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LETHAL EFFECTS OF NI -SIDEROPHORE COMPLEX ON ENZYMATIC FUNCTIONS IN *VIGNA RADIATA* UNDER BIOTIC AND ABIOTIC STRESSES

RAFIA AZMAT[1*] AND SUMEIRA MOIN

[1]*Department of Chemistry, University of Karachi, Karachi, Pakistan*
[2]*Department of Botany, University of Karachi, Karachi, Pakistan.*
[*]*Corresponding author's email: rafiasaeed200@yahoo.com*

Abstract

Bioremediation was employed as an innovative emerging area in the field of biotechnology; detoxify metal via complex formation with the pigments. The impact of metal, siderophore and complex were monitored separately and simultaneously both in aqueous and nutrient medium. The strains of *Pseudomonas aeruginosa* was selected, on the basis of their, siderophore secretion for the binding the Ni (50 & 100 ppm) from solution culture while Ni, chosen as a micronutrient, necessary for plant survival and growth, however toxic when in excesses. Bacterial responses were observed on seeds of *Vigna radiata* in Petri dish experiment in relation to plant growth and enzymes activity. In this regard, the enzymatic activity of roots of 4 d old seedling of *Vigna radiata* greatly influenced by the probable complex of Ni and siderophore of bacterial strain *P. aeruginosa.* Results demonstrated that altered activities of enzymes due to the presence of nickel and *P. aeruginosa* created an effect on growth parameters in early stages. The biomass of plant was found to be decreased in the presence of Ni and microbes when compared to uninoculated plants. The growth rate of seedling was more significant at a higher concentration of Ni which reduces in the presence of microbes that showed the apparent interaction of siderophore to that of Ni, due to which Ni was no more available for growth stimulation. The article highlights the other (biotic and abiotic) factors except for temperature and pH which influence the enzymatic functions of seedlings.

Key words: Bioremediation, Enzymatic activity, Ni, Siderophore.

Introduction

Enzymes are useful biomarkers of environmental stress. Regulation of enzymes activities is of vital rank in all biological systems including plants and animals particularly for the period of anxiety conditions. Enzyme regulation is crucial for plant's survival as enzymes, protein in nature having fine tertiary structure, act as catalysts to control the rate of metabolic reactions. Each enzyme on its surface has an active site usually refer as a pocket which readily combines with the substrate (Azmat, 2014) and forms enzyme-substrate complex to the active site. At that time the substrate is converted into product. The lock and key theory elucidate that only one substrate (the key) will adequate into the active site (lock). Enzymes lower the energy of activation for the metabolic reaction (Azmat *et al*., 2013). Their action is altered by temperature, pH, and substrate concentration. Generally, enzymes catalyzing property destroyed by the presence of organic chemicals or pollutants like metals. Krämer, (2005) observed that numerous enzyme activities governed by the presence of Ni ion which explains the positive effects of low concentration of Ni on plant growth and development in plants species like oil seed rape, zucchini, sweet pepper, cotton, tomato, potato, and Chinese hemp (Welch 1981, Gerendas, & Sattelmacher,1997a; Gerendas, and Sattelmacher 1997b; Gerendas and Sattelmacher 1999). Hence, spraying cotton plants with nickel sulfate solution (234.8 mg/kg) improved the quantities of buds and flowers, the rate of boll formation, and seed oil content (by 4.6%) (Andreeva *et al.,* 2001). Total decline of enzyme activities is sometimes observed due to decreased enzyme contents. Depending on its concentration, nickel ion can both stimulate and inhibit enzyme activities in plant tissues. Heavy metals accumulation inactivates enzymes via interacting with protein SH-groups; in this way, protein conformation is altered (Tabaldi *et al.*, 2007). Microbes sequestered from usual surroundings, polluted with heavy metals frequently reveal patience to several contaminants as they have adjusted to such environments (Jones *et al*., 1994; Gregerson *et al*., 1994). The vital role of microorganism in understanding the soil characteristics in heavy metal concentration is essential. The reduction of fresh plant weight and dry weight were noticed and reported by Zaidi *et al*. (2006) and Rajkumar & Freitas (2008). They stated that in Ni-amended soil condition, the value of growth parameters in *B. juncea* plants was decreased considerably with a 32% reduction in fresh weight and 51% reduction in dry weight.

The aims and objective of present research was to evaluate the significance of siderophore (from microbial strain) in trapping Ni from solution culture and monitor the impact on growth parameters and activity of enzymes as environmental biomarkers in seedlings

Materials and Methods

Seed germination: Eight Petri plates were prepared, each plate contained five healthy surfaces sterilized (using NaOCl and rinsed twice with sterilized distilled water) seeds of *Vigna radiata*. Following sets were prepared and incubated for four days at room temperature in the dark (Azmat, 2014).

1.Control 1: 10ml water
2. Control 2: 10ml Hoagland solution (HS)
3. 9ml H_2O + 1ml *Pseudomonas* culture
4. 9ml HS + 1ml *Pseudomonas* culture
5. 5ml HS + 4ml 50ppm $NiSO_4$ + 1ml H_2O
6. 5ml HS + 4ml 50ppm $NiSO_4$ + 1ml *Pseudomonas* culture
7. 5ml HS + 4ml 100ppm $NiSO_4$ solution and 1ml distill water
8. 5ml HS + 4ml 100ppm $NiSO_4$ solution and 1ml *Pseudomonas* culture

Identification of Microbial life: Microbes were sequestered from garden soil and identified morphologically and biochemically as described by Hayat *et al.* (2012).

Extract preparation from germinating plant roots: Sprouting plants were first washed with tap water and then with distilled water. Roots were separated, weighed and crushed in 3ml distill water and centrifuge at 3000 rpm for 20-25 minutes. The supernatant was taken and stored in Eppendorf at -20°C temperature.

Enzymes activities: The semi-automatic biochemical analyzer (Technicon, RA-XT, USA) with the Randox test kit (Randox Laboratories Ltd. 55 Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY) was employed for the determination of the activities of alkaline phosphatase (ALP), Aspartate aminotransferase (AAT), Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH). All experiments were performed in triplicate (Azmat *et al.,* 2013; Azmat, 2014).

Results and Discussion

The current research was conducted to observe the removal of Ni from solution culture via complex formation with the siderophore of microbial strain *P. aeruginosa.* For this purpose seeds of *Vigna radiata* were germinated in the presence of *P. aeruginosa* at different concentration of $NiSO_4$ (500ppm and 100ppm) in a Petri dish with three replicates. Root region of 4 d old seedlings was selected as a primary targeting zone of biotic (*P. aeruginosa*) and abiotic stress (Ni) Fig. 1. Roots size were measured after four days of incubation and root extract was prepared for the analysis of Lactate dehydrogenase (LDH), alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AAT). The Table 1 showed that in 1st and 2nd Petri dish no pigmentation was observed as there was no microbial strain. While in 3 & 4 experimental dish microbial strain secretes pigmentation i-e siderophore which showed lethal effects on root germination (Table 1) while in the rest of plates no color indicates that siderophore reduced in the presence of Ni and formed a complex with Ni that produced lethal effects on root hairs, length, and weight (Table 1).

Effect of Ni and microbes on growth parameters: Results of growth parameters were presented in Table 1, showed that the length and weight of the root were maximum in an aqueous medium as compared to Hoagland solution, separately and simultaneously with Ni and bacterial strain. An interesting observation was noticed in the current investigation that presence of Ni in the medium (at both 50 and 100ppm) showed promoting effects on plant growth parameters. The maximum root length (6cm) was recorded as compared to control (4.4cm), nutrient (2.02cm) and bacterial strains [3.39cm (50ppm) & 1.6cm (100ppm)] with reduced biomass production as compared to aqueous medium. The germination of seeds was tested and reported in Table 1, clearly indicated that the seeds germination greatly influenced by the presence of Ni in the medium and inhibited at both applied dose of Ni while restored in the presence of *P. aeruginosa*.. Moreover, root hairs are the main targeting zone of environmental factors which were damaged in the presence of Ni and *P. aeruginosa* separately and simultaneously in the early stages of developments.

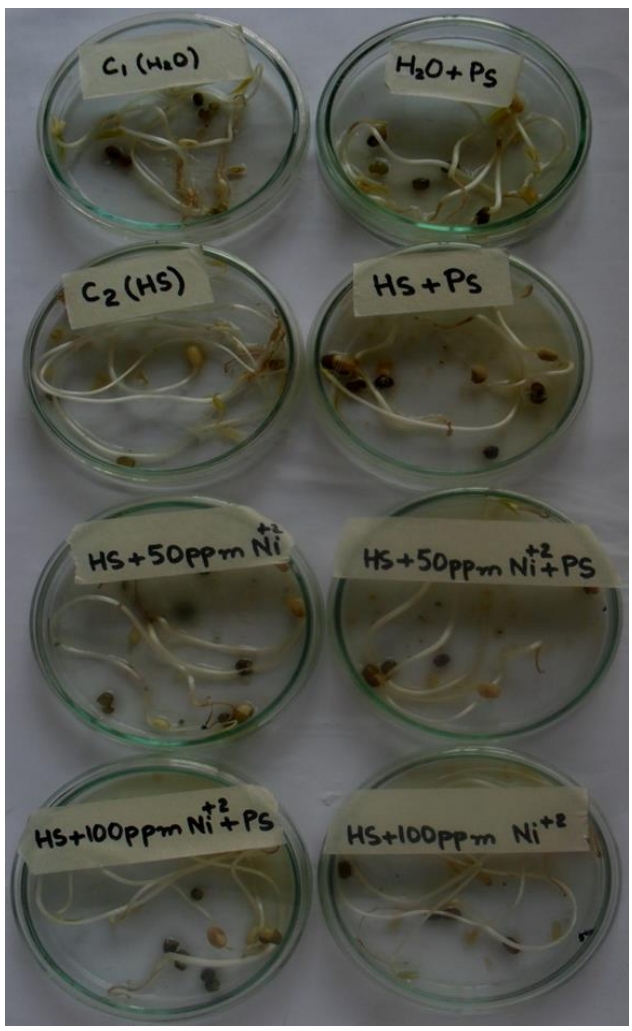

Fig. 1. Germination of *Vigna radiata* in different concentrations of $NiSO_4$ and *Pseudomonas aurginosa*.

Table 1. Effect of $NiSO_4$ and *Pseudomonas aurginosa* on seed germination.

Plates	Average root size (cm)	Seed germination	*Pseudomonas* pigmentation	Root hairs	Weight of roots (gm)
C1 (H_2O)	4.4	4/5	Nil	+	0.15
C2 (HS)	2.02	5/5	Nil	+	0.138
H_2O + *Pseudomonas*	3.32	5/5	+ (Slight)	+	0.098
HS + *Pseudomonas*	2.07	4/5	+ (Slight)	-	0.085
HS + 50ppm $NiSO_4$	3.9	3/5	-	+	0.084
HS + 50ppm $NiSO_4$ + *Pseudomonas*	1.63	3/5	-	-	0.055
HS +100ppm$NiSO_4$	6.1	3/5	-	-	0.089
HS+100ppm$NiSO_4$ + *Pseudomonas*	1.66	5/5	-	-	0.085

Effect of Ni and microbes on Enzymes activity: The enzymes activities in the roots of seedlings were monitored in the presence of Ni and microbial strain of *P. aeruginosa*. The results were presented in the Fig. 2. The variations in ALP activity of root was observed in all incubations such as aqueous medium (C1), nutrient medium(C1), an aqueous medium with microbial strain, *P. aeruginosa* (H_2O + PS), nutrient medium with *P. aeruginosa* (HS + PS), metal concentration (50ppm) with nutrient medium (HS), metal concentration (50ppm) with nutrient medium (HS) and microbial strain, metal concentration (100ppm) with nutrient medium and metal concentration (100ppm) with nutrient medium and microbial strain. It was 2.76 U/I in control whereas there is a significant increase in the ALP activity in the Hoagland nutrient medium (11.04 U/I) in 4 d old seedlings root and the lowest activity of ALP in 100 ppm of Ni (1.656 U/I). It suggested that decrease in ALP activity at a higher concentration of Ni indicates the disruption in the membrane system. The higher activity of ALP was noticed in 50ppm Ni (5.52 U/I) which was reduced to the value of control (2.76 U/I) at 50ppm $NiSO_4$ with *P. aeruginosa* when compared to other incubated experiments. The values of activity of ALP at 100ppm $NiSO_4$ with *P. aeruginosa* was enhanced when compared with control and all other inoculations. It was established that function of enzymes is also affected by environmental factors except than those of temperature and pH. The function of alkaline phosphatase (ALP) is hydrolyzed phosphate bound to cell surface phosphoproteins which modify their biological activities. Agoreyo, (2010) reported that the high activity at 100ppm might indicate hydrolysis of the organic phosphate in the roots. In plants, alkaline phosphatase activity in seeds intensified significantly throughout germination, which showed a probable part in phosphate metabolism and mobilization (Duff *et al*., 1994). *P. aeruginosa* helps in activation of ALP in roots, which indicated active phosphate metabolism. Present results at high concentration of metal are similar to that of Raimi *et al.,* 2011 in which the activity of ALP was inhibited at a high concentration of metal while a robust activation of ALP activity at 50ppm was similar to that of previous researchers like Moss *et al.,* (1985) & Zaidi *et al*. (2006). The value of ALP at 50 ppm of Ni and microbial strain reaches to the reasonable level indicated that siderophore of *P. aurigenosa* form complex with the metal due to which metal was not available for bioaccumulation and activity of enzymes becomes normal, but the complex which formed with metal was not appropriate for plant growth (Table 1). It suggested that although microbial strain successfully controls the mobility of Ni metal into the plant but siderophore complexation proved to be toxic.

Ni^{+2} act as a micronutrient at low concentration, but at high concentration, it has lethal effects on plant growth. LDH is an enzyme which transfers the hydride from one molecule to other and is a marker of joint injuries and disease. Commonly elevated level of this enzyme showed acute tissue damage. Activation and inhibition of LDH activity (Fig. 3) in the current investigation showed that LDH is sensitive towards environmental biotic and abiotic conditions. Values of LDH were increased in 50ppm $NiSO_4$ as compared by 50ppm $NiSO_4$ with *P. aeruginosa*. At 100ppm $NiSO_4$ LDH values were decreases but increases in the presence *P. aeruginosa* in 100ppm $NiSO_4$. This rise in LDH showed that roots faced execution in oxygen shortage, prone to damage conditions in roots. It was related to the formation of Ni siderophore complex as *P. aeruginosa* generates pigment in which Ni^{+2} is trapped consequently root growth is affected. This might be possible that aggregates formed with the organism in roots, creating slight environmental stress in seed germination which appeared as reduced root growth (Table 1).

Aspartate aminotransferase is a central enzyme involved in carbon and nitrogen metabolism. AAT catalyzes the reversible transfer of the amino group from aspartate to α-ketoglutarate, yielding oxaloacetate and glutamate (Gerendas & Sattelmacher, 1997a). Figure 4 showed that AAT value decreased at all inoculation except slight increased at 50ppm Ni. Results indicated that at both concentrations of Ni with microbial life, Ni siderophore complex produced lethal effects on carbon and nitrogen metabolism due to which there was significantly decreased in length and weight of roots (Table 1). Alteration in the activity of the aminotransferases within root tissues might result not only from abiotic and biotic stresses but also due to the Ni siderophore complex due to which the lowest value of enzyme was reported at 100pppm of Ni with *P. aeruginosa*. Thus, it is possible that activity changes in response to the complex impact on growth that could disturb amino acid and carbon metabolism (Sempruch *et al*., 2012).

Results indicate that in 50ppm $NiSO_4$ and 100ppm $NiSO_4$, Ni^{+2} acted as a heavy metal, and it created an oxygen shortage rendering to reduced roots of a plant due to which ALT values increased (Hoffman *et al*., 1986). Alanine aminotransferase (ALT) in the presence of *P. aeruginosa* showed the highest activity in all inoculation as compared to control. In 50ppm $NiSO_4$ and 100ppm $NiSO_4$, the value of ALT increases but in the presence of *P. aeruginosa* in 50ppm $NiSO_4$ and 100ppm $NiSO_4$, the of ALT decreased (Fig. 5). The induction of ALT during oxygen shortage has been reported for several plant species including *Hordeum vulgare* (barley) (Good and Crosby, 1989; Good and Muench, 1992). The results suggested that the high level of constitutive resistance of root towards the biotic and abiotic stress may be connected with higher ALT activity and lower AAT activity (Sas-Nowosielska *et al*., 2008)

There are extensive intrinsic reorganizations of primary and secondary metabolism adapted by the plants due to their immobility from the hostile extrinsic environment including respiration, alteration in enzymes activity and activated antioxidant system associated with the majority of plant defense responses. Mostly enzymes function is affected by pH and temperature, but in stress conditions, other factors like the concentration of substrates, coenzymes, and cofactors and the presence of activators and inhibitors show a crucial role. Low – molecular inhibitors or activators like malate or glucose - 6- phosphate or siderophore which is small molecular weight compounds strongly influenced the activity of the enzymes (Mishra & Dubey, 2008). Similar results were reported in a current investigation where Ni stimulate the activity of enzymes at a low concentration while decreases at higher concentration. The siderophore of *P.aurigenosa* also plays a pivotal rule in inactivation and inhibition of enzymes activities. Also, it was reported in the literature that final metabolic products or energetically rich compounds such as ATP and GTP often serve as inhibitors of enzymes (Doubnerova *et al*., 2009).

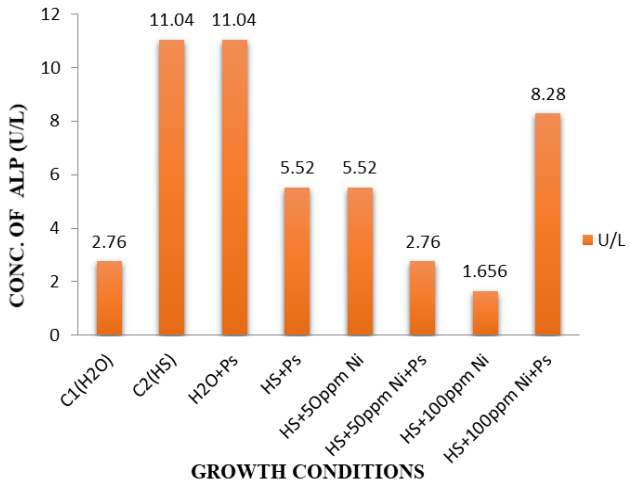

Fig. 2. Estimation of alkaline phosphatase (ALP) in the roots of *Vigna radiate.*

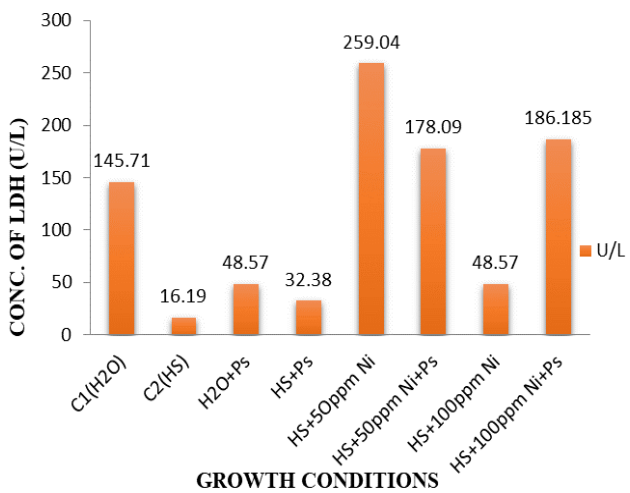

Fig. 3. Estimation of lactate dehydrogenase (LDH) in the roots of *Vigna radiate*.

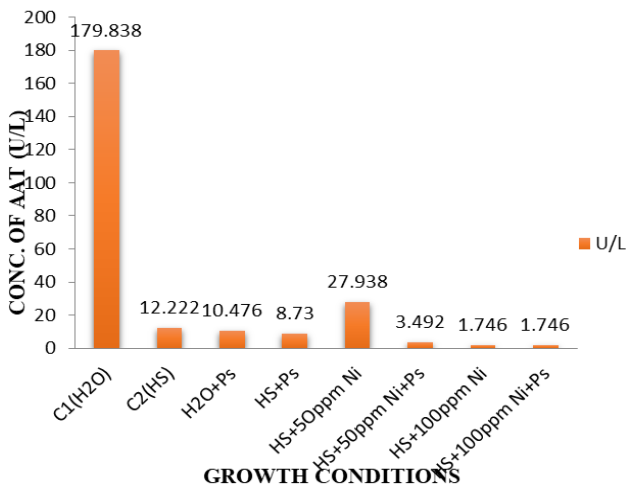

Fig. 4. Estimation of aspartate aminotransferase (AAT) in the roots of *Vigna radiate*.

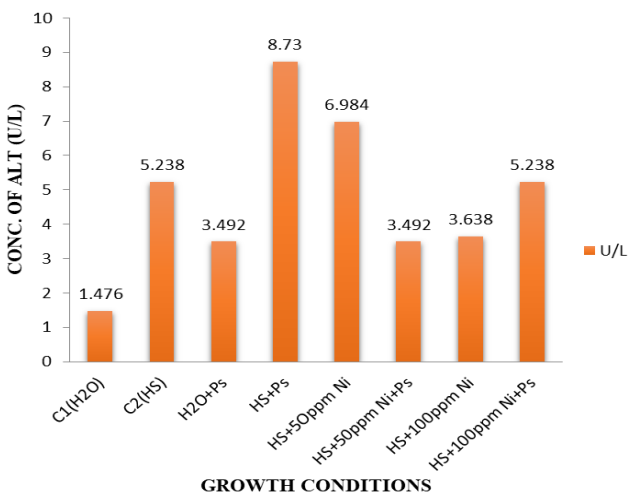

Fig. 5. Estimation of alanine aminotransferase (ALT) in the roots of *Vigna radiate*.

Conclusion

It was concluded that the activity of the enzyme is also altered by viable environmental conditions except than those of pH and temperature. The study showed that rhizosphere atmosphere played a crucial rule in early stages of development. Enzymes control the metabolic reactions; the variation in activity of enzymes in presence of Ni and microbial life displayed a lethal effect on roots hairs, length, and weight.

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