RESEARCH PAPER

Growth, Morphological, and Biochemical Responses of Groundnut (Arachis hypogea L.) Seedlings to Nickel Exposure

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ABSTRACT
This study evaluated the effects of nickel (Ni) toxicity across a gradient of concentrations (5, 10, 25, 50, 75, 100, 200, and 300 mg L^{-1}) on the early development, biochemical parameters like chlorophyll content, protein, aminoacid, sugar, and proline content of groundnut (Arachis hypogaea L.) seedlings. Seeds were subjected to ascending dosage levels to determine threshold concentration toxicity. Exposure to 10 mg L^{-1} Ni produced a stimulatory effect, yielding maximum values across growth parameters and biochemical activities. Conversely, progressive increases in nickel concentration beyond this threshold significantly inhibited seedling development, with the lowest values recorded at 300 mg L^{-1}. Overall, the results indicate a biphasic dose-response relationship, wherein low concentrations of nickel promote plant growth and metabolic activity, whereas elevated concentrations induce toxic and inhibitory effects.

Keywords: Nickel, Ground nut, morphological, protein, aminoacid, sugar and proline.

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INTRODUCTION

Nickel is a prime example of a ubiquitous trace metal. It enters the environment through both natural cycles and anthropogenic activities. Several specific compounds are central to industrial processes, including: Nickel Acetate, Nickel Carbonate, Nickel Hydroxide, Nickel Oxide. As these industrial byproducts accumulate in the ecosystem, they are readily assimilated by vegetation. Once these plants are consumed, the metals transition into the food chain, leading to deleterious physiological effects in both animals and humans (Bani *et al*., 2024). Nickel contamination in soil poses a serious threat to the growth, development, and survival of living organisms (Naqqash *et al*., 2024; Shahzad *et al*., 2018). Nickel is the 22nd most abundant element in the Earth's crust and occurs naturally in agricultural soils through both geological processes and anthropogenic activities (Shahzad *et al*., 2018). Elevated concentrations of Ni in the environment mainly result from industrial operations such as electroplating, metallurgical processing, cement manufacturing, mining, smelting, fossil fuel combustion, and vehicular emissions (Begum *et al*., 2022).

Groundnut (*Arachis hypogaea* L.) is one of the most important legume crops cultivated worldwide and ranks among the leading oilseed crops in terms of production. It is well adapted to semi-tropical and semi-arid tropical regions, where it serves as a significant source of edible oil, protein, and income for farmers. In India, the cultivation of groundnut is predominantly concentrated in a few major producing states. Recent analyses of the spatial distribution of groundnut cultivation, productivity, and production indicate that nearly 87% of the total cultivated area and about 85% of the country's groundnut production are contributed by six states, namely Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra, and Rajasthan (Mallikarjun *et al*., 2024).

MATERIALS AND METHODS

Germination Studies

Germination study was performed with Ground nut seeds treated with Nickel. The seeds of Ground nut were surface sterilized with 0.2 per cent of $HgCl_2$ for two minutes and subsequently exhaustively with tap water. The seeds were

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evenly positioned in synthetic containers packed with garden soil and they were treated with different concentrations (5, 10, 25, 50, 75, 100, 200 and 300 mg/L) of Nickel. The control set was maintained by using tap water. Five replicates were maintained for each treatment. On the fifteenth day, the germination percentage, shoot length, root length, total leaf area, seedling fresh weight, and seedling dry weight were taken. From these findings, the following values of vigour index, tolerance index and percentage of phytotoxicity were calculated. Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid), biochemical analyses such as reducing sugar, non-reducing sugar, amino acid, proline and protein were examined.

2.1.1.Germination percentage

The number of seeds sprouted in each concentration was counted on the 15th day and the germination percentage was calculated by using the subsequent equation:

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

2.1.2. Root and shoot length (cm seedling^{-1})

Twenty seedlings were arbitrarily selected on 15th day from each treatment to record the seedling development. The root and shoot length of ththe Ground nut seedlings were measured by using a centimetre scale and the values were recorded.

2.1.3. Total leaf area

The total leaf area was calculated by gauging the span and thickness of the foliage as detailed by Yoshida *et al.* (1972).

$$\text{Leaf area (cm}^2) = K \times \text{length} \times \text{breadth}$$

Where

K = Kemp's constant (for dicot leaves 0.66)

2.1.4. Fresh weight and dry weight (cm seedling^{-1})

A score of young plants were obtained, surface-dried, and their fresh weight was recorded. These identical seedlings were kept in a hot air oven at 80^0C for 24 hrs. Subsequently, the specimens were stored in drying chambers and their dry mass was determined by utilizing an electrical single pan balance.

2.1.5. Vigour index

Vigour index of the seedlings were calculated by using the equation suggested by Abdul-Baki and Anderson (1973).

Vigour index = Germination percentage × seedling length

2.16. Tolerance index

Tolerance index of the young plant was calculated by using formula presented by Turner and Marshal (1972).

$$\text{Tolerance index} = \frac{\text{Mean length of longest root in treatment}}{\text{Mean length of longest root in control}}$$

2.1.7. Percentage of phytotoxicity (Chou *et al*., 1978)

The percentage of phytotoxicity of Nickel response to ground nut was calculated by using the following formula:

$$\text{Percentage of Toxicity} = \frac{\text{Radicle length of control} - \text{Radicle length of test}}{\text{Radicle length of control}} \text{ X } 100$$

2.2 PHOTOSYNTHETIC PIGMENTS AND BIOCHEMICAL ANALYSIS

The photosynthetic pigments including chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid and the biochemical constituents such as amino acids, proline, protein and sugars were evaluated in the 15th day old seedlings reared under laboratory conditions.

2.2.1. Chlorophyll (Arnon, 1949)

Five hundred mg of raw foliar matter was pulverized with a morter and pestle with 10 ml of 80 per cent acetone. The homogenized mixture was centrifuged at 800 rpm for 15 min. The supernatant was saved. The sediment was processed again with 10 ml of 80 per cent acetone. The supernatant was retained and the absorbance values were measured at 645 and 663 nm in a UV-Spectrophotometer (Hitachi). The chlorophyll 'a', chlorophyll 'b', and total chlorophyll contents were calculated and reported in mg g^{-1} fresh weight basis.

2.2.2. Estimation of protein (Lowry *et al*., 1951)

Five hundred mg of plant tissues were measured and crushed in a mortar and pestle with 10 ml of 20 percent trichloroacetic acid. The homogenized liquid was centrifuged for 15 min at 600 rpm. The supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged for 5 min. The extract was retained and

diluted to 10 ml with 0.1 N NaOH. This solution was used for the quantification of protein.

2.2.3. Estimation of amino acids (Moore and Stein, 1948)
Five hundred mg of plant matter were weighed and macerated with a pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged for 10 min. The supernatant was saved. The extract was used for the analysis of amino acids.

2.2.4. Proline (Bates *et al*., 1973)
300 mg of fresh leaves were homogenized in 10 ml of 3% aqueous sulpho salicylic acid (3 g of sulpho salicylic acid was dissolved in 100 ml of distilled water). The homogenate was centrifuged at 9000 rpm for 15 min. The procedure was repeated with the residue and the filtrates were combined.

2.2.5 Estimation of reducing sugars and Non-reducing sugars (Nelson, 1944)
Five hundred mg of plant specimens were measured and pulverized in a mortar and pestle with 10 ml of 80 percent ethanol. The homogenized mixture was centrifuged for 10 min at 800 rpm. The supernatant was reserved, and the the ethanol was evaporated in a water bath at 50^0C The final volume was adjusted to 20 ml with distilled water, and the extract was utilized for the analysis of reducing sugar.

RESULTS

3.1.1. Germination studies
The germination studies of ground nut seeds grown under different concentrations of nickel were carried out and the results were recorded in 15 day old seedlings.

3.1.2. Morphological parameters
The germination percentage, root length, shoot length, fresh weight, dry weight, vigour index, tolerance index and percentage of phytotoxicity of ground nut seeds grown under different concentrations of nickel (5, 10, 25, 50, 75, 100, 200 and 300 mg/L) are given in Fig.1. The highest values of seed germination percentage (98), root length (2.6 cm/seedling), shoot length (7.8 cm/seedling), total leaf area (142.7 cm2/seedling), fresh weight (1.83 g/seedling) and dry weight (0.479 g /seedling) were recorded in seedlings treated with 10 mg/L concentration of nickel. At the same time, the minimum values of germination percentage (26), shoot length (3.7cm/seedling), root length (1.83 cm/seedling), total leaf area (69.65cm2/seedling), fresh weight (0.76 g/seedling) and dry weight (0.07g/seedling) were recorded in seedlings treated with 300 mg/L concentration of nickel.
The highest vigour index (3538.17) and tolerance index (1.55) were recorded in the seedlings treated with 10 mg/L concentration and also lowest percentage of phytotoxicity (0.13) in 5 mg/L concentration of nickel. The lowest vigour index (521.72) and tolerance index (0.18) and also highest percentage of phytotoxicity (1.65) were recorded in the seedlings treated with 300 mg/L concentration of nickel.

3.1.3. Photosynthetic pigments
The effect of different concentrations of nickel on photosynthetic pigments like chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll and carotenoid contents of ground nut seedlings are given in figure 2.The highest values of chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll and carotenoid contents (0.84, 0.43, 1.26 and 0.74 mg/g fr. wt.) of marigold seedlings were recorded at 10 mg/L concentration of nickel. These contents gradually decreased with increase of nickel concentrations. The lowest amount of chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll and carotenoid contents (0.043, 0.018, 0.058 and 0.036 mg/g fr. wt.) were recorded at 300 mg/L concentration of nickel treated ground nut seedlings.

3.1.4.Biochemical parameters
The effect of nickel on protein, amino acid, proline, reducing sugar and non-reducing sugar contents of ground nut seedlings are expressed in mg/g fr. wt. basis and they are presented in fig.3. The highest amount of protein (2.89 mg/g fr. wt.), amino acid (3.25 mg/g fr. wt.), reducing sugar (1.95 mg/g fr. wt.) and non-reducing sugar (2.32 mg/g fr. wt.) content were recorded at 10 mg/L concentration of nickel treated ground nut seedlings and the lowest amount of proline (1.75 mg/g fr. wt.) content was recorded in control seedlings. The lowest amont of protein (0.97 mg/g fr. wt.), amino acid (1.17 mg/g fr. wt.), reducing sugar (0.3 mg/g fr. wt.), non-reducing sugar (0.68 mg/g fr. wt.) and highest amount of proline content (4.41 mg/g fr. wt.) were observed at 300 mg/L concentration of nickel treated ground nut seedlings.

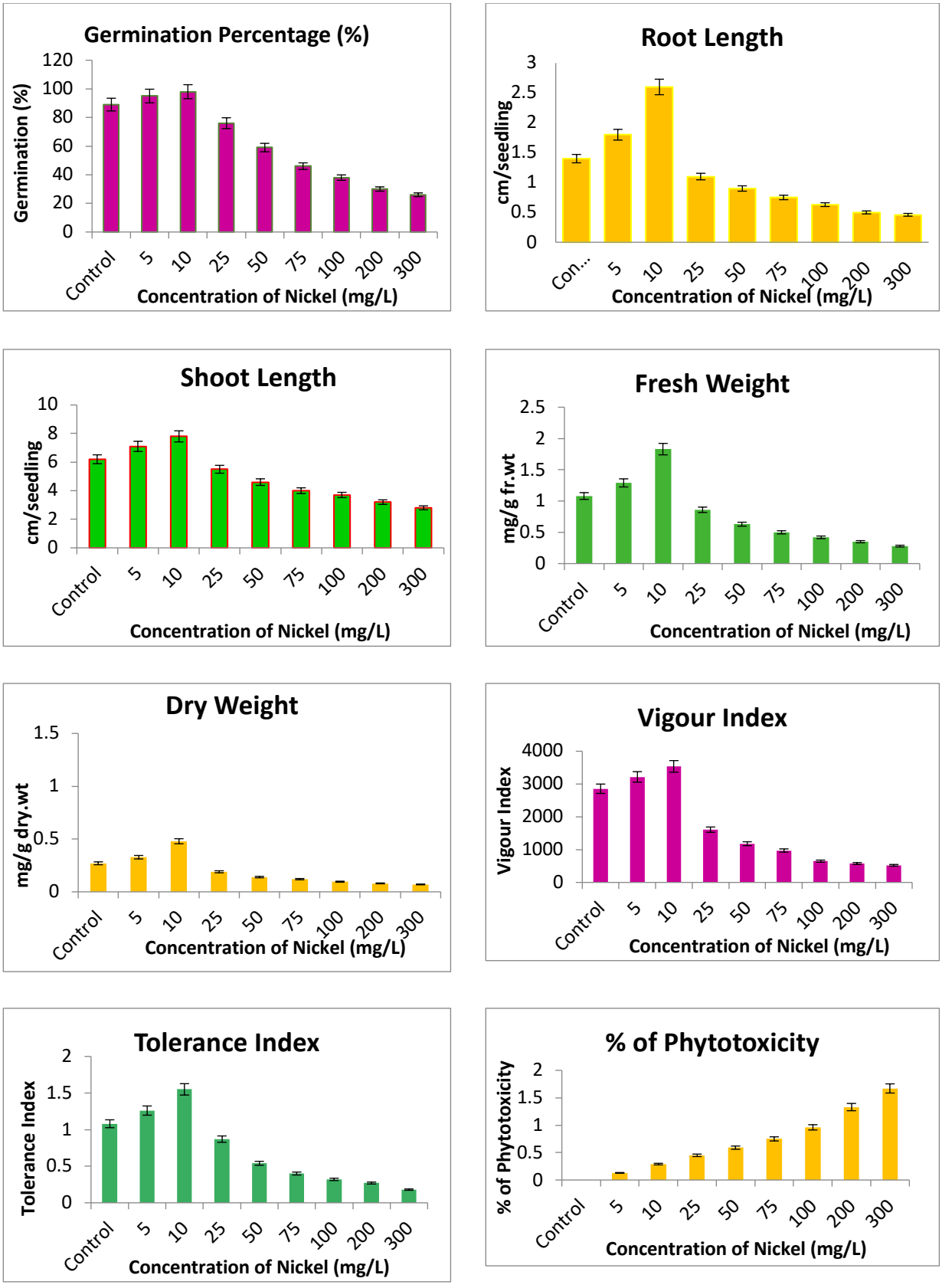


Fig.1. Effect of different concentrations of Nickel on seed germination percentage and morphological parameters of *Arachis hypogea* L. seedlings.

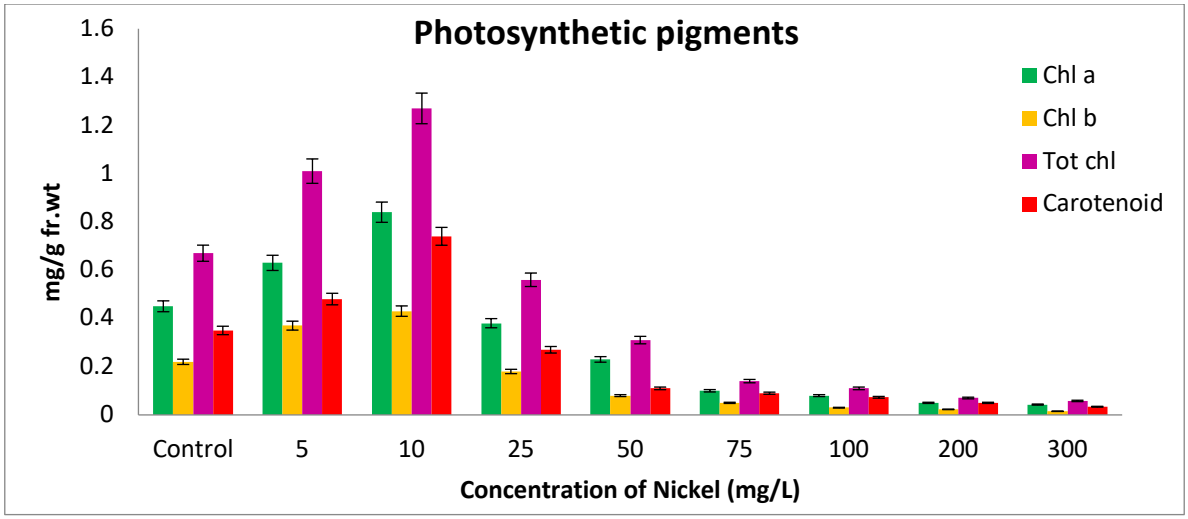


Fig.2. Effect of different concentrations of Nickel on Photosnthetic Pigmens of *Arachis hypogea* L. seedlings.

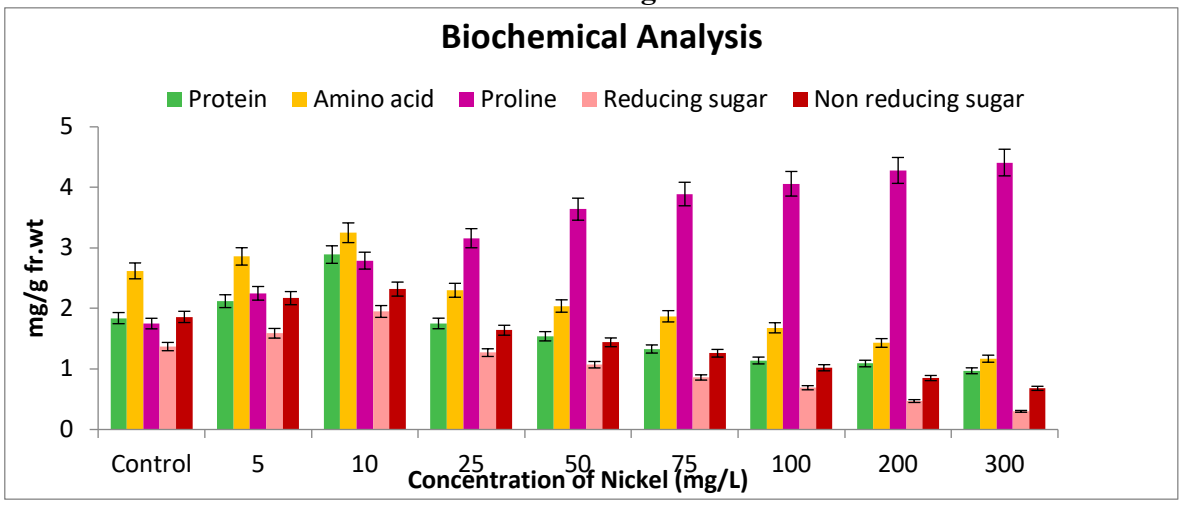


Fig.3. Effect of different concentrations of Nickel on Biochemical Analysis of *Arachis hypogea* L. seedlings.

DISCUSSION

As the initial bridge to plant establishment, seed germination is acutely sensitive to external ambient conditions (Besma and Mounir, 2010). This phase marks the resumption of metabolic pathways, where embryonic growth triggers the rupture of the protective seed coat and driving the emergence of the radicle. The timeline separating seed sowing from successful seedling establishment represents a critical developmental checkpoint. Exposure to severe environmental stressors during this interval degrades germination uniformity and prevents the formation of healthy seedlings. In the current trial, the germination success of groundnut seeds exhibited a steady, concentration-dependent decline as Ni levels escalated. The highest germination percentages occurred under the 10 mg/L concentration Ni treatment, whereas the lowest values were in 300 mg/L concentration of nickel treated seeds. These findings mirror historical reports showing that excessive Ni suppresses seed germination, retards vegetative development, and disrupts cellular mitotic activities (Rao and Sresty, 2000).

Plant growth and structural elongation are continuous biological processes determined by the interaction between the plant's genotype and its immediate soil environment (Shanker et al., 2005). In this study, the impact of various Ni treatments on the shoot and root lengths of Arachis hypogea was evaluated across successive growth stages. The maximum shoot and root lengths were consistently achieved in plants treated with the mild 10 mg/L Ni solution across all sampling intervals at 15, 30, 60 and 90 DAS. Conversely, the minimum growth metrics occurred under the 300 mg/L concentration. These results confirm that while trace amounts of nickel are required for basic metabolic operations (Sreekanth et al., 2013), high concentrations act as a powerful growth inhibitor (Pandey and Sharma, 2002; Gajewska and Sklodowska, 2007; Nagajyoti et al., 2010; Uruc Parlak, 2016). This inhibition is driven by disruptions in regular ionic uptake mechanisms within the root zone (Ouzounidou et al., 2006).

Similar growth restrictions have been documented across diverse cultivars, including field crops and leafy vegetables (Ain et al., 2016; Antonkiewicz et al., 2016).Our observations align with those of Brown et al., (1987) who noted a significant increase in root and shoot dry matter under trace metal applications, but a sharp drop at higher concentrations. This biomass collapse at elevated levels also mirrors the findings of Baccouch et al., (2001), where excessive nickel significantly reduced both root and shoot dry weights. High concentrations of NiCl2 caused a much larger drop in dry weight than lower concentrations, a pattern that matches the heavy metal toxicity responses described by Priyadarshanee et al.,(2022).

As the primary organ for photosynthesis, the leaf is highly sensitive to heavy metal stress. Total leaf area is determined by the cumulative interaction of leaf number, length, and width. In this study, the impact of graded Ni concentrations on the total foliage profile of groundnut was carefully recorded. The maximum leaf area occurred in the 10 mg/L Ni treatment across all sampling intervals, whereas the minimum leaf area was observed under the 300 mg/L treatment. While the low 10 mg/L treatment enhanced groundnut leaf extension, higher concentrations caused a steady decline in leaf development. These observations align with past evaluations of crops treated with heavy metal-bearing inputs (Barman et al., 2000; Sinha et al., 2006).

The seedling vigor index, calculated by multiplying the germination percentage by cumulative seedling length, reflects a plant's ability to establish itself in a stressed environment. In this study, the groundnut vigor index increased up to the 10 mg/L Ni treatment, but the value decreased with the increase of effluent concentration. This biphasic trend matches the vigor responses recorded across several other economic crops exposed to environmental contaminants (Rath et al., 2013; Ashagre et al., 2014; Vaithiyanathan and Sundaramoorthy, 2016).

The tolerance index is calculated by dividing the longest root length of treated seedlings by the longest root length of the 10 mg/L nickel treated plants. In this trial, the tolerance index decreased steadily as nickel concentrations increased. This downward trend matches the tolerance responses observed in Vigna radiata and Cicer arietinum under industrial chemical stress (Mehta and Bhardwaj, 2012), in black gram exposed to processing effluents (Vaithiyanathan et al., 2014), and in lady's finger under dyeing industry wastewater stress (David Noel and Rajan, 2015).

The percentage of phytotoxicity is determined by comparing the radicle lengths of seedlings in the control group with those in the nickel treatments. In this study, the phytotoxicity percentage increased gradually with increase nickel concentration. This concentration-dependent increase in toxicity matches the findings of Ashagre et al., (2014), David Noel and Rajan (2015), and Vaithiyanathan et al., (2016). In our groundnut trial, Ni concentrations above 5 to 300 mg/L clearly inhibited chlorophyll retention. This matches the findings of Gajewska et al., (2006), who noted that high Ni treatments reduced chlorophyll a and b contents in leaves, a localized response common in higher plants (Seregin and Kozhevnikova, 2006). Uruc Parlak (2016) also reported that specific Ni exposures cut chlorophyll content by up to 50% in wheat. The decreased chlorophyll content of leaves is driven by the direct suppression of chlorophyll biosynthesis. At high concentrations, Ni specifically targets and decreases chlorophyll a pigments in Triticum aestivum L.

Proteins serve as primary storage molecules that support early seedling establishment and ongoing plant development. In this study, the highest protein content of groundnut was recorded under the 10 mg/L Ni treatment, but the lowest protein content was recorded in nickel concentrations increased up to 300 mg/L. The elevated protein content under low nickel levels suggests that the plants successfully absorbed essential growth elements. For instance, maintaining optimal magnesium levels is crucial for stabilizing the structure of ribosomes and ribonucleoprotein complexes, which are essential for protein synthesis. This response pattern matches protein variations observed under heavy metal stress in crops like finger millet (Lakshmi and Sundaramoorthy, 2000), tomato (Ravindran et al., 2013), spinach (Kumar and Chopra, 2014e), eggplant (Kumar, 2015), and wheat (Mane et al., 2015a).

Proline is a specialized amino acid that accumulates heavily in organisms ranging from microbes to higher plants when exposed to abiotic stress (Saradhi et al., 1993). This accumulation is widely used as a reliable biochemical marker for stress tolerance (Ramasubramanian et al., 2006). In this trial, the proline content of groundnut was decreased in nickel concentration increased. This accumulation pattern matches observations of plants under environmental stress (Pant et al., 2011). The same trends of sugar variations recorded in radishes (Vijayaragavan et al., 2011), tomato (Rajan and Periyasamy, 2013), cluster bean (Jayakumar et al., 2014), and sugarcane (Kumar and Chopra, 2014c) under metal stress.

This decline indicates that high nickel concentrations impair starch metabolism and block the translocation of synthesized sugars to growing tissues. Furthermore, excessive chemical concentrations can disrupt the uptake of essential nutrients like magnesium, potassium, and phosphorus.The resulting magnesium deficiencies cause leaf chlorosis and depress the photosynthetic rate, which directly reduces the carbohydrate and starch reserves of the crop (Ayyasamy et al., 2008; Divyapriya et al., 2014).

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