

Physiological performance of *Linum usitatissimum* (Flax) in coal tailing-amended soils

Philisiwe F. Mhlanga

55859127@mynwu.ac.za

North-West University <https://orcid.org/0000-0002-4886-492X>

Marco Le Roux

North-West University <https://orcid.org/0000-0002-9330-426X>

Jacques M. Berner

North-West University <https://orcid.org/0000-0003-3947-7415>

Research Article

Keywords: Coal tailings, phytoremediation, land rehabilitation, chlorophyll fluorescence, OJIP curve, photosystem II, germination, biomass production

Posted Date: June 18th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-6914653/v1>

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Additional Declarations: The authors declare no competing interests.

Abstract

Coal tailings (CT), a by-product of coal mining, pose environmental risks due to their acidity and heavy metal content. This study assessed the potential of using coal tailings as a soil amendment for growing *Linum usitatissimum* (flax), focusing on germination, biomass production, and photosynthetic efficiency. Two coal tailings from the Witbank coalfield in Mpumalanga, South Africa (Coal A and Coal B) were tested individually and in combination with soil, with and without organic fertilizer. Pure CT treatments significantly reduced germination and seedling survival possibly due to high concentrations of lead and arsenic. However, mixing CT with soil improved germination (up to 80%) and seedling establishment by reducing metal toxicity. Chlorophyll *a* fluorescence analyses revealed improved photosynthetic performance in soil-amended and fertilized treatments, particularly where Coal A was mixed with soil. Fertilization enhanced biomass accumulation and photosystem II efficiency, with the highest performance indices observed in fertilized Coal A + Soil. These findings suggest that soil amendments and fertilization can partially mitigate CT toxicity, offering a potential strategy for CT reuse in vegetation establishment.

1 Introduction

Coal is a critical energy source and one of the most economical contributors to national development and energy security (Cardoso 2015; T. Liu and Liu 2020). Despite the increasing global shift toward renewable energy sources such as wind, solar, and biomass (Brockway et al. 2019; Smith et al. 2022; Devlin et al. 2023), coal still accounts for over 26% of the world's primary energy use, with a total consumption of 161.47 EJ in 2022 (Tang et al. 2024). As a cheap and abundant resource, coal has historically fuelled industrialisation in regions such as Europe, North America, and Asia (Pomeranz 2000). It continues to be used extensively in electricity generation, steel production, and other industries (Miller 2010). However, this longstanding reliance on coal has come at a significant environmental and health cost.

Coal mining and processing produce substantial volumes of waste, including more than 600 million tonnes of coal fly ash (CFA) and fine-grained coal tailings annually (Sun et al. 2017; Babla et al. 2022). These by-products contribute to global environmental degradation, land disturbance, pollution of air and water resources, and threats to public health (Hendryx et al. 2019; Rauner et al. 2020; Gasparotto and Da Boit Martinello 2021). Coal tailings present a serious concern due to their content of persistent toxic substances such as heavy metals (e.g., Pb, Cd, As), altered pH, and depleted nutrients (Artiola et al. 2019; Sikdar et al. 2020). These conditions compromise soil structure and microbial activity, reduce fertility, and impair plant development by inducing oxidative stress, disrupting photosynthetic pathways, and suppressing growth (Strasser et al. 2004). In addition, harmful emissions from mining operations, refineries, and power plants - including mercury, nitrogen oxides, sulphur dioxide, and fine particulate matter - further heighten ecological and health risks (Tchounwou et al. 2012; Hendryx et al. 2019).

Given the scale and severity of coal mining's environmental footprint, there is an urgent need for sustainable strategies to manage the resulting waste. Conventional reclamation methods, such as capping, chemical stabilization, and physical removal, are often prohibitively expensive, resource-intensive, and may cause secondary ecological damage (Liu et al. 2018; Human and Truter 2021). In light of these limitations, researchers have increasingly explored the potential of repurposing coal mining waste for soil conditioning and land rehabilitation. With the global coal industry generating substantial volumes of tailings, the sustainable reuse of this material presents an opportunity to align waste management with the United Nations Sustainable Development Goals. Among emerging strategies, the incorporation of coal tailings (CT) into agroecosystems as a soil conditioner has gained attention. This approach aims to utilise CT in a safe, environmentally sound, and cost-effective manner, offering a dual benefit of improving degraded soils while reducing the burden of industrial waste.

Several innovative strategies have been proposed to valorise coal waste, including the development of technosols from coal tailings (Firpo et al. 2021), the use of biochar-amended soils for phytoremediation (Lebrun et al. 2021), and the synthesis of ash-based geopolymers for construction materials (Shamsaei et al. 2021). While these technologies offer promising environmental benefits, their widespread adoption remains limited due to scalability challenges and market constraints, leaving the global coal waste burden largely unresolved. In the agricultural context, co-pelletisation of coal waste with agro-waste has shown potential as a soil conditioner (Hossain and Morni 2020), though its practical applications in farming systems are still constrained. A growing body of evidence supports the use of coal fly ash (CFA) as a soil amendment, given its abundance of essential macro- and micronutrients, such as Ca, Mg, K, S, P, Si, Al, Fe, and Cu, and its ability to improve key soil physicochemical properties. Studies have demonstrated that CFA can enhance soil pH, porosity, electrical conductivity, water-holding capacity, and biological activity (Panda and Biswal 2018; Tripathi et al. 2020; Shakeel et al. 2021). For instance, CFA application in wheat cultivation improved soil fertility and productivity (Panda and Biswal 2018). In chickpea, it increased water retention, soil porosity, moisture content, and nutrient levels (P, K, Mn, S), alongside enhanced microbial populations (Tripathi et al. 2020). Similarly, in carrot, CFA significantly improved pH, water-holding capacity, and plant physiological parameters, including nitrate reductase activity, photosynthetic pigments, proteins, and carbohydrate content (Shakeel et al. 2021). Moreover, coal tailings, when applied at low rates and mixed with soil, showed beneficial effects on tomato germination and nutrient uptake, attributed to improved carbon and essential nutrients (N, P, K, Mg, Ca) in leaves (Yong et al. 2022). Despite these promising developments, the direct application of coal tailings remains largely underexplored in agricultural or ecological restoration contexts (Zhou et al. 2019; Amoah-Antwi et al. 2020; Firpo et al. 2021). Further research is needed to assess their agronomic potential, particularly regarding their nutrient contribution, metal toxicity, and long-term environmental impacts.

Flax (*Linum usitatissimum*), a multipurpose crop valued for its fibre and oilseed properties (Chaikivskyi and Zbarzhevetska 2023; Ozhimkova et al. 2023), has shown resilience under various abiotic stress conditions, including heavy metal contamination (Lebrun et al. 2021; Zhao et al. 2022; Ilavská et al. 2024), which is characteristic of coal by-products such as coal tailings. Its ability to tolerate and accumulate heavy metals, combined with a relatively fast growth cycle and adaptability, makes flax a strong candidate for phytoremediation and land rehabilitation. While studies have confirmed its potential in soils contaminated with metals such as Cu, Cd, Pb, Ni, and Zn (Angelova et al. 2004; Hosman et al. 2017; Saleem et al. 2020), the physiological mechanisms underpinning its stress responses, especially in coal tailing-amended substrates, are not fully understood.

Chlorophyll *a* fluorescence analyses are known to be effective in monitoring changes in the photosynthetic apparatus under various environmental stressors. This non-invasive, rapid, and precise technique has proven to be a powerful diagnostic tool for assessing photosynthesis in a wide range of plant species (Strasser and Strasser 1995; Strasser et al. 2000; Strasser et al. 2004). Chlorophyll *a* fluorescence offers a sensitive framework for evaluating the functional status of photosystem II (PSII) and detecting early physiological stress induced by heavy metal toxicity, nutrient imbalances, and other harsh soil conditions. When dark-adapted photosynthetic material is exposed to light after stress exposure, it displays a characteristic rise in fluorescence within the first second, forming a distinct OJIP transient (Strasser et al. 2000). This rise follows a specific sequence of phases, from the initial fluorescence (F_0) to the maximum fluorescence (F_M), marked as O (20 μ s), J ($\sim$ 2 ms), I ($\sim$ 30 ms), and P ($\sim$ 300 ms) (Stirbet and Govindjee 2011). These steps correspond to the redox transitions within PSII and PSI, reflecting the efficiency of electron transport through the photosynthetic chain to the terminal electron acceptor on the PSI acceptor side. Key chlorophyll *a* fluorescence parameters such as the Performance Index (PI_{total}), the F_v/F_M ratio, and the shape of the OJIP curve provide highly sensitive and reliable indicators of photosynthetic efficiency and stress resilience. Although chlorophyll *a* fluorescence holds significant promise as a diagnostic tool for assessing plant vitality, its

application in monitoring plant health in degraded environments, such as coal-tailing-contaminated soils, has received limited research attention.

Despite promising results in controlled or hydroponic experiments, there is a gap in understanding how flax performs physiologically in actual coal-tailing-amended soils. This study seeks to fill that gap by integrating chlorophyll *a* fluorescence indices with biomass measurements to assess flax's adaptability to South African coal tailing–soil mixtures. By doing so, the research aims to evaluate flax's dual role as a phytoremediation agent and a vegetative cover crop for ecological restoration. The findings are expected to contribute to the development of cost-effective, sustainable strategies for rehabilitating post-mining landscapes and improving the functional quality of degraded soils.

2 Materials and methods

2.1 Experimental setup and plant treatments

Coal tailings (CT) of different sizes were collected from two mining sites in Witbank, South Africa. Prior to planting, elemental analyses (heavy metals, macronutrients and micronutrients) of the CT and vermicompost (herein referred to as soil) samples were performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at NviroTek Laboratory (Hartbeespoort, South Africa). The experiment was conducted in a glasshouse with a day/night air temperature of 26/18°C and a 13 h day photoperiod and a dark period of 11 h. Commercial *Linum usitatissimum* (flax) seeds were manually sown into 30 cm-diameter pots containing different treatments: vermicompost alone (control), Coal A (small size), Coal B (large size), Coal A + Soil (1:1), or Coal B + Soil (1:1), with or without organic fertilizer application. Twenty mL of liquid organic fertilizer, Agri-boost (1:1000) (Agri-boost (Pty) Ltd), was applied to the soil once a week. Treatment consisted of three replicates arranged in a randomized complete block design. Pots were manually watered every 2–3 days throughout the 8-week experimental period to ensure adequate soil moisture. Germination rates were recorded 10 days after sowing, and plants were maintained under uniform environmental conditions.

2.2 Chlorophyll *a* fluorescence: OJIP transient analysis

The *in vivo* analysis of the polyphasic chlorophyll *a* fluorescence kinetics, indicative of the quinone acceptor (QA) reduction in photosystem II (PSII), was performed using a Plant Efficiency Analyser (Handy PEA) (Hansatech Instrument Ltd., King's Lynn, UK). Leaves were dark-adapted for 1 hour before measurements. The chlorophyll *a* fluorescence was measured on the adaxial side of three fully expanded leaves per plant from three plants per treatment. Fluorescence was induced using red actinic light at an intensity of 3000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, delivered by three 5 mm-diameter light-emitting diodes with a 12-bit resolution and 1-second duration. Fluorescence transients were processed using PEA Plus software (version 1.10, Hansatech Instrument Ltd.).

Data points were captured at intervals spanning from 0.02 ms to 0.05 ms for the initial O step (F_0), 2 ms for the J step (F_J), 30 ms for the I step (F_I), and 300 ms for the P step (F_M). The OJIP fluorescence transient, plotted on a logarithmic timescale, reflected the typical rise from the minimal fluorescence (F_0 , where all PSII reaction centres are open) to the maximum fluorescence (F_M , where all PSII reaction centres are closed or reduced). Key parameters relevant to this study are below:

$PI_{total} = [\gamma_{RC}/(1-\gamma_{RC})] \cdot [\varphi_{Po}/(1-\varphi_{Po})] \cdot [\psi_{Eo}/(1-\psi_{Eo})] \cdot [\delta Ro/(1-\delta Ro)]$	The total performance index is an index (potential) for energy conservation from excitons to the reduction of PSI end electron acceptors.
$PI_{ABS} = [\gamma_{RC}/(1-\gamma_{RC})] \cdot [\varphi_{Po}/(1-\varphi_{Po})] \cdot [\psi_{Eo}/(1-\psi_{Eo})]$	The total performance index on an absorption basis
$RC/ABS = \gamma_{RC}/(1-\gamma_{RC}) = \varphi_{Po}$ (VJ/M0)	
$\psi_{Eo} = ET_0/TR_0 = (1 - VJ)$	The efficiency/probability that an electron moves further than QA^- into the electron transport chain.
$\delta Ro = RE_0/ET_0 = (1 - VI)/(1 - VJ)$	The efficiency/probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side (RE)
$\varphi_{Po} = TR_0/ABS = [1 - (F_0/F_M)]$	The maximum quantum yield of primary photochemistry.

2.3 Biomass determination

At the end of the growing cycle, the plants were uprooted from the pots, weighed and dried in an oven at 80°C for 3 days. The dry mass was recorded and used to determine total biomass: (dry mass/fresh mass) x 100

2.4 Data analysis

Data were analysed using SigmaPlot software (version 21). The normality of the data was tested prior to statistical analysis. A one-way analysis of variance (ANOVA) was performed to determine significant differences among the treatments. Post hoc tests were conducted where appropriate to identify pairwise differences between treatment means. Statistical significance was determined at $p < 0.05$.

3 Results

3.1 Properties of soil and coal tailing treatments

The chemical composition and heavy metal content of the soil and coal tailings varied across treatments (Table 1). The pH of the control soil (7.19) was neutral, whereas the coal tailings exhibited more acidic conditions, particularly in Coal A (pH 6.11) and Coal B + Soil (pH 5.98). The pH of the coal tailing-amended soils (Coal A + Soil and Coal B + Soil) remained lower than that of the control soil. The soil texture analysis revealed that the control soil contained the highest clay content (9%), while Coal A had the lowest (3%). Sand was the dominant fraction across all samples, exceeding 85%, classifying the materials primarily as sandy soils. The incorporation of soil into coal tailings (Coal A + Soil and Coal B + Soil) slightly increased clay content.

Elemental analysis indicated substantial differences in macronutrient and heavy metal content across treatments. Calcium (Ca) levels were significantly elevated in Coal A (600.10 mg/kg) compared to Coal B (154.46 mg/kg) and control soil (26.0 mg/kg). Adding soil to coal tailings reduced Ca concentrations, though Coal A + Soil still retained high levels (507.46 mg/kg). Magnesium (Mg) followed a similar trend, with Coal A containing the highest concentration (150.66 mg/kg), while Coal B had significantly lower Mg content (40.17 mg/kg). Iron (Fe)

concentrations were markedly higher in the coal tailings than in the control soil. Coal A had the highest Fe content (30,568.81 mg/kg). The blending of Coal A with soil reduced Fe concentrations (21,622.69 mg/kg), yet they remained elevated in Coal B + Soil (20,214.83 mg/kg). Among heavy metals, arsenic (As) was considerably higher in Coal B (26.65 mg/kg) compared to Coal A (4.57 mg/kg) and control soil (1.47 mg/kg). The mixture of coal tailings with soil mitigated As concentrations, though Coal B + Soil still contained elevated levels (6.79 mg/kg). Lead (Pb) also showed a significant increase in coal tailings, particularly in Coal B (30.74 mg/kg), with lower levels in Coal A (26.12 mg/kg) and control soil (2.79 mg/kg). Chromium (Cr) was abundant in the control soil (233.05 mg/kg), but substantially lower in Coal A (72.19 mg/kg) and Coal B (41.80 mg/kg). The mixture of Coal A + Soil and Coal B + Soil showed intermediate Cr levels (107.91 mg/kg and 113.10 mg/kg, respectively). Selenium (Se) and uranium (U) concentrations were elevated in the coal tailings, with Coal A exhibiting the highest Se levels (3.68 mg/kg), while Coal B had slightly lower values (3.42 mg/kg).

Table 1
Chemical properties and heavy metal content of soil and coal tailings from South African coal mining site.

	Soil	Coal A	Coal B	Coal A + Soil	Coal B + Soil
pH KCL	7.19	6.11	6.81	6.52	5.98
Clay (%)	9	3	6	7	9
Sand (%)	89	91	91	88	89
Silt (%)	2	6	3	5	2
Elemental content (mg/kg)					
As	1.47	4.57	26.65	2.79	6.79
Ca	26.0	600.10	154.46	507.46	76.07
Cd	< 0.04	0.29	0.18	0.13	0.28
Co	4.92	7.21	14.20	5.27	4.17
Cr	233.05	72.19	41.80	107.91	113.10
Cu	13.58	23.23	14.63	16.27	16.16
Fe	12582.77	30568.81	19447.22	21622.69	20214.83
Hg	0.28	0.53	0.66	0.42	0.50
Mg	15.38	150.66	40.17	96.11	30.79
Mn	198.45	126.97	82.28	197.02	216.01
Na	20.59	11.51	11.19	76.90	48.87
Pb	2.79	26.12	30.74	10.70	14.43
Se	0.56	3.68	3.42	1.56	2.02
U	0.28	3.78	3.02	1.55	1.49
V	27.52	56.97	23.42	40.70	31.63
As = arsenic, Ca = calcium, Cd = cadmium, Co = cobalt, Cr = chromium, Cu = copper, Fe = iron, Hg = mercury, Mg = magnesium, Mn = manganese, Na = sodium, Pb = lead, Se = selenium, U = uranium, V = vanadium					

The incorporation of soil reduced Se concentrations, yet Coal A + Soil (1.56 mg/kg) and Coal B + Soil (2.02 mg/kg) still contained higher levels than the control soil (0.56 mg/kg). Uranium followed a similar trend, with Coal A (3.78 mg/kg) and Coal B (3.02 mg/kg) showing elevated levels compared to soil (0.28 mg/kg), though mixing with soil reduced these values.

3.1 Effect of coal tailings on germination and seedling establishment

Flax germination was significantly affected by the presence of coal tailings, with pure coal tailing treatments (Coal A and Coal B) exhibiting markedly lower germination rates than the control (vermicompost soil). Seedlings in these treatments failed to survive beyond the 10-day germination phase (Fig. 1). However, the addition of soil to coal tailings (Coal A + Soil and Coal B + Soil) substantially improved germination rates to 70% and 80%, respectively, compared to the negligible rates observed in Coal A and Coal B alone. This suggests that soil amendments mitigated some of the adverse effects of coal tailings, likely by improving soil structure, buffering pH, and diluting heavy metal concentrations. The increase in clay content from 3% in Coal A to 7% in Coal A + Soil, and from 6% in Coal B to 9% in Coal B + Soil, may have enhanced water retention and root establishment. Additionally, the dilution effect likely reduced the bioavailability of toxic elements such as As, Cd, and Pb, improving conditions for seedling emergence.

3.2 Effect of coal tailings on OJIP transient

The rapid chlorophyll *a* fluorescence induction kinetics, depicted as OJIP transients (Fig. 2), provide a sensitive multiphasic signature reflecting the functional status of Photosystem II (PSII) electron transport. The characteristic O, J, I, and P steps within the transient correspond to specific biophysical events: initial fluorescence (F_0) at 20 μ s representing antenna excitation with open reaction centres (RCs); the J-step at approximately 2 ms reflecting the reduction of quinone A; the I-step around 30 ms indicating the reduction of the plastoquinone (PQ) pool; and the peak fluorescence (F_M) at approximately 300 ms signifying the maximal reduction of QA and the PQ pool (Strasser - Michael, and Srivastava 2004; Strasser et al. 2000; Strasser and Strasser 1995).

The OJIP curves (Fig. 2) revealed distinct patterns contingent on the soil amendment and fertilization. In the treatments without fertilizer, the Soil exhibited the highest fluorescence intensities across the O, J, I, and P steps. The unfertilized Coal A + Soil treatment showed intermediate fluorescence levels at the O, J, I, and P steps. Similarly, Coal B + Soil displayed a fluorescence pattern with O, J, and I steps comparable to the fertilized soil treatment, but with a lower P-peak. Upon fertilizer application, the treatments showed a steady rise in fluorescence. The fertilized Soil treatment had a P-peak higher than the unfertilized soil. The fertilized Coal A + Soil and fertilized Coal B + Soil treatments exhibited the highest fluorescence intensities across the O, J, I, and P steps approaching the highest observed values.

3.3 Effect of coal tailings on JIP test parameters

The Performance Indexes (PI_{total} and PI_{ABS}) are stress-sensitive indicators that provide a holistic view of photosynthetic performance, integrating energy capture and electron transport efficiencies. These revealed distinct responses to the coal tailing amendments (Fig. 3). In the absence of fertilizer, Coal A + Soil resulted in an increase in both the PI_{total} and PI_{ABS} compared to the control soil, *albeit* not significantly different ($p < 0.05$). The slight increase in PI_{total} and PI_{ABS} in Coal A + Soil treatments compared to the Soil control, in the absence of fertilizer, suggests that Coal A tailings may contain components that can independently enhance photosynthetic efficiency. Conversely, Coal B + Soil exhibited a decrease in PI_{total} and PI_{ABS} under unfertilized conditions ($p < 0.05$). The chemical analysis shows

that Coal B tailings also have an acidic pH (6.81) and contain high levels of As (26.65 mg/kg) and Pb (30.74 mg/kg). Upon fertilizer application, both Coal A + Soil and Coal B + Soil showed increases in the performance indexes.

Differences were observed between non-fertilized and fertilized treatments across all measured chlorophyll fluorescence parameters. The performance index on absorption basis (PI_{ABS}) increased consistently under fertilized conditions, with values ranging from 4.00 in Soil to 5.11 in Coal A + Soil and 4.61 in Coal B + Soil, compared to lower values under non-fertilized conditions, where PI_{ABS} ranged from 2.51 in Coal B + Soil to 3.50 in Coal A + Soil. Similarly, the total performance index (PI_{total}) was elevated in the presence of fertilizer, ranging from 2.31 in Soil to 2.91 in Coal A + Soil and 2.61 in Coal B + Soil, relative to non-fertilized treatments, which exhibited values of 1.64, 1.835, and 1.40 for Soil, Coal A + Soil, and Coal B + Soil, respectively.

The maximum quantum yield of primary photochemistry (ϕPo) also followed a similar trend, increasing from 4.286 in non-fertilized Soil to 5.660 under fertilized conditions, with the highest value recorded in fertilized Coal A + Soil (7.430), in contrast to the lowest in non-fertilized Coal A + Soil (3.554) (Fig. 4). The density of active reaction centres per absorbed light (RC/ABS) was higher across fertilized treatments, with values ranging from 0.55 in Soil to 0.80 in Coal A + Soil and 0.72 in Coal B + Soil, whereas non-fertilized treatments exhibited lower RC/ABS values of 0.65, 0.40, and 0.37 for Soil, Coal A + Soil, and Coal B + Soil, respectively. A comparable pattern was evident in the efficiency of electron transport beyond QA^- (ψEo), which increased from 1.741 in non-fertilized Soil to 2.5 in fertilized Soil, with the highest value again in Coal A + Soil (4.0) under fertilized conditions.

3.4 Effect of coal tailings on plant biomass production

Biomass accumulation showed clear differences between fertilized and non-fertilized treatments (Fig. 5). Notably, no significant difference in biomass was observed between the non-fertilized Soil (5.0 g) and fertilized Soil (7.5 g) treatments. Similarly, no significant difference was found between fertilized Coal A + Soil (9.6 g) and fertilized Coal B + Soil (6.7 g). Within each coal tailing treatment, significant increases in biomass were observed following fertilizer application. Specifically, the Coal A + Soil treatment exhibited a pronounced increase in biomass from 3.7 g under non-fertilized conditions to 9.6 g when fertilized ($p < 0.05$). Likewise, the Coal B + Soil treatment demonstrated a significant increase in biomass from 2.8 g to 6.7 g upon fertilizer application ($p < 0.05$).

The spider plots represent a comparative visualization of JIP-test parameters for plants grown under different coal tailing treatments with and without fertilizer (Fig. 6). For the non-fertilized treatments, there was a relatively compact and uneven shape, indicating moderate to low values across most of the tested parameters. The Soil only treatment shows a relatively balanced polygon, with moderately high values across all parameters. The Coal A + Soil treatment exhibits a more contracted shape, particularly on the RC/ABS and ϕPo axes, Coal B + Soil shows similar contraction, especially in PI_{total} , RC/ABS, and PI_{ABS} . The fertilized treatments showed larger and more outward-stretching polygons, reflecting enhanced photosynthetic performance. All treatments demonstrated increases in PI_{total} and PI_{ABS} , with Coal A + Soil treatment exhibiting the most pronounced expansion across all axes, especially in ψEo and ϕPo . Fertilized soil and fertilized Coal B + Soil also showed improved performance, though Coal B + Soil remains slightly less expansive compared to Coal A + Soil. Notably, RC/ABS values increased across all fertilized treatments.

4 Discussion

Coal tailings (CT), a by-product of coal mining, pose significant environmental risks when poorly managed, including dam failures, toxic leachate contamination, and spontaneous combustion due to self-ignition (Hatje et al. 2017; Park et al. 2019). However, under controlled conditions, CT may hold potential as a soil amendment due to its content of

organic carbon, calcium/magnesium carbonate compounds, and in certain cases, plant-available nutrients. These compounds may contribute to improved soil structure, reduced metal mobility, and pH buffering (Spain and Tibbett 2012; Wang et al. 2015). Nonetheless, the agronomic value of CT remains uncertain, particularly considering variability in chemical composition and potential phytotoxicity. This study explored the capacity of *Linum usitatissimum* (flax), a versatile and stress-tolerant crop, to germinate and maintain physiological performance in coal tailing-amended soils. The results provide insight into the effects of CT composition, soil amendment, and nutrient input, biomass production, and chlorophyll *a* fluorescence-derived photosynthetic parameters.

Linum usitatissimum germination was significantly affected by the presence of coal tailings. Pure coal tailing treatments (Coal A and Coal B) had lower germination than the control (vermicompost soil), with seedlings in these treatments failing to survive beyond the first 10 days (Fig. 1). This suppression of germination is likely due to a combination of low pH (Coal A = 6.11; Coal B = 6.81) and elevated levels of heavy metals, particularly arsenic and lead in Coal B, and selenium and uranium in both tailings (Table 1). Such conditions can impair nutrient solubility and uptake (Appel and Ma 1998; Yang et al. 2005), while the acidic environment further increases the mobility of toxic metals (Kicińska et al. 2022). Comparable results have been observed in other plant species, such as tomato, where high-metal-content coal tailings hindered early growth and development despite pH variability (Yong et al. 2022). In this study, the high concentrations of iron and lead in both tailings (e.g., Fe at 30 568.81 mg/kg in Coal A) may have exacerbated nutrient imbalances, resulting in further phytotoxicity. These findings also emphasize the role of soil properties in moderating soil contaminant behaviour. Soil pH and texture, particularly clay content, significantly influence heavy metal retention and transport. While clay-rich soils tend to adsorb and retain metals more effectively due to their surface charge properties and mineral composition, sandy soils facilitate faster migration of contaminants (Sherene 2010). In acidic environments, protonation of clay and oxide surfaces can enhance anion retention while reducing nutrient mobility, thereby limiting plant access to essential elements and exacerbating toxic metal availability (Sherene 2010; Kicińska et al. 2022).

The addition of soil to coal tailings (Coal A + Soil and Coal B + Soil) improved germination rates to 70% and 80%, respectively, compared to the negligible rates observed in Coal A and Coal B alone (Fig. 1). This suggests that soil amendments potentially mitigated the adverse effects of coal tailings by diluting heavy metal concentrations (Alam et al. 2020), thus improving conditions for seedling emergence. The increase in clay content from 3% in Coal A to 7% in Coal A + Soil, and from 6% in Coal B to 9% in Coal B + Soil, may have enhanced water holding capacity (Sherene 2010) and consequently root establishment. To further ameliorate the possible toxic effects of coal tailings and support plant growth beyond the germination stage, organic fertilizer was applied to all treatments for the remainder of the experimental period. Applying organic amendments was expected to enhance microbial activity, improve nutrient availability, and promote overall plant resilience against potential heavy metal stress.

Chlorophyll *a* fluorescence measurements provided critical insights into the structural and functional responses of PSII to different coal tailing amendments and fertilizer. Variations in the shape and amplitude of the OJIP curves across treatments (Fig. 2) indicated that both tailing composition and fertilization substantially influenced photosynthetic efficiency. In the absence of fertilizer, Soil and Coal A + Soil treatments exhibited higher J-P phase fluorescence intensities, suggesting efficient energy trapping and electron accumulation within the PSII complex. This may indicate a well-regulated photosynthetic apparatus that maintains high electron transport capacity under baseline or moderately altered soil conditions. By contrast, the Coal B + Soil treatment showed reduced fluorescence amplitudes and a diminished OJIP curve, potentially reflecting stress-induced energy dissipation mechanisms or limitations caused by the physicochemical characteristics of the Coal B-amended soil. JIP-test parameters corroborated these observations. Under non-fertilized conditions, Coal A + Soil showed a slight increase in performance indexes (PI_{total} and PI_{ABS}) compared to the Soil control (Fig. 3), possibly due to increased micronutrient

availability, particularly iron (30 568.81 mg/kg), which plays a pivotal role in the electron transport chain. However, the simultaneous presence of phototoxic arsenic (4.57 mg/kg) complicates this outcome, emphasizing the dual role of trace elements as both essential nutrients and potential toxins depending on concentration and bioavailability (Farooq et al. 2013). In contrast, Coal B + Soil significantly suppressed PI_{total} and PI_{ABS} values under nutrient-low conditions. This suppression is likely attributable to the higher concentrations of arsenic (26.65 mg/kg) and lead (30.74 mg/kg) in Coal B, which are well-documented inhibitors of PSII efficiency due to their oxidative stress-inducing and electron transport-disrupting properties (Beneragama et al. 2014; Saleem et al. 2020). The associated declines in ϕPo and RC/ABS further support the hypothesis that Coal B tailings potentially exerted a phytotoxic effect on PSII function in nutrient-limited conditions.

Fertilizer application markedly improved photosynthetic performance across all treatments, as demonstrated by significant increases in PI_{total} and PI_{ABS} . The most pronounced improvement was recorded in the fertilized Coal A + Soil treatment, suggesting a potential synergistic interaction between the Coal A properties and fertilizer application. Elevated values of ϕPo (7.43), ϕEo (4.0), and RC/ABS in this treatment (Fig. 4) indicate improved primary photochemistry, enhanced electron flow beyond QA^- , and increased availability or regeneration of PSII reaction centres. These results indicate a favourable modulation of PSII structure and function by fertilization, possibly through enhanced nutrient translocation and reduced metal toxicity. While fertilized Coal B + Soil also showed improved photosynthetic indexes, its performance remained slightly inferior to that of Coal A + Soil. This is possibly due to residual heavy metal toxicity, especially under acidic pH conditions (5.98), which can maintain high metal mobility and uptake (Brunetti et al. 2011), thereby limiting the full benefits of nutrient supplementation. Nonetheless, these findings emphasize the capacity of fertilization to mitigate, at least partially, the adverse effects of coal tailings on plant vitality by promoting more favourable physiological conditions. Soil amendments, such as fertilizers, have been reported to improve nutrient availability in crops.

Beyond physiological parameters, biomass accumulation patterns mirrored trends in JIP parameters (Fig. 5). Fertilizer application significantly increased biomass in both Coal A + Soil and Coal B + Soil treatments, rising from 3.7 g to 9.6 g and from 2.8 g to 6.7 g, respectively. This aligns with previous studies demonstrating that improved nutrient availability through fertilizer application (Nkebiwe et al. 2016) enhances carbon assimilation and resource allocation to growth (Hawkesford 2012). This positive effect of coal tailings combined with fertilizer must be weighed against the potential negative impacts of introducing heavy metals into the soil. Interestingly, no significant biomass difference was observed between fertilized Soil and fertilized Coal B + Soil, despite notable disparities in their JIP parameter profiles. This could indicate that biomass production alone may not fully capture the physiological nuances revealed by chlorophyll fluorescence analysis, emphasizing the value of integrative physiological diagnostics in stress assessment.

The radar plot analysis offers a holistic visualization of the JIP test response under different treatments (Fig. 6). In non-fertilized conditions, the contracted polygons in Coal A + Soil and Coal B + Soil highlight impaired photosynthetic performance, particularly along RC/ABS and ϕPo axes. This visual contraction signifies restricted energy trapping and reaction centre density, consistent with stress-imposed photosynthetic limitations. In contrast, fertilized treatments showed more expansive and symmetrical polygons, reflecting broad-based enhancement in photochemical parameters. Coal A + Soil demonstrated the most pronounced radial expansion, indicative of a robust and integrated photosynthetic response.

5 Conclusion

This study demonstrates that *Linum usitatissimum* (flax) can establish and grow in coal-tailing–amended soils when supplemented with organic fertilizer, offering a potential strategy for rehabilitating degraded substrates. Due to their acidity and elevated heavy metal content, pure coal tailings significantly inhibited seed germination and early seedling survival. However, these adverse effects were mitigated when combined with soil, allowing for improved plant establishment. Adding organic fertilizer further enhanced photosynthetic efficiency and biomass accumulation, as reflected by improvements in OJIP chlorophyll fluorescence parameters and elevated performance indices (PI_{total} and PI_{ABS}). Among the treatments, fertilized Coal A + Soil yielded the most favourable physiological responses, suggesting comparatively lower phytotoxicity and greater micronutrient availability than Coal B.

Notably, chlorophyll *a* fluorescence emerged as a powerful, non-invasive tool for evaluating plant responses to coal tailing stress. Its high sensitivity to PSII disruptions enabled the detection of early physiological disturbances caused by heavy metal toxicity and nutrient imbalances. This highlights the value of chlorophyll fluorescence in assessing plant vitality and functional integrity in contaminated or marginal soils. The study also emphasizes integrating physiological indicators, such as chlorophyll fluorescence, with conventional biomass measurements to gain a more comprehensive understanding of plant stress responses. Nevertheless, the elevated concentrations of heavy metals, particularly in Coal B, present long-term risks of bioaccumulation and environmental contamination. These risks must be carefully considered when using coal tailings for agricultural or ecological purposes. Overall, the findings support the feasibility of using coal tailing–soil mixtures and organic amendments- to promote plant growth on contaminated substrates. However, long-term investigations are necessary to evaluate the sustainability, safety, and environmental implications of such practices. Strategic management of soil amendments, coupled with the selection of tolerant crop species, may facilitate the reclamation of post-mining landscapes while mitigating ecological risks.

Declarations

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Authors' Contributions

Marco Le Roux and Jacques M. Berner contributed to the study conception and design. Experimentation, data collection and analysis were performed by Philisiwe F. Mhlanga. The first draft of the manuscript was written by Philisiwe F. Mhlanga and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose

Data Availability Statement

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request

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Figures

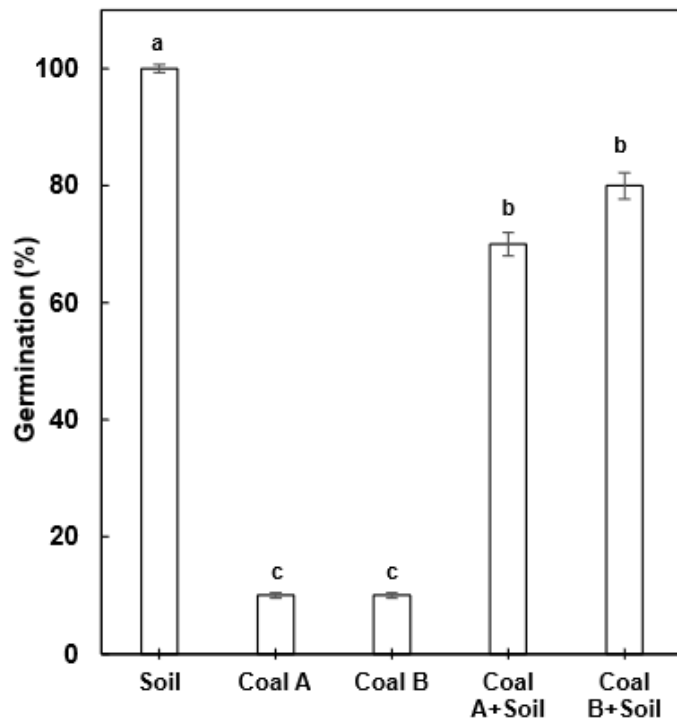


Figure 1

Germination rates of *Linum usitatissimum* in different soil treatments. Germination rates of flax seeds were recorded 10 days after sowing in soil-only (control), Coal A, Coal B, Coal A+Soil (1:1), and Coal B+Soil (1:1) treatments. Each bar represents the means $\pm$ SD. Values with similar alphabets are not significantly different (ANOVA, $p \leq 0.05$).

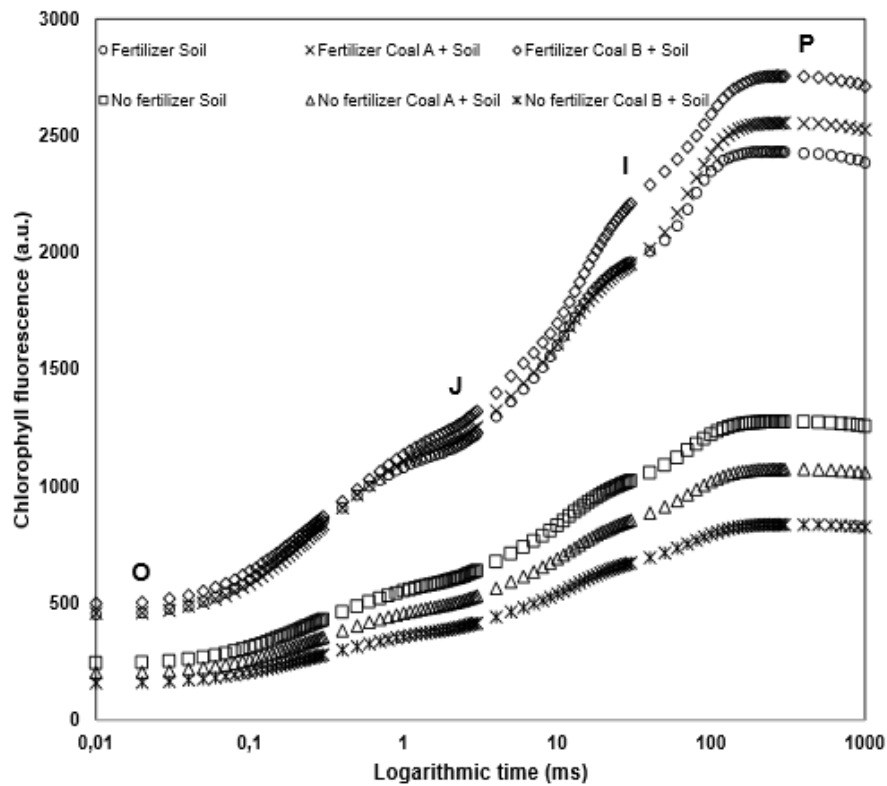


Figure 2

OJIP chlorophyll a fluorescence induction curves of *Linum usitatissimum* grown under different soil and coal treatments with or without fertilization. Polyphasic rise kinetics of prompt chlorophyll fluorescence, representing the reduction of QA in PSII, were measured in leaves of flax plants grown in control (soil-only), Coal A+Soil, and Coal B+Soil treatments.

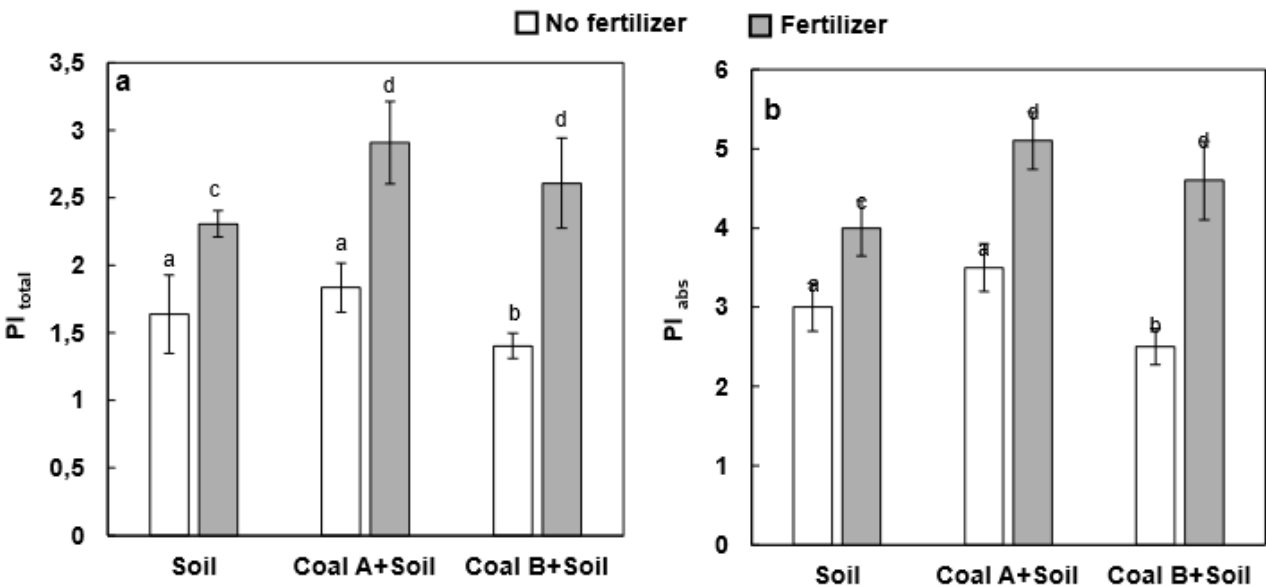


Figure 3

Photosynthetic performance parameters of *Linum usitatissimum* in non-fertilized and fertilized soil treatments with coal tailings (soil = control, Coal A+Soil, and Coal B+Soil) treatments 10 weeks after sowing. Each bar represents the means $\pm$ SD. Values with similar alphabets are not significantly different (ANOVA, $p \leq 0.05$).

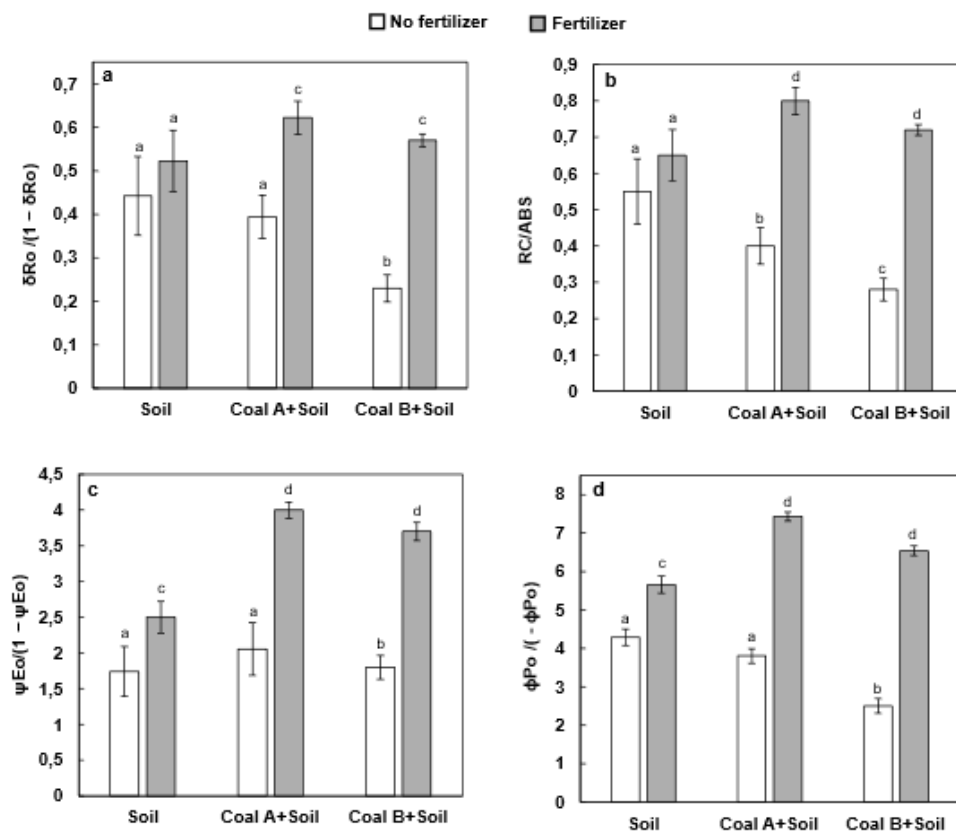


Figure 4

Effects of coal tailing treatments on the probability to reduce the end electron acceptors at the PSI acceptor side (δRo), RC density on a chlorophyll basis (RC/ABS), the efficiency with which an electron moves into the electron transport chain further than QA^- (ψEo) and the quantum yield of primary photochemistry (ϕPo) of *Linum usitatissimum*. Each bar represents the means $\pm$ SD. Values with similar alphabets are not significantly different (ANOVA, $p \leq 0.05$).

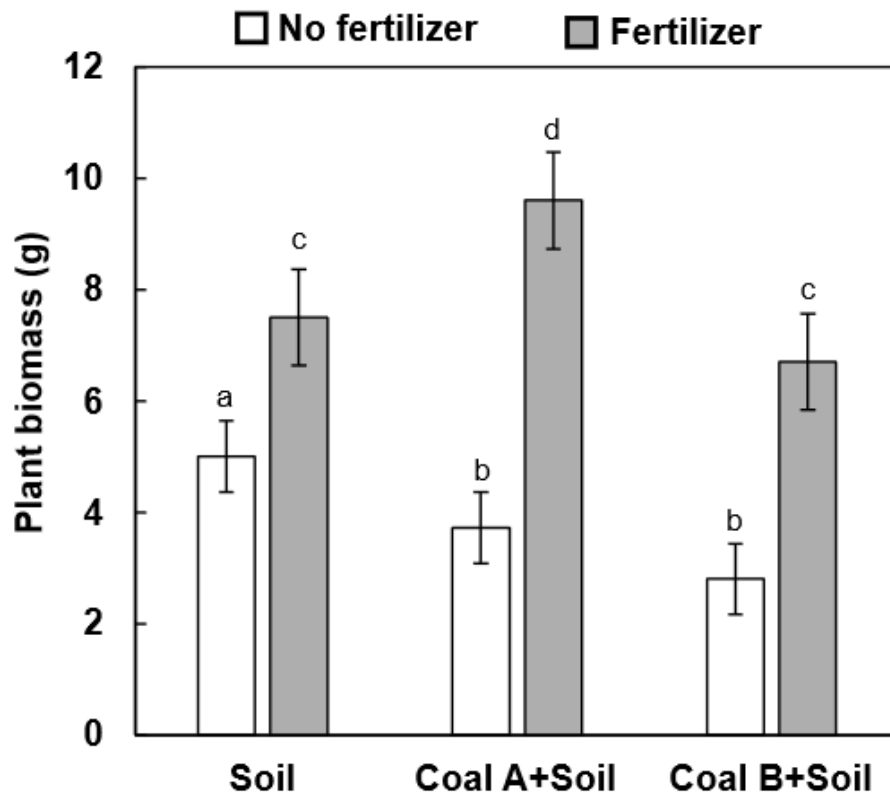


Figure 5

Effects of coal tailing treatments on the biomass production in *Linum usitatissimum* after eight weeks of growth. Each bar represents the means $\pm$ SD. Values with similar alphabets are not significantly different (ANOVA, $p \leq 0.05$).

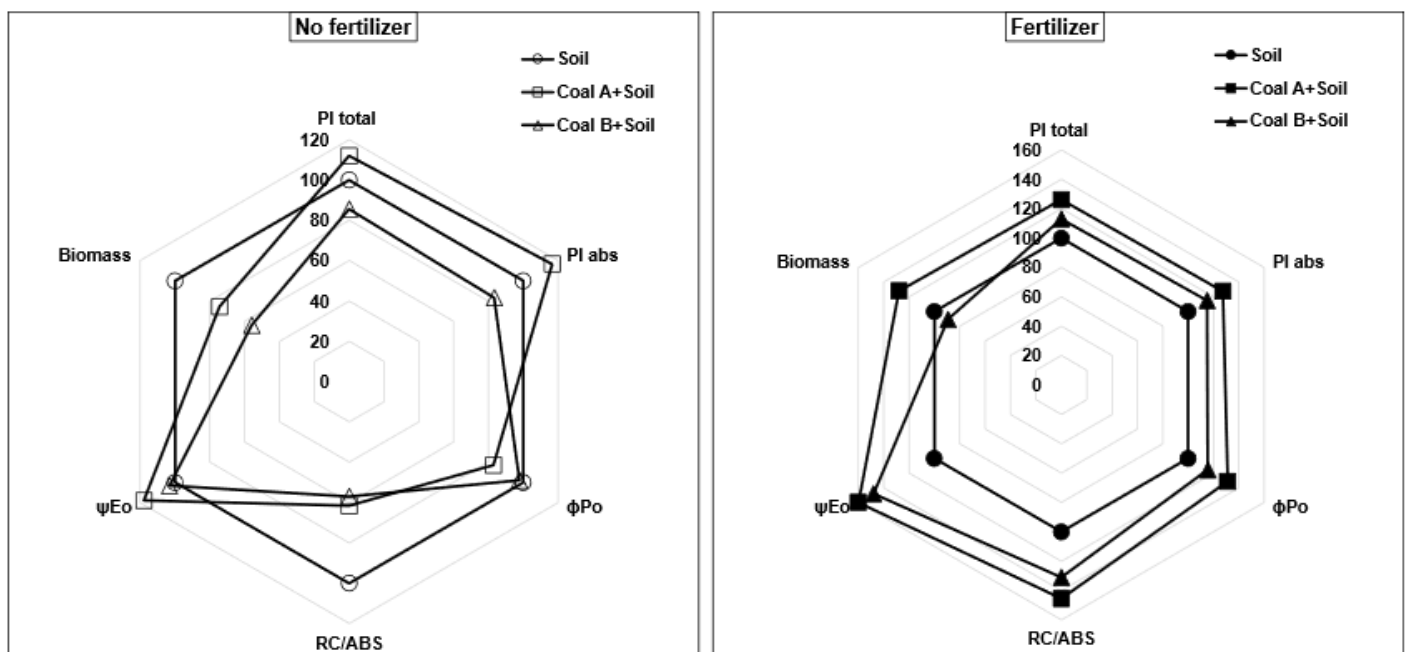


Figure 6

Radar plot showing various JIP-test parameters quantifying PSII functionality of the photosynthetic apparatus in *Linum usitatissimum* under different coal tailing and soil treatments after eight weeks of growth