Cadmium accumulation and physiological response of sunflower plants to Cd during the vegetative growing cycle

S. De Maria[1], M. Puschenreiter[2], A.R. Rivelli[1]

[1]*School of Agricultural, Forest, Food and Environmental Sciences,*
  University of Basilicata, Potenza, Italy
[2]*Department of Forest and Soil Sciences, University of Natural Resources*
  and Life Sciences, Vienna, Austria

ABSTRACT

The effects of soil cadmium (Cd) contamination on Cd accumulation and distribution, growth and physiological responses of sunflower plants were investigated. Plants were subject to six levels of soil contamination (from 2.5 to 15 mg Cd kg/soil) with an untreated control, from the emergence of the cotyledon leaves until the harvest, when plants were at the flower bud stage. An overall increase of Cd concentration was found in all tissues of the plants (roots, stem, young, mature and old leaves) by increasing the Cd contamination in the soil. Regardless of treatments, Cd concentration in roots always exceeded those in the aboveground dry matter with a low translocation from roots to shoots. At early stage of growth, Cd concentration in plants was higher than at the flower bud stage. Soil Cd contamination did not affect plant growth, relative water content and gas exchange parameters. Negative and significant correlation was only found between Cd concentration in the young leaves and chlorophyll concentration at the end of vegetative growing stage. Roots and old leaves are the main metal sinks suggesting a defense or tolerance mechanism of the plants to avoid toxic levels in physiologically most active apical tissues. These results should be tested in open field to verify the suitability of sunflower in the area of phytotechnologies.

Keywords: trace element; phytoremediation; *Helianthus annuus* L.; physiological parameters; translocation factor; growth

Cadmium (Cd) is a highly toxic pollutant released into the environment by both anthropogenic and natural resources. Its presence in the soil, including agricultural lands, is considered a serious environmental issue mainly due to its entry in the food human chain with dangerous effects on living organisms. Although not essential for plant growth, Cd is readily taken up by roots and accumulated in plant tissues also to high levels (Prasad 1995). At the root surface, Cd competes with several essential nutrients for the same transmembrane carriers and, once taken up, it can induce a series of toxicity effects. Extensive literature is available about heavy metal accumulator plants, from the wild to the cultivated species,

suitable for phytotechnology procedures. In the last decades, in field, potted and hydroponic trials, the capacity to accumulate and/or stabilize Cd in several crops, including sunflower (Simon 1998, Madejón et al. 2003, Turgut et al. 2005) was evaluated. In sunflower highly contrasting are the results about distribution of Cd in plant tissues and its effect on growth and physiological parameters. Indeed, some authors report that Cd can cause many morphological, physiological and biochemical changes in sunflower plants (Hammami et al. 2004, Azevedo et al. 2005, Turgut et al. 2005) while others highlight no significant Cd toxicity effects (Simon 1998, Rivelli et al. 2012). These contrasting responses could be ascribed to several factors

including cultivar, development stage and level of soil Cd contamination. The aim of this study was to investigate the effects of soil Cd contamination, evaluated as concentration- and time-response on Cd accumulation and distribution in different portions of sunflower plants, growth and physiological responses during the vegetative growing cycle.

MATERIAL AND METHODS

Plant material and experimental layout. The experiments were carried out on sunflower plants (*Helianthus annuus* L., cv. Oleko) grown in plastic pots filled with 10 kg of soil, under natural light in a temperate-controlled glasshouse maintained at 26/18°C (day/night). Plants were subject to six levels of soil contamination corresponding to 2.5, 5, 7.5, 10, 12.5, 15 mg Cd kg/soil (referred to as $Cd_{2.5}$ through Cd_{15}) with an untreated soil as a control (Cd_0). The experimental soil was air-dried and homogenized before use; its characteristics are shown in Table 1. The pots, after filling, were sealed at the base to prevent loss of water and divided into 7 groups to which, except the untreated control, a $CdSO_4$ solution (containing 43.25, 86.50, 129.74, 172.99, 216.24, 259.49 mg of $CdSO_4$, respectively) was applied bringing the soil to the maximum water holding capacity. Seeds were pre-germinated and then planted one per pot. Seventy-six pots were set up in a completely randomized design and each treatment was replicated 4 times; 48 pots, only for 3 treatments (Cd_0, Cd_5, Cd_{10}), were harvested during the growing cycle at 24, 32, 38, 46 days after emergence (DAE); 28 pots, for all 7 treatments, were collected at the end of the vegetative growing cycle, 54 DAE, when plants were at the flower bud stage.

Sampling and measurements. Physiological parameters, total dry matter and Cd concentration in the tissues samples were determined for 4 plants of each treatment at 54 DAE; Cd concentration of the tissues were also analysed during plant growth at 24, 32, 38, 46 DAE for the only treatments Cd_0, Cd_5 and Cd_{10}. Gas exchange parameters (net CO_2 assimilation rate – A; stomatal conductance – g_s; transpiration – T; sub-stomatal CO_2 concentration – C_i) were recorded on the youngest fully expanded leaves exposed to high light intensity (PAR > 1200 µmol/m^2/s) by using LI-6400 portable gas exchange systems (Li-Cor, Lincoln, USA). During measurements, leaf temperature was maintained

at 22 ± 1°C and CO_2 was set at 400 µmol/mol. Chlorophyll concentration (Chl) was determined according to Porra et al. (1989). Total leaf water potential (Ψ) and osmotic potential (Ψπ) were measured using a Peltier cooled thermocouple psychrometer (Tru Psi SC10X, Decagon Devices, Pullman, USA). Leaf discs were removed and sealed in the psychrometer chambers in less than 15 s. Samples were allowed to equilibrate for 2 h before Ψ readings were made. Tissue Ψπ was measured at the same samples after freezing the tissue in liquid nitrogen and re-equilibrating the psychrometer at 20°C for 4 h. Relative water content (RWC) was determined using 1 cm^2 segments of leaf tissue, which were weighed to record fresh weight (FW), floated in distilled water for 5 h to determine turgid weight (TW) then oven-dried at 70°C for 48 h to measure dry weight (DW). RWC was calculated as (FW – DW)/(TW – DW). After physiological measurements, plants were harvested and partitioned into stem, leaves (divided in young, mature and old) and roots. Roots were washed, sonicated

Table 1. Experimental soil properties

Characteristics	Units	Value
Sand	g/kg	361
Silt	g/kg	287
Clay	g/kg	353
CEC	mmol$_+$/kg	360
pH ($CaCl_2$)		7.1
EC	mS/cm	0.14
C_{org}	g/kg	11.6
N_{tot}	g/kg	1.2
Exchangeable cations		
K	mg/kg	281
Ca	mg/kg	6670
Mg	mg/kg	219
Extractable cations		
Zn	mg/kg	0.9
Fe	mg/kg	26.2
Cu	mg/kg	3.7
Cd concentration		
Total	mg/kg	0.270
Exchangeable	mg/kg	0.006

in 0.05 mol/L $CaCl_2$ for 10 min in an ultrasonic bath (Transsonic T 460/H, Elma, Germany) and rinsed with deionised water. All samples were oven-dried (70°C for 48 h), weighed to determine the dry matter (DM) and ground in a stainless box mill. Subsamples of 0.5 g were digested for 32 min in a microwave digestion unit (Milestone 1200 MEGA, Bergamo, Italy) by using 5 mL HNO_3 and 1 mL H_2O_2; after that 50 mL of distilled water was added to the sample volume. The resulting solutions were analyzed for Cd concentration by using the ICP/OES spectrometer (Thermo Scientific iCAP 6000 Series Cambridge, UK). Certified reference material was digested and analyzed together with the sample for quality assurance. Total leaf Cd concentration was determined dividing the total leaf Cd content by the total leaf DM. The translocation factor (TF) was calculated as Cd concentration in the epigeous dry matter divided by Cd concentration in the roots.

Statistical analysis was performed by R software (version 2.10.1 Vienna, Austria). All variables were tested with one way ANOVA followed by the Duncan's test.

RESULTS AND DISCUSSION

Cadmium accumulation and partitioning in plant. At 54 DAE an overall increase of Cd in plant tissues was observed with increasing of Cd in soil. In particular, Cd increased of about 10-fold in leaves (from 0.06 of $Cd_{2.5}$ to 1 mg Cd/kg DM of Cd_{15} treatment) (Figure 1A) and of about 3-fold in roots (from 3.6 of $Cd_{2.5}$ to 10 mg Cd/kg DM of Cd_{15} treatment) (Figure 1B). Instead, in stem and flower bud Cd highly increased only from $Cd_{2.5}$ (0.06 mg Cd/kg DM, on average) to Cd_5 treatment (0.6 mg Cd/kg DM, on average), afterwards it remained almost constant in flower bud and declined in stem to 0.35 mg Cd/kg DM in Cd_{15} treatment (Figures 1A,C). Regardless of treatments, Cd was accumulated more in the roots than in the epigeous portions, in which the highest accumulation was found in the leaves, mainly in the old ones (Figure 2). Many species, including sunflower, accumulate toxic metals mainly in the roots (Groppa et al. 2008, Vamerali et al. 2012); according to Wu (1990) about 70–85% of the absorbed Cd by various plants remains in the
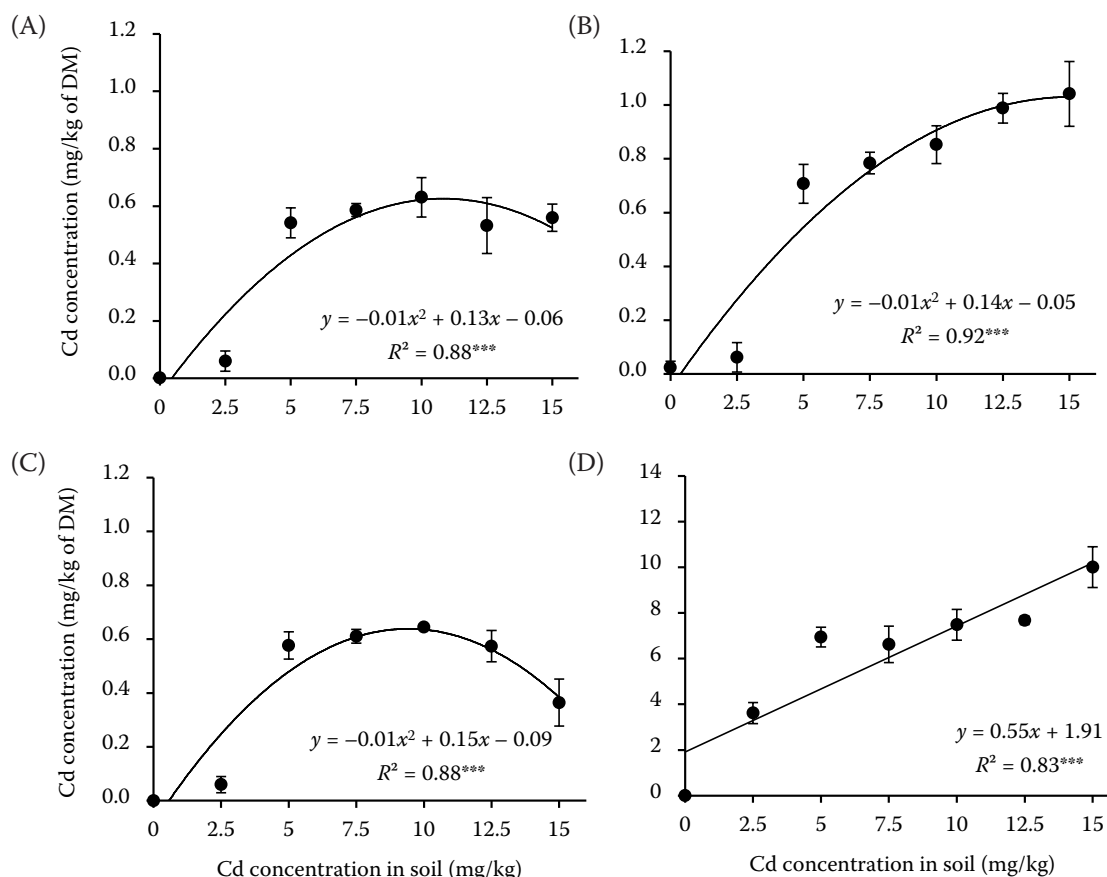

Figure 1. Relationships between Cd concentration in the soil and Cd concentration in sunflower flower bud (A); leaves (B); stem (C), and roots (D). Values are means (*n* = 4) ± SE; DM – dry weight
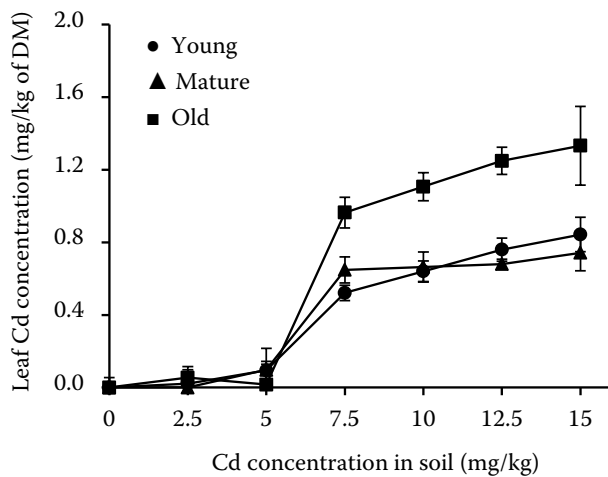
Figure 2. Cd concentration in young, mature and old sunflower leaves grown at different Cd concentration levels. Values are means (*n* = 4) ± SE; DM – dry weight

roots. However, the activities of metal seque-stering pathways in root cells and the transport efficiency through the xylem seem to play a key role in determining the rate of translocation to

the different aerial parts (Clemens 2006). The high Cd concentration, found mainly in roots and old leaves, suggested that sunflower tend to avoid toxicity in the physiologically most active portions of the plants by reducing Cd translocation to the epigeous portion, and by promoting the re-translocation of toxic metals from shoots to the roots. This mechanism of intra-plant allocation was described also in other species subject to Zn stress (Di Baccio et al. 2009), and it is similar to those found for sunflower as effect of Cl stress in salinity conditions (Rivelli et al. 2010).

Considering the vegetative growing phase, Cd concentration declined with time in all portions of the plant (leaves, stem and roots) (Figure 3). In particular, in both leaves and stem of the treated plants, Cd was reduced over time of about 70% passing from the early stage (24 DAE) to the end of the vegetative growing phase (54 DAE); whereas in roots it declined by about 57%. Reduction in Cd concentration over time in sunflower plants was also observed by Madejón et al. (2003). As a result of the highest Cd concentration in
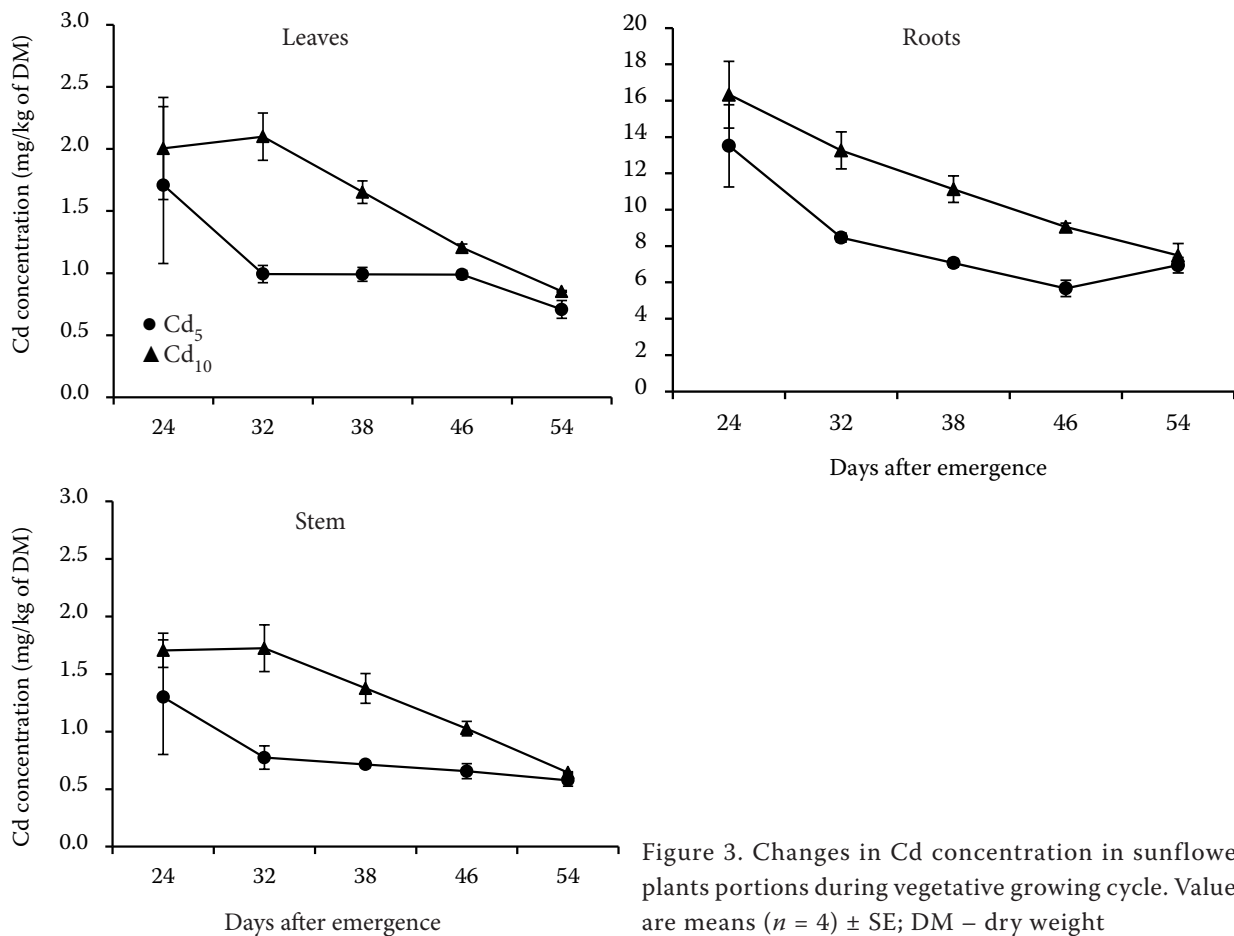

Figure 3. Changes in Cd concentration in sunflower plants portions during vegetative growing cycle. Values are means (*n* = 4) ± SE; DM – dry weight
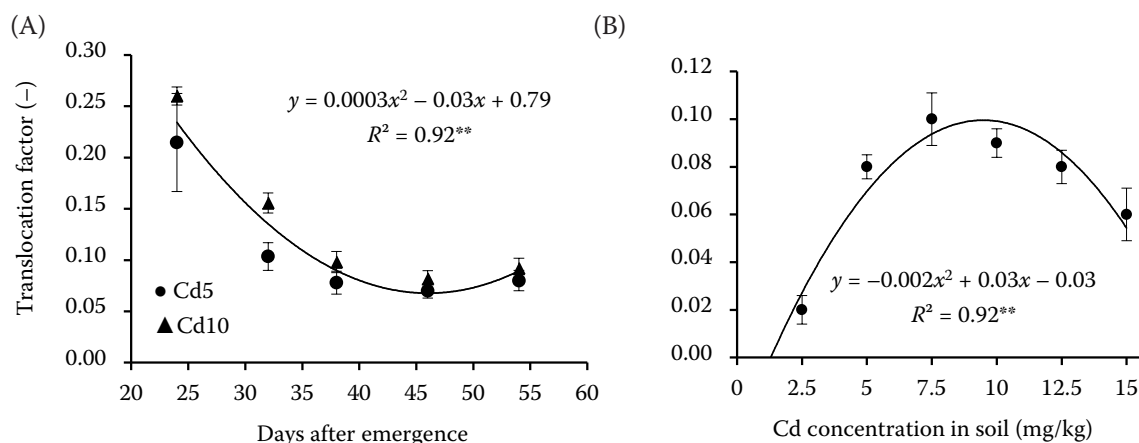
Figure 4. Cd translocation factor over time (A) and in relation to Cd concentrations in the soil at the end of the vegetative growing phase (B). Values are means (n = 4) ± SE

the roots, the TF was very low and less than 1, and it further showed a significant (r^2 = 0.92) decreasing during the time (Figure 4A). Probably, at the early stage of growth, the most likely first response of the plant is the metal accumulation (with consequent production of complexes, i.e. phytochelatins, that immobilize Cd in the cells), whereas later the plants reduce and/or block the uptake and the translocation of the toxic metal. Nevertheless, the TF significantly increased by increasing the levels of Cd in the soil (Figure 4B), and declining only in the most contaminated treatment, probably as an effect of Cd toxicity.

Plant growth and physiological response. The Cd levels applied and its accumulation in plants produced only few significant negative effects on physiological response of the plants. Among water

relation parameters, only leaf $\Psi\pi$ significantly decreased from –1.17 MPa in $Cd_{2.5}$ to –1.34 MPa in Cd_{15} by increasing the levels of Cd in the soil, whereas RWC (Table 2) and Ψ (data not shown) were not significantly affected. Similar results were found also for the gas exchange parameters (A (Table 2), g_s, T, C_i (data not shown)). There are no univocal reports on the relationships between Cd stress and water relations since Cd can interfere in several ways on the parameters that affect leaf water potential (Poschenrieder and Barceló 2004). According to Barcelò et al. (1986), Cd can alter the water relations by disturbing water balance throughout the effects on stomatal conductance, water transport and cell wall elasticity. In previous studies on sunflower (Rivelli et al. 2012) we found that Cd can compete with several essential

Table 2. Relative water content (RWC); leaf osmotic potential ($\Psi\pi$); net CO_2 assimilation rate (A), and total chlorophyll concentration (Chl) at 54 days after emergence in sunflower leaves

Treatment	RWC (%)	$\Psi\pi$ (MPa)	A (μmol CO_2 /m^2/s)	Chl (mg/m^2)
Cd_0	78.8 ± 0.9	–1.08 ± 0.01[c]	32.4 ± 0.9	315 ± 22[a]
$Cd_{2.5}$	82.4 ± 2.1	–1.17 ± 0.01[bc]	32.5 ± 0.9	265 ± 9[b]
Cd_5	79.2 ± 1.6	–1.20 ± 0.04[b]	31.2 ± 0.8	280 ± 23[ab]
$Cd_{7.5}$	81.2 ± 1.1	–1.24 ± 0.03[ab]	28.6 ± 0.4	252 ± 11[b]
Cd_{10}	81.1 ± 0.7	–1.24 ± 0.04[ab]	31.0 ± 1.3	256 ± 9[b]
$Cd_{12.5}$	82.5 ± 0.6	–1.33 ± 0.05[a]	31.4 ± 0.8	246 ± 27[b]
Cd_{15}	77.7 ± 1.6	–1.34 ± 0.04[a]	31.9 ± 1.3	230 ± 12[b]
F probability	ns	$P \le 0.01$	ns	$P \le 0.01$

Values are means (n = 4) ± SE, means followed by the same letters are not significantly different for $P \le 0.05$ according to the Duncan's test; ns – not significant. Cd_0 – 0 mg Cd kg/soil; $Cd_{2.5}$ – 2.5 mg Cd kg/soil; Cd_5 – 5 mg Cd kg/soil; $Cd_{7.5}$ – 7.5 mg Cd kg/soil; Cd_{10} – 10 mg Cd kg/soil; $Cd_{12.5}$ – 12.5 mg Cd kg/soil; Cd_{15} – 15 mg Cd kg/soil
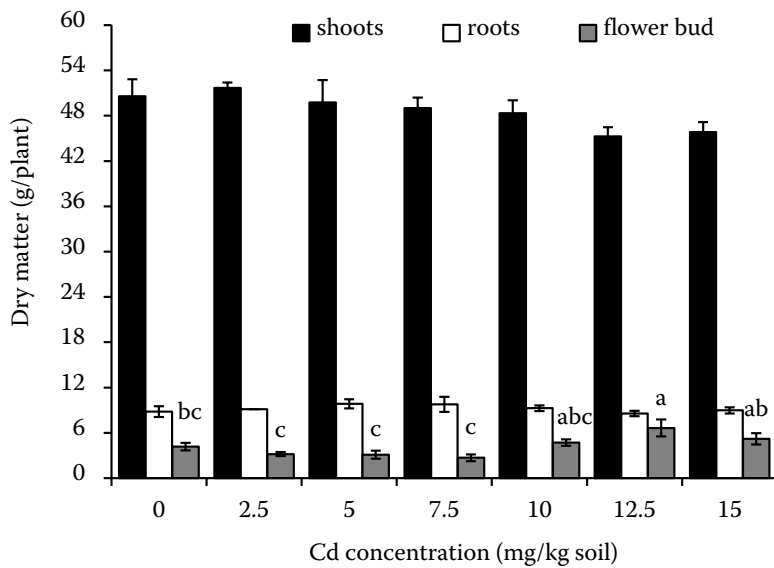
Figure 5. Effect of different levels of Cd concentration on sunflower plant dry matter at the end of the vegetative growing phase. Value are means ($n = 4$) ± SE, bars followed by the same letters are not significant different for $P \leq 0.05$ according to the Duncan's test

nutrients (e.g. Ca, K) altering their concentration in tissues. The effect on osmotic potential could be ascribed to dysfunctions of the membrane in-tegrity caused by displacement of Ca from the cell surface by Cd or, as suggested by Poschenrieder and Barceló (2004) by the increase of solutes in
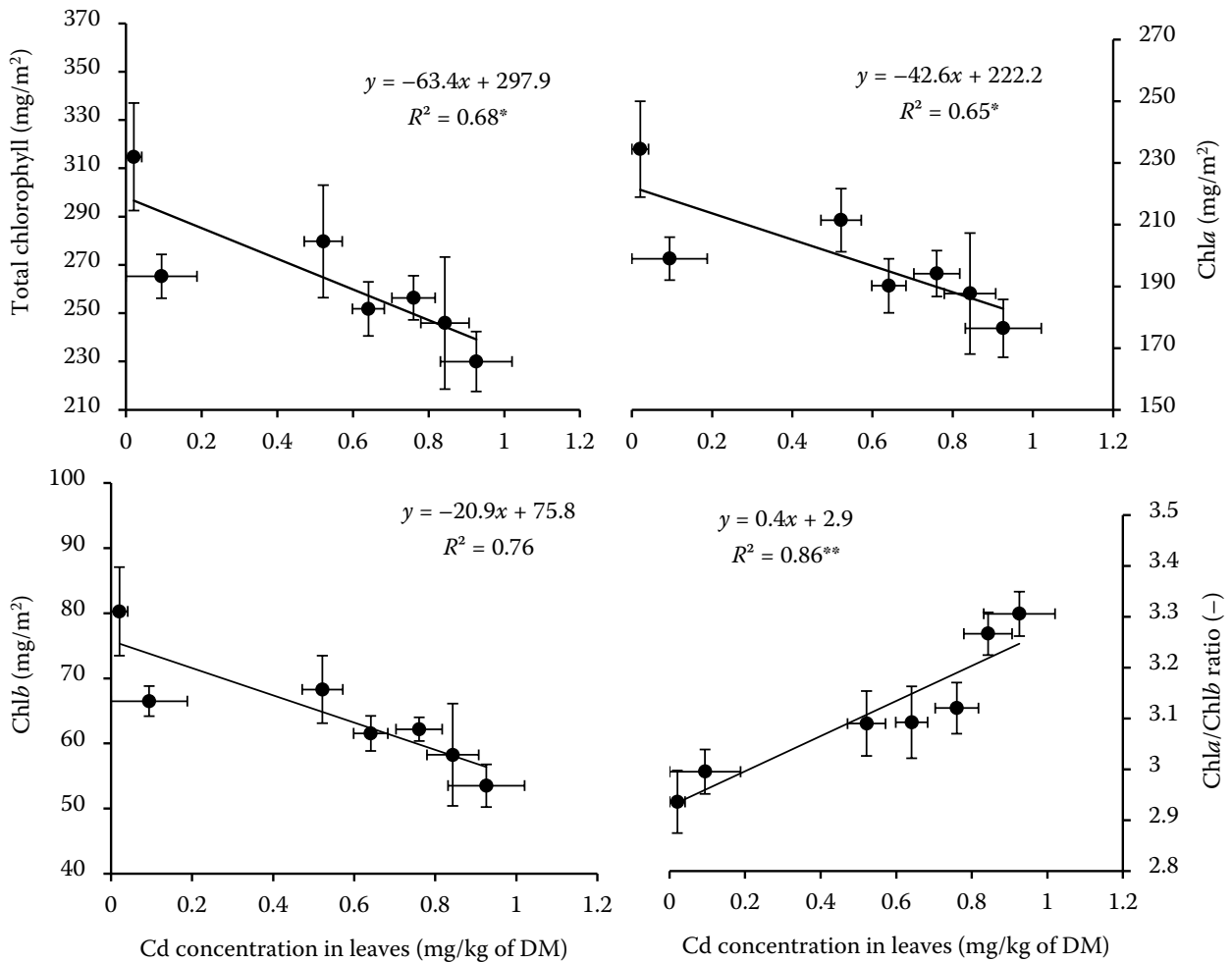

Figure 6. Relationships between total chlorophyll, Chl*a* and Chl*b* concentrations, Chl*a*/Chl*b* ratio and Cd concentration in the young sunflower leaves at the end of the vegetative growing phase. Values are means ($n = 4$) ± SE; DM – dry matter

cells, probably in the vacuoles, that store Cd-complexes. Conversely to gas exchanges, total Chl (Table 2) and Chl*b* concentrations (data not shown) were significantly affected by Cd levels in the soil. Furthermore, significant negative relationships were found between Cd concentrations in the young leaves and Chl, Chl*a* and Chl*b* concentrations (Figure 6). Reduction in Chl is not always associated with chloroplast photo-functioning and consequently with light absorption, thus, the fraction of incident light absorbed can remain high even at very low leaf Chl concentration, preserving the overall photosynthetic activity (Baryla et al. 2001). Anyway, loss of chlorophyll could precede the inhibition of photosynthesis (Baszynski et al. 1980), and can be due to Chl degradation and/or disorders in its biosynthesis, reduction of thylakoid membrane integrity (Santos et al. 2001) and/or substitution of the central Mg ion (Küpper et al. 1998). Focusing on the Chl*a*/Chl*b* ratio (Figure 6), results showed that it linearly and significantly increased by increasing leaf Cd concentration due to the higher reduction of Chl*b* than Chl*a* in Cd-treated plants. Differences in the percentage of reduction in Chl*a* and Chl*b* could be due to preferential degradation of Chl*b* and its conversion in Chl*a*, as suggested by Fang et al. (1998). The weak effects on physiological parameters were reflected on plant growth response and dry matter production; indeed, no significant differences were observed in shoot and root DM (Figure 5). Only flower bud DM significantly increased by increasing Cd in the soil (Figure 5) particularly in the most contaminated treatments. Such response could be associated to an early flowering induction observed at the end of the experiment, as a probable effect of Cd stress. Arteca and Arteca (2007) reported that the exposure to Cd stress induces the ethylene production, a hormone which regulates growth and several physiological processes including flowering induction.

In conclusion, our results indicate that sunflower accumulates increasing amounts of Cd in the tissues at increasing Cd contamination in soil, without negative effects on growth and dry matter production. Roots and old leaves are the main metal sinks due to a low translocation from roots to the shoot and a probable re-translocation toward old leaves, suggesting a defense or tolerance mechanism to avoid toxic levels in physiologically most active apical tissues. Sunflower, a fast-growing crop, which produces an appreciable dry matter production while accumulating Cd in the tissues, seems to be interesting in view of the phytoremediation technologies, to gra-dually remediate the soil at low cost, while producing harvestable biomass usable for industrial purposes. However, results should be investigated in open field, by using the standard agronomic practices, in order to test and improve the removal/stabilization of heavy metal from contaminated soils.

REFERENCES

Arteca R.N., Arteca J.M. (2007): Heavy-metal-induced ethylene production in *Arabidopsis thaliana*. Journal of Plant Physiology, *164*: 1480–1488.

Azevedo H., Pinto C.G.G., Fernandes J., Loureiro S., Santos C. (2005): Cadmium effects on sunflower growth and photosynthesis. Journal of Plant Nutrition, *28*: 2211–2220.

Barcelò J., Poschenrieder C., Andreu I., Gunsè B. (1986): Cadmium-induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv. Contender). I. Effects of Cd on water potential, relative water content and cell wall elasticity. Plant Physiology, *125*: 17–25.

Baryla A., Carrier P., Franck F., Coulamb C., Sahut C., Havaux M. (2001): Leaf chlorosis in oilseed rape plant (*Brassica napus*) grown on cadmium-polluted soil: Causes and consequences for photosynthesis and growth. Planta, *212*: 696–709.

Baszynski T., Wajda L., Krol M., Wolinska D., Krupa Z., Tukendorf A. (1980): Photosynthetic activities of cadmium-treated tomato plants. Physiologia Plantarum, *48*: 365–370.

Clemens S. (2006): Evaluation and function of phytochelatin synthases. Journal of Plant Physiology, *163*: 319–332.

Di Baccio D., Tognetti R., Minnocci A., Sebastiani L. (2009): Responses of the *Populus × euramericana* clone I-214 to excess zinc: Carbon assimilation, structural modification, metal distribution and cellular localization. Environmental Experimental Botany, *67*: 153–163.

Fang Z., Bouwkamp J.C., Solomos T. (1998): Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *Phaseolus vulgaris* L. Journal of Experimental Botany, *49*: 503–510.

Groppa M.D., Zawoznik M.S., Tomaro M.S., Benavides M.P. (2008): Inhibition of root growth and polyamine metabolism in sunflower (*Helianthus annuus*) seedling under cadmium and copper stress. Biological Trace Element Research, *126*: 246–256.

Hammami S.S., Chaffai R., Ferjiani E.E. (2004): Effect of cadmium on sunflower growth, leaf pigment and photosynthetic enzymes. Pakistan Journal of Biological Science, *7*: 1419–1426.

Küpper H., Küpper F., Spiller M. (1998): In situ detection of heavy metal substituted chlorophylls in water plants. Photosynthesis Research, *58*: 123–133.

Madejón P., Murillo J.M., Marañón T., Cabrera F., Soriano M.A. (2003): Trace element and nutrient accumulation in sunflower

plants two years after the Aznalcóllar mine spill. Science of the Total Environment, *307*: 239–257.

Porra R.J., Thompson W.A., Kriedemann P.E. (1989): Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: Verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica and Biophysica Acta, *975*: 384–394.

Poschenrieder C., Barceló J. (2004): Water relations in heavy metal stressed plants. In: Prasad M.N.V. (ed.): Heavy Metals Stress in Plants: From Biomolecules to Ecosystems. 2nd Edition. Springer Verlag, Berlin, 249–270.

Prasad M.N.V. (1995): Cadmium toxicity and tolerance in vascular plants. Environmental Experimental Botany, *35*: 525–545.

Rivelli A.R., De Maria S., Pizza S., Gherbin P. (2010): Growth and physiological response of hydroponically-grown sunflower as affected by salinity and magnesium levels. Journal of Plant Nutrition, *33*: 1307–1323.

Rivelli A.R., De Maria S., Puschenreiter M., Gherbin P. (2012): Accumulation of cadmium, zinc and copper by *Helianthus annuus* L.: Impact on plant growth and uptake of nutritional elements. International Journal of Phytoremediation, *14*: 320–334.

Santos V.C.L., Campos A., Azevedo H., Caldeira G. (2001): *In situ* and *in vitro* senescence induced by KCl stress: Nutritional imbalance, lipid peroxidation and antioxidant metabolism. Journal of Experimental Botany, *52*: 351–360.

Simon L. (1998): Cadmium accumulation and distribution in sunflower plant. Journal of Plant Nutrition, *21*: 341–352.

Turgut C., Pepe M.K., Cutright T.J. (2005): The effect of EDTA on *Helianthus annuus* uptake, selectivity, and translocation of heavy metals when grown in Ohio, New Mexico and Colombia soils. Chemosphere, *58*: 1087–1095.

Vamerali T., Marchiol L., Bandiera M., Fellet G., Dickinson N.M., Lucchini P., Mosca G., Zerbi G. (2012): Advances in agronomic management of phytoremediation: Methods and results from a 10-year study of metal polluted soils. Italian Journal of Agronomy, 7:e42: 323–330.

Wu L. (1990): Colonisation and establishment of plants in contaminated sites. In: Shaw A.J. (ed.): Heavy Metal Tolerance in Plants: Evolutionary Aspects. CRC Press, Boca Raton, 269–284.

Corresponding author:

Prof. Anna Rita Rivelli, University of Basilicata, School of Agricultural, Forest, Food and Environmental Sciences, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy
phone: + 39 0971 205 382, fax: + 39 0971 205 378, e-mail: annarita.rivelli@unibas.it