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The Impact of Abiotic Environmental Stressors on Fluorescence and Chlorophyll Content in *Glycine max* (L.) Merrill

Judita Tomaškinová [1,*], Ján Tomaškin [2], Marek Drimal [3] and Jesus Bellido [4]

[1] Applied Research and Innovation Centre, MCAST Resource Centre, Malta College of Arts, Science and Technology, Main Campus, Triq Kordin, PLA 9032 Paola, Malta
[2] Department of Biology and Environmental Studies, Faculty of Natural Sciences, University of Matej Bel in Banska Bystrica, Tajovskeho 40, 974 04 Banska Bystrica, Slovakia; jan.tomaskin@umb.sk
[3] Department of Engineering and Technology, Faculty of Natural Sciences, University of Matej Bel in Banska Bystrica, Tajovskeho 40, 974 04 Banska Bystrica, Slovakia; marek.drimal@umb.sk
[4] IUCN Spanish National Committee, Av. Costa Rica 150, 09001 Burgos, Spain; comite@uicn.es
* Correspondence: judita.tomaskinova@mcast.edu.mt; Tel.: +421-915952783

Abstract: In this study, we present the results of the impact of abiotic environmental (chemical) stressors in the soil environment (salinity, acidification, inorganic elements from industry—red mud waste containing Al) on the content and fluorescence of chlorophyll in the assimilating tissues of *Glycine max* (L.) Merrill, cv. ES Mentor. Under controlled conditions of a pot experiment during the 2023 growing season, we applied graded doses of these stressors (salinity—doses of 20, 30, and 60 g NaCl per 2 dm^3 of water used for plant irrigation; acidity—pH 6, pH 5, and pH 4; red mud—200 g, 400 g, and 600 g per pot) and assessed their impact on the effective and maximum quantum yield of photosystem II (PSII), yield Y(II), or the ratio of variable to maximum fluorescence—the Fv/Fm test. These tests are used to detect plant stress. The Y(II) test yielded values in the range of 0.627–0.800. Significant differences (variance analysis, 95% Least Significant Difference—LSD, post hoc test of analysis of variance—ANOVA) in reducing PSII chlorophyll fluorescence (Y(II)) were found between the medium and high doses of all three stressors compared to the control, indicating plant stress response. The Fv/Fm test yielded values between 0.668 and 0.805 and similarly detected stress responses in plants to all medium and high doses of stressors. The evaluated cultivar showed tolerance to moderately increased salt (NaCl) content and red mud levels. This was also confirmed by the chlorophyll content expressed as the Chlorophyll Content Index (CCI). The highest (significantly confirmed) chlorophyll content was found in the control variant and the variants with lower salt content and a soil pH of 6, with values of 35.633–37.467 CCI, compared to variants with higher red mud content (15.533–18.133 CCI) and higher soil acidity with pH 4 (22.833 CCI). Based on the results obtained, we conclude that the ES Mentor cultivar is tolerant to lower doses of the assessed stressors and can be cultivated in agricultural practice. However, medium to high doses of stressors trigger a strong stress response in plants and, therefore, we do not recommend cultivating this variety in contaminated environments.

Keywords: effective quantum yield PSII; environmental stress; Fv/Fm test; *Glycine max*; chemical stressor; chlorophyll fluorescence; red mud; soil acidification; soil salinization; soybean; Y(II) yield

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Received: 10 December 2024
Revised: 16 January 2025
Accepted: 18 January 2025
Published: 22 January 2025

Citation: Tomaškinová, J.; Tomaškin, J.; Drimal, M.; Bellido, J. The Impact of Abiotic Environmental Stressors on Fluorescence and Chlorophyll Content in *Glycine max* (L.) Merrill. *Agronomy* **2025**, *15*, 263. https://doi.org/10.3390/agronomy15020263

1. Introduction

Soil degradation and contamination are significant environmental challenges, with salinization, acidification, and industrial pollution acting as major abiotic stressors that disrupt plant physiology and reduce agricultural productivity [1,2]. These stressors affect water uptake, induce ion toxicity, and cause oxidative damage, leading to impaired photosynthesis, reduced growth, and yield loss [3,4]. Salinity alone affects more than 7% of global soils, particularly in arid and semi-arid regions, and is projected to increase [5,6]. Soybean (*Glycine max*), a strategic crop globally valued for its high-quality protein and oil content [7–10], is moderately tolerant to salinity, with yield reductions occurring at salinity thresholds of 2–5 $dS{\cdot}m^{-1}$ [11–13]. In this paper, we assess the impact of chemical soil degradation, specifically salinization, acidification, and industrial contamination by inorganic elements (red mud waste containing aluminum), on the assimilatory tissues of plants. The effects of these factors were evaluated under controlled conditions in a pot experiment, where *Glycine max* (L.) Merrill, cv. ES Mentor was chosen as the model plant species. The ES Mentor variety is unequivocally the most widely cultivated and most versatile medium-early cultivar in Central Europe [14]. This consideration led us to select this particular variety for our experiment. Consequently, the results obtained can serve as a basis for recommendations that extend to a much broader range of agricultural practices than would be possible with a less commonly grown variety.

Biotic or abiotic factors that negatively affect the growth and development of plants in their natural environment are considered stress factors. Salinization, acidification, and industrial pollution represent abiotic chemical stressors that often have a detrimental impact on vegetation health, which can ultimately lead to the death of individual plants or even entire populations [3]. Salinity is a major abiotic stress factor that reduces plant productivity in both irrigated and non-irrigated agricultural lands, especially in semi-arid and arid regions around the world [15,16]. More than 7% of the world's soil is affected by sodicity or salinity [5]. Liu et al. [6] report that the area of saline soil worldwide is as large as 954 million hectares, spanning all continents. In recent years, soil salinization has become a growing global trend [17].

Soil salinity is a major factor disrupting plants' water management. Plants expend more energy and absorb less water during periods when salt concentration remains high in the root zone. This results in yield loss and diminished quality in terms of crop production. To mitigate these losses in saline soils, resistant plant species and cultivars are needed [18]. It is well known that some plants tolerate mild saline conditions, while many are adversely affected, even by low levels of salt. Salinity negatively impacts plant growth and productivity at all stages of development [19]. Oral et al. [20] recommend that in cases where soil salinity cannot be resolved in the short term, the most fundamental approach is to identify salt-tolerant species (which was also our objective in testing the ES Mentor cultivar). Soybean species demonstrate some salt tolerance, though high salt concentrations can adversely affect their entire life cycle, with tolerance levels varying across developmental stages. Soil salinity has been shown to significantly disrupt soybean growth and yield, primarily due to increased Na^+ content in shoots, which induces ion toxicity [13,21–23]. Salt stress negatively impacts plant growth and productivity by inducing osmotic and ionic stress, primarily due to excessive Na^+ and Cl^- accumulation in chloroplasts, which disrupts electron transport and photosystem II (PSII) activity. Elevated salinity, often caused by NaCl, limits water potential, induces oxidative stress, and hinders essential cellular functions. Consequences include reduced shoot length, biomass production, chlorophyll, and carotenoid content due to cell membrane damage and enzymatic disruption. Plants in saline soils experience slowed leaf expansion, stomatal closure, and decreased photosynthesis and respiration rates. Collectively, these effects alter plant physiology, morphology, and biochemistry, resulting in diminished growth and yield [4,16,24–40].

Another factor evaluated for its effect on fluorescence and chlorophyll content in soybeans was soil pH. The optimal soil pH for soybean cultivation should be within the range of 6 to 8. Acidic soils (pH < 5.5) are unsuitable because they do not support the formation of a symbiotic community necessary for successful root nodulation. The same applies to compacted and saline soils, i.e., soils with a pH higher than 8 [40]. Generally, soybeans prefer slightly acidic soils, with a pH range of 6 to 7 [41,42].

Metals, including aluminum, are significant abiotic stressors in plants, particularly in contaminated or acidic soils. Aluminum, which constitutes approximately 7–8.3% of the Earth's crust, typically exists in non-toxic forms but becomes toxic as Al^{3+} ($[Al(H_2O)_6]^{3+}$) under acidic conditions, affecting nearly 40% of the world's arable land. Toxic Al^{3+} reduces plant vigor, yield, and photochemical efficiency, inhibiting photosynthesis and pigment synthesis. However, low concentrations of aluminum may have neutral or even positive effects on plant growth, suggesting potential for managing aluminum-contaminated soils. [43–45].

Critical tolerance thresholds for Al^{3+} toxicity vary by crop and genotype, with values such as 5.2 cmol/kg for soybeans and 4.0 cmol/kg for wheat. Aluminum's impact also shifts across growth stages and genotypes. Studies on soybean varieties reveal significant differences in aluminum tolerance, with some varieties exhibiting resilience, while others show pronounced sensitivity [46,47]. This highlights the importance of varietal selection in mitigating aluminum-induced stress and advancing agricultural practices.

In the past, most studies investigating the effects of stress on plants have utilized biochemical indicators, biomass information, and photosynthetic parameters, while only a few have employed chlorophyll fluorescence kinetics. Chlorophyll fluorescence kinetics refers to the technical means of analyzing light energy distribution in plant leaves through chlorophyll fluorescence, allowing for rapid, non-destructive, and precise detection and analysis of photosynthetic organs, as well as the overall health of the plant itself [48,49]. Our objective was also to assess the alterations in chlorophyll fluorescence and content in response to abiotic stressors. One of the earliest and most critical indicators of stress in plants is damage to the assimilation system, particularly Photosystem II (PSII). By measuring chlorophyll fluorescence, we can gauge the efficiency of PSII in absorbing and utilizing photosynthetically active radiation. The chlorophyll content and fluorescence parameters serve as reliable indicators for assessing the impact of abiotic chemical stressors on plants [50]. Several studies conducted in Slovakia further corroborate the thesis that environmental abiotic factors contribute to variations in chlorophyll content in plant leaves [51–53].

The aim of this study was to evaluate the effects of selected abiotic chemical stressors on chlorophyll fluorescence parameters and overall vitality in *Glycine max* (L.) Merrill, cv. ES Mentor.

2. Materials and Methods

During the 2023 growing season, we conducted a pot experiment at the Department of Biology and Environmental Studies, Faculty of Natural Sciences, Matej Bel University, Banská Bystrica, Slovakia. This study aimed to assess the effects of simulated salinity, acidification, and industrial contamination by inorganic elements from red mud waste, produced during the processing of bauxite and aluminum oxide (or aluminum) production at Žiar nad Hronom (Central Slovakia), on the chlorophyll content and fluorescence in *Glycine max* (L.) Merrill, cv. ES Mentor. Each stressor was prepared in three graded doses. Salinity in the soil was simulated by preparing saline solutions with 20, 30, and 60 g of NaCl dissolved in two liters of water. Acidification was simulated by preparing acidic solutions (based on nitric acid) with pH levels of 6, 5, and 4, also in two liters of water. The red mud waste was obtained from the vicinity of the Slovalco plant in Žiar nad Hronom (48°33′ N, 18°50′ E), located in the western part of Central Slovakia. This plant processes bauxite to produce aluminum, and red mud, a byproduct of production, is stored in the area and spread by wind into the surrounding agricultural and residential regions. The red mud was applied in doses of 200, 400, and 600 g, mixed with the corresponding amount of horticultural substrate (producer: AGRO CS a.s., Říkov, Czech Republic). The manufacturer provides the following chemical and physical specifications for the substrate: pH range 6.5–7.0, total nitrogen (N) content up to 1.9%, total phosphorus (P) as P_2O_5 up to 0.5%, total potassium (K) as K_2O up to 0.9%, particle size above 20 mm not exceeding 5%, moisture content up to 65%, combustible substance content at a minimum of 50%, and electrical conductivity not exceeding 1.2 $mS \cdot cm^{-1}$. The red mud used in the experiment contains the following components: Fe_2O_3 (45%), Al_2O_3 (15%), SiO_2 (13%), Na_2O (7.5%), TiO_2 (6%), and CaO (2%) [54]. The control treatment consisted solely of 4.0 dm^3 of the gardening substrate. The experimental treatments, including the control, are detailed in Table 1.

Table 1. Experimental treatments used in this study.

Treatment	Abiotic Chemical Stressor	Dose of Chemical Stressor	Volume of Gardening Substrate (dm^3)
A1	Salinity	20 g NaCl/2 dm^3 H_2O = 10‰	4.0
A2		30 g NaCl/2 dm^3 H_2O = 15‰	4.0
A3		60 g NaCl/2 dm^3 H_2O = 30‰	4.0
B1	Acidity	pH 6	4.0
B2		pH 5	4.0
B3		pH 4	4.0
C1	Red mud	200 g	3.8
C2		400 g	3.6
C3		600 g	3.4
Control	-	-	4.0

The effects of the specified abiotic environmental stressors on chlorophyll content and fluorescence were assessed in photosynthetically mature leaves of *Glycine max* (L.) Merrill, cv. ES Mentor. The plant material was pre-grown from seeds obtained from SAATBAU Slovensko, s.r.o., Trnava Slovakia (a producer and distributor of certified field crop seeds). The seeds were sown in horticultural substrate on 18 April 2023, at a soil depth of 40 mm. On 26 June 2023, the mature soybean plants were transplanted (two plants per pot) into large-volume pots with the appropriate soil substrate volumes (Table 1). For variants A1–A3 and B1–B3, from 3 July to 31 July, prepared stressor solutions were applied weekly in the amount of 0.5 dm^3 per variant. Plants in variants C1–C3 were planted in soil contaminated with red mud, while control plants were planted in 4.0 dm^3 of clean horticultural substrate. During the growing season, three measurements of chlorophyll content and fluorescence were taken on 19 July, 26 July, and 2 August 2023.

In recent years, chlorophyll fluorescence analysis has emerged as a quick, reliable, and cost-effective alternative to traditional biochemical methods for evaluating the status of Photosystem II (PSII) and assessing photosynthetic electron transport [55–57]. Chlorophyll fluorescence, emitted by the chlorophyll molecule after excitation by light, serves as a non-destructive biomarker for determining stress effects on PSII [58]. In this study, chlorophyll fluorescence was measured using a yield Y(II) test and Fv/Fm test with the OS5p chlorophyll fluorometer (Opti-Sciences, Inc., Hudson, NH, USA). These tests are widely accepted for detecting and quantifying plant stress levels. The effective quantum yield of PSII (Y(II)) was measured during daylight hours, while the maximum quantum yield of PSII (Fv/Fm) was measured after 30 min of leaf shading. For most plant species, optimal Fv/Fm values range from 0.79 to 0.84, with lower values indicating the presence of plant stress [50]. Linda et al. [59] also note that the Fv/Fm ratio decreases as the intensity of stress increases, with an Fv/Fm value below 0.7 generally indicating the onset of stress conditions. Values around 0.6 or lower suggest the presence of severe stressors, often accompanied by visible damage in many cases. Similarly, Malinská et al. [60] report that measuring leaf fluorescence serves as an effective tool for detecting plant stress in vivo. As a non-destructive evaluation method, chlorophyll fluorescence provides an accurate and rapid assessment of plant health and offers insights into photosynthetic electron transport under stress conditions [61].

The fluorescence results are supplemented by chlorophyll content measurements. Pavlović et al. [62] emphasize the significance of chlorophyll in detecting plant health. They note that chlorophyll, as a key plant pigment, plays a crucial role in photosynthesis for the synthesis of organic compounds and biomass production. Chlorophyll is highly sensitive to various stress factors, responding by reducing its content and altering fluorescence (a decrease in Fv/Fm). We measured the chlorophyll content in leaf values using the CCI (Chlorophyll Content Index) with a CCM-200 plus chlorophyll meter (Opti-Sciences, Inc., Hudson, NH, USA). In some cases, the relative chlorophyll content is also reported using SPAD values, as referenced in some studies mentioned in the discussion. The CCI and SPAD values are essentially identical, with the difference lying in the use of instrument technology from two renowned manufacturers, both utilizing the same measurement principle. For instance, the SPAD-502Plus chlorophyll meter by Konica Minolta measures chlorophyll content in SPAD units, while the CCM-200 Plus by Opti-Sciences, Inc., USA, provides values in CCI. Both devices measure chlorophyll content at nearly identical wavelengths, resulting in closely correlated data. Parry et al. [63] report determination coefficients of r^2 for SPAD-CCI resp. r^2 CCI-SPAD as 0.99. Higher CCI values indicate higher chlorophyll concentration. Fluorescence and chlorophyll content measurements were conducted in the morning hours (10:00–11:00 am) with 10 repetitions (5 measurements per plant) on photosynthetically mature leaves (the average results are presented in this paper).

Chlorophyll content and fluorescence data were analyzed using a two-way ANOVA with LSD at a significance level of α 0.05. The statistical analysis was performed using Statgraphics 19® centurion (Statgraphics Technologies, Inc., Virginia, MN, USA).

3. Results and Discussion

The measured quantum yield values of PSII in the assimilating apparatus of soybean leaves, for both the evaluated variants of abiotic environmental stressors and the control variant during the 2023 growing season, are presented in Figures 1 and 2. The chlorophyll content is shown in Figure 3.

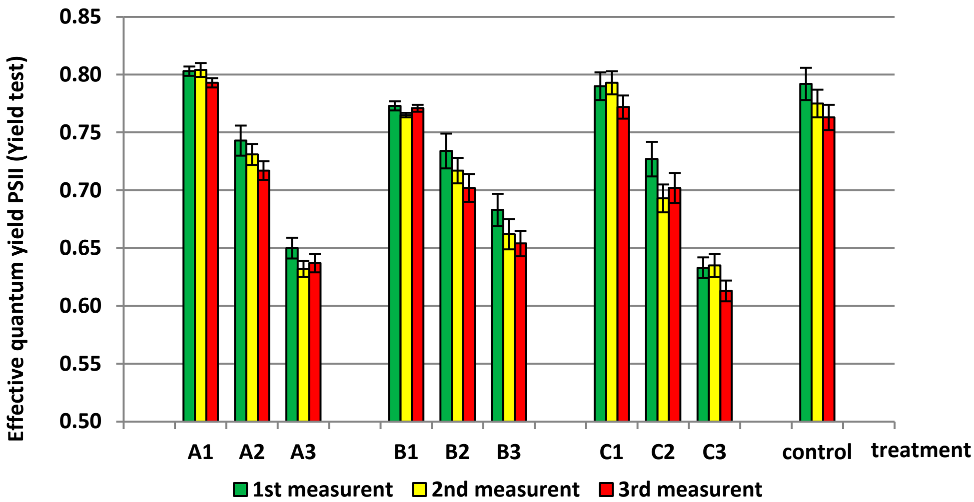

Figure 1. Effective quantum yield of the PSII Y(II) test in *Glycine max* (L.) Merrill, cv. ES Mentor.

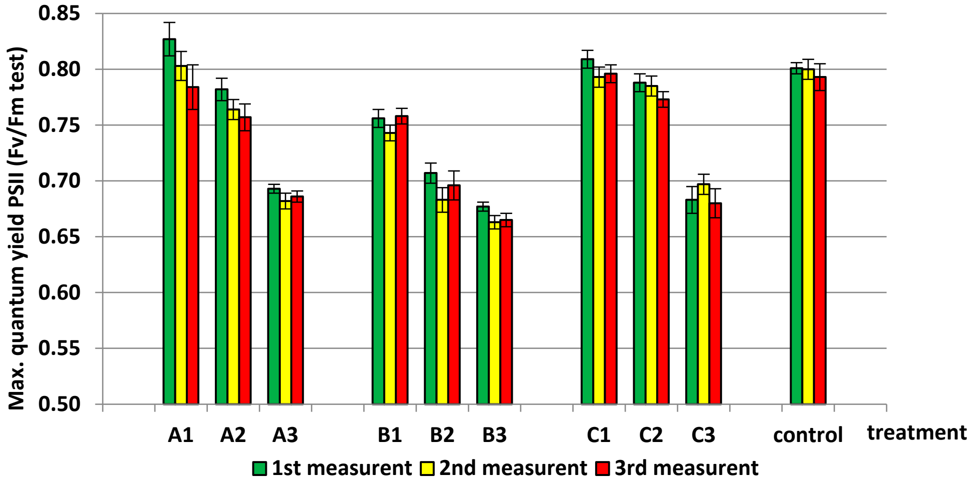

Figure 2. Maximum quantum yield of the PSII Fv/Fm test in *Glycine max* (L.) Merrill, cv. ES Mentor.

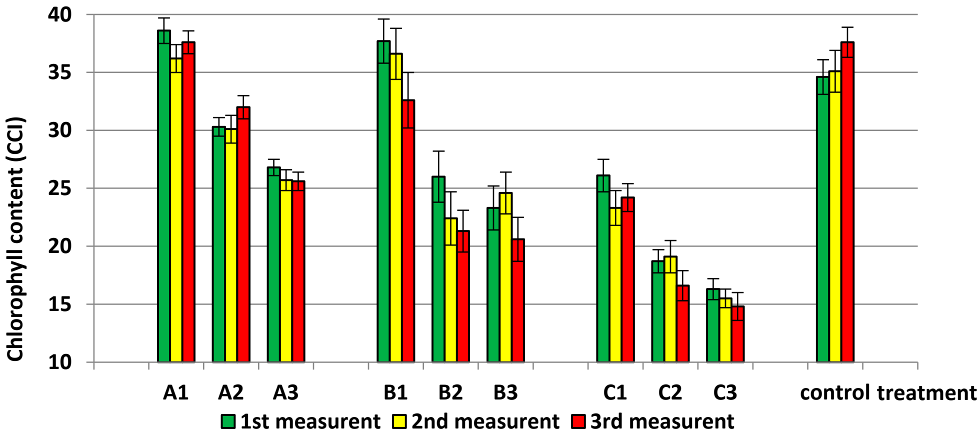

Figure 3. Chlorophyll content (CCI) in *Glycine max* (L.) Merrill, cv. ES Mentor.

The effects of abiotic stressors on soybean plants (incremental doses plus a control treatment) are illustrated in Figures 4–6 (these photographs were taken on 2 August 2023 during the third round of chlorophyll parameter measurements).

Figure 4. The effect of salinity (including the control treatment) on soybean plants.

Figure 5. The effect of acidity (including the control treatment) on soybean plants.

Figure 6. The effect of red mud (including the control treatment) on soybean plants.

The results of PSII quantum yields and chlorophyll content were statistically analyzed using a multifactor analysis of variance (ANOVA) at a 95% confidence level with the LSD test. The impact of three levels of chemical stressors (salinity, acidity, red mud) plus the control variant, as well as three measurement time points, were evaluated as factors (Table 2).

Table 2. The impact of environmental stressors on quantum yields of PSII and chlorophyll content in *Glycine max* (L.) Merrill, cv. ES Mentor.

Factor		**Quantum Yields PSII**		**Chlorophyll Content (CCI)**
		Y(II)	**Fv/Fm**	
Treatment	A1	0.800 ± 0.006 g	0.805 ± 0.022 f	37.467 ± 1.206 e
	A2	0.730 ± 0.013 d	0.768 ± 0.013 d	30.800 ± 1.044 d
	A3	0.640 ± 0.009 a	0.687 ± 0.006 b	26.033 ± 0.666 c
	B1	0.770 ± 0.004 e	0.752 ± 0.008 c	35.633 ± 2.684 e
	B2	0.718 ± 0.016 cd	0.695 ± 0.012 b	23.233 ± 2.458 b
	B3	0.666 ± 0.015 b	0.668 ± 0.008 a	22.833 ± 2.040 b
	C1	0.785 ± 0.011 f	0.797 ± 0.009 ef	24.533 ± 1.429 bc
	C2	0.707 ± 0.018 c	0.782 ± 0.008 de	18.133 ± 1.343 a
	C3	0.627 ± 0.012 a	0.687 ± 0.009 b	15.533 ± 0.751 a
	Control	0.777 ± 0.015 ef	0.798 ± 0.004 f	35.767 ± 1.607 e

Table 2. *Cont.*

Factor		Quantum Yields PSII		Chlorophyll Content (CCI)
		Y(II)	Fv/Fm	
	LSD $\alpha_{0.05}$	0.01326	0.01467	2.63611
Measurement	1	0.733 ± 0.060 c	0.752 ± 0.057 b	27.840 ± 7.525 b
	2	0.721 ± 0.064 b	0.741 ± 0.055 a	26.860 ± 7.369 ab
	3	0.712 ± 0.063 a	0.739 ± 0.051 a	26.290 ± 8.275 a
	LSD $\alpha_{0.05}$	0.00727	0.00803	1.44386

Statistical method: multifactor ANOVA—95.0% LSD test ($\alpha = 0.05$). The values in the same column with different letters (a–g) are significantly different at the $p < 0.05$ level; there are no significant differences between values with the same letters. Values are presented as means ± SD (standard deviation).

3.1. The Effect of Soil Salinity on Chlorophyll Fluorescence and Content in Soybean

The lowest effective quantum yield of PSII (Y(II)) was observed in the high salinity variants A3 and A2, where salt was applied to the soil. Both values were significantly lower than the control and the variant with the lowest salinity A1 (0.800). It can be concluded that soybean plants responded very sensitively to higher doses of salinity stress, whereas the lowest dose was tolerated without any negative reaction. A similar pattern was observed when evaluating chlorophyll fluorescence using the Fv/Fm test. The maximum quantum yield of PSII (Fv/Fm) in the assimilating apparatus of soybean leaves in variants A3 and A2 was significantly lower than in the control and variant A1. These findings confirm that higher salt doses led to reduced chlorophyll fluorescence and induced a stress response in the plants, while the lowest salt dose was tolerated. Chlorophyll content is another objective indicator of plant response to applied stressors. The lowest chlorophyll content was again recorded in the variant with the highest salt content, A3, followed by variant A2. Both variants had significantly lower chlorophyll content than the control and variant A1, which had the highest chlorophyll content, although this increase compared to the control was not significant.

Soil salinity is a major limitation of legume production in many areas of the world. The use of saline irrigation water and the application of fertilizers are the primary factors responsible for increasing soil salinity [64]. Salt stress is one of the major abiotic factors that reduce soybean productivity worldwide [65]. Saline soils currently account for up to 20% of agricultural land, with projections suggesting that this could rise to 50% by the end of the 21st century [66]. However, Oyebowale et al. [22] estimate that this increase may occur sooner, by as early as 2050. Cultivating salt-tolerant crops or varieties is one of the more cost-effective strategies for managing soil salinity, which is a significant factor impacting crop production and agricultural sustainability in arid regions. Sheteiwy et al. [67] highlight that soybean (*Glycine max* L.) is a vital leguminous crop, serving as a major source of protein, oil, and essential nutrients globally, including in China. However, its production has been significantly impacted by environmental stresses, particularly salinity stress [68]. In saline soil conditions, soybeans exhibit stress through symptoms such as leaf chlorosis, stunted growth, and reduced yields. Joshi et al. [32] note that one of the initial responses to salt stress is a decrease in the rate of leaf surface expansion, followed by stomatal closure and a reduction in photosynthesis. Additionally, a decline in chlorophyll content, chloroplast function, and overall plant growth is also observed.

Sheteiwy et al. [67] identified notable varietal differences in soybean responses to soil salinity. In their study, the cultivar Nannong 99-6 demonstrated salt tolerance, while Lee 68 was highly sensitive under 100 mM NaCl stress. These responses were quantified using the Fv/Fm test, a measure of PSII quantum yield, with Nannong 99-6 recording a higher value of 0.30 (indicative of salt tolerance) compared to 0.12 for Lee 68 (indicative of salt sensitivity). The ES Mentor cultivar we evaluated showed tolerance to lower doses of salt; however, it became sensitive to higher salt concentrations, as indicated by changes in both chlorophyll fluorescence and content. The A3 variant recorded an Fv/Fm value of 0.687, below 0.7, which signifies the onset of a stress response in the plants.

Luo et al. [38] investigated the impact of increasing NaCl levels in the soil on chlorophyll fluorescence parameters (Fv/Fm—maximal photochemical efficiency of PSII in the dark, and Y(II)—actual photochemical efficiency of PSII in the light) in two soybean cultivars. Initially, salt stress led to a temporary increase in Fv/Fm (maximal photochemical efficiency of PSII) and Y(II) (actual photochemical efficiency of PSII) during the first 5–10 days, with Fv/Fm rising from 0.8 to 0.85 and Y(II) from 0.40 to 0.45 at lower salt doses. However, at higher salt concentrations, both parameters declined significantly. Fv/Fm dropped to 0.76 under severe salinity, while Y(II) decreased to 0.35–0.30 within 5–15 days, highlighting a marked reduction in the photosynthetic capacity of soybean seedlings under prolonged salt stress. Our results are consistent with this assessment, confirming that high doses of salt reduce chlorophyll fluorescence parameters. Luo et al. [38] further note that these findings are aligned with reports on the effects of heavy metal stress [69] and drought stress [70] on soybean plants. Yan [12] also noted that short-term salt stress had no significant effect on soybean chlorophyll fluorescence, but prolonged or severe stress reduced PSII photochemical efficiency and increased energy dissipation. Phenotypically, salt stress led to reduced stem elongation, leaf expansion, and biomass accumulation. Similarly, Calzada et al. [29] studied the effects of NaCl stress (0, 50, and 100 mmol L^{-1}) on PSII maximum quantum efficiency (Fv/Fm) in soybean plants (M-Soy 8222). While optimal Fv/Fm values range from 0.75 to 0.85, they recorded a decrease from 0.8 at 0 mmol L^{-1} to 0.65 and 0.60 under elevated salinity, reflecting the stress response. These findings are consistent with our results, confirming the sensitivity of soybean photosynthetic efficiency to salt stress.

Alharbi et al. [71] also investigated the impact of salt stress on soybean plants. Their results show that salt stress reduced growth, biomass accumulation, photosynthesis, chlorophyll and carotenoid content, and PSII efficiency. Compared to the control variant, salt stress (100 mmol NaCl/L) significantly decreased the maximum PSII efficiency (Fv/Fm) from 0.775 to 0.530. Chlorophyll content also significantly decreased from 0.950 mg/g FW to 0.576 mg/g FW under salt stress. Calzada et al. [29] studied the effects of salt stress on chlorophyll content (Chlorophyll Content Index, CCI) in soybean plants (M-Soy 8222 cultivar). In the 0 mmol L^{-1} NaCl variant, they recorded a CCI of 21, while increased salinity levels (50 and 100 mmol L^{-1} NaCl) led to a significant reduction in chlorophyll content (CCI = 17 and 16, respectively). In our study, we observed a similar trend of reduced chlorophyll content under higher salinity levels. For reference and potential comparison with our data, we mention the study by Buczek et al. [72], which assessed the chlorophyll content in soybean plants (cv. Merlin) in a field experiment (in Boguchwała, Poland). These plants were not exposed to targeted abiotic stress, and the factors influencing the results were the cultivation years (2017–2019) and the tillage systems adopted. The aforementioned authors recorded chlorophyll content in the range of 38.4 to 53.8 SPAD values. The cultivar we evaluated in the control variant and the variant with the lowest salt dose (A1) reached the lower limit of this range, with values of 35.767 CCI and 37.467 CCI, respectively.

3.2. The Effect of Soil pH on Chlorophyll Fluorescence and Content in Soybean

Another stress factor evaluated for its impact on chlorophyll fluorescence and content in soybeans was soil pH. The lowest effective quantum yield of PSII (Y(II)) was recorded in variants B3 and B2, while significantly higher values were observed in variant B1 and the control. Soybean plants were highly sensitive to low soil pH (variant B3 = pH 4 and B2 = pH 5), whereas variant B1 (pH 6) proved to be suitable for soybean cultivation, with no negative effects on the photosystem. A similar plant response was observed in the evaluation of chlorophyll fluorescence using the Fv/Fm test. The maximum quantum yield of PSII (Fv/Fm) in the assimilating apparatus of soybean leaves in variants B3 and B2 was significantly lower than in variant B1 and the control. This confirms that higher soil acidity (pH 4 and 5) reduced chlorophyll fluorescence and induced a stress response, while plants tolerated soil with pH 6. Chlorophyll content is another objective indicator of plant response to the applied stressor. The lowest chlorophyll content was recorded in the variant with the highest soil acidity, B3, followed by variant B2. Both variants had significantly lower chlorophyll content than variant B1 and the control. Soybean plants of the cv. ES Mentor tolerated a soil pH of 6 (variant B1) very well, as their chlorophyll content was identical to that of plants in the control variant.

Unfavorable soil pH is one of the most critical factors negatively affecting various stages of nodulation and nitrogen fixation, including rhizobium survival, infection processes, and overall nodulation and N fixation efficiency [73]. Globally, soil acidity below a pH of 5.5 can significantly hinder soybean growth and nodulation across different regions. In acidic soils, multiple stress factors coexist, including a high concentration of H+ ions, aluminum toxicity, and reduced phosphorus availability. To mitigate the adverse effects of soil stress on soybean growth and yield, common approaches include the use of tolerant plant and bacterial species, as well as enhancing soil properties through biotechnological and biological interventions [74].

Evans et al. [75] observed that acid rain reduces soybean yields, with a 9% average decrease in seed yield across cultivars exposed to simulated rainfall at pH 4.1 compared to the control (pH 5.6). Similarly, Pham et al. [76] studied the effects of simulated acid rain on soybean growth and yield in northern Vietnam, reporting significant declines in soybean growth parameters under acidic conditions (pH 3.5 and 3.0). Both chlorophyll content and the leaf area index (LAI) decreased as acidity intensified. Their findings emphasize that agricultural crops, including soybeans, are more sensitive to acid rain than natural plant species. Optimal soybean growth occurs at a pH range of 6.0–7.0, as soybeans are particularly vulnerable to both acidic and alkaline soils [76]. Acid rain can also affect the structure of plant leaves as well as disrupt normal physiological and biochemical processes, ultimately impacting plant growth and development. For example, acid rain with a pH of 3.0 or lower can damage the integrity of chloroplasts and reduce chlorophyll content in plants [77].

Hu et al. [77] describe the effects of acid rain on chlorophyll content and chlorophyll fluorescence response in soybean plants. They found that both chlorophyll content and maximum photochemical efficiency (Fv/Fm) were significantly reduced compared to the control. At pH 7.0 (control), they recorded an Fv/Fm value of 0.81, while at pH levels of 4.5, 3.5, and 2.5, there was a significant decrease to 0.78, 0.77, and 0.78, respectively. Similarly, chlorophyll content also declined. At pH 7.0, the chlorophyll content was 0.91 $mg.g^{-1}$, but at pH levels of 4.5, 3.5, and 2.5, there was a gradual and significant decrease to 0.60, 0.55, and 0.18 $mg{\cdot}g^{-1}$. Shamsi et al. [78] investigated the effect of soil pH (4.0 and 6.5) on the chlorophyll content of two Chinese soybean cultivars, L1 and Z2. At pH 6.5, the chlorophyll content of L1 and Z2 was 33.7 and 35.6 SPAD units, respectively, but, at acidic pH 4.0, both cultivars showed a significant decrease in chlorophyll content to 31.8 resp. 31.4 SPAD units. It can be concluded that acidic soil conditions had a pronounced impact on reducing chlorophyll content. Other growth-related parameters (stem and root length, biomass production) were also reduced under acidic soil conditions. Pham et al. [76] observed an identical trend in the reduction in chlorophyll content under acidic rain conditions. At the onset of soybean flowering, the chlorophyll content for the control variant (pH 6.0) was determined to be a 22.46 SPAD value. As the pH gradually decreased (5.0, 4.0, 3.0), the chlorophyll content significantly dropped to 19.37, 16.61, and 13.35 SPAD values, respectively. A similar declining trend in chlorophyll content was also recorded at the pod formation stage (control variant—35.82, pH 5–30.15, pH 4–25.20, pH 3–21.01 SPAD value). These trends in the effects of acidic conditions on the photosynthetic apparatus of soybeans are consistent with the results observed in our research.

3.3. The Effect of Red Mud (Al) on Chlorophyll Fluorescence and Content in Soybean

We assessed the impact of red mud waste, rich in aluminum, on chlorophyll fluorescence and content in soybeans. The lowest effective quantum yield of PSII (Y(II)) was recorded in variants C3 and C2. Significantly higher values were observed in the control and variant C1. Soybean plants exhibited high sensitivity to the elevated levels of red mud, whereas variant C1 was suitable for soybean growth and did not trigger any negative response in the photosystem. A similar plant response was observed when evaluating chlorophyll fluorescence using the Fv/Fm test. The maximum quantum yield of PSII (Fv/Fm) in the assimilating apparatus of soybean leaves was significantly lower in variant C3 compared to both contaminated variants, C2 and C1, and the control. We observed that the variant most contaminated with red mud (C3) led to a reduction in chlorophyll fluorescence and induced a stress response in the soybean plants. The Fv/Fm value of this variant, 0.687, is below the threshold of 0.7 generally reported in the literature for the onset of stress conditions [59]. Soil with medium (C2) and the lowest (C1) levels of red mud contamination was relatively well tolerated by the plants. However, plants showed high sensitivity to higher doses of red mud in the soil, as reflected by a reduced chlorophyll content. The lowest chlorophyll content was recorded in variant C3, which had the highest level of red mud contamination, and a very low chlorophyll content was also observed in variant C2. Both variants had significantly lower chlorophyll content compared to variant C1 and the control. Soybean plants in all three variants contaminated with red mud had significantly lower chlorophyll content than the control. Among all the stressors evaluated, chlorophyll content in soybeans was the lowest in the variants contaminated with red mud.

The results of the influence of red mud on fluorescence and chlorophyll content in soybeans are reported only sporadically in the scientific literature. From the chemical composition, which we present in the methodology, it is clear that red mud is rich in aluminum content (15% Al_2O_3), and the influence of aluminum as a possible stressor is mentioned much more often. Studies on soybean growth have revealed that toxic metals/metalloids inhibit normal plant growth. Javor et al. [79] state that soy easily absorbs heavy metals from the air and soil; therefore, it is not suitable for food and fodder use in the vicinity of chemical plants and power plants. We also noted the mentioned risk in our container vegetation experiment through the stress reaction of plants to the application of waste red sludge containing aluminum. Both chlorophyll fluorescence tests, yield Y(II) and Fv/Fm, significantly detected the stress response of plants, especially to higher doses of waste red sludge (variant C3). This fact is also illustrated by the significantly lower chlorophyll content, which we recorded among all the evaluated stressors on variants C3 and C2.

Shamsi et al. [43,78] report that aluminum stress (variant with 150 µmol/L Al in a soil substrate with pH 4.0) over 20 days significantly reduced root length, shoot height, and plant dry weight compared to the control. The reduction in growth under metal stress can occur due to the inhibition of photosynthesis. Aluminum-induced stress led to a significant decrease in chlorophyll content and also reduced the rate of photosynthesis [43,78]. This reduction in chlorophyll content was observed in two evaluated genotypes, Liao and Zhechun, with Zhechun showing high sensitivity to aluminum. The control variant had a chlorophyll content of 31.4 SPAD units, whereas the variant exposed to aluminum stress exhibited a significantly lower chlorophyll content of 25.5 SPAD units. The effects of higher aluminum concentrations on chlorophyll content in soybeans were also studied by Davarpanah et al. [80] in two cultivars, Williams and Katoul. The soybean cultivars responded differently to higher concentrations of aluminum in the soil environment. In the Katoul cultivar, chlorophyll content remained relatively unchanged, whereas the Williams cultivar exhibited a stress response at higher aluminum concentrations. Chlorophyll content significantly decreased from 2.6 mg·g^{-1}. FW^{-1} (FW = fresh weight) (in the control variant) to 1.4 mg·g^{-1}·FW^{-1} (at a concentration of 700 μM $AlCl_3$). Similarly, chlorophyll b content significantly dropped from 1.1 mg·g^{-1}. FW^{-1} to 0.58 mg·g^{-1}. FW^{-1}. Based on the results of the authors, it can be concluded that the sensitivity of chlorophyll to aluminum may be determined by the genetic predisposition of individual soybean cultivars. Ying and Liu [81] investigated the impact of varying aluminum concentrations on the photosynthetic properties of three soybean cultivars (Glycine max): Zhechun No. 2, Zhechun No. 3, and 9703. The results indicated that increasing Al concentrations reduced leaf chlorophyll content by 5% to 35%, while stomatal resistance increased by 10% to 35% and stomatal conductance decreased by 10% to 40%. Photosynthetic and transpiration rates declined by 5% to 40% and 20% to 50%, respectively, and water use efficiency dropped by 15% to 50%. Al stress was found to inhibit photosynthesis in soybean leaves, with more pronounced effects during the vegetative growth stage compared to the reproductive stage. Additionally, this study highlighted genetic differences among the cultivars in their responses to Al toxicity. Shamsi et al. [43] present experimental findings on the chlorophyll a (Chl a) and chlorophyll b (Chl b) content in the leaves of two soybean genotypes (Huachun No. 18 and Zhechun No. 3), whose soil environment (excluding the control variant) was contaminated with four increasing doses of aluminum in the form of $Al_2\ (SO_4)_3$. 18 H_2O. The authors report that as Al concentration increased, Chl a and Chl b content in soybean plants progressively and significantly decreased. In the Zhechun No. 3 cultivar, Chl a content dropped by 18.6% to 32.2% compared to the control, with the decline being slightly more pronounced in Huachun No. 18. (For Huachun No. 18, the soybean leaves had a chlorophyll content of 2.5 mg·g^{-1} FW in the control variant, which progressively and significantly decreased to 1.7 mg·g^{-1} FW at the highest aluminum contamination level.) This study further notes that Chl b content followed a similar pattern of decline to Chl a under Al treatment. We conclude that our results are consistent with these findings.

The second evaluated factor influencing chlorophyll fluorescence and content in soybeans was the timing of the measurements across three different dates. The highest values for quantum yields of PSII (yield Y(II), Fv/Fm test) and chlorophyll content were recorded during the first measurement on 19 July 2023. Subsequently, these values declined during the second measurement on 26 July 2023, and the lowest, statistically significant values were observed during the third measurement on 2 August 2023. This chronological progression of changes in the evaluated parameters appears logical, as by the third measurement date, the plants had sufficient time to respond to the applied doses of chemical stressors and to the varying soil conditions of the cultivation environment.

In the previous section, we presented the evaluation of chlorophyll fluorescence and content in soybeans under the individual effects of salinity, acidity, and red mud. These results can also be interpreted collectively for all applied stressors. The effective quantum yield of PSII (Y(II)) was lowest in the following variants: C3, A3, B3, C2, B2, and A2. The control variant, along with variants B1, C1, and A1, had significantly higher values, demonstrating a certain adaptability and resilience of the evaluated soybean cultivar to the lowest doses of stressors and a soil pH of 6. Similarly, the maximum quantum yield of PSII (Fv/Fm) in the assimilating apparatus of soybean leaves was lowest in variants B3, C3, A3, B2, B1, A2, and C2. The control variant, along with variants C1 and A1, had significantly higher values, again indicating some adaptability and resilience of the soybean cultivar to the lowest doses of stressors and soil pH of 6. The interpretation of the results is similar when it comes to chlorophyll content. The lowest chlorophyll content was observed in variants with higher doses of stressors or a soil pH of 4 and 5 in the following order: C3, C2, B3, B2, C1, A3, and A2. Plants in variants with the lowest doses of stressors—B1, control, and A1—had significantly higher chlorophyll content.

The mechanisms of stress responses are specific to each plant taxon and the environmental stressor involved. Common defense mechanisms employed by plants against stress factors typically include [74,82] the synthesis of stress proteins, the generation and scavenging of reactive oxygen species, hormonal level adjustments, the synthesis of protective antioxidant enzymes (e.g., glucanase, chitinase, catalase, peroxidase, superoxide dismutase), the production of phytohormones, the synthesis of antimicrobial volatile compounds, and the activity of ACC (1-aminocyclopropane-1-carboxylate) deaminase. Additionally, plant growth-promoting bacteria enhance nutrient uptake, modulate hormone levels, and release bioactive substances involved in stress mitigation (e.g., gibberellins, indole-3-acetic acid). Other strategies include the uptake or synthesis of osmoregulatory compounds (e.g., salts, sugars, amino acids, polyols), the production of compounds that lower the freezing point of water (e.g., glycerol), and the synthesis of substances that neutralize toxic compounds (e.g., organic acids precipitating aluminum). A comprehensive and current overview of plant strategies to overcome abiotic and biotic stresses is provided by Du et al. [83]. To optimize metabolic efficiency and minimize the energetic costs associated with stress defense, plants prioritize first-line defense strategies in the apoplastic space. These include ascorbate, defensins, small peptides, and secondary metabolites, which act before cellular processes are significantly impacted. Additionally, plants deploy a variety of symplastic mechanisms to bolster stress defense, such as chemical antioxidants, antioxidative enzymes, secondary metabolites, defensins, peptides, and proteins. Both symplastic and apoplastic defense systems rely on specialized transporters to facilitate the exchange of compounds across membranes; however, many of these transporters remain unidentified, and the processes involved in regenerating defense compounds are not yet fully understood. Beyond cellular-level responses, plants employ defense and compensation strategies at the organ and whole-plant levels. These include stomatal regulation and hypersensitive and systemic responses aimed at limiting or preventing the spread of stress effects throughout the plant. At the ecosystem level, plants contribute to stress mitigation by releasing signaling molecules through root exudates and emitting volatile organic compounds into the rhizosphere or aboveground atmosphere. These compounds can influence plant interactions with their environment either directly or indirectly. Despite significant advances, the mechanisms governing the production, regulation, and perception of these compounds under stress conditions remain poorly understood.

4. Conclusions

We evaluated the effects of increasing doses of abiotic environmental stressors in the soil environment (salinization, acidification, inorganic elements from industrial waste—red mud containing aluminum) on chlorophyll content and fluorescence (quantum yields of PSII) in *Glycine max* (L.) Merrill, cv. ES Mentor.

Based on the results obtained, we conclude that the species evaluated showed the highest sensitivity to acidic soil conditions (pH 4), the highest doses of red mud waste (variant C3), and the highest level of soil salinization (variant A3). This is documented by the lowest recorded Fv/Fm values in the B3, C3, and A3 variants. These low values indicate a stress response in the plant's assimilatory tissues and are significantly lower than in variants C1, the control, and A1, where no stress response was observed. We can conclude that the evaluated cultivar is tolerant to the lowest levels of applied stressors, specifically salinity and red mud. The Fv/Fm test values are significantly higher than the threshold for the onset of a stress response and are similar to those of the control variant. The lowest chlorophyll content was recorded in variants with higher doses of red mud, C3, and C2, respectively, in the variants with acidic soil conditions, such as pH 4 and 5. Significantly higher chlorophyll content was found in plants from variants with the lowest doses of stressors, B1, control, and A1.

Based on the results obtained, we conclude that the ES Mentor cultivar is tolerant to lower doses of the evaluated stressors. The variety does not exhibit destruction of the photosynthetic apparatus, and it is likely that it can be cultivated even in moderately contaminated soils. However, medium to high doses of stressors trigger a strong stress response in the plants, and, therefore, we do not recommend cultivating this variety in contaminated environments.

Author Contributions: Conceptualization, J.T. (Judita Tomaškinová) and J.T. (Ján Tomaškin); methodology, J.T. (Judita Tomaškinová) and J.T. (Ján Tomaškin); software, J.T. (Ján Tomaškin); validation, J.T. (Judita Tomaškinová) and J.T. (Ján Tomaškin); formal analysis, J.T. (Judita Tomaškinová) and J.T. (Ján Tomaškin); investigation, J.T. (Ján Tomaškin); resources, J.T. (Judita Tomaškinová) and J.T. (Ján Tomaškin); data curation, J.T. (Ján Tomaškin); writing—original draft preparation, J.T. (Judita Tomaškinová), J.T. (Ján Tomaškin), M.D. and J.B.; writing—review and editing, J.T. (Judita Tomaškinová), J.T. (Ján Tomaškin), M.D. and J.B.; visualization, J.T. (Judita Tomaškinová) and J.T. (Ján Tomaškin); supervision, J.T. (Ján Tomaškin); project administration, J.T. (Judita Tomaškinová) and J.T. (Ján Tomaškin); funding acquisition, J.T. (Judita Tomaškinová) and J.T. (Ján Tomaškin). All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the scientific project VEGA 'Bioavailability of potentially toxic elements in abandoned Cu-deposits', grant number 1/0220/23, and *part-financed by the International Partnership Awards Scheme Plus (IPAS+) 2023 of the Malta Council for Science and Technology* (GA: IPAS-2023-030). *Article Processing Charges financed by the Research Networking Scheme (RNS) 2024 of Xjenza Malta* (GA: RNS-2024-014).

Data Availability Statement: Data are available from the corresponding author upon request.

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.

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