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Analysis of urban runoff remediation potential of zinc with Juncus effusus L. and Iris pseudacorus L. plants

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Abstract

Phytoremediation is a sustainable technology that uses vegetation to remediate contaminants from water, soil and sediments. In this paper, two plants, *Juncus effusus* L. and *Iris pseudacorus* L. were used in an experimental study of phytoremediation of water contaminated with zinc (Zn) within bioretention systems (BRs). Plants were grown hydroponically in jars with Hoagland nutrient and under different Zn treatments (T1-T2). Four plants of each species were maintained in jars containing only Hoagland nutrient (T1), while for other Zn concentrations were: 0.5 mg/L (T2), 1 mg/L (T3), 2 mg/L (T4) and 4 mg/L (T5). Total Zn concentration in *Juncus* plants range from 36.2 mg/L to 264.59 mg/L for leaves and 53.20 mg/L for roots, while the concentration of Zn in *Iris* plants was in the ranged from 45.85 mg/L to 193.05 mg/L. There was a significant difference ($p < 0.05$) between the samples of *Juncus effusus* L. leaves and *Juncus effusus* L. roots as well as between the samples of *Juncus effusus* L. leaves and *Iris pseudacorus* L. roots. *Juncus effusus* L. plants showed significant plant growth under both, low and high Zn concentrations and a high uptake capacity of water. On the contrary, *Iris pseudacorus* L. plants were affected by Zn treatments, in both leaves and roots and at the end of the experiment, the decrease in the leaf biomass was noted. However, the overall results have confirmed that both plant species have the high accumulation capacity for Zn uptake and therefore, have a great potential for phytoremediation of polluted waters.

1 Introduction

Urban runoff has become an emergent ecotoxicity risk for the environment and recognized as a substantial source of pollutants to receiving waters (Davis et al. 2001; Müller et al. 2020). Runoff contains various substances, including heavy metals (HMs) like nickel (Ni), lead (Pb), chromium (Cr), cadmium (Cd), copper (Cu), zinc (Zn), mercury (Hg) and others (Reddy et al. 2014). Heavy metals (HMs) or "trace metals" are defined as a group of elements that are associated with environmental pollution (Prasad 2004). Although, some of the mentioned HMs like Zn represent the so-called micronutrients, which contribute to several cellular and physiological activities of plants and promote plant growth and development. Their presence in water in concentrations higher than necessary can have a toxic effect on flora, fauna, and humans (Hamzah et al. 2022). Sources of Zn in stormwater runoff are mostly derived from human activities, roads related to traffic, fluid leaks, brake and tire wear, paints, coatings, galvanized materials, etc. spills and leachates, various surface deposits and atmospheric deposits (Davis 2007; Müller et al. 2020). Exposure and risk of Zn toxicity on surface waters have been widely conducted, within a number of investigators that have found significant levels of Zn in runoff, in both residential and industrial areas (Davis et al. 2001; Mosley and Peake 2001; Joshi and Balasubramanian 2010; Li and Zuo 2013; Shajib et al. 2019). With increasing emissions, Zn might pose great ecological risk to aquatic organisms, which are sensitive to this metal, especially by the fact that the global production of Zn in aquatic environments is ranked in the top three compared to other metals (Fu et al. 2016).

Mentioned concerns over water contamination have prompted environmental monitoring and sustainable environmental management technologies. Phytoremediation is a cost effective, noninvasive, and publicly

acceptable method of removing environmental contaminants than most chemical and physical methods, since it uses vegetation to remediate contaminants from water, soil and sediments (Ruby and Appleton 2010; Yan et al. 2020). Five types of phytoremediation technologies have often been employed for decontamination: phytostabilization, phytodegradation, rhizofiltration, phytoextraction and phytovolatilization (Nedjimi, 2021). The use of metal-accumulating plants to clean soil and water contaminated with toxic metals is the most rapidly developing component of this environmentally friendly and cost-effective technology (Raskin et al. 1997). Along with constructed wetlands (CWs), bioretention systems (BRs) are known as one of the low cost alternatives to conventional cleanup techniques, positively improving remediation of contaminants, storing runoff water, reducing peak-flow, nutrient cycling, sequestering HMs and also providing supplementary benefits such as recreational facilities (Malaviya et al. 2019; Morash et al. 2019). Their performance and high effectiveness in removing pollutants found in urban runoff has been confirmed both by laboratory and field research (Davis et al. 2003; Hunt et al. 2006; Davis 2007; David et al. 2014) suggesting that BRs are effective for urban water quality improvement.

In BRs, plants are exposed to high levels of diverse pollutants. The selection of vegetation for phytoremediation of urban runoff with BRs is, therefore, primarily based on their ability to survive in potentially stressful growing conditions and their ability to absorb nutrients (nitrogen, phosphorus, etc.), HMs (Pb, Zn, Cu) and other pollutants (Hunt et al. 2015; Dagenais et al. 2018). A range of plant species has been investigated for phytoremediation, both soil and water (Zhang et al. 2012; Figueira et al. 2012; Ladislas et al. 2013; Pérez-Sirvent et al. 2017). For example, *Typha angustifolia*, *Colocasia esculenta* and *Cyperus malaccensis*, are known as plants for phytoremediation of Cd and Zn. All three species under hydroponic conditions for 15 days showed 100% of survival (Chayapan et al. 2015). *Phragmites australis* and *Typha latifolia* grown in the CWs that used to treat highway runoff in Ireland have accumulated a considerable amount of different HMs in sediment and plants, associated with road runoff, whereas, the highest removal efficiencies in the CW were for 86% for Zn (Gill et al. 2017). Species *Phragmites australis*, *Iris pseudacorus*, *Canna indica*, *Alissma plantago*, *Menianthes trifoliata* and *Iris sibirica* showed a potential for accumulation of Cr and Ni in their below-ground parts grown in floating treatment wetlands (Čule et al. 2021).The results clearly indicated that *Canna indica*, *Pontederia cordata* and *Thalia dealbata* are among species that were the most suitable species for CWs (Barco and Borin 2020). In a study with an artificial pond constructed with two topsoils collected from the Portman Bay (SE Spain), species *Juncus effusus* L. and *Iris pseudacorus* L. were highly recommended for remediation purposes.

Hydroponic experiments are one of the desirable alternative methods for examining the tolerance characteristics of plants to HMs on short timescales, providing HMs accumulation in plant species with greater precision than soil studies (Chayapan et al. 2015; Liu et al. 2018). In this study, we investigated the accumulation of Zn in two different plant species, namely *Juncus effusus* L. and *Iris pseudacorus* L. Plants were grown hydroponically in jars with Hoagland nutrient and different Zn concentrations, in order to provide a contribution in determining plants suitable for phytoremediation of urban runoff. In this study, Zn was selected for phytoremediation, based on previous research on the quality of the runoff samples at the analyzed locations in the city of Novi Sad, Serbia, which were singled out as locations

with the problem of urban flooding (Greksa et al. 2019). The results of this analysis demonstrated the presence of high Zn concentrations in urban runoff, which can be identified as potential threats to natural aquatic ecosystems. The aim of this work is therefore, to recommend plants as a part of BRs for phytoremediation of urban runoff as well as provide information of plant species which might be useful for phytoremediation of polluted waters in general.

2 Materials and methods

2.1 Experimental setup

For the purpose of the experiment, *Juncus effusus* L. and *Iris pseudacorus* L. plants were taken. *Juncus effusus* L. is a perennial in the rush family (*Juncaceae*). It is a common CWs species (macrophyte) with a broad, nearly global distribution and a wide ecological tolerance, which is evidenced by a high resistance to several toxic metal(loid)s (Müller et al. 2020). Likewise, *Iris pseudacorus* L. is an ornamental plant that can be grown for both urban landscaping and wastewater remediation. This plant tolerates polluted environments and is useful for water treatment purposes, grows best in very wet conditions and is also common in CWs, where it tolerates submersion, low pH, and anoxic soils and it also displays a high rate of biomass production (Caldelas et al. 2012; Gallego-Tévar 2022).

The plants for the experiment were obtained from a local nursery in the city of Novi Sad and they were around 12 to 24 months old. A total of 40 plants were selected, that is, 20 plants per species. Before setting up the experiment, the plants were removed from the pots, cleaned of soil and their roots were washed several times with distilled water, after which the plants were left to dry. Morphometric characteristics such as weight and height were measured for each plant after which the plants were placed in 720 ml glass jars with distilled water and Hoagland nutrient solution containing $Ca(NO_3)_2 \times 4H_2O$, 1250 mg L^{-1}; KNO_3, 410 mg L^{-1}; $NH_4H_2PO_4$, 280 mg L^{-1}; $MgCl_2 \times 6H_2O$, 624 mg L^{-1}; $FeSO_4 \times 7H_2O$, 60 mg L^{-1}; EDTA-Na^2, 80 mgL^{-1}; H_3BO_3, 6 mg L^{-1}; $MnCl_2 \times 4H_2O$, 4 mg L^{-1}; $ZnSO_4 \times 7H_2O$, 0.04 mg L^{-1} and $CuSO_4 \times 5H_2O$, 0.04 mg L^{-1} (Zhang et al. 2017). Four plants of each species were placed in jars containing only Hoagland nutrient without Zn concentration (herein referred as control jars) while other plants were placed in jars containing different Zn concentrations. There were four replicates (plants) for each concentration. According to the Serbian Regulation on limit values in water and deadlines for their achievement (**Official Gazette of the RS 50/2012**), the maximum allowed concentration of Zn is 2 mg/L. We adjusted these values to concentrations corresponding to lower and higher values than this maximum allowed concentration. Final concentrationc of Zn were as follows:

(a) Untreated control (Plants were only in a Hoagland nutrient solution) – treatment T1
(b) Plants in a Hoagland nutrient solution containing 0.5 mg/L of Zn in each jar - treatment T2
(c) Plants in Hoagland nutrient solution containing 1 mg/L of Zn in each jar - treatment T3
(d) Plants in Hoagland nutrient solution containing 2 mg/L of Zn in each jar - treatment T4
(e) Plants in Hoagland nutrient solution containing 4 mg/L of Zn in each jar - treatment T5

During the experiment, plants were placed in a simple, constructed growth chamber (Fig. 1), containing an LED bar and reflective Mylar film described at Žunić et al. (2022). A hydroponic LED light bar with 12:12 (day:night) photoperiod and light intensity of 200 µmol photons m^{-2} s^{-1} was used with dominant wave lengths (blue and red spectrum) at 460 nm and 630 nm, respectively. This spectrum delivers a uniform dispersion of the necessary wave lengths for propagation, as it accelerates plant cell multiplication. Inside the growing chamber, temperature was maintained at around 26 ± 2°C.

Plants were under growth chamber for 4 weeks, from November to December and they were regularly tested on their general condition - changes in the colour of the leaves or roots damages, changes in plant growth etc. Water volume in jars was kept at the same level by adding distilled water to the jars with noticable amount of evaporated water. During experiment setup, jars were filled two times with new Hoagland's solution and mentioned Zn concentrations, respectively, on the 14th and 28th days after experiment setup. With each addition of the new water solution and Zn, the pH and EC values of water samples were measured by a pH meter (EcoScan 6; Eutech, Instruments, Netherlands) and an EC meter (Testo 206 pH2; Testo, Germany) and also, the growth of plants in jars was recorded. The aim of adding new concentrations of Zn was to replicate the natural conditions in BRs after rain, namely it was hypothesized that runoff inflow will contain new concentrations of Zn directed to the plants after every storm event.

2.2 Plant preparation for Zn analysis

At the end of the experiment, all plants were taken out of the jars and left to dry naturally on for 20 days using filter paper. After drying, they were divided into root and shoot portions, and their dry weights were recorded, respectively before they were prepared for Zn analysis. Collected plant samples were subjected to chemical analysis. Dried plant samples (1g) were grounded in a blender to a powdery fraction and digested with a mixture of nitric (HNO_3) and perchloric acid ($HClO_4$) for the determination of Zn (nitric - perchloric acid wet digestion in an open vessel (Kalra 1998). The measuring was performed by atomic absorption spectrophotometer (Shimadzu 6300, Japan), using the flame technique. A total of 60 plant material samples was tested. Control jars for both *Juncus* and *Iris* plants were analyzed as one common sample (marked as $J1_{R,L}$-$J4_{R,L}$ and $IR1_{R,L}$–$IR4_{R,L}$). Accuracy was evaluated with the certified reference material LGC6175 (LGC, UK). Calibration curves for the determination of Zn were processed with different dilutions of the standard stock solutions conc. 1,000 mg/L (J.T: Baker, The Netherlands). The linear regression lines were obtained with $R \geq 0.95$.

2.3 Data analysis

The chemometric analysis included the application of Wilcoxon matched pairs test (WMPT), as a non-parametric statistical test. It is considered an alternative to *t*-test for dependent samples. Also, principal component analysis (PCA) was applied as a chemometric pattern recognition technique. The WMPT and PCA were carried out done by using statistics v.14.0.0.15 program (TIBCO Software Inc 2020). The chemometric analysis was carried in the natural (non-standardized) data, considering the fact that all the processed data was comparad and on the same scale.

In order to show metal translocation properties from roots to shoots, the translocation factor (TF) or mobilization ratio (Eq. 1) for Zn within plants was calculated. Translocation factor (TF) defines the link between the chemical composition of the leaves and their root content, which provides information on plant efficiency while transporting metals from root to leaves (Sharma et al. 2012) thus contributing to phytoextraction (Mayerová et al. 2017).

TF = Metal concentration in shoots / Metal concentration in roots (1)

3 Results

3.1 Accumulation of Zn in plants

Concentrations of Zn in *Iris* and *Juncus* plants in different treatments are presented in Table 1. As it can be noted, the maximum measured Zn concentration was found in *Juncus effusus* L. plant root (plant marked as $J19_r$), amounting 264.59 (mg/L). Similar high concentrations were also detected in *Juncus* plants marked as $J16_r$, $J18_r$ and $J19_r$. These *Juncus* plants were maintained in jars that were filled with solution amended with the highest Zn concentrations, respectively, 4 mg/L of Zn per water solution in the jar (T5). Average Zn concentration in *Juncus* plants was 76.12 mg/L for leaves and double higher for roots, which is 144.78 mg/L, while average Zn concentration for *Iris* roots was 134.53 mg/L. The lowest concentration of Zn was detected in control plants (for *Juncus* leaves J_{1L}-J_{4L}), and *Juncus* root of plant J_{7r}. For *Iris pseudacorus* L. roots, the plant marked as IR_7 was detected with the smallest Zn concentration (45.85 mg/L). Average accumulated Zn in *Iris* plants was 191.27 mg/L. In general, the concentration of Zn in rhizome and root tissues increased with increasing Zn concentrations in the growth media. As for the control jars, total Zn ranged from 36.2 mg/L for *Juncus* leaves and 110 mg/L for *Iris* roots.

Starting from the lowest to the highest, the concentration of Zn, in plants was in the following order:

Juncus effusus leaves < *Juncus effusus* roots < *Iris pseudacorus* roots

Table 1
Concentration of Zn in *Iris* and *Juncus* plants in different treatments (T1-T5)

Zn treatments in jars (mg/L)	Zn concentration in *Juncus efussus* L. leaves (mg/L)	Zn concentration in *Juncus efussus* L. roots (mg/L)	Zn concentration in *Iris pseudacorus* L. roots (mg/L)
Control jars (T1)	36.2	110.93	191.27
T2 (0.5 mg/L)	49.95	108.65	112
	59.76	60.20	102.5
	48.63	53.20	45.85
	77.36	89.60	103.57
T3 (1 mg/L)	56.37	81.21	102.07
	55.94	113.48	51.57
	57.58	150.92	177.82
	59.12	134.11	87.41
T4 (2 mg/L)	111.42	196.53	150.73
	68.18	156.71	186.96
	59.96	121.65	154.66
	111.83	205.49	186.47
T5 (4 mg/L)	68.26	118.07	147.11
	92.83	262.98	167.4
	113.17	264.59	193.05
	167.5	232.93	126.59
$\overline{x}$	**76.12**	**144.78**	**191.27**

3.2 Physicochemical properties of water solutions in jars

The pH of the nutrient solution was in the range from acidic to alkaline, ranging from 3.4 to 7.18 for *Iris* plants, with an average value of 6.35 during the first measurement (after 8 days of the experiment), from 7.63 to 9.12 with the average values of 8.22 for the second measurement (after 20 days of the experiment) and from 6.6 to 8.6 with average value of 7.65 (at the end of experiment). The lowest value of pH was measured in *Iris* plant marked as $IR_{18,}$ placed in a jar with a water solution with the highest Zn concentration. The values of pH for *Juncus* plants were in the range from 6.5 to 7.15, after first measurement, from 6.57 to 8.05 for second measurement and from 6.77 to 7.5 for third measurement.

Much greater variability in pH values was noted for *Iris* plants, where the values of pH ranged from 3.11 to 9.12. The electric conductivity (EC) of water varied from 870 to 1929 $\mu S/m^{-1}$ for *Iris pseudacorus* L. and from 744 to 1960 for *Juncus effusus* L. The maximum EC of water solution amounting 1960 $\mu S/cm^{-1}$ was noted for plants under the highest treatment of Zn, which is 4 mg/L (T5). This value was measured at the end of the experiment. The lowest EC values were also noted for *Juncus* plants that were in water solution with the highest treatment of Zn during the third measurement, obtained after three weeks from the beginning of the experiment.

The change of average conductivity and pH during the experiments measured at defined sampling terms are presented in Fig. 2.

3.3 General conditions of plants during the experiment-biomass production and plant growth

Parameters measured to assess the plant´s tolerance to different concentrations of Zn in different concentrations were vegetative growth and biomass. These parameters were recorded at the beginning, during, and at the end of the experiment. Leaf growth measurements of *Juncus efussus* L. and *Iris pseudacorus* L. were made by measuring the length of the leaves with a ruler from the top to the bottom of leaves. Roots measurements were done at the end of the experiment when plants were left to dry from the initial point of growth to the endpoint of the roots. *Juncus effusus* L. plants showed significant plant growth under both, low and high Zn concentrations. Leaf biomass was significantly bigger and plants were more robust, actively growing and without any damages. It was also noticed that the root system in water solution was developing new adventive roots soon after the beginning of the experiment (6 days). Moreover, *Juncus* roots demonstrated high uptake capacity of water, which was noted based on water infiltrated from the top of the jars containing *Juncus* plants. On the contrary, *Iris pseudacorus* L. plants were affected by Zn treatments, in both leaves and roots. The leaves have become dry and yellow and the roots had brown spots on the roots, mucus appear on the surface of the jar after 7 days after placing the plants in jars and there was observable algal growth in all treatments. At the end of the experiment, the decrease in the leaf biomass was noted, so the leaves were not used for the analysis of Zn. However, the presence of new, developing roots was also noted during the experiment. Relative to the initial weight, plants of *Juncus effusus* L. have gained weight from 8.96–62.78% with an average biomass increase amounting to 32.01% (Table 2). For some *Iris pseudacorus* L. plants, there were reductions in plant weight, ranging from 2.98–23.15% compared to the initial one, due to loss of leaf mass. The development of new adventitious roots has contributed to a biomass increase of *Iris pseudacorus* L. plants resulting in an average weight increase of 63.88%. Compared with the control treatments, *Juncus* plants that were grown in solution with the increased content of Zn grew up to 53.82% in average, that is, the highest obtained biomass calculated for a plant that grew up in nutrient solution with Zn concentrations of 4 mg/L (plant marked as J_{17}).

Table 2
The summary statistics (ranges, means, standard deviations) differences between the observed morphometric parameters among *Juncus* and *Iris* plants before and at the end of experiment

	Juncus effusus L.				*Iris pseudacorus* L.			
Value	**Weight[a] (g)**	**Height (cm)[a]**	**Weight (g)[b]**	**Height (cm)[b]**	**Weight[a] (g)**	**Height (cm)[a]**	**Weight (g)[b]**	**Height (cm)[b]**
Min	25	40	39.3	59	4.7	9.5	7.8	0
Max	103.6	83	120	104	74.6	3.6	96.5	6
Mean ± SD	65.95 ± 22.21	55.65 ± 8.60	83.98 ± 22.03	80.65 ± 13.35	24.40 ± 19.13	18.88 ± 9.18	32.43 ± 23.91	0.75 ± 1.76
a-before the experiment; b-after the experiment								

3.4 Translocation factor (TF) of Zn

Due to decreased leaf biomass of *Iris pseudacorus* L. at the end of the experiment, the translocation factor (TF) was calculated only for *Juncus effusus* L. The levels of translocation factor were TF < 1.0, ranging from 0.33 to 0.99 (Fig. 3). The average calculated TF value was 0.60. This finding suggest that Zn can be easily accumulated by *Juncus* plants and transported to their aboveground parts respectively, that *Juncus effusus* L. meets the requirements for phytostabilization of urban ecosystem soil contamination with HMs (Yakovyshyna 2021).

3.5 Results of chemometric analysis

The results of the WMPT are presented in Table 3. The data indicate that in terms of Zn concentration there is a significant difference between the samples of *Juncus effusus* L. leaves and *Juncus effusus* L. roots as well as between the samples of *Juncus effusus* L. leaves and *Iris pseudacorus* L. roots. These tests are significant at $p < 0.05$. However, there is no significant difference between the samples of *Juncus effusus* L. roots and *Iris pseudacorus* L. roots.

Table 3
The results of the WMPT

Zn concentration in:	Wilcoxon Matched Pairs Test - Marked tests* are significant at $p < 0.05$			
	Valid N	T	Z	*p*-value
Juncus effusus L. leaves & *Juncus effusus* L. roots*	17	0	3.62	0.00029
Juncus effusus L. leaves & *Iris pseudacorus* L. roots*	17	9	3.20	0.00140
Juncus effusus L. roots & *Iris pseudacorus* L. roots	17	64	0.59	0.55403

In Fig. 4, there is a box-whisker plot of the concentration of Zn determined in twenty samples of each plant. The box-whisker plot presents the mean value, deviations (SD) limit and 1.96 SD limit (the whisker).

As it can be seen, the lowest mean and standard deviation are observed in *Juncus effusus* L. leaves. Higher Zn concentrations are present in *Juncus effusus* L. roots and *Iris pseudacorus* L. roots. The high concentration and standard deviation range are noticed for *Juncus effusus* L. roots.

The PCA was carried out on the data that include the conductivity and pH values, separately. The results of the PCA of conductivity data are given in Table 4. The graphical representation of the results is given in Fig. 5. The 4-PC model was obtained so that first two PC cover 76.6% of total variance. These PCs have the Eigenvalues higher than 1, therefore the 2-PC model was taken into consideration.

Table 4
The PCA model based on conductivity data of the samples

PC	Eigenvalue	% of Total variance	Cumulative Eigenvalue	Cumulative %
1	1.67	41.68	1.67	41.68
2	1.40	34.91	3.06	76.60
3	0.70	17.41	3.76	94.00
4	0.24	6.00	4.00	100.00

In Fig. 5, there are scores and loadings plots of the 2-PC model. The score plot indicates that the *Iris pseudacorus* L. samples (IR) and *Juncus effusus* L. samples (J) are clearly separated by the PC2 = 0 line. The J samples are described by the positive PC2 values, while the IR samples have negative PC2 values. The loadings plot indicates that this distribution is mostly induced by ST1 and ST4 measurements (first

and the last day of the measurement). Along the PC1 axis there is no separation of the samples. Therefore, the IR and J samples mostly differ on the first and the last day of the measurements.

The PCA based on pH values resulted in 4-PC model in which first two PCs cover 83.27% of total variance and have the Eigenvalues higher than 1 (Table 5).

Table 5
The PCA model based on pH data of the samples

PC	Eigenvalue	% of Total variance	Cumulative Eigenvalue	Cumulative %
1	1.82	45.53	1.82	45.53
2	1.51	37.74	3.33	83.27
3	0.53	13.33	3.86	96.59
4	0.14	3.41	4.00	100.00

The score plot, presented in Fig. 6, indicates the significant separation of the J sample along PC2 axis. All the J samples have positive PC2 values, including two outliers from IR samples (IR18 and IR19). This separation is achieved based on ST2 and ST3 measurements since those two variables have the highest influence on the distribution along PC2 axis, as it can be seen on the loadings plot in Fig. 6. Therefore, the pH could be a discriminating factor for the IR and J samples measured on ST2 and ST3. The IR and J samples cannot be separated along the PC1 axis, except the IR17, IR18 and IR19 samples that could be considered to be the outliers since they are placed further from the rest of the samples (the lowest pH).

4 Discussion

4.1 Effects of Zn on *Juncus effusus* L. and *Iris pseudacorus* L. survival and growth parameters

According to Balafrej et al. (2020) Zn's toxic effects depend on its external bioavailable concentration, exposure time, plant genotype, and step of plant development, whereas Zn toxicity increases with its bioavailability. The most obvious symptoms of Zn toxicity reported in plants are the inhibition of growth, chlorosis of young leaves, mainly in new leaves, depressed plant growth, curled leaf tips, reduced photosynthetic rate, nutrient imbalance and membrane degradation (Kabata-Pendias 2000; Balafrej et al. 2020; Hamzah et al. 2022). Moreover, the phenomenon of rotted roots is usually taken as one of the indicators of the growth status of plants under different HMs concentration (Wu et al. 2022). Morphological and growth indicators including root length and shoot height are the most common indicators to describe plant tolerance (Liu et al. 2018). In this study, *Juncus effusus* L. plants did not show any visible Zn toxicity symptoms since all plants survived, even at the high Zn concentrations (Fig. 7). All plants reached high biomass, on average; they grew up from 18–44 mm after 40 days of the experiment. A significant increase in the weight of *Juncus* plants was also noted. Relative to the initial weight, at the end of the experiment, average plant weight increased from 7.79–62.78%. Based on root

growth of *Juncus effusus* L. in the present study, it can be concluded that from the aspect of root development, Zn had also a positive effect on *Juncus effuses* L. roots. For *Iris* plants, the increasement of weight varied from 3.07–57.14%. It was noted that the highest recorded percentage of plant weight of both *Iris* and *Juncus* plants, was calculated for the plants that were grown in jars with the Zn concentration of 4 mg/L (plants marked as J17 and IR17). These results support the statements that Zn was involved in plant growth under water stress and that moderate Zn improved plant root biomass, promoted root growth; maintained root and leaf anatomy structural integrity of maize (*Zea mays* L.) in the nutrient solution (Zhang et al. 2021) and gave high shoot biomass for species A. *halimus* and M. *lupulina* grown in Hoagland's solutions for both low and high levels of concentrations of Pb and Zn (5, 10, 25, 50, and 100 mg/L) (Amer et al. 2013).

Similar responses of *Juncus* and *Iris* plants' to different Zn exposure were also reported by several authors. For the populations of *Juncus*, *Juncus effuses* L. and *Juncus articulatus* L., studies reported the tolerance on concentrations of Zn of up to 100 µmol L^{-1}, but growth was inhibited at the higher treatment levels (Matthews et al. 2004). On the contrary, Mateos-Naranjo et al. (2014) showed that Zn excess (concentrations up to 100 $mmol^{-1}$) did not cause toxicity symptoms such as chlorosis, necrosis or affected photosynthesis suggesting that *Juncus acutus* L. is hypertolerant species to Zn. The growth of *Juncus effusus* L. was in very good condition due to the high number of new shoots after an experimental hydroponic study of phytoremediation conducted by Wu et al. (2022). In the present study, there were notable morphological changes trough biomass reductions after 7 days of treatment at all Zn concentrations for the *Iris* plants (Fig. 8). These findings are similar to the results obtained by Gariepy E R (2020) for species *Acorus calamus* L. which is comparable to species *Iris pseudacorus* L. with respect to its morphology and lifestyle. After 30 days of growth in nutrient solution, the plants showed yellowing of leaves in all treatments, which could have been due to nutrient overloading or natural senescence patterns. Furthermore, this could be also explained with a natural cycle in which leaves would grow, become yellow, and then the plant would produce new shoots to replace the old, which was also observed in *Acorus* plants in the mentioned study by Gariepy E R (2020), because our experiment was set in the fall (November-December). The outer leaves of *Iris pseudacorus* L. showed damage after 1–2 weeks under anoxia, with the tips turning soft and brown and after 28 d with the total soluble carbohydrate (TSC) content of leaves reduced by 60%, which was 45% higher than for the species *Acorus calamus* L. (Schlüter and Crawford 2001). In addition, *Iris wilsonii* plants decreased after 60 days of an experimental hydroponic study under acid mine drainage which was indicated based on the decay of the roots of some plants (Wu et al., 2022). The concentration of Ni markedly caused serious toxicity symptoms of *Iris sibirica* L. Both the root and shoot biomass appeared erratic with the increasing concentrations of Ni and eventually, they died (Wan et al. 2021).

On the contrary, Caldelas et al. (2012) reported high rate of surviving and continued growth of *Iris pseudacorus* L. plants in nutritive solution with $ZnCl_2$, along with remarkable accumulation of Zn in roots and leaves. For other HMs, like cadmium (Cd) *Iris pseudacorus* L. showed to be tolerant with medium (2 mg/L) and high (4 mg/L) Cd dose treatment (Mohsin et al. 2023). Similar results were obtained by

Branković et al. (2015) in floating wetland. *Phragmites australis* and *Iris pseudacorus* L. displayed 100% survival with no toxicity symptoms such as withering, necrosis or chlorosis according to visual observations throughout the growth period. *Iris pseudacorus* L. was also associated with the high efficiency in the removal of silver nanoparticles (approximately 96%) in a vertical flow wetland system and the reduction of COD compounds (83%), total N (61%), ammonia (42%), and total P (70%) (Huang et al. 2019).

4.2 Changes in physicochemical properties of water solutions in jars

The water quality changes, plant growth, and microbial reproduction can be affected by the pH of the water (Aoyagi et al. 2017). Increased mobility of Zn and Mn is most effective with pH = 6 (Parzych et al. 2016). According to Kabata-Pendias (2000) at a high pH value (7.2–7.8), Zn uptake by barley is also closely correlated with its content in soils and therefore, an increase in pH is important for the removal of HMs from the water since the acidity of the solution allows the metals to be transported in the most soluble form. It has been also reported that Zn is more bioavailable at an acid pH than at an alkaline pH (Imran et al., 2014). Thus, pH goes down due to the release of organic acids and H + which facilitates Zn solublization and uptake by plants. Our study revealed that the highest variation in the pH values of the solution for *Iris pseudacorus* L. was noted after second treatment with Zn, namely 10 days after replacing the nutrient solution + Zn with new solution and the same Zn concentrations. For example, the pH of *Iris pseudacorus* L. plants grown in T5 Zn treatment (4 mg/L) increased from 3.4 to 8.05 and from 3.69 to 8.6. Until the end of the experiment, the pH of water decreased the most since the beginning of the experiment for plants under all treatments. For the species *Juncus effuses* L. the same trend of the pH changes was noted. In general, the average pH values of water for *Iris* plants were higher than for the *Juncus* plants. This results are similar to those published by Wu et al. (2022). The pH of the experimental water increased from 4 to more than 7 after 20 days of growing the plants in water, which means that the water changed from acidic to weakly alkaline, and the pH of the water was stabilized at 7–8 in the later stages of the experiment (Wu et al. 2022). The increasement of pH of water solution might be caused by the presence of the amino functional groups in the root of aquatic plants like *Acorus calamus* L. and *Juncus effusus* L., which can form complexes with the heavy metal ions of Cu, resulting in an increase of pH of the solution (Lu et al. 2018).

Optimum growing conditions for hydroponic plants are also optimum for the growth of algae (Coosemans 1993). The rapid increase in algal biomass was linked with an increase in pH for all treatments, affecting EC and in increasing the pH of the water solution. Like in our study, there was also observable algal growth in all hydroponically grown *Iris* plant treatments with no apparent preference for any specific Zn concentration (West et al. 2017; Gariepy E R 2020). The high variability in pH and EC can be explained by nutrient uptake by the plants (Lv et al. 2016). It was reported that *Iris* and *Phragmites* plants reduced the ions in the water column to the greatest degree, possibly due to enhanced direct nutrient uptake. Furthermore, nutrient solution at a constant EC can increase the EC in the root environment in nutrient solution (Schwarz and Grosch 2003).

The electrical conductivity (EC) is an index of salt concentration and an indicator of its salt content, ionic content, impurity content (Ding et al. 2018; Wu et al. 2022). A higher EC means higher salt concentration, while a lower EC means a lower salt concentration. The mean nutrient solution EC in our study ranged between 1087 μS/ cm^{-1} and 1569 μS/ cm^{-1} for *Juncus* plants and between 1121 μS/ cm^{-1} and 1218 μS/ cm^{-1} for *Iris* plants. The lowest single values of EC were recorded for *Juncus* plants, with the lowest value of 744 μS/ cm^{-1} obtained from *Juncus* plant grown in T5 Zn treatment (4 mg/L), while the lowest average EC values were measured for *Iris* plants, both during third measurement. Very high EC values were also noted at the end of the experiment, for plants under T5 treatment, both for *Iris* and *Juncus* plants. Changes in the conductivity of water might be caused with the increasement of the Zn concentrations in solution, also accumulated in plants. The EC of the water samples of the remaining four plants increased to different degrees, which was mainly due to some root rot of these four plants in the late stage of the experiment, resulting in the dissolution and release of internal plant components (Wu et al. 2022). The presence of high EC levels can negatively affect biomass of plants (Salimi et al. 2012). On the contrary, the salinity was found to primarily enhance the mobility of the Cd and its uptake by *Lemna minor* (Laing et al. 2008).

4.3 Uptake and accumulation of Zn by plants

Average concentrations of Zn were higher in the roots of *Iris pseudacorus* L. plants than in the roots of *Juncus effusus* L. which indicates that *Iris pseudacorus* L. can accumulate higher concentrations of Zn than the *Juncus effusus* L. However, the overall results showed that both species significantly accumulated dissolved Zn. Zinc concentrations were mostly distributed in root tissues of *Juncus* and *Iris* plants, suggesting that the Zn distribution in plant parts usually follow this pattern: roots > foliage > branch > trunk (Hong et al. 2005), respectively concentration sequence of the HMs are mostly as follows: rhizosphere soils > roots > shoots (Dołęgowska et al. 2022). The biologically active, high surface area formed by plant roots that are rapidly growing, can be extremely active in absorbing pollutants from water (Raskin et al. 1997). These features make *Juncus* plant an ideal plant for rhizofiltration as a mechanism to remove pollutant from water whereas, the most of the pollutants are retained in the root system. Metals that are commonly present in roots are the main target of rhizofiltration technique including Zn. In the indigenous zinc smelting area in the northwest region of Guizhou Province, China, authors Peng et al. (2018) pointed out that *Juncus effusus* L. could be used for phytostabilization or as a pioneer plant for phytoremediation of potentially toxic HMs. In deciding whether an accumulator can be used for phytoextraction or phytostabilization purposes, the heavy-metal accumulation in roots or aboveground shoots is a major factor (Liu et al. 2018). Phytoremediation potential of the plants is generally evaluated by determining Bioconcentration Factor (BCF) (Kafle et al. 2022). Bioconcentration values > 2 are considered to be higher values. Plants can be used as phytostabilizers if they have bioconcentration factors > 1. According to Takarina and Pin (2017) and Sharma et al. (2022), plants with BCF > 1 and TF < 1 are reported to demonstrate the possibility of phytostabilization. BCF value higher than 1 indicates that the plant is a hyperaccumulator for the special pollutant. Based on so far studies, *Juncus* plants demonstrated very high BCF values. A study conducted by Ladislas et al. (2013) showed very high concentrations of Zn in *Juncus* plants collected from a detention pond receiving highway

runoff that was also confirmed with very high bioconcentration factor (BCF = 4.8) by *Juncus* sp. Our study also revealed that based on the calculated TF values, *Juncus* can be used for remediation.

Our study did not examine the concentration of Zn in *Iris pseudacorus* L. leaves, but as previously reported by several authors (e.g. Schlüter and Crawford 2001; Galletti et al. 2010; Branković et al. 2015; Vymazal 2016; Mohsin et al. 2023) the roots/rhizomes were the primary organs for HMs accumulation and concentrations of HMs in wetland plants like *Iris pseudacorus* L. compared to stems, leaves, and flowers, so we hypothesized that the concentration of Zn would be much lower in *Iris* leaves if they were included in the analysis. According to Hamzah Saleem et al. (2022), the majority of Zn in plants is found in its roots, whereas a small percentage is transported to the leaves and stems and even small lateral roots can retain more Zn than other vegetation components. For example, the content of HMs in the roots was two- to five-fold higher than in the shoots of the *Azolla* species (Sela et al. 1989). Under both Zn stress and combined lead–zinc stress, the root was the main organ that accumulated heavy metals in *Sasa argenteostriata* (Regel) E.G. Camus. The presence of Zn additionally, promoted the absorption of lead (Pb) by the root (Cardwell et al. 2002). Moreover, roots are relatively sensitive to oxygen deprivation, and the survival capacity of most tolerant species resides in the shoots or rhizomes (Schlüter and Crawford, 2001). However, as reported by Gupta et al. (2012) Zn is among easily biologically available metals in plants respectively, soluble forms of Zn are readily available to plants.

5 Conclusion

The aim of this study was to investigate the Zn tolerance and accumulation characteristics of the selected plant species, *Juncus effusus* L. and *Iris pseudacorus* L. The results confirmed so far published facts that high Zn concentration did not implicate phytotoxic manifestation on growth performances and vitality of *Juncus* plants. All plants reached high biomass and the development of new roots was noted, while for the *Iris* plants, despite to the high Zn accumulation in roots, during the experiment there were some changes in morphology. In general, the concentration of Zn in rhizome and root tissues increased with increasing Zn concentrations in the water solution. Uptake and translocation of Zn values confirmed the theory that *Juncus* and *Iris* plants are a good candidate for water remediation. The highest concentration of Zn was accumulated in roots, suggesting that this plants can be used for phytofiltration by using hydroponically cultured plants to absorb or adsorb heavy metal ions from water.

Treatment levels of Zn used in our experiment were above the limit values in water and deadlines for their achievement according to the Serbian Regulation on limit values, so this study showed that both *Juncus* and *Iris* plants have a potential to effectively remove high concentrations of Zn from contaminated water (2 mg/L, 4 mg/L) respectively, to improve the quality of urban runoff at the previously analyzed locations and therefore, prevent or mitigate further HMs contamination. Although, *Iris* plants showed biomass reduction and slow growth rate, which can be a sign of limited efficiency for remediation of Zn. However, one of the key challenges in selecting species for remediation lies in incorporating different species based solely on laboratory testing. While laboratory tests can provide valuable initial insights, further

analysis of the adaptability of these plants to a variety of environmental conditions is essential. Therefore, conducting comprehensive field studies becomes crucial to enhance the selection process.

Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Authors' contributions

All authors contributed to the study conception and design.

Conceptualization: Amela Greksa; Methodology: setting up the experiment, laboratory analysis and investigation were performed by Amela Greksa, Vladimira Žunić and Ivana Mihajlović; Chemical analysis were performed by Mirjana Vijuk and Mirna Štrbac, Data analysis were performed by Boško Blagojević, Sanja Podunavac-Kuzmanović and Strahinja Kovačević. The first draft of the manuscript was written by Amela Greksa and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics declarations

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate

The authors confirm that the final version of the manuscript has been reviewed, approved, and consented to for publication to Environmental Processes Journal by all authors.

Consent for publication

This manuscript has not been published elsewhere and is not under consideration by another journal.

Conflict of interest

The authors declare no competing interests.

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Amer N, Chami Z A, Bitar L A, Mondelli D, Dumontet S (2013) Evaluation of Atriplex halimus, Medicago lupulina and Portulaca oleracea for phytoremediation of Ni, Pb, and Zn. Int. J Phytoremediation 15(5) 498-512. https://doi.org/10.1080/15226514.2012.716102
2. Aoyagi T, Hamai T, Hori T, Sato Y, Kobayashi M, Sato Y, Inaba T, Ogata A, Habe H, Sakata T (2017) Hydraulic retention time and pH affect the performance and microbial communities of passive bioreactors for treatment of acid mine drainage. Amb Express 7 1-11. https://doi.org/10.1186/s13568-017-0440-z
3. Balafrej H, Bogusz D, Triqui ZA, Guedira A, Bendaou N, Smouni A, Fahr M (2020) Zinc hyperaccumulation in plants: A review. Plants 9(5) 562. https://doi.org/10.3390/plants9050562
4. Barco A, Borin M (2020) Ornamental plants for floating treatment wetlands: Preliminary results. Ital J Agron 15(2) 109-120. https://doi.org/10.4081/ija.2020.1602
5. Branković S, Glišić R, Topuzović M, Marin M (2015) Uptake of seven metals by two macrophytes species: potential for phytoaccumulation and phytoremediation. Chem Ecol, 31(7), 583-593. https://doi.org/10.1080/02757540.2015.1077812
6. Caldelas C, Araus J L, Febrero A, Bort J (2012) Accumulation and toxic effects of chromium and zinc in Iris pseudacorus L. Acta Physiol Plant 34 1217-1228. https://doi.org/10.1007/s11738-012-0956-4.
7. Cardwell A J, Hawker D W, Greenway M (2002) Metal accumulation in aquatic macrophytes from southeast Queensland, Australia. Chemosphere 48(7) 653-663. https://doi.org/10.1016/S0045-6535(02)00164-9
8. Chayapan P, Kruatrachue M, Meetam M, Pokethitiyook P (2015) Phytoremediation potential of Cd and Zn by wetland plants, L. Schott., Lam., and Typha angustifolia L. grown in hydroponics. J

Environ Biol 36(5) 1179-1183.

9. Coosemans J (1993, September). Control of algae in hydroponic systems. In IV International Symposium on Soil and Substrate Infestation and Disinfestation 382, pp. 263-268.
10. Čule N, Lucic A, Nesic M, Veselinovic M, Mitrovic S, Sredojevic Z, Brasanac-Bosanac L. (2021) Accumulation of chromium and nickel by Canna indica and decorative macrophytes grown in floating treatment wetland. Fresenius Environ Bull 30(6b) 7881-7890.
11. Dagenais D, Brisson J, Fletcher T D (2018) The role of plants in bioretention systems; does the science underpin current guidance? Ecol Eng 120 532-545. https://doi.org/10.1016/j.ecoleng.2018.07.007
12. David N, Leatherbarrow J E, Yee D, McKee L J (2014) Removal efficiencies of a bioretention system for trace metals, PCBs, PAHs, and dioxins in a semiarid environment. J Environ Eng 141(6) 04014092. https://doi.org/10.1061/(ASCE)EE.1943-7870.000092
13. Davis A P, Shokouhian M, Ni S (2001) Loading estimates of lead, copper, cadmium, and zinc in urban runoff from specific sources. Chemosphere 44(5) 997-1009. https://doi.org/10.1016/S0045-6535(00)00561-0
14. Davis A P, Shokouhian M, Sharma H, Minami C, Winogradoff D (2003) Water quality improvement through bioretention: Lead, copper, and zinc removal. Water Environ Res 75(1) 73-82. doi: 10.2175/106143003x140854
15. Davis A P (2007) Field performance of bioretention: Water quality. Environ Eng Sci 24(8) 1048-1064. https://doi.org/10.1089/ees.2006.0190
16. Ding X, Jiang Y, Zhao H, Guo D, He L, Liu F, et al (2018) Electrical conductivity of nutrient solution influenced photosynthesis, quality, and antioxidant enzyme activity of pakchoi (Brassica campestris L. ssp. Chinensis) in a hydroponic system. PloS one, 13(8), e0202090. https://doi.org/10.1371/journal.pone.0202090
17. Dołęgowska S, Gałuszka A, Migaszewski Z M, Krzciuk K (2022) Bioavailability of selected trace and rare earth elements to Juncus effusus L.: the potential role of de-icing chlorides in the roadside environment. Plant Soil 472(1-2) 641-658. https://doi.org/10.1007/s11104-021-05278-0
18. Figueira E, Freitas R, Pereira E, Duarte A (2012) Mercury uptake and allocation in Juncus maritimus: implications for phytoremediation and restoration of a mercury contaminated salt marsh. J Environ Monit 14(8) 2181-2188. doi: 10.1039/c2em30076a
19. Fu Z, Wu F, Chen L et al (2016) Copper and zinc, but not other priority toxic metals, pose risks to native aquatic species in a large urban lake in Eastern China. Environ Pollut 219, 1069-1076. https://doi.org/10.1016/j.envpol.2016.09.007
20. Galletti A, Verlicchi P, Ranieri E (2010) Removal and accumulation of Cu, Ni and Zn in horizontal subsurface flow constructed wetlands: Contribution of vegetation and filling medium. Science of the Total Environment, 408(21) 5097-5105. https://doi.org/10.1016/j.scitotenv.2010.07.045
21. Gallego-Tévar B, Grewell B J, Whitcraft C R, Futrell J C, Bárcenas-Moreno G, Castillo J M (2022) Contrasted impacts of Yellow Flag Iris (Iris pseudacorus) on plant diversity in tidal wetlands within

Its native and invaded distribution ranges. Diversity 14(5) 326. https://doi.org/10.3390/d14050326

22. Gariepy E R (2020) Phytoremediation of vanadium and nickel from wastewater using Acorus calamus. Dissertation, University of Alberta. https://doi.org/10.7939/r3-0e26-jt86
23. Gill L W, Ring P, Casey B, Higgins N M, Johnston P M (2017) Long term heavy metal removal by a constructed wetland treating rainfall runoff from a motorway. Sci Total Environ 601, 32-44. https://doi.org/10.1016/j.scitotenv.2017.05.182
24. Greksa A, Novaković M, Petrović M, Mihajlović I, Sekulić M, Grabić J (2019) Assessment of heavy metals (Cu, Pb, Zn and Ni) in the stormwater runoff from the main streets of the city of Novi Sad. In : 12th international scientific conference Contemporary Materials 2019; 1–3 September 2019; Banja Luka, Republic of Srpska, Bosnia and Herzegovina. p. 41–50.
25. Gupta D, Singh L K, Gupta A D, Babu V (2012) Phytoremediation: An efficient approach for bioremediation of organic and metallic ions pollutants. Bioremediation and Sustainability: Research and Applications (eds R. Mohee and A. Mudhoo). Hoboken, NJ: John Wiley & Sons, Inc, pp 213-240.
26. Hamzah Saleem, M, Usman K, Rizwan M, Al Jabri H, Alsafran M (2022) Functions and strategies for enhancing zinc availability in plants for sustainable agriculture. Front. Plant Sci 13 1033092. https://doi.org/10.3389/fpls.2022.1033092
27. Hong D (2005) Metal (Pb, Zn, Cu, Cd, Fe) uptake, tolerance and radial oxygen loss in typical wetland plants. Dissertation, Hong Kong Baptist University.
28. Hunt W F, Jarrett A R, Smith, J T, Sharkey L J (2006) Evaluating bioretention hydrology and nutrient removal at three field sites in North Carolina. J Irrig Drain Eng 132(6) 600-608. https://doi.org/10.1061/(ASCE)0733-9437(2006)132:6(600)
29. Hunt W F, Lord B, Loh B, Sia A (2015). Plant selection for bioretention systems and stormwater treatment practices. Springer Nature, p. 59. https://doi.org/10.1007/978-981-287-245-6
30. Huang J, Yan C, Liu J et al (2019) Feasibility Study of Vertical Flow Constructed Wetland for Tertiary Treatment of Nanosilver Wastewater and Temporal-Spatial Distribution of Pollutants and Microbial Community. J Environ Manag 245, 28–36. https://doi.org/10.1016/j.jenvman.2019.04.128
31. Imran M, Arshad M, Khalid A, Kanwal S, Crowley D E (2014) Perspectives of rhizosphere microflora for improving Zn bioavailability and acquisition by higher plants. Int J Agric Biol 16(3).
32. Joshi U M, Balasubramanian R (2010). Characteristics and environmental mobility of trace elements in urban runoff. Chemosphere, 80(3), 310-318. https://doi.org/10.1016/j.chemosphere.2010.03.059
33. Kabata-Pendias A (2000) Trace elements in soils and plants. 3rd edn, CRC press
34. Kafle A, Timilsina A, Gautam A, Adhikari K, Bhattarai A, Aryal N (2022) Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. Environ Adv 100203. https://doi.org/10.1016/j.envadv.2022.100203
35. Kalra Y (1998) Handbook of reference methods for plant analysis. Boca Raton CRC Press.
36. Ladislas S, Gerente C, Chazarenc F, Brisson J, Andres Y (2013) Performances of two macrophytes species in floating treatment wetlands for cadmium, nickel, and zinc removal from urban stormwater

runoff. Water Air Soil Pollut 224, 1-10. https://doi.org/10.1007/s11270-012-1408-x

37. Laing G, De Vos R, Vandecasteele B, Lesage E, Tack F M, Verloo M G (2008) Effect of salinity on heavy metal mobility and availability in intertidal sediments of the Scheldt estuary. Estuar Coast Shelf Sci 77(4) 589-602. https://doi.org/10.1016/j.ecss.2007.10.017
38. Li H, Zuo X J (2013) Speciation and size distribution of copper and zinc in urban road runoff. Bull Environ Contam Toxicol 90, 471-476. https://doi.org/10.1007/s00128-012-0953-8.
39. Liu J, Xin X, Zhou Q (2018) Phytoremediation of contaminated soils using ornamental plants. Environ Rev 26(1) 43-54. https://doi.org/10.1139/er-2017-0022
40. Lu D, Huang Q, Deng C, Zheng Y (2018). Phytoremediation of Copper Pollution by Eight Aquatic Plants. Pol J Environ 27(1). https://doi.org/10.1139/er-2017-0022
41. Lv T, Zhang Y, Casas M E et al (2016) Phytoremediation of imazalil and tebuconazole by four emergent wetland plant species in hydroponic medium. Chemosphere, 148, 459-466. https://doi.org/10.1016/j.chemosphere.2016.01.064
42. Malaviya P, Sharma R, Sharma P K (2019) Rain gardens as stormwater management tool. In: Sustainable green technologies for environmental management. Springer, Singapore pp 141-166. https://doi.org/10.1007/978-981-13-2772-8_7
43. Matthews D J, Moran B M, Otte M L (2004) Zinc tolerance, uptake, and accumulation in the wetland plants Eriophorum angustifolium, Juncus effusus, and Juncus articulatus. Wetlands, 24(4), 859-869. https://doi.org/10.1672/0277-5212(2004)024[0859:ZTUAAI]2.0.CO;2
44. Mateos-Naranjo E, Castellanos E M, Perez-Martin A (2014). Zinc tolerance and accumulation in the halophytic species Juncus acutus. Environ Exp Bot, 100, 114-121. https://doi.org/10.1016/j.envexpbot.2013.12.023
45. Mayerová M, Petrová Š, Madaras M, Lipavský J, Šimon T, Vaněk T (2017) Non-enhanced phytoextraction of cadmium, zinc, and lead by high-yielding crops. Environ Sci Pollut Res 24 14706-14716. https://doi.org/10.1007/s11356-017-9051-0.
46. Morash J, Wright A, LeBleu C, Meder A, Kessler R, Brantley E, Howe J (2019) Increasing sustainability of residential areas using rain gardens to improve pollutant capture, biodiversity and ecosystem resilience. Sustainability, 11(12), 3269. https://doi.org/10.3390/su11123269.
47. Mohsin M, Nawrot N, Wojciechowska E, Kuittinen S, Szczepańska K, Dembska G, Pappinen, A (2023) Cadmium accumulation by Phragmites australis and Iris pseudacorus from stormwater in floating treatment wetlands microcosms: Insights into plant tolerance and utility for phytoremediation. J Environ Manage 331 117339. https://doi.org/10.1016/j.jenvman.2023.117339
48. Mosley L M, Peake B M (2001) Partitioning of metals (Fe, Pb, Cu, Zn) in urban run-off from the Kaikorai Valley, Dunedin, New Zealand. N Z J Mar Freshw Res 35(3) 615-624.
49. Müller A, Österlund H, Marsalek J, Viklander M (2020) The pollution conveyed by urban runoff: A review of sources. Sci Total Environ 709 136125. https://doi.org/10.1016/j.scitotenv.2019.136125
50. Nedjimi B (2021). Phytoremediation: a sustainable environmental technology for heavy metals decontamination. SN Appl Sci 3(3), 286. https://doi.org/10.1007/s42452-021-04301-4

51. Pérez-Sirvent C, Hernández-Pérez C, Martínez-Sánchez M J, García-Lorenzo M L, Bech, J (2017). Metal uptake by wetland plants: implications for phytoremediation and restoration. J. Soils Sediments, 17, 1384-1393. https://doi.org/10.1007/s11368-016-1520-4
52. Prasad M N V (Ed.) (2004) Heavy metal stress in plants: from biomolecules to ecosystems, 2nd edn. Springer Science & Business Media.
53. Parzych A, Cymer M, Macheta K (2016) Leaves and roots of Typha latifolia L. and Iris pseudacorus L. as bioindicators of contamination of bottom sediments by heavy metals. Limnol Rev, 16(2), 77-83. https://doi.org/10.1515/limre-2016-0008
54. Peng Y, Chen J, Wei H, Li S, Jin T, Yang R (2018) Distribution and transfer of potentially toxic metal (loid) s in Juncus effusus from the indigenous zinc smelting area, northwest region of Guizhou Province, China. Ecotoxicol Environ Saf 152, 24-32. https://doi.org/10.1016/j.ecoenv.2018.01.026
55. Raskin I, Smith R D, Salt D E (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8(2) 221-226. https://doi.org/10.1016/S0958-1669(97)80106-1
56. Reddy K R, Xie T, Dastgheibi S (2014) Removal of heavy metals from urban stormwater runoff using different filter materials. J Environ Chem Eng 2(1), 282-292. https://doi.org/10.1016/j.jece.2013.12.020
57. Regulation on emission limit values of polluting substances in surface and groundwaters and deadlines for their achievement (Official Gazette of the RS 50/2012). https://www.ekologija.gov.rs/. Accessed 16 June 2023 (in Serbian).
58. Ruby M, Appleton B (2010) Using landscape plants for phytoremediation. In Low Impact Development 2010: Redefining Water in the City , pp. 323-332 https://doi.org/10.1061/41099(367)29
59. Salimi M, Amin M M, Ebrahimi A, Ghazifard A, Najafi P (2012) Influence of electrical conductivity on the phytoremediation of contaminated soils to Cd 2+ and Zn 2+. Int J Env Health Eng 1(1) 11. https://doi.org/10.4103/2277-9183.94395
60. Schwarz D, Grosch R (2003) Influence of nutrient solution concentration and a root pathogen (Pythium aphanidermatum) on tomato root growth and morphology. Sci Hortic, 97(2), 109-120. https://doi.org/10.1016/S0304-4238(02)00143-7
61. Sela M, Garty J, Tel-Or E (1989). The accumulation and the effect of heavy metals on the water fern Azolla filiculoides. New Phytol 112(1), 7-12.
62. Schlüter U, Crawford R M (2001) Long-term anoxia tolerance in leaves of Acorus calamus L. and Iris pseudacorus L. J Exp Bot 52(364) 2213-2225. https://doi.org/10.1093/jexbot/52.364.2213
63. Shajib M T I, Hansen H C B, Liang T, Holm P E (2019) Metals in surface specific urban runoff in Beijing. Environ. Pollut 248 584-598. https://doi.org/10.1016/j.envpol.2019.02.039
64. Sharma P, Singh S P, Ton, Y W (2022) Phytoremediation of metals: Bioconcentration and translocation factors. In Current Developments in Biotechnology and Bioengineering Elsevier, pp. 19-37. https://doi.org/10.1016/B978-0-323-99907-6.00002-5

65. Sytar O, Ghosh S, Malinska H, Zivcak M, Brestic M (2021) Physiological and molecular mechanisms of metal accumulation in hyperaccumulator plants. Physiol Plant, 173(1) 148-166. https://doi.org/10.1111/ppl.13285
66. Takarina N D, Pin T G (2017) Bioconcentration factor (BCF) and translocation factor (TF) of heavy metals in mangrove trees of Blanakan fish farm. Makara J Sci, 21(2) 4. https://doi.org/10.7454/mss.v21i2.7308
67. TIBCO Software Inc (2020) Data Science Workbench, version 14. http://tibco.com
68. Vymazal J (2016) Concentration is not enough to evaluate accumulation of heavy metals and nutrients in plants. Sci Total Environ 544 495-498. https://doi.org/10.1016/j.scitotenv.2015.12.011
69. Wan S, Pang J, Li Y, et al (2021) Hydroponic Phytoremediation of Ni, Co, and Pb by Iris Sibirica L. Sustainability, 13(16), 9400. https://doi.org/10.3390/su13169400
70. West M, Fenner N, Gough R, Freeman C (2017) Evaluation of algal bloom mitigation and nutrient removal in floating constructed wetlands with different macrophyte species. Ecol. Eng 108 581-588. https://doi.org/10.1016/j.ecoleng.2017.07.033
71. Wu A, Zhang Y, Zhao X et al (2022) Experimental Study on the Hydroponics of Wetland Plants for the Treatment of Acid Mine Drainage. Sustainability 14(4) 2148. https://doi.org/10.3390/su14042148
72. Yakovyshyna T (2021) Integrated Approach of Phytostabilization for Urban Ecosystem Soils Contaminated with Lead. Environ Res Eng Manag 77(2) 43-52. https://doi.org/10.5755/j01.erem.77.2.28633
73. Yan A, Wang Y, Tan S N, Mohd Yusof M L, Ghosh S, Chen Z (2020) Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. Front Plant Sci 11 359. https://doi.org/10.1016/j.geoderma.2012.01.020
74. Zhang L, Yan M, Ren Y, Chen Y, Zhang S (2021) Zinc regulates the hydraulic response of maize root under water stress conditions. Plant Physiol Biochem 159 123-134. https://doi.org/10.1016/j.plaphy.2020.12.014
75. Zhang J, Wang X, Zhou Q (2017) Co-cultivation of Chlorella spp. and tomato in a hydroponic system. Biomass Bioenergy 97:132–138. https://doi.org/10.1016/j.biombioe.2016.12.024
76. Zhang Z, Rengel Z, Chang H, Meney K, Pantelic L, Tomanovic R (2012) Phytoremediation potential of Juncus subsecundus in soils contaminated with cadmium and polynuclear aromatic hydrocarbons (PAHs). Geoderma, 175, 1-8. https://doi.org/10.1016/j.geoderma.2012.01.020
77. Žunić V, Jafari T H, Grabić J, Đurić S, Stamenov D (2022) Hydroponic systems: exploring the balance between co-cultivation of Chlorella vulgaris and Swiss chard (Beta vulgaris L. subsp. cicla). J Appl Phycol 34(2) 903-913. https://doi.org/10.1007/s10811-021-02673-z.

Figures

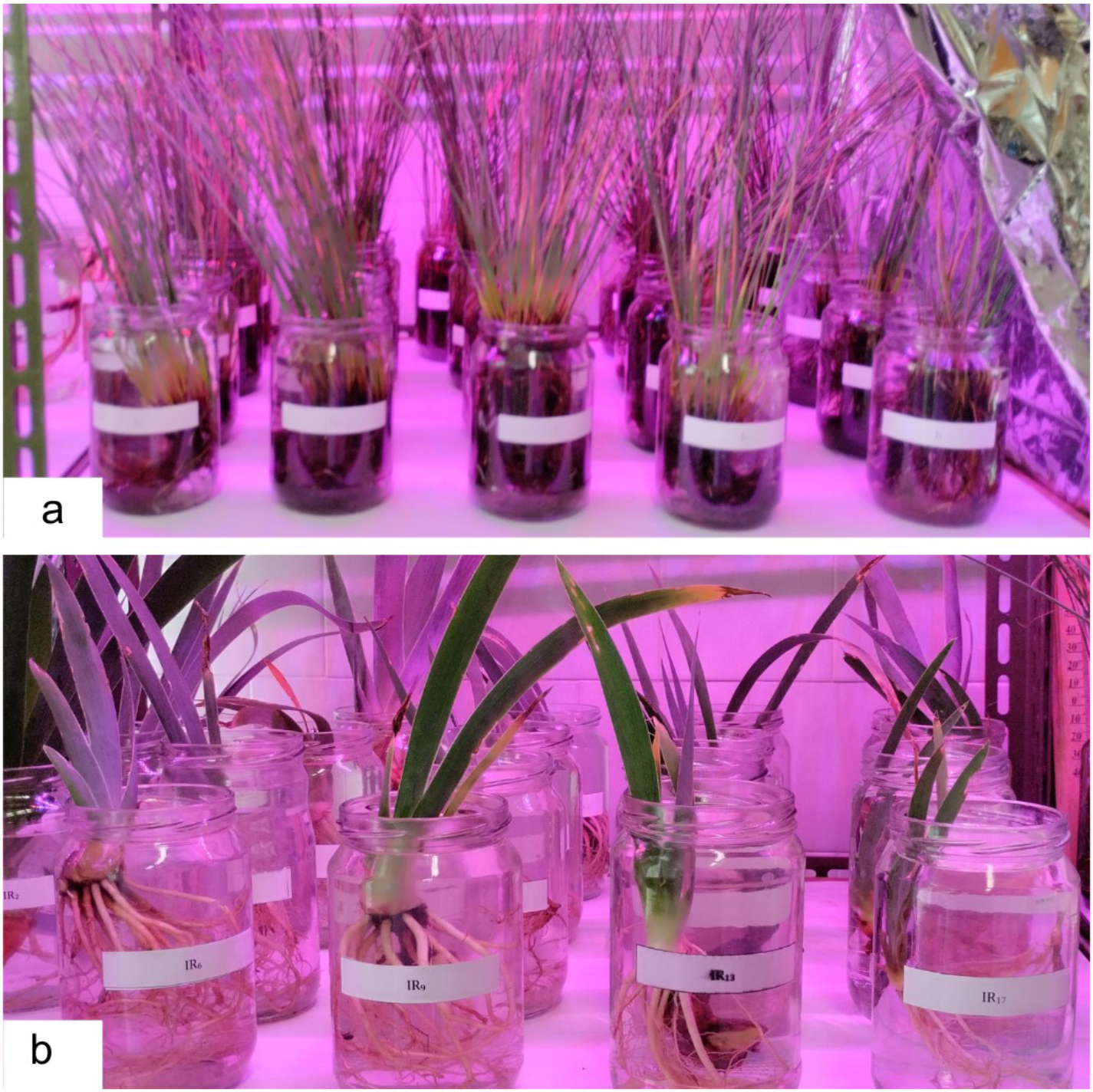


Figure 1

Experimental jars with *Juncus effusus* L. and *Iris pseudacorus* L. plants grown hydroponically in a constructed growth chamber

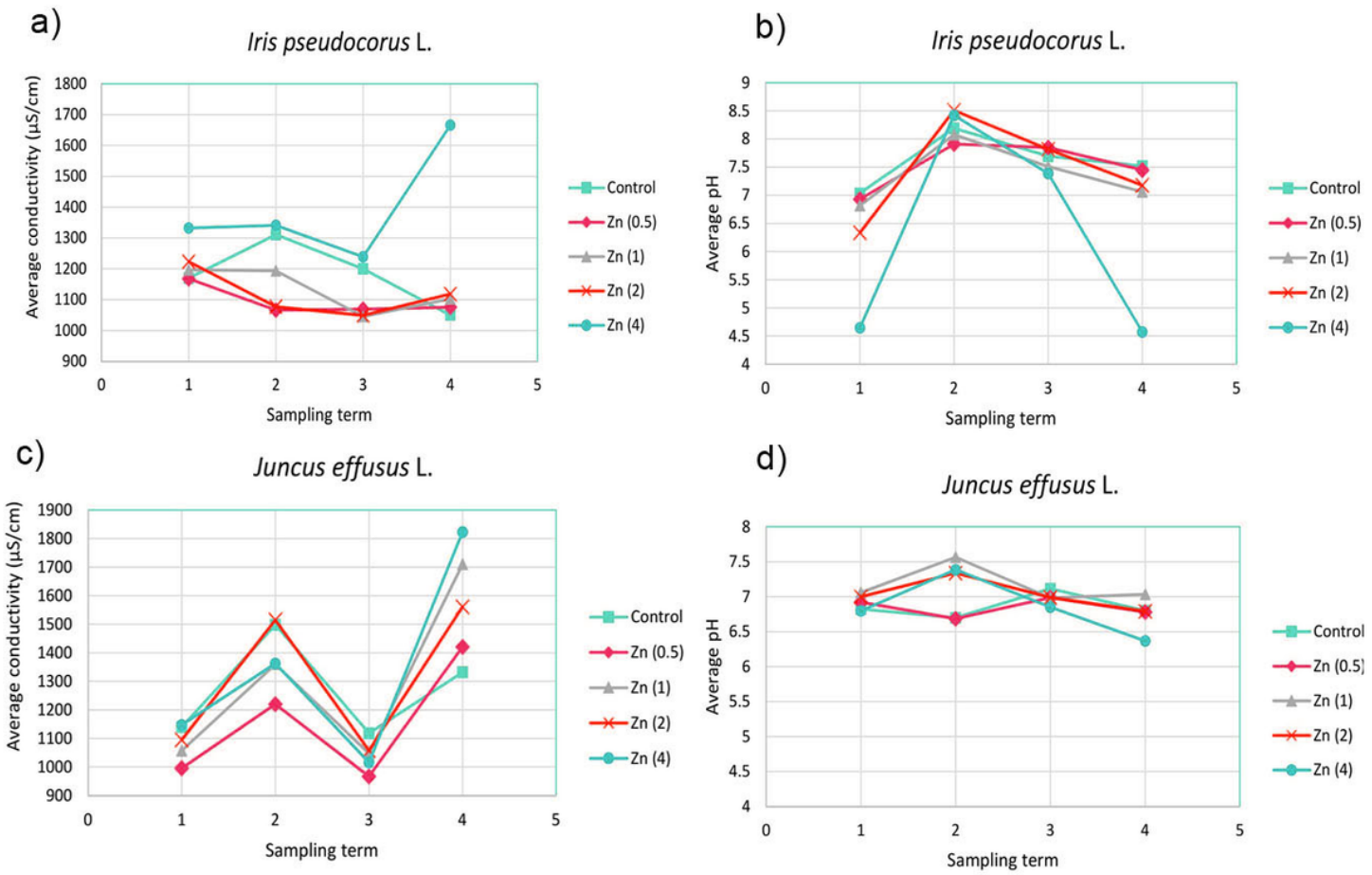


Figure 2

The change of average conductivity and pH during the experiments measured at defined sampling terms: a) and b) for *Iris pseudacorus* L. and c) and d) for *Juncus effusus* L. Average conductivity and average pH were calculated based on four samples in the experimental set.

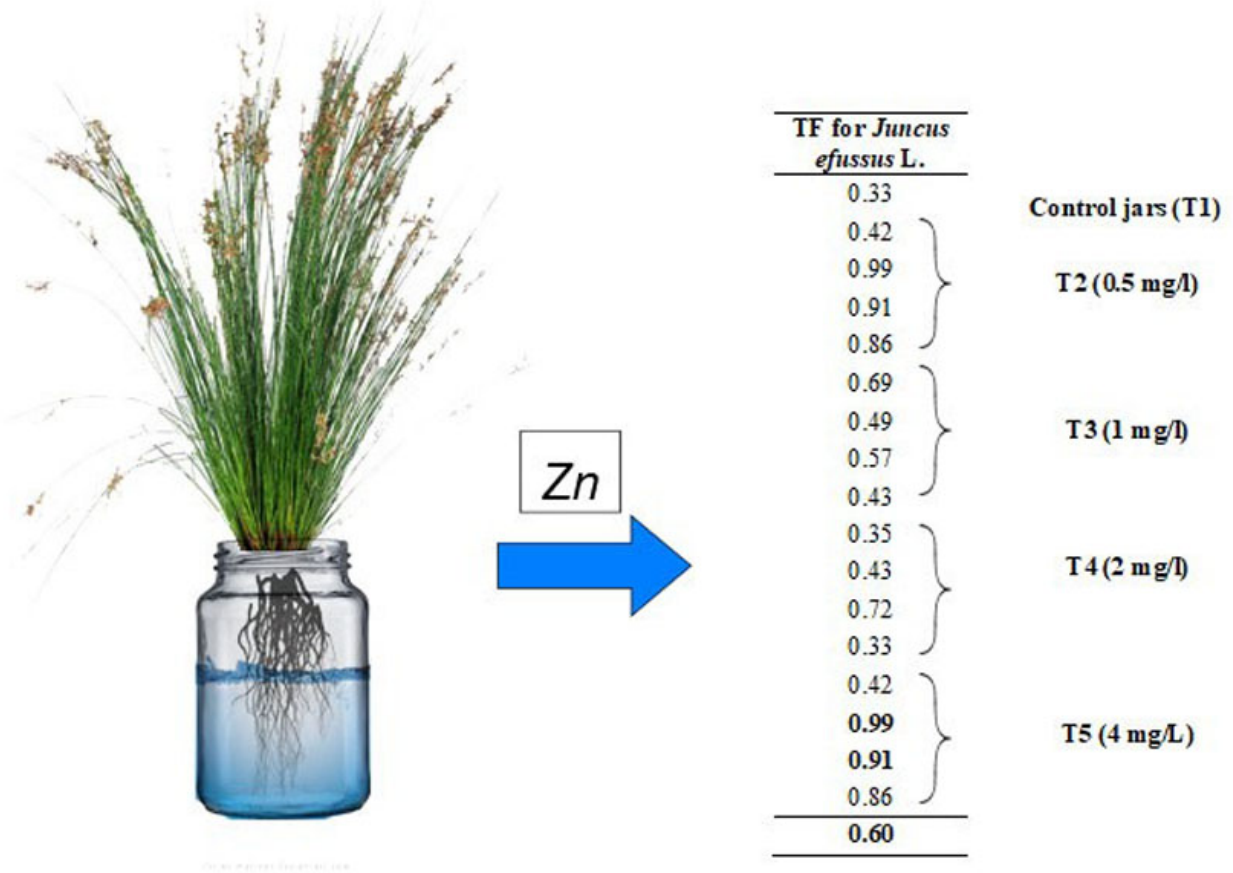


Figure 3

Translocation factor (TF) for *Juncus* plants

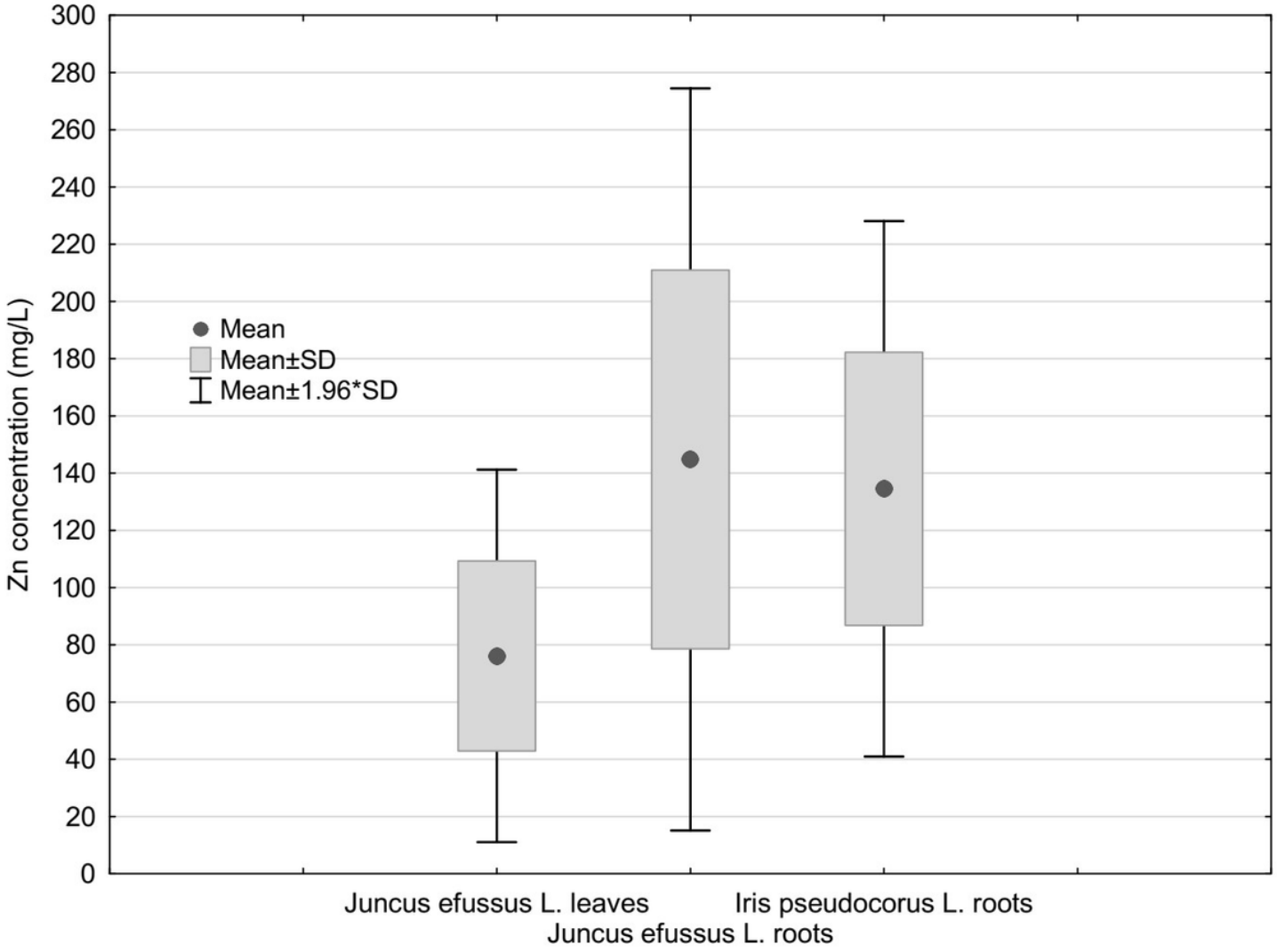


Figure 4

Box-Whisker plot of the Zn concentrations in the samples of *Juncus effusus* L. leaves and roots and *Iris pseudacorus*L. roots. Each box-whisker graph refers to twenty measurements (including control measurements) of the specified plant samples.

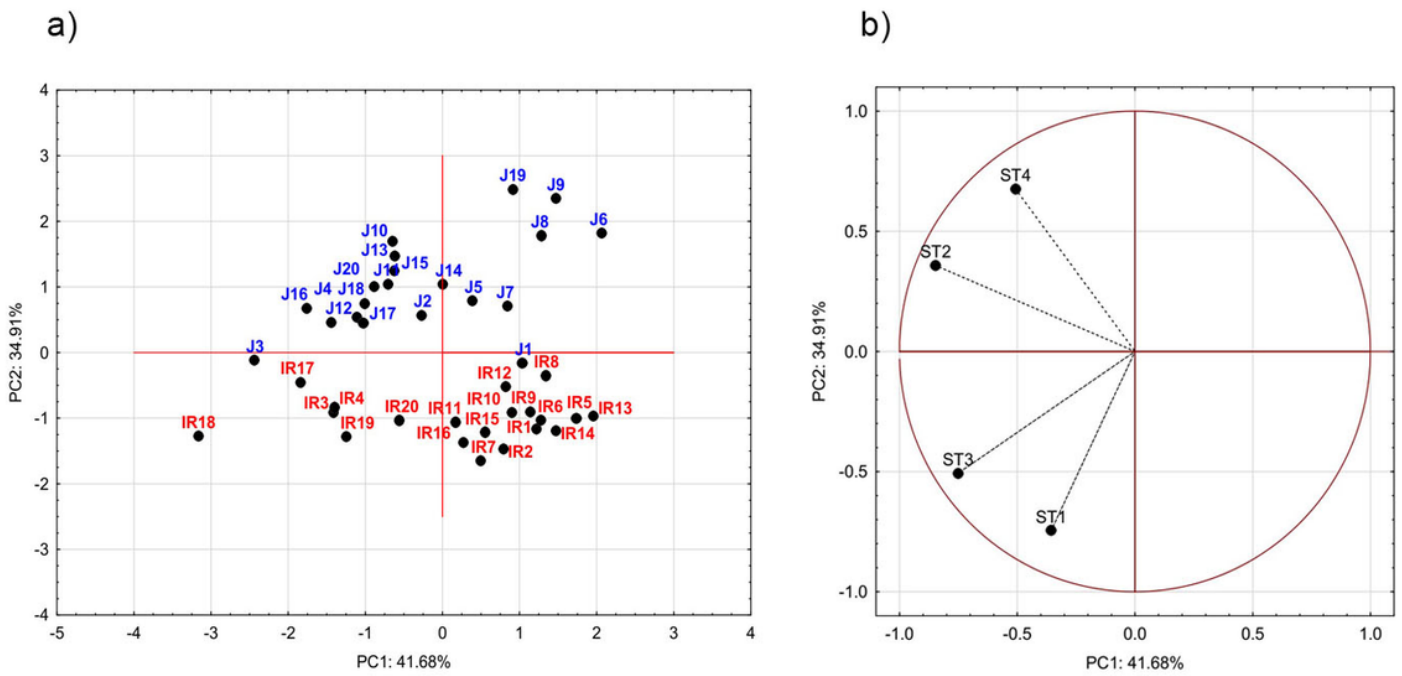


Figure 5

Score (a) and loadings (b) plot based on the conductivity data (IR - *Iris pseudacorus*samples; J - *Juncus effusus* samples; ST – sampling term).

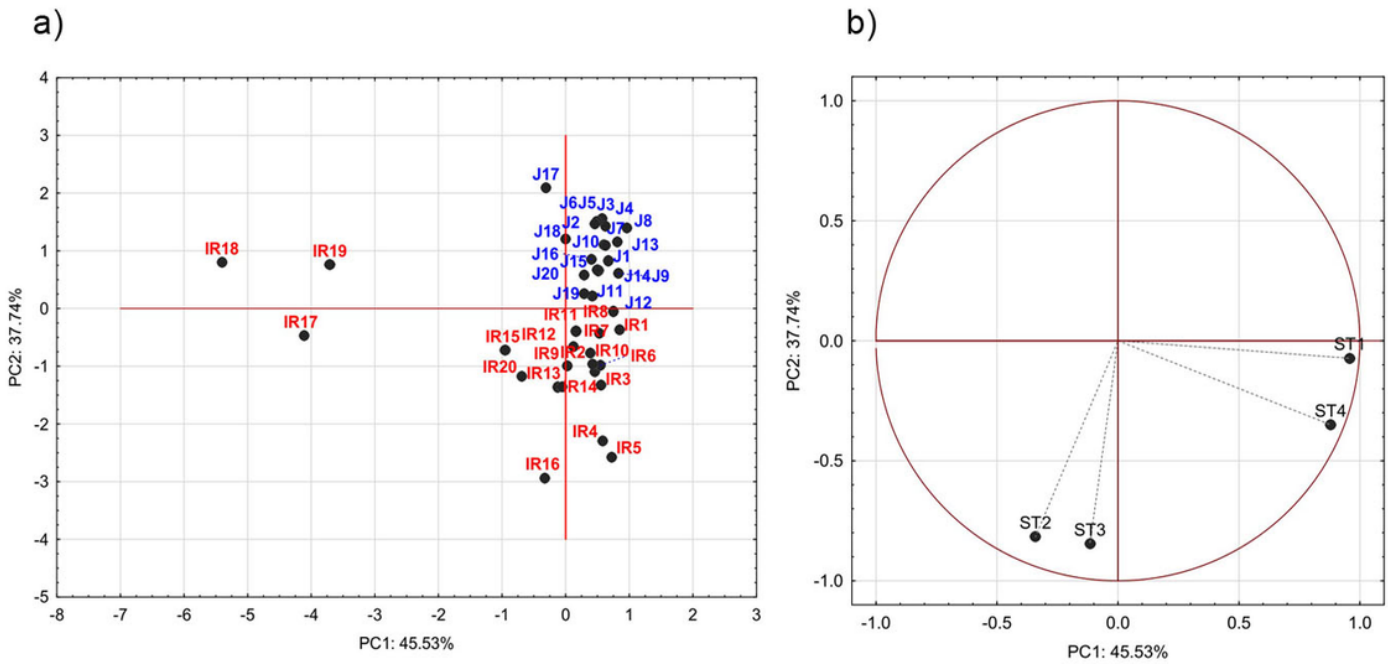


Figure 6

Score (a) and loadings (b) plot based on the pH data (IR - *Iris pseudacorus*samples; J - *Juncus effusus* samples; ST – sampling term).

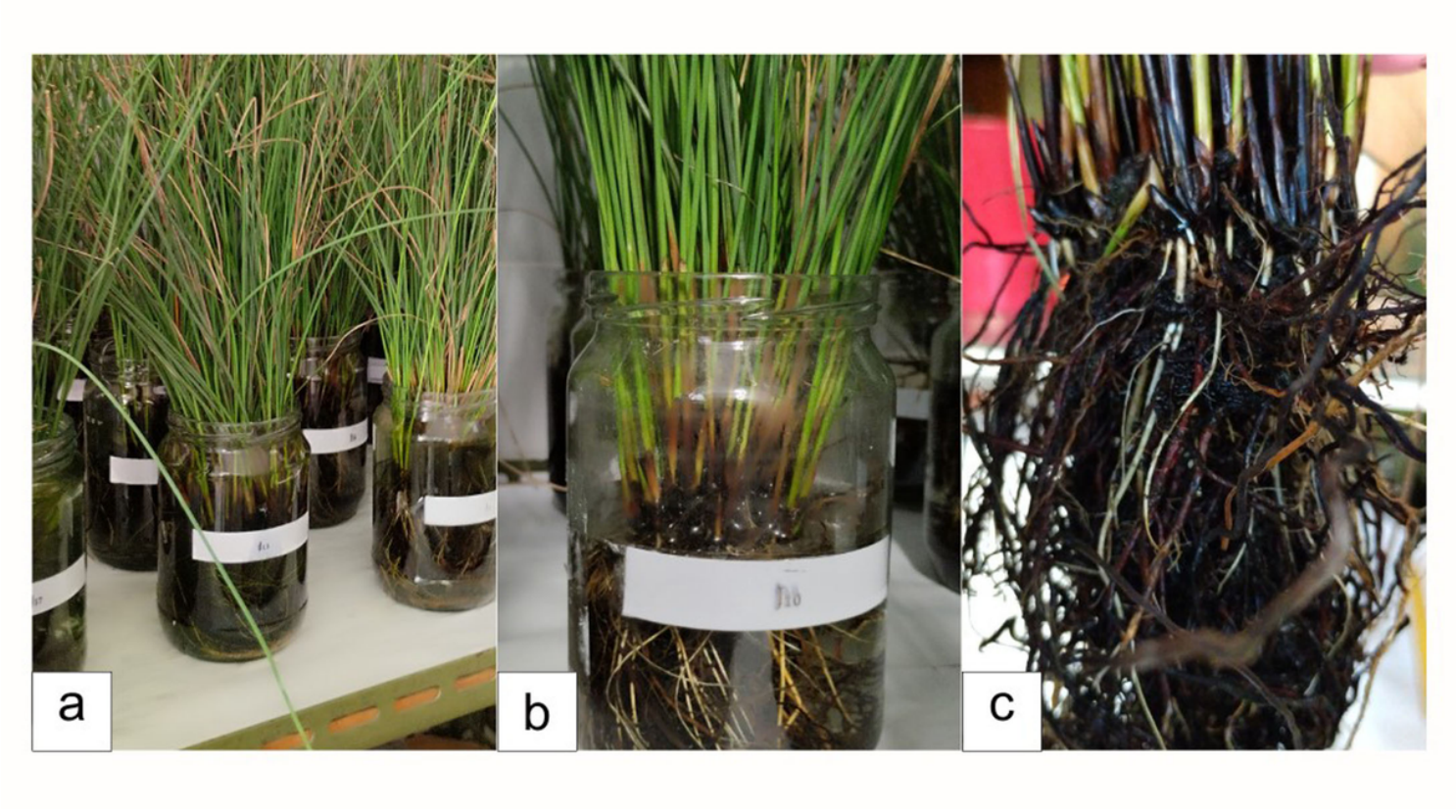


Figure 7

Growth responses of *Juncus effusus* L. plants during the experiment (a) biomass increasement, (b) infiltration of the water via roots, (c) development of new, adventive roots.

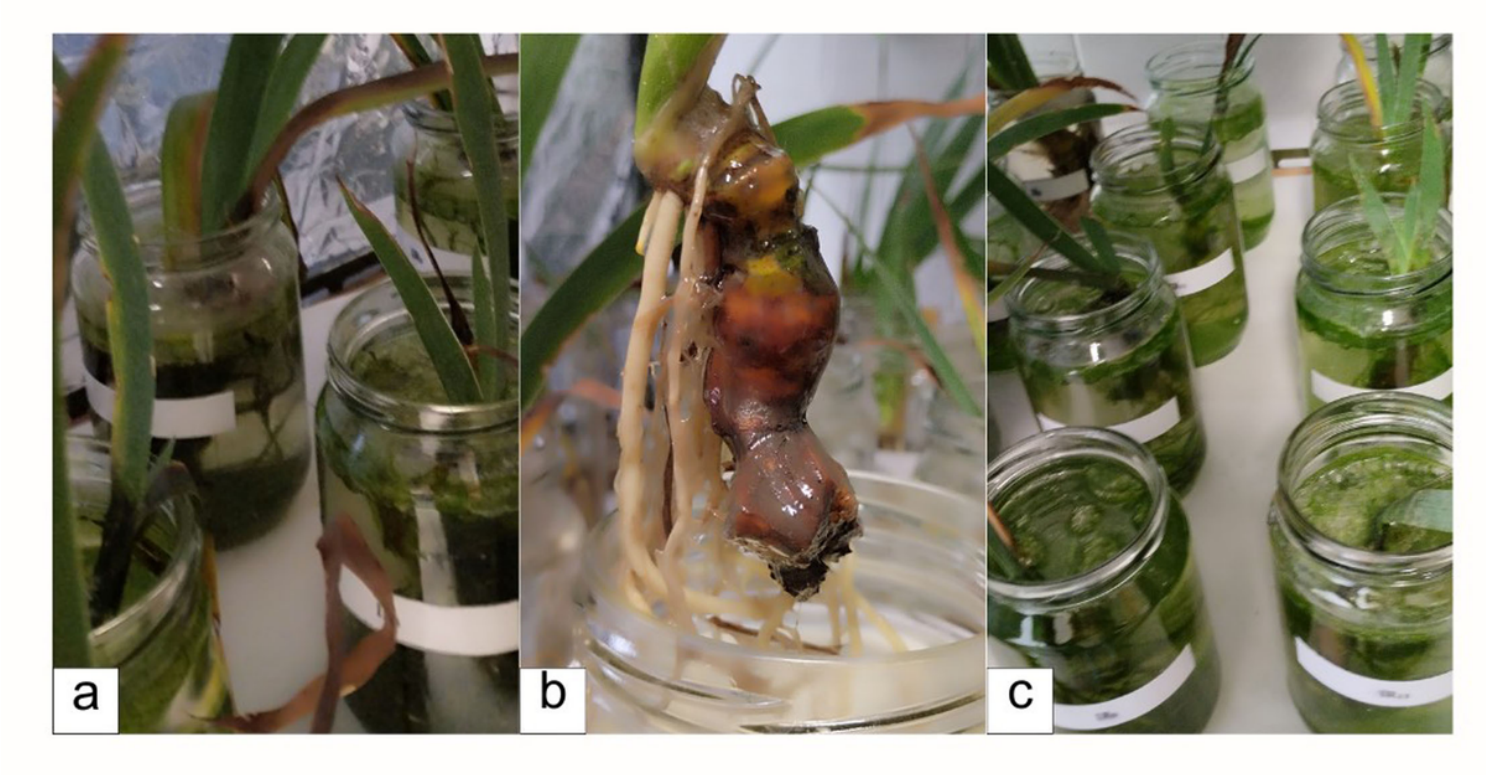


Figure 8

Growth responses of *Iris pseudacorus* L. plants during the experiment (a) reduction of leaf biomass, (b) brown spots on the rhizomes/roots with the new adventive root development, (c) algal growth in all jars with different Zn concentrations