Effect of Chromium VI on edible plants and their health risks: case of Radish (*Raphanus sativus* L.)

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Abstract. Radish (*raphanus sativus* L) is a vegetable very rich in vitamin C and fiber, this plant belonging to the family Brassicacae characterized by their great capacity to accumulate heavy metals such as Chromium. The aim of our work is the study of the effect of Chromium VI on the morpho-physiological parameters of radish and the assessment of health risk related to the bioaccumulation of Cr in the edible parts. The plantation of radish was made on a soil artificially contaminated by 4 concentrations of Cr(VI) (10, 20, 40 and 60ppm). After comparing the results obtained with the results of non-contaminated soil, it is observed that the Cr affects negatively the growth, yield and the content of chlorophyll, On the other hand, it is noticed that there is a slight increase of sugars, proteins and Proline content with the increase of CrVI concentration in the soil, we can explain this increase by the development of defense mechanisms by the radish plant against the stress caused by CrVI. Regarding the bioaccumulation of Cr we found that the concentration of Cr in different parts of radish is too high compared to the recommended daily dose (120µg), so it is not recommended to consume radish grown in areas contaminated by Cr.

1 Introduction

The human and industrial activities have led to contamination of soils by various pollutants such as heavy metals, contamination of agricultural soils leads to losses in agricultural production and yield, and an hazardous effects on humain and animals health. Large soils all over the world are contaminated with heavy metals [1], the strong global demand for agricultural production can lead to use the contaminated or marginal soils and increase the risk of food contamination, each metal act in a different way depending on their role in cell metabolism. Microelements, such as zinc, are essential and participate in many physiological processes [2]. However, at optimal doses they are highly toxic and inhibit plant growth. Heavy metals such as chromium are toxic even at low concentrations [3]. The main sources of chromium contamination in leather processing, landfill leachate, Anthropogenic activities, industrial effluents, automobile exhausts, waste incinerators, metal plating and finishing operations, pesticides and fertilizers manufacturing, cement factories, wood processing, metallization, oxidative dyeing, metal finishing, tobacco emissions, acid manufacture chromic, cement plants and paper production [1, 4, 5]. metal stress-induced reductions in plant growth parameters may be ascribed to low water potential, nutritional imbalance, and reactive oxygen species (ROS) mediated oxidative stress [6]. [7] argued that plants exposed to high concentrations of trace elements synthesized less chlorophylls, carotenoids, and other photosynthetic pigments. In addition, as a self-defense mechanism, plants have antioxidant systems to remove ROS. However, in the case of severe toxicity, this defense system may not be able to mitigate the toxic effects that have resulted in reduced plant functioning and normal growth [8]. Some edible plants, including family of Brassica plants, are characterized for their ability to accumulate large quantities of heavy metals such as radish. This has led to the search for species that can be used for phytoremediation of sole, with the following main features [9] (i) the ability to accumulate heavy metals in non-edible parts; (ii) tolerance to high doses of metals; (iii) rapid growth and high accumulation biomass; (iv) easy to grow as an agricultural crop and easily harvested.

In this study, we evaluated the effect of chromium metal concentrations (10 to 60 ppm) on growth parameters, chlorophyll content, TSS content, proteins content and proline content of Radish (Raphanus sativus L.)

2 Materials and Methods

2.1 Germination seeds

Dry seeds were washed with sodium hypochlorite 20% for 5 min, and then rinsed with distilled water. The seeds were placed on double layered the seeds were placed glass Petri dishes with double layer filter papers, soaked with 15 ml of H2O for the control and for the treatment soaked with 5ml of chromium solution [10].

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2.2 Growth parameters

The Radish plants were harvested after 1 month. The plants were oven dried at 80°C for 24h to determine the dry weight of root, shoot and hypocotyl of radish. The content of chlorophyll was estimated using the method of [11]. Pigment concentrations are expressed as mg/g FW (Fresh Weight).

2.3 Chlorophyll content

Leaf pigment contents including chlorophyll a andb, were extracted as described by [11].160 mg leaf samples were broyed in 4 ml of acetone (80%). The mixture was centrifuged for 10 min at 5000×g, and the absorbance ofthe supernatant was noted at 662 and 645 nm using a UV–Vis spectrophotometer (Lambda 25, PerkinElmer, Inc. USA).The pigment contents were calculated using theequations and coefficients given by [11].

$$\text{Chlorophyll } a \text{ (mg/g)} = 11{,}24 \times DO_{662} - 2{,}04 \times DO_{645}$$

$$\text{Chlorophyll } b \text{ (mg/g)} = 20{,}13 \times DO_{645} - 4{,}19 \times DO_{662}$$

$$\text{Total chlorophyll} = Chl\ a + Chl\ b$$

The pigment fractions were calculated as mg g-1 fresh leaf weight.

2.4 Total soluble sugars (TSS) content

Total soluble sugars (TSS) were estimation by phenol sulfuric acid method [12]. The samples were measured spectrophotometrically at 485 nm by spectrophotometer (Palo Alto, California, USA). we used D-glucose as a standard.

2.5 Proteins content

Protein content in plant (50 mg) was determined by using BSA as standard protein following the method of [13].

2.6 Proline content

Proline content in Radish plant (250 mg FW) was measured by spectrophotometer (Palo Alto, California, USA) according to the method of [14]. A standard curve was obtained from known concentrations of proline.

2.7 Chromium accumulation

The standardized color method [15] was used to determine the concentration of Cr (VI) which forms a red-violet complex measured by spectrophotometry at 540 nm using a JENWAY 6300 spectrophotometer with 1.5-diphnylcarbazide (DPC).

2.8 Statistical analysis

All the measurements were triplicates. The analysis of variance (ANOVA) was carried out using a version 24 of SPSS and by Tukey's post-hoc test between treatment means [16].

3 Results and Discussion

3.1 Germination seeds

The chromium effect on radish seeds germination was shown in Fig. 1. Our results showed a decrease in germination rate by 1.65%, 3.3%, 13.3% and 38.3% for 10ppm, 20ppm, 40ppm, and 60ppm of Chromium respectively, as compared to control plant.

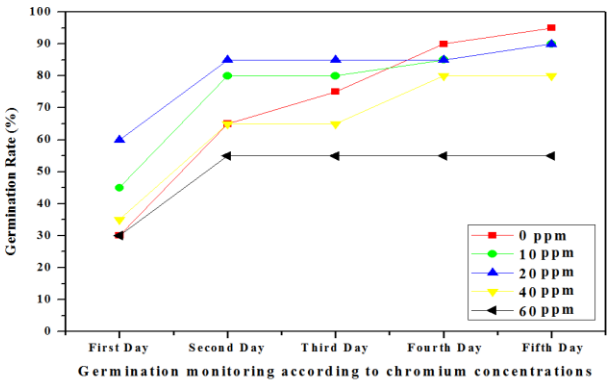

Fig. 1 Germination rate of radish seeds exposure to Chromium

3.2 Growth parameters and Chlorophyll content

The results showed that chromium have a harmful effect on growth parameters, chlorophyll content, TSS content, proteins rate and proline content Table 1. In general, chlorophyll content decrease by 23%, 41%, 67%, and 75% for 10ppm, 20ppm, 40ppm, and 60ppm of chromium respectively, as compared to control plant.

The dry weight of shoot biomass of Raphanus sativus was influenced by the chromium, the metal caused a reduction of about 3%, 6%, 25%, 66.5% and in presence of 10ppm, 20ppm, 40ppm and 60ppm respectively, as compared to control plant. The dry weight of root of *Raphanus sativus* was affected by the metal.

The DW of root decreased by 25%, 41%, 83%, 91% and in presence of 10ppm, 20ppm, 40ppm and 60ppm respectively, as compared to control plant.

The dry weight of hypocotyl was affected by the chromium, the metal caused a reduction of about 2%, 5.3%, 55.7%, 79% for 10ppm, 20ppm, 40ppm and 600ppm of metal respectively, as compared to control plant. In addition, However, we observed that TTS, total proteins and proline in Radish an increased slightly with the elevation of Cr (VI) concentration.

Table 1 Chlorophyll content, dry weight of shoot, root hypocotyl, TSS content, proteins and proline content of Radish plant

Parameter	Cr (ppm)				
Treatment	Control	10	20	40	60
Chl a + b	12.619±0.003	9.716±0.02	7.446±0.05	4.164±0.045	3.154±0.06
Shoot (g/plant)	0.74±0.002	0.72±0.01	0.70±0.001	0.55±0.013	0.33±0.005
Root (g/plant)	0.12±0.045	0.09±0.12	0.07±0.46	0.02±0.001	0.01±0.03
Hypocotyl DW(g/plant)	0.95±0.1	0.93±0.07	0.90±0.057	0.42±0.006	0.20±0.08
total sugars (mg/g FW)	0.176±0.04	0.182±0.075	0.190±0.8	0.209±0.165	0.219±0.065
Proteins (mg/g FW)	5.16±0.12	5.31±0.104	5.43±0.04	6.15±0.065	6.3±0.08
Proline (mg/g FW)	0.23±0.156	0.245±0.05	0.253±0.084	0.287±0.07	0.306±0.052

3.3 Total sugars, Proteins and Proline content

The results in Table 1 showed that exposure of radish plants to chromium slightly increases the production of soluble sugars, protein and proline. this increase is proportional to the increase in chromium concentration in the culture medium. it can be noted that the maximum production is recorded at the high chromium dose (60ppm) with an increase of 24%, 22%, 33% for SST, protein and proline respectively compared to the control plant.

3.4 Chromium accumulation

The variability of metal concentrations affected the chromium accumulation, Table 2 Showed that the accumulation in tissues increases with the increase of chromium concentrations in soil.

Table 2 Chromium accumulation in shoot and root of Radish (*Raphanus sativus)*

Part	Treatment				
	Control	10ppm	20ppm	40ppm	60pmm
Shoot	0	0.47±0.003	1.2±0.01	1.5±0.021	2.2±0.02
Root	0	7.5±0.01	16±0.05	19±0.035	21±0.04

The germination step is the first exchange interface with the surrounding medium and accordingly it is relatively sensitive to changing environmental conditions [17]. The present study showed that the Chromium metal reduced the germination rate, chlorophyll content, root and shoot dry weight of Radish (Raphanus sativus) as compared to control plant. These results correspond with that of [18] who observed that Chromium decreased the rate germination of Alfalfa seeds (*Medicago sativa* L.) by more than 50% and decrease the root and shoot weight Alfalfa plant. In addition [19] reported that the heavy metals reduced the root elongation of lettuce, broccoli, tomato and radish plant. However [20] noted that the germination of Coronilla varia was decreased by Ni, Cu, and Cd. and the inhibitory effect on the belowground growth was greater than on the aboveground growth. [21] observed that the Cd2+reduced the shoot and root dry weight of Okmass plants and decreased the Chlorophyll a (Chl.a) content. [22] noted that Exposure to Cd decreased the transcription levels of three upstream genes in the Chl biosynthesis pathway in leaf of Arabidopsis, The damage induced by toxic amounts of heavy metals has been attributed to different causes that usually act together, and can include direct metal damage and indirectly via induced oxidative stress [23]. reported effects include reduction in chlorophyll, altered water balance, decreases activity of various enzymes, stomatal closure, slowing down of photosynthesis rate and reduced uptake of essential mineral nutrients [1]. Soluble sugars are major products of photosynthesis and a main osmoregulation substance. Our results showed a slightly increase in soluble sugar with the elevation of Cr. [24] reported that the total proteins, soluble sugars, and malondialdehyde in *T. angustifolia* increased when the concentration of Cr (VI) increased from 9 to 30 mg L-1. Accumulated total soluble sugars act as osmolytes to protect cells from damage and maintain the original physiological processes [25]. The total proteins in *Raphanus sativus* increased slightly with the increase of chromium concentrations. This may be that the oxidative stress caused by chromium metal induced an activation of enzyme activity in Raphanus sativus. Some studies also showed that some polypeptides (like phytochelatins and metallothioneins), which can bind with heavy metals to reduce their toxicity, can be synthesized by plants [26]. The increase in total soluble protein content under heavy metal stress may be related to the induced synthesis of stress proteins such as enzymes involved in Krebs cycle, glutathione and phytochelatin biosynthesis and some heat shock proteins [27]. The results of [28] showed that the heavy metals (lead, chromium, nickel, cadmium, zinc) affected the growth and performance of common bean (*Phaseolus vulgaris* L. cv. Nebraska), all heavy metals lowered the leaf contents of the photosynthetic pigments (chlorophyll a, b and carotenoids), also chemical analysis of carbohydrates showed increases in the contents of reducing sugars and total soluble sugars, and proteins content in response to heavy metals. In addition we reported that root of radish accumulated more of chromium than the leaves. The study of [29] revealed that as the contamination level increased significant reductions were observed in biomass, photosynthetic rate and chlorophyll a and b contents as compared to control plants. However, plant's stress tolerance mechanisms, including proline content and activity of antioxidant enzyme increased under different treatments. [30] observed an increase in proline concentration in roots and shoots that was triggered by Cu and Cd exposure was partly reversed. [31] Several species from the Brassica genus are very important agricultural crops in different parts of the world and are also known to be heavy metal accumulators. Cr-tolerant plants generally accumulated higher Cr concentrations in their roots than in their leaves [32]. Finally, we can concluded that the most of the absorbed chromium was accumulated in the roots of Radish, and small portions of Cr were transported to the rest of the plant by active transportation.

4 Conclusion

The present study revealed that growth parameters and chlorophyll contents were reduced under the influence of chromium treatments, while other parameters such as total soluble sugar, proteins content and proline content were slightly increased under similar conditions. Data indicated that Radis is moderately tolerant to chromium. it's could possibly be used with success in polluted soils and the extraction of heavy metals could be maintained at satisfying levels.

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