

Translocation and bioconcentration of trivalent chromium in green beans grown on bioponics

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Abstract: The increasing number of cases of soil contamination by trace elements have affected crop production, and represents a risk threatening the quality of our food products. Some of these contaminants, such as trivalent chromium Cr (NO₃)₃, which is similar to micronutrients, can, therefore, be absorbed by plants and whose phytotoxicity has long been considered negligible, and largely underestimated. The purpose of this work was to study the transfer of trivalent chromium from nutrient solution to green beans *Phaseolus vulgaris L* grown on bioponics; the contamination responses were determined in terms of growth parameters, yield, and dry matter production; at various concentrations (5, 10 and 20 ppm). Chromium trivalent effects have also been studied in tissues plant. Results showed that the absorption of trivalent chromium from the nutrient solution and its translocation to the aerial tissues plants had no adverse effects on growth parameters, and also on beans yield. Results also showed that chromium accumulates in roots rather than in the other tissues, and did not reduce the dry matter production, in terms of translocation and bioconcentration. The transfer factor is low and green beans cannot be defined as a hyperaccumulator of chromium.

Keywords: Bioconcentration; Green beans; Nutrient solution; Translocation; Trivalent Chromium.

1. Introduction

Chromium toxicity and its negative effects on plants were widely observed in many studies on different plants. Naturally, chromium is found in many oxidation states, in which trivalent (Cr III) and hexavalent (Cr VI) are considered the most stable. Many studies declared that Cr (VI) is regarded as the most toxic for plants, because of its mobility and bioavailability, when its concentration exceeds the limits it leads to a decrease in the biomass, crop quality and yield production, low chlorophyll content and high production of reactive oxygen species (ROS) ¹. At the same time, Cr (III) appears to be relatively non-toxic, but at high pH and temperature, trivalent can be converted to the hexavalent chromium. Chromium (III) is a natural element found in nature, in rocks, soil, volcanic dust and gases, It is used in several industrial applications such as tanning industry, leather processing electroplating, resulting in the discharge of chromium-containing effluents. This ultimately causes a significant elevation in Cr contents in the environment ². Due to its high solubility in water and agricultural soils, chromium is regarded as a hazardous ion that contaminates groundwater and

can be transferred through the food chain ³, and cause serious health problems.

Green beans are the third most significant feed legume after soybean and pea are an essential pulse crop. It is considered one of the major food legume crops in the Latin America, Middle East, and the Mediterranean region, it is adapted to grow in a broad zone of climates, and it is a perfect rotation crop because its roots are associated with nitrogen-fixing bacteria ⁴.

Therefore, using bioponics as a cultivation method, this work allowed us to study and analyze the different aspects of responses of green bean plants, contaminated through their root system in contact with trivalent chromium in a nutrient solution. The roots are physically supported by an inert medium and a thin layer of organic potting soil; it is a selective radiation interface in which free root hair allows better hydration of the plant and sustained growth. The biotop system promotes better bioavailability of mineral elements by optimizing the living environment of beneficial microorganisms. The nutritive solution is a suitable environment for the availability of metals, favoring their transportation to the cultures. Therefore it allows

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DOI: <http://dx.doi.org/10.13171/mjc1070200815051a>

Received June 22, 2020

Accepted July 9, 2020

Published August 4, 2020

more robust studies on translocation, absorption kinetics, and redistribution of minerals in plants. Moreover, to our knowledge, this method cannot be found in the literature. The present study was conducted to assess the influence of chromium on development, yield and dry matter production of *Phaseolus vulgaris* L., and to assess the residual content of this metal in tissues plant, to evaluate translocation and bioconcentration of trivalent chromium in beans plant.

2. Materials and Methods

2.1. Experimental design and treatments

Plants were grown in a greenhouse, utilizing biotop device, during 65 days, relating to the vegetative cycle of bean plants. After a week of development in a pot filled with potting soil, seedlings of the same length are transplanted into biotop boxes, filled with a layer of experiment soil (5 liters of potting soil per box), previously positioned on 4 liters of the inert medium which is cork granulate (Figure 1).



Figure 1. Biotopics system used to grow beans

Plants were daily irrigated based on their water needs with a nutrient solution prepared once every week using distilled water and nutrient solution, and the irrigation was practiced once a week. The pH value and the electrical conductivity of the nutrient solution used were 6.2 and 2.4 (ms/cm) respectively, while its mineral composition was as follows:

K₂O (potassium oxide); P₂O₅ (phosphoric anhydride): 6% for each and both are soluble in water. 6% total nitrogen (N) of which: 2.1% nitric nitrogen (N); 1.4% ammoniacal nitrogen; 2.5% urea nitrogen (N). Water soluble trace elements: 0.01% Boron (B); 0.002% Copper (Cu) chelated by EDTA; 0.05% Iron (Fe) chelated by EDTA; 0.02% Manganese (Mn) chelated by EDTA; 0.001% Molybdenum (Mo); 0.0125% of Zinc (Zn) chelated by EDTA. 6.6.6).

The treatments constituted of increasing concentrations of chromium (5, 10 and 20 ppm) added to the nutrient solution. We used a chromium nitrate as a contaminant Cr(NO₃)₃. The protocol consists in adding the increasing concentration of chromium to nutrient solution; these contents (5, 10, and 20 ppm) allow us to obtain enough vegetal material for our laboratory experience. These values intoxicate plants without eliminating plants during its development. Control treatment plants were sprayed with nutrient solution solely.

We used 8 biotop devices in total; every two devices are reserved for the same concentration (5, 10, or 20 ppm), each device contains 2 plants of green bean, and the two terminal devices are reserved for

the control. The experiment was carried out in replicates of three (Figure 2).

Contaminated plants, as well as control plants, are measured using a meter rule, from the first week of transplantation until the fruit harvest. The same goes for branches and leaves; their number was counted at transplantation and 1-week interval for 4 weeks when plants were harvested. Regarding fruits, their number was immediately counted at onset flowering and fruit production. Each treatment was independently run in triplicate of three.

2.2. Sample preparation

Samples of water and nutrient solution were placed in polyethylene bottles recently washed with soap. An acid (10% HCl), lastly, flushed a few times with distilled water. After that, samples were fixed with 2% HNO₃, and afterward, they are filtered with Whatman 40 filter paper. The filtrate has been put away at 4°C until the metals analysis.

Samples of potting soil were extracted from the surface, dried in an oven (105°C.) for 24 hours, and then ground in Agathe's mortar and homogenized. And in Teflon bombs of 30 ml, we add 1 ml of the hydrochloric acid (30%) - nitric acid (65%), 3/1; V/V, and 6 ml of pure Norma hydrofluoric acid to 0.2 g of soil. Lastly, all bombs remain intact for 24 hours for pre-digestion. They are then heated at 120°C in a thermostatic sand bath for 4 hours. After cooling, using bidistilled water, the volume is completed to 50 ml in dilution tubes containing 2.7 g of boric acid ⁵.

Green bean plants were rinsed beforehand with deionized water; the different parts (roots, stem, leaves, and fruits) of all plants are separated, cut into pieces, and then dried in an oven at 65-70°C until constant mass. The material was ground and digested using a 2:1 (v/v) nitroperchloric solution [nitric acid (HNO₃): perchloric acid (HClO₄)]. For all plants including control plants, The contents of chromium

in dry matter of root system (DMR), of the stem (DMS), of leaves (DML), and fruits (DMF), were quantified using a Varian flame absorption spectrophotometer, with acetylene flame and hollow cathode lamp. Concerning the metal analysis of all samples it was performed using ICP AES atomic emission spectrometry (Ultima 2 – Jobin Yvon).



Figure 2. Green bean plants in biotop device

2.3. Statistical analysis

Statistical analysis was carried out using the software package SPSS v21.0, and the comparison of averages of each treatment was based on the analysis of variance (ANOVA) and Duncan's multi-range test at significance level 5%. According to DMRT at $p \geq 0.05$, in a column, the means followed by the same letter do not differ significantly.

3. Results and discussion

3.1. Effects of trivalent chromium on growth parameters of green beans

The organic potting soil is the type of soil that was used in this experiment, whose physicochemical

characteristics are presented in Table 1, the values of all parameters indicating that the soil was suitable for the cultivation of beans plants, according to AFNOR standard NF U 44-051. The results of the impacts of chromium contamination on green beans execution estimated for about a month (4 weeks) after including contaminants appear in Tables 2, 3, and 4.

It is well known that chromium is not an essential element for plant growth, nonetheless, above specific concentrations; it becomes toxic and gets harmful for most plant species⁶. According to this research, growth parameters were not statistically significant compared with control samples.

Table 1. Physicochemical characteristics of potting soil.

Parameter	Value	Standards (Afnor NF U 44-051)
N total (%)	2,50	<3
P ₂ O ₅ (%)	0,72	<3
K ₂ O (%)	1,68	<3
CEC (meq/100 g)	34,67	-
Organic matter (%)	80	≥ 30
pH (H ₂ O)	6,4	-
Calibration (mm)	0-20	-
Water content (%)	54	-
EC (ms/m)	45	-

Table 2. Trivalent chromium effect on stem height.

	1 st week	2 nd week	3 rd week	4 th week
5 ppm	5.30 ± 1.18a	12.07 ± 1.56a	15.21 ± 3.12a	15.21 ± 3.12a
10 ppm	6.07 ± 1.74b	13.44 ± 1.66b	16.01 ± 3.17b	16.01 ± 3.17b
20 ppm	5.15 ± 1.32a	12.08 ± 1.22a	15.03 ± 2.97a	15.03 ± 2.97a
C	5.32 ± 1.40a	12.12 ± 1.77a	15.33 ± 3.03a	15.33 ± 3.03a

Table 3. Trivalent chromium effect on leaves number.

	1 st week	2 nd week	3 rd week	4 th week
5 ppm	8.54 ± 2.12a	12.44 ± 2.77a	14.32 ± 3.55a	14.32 ± 3.55a
10 ppm	8.02 ± 2.07a	13.35 ± 2.79b	15.33 ± 3.82b	15.33 ± 3.82b
20 ppm	8.42 ± 1.69a	12.90 ± 2.67a	14.05 ± 1.82a	14.05 ± 1.82a
C	8.22 ± 2.34a	13.11 ± 2.65b	14.67 ± 4.22a	14.67 ± 4.22a

Table 4. Trivalent chromium effect on branches number.

	1 st week	2 nd week	3 rd week	4 th week
5 ppm	13.43 ± 1.07a	14.55 ± 3.02a	14.76 ± 3.02a	14.76 ± 3.02a
10 ppm	13.32 ± 1.02b	15.78 ± 3.71b	15.07 ± 3.71b	15.07 ± 3.71b
20 ppm	13.40 ± 0.98a	14.32 ± 3.14a	14.34 ± 3.14a	14.34 ± 3.14a
C	13.21 ± 1.30a	14.78 ± 3.23b	15.16 ± 3.23b	15.16 ± 3.23b

The mechanisms induced by plants in response to the exposure to trivalent chromium showed that stem height, number of leaves, and branches were not statistically significant compared with control samples (Tables 2, 3, and 4). On the one hand, it was quoted that some plants can tolerate chromium stress below and show no significant effects. On the other hand, chromium shows physiological, biochemical, length, fresh weight, dry weight, and leaf area decrease. This was observed in many plants, such as *Brassica napus* ⁷. The same results were also confirmed by ⁸; chromium affected growth of leaves of *C. procerca* the main photosynthetic plant organ, increasing chromium concentration leads to a significant reduction in the leaf area and leaf biomass. In general, the reduction in plant height could be fundamentally due to reduced root growth

and the transport of nutrients and water to the aerial parts of the plant. Also, the transport of chromium to the aerial part of the plant can directly affect the cellular metabolism of shoots contributing to the reduction of plant height. As chromium is a nonessential element, no specific mechanism is associated with it in plants. It possesses the high potential to disturb nutrient uptake by plants. Cr (III) usually involves a passive mechanism, and plants require no energy for this phenomenon ⁹. It is supposed that a competitive effect (antagonism) between chromium and some essential elements. The amounts of chromium utilized facilitate a higher absorption of Fe, which remains necessary for the synthesis of chlorophyll and forming a lot of vegetative tillers; that is what explains the high number of leaves in 10 ppm compared to the control.

Table 5. Trivalent chromium effect on fruits number.

	Harvest 1	Harvest 2	Harvest 3
5 ppm	7.20 ± 1.72a	4.11 ± 2.20a	2.89 ± 1.53a
10 ppm	7.02 ± 1.07b	5.13 ± 2.07a	3.55 ± 0.42b
20 ppm	7.09 ± 1.42a	4.20 ± 2.04a	3.02 ± 0.65b
C	7.46 ± 1.66a	5.22 ± 2.31a	3.31 ± 1.75b

3.2. Effects of trivalent chromium on the yield of green beans

Since varieties of seeds have differing maturity rates, it is not a hard and fast rule you can rely on it.

However, in most cases, bean pods will begin to appear about two weeks after the plant sets blossoms. All pods ripen almost at the same time, with an interval of 2 or 3 days between the three

harvests, the results showed that there was no significant difference in fruits number for all treatments compared to the control. It is realized that Cr (III) is kinetically inert at low portions, which reduces its role in the development of harmful impacts especially in shoots; this has been affirmed by ¹⁰. That more than 95 % of chromium is absorbed and stays in roots, and a low concentration reaches fruit, which had no harmful effect on the plant's yields (Table 5).

3.3. Translocation and bioconcentration of chromium in green beans

The potential of plants in uptaking chromium from the nutritive solution was estimated by the phytoextraction coefficient or transfer factor, t , utilizing the following relation:

$$t = \frac{\text{total metal in plant}}{\text{metal in nutrient solution}}$$

The calculations were performed by considering the contents of chromium in dry matter of (roots, stem, leaves, and fruits) DMR+DMS+DML+DMF, in all

concentrations used; the larger this factor, the larger the contaminant absorption ¹¹. The relative production index (RP), related to the influence of the metal on the variation of dry matter production (DMR+ DMS+ DML), is obtained by:

$$RP(\%) = \left(\frac{\text{dry matter produced using a given metal content}}{\text{dry matter produced with metal absent}} \right) \times 100$$

The average results were submitted to Tukey's test (5% probability).

The first essential for more significant returns in quite a while is an increase in the production of biomass in terms of dry matter. Carbon compounds represent 80 to 90% of the total dry matter produced by plants. A larger source size and an increased photosynthetic process have proven to be the basis for the accumulation of organic substances and the production of dry matter under the stress of trace elements in general and chromium in particular ¹².

Table 6. Production of dry matter and relative production index in tissues plant in the presence of trivalent chromium.

Treatment (ppm)	DM (weight in g)				RP index (%)			
	DMR	DMS	DML	DMF	DMR	DMS	DML	DMF
5	2.58	3.08	3.16	2.50	93.81	100.00	98.41	114.67
10	2.46	3.11	3.17	2.63	89.45	100.31	98.73	120.64
20	2.32	3.02	3.03	2.13	84.36	98.37	95.88	97.70
C	2.75	3.07	3.16	2.18	100	100	100	100

According to the results of Table 6, which represents the production of dry matter in plant tissues of green beans in the presence of chromium, the control treatment presented the best development. The highest fruit production was obtained at 10 ppm Cr, which is about 14% higher than the control treatment, the 5 and 10 ppm have positively influenced the productivity of the green bean plants, increasing by 14% and 20% compared to the control

treatment. The production of the aerial parts did not change for 5 and 10 ppm compared to the control plants. For the highest dose (20 ppm), the dry matter of the aerial portion was not negatively affected (Figure 3). According to ¹³, the contents of Fe, Mg, and Mn (essential elements) increased both in the roots and the shoots of *Pennisetum sinense* under treatments with chromium between 0,9 ppm and 18 ppm.

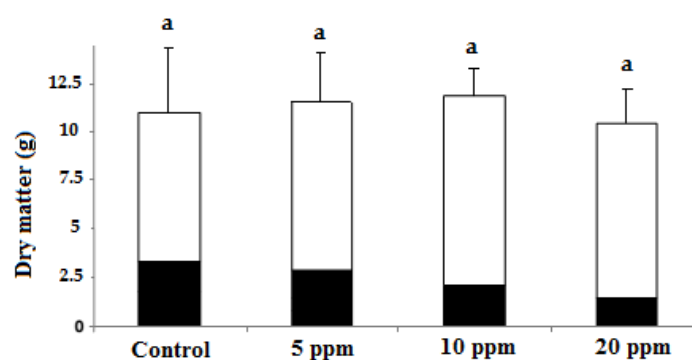


Figure 3. Dry matter production of green beans

But the increase of Mg and Mn was not significant; the authors also showed that there was no visible effect in the shoots under all chromium treatments from 0,9 ppm to 36 ppm. The phenomenon whereby low concentrations of a heavy metal promote plant growth is defined as hermetic effect and frequently occurs in treatments with trace elements ¹⁴

The translocation index (TI) gives the capability of species in translocating trivalent chromium from root to the aerial portion ¹⁵.

$$TI = [DMAP / (DMR + DMS + DML + DMF)] \times 100$$

$$TI = [DMF / (DMR + DMS + DML + DMF)] \times 100$$

DMAP: dry matter in aerial portion (stem+leaves+fruits); DM: dry matter production; R: roots; S: stem; L:leaves; F:fruits.

According to the results, it can be concluded that chromium moves very poorly in all green bean plants, given the significant difference in contaminant found in the three compartments of the plant. For the aerial parts, the higher the concentration increases the more the amount of chromium translocated decreases with 42.96%, 38.88, 27.18%, unlike the leaves and fruits whose concentration increases with 7.60%; 12.64%; 9.40%, for leaves and 3.80%; 4.21%; 6.58% for fruits, and this for 5, 10 and 20 ppm respectively. The highest translocation index recorded for the fruits was calculated at 20 ppm (6.58%), followed by 10 ppm (4.21%), and 5 ppm (3.80%). The greatest translocation to fruit was found for the high concentration applied (20 ppm), decreasing with the decrease in the availability of the metal. The transfer factor *t* had a direct relationship with TI for fruit (0.75) (Table 7).

Table 7. Translocation of chromium from roots to aerial parts of green beans.

Treatment (ppm)	Content (mg/kg)				TI (%)			
	Roots	Stem	Leaves	Fruits	Stem	Leaves	Fruits	t (%)
5	0.036	0.0339	0.006	0.003	42.96	7.60	3.80	1.19
10	0.042	0.0369	0.012	0.004	38.88	12.64	4.21	1.25
20	0.0604	0.0289	0.010	0.007	27.18	9.40	6.58	0.75
C	0	0	0	0	0	0	0	0

Depending on the specific metal being translocated, green beans could not be considered as a high potential phytoextractor, since the *t* values obtained were less than 1,7 ¹⁶. Researchers have found that increased xylem loading is the main factor affecting the absorption and translocation of trace elements from root to shoot. After absorption by root hairs, chromium is poorly transported to other parts of the plant. It is stored mainly in roots as an essential protection, making phytoextraction difficult for some plants such as beans ¹⁰.

Quantity of heavy metals absorbed by the whole plant from nutrient solution, was estimated by using the bioconcentration factor (BCF), This is an index of the capacity of the plant to accumulate a specific metal regarding its concentration in the nutrient solution and is obtained by ¹⁷:

BCF = Concentration of metal in the tissues of the whole plant / Initial concentration of metal in nutrient solution

The higher the BCF value, the more appropriate is the plant for phytoextraction. BCF values > 2 were viewed as high values.

The ability of green beans to absorb chromium was not visible even at a high concentration (20 ppm). In our study, the ability of plants to uptake chromium from nutrient solution and translocate it from roots to the aerial parts was limited with a BCF, which is less than 2 (Table 8). The study also shown that most chromium absorbed was stored in the roots, and a minimal amount was transferred to the aerial parts (Figure 4). These results are in agreement with those of ¹⁸; the authors found that water lettuce can tolerate concentrations that can reach the value of 80 ppm in the presence of chromium (III).

Table 8. Bioconcentration of chromium in beans plants.

concentration (ppm)	Roots	Stem	Leaves	Fruits	BCF
5	0.036 ± 0.04	0.033 ± 0.10	0.006 ± 0.04	0.003 ± 0.03	0.015
10	0.042 ± 0.05	0.036 ± 0.06	0.012 ± 0.03	0.004 ± 0.12	0.009
20	0.060 ± 0.01	0.028 ± 0.11	0.010 ± 0.16	0.007 ± 0.01	0.005

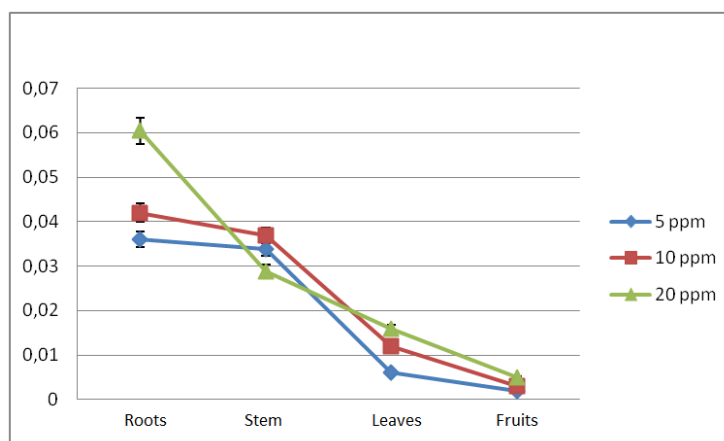


Figure 4. Bioconcentration of chromium in roots, stem, leaves, and fruits of green beans

Many studies have shown that silicon treatment shows positive behavior against chromium toxicity by reducing chromium uptake, improving the antioxidant defense¹⁹. Other authors demonstrated that phyto-melatonin considered as an antioxidant under abiotic stresses, including heavy metal exogenously applied 50 $\mu\text{mol/kg}$ and 100 μmol to *Galinsoga parviflora* showed improved growth and photosynthetic efficiency^{20,21}. Many studies also showed that mycorrhizae *Arbuscular mycorrhizal* are capable of surviving and proliferating in chromium contaminated soil. Its symbiosis lowers chromium uptake by plants even at deficient concentrations in soil²², and mycorrhizal symbiosis may also reduce chromium transport from lateral roots to main roots, and decreased chromium translocation factor²³.

4. Conclusion

In this study, we found that the growth of green beans was not inhibited by relatively high concentrations of chromium, indicating that *Phaseolus vulgaris L* had a relatively high tolerance to chromium. The metal is sequestered and transported to the aerial parts. The translocation factor (TF) of trivalent chromium in each of the three different concentrations (5, 10, and 20 ppm) showed that chromium accumulates and translocates to the shoots. Still, the higher contents were only stored in the roots, while fruits collected the lowest contents. In terms of bioconcentration, this study shows that green beans cannot be defined as a hyperaccumulator of chromium since the absorption of this metal by plants was limited with a value of BCF <2. The present study has also demonstrated that in bioponics, trivalent chromium has no adverse effect on performance. On the production of green beans, the stem height, the number of leaves, branches, and fruits are not affected significantly.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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