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Cover Page Footnote

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Effect of Chromium Metal Accumulation on the Magnesium Absorption and Chlorophyll Content in Vegetables

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Abstract

This study analyzed the effect of chromium metal accumulation on magnesium absorption and chlorophyll content in vegetables. The effect of accumulation was determined by performing controlled experimental methods on planting media supplemented with chromium and by directly observing vegetables grown in chromium-polluted areas, such as mountain, rice field, street, and industrial areas. The controlled experiments were carried out by varying the chromium contamination (1 and 3 ppm) and magnesium nutrition (0.4, 0.6, and 0.8 g/L) in planting media. The controlled experiment was compared with the results of field observation in several chromium-polluted areas. The effect of the treatment was analyzed based on the chlorophyll and magnesium levels in the leaves in comparison with the chromium levels in the planting medium. The results of observation and controlled experiments showed that the accumulation of chromium in plants affected the absorption of magnesium, which also affected chlorophyll formation and thus disrupted plant growth. The high chromium level (3 ppm) and magnesium level in planting media can reduce the accumulation of chromium in kale stems and leaves by 19% and 33%, respectively, increase magnesium absorption on kale stems and leaves by 7% and 5%, respectively, and increase chlorophyll formation on kale stems and leaves by 12% and 11%, respectively. Field observation in several chromium-polluted areas showed that spinach has a better chromium accumulation tolerance than kale in terms of magnesium absorption. The type of planting media, plant species, and the presence of other metal contaminants also affect chromium accumulation, magnesium absorption, and chlorophyll level.

Keywords: accumulation, chlorophyll, chromium, magnesium

Introduction

Plants grow well if the macro and micronutrients essential for growth are provided in planting medium. Nutrient deficiency can disrupt plant growth [1][2]. Many factors affect the absorption of minerals needed by plants from growth media through the roots. One factor is the existence of species, such as heavy metals, that are unnecessary in plant metabolic processes [3]. When the roots absorb the minerals they need, heavy metals around the roots can also accumulate into plants through the roots. According to the Hard–Soft Acid–Base (HSAB) theory proposed by Pearson, Cr^{3+} has the same properties as Mg^{2+} [4]. Therefore, the presence of Cr^{3+} in the planting medium can increase the competition of Mg^{2+} absorption by the roots so that the absorption of Mg^{2+} metals becomes disrupted. Metabolic processes and reactions that are influenced by magnesium are photoposporylation, CO_2 fixation in photosynthesis, synthesis protein, chlorophyll biosynthesis, phloem loading, partition and utilization photoacimilation, formation of reactive oxygen species, and photooxidation on leaf tissue [5].

Chou *et al.* [6] and Liu *et al.* [7] compared the effect between nutrient deficiency and cadmium toxicity. Chou reported that deficiency magnesium can increase the activity of antioxidant enzymes. Deficiency in magnesium can protect rice seedlings from cadmium-induced stress. However, chlorophyll damage is greater in leaves with sufficient magnesium than in leaves with deficient magnesium. Magnesium also exerts no effect on cadmium absorption through the roots because cadmium levels in plant stems and roots remain high when the growing media are magnesium deficient. Potassium deficiency can increase the activity of antioxidant enzymes but cannot inhibit cadmium uptake from the environment [7]. According to Gill [8], the toxicity of heavy metals in plants depends on the type of plants and heavy metals. Thus, the effect of Cr accumulation on Mg absorption must be observed. Chlorophyll content studies are needed to establish the correlation between Mg and chlorophyll biosynthesis.

Heavy metals such as chromium are not needed in plant growth. High chromium concentrations are usually

toxic to plants. Chromium metal that accumulates in plants can disrupt metabolism during growth [5] [9]. Chromium can trigger oxidative stress, which causes degradation of photosynthetic pigments, such as chlorophyll a and chlorophyll b, thus inhibiting the growth process [10]. Chromium metal can inhibit the germination, growth, and vigor index of seedlings in *Hibiscus esculentus* [11]. The metabolic processes in plants occur with the help of chlorophyll. Mg^{2+} metal plays an important role in chlorophyll biosynthesis. Mg^{2+} metal is a complexing ion in the chlorophyll structure. Thus, when the absorption of Mg^{2+} metal is disturbed, chlorophyll biosynthesis can also be disrupted.

Magnesium ion has the same properties as ion chromium. Chromium in nature exists in Cr^{3+} as a non-toxic ion and Cr^{6+} as a toxic ion. Thus, studies focusing on chromium in plants are rarely discussed. Arranging magnesium ion levels in planting media is expected to reduce chromium accumulation in plants. In this study, the effect of magnesium absorption was analyzed in controlled planting media contaminated with chromium. Results of the controlled experiment were compared with those of field observation, which indicated contamination with chromium metal in Lampung. The determination of chlorophyll levels was analyzed to compare its relationship with magnesium adequacy. In the controlled experiment, water was used as planting media to regulate its nutritional levels easily. Kale was used as an object in the controlled experiment. Kale and spinach were used as study objects in the field observation because they are common types of vegetables easily cultivated in the community.

Methods

Tools and Materials. The equipment used in this study included a trial bucket, glassware, and a visible spectrophotometer. Materials used in this study were kale, spinach, and planting media (water and sediment). Samples were taken from several locations contaminated with chromium metal in Lampung, including a mountain area in Batu Kramat, a rice field area in Pekalongan East Lampung, a street area in Pekalongan, East Lampung, and an industrial area in Tanjung Bintang South Lampung. Samples obtained in an uncontaminated area in Tanggamus served as control. Chemical materials used in planting media were $Cr(NO_3)_2$, $Cd(NO_3)_2$, and $Pb(NO_3)_2$ as chromium, cadmium, and lead metals, respectively. Hoagland media contained the following [12]: $Ca(NO_3)_2 \cdot 4H_2O$, KNO_3 , KH_2PO_4 , $MgSO_4 \cdot 7H_2O$, ferri-tartart, H_3BO_3 , $MnCl_2 \cdot 4H_2O$, $ZnSO_4$, $CuSO_4 \cdot 5H_2O$, $H_2MoO_4 \cdot 4H_2O$.

Controlled Experiment. The treatment was composed of 1 ppm Cr (A1)-contaminated media and 3 ppm Cr (A2)-contaminated media with Mg contents of 0.4 g/L (X1), 6 g/L (X2), and 0.8 g/L (X3). Cr and Mg were

poured into Hoagland media. Acclimatized kale was planted on A1X1, A2X1, A1X2, A2X2, A1X3, and A2X3 media, with two replications for sampling and instrumental analyses. Kale acclimatized for 3 days was planted with Hoagland media by regulating the magnesium level in chromium-contaminated media. Planting was carried out for 9 days.

Experiments were carried out with variations in growing media to determine the effects of other heavy metals through Mg and chlorophyll levels. Kale was grown on media with different levels of heavy metal contamination. In specific, Media B was contaminated with 4 ppm Cr; Media D with 20 ppm Cd, 4 ppm Cr, and 8 ppm Pb; and Media E was uncontaminated. Planting was carried out for 10 and 15 days.

Vegetable Sampling on Field Observation. Purposive sampling was performed by referring to the type of location where kale and spinach were planted: 1) the mountains as a positive control, 2) rice fields, 3) the edge of the highway (street), and 4) the industrial area.

Chlorophyll Analysis. Samples of the crushed 0.5 g dried vegetables were dissolved in 20 mL of 85% acetone for 4 h, and then the solution was filtered. The filtrate was diluted with acetone until 25 mL. The filtrate was measured for absorbance at wavelengths of 663 and 645 nm with a visible spectrophotometer. Chlorophyll content was calculated using the following formula:

$$\text{Chlorophyll}(ppm) = \frac{17,30 \times A_{645} + 7,18 \times A_{663}}{\text{mass of sample} \times \text{filtrate}} \times 1000$$

Chromium Analysis (Vogel Method [13]). Samples of the vegetable section were digested with 50 mL of H_2SO_4 for 50 mL at 110 °C. Then, 10 mL of the sample was reacted with 5 mL of 0.25% 1,5-diphenylcarbazide complexing reagent to form a violet-colored complex. The complex was diluted until 25 mL. The absorbance of the violet-colored complex was obtained at a wavelength of 365 nm with a visible spectrophotometer after 10 min.

Magnesium Analysis (Vogel Method [13]). The sample of the vegetable section was dried with the furnace and then dissolved with 10 mL of 1 M HCl. The sample was added with 80 mL of buffer solution pH 10 (0.75% w/v ammonium chloride solution dissolved in ammonia solution) and then reacted with 10 mL of 0.1% solochrome black complexing reagent in methanol to form a red complex by conditioning the solution pH with a buffer of pH 10. The absorbance of the red complex was obtained at a wavelength of 520 nm with a visible spectrophotometer not more than 20 min.

Data Analysis. Data from the observation of variations in chromium contamination and magnesium nutrition through chlorophyll levels in the stem and leaves were analyzed using two-way ANOVA ($p < 0.05$) with variations in planting contaminated media of chromium (X1) and variation of magnesium nutrition levels (X2).

Results

The effect of chromium metal accumulation on magnesium absorption and chlorophyll content was observed through experiments and direct observation of plants in areas contaminated with chromium. Observation through experiments was carried out to determine the effect of chromium metal accumulation on magnesium absorption and chlorophyll content under controlled contamination conditions. Meanwhile, direct observation of vegetables that grew at indicated locations contaminated with chromium was performed to determine the pattern of its effect significantly. The standard deviation values of the analyzed data were 2–6 for chromium, 0.2–1.6 for magnesium, and 27–110 for chlorophyll.

As shown in Figure 1, in 1 ppm chromium-contaminated planting media, increased levels of magnesium in planting media (0.4–0.8 g/L) decreased chromium accumulation in kale stems by 15%, increased magnesium absorption by 3%, and increased chlorophyll formation by 6%. In 3 ppm chromium-contaminated planting media, chromium accumulation decreased by 19%, which increased magnesium absorption in kale stems by 7% and chlorophyll formation by 12%. Chromium accumulation affects the chlorophyll content in kale stems. The magnesium content in the stem was proportional to the chlorophyll level because magnesium is a complex part of chlorophyll. Thus, an increase in magnesium level was proportional to an increase in chlorophyll level. Increased levels of magnesium (X1, X2, and X3) in 1 ppm (A1) or in 3 ppm (A2) chromium-polluted growing media increased the chance of magnesium absorption through the roots and reduced the chance of chromium absorption through the roots. The high concentration of magnesium in plant stems was proportional to the chlorophyll content in kale stems.

The high chromium contamination on planting media (A2 = 3 ppm) in this study induced higher magnesium and chlorophyll levels than the chromium contamination of 1 ppm (A1). This result indicates that high chromium stress in planting media can increase the ability of plants to survive. The absorption of magnesium when chromium contamination was 3 ppm was greater than that when chromium contamination was only 1 ppm.

The same phenomenon was observed in kale leaves. The accumulation of chromium in kale affected the

chlorophyll content in kale leaves. As shown in Figure 2, at low concentrations of chromium contamination (A1) by adjusting the levels of magnesium (X1, X2, and X3), the magnesium concentration in kale leaves was higher at the magnesium regulation of 0.8 g/L (X3) compared with 0.6 g/L (X2) and 0.4 g/L (X3). The same thing occurred with high chromium contamination (A2). As shown in Figure 2, the magnesium regulation in 3 ppm chromium-polluted (A2) planting media had higher concentration than that in 1 ppm chromium-polluted planting media.

In planting media contaminated with 1 ppm chromium and magnesium levels at 0.4–0.8 g/L, chromium accumulation decreased in kale leaves by 28%, which increased magnesium absorption by 2% and chlorophyll formation by 5%. In planting media contaminated with 3 ppm chromium, chromium accumulation decreased by 33%, which increased magnesium absorption by 5% and chlorophyll formation by 11%.

Table 1 and Table 2 show the two-way ANOVA results of chlorophyll levels ($p < 0.05$) with variations in planting contaminated media of chromium (X1) and variation of magnesium nutrition levels (X2). Chromium contamination on kale planting media significantly affected the chlorophyll level in the stems and leaves, as evidenced by sig value $X1 < 0.05$. Regulation of magnesium nutrition levels in planting media significantly influenced chlorophyll level in the stems and leaves, as evidenced by sig value $X2 < 0.05$. Regulation of magnesium nutrition levels and varying chromium contamination in planting media affected chlorophyll level, as evidenced by sig value $X1 * X2 < 0.05$.

Fields observation of vegetables grown in locations with chromium contamination was performed to determine the influence pattern of chromium accumulation on magnesium absorption and chlorophyll content. As shown in Figure 3, high chromium accumulation in plant parts corresponded to low levels of magnesium

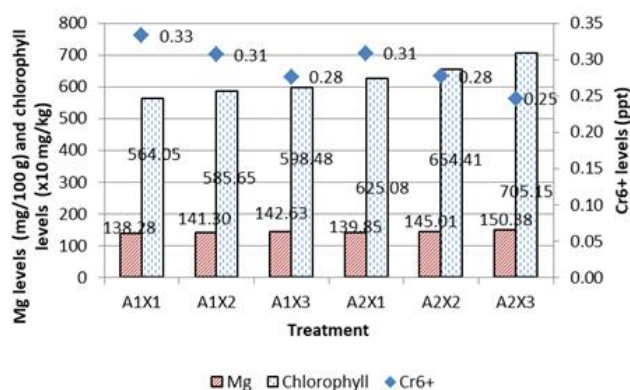


Figure 1. Comparison of Cr⁶⁺, Mg, and Chlorophyll Levels in Kale Stems

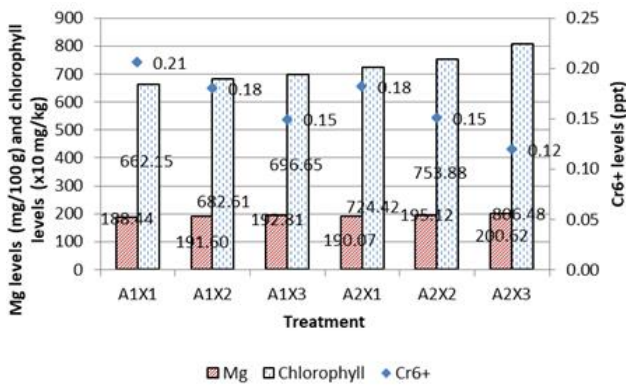


Figure 2. Comparison of Cr⁶⁺, Mg, and Chlorophyll Levels in Kale Leaves

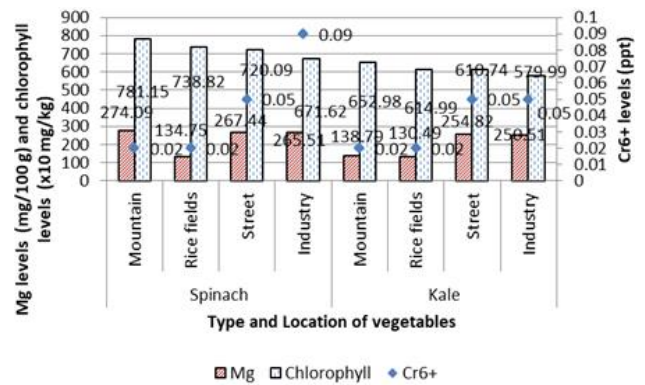


Figure 3. Comparison of Cr⁶⁺, Mg, and Chlorophyll Levels in the Leaves of Spinach and Kale

Table 1. Two-way ANOVA Test of Chlorophyll Levels in Kale Stems

Dependent Variable: Chlorophyll levels						
Source	Type III Sum of Squares	df	Mean Source	F	Sig.	Partial Eta Squared
Corrected Model	2.641E6 ^a	5	528234.368	134.947	.000	.991
Intercep	4.645E8	1	4.645E8	1.187E5	.000	1.000
X1	1863612.202	1	1863612.202	476.095	.000	.988
X2	658246.174	2	329123.087	84.081	.000	.966
X1 * X2	119313.465	2	59656.733	15.240	.004	.836
Error	23486.236	6	3914.373			
Total	4.671E8	12				
Corrected Total	2664658.077	11				

a. R Squared = .991 (Adjusted R Squared = .984)

Table 2. Two-way ANOVA Test of Chlorophyll Levels in Kale Leaves

Dependent Variable Chlorophyll levels						
Source	Type III Sum of Squares	df	Mean Source	F	Sig.	Partial Eta Squared
Corrected Model	2.786E6 ^a	5	557208.711	108.182	.000	.989
Intercep	6.239E8	1	6.239E8	1.211E5	.000	1.000
X1	1974322.900	1	1974322.900	383.313	.000	.985
X2	684078.124	2	342039.062	66.407	.000	.957
X1 * X2	127642.528	2	63821.264	12.391	.007	.805
Error	30904.088	6	5150.681			
Total	6.267E8	12				
Corrected Total	2816947.640	11				

a. R Squared = .989 (Adjusted R Squared = .980)

and chlorophyll in spinach leaves. This result indicates that chromium accumulation in plants affects magnesium absorption, which also affects chlorophyll formation and plant metabolism.

Plant species also influence the effect of chromium metal accumulation on magnesium absorption and chlorophyll content. Chromium accumulation was greater in spinach than in kale, indicating that the type

of plant affects chromium accumulation. Spinach exhibited better survival ability in conditions polluted with chromium than spinach, as shown in Figure 3, Figure 4, and Table 1. Under the same environmental condition and presence of other contaminants, kale accumulated less Cr than spinach. However, although the accumulation of chromium in kale was lower than the accumulation of chromium in spinach in the industrial area, the chlorophyll content in kale leaves was lower than that in spinach. The chromium accumulation in the industrial area was the highest among the sampled areas. In the industrial area, the chromium accumulation reached 0.09 ppt in spinach leaves and 0.08 ppt in spinach stems, whereas the chromium accumulation on kale was only 0.05 ppt. However, the chlorophyll level in spinach was higher than that in kale. This condition also occurs in mountain, rice field, and street areas, where the chromium accumulation was almost the same. This finding proves that under chromium stress, spinach exerts better magnesium absorption than kale. Plants species affect chromium accumulation, magnesium absorption, and chlorophyll formation.

As shown in Figure 4, the magnesium and chlorophyll levels in kale and spinach leaves were low when the chromium accumulation in the plant parts was high, except on the kale stem at the industrial area. This condition is generally almost the same as the analysis results of spinach and kale. Therefore, the chromium accumulation in plants influences the magnesium absorption and chlorophyll levels further.

Chromium accumulation in plants affects magnesium absorption and chlorophyll formation, which can also be observed in several chromium-contaminated areas. High levels of magnesium and chlorophyll were found in the leaves and stems of spinach from the mountain area, which had low chromium accumulation. The high chromium accumulation of 0.09 ppt on spinach leaves and 0.08 ppt on spinach stems in the industrial area showed the lowest levels of magnesium and chlorophyll in spinach. However, this condition was still higher than the magnesium levels in the leaves and stems of spinach in the rice field area.

The chromium accumulation on spinach leaves and stems in rice fields was 0.02 ppt, but its magnesium levels were lower than those in the industrial area where chromium accumulation was the highest. This condition also can be observed in the leaves and stems of kale in rice fields. The chlorophyll level on spinach and kale in the rice field area was still higher than that in the industrial area. This phenomenon may be due to the lack of magnesium in rice fields, but its deficiency did not cause chlorophyll levels in spinach and kale to be lower than those in the industrial area.

Different patterns were observed in the magnesium and chlorophyll levels of kale leaves and stems. The highest magnesium level occurred on kale in the industrial area, which had the highest chromium accumulation. The magnesium level on kale in the industrial area was inversely proportional to the chlorophyll level formed on kale leaves and stems. When the chromium accumulation in kale was higher, the magnesium levels increased but the chlorophyll levels decreased. This condition is different from the conditions of kale that were observed through controlled experiment.

This condition can be explained through the data in Table 3, which is the result of chromium and several other metal pollutants, such as cadmium and lead, in planting media of spinach and kale in several chromium-contaminated areas. As listed in Table 3, the levels of chromium, cadmium, and lead in water were lower than those in sediment/soil. Kale was grown in water while spinach was grown in sediment/soil in all indicated chromium-contaminated areas. Thus, the chromium accumulation in spinach grown in sediment/soil was higher than that in kale. Magnesium has a lower molecular weight than chromium, cadmium, and lead. Thus, magnesium is more likely to dissolve in water. This phenomenon causes the absorption of magnesium to be higher in kale than in spinach. The presence of other heavy metals in the planting media and the type of planting media also influences the levels of magnesium and chlorophyll in vegetable.

The presence of other heavy metals in the growing media also affected Mg and chlorophyll levels in kale leaves, as shown in Figure 5. After 15 days, the presence of other heavy metals such as Cd and Pb in Media D reduces Mg and chlorophyll levels in kale leaves. In 10 days, where the chromium accumulation and magnesium absorption in kale leaves in media B and D were the same, the chlorophyll level on kale leaves in media D was lower than that in media B. This result indicated that the presence of other metal pollutants

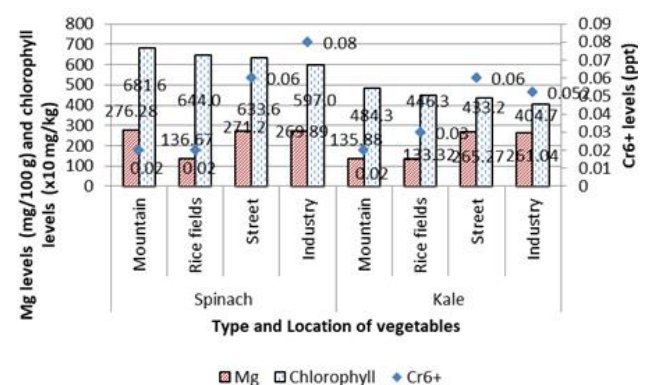


Figure 4. Comparison of Cr6+, Mg, and Chlorophyll Levels in Stems of Spinach and Kale

Table 3. Cd, Cr, and Pb Heavy Metal Analysis Data at Sampling Locations

Parameter	Location			
	Mountain	Rice fields	Street	Industry
Cd (mg/L) in water	0.01	0.02	0.04	0.09
in sediments	0.12	0.88	1.6	1.82
Cr (mg/L) in water	0.01	0.03	0.06	0.21
in sediments	0.11	0.69	1.01	2.17
Pb (mg/L) in water	0.01	0.03	0.07	0.25
in sediments	0.19	0.62	1.57	2.49

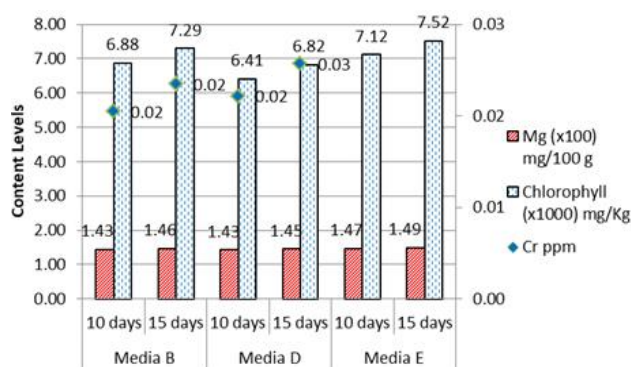


Figure 5. Comparison of Cr6+, Mg, and Chlorophyll Levels of Kale Leaves in Various Media

affected chlorophyll formation. In addition, the presence of other metal pollutants also affected the chromium accumulation on kale leaves. In 15 days, the chromium accumulation on kale leaves in media B only accumulated 0.02 ppm, whereas that in media D reached 0.03 ppm. In uncontaminated media (Media E), the absorption of Mg was optimal. Thus, the chlorophyll content in the leaves was also optimal. The presence of one or more heavy metals reduced the absorption of Mg. The large amount of heavy metals in growing media affected the growth of kale.

Discussion

The decrease in chlorophyll content in this study was due to competition in the absorption of chromium and magnesium. Magnesium a mineral needed for chlorophyll biosynthesis in addition to Fe and N. The presence of chromium in the planting medium reduces the absorption of magnesium by the roots. Shanker *et al.* [14] reported that Cr's first interaction with plants was during absorption. Cr is a toxic element not needed by plants; hence, it Cr does not have a special mechanism in absorption. Therefore, the absorption of Cr follows the absorption path of nutrients needed for plants. Cr toxicity depends on the form of the chromium species, which then affects its absorption, translocation, and accumulation [15].

Magnesium regulation through increased levels in this study exerted a positive effect on chlorophyll content in the stems and leaves. Increased magnesium levels increase the absorption of magnesium by chromium, which can be demonstrated by the ability of kale to maintain chlorophyll content in the stems and leaves. Shanker *et al.* [14] reported that chromium can cause the inactivation of enzymes involved in chlorophyll biosynthesis and thus reduce chlorophyll level in plants. The decrease in chlorophyll levels due to chromium can be caused by the destabilization and degradation of proteins in the peripheral part [14].

The absorption of magnesium by kale from the planting medium when the chromium contamination was 3 ppm in this study was greater than that under 1 ppm. This result may be ascribed to the fact that kale adapts to environmental conditions, which increase its ability to absorb magnesium. Plants develop self-defense mechanisms to continue physiological processes. Widowati *et al.* [16] also explained that plants are tolerant of polluted environmental conditions through antioxidant cells and enzymes. Tandon and Vikram [17] mentioned that low chromium concentrations (0.25–0.5 mM) increase antioxidant enzyme activity.

Total chlorophyll level decreases under chromium stress in *Catharanthus roseus* [18] and *Pistia stratiotes* L. [19]. Chromium accumulation can inhibit chlorophyll biosynthesis [20]. Plants respond to heavy metals in various ways, including immobilization, exclusion, chelation, and compartmentalization of metal ions. It can also express responses to stress, such as release of ethylene and stress proteins. Hayat *et al.* reported that chromium can inhibit the action of δ -aminolevulinic acid dehydratase [22].

The effect of chromium accumulation on magnesium absorption and chlorophyll content was also influenced by the type of vegetables and the presence of other heavy metals in the planting media environment. This result is consistent with the report of Gill [8] that the toxicity of heavy metals in plants depends on the type of plant, heavy metals, levels, chemical form/species, composition, and soil pH.

Conclusion

The observations and discussion above show that the accumulation of chromium in plants, such as spinach and kale, decreases magnesium absorption and thus affects the chlorophyll level. Increased levels of magnesium (0.4–0.8 g/L) in growing media decrease Cr accumulation by 15%–19% in kale stems and 28%–33% in kale leaves. Plant species, the presence of other heavy metals, and the type of planting media also affect the magnesium absorption and chlorophyll content by plants grown on chromium-contaminated media. Under chromium stress, spinach exhibits better magnesium absorption than kale.

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