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Original Paper

Responses of growth and antioxidative enzymes to different Ni concentrations in castor bean (*Ricinus communis*)

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Abstract

A pot culture experiment was conducted to investigate the effect of different levels of nickel on growth and biochemical parameters of castor bean (*Ricinus communis* L.). Castor seedlings were grown in control conditions or in soil amended with 50, 100, 300 and 500 mg kg^{-1} of Ni. Plants were harvested after 60 days of sowing. A significant decrease in growth, and concentration of chlorophyll and carotenoids were observed with increasing concentrations of Ni. Leaf proline and malondialdehyde concentration increased considerably in response to Ni application. It was observed that the protein concentration in the leaves of castor plants decreased significantly with the increase in Ni concentrations in soil. An increase in protein content and enzyme activities was observed at increasing Ni concentration in soil. Significant changes in the antioxidative enzyme activities were observed in *R. communis* treated with varying Ni doses. It was found that with increasing concentrations of Ni in soil, the activity of the enzymes also increased. This suggests that the increase of enzyme activity due to Ni toxicity might play a role in the defence response of *R. communis*.

Key words: antioxidative enzymes, chlorophyll, growth, nickel, *Ricinus communis*.
Abbreviations: CAT, catalase; DAS, days after sowing; MDA, malondialdehyde; POD, peroxidase; ROS, reactive oxygen species; RWC, relative water content; SOD, superoxide dismutase; TI, tolerance index..

Introduction

Nickel has gained considerable attention in recent years, due to its rapid increasing concentrations in air, water and soil. In polluted soil, Ni concentration ranges from 200 to 26 000 mg kg^{-1}, in comparison to the overall range of 10 to 1000 mg kg^{-1} found in natural soil (Izosimova 2005). In low concentration Ni is an essential element for plant growth and development (Chen et al. 2009); however, it becomes toxic at higher concentration (Dubey, Pandey 2011). Generally, plants uptake Ni through roots either by passive diffusion or by active transport (Seregin, Kozhevnikova 2006). The uptake mechanism of Ni varies within plant species and soil mineralogy, acidity of soil, presence of other minerals etc. The availability of Ni depends on soil pH, cation exchange capacity, presence of other metal ions and organic matter content in soil (Chen et al. 2009). An elevated level of Ni in plants alters many physiological and biochemical processes, leading to inhibition of germination and growth (Khan, Khan 2010), chlorosis and necrosis of leaves (Zornoza et al. 1999; Seregin, Kozhevnikova 2006) and inhibition of chlorophyll biosynthesis (Singh, Pandey 2011). Plants grown in high Ni-containing soil show impairment of nutrient balance and disorder of cell membrane functions, and its toxicity causes inhibition of shoot growth and reduction of root and shoot biomass (Gajewska et al. 2006).

Nickel toxicity may induce formation of reactive oxygen species (ROS), which can cause oxidative stress in plants. These ROS are harmful for plant cells and lead to inhibition of photosynthetic activity, reduction of ATP production, initiation of lipid peroxidation, and DNA damage (Ruley 2004). Plants have a well developed enzymatic antioxidative system, including the enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), for scavenging of ROS (Cakmak, Horst 1991). As an initial ROS scavenger, SOD catalyses the dismutation of superoxide anion radical to H_2O_2 and molecular oxygen. Further, H_2O_2 is detoxified by CAT and/or POD to water and oxygen (Chelikani et al. 2004). In several plants, Ni-induced changes in activity of ROS-scavenging enzymes, including SOD, POD and CAT, have been detected (Gajewska, Skłodowska 2007).

The remediation of nickel contaminated soils by mechanical and chemical methods is not suitable for agricultural lands, as these methods are often expensive and incompatible to soil structure and fertility (Gaur, Adholeya 2004). Phytoremediation, the use of plant systems for soil decontamination, gained enormous momentum since it is

cost-effective, environment-friendly approach and can be applied to large areas. As the inclusion of pollutants into the human food chain is a primary concern, it becomes imperative to grow non-edible industrial plants, even those that are not consumed by animals, which can clean up a polluted site. A suitable plant for phytoremediation should ideally have high and fast biomass production, as well as a capacity of accumulation of pollutants into above-ground parts (Cunningham, Ow 1996). Castor (*Ricinus communis* L.) is an economically important, non-edible oil seed crop of the spurge family, Euphorbiaceace. It is a large robust shrub, has a rapid growth rate, high biomass production and wide deep root system (Huang et al. 2011; Baudh, Singh 2015).

Previous studies have shown that *R. communis* grows well in metal polluted soil and has an excellent capacity to accumulate metal ion in plant tissue (Shi, Cai 2009; Yi et al. 2014; Kiran, Prasad 2017). However, relatively few reports have been published on the oxidative metabolism of castor plants under Ni stress. Therefore, this study aims to further investigate Ni-induced toxic effects on morpho-physiological attributes and antioxidative responses to varying Ni concentrations under greenhouse conditions, and to evaluate the overall phytoextraction ability of *R. communis*.

Materials and methods

Plant cultivation and treatment

Healthy and mature seeds of castor were obtained from the Botanical Garden of CSIR-North East Institute of Science and Technology, Jorhat, Assam. In order to avoid microbial contamination, seeds were surface sterilized with 0.1% mercuric chloride for 5 min and then subsequently washed with sufficient distilled water.

Random soil samples were collected from the agricultural farm of the Institute and a composite sample was made. Physicochemical properties of the soil were analysed prior to use. Soil was sandy clay loam with pH 5.5 to 6.0, organic matter 0.88%, nitrogen 0.085%, phosphorus 0.0007% and potassium 0.0032%. The soil was spiked with selected concentrations of Ni (0, 50,100, 300 and 500 mg kg^{-1}) by using $NiNO_3$ salt. The amended soil was then allowed to stabilize for two weeks before sowing of castor seeds.

Earthen pots were packed with sieved soil with a final weight of 6 kg of soil per pot. Ten healthy seeds were sown in each pot and after germination were thinned to five seedlings with uniform size. Each treatment including the control was replicated three times. Seedlings were watered every alternate day to maintain soil moisture content. The pots were kept directly in a naturally illuminated greenhouse with temperature ranging between 18 and 25 °C and relative humidity 70 to 86%. All the measurements were performed at 60 days after sowing (DAS). The plants were sampled 60 DAS and the morphological, biochemical and physiological parameters were measured. Three plant samples were randomly collected from each replicate of a pot and were analysed for its various parameters and the average was calculated. The intact plants were carefully removed from the pots and the adhering soil was removed by washing with distilled water. All plants were divided into roots, stem and leaves for further analysis.

Morphological parameters

Fresh biomass of plants was determined using an electronic balance. The samples were kept in oven at temperature 70 °C for 72 h and dry biomass was measured. The root and shoot length were measured using a meter scale. Relative water content (RWC) was determined according to the method by Chen et al. (2009) and calculated by the following formula:

$$RWC = (FW - DW) / FW \times 100,$$

where *FW* is fresh weight and *DW* is dry weight.

Leaf area was determined by multiplying leaf length with leaf width and using a correction coefficient 0.72 as proposed by Hoyt and Bradfield (1962). A tolerance index (TI) was calculated according the method of Baker et al. (1994) as the ratio of plant biomass (dry weight) in heavy metal contaminated soil to that of control plant biomass (dry weight):

$$\textit{Tolerance index} = \textit{Plant biomass of treated plants} / \textit{Plant biomass of control plants}.$$

Photosynthetic pigment concentration

The photosynthetic pigments of castor bean leaves were determined according to the method described by Arnon (1949). A sample of fresh leaves (200 mg) was ground in a mortar and pestle, and then extracted with 10 mL of 80% acetone. The homogenate was centrifuged at 2500 × *g* and pigment concentration was evaluated in the supernatant by measuring absorbance at 645, 663, 480 and 510 nm with a UV-Vis spectrophotometer (Thermofisher, Evolution 201). Pigment concentrations were calculated using the following formulas:

$$\text{Chlorophyll } a \text{ (mg g}^{-1}) = (12.7 \times A_{663} - 2.69 \times A_{645}) \times V / W \times 1000,$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}) = 22.9 \times A_{645} - 4.68 \times A_{663}) \times V / W \times 1000,$$

$$\text{Total carotenoids (mg g}^{-1}) = (7.6 \times A_{480} - 1.49 \times A_{510}) \times V / W \times 1000,$$

where, *A* is absorbance of the leaf extract at wavelengths (645, 663, 480 and 510 nm), *V* is final volume of chlorophyll extract in 80% acetone, *W* is fresh weight of leaf tissue (mg).

Measurement of proline concentration

The proline concentration was estimated by the method of Bates et al. (1973). Fresh leaf samples (0.5 g) were collected and ground in a mortar using pestle and then extracted in 10 mL 3% sulfosalicylic acid. The extract was filtered through Whatman No. 1 filter paper and the filtrate was used for estimation of proline. Sample extract (2 mL) was taken

and 2 mL each of glacial acetic acid and acid ninhydrin were added. The reaction mixture was added to a test tube and then heated for 1 h in boiling water bath and finally terminated by transferring the test tube to an ice bath. Then 4 mL of toluene was added to the reaction mixture and vigorously shaken for 20 to 30 s. The chromophore (toluene) layer was aspirated and warmed to room temperature. The absorbance having red colour was read at 520 nm against a reagent blank on a spectrophotometer (Thermofisher, Evolution 201). The amount of proline in the sample was calculated by using a standard curve prepared from pure proline range (0.1 to 36 µmol) and expressed on a fresh mass basis of the sample.

Measurement of malondialdehyde concentration

Malondialdehyde (MDA) concentration was estimated by the content of 2-thiobarbituric acid-reactive substances according to the method of Cakmak and Horst (1991). Fresh leaf samples (0.2 g) were ground in a mortar and pestle with 5 mL of 0.1% (w/v) trichloroacetic acid at 4 °C. Following centrifugation at 12 000 × *g* for 5 min, an aliquot of 1 ml from the supernatant was added to 4 mL of 0.5 % (w/v) thiobarbituric acid in 20% (w/v) trichloroacetic acid. The samples were heated at 90 °C for 30 min. Then, the reaction was stopped in an ice bath. Centrifugation was performed at 10 000 × *g* for 5 min, and absorbance of the supernatant was recorded at 532 nm on a UV-Vis spectrophotometer and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. Absorption coefficient (ε = 155 mm cm^{-1}) was used to calculate MDA concentration expressed as nmol per g of fresh mass.

Leaf protein concentration and antioxidative enzyme activity

Fresh leaf tissues (0.2 g) were homogenized separately in a pre-cooled mortar and pestle under ice-cold conditions with 2.0 mL of extraction buffer [50 mM phosphate buffer (pH 7.5), 0.5 mM ascorbate and 1 mM EDTA]. The homogenate was centrifuged at 10 000 × *g* for 15 min. The supernatant was used for the measurement of SOD and CAT enzyme activity. The protein content was measured according to the method of Bradford (1976), using bovine serum albumin as a standard.

Superoxide dismutase was determined by the method of Beauchamp and Fridovich (1971) by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium. One unit of SOD activity equalled to the amount required to inhibit photo-reduction of nitroblue tetrazolium by 50%. The reaction mixture (3 mL) was prepared with 100 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 2.25 mM nitroblue tetrazolium, 60 µM riboflavin and enzyme extract. After mixing, the mixture in the cuvette was illuminated (40 W) for 10 min at 25 °C. Enzyme extract kept in the dark served as a blank, while buffer with no enzyme extract kept in the light served as a control. The absorbance was measured at 560 nm against a blank using a UV-Vis spectrophotometer. Nitroblue tetrazolium reduction in the light was measured in the presence and absence of enzyme extract.

The method proposed by Reddy et al. (1995) was adopted for assaying the activity of POD. Leaf tissues (0.2 g) were homogenized in 0.1 M phosphate buffer (pH 6.5), centrifuged at 12 000 × *g* and the supernatant was used for the assay. The reaction mixture contained 3.0 mL of pyrogallol solution and 0.1 mL of the enzyme extract. The spectrophotometer was adjusted to read zero at 430 nm. The reaction mixture was further mixed with 0.5 mL of H_2O_2 in the test cuvette. The change in absorbance was recorded every 30 s up to 2 min in a UV-Vis spectrophotometer. One unit of peroxidase was defined as the change in absorbance per minute at 430 nm.

CAT activity was determined by measuring the decomposition of hydrogen peroxide according to the method of Aebi (1984). About 100 µL of enzyme extract was added in a reaction mixture containing 50 mM phosphate buffer (pH 7.0) and 33 mM H_2O_2. The decrease of the absorbance at 240 nm was recorded. Activity was calculated using an extinction coefficient of 40 mM^{-1} cm^{-1}. One unit of CAT activity was defined as the amount required for decomposing 1 µmol of hydrogen peroxide min^{-1}.

Ni concentration, bioaccumulation factor and translocation factor

After harvesting, castor bean plants were separated into root, stem and leaves. The plant parts were oven dried at 70 °C and ground into powder using a mortar and pestle. Dried plant material (0.5 g) was digested in 10 mL of nitric acid and hydrogen peroxide (HNO_3/H_2O_2) mixture. After the disappearance of brown fumes, the samples were heated (110 °C) to near dryness. The sample volume was adjusted to 25 mL with deionized water. The nickel concentration in each sample was measured by an atomic absorption spectrophotometer (Perkin Elmer A=Analyst 700).

The phytoextration ability of *R. communis* plants was assessed using the parameters bioaccumulation factor (BAF) and translocation factor (TF) as follows:

BAF = Ni concentration in plant tissues / Ni concentration in soil,

TF = Ni concentration in shoot / Ni concentration in root.

Statistical analysis

Data were presented as mean value ± SD. The significance of differences ($p < 0.05$) among the treatments was determined by Duncan's multiple range test.

Results

Effects of Ni on plant growth

Castor bean plants showed significant decrease in growth of roots, shoots and leaves at the growth stage of 60 DAS due to exposure with different Ni concentrations in soil (Table 1). For the highest treatment level of Ni (500 mg kg^{-1}), there

was 52% reduction in biomass for stems, 48% reduction in biomass of roots and 60% reduction in biomass of leaves. The effect of Ni on leaf growth was more pronounced compared to root and stem growth.

The inhibitory effect of Ni on plant biomass, relative water content and tolerance index under different Ni treatments is shown in Table 1. A significant decrease in plant biomass of castor bean was observed at increased levels of Ni in the soil compared to the control. Treatment with Ni at 500 mg kg^{-1} significantly decreased the fresh and dry plant biomass as compared to the control, with 37% reduction. The tolerance index of the examined castor bean plants also differed significantly due to Ni stress. The highest sensitivity was observed in the Ni 500 treatment level (67.43 %).

Effect on Ni on photosynthetic pigments

The results obtained in the analysis of photosynthetic pigments of the fresh leaf tissues for plants cultivated at different concentration of Ni are presented in Table 2. Chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid concentration in castor bean plants increased significantly on application of 50 mg kg^{-1} Ni in soil, whereas a significant decrease was noticed in the photosynthetic pigment concentration at higher Ni treatment levels (100 to 500 mg kg^{-1}). At the highest Ni treatment level (500 mg kg^{-1}), the reduction in chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid concentration was observed to be 25, 44, 26 and 25%, respectively. It was also noted that the impact of Ni was more visible in the case of chlorophyll *b*.

Effect of Ni on biochemical parameters

The results obtained in the analysis of castor bean leaves for protein concentration, lipid peroxidation as indicated by MDA and proline concentration are presented in Table 2. Leaf protein concentration at lower Ni treatment (50 mg kg^{-1}) was higher than that of the control, but at higher concentrations of Ni in soil, the protein concentration decreased significantly. Increasing levels of Ni (50 to 500 mg kg^{-1}) induced increase in concentration of MDA and proline in the leaves of castor bean as compared to the control. In terms of the relative increase, it was 41, 56, 72 and 102% for MDA, showing near-linear increase with increasing substrate Ni level in comparison to control. Proline concentration also showed continuous increase with increasing Ni concentration, being 39, 116, 134 and 158% at soil Ni concentrations of 50, 100, 300 and 500 mg kg^{-1}, respectively.

The results of the antioxidative enzyme activity analysis showed a concentration-dependent increase in activity in Ni-treated castor bean plants when compared to the control. The enzyme activity increased in the order POD > SOD > CAT, with 120, 112 and 94% increase, respectively, at 500 mg kg^{-1} Ni treatment compared to the control (Table 3).

Ni accumulation in plant tissues

Accumulation of Ni among different tissues (root, stem and leaf) of castor bean plants treated with varying doses of Ni in soil are summarized in Fig. 1A. A significant increase in the amount and accumulation of Ni in castor bean plants with increasing Ni concentration from 50 to 500 mg kg^{-1} in the soil as compared to control was evident. Relatively higher Ni concentration and accumulation at all doses was found in roots of castor bean than in aerial parts. Accumulation in different plant parts was in the order roots > leaves > stems. For the maximum treatment level (500 mg kg^{-1}) of Ni, accumulation in root, stem and leaf was 152, 13.72 and 16.67 mg kg^{-1}, respectively.

The bioaccumulation factor (BAF) and translocation factor (TF) are important indicators for the evaluation of metal bioaccumulation and translocation in plant tissues. BAF expresses the ability to accumulating metal in the roots while the TF determines the ability of plant to translocate the metal from roots to its aerial parts. In the present study, the BAF and TF of Ni in castor bean is shown in Fig. 1B and C. At increasing concentration of Ni (50 to 500 mg kg^{-1}) in soil, the root BAF was 0.97, 1.38, 2.03 and 1.64, whereas the shoot BAF was 0.74, 0.35, 0.34 and 0.32. The TF of all treatments was less than one indicating that *R. communis* preferentially accumulated Ni in the root.

Table 1. Effect of Ni stress on growth, biomass, relative water content and tolerance index of castor bean plants. Values represents mean ± SD (n = 3). Different letters in the same column show significant differences ($p < 0.05$) among different treatments for a specific parameter

Treatment (mg kg^{-1})	Root length (cm)	Shoot length (cm)	Leaf area (cm^2)	Biomass change (%)			Biomass (g per plant)		Relative water content (%)	Tolerance index (%)
				Root	Stem	Leaf	Fresh weight	Dry weight		
Control	21.0 ± 0.4 a	51.3 ± 0.5 a	280 ± 9 a	100	100	100	10.9 ± 0.3 a	1.74 ± 0.05 a	84.0	100
Ni 50	18.4 ± 0.5 ab	45.2 ± 0.5 b	243 ± 5 a	87.6	88.1	86.8	10.4 ± 0.3 a	1.52 ± 0.04 b	85.3	87.4
Ni 100	17.0 ± 0.2 b	41.1 ± 0.4 b	201 ± 7 a	81.1	80.2	71.6	8.2 ± 0.2 b	1.36 ± 0.04 c	83.4	78.2
Ni 300	12.6 ± 0.4 c	31.1 ± 0.4 c	147 ± 7 b	59.94	60.7	52.4	8.0 ± 0.2 b	1.34 ± 0.05 c	83.2	76.8
Ni 500	10.8 ± 0.3 c	24.6 ± 0.6 d	112 ± 9 b	51.51	48.1	39.9	6.8 ± 0.3 b	1.17 ± 0.15 d	82.7	67.4

Table 2. Effect of Ni stress on the biochemical parameters of castor bean seedlings. Values represents mean ± SD ($n = 3$). Different letters in the same column show significant differences ($p < 0.05$) among different treatments for a specific parameter

Treatment (mg kg^{-1})	Chlorophyll *a* (mg g^{-1} FM)	Chlorophyll *b* (mg g^{-1} FM)	Total chlorophyll (mg g^{-1} FM)	Carotenoids (mg g–$^{-1}$ FM)	Protein (mg g^{-1} FM)	MDA (µmol g^{-1} FM)	Proline (µmol g^{-1} FM)
Control	1.75 ± 0.02 a	1.18 ± 0.02 b	2.82 ± 0.02 b	0.76 ± 0.02 b	4.58 ± 0.09 b	0.54 ± 0.03 e	2.06 ± 0.05 d
Ni 50	1.77 ± 0.03 a	1.33 ± 0.02 a	3.08 ± 0.06 a	1.00 ± 0.02 a	5.19 ± 0.09 a	0.76 ± 0.01 d	2.86 ± 0.05 c
Ni 100	1.60 ± 0.02 a	0.95 ± 0.02 c	2.54 ± 0.03 bc	0.67 ± 0.02 c	4.47 ± 0.05 b	0.84 ± 0.04 c	4.45 ± 0.04 b
Ni 300	1.52 ± 0.01 b	0.72 ± 0.01 d	2.24 ± 0.03 c	0.64 ± 0.02 c	4.18 ± 0.06b c	0.93 ± 0.01 b	4.82 ± 0.06 b
Ni 500	1.32 ± 0.02 c	0.66 ± 0.02 e	2.10 ± 0.01 c	0.57 ± 0.03 d	3.25 ± 0.05 c	1.16 ± 0.04 a	5.32 ± 0.08 a

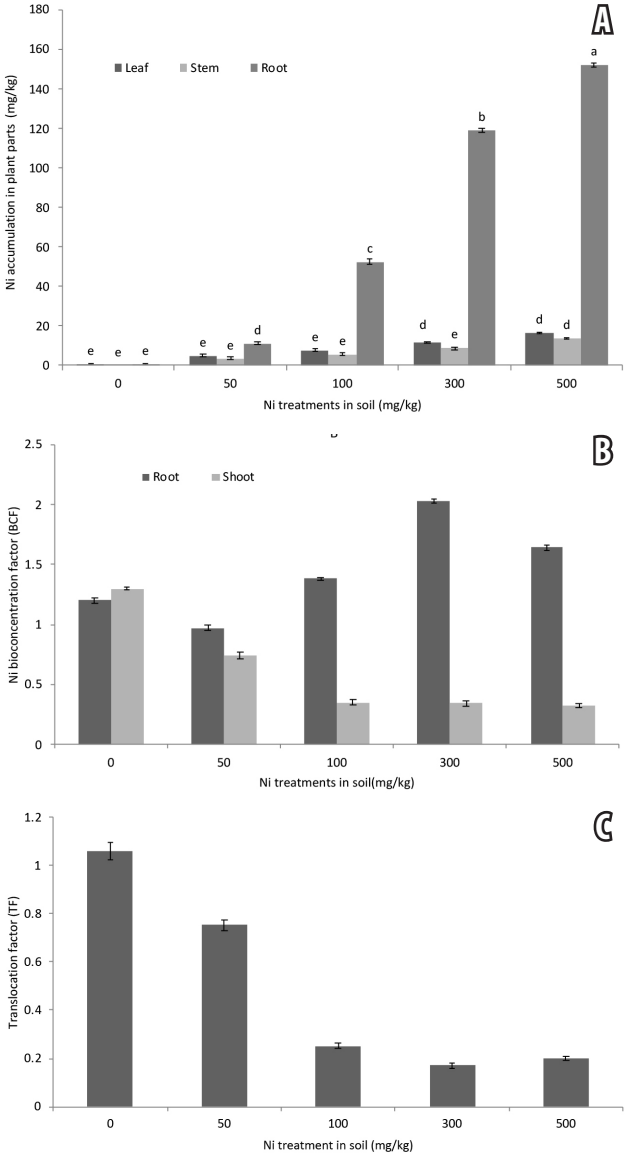

Fig. 1. A, accumulation of Ni in different parts of *R. communis* grown on soil amended with varying doses of Ni. B, root and shoot Ni Bioconcentration factor. C, Ni translocation factor. Data presented are means of three replicates ± SD. In graph A, mean values with identical letters are not significantly different at $p < 0.05$, according to Duncan's test.

Discussion

Nickel is an essential micronutrient for plant growth and development, but it becomes toxic at higher concentration. The increasing concentration of Ni showed harmful effects on castor bean plants and ultimately reduced biomass production and plant growth (Table 1). In the present study, the observed inhibition of root and shoot growth and leaf area caused reduction of fresh and dry biomass of *R. communis*. It has been reported that higher concentration of Ni in plants can reduce growth parameters such as plant height, biomass production in several agricultural crops (Seregin, Kozhevnikova 2006; Yusuf et al. 2011). Inhibitory effect on plant growth might be due to Ni-induced alterations of fundamental metabolic processes, such as photosynthesis and transport of photoassimilates from leaves (Gazewska et al. 2006). The higher relative leaf water content in some treatments may be attributed to stomatal closure due to atmospheric carbon fixing activities that were compromised as a consequence of Ni stress (Brunet et al. 2008). The decrease in leaf area in response to Ni treatment may be related to accumulation of Ni in leaves. The results of the present study coincide with the results for *Vigna radiata* plants (Pandey, Pathak 2006), where the size of gram plants declined and they developed chlorosis when treated with excess Ni concentrations. Compared with control plants, a decrease in plant biomass was observed in the treated plants. The decrease of biomass was also been reported in cabbage (Pandey, Sharma 2002) and wheat plants (Gajewska et al. 2006). Increasing concentrations of Pb to 800 mg kg^{-1} decreased root and shoot length and biomass of *R. communis* (Ananthi et al. 2012). There was no such significant change in relative water content in the case of Ni-treated plants. During the experiment visible changes occurred in the appearance of the aerial parts.

At an elevated level, Ni causes reduction in photosynthetic activity (Hussain et al. 2013). It has been observed that concentration of pigments chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids in *R. communis* increased at low Ni concentration (50 mg kg^{-1}) and further gradually decreased with increasing levels of Ni in substrate (100 to 500 mg kg^{-1}). Increase in pigment

Table 3. Effect of Ni on the antioxidative enzyme activity in fresh leaf tissues of castor bean against different concentration of Ni in soil. Values represents mean ± SD (n = 3). Different letters in the same column show significant differences ($p < 0.05$) among different treatments for a specific parameter

Treatment (mg kg^{-1})	SOD activity (U g^{-1} protein)	POD activity (U g^{-1} protein)	CAT activity (U g^{-1} protein)
Control	12.84 ± 0.10 e	4.71 ± 0.10 d	0.50 ± 0.02 c
Ni 50	17.34 ± 0.14 d	7.54 ± 0.10 c	0.73 ± 0.01 b
Ni 100	20.27 ± 0.06 c	7.96 ± 0.14 c	0.84 ± 0.02 b
Ni 300	24.86 ± 0.07 b	8.43 ± 0.08 b	0.90 ± 0.01 a
Ni 500	27.24 ± 0.09 a	10.35 ± 0.13 a	0.97 ± 0.02 a

concentration could be regarded as a metal-specific response that may have resulted in chlorophyll upgradation and synthesis of photosynthates (Bazzaz et al. 1974). Also, Ni is an essential element for plants at low level. The decrease in photosynthetic pigments has also been documented in other plants grown in soil treated with high Ni concentrations (Pandey, Sharma 2002; Gazjewska et al. 2006). Carotenoid concentration also decreased due to Ni toxicity (Gazjewska et al. 2006; Singh et al. 2012). Protein concentration significantly decreased when compared to control except at 50 mg kg^{-1} Ni treatment. Similar results regarding the influence of nickel on protein concentration was observed in *R. communis* (Adhikari, Kumar 2012) and *Phaseolus mungo* (Selvaraj 2015). Higher concentration of proteins under low metal exposure is likely due to stimulation of stress response protein synthesis (Srivastava et al. 2005). However, the reduction may be due to the degradation by proteases (Prasad 1996; Romero-Puertas et al. 2002;).

The level of malondialdehyde (MDA; a component of lipid peroxidation) in leaves was determined as content of thiobarbituric acid-reactive substances. A rapid increase of MDA in leaves with increasing Ni concentration was observed compared to the control. Similarly, increasing concentration of substrate Cd and Pb increased MDA concentration in leaves of *R. communis* (Chen 2015; Kiran, Prasad 2017). High MDA content due to Ni stress results from a high level of production of ROS leading to destabilization of membrane integrity, affecting their functionality and resulting in disruption of ionic balance in the cytoplasm. Proline is an α-amino acid derivative compound, and it plays diverse role in plant growth, development, and stress physiology. Proline accumulation results in the formation of phytochelatins and alleviates metal toxicity in plants (Sharma, Dubey 2005). In the present study, proline concentration in leaves of castor bean was found to be increased with the increase in nickel concentration in soil, similarly as in a study reported by Bauddh and Singh (2015). Nickel induced proline accumulation in plants was shown also for wheat (Pandey, Sharma 2002) and cabbage (Parlak 2016).

Antioxidants in plants play unique roles in preventing cellular damage to stress conditions. Under metal stress conditions, including excess nickel exposure, an imbalance between generation and removal of ROS arises in plant tissues (Gratao et al. 2005). These ROS are harmful for plant cell and lead to inhibition of photosynthetic activity, inhibition of ATP production, lipid peroxidation and DNA damage (Ruley 2004). The detoxification of ROS is done through antioxidative enzymes, such as SOD, CAT and POD in plants. In this study, the results of the SOD, CAT and POD assay showed increased activity of all the three enzymes. Many studies have assessed the antioxidant activity in *R. communis* under metal stress (Bauddh et al. 2016; Kiran, Prasad 2019). Activities of the enzymes CAT and SOD are upregulated in the roots and leaves of *R. communis* following increased Cd treatment levels (Bauddh et al. 2016). In another study, the increasing levels of Pb significantly increased CAT and POD activity, while SOD, ascorbate peroxidase activity and the redox ratio of glutathione decreased with increasing Pb stress (Kiran, Prasad 2019). In seedlings of *Cajanus cajan*, the activity of SOD has been shown to be increase in response to Ni treatment (Rao, Sresty 2000). The activities of the antioxidative enzymes CAT, POD and SOD were also reported to increase in *Brassica juncea* due to Ni stress (Sharma et al. 2008).

The increase in SOD activity in response to Ni stress is possibly attributed to the de novo synthesis of the enzymic protein (Cakmak, Horst 1991). Catalase is another important enzyme that detoxifies H_2O_2 directly in the cell. The increase of CAT could be attributed to increased H_2O_2 production, leading to increased capacity of H_2O_2 degradation. In the plant kingdom, peroxidases are widely distributed and are the principal enzymes involved in the elimination of ROS under stress. POD decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants (Blikhina et al. 2003). Therefore, the elevation of POD activity in the present study suggests its role in the detoxification of H_2O_2 (Farooqi et al. 2009). The enhanced activity of POD in excess nickel treated plants resulted in the elimination of ROS and improvement of stress condition in plants. Therefore, the increased activities of all the enzymes suggest their key role in ameliorating Ni toxicity in *R. communis*.

Nickel is very mobile and can be accumulated in vegetative and reproductive parts of plants (Soon et al. 1980). The uptake of Ni in plants is mainly carried out through the root system via diffusion and active transport process (Seregin, Kozhevnikova 2006). Concerning *R. communis* Ni

accumulation, overall data across all treatments indicates that the plant preferentially concentrates Ni in the root (up to 152 mg kg^{-1}). The Ni accumulation pattern in the castor bean plant is in the order roots > leaves> stem. The results of the present study on accumulation of Ni by castor bean was rather similar to those reported in other studies (Baudh et al. 2015; Parlak 2016). It was observed that the concentration of Ni in roots of castor plant ranges from trace (control) to 455 mg kg^{-1}, which was directly related to soil Ni concentration (Adhikari, Kumar 2012). In another study, it was found that about 50% of Ni absorbed by plants was in the root system (Cataldo et al. 1978). Additionally, the root BAF was higher than that of the shoot across all the Ni treatment levels (Fig 1B).

Some of the factors that affect metal bioavailability and uptake include plant and metal interaction, specific metal and concentration, species-specific characteristics, environmental efficacy and disposal route. Plants with a BAF value > 1 are considered to be promising phyto-extractors and are suitable for phytoremediation of contaminated soil (Kamari et al. 2012; Amin et al. 2018). The translocation factor (TF) is an efficient measure to determine the phytoextraction of metal from soils (Kamari et al. 2012). It is the ratio of metal concentration in plant shoot to that in plant roots. Three categories of plants are identified based on their translocation factor – accumulator (TF > 1), excluder (TF < 1), and indicator (TF near 1) (Baker 1981; Ghosh, Singh 2005). Our results revealed that TF of all treatments was less than one (Fig. 1C), which indicates that *R. communis* preferentially accumulates Ni in its root system. The study showed that the plant has high accumulation of Ni in roots and low translocation in shoots; however, it could be used for phytoextraction of Ni contaminated soil.

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