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Nickel induced exposure analysis for toxic changes in growth and antioxidative enzymes in sesban eliciting biochemical sensitivity

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Abstract

Nickel (Ni) exposure in plants leads to severe toxicity problems, depending on its exposure concentration. The present pot culture investigation assesses the phytotoxic effects of different Nickel (Ni) concentrations on various biochemical parameters of *Sesbania*. The study involves applying Nickel at 50, 100, 200, and 300 ppm along with a control group at 0 ppm for 30 days. Results revealed retarded growth, reduced pigment content, and enhanced antioxidative enzyme activity as nickel concentration increased. Exposure to Ni (100 ppm) and above severely affects seed germination, plant growth, and biomass production. Furthermore, relative phytotoxicity was evident from a 15% reduced germination rate and a fall in germination index from 10 to 8.5. The seedling vigour index was drastically reduced from 960 (control) to 93.5(300 ppm Ni). In addition to these, plant growth retardation was striking with root length stunted by 70% and shoot length by 50% in response to Ni (300 ppm). Chlorophyll and carotenoid contents decreased by nearly 50% with the 300-ppm nickel treatment. Although protein levels and antioxidant enzyme activity (catalase and peroxidase) showed a stimulatory response to 100 and 200 ppm Ni treatments, both suffered a sharp decline due to toxic stress at 300 ppm Ni. This study explicitly highlights the harmful effects of high doses of Ni, highlighting the sensitivity of various morphometric and biochemical parameters to Ni toxicity. These findings highlights the need to mitigate environmental contamination and adopt measures to protect plant health.

Keywords

carotenoid; catalase; chlorophyll; germination index; nickel phytotoxicity; peroxidase

Introduction

Heavy metal contamination is a significant environmental issue that jeopardizes agriculture and plant diversity. Heavy metals occur naturally in the earth's crust, but anthropogenic activities like manufacturing, mining, traffic, burning fossil fuels, industrial production like foundries, chemical industry, petrochemical plants, oil refineries, smelters, and the use of fertilizers and pesticides, have escalated their concentration in the soil to hazardous levels. Their impact of heavy metals on the growth and development of plants involves disruptions to physiological and biochemical processes, including photosynthesis, respiration, nutrient uptake, enzyme activity, and gene expression (1). While metals such as copper, zinc, iron, nickel, and

manganese serve as essential micronutrients for plants, their concentrations beyond optimal ranges can lead to toxicity (2). Conversely, heavy metals like lead, cadmium, mercury, and arsenic have no recognized biological role and can be harmful even at low levels (3). Depending on their chemical form and concentrations in the soil, heavy metals can enter plant cells through the roots or leaves. Some heavy metals can form compounds with organic matter or clay minerals in the soil, decreasing their movement and bioavailability. Other heavy metals can be dissolved by acid rain or irrigation water, thereby increasing their drainage and absorption by plants. Once inside plant cells, heavy metals can disrupt various metabolic processes by generating reactive oxygen species (ROS), which induce oxidative stress. ROS has the potential to harm cellular components such as lipids, proteins, and DNA, leading to cell death or mutations (4-5).

Ni is a crucial element mainly because it serves as an indispensable component of the urease enzyme, which catalyses the hydrolysis of urea-nitrogen. Plants cannot use urea derived nitrogen unless it is hydrolysed into carbon dioxide (CO_2) and ammonia (NH_3). As a result, plants grown in urea-based system are extremely susceptible to inadequate Ni supply (6). Ni was initially recognised as vital for legumes (7-8) and later found to be essential for several temperate cereal crops as well (9-11). However, an excess of Ni ions interferes with enzyme functions and other plant biochemical reactions such as pigment synthesis and photosynthesis (12). Generally, exposure to more than 100 ppm of nickel leads to plant toxicity.

The flowering plant *Sesbania* is the sole genus of the pea family, Fabaceae, within tribe Sesbanieae. This genus comprised over 60 species, including a few species to Asian countries such as India, Malaysia, and Indonesia. *Sesbania* is distinguished by its exceptionally rapid growth rate, highly efficient nitrogen-fixing root systems, ability to thrive in extremely arid regions, capacity for hyperaccumulation of other metals, and remarkable tolerance to a wide range of heavy metal contaminated sites (e.g. Zn, Cd, Pb, Cr, etc.). Notably, species such as *Sesbania cannabina* and *Sesbania virgata*, exhibit remarkable adaptability to extremely harsh conditions including arid environments and heavy metal-contaminated soils. Their resilience is largely attributed symbiotic relationships with rhizobia, which enhance their growth and stress tolerance (13). Furthermore, *S. virgate* has demonstrated the ability to stabilize metals, particularly chromium, indicating its potential for phytoremediation despite not being classified as a high bio accumulator (14). In addition to their environmental benefits, *Sesbania* species are valuable for producing fodder, firewood, pulp and paper, food, green manure, and landscape decorations. Cultivating *Sesbania* as a leguminous agroforestry plant not only enriches the soil by increasing nitrogen content but also contributes to environmental sustainability removing toxins from the terrestrial and aquatic ecosystems. The present experiment was designed to investigate nickel toxicity in *Sesbania* species, focusing on its effects on morphological and biochemical characteristics.

Although similar research has been conducted on heavy metals, exploring the biochemical toxicity of nickel (Ni) in Sesbania represents a novel and original approach. This study examined various biochemical parameters as markers for assessing the severity of Ni toxicity. In this research, *Sesbania* served as the test plant and demonstrated resilience to toxic effects of Ni through changes in antioxidant enzyme. Additionally, this adaptability paves the way for leveraging Sesbania in bioaccumulation and green mining efforts to remediate contaminated areas using this leguminous plant.

Materials and Methods

Pot culture studies were conducted using a completely randomised design. *Sesbania* seeds (dry and graded) were obtained from Odisha State Seeds Corporation, Bhubaneswar. The seeds were surface sterilized with 0.1% mercuric chloride (w/v) for 5 minutes, followed by thorough washing with distilled water.

Germination study

Seed germination is the first physiological process, serves as an indicator of tolerance to Ni. The ability of seeds to germinate in a medium containing Ni reflects their level of tolerance to this metal. About 20 uniform healthy presoaked seeds were placed in five Petri-plates lined with cotton pads which were saturated with different concentrations of Ni^{2+} viz. 0 (control), 50, 100, 200, and 300 ppm. The plates were incubated at 25 ± 2°C for 48-72 hours. After two days (48 hours) the number of germinated seeds (defined by emergence of a 2 mm radicle) in each treatment of Ni^{2+} was counted. The percentage germination and the percentage inhibition (relative toxicity) were calculated to access the effects of Ni^{2+} on seed germination.

Pot culture study

Pots containing 5 kg of air-dried soil were prepared for seedling plantation. The seedlings were grown in pots with control soil (without nickel) and in pots containing soil to which different concentrations of nickel solution were applied (50, 100, 200, and 300 ppm). Trays were placed at the bottom of the pots to collect the runoff, which was subsequently returned to the respective pot. $NiCl_2$ was used as the source salt for preparing the nickel solution with distilled water. The nickel solutions were applied to the soil surface and thoroughly mixed. Each pot was planted with ten seeds. Daily irrigation was provided to all the pots. Each treatment, including the control, was replicated three times. Periodic monitoring and analysis of growth rates and morphological factors were conducted over 30 days. Plant samples were collected 30 days post-treatment (30 DAT) to analyse distinct biochemical components and assess antioxidant enzyme activity. Chlorophyll was extracted using 80% (v/v) cold acetone and quantified following the techniques of Arnon (15) and Litchenthaler (16), with slight modifications. Protein quantification was performed using the Bradford (17) technique. Purification and analysis of proteins and enzymes were conducted at 4°C. The assay and activity of catalase (CAT) and peroxidase (POD) enzymes were determined following the procedure described by Chance and Maehly (18).

Results and Discussion

Effect on seed germination

As illustrated in Fig. 1, seed germination exhibited gradual inhibition with the increase in Ni^{2+} concentration. Also, higher Ni^{2+} concentration caused a delay in the germination process. Table 1 demonstrates that seeds exposed to varying doses of Ni^{2+} exhibited decreased germination % compared to the control. The degree of inhibition or relative toxicity increased proportionally with the rise in Ni^{2+} levels (19).

Notably, the toxic effect of 300 ppm Ni^{2+} was particularly to detrimental, as evidenced by a substantial reduction in root length compared to the control. Root length and shoot length of Sesbania seedlings showed high sensitivity to Ni toxicity. Previous studies have documented comparable findings by several other workers in other plants (21).

Effect of Ni^{2+} concentration on chlorophyll

There was a notable decline in the chlorophyll concentration of *Sesbania* leaves as the supply of Ni^{2+} increased over

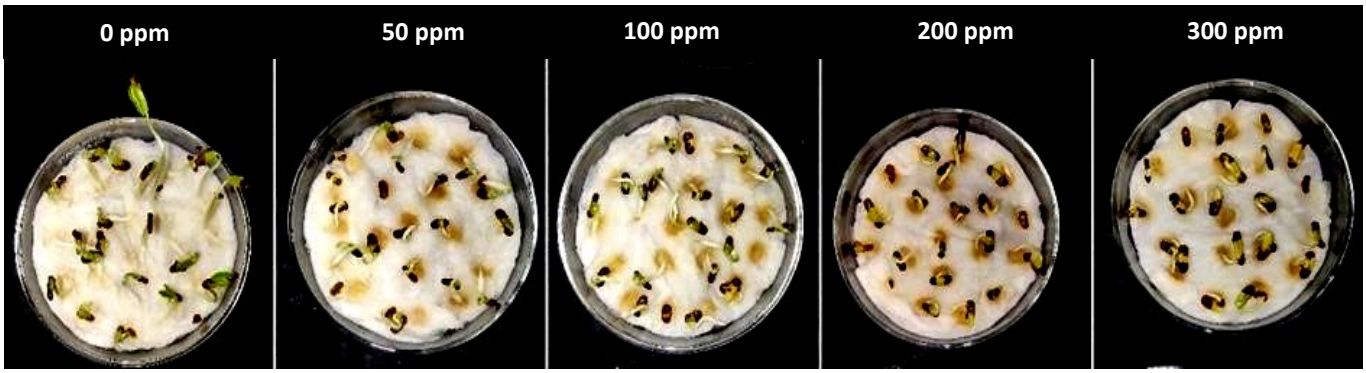

Fig. 1. Effect of different concentrations of Ni^{2+} on the germination of *Sesbania* sps seeds.

Table 1. Effect of nickel on the germination, relative toxicity, and seedling vigour index (SVI-I) on the seeds of *Sesbania* sp.

Conc. of Ni (II) in ppm	Total no. of seeds placed in Petri plate	No. of seeds germinated	% of seeds germinated	Relative Toxicity (% of inhibition)	Germination Index	Seedling Vigour Index
Control (0)	20	20	100	0	10	960
50	20	19	95	5	9.5	304
100	20	18	90	10	9	198
200	20	18	90	10	9	162
300	20	17	85	15	8.5	93.5

Inhibition of growth in pot culture study after 30 DAT

Conspicuous alterations were noted in the roots and shoots lengths of treated plants compared to the control group (Fig. 2 and Table 2). Significant reductions in root and shoot length were observed with treatment starting at 100 ppm. The consistent decline in growth parameters supports the conclusions of previous studies conducted on several plants about Nickel (20). After 30 days of growth, *Sesbania* seedlings exposed to hazardous Ni^{2+} concentrations showed substantially reduction compared to control.

Table 2. Root and shoot length of *Sesbania* plants exposed to different concentrations of Ni^{2+}

Ni Treatment (in ppm)	Root Length	Shoot Length
Control	9.5	16.4
50	7.8	13.3
100	6.4	12.6
200	4.8	10.5
300	3.5	8.1

Fig. 2. Effect of different concentrations of Ni2+ on the growth of *Sesbania* sps plants. [From left to right the treatments of Ni2+ concentrations (ppm): Control (0), 50, 100, 200, 300].

the 30-day growth period (Fig. 3). A notable dip in the total chlorophyll content was recorded at a 50-ppm concentration, which then surged at 100 ppm, followed by gradual decline beyond this concentration. The amount of chlorophyll b was lower than control across all the treated plants. In addition to total chlorophyll content, carotenoid content were significantly reduced at 300 ppm compared to the control plants. Similar findings on the impacts of metal toxicity have been reported in various plants species (22-24). Nickel toxicity decreases chlorophyll and carotenoid content in plants, leading to reduced photosynthetic efficiency and potential damage to the photosynthetic system (25). Heavy metals are known to interfere with chlorophyll synthesis, either directly or by inhibiting an enzyme indirectly, by inhibiting enzymes or causing a nutrient deficiencies (26-27). It has been suggested that heavy metal interfere with the plant pigment formation process (28).

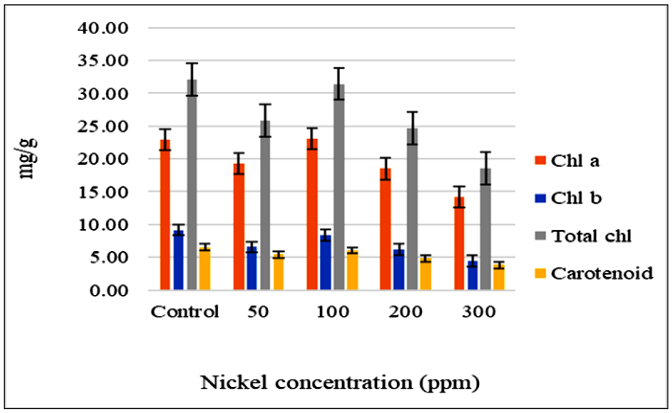

Fig. 3. Effect of different concentrations of Ni^{2+} on the photosynthetic pigments of *Sesbania* leaf.

Effect of the concentration of Ni^{2+} on the total protein content of Sesbania

The impact of nickel (Ni) on the overall protein content of treated *Sesbania* leaves was significant. As the concentration of this heavy metal increased, the protein content initially exhibited a slight ris. However, with further increases in nickel concentration, the total protein content began to decline, reaching its lowest point at 300 ppm (Fig. 4). This decline can be attributed to protein degradation and reduced amino acid production (29), plant undergoes cellular detoxification (30). At moderate Ni^{2+} concentrations of 100 and 200 ppm, a slight increase in overall protein content was observed, likely due to the synthesis of various stress proteins. However, at the higher concentration of 300 ppm, the protein content fell below that of the control plant, indicating a significant increase in protein degradation (31).

Effect of Ni^{2+} concentration on catalase and peroxidase activity

Under stress conditions, plants release reactive oxygen species (ROS), which can cause oxidation and cellular damage. To counteract this, catalases and peroxidases play a crucial role in eliminating ROS, thereby protecting plants. In *Sesbania* seedlings treated with 100 and 200 ppm of Ni^{2+}, catalase activity significantly increased. However, at 300 ppm of Ni^{2+}, catalase activity dropped to less than 50% of its previous levels (Fig. 5). Meanwhile, peroxidase (GPX) activity showed a notable increase with rising Ni^{2+} concentrations (Fig. 6). Significant differences were observed in catalase and peroxidase activities among plants exposed to varying levels of Ni^{2+}. Both peroxidase and catalase are effective scavengers of H_2O_2, reducing its accumulation and preventing oxidative damage by limiting its movements across cell membranes. The observed increase in catalase activity likely enhanced H_2O_2 scavenging in treated plants (19). Peroxidase activity peaked in plants treated with 50 ppm Ni^{2+}, but declined at higher concentrations, possibly due to excessive ROS production, which indicates cytotoxic effects (32). Despite this decline, peroxidase activity remained consistently higher in all Ni^{2+} treatments compared to control plants.

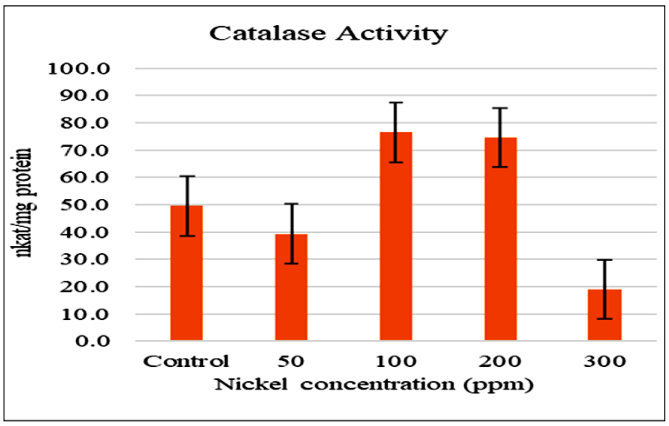

Fig. 5. Effect of Ni^{2+} on the catalase activity of plants under different concentrations.

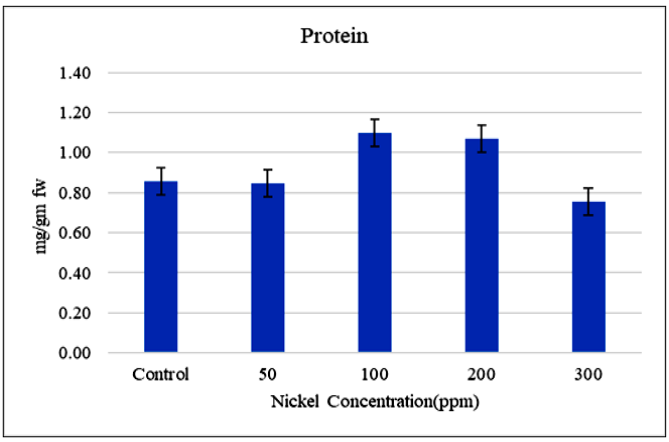

Fig. 4. Effect of different concentrations of Ni^{2+} on the total protein content of *Sesbania* leaf.

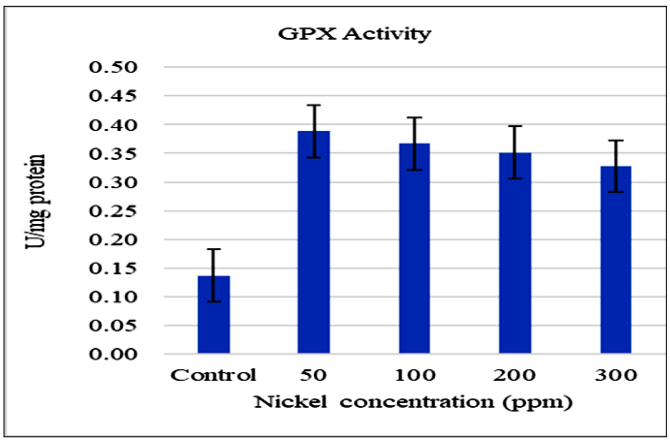

Fig. 6. Effect of Ni^{2+} on the peroxidase (GPX) activity of plants under different concentrations.

Conclusion

This study examined the harmful effects of different levels of Ni^{2+} on germination, root and shoot length, photosynthetic pigments, and antioxidant enzymes, assessing its phytotoxicity and tolerance capacity of *Sesbania*. The findings of the study highlight the detrimental impacts of nickel pollution and emphasize the need to safely remove toxic nickel from the environment. In conclusion, the study demonstrates that *Sesbania* plants can thrive under nickel stress and protect themselves against phytotoxicity by altering various metabolic processes.

The study explored the negative effects of different concentrations of Ni^{2+} on germination, root and shoot length, photosynthetic pigments, and antioxidant enzymes in *Sesbania*. These investigations underscore the seriousness of nickel contamination and the necessity of addressing it effectively. The results indicate that *Sesbania* plants have the capacity to adapt to nickel stress and mitigate its harmful effects through metabolic adjustments.

Furthermore, empirical studies are recommended to explore the influence of environmental factors such as temperature, light, pH, and soil quality on these laboratory findings. The outcomes of this study will support the implementation of advanced nickel phytoremediation techniques in real-world field settings.

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Authors' contributions

MM has conceptualized, designed the study, supervised and corrected the draft article. SM carried out the work, performed the statistical analysis and prepared the draft article. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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