



Article

Iron Nitrates Stimulate Phenolic Compound Production by *Melissa officinalis* L.

Dorota Adamczyk-Szabela *, Wojciech M. Wolf and Dawid Krakowiak

Institute of General and Ecological Chemistry, Lodz University of Technology, Zeromskiego 116, 90-924 Lodz, Poland; wojciech.wolf@p.lodz.pl (W.M.W.); dawid.krakowiak@dokt.p.lodz.pl (D.K.)

* Correspondence: dorota.adamczyk@p.lodz.pl

Abstract

Iron plays a key role in plant chemical processes and photosynthesis. The aim of this study was to investigate the effect of various iron concentrations on total phenolic compound levels and photosynthetic efficiency in *Melissa officinalis*. It is a very popular herb, widely cultivated worldwide for its medicinal properties. Herbs were grown under controlled laboratory conditions. Gas exchange parameters, chlorophyll index, and total content of phenolic compounds were determined. Additionally, iron, manganese, copper, and zinc concentrations in either soil or plant tissues were analyzed. The hypothesis that iron supplementation significantly affects photosynthesis and total phenolic compounds in lemon balm was confirmed by one-way analysis of variance. In particular, the beneficial biofortification at 100 and 200 µg/g iron doses generates a moderate increase in phenolic compounds and simultaneously does not hamper plant growth and photosynthetic efficiency. However, iron doses above 500 µg/g induce plant stress and trigger defense mechanisms that significantly increase phenolic compound production. This is accompanied by a decrease in biomass and photosynthetic efficiency.

Keywords: heavy metals; iron supplementation; photosynthesis; herbs; total phenolic compounds

1. Introduction

Melissa officinalis L. (lemon balm) is a perennial herb from the mint family, extensively cultivated worldwide and in Poland [1]. The chemical compounds present in the plant are effective in treating nervous agitation, stress, chronic fatigue syndrome, anxiety, vegetative neuroses, and digestive problems [2]. Biologically active compounds contained in lemon balm leaves give it antibacterial, antifungal, and antiviral properties [3,4]. Furthermore, the herb contains phenolic compounds, which are responsible for its antioxidant properties.

In stress conditions induced by abiotic factors, including heavy metals, plants moderate their response by increasing the production of phenolic compounds. Phenolic compounds reduce oxidative stress not only by directly scavenging free radicals but also by inhibiting the activity of enzymes responsible for their formation and lipid peroxidation. Specifically, they inhibit NADPH and xanthine oxidases which generate reactive oxygen species, as well as lipoxygenase and cyclooxygenase [5]. The latter two enzymes participate in lipid peroxidation. Phenolic compounds also increase the activity of important antioxidant enzymes, i.e., superoxide dismutase and catalase [6]. Stress in

Academic Editor: Oana Lelia Pop

Received: 14 May 2026

Revised: 8 July 2026

Accepted: 10 July 2026

Published: 11 July 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

plants can be induced not only by an excess of metals in the environment but also by a deficiency of nutrients. Iron can serve as an example, as it plays a key role in plant development.

Approximately 40% of the world's cultivated areas are affected by soil iron deficiency [7], which negatively impacts crop quality. While the iron total content in soils fluctuates between 0.5 and 5% [8] the soluble iron levels are usually exceptionally low [4,9]. Following Korzeniowska et al. [10], iron content below 700 mg/kg of dry soil is considered as low while 700–3800 mg/kg and 3800 mg/kg concentrations are referred to as medium and high levels, respectively. Iron solubility in soil is dependent on many reactions, particularly hydrolysis and the content of complex compounds [11]. Furthermore, iron mobility in soil is largely controlled by the solubility of amorphous hydrated Fe^{3+} and Fe^{2+} oxides and the formation of other iron compounds [12]. Iron participates in numerous processes [13], including protein synthesis, DNA replication, photosynthesis, and plant respiration [3,14–17]. Iron content in plants is usually in the range from 50 to 200 mg/kg of dry weight and depends on several factors such as soil pH, organic matter, moisture, temperature, and interactions with other elements [18,19]. Iron uptake by plants is metabolically controlled, although it can be absorbed as Fe^{3+} , Fe^{2+} or Fe-chelates [17]. Separation of chelated Fe prior to absorption appears to require reduction of Fe^{3+} to Fe^{2+} at the root surface [20–22]. In xylem exudates, iron occurs in an unchelated form, but its transport is primarily mediated by citrate chelates [23–26]. Iron deficiency causes metabolic disturbances in plants, leading to decreased chlorophyll index and reduced photosynthesis [27,28]. The primary sign of iron deficiency in plants is chlorosis, which occurs due to the degradation of chlorophyll [29]. In agricultural practice, iron deficiencies in plants are not related to low levels of iron in the soil, but rather to its limited availability to plants due to soil conditions. This is often caused by intensive liming, overfertilization, or periodic flooding. Iron fertilization requires appropriate soil conditioning to ensure that the element is absorbed most effectively. It is essential to select the appropriate dose to suit the individual needs of the plant. Furthermore, iron cations play a crucial role in regulating the mobility of macronutrients and trace elements in plants. During soil overfertilization, high iron concentrations can be potentially toxic to plants and lead to the formation of reactive oxygen species. The available literature primarily focuses on soil iron deficiency, which directly stimulates the biosynthesis and secretion of phenolic compounds in plants [30,31]. There are only a few reports on iron fertilization of plants. However, there is an astonishing lack of data on herbs supplemented with different doses of iron.

The aim of this study was to investigate the response of lemon balm to various levels of Fe^{3+} supplementation. We focused on the determination of a plant-safe dose that would enrich lemon balm with iron and additionally increase the level of phenolic compounds in the plant's tissues. This should enhance the pharmaceutical value of this herb.

In Central Europe's climate zone, supplementation with phenolic compounds is often necessary for people consuming a diet low in these compounds. According to the available literature, consuming foods rich in phenolic compounds reduces the likelihood of cardiovascular disease, heart disease, and cancer [32–34]. Various preparations containing freeze-dried extracts, such as those from bergamot fruit, with standardized concentrations of phenolic compounds are available on the market [35,36].

Nevertheless, there is a clear need to expand the range of plants from which phenolic extracts can be successfully obtained. It is well known that lemon balm extracts are considered safe, and allergic reactions induced by them are extremely rare [37,38].

It is generally known that phenolic acids can bind Fe^{3+} and Fe^{2+} ions, modifying their solubility and mobility [39]. Not all plant species exhibit such a clear, measurable response, making lemon balm an excellent experimental model. However, further research on the antioxidant activity of plants is clearly needed. This is particularly due to

the rising consumer concerns over novel antioxidants which are likely to enter the market [40].

Our preliminary results indicated that low iron supplementation enhanced photosynthesis by increasing chlorophyll production in herbs. The opposite effect was observed with high iron doses. In this work, we extend this observation over lemon balm cultivated in several carefully controlled conditions.

2. Results

2.1. Analysis of Soil Used for Plant Cultivation

The analysis of untreated soil showed that it belongs to acidic, organic soils [41,42]. According to international standards, the soil was not contaminated with iron, manganese, copper or zinc [43,44] (Table 1).

Table 1. Analysis of untreated soil.

pH	5.82			
Organic matter	25%			
Metal content ($\mu\text{g/g}$)	Fe^{3+}	Mn^{2+}	Cu^{2+}	Zn^{2+}
Total forms	3452 ± 11	168 ± 5	29.6 ± 0.6	94 ± 3
Bioavailable forms	442 ± 5	93.8 ± 1.1	18.5 ± 0.4	48.2 ± 0.9

2.2. Lemon Balm Plant Analysis

2.2.1. The Effect of Iron Supplementation on Heavy Metal Contents in Plants

Metal contents in the above-ground parts and roots of lemon balm are shown in Figure 1. Lemon balm grown in soil without any additives (control) accumulates iron, copper and zinc in the roots and manganese in the above-ground parts. The null hypothesis was whether iron application affects the migration of metals from soil to plant (Table 2). In all tested plants, the increase in iron content hindered the uptake of all the determined metals from the soil and their transport to the roots and above-ground parts.

Table 2. The one-way ANOVA for iron, manganese, copper and zinc concentrations in lemon balm plants across iron supplementation. Critical Snedecor's F value is $F_{\text{crit.}(5,24)} = 2.620$; probability level $p = 0.05$.

	Mn^{2+}	Cu^{2+}	Zn^{2+}
Roots	$F = 343.636$	$F = 295.300$	$F = 432.119$
	$p = 1.66 \times 10^{-21}$	$p = 9.94 \times 10^{-21}$	$p = 1.10 \times 10^{-22}$
Above-ground parts	$F = 393.066$	$F = 166.168$	$F = 132.868$
	$p = 3.39 \times 10^{-22}$	$p = 8.36 \times 10^{-18}$	$p = 1.11 \times 10^{-16}$

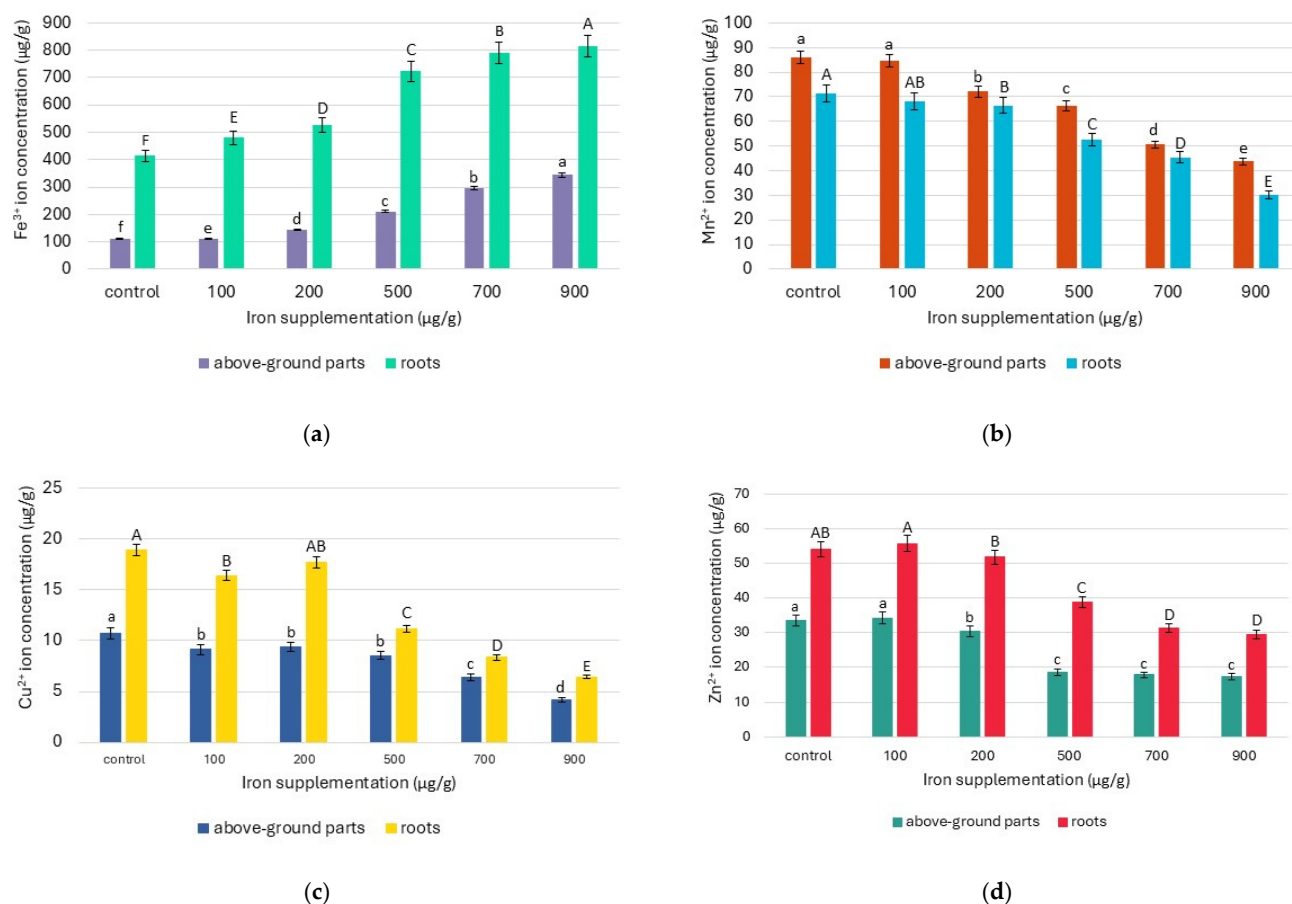


Figure 1. Iron (a), manganese (b), copper (c) and zinc (d) ion concentration in above-ground parts and roots of herbs cultivated in soil under iron supplementation. The number of samples $n = 5$. Within each plant part, values associated with different letters are significantly different according to Tukey's HSD test at $p = 0.05$.

Metal uptake by plants can be estimated using bioaccumulation factors (BAFs), translocation factors (TFs) and transfer coefficients (TCs) (Table 3). BAF is defined as the ratio of the metal concentration in the above-ground parts of the plant to its content in the soil, TF is the ratio of the metal concentrations in the above-ground parts and roots of the plant, respectively, while TC is the ratio of metal content in roots to its content in the soil [45].

Table 3. Bioaccumulation factors (BAFs), translocation factors (TFs) and transfer coefficients (TCs) calculated for lemon balm. Elements are shown in decreasing order of particular factor. Each value is the average of the data from five replicates.

Samples	BAF	TF	TC
control	Mn(0.51) > Zn(0.36) > Cu(0.36) > Fe(0.03)	Mn(1.21) > Zn(0.62) > Cu(0.57) > Fe(0.26)	Cu(0.64) > Zn(0.58) > Mn(0.42) > Fe(0.12)
100 µg/g	Mn(0.50) > Zn(0.36) > Cu(0.31) > Fe(0.03)	Mn(1.24) > Zn(0.61) > Cu(0.56) > Fe(0.23)	Zn(0.59) > Cu(0.55) > Mn(0.41) > Fe(0.14)
200 µg/g	Mn(0.43) > Zn(0.32) > Cu(0.32) > Fe(0.04)	Mn(1.08) > Zn(0.59) > Cu(0.53) > Fe(0.28)	Cu(0.60) > Zn(0.55) > Mn(0.40) > Fe(0.15)
500 µg/g	Mn(0.40) > Cu(0.29) > Zn(0.20) > Fe(0.06)	Mn(1.26) > Cu(0.76) > Zn(0.48) > Fe(0.29)	Zn(0.41) > Cu(0.38) > Mn(0.31) > Fe(0.21)
700 µg/g	Mn(0.30) > Cu(0.22) > Zn(0.19) > Fe(0.09)	Mn(1.11) > Cu(0.77) > Zn(0.57) > Fe(0.37)	Zn(0.33) > Cu(0.28) > Mn(0.27) > Fe(0.23)
900 µg/g	Mn(0.26) > Zn(0.19) > Cu(0.14) > Fe(0.10)	Mn(1.44) > Cu(0.65) > Zn(0.59) > Fe(0.42)	Zn(0.31) > Fe(0.24) > Cu(0.22) > Mn(0.18)

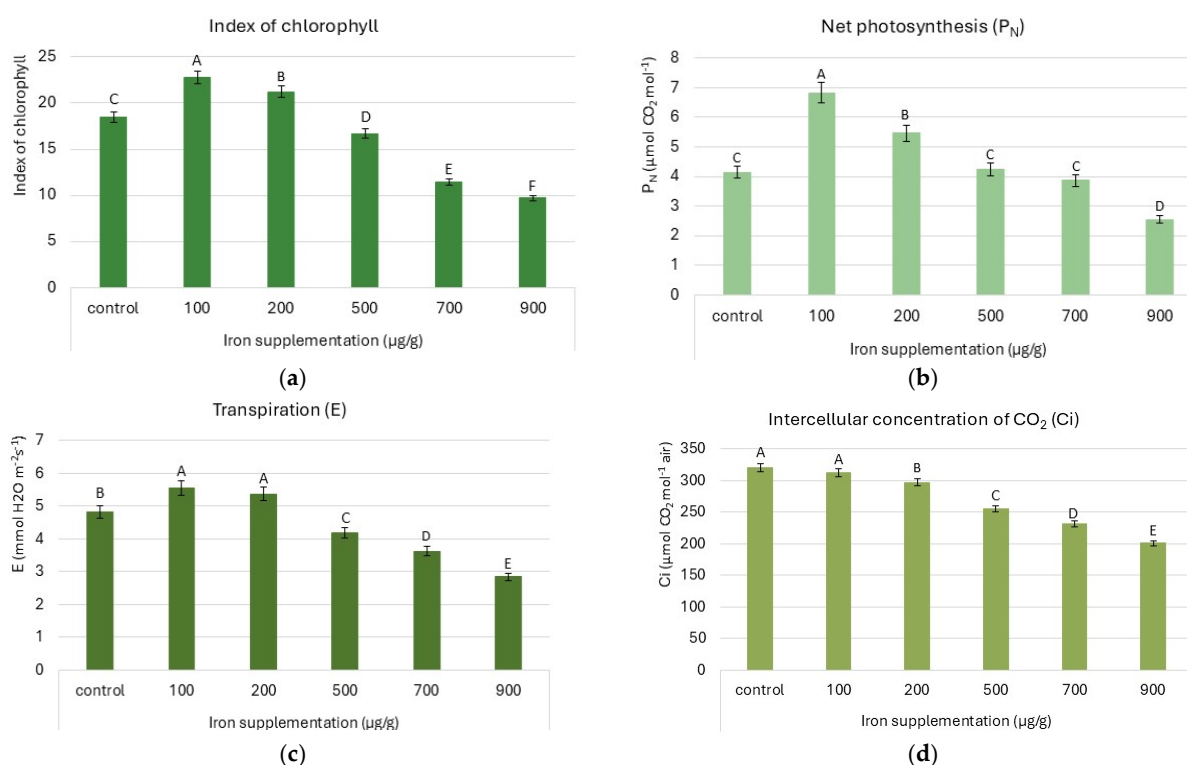
2.2.2. The Effect of Iron Supplementation on Plant Growth and Photosynthesis

The gas exchange parameters, which illustrate the photosynthesis efficiency, i.e., index of chlorophyll, net photosynthesis (P_N), transpiration (E), stomatal conductance (G_s), intercellular CO_2 (C_i) and biomass of above-ground parts of plants, are shown in Figure 2. Low doses (100 and 200 µg/g) of iron increased photosynthesis yield

substantially. Supplementations 500 and 700 $\mu\text{g/g}$ stabilized P_N at the control level while the largest iron dose (900 $\mu\text{g/g}$) drove it to the lowest value observed. This situation is reflected by the chlorophyll index. The chlorophyll index in herbs treated with 100 and 200 $\mu\text{g/g}$ Fe was significantly higher than control, as shown in Figure 2. However, higher doses of iron (500; 700; 900 $\mu\text{g/g}$) caused a gradual decrease in the chlorophyll index (10%; 38%; 48% respectively) in plants. Iron excess did not affect the intercellular CO_2 significantly. A gradual decrease in C_i content was observed along the increasing concentration of the supplemented iron dose. Iron doses of 100 and 200 $\mu\text{g/g}$ increased transpiration and stomatal conductance. However, supplementation with higher Fe^{3+} doses caused a significant decrease in transpiration and stomatal conductance compared to control. Plants control iron uptake by regulating water transport through stomatal opening. This affects transpiration, which lags behind net photosynthesis and reduces the intercellular CO_2 concentration in the plant samples for which the highest photosynthetic efficiency was observed. Low doses enhanced the photosynthetic process while iron supplementation above 100 $\mu\text{g/g}$ significantly inhibited lemon balm mass (Figure 2). To assess the effect of iron supplementation on photosynthesis, one-way ANOVA analysis was used at the probability level of $p = 0.05$ (Table 4).

Table 4. One-way ANOVA for index of chlorophyll, net photosynthesis, transpiration, intercellular concentration of CO_2 , stomatal conductance and dry mass of lemon balm across iron supplementation. Critical Snedecor's F value is $F_{\text{crit.}(5,24)} = 2.620$; probability level $p = 0.05$.

Index of Chlorophyll	Net Photosynthesis	Transpiration	Intercellular Concentration of CO_2	Stomatal Conductance	Dry Mass
$F = 811.055$ $p = 6.20 \times 10^{-26}$	$F = 262.826$ $p = 3.90 \times 10^{-20}$	$F = 238.386$ $p = 1.20 \times 10^{-19}$	$F = 555.339$ $p = 5.62 \times 10^{-24}$	$F = 132.980$ $p = 1.10 \times 10^{-16}$	$F = 687.045$ $p = 4.50 \times 10^{-25}$



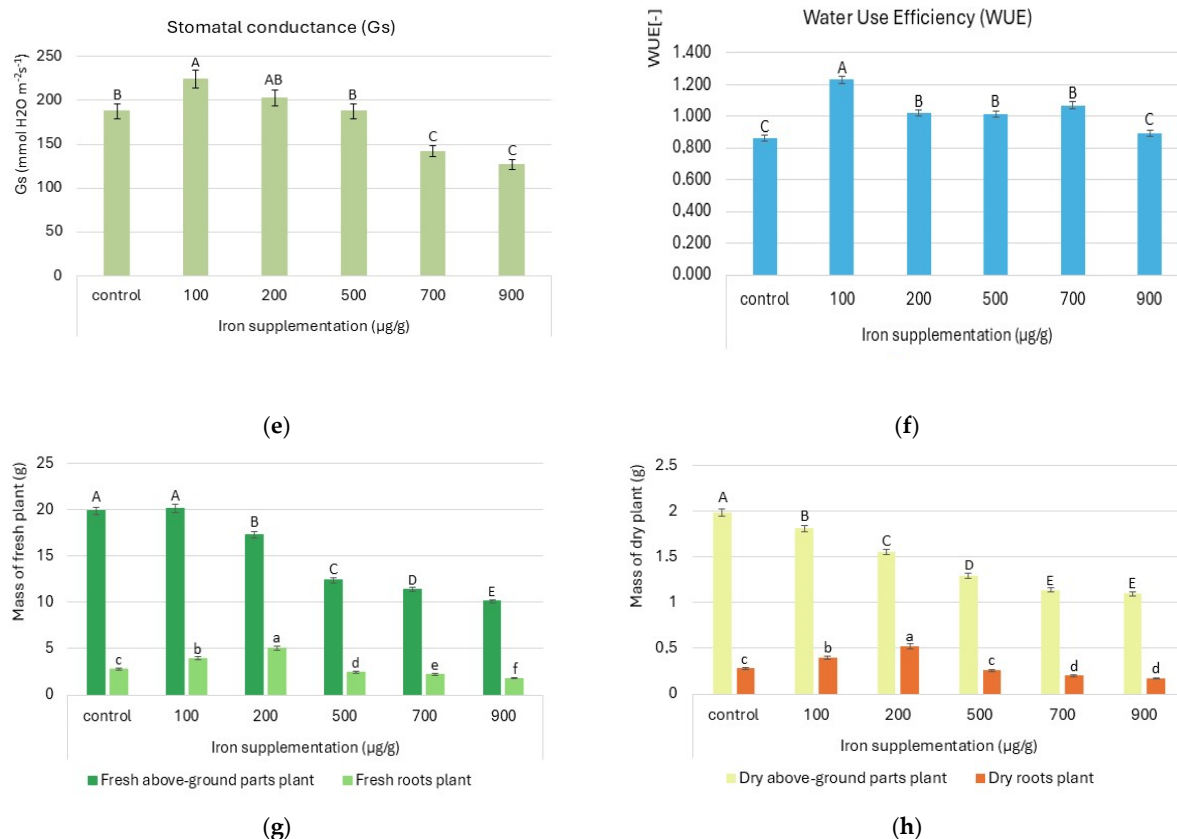


Figure 2. Index of chlorophyll (a), net photosynthesis (b), transpiration (c), intercellular concentration of CO_2 (d), stomatal conductance (e) water use efficiency (f), fresh plant mass (g) and dry plant mass (h) determined for lemon balm with relevant standard deviations ($n = 5$). Within each plant part, values associated with different letters are significantly different according to Tukey's HSD test at $p = 0.05$.

2.2.3. The Effect of Iron Supplementation on the Total Phenolic Compounds

The levels of total phenolic compounds (TPCs), which were determined by the Folin–Ciocalteu procedure, are summarized in Figure 3. Extracts of lemon balm were characterized by a diversified content of total phenolic compounds. The highest amount was found in lemon balm supplemented with 900 µg/g iron (over 200%). Iron supplementation had a statistically significant effect on the total phenolic content in lemon balm ($F = 240.063$; $p = 1.14 \times 10^{-19}$; $F_{\text{crit.}(5,24)} = 2.620$; probability level $p = 0.05$).

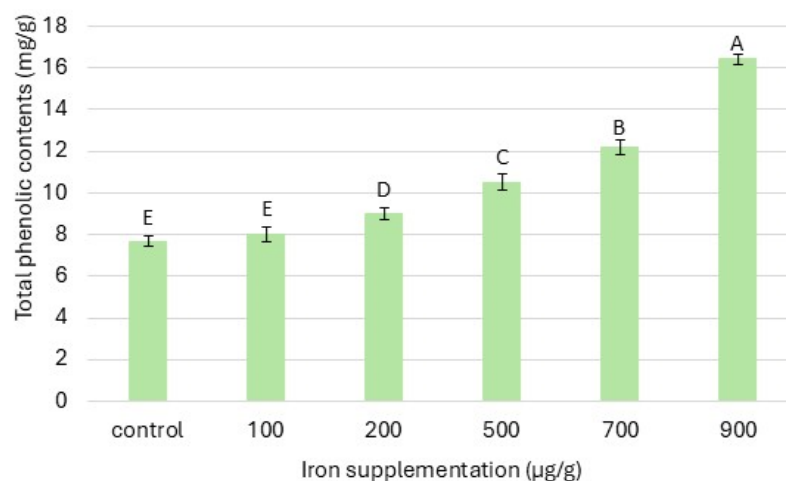


Figure 3. The total phenolic contents in above-ground parts of lemon balm as determined for control and iron supplementation accompanied by relevant standard deviations ($n = 5$). Within each plant part, values associated with different letters are significantly different according to Tukey's HSD test at $p = 0.05$.

3. Discussion

The availability of iron in the soil is often limited. In this respect, the application of fertilizers containing iron is the simplest way to deal with the symptoms of iron deficiency in plants. However, iron supplementation at high doses may be a stress factor which limits yield of cultivated herbs. A wide range of iron concentrations (100–900 $\mu\text{g/g}$) was used in our experiment. We aimed to encompass levels either close to the natural content of available iron in soil or elevated values that may cause physiological changes in plants.

Our results clearly demonstrate that high iron contents in the soil affect the manganese, zinc, and copper uptake and their subsequent migration within the lemon balm plant. The translocation factors calculated for plants grown in reference soil (control) are in the order $\text{Mn} > \text{Zn} > \text{Cu} > \text{Fe}$. Iron supplementations above 500 $\mu\text{g/g}$ prompted a shift in the order between zinc and copper in the row. The transfer coefficients calculated for lemon balm cultivation in control soil are arranged in the following series: $\text{Cu} > \text{Zn} > \text{Mn} > \text{Fe}$. The addition of iron changed that order for all investigated metals. The largest decreases in TC values were recorded for copper after iron supplementation at a dose of 500 $\mu\text{g/g}$ and for manganese at an iron concentration of 700 $\mu\text{g/g}$. Bioaccumulation factors for untreated soil followed the order $\text{Mn} > \text{Zn} > \text{Cu} > \text{Fe}$. Only zinc and copper changed their position within the series after 500 and 700 $\mu\text{g/g}$ supplementations.

Iron supplementation decreased the BAF values for all investigated elements but did not affect their orders in respective series. According to data in the literature, Mn-Fe, Cu-Fe and Zn-Fe antagonisms are commonly known. This is due to the fact that zinc, manganese, copper and iron use similar protein transporters (ZIP, NRAMP and YSL) and compete for their active sites during the uptake [14,46]. This leads to nutrient imbalances and stunted plant growth. Furthermore, excess iron initiates ionic antagonism, in which Fe inhibits the uptake of Mn, Zn, and Cu through competitive interactions in the root zone. Furthermore, when plants absorb high doses of iron, they can secrete compounds in the root zone which alter the solubility of other metals and make them less available to the plant [47].

Iron is a key trace element for chlorophyll biosynthesis and the proper functioning of the photosynthetic apparatus. Iron deficiency leads to the inhibition of chlorophyll synthesis by blocking steps in the porphyrin pathway and results in leaf chlorosis [48]. Our results show that iron concentrations of 100 $\mu\text{g/g}$ and 200 $\mu\text{g/g}$ significantly increased chlorophyll content in lemon balm. Supplementations with applicable doses of iron increase the activity of biosynthetic enzymes and the level of chlorophyll precursors. Finally, they are likely to increase the chlorophyll content in plant tissues.

Furthermore, iron is a key cofactor of ferredoxin and plays a crucial role in electron transport during photosynthesis [49]. However, at high levels of supplementation, we observed a reduction in chlorophyll content in plants. Excess iron induced oxidative stress [50] by generating reactive oxygen species via the Fenton reaction [51], leading to chlorophyll degradation and chloroplast damage [52]. Furthermore, it affected the cellular ionic balance, disrupted chlorophyll biosynthesis and limited the synthesis of this pigment. In summary, either iron deficiency or iron excess reduced photosynthetic efficiency and prompted chlorotic changes in leaves [52].

Our results clearly showed an increase in net photosynthesis at iron concentrations of 100 and 200 $\mu\text{g/g}$. This was accompanied by a decrease in the intercellular CO_2 which was likely due to the closure of stomata responsible for water transport regulation. The water use efficiency index and stomatal conductance approximately followed the patterns

observed for net photosynthesis and transpiration. However, direct comparison of numerical data indicates that increased photosynthesis efficiency was accompanied by better use of water. This means that a higher proportion of water was directly used in photosynthesis. On the other hand, the reduction in water uptake was due to the stress effects stimulated by iron at high concentrations, which limited the growth of cultivated herbs. In our studies, a decrease in the fresh mass of the above-ground parts of the plant was observed after all iron applications. We therefore speculate that the latter affects the plant strategy and stimulated the investment of more energy into root system development. This hampers the growth of its green parts. There was a clear correlation between increased phenolic content and reduced plant weight, especially at iron doses above 500 µg/g. Plants treated with iron at a concentration of 900 µg/g showed the highest content of phenolic compounds. Two opposing processes therefore occurred. Beneficial biofortification with lower Fe doses generated a moderate increase in phenolic compounds and therefore did not negatively impact the plants' growth or their photosynthetic efficiency. However, high Fe doses (above 500 µg/g Fe) induced plant stress and triggered defense mechanisms, which finally enhanced phenolic production. This was accompanied by a decrease in biomass and photosynthetic efficiency. We therefore reason that the limitation of photosynthesis at higher iron doses is due to the limited water availability as indicated by the C_i values. Under normal plant growth conditions, there is a balance between the production and detoxification of reactive oxygen species due to the metabolic processes in plants. However, under stress conditions, the free radical balance is disturbed, leading to the accumulation of reactive oxygen species. This results in the peroxidation of membrane lipids, which can initiate cell membrane destruction [53]. Plants may respond to this stress by increasing the activity of their antioxidant system. Antioxidant enzymes capture free radicals and prevent their formation [54]. Phenolic compounds are secondary metabolites with distinct free radical scavenging ability which increase plant tolerance to environmental stress. Plants adopt a plethora of diverse strategies to mitigate heavy metal toxicity. The first line of defense lies in the rhizosphere where root exudates interfere with metal ions and affect their uptake by plant tissues [55,56]. In particular, metal chelation hampers their entry into the symplast [57]. Root exudation of phenolic compounds is linked with metal concentration in the rhizosphere and differs substantially among diverse plant species [58,59]. Phenolic compounds play an essential role in the cultivation of lemon balm in iron-polluted soils. Our study shows that the total levels of phenolic compounds were systematically increased in all lemon balm cultivations supplemented with Fe^{3+} . Unfortunately, biomass production consequently decreased. We would like to emphasize that herbal products with increased phenol content typically have a higher market value than the original herbs. Therefore, selecting an appropriate Fe^{3+} dose to increase phenolic content without compromising plant biomass is crucial. However, this requires further in-depth research and large-scale-cultivation field studies.

4. Materials and Methods

4.1. Soil Samples

Soil samples were collected from a farm located in Lagiewniki (51°51' N, 19°28' E; unfertilized and pesticide-free for two years from the beginning of the experiment; located away from traffic) according to PN-ISO 10381-4: 2007 [60] in August 2024. The soils were dried in an air-circulating room and passed through a 2 mm stainless steel sieve. Soil pH was measured in 1 mol/L KCl solution according to PN-ISO 10390:1997 by the potentiometric method [61]. Organic matter content in soil was determined by the gravimetric method [62,63]. The bioavailable forms of metals in soil were extracted by 0.5 mol/L HCl solution. A mixture of concentrated HNO_3 (6 mL) and HCl (2 mL) was applied

to soil (0.5 g) decomposed by minerals with the Anton Paar Multiwave 3000 closed system instrument (Graz, Austria). Metal contents were measured in soil samples by HR-CS FAAS with contraAA 300 spectrometer (Analytic Jena, Germany). Five replications were conducted for each sample.

4.2. Cultivation of Lemon Balm Material and Experimental Protocol

All herbs were cultivated under laboratory conditions by the pot method from March to July 2024. Lemon balm seeds (P.H. Legutko, Rawicz, Poland) were placed in 12 cm diameter plastic containers (0.3 L) filled with 500 g of soil. After germination, each container contained five plants. After one month of growth, lemon balm plants were treated with 5, 10, 25, 35, or 45 mL of 10 mg/mL $\text{Fe}(\text{NO}_3)_3$ solution, giving iron concentrations in the pots of 100, 200, 500, 700, and 900 $\mu\text{g/g}$, respectively. We used five parallel samples (i.e., five pots) in each cultivation series. The mean value was calculated from five independent pots. The cultivation was conducted in a greenhouse under controlled conditions: the temperature was 23 ± 2 °C and 16 ± 2 °C for day and night, respectively; the relative humidity was limited to 65–70%; the photosynthetic active radiation (PAR) during the 16 h photoperiod was restricted to 400 $\mu\text{mol m}^{-2}\text{s}^{-1}$. All plants were regularly watered with deionized water. The herbs were grown for four months. Then, the plants were cut and the above-ground parts and roots were separated, washed, dried at 45 °C to a constant weight and homogenized.

4.3. Morphological and Physiological Parameters of Plants

Measurements of photosynthesis parameters were made four months after sowing the seeds, just before cutting the plants. The index of chlorophyll was measured using the Konica Minolta SPAD-502Plus (Tokyo, Japan) chlorophyll meter. Net photosynthesis (P_N), stomatal conductance (G_s), intercellular concentration of CO_2 (C_i), and transpiration (E) were determined with the gas analyzer CIRAS-3 (Portable Photosynthesis System, Amesbury, MA, USA) (Table 5). All measurements were made five times on separate plants without damaging the plant material.

Table 5. Conditions for measuring photosynthesis parameters.

Parameter		Value
Chamber CO_2 concentration	CO_{2r}	254.7 $\mu\text{mol}\cdot\text{mol}^{-1}$
Light intensity	PAR_i	1737 $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$
Leaf temperature	T_{leaf}	28.9 °C
Vapor pressure deficit	VPD	2.2 kPa

4.4. Determination of Heavy Metals in Herbs

Approximately 0.5 g of dried roots and 0.5 g aerial parts of the plants were weighed, 6 mL of concentrated HNO_3 and 1 mL of concentrated HCl were added, and the whole was subjected to microwave mineralization in a closed system of the Anton Paar Multiwave 3000 apparatus (Graz, Austria). Metal contents were determined by HR-CS FAAS with a contraAA 300 spectrometer (Analytic Jena, Germany). Quality assurance and quality control (QA/QC) for metals in plant samples were estimated by determining metal contents in the certified reference material INCT-MPH-2, containing a mixture of selected Polish herbs [64] (Table S1).

4.5. Determination of Total Phenolic Content in Lemon Balm

The total phenolic content was determined by the Folin–Ciocalteu spectrophotometric method, with gallic acid applied as the standard [65]. Dried and

ground herbs (1 g) were extracted with 40 mL of methanol (70%). The mixture was then centrifuged at 3000 rpm for 10 min and poured into plastic bottles. An amount of 0.1 mL of that extract, 6 mL of distilled water, and 0.5 mL of the Folin–Ciocalteu reagent were placed in a 10 mL volumetric flask and thoroughly mixed. After 3 min, 1.5 mL of saturated sodium carbonate solution was added and made up to the mark with distilled water. The solution was subsequently placed in a thermostat at 40 °C for 30 min. The absorbance was measured at a wavelength of 765 nm using a Specol 11 spectrophotometer (Carl Zeiss, Jena, Germany).

4.6. Statistical Analysis

Each determination was performed five times for independent samples. The normality of the data distribution was assessed using the Shapiro–Wilk test [66,67]. One-way analysis of variance (ANOVA) was used to identify significant differences in the phenolic content of iron-supplemented herbs. Tukey’s HSD post hoc test was used to assess statistically significant differences between the mean values within each variable.

Metal uptake by iron-treated herbs was assessed by one-way ANOVA at the 0.05 probability level (Table 2) and followed by post hoc Tukey’s test.

5. Conclusions

Low or moderate $\text{Fe}(\text{NO}_3)_3$ supplementation stimulated photosynthesis in lemon balm leaves through elevated chlorophyll synthesis. In particular, the increased photosynthesis efficiency prompted better use of water which, to a large extent, was directly used in photosynthesis. On the other hand, high Fe^{3+} levels hampered plant metabolism and increased phenolic compound production. The reduction in water uptake in this condition is due to the stress effects stimulated by iron at high concentrations. This limits the yield of cultivated herbs. In our studies, a decrease in the fresh mass of the above-ground parts of the plant was observed in all iron applications. We reason that the latter affects the plant growth strategy and prompts more energy to be invested into root system development. This hampers the growth of its green parts. Further studies are clearly needed for the determination of the $\text{Fe}(\text{NO}_3)_3$ levels which will elevate phenolic compound production in lemon balm while not substantially limiting plant metabolism.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms27146211/s1>.

Author Contributions: Conceptualization, D.A.-S. and W.M.W.; methodology, D.A.-S. and D.K.; software, D.A.-S. and D.K.; validation, D.A.-S. and D.K.; formal analysis, D.A.-S.; investigation, D.A.-S.; resources, D.A.-S. and W.M.W.; data curation D.A.-S. and W.M.W.; writing—original draft preparation, D.A.-S. and D.K.; writing—review and editing, D.A.-S. and W.M.W.; visualization, D.A.-S.; supervision, D.A.-S. and W.M.W.; project administration, D.A.-S. and W.M.W.; funding acquisition, D.A.-S. and W.M.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article and Supplementary Materials. Further inquiries can be directed to the corresponding author.

Acknowledgments: The European University Foundation is acknowledged for advising on the legal and social dimensions of this study, and Jakub Kubicki for advice and help during heavy metal determinations.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

- Seidler-Lozykowska, K.; Mordalski, R.; Kucharski, W.; Kedzia, E.; Nowosad, K.; Bocianowski, J. Effect of organic cultivation on yield and quality of lemon balm herb (*Melissa officinalis* L.). *Acta Sci. Pol. Hortorum Cultus* **2015**, *14*, 55–67.
- Awlqadr, F.H.; Altemimi, A.B.; Qadir, S.A.; Mohammed, O.A.; Saeed, M.N.; Hesarinejad, M.A.; Lakhssassi, N. Bioactive Compounds, Medicinal Benefits, and Contemporary Extraction Methods for Lemon Balm (*Melissa officinalis*). *Food Sci. Nutr.* **2025**, *13*, e70864.
- Connorton, J.M.; Balk, J.; Rodriguez-Celma, J. Iron homeostasis in plants—A brief overview. *Metallomics* **2017**, *9*, 813–823.
- Kim, S.A.; Guerinot, M.L. Mining iron: Iron uptake and transport in plants. *FEBS Lett.* **2007**, *581*, 2273–2280.
- Fragaa, C.G.; Oteiza, P.I.; Hida, E.J.; Galleano, M. Polyphenols and the regulation of NADPH oxidases. *Red. Biol.* **2023**, *67*, 102927.
- Yahfoufi, N.; Alsadi, N.; Jambi, M.; Matar, C. The immunomodulatory and anti-inflammatory role of polyphenols nutrients. *Nutrients* **2018**, *10*, 1618.
- Mori, S. Iron acquisition by plants. *Curr. Opin. Plant Biol.* **1999**, *2*, 250–253.
- Kabata-Pendias, A.; Pendias, H. *Trace Elements in Soils and Plants*, 3rd ed.; CRC Press: Boca Raton, FL, USA, 2001.
- Velez-Bermudez, I.C.; Schmidt, W. Iron sensing in plants. *Front. Plant Sci.* **2023**, *14*, 1145510.
- Korzeniowska, J.; Stanisławska-Glubiak, E.; Jadczyzyn, T.; Lipiński, W. *Fertilizing Agricultural Crops with Microelements*; National Centre for Research and Development, IUNG-PIB: Pulawy, Poland, 2021.
- Schwertmann, U. Solubility and dissolution of iron oxides. *Plant Soil* **1991**, *130*, 1–25.
- Lindsay, W.L. Iron oxide solubilization by organic matter and its effect in iron availability. *Plant Soil* **1991**, *130*, 27–34.
- Krishna, T.P.A.; Ceasar, S.A.; Maharajan, T. Biofortification of crops to fight anemia: Role of vacuolar iron trans-porters. *J. Agric. Food Chem.* **2023**, *71*, 3583–3598.
- Takanori, K.; Tomoko, N.; Naoko, K.N. Iron transport and its regulation in plants. *Free Radical Biol. Med.* **2018**, *133*, 11–20.
- Rout, G.R.; Sahoo, S. Role of iron in plant growth and metabolism. *Rev. Agric. Sci.* **2015**, *3*, 1–24.
- Skiba, E.; Adamczyk-Szabela, D.; Wolf, W.M. Metal-based nanoparticles' interactions with plants. In *Plant Responses to Nanomaterials Recent Interventions and Physiological and Biochemical Responses*; Singh, V.P., Singh, S., Tripathi, D.K., Prasad, S.M., Chauhan, D.K., Eds.; Springer: Berlin/Heidelberg, Germany, 2021; pp. 145–169.
- Ning, X.; Lin, M.; Huang, G.; Mao, J.; Gao, Z.; Wang, X. Research progress on iron absorption, transport, and molecular regulation strategy in plants. *Front. Plant Sci.* **2023**, *14*, 1190768.
- Johnson, D.B.; Kanao, T.; Hedrich, S. Redox transformations of iron at extremely low pH: Fundamental and applied aspects. *Front. Microbiol.* **2012**, *3*, 96.
- Miller, C.N.; Busch, W. Using natural variation to understand plant responses to iron availability. *J. Exp. Bot.* **2021**, *72*, 2154–2164.
- Ishimaru, Y.; Suzuki, M.; Tsukamoto, T.; Suzuki, K.; Nakazono, M.; Kobayashi, T.; Wada, Y.; Watanabe, S.; Matsushashi, S.; Takahashi, M.; et al. Rice plants take up iron as an Fe³⁺ phytosiderophore and as Fe²⁺. *Plant J.* **2006**, *45*, 335–346.
- Hamzah, E.A.R.; Abdullah, N.A.; Hussein, H.S. Iron toxicity in plants: A Review. *EAS J. Pharm. Pharmacol.* **2024**, *6*, 4.
- Aung, M.S.; Masuda, H. How does rice defend against excess iron?: Physiological and molecular mechanisms. *Front. Plant Sci.* **2020**, *11*, 1102. Correction in *Front. Plant Sci.* **2020**, *11*, 601527. doi: 10.3389/fpls.2020.601527.
- Xiong, Y.; Contento, A.L.; Nguyen, P.Q.; Bassham, D.C. Degradation of oxidized proteins by autophagy during oxidative stress in Arabidopsis. *Plant Physiol.* **2007**, *143*, 291–299.
- Dufey, I.; Hakizimana, P.; Draye, X.; Lutts, S.; Bertin, P. QTL mapping for biomass and physiological parameters linked to resistance mechanisms to ferrous iron toxicity in rice. *Euphytica* **2009**, *167*, 143–160.
- Pereira, E.G.; Oliva, M.A.; Rosado-Souza, L.; Mendes, G.C.; Colares, D.S.; Stopato, C.H.; Almeida, A.M. Iron excess affects rice photosynthesis through stomatal and non-stomatal limitations. *Plant Sci.* **2013**, *201*, 81–92.
- Durrett, T.P.; Gassmann, W.; Rogers, E.E. The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation1[OA]. *Plant Physiol.* **2007**, *144*, 197–205.

27. Dey, S.; Chowardhara, B.; Regon, P.; Kar, S.; Saha, B.; Panda, S.K. Iron deficiency in blackgram (*Vigna mungo* L.), Redox status and antioxidant activity. *Plant Biosyst.* **2020**, *156*, 1–16.
28. Riaz, N.; Guerinot, M.L. All together now: Regulation of the iron deficiency response. *J. Exp. Bot.* **2021**, *72*, 2045–2055.
29. Schmidt, W.; Thomine, S.; Buckhout, T.J. Editorial: Iron nutrition and interactions in plants. *Front. Plant Sci.* **2020**, *10*, 1670.
30. Jin, C.W.; You, G.Y.; He, Y.F.; Tang, C.; Wu, P.; Zheng, S.J. Iron deficiency-induced secretion of phenolics facilitates the reutilization of root apoplastic iron in red clover. *Plant Physiol.* **2007**, *44*, 278–285.
31. Jin, C.W.; You, G.Y.; Zheng, S.J. Iron deficiency-induced phenolics secretion plays multiple important roles in the plant iron acquisition underground. *Plant Signal. Behav.* **2008**, *3*, 60–61.
32. Li, Y.; Yan, X.; Xu, Y.; Pope, R.; Spector, T.D.; Falchi, M.; Steves, C.J.; Bell, J.T.; Small, K.S.; Menni, C.; et al. Higher adherence to (poly)phenol-rich diet is associated with lower CVD risk in the Twins UK cohort. *BMC Med.* **2025**, *23*, 645.
33. Williamson, G. The role of polyphenols in modern nutrition. *Nutr. Bull.* **2017**, *42*, 226–235.
34. Grosso, G.; Godos, J.; Currenti, W.; Micek, A.; Falzone, L.; Libra, M.; Giampieri, F.; Forbes-Hernández, T.Y.; Quiles, J.L.; Battino, M.; et al. The effect of dietary polyphenols on vascular health and hypertension: Current evidence and mechanisms of action. *Nutrients* **2022**, *14*, 545.
35. Carresi, C.; Gliozzi, M.; Musolino, V.; Scicchitano, M.; Scarano, F.; Bosco, F.; Nucera, S.; Maiuolo, J.; Macrì, R.; Ruga, S.; et al. The effect of natural antioxidants in the development of metabolic syndrome: Focus on bergamot polyphenolic fraction. *Nutrients* **2020**, *12*, 1504.
36. Vedova, D.L.; Gado, F.; Vieira, T.A.; Grandini, N.A.; Palácio, T.L.N.; Siqueira, J.S.; Carini, M.; Bombardelli, E.; Correa, C.R.; Aldini, G.; et al. Chemical, nutritional and biological evaluation of a sustainable and scalable complex of phytochemicals from bergamot by-products. *Molecules* **2023**, *28*, 2964.
37. Cimpeanu, I.-A.; Boru, C.; Dehelean, C.A.; Liga, S.; Cosoroabă, R.M.; Ardelean, S.; Popescu, R.; Vlad, D. *Melissa officinalis* L. (Lemon Balm): An integrative review of phytochemistry and evidence from preclinical research to clinical studies. *Plants* **2026**, *15*, 650.
38. Mathews, I.M.; Eastwood, J.; Lampion, D.J.; Cozannet, R.L.; Fanca-Berthon, P.; Williams, C.M. Clinical Efficacy and Tolerability of Lemon Balm (*Melissa officinalis* L.) in Psychological Well-Being: A Review. *Nutrients* **2024**, *16*, 3545.
39. Nowak, M.; Sasak, K.; Włodarczyk, A.; Grabska-Kobylecka, I.; Sarniak, A.; Nowak, D. A Modified Fenton's system Fe²⁺-EGTA-H₂O₂ reveals that redox activities of simple polyphenols are suppressed in binary mixtures. *Molecules* **2025**, *30*, 2269.
40. Yunusa, A.K.; Rohin, M.A.K.; Bakar, C.A.A. Free radical scavenging activity of polyphenols. *J. Chem. Pharm. Res.* **2015**, *7*, 1975–1980.
41. Fotyma, M.; Mercik, S. *Agricultural Chemistry*; PWRL: Warsaw, Poland, 2003.
42. Dobrzański, B.; Zawadzki, S. *Soil Science*; PWRL: Warsaw, Poland, 1995.
43. Council Directive 86/278/EEC of 12 June on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. *Off. J. L* **1986**, *181*, 6–12.
44. IUSS Working Group WRB. *World Reference Base for Soil Resources World Soil Resources Reports No. 103*; FAO: Rome, Italy, 2006.
45. Adamczyk-Szabela, D.; Romanowska-Duda, Z.; Lisowska, K.; Wolf, W.M. Heavy metal uptake by herbs. V. Metal accumulation and physiological effects induced by thiuram in *Ocimum basilicum* L. *Water Air Soil Pollut.* **2017**, *228*, 334.
46. Thomine, S.; Lanquar, V. Iron transport and signaling in plants. In *Transporters and Pumps in Plant Signaling*; Geisler, M., Venema, K., Eds.; Springer: New York, NY, USA, 2011.
47. Seregin, I.V.; Kozhevnikova, A.D. Plant responses to heavy metals: From deficiency to excess. *Int. J. Mol. Sci.* **2025**, *26*, 2064.
48. Hu, G.; Zhou, X.; Chao, M.; Hu, H. Roles of exogenous 5-aminolevulinic acid and dihydroporphyrin iron in chlorophyll precursor synthesis and chlorophyll-related enzyme activities in wheat under different light intensities. *Agronomy* **2024**, *14*, 2464.
49. Spiller, S.C.; Castelfranco, A.M.; Castelfranco, P.A. Effects of iron and oxygen on chlorophyll biosynthesis. *Plant Physiol.* **1982**, *69*, 107–111.
50. Xu, Y.L.; Guo, J.Y.; Zhang, Z.; Ma, R.R.; Ma, H.; Zhang, Y.; Yang, Y.L. Chloroplast antioxidant reactions associated with zinc-alleviating effects on iron toxicity in wheat seedlings. *Photosynthetica* **2024**, *62*, 381–392.
51. Kastanek, F.; Spacilova, M.; Krystynik, P.; Dlaskova, M.; Solcova, O. Fenton reaction—unique but still mysterious. *Processes* **2023**, *11*, 432.
52. Li, J.; Cao, X.; Jia, X.; Liu, L.; Cao, H.; Qin, W.; Li, M. Iron deficiency leads to chlorosis through impacting chlorophyll synthesis and nitrogen metabolism in *Areca catechu* L. *Front. Plant Sci.* **2021**, *12*, 710093.
53. Shabala, S.; Shabala, L.; Barcelo, J.; Poschenrieder, C. Membrane transporters mediating root signaling and adaptive responses to oxygen deprivation and soil flooding. *Plant Cell Environ.* **2014**, *37*, 2216–2233.
54. Wang, Y.; Kang, Y.; Zhong, M.; Zhang, L.; Chai, X.; Jiang, X.; Yang, X. Effects of iron deficiency stress on plant growth and quality in flowering Chinese cabbage and its adaptive response. *Agronomy* **2022**, *12*, 875.

55. Bali, A.S.; Sidhu, G.P.S.; Kumar, V. Root exudates ameliorate cadmium tolerance in plants: A review. *Environ. Chem. Lett.* **2020**, *18*, 1243–1275.
56. Montiel-Rozas, M.M.; Madej'on, E.; Madej'on, P. Effect of heavy metals and organic matter on root exudates (low molecular weight organic acids) of herbaceous species: An assessment in sand and soil conditions under different levels of contamination. *Environ. Pollut.* **2016**, *216*, 273–281.
57. Nigam, R.; Srivastava, S.; Prakash, S.; Srivastava, M.M. Cadmium mobilization and plant availability—the impact of organic acids commonly exuded from roots. *Plant Soil* **2001**, *230*, 107–113.
58. Ali, M.B.; Singh, N.; Shohael, A.M.; Hahn, E.J.; Paek, K.Y. Phenolics metabolism and lignin synthesis in root suspension cultures of *Panax ginseng* in response to copper stress. *Plant Sci.* **2006**, *171*, 147–154.
59. Anjitha, K.S.; Sameena, P.P.; Puthur, J.T. Functional aspects of plant secondary metabolites in metal stress tolerance and their importance in pharmacology. *Plant Stress* **2021**, *2*, 100038.
60. *PN-ISO 10381-4*; Soil Quality—Sampling—Part 4: Rules for Procedure During the Research Areas of Natural, Semi-Natural and Cultivated. The International Organization for Standardization (ISO): Geneva, Switzerland, 2007.
61. *PN-ISO 10390*; Agricultural Chemical Analysis of the Soil. Determination of pH. The International Organization for Standardization (ISO): Geneva, Switzerland, 1997.
62. *ASTMD2974-00*; Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils. American Society for Testing and Materials: West Conshohocken, PA, USA, 2000.
63. Schumacher, B.A. *Methods for the Determination of Total Organic Carbon (TOC) in Soils and Sediments*; United States Environmental Protection Agency, Environmental Sciences Division, National Exposure Research Laboratory: Las Vegas, NV, USA, 2002.
64. Dybczyński, R.; Danko, B.; Kulisa, K.; Maleszewska, E.; Motrenko, H.P.; Samczynski, Z.; Szopa, Z. Preparation and preliminary certification of two new Polish CRMs for inorganic trace analysis. *J. Radioanal. Nucl. Chem.* **2004**, *259*, 409–413.
65. Ndhlala, A.R.; Kasiyamhuru, A.; Mupure, C.; Chitindingu, K.; Benhura, M.A.; Muchuweti, M. Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*. *Food Chem.* **2007**, *103*, 82–87.
66. Goodson, D.Z. *Mathematical Methods for Physical and Analytical Chemistry*; Wiley: Hoboken, NJ, USA, 2011.
67. Razali, N.M.; Wah, Y.B. Power comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests. *J. Stat. Model. Anal.* **2011**, *2*, 21–33.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.