

Research Article

Impaired Growth with declined pigment content and biochemical toxicity induced by Nickel (Ni^{+2}) in sixty days exposed *Sesbania sesban* seedlings

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Abstract

Heavy metal contamination, driven by both natural and human activities, poses significant environmental risks. Nickel, a key heavy metal, harms plants and animals when its concentration exceeds safe levels. This study investigates the phytotoxic effects of various nickel concentrations on the biochemical parameters such as chlorophyll, carotenoid, proline, protein and catalase activity of *Sesbania* species. A pot experiment was conducted with nickel concentrations of 50, 100, 200, and 300 ppm alongside a control group (0 ppm), using NiCl_2 as the source of nickel and the observations were made over 60 days. Results showed a significant decline in shoot length, from 63.4 cm to 12.2 cm, with growth inhibition observed at 200 ppm to 300 ppm. Chlorophyll and carotenoid concentrations decreased 6-7 times in plants exposed to 300 ppm nickel treatments compared to control. Proline accumulation was doubled with 100 ppm nickel treatments, which gradually declined to nearly 70% in plants with 300 ppm Ni treatment due to retarded growth. The protein concentration was decreased from 10.1 mg/gm fr. wt. to 7.9 mg/gm fr. wt. with increased Ni treatment from 100 ppm to 200 ppm whereas catalase activity was decreased from 6.4 nKat/mg protein to 2.2 nKat/mg protein with increased Ni treatment of 50 ppm to 100 ppm. Common *sesban* seedlings' protein and proline content showed a strong positive correlation ($r=0.895$). Overall, the study highlights the harmful effects of elevated nickel concentrations on *Sesbania sesban*, demonstrating its negative impact on plant growth and biochemical parameters.

Keywords: Carotenoid, Catalase, Chlorophyll, Heavy metal, Phytotoxicity, Proline**INTRODUCTION**

Heavy metals are naturally present in the earth's crust; however, anthropogenic activities such as manufacturing, mining, transportation, fossil fuel combustion, industrial processes including foundries, the chemical industry, petrochemical facilities, oil refineries, smelters, and the application of fertilizers and pesticides have significantly increased their concentration in the soil to hazardous levels. Heavy metal pollution is a critical environmental concern threatening agriculture and plant biodiversity. Their influence on plant growth and development includes modifying physiological and bio-

chemical processes, including photosynthesis, respiration, food absorption, enzyme activity, and gene expression (Dutta *et al.*, 2018). Certain heavy metals can form compounds with organic matter or clay minerals in the soil, thereby reducing their mobility and availability. Other heavy metals may be solubilized by acid rain or irrigation water, thereby enhancing their leaching and uptake by plants. Heavy metals can infiltrate plant cells via the roots or leaves, contingent upon their chemical composition and soil presence (Angon *et al.*, 2024). Heavy metals can interfere with numerous metabolic processes upon entering plant cells by generating reactive oxygen species (ROS), causing oxidative stress.

Reactive oxygen species (ROS) can damage cellular constituents, including proteins, lipids, and DNA, resulting in mutations or cell death (Gajewska *et al.*, 2024; Atta *et al.*, 2024; Kumar *et al.*, 2022; Sharma *et al.*, 2012).

Nickel is considered a vital element primarily due to its essential role as a urease enzyme component, which facilitates urea nitrogen breakdown. Plants are unable to utilize urea nitrogen unless it is hydrolyzed into carbon dioxide and ammonia. Nickel was subsequently identified as essential for legumes and later for various temperate cereal crops. The deficiency of nickel impairs plant growth and development in multiple ways like, inducing senescence, altering nitrogen absorption and iron uptake, and chlorosis, particularly in young leaves, culminating in meristematic necrosis (Shahzad *et al.*, 2018; Mustafa *et al.*, 2023; Kovacik and Vydra, 2024). Excessive Ni ions disrupt enzyme activities and other biochemical processes in plants, including pigment formation and photosynthesis. Nickel concentrations in contaminated aquatic and terrestrial environments can reach 0.2 mg L^{-1} and 26 g kg^{-1} , significantly exceeding levels found in uncontaminated resources (Chen *et al.*, 2009). Typically, exposure of plants to concentrations over 100ppm of nickel metal manifests hazardous symptoms (Helaoui *et al.*, 2022). Excessive nickel (Ni) in plants has been associated with diminished germination, stunted growth, impaired cell division, reduced biomass production, compromised nutrient absorption, and adverse effects on photosynthesis and transpiration, as well as chlorosis in leaves and necrosis in cells and tissues, among other consequences (Shahzad *et al.*, 2018; Banerjee and Roychoudhury, 2020; Mustafa *et al.*, 2023; Yu *et al.*, 2024; Kovacik and Vydra, 2024). Nickel exposure in the workplace is associated with various health issues, including respiratory diseases, allergies, and increased risk of cancers, as evidenced by the occupational pathology data from nickel industry workers (Syurin and Nikanov., 2024). Nickel is known to be haematotoxic, immunotoxic, neurotoxic, nephrotoxic, and hepatotoxic and can adversely affect various organs, including the brain, kidneys, lungs, and liver (Khan *et al.*, 2022). Various techniques have been explored for the remediation of nickel contamination in aquatic systems and agricultural soils (Chen *et al.*, 2019; El Naggat *et al.*, 2021; Vischetti *et al.*, 2022).

Sesbania is the exclusive genus of the Fabaceae family, located within the tribe Sesbanieae. The plant's primary distinguishing features include its rapid growth rate, efficient nitrogen-fixing root systems, adaptability to arid environments, hyperaccumulation of various metals, and significant tolerance to numerous heavy metal-contaminated sites (Zn, Cd, Pb, Cr, etc.). *S. virgata* can stabilize metals, especially chromium, suggesting its potential for phytoremediation, although it is not categorized as a strong bioaccumulator (Rodriguez

et al., 2023). Cultivating *Sesbania* species as a leguminous agroforestry plant enhances the soil by increasing nitrogen levels and contributes to environmental sustainability by eliminating pollutants from terrestrial and aquatic ecosystems. The objective of the present investigation was to find the nickel-induced morphological and physiological toxicity in *Sesbania sesban* seedlings.

MATERIALS AND METHODS

Experimental design

A fully randomised strategy was used to conduct the pot culture studies. The seeds of *Sesbania sesban* (L.) Merr. (common sesban) were cleaned with distilled water after being surface-sterilized with 0.1% mercuric chloride (w/v) for 5 minutes. The seeds were sourced from the Odisha State Seeds Corporation in Bhubaneswar.

Pot culture study

NiCl_2 was utilized as the salt source to prepare a nickel solution using distilled water. Pots (diameter of 30 cm and height of 50 cm) were prepared for seedling plantation, each containing 5 kg of air-dried soil. The physicochemical properties of the soil are mentioned in Table 1. The seedlings were cultivated in pots in the garden of Rama Devi Women's University with a temperature range of 25 to 38°C and humidity range 50% - 60% taking control soil (devoid of nickel) and in pots containing soil administered with varying amounts of nickel solution (50, 100, 200, and 300 ppm) in the garden of the Rama Devi Women's University. Trays were positioned at the base of the pots to collect runoff, which was subsequently returned to the corresponding pot. Each pot included ten seeds. All pots received daily irrigation. Each treatment, including the control, was replicated three times.

Table 1. Physicochemical parameters of Garden soil of Rama Devi Women (Data presented as Mean $\pm$ SEM)

Physicochemical parameters	Quantity
pH	6.2 ± 0.02
EC(dS/m)	0.005 ± 0.001
Soil Organic Carbon (%)	5.31 ± 0.25
Avl. N (kg/ha)	245 ± 0.85
Avl. P (kg/ha)	$636 \pm 0.98 \pm$
Avl. K(kg/ha)	498 ± 1.02
S	38.5 ± 0.56
Zn	28.3 ± 0.41
B	0.41 ± 0.09
Ni	0.05 ± 0.008

Morphological and biochemical estimations

Periodic monitoring and study of growth rate and morphological parameters (Root shoot length, biomass content) were performed over 60 days, during which plant samples were collected to analyze specific biochemical components and evaluate antioxidant enzyme activity. Sixty days post-treatment (60 DAT), leaf pigments were extracted using 80% (v/v) cold acetone and chlorophyll a, chlorophyll b, total chlorophyll and carotenoid quantified according to the methods of Arnon (1949) and Lichtenthaler (1987), with minor modifications. Proline was estimated as per Bates *et al.* (1973) method. Protein and enzyme purification and analysis were performed at a temperature of 4°C. Protein quantitation was conducted by the Bradford assay. The assay and activity of the catalase (CAT) enzyme were assessed according to the methodology outlined by Chance and Maehly (1936).

Statistical analysis

The data were taken in triplicates, and SEM (Standard error of mean) was calculated. Pearson's correlation coefficient matrix was calculated using Ms-Excel for different biochemical parameters.

RESULTS AND DISCUSSION

Growth parameter study

The toxic impact of 300 ppm Ni²⁺ on plants was very adverse, as demonstrated by a marked decrease in root and shoot length relative to the control group, as shown in Fig. 1. The study found that when treated with 200 ppm, both root and shoot length were significantly reduced as compared to control, 50 and 100 ppm. The impact of hazardous Ni²⁺ concentrations on the root and shoot length of *Sesbania* seedlings was significant in comparison to the control group after 60 days of growth. The root and shoot lengths of *Sesbania* seedlings exhibited significant sensitivity to nickel poisoning. Javeed *et al.*, (2024) studied the effects of salinity and Ni stress on *Citrullus colocynthis* growth. They discov-

ered that there were no negative effects on seed germination, radical and plumule length, and fresh and dry seedling weight at low concentrations. Similar results were also observed in our study, where up to a treatment concentration of 100 Ni, there was no significant reduction in the root or shoot length, whereas at 200 ppm and 300 ppm, there was a sharp decline. Reduction in growth at high concentrations may be attributed to the reduced water potential, as reported by several researchers (Kahlon *et al.*, 2018; Javeed *et al.*, 2024). There was a 50% decline at 200 ppm treatment and 75% decline at 300 ppm in root and shoot length as compared to 100 ppm treatment.

Effect on chlorophyll and carotenoid

Sesbania leaves markedly decreased chlorophyll concentration throughout a 60-day growth period when Ni²⁺ supply increased. The chlorophyll content decreased at a 50 ppm concentration and continued to decline. The chlorophyll a and b concentrations were lower than the control in all treated plants. In addition to total chlorophyll content, carotenoid content exhibited a comparable diminishing trend to chlorophyll (Fig. 2). The carotenoid concentration was markedly reduced at 300 ppm compared to the control plants. Heavy metals may disrupt the formation of plant pigments, as indicated by Van Assche and Clijsters (1990). Heavy metals are recognized for their interference with chlorophyll synthesis through direct interaction or by indirectly inhibiting an enzyme, resulting in a nutritional deficit (Meers *et al.*, 2010). Nickel toxicity reduces chlorophyll and carotenoid levels in plants, resulting in diminished photosynthetic efficiency and possible harm to the photosynthetic system (Doğru *et al.*, 2021). Various studies document the effects of metal toxicity across different plant species (Satpathy and Samantaray, 2023). Reddy *et al.* (2024) examined the impact of heavy metals, including Cd, Cu, Zn, Pb, and Ni in *Vigna radiata* and found that low concentrations of Ni had a negligible effect on chlorophyll content. As concentration increases, chlorophyll content declines as a

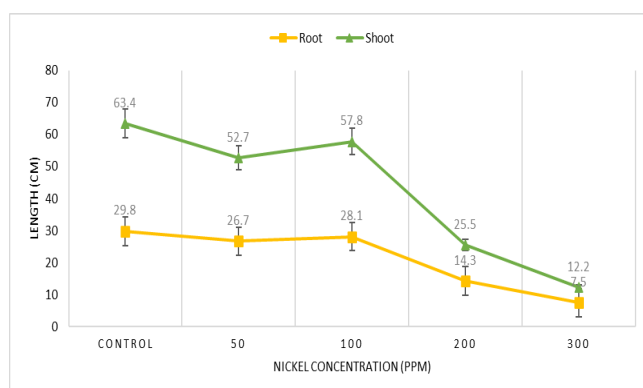


Fig. 1. Effect of various concentrations of nickel on growth of sesban (data presented as Mean $\pm$ SEM)

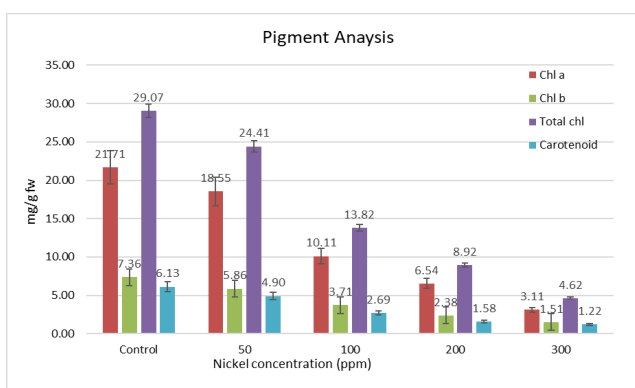


Fig. 2. Effect of nickel on chlorophyll and carotenoid content (Data presented as Mean $\pm$ SEM)

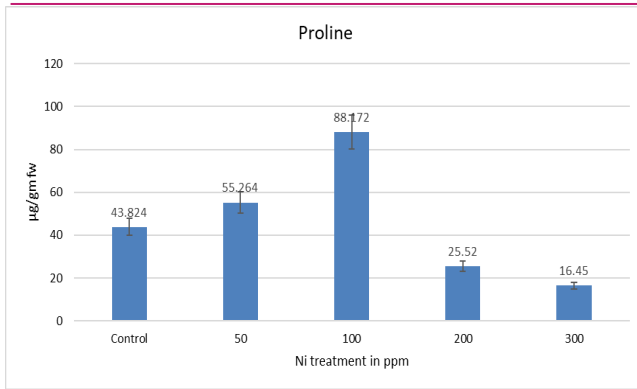


Fig. 3. Effect of nickel on Proline content of sixty days grown sesban seedlings exposed to varying concentrations (Data presented as Mean $\pm$ SEM)

result of oxidative stress in black gram (Dubey and Pandey, 2011). From the correlation table (Table 2), it was observed that there was a negative correlation (-0.408) between total chlorophyll and protein content that maybe due to an elevated accumulation of protein induced under stress conditions.

Effect on proline content

An outstanding osmolyte, proline shields plants from stress, membrane permeabilization, reactive oxygen species (ROS) toxicity, and protein instability (Satpathy and Samantaray, 2023; Reddy *et al.*, 2024; Dubey and Pandey, 2011; Lin and Kao, 2007; Atta *et al.*, 2024; Aslam, 2017). It facilitates molecular recognition in intracellular signalling, which activates specific gene expression and aids in the recovery of plants from stress. In the present study, the total proline accumulation in nickel-treated plants was higher in 100 ppm than in the control group. The proline content increased with an increase in Nickel concentration to 100 ppm, after which it began to decline, reaching its lowest point at 300 ppm (Fig. 3). Proline regulates antioxidant enzyme activities, thereby enhancing the plant's capacity to manage oxidative stress induced by nickel (Atta *et al.*, 2024; Aslam, 2017). The increase in proline levels is associated with decreased proline dehydrogenase activity, suggesting a transition towards proline synthesis as a response to nickel exposure (Lin and Kao, 2007). Proline levels rise dramatically in plants exposed to nickel, as demonstrated in studies with wheat and mus-

tard, where increased nickel concentrations were associated with elevated proline content and oxidative stress markers (Atta *et al.*, 2024; Gopal and Nautiyal, 2012). Proline treatment in *Atropa belladonna* decreased nickel accumulation and lipid peroxidation, indicating its protective function against nickel toxicity (Stetsenko *et al.*, 2011). The established correlation between proline accumulation and nickel toxicity in diverse plant species indicates its viability as a biomarker for evaluating nickel stress in plants (Atta *et al.*, 2024; Reddy *et al.*, 2024; Satpathy and Samantaray, 2023; Gopal and Nautiyal, 2012; Lin and Kao, 2007). Monitoring proline levels may offer insights into the degree of nickel contamination in agricultural environments.

Effect on protein content

Ni had a notable effect on the total protein level of the treated *Sesbania* leaves. With the escalation of heavy metal concentration, a marginal reduction in protein content was seen at 50 ppm, followed by a little rise at 100 ppm (Fig. 4). Nonetheless, when the heavy metal concentration increased, the sample's overall protein content dropped. The protein level further diminished, reaching a minimum of 300 ppm concentration of nickel. The impact of heavy metal stress on protein content and amino acid metabolism in plants is multifaceted. Initially, low concentrations of nickel (Ni^{2+}) can stimulate the synthesis of stress proteins and amino acids, such as proline and glutathione, leading to a slight increase in total protein content due to cellular detoxification mechanisms (Jablonkai, 2022; Hall, 2002). However, at higher concentrations (300 ppm), the degradation of proteins surpasses synthesis, resulting in a net decrease in protein content, which indicates increased protein degradation (Scornik, 1984). The synthesis of stress proteins is a key response to heavy metal exposure, enhancing the plant's ability to cope with toxicity (Ningombam *et al.*, 2024). Heavy metals alter amino acid profiles, with some amino acids increasing while others decreasing, reflecting stress-related metabolic adjustments (Bohuslavska *et al.*, 2022).

Effect on catalase activity

To protect themselves from oxidation and stress, plants produce reactive oxygen species (ROS), which en-

Table 2. Pearson's correlation coefficient across treatments

Variables	Total Chl	Carotenoid	Protein	Catalase Activity	Proline
Total Chl	1.000	0.997	-0.408	-0.067	-0.274
Carotenoid	0.997	1.000	-0.392	-0.102	-0.263
Protein	-0.408	-0.392	1.000	0.775	0.895
Catalase Activity	-0.067	-0.102	0.775	1.000	0.712
Proline	-0.274	-0.263	0.895	0.712	1.000

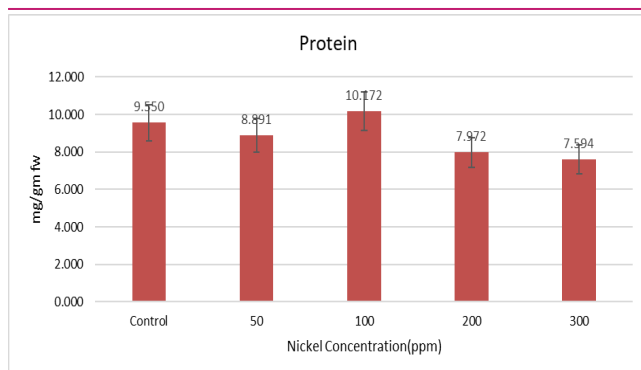


Fig. 4. Effect of nickel on total protein content (Data presented as Mean $\pm$ SEM)

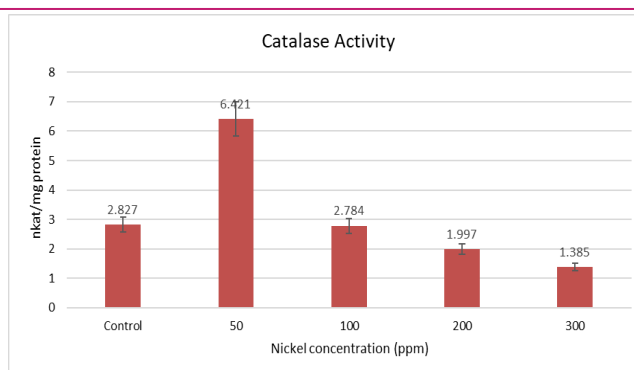


Fig. 5. Effect of nickel on catalase activity (Data presented as Mean $\pm$ SEM)

zymes like catalases and peroxidases can neutralize (Pandhair and Sekhon, 2006; Das and Roychoudhury, 2014). After a dramatic increase at 50 ppm Ni^{2+} , the catalase activity in *Sesbania* plants dropped to below 50% at 300 ppm Ni (Fig. 5). The effect of nickel (Ni) on catalase activity in plants, including *Sesbania*, reveals a complex relationship where Ni acts as both a micronutrient and a potential toxin. Ni is essential for various physiological processes at low concentrations (Preferably below 10mg per kg biomass), including the synthesis of Urease, phytoalexins, and Nitrogen assimilation (Rizwan *et al.*, 2024; Pudake *et al.*, 2019). But elevated levels of Ni lead to oxidative stress and reduce catalase activity, which is crucial for detoxifying hydrogen peroxide in plants (Rizwan *et al.*, 2024) which may be probably due to its binding to thiol groups of enzyme proteins and disrupting the structure and function of proteins and release of ROS (reactive oxygen species (Kumar *et al.*, 2022).

In the present study, catalase activity showed a 3-fold increase at 50pppm Ni treatment, a stimulatory response. The higher activity of catalase could be a reason to protect the plant through scavenging the increased ROS during higher Ni exposure (Baran and Ekmekçi 2022). It is also found that there is increased synthesis of abscisic acid (ABA) and ethylene at high Ni concentrations. Higher ABA may control stress by inducing the synthesis of antioxidant enzymes and promoting increased scavenging of ROS (Baran and Ekmekçi 2022). Similarly, ethylene causes activation of NADPH oxidase. Ni treatment at 300 ppm showed 50% reduction in catalase activity in common sesban seedlings as compared to control. Increased Ni concentrations generally result in decreased catalase activity across various plant species, including Fabaceae trees and wheat (Rizwan *et al.*, 2024; Khudhur and Albarzinji, 2022; Gajewska *et al.*, 2006). For instance, in black gram, catalase activity diminished with rising Ni levels, indicating oxidative damage (Dubey and Pandey, 2011). Similarly, green gram exhibited reduced catalase activity alongside increased oxidative stress markers when exposed to excess Ni (Pandey and

Pathak, 2006). Plants respond to Ni toxicity by enhancing other antioxidant enzymes, such as superoxide dismutase (SOD) and peroxidase, while catalase activity declines (Pandey and Pathak, 2006) (Gajewska *et al.*, 2006). This shift suggests a compensatory mechanism where plants prioritize other pathways to mitigate oxidative stress caused by Ni accumulation (Helaoui *et al.*, 2023; Baran and Ekmekçi 2022; Riizwan *et al.*, 2024). In contrast, some studies suggest that low levels of Ni can stimulate certain enzymatic activities, indicating that the relationship between Ni and catalase is concentration-dependent. Thus, while Ni is essential at trace levels, its toxicity at higher concentrations (More than 100 ppm) poses significant challenges to plant health and metabolism. Increased catalase activity with stress response indicated an oxidative stress response and is evident from the strong positive correlation (0.775) between catalase and protein.

Statistical interpretation of biochemical parameters

There was a strong positive correlation (0.997) between total chlorophyll and carotenoid content among all the treatments of Ni, suggesting that the carotenoid content with decreasing chlorophyll concentration also declined. These pigments were linked with each other under increasing nickel stress. From the strong positive correlation value of 0.895 between protein and proline content of common sesban seedlings, it was interpreted that both were strongly linked and proline accumulation is a protective mechanism under stress as revealed by several researchers (Atta *et al.*, 2024; Reddy *et al.*, 2024; Satpathy and Samantaray, 2023).

Conclusion

The study explains the potential of common sesban plants to thrive and defend against different toxic levels of nickel. The lower nickel concentration, i.e. 50 ppm, stimulated the root and shoot length, whereas the treatment concentration above 100 ppm was detrimental to the root and shoot growth. The higher synthesis of protein and proline at 100 ppm of Ni was an important bi-

omarker that declined almost half in the 200 ppm Ni treatment. The study sheds light on the potential ability of the common sesban plant to move towards different nickel concentrations. The reports above elucidate the phytotoxic impacts of Ni in common sesban and address the potentiality of common sesban to eliminate the toxicity of nickel from the polluted environment by a phytoremediation approach. Thus, the study demonstrates that *Sesbania* plants can thrive in moderate nickel stress and defend themselves against phytotoxicity by modifying several metabolic processes. An empirical investigation is advised to determine the impact of ambient factors (such as temperature, light, pH, soil quality, etc.) on the aforementioned laboratory-based findings. The present study results advise implementing the advanced nickel phytoremediation method in real-world field settings.

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Conflict of interest

The authors declare that they have no conflict of interest.

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