

## CHROMIUM AND WHEAT STRAW EFFECTS ON GROWTH AND PHYSIOLOGICAL CHARACTERISTICS OF SWISS CHARD (*Beta vulgaris* L.)

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### ABSTRACT

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Swiss chard (*Beta vulgaris* L.) is a widely spread promising leafy crop in Iraq, where majority of farmers utilized sewage water for irrigating chard, resulting in health hazards, particularly from heavy metal contaminants including chromium (Cr). This study employed a factorial experimental design to examine the effects of Cr concentrations (0, 5, 10, or 15mg.kg<sup>-1</sup> soil) using potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) as a source of the Cr, combined with different concentrations of wheat straw (WS) (0, 1, and 2% w/w) on the growth and certain physiological and biochemical traits of the plant. The results showed that the administration of Cr at 10mg.kg<sup>-1</sup> concentration significantly increased the percent of seed germination (83.33%), while the use of WS reduced the germination rate. Most vegetative growth characteristics responded inversely to increasing wheat straw percentage, but root performance was enhanced with Cr application of Cr. A high straw percent of 2% diminishes the performance of both roots and shoots in comparison to plants without additional WS, with the exception of leaf thickness. Shoot and root dry matter was higher in Cr15mg.kg<sup>-1</sup>. Photosynthetic pigments non-significantly affected by application of Cr and significantly decreased by added straw. Applications of Cr and WS reduced the activity of peroxidase enzyme, ascorbic acid, and the percent of total carbohydrate significantly relative to no Cr and WS-treated plants, with the exception of proline levels. This study has indicated that Cr and the phytotoxic effects of wheat straw on chard can be further utilized for sustainable chard cultivation.

**KEYWORDS:** Antioxidants, Chromium, Soil Amendment, *Beta vulgaris*, Vitamin C.

### 1. INTRODUCTION

Swiss chard (*Beta vulgaris* L.) is an important annual plant that grows rapidly and belongs to the Amaranthaceae family, which comprises the most diversified lineage with over 71 genera and 900 species. It is commonly utilized as a vegetable in Iraq for many traditional dishes, which are extensively cultivated in Iraq and the Kurdistan Region. The majority of them are irrigated by wastewater, resulting in elevated levels of various nonessential and hazardous elements such as lead, cadmium, and chromium (Dlamini *et al.*, 2020; Bzhen *et al.*, 2022).

The management of wastewater in Iraq has significantly influenced agricultural practices and food production, with around 580 million cubic meters of processed sewage waste. Most Iraqi farmers employ untreated wastewater for the irrigation of diverse food crops, such as lettuce, chard, and cabbage (Todd, 2023). Around 225 hectares of area in Erbil Governorate are irrigated with untreated sewage water for the cultivation of various vegetables used by nearly two million people as part of their diet. Some of leafy and tuberous vegetables accumulate higher concentrations of heavy metals compared to grains and fruits, particularly those irrigated with

wastewater. Leafy vegetables are recognized for absorbing heavy metals from the soil. This capability poses a considerable health hazard due to the prolonged effects of metals absorbed through the foliage, posing risks to the community (Ahmed *et al.*, 2024). Many studies were conducted in the Iraqi Kurdistan Region on the effects of heavy metals and wastewater on various plants (Abdullah & Albarzinji, 2023), including leafy crops such as lettuce (Talabany & Albarzinji, 2023) and celery (Saeed, 2023).

Heavy metals are defined as metals possessing a density over 5g.cm<sup>-3</sup> and their atomic number greater than 20. Heavy metals, including chromium (Cr), lead (Pb), mercury (Hg), arsenic (As), cobalt (Co), and cadmium (Cd), are currently significant hazardous trace contaminants that can profoundly affect the health of humans, animals, and plants (Ali *et al.*, 2023). Chromium is highly recommended as a non-essential metal due to its lack of significant biological functions (Ali *et al.*, 2020). Chromium, as a heavy metal, significantly contaminates soil, sediment, and groundwater worldwide, originating from both natural sources like chromite, rock weathering, and volcanic activity, as well as anthropogenic activities including tanning, mining, electronic waste disposal, and atmospheric deposition, which release substantial quantities of chromium into the

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environment (Singh *et al.*, 2013). Moreover, chromium deposition in agricultural soils is a worldwide issue due to its non-biodegradable nature, as chromium toxicity in plants impedes enzyme function, seed germination, root development, photosynthesis, and photophosphorylation (Joseph *et al.*, 2023). Many studies were conducted in Iraqi Kurdistan to mitigate the detrimental effects of Cr stress on plants; many methods have been employed, including physical, chemical, and biological methods. Specific approaches, while effective at reducing toxicity, may also cause secondary damage to the plant. Remarkably, the bio-sorption strategy is widely used because of its low cost, stability, high efficiency, and selectivity towards different heavy metal ions, as well as its capacity to reduce the adverse effects of the toxicity of heavy metals in plants. This is by application of several soil amendments such as straw, charcoal, humus, and compost (Sarwar *et al.*, 2023). Wheat straw is a byproduct of wheat, produced in thousands of tons during the harvesting process.

Consequently, the present study is a trial to employ this material in the Kurdistan Region of Iraq, specifically targeting soils contaminated by heavy metals. These contaminants frequently stem from wastewater irrigation, industrial activities, and the petroleum industry (Ali *et al.*, 2023). Previous studies have aimed to use wheat straw, a primary biomass feedstock with extensive geographic distribution and substantial availability, to absorb and reduce the concentrations of toxic metals in soil and water (Cao *et al.*, 2019). The present study aims to assess the effects of different chromium concentrations and wheat straw percentages on growth characteristics, development, and physiological and biochemical properties of Swiss chard, and to determine the ability of wheat straw to reduce Cr toxicity in Swiss chard cultivated in a Cr-polluted area.

## 2. MATERIALS AND METHODS

### Study Site, Cultivation, and Treatments:

In this study, a completely randomized design (CRD) as a factorial experiment using three replications was performed in the open field in Koya district ( $44^{\circ} 38' E$ ,  $36^{\circ} 4' N$  and 570 m) of altitude above sea level, Erbil, Iraq, during the growing season from September to December 2024. Twenty local chard seeds for smaller than 5 cm, plant leaf area ( $cm^2$ ) calculated by the method of (Getman-Pickering *et al.*, 2020), leaf thickness (mm) measured by using a caliper micrometer (J0006, size 0-25mm, China) (Dupuis *et al.*, 2017), plant shoot and root length (cm) were measured by metric tapeline, fresh weight and the percent of shoot and root dry matter were measured as it is mentioned by Tudela *et al.* (2017).

The method mentioned by Wellburn (1994) was used for estimating chlorophyll a (Chl.a), chlorophyll b (Chl.b), and total carotenoids by using 80% acetone. The content of peroxidase enzyme activity (POD) (units.  $g^{-1}$  fresh weight) was calculated (Müftügil, 1985), spectrophotometrically at 420 nm using guaiacol and  $H_2O_2$  (Aebi, 1974). Ascorbic acid (AA) ( $mg.100g^{-1}$  fresh weight) content in leaves was measured by the method conducted by Hussain *et al.* (2010), using ammonium molybdenum powder spectrophotometrically which determines the absorption at 760 nm. Using the spectrophotometer at 520 nm, calibrated with a proline standard curve of proline according to the work of Bates *et al.* (1973), the proline (Pr) content ( $\mu g. g^{-1}$  fresh weight) was determined using sulfosalicylic acid and

each experimental unit were planted on September 15, 2024, the experimental units were arranged in three groups 60 cm between each., in a silty loam soil with pH 7.0, EC 0.52 ds.m<sup>-1</sup> and O.M 2.42%, 5mm polythene sheets were used for lining the boxes (34 cm width, 54 cm length and 26 cm height), that filled with 40 kg soil free from weed seeds, to prevent loss of nutrients and trace elements out of the boxes, un-perforated polythene sheets were placed under each box separately in order to leachates collected were come back to the respective box. The average of the environmental conditions during the growing season varied from 11.84-30.09 °C for temperature, 27.4 – 55% for relative humidity, 0- 1.23mm for precipitation, and 5.07-2.61 km.h<sup>-1</sup> for the average of wind speed. Different agricultural services were done, including weed control and irrigation, according to the plant's needs. 2.6 g of chemical fertilizer (30N 10P 10K) was mixed with 3 liters of water and added to each experimental unit on October 8, 2024. After one month, seedling thinning was conducted to 5 plants per box, ensuring the survival of the seedlings. Between November 24 and 30, 2024, sampling was conducted, and samples were returned to the laboratory as soon as possible to determine Chlorophylls and total carotenoid pigments, as well as enzymatic and non-enzymatic antioxidants. On December 25, 2024, harvesting was done, and different shoot and root characteristics were measured. Samples for mineral constituents were also taken.

The study consisted of two factors, the first was three levels of Cr (5, 10, and 15mg.kg<sup>-1</sup> soil), in addition to the control treatment (no Cr added), where potassium dichromate ( $K_2Cr_2O_7$ ) was used as a source of the Cr, they mentioned as (Cr0, Cr5, Cr10, and Cr15), respectively and the second factor was adding wheat straw (0.1 and 2% w/w) which was applied prior to sowing. The soil of each experiment unit was mixed well with the added Cr and straw at the required concentrations according to the treatments.

### Studied Characteristics:

From each experimental unit, three plants were chosen randomly to determine all studied characteristics as follows: percent of germination was recorded every day for 15 days (Ahmadloo *et al.*, 2012), for the number of leaves and area of all leaves were counted, except those measuring ninhydrin. The soluble total carbohydrate percentage was determined spectrophotometrically at 488 nm using concentrated sulfuric acid ( $H_2SO_4$ ) and 5% phenol, as described by Herbert *et al.* (1971).

### Statistical Analysis:

Analysis of variance and Duncan's multiple-range test at a probability level of  $p \leq 0.05$  were used for the statistical analysis of the data and to compare experimental means using SAS version 9.1 of the SAS program.

## 3. RESULTS

### Seeds Germination Velocity and Percent:

The results in Table 1 show non-significant effects of Cr concentrations and straw percent on seed germination velocity. In the interaction treatments, the control and Cr15xSt1 treatments showed a significant effect, with the control and Cr15xSt1 treatments giving significantly earlier seed germination (4.17 and

4.22 days, respectively) compared to the interaction Cr0xSt1 only, which showed the longest germination velocity (4.69 days).

Regarding the effects of Cr concentration on seed germination percent (Table 1), increasing the concentration of Cr to 10mg.kg<sup>-1</sup> resulted in a significant increase in the germination percent of Swiss chard seed germination to 83.33%, compared with all other treatments. Seed germination percent decreased significantly with increasing straw percent, with values of 86.66,

72.08, and 71.66% for the control, St1, and St2, respectively. The interaction between Cr concentration and straw percent significantly affected seed germination percentage, with the highest percentage (98.33%) recorded in the control treatment and the lowest (61.66%) in the (Cr0xSt2) treatment. The effects of straw at 2% was more when no Cr or low concentration was used, whereas for the highest concentrations of Cr, its effect was less.

**Table 1:** Effects of chromium, straw, and their interactions on germination performance of Swiss chard (*Beta vulgaris*).

Treatments	Germination velocity (days)	Germination percent (%)
<b>Cr concentration (mg.kg<sup>-1</sup> soil)</b>		
Cr0	4.48 <sup>a*</sup>	74.44 <sup>b</sup>
Cr5	4.30 <sup>a</sup>	73.33 <sup>b</sup>
Cr10	4.43 <sup>a</sup>	83.33 <sup>a</sup>
Cr15	4.29 <sup>a</sup>	76.11 <sup>b</sup>
<b>Straw (%)</b>		
St0	4.26 <sup>a</sup>	86.66 <sup>a</sup>
St1	4.41 <sup>a</sup>	72.08 <sup>b</sup>
St2	4.45 <sup>a</sup>	71.66 <sup>c</sup>
<b>Interactions between Cr and Straw</b>		
Cr0 x St0	4.17 <sup>b</sup>	98.33 <sup>a</sup>
Cr0 x St1	4.69 <sup>a</sup>	63.33 <sup>de</sup>
Cr0 x St2	4.59 <sup>ab</sup>	61.66 <sup>e</sup>
Cr5 x St0	4.38 <sup>ab</sup>	83.33 <sup>b</sup>
Cr5 x St1	4.23 <sup>ab</sup>	68.33 <sup>de</sup>
Cr5 x St2	4.28 <sup>ab</sup>	68.33 <sup>de</sup>
Cr10 x St0	4.26 <sup>ab</sup>	86.66 <sup>b</sup>
Cr10 x St1	4.52 <sup>ab</sup>	85.00 <sup>b</sup>
Cr10 x St2	4.51 <sup>ab</sup>	78.33 <sup>bc</sup>
Cr15 x St0	4.23 <sup>ab</sup>	78.33 <sup>bc</sup>
Cr15 x St1	4.22 <sup>b</sup>	71.66 <sup>ed</sup>
Cr15 x St2	4.42 <sup>ab</sup>	78.33 <sup>bc</sup>

\*Means followed by the same letter for each factor and their interactions separately are not significantly different at  $p \leq 0.05$  according to Duncan's multiple range test, and vice versa.

#### Vegetative Growth:

Results in Table 2 show that each of the leaf number, leaf thickness, branch number, shoot length, stem diameter, and shoot fresh weight responded non-significantly to the increase of Cr concentration. In contrast, plant leaf area and shoot dry weight significantly decreased at 15mg.kg<sup>-1</sup> of Cr, measuring (295cm<sup>2</sup>) and (36.65g), respectively, compared to the control treatment (402cm<sup>2</sup> and 44.16g). An inverse response was observed in shoot dry matter, with an increase in Cr concentration of Cr to 15 mg.kg<sup>-1</sup>, leading to a significant increase in this property to 10.90% compared to the control treatment (9.71%).

Regarding the effects of straw percent on the performance of vegetative growth, the number of leaves number, area, and thickness, branch number, shoot length, stem diameter, shoot fresh and dry weight, and shoot dry matter were decreased significantly with increasing straw percent from 0-2% compared to the plants without straw incorporation. Leaf thickness

responded positively to the increase of straw percent, where St2 increased leaf thickness significantly to 0.89 mm compared to each of the control and St1 treatments (Table 2).

For the interactions between Cr concentrations and straw percentage, effects on the vegetative growth differ according to the studied characteristics, where only the interactions of St0 with different Cr concentrations enhanced leaf number and area, branch number, shoot length, stem diameter, shoot fresh and dry weight, and shoot dry matter when compared to other straw treatments. The exception was the amount of shoot dry matter, which noticeably increased with Cr at 15mg.kg<sup>-1</sup> across all straw rates. The highest value (11.48%) was recorded in the Cr15xSt0 treatment, whilst the lowest (7.73%) was reported in the Cr5xSt2 treatment. Regarding the plant leaf area, the highest value was recorded in the control treatment (694 cm<sup>2</sup>), whereas Cr15 xSt0 showed (513

cm<sup>2</sup>) a notable variation in leaf area, which is ascribed to elevated chromium concentrations.

**Table 2:** Effects of chromium, straw, and their interactions on some plant vegetative growth properties of Swiss chard (*Beta vulgaris*).

Treatments	Leaf number	Leaf area (cm <sup>2</sup> )	Leaf thickness (mm)	Branch number	Shoot length (cm)	Stem diameter (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot dry matter (%)
<b>Cr concentration (mg.kg<sup>-1</sup> soil)</b>									
Cr0	8.43 <sup>a*</sup>	402 <sup>a</sup>	0.84 <sup>a</sup>	1.68 <sup>a</sup>	43.60 <sup>a</sup>	12.76 <sup>a</sup>	396 <sup>a</sup>	44.16 <sup>a</sup>	9.71 <sup>b</sup>
Cr5	8.11 <sup>a</sup>	355 <sup>b</sup>	0.84 <sup>a</sup>	1.24 <sup>a</sup>	44.76 <sup>a</sup>	13.94 <sup>a</sup>	356 <sup>a</sup>	35.86 <sup>b</sup>	9.49 <sup>b</sup>
Cr10	8.37 <sup>a</sup>	335 <sup>b</sup>	0.82 <sup>a</sup>	2.15 <sup>a</sup>	45.00 <sup>a</sup>	14.78 <sup>a</sup>	384 <sup>a</sup>	39.36 <sup>ab</sup>	9.67 <sup>b</sup>
Cr15	7.86 <sup>a</sup>	295 <sup>c</sup>	0.83 <sup>a</sup>	2.35 <sup>a</sup>	41.66 <sup>a</sup>	13.81 <sup>a</sup>	326 <sup>a</sup>	36.65 <sup>b</sup>	10.90 <sup>a</sup>
<b>Straw %</b>									
St0	10.72 <sup>a</sup>	622 <sup>a</sup>	0.80 <sup>b</sup>	3.58 <sup>a</sup>	58.50 <sup>a</sup>	23.35 <sup>a</sup>	689 <sup>a</sup>	73.63 <sup>a</sup>	10.73 <sup>a</sup>
St1	7.93 <sup>b</sup>	318 <sup>b</sup>	0.81 <sup>b</sup>	1.70 <sup>b</sup>	45.67 <sup>b</sup>	18.12 <sup>b</sup>	318 <sup>b</sup>	35.54 <sup>b</sup>	10.31 <sup>a</sup>
St2	5.93 <sup>c</sup>	101 <sup>c</sup>	0.89 <sup>a</sup>	0.30 <sup>c</sup>	27.10 <sup>c</sup>	0.00 <sup>c</sup>	90 <sup>c</sup>	7.85 <sup>c</sup>	8.78 <sup>b</sup>
<b>Interactions between Cr and Straw</b>									
Cr0 x St0	11.16 <sup>a</sup>	694 <sup>a</sup>	0.81 <sup>d</sup>	4.30 <sup>a</sup>	59.43 <sup>a</sup>	24.70 <sup>a</sup>	763 <sup>a</sup>	83.18 <sup>a</sup>	10.96 <sup>ab</sup>
Cr0 x St1	8.40 <sup>b</sup>	358 <sup>d</sup>	0.83 <sup>cd</sup>	0.76 <sup>bc</sup>	46.63 <sup>bc</sup>	13.60 <sup>b</sup>	273 <sup>bc</sup>	36.36 <sup>c</sup>	9.68 <sup>b-d</sup>
Cr0 x St2	5.73 <sup>d</sup>	155 <sup>f</sup>	0.89 <sup>ab</sup>	0.00 <sup>c</sup>	24.73 <sup>d</sup>	0.00 <sup>c</sup>	151 <sup>cd</sup>	12.94 <sup>d</sup>	8.51 <sup>de</sup>
Cr5 x St0	10.33 <sup>a</sup>	661 <sup>a</sup>	0.79 <sup>d</sup>	2.20 <sup>a-c</sup>	59.76 <sup>a</sup>	22.26 <sup>a</sup>	674 <sup>a</sup>	65.79 <sup>b</sup>	9.76 <sup>b-d</sup>
Cr5 x St1	7.63 <sup>bc</sup>	312 <sup>e</sup>	0.83 <sup>b-d</sup>	1.00 <sup>b-c</sup>	45.00 <sup>bc</sup>	19.56 <sup>ab</sup>	325 <sup>bc</sup>	36.40 <sup>c</sup>	10.98 <sup>ab</sup>
Cr5 x St2	6.36 <sup>d</sup>	92 <sup>g</sup>	0.89 <sup>ab</sup>	0.53 <sup>bc</sup>	29.53 <sup>d</sup>	0.00 <sup>c</sup>	70 <sup>d</sup>	5.38 <sup>d</sup>	7.73 <sup>e</sup>
Cr10 x St0	10.96 <sup>a</sup>	618 <sup>b</sup>	0.80 <sup>d</sup>	3.96 <sup>a</sup>	59.93 <sup>a</sup>	23.93 <sup>a</sup>	726 <sup>a</sup>	77.74 <sup>a</sup>	10.73 <sup>ab</sup>
Cr10 x St1	8.40 <sup>b</sup>	300 <sup>e</sup>	0.77 <sup>d</sup>	2.50 <sup>ab</sup>	43.76 <sup>c</sup>	20.42 <sup>ab</sup>	354 <sup>b</sup>	34.19 <sup>c</sup>	9.61 <sup>bd</sup>
Cr10 x St2	5.76 <sup>d</sup>	86 <sup>g</sup>	0.88 <sup>ac</sup>	0.00 <sup>c</sup>	31.30 <sup>d</sup>	0.00 <sup>c</sup>	71 <sup>d</sup>	6.15 <sup>d</sup>	8.67 <sup>ce</sup>
Cr15 x St0	10.43 <sup>a</sup>	513 <sup>c</sup>	0.80 <sup>d</sup>	3.86 <sup>a</sup>	54.86 <sup>ab</sup>	22.50 <sup>a</sup>	592 <sup>a</sup>	67.81 <sup>b</sup>	11.48 <sup>a</sup>
Cr15 x St1	7.30 <sup>bd</sup>	300 <sup>e</sup>	0.81 <sup>d</sup>	2.53 <sup>ab</sup>	47.30 <sup>bc</sup>	18.93 <sup>ab</sup>	319 <sup>bc</sup>	35.21 <sup>c</sup>	10.98 <sup>ab</sup>
Cr15 x St2	5.86 <sup>d</sup>	72 <sup>g</sup>	0.90 <sup>a</sup>	0.66 <sup>bc</sup>	22.83 <sup>d</sup>	0.00 <sup>c</sup>	68 <sup>d</sup>	6.94 <sup>d</sup>	10.24 <sup>ac</sup>

\*Means followed by the same letter for factor and their interactions separately are not significantly different at  $p \leq 0.05$  according to Duncan's multiple range test, and vice versa.

#### Root Growth:

The results in Table 3 illustrate that chromium concentration significantly influenced root growth characteristics in Swiss chard. An increase in concentration led to a significant increase in root length and root fresh weight, reaching 34.51 cm and 76.75 g, respectively, compared with the control treatment (28.96 cm and 62.38 g). In contrast, Cr had no significant effect on root

diameter or on fresh and dry weight. Regarding root dry matter content, which increased with increasing the Cr concentration to 15mg.kg<sup>-1</sup> significantly to (24.19%) compared to 5mg.kg<sup>-1</sup> (20.73%).

According to the obtained results, Swiss chard root growth, as represented by the root length, root diameter, root fresh weight, and root dry weight, effectively decrease by addition of wheat straw to the soil, especially in treatments with elevated straw

content, with the exception of root dry matter, where there was no significant effect from raising the straw rate.

The interactions between Cr and straw levels exhibited significant trends, with the most favorable root performance recorded in the St0 treatments at Cr0, Cr5, Cr10, and Cr15mg.kg<sup>-1</sup>, indicating that ideal Cr concentrations along with the absence of straw produced the most vigorous root development.

Conversely, combinations with St2 consistently yielded the lowest values for all root characteristics, regardless of Cr concentration. The dry matter percentage of the roots was not significantly influenced by most treatments of either Cr or St as compared to the control plants. The highest value was identified at Cr0xSt2, whilst the lowest value was observed at Cr5xSt2 treatment by 25.24 and 16.95 %, respectively.

**Table 3:** Effects of chromium, straw, and their interactions on some root growth properties of Swiss chard (*Beta vulgaris*).

Treatments	Root Length (cm)	Root Diameter (mm)	Root Fresh Weight (g)	Root Dry Weight (g)	Root Dry Matter (%)
<b>Cr concentration (mg.kg<sup>-1</sup> soil)</b>					
Cr0	28.96 <sup>b*</sup>	16.21 <sup>a</sup>	62.38 <sup>c</sup>	14.21 <sup>a</sup>	23.05 <sup>ab</sup>
Cr5	32.25 <sup>ab</sup>	18.47 <sup>a</sup>	68.25 <sup>b</sup>	15.31 <sup>a</sup>	20.73 <sup>b</sup>
Cr10	33.85 <sup>a</sup>	16.38 <sup>a</sup>	76.75 <sup>a</sup>	17.19 <sup>a</sup>	21.58 <sup>ab</sup>
Cr15	34.51 <sup>a</sup>	16.50 <sup>a</sup>	70.05 <sup>b</sup>	16.27 <sup>a</sup>	24.19 <sup>a</sup>
<b>Straw (%)</b>					
St0	37.90 <sup>a</sup>	22.42 <sup>a</sup>	118.36 <sup>a</sup>	27.19 <sup>a</sup>	22.89 <sup>a</sup>
St1	35.90 <sup>a</sup>	17.89 <sup>b</sup>	75.55 <sup>b</sup>	17.19 <sup>b</sup>	22.65 <sup>a</sup>
St2	23.37 <sup>b</sup>	10.36 <sup>c</sup>	14.16 <sup>c</sup>	3.20 <sup>c</sup>	21.63 <sup>a</sup>
<b>Interactions between Cr and Straw</b>					
Cr0 x St0	35.76 <sup>a</sup>	23.56 <sup>a</sup>	122.80 <sup>b</sup>	28.47 <sup>a</sup>	23.03 <sup>ab</sup>
Cr0 x St1	28.73 <sup>b</sup>	14.80 <sup>c-e</sup>	45.10 <sup>e</sup>	9.33 <sup>d-e</sup>	20.86 <sup>ac</sup>
Cr0 x St2	22.40 <sup>b</sup>	10.26 <sup>e-g</sup>	19.26 <sup>f</sup>	4.85 <sup>e-f</sup>	25.24 <sup>a</sup>
Cr5 x St0	36.30 <sup>a</sup>	21.55 <sup>ab</sup>	95.76 <sup>c</sup>	20.73 <sup>c</sup>	21.53 <sup>a-c</sup>
Cr5 x St1	37.16 <sup>a</sup>	20.53 <sup>ab</sup>	100.70 <sup>c</sup>	23.85 <sup>b-c</sup>	23.71 <sup>ab</sup>
Cr5 x St2	23.30 <sup>b</sup>	13.33 <sup>d-f</sup>	8.30 <sup>g</sup>	1.35 <sup>f</sup>	16.95 <sup>c</sup>
Cr10 x St0	37.96 <sup>a</sup>	21.50 <sup>ab</sup>	119.90 <sup>b</sup>	27.83 <sup>ab</sup>	23.25 <sup>ab</sup>
Cr10 x St1	41.10 <sup>a</sup>	19.43 <sup>ac</sup>	96.73 <sup>c</sup>	20.95 <sup>c</sup>	21.50 <sup>ac</sup>
Cr10 x St2	22.50 <sup>b</sup>	8.23 <sup>g</sup>	13.63 <sup>fg</sup>	2.79 <sup>f</sup>	20.00 <sup>bc</sup>
Cr15 x St0	41.60 <sup>a</sup>	23.06 <sup>a</sup>	135.00 <sup>a</sup>	31.74 <sup>a</sup>	23.73 <sup>ab</sup>
Cr15 x St1	36.63 <sup>a</sup>	16.80 <sup>bd</sup>	59.70 <sup>d</sup>	14.63 <sup>d</sup>	24.52 <sup>ab</sup>
Cr15 x St2	25.30 <sup>b</sup>	9.63 <sup>fg</sup>	15.46 <sup>fg</sup>	3.80 <sup>ef</sup>	24.32 <sup>ab</sup>

\*Means followed by the same letter for factor and their interactions separately are not significantly different at  $p \leq 0.05$  according to Duncan's multiple range test, and vice versa.

#### Photosynthetic Pigments, Enzymatic and Non-enzymatic Antioxidants:

The results shown in Table 4 illustrate that the application of chromium at varying concentrations (C0, C5, C10, C15mg.kg<sup>-1</sup>) did not cause any significant changes in chl.a, chl.b, or total carotenoids, in agreement with our data. According to the results in Table 4, the effect of straw was significant on chlorophyll a, b, and total carotenoids. Chl. a and b decreased significantly when wheat straw was added to the soil, particularly at high straw percent. Inversely, total carotenoids increased significantly with the addition of 1% and 2% straw compared to the control treatment.

The interaction between Cr concentration and straw percent significantly influenced chlorophyll a and b levels, with the

highest levels observed in treatments without straw (St0), regardless of Cr concentration. This suggests that chromium application alone, in the absence of straw, exerts a less detrimental impact on pigment levels, whereas the presence of straw, particularly at 2%, markedly diminishes chlorophyll a and b content.

The results presented in Table 4 show that the effects of Cr concentration significantly affected some of the enzymatic and non-enzymatic antioxidants. The POD and Pr significantly decreased at Cr15mg.kg<sup>-1</sup> (196.11 units. g<sup>-1</sup> fresh weight) and (0.35 µg. g<sup>-1</sup> fresh weight) compared to the control treatment (205.33 units. g<sup>-1</sup> fresh weight) and (0.56 µg. g<sup>-1</sup> fresh weight). This is while the highest level of Carbohydrates (6.71%) was recorded from Cr15 treatments as compared to the other treatments, whereas Cr had a non-significant effect on AA.

The effect of straw was significant on some of the enzymatic and non-enzymatic antioxidants. The POD, AA, and CHO decreased with increasing straw percent compared to the control treatment. The proline increased significantly to  $0.47 \mu\text{g.g}^{-1}$  fresh weight with increasing the straw percent to 2% compared to the control and St1 treatments.

The interaction impact of Cr concentration and straw percent was found to be significant in increasing both Cr and

straw, which significantly reduced the levels of POD, AA, and CHO. However, the application of the highest straw percent (2%) further decreased POD activities regardless of the Cr concentration; the highest value of POD (238.66 units.  $\text{g}^{-1}$  fresh weight) was recorded from Cr5xSt0 treatment as compared to the other treatments. In contrast, the lowest values (156.33 units.  $\text{g}^{-1}$  fresh weight) was recorded from the Cr15xSt2. Presence of Cr and St caused a decrease in POD enzyme activity.

**Table 4:** Effects of chromium, straw, and their interactions on some photosynthetic pigments and enzymatic and non-enzymatic antioxidants of Swiss chard (*Beta vulgaris*).

Treatments	Chlorophyll a	Chlorophyll b	Total carotenoids	Enzymatic and non-enzymatic antioxidants				
	Pigments ( $\text{mg.g}^{-1}$ fresh weight)				Peroxidase absorbing units/ $\text{g}$ fresh weight	Ascorbic acid ( $\text{mg.100 g}^{-1}$ fresh weight)	Proline ( $\mu\text{g.g}^{-1}$ fresh weight)	Total soluble carbohydrate (%)
<b>Cr Concentration (<math>\text{mg.kg}^{-1}</math> soil)</b>								
Cr0	0.70 <sup>a*</sup>	0.39 <sup>a</sup>	0.15 <sup>a</sup>	205.33 <sup>a</sup>	28.33 <sup>a</sup>	0.56 <sup>a</sup>	5.32 <sup>b</sup>	
Cr5	0.71 <sup>a</sup>	0.40 <sup>a</sup>	0.14 <sup>a</sup>	203.55 <sup>ab</sup>	27.11 <sup>a</sup>	0.51 <sup>b</sup>	5.19 <sup>b</sup>	
Cr10	0.69 <sup>a</sup>	0.40 <sup>a</sup>	0.14 <sup>a</sup>	201.55 <sup>ab</sup>	25.44 <sup>a</sup>	0.38 <sup>c</sup>	5.04 <sup>b</sup>	
Cr15	0.70 <sup>a</sup>	0.37 <sup>a</sup>	0.15 <sup>a</sup>	196.11 <sup>b</sup>	27.11 <sup>a</sup>	0.35 <sup>d</sup>	6.71 <sup>a</sup>	
<b>Straw %</b>								
St0	0.84 <sup>a</sup>	0.64 <sup>a</sup>	0.12 <sup>b</sup>	227.41 <sup>a</sup>	34.66 <sup>a</sup>	0.43 <sup>b</sup>	7.49 <sup>a</sup>	
St1	0.68 <sup>b</sup>	0.30 <sup>b</sup>	0.16 <sup>a</sup>	208.66 <sup>b</sup>	25.25 <sup>b</sup>	0.45 <sup>b</sup>	5.54 <sup>b</sup>	
St2	0.58 <sup>c</sup>	0.23 <sup>b</sup>	0.15 <sup>a</sup>	168.83 <sup>c</sup>	21.08 <sup>c</sup>	0.47 <sup>a</sup>	3.66 <sup>c</sup>	
<b>Interactions between Cr and Straw</b>								
Cr0 x St0	0.85 <sup>a</sup>	0.63 <sup>a</sup>	0.13 <sup>ab</sup>	228.66 <sup>ab</sup>	41.33 <sup>a</sup>	0.75 <sup>a</sup>	8.69 <sup>a</sup>	
Cr0 x St1	0.74 <sup>b</sup>	0.35 <sup>b</sup>	0.16 <sup>a</sup>	200.66 <sup>e</sup>	24.33 <sup>c</sup>	0.35 <sup>f</sup>	3.72 <sup>e</sup>	
Cr0 x St2	0.53 <sup>e</sup>	0.20 <sup>b</sup>	0.15 <sup>a</sup>	186.66 <sup>f</sup>	19.33 <sup>c</sup>	0.57 <sup>b</sup>	3.56 <sup>e</sup>	
Cr5 x St0	0.84 <sup>a</sup>	0.71 <sup>a</sup>	0.08 <sup>b</sup>	238.66 <sup>a</sup>	34.66 <sup>ab</sup>	0.32 <sup>f</sup>	6.64 <sup>bc</sup>	
Cr5 x St1	0.66 <sup>cd</sup>	0.29 <sup>b</sup>	0.16 <sup>a</sup>	204.33 <sup>de</sup>	24.33 <sup>c</sup>	0.50 <sup>c</sup>	5.85 <sup>bd</sup>	
Cr5 x St2	0.64 <sup>cd</sup>	0.21 <sup>b</sup>	0.18 <sup>a</sup>	167.66 <sup>g</sup>	22.33 <sup>c</sup>	0.71 <sup>a</sup>	3.10 <sup>e</sup>	
Cr10 x St0	0.84 <sup>a</sup>	0.64 <sup>a</sup>	0.12 <sup>ab</sup>	218.33 <sup>bd</sup>	27.33 <sup>bc</sup>	0.48 <sup>cd</sup>	6.47 <sup>bc</sup>	
Cr10 x St1	0.65 <sup>cd</sup>	0.32 <sup>b</sup>	0.14 <sup>a</sup>	221.66 <sup>bc</sup>	27.33 <sup>bc</sup>	0.49 <sup>cd</sup>	5.66 <sup>cd</sup>	
Cr10 x St2	0.59 <sup>e-e</sup>	0.25 <sup>b</sup>	0.15 <sup>a</sup>	164.66 <sup>g</sup>	21.66 <sup>c</sup>	0.18 <sup>g</sup>	2.98 <sup>e</sup>	
Cr15 x St0	0.84 <sup>a</sup>	0.60 <sup>a</sup>	0.14 <sup>a</sup>	224.00 <sup>b</sup>	35.33 <sup>ab</sup>	0.18 <sup>g</sup>	8.18 <sup>a</sup>	
Cr15 x St1	0.67 <sup>b<sup>c</sup></sup>	0.26 <sup>b</sup>	0.18 <sup>a</sup>	208.00 <sup>c-e</sup>	25.00 <sup>c</sup>	0.45 <sup>de</sup>	6.93 <sup>b</sup>	
Cr15 x St2	0.58 <sup>d<sup>e</sup></sup>	0.26 <sup>b</sup>	0.13 <sup>ab</sup>	156.33 <sup>g</sup>	21.00 <sup>c</sup>	0.43 <sup>e</sup>	5.02 <sup>d</sup>	

\*Means followed by the same letter for factor and their interactions separately are not significantly different at  $p \leq 0.05$  according to Duncan's multiple range test, and vice versa.

#### 4. DISCUSSION

##### Seed Germination Velocity and Percent:

Generally, the interaction of chromium and straw treatments decreased seed germination which is accompanied by germination condition especially the high wind speed ( $13.15 - 0.56 \text{ h.km}^{-1}$ ), and high temperature, where the average temperature during the germination period (September) was ( $35.87-24.31^\circ\text{C}$ ) that is more than the optimal temperature for

Swiss chard seed germination ( $7^\circ\text{C}$  to  $24^\circ\text{C}$ ) (Abdulmalek, 2014). In this study, only the  $10\text{mg.kg}^{-1}$  soil of Cr increased the percent of seed germination, in contradictory to the results of radish seed germination percent which increased to 1.65, 3.3, 13.3 and 38.3% for Cr concentration of 10, 20, 40, and 60ppm respectively (Mohammed *et al.*, 2021). At the Cr concentration of 8 ppm, the percent of lettuce and Swiss chard germination was 90 and 30%, respectively (Escudero-Villa *et al.*, 2024). Direct contact of metals with the developing seedling tissues was noticeable after protrusion, whereas before the protrusion,

embryo tissues were protected by the seed coat from the metals (Bautista *et al.*, 2013). All treatments of 2% straw decreased significantly the germination of seeds, which may be due to straw releasing different allelochemicals that reduce water absorption and disturb enzyme activity in seeds, resulting in reduced or delayed germination rates. Consequently, an increase in straw quantities may result in a reduction in both germination percent and speed (Li *et al.*, 2024). The incorporation of straw, particularly at elevated percentages, diminished both the rate and efficacy of germination; hence, the use of straw did not have any beneficial impact on these parameters for Swiss chard plants. In the study of Alghamdi *et al.* (2022), the allelopathic activity of the straw of wheat was recognized to hydroxamic acids and other related chemicals, along with phenolic acids that contribute to minimize the percent of germination, where allelochemicals penetrate the seeds internal tissues, resulting in a reduction of gibberellin and the activity of  $\alpha$ -amylase, while at the same time it enhances antioxidant enzyme activity (Li *et al.*, 2024).

### Vegetative Growth:

The decrease in the plants' leaf area was significantly due to increasing concentration of Cr, which was parallel to the finding of Ali *et al.* (2020) on wild cabbage *Brassica oleracea* L. leaf area, which decreased by 29% as compared with controls, caused by high Cr concentration. Similarly, in green amaranth (*Amaranthus viridis*), increased Cr concentration showed significantly inhibited leaf number (Joseph *et al.*, 2023).

In this study all vegetative growth characteristics decreased with increasing straw percent, where this finding contradicts the previous results of Sarwar *et al.* (2023), where the application of compost improved fresh and dry weights of shoot (33 and 42%) and roots (77 and 33 %), of spinach (*Spinacia oleracea*) respectively, except for the percent of shoot dry matter, which considerably increased with the use of 1% straw. The reduction in these characteristics arises from the primary drawback of straw. When the quantity of straw returned is excessive, its comparatively high ratio of carbon to nitrogen (C/N) leads to antagonism with nitrogen during decomposition. Soil microorganisms utilize nitrogen to decompose the carbon-rich straw, thereby diminishing nitrogen availability for plant growth (Jin *et al.*, 2020).

The incorporation of high straw percent resulted in enhanced leaf thickness, epidermal cell size, and cuticle layers that may undergo thickening as a defensive mechanism to mitigate drought stress (Khaliq *et al.*, 2011). The reverse effect of straw may be due to delayed straw breakdown and restricted nutrient release resulting from an inadequate decomposition period, similar to the findings reported by Alghamdi *et al.* (2022).

The reduction in shoot length may result from chromium-induced ultrastructural damage to leaf mesophyll cells, ultimately leading to decreased shoot growth and, in turn, smaller leaf area (Ahmad *et al.*, 2020). These results agree with previously reported findings, which indicated the highest reduction in leaf number with Cr supply at 20mg kg<sup>-1</sup> in green amaranth (*Amaranthus viridis*) (Joseph *et al.*, 2023). Leaf thickness showed a positive correlation with increased levels of straw percent across all treatments compared with the control, possibly as a response to stress conditions induced by the percent of straw, as indicated by the results; it was high in the northern part of Iraq. The reduction of dry biomass with increased Cr concentrations can be ascribed to diminished CO<sub>2</sub> assimilation resulting from a

metal-induced drop in photosynthetic pigments. In the existence of Cr, the absorption of each of P, Ca, and Mg in roots and shoots was reduced to 49 and 71 % for Ca, 39 and 44% for Mg, and 57 and 77% for P in roots and shoots, respectively as compared to the control conditions (Sarwar *et al.*, 2023).

### Root Growth:

Increasing root length and fresh weight by increasing Cr concentration, as shown in Table 3 was not in agreement with the applied Cr in 4, 6, and 8 ppm for Swiss chard and lettuce, which demonstrated restricted radicle development, presumably because of the heightened sensitivity of the radicle to metallic solutions of Cu, Cr, and Cd (Escudero-Villa *et al.*, 2024). These results differ from those reported by Ali *et al.* (2020), who found that at a dose of 200  $\mu$ M, Cr stress reduced root length by 32% in *Brassica oleracea* L. compared to the control. Similarly, Cr supply at 5 and 10mg.kg<sup>-1</sup> resulted in a decrease in the root length in green amaranth (*Amaranthus viridis*) (Joseph *et al.*, 2023). An increase in dry matter production enhanced plant yield, increased source size, and enhanced photosynthetic activity were identified as the primary factors underlying increased dry matter under heavy metal stress, specifically Cr (Hayat *et al.*, 2012). Excessive straw absorption increases stress or diminishes the availability of nutrients. The roots and leaves of *Brassica oleracea* exhibited decreased growth as a result of Cr stress; root dry weight declined up to 43% at the treatment of 200  $\mu$ M of chromium.

Decreases in all root growth characteristics with straw application are similar to the results reported by Alghamdi *et al.* (2022). The opposing effects of wheat straw may be attributed to allelopathic chemicals or to nitrogen immobilization during breakdown, which can hinder Swiss chard growth. Alternatively, the brief growth cycle of the plants is due to the potential drawbacks of straw relative to composts over time (Khaliq *et al.*, 2011). The tolerance efficiency will depend on the species of the plant, indicating that elevated concentrations of Cr, along with high straw rates, may intensify toxicity effects, resulting in reduced biomass (Alghamdi *et al.*, 2022). This result supports a previous study indicating that organic additions can alleviate heavy metal stress by augmenting soil microbial activity and modifying metal speciation (Sarwar *et al.*, 2023).

### Photosynthetic Pigments, Enzymatic and Non-enzymatic Antioxidants:

The study may not have affected the upper parts of plants, especially the leaves, due to the application of powdered chromium mixed with soil. At the highest Cr concentration (Cr15mg.kg<sup>-1</sup>), Swiss chard has kept a stable pigment content. In spite of the accumulated Cr in roots and slow translocation to the aerial parts of the plant, plants showed different levels of Cr tolerance, uptake, and accumulation (Singh *et al.*, 2013). Our results are not in line with those obtained by Ali *et al.* (2020), which at concentration (200  $\mu$ M), chromium stress decreased the photosynthetic pigments leaves of (*Brassica oleracea*), also, for 10ppm, 20ppm, 40ppm, and 60ppm of chromium due to decreasing chlorophyll content by 23%, 41%, 67%, and 75% respectively, as compared to control radish (*Raphanus sativus L*) plant (Mohammed *et al.*, 2021). These results are also not consistent with the results previously reported for lettuce and Swiss chard under Cr exposure effects on the photosynthetic process, leading to decreased photochemical activity, structural

damage, and alterations in the levels of photosynthetic pigments (Escudero-Villa *et al.*, 2024).

Parallel results were found by Alghamdi *et al.* (2022). The high straw percent may directly damage the photosynthetic apparatus of the leaf in several ways, where the central core of the chlorophyll molecule is magnesium; thus, if Mg is deficient, the lack of chlorophyll synthesis results in stunted plant growth; in addition, nutrients like iron and manganese are needed for the synthesis of chlorophyll. Therefore, their lower absorption rates can reduce the synthesis of chlorophyll at high straw percent as reported by Khaliq *et al.* (2011). It is clarified that increasing the straw percent significantly decreased chlorophyll contents; this may be due to increased immobilization of nutrients or changes in soil microbial activity that affect nutrient uptake, as found by Jin *et al.* (2020). These results differ from previously reported results for lettuce treated with straw application (Liang *et al.*, 2019). In plant pigments, carotenoids function as non-enzymatic antioxidants, as they protect organs of plants from stresses (Sinha *et al.*, 2005). The total carotenoid content in this plant was elevated by augmenting the straw ratio to 2% relative to the control treatment. Wheat allelochemicals may have suppressed chlorophyll in chard by disrupting the manufacture of photosynthetic pigments or accelerating their breakdown through the induction of oxidative stress via reactive oxygen species, or by a combination of both mechanisms (Alghamdi *et al.*, 2022). Furthermore, Swiss chard is rich in phytopigments; chlorophylls and carotenoids which enhance antioxidant, detoxifying, and immune defense mechanisms derived from the diet (Libutti *et al.*, 2023). Numerous prior studies have shown a reduction in protein and sugar levels with increasing straw extract concentration, and thus, attributed the elevated free amino acid content to the breakdown of prevalent proteins (Alghamdi *et al.*, 2022).

Decreasing each of the POD enzyme activity and Pr content contrasts with those found by Mohammed *et al.* (2021), who showed that raised chromium levels improved the plant's mechanisms of stress tolerance, including Pr concentration and activities of the antioxidant enzymes in radish (*Raphanus sativus* L.), where Swiss chard has a high tolerance to stresses (Abdulmalek, 2014). Also, it may be due to the low concentrations of Cr used in this study, which has a stimulate hermetic effects on the plant, where exposure to the same elements like Cd, improves the performance of different plant species, despite accumulation of Cd in their roots and shoots, their defensive strategies have improved these plants to minimize Cd toxicity or such mechanisms that consider Cd as beneficial element (Carvalho *et al.*, 2020). Antioxidants play an important role in encouraging plants' resistance to metals by protecting labile macromolecules from damage caused by free radicals. Similar results were reported by Ali *et al.* (2020), who showed that the activity of antioxidative enzymes (SOD, CAT, and POD) was reduced in the roots and leaves of (*Brassica oleracea*) as a result of chromium stress. Excessive Cr stress at a concentration of 200  $\mu$ M of chromium reduces antioxidant synthesis, leading to damage in the growth and some physiological parameters of the green amaranth plant (*A. viridis*) (Joseph *et al.*, 2023).

Decreasing POD, AA, and CHO with increasing straw percent was similar to the results of Khaliq *et al.* (2011), who indicated that the antioxidant activity is related to polyphenolic chemicals, counting those found in Swiss chard, which can mitigate and reduce oxidative stress according to their chemical properties (Libutti *et al.*, 2023). Cr can inhibit antioxidant

enzymes, such as POD, by altering their structure or interfering with their synthesis at the genetic level. Simultaneously, excessive straw incorporation into the soil can release phenolic compounds during decomposition that act as natural enzyme inhibitors (Abdellah *et al.*, 2024). The interaction between Cr and straw percent had significant effect and decreased the CHO at Cr10xSt2 (2.98%) as compared to the control (8.69%), a previous study indicated that exposure to chromium and compost significantly enhanced the production of several antioxidants in spinach (Sarwar *et al.*, 2023) which is also true for total carbohydrate content by (Alghamdi *et al.*, 2022).

## CONCLUSION

The present study concluded that Swiss chard could grow under chromium-amended nutrient conditions. The results showed that the use of Cr at 15mg.kg<sup>-1</sup> soil led to a significant increase in the percent of seed germination, and application of wheat straw at 1% and 2% decreased the percent of seed germination. Most vegetative growth characteristics responded inversely to the increasing wheat straw. A high straw percent of 2% w/w decreases most root and shoot performance, compared to no straw added plants, except for the leaf thickness. Photosynthetic pigments were affected non-significantly by the application of Cr and significantly decreased by the addition of straw. Applications of Cr and wheat straw decreased enzymatic and non-enzymatic activity; thus, the application of wheat straws was un-conducive to the normal growth of Swiss chard. Further research is recommended to understand how different types, rates, and application methods of wheat straw impact soil health, crop growth, yield, and quality. This includes exploring how these factors affect soil structure, nutrient composition, and microbial activity, which are crucial for sustainable crop production.

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## Ethical Statement:

This study was conducted on plants, which do not require a consent approval form.

## Author Contributions:

B. E. A. conducted the experiment, took the required measurements and laboratory part, and wrote the manuscript. I. M. A. was the supervisor of the study who designed the study concept, statistical analysis, and laboratory studies, and reviewed the manuscript.

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