MORPHO-PHYSIOLOGICAL UNDULATIONS OF *CUCUMIS MELO AGRESTIS* TO COMBINED NACL AND NICL$_2$ STRESSES

Hassan Raza Javeed[1], Nargis Naz [1], Saqib Ullah[2*]

[1]Department of Botany, The Islamia University of Bahawalpur, Punjab, Pakistan
[2]Department of Botany, Islamia College Peshawar, Khyber Pakhtunkhwa, Pakistan

Abstract: Salinity and heavy metals in soil are causing decline in plant seed germination, development and growth. Phytoremediation is an eco-friendly approach for the rehabilitation of contaminated soil. Medicinal weeds can provide multifaceted benefits whenever used for phytoremediation. Morphological and physiological behavior is an adequate criterion to assess the ramification of stress. A medicinal cucurbit weed *Cucumis melo agrestis* L. was given exposure to a series of combined (NaCl and NiCl$_2$) levels i.e. Tc=control, T1=100mM NaCl+50uM NiCl$_2$, T2=400mM NaCl +50uM NiCl$_2$, T3=100mM NaCl+ 200uM NiCl$_2$, and T4=400mM NaCl+ 200uM NiCl$_2$. Germination percentage, root and shoot length, fresh and dry weight, Number of branches and leaf area index, Na$^+$, K$^+$, Ca^{++}, Cl$^-$, Ni+, chlorophyll a and b, total soluble sugars, proline, catalase and superoxide dismutase were evaluated. A positive response was being observed in low salt combined with low metal. Low salt level (100mM NaCl) moderated the adverse effects of high heavy metal (200uM NiCl$_2$) and low heavy metal (50uM NiCl$_2$) ameliorated the high salt complications (400mM NaCl) while high salt+ high metal (400mM NaCl+ 200uM NiCl$_2$) severely ($p \leq 0.05$) affected the *C.melo agrestis*. Growth factors like length of shoot, root, fresh and dry weight, branches, leaf area index, chlorophylls, Ca$^{++,}$ and K$^+$ were affected adversely at high salt+ high metal while some factors like free sugars, proline, antioxidants like superoxide dismutase and catalase, Na$^+$ and Cl$^-$ increased. *C. melo agrestis* has proved successful against combined stresses of salt and heavy metal in the present research and can be considered for low-cost phytoremediation of salt and heavy metals.

Index-terms; Salinity stress, Phytoremediation, Physiology, *Cucumis,* Combined stress

I. INTRODUCTION

Soil salinity is a severe problem for agricultural production (Majeed & Muhammad, 2019) contaminating agricultural lands, and a frightening risk to the health of the plant, humans and animals recently become a severe ecological crisis of soil in the world (Saxena, Purchase, Mulla, Saratale, & Bharagava, 2020). It was observed that the combined effects of heavy metal with salinity were more blatant as compared to single or independent stress (Akeel & Jahan, 2020). Soil salinity leads to a decline in plant growth and overall yield as it is a hazardous agent causing osmotic and oxidative stresses (A. Kumar, Singh, Gaurav, Srivastava, & Verma, 2020). Among toxic salts, one of the most toxic salt is sodium chloride (NaCl) which comprises 50% of the entire soluble salts responsible for the addition of Na$^+$ and Cl$^-$ which are dominating in saline soils (Subba Rao et al., 2017). It is documented that the stress trigger in most plants is Na$^+$, but, in several cases, Cl$^-$ defeats Na$^+$ in toxicity (Alsaeedi et al., 2017).

Along with other heavy metals Nickel (Ni) and lead (Pb), are regarded as the most important environmental pollutants (Rinklebe & Shaheen, 2014). Presently, the global level of Nickel has exceeded about twenty to thirty folds more than the total range (Barraza et al., 2018). A slight amount of Nickel (Ni) is required for normal plants as its deficiency is rarely reported whereas its toxicity is a concerning issue (Morales-Díaz et al., 2017). Nickel, an important metal contaminant has crucial apprehension because of its rapidly rising levels in the soils of various areas of the world (Kamran et al., 2016). The environment is being polluted with Nickel by varied natural as well as anthropogenic sources and accordingly going to become strange in concentration. Natural sources of Ni may be weathering of rocks while anthropogenic sources are industries in which various Nickel compounds like Nickel (chloride, acetate, carbonate, oxide & hydroxide) are being used (Ghazanfar et al., 2021). Nickel compounds are accumulated in the soil, available for the plants, taken up while absorption of water, and ultimately enter in food chain producing injurious effects on animal and human lives (Okereafor et al., 2020).

The toxic effects of accumulated sodium(Na^+) and chloride(Cl^-) ions cause an increase in the production of reactive oxygen species (ROS) (Agarwal et al., 2021) resulting in decreased activity of stomata and electron transport system (Negrão, Schmöckel, & Tester, 2017; Stępień & Kłbus, 2006). The excessive production of ROS (SOD & CAT etc.) can cause cell death due to the oxidation of lipids, proteins, carbohydrates, nucleic acids, and chlorophyll (Sajjad Hussain et al., 2019). Adaptive defense mechanisms of plants to produce compatible solutes, ROS scavenging system, and compartmentalization of toxic ions combat salinity (Shah et al., 2017). Regardless of toxication, Cl^- (chloride ions) has a dogmatic role in the turgor generation, pH, enzyme stability, and balance of charge (Delpire & Staley, 2014). Modification in membrane potential, osmoregulation, volume control, and stomatal conductance leads to preventing water by minimizing its loss & use and the photosynthetic competence of the plant (Sharma et al., 2020). The presence of ions in a lower limit is beneficial but Surplus salt ions either in soil or in water cause considerable alterations in the morpho-physiological characteristics of plants. During salinity stress, plants absorb excess Na^+ at the expense of K^+ & Ca^{++} consequential in extra Na+ contents in plant parts like leaves and stems, which is increased, leading to nutritional disproportion resulting in decreased plant growth, inhibited physiological activities, and reduced dry matter (M. N. Khan et al., 2021).

Different plant organs accumulate Na+ and Cl^- under salt stress (Ulas, Aydin, Ulas, Yetisir, & Miano, 2020). Many researchers testified that long-term salt stress causes water deficiency and ion toxicity in older leaves while carbohydrate deficiency in young leaves (Kurtar, Balkaya, & Kandemir, 2016). Therefore, the Adaptive strategies of the plant under stress conditions determine the ability of salt resistance (Ors & Suarez, 2017). Different plants could be different in physiological and biochemical tolerance mechanisms either at the complete plant level or at the cellular (Shahzad et al., 2018).To select salt-tolerant cell lines, salinity tolerance expressed at the cellular level may be helpful to understand the mechanisms of salinity tolerance (Shelke et al., 2019). The degree of difference in responses to salinity and heavy metals in plants can be determined by measurement of altered germination percentage, growth parameters, and production of various compatible organic and inorganic solutes like antioxidants, etc (Handa et al., 2018). All the life events of a plant depend upon the germination of the seed. Increasing salinity and heavy metal stress inhibit seed germination in glycophytes, while elevates halophytes developing adaptations, for example seed germination in *Kochia scoparia* is increased in high salinity stress (Kafi, Asadi, & Ganjeali, 2010).

It has been acknowledged that harmful effects of salinity stress can be minimized or even ameliorated by the appliance of special micronutrients to plants, such as barley enhanced yield under salinity stress when provided Si micronutrient (Noreen, Fatima, Ahmad, Athar, & Ashraf, 2018). Nickel being a micronutrient can be used as a nanomaterial to minimize the toxic effects of salts as many others are being used for this purpose (Ni, Ni, Yang, & Wang, 2013).

C. melo agrestis L. is a medicinal cucurbit plant commonly known as 'Naud' (Swamy, 2017) and has multiple medicinal, nutritional, and biological values (Tang, Zhang, Cao, Wang, & Qi, 2015). The seeds of *C. melo agrestis* contain tocopherols, sterols, and fatty acids (Mariod & Matthaeus, 2008). *C. melo agrestis* is a weed-infesting crop of cotton and sorghum (Johnson & Mullinix, 2002). C.melo agrestis is also used in preparing food items like soya sauce, condiments, and garnishes as well as oils obtained from it are used as thickeners in soups, fat binders, and raw feeds (Noreen et al., 2018; Tzortzakis, Chrysargyris, & Petropoulos, 2018).

Recognition and understanding of plant abilities to salinity and heavy metal tolerance and resistance are of comprehensible attention in varietal improvement for reclamation of saline and heavy metal contaminated soils. Moreover, the use of heavy metals as micronutrients may prove helpful to the plants to ameliorate salinity stress and improve plant growth in a saline environment. Whereas soil contaminated with heavy metal may be turned into greenland if the plants are provided salts in lower concentrations (Zulfiqar & Ashraf, 2021). Therefore this study was conducted to evaluate morphological and physiological responses of *C. melo agrestis* under various combinations of salt and heavy metal concentrations to asses its reliability for the rehabilitation of contaminated soils in a low-cost and eco-friendly way.

II. MATERIALAS AND METHODS

The experiment was carried out to study the adaptations against variable levels of independent salt or heavy metal as well as combined stresses in cucurbit weed *C. melo agrestis.* The practical conduct of the experiment was done in July 2020 in the Research area, Botanical Garden, Botany Department, The Islamia University of Bahawalpur.

2.1 Collection of seeds

Seeds of *C. melo agrestis* were collected from different sites of the thal desert of district Layyah (71.4774 E & 30.9057 N) for assessment of the response of species under salt and heavy metal stresses. The ripened fruits of the species were collected and seeds were obtained by removing the dried pulp. Healthy seeds were selected for further experiments.

2.2 Levels of salt and heavy metal stresses

To assess the tolerance potential and adaptive strategies of *C. melo agrestis* L. variable levels of combined NaCl and $NiCl_2$ were selected and added to the corresponding petri dishes to create 5 treatments. Control plants were grown without salt and metal while experimental groups include (T0)=Distilled water, T1=100mM NaCl+50uM $NiCl_2$ (low salt+low metal), T2=400mM NaCl +50uM $NiCl_2$(High salt+low metal), T3=100mM NaCl+ 200uM $NiCl_2$ (low salt+High metal), and T4=400mM NaCl and 200uM $NiCl_2$(High salt+ High metal).

2.3 Experimental design

Completely randomized design (CRD) for the conduction of the experiment with the following factors (weed species, salinity, Heavy metal, and salinity+ Heavy metal) with three replicates.

2.4 Laboratory experiment

To evaluate the seed germination percentage, the experiment was conducted in the lab. in 9cm diameter Petri dishes. Each petri-plate was provided with Whatman No.43 filter paper and twenty seeds were placed in each petri plate after soaking seeds for 24 hours in a 10% solution of sulphuric acid for breaking of the seed coat. All the petri plates were provided with Hoagland solution to maintain other nutrients. Petri plates were covered to protect from external irrelevant disturbances. The seed germination percentage was recorded according to the formula of Coolbear. Germination percentage $GP = \frac{\Sigma G}{N} \times 100$

Fresh and dry weights were calculated by electronic balance. The plant samples were preserved in a properly labeled vial containing preservatives (FAA for anatomy), and paper bags (for taking dry weight).

2.5 Pot experiment

2.5.1 Soil analysis and sowing of seeds

The soil samples for the potted experiment were taken to be analyzed for physio-chemical characteristics which were EC1.97 ds/m, pH 8.1, soil texture sandy loam, organic matter 0.51%, available phosphorus 6ppm, and potassium 113ppm using EC meter, pH meter, hydrometer, loss on ignition, PFP-7 Jenway flame photometer (Jenway, PFP-7) using 200g dried soil for analysis. Seeds of *C.melo agrestis* were soaked in a 10% sulphuric acid solution in water for 24 hours to soften the seed coat. Twenty seeds of *C. melo agrestis* were placed in each plastic pot (15cm diameter) containing soil with 40% sand, 30%silt and 30% clay to a depth of 1- 2 cm.

2.6 Morphological parameters Pots were treated with various levels of (salt NaCl+ heavy metal $NiCl_2$) according to the method described above. Pots were placed in the research area and left open. Adequate moisture was kept by providing water to the pots. After germination pots were given the treatment of salt, heavy metal, and combined salt+ heavy metal weekly. Morphological data were collected after four weeks at the vegetative stage. Data was collected for fresh weight/mass (mg), dry weight/mass (mg), root and shoot length (cm), branches count and leaf area index (cm^2).

2.7 Biochemical and physiological parameters

2.7.1 Determination of ions; Jenway flame photometer for the determination of Na^+, $K^+$and Ca^{++}. Results were computed after a comparison of curves with a standard curve. Jenway PCLM chloride meter was used for the determination of chloride (Cl^-). Ni^+ (aq) reacts with Cl^- ion in the presence of ethanol and gives tetrachloronickel (ion/ $NiCl_4$ $)^{2-}$, a blue color solution is obtained.

2.7.2 Estimation of Chlorophylls; Arnon method (Arnon, 1949) was used to determine chlorophyll a and b. Reading for absorbance of the supernatant using a spectrophotometer (Hitachi, Japan), was taken at 645 and 663nm. Formulae chlorophyll a and chlorophyll b are;

Chl. **a** (mg/g) = [12.7 (O D 663) - 2.69 (O D 645) × V/1000 × W]

Chl. **b** (mg/g) = [22.9 (O D 645) - 4.68 (O D 663) × V/1000 × W]

Where W= Fresh weight of sample V = volume of the sample

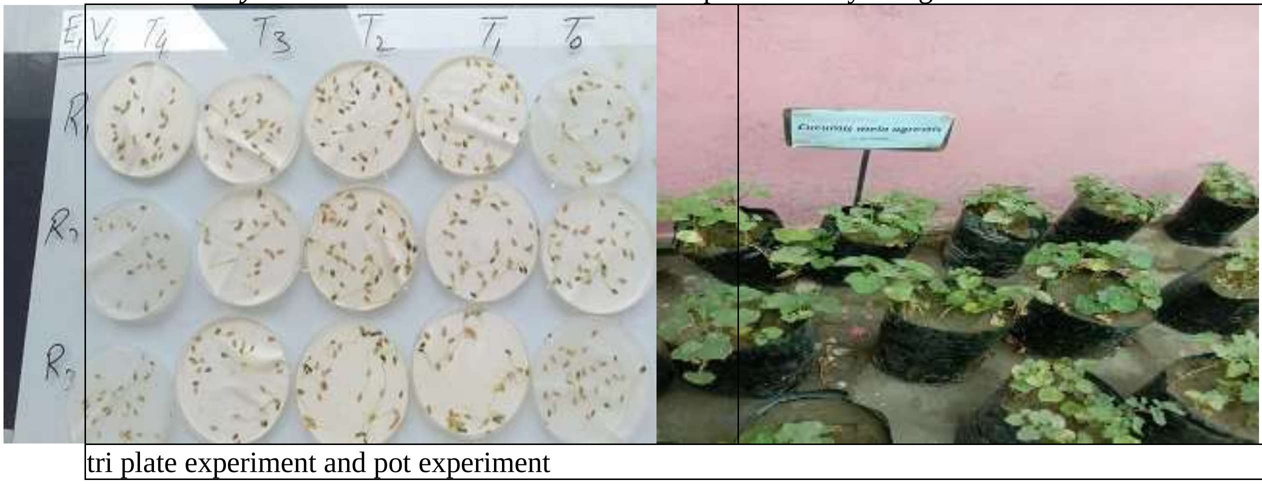
2.7.3 Determination of soluble sugars; Yemm & Willis method (Yemm & Willis, 1954) for determination of soluble sugars. Reading of optical density was taken at 625 nm on a spectrophotometer of Hitachi 220, Japan.

2.7.4 Determination of Proline; (Bates, Waldren, & Teare, 1973) method for the determination of proline. The standard curve comparison method is used for the determination of proline concentration and calculated as follows:-

µmole proline g-1 fresh weight = (µg proline ml-1 x ml of toluene/115.5) / (g of sample)

2.7.5 Determination of CAT & SOD; Chance & Maehly's (1955) method was used for the determination of Catalase while SOD activity was analyzed by Giannopolitis & Ries (1977).

2.7.6 Statistical analysis ANOVA and LSD of the data was performed by using STATISTIX 8.1 software.



tri plate experiment and pot experiment

III. RESULTS

In comparison to the control, *C. melo agrestis* L. showed a significant escalation in germination percentage at the combination of lower salt and lower Heavy metal (100mM NaCl+ 50uM $NiCl_2$), a slight decrease in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) and high salt+ lower heavy metal level(400mM NaCl+50uM $NiCl_2$), while the considerable decrease in germination at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$).

C. melo agrestis L. showed a significant gain in root length at joint lower salt and lower Heavy metal (100mM NaCl+ 50uM $NiCl_2$), slight decrease in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) and high salt+ lower heavy metal level(400mM NaCl+50uM $NiCl_2$), while the considerable decrease in root length at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$).*C. melo agrestis* L. showed a significant rise in shoot elongation at joint lower salt and lower Heavy metal (100mM NaCl+ 50uM $NiCl_2$), slight decrease in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) and high salt+ lower heavy metal level(400mM NaCl+50uM $NiCl_2$), while a considerable decrease in shoot length at the high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$) in comparison with control.

C. melo agrestis L. showed a significant rise in fresh weight at amalgamated lower salt and lower Heavy metal (100mM NaCl+ 50uM $NiCl_2$), slight decrease in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) and high salt+ lower heavy metal level(400mM NaCl+50uM $NiCl_2$), while a considerable decrease in fresh weight at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$) in comparison with control. *C. melo agrestis* L. showed a significant escalation in dry weight at pooled low salt with low metal (100mM NaCl+ 50uM $NiCl_2$), slight decrease in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) and high salt+ lower heavy metal level(400mM NaCl+50uM $NiCl_2$), while a considerable decrease in dry weight at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$) in comparison with control.

C. melo agrestis L. showed a significant increase in branching at collective low salt with low metal (100mM NaCl+ 50uM $NiCl_2$), slight decrease in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$), and high salt+ lower heavy metal level(400mM NaCl+50uM $NiCl_2$), while a considerable decrease in dry weight at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$). In comparison with control, *C. melo agrestis* L. showed a significant increase in leaf area index at mixed low salt with low metal (100mM NaCl+ 50uM $NiCl_2$), slight increase in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) and high salt+ lower heavy metal level(400mM

NaCl+50uM $NiCl_2$), while a considerable decrease in leaf area index at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$).

An increase in potassium ion level was determined in *C. melo agrestis* L. grown under low salt with low metal (100mM NaCl+ 50uM $NiCl_2$). A slight decrease of potassium ions was observed in plants grown in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) and high salt+ lower heavy metal level(400mM NaCl+50uM $NiCl_2$), while the considerable decrease in Potassium ions content was observed at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$). A decrease in sodium ion concentration was determined in *C. melo agrestis* L. grown under low salt with low metal (100mM NaCl+ 50uM $NiCl_2$). A slight increase of sodium ions was observed in plants grown in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) and high salt+ lower heavy metal level(400mM NaCl+50uM $NiCl_2$), while the considerable increase in sodium ions content was observed at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$).

An increase in Calcium ion level was detected in *C. melo agrestis* L. grown under low salt added to low metal (100mM NaCl+ 50uM $NiCl_2$). A slight decrease of calcium ions was observed in plants grown in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) and high salt+ lower heavy metal level(400mM NaCl+50uM $NiCl_2$), while a considerable decrease in Calcium ions content was observed at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$).In comparison with the control a minor decrease in chloride ion level was measured in *C. melo agrestis* L. grown under low salt mixed with low metal (100mM NaCl+ 50uM $NiCl_2$). A slight increase of chloride ions was observed in plants grown in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) and high salt+ lower heavy metal level(400mM NaCl+50uM $NiCl_2$), while the considerable increase in chloride ions content was observed at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$). A minor increase in Nickel level was determined in *C. melo agrestis* L. grown under low salt mixed with low metal (100mM NaCl+ 50uM $NiCl_2$). A slight increase of nickel ions was observed in plants grown in high salt+ lower heavy metal level(400mM NaCl+50uM $NiCl_2$), while the considerable increase of nickel content was detected at low salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) and in a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$).

Significant increase in chlorophyll a was observed in *C. melo agrestis* L. grown under low salt with low metal (100 mM NaCl+ 50uM $NiCl_2$). A minor rise in chlorophyll a was perceived in plants developed in high salt+ lower heavy metal level (400mM NaCl+50uM $NiCl_2$), A slight decrease of chlorophyll a was observed in plants grown in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) while a considerable decrease in Chlorophyll a was detected at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$). In comparison with the control the significant increase in chlorophyll b was observed in *C. melo agrestis* L. grown under low salt with low metal (100mM NaCl+ 50uM $NiCl_2$). A minor rise in chlorophyll b was observed in plants under high salt+ lower heavy metal level (400mM NaCl+50uM $NiCl_2$), A slight decrease of chlorophyll b was observed in plants grown in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$) while a considerable decrease in Chlorophyll a was observed at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$).

A slight increase in soluble sugars was observed in *C. melo agrestis* L. grown under low salt combined with low metal (100 mM NaCl+ 50uM $NiCl_2$). A substantial rise in soluble sugars was detected in plants grown under high salt+ lower heavy metal level(400mM NaCl+50uM $NiCl_2$) and in plants grown in lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$). The maximum increase of soluble sugars was observed at a high level of salt + a high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$). In comparison with the control slight upsurge in proline, value was observed in *C. melo agrestis* L. grown under low salt with low metal (100 mM NaCl+ 50uM $NiCl_2$). A significant increase in proline was observed in plants under high salt+ lower metal level (400mM NaCl+50uM $NiCl_2$) and in plants grown at lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$). The maximum increase of proline was observed at a high level of salt + high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$).

A trivial increase in catalase content was observed in *C.melo agrestis* L. grown under low salt with low metal (100mM NaCl+ 50uM $NiCl_2$). A noteworthy rise in catalase was observed in plants under high salt levels combined with low metal levels (400mM NaCl+50uM $NiCl_2$) and in plants grown at lower salt level + high heavy metal level (100mM NaCl + 200*u*M $NiCl_2$). The maximum increase of catalase was observed at a high level of salt + a high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$). In comparison with the control slight increase in Superoxide dismutase (SOD) content was observed in *C. melo agrestis* L. grown under low salt combined with low metal (100mM NaCl+ 50uM $NiCl_2$). A significant increase in Superoxide dismutase (SOD) was observed in plants under high salt+ lower heavy metal levels s (400mM NaCl+50uM $NiCl_2$) and in plants grown in lower salt level + high heavy metal levelss (100mM NaCl + 200*u*M

$NiCl_2$. The maximum increase of Superoxide dismutase (SOD) was observed at the high level of salt + a high level of heavy metal (400mM NaCl + 200*u*M $NiCl_2$).

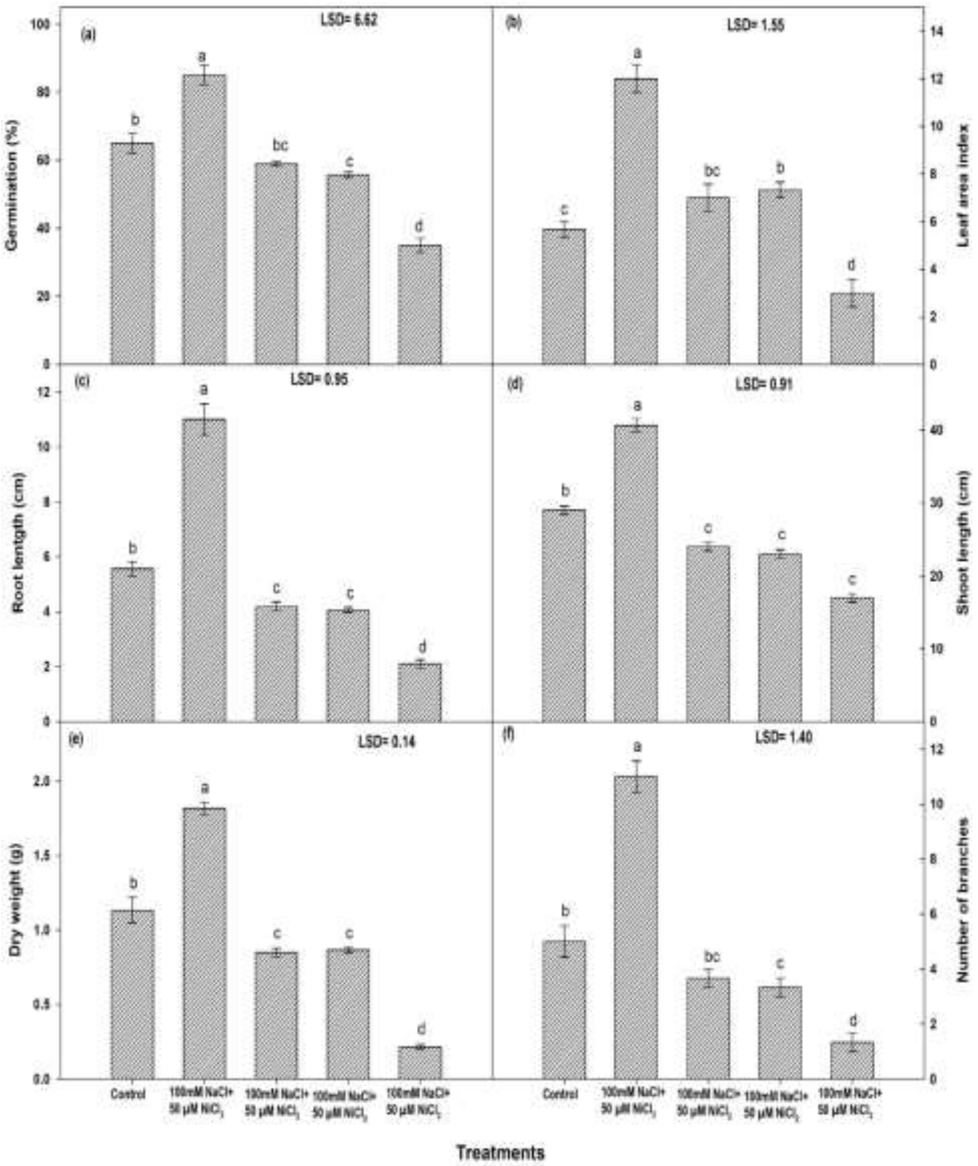


Fig. 1. Graphs showing germination percentage and morphological response of *Cucumis melo agrestis* under combined stress levels of salt and heavy metal (NaCl+NiCl2)

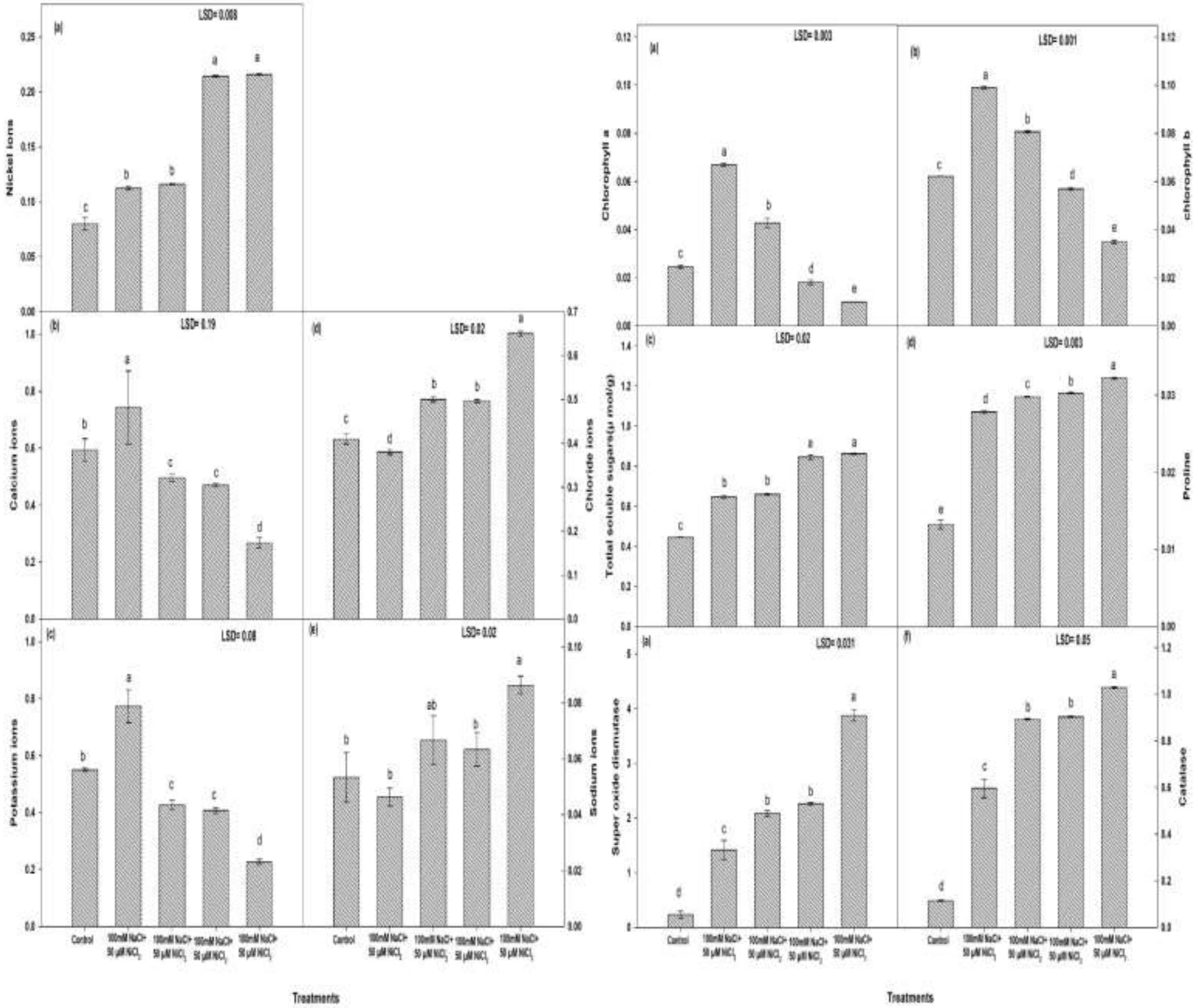


Fig. 2 & 3. Graphs showing biochemical ions & physiological parameters of *Cucumis melo agrestis* under combined stress levels of salt and heavy metal (NaCl+NiCl2).

Table 1- ANOVA for the effect of combined Salt + metal (NaCl + $NiCl_2$) Stresses on germination & morphological factors of *Cucumis melo agrestis* L.

Sources of variation	Degree of freedom	Mean sum of squares						
		GP %	RL	SL	FW	DW	NB	LAI

Treatment	9	971.067	34.1210	236.567	4.40333	1.00192	40.4333	32.1667
Error	20	13.267	0.2733	1.267	0.02867	0.00667	0.6000	0.7333
Grand Mean		59.933	5.3867	26.733	2.000	0.9767	4.8667	7.0000
CV		6.08	9.71	4.21	8.47	8.36	15.92	12.23
F		73.2	125	187	154	150	67.4	43.9

Table 2- ANOVA for the effect of combined Salt + metal (NaCl + $NiCl_2$) Stresses on ions (K^+, Na^+, Ca^{++}, Cl^- and Ni^{++}) in *Cucumis melo agrestis* L.

Sources of variation	Degree of freedom	Mean sum of squares				
		K^+	Na^+	Ca^{++}	Cl^-	Ni^+
Treatment	9	0.12247	6.968	0.09172	0.03315	0.01206
Error	20	0.00229	1.271	0.01109	0.00015	0.00002
Grand Mean		0.4767	0.0632	0.5140	0.4873	0.1479
CV		10.03	17.84	20.49	2.48	3.27
F		53.6	5.48	8.27	227	517

Table 3- ANOVA for the effect of combined Salt and metal (NaCl + $NiCl_2$) Stresses on Physiological characteristics of *Cucumis melo agrestis* L.

Sources of variation	Degree of freedom	Mean sum of squares					
		Chl. a	Chl. b	Total soluble sugars	Proline	CAT	SOD
Treatment	15	0.00155	0.00177	0.08706	1.250E-05	0.40528	5.29214
Error	32	3.111E-06	7.067E-07	0.00013	3.800E-08	0.00106	0.02917
Grand Mean		0.0324	0.0668	0.6916	0.0294	0.7071	1.9735
CV		5.44	1.26	1.64	0.66	4.61	8.65
F		499	2504	675	329	382	181

IV. DISCUSSION

Responses to salinity and heavy metals vary in different plants (Fernandes, Mucha, Francisco, Gomes, & Almeida, 2017) either the same or dissimilar species or genera (Sanjosé et al., 2021). The over-concentration of Na^+ and Cl^- influence plant morphology and some physiological characteristics (D. Kumar, Malik, Patel, & Gupta, 2019). Plant characteristics either morphology or physiology as well as biochemistry vary under varying salinity or metal stress (Kusvuran & Dasgan, 2017).

In present studies, *C.melo agrestis* L. showed a positive response of seed germination, root or shoot length, fresh or dry weight, number of branches, and leaf area index in plants developed under combined low salt and low metal (100mM NaCl+ 50uM NiCl2). Parallel results were reported by (López, Peralta-Videa, Benitez, & Gardea-Torresdey, 2005) in *Medicago sativa* and Sultana (Sultana, Adnan, & Ali, 2019) in wheat *Triticum aestivum*. In the present study, germination of *C. melo agrestis* was lowered in high combined high salt levels and high heavy metal levels (400mM NaCl + 200uM $NiCl_2$) which is supported by (Essa, 2002) in Soybean, (Chandraju, Basavaraju, & Kumar, 2008) in pulses, (Jan, Heck, Laskin, & Laskin, 2020) in mustard, (Nadia, Emanuel, & Robinson, 2021) in Amaranthus and (Anaya, Fghire, Wahbi, & Loutfi, 2018) in *Vicia faba*. The root length and shoot length of *C. melo agrestis* L. in the present study were highly retarded at combined high salt and high heavy metal (400mM NaCl and 200uM $NiCl_2$). Reduction of the shoot and root length under high salinity and heavy metals has been testified by many investigators (Adibah, Jahan, & Fatihah, 2020). Excess salinity and heavy metal stresses minimize water uptake by the plant inducing a negative impact on root length (Emamverdian, Ding, Mokhberdoran, & Xie, 2015). Moreover, salinity and heavy metal stress inhibit metabolic steps during cell division and elongation in high concentrations main cause of which may be the osmotic effect disturbed due to higher salts and heavy metals. The fresh & dry weight of *C. melo agrestis* was amplified at collective low salt and low metal (100mM NaCl and 50uM $NiCl_2$) same as observed by (Çimrin, Türkmen, Turan, & Tuncer, 2010; Emamverdian et al., 2015). The fresh and dry weight of *C. melo agrestis* in our studies was decreased at combined high salt and high heavy metal (400mM NaCl and 200uM $NiCl_2$). Our results are also supported by many reports (Jusiak, Cleto, Perez-Piñera, & Lu, 2016; Yasmeen & Siddiqui, 2018). Declined fresh weight was the result of reduced water intake, which as a result could cause decreased water content in plant tissue (Kahlon et al., 2018; Taghipour & Jalali, 2019). Sodium chloride absorbed in plants increased toxic ion concentrations resulting in disturbance of the ionic balance of plant tissues (Shahid et al., 2020; Turan, Elkarim, Taban, & Taban, 2010).

The leaf area index of *C. melo agrestis is* L showing a decrease in combined high salt and low metal (400mM NaCl + 50uM $NiCl_2$) and combined high salt and high metal (400mM NaCl + 200uM $NiCl_2$). Similar findings have been reported (Amjad et al., 2020) in tomatoes and Rhodopseudomonas palustris (Kanwal et al., 2020). As the salt and heavy metal are accumulated in the root and shoot, the leaf area cannot expand due to inhibited cell division (Alipour, Saharkhiz, Niakousari, & Damyeh, 2019). The point to ponder is that coeffect of heavy metals and salinity stress when in high concentration are comparatively more severe (Ermolenko et al., 2020; Gul, Nawaz, & Azeem, 2016). *C. melo agrestis is* L. revealed a considerable decrease in a number of branches combined with high salt and high heavy metal (400mM NaCl and 200uM $NiCl_2$). This is similar to (Kotagiri & Kolluru, 2017) who noticed reduced plant growth under salinity stress. Reduction in leaves under salinity and heavy metals stresses was also reported (Al Murad, Khan, & Muneer, 2020; Taha & Abd El-Samad, 2022). It may be due to the accumulation of salt and heavy metal in the shoot decreasing the cell division and elongation as studied in *Calendula officinalis* L. (Ebrahimi, Zamani, & Alizadeh, 2017). Branching of the shoot depends upon mitotic activity in the meristematic cell which is distressed by salinity and metals (Yadav, Kumar, Kumar, Kumar, & Arya, 2022).

C. melo agrestis L. exposed a noteworthy upsurge in Chloride substances at all treatment levels as compared to control. The chloride content in plants may cause a reduction in plant characteristics and is critical for saline acceptance (Geilfus, 2018). High levels of chlorine are correlated with severe physiological dysfunction (Bazihizina, Colmer, Cuin, Mancuso, & Shabala, 2019; Geilfus, 2018; Riaz et al., 2020; Shamizadeh, Alinejad Shahabi, & Arjmand, 2018) positively in some plants and negatively in others (Bazihizina et al., 2019; KAOUTAR, CHETTO, BENIKEN, BENKIRANE, & HAMID, 2021; Van Zelm, Zhang, & Testerink, 2020). Potassium ions were increased in plants under combined low salt and low metal (100mM NaCl and 50uM $NiCl_2$), and decreased potassium level was observed in combined high salt and low metal (400mM NaCl and 50uM $NiCl_2$), combined low salt and high metal (100mM NaCl and 200uM $NiCl_2$) and combined high salt and high metal(400mM NaCl and 200uM $NiCl_2$) while sodium ion was increased by increasing level of salt. Cytosolic homeostasis and the ability of various plant tissues have been reported by retention of K^+ (Kader & Lindberg, 2010; Kumari, Chhillar, Chopra, Khanna, & Khan, 2021). Calcium ions were antagonistic to nickel ions in *C. melo agrestis* L. where Nickel was low their calcium level was increased because calcium ions strongly affect the uptake of heavy metals from the soil as was demonstrated (Ouzounidou, Moustakas, Symeonidis, & Karataglis, 2006). Calcium level was decreased in the trials where a substantial volume of nickel was observed at combined low salt and high metal (100mM NaCl + 200uM $NiCl_2$) and combined high salt and high heavy metal (400mM NaCl and 200uM $NiCl_2$). (Amin, Sarwar, Saleem, Latif, & Opella, 2019) also suggested that a common trans-membrane transporter may be found in the plants for the uptake of heavy metals like Ni, Cd, and Cu, etc. Transport of Nickel by active and passive systems takes place in spruce and soybean (Boyd, 2020). Plants accumulate solutes for osmotic adjustment under the salinity and heavy metal stress, the combined effect of salts is more severe than single salt or heavy metal (Gul et al., 2016).

C. melo agrestis L. revealed *a* significant decrease in chlorophyll a and b under combined high salt and heavy metal (400mM NaCl and 200uM $NiCl_2$) similar to photosynthetic pigments of *Vigna mungo* were seriously decreased under various levels of Nickel chloride studied (Aqeel et al., 2021). The effect of nickel in cabbage (*Brassica oleracea* L.) diminished the chlorophyll (Molas, 1998). Total soluble sugars were increased in increased levels of salt as well as heavy metals. The lower amount of total soluble sugars were detected in plants under lower salt and heavy metal concentrations. Soluble sugars produced during stress conditions can work as signaling indicators, work in association;ion with a plant growth regulators, the sugar form, and establish a multifaceted network in plants (Saleem, Fariduddin, & Janda, 2021). As compared to starch, the total soluble sugar concentration increase under salt and heavy metal stress but under severe condition, the sugar content also decreases. It was confirmed that the deposition of sugars plays a basic role in hassle tolerance (N. Khan et al., 2020; Vishal, Krishnamurthy, Ramamoorthy, & Kumar, 2019). Soluble sugars are associated with many biochemical procedures and structural components of the cell and act as a metabolic resource [85]. Soluble sugars are an essential part of the signaling cascade, which communicates the stress pathways that form a complex system and control the metabolic responses of plants (Rosa et al., 2009). Soluble sugars may support stress tolerance and may directly work as adverse signals or adjust the cellular pathways to encourage stress response indications and rise resistance to stress (Gangola & Ramadoss, 2018). The level of soluble sugars is normally amplified by high salinity and heavy metal. The actions of soluble sugars depend on vegetal species and the strength of stress (El-Esawi et al., 2020). The present research indicates that the increased level of soluble sugars and other osmolytes significantly improve plant acceptance to salinity and metal stress as described earlier (Karami Mehrian, Heidari, & Rahmani, 2015).

In our studies on *C.melo agrestis* L. proline augmented employing the cumulative salinity as well as metal stress. There was a gradual increase in proline by increasing levels of salinity and metal stresses. All-out quantity of proline was documented in combined high salt and high metal levels (400mM NaCl and 200uM $NiCl_2$). Parallel conclusions stated that proline and free amino acids are increased in plants under stress due to the biosynthesis of amino acids and the absence of translational factors (Hayat et al., 2012). The increase of proline is an adaptive retort to salinity and metal stress [90]. Levels of proline have been revealed to enhance salt-tolerating flora in erratic heights of salinity. In this study, levels of proline increased in the experimented plants with increasing salinity and heavy metal same as in species of Chenopodiaceae was observed higher proline under stress (Di Martino, Delfine, Pizzuto, Loreto, & Fuggi, 2003).

Salt tolerance is regulated by the synchronized action of variable gene families involved in the initiation of a variety of mechanisms such as water conservation strategies, the sequestration of toxic ions, adjustment of toxic metabolites, and antioxidative defense (Le Saux et al., 2020). The elevated levels of salts cause reactive oxygen production (ROS) production including superoxide radicals and hydroxyl radicals (Liu, Zhao, & Wang, 2021). It has been observed in our experiment on *C. melo agrestis*, antioxidant enzyme actions amplified with the growing level of salinity level as well as heavy metal. Superoxide dismutase was increased in all levels of salt, heavy metal, and combined salt and heavy metals in equated to control. An all-out increase in Superoxide dismutase was documented in plants under high salt combined with high heavy metal stress (400mM NaCl + 200uM $NiCl_2$) and the least SOD level was detected in the plants in lower salt combined with lower metal (100mM NaCl + 50uM $NiCl_2$). Catalase antioxidant enzyme activities increased with an increase in combined salt and metal stress levels. A maximum increase in catalase was recorded in plants grown-up in high metal stress combined with high salt (400mM NaCl + 200uM $NiCl_2$). Oxidative stress in salt-tolerant plants was directly related to characteristics such as catalase and superoxide dismutase activity. The augmented commotion of the enzymes like CAT and SOD at higher salinities showed a significant correlation between plant tolerance levels and these antioxidant systems (Saddam Hussain, Khan, Hussain, &

Nie, 2016). Metal toxicity is allied with oxidative stress indicated by the boost in the quantity of hydroxyl radicals (OH^-), superoxide dismutase, and catalase (Zhang et al., 2017).

CONCLUSION

C. melo agrestis L. can tolerate the stresses exerted by salinity and heavy metals combined stress. Lower levels of stress positively affect *C. melo agrestis* L. by enhancing its morphological and physiological parameters. However higher stress levels adversely affect the germination, morphology, and physiology of plant *C. melo agrestis* L. Lower levels of salts (100mM) and Nickel (50uM) were beneficial for the growth parameters. Production of ROS like superoxide dismutase (SOD) and catalase (CAT) indicates its strategy for the amelioration of negative effects of salts and heavy metals. *C. melo agrestis* L. may be suggested for the removal of salt and heavy metals from contaminated soils after a complete study of its allelopathic effects. This research will open a gateway to the researchers to work on allelopathic impacts of *C. melo agrestis* under control and stress conditions.

Acknowledgements Author is thankful to all concerned for technical support and assistance in the conductance of the experiment and completion of the research work. This manuscript is part of the Ph.D thesis of Mr. Hassan Raza Javeed.

Conflict of interest Authors has no conflict of interest.

REFERENCES

Adibah, F. S. F., Jahan, M. S., & Fatihah, H. N. N. (2020). Betaine-rich Nano fertilizer improves growth parameters of Zea mays var. saccharata and Arabidopsis thaliana under salt stress. *Bulg. J. Agric. Sci*, *26*(1), 177-185.

Agarwal, P., Baraiya, B. M., Joshi, P. S., Patel, M., Parida, A. K., & Agarwal, P. K. (2021). AlRab7 from Aeluropus lagopoides ameliorates ion toxicity in transgenic tobacco by regulating hormone signaling and reactive oxygen species homeostasis. *Physiologia Plantarum*, *173*(4), 1448-1462.

Akeel, A., & Jahan, A. (2020). Role of cobalt in plants: its stress and alleviation. *Contaminants in Agriculture: Sources, Impacts and Management*, 339-357.

Al Murad, M., Khan, A. L., & Muneer, S. (2020). Silicon in horticultural crops: cross-talk, signaling, and tolerance mechanism under salinity stress. *Plants*, *9*(4), 460.

Alipour, M., Saharkhiz, M. J., Niakousari, M., & Damyeh, M. S. (2019). Phytotoxicity of encapsulated essential oil of rosemary on germination and morphophysiological features of amaranth and radish seedlings. *Scientia Horticulturae, 243*, 131-139.

Alsaeedi, A. H., El-Ramady, H., Alshaal, T., El-Garawani, M., Elhawat, N., & Almohsen, M. (2017). Engineered silica nanoparticles alleviate the detrimental effects of Na+ stress on germination and growth of common bean (Phaseolus vulgaris). *Environmental Science and Pollution Research*, *24*, 21917-21928.

Amin, A., Sarwar, A., Saleem, M. A., Latif, Z., & Opella, S. J. (2019). Expression and purification of transmembrane protein MerE from mercury-resistant Bacillus cereus.

Amjad, M., Ameen, N., Murtaza, B., Imran, M., Shahid, M., Abbas, G., . . . Jacobsen, S. E. (2020). Comparative physiological and biochemical evaluation of salt and nickel tolerance mechanisms in two contrasting tomato genotypes. *Physiologia Plantarum, 168*(1), 27-37.

Anaya, F., Fghire, R., Wahbi, S., & Loutfi, K. (2018). Influence of salicylic acid on seed germination of Vicia faba L. under salt stress. *Journal of the Saudi Society of Agricultural Sciences, 17*(1), 1-8.

Aqeel, M., Khalid, N., Tufail, A., Ahmad, R. Z., Akhter, M. S., Luqman, M., . . . Hashem, M. (2021). Elucidating the distinct interactive impact of cadmium and nickel on growth, photosynthesis, metal-homeostasis, and yield responses of mung bean (Vigna radiata L.) varieties. *Environmental Science and Pollution Research*, *28*, 27376-27390.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant physiology, 24*(1), 1.

Barraza, F., Maurice, L., Uzu, G., Becerra, S., López, F., Ochoa-Herrera, V., . . . Schreck, E. (2018). Distribution, contents and health risk assessment of metal (loid) s in small-scale farms in the Ecuadorian Amazon: An insight into impacts of oil activities. *Science of the Total Environment, 622*, 106-120.

Bates, L., Waldren, R. a., & Teare, I. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil, 39*, 205-207.

Bazihizina, N., Colmer, T. D., Cuin, T. A., Mancuso, S., & Shabala, S. (2019). Friend or foe? Chloride patterning in halophytes. *Trends in plant science, 24*(2), 142-151.

Boyd, M. (2020). *Effects of nickel toxicity on expression of genes associated with nickel resistance in white spruce (Picea glauca): Nickel translocation in plant tissues.* Laurentian University of Sudbury,

Chandraju, S., Basavaraju, H., & Kumar, C. C. (2008). Investigation of impact of irrigation of distillery spentwash on the nutrients of pulses. *Asian Journal of Chemistry, 20*(8), 6342.

Çimrin, K. M., Türkmen, Ö., Turan, M., & Tuncer, B. (2010). Phosphorus and humic acid application alleviate salinity stress of pepper seedling. *African Journal of Biotechnology, 9*(36).

Delpire, E., & Staley, K. J. (2014). Novel determinants of the neuronal Cl− concentration. *The Journal of physiology, 592*(19), 4099-4114.

Di Martino, C., Delfine, S., Pizzuto, R., Loreto, F., & Fuggi, A. (2003). Free amino acids and glycine betaine in leaf osmoregulation of spinach responding to increasing salt stress. *New Phytologist, 158*(3), 455-463.

Ebrahimi, M., Zamani, G. R., & Alizadeh, Z. (2017). Investigation of qualitative traits and evaluation of flower yield of pot marigold (Calendula officinalis L.) during its growth period under drought stress. *Environmental Stresses in Crop Sciences, 10*(2), 293-306.

El-Esawi, M. A., Elkelish, A., Soliman, M., Elansary, H. O., Zaid, A., & Wani, S. H. (2020). Serratia marcescens BM1 enhances cadmium stress tolerance and phytoremediation potential of soybean through modulation of osmolytes, leaf gas exchange, antioxidant machinery, and stress-responsive genes expression. *Antioxidants, 9*(1), 43.

Emamverdian, A., Ding, Y., Mokhberdoran, F., & Xie, Y. (2015). Heavy metal stress and some mechanisms of plant defense response. *The scientific world journal, 2015*.

Ermolenko, A., Vikulova, M., Shevelev, A., Mastalygina, E., Ogbuna Offor, P., Konyukhov, Y., . . . Burmistrov, I. (2020). Sorbent based on polyvinyl butyral and potassium polytitanate for purifying wastewater from heavy metal ions. *Processes, 8*(6), 690.

Essa, T. (2002). Effect of salinity stress on growth and nutrient composition of three soybean (Glycine max L. Merrill) cultivars. *Journal of Agronomy and Crop science, 188*(2), 86-93.

Fernandes, J. P., Mucha, A. P., Francisco, T., Gomes, C. R., & Almeida, C. M. R. (2017). Silver nanoparticles uptake by salt marsh plants–Implications for phytoremediation processes and effects in microbial community dynamics. *Marine pollution bulletin, 119*(1), 176-183.

Gangola, M. P., & Ramadoss, B. R. (2018). Sugars play a critical role in abiotic stress tolerance in plants. In *Biochemical, physiological and molecular avenues for combating abiotic stress tolerance in plants* (pp. 17-38): Elsevier.

Geilfus, C.-M. (2018). Chloride: from nutrient to toxicant. *Plant and Cell Physiology, 59*(5), 877-886.

Ghazanfar, S., Komal, A., Waseem, A., Hassan, W., Iqbal, R. J., Toor, S., . . . Tarar, Z. H. (2021). Physiological effects of nickel contamination on plant growth. *NVEO-NATURAL VOLATILES & ESSENTIAL OILS Journal| NVEO*, 13457-13469.

Gul, S., Nawaz, M. F., & Azeem, M. (2016). Interactive effects of salinity and heavy metal stress on ecophysiological responses of two maize (Zea mays L.) cultivars. *FUUAST Journal of Biology, 6*(1), 81-87.

Handa, N., Kohli, S. K., Kaur, R., Sharma, A., Kumar, V., Thukral, A. K., . . . Bhardwaj, R. (2018). Role of compatible solutes in enhancing antioxidative defense in plants exposed to metal toxicity. *Plants under metal and metalloid stress: responses, tolerance and remediation*, 207-228.

Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J., & Ahmad, A. (2012). Role of proline under changing environments: a review. *Plant signaling & behavior, 7*(11), 1456-1466.

Hussain, S., Khan, F., Hussain, H. A., & Nie, L. (2016). Physiological and biochemical mechanisms of seed priming-induced chilling tolerance in rice cultivars. *Frontiers in plant science, 7*, 116.

Hussain, S., Rao, M. J., Anjum, M. A., Ejaz, S., Zakir, I., Ali, M. A., . . . Ahmad, S. (2019). Oxidative stress and antioxidant defense in plants under drought conditions. *Plant abiotic stress tolerance: Agronomic, molecular and biotechnological approaches*, 207-219.

Jan, Y.-H., Heck, D. E., Laskin, D. L., & Laskin, J. D. (2020). DNA damage signaling in the cellular responses to mustard vesicants. *Toxicology letters, 326*, 78-82.

Johnson, W. C., & Mullinix, B. G. (2002). Weed management in watermelon (Citrullus lanatus) and cantaloupe (Cucumis melo) transplanted on polyethylene-covered seedbeds. *Weed Technology, 16*(4), 860-866.

Jusiak, B., Cleto, S., Perez-Piñera, P., & Lu, T. K. (2016). Engineering synthetic gene circuits in living cells with CRISPR technology. *Trends in biotechnology, 34*(7), 535-547.

Kader, M. A., & Lindberg, S. (2010). Cytosolic calcium and pH signaling in plants under salinity stress. *Plant signaling & behavior, 5*(3), 233-238.

Kafi, M., Asadi, H., & Ganjeali, A. (2010). Possible utilization of high-salinity waters and application of low amounts of water for production of the halophyte Kochia scoparia as alternative fodder in saline agroecosystems. *Agricultural water management, 97*(1), 139-147.

Kahlon, S. K., Sharma, G., Julka, J., Kumar, A., Sharma, S., & Stadler, F. J. (2018). Impact of heavy metals and nanoparticles on aquatic biota. *Environmental chemistry letters, 16*, 919-946.

Kamran, M. A., Eqani, S. A. M. A. S., Bibi, S., Xu, R.-k., Monis, M. F. H., Katsoyiannis, A., . . . Chaudhary, H. J. (2016). Bioaccumulation of nickel by E. sativa and role of plant growth promoting rhizobacteria (PGPRs) under nickel stress. *Ecotoxicology and environmental safety, 126*, 256-263.

Kanwal, F., Tahir, A., Shah, S. A. Q., Tsuzuki, T., Nisbet, D., Chen, J., & Rehman, Y. (2020). Effect of phyto-fabricated nanoscale organic-iron complex on photo-fermentative hydrogen production by Rhodopseudomonas palustris MP2 and Rhodopseudomonas palustris MP4. *Biomass and Bioenergy, 140*, 105667.

KAOUTAR, K., CHETTO, O., BENIKEN, L., BENKIRANE, R., & HAMID, B. (2021). SCREENING TEST In vitro BY THE APPLICATION OF SALT STRESS ON FRIABLE CALLUSES OUTCOME FROM FOUR GENOTYPES OF CITRUS ROOTSTOCKS. *PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY*, 8-20.

Karami Mehrian, S., Heidari, R., & Rahmani, F. (2015). Effect of silver nanoparticles on free amino acids content and antioxidant defense system of tomato plants. *Indian Journal of Plant Physiology, 20*, 257-263.

Khan, M. N., Siddiqui, M. H., Mukherjee, S., Alamri, S., Al-Amri, A. A., Alsubaie, Q. D., . . . Ali, H. M. (2021). Calcium-hydrogen sulfide crosstalk during K+-deficient NaCl stress operates through regulation of Na+/H+ antiport and antioxidative defense system in mung bean roots. *Plant Physiology and Biochemistry, 159*, 211-225.

Khan, N., Ali, S., Zandi, P., Mehmood, A., Ullah, S., Ikram, M., . . . Babar, M. (2020). Role of sugars, amino acids and organic acids in improving plant abiotic stress tolerance. *Pak. J. Bot, 52*(2), 355-363.

Kotagiri, D., & Kolluru, V. C. (2017). Effect of salinity stress on the morphology and physiology of five different Coleus species. *Biomedical and Pharmacology Journal, 10*(4), 1639-1649.

Kumar, A., Singh, S., Gaurav, A. K., Srivastava, S., & Verma, J. P. (2020). Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. *Frontiers in Microbiology, 11*, 1216.

Kumar, D., Malik, D., Patel, S. L., & Gupta, V. (2019). Human health risk assessment and mitigation of heavy metal pollution in agriculture and environment. *Contaminants in Agriculture and Environment: Health Risks and Remediation, 1*, 66-75.

Kumari, S., Chhillar, H., Chopra, P., Khanna, R. R., & Khan, M. I. R. (2021). Potassium: A track to develop salinity tolerant plants. *Plant Physiology and Biochemistry, 167*, 1011-1023.

Kurtar, E. S., Balkaya, A., & Kandemir, D. (2016). Evaluation of haploidization efficiency in winter squash (Cucurbita maxima Duch.) and pumpkin (Cucurbita moschata Duch.) through anther culture. *Plant Cell, Tissue and Organ Culture (PCTOC), 127*, 497-511.

Kusvuran, S., & Dasgan, H. Y. (2017). Drought induced physiological and biochemical responses in Solanum lycopersicum genotypes differing to tolerance. *Acta scientiarum polonorum hortorum cultus, 16*(6), 19–27-19–27.

Le Saux, A., David, E., Betoulle, S., Bultelle, F., Rocher, B., Barjhoux, I., & Cosio, C. (2020). New insights into cellular impacts of metals in aquatic animals. *Environments, 7*(6), 46.

Liu, Y., Zhao, Y., & Wang, J. (2021). Fenton/Fenton-like processes with in-situ production of hydrogen peroxide/hydroxyl radical for degradation of emerging contaminants: Advances and prospects. *Journal of Hazardous Materials, 404*, 124191.

López, M. L., Peralta-Videa, J. R., Benitez, T., & Gardea-Torresdey, J. L. (2005). Enhancement of lead uptake by alfalfa (Medicago sativa) using EDTA and a plant growth promoter. *Chemosphere, 61*(4), 595-598.

Majeed, A., & Muhammad, Z. (2019). Salinity: a major agricultural problem—causes, impacts on crop productivity and management strategies. *Plant abiotic stress tolerance: Agronomic, molecular and biotechnological approaches*, 83-99.

Mariod, A., & Matthaeus, B. (2008). Fatty acids, tocopherols, sterols, phenolic profiles and oxidative stability of Cucumis melo var. agrestis oil. *Journal of Food Lipids, 15*(1), 56-67.

Molas, J. (1998). Changes in morphological and anatomical structure of cabbage (Brassica oleracea L.) outer leaves and in ultrastructure of their chloroplasts caused by an in vitro excess of nickel. *Photosynthetica, 34*, 513-522.

Morales-Díaz, A. B., Ortega-Ortíz, H., Juárez-Maldonado, A., Cadenas-Pliego, G., González-Morales, S., & Benavides-Mendoza, A. (2017). Application of nanoelements in plant nutrition and its impact in ecosystems. *Advances in Natural Sciences: Nanoscience and Nanotechnology, 8*(1), 013001.

Nadia, D., Emanuel, M., & Robinson, D. (2021). The efficacy of alternative (biorational) insecticides in suppressing damage caused by insect pests affecting callaloo, Amaranth xanthosoma and pak choy, Brassica rapa, production in Jamaica. *Horticult Int J, 5*(2), 51-59.

Negrão, S., Schmöckel, S., & Tester, M. (2017). Evaluating physiological responses of plants to salinity stress. *Annals of botany, 119*(1), 1-11.

Ni, S.-Q., Ni, J., Yang, N., & Wang, J. (2013). Effect of magnetic nanoparticles on the performance of activated sludge treatment system. *Bioresource technology, 143*, 555-561.

Noreen, S., Fatima, Z., Ahmad, S., Athar, H.-u.-R., & Ashraf, M. (2018). Foliar application of micronutrients in mitigating abiotic stress in crop plants. *Plant nutrients and abiotic stress tolerance*, 95-117.

Okereafor, U., Makhatha, M., Mekuto, L., Uche-Okereafor, N., Sebola, T., & Mavumengwana, V. (2020). Toxic metal implications on agricultural soils, plants, animals, aquatic life and human health. *International journal of environmental research and public health, 17*(7), 2204.

Ors, S., & Suarez, D. L. (2017). Spinach biomass yield and physiological response to interactive salinity and water stress. *Agricultural water management, 190*, 31-41.

Ouzounidou, G., Moustakas, M., Symeonidis, L., & Karataglis, S. (2006). Response of wheat seedlings to Ni stress: effects of supplemental calcium. *Archives of Environmental Contamination and Toxicology, 50*, 346-352.

Riaz, M. U., Ayub, M. A., Khalid, H., ul Haq, M. A., Rasul, A., ur Rehman, M. Z., & Ali, S. (2020). Fate of micronutrients in alkaline soils. *Resources use efficiency in agriculture*, 577-613.

Rinklebe, J., & Shaheen, S. M. (2014). Assessing the mobilization of cadmium, lead, and nickel using a seven-step sequential extraction technique in contaminated floodplain soil profiles along the central Elbe River, Germany. *Water, Air, & Soil Pollution, 225*, 1-20.

Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J. A., Hilal, M., & Prado, F. E. (2009). Soluble sugars: Metabolism, sensing and abiotic stress: A complex network in the life of plants. *Plant signaling & behavior, 4*(5), 388-393.

Saleem, M., Fariduddin, Q., & Janda, T. (2021). Multifaceted role of salicylic acid in combating cold stress in plants: A review. *Journal of Plant Growth Regulation, 40*, 464-485.

Sanjosé, I., Navarro-Roldán, F., Infante-Izquierdo, M. D., Martínez-Sagarra, G., Devesa, J. A., Polo, A., . . . Muñoz-Rodríguez, A. F. (2021). Accumulation and effect of heavy metals on the germination and growth of Salsola vermiculata L. seedlings. *Diversity, 13*(11), 539.

Saxena, G., Purchase, D., Mulla, S. I., Saratale, G. D., & Bharagava, R. N. (2020). Phytoremediation of heavy metal-contaminated sites: eco-environmental concerns, field studies, sustainability issues, and future prospects. *Reviews of Environmental Contamination and Toxicology Volume 249*, 71-131.

Shah, Z. H., Rehman, H. M., Akhtar, T., Daur, I., Nawaz, M. A., Ahmad, M. Q., . . . Chung, G. (2017). Redox and ionic homeostasis regulations against oxidative, salinity and drought stress in wheat (a systems biology approach). *Frontiers in genetics, 8*, 141.

Shahid, M. A., Sarkhosh, A., Khan, N., Balal, R. M., Ali, S., Rossi, L., . . . Garcia-Sanchez, F. (2020). Insights into the physiological and biochemical impacts of salt stress on plant growth and development. *Agronomy, 10*(7), 938.

Shahzad, B., Tanveer, M., Rehman, A., Cheema, S. A., Fahad, S., Rehman, S., & Sharma, A. (2018). Nickel; whether toxic or essential for plants and environment-A review. *Plant Physiology and Biochemistry, 132*, 641-651.

Shamizadeh, H., Alinejad Shahabi, R., & Arjmand, M. (2018). Modeling the Chlorine Gas Dispersion in the Water Treatment Plant. *Journal of Chemical Health Risks, 8*(2).

Sharma, A., Kumar, V., Shahzad, B., Ramakrishnan, M., Singh Sidhu, G. P., Bali, A. S., . . . Khanna, K. (2020). Photosynthetic response of plants under different abiotic stresses: a review. *Journal of Plant Growth Regulation, 39*, 509-531.

Shelke, D., Nikalje, G., Nikam, T., Maheshwari, P., Punita, D., Rao, K., . . . Suprasanna, P. (2019). Chloride (Cl−) uptake, transport, and regulation in plant salt tolerance. *Molecular plant abiotic stress: biology and biotechnology*, 241-268.

Stępień, P., & Kłbus, G. (2006). Water relations and photosynthesis in Cucumis sativus L. leaves under salt stress. *Biologia Plantarum, 50*, 610-616.

Subba Rao, N., Marghade, D., Dinakar, A., Chandana, I., Sunitha, B., Ravindra, B., & Balaji, T. (2017). Geochemical characteristics and controlling factors of chemical composition of groundwater in a part of Guntur district, Andhra Pradesh, India. *Environmental earth sciences*, *76*, 1-22.

Sultana, R., Adnan, M. Y., & Ali, H. (2019). Salicylic acid seed priming modulates some biochemical parametrs to improve germination and seedling growth of salt stressed wheat (Triticum aestivum L.). *Pak. J. Bot, 51*(2), 385-391.

Swamy, K. (2017). Origin, distribution and systematics of culinary cucumber (Cucumis melo subsp. agrestis var. conomon). *Journal of Horticultural Sciences, 12*(1), 1-22.

Taghipour, M., & Jalali, M. (2019). Impact of some industrial solid wastes on the growth and heavy metal uptake of cucumber (Cucumis sativus L.) under salinity stress. *Ecotoxicology and environmental safety, 182*, 109347.

Taha, R. M., & Abd El-Samad, H. M. (2022). The Impact of Minerals on Wheat Plants Grown under Salinity Stress. *American Journal of Plant Sciences, 13*(4), 541-556.

Tang, Y., Zhang, C., Cao, S., Wang, X., & Qi, H. (2015). The effect of CmLOXs on the production of volatile organic compounds in four aroma types of melon (Cucumis melo). *Plos one, 10*(11), e0143567.

Turan, M. A., Elkarim, A. H. A., Taban, N., & Taban, S. (2010). Effect of salt stress on growth and ion distribution and accumulation in shoot and root of maize plant. *African Journal of Agricultural Research, 5*(7), 584-588.

Tzortzakis, N., Chrysargyris, A., & Petropoulos, S. (2018). Phytochemicals content and health effects of cultivated and underutilized species of the cucurbitaceae family. *Phytochemicals in vegetables: A valuable source of bioactive compounds, 99*.

Ulas, A., Aydin, A., Ulas, F., Yetisir, H., & Miano, T. F. (2020). Cucurbita rootstocks improve salt tolerance of melon scions by inducing physiological, biochemical and nutritional responses. *Horticulturae, 6*(4), 66.

Van Zelm, E., Zhang, Y., & Testerink, C. (2020). Salt tolerance mechanisms of plants. *Annual review of plant biology, 71*, 403-433.

Vishal, B., Krishnamurthy, P., Ramamoorthy, R., & Kumar, P. P. (2019). Os TPS 8 controls yield-related traits and confers salt stress tolerance in rice by enhancing suberin deposition. *New Phytologist, 221*(3), 1369-1386.

Yadav, N., Kumar, A., Kumar, N., Kumar, S., & Arya, S. (2022). Impacts on Plant Growth and Development Under Stress. In *Plant Stress Mitigators: Action and Application* (pp. 61-100): Springer.

Yasmeen, R., & Siddiqui, Z. S. (2018). Ameliorative effects of Trichoderma harzianum on monocot crops under hydroponic saline environment. *Acta Physiologiae Plantarum, 40*, 1-14.

Yemm, E., & Willis, A. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemical journal, 57*(3), 508.

Zhang, J., Hao, H., Chen, M., Wang, H., Feng, Z., & Chen, H. (2017). Hydrogen-rich water alleviates the toxicities of different stresses to mycelial growth in Hypsizygus marmoreus. *Amb Express, 7*, 1-11.

Zulfiqar, F., & Ashraf, M. (2021). Nanoparticles potentially mediate salt stress tolerance in plants. *Plant Physiology and Biochemistry, 160*, 257-268.

AUTHORS

Corresponding Author: Saqib Ullah, M.Phil. Department of Botany, Islamia College Peshawar, Pakistan.
First Author – Hassan Raza Javeed, Ph.D. Department of Botany, The Islamia University of Bahawalpur, Punjab, Pakistan.
Second Author – Nargis Naz, Ph.D. Professor, Department of Botany, The Islamia University of Bahawalpur, Punjab, Pakistan
Third Author – Saqib Ullah, M.Phil. Department of Botany, Islamia College Peshawar, Pakistan.