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The effects of cadmium and zinc interactions on the concentration of cadmium and zinc in pot marigold (*Calendula officinalis* L.)

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Abstract

Experiments were conducted on pot marigold (*Calendula officinalis L.)* plants grown under glasshouse conditions to study interactions between Cd and Zn and the effects of these on the respective concentrations of these metals in plant tissues (leaves and petals). A factorial experiment with two factors (Cd and Zn) at five concentrations (0, 1, 5, 10, 15 mg kg^{-1}) was carried out. Cadmium was applied as $CdSO_4{\cdot}8/3H_2O$ and Zn as $ZnSO_4{\cdot}7H_2O$. Increasing Cd and Zn additions to the soil resulted in an increase in the concentration of Cd and Zn in plant tissues, as well as in the amounts of Cd and Zn extracted by diethylene triamine penta-acetic acid–triethanol amine (DTPA–TEA). Significant inhibitory effects of Zn on Cd concentration in the leaves occurred at levels above 5 mg Zn kg^{-1} soil. Zinc concentrations in the leaves decreased, while those in the petals increased with increasing rates of applied Cd. Cd application increased the Cd/Zn ratio and Zn application reduced the Cd/Zn ratio of plant tissue. DTPA–TEA-extractable Cd and Zn significantly correlated with the Cd and Zn concentrations within the leaves and petals, indicating that such determinations could be used to predict Cd and Zn concentrations in the plant tissues of pot marigold.

Keywords: Cadmium; Cd–Zn interactions; DTPA–TEA; *Calendula officinalis* (L); Pot marigold; Zinc.
Abbreviations: AAS, Atomic absorption spectrophotometry; DTPA–TEA, diethylene triamine penta-acetic acid–triethanol amine.

Introduction

Cadmium (Cd) is a widespread pollutant and one of the most toxic heavy metals in the environment due to its high mobility and toxicity at low concentration (Adriano 1986; Farmer and Farmer 2000; Wagner 1993). Cadmium contamination in soils has been reported to be the main constraint for food safety and agricultural land quality (Atafar et al. 2010). Cadmium is an abiotic stress responsible proteins (Kamal et al. 2010). Zinc (Zn) is an essential trace element for plants and animals, but is toxic when present at high levels. Cadmium and Zn are elements having similar geochemical and environmental properties; their chemical similarity can lead to interaction between Cd and Zn during plant uptake, transport from roots to the aerial parts, or accumulation in edible parts (Das et al. 1997). Antagonistic effects have been reported (McLaughlin and Singh 1999). It is generally accepted that Zn status in soils and plants plays an important role in Cd accumulation in crop plants (Grant and Bailey 1997; Oliver et al. 1997; Sarwar et al. 2010). Interactions between Cd and Zn and their accumulation in plant parts in solution culture or in pot experiments have been reported (Coughtrey et al. 1979; Smilde et al. 1992; Mckenna et al. 1993; Moraghan 1993; Dudka et al. 1994; Long et al. 2003; Chizzola and Mitterenger 2005; Mohammad and Moheman 2009). Several medicinal plants manifest a tendency to take up higher amounts of Cd than do other useful plants. Pot marigold *(Calendula officinalis)* is a medicinal plant and is used for the treatment of skin disorders and pain, as well as a bactericide, antiseptic and anti-inflammatory (Isaac 2000). The objectives of this research were to examine the interactions of Cd and Zn and the effects on their respective concentrations in leaves, and petals of pot marigold

Materials and methods

Pot experiments

Pot experiments were conducted under glasshouse conditions at the Agricultural University of Athens to study the interaction between Cd and Zn on the concentration of these metals in leaves and petals of pot marigold as affected by different applications of Cd and Zn to the soil. The experiment was carried out twice, in the spring and early summer of 2008 (four months) and then in the spring and early summer of 2009 (four months). For the experiments, pot marigold seeds of the variety Orange King were used. The seeds were sown in seed trays of 20 compartments, in a peat and perlite medium (1:1 v/v). After three weeks, when the plants had reached approximately 5 cm in length (with four leaves each), they were transplanted into the experimental pots. The pots were black plastic containers 14 cm in diameter and volume 0.5 L, filled with peat and perlite

Table 1. Sources of variance in Cd and Zn concentration, in leaves and petals in Cd- and Zn-treated pot marigold plants

	Leaves		Petals	
	Cd	Zn	Cd	Zn
Main effects				
Cd application	***	***	***	***
Zn application	***	***	***	***
Interaction	-	-	-	-
Cd × Zn	***	***	***	***

*** Significant F at $p \leq 0.0001$

medium (1:1 v/v), with organic matter content 90% - 95%, electrical conductivity 0.3 S m^{-3}, pH 5.6, and 0.5 mg kg^{-1} Cd extractable by diethylene triamine penta-acetic acid–triethanol amine (DTPA-TEA). This medium was used in all treatments. A factorial experiment with a randomized complete block design with two factors (Cd and Zn) and five levels (0, 1, 5, 10, 15 mg kg^{-1}) for each factor was conducted. This resulted in there being 25 different combinations for the plant treatments. Each treatment was replicated five times (125 pots totally). Cadmium was applied as $CdSO_4 \cdot 8/3H_2O$ and Zn as $ZnSO_4 \cdot 7H_2O$. Twice a week after transplanting, Cd and Zn were added in a volume of 20 ml per pot. Fertilization of the pots was performed approximately every two weeks, using a commercial fertilizer (NUTRILEAF-60) with 2 mg N, 2 mg P_2O_5, and 2 mg K_2O for each pot.

Plant analysis

The plants began to flower the third week after transplantation. During the blooming period, flowers were clipped off then petals were removed from the flowers and oven dried at 50°C to constant weight and ground in a stainless steel Wiley mill. The collection of flowers continued for approximately 10 weeks. At the end of the experiment, approximately 4 months after transplanting, the aerial parts of the plants were harvested. Leaves were separated from stems, oven-dried at 50°C to constant weight and ground in a stainless steel Wiley mill. Dry ground plant tissues (leaves and petals) (0.5 g) were placed in beakers and ashed at 450°C. The residue was dissolved in 5 ml of 6N HC1. Zinc and Cd were determined by flame atomic absorption spectrophotometry (AAS) (Varian, A-300; Varian Techtron Pty. Limited, Australia) at 213.9 nm and 228.8 nm wavelength, respectively, using an air-acetylene flame (Baker and Amacher, 1982). Deuterium background correction was used.

Soil analysis

At the end of each experiment, samples of air-dried soil from each pot were passed through a 500 μm plastic sieve and analyzed for extractable Cd and Zn using DTPA−TEA method following the procedure of Lindsay and Norvell (1978). 0.005M DTPA, 0.01M.1M TEA and $CaCl_2$, was adjusted to 7.3 with hydrochloric (HCl). Ten grams of soil and 20 ml DTPA-TEA extracting solution were placed in stoppered polyethylene flasks and placed on a horizontal shaker (240 oscillations/minute) for 2 hours. The suspensions were then filtered by gravity through Whatman no. 42 filter paper and the filtrates were analyzed for Cd and Zn.

Statistical analysis

Statistical analysis was carried out with STATISTICA™ version 8.0 (StatSoft 2008) for all the parameters studied. All data were subjected to Duncan's Multiple Range Test to determine statistical significance of the effects due to treatments with Cd, Zn and their interaction. Bartlett's chi-squared test showed that combining the data from both cultivated periods was acceptable. In the analysis that follows, all values given are the average of the data of the two experimental periods combined.

Results

An overview of the influence of Cd, Zn and their combination according to a factorial ANOVA is given in Table 1.

Effects of Cd and Zn interaction on the concentration of Cd in leaves and petals

Cadmium concentrations in the leaves and petals of pot marigold are shown in Fig. 1. Cadmium ranged between 12 and 13 mg kg^{-1} in leaves and between 4 and 6 mg kg^{-1} in petals in plants not treated with this element. The addition of Cd to the soil led to a significant increase of the concentration of this metal to 372 mg kg^{-1} in leaves with 15 mg Cd kg^{-1} and 0 mg Zn kg^{-1} treatment and to 90 mg kg^{-1} in petals with 15 mg Cd kg^{-1} and 5 mg Zn kg^{-1} treatment. Fig. 1 reveals that the Cd concentration in leaves was much higher than in petals. This difference ranged from 40% to 87% under different Cd and Zn applications. A significant reduction of Cd concentration in leaves under various Zn treatments was observed at 15 mg Cd kg^{-1} . In general, the Cd concentration in leaves decreased significantly with increasing Zn application (Fig. 1). Specifically, at 1 mg Cd kg^{-1}, the Cd concentration in leaves significantly decreased with Zn applications above 5 mg kg^{-1}. At 5, 10, 15 mg Cd kg^{-1}, any addition of Zn significantly reduced the Cd concentration in leaves. At 15 mg Cd kg^{-1}, all applications of Zn significantly reduced the Cd concentration in leaves to the same extent.

Effects of Cd and Zn interaction on the concentration of Zn in leaves and petals

Fig. 2 shows Zn concentration in the pot marigold plants. Zinc concentration ranged between 102 and 119 mg kg^{-1} in leaves and between 62 and 88 mg kg^{-1} in petals not treated with this element. The addition of Zn increased the concentration of this metal to 373 mg kg^{-1} in the leaves with 15 mg Zn $kg^{-1}$and 0 mg Cd kg^{-1} treatment and to 205 mg kg^{-1} in the petals with 15 mg Cd kg^{-1} and 5 mg Zn kg^{-1} treatment. The Cd concentration in leaves with 0 mg Cd kg^{-1}, 1 mg Cd kg^{-1} and 5 mg Cd kg^{-1} treatments was significantly reduced at 10 mg Zn kg^{-1} and 15 mg Zn kg^{-1} application rates (Fig. 2). With 15 mg Cd kg^{-1} treatment, the Zn concentration in leaves at the 15 mg Zn kg^{-1} application rate increased significantly compared to the Zn concentration at the same Zn application rate but with the 5 mg Cd kg^{-1} treatment.

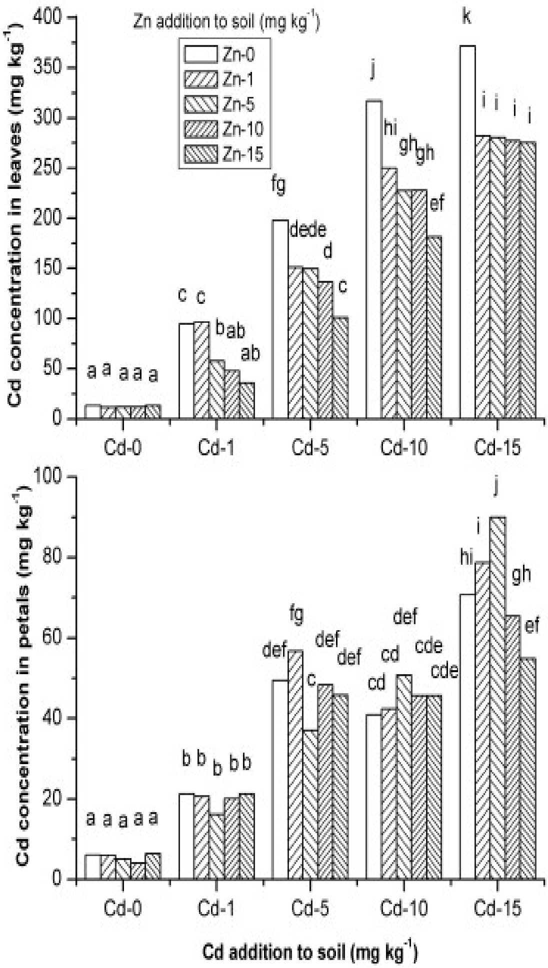

Fig 1. Cd concentration in leaves and petals of pot marigold plants with different additions of Cd and Zn to soil medium. Different letters indicate a significant difference at p<0.05 according to Duncan's multiple range test.

A different pattern of the effect of Cd application on Zn concentration in petals was detected. Zinc concentrations in petals generally increased with increasing Cd application as presented in Fig 2.

Application of Cd increased the Cd/Zn ratio in plant tissues (leaves and petals) of pot marigold plants (Fig. 3), while application of Zn reduced the Cd/Zn ratio. The Cd/Zn ratio with the high Cd rate (15 mg Cd kg^{-1}) was 3.5 at 0 mg Zn kg^{-1} in leaves and 0.40 at 15 mg Zn kg^{-1} in petals.

Cd/Zn ratio in leaves and petals

Increasing the applied Cd/Zn ratio in the soil resulted in a corresponding increase in the Cd/Zn ratio in leaves and petals, as indicated in Fig 3. The results show that when the applied Cd/Zn ratio approached unity, the Cd/Zn ratio ranged from 0.64 to 0.89 in leaves (not statistically different) and from 0.24 to 0.40 in petals (not statistically different).

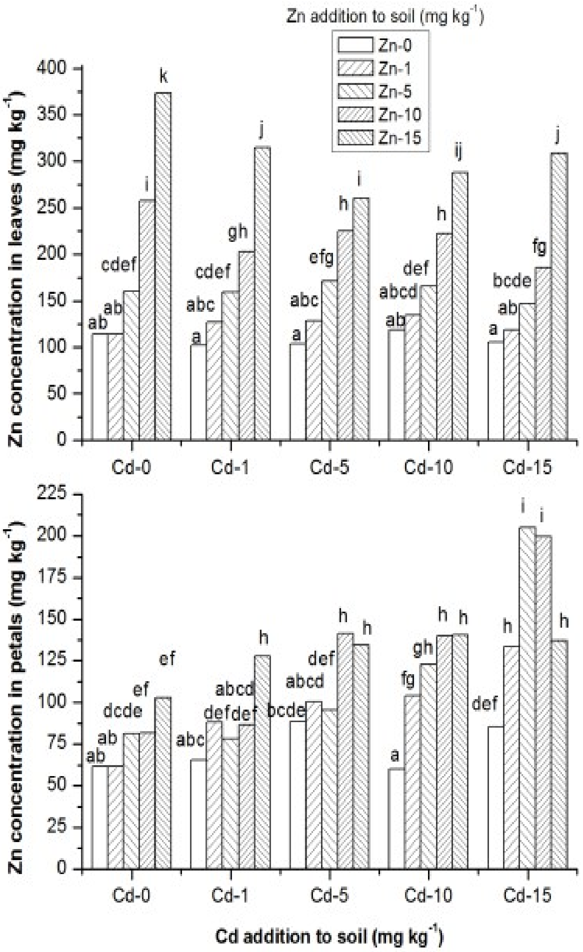

Fig 2. Zn concentration in leaves and petals of pot marigold plants with different additions of Cd and Zn to soil medium. Different letters indicate significant difference at p<0.05 according to Duncan's multiple range test.

Effects of Cd and Zn interactions on extractable Cd and Zn

The addition of Cd led to a significant increase of extractable Cd by DTPA-TEA, with a value of 237 mg kg^{-1} at 15 mg Cd kg^{-1} and 0 mg Zn kg^{-1} (Fig 4).

No significant differences were detected in extractable Cd with Cd at 0 mg Cd kg^{-1} and 1 mg Cd kg^{-1} between any of the Zn application rates. At 5 mg Cd kg^{-1}, 10 mg Cd kg^{-1} and 15 mg Cd kg^{-1} treatments, the extractable Cd was significantly reduced.

The addition to the soil of Zn led to significant increase of extractable Zn by DTPA-TEA, with a value of 162 mg kg^{-1} at 0 mg Cd kg^{-1} and 15 mg Zn kg^{-1} (Fig 4).

Discussion

Visual examination of treated pot marigold plants did not reveal symptoms of toxicity or nutrient deficiency of plants over the two growing periods studied. Plants grown on

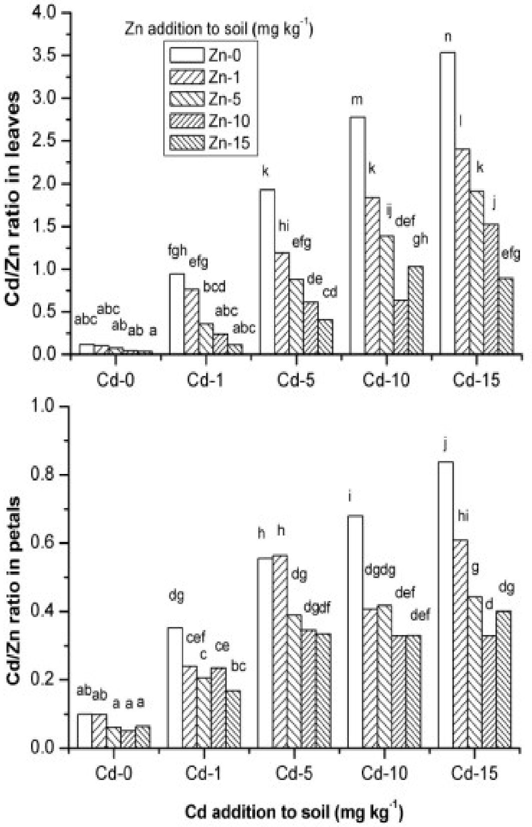

Fig 3. Cd/Zn ratio with different additions of Cd and Zn to soil medium. Different letters indicate significant difference at $p<0.05$ according to Duncan's multiple range test.

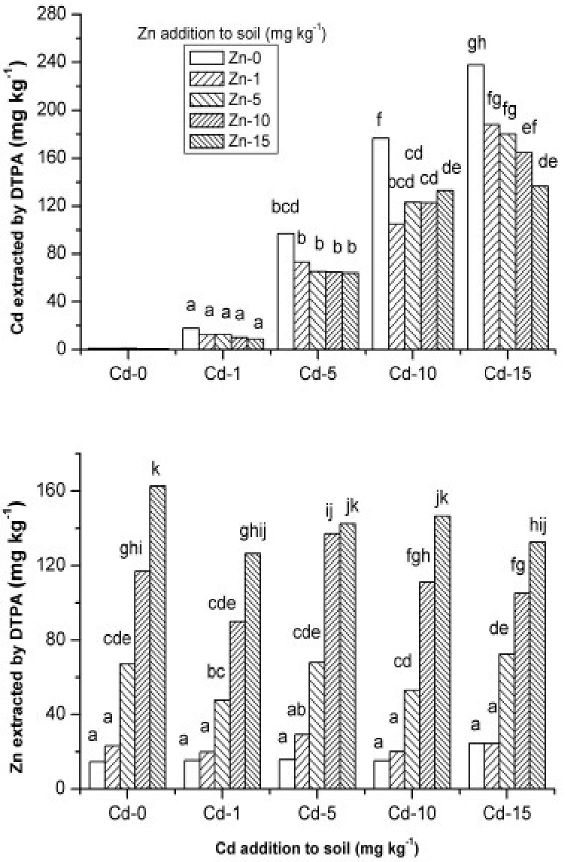

Fig 4. Extractable Cd and Zn by DTPA-TEA with different additions of Cd and Zn to soil medium. Different letters indicate significant difference at $p<0.05$ according to Duncan's multiple range test.

uncontaminated soils usually contain less than 0.2 mg kg^{-1} Cd and maximum tolerable levels for Cd in agricultural soils proposed in various countries range from 1.6 to 3 mg Cd kg^{-1} (Kabata-Pendias and Pendias 1992). The interaction of Cd and Zn has been reported to be antagonistic by some researchers (Li et al. 1990; Long et al. 2003) but synergistic by others (Piotrowska et al. 1994; Salt et al. 1995; Nan et al. 2002). Perronnet et al. (2003) reported that Cd and Zn were distributed differently within the hyperaccumulating plant *Thlaspi caerulescens* and that the partitioning of these elements varied with plant age and organ. In wheat (*Triticum aestivum* L. and *T. turgidum* L. var. *durum*) at the level of the root cell membrane, Cd and Zn show a competitive interaction, indicating a common transport system (Hart et al. 2002). Various results have been reported concerning the interactions between the accumulation of Cd and Zn. Cadmium accumulation may or may not be influenced by increasing Zn supply. Great differences occur among species and even between different varieties of the same species (Grant and Bailey 1997). Some researchers found that Zn supply can inhibit Cd adsorption and thereby cause a low Cd concentration in plants (Adriano 1986; Nan et al. 2002). Results from the present work showed that Cd concentration in the studied plant tissues of pot marigold were largely dependent on the Zn level. Cadmium concentration in leaves decreased significantly with increasing Zn application to the soil (Fig. 1). However, no growth depression was detected, although considerable Cd concentrations were found in the plants. Therefore, this heavy metal was highly mobile and available to the plants, and thus pot marigold can be considered a Cd-accumulating plant. Low soil pH is a major factor favouring the uptake of heavy metals (Kabata-Pendias and Pendias 1992). In the present study, as the pH was 5.6, the availability of Cd could also be explained by a soil pH effect because the Cd was added to the soil throughout the growing period. The Zn status seems to be important for Cd accumulation and partition in the plant in that Zn deficiency favours Cd accumulation by the plant (Grant and Bailey 1997; Welch et al. 1999). The Zn levels in leaves and petals of our pot marigold plants were certainly sufficient, as they were within a normal range for plants not supplied with additional Zn and the plants showed vigorous growth. Cadmium and Zn might be considered chemically similar elements because they have similar ionic structure and electronegativities, and may influence each other in plant uptake and accumulation, but they play quite different roles in the plant's metabolism. Zinc is a micronutrient, whereas Cd is toxic and ordinarily is found at very low concentrations in the plant; usually, the Zn concentration is more than 100 times the Cd level (Chaney et al. 1999). However, they have

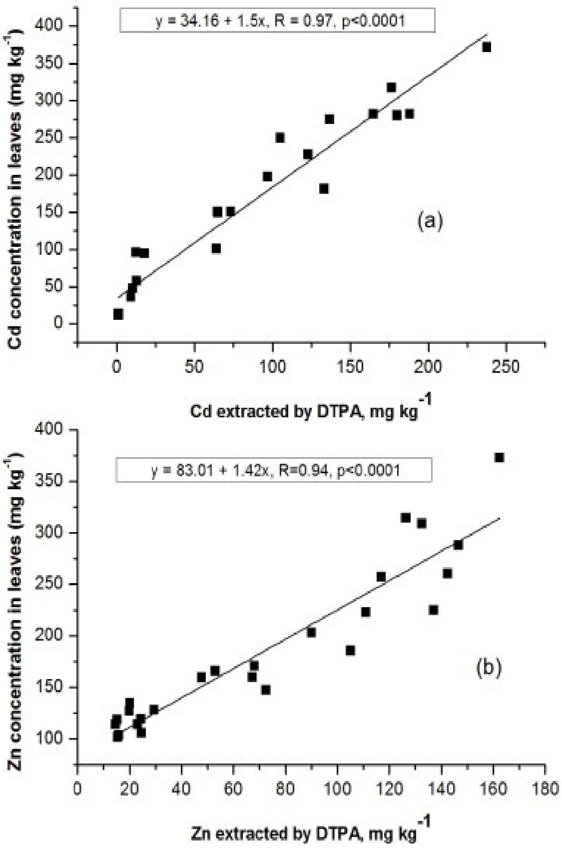

Fig 5. The relationship between Cd (a) and Zn (b) extracted by DTPA-TEA and their respective concentrations in pot marigold leaves.

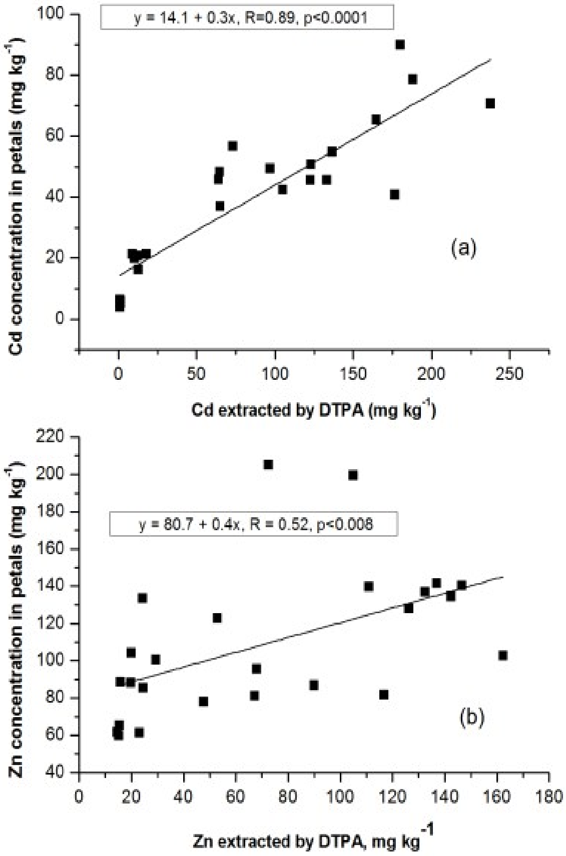

Fig 6. The relationship between Cd (a) and Zn (b) extracted by DTPA-TEA and their respective concentrations in pot marigold petals.

different ionic radii (Zn^{2+} = 0.074 nm, Cd^{2+} = 0.097 nm); this difference may play a role in plant selectivity for Zn. In other words, the reduced uptake of Cd as a result of the addition of Zn addition in our work might result from competitive transport and absorption interaction between these two ions. Zinc levels usually range between 20 to 100 mg kg^{-1} and maximum tolerable levels for Cd in agricultural soils proposed in various countries ranged from 150 to 300 mg Zn kg^{-1} (Kabata-Pendias and Pendias 1992). In *Thlaspi* species, the Zn concentrations were not affected by Cd supply (Ozturk et al. 2003). In short-term experiments in young poppy plants (*Papaver somniferum* L.), the simultaneous addition of Cd and Zn to the nutrient solution or to the soil did not decrease the Cd content of the plants (Chizzola 1997). In sunflower (*Helianthus annuus* L.), even a supply of 10 mg kg^{-1} Cd in the substrate did not influence the Zn content in the aerial organs (Simon 1998). Grejtovsky and Pirc (2000) reported that the addition of Cd resulted in a suppressed Zn content in the diploid chamomile variety 'Novbona' but not in the tetraploid 'Lutea'. On the other hand, in barley (*Hordeum vulgare* L.) grown in nutrient solution, the presence of Cd enhanced the shoot Zn concentration (Girling and Peterson 1981), presumably as a consequence of growth inhibition and a subsequent dilution effect. Our study failed to record a reduced Zn concentration with increasing Cd supply in leaves and petals, except in leaves at rates of Cd lower than 5 mg kg^{-1}. Extractable Cd and Zn by DTPA-TEA was highly correlated with Cd and Zn concentration in leaves and petals, indicating that this extractant may be useful for predicting Cd and Zn concentrations in leaves and petals of pot marigold plants (Fig. 5, 6). Moustakas et al. (2001) and Akoumianakis et al. (2008) reported that this extractant may also be used for predicting Cd concentrations in lettuce, radish, cucumber, endive and rocket plants.

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