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NICKEL ACCUMULATION, GROWTH PERFORMANCE AND YIELD ATTRIBUTES OF *ORYZA SATIVA* L. CV. HUR 105 CROP EXPOSED TO NICKEL POLLUTED SOIL

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ABSTRACT

Nickel (Ni) is essential heavy metals for plant metabolism but is dangerous to most plants when present in excess concentrations as well as poses a threat to agricultural sustainability and food security. The present study investigates the effects of elevated Ni in soil on its accumulation, transfer, and growth performance and yield attributes of rice plants (*Oryza sativa* L. cv HUR 105) under tropical environment of Indian Indo-Gangetic plains. The results showed significantly higher Ni accumulation with maximum in roots followed by leaves, stems and least in seeds (54.7, 38.1, 18.3 and 2.4µg g^{-1}dw, respectively) of *O. sativa* plants grown in Ni polluted soil as compared to control (1.6, 0.8, 0.65 and 0.16µg g^{-1}dw, respectively). Elevated Ni in soil adversely affected growth and yields of *O. sativa* plants, resulting in significant reduction as 26, 31.8, 61.9, 52.6, 32.8 and 40%, respectively at vegetative stage and 35.5, 13.7, 47.6, 54.9, 36.5 and 34.8%, respectively at reproductive stage in leaf area, plant height, biomass accumulation, total chlorophyll content, photosynthetic rate and Fv/Fm ratio ($p<0.05$). Furthermore, biochemical analyses showed significant enhancement in lipid peroxidation and enzymatic activities in leaf tissues of *O. sativa* plant ($p<0.05$). The present study concludes that long term cultivation of *O. sativa* crops grown in Ni polluted soil could not be safe for the consumption as well as pose risk to both agricultural sustainability and food safety.

Key words : Ni accumulation, Growth, Yield, Food safety, Sustainability.

Introduction

Nickel (Ni) contaminated soil presents a significant hazard to the health and survival of organisms (Naqqash *et al*., 2024; Shahzad *et al*., 2018). Niis naturally abundant, ranking as the 22nd most common element in the Earth's crust, and is frequently found in agricultural soils due to natural processes or human activities (Shahzad *et al*., 2018). The presence of toxic levels of Ni in the environment is largely attributed to emissions from various industries, including electroplating, metallurgy, and cement production, as well as activities such as fossil fuel combustion, vehicle emissions, smelting and mining (Begum *et al*., 2022). Ni serves as a crucial micronutrient essential for the normal development and growth of plants (Doria-Manzur *et al*., 2023). It facilitates seed germination and activates a variety of enzymes, including urease, peptide deformylases, glyoxalases, superoxide dismutase (SOD) and hydrogenase within plant systems (Lavres *et al*., 2016).

Ni requirement for optimal plant growth varies depending on the specific plant species and environmental conditions. However, its optimal range in plants falls between 0.05 - 10 mg kg^{1}dw based on plant dry weight (Yusuf *et al*., 2011). Elevated levels of Ni beyond this range can become detrimental to plant health (Abd-Allah *et al*., 2019). Excessive Ni exposure has been noted to impede various crucial processes in plants, including seed germination, shoot and root growth, biomass accumulation, photosynthesis, transpiration, nutrient uptake and disrupt plant water relations. This can manifest in

symptoms like chlorosis and necrosis, ultimately leading to reduced crop yield and quality (Altaf *et al*., 2021).

Under Ni stress, plants experience oxidative stress due to the increased production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$) and hydroxyl ($^{\bullet}OH$) radicals, which can cause damage to essential cellular components like lipids, nucleic acids, proteins and enzymes (Jahan *et al*., 2020). Plants employ strategies to counteract oxidative damage to cellular structures by enhancing both enzymatic and non-enzymatic antioxidant systems (Shad *et al*., 2022). The objective of present study is to investigate the Ni uptake, consequent responses and yield attributes of *O. sativa* plants grown in Ni-polluted soil under tropical environment of Indian Indo-Gangetic plains.

Materials and Methods

Study site, experimental soil and plant

The current research was conducted at the Botanical Garden of the Department of Botany, Banaras Hindu University, Varanasi (latitude: 25°16′ 14.1′′ N, longitude: 82°59′ 20.9′′ E and altitude: 76 meters above mean sea level), from June to November 2022. The average maximum and minimum temperature were 39.27°C and 13.03°C, respectively with a rainfall 56.78 mm during the experimental period. The experimental soil is characterised by typical alluvial type with pH, EC, P, N, Na, Ca, Mg, K, Ni and organic carbon (7.6, 0.26 dS m^{-1}, 66.43 mg kg^{-1}, 0.24%, 148.1 mg kg^{-1}dw, 276.1mg kg^{-1}dw, 987.2mg kg^{-1}dw, 5.3mg kg^{-1}dw and 0.54%, respectively). The rice cultivars used (*Oryza sativa* L. cv. HUR 105) were procured from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. *O. sativa* is highly scented, an erect, semi dwarf (100–110 cm in height) variety with 125-130 days to maturity and high yielding potential in Northern India.

Experimental design

A pot based experimental study using soil and *O. sativa* plant was conducted at the Botanical Garden of the Department of Botany, Banaras Hindu University, Varanasi. The soil air dried and uniformly mixed with nitrogen (N), phosphorus (P) and potassium(K) at recommended doses (120:80:40 kg ha^{-1}), supplied as urea, ammonium phosphate, and potassium muriate, respectively, along with farmyard manure (20 t ha^{-1}). The soil was divided into two groups, one was kept as such and treated as control (C) and other was supplied with Ni at rate of 100 mg kg^{-1} as $NiCl_2.6H_2O$, representing elevated Ni (eNi). Ten pots (each pot have 28.2 cm, 20 cm and 625 cm^2, diameter, depth and area, respectively) from each group were filled with soil at the rate of 5 kg pot^{-1}. Ten healthy *O. sativa* seeds were manually sown at a depth of 2 cm with consistent spacing. To ensure uniform moisture levels, equal volumes of ground water were applied to each pot. Ten days' post-germination, the plants were thinned to five per pot, and manual weeding was performed as and when required throughout the experimental period.

Sampling and analysis

Morphological measurements

Five *O. sativa* plants were randomly collected from each treatment pots at both vegetative and reproductive stages. To ensure the removal of any soil or debris adhering to the samples, samples were thoroughly washed under running tap water were dried using blotting paper. The samples underwent analysis to assess various mrophological parameters such as leaf number, leaf area, root length, shoot length, total plant length, root dry weight, shoot dry weight and total dry weight. Leaf number and leaf area were determined manually and using a leaf area meter (Model 211, Systronics, India), respectively. Root and shoot lengths were manually measured using a meter scale, and their values were sumed to obtain the total plant length. Plant were sebrerated into roots, stems, and leaves, chopped and were subjected to oven drying at 80°C until a constant weight. The total plant biomass was then calculated by adding the dry weights of all plant components.

Physiological and biochemical measurements

Portable photosynthetic system (CIRAS-3, PP system, Haverhill, Amesbury, MA, United States) and plant efficiency analyzer (PAM-2500, WALZ, Germany) were used to measure the photosynthetic rate and Fv/Fm ratio in the fourth leaf from the top, respectively, under ambient climatic conditions. The total chlorophyll content in leaf tissues was assessed using a portable chlorophyll meter (SPAD-502, Konica Minolta, Japan). Biochemical parameters were analysed in triplicates of fresh leaf samples at both vegetative and reproductive stages. Malondialdehyde content in the leaf tissues of tested plants was determined to extent lipid peroxidation (MDA) following the protocol outlined by Heath and Packer (1960). Superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) activities were evaluated in the leaf tissues using the methodologies described by Fridovich (1974), Aebi (1984) and Anderson (1996), respectively.

Yield attribute measurements

Ten replicates of plant samples were randomly collected from each treatment for the analysis of yield

characteristics of the *O. sativa* plant. Yield attributes were analysed in terms of number of panicles (plant^{-1}), number of seeds (panicle^{-1}), number of seeds (plant^{-1}), panicle weight (plant^{-1}), seed weight (plant^{-1}) and test weight (g 1,000 seeds^{-1}). Harvest index (HI) indicating the ratio of grain weight to above-ground biomass at maturity was also calculated to assess the biomass allocation in the edible parts of tested crops.

Ni accumulation and soil-plant transfer

A total of 0.25 g of air-dried soil and 0.1 g of dried powder from each part of the *O. sativa* plant (root, stem, leaf and seed) were mixed with 10 ml of tri-acid mixture (HNO_3, H_2SO_4 and $HCLO_4$ in 5:1:1 ratio) to determine the total concentration of Ni, following the procedure as described by Allen *et al*. (1986). The digested samples were then filtered using Whatman No. 42 filter paper, and the resulting filtrates were adjusted to a final volume of 25 ml with DDW. The Atomic Absorption Spectrophotometer (Model 2380, Perkin Elmer, USA) with a detection limit of 0.003 µg mL^{-1} was used to determine the Ni content in the filtrates of different samples. Soil-plant transfer of Ni in each treatment was computed as bioaccumulation factor (BAF), a ratio of the heavy metal concentration in the plant to that in the soil (Pietrelli *et al*., 2022).

Statistical analysis

The significance of variations among treatment means was assessed through statistical analyses using Duncan's multiple range tests at a 0.05 probability level, using SPSS software version 16. The results were presented as means ± standard error (SE) from n replicates.

Results and Discussion

Ni accumulation and soil-plant transfer

The roots, stems, leaves and seeds of *O. sativa* plants exposed to eNi has demonstrated significantly higher Ni accumulation as compared to the control. Specifically, in the eNi treatment, Ni concentration in the roots, leaves, stems, and seeds were maximum to minimum as 54.75, 38.08, 18.29 and 2.41 µg g^{-1} dw, respectively and 1.56, 0.81, 0.65, and 0.16 µg g^{-1} dw, respectively in the control (Fig. 1). Root tissues exhibited the highest concentration of Ni followed by leaves, stems, and lowest in seeds, respectively (Fig. 1). These findings are consistent with previous studies of Rizwan *et al*. (2017) in on *O. sativa* and Siddiqui *et al*. (2011) in *Triticum aestivum* L. plants indicating a substantial sequestration of toxic metals in root tissues (Sharma and Dubey, 2007). The concentration of Ni in harvested soil from control eNi treatments were found to be 2.87 and 53.18 µg g^{-1} dw, respectively. The

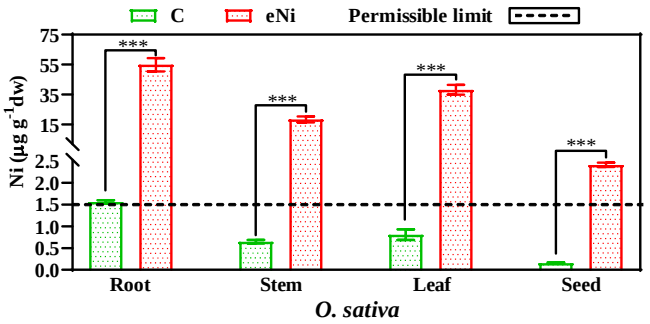

Fig. 1 : Ni accumulation in different parts of *O. sativa* crop grown in Ni polluted soil. Level of significance: ***p< 0.001

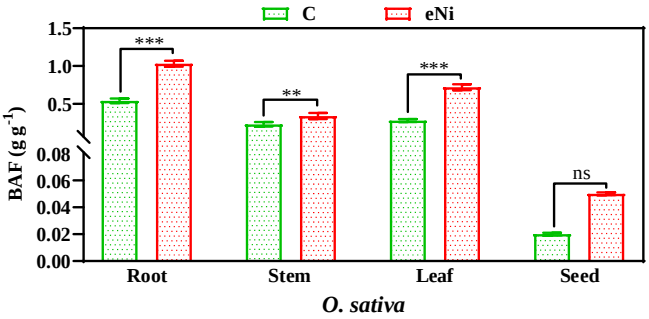

Fig. 2 : Ni bioaccumulation factor computed for different parts of *O. sativa* crops grown in Ni polluted soil. Level of significance: **p< 0.01; ***p< 0.001; ns = not significant.

BAF indicating the Ni uptake capacity of *O. sativa* plants exposed to Ni-polluted soil. Results indicated that the BAF value was found highest for the roots, followed by the leaves, stems and lowest in the seeds of the *O. sativa* in both the treatments (Fig. 2). The percent increase in BAF of *O. sativa* plants in eNi was found most pronounced in the roots, followed by the leaves, stems and seeds, *i.e.* 90.7%, 63.6%, 47.8% and 33.4%, respectively. Infante *et al*. (2021) observed that when the BAF exceeds a unit it indicates a higher concentration of heavy metals in the plant than in the soil. The results indicate that plant absorbs more heavy metal and accumulates in various plant tissues, potentially posing human health risks when edible parts of the plant are consumed. Conversely, when the BAF is less than 1, it suggests that the plant absorbs heavy metals without significant accumulation in different plant tissues (Satpathy *et al*., 2014). Similar findings have been reported for leaves, stems and seeds, except for roots by Infante *et al*. (2021).

Morphological, physiological and biochemical responses of *O. sativa* plants

The effects of elevated Ni in soil on morphological, biochemical and physiological responses of *O. sativa* plants as compared to control are presented in Table 1. The results demonstrated that eNi treatment caused a significant decline in growth performance, including the

Table 1 : Morphological, physiological and biochemical responses of *O. sativa* crop exposed to Ni polluted soil.

Plant traits/treatments	*O. sativa* cv. HUR 105			
	Vegetative age		Reproductive age	
	C	eNi	C	eNi
No. of leaves plant^{-1}	23.66 ± 4.0**	10.33 ± 0.6**	24 ± 2.1*	14.66 ± 3.1*
Leaf area (cm^2 plant^{-1})	212.67 ± 14.0***	157.33 ± 9.3***	235.67 ± 16.0***	152 ± 9.7***
Root length (cm plant^{-1})	9.61 ± 0.3**	4.88 ± 0.5**	20.03 ± 0.8**	15.03 ± 0.5**
Shoot length (cm plant^{-1})	40.47 ± 0.4***	29.29 ± 0.6***	87.36 ± 4.1**	77.63 ± 1.4**
Total length (cm plant^{-1})	50.09 ± 0.6***	34.18 ± 1.0***	107.40 ± 5.1***	92.66 ± 2.0***
Root dry weight (g plant^{-1})	1.92 ± 0.2**	0.41 ± 0.02**	7.64 ± 0.3**	5.11 ± 0.5**
Shoot dry weight (g plant^{-1})	12.28 ± 0.4**	5.01 ± 0.4**	32.78 ± 3.7***	16.08 ± 0.4***
Total dry weight (g plant^{-1})	14.21 ± 0.2***	5.41 ± 0.2***	40.42 ± 3.4***	21.19 ± 2.2***
Total chlorophyll(mg g^{-1} fw)	22.31 ± 2.1***	10.58 ± 1.2***	19.41 ± 1.3***	8.76 ± 0.8***
LPO (n mol ml^{-1})	0.78 ± 0.2***	2.01 ± 0.1***	0.66 ± 0.05**	1.96 ± 0.2**
SOD (unit g^{-1} fw)	0.34 ± 0.01**	0.20 ± 0.02**	0.23 ± 0.01*	0.17 ± 0.02*
CAT (µmol H_2O_2 oxidised min^{-1}g^{-1} fw)	8.21 ± 1.1**	2.81 ± 0.3**	3.75 ± 0.3**	1.43 ± 0.2**
GR (µmol purpurogallin formed min^{-1}g^{-1} fw)	25.43 ± 1.4**	43.06 ± 0.7**	10.11 ± 1.4**	21.61 ± 1.7**
Photosynthetic rate(µ mol CO_2 m^{-2} s^{-1})	18.93 ± 0.6***	12.73 ± 0.3***	16.43 ± 0.5***	10.43 ± 0.6***
Fv/Fm ratio	0.70 ± 0.01***	0.42 ± 0.03***	0.69 ± 0.04***	0.45 ± 0.02***

Values are mean ± SE for three replicates. Levels of significance: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$
C = control eNi = elevated Ni

number of leaves, leaf area, root length, shoot length, total plant length, root dry weight, shoot dry weight and total dry weight in *O. sativa* plants as compared to the control ($p < 0.05$). Specifically, during the vegetative stage, eNi treatment exhibited significant reductions in above parameters by 56.3%, 26.0%, 49.2%, 27.6%, 31.8%, 78.6%, 59.2% and 61.9%, respectively and 38.91%, 35.5%, 24.9%, 11.3%, 13.7%, 33.1%, 50.9% and 47.7%, respectively during reproductive stage as compared to the control (Table 1). The results of present study showed the eNi has maximum effects of total dry matters during the growth and development of *O. sativa* plants. Similar findings have been reported in *O. sativa* (Rizwan *et al*., 2022), *Gossypium hirsutum* L. (Khaliq *et al*., 2016) and *Lycopersicon esculentum* L. (Mosa *et al*., 2016) exposed to Ni contamination.

In comparison to the control plants, eNi in the soil significantly increased lipid peroxidation and GR levels in leaf tissues of *O. sativa*, while simultaneously decreasing SOD, CAT, total chlorophyll content, photosynthesis rate, and Fv/Fm ratio by 67.9%, 69.3%, 41.2%, 65.8%, 52.6%, 32.8% and 40%, respectively at the vegetative stage, and 75.7%, 59.3%, 26.1%, 61.9%, 54.9%, 36.5% and 34.8%, respectively at the reproductive stage over control (Table 1). Rizwan *et al*. (2022) and Mosa *et al*. (2016) have shown decline in photosynthesis rate and total chlorophyll significantly in *O. sativa* crops under Ni stress which is consisted with the present findings. In the present experiment, MDA content of leaf tissue in *O. sativa* crops was significantly higher under eNi stress as compared to the control plants. The present results indicated that Ni toxicity enhances MDA content in *O. sativa* plants (Mostofa *et al*., 2021; Yadav *et al*., 2021), *T. aestivum* (Gajewska *et al*., 2012) and *Sorghum bicolor* L. (Pandian *et al*., 2020). The resent study found that *O. sativa* plants exposed to higher Ni concentrations experienced a decline in SOD and CAT activities, consistent with previous observations in *O. sativa* plants reported by Rizwan *et al*. (2017). Moreover, the present results also revealed that exposure of *O. sativa* plants to eNi led to increased GR activity, in line with findings reported in *S. bicolor* (Pandian *et al*., 2020). The results of two-way ANOVA analysis indicated significant variations in SOD, photosynthetic rate, and total plant length attributed to both age and treatment, while variations in leaf area and total dry weight were significantly influenced by age, treatment and their interaction (Table 2).

Yield attributes

The results of the present study showed a significant decrease in yield attributes of *O. sativa* plants under eNi treatment as compared to control, with reductions of 60.1%, 40%, 75.9%, 67.8%, 66.3%, 31.2% and 87.7% for the number of panicles plant^{-1}, number of seeds

Table 2 : Two-way ANOVA results for physiological, biochemical and growth performance of *O. sativa* crop exposed to Ni polluted soil.

Parameters	Variables		
	Age (A)	Treatments (T)	A × T
No. of leaves	16.6^{**}	234.7^{***}	12.5^{**}
Leaf area	35.4^{***}	952.3^{***}	23.6^{***}
Root length	297^{***}	66.4^{***}	0.05^{ns}
Shoot length	2384^{***}	114.9^{***}	0.55^{ns}
Total length	1918^{***}	134.3^{***}	0.19^{ns}
Root dry weight	343.9^{***}	51.9^{***}	3.7^{ns}
Shoot dry weight	1379^{***}	795.7^{***}	122.8^{***}
Total dry weight	1134^{***}	505.2^{***}	70.3^{***}
Total chlorophyll	13.6^{**}	307.2^{***}	0.72^{ns}
LPO	0.03^{ns}	65^{***}	0.04^{ns}
SOD	25^{***}	34.7^{***}	10.4^{*}
CAT	11.4^{**}	20.4^{**}	3.2^{ns}
GR	185.6^{*}	116.7^{***}	4.9^{*}
Photosynthetic rate	21.7^{**}	139.9^{***}	0.04^{ns}
Fv/Fm ratio	0.3^{ns}	632^{***}	3.1^{ns}

Levels of significance: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns= not significance.

Table 3 : Yield attributes of *O. sativa* crop exposed to Ni polluted soil.

Yield attributes/ treatments	*O. sativa*	
	C	eNi
Number of panicle (plant^{-1})	$3.33 \pm 0.3^{**}$	$1.33 \pm 0.1^{**}$
Number of seeds (panicle^{-1})	$123.33 \pm 5.3^{**}$	$74 \pm 2.3^{**}$
Number of seeds (plant^{-1})	$412 \pm 16.12^{**}$	$99.33 \pm 5.4^{**}$
Seeds weight (plant^{-1})	$15.26 \pm 1.0^{***}$	$4.91 \pm 0.2^{***}$
Panicle weight (plant^{-1})	$18.30 \pm 2.1^{**}$	$6.17 \pm 0.4^{**}$
Test weight (g 1000 seeds^{-1})	$20.03 \pm 1.5^{***}$	$13.79 \pm 1.4^{***}$
Harvest index (%)	$46.55 \pm 6.3^{***}$	$5.65 \pm 0.2^{***}$

Values are mean ± SE for three replicates.
Levels of significance: $^{**}p < 0.01$, $^{***}p < 0.001$

panicle^{-1}, number of seeds plant^{-1}, panicle weight plant^{-1}, seed weight plant^{-1}, test weight (g^{-1}1000 seeds) and harvest index (%), respectively, at a significance level of $p < 0.05$ (Table 3). Nowwar *et al*. (2022) reported a similar trend in both growth and yield parameters, including the number of pods and seeds plant^{-1}, weight of seeds plant^{-1} and weight of 1000 seeds, in jute mallow (*Corchorus olitorius* L.) plants under untreated wastewater irrigation as compared to those under fresh water irrigation as a control. The reduction in yield parameters due to wastewater irrigation may be attributed to elevated levels of heavy metals in soil and their transfer to plants (Kanwal *et al*., 2020; Bhat *et al*., 2019).

Conclusion

The present study demonstrated that Ni stress, particularly at elevated concentration in soil has severely inhibited growth and photosynthetic parameters of *O. sativa* plants with increased levels of antioxidant activities in response to high accumulation of Ni in leaf tissues of *O. sativa* plants. Significant variations were observed in Ni accumulation across roots, stems, leaves and seeds, with the root exhibiting the highest Ni content. Elevated levels of Ni also induced oxidative stress that led to increased production of ROS and lipid peroxidation in *O. sativa* plants. The biochemical parameters such as SOD, CAT and GR activities reflect the ability of *O. sativa* plants to better endure Ni-induced oxidative stress. Ni accumulation in seeds exceeds its permissible limit suggested that seeds of *O. sativa* plants cultivated in high Ni contaminated area is not safe for consumption.

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