The Influence of Heavy Metals Contained in Sludge Used as Fertilizer for the *Dahlia Variabilis* Plant on Photosynthetic Traits

DENISA BACALU-RUS[1], IOAN BOTH[2], FLAVIA BORTES[1,3], ANDREEA LUPITU[3], DANA COPOLOVICI[3], LUCIAN COPOLOVICI[1,3]
[1]Interdisciplinary School of Doctoral Studies,
"Aurel Vlaicu" University, Romania,
2 Elena Drăgoi, Arad 310330,
ROMANIA

[2]Faculty of Civil Engineering,
Politehnica University of Timişoara,
1st Ioan Curea, Timişoara, 300226,
ROMANIA

[3]Faculty of Food Engineering, Tourism, and Environmental Protection,
Institute of Interdisciplinary Research, "Aurel Vlaicu" University,
2 Elena Dragoi, Arad 310330,
ROMANIA

Abstract: - This study examines the effects of sewage sludge and wastewater on soil parameters and photosynthetic traits in *Dahlia variabilis*. Applying sewage sludge to soil reduces pH, increases electrical conductivity, and enhances soil fertility by boosting nitrogen (N) and phosphorus (P) availability. Plants treated with sludge show increased assimilation rates, higher stomatal conductance, and elevated chlorophyll content, particularly at sludge levels above 8 g/kg soil. However, zeaxanthin levels, which contribute to photoprotection, decrease with high sludge treatment while increasing in wastewater-treated plants, indicating stress. This study highlights the complex relationship between nutrient availability and photosynthetic efficiency in *Dahlia variabilis*.

KeyWords: - wastewater, sludge, Dahlia veritabilis, photosynthetic parameters, chlorophylls, zeaxanthin.

Received: September 2, 2024. Revised: March 8, 2025. Accepted: April 13, 2025. Published: May 14, 2025.

1 Introduction

Starting from the environmental problems related to climate change, which produce droughts in more areas of Romania, wastewater reuse represents a specific measure for water management. Treated wastewater becomes an important source of water and nutrients for certain agricultural crops.

To reduce the large amounts of sewage sludge generated by wastewater treatment plants, the composting of sludge by mixing it with green waste, followed by anaerobic decomposition, resulting in a nutrient-rich product that can be used as a natural fertilizer for plants was investigated in [1]. Many synthetic nitrogen-based fertilizers pollute the environment due to greenhouse gases emitted into the atmosphere. Thus, using sludge as a natural fertilizer helps reduce greenhouse gas (GHG) emissions, [2].

Research on this subject was carried out at the M'zar plant in Morocco, [3]. In their study, based on the physicochemical analyses of the treated water, they found that the pH, COD, and BOD_5 values were within the limits allowed by Moroccan standards, and wastewater contributed to significant savings in fertilization.

In addition to nutrients, treated water and sludge from treatment plants also contain heavy metals. These metals were investigated in the soil of the industrial city of Alborz in Iran, [4]. A field cultivated with ornamental sunflowers was irrigated with treated industrial wastewater, and based on the analyses, they found that the soil was contaminated with heavy metals. The experiment consisted of treating the soil with cow manure and biosolids in three weight ratios, concluding that its treatment can improve the ornamental capacity of the sunflower to accumulate heavy metals from the soil.

Pollution assessment and potential ecological risk of seven heavy metals (Cd, Cr, Cu, Hg, Ni, Pb, and Zn) in sewage sludge collected from a wastewater treatment plant in a highly industrialized area in Poland, [5]. The research demonstrated that zinc, cadmium, and mercury have the highest impact on the plants through risk assessment.

Also in Poland, [6] a research on the content of heavy metals in a sewage treatment plant was performed, reaching the conclusion that in addition to the contained heavy metals, this sludge also has beneficial compounds for natural management and only at one treatment plant, the concentration of studied heavy metals exceeded the allowed limit.

A study that consisted of investigating the potential of native plants for the phytoremediation of soils contaminated with lead, zinc, cadmium, and nickel in the pastures around the lead and zinc factory in Zanjan, Iran, was carried out, [7]. Lead, zinc, cadmium, and nickel were extracted from plants and soil samples. The results showed no Pb and Zn hyperaccumulators were identified in the area.

In South Africa, the content of heavy metals in sewage treatment plants was studied , especially Cd, concluding that the carcinogenic risks for humans and the aquatic environment are low, but they do not recommend treated water for irrigation, [8].

Sludge from wastewater treatment plants is rich in nutrientsThe investigations on the sludge from Machakos town's decentralized sewage treatment plant lead to the conclusion that it is a potential fertilizing agent that can stimulate plant growth in agricultural farms, [9].

The activated sludge from sewage treatment plants contains nutrients such as phosphorus, potassium, and nitrogen, which is of particular importance when considering using the sludge as an agricultural fertilizer or soil conditioning agent. In this sense, A study on the effect of nitrogen (N) on vegetables using two alternative methods: a traditional one and a sewage sludge method, was performed in [10]. It was concluded that sewage sludge supported production without needing inorganic fertilization for three years.

Sewage sludge contains beneficial nutrients for plant growth. Another research consists in the comparison between the plants grown with soil mixed in different proportions with manure, respectively, sludge, [11] . In this study, they discovered that after 28 days, the highest growth of the plants was those grown with a 50% soil-sludge mixture.

In addition to the necessary nutrients K, N, and P, also need microelements to grow, so we considered using the sludge from the sewage treatment plant as fertilizer, [12]. Some cereal crops grown on deficient soils are occasionally treated with the addition of Cu, [13].

Dahlia variabilis is an ornamental and medicinal plant widely cultivated in China. In a study performed in China on this Dalia, the symptoms of leaf spots and deformations in plants collected in Hohhot, China, were found, [14]. Phylogenetic analysis of isolates based on genome sequence grouped four DCMV isolates into a branch most closely related to the dahlia mosaic virus (DMV).

The research in [15] was conducted to observe the performance of Dahlia (*Dahlia variabilis* L.) genotypes. They studied 35 genotypes, measuring their height (116.69 cm), plant spread (43.93 cm), leaf area index (3.63), stem circumference (12.70 mm), and chlorophyll (54,19). The study showed that some genotypes did not show any incidence of mold, and in others, no incidence of aphids and mites was observed.

The effect of rooting hormones (IBA and NAA) on the propagation of Dahlia Kenya Blue and Kenya Yellow mutants was studied in [16], who concluded that plants treated with IBA-500 ppm had excellent results on Kenya Blue, which performed superior in most flowering traits compared to Kenya Yellow which performed best in other flowering characteristics.

The study conducted on several varieties of *Dahlia variabilis* shows that high thermal stress (40°C) leads to a significant decrease in chlorophyll content, transpiration rate, net photosynthetic rate, and water potential, [17].

The roots of red Dahlia have a high carbohydrate and inulin content [18], which is a medicinal plant also used in the food industry for dye extraction, [19]. The study also explored the potential use of phytochemicals from *Dahlia pinnata* against bacterial infections, [20].

To highlight the potential use of Dahlia as a medicinal plant, the current study also examines the content of heavy metals present in the sludge added as fertilizer to the soil for plant growth, as well as their content in the soil after the plant's growth. The impact of using treated wastewater and sludge on the photosynthetic parameters and chlorophyll pigments of *Dahlia variabilis* plants is presented.

2 Materials and Methods

Dahlia (*Dahlia variabilis L.*) seeds were sown in 0.3 l plastic containers containing a mixture of commercial garden soil and sewage sludge, Figure 1. The light intensity at the plant level was 800 mol/(m^2 s^1) and was supplied by LED lamps (Hoff, Nürnberg, Germany). Temperatures and relative humidity were maintained at 25/22 °C and 65%, respectively. The vegetation was irrigated daily to the capacity of the soil field. The investigations utilized seven-week-old, non-bolting plants with at least four levels ofdeveloped leaves. Randomization ensured that all plants received the same amount of light.

Fig. 1: Experimental containers with soil and sewage sludge mixture

Treated wastewater and sludge were collected from the final sedimentation basin at the Domestic and Industrial Water Treatment Plant in the Municipality of Arad, Romania, for the plant's treatment. Plants have been treated with varying quantities of sludge fertilizer, ranging from 4 g to 15 g/kg of soil and wastewater.

As previously reported, a portable gas exchange system (GFS-3000, Waltz, Effeltrich, Germany) was used to determine the photosynthetic parameters, [21]. A leaf was enclosed in the measurement chamber, and the following parameters were maintained: carbon dioxide concentration: 410 ppm, light: 1000 µmol m^{-2} s^{-1}, humidity: 65%, temperature: 25°C. The steady-state values of net assimilation (A) and stomatal conductance to water vapor (gs) were calculated.

The pigments (chlorophyll a, chlorophyll b, and beta-carotene) were extracted in acetone according to the technique reported before [22], and the quantitative analyses were carried out utilizing the UHPLC-DAD equipment (NEXERA 8030, Shimadzu, Kyoto, Japan) following the method that was previously published, [23]. Using the pure chromatographic standards developed by Merck in Darmstadt, Germany, a calculation was made to determine the chlorophyll *a*, chlorophyll *b*, and zeaxanthin levels.

3 Results and Discussions

Repeated reports indicate that applying sewage sludge to soil leads to changes in soil parameters, such as a reduction in pH and an increase in electrical conductivity (EC), [24], [25], [26]. The addition of sewage sludge also enhances soil fertility by increasing the accessibility of certain nutrients, such as nitrogen (N) and phosphorus (P), [27]. The assimilation rates increase for plants treated with sludge, but wastewater does not affect them. The enhanced carbon sink capacity resulting from N_2 fixation was due to the heightened activity of sucrose phosphate synthase (SPS) in sludge-fertilized plants. This led to the storage of more carbohydrates in the leaves, [28]. Even more, the increased nutrient content, particularly nitrogen (N), in leaves due to the addition of sludge can be directly and positively linked to alterations in leaf biochemical and structural characteristics. This includes an increase in leaf stomatal conductance which leads to a greater influx of CO_2 through the sub-stomatal chamber and subsequently enhances the rate of CO_2 assimilation [29] as observed in Figure 2.

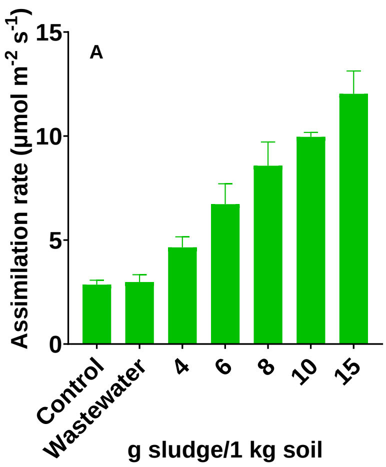

Fig. 2: Assimilation rate for plants treated with sludge and wastewater

Stomatal conductance to water vapor increases to a maximum with a sludge treatment of up to 6g/kg, Figure 3. This behavior can be explained by the differences in stomatal density in plants grown with high nitrogen fertilizer. The regulation of stomatal aperture is complex and is influenced by several factors, including leaf hydration status and the concentration of abscisic acid. Additionally, the stability of stomatal conductance is primarily influenced by the water supply available to the plant within a specific environmental context. The balance between these factors ensures that stomatal conductance remains optimized for the plant's physiological needs, reflecting the interplay between nutrient availability, hydration status, and hormonal signaling, [30].

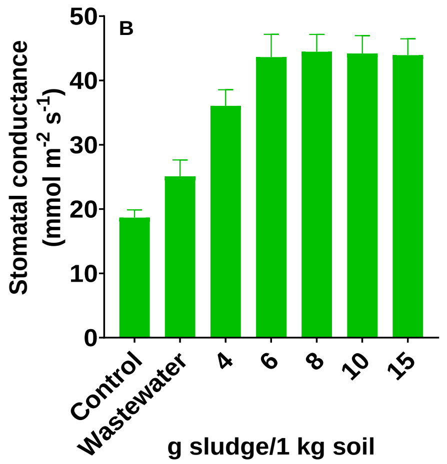

Fig. 3: Stomatal conductance for plants treated with sludge and wastewater

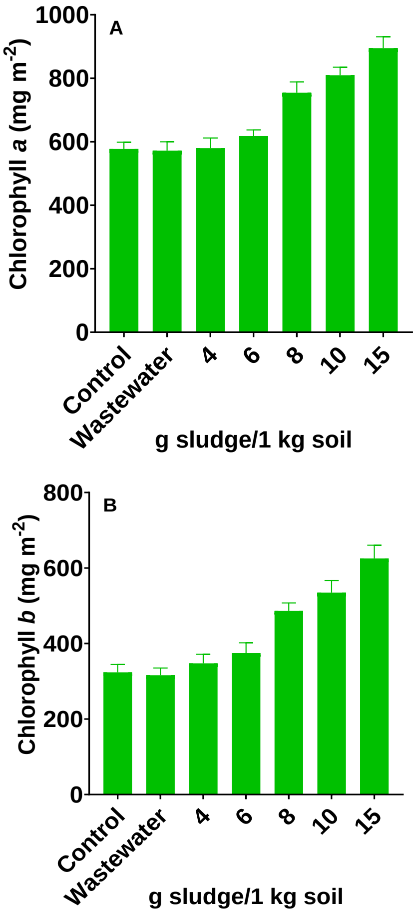

Fig. 4: Chlorophylls content in the leaves of *Dahlia variabilis* plants treated with wastewater and sludge

The increased chlorophyll content observed in *Dahlia variabilis* leaves treated with sludge can likely be attributed to the higher nitrogen availability within the leaves, Figure 4.

In our cases, the concentrations of chlorophylls were significantly higher only for plants treated with more than 8 g sludge/kg soil, and no significant differences were found for plants treated with wastewater, Figure 4.

The application of fertilizer increased chlorophyll a and b content, suggesting that nitrogen had a crucial effect on enhancing plant growth and photosynthetic efficiency, [31]. Chlorophyll, a nitrogenous molecule, needs a sufficient nitrogen source for production. Furthermore, the enzymes implicated in nitrogen metabolism are essential for chlorophyll synthesis. These enzymes, by enabling the transformation of nitrogen into usable forms, directly affect chlorophyll production and accumulation. In addition to nitrogen availability, many metabolic variables also influence chlorophyll synthesis. The equilibrium of micronutrients, especially critical elements like magnesium, iron, and manganese, is vital, as these nutrients function as cofactors for enzymes that facilitate chlorophyll formation and photosynthesis. Water availability is essential for maintaining cellular turgor, facilitating nutrient transport, and avoiding stomatal closure, which might restrict CO_2 absorption and diminish photosynthetic efficiency. Moreover, oxidative stress, resulting from an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defenses, can deteriorate chlorophyll molecules and hinder photosynthesis. Efficient antioxidant systems, including enzymes like superoxide dismutase and catalase, alleviate oxidative stress, therefore preserving chlorophyll stability. Thus, the improved nitrogen availability from sludge treatment, optimal micronutrient levels, sufficient water supply, and regulated oxidative stress collectively facilitate the enzymatic activities required for chlorophyll synthesis, leading to increased chlorophyll content in the leaves, [32].

The concentration of the photosynthetic pigment zeaxanthin, which contributes to the photoprotective effect, decreases in plants treated with more than 8 g of sludge per kg, Figure 5.

Additionally, the content of this pigment increases in wastewater-treated plants, suggesting a stress factor, [33]. N-deficient plants often squander a more significant proportion of absorbed solar energy than N-adequate plants. This disparity is linked to concomitant alterations in xanthophyll cycle pigments. In nitrogen-deficient plants, the total xanthophyll pigments mainly consist of zeaxanthin and antheraxanthin. N-adequate plants demonstrate a distinct distribution of xanthophyll pigments, characterized by reduced zeaxanthin and antheraxanthin.

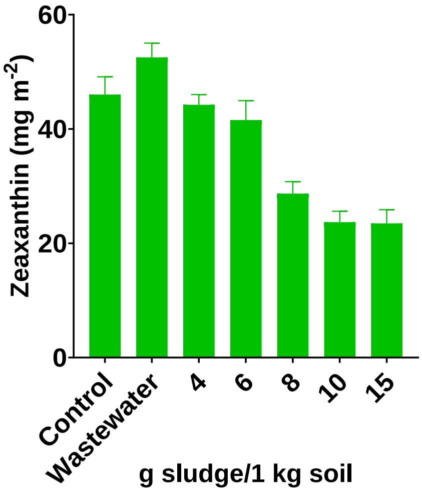

Fig. 5: The zeaxanthin content in the leaves of *Dahlia variabilis* plants treated with wastewater and sludge

This alteration in pigment composition indicates that nitrogen-deficient plants may depend more significantly on the xanthophyll cycle for photoprotection, turning increased amounts of violaxanthin into zeaxanthin and antheraxanthin to dissipate surplus light energy and safeguard against photodamage. The discourse on zeaxanthin emphasizes its function in photoprotection during nitrogen-deficient circumstances. This work lacks precise biochemical or physiological data; nonetheless, the postulated mechanism aligns with previous literature about the xanthophyll cycle and its role in energy dissipation. The increased conversion of violaxanthin to zeaxanthin and antheraxanthin is a well-documented reaction to light stress in plants, and nitrogen shortage is recognized to intensify this stress. This rationale corresponds with the observed alterations in pigment composition, indicating that nitrogen-deficient plants engage the xanthophyll cycle to alleviate possible photodamage. Subsequent research may examine these mechanisms more thoroughly, although the facts given corroborate the idea as a credible interpretation of the results, [34].

The determination of the metals in the activated sludge from the treatment plant was done by atomic absorption spectrometry, [34]. Several heavy metals were analyzed, but Cu, Zn, and Cr had the largest share. From the research carried out, as seen in Table 1, the content of Hg is below the detection limit.

Table 1. Content of Cu, Zn, Cr, and Hg in the sludge used as fertilizer

Element	UM	Results
Cu	ppm	500 ± 50
Zn	ppm	900 ± 50
Cr	ppm	400 ± 50
Hg	ppm	bdl

In each pot containing 420 g of soil, 4, 6, 8, 10, and 15 g of sludge from the sewage treatment plant in Arad, used as fertilizer, was added, sample P4-420, P6-420, P8-420, P10-420, and P15-420, respectively, in Table 2.

After the plants' growth, the soil, which was mixed in different proportions with the fertilizer, was analyzed by atomic absorption spectroscopy. The metals contained in the sewage sludge were also detected in the soil-sewage sludge mixture, in quantities presented in Table 2. The last column of Table 2 represents the allowable limit according to the Romanian regulations.

Table 2. The concentrations of Cu, Zn, Cr, and Hg in the soil mixed in different amounts with sewage sludge

Elem	UM	Results						Limit value [mg/kg]
		PC	P4-420	P6-420	P8-420	P10-420	P15-420	
Cu	ppm	18 ±5	19 ±5	21 ±5	23 ±5	43 ±5	71 ±5	100
Zn	ppm	62 ±5	75 ±5	84 ±5	80 ±5	128 ±5	214 ±5	300
Cr	ppm	< 200	< 200	< 200	< 200	< 200	< 200	100
Hg	ppm	< 6	< 6	< 6	< 6	< 6	< 6	1

The heavy metals Cu, Zn, and Cr were detected in the soil containing sewage sludge from the domestic and industrial wastewater sewage plant in larger quantities than in the soil without any sewage sludge content in the control soil (PC), similar to the determination in the literature, [11].

In Figure 6, the largest amount of sewage sludge mixed with the soil is 15g sludge/420g soil, 3.57%. The Zn content is the highest compared to the other metals contained in the soil, similar to the findings in [35].

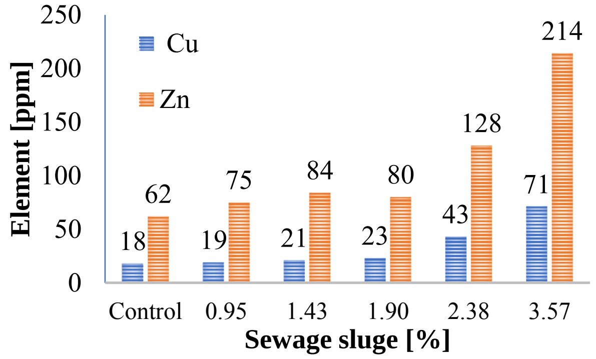

Fig. 6: The concentrations of Cu, Zn, in the soil mixed in different amounts with sewage sludge

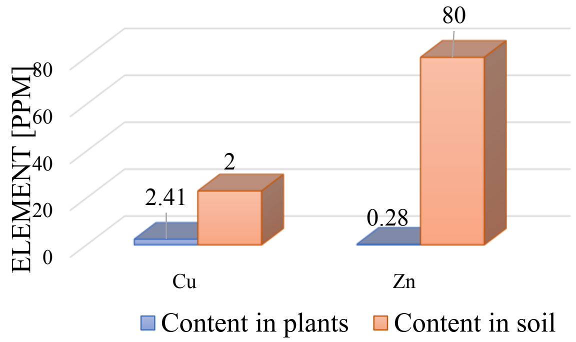

Fig. 7: Content of Cu, Zn in plants and sludge at the proportion of 8g sludge (1.90%)

Zn is a metal found naturally in the soil (about 70 mg kg^{-1} in crustal rocks), [11]. It is a metal that both plants and the human body need. According to data from Table 2, which are taken from the standards in force, it is within the limits allowed.

The amounts of heavy metals constantly increase with the addition of residual sludge to the soil but remain within the allowed limits.

The determination of heavy metals in the leaves of *Dahlia variabilis* by AAS atomic absorption spectroscopy, [36].

The determination was made at an interval of 15 weeks after planting on the leaves of the plants treated with sludge from the sewage treatment plant in the proportion of 8g of sewage sludge to 420g of commercial soil(1.90%). The content of Cu and Zn was determined, and the heavy metals were detected both in the sludge from the sewage treatment plant and the commercial soil mixed with it after the growth of the plants. The determination of heavy metals from *Dahlia variabilis* leaves was performed on the sample with a concentration of 8g of fertilizer due to the sudden increase in the assimilation rate (Figure 2) and chlorophyll a and b (Figure 4) and a decrease in zeaxanthin pigments (Figure 5), appeared at this concentration.

Figure 7 shows the difference in the concentration of Cu and Zn metals between the commercial soil mixed with sewage sludge and the leaves after growing *Dahlia variabilis*.

The concentration of Cu and Zn decreased considerably, meaning the plant needs metals such as Cu and Zn for growth. The plant extracted zinc in a much lower amount than Copper.

Copper is necessary for many redox reactions in plants, participating in the activity of cupro-enzymes and being an essential element for the production of chlorophyll, [37].

The samples, soil, and plants, are presented in Figure 8 showing an influence on the development of the plant depending on the sewage sludge mixtures.

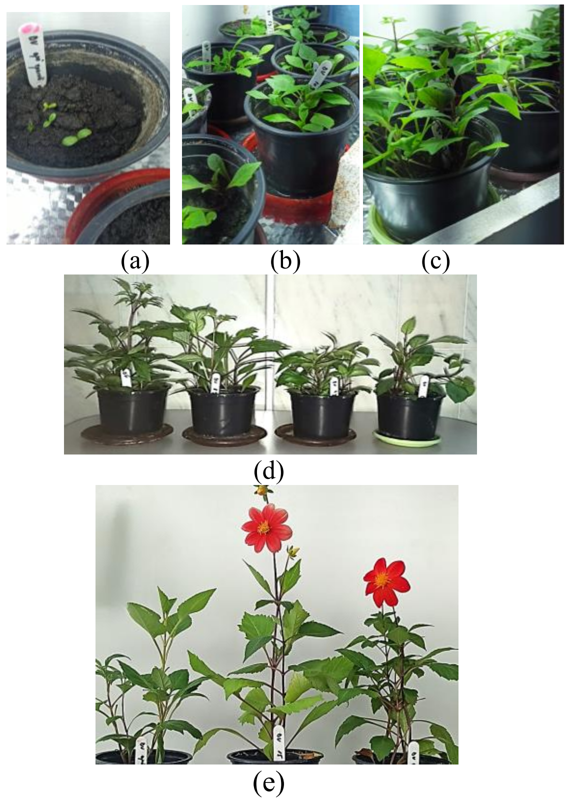

Fig. 8: Stages of plant growth of *Dahlia variabilis:* a) 4 days, b) 18 days, c) 29 days, d) 40 days, e) 77 days

4 Conclusion

The study concludes that applying sewage sludge to soil improves soil fertility by increasing nitrogen (N) and phosphorus (P) availability, though it reduces pH and increases electrical conductivity. Sludge treatment enhances plant assimilation rates, stomatal conductance, and chlorophyll content, particularly at levels above 8 g/kg, due to heightened sucrose phosphate synthase (SPS) activity and increased nitrogen availability. This results in greater carbohydrate storage in leaves and improved CO_2 assimilation. However, zeaxanthin levels, crucial for photoprotection, decrease with high sludge treatment while increasing in wastewater-treated plants, indicating a stress response. Overall, sewage sludge positively impacts soil fertility and photosynthetic efficiency in *Dahlia variabilis*, but also prompts a stress-related increase in zeaxanthin under certain conditions.

Copper and Zinc are essential metals for plant growth, they have an important role in the metabolism of the protective cell wall against oxidative stress. Zinc is an essential element in the biochemical processes of plants, meaning that they are indispensable for plants.

From the research presented in this article, it appears that copper was accumulated by plants in a larger quantity than zinc. Copper is considered an antifungal agent, but maybe in larger quantities it reduces the availability of zinc for plants, but in our case, it is in the concentrations allowed by Romanian standards and does not present a risk for plants.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process

The authors wrote, reviewed and edited the content as needed and they have not utilised artificial intelligence (AI) tools. The authors take full responsibility for the content of the publication.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Conceptualization: Denisa Bacalu-Rus and Ioan Both; Data curation: Denisa Bacalu-Rus and Lucian Copolovici; Investigation: Denisa Bacalu-Rus, Flavia Bortes; Methodology: Dana Copolovici; Resources: Andreea Lupitu; Supervision: Ioan Both and Lucian Copolovici; Validation: Dana Copolovici; Writing – original draft, Denisa Bacalu-Rus and Lucian Copolovici; Writing – review & editing, Ioan Both. All authors have read and agreed to the published version of the manuscript.

Sources of Funding for Research Presented in a Scientific Article or Scientific Article Itself

No funding was received for conducting this study.

Conflict of Interest

The authors have no conflicts of interest to declare.