Severe nickel stress constrains phenotypic plasticity and induces developmental canalisation in Catharanthus roseus

Antonia Valdirene Teixeira Miranda Franco
Federal University of Minas Gerais: Universidade Federal de Minas Gerais
Millena Miranda Franco
millena@usp.br
University of Sao Paulo: Universidade de Sao Paulo https://orcid.org/0000-0002-0292-0797
Iasmim Miranda Franco
Federal University of Minas Gerais: Universidade Federal de Minas Gerais

Research Article

Keywords: Phenotypic plasticity, Developmental canalisation, Nickel toxicity, Plant allometry, Catharanthus roseus

Posted Date: March 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8757682/v1

Additional Declarations:
Author Contribution

Antônia Valdirene Teixeira Miranda Franco: Writing — original draft, Supervision, Conceptualisation, Review and editing, Experimentation; **Millena Miranda Franco**: Data analysis and Software, Writing — Original draft preparation; **Iasmim Miranda Franco**: Experimentation, Writing — Original draft preparation and revision. All authors have read and agreed to the published version of the manuscript.

Severe nickel stress constrains phenotypic plasticity and induces developmental canalisation in *Catharanthus roseus*

Antônia Valdirene Teixeira Miranda Franco
Master's student in Food and Health at the Institute of Agricultural Sciences, Federal University of Minas Gerais (UFMG). Biologist graduated from the State University of Montes Claros (UNIMONTES), Minas Gerais, 39401102, Brazil.
Millena Miranda Franco
PhD student in Education at the University of São Paulo (USP). São Paulo, 06343000, Brazil.
Iasmim Miranda Franco
Undergraduate student in Agronomy at the Institute of Agricultural Sciences, Federal University of Minas Gerais (UFMG). Minas Gerais, 39401102, Brazil.

Abstract

Phenotypic plasticity is a key strategy for plant acclimatisation; however, its limits under lethal stress remain poorly understood. This study investigated the morphophysiological responses and changes in the developmental architecture of *Catharanthus roseus* subjected to acute nickel stress (70 and 130 mg kg^{-1}). While the intermediate dose induced a succulence-by-dilution mechanism and an avoidance syndrome (etiolation), severe stress caused metabolic collapse, evidenced by a 28% reduction in chlorophyll and stagnation of the absolute growth rate. Nevertheless, allometric analysis revealed an unexpected phenomenon of developmental canalisation under maximum toxicity. In contrast to the control plants, which exhibited high phenotypic plasticity (high residual variability), plants under 130 mg kg^{-1} Ni adopted a strictly coordinated and mathematically predictable growth pattern (R^2= 0,93; MAPE = 3.97%). These results suggest that, faced with the energy scarcity imposed by severe toxicity, *C. roseus* sacrifices its developmental plasticity, channeling resources toward a rigid survival phenotype. This study provides new perspectives on plant allometry under stress, demonstrating that extreme toxicity can act as a canalising force that reduces developmental "noise".

Keywords: Phenotypic plasticity; Developmental canalisation; Nickel toxicity; Plant allometry; *Catharanthus roseus*.

1 Introduction

Catharanthus roseus (family Apocynaceae), popularly known as Madagascar Periwinkle or Vinca, is a species native to Madagascar that is currently widely acclimatised in various regions of the world, including Brazil—where it holds the status of a cultivated plant (Jyothsna, Sowmithri, Bhagyasri et al., 2025). It is a perennial herbaceous dicotyledon with an erect, branched, and latescent stem, featuring vascular bundles arranged in a ring—a typical characteristic of the family. Its leaves are simple, dark green, with reticulate venation, flexible, and rich in white latex (World Flora Online, 2026; Missouri Botanical Garden, 2026). The root system is taprooted, with a well-developed primary root and lateral branches provided with root hairs responsible for the absorption of water and nutrients (Rani, 2022). The flowers are simple, axillary, and pentamerous, with a gamopetalous corolla varying in colour from white

to pink; this, combined with continuous flowering, promotes its extensive use in urban landscaping (Royal Botanic Gardens, Kew, 2026; Missouri Botanical Garden, 2026). The fruits are follicles, completing its reproductive cycle (World Flora Online, 2026; Rani, 2022).

Beyond its ornamental and ethnobotanical relevance, the species exhibits high hardiness, tolerating arid climates and low water availability, as well as attracting a diverse range of pollinators. From a biological perspective, it is notable for its highly active secondary metabolism, which is responsible for the production of alkaloids of pharmacological interest, such as vinblastine and vincristine (Duffin, 2000). The medicinal aspects of the species are extensively elucidated by Banyal et al. (2023), Chuah *et al.* (2024), Cordeiro (2019), Ferreira *et al.* (2004), Sarkar & Sharma (2025), Spagnuolo *et al.* (2010), and Wright (2002).

This physiological plasticity is also manifest in the species' ability to accumulate and translocate chemical compounds and metals across different plant organs (Soumya & Kiranmayi, 2023), underpinning its biotechnological potential and applications in phytoremediation (Srivastava & Srivastava, 2010). Studies indicate that environmental factors directly influence its chemical composition Arefifard *et al.* (2014); however, gaps remain regarding the effects of abiotic stress on alkaloid biosynthesis. Within this context, *C. roseus* has been investigated for its tolerance to and accumulation of heavy metals (Kumar, Singh & Singh, 2022; Srivastava & Srivastava, 2010), such as nickel. Although nickel is an essential micronutrient at low concentrations, it becomes phytotoxic at high levels—a common occurrence in industrial soils and mining areas, where it can compromise photosynthesis and plant growth.

In response to environmental variations, phenotypic plasticity allows plants to adjust their morphology and physiology (Pérez-Ramos *et a*l., 2019), for instance, by developing thicker leaves to tolerate water stress, or by reducing growth rates or seed production (Valladares *et al.*, 2007). Thus, under conditions of extreme stress, trade-offs are expressed through a decrease in phenotypic variability (Burress & Muñoz, 2023), the canalisation of resources into rigid and predictable growth patterns, and the prioritisation of immediate survival over plasticity. Through the reduction of developmental "noise", a stricter allometric coordination is imposed (Andrade *et al.*, 2013). From an evolutionary perspective, such responses reveal how intense environmental pressures can shape allometry and reduce phenotypic variability, influencing the dynamics of plant populations and communities (Silva, 2021). Recent genomic studies corroborate this view, demonstrating that variation in scaling

exponents is correlated with genes for abiotic stress resistance and local adaptation, reflecting evolutionary trade-offs between growth and survival (Vasseur *et al*., 2018).

Phenotypic plasticity refers to the ability of a genotype to express different phenotypes in response to environmental variations (Vaz de Lima & Langaro, 2019). Although it serves as a survival strategy, it incurs energy costs and may compromise other vital functions, such as reproduction (Nascimento, 2019). In contrast, canalisation—a concept introduced by Waddington (1942)—refers to the tendency of a genotype to produce consistent and stable phenotypes, even in the face of environmental or mutational variations (Flatt & Wagner, 2018). Canalisation can be advantageous in extreme environments where variability would be energetically costly or unfeasible (Wang & Callaway, 2024).

When a plant is dying, complete disorganisation is expected. However, studies show that even during metabolic collapse, certain species maintain predictable proportions between organs. This occurs because developmental programmes are strictly regulated by genetic and hormonal networks that continue to operate until the limit of viability is reached (Niklas & Enquist, 2001). Under lethal stress, phenotypic plasticity tends to vanish, which includes preserving allometric proportions; for instance, despite a reduction in biomass, the root-to-shoot ratio may remain constant, reflecting an internal resource allocation rule (Poorter *et al.*, 2012).

Within this context, the present study investigates the limits of phenotypic plasticity and alterations in the developmental architecture of *Catharanthus roseus* under nickel acetate stress. The objective is to characterise not only the losses in biomass and photosynthetic efficiency but, primarily, the allometric strategies adopted by the species to survive toxicity. We seek to answer the following questions: how does the metabolic scarcity induced by the metal remodel the coordination between vertical growth and leaf emergence? And, specifically, whether severe stress acts as a canalising force, restricting developmental variability in favour of a more rigid survival phenotype.

2 Materials and Methods

An overview of the experimental design, ranging from seedling establishment and the application of nickel stress treatments to the stages of biometric monitoring and final physiological analyses, is outlined in Fig. 1.

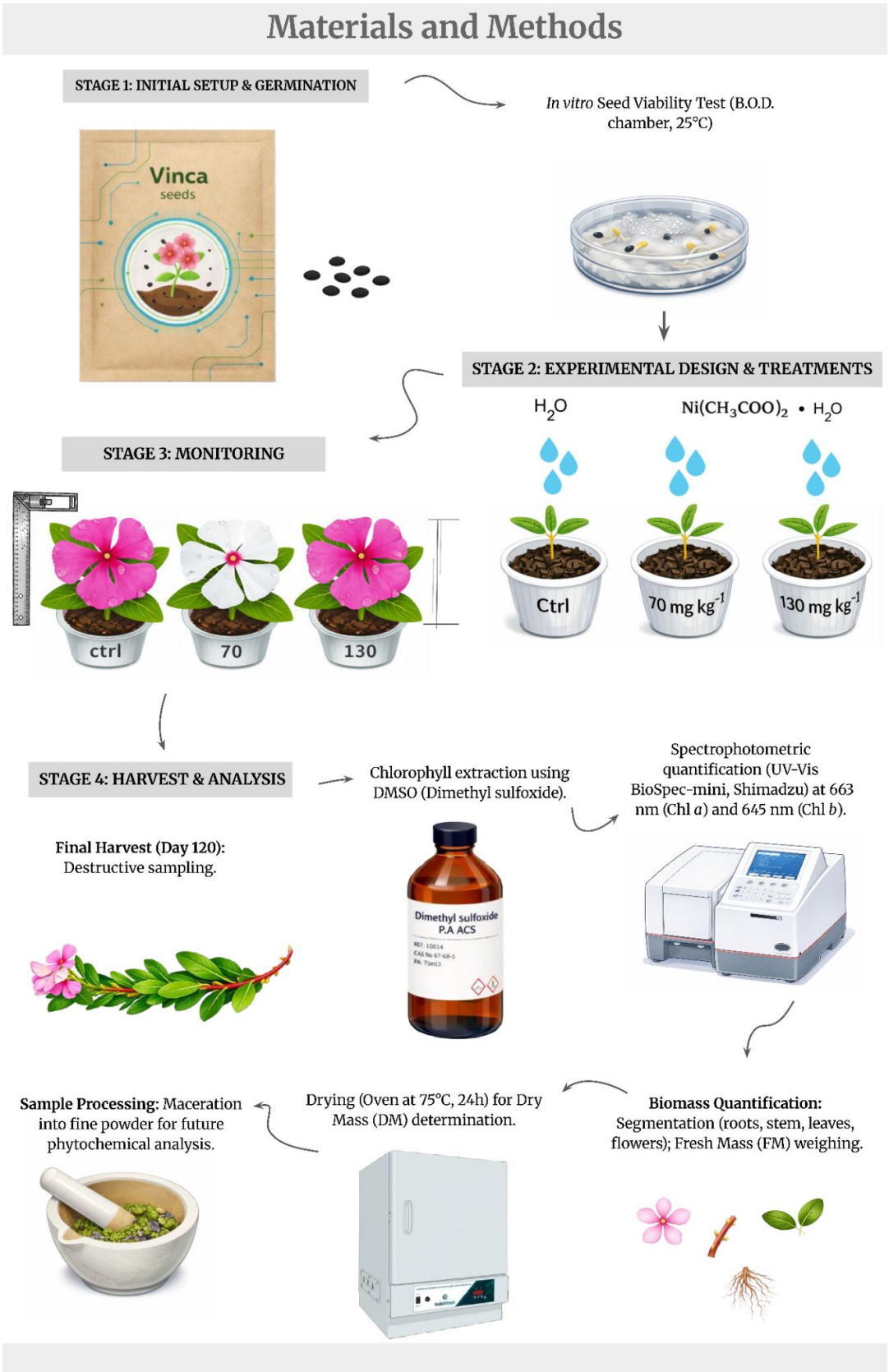

Fig. 1. Schematic representation of the experimental design and methodological workflow employed to evaluate the responses of *Catharanthus roseus* to nickel stress. (Stage 1) Preliminary seed viability (in vitro) and germination tests. (Stage 2) Establishment of the three experimental groups in a greenhouse ($n = 3$): Control (distilled water), 70 mg kg^{-1}, and 130

mg kg^{-1} of Nickel Acetate. (Stage 3) Fortnightly biometric monitoring of plant development. (Stage 4) Final harvest on day 120, followed by physiological analyses (chlorophyll quantification via DMSO extraction) and biomass determination (drying and maceration).

2.1 Experimental site and biological material

The present study was conducted over a period of 120 days in a greenhouse under controlled conditions, located in the municipality of Montes Claros, Minas Gerais, Brazil (16°43'14.6" S, 43°52'49.2" W). *Catharanthus roseus* was used as the biological material. Natural seeds — non-transgenic and pesticide-free — were purchased commercially from a specialised establishment within the municipality. Cultivation was performed using a commercial substrate, the initial physicochemical characteristics of which were previously analysed. Initially, the seeds were sown in containers containing 40 g of substrate. Following germination and initial stabilisation, the seedlings were transplanted into final containers containing 400 g of the same substrate.

2.2 Viability and Germination Test

Initially, a preliminary in vitro seed viability test was conducted. Seeds were sown in sterilised Petri dishes lined with filter paper and hydrophilic cotton wool moistened with distilled water. The set was maintained in a germinator (B.O.D. incubator) at a controlled temperature of 25 °C under a dark photoperiod.

For the main experiment, properly labelled plastic containers (cups) filled with substrate were used. Three seeds were sown per container. During the initial establishment phase (the first 7 days), the containers were covered with moist cotton cloth to maintain humidity and protect against excessive desiccation. After seedling emergence, the cloth was removed, and watering was performed with 3 mL of distilled water on alternate days, with adjustments made according to the plants' water demand.

Fifteen days after emergence, thinning was carried out, leaving only the most vigorous individual per container to avoid intraspecific competition for resources (water, light, and nutrients).

2.3 Experimental Design and Treatments

The experiment was conducted in triplicate, using a Completely Randomised Design (CRD), and consisted of three experimental groups: the control group (CG), comprising plants

irrigated solely with contaminant-free distilled water; treatment 1 (T1), containing plants subjected to nickel acetate ($Ni(CH_3COO)_2 \cdot 4H_20$) contamination at a concentration of 70 mg kg^{-1}; and treatment 2 (T2), formed by plants subjected to nickel acetate contamination at a concentration of 130 mg kg^{-1}.

The metal concentrations in the compound were defined based on Brazilian prevention and investigation guidelines for soils, established by the National Environment Council (CONAMA) Resolution No. 420/2009.

2.4 Biometric and Growth Analyses

Plant development was monitored over a 120-day period. Non-destructive measurements were taken fortnightly, using a millimetre ruler to assess shoot height (from the plant collar to the apical meristem) and manual counting of the number of leaves, flower buds, and open flowers. The data were recorded in monitoring spreadsheets.

2.5 Harvest and Physiological Analyses

At the end of day 120, a destructive harvest of the individuals was performed, following a random selection within each experimental group. Prior to removing the plants from the substrate, the concentration of photosynthetic pigments was determined using dimethyl sulfoxide (DMSO) as the extraction solvent—a method validated by Magalhães *et al.* (2010) and Colombo *et al.* (2018) due to its high tissue diffusion capacity.

For extraction, leaf discs (totalling approximately 100 mg of fresh mass per sample) were cut into smaller fragments and incubated in test tubes containing 5 mL of DMSO. The samples were kept in a boiling water bath for 20 minutes to ensure complete leaching of the pigments. After cooling to room temperature, the absorbance of the extract was measured using a UV-Vis spectrophotometer (BioSpec-mini, Shimadzu Corporation, Kyoto, Japan) at wavelengths of 645 nm and 663 nm, using pure DMSO as a blank. The concentrations of chlorophyll *a*, *b*, and total chlorophyll (expressed in mg g^{-1} of fresh mass) were calculated according to the equations of Arnon (1949):

$$Total\ Chlorophyll = 20.2 \times A_{645} + 8.02 \times A_{663}$$

$$Chlorophyll\ a = 12.7 \times A_{663} - 2.69 \times A_{645}$$

$$Chlorophyll\ b = 22.9 \times A_{645} - 4.68 \times A_{663}$$

Subsequently, the plants were removed from the containers and carefully washed with running water, followed by distilled water, to remove soil particles adhering to the roots. Excess moisture was removed using paper towels. The plants were segmented into roots, stems, leaves, and flowers, and the Fresh Mass (FM) of each organ was determined using a precision analytical balance. Finally, the plant material was placed in properly identified paper bags and subjected to drying in a forced-air circulation oven at 75 °C for 24 hours (or until a constant weight was achieved) to obtain the Dry Mass (DM).

2.6 Maceration and Storage

After drying and weighing, the root, stem, leaf, and flower samples were individually macerated using a mortar and pestle until a fine powder was obtained. The pulverised material was weighed, labelled, and stored in airtight flasks, protected from light, for subsequent phytochemical analyses. Additionally, 100g soil samples from each treatment (Control, T1, and T2) were collected for a posteriori chemical analyses.

2.7 Statistical Analysis

The experimental data were initially subjected to descriptive statistics to obtain measures of central tendency (mean) and dispersion (standard deviation). For the comparison between groups (Control, 70 ppm, and 130 ppm) regarding biomass variables, water content, and photosynthetic pigments, a one-way Analysis of Variance (ANOVA) was employed. Statistical significance was defined at $p < 0{,}05$. Furthermore, simple linear regression analysis was performed to model plant development under the highest contaminant concentration (130 ppm), with the quality of the fit evaluated by the coefficient of determination (R^2).

To investigate the relationship between growth variables (height, leaf number, and mean germination rate), Pearson's correlation coefficient (r) was applied, as detailed below, where x_i and y_i are the individual values of the variables, and $\underline{x}$ and $\underline{y}$ are their respective means. Values of $|r|$ close to 1 indicate a strong linear association:

$$r = \frac{\Sigma\,(x_i - \underline{x})(y_i - \underline{y})}{\sqrt{\Sigma(x_i - \underline{x})^2\,\Sigma(y_i - \underline{y})^2}}$$

The predictive model was validated by calculating the Mean Absolute Percentage Error (MAPE) using the leave-one-out cross-validation method. Additionally, to test the environmental canalisation hypothesis, the heterogeneity of residual variances among treatments was statistically compared using Levene's Test, with significance set at $p < 0,05$. The Absolute Growth Rate (AGR) was calculated as the ratio between the height increment and the time interval ($\Delta P/\Delta t$), where P represents height and t represents time. Statistical analyses and graphical representations were performed using the R statistical programming language (R Core Team, 2024) and the ggplot2 package for data visualisation.

3 Results and Discussion

3.1 Costly water homeostasis and inhibition of metabolic recovery

Exposure to nickel did not compromise the hydration status of the established leaf tissues. The analysis of water content revealed statistically invariant means (ANOVA, $p = 0.43$), ranging between 81.56% (Control) and 83.38% (70 mg kg^{-1}). However, the maintenance of this water homeostasis under contaminated soil conditions suggests a hidden metabolic cost. The preservation of turgidity in environments with reduced osmotic potential (due to the presence of metallic salts) generally requires the active synthesis of compatible osmolytes (such as proline and soluble sugars) and the vacuolar sequestration of ions—both of which are energetically onerous processes (Jalmi *et al.*, 2018).

This energy investment in water retention — possibly acting as a mechanism for diluting cytosolic toxicity (the succulence effect) — competes directly with carbon allocation for cell wall synthesis and structural growth. Therefore, the water stability observed here should be interpreted not merely as passive tolerance, but as a physiological trade-off: *C. roseus* prioritises the maintenance of cellular hydration to enable survival and basal metabolism, sacrificing, in return, the accumulation of dry biomass (discussed in Section 3.3).

In contrast to the stability observed in adult tissues, the seedling establishment phase proved to be the window of greatest vulnerability to the contaminant. Germination dynamics suffered a severe and dose-dependent delay induced by the metal (Fig. 2A). While the control group exhibited maximum synchrony and rapid establishment (100% by the 5th day), the presence of nickel imposed a chemical barrier to the resumption of growth, extending the process until the 20th day at a concentration of 130 mg kg^{-1}.

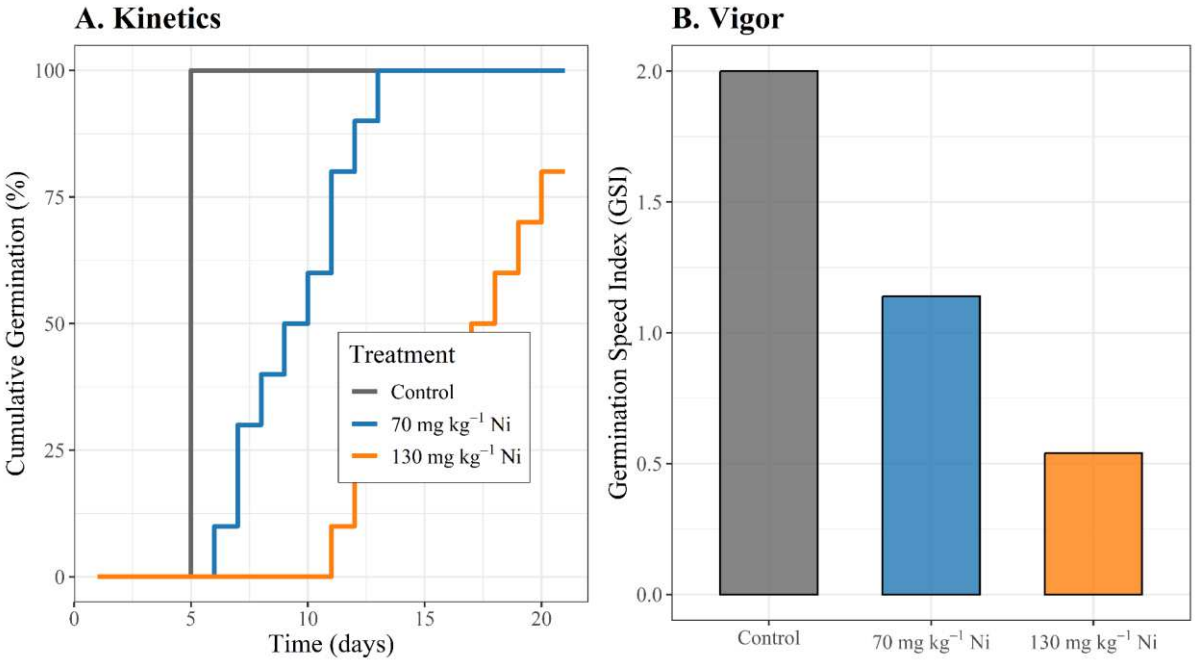

Fig. 2. Effect of Nickel (Ni) on the germination behaviour of *Catharanthus roseus*. (A) Cumulative germination kinetics over 21 days; (B) Germination Speed Index (GSI). The control group showed rapid and synchronous germination, while Ni treatments induced severe temporal delays and reduced seed vigor in a dose-dependent manner.

This delay was drastically reflected in the Germination Speed Index (GSI), with decreases of 43% and 73% in the 70 and 130 mg kg^{-1} treatments, respectively (Fig. 2B). The observed dissociation between the unaltered water content (in the adult plant) and the severe inhibition of germination (in the embryo) suggests that nickel acts by blocking the mobilisation of essential reserves for radicle protrusion. Unlike adult tissue, which possesses established compartmentalisation mechanisms, the germinating embryo exposes its meristematic tissues directly to the soil solution, resulting in systemic metabolic inhibition even before water relations can be established (Olzacki *et al.,* 2025).

3.2 Biometric analysis and growth dynamics

3.2.1 Absolute Growth Rate

The temporal dynamics of the Absolute Growth Rate (AGR) revealed a contrasting response pattern between the stress conditions and the control (Fig. 3). While the Control group showed a peak of accelerated growth in the 77–91 day interval (0.21 cm day^{-1}), the treatment under severe stress (130 mg kg^{-1} Ni) demonstrated a progressive suppression of vitality.

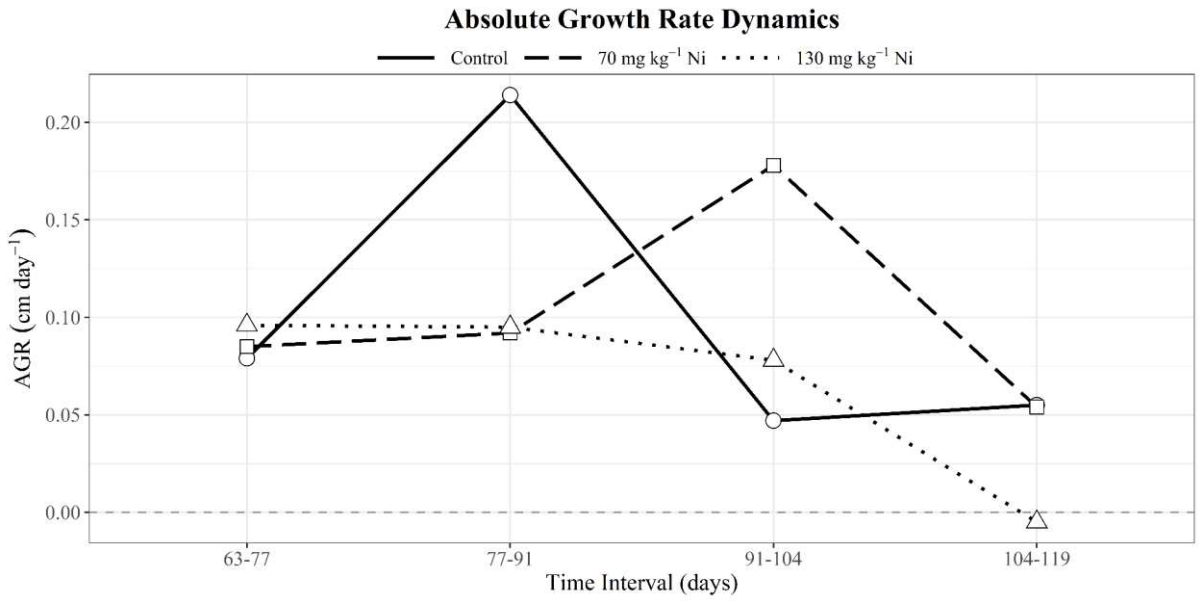

Fig. 3. Dynamics of the Absolute Growth Rate (AGR) of *Catharanthus roseus* under different nickel concentrations throughout the experimental period.

Notably, in the final experimental interval (104–119 days), the 130 mg kg^{-1} group crossed the survival threshold, recording negative rates (–0.01 cm day^{-1}). This decline below zero, visualised by the break in linearity of the dotted curve (Fig. 3), indicates not only stagnation but also biomass loss and senescence induced by acute metal toxicity.

3.2.2 Global Allometric Trajectory

The global allometric correlation analysis ($n = 150$), integrating the entire developmental period (days 63 to 119), revealed that nickel stress fundamentally altered the plant's resource allocation strategy (Fig. 4).

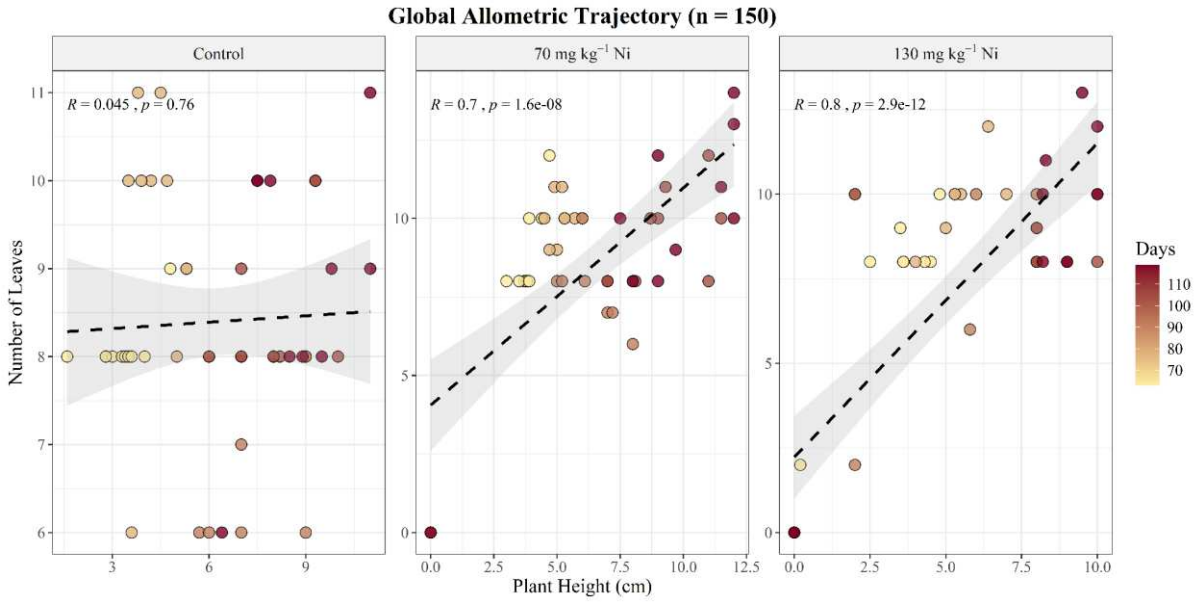

Fig. 4. Global allometric trajectory ($n = 150$) relating plant height and leaf number in *Catharanthus roseus*. The colour gradient (lighter to darker) represents the passage of time (days 63 to 119). Dashed lines indicate the linear regression model for each treatment, with their respective Pearson correlation coefficients (R) and p values.

In the Control group, high data dispersion and an absence of significant correlation between height and leaf number were observed ($R = 0.045$; $p = 0.76$). This suggests that, under ideal conditions, *C. roseus* exhibits high phenotypic plasticity, investing in height or leaves independently and variably according to immediate requirements.

In contrast, exposure to nickel induced a strong "canalisation" of growth. In the 70 mg kg^{-1} and 130 mg kg^{-1} treatments, the correlation became positive, linear, and highly significant ($R = 0.7$ and $R = 0.8$, respectively; $p < 0.001$). As evidenced by the clustering of data points around the regression line in Fig. 4, severe stress forced the plant into a rigid and coordinated developmental pattern: each increment in height was strictly accompanied by proportional leaf emergence, reflecting a conservative survival strategy in which plasticity is sacrificed in favour of maintaining basic vital proportions.

3.3 Biomass accumulation

The initial analysis of fresh biomass revealed, primarily, a defence strategy based on water homeostasis (Fig. 5). The intermediate treatment of 70 mg kg^{-1} Ni presented a higher water content (84.1%) compared to the Control (82.4%). This increase in turgidity suggests the

activation of a "succulence-by-dilution" mechanism, a situation in which the plant maximises water uptake in an attempt to reduce the cytosolic concentration of the toxic metal.

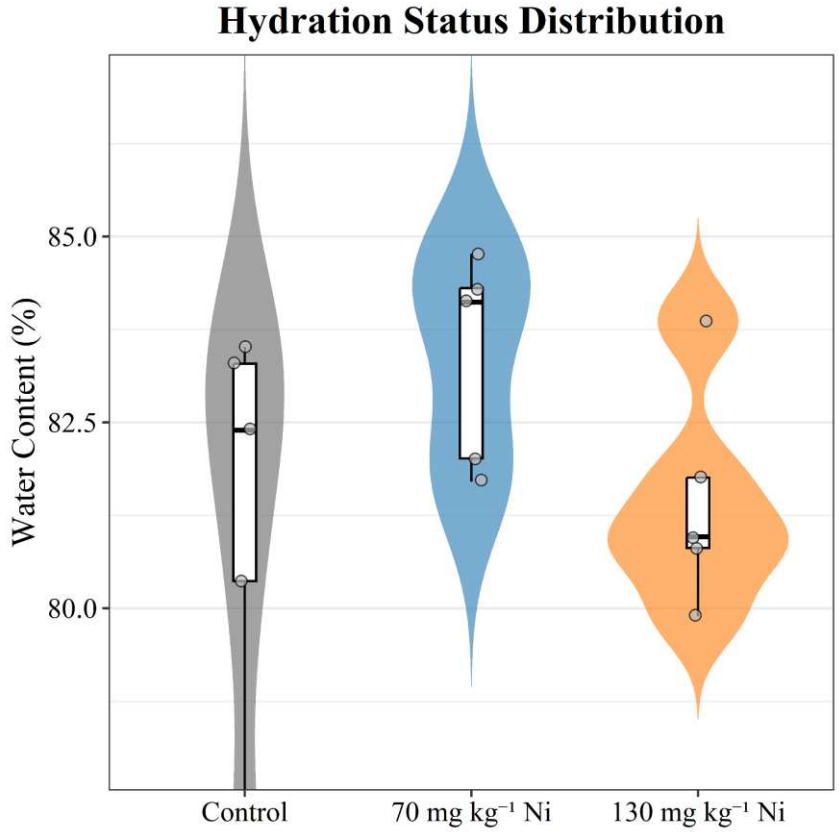

Fig. 5. Hydration status (Relative Water Content) of *Catharanthus roseus* at the end of the experimental period (day 119). The 70 mg kg^{-1} treatment exhibits the highest percentage of water retention, indicating a succulence mechanism for diluting the contaminant. Bars represent mean ± standard error ($n = 5$).

However, the quantification of dry biomass revealed the high metabolic cost of this strategy (Fig. 6). Upon removing the water component, it was found that nickel exposure severely impacted the capacity to convert carbon into structural tissue. Although the 70 mg kg^{-1} group presented a mean height greater than that of the control, its dry biomass (0.42 ± 0.11 g) was lower than that of the Control group (0.57 ± 0.31 g). This contrast confirms a state of "pseudo-growth": the plant invested energy in cell elongation (turgidity and primary wall) at the expense of tissue filling and robustness (true growth).

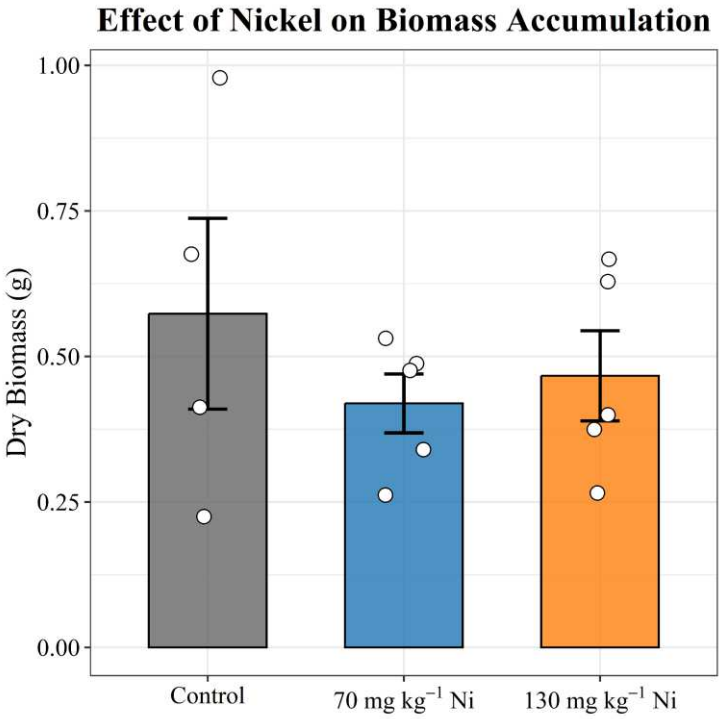

Fig. 6. Dry biomass accumulation in *Catharanthus roseus* after 120 days of cultivation under different nickel concentrations. Bars represent the mean ($n = 5$), and vertical lines indicate the standard error of the mean. White dots represent the dispersion of individual data points for each treatment.

To understand the nature of this dysfunctional growth, an integrated analysis of final height and dry matter accumulation was conducted (Fig. 7). The results reveal a dissociation in morphological strategies: while the Control group is positioned within the 'robustness' quadrant (lower height, higher density), the 70 mg kg^{-1} treatment shifts towards the 'etiolation' zone.

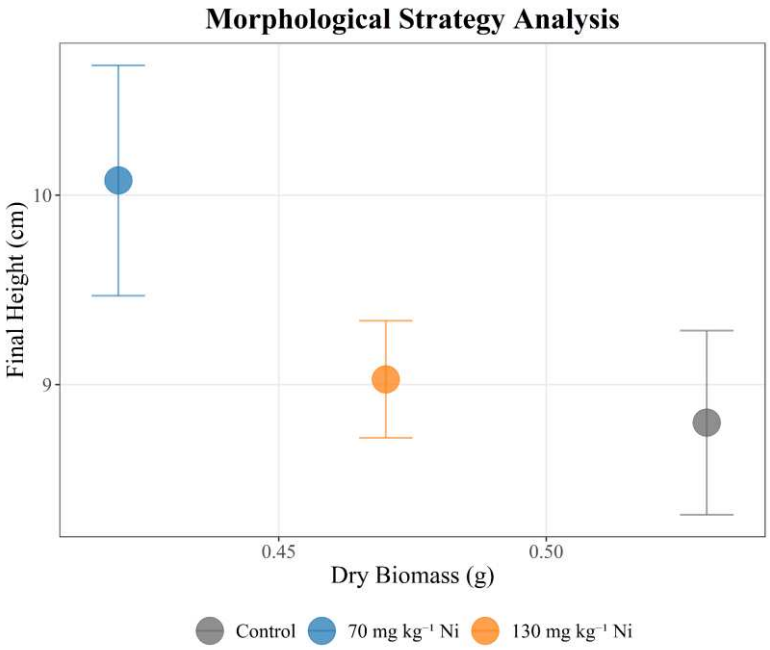

Fig. 7. Analysis of the morphological strategy of *Catharanthus roseus* under nickel stress. The graph relates final plant height to accumulated dry biomass. The shift of the 70 mg kg^{-1} group (orange) to the upper-left quadrant highlights the phenomenon of etiolation: high investment in vertical elongation with low gains in structural mass. Bars represent the standard error of the mean ($n = 5$).

This behaviour mimics a Shade Avoidance Syndrome-like response. Under moderate abiotic stress, disturbances in auxin and ethylene homeostasis, coupled with the production of Reactive Oxygen Species (ROS), can induce a Stress-Induced Morphogenic Response (SIMR) (Potters *et al.*, 2007). In this scenario, the plant interprets the chemical stress from nickel as an adverse environmental signal necessitating "escape", thus prioritising vertical stem elongation. However, this constitutes maladaptive plasticity: the metal induces the plant to allocate its scarce energy resources to stem elongation — an "escape to nowhere" — leaving little carbon available for leaf and root development, resulting in a fragile and hydrotic plant architecture (Ballaré & Pierik, 2017).

In contrast, under the severe treatment of 130 mg kg^{-1}, a collapse of both strategies (water defence and morphological escape) was observed, with a consistent reduction in both height and dry biomass, evidencing the metabolic failure induced by acute toxicity.

3.4 Photosynthetic Pigments (Chlorophyll)

Total chlorophyll content was quantified using the spectrophotometric method of Arnon (1949), following extraction with DMSO and absorbance readings at 663 nm

(chlorophyll a) and 645 nm (chlorophyll b). Exposure to nickel significantly reduced the levels of this pigment (one-way ANOVA: $F(2.12) = 10.07$; $p = 0.003$) Tukey's post-hoc analysis ($p < 0,05$) revealed that both treatments (70 and 130 mg kg^{-1} Ni) differed from the control group but not from each other (Fig. 8A).

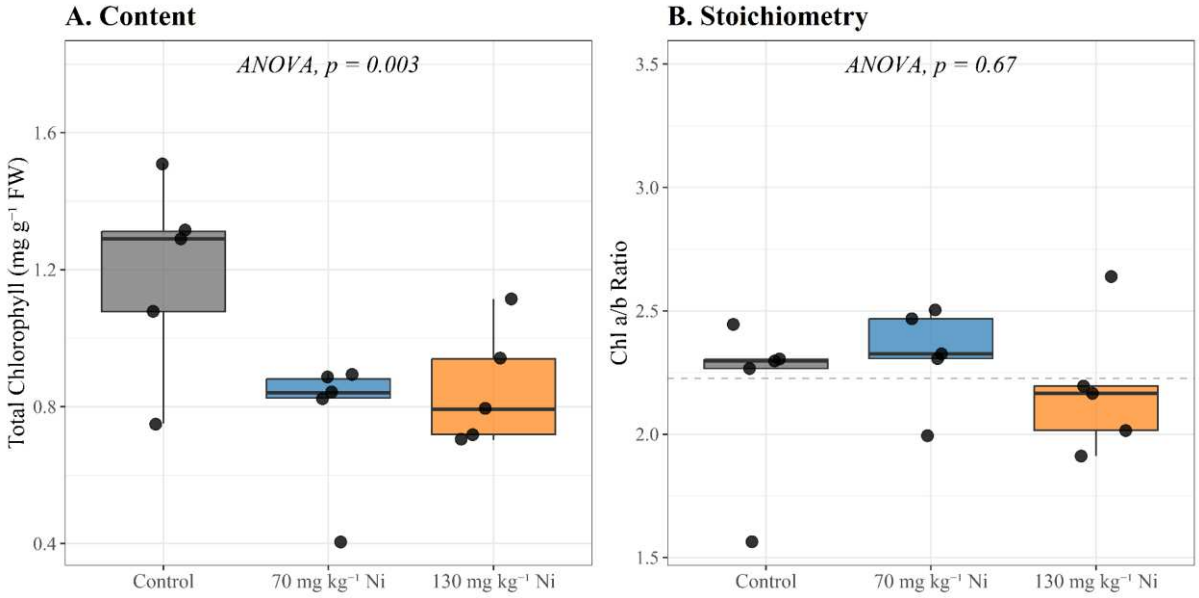

Fig. 8. Variation in photosynthetic pigments of *C. roseus* exposed to different nickel concentrations. (A) Total Chlorophyll content (mg g^{-1} fresh mass); (B) Stoichiometric ratio between Chlorophyll a and b. The boxplots represent the data distribution ($n = 5$), where the central line indicates the median, the boxes indicate the interquartile range, and the black dots represent individual observations. p values refer to the one-way ANOVA. The dashed line in (B) indicates the global mean of the a/b ratio.

In percentage terms, a reduction of 35.1% in chlorophyll content was observed in the 70 mg kg^{-1} treatment and 28.1% in the 130 mg kg^{-1} treatment relative to the control. The drastic and significant reduction in chlorophyll levels constitutes a classic biochemical marker of metal stress (Ahanger *et al.*, 2022; Sharma *et al.*, 2020) and is visually correlated with the observed chlorosis symptoms. The underlying mechanism likely involves ionic competition: the excess Ni^{2+} cation competes with Mg^{2+} — the central ion of the chlorophyll molecule — potentially inhibiting its biosynthesis or leading to the degradation of the molecule (Seregin & Kozhevnikova, 2006). The fact that there was no significant difference between the chlorophyll levels of the 70 and 130 mg kg^{-1} treatments (Fig. 8A) suggests that, under the conditions of this experiment, a concentration of 70 mg kg^{-1} of Ni acetate was already sufficient to reach a maximum threshold of negative impact on the biosynthesis of this pigment. This impairment of the photosynthetic apparatus represents a significant metabolic

cost for the plant under stress from this metal, even at concentrations considered intermediate.

On the other hand, the qualitative assessment of the photosynthetic apparatus revealed an important functional stability. The stoichiometric ratio between Chlorophyll a and b remained unaltered across the groups, staying constant at approximately 2.2 regardless of the nickel concentration (Fig. 8B; ANOVA, $p = 0.67$). This result indicates that, despite the quantitative reduction in pigment biomass (down-regulation), *C. roseus* preserved the coordination between the antenna complexes and the remaining reaction centres, suggesting a survival mechanism that avoids the disordered dismantling of the photosystems.

Additionally, the reduction in biomass proved to be strongly dependent on the photosynthetic status of the plant (Fig. 9). A direct linear correlation was observed between the total chlorophyll content and the accumulated dry biomass. The positioning of the treated groups (blue and orange) at the lower end of the regression line suggests that nickel toxicity acted primarily by destroying pigments or inhibiting chlorophyll biosynthesis, thereby limiting carbon fixation capacity and, consequently, preventing mass gain, regardless of water availability.

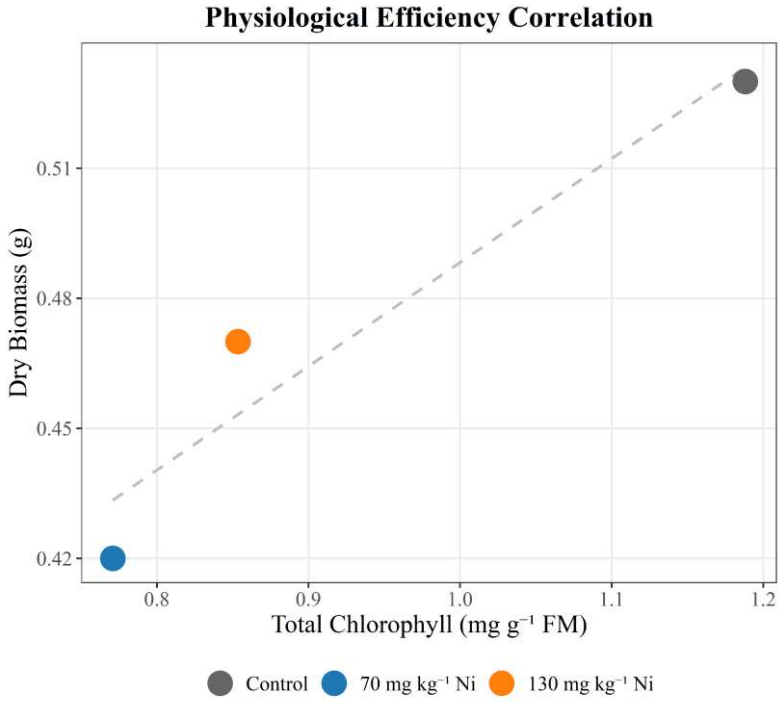

Fig. 9. Physiological correlation between the integrity of the photosynthetic machinery (Total Chlorophyll) and final productivity (Dry Biomass). The dashed line indicates a positive linear trend, demonstrating that biomass loss in the nickel-treated groups is directly associated with pigment degradation.

3.5 Predictive model under severe stress

Linear regression analysis revealed a strict allometric relationship between plant height and leaf number in the 130 mg kg^{-1} Ni treatment. The resulting model (Leaves = *1.024* × Height + *0.385*) explained 93% of the data variability (Fig. 10, $R^2 = 0.93, p < 0.01$). The statistical credibility of this model was visually confirmed by the proximity of the data points to the 1:1 line in Figure 11A and validated by the leave-one-out method, which yielded a Mean Absolute Percentage Error (MAPE) of only 3.97%. This demonstrates that, under severe toxicity, height becomes a high-accuracy predictor of leaf number.

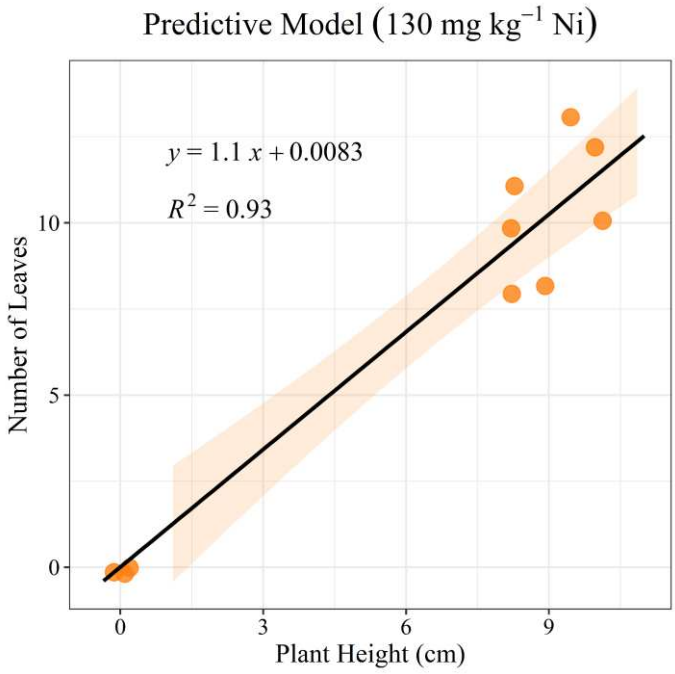

Fig. 10. Linear regression model estimating leaf number based on plant height for *Catharanthus roseus* exposed to 130 mg kg^{-1} Ni ($n = 10$). The inclusion of non-surviving plants (points at origin) highlights the populational response to toxicity. The black line represents the linear fit ($R^2 = 0.93$), and the orange shaded area indicates the 95% confidence interval.

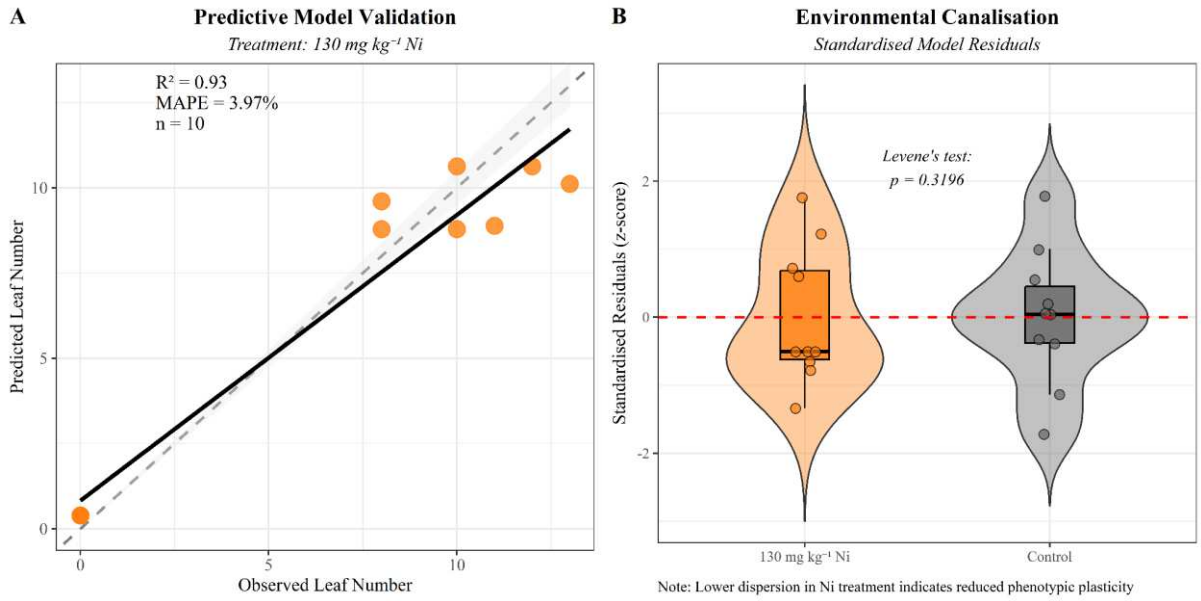

Fig. 11. Validation of the allometric model and analysis of phenotypic plasticity in *Catharanthus roseus*. (A) Validation plot comparing observed vs. predicted values (dashed line = perfect 1:1 prediction; $R = 0.97$). (B) Comparison of residual variability between Ni treatment and Control. Lower dispersion in the nickel treatment evidences environmental canalisation (Levene's test: $p < 0.05$).

The mathematical ordering is remarkable, especially considering the growth stagnation (negative AGR) observed in this treatment. This result suggests that although nickel suppressed overall biomass gain, the allometric coordination between organs was preserved. The plants, despite their limited development, maintained consistent morphophysiological proportions.

This high predictability at the maximum dose contrasts sharply with the greater variability observed in the control plants. As evidenced in Figure 11B, the control group exhibited higher dispersion in allometric residuals — an indicator of phenotypic plasticity, where the individual possesses the "freedom" to adjust its architecture. In contrast, plants under 130 mg kg^{-1} Ni presented significantly smaller residuals (Levene's test, $p < 0.05$), indicating a drastic reduction in this plasticity.

These findings corroborate the hypothesis of Environmental Canalisation. Under severe toxic stress, the high metabolic cost of dealing with nickel appears to restrict the plant's developmental pathways. Lacking surplus resources to vary its architecture, the organism is forced into a strict and mathematically predictable survival phenotype. This constitutes a unique finding, as while most abiotic stresses induce 'developmental noise' (an increase in instability and variation), acute toxicity at 130 mg kg^{-1} imposed a biological 'silence',

suppressing individual deviations. Paradoxically, the same toxicity that threatens the plant's survival is the factor that makes its morphological behaviour extremely ordered.

Conclusions

This study has demonstrated that nickel exposure induces a complex transition in *Catharanthus roseus* between strategies of phenotypic plasticity and allometric rigour. The response was strictly dose-dependent: while germination was severely inhibited (GSI of 0.54 at 130 mg kg^{-1} versus 2.0 in the control) and chlorophyll levels were reduced by up to 35%, the integrated biomass analysis revealed that the species' survival depends on a delicate balance between water maintenance and architectural coordination.

In the intermediate treatment (70 mg kg^{-1}), a succulence-by-dilution mechanism was identified, in which the plant maintained turgidity and stem elongation (etiolation), albeit with low dry biomass accumulation. This indicates an energy trade-off: the species prioritised water uptake to dilute the metal's toxicity, sacrificing structural robustness. Conversely, under severe stress (130 mg kg^{-1}), a collapse of this strategy was observed, with a simultaneous reduction in water content and growth stagnation (negative AGR).

Nevertheless, the most relevant finding was the high predictability of the allometric development. Even under extreme toxic conditions, the relationship between height and leaf number remained strongly correlated($r = 0{,}97$ on day 119), allowing for the construction of an accurate predictive model ($R^2 = 0{,}93$; $EPM = 3{,}97\%$). This suggests that *C. roseus* does not lose its morphofunctional integration but instead canalises its resources proportionally, preserving the best possible plant architecture within the limitations imposed by the stress.

For future studies, it is recommended to: (i) investigate osmoregulation mechanisms and stomatal conductance to deepen the understanding of the succulence strategy; (ii) quantify alkaloids (vinblastine and vincristine) to verify whether stress stimulates the production of secondary metabolites; and (iii) analyse nickel compartmentalisation to elucidate the physiological basis of the observed tolerance. Such data will complement the understanding of how *C. roseus* modulates its architecture and metabolism at the threshold of survival.

Acknowledgements

This study was developed with the support of a research scholarship from the State University of Montes Claros (UNIMONTES) and, in part, by the São Paulo Research Foundation (FAPESP), Brazil (Grant Number 2025/11544-9). We also thank the UNIMONTES Bioproducts Laboratory; Professor Sônia Ribeiro Arrudas, PhD, for providing the seeds and laboratory facilities; and Rozalio Rodrigues Franco for his assistance with plant monitoring.

References

Ahanger, M.A., Aziz, U., Alsahli, A.A., Alyemeni, M.N., & Ahmad, P., 2022. Influence of Exogenous Salicylic Acid and Nitric Oxide on Growth, Photosynthesis, and Ascorbate-Glutathione Cycle in Salt Stressed Vigna angularis. Biomolecules, 12(1), 42. https://doi.org/10.3390/biom12010042

Andrade, E.A., Barbosa, M.E.A., & Demetrio, G.R.., 2013. Density-dependent morphological plasticity and trade-offs among vegetative traits in Eichhornia crassipes (Pontederiaceae). Acta Amazonica, 43(4), 455–459. https://doi.org/10.1590/S0044-59672013000400007

Arefifard M, Mahdieh M, Amirjani M., 2014. Study of the effect of nickel heavy metals on some physiological parameters of *Catharanthus roseus*. Nat Prod Res. 2014;28(18):1499-502. doi: 10.1080/14786419.2014.913240. Epub 2014 May 28. PMID: 24870880. https://doi.org/10.1080/14786419.2014.913240

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol. 24(1), 1–15. https://doi.org/10.1104/pp.24.1.1

Ballaré CL, Pierik R., 2017 Nov. The shade-avoidance syndrome: multiple signals and ecological consequences. Plant Cell Environ. 40(11):2530-2543. doi: 10.1111/pce.12914. Epub 2017 Mar 1. PMID: 28102548. https://onlinelibrary.wiley.com/doi/10.1111/pce.12914

Banyal, A., Tiwari, S., Sharma, A., Chanana, I., Patel, S.K.S., Kulshrestha, S., Kumar, P., 2023. Vinca alkaloids as a potential cancer therapeutics: recent update and future challenges. 3 Biotech 13, 211. https://doi.org/10.1007/s13205-023-03636-6

Burress E.D., Muñoz M.M., 2023 May 19. Functional Trade-Offs Asymmetrically Promote Phenotypic Evolution. Syst Biol. 72(1):150-160. doi: 10.1093/sysbio/syac058. PMID: 35961046. https://pubmed.ncbi.nlm.nih.gov/35961046/

Chakraborty, D., & Jha, L., 202). Unlocking the therapeutic potential of *Catharanthus roseus*: A modern pharmacological perspective on a historic medicinal plant. International Journal for Multidisciplinary Research (IJFMR), 7(4). https://pubmed.ncbi.nlm.nih.gov/40676081/

Chuah, Y.Y., Lee, Y.Y., Chou, C. K., & Chang, L.J., 2024. *Catharanthus roseus* intoxication mimicking acute cholangitis. BMC Complementary Medicine and Therapies, 24 (1), 139. https://doi.org/10.1186/s12906-024-04441-1

Colombo, R., Rossini, M., Meroni, M., Panigada, C., Cogliati, S., Julitta, T., Guanter, L., 2018. Variability of sun-induced chlorophyll fluorescence in loblolly pine forests. Glob. Chang. Biol., 24(7), 2980–2996. https://doi.org/10.1111/gcb.14081

Cordeiro, SZ., 2019. *Catharanthus roseus* (L.) G. Don. Universidade Federal do Estado do Rio de Janeiro (UNIRIO). Recuperado de

https://www.unirio.br/ccbs/ibio/herbariohuni/catharanthus-roseus-l-g.don

Duffin J, 2000. Poisoning the spindle: serendipity and discovery of the anti-tumor properties of the Vinca alkaloids. Can Bull Med Hist. 2000 Nov;17(1-2):155-92. doi: 10.3138/cbmh.17.1.155. PMID: 14526803. https://doi.org/10.3138/cbmh.17.1.155

Ferreira, MM., Motta, MB., Pinto, JEBP., & Castro, EM., 2004. Crescimento e alocação de biomassa de plantas de vinca (*Catharanthus roseus (L.) G. Don*) em função da adubação orgânica e época de colheita. Revista Brasileira de Plantas Medicinais, 6(2), 72–76. Recuperado de https://www.sbpmed.org.br/admin/files/papers/file_weqpNq0ZfHmt.pdf

Flatt, T., & Wagner, G., 2018. Canalization and robustness. In Evolutionary Biology (Oxford Bibliographies). Oxford University Press. https://doi.org/10.1093/OBO/9780199941728-0109

Jalmi, SK., Bhagat, PK., Verma, D., Noryang, S., Tayyeba, S., Singh, K., Sharma, D., & Sinha, AK., 2018. Traversing the links between heavy metal stress and plant signaling. Front. Plant Sci., 9, 12. https://doi.org/10.3389/fpls.2018.00012

Jyothsna, K., Sowmithri, Ch., Bhagyasri, H., & Kalpana, T., 2025. Role, toxicity and remediation of nickel in plants: A review. Journal of Integral Sciences, 8 (2), 11–23. https://doi.org/10.37022/jis.v8i2.106

Kumar, S., Singh, B., & Singh, R., 2022. *Catharanthus roseus (L.) G. Don*: A review of its ethnobotany, phytochemistry, ethnopharmacology and toxicities. Journal of Ethnopharmacology, 284, 114647. https://doi.org/10.1016/j.jep.2021.114647

Magalhães, NS., Marenco, RA., & Mendes, KR., 2010. Aclimatação de mudas de acariquara à alta irradiância. Pesquisa Agropecuária Brasileira, 44(7), 687–694. https://doi.org/10.1590/S1678-3921.pab2009.v44.2064

Missouri Botanical Garden., 2026. *Catharanthus roseus* – Plant Finder. Retrieved January 25, 2026, from https://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kempercode=a569

Nascimento, KC., 2019. Tendências evolutivas da plasticidade fenotípica em plantas diante de distintos cenários de mudanças globais (Doctoral dissertation). Universidade Estadual de Maringá, Maringá, Brasil. http://repositorio.uem.br:8080/jspui/handle/1/4889

National Environmental Council [CONAMA]., 2009, December 28. Resolução nº 420, de 28 de dezembro de 2009: Dispõe sobre critérios e valores orientadores de qualidade do solo quanto à presença de substâncias químicas e estabelece diretrizes para o gerenciamento ambiental de áreas contaminadas por essas substâncias em decorrência de atividades antrópicas [Resolution No. 420, December 28, 2009: Establishes criteria and guiding values for soil quality regarding the presence of chemical substances and sets guidelines for environmental management of contaminated areas resulting from anthropic activities]. Diário Oficial da União, Brasília, DF. https://conama.mma.gov.br/?option=com_sisconama&task=arquivo.download&id=589

Niklas, KJ., & Enquist, BJ., 2001. Invariant scaling relationships for interspecific plant biomass production rates and body size. Proceedings of the National Academy of Sciences, 98(5), 2922–2927. https://doi.org/10.1073/pnas.041590298

Olzacki, M., Bosiacki, M., & Świerczyński, S., 2025. The effect of increasing doses of heavy metals on seed germination of selected ornamental plant species. Agronomy, 15(6), 1262. https://doi.org/10.3390/agronomy15061262

Pérez-Ramos, I.M., Matías, L., Gómez-Aparicio, L., Godoy, Ó., 2019. Functional traits and phenotypic plasticity modulate species coexistence across contrasting climatic conditions. Nat Commun 10, 2555. https://doi.org/10.1038/s41467-019-10453-0

Poorter, H., Niklas, KJ., Reich, PB., Oleksyn, J., Poot, P., & Mommer, L., 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist, 193(1), 30–50. https://doi.org/10.1111/j.1469-8137.2011.03952.x

Potters G, Pasternak TP, Guisez Y, Palme KJ, Jansen MA, 2007. Stress-induced morphogenic responses: growing out of trouble? Trends Plant Sci. 2007 Mar;12(3):98-105. doi: 10.1016/j.tplants.2007.01.004. Epub 2007 Feb 6. PMID: 17287141. https://doi.org/10.1016/j.tplants.2007.01.004

Rani, N., 2022. Botanical features of periwinkle, *Catharanthus roseus*. In The Catharanthus Genome (pp. 1–14). Springer Nature. https://doi.org/10.1007/978-3-030-89269-2_1

Royal Botanic Gardens, Kew., 2026. *Catharanthus roseus (L.) G.Don*. Plants of the World Online. Retrieved January 25, 2026, from https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:80634-1

Sarkar, S., & Sharma, S., 2025. Therapeutic insights into *Catharanthus roseus*: Advances in vinca alkaloid production and cancer treatment. South African Journal of Botany, 184, 780–793. https://doi.org/10.1016/j.sajb.2025.06.045

Seregin, IV., & Kozhevnikova, AD., 2006. Physiological role of nickel and its toxic effects on higher plants. Russian Journal of Plant Physiology, 53(2), 257–277. https://doi.org/10.1134/S1021443706020178

Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M., 2020. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. Journal of Botany, 2020, 1-26. https://doi.org/10.1155/2012/217037

Silva, N., 2021. Changes in the growth-survival trade-off in a Brazilian tropical basin (Master's thesis). Universidade Federal de Viçosa, Viçosa, MG. https://locus.ufv.br//handle/123456789/28102

Soumya, V., Basira, H. & Kiranmayi, P., 2023. Potential of *Catharanthus roseus* applied to remediation of disparate industrial soils owing to accumulation and translocation of metals into plant parts. International Journal of Phytoremediation, 25 (6), 746–758. https://doi.org/10.1080/15226514.2022.2106183

Spagnuolo PA, Hu J, Hurren R, Wang X, Gronda M, Sukhai MA, Di Meo A, Boss J, Ashali I, Beheshti Zavareh R, Fine N, Simpson CD, Sharmeen S, Rottapel R, Schimmer AD., 2010. The antihelmintic flubendazole inhibits microtubule function through a mechanism distinct from Vinca alkaloids and displays preclinical activity in leukemia and myeloma. Blood. 2010 Jun 10;115(23):4824-33. doi: https://doi.org/10.1182/blood-2009-09-243055. Epub 2010 Mar 26. PMID: 20348394.

Srivastava NK, Srivastava AK., 2010. Influence of Some Heavy Metals on Growth, Alkaloid Content and Composition in *Catharanthus roseus L*. Indian J Pharm Sci.

Nov;72(6):775-8. doi: https://doi.org/10.4103/0250-474x.84592. PMID: 21969751; PMCID: PMC3178980.

Stearns, Stephen C., 1998. The Evolution Of Life Histories. Oxford University Press, Oxford. https://doi.org/10.1093/oso/9780198577416.001.0001, accessed 22 Jan. 2026.

Valladares F, Gianoli E, Gómez JM., 2007. Ecological limits to plant phenotypic plasticity. New Phytol. 176(4):749-763. doi: 10.1111/j.1469-8137.2007.02275.x. PMID: 17997761. https://pubmed.ncbi.nlm.nih.gov/17997761/

Vasseur, F., Exposito-Alonso, M., Ayala-Garay, O.J., Wang, G., Enquist, B.J., Vile, D., Violle, C., & Weigel, D., 2018. Adaptive diversification of growth allometry in the plant Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America, 115(13), 3416–3421. https://doi.org/10.1073/pnas.1709141115.

Vaz de Lima, D.M., & Langaro, A.P., 2019. Plasticidade fenotípica: revisão de conceitos, metodologias mais utilizadas e aplicações. Universidade Estadual de Mato Grosso do Sul. https://anaisonline.uems.br/index.php/enic/article/view/2486

Waddington, C.H., 1942. Canalization of development and the inheritance of acquired characters. Nature, 150, 563–565. https://doi.org/10.1038/150563a0

Wang, S., & Callaway, R.M., 2024. Associations between developmental stability, canalization, and phenotypic plasticity in response to heterogeneous experience. Ecology and Evolution, 14(9), e11234. https://doi.org/10.1002/ece3.70436

World Flora Online., 2026. *Catharanthus roseus (L.) G.Don*. Retrieved January 25, 2026, from http://www.worldfloraonline.org/taxon/wfo-0000819442

Wright, J.R., 2002. Almost famous: E. Clark Noble, the common thread in the discovery of insulin and vinblastine. CMAJ, 167(12), 1391–1396. https://www.cmaj.ca/content/167/12/1391