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Use of seed priming to improve Cd accumulation and tolerance in *Silene sendtneri*, novel Cd hyper-accumulator

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ABSTRACT

Changes in the environment as a result of industrialisation and urbanisation impact negatively on plant growth and crop production. Cadmium (Cd) is one of the most dangerous metals that enters the food chain, with toxic effects on plants and human health. This study evaluated the potential of *Silene sendtneri* as a novel hyper-accumulator and the role of seed priming in tolerance and accumulation rate of Cd. The effect of different priming agents on germination performance, root growth, seedling development, metal uptake and accumulation, antioxidant defences including enzymatic and non-enzymatic antioxidants has been assessed. Seed priming using silicic acid, proline alone or in combination with salicylic acid- enhanced germination, seedling development, and root growth under Cd stress. The same priming treatments induced an increase of water content in shoots and roots when plants were exposed to Cd. The enzymatic antioxidant response was specific for the priming agent used. An increase in ferulic acid and rutin in shoots was related to the increase of Cd concentration in the medium. The concentration of malic and oxalic acid increased significantly in shoots of plants grown on high Cd concentrations compared to low Cd concentrations. *Silene sendtneri* can accumulate significant levels of Cd with enhanced accumulation rate and tolerance when seeds are primed. The best results are obtained by seed priming using 1% silicic acid, proline and salicylic acid.

1. Introduction

Industrialisation and urbanisation have brought different environmental challenges all around the globe, such as heavy metal pollution, which is now considered as a widespread problem due to the risk of non-biodegradable, bioaccumulation, and high toxic properties of heavy metal (Yu et al., 2010). When these metals are present in the environment, they can be further absorbed by plants and accumulated in edible parts of crops, thus entering the food chain. Cadmium (Cd) poses a great risk for human health (Boularbah et al., 2006; Xin et al., 2010; Wang et al., 2011), and it is one of the main pollutants as a result of industrialisation and agrochemicals. Once Cd enters the food chain it can accumulate persistently in the body impairing liver and kidney function, causing osteoporosis, bone atrophy, and deformations (Klaassen et al., 1999; Patrick, 2003). World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO) recommend a provisional tolerable weekly intake (PTWI) for Cd of no more than 7 mg/kg of body weight (Muñoz et al., 2005).

Heavy metals present in excess are toxic for plants as well. They have evolved different strategies to avoid the toxic effects of metal including chelation, sequestration, storage, and efflux (Sharma and Dietz, 2006; Francini and Sebastiani, 2010; Kováčik et al., 2011). Plant defence also includes the synthesis of diverse arrays of secondary metabolites (Kováčik et al., 2008, 2012) suggesting mechanisms that include regulation of both phenylpropanoid and terpenoid biosynthesis (Kang et al., 2014; Hojati et al., 2016). The root is the first plant organ that encounters toxic levels of heavy metals, and in non-hyper-accumulating

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Nomenclature

23HBA	2,3-dihydroxybenzoic acid
3HBA	3-hydroxybenzoic acid
4HBA	4-hydroxybenzoic acid
5HFA	5-hydroxyferulic acid
ABA	abscisic acid
BCF	bioconcentration factor
CA	caffeic acid
Cd	cadmium
DW	dry mass
GA	gibberellic acid
FA	ferulic acid
FAO	food and agriculture organization of the United Nations
Fe	iron
FW	fresh mass
GAL	galangin
HES	hesperetin
MYR	myricetin
NAR	naringenin
pCA	p-coumaric acid
pMeCA	p-coumaric acid methyl ester
POD	Guaiacol peroxidase activity
Pro	proline
PVP	Polyvinylpyrrolidone
QUE	quercetin
RaA	rosmarinic acid
RUT	rutin
ROS	reactive oxygen species
SaA	salicylic acid
SaAG	salicylic acid glucoside
SD	standard deviation
SiA	1% silicic acid
SiA	syringic acid
SyA	synapic acid
TF	Translocation factor
TP	total protein content
WC	water content
WHO	world health organization

plants, roots are involved in restriction of metal translocation, protecting the plant from its toxic effects (Lefèvre et al., 2016). On the other hand, in hyper-accumulating plant species translocation from the root to the shoot is increased and the metal is accumulated in the aerial parts of the plants, thus different protective systems are activated.

Most plants are sensitive to even low concentrations of Cd and often exhibit various symptoms including leaf chlorosis, inhibition of photosynthesis, growth retardation and root damage (Das et al., 1997), the disruption of homeostasis of essential microelements, especially iron (Fe), (Siedlecka and Baszynski, 1993; Schützendübel and Polle, 2002; Clemens, 2006; Wu et al., 2012). One of the methods for revitalisation of soil contaminated by heavy metals is phytoremediation. Accumulation and tolerance of heavy metals are different for various plant species (Liu et al., 2005a; Grant et al., 2008), and it varies not only across species but also within the varieties of each species (Liu et al., 2005b; Ding et al., 2013). Different approaches have been tested for Cd removal from contaminated soil, and phytoextraction has proven to be one of the most cost-effective and eco-friendly solutions. (Mahar et al., 2016). The selection of adequate plant species is therefore a fundamental aspect for soil phytoremediation. The selected plant must be a perennial plant, with the ability to adapt to a different environmental condition and high biomass production (Pilon-Smits, 2005).

Silene sendtneri Boiss. belongs to the family of the Caryophyllaceae and it is endemic for the Dinaric Alps with *locus classicus* in Bosnia and Herzegovina. This species is well adapted and it can grow in both shallow and deeper soils, on silicates and limestone soils (Šilić, 1990). This plant has been selected as a model in this study, due to high production of seeds, the longevity of seeds (germination rate over 80% after 10-year storage; personal observation), high biomass production, It is a perennial plant which is a close relative to the highly investigated well known hyperaccumulating species *Silene vulgaris*. Literature data suggests that *Silene vulgaris* has many natural populations (ecotypes) and they can belong to heavy metal sensitive or highly tolerant populations (Chardonnens et al., 1999). Tolerant ecotypes include ecotypes that can accumulate zinc and cadmium (Ernst and Nelissen, 2000), copper (Verkleij et al., 2001), lead and nickel (Muszyńska et al., 2019) and arsenate (Sneller et al., 2000). The plant used in this investigation can grow in different soil types including serpentine soils and is considered to be tolerant to heavy metals but no investigations have been performed yet.

Plant tolerance of heavy metals in the soil is crucial in the first stages of germination upon radicle emergence, and usually, the most pronounced effect of heavy metal toxicity is the failing of plant germination not producing seedlings and mature plants (Akar and Atis, 2018). It has been recorded that seed priming, a method usually used for the improvement of seed performance, germination speed, and uniformity (Ashraf and Foolad, 2005; Krishnotar et al., 2009; Srivastava and Bose, 2012; Karalija et al., 2019), can be beneficial for heavy metal tolerance during the plant development (Karalija and Selović, 2018; Šabanović et al., 2018;).

To evaluate the full potential of *Silene sendtneri* as a novel Cd-hyperaccumulator, we hypothesized that seed priming will enhance Cd accumulation in the aboveground plants through an increase in plants resistance by affecting secondary metabolism and antioxidant potential. Hence, the effect of different priming agents on the performance of *Silene sendtneri* under Cd stress as well as the changes in Cd tolerance and accumulation have been assayed. The germination response, growth parameters, Cd accumulation and translocation rate, production of secondary metabolites, organic acids, and activity of antioxidant enzymes have been investigated.

2. Materials and Methods

2.1. Plant material, priming treatments

The seeds of *Silene sendtneri* were collected during July, 2018 in the vicinity of Pjeskovita ravan on Mt. Ozren (43°54'191'' N, 18°27'170'' E; 1302 m.s.m.). All seeds were kept at 4 °C in the Laboratory for Plant physiology until their use. Seed priming was performed by submerging seeds in the appropriate solution for 24 h at 4 °C. The agents used for seed priming were distilled water (hydro-primed seeds), 1% silicic acid (SiA); 0.5 m*M* salicylic acid (SaA), 1.0 m*M* SaA, 10 m*M* proline (Pro), 20 m*M* Pro, 0.5 m*M* SaA + 10 m*M* Pro, 0.5 m*M* SaA + 20 m*M* Pro, 1.0 m*M* SaA + 10 m*M* Pro, 1.0 m*M* SaA + 20 m*M* Pro. Non-primed seeds were used as control. After 24 h, all seeds were rinsed twice with sterile distilled water and air-dried for 72 h. Seeds were packed in tubes containing silica gel to maintain low moisture and stored at 4 °C until cultivation.

2.1.1. Cultivation and growth conditions

The media used for seed cultivation was prepared according to mineral composition given by Murashige and Skoog (1962) recapture with the addition of vitamins and 3% (w/v) sucrose. Cadmium was added to the media in two concentrations (0.25 and 0.5 m*M* Cd) in the

form of $Cd(NO_3)_2$. Medium without cadmium served as control. For all media pH was adjusted to 5.8 using KOH/HCl before the addition of agar (w/v; 0.8%). All media were autoclaved and poured into marked Petri dishes, cooled and sealed with parafilm. Media was stored at room temperature for 48 h before cultivation for contamination check.

Primed seeds were cultivated using an aseptic technique under laminar air-flow into prepared media. All cultures were kept in a growth chamber under the light (16 h photoperiod; 2000 lux) provided by Osram Fluora 18 W, the temperature of 23 °C (± 2 °C) and 70% humidity. Plants were analysed after 4 weeks of culture.

2.1.2. Germination analysis

For the evaluation of germination rate 30 seeds per petri dish were cultivated, i.e. for each seed priming 5 Petri dishes for each cadmium concentration were cultivated. The effect of seed priming on the germination rate of *Silene sendtneri* under cadmium stress was evaluated after 15 days of cultivation. The percentage of the fully grown seedlings formed out of germinated seeds was evaluated after 30 days of cultivation.

2.2. Root growth analysis

The effect of priming on the root growth rate and average daily growth rate of *Silene sendtneri* under Cd stress was evaluated for 3-time periods or at 3-time points, e.g after 7, 15, and 30 days of cultivation. For the evaluation of root growth, seeds were placed in one line in the Petri dish and stored vertically to observe gravitationally oriented root growth. The length of the root was estimated each day by marking the root tip on the back of the petri dish, photographing against millimeter paper and measuring in ImageJ software. For each seed priming and each cadmium concentration, 10 roots were measured for each time interval.

2.2.1. Seedling growth analysis

The effect of seed priming on a fresh and dry shoot and root mass of *Silene sendtneri* growing under cadmium stress was evaluated. Ten representative shoots for each treatment were selected, roots were washed thoroughly to eliminate agar residues and blotted with filter paper to remove water excess. The root and the shoots were separated using a plastic scalpel and placed in separate Petri dishes. The fresh mass was measured and the samples were placed in the oven overnight at 60 °C, after which the dry mass was recorded. The fresh and dry weight was expressed in mg/plant. The water content was calculated according to the difference between fresh and dry weight.

2.3. Cadmium concentration analysis by flame atomic absorption spectrometry

Aerial plant tissues dried at 70 °C to were finely grounded and submitted to acid digestion in a mixture of HNO_3 and 30% H_2O_2 (Kalra and Maynard, 1998). After digestion, the solutions were analysed for cadmium by FAAS under optimised measurement conditions.

2.3.1. Bioconcentration and translocation factor

Cadmium concentrations determined for the root, and the shoot samples were used for the calculation of bioconcentration and the translocation factor. The bioconcentration factor (BCF) represents the ability of plants for elemental accumulation from the substrate/media (Wu et al., 2011), and it was calculated according to the equation:

$$BCF = \frac{Metal\ concentration\ (plant\ \ part)}{Metal\ concentration\ (media)}$$

The translocation factor (TF) was calculated from the ratio of element's presence in the plant's shoots compared to the plant's roots (Wu et al., 2011) using the equation:

$$TF = \frac{Metal\ concentration\ (shoots)}{Metal\ concentration\ (roots)}$$

2.4. The analysis of antioxidant enzymes

Enzyme extraction was performed by grinding fresh plant samples (taken from the root and shoot) in the presence of 0.5 m*M* phosphate buffer (pH = 7.0) and 0.1% PVP (Polyvinylpyrrolidone). Homogenate was centrifuged at 10.000 rpm and 4 °C for 30 min. The supernatant was collected for enzyme analysis.

Guaiacol peroxidase activity (POD, EC 1.11.1.7) was evaluated according to the modified method of Angelini et al. (1990) for plant extracts diluted 500 times in 0.1 *M* phosphate buffer (pH = 7.0) containing 5 m*M* guaiacol (Fluka, Japan) and 0.1 *M* H_2O_2. The activity was calculated as the change in absorbance in 120 s time intervals regarding to protein content. The results were calculated using the extinction coefficient of guaiacol dehydrogenation product tetraguaiacol ($\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$) and expressed as the units of enzyme per mg of protein. One unit of enzyme represents the amount of enzyme catalysing the oxidation of 1 μ*M* of guaiacol per min.

Total protein content was determined using bovine serum albumin as a protein standard according to the Bradford method (1976).

2.5. UHPLC-MS/MS analysis

UHPLC-MS/MS was performed on UltiMate™ 3000 liquid chromatographic system consisting of binary pumps, an autosampler, and a column thermostat coupled to a TSQ Quantum Access Max triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation was performed on an Acquity BEH C18 (150 × 3.0 mm; 1.7 μm particle size) UHPLC column (Waters Corp., Milford, MA, USA) kept at 40 °C. The mobile phase consisted of 10 m*M* formic acid in water (component A) and acetonitrile (component B). Totally 32 compounds were separated using a binary gradient starting at 5% B for 0.8 min, increasing to 10% B in 0.4 min with an isocratic run for 0.7 min, then increasing to 15% B for 0.5 min and an isocratic run for 1.3 min, then increasing to 20% B for 0.3 min and isocratic for 1.2 min, then increasing to 25% B for 0.5 min with next increase to 35% B within 2.3 min, then increasing to 70% B for 2.5 min, then the further increase to 100% B for 1 min, with an isocratic run for 1 min, and then back to 5% B for 0.5 min. Finally, the equilibration to the initial conditions took 3.3 min, with the total chromatographic run of 16 min. The flow rate was 0.4 mL/min and the injection volume 10 μL.

All analytes were detected in the negative ionization mode ESI-. The multiple reaction monitoring (MRM) mode was used for their quantification. The spray voltage was 3 kV, and the vaporizer the ion transfer tube temperatures were 320 °C.

Standard solutions of 32 target compounds (apigenin, 2.3-dihydroxybenzoic acid, caffeic acid, carnosic acid, catechin, chlorogenic acid, chrysin, ferulic acid, galangin, gallic acid, hesperetin, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 5-hydroxyferulic acid, kaempherol, methyl *p*-coumarate, morin, myricetin, naringenin, naringin, *p*-coumaric acid, pinocembrin, quercetin, quercitrin, rosmarinic acid, rutin, salicylic acid, salicylic acid glucoside, sinapic acid, syringic acid, *trans*-cinnamic acid, and vanillic acid), purchased from Sigma Aldrich Company, Germany, were firstly prepared in methanol at 1 m*M* concentrations, and the solutions were gradually diluted in the mobile phase to the working concentrations that ranged from 0.01 to 50 μ*M*. Quantification was performed by an isotope diluting method using *p*-coumaric acid-d_6 and salicylic acid-d_4. The analysis was performed only for shoot samples.

2.6. Analysis of organic acids

The quantitative analysis of organic acids was carried on an automatic system for capillary electrophoresis Agilent Technologies

G1600AX. Separations were performed on fused silica capillary (80.5 cm total length, 72 cm effective length, 75 μm I.D.). The detection wavelength was set to 250 nm and 214 nm for the reference. The commercially available kit (CElixirOA™ by MicroSolv) consisting of two solutions was employed. Indirect UV detection is based on using 2,6-pyridinedicarboxylic acid as a background electrolyte (Soga and Ross, 1997). Before the use, a new capillary was flushed for 10 min with 1 *M* NaOH and 10 min with water (Milli-Q). Preconditioning before each analysis consisted of four washing steps: 90 s 0.1 *M* NaOH, 90 s water (Milli-Q), 90 s Initiator (first part of the kit) and 90 s Accelerator (second part of the kit). To achieve injection pressure of 50 mbar was applied for 5 s. The accelerator solution was used as background electrolyte. The applied voltage of − 30 kV (ramp −1 kV/s) resulted in an electrophoretic current of approximately − 18 μA. The temperature was maintained at 25 °C. The Content of buffer vials was changed after five consecutive analyses. The identification of analytes was performed by comparison of retention times of authentic standards obtained from Sigma Aldrich Chemical Company (Germany), while their quantities were calculated according to the calibration curves ranged 0.28–5.55 m*M* for oxalate, 0.13–2.60 m*M* for malate, and 0.19–3.73 m*M* for citrate.

2.7. Statistical analysis

All data were analysed using the STATISTICA 10.0 software (Statsoft Inc.). Experimental results were presented as the mean ± standard deviation of three independent replications. The obtained data were subjected to variance analysis (ANOVA) and the Newman-Keuls post hoc test was carried out to identify significant differences between the extract types. Mean values with $p < 0.01$ were considered statistically significant. Pearson correlations were performed to observe the possible correlation between the phenolic profile, antioxidant capacity, and detected antimicrobial activity at the level of significance $p < 0.01$.

3. Results

3.1. Effect of seed priming on seed germination of Silene sendtneri under Cd stress

A non-primed seed showed a strong reduction of germination rate related to the increasing Cd concentration. All priming treatments improved the germination rate for non-stressed and Cd stressed conditions. For control media significant improvement of germination compared to control was recorded for all priming treatments (except for 0.5 m*M* SaA), with germination percentage ranging from 85.19% ± 6.76 (0.5 m*M* SaA + 20 m*M* Pro) to 100% ± 0.00 (1% SiA). Under moderate cadmium stress (0.25 m*M* Cd) the best performance was recorded for 0.5 and 1.0 m*M* SaA (82.83% ± 0.87; 81.48% ± 6.42, respectively). In conditions of high Cd stress (0.5 m*M* Cd) the highest recorded germination rate was for treatment with 1% SiA (93.33% ± 11.55). The germination rate gives only the information regarding the first stage of germination. Therefore, the percentage of seedling development from seeds germinated in the presence of Cd was also evaluated. For most of the priming treatments seedlings developed at 100% rate. Under Cd stress, there were noticeable differences between the treatments. For 1% SiA treatment, a high rate of seedling development was recorded when exposed to 0.25 and 0.5 m*M* Cd (100% ± 0.00% and 90% ± 10.00, respectively). Hydro-priming showed a high percentage of seedling development under Cd stress, as well as treatments that combined SaA and Pro as priming agents (Table 1).

Table 1
Effect of priming on germination of *Silene sendtneri* under cadmium stress.

Priming agent	Cd (m*M*)	Germination rate (%)	Fully formed seedlings (% of germinated seeds)
Non-primed	0	$79.70^{bc} \pm 5.24$	$100.00^{a} \pm 0.00$
	0.25	$84.18^{b} \pm 4.08$	$92.59^{ba} \pm 6.42$
	0.50	$51.52^{c} \pm 18.89$	$75.79^{b} \pm 9.55$
Hydro-primed	0	$93.94^{b} \pm 10.50$	$100.00^{a} \pm 0.00$
	0.25	$69.70^{bc} \pm 5.25$	$86.90b^{a} \pm 1.03$
	0.50	$78.79^{bc} \pm 5.25$	$76.85^{b} \pm 1.60$
1% SiA	0	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$
	0.25	$67.27^{bc} \pm 12.60$	$100.00^{a} \pm 0.00$
	0.50	$93.33^{b} \pm 11.55$	$90.00^{a} \pm 10.00$
0.5 m*M* SaA	0	$73.33^{bc} \pm 5.77$	$95.24^{a} \pm 8.25$
	0.25	$82.83^{b} \pm 0.87$	$82.96^{ba} \pm 5.13$
	0.50	$90.91^{b} \pm 9.09$	$53.37^{cd} \pm 2.96$
1.0 m*M* SaA	0	$93.33^{b} \pm 11.55$	$95.83^{a} \pm 7.22$
	0.25	$81.48^{b} \pm 6.42$	$73.21^{bc} \pm 11.71$
	0.50	$79.26^{bc} \pm 20.04$	$59.45^{cd} \pm 6.38$
10 m*M* Pro	0	$83.22^{b} \pm 7.10$	$100.00^{a} \pm 0.00$
	0.25	$58.18^{c} \pm 3.15$	$88.89^{ba} \pm 9.62$
	0.50	$79.26^{bc} \pm 1.28$	$82.74^{ba} \pm 6.76$
20 m*M* Pro	0	$85.93^{b} \pm 7.06$	$100.00^{a} \pm 0.00$
	0.25	$79.26^{bc} \pm 1.28$	$73.81^{bc} \pm 2.06$
	0.50	$93.33^{b} \pm 5.77$	$46.30^{d} \pm 3.21$
0.5 m*M* SaA + 10 m*M* Pro	0	$85.19^{b} \pm 12.83$	$96.30^{a} \pm 6.42$
	0.25	$60.10^{bc} \pm 3.06$	$80.95^{ba} \pm 8.25$
	0.50	$85.78^{b} \pm 13.67$	$57.20^{cd} \pm 4.59$
0.5 m*M* SaA + 20 m*M* Pro	0	$85.56^{b} \pm 6.76$	$100.00^{a} \pm 0.00$
	0.25	$65.76^{bc} \pm 3.67$	$90.48^{a} \pm 8.25$
	0.50	$90.77^{b} \pm 10.09$	$67.30^{c} \pm 5.14$
1.0 m*M* SaA + 10 m*M* Pro	0	$79.55^{bc} \pm 3.94$	$96.30^{a} \pm 6.42$
	0.25	$67.93^{bc} \pm 8.31$	$91.67^{a} \pm 7.22$
	0.50	$52.78^{c} \pm 4.81$	$73.81^{bc} \pm 8.58$
1.0 m*M* SaA + 20 m*M* Pro	0	$94.44^{b} \pm 4.81$	$100.00^{a} \pm 0.00$
	0.25	$63.10^{bc} \pm 10.31$	$92.13^{a} \pm 6.85$
	0.50	$63.89^{bc} \pm 4.81$	$56.55^{cd} \pm 6.27$

The data represent means of three replicates (± standard deviation). The values within one column followed by the same letter do not differ significantly after the Factorial ANOVA post hoc Newman-Keuls analysis at significance level of $p < 0.01$.
SiA – silicic acid; SaA – salicylic acid; Pro - proline.

3.1.1. Effect of seed priming on root growth of Silene sendtneri under Cd stress

The seedling formation does not necessarily mean that the plant will grow. Different problems/conditions and defects in root and/or shoot development can slow down or stop further development towards the adult plant. The lowest root lengths were recorded for non-primed plants (Table 2). The longest root after 30 days of cultivation was recorded on control media for plants primed with 10 m*M* Pro (85.82 mm ± 2.35). For plants grown under moderate Cd stress (0.25 m*M* Cd) plant primed with 0.5 m*M* SaA in combination with 10 m*M* Pro, and 1.0 m*M* SaA in combination with 20 m*M* Pro had the longest root (35.78 mm ± 5.35 mm and 35.15 ± 5.36, respectively). The highest concentration of Cd induced significant reduction of root growth with most resistant plants primed with 10 m*M* and 20 m*M* Pro (19.35 mm ± 5.54 and 18.14 mm ± 2.90, respectively).

Evaluation of root growth revealed that the growth rate was reduced regarding to Cd concentration in the medium (Table 2). The highest growth rate was recorded for plants primed with 1 m*M* SaA combined with 20 m*M* Pro and 20 m*M* Pro alone for control medium (2.39 ± 0.24 and 2.86 mm/per day ± 0.08, respectively). Under stress, high root growth rate was recorded for plants primed with 1.0 m*M* SaA, 0.5 m*M* SaA with 10 m*M* Pro and 1.0 m*M* SaA with 20 m*M* Pro (0.96 ± 0.12; 1.19 ± 0.18 and 1.17 ± 0.18 mm/per day, respectively). Under high Cd stress (0.5 m*M* Cd) plants primed with 10 m*M* and 20 m*M* Pro showed the highest root growth rate (0.65 ± 0.18 and 0.60 ± 0.10 mm/per day, respectively).

3.1.2. Effect of seed priming on growth of Silene sendtneri seedlings under Cd stress

Reduction in fresh and dry weight for all primed plants was recorded regarding to Cd concentration in the media (Table 3). Non-primed plants grown under the highest Cd concentration showed growth retardation and it was not possible to collect samples for the analysis, while all

Table 2
Effect of priming on root growth of *Silene sendtneri* under cadmium stress.

Priming agent	Cd (m*M*)	Root length (mm)			Average daily growth rate (mm/day)
		7 days	15 days	30 days	
Non-primed	0	$5.16^{ef} \pm 0.22$	$9.36^{f} \pm 1.10$	$23.82^{fg} \pm 1.05$	$0.79^{f} \pm 0.04$
	0.25	$4.06^{ef} \pm 1.24$	$11.15^{ef} \pm 0.89$	$21.89^{fg} \pm 1.02$	$0.73^{f} \pm 0.03$
	0.50	$5.72^{ef} \pm 0.16$	$6.63^{g} \pm 1.45$	$11.69^{hi} \pm 0.90$	$0.39^{g} \pm 0.03$
Hydro-primed	0	$15.62^{bc} \pm 2.55$	$23.74^{c} \pm 3.83$	$30.47^{e} \pm 3.46$	$1.02^{e} \pm 0.12$
	0.25	$7.02^{de} \pm 0.53$	$14.65^{e} \pm 0.94$	$23.52^{fg} \pm 1.15$	$0.78^{f} \pm 0.04$
	0.50	$6.31^{e} \pm 0.42$	$11.71^{ef} \pm 0.44$	$12.84^{h} \pm 1.02$	$0.43^{g} \pm 0.03$
1% SiA	0	$11.94^{d} \pm 2.13$	$38.13^{a} \pm 1.56$	$52.22^{c} \pm 4.80$	$1.74^{c} \pm 0.16$
	0.25	$8.73^{de} \pm 0.61$	$20.60^{cd} \pm 2.24$	$15.53^{h} \pm 1.93$	$0.52^{fg} \pm 0.06$
	0.50	$8.29^{de} \pm 1.68$	$10.93^{f} \pm 1.22$	$11.96^{hi} \pm 1.56$	$0.40^{gh} \pm 0.05$
0.5 m*M* SaA	0	$8.61^{de} \pm 1.00$	$19.92^{d} \pm 0.69$	$42.17^{d} \pm 5.26$	$1.41^{d} \pm 0.18$
	0.25	$6.03^{e} \pm 1.60$	$14.73^{e} \pm 2.14$	$19.25^{g} \pm 1.38$	$0.64^{f} \pm 0.05$
	0.50	$5.74^{ef} \pm 0.15$	$8.01^{fg} \pm 2.16$	$9.48^{i} \pm 1.77$	$0.32^{g} \pm 0.06$
1.0 m*M* SaA	0	$14.73^{bc} \pm 0.88$	$31.05^{b} \pm 2.25$	$40.94^{d} \pm 5.48$	$1.36^{d} \pm 0.18$
	0.25	$10.03^{d} \pm 2.05$	$23.16^{c} \pm 3.11$	$28.76^{ef} \pm 3.58$	$0.96^{e} \pm 0.12$
	0.50	$10.17^{d} \pm 1.63$	$9.17^{f} \pm 1.65$	$14.53^{h} \pm 1.31$	$0.48^{fg} \pm 0.04$
10 m*M* Pro	0	$23.64^{a} \pm 1.14$	$41.88^{a} \pm 6.39$	$85.82^{a} \pm 2.35$	$2.86^{a} \pm 0.08$
	0.25	$8.90^{de} \pm 1.70$	$13.80^{e} \pm 3.50$	$19.30^{g} \pm 5.40$	$0.60^{f} \pm 0.18$
	0.50	$6.69^{e} \pm 1.27$	$13.81^{e} \pm 3.65$	$19.35^{g} \pm 5.54$	$0.65^{f} \pm 0.18$
20 m*M* Pro	0	$19.85^{ab} \pm 0.35$	$41.57^{a} \pm 1.76$	$47.49^{c} \pm 3.88$	$1.58^{d} \pm 0.13$
	0.25	$10.93^{de} \pm 2.26$	$16.51^{e} \pm 1.40$	$23.51^{f} \pm 4.54$	$0.78^{f} \pm 0.15$
	0.50	$11.12^{de} \pm 5.16$	$14.45^{e} \pm 4.93$	$18.14^{g} \pm 2.90$	$0.60^{f} \pm 0.10$
0.5 m*M* SaA + 10 m*M* Pro	0	$11.93^{de} \pm 2.34$	$24.85^{c} \pm 1.39$	$44.63^{d} \pm 3.06$	$1.49^{d} \pm 0.10$
	0.25	$10.44^{de} \pm 2.69$	$20.39^{cd} \pm 1.44$	$35.78^{e} \pm 5.35$	$1.19^{e} \pm 0.18$
	0.50	$4.05^{ef} \pm 0.34$	$8.18^{fg} \pm 1.11$	$15.19^{h} \pm 1.55$	$0.51^{fg} \pm 0.05$
0.5 m*M* SaA + 20 m*M* Pro	0	$19.28^{ab} \pm 0.43$	$23.57^{c} \pm 1.93$	$33.35^{e} \pm 3.32$	$1.11^{e} \pm 0.11$
	0.25	$7.85^{e} \pm 0.17$	$17.63^{cd} \pm 0.55$	$26.65^{f} \pm 4.10$	$0.89^{ef} \pm 0.14$
	0.50	$3.78^{f} \pm 0.29$	$8.56^{fg} \pm 0.59$	$10.39^{i} \pm 1.05$	$0.35^{h} \pm 0.03$
1.0 m*M* SaA + 10 m*M* Pro	0	$15.18^{c} \pm 1.87$	$29.03^{b} \pm 2.19$	$50.50^{c} \pm 3.10$	$1.68^{c} \pm 0.10$
	0.25	$10.43^{e} \pm 1.17$	$13.63^{e} \pm 2.71$	$13.63^{h} \pm 1.48$	$0.61^{f} \pm 0.05$
	0.50	$4.67^{ef} \pm 0.64$	$7.56^{g} \pm 1.30$	$12.49^{hi} \pm 1.25$	$0.42^{g} \pm 0.04$
1.0 m*M* SaA + 20 m*M* Pro	0	$18.16^{bc} \pm 3.85$	$27.43^{b} \pm 5.56$	$71.61^{b} \pm 7.34$	$2.39^{b} \pm 0.24$
	0.25	$13.42^{d} \pm 2.89$	$19.46^{d} \pm 6.38$	$35.15^{e} \pm 5.36$	$1.17^{e} \pm 0.18$
	0.50	$10.27^{d} \pm 2.77$	$10.77^{ef} \pm 2.42$	$14.45^{h} \pm 3.05$	$0.48^{g} \pm 0.10$

The data represent means of three replicates (± standard deviation). The values within one column followed by the same letter do not differ significantly after the Factorial ANOVA post hoc Newman-Keuls analysis at significance level of $p < 0.01$.
SiA – silicic acid; SaA – salicylic acid; Pro - proline.

priming treatments enabled the development of the seedlings under the highest used Cd concentration. The highest fresh weight of shoots recorded under high Cd concentration (0.5 m*M* Cd) was for the plants primed with 1% SiA and 1.0 m*M* SaA (16.67 ± 1.88 and 14.11 ± 1.27 mg/plant, respectively). The same trend was recorded for the dry mass of the shoots with a significant reduction of mass according to increasing Cd concentration, the highest recorded shoot dry mass for medium containing 0.5 m*M* Cd was for 20 m*M* Pro (4.43 ± 0.52 mg/plant). Water content in shoots showed an increase related to Cd concentration when control was compared to Cd stress plants with highest water content for plants grown on the medium containing 0.5 m*M* Cd, ranging from 91.09% ± 8.66 (0.5 m*M* SaA combined with 20 m*M* Pro) to 62.63% ± 1.26 (20 m*M* Pro).

For roots, similar trends were observed, with the reduction of fresh and dry weight and increase of water content according to the increase of Cd concentration in the medium. Under high Cd stress conditions (0.5 m*M* Cd) the highest root fresh weight per plant was recorded for plants primed with 1.0 m*M* SaA and 0.5 m*M* SaA combined with 20 m*M* Pro (2.15 ± 0.32 and 2.04 ± 0.15 mg/plant, respectively). The plants grown under this Cd concentration also showed high water content in roots, especially plants primed with 1.0 m*M* SaA combined with 10 m*M* Pro (90.89% ± 3.71).

3.2. Effect of seed priming on Cd adsorption, accumulation, and translocation in Silene sendtneri seedlings

For a plant to be hyper-accumulator it is expected that the above-ground concentration of Cd (in aerial arts of the plat) exceeds 1% of plant dry weight. Since before this research there was no data regarding Cd accumulation in *Silene sendtneri* we analysed the concentration of accumulated Cd in shoots and roots and determined bioconcentration and translocation factors (Table 4). Since Cd is added to the medium in very small concentrations as a micronutrient, we also included control plants in the analysis of Cd absorption from the media. For control plants low concentration of Cd was recorded in shoots ranging from 1.22 ± 0.17 (0.5 SaA combined with 20 m*M* Pro) up to 8.08 ± 0.00 mg/kg DW (1.0 m*M* SaA) and from 3.98 ± 0.19 (1.0 m*M* SaA combined with 20 m*M* Pro) up to 22.78 ± 0.28 mg/kg DW (20 m*M* Pro) for roots. Under moderate Cd stress (0.25 m*M* Cd) the highest Cd concentration for shoots was recorded for plants primed with 1% SiA (1465.40 ± 20.00 mg/kg DW), while the lowest recorded was for plants primed with 0.5 m*M* SaA combined with 20 m*M* Pro (992.03 ± 10.02 mg/kg DW). For the roots highest Cd concentration was recorded for hydro-primed plants (783.22 ± 7.11 mg/kg DW) and the lowest for 0.5 m*M* SaA (490.97 ± 2.96 mg/kg DW). When the plants were subjected to high dosages of Cd (0.5 m*M* Cd) the concentration of Cd in the shoots increased to the maximum of 2156.10 ± 1.10 mg/kg DW for plants primed with 1.0 m*M* SaA. While for roots the highest concentration of Cd was recorded for plants primed with 10 m*M* Pro (1795.84 ± 18.14 mg/kg DW), (Table 4).

The bioaccumulation factor for shoots of plants grown under moderate Cd stress (0.25 m*M* Cd) ranging from 0.14 up to 0.25 (non-primed plants and 1% silicic acid, respectively), and for the roots from 0.8 up to 0.14 (0.5 m*M* SaA and 1.0 m*M* SaA, respectively). Under high Cd stress the bioconcentration factor for the shoots ranging from 12.00 (0.5 SaA combined with 20 m*M* Pro) to 18.00 (1.0 m*M* SaA), and for the roots from 0.09 (1% silicic acid) to 0.15 (1.0 m*M* SaA combined with 10 m*M* Pro), (Table 4).

The highest translocation factor of 2.29 was recorded for the plants primed with 1% silicic acid and grown under moderate Cd stress (0.25 m*M* Cd). The translocation factor above 1.5 was recorded for several priming treatments growing under moderate Cd stress, the highest used dosage of Cd-induced reduction of translocation factor in correlation to reduction of fresh and dry weight (Table 4).

Table 3
Effect of seed priming on fresh and dry weight and water content in *Silene sendtneri* seedlings under cadmium stress.

Priming agent	Cd (m*M*)	Roots			Shoots		
		FW (mg/plant)	DW (mg/plant)	WC (%)	FW (mg/plant)	DW (mg/plant)	WC (%)
Non-primed	0	$2.85^{b} \pm 0.47$	$0.63^{d} \pm 0.16$	$78.08^{bc} \pm 1.81$	$27.65^{a} \pm 1.56$	$8.00^{b} \pm 1.46$	$71.85^{c} \pm 5.55$
	0.25	$1.99^{c} \pm 0.24$	$0.32^{ef} \pm 0.06$	$83.77^{b} \pm 3.09$	$16.21^{e} \pm 1.74$	$2.83^{f} \pm 0.63$	$82.61^{bc} \pm 1.37$
	0.50	lc	lc	lc	lc	lc	lc
Hydro-primed	0	$3.74^{a} \pm 0.48$	$1.09^{a} \pm 0.12$	$70.71^{cd} \pm 4.10$	$25.97^{b} \pm 1.09$	$10.49^{a} \pm 1.33$	$58.27^{de} \pm 2.72$
	0.25	$2.41^{bc} \pm 0.66$	$0.46^{e} \pm 0.11$	$79.00^{bc} \pm 1.14$	$18.03^{e} \pm 1.41$	$2.66^{fg} \pm 0.62$	$84.50^{b} \pm 6.49$
	0.50	$1.99^{c} \pm 0.49$	$0.22^{g} \pm 0.01$	$88.48^{ab} \pm 2.68$	$11.21^{g} \pm 1.47$	$1.20^{gh} \pm 0.49$	$88.88^{ab} \pm 5.86$
1% SiA	0	$2.55^{bc} \pm 0.82$	$0.61^{d} \pm 0.27$	$76.90^{bc} \pm 4.64$	$21.87^{cd} \pm 1.31$	$8.69^{ab} \pm 1.10$	$61.91^{d} \pm 7.67$
	0.25	$1.70^{c} \pm 0.42$	$0.41^{e} \pm 0.01$	$74.50^{c} \pm 7.50$	$16.40^{e} \pm 1.87$	$4.30^{de} \pm 1.15$	$72.66^{c} \pm 1.70$
	0.50	$1.91^{c} \pm 0.18$	$0.32^{ef} \pm 0.07$	$83.17^{b} \pm 2.25$	$16.67^{e} \pm 1.88$	$3.89^{e} \pm 0.80$	$76.38^{c} \pm 6.14$
0.5 m*M* SaA	0	$3.19^{ab} \pm 0.53$	$0.80^{bc} \pm 0.19$	$74.17^{c} \pm 1.27$	$26.00^{ab} \pm 2.81$	$10.06^{a} \pm 1.40$	$58.92^{de} \pm 6.98$
	0.25	$1.50^{c} \pm 0.17$	$0.47^{e} \pm 0.19$	$67.59^{d} \pm 2.21$	$14.10^{f} \pm 1.83$	$4.83^{d} \pm 0.25$	$63.96^{d} \pm 1.64$
	0.50	$1.94^{c} \pm 0.20$	$0.45^{e} \pm 0.04$	$76.87^{bc} \pm 1.62$	$11.88^{g} \pm 1.99$	$3.55^{e} \pm 1.65$	$68.82^{cd} \pm 3.33$
1.0 m*M* SaA	0	$3.14^{ab} \pm 0.69$	$0.79^{cd} \pm 0.13$	$74.51^{c} \pm 1.94$	$14.71^{f} \pm 1.44$	$1.69^{gh} \pm 0.77$	$88.84^{ab} \pm 1.64$
	0.25	$2.19^{bc} \pm 0.54$	$0.43^{e} \pm 0.10$	$80.07^{b} \pm 1.65$	$23.94^{c} \pm 1.54$	$3.72^{e} \pm 0.48$	$80.79^{bc} \pm 1.18$
	0.50	$2.15^{bc} \pm 0.32$	$0.36^{ef} \pm 0.08$	$82.93^{b} \pm 1.62$	$14.11^{f} \pm 1.27$	$3.71^{e} \pm 0.52$	$73.50^{c} \pm 3.62$
10 m*M* Pro	0	$3.04^{ab} \pm 0.46$	$0.84^{bc} \pm 0.26$	$71.19^{cd} \pm 4.14$	$21.13^{cd} \pm 1.08$	$9.10^{a} \pm 1.45$	$57.12^{de} \pm 2.86$
	0.25	$2.31^{bc} \pm 0.62$	$0.39^{ef} \pm 0.13$	$81.16^{b} \pm 1.65$	$20.70^{d} \pm 1.93$	$4.12^{de} \pm 1.07$	$79.99^{c} \pm 1.91$
	0.50	$1.80^{c} \pm 0.17$	$0.23^{f} \pm 0.02$	$87.24^{ab} \pm 1.55$	$10.18^{g} \pm 0.18$	$2.95^{f} \pm 0.96$	$70.94^{c} \pm 5.93$
20 m*M* Pro	0	$1.88^{c} \pm 0.05$	$0.44^{e} \pm 0.06$	$76.55^{bc} \pm 2.32$	$17.97^{e} \pm 0.71$	$6.99^{c} \pm 1.72$	$61.21^{d} \pm 4.08$
	0.25	$2.17^{bc} \pm 0.55$	$0.44^{e} \pm 0.05$	$79.23^{bc} \pm 3.01$	$21.36^{c} \pm 1.46$	$6.70^{c} \pm 0.89$	$68.19^{cd} \pm 5.89$
	0.50	$1.64^{c} \pm 0.12$	$0.34^{ef} \pm 0.02$	$78.99^{bc} \pm 4.14$	$11.92^{g} \pm 1.50$	$4.43^{de} \pm 0.52$	$62.63^{d} \pm 1.26$
0.5 m*M* SaA + 10 m*M* Pro	0	$2.17^{bc} \pm 0.23$	$0.52^{e} \pm 0.13$	$76.33^{bc} \pm 2.45$	$16.62^{e} \pm 1.86$	$6.30^{c} \pm 1.07$	$62.77^{d} \pm 2.36$
	0.25	$2.06^{bc} \pm 0.05$	$0.55^{ed} \pm 0.15$	$73.36^{c} \pm 4.10$	$20.05^{c} \pm 1.36$	$5.96^{cd} \pm 1.07$	$70.68^{c} \pm 1.01$
	0.50	$0.79^{d} \pm 0.70$	$0.07^{g} \pm 0.06$	$94.07^{a} \pm 1.69$	$3.54^{h} \pm 0.24$	$0.62^{h} \pm 0.54$	$83.18^{b} \pm 7.84$
0.5 m*M* SaA + 20 m*M* Pro	0	$3.08^{ab} \pm 0.01$	$0.61^{d} \pm 0.00$	$80.38^{b} \pm 5.33$	$25.14^{b} \pm 0.00$	$4.10^{de} \pm 0.03$	$83.69^{b} \pm 2.55$
	0.25	$2.43^{bc} \pm 0.21$	$0.44^{e} \pm 0.06$	$81.98^{b} \pm 2.11$	$16.16^{e} \pm 1.53$	$2.59^{f} \pm 1.35$	$84.07^{b} \pm 1.76$
	0.50	$2.04^{bc} \pm 0.14$	$0.27^{f} \pm 0.00$	$86.57^{ab} \pm 2.96$	$9.99^{g} \pm 0.99$	$0.91^{h} \pm 0.36$	$91.09^{a} \pm 8.66$
1.0 m*M* SaA + 10 m*M* Pro	0	$2.10^{bc} \pm 0.36$	$0.74^{bc} \pm 0.07$	$64.32^{d} \pm 1.05$	$18.43^{e} \pm 1.42$	$10.02^{a} \pm 1.02$	$45.22^{e} \pm 3.75$
	0.25	$2.85^{b} \pm 0.21$	$0.45^{e} \pm 0.10$	$84.34^{b} \pm 4.36$	$24.03^{bc} \pm 0.56$	$7.31^{cb} \pm 1.06$	$69.44^{cd} \pm 1.42$
	0.50	$1.76^{c} \pm 0.26$	$0.15^{f} \pm 0.07$	$90.89^{a} \pm 3.71$	$11.80^{g} \pm 1.39$	$1.23^{gh} \pm 0.17$	$89.09^{ab} \pm 6.03$
1.0 m*M* SaA + 20 m*M* Pro	0	$3.26^{ab} \pm 0.18$	$0.80^{bc} \pm 0.23$	$75.33^{bc} \pm 5.31$	$19.50^{d} \pm 1.43$	$6.00^{c} \pm 0.49$	$70.26^{c} \pm 2.54$
	0.25	$1.96^{c} \pm 0.54$	$0.49^{e} \pm 0.06$	$73.91^{c} \pm 7.50$	$17.05^{e} \pm 1.10$	$3.90^{ef} \pm 0.84$	$76.40^{bc} \pm 1.92$
	0.50	$1.98^{c} \pm 0.31$	$0.25^{f} \pm 0.14$	$87.73^{ab} \pm 6.92$	$11.58^{g} \pm 1.10$	$1.89^{gh} \pm 0.53$	$83.74^{b} \pm 1.86$

FW- fresh weight; DW- dry weight; WC- water content; lc - lethal concentration (too small plants to be harvested for analysis). Data represent means of three replicates (± standard deviation). The values within one column followed by the same letter do not differ significantly after the Factorial ANOVA post hoc Newman-Keuls analysis at significance level of $p < 0.01$.
SiA – silicic acid; SaA – salicylic acid; Pro - proline.

3.3. Effect of seed priming on peroxidase activity in shoots and roots of Silene sendtneri seedlings grown under Cd stress

The analysis of peroxidase activity showed lower activity in shoots compared to the activity in roots for all primed plants, although this trend was not observed for non-primed plants (Table 5). The variation of peroxidase activity in the shoots showed no correlation with the concentration of Cd. Identified variation was related to priming, where different priming treatments resulted in different peroxidase activity responses to an increase in Cd concentration. For some treatments an increase in Cd concentration induced an increase in peroxidase activity (10 m*M* Pro), while for most of the treatments the highest Cd concentration induced a drop in peroxidase activity in the shoots. In the roots, significant increases in peroxidase activity were measured for the plants primed with 0.5 m*M* SaA combined with 20 m*M* Pro and 1.0 m*M* SaA combined with 20 m*M* Pro for all used Cd concentrations. In the first case significant increase of peroxidase activity was recorded in the roots of plants grown under the highest Cd stress, while the latter was associated with the drop of peroxidase activity in the roots of plants grown under the highest Cd stress.

The concentration of protein varied significantly in relation to priming and Cd concentration as well, ranging from 2.45 ± 0.12 mg/g FW to 9.74 ± 0.43 mg/g FW for the shoots and from 0.75 ± 0.01 mg/g FW to 5.19 ± 0.54 mg/g FW for the roots (Table 5). For several priming treatments rise of protein content in shoots was related to the increase in Cd concentration in the medium (1.0 m*M* SaA combined with 10 m*M* proline; 10 m*M* Pro etc.), while in treatment with 1% silicic acid the decrease of protein content was recorded. A similar trend was recorded for protein content in roots, with no obvious trend of concentration change for all priming treatments.

3.3.1. Effect of seed priming on the production of secondary metabolites in shoots of Silene sendtneri seedlings grown under Cd stress

The UHPLC/MS-MS analysis of composition and concentration of phenolic acids and flavonoids in shoots of *S. sendtneri* seedlings showed differences between priming treatments as well as between the differences in Cd concentration used (Fig. 1; Supplement 1). For the large number of priming treatments, a trend of decrease in 4-hydroxybenzoic acid, caffeic acid and salicylic acid glucoside was recorded, with an increase in ferulic acid content related to an increase of Cd concentration in the medium. The increase of ferulic acid within one priming treatment ranged from 21.59 ± 0.23 (0 m*M* Cd) to 119.59 ± 0.06 (0.5 m*M* Cd), as recorded for shoots of plants primed with 0.5 m*M* SaA. For flavonoids, a high increase of rutin for plants primed with salicylic acid and proline compared to the non-primed and other primed plants was recorded.

3.3.2. Effect of seed priming on the production of organic acids in shoots of Silene sendtneri seedlings grown under Cd stress

The analysis of organic content showed some interferences during the measurement of oxalic acid and some of the data are missing (Table 6). The relationship between the concentration of organic acid and Cd concentration in the medium was different between different priming treatments. In a case of non-primed plants and plants primed with 1% SiA, 10 m*M*, and 20 m*M* Pro the drop in the concentration of oxalic, malic, and citric acid was recorded when plants were exposed to

Table 4
Effect of seed priming on cadmium concentration, bioconcentration and translocation factor in *Silene sendtneri* seedlings.

Priming agent	Cd (m*M*)	Cd roots (mg/kgDW)	Root BCF	Cd shoots (mg/kgDW)	Shoot BCF	TF
Non-primed	0	$6.65^{k} \pm 0.37$	–	$4.96^{k} \pm 0.06$	–	$0.71^{k} \pm 0.04$
	0.25	$691.45^{i} \pm 6.98$	$0.12^{d} \pm 0.01$	$1235.70^{g} \pm 0.00$	$0.14^{g} \pm 0.00$	$1.18^{g} \pm 0.02$
	0.50	Lc	Lc	Lc	Lc	Lc
Hydro-primed	0	$16.43^{k} \pm 0.22$	–	$7.37^{k} \pm 0.36$	–	$0.46^{o} \pm 0.02$
	0.25	$783.22^{h} \pm 7.11$	$0.13^{c} \pm 0.00$	$1061.07^{i} \pm 50.02$	$0.19^{c} \pm 0.01$	$1.41^{f} \pm 0.05$
	0.50	$1580.26^{d} \pm 14.35$	$0.14^{b} \pm 0.00$	$1399.56^{f} \pm 118.52$	$0.13^{g} \pm 0.01$	$0.95^{i} \pm 0.07$
1% SiA	0	$11.01^{k} \pm 0.59$	–	$5.30^{k} \pm 0.04$	–	$0.46^{o} \pm 0.02$
	0.25	$641.07^{i} \pm 6.48$	$0.11^{e} \pm 0.00$	$1465.40^{e} \pm 20.00$	$0.25^{a} \pm 0.00$	$2.29^{a} \pm 0.03$
	0.50	$1047.42^{g} \pm 10.58$	$0.09^{g} \pm 0.00$	$1877.40^{c} \pm 25.17$	$0.16^{f} \pm 0.00$	$1.78^{c} \pm 0.04$
0.5 m*M* SaA	0	$14.99^{k} \pm 0.64$	–	$4.82^{k} \pm 0.37$	–	$0.28^{r} \pm 0.03$
	0.25	$490.97^{j} \pm 2.96$	$0.08^{h} \pm 0.00$	$1182.10^{h} \pm 6.96$	$0.20^{bc} \pm 0.00$	$2.39^{a} \pm 0.02$
	0.50	$1266.71^{f} \pm 12.79$	$0.11^{e} \pm 0.00$	$1949.20^{b} \pm 17.61$	$0.16^{f} \pm 0.00$	$1.51^{e} \pm 0.03$
1.0 m*M* SaA	0	$13.93^{k} \pm 0.43$	–	$8.08^{k} \pm 0.00$	–	$0.56^{n} \pm 0.02$
	0.25	$793.16^{h} \pm 8.01$	$0.14^{b} \pm 0.00$	$1226.00^{g} \pm 0.03$	$0.21^{b} \pm 0.00$	$1.53^{e} \pm 0.02$
	0.50	$1479.13^{e} \pm 11.93$	$0.13^{c} \pm 0.00$	$2156.10^{a} \pm 1.10$	$0.18^{cd} \pm 0.00$	$1.45^{f} \pm 0.01$
10 m*M* Pro	0	$3.99^{k} \pm 0.64$	–	$1.76^{k} \pm 0.17$	–	$0.30^{p} \pm 0.26$
	0.25	$672.20^{g} \pm 8.16$	$0.12^{d} \pm 0.00$	$1050.09^{i} \pm 13.83$	$0.18^{c} \pm 0.00$	$1.56^{e} \pm 0.00$
	0.50	$1795.84^{a} \pm 18.14$	$0.15^{a} \pm 0.00$	$1687.11^{d} \pm 18.76$	$0.14^{g} \pm 0.00$	$0.94^{i} \pm 0.00$
20 m*M* Pro	0	$22.78^{k} \pm 0.28$	–	$5.10^{k} \pm 0.00$	–	$0.22^{r} \pm 0.00$
	0.25	$587.19^{j} \pm 5.93$	$0.10^{f} \pm 0.00$	$1107.80^{h} \pm 12.22$	$0.19^{c} \pm 0.00$	$1.86^{c} \pm 0.02$
	0.50	$1533.59^{d} \pm 12.37$	$0.13^{c} \pm 0.00$	$1860.00^{c} \pm 39.10$	$0.16^{f} \pm 0.00$	$1.20^{g} \pm 0.03$
0.5 m*M* SaA + 10 m*M* Pro	0	$8.97^{k} \pm 0.38$	–	$6.28^{k} \pm 0.34$	–	$0.71^{k} \pm 0.01$
	0.25	$591.91^{j} \pm 5.38$	$0.10^{f} \pm 0.00$	$1032.44^{i} \pm 9.38$	$0.18^{cde} \pm 0.00$	$1.74^{c} \pm 3.56$
	0.50	$1622.23^{c} \pm 13.08$	$0.14^{b} \pm 0.00$	$1607.45^{d} \pm 14.60$	$0.14^{g} \pm 0.00$	$0.99^{i} \pm 0.00$
0.5 m*M* SaA + 20 m*M* Pro	0	$16.34^{k} \pm 1.40$	–	$1.22^{k} \pm 0.14$	–	$0.08^{s} \pm 0.00$
	0.25	$650.91^{i} \pm 6.57$	$0.11^{e} \pm 0.00$	$992.03^{j} \pm 10.02$	$0.17^{e} \pm 0.00$	$1.52^{e} \pm 0.00$
	0.50	$1526.53^{d} \pm 15.42$	$0.13^{c} \pm 0.00$	$1375.46^{f} \pm 12.49$	$0.12^{h} \pm 0.00$	$0.90^{i} \pm 0.00$
1.0 m*M* SaA + 10 m*M* Pro	0	$5.93^{k} \pm 0.29$	–	5.30k ± 0.00	–	$0.85^{j} \pm 0.04$
	0.25	$762.83^{h} \pm 6.93$	$0.13^{c} \pm 0.00$	$1250.00^{g} \pm 0.00$	$0.21^{b} \pm 0.00$	$1.62^{d} \pm 0.02$
	0.50	$1700.51^{b} \pm 17.18$	$0.15^{a} \pm 0.00$	$2105.80^{a} \pm 0.00$	$0.18^{cd} \pm 0.00$	$1.23^{g} \pm 0.01$
1.0 m*M* SaA + 20 m*M* Pro	0	$3.98^{k} \pm 0.19$	–	$2.77^{k} \pm 0.11$	–	$0.69^{m} \pm 0.01$
	0.25	$504.77^{j} \pm 1.43$	$0.10^{f} \pm 0.00$	$1219.33^{g} \pm 12.32$	$0.21^{b} \pm 0.00$	$2.04^{b} \pm 0.01$
	0.50	$1436.24^{e} \pm 11.58$	$0.12^{d} \pm 0.00$	$1590.43^{d} \pm 14.45$	$0.14^{g} \pm 0.00$	$1.11^{h} \pm 0.00$

The data represent means of three replicates (± standard deviation). The values within one column followed by the same letter do not differ significantly after the Factorial ANOVA post hoc Newman-Keuls analysis at significance level of $p < 0.01$.
SiA – silicic acid; SaA – salicylic acid; Pro - proline.

Cd. For hydropriming, and all priming treatments containing SaA except for 0.5 mM SaA a drop of oxalic acid was recorded but the concentration of malic and citric acid increased in the shoots of plants grown on high Cd concentrations compared to lower Cd concentrations (Table 6).

4. Discussion

Germination as a crucial physiological process is the first event in plant development that is under the direct effect of toxic Cd in the soil. Different priming agents and techniques can be used to alleviate some of the adverse effects of heavy metals on the germination process (Karalija and Selović, 2018; Šabanović et al., 2018; Rao et al., 2019). The presented study showed that some SaA concentrations (0.5 m*M*) can inhibit seed germination while others (1.0 m*M*), stimulate it. Selection of priming agent used and its concentration was based on preliminary studies that included testing wider range of concentrations of all priming agents in the study, and to evaluate their effect on plant growth. Concentrations with minimal negative effect or stimulating effect on plant growth were selected to evaluate priming effect on Cd accumulation. Processes that precede radicle emergence are delicate and precisely controlled, several phytohormones play a crucial role in this process. Ratio of ABA and GA can be influenced by the addition of SaA. In cases where SaA inhibited germination, it could be connected to enhanced ABA synthesis which can stop germination, while increased SaA concentration could activate different mechanisms such as stress-induced response in germinated seeds. For different species, different SaA inhibitory concentrations have been recorded indicating that this process is species-specific (Anandhi and Ramanujam, 1997; Negi and Prasad, 2001; Chandra et al., 2007). The presented study indicated that priming can be effective in the alleviation of adverse effects of Cd on the germination process itself and further to increase tolerance of emerging seedlings to Cd. Not only the type of the priming agent but also the dosage applied had a diverse effect on the level of Cd tolerance during the germination phase and the emergence of seedlings. A decisive role of a dose of priming agent was also noticed in *Urtica dioica* (Kalaycioglu, 2005). Moreover, it was pointed out that priming agents could increase germination in polluted soils but can be less effective in non-polluted soils (Galhautet al, 2014). However, bearing in mind the results of our research where several priming treatments increased the germination rate of control seeds as well as under Cd stress the phenomenon is not general, and is dependent on the chemical nature of the priming agent.

The root is the first plant organ that senses heavy metals in the soil and is strongly affected by Cd which results in inhibition of primary root growth, and often stimulation of lateral root formation (Xu et al., 2010; Yuan and Huang, 2016; Akar and Atis, 2018). In this research we showed a general increase of root growth in the primed plants related to non-primed seed, cultivated on the same Cd supplemented media. Priming with Pro was the most effective in stimulation of root growth under high Cd stress. Accumulation of Pro in plants as a response to exposure to heavy metals has been widely reported (Costa and Morel, 1994; Sun et al., 2007; Tamas et al., 2008; Hossain et al., 2010; Karalija and Selović, 2018). Exogenously applied Pro can induce enhanced tolerance of biotic oxidative stress (Park et al., 2006; Hoque et al., 2007; Huang et al., 2009; Hossain and Fujita, 2010; Islam et al., 2010), and using Pro as a priming agent is one of the methods to induce this enhanced tolerance. Since the root is the first organ that is affected by heavy metal toxicity it is also the first line of defence responsible for significantly contributing to heavy metal tolerance. It is debated that plants' ability to tolerate heavy metals is not the result of an accumulation of different phytochemicals in the shoot but rather is the result of defending mechanisms in the root cells themselves (Ernst, 1994).

The negative effects of adsorbed Cd on plant growth are usually

Table 5
Effect of seed priming on protein content and peroxidase activity in *Silene sendtneri* under cadmium stress.

Priming agent	Cd (m*M*)	Root		Shoot	
		TP (mg/gFW)	Peroxidase activity (UNITS/mgprot/min)	TP (mg/gFW)	Peroxidase activity (UNITS/mgprot/min)
Non-primed	0	$2.41^{f} \pm 0.12$	$7.88^{n} \pm 0.34$	$5.37^{d} \pm 0.42$	$18.16^{jk} \pm 0.44$
	0.25	$2.51^{f} \pm 0.05$	$37.65^{jk} \pm 7.44$	$6.96^{bc} \pm 0.37$	$37.24^{h} \pm 5.86$
	0.50	Lc	Lc	Lc	Lc
Hydro-primed	0	$1.53^{j} \pm 0.11$	$64.69^{h} \pm 7.45$	$6.12^{c} \pm 0.70$	$13.04^{l} \pm 2.59$
	0.25	$1.1^{l} \pm 0.12$	$88.19^{g} \pm 12.38$	$3.83^{ef} \pm 0.24$	$39.07^{h} \pm 1.73$
	0.50	$1.44^{jk} \pm 0.26$	$97.86^{fg} \pm 11.46$	$9.00^{a} \pm 0.17$	$17.19^{jkl} \pm 1.79$
1% SiA	0	$2.02^{h} \pm 0.18$	$129.99^{d} \pm 20.54$	$6.37^{c} \pm 0.42$	$11.40^{m} \pm 1.45$
	0.25	$1.47^{jk} \pm 0.09$	$106.98^{e} \pm 20.28$	$4.29^{e} \pm 0.21$	$59.55^{e} \pm 3.00$
	0.50	$2.70^{e} \pm 0.09$	$14.38^{lmn} \pm 2.85$	$4.00^{e} \pm 0.64$	$22.12^{i} \pm 5.70$
0.5 m*M* SaA	0	$3.66^{c} \pm 4.71$	$11.40^{lmn} \pm 8.431$	$2.86^{g} \pm 0.13$	$5.50^{n} \pm 0.07$
	0.25	$0.97^{l} \pm 0.03$	$31.08^{jk} \pm 1.05$	$2.46^{g} \pm 0.12$	$9.36^{n} \pm 0.65$
	0.50	$1.17^{l} \pm 0.08$	$61.62^{hi} \pm 13.50$	$5.46^{d} \pm 0.22$	$24.76^{i} \pm 2.74$
1.0 m*M* SaA	0	$1.49^{j} \pm 0.05$	$89.97^{g} \pm 10.72$	$4.96^{de} \pm 0.36$	$16.78^{jkl} \pm 1.16$
	0.25	$1.35^{k} \pm 0.03$	$24.82^{klm} \pm 0.51$	$4.38^{e} \pm 0.14$	$20.78^{i} \pm 0.51$
	0.50	$0.99^{l} \pm 0.09$	$57.22^{hi} \pm 4.93$	$5.37^{d} \pm 0.22$	$23.92^{i} \pm 0.51$
10 m*M* Pro	0	$5.19^{a} \pm 0.54$	$27.38^{kl} \pm 3.14$	$4.68^{e} \pm 0.08$	$62.36^{d} \pm 8.52$
	0.25	$1.92^{i} \pm 0.08$	$74.29^{h} \pm 3.57$	$5.65^{d} \pm 0.19$	$22.84^{i} \pm 3.10$
	0.50	$1.12^{l} \pm 0.02$	$58.29^{hi} \pm 1.00$	$5.87^{cd} \pm 0.23$	$80.48^{a} \pm 1.86$
20 m*M* Pro	0	$3.05^{d} \pm 0.26$	$61.84^{hi} \pm 5.95$	$6.28^{c} \pm 0.39$	$21.68^{i} \pm 1.71$
	0.25	$1.69^{j} \pm 0.19$	$62.33^{hi} \pm 5.01$	$6.05^{cd} \pm 0.12$	$25.19^{i} \pm 1.38$
	0.50	$2.32^{g} \pm 0.09$	$16.53^{lmn} \pm 0.70$	$9.18^{a} \pm 0.66$	$16.19^{jk} \pm 1.11$
0.5 m*M* SaA + 10 m*M* Pro	0	$1.52^{j} \pm 0.09$	$96.29^{fg} \pm 34.72$	$5.48^{d} \pm 0.20$	$24.97^{i} \pm 1.59$
	0.25	$1.31^{k} \pm 0.07$	$64.96^{h} \pm 16.55$	$5.00^{d} \pm 0.26$	$17.66^{jk} \pm 1.89$
	0.50	$3.04^{d} \pm 0.18$	$51.10^{hi} \pm 0.29$	$9.74^{a} \pm 0.43$	$19.95^{ij} \pm 1.01$
0.5 m*M* SaA + 20 m*M* Pro	0	$0.96^{l} \pm 0.04$	$191.22^{b} \pm 7.89$	$4.00^{e} \pm 0.04$	$73.62^{b} \pm 2.11$
	0.25	$2.05^{h} \pm 0.10$	$100.11^{fg} \pm 6.16$	$7.35^{b} \pm 0.27$	$52.12^{f} \pm 0.96$
	0.50	$1.17^{l} \pm 0.01$	$250.50^{a} \pm 5.97$	$4.46^{e} \pm 0.14$	$19.99^{ij} \pm 0.61$
1.0 m*M* SaA + 10 m*M* Pro	0	$4.79^{b} \pm 0.14$	$67.10^{h} \pm 2.36$	$4.81^{de} \pm 0.21$	$20.35^{i} \pm 1.18$
	0.25	$1.00^{l} \pm 0.09$	$38.77^{j} \pm 5.73$	$4.81^{de} \pm 0.18$	$47.82^{g} \pm 3.37$
	0.50	$0.75^{m} \pm 0.01$	$43.02^{ij} \pm 4.13$	$5.09^{d} \pm 0.35$	$50.53^{f} \pm 4.03$
1.0 m*M* SaA + 20 m*M* Pro	0	$2.32^{g} \pm 0.04$	$117.03^{de} \pm 16.06$	$5.27^{d} \pm 0.03$	$18.94^{jk} \pm 1.00$
	0.25	$0.78^{m} \pm 0.03$	$198.28^{b} \pm 8.92$	$4.96^{de} \pm 0.08$	$59.70^{e} \pm 1.03$
	0.50	$2.02^{h} \pm 0.06$	$169.02^{c} \pm 4.78$	$4.93^{de} \pm 0.14$	$66.19^{c} \pm 1.85$

The data represent means of three replicates (± standard deviation). The values within one row (one phenol type) followed by the same letter do not differ significantly after Factorial ANOVA post hoc Newman-Keuls analysis at significance level of $p < 0.01$.
TP – total protein content; SiA – silicic acid; SaA – salicylic acid; Pro – proline.

manifested by inhibition of photosynthesis, oxidative stress (Asgher et al., 2015; Zhang et al., 2015), but also through the reduction of plant growth and biomass production. Reduction of plant growth was also recorded for *S. sendtneri* especially when the plant was exposed to the highest dosages of Cd. A decrease in dry matter weight under Cd stress was recorded previously. The application of various priming agents significantly induced an increase in dry mass of Cd stressed plants related to non-primed seed, although the recorded mass was still lower than the mass recorded for the shoots and root of plants growing on the media without Cd.

Cd exposure induces a decrease in water uptake which was evident for non-primed plants, especially for the highest lethal dosage of Cd for non-primed plants. Cd mimics drought stress in plants by suppressing the short distance water transport (Rucińska-Sobkowiak, 2016). Priming with Pro and SaA seems to counteract this effect and increases the content of water in shoots, reaching 91% in shoots of plants primed with 0.5 m*M* SaA and 90.89% in the roots of plants primed with 1.0 m*M* SaA combined with 10 m*M* Pro. SaA as a signalling molecule engages a wide array of metabolic responses in plants that affect plant water relations (Hayat et al., 2010).

For a plant to be classified as a Cd hyper-accumulator concentration of Cd in shoot must exceed 0.01% of the dry weight of the plant. The concentration of Cd in the shoots of *S. sendtneri* of non-primed plants grown on a medium containing 0.25 m*M* Cd was 1235.70 mg/kg DW (1.2% of the dry weight) and reached an astonishing 2156.10 mg/kg DW for the plants primed with 1.0 m*M* SaA (2.15% of the dry weight). A plant species can be considered as an efficient phytoremediator if it possesses a translocation factor greater than 1.5, and for *S. sendtneri* the highest translocation factor was 2.29. Some studies have suggested that exogenous application of Pro plays a role in the control of the influx of Cd (Zouari et al., 2016). Our results suggest that Cd translocation is not affected by the use of Pro as a priming agent, proposing that other mechanisms are activated. Pro priming is known to trigger the accumulation of osmoprotectants (Karalija and Selović, 2018) which could play a role together with phytochelatins in overcoming Cd toxicity on a cellular level (Hassan and Mansoor, 2017). The pH value is an important factor for Cd bioavailability and the transport of Cd, and values above 6 pH induce the formation of insoluble compounds such as $Cd_3[PO_4]_2$, while increased uptake of Cd is recorded for pH values bellow 4 (Banabid and Ghorab, 2014), for this reason, pH of media in this study were adjusted to 5.8 to make sure that bioavailability of Cd in the media is not compromised.

Metals, such as Cd, alter the activity of antioxidant enzymes, such as guaiacol peroxidases, by triggering metabolic profiles in a specific manner. An increase in antioxidant enzymatic activity has been recorded previously (Yang et al., 2018). Adding priming treatments to the mix induces separate triggering of plant defence responses resulting in a diverse responses of *S. sendtneri* primed seeds exposed to different levels of Cd. Overall, it seems that SaA and Pro trigger similar responses in *S. sendtneri* inducing an increase in peroxidase activity under Cd stress, in relation to 1% SiA. Cd is not a redox-active metal, and has no role in Fenton nor Haber-Weiss reaction, but it is associated with lipid peroxidation and considered to trigger oxidative stress in plants through binding of Cd to GSH and formation of GSH derived phytochelatins (Schützendübel and Polle, 2002). The role of Pro in the activation of antioxidant response is associated to activation of Pro synthesis genes under proline accumulation (Charest and Ton Phan, 1990). Proline can act as a preserving agent for enzymes (Szabados and Savouré, 2010) and

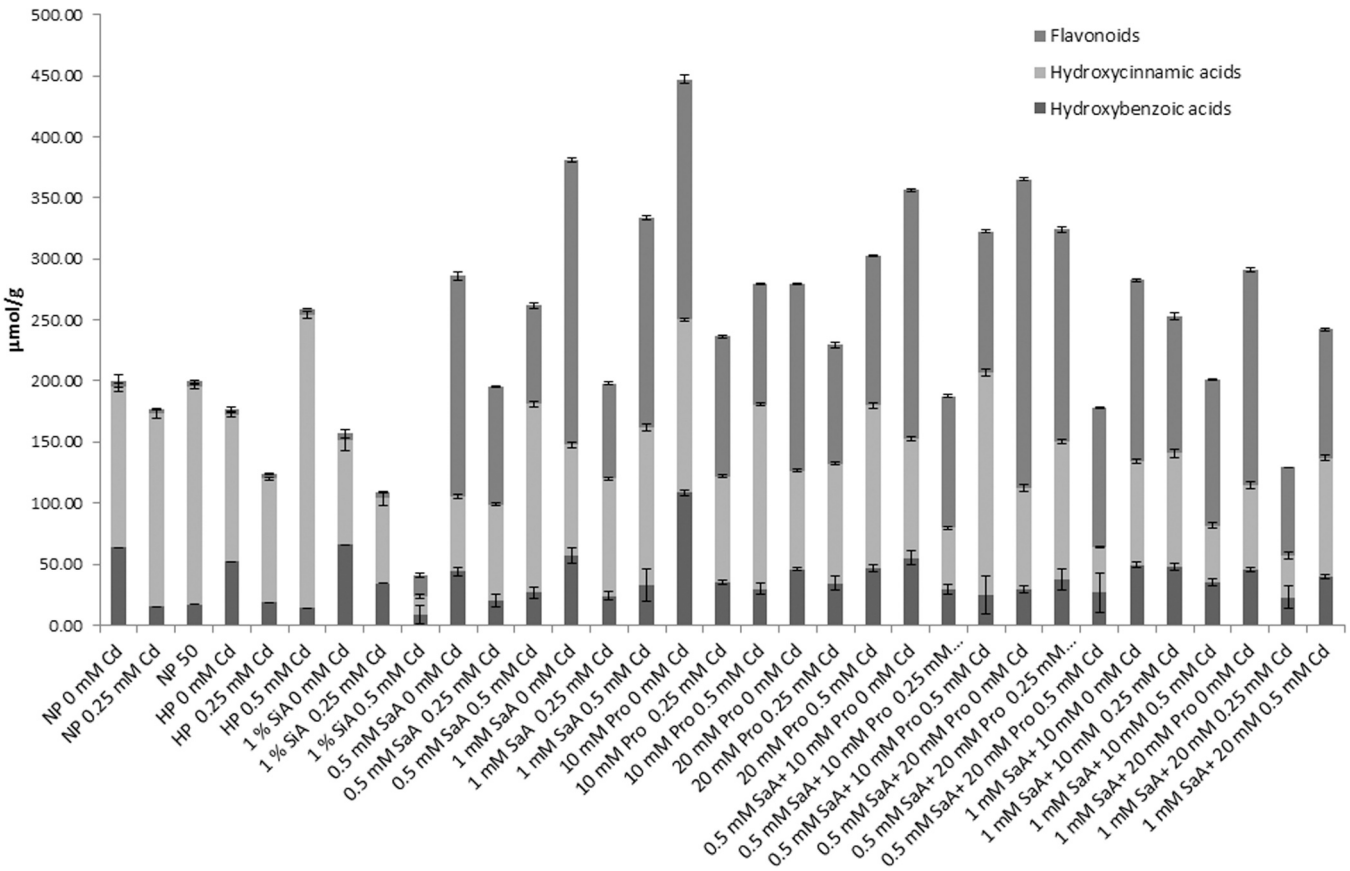

Fig. 1. Effect of seed priming on phenolic composition in shoots of *Silene sendtneri* grown under cadmium stress.

act as a singlet oxygen quencher (Matysik et al., 2002; Szabados and Savouré, 2010), thus minimizing the oxidative damage by heavy metals.

Cd profoundly affects the homeostasis in plant cells by destroying protein structure, thereby interfering with essential physiological processes such as photosynthesis. Total soluble protein content usually decreases during the heavy metal stress, but an increase upon exposure of plants to Cd was also recorded in the shoots and the roots of several primed plants (Shinwari et al., 2015). An increase in protein content after SaA priming can be attributed to the role of SaA in protein degradation (Shinwari et al., 2015) and the activation of different stress response pathways under exposure to SaA (Agastian et al., 2000; Ali et al., 2012). The relationship between an increase of protein content and drop in peroxidase activity has been previously reported and it is related to growth potential (Kraus et al., 1981). Drop in peroxidase activity is often associated with the synthesis of different anionic forms particularly affecting their role in cell wall function and lignification (Lamport, 1986).

For all plants (primed and non-primed ones) the reduction of the majority of phenolic acids (except ferulic acid) in relation to an increase of Cd concentration was recorded. A similar decrease of phenolic compounds was also observed for *Lepidium sativum* exposed to Cd (Elguera et al., 2013). The increase in the content of ferulic acid was recorded regarding to the Cd level. Literature data reveal that ferulic acid has a strong effect against heavy-metal induced oxidative stress in rats (Kelainy et al., 2019) with no data available for plants. Ferulic acid is involved in cell wall formation and rigidification because it can be cross-linked with lignins, proteins, and cell wall carbohydrates. Moreover, cell wall structures can bind metal ions, thus preventing their entrance in the cell (Schützendübel and Polle, 2002; Krzesłowska, 2011). Our result suggests that an increase in ferulic acid was a response to increased oxidative stress induced by the accumulation of Cd in shoots. Ferulic acid is a potent scavenger of free radicals and it attenuates oxidative stress (Alam, 2019). In primed plants a high spike of rutin was recorded, especially for SaA acid and Pro primed plants grown under Cd stress. The antioxidative role of flavonoids is well known (Aherne and O'Brien, 2000; Havsteen, 2002; Rusak et al., 2005) and they can protect the plant from heavy metal stress (Tumova and Ruskova, 1998; Kim et al., 1999; Michalak, 2006). Since cadmium can induce inhibition of antioxidant enzymes (Schützendübel and Polle, 2002), alternative non-enzymatic antioxidants, flavonoids, are produced (Tumova and Ruskova, 1998; Kim et al., 1999; Michalak, 2006). The increase of flavonoids results in complexation with heavy metals and an increase of defensive response to heavy metals in plants (Brown et al., 1998; Michalak, 2006; Korkina, 2007; Aherne and O'Brien, 2000; Soczynska-Kordala et al., 2001). Rutin is also known for its protective role against DNA single-strand breaks which could be a consequence of ROS generated during the Cd stress. (Aherne and O'Brien, 2000).

Organic acids such as malate, citrate, and oxalate are directly involved in the detoxification of metals on the cellular level (Anjum et al., 2015). Heavy metal binding in cell cytosol involving organic ligands such as organic acids and their sequestration and immobilization in the vacuoles is considered as one of the most important mechanisms of metal tolerance and hyperaccumulation in plants (Chai et al., 2014). Our investigation suggests an important role of these acids in above ground detoxification and accumulation of metals in primed plants. Regarding elevation in organic acid concentration, the most prominent effect of priming was observed for malic acid. This is in concordance with the previous study of hyperaccumulating plant *Sedum alfredii,* where up to 85% Cd present in shoots is associated with malate and only 15% with citrate (Lu et al., 2013). Additionally, in this work a positive correlation was found between oxalate concentration and Cd concentration in the leaves of plants primed with both SaA and Pro, suggesting their synergistic effect on the higher accumulation of Cd in the leaves of this plant and the plants' resistance to it. Keeping in mind these results,

Table 6
Effect of seed priming on organic acid content in shoots of *Silene sendtneri* grown under Cd stress.

Priming agent	Cd (m*M*)	Acid concentration μmol/g)		
		Oxalic	Malic	Citric
Non-primed	0	$13.34^{b} \pm 1.29$	$18.02^{bc} \pm 0.36$	$6.92^{cd} \pm 0.92$
	0.25	INTERF.	$6.02^{gh} \pm 0.10$	$5.05^{d} \pm 0.07$
	0.50	$3.97^{fg} \pm 0.05$	$2.81^{j} \pm 0.05$	$2.24^{gh} \pm 0.02$
Hydro-primed	0	$16.25^{a} \pm 0.65$	$21.43^{b} \pm 0.31$	$14.84^{a} \pm 0.13$
	0.25	INTERF.	$3.64^{ij} \pm 0.35$	$2.49^{gh} \pm 0.20$
	0.50	$2.89^{g} \pm 0.05$	$8.92^{e} \pm 0.61$	$5.11^{d} \pm 0.18$
1% SiA	0	$9.90^{d} \pm 0.17$	$6.85^{gh} \pm 0.07$	$3.51^{fg} \pm 0.05$
	0.25	INTERF.	$5.22^{hi} \pm 0.02$	$2.75^{gh} \pm 0.05$
	0.50	INTERF.	$5.57^{hi} \pm 0.07$	$2.39^{gh} \pm 0.10$
0.5 m*M* SaA	0	$12.69^{bc} \pm 0.24$	$17.10^{c} \pm 0.55$	$8.24^{c} \pm 0.21$
	0.25	$3.34^{fg} \pm 0.35$	$3.69^{ij} \pm 0.08$	$1.93^{h} \pm 0.03$
	0.50	$4.23^{f} \pm 0.18$	$3.88^{ij} \pm 0.27$	$1.86^{h} \pm 0.07$
1.0 m*M* SaA	0	$10.06^{cd} \pm 0.56$	$14.38^{d} \pm 0.15$	$7.05^{c} \pm 0.01$
	0.25	$2.10^{g} \pm 0.40$	$4.32^{i} \pm 0.24$	$1.63^{h} \pm 0.04$
	0.50	$3.79^{f} \pm 0.08$	$9.14^{e} \pm 0.12$	$3.83^{fg} \pm 0.08$
10 m*M* Pro	0	$13.68^{b} \pm 0.55$	$35.50^{a} \pm 0.83$	$15.51^{a} \pm 0.14$
	0.25	$2.39^{g} \pm 0.37$	$7.08^{f} \pm 0.50$	$3.78^{fg} \pm 0.39$
	0.50	$2.63^{g} \pm 0.04$	$4.86^{i} \pm 0.49$	$2.49^{gh} \pm 0.22$
20 m*M* Pro	0	$7.92^{d} \pm 0.04$	$12.40^{d} \pm 0.07$	$7.98^{c} \pm 0.37$
	0.25	$2.65^{g} \pm 0.48$	$8.82^{ef} \pm 0.31$	$4.82^{f} \pm 0.28$
	0.50	INTERF.	$5.31^{hi} \pm 0.23$	$2.34^{gh} \pm 0.09$
0.5 m*M* SaA + 10 m*M* Pro	0	$8.09^{de} \pm 0.55$	$9.63^{e} \pm 0.05$	$4.53^{f} \pm 0.09$
	0.25	$4.47^{f} \pm 0.06$	$4.96^{i} \pm 0.11$	$2.87^{gh} \pm 0.28$
	0.50	$3.96^{fg} \pm 0.07$	$6.76^{gh} \pm 0.73$	$4.16^{f} \pm 0.24$
0.5 m*M* SaA + 20 m*M* Pro	0	$13.35^{b} \pm 0.74$	$6.98^{gh} \pm 0.18$	$2.62^{gh} \pm 0.16$
	0.25	$6.26^{f} \pm 0.58$	$9.29^{e} \pm 0.01$	$6.53^{c} \pm 0.14$
	0.50	$11.34^{c} \pm 0.44$	$4.03^{i} \pm 0.51$	$3.58^{fg} \pm 0.39$
1.0 m*M* SaA + 10 m*M* Pro	0	$15.69^{a} \pm 0.40$	$18.93^{bc} \pm 2.69$	$12.42^{b} \pm 1.38$
	0.25	$3.39^{fg} \pm 0.33$	$7.65^{fg} \pm 0.01$	$3.45^{fg} \pm 0.06$
	0.50	$6.34^{e} \pm 0.96$	$5.59^{hi} \pm 0.14$	$2.73^{gh} \pm 0.04$
1.0 m*M* SaA + 20 m*M* Pro	0	$8.40^{de} \pm 1.35$	$7.64^{fg} \pm 0.31$	$4.77^{ef} \pm 0.06$
	0.25	$3.48^{fg} \pm 0.04$	$5.21^{hi} \pm 0.25$	$2.45^{gh} \pm 0.23$
	0.50	$3.63^{fg} \pm 0.28$	$5.91^{hi} \pm 0.00$	$2.50^{gh} \pm 0.26$

The data represent means of three replicates (± standard deviation). The values within one column followed by the same letter do not differ significantly after the Factorial ANOVA post hoc Newman-Keuls analysis at significance level of $p < 0.01$; SiA – silicic acid; SaA – salicylic acid; Pro – proline.

the accumulation of organic acids in shoots could be one of the possible mechanisms of seed priming influence on the enhanced accumulation of Cd in *S. sendtneri* plants.

5. Conclusions

The presented study was focused on priming effects on tolerance and accumulation rate of Cd in *Silene sendtneri*, a plant that could potentially be used as a phytoremediator for Cd removal from contaminated soils. The lethal dosage for *S. sendtneri* in this study was 0.5 mM $Cd(NO_3)_2$ where reduction of growth was too severe for plants to be harvested for analysis. Enhancement of tolerance to this concentration was recorded for primed plants with various degree of tolerance. An increase in germination rate, followed by a high seedling developmental rate was recorded for silicic acid, proline, and salicylic acid priming. Priming with Pro was most effective in the stimulation of root growth under high Cd stress. Reduction of fresh and dry mass and water content was recorded for non-primed plants and in some cases for primed plants as well. In non-primed plants severe drop in water content was recorded, while priming with Pro and SaA seems to counteract drought effects induced by heavy metals and increases the content of water in shoots and roots. Accumulation of Cd in shoots was significant and reached the astonishing 2156.10 mg/kg DW for plants primed with 1.0 m*M* SaA with the highest translocation factor of 2.29. Results indicate that SaA and Pro trigger similar responses in *S. sendtneri* inducing an increase in peroxidase activity under Cd stress, related to 1% SiA. Total soluble protein content usually decreases during the heavy metal stress, but an increase upon exposure of plants to Cd was also recorded in the shoots and the roots of several primed plants. Increase in content of ferulic acid and rutin was recorded referring to Cd level suggesting its role in scavenging of free radicals. Priming with SaA as well as SaA combined with Pro, induced an increase in malic and oxalic acid respectively, suggesting their role in chelation of Cd and vacuolar sequestration in shoots as well. As a result, *Silene sendtneri* could be considered as an efficient phytoremediator for Cd removal from contaminated soil. Additionally, our results suggest seed priming as a promising method for enhancement of Cd tolerance and consequently accumulation potential of the species. The study could be taken forward towards investigating molecular mechanisms underlying enhanced tolerance of primed plants to Cd, with special emphasis on transcriptional and posttranscriptional regulation of gene expression in primed plants under Cd stress. *S. sendtneri* could serve as a model plant and a tool for identifying genes involved in heavy metal tolerance and accumulation. Future research, which will include transcriptome analysis, may uncover a number of novel metal resistance mechanisms in plants.

CRediT authorship contribution statement

Erna Karalija: Conceptualization, Methodology, Writing - original draft. **Alisa Selović**: analysis of heavy metal content, revision of draft. **Sabina Dahija**: biomass related analysis, growth analysis. **Arnela Demir**: in vitro cultivation and analysis. **Jelena Samardžić**: Writing - review & editing. **Ondřej Vrobel**: organic acid analysis. **Sanja Ćavar Zeljković**: secondary metabolite analysis, writing and reviewing. **Adisa Parić**: physiological analysis and revision of draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2020.111882.

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