Chapter 2

Enzymatic Antioxidant Responses of Plants in Saline Anthropogenic Environments

Piotr Kamiński, Beata Koim-Puchowska, Piotr Puchowski, Leszek Jerzak, Monika Wieloch and Karolina Bombolewska

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/51149

1. Introduction

Plants under natural conditions are frequently exposed to combined stressors including drought stress and desiccation, salt stress, chilling, heat shock, heavy metals, ultraviolet, radiation, air pollutants such as ozone and SO_2, mechanical stress, nutrient deprivation, pathogen attack and high light stress [1]. A common result of most abiotic and biotic stresses is an increased production of reactive oxygen species (ROS), which frequently result in oxidative stress [2], [3]. The production of ROS results from pathways such as photorespiration, from the photosynthetic apparatus and from mitochondrial respiration [1].

The reduction of molecular oxygen to H_2O yields to intermediates reactive oxygen species (superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), hydroperoxyl radical ($HO_2^{\bullet}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2^{\bullet -}$), which are potentially toxic because they are relatively reactive compared with O_2 [4]. Reactive oxygen species may lead to the unspecific oxidations of proteins, membrane lipids or DNA injury. Malonodialdehyd (MDA) content, as a product of lipid peroxidation, has been considered as an indicator of oxidative damage in the cell membrane, resulting in disruption of metabolic function and loss of cellular integrity [5], [6]. Many reports confirm lipid peroxidation has been associated with damages provoked by a variety of environmental stresses [7], i.e. with salt stress [8], [9], [10] and heavy metals [11], [12].

However, there is evidence that ROS also plays important roles in the plant's defence system against pathogens and any pathogenic factors, mark certain development stages such as tracheary element formations, lignification and other cross linking process in the cell wall and act as intermediate signalling molecules to regulate the expression of genes [4]. Thus it is very important for cells to control of level of reactive oxygen species but not eliminate them completely. Defence mechanisms against free radical-induced oxidative stress involve: (1) preventive mechanisms, (2) repair mechanisms, (3) physical defences, and

(4) antioxidant defences [13]. The plants defend against these ROS by the induction of activities of certain antioxidative enzymes such as catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and superoxide dismutase (SOD), which scavenge reactive oxygen species [14], [15].

Superoxide dismutase catalyses the reaction of dismutation of superoxide radicals: $2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$. SOD is a metaloprotein with metals Cu, Zn, Mn, and Fe as co-factor [16], Stroiński 1999). Cu-Zn-SOD (homodimer; 31-33 kD) is present in cytosol and chloroplasts. Mn-SOD (tetramer 90kD) was found in the matrix mitochondrium [17], [18], whilst Fe-SOD, commonly occurring in *Procaryota*, was detected only in some plants [19], [18]. On the other hand catalase catalyses the reaction: $2\ H_2O_2 \rightarrow 2H_2O + O_2$. This enzyme contains three isoenzymatic forms (CAT-l, CAT-2, and CAT-3). CAT-1 and CAT-2 occur mainly in glyoxisomes, peroxysomes, and cytosol, while CAT-3 in mitochondria and cytosol [20], [18].

Another important factor decomposing H_2O_2 is peroxidase of ascorbic acid (APOX) (m.w. 28-34 kD) which contains protoheme as a prosthetic group and 4 cysteines responsible for sensitivity of the enzyme to compounds blocking a thiol group [21], [18]. It occurs in chloroplasts and cytosol [22], [18], vacuole [23], [18], and in apoplasts [24], [18]. APOX, in particular the chloroplast isoenzyme, becomes labile if the concentration of ascorbic acid drops significantly and it decomposes under the influence of its own radical product. Under such circumstances both monodehydroascorbate (MDHAA) and dehydroascorbate (DHAA) are easily reduced along the Halliwell-Asada path [18], [25]. The cell defensive mechanism also depends on small-cellular antioxidants such as glutathion (GSH), proline (Pro) or ascorbic acid (AA) which also react with radicals generated in the oxidative-stress [18].

The origin of oxidative stress under salinity conditions is well documented in leaves, where the inhibition of Calvin cycle results in over-reduction of oxygen and formation of superoxides [26]. Salt stress limits also gas exchange and thereby CO_2 supply to the leaf. One consequence is the over-reduction of photosynthetic-chain electron transport. This induces the generation of ROS [15]. Little is known about the origin of oxidative stress in roots, although salt stress-related impairment of mitochondrial function is likely to be involved [27]. Recent studies have revealed that salt-induced oxidative stress occurs in root mitochondria in a wild salt-tolerant tomato species as indicated by increased concentrations of H_2O_2 and MDA [28], [27]. It is now widely accepted that superoxide radicals produced during respiration, in response to salt stress, are the main precursors of mitochondrial H_2O_2 and make an important contribution to the oxidative load experienced by the cell [26]. Root mitochondrial ROS production is increased as a result of high salinity as constraints are imposed on electron transport through mitochondrial complexes I and II [27].

Calcium as macroelement and important physiological chemical element reacts with heavy metals and is required for various roles in plant cell [29], [30]. It is implicated in the movement of cellular organelles such as the spindle apparatus and secretory vesicles, and may play a key role in integrating plant cell metabolism [31], [32]. However, the homeostasis of Ca is maintained principally by the action of extrusion proteins. The cytosol is strongly buffered against high concentrations of calcium by numerous ranges of calcium-

binding proteins such as calmodulins and calmodulin-binding proteins [2]. Reports showed close interaction between intracellular H_2O_2 and cytosolic calcium in response to biotic and abiotic stresses -increase in cytosolic calcium boosts the generation of H_2O_2. The protein calmodulin binds to activates plant catalases in the present of calcium. It indicated a dual function of Ca in regulating H_2O_2 homeostasis [30].

Metals are involved in the direct or indirect generation of free radicals (FR) and reactive oxygen species in the following ways: 1. direct transfer of electron in the single electron reduction; 2. disturbance of metabolic pathways resulting in an increase in the rate of FR and ROS formation; 3. inactivation and down regulation of the enzymes of the antioxidative defence system, and 4. depletion of low molecular weight of antioxidants [33]. Microelements such as Fe, Zn, Cu and Mn fulfil various roles in the metabolism of plant organism and are necessary for the regularity of physiological processes, however the excess and deficiency of these elements leads also to disturbance of ionic homeostasis [34]. Fe, Mn, Cu, and Zn as transition metals have frequently unpaired electrons and they are, therefore, very good catalysts of oxygen reduction. In aqueous solutions at neutral pH, $O_2^{\bullet -}$ can generate H_2O_2, which can subsequently decompose to produce $^{\bullet}OH$ by the Haber-Weiss reaction in which Cu and Fe being involved: $O_2^{\bullet} + H_2O_2 \rightarrow O_2 + OH^- + {}^{\bullet}OH$. When iron is the transition metal in the Haber-Weiss reaction, it is called the Fenton reaction [35], [33].

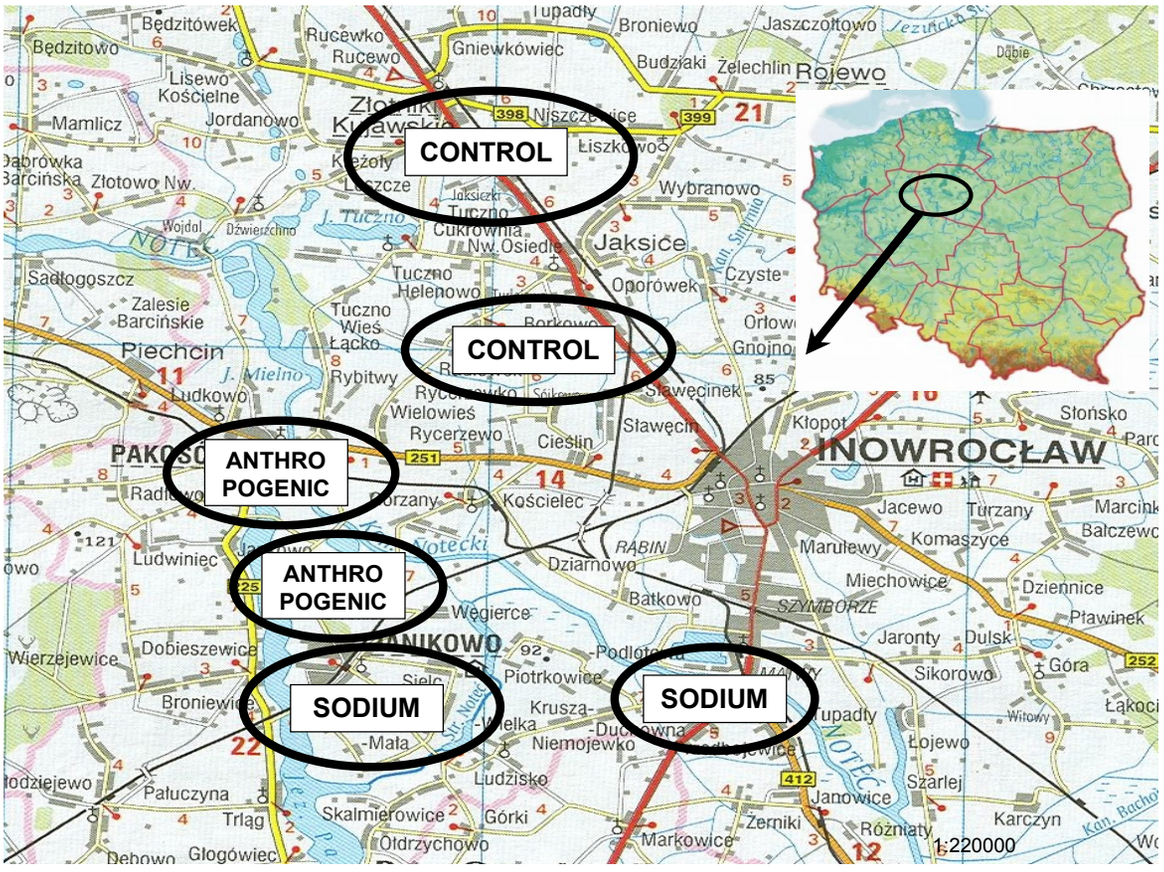
Toxic heavy metals Cd an Pb are considered as not essential metals for plant metabolism and stimulate formation of free radicals and reactive oxygen species [36], [37], [38]. The ROS formation by toxic metals is indirectly rather than directly [39]. The toxicity of Cd may result from its binding to sulfhydryl groups (S-H) of proteins leading to inhibition of activity and disruption of structure, due to perturbations in the nutrient balance and disturbance of cellular redox control 40]. Due to a Cd inhibitory effect on the Calvin cycle, there is a decrease in NADPH utilization, resulting in the one-electron reduction of a large number of oxygen molecules on the reducing side of PSI [39]. The reports of [41], [42], [43], [44], [45], [46], [47], and [48] have concluded that an oxidative stress could be involved in Cd toxicity, by either inducing oxygen free radical production, or by decreasing enzymatic and non-enzymatic antioxidants.

The subject of numerous studies for at least the past four decades has been physiological responses of plants for salinity and heavy metals in the controlled laboratory conditions. However, many of these determinations are concentrated to one type of pollutant, whilst plants in the natural conditions are subjected to many stressful differentiated ecophysiological sources and factors. Therefore there still remains a need for research on the interdependencies of plants with multiple biotic and abiotic factors in their natural habitats, their adaptation mechanisms and responses. The aim of this paper was thus to investigate the enzymatic antioxidant mechanisms and responses in plants subjected to destabilization of chemical elements management in the natural conditions. We thus studied antioxidant enzymes SOD, CAT, and APOX, and the content of malondialdehyde (MDA) variations in different ecological groups of glycophytes Creeping thistle *Cirisium arvense*, Common nettle *Urtica dioica*, Yarrow *Achillea millefolium*, and Burdock *Arctium lappa* in various types of environments: salted and alkaline anthropogenic environments, agricultural environments

and also pollution free environments of the Pomeranian region of Poland. We also investigated the halophyte Common glasswort *Salicornia europaea* from only salted environments. Simultaneously, we examined the levels of chemical elements Na, Ca, Fe, Zn, Cu, Mn, Cd, and Pb in roots and green parts of plants as probably the factor generated reactive oxidant species and thus activated enzymatic antioxidant mechanisms. We compare environmental and ecophysiological determinations of plant groups under salinity and acidity and their adaptation strategies. These studies were planned in order to understand whether the consideration of particular relationships between destabilization of free radicals homeostasis are connected with the excess of Na and Ca and pro-antioxidant balance.

2. Study area

The study area is situated in the Pomeranian Kujawy region (52-53°N, 18-20°E, central Poland) connected with Permian rock-salt uplifted in the form of salted domes and associated salt springs and saline ground waters, which had influence on the development of a sodium industry in districts of Janikowo and Inowrocław and non-polluted and industrialized area of the Tuchola Forestry complex (54-53°N and 17°30′-18°30′ E); Fig. 1.



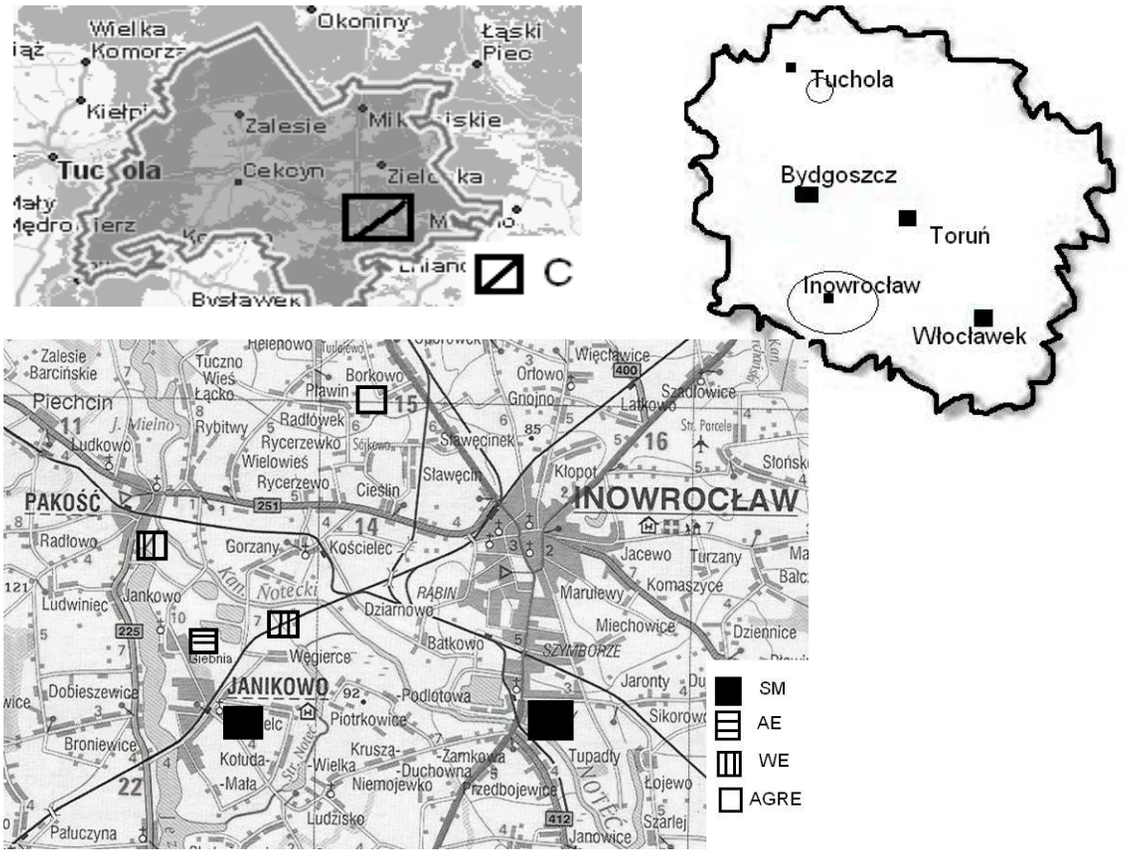


Figure 1. Study area. Environments: C – control, AGRE – agricultural, WE – wetlands, AE – anthropogenic, SM – sodium manufactures (http://www.maps.google.pl/kujawsko-pomorskie, modified).

The mainly human management caused secondary salinity and consequently alkalization of Kujawy areas as a result of inappropriate tightening of sediment traps causing wastes to infiltrate into the soil and grassland irrigation. Our research was done in selected habitats with a variety of anthropogenic disturb: 1) "Sodium manufactures" (SM) - Inowrocław and Janikowo district, closely by sodium factories; 2) "Anthropogenic" (AE) waste dumping sites near Giebnia and neighboured polluted areas; 3) "Wetlands" (WE) floodplains near Noteć Channel and Pakoskie Lakeland; 4) "Agriculture" (AGRE) – agriculture areas near Borkowo and 5) "Control" – in Tuchola Forestry named "Bory Tucholskie" (see Fig. 1).

3. Material and methods

Field studies were carried out in 2008-2010 during summer (May-June-July). Plant species were estimated on the basis of the work by [49] and were divided onto glycophytes - Creeping thistle *Cirisium arvense*, Common nettle *Urtica dioica*, Yarrow *Achillea millefolium*, Burdock *Arctium lappa* and obligatory halophyte: Common glasswort *Salicornia europea*. Common glasswort appears only in salted habitats and is known as succulent [50]. It is one of the most saline tolerant plants in general and is capable to grow under highly saline conditions in the lowest part of salt marshes [51], [52]. Samples of roots and green parts of

plants: leaves from glycophytes (No of samples N=220), and shoots of Common glasswort *Salicornia europea* (No of samples N=150), and samples of soils (No of samples N=300) from rhizosphera were collected from all studied environments (glycophytes) and only from anthropogenic salted areas (halophytes), because of their natural distribution.

Samples of soil were air-dried to a constant mass up to 65^0C, homogenized and sieved through 1 mm mesh. The electrolythical conductivity (expressed as mS or μS) of soil (Ec) was measured by conductivity meter (Elmetron CC-401). Instead the soil acidity (pH) was determined in bidistilled water at soil solution ratio of 1:2.5 with a potentiometric glass electrode by used pH-meter (Elmetron C-501).

Samples were collected from randomly selected plants at midday, and were kept cool in freezer bags for transfer to the laboratory. Green parts samples were then washed three times with deionized water; roots were gently separated from soils, then abundantly washed with tap water to eliminate soil particles, and also rinsed three times with deionized water. Root and washed shoot samples were divided into two parts. One was immediately stocked at −80°C until further biochemical analysis, while a second was cut into small pieces and dried in oven for 24 h [53], [54]. Mineralized samples of soil and plants were analyzed for Na, Ca, Fe, Zn, Cu, Mn, Cr, Ni, Cd, and Pb using inductively coupled plasma mass spectrometry ICP-MS (AGILENT 7500 CE; plasma ICP-MS spectrophotometer from Agilent Technologies Inc. (Palo Alto, CA, USA). The results were interpreted comparatively with standard well-known concentration, i.e. in relation to the analysis of reference materials. Parallel measurements were taken in the blind trials. The results were given in $mg*kg^{-1}$ of dry weight.

To determine the levels of superoxide dismutase (SOD) and catalase (CAT) roots and green parts of plants (1g) were homogenized in a 0.05 M phosphate buffer (pH 7.0) containing 1mM EDTA (3 ml). During homogenization polyvinyl-pyrrolidone (PVP) (0.25 g) was added (modified by [40]). The homogenate was centrifugated twice for 10 min first at 10 000 x g and second at 20 000x g. For ascorbate peroxidase (APOX) assay the roots and green parts (1g) were homogenized according to [54], i.e. by adding 0.05 M phosphate buffer (pH 7.5) containing 1 mM EDTA, 1 mM sodium ascorbate, 1 mM DTT and 4.0% (w/v) polyvinyl-pyrrolidone (PVP), 1 mM EDTA (5 ml). The homogenate was centrifugated at 15 000x g for 20 min. Sodium ascorbate was added only in case of green parts. All assays were conducted at 4^o C. The protein content in the supernatant was measured according to [55].The activity of all enzymes was expressed in units of mg^{-1} of protein.

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of NBT, adopting the method of [56]. The reaction mixture consist of 50 mM phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 120 μM riboflavine, 97.5 mM methionine, 2.25 mM NBT. After the addition of plant homogenate switching on a UV lamp for one minute the start of reaction is begin. The absorbance at 560 nm of wave length was measured. Fifty percent reduction in color was considered as one unit of enzyme activity.

The activity of CAT was assayed according [57], [58] in reaction solution (3 ml) composed of 0.1 M phosphate buffer (pH 7.0) to which 30% (w/v) H_2O_2 was added until reaching an absorbance at 240 nm between 0.520 and 0.550. The reaction was initiated by the addition of 10 μl of plant extract and the activity of CAT was monitored by the decreasing absorbance at 240 nm at for 2 min. One unit of enzyme was the amount necessary to decompose 1 μmol of H_2O_2 per min at 25ºC (extinction coefficient 43.6 $M^{-1}*cm^{-1}$).

The activity of APOX was measured according method of [59]. The soluble protein extract (25 μl) was added to 1.975 ml reaction mixture of 0.05 M phosphate buffer (pH 7.0), 0.5 mM sodium ascorbate, 0.1 mM H_2O_2 and the decrease absorbance of ascorbate at 290 nm for 2 min was used to calculate APOX activity. Enzyme activity was expressed in enzyme unit of mg^{-1} protein. One unit of enzyme was the amount necessary to decompose 1 μmol of substrate per min at 25ºC (extinction coefficient 2.8 $mM^{-1}*cm^{-1}$).

The intensity of lipid peroxidation was estimated following the method by [60]. Approximately 0.5 g of frozen plant tissue samples was cut into small pieces, homogenized with 2.5 ml of 5% trichloroacetic acid, and then centrifuged at 10000 g for 15 min. at room temperature. The equal volumes of supernatant and 0,5% thiobarbituric acid in 20% trichloroacetic acid were added in a new tube and incubated in 96°C for 25 minutes and then quickly cooled in an ice bath. After centrifugation at 8 000 g for 5 min, the absorbance of supernatant was recorded at 532 and 600 nm. The value for non-specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using coefficient of absorbance 155 mM-1 cm-1 and was expressed as μmol g^{-1} FW.

3.1. Statistical analysis

Arithmetic means and descriptive statistics of activation of antioxidant enzymes: SOD, CAT, APOX and concentrations of Na, Ca, Fe, Zn, Cu, Mn, Pb, and Cd in roots and green parts of plants were calculated. We also calculated arithmetic mean of concentrations of the same elements in soils from root zone of plants. The data did not show normal distribution, so non parametric tests were used. We used ANOVA Kruskal-Wallis test, followed by multiple Kruskal-Wallis comparison and U-Mann-Whitney test to estimate the significance of differences in the activation of antioxidant enzymes and concentrations of elements in roots and green parts from different environments, between organs and also groups of plants from the same environments (significance level at $p<0.05$), and also the differences between concentrations of metals in soil in particular environments. The dependence of activity of antioxidant enzymes and concentrations of elements in the roots and green parts of plants from different environments were calculated by correlation coefficient (r) according to the ranks of Spearman test (significance level at $p<0.05$) [61].

This work required permits from General and Regional Nature Conservation Dpts. These were obtained and had the following respective numbers: DOPozgiz-4211/I-14/995/09/ep, RDOS.PN.6631/2/08/KLD, and RDOS.04.PN.6631/37/09/KLD.

4. Results

4.1. Soil parameters

As shown in Table 1, the soils at Kujawy region were in the various degree of alkalinity (sodium manufactures; strong alkaline), anthropogenic environments, wetlands, agriculture environment (moderate alkaline), whilst pH of soils from control was acid. The electrical conductivity of soils was higher in sodium manufactures and anthropogenic environments than in control (Table 1).

As soils collected in SM and AE environments mainly consisted of tailings from nearby sodium factory and AGRE environment near field used agriculturally, heavy metal contents in the sample of soils were high and varied greatly in comparison with control and wetlands. The concentration of Na, Ca, Fe, Zn, Cu, and Mn were higher in soils at disturbed than control environments but in case of the same elements also from wetlands. However, concentration of toxic metals Pb, and Cd were higher in Tuchola Forestry than in Wetlands and did not differ with concentrations in soils from remaining environments (Table 1).

4.2. Chemical elements in roots and green parts of glycophytes and Common glasswort *Salicornia europaea*

4.2.1. Glycophytes

We found lower concentrations of Na, Ca, Fe, Cu, and Pb in roots in control in compare with environments in Kujawy region in opposite to level of Zn (higher in control than in agricultural and did not differ in the remaining environments). Similar to Zn concentration, Cd level was also higher in non-polluted environment (control) in comparison with factory disturbed sodium manufactures and anthropogenic (Table 2). Simultaneously, sodium level in glycophytes from anthropogenic environments was higher than in those from remaining areas.

Concentrations of Fe and Zn were lower in leaves from control but only in comparison with wetlands. Simultaneously, Pb level was lower in control areas than in wetlands and agriculture fields. It is interesting that the level of Cd, in opposite to Pb, was higher in the unpolluted environment (C) compared with all environments studied in Kujawy region, except anthropogenic areas. Similar concentration of Mn was also higher in the control (C) as compared with industrial disturbed SM and AE and also wetlands (Table 2). We did not find significant differences in the concentrations of Mn in roots and the level of Ca, and Cu in leaves among investigated environments (Table 2).

4.2.2. Common glasswort Salicornia europaea

We found only higher concentrations of Na in roots and Mn in leaves from anhropogenic than from sodium manufactures. Simultaneously, the roots and green parts of plants had higher concentrations of Pb in sodium manufactures than in the anthropogenic. Concentrations of Ca, Fe, Zn, Cu and Cd did not differ between both organs and amongst environments studied (Table 3).

	SM (N=35)		AE (N=55)		WE (N=85)		AGRE (N=50)		C (N=75)		H	p	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
pH	8,78	1,713	8,25	0,509	7,84	0,181	7,83	0,114	5,00	1,518	24,66	0,000	C<SM, AE, WE
EC	33,90	28,625	7,55	8,504	1,68	1,156	1,45	0,354	1,31	0,980	14,30	0,006	C<SM, AE
Na	821,81	823,663	451,04	412,291	52,02	33,097	67,00	4,282	51,77	42,593	19,67	0,001	C, WE<AE
Ca	83778,73	104267,017	40552,16	38357,177	5472,83	3456,820	32691,09	4396,396	2121,80	1854,719	29,39	0,000	C<SM, AE, AGRE; WE<AE
Fe	5724,14	1541,227	9312,59	2272,352	4955,36	2448,168	7211,14	221,338	2035,80	272,179	28,94	0,000	C<AE, AGRE; WE<AE
Zn	42,79	32,909	72,82	46,669	14,65	5,187	31,21	1,201	17,42	7,450	30,39	0,000	C, WE<AE; WE<AGRE
Cu	8,79	5,318	20,28	16,444	3,33	1,368	9,21	0,085	1,90	0,820	33,83	0,000	C<SM,AE,AGRE; WE< AE, AGRE
Mn	200,83	33,028	220,05	45,325	158,46	70,534	287,31	28,798	138,75	98,082	19,42	0,001	C,WE<AGRE
Pb	17,78	10,227	59,20	39,063	8,95	3,424	13,64	0,787	13,23	3,849	30,31	0,000	WE<AE,C
Cd	0,13	0,074	0,16	0,098	0,06	0,025	0,09	0,001	0,14	0,058	11,75	0,019	C>WE

Table 1. Soil characteristics of sodium manufacture (SM), anthropogenic environments (AE), wetlands (WE), agricultural environments (AGRE) and control (C); mean ± SD. Significant differences in the concentrations of Na, Ca, Fe, Zn, Cu, Mn, Pb, and Cd in soil among environments ($p<0.05$).

	SM (N=34)		AE (N=49)		WE (N=72)		AGRE (N=34)		C (N=31)				
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	H	p	
roots													
SOD	83,83	52,06	119,19	79,51	96,25	64,43	143,73	87,06	96,80	37,29	13,54	0,009	AGRE> SM, WE
CAT	0,35	0,49	0,23	0,24	0,19	0,13	0,21	0,19	0,19	0,10	1,12	0,891	
APOX	34,09	22,73	41,42	37,50	46,72	51,18	48,73	32,82	38,04	20,87	4,99	0,289	
MDA	11,40932	6,507260	11,12850	5,715258	10,59186	6,015241	5,86504	2,785663	11,12856	8,162991	25,68	0,000	AGRE<:SM, AE, WE, C
Na	2028,65	2193,52	2249,29	2193,05	572,37	726,67	797,09	693,15	663,97	981,73	42,29	0,000	C<SM, AE ; WE<SM, AE
Ca	13322,53	8226,41	9033,28	5182,81	8 175,14	3716,05	9141,37	7099,62	6098,64	2384,96	28,76	0,000	SM>AE, WE, AGRE, C
Fe	675,88	713,57	461,23	327,59	579,35	411,30	600,60	464,30	364,68	346,99	10,99	0,027	C<WE
Zn	24,67	10,64	23,72	10,89	18,51	11,59	15,05	3,40	26,27	18,09	29,32	0,000	AGRE<SM, AE, C; WE<AE, SM
Cu	13,67	9,60	10,45	12,93	9,57	8,98	7,37	5,06	9,72	11,14	12,98	0,011	SM>WE, AGRE, C
Mn	40,91	29,92	40,66	37,82	45,90	41,03	57,59	30,33	77,61	75,52	10,01	0,402	
Pb	4,15	4,15	3,89	6,55	1,54	1,48	1,16	0,91	1,18	0,74	39,67	0,000	AE>AGRE, C, WE; SM>AGRE, C, WE
Cd	0,17	0,11	0,15	0,11	0,19	0,24	0,10	0,04	0,25	0,22	16,31	0,003	AGRE<C, SM
green parts													
SOD	120,21	89,97	140,63	104,34	73,02	42,80	169,19	161,18	123,77	90,81	29,86	0,000	WE<SM, AE, AGRE, C
CAT	0,34	0,34	0,38	0,64	0,19	0,16	0,25	0,18	0,46	0,64	8,72	0,069	
APOX	57,52	39,03	45,00	36,42	55,74	52,06	59,92	40,74	48,03	47,10	6,90	0,141	
MDA	9,51898	3,440164	14,53614	4,554324	10,01070	4,226993	9,79719	4,854250	11,04482	5,097336	34,35	0,000	AE>SM, WE, AGRE, C
Na	171,24	209,12	1423,17	2379,17	193,29	371,57	147,09	538,10	80,85	122,73	78,24	0,000	C<SM, AE, WE ; AGRE<SM, AE, WE ; AE>SM, WE
Ca	21754,94	14162,79	18801,86	11008,24	20 838,65	12124,47	25354,39	14019,17	21597,36	14022,93	3,69	0,449	
Fe	178,51	97,44	217,27	164,10	224,47	173,91	185,20	116,29	152,36	99,02	10,80	0,029	C<WE
Zn	33,27	21,09	27,46	13,26	24,06	10,40	26,96	15,56	35,12	20,39	11,15	0,025	C<WE
Cu	7,08	4,31	7,46	2,97	7,88	6,03	7,44	2,77	7,25	3,59	3,11	0,540	
Mn	37,38	46,00	54,90	74,88	72,79	112,25	53,04	24,37	128,35	197,60	48,60	0,000	SM<AGRE, WE; C>SM, AE, WE
Pb	0,82	0,73	0,87	0,50	0,68	0,38	0,54	0,34	0,51	0,53	30,07	0,000	C<AE, WE ; AE>AGRE
Cd	0,06	0,05	0,07	0,05	0,17	0,43	0,06	0,04	0,19	0,22	13,53	0,009	C>SM, WE, AGRE

Table 2. Elements concentrations, activity of antioxidant enzymes, and degree of lipoperoxidation in organs of populations of glycophytes in sodium manufacture (SM), anthropogenic environments (AE), wetlands (WE), agricultural environments (AGRE) and control (C); mean ± SD. Significant differences among environments ($p<0.05$).

	SM (N=70)		AE (N=80)			
	Mean	**SD**	**Mean**	**SD**	**Z**	**p**
roots						
SOD	152,34	207,76	127,13	104,47	0,00	1,000
CAT	0,25	0,30	0,17	0,14	0,03	0,975
APOX	81,59	42,20	98,08	77,09	-0,03	0,975
MDA	5,19	1,73	5,08	0,91	-0,10	0,922
Na	10266,67	6637,63	18772,32	7713,88	-2,14	0,032
Ca	6112,00	5671,56	3900,34	1462,37	-0,09	0,926
Fe	467,42	391,22	470,88	274,67	-0,59	0,555
Zn	19,49	13,36	17,39	7,22	0,28	0,780
Cu	5,80	5,09	6,33	3,01	0,28	0,780
Mn	30,46	19,87	24,72	7,81	0,96	0,335
Pb	2,64	1,71	1,01	0,29	3,13	0,002
Cd	0,16	0,24	0,14	0,09	-0,84	0,401
green parts						
SOD	110,04	100,51	171,65	146,89	-1,93	0,053
CAT	0,39	0,32	0,15	0,14	1,88	0,061
APOX	34,44	34,77	21,68	17,38	0,55	0,583
MDA	2,48	0,69	4,04	0,98	-3,64	0,000
Na	44235,06	32261,31	49327,20	22546,83	-0,78	0,436
Ca	22766,03	20946,63	25326,90	12844,86	-0,49	0,624
Fe	378,85	482,21	239,69	159,86	-0,38	0,707
Zn	19,98	10,44	13,38	6,51	1,82	0,069
Cu	8,25	18,29	4,07	1,62	-0,89	0,371
Mn	36,92	36,70	127,42	84,06	-3,20	0,001
Pb	1,32	0,55	0,27	0,08	4,13	0,000
Cd	0,20	0,29	0,20	0,18	-0,49	0,624

Table 3. Elements concentrations, activity of antioxidant enzymes, and degree of lipoperoxidation in the organs of populations of Common glasswort *Salicornia europaea* in sodium manufacture (SM), and anthropogenic environments (AE); mean ± SD. Significant differences among environments at $p<0.05$.

4.3. Antioxidant enzymes activity and lipoperoxidation

Our results indicated significant differences only in the activity of SOD (glycophytes) in both organs. SOD activity was higher in roots from agricultural environments comparatively with sodium manufactures and wetlands. Simultaneously, SOD activity was lower in leaves from wetlands in comparison with remaining environments. We also found lower MDA content in roots from agricultural environments than from remaining examined areas. Instead the highest degree of lipid peroxidation was in the green parts of plants from anthropogenic environments (Table 2). We did not found differences in the biochemical parameters in organs of Common glasswort *Salicornia europaea* except of MDA content in the green parts of plants (higher level in the AE than in the SM) from different environments (Tables 4, 5).

4.4. The impact of metals on the antioxidant enzymes activity in roots and leaves of plants

Spearman coefficient analyses were performed with concentrations of each of the metals studied in roots and green parts of glycophytes and Common glasswort *Salicornia europaea* versus the activity of antioxidant enzymes and degree of lipoperoxidation. The activity of enzymes was also correlated with MDA content. However, these relationships were depending on the type of environment (Tables 4-6).

4.4.1. Glycophytes

We found more relationships between activity of antioxidant enzymes and metals in the green parts than in roots, depending on the type of environment. However, concentrations of Na (SM), Fe (AE, WE), Cu (SM, AE, WE, C) and Pb (AE) were positively correlated with SOD activity. Instead, CAT activity had also positive relationships with Na, Cu (AE) and Zn, Mn (C). Simultaneously, the activity of another enzyme, APOX, was positively related with level of Na (SM, AGRE), Zn, and Mn (AE) and negatively – with Cu (C), and Cd (AGRE, C).

Our results indicated positive correlations of SOD activity with concentrations of Zn (SM, AGRE), Cu (SM) and Cd (SM, WE, AGRE) but also negative with Mn (AGRE, C) and Pb (AGRE). Concentrations of Na were also related with SOD activity, but were positive (AE) or negative (C). We found negative relationships between CAT activity and concentrations of Ca (WE), Fe (SM, WE, AGRE), Mn (SM), and Pb (WE, AGRE) but also positive with Zn (AGRE). The level of Cu was positive related with activity of CAT in the leaves of plants from sodium manufactures but also negative in the leaves from anthropogenic areas. APOX activity was positively correlated with Ca concentrations in the leaves of plants from all environments and with Fe in the leaves collected at wetland and agriculture environments. However, the activity of this enzyme was negatively related with Na, Zn (C) and Cd (SM, AE, C). Concentrations of Cu had both positive (WE) and negative (AE) relationships with APOX in the leaves.

We found positive relations between concentrations of Na (AE), Cu, (AE, C), Pb (WE) and Cd (WE, C) and degree of lipoperoxidation processes in roots. However, we also found correlations between Na, Zn, Mn, and Cd but in the control environment (Na), AE, WE (Ca), AE (Zn, Cd), and SM (Mn). It should be emphasized that we did not state relation between concentrations of Na, and also Mn, and Pb and MDA content in the leaves. Concentrations of Ca were positively correlated with level of MDA (AE, WE) in the green parts in opposite to metals – Zn and Cd (AE, AGRE). Iron and copper stimulated lipoperoxidation (Fe in AGRE, Cu in WE) and was mutually negatively correlated (SM, SM, C) with content of MDA but it depend on the type of environment (Tables 4, 5, Figs. 2, 3). MDA content was positively correlated with CAT activity in roots (AE, AGRE) and APOX in leaves (AE, WE, AGRE). On the other hand, we found negative relations between MDA and the activity of APOX in roots (AE) and SOD, and CAT in leaves (AGRE); Table 4.

SM (N=34)			AE (N=49)			WE (N=72)			AGRE (N=34)			C (N=31)		
relations	R	p	relations	R	p	relations	R	p	relations	R	p	relations	R	p
roots														
SOD & Cu	0,43	0,011	SOD & Fe	0,46	0,001	SOD & Fe	0,29	0,015	APOX & Cu	-0,37	0,031	SOD & Cu	0,364	0,044
SOD & Na	0,61	0,000	SOD & Cu	0,59	0,000	SOD & Cu	0,42	0,000	APOX & Cd	-0,51	0,002	APOX & Cu	-0,565	0,001
APX & Na	0,36	0,036	SOD & Pb	0,34	0,016	MDA & Cu	0,37	0,001	APOX & Na	0,61	0,000	APOX & Cd	-0,360	0,040
MDA & Mn	-0,35	0,040	APX & Mn	0,32	0,024	MDA & Cd	0,39	0,001	CAT & MDA	0,63	0,000	CAT & Mn	0,421	0,040
			APX & Zn	0,32	0,025	MDA & Pb	0,33	0,005				CAT & Zn	0,528	0,040
			CAT & Cu	0,43	0,002	MDA & Na	0,32	0,006				MDA & Cu	0,498	0,040
			CAT & Na	0,34	0,018	MDA & Ca	-0,27	0,021				MDA & Cd	0,720	0,040
			MDA & Cu	0,35	0,014							MDA & Na	-0,510	0,040
			MDA & Zn	-0,43	0,002									
			MDA & Cd	-0,32	0,023									
			MDA & Na	0,49	0,000									
			MDA & Ca	-0,41	0,003									
			APOX & MDA	-0,51	0,000									
			CAT & MDA	0,55	0,000									
green parts														
SOD & Cu	0,34	0,047	SOD & Na	0,35	0,013	SOD & Cd	0,42	0,000	SOD & Mn	-0,41	0,017	SOD & Mn	-0,521	0,003
SOD & Zn	0,38	0,025	APOX & Cd	-0,45	0,001	APOX & Fe	0,34	0,003	SOD & Zn	0,60	0,000	SOD & Na	0,411	0,022
SOD & Cd	0,35	0,044	APOX & Ca	0,59	0,000	APOX & Cu	0,29	0,014	SOD & Cd	0,57	0,000	APOX & Zn	-0,473	0,007
APOX & Cd	-0,36	0,036	CAT & Cu	-0,35	0,012	APOX & Ca	0,31	0,008	SOD & Pb	-0,48	0,004	APOX & Cd	-0,649	0,000
APOX & Ca	0,41	0,017	MDA & Zn	-0,43	0,002	CAT & Fe	-0,33	0,004	APOX & Fe	0,39	0,024	APOX & Na	-0,367	0,043
CAT & Mn	-0,50	0,002	MDA & Cd	-0,43	0,002	CAT & Pb	-0,31	0,007	APOX & Ca	0,38	0,029	APOX & Ca	0,545	0,002
CAT & Fe	-0,40	0,020	MDA & Ca	0,50	0,000	CAT & Ca	-0,23	0,049	cat & Fe	-0,47	0,005	MDA & Cu	-0,628	0,000
CAT & Cu	0,38	0,028	APOX & MDA	0,54	0,000	MDA & Cu	0,24	0,040	CAT & Zn	0,50	0,002	MDA & Zn	-0,378	0,036
MDA & Fe	-0,46	0,006				MDA & Ca	0,40	0,000	MDA & Mn	0,34	0,048			
MDA & Cu	-0,60	0,000				APOX & MDA	0,42	0,000	MDA & Fe	0,59	0,000			
									MDA & Zn	-0,52	0,002			
									MDA & Cd	-0,61	0,000			
									MDA & Cd	-0,61	0,000			
									SOD & MDA	-0,49	0,003			
									APOX & MDA	0,51	0,002			
									CAT & MDA	-0,63	0,000			

Table 4. Concentration of element–activity of enzyme, content of MDA interactions and content of MDA–activity of enzyme interaction related changes of elements level and antioxidant enzymes activity, the level of lipoperoxidation in roots and green parts of glycophytes in different environments.

SM (N=70)			AE (N=80)		
Relations	R	p	relations	R	p
Roots					
SOD & Fe	0,66	0,036	CAT & Fe	-0,77	0,002
SOD & Cu	0,64	0,046	CAT & Cu	-0,76	0,003
SOD & Zn	0,74	0,015	CAT & Zn	-0,62	0,023
APOX & Cd	-0,82	0,004	CAT & Cd	-0,67	0,012
APOX & MDA	-0,64	0,048	CAT & Na	-0,71	0,006
green parts					
SOD & Zn	0,65	0,023	ns		
SOD & Cd	0,82	0,001			
APOX & Fe	0,59	0,044			
APOX & Cu	0,65	0,023			
APOX & Cd	0,79	0,002			
APOX & Ca	0,60	0,041			
CAT & Pb	0,60	0,039			

Table 5. Concentration of element–activity of enzyme, content of MDA interactions and content of MDA–activity of enzyme interactions related changes of elements level and antioxidant enzymes activity, level of lipoperoxidation in roots and green parts of Common glasswort *Salicornia europaea* in different environments.

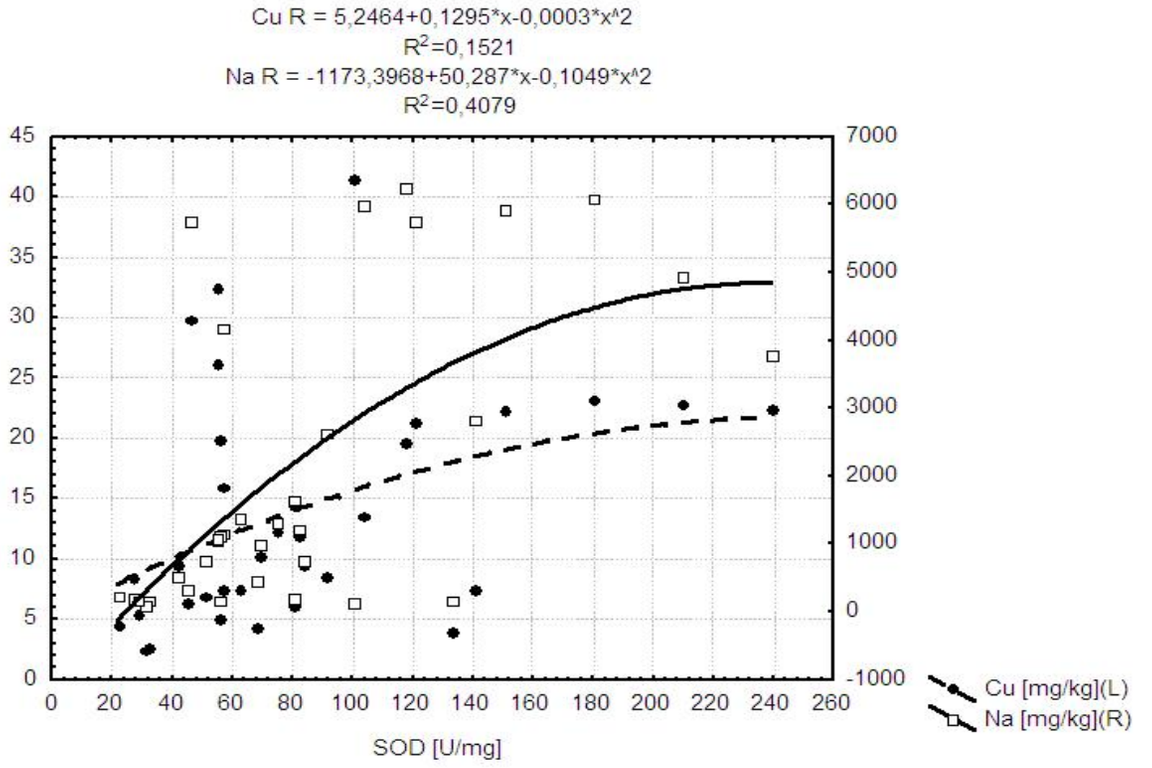


Figure 2. Interrelationships between SOD activity and the concentration of Cu, and Na (mg*kg^{-1}) in roots of glycophytes from sodium manufacture.

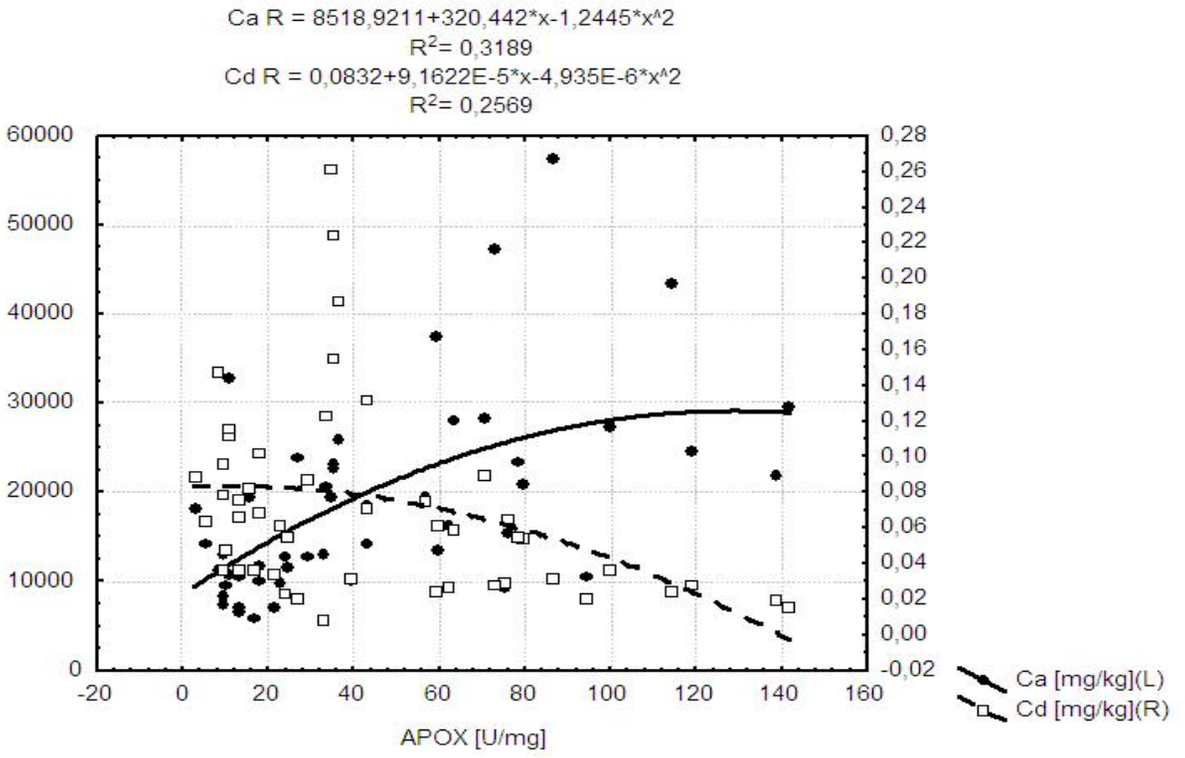


Figure 3. Interrelationships between APOX activity and the concentration of Ca, and Cd in (mg*kg^{-1}) the green parts of glycophytes from anthropogenic environment.

4.4.2. Common glasswort Salicornia europaea

Our results indicated relationships between activity of antioxidant enzymes and concentrations of metals in roots of plants in both environments and only in shoots from sodium manufactures. Concentrations of Fe, Cu, and Cd were positively related with SOD activity in the roots of plants from sodium manufactures in opposite to activity of CAT in roots of plants from anthropogenic areas. However, Na and Cd concentrations were also negatively correlated with CAT activity in shoots of plants from anthropogenic environments. We also found negative relations between concentrations of Cd and APOX activity in roots of plants from sodium manufactures. On the other hand, the activity of antioxidant enzymes was positively correlated with concentrations of Zn, Cd (SOD), Pb (CAT), Ca, Fe, Cu, and Cd (APOX) in shoots of plants. We did not find relations between MDA content and chemical elements concentration in both studied organs of Common glasswort *Salicornia europaea* (Tables 4, 5, Figs. 4, 5). However, we found negative relations between MDA content and APOX activity in the roots of plants from sodium manufactures.

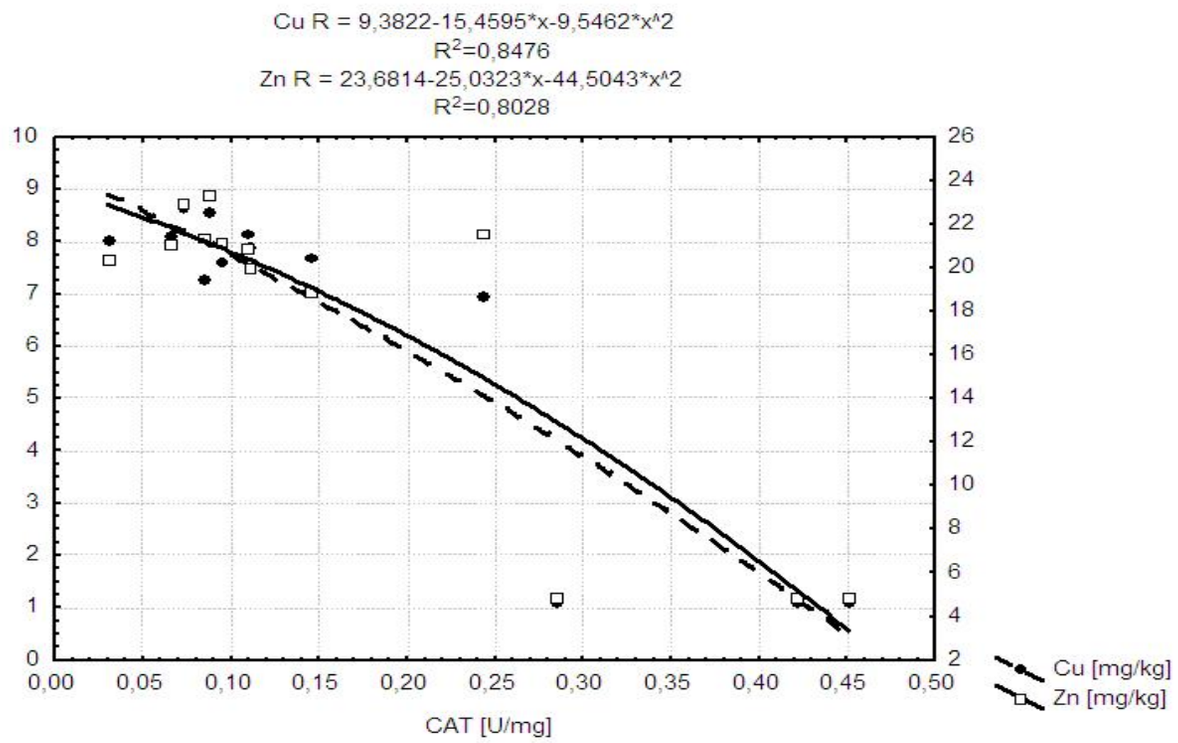


Figure 4. Interrelationships between CAT activity and the concentration of Cu, and Zn in roots ($mg*kg^{-1}$) of Common glasswort *Salicornia europaea* from anthropogenic environment.

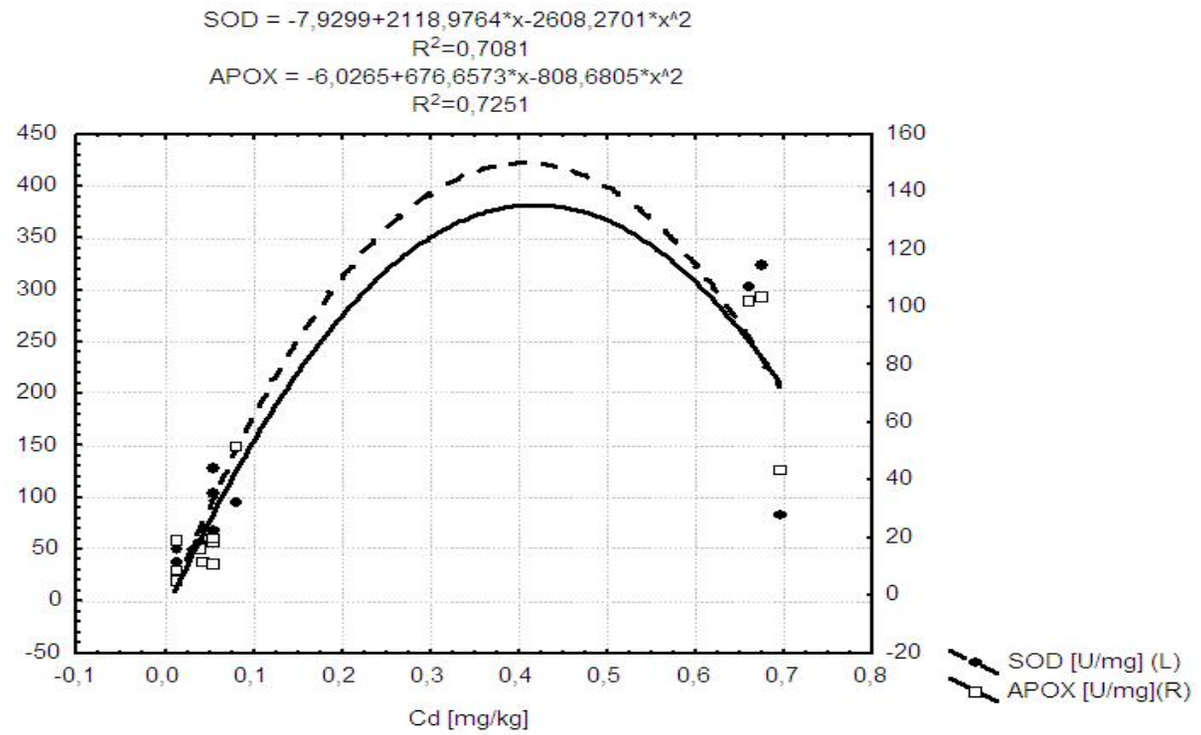


Figure 5. Interrelationships between Cd concentration (mg*kg^{-1}) and SOD, and APOX (U/mg) activity in the green parts of Common glasswort *Salicornia europaea* from sodium manufacture.

4.5. Differences between glycophytes and Common glasswort *Salicornia europaea* in their natural environments

We found significant higher activity of APOX in roots of glycophytes than in Common glasswort *Salicornia europaea* from both examined environments (SM, AE) in opposite to green parts. CAT activity was similarly higher in the green parts of glycophytes than in the halophyte (Common glasswort *Salicornia europaea*) but only in the anthropogenic environments. Instead, the level of MDA was higher in the glycophytes except green parts in the anthropogenic areas. Common glasswort *Salicornia europea* cumulated more sodium in both organs in all environments studied and also had higher level of Zn, Cu, Mn, and Cd in the green parts from AE than glycophytes. However, Cu and Pb concentrations were also higher in the green parts of this halophyte in sodium manufactures. On the other hand, glycophytes accumulated more calcium in roots from both environments, and more copper in roots from sodium manufactures and also Pb in both organs from anthropogenic environments than Common glasswort *Salicornia europaea*. The differences between Fe concentration and SOD activity in the organs of examined plants from two various environments did not differ (Table 6).

	SM		AE		SM		AE	
	roots				green parts			
	Z	p	Z	p	Z	p	Z	p
SOD	ns		ns		ns		ns	
CAT	ns		ns		ns		-2,02	*
APX	3,23	**	3,54	***	-2,14	*	-2,06	*
MDA	-3,25	**	-4,00	***	-5,20	***	-5,82	***
Na	4,19	***	5,24	***	5,09	***	5,32	***
Ca	-2,53	*	-3,86	***	ns		ns	
Fe	ns		ns		ns		ns	
Zn	ns		ns		ns		-3,66	***
Cu	-2,53	*	ns		-2,51	*	-3,53	***
Mn	ns		ns		ns		3,86	***
Pb	ns		-3,10	***	3,01	**	-5,00	***
Cd	ns		0,14		ns		2,22	***

Table 6. Differences among biochemical parameters: activity of enzymes (SOD, CAT, APOX), content of MDA, concentrations of elements between root and green part of glycophytes and Common glasswort *Salicornia europaea* from different environments ($p<0.005$).

4.6. Differences between enzymes activity, lipoperoxidation and concentrations of elements in plants

We noted higher activity of SOD and APOX in the green parts of glycophytes from sodium manufactures. However MDA level was significantly higher only in green parts of glycophytes than in roots from AE and AGRE. On the other hand, APOX activity in shoots of Common glasswort *Salicornia europaea* was lower, similarly to MDA level of these plants from SM, but the intensity of lipoperoxidation was significantly higher in roots also at AE. Concentrations of Na were higher in roots of glycophytes in opposite to halophyte. Simultaneously, Ca concentrations were higher in leaves of both glycophytes and Common glasswort in opposite to level of Fe, which was rather cumulated in roots (not significant at SM in Common glasswort *Salicornia europea*). Simultaneously, Zn was found in higher concentrations only in glycophytes but in non salted environments (WE, AGRE, C). Cu and Mn were rather mobile metals but we found significant higher Cu concentrations in roots of glycophytes (SM) and – for both elements – in roots of Common glasswort (AE). Lead, as toxic metal was accumulated in roots of plants. Glycophytes also accumulated Cd in roots but it is interesting that we did not find the differences among organs in plants from control environments. Instead, concentrations of Cd were not differs amongst organs of Common glasswort *Salicornia europea* from both environments (Table 7).

glycophytes										Common glasswort			
	SM		AE		WE		AGRE		C		SM		AE	
SOD	-1,98	*	ns		ns		ns		ns		ns		ns	
CAT	ns		ns		ns		ns		ns		ns		ns	
APX	-2,46	*	ns		ns		ns		ns		2,67	*	3,78	***
MDA	ns		-3,26	**	ns		-4,02	***	ns		3,73	***	2,38	*
Na	5,61	***	3,091	**	6,41	***	6,38	***	4,55	***	-3,13	**	-2,75	**
Ca	-2,59	**	-5,492	***	-7,79	***	-5,01	***	-5,14	***	-2,14	*	-4,22	***
Fe	5,17	***	5,372	***	5,86	***	4,63	***	3,01	**	ns		2,09	*
Zn	ns		ns		-4,25	***	-4,94	***	-2,25	*	ns		ns	
Cu	3,05	**	ns		ns		ns		ns		ns		2,26	*
Mn	ns		ns		ns		ns		ns		ns		-4,16	***
Pb	5,83	***	5,642	***	4,26	***	2,39	*	3,63	***	2,27	*	4,22	***
Cd	5,15	***	4,860	***	4,37	***	3,72	***	ns		ns		ns	

Table 7. Differences among the content of biochemical parameters: MDA, proline and concentration of elements in organs of plant groups from the same environment ($p<0.005$).

5. Discussion and conclusions

The presence of a sodium factory and industrialization of Kujawy region affected the salinity, alkalinity and higher content of Na, Ca but also Fe, Cu, and Zn in the environments of this region, especially neighbored with sodium manufactures and waste dumping sites. On the other hand, higher concentration of microelements in the agricultural environments than in controls was probably reflected the use of fertilizers in the agricultural practices. It should be emphasized that we did not find differences in the concentration of toxic elements (Pb, Cd) in plants and soils between control and disturbed environments (SM, AE); Table 1. However, concentration of chemical elements studied in plants differed among environments but relations were not directly similar to those occurred in soils (Tables 2, 3). It probably depends on the environmental conditions. We should consider that bioavailability of chemical elements by plant is affected by numerous basal environmental characteristics of soil: pH, red-ox potential, salinity, the content of organic matter, etc. [62], [63]. Simultaneously, different factors than edaphic conditions of soils, i.e. seasonal physiology, the condition of plants, species-species capacities for uptake, translocation and compartmentalization, may contribute to the differential bioaccumulation of elements [64]. The roots of glycophytes accumulated about 3 fold higher concentrations of Na than collected at control environments, whilst green parts of plants – even 17 fold higher (Table 2). We can thus conclude that at sodium manufactures, especially anthropogenic excess of sodium affects ionic balance and primarily impacts upon metabolic processes [65]. Instead, calcium concentration in the organs of plants do not exceed average concentrations recommended by [66]; 0.2-5% d.w. However, the higher Ca concentrations, especially in salted environments, could be related with higher level of this element in these environments. On the other hand, increased intake of calcium could be connected with excess of sodium and mobilization of defence mechanisms [67]. Simultaneously, our results indicated that concentration of Zn and Cu in roots and leaves of examined plants do not exceed the phytotoxic range recommended by [68] and [69], i.e., 100-400 $mg*kg^{-1}$; 30 $mg*kg^{-1}$,

respectively). Similar level of Pb, and Cd in plant's organs in our study was below ranges of 30-300 $mg*kg^{-1}$, and 5-30 $mg*kg^{-1}$, respectively, considered by [68] as phyototoxic. However, iron and manganese concentration in the range of 40-500 $mg*kg^{-1}$ and 50-500 $mg*kg^{-1}$, respectively, according to [70] are found as toxic for plants, so our result indicate that Fe level is high in both organs of plants, but high concentrations of Mn in roots and green parts of glycophytes was connected with environmental factors.

Stressful factors (acidity, salinity, toxic heavy metals) adversely affects the associated ecological balance and increases the level of free radicals and reactive oxygen species (ROS), which is related with changes in activities of antioxidant enzymes [71], [72], [73]. Most studies show that the higher activities of these enzymes were positively correlated with plant stress-resistance [74], [6], [73]. However, the changes of enzyme activities also depend on plant genotypes and stress intensity [74], [75].

Based on the results obtained by us, we can conclude that physiological activity of important antioxidant enzymes can maintain oxidative homeostasis and also reduce the membrane damages in the plant organism (mainly by lipoperoxidation). Moreover, SOD, CAT, and APOX is related with concentration of examined elements (Na, Ca, Fe, Zn. Cu, Mn, Pb, Cd), depending on the status of the environment. These relations were positive and negative, and they depend on the ecological group of plants, the type of organ and on stress intensity (various degree of anthropogenic impact). Glycophytes studied by us were subjected on many stressors at disturbed environments in Kujawy region. These plant species strive to maintain their oxidative homeostasis and develop efficient antioxidative responses. Thus we found positive relationships between sodium concentrations and the content of MDA as indicator of oxidant injury, but also between CAT activity in roots of plants from anthropogenic environments, and also similar between calcium concentrations and the level of lipoperoxidation and APOX in green parts of plants from anthropogenic and wetland environments. Copper concentration was also related with APOX activity and MDA level in the wetlands. Instead, Fe was correlated with MDA and APOX in the agricultural areas (Table 4). We can thus concluded that Na, Ca, Cu and also Fe stimulated lipoperoxidation processes but also mobilization antioxidant mechanisms of glycophytes in Kujawy region. Furthermore, negative relation of Cd with APOX in both organs from controls and also positive correlations with degree of lipoperoxidation in roots (Table 4) indicate that discrimination of one of important enzymes involved in antioxidant mechanism and impact on ROS generation. Similar to Cd, also Cu stimulated higher level of MDA and negatively affect the activity of APOX in opposite to activity of SOD in roots of glycophytes in control sites (Table 4). In the organs of Common glasswort *Salicornia europea* we did not find these relations (Table 5). Reports of [8], [76], and [6] showed correlations between stress level and MDA content. Moreover, lipid peroxidation and H_2O_2 levels, and also SOD, CAT, APOX and GR activities increased in pea roots and leaves under Cd stress [77], whilst APOX and CAT decreased at high Cd concentrations [78]. Also [47] reported that Cd-induced oxidative stress in *Arabidopsis* is due to H_2O_2 accumulation.

It should be emphasized that we found more correlations between concentrations of chemical elements and studied biochemical parameters (Tables 4, 5), which indicate the

complexity of antioxidative processes and simultaneously the participation of chemical elements in stimulation and modification of lipoperoxidation processes and SOD, CAT or APOX activities. Simultaneously, the same elements could impact upon the activation of many oxidative pathways in organs of glycophytes (Fig. 5) and it depends on the environmental conditions. In disturbed sodium manufactures the level of Na stimulated activity of SOD and APOX, but in the other environments it stimulated the activity of CAT. Similarly, copper concentration is positively correlated with CAT in roots but negatively in the green parts of plants from anthropogenic environments. Furthermore, Na and Ca concentrations stimulated activity of antioxidant mechanisms, especially in disturbed environments. On the other hand, calcium is also related with APOX activity in all studied environments. Simultaneously, concentrations of transition metals could impact the increase or decrease of antioxidant enzymes activity (Figs. 2-5). We suggest it depends on the interactions amongst elements, the intensity of stressors or impact of other not examined factors. However, more analysis will require for estimation these problems. We also should consider that Cu, Zn, Mn, and also Fe are cofactors of metalloprotein, i.e. SOD. Simultaneously, cadmium and lead as toxic metals especially defected the activity of APOX and CAT, respectively, in the organs of glycophytes, which is in opposite to SOD activity (Tab. 4). Similarly, Fe, Zn, and Cu stimulate activity of SOD in sodium manufactures in roots and shoots of Common glasswort *Salicornia europea*. Moreover, the same metals decrease CAT activity in roots from anthropogenic environments. Cadmium and lead concentration are also defected the activity of APOX and CAT and impacted the increase of SOD. We also found correlations between APOX and Ca concentrations in shoots of Common glasswort *Salicornia europaea* in sodium manufactures, alike as in the green parts of glycophytes. Moreover, sodium concentrations influence the decrease of CAT in roots of this halophyte (Table 5). Similarly, CAT activity is also higher in green parts of glycophytes than in halophytes. This indicate that glycophytes are better adaptation to stressful conditions, however there are many indistinct questions required to estimation.

There is much evidence that salinity and heavy metals enhanced or decreased activity of antioxidant enzymes but usually under control conditions. Both increase as well as decrease in the activity of SOD has been reported in plants in response to salinity stress [8], [79]. It appears that the activity of SOD under salinity varies depending upon plant species, organ analyzed as well as upon the level of salinity [80], [81]. [9] showed that in Cotton *Gossypium hirsutum* L. under salted NaCl stress the increases of SOD activities occurs, as well as decreases of the activities of catalase and ascorbate peroxidase. In the leaves of rice plant, salt stress preferentially enhances the content of H_2O_2 and the activities of SOD, APOX, whereas it decreases catalase activity [82]. These authors reported that NaCl treatment increases the activities of catalase but does not affected the activity of SOD in cucumber plants. The tomato under high salt concentration showed higher among others antioxidant enzyme activities such as SOD, catalase, and ascorbate peroxidase [83]. Similarly, [84] showed that activity of cytosolic CuZn-SOD II, chloroplastic CuZn- SOD II, and mitochondrial and/or peroxisomal Mn-SOD were correlated with increasing concentration of NaCl in pea at the higher NaCl concentrations.

We can consider that heavy metals toxicity is occurred in the ability to bind strongly the oxygen, nitrogen and sulphur ions. This process is related with the free enthalpy of the formation of the product of metals and ligands. By this features heavy metals can inactivate the enzymes by binding to cysteine residues. Heavy metals can also displace one metal with another, which can lead to inhibition or loss the enzyme activities [4], [13]. On the other hand, [85] examined the role of antioxidative enzyme system. They investigated the relation to Cd stress in hyperaccumulator plants of the genus *Alyssum*. In both species superoxide dismutase activity was elevated at high cadmium concentrations, whilst ascorbate peroxidase activity remained unchanged [7], whilst [86] indicated that activity of antioxidant enzymes was estimated as a function of time and concentrations of Pb in roots of lupin. The results of [25] suggested that lead induces oxidative stress in growing rice plants and that SOD and APOX could serve as important components of antioxidative defence mechanism against Pb induced oxidative injury in rice. They observed a Pb dependent increase in the activities of SOD from root tip extract, whilst CAT and APOX activities decreased at higher lead concentrations. Cadmium treatment induced lipooxygenase with simultaneous inhibition of antioxidative enzymes, SOD and CAT [41]. In particular, CAT activity often decreased following exposure to elevated cadmium concentrations [78], [46]. On the other hand, [87] indicated that CAT activities and specific isoenzymes of SOD increased in the leaves and roots of a resistant variety of radish, following exposure to increasing (between 0.25 and 1 mM) concentrations of cadmium. A severe suppression of SOD and CAT, and almost complete loss of APOX activities after 48 h of exposure to 50 μM Cd was observed in pine roots [88]. Cd-induced inhibition of APOX and CAT was also associated with H_2O_2 accumulation and growth retardation in the poplar roots [4]. Also [89] studied the involvement of H_2O_2 and O_2^{-} in the signaling events that resulted in variation of the transcript levels of CAT, GR and Cu-Zn-SOD in pea plants under Cd stress.

Considering the influence of chemical elements on the defence mechanisms of plants [90] we can conclude the unique importance of Ca^{2+} for stabilization of membranes. High salinity results in increased cytosolic Ca^{2+}, which is transported from the apoplast and the intracellular compartments [91]. This transient increase in the cytosolic Ca^{2+} initiates stress-signal transduction leading to salt adaptation. Adequate levels of calcium are necessary for the membrane to its normal function [31].

Most of the interest in calcium participation in plants has centered on its role in the cytoplasm in controlling the developmental processes [92]. [32] reported the effects of calcium chloride on the sodium chloride-stressed plants of Mung bean *Vigna radiatae* (L.). We could confirm that when $CaCl_2$ was combined with NaCl, $CaCl_2$ altered the overall plant metabolism to ameliorate the deleterious effects of NaCl stress and increased the vegetative growth of plants. [93] indicated that Ca^{2+} prevented Cd-induced increasing of the activity of SOD and restored CAT activity. These results suggested that exogenous application of Ca^{2+} could be advantageous against Cd^{2+} toxicity, and could confer tolerance to heavy metal's stress in plants.

Our results confirm also the disruption of oxidative homeostasis related with environments of glycophytes through higher degree of lipid peroxidation in leaves in the anthropogenic

environments and lower level of MDA in roots of plants from wetlands than from remaining environments. However, we found a higher SOD activity in the roots of glycophytes from agriculture areas in comparison with sodium manufactures and wetlands. Simultaneously, the activity of the same enzymes in green parts of plants was lower in wetland areas than in remaining environments (Table 2). All of these results indicated that plants probably can mitigate the oxidative damage initiated by ROS (especially at agriculture) by the complex of defensive antioxidative system [71]. Moreover, [15] indicated that both roots and shoots of plants had malonyldialdehyde (MDA) contents cultivated at the optimal salt concentration (50 mM NaCl) were lower than in the control areas. This was related to enhanced activities of antioxidant enzymes, like superoxide dismutase, catalase and peroxidase, especially in shoots. SOD and CAT activities have been reported to be negatively correlated with the degree of damage to plasmalemma, chloroplasts and mitochondrial membrane systems and positively correlated with stress resistance indices [7], [32]. Simultaneously, we can not exclude other factors as draught or climate conditions, which could generate free radicals or reactive oxygen species (i.e., draught, climate conditions) and thus impact on high activity of antioxidant enzymes in organs in the control environments (C). We should also note that the level of cadmium was higher in leaves of plants from Tuchola Forestry than from remaining environments. Similarly, in roots of plants from these areas, cadmium concentrations did not differ from other environments, but was higher than in the agricultures. Cadmium is probably a factor, which can indirectly upset cell membrane. Simultaneously, we found relationships between biochemical parameters and concentrations of Na, Ca, Cu, Zn and Mn, which stimulated the activity of CAT, and Na-SOD and Ca-SOD and APOX. Instead, we found differences between the content of MDA (higher in shoots of plants from anthropogenic than from sodium manufactures) but the activity of SOD, CAT and APOX did not differ in the organs of Common glasswort *Salicornia europaea* between two examined environments. It could indicate that Common glasswort, as obligatory halophyte, is adapted to high salted environments (sodium manufactures). Thus mutual and differentiated relationships between chemical elements and antioxidant enzymatic mechanisms as plant responses to the environmental disturbs, are probably another factor influenced the higher level of lipoperoxidation in anthropogenic environments.

We also found that Na, Fe, and Pb are accumulated in higher level in roots of glycophytes in opposite to Ca. Zn, Cu and Mn at all examined environments and they showed more mobility, which depends on the type of environment. Interestingly, the concentrations of Cd and Pb, as toxic elements were also higher in roots of plants at all environments of Kujawy region but not at the control sites, whilst the activity of antioxidant enzymes did not differ significantly between organs of plants in the examined environments, except of SOD and APOX at SM. We found higher level of these enzymes in the leaves than in roots of plants. It was probably related with the concentrations of Ca and also Cu, Zn, and Cd. We could also conclude that Zn and Cu are co-factors of SOD so further analyses are needed. However, the location of chemical elements rather not deflected on the activity of enzymes in glycophytes. On the other hand, the concentrations of Na were higher in shoots than roots of Common glasswort *Salicornia europaea*, which is connected with adaptation to life in high salinity (e.g.,

osmoregulation); [13]. It is interesting we found the higher level of MDA and higher lead concentrations in roots, similar to activity of APOX. However, we could not confirm relations among these parameters because we did not find significant correlation. Furthermore, the roots are more markedly affected by saline conditions than leaves, because of being the first part at the plant to encounter soil salinity [94]. [81] confirm that roots of halophyte Sea fennel *Crithmum maritimum* were distinguished from leaves by malondialdehyde concentration, however activity of APOX was higher in leaves, despite the fact that stress factor was only a high salinity. Simultaneously, [95] and [96] stated that root tissues markedly exhibited by higher APOX activity in comparison of shoots of plants under salinity stress.

The mechanisms of salt tolerance are of two main types: those minimizing the entry of salt into the plant, and those minimizing the concentration of salt in the cytoplasm. Halophytes, as naturally salt tolerant plants, have both types of mechanisms. They exclude salt well, but effectively compartmentalize in vacuoles the salt that inevitably gets in. This allows them to growth of long period of time in saline soil. Instead, glycophytes avoid sodium to maintain ionic homeostasis [13], [15]. We can thus conclude basing also on our results showed in Table 5 that higher concentrations of sodium in roots and leaves of Common glasswort *Salicornia europea* than in glycophytes are resulting from the saline determinations of the environment and predominantly from the interactions with sodium. We also conclude that glycophytes are subjected on higher salinity stress, which cause probably higher level of ROS, and lipoperoxidation in the organs (except green parts at AE) than in halophytes. On the other hand, activity of APOX is higher in the roots of halophyte than in glycophytes in both environments in opposite to green parts. We could suspect that this were connected with higher concentrations of Ca, elevated salinity effect [32] and indicated the activity of APOX in the green parts of plants more sensitive on salt damages than roots [97]. Similar activity of CAT is also higher in green parts of our glycophytes than halophyte. It is indicated that glycophytes are well adapted to stressful conditions however there are still many indistinct topics, required to estimation.

The area of mutual relationships: plants-environmental stressors and conducted research in this field has been gaining ground in the recent years. However, in the view of considerable variations in the protective mechanisms against activated oxygen species in different plant species, the further work, especially in natural conditions under many stressors is required to establish the general validity of various regularities and processes in salinity tolerance and also disturbed ionic homeostasis.

The positive and negative correlations between the level of chemical elements and antioxidative responses of plants indicate the complexity of enzymatic antioxidative processes and simultaneously the participation of chemical elements, which does not influence lipid peroxidation but stimulate or injure SOD, CAT or APOX activity. However, the concentrations of Na, Ca, Cu and Fe stimulate lipoperoxidation but can also mobilize antioxidant responses of glycophytes in natural salted Pomeranian region, whilst Cd in the control environment influence MDA content and increasing APOX activity in roots of glycophytes. Simultaneously this toxic metal may impact the activation of many oxidant ways in organs of glycophytes and it depend on the environmental conditions (pH, Ec). Na

and Ca concentrations rather stimulate enzymatic antioxidant mechanisms activity, especially in disturbed environments, and they can also stimulate the transition metals which causing increase or decrease of antioxidant enzymatic activity. However, Cd and Pb can defect the activity of APOX and CAT in organs of glycophytes, which is opposite to SOD activity. Similarly, in the organs of Common glasswort *Salicornia europaea*, Fe, Zn, and Cu level can stimulate SOD activity but they can also inhibit CAT activity. These processes depend on the environmental factors (pH, Ec) and types of plant organs and toxic heavy metals Cd and Pb concentration. They defect APOX and CAT activity but influence the increase of SOD. We also found correlation between APOX and calcium concentration (alcalinity) in shoots of Common glasswort and in green parts of glycophytes.

The differences in MDA content and SOD activity either in roots or in green parts of glycophytes indicate that plants can probably mitigate the oxidative damage initiated by reactive oxygen species (ROS) by complexes of defensive enzymatic antioxidative system. Instead we found only differences between MDA content (higher in shoots from anthropogenic than in more salted environments) but the activity of SOD, CAT and APOX didn't differ in organs of Common glasswort *Salicornia europaea*, which indicates that the impact of other factors than examined elements influenced higher level of lipoperoxidation in anthropogenic environments. Antioxidant enzymatic activity didn't differ significantly between plants organs in examined environments except of SOD and APOX in strong salted environment (SM). We found higher level of these enzymes in leaves than in roots of plants studied. It was probably related with Ca and also Cu, Zn, and Cd concentrations. However, the level of lipoperoxidation was significant higher in green parts of glycophytes (anthropogenic, agricultural environments), which is opposite to common glasswort (both environments). Instead in glycophytes avoiding sodium to maintain ionic homeostasis we found higher Na concentrations in roots and leaves of halophytes (Common glasswort *Salicornia europaea*) than in glycophytes. We can conclude that glycophytes subjected to higher salinity stress develop probably higher level of ROS, thus lipoperoxidation in their organs (except of green parts in AE) is higher than in halophytes. On the other hand, APOX activity is higher in roots of halophytes than in glycophytes in both environments in opposite to their green parts. We thus can suspect that this is connected with higher concentrations of Ca (alcalinity) and elevated salinity effect indicated APOX activity in green parts of plants more sensitive on salt damages than roots. Similarly, CAT activity is also higher in green parts of glycophytes than in halophytes. This indicate that glycophytes are better adaptation to stressful conditions, however there are many indistinct questions which require further study.

Author details

Piotr Kamiński, Beata Koim-Puchowska, Monika Wieloch and Karolina Bombolewska
Nicolaus Copernicus University, Collegium Medium in Bydgoszcz, Department of Ecology and Environmental Protection, Bydgoszcz, Poland

Piotr Kamiński
University of Zielona Góra, Faculty of Biological Sciences, Institute of Biotechnology and Environment Protection, Department of Biotechnology, Zielona Góra, Poland

Piotr Puchowski
Government Forestry in Toruń; Zamrzenica Forestry District, Bysław, Poland

Leszek Jerzak
University of Zielona Góra, Faculty of Biological Sciences, Institute of Biotechnology and Environment Protection, Department of Environmental Protection and Biodiversity, Zielona Góra, Poland

Acknowledgement

We thank Professor Brendan P. Kavanagh (Royal College of Surgeons in Ireland, Medical University of Bahrain) for his help with improving English language of the paper.

6. References

[1] Mittler R. 2002. Oxidative stress, antioxidant and stress tolerance. Trends Plant Sci. 7: 405-410.
[2] Bowler C., Fluhr R. 2000. The role of calcium and activated oxygens as signals for controlling cross-tolerance. Trends Plant Sci. 5: 241-246.
[3] Panda S.K., Khan M.H. 2004. Changes in growth and superoxide dismutase activity in *Hydrilla verticillata* L. under abiotic stress. Braz. J. Plant Physiol., 16(2):115-118.
[4] Schutzendubel A., Polle A. 2002. Plant responses to abiotic stresses: heavy-metal induced oxidative stress and protection by mycorrhization. J. Exp. Bot. 53: 1351-1365.
[5] Scandalios J.G. 1993. Oxygen stress and superoxide dismutase. Plant Physiol. 101: 7-12.
[6] Sairam R.K., Srivastava G.C. 2002. Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Sci. 162: 897-904.
[7] Elkahoui S., Hernandez J.A., Abdelly Ch., Ghrir R., Limam F. 2005. Effects of salt on lipid peroxidation and antioxidant enzyme activities of *Catharanthus roseus* suspension cells. Plant Sci. 168: 607-613.
[8] Dionisio-Sese M.L., Tobita S. 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135: 1-9.
[9] Gossett D.R., Millhollon E.P., Lucas M.C. 1994. Antioxidant responses toNaCl stress in salt tolerant and salt-sensitive cultivars of cotton, Crop Sci. 34: 706-714.
[10] Sreenivasulu N., Ramanjulu S., Ramachandra-Kini K., Prakash H.S., Shekar-Shetty H., Savitri H.S., Sudhakar C. 1999. Total peroxidase activity and peroxidase isoforms as modified by salt stress in two cultivars of fox-tail millet with differential salt tolerance. Plant Sci. 141: 1–9.
[11] Loureiro S., Santos C., Pinto G., Costa A., Monteiro M., Nogueira A.J.A., Soares A.M.V.M. 2006. Toxicity assessment of two soils from Jales Mine (Portugal) using plants: growth and biochemical parameters. Arch. Environ. Contam. Toxicol. 50: 182-190.
[12] Bidar G., Pruvot Ch., Garçon G., Verdin A., Shirali P., Douay F. 2009. Seasonal and annual variations of metal uptake, bioaccumulation, and toxicity in *Trifolium repens* and

Lolium perenne growing in a heavy metal-contaminated field. Environ. Sci. Pollut. Res. 16: 42-53.

[13] Przybył K., Woźny A. 2004. Komórka w warunkach stresu środowiskowego. t 2. Wyd. UAM, Poznań, 172 pp.

[14] Mittova V., Tal M., Volokita M., Guy M. 2003. Up-regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species *Lycopersicon pennellii*. Plant Cell Environ. 2: 845-856.

[15] Ben Amor N., Ben Hamed K., Debez A., Grignon C., Abdelly C. 2005. Physiological and antioxidant responses of the perennial halophyte Crithmum maritimum to salinity. Plant Sci. 168, 889-899.

[16] Bowler C., Van Montagu M., Inze D. 1992. Superoxide dismutase and stress tolerance. Ann. Rev. Plant Physiol. Plant Mol. Biol. 43: 83-116.

[17] Baum J.A., Scandalios J.G. 1981. Isolation and characterization of cytosolic and mitochondrial superoxide dismutases of maize. Arch. Biochem. Biophys. 206, 249-261.

[18] Stroiński A. 1999. Some physiological and biochemical aspects of plant resistance to cadmiumeffect. I. Antioxidative system. Acta Physiologiae Plantarum 21 (2): 999:175-188.

[19] Duke M.V., Salin M.L. 1985. Purification and characterization of an iron-containing superoxide dismutase from eukaryote, *Ginko biloba*. Arch. Biochem. Biophys. 243: 305-314.

[20] Scandalios J.G. 1990. Response of plant antioxidant defence genes to environmental stress. Adv. Genet. 28: 1-41.

[21] Chen G.X., Asada K. 1989. Ascobate peroxidase in tea leaves. Occurrence of two isozymes and their differences in enzymatic and molecular properties. Plant Cell Physiol. 30: 987-998.

[22] Asada K. 1992. Ascorbate peroxidase - a hydrogen peroxide-scavenging enzyme in plants. Physiol. Plant. 85: 235-241.

[23] Barcelo A.R., Ferrer M.A., Florenciano E.G., Munoz R. 1991. The tonoplast localization of two basic isoperoxidases of high pI in Lupinus. Bot. Acta 104: 272-278.

[24] Takahama U., Oniki T. 1992. Regulation of peroxidase-dependent oxidation of phenolics in the apoplast of spinach leaves by ascorbate. Plant Cell Physiol. 33: 379-387.

[25] Verma S., Dubey R.S. 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci. 164: 645-655.

[26] Grassmann J., Hippeli S., Elstner E.F. 2002. Plant's defence and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. Plant Physiol. Biochem. 40: 471-478.

[27] Hamilton III E.W., Heckathorn S.A. 2001. Mitochondrial adaptations to NaCl stress: Complex I is protected by anti-oxidants and small heat shock proteins, whereas Complex II is protected by proline and betaine. Plant Physiol. 126: 1266-1274.

[28] Mittova V., Guy M., Tal M., Volokita M. 2004. Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. J. Exp. Bot. 55: 1105-1113.

[29] Banuls J., Legaz F., Primo-Millo E. 1991. Salinity-calcium interactions on growth and ionic concentrations of citrus plants. Plant Soil, 133: 39-46.
[30] White P.J., Broadley M.R. 2003. Calcium in plants. Ann. Bot. 92: 487-511.
[31] Jaleel C.A., Manivannan P., KishoreKumar A., Sankar B., Panneerselvam R. 2007. Calcium chloride effects on salinity-induced oxidative stress, proline metabolism and indole alkaloids accumulation in *Catharanthus roseus*. C.R. Biol. 330: 674-683.
[32] Manivannan P., Jaleel C.A., Sankar B., Somasundaram R., Murali P.V., Sridharan R., Panneerselvam R. 2007. Salt stress mitigation by calcium chloride in *Vigna radiate* (L.) Wilczek. Acta Biol. Cracov. Ser. Bot. 49, 2: 105-109.
[33] Nagajyoti P.C., Lee K.D., Sreekanth T.V.M. 2010. Heavy metals, occurrence and toxicity for plants: a review. Environ. Chem. Lett. 8: 199-216.
[34] Kabata-Pendias A., Mukherjee A.B. 2007. Trace Elements from Soil to Human. Springer-Verlag, Berlin-Heidelberg-New York, 550 pp.
[35] Bartosz G. 2006. Druga twarz tlenu. Wolne rodniki w przyrodzie. PWN-Pol. Sci. Publ., Warszawa, 447 pp.
[36] Kaznina N.M., Laidinen G.F., Titov A.F., Talanov A.V. 2005. Effect of lead on the photosynthetic apparatus of annual grasses. Izv. Akad. Nauk Ser. Biol. 2: 184-188.
[37] Andra S.S., Datta R., Sarkar D., Makris K.C., Mullens C.P., Sahi S.V., Bach S.B.H. 2010. Synthesis of phytochelatins in vetiver grass upon lead exposure in the presence of phosphorus. Plant Soil. 326: 171-185.
[38] Singh R., Tripathi R.D., Dwivedi S., Kumar A., Trivedi P.K., Chakrabarty D. 2010. Lead bioaccumulation potential of an aquatic macrophyte Najas indica are related to antioxidant system. Bioresource Technol. 101: 3025-3032.
[39] Pal M., Horvath E., Janda T., Paldi E., Szalai G. 2006. Physiological changes and defense mechanisms induced by cadmium stress in maize. *J. Plant Nutr. Soil Sci.* 2006 (169)239–246.
[40] Mishra S., Srivastava S., Tripathi R.D., Dwivedi S., Shukla M.K. 2008. Response of Antioxidant Enzymes in Coontail (*Ceratophyllum demersum* L.) Plants Under Cadmium Stress. Environ. Toxicol. 23: 294-301.
[41] Somashekaraiah B.V., Padmaja K., Prasad A.R.K. 1992. Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): involvement of lipid peroxides in chlorophyll degradation. Physiol. Plant. 85: 85-89.
[42] Stohs S.J., Bagchi D. 1995. Oxidative mechanisms in the toxicity of metal ions. Free Rad. Biol. Med. 18: 321-336.
[43] Shaw B.P. 1995. Effect of mercury and cadmium on the activities of antioxidative enzymes in the seedlings of *Phaseolus aureus*. Biol. Plant. 37: 587-596.
[44] Gallego S.M., Benavides M.P., Tomaro M.L. 1996. Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. Plant Sci. 121: 151-159.
[45] Balestrasse K.B., Gardey L., Gallego S.M., Tomaro M.L. 2001. Response of antioxidant defence system in soybean nodules and roots subjected to cadmium stress. Aust. J. Plant Physiol. 28: 497-504.
[46] Fornazier R.F., Ferreira R.R., Vitória A.P., Molina S.M.G., Lea P.J., Azevedo R.A. 2002. Effects of cadmium on antioxidant enzyme activities in sugar cane. Biol. Plant. 45: 91-97.

[47] Cho U., Seo N. 2004. Oxidative stress in Arabidopsis thaliana exposed to cadmium is due to hydrogen peroxide accumulation, Plant Sci. 168: 113-120.
[48] Benavides M.P., Gallego S.M., Tomaro M.L. 2005. Cadmium toxicity in plants. Braz. J. Plant Physiol. 17(1): 21-34.
[49] Szafer W., Kulczyński S., Pawłowski B. 1986. Polish Plants. PWN-Pol. Sci. Publ., Warszawa.
[50] Balnokin Yu.V., Myasoedov N.A., Shamsutdinov Z.Sh., Shamsutdinov N.Z. 2005. Significance of Na^+ and K^+ for sustained hydration of organ tissues in ecologically distinct halophytes of the family Chenopodiaceae. Russ. J. Plant Physiol. 52, 6: 779-787.
[51] Ungar I.A. 1977. The relationship between soil water potential and plant water potential in two inland halophytes under field conditions. Bot. Gazette, 138: 498-501.
[52] Egan T.P., Ungar I.A. 2000. Mortality of the Salt Marsh Species Salicornia europaea and *Atriplex prostrata* (*Chenopodiaceae*) in Response to Inundation. Ohio J. Sci. 100, 2: 24-27.
[53] Demirezen D., Aksoy A. 2006. Common hydrophytes as bioindicators of iron and manganese pollutions. Ecol. Indicators, 6: 388-393.
[54] Juszczuk I., Malusà E., Rychter A.M. 2001. Oxidative stress during phosphate deficiency in roots of bean plants (*Phaseolus vulgaris* L.) J. Plant Physiol. 158: 1299-1305.
[55] Bradford M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72: 248-254.
[56] Beauchamp C., Fridovich I. 1971. Superoxide dismutase: improved assaysand applicable to acrylamide gels. Anal. Biochem. 44: 276-287.
[57] Aebi H. 1984. Catalase in vitro. Method. Enzymol. 105: 121-126.
[58] Scebba F., Sebastiani L., Vitagliano C. 1998. Changes in activity of antioxidative enzymes in wheat (*Triticum aestivum*) seedlings under cold acclimation. Physiol. Plant. 104: 747-752.
[59] Nakano Y., Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. Plant Cell Physiol. 22: 867-880.
[60] Okhawa H., Ohishi N., Yagi Y. 1979. Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. Analyt. Biochem. 95: 351-358.
[61] Stanisz A. 2006. Przystepny kurs statystyki z zastosowaniem STATISTICA PL na przykładach z medycyny. Wyd. Statsoft Polska Sp. z o.o., 531 pp.
[62] Samecka-Cymerman A., Kempers A.J. 2001. Concentrations of heavy metals and plant nutrients in water, sediments and aquatic macrophytes of anthropogenic lakes (former open cut brown coal mines) differing in stage of acidification. Sci. Total Environ. 28: 87-98.
[63] Sundareshwar P.V., Morris J.T., Koepfler E.K., Fornwalt B. 2003. Phosphorous limitation of coastal ecosystem processes. Science, 299: 563-565.
[64] Bargagli R. 1998. Trace Elements in Terrestrial Plants. An Ecophysiological Approach to Biomonitoring and Biorecovery. Springer, Berlin, 324 pp.
[65] Kłosowska K. 2010. Reakcje roślin na stres solny. KOSMOS, Probl. nauk biol. 59: 539-549.
[66] Kopcewicz J., Lewak S. 2002. Fizjologia roślin. PWN-Pol. Sci. Publ., Warszawa, 806 pp.

[67] Wrochna M., Gawrońska H., Gawroński S.W. 2006. Wytwarzanie biomasy i akumulacja jonów Na+, K+, Ca2+, Mg2+, Cl- w warunkach stresu solnego, przez wybrane gatunki roślin ozdobnych. Acta Agrophysica, 134: 775-785.

[68] Kabata-Pendias A., Pendias H. 1984. Trace elements in soils and plants. CRC Press, Boca Raton, 315 pp.

[69] Baker D.E., Senft J.P. 1995. Copper. In: Alloway B.J. (Ed.). Heavy Metals in Soils. 2nd ed. Blackie Acad. Professional, London, pp. 179-205.

[70] Allen S.E. 1989. Chemical Analysis of Ecological Materials. 2nd ed. Blackwell Sci. Publ., Oxford.

[71] Foyer C.H., Noctor G. 2000. Oxygen processing in photosynthesis: regulation and signalling. New Phytol. 146: 359-388.

[72] Reddy A.R., Chaitanya K.V., Vivekanandan M. 2004. Droughtinduced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol. 161: 1189-1202.

[73] Kim S.Y., Lim J.H., Park M.R., Kim Y.J., Park T.I., Seo Y.W., Choi K.G., Yun S.J. 2005. Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barlay roots under saline stress. J. Biochem. Mol. Biol. 38: 218-224.

[74] Li L., Van Staden J. 1998. Effects of plant growth regulators on the antioxidant system in callus of two maize cultivars subjected to water stress. Plant Growth Regul. 24: 55-66.

[75] Chen K.M.,1, Gong H.J.,, Wang S.M., Zhang C.L., 2007. Antioxidant defense system in *Phragmites communis* Trin. ecotypes. Biologia Plantarum 51 (4): 754-758

[76] Sudhakar C., Lakshmi A., Giridarakumar S. 2001. Changes in the antioxidant enzymes efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Sci. 161: 613-619.

[77] Dixit V., Pandey V., Shyam R. 2001. Differential oxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L cv. Azad). J. Exp. Bot. 52: 1101-1109.

[78] Sandalio L.M., Dalurzo H.C., Gomez M., Romero-Puertas M.C., del Río L.A. 2001. Cadmium- induced changes in the growth and oxidative metabolism of pea plants. J. Exp. Bot. 52: 2115-2126.

[79] Shalata A., Tal M. 1998. The effect of salt stress on lipid peroxidation and antioxidant in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. Physiol Plant. 104: 169-174.

[80] Chaparzadeh N., D'Amico M.L., Khavari-Nejad R.A., Izzo R., Navari-Izzo F. 2004. Antioxidatve responses of *Calendula officinalis* under salinity conditions. Plant Physiol. Biochem. 42: 695-701.

[81] Ben Hamed K., Castagna A., Salem E., Ranieri A., Abdelly Ch. 2007. Sea fennel (*Crithmum maritimum* L.) under salinity conditions: a comparison of leaf and root antioxidant responses. Plant Growth Regulation, 53:185-194.

[82] Lechno S., Zamski E., Telor E. 1997. Salt stress-induced responses in cucumber plants. J. Plant Physiol. 150: 206-211.

[83] Rodriguez-Rosales M.P., Kerkeb L., Bueno P., Donaire J.P. 1999. Changes induced by NaCl in lipid content and composition, lipoxygenase, plasma membrane H+ATPase and

antioxidant enzyme activities of tomato (*Lycopersicon esculantum* Mill.) calli. Plant Sci. 143: 143-150.

[84] Hernandez J.A., Campillo A., Jimenez A., Alacon J.J., Sevilla F. 1999. Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. New Phytol. 141: 241-251.

[85] Schickler H., Caspi H. 1999. Response of antioxidant enzymes to nickel and cadmium stress in hyperaccumulator plants of the genus Alyssum. Physiol. Plant. 105: 39-44.

[86] Rucińska R., Waplak S., Gwóźdź E. 1999 Free radical formation and activity of antioxidant enzymes in lupin roots exposed to lead. Plant Physiol. Biochem. 37: 187-194.

[87] Vitoria A.P., Lea P.J., Azevedo R.A. 2001. Antioxidant enzymes responses to cadmium in radish tissues. Phytochemistry 57: 701-710.

[88] Schutzendubel A., Schwanz P., Teichmann T., Gross K., Langenfeld-Heyser R., Godbold A., Polle A. 2001. Cadmium-induced changes in antioxidative systems, H_2O_2 content and differentiation in pine (*Pinus silvestris*) roots. Plant Physiol. 127: 887-898.

[89] Romero-Puertas M.C., Rodríguez-Serrano M., Corpas F.J., Gomez M., del Rio L.A., Sandalio L.M. 2004. Cadmium-induced subcellular accumulation of $O_2^{\cdot-}$ and H_2O_2 in pea leaves. Plant Cell Environ. 27: 1122-1134.

[90] Demiral T., Turkan I. 2006. Exogenous glycine-betain affects growth and proline accumulation and retards senescence in two rice cultivars under NaCl stress. Environ. Exp. Bot. 56: 72-79.

[91] Knight H., Trewaves A.J., Knight M.R. 1997. Calcium signalling in Arabidopsis thalliana responding to drought and salinity. Plant J. 12: 1067-1078.

[92] Arshi A., Ahmad A., Aref I.M., Iqbal M. 2010. Effect of calcium against salinity-induced inhibition in growth, ion accumulation and proline contents in *Cichorium intybus* L. J. Environ. Biol. 31(6): 939-944.

[93] Di H, Yun-guo L, Yu-e H, , Xiang-jin L, Wei Z (2007) Effects of calcium on chlorophyll and antioxidant enzymes in *Phragmites australis* under cadmium stress. J. Agro-Environ. Sci., 2007-2001.

[94] Di Baccio D., Navari-Izzo F., Izzo R. 2004. Seawater irrigation: antioxidant defence responses in leaves and roots of a sunflower (*Helianthus annuus* L.) ecotype. J. Plant Physiol. 161: 1359-1366.

[95] Meneguzzo S., Navario-Izzo F., Izzo R. 1999. Antioxidative responses of leaves and roots of wheat to increasing NaCl concentrations. J. Plant Physiol. 155: 274-280.

[96] Bandeoglu E., Eyidogan F., Yucel M., Oktem H.A. 2004. Antioxidant responses of shoots and roots of lentil to NaCl salinity stress. Plant Growth Regulation, 42: 69-77.

[97] Tester M., Davenport R. 2003. Na^+ tolerance and Na^+ transport in higher plants. Ann. Bot. 91: 503-527.