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ORIGINAL PAPER

EFFECT OF MANGANESE ON YIELD AND QUALITY OF HYDROPONICALLY GROWN LETTUCE*

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Abstract

Hydroponic production of leafy vegetables sustains consumption of fresh produce all year round. However, cultivation of leafy vegetables in a growing medium prepared from water contaminated with trace elements, particularly manganese (Mn), may affect both the plant products and their consumers. Therefore, an attempt was made in the present study to evaluate the effect of treating two cultivars of lettuce (cv. Locarno and cv. Satine) with Mn (0.5 – control, 5, 25 and 50 mg dm^{-3}) on (i) the concentration of Mn and other elements, (ii) biomass accumulation, (iii) the efficiency of the photosynthetic apparatus, (iv) the levels of ROS (reactive oxygen species), (v) the activity of antioxidative enzymes, and (vi) the content of phenolic compounds. Increased concentrations of Mn in the growing medium led to a significantly higher concentration of this element in lettuce leaves (up to a 9.4-fold increase), but concentrations of potassium, calcium and magnesium were almost unchanged. Although the intensity of photosynthesis was lower in both of the examined cultivars, biomass accumulation was slightly reduced only in cv. Locarno. The recorded toxic effect of Mn most probably resulted from the induced generation of ROS, levels of which usually increase *albeit* insignificantly in plants treated with the tested metal. Higher generation of ROS was to some extent counteracted by the increased activity of antioxidative enzymes (ascorbate peroxidase and catalase) and a higher content of phenolic compounds. The results of this study demonstrate that increased accumulation of Mn in lettuce not only impairs the plant growth, but may also pose a risk to human health, as these plant products may become dietary sources of Mn and elevated levels of ROS.

Keywords: biomass accumulation, elements concentration, lettuce, manganese, oxidative stress, photosynthetic apparatus.

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INTRODUCTION

Over the past few decades, agronomists and policy makers have focused on increasing crop yielding (White, Broadley 2009). A negative side effect of this strategy is that the concentration of nutrients in crops is often decreased (Rosanoff 2013), leading to, among others, a deficiency of nutrients in half of the world's population (Broadley et al. 2008). Hence, a growing interest in producing plants that have been enriched with essential elements. However, under unfavourable conditions, modification of a plant's mineral composition, while improving its nutritional properties, may result in trace elements exceeding the permissible concentrations, thus making the food products obtained harmful to consumers.

Lettuce (*Lactuca sativa* L.) is an easy-to-grow vegetable, rich in compounds that have a positive effect on health, e.g. phenolic compounds and inulin (Llorach et al. 2008, Di Bartolomeo et al. 2013). It is also presents a promising target for enrichment with trace elements (Smoleń et al. 2014). Growing lettuce hydroponically provides a year-round source of fresh vegetables in human diets. However, there may be a risk when low quality water is used to prepare the growing medium. According to Kleiber (2014), about 5% of water used in greenhouse production contains manganese (Mn) in elevated concentrations up to 1-4.5 mg dm^{-3} and sometimes even higher.

Manganese is an essential micronutrient in plants as it participates in redox reactions and acts as a co-factor for many enzymes (Marschner 1995, González et al. 1998), including the antioxidant superoxide dismutase (Alscher et al. 2002). Mn is also involved in the water-splitting system that provides electrons to PSII (Dau, Haumann 2007). However, an excess of Mn is toxic for plants. It causes the formation of brown spots on leaves, followed by chlorosis, necrosis and leaf shedding (Fecht-Christoffers et al. 2003). High concentrations of Mn interfere with the absorption, translocation and utilisation of other mineral elements, stimulates the phenolic metabolism, affects the energy metabolism, decreases photosynthesis and respiration rates, and causes oxidative stress (Demirevska-Kepova et al. 2004, St. Clair, Lynch 2004, Li et al. 2010, 2011, Srivastawa, Dubey 2011).

Similarly to some other trace elements, Mn is easily accumulated in aerial parts of leafy vegetables (Ducic, Polle 2005, Kleiber 2014), possibly reaching levels that can negatively affect consumer health. In humans, Mn may cause a clinical condition resembling Parkinson's disease (Aschner, Aschner 2005). Moreover, Mn-induced formation of ROS in crops may result in their elevated levels in human diets.

This study attempted to evaluate the effects of Mn treatment of two hydroponically grown cultivars of lettuce on the yield and quality of the food products obtained, based on (i) the concentration of Mn and other elements (K, Ca, Mg), (ii) the efficiency of the photosynthetic apparatus and biomass

accumulation, (iii) levels of ROS, (iv) the activity of antioxidative enzymes and (v) the content of phenolic compounds.

MATERIALS AND METHODS

Plant material and growing conditions

For this study, two cultivars of lettuce (*Lactuca sativa* L.) Satine and Locarno, which are popular in greenhouse production, were chosen. Seeds (Riij Zwaan, Netherlands) were sown onto rockwool plugs (Grodan, Netherlands) and germinated in a growing chamber (Sanyo MLR-350H, Japan) at 16°C and 8/16 h day/night. Uniform, two-week-old seedlings were transplanted into a hydroponics system (containers with 1.2 dm^3 of growing medium changed weekly) and cultivated for five weeks at 20/14°C, 10/14 h day/night, light intensity on the plant level of 200 μmol m^{-2} s^{-1}, pH of 6.2 and aeration for 12 h day^{-1}, in a cycle: 2 h of aeration followed by 2 h without aeration. The growing medium contained N-NO_3 – 200, P – 30, K – 210, Ca – 240, Mg – 40, Mn – 0.5, B – 0.3, Fe – 12, Cu – 0.1, Zn – 0.1 and Mo – 0.16 mg dm^{-3}. During the first week of cultivation, plants were acclimated to the growing medium using half-strength nutrient solution. Additional Mn was applied after the acclimation period to the full-strength growing medium as $MnSO_4$ in order to obtain the concentrations of 5, 25 and 50 mg dm^{-3}, and the plants were grown for further four weeks until they reached the commercial value. Control plants were grown in a growing medium containing the recommended 0.5 mg Mn dm^{-3}.

Ions concentration

Concentrations of selected ions were determined at harvest. Before drying (105°C for 2 h and then at 75°C for 48 h), the leaves were rinsed twice in tap H_2O and then distilled H_2O to purify their surface. The dried material was ground in a laboratory mill and wet mineralised with nitric acid. Atomic absorption spectrometry (Solaar M6, Thermo scientific, USA) was used to determine the amounts of Mn, K, Ca and Mg.

Efficiency of the photosynthetic apparatus and biomass accumulation

The efficiency of the photosynthetic apparatus was assessed 14 days after the treatment with additional Mn on fully developed, undamaged leaves from the middle part of a rosette. Plant gas exchange was evaluated using a LICOR 6400 Photosynthesis System (LI-COR, Inc., USA) equipped with a 6400-40 Leaf Chamber Fluorometer and a 6400-01 CO_2 mixer. Measurements of net photosynthesis and stomatal conductance were taken at the reference of CO_2 400 μmol mol^{-1}, PPFD (photosynthetic photon flux density) 800 μmol m^{-2} s^{-1}, flow rate 400 μmol m^{-2} and humidity of 40-50%. The chloro-

phyll content was measured using a chlorophyll meter CCM-200 (Opti-Sciences, USA). Chlorophyll *a* fluorescence was determined with a continuous excitation fluorometer (Handy PEA, Hansatech Instruments, UK) in leaves adapted to darkness for 45 min. The saturating light pulse intensity was 1500 μmol m^{-2} s^{-1} and its duration was 1 sec. The basic fluorescence parameters, minimal fluorescence (Fo), maximum fluorescence (Fm) and variable fluorescence (Fv=Fm-Fo), were recorded automatically and used for calculations of the maximum quantum efficiency of PSII (Fv/Fm). Additionally, the performance index (PI=Abs/CS*TRo/CS*ETo/CS, where Abs/CS = absorption flux per cross section, TRo/CS = trapped energy flux per cross section and ETo/CS = electron transport flux per cross section) was determined.

At harvest, the fresh weight and dry matter (drying at 105°C for 2 h and then at 75°C for 48 h) of leaves and roots were recorded.

Levels of ROS and activity/content of selected components of the antioxidative system

The generation of ROS, activity of antioxidative enzymes and the content of phenolic compounds were assessed spectrophotometrically (Spectrometer UV/VIS U2900, Hitachi, Japan) at harvest. The levels of superoxide anion-radical ($O_2^{\bullet-}$) and hydroxyl radical (OH°) were determined in freshly ground leaves and roots at wavelengths of 580 nm and 540 nm respectively (Chaitanya, Naithani 1994). Samples for the determination of antioxidative enzyme activity and phenolic compounds were stored at -80°C until analysis. The activity of enzymes was measured in leaves and roots at wavelengths of 240 nm and 290 nm respectively for ascorbate peroxidase (APX) and catalase (CAT), as described by Nakano and Asada (1987) and Beers and Sizer (1952), both modified by Łata et al. (2005). The content of phenolic compounds was assessed in leaves at a wavelength of 420 nm with gallic acid (GA) used as standard (Medina 2011).

Statistics

Data were subjected to analysis of one factorial ANOVA using Statgraphics Plus 4.1. (Statpoint Technologies Inc., Warrenton, VA, USA). The Shapiro-Wilk test was used to examine the normality of distribution, while the Bartlett's test verified the homogeneity of variances. Differences between means of combinations were evaluated by *post-hoc* Tukey's Honestly Significant Difference (HSD) test at $\alpha = 0.05$.

The data are means ± SE. The number of biological replications (a single plant in a container) was seven. The number of replications (separate sampling) for a given parameter ranged between three and ten. Two experiments were performed. Since the results obtained in both showed similar trends, the data presented here are from the experiment with a wider range of Mn concentrations and more parameters measured.

RESULTS

Ions concentration

Growing lettuce in the presence of elevated concentrations of Mn led to a significant increase in the content of this element in leaves (Table 1). In cv. Locarno lettuce, concentrations of Mn were greater than in the control

Table 1

Content of selected ions in leaves of lettuce plants grown in the presence of elevated concentrations of Mn in growing medium. Data are mean ±SE, n = 3.

Cv.	Mn (mg dm^{-3})	Mn	K	Ca	Mg
		(mg g^{-1} DM)			
Locarno	0.5 (control)	0.141±0.002*a**	39.74±0.66	12.23±0.04	7.27±0.18
	5	0.266±0.007*a*	39.64±0.60	12.15±0.04	6.11±0.37
	25	0.776±0.052*b*	38.11±0.67	12.17±0.06	6.73±0.29
	50	1.328±0.024*c*	37.12±1.31	12.18±0.03	7.71±0.31
Satine	0.5 (control)	0.131±0.008*a*	38.76±1.59	11.49±0.14	9.57±0.05
	5	0.267±0.003*b*	45.48±0.25	12.00±0.06	10.62±0.44
	25	0.658±0.001*c*	45.22±0.38	12.08±0.07	11.18±0.29
	50	1.144±0.022*d*	41.38±1.14	11.75±0.03	8.84±0.50

* Data followed by the same letter do not differ significantly.

by 89-842%, and in cv. Satine they exceeded the control by 104-773%, with the lowest and highest values found in plants grown in 5 and 50 mg Mn dm^{-3}, respectively. When comparing the cultivars, concentrations of Mn were almost the same, irrespective of the Mn concentration used (Table 1).

The effect of Mn treatments on the concentrations of the other examined elements was almost unnoticeable (Table 1). The addition of Mn did not change concentrations of Ca and Mg in either cultivar. Concentrations of K were insignificantly increased in Satine (by 7-17%), while in Locarno, same as the concentrations of Ca and Mg, they remained unchanged (Table 1).

Efficiency of photosynthetic apparatus and biomass accumulation

Irrespective of the cultivar, the addition of Mn insignificantly decreased gas exchange in the examined plants (Figure 1*a*, *b*). The intensity of photosynthesis was reduced by 14-24% in cv. Locarno and by 15% in cv. Satine (Figure 1*a*). For the stomatal conductance, these values amounted to 18-31% and 9-19% for the cultivars Locarno and Satine, respectively (Figure 1*b*).

Elevated levels of Mn usually have a positive effect on the chlorophyll content, and this was especially evident in cv. Locarno, in which a significant increase of 24-147% was noted (Table 2). Regardless of the Mn concentration

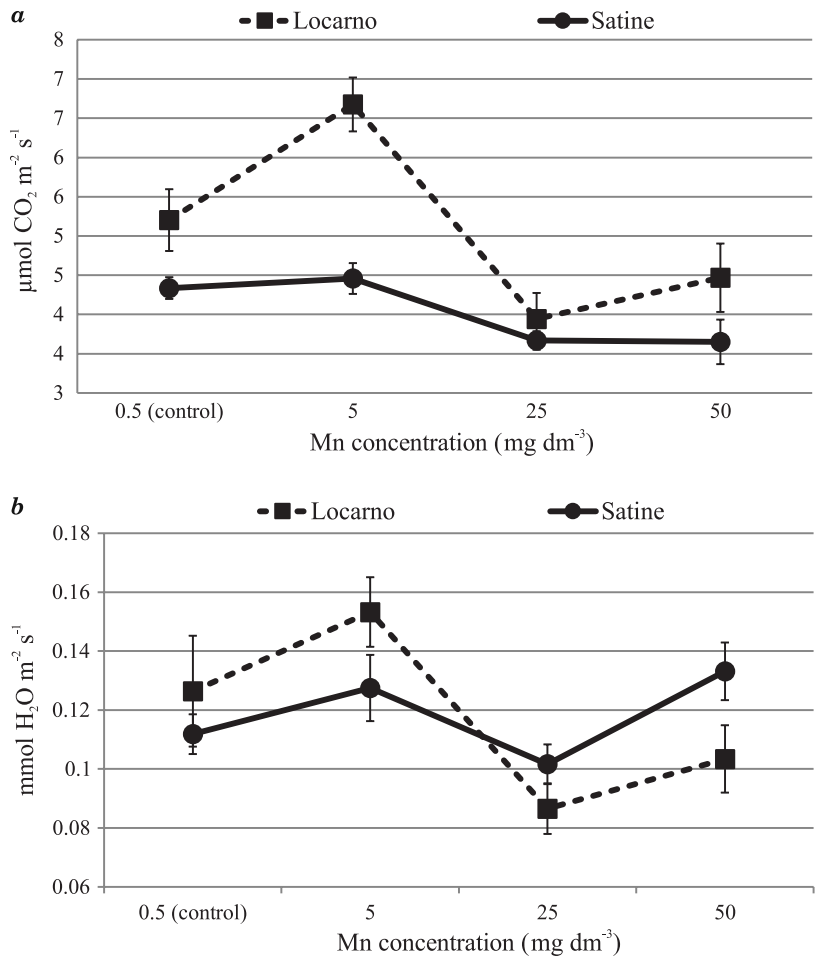

Fig. 1. Intensity of photosynthesis (*a*) and stomatal conductance (*b*) in lettuce plants grown in the presence of elevated concentrations of Mn in growing medium. Data are mean ±SE, $n = 5$

and cultivar tested, Fv/Fm was at the optimal level. The treatment with Mn did not affect the PI in cv. Locarno, while in cv. Satine the values of this parameter increased insignificantly (by 14-28%) – Table 2.

The impact of elevated Mn concentrations in the growing medium on biomass accumulation was slight in most cases, however some trends were recorded (Table 3). In cv. Locarno the addition of Mn insignificantly decreased fresh weight (by 4-23%) and dry matter (4-29%) in leaves, while it had no effect on the biomass accumulation in roots. Treatments with Mn usually did not change the biomass accumulation in cv. Satine; the only exceptions were an insignificant increase in the fresh weight in leaves of the plants grown in the presence of 5 mg Mn dm^{-3} and a decrease of dry matter in roots of the plants exposed to 5 mg Mn dm^{-3} (Table 3).

Table 2

Chlorophyll content and selected parameters of chlorophyll *a* fluorescence of lettuce plants grown in the presence of elevated concentrations of Mn in growing medium. Data are mean ±SE, n = 10

Cv.	Mn (mg dm^{-3})	Chlorophyll content (CCI)	Fv/Fm	P.I.
Locarno	0.5 (control)	3.12±0.25*a**	0.861±0.002	2.97±0.13
	5	7.70±0.03*c*	0.851±0.001	3.43±0.15
	25	3.88±0.21*ab*	0.859±0.001	2.83±0.11
	50	6.30±0.62*bc*	0.858±0.001	2.66±0.09
Satine	0.5 (control)	5.20±0.23	0.857±0.001	3.38±0.08
	5	5.32±0.20	0.856±0.001	3.84±0.17
	25	4.92±0.22	0.854±0.001	4.31±0.19
	50	6.06±0.30	0.853±0.001	3.96±0.09

* Data followed by the same letter do not differ significantly.

Table 3

Fresh weight (FW) and dry matter (DM) of leaves and roots of lettuce plants grown in the presence of elevated concentrations of Mn in growing medium. Data are mean ±SE, n = 5

Cv.	Mn (mg dm^{-3})	FW g plant^{-1}		DM g plant^{-1}	
		leaves	roots	leaves	roots
Locarno	0.5 (control)	92.37±5.30	7.59±0.54	4.38±0.22	0.36±0.01
	5	88.64±13.9	8.51±1.62	4.19±0.63	0.42±0.08
	25	70.69±4.77	8.04±0.26	3.13±0.25	0.38±0.02
	50	70.88±7.68	7.58±0.76	3.22±0.37	0.33±0.04
Satine	0.5 (control)	87.78±2.08	5.82±0.29	3.96±0.06	0.32±0.02
	5	101.5±2.04	6.36±0.09	4.37±0.11	0.24±0.01
	25	82.93±4.45	5.38±0.41	3.68±0.18	0.22±0.02
	50	84.02±5.41	4.58±0.27	3.79±0.24	0.21±0.01

Levels of ROS and activity/content of selected components of the antioxidative system

Increased concentrations of Mn in the growing medium stimulated generation of ROS in the leaves and roots of both cultivars, although recorded changes were insignificant (Table 4). In leaves the levels of $O_2^{\bullet-}$ were higher than in the control by 8-43% and 50-263%, while OH° was higher by 17-77% and 52-129% in Locarno and Satine, respectively. In roots, increased ROS production after the treatment with Mn was recorded only in cv. Locarno (by 2-7% and 54-73%, $O_2^{\bullet-}$ and OH° respectively), while the levels of the examined ROS in cv. Satine were usually slightly reduced. Of the tested cultivars, higher levels of $O_2^{\bullet-}$ were always recorded in cv. Locarno, while OH° was higher in the leaves of cv. Satine and in the roots of cv. Locarno (Table 4).

Table 4

Content of total phenolic compounds, levels of anion-radical ($O_2^{\bullet-}$) and hydroxyl radical (OH°), and activity of ascorbate peroxidase (APX) and catalase (CAT) in lettuce plants grown in the presence of elevated concentrations of Mn in growing medium. Data are mean ±SE, $n = 3$

Cv.	Mn (mg dm^{-3})	Total phenols (µg GA g^{-1} FW)	$O_2^{\bullet-}$		OH°		APX		CAT	
			relative value				n kat g^{-1} FW			
			leaves	roots	leaves	roots	leaves	roots	leaves	roots
Locarno	0.5 (control)	60.78±1.29	0.327±0.039	0.351±0.013	0.035±0.003	0.041±0.001	12.31*a** ±0.221	11.37±0.83	0.129±0.004	0.24±0.021
	5	67.45±1.57	0.467±0.017	0.375±0.018	0.041 ±0.004	0.065±0.007	11.81*a*±0.463	11.47±0.59	0.137±0.005	0.25±0.007
	25	67.28 ±1.00	0.354 ±0.009	0.357±0.220	0.042±0.001	0.063±0.003	13.71*a*±0.277	11.30±0.84	0.124±0.000	0.21±0.019
	50	70.59±2.58	0.270±0.023	0.316±0.010	0.062±0.004	0.071±0.001	18.95*b*±0.636	10.91±0.29	0.161±0.010	0.29±0.015
Satine	0.5 (control)	81.97±1.49	0.111±0.013	0.325±0.014	0.048±0.005	0.050±0.004	11.25±0.808	12.87±0.80	0.081±0.007	0.31±0.008
	5	84.64±3.13	0.403±0.012	0.340±0.014	0.110±0.010	0.049±0.004	11.81±0.144	14.97±0.31	0.100±0.008	0.30±0.027
	25	76.50±2.96	0.236±0.050	0.298±0.005	0.073±0.002	0.048±0.003	12.22±0.190	14.60±0.30	0.072±0.004	0.29±0.014
	50	89.37 ±1.67	0.167±0.008	0.312±0.013	0.094±0.007	0.028±0.000	13.39±0.384	14.83±0.65	0.085±0.011	0.29±0.010

* Data followed by the same letter do not differ significantly.

Irrespective of the examined cultivar, the activity of APX in leaves was greater in lettuce exposed to Mn (by 11-54% and 5-19% in Locarno and Satine, respectively), but significantly only when cv. Locarno plants were grown in the presence of Mn at a concentration of 50 mg dm^{-3} (Table 4). The effect of Mn on the activity of CAT was less unidirectional, as in both cultivars it increased at 5 and 50 mg Mn dm^{-3} and was reduced at 25 mg Mn dm^{-3}. In roots, changes in the activity of antioxidative enzymes caused by Mn were much weaker than in leaves. An insignificantly higher activity of APX was noted in cv. Satine (Table 4).

An increased content of phenolic compounds in plants exposed to additional Mn was noted in both cultivars, but the recorded changes were insignificant (by 11-16% and 3-9% in the cultivars Locarno and Satine, respectively) – Table 4.

DISCUSSION

Hydroponically grown lettuce can be an excellent source of leafy vegetables in the winter, when other fresh plant products are not available on the market or are more expensive. Lettuce also represents a highly promising target for enrichment with trace elements (Smoleń et al. 2014). However, there may be a risk to human health when water used for the preparation of growing medium contains high concentrations of potentially toxic compounds, as can often happen in the case of Mn (Ducic, Polle 2005, Kleiber 2014). Mn is easily taken up from the growing medium and subsequently translocated to aerial organs of plants, which are edible parts of leafy vegetables. According to the National Academy of Sciences, an adequate intake of Mn for adult men and women is 2.3 and 1.8 mg day^{-1} respectively (Aschner, Aschner 2005). Based on the results of this study, a whole lettuce plant of cv. Locarno grown in Mn at 5 mg dm^{-3} provides 48% and 62% of the recommended daily intake of this element for men and women, respectively. For cv. Satine, these values amounted to 51% and 65%. If the concentration of Mn increased to 25 mg dm^{-3}, the recommended daily intake is exceeded, irrespective of the cultivar. In hydroponics, excessive accumulation of this element can be avoided by monitoring Mn levels in water used to prepare the growing medium and correctly adjusting its pH because the availability of Mn increases with a decreasing pH (Ducic, Polle 2005). Nevertheless, the risk associated with Mn concentration in plant products is often underestimated.

An excess of metal(s) in the growing medium often leads to competitive interference with other elements, which in the case of Mn are Fe, Mg, Ca and K (St. Clair, Lynch 2004). In this study, concentrations of K, Ca and Mg in plants treated with Mn changed to a very small degree. In contrast to the above, Lee et al. (2011) and Kleiber (2014) demonstrate that elevated concentrations of Mn decrease the contents of K, Ca, Mg, Fe, Zn, and Cu.

Mn is an essential metal (Marschner 1995), but an excess of it has negative effects on plants (Fecht-Christoffers et al. 2003, Demirevska-Kepova et al. 2004, St. Clair, Lynch 2004, Li et al. 2010, Lee et al. 2011, Srivastawa, Dubey 2011). In this study, biomass accumulation decreased in cv. Locarno plants grown in the presence of Mn at concentrations exceeding 5 mg dm^{-3}, although the recorded changes were insignificant. In contrast, Rezai and Forboodnia (2008) show that the growth of pea is not disturbed by Mn in concentrations below 50 mg dm^{-3} and toxicity is only recorded at 100 mg Mn dm^{-3}. Higher toxicity of Mn in aerial parts than in roots is a characteristic shared by many species (Rezai, Forboodnia 2008, Li et al. 2010). It can result from the fact that the inhibition of Mn uptake or its retention in roots is not a common strategy for maintaining normal growth under Mn excess (Horst 1988). In the present study, this was true for cv. Locarno, but not for cv. Satine.

A slight reduction in the biomass accumulation in lettuce was preceded by a decrease in the efficiency of the photosynthetic apparatus, which is in line with the findings of Li et al. (2010) and Lee et al. (2011). Plants grown in two higher Mn concentrations photosynthesised with less intensity, but the level of this decline depended on a cultivar. A greater decrease in the intensity of photosynthesis was recorded in cv. Locarno. The reduced intensity of photosynthesis corresponded well with changes in stomatal conductance, which were usually lower in plants treated with Mn. Reduced stomatal conductance makes the CO_2 flow to a chloroplast more difficult and, at least partly, explains the decreased intensity of photosynthesis in plants grown in the presence of elevated Mn. On the other hand, Gonzalez and Lynch (1997), Lidon et al. (2004) and Li et al. (2010) show that Mn-mediated inhibition of photosynthesis barely implicates stomatal conductance, unless chlorosis is severe. The photosynthetic apparatus might also be impaired due to a reduction in the number of chloroplasts and a decrease in the chlorophyll content resulting from excessive Mn accumulation (Gonzalez, Lynch 1997, Gonzalez, Lynch 1999, Demirevska-Kepova et al. 2004), and enhanced production of carotenoids, leading to symptoms similar to those triggered by photoinhibition (Doncheva et al. 2009). In contrast to the above, in the present study and in the work of Li et al. (2010), a negative effect of Mn on the chlorophyll content was not recorded. Another possible explanation is the competition between Mn and other elements required for photosynthesis. St. Clair and Lynch (2004) demonstrate that excess Mn decreases concentrations of Fe, Mg and K, which are involved in photosynthetic electron transport, activity of RuBisCO and regulation of stomatal closing/opening. Since concentrations of K and Mg barely changed in plants exposed to Mn in the present study, competition between elements did not seem to be the major factor leading to a decrease in the intensity of photosynthesis under the conditions tested in this work.

Another parameter describing the efficiency of the photosynthetic apparatus, which is often cited as being sensitive to non-optimal Mn concentra-

tions, is chlorophyll *a* fluorescence (Doncheva et al. 2009, Li et al. 2010, Lee et al. 2011). In this study, Mn treatments did not affect Fv/Fm in either cultivar or PI in cv. Locarno. In cv. Satine, there was a slight increase in PI. Lidon et al. (2004) did not record any significant changes in the parameters of chlorophyll *a* fluorescence in plants exposed to increased concentrations of Mn and suggested that the accumulation of photosynthetic electron carriers are a major factor affecting photosynthesis in Mn-excess conditions.

The results of the present study indicate that excessive accumulation of Mn in lettuce most probably generated oxidative stress through the formation of ROS, as previously demonstrated by Demirevska-Kepova et al. (2004) and Srivastawa and Dubey (2011). Elevated levels of ROS were recorded in both cultivars and their production increased to a greater extent in leaves. Mn is a transition metal involved in the production of ROS *via* Fenton reaction (Fitsanakis et al. 2010), even though it is less likely than Fe to undergo spontaneous redox cycling (Gregus 2008). Srivastawa and Dubey (2011) show increased generation of $O_2^{\bullet-}$, elevated levels of H_2O_2 and thiobarbituric acid reactive substances, and a decline in protein thiols in Mn-treated plants. An increased level of ROS in lettuce accumulating an excessive amount of Mn is probably the main reason for a decline in the intensity of photosynthesis and consequently in the plant growth. These plants may also become a dietary source of ROS, thus negatively affecting human health, a consequence which until now has been underestimated in literature.

According to Demirevska-Kepova et al. (2004), the degree of cell damage after treatment with Mn depends on the rate of ROS formation and the efficiency and capacity of their detoxification/repair mechanisms. In this study, greater ROS generation was counteracted to some degree by the increased activity of APX and CAT and a higher content of phenols. An increase in the antioxidant activity/content was more evident in leaves, which corresponds well with stronger ROS formation especially in aerial parts of plants. These results are in line with the findings of St. Clair and Lynch (2004) and Li et al. (2010). According to Srivastawa and Dubey (2011), the antioxidative enzymes such as superoxide dismutase, glutathione peroxidase, APX and glutathione reductase appear to play a key role in scavenging $O_2^{\bullet-}$ and H_2O_2 under Mn excess. Li et al. (2010) claim that the antioxidant systems in Mn-excess plants provide sufficient protection against oxidative damage. Demirevska-Kepova et al. (2004) are more sceptical, pointing to the ambiguous response of the antioxidative system to high Mn concentrations. In our opinion, the oxidative stress under Mn toxicity is a consequence of the depletion of low-molecular antioxidants as a result of their involvement in detoxification processes and of the misbalance among antioxidative enzymes. APX is particularly sensitive to Mn excess (Demirevska-Kepova et al. 2004) as this enzyme is rapidly inactivated in the absence of ascorbic acid, which occurs under Mn toxicity conditions (González et al. 1998, Srivastawa, Dubey 2011). Since not all ROS and antioxidants were measured in this study, it is difficult to state unambiguously whether the increase in antioxidative activity

was sufficient to mitigate the negative effects of ROS, and therefore higher ROS generation should be regarded as a threat to both plant performance and human health.

CONCLUSIONS

1. An excess of Mn in the growing medium led to a higher content of this element in lettuce.

2. Despite the fact that the intensity of photosynthesis decreased in both cultivars examined, a slight reduction in biomass accumulation was recorded only in cv. Locarno.

3. A higher concentration of Mn in leaves had no effect on the concentrations of K, Ca and Mg.

4. The toxic impact of Mn is most probably due to higher levels of ROS and consequently the exacerbation of oxidative stress.

REFERENCES

Alscher R.G., Erturk N., Heath L.S. 2002. *Role of superoxide dismutases (SODs) in controlling oxidative stress in plants*. J. Exp. Bot., 53: 1331-1341.

Aschnera J.L., Aschnera M. 2005. *Nutritional aspects of manganese homeostasis*. Mol. Aspects. Med., 26(4-5): 353-362.

Beers R.F., Sizer I. 1952. *A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase*. J. Biol. Chem., 195(1): 133-140.

Broadley M.R., Hammond J.P., King G.J., Astley D., Bowen H.C., Meacham M.C., Mead A., Pink D.A., Teakle G.R., Hayden R.M., Spracklen W.P., White P.J. 2008. *Shoot calcium (Ca) and magnesium (Mg) concentrations differ between subtaxa, are highly heritable, and associate with potentially pleiotropic loci in Brassica oleracea*. Plant Physiol., 146: 1707-1720.

Chaitanya K.S.K., Naithani S.C. 1994. *Role of superoxide lipid peroxidation and superoxide dismutase in membrane perturbation during loss of viability in seeds of Shorea robusta* Gaertn F. New Phytol., 126: 623-627.

Dau H., Haumann M. 2007. *Eight steps preceding O-O bond formation in oxygenic photosynthesis-A basic reaction cycle of the Photosystem II manganese complex*. Biochim. Biophys. Acta., 1767(6): 472-83.

Demirevska-Kepova K., Simova-Stoilova L., Stoyanova Z., Holzer R., Feller U. 2004. *Biochemical changes in barley plants after excessive supply of copper and manganese*. Environ. Exp. Bot., 52: 253-266.

Di Bartolomeo F., Startek J.B., Van den Ende W. 2013. *Prebiotics to fight diseases: reality or fiction?* Phytother. Res., 27: 1457-1473.

Doncheva Sn., Poschenrieder C., Stoyanova Zl., Georgieva K., Velichkova M., Barceló J. 2009. *Silicon amelioration of manganese toxicity in Mn-sensitive and Mn-tolerant maize varieties*. Environ. Exp. Bot., 65: 189-197.

Ducic T., Polle A. 2005. *Transport and detoxification of manganese and copper in plants*. Braz. J. Plant Physiol., 17(1): 103-112.

Fecht-Christoffers M.M., Braun H-P., Lemaitre-Guillier Ch. 2003. *Effect of manganese toxicity on the proteome of the leaf apoplast in cowpea*. Plant. Physiol., 133(4): 1935-1946.

Fitsanakis V.A., Zhang N., Garcia S. Aschner M. 2010. *Manganese (Mn) and iron (Fe): interdependency of transport and regulation*. Neurotox. Res., 18: 124-131.

Gonzalez A., Lynch J.P. 1997. *Effects of manganese toxicity on leaf CO_2 assimilation of contrasting common bean genotypes*. Physiol. Plantarum., 101: 872-880.

Gonzalez A., Lynch J.P. 1999. *Subcellular and tissue Mn compartmentation in bean leaves under Mn toxicity stress*. Aust. J. Plant Physiol., 26: 811-822.

Gonzalez A., Steffen K.L., Lynch J.P. 1998. *Implications for oxidative stress in common bean*. Plant Physiol., 118: 493-504.

Gregus Z. 2008. *Mechanisms of toxicity*. In: *Casarett and Doull's Toxicology: The Basic Science of Poison*. Klassen C.D. (ed), McGraw-Hill Medical, New York, USA.

Horst W.J. 1988. *The physiology of manganese toxicity*. In: *Manganese in Soil and Plants*. Graham R.D., Hannam R.J., Uren N.C. (Eds.), Kluwer Academic Publ., Dordrecht, The Netherlands.

Kleiber T. 2014. *Effect of manganese nutrition on content of nutrient and yield of lettuce (Lactuca sativa* L.*) in hydroponic*. Ecol. Chem. Eng., 21(3): 529-537.

Łata B., Przeradzka M., Binkowska M. 2005. *Great differences in antioxidant properties exist between 56 apple cultivars and vegetation seasons*. J. Agric. Food. Chem., 53(23): 8970-8978.

Lee T.J., Luitel B.P., Kang W.H. 2011. *Growth and physiological response to manganese toxicity in Chinese cabbage (Brassica rapa* L. ssp. *campestris)*. Hort. Environ. Biotechnol., 52(3): 252-258.

Li Q., Chen L-S., Jiang H-X., Tang N., Yang L-T., Lin Z-H., Li Y., Yang G-H. 2010. *Effects of manganese-excess on CO_2 assimilation, ribulose-1,5-bisphosphate carboxylase/oxygenase, carbohydrates and photosynthetic electron transport of leaves, and antioxidant systems of leaves and roots in Citrus grandis seedlings*. BMC Plant. Biol., 10(42): 1-16.

Lidon F.C., Barreiro M.G., Ramalho J.C. 2004. *Manganese accumulation in rice: implications for photosynthetic functioning*. J. Plant Physiol., 161(11): 1235-1244.

Llorach R., Martinez-Aanchez A., Tomas-Barberan T.A., Gil M.I., Ferreres F. 2008. *Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole*. Food Chem., 108: 1028-1038.

Marschner H. 1995. *Mineral Nutrition of Higher Plants*. Academic Press, London, UK.

Medina M.B. 2011. *Simple and rapid method for the analysis of phenolic compounds in beverages and grains*. J. Agric. Food. Chem., 59: 1565-1571.

Nakano Y., Asada K. 1987. *Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical*. Plant Cell Physiol., 28(1): 131-140.

Rezai K., Farboodnia T. 2008. *The response of pea plant to manganese toxicity in solution culture*. Agric. J., 3(3): 248-251.

Rosanoff A. 2013. *Changing crop magnesium concentrations: Impact on human health*. Plant Soil, 368: 139-153.

Smoleń S., Kowalska J., Sady W. 2014. *Assessment of biofortification with iodine and selenium of lettuce cultivated in the NFT hydroponic system*. Sci Hortic., 166: 9-16.

Srivastava S., Dubey R.S. 2011. *Manganese-excess induces oxidative stress, lowers the pool of antioxidants and elevates activities of key antioxidative enzymes in rice seedlings*. Plant Growth Regul., 64: 1-16.

St. Clair S., Lynch J.P. 2004. *Photosynthetic and antioxidant enzyme responses of sugar maple and red maple to excess manganese in contrasting light environments*. Funct. Plant. Biol., 31: 1005-1014.

White P.J., Broadley M.R. 2009. *Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine*. New Phytol., 182: 49-84.