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Original Article

Beyond survival: unraveling the adaptive mechanisms of cucurbit weeds to salt and heavy metal stress through biochemical and physiological analyses

Além da sobrevivência: desvendando os mecanismos adaptativos de ervas daninhas cucurbitáceas ao sal e estresse de metais pesados por meio de análises bioquímicas e fisiológicas

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Abstract

Salt stress and heavy metal are instigating hazard to crops, menace to agricultural practices. Single and combined stresses affecting adversely to the growth and metabolism of plants. To explore salt and heavy metal resistant plant lines as phytoremediants is a need of time. Physiological responses are main adaptive responses of the plants towards stresses. This response varies with species and ecotype as well as type and level of stress. Two cucurbit weeds from two ecotypes were selected to evaluate their physiological adaptations against independent and combined stresses of various levels of salt (NaCl) and heavy metal ($NiCl_2$). Various physiological parameters like water potential, osmotic potential, pressure potential, CO_2 assimilation rate, stomatal conductance, chlorophyll *a* and *b*, carotenoids, and production of adaptive chemicals like SOD, CAT, proteins, sugars and proline were studied. *Citrullus colocynthis* showed more adaptive response than *Cucumis melo agrestis* and desert ecotype was more successful than agricultural ecotype against stresses.

Keywords: physiology, stress, *Citrullus cucumis*, heavy metal, salt.

Resumo

O estresse salino e os metais pesados são um perigo instigante para as plantações e uma ameaça para as práticas agrícolas. Estresses simples e combinados afetam adversamente o crescimento e o metabolismo das plantas. Explorar linhagens de plantas resistentes a sais e metais pesados como fitorremediantes é uma necessidade de tempo. As respostas fisiológicas são as principais respostas adaptativas das plantas aos estresses. Essas respostas variam de acordo com a espécie e o ecótipo, bem como com o tipo e o nível de estresse. Duas plantas daninhas cucurbitáceas de dois ecótipos foram selecionadas para avaliar suas adaptações fisiológicas frente a estresses independentes e combinados de vários níveis de sal (NaCl) e metal pesado (NiCl2). Vários parâmetros fisiológicos, como potencial hídrico, potencial osmótico, potencial de pressão, taxa de assimilação de CO2, condutância estomática, clorofila a e b, carotenoides e produção de produtos químicos adaptativos, por exemplo, SOD, CAT, proteínas, açúcares e prolina, foram estudados. *Citrullus colocynthis* apresentou resposta mais adaptativa do que *Cucumis melo agrestis* e o ecótipo deserto foi mais bem-sucedido do que o ecótipo agrícola contra estresses.

Palavras-chave: fisiologia, estresse, *Citrullus cucumis*, metais pesados, sal.

1. Introduction

The salinity that affects agricultural productivity with a massive loss (Sharma and Singh, 2015) may be due to irrigation or dry-land salinity characterized by Rengasamy (2016). The salinity stress has affected both the water absorption and the biochemical processes which lead to reduced vegetation (Safdar et al., 2019). Stress due to soil salinity is major environmental stress that limits global agronomic efficiency.

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Having a relation to semi-arid or arid regions, salinity effects are exaggerated by the coordinated pattern of xerothermic features like aridity and elevated temperature (Waqas et al., 2019). To stop the process of soil degradation and desertification and reclaim the affected soils, it is necessary to understand salt tolerance, its avoidance, and adaptations by the plants (Hanin et al., 2016). 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).

The native plants of their locations thrive well under such unfavorable conditions developing specific adaptive physiognomies in their morpho-anatomical features (Fatima et al., 2021). Plants galvanize all metabolical and physiological shield schemes to survive under tough climatic circumstances, along with particular fundamental adaptations (Rashid and Al-Marzoql, 2019). Among the morphological features that are usually inhibited by mineral stress during plant growth, root growth sometimes becomes an important parameter for the analysis of plants that tolerate mineral stress (Hamim et al., 2018).

Plants adapt modifications in morpho-physiological, biochemical, and anatomical characteristics to cope with salt stress, e.g. stimulate the generation of reactive enzymes, that deteriorate biomolecules (like proteins, lipids, and carbohydrates) and modify redox homeostasis (Chaudhry and Sidhu, 2022). The salt stress cause decline in the osmotic potential of the growth medium resulting in physiological drought (Borde et al., 2017), causes harm to fundamental cell organelles by gathering poisonous ions such as Na^+ and Cl^- interfering with vivacious cellular procedures (Meddich et al., 2018), also triggering nutrient disparity and ion scarcities in plants due to extensive potassium escape by depolarization of root membrane (Bazihizina et al., 2019).

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 needs results in poisonous effects (Kalaivanan and Ganeshamurthy, 2016). Effects of heavy metals contamination are devastating on 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).

Plants principally depend on soil solutions to obtain nutrients for their maintenance, growth, and development. The increased contamination of arable or arid lands with heavy metals is the most important reason for loss in crop efficiency (Ungureanu et al., 2020). Metal content in some areas of the earth is high (Vodyanitskii, 2012) e.g. ultramafic bed in Sulawesi, Indonesia, has Mg, Fe, and Ni in excess (Vicente et al., 2021). Low content of macronutrients such as N, P, K and Ca, while high content of micronutrients such as Ni is so high that toxicity resists plant growth (Kumar et al., 2016).

Heavy metal stress has converted into a major concern in several terrestrial global ecosystems. Presently, extensive industrialization divulges detrimental effects on the atmosphere, lithosphere, and crop productivity by heavy metals 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, and energy, and fuel production (Saxena, 2021).

Vegetable leaves contain more Ni and Fe than other edible parts (Rehman et al., 2013). Edible parts of various vegetables have an ordinary absorption of Ni, Pb, Cd, and Cr (Latif et al., 2018). Moreover, such abnormalities also give rise to the synthesis of ROS e.g., (O^{2-}), (OH^-), and H_2O_2 resulting in disturbance of the redox homeostasis (Xie et al., 2019).

The plants belonging to Family Cucurbitaceae are widespread and its many species are distributed all over the world. Almost 100 genera and over 750 species of this family are available worldwide (He et al., 2021). The fruits of the cucurbit plants are a source of carbohydrates, while the seeds of these plants yield oils that have similar properties to commercial fats (Karrar et al., 2019). Cultivated plants have tactics for resistance against salts and heavy metals which allow them to grow in good conditions without any toxicity. They may develop proteins that seize metals and trim down their toxic effects as well as modification in cell wall bonding for regulation of ion transports (Sheng et al., 2018). The present study focused on the exploration of physiological mechanisms of adaptation of *Citrullus colocynthis* L. and *Cucumis melo* L. under salt and Ni stresses.

2. Methodology

Twenty seeds of each ecotype of *C. melo agrests* and *Citrullus colocynthis* L. were placed in each pot with soil (30% clay, 30% silt, and 40% sand) to a depth of 1- 2 cm in 15 cm diameter plastic pots. Variable combined levels of NaCl and $NiCl_2$ were selected and added to the corresponding petri plates (Figure 1).

1. Control plants (Tc) were grown without salt and metal for investigation of the combined effects of salt and heavy metal, nine treatments were maintained for combined levels of NaCl and $NiCl_2$ in the present experiment i.e.
2. T7=(T1+T4) (least salt stress level + least nickel stress level)
3. T8=(T2+T4) (moderate salt stress level + least nickel stress level)
4. T9=(T3+T4) (severe salt stress level + least nickel stress level)
5. T10=(T1+T5) (least salt stress level + moderate nickel stress level)
6. T11=(T2+T5) (moderate salt stress level + moderate nickel stress level)
7. T12=(T3+T5) (severe salt stress level + moderate nickel stress level)
8. T13=(T1+T6) (least salt stress level + moderate nickel stress level)
9. T14=(T2+T6) (moderate salt stress level + moderate nickel stress level)
10. T15=(T3+T6) (severe salt stress level + moderate nickel stress level)

Physiological parameters

Measurements of the water potential, solute potential and turgor potential were done on fully expanded leaf of four plants of each replicate were done by scholander type pressure chamber and measurement was recorded at 7:00 am to 10:00 am. Osmotic potential was recorded on frozen leaf extract using vapor pressure Osmometer (Wescor 5500). Turgor pressure was calculated by using formula $\Psi_p = \psi_w - \psi_s$.

Photosynthetic parameters such as net assimilation rate of CO_2, rate of transpiration and stomatal conductance (gs) were made using an LCA-4 ADC portable infrared gas analyzer (IRGA). Measurements on fully expanded leaf were done during 10:00 am to 12:00 pm recorded at PAR of 1300 µmol m^{-2} s^{-1}, 30± 2 Ċ temperature, 350µmol CO_2 concentration, 99 Kpa Atmospheric pressure and 6±2m Bar of water vapor pressure. For the determination of organic solutes/ osmotica, Total amino acids were estimated by Moore and Stein (1948) method. The chlorophyll *a*, *b* and carotenoids were determined according to the method of Arnon (1949). Total soluble sugars were determined according to the method of Yemm and Willis (1954). Total soluble proteins are determined using the method of Lowry et al. (1951). Free amino acids in the sample were evaluated by Hamilton and Van Slyke (1943). Proline was estimated according to the method of Bates et al. (1973). CAT actions were measured by using the method of Chance and Maehly (1955). The activity of SOD was analyzed by the method used by Giannopolitis and Ries (1977) by measuring the capacity of the enzyme to inhibit the photochemical reduction of nitrobluetetazolium (NBT).

3. Results

3.1. Under independent NaCl stresses

3.1.1. CO_2 assimilation rate (u mol m^{-2} s^{-1})

Cucumis melo agrestis (S1) At low and moderate salt stress level, an increase in CO_2 assimilation was observed while no effect on high salt level. In agricultural ecotype a significant increase in assimilation at low and moderate while decrease at high salt level was observed. *Citrullus colocynthis* L. (S2) In desert ecotype a slight increase at low and moderate salt level while decrease at high salt level while in agricultural ecotype a significant increase at low salt was observed (Table 1, Figure 2).

3.1.2. Catalase

Cucumis melo agrestis (S1) Catalase under single stress of salt showed significant increase at high level (400 mM NaCl). In desert ecotype slight increase at low and moderate salts. The maximum increase in the desert ecotype under high salt level (400 mM NaCl) was observed. *Citrullus colocynthis* L. (S2) In comparison with control, the desert ecotype of *Citrullus colocynthis* L. catalase enzyme was increased by increasing stress levels. Catalase under single stress of salt showed slight increase at low level (100 mM NaCl). Catalase under single stress of salt showed significant increase at moderate and high salt level (200 and 400 mM NaCl) (Table 1, Figure 2).

Figure 1. A click of pot experiment.

3.1.3. Proline

Cucumis melo agrestis (S1) Proline level was increased with increasing salt level. The maximum proline level was observed at high salt level 400mM of NaCl in both ecotypes. *Citrullus colocynthis* L. (S2) In comparison with control, Proline under single stress of salt showed slight increase at low, moderate and high salt level (100, 200 and 400 mM NaCl) (Table 1, Figure 2).

3.1.4. Rate of transpiration (u mol m^{-2} s^{-1})

Cucumis melo agrestis (S1) In desert ecotype a slight increase at low and moderate salt, no change at high salt level while in the agricultural ecotype a significant increase at low and moderate salt and no change at high salt was observed. *Citrullus colocynthis* L. (S2) In desert ecotype a slight increase at low salt and moderate salt level while decrease at high salt level while in the agricultural ecotype a significant increase at low salt and slight increase at moderate and no change at high salt was observed (Table 1, Figure 2).

3.1.5. Stomatal conductance (u mol m^{-2} s^{-1})

Cucumis melo agrestis (S1) In both ecotypes, an increase in stomatal conductance at low salt was observed while it gradually decreased by increasing salt level. *Citrullus colocynthis* L. (S2) In desert ecotype a slight increase at low salt, no change at moderate salt level while decrease at high salt level while in agricultural ecotype a significant increase at low salt and gradual decrease at moderate and high salt was observed (Table 1, Figure 2).

3.1.6. Superoxide dismutase

Cucumis melo agrestis (S1) A gradual increase in superoxide dismutase was observed with increasing salt level in both ecotypes. The maximum amount of superoxide dismutase was observed at high salt level 400mM of NaCl. *Citrullus colocynthis* L. (S2) In comparison with control, super oxide dismutase enzymes (SOD) was increased by increasing stress levels in both ecotypes. Super oxide dismutase enzymes (SOD) under single stress of salt showed significant increase at low, moderate and high salt level (100, 200 and 400 mM NaCl) (Table 1, Figure 2).

3.1.7. Total soluble proteins

Cucumis melo agrestis (S1) Total soluble proteins were increased under salt stress. The maximum soluble proteins were observed in the desert ecotype at 100mM of salt. However 400Mm of salt level has no effect on total soluble proteins. *Citrullus colocynthis* L. (S2) In comparison with control, the desert ecotype amount of soluble proteins under single stress of salt showed increase at high salt level (400 mM NaCl) while decreased at low salt level. Agricultural ecotype showed significant increase in soluble proteins at 100mM, slight increase at 200mM and decrease at high salt levels (400 mM NaCl) (Table 2, Figure 2).

3.1.8. Chlorophyll a

Cucumis melo agrestis (S1) Chlorophyll a level increased at lower and moderate salt levels (100 and 200 mM), while a slight decrease at high level 400mM of salt while in agricultural ecotype a significant increase in chlorophyll a at low salt 100mM and slight increase at moderate level while decrease at high salt level. *Citrullus colocynthis* L. (S2) Desert ecotype plants under single stress of salt showed increased level of chlorophyll a at low salt level (100 mM NaCl) and gradual decrease at moderate and high salt levels (200 and 400 mM NaCl). Agricultural ecotype plants showed increased level of chlorophyll a at low salt level (100 mM NaCl) and gradual decrease at moderate and high salt levels (200 and 400 mM NaCl) better as compared to desert ecotypes (Table 2, Figure 2).

3.1.9. Chlorophyll b

Cucumis melo agrestis (S1) amount of chlorophyll b under single stress of salt showed increase at low salt level (100 mM NaCl) and gradual decrease at moderate and high salt levels (200 and 400 mM NaCl).

Table 1. Analysis of variance for Physiological parameters of *Cucumis melo agrestis* and *Citrullus colocynthis* from two habitats under different levels of Salt (NaCl) Stress in pot experiment.

Source of Variance	d.f.	Net Assimilation of CO2 (μmol $m^{-2}s^{-1}$)	Catalase	Proline Contents (μg/g f.wt.)	Rate of Transpiration (mmol $m^{-2}s^{-1}$)	Stomatal Conductance (mmol $m^{-2}s^{-1}$)	Superoxide Dismutase
Species	1	409.022	12.480***	0.13490**	3.38035*	4.6875**	4.02521**
Habitat	1	24.350**	77.894	0.00016	0.20124**	2.5208	3.25521
Species × Habitat	1	44.467**	0.350***	0.00104**	0.36750**	0.5208**	2.85187***
Salt	3	299.315	342.811	0.06567	2.47367	97.6875	456.098
Species × Salt	3	36.133**	5.092**	0.00982**	0.15995**	1.6319**	2.083E-04**
Habitat × Salt	3	19.354**	12.898**	0.00227**	0.29862	0.5764**	0.00132
Species × Habitat × Salt	3	11.785***	0.079***	0.00196***	0.09739**	0.1319***	2.083E-04**
Errors	30	0.450	0.061	0.00010	0.00372	0.9875	0.22718
Total	47						

Significance levels * = 0.05 ** = 0.01 *** = 0.001 d.f. = degrees of freedom.

The maximum increase was observed in the agricultural ecotype at 100mM, while the maximum decrease of chlorophyll b in the desert ecotype at 400mM of salt. *Citrullus colocynthis* L. (S2) In comparison with control, both ecotypes amount of chlorophyll b under single stress of salt showed increase at low salt level (100 mM NaCl) and gradual decrease at moderate and high salt levels (200 and 400 mM NaCl) (Table 2, Figure 2).

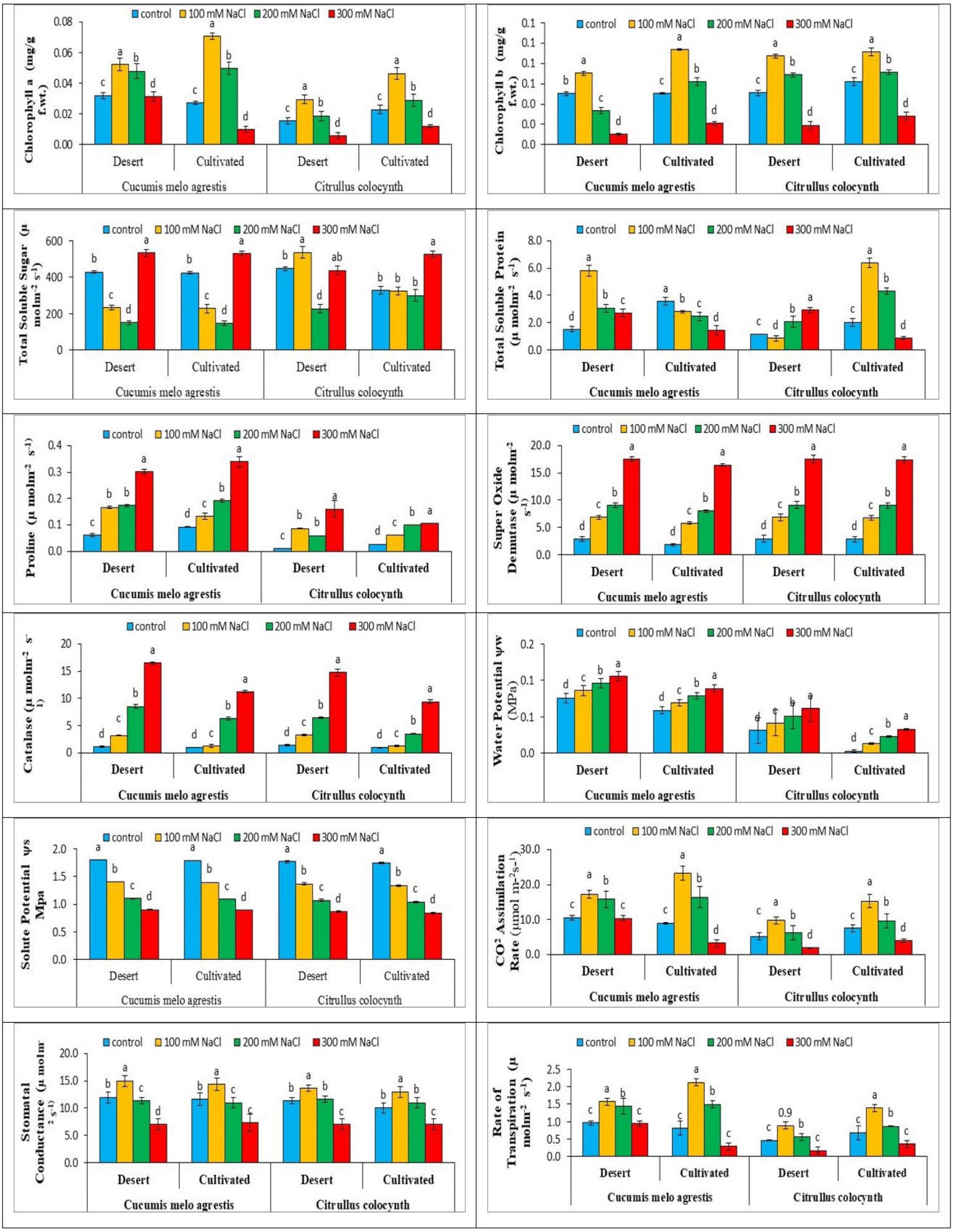

Figure 2. Effect of salt (NaCl) stress on physiological parameters of two ecotypes of *Cucumis melo agrestis* and *Citrullus colocynthis.*

3.1.10. Total soluble sugars

Cucumis melo agrestis (S1) Total soluble sugars significantly increased at high level of salt in both ecotypes, while decreased at low level of salt in both ecotypes. The maximum increased soluble sugars were shown in 400 Mm of salt. *Citrullus colocynthis* L. (S2) In comparison with control, the desert ecotype of *Citrullus colocynthis* L. amount of soluble sugars under single stress of salt showed increase at low salt level (100 mM NaCl) and and high salt levels (400 mM NaCl). Agricultural ecotype showed increased soluble sugars at high level of salt (Table 2, Figure 2).

3.1.11. Water potential (Mpa)

Cucumis melo agrestis (S1) In both ecotypes a gradual decrease at low, moderate and high salt level was observed as compared to control. *Citrullus colocynthis* L. (S2) In both ecotypes a gradual decrease in water potential at low, moderate and high salt level was observed as compared to control (Table 2, Figure 2).

3.1.12. Solute potential (Mpa)

Cucumis melo agrestis (S1) In both ecotypes a gradual decrease at low, moderate and high salt level was observed as compared to control. *Citrullus colocynthis* L. (S2) In both ecotypes a gradual decrease in solute potential at low, moderate and high salt level was observed as compared to control (Table 2, Figure 2).

3.2. Under independent heavy metal ($NiCl_2$) stresses

3.2.1. Proline (u mol $m^{-2}s^{-1}$)

Cucumis melo agrestis (S1) Proline level was increased with increasing heavy metal level. Maximum proline level was observed at high heavy metal level 50uM of $NiCl_2$ in both ecotypes. *Citrullus colocynthis* L. (S2) In comparison with control, Proline under single stress of heavy metal showed slight increase at low, moderate and high heavy metal level (50, 100 and 200 uM $NiCl_2$) (Table 3, Figure 3).

Table 2. Analysis of variance for Physiological parameters of *Cucumis melo agrestis* and *Citrullus colocynthis* from two habitats under different levels of Salt (NaCl) Stress in pot experiment.

Source of Variance	d.f.	Total Soluble Sugar (µg/g f.wt.)	Chlorophyll a (mg/g f.wt.)	Solute Potential (MPa)	Water Potential (MPa)	Total Soluble Protein (µg/g f.wt.)	Chlorophyll (mg/g f.wt.)
Species	1	38116**	0.00366**	0.02200***	0.03052**	4.97083	0.00144**
Habitat	1	6473**	2.221E-04	0.00353	0.00622***	6.54835	0.00152**
Species × Habitat	1	4650	4.028E-04***	0.00132***	3.991E-04	8.81770	2.517E-04
Salt	3	193792**	0.00273**	1.84000**	0.00200***	7.72574	0.00889***
Species × Salt	3	44556**	1.683E-04**	3.080E-31	5.296E-34***	4.39290	1.801E-04
Habitat × Salt	3	16519***	1.083E-04	8.932E-33**	6.821E-35**	1.74839	5.660E-05**
Species × Habitat × Salt	3	16301**	4.238E-06**	4.303E-32***	7.448E-35***	3.08229	2.144E-04**
Errors	30	37	3.295	8.239E-05	6.857E-05	0.02584	2.704E-07
Total	47						0.00144**

Significance levels ** = 0.01 *** = 0.001 d. f. = degrees of freedom.

Table 3. Analysis of variance for Physiological parameters of *Cucumis melo agrestis* and *Citrullus colocynthis* from two habitats under different levels of Salt (NiCl2) Stress in pot experiment.

Source of Variance	d.f.	Catalase	Chlorophyll b (mg/g f.wt.)	Net Assimilation of CO2 (µmol $m^{-2}s^{-1}$)	Proline Contents (µg/g f.wt.)	Rate of Transpiration (mmol $m^{-2}s^{-1}$)	Stomatal Conductance (mmol $m^{-2}s^{-1}$)	Superoxide Dismutase
Species	1	8.550**	0.00248**	246.468	0.15292**	3.38035***	20.0208	4.02231*
Habitat	1	7.449**	0.00223***	45.876**	1.068E-04	0.20124	7.5208***	3.25156**
Species × Habitat	1	2.633	2.926E-04	16.780	3.764E-06	0.36750**	2.5208**	2.82512**
Salt	3	201.586	0.00810**	345.406	0.07065**	2.47367**	79.3542	908.401**
Species × Salt	3	1.846**	1.667E-04	37.437**	0.00148	0.29862	0.1319**	2.552E-05
Habitat × Salt	3	2.816**	2.426E-04	26.591	0.00883**	0.15995**	1.1875**	2.002E-05
Species × Habitat × Salt	3	1.708**	2.152E-04	11.863**	0.00375***	0.09739*	0.2431**	4.687E-06
Errors	30	0.082	2.610E-07	0.161	9.083E-05	0.00372	0.8625	0.22891
Total	47							

Significance levels * = 0.05 ** = 0.01 *** = 0.001 d. f. = degrees of freedom.

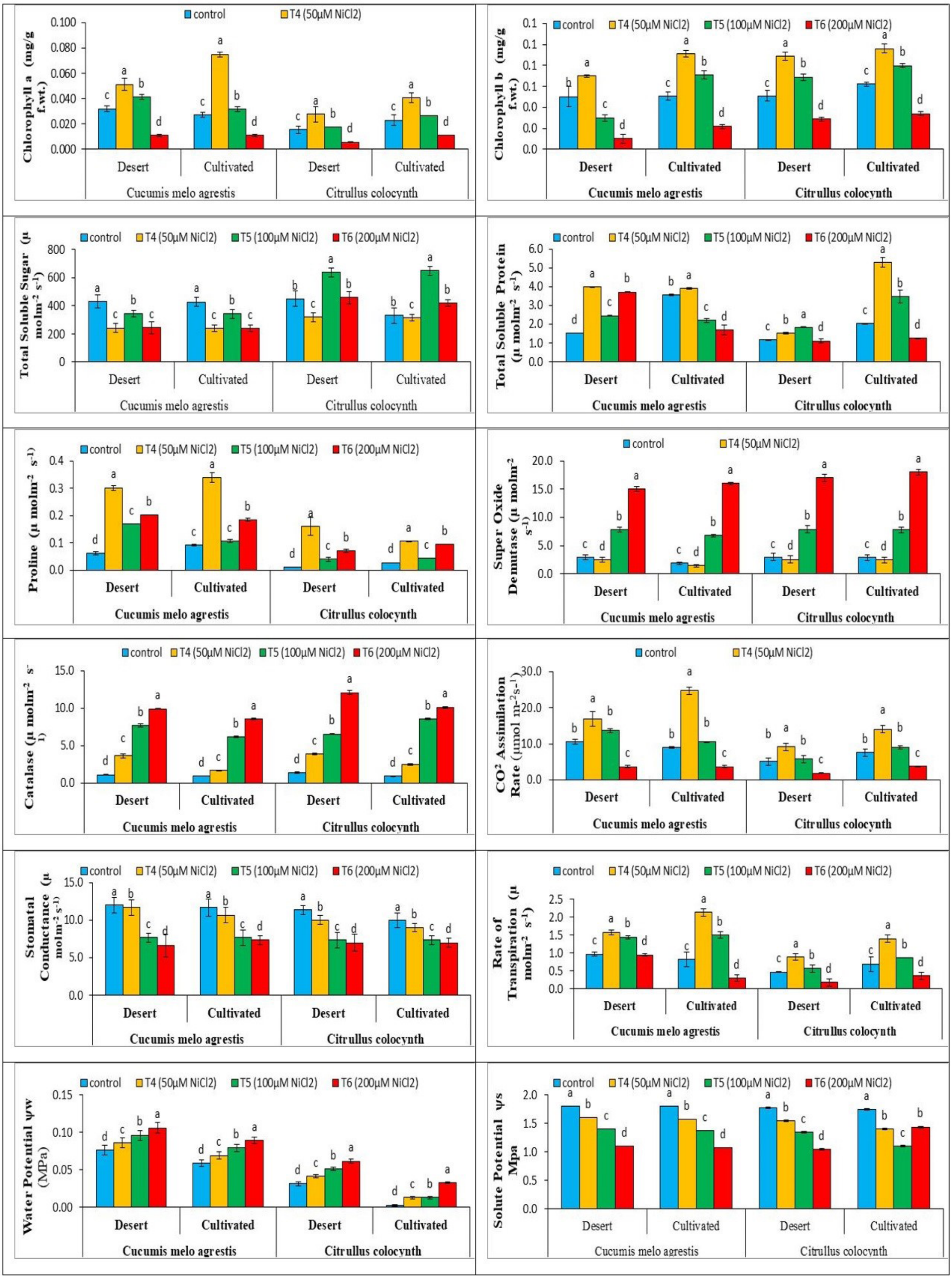

Figure 3. Effect of heavy metal ($NiCl_2$) on physiological parameters of two ecotypes of *Cucumis melo agrestis* L.and *Citrullus colocynthis* L.

3.2.2. Catalase (u mol $m^{-2}s^{-1}$)

Cucumis melo agrestis (S1) Catalase under single stress of heavy metal showed gradual increase with increasing heavy metal level. Maximum increase in both ecotype under high heavy metal level (200 uM $NiCl_2$) was observed. *Citrullus colocynthis* L. (S2) In comparison with control, the desert ecotype of *Citrullus colocynthis* L. catalase enzyme was increased by increasing stress levels.

Catalase under single stress of heavy metal showed slight increase at low level (50 uM $NiCl_2$). Catalase under single stress of heavy metal showed significant increase at moderate and high heavy metal level (100 and 200 uM $NiCl_2$) (Table 3, Figure 3).

3.2.3. Superoxide dismutase (u mol $m^{-2}s^{-1}$)

Cucumis melo agrestis (S1) A gradual increase in superoxide dismutase was observed with increasing heavy metal level in both ecotypes. Maximum amount of superoxide dismutase was observed at high heavy metal level 200uM of $NiCl_2$. *Citrullus colocynthis* L. (S2) In comparison with control, super oxide dismutase enzymes (SOD) was increased by increasing stress levels in both ecotypes. Super oxide dismutase enzymes (SOD) under single stress of heavy metal showed significant increase at low, moderate and high heavy metal level (50, 100 and 200 uM $NiCl_2$) (Table 3, Figure 3).

3.2.4. CO_2 assimilation rate (u mol m^{-2} s^{-1})

Cucumis melo agrestis (S1) At low and moderate heavy metal stress level, an increase in CO_2 assimilation was observed while decrease on high heavy metal level. In agricultural ecotype a significant increase in assimilation at low and no change on moderate while decrease at high heavy metal level was observed. *Citrullus colocynthis* L. (S2) In desert ecotype a slight increase at low and moderate heavy metal level while decrease at high heavy metal level while in agricultural ecotype a significant increase at low heavy metal was observed (Table 3, Figure 3).

3.2.5. Stomatal conductance (u mol m^{-2} s^{-1})

Cucumis melo agrestis (S1) In both ecotypes, a gradual decrease in stomatal conductance was observed by increasing heavy metal level. *Citrullus colocynthis* L. (S2) In both ecotypes, a gradual decrease in stomatal conductance was observed by increasing heavy metal level (Table 3, Figure 3).

3.2.6. Rate of transpiration (u mol m^{-2} s^{-1})

Cucumis melo agrestis (S1) In desert ecotype a slight increase at low and moderate heavy metal, no change at high heavy metal level while in agricultural ecotype a significant increase at low and moderate heavy metal and decrease at high heavy metal was observed. *Citrullus colocynthis* L. (S2) In desert ecotype a slight increase at low heavy metal and moderate heavy metal level while decrease at high heavy metal level while in agricultural ecotype a significant increase at low heavy metal and slight increase at moderate and decrease at high heavy metal was observed (Table 3, Figure 3).

3.2.7. Chlorophyll a

Cucumis melo agrestis (S1) Chlorophyll *a* level increased at lower and moderate heavy metal levels (50 and 100 uM) while a slight decrease at high level 200uM of heavy metal while in the agricultural ecotype a significant increase in chlorophyll *a* at low heavy metal 50uM and slight increase at moderate level while decrease at high heavy metal level. *Citrullus colocynthis* L. (S2) Desert ecotype plants under single stress of heavy metal showed increased level of chlorophyll a at low heavy metal level (50 uM $NiCl_2$) and gradual decrease at moderate and high heavy metal levels (100 and 200 uM $NiCl_2$). Agricultural ecotype plants showed increased level of chlorophyll a at low heavy metal level (50 uM $NiCl_2$) and gradual decrease at moderate and high heavy metal levels (100 and 200 uM $NiCl_2$) better as compared to desert ecotype (Table 4, Figure 3).

3.2.8. Chlorophyll b

Cucumis melo agrestis (S1) Amount of chlorophyll *b* under single stress of heavy metal showed increase at low heavy metal level (50 uM $NiCl_2$) and gradual decrease at moderate and high heavy metal levels (100 and 200 uM $NiCl_2$). The maximum increase was observed in agricultural ecotype at 50uM, while the maximum decrease of chlorophyll *b* in desert ecotype at 200uM of heavy metal.

Table 4. Analysis of variance for Physiological parameters of *Cucumis melo agrestis* and *Citrullus colocynthis* from two habitats under different levels of heavy metal ($NiCl_2$) Stress in pot experiment.

Source of Variance	d.f.	Total Soluble Protein (μg/g f.wt.)	Chlorophyll a (mg/g f.wt.)	Solute Potential (MPa)	Water Potential (MPa)	Total Soluble Sugar (μg/g f.wt.)
Species	1	4.97083**	0.00001	0.00353***	0.00692***	215603**
Habitat	1	6.54835**	0.00143***	0.02200	0.03205	4985**
Species × Habitat	1	8.81770**	0.00004	0.00132**	0.00192**	3545**
Salt	3	7.72574**	0.00266**	1.07000**	0.00059**	101328
Species × Salt	3	4.39290	0.00008	1.631E-32	0.00002	2665***
Habitat × Salt	3	1.74839**	0.00006**	3.119E-31	0.00002***	65066
Species × Habitat × Salt	3	3.08229**	0.00026***	1.692E-32	0.00002**	2422***
Errors	30	0.02584	0.00011	8.239E-05	0.00007	80
Total	47	3				

Significance levels ** = 0.01 *** = 0.001 d. f. = degrees of freedom.

Citrullus colocynthis L. (S2) In comparison with control, both ecotypes amount of chlorophyll *b* under single stress of heavy metal showed increase at low heavy metal level (50 uM $NiCl_2$) and gradual decrease at moderate and high heavy metal levels (100 and 200 uM $NiCl_2$) (Table 4, Figure 3).

3.2.9. Total soluble proteins (u mol $m^{-2}s^{-1}$)

Cucumis melo agrestis (S1) Total soluble proteins were increased under heavy metal stress. The maximum soluble proteins were observed in desert ecotype at 50uM of heavy metal. However 200uM of heavy metal level has no effect on total soluble proteins in agricultural ecotype. *Citrullus colocynthis* L. (S2) In comparison with control, the desert ecotype amount of soluble proteins under single stress of heavy metal showed increase at low heavy metal level (50 uM $NiCl_2$) while decreased at high heavy metal level. Agricultural ecotype showed significant increase in soluble proteins at 50uM, slight increase at 100uM and decrease at high heavy metal levels (200 uM $NiCl_2$) (Table 4, Figure 3).

3.2.10. Total soluble Sugars (u mol $m^{-2}s^{-1}$)

Cucumis melo agrestis (S1) Total soluble sugars decreased at low and high level of heavy metal in both ecotypes while decreased at low level of heavy metal in both ecotypes. Maximum increased soluble sugars were shown in 100 uM of heavy metal. *Citrullus colocynthis* L. (S2) In comparison with control, the desert ecotype of *Citrullus colocynthis* L. amount of soluble sugars under single stress of heavy metal showed decrease at low heavy metal level (50 uM $NiCl_2$) and increase at moderate and high heavy metal levels (100 and 200 uM $NiCl_2$). Agricultural ecotype showed increased soluble sugars at moderate level of heavy metal (Table 4, Figure 3).

3.2.11. Water potential (Mpa)

Cucumis melo agrestis (S1) In both ecotypes a gradual decrease at low, moderate and high heavy metal level was observed as compared to control. *Citrullus colocynthis* L. (S2) In both ecotypes a gradual decrease in water potential at low, moderate and high heavy metal level was observed as compared to control(Table 4).

3.2.12. Solute potential (Mpa)

Cucumis melo agrestis (S1) In both ecotypes a gradual decrease at low, moderate and high heavy metal level was observed as compared to control. *Citrullus colocynthis* L. (S2) In both ecotypes a gradual decrease in solute potential at low, moderate and high heavy metal level was observed as compared to control (Table 4, Figure 3).

3.3. Under combined stresses of salt (NaCl) and heavy metal ($NiCl_2$)

3.3.1. Proline (ug/g)

Cucumis melo agrestis (S1) Proline level increased with increasing salt or heavy metal stress levels. Minimum proline was recorded at T7 and T8. A slight increase at T9 while significant increase at T10, T11, T12, T13, T14 and T15 in desert ecotype. While in agricultural ecotype minimum proline was observed at T7 and T10. An increase in proline at T8 and T11 while a considerable increase of proline at T9, T12, T13, T14 and T15 was noticed. *Citrullus colocynthis* L. (S2) Proline level increased with increasing stress levels. Low proline recorded at T7, T8, T9, T10, T11 and T12 while increased proline at T13, T14 and T15 was recorded in desert ecotype. In case of agricultural ecotype enhanced proline at T9, T12, T13, T14 and T15 while low proline at T7, T8, T10 and T11 was observed (Table 5, Figure 4).

3.3.2. Catalase

Cucumis melo agrestis (S1) Catalase level increased with increasing stress level of salt or heavy metal. Low level of catalase at T7, T8, T10 and T11 while increased catalase at T9, T12, T13, T14 and T15 was observed iin both ecotypes.

Table 5. Analysis of variance for Physiological parameters of *Cucumis melo agrestis* and *Citrullus colocynthis* from two habitats under different levels of combined Salt (NaCl + NiCl2) Stress in pot experiment.

Source of Variance	d.f.	Catalase	Chlorophyll b (mg/g f.wt.)	Net Assimilation of CO2 (μmol $m^{-2}s^{-1}$)	Proline Contents (μg/g f.wt.)	Rate of Transpiration (mmol $m^{-2}s^{-1}$)	Stomatal Conductance (mmol $m^{-2}s^{-1}$)	Superoxide Dismutase
Species	1	15.910**	0.01358***	254.620	0.40563**	6.87916	56.3767**	7.352***
Habitat	1	10.527	7.500E-07	159.812***	0.00010	5.38908**	10.1017	9.005**
Species × Habitat	1	8.591***	7.500E-07	80.147**	0.00106**	0.12840	0.4498**	6.373**
Salt	3	38.168	6.460E-04	159.812	0.00773	1.09804**	9.1128**	29.025**
Species × Salt	3	141.481	0.00112***	139.125	0.01003**	1.85373**	29.0216	129.149
Habitat × Salt	3	96.856***	0.00240	74.841**	0.01414**	1.32925	23.2193**	61.438**
Species × Habitat × Salt	3	146.098**	0.00254***	163.423**	0.01537*	2.73765**	32.8649*	200.623**
Errors	30	0.277	3.721E-07	0.027	0.00010	0.91713	0.7114	0.218
Total	47							

Significance levels * = 0.05 ** = 0.01 *** = 0.001 d. f. = degrees of freedom.

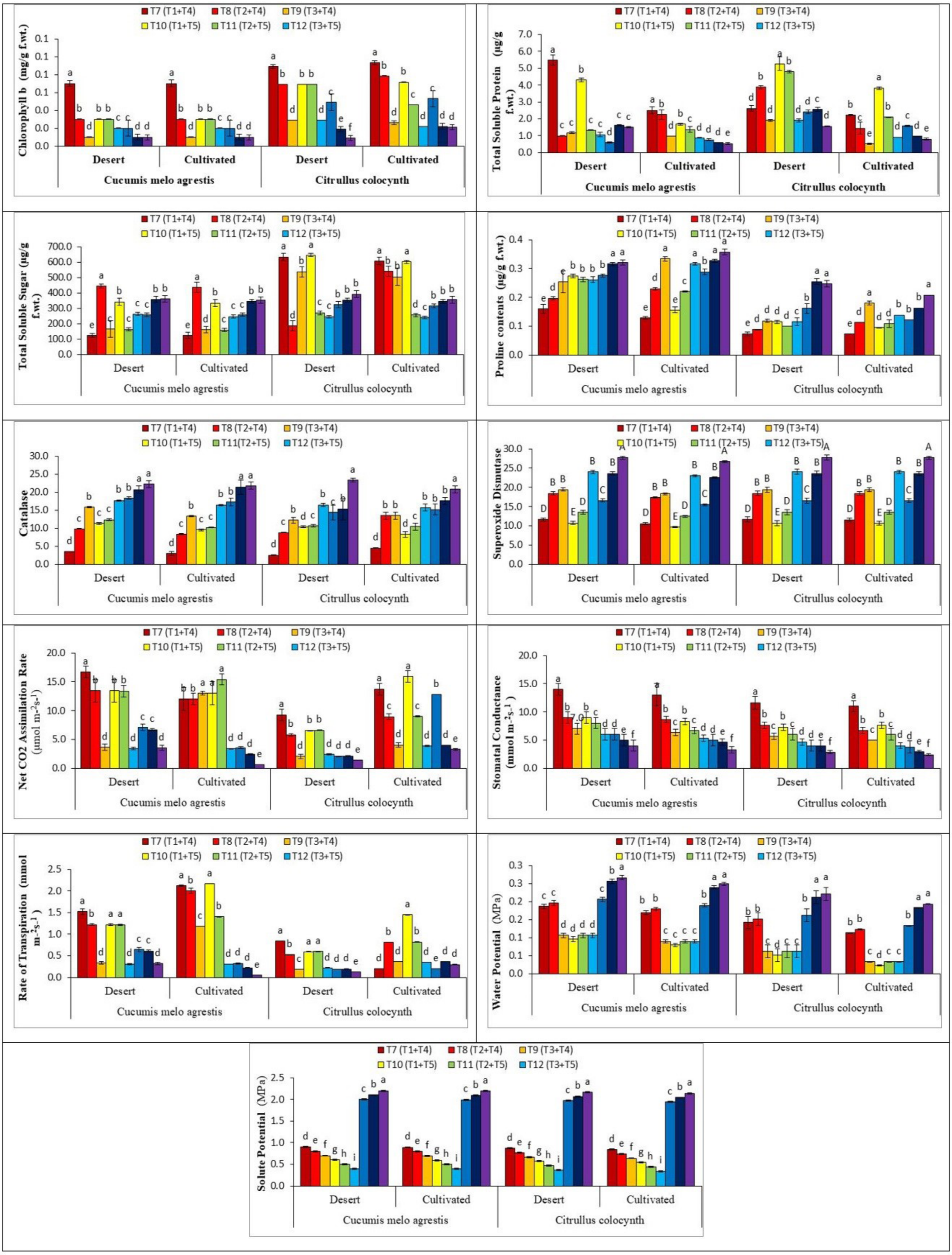

Figure 4. Effect of combined saalt (NaCl) with heavy metal ($NiCl_2$) on *Cucumis melo agrestis* L. and *Citrullus colocynthis* L. from two habitats.

Desert ecotype has more value of catalase than agricultural ecotype. *Citrullus colocynthis* L. (S2) Catalase level increased with increasing stress level of salt or heavy metal. Minimum catalase at T7, T8, T10, T11 and T13. A significant increase of catalase was observed at T9, T12, and T14. Maximum increase of catalase was observed at T15 where high salt was combined with high metal level. Desert ecotype has more value of catalase than agricultural ecotype (Table 5, Figure 4).

3.3.3. Superoxide dismutase

Cucumis melo agrestis (S1) Superoxide dismutase level increased with increasing stress level of salt or heavy metal. Low level of SOD at T7, T10 and T13 while increased SOD at T8, T11 and T14 was observed. A significant increase of SOD at T9, T12 and T15 was observed in both ecotypes. Desert ecotype has more value of SOD than agricultural ecotype. *Citrullus colocynthis* L. (S2) Superoxide dismutase level increased with increasing stress level of salt or heavy metal. Minimum SOD was recorded at T7, T10, T11 and T13. A significant increase of SOD was observed at T8, T9, T12, T14 and T15. Maximum increase of SOD was observed at T15 where high salt was combined with high metal level. Desert ecotype has more value of SOD than agricultural ecotype (Table 5, Figure 4).

3.3.4. CO_2 assimilation rate (u mol m^{-2} s^{-1})

Cucumis melo agrestis (S1) Assimilation rate of CO_2 level increased at low stress level of salt or heavy metal. Increased assimilation at T7, T8, T10 and T11 while decreased assimilation at T9, T12, T13, T14 and T15 was observed iin both ecotypes. Agricultural ecotype has more value of assimilation than desert ecotype. *Citrullus colocynthis* L. (S2) Assimilation rate of CO_2 level increased at low stress level of salt or heavy metal. Maximum rate of assimilation at T7, T8, T10, T11 and T13. A significant decrease of assimilation rate was observed at T9, T12, T14 and T15. Agricultural ecotype has more assimilation rate than desert ecotype (Table 5, Figure 4).

3.3.5. Stomatal conductance (u mol m^{-2} s^{-1})

Cucumis melo agrestis (S1) Stomatal conductance increased at low stress level of salt or heavy metal. Increased conductance at T7, T8, T10 and T11 while decreased at T9, T12, T13, T14 and T15 was observed iin both ecotypes. Agricultural ecotype has more value of stomatal conductance than desert ecotype. *Citrullus colocynthis* L. (S2) Stomatal conductance level increased at low stress level of salt or heavy metal. Maximum rate of assimilation at T7, T8, T10, T11 and T13. A significant decrease of assimilation rate was observed at T9, T12, T14 and T15. Agricultural ecotype has more assimilation rate than desert ecotype (Table 5, Figure 4).

3.3.6. Rate of transpiration (u mol m^{-2} s^{-1})

Cucumis melo agrestis (S1) Transpiration increased at low stress level of salt or heavy metal. Increased transpiration at T7, T8, T10 and T11 while decreased at T9, T12, T13, T14 and T15 was observed iin both ecotypes. Agricultural ecotype has more value of transpiration rate than desert ecotype. *Citrullus colocynthis* L. (S2) Rate of transpiration increased at low stress level of salt or heavy metal. Maximum rate of transpiration at T7, T8, T10, T11 and T13. A significant decrease of transpiration rate was observed at T9, T12, T14 and T15. Agricultural ecotype has more transpiration rate than desert ecotype (Table 5, Figure 4).

3.3.7. Chlorophyll a

Cucumis melo agrestis (S1) Chlorophyll a level increased at T7, T10 and T13 where low salt was combined with low, moderate and high metal levels. A slight decrease was observed at T8, T11 and T14 where moderate salt was combined low, moderate and high metal levels. A significant decrease of chlorphyll was observed at T9, T12 and T15 where high salt level was combined with low, moderate and high metal levels. Agricultural ecotype has more value of chlorophyll a than desert ecotype. *Citrullus colocynthis* L. (S2) Chlorophyll a level increased at T7, T10 and T13 where low salt was combined with low, moderate and high metal levels. A slight decrease was observed at T11 and T14 where moderate salt was combined moderate and high metal levels. A significant decrease of chlorphyll was observed at T9, T12 and T15 where high salt level was combined with low, moderate and high metal levels. Maximum increase of chlorophyll a was observed at T8 where moderate salt was combined with low metal level. Desert ecotype has more value of chlorophyll a than agricultural ecotype (Table 6 , Figure 4).

Table 6. Analysis of variance for Physiological parameters of *Cucumis melo agrestis* and *Citrullus colocynthis* from two habitats under different levels of combined Salt (NaCl + NiCl2) Stress in pot experiment.

Source of Variance	d.f.	Total Soluble Protein (μg/g f.wt.)	Chlorophyll a (mg/g f.wt.)	Solute Potential (MPa)	Water Potential (MPa)	Total Soluble Sugar (μg/g f.wt.)
Species	1	11.3766**	0.00003***	0.04949	0.06868***	505518
Habitat	1	30.0934	0.00003**	0.00794**	0.01399	1087**
Species × Habitat	1	2.9958**	0.00890**	0.00297	0.00090**	4643**
Salt	3	5.7325**	0.00251	0.40125**	0.00236	62211**
Species × Salt	3	5.2919**	0.00445**	2.92125	0.00244**	37259**
Habitat × Salt	3	3.5044**	0.00241	1.18875**	0.02914	68369**
Species × Habitat × Salt	3	3.9805***	0.00438**	2.08875***	0.02096**	50545***
Errors	30	0.0162	0.00199	0.00008	0.00007	34
Total	47					

Significance levels ** = 0.01 *** = 0.001 d.f. = degrees of freedom.

3.3.8. Chlorophyll b

Cucumis melo agrestis (S1) Chlorophyll *b* level increased at T7, T10 and T13 where low salt was combined with low, moderate and high metal levels. A slight decrease was observed at T8, T11 and T14 where moderate salt was combined low, moderate and high metal levels. A significant decrease of chlorophyll was observed at T9, T12 and T15 where high salt level was combined with low, moderate and high metal levels. Agricultural ecotype has more value of chlorophyll a than desert ecotype. *Citrullus colocynthis* L. (S2) Chlorophyll *a* level increased at T7, T10 and T13 where low salt was combined with low, moderate and high metal levels. A slight decrease was observed at T8, T11 and T14 where moderate salt was combined moderate and high metal levels. A significant decrease of chlorophyll was observed at T9, T12 and T15 where high salt level was combined with low, moderate and high metal levels. Desert ecotype has more value of chlorophyll a than agricultural ecotype (Table 6, Figure 4).

3.3.9. Total soluble proteins

Cucumis melo agrestis (S1) Total soluble proteins increased at T7 and T10 where low salt was combined with low and moderate metal levels. A slight decrease was observed at T8, T9, T11, T12, T13, T14 and T15 as the concentration of heavy metal and salt was increased. Where moderate salt was combined low, moderate and high metal levels. A significant decrease of total soluble protein was observed at T15 of agricultural ecotype where high salt level was combined with high metal levels. Agricultural ecotype has less value of total soluble proteins than desert ecotype. *Citrullus colocynthis* L. (S2) Total soluble protein level increased at T7, T8, T10 and T11 while decreased at T9, T12, T13, T14 and T15 as the level of salt or heavy metal was increased in the combination. A significant decrease of total soluble protein was observed at T15 where high salt level was high metal levels. Maximum increase of total soluble protein was observed at T10 where moderate salt was combined with moderate metal level. Desert ecotype has more value of chlorophyll a than agricultural ecotype (Table 6, Figure 4).

3.3.10. Total soluble sugars

Cucumis melo agrestis (S1) Total soluble sugars level increased at T8, T10, T14 and T15 while low value of total soluble sugars was observed in plants under T7, T9, T11, T12 and T13. A significant decrease of total soluble sugars was observed at T7 where low salt level was combined with low metal level. Agricultural ecotype has more value of total soluble sugars than desert ecotype. *Citrullus colocynthis* L. (S2) Total soluble sugars level increased at T7, T9 and T10 while slight decrease at T13, T14 and T15 while significant decrease at T8, T11 and T12 of desert ecotype. In case of agricultural ecotype total soluble sugars were increased at T7, T8, T9, and T10 while decreased at T11, T12, T13, T14 and T15. Desert ecotype has more value of total soluble sugars than agricultural ecotype (Table 6, Figure 4).

3.3.11. Water potential (Mpa)

Cucumis melo agrestis (S1) In both ecotypes a gradual decrease in water potential was observed with gradual increase of low, moderate and high salt combined with low, moderate and high heavy metal levels respectively. *Citrullus colocynthis* L. (S2) In both ecotypes a gradual decrease in water potential at low, moderate and high salt level combined with low, moderate and high heavy metal level respectively (Table 6, Figure 4).

3.3.12. Solute potential (Mpa)

Cucumis melo agrestis (S1) In both ecotypes a gradual decrease in solute potential was observed with gradual increase of low, moderate and high salt combined with low, moderate and high heavy metal levels respectively. *Citrullus colocynthis* L. (S2) In both ecotypes a gradual decrease in solute potential at low, moderate and high salt level combined with low, moderate and high heavy metal level respectively (Table 6, Figure 4).

4. Discussion

Citrullus colocynthis L. resulted in adaptable outcomes in variable stresses. In association with control, the extent of chlorophyll was decreased by increasing stress levels of salinity and heavy metal. Plants treated with higher levels of salt and heavy metals resulted in decreased chlorophyll levels and vice versa. The quantity of chlorophyll b was also decreased by rising levels of salinity and heavy metal stresses. The plants retort to hassles at the bimolecular level can be linked with the sensitivity of environmental indicators and their diffusion to the adjusting machinery of the cell to stimulate definite adaptive mechanisms (Xian et al., 2015). Photosynthetic pigments of *Vigna mungo* were seriously decreased under various levels of Nickel chloride (Gurpreet et al., 2012).

It has been observed that amount of sugars decreased at high levels of salts and heavy metals. Jan et al. (2019), demonstrated that the sugar levels of *Oryza sativa* decreased in higher levels of Nickel chloride. Stress signal adversely affect the mechanisms in cells and biochemical reactions (Xian et al., 2015). It has been observed that amount of proteins decreased at low levels of salt and heavy metals. Victor et al., 2016 observed a reduction in the protein content of Lettuce and Hyacinth plants under chromium stress. Free amino acids were increased by increasing level of stress in *C. colocynthis* and *C. melo agrestis*. It has also been reported earlier that free amino acids are increased instead of proteins under stress conditions (Amraee et al., 2020). According to Dong et al. (2017), free amino acids are increased in the plants under stress due to the biosynthesis of amino acids and the absence of translational factors. Proline amplified with the escalating level of salinity level as well as heavy metal. The maximum extent of proline was chronicled in combined high $NaCl^{+}$ high $NiC1_2$ was 0.3285 u mole/g in Citrullus desert, 0.3488 u mole/g in citrullus agricultural ecotype in comparison with 0.1350 u mole/g of control. 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 accomplishments augmented with the increasing level of salinity level as well as heavy metals in both of our plants. Combined stresses elevated production of superoxide dismutase and catalase. The maximum amount of Superoxidase dismutase (SOD) 21.55 u/mg of protein was recorded in high NaCI high $NiC1_2$ in comparison to 2.18 u/mg in control. Similar results were observed for Catalase (CAT) antioxidant enzyme activities. The maximum amount of catalase (CAT) 18.45 u/mg of protein was recorded in comparison to 0.46 u/mg of control under high NaCI high $NiC1_2$. Oxidative stress becomes an important tool to categorize the salt tolerance level of the plants. Oxidative stress in salt-tolerant plants was directly related to characteristics such as catalase (CAT) and superoxide dismutase (SOD) activity (Wu et al., 2012). Salinity tolerance is controlled by the coordinated action of variable gene involved in the initiation of a diversity of contraptions such as the confiscation of toxic ions, regulation of toxic metabolites, and antioxidative defense (Yasmeen 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 (Rizvi et al., 2018).

Cucumis melo agrestis L. has variable results in variable stresses. Plants treated with higher levels of salt and heavy metals resulted in decreased chlorophyll levels. Chlorophyll a level notably decreased at high NaCl, high $NiCl_2$ and combined high levels with other levels of salt and metal. In comparison with control, the amount of chlorophyll b was significantly decreased by increasing salinity and heavy metal stress levels. Photosynthetic pigments of *Vigna mungo* were seriously decreased under various levels of Nickel chloride (Aqeel et al., 2021). It has been observed that amount of sugars in present studies decreased in high levels of salts and heavy metals and combined high level treatments with other levels. Jan et al. (2019), demonstrated that the sugar levels of *Oryza sativa* decreased in higher levels of Nickel chloride. It has been observed in this study that the amount of proteins increased at moderate and high levels of salt and heavy metals significantly increased at low concentrations of salt and heavy metals. 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. A decrease in proteins was also recorded in this study at high levels of salts and heavy (Victor et al., 2016).

Free amino acids were gradually increased with increasing levels of stresses in this study. According to Amraee et al, (2020), free amino acids are increased in the plants under stress due to the biosynthesis of amino acids and the absence of translational factors. and free amino acids are increased instead of proteins under stress conditions(Zhang et al., 2014). Proline gradually amplified with the escalating level of salinity level as well as heavy metal. The maximum amount of proline was recorded in high NaCI high $NiC1_2$. The accumulation of Proline is also an adaptive response to salinity and heavy metal stress (Hayat et al., 2022).

It has been observed in present study that antioxidant enzyme activities increased with the increasing level of salinity as well as heavy metals. The maximum amount of super oxidase dismutase (SOD) was recorded in combined high (400 mM) NaCl with high (200 uM) $NiC1_2$. Salt tolerance is regulated by the synchronized action of variable gene involved in the initiation of a variety of mechanisms such as antioxidative defense (Yang et al., 2022). Similar results were observed for catalase (CAT) antioxidant enzyme activities. The maximum amount of catalase (CAT) was recorded at combined high NaCI with high $NiC1_2$. Oxidative stress becomes an important tool to categorize the salt tolerance level of the plants. Oxidative stress in salt-tolerant plants was directly related to characteristics such as catalase (CAT) and superoxide dismutase (SOD) activity (Wu et al., 2012). 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 (Hasanuzzaman et al., 2022). Metal toxicity is allied with oxidative stress (Jiang et al., 2018). These scenarios change physiological processes and consequences decreasing crop quality and yield (Bisbis et al., 2018).

5. Conclusion

Phytoremediation of salinity and heavy metal polluted soils is a reliable technique. For this purpose screening of plant lines is a preliminary requirement. Those plant lines having economic importance especially medicinal importance are very useful for the purpose. Those plants which require low water can be preferred. The plants adapt various physiological adaptations to tolerate and resist the stress produced due to salt and heavy metals. In this study two medicinal cucurbit weeds *Citrullus colocynth* and *Cucumis melo agrestis* from two ecotypes were treated against single independent and combined stresses of salt and heavy metal to study their physiological response. The results and discussion concludes that both of these medicinal cucurbit weeds have shown tolerance against stress levels. *Citrullus colocynth* is more resistant against stresses than the *Cucumis melo* and desert ecotype is more resistant as compared to agricultural ecotype. These plants have minor effect on their physiology but both of these produced resisting chemicals against stresses like SOD and catalase.

6. Recommendation

Phytoremediation is low cost pollution free technique for improvement of salinity and heavy metal affected soils. Among many other plant lines, medicinally important species are valuable to improve our soil bed. Cucurbit weeds *Citrullus colocynth* and *Cucumis melo agrestis* are proved to be resistant and tolerant against independent and combined salt and heavy metal stresses. These may be good source of soi soil remediation from pollution. *Citrullus colocynth* is more tolerant than *Cucumis melo agrestis* while desert species is more resistant than agricultural species.

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