# Assessment of cadmium uptake and nutrient content in sunflower plants grown under Cd stress

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

[1]*School of Agricultural, Forestry, 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**

Sunflower plants were grown on soil contaminated with several levels of Cd (from 0.3 to 15 mg Cd kg/soil). Cd and nutrient (Cu, Zn, Fe, Ca, K, Mg) contents were evaluated in root and aboveground biomass during the vegetative growth period (from the emergence of the cotyledon leaves to the flower bud phase). An overall increase of Cd content was found in the plants during the growing cycle. Significant interactions were found between Cd contamination levels and phenological stages for Cu, Fe, Ca and Mg content in the whole plant. Cd levels involved significant increases of such nutrients only at the flower bud phase. At this stage, significant interactions between Cd levels in soil and plant portions (root and shoot) were found only for micronutrients (Zn, Cu, Fe). The translocation of micronutrients from root to the shoot was negatively affected by Cd, which probably interferes with the mechanisms of the element transport in the plant. Root stored about 70% of the total Cd and showed greater imbalance in nutrient content compared to the aboveground portion. Cadmium affects plant nutritive status in a different way depending on Cd contamination level, nutrient considered, plant portion and phenological stage.

**Keywords**: *Helianthus annuus* L.; metal translocation; nutrient uptake; nutrient imbalances; phytoremediation

In the last years, sunflower (*Helianthus annuus* L.), an increasingly important source of vegetable oil and biomass, usefully employed for chemical, energy and industrial purposes (Riva and Calzoni 2004), gained growing interest for phytoremediation of organic pollutants and heavy metals. It accumulates several metals in plant tissues, although metal uptake and distribution pattern seem to vary greatly with cultivars (Li et al. 1997), phenological stage (Madejón et al. 2003), level of contamination (Simon et al. 1998) and combination of metals (Niu et al. 2012). Cadmium, a non-essential metal, is taken up by membrane transporters of essential elements (Clemens 2006) and can interfere with the uptake, transport and physiological function of several elements (Das et al. 1997). It is usually stored in large amounts in the roots without causing macroscopic stress symptoms in sunflower plants (Simon et al. 1998, Rivelli et al. 2012). It was reported that Cd differently influences nutrient content depending on species, varieties, growth stages and plant portion (Liu et al. 2003, Gonçalves et al. 2009). In sunflower only few studies are available about the effect of toxic metals on nutrient uptake and translocation from root to the aboveground parts, and contradictions exist between the results of the experiments. Simon et al. (1998) showed that nutrient content in plant was unaffected by Cd soil contamination (1 and 10 mg Cd/kg soil) with only exception of Ca, Mn and Fe in head. Instead, Rivelli et al. (2012) found that K, Mg and Fe increased in root whereas Ca decreased in leaves of sunflower as effect of low level of Cd contamination (5 mg Cd/kg soil). Furthermore, toxic metal and nutrient contents in sunflower plants grown on pluri-contaminated soil varied depending on plant age (Madejòn et al. 2003, De Maria and Rivelli 2013). The aim of this study was

to evaluate the effects of a wide range of soil Cd contamination levels (from 0.3 to 15 mg Cd/kg soil) on Cd and nutrient (Cu, Zn, Fe, Ca, Mg and K) content and their interaction in root and aboveground biomass of sunflower. Plant growth and physiological response to Cd stress were reported in a previous paper (De Maria et al. 2013).

## MATERIAL AND METHODS

Sunflower (*Helianthus annuus* L., cv. Oleko) was subjected to 6 levels of Cd 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 plants were grown in controlled glasshouse in plastic pots (each of 20 cm in diameter and 80 cm in height) filled with 10 kg of soil (whose characteristics are shown in Table 1) and divided into 7 groups to which, except the untreated control, $CdSO_4$ solutions were applied bringing the soil to the maximum water holding capacity. Seeds were pregerminated 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 $Cd_0$, $Cd_5$ and $Cd_{10}$ treatments) were harvested during the vegetative growing cycle at the stages V-4, V-8, V-12, V-16 (corresponding respectively to 4, 8, 12, 16 true leaves at least 4 cm in length); 28 pots (for all 7 treatments) were collected at the flower bud stage R-1 (when the terminal bud forms a miniature floral head rather than a cluster of leaves); the phenological stages were classified according to Schneiter and Miller (1981). At each harvesting time, plant biomass was divided in shoot and root. Roots were sonicated in 0.05 mol/L $CaCl_2$ for 10 min in an ultrasonic bath (Elma Transsonic T 460/H, Hameln, Germany) and rinsed with deionised water. All samples were oven-dried (70°C for 48 h), weighted to determine the dry matter (DM) and ground in a stainless box mill.

Cd content in root and shoot was determined at stages V-4, V-8, V-12, and V-16 in selected treatments ($Cd_0$, $Cd_5$ and $Cd_{10}$) and at the flower bud stage R-1 in all 7 treatments. Nutrient content (Cu, Zn, Fe, Ca, K, Mg) was determined at V-8 stage (in $Cd_0$, $Cd_5$, $Cd_{10}$ treatments) and at R-1 stage (in $Cd_0$, $Cd_5$, $Cd_{10}$, $Cd_{15}$ treatments). 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 were added to the sample volume. Then Cd and nutrient concentration of all samples were determined by a spectrometer (ICP-OES, Thermo Scientific iCAP 6000 Series, Cambridge, UK).

Soil samples were processed to determine extractable and total metal fraction. For measurement of extractable metal fraction, 20 g of dry soil were shaken with 1 mol/L of $NH_4NO_3$ solution, according to the DIN V 19730 (1993) procedure. For total metal fraction, 0.5 g of soil were digested with aqua regia and $H_2O_2$ in a microwave digestion unit (Milestone 1200 MEGA) as described by Leita and Petruzzelli (2000). The elemental concentration of soil samples were determined by an ICP-OES spectrometer.

Certified reference material was always digested and analyzed together with the soil and tissue samples for quality assurance.

The element content was determined by multiplying the concentration of each tissue by the respective DM. The translocation factor (TF) was

Table 1. Experimental soil properties

| Characteristics | Unit | 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 |

CEC – cation exchange capacity; EC – electrical conductivity

calculated as ratio between content of elements (Cd, Cu, Zn, Fe) in shoot and root. Values of TF were normalized through *z* scores standardization. The *z* score value is given by:

$$z = (x - \mu)/\sigma$$

Where: $x$ – TF value of each sample; $\mu$ and $\sigma$ are average and standard deviation of TF values for each element, respectively.

Statistical analysis was performed by the R software (version 2.10.1, R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS AND DISCUSSION

During the vegetative growth period, $Cd_5$ and $Cd_{10}$ treatments did not differ from each other regarding the content of Cd in total plant, root and shoot; as a consequence, only three regression lines were displayed in Figure 1. Cd significantly and linearly increased over time in plants passing, on average, from 0.005 mg Cd/plant at V-4 stage to 0.1 mg Cd/plant at R-1 stage. During the vegetative stages V-4 and V-8, root and shoot showed similar Cd amount, after that root retained almost 70% of the total Cd. Similarly, in each stage considered, no differences among treatments were found in dry matter production, that was in shoot and root, respectively, 1.2 and 0.19 g/plant at V-4, 5.1 and 0.95 at V-8, 18.7 and 4.8 at V-12, 34.8 and 8.0 at V-16 and 53.6 and 9.5 at R-1 (on average of the treatments). Therefore, a significant positive relationship was found between the dry matter production and Cd content in plant ($R^2$ = 0.95; $P \leq 0.001$, data not shown). In the preview paper, we reported that, regardless of the Cd levels in the soil, the concentration of Cd in shoot was reduced over time of about 70% passing from the early stage to the end of the vegetative growing phase, whereas in root it declined by about 57% (De Maria et al. 2013). The differences observed between content and concentration in tissues could indicate that although Cd continues to enter into the plant during the vegetative growing cycle, the uptake rate is reduced with time relative to the biomass increase. As recently summarized by Hossain et al. (2012), plants employ various strategies to cope with toxic effects of heavy metals, and the resistance to heavy metal stress can be achieved by reducing the concentration of metals entering the cell by extracellular precipitation, biosorption to cell walls, reduced uptake, or increased efflux. Furthermore, plants have a range of detoxification and tolerance mechanisms that appear to be involved primarily in avoiding toxic concentrations at sensitive sites within the cells, thus preventing damaging effects (Hall and Williams 2003). As a result, metals in excess are often stored in roots, where physiological processes are less affected. Accordingly, De Maria et al. (2013) found that sunflower grown on contaminated soil tends to accumulate Cd mainly in root and old leaves without significant detrimental effects on plant growth and physiological parameters, except for chlorophyll content. It is well established that Cd can interfere with the uptake, transport and physiological function of several elements (Das et al. 1997). In our study the nutrient content in plant increased over time but, unlike expected, it significantly varied among the treatments only at R-1 stage, whereas no differences were observed at V-8 stage, except for Ca content (Figure 2). Significant interactions between phenological stages and soil Cd levels were found for all nutrients, except for Zn and K. Instead, Cu, Fe and Ca seem to be similarly affected by Cd, as their content increased with respect to the uncontaminated control. Recently, Rezvani et al. (2012) reported that Cd supply increased macronutrients and decreased micronutrients concentrations in plant of *Aeluropus littoralis*. Specific mechanisms seem to be involved to maintain homeostasis, i.e.

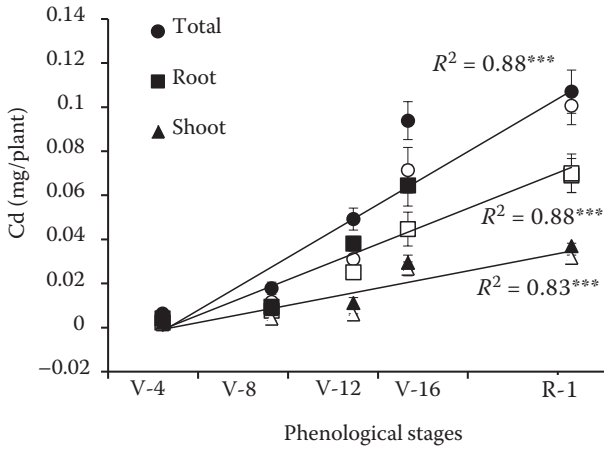


Figure 1. Cd content in total plant, root and shoot of sunflower subjected to two levels of Cd soil contamination ($Cd_5$, white symbols; $Cd_{10}$, black symbols) during the vegetative growing cycle (V-4, V-8, V-12, V-16) and flower bud stage (R-1). Values are means ($n$ = 4) ± S.E.; the asterisks indicate the significance level of *F*-ratio: ***$P \leq 0.001$

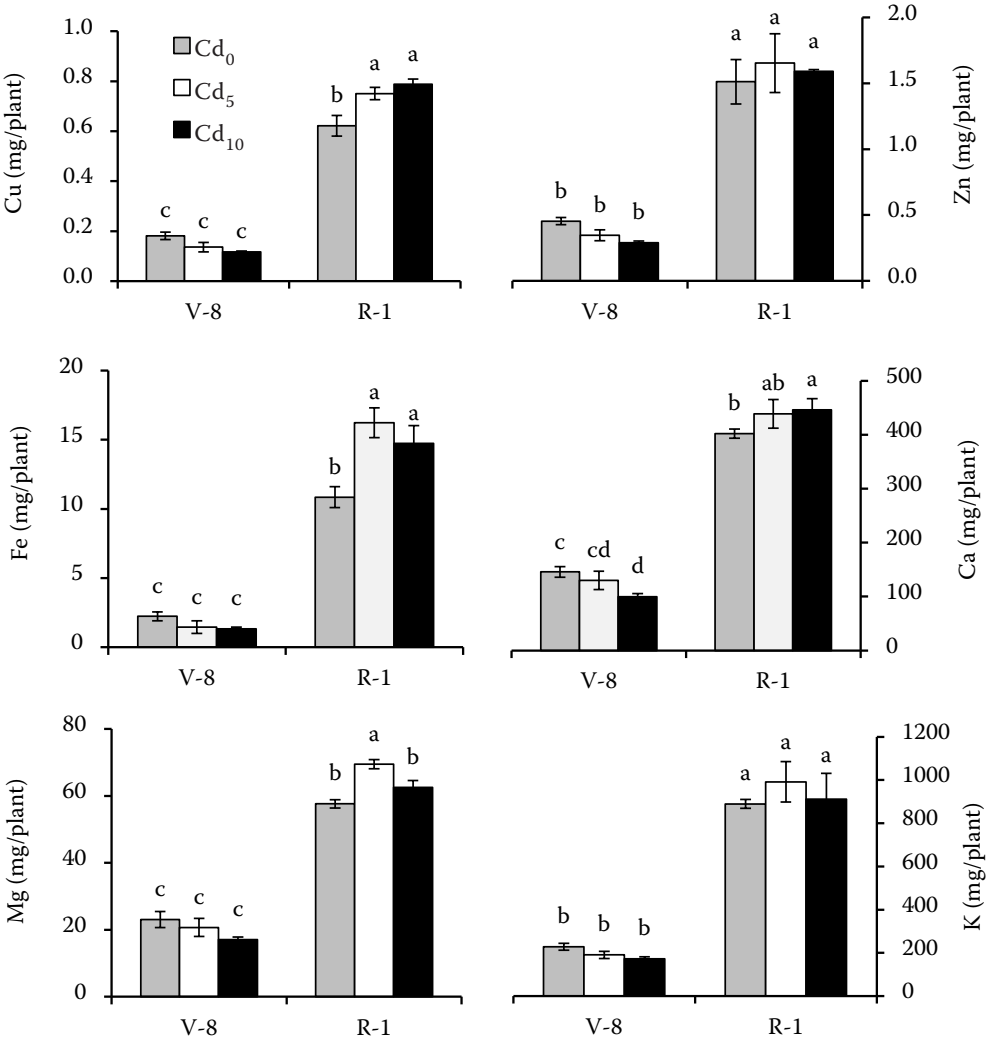


Figure 2. Nutrient content (Cu, Zn, Fe, Ca, Mg, K) in sunflower plants at two phenological stages (V-8 and R-1). Values are means ($n$ = 4) ± S.E.; for each element, bars followed by the same letters are not significantly different for $P \leq 0.05$ according to the Duncan's test

a balance between having enough essential metals available for metabolic functions and at the same time avoiding toxicity and to keep nonessential metals below their toxicity thresholds (Clemens 2006). De Maria and Rivelli (2013) found that passing from flowering to maturity stage of sunflower plants, Cd, Cu and Zn (given at toxic concentrations in soil) increased in the older leaves and stem decreasing in head, suggesting a possible effect of metal retranslocation in plant to preserve reproductive and photosynthesizing tissues. In the same study, it was shown that in sunflower seeds the concentration of toxic metals never exceed the toxicity threshold values considered for livestock food. Clemens (2006) summarized processes involved in the uptake, sequestration and translocation of Cd in plant roots, highlighting that Cd could be taken up into plant cells by Fe and Zn transporters and then stored mainly in the vacuoles through metal binding peptides, i.e. phytochelatins (PCs). It was shown that the levels of PCs in sunflower increase as effect of increasing Cd levels and time of exposure (Yurekli and Kucukbay 2003). PCs are also involved in the homeostasis of Zn and Cu by providing a transient storage form for the ions (Thumann et al. 1991). Considering our results, the variation in nutrient content as effect of Cd could be due to a series of defence mechanisms, e.g. PCs production, expressed by sunflower to avoid toxicity.

At the flower bud stage (R-1), considering all treatments tested, Cd significantly increased in

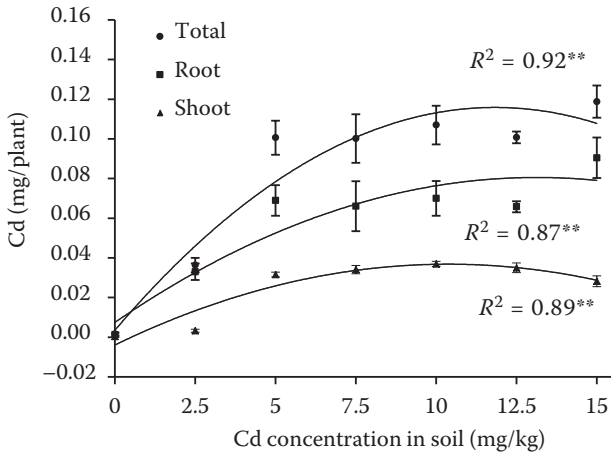


Figure 3. Cd content in total plants, root and shoot of sunflower subjected to several levels of Cd soil contamination at the flower bud stage (R-1). Values are means ($n$ = 4) ± S.E.; the asterisks indicate the significance level of $F$-ratio: $^{**}P \leq 0.01$

plants as effect of increased levels of Cd in soil (Figure 3); in particular, in the whole plant Cd increased from 0.04 mg Cd/plant in $Cd_{2.5}$ to 0.12 mg Cd/plant in $Cd_{15}$. The highest increase in the Cd content was observed passing from $Cd_{2.5}$ to $Cd_5$ treatment and it remained almost constant for higher levels of Cd contamination (from $Cd_5$ to $Cd_{15}$). Considering all treatments, about 70% of total Cd content was found in root, except for $Cd_{2.5}$ treatment wherein Cd was retained almost to 100% in root. Therefore, the translocation of Cd from the root to the shoot occurred when Cd contamination exceeded 2.5 mg/kg of soil. Still at R-1 stage, regardless of the treatments, the highest contents of Ca, K, Mg, Cu and Zn occurred in the shoot whereas, as observed for Cd, the Fe content was higher in the root (Table 2). Almost all analysed nutrients were affected by Cd supply, but significant interactions between Cd contamination and nutrient contents in root and shoot were found only for Cu, Zn and Fe (Table 2). Such results indicate that Cd affected the translocation of Cu, Zn and Fe from the root to the shoot. To facilitate comparisons between Cd and nutrient translocation, the values of translocation factor were normalized as $z$ scores (Figure 4). As expected, Cd value in the $Cd_0$ treatment is below zero (which is the level corresponding to the mean value of translocation of each element). In the same treatment the values of Zn, Cu and Fe are higher compared with values of the contaminated treatments. Interestingly, the values of Cd were always in reverse order with respect to those of the nutrients, indicating that when Cd is translocated to the shoot, the translocation of nutrients is reduced. Differences in absorption and

Table 2. Nutrient content in shoot and root of sunflower plants subjected to several Cd contamination levels

| Portion | Treatment | Nutrient (mg/plant) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cu | Zn | Fe | Ca | Mg | K |
| Shoot | $Cd_0$ | 0.47$^a$ | 1.18$^a$ | 2.67$^c$ | 313.3 | 39.8 | 509.2 |
| | $Cd_5$ | 0.45$^a$ | 0.99$^a$ | 2.47$^c$ | 320.9 | 39.8 | 504.2 |
| | $Cd_{10}$ | 0.50$^a$ | 1.04$^a$ | 2.30$^c$ | 354.8 | 40.3 | 558.4 |
| | $Cd_{15}$ | 0.35$^b$ | 1.03$^a$ | 2.06$^c$ | 285.2 | 37.3 | 460.7 |
| Root | $Cd_0$ | 0.16$^d$ | 0.34$^c$ | 8.17$^b$ | 88.8 | 17.8 | 380.5 |
| | $Cd_5$ | 0.30$^{bc}$ | 0.66$^b$ | 13.76$^a$ | 118.2 | 29.6 | 487.3 |
| | $Cd_{10}$ | 0.29$^{bc}$ | 0.55$^{bc}$ | 12.43$^a$ | 91.7 | 21.7 | 352.8 |
| | $Cd_{15}$ | 0.24$^c$ | 0.38$^c$ | 11.52$^a$ | 86.5 | 20.7 | 407.9 |
| Significance | | | | | | | |
| Portion (P) | | $P \leq 0.01$ | ns | $P \leq 0.05$ | $P \leq 0.05$ | $P \leq 0.05$ | ns |
| Treatment (T) | | $P \leq 0.001$ | $P \leq 0.001$ | $P \leq 0.001$ | $P \leq 0.001$ | $P \leq 0.001$ | $P \leq 0.05$ |
| T × P | | $P \leq 0.05$ | $P \leq 0.05$ | $P \leq 0.05$ | ns | ns | ns |

Values are means ($n$ = 4). Data were analyzed independently by two way ANOVA to evaluate the effects of portion, treatment and the interaction between them on nutrient content. The significance level of $F$-ratio is given for all factors and interactions. For each nutrient values followed by the same letters are not significantly different for $P \leq 0.05$ according to the Duncan's test; ns – not significant

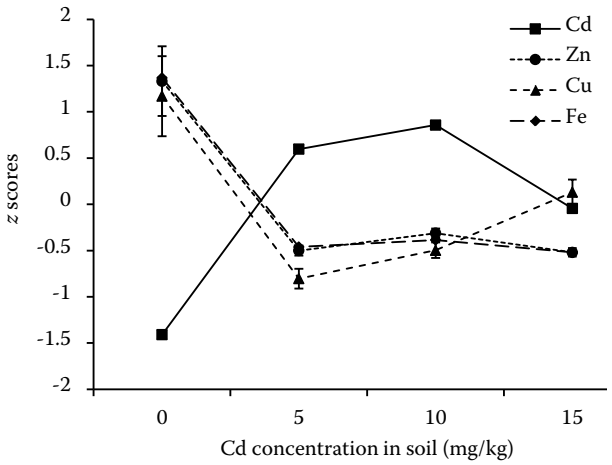


Figure 4. *Z* scores values performed on translocation factor of Cd and micronutrients (Zn, Cu and Fe). Values are means ($n$ = 4) ± S.E.

translocation capacities of nutrients in plants as effect of the interactions among Cd and nutrients were already reported by Herrero et al. (2010). Liu et al. (2003) showed that the effects of Cd on the mineral nutrients in the roots and leaves of rice were mostly significant but varied with metal elements, organs and growing stages. Metal uptake and partitioning to plant organs and cell types, as well as metal storage and re-mobilization all require the operation of transition metal transporters (Krämer et al. 2007). A lack of specificity of uptake and distribution systems also leads to the accumulation of non-essential metals as Cd (Clemens 2006). For example, the nicotianamine (a non-protein amino acid found in all plants), that is linked with Fe, Cu and Zn homeostasis in plant and is involved in maintaining the mobility of such metal ions in vascular tissues and between cells (Krämer et al. 2007), seems to be involved also in Cd chelation, transport and detoxification (Sharma and Dietz 2006). Liu et al. (2003) found that the interactions of Cd and Fe, Zn and Cu are synergetic in uptake and translocation from root to shoot by rice plants. Our results showed that Cu, Zn and Fe contents in sunflower root generally increased by increasing the level of Cd soil contamination but their translocation from root to the shoot was reduced, suggesting that complex interactions can be involved between toxic and nutrient elements with synergistic or antagonistic effects between them, depending on nutrient element and plant portion. Despite symptoms of Cd toxicity were related to interactions between the uptake and translocation of nutrients in plant (Rezvani et al. 2012), we did not find any visible symptom of Cd toxicity probably because the content of nutrients remained in the normal range. Even though the results on metal-induced plant stress were frequently reported, there is still a need for answering further questions on the factors that modulate the signaling pathways in response to Cd stress (Azevedo et al. 2012). Our results, although they are to be tested in open field, provide useful information about the interaction between Cd and essential elements in sunflower plants; as highlighted by Liu et al. (2003) the interactions between Cd and other elements may provide clues to explain the nature of Cd accumulation in crops. In addition, according to Dickinson et al. (2009), it is important to treat skeptically any data obtained from hydroponics, pot experiments, or spiked soils on the grounds that there is a high probability these methods will not accurately reflect concentrations in field-grown plants.

In conclusion, the effect of Cd contamination on nutrient content in sunflower plants varied depending on the Cd contamination level, nutrient considered, plant portion and phenological stage. Based on the responses to Cd contamination in controlled condition, sunflower appears promising for phytoremediation. Considering that the time of remediation is often a limiting factor in phytoremediation technologies, sunflower biomass can be used to generate an alternative income for the farmer while remediating at the same time the soil, thus rendering the time constraint less important.

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*Corresponding author:*

Susanna De Maria, Ph.D., University of Basilicata, School of Agricultural, Forestry, Food and Environmental Sciences, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy
phone: + 39 0971 205 382, fax: + 39 0971 205 378, e-mail: demariasusanna@libero.it