International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.4, pp 1710-1720, 2015

Impact of Nickel on Enzymes Activity in leaves of Paddy Plant *Oryza sativa* L.

[1]Waqeed Mahdi Hadif, [1]Sahibin Abd Rahim, [1]Ismail Sahid, [1]Atiqur Rahman and [2]Izyanti Ibrahim

[1]School of Environmental and Natural Resource Sciences,
[2]School of Biosciences and Biotechnology, Faculty of Science and Technology,
Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

Abstract: *Oryza sativa* plants were grown near Ultrabasic soil. Two fields designated as field 1 and field 2 were located in Ranau, Sabah, and the other field (field 3) used as control was at the UKM experimental plot for paddy plants in Peninsular Malaysia for the year 2014. The accumulation of nickel in paddy plants were determined and the influence of nickel on antioxidants enzymes ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) of paddy leaves were examined at (17, 47, 77, 107 and 145) days post planting date. The paddy plant species used in the present investigation is Sarawak merah. It was determined that paddy could accumulate appreciable amounts of Ni in leaves. Under stressful conditions, the antioxidant enzymes were up regulated compared to the control. Our results showed that paddy had the capacity to overcome nickel induced stress to a certain extent. The maximum enzyme activities were observed at different nickels concentrations. An increase in all enzyme activities was observed at high concentration of nickel at age 47 days post planting in Ranau fields followed by a decline in these enzyme activities with the increase of nickel contents in paddy leaves.
Key words: Enzyme Activity, Nickel, Paddy.

Introduction

Nickel which is often referred to as Ni, is a transition metal presents in natural soils at trace levels excluding ultramafic or serpentine soils where unusually higher concentration exists. However, certain areas have witnessed an increasing concentration of Ni owing to human activities such as emission of smelters, burning of coal and oil, mining work, sewage, pesticides and phosphate fertilisers[18]. The concentration of Ni in polluted soil may range from 200 – 26,000 mg/kg which is 20 to 30 folds higher than the normal concentration found in natural soils which is 10 – 1,000 mg/kg[24]. Nickel is a toxic heavy metal usually found in high concentrations in waste water due to heavy industrial wastes such as electroplating, dye manufacturing, and steam electric power plants[37]. Different plants species found in soil with excess of Ni usually suffer several physiological derangements with numerous toxicity symptoms including necrosis and chlorosis[15, 57, 41].

Nickel is responsible for some severe health challenges including dermatitis[10], allergic sensitization[54] and lungs and nervous derangement[20]. Ni content of surface water was observed to be about 0.01 – 0.02 mg/L[26]. Even though Ni at low concentration is known to be essential for plants, toxicity can occur at high concentration[42,8,16]. Nickel concentration ranges from 0.1– 5.0 µg/g of dry matter has been observed in plants[32]. Ni deficiency is uncommon compared to its excess, which is

largely as a result of metal mining and smelting. Dosage and exposure time have reported to be directly related to the negative effect of Ni on plants[28,39], and this effect varies according to the species of plants and conditions[21]. Anti-oxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase play vital roles in scavenging free radicals and peroxides that are produced under metal-induced stress[52]. Moderate exposure to Ni (100 µmol/L $NiSO_4$) has been demonstrated to induce significant increase in H_2O_2 concentration as well as antioxidant enzyme activities in maize leaves[29].

On the other hand, Ni has been recognized as an essential trace element for living organisms. It is also known to be an essential component of urease enzyme, necessary for its functions, thereby making it a component for good health in animals. Plants that grow in high Ni-containing soil have been shown to have nutritional in balance which consequently leads to functional disorder of the cell membrane. Thus lipid composition and H-ATPase activity in the plasma membrane of *Oryza sativa* L. shoots have been reported to be affected by Ni[44]. Wheat exposed to high level of Ni has been reported to induce increased concentration of malondialdehyde (MDA). The concentration of MDA has also been shown to be higher in Ni-sensitive plants as against Ni-tolerant plants[19]. These changes in Ni concentration could induce disturbances in membrane functionality and ion balance, particularly potassium (K^+), which is the most mobile ion across plant cell membrane.

Other alterations observed in Ni-treated plants were associated with changes in water balance. High uptake of Ni has been shown to induce a decline in the water content of both monocot and dicot plant species and the decrease in the water uptake is employed as an indicator, signaling the progression of Ni toxicity in plants[17]. Deteriorations of the environment has generated an increase in stress levels of all forms of life. Since agriculture is the lifeline of the global society, stress on agricultural crops is of paramount significance. Growth and grain yield of paddy plant has been observed to be adversely affected by abiotic stress factors such as salinity stress, water stress and high temperature[38]. Aside these stress factors, toxic heavy metals have become an emerging and more dangerous stress factor for major agricultural crops. Soil and water arising from toxic heavy metals is said to be mostly of anthropogenic origin and several reports have been documented that agricultural lands adjacent to industrial areas are usually polluted with varied degrees of toxic heavy metals[43]. Different plant species differ in the amount of toxic and non-toxic metal uptake from the soil and water. There are also variations in the extent and where these toxic metals are accumulated in the different parts of the plants[14].The bioaccumulation of toxic heavy metals by several agricultural plants has constituted a significant health hazard as documented by numerous workers [33]. Under stressful conditions, excessive ROS are generated in the young senescing leaf cells and these ROS are scavenged by complex enzymatic (α-tocopherol, ascorbate, glutathione) and non-enzymatic antioxidant systems (SOD, APX, CAT, GPX, GR etc)[40]. Different plants have developed effective antioxidant pathways that are capable of protecting them from oxidative damage during periods of moderate stress and normal growth[22]. However, under severe stressful conditions, the generation of ROS can exceed the neutralizing capacity of the antioxidant system, subsequently leading to oxidative damage. Heavy metals are known to induce increased generation of free radicals[6] and this eventually leads to oxidative damage in senescing leaf cells under light. Heavy metals such as Zn and Cu serve either as cofactors or activators of enzyme reactions such as the formation of enzyme/substrate metal complex[31] or induce catalytic reactions such as prosthetic group in metallo proteins. These essential micronutrients play some roles in redox reactions, structural functions and electron transfer in nucleic acid metabolism. Some heavy metals like Hg, Cd and As are highly poisonous to metal-sensitive enzymes which subsequently lead to inhibited growth and death of the organisms. Based on their coordination chemistry, heavy metals are alternatively classified as Class B metals under non-essential trace elements which include highly toxic elements such as Ag, Pb, Hg and Ni[36]. The exact mechanism through which reactive oxygen species (ROS) are generated and the factors affecting the generation of the ROS in Ni treated plants are still largely unknown. Even though Nickel officinal metal accumulation properties have been extensively investigated[58, 45], little attention has been given to the role of antioxidant responses and the resultant effect of accumulation in this plant. The objective of this study was to investigate the impact of accumulation of nickels in leaves of paddy plants on the antioxidant enzymes SOD, CAT and APX activities.

Materials and Methods

Soil analysis

The Paddy plants and soil were collected from three fields of paddy, two fields in Ranau, Sabah (Field 1 and Field 2) and the other field in UKM Paddy field (Field 3), for the year 2014. The soil samples were air dried and ground to pass through a 63-µm sieve. The wet digestion method was adopted to extract heavy metals from soil[55.] One gram of each sample was weighed into a conical flask, then 15 mL of HNO_3 was added followed by 5 mL of $HClO_4$(3:1) and left for 2-3 h in a sand bath. The digested samples were filtered through 0.45µm pore size Millipore filter paper and made up to 50 mL with deionised water before the metal contents were determined with ICP-Mass spectrometer model EIAN 9000. Soil pH was determined in soil, water ratio of (1:2.5)[5] method, whereas total organic carbon was determined according to Walkley and Black (1934)[53].

Table1 Mean values of soil pH, organic matter, Ni concentration and soil texture for different sites

soil properties	Ranau/field 1	Ranau/field 2	Control/field3
pH	5.28±0.11	5.75±0.16	4.25±0.08
Organic matter	10.89±0.16	6.48±0.06	2.91±0.03
Ni	2050.40±228.36	186.79± 15.01	5.76±0.62
Soil texture	Silty clay	Clay loam	Clay loam

Determination of nickel in leaves of paddy plant

Plant samples were washed with tap water, and then washed thoroughly thrice with distilled water and thrice with distilled deionised water and then dried with tissue paper. The samples were divided in to two parts, one for enzyme assay which was kept in refrigerator -80˚C, and the other for nickel determination. The leaves samples were sliced into small pieces before being oven dried at 70˚C for 72 hours, and then ground using an agate pestle. One gram of sample was weighed and put through a wet digestion procedure using HNO_3:$HClO_4$ (3:1) in a conical flask for 2-3 hours on a sand bath[3]. Afterwards, 10 mL of hydrochloric acid (HCl) was added to dissolve inorganic and oxide salts[27]. The digested samples were then filtered using a 0.45 µm pore size cellulose nitrate membrane filter paper (Millipore). The volume was then made up to 50 mL using deionised water. The concentrations of nickel in leaves of paddy plants were determined by using ICP-Mass spectrometer (EIAN 9000) model.

Enzymes extractions and assays

Fresh leaves tissue of 0.2 g were homogenized in an ice –cooled mortar under liquid nitrogen. Subsequently soluble proteins were extracted by mixing the powder in an extraction mixture consisting with 5 mL of 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% (w/v) polyvinyl pyrrolidone. The homogenate was centrifuged at 15000 g for 15 min at 4°C[34]. The supernatant was used for enzyme activity determination[49]. After which the supernatant was transferred to a new tube and kept at −20˚C for later determinations of enzyme activities of APX, CAT and SOD.

Protein assay

Protein concentration was measured following the procedure of Bradford assay (1976)[12] using bovine serum albumin as a standard protein.

Ascorbate Peroxidase (EC 1.11.1.7) assay

Ascorbate peroxidase (EC 1.11.1.7) was assayed using the method of Nakano and Asada (1981) with minor modification[35]. The reaction mixture contained 50 mmol/L potassium phosphate, pH 7.0, 1 mmol/L ascorbic acid, 2.5 mmol/L H_2O_2 and enzyme sample (15µg protein) in a final volume of 1 mL at 25˚C. Ascorbate oxidation was followed spectrophotometrically by a decrease of A_{290} and using the absorption coefficient of 2.8 mM^{-1} cm^{-1}.

Catalase (EC 1.11.1.6) assay

Catalase (EC 1.11.1.6) activity was determined by directly measuring the decomposition of H_2O_2 at 240 nm (0.04 mM^{-1} cm^{-1}) in 50 mmol/L potassium phosphate, pH 7.0, containing 10 mmol/L H_2O_2 and enzyme sample (15 µg protein) in a final volume of 1 mL at 25˚C as described by Aebi (1983)[1].

Superoxide dismutase (EC1.15.1.1)

Superoxide dismutase (EC1.15.1.1) activity assay was based on the method of Beauchamp and Fridovich (1971)[9], who measured the inhibition of the photochemical reduction of nitro blue tetrozulium (NBT) at 560nm, with some modifications. One milliliter of reaction mixture contained 50 mM phosphate buffer, pH 7.8, 0.1 mmol/L EDTA, 13 mmol/L methionine, 75 µmol/L NBT, 16.7 µmol/L riboflavin and enzyme extract (20 µg protein). Riboflavin was added last, then the test tubes were shaken and enzyme reaction was initiated by placing the tubes (30 cm) under the light of two 15-W fluorescent lamps, equivalent to 320 $\mu molm^{-2}\ s^{-1}$. The reaction was terminated after 10 min by removing the reaction mixture from the light source. An illuminated blank without protein gave the maximum reduction of NBT, and therefore, the maximum absorbance at 560nm. SOD activity is presented as absorbance of sample divided by absorbance of blank, giving the percentage of inhibition. In this assay, one unit of SOD is defined as the amount required to inhibit the photo reduction of NBT by 50%. The specific activity of SOD was expressed as unit mg^{-1} protein.

Statistical analysis

All samples were processed in triplicate and the mean were calculated. Statistical analysis was performed using SPSS (version 20). Two-way ANOVA was employed in the data analysis and statistical significance was determined at $P < 0.05$. All data were expressed as mean with standard error ± SD^{50}.

Results and discussion

Nickel Concentration in Leaves

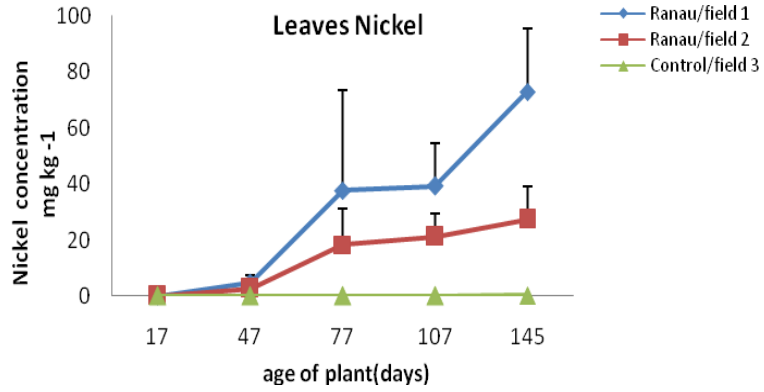
The data obtained from this study has shown significant differences ($P < 0.05$) in nickel concentration between different sites (Table 2), Ranau field 1 was observed to have higher concentration of Nickel metal (30.89 mg kg^{-1}) compared to the concentration in the control plot (0.06 mg kg^{-1}). In a similar manner, there was significant differences ($P < 0.05$) between paddy plant ages in Nickel metal concentration. Nickel metal concentration was observed to increase with increase in age of paddy plants (0.00, 2.48, 18.68, 20.18, 33.50) mg kg^{-1} for ages of paddy (17, 47, 77, 107, 145) days respectively.

There was also significant differences ($P < 0.05$) from the interactions between sites and age as shown in Table 2, The highest content of Nickel was recorded from the interactions between Ranau filed 1 and the age of 145 days (72.84 mg kg^{-1}), while the lowest Nickel concentration was obtained from the interactions between the control field 3 and the ages of 17,47,77,107 days (0.00 mg kg -1) and there was no significant differences between these 4 ages.

Table 2 Effect of Sites and Ages of Plant on Nickel Content Concentration in leaves (mg kg^{-1}).

Sites	Age of plant (days)					Sites mean
	17	**47**	**77**	**107**	**145**	
Ranau (Field 1)	0.00 ±0.00	4.62 ±2.68	37.72 ±35.52	39.28 ±15.26	72.84 ±22.68	30.89_a ±32.29
Ranau (Field 2)	0.00 ±0.00	2.83 ±1.91	18.31 ±12.71	21.25 ±8.23	27.36 ±11.90	13.95_b ±13.26
Control (Field 3)	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.31 ±0.23	0.06_c ±0.16
Age of plant mean	0.00_c ±0.00	2.48_c ±2.60	18.68_b ±24.95	20.18_b ±19.11	33.50_a ±34.23	

Note: Means within the sites column, age of plant row followed by the same letter are not significantly different to each other at p> 0.05.



Effect of Nickel on Antioxidant Enzymes

Ascorbate Peroxidase

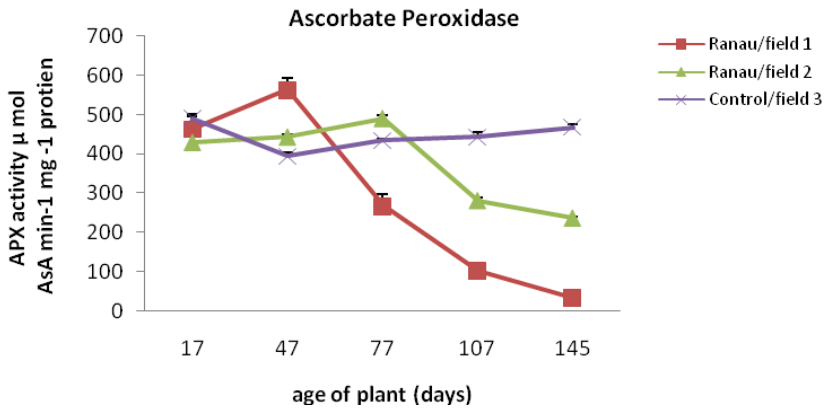
The APX activity observed in this study is presented in Table 3. APX enzyme activity vary significantly ($P < 0.05$) between sites, with the control field 3 having the highest activity (445.86 µmol min^{-1} mg^{-1}) while the lowest APX activity (285.99 µmol min^{-1} mg^{-1}) was recorded in Ranau field 1. There was strong correlation (-1.00*) between APX activity and Ni concentration in these sites. Similar trend was also observed in the age of the plant with the maximum level of APX activity (466.72 µmol min^{-1} mg^{-1}) recorded at 47 day post date of planting. However, APX activity did not vary significantly (461.63 µmol min^{-1} mg^{-1}) at the age of 17 days and the minimum level (246.23 µmol min^{-1} mg^{-1}) was recorded at the age of 145 days. Correlation coefficient with Ni concentration was -0.92 *.

The interactions site × age in terms of APX activity showed a significant variation ($P < 0.05$), with the highest APX activity (563.12 µmol min^{-1} mg^{-1}) recorded from the interaction between Ranau field 1 and the age of 47 days while the interactions between the same site and age 145 days recorded the lowest APX activity (33.15µmol min^{-1} mg^{-1}).

Table 3 Effect of Nickel on Ascorbate Peroxidase Activity (µmol min^{-1} mg^{-1}).

Sites	Age of plant (days)					Mean	r sites & leaves nickel
	17	**47**	**77**	**107**	**145**		
Ranau/Field 1	463.72 ±30.79	563.12 ±31.13	266.72 ±31.54	103.23 ±6.11	33.15 ±3.95	285.99_c ±211.15	-1.00 *
Ranau/Field 2	430.16 ±16.24	443.63 ±6.96	491.00 ±8.15	281.69 ±5.88	236.97 ±4.20	376.69_b ±102.72	
Control/Field 3	491.00 ±10.77	393.42 ±10.71	433.78 ±5.54	442.54 ±11.58	468.56 ±5.92	445.86_a ±35.09	
Age of plant mean	461.63_a ±32.07	466.72_a ±77.35	397.17_b ±102.27	275.82_c ±147.17	246.23_d ±188.71		
r ages & leaves nickel	-0.92 *						

Note: Means within the sites column, age of plant row followed by the same letter are not significantly different to each other at p> 0.05.
Note: r * Correlation is significant at the 0.05 level, r ** significant at the 0.01 level.



Catalase

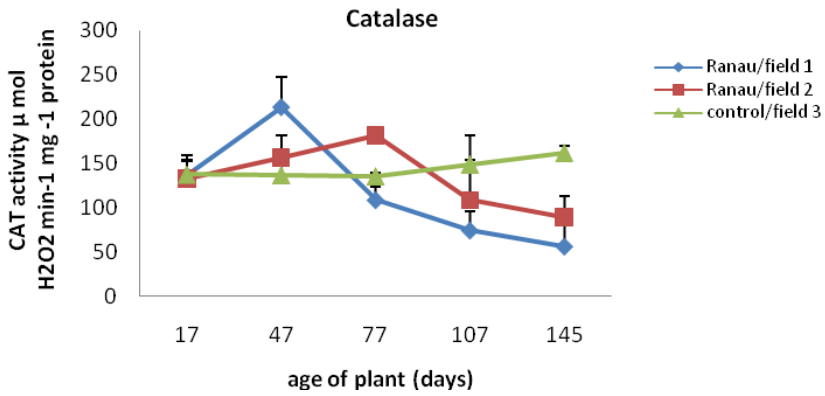
The CAT activities as recorded in this study are shown in Table 4. There were significant differences ($P < 0.05$) between sites, with the maximum CAT activity (143.91 µmol min $^{-1}$ mg $^{-1}$) being observed at control field 3 while Ranau field 1 recorded the least activity (117.60 µmol min $^{-1}$ mg $^{-1}$). The correlation coefficient between CAT activity and Ni concentration in these sites was -0.99 *. Increase in the age of paddy plant was observed to be directly associated with increase in CAT activity through the age of 47 days from where the enzyme activity was observed to decline in the order; 135.33, 168.75, 141.68, 110.34 and 102.23 µmol min $^{-1}$ mg $^{-1}$ for ages 17, 47, 77, 107 and 145 days respectively. The correlation coefficient of the accumulated Ni concentration in the leaves of the plant and CAT activity was -0.78.

Site ×age interaction showed significant difference ($p<0.05$) and the highest CAT activity (213.32 µmol min $^{-1}$ mg $^{-1}$) was recorded at Ranau field 1 at age 47 days, while the lowest activity (56.06 µmol min $^{-1}$ mg $^{-1}$) was at the same site but at the age of 145 days.

Table 4: Effect of Nickel on Catalase Activity µmol min $^{-1}$ mg $^{-1}$.

Sites	Age of plant (days)					Mean	r sites&leaves nickel
	17	**47**	**77**	**107**	**145**		
Ranau/Field 1	136.15 ±22.93	213.32 ±34.61	108.37 ±15.23	74.09 ±21.70	56.06 ±24.61	117.60 $_{b}$ ±60.87	-0.99 *
Ranau/Field 2	132.45 ±20.00	156.11 ±24.94	181.25 ±8.87	108.41 ±73.11	89.27 ±23.22	133.50 $_{ab}$ ±46.37	
Control/Field 3	137.38 ±16.64	136.83 ±28.81	135.43 ±4.36	148.53 ±4.94	161.37 ±8.50	143.91 $_{a}$ ±16.73	
Age of plant mean	135.33 $_{b}$ ±17.48	168.75 $_{a}$ ±43.00	141.68 $_{b}$ ±33.17	110.34 $_{c}$ ±50.01	102.23 $_{c}$ ±49.78		
r ages & leaves nickel	-0.78						

Note: Means within the sites column, age of plant row followed by the same letter are not significantly different to each other at p> 0.05.
Note: r * Correlation is significant at the 0.05 level, r ** significant at the 0.01 level.



Superoxide dismutase

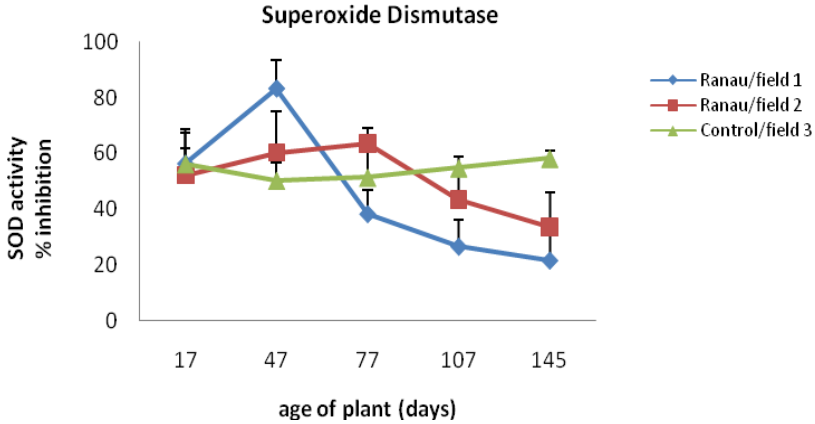
The increase in Ni content of paddy plants depends on the sites and this was observed to have no significant effect (P < 0.62) on SOD activity Table 5. However, increase in the age of paddy plant was observed to induce decrease in SOD activity. Depending on the concentration of Ni, the order of SOD activity was 54.90%, 64.60%, 51.03%, 41.55%, and 37.79% for ages 17, 47, 77, 107, 145 days respectively and the correlation coefficient with the Ni concentration was -0.88 *.

Sites × ages interactions indicated significant differences (P < 0.05), with the highest SOD activity (83.26%) was obtained at Ranau field 1 and age 47 days and the lowest activity (21.59%) was obtained at the same site but in the age of 145 days.

Table 5 Effect of Nickel on Superoxide Dismutase Activity (%).

Sites	Age of plant (days)					Mean	r sites &leaves nickel
	17	**47**	**77**	**107**	**145**		
Ranau/Field 1	56.34 ±12.47	83.26 ±10.46	38.27 ±8.64	26.56 ±9.79	21.59 ±10.09	45.20 n.s ±24.86	-0.99 *
Ranau/Field 2	52.08 ±9.61	60.24 ±15.00	63.46 ±5.58	43.32 ±11.08	33.59 ±12.45	50.54 n.s ±14.79	
Control/Field 3	56.27 ±11.01	50.30 ±6.38	51.37 ±9.82	54.78 ±4.07	58.20 ±2.65	54.18 n.s ±7.05	
Age of plant mean	54.90 b ±9.84	64.60 a ±17.55	51.03 b ±13.02	41.55 c ±14.48	37.79 c ±18.09		
r ages & leaves nickel	-0.88 *						

Note: Means within the sites column, age of plant row followed by the same letter are not significantly different to each other at p> 0.05.
Note: r * Correlation is significant at the 0.05 level, r ** significant at the 0.01 level.



Discussion

This study, demonstrated that paddy plant can grow in soils contaminated with Ni and this is an indication that paddy plant can tolerate higher concentrations of Ni, possibly higher than Ni concentrations in contaminated areas. Paddy plant was also observed to accumulate appreciable amount of Ni in the leaves. The high accumulation of Ni in the different parts of the paddy plants could be due to its role as a trace element[4]. In this study, we observed that the uptake of Ni by paddy plant is concentration dependent. These findings are similar with the results obtained in earlier studies[28, 42]. Plants with more than 1000 mg/kg of Ni content in their tissues are considered as Ni-hyper accumulator[13].

The results obtained from this study has given indication of the paddy plant as a Ni hyper accumulator. A previous related study [30] has reported the accumulation of more than 1000 mg/kg of Ni by aquatic plants. Ni can be rapidly taken up via the root system of paddy plants[2].

These results also suggests that there may be protective barriers that are responsible for preventing the Ni from the roots to the leaves[23]. In a previous study, it was stated that high Ni concentrations could be responsible for weak plant growth, consequently leading to depression, metabolic disorders and sometimes chlorosis. It was observed that low concentration of Ni was capable of inducing increased biomass. This increase in biomass could be due to an increase in low molecular weight stress proteins such as antioxidant enzymes[49]. Decline in cell mass and growth was observed to be associated with increase in concentration of Ni and increase in duration of exposure usually lead to degradation of the cell membrane[25]. Protein content of plants may be considered as reliable indicators of oxidative metal stress. The differences in the antioxidative enzyme activities of the leaves of the paddy plant as observed in this study could explain the difference in the tolerance levels of the leaves.

This study also indicated that the accumulation of Ni through the age of 145 days and the activities of the antioxidant enzymes were also affected at different extents. It was observed that all the antioxidant enzymes exhibited similar response curves, with increased activities at a low concentration of Ni while the activities decreased at lower concentration of Ni. Several studies on related metals such as copper, arsenic and chromium have shown similar findings[34, 49]. The reduction in the activities of SOD, CAT and APX at higher Ni concentration seen in this study could be due to enzyme modulation by stress-related effect or molecules[51]. Superoxide dismutase play vital role in cellular defence mechanisms against ROS by breaking down the toxic reactive oxygen species into hydrogen peroxide which is less toxic to the system. Superoxide dismutase reduces the risk of OH radical formations which is responsible for severe damages to membranes, proteins and DNA[56]. The activities of SOD increased significantly in response to exposure to low levels of Ni and decline at high concentrations of Ni. These findings are in accord with earlier findings[7], which reported an initial increase in SOD activities subsequently followed by a decrease in the enzyme activities in *Zea mays* L. shoots exposed with 250 µmol/L Ni.

However, contrary to our findings, [8] an earlier study had shown that SOD activities of *Zea mays* L. roots were not altered even when exposed to 250 µmol/L Ni for 5 days. The increase in SOD activities of paddy plants at low concentrations of Ni is an indication of paddy plants tolerance on Ni via effective neutralization of oxidative stress. On the other hand, the reduction in SOD activities in paddy plant leaves could be due to an increase in the accumulation of Ni which eventually overwhelmed the scavenging capacity of the SOD[11]. The H_2O_2 that is generated by SOD is less toxic and is further converted to H_2O and oxygen by other antioxidant enzymes such as CAT[56]. Similar to our findings on SOD activity, CAT activity increased in response to Ni exposure in leaves up to the age of 47 days. This observation on CAT activity is consistent with the findings of Gajewska and Skłodowska (2007)[16] who made similar observation while studying the effect of Ni exposure on oxidative enzyme activities in wheat leaves. The increase could be consequential to the increase in the amount of CAT substrate. This is an indication that paddy plant employs an adaptive mechanism in maintaining the level of H_2O_2 in the system. However, at higher concentrations, the accumulated H_2O_2 over powered the capacity of the CAT enzymes, thereby reducing its level and or activities. This was observed especially after the age of 47 days. This results is an indication that beyond this age (47 days), the adaptive mechanism is compromised, making the system unable to cope with increasing H_2O_2 levels. One of the most important H_2O_2 scavenging enzyme is APX. The enzyme has several

physiological functions in plant cells and it participates in many biochemical reactions. Stimulation of this enzyme can induce functional dysregulation of the cell wall, leading to decreased growth rate. Just like the CAT enzymes, APX also breaks down H_2O_2 into water and oxygen. However, the APX affinity towards H_2O_2 is higher than that of CAT[48]. The APX enzyme activity in leaves of paddy at age 47 days was observed to be higher than those of other ages. The increase in APX in response to Ni suggests that APX is an activity scavenger of H_2O_2 and can effectively convert the H_2O_2 to water and oxygen. Therefore, our findings are in agreement with the report of (Kumar et al., 2007)[29], who investigated the effect of Ni on antioxidative enzyme in corn leaves. The said study observed that SOD activities first increase following exposure to Ni and then decreased with higher concentration of Ni.

Conclusion

The result of this study has shown that paddy plants can accumulate a significant amount of Ni and can effectively combat Ni-induced oxidative damages. Our results have demonstrated that the effect of nickel on all the enzymes investigated in leaves of paddy depends on the age of the plant and highest enzyme activities were recorded in Ranau fields that had higher concentrations of nickel at age 47 days which then declined due to increase in the age of the plant and nickel concentration. Based on the findings of this study, it was concluded that the nickel concentration in the leaves of paddy plant varies between fields and age of the plants. It was further concluded that paddy plant has effective antioxidant enzyme activities that enables it to combat relatively high concentrations of nickel

Acknowledgement

This research was supported by research grant RGS/2/2013/STWN01/UKM/01/2. We thank the Faculty of Science and Technology, The National University of Malaysia for providing the research facilities to carry out this project.

References

1. Aebi HE(1983). Catalase. In: Bergmeyer HU, ed. Methods of Enzymatic Analyses, Vol. 3. Verlag Chemie, Weinheim. pp. 273–282.
2. Ali, B., et al. (2008). "24-Epibrassinolide protects against the stress generated by salinity and nickel in Brassica juncea." Chemosphere 72(9): 1387-1392.
3. AOAC. Official Method of Analysis. 14th Edn. Sidney William. Association of Official Chemists, Inc. Virginia, USA. 1984.
4. Assunção, A. G., et al. (2003). "Thlaspi caerulescens, an attractive model species to study heavy metal hyperaccumulation in plants." New Phytologist 159(2): 351-360.
5. Avery B. W., Bascomb C. L. (1982) Soil survey laboratory meth- ods. Technical Monograph No. 6. Soil Survey, Harpenden, UK.
6. Aust SD, Marehouse CE, Thomas CE (1985) Role of metals in oxygen radical reactions. J Free Radi Biol Med 1:3–25.
7. Baccouch, S., et al. (1998). "Nickel-induced oxidative damage and antioxidant responses in Zea mays shoots." Plant Physiology and Biochemistry 36(9): 689-694.
8. Baccouch, S., et al. (2001). "Nickel toxicity induces oxidative damage in Zea mays roots." Journal of plant nutrition 24(7): 1085-1097.
9. Beauchamp, C. and I. Fridovich (1971). "Superoxide dismutase: improved assays and an assay applicable to acrylamide gels." Analytical biochemistry 44(1): 276-287.
10. Bocca, B., et al. (2007). "Levels of nickel and other potentially allergenic metals in Ni-tested commercial body creams." Journal of pharmaceutical and biomedical analysis 44(5): 1197-1202.
11. Boominathan, R. and P. M. Doran (2002). "Ni‐induced oxidative stress in roots of the Ni hyperaccumulator, Alyssum bertolonii." New Phytologist 156(2): 205-215.
12. Bradford, M. M. (1976). "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding." Analytical biochemistry 72(1): 248-254.
13. Brooks, R., et al. (1977). "Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants." Journal of Geochemical Exploration 7: 49-57.

14. Chambers JC, Sidle RC. Fate of heavy metal in abandoned lead, zinc tailing ponds: I Vegetation. J. Environ. Qual. 1991; 20: 745-748.
15. Das P, Samantaray S, Rout GR (1997) Studies on cadmium toxicity in plants: a review. Environ Pollut 98:29–36.
16. Gajewska, E. and M. Skłodowska (2007). "Effect of nickel on ROS content and antioxidative enzyme activities in wheat leaves." Biometals 20(1): 27-36.
17. Gajewska E, Sklodowska M, Slaba M, Mazur J (2006) Effect of nickel on antioxidative enzymes activities, proline and chlorophyll contents in wheat shoots. Biol Planta 50:653–659.
18. Gimeno-Garcia E, Andreu V, Boluda R (1996) Heavy metals incidence in the application of inorganic fertilizers and pesticides to rice farming soils. Environ Pollu 92:19–25.
19. Gonnelli C, Galardi F, Gabbrielli R (2001) Nickel and copper tolerance in three Tuscan populations of Silene paradoxa. Physiol Planta 113:507–514.
20. Haber, L., et al. (2000). "Hazard identification and dose response of ingested nickel-soluble salts." Regulatory Toxicology and Pharmacology 31(2): 231-241.
21. Hao, F., et al. (2006). "Involvement of plasma-membrane NADPH oxidase in nickel-induced oxidative stress in roots of wheat seedlings." Plant science 170(1): 151-158.
22. Hauptmann N, Cadenas E. The oxygen paradox: Biochemistry of active oxygen. In: Sandalios JG (ed) Oxidative Stress and Molecular Biology of Antioxidant Defense. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1997; 1-20.
23. Hozhina, E., et al. (2001). "Uptake of heavy metals, arsenic, and antimony by aquatic plants in the vicinity of ore mining and processing industries." Journal of Geochemical Exploration 74(1): 153-162.
24. Izosimova A (2005) Modelling the interaction between calcium and nickel in the soil-plant system. FAL Agric Res Special issue 288:99.
25. Kabała, K., et al. (2008). "Comparison of heavy metal effect on the proton pumps of plasma membrane and tonoplast in cucumber root cells." Journal of plant physiology 165(3): 278-288.
26. Karadede, H. and E. Ünlü (2000). "Concentrations of some heavy metals in water, sediment and fish species from the Atatürk Dam Lake (Euphrates), Turkey." Chemosphere 41(9): 1371-1376.
27. Khairiah J., Zalifah M.K., Yin Y.H., Aminah A., 2004. The uptake of heavy metals by fruit type vegetables grown in selected agricultural areas. 7(8):1438-1442.
28. Kováčik, J., et al. (2009). "Physiology of Matricaria chamomilla exposed to nickel excess." Ecotoxicology and environmental safety 72(2): 603-609.
29. Kumar, P., et al. (2007). "Excess nickel–induced changes in antioxidative processes in maize leaves." Journal of Plant Nutrition and Soil Science 170(6): 796-802.
30. Maleva, M. G., et al. (2009). "Ecophysiological tolerance of Elodea canadensis to nickel exposure." Chemosphere 77(3): 392-398.
31. Mildvan AS (1970) Metal in enzymes catalysis. In: Boyer DD (ed) The enzymes, vol 11. Academic Press, London, pp 445–536.
32. Mishra, D. and M. Kar (1974). "Nickel in plant growth and metabolism." The botanical review 40(4): 395-452.
33. Mishra SN, Singh DB. Accumulation of lead and cadmium in upper parts of mustard (Brassica juncea) seedlings in response to putrescine. Indian J. Expt. Biol. 2000; 38: 814-
34. Mishra, S., et al. (2006). "Phytochelatin synthesis and response of antioxidants during cadmium stress in Bacopa monnieri L◊." Plant Physiology and Biochemistry 44(1): 25-37.
35. Nakano, Y. and K. Asada (1981). "Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts." Plant and cell physiology 22(5): 867-880.
36. Nieboer E, Richardson DHS (1980) The replacement of the nondescript term heavy metals by a biologically and chemistry significant classification of metal ions. Environ Pollut Series B 1:3–26.
37. Padmavathy, V. (2008). "Biosorption of nickel (II) ions by baker's yeast: Kinetic, thermodynamic and desorption studies." Bioresource Technology 99(8): 3100-3109.
38. Pareek A, Singla SL, Grover A. Analysis of stress proteins at four different developmental stages in field grown rice, Oryza sativa L. (cv Pusa 169) plants. Curr. Sci. 1999; 76: 81-86.
39. Poulik, Z. (1999). "Influence of nickel contaminated soils on lettuce and tomatoes." Scientia horticulturae 81(3): 243-250.
40. Prochazkova D, Sairam RK, Srivastava GC, Singh DV. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. Plant Sci 2001; 161: 765-771.

41. Rahman H, Sabreen S, Alam S, Kawai S (2005) Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrient solution. J Plant Nutri 28:393–404.
42. Rao, K. M. and T. Sresty (2000). "Antioxidative parameters in the seedlings of pigeonpea (Cajanus cajan (L.) Millspaugh) in response to Zn and Ni stresses." Plant science 157(1): 113-128.
43. Rao Ramani IV. Measurement and characterization of some heavy metals Hg, Pb, Cd, and Cu in the aquatic environment of the Kalu River. 1979. M.Sc. Thesis of the University of Bombay.
44. Ros R, Cook DavidT, Picazo CarmenMartinez-CortinaIsabel (1992) Nickel and cadmium-related changes in growth, plasma membrane lipid composition, atpase hydrolytic activity and protonpumping of rice (Oryza sativa L. cv. Bahia) Shoots. J Exp Bot 43:1475–1481.
45. Saygideger S, Dogan M, 2005. Influence of pH on lead uptake, chlorophyll and nitrogen content of Nasturtium officinale R. Br. and Mentha aquatica L. Journal of Environmental Biology, 26: 753–759.
46. Sheoran, I., et al. (1990). "Effect of cadmium and nickel on photosynthesis and the enzymes of the photosynthetic carbon reduction cycle in pigeonpea (Cajanus cajan L.)." Photosynthesis Research 23(3): 345-351.
47. Shri, M., et al. (2009). "Effect of arsenic on growth, oxidative stress, and antioxidant system in rice seedlings." Ecotoxicology and environmental safety 72(4): 1102-1110.
48. Siedlecka, A. and Z. Krupa (2002). Functions of enzymes in heavy metal treated plants. Physiology and biochemistry of metal toxicity and tolerance in plants, Springer: 303-324.
49. Srivastava, S., et al. (2006). "Copper-induced oxidative stress and responses of antioxidants and phytochelatins in Hydrilla verticillata (Lf) Royle." Aquatic Toxicology 80(4): 405-415.
50. Starkings, S. (2012). "Quantitative Data Analysis with IBM SPSS 17, 18 & 19: A Guide for Social Scientists by Alan Bryman and Duncan Cramer." International Statistical Review 80(2): 334-335.
51. Takahashi, H., et al. (1997). "Development of necrosis and activation of disease resistance in transgenic tobacco plants with severely reduced catalase levels." The Plant Journal 11(5): 993-1005.
52. TanyolacD,EkmekciY,UnalanS,2007.Changesinphotochemical and antioxidant enzyme activities in maize (Zea mays L.) leaves exposed to excess copper. Chemosphere, 67: 89– 98.
53. Walkley, A. and I. A. Black (1934). "An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method." Soil science 37(1): 29-38.
54. Wilhelm, M., et al. (2007). "Influence of industrial sources on children's health–hot spot studies in North Rhine Westphalia, Germany." International journal of hygiene and environmental health 210(5): 591-599.
55. Williams, S. (1984). "Official Methods of Analysis of the AOAC." Inc., Arlington, VA.
56. Zhang, F.-Q., et al. (2007). "Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (Kandelia candel and Bruguiera gymnorrhiza)." Chemosphere 67(1): 44-50.
57. Zornoza P, Robles S, Martin N (1999) Alleviation of nickel toxicity by ammonium supply to sunflower plants. Plant Soil 208: 221–226.
58. Zurayk, R., et al. (2001). "Chromium phytoaccumulation from solution by selected hydrophytes." International Journal of Phytoremediation 3(3): 335-350.

***** *****