Cadmium accumulation and toxicity in watercress (*Barbarea verna*), chicory (*Cichorium endivia*) and rocket (*Eruca sativa*) plants[1]

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ABSTRACT

Some vegetables have the ability to accumulate cadmium (Cd) at levels that are toxic to most other plants. The objective of this study was to evaluate the Cd accumulation and its toxic effects on edible vegetables. Three species were tested: watercress (*Barbarea verna)*; chicory (*Cichorium endivia)*; rocket (*Eruca sativa*) and four Cd doses (0; 1.5; 3.0; 6.0 mg kg^{-1}), with three replications. We evaluated Cd concentration, fresh and dry mass and SPAD index. We calculated tolerance index (TI), translocation index (TR) and the transfer coefficient (TC). Rocket, chicory and watercress are susceptible to Cd contamination and chicory presented visual symptoms of toxicity (chlorosis and mass loss). The Cd contents above those allowed were 0.6; 0.56 and 1.03 mg kg^{-1} in rocket, watercress and chicory, respectively, these values were reached by applying doses from 1.5 mg kg^{-1} for rocket and 3 mg kg^{-1} for watercress and chicory.

Keywords: contamination; heavy metal; translocation; tolerance.

INTRODUCTION

Cd is one of the major environmental pollutants and it is known for contaminating the food chain. Anthropogenic activities such as industrialization, intensive farming and inadequate mining practices have resulted in an increase in inorganic contaminants such as Cd (Sidhu *et al.*, 2017). The knowledge of Cd mobility in the soil-plant system is essential, as it may cause a series of deleterious effects in plants and mammals. (Sharid *et al.*, 2017).

The ingestion of Cd through food is the main source of exposure for animals and humans in an environment not contaminated by this metal (Sharid *et al.*, 2017). Among foods, leafy vegetables were identified as the main access way to the body (Huang *et al.*, 2017). It is generally toxic to non-tolerant plants, even at low concentrations. In leaves, contents between 5 and 10 mg kg^{-1} of Cd, result in toxic effects for most plants (Gallego *et al.*, 2012). However, some species of plants grown in high concentrations of this element have normal biological functions, as they have developed ways to adapt to this environment (Prasad & Freitas, 2003).

According to Huang *et al.* (2020), edible plants are considered the main source of Cd exposure for the general population, through consumption of grains and leafy vegetables. The National Health Surveillance Agency (Brasil, 2013) establishes that the maximum level of Cd allowed for leafy vegetables is 0.2 mg kg^{-1}. The ability of plants to absorb it depends on the concentration in the soil, its accumulation and translocation capacity, among other factors.

There are studies of Cd accumulation in watercress and chicory, especially *Nasturtium offinale* and *Cichorium intybus*, respectively. However, there are other species of

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watercress and chicory that are widely consumed in Brasil but little studied, such as *Barbarea verna* and *Cichorium endivia*, respectively. Knowing the toxic effects of Cd in edible plants and whether the concentration is in accordance with the legislation becomes important to avoid contamination of the food chain.

The aim of this study was to evaluate the accumulation of Cd and its toxic effects in watercress (*Barbarea verna*), chicory (*Cichorium endivia*) and rocket (*Eruca sativa*) and compare with current legislation.

MATERIAL AND METHODS

The experimental design used was a completely randomized in a factorial scheme, using three species of edible vegetable (watercress-*Barbarea verna* var. Da Terra; chicory - *Cichorium endivia* var. Escarola Lisa; rocket-*Eruca sativa* var. Cultivada Gigante Folha Larga) and four doses of Cd (0; 1.5; 3.0; 6.0 mg kg^{-1}), with three replicates. The experimental units consisted of plastic trays, with 1.8 kg of soil.

A dystrophic Red-Yellow Argisol collected in the city of Lauro Muller -SC (28° 23' 25,29''S; 49° 22' 5,27'' W), in the 0-20 cm depth layer, in an area free from anthropic activities was used. This soil was used because it has a sandy texture and low clay content to avoid Cd adsorption. The soil was air-dried, ground, homogenized and sieved in a 2mm mesh. The characterization of the soil used in the experiment is shown in Table 1. The soil granulometry was performed as described by Gee & Bauder (1986). Chemical attributes were determined according to Tedesco *et al.* (1995).

Table 1: Chemical characterization and granulometry of a Red-Yellow Argisol used under natural conditions (0-20 cm layer)

Sand	Silt	Clay	pHH_2O	pHSMP	Ca	Mg	Al	Ef CEC	CEC ph7
-----------g kg^{-1}---------						-----------------cmolc dm^{-3}--------------------			
640	200	160	4.7	5.1	1.15	0.67	2.48	4.62	14.44

Base sat	Al sat	OM*	OC*	P	Na	K	Fe	Cu	Zn	Cd
-------%-------		----g kg^{-1}-----			----------------------------mg kg^{-1}----------------------------					
14.84	53.68	22.00	12.80	0.50	52.00	126.00	139.60	0.90	1.20	0.018

*OM=Organic matter; OC=Organic carbon.

The Cd doses were applied in the form of a solution prepared with Cd $(NO_3)_2$ following the adopted concentrations (0; 1.5; 3.0; 6.0 mg kg^{-1}), with the soil remaining incubated for 15 days. The doses were defined considering the reference value for Cd in agricultural soils (3 mg kg^{-1}) according to Brasil (2009).

After this period, the soil was corrected to pH 5.5, with the addition of 5.4 g $tray^{-1}$ of PRNT 100% limestone. Was also applied 0.42g of phosphorus (P) in the form of triple superphosphate and 0.13g$tray^{-1}$ of potassium (K) in the form of potassium chloride as indicated by the soil analysis (CQFS, 2004). Finally, 0.39g$tray^{-1}$ of nitrogen (N) was applied in the form of urea, divided into three applications: 0, 15 and 30 days after planting.

For all species, the seedlings used in the experiment were produced in a greenhouse. Sowing was carried out in Styrofoam trays containing Tecnomax commercial substrate. NPK fertilization was performed on the substrate, which was disinfected with sodium hypochlorite, at the 1:15 proportion (hypochlorite: water). The Styrofoam trays remained immersed in distilled water and covered with 50% shading.

After 30 days, the seedlings were transplanted to 31 x 20.5cm plastic trays. Six seedlings were planted in each tray, which remained in a greenhouse for 45 days. Moisture was maintained at 50% of the field capacity, monitored by means of weighing. Trays were rotated every 10 days.

SPAD index

At 30 days after transplanting, the relative chlorophyll content was determined using a SPAD-502 chlorophyll meter (Konica Minolta®, Tokyo, Japan). Readings were performed on the first fully expanded leaves, at five points/leaf, three leaves/plant and three plants/ plot.

Dry and fresh matter

Harvest was performed 45 days after transplantation. After harvesting, the roots were immersed in a 5mM $CaCl_2$ solution for 10 minutes in order to remove the Cd from the outer part of the root. Then the plants were washed in distilled water, dried on paper towels, where the roots were separated from the aerial part and the fresh mass was determined. Next, they were packed in paper bags and taken to the oven at 65 ºC until they reached constant mass. At the end of this period, the dry matter was determined and then the samples were ground in an IKA-analytical mill, model A-11.

Cd quantification

Plant samples were opened according to the USEPA 3051A method (USEPA, 2007). Cd was quantified using high-resolution atomic absorption spectrometry with electrothermal atomization in ContrAA equipment (Analytic Jena, Jena, Germany).

The reliability of the analytical method used to determine Cd was evaluated using a control sample of plants SRM 1573a (tomato leaves) certified by the National Institute of Standards and Technology (NIST). The recovery rate (RcR) is obtained using the equation:

$$RcR(\%) = \left(\frac{found\ value}{certified\ value}\right) \times 100$$

The amount of 1.03 mg kg^{-1} of Cd was obtained, with an expected 1.52 ± 0.04 mg kg^{-1}, thus, a recovery rate of 67.76%.

Transfer coefficients, translocation and tolerance index

The transfer coefficients of the Cd in the soil (TC), translocation index (TR) and tolerance index (TI) were determined using the following equations:

$$TC = \frac{Cd\ content\ plant\ fresh\ weight\ (mg\ kg^{-1})}{available\ Cd\ content\ soil\ (mg\ kg^{-1})}$$

$$TR = \left(\frac{quantify\ accumulated\ in\ the\ aerial\ part\ (g\ pot^{-1})}{quantify\ accumulated\ in\ the\ plant\ (g\ pot^{-1})}\right) \times 100$$

$$TI = \left(\frac{dry\ mass\ of\ the\ plant\ at\ the\ dose\ (g)}{control\ dry\ mass\ (g)}\right) \times 100$$

Statistical analysis

The results were statistically analyzed based on a linear model of analysis of variance according to a completely randomized design in a factorial arrangement. Data normality was analyzed using the Shapiro-Wilk test, while the homogeneity of variances was tested using the Levenne test. In order to meet the theoretical assumptions of the tests, the logarithmic transformation was applied to the variables related to Cd contents in fresh mass and dry mass of aerial parts and roots. Considering the differential effect of species on the dynamics of responses to Cd doses, has been adjusted polynomial regression equations to Cd doses in each of the species tested. All analyses were performed with a minimum level of significance of 5% and conducted using the software R (R Development Core Team, 2012).

RESULTS AND DISCUSSION

There was an interaction effect between species and Cd doses in all analyzed variables (Table 2). Considering this behavior and for a better perception of the dynamics of responses to Cd doses, was shown and discussed the variations in the values of the variables in relation to the doses within each of the species tested.

Cd accumulation in dry mass

In control doses of rocket, watercress and chicory, Cd concentration was below the detection limit (DL), that is, smaller than 0.006 mg kg^{-1}. The levels of Cd in dry mass increased in aerial part of the 3 species with the increase of doses, however, was higher in chicory and rocket, which presented similar values in all doses. In the highest applied dose (6 mg kg^{-1}), the levels of Cd were 6.03; 29.75 and 28.46 mg kg^{-1} in watercress, chicory and rocket, respectively (Figure 1a).

Chicory (*Cichorium* sp) has been reported as a species capable of accumulating Cd. Stafford *et al.* (2016) investigated the accumulation of Cd in 12 plant species (fertilized and corrected pH soil). *Cichorium intybus* was the species that showed the highest concentration of Cd, 1.63mg kg^{-1}indry mass with 0.43 mg kg^{-1}of Cd in the soil. Cd bioavailability in the soil depends on its physical and chemical properties such as: pH, texture, cation exchange capacity (CEC), organic carbon, levels of Fe, Al and Mg (Dziubanek *et al.*, 2017) and biological properties (Baldantoni *et al.*, 2015).

Reis *et al.* (2014) evaluated the influence of chemical, granulometric and mineralogical attributes of the soil on the adsorption of Cd in 12 types of Oxisols. Of the 6 types of Oxisols collected in native field, in 4 of these Oxisols the adsorption was greater in the native field than in the same type of soil cultivated. Soils with higher goethite/hematite ratio, CEC and pH have a higher maximum adsorption capacity, so the Cd is less available to be absorbed by the plants.

Table 2: Mean squares and coefficients of variation (CV) referring to the analysis of variance of the studied variables

Causes of Variation	DF	APDM	RDM	APFM	RFM	CdAPDM	CdRDM
		Mean squares					
Species	2	12.856*	0.091*	846.640*	1.244*	1.432*	6.995*
Dose	3	0.478*	0.031*	8.870*	0.895*	13.328*	20.914*
Species x Dose	6	0.486*	0.008*	10.590*	0.402*	0.455*	2.242*
Residue	24	0.046	0.002	2.370	0.062	0.076	0.044
CV (%)		11.74	24.60	11.55	19.83	17.76	10.51

Causes of Variation	DF	CdAPFM	CdRFM	TC	TI	TR	SPAD
		Mean squares					
Species	2	0.499*	6.280*	68.911*	4218.900*	1405.500*	477.480*
Dose	3	3.189*	4.437*	25.797*	732.200*	2635.400*	2.370^{NS}
Species x Dose	6	0.245*	0.799*	9.849*	1310.500*	461.000*	5.490*
Residue	24	0.022	0.020	0.454	228.00	56.800	1.380
CV (%)		26.54	15.47	26.65	16.96	29.69	4.46

APDM – Aerial Part Dry Mass; RDM – Root Dry Mass; APFM – Aerial Part Fresh Mass; RFM – Root Fresh Mass; CdAPDM – Cd in Aerial Part Dry Mass; CdRDM - Cd in Root Dry Mass; CdAPFM – Cd in Aerial Part Fresh Mass; CdRFM - Cd in Root Fresh Mass; TC – Transfer Coefficient; TI – Tolerance Index; TR – Translocation Index; SPAD –SPAD Index; DF - Degrees of Freedom; CV - Coefficient of variation * Significativo (P < 0,05).

Guerra *et al.* (2014) estimated Cd concentrations of 36.88 and 101.5 mg kg^{-1} in Typic Hapludox (TH) and Typic Quartzipsamment (TQ), respectively, at a dose of 6 mg kg^{-1} in *Eruca sativa* (rocket). The contents for both soils were higher than in this work (28.46 mg kg^{-1}), even the TH and TQ with higher clay contents. In general, Cd adsorption is greater in clayey soils, consequently less available to plants. It's noteworthy that the soil was fertilized and pH corrected (as in this work) and the CEC and organic matter (OM) values in this work (4.62 cmol$_c$ dm^{-3} and 22 g kg^{-1}) were higher than TQ (1.92 cmol$_c$ dm^{-3} and 7 g kg^{-1}) and similar to TH (5.52cmol$_c$ dm^{-3} e 23 g kg^{-1}), respectively.

Kamran et al. (2015) used bacteria (*Pseudomona putida*) to improve the accumulation capacity and tolerance of Cd in rocket (*Eruca sativa*) for phytoremediation purposes. The doses of Cd were 150, 250 and 500 mg kg^{-1}. It was verified that the use of bacteria increased the absorption of Cd by 23, 28, 29 and 27% in T0, T1, T2, T3, respectively, when compared to the uninoculated treatment, what characterizes the biological influence on Cd absorption.

The Cd contents in the dry mass of root increased in the three species with the Cd doses (Figure 1b). The highest contents were obtained in rocket and were higher in the root than in the aerial part in watercress and rocket for all doses. Contrary to this study, Baldantoni *et al.* (2015) obtained twice as much Cd in aerial part as in roots of *Lactuca sativa* (lettuce) and *Cichorium endivia* (chicory). The behavior may differ between different cultivars. The levels of Cd accumulated in the roots are not relevant for this study, as it is not intended for phytoremediation.

Cd accumulation in fresh mass

For the dose 3 mg kg^{-1}, which is the investigation reference value for Cd in agricultural soils (Brasil, 2009), Cd values in the fresh mass of aerial part were 0.56; 1.03; 0.81 mg kg^{-1} in the watercress, chicory and rocket, respectively (Figure 2). These values are 2.5; 5 and 4 times greater than those allowed by the regulatory agency (Brasil, 2013). The European Union (2011) and the National Health Surveillance Agency (Brasil, 2013) set that the maximum allowed level of Cd for leaf vegetables and fresh herbs is 0.2 mg kg^{-1} of fresh mass. At 1.5 mg kg^{-1} Cd, the levels were 0.2; 0.2 and 0.6 mg kg^{-1} in watercress, chicory and rocket, respectively. Although the dose is half of the guiding value, rocket has Cd levels that exceed three times the maximum allowed value. At 6 mg kg^{-1}, twice the guiding value in soils, which simulates a contamination condition, the Cd contents were 1.0; 3.4 and 4.9 mg kg^{-1}, for watercress, chicory and rocket, respectively. These levels are 5; 17 and 24.5 times higher than the allowed.

Other studies have also found Cd levels above those allowed in vegetables. Khan *et al.* (2010) found high concentrations of toxic elements in the edible parts of *Spinacia oleraceae* (spinach), *Brassica campestris* (mustard), *Brassica oleraceae* (cabbage) and *Chichorium intybus* (chicory). In soils with Cd contents between 0.3 to 2.3 mg kg^{-1}, the Cd concentration remained between 0.24 and 2.10 mg kg^{-1}, exceeding the limits (0.1 - 0.2 mg kg^{-1}) established by the States Environmental Protection Administration in China (SEPA). Bester *et al.* (2013) determined the Cd concentration in 9 plant species in soils with an average Cd concentration of 2.6 mg kg^{-1}. The highest concentrations of Cd were found in the roots (beets and carrots) and leafy vegetables (chicory and endive). The highest content was found in chicory, 1.2 mg kg^{-1} fresh mass.

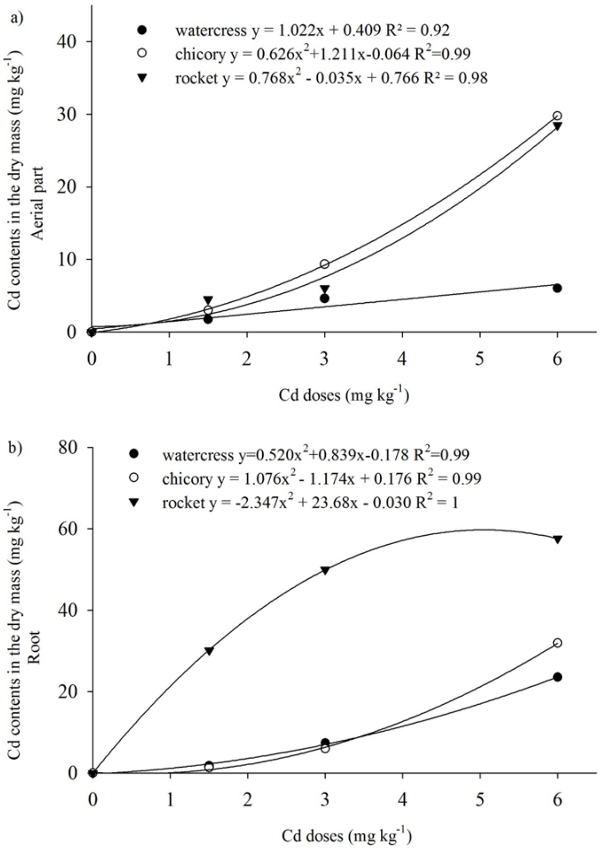

Figure 1: Average Cd contents in dry mass of aerial part (a) and root (b) in watercress, chicory and rocket with increasing Cd doses.

Translocation index

The TR increased up to the dose of 3 mg kg^{-1} (around 45%) and decreased after that dose for watercress and chicory (Figure 3). For rocket, there was an increase in translocation with increasing the doses, although the values were lower than watercress and chicory in all doses. Therefore, despite showing different behaviors, the three species were able to translocate Cd.

According to Gallego *et al.* (2012), the translocation of metals from the roots to the aerial part through the xylem is the main characteristic that determines the phenotype of accumulating species. For Rascio & Navari-Izzo (2011), non-accumulating species can retain in the root cells most of the heavy metals absorbed from the soil, detoxifying them through chelation in the cytoplasm or storing them in the vacuoles. However, the accumulating species rapidly and efficiently transfer these elements to the aerial part through the xylem.

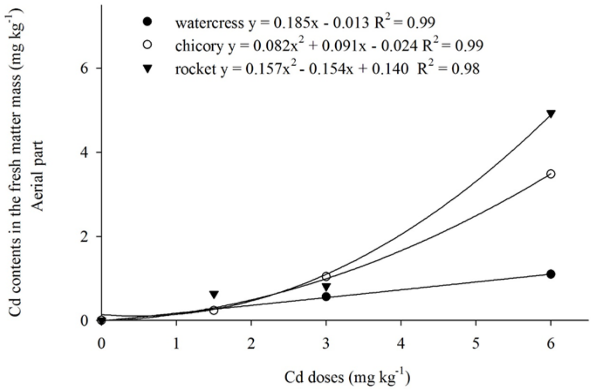

Figure 2: AverageCd contents in fresh mass of aerial part in watercress, chicory and rocket with increasing Cd doses.

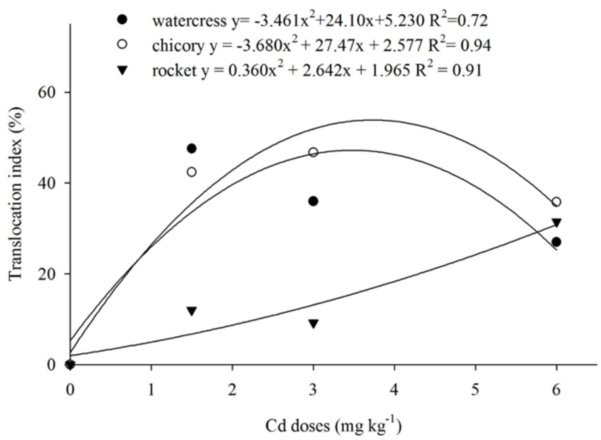

Figure 3: Translocation index in watercress, chicory and rocket with increasing Cd doses.

Transfer coefficient

In rocket, there was an increase in the TC values with increased doses. In the watercress and chicory, the increment in the doses had no effect on the TC. The highest TC values were found in the rocket, with the maximum around 7.0 at 3 and 6 mg kg^{-1} (Figure 4). The TC assesses the capacity of the species in absorbing the element from the soil.

According to Magna *et al.* (2013), TC values for Cd are within the 1-10 range. The plant genotype is one of the factors that have the greatest influence on Cd absorption, which would explain the variability between species.

Khan *et al.* (2008) observed that in different plants, the TC remained in the order Cd > Ni > Cu > Zn > Cr > Pb and high TC values especially in leafy vegetables. According to Melo *et al.* (2014), TC values greater than 1.0 indicates an accumulating species.

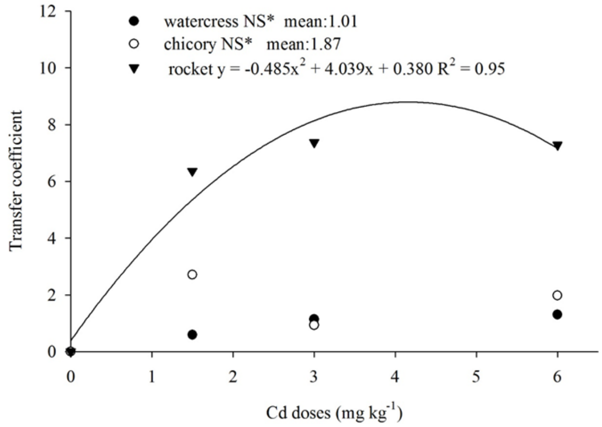

Figure 4: Transfer coefficient in watercress, chicory and rocket with increasing Cd doses.
*Not signficant.

Dry and fresh mass

In watercress there was no loss of mass in the aerial part and root (Figures 5a, 5b, 5c, 5d). Rocket showed loss of fresh and dry mass only in the roots (Figures 5b, 5d). There was a decrease in the fresh mass of aerial part only chicory. The decrease was 5.3 times in the highest dose applied, in comparison to the control (Figure 5a).

According to Rizwan *et al.* (2017) the reduction in the plant growth and biomass under stress by Cd depends on the plant species, as well as on the dose and period of exposure to the metal. The decrease in fresh mass of the aerial part and root was also found in the genus *Cichorium* by Khateeb (2014), who observed a reduction in fresh weight in *Cichorium pumilum* (dwarf chicory) and root growth using Cd doses of 0 to 1600 µM (180 mg kg^{-1}).

For rocket, Kamran *et al.* (2015) found a reduction in fresh weight, a decrease in the length of the root and aerial part with Cd doses of 0 to 500 mg kg-[1]. Yildirim *et al.* (2019) found a reduction of 44.7; 51.4; 27.8 and 36.8% of fresh and dry mass of aerial part, fresh and dry mass of root for rocket to 200 mg kg^{-1} of Cd, when compared to the control, respectively.

Tolerance index

This index decreased with the increase of Cd doses only in chicory (Figure 6), meaning less tolerance of the species to the metal. The decrease in the growth is one of

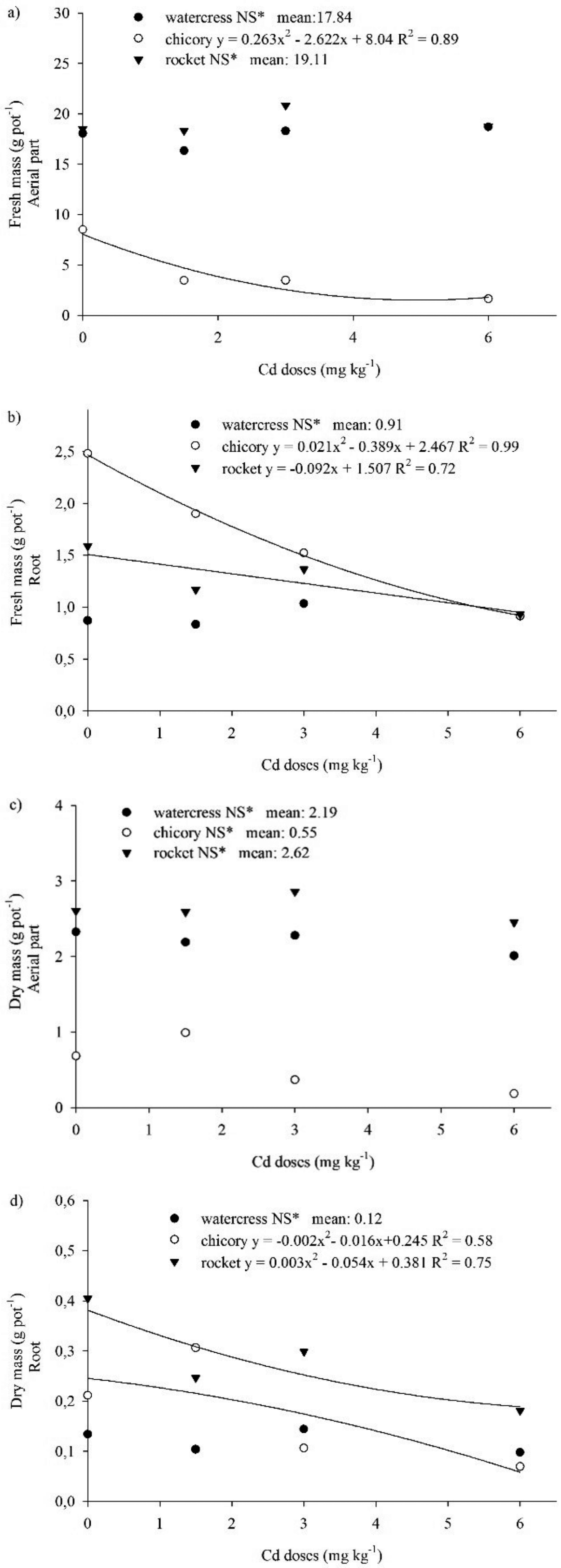

Figure 5: Fresh mass of aerial part (a) and roots (b), dry mass of aerial part (c) and roots (d) in watercress, chicory and rocket with increasing Cd doses.

the major symptoms of toxicity for species not tolerant to Cd. Therefore, considering TI, *Eruca sativa* (rocket) and *Barbarea verna* (watercress) would fit as species tolerant to Cd and chicory (*Chicorium endivia*) as a species sensitive to this metal.

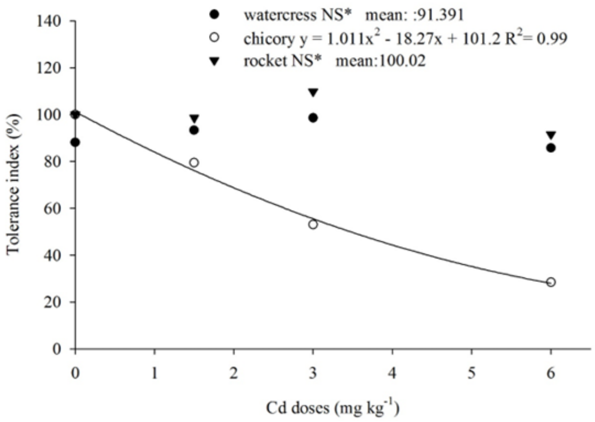

Figure 6: Tolerance index in watercress, chicory and rocket with increasing Cd doses.
*Not significant.

SPAD index

The incrementin Cd doses had an effect on the relative chlorophyll content (SPAD Index) for chicory and rocket (Figure 7). The SPAD index is related to the chlorophyll content in the plant or to the green intensity of the leaf. The decrease in the relative chlorophyll content was more pronounced in chicory. In this species, the SPAD Index was 21.45 in the control to 18.05 at the highest dose (6 mg kg^{-1}), which is equivalent to 84.1% of green intensity, compared with the control. For rocket, the reduction was greater in the 1.5 mg kg^{-1} dose, equivalent to 91% of the green color.

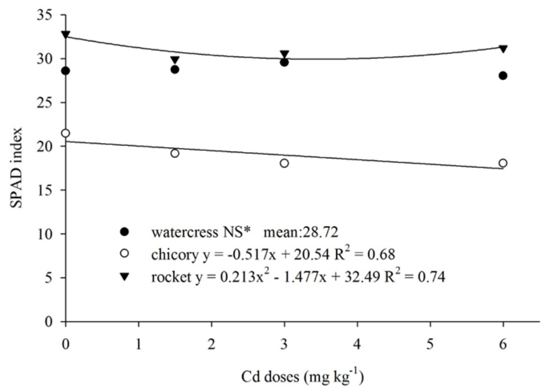

Figure 7: SPAD Index in watercress, chicory and rocket with increasing Cd doses.

Yildirim *et al.* (2019) using doses 100, 150 and 200 mg kg^{-1} also observed a decrease in the SPAD Index in rocket. Cd provides the reduction in the chlorophyll content, as well as the deficiency of Mg and Fe also caused by Cd, may have contributed to the reduction in the chlorophyll biosynthesis. However, Kamran *et al.* (2015) found a decrease in the SPAD Index in rocket only at the dose of 500 mg kg^{-1}, which is about 80 times higher than those used in this study.

CONCLUSIONS

The three species present risks if grown in contaminated soils, due to Cd levels being above the permitted in plant issues (0.2 mg kg^{-1}) by the regulatory agency in the guiding value of Cd for agricultural soils (3 mg kg^{-1}).

The rocket showed levels of Cd (0.6 mg kg^{-1}) above the allowed from the dose 1.5 mg kg^{-1} and watercress and chicory (0.56 and 1.03 mg kg^{-1}) from dose 3 mg kg^{-1}. Cd caused toxic effects in chicory (chlorosis and mass decrease) from the dose 1.5mg kg^{-1}.

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