TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2021) 45: 553-562
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doi:10.3906/bot-2107-52

Effect of zinc on phytoremediation potential and carbonic anhydrase and polyphenol oxidase activities of *Lythrum salicaria* L.

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Received: 27.07.2021 • **Accepted/Published Online:** 06.11.2021 • **Final Version:** 30.12.2021

Abstract: In this study, *Lythrum salicaria* plant was tested in hydroponic culture to demonstrate its zinc accumulating capacity and tolerance to different zinc levels. *Lythrum salicaria* seedlings were grown in 10% Hoagland solution containing 0, 5, 10, 20, 30, 40, 50, 75, and 100 mg/L zinc, and 30 mg/L zinc with different pH levels (5, 6, and 7). Following this, the seedlings were harvested after 1st, 2nd, 4th and 7th days. Zinc caused significant decreases in the relative values of the mean root-shoot length and fresh weight of the plant (92 to 75%; 100 to 92%; 64.2 to 41.2%, respectively), and contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in the leaf (0.54 to 0.43; 0.16 to 0.11; 0.65 to 0.54; 0.097 to 0.080 mg/g fresh weight, respectively). There were significant increases in zinc accumulation parallel to zinc increase and pH in solution in plant tissues, zinc accumulation in roots (13,659.7 mg/kg dry weight) was higher than in shoots. Leaf protein content (0.31 to 0.49 mg/g fresh weight), and polyphenol oxidase (0.80 to 1.95 mg/g fresh weight), and carbonic anhydrase activities in the roots increased (6.7 to 17.87 mg/g fresh weight).These data indicate that *Lythrum salicaria* has a high ability to accumulate zinc, so that it may have the potential to be used in zinc remediation projects.

Key words: Accumulation, chlorophyll, enzyme activity, *Lythrum salicaria*, protein, zinc

1. Introduction

Heavy metal release from mining areas is a serious environmental problem and heavy metals have become one of the most important hazardous pollutants all over the world (Sytar et al., 2019). Natural and anthropogenic activities, including urban sewage, tanneries, and the textile industry, have contaminated freshwater with various metals including Zn, As, Pb, Ni, Cr, and Cd. Because of these activities, heavy metal levels in freshwater are a great concern in Turkey. Kütahya is one of the rich cities for mineral resources in Turkey and it contains minerals of strategic importance for Turkey within its borders such as boron, magnesite, silver, chromium, antimony, zinc, iron, manganese, magnesite (Hastorun, 2017). In addition, since Turkey's largest silver deposit is located in Kütahya, silver mining activities have caused As, Pb, Sb, and Zn pollution in soil and surface waters in the region. Studies have revealed that the soil, freshwater, and sediment of Kütahya province contain high amounts of zinc (75.80 mg/kg – 4993 ppm, 31 to 65 µg/L and 133.98 to 215.88 mg/kg, respectively) (Hastorun, 2017; Saleh, 2017; Özkul et al., 2018; Akın and Bingöl, 2019). Many countries have developed physical, biological, and chemical techniques to remove contaminants from polluted sites, and implemented these techniques in the field. In recent years, phytoremediation, one of the most cost-effective and ecofriendly techniques, has been used to remove toxic metals from contaminated waters by roots of green plants. Only 0.2% of known plant species have been identified as heavy metal hyperaccumulators including *Alyssum bertolonii*, *Pteris cretica*, *Thlaspi caerulescens*, *Azolla pinnata*, and *Lemna minor* (Rascio and Navari- Izzo, 2011; Tangahu et al., 2011; Ali et al., 2013; Thayaparan et al., 2015).

Zinc (Zn), one of the heavy metals that enter the environment through anthropogenic activities including industrial wastes, sewage sludge, and acid rains, is an important micronutrient for plant growth but it can become a toxic element for plants when present in large quantities (Rout and Das, 2003; Nardis et al., 2018). Zn is involved in several plant metabolic processes such as enzyme activation, protein synthesis, and carbohydrate and lipid metabolism (Escudero-Almanza et al., 2012; Tsonev and Lidon, 2012; Ackova, 2018). Even though Zn

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has an important role in this metabolism in plants, can also betoxic and cause chlorosis at high concentrations (Tripathi et al., 2015). Several Zn-dependent processes can be associated with the active Zn level in plant tissues. Zn-dependent enzymes catalyze many important metabolic processes. Zinc is the cofactor of carbonic anhydrase (CA) in plants. CA, a metalloenzyme, is one of the zinc-dependent enzymes that catalyze the conversion of carbon dioxide and water to bicarbonate and a proton (Escudero-Almanza et al., 2012; Soltangheisi et al., 2014) and also helps to raise the CO_2 concentration within the chloroplast in plants (Escudero-Almanza et al., 2012; Bhat et al., 2017). Many researchers have described the role of CA under stress conditions. According to Wei-Hong et al. (2014), the expression of CA was related to environmental factors such as light intensity, salinity, availability of Zn, and CO_2 concentration.

Polyphenol oxidase (PPO) is one of the important enzymes that catalyze phenols to quinones in plants. Some researchers mentioned that environmental factors and stress conditions also enhance the amount of phenolic compounds in plants (Michalak, 2006; Araji et al.,2014; Taranto et al., 2017). Several studies have found a relationship between PPO activity and diseases (Araji et al., 2014; Taranto et al., 2017), wounding (Pinto et al., 2008), hormonal regulation (Araji et al., 2014), enzymatic browning (Kocaçalışkan, 2004; Pinto et al.,2008), and germination (Kocaçalışkan et al., 1995) in plants.

Lythrum salicaria L. (Lythraceae) (purple loosestrife), an herbaceous perennial plant geographically originating from Eurasia, grows up to 20–180 cm tall in wetlands between 0 and 1400 m altitudes. The generic name of Lythrum is the Greek Word "luthron", meaning blood, referring to its ability to stop bleeding. The main compounds of the plant are tannins, flavones, and anthocyanins. *Lythrum salicaria* was used medicinally to treat diarrhea, varicosis, hemorrhoid, bleeding gums, and eczema (Rauha et al., 2001; Humadi and Istudor, 2009). Mal et al. (2002) found that fluctuating asymmetry in *L. salicaria* may be used as an ecological indicator to identify environmental stress caused by Pb. It also has the ability to accumulate heavy metals such as Cr, Cu, Zn, Fe, Ni, and Pb mostly in its roots, and thus it has been used for phytoremediation of heavy metals (Sun et al., 2013; Bingöl et al., 2017).

Factors such as the level of heavy metal that the plant can tolerate, temperature, pH, redox potential, and salinity affect the phytoremediation potential of wetland plants (Sood et al., 2012). Plants exposed to these factors show visible toxicity symptoms such as decreased growth rate, chlorosis, blackish roots, a decline in root length, and death. Thus, plants have developed various mechanisms to fight heavy metal stress (Ghori et al., 2019). As far as we know, there has been no previous study about changes in PPO and CA enzyme activities of *L. salicaria* under zinc stress. Even though there are plant species that can grow in zinc contaminated areas and tolerate the contaminant, it is very important to investigate new plant species with a phytoremediation potential for cleaning zinc contaminated areas. Species such as *Eichhornia crassipes*, *Roemeria hybrida* subsp. *dodecandra*, and *Tussilago farfara*, have recently been used to remove zinc from the environment due to their highly competitive and hyperaccumulation abilities (Tel-or and Forni, 2011; Mazumdar and Das, 2015; Hesami et al., 2018; Wechtler et al., 2019).

The present study was designed to search the zinc accumulation potential of *L. salicaria* grown in different concentrations of zinc and pH in hydroponic culture and to determine the zinc tolerance level exhibited by the plant. The zinc accumulation potential of the *L. salicaria* and response to elevated zinc concentrations were evaluated with the below references: (i) changes in mean relative values (%) of root-shoot length, and fresh weight; (ii) changes in protein and pigment contents; (iii) changes in CA and PPO activities.

2. Materials and methods

2.1. Plant material

Purple loosestrife capsules were collected from populations growing along the Porsuk River, Kütahya, Turkey (39°20'59.61"N – 30°02'16.91"E, 39°22'47.43"N – 30°04'00.07"E). The seeds were separated from the capsules before the experiment. Healthy seeds were planted in pots filled with soil containing 54% organic matter, pH 5.5–6.8, and purity 95%, produced by Mixflor. The pots were kept in pools filled with 10 cm deep water in a greenhouse until the seedlings reached a height of about 15 cm (about 2 months old seedlings). After that, the seedlings were transferred to hydroponic culture pots containing 10% Hoagland solution (pH 6.2) for adaptation and kept in hydroponic conditions for seven days (Hoagland and Arnon, 1950). Each experiment was repeated three times.

2.2. Phytoremediation experiments

Experiments were performed in pots (2.5 L capacity) of a hydroponic culture system. The experiments took place in three steps. In the first step, after the adaptation period, to determine the concentration of Zn at which the plant accumulated the most zinc, purple loosestrife plants were grown in 10% Hoagland solution with nine different Zn (II) concentrations, prepared by using $ZnSO_4$ (0 as the control group, 5, 10, 20, 30, 40, 50, 75 and 100 mgZn/L) for seven days. In the second step, after calculating the zinc concentration that the plant accumulated the most zinc (30 mg Zn/L), the appropriate pH level was determined for zinc accumulation. For this, *L. salicaria* seedlings were kept in 10% Hoagland solution containing 30 mg Zn/L at

5, 6, and 7 pH for seven days. pH < 5 and > 7 could not be determined because plants died at pH 4 and, above pH 7, zinc precipitated as zinc hydroxide. In the last step, to determine the distribution of Zn in the root, shoot, and leaves of *L. salicaria*, the seedlings were placed in 10% Hoagland solution containing 30 mg Zn/L at pH 7 and harvested at 1, 2, 4, and 7 days. Zn accumulation in all groups was measured at the end of these exposure periods.

2.3. Growth measurements

At the end of the experiment, seedlings were harvested and their roots were rinsed in Na-EDTA (1%) and ultrapure water to remove any heavy metal contaminants. The relative lengths (root, shoot) and fresh weight were calculated as:

$$\text{Relative growth parameters (\%)} = \frac{\text{Growth parameters in zinc solutions}}{\text{Growth parameters in control solution}} \times 100$$

2.4. Measurement of zinc content

Root, shoot, and leaf parts of *L. salicaria* seedlings were dried at 70 °C for 48 h. The dried plant parts were weighed and recorded as dry weight (DW). The Zn content of the plant parts was analyzed by Atomic Absorption Spectrometer (Analytik Jena ContrAA 300) at the Advanced Technologies Centre of Dumlupınar University by using Flame Atomic Absorption Spectrometry. For this process, 0.1 g dried plant samples were digested by a wet digestion method using nitric acid and hydrogen peroxide (Kaçar, 2008).

2. 5. Measurement of the enzyme activities and protein and chlorophyll contents

PPO activity was determined in fresh roots and leaves of *L. salicaria* by measuring the absorbance of the samples containing 0.2 mL of enzyme solution at 420 nm using a spectrophotometer (Jennings and Duffus, 1977). The activity of CA was estimated in fresh roots and leaves of *L. salicaria* in a solution consisting of 2 mL of 25 mM veronal buffer, 0.2 mL of bromthymol blue, and 0.8 mL of enzyme solutions, and 2.0 mL of a cold saturated CO_2 solution was added. The experiment recorded the time from the moment of adding the solution to the color change of the indicator from blue to a greenish-yellow. CA activity was calculated in enzyme units (EU) (Wilbur and Anderson, 1948; Rickli et al., 1964). Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content) were extracted from fresh leaves of *L. salicaria* using acetone and the absorbance of the supernatant was read at 450, 647, and 663 nm using a spectrophotometer (Optizen POP) (Arnon, 1949). Protein was extracted from fresh leaves (0.5 g) with 5 mL of a phosphate buffer. The homogenate was then centrifuged at 20000 rpm for 20 min. The protein content in a 0.1-mL supernatant sample was recorded at 595 nm with bovine serum albumin used as a standard (Bradford, 1976).

2. 6. Statistical analysis

The experiments were conducted using a completely randomized design with three replicates. ANOVA and Tukey HSD multiple samples at the $p = 0.05$ level were used to demonstrate statistically the relationship between zinc concentration and average zinc accumulation, root length, shoot length, fresh weight, chlorophyll a, chlorophyll b, total chlorophyll, carotenoid and protein contents, and the activities of polyphenol oxidase and carbonic anhydrase. All statistical analyses were performed using JMP6 SAS (2005).

3. Results and discussion

The results of this study indicate that *L. salicaria* has the potential to accumulate a considerable amount of zinc in the whole plant under hydroponic conditions. However, the seedlings started to decay in high concentrations of zinc (75 and 100 mg/L), and the above and below-ground parts of all seedlings died after seven days of exposure. Zinc accumulation in *L. salicaria* increased with increasing zinc concentration in solution ($F = 1{,}600.9$; $p < 0.05$), reaching maximum zinc accumulation (12,098.3 mg/kg DW) in 30 mg/L Zn (Figure 1a). Zn uptake at 30 mg/L Zn was about 60 times higher than the control group.

Lythrum salicaria showed some toxicity symptoms at 40–50 mg/L Zn concentrations such as necrosis in the leaves and blackening in the roots. High zinc concentrations can cause toxicity in plants (Rout and Das, 2003; Mirshekali et al., 2012), but zinc phytotoxicity varies according to plant species, age of the plant, environmental conditions, and the combination of zinc with other metals (Tsonev and Lidon, 2012). Still, our results indicated that *L. salicaria* has a high zinc accumulation capacity compared with *Roemeria hybrida* subsp. *dodecandra*, *Tussilago farfara,* and other wetland plants (Mazumdar and Das, 2015; Hesami et al., 2018; Wechtler et al., 2019).

Metal accumulation in plants can be affected by several abiotic factors such as pH, redox potential, organic content, light intensity, and temperature in aquatic systems (Tangahu et al., 2011; Morkunas et al., 2018). The results of this experiment showed that Zn accumulation at 30 mg/L increased as pH values in nutrient solution increased with the highest Zn accumulation at pH 7(13,893.5 mg/kg DW; $F = 1163{,}6$; $p < 0.05$; Figure 1b). This result shows that pH is an important parameter in Zn uptake. Aisien et al. (2010) also found that the amount of zinc accumulated by *E. crassipes* increased depending on pH, with the highest amount of zinc accumulated in its roots at pH 8.5. However, another study found that the removal efficiency of zinc by *E. crassipes* decreased with decreasing pH (Swarnalatha and Radhakrishnan, 2015). Deng et al. (2004) noted a negative correlation between Zn accumulation in the underground tissues of *Juncus effusus* and pH.

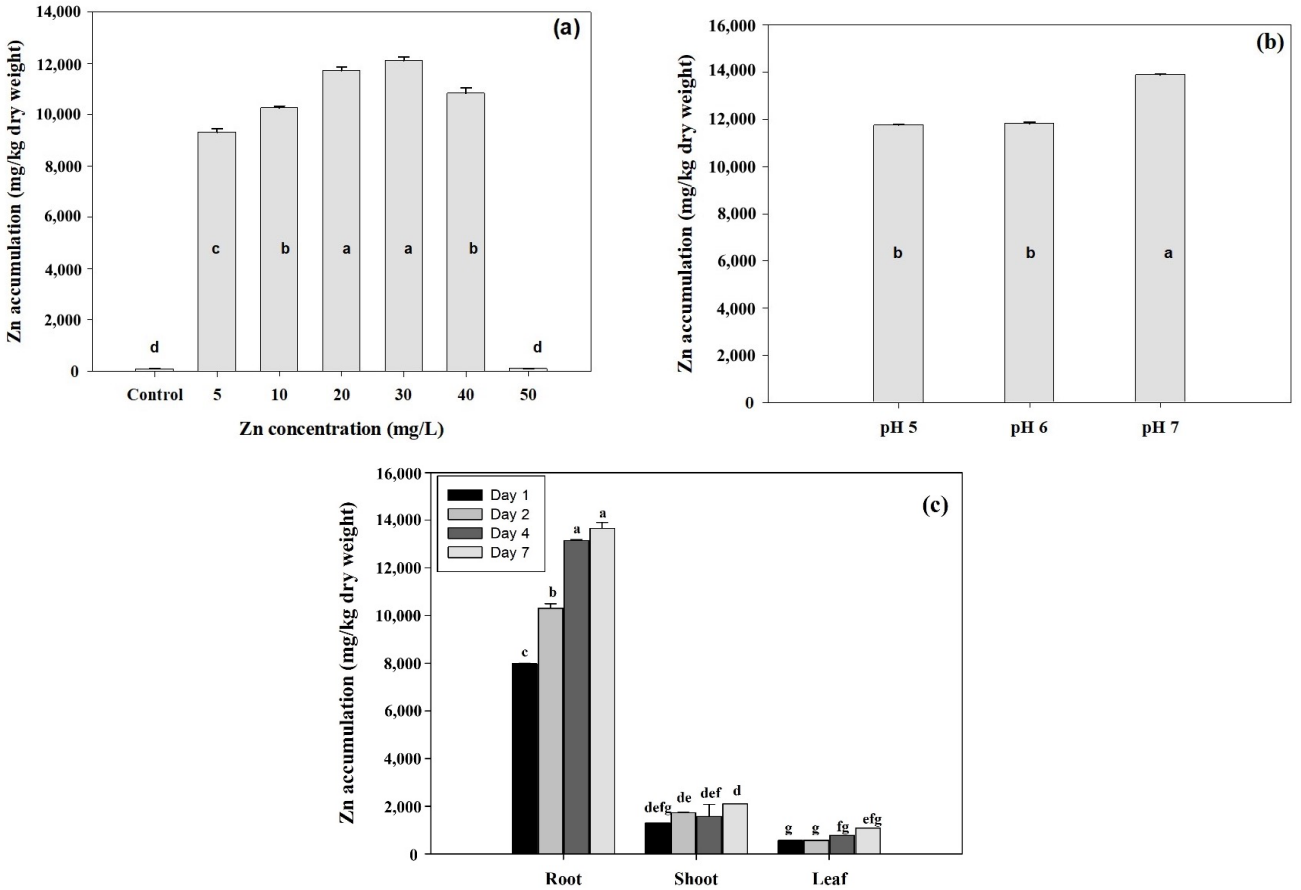

Figure 1. The accumulation of Zn (II) (mg/kg DW) in *L. salicaria* at **(a)** different Zn concentrations at pH 6.2 in the whole plant on the7th day of treatment, **(b)** different pH levels at 30 mg Zn/L and the 7th day of treatment, **(c)** Zn (II) accumulation in different organs of *L. salicaria* during the exposure time for each treatment (1st day, 2nd day, 4th day, 7th day) at 30 mg Zn/L and pH 7. The vertical bar represents standard error values. According to the Tukey HSD test, groups with the same letter are not significantly different at $p \leq 0.05$ ($n = 21$).

Total zinc accumulations in the root, shoot, and leaves of *L. salicaria* were measured after 1, 2, 4, and 7 days (9,828.8; 12,612.5; 15,499.3; 16,840.2 mg/kg DW, respectively) of the experiments at 30 mg Zn/L and pH 7. Comparing the 1st and 7th days of the experiment, 50% of total zinc accumulation occurred on the 1st day of the experiment (Figure 1c). When the zinc accumulation in three organs on the 7th day of the experiment was compared to each other, according to the Tukey HSD test, the roots had higher zinc accumulation than the shoots and leaves (13,659.7; 2,095.8 and 1,084.7 mg/kg DW, respectively; $F = 1,211.57$; $p < 0.05$). Thus, maximum zinc accumulation in the roots on the 7th day of the experiment was approximately 6.5 times more than in the shoots and 12.5 times more than in the leaves (Figure 1c). One of the most important problems encountered in phytoremediation studies, depending on the heavy metal type, is the harvesting of plants contaminated with pollutants at the end of the growing season. However, plants that accumulate heavy metals in their roots can be harvested and disposed of easily and cost-effectively (Ali et al., 2020). Ahmad et al. (2014) investigated whether the metals accumulated in the roots of *Phragmites australis* were transferred to the aboveground organs and found that zinc was the lowest transferred metal among 11 metals measured. These authors concluded that *Phragmites australis* retains Zn by rhizofiltration. In our study, we also found that the roots of *L. salicaria* are more effective than aboveground parts in the removal of Zn from hydroponic solutions, which is in agreement with the results of other studies. Bingöl et al. (2017) found that *L. salicaria* also accumulated Ni more in its roots than its shoots and leaves. A similar result has been reported by Panyakhan et al. (2006) and they also showed that metal accumulation in *Hydrocotyle umbellata* organs (roots and shoots) significantly increased as both zinc concentration and the exposure time were increased. A study investigating the usability of ten macrophyte plant species, including *Lythrum salicaria*, in the treatment of

heavy metal contaminated wastewater with plants in small-scale wetlands found that, for most of the studied species, higher Zn accumulation was obtained in the underground parts of the plants compared to the aboveground parts with Zn accumulation in the roots of *L. salicaria* reaching 18,410 mg/kg DW on the 15th day of the experiment (Sun et al., 2013).

Plants, faced with heavy metal stress, show some changes in their growth (Ackova, 2018). Similarly, we saw a significant relation between the growth parameters and zinc concentration. The mean relative root length, shoot length and fresh weight of *L. salicaria* decreased relative to increasing zinc concentrations from 5 to 50 mg/L in solutions (92 to 75%; 100 to 92%; 64.2 to 41.2%, respectively; Figure 2). In fact, the final root lengths of seedlings grown in solutions containing 40 and 50 mg/L Zn were shorter than their initial root lengths as a result of zinc heavy metal stress. In another study related to *L. salicaria*, Zn was shown to decrease root length but biomass weight increased (Sun et al., 2013). Tsonev and Lidon (2012) noted that increasing zinc doses negatively affected root and shoot growth of *Artemisia annua* and sugarcane plants. Plants grown in high metal concentrations showed reduced growth with their leaves turning dark red in color (Al-Chami et al., 2015). These authors also noted that roots of *Sorghum bicolor* and *Carthamus tinctorius* exhibited blackening, and reduced biomass. Root growth is the product of both cell division and elongation. In this context, there is a decrease in mitotic activity as a result of exposure to high concentrations of heavy metals in many plant species, and as a result, root growth is suppressed (Singh et al., 2016).

Chlorophylls a and b, total chlorophyll, and carotenoid amounts were determined to demonstrate the response of plants to zinc. The amount of chlorophyll a was the highest at 5 mg Zn/L (0.54 mg/g leaf, fresh weight (FW)) whereas, in control, it was found 0.51 mg/g leaf, FW. A gradual decrease was observed in chlorophyll a content when Zn concentration was further increased. In addition, the amount of chlorophyll b was found highest (0.16 mg/g leaf, FW) at 5 mg Zn/L concentration. However, the lowest chlorophyll b amount (0.11 mg/g leaf, FW) was obtained at 50 mg Zn/L concentration. A similar result was obtained in total chlorophyll, higher (0.70 mg/g leaf, FW) at 5 mg Zn/L concentration and decreased as the concentration increased. Total carotenoid was found to be maximum (0.097 mg/g leaf, FW) at 5 mg Zn/L while the control showed 0.084 mg/g leaf, FW (Figure 3). This pattern of Zn accumulation in leaves may be due to the inhibition of chlorophyll biosynthesis (Shakya et al., 2008). Even though Zn is important for plant growth and photosynthesis (Tripathi et al., 2015; Sharma et al., 2020), excess Zn causes a decrease in photosynthetic pigment synthesis, damaging the photosynthetic apparatus. As a result, the plant develops leaf chlorosis (Shakya et al., 2008; Tripathi et al., 2015). Similarly, decreasing chlorophyll content has been found in many plants under heavy metal stress (Shakya et al., 2008; Chandra and Kang, 2016).

Protein content was the lowest in the control group while plants grown at 30 mg/L Zn concentration had the highest protein content (0.25 and 0.61 mg/g leaf, FW, respectively). However, while a linear increase in the protein content of *L. salicaria* was observed up to 30 mg/L Zn concentration, after this concentration the protein content of the plant

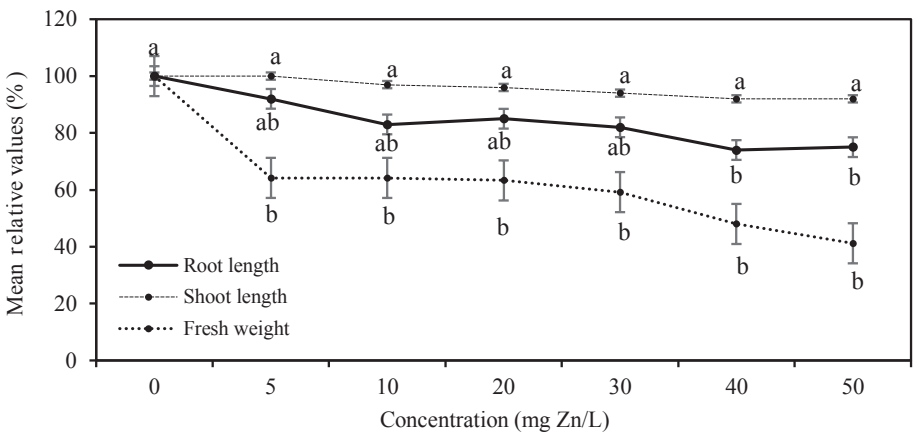

Figure 2. The effects of Zn (II) concentrations on mean relative values (%), root lengths ($F = 3.67$; $p = 0.02$), shoot lengths ($F = 1.28$; $p = 0.33$) and fresh weight ($F = 12.40$; $p < 0.0001$) of *L. salicaria* at 30 mg Zn/L, pH 7 and on the 7th treatment day. The vertical bar represents standard error values. According to the Tukey HSD test, groups with the same letter are not significantly different at $p \leq 0.05$ ($n = 21$).

decreased with increasing zinc concentration (Figure 4). Zinc plays an important role in DNA, protein synthesis, and the structure of some enzymes. Several studies found a relation between zinc concentration and protein content in plants (MacDonald, 2000; Tsonev and Lidon, 2012). For example, Jayasri and Suthindhiran (2017) found that the soluble protein content in *Lemna minor* decreased with increasing zinc concentration.

Zinc had a positive effect on PPO activities in both leaf and root tissues, increasing by 1.95 and 25.95 A_{420} g, respectively in the roots and leaves at 50 mg Zn/L (Figure 5). Our results indicated that the PPO enzyme is present in larger amounts in the leaves than roots, which confirms the finding that the PPO enzyme exists in both chloroplasts and mitochondria and plays a role in respiration (Bidwell, 1979). PPO activity can increase as a response to abiotic stresses (Taranto et al., 2017), as has been reported in previous studies for several plant species, but the stress responses differed according to the plant species (Ortega-Gracı´a and Perago´n, 2009; Boeckx et al., 2015). Polyphenol oxidase enzyme is located in the chloroplasts and may have a role in either acclimation or short-term response to stress. Thipyapong et al. (2004) reported that PPO suppression improved plant-water relations and delayed photoinhibition and photooxidative damage in tomato plants with different levels of PPO expression subjected to water stress. PPO oxidizes some phenols to quinones (Kocaçalışkan, 2004; Taranto et al.,

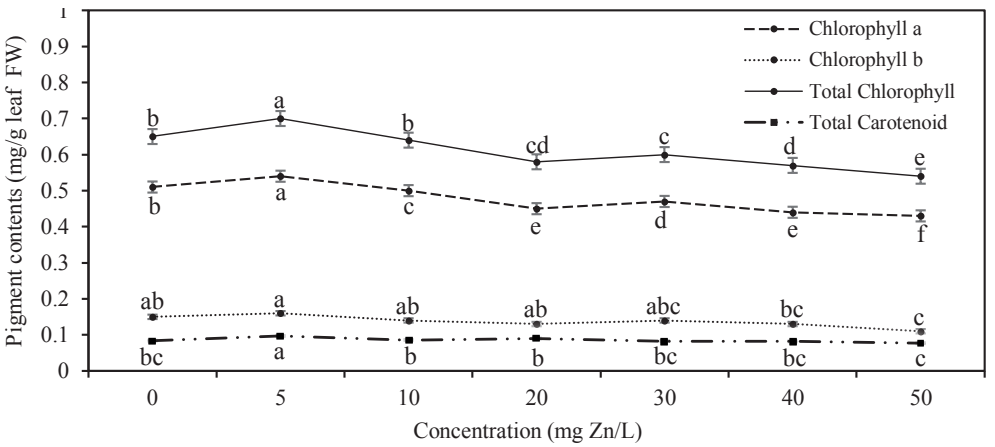

Figure 3. The effects of Zn (II) concentrations on chlorophyll a (F = 587.5; p = 0.0001), chlorophyll b (F = 26.5; p = 0.0001), total chlorophyll (F = 207.3; p = 0.0001) and total carotenoid (mg/g leaf) (F = 41.3; p = 0.0001) of *L. salicaria* at 30 mg Zn/L, pH 7 and on the 7th treatment day. The vertical bar represents standard error values. According to the Tukey HSD test, groups with the same letter are not significantly different at $p \leq 0.05$ (n = 21).

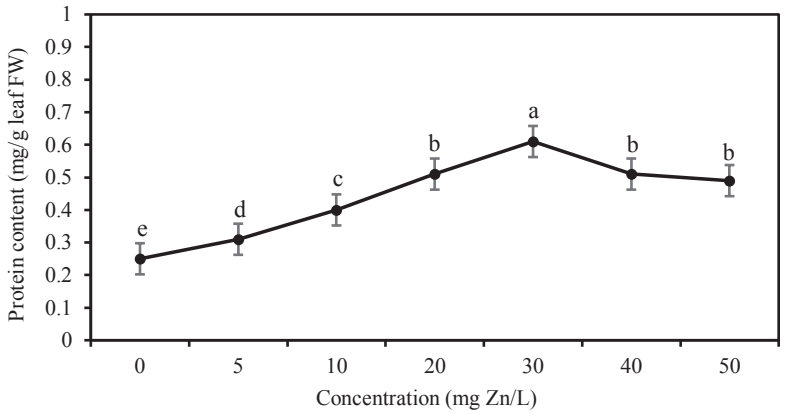

Figure 4. The effects of Zn (II) concentrations on leaf protein contents of *L. salicaria* at pH 7 and on the 7th treatment day (F = 454.7; p = 0.0001). The vertical bar represents standard error values. According to the Tukey HSD test, groups with the same letter are not significantly different at $p \leq 0.05$ (n = 21).

2017), which play a role in photosynthesis (plastoquinone) and respiration (ubiquinone). For example, plastoquinone (PQ) and ubiquinone (UQ) are two important quinones that function as electron transporters in plants (Liu and Lu, 2016). In our study, we found that although there were some fluctuations in the PPO activity with increasing zinc concentrations, the zinc concentrations were lower in the leaves, whereas the PPO activity, on the contrary, was higher. This situation indicates that there may be some factors other than zinc that affect PPO activity in the leaves (Figure 5). For example, it is known that the PPO enzyme is mainly located in leaf chloroplasts. Therefore, the high levels of PPO activity in the leaves may be the result of the metabolic effect of photosynthesis (Boeckx et al., 2015).

CA activity (U mg^{-1} protein) in both the leaves and roots was enhanced with increasing Zn concentrations (7.19 to 38.41 and 6.94 to 17.87 U mg^{-1} protein, respectively; Figure 6). Zn is the cofactor of the CA enzyme and CA requires Zn for its activity. The relation between CA enzymatic activity and Zn was reported years ago in crops such as *Pisum sativum*, *Lactuca sativa*, *Petroselinum crispum*, *Spinacia oleracea,* and *Oryza sativa* (Escudero-Almanza et al., 2012; Bhat et al., 2017). In this experiment, it was expected that the activity of the CA enzyme should increase with increasing Zn concentration, which is what we found. CA activity in the leaf was found to be higher than in the roots. Therefore, this enzyme is more abundant in leaves than in the roots. Because photosynthesis occurs in the leaves, CA enzyme activity is higher here due to metabolic activities. CA activity varies according to plant species. Bhat et al. (2017) observed that CA activity is higher in tobacco leaves than in the stem, pods, roots, or fruits. Soltangheisi

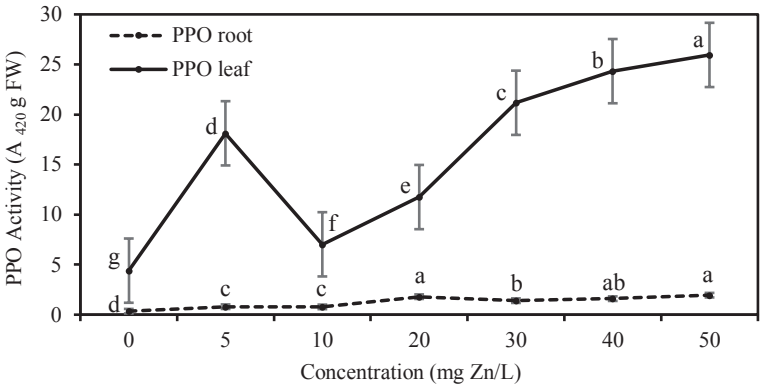

Figure 5. The effects of Zn (II) concentrations on PPO activity (A_{420} g) in the roots (F = 61.7; p = 0.0001) and leaves (F = 11,125.7; p = 0.0001) of *L. salicaria* at pH 7 and on the 7th treatment day. The vertical bar represents standard error values. According to the Tukey HSD test, groups with the same letter are not significantly different at p ≤ 0.05 (n = 21).

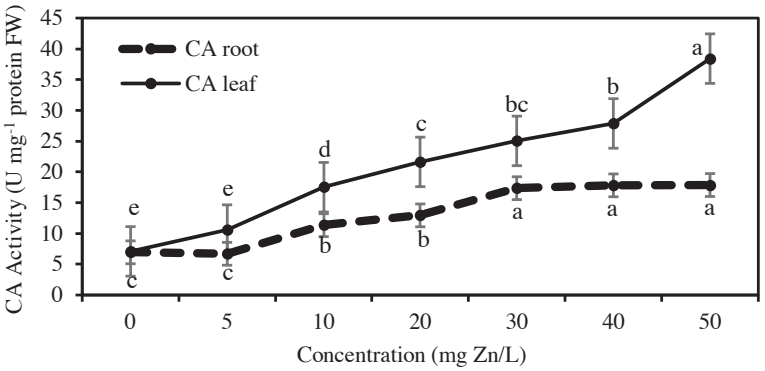

Figure 6. The effects of Zn (II) concentrations on CA activity (U mg^{-1} protein) in the roots (F = 16.0; p = 0.0001) and leaves (F = 178.6; p = 0.0001) of *L. salicaria* at pH 7 and on the 7th treatment day. The vertical bar represents standard error values. According to the Tukey HSD test, groups with the same letter are not significantly different at p ≤ 0.05 (n = 21).

et al. (2014) also showed that, with increasing Zn supply, CA activity in leaves increased in sweet corn plants. Zinc deficiency affects the catalytic activity of enzymes such as alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase, and thus the metabolic pathways in which they are involved. In some plants, Zn deficiency can lead to reduced carbonic anhydrase activity (Escudero-Almanza et al., 2012; Castillo-González et al., 2018). The results obtained from this study are in agreement with those of these other studies and revealed that excess Zn increased the CA activity in the roots and leaves of *L. salicaria*. Overall, CA activity has been suggested to be a better indicator of Zn nutritional status than Zn concentration alone.

4. Conclusion

Lythrum salicaria is originally from Eurasia, it has been defined as an invasive plant species in North America and Australia. *Lythrum salicaria* is a plant species which can survive Zn heavy metal toxicity and accumulates Zn in the roots. The response of *L. salicaria* to zinc stress and Zn accumulation in different tissues was comprehensively reported for the first time in this study. The root of *L. salicaria* was more effective than above-ground parts in the removal of Zn, and increasing pH level in solution increased the accumulation of zinc by *L. salicaria*. While the growth parameters and pigment contents of *L. salicaria* were negatively affected by high zinc concentrations, protein contents and enzyme activities increased. Considering the great ability of *L. salicaria* to accumulate zinc and tolerate high zinc levels, *L. salicaria* may be a suitable species for phytoremediation. This research will be a good example of similar phytoremediation studies, examining the possible use of *L. salicaria*, a native plant in Turkey but highly invasive in North America and Australia.

Acknowledgments

We gratefully acknowledge Kütahya Dumlupınar University Research Fund (Project No: 2016-63) for supporting part of this research financially. We also thank Dr. Keith Edwards (University of South Bohemia in České Budějovice, Branišovská) for linguistic and editing help.

Contribution of authors

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by [Nüket Akanıl Bingöl], [Betül Akın], [İsmail Kocaçalışkan], [Barbaros Nalbantoğlu] and [Onur Meşeli]. The first draft of the manuscript was written by [Nüket Akanıl Bingöl] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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