Genetics and Plant Physiology – 2014, Volume 4 (3–4), pp. 131–139
Special Issue (Part 2) – Conference "Plant Physiology and Genetics – Achievements and Challenges"
24 - 26 September 2014 – Sofia, Bulgaria

Available online at http://www.ifrg-bg.com

ACCUMULATION OF Cd, Pb, Zn AND ANTIOXIDANT RESPONSE IN CHAMOMILE (*MATRICARIA RECUTITA* L.) GROWN ON INDUSTRIALLY POLLUTED SOIL

Geneva M.[1*], Yu. Markovska[2], I. Todorov[1], I. Stancheva[1]

[1]Institute of Plant Physiology and Genetics – Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21,1113 Sofia, Bulgaria

[2]Sofia University "St. Kliment Ohridski", Faculty of Biology, 8 D. Tzankov Blvd., 1164 Sofia, Bulgaria

Received: *06 October 2014* Accepted: *05 December 2014*

Summary: This study evaluates the extent of accumulation of Cd, Pb and Zn in *Matricaria recutita* and the effect of heavy metals uptake on plant biomass, antioxidant potential and essential oil yield and quality. *Matricaria recutita* L plants were grown as a pot experiment on soil collected from the vicinities (1 km) of a Non-Ferrous Metals Combine and on non-contaminated control soil. If we take into account the high content of Cd, Pb and Zn in the plant organs only small plant growth retention occurred in treated plants. Therefore, this plant species exhibited tolerance to high levels of Cd, Pb and Zn in the soils. Accumulation of low molecular antioxidants such as ascorbate, glutathione and flavonoids in the above ground parts of treated plants was observed. Enzymatic antioxidant defence was activated in the plants grown on contaminated soil, mainly on the account of ascorbate peroxidase in the above ground parts, glutathione peroxidase and quaiacol peroxidase in the leaves and dehydroascorbate reductase and glutathione-S-transferase in the flowers. Despite the accumulation of Cd, Pb and Zn in the plant organs, essential oil yield and quality did not change significantly.

Citation: Geneva M., Yu. Markovska, I. Todorov, I. Stancheva, 2014. Accumulation of Cd, Pb, Zn and antioxidant response in camomile (*Matricaria recutita* L.) grown on industrially polluted soil. *Genetics and Plant Physiology*, Conference "Plant Physiology and Genetics – Achievements and Challenges", 24-26 September 2014, Sofia, Bulgaria, Special Issue (Part 2), 4(3–4): 131–139.

Keywords: Antioxidant metabolites and enzymes; essential oil; heavy metals; *Matricaria recutita* L.

INTRODUCTION

Heavy metal (HM) pollution of agricultural soils is a major environmental problem that can affect plant productivity, food quality and human health (Kabata-Pendias, 2001). Remediation of contaminated soils using phytoextraction methods represents a low-cost technology based on the solar driven uptake and

*Corresponding author: boykova2@yahoo.com

accumulation of metals in harvestable shoots of the HM tolerant plants.

The influence of industrial pollution in the vicinities of a Non-Ferrous Metals Combine near Plovdiv was investigated on a large number of medicinal and aromatic plants (MAP) (Geneva et al., 2010; Markovska et al., 2009). Although plants do not differ in their phytoextraction potential, they showed different tolerance to higher quantity of HM in the soil. HM accumulation in their different organs did not affect the quality and quantity of essential oils (Geneva et al., 2010; Stancheva et al., 2010a). That is why, the HM tolerant MAPs provide a possibility for economically advantageous soil purification in comparison with conventional expensive technologies, without deteriorating effects on the quality and quantity of active compounds, usable in cosmetics and pharmaceutic industries.

An important property of extracts and essential oils prepared from MAPs is their antioxidant activity (Zakaria et al., 2008). It is well known that antioxidant defense system in plants is responsible for detoxification of harmful reactive oxygen species resulting from HM pollution. The antioxidant response of plants includes mobilization of non-enzymatic antioxidants - ascorbate (ASC), glutathione (GSH), α-tocopherol, carotenoids, phenolic compounds and enzymatic antioxidants – superoxide dismutase (SOD), different specific peroxidases, catalase (CAT) and enzymes of ascorbate-glutathione cycle (AGC) (Sharma et al. 2010).

Chamomile (*Matricaria recutita* L.) is one of the most important medicinal herbs, frequently cultivated in Europe. Its anthodia contain specific secondary metabolites with anti-inflammatory and spasmolytic action. The pharmacological effect of chamomile is due to the combined action of inherent substances: sesquiterpenes, flavonoids, polyacetylenes, coumarins, mucilages, etc**.** (Schilcher, 1987)**.** This medicinal plant has the ability to accumulate high levels of toxic metals in the shoots and has been classified as Cd hyperaccumulator (Grejtovský and Pirč, 2000) and Zn accumulator (Grejtovský et al., 2006).

The aim of this study was to evaluate the extent of accumulation of Cd, Pb and Zn in *Matricaria recutita* L. grown on HM contaminated soil and the effect of HM uptake on plant biomass, antioxidant capacity, quality and quantity of essential oils.

MATERIAL AND METHODS

Chamomile (*Matricaria recutita* L.) plants were grown starting from seeds in a climatic chamber at 12 h photoperiod, day/night temperature 25/18°C and photon flux density of 95 mmol $m^{-1}s^{-1}$ until 60^{-th} day. Two-month-old seedlings were transferred to 5 kg plastic pots (10 plants per pot) and were grown 3.5 months until flowering stage on the soil/sand substrate in a ratio 3:1. Water was added to make up about 60% of water holding capacity. The soil was collected from the vicinities (1 km) of a Non-Ferrous Metals Combine with $pH_{(H_2O)}$ – 7.35 and the content of HMs (mg kg $soil^{-1}$): Cd - 9.02, Cu - 82.10, Pb - 301.75, Zn - 641.60. Because the Bulgarian permissible limit concentrations (PLC) at $pH_{(H_2O)}$ – 7.35 are Cd - 3.0, Cu < 260, Pb < 80 and Zn < 340 mg per kg, the soils are heavily polluted with Cd,

Pb and Zn. For the control non-polluted leached cinnamonic forest soil (Chromic Luvisols – FAO) was used ($pH_{(H_2O)}$ - 6.2) with the content of HMs (mg kg $soil^{-1}$): Cd – 0.25, Cu - 22.83, Pb - 16.00, Zn - 46.03. Because of the long duration of the experiment two formulations of foliar fertilizers (Agroleaf®, Scotts Company, Wooster, Ohio, USA) were applied to avoid starvation. Agroleaf® total - N:P:K=20:20:20 + microelements (N – NH_4^++NO_3^-, P - P_2O_5, K - K_2O), was applied twice during the vegetative growth stage on a 20-d interval until the bud formation phase. Agroleaf® with high P - N:P:K=12:52:5 + microelements, was applied before the blooming stage. Microelements in chelated form were present in concentrations: 0.1% Fe, 0.06% Mn, 0.06% Cu, 0.06% Zn, 0.02% B. Agroleaf® was applied by spraying at rates 0.3% solution, recommended by Scotts Company.

Chamomile anthodia were collected at the stage when ½ of tubular flowers were in full flower. Cultivation was terminated on day 110 after potting the seedlings when the age of plants reached 170 days. The drug (*Matricariae flos*) yield was determined by weighing the anthodia and shoots air-dried at room temperature. The production of root dry matter was determined after drying at 80°C for 72 h. The plant samples were digested in a 3:1 (v/v) HNO_3:$HClO_4$ solution. The samples were heated on a heating block at 200°C to evaporate them to dryness. The residue was taken up in 25 ml of 1N HCl. Metal concentrations were determined on an inductively – coupled Plasma Mass Spectrometer (CCD Simultaneus ICP OES, Varian, Austria).

Crude extracts for determination of enzyme activities were obtained according to Stancheva et al. (2010b). All enzymes were assayed spectrophotometrically according to the following authors: GPX (EC 1.11.1.9) (Edwards 1996), GST (EC 2.5.1.18) (Li et al. 1995), GR (EC 1.6.4.2) (Sherwin and Farrant 1998), MDHAR (EC 1.6.5.4.) (Miyake and Asada 1992), DHAR (EC 1.8.5.1.) (Doulis et al. 1997), CAT (EC 1.11.1.6) (Beers and Sizer 1952), APX (EC 1.11.1.11) (Nakano and Asada 1984) and GPO (EC 1.11.1.7) (Dias and Costa 1983). Protein content was determined by the method of Lowry et al. (1951). The concentrations of reduced (GSH) (Grifith 1980) and oxidized (GSSG) (Fadzilla et al. (1997) glutathione were determined using an enzyme recycling assay. ASC content was assayed after Foyer et al. (1983). The level of lipid peroxidation, chiefly malondialdehyde (MDA) and H_2O_2 content were determined according to Heath and Packer (1968). The content of phenolic compounds was determined spectrophotometrically using Folin-Ciocalteu reagent (Pfeffer et al., 1998). Flavonoids in plant tissues were measured using the method of Zhishen et al. (1999). Essential oil was determained following Stancheva et al. (2010a).

Data are expressed as means ±SE, where n = 3. Comparison of means was performed by Fisher's LSD test ($P \leq 0.05$) after performing ANOVA analysis (Statgraphics Plus, v. 2.1).

RESULTS AND DISCUSSION

An increase of shoot (17%) together with a small reduction of root dry biomass (7%) was observed in chamomile plants grown on industrially polluted soil

Table 1. Changes in shoot, root and anthodia dry biomass, plant height and diameter of anthodia in chamomile plants grown on heavy metal polluted (HM) and control unpolluted (C) soil.

	Shoot DW [g plant^{-1}]	Root DW [g plant^{-1}]	Anthodia DW [g plant^{-1}]	Number anthodia [plant^{-1}]	Anthodia diameter [cm]	Height [cm]
Control plants	0.153±0.008^{a}	0.189±0.009^{a}	0.042±0.002^{a}	3.57±0.18^{a}	1.74±0.87^{a}	25.00±1.25^{b}
Treated plants	0.179±0.009^{b}	0.175±0.008^{a}	0.045±0.002^{a}	4.03± 0.20^{b}	1.67±0.84^{a}	21.38±1.07^{a}
LSD (P≤0.05)	0.019	0.019	0.0045	0.431	0.19	2.63

Values are means ± SE by using 10 replications. The comparisons of means were determined by Fisher LSD test after performing ANOVA analysis. The different letters indicate significant differences assessed at (P≤ 0.05).

(Table 1). The number of flowers per plant increased while the flowers dry biomass did not change significantly due to HM contamination of the soil (Table 1). The height of treated plants was smaller in comparison with control plants, but the diameter of anthodia did not change. Plants grown on HM contaminated soil showed minor changes regarding biometrical parameters. Grejtovský et al. (2006) showed a weak positive effect of the increasing concentrations of Zn in the soil on the production of plant biomass and only small inhibition of growth in Cd-treated chamomile plants (Grejtovsky and Pirc, 2000).

The content of Cd, Cu, Pb and Zn significantly increased in chamomile plants grown on industrially contaminated soil (Table 2). Chamomile plants accumulated Cd predominantly in the above ground parts, while Cu, Pb and Zn are accumulated mainly in the roots. In general, the above ground plant parts accumulated 25 times more Cd and 1.5 times more Zn. The content of Pb and Zn in the roots of treated plants was increased 21 and 3.5 times, respectively,

Table 2. Distribution of heavy metals in shoots and roots of *Matricaria recutita* L. grown on heavy metal polluted (HM) and control unpolluted (C) soil.

Treatments	Cd [µg g^{-1} DW]	Cu [µg g^{-1} DW]	Pb [µg g^{-1} DW]	Zn [µg g^{-1} DW]
C - shoot	2.133±0.110^{a}	12.800±0.640^{a}	3.330±0.167^{a}	184.110±9.206^{a}
C - root	1.466±0.073^{a}	17.330±0.866^{a}	9.990±0.500^{a}	140.450±7.023^{a}
HM - shoot	54.260±2.710^{b}	11.670±0.583^{a}	11.330±0.567^{b}	286.570±14.328^{b}
HM - root	17.930±0.897^{b}	60.330±3.017^{b}	213.310±10.670^{b}	496.820±24.840^{b}
LSD - shoot	4.347	1.376	0.947	27.302
LSD - root	1.443	5.032	17.123	41.378

Values are means ± SE by using 3 replications. The comparisons of means were determined by FisherLSD test after performing ANOVA analysis. The different letters indicate significant differences assessed at (P≤ 0.05).

when compared with controls.

The level of antioxidant metabolites (Fig. 1) and enzymes (Fig. 2) indicated the antioxidant potential of chamomile plants. Toxic oxygen species can initiate lipid peroxidation and thus, increase MDA levels. A reduced level of MDA and total phenols in treated plants was observed both in the flowers and leaves (Fig. 1). Similar results regarding the oxidative status of chamomile related to Cd and Cu uptake were obtained by Kováčik and Bačkor (2007). The levels of H_2O_2, GSSG and DHASC increased significantly only in the flowers of treated plants. On the other hand, ASC, GSH and flavonoids increased in the leaves as a result of HM contamination. Therefore, higher accumulation of low molecular antioxidants in the above ground parts of treated plants was observed in comparison with control plants.

Despite the increased H_2O_2 content, CAT and GPO activities (potential scavengers of H_2O_2) decreased in the flowers of plants grown on HM contaminated soil (Fig. 2). Only APX – another scavenger of H_2O_2 increased in treated chamomile flowers. Among the enzymes of AGC, the activities of GST and DHAR increased in the flowers, APX both in the flowers and leaves and the activities of GPX and GPO increased in the leaves of treated plants. The small increase of reduced glutathione in the leaves of plants grown on polluted soil

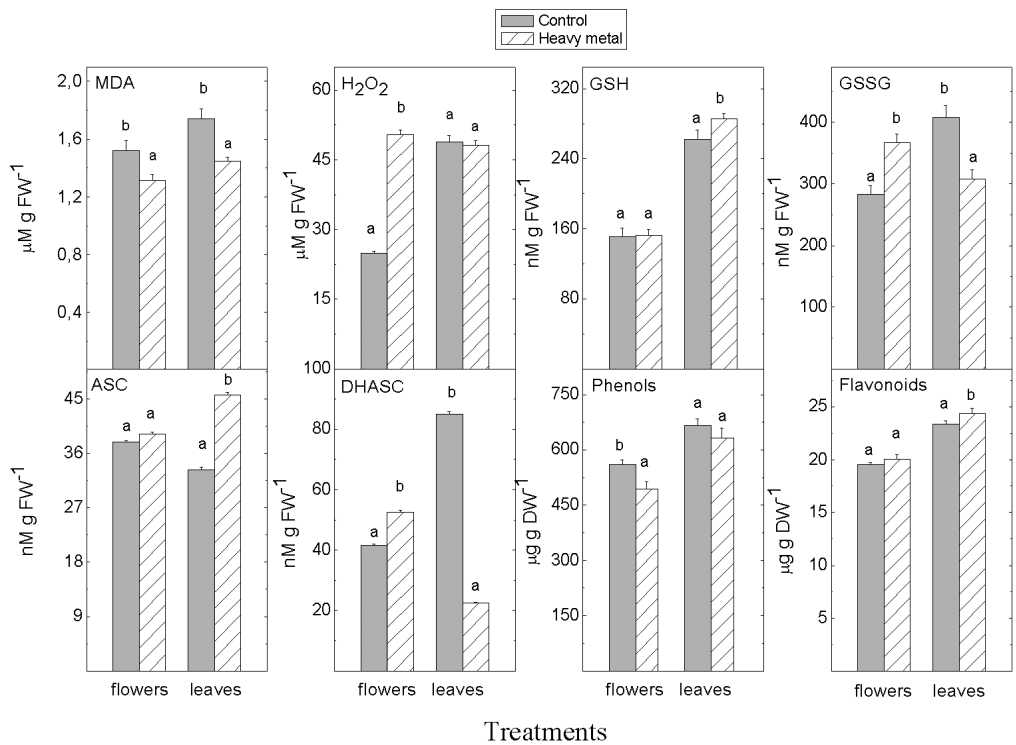

Figure 1. Content of MDA, H_2O_2, GSH, GSSG, ASC and DHASC, phenols and flavonoids in flowers and leaves of *Matricaria recutita* plants grown on industrially polluted (HM) and control unpolluted (C) soil. Values are means ± SE, $n = 3$; different letters indicate significant differences assessed by Fisher LSD test ($P \leq 0.05$) after performing ANOVA multifactor analysis.

(Fig. 1) could be due to high GPX activity, compensated with unchanged GR. High GSSG in the flowers (Fig. 1) corresponded to the elevated DHAR activity (Fig. 2). The MDHAR activity in the treated plants did not play a significant role in ASC levels regeneration. According to Smirnoff (1993) the antioxidant systems can be divided into two categories: one that reacts with ROS and keeps them at low levels (peroxidases, SOD, and CAT), and one that regenerates the oxidized antioxidants (MDHAR, DHAR, APX and GR). The antioxidant potential of treated chamomile plants was determinated by the increased content of low molecular antioxidants such as ASC, GSH and flavonoids as well as the higher activity of antioxidant enzymes such as APX. It has already been reported that the antioxidant mechanism of flavonoids may also come from their interaction with transition-metal ions to produce complexes that keep metal ions from participation in free-radical generation (Zhishen et al., 1999).

Chamomile anthodia (*Matricariae flos* drug) contain a range of pharmacologically effective secondary metabolites with anti-inflammatory and spasmolytic action. While α-bisabolol and chamazulene have proved to have the most bioactive properties, flavonoids such as apigenin and luteolin exhibit anti-inflammatory activity (Šalamon, 1992). According to the chamomile

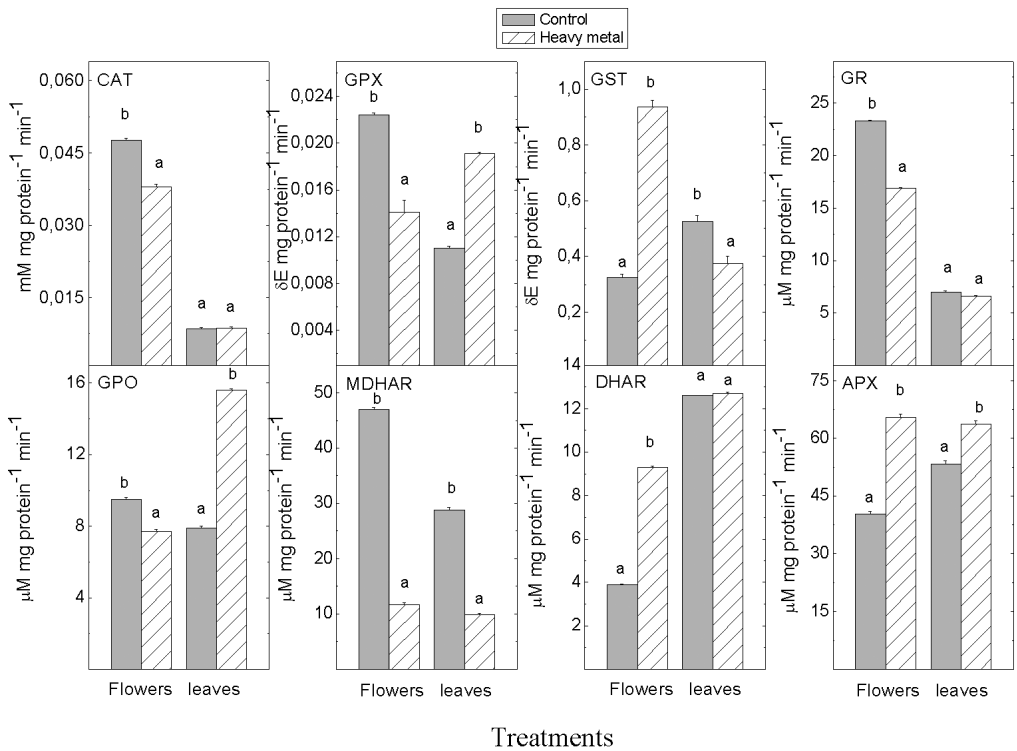

Figure 2. Activity of CAT, GPX, GST, GR, GPO, MDHAR, DHAR and APX in flowers and leaves of *Matricaria recutita* plants grown on industrially polluted (HM) and control unpolluted (C) soil. Values are means ± SE, $n = 3$; different letters indicate significant differences assessed by Fisher LSD test ($P \leq 0.05$) after performing ANOVA multifactor analysis.

pharmacological properties sesquiterpens α-bisabolol, chamazulene and farnesene are the most valuable constituents of the chamomile essential oil. The reports on the influence of HM on the active substances in chamomile plants are limited (Grejtovský et al., 2001, 2006).

Growing chamomile on contaminated with HM soil did not cause significant changes of the essential oil yield and composition (Table 3). Only chamazulene in the treated plants was undetectable and the content of farnesene slightly decreased. Our observation was in agreement with Grejtovský et al. (2006), who failed to show any changes in the content of chamomile essential oil substances even after application of a considerable quantity of Zn into the soil. In the study performed by Grejtovský et al. (2001) application of toxic Cd only to the soil caused a significant decrease in the content of essential oil including chamazulene and α-bisabolol and an increase in farnesene and polyacetylenes ene-yne-dicycloethers. These results differed from ours probably because of the complex interaction of several pollutants in the process of their uptake and distribution in the plants.

An increase in the above ground shoot biomass and number of flowers, together with the absence of significant changes in root and flower biomass and only a slight decrease in plant height were observed in chamomile plants grown on HM contaminated soil, indicating that plants were tolerant to the increased levels of Cd, Pb and Zn in the soil. Chamomile plants accumulated Cd predominanly in the above ground parts, while Cu, Pb and Zn were accumulated mainly in the roots. The antioxidant potential of treated plants was demonstrated by the elevated levels of antioxidants such as ASC, GSH and flavonoids in the above ground parts. The enzymatic antioxidant defence was activated in the plants grown on contaminated soil, mainly on the account of ascorbate peroxidase in the above

Table 3. Effect of heavy metals on the yield and levels of main essential oil components in chamomile anthodia extract.

	Control plants	Treated plants
Extracted compounds in % per DW	35±1%	35±1%
Apigenin – glycoside (HPLC)	0.8%±0.1% - in the drug 2.3±0.1% from extr. comp.	0.7%±0.1% - in the drug 2.0±0.1% from extr. comp.
Yield of essential oil	0.8±0.1%	0.7±0.1%
α-bisabolol	28-30%	30-32%
chamazulene	2-3%	trace
α-bisabolol oxid A	3-4%	5-6%
α-bisabolol oxid B	7-8%	5-6%
cis-spiroeter	28-30%	30-32%
dioxa-spiroeter	9-10%	8-9%
farnesene	16-18%	13-15%

ground parts, glutathione peroxidase and quaiacol peroxidase in the leaves and DHAR and GST in the flowers. The established levels of the essential oil compounds in chamomile plants grown on HM contaminated soil did not indicate deterioration of the essential oil quality.

REFERENCES

Beers RF, IW Sizer, 1952. A spectrophotometric method for measuring breakdown of hydrogen peroxide by catalase. J Biol Chem, 195: 133–140.

Dias A, M Costa, 1983. Effect of low salt concentration on nitrate reductase and peroxidase of sugar beet leaves. J Exp Bot, 34: 537–543.

Doulis AG, N Debian, AH Kingston-Smith, CH Foyer, 1997. Differential localization of antioxidants in maize leaves. Plant Physiol, 114: 1031–1037.

Edwards R, 1996. Characterization of glutathione transferases and glutathione peroxidases in pea (*Pisum sativum*). Physiol Plant, 98: 594–604.

Foyer CH, Rowell J, Walker D, 1983. Measurement of the ascorbate content of spinach leaf, protoplasts and chloroplasts during illumination. Planta, 157: 239–245.

Geneva M, Yu Markovska, I Stancheva, 2010. Metal uptake by Saint John`s wort (*Hypericum perforatum* L.) grown on industrially polluted soil. In: Proccedings of 6th Conference on Aromatic and Medicinal Plants of Southeast European Contries, Anthalia, 722–731.

Grejtovský A, R Pirč, 2000. Effect of high Cd concentrations in soil on growth, uptake of nutrients and some HMs of *Matricaria recutita* (L.). Rauschert. J Appl Bot, 74: 169–174.

Grejtovský A, M Repčák, A Eliašová, K Markušova, 2001. Effect of cadmium on active principle contents of *Matricaria recutita* L. Herba Pol, 47: 203–208.

Grejtovský A, K Markušova, A Eliašová, 2006. The response of chamomile (*Matricaria recutita* L.) plants to soil zink supply. Plant Soil Environ, 52: 1–7.

Heath RL, L Packer, 1968. Photoperoxidation in isolated choroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys, 125: 189–190.

Kabata-Pendias A, 2001. Trace elements in soils and plants. 3rd ed. LLC, Boka Raton, CRC Press.

Kováčik J, M Bačkor, 2007. Phenylalanine ammonia-lyase and phenolic compounds in chamomile tolerance to cadmium and copper excess. Water Air Soil Pollut, 185: 185–193.

Li Z-S, R-G Zhen, PA Rea, 1995. 1-chloro-2,4-dinitrobenzene elicited increase in vacuolar glutathione-S-conjugate transport activity. Plant Physiol, 109: 177–185.

Lowry OH, NJ Rosenbough, AL Farr, RJ Randall, 1951. Protein measurement with the Folin reagent. J Biol Chem, 193: 265–275.

Markovska Yu, Z Uzunov, M Nedkovska, N Gorinova, 2009. Antioxidative responses of *Mentha piperita* and *Mentha spicata* grown in HM contaminated soils, J Balkan Ecol, 12: 147–158.

Miyake C, K Asada, 1992. Thylakoid-

bound ascorbate peroxidase in spinach chloroplasts and photoprotection of its primary oxidation product MDA radicals methyloxids. Plant Cell Physiol, 33: 541–553.

Nakano Y, K Asada, 1984 Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol, 22: 867–880.

Pfeffer H, F Dannel, V Römheld, 1998. Are there connection between phenol metabolism, ascorbate metabolism and membrane integrity in leaves of boron-deficient sunflower plants? Physiol Plant 104: 479–485.

Šalamon I, 1992. Chamomile: a medicinal plant. Herb Spice Med Plant Digest 10: 1–4.

Schilcher H, 1987. Die Kamille. Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Sharma P, AB Jha, RS Dubey, 2010. Oxidative stress and antioxidative defense systems in plants growing under abiotic stresses. In: Pessarakli M, editor. Handbook of plant and crop stress. 3th edn. CRC Press, pp. 89–139.

Sherwin HW, JM Farrant, 1998. Protection mechanisms against excess light in resurrection plants *Craterostigma wilmsii* and *Xerophyta viscosa*. Plant Growth Regul, 24: 203–210.

Smirnoff N, 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist, 125: 27–58.

Stancheva I, M Geneva, G Georgiev, M Todorova, L Evstatieva, 2010a. Essential oil variation of *Salvia officinalis* leaves during its vegetation after treatment with foliar fertilization and thidiazuron. Comm Soil Sci Plant Anal, 41: 244–249.

Stancheva,I, M Geneva, M Hristozkova, Yu Markovska, I Salamon, 2010b. Antioxidant capacity of sage grown on heavy metals polluted soil. Russ J Plant Physiol, 57: 799–805.

Zakaria Z, YL Lachimanan, S Sreenivasan, X Rathinam, 2008. Antioxidant activity of *Coleus Blumei*, *Orthosiphon Stamineus*, *Ocimum basilicum* and *Mentha arvensis* from *Lamiaceae* family. Int J Nat Eng Sci, 2: 93–95.

Zhishen J, T Mengcheng, W Jianming, 1999. The Determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem, 64: 555–559.