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Ameliorate the cadmium toxicity in *Solanum tuberosum* L. plants with selenium and silicon application

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Abstract: The present study aimed to prove the efficiency of Se or Si as relievers of the Cd toxicity in potato plants. *Solanum tuberosum* plants, Asterix genotype, from *in vitro* propagation were placed in pots with sand and irrigated with complete nutrient solution for 60 days under six treatments: T1: Control (nutrient solution); T2: 2.5 µM Se; T3: 2.5 mM Si; T4: 50 µM Cd; T5: 50 µM Cd + 2.5 µM Se; T6: 50µM Cd + 2.5 mM Si. The treatments were arranged in completely randomized design, with four replicates for each treatment and six plants per replicate. The plants were collected at 30 and 60 days after application of the treatments. Cadmium was highly toxic in all parameters (dry and fresh weight, plant height, leaf number, leaf area, root and photosynthetic parameters), in both assessments. However, Se and Si were effective in mitigating Cd toxicity in all parameters, although Si has been shown to be more efficient than Se in dry weight and plant height parameters. Thus, from data obtained in this study, it is clear that the beneficial elements tested have power to ameliorate Cd toxicity.

1. Introduction

Plants differ in their ability to absorb, accumulate and tolerate heavy metals, including cadmium (Cd), and toxic levels of heavy metals affect a variety of plant processes (Gupta *et al.,* 2013). Cadmium is one of most toxic heavy metals, having a high mobility in environment (Tang *et al.*, 2015), being absorbed by roots and transported to shoot of many plant species (Shi *et al.,* 2005). Although Cd has no known biological function in plants (Pence *et al*., 2000; Pereira *et al*., 2016), it can be easily absorbed and transported by xylem (Lux *et al.,* 2011), since it has an electronic configuration and state of zinc-like valence (Nan *et al.,* 2002). This heavy metal is toxic even at low concentrations, inducing stress responses in plants from 5-10 µg g^{-1} soil (While and Brown, 2010). Several studies have reported significant reductions in biomass accumulation in plants exposed to Cd (Farooq *et al.,* 2013; Said *et al.,* 2014; Vaculík *et al.,* 2015), and this inhibition in biomass production can occur in short time of exposure to this heavy metal (Han *et al.,* 2015). In contrast to others toxic heavy metals, Cd present in soil is easily absorbed by plant roots, particularly in acidic soils (Guimarães *et al.,* 2008). The plant development stage and time of exposure to heavy metal affect the absorption and distribution of Cd in different parts of plant (Gonçalves *et al.,* 2009 a).

Thus, it is necessary to develop strategies that result in lesser absorption of these toxic elements present in soil by plants, optimizing the use of natural resources and production of safe food, especially when it comes to a plant used in food such as potatoes. Potato (*Solanum tuberosum* L.) is one of main foods for mankind, consumed by more than one billion people worldwide, due to its composition, gastronomic and technological versatility, as well as the low market price of tubers (Coelho *et al.,* 1999; Dorneles *et al.,* 2016). According to Birch *et al.* (2012), potatoes are third most important crop in world, behind only rice and wheat. The potato is susceptible to Cd, and this sensitivity can be accentuated by level of Cd in soil, time of exposure and cultivar (Gonçalves *et al.,* 2009 b).

One of options sought to solve this problem with Cd in plant growth is use of beneficial elements, which when used in low concentrations can alleviate the damaging Cd effects. In this sense, selenium (Se) and silicon (Si) are recognized as beneficial elements for growth of some plants, and can increase the tolerance of plants to abiotic stresses. These elements are recognized as being capable of mitigating metal toxicity in plants (Wu *et al.,* 2017). However, it is necessary to evaluate the potential use of these mitigating elements in the presence of Cd, since some studies show that these may not be effective to reduce the toxicity of this element in some species (Khattab, 2004; Liu *et al.,* 2013). However, Si has been shown to be effective in alleviating Al toxicity in potato plants (Dorneles *et al.,* 2016) and Cd in species such as peanuts (Shi *et al.,* 2010), Chinese cabbage (Song *et al.,* 2009), rice (Tripathi *et al.,* 2013), maize (Lukacová *et al.,* 2013), wheat (Khan *et al.,* 2015) and sunflower (Said *et al.,* 2014). Selenium, however, has stress-alleviating properties that are more focused on biochemical mechanisms activation (Kumar *et al.,* 2012; Feng *et al.,* 2013; Tamaoki and Maruyama-Nakashita, 2017). However, it already has great potential in use to relieve stresses by metals (He *et al.,* 2004; Pezzarossa *et al.,* 2012). However, this element, to date, has not been tested as potential to relieve the stress caused by Cd toxicity in potato plants. Thus, the aim of this work was to test the possibility of using Se or Si as reliever of Cd toxicity in potato plants.

2. Materials and Methods

Plants of *Solanum tuberosum* L., Asterix genotype, were used for the experiment, which were propagated *in vitro* from nodal segments for 25 days in MS culture medium (Murashige and Skoog, 1962).

After this period, plants were transferred to plastic vessels containing 1 plant each and 5 kg of sand, being watered daily with complete nutritive solution. This solution had the following composition (mg L^{-1}): 85.31 N; 7.54 P; 11.54 S; 97.64 Ca; 23.68 Mg; 104.75 K; 176.76 Cl; 0.27 de B; 0.05 Mo; 0.01 Ni; 0.13 Zn; 0.03 Cu; 0.11 Mn and 2.68 Fe ($FeSO_4$/Na-EDTA). After five-day of acclimation period, treatments were applied, which consisted of following combinations: Treatment 1: Complete nutritional solution and absence of Cd, Se and Si; Treatment 2: Complete nutritional solution + 2.5 µM Se; Treatment 3: Complete nutritional solution + 2.5 mM Si; Treatment 4: Complete nutritional solution + 50 µM Cd; Treatment 5: Complete nutritional solution + 50 µM Cd + 2.5 µM Se; Treatment 6: Complete nutritional solution + 50 µM Cd + 2.5 mM Si. These solutions were applied daily maintaining 80% of the vessel capacity, which was monitored by daily determining the weight of the vessel. The treatments were arranged in a completely randomized design, with four replicates for each treatment and six plants per replicate. The pH of the solutions was adjusted daily (4.5±0.1).

Two collects were performed, the first collect being at 30 days of exposure to treatments and the second at 60 days. In both collections, the following evaluations were carried out:

- Photosynthetic parameters were evaluated in fourth fully expanded leaf of four plants per replicate: the photosynthetic rate (A - µmol CO_2 m^{-2} s^{-1}), the stomatal conductance of water vapors (Gs - mol H_2O m^{-2} s^{-1}), internal CO_2 concentration (Ci - µmol

m^{-2}), water use efficiency (WUE - mol CO_2 mol H_2O^{-1}), and the transpiration rate (Tr mmol - mmol H_2O m^{-2} s^{-1}) obtained by the ratio between the amount of CO_2 fixed by photosynthesis and the internal CO_2 concentration. The evaluations were carried out in period between 8 and 11h with use of IRGA portable meter, brand LI-COR, model LI-6400XT.
- Growth parameters: fresh and dry biomass, height, leaf area, number of leaves, and morphological parameters of the root system (length, diameter, volume and number of root branches), according to methodology described by Dorneles *et al.* (2016).

For statistical analysis of data, it was verified the normality of error distribution through Anderson-Darling test and homogeneity of error variances through the Bartlett test (Estatcamp, 2012) for all variables of experiment. The averages were submitted to analysis of variance and compared by the Scott-Knott test, with 5% significance, using Sisvar (Ferreira, 2011). The graphic program used was SigmaPlot 12.5.

3. Results

For experimental conditions tested, cadmium (Cd) presented great toxicity to potato plants both at 30 and 60 days of growth. This is evidenced by averages of fresh weight accumulation obtained by plants exposed to Cd, which did not increase after 30 days of cultivation, remaining unchanged up to 60 days (Fig. 1) both being statistically smaller than in the control treatment. Besides, in general, when applied in isolation, Si and Se did not promote greater accumulation of fresh and dry weight in comparison to control plants (Figs. 1, 2). However, both elements tested proved to be effective in mitigating Cd toxicity from first collect, inducing a higher accumulation of fresh weight in both tissues, compared to the treatment where only Cd was applied. The enhancing effects of Se and Si are more evident in shoot, where plants exposed to Cd combined with Se or Si maintained some growth in second collect (Fig. 1 A). The roots of potato plants (Fig. 1 B) also show a significant improvement in presence of both beneficial elements. This probably contributed to growth of shoot, considering that roots are the first tissue to come into contact with Cd.

For dry weight, potato plants showed similar behavior to that observed for fresh weight in both tissues (Fig. 2). Cadmium showed to be toxic to both tissues for dry weight, inhibiting this accumulation until last collect. However, Se and Si induced biomass accumulation similar to control plants, even when applied together with Cd. Silicon induced greater accumulation of shoot dry weight when compared to Se (Fig. 2 A). In roots, Cd prevented the dry weight increase, while plants treated with Se and Si presented increase in root dry weight in second collect (Fig. 2 B), that is, Se and Si alleviated the toxic effects of cadmium.

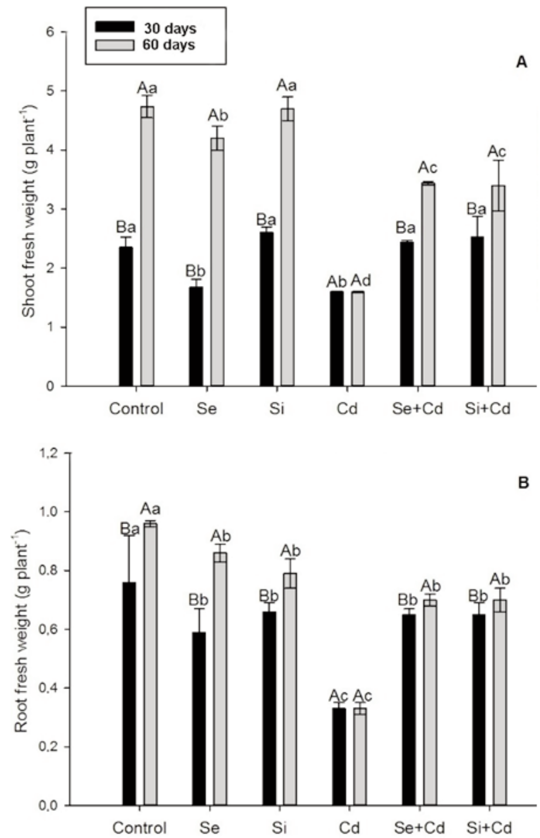

Fig. 1 - Effect of selenium (2.5 µM) or silicon (2.5 mM) on shoot (A) and roots fresh weight (B) of potato plants grown in presence of cadmium (50 µM) at 30 and 60 days after application of the treatments. Different lowercase letters indicate significant differences between treatments in same collect. Different uppercase letters indicate significant differences between collects for same treatment.

In addition to affecting the production of fresh and dry biomass, Cd inhibited the production and expansion of leaves, as well as the growth in height of potato plants. However, plants that received Si and Se together with Cd obtained higher means even in comparison to control plants (Figs. 3 A, B). In addition, the number of leaves and height of plants exposed to Cd were higher when applied Si, in comparison to plants treated with Se as amendment. Both Se and Si were effective in reducing the toxicity of cadmium from first collect, inducing higher height, number of leaves and leaf area than plants with only

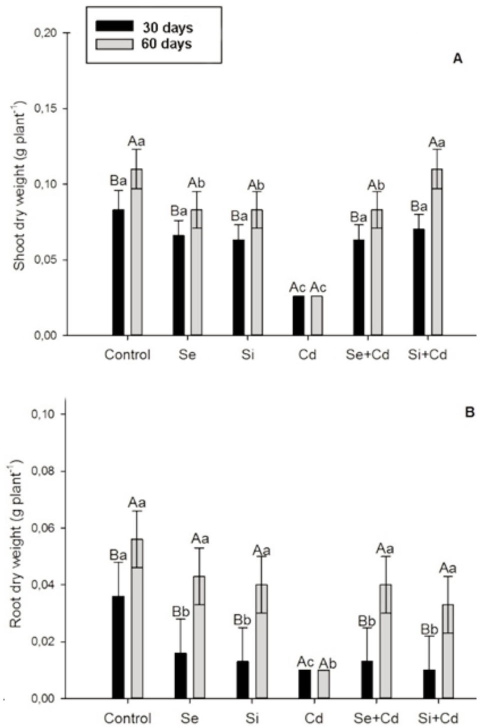

Fig. 2 - Effect of selenium (2.5 µM) or silicon (2.5 mM) on shoot (A) and roots dry weight (B) of potato plants grown in cadmium presence (50 µM) at 30 and 60 days after application of the treatments. Different lowercase letters indicate significant differences between treatments in same collect. Different uppercase letters indicate significant differences between collects for same treatment.

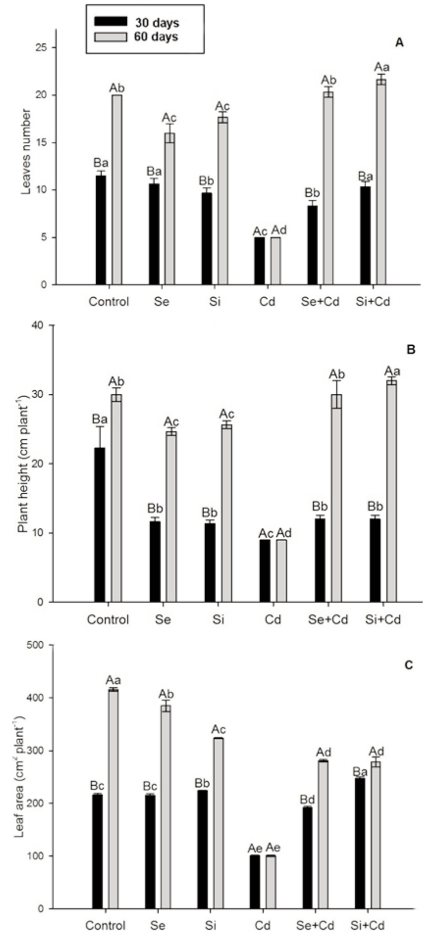

Fig. 3 - Effect of selenium (2.5 µM) or silicon (2.5 mM) on leaves number (A), plants height (B) and leaf area (C) of potato plants grown in cadmium presence (50 µM) at 30 and 60 days after application of the treatments. Different lowercase letters indicate significant differences between treatments in same collect. Different upper case letters indicate significant differences between collects for same treatment.

Cd in nutrient solution.

There was no statistical difference between treatments for root length and root diameter at 30 days, but plants treated with Cd presented root length 45% lower than the control (Table 1). At 60 days, plants exposed to Cd showed root length 16% and root

Table 1 - Length, diameter, volume and number of branch roots of *Solanum tuberosum* plants grown in presence of selenium (2.5 µM), silicon (2.5 mM) and cadmium (50 µM) at 30 and 60 days after application of the treatments

Collect	Treatments	Root length (cm)	Root diameter (mm)	Root volume (cm^3)	Branch number
30 days	Control	798±0.24 Aa	0.35±0.02 Ba	1.64±0.49 Aa	715±68 Aa
	Se	776±18.3 Aa	0.38±0.04 Ba	0.91±0.25 Ab	394±18 Bb
	Si	743±9.24 Aa	0.35±0.02 Ba	0.84±0.19 Ab	401±20 Bb
	Cd	438±3.76 Ba	0.34±0.02 Aa	0.37±0.07 Ac	201±13 Ad
	Se + Cd	650±4.62 Aa	0.36±0.00 Ba	0.65±0.05 Ab	323±35 Bc
	Si + Cd	711±3.43 Aa	0.41±0.03 Aa	0.83±0.08 Ab	318±38 Bc
60 days	Control	998±0.57 Aa	0.45±0.02 Aa	1.83±0.21 Aa	790±48 Aa
	Se	884±21.6 Aa	0.45±0.01 Aa	0.85±0.03 Ab	794±58 Aa
	Si	862±25.2 Aa	0.45±0.02 Aa	0.76±0.08 Ab	790±62 Aa
	Cd	839±10.11 Ab	0.34±0.02 Ab	0.34±0.02 Ac	253±24 Ac
	Se + Cd	795±5.50 Aa	0.46±0.00 Aa	0.75±0.04 Ab	423±21 Ab
	Si + Cd	791±6.11 Aa	0.44±0.02 Aa	0.85±0.07 Ab	452±22 Ab

Different lowercase letters indicate significant differences between treatments in same collect. Different upper case letters indicate significant differences between collects for same treatment.

diameter 25% lower, compared to the control. On the other hand, all treatments presented lower root volume compared to control, but this effect was more significant in the treatment containing only Cd in the growth medium, both at 30 and 60 days of cultivation. This same behavior was observed for the number of branches at 30 days. At 60 days, branch numbers was lower in the treatments containing Cd, Se + Cd and Si + Cd, and this effect was more significant in the treatment containing only Cd in the growth medium. Cadmium showed to be highly toxic, reducing volume of roots and ramifications to less than half of values presented by control plants.

However, Si and Se presented an amendment effect on all root variables, mainly for total length and diameter, both at 30 and 60 days of cultivation. In these variables, plants exposed to Cd treated with both Si and Se were statistically the same as control plants.

Plants exposed to Cd had photosynthetic rate (A) reduced by 37% compared to control plants at first collect, and 42% at second collect (Table 2). Besides, Cd caused significant reductions in stomatal conductance (GS), internal CO_2 concentration (Ci) and transpiratory rate (Trmmol) in both collects. The water use efficiency (WUE) in both collects was higher only for the treatment with Se + Cd. However, in both collects Si and Se were effective in mitigation of Cd toxicity for all variables except for WUE (Table 2).

4. Discussion and Conclusions

In the present study, Cd promoted a significant reduction in biomass production in potato plants (Fig. 1). The reduction in biomass accumulation caused by Cd may be due, in part, to its effect on the inhibition of nutrient uptake by roots (Cao *et al.,* 2014; Li *et al.,* 2016).

The effects of Cd on absorption of nutrients may be due to damage caused by this element in the roots. Since root tissues are first to come into contact with the Cd present in solution, they are also the most affected. Several studies have already reported negative effects of Cd on cellular level of root tissues (Benavides *et al.,* 2005; Lux *et al.,* 2011; Martinka *et al.*, 2014).

The data presented in this work show the significant reduction in root parameters caused by Cd. This behavior may be due to effect of Cd on degradation of membranes and nucleotides of root cells (Han *et al.,* 2015). This degradation of membranes and organelles can be explained by the increase in concentration of reactive oxygen species (ROS) induced by Cd (Chou *et al.,* 2012; Farooq *et al.,* 2013; Said *et al.,* 2014; Han *et al.,* 2015). It is possible that this effect of Cd on increase of ROS, reported in these studies, may inhibit cell division of roots (Said *et al.,* 2014) In addition, the Cd has affinity for phosphates and some amino acids components of enzymes and

Table 2 - Effect of selenium (2.5 µM) or silicon (2.5 mM) on photosynthetic rate (A-µmol CO_2 m^{-2} s^{-1}), stomatal conductance (GS - mol H_2O m^{-2} s^{-1}), internal CO_2 concentration (Ci - µmol m^{-2} s^{-1}), transpiration rate (Trmmol - H_2O mmol m^{-2} s^{-1}),and water use efficiency (WUE - CO_2 mol H_2O mol^{-1}) of *Solanum tuberosum* plants grown in cadmium presence (50 µM) at 30 and 60 days after application of the treatments

Collect	Treatment	A	GS	Ci	Trmmol	WUE
30 days	Control	9.47±0.13 Ba	0.20±0.07 Aa	288±0.48 Aa	9.47±0.13 Ba	192±4.59 Bb
	Se	8.39±0.32 Bb	0.16±0.04 Aa	295±11.8 Ba	8.39±0.32 Bb	178±11.8 Bb
	Si	8.92±0.31 Bb	0.04±0.00 Ac	272±2.44 Bb	8.92±0.31 Bb	200±20.3 Bb
	Cd	5.92±0.27 Bc	0.10±0.00 Ab	257±8.54 Ac	5.92±0.27 Bc	228±8.63 Ab
	Se + Cd	8.73±0.07 Bb	0.06±0.03 Ac	297±5.57 Aa	8.73±0.07 Bb	308±3.05 Aa
	Si + Cd	8.26±0.03 Bb	0.10±0.00 Ab	255±9.29 Ac	8.26±0.03 Bb	236±12.6 Ab
60 days	Control	12.5±0.14 Aa	0.20±0.03 Aa	289±0.24 Aa	12.5±0.14 Aa	205±0.25 Ab
	Se	10.3±0.32 Ab	0.14±0.01 Aa	268±14.6 Ab	10.3±0.32 Ab	215±0.24 Ab
	Si	10.1±0.06 Ab	0.06±0.00 Ab	298±5.53 Aa	10.1±0.06 Ab	236±0.02 Ab
	Cd	7.41±0.31 Ad	0.04±0.00 Bc	269±8.13 Ab	7.41±0.31 Ad	220±0.26 Ab
	Se + Cd	9.08±0.72 Ac	0.08±0.00 Ab	257±8.54 Bc	9.08±0.72 Ac	298±0.14 Aa
	Si + Cd	8.98±0.61 Ac	0.10±0.00 Ab	255±9.29 Ac	8.98±0.61 Ac	245±0.22 Ab

Different lowercase letters indicate significant differences between treatments in same collect. Different upper case letters indicate significant differences between collects for same treatment.

proteins, which leads, in addition to damage to membranes, genetic damage and can disrupt oxidative phosphorylation in exposed tissues (Hasanuzzaman and Fujita, 2012; Nahar *et al.,* 2016).

The present study also shows the effect of Cd under photosynthetic parameters, in which there was a significant reduction in plants exposed to this heavy metal. This reduction in photosynthetic parameters may be a consequence of the degradation of chlorophylls caused by Cd (López-Millán *et al.,* 2009). Furthermore, Cd causes disorder in arrangement of grana and thylakoids, which limits the efficiency of most photosynthetic parameters (Han *et al.,* 2015; Bayçu *et al.,* 2016).

The potato plants used in present study, treated with Si or Se as amendments of Cd toxicity, showed higher biomass production than plants exposed to only Cd. In addition, Se and Si significantly reduced the damage caused by Cd in photosynthetic parameters. These results of biomass and photosynthetic rate are related, since the beneficial effects of Si and Si on the Cd toxicity in photosynthetic parameters are expressed, to a greater or lesser degree, in higher production of biomass by plants.

Each element used as amendment in this work (Se or Si) act in different ways in the plant. Silicon have been proven to be effective in easing stress by Cd in different species such as cucumber (Feng *et al.,* 2010), corn (Malčovská *et al.,* 2014; Vaculík *et al.,* 2015) and cotton (Farooq *et al.,* 2016). This effect of Si may be due to its deposition on cell wall which increases its plasticity and elasticity (Vaculík *et al.,* 2009). The Si deposited on cell wall of the plants may increase Cd retention in apoplast (Lukačová *et al.,* 2013; Vaculík *et al.,* 2015), which may reduce the availability and translocation of Cd. Due to these cell wall modulations, Si can inhibit Cd uptake (Liu *et al.,* 2013). These effects of Si can explain the more evident amelioration of Cd in shoot than in roots for dry weight in potato plants (Fig. 2). In addition, silicon has photoprotective properties when deposited on leaves (Tripathi *et al.,* 2017), which may explain the mitigation of Cd toxicity in gas exchange parameters.

There is a chance that Se also has the property of forming complexes with toxic or heavy metals, but were only found complexes of Se-mercury (Hg) in plants (Said *et al*., 2014). However, there are reports that selenium reduces Cd accumulation in tissues (*Lactuca sativa* L.) (He *et al.,* 2004). While Si is recognized for increasing the enzymatic antioxidants activity (Debona *et al.,* 2017), Se has the property of inducing resistance by activating routes of synthesis of hormones linked to stress response and antioxidant activities (Freeman *et al.,* 2010; Feng *et al.,* 2013; Tamaoki and Maruyama-Nakashita, 2017). Thus, the mechanism of mitigation of Se may be more directly related to antioxidant system. Many studies have reported this effect of Se under plant antioxidant enzymes, as well as its effect on direct removal of ROS (Cartes *et al.,* 2010; Zembala *et al.,* 2010). In present study, the Cd toxicity was reduced in most of evaluated parameters, mainly under photosynthetic parameters. The possible effect of Se under enzyme activity may help to explain its most evident effect under these parameters. Selenium was more effective in reducing the effects of Cd on photosynthetic parameters than for other parameters evaluated.

For potato plants under experimental conditions used in this study, Si and Se elements proved to be effective in mitigating Cd toxicity. Thus, these elements have potential for use in fertilizers applied to contaminated soils. Future biochemical, histological and molecular analyzes may complement the elucidation of action mechanisms these elements.

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