Pesticide Biochemistry and Physiology 130 (2016) 17–21

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Antioxidant responses in soybean and alfalfa plants grown in DDTs contaminated soils: Useful variables for selecting plants for soil phytoremediation?

Francesca M. Mitton [a], Josencler L. Ribas Ferreira [b], Mariana Gonzalez [a], Karina S.B. Miglioranza [a,*], José M. Monserrat [b]

[a] Laboratorio de Ecotoxicología y Contaminación Ambiental, Instituto de Investigaciones, Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMdP) — Consejo Nacional de Investigaciones, Científicas y Técnicas (CONICET), Dean Funes 3350, Mar del Plata 7600, Argentina
[b] Universidade Federal de Rio Grande — FURG, Instituto de Ciências Biológicas (ICB), Rio Grande do Sul, Brazil

ARTICLE INFO

Article history:
Received 13 February 2015
Received in revised form 16 November 2015
Accepted 15 December 2015
Available online 18 December 2015

Keywords:
Soybean
Alfalfa
DDTs
Phytoremediation
Oxidative stress

ABSTRACT

Phytoremediation is a low-cost alternative technology based on the use of plants to remove pollutants from the environment. Persistent organic pollutants such as DDTs with a long half-life in soils are attractive candidates for remediation. This study aimed to determine the potential of antioxidant response use in the evaluation of plants' tolerance for selecting species in phytoremediation purposes. Alfalfa and soybean plants were grown in DDT contaminated soils. After 60 days, growth, protein content, antioxidant capacity, GST activity, concentration of proteic and non-proteic thiol groups, chlorophyll content and carotenoid content were measured in plant tissues. Results showed no effect on alfalfa or soybean photosynthetic pigments but different responses in the protein content, antioxidant capacity, GST activity and thiol groups on roots, stems and leaves, indicating that DDTs affected both species. Soybean showed higher susceptibility than alfalfa plants due to the lower antioxidant capacity and GST activity in leaves, in spite of having the lowest DDT accumulation. This study provides new insights into the role of oxidative stress as an important component of the plant's response to DDT exposure.
© 2015 Elsevier Inc. All rights reserved.

1. Introduction

The organochlorine pesticide dichlorodiphenyltrichloroethane (DDT) and its metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) belong to persistent organic pollutants (POPs), regulated by the Stockholm Convention and characterized by a long half-life, bioaccumulative behavior and ability to produce chronic adverse effects on humans and animals. DDT was widely used throughout the world to control arthropod disease-vectors and agricultural pests before it was banned. Consequently, the residues of DDT and metabolites are widely distributed in different environmental compartments [1,2]. Nowadays, DDT is still in use for malaria control in developing countries [3]. Considering the physico-chemical properties of DDTs (DDT + DDD + DDE) and their bioaccumulation potential, phytoremediation is a likely tool to clean soils contaminated by DDTs. This technique is defined as the use of green plants to remove pollutants from the environment or to render them harmless [4]. It has been well-demonstrated that some crops incorporate organochlorine pesticides from soil, depending on plant species, soil type and involved insecticide [5,6]. In this sense, previous studies showed that soybean and alfalfa plants grown in DDT polluted soils (500 ng g^{-1} dry weight) bioconcentrate pesticides in roots reaching values of 830 and 1120 ng g^{-1} dry weight of DDTs, respectively [7].

However, the extent of phytoremediation success is conditioned by two main factors: the pollutant availability that would have a direct consequence on the soil-root transfer [8], and the toxicity, that might limit the plant growth affecting uptake and translocation processes. Moreover, each plant species will also influence those processes by modifying the soil–root environment with root exudates and specific rhizospheric interactions as well as having different levels of tolerance towards the contaminants [9]. Identification and selection of suitable plants for pollutant removal from the environment require a broad knowledge of the physiological and biochemical features of the different plant species. Edwards [11] defined the xenome as “the biosystem responsible for the detection, transport and metabolism of xenobiotics within the plant tissues”. Pollutants induce plant stress because they may elicit toxic effects by disrupting membrane integrity or metabolic pathways, making it necessary to safely sequester, extrude or detoxify the plants rapidly through biotransformation. During severe and persistent stress conditions, reactive oxygen species (ROS) accumulate

* Corresponding author at: Laboratorio de Ecotoxicología y Contaminación Ambiental, FCEyN, UNMdP, Funes 3350, Mar del Plata 7600, Argentina.
E-mail addresses: fmmitton@mdp.edu.ar (F.M. Mitton), kmiglior@mdp.edu.ar (K.S.B. Miglioranza).

http://dx.doi.org/10.1016/j.pestbp.2015.12.005
0048-3575/© 2015 Elsevier Inc. All rights reserved.

causing several damages including membrane and protein modifications if they are not detoxified by cell mechanisms. The oxidative stress in several plant species is indicated by an enhancement of lipid peroxidation, protein oxidation and activation of the antioxidant system [11].

However, plants cells are equipped with both non-enzymatic antioxidants and enzymatic ROS scavengers to protect themselves from oxidative damage [13]. In classical oxidative stress studies, the variation of levels or activities of individual antioxidants is used to indicate ROS mediated toxicity. Particularly, GSTs are a family of very abundant and ubiquitous enzymes present in aerobic organisms that catalyze the conjugation of GSH to a wide variety of hydrophobic and electrophilic compounds to form less- or non-toxic derivatives [16]. This conjugation reaction is involved in the detoxification and processing of various xenobiotics, which after glutathionylation are rapidly transported to the vacuole [17]. The role of glutathione-S-transferase (GST) during various stress conditions in plants has been reported [15].

The redox state of thiol (SH) groups affects the activity and structure of many enzymes, receptors and transcription factors, and organisms maintain it in proteins and low-molecular-mass thiols with complex regulatory machinery [18]. Oxidation of cysteine SH groups can cause intermolecular protein cross-linking and enzyme inactivation, leading eventually to cell death. The protein S-thiolation is a process in which protein-SH groups form mixed disulfide with low-molecular-mass thiols such as GSH [19]. Moreover, it represents a post-translational modification that possesses an antioxidant role in the protection against irreversible oxidation, or may alternatively serve in a regulatory role, analogous to other post-translational modifications such as protein phosphorylation [20]. The measurements of a limited number of antioxidants do not consider that the antioxidant systems can act in a cooperative way [14]. Therefore, a more holistic determination of total antioxidant capacity will provide a better understanding of an organism's resistance to toxicity caused by ROS. Additionally, the determination of pigment concentration [12] has also been employed as a marker to assess plant damage by pollutant exposure.

The present study investigates the GST activity, total antioxidant capacity, and the concentration of proteic and non-proteic thiol groups as useful biomarkers for selecting plant species to remediate soils contaminated with DDTs.

2. Materials and methods

2.1. Plant growth

Plants were grown in rectangular pots of 6000 cm^3 filled with 1000 g of dry polluted soil (455.3 and 63.5 ng g^{-1} dry weight of DDE and DDT, respectively), obtained from a typical apple and peach field settled in Villa Regina city in the Upper Valley of the Rio Negro basin, Argentina (S 39°04.9′14″, W 67°02.9′59″) [21].

Seeds of *Glycine max* "soybean" (5) and *Medicago sativa* "alfalfa" (50) were placed in three separate pots and kept in a greenhouse at a temperature of 10–26 °C under natural sunlight (light:dark cycle 14:10 h). Planted control pots with non-polluted soil were also established. All pots were weeded on demand and watered weekly with tap water.

2.2. Plant sampling

Soybean and alfalfa plants were destructively harvested at 60 days after germination (appearance of the first true leaves). Roots, stems and leaves were separated and washed to remove attached soil particles. For soybean plants, roots, stems and leaves were obtained, while for alfalfa plants, due to the small size of each individual, the aerial tissues (stems + leaves) were pooled. Samples from each pot were composited and individually analyzed. All samples were kept in a freezer at −80 °C until biochemical analysis.

2.3. Tissue homogenization

For measurements of protein content, total antioxidant capacity, GST activity and proteic and non-proteic sulfhydryl groups, roots and aerial tissues were homogenization following the method described by Martinez-Dominguez et al. [22], with some modifications. Briefly, the tissues were prepared in liquid nitrogen and homogenized (1:2 *w/v*) in ice-cold 0.1 M sodium phosphate buffer containing 20% glycerol, 14 mM dithrothreitol (DTE), 1 mM phenylmethylsulfonylfluoride (PMSF) and 1 mM (ethane-1,2-diyldinitrilo) tetra acetic acid (EDTA) and adjusted pH to 6.5. All reagents were obtained from Sigma-Aldrich. Homogenates were centrifuged at 15,000 ×*g* for 20 min (4 °C) and the supernatants were collected and stored at −80 °C for later use.

2.4. Protein determination

Protein concentration was assayed with bovine serum albumin (BSA; Sigma-Aldrich) as standard protein according to the Bradford method [23].

2.5. Determination of antioxidant capacity

Antioxidant capacity was assayed according to the method described by Amado et al. [24] which is based on the detection of ROS by fluorometry (ex/em: 485/520 nm). The assay was performed with some modifications of Vianna [25], which allows their use in samples with low protein content. Peroxyl radicals were generated in the analyzed samples by thermal decomposition at 37 °C of 2,2′-azobis(2-methylpropionamidine) dihydrochloride (ABAP, Sigma-Aldrich), resulting in the emission of a fluorescent signal caused by the reaction between ROS and 2′,7′-dichlorodihydrofluorescein (H_2DCF) probe, that resulted in the previous cleavage of 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA, Invitrogen) by alkaline hydrolysis for 30 min. The blanks were prepared with the buffer of homogenization and with and without ABAP or probe addition. The reaction buffer, containing 30 mM HEPES (pH 7.2), 200 mM KCl, and 1 mM $MgCl_2$, was added to the samples. Then, ABAP (10 mM) was added to three wells of each sample, while the same volume of ultrapure water (Milli-Q) was added to the three remaining wells. Immediately before the microplate reading, the hydrolyzed probe was added to the wells at a final concentration of 40 μM and lectures were performed in a fluorescence microplate reader (Victor2 D, Perkin Elmer, Waltham, MA, USA). The oxidation of non-fluorescent H_2DCF by the ROS generated by thermal decomposition of ABAP into a fluorescent compound (DCF) was detected at 485 (excitation) and 520 (emission) wavelengths (nm), every 5 min for 30 min.

Total fluorescence production was calculated according to Eq. (1), and the results were expressed in percentage of antioxidant capacity (%AC).

$$\%AC = (\Delta Blank - \Delta Sample)/\Delta Blank \times 100 \quad (1)$$

ΔBlank = NF Blank with ABAP − NF Blank without ABAP; ΔSample = NF Sample with ABAP − NF Sample without ABAP; NF (Net fluorescence) = AF with H2DCF − AF without H2DCF; AF = average fluorescence, calculated from each triplicate.

2.6. Measurement of glutathione-S-transferase (GST) activity

GST activity was based on methodology described by Habig and Jakoby [26] where the absorbance generated by the conjugation of 1 mM glutathione (GSH, Sigma-Aldrich) with 1 mM of 1-chloro-2,4-dinitrobenzene (CDNB, Sigma-Aldrich) was monitored at 340 nm during 1 min at 25 °C.

2.7. Measurement of proteic (P-SH) and non proteic (NP-SH) sulfhydryl groups

The measurement of P-SH and NP-SH sulfhydryl groups was based on Sedlak and Lindsay [27] and Ferreira Cravo [28] methods. Determination of total sulfhydryl content was measured before deproteinization of homogenates with trichloro acetic acid (TCA, 50%). Total and NP-SH content was detected using 2,3 naphthalene carboxaldehido (NDA) (10 mM; Sigma). Fluorescence readings (485 and 530 nm) were done using a fluorescence microplate reader (Victor2 D, Perkin Elmer, and Waltham, MA, USA). P-SH was estimated as the difference between total and NP-SH content. Both P-SH and NP-SH were referred to the glutathione (GSH) concentration curve.

2.8. Measurement of chlorophyll content and carotenoid content

Chlorophyll content and carotenoid content in leaves were spectrophotometrically determined. Samples were homogenized with acetone (0.25:5 *w*/*v*) and incubated for 1 h at 4 °C in darkness, centrifuged at 590 × *g* for 5 min at 4 °C and measured at the wavelength of 663, 646 and 470 nm. The chlorophyll (*a*, *b*) and carotenoid concentrations were estimated according to the absorbance coefficients determined by Lichtenthaler [29] and results expressed as mg g^{-1} of dry weight.

2.9. Statistical analysis

The protein content, GST activity, antioxidant capacity against peroxyl radicals, proteic (P-SH) and non-proteic (NP-SH) sulfhydryl groups, and chlorophyll and carotenoid content results represent the mean of three independent determinations in different plant tissues. All variables were analyzed by means of parametric one-way ANOVA [30]. Previously, normality and variance homogeneity were verified and mathematical transformation applied if at least one assumption was violated. In all cases, the significance level was fixed at 0.05.

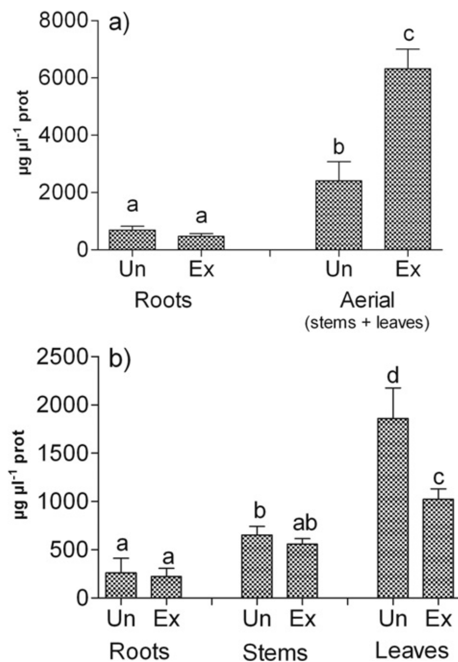

Fig. 1. Protein content in roots and aerial tissues of 60-days old alfalfa (a) and soybean (b) plants grown in polluted (Ex) and unpolluted (Un) soils. Different letters indicate significant differences ($p \leq 0.05$).

3. Results and discussion

3.1. Protein content

Plant protein content might be affected by several stressors including xenobiotics [31,32]. Fig. 1 shows the protein content in aerial and root tissues of exposed and unexposed soybean and alfalfa plants. The DDT exposure did not affect the general pattern of protein content being "roots < leaves". However, when comparing exposed and control plants, a protein depletion in soybean leaves and an increment in alfalfa was observed in the exposed plants (Fig. 1 a and b). The results observed in soybean plants might indicate either a reduction or inhibition in the synthesis of or an enhancement in the degradation of proteins. Roots of both species did not show changes in the protein content after DDT exposure (Fig. 1). Some authors showed that pesticides decrease the soluble protein content in many plants, such as trigonella [33] and sunflower [34]. Protein synthesis inhibition during shoot emergence by the exposure to the herbicide Butachlor was reported for rice by Janardhan [35] and Noviel [36]. Moreover, Sharma [37] reported a decrease in the protein content when rice seedlings were exposed to the insecticide imidacloprid and, Sammaiah [10] reported a dose-dependent effect, with increased protein content in *Solanum melongena* (eggplants) exposed to 500–1000 ppm of Endosulfan and reduction at higher concentrations. These authors proposed that low pesticide doses have a positive effect on the germination and growth of the seedling but at higher levels it becomes phytotoxic. On the other hand, it was also proposed that the increasing of protein content can be connected with an increase in the nitrogen content and in this sense some insecticides increase the nitrate reductase activity [38].

3.2. Antioxidant and metabolic responses

3.2.1. Total antioxidant capacity against peroxyl radical (AC)

This parameter is used to evaluate the overall resistance of organisms to ROS toxicity. The methodology used in this study allows the comparison of responses among different tissues and species, and results showed the differences in the susceptibility of the studied species, showing specie and tissue-specific responses to DDTs. Plant exposure leads to reduced AC in alfalfa aerial tissues and soybean leaves (Fig. 2) and an increase of AC in soybean roots and stems. The depletion of the AC indicates the pro-oxidant condition elicited by DDT exposure followed by damage in the antioxidant system and/or to the use of antioxidants to cope with this stress. The increased AC in roots and stems of soybean plants suggested a mild pro-oxidant condition that usually promotes the expression of antioxidant genes [13].

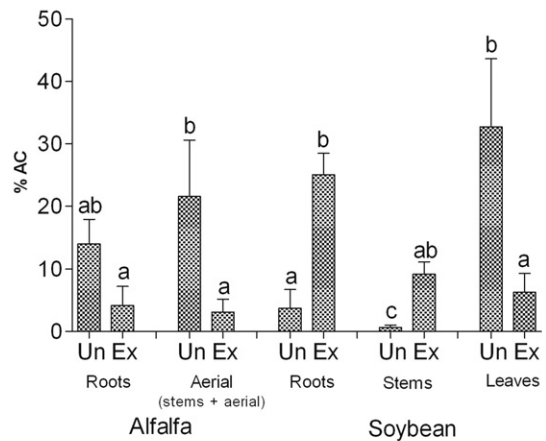

Fig. 2. Antioxidant capacity (%AC) of roots and aerial tissues of 60 days-old alfalfa and soybean plants grown in polluted (Ex) and unpolluted (Un) soils. Different letters indicate significant differences ($p \leq 0.05$).

Together with the biochemical responses, it is important to consider that alfalfa and soybean plants differ in their pesticide uptake ability [7]. The observed species-specific differences, might be attributed to the higher DDTs bioconcentration by alfalfa tissues (root: 1100 ng g^{-1}; aerial: 840 ng g^{-1}), creating a more pro-oxidant condition where the spending of antioxidants (or damage to the antioxidant system) is observed instead of antioxidant inductions, as observed in soybean roots and stems which accumulate 100 ng g^{-1} and 15 ng g^{-1} of DDTs, respectively [7]. However, the lower antioxidant capacity in exposed soybean leaves indicates different tissue susceptibility to DDTs.

3.2.2. GST activity

The DDT exposure modified GST activity in all plants (Fig. 3 a and b) with increased activity in alfalfa roots and significant depletion in soybean leaves. GSTs represent more than 1% of soluble proteins in plant cells, therefore this lower activity in soybean leaves also correlated well with the reduced protein content (Fig. 1). Results from this work are in agreement with previous reports on other species and compounds with roots showing the highest GST activity and its induction by pesticide exposure [39]. The close relation between roots and soil matrix that leads to a main DDT uptake route is also contributing to the generation of biochemical responses in this organ. In this sense, GST induction in soybean and alfalfa roots might be linked to a metabolic detoxification response to DDT accumulation.

3.3. Concentration of sulfhydryl groups

Emerging evidence indicates that abiotic stress induces changes in the cellular redox status that can be sensed by oxidative modifications of protein redox sensitive cysteines [39]. The increased levels of SH-non proteic groups found in alfalfa roots and soybean leaves (Fig. 4 a and b, respectively) could indicate a general mechanism of redox regulations by increasing levels of low molecular weight thiols, like glutathione. On the other hand, SH-proteic did not vary between exposed or unexposed plants, except for alfalfa aerial tissues that showed a decrease in this parameter. This result might indicate that DDT presence in alfalfa roots could enhance the oxidant environment in cells leading to protein oxidation.

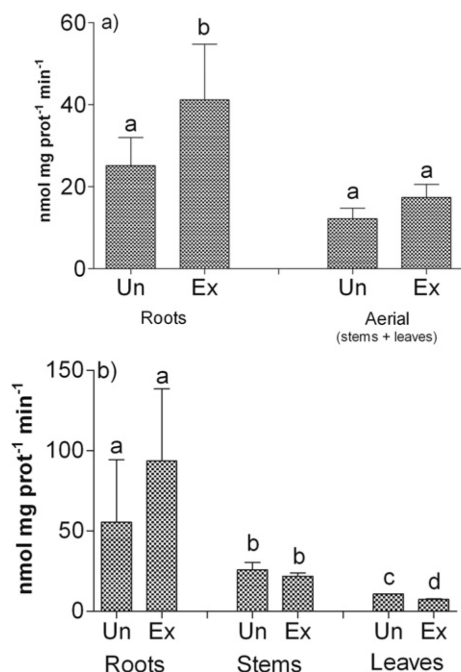

Fig. 3. GST-activity in roots and aerial tissues of 60 days-old alfalfa (a) and soybean (b) plants grown in polluted (Ex) and unpolluted (Un) soils. Different letters indicate significant differences ($p \le 0.05$).

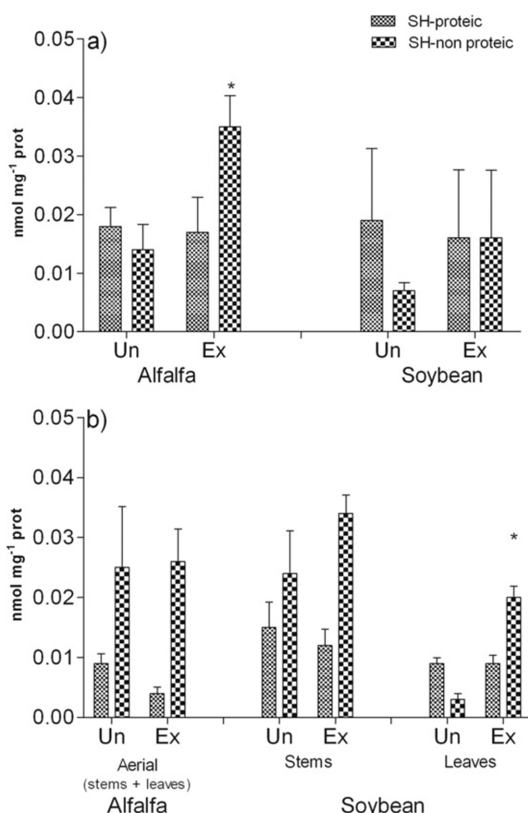

Fig. 4. SH-proteic and SH-non proteic groups in roots (a) and aerial tissues (b) of 60 days-old alfalfa and soybean plants grown in polluted (Ex) and unpolluted (Un) soils. * indicates significant differences ($p \le 0.05$).

Additionally, previous works showed increased lipid peroxidation levels in alfalfa aerial tissues grown in polluted soils [7]. Therefore, this effect biomarker can be linked to the results of SH-proteic groups of this work, suggesting that despite the increased GST activity of alfalfa plants, the AC decreased, indicating that the defense response might not be enough to avoid oxidative damage.

3.4. Pigment content

Pollutant effects on chlorophyll content might result in varying responses depending on plant age and specie and exposition time [40].

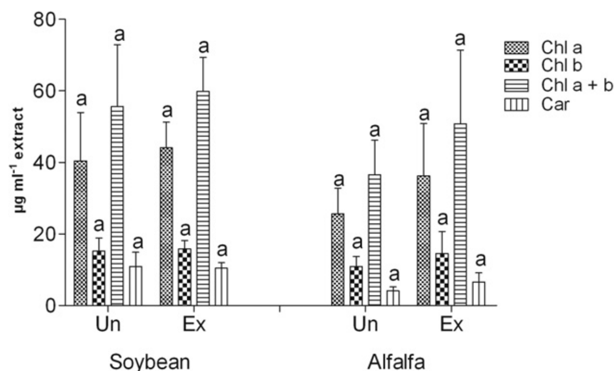

Fig. 5. Chlorophyll content (*a*, *b*, and total chlorophyll) and carotenoid levels in aerial tissues of 60 days-old alfalfa and soybean plants grown in polluted (Ex) and unpolluted (Un) soils. Different letters indicate significant differences ($p \le 0.05$).

Results showed that neither chlorophyll nor carotenoid contents were affected by DDT exposure (Fig. 5) indicating that DDTs have no effect on the pigment content on 60 day-old plants.

4. Conclusions

The biochemical parameters analyzed in this work: protein content, antioxidant capacity, GST activity and SH group content, could be suitable endpoints for the assessment of DDT exposure in soybean and alfalfa plants. However, the studied species presented different responses against a similar DDT exposure. The highest sensitivity of soybean plants to DDTs was expressed on the basis of the antioxidant responses found in this study. Comparing these results with previous works, higher bioconcentration seems to be linked to higher plant ability to pesticide uptake.

The protein content, antioxidant capacity, GST activity and SH group content could be used as complements to chemical analysis in the selection of candidates for phytoremediation purposes. Moreover, results are of concern for the understanding of the response mechanism of plants to persistent organic pollutants. Further studies could be linked to gain some insights into the study of these responses at earlier stages of growth in alfalfa and soybean plants. The knowledge about how crops could be affected by current and legacy pesticides such as DDTs and how they deal with it, is a topic of concern from the point of view of food production and agro-based economies that should be more deeply investigated.

Acknowledgments

This work was funded by grants from the Universidad Nacional de Mar del Plata (EXA 516/10–EXA 609/12) and ANPCyT (PICT-07/410). This work is part of the PhD thesis of the first author (Mitton, F. M).

References

[1] Z.M. Gong, S. Tao, F.L. Xu, R. Dawson, W.X. Liu, Y.H. Cui, J. Cao, X.J. Wang, W.R. Shen, W.J. Zhang, B.P. Qing, R. Sun, Level and distribution of DDT in surface soils from Tianjin, China, Chemosphere 54 (2004) 1247–1253.
[2] K.S.B. Miglioranza, M. Gonzalez, P.M. Ondarza, V.M. Shimabukuro, F.I. Isla, G. Fillmann, J.E. Aizpún, V.J. Moreno, Assessment of Argentinean Patagonia pollution: PBDEs, OCPs and PCBs in different matrices from the Río Negro basin, Sci. Total Environ. 452-453 (2013) 275–285.
[3] H. Nakataa, Y. Hirakawaa, M. Kawazoea, T. Nakabob, K. Arizonoc, S.-I. Abea, T. Kitanoa, H. Shimadad, I. Watanabee, W. Lif, X. Ding, Concentrations and compositions of organochlorine contaminants in sediments, soils, crustaceans, fishes and birds collected from Lake Tai, Hangzhou Bay and Shanghai city region, China, Environ. Pollut. 133 (2005) 415–429.
[4] D.E. Salt, R.D. Smith, I. Raskin, Phytoremediaton, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49 (1998) 643–668.
[5] M. Gonzalez, K.S.B. Miglioranza, J.E. Moreno, J.V. Moreno, Occurrence and distribution of organochlorine pesticides (OCPs) in tomato (*Lycopersicon esculentum*) crops from organic production, J. Agric. Food Chem. 51 (2003) 1353–1359.
[6] A. Nurzhanova, S. Kalugin, K. Zhambakin, Obsolete pesticides and application of colonizing plant species for remediation of contaminated soil in Kazakhstan, Environ. Sci. Pollut. Res. 20 (2013) 2054–2063.
[7] F.M. Mitton, K.S.B. Miglioranza, M. Gonzalez, V.M. Shimabukuro, J.M. Monserrat, Assessment of tolerance and efficiency of crop species in the phytoremediation of DDT polluted soils, Ecol. Eng. 71 (2014) 501–508.
[8] E. Hoballah, M. Saber, I. Matter, A. Zaghloul, Bioremediation of polychlorinated biphenyl (PCBs) in a sewaged soil by certain remediative amendments followed by phytoremediation, RJPBCS 5 (2014) 91–103.
[9] P. Krupa, J. Kozdro, Accumulation of heavy metals by ectomycorrhizal fungi colonizing birch trees growing in an industrial desert soil, World J. Microbiol. Biotechnol. 20 (2004) 427–430.
[10] D. Sammaiah, C. Chandra Shekar, V. Ramkrishna Prasad, K. Jaganmohan Reddy, Pesticides induced alterations in physiological responses in *Solanum melongena* L, Int. J. Pharm. Bio. Sci. 2 (2011) 374–384.
[11] R. Edwards, M. Brazier-Hicks, D.P. Dixon, I. Cummins, Chemical manipulation of antioxidant defenses in plants, Adv. Bot. Res. 42 (2005) 1–32.
[12] Ö. Aksoy, A. Deveci, S. Kızılırmak, G. Billur Akdeniz, Phytotoxic effect of quizalofop-p-ethyl on soybean (*Glycine max* L.), J. Biol. Environ. Sci. 7 (2013) 49–55.
[13] C.H. Foyer, G. Noctor, Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses, Plant Cell 17 (2005) 1866–1875.
[14] G.W. Winston, F. Regoli, A.J. Dugas, J.R. Jessica, H. Fong, K.A. Blanchard, A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids, Free Radic. Biol. Med. 24 (1998) 480–493.
[15] A. Wyrwicka, S. Steffani, M. Urbaniak, The effect of PCB-contaminated sewage sludge and sediment on metabolism of cucumber plants (*Cucumis sativus* L.), Ecohydrol. Hydrobiol. 14 (2014) 75–82.
[16] T.D. Dalton, H.G. Shertzer, A. Puga, Regulation of gene expression by reactive oxygen, Annu. Rev. Pharmacol. 39 (1999) 67–101.
[17] R. Mittler, Oxidative stress, antioxidants and stress tolerance, Trends Plant Sci. 7 (2002) 405–410.
[18] O. Carmel-Harel, G. Storz, Roles of the glutathione- and thioredoxin-dependent reduction systems in the *Escherichia coli* and *Saccharomyces cerevisiae* responses to oxidative stress, Annu. Rev. Microbiol. 54 (2000) 439–461.
[19] K. Bhoomika, S. Pyngrope, R.S. Dubey, Effect of aluminum on protein oxidation, non-protein thiols and protease activity in seedlings of rice cultivars differing in aluminum tolerance, J. Plant Physiol. 171 (2014) 497–508.
[20] E. Kuzniaka, A. Kazmierczak, M. Wielanek, R. Glowacki, A. Kornas, Involvement of salicylic acid, glutathione and protein S-thiolation in plant cell death-mediated defence response of *Mesembryanthemum crystallinum* against *Botrytis cinerea*, Plant Physiol. Biochem. 63 (2013) 30–38.
[21] M. Gonzalez, K.S.B. Miglioranza, J.E. Aizpún, F.I. Isla, A. Peña, Assessing pesticide leaching and desorption in soils with different agricultural activities from Argentina (Pampa and Patagonia), Chemosphere 81 (2010) 351–358.
[22] D. Martínez-Domínguez, M.A. de las Heras, F. Navarro, R. Torronteras, F. Córdoba, Efficiency of antioxidant response in *Spartina densiflora*: an adaptative success in a polluted environment, Environ. Exp. Bot. 62 (2008) 69–77.
[23] M. Bradford, A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[24] L.L. Amado, M.L. Garcia, P.B. Ramos, R.L. Freitas, B. Zafalon, J.L. Ferreira, J.S. Yunes, J.M. Monserrat, A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity, Sci. Total Environ. 407 (2009) 2115–2123.
[25] D.R. Vianna, G. Bubols, G. Meirelles, B.V. Silva, A. da Rocha, M. Lanznaster, J.M. Monserrat, S.C. Garcia, G. von Poser, V.L. Eifler-Lima, Evaluation of the antioxidant capacity of synthesized coumarins, Int. J. Mol. Sci. 13 (2012) 7260–7270.
[26] W.H. Habig, W.B. Jakoby, Assays for differentiation of glutathione-S-transferases, Methods Enzymol. 77 (1981) 398–405.
[27] J. Sedlak, R.H. Lindsay, Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent, Anal. Biochem. 1 (1968) 192–205.
[28] M. Ferreira-Cravo, F. Reinhardt Piedras, T. Barros Moraes, J.L. Ribas Ferreira, D.P. Salomao de Freitas, M. Dornelles Machado, L.A. Geracitano, J.M. Monserrat, Antioxidant responses and reactive oxygen species generation in different body regions of the estuarine polychaeta *Laeonereis acuta* (Nereididae), Chemosphere 66 (2007) 1367–1374.
[29] H.K. Lichtenthaler, Chlorophylls and carotenoids: pigments of photosynthetic membranes, Methods Enzymol. 148 (1987) 350–382.
[30] J.H. Zar, Biostatistical Analysis, second ed. Prentice-Hall, Englewood Cliffs, New Jersey, 1984.
[31] S.M. Bayramov, H.G. Babayev, M.N. Khaligzade, N.M. Guliyev, C.A. Raines, Effect of water stress on protein content of some Calvin cycle enzymes in different wheat genotypes, Proceedings of ANAS (Biological Sciences), 65 2010, pp. 106–111.
[32] B.N. Djebar, M. Reda, Z. Noureddine, B. Houria, Differential response to treatment with herbicide chevalier induced oxidative stress in leaves of wheat, Ann. Biol. Res. 5 (2014) 1–7.
[33] A.B. Kamble, A.B. Sabale, Influence of Bavistin and Monocrotohos seed germination and seedling growth to *Trigonealla foenumgraecum* L, Pollut. Res. 18 (1999) 61–65.
[34] A.K. Panduranga, G. Murthy, S. Leelavathi, Effect of some xenobiotics on early seedling growth and chlorophyll content in sunflower CV. "morden", during seed germination, Eco. Env. Cons. 8 (2002) 129–131.
[35] K.V. Janardhan, Nucleic Acid and Protein Synthesis, Agricultural Research Station, Nave Shigmosa, 1988.
[36] L.M. Noviel, Effect of butacholr on the protein content of C-168 and IR-36 rice cultivars as different growth stages, Weed Abstr. 32 (1989) 85.
[37] I. Sharma, R. Bhardwai, P.K. Pati, Stress modulation response of 24-epibrassinolide against imidacloprid in an elite indica rice variety Pusa Basmati-1, Pestic. Biochem. Physiol. 105 (2013) 144–153.
[38] S.K. Ries, R.P. Larsen, A.L. Kenworthy, The apparent influence of simnazine on nitrogen nutrition of peach and apple trees, Weeds 11 (1963) 270–273.
[39] G. Copkun, F. Zúhnúoúlu, Effect of some biocides on glutathione-s-transferase in barley, wheat, lentil and chickpea plants, Turk. J. Biol. 26 (2002) 89–94.
[40] M. Faure, A. San Miguel, P. Ravanel, M. Raveton, Concentration responses to organochlorines in *Phragmites australis*, Environ. Pollut. 164 (2012) 188–194.