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TOXICOLOGICAL CHANGES IN RICE UNDER NICKEL STRESS

Jyotirmay Mathan[1], Monalisa Mohanty[2], Chinmay Pradhan[3]* and Hemanta Kumar Patra[4]

[1,2,3,4] Laboratory of Plant Biochemistry and Environmental Biotechnology,
Post Graduate Department of Botany. Utkal University, Bhubaneswar-751004

E-mail: chinmay.uubot@gmail.com

ABSTRACT

Nickel is an important mineral trace nutrient found at low concentrations in most natural soils as a heavy metal. However, it may reach toxic levels in certain areas and affect a number of biochemical and physiological processes in plants. The present study reports nickel (Ni) induced phytotoxic alterations in plant growth, physiology and antioxidative enzyme activities in rice (*Oryza sativa* L cv. NDR-97) seedlings under applications of Ni^{2+} ions. Hydroponically grown 7 days old rice seedlings were studied for the impact of different concentrations of Ni^{+2} (10µM to100 µM). The seedlings showed stimulated growth when supplemented with 10µM of Ni^{+2} and where as growth retardation was observed beyond 10µM of Ni^{+2} treatments which were evidenced from their respective total chlorophyll values. Catalase and peroxidase activity exhibits a concentration dependent increase with elevated Ni^{+2} treatments. Ni Phytotoxicity assessment in developing rice crops will reveal the extent of negative impacts of Ni in response to its physiological and biochemical parameters.

Key words: Catalase; Chromium; Chlorophyll; Growth response; Nickel; Peroxidase

INTRODUCTION

Heavy metals have recently received the attention of researchers all over the world, mainly due to their harmful effects on plant and animals (Alloway, 1995; Dudka and Adriano, 1997; Kabata-Pendias and Pendias, 2001; Wong et al., 2002; Girard et al., 2005; Selinus et al., 2005; Kribek et al., 2010). Severity in crop loss due to heavy metal toxicity in the environment draws the attention of researchers for its possible remediation through innovative techniques. Nickel and its compounds are released into the atmosphere from both natural and anthropogenic sources. Ni is reported as an essential trace element (0.01-5µg/g dry wt.) for some plants (Seregin and Kozhevnikova, 2006). Although Nickel is an essential element (micronutrient) to plants, there has been much more concern about the toxicity of Ni than about Ni deficiency. According to World Health Organisation (WHO), 1991**,** the chemical and physical forms of Nickel and its salt strongly influence bioavailability and toxicity. Ni is considered as an essential micronutrient for plants but is strongly phytotoxic at high concentrations and has a destructive effect on growth, mineral nutrition, photosynthesis and membrane function (Woolhouse, 1983; Pandolfini *et al.*, 1992; Rao and Sresty, 2000; Peralta *et al.*, 2000; Reichman, 2002). Rice is one of the most important staple food consumed world wide. It is a major crop of Odisha as well as in India. The present study encompasses the phytotoxic effects of varying concentrations of nickel on physiological and toxicological changes in rice seedlings after 7 days exposure.

MATERIALS AND METHODS

Plant Material:
Graded dry seeds of rice (*Oryza sativa* L. cv. NDR-97) were obtained from Central Rice Research Institute, Cuttack (India).

Germination Study:
Uniform sized seeds surface sterilized with 0.1% mercuric chloride ($HgCl_2$) were placed in sterilized petriplates over saturated tissue paper for germination under varying concentrations of Ni^{+2} (0 µM as Control, 10µM, 25 µM, 50µM, 75µM and 100µM) in different petriplates. The seeds were germinated under controlled condition at 25º C in darkness for two days. After two days, emergence of 2 mm radicle was used as the operational definition of germination. Germination percentage, Vigor Index (VI) and Tolerance indices (TI) were determined by following the formula as given by Iqbal and Rahmati (1992) (Pattnaik et al., 2012).

% of Germination = No. of seeds germinated X 100
Total no. of seeds taken VI = Seedling Length (cm) × Germination percentage

TI = (Mean root length of Ni treated seedlings/Mean root length of seedlings grown in control) × 100

Seedling Growth and Growth Parameter Study:
Two-days-old uniform surface sterilized germinated seeds were transferred to well aerated Hoagland's nutrient solution (half strength) as control and Hoagland's solution supplemented with varying concentrations of nickel (Source: $NiCl_2$) placed in hydroponic culture vessels for seedling growth. The seedlings were grown in the growth chamber and the white light was provided (12h photo period) by white fluorescent tubes (36 W Philips TLD) with a photon flux density of 52 µ /m^2s (PAR). Seven days old rice seedlings supplemented with different nickel concentrations were used for study of various growth parameters like root length, shoot length, fresh matter and dry matter. Seedlings supplemented with Hoagland media and Ni^{+2} concentrations from (10µM) to (100µM) were analysed for their different growth parameters. Both nickel treated and control seedlings were kept in an oven at 80º C for a period of 3 days or more (till constant weight was attained) for dry mass determination.

Analysis of Chlorophyll Content:
The extraction and analysis of leaf chlorophyll content was conducted by using 10 ml of 80% cold acetone following the method of Porra (2002). The leaf samples from different culture pots were kept in dark in refrigerator for 48 hours at 4^0C. The absorbance of extracted liquid was observed at 663.6 nm, 646.6 nm and 470 nm for Chlorophyll-a, Chlorophyll-b and carotenoid respectively.

Analysis of Antioxidative Enzyme Activity:
Fresh leaves (0.5 gm) from seven days old seedlings of six different treatments vessels were separately chopped into small pieces. The chopped material was homogenized with the help of pre chilled mortal and pestle in the presence of phosphate buffer and EDTA. The total volume of buffer and the EDTA was 3ml. (2.7ml buffer + 0.3ml EDTA). PMSF and 10% w/v PVP were added prior to homogenization. The slurry was collected in separate eppendorf tubes and centrifuged at 14,000 rpm for 15 min. The centrifugation was repeated for two times. The supernatants as collected were stored at 4˚C for the estimation.

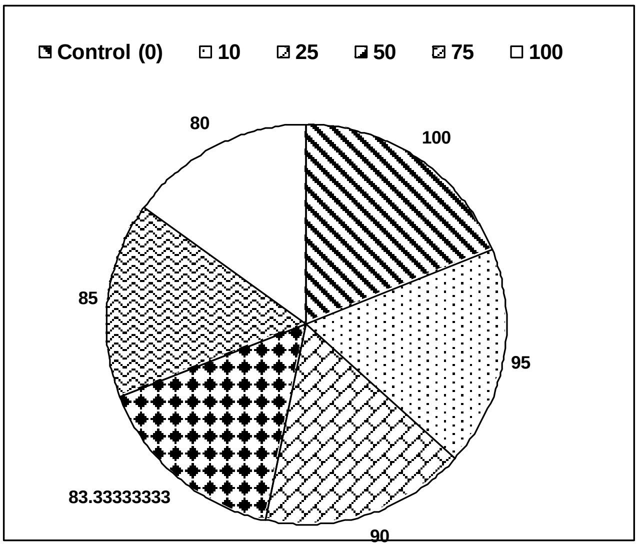
Estimation of Catalase activity:
For catalase assay 2 ml of sodium phosphate buffer and 0.5 ml of 12 mM of H_2O_2 was added to 0.5 ml of the plant leaf extract and the O.D was taken at 240 nm. Catalase activity was assayed by measuring the initial rate of disappearance of H_2O_2 (Kato and Shikizu, 1987). The decrease in H_2O_2 was followed as the decline in absorbance at 240 nm; activity was calculated using the extinction coefficient (40mM- cm- at 240nm) for H_2O_2(Mohanty and Patra, 2012).

Estimation of Peroxidase activity:
For assay of peroxidase activity, 2.8 ml of potassium phosphate buffer (pH = 7.0), 50µl of 10 mM H_2O_2 and 50µl of guaiacol was added to diluted leaf extract. The mixture was mixed properly and absorbance value was recorded at 436 nm. Activity was calculated using the extinction coefficient (25.5 mM^{-1} cm^{-1} at 436 nm) for tetraguaiacol (Mohanty and Patra, 2012).

RESULTS AND DISCUSSION

Maximum inhibition of seed germination was observed at 100 µM of Ni, which resulted in 20 % inhibition in seed germination whereas minimum was at 10 µM Ni treatment resulting in 5 % inhibition of seed germination (Figure.1).

Figure-1. Impact of Ni^{+2} treatments (µM) on germination % of rice seeds



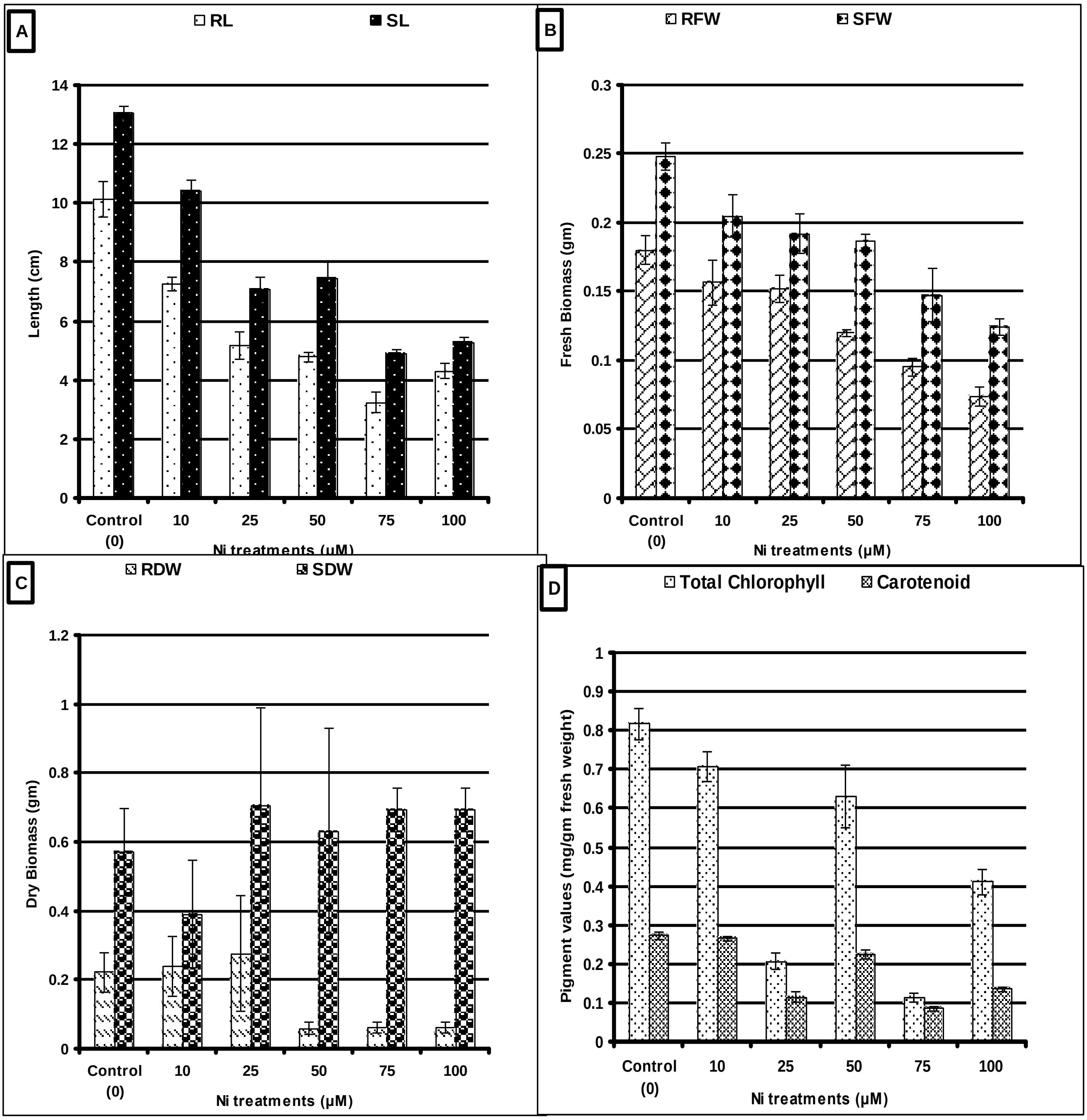
Changes in growth parameters:
Treatment of different concentrations of Ni^{2+} showed considerable changes in different growth parameters of seven days grown rice seedlings (Figure. 2A, Fig. 2B and Fig. 2C). Shoot length decreased markedly with increase in Ni^{2+} concentrations (10µM to 100µM). The shoot length of the seedlings treated with Ni^{2+} (10µM) was noted highest as compared to all other treatments of Ni^{2+}. The seedlings treated with 100µM-Ni^{2+} had minimum length as shown in Figure. 2A. The trend shows similarity with the findings of others in different plants (Mohanty and Patra, 2012; Mohanty and Patra, 2013). The root length also markedly decreased with increasing concentrations of $NiCl_2$ in the growth medium. The seedlings treated with $NiCl_2$ metal ions showed relative decrease in the root growth (measured in terms of root length) in the similar order as observed in shoot growth study.

The shoot fresh weight and fresh root weight gradually decreased with increase in the concentrations of $NiCl_2$. Similar growth trend values were found for dry biomass production as compared to fresh weight values (Fig. 2B and Figure. 2C). The rice seedlings were examined for tolerance in different concentrations of Ni. It was observed that tolerance was gradually decreased by increasing concentrations of Ni treatments (figure 3). Ni treatment at 10 µM showed high percentage (71.47%) of Tolerance Index in rice seedlings which get subsequently decreased with elevated levels of Ni treatments. But there was a sudden increase in Ti values beyond 100 µM of Ni^{+2}. The seedling vigour index and tolerance index values' giving a phytotoxic interpretation of Ni as illustrated in Figure. 3.

Analysis of Chlorophyll and Carotenoid Content:
The decrease in chlorophyll-a content was found with the increase in concentration of $NiCl_2$ except 10 µM. The chlorophyll-a content was found maximum with 10µM-$NiCl_2$ treatment and minimum in 100µM-$NiCl_2$. There was a similar trend of increase/decrease in chlorophyll-b and carotenoid content of the seedlings treated with different concentrations of $NiCl_2$ as shown Chlorophyll-a. The chlorophyll-b content decreased with increasing concentration of $NiCl_2$. The total chlorophyll content was the highest in the seedlings treated with $NiCl_2$ (10µM) and lowest in seedlings treated with $NiCl_2$ (100µM) (Figure 2D).

Alterations in Antioxidative Enzyme activity:
The antioxidants (catalase, peroxidase activities and carotenoids content) showed variable responses to different nickel concentrations (Fig.4). The activity of peroxidase (POX) increased with increase in nickel-stress. Catalase

Figure-2. Phytotoxic Impact of Ni^{+2} treatments (µM) on A: Root and Shoot length, B: Fresh Biomass, C: Dry Biomass and D: Total Chlorophyll and Carotenoid Content of & days old rice seedlings.

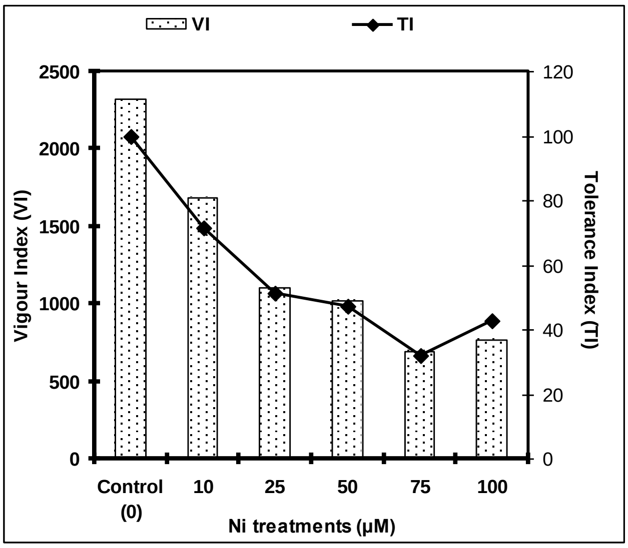


NB: RL-Root length; SL-Shoot length; RFW-Root Fresh Weight; SFW-Shoot Fresh Weight; RDW-Root Dry Weight; SDW-Shoot Dry Weight

(CAT) activity also showed increasing trend with increased nickel concentrations up to 100µM, which decreased at 10µM nickel exposure. Under metal stress conditions, including excess nickel exposure, an imbalance between generation and removal of ROS arise in plant tissues (Grataq et al., 2005).

POX and CAT activities are essential components of plant antioxidant defence system.

Figure-3. Phytotoxic interpretations (VI and TI values) of Ni^{+2} treatments (µM) on 7 days old rice seedlings



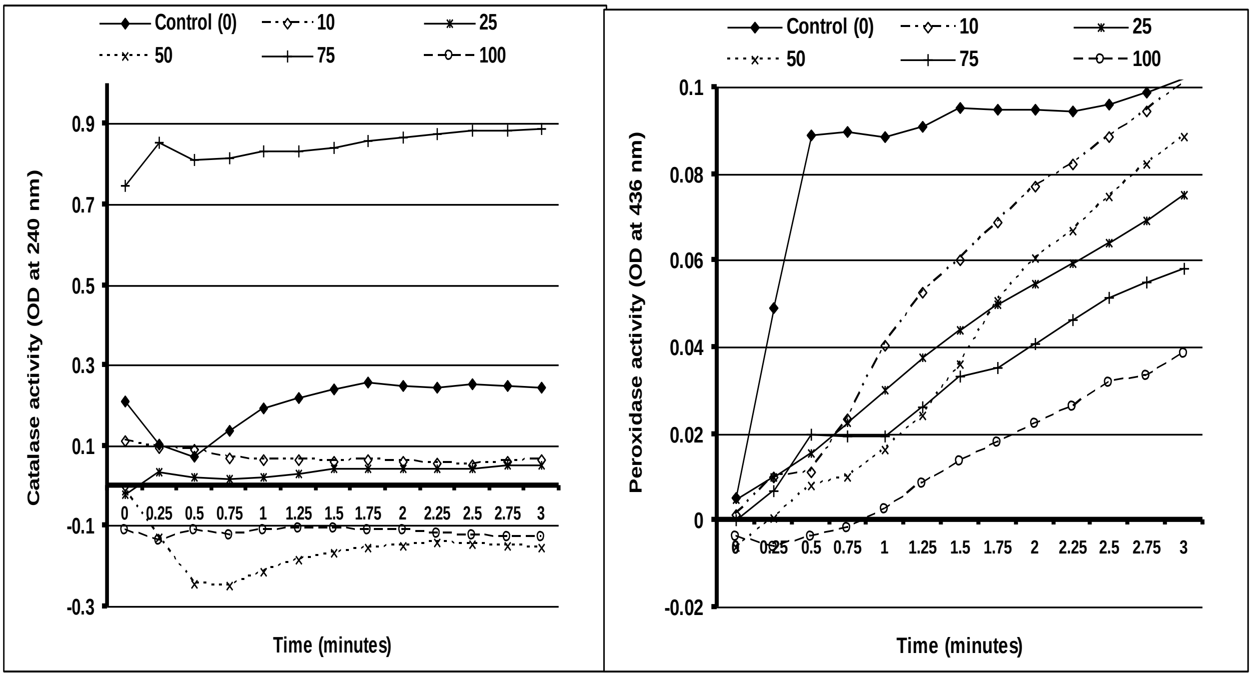
Elevation in POX activity in nickel treated *O. sativa*, suggests its role in the detoxification of H_2O_2 (Dey et al., 2009). The enhance activity of POX in excess nickel treated plants might result either in peroxidative damage of the thylakoid membrane or lower auxin and protein contents in tissues (Sandman and Boger, 1980). The activities of CAT and POX protect the metabolism in plant cells (Pandey, 2008).

A stable catalase activity was noticed in a three minute reaction for all the Ni treatment plant samples (Fig 4) where as a sharp linear rise in peroxidase (GPX) activity was observed with time as evident from the graph. The scavenging activity was enhanced by peroxidase with progressive increase in its activity with time.

DISCUSSION

A massive loss in agricultural yield and potential hazardous health effects are most possible outcome of heavy metal contamination in soil which has now become a worldwide problem and needs implementation of mitigation measures. Heavy metals are detrimental because of their non-biodegradable nature, long biological half-life and their potential to accumulate in different body parts (Behbahaninia *et al.*, 2009; Pattnaik et al., 2012; Mohanty and Patra, 2013). Nickel has been considered as an essential micronutrient but at high concentrations it causes adverse effects in plants as evidenced by several workers (Welch 1995; Pattnaik et al., 2012). The study shows detrimental effects of nickel on growing rice seedlings at elevated concentrations. The study also depicts significant reduction in germination of seeds with increasing supply of $NiCl_2$.

Figure-4. Rate of Change in Catalase (CAT) and Peroxidase (GPX) activities of 7 days grown rice seedlings in response to Ni stress



This may be due to inhibition of biosynthetic activity. Growth parameters viz. plant height and biomass of developing rice seedlings showed noticeable decline in response to increasing concentrations of $NiCl_2$. The findings of the present study corroborates that of other researchers in plants like green gram, wheat, rice in response to various heavy metals like Cr, Ni etc. (Pattnaik et al., 2012; Mohanty and Patra, 2012; Mohanty and Patra, 2013). However, at 10 μM $NiCl_2$ the growth was slightly stimulated. The increased antioxidative enzyme indicates potentiality of seedlings for scavenging Ni stress induced reactive oxygen species.

CONCLUSION

Tolerance to heavy metals in plants may be defined as the ability to survive in soil that is manifested by an interaction between a genotype and its environment (Macnair *et al.*, 2000). Tolerance to Ni treatments in rice was lower as compared to control but it was also noticed that the seedlings treated with 100 μM of Ni^{+2} showed high tolerance index in comparison to 75 μM of Ni^{+2} treatments. This information can be considered a contributing step in exploring and finding of tolerance limit of rice at different levels of treatment. In our study the enzyme responses to Ni^{+2} differed; elevated concentrations of Ni^{+2} resulted in increased peroxidase activity which might be due to increased rate of enzyme activity for scavenging the reactive oxygen species as induced by Ni stress.

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