Lyallpur Khalsa College
Jalandhar-144001, Punjab

**Research & Reviews in Biotechnology & Biosciences**
**Homepage: www.biotechjournal.in**
**Volume: 5. Issue: 2. Year: 2018 PP: 59-69**

*Review Article*

# Heavy metal ($Pb(NO_3)_2$)and Salt stress (NaCl) Alter the shoot length and activity of Amylase enzyme in *Vigna radiate*

***Harman Kaur and Harsharan singh****

***Post Graduate Department of Biotechnology, Lyallpur Khalsa College Jalandhar, Punjab, India***

***DOI: https://doi.org/10.6084/m9.figshare.8977367.v1***

*Article History*

Received: 25/07/2018
Revised: 03/09/2018
Accepted: 28/09/2018

***Corresponding Author:***
E-Mail: harsharan78@gmail.com

**Abstract**

The study was undertaken to identify the responses of amylase enzyme activities and the morphological changes in *Vigna radiate* cultivars under Heavy metal ($Pb(NO_3)_2$)and Salt stress (NaCl) conditions. Plants were grown under three different concentration regimes of $Pb(NO_3)_2$ and NaCl respectively. The Choosen concentrations of $Pb(NO_3)_2$ were 50mM, 100mM and 150 mM and for NaCl were 0.5 M, 1M and 1.5 M. These stress treatments preferentially decreased the shoot length in a concentration dependent manner. The Amylase activities was forund to be decreased after the application of $Pb(NO_3)_2$ and NaCl. The pattern of alternation of amylase activity was common for both $Pb(NO_3)_2$ and NaCl treatment suggesting that similar biochemical pathways might be triggered by $Pb(NO_3)_2$ and NaCl.

## Introduction

Plants often counter unfavorable abiotic factors, causing abiotic stresses - that play a major role in determining productivity of crop yields (1). Because of their sessile nature, plants must combat these conditions some examples of abiotic stresses that a plant may face include decreased water availability, extreme temperatures (heating or freezing), decreased availability of soil nutrients and/or excess of toxic ions, excess of light and increased hardness of drying soil that hamper roots growth (2). The ability of plants to adapt and/or acclimate to different environments is directly or indirectly related with the plasticity and resilience of photosynthesis, in combination with other processes, determining plant growth and development, namely reproduction (3). Plant adaptation Involve a variety of responses to acclimatize to environmental stresses. During evolution, plants have developed sophisticated mechanisms to sense the subtle changes of growth conditions, and trigger signal transduction cascades, which in turn activate stress responsive genes and ultimately lead to changes at the physiological and biochemical levels.

**Heavy metals** ($Pb(NO_3)_2$) can be highly toxic when their concentrations are exceeded threshold value. Otherwise heavy metals at low doses are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition for most of the plants species. A common feature of heavy metal is their ability for production of toxic oxygen derivatives (4). Reactive oxygen species (ROS) are continuously produced at low level during normal metabolic processes (4). But in biological systems, increasing the

synthesis of ROS is one of the initial responses to different stress factors (5).

High salinity (NaCl) in the soil and/or irrigation water becomes a common problem, and NaCl stress is a major factor limiting crop production since it affects almost all plant functions (6). Although the physiological and whole-plant responses to NaCl stress have been studied, the mechanisms which confer NaCl tolerance to non-halophytic plants are still poorly understood. The over salinity of the soil is one of the main factors that limits the spread of plants in their natural habitats. The property of salinity tolerance is not a simple attribute, but it is an outcome of various features that depend on different physiological interactions, which are difficult to determine. The morphological appearance presented by the plant in response to salinity, may not be enough to determine its effect so it is important to recognize other physiological and biochemical factors, including toxic ions, osmotic potential, lack of elements and other physiological and chemical disorders, as well as the interactions between these various stresses (7, 8,9 and 10).

*Vigna radiate,* Commonly known as the **moong bean**, **green gram**, or **mung** is a plant species in from the legume family. The *V. radiata* is mainly cultivated in India, China, Korea, and Southeast Asia. *V. radiata* beans are a high source of nutrients like: manganese, potassium, magnesium, folate, copper, zinc and various B vitamins. They are also a very filling food, high in protein, resistant starch and dietary fiber. Because of their high nutrient density, mung beans are considered useful in defending against several chronic, age-related diseases, including heart disease, cancer, diabetes and obesity. Keeping the above fact in mind, the present study was conducted in *V. radiata* to analyze morphological and biochemical changes induced by $Pb(NO_3)_2$ and NaCl.

**Material and method;**

***Plant Material***

Seeds of *V. radiata* were obtained from the local market of Jalandhar. Before start of experimentation seeds were thoroughly washed with water and surface- sterilized using 0.1% (*w/v*) solution of HgCl2 solution. The result presented in this study was obtained from three independent experiments with three replicate each.

***Germination of Seeds and imposition of stress***

Surface sterilized seeds were germinated in petri plate by placing on moist filter paper. Six days old seedlings was used for stress treatment. For NaCl treatment nine petri plates were taken, three plates were treated with 0.5 M NaCl, other three were treated with 1 M NaCl and last three was treated with 1.5 M NaCl

For $Pb(NO_3)_2$ treatment nine petri plates were taken, three plates were treated with 50mM $Pb(NO_3)_2$ , other three were treated with 100mM $Pb(NO_3)_2$ and last three was treated with 3 150mM $Pb(NO_3)_2$.

For the entire period of experimentation, plants were kept at 25°C with 8hr light and dark cycle.

***Extraction of protein:***

Protein was extracted using Sodium Phosphate Buffer (pH 7.5).For extraction of protein 3days, 6days and 9days old seedlings weighing 1g was taken and then the protein was extracted from each tissue (shoot, endosperm) separately. The total 10ml of extraction buffer was used per gram of tissue. The homogenate was centrifuged at 10,000 rpm for 20 minutes at 4˚C. The

resultant supernatant was collected and stored at -20˚C for protein estimation.

***Extraction of enzymes for enzymes assay:***

Tissue sample (1g) was homogenized in 10ml of extraction buffer (0.067M phosphate buffer) per gram of tissue. The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C. Supernatant was collected carefully without disturbing the pellet and either immediately used for assay or kept at -20°C.

***Enzymes assay:***

The α -amylase activity is measured using a colorimetric method with 3,5-dinitrosalicylic acid (DNS) reagent. In this method, starch by α – amylase is converted into maltose. Maltose released from starch is measured by the reduction of 3,5-dinitrosalicylic acid. Starch + H2O α-Amylase Maltose (reducing agent) 9 Maltose reduces the pale yellow coloured alkaline 3, 5-Dinitro salicylic acid (DNS) to the orange- red colored. The intensity of the color is proportional to the concentration of maltose present in the sample. This intensity change in color is measured using a spectrophotometer as the absorbance at 540nm wavelength. Wave length is set to 540 nm because it is the region where orange-red color absorbs. This procedure applies to all products that have a specification for α- amylase

The assay mixture contained 200µl of guaiacol, 250µl Tris-HCL, 50µl enzyme extract and 500µl of $H_2O_2$. The reaction was started with the addition of $H_2O_2$ and the absorbance at 470nm was recorded up to 5 min. The difference in absorbance was divided by the molar extinction coefficient 26.6/mM/cm and specific activity of enzyme is expressed as µmol of $H_2O_2$ oxidized/min/mg protein.

**Results and Discussion**

**Effect of lead nitrate on shoot length and amylase activity:**

Application of $Pb(NO_3)_2$ shows on germinating seedling resulted in decrease in shoot length in a concentration dependent manner during the entire period of experimentation**(Fig:1&2 )**. This decrease was 42.86% at 50mM, 48.57% at 100mM and 65.71% at 150mM during third day of germination. Similar trend was exhibited by 6 days and 9days treated seedlings of *V. radiata,* where the shoot length was decreased 77.77% and 80.74% respectively at conc. 150mM of $Pb(NO_3)_2$. It was also observed that with increasing exposure time and concentration decrease in shoot length also exaggerated.

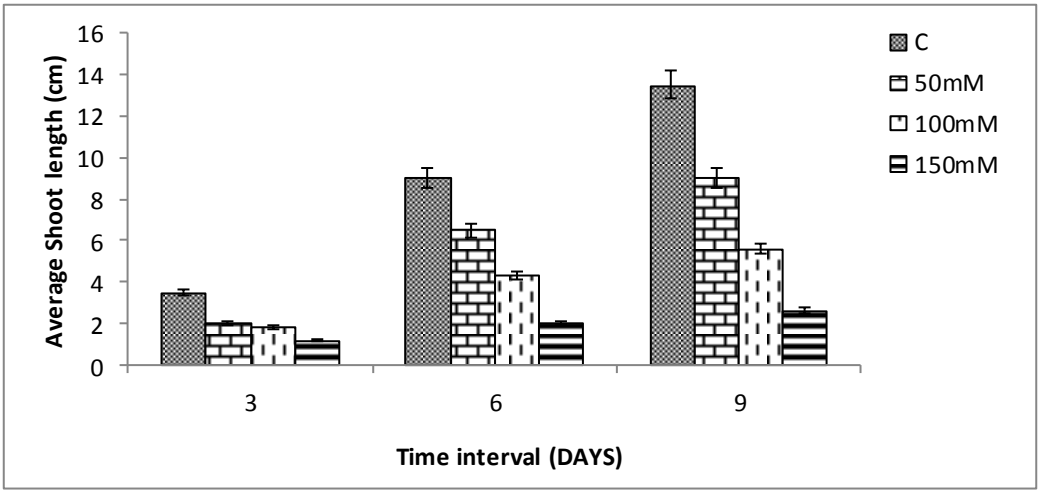


**Fig 1: Average shoot length of *V. radiata* seedlings treated with lead nitrate.. The seedlings were treated with different concentrations of $Pb(NO_3)_2$ i.e., 50mM, 100mM and 150mM. The shoot length was measured at 3, 6 and 9 days respectively. (Errors bars indicate mean ± SD (n=3) with P value ≤ 0.005).**

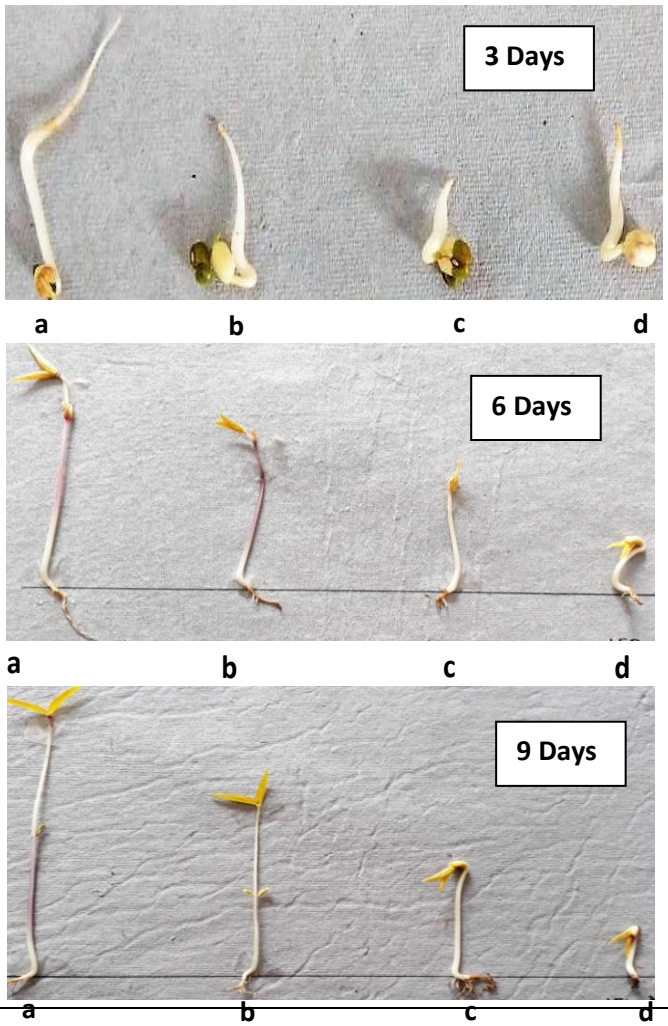

**Fig 2: Effect of lead nitrate on shoot length in seedlings of *V. radiata*. The seedlings were treated with different concentrations of $Pb(NO_3)_2$ i.e., 50mM, 100mM and 150mM herein represented as b, c and d in each panel. The respective control is represented by "a" in all the panels. The Different panels represent different time points as mentioned on each panel.**

The exact mechanism of this type of behaviour in shoot growth is not known, but it might be due in part to a quicker deposition of lead in the roots and shoots (11). Inhibition of seed germination by lead had been documented in *Spartiana alterniflora* (12), *Pinus helipensis* (13). Schulz – Baldes and Lewin (14) reported that Pb reduced cell division and uptake of crucial elements as it accumulate on the cell membrane and hence inhibited seedling growth. In the present study, application of different concentrations of $Pb(NO_3)_2$ in *V. radiata* seedlings adversely decreased their growth pattern (shoot length) as compared with normal growth conditions. Our studies were observed to be similar and consistent with previous studies reported in rice (*Oryza sativa. L)* by Sara *et al.,*(15), *Brassica campestris* by (16). Early seedling growth was also inhibited by lead in soya bean (17), rice (18), maize (19), barley (20) and certain legumes (21). The primary cause of cell growth inhibition arise from a lead induced simulation of indole- 3 acetic acid (IAA) oxidation.

Exposure of lead nitrate resulted in decrease of amylase activity. The value of this decrease at 3 days was 7.40%, 66.67% and 85.19% at conc. 50mM, 100mM and 150mM of $Pb(NO_3)_2$ as compared to untreated seedlings **(Table:1, Fig:3).**. The same trend was observed at 6 and 9 days of treatment. It was observed that higher the conc. of $Pb(NO_3)_2$ lower the activity of amylase. At highest conc. of $Pb(NO_3)_2$ 150mM the activity of amylase was decreased by 48.48% and 30.43% in 6 and 9 days seedlings respectively as compared to respective control.

**Table 1: Amylase activity in seedlings of *V.radiata* treated with lead nitrate. The seedlings were treated with different conc. of $Pb(NO_3)_2$ i.e., 50mM, 100mM and 150mM. Enzyme activity in unit (µmol/min/ml) determined by DNS assay at every 3 days interval.**

| TREATMENT | AMYLASE ACTIVITY (units) | | |
|---|---|---|---|
| | 3 DAYS | 6 DAYS | 9 DAYS |
| Control | 67.66±2.2 | 330.82±2.3 | 172.93±2.8 |
| 50mM | 62.65±2.5 | 273.18±2.6 | 137.84±2.8 |
| 100mM | 22.55±2.3 | 220.43±2.7 | 165.41±2.5 |
| 150mM | 10.02±2.3 | 170.42±2.7 | 120.30±2.8 |

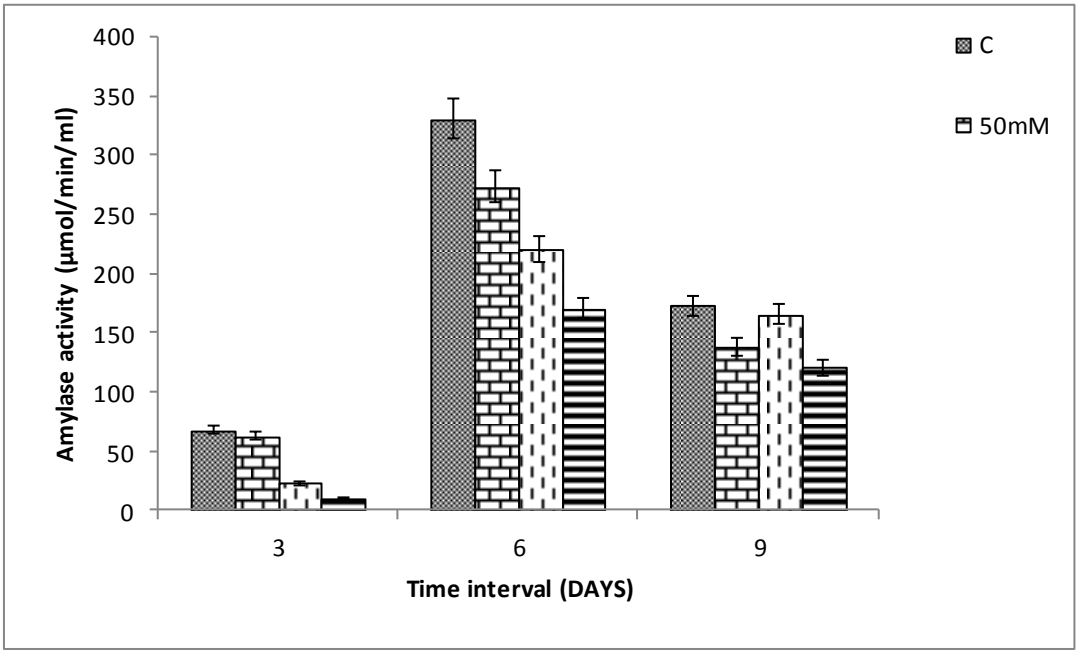


**Fig.3: Effect of lead nitrate on Amylase activity in seedlings of *V.radiata*. The seedlings were treated with different conc. of $Pb(NO_3)_2$ i.e., 50mM, 100mM and 150mM. Enzyme activity in unit (µmol/min/ml) determined by DNS assay at 3,6 and 9 days respectively. (Errors bars indicate mean ± SD (n=3) with P value ≤ 0.005)**

Enzymes associated with starch degradation, sucrose synthesis and utilization of glucose play a key role in seedling growth which further determine the final yield of the crop. **Amylase** (EC 3.2.1.1) catalyses hydrolytic cleavage of internal α-1,4-glucan bonds of starch releasing fragments that can be further broken down by β-amylase (EC 3.2.1.2) into maltose (22,23). In contrast to the antioxidant enzymes the heavy metals treatments caused significant reduction in the hydrolysis enzymes activity (amylase). This type of inhibitions might be correlated with impaired hydrolysis of storage products, which, in turn, leads to starvation of the germinating embryo (24). In the present study we observed that, Amylase enzyme activity in the control germinated grains increased with time during the study as it reached the highest value at 6 days seedlings, whereas in case of the heavy metals treatment this activity was highly inhibited than the control and decreased with increasing the age of the germinating grains the lowest activity of amylase was in case of the highest concentration of $Pb(NO_3)_2$. Reduced starch mobilization from reserve tissues was also observed in germinating *Phaseolus vulgaris L.* seedlings under cadmium (25) and copper toxicities (25 ) which was attributed to reduced amylase activities. Earlier, Karmous I, Khadija J *et al.*, reported the Pb decreased activity of proteases and α-amylases in rice endosperm (26).

**Effect of Sodium Chloride on shoot length and amylase activity:**

Application of NaCl also decreased shoot length of *V. radiate* **(Fig:4&5).** Decrease in the shoot length was increased with the increase in the concentration of NaCl as compared to respective controls except at 3 days of treatment. The effect of Exposure to 0.5M conc. of NaCl (3days) decreased the

shoot length by 40.91% as compared to control and then a sudden increase was observed 13.63% at 1.0M conc. which further increased 36.63% at the maximum conc. of NaCl i.e., at 1.5M.

In contrary to this, the shoot length decreased gradually for 6 and 9 days treated seedlings of *V. radiata*. The shortest shoot length was recorded at the highest conc. 1.5M of NaCl 42.23% for 6 days and 43.33% for 9 days.

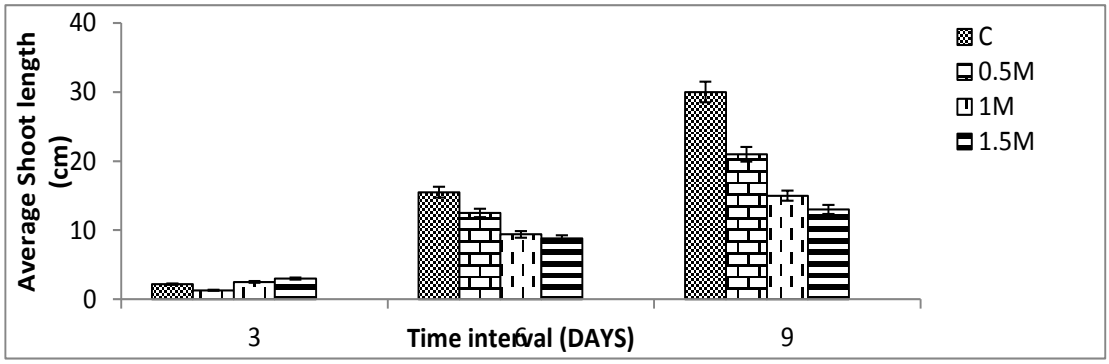


**Fig.4: Average shoot length of *V. radiata* seedlings treated with Sodium chloride. The seedlings were treated with different concentration of NaCl i.e., 0.5M, 1M and 1.5M. The shoot length was measured at 3, 6 and 9 days respectively.(Errors bars indicate mean ± SD (n=3) with P value ≤ 0.005).**

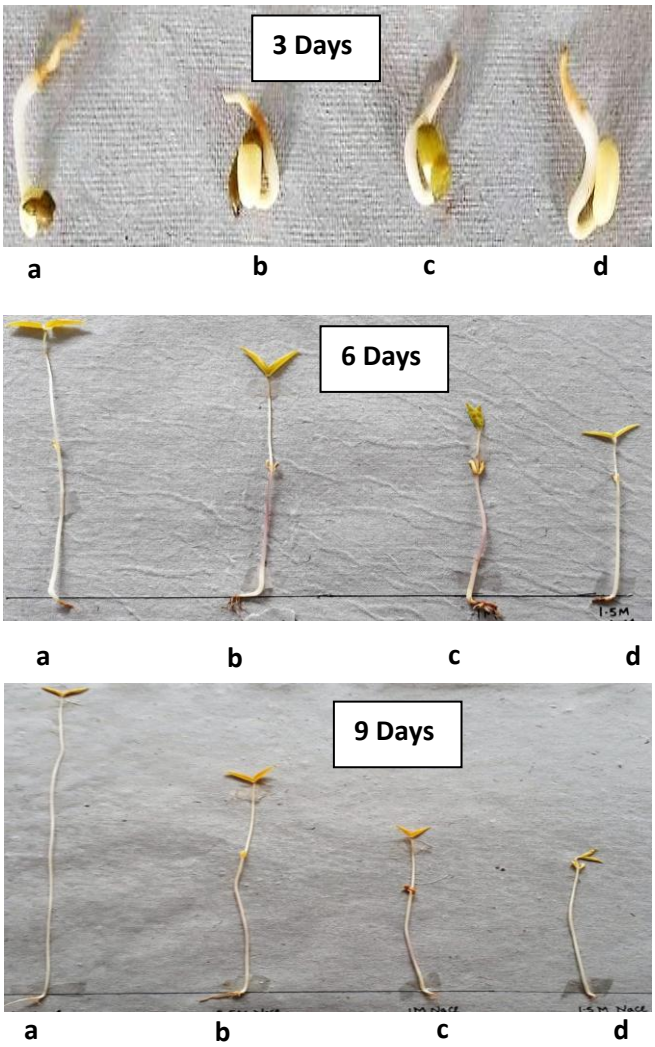

**Fig.5: Effect of Sodium chloride on shoot length in seedlings of *V. radiata.* The seedlings were treated with different concentrations of NaCl i.e., 0.5M, 1M and 1.5M herein represented as b, c and d in each panel. The respective control is represented by "a" in all the panels. The Different panels represent different time points as mentioned on each panel.**

Shoot length of *V. radiata* seedlings was greatly inhibited by NaCl. The degree of inhibition increased as the NaCl concentration level increased. In the present study, application of different concentration of NaCl in *V. radiata* seedlings adversely decreased shoot length as compared to respective control. These observations are similar and consistent with previous studies reported in *Zea mays* by Hamada (27). Our results and work by different Scientist around the globe suggested that there might be a connection between the decrease in plant length and increase in the concentration of NaCl (28, 29, 30, 31, 32, 33 and 34).

Application of NaCl decreased the activity of amylase except at 3 days seedlings of *V.radiata* **(Table:2, Fig:6).** Therefore It may be inferred that intial exposure of NaCl might elicit the activity of amylase the observed values at 3 days was increased by 1.24%, 2.40% and 4.30% at conc. 0.5M, 1.0M and 1.5M of NaCl as compared to respective control. On contary, the reverse trend was observed in 6 and 9 days old seedlings. It was observed that at 6 days of treatment activity of amylase decreased to 5.25%, 7.81% and 13.85% at conc. 0.5M, 1.0M and 1.5M as compared to untreated seedlings. At 9 days of treatment maximum decrease in activity of amylase was observed at conc. 1.5M of NaCl as compared to respective control.

**Table. 2: Effect of sodium chloride on Amylase activity in seedlings of *V. radiata.* The seedlings were treated with different concentrations of NaCl i.e., 0.5M, 1.0M and 1.5M. Enzyme activity in unit (µmol/min/ml) determined by DNS assay at every 3 days interval.**

| TREATMENT | AMYLASE ACTIVITY (units) | | |
|---|---|---|---|
| | 3 DAYS | 6 DAYS | 9 DAYS |
| Control | 262.29±2.3 | 256.02±2.3 | 250.25±2.6 |
| 0.5M | 265.56±2.5 | 242.56±2.4 | 240.21±2.8 |
| 1.0M | 268.59±2.6 | 236.00±2.5 | 229.16±2.9 |
| 1.5M | 273.57±2.8 | 220.56±2.5 | 215.60±2.7 |

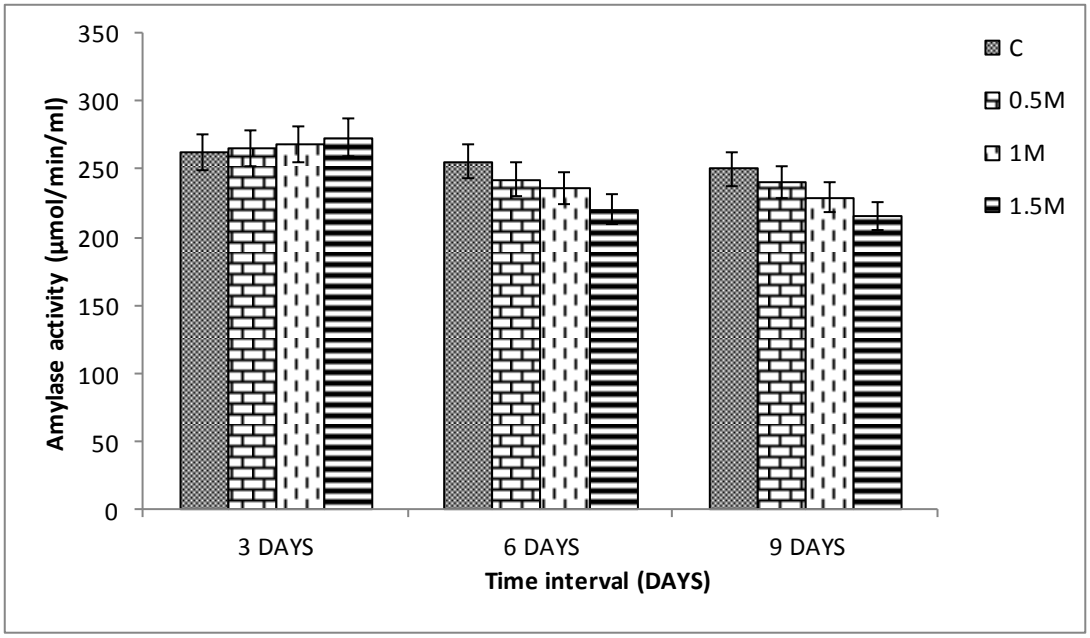


**Fig.6: Effect of sodium chloride on Amylase activity in seedlings of *V. radiata*. The seedlings were treated with different concentrations of NaCl i.e., 0.5M, 1.0M and 1.5M. Enzyme activity in unit (µmol/min/ml) determined by DNS assay at 3, 6 and 9 days respectively. (Errors bars indicate mean ± SD (n=3) with P value ≤ 0.005).**

In the present study, data suggested slight increase in amylase activity at 3 days after the application of NaCl, However after increase in duration of treatment with NaCl the amylase activity decreases as compared to untreated seedlings. Ahemad Adda *et al.* (2014) observed increase in amylase activity in two genotypes of *Phaseolus vulgaris* L. seedlings when exposed to salt stress, similar to present results obtained at 3 days interval(35). But with the increase in duration of germination the amylase activity decreases and sugar content increases. Similar results were found in NaCl stressed rice seeds by Kim SK *et al.,* (36). The higher concentration of NaCl induced significant increase in total carbohydrate content of black gram (37). The similar results were observed and reported that the salinity can affect the carbohydrate metabolism and overall production of carbohydrate (38, 39). The significant increase in carbohydrate fraction and decrease in amylase activity in Zea may was observed (Hassaein 40). Salt and water deficit stresses caused a significant increase in soluble carbohydrate content of barley leaves. With increasing salt doses, the rate of increase in soluble carbohydrate content was increased, indicating a role of soluble carbohydrate in the osmotic adjustment. The accumulation of sugars in plants under stress conditions might be involved in the osmotic adjustment was reported (41). A decrease in starch content and an increase in both reducing and nonreducing sugars is due to increase in amylase activity has been reported in leaves of *Bruguiera parviflora* (42).

It might be concluded from above study that heavy metal and salt stress exerted same type of effect on shoot length and amylase activity. It might be possible that these abiotic factor trigger the similar pathway of plant defense. To confirm these assumption further study is needed.

**ACKNOWLDGEMNTS**

Source of Support: Authors want to thank president governing council for this project work
Conflict of interest: No

## REFERENCES

1. Boyer JS, 1982. Plant Productivity and Environment. *Science*. 218 (4571), 443-448.
2. Versulues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu JK, 2006. Methods and Concepts in Quantifying Resistance to Drought, Salt and Freezing, Abiotic Stresses that Affect Plant Water Status. *The Plant Journal*. 45(4), 523-539.
3. Chaves MM, Costa JM, Saibo NJM, 2011. Recent Advances in Photosynthesis Under Drought and Salinity. In: Turkan I. (ed.) *Advances in Botanical Research*, 57, 50-83.
4. Arora A., Sairam R.K., Sriuastava G.C., 2002. Oxidative stress and antioxidative system in plants. Current Science, 82: 1227–1238.
5. Singh S, Sinha S, 2005 Accumulation of metals and its effects in Brassica juncea (L.) Czern (cv. Rohini) grown on various amendments of tannery waste. Ecotoxicol Environ Saf 62: 118-127.
6. Greenway H, Munns R, 1980. Mechanism of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology*. 31, 149-190.
7. Munns R, 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant Cell Environ*. 16, 15–24.
8. Munns R, 2002. Comparative physiology of salt and water stress. *Plant Cell Environ*. 25, 239–250.
9. Neumann P, 1997. Salinity resistance and plant growth revisited. *Plant Cell Environ*. 20, 1193–1198.
10. Hasegewa PM, Bressan RA, Zhu JK, Bohnert HJ, 2000. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Mol. Biol*. 51, 463–499.
11. Al-Helal A, 1995. Effect of Cadmium and Mercury on Seed Germination and Early Seedling Growth of Rice and Alfalfa. *J. Univ. Kuwait (Sci)*. 22, 76-83.
12. Morzck E Jr, Funicclli NA, 1982. Effect of lead and on germination of *Spartina alterniflora* Losiel seeds at various salinities. *Environ Exp Bot*, 23–32.
13. Nakos G, 1979. Lead pollution: fate of lead in soil and its effects on *Pinus haplenis.Plant Soil*.50, 159–161.
14. Schulz-Baldes, M. and Lewin, R. 1976. Lead Uptake in Two Marine Phytoplankton Organism. *Biol. Bull*. 150, 118-127.
15. Sara A, Jabeen M, Imran QM, Ullah F, Mehmood Z, Jahngir M and Jamil M, 2015. Effects of Lead Toxicity on Plant Growth and Biochemical Attributes of Different Rice (*Oryza Sativa* L.) Varieties.*Journal of Bio-Molecular Sciences (JBMS)*. 3(1), 44-55.
16. Singh A and Prasad SM (2011) Reduction of heavy metal load in food chain: technology assessment. *Rev Environ Sci Biotechnol*.10, 199-214.
17. Huang CV, Bazzaz FA, Venderhoef LN, 1974. The inhibition of soya bean metabolism by cadmium and lead. *Plant Physiol*.34, 122–124.
18. Mukherji S and Maitra P, 1976. Toxic effects of lead on growth and metabolism of germinating rice (*Oryza sativa L.*) root tip cells. *Ind. J. Exp. Biol*. 14, 519–521.
19. Miller JE, Hassete JJ, Koppe DE, 1975. Interaction of lead and cadmium of electron energy transfer reaction in corn mitochondria. *Physiol Plant*. 28, 166–171.
20. Stiborova M, Pitrichova M, Brezinova A, 1987. Effect of heavy metal ions in growth and biochemical characteristic of photosynthesis of barley and maize seedlings. *Biol Plant*.29, 453–467.
21. Sudhakar C, Symalabai L, Veeranjaveyuler K, 1992. Lead tolerance of certain legume species grown on lead or tailing. *Agri Eco Environ*. 41, 253–261.
22. Araga˜o VPM, Navarro BV, Passamani LZ, Macedo AF, Floh EIS, Silveira V and Santa-Catarina C, 2015. Free amino acids, polyamines, soluble sugars and proteins during seed germination and early seedling growth of *Cedrelafissilis Vellozo* (Meliaceae), an endangered hardwood species from the Atlantic Forest in Brazil. Theor. Exp. *Plant Physiol*. 27, 157–169.

23. Yang J, Kang S, Park S, Yoon H, Kim M, Heu S and Ryu S, 2004. Characterization of a novel amylolytic enzyme encoded by a gene from a soil-derived metagenomic library. *Appl. Environ. Microbiol*.70, 7229-7235.

24. Kuriakose SV and Prasad MNV, 2008. Cadmium stress affects seed germination and seedling growth in *Sorghum bicolor (L.)* Moench by changing the activities of hydrolyzing enzymes. *Plant Growth Regul*. 54, 143-156.

25. Sfaxi-Bousbih A, Chaoui A and El Ferjani E, 2010. Cadmium impairs mineral and carbohydrate mobilization during germination of bean seeds. *Ecotoxicol. Environ. Safety*. 73, 1123–1129.

26. Karmous I, Khadija J, El Ferjani, E and Chaoui A, 2014. Responses of Proteolytic Enzymes in Embryonic Axes of Germinating Bean Seeds under Copper Stress. *Biol Trace Elem Res*.160, 108–115.

27. Hamada AM, 1995. Alleviation of the adverse effects of NaCl on germination, seedling, growth and metabolic activities of maize plants by calcium salts. *Bull. Fac. Sci. Assiut Univ*.24, 211–220.

28. Beltagi MS, Ismail MA, Mohamed FH, 2006. Induced salt tolerance in Common Bean (*Phaseolas vulgaris* L.) by Gamma Irradation. *Pak. J. Biol. Sci*. 9(6), 1143-1148.

29. Mustard J, Renault S, 2006. Response of red-osier dogwood (Cornussericea) seedling to NaCl during the onset of bud break. *Can. J. Bot*. 84 (5), 844–851.

30. Gama PBS, Inanaga S, Tanaka K, Nakazawa R, 2007. Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. *Afr. J. Biotechnol*. 6 (2), 79–88.

31. Jamil M, Rehman S, Rha ES, 2007. Salinity effect on plant growth, ps11 photochemistry and chlorophyll content in sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleraceacapitata* L.). *Pak. J. Bot*.39(3), 753–760.

32. Houimli SIM, Denden M, Elhadj SB, 2008. Induction of salt tolerance in pepper by 24-epibrassinolide. *Eurasia J. Biol. Sci*. 2, 83–90.

33. Rui L, Wei S, Mu-xiang C, Cheng-jun J, Min W, Bo-ping Y, 2009. Leaf anatomical changes of Burguieragymnorrhiza seedlings under salt stress. *J. Trop. Subtrop. Bot*. 17(2), 169–175.

34. Memon SA, Hou X, Wang LJ, 2010. Morphological analysis of salt stress response of pak Choi. EJEAFChe 9 (1), 248–254.

35. Ahmed Adda, Zineb Regagba, Ahmed Latigui and Othmane Merah, 2014. Effect of Salt Stress on α-amylase Activity, Sugars Mobilization and Osmotic Potential of *Phaseolus vulgaris* L. Seeds Var. 'Cocorose' and 'Djadida' During Germination. *Journal of Biological Sciences, 14: 370-375.*

36. Kim S. K., Son T. K, et.al., 2006 Influences of gibberellin and auxin on endogenous plant hormone and starch mobilization during rice seed germination under salt stress. *Journal of Environmental Biology* 27: 181-186 .

37. Dheeba B, Selvakumar S, Kannan M, and Kannan K.,2015.Effect of Gibberellic Acid on Black Gram (Vigna mungo) Irrigated with Different Levels of Saline Water. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 6(6):709-720 ·

38. Boubaker M, 1996. Salt tolerance of durum wheat cultivar during germination and early Seedling growth. *Meditteranean Agriculture*. 126, 32 -39

39. Sultana N, Ikeda T, Itoh R, 2000. Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. *Environ. Exp. Bot*.42(3), 211–220.

40. Hassanein RA, Bassony FM, Barakat M, Khalil RR, 2009. Physiological effects of nicotinamide and ascorbic acid on Zea mays plant grown under salinity stress. 1 - Changes in growth, some relevant metabolic activities and oxidative defense systems. *Research Journal of Agricultural and Biological Sciences*.5(1), 72 – 81.

41. Perez- Lo pez U, Robredo A, Lacuesta M, Munoz-Rueda A, Mena - Petite A, 2010. Atmospheric CO2 concentration influences the contributions of osmolytes accumulation and cell wall elasticity to salt tolerance in barley cultivars. *Journal of Plant Physiology*. 167, 15-22.

42. Parida AK and Das AB, 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol Environ Saf* . 60, 324-349.