



## RESEARCH ARTICLE

## Response of Two Mint Cultivars Peppermint (*Mentha piperita* L.) and Curly Mint (*Mentha spicata* var. *crispa*) to Different Levels of Cadmium Contamination

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**ARTICLE INFO**

Received: Apr 24, 2024

Accepted: June 6, 2024

**Keywords**

Cadmium, chemical constituent, mint plants, soil contamination, vegetative growth

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**ABSTRACT**

The agricultural soils that are contaminated with heavy metals poses significant environmental challenges, impacting plant productivity and the safety of plant-based food and feed products. Cadmium (Cd) that is considered one of heavy metals enters the ecosystem by two sources natural and anthropogenic. Rising Cd level in edible plants has impact on human health. The primary objective of the present study was to investigate the influence of different levels of cadmium contamination in soil on the growth and chemical composition of two different mint cultivars, namely peppermint and curly mint as well as accumulation of Cd in mint leaves. The experiment was conducted using a randomized complete block design (RCBD) using three replications. Uniform rhizomes in weight were planted with a size of 17 cm in pots. After the rhizomes had developed to produce leaves, the plants were irrigated with different concentrations of cadmium in distilled water as solution (0, 15, 30, and 45 mg/kg), for control only distilled water was used. The results indicated that peppermint plants performed better than curly mint plants in terms of various vegetative and chemical parameters. On the other hand, the increased concentrations of cadmium from Zero to 45 mg/kg resulted in significant decrease in most morphological and chemical characters in both mint cultivars. The affected parameters were plant height decreased to (29.05 cm), fresh and dry vegetative weight (29.52-11.83 g), leaf area (190.1 cm<sup>2</sup>), number of leaves (31.17 leaf plant<sup>-1</sup>), total chlorophyll content (14.01 mg g<sup>-1</sup> F.W), and carbohydrate content in the leaves (7.41 %) compared to the control. Nevertheless, it has been noticed that the percentage of volatile oil and the amount of volatile oil per plant increased when the soil was treated with 15 mg/kg of cadmium in both cultivars (1.537%) for peppermint and (1.340%) for curly mint. Additionally, the accumulation of cadmium in the leaves also increased to (0.165 mg/kg) after the soil was treated by 30mg/kg of cadmium comparing to the control. This means that when the mint plants obtained from areas contaminated with Cd have high concentration of Cd in there structure and have impact on health when they have been eaten.

## INTRODUCTION

*Mentha* a member of the Lamiaceae family commonly recognized as a mint comprises 19 species as well as 13 hybrids (Teles *et al.*, 2013). Telci *et al.* (2011) highlighted that among all mint species, *Mentha canadensis*, *Mentha spicata*, and *Mentha piperita* have a significant economic importance because of their aromatic and medicinal properties. The essential oil derived from mint has a great role in the global essential oil market (Lawrence, 2006). Mint essential oil and extracts find applications in confectionery, perfumery, and pharmaceutical preparations. The utilization of medicinal plants in the pharmaceutical and food sectors is contingent upon the presence of biologically active compounds and their chemical composition (Lorenzi *et al.*, 2021).

The agricultural soils that are contaminated with heavy metals poses a significant environmental challenge, which has the potential to diminish the productivity of plants and compromise the safety of plant-based food and animal feed (Alloway, 1990& Kabata-Pendias, 2004). Over the past few years, there has been a rise in environmental pollution and ecological degradation, largely attributed to the swift advancement of industrialization as well as natural and human-induced activities. Of these concerns, soil polluted by heavy metals has emerged as a significant global environmental challenge (Prasad *et al.* 2014). Cadmium (Cd) is a hazardous metallic substance with a silver-white appearance, primarily due to its ability to dissolve in water, its fluid nature, and its toxic properties. The absorption of Cd by plant roots is effortless, which subsequently affects the functional and structural features of plants. Moreover, it hinders germination of seed and root elongation, resulting in a range of observable toxic indications, including stunted growth, yellowing of leaves, withering, and cellular demise (Daud *et al.*, 2009; Ali *et al.*, 2014 & Song *et al.*, 2017). Cd directly or indirectly hinders essential biological processes, including photosynthesis, respiration, water transportation, and exchange of gases, which cause a disruption of plant metabolism (Tang *et al.*, 2018). Furthermore, Cadmium has the potential to influence the metabolic processes and chlorophyll synthesis in plants (Jia *et al.*, 2015), as well as disrupt the antioxidant defense system through the elevation of reactive oxygen species ROS (Gallego & Benavides, 2019). It also plays a role in substance assimilation (Bertoli *et al.*, 2012) and division of cell (Potters *et al.*, 2007).

The normal growth of plants can be adversely affected by the absorption and accumulation of Cd. The presence of stress of Cd results in a reduction in the photosynthetic rate and biomass of plants, as well as causing oxidative damage and disrupting the balance of nutrient absorbance (Rizwan *et al.*, 2016 & Zou *et al.*, 2017). Plants possess the ability to alleviate the detrimental heavy metals effects by producing and combining different enzymatic and non-enzymatic antioxidants, chelating agents, and osmolytes (Haisel *et al.*, 2019). This is particularly significant as the cadmium accumulation in plants can lead to its transfer into the human body via the food chain, leading to chronic poisoning and posing a threat to human well-being (Xiong *et al.*, 2016).

Numerous studies have indicated that in the presence of high levels of specific heavy metals leads to a decline in both germination and plant growth parameters across various plant species (Marques *et al.*, 2007; Sengar *et al.*, 2008; Jun *et al.*, 2009). Chlorophyll synthesis inhibition and photosynthesis processes cause a reduction of plant biomass due to the increasing levels of Cd toxicity, as stated by Padmaja (1990). In contrast, Scora & Chang (1997) found that *Mentha peperita* L. planted in contaminated soil with Cd at concentrations ranging from 0.12 to 6.1 ppm did not exhibit any changes in essential oil components or biomass. However, Zheljazkov & Nielsen (1996) reported that higher concentrations of Cd, Pb, Cu, Zn, and Mn led to a decrease in both fresh yield and essential oil yield. Scavroni & colleagues (2005) provided evidence to support the cultivation of peppermint as an effective phytoremediator in contaminated soil. Their study revealed that peppermint plants were able to thrive and grow without accumulating harmful metals in their tissues. However, (Amirmoradi *et al.*, 2012), showed that with increasing concentration of Cd from

0 to 100 ppm on peppermint plants, fresh and dry vegetative weight, plants height, leaves area, the numbers of leaves, and leave essential oil significantly decreased when compared with control.

The main objective of the present study was to determine the effect soil contamination by different concentration of cadmium (Cd) mineral on vegetative growth and chemical constituent and production of volatile oil of two mint cultivar plants, and determine which cultivar has more tolerance to soil contamination by heavy metals.

## MATERIALS AND METHODS

The study was performed in 2023 at the nursery field located in Bagera city, Duhok, Kurdistan region, Iraq. The experimental setup involved varying concentrations of Cd in distilled water as a solution (0, 15, 30, and 45 mg/kg). The experiment was designed with 2 factors: 4 concentrations of cadmium and 2 mint cultivars, resulting in 8 treatments with 3 replications and 5 plants per treatment. Therefore, the total number of treated plants amounted to 120 (2\*4\*3\*5). The mint cultivars used in the present study because the people in Kurdistan Region of Iraq widely use them for eating and cooking purposes. The treatments were organized based on a randomized complete block design. Solutions containing Cd were prepared, while distilled water served as the control. The mint cultivars used, peppermints and curly mint, were grown from rhizomes of uniform weight harvested in Akre city, Kurdistan region, Iraq. Each rhizome contained 2 buds, and 2 rhizomes were planted in a medium consisting of sandy loam in 17 cm diameter plastic pots. Irrigation with Cd solutions was carried out at field capacity once all rhizomes had germinated; distilled water was used for the control treatment. After the irrigation with Cd solutions, all pots were subsequently irrigated with distilled water. In addition, the drained water from the bottom of the pots reused to maintain Cd levels in treatment pots. Nano NPK fertilizers were applied depending on the soil analysis results. Harvesting was conducted at the flowering stage at 15%. The key physicochemical properties obtained from this experiment are presented in **Table (1)**.

**Table (1). Some physicochemical properties of the planting medium.**

| Soil texture    | Bulk density<br>g cm <sup>3</sup> | Water retention % | Total Porosity % | Cadmium mg/kg | N %  | P %  | K %  | CaCo3 % | pH   | Total organic matter % |
|-----------------|-----------------------------------|-------------------|------------------|---------------|------|------|------|---------|------|------------------------|
| Sandy clay loam | 0.60                              | 59.53             | 65.37            | N.D           | 97.8 | 12.5 | 20.1 | 14.6    | 5.56 | 14.69                  |

\*N.D= Not Detected.

The following parameters were recorded at the end of the experiment

### **Vegetative characteristic:**

Plant height (cm), fresh and dry vegetative weight (g), Leaves area (cm<sup>2</sup>) according (Singh et al., 2021), and Number of leaves (leave plant<sup>-1</sup>).

### **Chemical characteristic:**

#### **Total chlorophyll content in leaves (mg g<sup>-1</sup> F.W)**

Fresh leaf samples were collected to analyse chlorophyll levels. Pigments were extracted successfully by repeating extraction process by acetone usage. The chlorophyll content was then quantified through spectrophotometric analysis to determine the total chlorophyll, chlorophyll a, and chlorophyll b contents (Bruinsma, 1963). Absorbance readings were taken at 663 and 645 nm, and the findings were reported as milligrams of chlorophyll per gram of fresh tissue. :

**Chlorophyll a =  $(12.7(N_{663}) - 2.69(645)) \times V / (1000 \times W)$**

**Chlorophyll b =  $(22.9(N_{645}) - 4.68(663)) \times V / (1000 \times W)$**

**Total Chlorophyll content = Chlorophyll a + Chlorophyll b**

**N<sub>663</sub> = Reading of absorbance at 663**

**N<sub>645</sub> = Reading of absorbance at 645**

**V = end of volume acetone**

**W = fresh weight of sample (g)**

**Carbohydrate content in leaves (%).**

Samples were taken from each plant leaves in the experiment based on the specific treatments, and subsequently subjected to drying at a temperature of 70°C using electric oven. This particular procedure involved obtaining a 0.5 g dry sample of crushed powder from the leaves and stem located at the center of the branches. The samples were then mixed with 10 ml of distilled water and centrifuged at a speed of 3000 rounds\minute for 15 minutes using a centrifuge instrument. Following this, 1 ml of the resulting sample was extracted and diluted in 9 ml of distilled water. From this diluted solution, 1 ml was further extracted and combined with 5 ml of concentrated H<sub>2</sub>SO<sub>4</sub> along with 1 ml of phenol 5%. The resulting mixture was then placed in a water bath at a temperature of 27°C for a period of 20 minutes. Finally, the mixture was measured using a spectrophotometer at a wavelength of 488 (Dubois *et al.*, 1956).

**Volatile oil in leaves (%), and its amount per plant (mL plant<sup>-1</sup>):**

The water distillation method, as described in Clevenger's (1928) study, was employed to determine the percentages of volatile oil in vegetative growth from each replicate of every treatment. The volatile oil percentage was calculated using the equation:

**Volatile oil (%) = volatile oil / weight of 100 g fresh plant material × 100. Subsequently, the amount of volatile oil was computed using the formula:**

**Amount of volatile oil (mL plant<sup>-1</sup>) = Total weight × Percentage of volatile oil.**

**Cadmium accumulation in leaves:**

The famous method via the digestion of samples by add Nitric acid (HNO<sub>3</sub>), Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and Perchloric acid (HClO<sub>4</sub>) (5:1:0.5). The sample boiled in acid solution in fume hood on hot plate till the digestion is complete. Thereafter, few drops of distilled water are add and allow to cool. The solutions were analyzed for the elements of interest utilizing Atomic Absorption Spectrometer AAS (Abou-Arab, 2000)

**Statistical analysis:**

The data has been analyzed by using the computer through the **SAS** (2013) program, and the means comparison was done by Duncan's Multiple Ranges Test (DMRT) below the probability level 5% which was claimed by (Arthur *et al.*, 2001).

## **RESULTS**

### **Vegetative Characteristics**

#### **Plant height (cm)**

Results in Table (2) showed that peppermint plants had a higher height 34.14 cm compared to curly mint plants, which reached 30.56 cm. whereas, increase the concentration of cadmium in the

soil increased to 45 mg/kg, the plant height decreased significantly reached 29.05 cm when compared with control reached 36.10 cm resulting in a decrease about 24.26%.

From the interactions between the two mint cultivars and different cadmium concentrations, the lowest plant height 25.74 cm was observed for curly mint plants under 45 mg/kg Cd concentration treatment compared with the highest plant height of 37.76 cm was recorded for peppermint plants when it was grown with 0 mg/kg.

**Table (2):- The influence of soil contamination by various concentrations of cadmium on the plant height (cm) of two different mint cultivars.**

| Mint Cultivars | Cadmium concentration |                     |                     |                     | Cultivar effect    |
|----------------|-----------------------|---------------------|---------------------|---------------------|--------------------|
|                | 0 mg/kg               | 15 mg/kg            | 30 mg/kg            | 45 mg/kg            |                    |
| Peppermint     | 37.76 <sup>a</sup>    | 33.74 <sup>ab</sup> | 32.70 <sup>ab</sup> | 32.36 <sup>ab</sup> | 34.14 <sup>a</sup> |
| Curly mint     | 34.45 <sup>ab</sup>   | 32.09 <sup>ab</sup> | 29.97 <sup>bc</sup> | 25.74 <sup>c</sup>  | 30.56 <sup>b</sup> |
| Cadmium Effect | 36.10 <sup>a</sup>    | 32.92 <sup>ab</sup> | 31.33 <sup>b</sup>  | 29.05 <sup>b</sup>  |                    |

The means sharing the same letter for each factor and interactions do not show significant differences at the 5% level according to the Duncan's Multiple Range Test (DMRT).

### Fresh vegetative weight (g)

Depending on the results in Table (3), it was observed that peppermint plants caused an increase in fresh vegetative weight significantly, reached 33.85 g. In contrast, curly mint plants resulted in a lower fresh vegetative weight reached 31.65 g. Furthermore, the study also found that increased cadmium concentrations in the soil, specifically at 45 mg/kg caused a significant decrease in fresh vegetative weight reached 29.52 g when comparing with the control that was 37.56 g.

**Table (3):- The influence of soil contamination by various concentrations of cadmium on the fresh vegetative Weight (g) of two different mint cultivars.**

| Mint cultivars | Cadmium concentration |                     |                     |                     | Cultivar effect    |
|----------------|-----------------------|---------------------|---------------------|---------------------|--------------------|
|                | 0 mg/kg               | 15 mg/kg            | 30 mg/kg            | 45 mg/kg            |                    |
| Peppermint     | 39.07 <sup>a</sup>    | 34.11 <sup>bc</sup> | 31.45 <sup>cd</sup> | 30.78 <sup>cd</sup> | 33.85 <sup>a</sup> |
| Curly mint     | 36.05 <sup>ba</sup>   | 33.10 <sup>bc</sup> | 29.20 <sup>d</sup>  | 28.25 <sup>d</sup>  | 31.65 <sup>b</sup> |
| Cadmium Effect | 37.56 <sup>a</sup>    | 33.61 <sup>b</sup>  | 30.33 <sup>c</sup>  | 29.52 <sup>c</sup>  |                    |

The means sharing the same letter for each factor and interactions do not show significant differences at the 5% level according to the Duncan's Multiple Range Test (DMRT).

The interactions between the two mint cultivar plants and the cadmium concentrations showed that the soil was treated with 45 mg/kg of cadmium concentration, it resulted in the lowest fresh vegetative weight, reaching only 28.25 g for curly mint plants. On the other hand, the highest fresh vegetative weight that was 39.07 g observed for peppermint plants when they interacted with 0 mg/kg of cadmium, indicating that cadmium had a lesser effect on this cultivar compared to curly mint plants.

### Dry vegetative weight (g)

Results in Table (4) showed that the use of peppermint plants resulted in a significantly higher dry vegetative weight reached 16.34 g compared to curly mint plants reached 13.34 g. However, increasing the cadmium concentration to 45 mg/kg caused a significant decrease in dry vegetative weight; the weight was reduced to 11.83 g comparing to the control reached 17.99 g.

The interactions between the two mint cultivar plants and cadmium, the lowest dry vegetative weight 11.00 g was observed for curly mint plants exposed to 45 mg/kg of cadmium, when compared with highest dry vegetative weight 19.00 g was perceived for peppermint plants when they were not exposed to any concentration of cadmium.

**Table (4):- The influence of soil contamination by various concentrations of cadmium on the vegetative dry weight (g) of two different mint cultivars.**

| Mint cultivars | Cadmium concentration |                     |                     |                     | Cultivar effect    |
|----------------|-----------------------|---------------------|---------------------|---------------------|--------------------|
| Peppermint     | 0 mg/kg               | 15 mg/kg            | 30 mg/kg            | 45 mg/kg            | 16.34 <sup>a</sup> |
|                | 19.00 <sup>a</sup>    | 17.49 <sup>a</sup>  | 16.22 <sup>ab</sup> | 12.67 <sup>cd</sup> |                    |
| Curly mint     | 16.97 <sup>ab</sup>   | 14.26 <sup>bc</sup> | 11.11 <sup>d</sup>  | 11.00 <sup>d</sup>  | 13.34 <sup>b</sup> |
| Cadmium Effect | 17.99 <sup>a</sup>    | 15.88 <sup>b</sup>  | 13.66 <sup>c</sup>  | 11.83 <sup>c</sup>  |                    |

The means sharing the same letter for each factor and interactions do not show significant differences at the 5% level according to the Duncan's Multiple Range Test (DMRT).

### Leaves area (cm<sup>2</sup>)

Results in Table (5) indicated that the peppermint plants had a significantly greater leaves area compared to curly mint plants. The leaf area of peppermint was 204 cm<sup>2</sup>, which was superior to the leaves area of curly mint plants, which reached 196.83 cm<sup>2</sup>. Furthermore, increasing the concentration of cadmium in the soil to 15 mg/kg caused a significant decrease in leaf area. The leaf area under this cadmium concentration was reduced to 190.1 cm<sup>2</sup>. In contrast, the control plants had a leaf area of 226.9 cm<sup>2</sup> the percentage decrease in leaf area due to the 15 mg/kg cadmium concentration was calculated to be 19.35 %.

**Table (5):- The influence of soil contamination by various concentrations of cadmium on the leaf area (cm<sup>2</sup>) of two different mint cultivars.**

| Mint Cultivars | Cadmium concentration |                    |                      |                    | Cultivar effect     |
|----------------|-----------------------|--------------------|----------------------|--------------------|---------------------|
| Peppermint     | 0 mg/kg               | 15 mg/kg           | 30 mg/kg             | 45 mg/kg           | 204.68 <sup>a</sup> |
|                | 231.67 <sup>a</sup>   | 195.2 <sup>b</sup> | 191.83 <sup>bc</sup> | 200 <sup>b</sup>   |                     |
| Curly mint     | 222.10 <sup>a</sup>   | 185 <sup>cd</sup>  | 199.00 <sup>b</sup>  | 181.2 <sup>d</sup> | 196.83 <sup>b</sup> |
| Cadmium Effect | 226.9 <sup>a</sup>    | 190.1 <sup>b</sup> | 195.4 <sup>b</sup>   | 190.6 <sup>b</sup> |                     |

The means sharing the same letter for each factor and interactions do not show significant differences at the 5% level according to the Duncan's Multiple Range Test (DMRT).

Based on the results of the interaction between the two mint cultivars and cadmium concentration, it appears that using 45 mg/kg cadmium had a significant effect on the leaf area. When curly mint was exposed to 45 mg/kg cadmium, it resulted in the lowest height value of 181.2 cm<sup>2</sup> compared

with peppermint plants were exposed to 0 mg/kg concentration of cadmium, it had a higher height value of 231.67 cm<sup>2</sup>.

### Number of leaves (leaf plant<sup>-1</sup>)

The results in Table (6) exposed that peppermint plants showed a significantly higher number of leaves reached 40.17 leaf plant<sup>-1</sup> compared to curly mint plants which reached 35.58 leaf plant<sup>-1</sup>. The peppermint plants had approximately 12.9% more leaves than the curly mint plants. However, the concentration of cadmium increased to 45 mg/kg, there was a decrease significantly in the leaf numbers reached 31.17 leaf plant<sup>-1</sup> when comparing to the control reached 47.67 leaf plant<sup>-1</sup>, and this represents a decrease of approximately 35.54% in the number of leaves compared to the control.

The interaction between 45 mg/kg cadmium concentration and curly mint plants resulted in the lowest number of leaves reached 30.00 leaf plant<sup>-1</sup> in contrast, the highest leaf numbers 53.67 leaf plant<sup>-1</sup> was observed when 0 mg/kg of cadmium interacted with peppermint plants.

**Table (6):- The influence of soil contamination by various concentrations of cadmium on the number of leaves (leaf plant<sup>-1</sup>) of two different mint cultivars.**

| Mint cultivars | Cadmium concentration |                     |                     |                     | Cultivar effect    |
|----------------|-----------------------|---------------------|---------------------|---------------------|--------------------|
| Peppermint     | 0 mg/kg               | 15 mg/kg            | 30 mg/kg            | 45 mg/kg            | 40.17 <sup>a</sup> |
|                | 53.67 <sup>a</sup>    | 39.00 <sup>bc</sup> | 35.67 <sup>d</sup>  | 32.33 <sup>ef</sup> |                    |
| Curly mint     | 41.67 <sup>b</sup>    | 36.00 <sup>cd</sup> | 34.67 <sup>de</sup> | 30.00 <sup>f</sup>  | 35.58 <sup>b</sup> |
| Cadmium Effect | 47.67 <sup>a</sup>    | 37.50 <sup>b</sup>  | 35.17 <sup>c</sup>  | 31.17 <sup>d</sup>  |                    |

The means sharing the same letter for each factor and interactions do not show significant differences at the 5% level according to the Duncan's Multiple Range Test (DMRT).

### Chemical Characteristic

#### Total chlorophyll content in leave (mg g<sup>-1</sup> F.W)

Results in Table (7) indicated that the peppermint plants had significantly higher total chlorophyll content in their leaves 21.54 mg g<sup>-1</sup> F.W compared to curly mint plants 18.51 mg g<sup>-1</sup> F.W. This indicates that peppermint plants are more efficient at producing chlorophyll. Additionally, increasing the cadmium concentration to 45 mg /kg caused a decrease significantly in total chlorophyll content in the leaves of both mint cultivars. For the control 0 mg/kg cadmium, the total chlorophyll content was 24.19 mg g<sup>-1</sup> F.W. However, when the cadmium concentration was increased to 45 mg/kg, the total chlorophyll content dropped to 14.01 mg g<sup>-1</sup> F.W. This indicates that high cadmium concentrations have a negative impact on chlorophyll production in mint plants.

The interaction between the two mint cultivars and different cadmium concentrations. The lowest total chlorophyll content 13.34 mg g<sup>-1</sup> F.W was observed in curly mint plants when exposed to 45 mg/kg of cadmium compared with peppermint plants had the highest total chlorophyll content 27.05 mg g<sup>-1</sup> F.W when they were grown without any concentration of cadmium.

**Table (7):- The influence of soil contamination by various concentrations of cadmium on the total chlorophyll content in leave ( $\text{mg g}^{-1}$  F.W) of two different mint cultivars.**

| Mint cultivars | Cadmium concentration |                     |                     |                     | Cultivar effect    |
|----------------|-----------------------|---------------------|---------------------|---------------------|--------------------|
| Peppermint     | 0 mg/kg               | 15 mg/kg            | 30 mg/kg            | 45 mg/kg            | 21.54 <sup>a</sup> |
|                | 27.05 <sup>a</sup>    | 24.24 <sup>ab</sup> | 20.20 <sup>bc</sup> | 14.67 <sup>de</sup> |                    |
| Curly mint     | 21.33 <sup>bc</sup>   | 21.34 <sup>bc</sup> | 18.00 <sup>cd</sup> | 13.34 <sup>e</sup>  | 18.51 <sup>b</sup> |
| Cadmium Effect | 24.19 <sup>a</sup>    | 22.79 <sup>a</sup>  | 19.10 <sup>b</sup>  | 14.01 <sup>c</sup>  |                    |

The means sharing the same letter for each factor and interactions do not show significant differences at the 5% level according to the Duncan's Multiple Range Test (DMRT).

### Total carbohydrate in leaves (%)

Based on Table (8), showed that in the carbohydrate percentage there were no significant differences between the two mint cultivar plants. Whereas, the concentration of cadmium in the soil was increased to 45 mg/kg caused a significant decrease in the percentage of carbohydrate content in leaves comparing to the control. It decreased to 7.42% in the presence of 45 mg/kg cadmium, while it was 11.89% in the control, representing a decrease of 60.46%.

**Table (8):- The influence of soil contamination by various concentrations of cadmium on the total carbohydrate in leaves (%) of two different mint cultivars.**

| Mint cultivars | Cadmium concentration |                     |                    |                    | Cultivar effect    |
|----------------|-----------------------|---------------------|--------------------|--------------------|--------------------|
| Peppermint     | 0 mg/kg               | 15 mg/kg            | 30 mg/kg           | 45 mg/kg           | 10.26 <sup>a</sup> |
|                | 12.90 <sup>a</sup>    | 10.74 <sup>ab</sup> | 9.37 <sup>bc</sup> | 8.04 <sup>bc</sup> |                    |
| Curly mint     | 10.87 <sup>ab</sup>   | 9.73 <sup>bc</sup>  | 8.08 <sup>bc</sup> | 6.79 <sup>c</sup>  | 8.87 <sup>a</sup>  |
| Cadmium Effect | 11.89 <sup>a</sup>    | 10.24 <sup>ab</sup> | 8.73 <sup>bc</sup> | 7.41 <sup>c</sup>  |                    |

The means sharing the same letter for each factor and interactions do not show significant differences at the 5% level according to the Duncan's Multiple Range Test (DMRT).

Furthermore, when considering the dual interactions between the two mint cultivar plants and cadmium, it was observed that using a concentration of 45 mg/kg of cadmium in the soil resulted in the lowest carbohydrate content value in leaves, reaching 6.79% for curly mint plants, compared with the highest carbohydrate value in leaves, reaching 12.90 %, was observed for peppermint plants that interacted with 0 mg/kg of cadmium.

### Volatile oil in leaves (%)

Based on the results presented in Table (9), it can be concluded that there were significant differences in the volatile oil content of leaves between the two mint cultivar plants. The peppermint plants had higher volatile oil content, measuring 1.318%, compared to curly mint plants, which had a volatile oil content of 1.175%. Furthermore, when the concentration of cadmium to the soil was increased to 15 mg/kg, there was a significant increase in the volatile oil content for both mint cultivar plants. The volatile oil content in leaves reached 1.438%, representing an increase of 29.54% compared to the control, which had a volatile oil content of 1.110%.

The interaction between the two mint cultivar plants and the concentration of cadmium in the soil showed that using 15 mg/kg of cadmium resulted in the highest value of volatile oil, reaching 1.537% for peppermint plants. In contrast, the lowest value of volatile oil 1.017% was observed for curly mint plants that were exposed to 0 mg/kg of cadmium.



**Table (9):- The influence of soil contamination by various concentrations of cadmium on the volatile oil in leaves (%) of two different mint cultivars.**

| Mint cultivars | Cadmium concentration |                    |                     |                     | Cultivar effect    |
|----------------|-----------------------|--------------------|---------------------|---------------------|--------------------|
| Peppermint     | 0 mg/kg               | 15 mg/kg           | 30 mg/kg            | 45 mg/kg            | 1.318 <sup>a</sup> |
|                | 1.203 <sup>b</sup>    | 1.537 <sup>a</sup> | 1.223 <sup>b</sup>  | 1.310 <sup>b</sup>  |                    |
| Curly mint     | 1.017 <sup>c</sup>    | 1.340 <sup>b</sup> | 1.163 <sup>bc</sup> | 1.180 <sup>bc</sup> | 1.175 <sup>b</sup> |
| Cadmium Effect | 1.110 <sup>c</sup>    | 1.438 <sup>a</sup> | 1.193 <sup>bc</sup> | 1.245 <sup>b</sup>  |                    |

The means sharing the same letter for each factor and interactions do not show significant differences at the 5% level according to the Duncan's Multiple Range Test (DMRT).

#### **Amount volatile oil (ml plant<sup>-1</sup>)**

The results in Table (10) designated that were significant differences in the amount volatile oil between the two mint cultivar plants. The peppermint plants had higher amount volatile oil to 0.352 mL plant<sup>-1</sup>, compared to curly mint plants, which had an amount volatile oil of 0.223 mL plant<sup>-1</sup>. Furthermore, when the concentration of cadmium in the soil was increased to 15 mg /kg, there was a significant increase in the amount volatile oil for both mint cultivar plants. The amount volatile oil reached 0.385 mL plant<sup>-1</sup>, representing an increase of 58.43% compared to 0.243 mLplant<sup>-1</sup> were plants exposed to 45 mg/kg of cadmium.

**Table (10):- The influence of soil contamination by various concentrations of cadmium on the Amount volatile oil (mL plant<sup>-1</sup>) of two different mint cultivars.**

| Mint cultivars | Cadmium concentration |                    |                    |                    | Cultivar effect |
|----------------|-----------------------|--------------------|--------------------|--------------------|-----------------|
| Peppermint     | 0 mg/kg-1             | 15 mg/kg           | 30 mg/kg           | 45 mg/kg           | 0.352a          |
|                | 0.332 <sup>bc</sup>   | 0.437a             | 0.362b             | 0.278c             |                 |
| Curly mint     | 0.169 <sup>d</sup>    | 0.332bc            | 0.181d             | 0.208d             | 0.223b          |
| Cadmium Effect | 0.251 <sup>b</sup>    | 0.385 <sup>a</sup> | 0.271 <sup>b</sup> | 0.243 <sup>b</sup> |                 |

The means sharing the same letter for each factor and interactions do not show significant differences at the 5% level according to the Duncan's Multiple Range Test (DMRT).

The interaction between the two mint cultivar plants and the concentration of cadmium in the soil showed that using 15 mg/kg of Cd resulted in the highest value of amount of volatile oil, reaching 0.437 mL plant<sup>-1</sup> for peppermint compared with the lowest value of amount volatile oil 0.169 mL plant<sup>-1</sup> was observed for curly mint plants that were exposed to 0 mg/kg of cadmium.

#### **Cadmium accumulation in leaves (mg/kg)**

The results in Table (11) can be summarized that there were significant differences in the Cadmium accumulation in leaves between the two mint cultivar plants. The peppermint plants had higher cadmium accumulation in leaves to 0.125 mg/kg, compared to curly mint plants, which had a cadmium accumulation in leaves of 0.094 mg/kg. Furthermore, when the concentration of cadmium in the soil was increased to 30 mg/kg, there was an increase significantly in the cadmium accumulation in leaves for mint cultivar plants. The amount cadmium accumulation in leaves reached 0.165 mg/kg, compared to control which not detected cadmium concentration in leaves.

The interaction between the two mint cultivar plants and the concentration of cadmium in the soil showed that using 30 mg /kg of cadmium resulted in the highest value of cadmium accumulation in leaves, reaching 0. 215 mg/kg for peppermint and 0.114 mg/kg for curly mint plant.

**Table (11):- The influence of soil contamination by various concentrations of cadmium on the cadmium accumulation in leaves (mg/kg) of two different mint cultivars.**

| Mint cultivars | Cadmium concentration |                    |                    |                    | Cultivar effect    |
|----------------|-----------------------|--------------------|--------------------|--------------------|--------------------|
|                | 0 mg/kg               | 15 mg/kg           | 30 mg/kg           | 45 mg/kg           |                    |
| Peppermint     | 0.0 <sup>d</sup>      | 0.116 <sup>c</sup> | 0.215 <sup>a</sup> | 0.167 <sup>b</sup> | 0.125 <sup>a</sup> |
| Curly mint     | 0.0 <sup>d</sup>      | 0.105 <sup>c</sup> | 0.114 <sup>c</sup> | 0.156 <sup>b</sup> | 0.094 <sup>b</sup> |
| Cadmium Effect | 0.0 <sup>c</sup>      | 0.111 <sup>b</sup> | 0.165 <sup>a</sup> | 0.162 <sup>a</sup> |                    |

The means sharing the same letter for each factor and interactions do not show significant differences at the 5% level according to the Duncan's Multiple Range Test (DMRT).

## DISCUSSION

Numerous researches have demonstrated that cadmium pollution poses a range of detrimental impacts on the growth and development of plants, making it a significant environmental concern globally (Pourghasemian *et al.*, 2013). Our study exposed that two mint cultivars, peppermint and curly mint, grew differently when exposed to varying concentrations of Cd minerals in the soil. In both vegetative and chemical criteria, peppermint cultivars outperform curly mints; these differences may be attributed to differences in their genetic composition and physiological traits. Various factors, including metal absorption, translocation, and detoxification systems, can impact a plant's capacity to tolerate and reduce the negative impacts of cadmium (Jia *et al.*, 2023).

However, the results demonstrate that the addition of 45 mg/kg of cadmium to the soil resulted in a considerable reduction in plant height, fresh and dry vegetative weight, the number of leaves, total carbohydrate percentage, and chlorophyll content in leaves. Moreover, considerable decrease in leaf area that plants treated with 15 mg/kg cadmium. Whereas, the percentage of volatile oil, the amount of volatile oil per plant increased when treated plants with 30 mg/kg of cadmium. While, the accumulation of cadmium in leaves all increased as the concentration of cadmium reached 45 mg/kg.

As previously indicated by other researchers, the decreased in plant height observed in these results could be explained that cadmium is a heavy metal that can be accumulated in the roots and shoots of plants. The cadmium accumulation has been found to disrupt their normal physiological processes in plants, leading to reduced growth and development, including a decrease in plant height, this nutrient imbalance can affect division of cell and elongation, resulting in stunted plant height (Gonçalves *et al.*, 2009; Choudhary *et al.*, 2018; & Hossain *et al.*, 2018). An alternative interpretation suggests that cadmium has the ability to trigger oxidative stress within plants. Upon exposure to cadmium, plants may generate reactive oxygen species (ROS) like superoxide radicals, hydroxyl radicals, and hydrogen peroxide. The excessive buildup of ROS can result in detrimental effects on cellular constituents, including proteins, lipids, and DNA. Consequently, these oxidative impairments can hinder the growth and division of cells, ultimately impacting the overall height of the plant (Gill *et al.*, 2013; & Ahmad *et al.*, 2015). Moreover, research has shown that exposure to cadmium can lead to changes in the quantities and functions of plant hormones, such as auxins, gibberellins, and cytokinins. These hormones are essential for the proper regulation of plant growth and development, including processes like

differentiation and elongation of cell. Consequently, any disruption in the hormonal balance caused by cadmium can ultimately lead to a decrease in plant height (Sharma *et al.*, 2020; & Singh *et al.*, 2020). When the current study outcomes were compared to those of earlier research, they concurred. (Mihalescu *et al.*, 2010) on maize plants, (Mehindirata *et al.*, 2000) on *Solanum melongena* L. and (Ali Khan & Siddhu, 2006) on *Vigna mungo* plants.

The decrease in both fresh and dry vegetative weight observed in this study could be attributed to either the diminished vegetative growth characteristics of the two mint cultivar plants caused by soil pollution with cadmium or the detrimental impact of this metal on the enzymatic activity involved in growth regulation and physiological processes. Greger (1989) ascribed the hindering impact of Cd on the growth of plant species to various factors. These include impeding water transportation to the shoot, diminishing the absorption of vital substances by the roots, and decreasing the opening of stomata and CO<sub>2</sub> uptake, thereby leading to a decline in photosynthesis. In a similar vein, Vassilev *et al.* (1998) documented that heavy metals impeded photosynthetic pigments, thereby affecting photosynthesis and impeding the growth and development of numerous plant species. The result was in conformity with the result of (Ghani, 2010) on *Mentha piperita* L., *Anethum graveolens* L. plants, and (Zheljazkov *et al.*, 2006) on *Ocimum basilicum* L. plants.

The decrease in leaf area was a result of both reduced leaf expansion and leaf aging. It appears that the diminishing leaf area of mint plants in our research can be attributed to the influence of heavy metals on structural alterations in the leaves (Al-Jobori & Kadhim, 2019). Barcelo *et al.* (1988) it was observed that the movement of water in beans was affected by a decrease in vascular rays and bundles due to exposure to cadmium, as well as changes in cell division, elongation, and differentiation of cambium cells. This aligns with a study conducted on *Matthiola chenopodiifolia* L. plants by (Ghaderin & Jamali Hajiani, 2011).

Nevertheless, there are various factors that can contribute to a decrease in the number of leaves in a plant. As you have highlighted, two significant reasons for this decline are the reduction in water uptake and the decrease in the synthesis of photosynthetic pigments, soluble sugars, and carbohydrates (Abdo *et al.*, 2012). Or it is possible that the negative relationship between certain growth traits like plant height ( $r=55.20$ -) and total carbohydrate ( $r=45.47$ -) could be the cause. This is evident in Table (11). This outcome aligns with the research conducted by (Jing *et al.*, 2005) on tomato plants, and (Siddhu & Khan, 2012) on *Phaseolus mungo* L. plants.

Conversely, the reduction in overall chlorophyll content within leaves could potentially be attributed to the disruptive effects of Cadmium on chloroplasts, the cellular organelles crucial for chlorophyll production and photosynthesis. This interference may result in the deterioration of thylakoid membranes, impaired electron transport, and diminished photosynthetic performance (Zhang *et al.*, 2014). Additionally, it is possible that the presence of Cadmium disrupts the process of chlorophyll synthesis by impeding the function of specific enzymes that play a role in the biosynthetic pathway. One such enzyme that can be affected by cadmium is delta-aminolevulinic acid dehydratase, which is involved in the initial stages of chlorophyll biosynthesis (Padmaja, *et al.*, 1990; & Popova *et al.*, 2009). Perhaps the reduction in chlorophyll synthesis and subsequent chlorosis can be attributed to the inhibitory impact of heavy metals on the uptake and transportation of iron

to the leaves of plants (Fodor *et al.*, 1995). Alternatively, the decline in chlorophyll concentration may be a consequence of an internal substitution within the plant tissues, where a magnesium atom in the chlorophyll molecule is replaced by heavy element atoms, leading to an essential breakdown mechanism in plants (Kupper *et al.*, 1998). The findings align with the results documented by (Gil *et al.*, 1995) on tomato (Padmaja *et al.*, 1990) on *Phaseolus vulgaris* L. and (Mehindirata *et al.*, 1999) on *Solanum melongena* L. plants

The reduction in the overall proportion of carbohydrates in the leaves of plants treated with cadmium can be explained to the deleterious impact of cadmium on pigments involved in photosynthesis, so impeding the process and consequently decreasing the production of sugars in plant tissues. Under cadmium stress, this interruption in the generation of carbohydrates can have detrimental effects on the plant's overall health and growth (Burzynski, 1987 & Poskuta *et al.*, 1988). Alternatively, the negative relationship observed among certain growth parameters like plant height ( $r=1.52-$ ), leaf area ( $r=4.74-$ ), number of leaves ( $r=45.47-$ ), and total chlorophyll ( $r=25.24-$ ) could be the reason for this reduction, as illustrated in Table (11). These findings align with the research conducted by (Fouda & Arafa, 2002) on soybean and (Pandey *et al.*, 2007) on *Catharanthus* plants.

While the increase in volatile oil percent and amount of volatile oil per plant can be attributed to the secondary metabolites, it is important to note that these metabolites do not directly contribute to the plants functioning. Nevertheless, they do play a crucial role in the plants' response to their environment and act as defense mechanisms. The composition and concentration of these secondary metabolites are greatly influenced by the growing conditions (Ramakrishna & Ravishankar, 2011). Secondary metabolites tend to accumulate in plants exposed to different stresses, elicitors, and signal molecules. Unfavorable environmental conditions can impact metabolic pathways and alter secondary metabolism. The biosynthesis and accumulation of potentially medicinally valuable chemicals in plants are influenced in unique ways when they are under heavy metal stress (De & De, 2011). Several studies have shown that heavy metals can have a positive impact on the production of key secondary metabolites, such as essential oils in medicinal plants (Maleki *et al.*, 2017). Which also high correlated with essential oil percentage in leaves plant with fresh weight of vegetative growth ( $r= 70.34^{**}$ ), number of leaves ( $r= 67.98^{**}$ ), and total carbohydrate percentage ( $r= 61.57^{*}$ ). Also, the amount of volatile oil had positive correlation with dry vegetative weight ( $r= 88.68^{**}$ ), Leaf area ( $r= 76.68^{**}$ ). These results are in agreement with those reported by (Azizollahi *et al.*, 2019) on *Satureja hortensis* L. plants

Accumulation of cadmium in leaves of two different mint cultivars has been found to increase significantly. This increase is influenced by various factors, including plant genetic characteristics. The accumulation and distribution of cadmium in plants vary depending on the species and even among different cultivars within the same species (Kuboi *et al.*, 1986 & Yang *et al.*, 1995). Furthermore, genetic selection plays a role in the tendency of certain species to accumulate cadmium, suggesting that plant breeding could be utilized to develop crop cultivars with lower concentrations of cadmium in their edible parts (Nriagu, 1979). Additionally, soil factors such as pH, salinity, organic matter content, and soil texture can also impact cadmium accumulation in plants (Malegus & Goh, 1995). Alternatively, the ease of transport of Cadmium from roots to plant tops may be attributed to its known properties (Kabata-Pendias, 2000). Cunningham *et al.* (1975) observed that the accumulation of Cd in

the roots increased while its translocation to the tops was suppressed when Cd ions in the solution were increased. This observation is further supported by the positive correlation between cadmium accumulation in leaves and fresh vegetative weight ( $r=60.53^*$ ) as well as the volatile oil percentage ( $r=80.63^{**}$ ). These findings align with the results reported by Wagner & Yeargan (1986) in their study on tobacco plants.

## CONCLUSION

The study found that peppermint and curly mint plants are affected by different levels of cadmium contamination in soil. While peppermint performed better than curly mint, both cultivars showed decreased growth and chemical composition with increasing cadmium levels. However, adding 15mg/kg of cadmium to the soil increased volatile oil production and accumulation in both cultivars. The study suggests that peppermint may be more suitable for areas with low to moderate Cd levels, while curly mint requires more careful selection and management to minimize the impact of Cadmium on plant growth and quality.

**Table (12): correlation relation between all studies characters of two mint cultivar plants.**

| characters                    | Plant height | Fresh vegetative weight | dry vegetative weight | Leaf area | No. of leaves | Total chlorophyll | Total carbohydrate | Volatile oil% | Amount volatile oil |
|-------------------------------|--------------|-------------------------|-----------------------|-----------|---------------|-------------------|--------------------|---------------|---------------------|
| Fresh vegetative weight       | 45.86-       |                         |                       |           |               |                   |                    |               |                     |
| dry vegetative weight         | 59.95*       | 45.02-                  |                       |           |               |                   |                    |               |                     |
| leaf area                     | 71.70*       | 55.47-                  | 76.87**               |           |               |                   |                    |               |                     |
| No. of leaves                 | 55.20-       | 78.34*                  | 40.46-                | 66.16*    |               |                   |                    |               |                     |
| Total chlorophyll             | 77.53*       | 48.08-                  | 66.87**               | 69.79*    | 57.49*        |                   |                    |               |                     |
| Total carbohydrate            | 1.52-        | 16.30-                  | 12.13-                | 4.74-     | 45.47-        | 25.24-            |                    |               |                     |
| Volatile oil%                 | 20.32-       | 70.34*                  | 6.44-                 | 15.22-    | 67.98*        | 37.86-            | 61.57*             |               |                     |
| amount volatile oil per plant | 61.92*       | 25.92-                  | 88.68**               | 76.68*    | 31.56-        | 54.28-            | 22.75-             | 33.97-        |                     |
| Cadmium in leaves             | 20.50-       | 60.53*                  | 13.69-                | 30.2-     | 55.39-        | 37.16-            | 37.81-             | 80.63*        | 22.13-              |

\*\*Significant at probability level (0.01)

\* Significant at probability level (0.05)

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