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EVALUATION OF THE TOXICITY OF CHROMIUM VI CONTAMINATED IRRIGATION WATER ON THE MINT (MENTHA SPICATA) CROP

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Abstract

The Human wastewater is used frequently in many areas of the world. With the rapid growth of population in developing countries that are also increasingly urbanized, the recycling of wastewater in agriculture is widely practiced in many countries but sometimes not enough attention is given to the contamination of these waters by pollutants such as heavy metals. Our objective is to study the effect of irrigating a mint crop with 10ppm of Chromium VI for 10 days and evaluate the toxicity of Chromium on this crop and the health risk related to their consumption. The results show that Cr affects the content of pigments (Chlorophyll a, b and carotenoids) and the relative water content (RWC), however there is a slight increase in the content of total soluble sugars, proteins and proline compared to control plants. Concerning the bioaccumulation of chromium we note that the Bioaccumulation factor is very important (more than 5) but the translocation factor (TF) is 1.17 (the majority of Cr is stored in the roots), despite the low TF we found that the daily dose of chromium (0.003 mg/kg/day) is strongly exceeded (0.1262 for men and 0.1767 mg/kg/day for women) so the risk of toxicity is highly probable. It is always necessary to control and check the purity of irrigation water to prevent the pollution of agricultural soils and subsequently the contamination of edible plants by humans and animals.

Keywords: *Mint; Chromium VI; Mentha spicata; Toxicity; Bioaccumulation*

Introduction

Heavy metal contamination of our environment is a very serious and important global problem. The industrial and anthropogenic activities such as galvanization, smelting, mining, excessive use of fertilizers and contaminated irrigation water have caused the emission of significant amounts of metals into our environment [1]. Contrary to organic substances, heavy metals are essentially non-biodegradable and accumulate in the environment. Accumulation of heavy metals in soils and waters poses a risk to the environment and human health. Heavy metals are largely diffused in the ecosystem and released by naturally and anthropogenic activities into the atmosphere, water and soil through which they are used by plants. Heavy metal levels in vegetables cannot be underestimated because vegetables and fruits are very nutritious and are mainly eaten [2;3]

Plant responses to heavy metal stress are the combined results of several mechanisms, Cr in particular Cr(VI), restricted root growth followed by reduced nutrient uptake, low water uptake and low productivity are the principal toxic signs caused by Cr in plants [4].

The mint (Mentha spicata) is a perennial plant of the family Labiatae. It is native to the Mediterranean [5]. It has been known for a long time in Morocco for the flavoring of tea. Its culture is located in the green belts of the cities which give to the cultivars their vernacular name: "mint of TIZNIT", "mint of Meknes", "mint of BROUJ" etc. The crop is grown all over Morocco, on small plots almost on every farm (for self-consumption); it is found everywhere. The national production is between 65,000 and 70,000 tons per year on an area of more than 4,000 hectares.

The objective of this work was to describe and study the effect of irrigating a mint crop with 10ppm of Chromium VI for 10 days and to evaluate the toxicity of Chromium on my physiological parameters of mint and the health risk related to their consumption

Methods

The mint seedlings (*Mentha spicata*) were planted in earthen pots of 10 cm diameter containing 100g of soil. For irrigation the seedlings were irrigated every day with 10ml of sterile water for 10 days, for the treatment we used a solution of chromium VI (potassium dichromate) with a concentration of 10ppm.

Pigments content

The extraction was realized under cold conditions with 4 ml of acetone from 160 mg of fresh leaf material. After 10 min of centrifugation at 5000 g, at 4°C, absorbance measurement was performed at 470, 645, and 662nm using a spectrophotometer. The pigment contents, expressed in in µg.mg-1 , are calculated from the following equations [6]:

$$\text{Chl a} = 11.24 \times DO_{662} - 2.04 \times DO_{645}$$

$$\text{Chl b} = 20.13 \times DO_{645} - 4.19 \times DO_{662}$$

$$\text{Carotenoids} = (1000 \times OD_{470} - (1.9 \times \text{chla}) - (63.14 \times \text{Chlb}))/214$$

Relative water content (RWC)

The water status of the plant was assessed by the relative content (RWC, %), measured according to the method of [7] and calculated according to the formula of [8]. It consists in a determination of the percentage of water present in the excised leaves. The leaves cut at the base of the leaf blade are immediately weighed to weighed to obtain their fresh weight (FW). They are then put in cell turgor in distilled water, in the dark, for 24 hours. in the dark for 24 hours. Afterwards, they are weighed to to obtain the turgor weight (WT). The samples are finally placed in an oven at 60°C for 48 hours, which allows to obtain the which allows to obtain the dry weight (DW). The relative water content is expressed as a percentage, it is given by the following formula:

$$RWC = [(FW - DW) / (WT - DW)] \times 100$$

Total soluble sugar content

The amount of sugar was determined by the phenol-sulfuric acid method described by [9] Extraction was done by 80% ethanol, grinding 200 mg of fresh material in 4 ml of ethanol. The resulting mixture was centrifuged at 5000 rpm for 10 min. A

volume of 1 ml of the supernatant was transferred to test tubes containing 1 ml of 5% phenol. After shaking, 5 ml of concentrated sulfuric acid was added rapidly to the mixture and the tubes were immediately placed in a water bath at 100°C for 20 min. After cooling the tubes in the dark for 30 min, the absorbance was measured using a spectrophotometer at 485 nm.

Sugar contents are expressed as mg/100 mg fresh material, with reference to a standard glucose range established by concentrations ranging from 0 to 100 µg/ml.

protein content

Proteins were quantified by the method of [10], which used Coomassie Brilliant Blue as a reagent and bovine serum albumin (BSA) as a standard. 200 mg of fresh material was cold ground in 4 ml of 0.1 M Tris-HCl buffer at pH 7.5. The grind then centrifuged at 15000 rpm for 20 min. The supernatant was collected for protein determination. The reaction medium consisted of 100 µl of the plant sample, 2 ml of Bradford reagent, and 100 µl of distilled water. After 10 minutes of reaction at room temperature optical density (OD) reading was taken at 595 nm, and protein levels were determined using a standard range established by bovine serum albumin (BSA) concentrations ranging from 0 to 100µg/ml.

proline content

Quantification of the proline ninhyidrin reaction was performed according to the method of [11]. 200 mg of plant material was ground in 2.5 ml of 95% ethanol followed by three rinses and washing with 2.5 ml of 70% ethanol each month. After centrifugation at 5000 rpm for 10 min, 5 ml of supernatant is removed and 2 ml of chloroform and 3 ml of distilled water are added. The closed tubes were left to stand overnight at 4°C. After decantation, 1 ml of the upper phase of the samples was taken and added to 0.33 ml of pure glacial acetic acid, 0.33 ml of distilled water and 0.33 ml of ninhyidrin (0.01g of ninhyidrin, 0.166 ml of 6M H 3 PO 4 and 0.25 ml of glacial acetic acid).After stirring and heating on a water bath at 100°C for 45 min, the mixture is cooled and 2 ml of toluene is added. After stirring with a vortex, two phases appear: the upper organic phase (toluene) contains proline and a lower phase without proline. The upper phase is taken and then left to rest for 30 min. The reading of the ptical density of the samples was carried out by the spectrophotometer at the wavelength of 520 nm. The standard range is prepared with proline concentrations between 0 and 20 µg/ml.

chromium VI content

The standardized colorimetric method [12] was used to determine the concentration of Cr (VI) which forms a red-violet complex with 1,5-diphnylcarbazide (DPC). measured spectrophotometrically at 540 nm using a JENWAY 6300.

Chromium accumulation and translocation

Translocation factor

The translocation of Cr from shoot to root was quantified by TF which is given below:

$$TF=C_{shoot}/Cr_{root}$$

C_{shoot} and C_{root} are the metal concentrations in the shoot(mgkg-1) and the plant root (mgkg-1), respectively. [13].

Bioaccumulation factor

The BAF of Cr was estimated as follows:

$$BAF=C_{plant}/C_{soil}$$

Cshoot and Csoil are the concentrations of metals in the plant shoot (mgkg-1) and soil (mgkg-1), respectively [14].

Estimation of daily metal intake

To assess the health risk associated with chromium VI contamination of the edible parts of mint, the daily metal intake was calculated using the following formula [15]

$$(DMI) = (VIR \times C)/ PC$$

VIR is the mint intake rate (mg person-1 day-1), C is the chromium concentration in the edible part (mg kg-1 , fresh weight), BW is the body weight assuming 70 kg for adult males and 50 kg for females.

Statistical analysis

The statistical analysis was conducted using SPSS (21.0) software. . Analysis was performed using

three replicates per combination per treatment for all parameter considered,Tukey's test was followed for comparison of means for the parameters considered [16].

Results

The results in Table 1 show that Cr affects pigment content (Chlorophyll a, b and carotenoids) and relative water content (RWC), however there is a slight increase in total soluble sugars, protein and proline content compared to control plants.

The application of 10ppm Cr in the soil reduced the chl a, b carotenoids and relative water content by 4.27% and 24.20%; 5.60% and 6.65%, respectively compared to the control plant.

In contrast to pigment content and RWC, the presence of Cr in the soil increases sugar, protein and proline content by 22.07%, 30.40% and 27.87%, respectively, compared to the control plant.

Concerning the accumulation of chromium we found (Table 2) that the root part accumulates much more chromium VI (about 94%) while the aerial part accumulates only 6%. For the bioaccumulation factor we noted that the factor is very important (x7) that means that the plant accumulates CrVI 7 times more compared to the concentration of CrVI in the soil, consequently we observe that the daily dose recommended for Cr is strongly exceeded.

Discussion

The presence of chromium VI in irrigation water affects the relative water content (RWC) and the pigments content (chlorophyll a, b and carotenoids) in mint leaves (*Mentha spicata*). These results are in accordance with those of [17;18]who found that Cr VI leads to a reduction of the chlorophyll content by 28.84% in maize plants. Similarly, the results of [19] showed that the chlorophyll a content in wild mint leaves decreased from 0.85 to 0.82 mg g1 , Chl b from 0.66 to 0.51 and carotenoids from 3.2 to 2.4 mg g1 under pollution stress. [20] found that plants irrigated with water containing 10 mg L-1 K2Cr2O7 had a lower concentration of photosynthetic pigments and suffered oxidative stress in their leaves. Results of [21] showed that shoot and root lenth, fresh and dry weight, chlorophyll and carotenoids content decreased under chromium VI application (0, 1 and 10 mM). On the other hand, [22] noticed that a gradual increase in soil Cr concentration led to a decrease in relative water content (RWC), seed vigor index (SVI) and tolerance index (TI). In addition, a significant decrease in relative leaf water content and an increase in membrane electrolyte leakage were observed under Cr contamination compared to plants grown in soil without Cr stress [23]. however, [24] observed a decrease in relative water content under heavy metal stress with an increase in soil Cr concentration.

In contrast to pigment content and RWC, our results showed that under chromium VI stress, there was a slight increase in the synthesis of soluble sugars, proteins and proline. Similar results were observed by [17], when maize plants were stressed with chromium VI, total soluble sugar and total protein in Zea mays plant exposed to CrVI was slightly increased by 6.56% and 2.66% respectively. [25] reported that proline and soluble sugar contents were significantly increased in henna plants grown in areas contaminated by heavy metals from an industrial complex. In addition to their potential as osmoprotectants, which maintain cell turgor, soluble sugars and proline detoxify free radicals and maintain redox homeostasis, thus inducing plant resistance to abiotic stress [26].

About the accumulation of Chromium VI in the different tissues of mint we notice that the majority of CrVI is stored in the roots, for the bioaccumulation factor (BCF) the whole plant accumulates 7 times more than the amount of CrVI exists in the soil. These results are in agreement with the results of [25] *Lawsonia inermis* accumulated high concentrations of Pb and Cd in the roots than the shoots. The same results were reported for *Armeria maritima* Willd. [27], *Zea mays* L. [17] and *Abelmoschus esculentus* L. [28]. The accumulation of heavy metals in roots may be a mechanism to protect the sensitive aerial parts from the toxic impacts of heavy metal stress [29].

Conclusion

The irrigation water Contamination is mainly due to the use of uncontrolled wastewater or contaminated groundwater. In Morocco few data are available on the assessment of the risks related to the ingestion of contaminated food, their causes

and their impacts on human health. Our study revealed that the contamination of irrigation water, even at low concentrations, can cause enormous damage to crops and increase the recommended daily intake of metals and consequently increase the risk of intoxication due to the ingestion of contaminated food, so it is necessary to control the quality of irrigation water to preserve the health of soils and food quality.

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Table 1: Effect of CrVI on chlorophyll (a and b), carotenoids, relative water content, protein, Sugar and Proline content in the mint plant (*mentha spicata*).

	control plant	treated plant
chlorophyll A (µg/mg FM)	0.919[a]	0.880[b]
chlorophyll B (µg/mg FM)	0.398[a]	0.302[b]
Carotenoids (µg/mg FM)	0.340[a]	0.321[b]
relative water content (%)	95.852[a]	89.474[b]
protein content (µg/mg FM)	17.580[a]	21.460[b]
Total Soluble Sugar content (µg/mg FM)	2.810[a]	3.664[b]
Proline content (µg/mg FM)	27.860[a]	35.625[b]

Table 2: Concentration of CrVI in different mint tissues, bioaccumulation factor, translocation factor and daily metal intake

	control plant	treated plant
Concentration of Cr Shoot (mg/Kg)	-	0.589
Concentration of Cr Root (mg/Kg)	-	62.651
Concentration of Cr Soil (mg/Kg)	-	8.838
translocation factor	-	0.940
Bioaccumulation factor	-	7.155
DMI male (mg/kg/day)	-	0.126
DMI Femele (mg/kg/day)	-	0.176