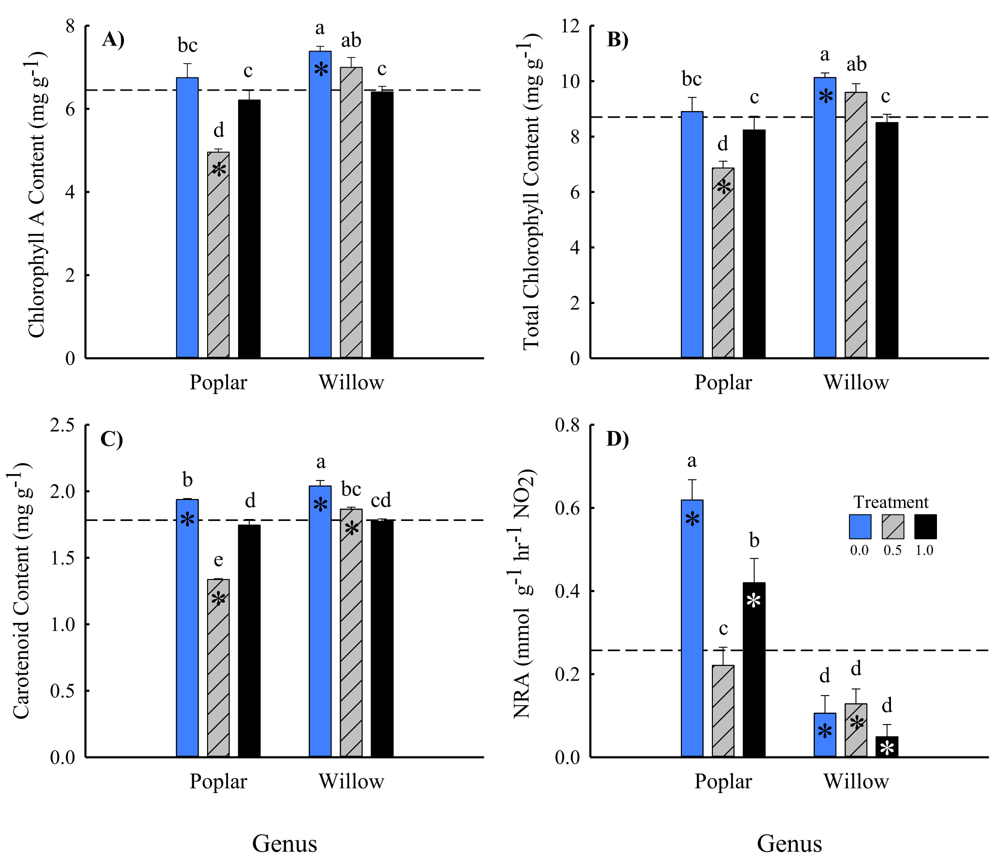

Fig. 2. Chlorophyll A content (A), total chlorophyll content (B), carotenoid content (C), and nitrate reductase activity (NRA) (D) for significant soil treatment × genus interactions (n = 12) following the first growing season (2014) in a study testing the capability of poplar clone 'Bora' (*Populus deltoides* Bartr. ex Marsh) and willow clone 'SV068' (*Salix viminalis* L.) for phytoextraction of heavy metals from contaminated river sediments. The three soil treatments included a control of alluvial soil without sediment amendments (i.e., 0 kg) and two amendments consisting of the addition of 0.5 and 1 kg of sediment per pot. The dashed line represents the overall mean, while bars with asterisks indicate means that differ from the overall mean at $P < 0.05$. Bars with the same letters were not different according to Fisher's protected LSD at $P < 0.05$.

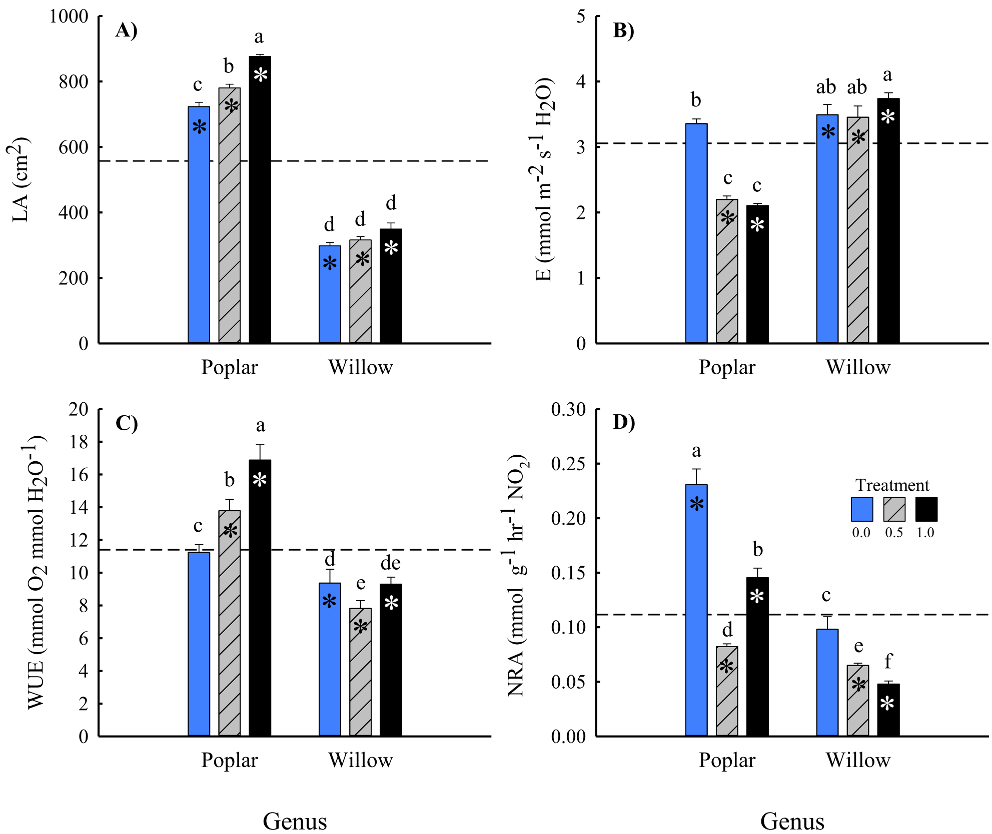

Fig. 3. Leaf area (LA) (A), transpiration rate (E) (B), water use efficiency (WUE) (C), and nitrate reductase activity (NRA) (D) for significant soil treatment × genus interactions (n = 12) following the second growing season (2015) in a study testing the capability of poplar clone 'Bora' (*Populus deltoides* Bartr. ex Marsh) and willow clone 'SV068' (*Salix viminalis* L.) for phytoextraction of heavy metals from contaminated river sediments. The three soil treatments included a control of alluvial soil without sediment amendments (i.e., 0 kg) and two amendments consisting of the addition of 0.5 and 1 kg of sediment per pot. The dashed line represents the overall mean, while bars with asterisks indicate means that differ from the overall mean at $P < 0.05$. Bars with the same letters were not different according to Fisher's protected LSD at $P < 0.05$.

1.0-kg amended soils, followed by the 0.5-kg treatment and the control. For willow, there were no differences among treatments for leaf area (Fig. 3). Similarly, soil treatments did not affect transpiration of willows, but transpiration of poplars grown in 0.5-kg and 1.0-kg soil amendment treatments was 50% and 57% less than the control, respectively. Additionally, the transpiration for these soil amendment treatments was ~30% less than the overall mean (Fig. 3). Furthermore, trends in WUE were similar to those for leaf area. Specifically, WUE of poplars was 1.6 times greater than willow, with poplar WUE of the 0.5- and 1.0-kg treatments having 21% and 48% greater WUE than the overall mean, respectively. For poplar, the greatest WUE was for trees grown in 1.0-kg amended soils, followed by the 0.5-kg treatment and the control. For willow, the control exhibited 21% greater WUE than the 0.5-kg treatment, and the 1.0-kg treatment was not different than either of the others (Fig. 3). The treatment × genus interaction for NRA was different than the previous three traits. The greatest NRA was for poplar control trees, which was 2.1 times higher than the overall mean and 1.5 times higher than the 1.0-kg treatment, which had the second best NRA. The 0.5-kg treatment was significantly less than its 1.0-kg counterpart and 27% less than the overall mean. For willow, NRA was greatest for the control treatment, followed by the 0.5-kg treatment that was 40% greater than the 1.0-kg treatment (Fig. 3).

Phenotypic correlations among growth and physiological parameters across genera ranged from −0.94 (poplar total dry mass-leaf area ratio) to 0.98 (willow WUE-A) (Table S6). Similar to 2014, there were distinct trends within and between genera; however, overall, there were a greater number of negative relationships in 2015. For both genera, leaf dry mass and shoot dry mass were positively correlated with aboveground dry mass, leaf area increased with increasing height, and leaf area ratio increased with increasing specific leaf area (Table S6). Leaf area ratio and root mass ratio were generally correlated with root dry mass and total dry mass, with the latter being negatively related to leaf area ratio. Similarly, with the exception of a non-significant specific leaf area-shoot dry mass correlation for willow, specific leaf area was negatively correlated with leaf, shoot, and aboveground dry mass for poplar, as well as leaf and aboveground dry mass for willow. In general, the remaining growth-growth correlations were significant for poplar but lacking in willow: total dry mass-specific leaf area; leaf area ratio with leaf dry mass, shoot dry mass, and aboveground dry mass; total dry mass with leaf, shoot, and aboveground dry mass (Table S6). For physiology-physiology correlations, the relationship between WUE and E was significant for both genera, yet poplar transpiration was inversely related to WUE while willow transpiration increased with increasing WUE. In addition, NRA was significantly related to A and E of poplar but not willow. In contrast, photosynthesis of willow was highly correlated with E and WUE but non-significant for poplar (Table S6). Growth-physiology correlations further corroborated differences between genera, with root and total dry mass being highly positively correlated with A, E, and WUE for willow but not poplar. In fact, the only significant correlation for poplar was total dry mass with A, with the two traits being inversely related (Table S6). Similarly, root mass ratio was highly positively correlated with A, E, and WUE for willow but not related for poplar. Relationships between A with shoot and aboveground dry mass, as well as leaf area with E and WUE, showed opposite trends, with correlations being significant for poplar but not willow (Table S6).

3.2. *Phytoremediation potential*

3.2.1. *Soil concentrations*

3.2.1.1. First growing season (2014). The treatment main effect was significant for the concentration of Cr, Cu, Ni, and Zn in soils, while genera differed for soil Ni concentrations (Table S7). The interaction between treatment and genus governed the soil concentrations of Ni and Zn (Table S7). All three treatments differed from one another for soil Cr concentrations, with the 1.0-kg treatment exhibiting 38.074 ± 2.512 mg Cr kg^{-1} and the 0.5-kg and control treatments having 22.958 ± 0.475 and 17.821 ± 0.444 mg Cr kg^{-1},

Table 2
Mean value (± standard error; mg kg^{-1}; n = 12) for **heavy metal concentrations** in soils for each combination of Treatment and Genus in a study testing the capability of poplar clone 'Bora' (*Populus deltoides* Bartr. ex Marsh) and willow clone 'SV068' (*Salix viminalis* L.) for phytoextraction of heavy metals from contaminated river sediments after being grown for the **2014 and 2015 growing seasons**. The three soil treatments included a control of alluvial soil without sediment amendments (i.e., 0 kg) and two amendments consisting of the addition of 0.5 and 1 kg of sediment per pot. Means with different letters within columns for Treatment × Genus interactions were different at $P < 0.05$. Interactions were not significant at $P < 0.05$ for means lacking LSD letters within columns.

Year	Genus	Treatment	Cd	Cr	Cu	Ni	Pb	Zn
2014	Poplar	0	0.085 ± 0.001	18.323 ± 0.535	20.607 ± 0.476	18.031 ± 0.650 w	6.909 ± 0.212	61.272 ± 0.257 w
		0.5	0.123 ± 0.013	22.770 ± 0.492	24.700 ± 0.708	19.213 ± 0.297 xw	7.029 ± 0.126	68.971 ± 0.490 x
		1	0.136 ± 0.010	42.268 ± 2.136	38.321 ± 1.390	22.797 ± 0.575 y	8.292 ± 0.289	85.369 ± 3.858 z
	Willow	0	0.092 ± 0.003	17.319 ± 0.086	21.051 ± 0.727	19.032 ± 0.251 xw	7.048 ± 0.307	64.268 ± 0.848 w
		0.5	0.120 ± 0.010	23.147 ± 0.443	29.376 ± 2.392	25.590 ± 0.558 z	7.357 ± 0.273	72.804 ± 0.697 y
		1	0.115 ± 0.005	33.880 ± 1.010	40.903 ± 3.950	20.887 ± 0.222 yx	7.009 ± 0.164	82.921 ± 2.877 z
2015	Poplar	0	0.141 ± 0.003 w	23.500 ± 0.509 c	21.825 ± 0.381 w	15.637 ± 0.119 c	8.095 ± 0.090 z	60.663 ± 0.398 e
		0.5	0.137 ± 0.003 w	30.293 ± 0.421 b	23.003 ± 0.354 xw	16.995 ± 0.335 c	7.747 ± 0.305 z	67.541 ± 0.430 de
		1	0.147 ± 0.003 yw	25.789 ± 0.342 bc	26.371 ± 0.649 x	25.067 ± 0.565 a	8.493 ± 0.091 z	87.000 ± 0.127 b
	Willow	0	0.132 ± 0.008 w	22.048 ± 0.524 c	23.255 ± 0.798 xw	22.356 ± 0.584 b	5.746 ± 0.067 y	63.321 ± 0.518 d
		0.5	0.175 ± 0.004 zy	23.933 ± 1.019 c	37.196 ± 0.408 y	25.650 ± 0.495 a	7.931 ± 0.025 z	73.412 ± 1.018 c
		1	0.187 ± 0.002 z	44.489 ± 0.265 a	51.841 ± 0.153 z	25.561 ± 0.341 a	7.891 ± 0.073 z	104.073 ± 0.374 a

respectively. Similarly, the 1.0-kg treatment exhibited the greatest soil Cu concentration (39.612 ± 3.017 mg Cu kg^{-1}), yet the remaining treatments were different from one another (0.5-kg = 27.038 ± 2.051 mg Cu kg^{-1}; control = 20.829 ± 0.622 mg Cu kg^{-1}).

Based on the treatment × genus interaction for heavy metal in the soil, willows exhibited 9% greater Ni concentrations than poplars, and trends in soil concentrations varied between genera. Specifically, for poplar, Ni soil concentrations were greatest for the 1.0-kg treatment that was significantly better than the 0.5-kg and control treatments, which were not different from one another. In contrast, for willow, soil Ni concentration was greatest for the 0.5-kg treatment, which was 23–34% greater than the 1.0-kg and control treatments, respectively (Table 2). In contrast, poplar and willow exhibited similar soil Zn concentrations and trends in treatment Zn levels. Specifically, Zn was greatest for the 1.0-kg treatment, followed by 0.5-kg and control, all of which were significantly different from one another within each genus (Table 2).

3.2.1.2. Second growing season (2015). Despite significant main effects for treatment and genus, the interaction between treatment and genus governed the soil concentrations of all heavy metals (Table S7). In general, based on treatment × genus interactions, willows exhibited greater overall soil heavy metal concentrations relative to poplars (Table 2). In particular, the percent advantage of willows versus poplars for soil concentrations of heavy metals ranged from 12% (Zn) to 58% (Cu). Specific comparisons among treatment × genus interactions were metal-specific and are shown in Table 2. Although not presented in manuscript, the increase of some heavy metals in soils of 1.0-kg treatment was also recorded, when compared to 2014.

3.2.2. Tissue concentrations

3.2.2.1. First growing season (2014). The treatment main effect was significant for Zn in leaves, as well as Cu, Ni, and Zn in shoots, while genera differed for Cd, Cr, Ni, and Zn in the leaves, and Cu and Zn in the shoots (Table S8). Despite significant treatment and genus main effects, the interaction between treatment and genus governed phytoextraction of Zn into leaves and Cu into shoots (Table S8). Across genera, phytoextraction of Ni into shoots was greatest for the 1.0-kg treatment though it was not significantly different than the 0.5-kg treatment, which had similar Ni uptake as the control. Overall, Ni concentration in the shoots of the 1.0-kg treatment was 18% greater than the overall mean and 37% greater than the control (Fig. 4). Similar trends were shown for uptake of Zn into the shoots, with the exception that both soil amendment treatments, which were not significantly different from one another, had greater concentrations of Zn in the shoots than the control. While neither amendment treatment differed from the overall mean, phytoextraction in the control was 22% less (Fig. 4).

Furthermore, phytoextraction potential of Cd, Cr, and Ni into leaves, as well as Zn into shoots, was 52%, 21%, 28%, and 30% greater for willow than poplar, respectively (Table 3). Based on the treatment × genus interaction for Cu phytoextraction into shoots, willows exhibited greater overall Cu concentrations than poplars, although the genera extracted Cu differently. Specifically, for poplar, Cu concentrations in the shoots were greatest for the 1.0-kg treatment that was significantly better than the 0.5-kg and control treatments, which were not different from one another. In contrast, for willow, phytoextraction was greatest for the control treatment, which was 24–35% greater than with amendments (Table 4). Similarly, willows exhibited 2.8 times greater overall Zn concentrations in leaves than poplars, yet the control treatment for both genera had significantly less Zn than the amendment treatments, which were not different from one another (Table 4).

3.2.2.2. Second growing season (2015). The treatment main effect was significant for Cu in leaves, Cr and Cu in roots, and Cu, Ni and Zn in shoots, while genera differed for Cd, Cu, and Zn in leaves, Cd, Cr, Cu, Ni, and Zn in roots, and Zn in shoots (Table S9). Despite significant treatment and genus main effects, the interaction between treatment and genus governed phytoextraction of Cd, Cu, Ni, and Zn into leaves, Cd, Cr, and Cu into roots, and Cd, Cu, Pb, and Zn into shoots (Table S9). Across genera, phytoextraction of Ni into shoots was greatest for the 0.5-kg treatment though it was not significantly different than the 0.5-kg treatment, which had similar Ni uptake as the control. Overall, Ni concentration in the shoots of the 0.5-kg treatment was 13% greater than the overall mean and 30% greater than the control. Significant differences for the main effect of genus showed that phytoextraction of Ni into willow roots was 48% and that of Zn 47% greater than for poplars. In general, based on treatment × genus interactions, willows exhibited greater overall phytoextraction potential relative to poplars (Table 4). In particular, the percent advantage of willows versus poplars for uptake of heavy metals ranged from 1% (shoot Pb) to 113% (leaf Zn). Specific comparisons among treatment × genus interactions were metal-specific and are shown in Table 4.

3.2.3. Bioconcentration factors

3.2.3.1. First growing season (2014). The treatment main effect was significant for the bioconcentration factor (BCF) of Cr, Ni, and Zn in leaves, as well as Cr, Cu, and Ni in shoots, while genera differed for Cd and Zn in the leaves, and Cu and Zn in the shoots (Table S8). The interaction between treatment and genus governed the BCF of Zn into leaves (Table S8). Across genera, the BCF of Cr followed similar trends

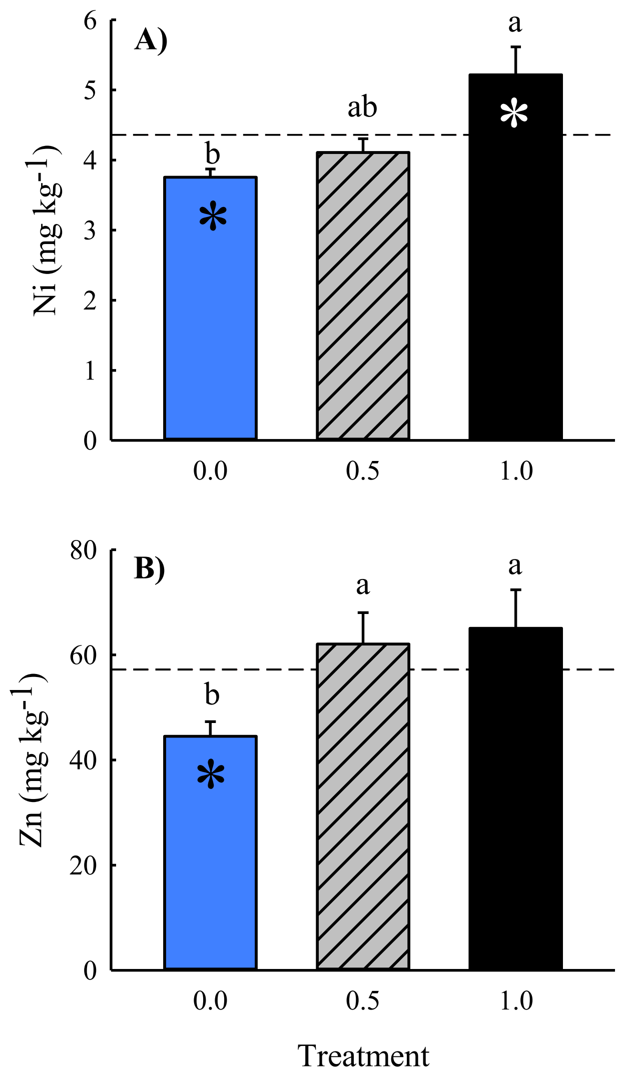

Fig. 4. Concentration of Ni (A) and Zn (B) in the shoots of trees for the soil treatment main effect (n = 24) in a study testing the capability of poplar clone 'Bora' (*Populus deltoides* Bartr. ex Marsh) and willow clone 'SV068' (*Salix viminalis* L.) for phytoextraction of heavy metals from contaminated river sediments after being grown for the 2014 growing season. The three soil treatments included a control of alluvial soil without sediment amendments (i.e., 0 kg) and two amendments consisting of the addition of 0.5 and 1 kg of sediment per pot. The dashed line represents the overall mean, while bars with asterisks indicate means that differ from the overall mean at $P < 0.05$. Bars with the same letters were not different according to Fisher's protected LSD at $P < 0.05$.

Table 3
Mean value (± standard error; n = 36) for heavy metal concentrations in tree tissues and bioconcentration factors of poplar clone 'Bora' (*Populus deltoides* Bartr. ex Marsh) and willow clone 'SV068' (*Salix viminalis* L.) grown for the **2014 growing season** in a study testing the capability for phytoextraction of heavy metals from contaminated river sediments. All means within a parameter were different at $P < 0.05$.

Parameter	Poplar	Willow
Leaf		
Cd (tree, mg kg^{-1})	0.869 ± 0.075 b	1.824 ± 0.105 a
Cd (bioconcentration factor)	8.005 ± 0.862 y	17.437 ± 1.685 z
Cr (tree, mg kg^{-1})	2.875 ± 0.143 b	3.621 ± 0.128 a
Ni (tree, mg kg^{-1})	5.768 ± 0.375 y	8.066 ± 0.321 z
Shoot		
Cu (bioconcentration factor)	0.343 ± 0.023 b	0.480 ± 0.070 a
Zn (tree, mg kg^{-1})	47.131 ± 2.844 y	67.316 ± 5.248 z
Zn (bioconcentration factor)	0.662 ± 0.038 b	0.910 ± 0.048 a

for leaves and shoots (Fig. 5). Specifically, the BCF of the control treatment was significantly greater than the 0.5-kg treatment, which itself was greater than the 1.0-kg treatment. The control was 21% and 36% greater than the overall mean for BCF of Cr in the leaves and shoots, respectively, while the 1.0-kg was 36% less than the overall mean for both tissues (Fig. 5). There were no treatment differences for the BCF of Ni in the leaves and shoots, nor were treatment values different from the overall mean, while Cu in the shoots showed similar trends as for Cr, with one exception. The BCF for the amendment treatments did not differ from one another, despite being 54% (0.5-kg) and 90% (1.0-kg) less than the control (Fig. 5). Furthermore, BCF of Cd in the leaves, as well as Cu and Zn in the shoots, was 54%, 29%, and 27% greater for willow than poplar, respectively (Table 3). Based on the treatment × genus interaction, willows exhibited a BCF that was 2.8 times greater than poplars for Zn in the leaves (Table 5). For both genera, the BCF of the control was significantly less than the amendment treatments, which were not different for poplar but the 0.5-kg treatment was 15% greater than the 1.0-kg treatment for willow (Table 5).

3.2.3.2. Second growing season (2015). The treatment main effect was significant for the BCF of Cd, Cr, and Cu in leaves, as well as Cr, Ni, and Zn in shoots, while genera differed for Cd, Cu, and Zn in leaves, Cd, Cr, and Zn in roots, and Ni in shoots (Table S9). The interaction between treatment and genus governed the BCF of Cd, Cr, Cu, and Zn in leaves, Cd, Cr, and Ni in roots, and Cu, Ni, and Pb in shoots (Table S9). Across genera, the BCF of Cr into shoots was greatest for the control treatment though it was not significantly different than the 0.5-kg treatment, which had similar Cr shoot BCF as the 1.0-kg treatment. Overall, Cr BCF in the shoots of the control was 16% greater than the overall mean and 34% greater than the 1.0-kg treatment. The response to treatments was different for the BCF of Zn in the shoots, whereby the 0.5-kg treatment was significantly greater than both of the other treatments that did not vary with one another. Overall, Zn BCF in the shoots of the 0.5-kg treatment was 24% greater than the overall mean and 38% and 44% greater than the 1.0-kg treatment and control, respectively. Across treatments, BCF of Zn in the roots was 36% greater for willow than poplar. In general, based on treatment × genus interactions, willows exhibited greater overall BCF relative to poplars (Table 5). In particular, the percent advantage of willows versus poplars for BCF of heavy metals ranged from 12% (shoot Pb) to 98% (leaf Zn). Specific comparisons among treatment × genus interactions were metal-specific and are shown in Table 5.

4. Discussion

4.1. Growth and physiology

We found that the effect of the application of dredged river sediments on the growth of tested poplars and willows varied considerably among application rate, genotype, and growing season. In the first growing season, the treatment of plants with sediments negatively affected growth in both poplars and willows, which was expressed by decreased aboveground biomass. In contrast, height and leaf area increased during the second year, but the relative increase was more pronounced in poplars than willows. Leaf area plays an important role in many remediation processes, especially due to its relationship to photosynthetic productivity. The relationships between leaf area and volume, and between leaf area and aboveground dry mass, are important for phytoremediation given the need for early prediction of potential remedial effectiveness (Zalesny et al., 2007).

When comparing growth differences between the tested genera, performance of poplars was more pronounced than willows during the entire study. The higher aboveground biomass production resulted in decreased root mass ratio for poplars. In most cases, the aboveground biomass of plants is 5–6 times heavier than roots (Harris, 1992), and the

Table 4
Mean value (± standard error; n = 12) for **heavy metal concentrations** in tree tissues for each combination of Treatment and Genus in a study testing the capability of poplar clone 'Bora' (*Populus deltoides* Bartr. ex Marsh) and willow clone 'SV068' (*Salix viminalis* L.) for phytoextraction of heavy metals from contaminated river sediments after being grown for the **2014 and 2015 growing seasons**. The three soil treatments included a control of alluvial soil without sediment amendments (i.e., 0 kg) and two amendments consisting of the addition of 0.5 and 1 kg of sediment per pot. Means with different letters within columns for Treatment × Genus interactions were different at $P < 0.05$. Interactions were not significant at $P < 0.05$ for means lacking LSD letters within columns. Roots were not harvested in 2014.

Year	Genus	Treatment	Leaf	Root	Shoot	Leaf	Root	Shoot
				Cd			*Cr*	
2014	Poplar	0	0.793 ± 0.023		0.719 ± 0.179	2.736 ± 0.081		2.927 ± 0.281
		0.5	0.736 ± 0.154		0.575 ± 0.030	3.047 ± 0.331		2.576 ± 0.093
		1	1.078 ± 0.098		0.543 ± 0.026	2.844 ± 0.323		2.924 ± 0.281
	Willow	0	2.102 ± 0.061		0.680 ± 0.045	3.347 ± 0.203		2.439 ± 0.144
		0.5	1.584 ± 0.098		0.585 ± 0.025	3.610 ± 0.093		2.445 ± 0.098
		1	1.787 ± 0.225		0.810 ± 0.161	3.906 ± 0.261		2.625 ± 0.321
2015	Poplar	0	0.833 ± 0.083 w	0.744 ± 0.220 b	0.564 ± 0.065 zyx	2.531 ± 0.115	12.120 ± 0.850 w	2.279 ± 0.074
		0.5	1.006 ± 0.065 x	1.468 ± 0.053 a	0.683 ± 0.035 zy	2.777 ± 0.095	13.922 ± 0.879 w	2.335 ± 0.090
		1	0.846 ± 0.046 w	0.777 ± 0.123 b	0.456 ± 0.025 x	2.767 ± 0.146	14.789 ± 0.548 w	2.138 ± 0.248
	Willow	0	1.354 ± 0.076 z	1.453 ± 0.093 a	0.563 ± 0.037 yx	2.688 ± 0.120	23.755 ± 0.624 y	2.115 ± 0.130
		0.5	1.232 ± 0.038 y	1.361 ± 0.134 a	0.733 ± 0.094 z	2.794 ± 0.130	19.541 ± 1.063 x	2.036 ± 0.043
		1	1.286 ± 0.005 zy	1.399 ± 0.106 a	0.693 ± 0.018 zy	2.371 ± 0.272	31.887 ± 0.135 z	2.712 ± 0.096
				Cu			*Ni*	
2014	Poplar	0	9.49 ± 1.029		8.459 ± 0.504 c	4.918 ± 0.089		3.506 ± 0.041
		0.5	11.584 ± 2.388		8.151 ± 0.725 c	5.901 ± 1.010		4.228 ± 0.150
		1	12.111 ± 0.705		10.874 ± 1.086 b	6.484 ± 0.184		5.368 ± 0.321
	Willow	0	13.006 ± 1.176		15.250 ± 1.071 a	8.371 ± 0.551		4.002 ± 0.078
		0.5	11.682 ± 0.978		11.264 ± 0.295 b	8.201 ± 0.707		3.986 ± 0.399
		1	12.533 ± 1.044		12.253 ± 0.356 b	7.625 ± 0.531		5.065 ± 0.817
2015	Poplar	0	17.024 ± 0.996 y	40.458 ± 10.714 c	8.884 ± 1.074 x	5.405 ± 0.334 c	14.211 ± 0.257	3.193 ± 0.184
		0.5	12.502 ± 1.020 x	53.803 ± 3.103 c	7.437 ± 0.428 x	5.145 ± 0.719 c	13.406 ± 0.785	4.204 ± 0.244
		1	37.584 ± 0.947 z	51.332 ± 6.457 c	20.275 ± 1.734 z	5.557 ± 0.507 c	12.945 ± 0.966	3.560 ± 0.180
	Willow	0	15.426 ± 0.499 yx	55.300 ± 3.628 c	13.706 ± 1.408 y	6.968 ± 0.145 a	21.385 ± 1.257	2.279 ± 0.370
		0.5	17.140 ± 0.490 y	75.287 ± 5.341 b	15.192 ± 0.152 y	6.714 ± 0.440 ab	18.065 ± 0.831	2.898 ± 0.246
		1	16.542 ± 1.278 y	116.675 ± 6.129 a	14.634 ± 0.228 y	5.980 ± 0.312 bc	20.746 ± 0.777	2.665 ± 0.058
				Pb			*Zn*	
2014	Poplar	0	2.085 ± 0.420		2.250 ± 0.281	44.573 ± 4.281 w		41.185 ± 4.428
		0.5	3.147 ± 1.265		3.407 ± 0.504	85.359 ± 7.992 x		50.423 ± 5.481
		1	2.277 ± 0.568		3.372 ± 0.168	117.316 ± 4.714 x		49.786 ± 4.557
	Willow	0	3.965 ± 1.071		2.887 ± 0.381	166.211 ± 6.640 y		47.857 ± 2.931
		0.5	2.990 ± 0.364		2.886 ± 0.503	268.396 ± 19.505 z		73.715 ± 3.622
		1	3.641 ± 1.378		2.354 ± 0.485	266.027 ± 23.381 z		80.377 ± 3.693
2015	Poplar	0	2.234 ± 0.036	4.626 ± 0.481	2.477 ± 0.056 yx	38.548 ± 3.283 c	33.784 ± 2.949	26.554 ± 0.898
		0.5	2.504 ± 0.242	6.318 ± 0.653	2.311 ± 0.106 yx	64.149 ± 1.820 b	55.895 ± 3.935	40.090 ± 2.673
		1	2.719 ± 0.098	6.602 ± 1.363	2.808 ± 0.152 zy	68.956 ± 1.048 b	57.115 ± 3.569	33.240 ± 2.169
	Willow	0	2.261 ± 0.131	6.260 ± 0.496	2.024 ± 0.179 x	118.458 ± 9.982 a	69.491 ± 9.436	23.711 ± 0.670
		0.5	2.684 ± 0.174	6.443 ± 0.756	3.057 ± 0.139 z	117.388 ± 3.623 a	67.624 ± 6.781	41.896 ± 1.106
		1	2.376 ± 0.356	5.829 ± 0.246	2.509 ± 0.229 yx	128.933 ± 4.433 a	78.904 ± 5.725	48.375 ± 2.378

proportion of total tree biomass accumulated in roots systems of poplars and willows declines during the first year of growth with a tendency towards stabilization during subsequent years (Dickmann and Hendrick, 1994; Dušek and Kvet, 2006). The values of root mass ratio in our study were in accordance with values for poplars and willows (Sebastiani et al., 2004; Sleight et al., 2015; Wullschleger et al., 2005). However, similar to the aforementioned studies, root mass ratio was greater for willows than poplars. More developed roots in willows can contribute to efficient phytoremediation because large root systems are more advantageous than their smaller counterparts for acquiring water and other belowground resources (Kramer, 1969). On the other hand, lower root-shoot ratios in poplars can be explained by higher assimilate accumulation in aboveground parts given that the root-shoot ratio shows the distribution of plant photosynthetic product (Haolin et al., 2008).

Under conditions of environmental variation and stress, evaluating the distribution pattern of photosynthetic carbon is more meaningful than that of the accumulation of photosynthate in plants (Yang et al., 2010). To address this, the difference in biomass allocation of the investigated genotypes can be explained by differences in physiological activity, where willows exhibited greater physiological responses than poplars. The higher distribution of roots in willows reflected its higher transpiration and lower water use efficiency in all treatments. Phenotypic correlations in willows also showed significant positive relationships between root mass and assimilation-related processes (i.e., photosynthesis, transpiration, water use efficiency) (Table S6). In contrast, poplars showed higher aboveground biomass production correlated with assimilation processes and higher water use efficiency which supported the conclusion of different survival strategies between poplars and willows when dealing with unfavorable conditions such as heavy-metal contaminated, dredged river sediments.

Analyses of physiological parameters in the current study showed more pronounced physiological responses in willows versus poplars. Significant alteration of physiological processes was not expressed by all parameters, with the most pronounced alterations for pigments content, water use efficiency, and nitrate reductase activity. Sensitivity of plants to heavy metals varies greatly, yet most plants show sensitivity to low Cd which alters the chloroplast ultrastructure and

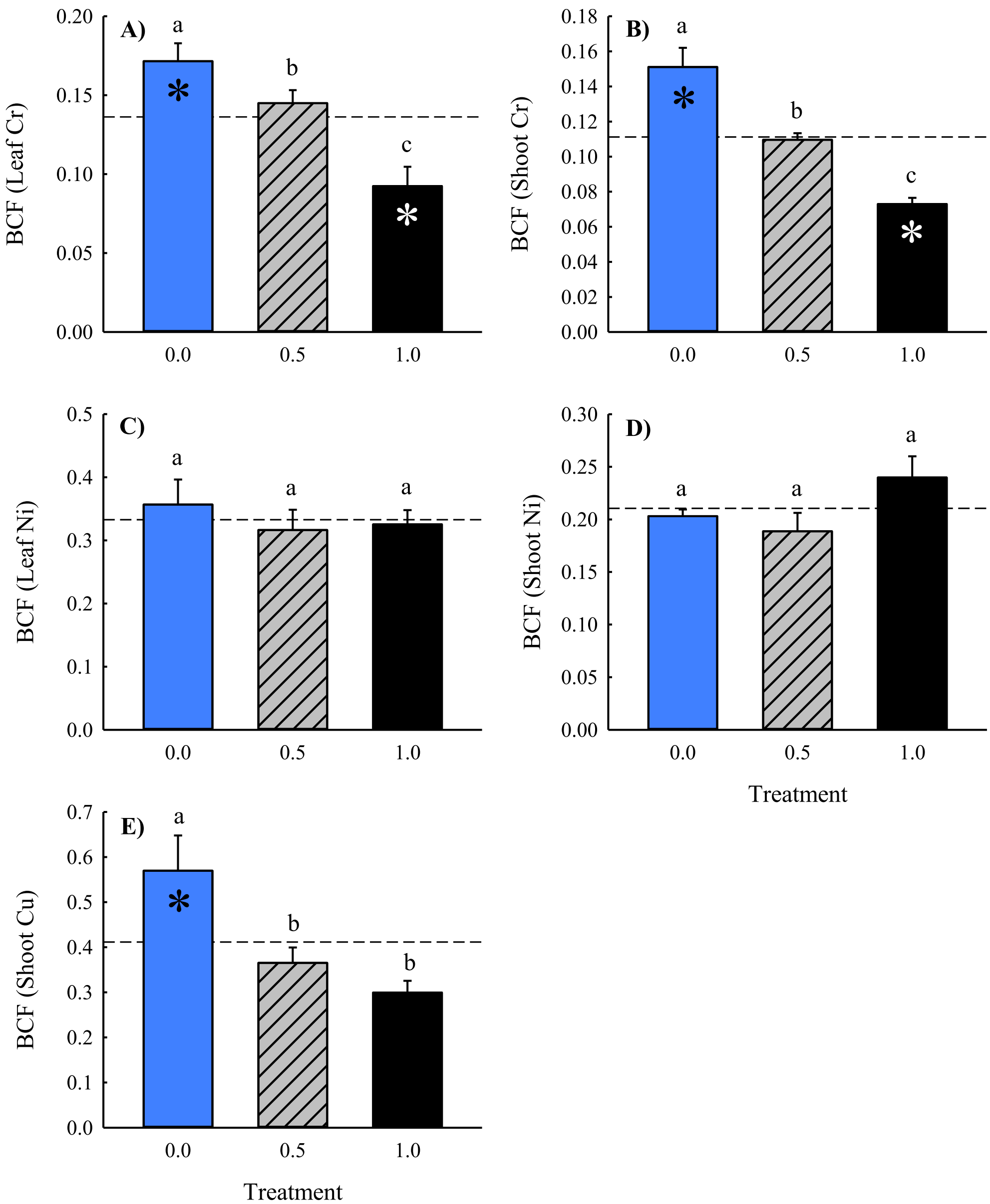

Fig. 5. Bioconcentration factor (BCF) of the uptake of Cr into tree leaves (A) and shoots (B), Ni into tree leaves (C) and shoots (D), as well as the uptake of Cu into shoots (E) for the soil treatment main effect (n = 24) in a study testing the capability of poplar clone 'Bora' (*Populus deltoides* Bartr. ex Marsh) and willow clone 'SV068' (*Salix viminalis* L.) for phytoextraction of heavy metals from contaminated river sediments after being grown for the 2014 growing season. The three soil treatments included a control of alluvial soil without sediment amendments (i.e., 0 kg) and two amendments consisting of the addition of 0.5 and 1 kg of sediment per pot. The dashed line represents the overall mean, while bars with asterisks indicate means that differ from the overall mean at $P < 0.05$. Bars with the same letters were not different according to Fisher's protected LSD at $P < 0.05$.

photosynthesis rate while disturbing the calvin cycle, nitrogen, sulfur and antioxidant enzymes, and the uptake and distribution of macro- and micro-nutrients (Gill et al., 2012). Other authors recorded decreased SPAD values in Cd-treated plants of *Pentas lanceolate*, indicating that this parameter can be used for determination of metal tolerant species (Chang et al., 2013; Chen et al., 2008). High Zn concentrations affect carbon assimilation, chlorophyll content, structural modifications of leaves, and biochemical processes (Di Baccio et al., 2005, 2009). Excess Cu and Cr in growing media affect photosynthetic processes, nitrogen metabolism, and antioxidant defense systems (Borghi et al., 2008; Farid et al., 2017; Trudić et al., 2013). Similarly, leaf chlorosis, disturbed water balance, and reduced stomatal opening are the major stress responses to toxic Ni concentrations (Clemens, 2006).

The net photosynthesis rate of our investigated trees was not directly altered by the application of river sediments, which corroborated the results of Tognetti et al. (2004), who reported a lack of significant decreases in photosynthetic rates of poplar clones grown in soils amended with industrial waste. In contrast, many other researchers recorded photosynthesis decreases (Borghi et al., 2008; Di Baccio et al., 2009; Nikolić et al., 2008). The effect of heavy metals on photosynthetic processes of poplars in these studies was pronounced through alteration of transpiration rate and water use efficiency, which was further shown for black locust (Župunski et al., 2016) and poplars and willows (Borišev et al., 2016; Polle et al., 2013). Severity of the stress may be indicated by changes in chlorophyll and carotenoid contents in plant leaves (Soudek et al., 2011). Our results showed no effect of heavy metals on SPAD values, although analysis of pigments content was affected by the presence of contaminated sludge. In particular, total chlorophyll and carotenoid content showed significant impacts of amendment treatments, genera, and their interactions, thus indicating the disturbance. The presence of heavy-metal contaminated sludge altered the content of photosynthetic pigments, especially chlorophyll A, total chlorophyll, and carotenoids in both genera. These results are in concordance with other authors who recorded significant effects of heavy metals on pigments content in poplars and willows. Nikolić et al. (2008) showed decreased chlorophyll content in poplars affected by Cd presence, Katanić et al. (2015) recorded decreased pigments content in *Populus alba* L. (white poplars) grown *in vitro* on growing medium containing 10^{-4} M of Ni. Since the assessment of pigments content was performed on each young, first fully-developed leaf (LPI = 5; Larson and Isebrands, 1971), and taking into account that the application of sediment was made on developed plants, it can be assumed that

Table 5
Mean value (± standard error; n = 12) for **bioconcentration factors of heavy metals** in tree tissues for each combination of Treatment and Genus in a study testing the capability of poplar clone 'Bora' (*Populus deltoides* Bartr. ex Marsh) and willow clone 'SV068' (*Salix viminalis* L.) for phytoextraction of heavy metals from contaminated river sediments after being grown for the **2014 and 2015 growing seasons**. The three soil treatments included a control of alluvial soil without sediment amendments (i.e., 0 kg) and two amendments consisting of the addition of 0.5 and 1 kg of sediment per pot. Means with different letters within columns for Treatment × Genus interactions were different at $P < 0.05$. Interactions were not significant at $P < 0.05$ for means lacking LSD letters within columns. Roots were not harvested in 2014.

Year	Genus	Treatment	Leaf	Root	Shoot	Leaf	Root	Shoot
				Cd			*Cr*	
2014	Poplar	0	9.369 ± 0.351		8.546 ± 2.216	0.150 ± 0.008		0.161 ± 0.021
		0.5	6.415 ± 2.030		5.013 ± 1.040	0.134 ± 0.015		0.113 ± 0.006
		1	8.230 ± 1.563		4.068 ± 0.404	0.069 ± 0.012		0.069 ± 0.003
	Willow	0	22.822 ± 0.501		7.364 ± 0.271	0.193 ± 0.011		0.141 ± 0.007
		0.5	13.756 ± 2.188		5.052 ± 0.705	0.156 ± 0.002		0.106 ± 0.005
		1	15.733 ± 2.514		7.172 ± 1.661	0.116 ± 0.008		0.077 ± 0.006
2015	Poplar	0	5.974 ± 0.724 y	5.441 ± 1.791 b	4.044 ± 0.549	0.108 ± 0.001 c	0.515 ± 0.015 x	0.097 ± 0.005
		0.5	7.420 ± 0.723 y	10.827 ± 0.857 a	5.039 ± 0.470	0.092 ± 0.006 c	0.459 ± 0.019 x	0.077 ± 0.005
		1	5.767 ± 0.218 y	5.386 ± 1.036 b	3.126 ± 0.264	0.108 ± 0.008 c	0.575 ± 0.031 x	0.084 ± 0.012
	Willow	0	10.463 ± 0.673 z	11.257 ± 1.033 a	4.474 ± 0.755	0.123 ± 0.011 a	1.085 ± 0.080 z	0.096 ± 0.004
		0.5	7.034 ± 0.094 y	7.787 ± 0.825 b	4.217 ± 0.624	0.118 ± 0.005 b	0.835 ± 0.112 y	0.086 ± 0.006
		1	6.873 ± 0.148 y	7.456 ± 0.422 b	3.710 ± 0.175	0.053 ± 0.006 d	0.717 ± 0.012 y	0.061 ± 0.002
				Cu			*Ni*	
2014	Poplar	0	0.464 ± 0.059		0.410 ± 0.015	0.274 ± 0.013		0.196 ± 0.010
		0.5	0.479 ± 0.115		0.333 ± 0.039	0.310 ± 0.061		0.221 ± 0.013
		1	0.320 ± 0.034		0.285 ± 0.030	0.286 ± 0.017		0.237 ± 0.022
	Willow	0	0.628 ± 0.091		0.729 ± 0.069	0.440 ± 0.027		0.211 ± 0.007
		0.5	0.405 ± 0.041		0.398 ± 0.057	0.323 ± 0.038		0.157 ± 0.018
		1	0.316 ± 0.042		0.314 ± 0.048	0.365 ± 0.026		0.243 ± 0.039
2015	Poplar	0	0.779 ± 0.028 y	1.853 ± 0.472	0.408 ± 0.048 yx	0.346 ± 0.025	0.909 ± 0.008 z	0.205 ± 0.014 a
		0.5	0.545 ± 0.050 xw	2.337 ± 0.091	0.323 ± 0.017 x	0.303 ± 0.044	0.793 ± 0.067 zy	0.247 ± 0.010 a
		1	1.431 ± 0.068 z	1.965 ± 0.298	0.771 ± 0.073 z	0.224 ± 0.031	0.522 ± 0.065 x	0.143 ± 0.011 b
	Willow	0	0.670 ± 0.052 yx	2.415 ± 0.292	0.600 ± 0.089 zy	0.313 ± 0.011	0.968 ± 0.110 z	0.102 ± 0.015 b
		0.5	0.461 ± 0.013 w	2.020 ± 0.108	0.409 ± 0.012 yx	0.262 ± 0.019	0.708 ± 0.054 y	0.113 ± 0.010 b
		1	0.319 ± 0.025 v	2.250 ± 0.111	0.282 ± 0.006 x	0.235 ± 0.019	0.812 ± 0.028 zy	0.104 ± 0.004 b
				Pb			*Zn*	
2014	Poplar	0	0.305 ± 0.028		0.330 ± 0.053	0.728 ± 0.074 v		0.671 ± 0.067
		0.5	0.456 ± 0.148		0.489 ± 0.086	1.235 ± 0.103 w		0.730 ± 0.073
		1	0.277 ± 0.030		0.408 ± 0.025	1.383 ± 0.069 w		0.586 ± 0.047
	Willow	0	0.555 ± 0.056		0.420 ± 0.083	2.584 ± 0.053 x		0.744 ± 0.032
		0.5	0.408 ± 0.013		0.387 ± 0.045	3.680 ± 0.218 z		1.012 ± 0.045
		1	0.533 ± 0.183		0.336 ± 0.066	3.197 ± 0.121 y		0.974 ± 0.057
2015	Poplar	0	0.276 ± 0.002	0.575 ± 0.073	0.306 ± 0.013 y	0.635 ± 0.049 e	0.557 ± 0.051	0.438 ± 0.011
		0.5	0.328 ± 0.047	0.833 ± 0.129	0.301 ± 0.020 y	0.950 ± 0.018 d	0.827 ± 0.049	0.595 ± 0.046
		1	0.321 ± 0.017	0.784 ± 0.175	0.331 ± 0.022 z	0.793 ± 0.014 d	0.656 ± 0.040	0.382 ± 0.024
	Willow	0	0.394 ± 0.022	1.088 ± 0.071	0.354 ± 0.039 zy	1.874 ± 0.178 a	1.099 ± 0.157	0.374 ± 0.005
		0.5	0.338 ± 0.020	0.814 ± 0.101	0.386 ± 0.020 z	1.601 ± 0.056 b	0.928 ± 0.118	0.572 ± 0.028
		1	0.300 ± 0.040	0.738 ± 0.018	0.317 ± 0.024 z	1.239 ± 0.049 c	0.758 ± 0.052	0.465 ± 0.026

presence of heavy metals affected chlorophyll synthesis in developing leaves rather than stimulated degradation that can occur in already-developed leaves after being exposed to heavy metal treatments (Nikolić et al., 2017).

Inactivation of nitrate reductase occurs in response to stress conditions including the loss of light, a decrease in CO_2 levels, an increase in cytosolic pH, or variations in photosynthetic activity (Kaiser et al., 1999). During the entire study, the effect of treatment, species, and their interaction were pronounced through nitrate reductase activity. The decrease of nitrate reductase activity in leaves of our trees can be explained by the hypothesis that the presence of heavy metals affected depletion of a continuous supply of nitrates through the xylem via the transpiration stream (Kawachi et al., 2002), because the main process of nitrate assimilation is located in leaves, while roots support nitrate deposition (O'Neill and Gordon, 1994). The decrease of nitrate reductase activity in plants can be attributed to the mutual effect of all heavy metals in river sediment present in higher concentrations (e.g., Zn, Cr, Cu, Ni, Cd). Vaypayee et al. (2000) reported that the decrease of chlorophyll content and nitrate reductase activity in *Nymphaea alba* L. was highly correlated with the presence of Cr in the growing medium, leading to the conclusion that there was a positive relationship between chlorophyll biosynthesis and nitrate reductase activity. These results support those of our study for poplar clone 'Bora' where there was a positive correlation between nitrate reductase activity and pigments content (i.e., chlorophyll A, total chlorophyll, carotenoids). The presence of Cd also negatively affected N metabolism by inhibiting nitrate uptake and reducing the activity of enzymes involved in the nitrate assimilation pathway (Chang et al., 2013). Sufficient application of N positively affects plant tolerance to Cd toxicity in poplars by unblocking the chlorophyll synthesis pathway and preventing the occurrence of toxicity symptoms (Zhang et al., 2014). Since our soils were not applied with any additional N, the increase of nitrate reductase activity in poplar leaves of plants treated with 1.0 versus 0.5 kg of sediment could be explained by the addition of 13.1 g N kg^{-1} from the sediment, especially during the first growing season. As previously mentioned, the effect of the mixture of heavy metals from the sediment likely affected nitrogen metabolism in various ways, sometimes even with synergistic effects.

The negative effect of contamination on nitrate reductase activity was more pronounced in poplars than willows, which supports the conclusion that there is a genetic pre-disposition of plants to synthesize nitrate reductase in response to external conditions (Katanić et al., 2015; Matraszek, 2008; Nikolić et al., 2017; Pilipović et al., 2005, 2012).

4.2. Phytoextraction and phytoremediation

The application of dredged river sediments increased concentrations of the investigated heavy metals in soils for both treatments in the current study. With the exception of Pb and Cd, the concentrations of heavy metals in the dredged sediments exceeded field regulatory thresholds, although its application to the trees did not exceed prescribed limits for poplar and willow. However, there was a slight increase of Cd, Cr, Cu and Zn over time, which may have resulted from migration of heavy metals from the topsoil to lower soil layers. With the exception of Cr and Zn, our measured concentrations were generally within the range reported by Mulligan et al. (2001) who stated that phytoremediation is most applicable for polishing of shallow soils with low levels of contamination ranging from 2.5 to 100 $mg\,kg^{-1}$. The concentrations of heavy metals in the sediments of the current study were higher or similar to some studies (Doni et al., 2015; Meers et al., 2005b; Vervaeke et al., 2003) and much lower than others (King et al., 2006; Vysloužilova et al., 2003).

During active growth stages, plants rapidly uptake mineral elements and also absorb organic and inorganic contaminants attached with them, from the growth medium (Farid et al., 2017). Phytoextraction of heavy metals is dependent upon many biotic and abiotic factors, and soil chemistry combined with plant specificity plays a significant role. The bioavailability of heavy metals strongly depends upon their extractability resulting from their bonds with different soil fractions. Therefore, results obtained from hydroponic screening of phytoextraction potential (Borišev et al., 2009; Dos Santos Utmazin et al., 2007) showed higher accumulation rates when compared to soil or field experiments, but the evidence of strong correlation in species/clone performance was present (Watson et al., 2003). To improve the phytoextraction potential of poplars and willows, studies were conducted to increase the bioavailability of heavy metals (Arsenov et al., 2017; Meers et al., 2005a; Mihucz et al., 2012; Robinson et al., 2000). High accumulation of metals in roots and low transport of heavy metals to the shoots was purported to be a key mechanism evolved to protect plant organs involved in photosynthesis (Landberg and Greger, 1996). Since that time, Pulford and Dickinson (2006) classified heavy metals according to mobility: 1) trace elements immobilized in roots (Al, Pb, Hg, Cr), and 2) mobile trace elements (As, B, Cd, Cu, Mn, Ni, Mo, Se, Zn).

The results of our current study showed higher accumulation of Cr, Pb, Ni, and Cu in roots of both genera, while accumulation of Zn and Cd was higher in aboveground tissues of the trees. The accumulation of Cr was greatest in roots of both poplar and willow in all of the investigated treatments. Although significant differences between treatments were recorded only in willow roots, accumulation followed a general decreasing pattern: roots > leaves > shoots. Similar results regarding distribution of Cr within plants were recorded in many studies. For example, the greatest root uptake was recorded in poplars (Giachetti and Sebastiani, 2006a, 2006b; Sebastiani et al., 2004; Tognetti et al., 2004) and willows (Giachetti and Sebastiani, 2007) treated with tannery waste containing high amounts of heavy metals, including Cr. Pulford et al. (2001) investigated uptake of Cr and Zn in different tree species (willows, birches, poplars, pines and alder) grown at contaminated sites (Cr processing waste and sewage sludge) versus hydroponics and reported poor Cr accumulation in aerial parts of plants when compared to Zn, which corroborated results from the current study. The Pb concentration in plant tissues reflected low concentrations of soils with the highest accumulation in roots and no differences between shoots and leaves. Meers et al. (2005b) reported the highest accumulation in leaves, although Pb content in their dredged sediment was 10–15 times higher than that of the current study. Other studies showed lower phytoextraction of Pb in aboveground tissues of trees (Vervaeke et al., 2003; King et al., 2006). Such high root accumulation of previously-discussed heavy metals may have resulted from natural processes in the root zone where Cr was reduced to non-bioavailable forms (Cr_{VII}) and where Pb formed lead phosphate and other chelates (Pulford and Dickinson, 2006).

The Cu accumulation in the current study showed the highest amount of Cu in roots of both poplars and willows followed by leaves and stems. However, the application of sediment treatments only increased accumulation in willow roots. On the other hand, increases in aboveground tissues were only recorded in poplar with the highest accumulation in leaves. Borghi et al. (2008) recorded similar accumulation of Cu in leaves and shoots of hybrid black poplars (*Populus × canadensis)* grown in hydroponics, while the highest accumulation was recorded in roots, thus supporting our current results. Sebastiani et al. (2004) also recorded the greatest root uptake of Cu in *Populus × euramericana* clone 'I-214', but there were no differences between stems and leaves. Increased Cu levels in leaves of poplars, when compared to stems, can be the result of Cu^{2+} ions binding with the sap organic molecules, rather than with xylem cell walls (Pulford and Dickinson, 2006).

Despite the increase of soil concentration due to the effect of sediment application, there were no significant changes in Ni accumulation for poplar or willow. Mertens et al. (2004) investigated willow phytoremediation potential for brackish river sediment with similar levels of Ni contamination and recorded lower accumulation than the current study, advocating to the consequence of low heavy metal bioavailability in these sediments.

Higher extractability of Zn by the soil solution (i.e., water) relative to Cu has indicated higher susceptibility of Zn to ion exchange mechanisms (Meers et al., 2005b), which may have regulated the higher Zn accumulation in the current study. The pattern of Zn accumulation in tissues of both poplar and willow followed that of previous studies: leaves > roots > shoots (Di Baccio et al., 2003; Sebastiani et al., 2004). In contrast, other researchers reported that the highest Zn accumulation was found in the roots (Di Baccio et al., 2009; Do Santos Utmazin et al., 2007). These results can be explained by the concentrations of applied Zn, growth stages of the plants, their clonal specificity, and the type of experiment (i.e., hydroponics, greenhouse, field), which considered together significantly affected bioavailability of elements and growth of the plants. The relatively high uptake of Cd in tissues of the clones in the current study (i.e., with a bioconcentration factor ranging from 3.13 to 22.82 mg) can be explained by lower Cd concentrations in the soils. The bioconcentration factor in fast growing species such as poplars and willows decreases with the increase of soil Cd (Dickinson and Pulford, 2005; references within), which was also observed in the current study. In addition, the increase of the Zn-Cd ratio and the Zn increase decreased uptake of Cd because Cd uses Zn transporters for uptake (He et al., 2017). Such decreases in Cd uptake were recorded in our study with the increase of Zn contamination by sediment application.

Overall, the phytoremediation potential of the clones tested in the current study showed different patterns that were dependent upon the heavy metal species and tree genotype. Most of the heavy metals tested showed the lowest accumulation in the stems of poplars and willows, which is likely the result of the fact that wood typically comprises 60% of tree biomass and woody species invest much of their energy in the production of support tissue with low heavy metal content in comparison to herbaceous species (Meers et al., 2005b). Thus, translocation of easily-mobile heavy metals such as Zn, Cd, and Cu to leaves and/or bark can be attributed to their long-term survival strategy, where excess of heavy metals are removed from perennial aboveground tissues. Such strategies have resulted in the decrease of accumulation in shoots of

Salix viminalis after one year of growth (Pulford et al., 2001), which was also recorded in the current study for some metals. Other metals (i.e., Pb, Ni and Cr) were mostly accumulated in the belowground tissues of trees indicating that the phytoremediation mechanism for these metals was phytostabilization. Similar results were recorded in other studies for phytoremediation of Cr, Pb, and Ni from contaminated sites (Borghi et al., 2008; Pulford et al., 2001; Roselli et al., 2003; Giachetti and Sebastiani, 2006a; Tognetti et al., 2004) or dredged sediments (Doni et al., 2015; King et al., 2006; Meers et al., 2005; Mertens et al., 2004; Vervaeke et al., 2003). In these studies, phytostabilization had greater potential than phytoextraction. In addition to heavy metal specific uptake and metabolic pathways, soil chemistry processes influenced phytoremediation potential. Although bioavailability of heavy metals was not analyzed in this study, other studies have shown that bioavailability was dependent upon the individual fraction of the heavy metal in the soil matrix (Doni et al., 2015), pH value of the substrate (Hammer et al., 2003), presence of $CaCO_3$ which immobilizes metals by increasing the pH, or the competitiveness of Ca^{2+} with heavy metals.

Based on the results of the current study, key components in the design of phytoremediation systems targeted for heavy metal-contaminated, dredged river sediments include: appropriate clonal selection based on the suite of heavy metals present, agronomic soil properties such as organic matter content and levels of macro- and micronutrients (to provide sufficient nutrition without affecting tree vitality), and knowledge of whether phytoextraction, phytostabilization, or both, are needed.

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Supplementary data

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