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Changes in the photosynthetic response of lettuce exposed to toxic element multicontamination under hydroponic conditions

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Abstract

The effect of toxic element multicontamination on photosynthetic responses was observed in a greenhouse hydroponic culture of lettuce plants (*Lactuca sativa* var. *capitata*). The experiment focused only on the combined effect of selected toxic elements without the influence of soil, due to the hydroponic conditions. Pre-cultivated (six-true-leaf stage) plants were grown in control and contaminated hydroponic culture for 14 d. The mix of toxic elements (As, Cd, Pb, and Zn) in the contaminated solution corresponded to the water-soluble fraction of soil from the anthropogenically contaminated Litavka River area, Czech Republic. The plant response was measured by determining the toxic element contents, dry biomass, and gas-exchange parameters. Lettuce accumulated toxic elements predominantly in the roots, with low translocation to the leaves. The uptake of toxic elements harmed photosynthesis and caused a decrease in net photosynthetic rate, transpiration rate, and stomatal conductance. Consequently, the whole dry biomass of the plants decreased. The results show that contamination in hydroponic conditions had an irreversible effect on plant fitness due to direct contact between the roots and contaminated solutions.

Keywords: solution; stress; toxic element; translocation factor; transpiration rate.

Introduction

Toxic elements such as arsenic (As), cadmium (Cd), lead (Pb), and zinc (Zn) have a strong influence on the environment, affect soil quality, and may accumulate in plant tissues (Khan *et al.* 2019). The mobility, bioavailability, and toxicity of toxic elements in soils depend on their specific chemical forms and their binding state (Khan *et al.* 2019, Wu *et al.* 2022). The water-soluble fraction together with exchangeable and carbonate-bound fractions is both labile and bioavailable, and may be directly absorbed by plants (Wu *et al.* 2022). Toxic elements are transported in different parts of the plants through different pathways, altering the physiological and

Highlights

- The combination of toxic elements has a negative effect on gas exchange in lettuce leaves
- A decrease in the rate of photosynthesis leads to a decrease in the overall dry biomass of the plant
- Direct contact of roots with contaminated solutions has a decisive effect on root fitness

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Abbreviations: C_i – intercellular CO_2 concentration; E – transpiration rate; g_s – stomatal conductance; P_N – net photosynthetic rate; TFs – translocation factors.

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metabolic activities of the plant, such as photosynthesis and antioxidant production (Soran *et al.* 2023). Among the abovementioned toxic elements, As, Cd, and Pb are considered to be toxic due to their extensive availability, intensity and persistence in soil (Soran *et al.* 2023). On the other hand, Zn is considered an essential element for plants, being an indispensable component in metabolic activities. However, its deficiency or surplus can be toxic for plants (Marschner 2012).

Toxic elements may accumulate in leafy vegetables from soil or irrigation water and have toxic effects on the plants (Corradini *et al.* 2018). Lettuce (*Lactuca sativa* var. *capitata*), as a leafy vegetable, is frequently used for plant toxicity studies (Tang *et al.* 2016, Lavres *et al.* 2019), is available worldwide, has a short growing period, and is very sensitive to the presence of toxic elements (Lavres *et al.* 2019, Bidar *et al.* 2020). Reactions to these elements are rapid (Gusman *et al.* 2013, Chen *et al.* 2022). For example, Cd is one of the most analysed toxic elements concerning lettuce physiology. Studies on the effect of Cd have reported a decrease in photosynthetically active pigments in leaves and in total chlorophyll and carotenoids (Wang *et al.* 2019, Gao *et al.* 2022), a decrease in stomatal conductance and transpiration rate (He and Ren 2009) as well as an increase in the Chl *a*/*b* ratio (Kaur and Jhanji 2016). This reduces the maximum photochemical efficiency of PSII and decreases the net CO_2 assimilation rate because Rubisco activity decreases (Dias *et al.* 2013, Chen *et al.* 2022). The effect of As on lettuce is similar to that of Cd stress, which is manifested as a decrease in photosynthesis, transpiration rate, and stomatal conductance and a change in the photosynthetically active pigment content (Gusman *et al.* 2013). On the other hand, Meng *et al.* (2022) found that the As content did not have any effect on the chlorophyll content in lettuce. Pb is less mobile within the plant and most of the element stays in the roots. Despite this, Shiyab (2013) reported a decrease in the chlorophyll content in the edible part of lettuce. Similarly, the carotenoid content decreases in the presence of Pb (Brengi and Abouelsaad 2019). Consequently, the net CO_2 assimilation rate may decrease. According to Ikkonen and Kaznina (2022), the increased ratio of respiration to photosynthesis reflects the shift in the carbon (C) balance of lettuce plants towards C losses under stress conditions of soil contamination with Pb. Nevertheless, the maximum quantum efficiency of PSII, represented by the F_v/F_m parameter, is not influenced by Pb (Silva *et al.* 2017). Studies on some plant species (*Lolium perenne*, *Beta vulgaris*, *Nicotiana tabacum*) focusing on excess zinc observed a decrease in the chlorophyll content and the net photosynthetic rate with an increasing Zn concentration (Monnet *et al.* 2001), and a negative effect of Zn excess on stomatal conductance (Sagardoy *et al.* 2010) and other leaf gas-exchange parameters (Pavlíková *et al.* 2014). Studies on the effect of surplus Zn on photosynthetic response in lettuce are scarce.

While the effect of individual toxic elements has been thoroughly examined, with a few exceptions, less is known about the effects of combinations of toxic elements, although combinations are common in the environment. In this study, we cultured lettuce hydroponically using a multicontaminant solution containing As, Cd, Pb, and Zn. The mix of toxic elements in the solution corresponded to the water-soluble fraction of As, Cd, Pb, and Zn in soil from the contaminated Litavka River area in the Czech Republic. This river drains out of one of the largest historical Czech ore regions near Příbram (Grygar *et al.* 2021). Floodplain soils of the Litavka River are heavily polluted as a result of combined historic lead–silver (Pb–Ag) mining and smelting activities as well as extreme flooding events that occurred between the years 1932 and 2002 (Kebonye *et al.* 2022). Many studies have investigated various aspects of toxic elements in this area, including their contents, speciation, distribution, and interaction with soil properties (Vaněk *et al.* 2008, Kotková *et al.* 2019, Kebonye *et al.* 2022). However, information about the effects of this contamination on plants is limited. Thus, the main objective of the study was to investigate the effect of multicontamination on toxic element accumulation and the response of lettuce at the photosynthetic level. We used hydroponic culture to enable us to focus only on the combined effect of toxic elements and exclude soil factors (such as buffering and sorption) that could affect the measurements (Antonkiewicz *et al.* 2017, Savvas and Gruda 2018).

Materials and methods

Plant material and hydroponic experiment: Lettuce (*Lactuca sativa* var. *capitata*) was grown under a greenhouse hydroponic culture. Nutrients were supplied by nutrient solution based on Hoagland growth medium (Hoagland and Arnon 1950). This medium contained macro- and microelements necessary for plant growth and development: 1,652.7 mg L^{-1} $Ca(NO_3)_2{\cdot}4H_2O$; 505.5 mg L^{-1} KNO_3; 272.2 mg L^{-1} KH_2PO_4; 493 mg L^{-1} $MgSO_4{\cdot}7H_2O$; 7.8 mg L^{-1} $FeSO_4{\cdot}7H_2O$, and 1 mg L^{-1} supplementary trace elements solution (2.8 g L^{-1} H_3BO_3; 1.8 g L^{-1} $MnCl_2{\cdot}4H_2O$; 0.2 g L^{-1} $ZnSO_4{\cdot}7H_2O$; 0.1 g L^{-1} $CuSO_4{\cdot}5H_2O$; 0.025 g L^{-1} $Na_2MoO_4{\cdot}2H_2O$). The growth conditions were as follows: temperature day/night of 23/19°C, 60% relative humidity, and light/dark period of 14/10 h.

Plants were precultivated to the six-true-leaf stage in Rockwool cubes (4 × 4 × 4 cm), subsequently transplanted to the 3-L pots, and divided into two treatments – Control and Contamination. The Control contained only the nutrient solution. In the Contamination treatment, 250 mL of contaminated solution was added to 2.75 L of nutrient solution per pot. A mix of toxic elements was created to reflect the water-soluble content of As, Cd, Pb, and Zn of contaminated gleyic fluvisol from the Litavka location (49°43'N, 14°0'E) and was added to the Contamination treatment. Contamination of soil was characterized by the pseudo-total content of elements determined by an inductively coupled plasma mass spectrometer (ICP–MS, *Agilent 7700x*, *Agilent Technologies Inc.*, USA; extraction by aqua regia) – As_{total}, Cd_{total}, Pb_{total}, and Zn_{total}, and the water-soluble content of elements determined by an inductively coupled plasma-

Soil in research location		Contaminant	Created solution for Contamination [mg L^{-1}]	Form
As_{total} [mg kg^{-1}]/As_{ws} [mg kg^{-1}]	286 ± 14/1 ± 0.2	As	4.92	$Na_2HAsO_4 \cdot 7H_2O$
Cd_{total} [mg kg^{-1}]/Cd_{ws} [mg kg^{-1}]	37 ± 0.8/0.2 ± 0.02	Cd	0.89	$CdCl_2$
Pb_{total} [mg kg^{-1}]/Pb_{ws} [mg kg^{-1}]	2,344 ± 57/5 ± 1	Pb	24.93	$PbCl_2$
Zn_{total} [mg kg^{-1}]/Zn_{ws} [mg kg^{-1}]	3,515 ± 54/16 ± 1	Zn	77.28	$Zn(NO_3)_2 \cdot 6H_2O$

optical emission spectrometer (ICP–OES, *Agilent 720*, *Agilent Technologies Inc.*, USA; 24-h extraction by demineralized water, 1:5 w/v) – As_{ws}, Cd_{ws}, Pb_{ws}, and Zn_{ws}.

The characteristics of the growth solutions (pH and electrical conductivity) were measured. The pH was 5.0 and 4.8 in the Control and Contamination, respectively. The electrical conductivity was 2.8 mS and 2.6 mS in the Control and Contamination, respectively.

Each treatment contained four pots, each with three plants. Plants in each pot were harvested as one replicate after 14 d of growth. During harvesting, the plants were divided into two parts – roots and leaves. The parts were oven-dried to a constant mass (3 d at 40°C) and homogenized for element analysis.

Analysis of elements: The content of As, Cd, Pb, Zn, Mg, and Mn was determined using ICP–OES (*Agilent 720*, *Agilent Technologies Inc.*, USA) following a previously described method (Lhotská *et al.* 2022). Translocation factors (TFs) were calculated as the mean ratio of the selected risk element content in the leaves and roots, according to Boechat *et al.* (2016).

Gas-exchange parameters: The net photosynthetic rate – P_N [μmol(CO_2) m^{-2} s^{-1}], transpiration rate – E [mmol(H_2O) m^{-2} s^{-1}], stomatal conductance – g_s [mol(H_2O) m^{-2} s^{-1}], and intercellular CO_2 concentration – C_i [μmol(CO_2) mol^{-1}] were measured using the *LCpro+* portable gas exchange system (*ADC BioScientific*, *Ltd.*, UK). All measurements were conducted between 8:00 and 12:30 h. The duration of each measurement was 10 min after the establishment of steady-state conditions inside the measurement chamber. The conditions in the chamber were as follows: 25°C, ambient CO_2 concentration of 550 ± 50 μl L^{-1}, air flow rate of 205 ± 30 μmol s^{-1}, and irradiance of 650 ± 50 μmol(photon) m^{-2} s^{-1} of photosynthetically active radiation. The fully expanded young leaves (leaves of medium age from the middle of the head of lettuce) were selected for measurement ($n = 3$). Each parameter was measured three times: (*1*) the day before the Contamination was enriched with toxic elements (a baseline), (*2*) 7 d after the addition of toxic elements, and (*3*) 14 d after the addition of toxic elements.

Statistical analysis: All data were analysed with *Statistica 12.0* software (*StatSoft*, USA) and checked for homogeneity of variance and normality using the *Levene* and *Shapiro–Wilk* tests. A one-way analysis of variance (*ANOVA*) was used to identify significant differences between treatments regarding element determination and dry biomass. A two-way *ANOVA* was used to identify significant differences between the treatments and time interaction for photosynthetic parameters.

Results

Accumulation of selected elements and dry biomass: The measured toxic elements were under the detection limit in the Control in both plant parts (roots, leaves), except for Zn (Table 1). The Zn content was low and did not differ significantly between the leaves and roots of lettuce in the Control. The content of all measured elements (As, Cd, Pb, Zn) was higher in the Contamination (Table 1), with greater accumulation in the roots than in the leaves, as indicated by the TFs (Table 1). In the case of Zn content in the roots and leaves, it was 131.8-fold and 25.8-fold higher in the Contamination, respectively, compared with the Control.

In addition to the mentioned toxic elements, Mg and Mn, which are important in photosynthesis, were also analysed (Fig. 1). The Mg content in the Contamination was 4.9 and 34.5% lower in the roots and leaves, respectively, compared with the Control. The content of micronutrient Mn followed a similar trend to that of Mg. The Mn content was 92.7% lower in the roots and 81.7% lower in the leaves in the Contamination compared with the Control.

A negative effect of toxic element accumulation was observed on plant dry biomass (Table 1). The dry biomass of lettuce leaves and roots was significantly lower in the Contamination compared with the Control. In the case of lettuce leaves it was 35.7% lower, while it was 84.2% lower in the case of lettuce roots.

Photosynthetic parameters: The uptake of the added toxic elements affected some gas-exchange parameters (Fig. 2). A decrease in E was observed in both treatments 7 d after the start of the experiment (Fig. 2*A*). The difference between the Control and Contamination was significant – 30.3%. After 7 d, the values of E in the Control were lower than at the start of the experiment (by about 26.7%) and the value of E in the Contamination decreased even more (by 48.9%), as shown in Fig. 2*A*. A considerable difference between the two treatments was observed after 14 d (61.7%). While E increased in the Control by 9.8% after 14 d compared with the values measured after 7 d, E decreased even further in the Contamination (by 39.1%). The overall decrease was 18.7 and 68.9% in the Control and Contamination, respectively, compared with the values at the start of the experiment.

Values of g_s (Fig. 2*B*) changed after 7 d in both treatments (by about 49.4%). However, the decrease was only significant in the Contamination (42.8%). The response of plants in the Control was not significant 7 d after the start of the experiment. After 14 d, the difference between treatments was still significant

Table 1. The content of toxic elements in the lettuce biomass of the analysed treatments. nd – not detected, the value was below the detection limit: As < 0.03 mg mL^{-1}, Cd < 0.001 mg mL^{-1}, Pb < 0.02 mg mL^{-1}, TFs – translocation factors. Values are means ± SD (n = 4). *Letters* indicate significant differences according to one-way *ANOVA* and *Fisher*'s LSD test ($p \leq 0.05$). Data with *the same letter* are not statistically different.

	Control			Contamination		
	Roots	Leaves	TFs	Roots	Leaves	TFs
As [mg kg^{-1}]	nd	nd	-	537.51 ± 7.21[b]	4.73 ± 1.07	0.008
Cd [mg kg^{-1}]	nd	nd	-	160.20 ± 8.59	16.31 ± 4.26	0.10
Pb [mg kg^{-1}]	nd	nd	-	2,653.27 ± 42.17	14.43 ± 5.03	0.005
Zn [mg kg^{-1}]	42.91 ± 0.49[a]	36.49 ± 7.62[a]	0.85	5,656.28 ± 235.13[b]	940.98 ± 92.38[b]	0.17
Dry biomass [g pot^{-1}]	0.48 ± 0.02[b]	10.8 ± 2.2[b]	-	0.08 ± 0.004[a]	6.9 ± 1.9[a]	-

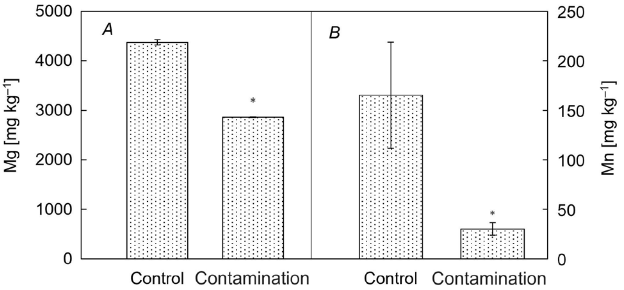

Fig. 1. The content of magnesium (*A*) and manganese (*B*) in the lettuce leaves of the analysed treatments. Values are means ± SD (n = 4). *Asterisks* indicate significant differences between treatments according to one-way *ANOVA* and *Fisher*'s LSD test ($p \leq 0.05$).

(64.3%). But compared with the values after 7 d, only the g_s of the Control increased after 14 d (21.9%). There was no significant difference between the g_s values of the Contamination after 7 and 14 d. The overall changes in g_s between the start of the experiment and 14 d later were as follows: in the Control, the overall increase was about 30.9% and in the Contamination, g_s was lower than at baseline, by 48.3%.

The changes in P_N also differed significantly between treatments (Fig. 2*C*). After 7 d, the difference between treatments was 30.8%. The P_N of the Contamination decreased by 32.5% after 7 d compared with the baseline. The values of the P_N in the Control did not change significantly. The difference between the two treatments was about 62.5% after 14 d. The values in the Contamination continued to decrease further, decreasing by 46.4% between 7 and 14 d. The changes in P_N values in the Control were not significant. The total decrease in P_N in the Contamination was 63.9%, compared with the values at the start of the experiment. The P_N in the Control did not change significantly over time, as shown in Fig. 2*C*.

C_i varied widely, as shown in Fig. 2*D*. A difference in C_i was observed between the two treatments after 7 d (22.4%). While C_i increased by 26% in the Control compared with the baseline values, there was no significant change in the Contamination over time. After 14 d, higher values were achieved in the Contamination and the difference between treatments was 11.8%. The C_i of the Control decreased by 12.5% compared with the values after 7 d in the same treatment. The opposite trend was observed after 14 d in the Contamination, where C_i increased by 20.7%. Overall, there was an increase in C_i values in the Control and Contamination by 18.3 and 32.2%, respectively, compared with the baseline values.

Discussion

The availability of toxic elements in vegetables depends on soil conditions, such as cation-exchange capacity, pH, and organic matter content (Novotná *et al.* 2015, Antoniadis *et al.* 2017, Yang *et al.* 2017). To avoid the confounding influence of these soil factors, many studies have investigated stress tolerance mechanisms and toxic element fate in plants using hydroponic solutions (Matraszek *et al.* 2016, Lavres *et al.* 2019, Rai *et al.* 2019, *etc*.). As shown in Table 1, the toxic element contents in plant biomass increased with their contents in solution. Bidar *et al.* (2020) showed significant positive correlations between the concentrations of toxic elements in lettuce leaves and their concentrations in the soil. Their results confirmed that the uptake, translocation, and accumulation mechanisms of plants differ depending on the toxic element. Lavres *et al.* (2019) confirmed the different degrees of Cd tolerance among lettuce genotypes. The degree of accumulation of toxic elements in lettuce was the highest for Zn and Cd in leaves and for Zn and Pb in roots in our experiment. The number of toxic elements accumulating in lettuce can be characterised using TFs (Gupta *et al.* 2021). In our experiment, Zn and Cd had the highest TFs in lettuce. The TFs for Cd could be attributed to an ineffective barrier in the root endodermis (Casparian band) and the symplastic pathway from the endodermis to the root stele, thus improving Cd load in the xylem and its transport to the shoots (Akhter *et al.* 2014). The behaviour of Cd might be influenced by the presence of other elements. Kummerová *et al.* (2010) commented that the effect of Cd in combination with Zn depends on the dose of each element. In our case, the contaminated solution had several-fold higher concentrations of Zn, in addition to Cd. There is an antagonistic relationship between Cd and Zn (Rivelli *et al.* 2014). Increased Zn content in plants may reduce

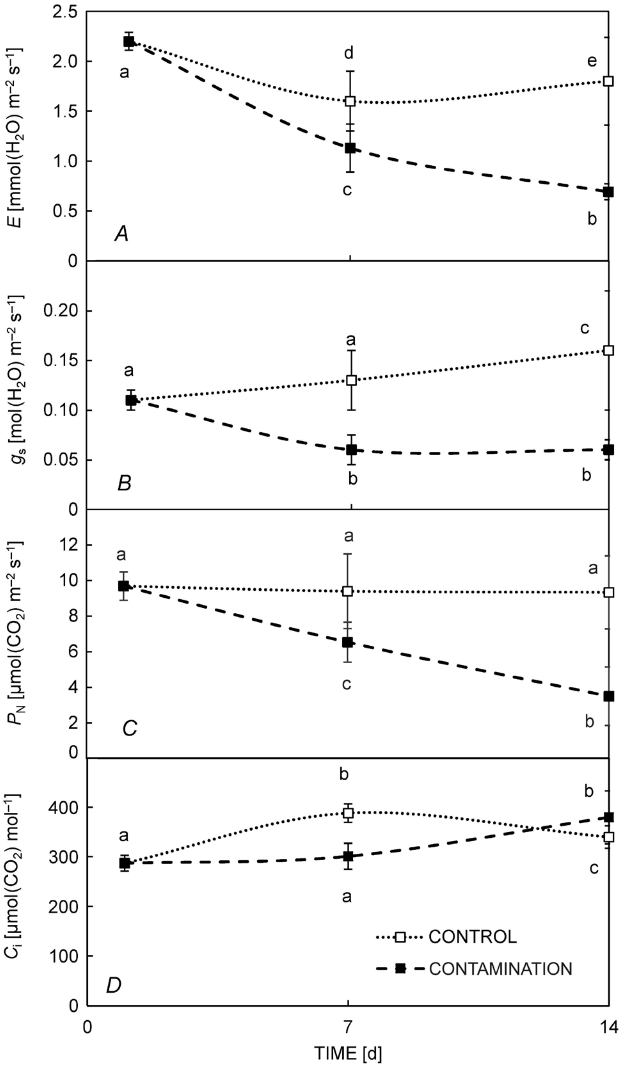

Fig. 2. Gas-exchange parameters in Control and Contamination treatments measured during the experiment (means ± SD). Measurements were recorded two times (7 and 14 d after the start of the experiment). *E* – transpiration rate (*A*); g_s – stomatal conductance (*B*); P_N – net photosynthetic rate (*C*); C_i – intercellular CO_2 concentration (*D*). *Letters* indicate significant differences and data with *the same letter* are not significantly different.

the translocation ratio from root to leaf for Ca, Fe, K, Mg, P, or Zn itself (Liščáková *et al.* 2022). In our experiment, most Pb and As accumulated in the roots, and shoot translocation was limited. Transport to the lettuce leaves was mainly affected by the formation of Pb complexes with organic acids (carboxylic and amino acids), promoting Pb mobility in plant vessels (Massaccesi *et al.* 2014). Lead availability is also affected by the presence of other toxic elements. Toxic elements such as Cd and Zn reduced Pb availability due to an antagonistic effect (Fahr *et al.* 2013).

The Mg and Mn contents decreased in both analysed plant parts (roots and leaves). Consistent with our results, Matraszek *et al.* (2016) and Jibril *et al.* (2017) reported a lower Mg content in lettuce leaves exposed to Cd solution in contrast to control plants. Jibril *et al.* (2017) and Dias *et al.* (2013) found a significant reduction in Mn content in leaves and roots, but only at the high Cd concentration. A higher Zn content in the soil can increase the Zn content in the roots, inhibit Fe and Mn accumulation, and reduce their concentration in the roots (Liščáková *et al.* 2022). According to these authors, imbalance in the nutrient contents and growth inhibition is a consequence of the competition between nutrients and toxic elements for binding sites in different compartments, for example, the plasma membrane and cell wall.

The damaging effects caused by the presence of toxic elements had a negative effect on the dry biomass of both leaves and roots in our study, similarly as observed in other studies (Saison *et al.* 2004, Antonkiewicz *et al.* 2017, Zemanová *et al.* 2021). In the latter studies, the toxic elements had a decisive influence primarily on plant roots. We presume that when roots were immersed directly in contaminated solutions, root damage led to changes in the physiology of the whole plant. Nazir *et al.* (2021) and Rabêlo *et al.* (2018) described the direct effect of Cd-enriched solutions on the morphology and anatomy of roots. Arsenic also affects root morphology (Zemanová *et al.* 2020, 2022; Burachevskaya *et al.* 2021). Zn is also associated with a reduced plant root system (Genc *et al.* 2007, Namdjoyan *et al.* 2017). The significant decrease in root dry biomass, as observed in the Contamination treatment in our experiment, is related to a decrease in cytokinin synthesis in root tips, a major site for cytokinin production (Rucińska-Sobkowiak 2016). The changes in root architecture (inhibition of the growth of primary and secondary roots, a reduction in hair surface area, a high root/shoot area ratio; Rucińska-Sobkowiak 2016) are induced by the action of auxins – key hormones that regulate all developmental processes that are produced in the presence of toxic elements (Riyazuddin *et al.* 2022, Ejaz *et al.* 2023). Plant stress caused by toxic elements leads to a higher auxin content and auxin/cytokinin ratio (Sofo *et al.* 2013). Pavlíková *et al.* (2023) showed that the amount of indole-3-acetic acid, the auxin growth hormone, in tubers of cherry radish varied under As stress, while the accumulation of toxic elements could increase abscisic acid (ABA) synthesis in roots (Ejaz *et al.* 2023). The root-derived ABA or ABA-induced signals may also play a role in leaf responses, such as stomatal conductivity (Rucińska-Sobkowiak 2016).

In general, the toxic elements harmed the measured photosynthetic parameters. During the experiment, plant guard cells were flaccid, leading to closed stomata in the Contamination treatment, as indicated by the value of g_s (Fig. 2*B*). The consequence of this was the decreased plant transpiration, as indicated by the *E* values (Fig. 2*A*). Similarly, He and Ren (2009) observed changes in stomatal conductance and the transpiration rate, which decreased as Cd doses increased (10–100 µmol L^{-1}). A decrease in both mentioned parameters was also observed in the multicontaminated soil (enriched with Pb, Zn, and Cu) (Cheng *et al.* 2017). Stomatal conductance can also decrease in response to excess Zn (10–300 µM) (Sagardoy *et al.* 2010). When plants have closed stomata,

CO_2 accumulates in mesophyll cells, as shown by the C_i (Fig. 2*D*). According to Sanz-Saez *et al.* (2019), an elevated CO_2 concentration can condition the fitness and productivity of plants. CO_2 can be used to assimilate and create C skeletons and energy molecules unless photorespiration occurs. During this process, plants limit assimilation because of stress conditions, nutrients and energy are redirected from the production of biomass towards the defensive process, as shown by Prasch and Sonnewald (2015). This was confirmed in the study of the wild type of *Nicotiana tabacum* (Pavlíková *et al.* 2014), where even though the intracellular CO_2 concentration increased, the dry matter yield decreased as the Zn concentration increased. The effect of decreased CO_2 content in mesophyll cells is also dependent on the type of toxic element. For example, Pb, in addition to Cd, did not affect the intracellular CO_2 concentration in leaves of *Coriandrum sativum* (Fattahi *et al.* 2021). In contrast, the multicontamination presented in our study had a significant influence on the net photosynthetic rate (Fig. 2*C*). The net photosynthetic rate decreased in the presence of toxic elements, in the Contamination treatment. Similarly, the net photosynthetic rate decreased after increasing the As dose (Pavlíková *et al.* 2020). The effect of toxic elements on the rate of photosynthesis was also investigated by Hattab *et al.* (2009). The authors stated that the presence of Cd and copper reduced the photosynthetic rate and content of photosynthetically active pigments. Cd decreases the net CO_2 assimilation rate by decreasing Rubisco activity (Dias *et al.* 2013, Chen *et al.* 2022). According to Sagardoy *et al.* (2010), photosynthesis can be disturbed by the depletion of CO_2 at the Rubisco carboxylation site, which consequently limits stomatal and mesophyll conductance under the effect of Zn deficiency.

The present study shows that in the presence of As, Cd, Pb, and Zn multicontamination, lettuce can accumulate high amounts of these elements in the roots and low amounts in the leaves. These results, together with the TFs and dry biomass, showed that the root system was adversely affected by multicontamination. A negative effect of multicontaminant treatments on Mg and Mn content was observed. These results, together with P_N, E, and g_s, demonstrated that multicontamination affected photosynthesis and transpiration despite the low accumulation of As, Cd, Pb, and Zn in the leaves. The lettuce plants were sensitive to multicontamination in hydroponic conditions due to direct contact between the roots and As, Cd, Pb, and Zn, such that plant fitness was irreversibly affected. This study confirmed that hydroponic culture is a good screening method for estimating the root uptake capacity of toxic elements as it avoids the confounding influence of soil. It is difficult to analyse the effect of multicontamination by toxic elements on mechanisms of plant gas-exchange parameters, and therefore there are still a lot of unanswered questions related to the synergistic and antagonistic effects of single elements in plants and the environment. Further studies will be needed to clarify the consequence of the interrelationship of toxic elements in multicontaminated environments on leaf gas exchange in plants.

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