RESEARCH ARTICLE

Hazardous Impact of Nickel in Alteration of Antioxidative Mechanism and Inhibition of Growth and Biomass Yield in Rice (*Oryza sativa* L.)

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Abstract

Many anthropogenic activities (smelters, mines and municipal waste) enhance the heavy metal toxicity in soil and water and it is gradually accumulated in plants, and finally through food chain it reaches to human beings. To investigate hazardous impact of excess nickel on growth, biomass, yield and metabolism of rice (Oryza sativa L) the plants were grown in refined sand at 0.0001mM (control), 0.05, 0.10, 0.20, 0.40, and 0.50 mM nickel (Ni) supply. After 16 days of treatment the excess supply of Ni(>0.50mM) reduced biomass, photosynthetic pigments (both chlorophyll a and b), Hill reaction activity, water soluble proteins, activity of catalase (CAT), and enhanced the activity of peroxidase (POX), ribonuclease and acid phosphatase in rice leaves. The antioxidative regulation was inhibited through CAT, although increased activity of POX supported the plants in overcoming the toxic effect of nickel. At 112 days (70 days after Ni treatment) the accumulation of iron in shoot and root was reduced. Phosphorus was significantly increased in both shoot and root. Sulphur accumulation was increased in panicle, leaves, shoot and root. Nickel accumulation in different plant parts was increased many fold (as in leaves it was increased about 25 times, in stem 18 times and in roots it was 17 times) in 0.50 mM Ni supply in comparision to control. Rice appeared very prone to nickel toxicity as Ni supply beyond 0.050 mM resulted into complete loss of economic yield due to inhibited flowering and poor panicle numbers.

Keywords: Catalase, Chlorophyll, Growth, Nickel, Photosynthetic pigment, Rice.

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Introduction

Rice has been among staple food of more than half of the world. Excess supply of heavy metal disturb plant metabolism and retard shoot and root growth and result into poor yield (Gajewska and Sklodowska, 2007). Long back bakane disease of rice resulted into a food scarcity in Japan which shows the importance of rice crop.

Solid is classified as hazardous, if it contains more than 50 mg kg^{-1} dry wt. of sludge which contains nickel. The biological significance of nickel as a possible micronutrient for higher plants has been established (Adriano, 2001) but it is considered as contaminant for surface soil and water when present in excess levels (Sasmaz and Yamen, 2006). Nickel (Ni) is an essential micronutrient for plants since it is the active center of the enzyme urease required for nitrogen metabolism in higher plants (Yan *et al.,* 2008). However, excess Ni is known to be toxic and many studies have been conducted concerning Ni toxicity of various plant species (Yan *et al*. 2008). As plant micronutrient nickel is found in the vegetative parts of most plants in the range of 1-10µg/g dry weight (Assuncao *et al*., 2003). Toxicity occurs at higher concentrations 10-50µg/g dry weight depending upon the crop species (Marschner, 2002). The toxicity of nickel is a major problem in agricultural soils due to amendment of soils with sewage and sludge often rich in nickel and waste disposal practices (Marschner, 2002; Barazani *et al*., 2004). The heavy metals enter the human body through food chain, and if accumulated beyond the threshold limit, become toxic and even lethal. Nickel uptake by plants from soil is largely a function of native or applied nickel in the soil and is absorbed in the ionic form (Ni^{+2}) (Adriano, 2001). The mobility of nickel within the plant is usually higher than other heavy metals (Gajewska and Sklodowska, 2007) in older leaves being the major sink.

Increase in nickel supply has been reported to increase nickel in different part of several crops (Robinson *et al*., 2003). The interveinal chlorosis and necrosis of leaves in dicots, and banded chlorosis in grasses are some common symptoms of nickel toxicity (Kukier and Chaney, 2004; Gratao *et al*., 2006). Schutzendubell and Polle (2002) listed three different mechanisms for heavy metal toxicity, such as production of reactive oxygen species (ROS) by antioxidation and Fenton reaction, blocking of essential functional groups in biomolecules and inactivating certain enzymes and displacement of essential metal ions from biomolecule inactivating enzymatic activity. Generally, it has been proposed to affect plants through oxidative damage to lipids, DNA and protein (Apel and Hirt, 2004). Although plants have antioxidants that quench ROS before they damage cellular structure. Antioxidant defence system comprises a variety of antioxidant molecules and

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enzymes (superoxide dismutase, catalase, peroxidase, glutathione reductase etc.). Soil nickel treatment decreases catalase activity but increases peroxidase and acid phosphatase activity (Pillay *et al*., 1996). Excess supply of nickel to plants also accelerates generation of reactive oxygen species (ROS) resulting into oxidative stress (Benavides *et al.,* 2005).

According to Shahzad *et al.,* (2018) the plants cannot endure their life cycle without adequate Ni supply. However, excessive Ni concentration can lead to induce ROS production affecting numerous physiological and biochemical processes such as photosynthesis, transpiration, as well as mineral nutrition and causes phytotoxicity in plants. They have also proposed different viable approaches for remediation of Ni-contaminated soils.

Studies suggested that Ni hyper accumulation has a protective function giants fungal and bacterial pathogens us many Nickelouphilous plants (Prasad, 2005)

Since nickel is found to be in varied range from as low as 0.1- 1000 $\mu g g^{-1}$ dry weight in plants. Therefore, in the present study an attempt has been made to identify hazardous level of nickel concentration inhibiting biomass, photosynthetic efficiency, pigment contents, certain enzymatic activities and accumulation of different elements in different plant parts of rice crop grown in sand pot culture.

Materials and Methods

Rice (*Oryza sativa* L. cv. Swarna Mansoori) plants were grown in acid washed sterile refined sand in a glass house under controlled conditions (Agarwala and Sharma, 1976) using polyethylene pots. In total 18 pots were used, and the experiment was performed in triplicate. Each pot had a central drainage hole covered with inverted watch glass lined with glass wool. After the emergence of the seedling, plants were supplied with complete nutrient solution daily for 43 days except on Sundays when pots were flushed with water to remove the accumulated salts.

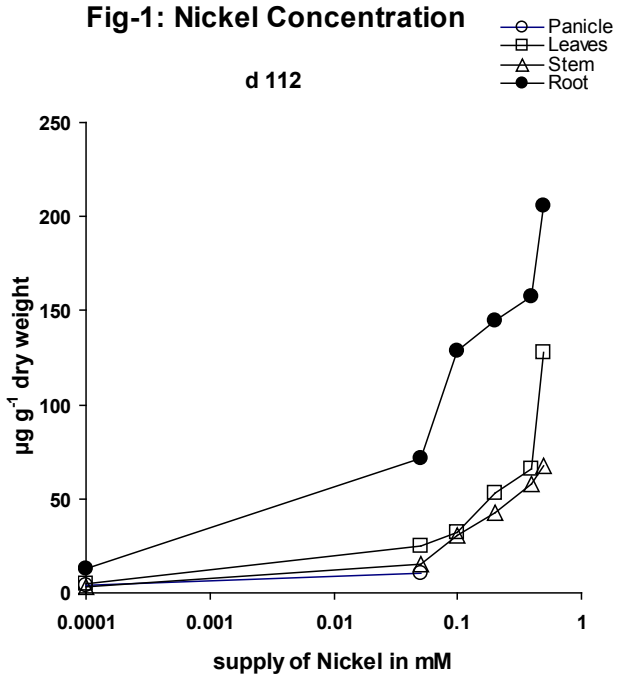

Fig. 1: Influence of excess nickel on concentration of nickel in different parts of rice.

The varying levels of nickel were supplied as $NiSO_4$ to induce the toxicity symptoms of nickel. On 44th day, pots with plants were separated into six lots. One lot was allowed to grow as such and treated as control. In remaining five lots 0.05, 0.10, 0.20, 0.40 and 0.50 mM of nickel sulphate was added. The purified nickel compound (nickel sulphate) was bought from CDH.

Apart from periodical record of foliar symptoms of Ni toxicity, plant samples were collected at d 59 and 112 for determination of biomass and tissue concentration after wet digestion by nitric-perchloric acid (10:1 v/v] and clear digest was estimated for phosphorus (Wallace, 1951), sulphur (Chesnin and Yien, 1951) and iron, zinc and nickel in different plant parts by Atomic absorption spectrophotometer. At d 60 (16 days after metal supply), chlorophyll contents (Arnon,1949), Hill reaction activity (Brewer and Jogendorf, 1965) and activity of some enzymes – catalase (Euler and Josephson, 1927), peroxidase (Luck, 1963) acid phosphatase (Schmidt, 1955) and ribonuclease (Tuve and Anfinson, 1960) were estimated in fresh leaf extracts. Soluble protein (Lowry *et al*., 1951) was also estimated in the enzyme extract to express specific activity of enzymes.

Catalase (CAT) and peroxidase (POD) were assayed in fresh leaf tissue extracts prepared by homogenizing samples in ice-cold glass distilled water (1:10) with a cold pestle and mortar at 4°C. The activity of CAT was assayed as described by Euler and Josephson (1927) in a reaction mixture (10 mL, standardized against 0.1 N $KMnO_4$) containing 500 µM of H_2O_2 and 1.0 mmol of potassium phosphate buffer (pH 7.0) was stabilized at 25°C. The reaction was allowed to proceed for 5 min. and was stopped by adding 2.0 mL of 2N H_2SO_4. The final reaction mixture was titrated against 0.1 N $KMnO_4$. The activity of POD was assayed by the method of Luck 1963). The reaction mixture (10 mL) contained 5 mL of 0.1 M of potassium phosphate buffer (pH 7.0), 1-mL of 0.01% H_2O_2, 1-mL of 0.5% *p*-phenyldiamine. The reaction was started by adding 1-mL suitably diluted enzyme extract and allowed to proceed for 5.0 min. The reaction was stopped by adding 2 mL of 5N H_2SO_4 and the color intensity was measured at 485 nm.

The activity of acid phosphatase was estimated by the method of Schmidt (1955). The reaction mixture contained 0.5 mL 0.1M sodium acetate buffer pH 5.0 and 0.4 mL suitably diluted enzyme extract in a centrifuge tube. The reaction was initiated by the addition of 0.1-mL 0.1M sodium β-glycerophosphate at 30°C and was stopped exactly after 20 min by addition of 1-mL 10% (w/v) cold trichloroacetic acid (TCA). The corresponding blanks were run simultaneously with added TCA before the addition of the substrate. The contents were centrifuged at 400 x g for 10 min at room temperature and amount of inorganic phosphorus (Pi) liberated was estimated in a suitable aliquot from the supernatant by the method of Fiske and Subbarow (1925). The results were expressed as µg inorganic phosphorus (Pi) liberated per 100 mg fresh weight or per mg protein during reaction.

The activity of ribonulcease was estimated by the method of Tuve and Anfinson (1960). An assay mixture contained 1-mL

0.1M citrate buffer pH 6.0 and 1-mL enzyme extract. The reaction was started by addition of 0.5 ml 0.5% (w/v) yeast RNA pH 7.0 and allowed to proceed for 30 minutes at 30°C. The reaction was stopped by the addition of 0.5 mL 0.75% (w/v) uranyl acetate in 25% (w/v) perchloric acid. Corresponding zero hour blanks were run simultaneously, in which uranyl acetate reagent was added before the addition of the substrate (RNA). In a suitably diluted supernatant after centrifugation at 4°C for 15 minutes, change in optical density was measured at 260 nm on a 'Milton Roy spectronic 1201'. The activity of the enzyme was expressed as the difference in optical density per minute per100mg fresh weight as well as per mg protein.

The soluble protein in the enzyme extracts were estimated by the method of (Lowry *et al* 1951). The protein of the enzyme extract was precipitated with 20% (w/v) chilled TCA and the mixture was allowed to stand for at least one hour at 4°C in a refrigerator. The supernatant was discarded and content were centrifuged. The residue was washed with glass distilled water to remove TCA. The water washed residue was treated with 80% (v/v) acetone to remove pigments. The clear residue was dried in vacuum and then dissolved in 1-mL of 0.5N NaOH at 80°C in a water bath for 10 min. and made the volume 5 ml**.** Freshly prepared alkaline copper reagent and 0.5 mL folin ciocalteau reagent was added**.** The optical density of the mixture was measured after half an hour on a spectrophotometer at 640 nm. The readings were referred to a standard calibrations curve prepared from crystalline Bovine serum albumin and the concentration of protein was expressed as percent in fresh weight.

The chlorophyll a and b concentration were estimated by the method of (Arnon, 1949). The fresh leaf lamina was chopped after removing the midrib and 100mg of this fresh tissue was ground in a clean mortar with pestle and in 10ml 80% (v/v) acetone. A pinch of $CaCO_3$ was added prior to grinding to prevent the denaturation of chlorophylls. The homogenate was centrifuged. The optical density of the chlorophyll 'a' and 'b' was measured in clear supernatant on Milton Roy spectronic 1201 spectrophotometer at 663 and 645 nm, respectively. The results were expressed as mg chlorophyll per g fresh weight.

The Hill reaction activity was measured as reduction of 2.6 dichlorophenol indophenol (DCPIP), spectrophotometrically at 620nm. The assay mixture consisted of 2ml Hill reagent containing 0.1 mM DCPIP, 0.01 mM KCl, 0.04 M phosphate buffer, pH 6.5 in a final volume of 6.6 ml and 0.4 ml of chloroplast suspension roughly equivalent to 40 mg chlorophyll. The whole mixture was exposed to direct sunlight or 50 Wm^{-2} light provided by a tungsten lamp for 10 minutes. Corresponding Zero hour blanks were also maintained in which inactivated boiled chloroplast suspension was added. Results were expressed as difference in optical density at 620 nm per 10 min per unit fresh weight.

Statistical Analysis

The experiment was conducted with three replicates using i.e., three pots of each treatment in randomized block design. The entire data presented in the table and figures were mean of three replicates and analyzed statistically and tested for significance by calculating LSD (least significance difference)at P=0.05, using sigma stat software program on computer.

Results and Discussion

The plants of rice were mildly depressed at d 55 owing to excess nickel at 0.5 mM concentration of Ni supply, which proved to be hazardous as the metabolism of the plants was altered and observed to be inhibitory of growth and biomass yield (Table 1). The decrease in plant growth at 0.1 and 0.2 mM Ni supply was not very much apparent but it seems that excess Ni affected the number of tillers (data not shown). At d 60 at 0.5 mM Ni, the young and middle leaves developed chlorosis in the form of bands on the middle portion (central portion) of the leaves (photo plate not shown). The upper and lower ends of lamina remained green. The observed symptoms in rice with less intensity were also present in plants at 0.2 and 0.1 mM concentration of Ni. With increase in age, the chlorosis intensified, exhibited necrotic areas and leaves rolled inward. Rice (cv. Swarna Mansoori) plants when exposed to variable level of nickel, developed visible characteristic symptoms which resembeled with those described earlier by Gopal et al. 2001 in

Table 1: Influence of excess nickel on biomass of rice at 59 (15DAMS) and 112 days growth (68DAMS) in Rice.

			mM Ni supply						
Days Growth	DAMS	Plant Part	Control	0.05	0.10	0.20	0.40	0.50	LSD P=0.05
			Dry weight : g $plant^{-1}$						
59	15	Y.L.	0.833	0.688	0.615	0.566	0.349	0.328	0.202
		O.L	0.610	0.337	0.363	0.375	0.317	0.276	0.196
		Stem	1.072	0.890	0.865	0.832	0.824	0.728	0.405
		Root	0.433	0.445	0.363	0.358	0.281	0.237	0.147
		Whole plant	2.947	2.360	2.205	2.131	1.772	1.569	0.620
112	68	Panicle	5.04	0.265	---	---	---	---	1.125
		Leaves	13.38	12.57	9.28	6.47	3.32	1.80	2.465
		Stem	14.72	13.20	10.18	8.28	4.61	2.27	1.521
		Root	5.01	3.99	3.40	2.58	2.20	1.36	1.089
		Whole plant	38.15	30.02	22.86	17.33	10.13	5.43	3.474

Y.L.= young leaves, O.L.= old leaves

rice and Kukier and Chaney 2004, in grasses. The leaf number and tillers were also reduced in excess nickel. The reduction in dry weight of rice owing to excess nickel was more pronounced at 0.4 and 0.5 mM (Table 1). The accumulation of nickel in different plant parts reduces carbohydrate and protein formation in leaves (Marschner, 2002; Kukier and Chaney, 2004).

The dry weight of rice decreased linearly with increasing level of nickel supply from 0.05 to 0.5 mM concentration at d 59 (15 days after metal supply). As compared to control dry weight of rice reduced by 40% and 47% owing to excess nickel at 0.40 and 0.50 mM, respectively. Reduction in yield was not exhibited significantly at concentration below 0.4 mM. At excess Ni supply the biomass of rice was decreased markedly at d 112 (68 DAMS). At this stage the photosynthetic decrease was pronounced at all levels of nickel supply, and no inflorescence was produced at any level (0.1 to 0.5 mM) of Ni concentration. However, the decrease in dry matter was obvious at 0.4 and 0.5 mM concentration of Ni (Table 1). At higher levels (>.05 mM) of nickel no panicle was produced thus zero yield and at lower level (<0.05 mM) also the seeds number were reduced and thus the seed weight. Ascribing the possibility of inhibition of photosynthesis as interpreted by reduced Hill reaction and chlorophyll contents (Pandey and Gopal, 2010).

At d 60 (16 DAMS) compared to the control the concentration of chlorophyll a, b and total chlorophyll in leaves was decreased with increase in nickel supply from 0.05 to 0.5 mM. The decrease in chlorophyll a was more than chlorophyll b at high concentration of nickel supply (Table 2). The higher concentration of nickel (0.4 and 0.5 mM) proved to be most hazardous as they degraded the photosynthtic pigments, resulting into chlorosis and necrosis.

The photosynthetic efficiency of the crop was measured in terms of Hill Reaction activity. Compared to the Hill reaction activity at control level, it was decreased in leaves of rice with an increase in nickel supply from 0.05 to 0.50 mM. The decrease was most pronounced at 0.50 mM nickel supply. The depression in Hill reaction activity at this level was about 78% as compared to that of normal leaves (Table 2). This hill reaction is a direct indicative of photosynthesis hence this reduced photosynthesis naturally led to inhibition in biomass yield of the crop. Since photosynthesis is a function of carbon dioxide and chlorophyll content, therefore any depression in chlorophyll (a, b, total concentration) decreased Hill reaction activity in leaves. The inhibition in Hill activity at 0.5 mM Ni was about 78% as compared to that of normal leaves. Very recently similar results are reported by several other workers (Pandey and Pathak., 2006) and (Gajewska and Skolowdska, 2007). The lowered concentration of chlorophyll in leaves with excess Ni absorption at higher Ni supply might be attributed to chlorophyll degradation or inhibition of chlorophyll biosynthesis and photosynthetic electron transport (Molas, 2002; Rahman *et al.,* 2005). It has been suggested that uptake of toxic amounts of nickel by a plant leads to quantitative changes in the structure of photosynthetic apparatus of plants (Kupper and Kroneck, 2007). The decrease in photosynthesis is also responsible for reduced biomass of crop plants in such condition. The alteration in the activity of antioxidative mechanism of certain enzymes was worked out. At d 60, (16 DAMS) on protein basis the activity of catalase in rice leaves was decreased as compared to control with an increase in nickel from 0.05 to 0.50 mM (Table 2). However, the activity of peroxidase and acid phosphatase on protein basis in

Table 2: Influence of excess Nickel on chlorophyll contents, Hill activity, antioxidative and certain other enzymes in rice

		mM Ni supply						
Days growth	DAMS	Control	0.05	0.10	0.20	0.40	0.50	LSD P=0.05
			Chlorophyll : mg/g fresh weight					
61	43	3.48	2.90	2.54	1.75	1.60	0.75	0.606
			b					
		1.50	1.24	1.08	0.85	0.52	0.35	0.261
			total					
		4.98	4.24	3.62	2.60	2.12	1.10	0.675
			Hill reaction activity: change in OD $100mg^{-1}$					
		2.52	2.48	2.30	1.85	1.60	0.55	0.436
			Catalase : µ moles H_2O_2 decomposed mg^{-1} protein					
		1107	1098	1059	706	707	560	137
			Peroxidase : Change in OD mg^{-1} protein					
		1.51	2.09	2.65	3.12	4.15	5.89	0.76
			Acid phosphatase : µg Pi liberated mg^{-1} protein					
		20.0	28.9	49.4	53.9	73.0	107.0	19.5
			Ribonuclease : Change in OD min^{-1} mg^{-1} protein					
		0.078	0.059	0.080	0.160	0.190	0.374	0.074
			Protein : % in fresh weight					
		3.50	3.30	2.88	2.69	2.26	1.73	0.442

rice leaves was increased with increase in Ni supply from 0.05 to 0.50 mM concentration as compared to control. The increase in peroxidase activity at 0.50 mM Ni was about four times higher than 0.05 mM (Table 2) concludes the most active response of antioxidative mechanism under the hazardous impact of the metal. Heavy metals are known to cause oxidative stress in plants which is ascribed to metal induced disturbances between reactive oxygen species (ROS) and antioxidant response enzyme system such as catalase (CAT) and peroxidase (POX). The enhanced activity of POX in excess Ni treated leaves might result either in peroxidative damages of the thylakoid membrane or lower the auxin and protein content in tissues, damage of photosynthetic apparatus, and eventually leading to reduced growth and yield in plants (Pandey and Gopal, 2010). The heavy metal like nickel is also shown to induce apoplastic peroxidase activity in plants grown in heavy metal containing nutrient solution (Rao and Sresty, 2000).The induced level of peroxidase has been considered as an indirect effect of excess metal supply. The activity of CAT in rice leaves decreased with an increase in nickel supply.CAT is considered as a key enzyme to decompose H_2O_2 produced during metabolic activities (Marschner, 2002). The decrease in CAT activity in turn increases H_2O_2 concentration creating oxidative stress. The decreased activity of catalase might be due to less iron availability for incorporation into the protein moiety of the enzyme as well as low protein content in excess Ni treated plants. In excess Ni the increase in acid phosphatase activity in rice might reflect poor utilization and incorporation of phosphorus in constitution parts of this crop for different metabolic pathways. Similar reports on increased acid phosphatase activity in sunflower (Pillay *et al.,* 1996) suggested that decrease in P content is correlated with this stimulation. Lower P concentration in rice leaves is also in agreement to these results. Ni similarly the activity of ribonuclease was minimum in leaves of rice at control level and it increased with an increase in nickel supply from 0.05 to 0.5 mM. Concentration of soluble protein was significantly reduced at excess levels of Ni supply (at 0.50mM Ni, protein concentration was reduced to 50% as compared to normal leaves.

In present study the low iron concentration in rice leaves might be a cause for decrease in CAT activity. The decreased enzyme activity suggests that heavy metal may induce changes in functional protein and ultimately the synthesis of enzyme (Adriano, 2001). As the hazardous effects of heavy metal toxicity also include the alteration in mineral nutrients uptake and accumulation. Some of the important macro and micro nutrient were also quantified. At d 112, sulphur concentration in leaves was increased with an increase in nickel supply from 0.05 to 0.50 mM. However, no consistency was observed in stem. At 0.50 mM nickel supply the sulphur was reported more in leaves as compared to other plant parts. Ni is taken up by the rice plant as Ni^{+2}, and its absorption in high concentration decreases the uptake of other divalent cations such as Fe^{2+}, Zn^{2+} and Cu^{2+} significantly. In panicles at 0.05 mM, the concentration of sulphur was more as compared to that of control level. These results are in contrast with the result of others (Gopal *et al*., 2001). Possibily S metabolism of plants get disturbed at excess nickel supply. The phosphorus metabolism is also reduced due to restricted mobility of phosphorus to upper parts. The lowering of phosphorus in leaves and greater accumulation in stem at excess Ni might suggest hindrances in the mobility of the nutrient. Similar observations on lower P content in excess Ni conditions have also been reported by (Miller *et al*. 2000), and may be interpreted with the influence of heavy metal on alteration of translocation. The phosphorus concentration was decreased in leaves and roots but increased in stem with an increase in nickel supply. In panicle at 0.05 mM, there was a decrease in phosphorus concentration from that of control plants (Table 3).The higher concentration of nickel was so hazardous that no panicle were developed at all.

The iron concentration in all parts of rice plants decreased in various plant parts (except in roots) with an increase in nickel supply. In roots there was a tendency of accumulation of iron with an increase in nickel supply. The iron concentration was lowest in leaves followed by stem at 0.5 mM Ni and its concentration was maximum in roots at this level (Fig. 2). The decrease of iron content in leaves and increase in roots of rice in excess nickel treated plants are in accordance with the results of Chatterjee and Chatterjee (2000) with respect to cauliflower. Interference of heavy metals including Ni with iron in plant metabolism is known to induce the disturbances creating physiological iron deficiency in plants.

Table 3: Influence of excess nickel on sulphur and phosphorus concentration of rice.

			mM Ni supply						
Days Growth	DAMS	Plant part	Control	0.05	0.1	0.2	0.4	0.5	LSD P=0.05
			Sulphur : % in dry weight						
112	68	Panicle	0.21	0.33	---	---	---	---	0.168
		Leaves	0.31	0.39	0.36	0.45	0.56	0.74	0.243
		Stem	0.26	0.29	0.53	0.50	0.50	0.64	0.137
		Root	0.44	0.41	0.43	0.54	0.53	0.50	0.146
			Phosphorus : % in dry weight						
		Panicle	0.310	0.192	---	---	---	---	0.096
		Leaves	0.370	0.320	0.280	0.260	0.245	0.130	0.069
		Stem	0.145	0.225	0.240	0.345	0.570	0.625	0.113
		Root	0.425	0.295	0.285	0.270	0.255	0.230	0.120

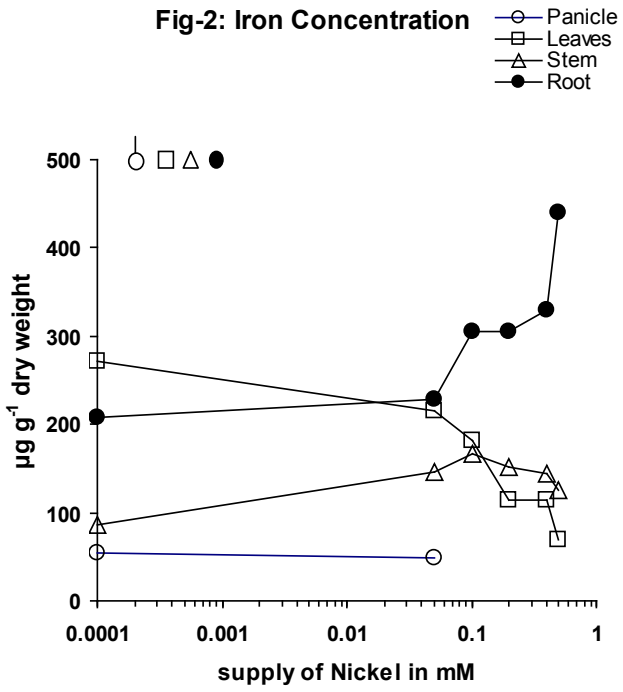

Fig 2: Influence of excess nickel on concentration of iron in different parts of rice.

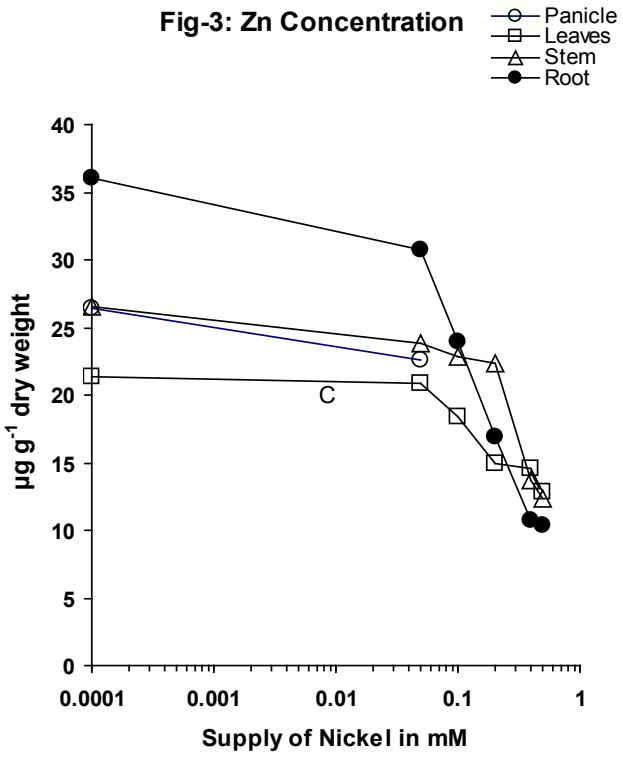

Fig 3: Influence of excess nickel on concentration of zinc in different parts of rice.

The Zinc concentration was increased in old leaves, but decreased in other plant parts (young leaves, stem and roots) with increasing Ni level from.05 to 0.5mM (Fig. 3). The decreased **z**inc concentration in leaves with an increase in levels of nickel absorption because it is reported that nickel share the same carrier in ion uptake required for zinc with an increase in variable levels of nickel is similar to the observation of Yang et.al.(2008) on maize. The decrease in zinc content in leaves might be suggested due to competition between zinc and nickel as the nickel is reported to Share with the same carrier in ion uptake which are required for zinc.

The concentration of nickel was increased in all plant parts with an increase in nickel supply as compared to control. The accumulation of nickel was highest in roots and the lowest in stem. The concentration of Ni was 127.8 µg.g-1 dry matter in leaves at 0.5 mM Ni supply (Fig. 1). The reason for this phenomenon may be ascribed as the tendency of nickel to displace several ions from physiologically important binding sites on root surface but its translocation thus reduces the uptake and mode of other heavy metals including iron (Mengel and Kirkby, 2001).

Conclusion

Nickel (Ni) is an essential micronutrient for plants since it is the active center of the enzyme urease although excess Ni is known to be toxic. Excess supply of heavy metal disturbed plant metabolism and retard shoot and root growth and resulted into poor yield. When rice (*Oryza sativa* L. cv. Swarna Mansoori) plants were grown in acid washed sterile refined sand in a glass house under controlled conditions. The varying levels of nickel were supplied as $NiSO_4$ to induce the toxicity symptoms of nickel.. As plant micronutrient nickel is found in the vegetative parts of most plants in the range of 1-10µg/g dry weight. Toxicity occurs at higher concentrations 10-50µg/g dry weight depending upon the crop species.

The metabolism of the plants was altered and observed to be inhibitory to of growth and biomass yield.Ni affected the number of tillers.. At d 60 at 0.5 mM Ni, the young and middle leaves developed chlorosis in the form of bands on the middle portion (central portion) of the leaves. The accumulation of nickel in different plant parts reduced carbohydrate and protein formation in leaves. The higher concentration of nickel (0.4mM and 0.5 mM) proved to be most hazardous as they degraded the photosynthtic pigments, resulting into chlorosis and necrosis. Ni concentration beyond 0.4mM depressed antioxidative mechanism and caused oxidative stress in the plants by inhibiting enzyme catalase and enhanced peroxidaes activity. At 0.5 mM Ni rice plant exhibited toxicity symptoms like, severe chlorosis and necrosis, altered physiology of nutrient P, S, Zn and Fe uptake and restricted the panicle formation ultimately resulted in to rice yield. The value of threshold of toxicity, and toxicity in leaves of rice were observed 14.0 and 40 ug^{-1} dry matter respectively.

Thus the present investigations confirmed that rice is one of the most susceptible plant for nickel supply if given in higher concentration. It proved hazardous if concentration is above 0.40 mM Ni. Future research is needed for developing the strategies for reducing Ni toxicity in soil treated with sewage sludge and other waste material containing high amount of Ni.

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