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PJMLS

Print ISSN: 2707-4471. Online ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v6i2.2987	Copyright © All rights are reserved by Corresponding Author
Vol. 6 No. 2, 2023: 161-164	
www.readersinsight.net/pjmls	**Revised:** June 06, 2023 **Accepted:** June 17, 2023
Submission: January 14, 2023	**Published Online:** June 30, 2023

FOLIAR SPRAY OF SALICYLIC ACID AND EDTA ON MORPHO-PHYSIOLOGICAL PARAMETERS OF *SILYBUM MARIANUM* UNDER ZINC AND COBALT STRESS

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Abstract

Heavy metal pollution is the main environmental concern that effects plant growth, and physiological and biochemical processes which are vital for plant development. Medicinal plants are more susceptible to environmental factors such as salt, drought and heavy metals. To explore the role of SA and EDTA under heavy metal stress an RCBD experiment was conducted at Botanical Garden University of Balochistan, Quetta. The Experiment was RCBD. The seed of the plant is sown, and after 15 days of germination heavy metal is applied (Zinc and Cobalt). After Two weeks of heavy metal application, the plant signalling molecules were applied as a foliar spray (SA and EDTA). Later 15 days foliar application plants were harvested for growth and photosynthetic parameters. Results showed that heavy metals affect plant growth and developmental parameters, however foliar spray of SA and EDTA positively improved plant growth and physiological attributes under heavy metal stress. It is suggested that plant signalling molecules play a vital role under stress and can be used in future to improve the medicinal value of plants under various stress conditions.

Keywords: *Heavy metals, Potential of chelating, Metabolomics, Silybum marianum*

INTRODUCTION

The escalating prevalence of heavy metals in soil, food, and water on a globalscale poses a significant environmental and health concern. The accumulation ofheavy metals in the environment can lead to soil contamination (1-3),affecting agricultural productivity and food safety (4-6). Page & Feller (7) reported heavy metals can find their way into the foodchain, posing risks to human health through the consumption of contaminatedfood and water. Therefore, there is an urgent need to discover effective methodsto remediate heavy metal pollution and safeguard ecosystems and human health. Theprocess of phytoremediation helps plants to mitigate environmental pollution.Phytoremediation involves the use of plants to extract, stabilize, and/ordegrade heavy metals, thereby reducing their concentration in the environment.This natural and cost-effective approach has gained attention as a sustainablesolution for cleaning up contaminated sites.Inaddition to their phytoremediation abilities, plants have also been shown torespond positively to exogenous treatments of phytohormones, such as salicylicacid (SA) (8, 9) and ethylenediaminetetraacetic acid(EDTA) (10, 11). Phytohormones have exhibited potentialin by improving growth parameters, particularly under stressful environmentalconditions. Salicylic acid, a phenolic compound, is a well-known signallingmolecule involved in plant defence responses (12, 13). It activates defence genes, booststhe synthesis of antioxidants (14-17), and regulates stress-related pathways,making plants more resilient to various environmental stressors, includingheavy metals.On the other hand,EDTA, a synthetic chelating agent, forms stable complexes with heavy metals,rendering them more soluble and easily transported (18)within the plant. Its remarkable efficacy in the mobilization andimmobilization of heavy metals has made it a popular choice in phytoremediationstudies. Silybum marianum (Milkthistle), a widely recognized medicinal plant (19, 20) known for itshepatoprotective properties. Milk thistle has been traditionally used for itshepatoprotective properties and is known to contain various bioactivecompounds, including flavonoids silymarin, and anthocyanins (21).

MATERIALS AND METHODS

An experiment done at Botanical Garden University of Balochistan, Quetta. The field was development by dividing the main plots into two subplots (for each heavy metal), i.e., one was control and the other one was Heavy metal (Cobalt and Zinc). After that, the subplots were being divided into four subplots [control, water spray, salicylic acid spray, and EDTA foliar spray]. The Design of Experiment was RCBD with 3 replications (Three rows). Seeds were sown at a depth of 1. Heavy metal was applied after 15 days of seed germination. Moreover, the foliar application of SA and EDTA was applied after 15 days of heavy metal treatment. Plants were harvested after 20 days of foliar treatment and growth and photosynthetic parameters were recorded.

SOURCE OF SEEDS AND MORPHOLOGICAL PARAMETERS

Seeds for the experiment ware provided by the BARCD, Quetta. Healthy and viable seeds used for the experiment. Morphological parameters of plants such as (number of leaves, leaf area, root and shoot length, root and shoot fresh and dry weight) were recorded.

ANALYSIS OF PHOTOSYNTHETIC PIGMENTS

To determine the chlorophyll contents, the Arnon method (22) was employed. Carotenoid content estimation was carried out by following the Kirk method (23).

STATISTICAL ANALYSIS

Data were subjected for statistics analysis using "STATISTICX 8.1" software from Analytical Software, Tallahassee, Florida. Mean and (SD) were calculated, and graphs were generated using MS EXCEL.

RESULTS

ROOT LENGTH (RL)

The data for root length of *Sylibum marianum* highest root length was observed when the plant was treated with water foliar spray under Zn stress while the least root length was observed with SA foliar spray treatment under Zn stress. Additionally, the application of SA treatment decreased root length compared to heavy metal stress.

In conclusion, the root length of *S. marianum* plants increased under SA treatment in heavy metal stress without foliar spray and EDTA foliar spray. However, SA foliar application was found to be the least effective in promoting root length. These findings emphasize the importance of different treatments in influencing the root length of *S. marianum* and provide insights for potential applications to enhance plant growth and stress tolerance.

SHOOT LENGTH (SL)

The data for shoot length in *Sylibum marianum*, the maximum shoot length was observed in Zn metal-stressed shoots treated with water foliar spray, while the minimum shoot length was recorded in shoots treated with SA foliar spray under Zn stress.

In conclusion, the overall results for shoot length showed that treatment with different heavy metals had a negative impact on the shoot length of *S. marianum,* demining its growth. However, the water treatment seemed to enhance the impact of Co and Zn treatments, resulting in the longest plants compared to all other treatments. These findings provide valuable insights into the influence of heavy metal stress and foliar treatments on shoot length, offering potential applications for demining plant growth and stress intolerant.

NUMBER OF LEAVES

The data for the number of leave recorded highest number of leaves was observed with NFS treatment under Control and Zn conditions. On the other hand, the minimum number of leaves was observed under Control condition with EDTA foliar spray.

In conclusion, the overall results for the number of leaves in the plant indicated that the absence of foliar treatment (control set) resulted in a lesser number of leaves in heavy metal treatments in addition to the foliar treated plants. These findings shed light on the influence of heavy metal stress and foliar treatments on leaf growth, providing valuable insights to avoid stress in plant development.

LEAF AREA

The data for leaf area in the plant showed maximum leaf area was observed under SA treatment under control conditions, while the minimum leaf area was noted when the plant was treated with EDTA with control condition.

In general, the overall results for leaf area in the plant indicated that SA foliar spray tended to enhance the leaf area, especially under control condition. However, EDTA foliar spray was found to be the least effective with control conditions. These findings provide insights into the impact of different treatments on leaf area and suggest potential applications for improving leaf growth and stress tolerance in plants.

SHOOT FRESH WEIGHT (SFW)

The data for shoot fresh weight (SFW) maximum shoot fresh weight was observed in plants treated with SA under control conditions, while the minimum shoot fresh weight was noted in plants treated with Zn and EDTA foliar application. Additionally, SFW showed a decrease under control condition and Zn stress, respectively, compared to *S. marianum* plants under Co stress.

Overall, the analysis of shoot fresh weight results revealed that *S. marianum* plants without foliar treatment had greater shoot fresh weight in control condition. These findings provide valuable insights into the impact of different treatments on shoot fresh weight and suggest potential applications for improving plant growth and stress tolerance.

ROOT FRESH WEIGHT (RFW)

The data for root fresh weight (RFW) highest root fresh weight was observed under Co treatment with NFS application, while the least root fresh weight was noted under Zn treatment with NFS and water foliar application. Comparing Co treatment to the NFS, RFW increased significantly under Co treatment but tended to decrease in the presence of any foliar treatment.

In conclusion, considering the root fresh weight of *S. marianum,* it was observed that SA foliar spray and EDTA foliar application demined root fresh weight under almost all conditions. These findings provide valuable insights into the impact of different treatments on root fresh weight and suggest potential applications decrease root growth and enhance stress in *S. marianum* plants.

SHOOT DRY WEIGHT (SDW)

The data for shoot dry weight (SDW) highest SDW was observed under Co treatment with EDTA foliar spray, while the lowest SDW was recorded under water spray with control conditions. Similar to root dry weight SDW showed a substantial reduction under heavy metal stress.

Overall, the results for shoot dry weight in *S. marianum* plants were consistent with those observed for almost all treatments. However, it is worth noting that plants treated with foliar supplements exhibited greater biomass, regardless of heavy metal treatment.

ROOT DRY WEIGHT (RDW)

Regarding the data for root dry weight (RDW) of *S. marianum* plants, statistically non-significant differences were observed ($P < 0.05$). The maximum RDW was noted under Co stress with NFS treatment, while the least RDW was recorded under control condition with NFS. RDW exhibited a considerable reduction under heavy metal treatments compared to plants under control conditions.

In conclusion, the overall results for root dry weight in *S. marianum* plants highlighted the beneficial impact of foliar supplementation with EDTA and SA under different conditions, leading to a decrease in root dry weight, especially with SA supplementation. However, plants under Co, regardless of foliar treatment, exhibited greater RDWs. These findings underscore the significance of foliar treatments in preventing root dry weight and suggest potential applications to improve root growth and stress tolerance in *S. marianum* plants.

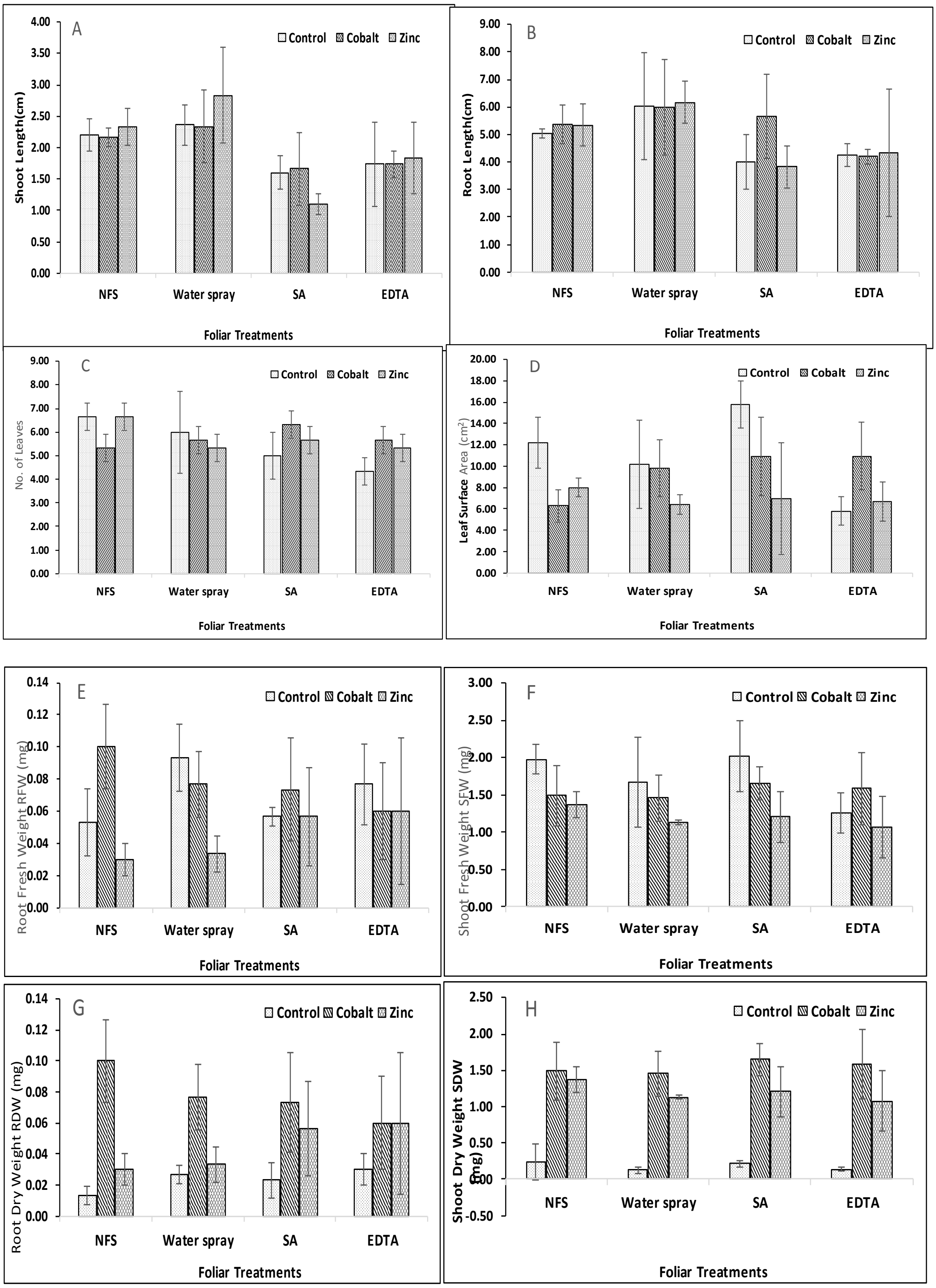

Fig. 1. Variation in plants under heavy metal stress in Shoot length A. root length B. No of leaves C. surface area D. root and shoot fresh weight E, F and root dry and shoot dry weight G.H, and foliar application of SA and EDTA alleviate negative impact.

PHOTOSYNTHETIC PIGMENTS

CHLOROPHYLL A

Data obtained for Chl A of *Silybum marianum* grown under heavy metal stress, under control conditions, SA led to the maximum Chl a (2.30 mg/g FWT) while SA treatment led to the minimum (2.12 mg/g FWT) content; however, under heavy metal stress, Chl a content was the highest and the lowest when

SA was applied . SA and EDTA foliar supplementation predominately increased Chl a content by about 80.40% and 26.42%.

CHLOROPHYLL B

Data obtained for Chl b maximum Chl b content (1.27 mg/g FWT), while minimum content (0.81 mg/g FWT) was observed in Zn (Con). Considering the overall improvements in *Silybum marianum* Chl b content, it was observed that heavy metal stress affected the Chl b content, while a foliar spray of SA and EDTA predominately imprecate the negative impact of heavy metal stress (Fig. 2B).

CAROTENOID

The carotenoid content in *Silybum marianum* was investigated EDTA treatment resulted in the slightly high carotenoid content with Zn as of 1.28 mg/g fresh weight (FWT). These findings indicate that SA and EDTA had similar effects on carotenoid levels under normal conditions. However, NFS had the least carotenoid content (1.01 mg/g FWT) under control condition.

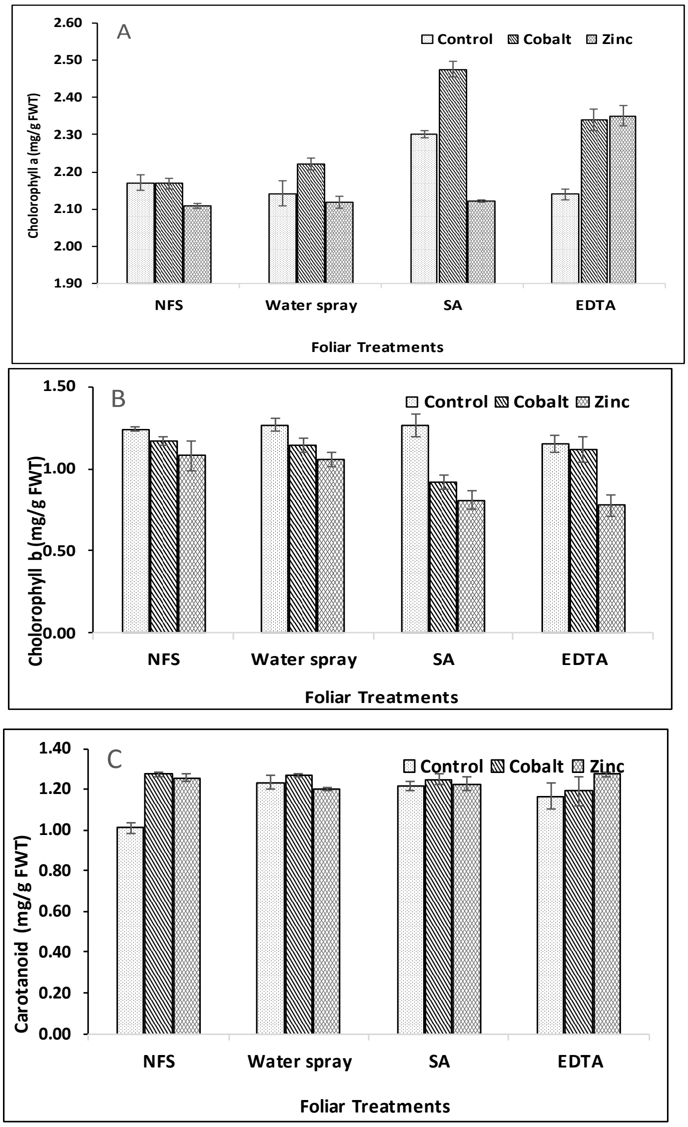

Fig. 2. Chl a (A), Chl b (B), Carotenoids (C) effect under heavy metal stress and application SA and EDTA improved plants photosynthetic pigments

DISCUSSION

The provided data delves into the repercussions of diverse treatments on the morphological attributes, photosynthetic pigments, and metabolites within *Silybum marianum* plants under controlled condition and heavy metal stress. The root system in plants is primary acceptor or barrier that exposed soil pollutant with heavy metals. The reduction in biomass and growth occurs under high level of Cd^{+2} uptakes (24). It was suggested from the study of Pal et al., (25) that cadmium translocation from roots of maize which are the first organs that directly exposed to stress of heavy metals, after that, passed through the other parts of plants that cause chlorosis which depicts in the low level of photosynthetic activity. Environment of Quetta, under water scar conditions, acted as sensors and transducer signals for the better growth and development (26). While Nizar et al., (27) documented that a decrease in particularly soil temperature limited the plants growth and several yield parameters as Quetta is at higher elevation the cooler environment challenged the survival of Silybum marianum (L.) Gaertn. While at low-altitude Turbat root development showed increasing trend in length due to normal and bearable temperature as compared to the high-altitude Quetta. The decline in root length also gets reduced by the application of Cd^{+2} treatments as compared to shoot length. Fresh weight of seedlings of milk thistle showed decreased in the growth by various concentrations of cadmium. (28-30). However, the study of Migahid et al., (31) reported that milk thistle showed highly significant results by minimizing the length of shoot/root ratio due to the seawater stress in milk thistle, the leaf area was also significantly decreased. While in the present study, it was obtained from the results that various treatments distinctly impacted the root length of *S. marianum*. The highest root length manifested under the application of a water foliar spray during Zn stress, while conversely, the lowest root length was observed when subjected to an SA foliar spray under Zn stress. Furthermore, SA treatment led to a reduction in root length compared to the imposition of heavy metal stress. Notable differences emerged in shoot length across the treatments. Shoots subjected to Zn stress, followed by water foliar spray, exhibited the maximal shoot length, whereas the lowest shoot length was recorded under Zn stress in the presence of an SA foliar spray.

It has been observed from the studies that attributes for growth in *Brassica juncea* such as shoot/root length, dry and fresh weight along with leaf area reduced by the application of Cd^{+2} (32). The study of Ahmad *et al*., (29) suggested that heavy metal Cd^{+2} affected the morphological parameters of plants with the interaction by up taking of minerals, plant water relations along with photosynthesis. The concentration of SA induces plant recovery exposed to Cd^{+2} toxicity by being best plant signaling molecule in mitigating the Cd^{+2} related toxicity symptoms. Nazarian et al., (33) reported that leaf area significantly affected under Cd^{+2} stresses. Likewise, in the current study, various treatments exerted influence on leaf area. The most expansive leaf area was witnessed under SA treatment within controlled conditions. Conversely, the smallest leaf area was observed under EDTA treatment within controlled circumstances. Treatment disparities were evident in the number of leaves. The most abundant leaf count was observed with NFS treatment under both Control and Zn conditions. Conversely, the fewest leaves were noted under the Control condition with an EDTA foliar spray.

Priming of seeds with SA has been very vital phenomenon for improving the morphological effectiveness i.e., Plant height, dry/fresh weights under un-stressed and stressed conditions Abdolahi & Shekari, (34). In the same manner, the study of Nizar et al., (35) reported that the altitudinal fields showed remarkable variations in the root/shoot fresh and dry weight by showing enhancement in both root and shoot fresh and dry weight at low-altitude (Tbt) as compared to Qta under priming and foliar treatment. While in the present study, shoot fresh weight enhanced under SA controlled conditions, while the least was observed in plants treated with Zn and subjected to EDTA foliar application. Root fresh weight underwent discernible changes due to the treatments. The highest root fresh weight was noted under Co treatment in combination with NFS application, whereas the lowest RFW was observed under Zn treatment accompanied by NFS and a water foliar application. SDW was subject to significant distinctions based on treatments. The greatest SDW was documented under Co treatment coupled with an EDTA foliar spray, whereas the lowest SDW was recorded under water spray treatment within control conditions. Treatment-induced disparities were evident in RDW. The utmost RDW was noted under Co stress with NFS treatment, whereas the least RDW was registered under the control condition coupled with NFS treatment.

Photosynthetic pigments, considered the most essential pigments in the growth and development of plants which is adversely affected by the heavy metal stress. Heavy metals toxicity affects the biosynthesis of chlorophylls and carotenoids that disturbs the membrane of chloroplasts including electron transport chain by impairing PS I and PS II (36, 37). However, Moussa *et al*., (38) reported that SA supplementation has widely been reported to restore the stress in photosynthetic traits caused by the heavy metal stress and improves the tolerance of plants in *Triticum aestivum*. Nizar et al., (35) reported that the Chl-*a* content was generally higher in both the altitudinal areas due to moderate temperature. It has been also reported that there was a considerable increase in milk thistle pigments (Chlorohpyll *a*, *b* and carotenoids) by applying H_2O_2 under Cd^{+2} stress Migahid *et al*., (31). Carotenoids are the potential antioxidants which serve as reactive oxygen species (ROS) scavenger against disruption of photo-oxidative in photosystems (39). The contents of carotenoids contents play a key role for plant survival under various environmental conditions Spano *et al*., (40). While in the current findings, Chl a content demonstrated susceptibility to both heavy metal stress and treatments. SA treatment under control conditions yielded the highest Chl content, whereas heavy metal stress combined, while in chl b content NFS treatment within control conditions yielded the maximal Chl b content, whereas Zn treatment within controlled circumstances was linked to the minimal content. Further, carotenoid content displayed sensitivity to treatments. Predominant enhancement in carotenoid content occurred under EDTA treatment during Zn concentration. NFS exhibited the most modest carotenoid content under control conditions.

CONCLUSION

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