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HEAVY METALS TREATED SOIL EFFECTS ON THE CONCENTRATION OF CHLOROPHYLL A, B, AND MINERAL ELEMENTS IN ALGAE

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SUMMARY

Soil treated with different concentrations of copper (75, 160, and 190 mg/kg soil) and cadmium (4, 8, and 10 mg/kg soil) had assessments for their effects on the growth of algae (*Cladophora* and *Spirogyra*). Soil treated with copper (190 mg/kg) and cadmium (10 mg/kg) has shown a significant impact, decreasing the dry weight of *Cladophora* (1.600 and 0.353 mg) and *Spirogyra* (1.500 and 0.189 mg). Chlorophyll a and b content also expressed decreased with copper application to the soil at 190 mg/kg. *Cladophora* and *Spirogyra* algae had concentrations of 0.082 and 0.007 mg/g fresh weight, respectively. In algae *Cladophora*, the cadmium (10 mg/kg soil) levels reached 0.073 and 0.008 mg/g of fresh weight, while *Spirogyra* had concentrations of 0.013 and 0.011 mg/g. In the nutrient concentration status of cladophora, soil treatment with cadmium (10 mg/kg) was the most beneficial for phosphorus, chloride, and sodium levels (0.170, 0.010, and 0.010 mg/g, respectively). Similarly, the phosphorus and sodium concentrations had declined with copper treatments in algae spirogyra (0.150 and 0.040 mg/g, respectively), while chloride concentration reduction was by cadmium (10 mg/kg soil).

Keywords: Algae *Cladophora* and *Spirogyra*, chlorophyll a and b, cadmium, copper, phosphorus, chloride, sodium, growth traits

Key findings: Soil treated with heavy elements has significantly decreased the fresh and dry weights of the algae *Cladophora* and *Spirogyra*. Observations also found that chlorophyll a and b, and chloride, sodium, and potassium levels, had decreased.

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INTRODUCTION

The academe and general community find environmental issues and their related fields interesting. After the soil, air, and water contamination in the 1970s, literature profusion and research devoted to ecological issues and problems thrived. Water and foodstuffs became contaminated with various types of natural, chemical, and biological materials, which contributed to boosting diseases and environmental components, causing diverse crop plant and animal species' eradication in the ecosystem. Some heavy elements have a high density and are toxic at low concentrations, such as, nickel, lead, cadmium, mercury, copper, and chromium (Karimpour *et al*., 2018).

Heavy metals affect all living organisms, microorganisms, invertebrates, plants, and animals due to higher toxicity (Mng'ong'o *et al*., 2021) and their ability to accumulate in the environment, then transmitted through the food chain (Xu *et al*., 2017). These minerals are also harmful to human health. A study noted that humans' exposure to increasing concentrations of these elements, especially nowadays, is due to the development of various industrial activities, raising soil, air, and water pollution (Luo *et al.*, 2020).

More observations added to mining operations and the expansion of pollution with various wastes in many regions (Masindi and Muedi, 2018; Zwolak *et al.*, 2019) the agricultural activities as sources that enhance the concentration of heavy metals. As a result, dense metal expansion in various regions worldwide caused a growing fear of using many agricultural lands because of contamination with countless elements and their transmission through edible crops (Feng *et al*., 2020). Therefore, numerous technologies used to get rid of heavy metals bloomed, such as, using algae as a bioprocessor to reduce heavy metal concentrations to lower levels in an environmentally friendly manner. Although algae share photosynthetic pigments with higher plants, their photosynthesis is more efficient, and the oxygen released by them into the environment and water bodies helps to degrade organic pollutants. Algae can convert waste into usable nutrients using enzymatic and metabolic processes (Kumar *et al*., 2021).

The heavy metal removal mechanisms include adsorption, absorption, precipitation, ketosis and anion exchange, microbiological activity, redox, and complex formation (Peng *et al*., 2018). Algae may remove heavy metals directly through two main mechanisms; the first is metabolism, which depends on cell absorption of heavy metals working well at low concentrations, but biosorption is the only one that is a physical-chemical property that leads to the removal of heavy metals through covalent bonds. Various functional groups, such as, SH, COO, R, RO, RNH, and OH, facilitate the absorption of metals (Ankit and Korstad, 2022).

These functional groups can exist on the surfaces of cells or in the cytoplasm, especially in the vacuoles. In algae, the components of the cell wall, such as fucoidan and ligand, are considered the primary bio-absorption-causing functional groups. Ions of lighter elements, i.e., sodium, calcium, and potassium, replace the heavier metal ions on cell surfaces (Ankit and Korstad, 2022). Cadmium, a non-essential dense element found in various environmental areas, its concentration has increased due to industrialization, mining, and fertilizer use (Fasih *et al*., 2021). Further reports said that enhanced soil acidity and root secretions increase cadmium solubility and, thus, increase its effect on living organisms (Abedi and Mojiri, 2020). The International Agency for Research on Cancer has also identified cadmium as a human carcinogen (Kim *et al*., 2020).

Copper is another toxic heavy element, although its low concentration may benefit living organisms. The environment also has copper polluting it in various ways, including mining and agricultural chemicals use. In the ecosystem, copper accumulation is a vital problem due to the difficulty of its removal and a source of concern globally (Petrovic and Krivokapic, 2020). Its increased concentration also causes vast damage to crop plants (La-Torre *et al*., 2018). The presented research aims to determine the toxicological impact of copper and cadmium on the growth and concentration of photosynthesis dyes and some elements that nourish algae *Cladophora* and *Spirogyra*.

MATERIALS AND METHODS

The soil for experimental purposes came from an agricultural area. Sun-drying them continued with sifting, using a special sieve to remove unwanted bodies. The dirt received treatments with three doses of two heavy metals, i.e., copper (75, 160, and 190 mg/kg soil) and cadmium (4, 8, and 10 mg/kg soil) after placing 3 kg of soil in each plant pot. Moss collection of the genus *Cladophora* (*Cladophora rupestris*) occurred at the Zummar Water Distribution Center, Iraq. For the genus *Spirogyra* (*Spirogyra* sp.), collection went on from various parts of Mosul, Iraq, where it had grown wild along the banks of the Tigris River, Iraq. Both collected algae samples underwent washing with the water of the Tigris River, with their soft mass added to the soil surface, and continued lightly mixing into the surface layer of the soil. Data recording followed after 30 days on the following variables:

The dry weight of algae

The algae's dry weight material estimation used an electric oven. Recording and measuring the weight of samples proceeded once the dry weight was stable after 48 h at 75 °C.

Chlorophyll a and b content

Algal chlorophyll content calculation followed the methodology of Abdul-Jaleel *et al*. (2009). The filled material's absorption measurement used a spectrophotometer at wavelengths of 645–663 nanometers.

Estimating nutrients

Crushing the algae's dried samples had the nutrients estimated. Analysis of a 0.5 g sample from each group had the following ions estimated (Chapman and Partt, 1961):

a. Chloride using the Mour method (Johnson and Ulrich, 1959)
b. Sodium using the Flam Photometer (Richard, 1954)
c. Phosphorus in a stannous chloride (APHA, 1998).

RESULTS AND DISCUSSION

Dry weight

Treating the soil with copper and cadmium at concentrations of 75, 160, and 190 mg/kg soil and 4, 8, and 10 mg/kg soil, respectively, the dry weight of *Cladophora* and *Spirogyra* significantly decreased (Figure 1). Copper decreased the algae species *Cladophora* and *Spirogyra* weights to 1.72, 1.69, and 1.60 g and 0.820, 0.660, and 0.353 g, respectively. Both algae species' dry weights also reduced after cadmium treatment with the said doses, having the weights 1.70, 1.66, and 1.50 g (cladophora) and 0.798, 0.781, and 0.189 g (spirogyra), respectively, compared with the control treatment. On dry weight, the data showed that the algae species *Spirogyra* was more vulnerable to the effects of heavy elements than *Cladophora*. Results also revealed the superior efficacy of cadmium with three concentrations on the dry weight of the two mosses.

After applying cadmium to the soil, the *Cladophora* and *Spirogyra* began to grow. Observations showed the highest effect on the dry weight of the two algae occurred when treated with cadmium at 10 mg/kg, compared with other treatments. These results were consistent with past findings revealing that plants with long periods of heavy elements led to a decrease in the vital mass of plants, which might be due to the toxic effects of cadmium (Jiyuan *et al*., 2021; AL-Rashedy, 2021a). The high concentrations of this element lead to damage in the plasma membranes and destroy the organs inside the cells (Abbas *et al*., 2017). Heavy metal ions in cadmium-contaminated *Scenedesmus incrassatulus* were

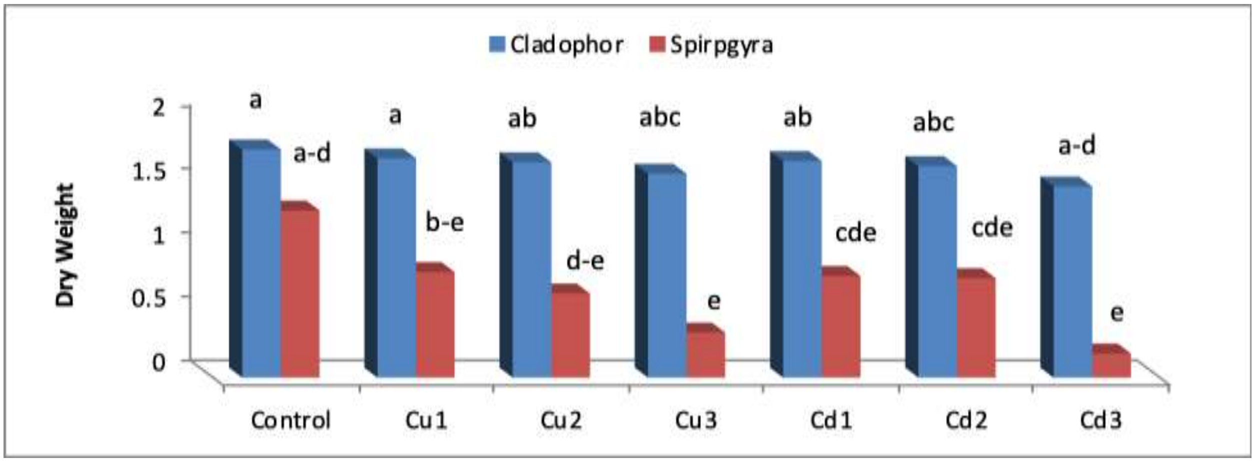

Figure 1. Effect of heavy metals on the dry weight (g) of algae species *Cladophora* and *Spirogyra.*

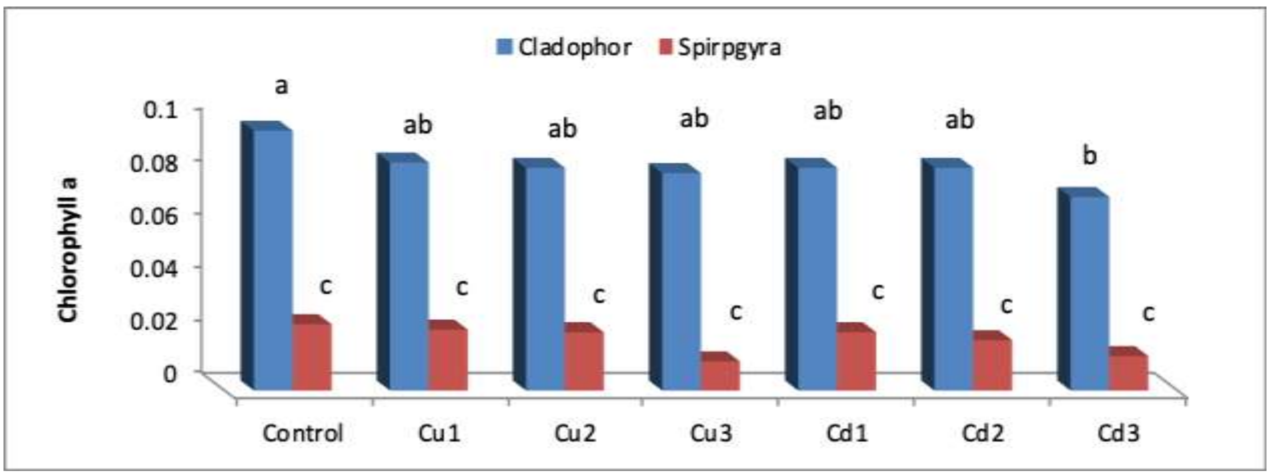

Figure 2. Effect of heavy metals on the concentration of chlorophyll a (mg/g fresh weight) in algae species *Cladophora* and *Spirogyra*.

harmful to cell density, biomass, growth rate, chlorophyll content, and absorption (Marinova *et al.*, 2018). Cadmium provided more significant effects compared with the copper treatments and the control. Therefore, the studies authenticated that cadmium inhibits growth more, reduces the size of cells, discourages photosynthesis, mineral nutrition, a water balance, and eventually ceases growth. Although small concentrations of copper are helpful for plants, exposure to their high concentrations leads to various changes in crop plants' physiological and morphological aspects (Daniele *et al.*, 2018)**.**

Chlorophyll a

Soil treatment with copper at the concentrations of 75, 160, and 190 mg/kg soil resulted in a significant decrease in chlorophyll a concentration in both algae species *Cladophora* (0.086, 0.084, and 0.082 mg/g wet weight) and *Spirogyra* (0.023, 0.022, 0.011 mg/g wet weight) (Figure 2). However, in contrast, cadmium at concentrations of 4, 8, and 10 mg/kg soil led to a decrease in chlorophyll a contents in both algae, i.e., 0.084, 0.084, and 0.073 in *Cladophora*, and 0.022, 0.019, and 0.013 mg/g wet weight in

Spirogyra, compared with the control treatment. Overall, the cadmium treatment had a better effect than copper on chlorophyll a concentration, with the algae species *Spirogyra* more affected by heavy metals than *Cladophora*. Al-Rashedy and Al-Mtewti (2022) also reported that treating the soil with heavy elements led to a decline in the concentration of carbohydrates, one of the products of photosynthesis that depend on chlorophyll concentrations. Ferati et al. (2022) noted that exposure to heavy metals affected the photosynthetic pigments in the plant leaves of *Salix purpurea*.

It is well known that cadmium is one of the elements known for its high mobility, after moving to plants through the roots, it moves to other plant parts (Dong *et al*., 2019). As a result of its accumulation within plant parts, it leads to a decrease in the biomass and photosynthesis rate and changes the uptake of nutrients (Hongyi *et al*., 2021). It may negatively affect the concentration of chlorophyll by affecting the rates of photosynthesis and absorption of magnesium, which plays a vital role in the synthesis of chlorophyll. The same findings also align with another study that the cadmium treatment led to decrease the chlorophyll content and nutrients in the algae *S. incrassatulus* (Marinova *et al*., 2018).

Chlorophyll b

After growing in heavy metal-treated soil, the chlorophyll b content also significantly decreased in the two algae species, *Cladophora* and *Spirogyra* (Figure 3). The chlorophyll b content in species *Cladophora* has reduced after treatment with copper and cadmium at rates of 75, 160, and 190 mg/kg, respectively. In spirogyra, it reached 0.021, 0.017, and 0.018 mg/g wet weight, and 0.018, 0.015, and 0.011 mg/g wet weight, compared with the control treatment (0.008, 0.008, and 0.007 mg/g wet weight). *Cladophora* is likewise more vulnerable to heavy metals than spirogyra. In the case of heavy metals, the copper element was more influential in the algae species *Cladophora*, while cadmium was more influential in algae species *Spirogyra* for chlorophyll b content. The lowest concentration of chlorophyll b appeared in algae cladophora, with a copper treatment at 190 mg/kg soil, and it reached 0.008 mg/g wet weight. These results were consistent with the previous findings showing a significant decrease in chlorophyll b and carotenoid content with a high cadmium concentration in *Chlorella vulgaris* (Cheng *et al*., 2017).

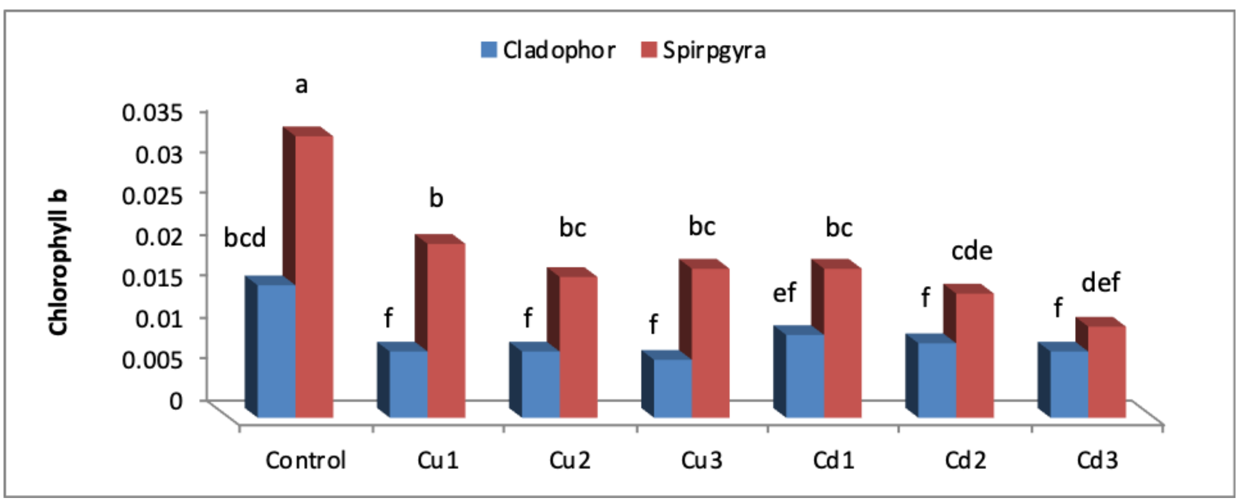

Figure 3. Effect of heavy metals on the concentration of chlorophyll b (mg/g fresh weight) in algae species *Cladophora* and *Spirogyra*.

The absorption of heavy metals and their distribution inside the plants and their impact on plants depend on the vegetable type, the duration of exposure to the heavy metals, and the metal concentration during growth (Ivakhnenko *et al*., 2021), and may also be due to the low magnesium concentration (AL-Rashedy and AL-Hamadany, 2021). The accumulation of cadmium inside the plant works to reduce the size of the cells, the rate of distribution of gaps in the skin, and low cell division, in addition to its effect on green plastids (Abbas *et al*., 2017). The high copper concentrations also prevent the synthesis of chlorophyll and carotenoid, delaying the addition of these dyes in the photosynthesis process (Anastasia *et al*., 2021).

Phosphorus

Copper and cadmium-treated soil showed a significant decrease in phosphorus concentration in the algae *Cladophora* (Figure 4). Copper (75, 160, and 190 mg/kg soil) and cadmium (4, 8, and 10 mg/kg soil) concentrations considerably reduced the phosphorus concentration in cladophora (0.249, 0.246, and 0.173 mg/g and 0.272, 0.257, and 0.150 mg/g, respectively), compared with the control treatment. The above doses of copper and cadmium also influenced a substantial decrease in the phosphorus concentration in *Spirogyra* algae (0.272, 0.257, and 0.150 mg/g and 0.278, 0.271, and 0.154 mg/g, respectively). Notably, compared with copper, the cadmium treatment had a superior effect on the phosphorus concentration in algae cladophora. The lowest concentration of phosphorus (0.150 mg/g) appeared in spirogyra moss treated with copper at 190 mg/kg soil. The decline in the phosphorus concentration in the two algae species as a result of treatment with heavy elements may be because these elements reduce the content of nitrogen, calcium, magnesium, and phosphorus in various plant parts (Zhang *et al*., 2019; Ankit and Korstad, 2022). Heavy elements affect the process of photosynthesis, causing changes in the water balance, which affects the plant's absorption of nutrients (Singh *et al*., 2016).

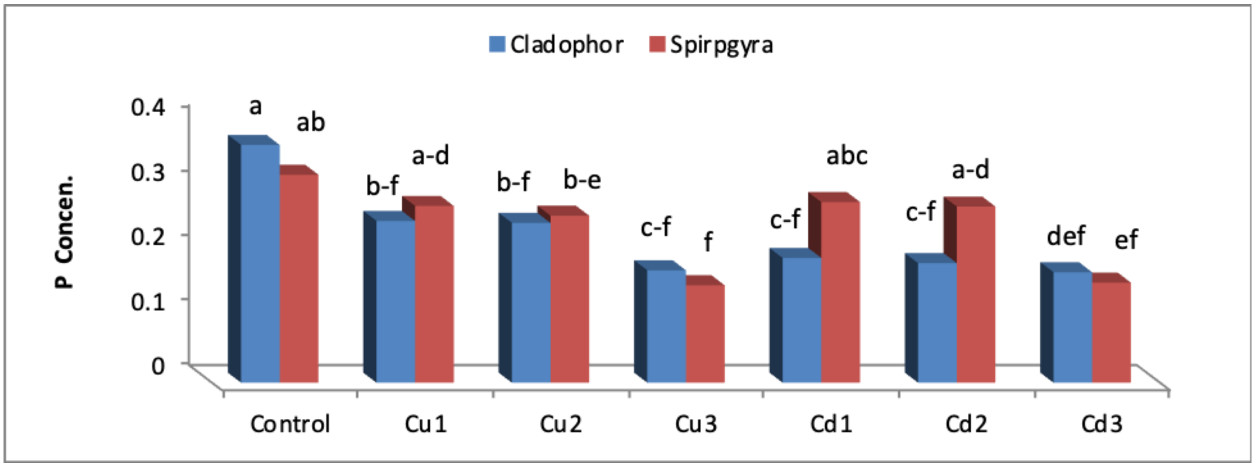

Figure 4. Effect of heavy metals on phosphorus concentration (mg/g) in algae species *Cladophora* and *Spirogyra*.

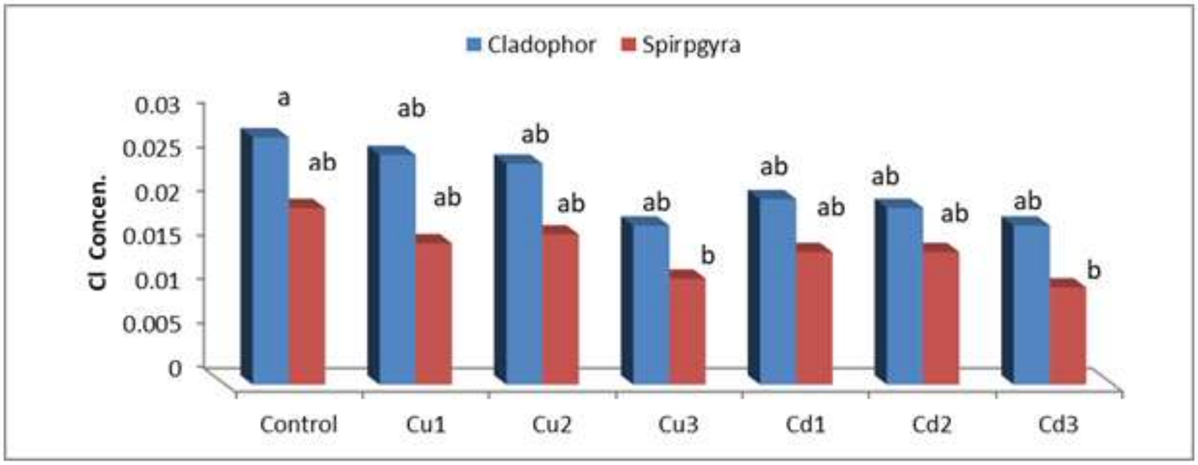

Figure 5. Effect of heavy metals on chloride concentration (mg/g) in algae species *Cladophora* and *Spirogyra.*

Chloride

Soil treatment with copper and cadmium concentrations of 160, 190, and 75 mg/kg, and 10, 8, and 4 mg/kg soil, respectively, led to a sizable decrease in the chloride concentration in algae species *Cladophora* at 0.026, 0.025, and 0.018 mg/g and 0.021, 0.020, and 0.018 mg/g (Figure 5). In algae *Spirogyra*, the chloride concentrations were 0.016, 0.017, and 0.012 mg/g and 0.015, 0.015, and 0.011 mg/g, respectively, compared with the control treatment. The results showed that copper and cadmium were more effective in reducing chloride concentration in algae *Spirogyra* than *Cladophora*. The lowest concentration of chloride (0.011 mg/g) emerged in *Spirogyra* grown in soil treated with cadmium (10 mg/kg soil) compared with the control and other treatments. The reduced chloride concentration from the treatment with copper and cadmium may be due to these elements competing with nutrients and replacing them with heavy elements. These results agree with the findings of Al-Rashedy *et al*. (2023), who concluded that treatment with heavy elements reduced the amount of chloride in various crop plant parts. Heavy metals also cause changes in enzymes, induce disturbances in photosynthesis, lessen carbon uptake, essential for energy production, and increase oxidation processes due to increased active oxygen (Küpper and Andresen, 2016).

Sodium

Treating soil with copper and cadmium at concentrations of 75, 160, and 190 mg/kg and 4, 8, and 10 mg/kg soil, respectively, reduced the sodium levels in algae species *Cladophora* (0.018, 0.015, and 0.013 mg/g and 0.013, 0.011, and 0.010 mg/g), while in *Spirogyra*, the sodium level reached 0.080, 0.060, and 0.040 mg/g and 0.080, 0.070, and 0.046 mg/g (Figure 6). Soil treatment with copper and cadmium led to a higher decrease in sodium concentration in algae *Cladophora* compared with *Spirogyra*, and cadmium treatment had more effect on the sodium concentration than copper. The lowest sodium concentration (0.010 mg/g) occurred in algae cladophora grown in cadmium-treated soils (10 mg/kg), and at percentage, it was lower than the control and other treatments. Al-Rashedy's (2021b) findings also revealed that salt content in the plant parts declined after treatment with heavy metals. Algae species *Cladophora* and *Spirogyra* also exhibited a drop in salt content after being treated with copper and cadmium. Heavy elements disrupt the plant's defense system and increase the concentration of ROS, which negatively affects various processes

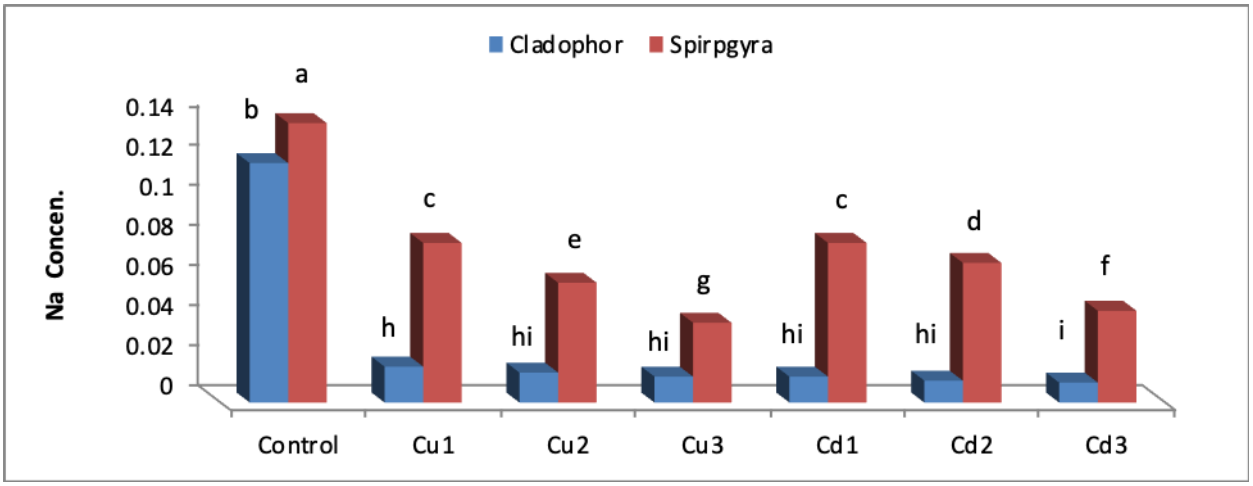

Figure 6. Effect of heavy metals on sodium concentration (mg/g) in algae species *Cladophora* and *Spirogyra.*

within plants, including absorbing nutrients vital to a plant's ability to carry out its essential functions (Gallego and Benavides, 2019).

CONCLUSIONS

Soil treated with copper and cadmium effectively decreased the dry weight of both algae species, *Cladophora* and *Spirogyra*. The accumulations of heavy metals were very high, which reflects the pollution of those species, leading to a decrease in the concentration of chlorophyll a and b and the content of crucial elements, i.e., chloride, sodium, and phosphorus, in both algae species. The results indicate that some algae species could be good bioindicator for pollution and heavy metal treatment.

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