



The impact of a seaweed-derived biostimulant (Kelpak®) on the tolerance of lettuce plants (*Lactuca sativa*) to cadmium pollution

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Lettuce is an important leafy vegetable containing beneficial bioactive compounds. Meanwhile, lettuce can absorb cadmium in areas of man-made soil contamination. Concentrations of 10 and 20 mg/L CdCl₂ in irrigation water resulted in noticeable changes in plant vital signs. The presence of cadmium in the irrigation water at a concentration of 10 mg/L had no significant effect on the productivity of the above-ground portion of lettuce. A 14% reduction in biomass was observed when the cadmium concentration in the irrigation water was doubled. Growth inhibition and a decrease in photosynthetic pigment content were observed. The chlorophyll *a* content of lettuce leaves decreased by 28.6% and 35.7%, respectively, due to an increase in cadmium chloride content in the irrigation water from 10 to 20 g/L. The carotenoid content of leaves decreased proportionally to the increase in cadmium content in irrigation water. At the same time, the presence of lipid peroxidation products in plant tissues increased. Foliar treatment with the seaweed-derived biostimulant in our case study had a visible effect only on plants growing in the soil, while under conditions of cadmium contamination of the soil, the weight of plants treated with Kelpak® was almost identical to the weight of untreated plants. Application of Kelpak® resulted in chlorophyll *a* and *b* content in the 20 mL/L CdCl₂+BS concentration variant slightly lower than in the 10 mL/L CdCl₂+BS concentration. An increase in carotenoid content by 15.5–27.3% in variants contaminated with cadmium indicates a mitigation of the negative impact of the toxicant on plants. Foliar treatment of plants with a biostimulant reduced the stress response to cadmium pollution in the form of decrease in the MDA production by 14–22%. A strong negative correlation was established between MDA content in lettuce leaves and biomass growth, chlorophyll *a*, and carotenoid content.

Keywords: lettuce; cadmium pollution; biostimulant Kelpak®; biomass; photosynthetic pigments; malondialdehyde.

Introduction

Soil contamination with cadmium is a critical environmental problem in agriculture. Its transfer into the soil can be caused by industrial emissions, mining, and the use of mineral fertilizers made from some phosphate rocks (Mar & Okazaki, 2012; Doležalová Weissmannová et al., 2019; Nekos et al., 2019). Military actions in Ukraine have resulted in serious chemical pollution of soils in areas damaged with explosions, burning military equipment, and industrial chemical spills (Petrushka et al., 2023; Krainiuk et al., 2025). Cadmium, along with lead, nickel and zinc, poses the greatest environmental hazard from soil contamination in and around blast craters. Cadmium is particularly dangerous because it is highly mobile and easily absorbed and accumulates in plants, inhibiting their growth and development. Furthermore, cadmium from contaminated plants is transmitted through the food chain and can pose a serious threat to human health. (Schaefer et al., 2020; Razanov et al., 2022). The contamination of crops with cadmium depends on climate, water, soil, and different uptake with aboveground biomass (Cavanagh et al., 2019; Kubier et al., 2019). Depending on the species, plants have different abilities to accumulate cadmium (van Lune & Zwart, 2001; Kulbachko et al., 2024). Tolerance to this element also varies between species. For example, in experiments by Azizian et al. (2011a; 2011b) it was shown that the presence of cadmium in irrigation water at doses of 10 and 20 mg/L had different effects on the yield of lettuce, corn and oats. Different genotypes within a single species can also have different sensitivity to cadmium. For example, this was demonstrated for sunflower in the experiment of Sacramento et al. (2018). Thus, indirect determination of chlorophyll content in lettuce leaves using a SPAD meter revealed a decrease in pigment levels under cadmium

stress (Zhou et al., 2023). Meanwhile, low concentrations of cadmium added to soil increased the SPAD value and yield of lettuce leaves. A strong negative correlation was found between cadmium concentration and the tolerance of lettuce roots and shoots. However, overt symptoms of dehydration, chlorosis, or necrosis may not appear even at high cadmium concentrations (Zorrig et al., 2013). Meantime, *Lactuca sativa* is able to extract nutrients from polluted soils (Dankaka et al., 2025). Different levels of cadmium increased the concentration of phytochemicals (phenolic compounds and malondialdehyde), but did not affect such nutrients and bioactive substances as vitamin C, flavonoids and proline (Jibril et al., 2017). Currently, various biobased byproducts are being actively studied that can reduce the toxicity of cadmium or decrease the bioavailability of the element for lettuce plants.

The use of some soil amendments is a direct way to reduce the entry of cadmium into the aboveground biomass of lettuce (Zheng et al., 2017; Zhang et al., 2025; Korkmaz et al., 2026). For example, the use of KMnO₄-modified biochar resulted not only in a significant reduction in Cd levels in lettuce roots and shoots but also in changes in the structure and abundance of soil microbes (Yin et al., 2022). The reduction in available cadmium in the soil confirmed the role of biochar in its passivation due to its large specific surface area (Fedeli et al., 2025). The use of cystamine-modified biochar improved soil properties, promoting the growth of beneficial bacteria and fungi essential for nutrient cycling and plant health. Furthermore, Cys-BC reduced cadmium bioavailability and effectively reduced oxidative stress by increasing antioxidant enzyme activity in lettuce tissue (Chen et al., 2024).

Plants have a cellular mechanism for cadmium detoxification due to its binding by cadmium phytochelatin and metallothionein pro-

teins (Li et al., 2023). Sulfur input is a key to enhancing plant metabolism and increasing their resistance to stress caused by heavy metals (Liu et al., 2020; Shi et al., 2020). Sulfur addition promoted growth, reduced cadmium accumulation, and increased the content of phytochelatins and their complexes with cadmium, limiting the movement of this element in hydroponic lettuce cultivation. A decrease in Cd content in organelles and increased sequestration in vacuoles and the cell wall were also observed (Chen & Lai, 2025). The watering of the lettuce plants with leonardite based humic substances revealed a reduction in cadmium in plants due to an increase in the amount of pigments, the activity of antioxidant enzymes and the biosynthesis of thiols (Atero-Calvo et al., 2025). Apparently, this is due to the ability of glutathione-S-transferase to form chelates of glutation (GSH) with cadmium (Mitra et al., 2024). The study of phenylpropanoid metabolism has paved the way for the use of ZnONPs- nanoparticles to reduce cadmium stress in lettuce by increasing the synthesis of phenolic acids (Zhang et al., 2024). Foliar treatment with salicylic acid (SA) concentration of 500 mg/L decreased peroxidase (POD) and superoxide dismutase (SOD) activity (by 15.5% and 19.9%), cadmium uptake and transport from the root system of lettuce to shoots by reducing the expression of Nramp5, HMA4 and SAMT (Tang et al., 2023). Cadmium content in lettuce shoots was significantly reduced after foliar treatment with melatonin, while cadmium content in roots was significantly increased, limiting cadmium transport into shoots (Tang et al., 2022). Application of abscisic acid inhibited lipid peroxidation, increasing the activity of antioxidant enzymes and the nutritional value of lettuce leaves (Dawuda et al., 2020). It was also established that protection from cadmium stress is possible due to the ability of exogenic γ -aminobutyric acids to enhance the antioxidant enzymes activity and reduce the increase in osmoregulatory compounds induced by cadmium stress (Tang et al., 2025). Foliar treatment of two *L. sativa* varieties with chitosan and spermidine reduced stress indicators, including relative membrane permeability (by 19–22%), H_2O_2 (by 26–27%) and malondialdehyde content by 6–7% (Rafique et al., 2025). It has been shown also that nitric oxide (NO) supplied via sodium nitroprusside plays a key role in mitigating cadmium-induced stress in lettuce (Samet et al., 2026). Currently, various biobased by products are being actively studied to reduce the toxicity of cadmium or decrease the bioavailability of the element for crops (Gao et al., 2024; Seyfferth et al., 2024; Maldonado-Mateus et al., 2025).

In addition, the potential of bioactive substances in seaweed extracts as biostimulants to improve growth and reduce stress in agricultural crops is being actively studied. (Mughunth et al., 2024). Seaweed extract can find a place in crop management programs. In addition to cytokinins, auxins and polyamines, 18 gibberellins, abscisic acid, brassinosteroids and castasterone were detected in varying concentrations in the extracts obtained from the brown seaweeds. Seaweed extracts have been shown to increase resistance to abiotic stress, improve crop yields, and extend the shelf life of agricultural products (Battacharyya et al., 2015; Pastor-Arbulú & Rodríguez-Delfin, 2025). Different seaweed extracts exhibit distinctly different mechanisms of action that can influence plant defense responses. In turn, studies aimed at identifying, isolating, and characterizing the bioactive compounds present in seaweed extracts can shed light on important aspects such as differences in metabolic reactions depending on the method or frequency of application (Stirk et al., 2020; Shukla et al., 2021). The use of foliar biostimulants (legume-derived protein hydrolysate and seaweed extract) stimulated antioxidant systems, improved the color of lettuce leaves and increased pigment content (Di Mola et al., 2020).

The main objective of this case study was to assess the impact of a seaweed-derived biostimulant (Kelpak®) on the tolerance of lettuce plants (*Lactuca sativa* L.) to cadmium pollution, evaluating fresh biomass and key indicators of physiological health, such as chlorophyll *a*, *b*, carotinoides and malondialdehyde content.

Materials and methods

The experimental design involved studying the effect of the biostimulant Kelpak® on lettuce plants (*Lactuca sativa* L.) exposed to

increasing concentrations of $CdCl_2$ in irrigation water (0, 10, and 20 mg/L, calculated as $CdCl_2 \cdot H_2O$). The experiment lasted 25 days. Lettuce seedlings were planted in 150 mL plastic pots filled with a mixture of compost and quartz sand in a ratio of 1:10. The plants were grown for 6 weeks in a greenhouse with a day/night cycle of 16/8 hour. The biostimulant application began three days after planting. Each plant was given three Kelpak applications with a dose of 1%, according to the manufacturing company indications at 7-day intervals. Treatments were arranged in factorial manner in a completely randomized design with four replicates.

The concentrations of chlorophyll (*Chl*) *a*, chlorophyll *b* and carotenoids were determined using a spectrophotometric method (Lichtenthaler & Wellburn 1983). Extinction was measured at 663, 646 and 470 nm (E_{663} , E_{646} , E_{470}) using the spectrophotometer Spectroquant® Prove 300 UV/VIS (Merck KGaA, Darmstadt, Germany). The content of pigments in the filtrate was calculated according to the following equations:

$$Chl\ a = 12.21 \cdot E_{663} - 2.81 \cdot E_{646}$$

$$Chl\ b = 20.13 \cdot E_{663} - 5.03 \cdot E_{646}$$

$$Carotenoids = (1000 \cdot E_{470} - 3.27 \cdot E_{663} - 104 \cdot E_{646}) / 229$$

The pigment content in leaves was calculated using the following expression:

$$Pigments = c (\mu g/mL) \cdot V (mL) / W (mg),$$

c – the concentration of pigments in the sample determined from the calibration curve, $\mu g/mL$; *V* – the volume of acetone, mL (10 mL); *W* – the weight of fresh plant material, g (200 mg).

Malondialdehyde (MDA) reacts with 2-thiobarbituric acid (TBA) to form a fluorescent product whose emission maximum is at 532 nm. The non-specific turbidity was taken into account by measuring at 600 nm (Heath & Packer, 1968). The molar concentration of MDA (mM or mmol/L) was calculated using the molar extinction coefficient ($155\text{ mM}^{-1}\text{ cm}^{-1}$) according to the equation: $c = (A_{532} - A_{600}) / 155\text{ mM}^{-1}\text{ cm}^{-1}$. For readability of the results, mM was converted to μM . Finally, to convert the molar concentration of MDA (μM) to μmol per 1 gram of fresh plant material, the following equation was used:

$$n = c (\mu mol/L) \cdot V (L) / W (g),$$

where *n* – the amount of MDA contained in 1 gram of fresh plant material, $\mu mol/g$; *c* – the molar concentration of MDA, $\mu mol/L$ calculated using the molar extinction coefficient, *V* – the volume of 2-thiobarbituric acid, L (0.005 L); *W* – the weight of fresh plant material, g (0.2 g).

All data statistical processing was performed in R Version 4.3.2 (R Core Team, 2023). The following packages were used for statistical analysis and data manipulation: readr, dplyr, stats, and multcompView (Graves et al., 2026; R Core Team, 2023). The analysis of variance for two factors (two-way ANOVA) was done using the aov function. The pairwise comparison of means was performed using Tukey's HSD test. The multcompLetters4 function was used to visualize letters indicating significant differences in pairwise comparisons (cdl – compact letter display). Pearson's correlation coefficients were calculated using the metan package (Olivoto & Lúcio, 2020). The plots were constructed using the packages: ggplot2 and ggthemes (Wickham et al., 2023).

Results

The presence of cadmium in irrigation water at a concentration of 10 mg/L had no significant effect on the productivity of above-ground plant material (Fig. 1). In the 20 mg/L treatment, a 14% weight reduction was observed. Treatment with a biostimulant produced a visible effect only for plants growing in cadmium-free soil: an increase in biomass of 11.5% was observed. In the cadmium-contaminated treatments, the weight of plants treated with Kelpak® was almost identical to that of untreated plants.

The results of this study showed that cadmium chloride in irrigation water reduced chlorophyll *a* content in lettuce leaves by 28.6–35.7%, depending on the toxicant concentration (Fig. 2). Treatment with Kelpak® increased pigment synthesis. In the cadmium-free variant, chlorophyll *a* content increased by 19%, and in the cadmium-

containing variants by 23.3–33.3%, compared to untreated samples. The greatest effect of the biostimulant was observed at a higher cadmium concentration. As a result, pigment content in the 20 mL/L CdCl₂+BS variant became virtually identical to that in the 10 mL/L CdCl₂+BS variant. No statistical differences were found between them. The effect of cadmium contamination on chlorophyll *b* was less pronounced than on chlorophyll *a* (Fig. 3). Its content in leaves decreased by 17.6–23.5%. No statistically significant differences were observed between treatments with different CdCl₂ concentrations. Treatment with a biostimulant increased chlorophyll *b* content by 12% in uncontaminated samples. In experiments with cadmium, pigment content increased by 14–15%, bringing this parameter closer to control values.

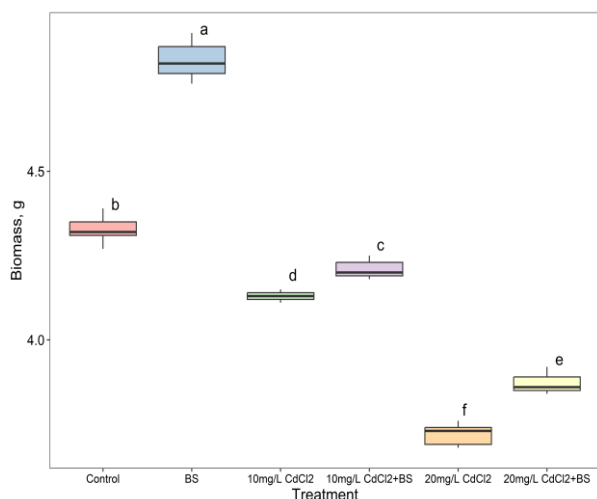


Fig. 1. Fresh lettuce biomass weight: ANOVA ($P < 0.01$); values are expressed as means $\pm$ standard error ($n = 5$)

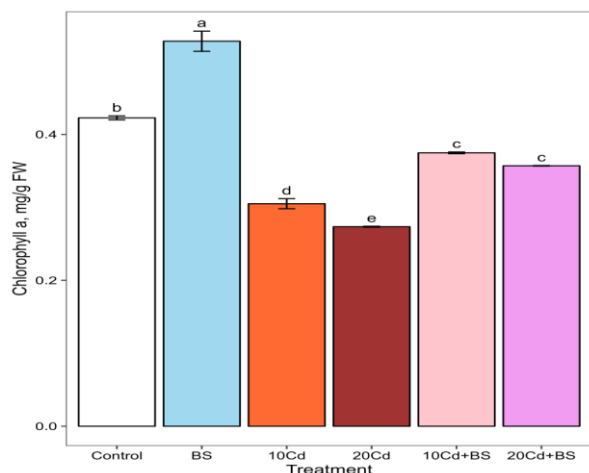


Fig. 2. Chlorophyll *a* content (mg/g FW) in lettuce leaves: ANOVA ($P < 0.01$); values are expressed as means $\pm$ standard error ($n = 4$)

The Chl *a*/Chl *b* ratio is a fairly stable indicator; the presence of cadmium and treatment with a biostimulant did not have a significant effect on it (Fig. 4).

Since cadmium pollution affects the biosynthesis of both chlorophyll *a* and chlorophyll *b*, the total chlorophyll content also changes significantly. A decrease in this indicator by 17.5% was noted in the 10 mL/L CdCl₂ and by 31.2% in the 20 mL/L CdCl₂ (Fig. 5).

Treatment with a biostimulant somewhat offset the negative impact of cadmium on the chlorophyll complex. The most pronounced positive effect of Kelpak[®] was observed in the uncontaminated variant and in the variant with the highest cadmium concentration. A negative impact of cadmium on carotenoid content in lettuce leaves was revealed (Fig. 6). Under less polluted conditions, a 32.6% decrease in pigment synthesis was observed. At higher cadmium concentrations, carotenoid content diminished by 43.1%. Biostimulant treatment had no significant effect on this parameter in the cadmium-free variant; no

statistically significant difference was found. In cadmium-contaminated experimental variants, an increase in pigment content by 15.5–27.3% was observed, which indicates partial compensation for the damage caused by the toxicant.

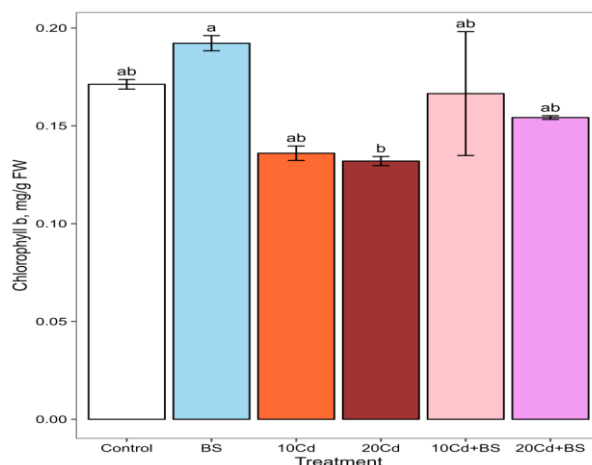


Fig. 3. Chlorophyll *b* content (mg/g FW) in lettuce leaves: ANOVA ($P < 0.01$); values are expressed as means $\pm$ standard error ($n = 4$)

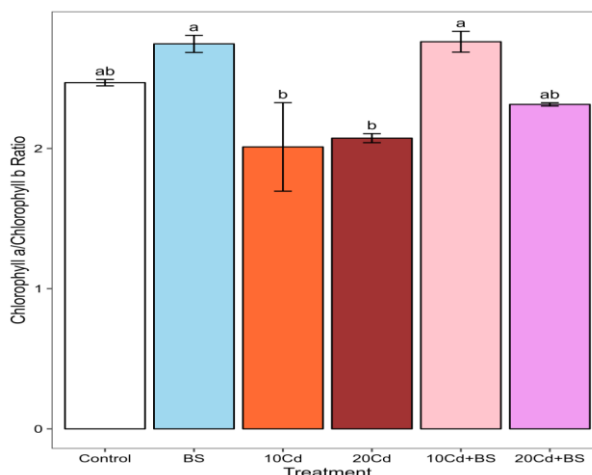


Fig. 4. Chl *a*/Chl *b* ratio in lettuce leaves: ANOVA ($P < 0.01$); values are expressed as means $\pm$ standard error ($n = 4$)

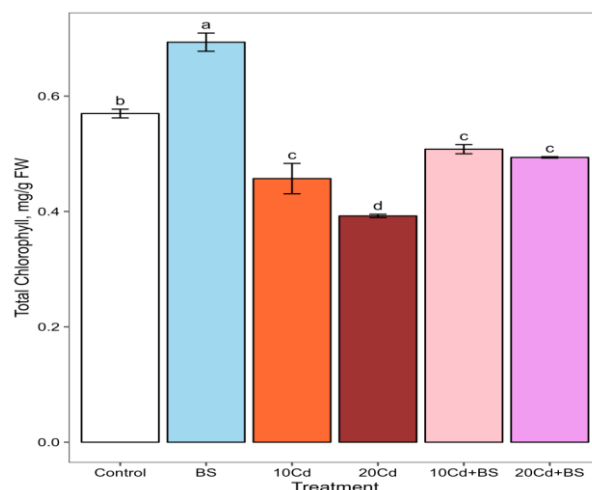


Fig. 5. Total chlorophyll content (mg/g FW) in lettuce leaves: ANOVA ($P < 0.01$); values are expressed as means $\pm$ standard error ($n = 4$)

Total chlorophyll/carotenoids ratio values did not differ significantly from each other in all experimental variants; no statistically significant difference was found (Fig. 7).

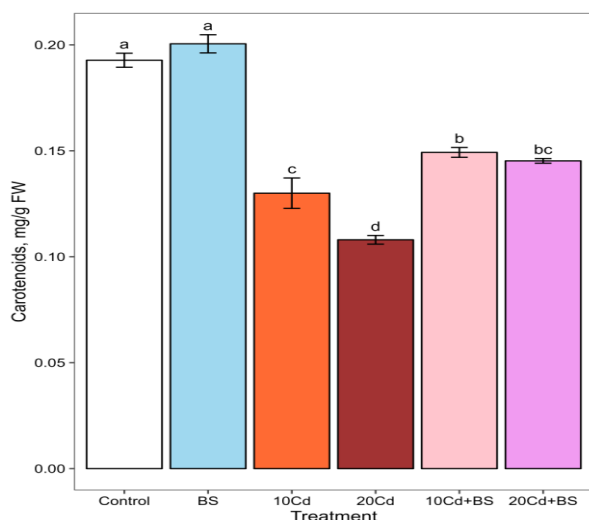


Fig. 6. Carotenoids content (mg/g FW) in lettuce leaves: ANOVA ($P < 0.01$); values are expressed as means $\pm$ standard error ($n = 4$)

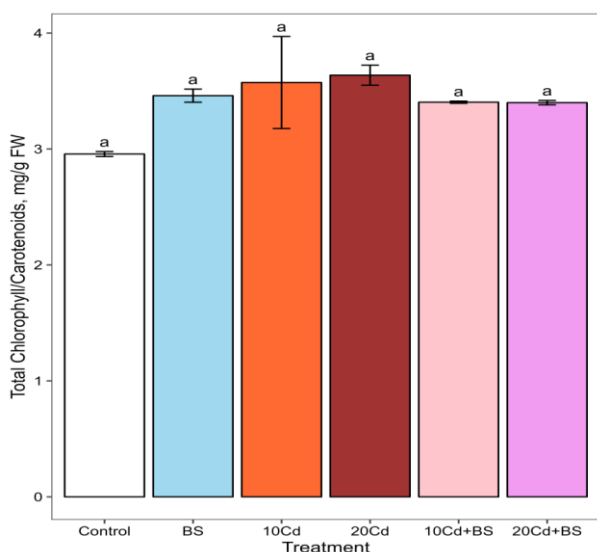


Fig. 7. Total chlorophyll/carotenoids ratio in lettuce leaves: ANOVA ($P < 0.01$); values are expressed as means $\pm$ standard error ($n = 4$)

In this experiment, the addition of 10 mg/L CdCl_2 increased MDA content by 18% compared to the control (Fig. 8). With 20 mg/L CdCl_2 , the effect was more pronounced: MDA content increased by 53.3%. Treatment with a biostimulant resulted in a decrease in MDA in plant tissues almost to the control level in the 10 mg/L CdCl_2 variant. At higher cadmium concentrations, the effect of Kelpak[®] was more pronounced: MDA content decreased by 21.8%. In the cadmium-free variant, the MDA level also declined, indicating stimulation of synthetic processes under the influence of Kelpak[®] biologically active substances.

A strong negative correlation was observed between MDA content in lettuce leaves and biomass growth, chlorophyll *a*, and carotenoid content (Fig. 9). The relationship between chlorophyll *b* and other parameters was less pronounced.

Discussion

Lettuce is one of the plants that actively absorbs cadmium. Bioinformatics research has shown that lettuce has two genes that, through splicing, produce phytochelatin synthase enzymes, which, in turn, determines the plant's ability to accumulate high levels of cadmium (Yazdi et al., 2019). Accumulation of the element mainly occurs in the early stages of ontogenesis (Tang et al., 2016). The results showed that Cd stress not only reduced the biometric indices of sunflower but also altered the characteristics of the number of PSII reactive centers per unit (Wang et al., 2024). For example, chlorophyll biosynthesis

can be destroyed due to the substitution of magnesium ions in the chlorophyll molecule by other divalent metals (Zn, Cu, Cd). Some heavy metals also affect enzymes involved in chlorophyll biosynthesis, disrupt electron transfer in light reactions, and affect various enzymes involved in dark reactions (Mysliwa-Kurdziel & Strzałka, 2002; Rai et al., 2016; Souri et al., 2019). Oxidative damage reduces pigment content and declines the rate of photosynthesis, which subsequently leads to decreased plant viability or even death (Albert et al., 2011; Aldesuquy, 2014; Sachdev, 2023). Our experiment revealed that cadmium pollution inhibits the biosynthesis of photosynthetic pigments in leaves. Moreover, the higher the cadmium concentration, the more pronounced the inhibitory effect is. These results are consistent with data obtained by other scientists in similar studies (Mobin & Khan, 2007; Parmar et al., 2013; Anjum et al., 2017; Chen et al., 2022).

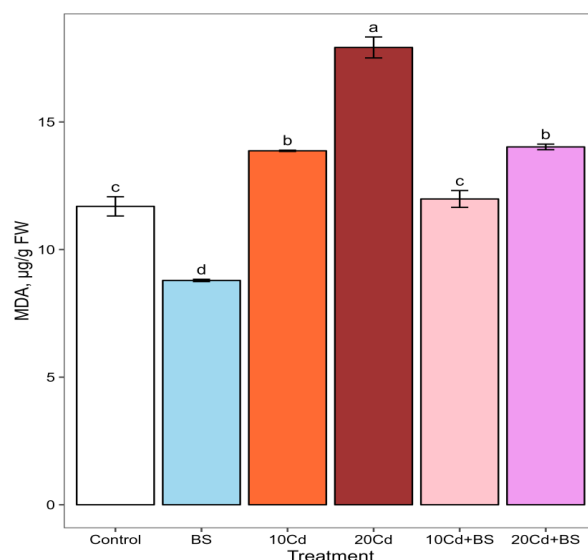
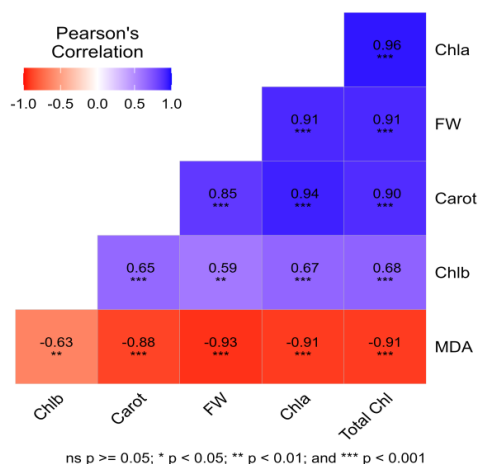


Fig. 8. MDA content (μg/g FW) in lettuce leaves: ANOVA ($P < 0.01$); values are expressed as means $\pm$ standard error ($n = 4$)



ns $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$

Fig. 9. Pearson's correlation matrix with P-values

An increase in malondialdehyde content can serve as an indicator of increased destructive processes in plant tissues under the influence of stress (Luna et al., 2000; Tommasino et al., 2012). In other words, MDA levels can be a good indicator of plant tolerance to cadmium stress. As MDA concentrations increase relative to optimal values, the level of oxidative damage rises. Similar findings have been demonstrated in numerous experiments with various plant species (Muradoglu et al., 2015; Jawad Hassan et al., 2020; Zhou et al., 2020; El-Okkiah et al., 2022). The doubling the cadmium chloride concentration in our pot experiment with lettuce resulted in an increase in MDA content 2.5 times greater than that observed with the addition of 10 mg/L CdCl_2 .

Recently, the effect of seaweed-based stimulants on plant resistance to various biotic and abiotic stresses has been actively studied

(Sujeeth et al., 2022; Raja & Vidya, 2023; Kumar et al., 2024; Velasco-Clares et al., 2025). In our study, Kelpak[®], an extract of the kelp *Ecklonia maxima*, was used to alleviate cadmium stress in lettuce plants. Unlike other seaweed extracts, the effects of this stimulant on various plants under stressful conditions have not yet been adequately studied. There is data that this preparation can reduce the negative effects caused by salt stress. Thus, in a study by Sogoni et al. (2024), it was demonstrated that *Spinacia oleracea* plants grown under saline conditions showed significant improvement in yield and reduced oxidative stress levels after treatment with 5% Kelpak[®]. In an experiment by Mohammed et al. (2024) with date palm exposed to salt stress, treatment of plants with different concentrations of Kelpak[®] improved nutrient uptake, increased the amount of photosynthetic pigments, and led to changes in biochemical markers associated with plant development and stress response. In a *Vigna unguiculata* experiment, treating plants grown under severe water deficit with a stimulant resulted in an increase in carbohydrate and photosynthetic pigment content in the leaves. At the same time, protein content, phenolic, and flavonoid concentrations decreased. This indicates stress mitigation and osmotic adaptation to water stress (Voko et al., 2022). Under conditions of mineral deficiency, Kelpak[®] is capable of changing both morphological and physiological characteristics to overcome nutrient deficiencies (Omoarelojie et al., 2025). There is very little information on the effect of Kelpak[®] on plants under conditions of pollution with heavy metals and other toxicants. According to Arthur et al. (2016), under conditions of severe Zn and Al pollution, the biostimulant had no positive effect on *Brassica juncea* plants. However, under moderate pollution, its use showed good results. This is consistent with the results of our experiment, where lettuce plants experienced moderate cadmium stress, and treatment with Kelpak[®] helped mitigate its negative effects. Given the lack of experimental data, further research is needed to determine the optimal doses of Kelpak to achieve maximum effect under polluted conditions. The mitigation effect was obtained in our case study as soon as lettuce seedlings watered with CdCl₂ were treated with the biostimulant Kelpak[®]. This yielded positive results. Resumption of growth was observed, and photosynthetic pigment content increased compared to untreated samples: chlorophyll *a* by 23–33%, chlorophyll *b* by 14–15%, and carotenoids by 15–27%. The use of the biostimulant helped reduce MDA formation by 14–22%.

Conclusion

The use of Kelpak[®] biostimulant promotes an increase in above-ground lettuce biomass under optimal conditions. Under cadmium contamination, the product's practical effect on lettuce productivity is negligible. Cadmium pollution reduces chlorophyll levels in lettuce leaves. Chlorophyll *b* is less sensitive to cadmium levels in irrigation water than chlorophyll *a*. A positive effect of biostimulant application on the chlorophyll complex has been noted. However, for chlorophyll *b*, the effect is less pronounced. Carotenoid biosynthesis in lettuce leaves is significantly reduced by cadmium contamination. Although treatment with Kelpak[®] increases pigment content, this only partially offsets the negative impact of the toxicant. As cadmium concentrations in the soil increase, MDA level in plant tissues rise proportionally. Kelpak[®]'s stimulating ingredients help mitigate the negative effects of cadmium pollution, resulting in a decrease in MDA content in lettuce leaves after treatment. Strong and moderate correlations were observed between pigments content, biomass, and MDA level in lettuce leaves.

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