

EVALUATION OF DIFFERENT NICKEL DOSES ON SOIL NUTRITIONAL ASPECTS AND SEEDLINGS GROWTH OF CANOLA

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ABSTRACT

Heavy metals found in industrial waste, particularly nickel, have the potential to impact soil fertility as well as crop yields. The primary goal of this study was to determine which specific nickel dosage had a detrimental impact on soil properties as well as canola production. The CRD approach was used to design the experiment with

three biological replications. The Ni dose @ 0, 1000 μM by 200 was applied to soil. The nickel at 1000 μM increased soil pH and Ec by 5.35% and 391.85% as compared to control. The increase in soil pH and Ec due to nickel at 1000 μM decreased the soil macronutrients including nitrogen, phosphorus and potassium by 82%, 79.57% and 80.04% and micronutrients including copper, zinc and magnesium by 84.72%, 55.35% and 91.28% as compared to control. The canola seedlings growth aspects including shoot/ root length, fresh weight and dry weight were decreased by 79.95%/61.94%, 61.02%/97.02% and 69.85%/96.99% in response to nickel at 1000 μM over control. The correlation analysis clearly indicated the soil-plant interaction under toxicity of highest dose of nickel. Future research work is needed on mitigation approaches for sustainable agriculture practices.

Keywords: Waste Management, Heavy Metal Stress, Soil Pollution, Fertility, Sustainability

Introduction

Soil contamination with industrial waste is considered to be a serious environmental issue (Aioub et al., 2019). Soil microbial activity and diversity are highly sensitive to soil contamination by organic and inorganic pollutants, including heavy metals (Gondal et al., 2021). It was found that both short-term and long-term exposures to heavy metals resulted in the reduction of soil microbial activity and diversity (Atkinson et al., 2021). Therefore, soil microbial activity and community structure are accepted as indicators of overall soil environmental quality. Among all the pollutants in soils, heavy metals are of significant concerns due to their inherent toxicity, bioaccumulation, persistence, and non-degradability (Mansoor et al., 2023). Heavy metals are considered as contaminants when their occurrences are unwanted, or in forms or concentrations resulting in detrimental effects to human or the ecosystem (Yaashikaa et al., 2022). Although heavy metals rarely occur at toxic levels naturally, intensive anthropogenic activities can drastically alter the natural biogeochemical cycling and balances of some heavy metals (Ishaq et al., 2024). They interfere with physiological activities of plants such as photosynthesis, gaseous exchange and nutrient absorption and cause reduction in plant growth, dry matter accumulation and yield (Saxena et al., 2020; Ghuge et al., 2023).

Soil is contaminated with heavy metals and is a worldwide problem, causing reduction in agricultural yield and perilous health effects when became part of food chain (Oyewo et al., 2020). In environment, Ni concentration is increasing due to over use of nickel and nickel compounds for commercial and industrial uses. Application of industrial waste to agricultural areas is contributing in mixing of trace elements in environment. Nickel level is increasing in soil due to this anthropogenic release and is responsible for collateral increase of nickel concentration in plants as well as in food chain (Haque et al., 2021; Shafiq et al., 2024). Many biochemical processes and physiological processes are affected in plants when nickel is up taken in toxic level. Chlorosis, inhibited photosynthesis and respiration are the most frequent symptoms (Ghori et al., 2019). In the list of heavy metals in earth crust, nickel has unique place. For plant growth, it is an essential micronutrient and is a component of urease enzyme which is necessary in higher plants for nitrogen metabolism (Shafiq et al., 2024).

Many physiological and biochemical processes are effected by Ni because of its essentiality and as toxic in toxic level. Respiration, chlorosis and inhibited photosynthesis are very common symptoms. Existence of nickel in soil is in different forms, like precipitate form, in the form of complexed or absorbed on cation exchange sites or absorbed on organic cation surfaces and also in the form of free ion or in soil solution, complex chelated metals (Giannakoula et al., 2021; Hafeez et al., 2024). There is still no research work on the toxicity of increasing dose of Ni on soil and plant aspects. Therefore, we hypothesized that evaluation of nickel toxicity on soil and plant aspects could help to plan the mitigation approaches. The main objective of this study was to check the different doses nickel on soil macro and micro nutrients and canola seedlings growth aspects.

Material and Methods

Seeds of canola variety viz. Chakwal sarson were obtained from Barani Agricultural Research Institute Chakwal, Pakistan. The seeds were surface sterilized with 0.1% sodium hypochlorite solution and rinsed thrice with double distilled water. The seeds were placed in the plastic pots (12 ×10) containing 7.5 kg of soil. The initial nutrients were applied to the soil during sowing time. The plants were allowed to grow and thinning were performed to maintain the proper seedlings. During the 2nd leaf stage, the nickel doses at 0 to 1000 µM by 200 were applied to the soil in the form of nickel chloride. All the agronomic practices were done throughout the experiment. After 2 weeks of stress imposition, plants were harvested for further analysis. The experiment was laid out in CRD-factorial approaches including three biological replications.

Soil Chemical Analysis

A soil sample was mixed with distilled water and a pH meter was used to measure the soil pH value. A saturated paste was prepared from the soil and water, and the EC of the soil was determined using an EC meter in Deci Siemens per meter. The soil N was measured by using the procedure of digestion, distillation and titration from khjedhal apparatus and method to follow the procedure was followed by (Bremner, 1960). Phosphorus was measured by wet digestion of soil sample filtrate, calibrated using running standards, and analyzed on a spectrophotometer at 410 nm wavelength (Hambleton, 1977). The digested filtrate was diluted and 'K' concentration was measured on a flame photometer. The process involved adding 1ml of soil sample, 5ml of distilled water, and 4ml of lithium chloride solution (Singh et al., 1983).

Seedlings Growth Aspects

The shoot and root samples from each treatments were harvested and used for the measurement. The shoot and root length were measured manually by using measuring rod. The fresh weight was measured by using weighing balance and their dry weight was also measured after air drying at 24 °C.

Statistical analysis

The soil and plant data were collected and analyzed by using one way ANOVA. The significant or non-significant difference among treatment means were checked by using Tukey HSD.test. The correlation analysis and principle component analysis

were done through R programming software.

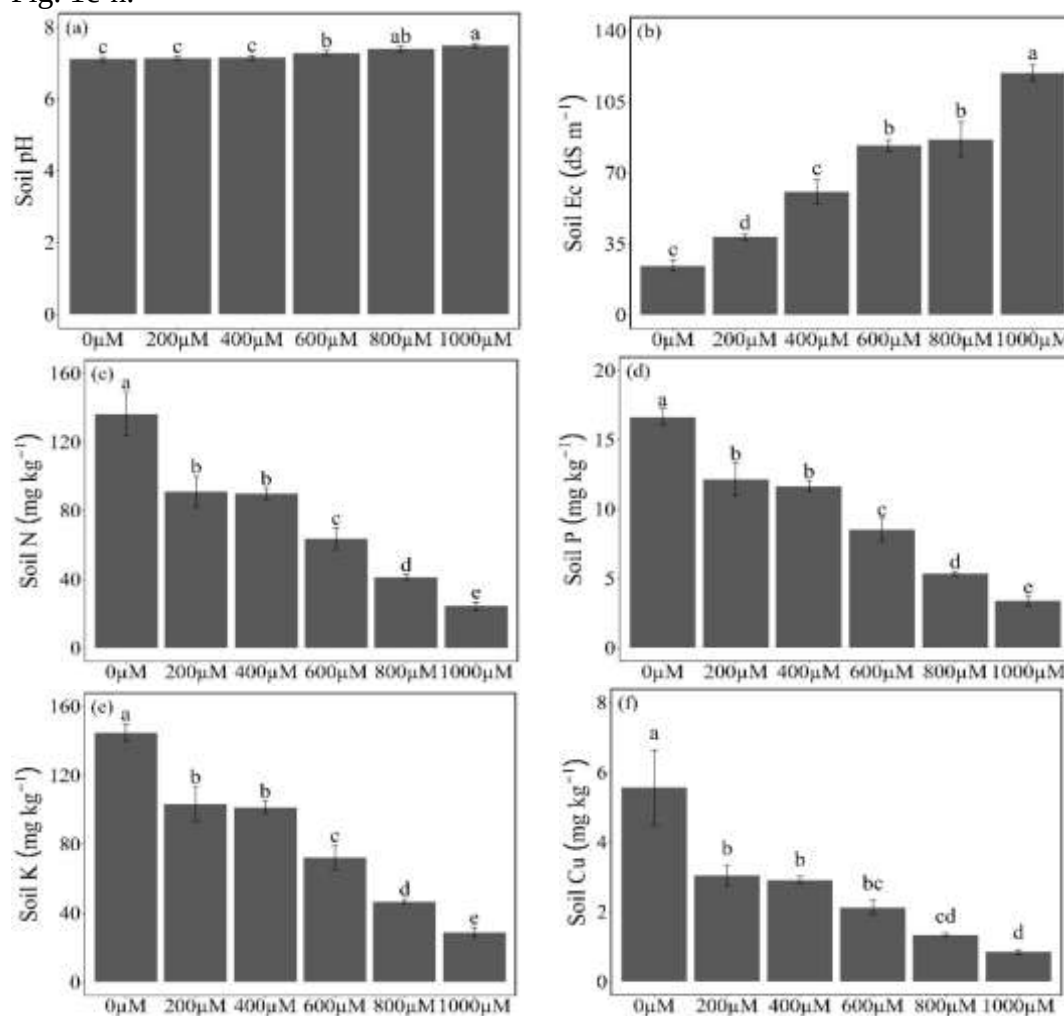
Results

Soil pH and Ec

Nickel (Ni) stress posed significant alterations in soil pH and electrical conductivity (EC) ($P < 0.05$). It was noticed that soil pH was maximum in response to Ni at 1000 μM by 5.35%, followed by 4.08% in response to 800 μM , as compared to the control as shown in Fig. 1a. However, the variation among Ni at 1000 μM was statistically non-significant. The Ni at 1000 μM significantly folded 3 times the soil EC as compared to other Ni treatments and the control as shown in Fig. 1b.

Soil Macro and Micro Nutrients

There is significant variation among all nickel (Ni) treatments in terms of soil macro nutrients including nitrogen (N), phosphorus (P), potassium (K) and micro nutrients including copper (Cu), zinc (Zn) and magnesium (Mg) ($p < 0.05$). It was noticed that higher dose of Ni gave maximum reduction in soil N, P, K, Cu, Zn, and Mg by 82%, 79.57%, 80.04%, 83.72%, 55.35%, and 91.28% as compared to control as shown in Fig. 1c-h.



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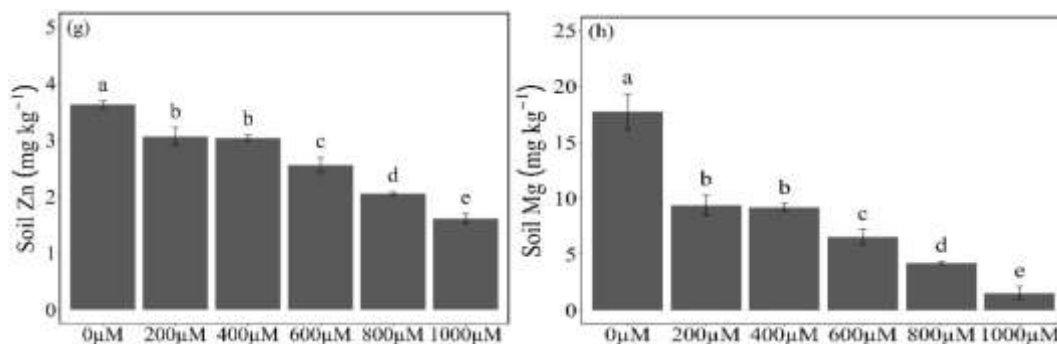
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Figure 1. Effect of different doses of nickel from 0 µM to 1000 µM on soil (a) nitrogen (b) phosphorus (c) potassium (d) copper (e) zinc (f) magnesium in canola growth. The bars are mean of three replications and error bar is standard deviation. The significant or non-significant difference among mean was presented through alphabet lettering on error bar.

Plant growth aspects

There is significant variation in plant growth aspects including shoot length (SL), root length (RL), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW) in response to nickel (Ni) stress ($p < 0.05$). The highest dose of Ni gave maximum reduction in all growth aspects of canola. The Ni at 1000 µM significantly decreased SL, SFW, SDW, RFW, and RDW by 79.95%, 61.92%, 69.85%, 97.02% and 96.99% as compared to control. However, the Ni at 1000 µM increased RL by 61.94% followed by 51.07% in response to Ni at 800 µM with no significant difference as shown in Table 1.

Table 1: Effect of different doses of nickel on seedling growth aspects of canola

Nickel	SL	RL	SFW	SDW	RFW	RDW
0µM	39.17±0.55 a	7.55±0.13 a	7.17±0.12 a	3.32±0.05 a	1.68±0.03 a	1.00±0.01 a
200µM	33.01±0.47 b	7.10±0.12 b	6.75±0.11 b	2.65±0.04 b	1.28±0.02 b	0.66±0.01 b
400µM	29.32±0.42 c	5.84±0.1c c	5.55±0.09 c	2.03±0.03 c	1.00±0.02 c	0.41±0.01 c
600µM	16.61±0.24 d	4.44±0.08 d	4.22±0.07 d	1.60±0.03 d	0.36±0.01 d	0.24±0.01 d
800µM	10.33±0.15 e	3.66±0.06 e	3.47±0.06 e	1.06±0.02 e	0.14±0e	0.11±0.00 e
1000µM		2.87±0.05 f	2.73±0.05 f	1.00±0.02 f		0.03±0.00 f
M	7.85±0.11f				0.05±0e	

The data are mean of three replications and standard deviation. The significant or non-significant difference among mean was presented through alphabet lettering. SL means shoot length; RL means root length; SFW means shoot fresh weight; SDW means shoot dry weight; RFW means root fresh weight and RDW means root dry weight.

weight.

Correlation analysis

Under normal conditions, the Pearson correlation analysis between soil parameters (pH, EC, N, P, K, Cu, Zn, and Mg) and plant growth aspects (SL, RDW, RFW, SDW, RL, SFW) revealed several significant associations as shown in Fig. 2a. Notably, Cu exhibited a strong and significant negative correlation with SL ($r = -0.92$), with all values statistically non-significant. Conversely, P showed a perfect positive correlation with EC ($r = 1.00$, $p < 0.001$), Zn ($r = 1.00$, $p < 0.01$), and Mg ($r = 1.00$, $p < 0.01$). EC and Zn were also positively and significantly correlated ($r = 1.00$, $p < 0.01$). Among soil plant interaction, N demonstrated a moderate positive but non-significant correlation with root and shoot parameters. Overall, a strong soil-plant interaction was observed between phosphorus-related soil properties and plant biomass traits, while copper exhibited a consistently negative association with plant growth aspects. Under nickel stress at 1000 μM , the correlation analysis revealed significant soil plant interactions. Soil pH exhibited a moderate positive correlation with SDW ($r = 0.60$, non-significant, $p > 0.05$) and plant height ($r = 0.60$, non-significant, $p > 0.05$) as shown in Fig. 2b. The EC, N, P, and K showed perfect positive correlation with zinc Zn and Cu contents (both $r = 1.00$, significant at $p < 0.001$). These soil parameters also demonstrated strong positive correlation with SL (r ranging from 0.87 to 1.00, significance varying from $p < 0.05$ to $p < 0.001$), RL (r ranging from 0.79 to 1.00, with significance levels between $p < 0.05$ and $p < 0.001$), SFW (r between 0.80 and 0.82, significant at $p < 0.05$), and RFW (r between 0.81 and 0.82, significant at $p < 0.05$). Among plant parameters, the Mg had strong negative correlation with RFW ($r = -1.00$, $p < 0.001$) and SFW ($r = -1.00$, $p < 0.001$), a moderate significant negative correlation with RL ($r = -0.62$, $p > 0.05$), and a moderate non-significant negative correlation with SL ($r = -0.46$, $p > 0.05$). Additionally, SDW was positively correlated with pH ($r = 0.60$, non-significant, $p > 0.05$) and Mg ($r = 0.35$, non-significant, $p > 0.05$), understanding the varied degrees of association between soil nutrients and plant growth traits under nickel stress.

Principle component analysis

The principle component analysis (PCA) of soil and plant parameters under different nickel stress levels revealed clear treatment-specific clustering and variable contributions. The first principle component (Dim1) accounted for 96.4% of the total variance, while the second component (Dim2) explained 2.1%, together capturing 98.5% of the total variation. The 0 μM Ni treatment grouped distinctly on the negative side of Dim1, closely associated with positive plant growth and nutrient parameters such as shoot dry weight (SDW), root length (RL), shoot length (SL), shoot fresh weight (SFW), root fresh weight (RFW), nitrogen (N), phosphorous (P), potassium (K), and zinc (Zn). In constant, higher Ni treatments (especially 1000 μM and 800 μM) were positioned on the positive side of Dim1, indicating their association with increased soil electrical conductivity (EC), pH, and copper (Cu) content, and a divergence from growth-related traits. The 600 μM treatment aligned with EC and pH vectors, while the 200 μM and 400 μM treatments clustered near intermediate positions along Dim1, suggesting dose-dependent impacts. Overall, PCA

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differentiated Ni stress levels based on coordinated changes in soil and plant parameters as shown in Fig. 2c.

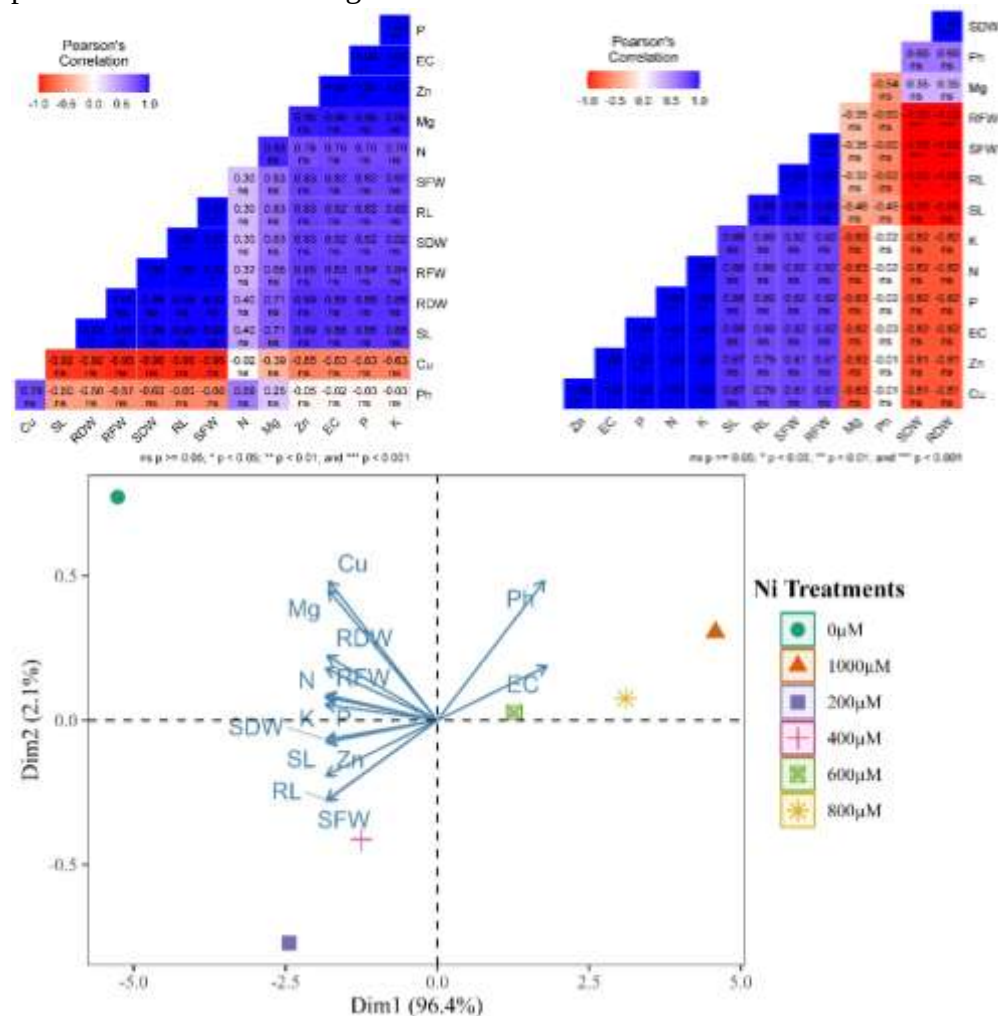


Figure 2. The Pearson correlation analysis among all soil and plant parameters under (a) Normal conditions (b) Nickel stress

Discussion

We observed that soil pH was increased under nickel (Ni) stress and increased by increasing Ni concentrations. The pH of the soil is influenced by nickel stress through both direct and indirect mechanisms. The pH of the effluent remains largely unchanged, yet it is more influenced by external factors. Plants may release alkaline root exudate particularly carboxylates to chelate Ni, reducing its bioavailability while increasing rhizosphere pH (Dixit et al., 2022). According to Turan et al. (2022), Ni forms less soluble hydroxides ($\text{Ni}(\text{OH})_2$) immobilization further increases pH. In present study, Ni stress has been observed to indirectly increased soil electrical conductivity (Ec) as shown in Fig. 1. Changes in soil properties due to heavy metals can influence Ec by altering the ionic composition of the soil solution (Beiyan et al., 2018). According to Ishaq et al. (2024), Ni toxicity decreases root cell integrity, causing leakage of intracellular ions (K^+ , Ca^+ , Mg^{2+}) into rhizosphere. In present

study, Ni stress decreased soil nitrogen as shown in Fig. 2. Nickel stress diminishes soil nitrogen availability by impairing nitrogen metabolism and decreasing the plant's nitrogen uptake (Faizan et al., 2024). Elevated nickel concentrations hinder the function of essential nitrogen fixation enzymes, including nitrate, phosphonitrate, and urease, thereby disrupting nitrogen transport within the soil (Rampazzo et al., 2022; Ishaq et al., 2024). Nitrogen accumulation in plant tissues leads to soil fertility decline, while toxicity restricts nitrogen availability, negatively impacting crop yield (Lu et al., ; Zhang et al., 2023). We observed that Ni stress was found to disrupt phosphorus bioavailability by reducing soil phosphorus availability. Elevated Ni levels can react with available phosphorus in soil, forming stable, partially soluble Ni-P compounds, decreasing phosphorus availability to plants. In addition, Ni toxicity decreases phosphorus soluble bacteria and adversely affects soil fertility and plant growth and development (Tian et al., 2021).

In present study, Ni stress may indirectly influence soil potassium availability by disrupting the nutrient uptake cycle in plants and affecting soil microorganisms (Chen et al., 2020). Elevated Ni concentrations can inhibit plant growth and root development, thereby restricting potassium absorption from the soil (Naveed et al., 2020). Additionally, Ni toxicity can disrupt nutrient cycling processes, which may decrease potassium availability for plant utilization (Hafeez et al., 2024). Our study found that soil copper (Cu) was decreased in response to Ni stress as shown in Fig. 2. Ni exhibits physicochemical similarities to Cu^{2+} , enabling it to compete for uptake via high-affinity transporters like COPT1 and ZIP2 (Thapa et al., 2021). The Ni interferes with Cu distribution by binding to transporters with high affinity, thereby inhibiting copper uptake into root cells (Mir et al., 2021). Nickel-induced Cu deficiency impacts electron transport chains, resulting in elevated reactive oxygen species (ROS), including hydroxyl radicals, due to incomplete electron transfer (Daulta et al., 2023). Plants may excessively produce phytochelators to sequester nickel, which inadvertently binds copper and increases its bioavailability (Roshid et al., 2023). In present study, the soil zinc (Zn) was decreased in response to Ni treatment as shown in Fig. 2. The Ni inhibits Zn^{2+} binding to NRAMP and ZIP family transporters in a competitive manner, as these transporters exhibit a lack of ion specificity (El-Samad & Taha, 2022). Zinc is crucial for the activity of copper/zinc superoxide dismutase enzymes, and deficiency can lead to hydrogen peroxide accumulation and lipid peroxidation (Verma et al., 2021; Natasha et al., 2022). Nickel-induced zinc deficiency modifies the auxin gradient, hinders root elongation, and diminishes the capacity of zinc to acquire resources from the soil (Thapa et al., 2021). We observed that Ni stress decreased soil magnesium (Mg) as shown in Fig. 2. The Ni^{2+} substitutes for Mg^{2+} in the tetrapyrrole rings of chlorophyll, resulting in a non-photosynthetic pigment (Tränkner et al., 2018). This substitution diminishes light-harvesting efficiency and enhances singlet oxygen production, thereby accelerating chloroplast degradation (Koç & Karayiğit, 2022).. Nickel displaces Mg^{2+} in ATP binding sites, including P-type ATPases, resulting in non-specific phosphorylation and depletion of cellular energy reserves (Cao et al., 2022; Rahim et al., 2025).

Under Ni stress, all soil macro and micro nutrients showed strong effects on canola seedlings growth aspects. It was found that high soil pH, Ec, N, P, and K were strongly associated with Zn and Cu contents, supporting effective seedlings growth at

lower Ni levels. Notably, increasing Ni levels disturbed positive correlations as shown in PCA clustering with higher Ni treatments linked to increased EC, pH, and Cu but reduced growth aspects of canola. Remarkably, Mg demonstrated strong negative correlation with fresh biomass, indicating antagonistic effects under Ni toxicity. Collectively, soil nutrient availability and balance were essential for sustaining canola seedling growth but their positive effect decreased as Ni stress increased.

Conclusion

The presence of heavy metals in industrial waste, such nickel in particular, has the potential to have an effect on the fertility of the soil as well as the yields of crops. The most important objective of this research was to identify the particular nickel dose that had a negative influence on the characteristics of the soil and the amount of canola that was produced. At a concentration of 1000 μM , the pH and Ec of the soil were elevated in comparison to the control. When compared to the control, the increase in soil pH and Ec caused by nickel at a concentration of 1000 μM resulted in a decrease in the quantities of essential nutrients found in the soil. These nutrients included nitrogen, phosphorus, potassium, copper, zinc, and magnesium. Exposure to nickel at a concentration of 1000 μM had an effect on the growth characteristics of canola seedlings, such as the length of the shoots and roots, the fresh weight, and the dry weight. This was discovered in comparison to the control group. Given the toxicity of the greatest amount of nickel, the correlation analysis revealed that there was an interaction between the soil and the plants. In the future, we need to do study on different mitigation strategies for environmentally responsible agricultural practices.

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