Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2017.0478

60 (2017) 329–334
January

Exogenous application of salt (NaCl) inhibit antioxidative system in Cd-treated watercress (*Nasturtium officinale* R. Br.)

Afef Nasraoui-Hajaji[a,b,*], Karine Pageau[c], Chiraz Haouari Chaffei[b], Houda Gouia[b]

[a]*Department of Biology, Faculty of Sciences and Arts, Khulais, Jeddah University, Kingdom of Saudi Arabia, Tel. +99541750905; email: hajajiafef@yahoo.fr*
[b]*Research Unit of Nitrogen Nutrition and Metabolism and Stress-related Proteins (99/UR/C 09-20), Tunisian Faculty of Sciences, University of Tunis El Manar, 1060 Tunisia, emails: hajajiafef@yahoo.fr (A. Nasraoui-Hajaji), chiraz_ch2001@yahoo.fr (C.H. Chaffei), aghouda@yahoo.fr (H. Gouia)*
[c]*Research Unit EA 3900 Plants Biology Innovation (BIOPI) - UFR of Sciences, 33 street saint Leu, 80039 Amiens Cedex, France, email: Karine.pageau@u-picardie.fr*

Received 21 April 2014; Accepted 14 July 2016

ABSTRACT

This research simulates conditions found in metal-contaminated sites also affected by a high concentration of salt. For these reasons, several exogenous doses of NaCl were used to treat Cd-stressed *Nasturtium officinale* R. Br. We have chosen *Nasturtium officinale* R. Br which is a hyper accumulator plant of metals (based on literature). Data suggested induction of oxidative stress under Cd treatment and demonstrated watercress's capacity to upregulate its antioxidative defense. Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (GPX) activities were stimulated by Cd. Supplementation of (100, 200, 300 mM NaCl) simultaneously with 1 MCd to six weeks aged *Nasturtium officinale* R. Br caused growth inhibition, reduction of photosynthetic pigment contents and a significant increase of Malonyldialdehyde (MDA) and hydrogen peroxide (H_2O_2) ones in leaves. Increase of NaCl dose reduced Cd tenor in *Nasturtium officinale* leaves. Moreover, a dramatic lost of antioxidant system efficiency was detected in these plants. Rise of salinity concentration significantly inhibited SOD, CAT, APX and GPX activities in Cd-treated *Nasturtium officinale* R. Br.

Keywords: Antioxidative enzymes; Cadmium; *Nasturtium officinale* R. Br.; Phytoremediation; Salinity

1. Introduction

Water pollution by heavy metals has become acute in recent years because metal ions from natural, domestic and industrial sources tend to concentrate in the organic residue at the sewage treatment works. Further, toxic metal pollution of waters constitutes a risk of agricultural soils contamination through irrigation. The problem is due, in particular, to the non-degradability of inorganic pollutants like heavy metals which are hazardous when discharged into a water body [1]. It is well suggested that excess concentrations of heavy metals in soils such as Cd have caused the disruption of natural aquatic and terrestrial ecosystems [2,3]. Salinity is another principal abiotic factor that affects crop productivity. Salt severely limits growth of glycophyte plants and is becoming more problematic due to the increase in irrigation around the world. Salt exposure also induces numerous physiological stress reactions in plants that alter the chemical composition of crops and inactivate plant enzymes [4].

Cd may occur with other abiotic stress factor at the same ecosystem, which lead to plants suffering from multiple abiotic stresses. For these reasons, study of simultaneous applications of Cd and salinity stress was taken into consideration.

Combination of salinity and Cd stresses resulted in more severe growth inhibition us suggested by Smýkalová et al. [5].

* Corresponding author.

Soil salinity has been shown to increase Cd concentration in crops grown on soils treated with phosphorous fertilizers generally containing Cd [6–8]. Mühling et al. [9] demonstrated that salt and Cd co-application not only disrupt cell membrane function and elicit lipid peroxidation, but also disturb redox homeostasis in plant cells which induce a burst of reactive oxygen species. The generation of ROS is one of the main causes of injuries in plants exposed to NaCl and/or Cd stresses [10,11]. Plants can scavenge ROS by antioxidant systems consisting of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), etc. [12]. It has also been reported that cross adaptation is an important strategy employed by plants to resist combined abiotic stresses [13,14]. However, very little is known about NaCl and Cd interactions.

Research of plants used as phytoremediator in moderately polluted terrestrial and aquatic ecosystems, is one of the main interest of Scientifics. These plant species have abnormally high capacities of trace element removal from water [15,16]. Soltan et al. [17] have shown that there was a correlation between metal concentration of hyperaccumulator plants and the water column. Moreover, the aquatic plants are often the first link in relation to metal contents of aquatic environments [18]. Submerged species have been found to accumulate relatively high heavy metal concentrations when compared with emergent species in the some area. Thus recently, there has been growing interest in the use of metal-accumulating roots and rhizomes of aquatic or semi aquatic vascular plants for the removal of heavy metals from contaminated aqueous streams. For example, water hyacinth (*Eichornia crassipes* Solms) [19], duckweed (*Lemna minor* L.), [20] watercress (*Nasturtium officinale*) [21], take up Pb, Cu, Fe, Cd and Ni from contaminated solutions. Metal accumulation properties of *Nasturtium officinale* have been also studied extensively [22–24]; however, little attention has been paid to the ensuing responses and resultant effect of other abiotic stress factor on metal accumulation. Namdjoyan et al. [25] suggested that exogenous NO could alleviate negative effects of arsenic on watercress plants through its ability to the stimulation of reactive oxygen species (ROS)-scavenging enzymes activity and/or direct scavenging of superoxide onion.

Our present study objective was to investigate effects of NaCl treatment on Nasturtium *officinale* ability to accumulate Cd. Changes of antioxidant compounds to Cd and salt interaction were investigated in order to obtain a better understanding of N*asturtium officinale* growth responses. Effects of both salinity and Cd stresses simultaneously applied was also analyzed through several antioxidant enzymes activities, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR).

2. Material and methods

2.1. Plant material and growth conditions

Seeds of *Nasturtium officinale* R. Br were germinated in petri dishes in the dark. Seedlings were transferred and grown under continuous aeration in a nutrient solution containing KH_2PO_4, 0.5 mM; $Ca(NO_3)_2$, 1.25 mM; KNO_3, 2 mM; $MgSO_4$, 0.5 mM; Fe-K-EDTA, 50 μM; $MnSO_4 4H_2O$, 5 μM; $ZnSO_4 7H_2O$, 1 μM; $CuSO_4 5H_2O$, 1 μM; H_3BO_3, 30 μM; $(NH_4)_6 Mo_7O_{24} 4H_2O$, 1 μM. The growth conditions of the plants were: hydroponic culture in a growth chamber under controlled conditions, a 16 h-light (150 μmol m^{-2} s^{-1} PAR)/8 h dark cycle, 23°C (light)/18°C (night) and 65% relatively humidity. After 6 weeks, seedlings were grown in control condition or transferred on modified nutrient solution containing 1 M $CdCl_2$ in absence or in presence of NaCl (100 mM, 200 mM, 300 mM). After 10-day long treatment, leaves were harvested. Plant tissues were dried for 3 d in an oven at 80°C for further determination of dry mass or immediately frozen in liquid nitrogen and stored at –80°C for analysis of enzymatic activity.

2.2. Dry weight determination, Cd, chlorophyll, carotenoid, MDA and H_2O_2 contents measurement

After 3 d at 80°C, the dry weight (DW) of leaves was measured for each treatment.

For determination of Cd content, dried plant material was powdered using a porcelain mortar and pestle and wet digested in a mixture of $HNO_3/HClO_4$ (4/1 v/v) at 160°C. The digested material was diluted with deionized water and Cd concentration was determined using an atomic absorption spectrophotometry (Perkin–Elmer, AAanalyst 300). Cd contents were expressed on the basis of DW of leaves.

The content of chlorophylls (Chla) and carotenoids (Car) was determined spectrophotometrically according to Lichtenthaler [26]. The pigment concentrations were calculated by equations allowing a simultaneous determination of Chla and carotenoids (mg/g FW).

MDA content was determined by measuring the concentration of thiobarbituric acid-reacting substances (TBARS), as described by Alia et al. [27]. The leaves were homogenized in 5% (w/v) trichloroacetic acid (TCA). After centrifugation, a sample of the supernatant was added to 20% TCA containing 0.5% (w/v) thiobarbituric acid (TBA). The mixture was incubated at 95°C for 30 min. The amount of MDA-TBA complex was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

The content of H_2O_2 in leaves was determined based on the modified method of, i.e., by a colorimetric method at A_{508}, using H_2O_2 (30% Sigma) (5–50 μM) as a standard.

2.3. Antioxydant enzymes assays

Enzyme extractions were carried out at 4°C. The plant tissues were powdered in liquid nitrogen and extracted at a ratio 1:3 (w/v) fresh weight in 50 mM potassium phosphate buffer (pH 7) containing 1 mM EDTA, 3 mM dithiothereitol (DTT) and 5% (w/v) insoluble polyvinylpoly-pyrrolidone (PVPP). The homogenate was centrifuged at 14,000 g for 30 min and the resulting supernatant was used for enzyme activity. Protein content was determined by Bradford [28] using bovine serum albumin (BSA) as standard.

SOD activity (EC 1.15.1.1) was assayed as the inhibition of the photochemical reduction of β-nitroblue-tretrazolium chloride (NBT). One unit of SOD was defined as the amount of enzyme, which induced a 50% inhibition of NBT reduction measured as absorbance decrease at 560 nm [29]. CAT activity (EC 1.11.1.6) was determined as the decomposition of H_2O_2 causing the decline in absorbance at 240 nm [30]. APX (EC 1.11.1.11) activity was measured according to

Chen et al. [31] by monitoring the rate of ascorbate oxidation at 290 nm (E = 2.8 mM^{-1} cm^{-1}). One unit of enzyme was the amount necessary to decompose 1.0 μmol of NADPH per minute at 25°C. GPX activity was measured by the increase in absorbance at 470 nm caused by guaiacol oxidation (E = 26.6 mM^{-1} cm^{-1}) according to Decleire et al. [32]. GR (EC 1.6.4.2) activity was determined by monitoring the GSH-dependent oxidation of NADPH at 340 nm (E = 6.2 mM^{-1} cm^{-1}) according to Rao et al. [33].

2.4. Statistical analysis

All values reported are the means of six replicates per treatment (± SE), each experiment being conducted in duplicate. The mean values ± SE are reported in figures and tables. Data were processed by the variance analysis (ANOVA) and significance levels were accepted at $P \leq 0.05$ at all tests.

3. Results and discussion

The present study deals with the effect of cadmium and salinity on phytoremediation efficiency of watercress (*N. officinale* R. Br) via analysis of plants growth, Cd, chlorophyll and carotenoid contents as well as several antioxidant enzymes activities in leaves. Pronounced damaging effects occurred in the watercress plants exposed to the Cd and high concentration of NaCl in comparison with plants exposed to Cd alone or Cd + 100 mM NaCl (Table 1). A reduction in the biomass Cd-treated leaves was observed as a function of salinity doses (22.51%, 62.12% and 71.8% successively by 100, 200 and 300 mM NaCl). Several reports are available and indicate that *N. officinale* R. Br has good tolerance and phytoremediation potential for Cd and other heavy metals [21,34,35]. However, no study was interested to investigate its effectiveness when salinity also exist with the metal contamination. The combination of NaCl and Cd stresses led to a significant decline in dry weight of leaves, but a slightly reduction (6.67%) was found between Cd treatment and control (Table 1). Same results were obtained in different plants seedlings growing on hydroponic nutrient solution simultaneously contaminated with Cd and NaCl such as, Oryza sativa [36], *Ricinus communis* and *Brassica juncea* [37]. According to several studies [38–41], growth inhibition was accompanied with a decrease of leaf protein tenor very pronounced when multiple abiotic stresses occurred simultaneously. In another way, growth inhibition caused by combination of NaCl and Cd application, seemed to be the result of mineral nutrition perturbation. However, rate of uptake and distribution of certain essential nutrients in plants treated with both Cd and salt stresses, may be responsible for mineral deficiencies/imbalance and depression of the plant growth [42–44].

Concerning bioaccumulation of Cd in tissues, results demonstrated that salinity reduced this parameter reflecting diminution of Cd translocation from soil to plants in *N. officinale*. Our data (Table 1) shown that the important tenor of Cd accumulated in plants was reduced by presence of salt (7.97%, 15.36% and 23.99%) successively by 100, 200 and 300 mM NaCl). When NaCl dose reached 300 mM, Cd tenor was more reduced (23.99%) compared with this in plants treated with Cd alone. An opposite effects of salinity on Cd accumulation was found in *R. communis* and *B. juncea.* Bauddh et al. [37] have demonstrated that salt provoke Cd absorption and accumulation in these species. Moreover, *R. communis* and *B. juncea* were proved still better phytoremediator of Cd than *N. officinale* when the contaminated soil is suffering from Cd and salinity stresses. Many other authors [37,45–47] showed that salinity increased Cd content in plant tissue and suggested that the formation of chloro-complexes increases Cd mobility in soils with a subsequent uptake of complexed Cd by plant roots [6].

In this study, the effect of varying stresses on leaf photosynthetic pigments (chlorophylla and carotenoid) contents of *N. officinale* was shown in Table 1. The total chlorophylla content as well as carotenoid one significantly decreased under the Cd+NaCl stresses compared with the controls (chlorophyll (6.86%, 15.68%, 59.11% and 69.41%); carotenoid (8.39%, 39.19% and 86.65%)). This decrease has been reported in many plants growing on salinity, drought or Cd stresses, alone or in combination. It is well suggested that mineral nutrition perturbation is in the base of photosynthesis activity lost resulting in growth inhibition of plants [46]. Moreover, nutritional disorders and loss of nutrient availability caused by salinity, resulted in reduction in biosynthesis of photosynthetic pigments [47]. Furthermore, displacement of Cd^{2+} by Na^{+} in cells roots disturbed membrane function by osmotic stress [48] and created a competition potential between the two ions. These were possible determinants for the observed decreased Cd tenor in plant tissue under salt condition [6,49]. Our results obtained in hydroponic conditions were at odds with those obtained in soil. In hydroponic culture, it might be possible that injured roots due to high salinity weaken the capacity of ion uptake or Na^{+} inhibits competitively Cd

Table 1
Dry weight (DW), cadmium (Cd), Chlorophyll a (Chla), carotenoids and malonyldialdehyde (MDA) contents in leaves of *Nasturtium officinale* treated with Cd alone or in combination with NaCl

	DW (mg)	Cd (μg/gDW)	Chla (mg/g FW)	Carotenoids (mg/g FW)	MDA (nmol/g FW)
Control	330 ± 10.02^{a}	0.012 ± 0.001^{d}	1.02 ± 0.01^{a}	0.893 ± 0.057^{a}	19.52 ± 1.01^{d}
Cd	308 ± 9.163^{b}	1.217 ± 0.12^{a}	0.95 ± 0.008^{a}	0.902 ± 0.062^{a}	20.07 ± 2.015^{d}
Cd+ 100 mM NaCl	255.71 ± 15.04^{b,c}	1.12 ± 0.153^{b}	0.861 ± 0.035^{b}	0.818 ± 0.01^{b}	25.75 ± 2.03^{c}
Cd+ 200 mM NaCl	125 ± 6.58^{c}	1.03 ± 0.184^{b}	0.417 ± 0.012^{c}	0.543 ± 0.038^{c}	29.13 ± 1.64^{b}
Cd+ 300 mM NaCl	93 ± 8.71^{d}	0.925 ± 0.0862^{b,c}	0.321 ± 0.11^{d}	0.12 ± 0.01^{d}	35.05 ± 2.96^{a}

Note: Each point represents the mean ± SD of six determinations. Different letters above bars indicate significant differences between treatments (ANOVA).

uptake [44]. However, *N. officinale* R. Br keeps its ability to take up and accumulate Cd in salinity condition, senescence symptoms were shown more pronounced compared with in plants treated with Cd alone.

In literature, it is well demonstrated that ROS increase engendered mitochondrial membrane lipid peroxidation characterized by MDA content [6,36,49], which can cause damage to these organelles. In our data, we showed that MDA content was not enhanced by treatment of Cd alone (Table 1). But, when (NaCl + Cd) stresses were applied, an increase of MDA level was detected with rise of NaCl concentration (Table 1). Similar response was detected in wheat seedlings treated with Cd and arsenate [50]. An increase in antioxidative activity is frequently an indication of unfavorable growth conditions and was found under many kinds of stress regimes [51,52]. In our study, activities of antioxidative enzymes were increased in the Cd treated plants (Figs. 1 and 2) which is consistent with the observations of Tao et al. [53]. In contrast, Shafi et al. [54] observed significant decline in CAT activity when wheat plants were exposed to Cd stress. Martin et al. [55] reported that SOD activity was unaffected in *Thlaspi arvense* plants exposed to Cd stress. These different results may be partly due to variation among species, developmental phase, Cd concentration, and exposure time. In our experiments, the combined Cd+NaCl stresses caused a marked decrease of the antioxidative activities in the plants in comparison with Cd alone (Figs. 1, 2). Maybe, the Cd and NaCl stresses showed antagonistic effects on oxidative stress. According to Guo et al. [56], combined Cd and elevated O3 had a significantly synergic effect on oxidative stress in wheat shoots. However, Welfare et al. [57] reported that NaCl and ozone in combination had an antagonistic effect on two chickpea cultivars. Moreover, Li et al. [58] showed that higher concentrations of Cd and As resulted in inhibition of SOD and POD activities. This suggests that there was insufficient increase in SOD and POD activities to scavenge excess $^{\bullet}O_2$ that accumulated in seedlings at high Cd and As concentrations. Therefore, further studies are required to understand the mechanisms of plant responses to multiple stresses. Concurrently to SOD and CAT activities decrease after (NaCl+Cd) exposure, the great increase in

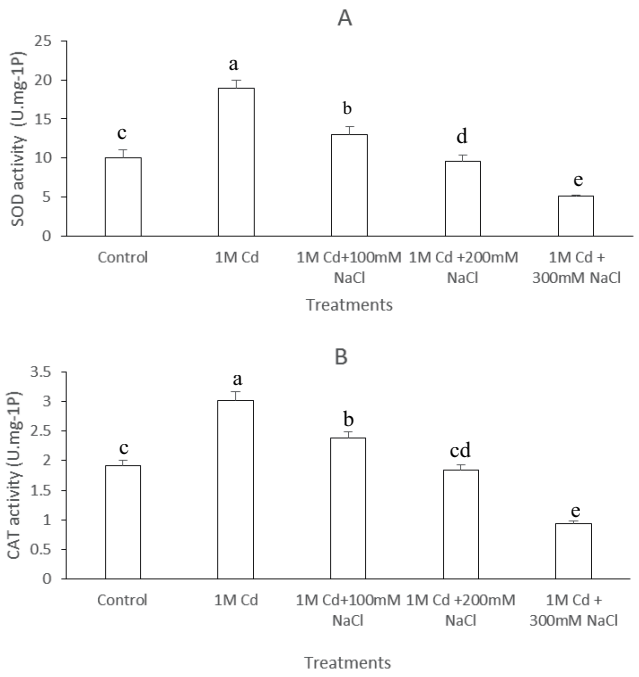

Fig. 1. (a) SOD and (b) CAT activities in leaves of *Nasturtium officinale* treated with non-supplemented nutrient solution (0 μM Cd), Cd alone or in combination with different concentration of NaCl (100, 200 or 300 mM).
Note: The data are means (SE) from six determinations. Different letters above bars indicate significant differences between treatments (ANOVA). SOD and CAT activities were expressed in U mg^{-1} protein.

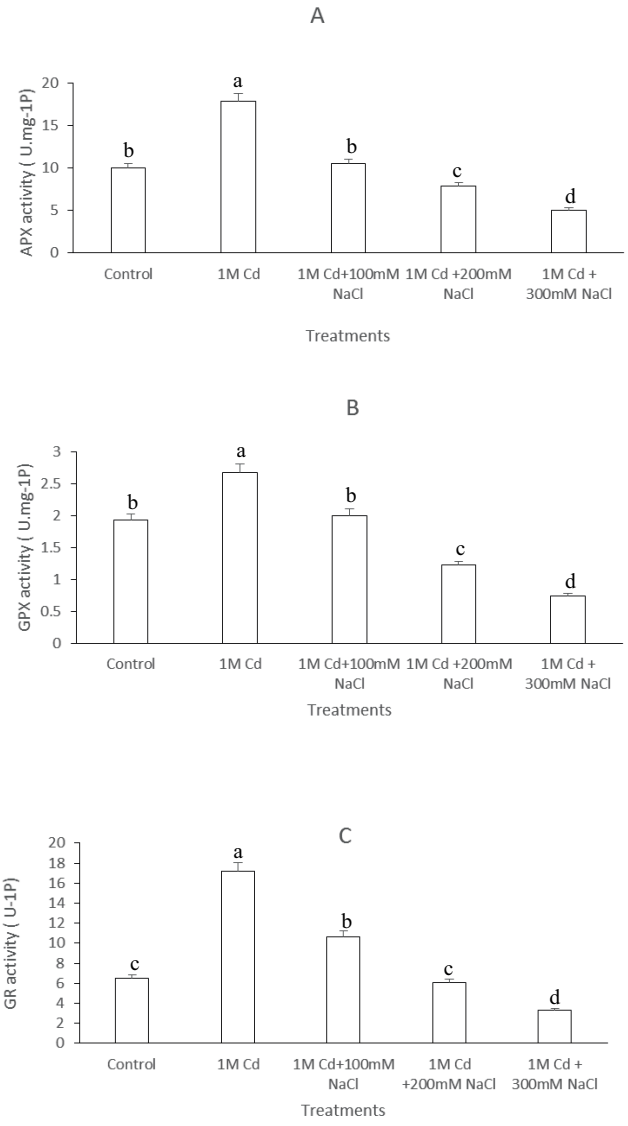

Fig. 2. (a) APX, (b) GPX, and (c) GR activities in leaves of *Nasturtium officinale* treated with non-supplemented nutrient solution (0 μM Cd), Cd alone or in combination with different concentration of NaCl (100, 200 or 300 mM).
Note: The data are means (SE) from six determinations. Different letters above bars indicate significant differences between treatments (ANOVA). APX was expressed in Asc mn^{-1} mg^{-1} protein. GPX activity was expressed in U mg^{-1} protein. GR was expressed in nmol NADPH mn^{-1} mg^{-1} protein.

MDA content and the start of visual toxicity symptoms were more pronounced in those plants (Table 1 and Fig. 1). These results confirmed the role of SOD activity in alleviation of oxidative stress by scavenging ROS from cell compartment. As (Cd+NaCl) stresses perturbed mineral nutrition, different important trace elements were not available. As consequence, there was a deficiency of different prosthetic groups (Fe, Cu/Zn or Mn) essential for catalytic action of SOD. Regarding the other enzymes of defense system, the levels of activities for APX, GPX and GR in different stressed plants changed in the same allure that SOD one (Fig. 2). Activation of the all different antioxidant enzymes in Cd-treated plants was reduced when salt is present simultaneously. In fact, SOD leads to the overproduction of hydrogen peroxide in the leaves of N. *officinale* plants subjected to Cd and salt stresses. However, the overproduction of hydrogen peroxide functions as the signal of stresses conditions, affected activities of the different antioxidant enzymes. These results put forward the major role of SOD which affected activities of all defense system enzymes resulting in growth inhibition. Far from, immobilization, exclusion, chelation and compartmentalization of metal ions are mechanisms adopted by plants to respond to heavy metal toxicity [59,60]. Salt et al. [61] suggested that the efficiency of these mechanisms were important in developing plants as agents for the phytoremediation of contaminated sites. However, interesting questions relating to the effect of antioxidative defense loss on the chelation of the metal by a ligand and, in some cases, the subsequent compartmentalization of the ligand-metal complex, remain to be answered in future studies.

4. Conclusion

Under acute Cd stress condition *N. officinale* R.Br growth was inhibited whereas the antioxidant enzymes were up-regulated compared with control. The combination of Cd and NaCl stresses showed that leaves Cd tenor was reduced by salt stress. But, N. *officinale* R.Br accumulation ability of Cd was saved in salt condition when NaCl dose was less than 300 mM. Our data demonstrated an antagonistic effect of stress combination on antioxidative enzyme activities which were decreased by increasing salt dose. As biomaterial for the efficient removal of cadmium, *N. officinale* R. Br may be used as a phytoremediator in polluted ecosystems by different abiotic pollutants (such as, Cd and salinity).

References

[1] R.K. Srivastav, S.K. Gupta, K.D.P. Nigam, P. Vasudevan, Treatment of chromium and nickel in wastewater by using aquatic plants, Water Resour., 28 (1994) 1631–1638.

[2] J.L. Gardea-Torresdey, L. Polette, S. Arteaga, K.J. Tiemann, J. Bibb, J.H. Gonzalez, Determination of the Content of Hazardous Heavy Metals on Larrea Tridentate Grown Around a Contaminated Area. Proc. Eleventh Ann. EPA Conf. on Hazardous Waste Research, L.R. Erickson, D.L. Tilison, S.C. Grant, J.P. McDonald, N.M. Albuquerque, 1996, pp. 660–672.

[3] R.B. Meagher, Phytoremediation of toxic elemental and organic pollutants, Curr. Opi. Plant Biol., 3 (2000) 153–162.

[4] H.A. Nasraoui, H. Gouia, H.C. Chaffei, Response of *Arabidopsis thaliana* seedlings to cadmium in relation to ammonium availability, Bull. Environ. Contam. Toxicol., 89 (2012) 1175–80.

[5] I. Smýkalová, B. Zámečníková, The relationship between salinity and cadmium stress in barley, Biol. Plant., 46 (2003) 269–273.

[6] M.J. McLaughlin, K.G. Tiller, T.A. Beech, M.K. Smart, Soil salinity causes elevated cadmium concentrations in field-grown potato tubers, J. Environ. Qual., 23 (1994) 1013–1018.

[7] E. Smolders, R.M. Lambrechts, M.J. McLaughlin, K.G. Tiller, Effect of soil solution chloride on Cd availability to Swiss chard, J. Environ. Qual., 27 (1997) 426–431.

[8] K. Weggler-Beaton, M.J. McLaughlin, R.D. Graham, Salinity increases cadmium uptake by wheat and Swiss chard from soil amended with biosolids, Aust. J. Soil. Res., 38 (2000) 37–46.

[9] K.H. Mühling, A. Läuchli, Interaction of NaCl and Cd stress on compartmentation pattern of cations, antioxidant enzymes and proteins in leaves of two wheat genotypes differing in salt tolerance, Plant Soil., 253 (2003) 219–231.

[10] K. Shah, R.G Kumar, S. Verma, R.S. Dubey, Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings, Plant Sci., 161 (2001) 1135–1144.

[11] R. Leyva, E. Sánchez-Rodríguez, J.J. Ríos, M.M. Rubio-Wilhelmi, L. Romero, J.M Ruiz, B. Blasco, Beneficial effects of exogenous iodine in lettuce plants subjected to salinity stress, Plant Sci., 181 (2011) 195–202.

[12] G.S. Shao, M.X. Chen, W.X. Wang, G.P. Zhang, The effect of salinity pretreatment on Cd accumulation and Cd-induced stress in BADH-transgenic and non transgenic rice seedlings, J. Plant Growth Regul., 27 (2008) 205–210.

[13] S. Boussiba, A. Rikin, A.E. Richmond, The role of abscisic acid in cross– adaptation of tobacco plants, Plant Physiol., 56 (1975) 337–339.

[14] C. Hafsi, A. Atia, A. Lakhdar, A. Debez, C. Abdelly, Differential responses in potassium absorption and use efficiencies in the halophytes catapodium rigidum and hordeum maritimum to various potassium concentrations in the medium, Plant Prod. Sci., 14 (2011) 135–140.

[15] A. Zayed, S. Gowthaman, N. Terry, Phytoaccumulation of trace elements by wetland plants: I. Duck weed, J. Environ. Qual., 27 (1998) 715–721.

[16] O.Y.L. Zhu, E.A.H. Pilon-Smits, L. Jouanin, N. Terry, Overexpression of glutathione synthetase in Indian mustard enhances cadmium accumulation and tolerance, Plant Physiol., 119 (1999) 73–79.

[17] M.E. Soltan, M.N. Rashed, Laboratory study on the survival of water hyacinth under several conditions of heavy metal concentrations, Adv. Environ. Res., 7 (2003) 321–335.

[18] A. Baker, R. Brooks, Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry, Biorecovery, 1 (1989) 81–126.

[19] S.H. Kay, W.T. Haller, L.A. Garrard, Effects of heavy metals on water hyacinths (*Eichornia crassipes*), Aquat. Toxicol., 5 (1994) 117–28.

[20] Y. Kara, Bioaccumulation of Cu, Zn and Ni from the wastewater by treated *Nasturtium officinale*, Int. J. Environ. Sci. Tech., 2 (2005) 63–67.

[21] Y. Kara, Bioaccumulation of copper from contaminated wastewater by using Lemna minor (Aquatic green plant), Bullet. Environ. Contam. Toxicol., 72 (2004) 467–471.

[22] R. Zurayk, B. Sukkariyah, R. Baalbaki, D.A. Ghanem, Chromium phytoaccumulation from solution by selected hydrophytes, Int. J. Phytoremed., 3 (2001) 335–350.

[23] M. Aslan, M.Y. Unlü, N. Türkmen, Y.Z. Yilmaz, Sorption of cadmium and effects on growth, protein content, and photosynthetic pigment composition of *Nasturtium officinale* R. Br. and Mentha aquatica L., Bull. Environ. Contam. Toxicol., 71 (2003) 323–329.

[24] S. Saygideger, M. Dogan, Influence of pH on lead uptake, chlorophyll and nitrogen content of *Nasturtium officinale* R. Br. and Mentha aquatica L., J. Environ Biol., 26 (2005) 753–759.

[25] S. Namdjoyan, S. Namdjoyan, H. Kermanian, Induction of phytochelatin and responses of antioxidants under cadmium stress in safflower (*Carthamus tinctorius*) seedlings, Turk. J. Bot., 36 (2012) 495–502.

[26] H.K. Lichtenthaler, Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, Method. Enzymol., 148 (1987) 350–382.

[27] H. Alia, H. Hayashi, A. Sakamoto, N. Murata, Enhancement of the tolerance of Arabidopsis to high temperatures by genetic engineering of the synthesis of glycinebetaine, Plant J., 16 (1998) 155–161.
[28] M.A. Bradford, Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing principles of protein-dye binding, Anal. Biochem., 72 (1976) 248–254.
[29] H. Costa, S.M. Gallego, M.L. Tomaro, Effects of UV-B radiation on antioxidant defense system in sunflower cotyledons, Plant Sci., 162 (2002) 939–945.
[30] I. Cakmak, D. Strbac, H. Marshner, Activities of hydrogen peroxide-scavenging enzymes in germinating wheat seeds, J. Exp. Bot., 44 (1993) 127–132.
[31] G.X. Chen, K. Asada, Ascorbate peroxidase in pea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties, Plant Cell Physiol., 30 (1989) 987–998.
[32] M.M. Decleire, Y.P. Honorze, G.V. Van Roey, Activité des peroxydase, catalase et glycolate oxydase après traitement avec divers herbicides, Weed. Res., 22 (1982) 85–88.
[33] M.V. Rao, G. Paliyath, D.P. Ormrod, Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*, Plant. Physiol., 110 (1996) 125–136.
[34] Y. Kara, D. Basaran, I. Kara, A. Zeytunluoglu, H. Genc, Bioaccumulation of nickel by aquatic macrophyta Lemna minor (duckweed), Int. J. Agr. Biol., 5 (2003) 281–283.
[35] D. Aydin, O.F. Coskun. Comparison of EDTA-enhanced phytoextraction strategies with *Nasturtium officinale* (watercress) on an artificially arsenic contaminated water, Pak. J. Bot., 45 (2013) 1423–1429.
[36] L.J. Ma, C.M. Yu, X.M. Li, L.L. Wang, C.Y. Ma, S.Y. Tao, N. Bu, Pretreatment with NaCl induces tolerance of rice seedlings to subsequent Cd or Cd + NaCl stresses, Biol. Plant., 57 (2013) 567–570.
[37] K. Bauddh, R.P. Singh, Growth, tolerance efficiency and phytoremediation potential of *Ricinus communis* (L.) and *Brassica juncea* (L.) in salinity and drought affected cadmium contaminated soil, Ecotox. Environ. Safety, 85 (2012) 13–22.
[38] N. Mohammadkhani, R. Heidari, Drought-induced accumulation of soluble sugars and proline in two maize varieties, World Applied Sci. J., 3 (2008) 448–453.
[39] S. Farouk, A.A. Mosa, A.A. Taha, M. Ibrahim Heba, A.M. El-Gahmery, Protective effect of humic acid and chitosan on radish (*Raphanus sativus*, L. var. sativus) plants subjected to cadmium stress, J. Stress Physiol. Biochem., 7 (2011) 391 100–116.
[40] A. Mafakheri, A. Siosemardeh, B. Bahramnejad, P.C. Struik, Y. Sohrabi, Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (*Cicer arietinum*) cultivars, Aust. J. Crop Sci., 5 (2011) 1255–1260.
[41] S. Muneer, J. Ahmad, M.I. Qureshi, Involvement of Fe nutrition in modulating oxidative stress and the expression of stress responsive proteins in leaves of *Vigna radiata* L., Aust. J. Crop Sci., 7 (2011) 1333–1342.
[42] G.P. Zhang, M. Fukami, H. Sekimoto, Influence of cadmium on mineral concentrations and yield components in wheat genotypes differing in Cd tolerance at seedling stage, Field Crops Re., 77 (2002) 93–98.
[43] G. Dražić, N. Mihailović, Z. Stojanović, Cadmium toxicity: the effect on macro- and micro-nutrient contents in soybean seedlings, Biol. Plant., 48 (2004) 605–607.
[44] Y. Huang, K. Wei, J. Yang, F.Z.D. Guo-Ping, Interaction of salinity and cadmium stresses on mineral nutrients sodium, and cadmium accumulation in four barley genotypes, J. Zhejiang Univ. Science. B., 8 (2007) 476–485.
[45] H.M. Helal, S.A. Haque, A.B. Ramadan, E. Schnug, Salinity–heavy metal interactions as evaluated by soil extraction and plant analysis, Commun. Soil Sci. Plant Anal., 27 (1996) 1355–1361.
[46] H.A. Nasraoui, H. Gouia. Photosynthesis sensitivity to NH4-N change with nitrogen fertilizer type, Plant Soil Environ., 60 (2014) 274–279.
[47] W.X. Wei, P.E. Bilsborrow, P. Hooley, D.A. Fincham, E. Lombi, B.P. Forster, Salinity induced differences in growth, ion distribution and partitioning in barley between the cultivar Maythorpe and its derived mutant Golden Promise, Plant Soil., 2 (2003) 183–191.
[48] S. Gallego, M. Benavides, M. Tomaro, Oxidative damage caused by cadmium chloride in sunflower (*Helianthus annuus* L.) plants, Phyt. Intern. J. Exp. Bot., 58 (1996) 41–52.
[49] K.H. Mühling, A. Läuchli, Interaction of NaCl and Cd stress on compartmentation pattern of cations, antioxidant enzymes and proteins in leaves of two wheat genotypes differing in salt tolerance, Plant Soil., 253 (2003) 219–231.
[50] X. Liu, S. Zhang, X.Q. Shan, P. Christie, Combined toxicity of cadmium and arsenate to wheat seedlings and plant uptake and antioxidative enzymes responses to cadmium and arsenate co-contamination, Eco. Environ. Safety, 68 (2007) 305–313.
[51] K.J. Dietz, Functions and responses of the leaf apoplast under stress, Progr. Bot., 58 (1996) 221–254.
[52] W. Maksymiec, Z. Krupa, The effects of short-term exposure to Cd, excess Cu ions and jasmonate on oxidative stress appearing in *Arabidopsis thaliana*, Environ. Exp. Bot., 57 (2006) 187–194.
[53] Y.M. Tao, Y.Z. Chen, T. Tan, X.C. Liu, D.L. Yang, S.C. Liang, Comparison of antioxidant responses to cadmium and lead in *Bruguiera gymnorrhiza* seedlings, Biol. Plant., 56 (2012) 149–152.
[54] M. Shafi, J. Bakht, M.J. Hassan, M. Raziuddin, G.P. Zhang, Effect of cadmium and salinity stresses on growth and antioxidant enzyme activities of wheat (*Triticum aestivum* L.), Bull. Environ. Contam. Toxicol., 82 (2009) 772–776.
[55] S.R. Martin, M. Llugany, J. Barceló, C. Poschenrieder, Cadmium exclusion a key factor in differential Cd resistance in Thlaspi arvense ecotypes, Biol. Plant., 56 (2012) 729–734.
[56] H.Y. Guo, R. Tian, J.G. Zhu, H. Zhou, D.P. Pei, X.R. Wang, Combined cadmium and elevated ozone affect concentrations of cadmium and antioxidant systems in wheat under fully open-air conditions, J. Hazard. Mater., 209–210 (2012) 27–33.
[57] K. Welfare, A.R. Yeo, T.J. Flowers, Effects of salinity and ozone, individually and in combination, on the growth and ion contents of two chickpea (*Cicer arietinum* L) varieties, Environ. Pollut., 120 (2002) 397–403.
[58] Y.M. Li, R.L. Chaney, A.A. Schneiter, Effect of soil chloride level on cadmium concentration in sunflower kernels, Plant Soil., 167 (1994) 275–280.
[59] L. Sanita di Toppi, R. Gabrielli, Response to cadmium in higher plants, Env. Exp. Bot., 41 (1999) 105–30.
[60] W. Ben Ammar, C. Mediouni, B. Tray, M.H. Ghorbel, F. Jemal, Glutathione and phytochelatin contents in tomato plants exposed to cadmium, Biol. Plant., 52 (2008) 314–320.
[61] D.E. Salt, R.D. Smith, I. Raskin, Phytoremediation, Annu. Rev. Plant Physiol. Plant Mol. Biol., 49 (1998) 643–668.