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GROWTH AND PHYTOREMEDIATION CAPACITY OF SUNFLOWER GROWN ON SOIL CONTAMINATED WITH CADMIUM

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ABSTRACT

The purpose of this study was to characterize sunflower (*Helianthus annuus* L. cv. Oleko) regarding its potential to be included in the phytoremediation programs for the contaminated areas of national interest of southern Italy. Therefore, Cd uptake, distribution and dose-response patterns were studied in plants subject to six levels of Cd contamination (from 2.5 up to 15 mg kg^{-1} of soil). No large differences were found on growth and physiological parameters as effect of Cd contamination. Regardless of treatments, Cd accumulated mainly in roots than in shoots, even at low concentration in soil. Moreover, Cd accumulation in roots and leaves progressively increased by increasing Cd in soil, while in stem and flower bud it remained almost constant above of 5 mg kg^{-1} of Cd in soil. The storage of heavy metal in roots and older leaves could be considered as a strategy of sunflower to avoid toxic accumulation in growing tissues.

1. INTRODUCTION

Plant uptake of heavy metals is dependent on several mechanisms that are both specie and metal specific. The accumulation/mobility of cadmium seems also to depend on plant growth stage, concentration of nutrients and/or metals combination [1]. The amount of Cd accumulated and translocated in plants varies with species and with cultivars within species [2] and can cause a series of physiological disorders depending on its concentration in the medium of growth and on plants time exposure. Cd is a highly toxic environmental pollutant that has been studied in numerous researches on phytoremediation technologies. Numerous are the plant species suitable for phytoremediation: from the wild hyperaccumulator to the cultivated species eligible for such purpose.
Currently numerous studies are oriented to combining phytoremediation with crops of commercial interest, with the aim of achieving soil decontamination at low cost by producing commercial resources usable for technical purposes (such as timber, bioenergy) and generating value added. Such approach has been recently known as phytomanagement [3]. In this contest, sunflower (*Helianthus annuus* L.) is an increasingly important source of vegetable oil and biomass, usefully employed for chemical, energetic and industrial purposes, and its importance as environmental crop for phytotechnologies to clean-up inorganic and organic contaminants is being increasingly recognized [4].
The aim of this experiment was to characterize sunflower (*Helianthus annuus* L. cv. Oleko) regarding its potential to be considered in the phytoremediation programs for the contaminated areas of national interest of southern Italy. For such purpose a dose-response relationship between Cd contamination from 2.5 up to 15 mg of Cd for kg of soil and Cd concentration in sunflower plants was study by evaluating in the same time the effects of Cd on growth and physiological parameters.

2. METHODS AND MATERIALS

2.1 Plant material and Experimental layout

The experiment was carried out in 2009 at the University of Basilicata, Italy (40°36'N, 15°48'E) in a naturally-lit and temperate-controlled glasshouse maintained at 26 °C during the day and 18 °C at night. Sunflower (*Helianthus annuus* L., cv. Oleko) was subject to six contamination levels of cadmium (referred to as $Cd_{2.5}$ through Cd_{15} and corresponding to: 2.5, 5, 7.5, 10, 12.5, 15 mg of Cd for kg of soil) and an untreated treatment as a control (C).
The soil sample is clay loam and it was collected from the top 20 cm layer of an area near the University, air-dried and homogenized before use. Then 35 plastic pots (20 Ø x 80 cm each) were filled with 10 kg of soil, sealed at the base to prevent loss of water and randomly divided into seven groups to each of which, except for the untreated control, an aqueous solution containing respectively: 43.3, 86.5, 129.7, 172.9, 216.2, 259.5 mg of $CdSO_4$ was applied to provide different degree of Cd stress.
Concurrently, seeds were preliminarily selected by weight (150-200 mg), surface sterilized with 1% sodium hypochlorite, pre-germinated in Petri dishes for 3 days and then planted one per each pot. During the growing cycle, seedlings were watered every 2-3 days to maintain maximum water holding capacity till the end of the experiment, 54

days after emergence (DAE) of cotyledon leaves, when plants were at the flower bud stage. At this stage physiological measurements, total dry matter, green leaf area and Cd concentration (in soil and tissues samples) were taken from five plants of each treatment.
Gas exchange parameters (net CO_2 assimilation rate, *A*; stomatal conductance, *gs*; transpiration, *T*; sub-stomatal CO_2 concentration, *Ci*) were recorded always on the youngest top leaf, fully expanded and exposed to high light intensity by using LI-6400 portable gas exchange systems (Li-Cor, Lincoln, NE, USA). During measurements, leaf temperature was maintained at 22 ± 1°C and CO_2 was set at 400 µmol mol^{-1}. Chlorophyll concentration was determined by extraction with 80% acetone as described by Porra *et al*. [5]. Total leaf water potential (Ψ) was measured using a Peltier cooled thermocouple psychrometer (Tru Psi SC10X, Decagon Devices, Pullman, WA, USA). RWC was calculated as (FW-DW)/ (TW-DW).

2.2 Laboratory Analysis

After physiological measurements, plants were harvested and partitioned into stem and leaves (divided in young, mature and old), and washed with tap water. Roots were quickly separated from the adhering soil by washing, then were sonicated for 10 min in 0.05 M $CaCl_2$ and rinsed with deionized water to remove extra metals from the apparent free space of the root tissues. All samples were dried at 70 °C for 48 hours, weighted to determine the dry matter weight (DM) and grounded in a stainless box mill. Subsamples of 0.5 g were digested for 30 minutes using a mixture of HNO_3/ H_2O_2 (5:1) in a high performance microwave digestion unit. The resulting solutions were analyzed for Cd concentration by using an ICP-OES. Certified reference material was always digested and analyzed together with the sample for quality assurance.
The translocation factor (TF) was calculated as element concentration in the shoots (stem + leaves) divided by the concentration in roots. Statistical analysis was performed by R software (version 2.10.1). All variables were tested with one way Analysis of Variance (ANOVA) followed by Duncan's test.

3. RESULTS AND DISCUSSION

The investigated levels of soil Cd contamination did not significantly affected neither dry matter of the individual components of the plants (roots and shoots) nor leaf area (Table 1) at the end of the vegetative growth stage (54 DAE), when plants were at the flower bud stage, although a small decrease of total dry biomass was observed by increasing the level of Cd in soil.

TABLE 1. Effect of several levels of Cd contamination on growth (roots and shoot dry matter weight and leaf area) and physiological parameters of sunflower at the end of the vegetative growing period (54 DAE).

Treatments	Roots DM	Shoot DM	Leaf area	A	Chl tot	RWC	Ψ
	g $plant^{-1}$		cm^2	µmol CO_2 m^{-2} s^{-1}	mg m^{-2}	%	MPa
C	8.8 ± 0.7	50.6 ± 2.2	2416 ± 55	32.4 ± 0.9	297 ± 28	80 ± 0.9	-1.09 ± 0.07
$Cd_{2.5}$	9.1 ± 0.0	52.5 ± 1.1	2359 ± 99	32.5 ± 0.9	242 ± 14	82 ± 2.1	-1.00 ± 0.05
Cd_{5}	9.9 ± 0.6	49.8 ± 2.9	2371 ± 96	31.2 ± 0.8	279 ± 23	79 ± 1.6	-1.10 ± 0.05
$Cd_{7.5}$	9.8 ± 1.0	49.0 ± 1.4	2268 ± 73	28.6 ± 0.4	251± 11	81 ± 1.1	-1.05 ± 0.03
Cd_{10}	9.3 ± 0.4	48.3 ± 1.7	2289 ± 56	31.0 ± 1.3	256 ± 10	81 ± 0.7	-1.14 ± 0.02
$Cd_{12.5}$	8.6 ± 0.4	45.3 ± 1.2	2289 ± 69	31.4 ± 0.8	245 ± 20	82 ± 0.6	-1.12 ± 0.02
Cd_{15}	8.9 ± 0.4	45.8 ± 1.3	2393 ± 85	31.9 ± 1.3	229 ± 12	78 ± 1.6	-1.19 ± 0.04
F probability	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*P≤0.05*	*n.s.*	*n.s.*

In plants of the most contaminated treatments, from 10 up to 15 mg of Cd kg^{-1} of soil, an early flowering induction was observed and flower bud were significantly larger than those of the less contaminated treatments (2.5 – 7.5 mg of Cd kg^{-1} of soil) (Figure 1). According to many authors, heavy metals, including Cd stress, stimulate ethylene production [6], which regulates several physiological and growth processes in plants, particularly senescence, plant growth and development [7], including flowering induction.
Nevertheless contrasting are the results reported in literature on the impact of Cd on biomass production: some authors reported a biomass reduction in numerous species, including sunflower [8] [9] [10], whereas no toxicity effect on dry matter production were found in several others studies [1] [3]. According to Madejón *et al.* [11] soil pollution significantly retarded early growth of sunflower without have significant effect on biomass at the final harvest.
Regarding the effect of Cd on physiological parameters no large differences were found on gas exchange parameters and water relations (Table 1), despite considerable amounts of Cd accumulated in different tissues of the plants (Figure 2 and 3).

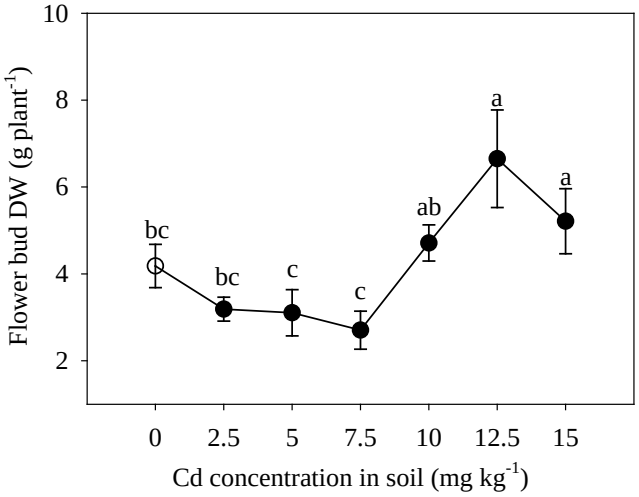

Figure 1. Effect of soil Cd contamination on flower bud dry matter at the end of vegetative growth stage (54 DAE). Values are means (n=5) ± s.e. Means with the same letter are not significantly different for $P \leq 0.05$ according to Duncan's *test*.

In particular, Table 1 shows the values of net CO_2 assimilation rate, relative water content (RWC), total leaf water potential (Ψ), which were not statistically different within the range of contamination tested, expect for the total chlorophyll content which significantly ($p \leq 0.05$) decreased by increasing the level of Cd in soil.

As widely reported in literature, Cd effects on plant species vary greatly depending on its concentration in the medium of growth and on plants time exposure [12] [13] [14]. Furthermore Wojcik and Tukiendorf [15] suggested that only the heavy metals which interfere with cellular metabolism have negative physiological effects. In our case the accumulation of Cd in sunflower tissues probably did not reach phytotoxic levels, maybe due to the low translocation of Cd from roots to the shoots. Considering the whole plant (roots, stem and leaves), Cd concentration in tissues increased by increasing the level of contamination in soil, passing from 0.6 mg kg^{-1} of DW in $Cd_{2.5}$ treatment up to 2.0 mg kg^{-1} of DW in Cd_{15} treatment (Figure 2).

Referring to the fractions of the plant, Cd concentrations in roots gradually increased passing from the less contaminated treatment $Cd_{2.5}$ to the most contaminated treatment Cd_{15} (passing from 3.8 up to 10 mg kg^{-1} of DM) while in shoots they increased from $Cd_{2.5}$ treatment (0.15 mg kg^{-1} of DM) to Cd_5 treatment (0.6 mg kg^{-1} of DM), after which values remained almost constant (Figure 3). Regardless of the treatments, Cd concentrations in roots always exceeded those in shoots, with a low translocation factor from roots to shoots (0.07 on average) (Figure 3).

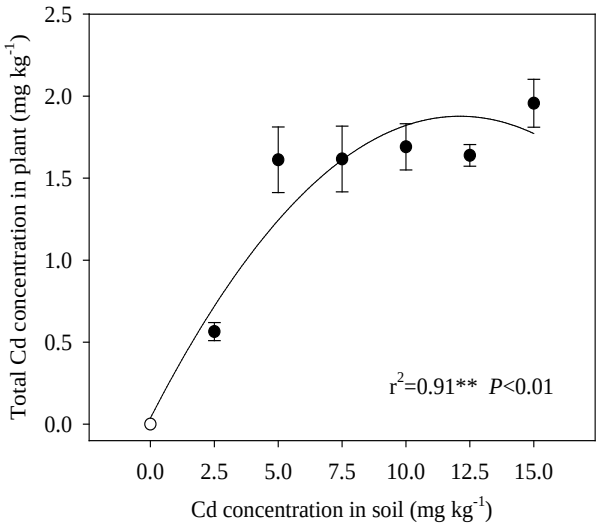

Figure 2. Dose-response relationship between Cd concentration in soil and total Cd concentration in plants. Values are means (n=5) ± s.e.

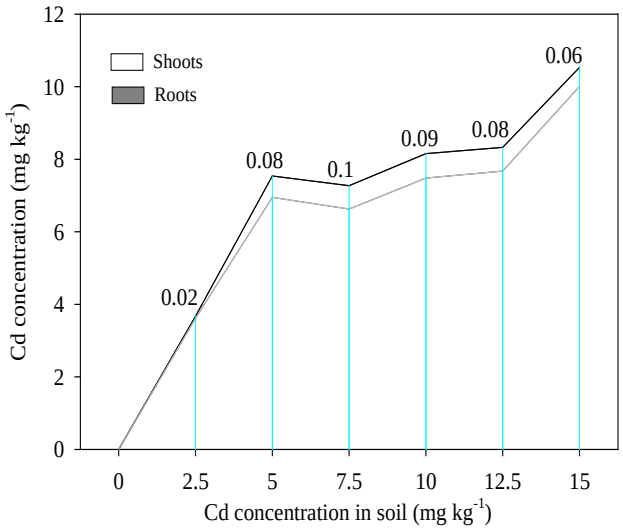

Figure 3. Cd concentration in roots and shoots of sunflower. Numbers above lines represent the Cd translocation factor for the different levels of contamination.

In addition, referring only to the leaves (data not shown) greater accumulation of Cd in almost all treatments was found in the older leaves with respect to the younger and mature ones.

The high storage of Cd in roots and older leaves could be considered as a strategy of sunflower to compartmentalise the potentially toxic element in the physiologically less active parts in order to preserve younger and metabolically more active apical tissues, as found by several authors in numerous species.
On the whole, such behaviour suggests that sunflower is tolerant to cadmium stress and differences in growth and metal uptake may depend upon crop species/genotypes, type and degree of contamination, soil characteristics and quality, and certainly a number of environmental factors.
The appreciable dry matter production in cadmium contaminated conditions and the accumulation of cadmium in tissues, particularly in roots, make this crop interesting for phytoremediation programs (including techniques as rhizofiltration or phytostabilization) for the contaminated areas of national interest in southern Italy.
Future experiments should be addressed in field conditions, and techniques to facilitate activity in pluri-contaminated environments should be developed. In addition, we should focus on accumulator species (e.g. fiber and energy crops) inserted into a crop rotation in order to effectively enhance the removal of heavy metals.

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