COMPARATIVE ANALYSIS OF SALT AND HEAVY METAL STRESS RESPONSES IN *CITRULLUS COLOCYNTHIS* (L.) SCHRAD AND *CUCUMIS MELO* SUBSPECIES *AGRESTIS* (NAUD) FOR PHYTOREMEDIATION APPLICATIONS

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Abstract. The pursuit of resistant plant varieties is indispensable for phytoremediation against salt and heavy metal stresses. *Citrullus colocynthis* (L.) Schrad and *Cucumis melo* subspecies *agrestis* are cucurbit weeds used as raw material in for medicines, papers and artificial cotton. Abiotic stresses like salt and heavy metal hassles are prodigious deterrents to early growth of plants. Nickel (Ni), in petite is a decisive micronutrient of plants but disturbs the proficiency of the plants under higher levels. However, response of various plant ecotypes varies under various concentrations of salinity and heavy metals. Germination, morphology, biochemistry and physiological properties are key to determining the fate of the plant. The present objective was to compare the impact of independent stresses by varying concentrations of salt (100, 200 and 400 mM NaCl) and heavy metal (50, 100 and 200 μM $NiCl_2$) on morphophysiological and biochemical parameters of *Citrullus colocynthis* (L.) Schrad and *Cucumis melo* subspecies *agrestis* from desert and agricultural ecotypes. It was a four-replica CRD trial. Germination percentage, radicle and plumule length, seedling fresh and dry weight, K^+, Ca^{++}, chlorophylls a and b showed a significat increase ($p \leq 0.05$) at lower level of stress (100 mM NaCl and 50 μM $NiCl_2$), slight decrease at moderate level of stress (200 mM NaCl and 100 μM $NiCl_2$) while significant decrease under higher stress levels (400 mM NaCl and 200 μM $NiCl_2$). An increase of Na^+, Cl^- , Ni^+ and secondary metabolites (total soluble sugars, total soluble proteins, catalase, superoxide dismutase and proline) with increasing stress levels was perceived. *Citrullus colocynthis* (L.) Schrad and *Cucumis melo* subspecies *agrestis* are adaptive to salt and heavy metal levels.Tolerance of salts and heavy metal metals in sequence *C. colocynthis* (L.) desert > *C. colocynthis* (L) cultivated > *C. melo* (L) *agrestis* desert > *C. melo* (L) *agrestis* agricultural was recorded and can be recommended for phytoremediation.

Keywords: *abiotic stress, biochemical systematics, Citrullus colocynthis, Cucumis melo agrestis, nickel, heavy metal, phytoremediation, catalase, thal desert, Cucurbitaceae, NaCl, $NiCl_2$*

Abbreviations. HMs: Heavy Metals; CRD: Completely Randomized Design; SOD: Superoxide dismutase; CAT: Catalase

Introduction

Globally, food crops are facing a severe challenge due to the dynamics of climate change (Skendzic et al., 2021; Ahmad et al.,2023). Increasing contagion of arable lands through with heavy metals is a crucial factor that not only affects the growth and yield of plants but also causes serious risks to human health (Rehman et al., 2018). During two decades (1990-2013), salinity in cultivated or irrigated land has amplified upto 37% (Qadir et al., 2014). Plants require an adequate supply of minerals for proper growth and to improve the depressing effects instigated by stress situations (Sharma et al., 2019). Saline soils have been estimated to be about 830 million hectares in the world (Rengasamy, 2016), and at least 20% of all cultivated irrigated lands are affected by salts (Shahid et al., 2018).

Seed germination of weeds is mediated by a variety of environmental factors such as osmotic and salt stresses (Rao et al., 2008; Kegode et al., 2010). Increasing salinity delayed and reduced the germination percentage of *Limonium emarginatum* and inhibited germination entirely above 2% salinity (Melendo and Giménez, 2019). While most plant types among crops show higher tolerance to salt stress at the time of germination. Plants respond to stresses variably, such as with modifications in plant morphology, growth configuration, and resistance mechanisms (Fahad et al., 2017; Zandalinas et al., 2018). Salinity stress induces oxidative damage to cellular macromolecules and disturbs redox homeostasis (Tahjib-Ul-Arif et al., 2022). The salinity that affects agricultural productivity with a massive loss (Sharma and Singh, 2015) may be due to irrigation or dry-land salinity (Rengasamy, 2016). The salinity stress has affected both the water absorption and the biochemical processes which lead to reduced vegetation (Safdar et al., 2019). The salinity, that influences seed germination through reduction or inhibition fluctuates with varieties, the genotype of plants, ecological circumstances, osmotic potential, and specific ion categories (Safdar et al., 2019). Depleted salt soil initiates and terminates germination effectively while the final germination rate is reduced or inhibited above 2% salt (Islam et al., 2022).

Among heavy metals, some (Fe, Ni, Cu, and Zn) are necessary for flora and fauna (Asati et al., 2016), their availability varies, and such metals are considered essential micronutrients (Khan et al., 2015), whose excess to the plant results in poisonous effects (Kalaivanan et al., 2016; Mbarki et al., 2022). The primary factors that impede plant growth are the absence of nutrients, reduced root development, and the generation of reactive oxygen species (Zaheer et al., 2021).The effects of heavy metal contamination have proved to be devastating to ecological parameters and the diversity of organisms (Xian et al., 2015). The presence of heavy metals and salt pollutants over a long period may accumulate in the body of organisms by various mechanisms to various extents and may be toxic or not (Chiarelli and Roccheri, 2014). Presently, extensive industrialization divulges detrimental effects on the atmosphere, lithosphere, and crop productivity by heavy metal accumulation (Kotecha et al., 2019). Heavy metals environmental contamination is a global disaster that is associated with anthropological activities and functions like mining, power transmission, smelting, sludge dumping, energy, and fuel production (Saxena, 2021). Although, the methods for cleaning up of contaminated environment including soil and water are usually expensive and do not give optimum results. Currently, phytoremediation is an effective and affordable technology used to remove inactive metals and metal pollutants from contaminated soil and water. It includes phytoextraction, rhizofiltration, phytostabilization, phytovolatization, and phytodegradation/ phytotransformation. This technology is ecofriendly and exploits the ability of plants to remediate pollutants from contaminated sites. More than 400 plant species have been identified to have potential for soil and water remediation. Among them, Thlaspi, Brassica, Sedum alfredii H., and Arabidopsis species have been mostly studied. Our paper aims to cover the causes of HM pollution and phytoremediation technology, including HM uptake mechanism and several reports describing its application at field level (Wani et al., 2012). The plants having the capability to repel heavy metals and adsorb high amounts may assist in the improvement of infected soils (Barra and Terenzi, 2021). Chemical Chelator like EDTA has the capability to boost up the uptake by dissolving components of metals and has proven to enhance the metal accumulation. Addition of EDTA results in increase in plant growth parameters, dry matter stress tolerance index and accumulation of different metals such as Cd, Zn and Pb. Chemically enhanced phytoremediation has been recognized as one of the most beneficial, effective and economically viable method of bioremediation (Mujahid et al., 2013).

The plants belonging to Family Cucurbitaceae the family of cucurbitaceae are widespread whose many species have dispersed all over the world (He et al., 2021). *Citrullus colocynthis* (L) Schrad is a member of the gourd family Cucurbitaceae (Shaheen et al., 2020), known as hindal (hanzal) in the Arabic language, bitter apple in the English language (Asid et al., 2015), indrain or tumma or tumba in common Hindi and Urdu versions (Kumar et al., 2009). In traditional folk medicines, *C. colocynthis* (L.) Schrad is widely used by rural inhabitants as a purgative, anti-neoplastic, anti-diabetic, anti-rheumatic, and anti-allergic (Shafaei et al., 2012). Dehydrated pulp and seed excerpt of *C. colocynthis* (L.) Schrad are used against constipation and diabetes (Akbar, 2020). *Cucumis melo* (L.) subspecies *agrestis* (Family Cucurbitaceae) is used in easy soy vinegar and sauce, grapevine, and as a raw material in papers and artificial cotton. *C. melo* (L.) subspecies *agrestis* is a branched, prostrate annual herb mostly permeating cotton, maize, pearl millet, and sorghum. One of the most critical phases in the life of this weed is its germination, emergence or seedling development (Shatpathy et al., 2018), and this is affected by several ecological factors such as osmotic and salt stress (Yang and Guo, 2018).

Salinity stress reduces the uptake and accumulation of nutrients in plants and results in nutritional imbalances (Paul and Lade, 2014; El Sabagh et al., 2021). Ion homeostasis and compartmentalization are crucial processes for growth and seedling development is also affected under salt stress (Arif et al., 2020). Biosynthesis and accumulation of compatible solutes like Proline (Sarker, 2018) and sugars (D'Amelia et al., 2018) under salinity stress in the plants are associated with the external osmolarity, which shields the structure of cells by maintaining osmotic balance through continuous water influx (Kunte, 2009). Variations in salt and metal also give rise to the synthesis of ROS (reactive oxygen species) e.g., superoxide radical (O^{2-}), (O_2^-), hydroxyl radical (OH^-), and H_2O_2 resulting in disturbance of the redox homeostasis (Xie et al., 2019). Antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD) are thought to positively minimize the

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salinity stress (Shah et al., 2021). SOD recruits the process of ROS cleansing by converting superoxide to hydrogen peroxide (Di et al., 2018).

As the salinity and heavy metal stress are the main abiotic stresses against germination and early development of the plant, phytoremediation is an eco-friendly way to mitigate the adverse impact of these pollutants, the experiment was conducted to explore the plant species have the ability to cope with the hazardous impacts of salts and heavy metals as well as reclaimation of polluted soils. *C. colocynthis* (L) Schrad and *C. melo* (L.) subspecies *agrestis* are medicinal cucurbit weeds. The experiment was carried out to study the germination, early growth, morphology, biochemistry and physiological adaptations against variable levels of salt (NaCl) and heavy metal ($NiCl_2$) stresses in different ecotypes inhabiting agricultural and desert areas.

Materials and Method

The site of trials was the research area of Botanical Garden, Department of Botany, The Islamia University Bahawalpur in July, 2018.

Collection of seeds

Seeds of *C. colocynthis* (L) Schrad and *C. melo* (L.) subspecies *agrestis* were collected from different sites in Thal Desert and agricultural areas of District Layyah (30.9 N and 70.5 E), Punjab Pakistan and were designated as desert ecotype and agricultural land ecotype. The ripened fruits of these species were collected and seeds were obtained by removing the dried pulp (*Table 1*).

Table 1. *Soil analysis for pot experiment*

EC (ds/m)	pH	Organic matter (%)	Available phosphorus (ppm)	Available potassium (ppm)	Texture
1.96	8	0.54	5.9	110	Sandy loam

Experimental design

Completely randomized design (CRD) with the following factors (weed species, ecotypes, salinity and Heavy metal) and four replicates. Seedling parameters of both species were studied in the petri plate experiment while biochemical and physiological were studied in the pot experiment. Twenty seeds of one species were placed in each petri plate. All the petri plates were provided with Whatman's filter papers to maintain moisture and Hoagland solution to maintain other nutrients necessary for the germination of seeds. Variable levels of NaCl and $NiCl_2$ were selected and added to the corresponding petri plates. T stands for treatment while c for control Tc= Control, T1=100 mMol NaCl, T2=200 mMol NaCl, T3=400 mMol NaCl, T4=50 uMol $NiCl_2$, T5=100 uMol $NiCl_2$, T6=200 uMol $NiCl_2$.

Morphological characteristics

The seed germination percentage was calculated by Coolbear et al. (1984). After germination radicle length and plumule length of protruded seedlings was measured by measuring scale. Fresh weights and dry weights were measured at end of the experiment with the help of electronic weight balance with 100% accuracy of 1 mg.

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Biochemical parameters

Na^+, K^+, and Ca^{2+} cations were determined by a flame photometer (Jenway, PFP-7), and Cl^- content by chloride meter (Jenway, PCLM 3). To determine nickel content EDTA technique was used. Ni^+ (aq) ion reacts with concentrated Cl^- ion in the presence of Ethanol medium to give tetrachloronickel (ii) ion/ Ni Cl_4 $)^{2-}$, a blue color solution is attained. The chlorophylls a and b by the method of Arnon (1949).

Total soluble sugars were determined according to the method of Yemm and Willis (1954). Total soluble proteins were determined using the method of Lowry et al. (1951). Proline by the method of Bates et al. (1973), CAT actions by the method of Chance (1955), and activity of SOD by Giannopolitis and Ries method (1977). Results were analysed by ANOVA and all pairwise comparison using STATISTIX 8.1 software.

Results

NaCl stress

Effects of various levels of salt (NaCl) stresses were observed on two ecotypes of *C. melo* (L.) subspecies *agrestis* and *C. colocynthis* (L) Schrad. The response was recorded in terms of selected morphological, physiological and biochemical features according to the increased concentration of stress. Germination percentage, radicle length, plumule length of seedlings (cm), fresh and dry mass of seedlings (g) of *C. melo* (L.) subspecies *agrestis* in desert ecotype increased at lower level of salt (100 mM NaCl) while decreased gradually with a rise in the levels of salt stress (200 and 400 mM NaCl) in ccomparison to the control plant. In the case of agricultural ecotype, a slight increase was noted at 100 mM while considerable decrease was observed at higher level (400 mM) of salt stress. In both ecotypes of *C. colocynthis* (L.) Schrad a maximum increase in germination percentage and morphological parameters was observed at 100 mM then it gradually decreased as the stress level increased. *C. colocynthis* (L.) Schrad showed better germination percentage, radicle length, plumule length of seedlings (cm), fresh and dry mass of seedlings against salinity levels as compared to *C. melo* (L.) subspecies *agrestis*. Similarly desert ecotype showed better results of the above parameters than agricultural ecotype (*Figure 1* and *Table 2*).

Calcium and Potassium contents (µg/g) in both ecotypes of *C. melo* (L.) subspecies *agrestis* gradually decreased with increasing levels of salt. In *C. colocynthis* (L) Schrad, both ecotypes under single stress of salt showed a gradual decrease in potassium and calcium with increasing salt levels. However more calcium and potassium was found in desert ecotype of *C. colocynthis* (L.) Schrad than *C. melo* (L.) subspecies *agrestis*. Sodium and Chloride content (µg/g) in both ecotypes of *C. melo* (L.) subspecies *agrestis* and *C. colocynthis* (L.) Schrad under single stress of salt showed a gradual increase with increasing salt level (*Table 2* and *Figure 3*). Chlorophyll content in both ecotypes of *C. melo* (L.) subspecies *agrestis* and *C. colocynthis* (L.) Schrad increased at lower and moderate salt levels (100 and 200 mM) while decreased at high level 400 mM of salt. However *C. colocynthis* (L.) Schrad showed more amount of chlorophyll than *C. melo* (L.) subspecies *agrestis* under same level T1 and T3 of salt stress (*Figure 5* and *Table 3*).

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Table 2. *Analysis of variance for morphological and biochemical parameters of C. melo (L.) subspecies agrestis and C.colocynthis (L) Schrad from two habitats under different levels of Salt (NaCl) Stresses*

SV	d.f.	GP	SPL (cm)	SRL (cm)	SFW (g)	SDW (g)	K^+ (mg/g d.wt.)	Na^+ (mg/g d.wt.)	Ca^{2+} (mg/g d.wt.)	Cl^- (mg/g d.wt.)	Ni^+ (mg/g d.wt.)
Species	1	0.521**	48.000	0.4256**	1.1656*	0.37808**	0.06021	0.00047**	6.933E-33	0.00935**	0.00441
Habitat	1	963.021	108.000*	1.8881	1.1041**	0.02901	0.01613***	0.00025**	0.00270***	0.01880**	0.00333**
Species × Habitat	1	20.021**	12.000**	5.8241**	6.8403	1.70253**	0.01613**	0.00025**	0.00270**	0.01880***	0.00086**
Salt	3	161.465**	218.722	13.3537	25.1357	6.19347***	1.29495**	2.32367	0.76915	0.24757***	0.00333**
Species × Salt	3	12.576	2.389**	0.1455**	0.0870**	0.02295	0.00112**	0.00215**	3.056*	0.00413**	0.00023
Habitat × Salt	3	29.410**	2.389	0.7162	0.2506	0.05690**	0.00470	0.00031**	3.722E-04	0.00440	0.00008***
Species × Habitat × Salt	3	9.299*	2.389**	0.3062**	0.1999**	0.02085**	0.00112**	0.00031*	3.722***	0.00440**	0.00032**
Errors	30	0.613	1.082	0.4793	0.9590	0.24887	0.01215	0.00634	0.01952	0.00895	0.00008
Total	47										

Abbreviation: SV=Source of variance, GP=Germination percentage, SPL=Seedlings plumule length, SRL=Seedlings radicle length, SFW=Seedlings fresh weight, SDW=Seedling dry weight, *, **, *** = significance levels at 0.05, 0.01and 0.001, respectively, ns = non-significant

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Table 3. *Analysis of variance for biochemical and physiological parameters of C. melo (L.) subspecies agrestis and C.colocynthis (L) Schrad from two habitats under different levels of Salt (NaCl) Stresses*

SV	d.f.	Chlo.a (mg/g f.wt.)	Chlo.b (mg/g f.wt.)	TSS (µg/g f.wt.)	TSP (µg/g f.wt.)	SOD	CAT	Pro. (µg/g f.wt.)
Species	1	0.00366**	0.00144**	38116**	4.97083	4.02521**	12.480***	0.13490**
Habitat	1	2.221E-04	0.00152**	6473**	6.54835	3.25521	77.894	0.00016
Species × Habitat	1	4.028***	2.517	4650	8.81770	2.85187***	0.350***	0.00104**
Salt	3	0.00273**	0.00889***	193792**	7.72574	456.098	342.811	0.06567
Species × Salt	3	1.683**	1.801	44556**	4.39290	2.083**	5.092**	0.00982**
Habitat × Salt	3	1.083E-04	5.660E-05**	16519***	1.74839	0.00132	12.898**	0.00227**
Species × Habitat × Salt	3	4.238**	2.144**	16301**	3.08229	2.083**	0.079***	0.00196***
Errors	30	3.295	2.704	37	0.02584	0.22718	0.061	0.00010
Total	47							

Abbreviations, SV=Source of Variance, d.f.=Degree of freedom, Chlo=Chlorophyll, CAT=Catalase, Pro=Proline, SOD=Superoxide dismutase, TSP=Total soluble proteins, TSS= Total soluble sugars, *, **, *** = significance levels at 0.05, 0.01and 0.001, respectively, ns = non-significant

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Total soluble proteins increased with increasing salt stress in both ecotypes of both species. Maximum soluble proteins were observed in desert ecotype of C. colocynthis (L) Schrad at 400 mM of salt. Amount of soluble protein was more in C. colocynthis (L) Schrad as compared to C. melo (L.) subspecies agrestis.

Similarly more amount of soluble protein in desert ecotype than agricultural ecotype was observed. Total soluble sugars significantly increased at high level of salt while decreased at low level of salt in both ecotypes of both species. Maximum increase in soluble sugars were recorded in higher level of salt. The desert ecotype of *C. colocynthis* (L.) Schrad produced more amount of soluble sugars under single stress of salt as compared to agricultural ecotype (*Figure 5* and *Table 3*). Proline in *C. melo* (L.) subspecies *agrestis* increased with increasing salt levels. Maximum proline level was observed at high salt level of NaCl in both ecotypes of both species. While in *C. colocynthis* (L.) Schrad more proline under single stress of salt than *C. melo* (L.) subspecies *agrestis*. Desert ecotype produced more proline than agricultural ecotype under salt stress (*Figure 5* and *Table 3*). Catalase and superoxide dismutase in both ecotypes of *C. melo* (L.) subspecies *agrestis* under single stress of salt showed a significant increase at high level (400 mM NaCl) while slight increase at low and moderate salt levels(100 and 200 NaCl). More increase in desert ecotype than agricultural ecotype under high salt level (400 mM NaCl) was observed. While in *C. colocynthis* (L.) Schrad desert ecotype catalase and superoxide dismutase enzyme was increased by increasing stress levels. A slight increase at a low level (100 mM NaCl) and significant increase at moderate and high salt level (200 and 400 mM NaCl). More amount of catalase and superoxide dismutase was produced in *C. colocynthis* (L.) Schrad than *C. melo* (L.) subspecies *agrestis* (*Figure 7* and *Table 3*).

Heavy metal stress ($NiCl_2$)

Germination percentage, radicle length of seedlings (cm), plumule length of seedlings (cm), and fresh and dry weight of seedlings of both ecotypes of both species increased at lower level (50 µM) of heavy metal while gradually decreased with increasing level (100 and 200 µM) of heavy metal stress as compared to the control plant. However, desert ecotype of *C. colocynthis* (L.) Schrad showed a maximum increase at lower level (50 µM) of $NiCl_2$ (*Figure 2* and *Table 4*). Calcium and Potassium (mg/g d.wt.) content in both ecotypes of both species gradually decreased with increasing levels of heavy metal. However, *C. colocynthis* (L.) Schrad showed less decrease than *C. melo* (L.) subspecies *agrestis* with increasing heavy metal at lower, moderate and high heavy metal levels (50, 100 and 200 µM $NiCl_2$). However less decrease in desert ecotype than agricultural ecotype (*Figure 4* and *Table 4*). Sodium, Chloride and Nickel (mg/g d.wt.) contents of *C. melo* (L.) subspecies *agrestis* desert ecotype under single stress of heavy metal showed a gradual increase with increasing heavy metal at lower, moderate and high heavy metal levels (50, 100 and 200 µM $NiCl_2$). While a huge increase was observed in agricultural ecotype under low, moderate and high levels of heavy metal. However, in *C. colocynthis* (L.) Schrad in shoot extract of both ecotypes under single stress of heavy metal showed a gradual increase with increasing heavy metal at lower, moderate and high heavy metal levels (50, 100 and 200 µM) of $NiCl_2$ (*Figure 4* and *Table 4*).

Chlorophyll a and b of *C. melo* (L.) subspecies *agrestis* increased at lower and moderate heavy metal levels (50 and 100 µM) while a slight decrease at high level (200 µM) of heavy metal. Desert ecotype performed more better than agricultural ecotype under heavy metal stress. In the case of *C. colocynthis* (L.) Schrad desert ecotype plants

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under single stress of heavy metal increased level of chlorophyll at low heavy metal level (50 µM $NiCl_2$) and gradual decrease at moderate and high heavy metal levels (100 and 200 µM $NiCl_2$). Desert ecotype plants showed more increased level of chlorophyll at low heavy metal level (50 µM $NiCl_2$) and lower decrease at moderate and high heavy metal levels (100 and 200 µM $NiCl_2$) better as compared to agricultural ecotype (*Figure 6* and *Table 5*).

Total soluble proteins in *Cucumis melo agrestis* increased by increasing stress level of heavy metal. Maximum soluble proteins were observed in desert ecotype at 200 uM of heavy metal. However amount of total soluble proteins in agricultural ecotype was lesser than desert ecotype. The desert ecotype of *C. colocynthis* (L) Schrad under single stress of heavy metal showed significant increase in amount of soluble proteins at higher level of heavy metal (200 uM $NiCl_2$). Agricultural ecotype of *C. colocynthis* (L.) Schrad showed increase in soluble proteins at 200 uM but lesser than desert ecotye (*Figure 6* and *Table 5*). Total soluble sugars in *Cucumis melo agrestis* increased with increasing level of heavy metal in both ecotypes while desert ecotype has produced more amount of total soluble sugas as compared to agricultural ecotype. In comparison with control, the desert ecotype of *Citrullus colocynthis* (L.) schrad produced more amount of soluble sugars under single stress of heavy metal showed increase with increasing stress levels (*Figure 6* and *Table 5*). The proline level of *C. melo* (L.) subspecies *agrestis* was increased with increasing heavy metal levels. Maximum proline level was observed at high heavy metal level (200 uM of $NiCl_2$) in both ecotypes but desert ecotype produced more amount of proline as compared to agricultural ecotype. While in *C. colocynthis* (L.) Schrad proline under single stress of heavy metal showed increase with increasing stress of heavy metal levels. *C. colocynthis* (L.) Schrad produced higher amount of proline as compared to *Cucumis melo agrestis* and desert ecotype produced more proline than agricultural ecotype (*Figure 6* and *Table 5*).

The superoxide dismutase (u mol $m^{-2}s^{-1}$) level of *C. melo* (L.) subspecies *agrestis* gradually increased with increasing heavy metal levels in both ecotypes. A maximum amount of superoxide dismutase was observed at a high heavy metal level of 200 uM of $NiCl_2$.

Similarly in *C. colocynthis* (L.) Schrad superoxide dismutase enzymes (SOD) were increased by increasing stress levels in both ecotypes. Superoxide dismutase enzymes (SOD) under single stress of heavy metal showed a significant increase at moderate and high heavy metal level (100, and 200 uM $NiCl_2$). However *C. colocynthis* (L.) Schrad produced more SOD than *C. melo* (L.) subspecies *agrestis* and desert ecotype more than agricultural ecotypeof both species (*Figure 8* and *Table 5*). Catalase (µ mol $m^{-2}s^{-1}$) level of *C. melo* (L.) subspecies *agrestis* under single stress of heavy metal showed a gradual increase with increasing heavy metal level. A maximum increase in both ecotypes under high heavy metal level (200 uM $NiCl_2$) was observed. While in *C. colocynthis* (L.) Schrad desert ecotype catalase enzyme was increased by increasing stress levels. Catalase under single stress of heavy metal showed a significant increase at moderate and high heavy metal levels (100 and 200 uM $NiCl_2$) more in *C. colocynthis* (L.) Schrad as compared to *C. melo* (L.) subspecies *agrestis* similarly more in desert ecotype than agricultural ecotype (*Figure 8* and *Table 5*).

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Table 4. *Analysis of variance for morphological, biochemical and physiological parameters of C.melo (L.) subspecies agrestis and C. colocynthis (L) Schrad from two habitats under different levels of heavy metal ($NiCl_2$) Stresses*

SV	d.f.	GP	SPL (cm)	SRL (cm)	SFW (g)	SDW (g)	Ca^{2+} (mg/g d.wt.)	Cl^- (mg/g d.wt.)	K^+ (mg/g d.wt.)	Na^+ (mg/g d.wt.)	Ni^+ (mg/g d.wt.)
Species	1	0.521	48.000	0.425**	1.1656	0.37808	0.001**	0.001***	0.015***	0.0017**	0.00002*
Habitat	1	963.021**	108.000	1.888**	1.104**	0.029**	0.0048	0.0056	1.171	0.0099	0.0015***
Species × Habitat	1	20.021**	12.000*	5.8241	6.8403	1.70253*	0.286**	0.026**	1.424**	0.001**	0.0002**
Salt	3	161.465**	218.7**	13.353	25.17	6.193**	0.0016*	0.202	8.333	0.002**	0.57082
Species × Salt	3	12.576**	2.389	0.145**	0.09***	0.023***	0.002**	0.0113**	0.00472**	0.0006**	0.00002**
Habitat × Salt	3	29.410***	2.389	0.7162*	0.2506	0.05690	0.00762**	0.00721**	0.00756	0.00112	0.00012**
Species × Habitat × Salt	3	9.299**	2.389**	0.3062	0.1999	0.0209**	0.0018*	0.004**	0.0043**	0.001***	0.00012
Errors	30	0.613	1.082	0.4793	0.9590	0.24887	0.01247	0.00876	0.00741	0.00019	0.00189
Total	47										

Abbreviation. SV=Source of variance, GP=Germination percentage, SPL=Seedlings plumule length, SRL=Seedlings radicle length, SFW=Seedlings fresh weight, SDW=Seedling dry weight, *, **, *** = significance levels at 0.05, 0.01and 0.001, respectively, ns = non-significant

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Table 5. *Analysis of variance for morphological, biochemical and physiological parameters of C.melo (L.) subspecies agrestis and C. colocynthis (L) Schrad from two habitats under different levels of heavy metal ($NiCl_2$) Stresses*

SV	d.f.	Chlo.b (mg/g f.wt.)	Chlo. a (mg/g f.wt.)	CAT	Pro. (µg/g f.wt.)	SOD	TSP (µg/g f.wt.)	TSS (µg/g f.wt.)
Species	1	0.0025**	0.00001	8.550**	0.1529**	4.0223*	4.97083**	215603**
Habitat	1	0.003***	0.00143***	7.449**	1.068	3.25156**	6.54835**	4985**
Species × Habitat	1	2.926	0.00004	2.633	3.764	2.82512**	8.81770**	3545**
Salt	3	0.0081**	0.00266**	201.586	0.0706**	908.401**	7.72574**	101328
Species × Salt	3	1.667	0.00008	1.846**	0.00148	2.552	4.39290	2665***
Habitat × Salt	3	2.426	0.00006**	2.816**	0.0088**	2.002	1.74839**	65066
Species × Habitat × Salt	3	2.152	0.00026***	1.708**	0.004***	4.687	3.08229**	2422***
Errors	30	2.610	0.00011	0.082	9.083	0.22891	0.02584	80
Total	47							

Abbreviations, SV=Source of Variance, d.f.=Degree of freedom, Chlo=Chlorophyll, CAT=Catalase, Pro=Proline, SOD=Superoxide dismutase, TSP=Total soluble proteins, TSS= Total soluble sugars, *, **, *** = significance levels at 0.05, 0.01and 0.001, respectively, ns = non-significant

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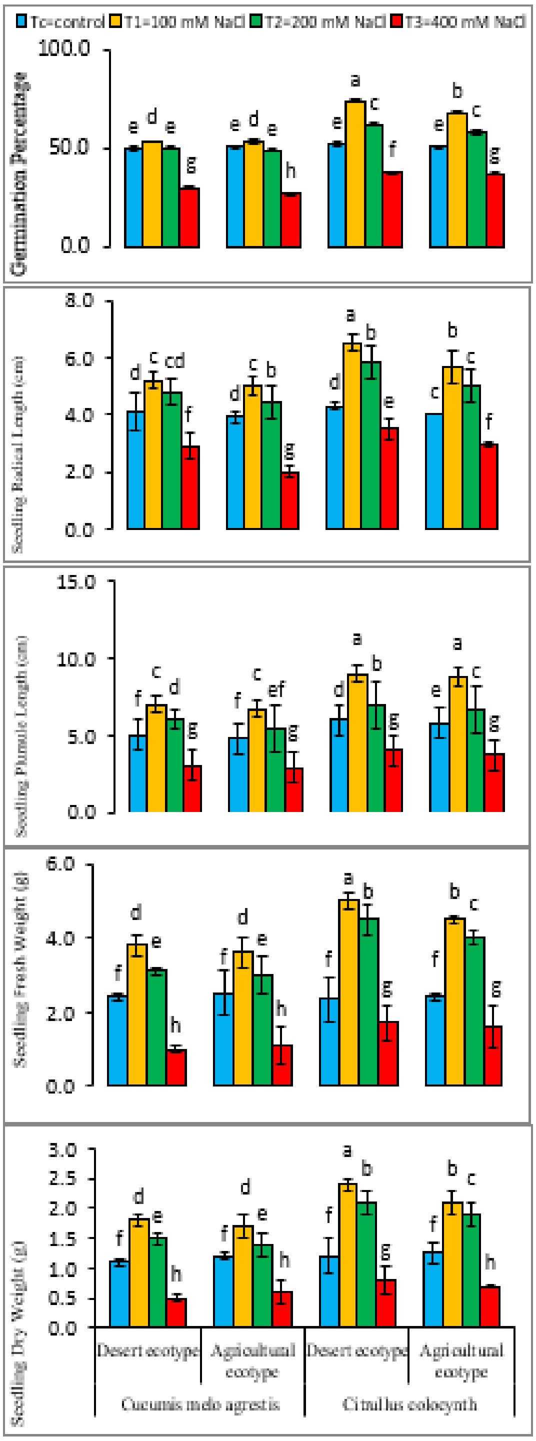

Figure 1. Effect of different levels of Salt (NaCl) Stress on germination and seedlings of C. melo (L.) subspecies agrestis and C. colocynthis (L.) Schrad from two habitats in Petri Dish Experiment. (p=0.05)

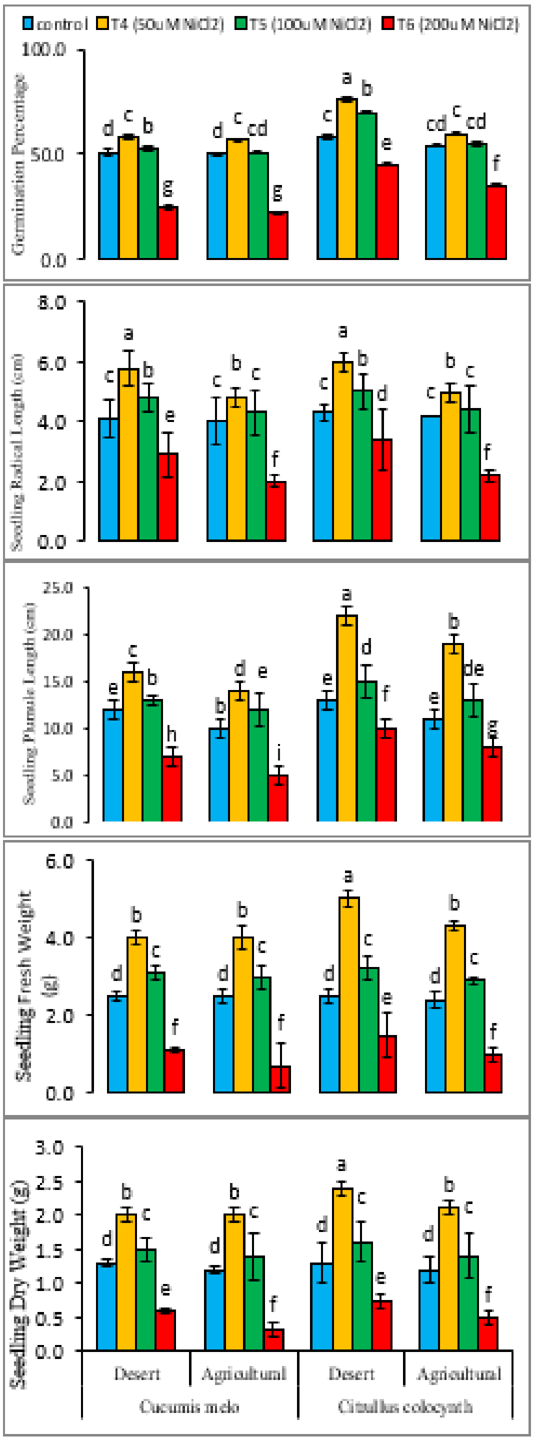

Figure 2. Effect of heavy metal ($NiCl_2$) stress on germination and seedling parameters of two ecotypes of C. melo (L.) subspecies agrestis and C.colocynthis (L.) Schrad (p=0.05)

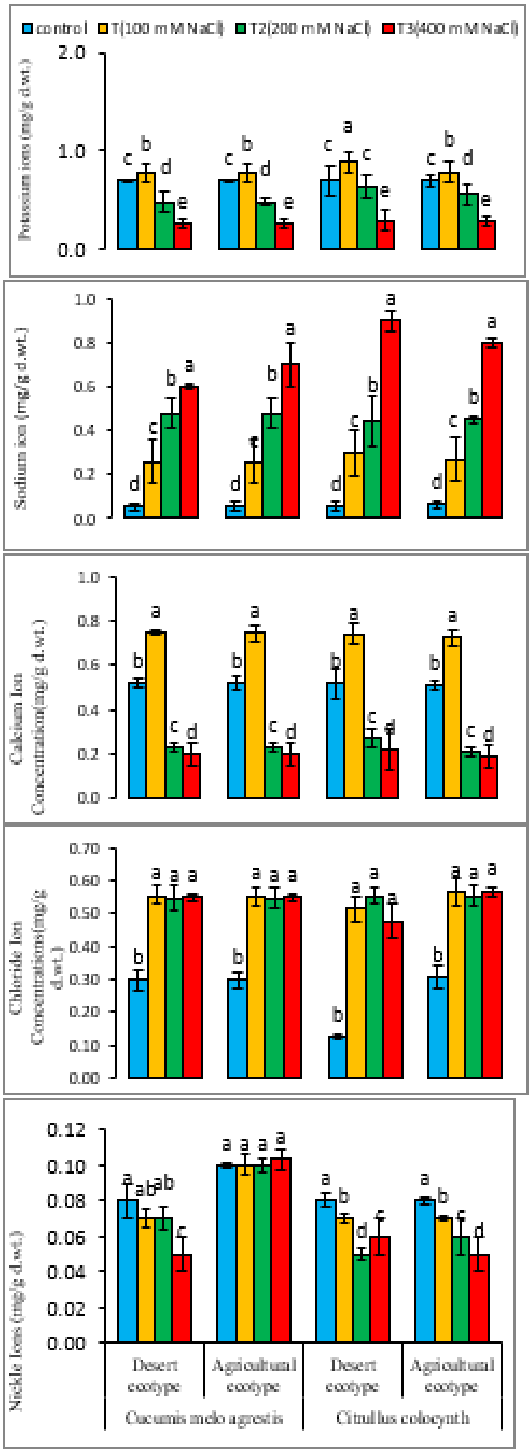

Figure 3. *Effect of different levels of Salt (NaCl) Stress on biochemical ions in seedlings of C.melo (L.) subspecies agrestis and C. colocynthis (L.) Schrad from two habitats. (p=0.05)*

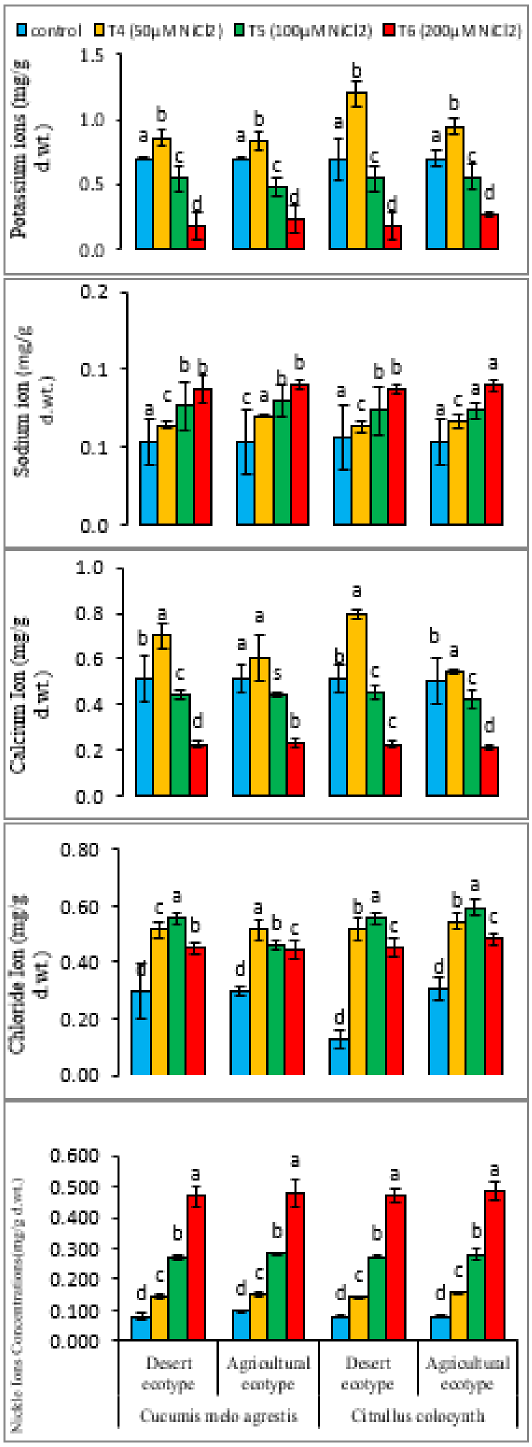

Figure 4. *Effect of heavy metal ($NiCl_2$) stress on biochemical ions of two ecotypes of C. melo (L.) subspecies agrestis and C. colocynthis (L.) Schrad. (p=0.05)*

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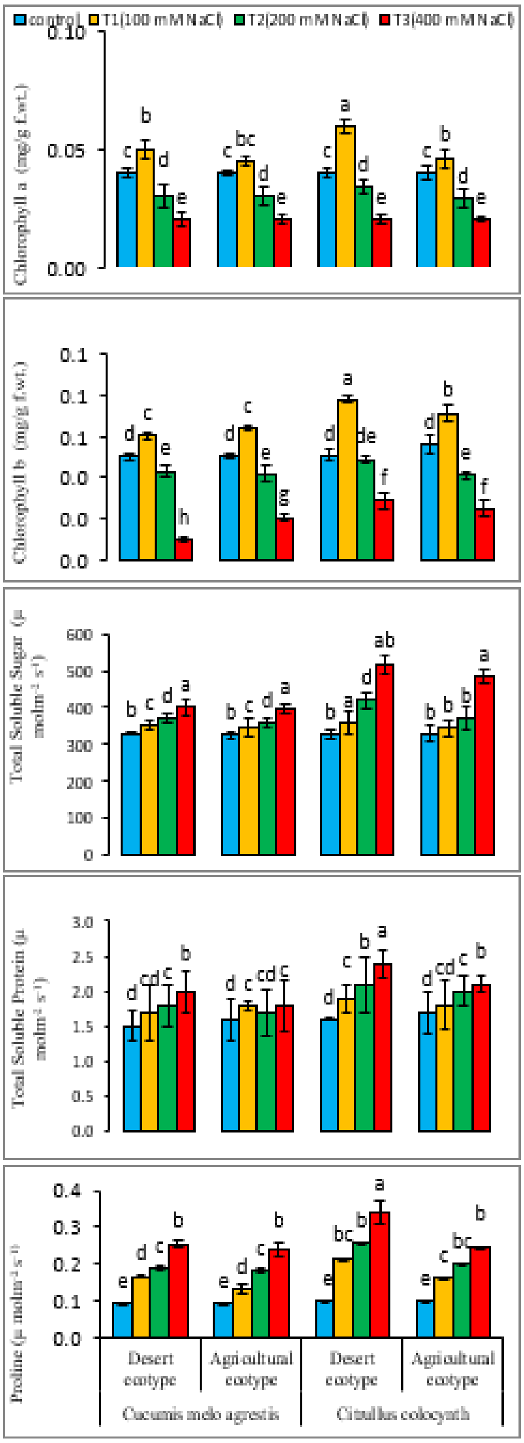

Figure 5. *Effect of salt (NaCl) on physiological parameters and secondary metabolites of C.colocynthis (L) Schrad and C.melo (L.). subspecies agrestis (p=0.05)*

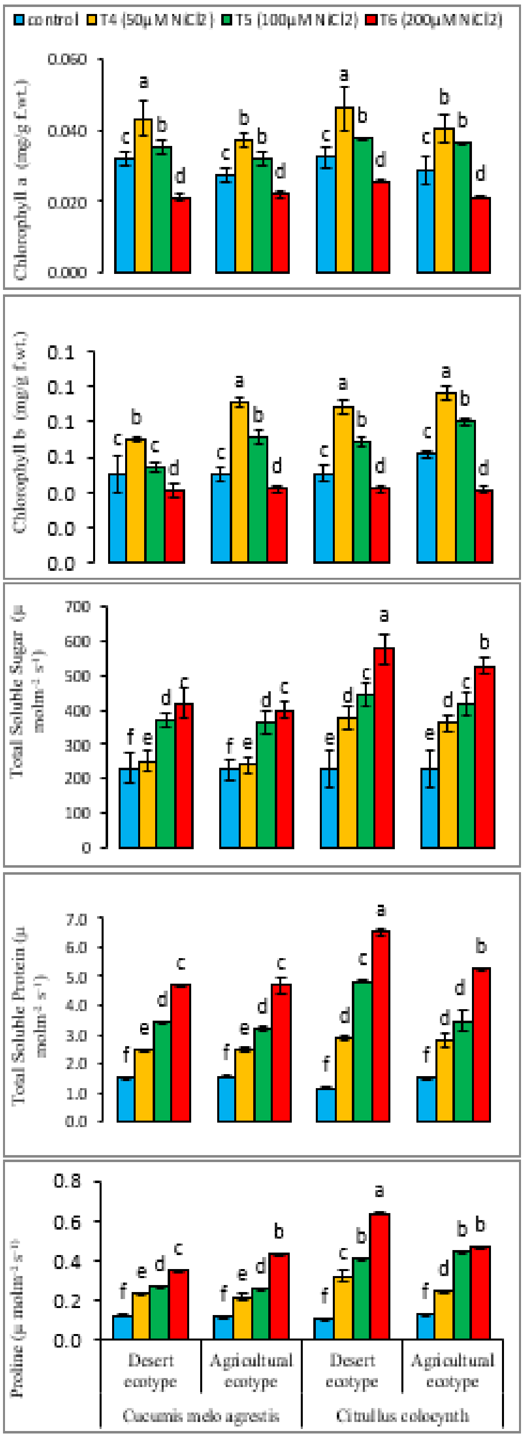

Figure 6. *Effect of heavy metal ($NiCl_2$) on physiological parameters and secondary metabolites of C.colocynthis (L) Schrad and C.melo (L.) subspecies agrestis (p=0.05)*

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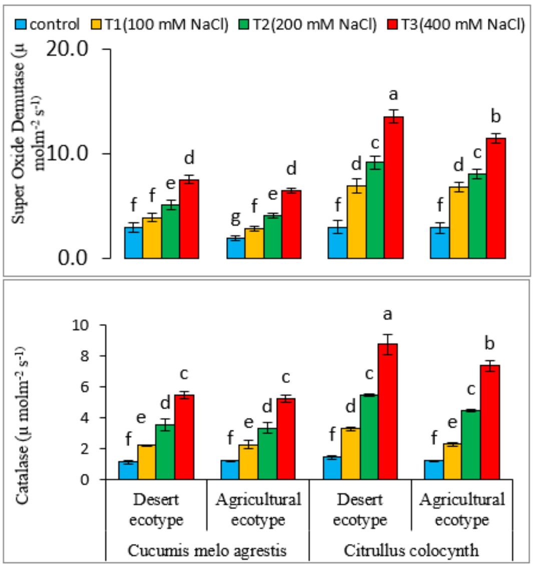

Figure 7. *Effect of salt (NaCl) on physiological parameters and secondary metabolites of C.colocynthis (L) Schrad and C.melo (L.) subspecies agrestis (p=0.05)*

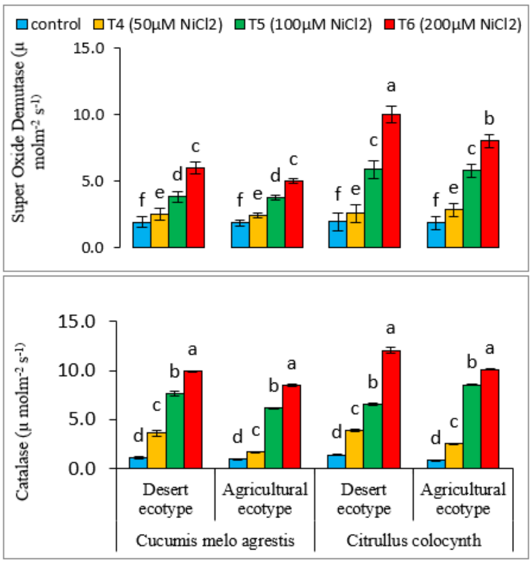

Figure 8. *Effect of heavy metal ($NiCl_2$) on physiological parameters and secondary metabolites of C.colocynthis (L) Schrad and C.melo (L.) subspecies agrestis (p=0.05)*

Discussion

Soil salinity is turning into a blatant problem for agricultural yield and production throughout the world (Jan et al., 2022). Nearby 20% of the global agricultural land and nearly 50% of the world's cultivated domains are damaged by salinity (Devkota et al., 2015). Diverse plant processes like seed sprouting, seedling progress, blossoming, and maturing are seriously affected by salinity, resulting in limited harvest and worth (Sharif et al., 2019). The issue of salinity in Pakistan is very common for irrigated farming where sufficient water flow is not supplied. Soil salinity in Pakistan is a consequence of original soil chemistry, climatic conditions, irrigation practices, and water table (Das et al., 2020).

Nickel supplements positively the morphophysiology of the plants as shown in the results of the present study. The importance of Ni is well known for higher plants but it harmfully affects plant morpho-physiology and biochemistry at higher applications (Farid et al., 2013; Asati et al., 2016). Nickel is an important part of the urease enzyme hydrolyzing urea and catalyzing the nitrogen metabolic process during germination and fertilization (Polaco et al., 2013). A lack of Nickel can clue to the disturbance of the metabolic rate of amino and organic acids in plants promoting its necessary role (De Macedo et al., 2016). The medium level of Ni in plants growing in uncontaminated soils ranges between 1 and 5 mg kg^{-1} and in general plants suffer from Ni toxicity at concentrations >100 mg (Kukier and Chaney, 2004).

In the present study, germination percentage of *C. colocynthis* (L.) Schrad. desert ecotype decreased at higher level of salt (400 mM NaCl) which was 38% as compared to control which is 52%. Germination percentage of agricultural ecotype of *C. colocynthis* decreased at higher level of salt (400 mM NaCl) which was 37%. as compared to control which was 51% Germination percentage of *C. melo* (L.) subspecies *agrestis* decreased at higher levels of salt (400 mM NaCl) which was 30% in desert and 27% in agricultural ecotype as compared to control which was 50 and 51%, respectively. It has been reported that higher salt levels have an adverse impact on the germination of seeds (Pandey et al.,

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2019). The root length of *C. colocynthis* desert ecotype increased at lower salt level (100 mM NaCl) which was 6.5 cm as compared to control of 4.3 cm. Shoot length of *C. colocynthis* (L.) Schrad desert ecotype at 100 mM NaCl was 9 cm as compared to 6 cm of control. Ain et al. (2016) studied growing wheat under Ni and salt stresses and determined that lower levels of salt and Ni stresses have a positive impact. Root length at higher levels of salt (400 mM NaCl) and heavy metal (200 uM $NiCl_2$) decreased. Thoroughgoing decrease of root length was recorded in agricultural ecotype of *C. melo* (L.) subspecies *agrestis* at 400 mM NaCl which was 2 cm as compared to 3.9 cm of control. Parallel conclusions that the salinity stress adversely affected the root length reported by Kotagiri and Kolluru (2017) and heavy metals had a significant hostile impact on root length of *S. robusta* (Pant and Tripathy, 2014). Excess HMs induce growth reduction of shoots and roots (Emamverdian et al., 2015). Paddy exposed to heavy metals ensued in reduced root length (Alfaraas et al., 2016) due to the accumulation of salt in roots (Chaves et al., 2011). Salinity and heavy metal stresses cause the reduction of water uptake decreasing the shoot length (Khan et al., 2015).

The fresh weight of desert ecotype and agricultural ecotypes of *C. colocynthis* (L.) Schrad was 5 and 4.5 gram as compared to 2.3 and 2.4 gram of control at lower salt 100 mM NaCl showed a gradual decrease in both ecotypes under increasing stress levels by salt whcich was 1.7 and 1.6 gram respectively at higher level of salt (400 mM NaCl). It appears that the abridged root and shoot fresh weight was due to the reduced water intake, resulted in decreased water in plant tissue (Sharma et al., 2005). Plant weight was reduced due to saline stress (Dolatabadian et al., 2011). The greatest dry and fresh weight decreases were observed in salinity associated with the control (Alam et al., 2016). Under heavy metal stress fresh weight of *C.melo agrestis* seedlings was decreased recorded 1.1 and 0.7 gram as compared to 2.5 and 2.5 gram of control. HMS significantly decreased the shoot fresh weight in Okmass cultivars (Xian et al., 2015). It has been reported that salinity and heavy metal stresses lead to a decrease in the uptake of water in the root which may cause reduced fresh weight (Eker et al., 2013). Total plant weight was reduced due to saline stress (Dolatabadian et al., 2011). Heavy metal significantly decreased the shoot dry weight in maize (Chen et al., 2021). It was determined that the effects of higher levels of heavy metal and salinity were more severe in comparison to the control (Gul et al., 2016).

With the extent of salinity in the soil, fluctuations affect plant's morpho-physiological (Mbarki et al., 2018) as well as biochemical behavior (Hassanpouraghdam, 2022). *C. colocynthis* (L.) Schrad and *C. melo* (L.) subspecies *agrestis* revealed a noteworthy rise in sodium and chloride content by increasing salinity level. The increased sodium and chloride content in plants may cause a reduction in plant characteristics and is crucial for salt tolerance (Zorb et al., 2019). High levels of chlorine are correlated with severe physiological dysfunction (Bazihizina et al., 2019) positively in some plants and negatively in others (Bazihizina et al., 2019; Van Zelm, 2020). High salinity and heavy metals may increase the ionic contents of plant tissues which in turn affect the water relations of the plant (Sun et al., 2018). *C. colocynthis* (L.) Schrad and *C. melo* (L.) subspecies *agrestis* revealed a low concentration of Potassium by increasing salt and heavy metal levels in comparison to the control. The plant's competence to adjust the cytosolic potassium (K) / sodium (Na) ratio becomes decisive in plant salinity tolerance by decreasing sodium uptake and its transport to shoot (Cuin and Shabala, 2008). There was an increase in potassium level under low levels of salt and metal. The increase in shoot K^+ at salinities is interpreted as the important role of this ion in an osmotic

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adjustment under such salty conditions (Hariadi et al., 2011). Cytosolic homeostasis and the ability of various plant tissues have been reported by the retention of K^+ (Shabala and Pottosin, 2014). *C. colocynthis* (L.) Schrad and *C. melo* (L.) subspecies *agrestis* exposed a momentous quantity of nickel at high $NiCl_2$ in contrast to the control. Viehwegar (2014) suggested that a common transmembrane transporter may be found in the plants that uptake heavy metals like Ni, was competitively repressed by uptake of K, Ca, and Mg. Transport of Nickel by active and passive systems takes place in spruce and soybean (Gall et al., 2014). However, this high level of nickel suppressed other plant growth parameters.

Chlorophyll a and b in both ecotypes of *C. colocynthis* (L.) Schrad *C. melo* (L.) subspecies *agrestis* was meaningfully upto 50% of the control reduced by increasing salinity and heavy metal stress levels. Photosynthetic pigments of *Vigna mungo* were seriously decreased under various levels of Nickel chloride (Islam et al., 2018). High levels of chlorine are correlated with severe physiological dysfunction (Bazihizina et al., 2019) positively in some plants and negatively in others (Bazihizina et al., 2019; Van Zelm, 2020). High salinity and heavy metals may increase ionic contents of plant tissues which in turn affect the water relations of the plant (Sun et al., 2009). High levels of salt reduces chlorophyll contents in the plants (Mir et al., 2022; Bachani et al., 2022).

It has been observed in this study that the amount of proteins increased at moderate and high levels of salt and heavy metals recorded 2.4 µg/g of fresh weight at 400 mM NaCl as compared to 1.6 µg/g of fresh weight in control of *C. colocynthis*. The reason behind this is that the plant retort to these stresses at the cellular and molecular level can be correlated with the perception of environmental signals and their transmission towards the regulatory machinery of the cell to activate specific adaptive mechanisms (Mantry et al., 2012). Proline gradually amplified with the escalating level of salinity and heavy metal in the present study recorded upto 0.3 µmol/m at 400 mM NaCl as compared to 0.1 µmol/m in control of *C.colocynthis*. The accumulation of proline is also an adaptive response to salinity and heavy metal stress (Hayat et al., 2022). It has been detected that antioxidant enzyme activities enhanced with the augmentation of salinity levels as well as heavy metals in both of our plants. Oxidative stress in salt-tolerant plants was directly related to increase in catalase (CAT) and superoxide dismutase (SOD) activity (Wu et al., 2012). Salt tolerance is regulated by the synchronized action of variable genes involved in the initiation of a variety of mechanisms such as the sequestration of toxic ions, adjustment of toxic metabolites, and antioxidative defense (Gouvera et al., 2020). The augmented level of the enzymes like CAT and SOD at higher salinities showed a significant correlation between plant tolerance levels and these antioxidant systems (Rasheed et al., 2016). Biosynthesis as well as accumulation of antioxidant enzymes, such as catalase (CAT), and superoxide dismutase (SOD), are considered to positively minimize the salinity stress (Shah et al., 2021). SOD recruits the process of ROS cleansing by converting superoxide to hydrogen peroxide (Di et al., 2018). 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 (Kibria et al., 2017).

Conclusion

In conclusion, this study provides a comprehensive understanding of how different levels of salt and heavy metal stress impact two cucurbit subspecies, *Cucumis melo* (L.) subspecies agrestis and *Cucumis colocynthis* (L.) Schrad, as well as their desert ecotype.

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The results reveal that while lower stress levels might offer some benefits, higher stress levels have divergent effects on the subspecies. Notably, *C. melo* (L.) subspecies agrestis shows heightened vulnerability, whereas *C. colocynthis* (L.) Schrad displays remarkable resilience. The standout feature is the desert ecotype's consistent tolerance across stress levels, highlighting its suitability for phytoremediation purposes. This study advocates for leveraging these plants, particularly the desert ecotype, to address salt- and heavy metal-contaminated soils through ecologically friendly phytoremediation techniques. This research not only contributes to environmental restoration but also offers insights into the integration of traditional medicine practices with modern environmental solutions. In essence, these findings underscore the intersection of plant adaptability, environmental health, and medicinal utility, emphasizing the value of holistic approaches in addressing pressing ecological challenges.

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Consent for Publication. All authors provide consent for publication.

Availability of Data. Data available on demand.

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