Soil Dynamics of Cr(VI) and Responses of *Portulaca Oleracea* Grown in a Cr(VI)-Spiked Soil under Different Nitrogen Fertilization Regimes

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Abstract

The reduction potential of the highly toxic Cr(VI) to the inert Cr(III) in an alkaline soil was studied during a 50-day experiment with *Portulacaoleracea* grown in pots. We aimed at assessing whether our test species can be a phytoremediation candidate for Cr(VI)-contaminated soils. We measured the Cr(VI) reduction rate in soil, determined the Cr(VI) and Cr(III) concentrations in aerial and root *P. oleracea* tissues, calculated the Transfer coefficient (TC=metal in plant over metal in soil) and the Translocation factor (TF=metal in aerial biomass over metal in roots) in order to assess Cr(VI) uptake and distribution in plant tissues, while we also studied the effect of added nitrogen in the studied parameters. We added five different Cr(VI) levels,reaching 148.6 mg Cr(VI) kg^{-1}soil and also had two N levels (equivalent to 0 and 200kg ha^{-1}). The results indicated that Cr in plant tissues was mainly found in its reduced form (Cr(III)) and only a minor fraction of Cr was detected in its oxidized form (Cr(VI)). The main remediation mechanism was found to be that of the naturally occurring Cr(VI) reduction that effectively produced Cr(III), followed by the uptake of Cr(VI) from our test plants. We also found that Cr(VI) in *P. oleracea* tissues was mainly found in roots and relatively low Cr(VI) concentrations were found in the above ground tissues. We concluded that *P. oleracea*is a tolerant plant species, especially if assisted with a sufficient level of N fertilization, although it failed to approach the threshold of being categorized as an accumulator species.However, before reaching more conclusive suggestionsabout *P. oleracea* as a potential phytoremediation species, further investigation is necessary.

1. Introduction

Metal ions can be introduced to surface soils by natural or anthropogenic processes and their environmental impact and availability is greatly affected by soil mineralogical and geochemical properties (Garret 2000). Chromium has several oxidation states ranging from − 2 to + 6. In the environment, Cr occurs primarily in two valence states, + 3 (chromite (Cr(III)) and + 6 (chromate Cr(VI)) and in natural soil conditions Cr is found predominantly in its trivalent state (Shanker et al. 2005). Geogenic derivation of hexavalent Cr is unusual, and its presence is rather entirely anthropogenic. Hexavalent Cr is commonly found in wastes of industrial activities related to metal plating, stainless steel production, chromic acid and Cr-pigment production, leather tanning, wood preservation, as well as in cement production. Thus Cr(VI) can be found mainly in the locality of industrial zones, but if discharged through streams, it may be found even kilometers down the stream, polluting adjacent surface soils and groundwater (Lilli et al. 2015; Megremi et al. 2019; Shanker et al. 2005).

The mobility of Cr species in soil varies greatly in respect to bioavailability and sorption characteristics. Several soil factors, such as pH, Cr speciation and attributes of the soil colloidal phase have a major influence on Cr availability (Ertani et al. 2017; Shi et al. 2020). Cr(III) is of low mobility in soil. In pH < 4, insoluble inorganic compounds are formed (i.e complexes of Cr^{3+} and Fe oxides) and as pH increases, trivalent Cr is mainly found in its hydrolyzed form (which species are of the general form of $Cr(OH)_n^{3-n}$, with $n = 1–3$); these species tend to form organic and inorganic complexes with fluoride, ammonium,

cyanide, thiocyanate, oxalate, and sulfate, with inorganic ligands being of much lower solubility compared to the organic (Ertani et al. 2017; Jobby et al. 2018; Shahid et al. 2017;). On the other hand, Cr(VI) can be retained more strongly than Cl^- and SO_4^{2-} ions and its retention strength can be compared to that of phosphates on hydrous Fe and Al oxides surfaces (Fendorf et al. 1997; Jobby et al. 2018; Shi et al. 2020).

Hexavalent chromium, even in well aerated soils, is expected to be readily reduced to the inert, less toxic and of lower mobility trivalent form (Cr(III)). Cr(VI) reduction, thus, acts as a natural, self-remediation process, that takes place even in the presence of particularly weak electron donors, such as H_2O (Antoniadis et al. 2017b; Antoniadis et al. 2018; Chen et al. 2015). Cr(VI) reduction is commonly encouraged by various reduced soil carbon compounds as follows:

1) Cr(VI) reduction from soil carbon compounds:

$$C_6H_6O_2 + CrO_4^{2-} + 2H_2O \rightarrow 0.5Cr_2O_3 + 1.5C_6H_4O_2 + 2.5H_2O + 2OH^-$$

2) Redox reactions resulting in Cr(VI) reduction in soil (electron donor: H_2O)

Reduction half reaction: $Cr^{VI}O_4^{2-} + 3e^- \rightarrow Cr^{3+} + 4H_2O$

Oxidation half reaction: $2\ H_2O^{-II} \rightarrow O^0_2 + 4H^+ + 4e^-$

Resultant redox reaction: $CrO_4^{2-} + 5H^+ \rightarrow Cr^{3+} + 2.5H_2O + 0.75O_2$

(Antoniadis et al. 2017b; Jobby et al. 2018)

Some indices are frequently used to assess trace element toxicity in plants: 1) Soil-to-plant element mobility (Transfer coefficient, TC), equal to the ratio of metal concentration in plant tissues (C_p) over the total concentration in soil (C_s) ($TC = C_p/C_s$), which shows the potential of an element to be transferred from soil to the plant tissues. Plant species, Cr bioavailability and Cr soil concentration are the major factors governing plant tissue Cr content and affecting TC. This index is taken into consideration when assessing a plant species for its phytoremediation potential, with values close or greater than unity being desirable (Antoniadis et al. 2017a; Antoniadis et al. 2021; Moral et al. 1995; Nagarajan and Sankar Ganesh 2014). An often used variant of this index is BAI (bioavailability index), with the same numerator but extractable soil Cr(VI) as denominator (instead of total soil Cr(VI)). 2) Translocation factor (TF), equal to the ratio of metal concentration in aerial plant tissues (C_{aerial}) over that in roots (C_{root}) ($TF = C_{aerial}/C_{root}$). Translocation factor is indicative of the plant species capacity to control toxic element translocation to the aerial biomass where metabolic activity is more intense. Cr accumulates

preferentially in roots, and minimal Cr concentrations are found in above ground plant tissues. Cr distribution in plant tissues (roots, stems and leaves) follow a stable plant species specific pattern that appears to be independent of the soil Cr concentration and bioavailability. Desirable TF values for a plant with phytoremediation potential are over unity (Antoniadis et al. 2017a; Antoniadis et al. 2021; Ertani et al. 2017; Moral et al. 1995). Our test plant, *Portulaca oleracea*, is a plant of high added value when commercially cultivated and well-known for its tolerance towards harsh abiotic stresses, e.g., salinity and draught (Alam et al. 2014; Karkanis and Petropoulos 2017; Petropoulos et al. 2016; Ozturk et al. 2020). However, tests assessing its potential as a possible phytoremediation species for Cr(VI)-laden soils are rare. Also, it is known that well-fertilized plants are more robust in addressing environmental stresses. However, although the effects of fertilizers on chemical composition and plant growth have been evaluated (Alam et al. 2014; Disciglio et al. 2017; Montoya-García et al. 2018; El-Sherbeny et al. 2015), the effect of sufficient levels of added N in the behavior of *P. oleracea* in addressing Cr(VI) stress is never before explored and needs to be investigated.

The aim of this work was to assess Cr(VI) dynamics in soil, and the responses of *P. oleracea* concerning its ability for Cr(VI) uptake, and the Cr speciation in plant tissues, as well as the distribution of Cr(VI) in plant tissues, under different N fertilization regimes. Based on the used phytoremediation indices, we also explored a practical issue, i.e., the number of *P. oleracea* harvests required to annihilate the spiked soil Cr(VI) levels, an approach that indicates the novelty of this study.

2. Materials And Methods

2.1. Soil properties, Cr(VI) spiking and nitrogen application

A pot experiment was established using an alkaline soil obtained from an area away from any known source of pollution, Velestino (39.394930 N, 22.757112 E), nr. Volos, central Greece. The soil was sieved through a 2-mm mesh sieve and three samples randomly acquired were analysed for selected physiochemical parameters (Table 1) according to established protocols (Koutroubas et al. 2020). For our pot experiment, we prepared a Cr(VI)-spiking solution, prepared at a concentration of 10,000 mg L^{-1}, by dissolving 19.22 g of CrO_3 in 1000 mL of double distilled water. The spiking solution was added to soil, resulting in 5 different Cr(VI) soil concentrations, namely: T-0: 0.0 mg Cr(VI) kg^{-1} soil (control), i.e., unamended soil; T-1: 20 mg Cr(VI) kg^{-1} soil, by adding 2 mL of the spiking solution per kg soil; T-2: 50 mg Cr(VI) kg^{-1} soil (with 5 mL of spiking solution kg^{-1} soil); T-3: 100 mg Cr(VI) kg^{-1} soil (10 mL of spiking solution kg-1 soil); and T-4: 150 mg Cr(VI) kg^{-1} soil (15 mL of spiking solution kg^{-1} soil) (Table 2).

Table 1
Properties and Cr content for the soil used in the experiment.

Properties	Unit	Value
pH		7.8
Electrical conductivity	$\mu S\ cm^{-1}$	850
$CaCO_3$	%	10.4
Organic carbon	%	1.5
Sand	%	45.2
Silt	%	38.8
Clay	%	16.0
Soil (untreated) Cr content		
Total Cr(III)	$mg\ kg^{-1}$	221.3
Cr(VI)	$mg\ kg^{-1}$	n.d
n.d. Non detected.		

Table 2
Initial soil Cr(VI) on Day 0, i.e., the day of the commencement of the experiment (14 October 2019) and final Cr(VI) concentration on the harvest day (termination of the experiment; 4 December 2019) during the growth period of *Portulaca oleracea*.

Treatments		Cr(VI)-Initial ($mg\ kg^{-1}$)	Cr(VI)-Final ($mg\ kg^{-1}$)	% of Cr(VI) reduced
No N	T-0	-	-	-
	T-1	20.65 ± 3.41^{a}	9.7 ± 1.93^{a}	53.3 ± 5.6
	T-2	49.92 ± 7.71^{b}	16.0 ± 0.50^{b}	67.9 ± 2.0
	T-3	106.43 ± 4.25^{c}	45.5 ± 3.19^{c}	57.2 ± 4.2
	T-4	148.62 ± 0.28^{d}	50.7 ± 4.48^{d}	65.88 ± 2.7
Added N	T-0	-	-	-
	T-1	20.65 ± 3.41^{a}	4.3 ± 0.63^{a}	79.2 ± 3.9
	T-2	49.92 ± 7.71^{b}	13.9 ± 1.96^{b}	72.2 ± 1.42
	T-3	106.43 ± 4.25^{c}	35.8 ± 2.60^{c}	66.3 ± 1.9
	T-4	148.62 ± 0.28^{d}	57.9 ± 1.53^{d}	61.1 ± 1.13
Treatment effect		$p < 0.001$	$p < 0.001$	$p = 0.453$
Cr effect		$p < 0.001$	$p < 0.001$	$p = 0.349$
Nitrogen effect		$p = 1.000$	$p = 0.371$	$p = 0.118$

Values are reported as mean ± standard error of all measured replicates. Different letters within columns denote significant ($p < 0.05$) differences among means in columns according to Duncan's multiple range test.

Also, we added nitrogen to half of the replicates of each of the established treatments, while the other half did not receive any additional N. Nitrogen was added in rates equivalent to 200 kg per hectare (considering an effective rhizosphere depth of 15 cm and dry bulk density of 1.33 g cm^{-3}), by applying 20 mL per kg of soil of a solution containing 14.3 g NH_4NO_3 L^{-1} (equal to 5 g N L^{-1}) The non-added-N-treatments are thereafter named N-0, while those that received N, N-1. Overall, the experimental design resulted in 10 treatments (5 Cr(VI) rates x 2 nitrogen rates). The spiked soils were placed into 2-L pots, with each pot containing 1 kg of soil; each treatment consisted of 10 replicates ($n = 10$). Pots were irrigated to 65% of their water holding capacity and left to equilibrate for 20 days. During the equilibration period, pots were thoroughly mixed every second day and water was added to compensate moisture loss. At the end of the equilibration period (considered as Day 0 of the experiment), four samples per Cr(VI)

treatment were obtained from the pots, air dried, passed through a 2-mm sieve in order to determine the initial hexavalent chromium (Cr(VI)) concentration. After this initial sampling, plants on Day 0 were transplanted into the pots as explained in the subsequent section.

2.2. Plant establishment, measurements and soil and plant analyses

On Day 0, *P. oleracea* plants, already sown in peat-filled seedling trays 25 days before Day 0, were transplanted in the pots; when transplanted, they had reached a height of 12 cm. Pots were then placed in an unheated greenhouse. During the growth period plants were watered according to their needs, and positions of pots were exchanged regularly, to compensate for possible light and temperature differences. In the samples obtained on Day 0, Cr(VI) was extracted using 0.01 M KH_2PO_4, colour was developed with the diphenyl carbazide method and absorption values were determined using a Biochrom Libra S11 spectrophotometer at 540 nm (Brozou et al. 2018). Soil total chromium (Cr(III) + Cr(VI)) was extracted with aqua regia (HCl/HNO_3, 3/1) after a digestion for 2h at 180 ^oC in a Velp DK 20 digestion unit (Golia et al. 2020), and total chromium concentrations were determined using a flame atomic absorption photometer (Perkin Elmer 3300). Trivalent chromium (Cr(III)) concentrations were calculated by subtracting the hexavalent chromium concentration from total chromium (Cr(III) = Cr(III + VI) – Cr(VI)) (Molla et al. 2012).

The growth period lasted for 50 days, from 14 October 2019 (Day 0—commencement of the experiment) to 4 December 2019 (harvest day). On the harvest day, we measured the weight of stems and the leaf area per plant. The plants were cut 2 cm above the soil surface, and the weight of fresh leaves was recorded, as well as the total leaf area. Then the aerial plant tissues were washed with deionized water and placed in a draught-forced oven at 70 ^oC. Roots were meticulously washed so that no soil particles remained attached, rinsed with deionized water, and likewise placed at 70 ^0C. Plant tissues remained in the oven until no further weight loss was recorded, i.e., for 96 h, after which both aerial and root tissues were weighted and pulverized. Then, 1.00 g of plant tissue was dry-ashed at 500 ^oC for 4 hours and extracted with 10 mL of 20% HCl. For plant tissues, K, P, Cr(VI) and Cr (III + VI) content was estimated.

Also, on the harvest day, four soil samples per pot were obtained; the samples were obtained throughout the whole depth of the pots and mixed into one composite sample per pot, so that samples may be as much representative for each particular pot as possible. Samples were then extracted for Cr(VI) and Cr(III + VI) concentrations. Available Cr(III) concentrations were determined with extraction with DTPA-TEA-$CaCl_2$ pH 7.3 (Lindsay and Norwel 1978), and residual soil N (assumed to be in the form of NO_3-N) was determined (Norman et al. 1985). As for the determination of Cr(VI) and Cr(III + VI) in soil, this was performed as described for the soil samples of the Day 0.

2.3. Indices

Apart from soil and plant tissue analysis, Transfer coefficient, Translocation factor, and Bioavailability index were assessed (Antoniadis et al. 2017a, Antoniadis et al. 2018, Buscaroli 2017, Levizou et al. 2019).

For brevity, full details concerning their calculation are reported in Appendix A (Table A1).

2.4. Quality assurance and statistical analysis

For data quality control assurance, certified soil (CRM051 and CRM042 - Labmix24 GmbH, Germany) and in-house plant and soil reference materials were used. Recovery rates of the reference materials were within the range of 95 to 105% of the certified value. In every extraction batch, blank samples were included, in order to rule out any case of cross-contamination. For Cr calibration curves, standard solutions from Merck were used. Every sample was measured in triplicate and samples with coefficient of variation of greater than 15% were discarded and measured again. Statistical analysis of the data was performed using IBM SPSS Statistics 26 and Excel 2019. To identify statistically significance among differences of all treatments, two-way ANOVA and post-hoc Duncan's multiple range tests were performed.

3. Results

3.1. Soil characteristics and Cr(VI) reduction rate

In this experiment we used an alkaline (pH 7.8) soil with high $CaCO_3$ content (10.4%) and an intermediate clay content of 16%. Indigenous Cr(III) concentration was of geogenic origin was 221.3 mg kg^{-1} and that of Cr(VI) below detection limit (Table 1). The evolution of Cr(VI) concentration in soil throughout the growing period is presented in Table 2. In aerated soils, Cr(VI) reduction to Cr(III) is favoured as part of a dynamic system affected from a series of parameters (Antoniadis et al. 2018; Bartlett 1991; Ertani et al. 2017), the most important of which are pH and redox potential. Because of the naturally occurring Cr(VI) reduction process, at the end of our 50-day growing period, Cr(VI) soil concentrations were reduced to 9.72 at T-1, to 15.95 at T-2, to 45.47 at T-3, and to 50.71 at T-4 (units in mg Cr(VI) kg^{-1} of soil; Table 2). In order to illustrate the efficiency of Cr(VI) reduction, we calculated the percentage of Cr(VI) remaining in soil at the end of the experiment in relation to the Cr(VI) concentration on Day 0. We found that in the lower added Cr(VI) rate (T-1, no N), almost half of the added Cr(VI) remained in soil, while the other half evolved to the reduced Cr(III). With increasing added Cr(VI), the percentage of Cr(VI) remaining in soil in the hexavalent species decreased, to the extent that almost 2/3 of added Cr(VI) was reduced to Cr(III). Thus we assume that the higher the added Cr(VI) concentration, the higher the soil potential to cause the reduction of Cr(VI) to Cr(III). As for the added-N treatments, no significant differences ($p = 0.118$) were noticed concerning soil Cr(VI) dynamics, indicating that added N in the form of NH_4^+ (NH_4NO_3) was not in any way in sufficient concentration to become effective electron donor for Cr(VI) reduction, and thus it did not affect Cr(VI) reduction rates.

3.2. Plant uptake of Cr(VI)

In order to assess the potential of *P. oleracea* to be used as a phytoremediation species, we measured the overall Cr(VI) uptake per individual plant. If our assumptions were based solely on the absolute concentration of Cr(VI) in plant tissues, leaving out of the discussion the produced plant biomass under

Cr(VI) stress, the uptake potential of the plant species would not be securely assessed. Thus we assessed the product of the multiplication of Cr(VI) concentration and plant biomass, i.e., Cr(VI) uptake (units in μg Cr(VI) in plant per pot or per kg of soil). A rather expected, the increasing soil Cr(VI) concentrations resulted in higher Cr(VI) uptake both for aerial and root biomass. More specifically, in the treatments of added Cr(VI) beyond T-2, the increase in Cr(VI) uptake was remarkable and nitrogen application at the highest Cr(VI) level (T-4) resulted in a further significant increase ($p = 0.004$), both for aerial (225.9 vs. 125.1 μg Cr(VI) pot^{-1}) and root biomass ($p < 0.001$) (610 vs. 166.8 μg Cr(VI) pot^{-1}) (Table 3).

Table 3
Cr(VI) uptake to aerial and root tissues (μg Cr(VI) per pot) and number of harvests required to annihilate Cr(VI).

Treatments		Aerial tissues	Root tissues	Num. of harvests
No N	T-0	NA	NA	—
	T-1	19.87^{a}	4.44^{a}	489
	T-2	42.06^{a}	22.54^{a}	379
	T-3	68.49^{a}	125.04^{a}	664
	T-4	125.12^{ab}	166.81^{a}	405
				Mean = 484
Added N	T-0	NA	NA	—
	T-1	26.73^{a}	14.30^{a}	161
	T-2	35.37^{a}	12.90^{a}	392
	T-3	90.56^{a}	152.25^{a}	395
	T-4	225.95^{b}	609.98^{b}	256
Treatment effect		$p = 0.008$	$p < 0.001$	Mean = 301
Cr effect		$p = 0.001$	$p < 0.001$	
Nitrogen effect		$p = 0.276$	$p = 0.031$	

Mean ± S. E. Different superscripts denote significant ($p < 0.05$) difference between means in columns according to Duncan's multiple range test. NA: Non applicable.

3.3. Remediation time

The time required for the studied plant to annihilate the added soil Cr(VI) was also calculated. For simplicity and in order to make more conservative estimations, the evolved Cr(III) was not taken into consideration and we thus proceeded estimating the number of harvests required for purslane plants to

fully extract the added soil Cr(VI) (calculated as current soil Cr(VI) concentration at the end of the experiment divided by Cr(VI) uptake). The efficiency of Cr(VI) extraction at the highest Cr(VI) concentration applied to the soil (T-4, 150 mg Cr(VI) kg^{-1} soil) was remarkably high compared to the lower concentrations: 405 harvests were required for the complete extraction of Cr(VI) from soil in the no-N-added treatment vs. 256 harvests for the N-added treatment. Overall, nitrogen addition greatly reduced the number of harvests required for the complete extraction of soil Cr(VI) (Table 3).

3.4. Indices of soil-to-plant mobility (TC), bioavailability index (BAI) and translocation factor (TF)

We calculated the soil-to-aerial plant tissues Cr(VI) transfer coefficient, which, for reasons of clarity, is reported multiplied with a factor of 1000. We found that the upward transfer from soil to the aerial tissues of purslane was significantly affected from Cr(VI) soil concentrations ($p = 0.004$), with the highest index values being observed at T-1 and the lowest at T-3 (Fig. 1a); the effect of nitrogen was not significant ($p = 0.854$). The values of soil-to-root transfer index were 10-to-100-fold greater than those of the aerial tissues (TC_{aerial}); with increasing Cr(VI) soil concentrations, TC_{root} gradually increased ($p < 0.001$), irrespective of added N ($p = 0.302$). On the combined effect of Cr(VI) and N on TC_{root}, it is evident that at T-4, roots absorbed more Cr(VI) and upon nitrogen application TC_{root} values was almost doubled ($p < 0.001$) (Fig. 1b). These results indicate that Cr(VI) uptake from roots is concentration-dependent and nitrogen addition had a positive effect on Cr(VI) root absorbance, as it evidently increases plant vigor and thus its ability to absorb Cr(VI).

Bioavailability index (BAI) indicated that neither Cr(VI) soil concentration ($p = 0.116$) nor nitrogen addition ($p = 0.153$) exerted significant effect in it. Our data suggest a decreasing trend with increasing Cr(VI) concentrations from T-0 to T-3, indicating that plants effectively limited the uptake of Cr(VI) up to the level of added Cr(VI) corresponding to T-3, while in higher Cr(VI) levels plant mechanisms that limit Cr(VI) uptake proved rather insufficient (Fig. 1c). Translocation of Cr(VI) from roots to shoots (reported for clarity in values multiplied with 1000, similar to transfer coefficient) was notably lower at T-3 and T-4 compared to T-1 and T-2. In the highest Cr(VI) soil concentrations, TF was remarkably low, indicating that *P. oleracae* under high Cr(VI) stress manages to effectively limit Cr(VI) translocation from root to the aerial tissues. The effect of nitrogen on TF values was not significant ($p = 0.128$) (Fig. 1d).

3.5. Chromium speciation in plant

In aerial plant tissues, low values of the Cr(VI)/Cr(VI + III) ratio were observed, indicating that Cr was almost exclusively found in its trivalent form. In the high added Cr(VI) concentrations (T-3 and T-4), a decreasing trend was noticed for Cr(VI)/Cr(VI + III) ratio, indicating that defense mechanisms resulting in Cr(VI) reduction, were triggered in response to excessive Cr(VI) stress. On the other hand, the Cr(VI)-to-total Cr ratio in roots reached noticeably higher values. Contrary to what was found for the aerial parts, increasing added soil Cr(VI) concentrations resulted in higher Cr(VI)/Cr(VI + III) ratio values ($p < 0.001$) and

nitrogen amendment also resulted in significantly higher values when combined with the highest (T-4) Cr(VI) soil concentration ($p < 0.001$); otherwise, nitrogen had a non-significant effect ($p = 0.117$) on the plant Cr(VI)/Cr(VI + III) ratio (Fig. 2).

3.6. Soil residual nitrogen

As added Cr(VI) increased, soil concentrations resulted in lower N uptake ($p < 0.001$) (Table 4), resulting in the accumulation of N at the end of the experiment. Low N uptake from plants under Cr(VI) stress may be due to the reduced plant growth rate that subsequently led to lower N demands.

Table 4
Residual soil concentration at harvest.

Treatments		Soil residual N (as NO_3^--N) (mg kg^{-1})
No N	T-0	11.6 ± 0.93^{a}
	T-1	45.9 ± 2.53^{b}
	T-2	39.9 ± 2.28^{b}
	T-3	56.0 ± 1.11^{bc}
	T-4	55.7 ± 2.96^{bc}
Added N	T-0	17.3 ± 1.59^{a}
	T-1	75.8 ± 3.62^{cd}
	T-2	81.2 ± 5.99^{d}
	T-3	96.5 ± 6.15^{d}
	T-4	122.4 ± 11.15^{e}
Treatment effect		$p < 0.001$
Cr effect		$p < 0.001$
Nitrogen effect		$p < 0.001$

Mean ± S. E. Different superscripts denote significant ($p < 0.05$) difference between means in columns according to Duncan's multiple range test.

4. Discussion

Cr(VI) reduction to the less toxic Cr(III) during our 50-day growing period of *P. oleracea* was as high as 79.2% of the initial added Cr(VI) soil concentration. This is in agreement with various works confirming that after spiking, Cr(VI) is reduced under normal soil conditions relatively fast. Soil Cr(VI) reduction rates have been reported to be largely dependent on soil conditions such as moisture, temperature pH and redox potential (Bartlett 1991; Kozuh et al. 2000).

Cr(VI) found in aerial and tissues increased with rising soil added Cr(VI) concentrations and N addition seemed to have a positive effect in plant uptake of Cr(VI). The fact that root tissues of the well-fertilized *P. oleracea* absorbed significantly higher Cr(VI) quantities from the soil compared to the non-fertilized may indicate an enhanced ability of the test plant to accumulate more Cr(VI) when nitrogen is applied (Marciol et al. 2007; Martinez-Trujillo and Carreón 2015; Yao et al. 2020). Nitrogen alleviating effects of Cr(VI) stress have also been reported in a work concerning *Arabidopsis thaliana* plants (Ortiz Castro et al. 2007).

Our results also indicated that our test plant effectively extracted soil Cr(VI) even at the highest added soil concentration (T-4), despite the fact that Cr(VI) stress significantly reduced growth rate. Nitrogen amendment had a positive effect in Cr(VI) uptake per plant that was largely attributed to the higher levels of biomass production. These results are in agreement with the findings of other similar works (Marciol et al. 2007; Martinez-Trujillo and Carreón 2015; Nagarajan and Sankar Ganesh 2014).

Compartmentalization of potentially toxic elements in plant tissues is of great importance, contributing to the plant capacity to address stress induced by toxic elements (Ertani et al. 2017; Shanker et al. 2005). In the present study, we observed that the translocation of Cr(VI) from roots to shoots (as noted in the TF) was notably lower at T-3 and T-4 compared to those at T-1 and T-2. In the highest Cr(VI) soil concentrations, low TF index values indicated that our test plant under high Cr(VI) stress manages to effectively limit Cr(VI) translocation from roots to the aerial tissues, while the effect of N on TF values was found to be non-significant (Fig. 1). As for the TC for aerial tissues, it was orders of magnitude lower than the corresponding values of roots. These findings indicate that plants effectively managed to limit the influx of Cr(VI) to the plant tissues and to retain the absorbed Cr(VI) to roots, although under severe Cr(VI) stress. The TF values confirm the results of TC, indicating that purslane defense mechanisms prohibited Cr(VI) translocation to aerial tissues, even though Cr(VI) was readily absorbed by root tissues. Cr accumulation in roots and the minimal transport to aerial tissues has been reported as a defense mechanism for Cr toxicity in a number of other similar studies concerning other plant species (Antoniadis et al. 2017a; Marciol et al. 2007; Moral et al. 1995; Shanker et al. 2005; Singh et al. 2013). These findings render the use of *P. oleracea* as an attractive option for phytoremediation, although both TC and TF were much lower than unity, contrary to the requirement of some researchers demanding TC and TF values of higher than unity so that a plant species may be categorized as accumulators (Antoniadis et al. 2017a; Antoniadis et al. 2021; Buscaroli 2017; Ertani et al. 2017; Moral et al. 1995).

As for the Cr speciation in plant tissues, our results indicate that in aerial tissues, increasing soil Cr(VI) concentrations seem to activate plant enzymatic and non-enzymatic mechanisms that effectively reduce Cr(VI) to the less toxic Cr(III). In soil, the extractability of Cr(III) with DTPA was very low. The available

concentration was 1.0 mg Cr(III) kg^{-1}, while total (aqua regia) Cr concentration was 287 mg Cr kg^{-1}. The control plants had non-detectable Cr levels. The aforementioned low Cr(III) phytoavailability combined with the known minimal absorption of Cr(III) from plants can lead us to the assumption that Cr(III) found in plant tissues was almost exclusively absorbed as Cr(VI) and subsequently reduced to Cr(III) within the plant. In root tissues, rising soil Cr(VI) concentrations resulted in higher Cr(VI) concentrations, indicating that the reduction capacity of roots was surpassed, resulting in an increased Cr(VI)-to-Cr(VI + III) ratio compared those of the aerial parts. These findings are in accordance with a series of other similar works reporting that enzymatic and non-enzymatic mechanisms in plant cells readily reduce Cr(VI) (Antoniadis et al. 2018; Ertani et al. 2017; Levizou et al. 2019; Paiva et al. 2008).

Concerning residual N, our results are in agreement with previous findings which suggest that Cr(VI) toxic effects result in reduced root growth that subsequent limits nutrient uptake. Furthermore, it is known that Cr(VI) interferes with nitrate transporters and through antagonistic effects may result in decreased N uptake from plants (Sundaramoorthy et al. 2010, Ortiz Castro et al. 2007).

5. Conclusions

- The potential of *P. oleracea* to absorb Cr(VI) increased with added N, likely as a result of the enhanced plant vigor due to sufficient fertilization.
- Nitrogen, except for the overall benefit of increased plant vigor, resulted in higher efficiency of Cr(VI) plant uptake. This was reflected in enhanced uptake (measured as quantity in μg of Cr(VI) in plant per pot, rather than tissue concentration); this meant that a complete site clean-up could be achieved within 256 harvests in the high Cr(VI) added rate at T-4 (vs. 405 harvests at non-N-added T-4).
- *P. oleracea* tolerated relatively high Cr(VI) concentrations and effectively remediated the Cr(VI)-spiked soil despite being under severe stress. However, plants failed to meet the requirements frequently used in assessing plants as accumulators (concerning TC and TF).
- Soil Cr(VI) was readily reduced to Cr(III), resulting in a decreased estimated remediation time. It is not fully known if the rate of reduction of Cr(VI) towards Cr(III) will continue past our 50-day growing period due to stress symptoms. Thus, more research is necessary in order to assess the Cr(VI) long-term soil dynamics and to evaluate the required time needed for Cr(VI) remediation of a contaminated site.
- Taking into consideration (a) plant Cr(VI) uptake and (b) Cr(VI) reduction naturally occurring in soil, we conclude that the latter is the predominant mechanism of soil Cr(VI) elimination.
- We conclude that *P. oleracea* may be used as a tolerant plant species in the process of remediating a Cr(VI)-contaminated soil, especially if assisted with a sufficient level of N fertilization, although the plant falls short from the threshold of being categorized as an accumulator species.

6. Declarations

Ethical Approval: Notapplicable

Consent to Participate: Not applicable

Consent to Publish: Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions: Author Contributions: Conceptualization, V.A. and S.A.P.; methodology, V.A. and S.A.P.; software, G.T and E.N.; validation, G.T and E.N.; formal analysis, V.A. and S.A.P.; investigation, G.T and E.N.; resources, G.T and E.N.; data curation, G.T and E.N.; writing—original draft preparation, G.T and E.N.; writing—review and editing, V.A. and S.A.P.; visualization, G.T and E.N.; supervision, V.A. and S.A.P.; project administration, V.A.; funding acquisition, V.A. and S.A.P. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: "The authors declare no conflict of interest."

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Figures

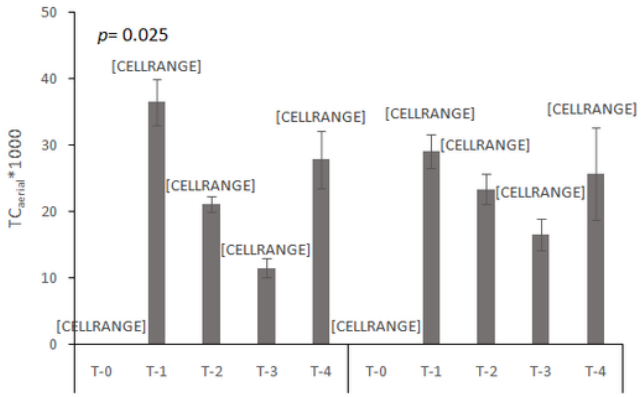

Figure 1a

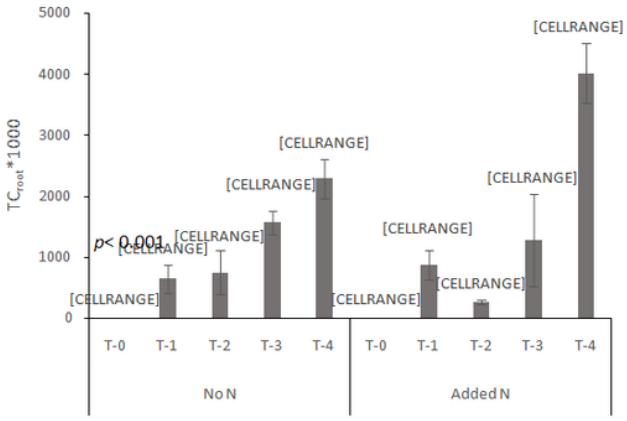

Figure 1b

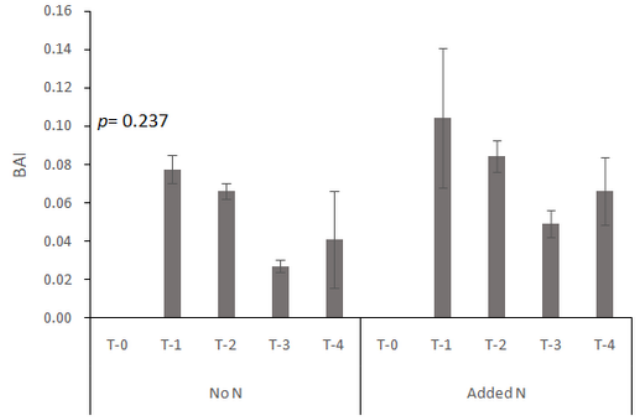

Figure 1c

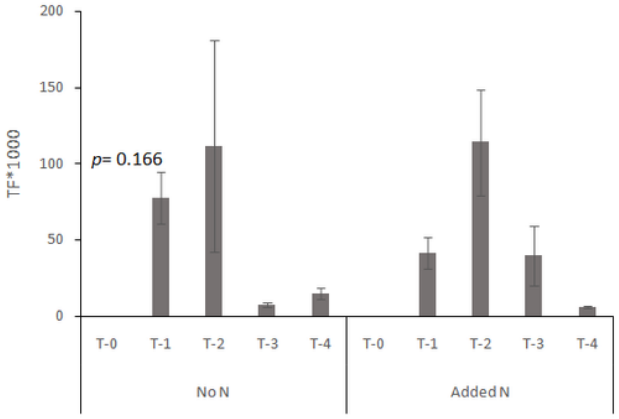

Figure 1d

Figure 1

Indices concerning Cr(VI) mobility in soil, root and aerial tissues: (a) TCaerial x 1000; (b) TCroot x 1000; (c) BAI; and (d) TF x 1000. Different letters denote significant ($p<0.05$) differences among means in columns according to Duncan's multiple range test.

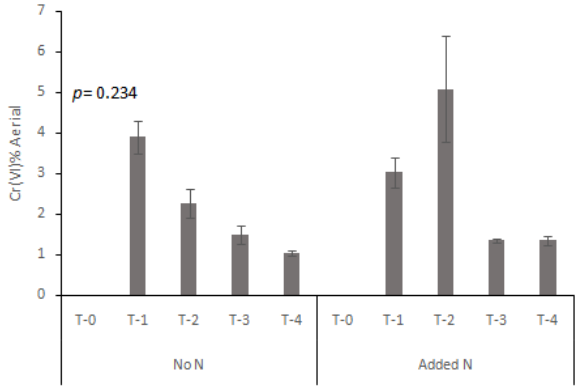

Figure 2a

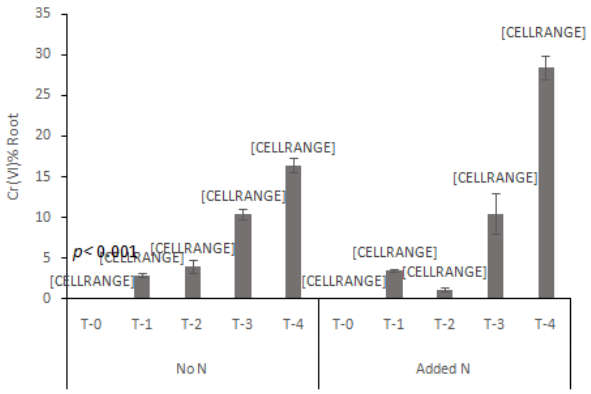

Figure 2b

Figure 2

Percentage of Cr(VI) relative to Cr(III)+Cr(VI) (100x(Cr(VI)aerial/Cr(VI+III)aerial), (a) in aerial plant tissues, and (b) in root tissues (100x(Cr(VI)root/Cr(VI+III)root). Different letters denote significant ($p<0.05$)

differences among means in columns according to Duncan’s multiple range test.

Supplementary Files

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