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## Potassium Chloride (KCl) Modulate biochemical Characteristics of Rice (*Oryza sativa*) under Salt (NaCl) Stress

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### Abstract

Salinity is a main factor of abiotic stresses to reduced crop production throughout the world. Current study investigates the effect of potassium chloride on biochemical parameter of two rice varieties; Basmati 385 (B-385) and SB (SB) grown under different concentration of sodium chloride (0, 50, 100, and 150mM). Salinity has negatively effect on biochemical characteristics of rice. In addition, photosynthetic pigment (Chl a, Chl b and carotenoids) lowered at different concentration of salt NaCl but significant increase was occurred at increasing concentration of KCl upto 100mM in both varieties. In addition, too, Proline accumulation increased as salinity increased but decreased as the concentration of KCl increased upto 100mM in both varieties. Salinity increased sodium ion concentration followed by decreased in Ca<sup>2+</sup> and K<sup>+</sup> as in KCl concentration the sodium ions decreased in shoot and root of both varieties. Furthermore, a possible decrease was observed in nitrogen and protein by increasing salinity level as compared to control. Unlikely, protein and nitrogen were increased with KCl concentration. Similarly, micro element (Cu, Fe and Mn) in roots and shoots of both varieties were decreased as level of NaCl increased though increased as the concentration of KCl increased upto 100mM. While zinc concentration increased as concentration of NaCl increased but decreased as KCl concentrations increased. The cadmium, lead and nickle were reduced as salinity increased but increased at 100mM of KCl.

**Keywords :** A biotic; Potassium Chloride; Sodium Chloride; Salinity; Stresses

### Introduction

Rice (*Oryza sativa* L.) is among the main staple food crop being used worldwide including Asian countries. Among abiotic stresses, salinity is one of the main factors in Asia, particularly in Pakistan that decline both productivity and quality of rice [1,2]. Soil Salinity is a major problem that effect crop productivity in both dry and semi-dry area. There are various types of salts in soils that turn down the productivity of crops, but among them NaCl is the most important one because it's involved in disruption of plants and form unnecessary complex with other ions present in soil or plant [3]. The osmotic effect, ionic toxicity and ionic imbalance in plant is due to the effect of salt [4,5]. The prevention of plant

from the absorption of toxic ions i.e. Na<sup>+</sup> and Cl<sup>-</sup> occur by certain mechanism- together with salt elimination and salt diffusion from leaves to roots [6,7]. It has been predictable that more than half of the yield possible of major crops is generally lost due to unfavorable growth circumstance such as drought or high salinity [3].

Potassium is one of important macronutrient, which playing a vital role in growth and development of plant in the whole life cycle [1]. Potassium is a major component of protein synthesis, enzyme activation, the pH gradient, phloem uploading, turgor pressure regulation, stomatal functioning and the photosynthetic system [8], as well as

controlling osmotic pressure and ionic balance during the disclosure of plants to salinity conditions [9]. Exogenous potassium applications, such as potassium chloride (KCl), monopotassium phosphate (KH<sub>2</sub>PO<sub>4</sub>), potassium nitrate (KNO<sub>3</sub>), as well as potassium sulfate (K<sub>2</sub>SO<sub>4</sub>) which act as a fertilizers, are well-known for aiding nutrient uptake, water use efficiency, photosynthesis and growth performance in order to reduce salt stress [10-12]. The application of exogenous potassium fertilizers, which alleviates salt damage by enhancing K<sup>+</sup> uptake and reducing Na<sup>+</sup> uptake, leading to a decrease in the sodium Na<sup>+</sup>/K<sup>+</sup> ratio, has been studies in many crop plants, i.e. pepper [13], sunflowers [11], ryegrass [14], cucumbers [15], strawberries [12] as well as rice [16].

## Materials and Methods

### Rice Seed collection and Processing

Rice seeds (*Oryza sativa* L.) of two varieties B-385 and SB were collected from National Agricultural Research Center (NARC) Islamabad, Pakistan. Seeds were surface sterilized through sodium hypochlorite (NaOCl) (3.5% (v/v)) for 3 or 4 minutes. Then seeds were rinsed through distilled water.

### Hydroponic Cultrig

After ten days of germination in controlled condition, the seedling was transferred to plastic pots containing 3 Liters of Hoagland solutions in green house [17]. After 30 days harvested plant sample were oven dry for biochemical analysis such as Elemental analysis (Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>++</sup>) were analyzed by flame photometer by following [18] method using 100ppm standard solution of Na, K, Ca. Micro element (Cu, Fe, Zn, Mn) and heavy metal (Ni, Cd, Pb) were

determined by atomic adsorption spectrophotometer (Perkin ELMER, 2380) with air acetylene flame via hollow cathode lamps against the standard. Photosynthetic pigment determination occurs by [19] method. Determined of Proline occur by [20] method with a minor amendment. Total nitrogen and protein content determination occur through micro-Kjeldahl techniques by following [21] method with little bit modifications.

### Data analysis

Statistical analyses of data occur by using Ms- Excel (statistic 9.1 versions) Statistic software. Each experiment has a replicate and its means value were compare with SD.

## Results

Potassium Chloride (KCl) modulate biochemical characteristics in Salt (NaCl) Stressed Rice (*Oryza sativa*) Varieties.

### Enricriment of Photosynthetic Pigments

The Chlorophylls and carotenoid contents of both varieties: B-385 and SB showed negative correlation with increasing NaCl concentration. In contrast with increasing NaCl+KCl concentration it showed positive correlation up to certain limit. In addition, the chlorophyll a, b and carotenoid contents at 100mM of NaCl declined by 0.76 mg g<sup>-1</sup>, 0.39 mg g<sup>-1</sup> and 12.40 mg g<sup>-1</sup>, respectively, over the control. At control, the overall performance of both varieties was good and chlorophyll a, b and carotenoid contents were 0.98 mg g<sup>-1</sup>, 0.62 mg g<sup>-1</sup> and 19.20 mg g<sup>-1</sup>, respectively, which reduced to 0.76 mg g<sup>-1</sup>, 0.39 mg g<sup>-1</sup> and 12.40 mg g<sup>-1</sup> at 100mM of NaCl. In addition, too, the chlorophyll a, b and carotenoid contents at 100mM of NaCl+KCl in B-385

Table 1: Coorelation of KCl and NaCl on photosynthetic pigment of rice varieties under Salinity (NaCl) condition.

Varieties	Variables	Control	50mM (NaCl)	100mM (NaCl)	150mM (NaCl)	50mM (NaCl +KCl)	100mM (NaCl +KCl)	150mM (NaCl + KCl)
	Chlorophyll (a)	0.98(±0.01)	0.87(±0.06)	0.76(±0.1)	0.64(±0.13)	0.68 (±0.15)	0.83(±0.15)	0.76(±0.13)
	Chlorophyll (b)	0.62(±0.01)	0.45(±0.02)	0.39(±0.02)	0.29(±0.03)	0.37(±0.04)	0.50(±0.05)	0.46(±0.05)
	Carotenoids	19.20(±1.0)	16.50(±1.76)	12.40(±2.4)	7.60(±3.0)	11.80(±3.5)	14.60(±3.4)	12.40(±3.5)
	Chlorophyll (a)	0.62(±0.02)	0.45(±0.04)	0.25(±0.05)	0.12(±0.05)	0.32(±0.03)	0.40(±0.02)	0.36(±0.02)
	Chlorophyll (b)	1.12(±0.01)	0.74(±0.04)	0.43(±0.05)	0.14(±0.05)	0.45(±0.02)	0.31(±0.02)	0.46(±0.02)
	Carotenoids	103 (±0.94)	92.16(±2.5)	44.30(±3.5)	35.92(±4)	42.50(±4.2)	58.02(±4.2)	52.73(±4.5)

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Table 2: Combined effect of NaCl and KCl on Proline, Protein and Nitrogen contents of rice varieties under salt (NaCl) stress.

Varieties	Variables		Control	50mM (NaCl)	100mM (NaCl)	150mM (NaCl)	50mM (NaCl +KCl)	100mM (NaCl +KCl)	150mM (NaCl + KCl)
B-385	Proline		0.02(±0.003)	0.09(±0.01)	0.098(±0.01)	0.12(±0.08)	0.108 (±0.01)	0.075(±0.004)	0.115(±0.009)
	Protein	Shoot	0.031(±0.005)	0.03(±0.002)	0.020(±0.006)	0.016(±0.002)	0.020 (±0.002)	0.026 (±0.003)	0.023 (±0.002)
		Roots		0.04(±0.003)	0.034(±0.002)	0.026(±0.002)	0.027 (±0.004)	0.032 (±0.001)	0.024(±0.002)
	Nitrogen	Shoot	0.004 (±0)	0.003 (±0)	0.003 (±0)	0.002 (±0)	0.003 (±0)	0.004 (±0)	0.003(±0)
		Roots		0.006(±0.00)	0.005 (±0)	0.004 (±0)	0.005(±0)	0.005 (±0)	0.004(±0)
	SB	Proline		0.030(±0.01)	0.063(±0.01)	0.074 (±0.01)	0.093 (±0.01)	0.082 (±0.008)	0.068 (±0.002)
Protein		Shoot	0.046(±0.022)	0.043(±0.01)	0.041(±0.005)	0.039(±0.008)	0.043 (±0.004)	0.045 (±0.004)	0.041(±0.011)
		Roots		0.07(±0.009)	0.057(±0.005)	0.053(±0.003)	0.059 (±0.005)	0.063 (±0.006)	0.049(±0.009)
Nitrogen		Shoot	0.007(±0.003)	0.007 (±0)	0.006 (±0)	0.006(±0.001)	0.007 (±0)	0.012 (±0.001)	0.006 (±0.002)
		Roots		0.01(±0.001)	0.009 (±0)	0.009 (±0)	0.010(±0)	0.010 (±0.002)	0.008(±0.001)

was enhanced i.e. 0.83 mg g<sup>-1</sup>, 0.50 mg g<sup>-1</sup> and 14.60 mg g<sup>-1</sup>, correspondingly. Similarly, in case of SB the chlorophyll a, b and carotenoid contents at 100mM of NaCl declined by 00.25mg/g, 0.43mg/g and 44.30mg/g, respectively, over the control. However, increased to 0.40mg/g, 0.31mg/g and 58.02mg/g at 100mM of NaCl+KCl as shown in Table 1.

#### Potassium Chloride modulate Proline-amino acid and Total Protein contents in Rice

The amino acid proline accumulation in two varieties of rice; B-385 and SB were markedly increased as NaCl concentration increased, over the control, but the mutual effect of both NaCl+KCl decrease amino acid proline accumulation up to certain extent. The results showed that level of proline amount (0.098 mg g<sup>-1</sup>) elevated in response to 100mM of NaCl, over the control (0.042mg g<sup>-1</sup>). However, reduction was observed for B-385 in proline concentration (0.075mg g<sup>-1</sup>) at 100mM of NaCl+KCl. Similarly, in case of SB proline concentration (0.077mg g<sup>-1</sup>) is enhanced at 100mM of NaCl, over the control (0.0303mg g<sup>-1</sup>) but reduced to 0.068mg/g at 100mM of NaCl+KCl. Therefore, increase of proline amount treated with stress could be attributed to enhance proline amount, but potassium reduced sodium stress mention as Table 2.

In shoot and root of both varieties; B-385 and SB,

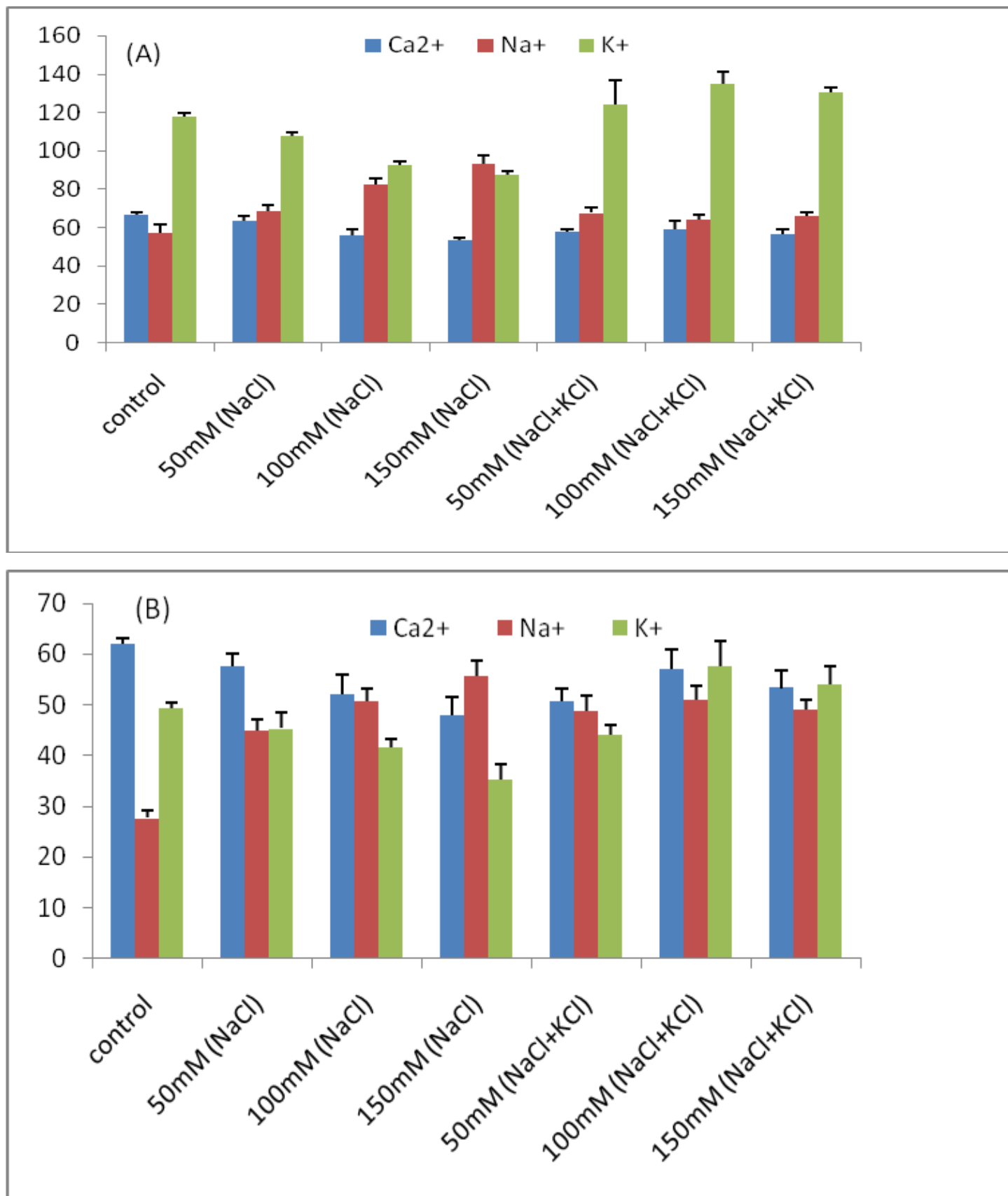
nitrogen and protein contents were decreased as the NaCl concentration increased, while increased as concentration of NaCl+KCl increasing upto 100mM. The results showed that a possible reduction was observed in shoot of B-385 and (0.0030g and 0.0200g) at 100mM of NaCl concentration, over control (0.0037g and 0.0313g) as increased to 0.0036g and 0.0261g at 100mM of NaCl+KCl. Likewise shoots, a possible reduction was observed in roots (0.0057g and 0.0344 g) at 100mM of NaCl concentration, over control (0.0076g and 0.0452g) whereas increased to 0.0054g and 0.0322g at 100mM of NaCl+KCl.

Data regarding SB shoots showed that plants treated with 100mM of NaCl concentration recorded minimum nitrogen and protein content (0.0069g and 0.0411g) as compared to control (.0077g and 0.0467g) whilst increased to 0.0106g and 0.0458g at 100mM of NaCl+KCl. Likewise shoots, roots of plants treated with 100mM of NaCl concentration recorded minimum nitrogen and protein content (0.0096g and 0.0575g) as compared to control (0.0128g and 0.0763g) whilst increased to 0.0107g and 0.0638g at 100mM of NaCl+KCl shown as Table 2.

#### Antagonistic profiling of Cellular Electrolytes (Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup>) during Salt stress

Significant variations were notified in electrolytes (Na<sup>+</sup>,

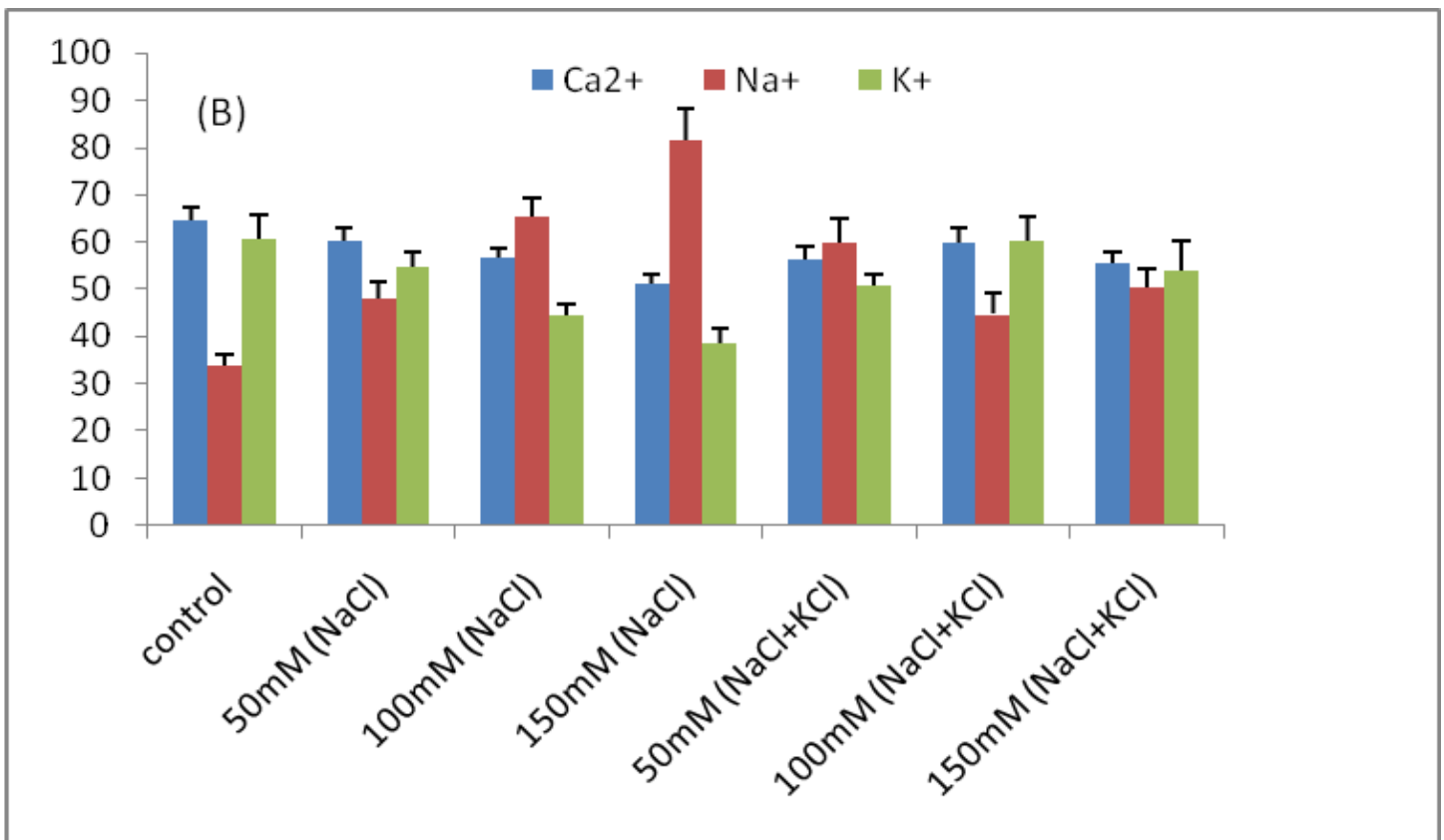
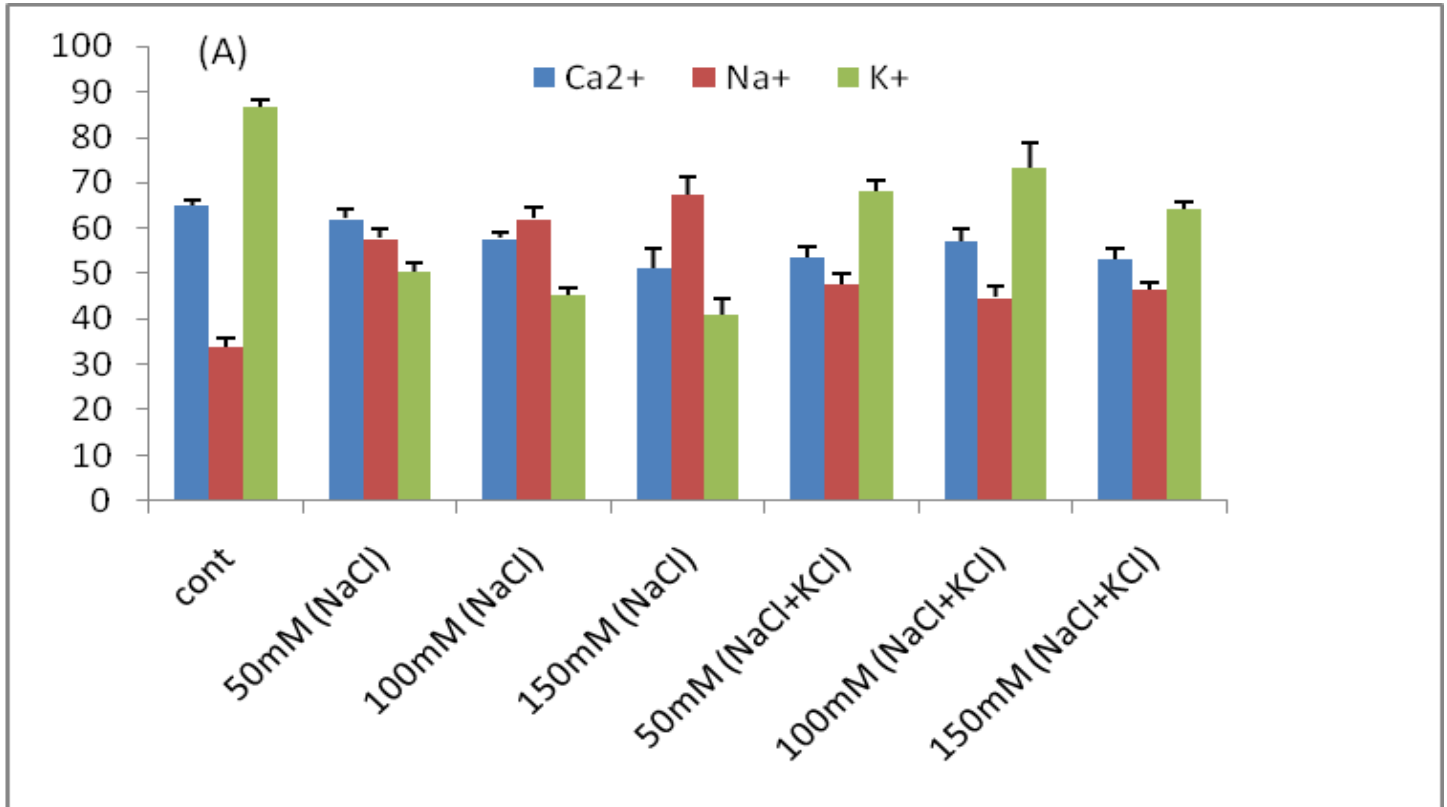
Figure 1: Effect of NaCl+KCl on Calcium, Sodium and Potassium ions in shoots (A) and roots (B) of B-385 under various concentration of NaCl.



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Figure 2: Effect of NaCl+KCl on Calcium, Sodium and Potassium ions in shoots (A) and roots (B) of SB under various concentration of NaCl.



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K<sup>+</sup> and Ca<sup>2+</sup>) content in two varieties of rice i.e. B-385 and SB at different concentrations of NaCl. In present study, Na<sup>+</sup> significantly increased and K<sup>+</sup> and Ca<sup>2+</sup> decrease with increasing NaCl in saline medium. While k<sup>+</sup> and Ca<sup>2+</sup> increased and a decline was observed for Na<sup>+</sup> content with increasing NaCl+KCl. In shoot maximum K<sup>+</sup>, Ca<sup>2+</sup> and Na<sup>+</sup> contents were recorded for control (118 ppm, 66.66 ppm, and 57.33 ppm) as minimum were observed for 100 mM of NaCl concentration (92.83ppm, 56ppm and 82.33ppm), respectively. As at 100mM of NaCl+KCl values were 135ppm, 59.33ppm and 64ppm for Basmati-385. Data regarding roots of B-385 showed that highest values were recorded for control (49.33ppm, 62ppm and 27.66ppm) as lowest were observed for 100mM of NaCl concentration (41.66ppm, 52ppm and 50.66ppm). At 100mM of NaCl+KCl were 57.66ppm, 57ppm and 51ppm, respectively in Figure 1.

In shoot of SB K<sup>+</sup>, Ca<sup>2+</sup> and Na<sup>+</sup> contents at control were (86.66ppm, 65ppm and 34ppm) as at 100mM of NaCl concentration were (73.33ppm, 57ppm and 44.66ppm). But at 100mM of NaCl+KCl observed values were (57.66ppm 45.33ppm and 62ppm). While in roots K<sup>+</sup>, Ca<sup>2+</sup> and Na<sup>+</sup> contents at control were 60.66ppm, 64.66ppm and 33.66ppm, while at 100mM of NaCl concentration 44.33ppm, 56.66ppm and 65.33ppm, But at 100mM of NaCl+KCl 60.33ppm, 60ppm and 44.66ppm Figure 2.

### **Mudulation of Macro and Micro Elements**

In Shoot and root of both varieties of rice; B-385 and SB Zinc concentration showed positive correlation with increasing NaCl concentration. But showed negative correlation with increasing of NaCl+KCl upto 100mM. In shoot and root of B-385 at control the zinc concentration was 1.57ppm, and 3.49ppm which elevated to 3.13ppm and 5.27ppm at 100mM of NaCl, but reduced to 2.17ppm and 4.08ppm at 100mM of NaCl+KCl. In shoot and roots of SB at control zinc concentration was 0.171ppm and 2.64ppm which enhanced to 1.32ppm and 3.49ppm at 100mM of NaCl, but reduced to 1.11ppm and 3.10ppm at 100mM of NaCl+KCl.

In shoot of B-385 at control the Copper, Iron and Manganese concentration was 0.098ppm, 1.67ppm and 2.22ppm which reduced to 0.07ppm, 1.06ppm and 1.106ppm at 100mM of NaCl, but enhance to 0.91ppm, 1.15ppm and 1.85ppm at 100mM of NaCl+KCl. While in root at control the Copper, Iron and Manganese concentration was 0.21ppm, 1.26ppm and 1.90ppm which reduced to 0.11ppm, 0.73ppm and 1.02ppm at 100mM of NaCl, but increased to 0.26ppm, 1.15ppm and 1.35ppm at 100mM of

NaCl+KCl.

In Shoot of SB at control Copper, Iron and Manganese concentration was 0.17ppm, 2.25ppm and 2.20ppm which reduced to 0.12ppm, 1.02ppm and 1.20ppm at 100mM of NaCl, but increased to 0.18ppm, 1.26ppm and 2.53ppm at 100mM of NaCl+KCl. While in root at control Copper, Iron and Manganese concentration was 0.31ppm, 2.27ppm and 1.13ppm which reduced to 0.16ppm, 1.42ppm and 0.48ppm at 100mM of NaCl, but increased to 0.25ppm, 1.47ppm and 1.55ppm at 100mM of NaCl+KCl.

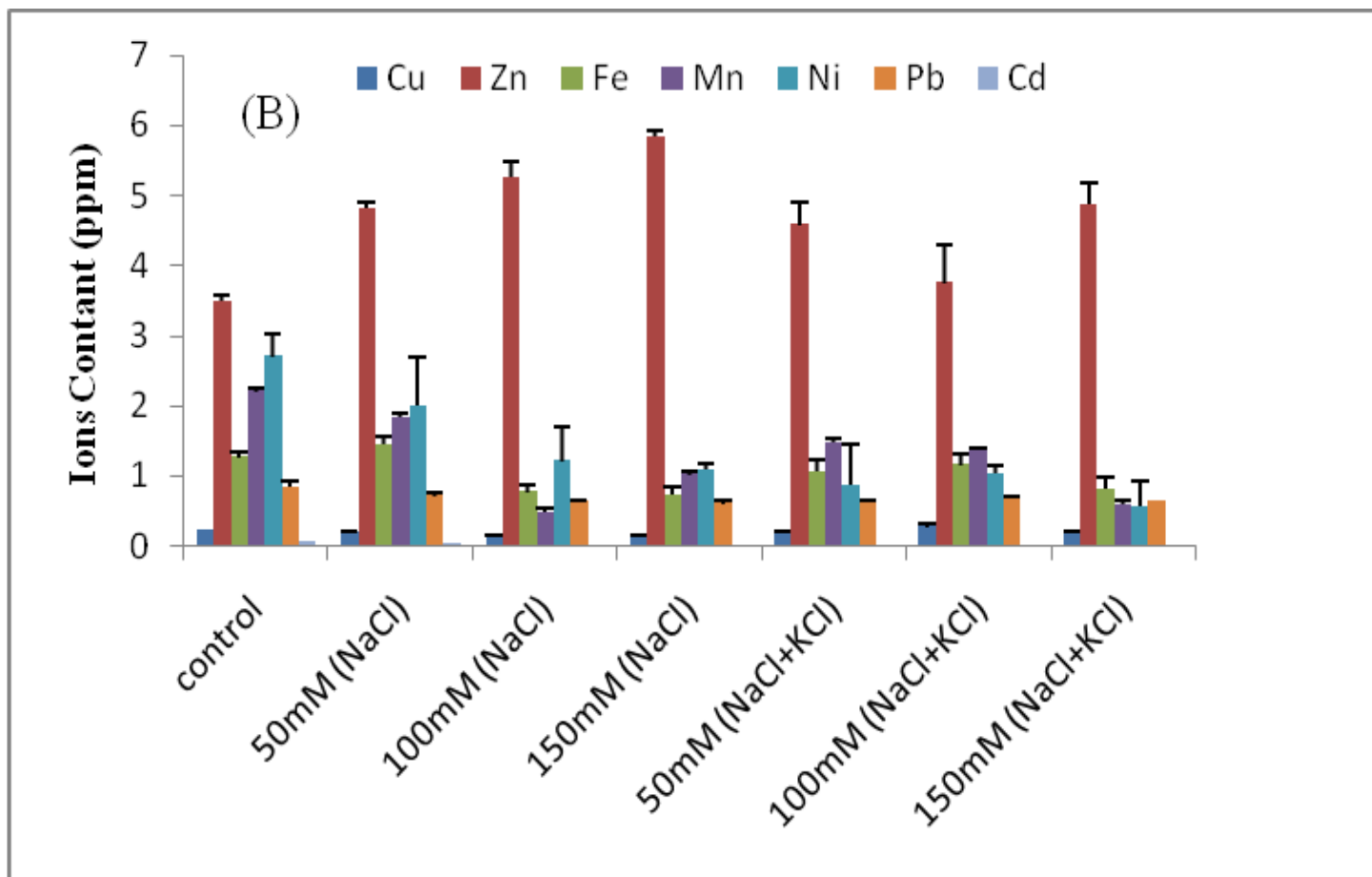
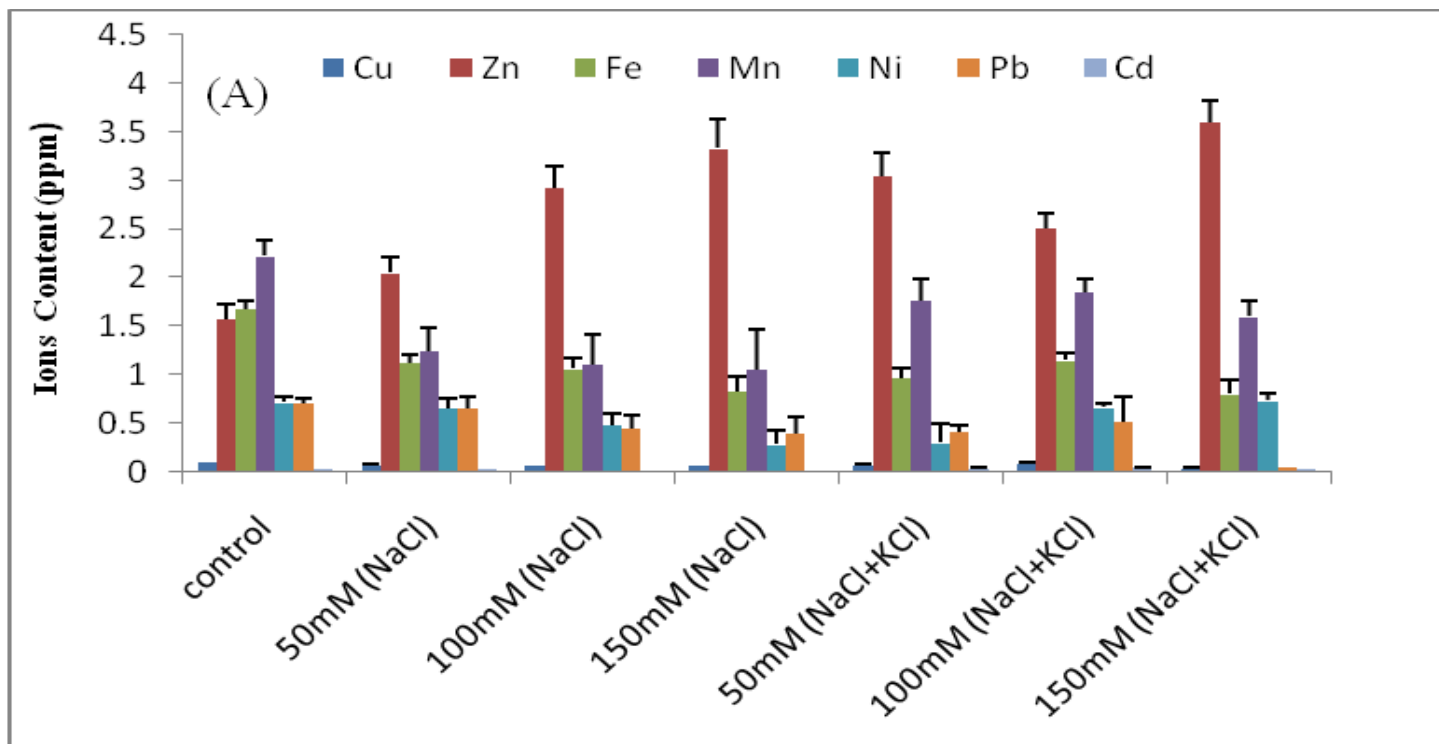
Cadmium, Lead and Nickle concentration in shoot and root of both varieties; B-385 and SB were significantly reduced by increasing NaCl concentration and show negative correlation with sodium chloride. While in case of NaCl+KCl concentration cadmium, lead and nickle concentration increased Figures 3 & 4. In shoot of B-385, at control the cadmium, lead and Nickle concentration were 0.23ppm, 0.70ppm and 0.716ppm which reduced to 0.018ppm, 0.44ppm and 0.484ppm at 100mM of NaCl, but 0.035ppm, 0.513ppm and 0.665ppm at 100mM of NaCl+KCl. While in case of root at control the cadmium, lead and Nickle concentration were 0.09ppm, 0.83ppm and 2.7ppm which reduced to 0.034ppm, 0.06ppm and 1.20ppm at 100mM of NaCl, but 0.028ppm, 0.068ppm and 1.320ppm at 100mM of NaCl+KCl.

In shoot of SB cadmium, lead and Nicle concentration were 0.054ppm, 1.30ppm and 1.39ppm at control and 0.021ppm, 0.78ppm and 0.739ppm at 100mM of NaCl, but 0.048ppm, 0.91ppm and 0.784ppm at 100mM of NaCl+KCl. While in case of root cadmium, lead and Nickle concentration were 0.053ppm, 0.034ppm and 1.953ppm at control and 0.031ppm, 0.35ppm and 0.846ppm at 100mM of NaCl, but 0.043ppm, 0.37ppm and 0.85ppm at 100mM of NaCl+KCl.

### **Discussion**

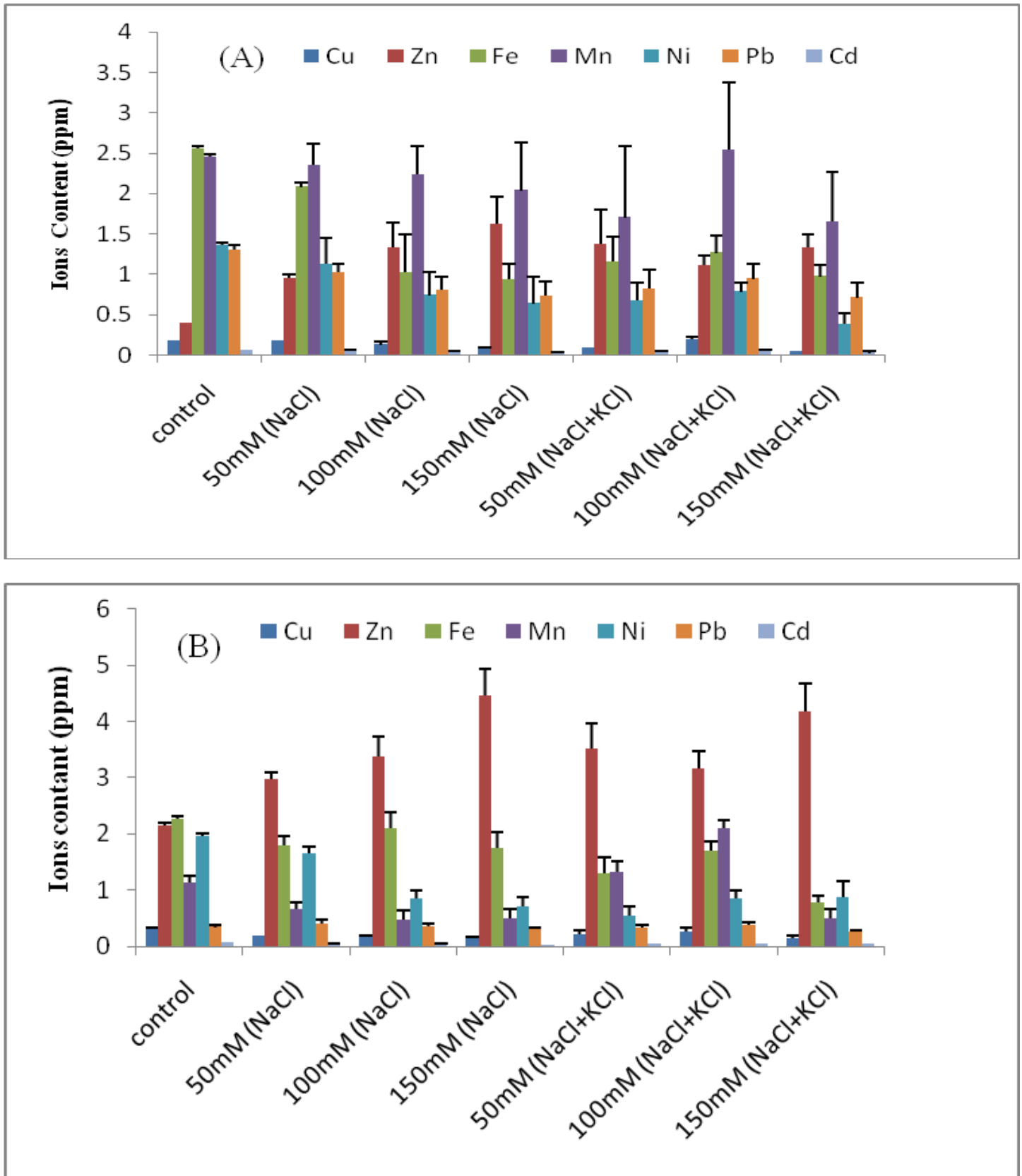
Photosynthetic pigments which may be chlorophyll (a, b) & carotenoid were declined with higher salinity NaCl but NaCl+KCl concentration increased both chlorophyll (a, b) as well as carotenoid (Table 2). So it may be due to reason that Potassium chloride might have some role with enzyme pool responsible for the process of photosynthesis which gives the plant strength to overcome the stress. [22] investigated that higher salinity suppressed enzymes which are mainly involve in chlorophyll synthesis and due to this the chlorophyll content reduced. Salinity stress mainly involve in the disruption of chloroplast as well as instability of pigment protein complex. But the chlorophyll contents reduced at higher concentration of NaCl salinities

Figure 3: Effect of NaCl+KCl on Copper, Zinc, Iron, manganese, Nickel, Lead, and Cadmium ions in a shoot (A) and root (B) of B-385 under various concentration of NaCl.



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Figure 4: Effect of NaCl+KCl on Copper, Zinc, Iron, manganese, Nickel, Lead, and Cadmium ions in a shoot (A) and root (B) of SB under various concentration of NaCl.



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[23]. These results were closely related to observation of that salt stress has negative effect on growth as well as on photosynthetic pigment of bean plants. The chlorophyll synthesis in the leaves of *E. agallocha* was increased by NaCl+KCl salinities.

Proline act as one of the defense mechanisms in plant to reduced salt stress. Shamseddin-saeid and Farahbakhsh, studies that in respect to salinity or various other kind of abiotic stresses proline accumulation occurs in plant leaves. Highly level of NaCl concentration in the medium increased proline accumulation in respect to control, but decreased as concentration of NaCl+KCl stress increased. So it might be a consequence of injury rather than being involved in the stress tolerance. Additional role of osmo-protectant, plant hormones provided through seeds or other way is a basic and alternate way to declined salinity stress [24]. The present studies investigate that the proline accumulation provides stability to plant under abiotic stresses [23]. According to Ueda [25] insertion of proline rich protein occurs under higher salinity stress. Our observation was closely related to the work of Shamseddin-saeid and Farahbakhsh [26].

The absorption of  $K^+$  ion in plant was suppressed by  $Na^+$  ion during salt stress was studied by [27,28]. While providing KCl to a plant lead to enhance  $K^+$  concentration in the roots and shoots more than normal condition [29]. But in our experiment the  $Ca^{2+}$  and  $K^+$  ions were reduced by higher salinity NaCl but increased by increasing NaCl+KCl stress up to moderate concentration (Figures 2 & 3). Due to the reason that observed increased was due to adding of KCl to root environment and potassium has negative correlation with sodium ions which has crucial processes of plant cells under salinity stresses. For osmotic adjustment of plant potassium ion is one of the most important and approximately 50 cytosolic enzymes have been activated by  $K^+$  ion [28]. Our Result was closely related to the result of. The result of [30] indicated that  $Ca^{2+}$  concentration in plant cell is one of potential marker for tolerance of salt in plant. They said that salt susceptible plants have reduced its ability to accumulate  $Ca^{2+}$  ions under high salinity stress. Applying KCl to root environment caused to decline accumulation of  $Na^+$  in plant which may led to enhance dry weight. Hence proved that there was a inversely relationship between  $Na^+$  and  $Ca^{2+}$ . But the result of our experiment also supported the result of [30]. It might due the fact that  $Ca^{2+}$  ion act as an important messenger in signaling pathway, and correlated with  $K^+$  ion which act as chemical fertilizer. The reduction of  $Ca^{2+}$  content was due to increasing  $Na^+$  content in root environment in salt salinity plants.

Jamil [31] observed that high level of salt in soil has

negative effect on protein contents of plants, but NaCl+KCl reduced these negative effects produced by salinity and enhanced total protein as well as nitrogen contents. This may be due to the fact that salt stress increases protein synthesis in cereals and facilitate the transfer of nitrogen into protein. In our results total nitrogen and protein content in rice plant decreased with increasing salinity NaCl but increasing of NaCl+KCl concentration would increase total nitrogen and protein. Potassium is a major component of protein synthesis, enzyme activation, the pH gradient, phloem uploading, turgor pressure regulation, stomata functioning, and the photosynthetic system, as well as controlling osmotic pressure and ionic balance during the exposure of plants to salt stress conditions. Our study is correlated with result of.

The concentration of Zn, Fe were significantly reduced in root, stem and leaf, Mn in stem and root in response to water deficient and salinity. The concentration of Cu significantly decreased in leaf with higher salinity. Moreover, a negative relationship was obtained between salinity and concentration for Fe, Mn, Cu, and Zn in leaf [32]. In our results the Cu, Fe and Mn were decreased as salinity NaCl increased. But increased with increasing of NaCl+KCl concentration (Figures 4 & 5), which correlated with result of [32]. While the Zn concentration increased with higher salinity NaCl but reduce as concentration of NaCl+KCl stress increased (Figures 4 & 5). Cadmium and lead contents decline in leaves of the plant in response to increasing soil salinity, because sodium ion concentration increased which may disturb the absorption of cadmium and lead. Other work also indicates that increased level of salinity cause a reduction in the concentration of elements absorbed by the plants [33]. In our studies cadmium and lead contents reduced with increasing NaCl concentration but increased with increasing NaCl+KCl concentration (Figures 4 & 5). Our finding is strongly supported with the work of [34- 36]. NaCl+KCl stress reduced the salinity stress (NaCl) in response of heavy metals absorption it shows us that NaCl+KCl play a crucial role in reducing the absorption of toxic elements like Na which assist the plant in the absorption of other essential compounds.

## Conclusion

It was concluded that potassium alleviate sodium stress in biochemical parameter of rice including photosynthetic pigments, elemental uptake ( $K^+$ ,  $Ca^{2+}$ , Cu, Fe, Mn, Pb, Cd), nitrogen and protein content and decline proline accumulation and ( $Na^+$ ,Zn) in B-385 and SB. However, potassium chloride showed antagonistic relationship with NaCl concentration.

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