Determination of endogenous concentration of Sodium potassium Cadmium Lead and Cadaverine correlating with physio-chemical parameters in *Lycopersicon* e*sculentum* under multiple stress conditions

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ABSTRACT Agricultural production is under serious threat from abiotic stresses. The effects of polyamines (PA) in this context are well documented and several mechanisms have been established to increase stress factors such as resistance to agricultural salt and heavy metal stresses. A chemically similar diamine putrescine, cadaverine (Cad), appears as a growth inhibitor and acts similarly. This research, which examined the effect of salt and heavy metals on three different tomato cultivars (Lycopersicon esculentum (L.) em. Thell): Pusa Rohini, Pusa Ruby and Pusa Sadabahar, was conducted in relation to this. Their large size significantly hinders plant development and carbon production. Cadmium reduces the toxic effects of heavy metals and acts as a determinant of plant development, stimulating the antioxidant defense system. This study is undertaken to prove that Cad had a varying effect on the Na^+/ K^+ levels of tomato seedlings under different stress and it was more efficient in mitigating salinity stress and heavy metal stress in Pusa Rohini and Pusa Sadabahar and Cad maintained the Na^+/ K^+ homeostasis but in Pusa Ruby Cad only in combination with NH_4NO_3 caused an increase in k+ under salinity stress. Similarly, Cad supplementation did not have any effect on Pusa Ruby under heavy metal stress but significantly reduced endogenous contents of lead and cadmium in Pusa Rohini and Pusa Sadabahar which shows that the role of Cad in amelioration of multiple stresses such as salinity and heavy metal varies greatly depending on different species. In this study, Pusa Sadabahar has the highest stress tolerating capacity especially in the presence of Cad.

Keywords: Cadaverine, Heavy metals, Lycopersicon esculentum, Na+/ K+, Salinity

1. INTRODUCTION

The aim of this research is to find new opportunities in bioengineered agriculture considering the soil nutrient problems currently occurring in many Indian states. India has 18% of the world's population and 15% of the world's livestock, but it has only 2.5% of the world's land area, a very precarious situation.

The primary factor causing soil degradation is stress, both biotic and abiotic. Pollution from heavy metals and salinity is a serious threat to the productivity of the land. Salts in soil and water inhibit plant growth. The amount of sodium in the soil solution reduces the plant's ability to absorb water, which slows its growth rate. At the same time, anthropogenic sources allow heavy metals to infiltrate into soil and plant ecosystems. If these chemicals are used in excess, they can have a negative impact on the earth's production, plants, organisms and the entire ecosystem. Cadmium is one of the most toxic heavy metals to plants and mammals. Cadmium compounds are more liquid than other heavy metals, which makes them more readily absorbed by plants, where they accumulate in edible plant parts. On the other hand, lead is mainly found in the topsoil due to accumulation of vehicle exhaust fumes in the air.

Similarly, some natural processes such as erosion and accumulation of sea salt by wind and rain are major causes of soil salinity. The balance of soil water logic is altered by human activities such as field clearance and irrigation, which are other causes of salinization. As a result, the amount of dissolved ions in the soil water increases to the point where plant growth is inhibited.

Plants are affected by several biotic and abiotic stresses (salinity, drought, heavy metals, extreme temperatures, and oxidative stress) during their lifespan. Their growth and development get hampered because of such environmental stresses [1]. This leads to a loss of crop productivity and so the food stock and economy in several countries around the globe per year [2]. This situation is getting worse day by day because of severe and frequent changes in climatic conditions like global warming, etc. India, being a populated country needs to develop certain tolerant crops to cope with such diverse situations.

Tomato being an economically pivotal and popular food crop in the world as it contains ―lycopene and certain bioflavonoids that possess anti-cancerous and anti-oxidative properties, and so the production of this crop is constantly increasing [3]. About 0.458 million hectares area is used for tomato cultivation, with a productivity of about 15.9 metric tons per hectare. Being a tropical plant, it adapts to all types of climates and can be cultivated anywhere around the globe; however, the original field potential of this crop can only get affected through these stresses. So far, stress tolerance in cultivated tomato plants has been achieved through some genetic approaches, using the general knowledge about plant adaptation. The biotechnological methods produce the upgraded version of tomato plants with stress tolerance capacity through the transfer of genetic information of many stress mitigating genes or genes involved in different regulatory pathways or signalling [4].It is one of the most significant food crops of India. It is cultivated in 0.458 Production of 7.277 million metric tons was produced in 1 million hectares with a yield of 15.9 metric tons per hectare. Although efforts have been made to create stress tolerant mutant plants, the management of stress conditions remains an area that requires further research in plant studies. In this regard, tomato cultivars Pusa Ruby, Pusa Rohini and Pusa Sadabahar (Lycopersicon esculentum Mill.) were used in this research under different abiotic stresses including salt and heavy metals. IARI, New Delhi, launched Pusa Ruby. It is an early-growing variety that has uniformly ripening fruit and a golden, slightly wrinkled stem end. It can be planted both in spring and summer and in autumn and winter. The yield averages 32.5 tonnes per hectare. In India, this variety is highly appreciated. Pusa Rohini was first announced by the Indian Agricultural Research Institute in the year 2005. These types of crops stay fresh for a long time. They bear medium-sized fruits with thick scarlet skin. It takes 80 days to reach maturity. For NCR, this type is recommended. Another format developed by the Indian Agricultural Research Institute and released in 2004 is called Pusa Sadabahar. This variety is also recommended for NCR and produces more tomatoes.

Many compounds use small, positively charged molecules called polyamines in various metabolic processes such as flower initiation, crop development, ripening, organ development, pollen tube growth, leaf senescence, and reduction. These compounds are also involved in responses to biological and abiotic stresses and contain plant hormones.

Diamine [NH2(CH2)4NH2] acts as a precursor to triamine Spd [NH2(CH2)3NH(CH2)4NH2] and tetraamine Spm [NH2 (CH2) 4 NH (CH2) 3 NH (CH2) 4 NH (CH2) 3 NH2], which are aliphatic and simple in nature. These multiple amines are distinct from each other and are among the biopositive ions. Functionally, these compounds are involved in various cellular processes.

In recent years, molecular genomics research has made significant progress in understanding how enzymes change during PA production and their potential biochemical activities in plants. Previous studies have identified putrescine (But), spermidine (Spd), and spermine (Spm) as three essential amino acids, along with their derivatives. Cadaverine has a unique molecular structure and belongs to the class of polyamines, which are believed to act as inhibitors of growth and persistence. In addition, it is often found in higher plants and is involved in reducing both energetic and abiotic stresses. The molecular equivalent of putrescine does not form in the five-carbon chain diamine.

or Arabidopsis. Polyamines (PA) are abbreviated polyamines, which are now recognized as amino molecules for people and plants. Examples of PAs are putrescine (diamine), cadaverine (diamine), spermidine (triamine) and spermine (tetramine). Cadaverine is a diamine that differs chemically from mod using a special pathway to produce it. It belongs to the same class as polyamines and has similar activity. Under various biotic and abiotic stresses, they are also integrated in higher plants, despite their unclear stress-relieving function. The molecular equivalent of putrescine does not form in the five-carbon chain diamine.

or Arabidopsis. The function of cadaverine (Cad), one of two growth-modifying polyamine diamines made entirely from a different pathway of other groups, in plant development is unclear. This diamine is found in the body as intermediate molecules in various forms of organic nitrogen. They act as building blocks for macromolecules, changing the organism's metabolism in the process. According to the literature review, diamine putrescine has been mainly discussed by plant physiologists, biochemists and molecular biologists for a variety of applications in crop improvement strategies. Diamine's role as a precursor to spermine and spermidine has also attracted the interest of researchers. Moreover, it has been found that cad promotes plant growth under multiple stresses, and proteins activated in response to stress in the presence of cadmium support plant growth under multiple stresses [8].

Casaverine diamine has been reported to have no effect on senescence in barley [10], induce asymmetric alkaloid production [11], or increase mitochondrial membrane porosity, although multiple amines are involved in antiaging interactions [9]. Very little research has been done on the metabolic function of elevated polyamines under stress [12]. Management of exogenous dead bodies under stress provides insight into the process of plant development, especially when tandem stressors are present [13].

2. LITERATURE REVIEW

Due to agricultural irrigation with polluted water, one of the main abiotic stresses is the accumulation of heavy metals, which inhibit nitrate reductase activity and reduce nitrogen and organic protein levels in leaf tissues [14]. According to reports, concentrations of heavy metals are higher in the upper parts of plants like crops than the lower parts like leaves, stems etc. [15]. The amount of cadmium absorbed by plants ranges from 0.1 to 2.4 mg/kg2.

Excessive amounts severely inhibit plant growth and carbon production.

When tomato plants were exposed to cadmium, Grato et al. (2008) reported increased lipid peroxidation, catalase activity [16], glutathione reductase activity and decreased glutathione peroxidase activity [15]. By protecting molecules, cad strongly supports the development of stressed plants [17].

Heavy metals are described as solid chemical elements that have a very high density (between 4 and 5 g/cm3) and are toxic even in small amounts. Toxic levels of heavy metals endanger ecosystems and have a significant impact on the nutritional, climatic and genetic characteristics of an area. In the 20th century, the industrial revolution led to a rapid increase in heavy metal pollution, causing severe pollution of land, water and air. Heavy metals are difficult to decompose and remain in soil for a long time, which reduces their quality [18].

2.1. Ionic Composition in Plants

Several researchers have explained certain changes in the amounts of inorganic ions under salt. Different responses to an increase in Na+ were observed, including a reduction in K+, Mg+, and Ca2+ ions, as well as Cl- ions [19].

An excessive accumulation of Na+ ions can be toxic to a plant's metabolic functions, which can lead to a decrease in the amount of available water. Soil-tolerant plants can balance the uptake of Na+ ions in their roots, while plants that are unable to do so were labelled as susceptible. Some studies have suggested that maintaining an optimum K+/Na+ ion ratio is important in controlling plant function in saltier environments. Furthermore, salt has been shown to increase Zn, Mn, and N concentrations, as well as Na+ and Cl- ions, but the amounts of P, S, and Ca2+ remain constant. However, under saline stress circumstances, the concentrations of K+, Mg+, Fe, and Cu ions can be affected [20]. Leidi and Saiz reported more granular matter, water's constituents, and other variables in 1997.

In salt-tolerant genotypes, there is greater Na+ ion buildup in the foliage compared to weaker genotypes [21]. These studies suggest that increased Na+ ion absorption and water quantity and tolerance processes are interconnected. Additionally, sufficient but regulated ion absorption is necessary for correcting halophytes, which is similar to effective ion reallocation and compartmentalization, and this can improve the development rate and the ability for water absorption.

When Na+ blocks the entry of nutrients inside plant sections, ion unbalancing can occur, competing with other minerals like Ca2+, K+, and NO3-. This can lead to a decrease in K+, Ca2+, Mg2+, N, and P concentrations in foliage and stems. Although many studies have characterized the constant amounts of K+, Ca2-, and S in the leaves, a reduced K+/Na+ or K+/Ca+ ratio can result [22]. In 1980, Thomas noted a rise in Mg+ and Ca+ ion concentrations in foliage. Therefore, it is essential to recognize these dietary problems to reduce the detrimental impacts of sodium stress on plant output [23].

Recent research has highlighted the impact of excess salt content on plant tissue reactions, resulting in decreased plant yield and development [24]. The buildup of salt molecules in the soil creates osmotic pressure, which lowers water absorption by plant roots and causes an abnormal buildup of reactive oxygen species (ROS) [25]. Root membranes are damaged as a result [26]. The higher quantity of Na+ that enters the root cells during stress results in the release of cytoplasmic K+, causing an ion excess and biochemical diseases brought on by lack of focus [27]. In 2014, Liu et al. found that PAs regulate salt-dependent ROS balance by causing the antioxidant protection system to become active in response to stress. Within the transmission of stress messages, receptors detect the signals, which are typically located at the cytomembrane, causing several secondary processes to be unleashed or activated, including stress-related molecules like calcium, ROS, and inositol phosphates. Secondary components, such as protein kinases and phosphatases, are signaled and stimulated [28, 29, 30, 31, 32, 33, 34].

There is a need to consider numerous agronomy problems with regard to environmental changes, organic processes, and crop quality. Plants and vegetables will benefit from taking the polyamine route into account. Lysine is catalyzed by lysine decarboxylase, as proven by Tomar et al. in 2013. This metabolism is controlled by maturation [35]. Increased salt and drought resistance in Burma are due to PA binding in Cynodondactylon, or grass [36]. The varied abundance of Cad in the plant is suggested as a potential contender for the taxonomy identifier by parts/products, as well as for commercial modification in conjunction with plant development and growth. Studies on the function of Cad have received less attention than those of other PAs. According to [37], the abundance of Cad rose during lupine, chickpea, and wide bean sprouting. Garcia-Garcia et al. noted in 2000 that after three months of storage, there were trace amounts of cadaverine in table olives. Brining [38] showed Cad in tomato, pea, and maize coleoptiles, as well as Datura. Legaz et al. established the reliance of cadaverine titers on plant age and came to the conclusion that Cad diminishes over time [39]. Tomar and co. discovered that immature orange species seeds contained an increased level of Cad in 2013 [25]. Inquiries and research have also discovered a high amount of Cad linkage under hyper-hydric plants in 2002 [40]. Shi et al. discovered the presence of cadaverine in the hairy roots of Brugmansia candida in 2021 after observing the increased production of cadaverine in Nicotiana tobacum hairy root cultures [42]. Application of cadaverine may cause anchoring in Scot pine containing ectomycorrhizal fungus [43]. It might impact by altering the bacteroid, nodule metabolism [44].

Pathogenesis-related protein 1b1 (PR1b1) was discovered by Goyal et al. in 2016 to be a key tomato plant protein that responds to cooling and is up-regulated in high genetics with recombinant polyamines [45]. In 2012, Nambeesan et al. proposed a link between tomato polyamine-mediated susceptibility to Botrytis cinerea and invasion with ethylene's functions in its protection. The biochemical changes associated with maturation are both independently of and in response to ethylene, and the apple metabolome is regulated by numerous regulators, including PA andethylene [46]. These findings suggest that PAs play a crucial role in plant defense against biotic and abiotic stresses.

Research has also shown that PAs work with ion channels and contribute to ion channel function, which is essential for housekeeping [28, 29, 30]. In addition, PAs can regulate plant growth and development, as well as stress responses, by affecting various signaling pathways [31, 32, 33, 34]. Therefore, understanding the roles and mechanisms of PAs in plants can help improve crop productivity and resilience to environmental stress.

It is important to note that while PAs have shown promise in promoting plant growth and stress tolerance, their application must be carefully controlled. Excessive PA application can lead to toxicity and negative effects on plant growth and development. Therefore, more research is needed to determine optimal PA application rates and methods to maximize their benefits without causing harm.

PAs are a vital component of plant growth and stress response mechanisms. Their roles in ion channel function, signaling pathways, and stress tolerance make them promising targets for improving crop productivity and resilience to environmental stress. However, careful control of their application is necessary to ensure their effectiveness and safety. Further research is needed to fully understand their mechanisms of action and optimize their use in agriculture.

Every year, agricultural production suffers huge losses due to prevailing weather conditions. Abiotic stress, especially cadmium salt and cadmium from human activities, exacerbates this. Crop production and human health are severely affected by many stresses on vegetation. Abiotic stresses hinder plant development by inhibiting changes in germination, dry mass accumulation, and biomass partitioning because they disrupt physical and chemical processes that simultaneously cause growth and decline in associated compounds.

Salinity affects agricultural growth and production worldwide, reducing the efficiency of many plants. In the next 25 years, salt stress, one of the biggest threats to food security, is expected to affect 30% of the agricultural area. To analyze the adverse effects of salt stress on production and growth and development of edible plants, several procedures have been established. For example, (1) low water potential in the root zone leads to water deficit in plant tissues as a result of salts, (2) Na^+ and Cl^- ions cause ionotoxicity in plants, and (3) reduced absorption of aggregates and vitamins. Roots may result in salt residues resulting in an ion imbalance [47].

Heavy metals are dangerous to plants as well as humans. Environmental pollution is one of the major reasons for increasing the toxicity of heavy metals in plants. This affects the human health and yield of the plants [48,49]. Cadmium and lead grabbed the attention of researchers because of their production and accumulation in the soil and causingtheir eradication. The plants can easily take these up as they tend to sustain in soilsolutions, being mobile and non-degradable [50,51].While, it was reported that a minimal quantity of lead affects plants by reducing themetabolic activities of the plants like cell division, germination, photosynthesis andnitrogen assimilation, etc. [52]. Further lead accumulation was foundin several medicinal plants like aromatic rice [53]. These are one of the environmental contaminants. Specific methods havealready been used to scavenge such contaminants from the environment, but they areexpensive and difficult to get optimal results. Natural and all human activitiescontribute to polluting the environment with heavy metals. Dusting or leaching of soilcauses the migration of these heavy metals to the non-contaminated areas resulting inecosystem contamination [54]. Several modern technologies are expensive and produce a large quantity of volumetric sludge [55]; other thermal and clinical methods are challenging, laborious, and costly, andthese may deteriorate the important components [56]. Generally, soil remediation of heavy metals includes onsite management orscraping off, followed by disposal to a landfill area. This process of disposing of individually moves the contaminants to some other area with the hazardous heavy metals related to the environment. Washing of soil is another alternative method to remove the contaminated soil and disposing of it off to the landfill. But this method is yet again expensive, and a residue remains rich in heavy metals that need to be treated further. Additionally, such physio-chemical methods used for remediation of soil provide the land for plant growth as they abolish all biological activities. Heavy metals may reside in the soil for thousands of years that can cause health danger to higher organisms in several ways [54].

Several researchers have revealed the uptake of heavy metals by plants. This can be used to standardize the parameters to improvise the functionality of plant uptake. Sinha and his team membersreported that plants are ―excluders‖ and ―accumulators [57].

Plants now enable to solubilize with the help of roots facilitated by chelating agents produced by the plants. These plants can directly trans-locate and reserve micronutrients. Similarly, these processes are also indulged in reserve, trans-locate, and take-up of contaminants whose chemical characteristics resemble those of essential elements.

Several proteins or transport processes are known which are embedded in the plasma membrane indulged in the uptake of ions they trans-locate (i) protein pumps, (ii) co-and anti-transporters, and (iii) channels. Each mechanism takes up the ions. The primary concern is the ion interaction during the uptake of several contaminants. Translocation in the shoots is prudent after the uptake by roots as the harvest of biomass of roots is not possible.

Plants do not deposit trace elements unless they have their metabolic requirements. So, they have an uptake-translocation process that seems to be closely related. All these needs may range from 10-15 ppm of certain trace elements. Another concern is the form of contaminants in which heavy metals are reserved in the plants and the mechanisms by which they avoid metal toxicity. Storage in the vacuole is one of the processes of accumulation of heavy metals in plants. Such plants are also termed ―hyper-accumulators, which remove the contaminants from the plants which trans-locate from roots to the shoots leaving the soil undisturbed [58].

2.2. Localization of Cd/Pb in Plants

These heavy metals possess a significant threat to human and environment as these are extracted from the soil andtransferred to the human beings via plants. These are considered to enter into the plantroot cells as they chelate via trans-membrane transporters [59] or asions through cation channels. They can also pass through the roots by apoplastic orsymplastic pathways before being trans-located into the shoot and the xylem. Even at low concentrations, these are toxic to several plants. Significant symptoms of their toxicity are leaf rolling, stunting, and chlorosis [60]. Many physiological and structural changes of plants are interrelated to heavy metal toxicity. For example, deterioration of phloem sieve tubes, cell wall saturation by phenolic compounds, retarded cambial activity, chloroplast alterations, stiffening of all walls, etc. [61]. Additionally, these heavy metals in higher concentrations can be proven carcinogenic or mutagenic for several animal species [62]. Hence, soils contaminated with heavy metals require a cost-effective solution. To withstand the higher concentrations of heavy metals, certain species of plants have developed a complicated process to control the take-up, deposition, or detoxification of metal traces. Qualitative root properties should also be studied concerning phytoextraction with qualitative parameters for biomass production, as modified root morphology can affect the water absorption and growth and development of the plant directly. On evaluating the Cd content, it is evident that exposure of plants to differentconcentrations leads to varying levels of Cd accumulation and hence causing theplasmolytic shrinkage leads to hindering precise sub-cellular Cd localization and so thecondensation of the cytoplasm [63].

2.3. Plant Growth and Development under Metal stress

In angiosperms, changes in the size and ratio of root tissues are observed

during their exposure to heavy metals [64, 65, 66]. In this study, they exposed plants to different concentrations of CdCl2 (0, 30, 60, and 100 μM) to evaluate plant heavy metal stress tolerance. She showed that P. vittata absorbs, transports, and deposits cadmium in the low bioactive tissues of fronds, trichomes, and scales. Heavy metal tolerance in this particular plant correlates with the metal-induced morphological response in the roots. The plants also showed changes in root apex size, suberin endododermal lamellae, developmental patterns of root hairs, etc.

This study demonstrated that this particular plant can tolerate a maximum of 60 µmCadmium concentration which by contrast improves the root system by allowingGood transport and deposition of metallic traces in the fronds.Moreover, Jiang et al., in 2016, experimented with observing the varying effects of cadmiumconcentrations in different rice cultivars. The plant growth parameters observed werePlant growth, photosynthesis, biomass, cadmium uptake, and root/shoot Plant antioxidant stress activity. They revealed that the CD negatively affected thePlant growth and photosynthesis, but correspondingly increased cadmium concentrations

Increased cadmium uptake and oxidative stress in all rice cultivars. Cadmium interferes with the absorption of ions, causing an imbalance of nutrients. The plant's mineral content could be reflected by the availability of cadmium in the growth medium. The plant faces dangerous and harmful effects when the cadmium content in the soil increases. Root elongation of plants subjected to heavy metal stress provides us with the basis for evaluating the tolerance of plants against heavy metal stress [25].

Aliphatic polycations called polyamines, such as cadaverine (Cad), have been found to accumulate in biotic and abiotic stressors, leading to the overexpression of stress endurance genes in larger plants. Physiological evaluations were conducted to track the effects of Cad on biomass accumulation and the increase in photosynthetic biomolecules, nitrate reductase activity, total soluble proteins, organic nitrogen, and colours, which have a strong correlation with the development stress of the tomato (Lycopersicon esculentum) variety. Pusa Ruby, Pusa Sadabahar, and Pusa Rohini were subjected to all treatments (T0 = control, T1 = NaCl, T2 = NH4NO3, T3 = Cd, T4 = Cd+NH4NO3, T5 = Cd+NaCl, T6 = Pb+NaCl, T7 = Cd+NaCl+NH4NO3, T8 = Pb+NaCl+NH4NO3, T9 = Pb+Cd, and T10 = Pb+NH4NO3, T11 = Cd+NH4NO3). Comparison studies provided comprehensive insights and an improved understanding of Cad's mechanism, which improves tomato resistance to various stresses.

The poorly understood external release of Cad under stress is expected to have beneficial effects on stressed tomato plants. The current research aims to shed some light on Cad's regulatory mechanism on tomato plant development and its ability to reduce the negative reactions brought on by various stressors. Under various stressor conditions, Cad also determines the natural content of Na+/K+, Cd, or Pb ions corresponding to physio-chemical factors. Plants may benefit from appropriate development even in stressful environments due to the proteins produced in response to stress in the presence of Cad [67]. This study was conducted to demonstrate that Cad had a variable impact on the Na+/K+ levels of tomato seedlings under various stresses. It was more effective in reducing salinity and heavy metal stress in the Pusa Rohini and Pusa Sadabahar regions and preserved Na+/K+ homeostasis there, but in the Pusa Ruby region, Cad only increased K+ under salinity stress when combined with NH4NO3.

3. MATERIALS AND METHODS

Tomato seeds (Lycopersicon esculentum var. Pusa rubi, Pusa rohini and Pusa sadabahar) were sterilized with 0.1% sodium hypochlorite solution for 2–3 min, followed by three rinses with twice distilled water. Seeds were planted in Petri dishes lined with Whatman filter paper. In accordance with the study design, plants were grown under controlled conditions (75 W light, 252 °C, 65% RH), irrigated with a full-strength Hoagland's nutrient solution containing 50 mM NaCl, 1 mM Pb, and 1 mM Cad. . The pH of 6.4 of nutrient solution containing salts and Cd was constant for all treatments (T0 = control, T1 = NaCl, T2 = NH4NO3, T3 = NaCl + NH4NO3, T4 = Cd, T5 = Pb, T6 = Cd + NaCl, T7 = Pb + NaCl, T8 = Cd + NaCl + NH4NO3, T9 = Pb + NaCl + NH4NO3, T10 = Pb + Cd, T11 = Pb + NH4NO3, T12 = Cd + NH4NO3) with and without cad. Pb and Cd were determined using atomic absorption spectrometer, while Na and K were estimated using digital flame photometer. Statistics are mean of three samples with standard deviation. Student's t test of significance of the intervention and data analysis was applied.

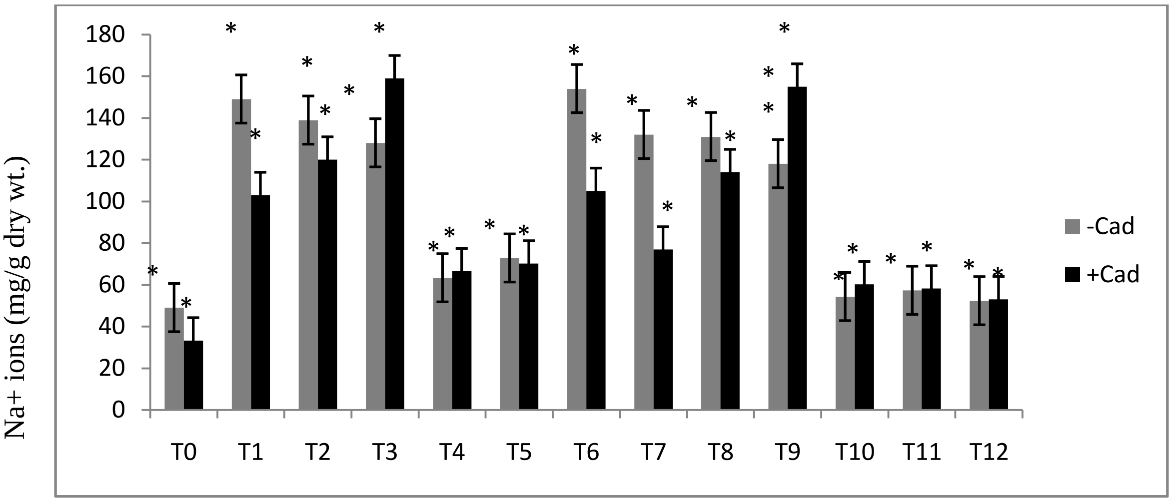
4. RESULTS AND DISCUSSION

4.1 Na^+ and K^+ in Tissues with or without Cad

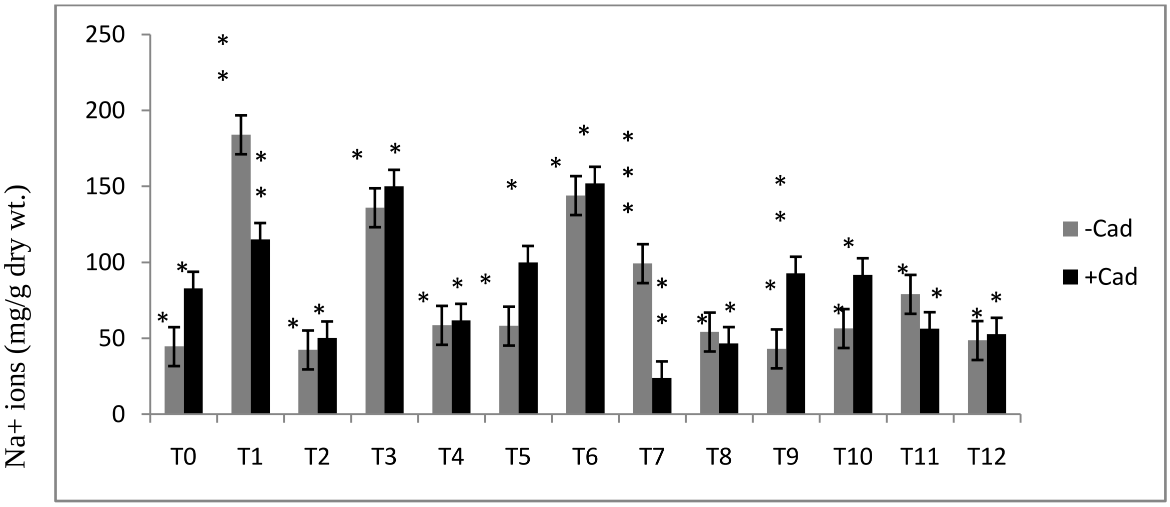
Under salt stress, nearly 3-fold increases in endogenous concentrations of Na^+were observed in Pusa Ruby. When compared to that of control, more than 3-fold increase was observed in Pusa Rohini whereas a significant decrease in Na^+ levels was noticed in Pusa Sadabahar. Similarly, under combined stress (Salt and heavy metals), elevated levels of Na^+were observed in Pusa Ruby and Pusa Rohini whereas in Pusa Sadabahar the Na^+ levels remained unchanged under cadmium and salt stress and decreased slightly under lead and salt stress. However, decreased endogenous Na^+ levels were observed in all other treatments of Pusa Sadabahar (Figure 1).

When compared to control, NH_4NO_3 treatment led to more than 2-fold increases in Na^+concentrations in Pusa Ruby, caused no change in Pusa Rohini, and decreased drastically in Pusa Sadabahar. When NaCl was coupled with $NH_4NO_{3,}$ Na^+ levelwas found to be decreasing over NaCl treatment alone in all three varieties. In Pusa Ruby Cad supplementation caused a significant decrease in the Na^+ levels under salt stress in most of the cases except in the presence of NH_4NO_3. However, neither Cad nor NH_4NO_3 had much influence on the Na^+ levels. In the case of Pusa Rohini, Cad supplementation leads to a significant decrease in NA^+ levels under cases of salinity stress and Pb combination of salinity stress. Pusa Sadabahar seedlings, Cad supplementation reduced Na^+levels significantly in almost all stressed conditionsbut the much-pronounced effect of Cad was seen with salinity stress where the NA^+levels reduced drastically.

Endogenous K^+ concentrations were also analysed in the seedlings of these three different tomato varieties exposed to different types of stresses. The results showed that the K^+levels reduced under all conditions of stresses as compared with control in Pusa Ruby. Levels of K^+ were lowest in all the multiple stress conditions followed by salinity stress. Similarly in Pusa Sadabahar, K^+ levels decreased under all stress conditions except for cadmium with NaCl exposed seedlings where the K^+ levels remained unchanged when compared to that of control. In contrast to these two varieties of tomato, Pusa Rohini showed a high increase in the K^+ levelsunder different stress conditions such as salt stress, salt and lead stress, salt stress with NH_4NO_3, and lead stress with $NH_4NO_{3.}$ A slight increase in K^+ levelswas observed under metal stress. In the presence of Cad, further reduction in the K^+ levelsoccurred in Pusa Ruby seedlings exposed to $NH_4NO_{3,}$ Cadmiumwith NaCl, Lead with NaCl, cadmium with NH_4NO_3,and lead with NH_4NO_3 whereas Cad supplementation leads to a high increase in K^+ levels under other conditions of stress such as NaCl with NH_4NO_3 and heavy metal stress either alone or in combination. In Pusa Rohini, Cad caused a decrease in K^+ levels under salinity stress, lead and salinity stress and lead with NH_4NO_3. In other conditions either a very slight increase in the K^+ levels was observed or there was not much change in the levels of K^+. In contrast to Pusa Ruby and Pusa Rohini, Pusa Sadabahar showed a heavy decrease in the K^+ levels under salinity stress and a remarkable increase under combined lead and cadmium stress (Figure 2).



Treatments (Pusa Rohini)



Treatments (Pusa Ruby)

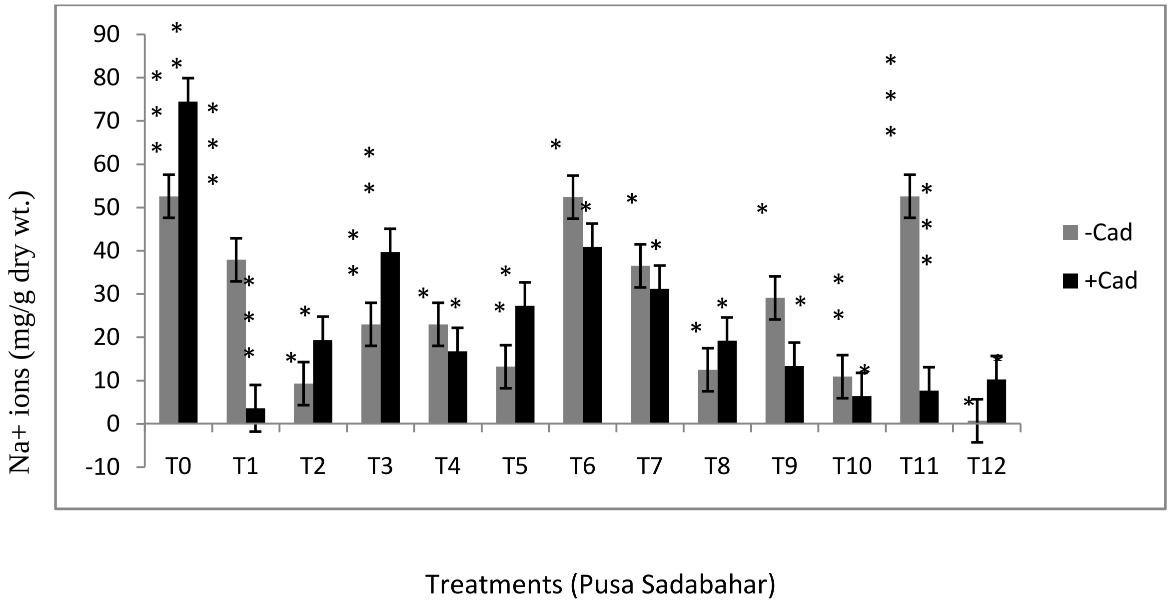
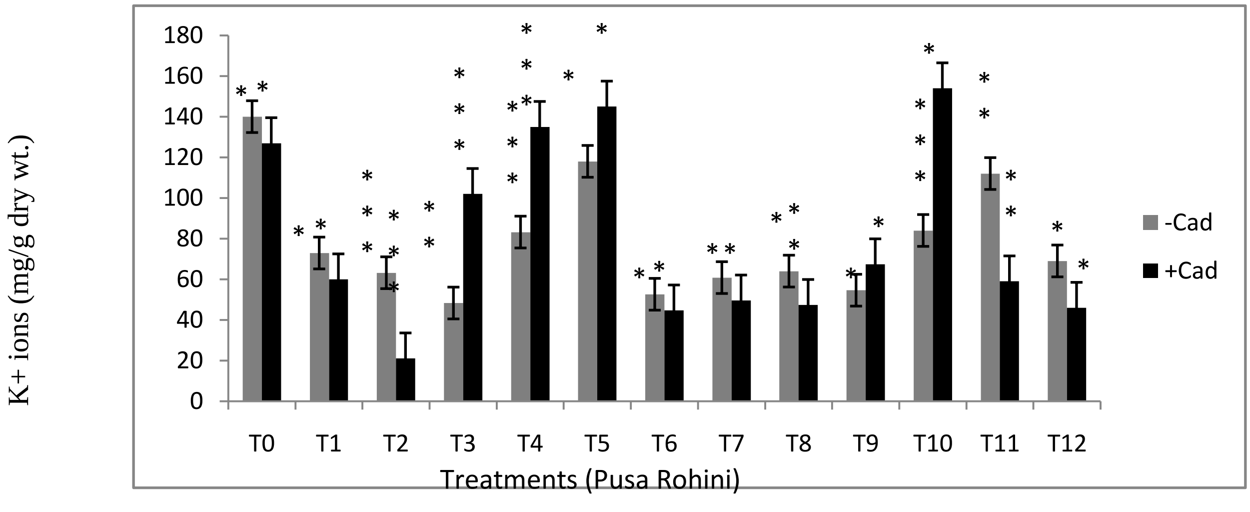


Figure 1: Na+ content of 7-day-old seedlings under multiple stresses with or without Cad. Here, T0= control, T1 = NaCl, T2 = NH 4NO 3, T3 = NaCl+NH 4NO3, T4 = Cd, T5 = Pb, T6 = Cd+ NaCl, T7= Pb+NaCl, T8 = Cd+NaCl+ NH 4NO 3 , T9 = Pb+NaCl+ NH 4NO 3, T10 = Pb+Cd, T11= Pb+NH 4NO 3, T12 = Cd+NH 4NO 3. Data are mean values of replicates with (n=3) ± SD. Asterisks p<0.05% (*) possibly significant, p<0.01 (**) definitely significant, p<0.001 (***) highly significant and no asterisks indicate significance of difference at p> = 0.05.



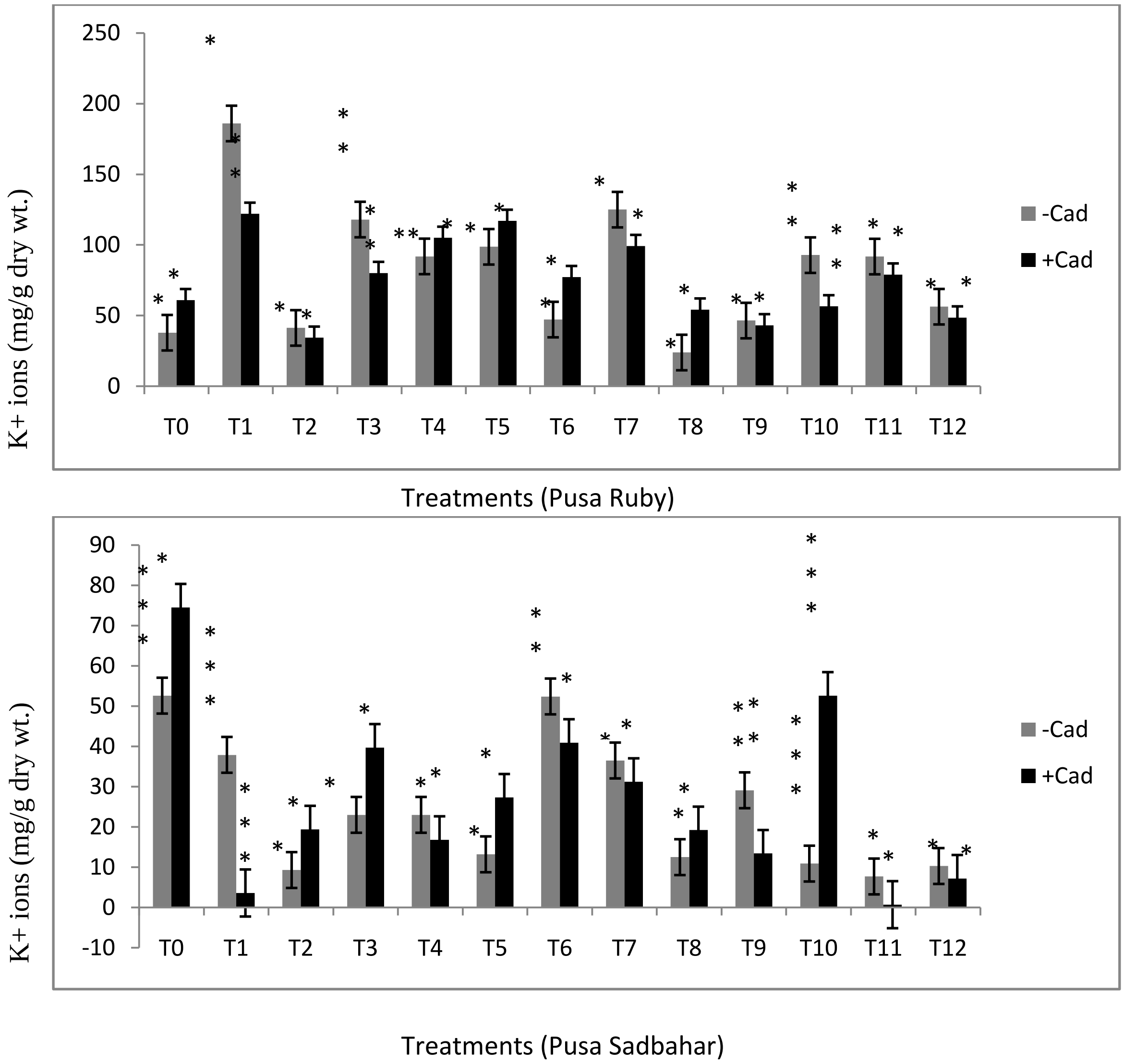
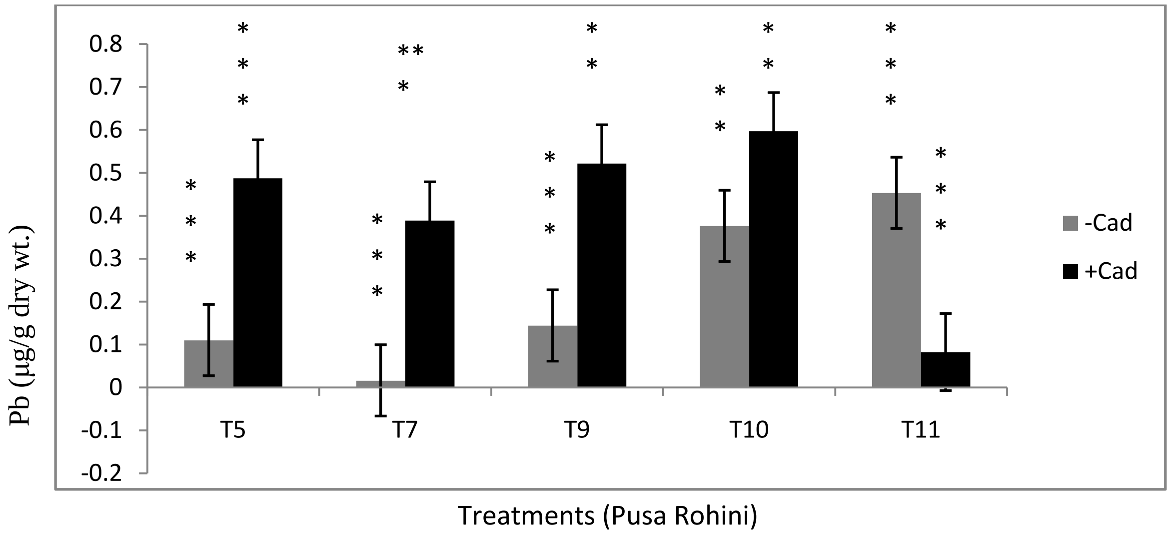


Figure 2: K^+ Content in 7-day old seedlings under multiple stress with or without Cad. Rest legend same as in Figure 1.

4.2 Cd/Pb Level under Multiple Stresses with or without Cad

In Pusa Ruby, the Pb content increased in all kinds of stress treatment except in the case of combined salt and metal stress where the Pb content was the lowest when compared to other treatments. Endogenous Pb content was highest on treatment with Pb and NH_4NO_3 which was greatly reduced on supplementation with Cad. However, in other treatments, Cad caused a further increase in Pb contents when compared to those without Cad. The endogenous load of Pb was higher in Pusa Rohini when compared with the other two varieties and the highest lead contents were observed in salt and metal stress along NH_4NO_3.Moreover,the lead contents under all the stress conditions were reduced to a significant amount by Cad, Combined heavy metal stress (Pb+ Cd) being the only exception where Cad caused an increase in Pb contents. In contrast to this, lead content in Pusa Sadabahar under combined heavy metal stress was reduced by Cad. (Figure 3) lead contents were also reduced drastically under metal and salinity stress whereas increased lead contents were observed under Pb stress and Pb and salt stress with NH_4NO_3 in the presence of Cad.

Under cadmium stress and cadmium along with salinity stress, the endogenous load of cadmium was more or less the same for all three varieties of tomato. Cad supplementation leads to an increase in Cd levels in Pusa ruby, a slight increase in Pusa Rohini, and a decrease in Pusa Sadabahar supplemented with Cd. Under conditions of multiple stress (Cd + NaCl), Cad did not have any significant effect on Cd contents Pusa Ruby whereas it reduced the Cd contents in Pusa Rohini and Pusa Sadabahar. The highest endogenous Cd contents were observed under Cd stress with NH_4NO_3 in all the varieties which were further reduced to a certain amount by Cad. Surprisingly, Cd was not detected in Pusa Sadabahar treated with Cd+NaCl+ NH_4NO_3 and Pb+Cd (Figure 4).



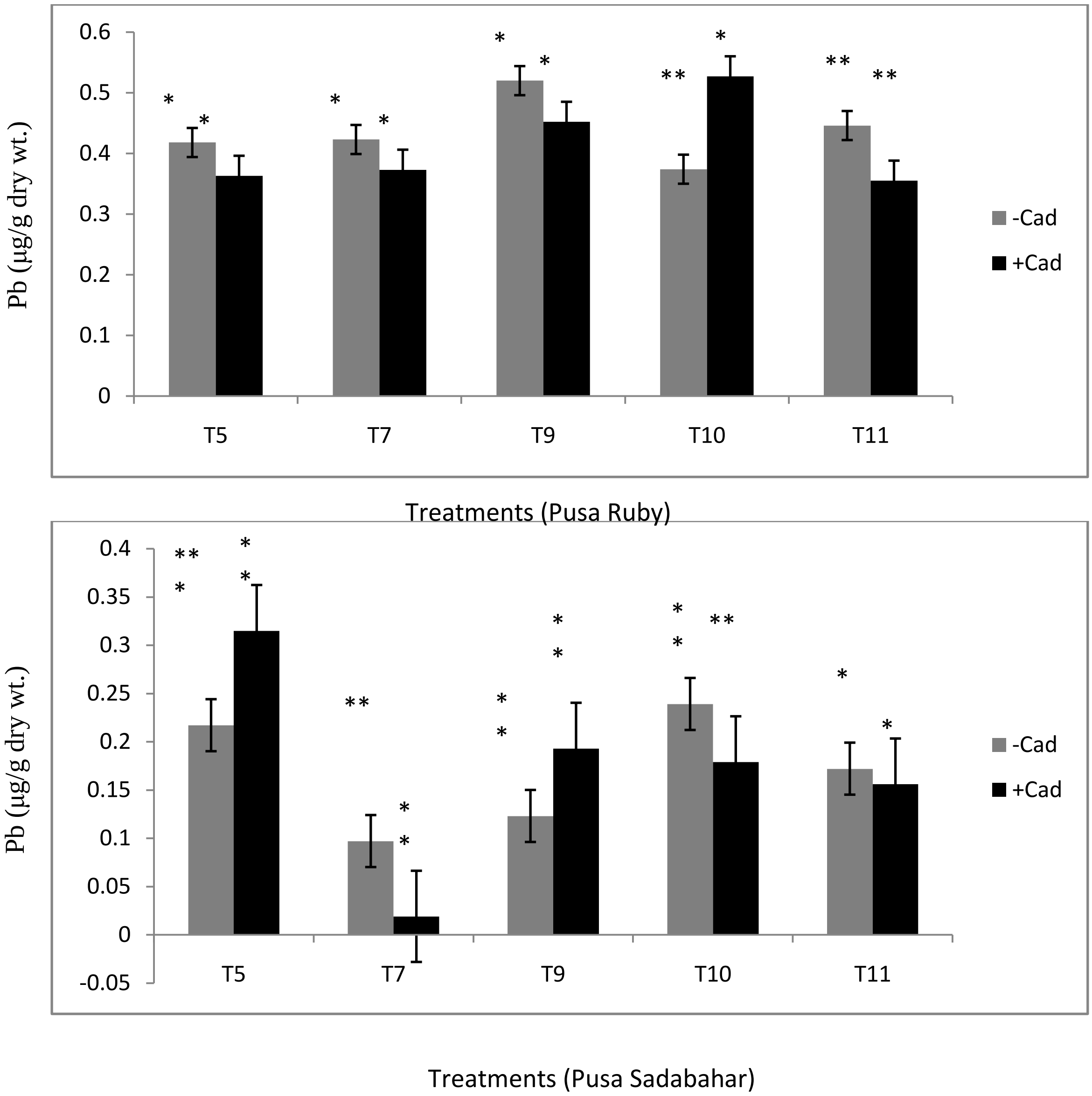
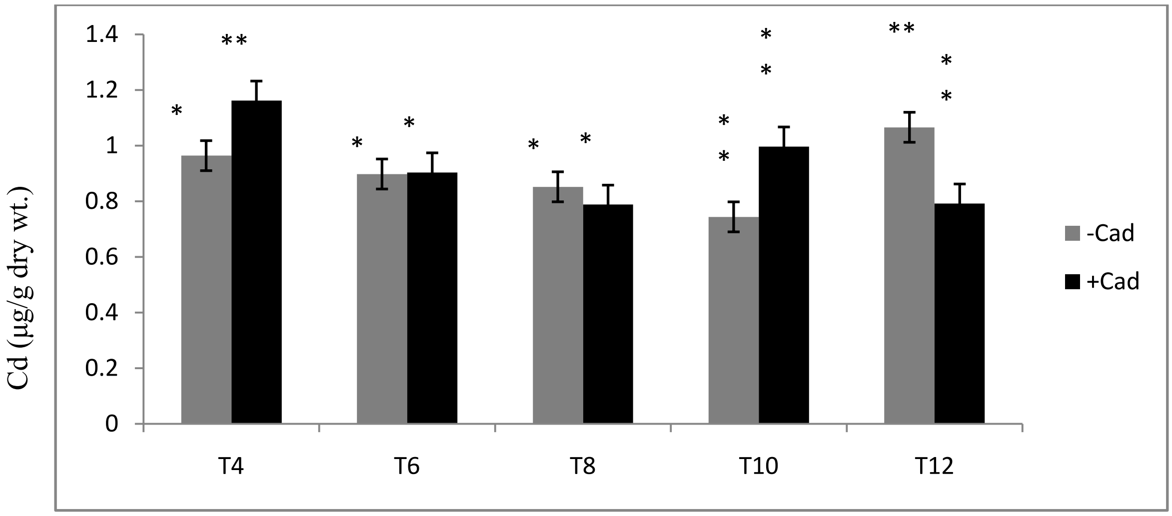
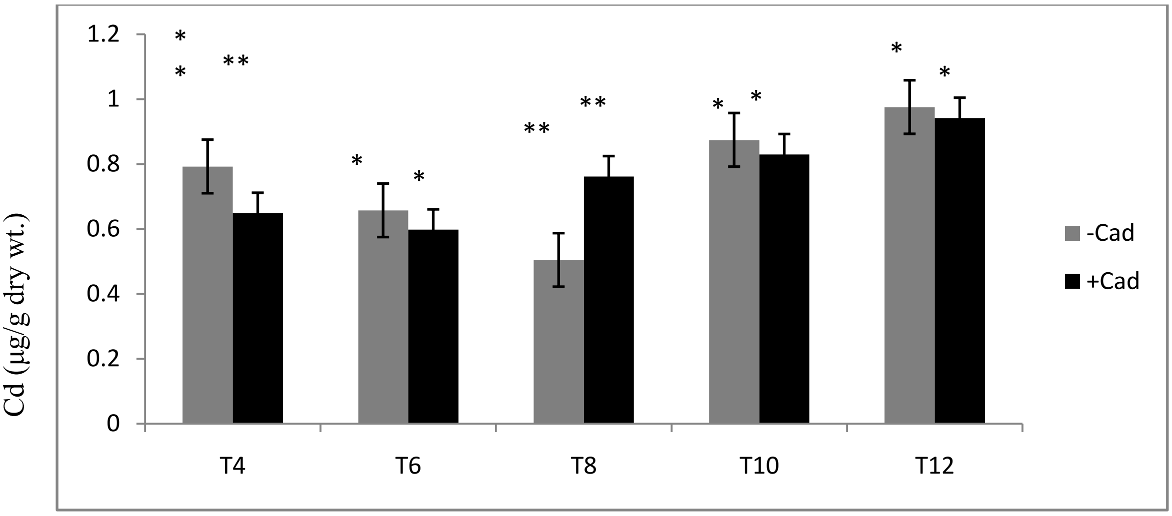


Figure 3: Pb content in 7-day old seedlings under multiple stress with or without Cad.

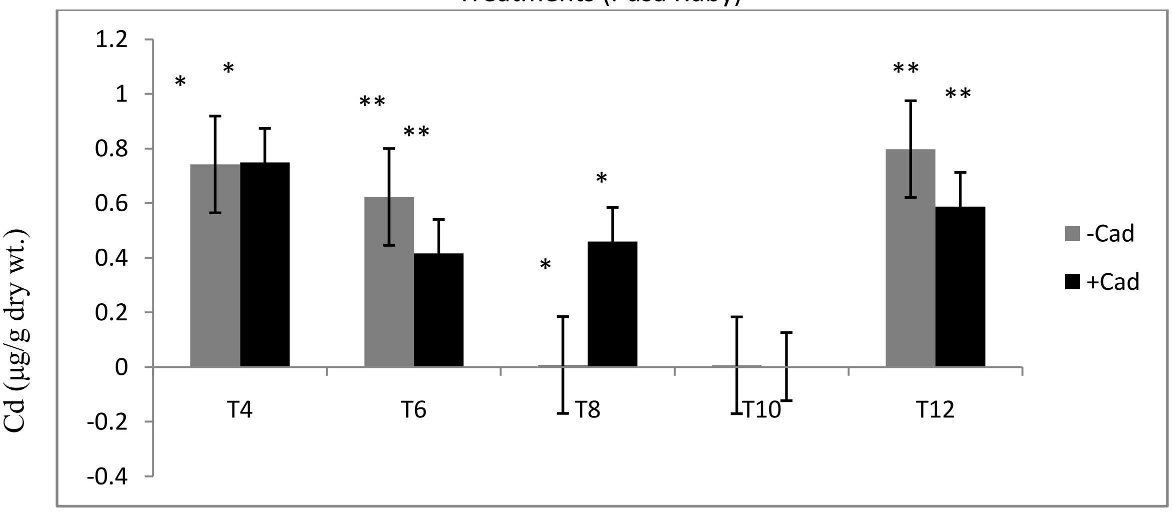
Rest legend same as in Figure 1.



Treatments (Pusa Rohini)



Treatments (Pusa Ruby)



Treatments (Pusa Sadabahar)

Figure 4: Cd Content in 7-day old seedlings under multiple stress with or without Cad. Rest legend same as in Figure 1.

5. CONCLUSION

The polyamines spermidine and spermine, as well as the diamines putrescine and cadaverine, are potential communication networks utilized by the body to protect against stress. These compounds are recognized for their anti-senescence and anti-stress benefits, as they possess antioxidant and acid-neutralizing properties and can stabilize membranes and cell walls. Other studies suggest that the addition of external polyamines to plants under salt stress can improve ionic (K^+/Na^+) interactions, which is important for regulating development in stressed plants [68, 69].

During embryonic development, an effective antioxidant process is thought to occur in the presence of these compounds. Under stressful conditions, the overall antioxidant capacity of polyamines was consistently greater than that of water. In addition, ionic and osmotic stress can impact germination and development. In 2020, Abdel-Faried et al. observed the development of seeds in Cucumis sativus and Lycopersicon esculentum plants in various concentrations of NaCl (25, 50, 100, and 200 mM) both in vivo and in vitro. They found observable delays in metabolic reaction and photosynthesis substance, as well as significant drops in fresh and dry weight. Even with only 200 mM of salt content, all interventions resulted in shorter roots, and the reduction of photosynthetic compounds in tomato was greater than that in cucumber. They also demonstrated that salt had an impact on the biochemical analysis of stressed plants, but a few beneficial secondary compounds were increased in tomato and cucumber crops [70].

In this study, the pattern of Na^+/K^+ level changes for various tomato types under different stress conditions differed significantly, indicating that cadaverine had no particular effect but a variable effect on ion balance. In Pusa Ruby, NH_4NO_3 and cadaverine alone or together did not affect Na^+ contents in response to heavy metal stress, but cadaverine along with NH_4NO_3 caused an increase in K^+ contents under salinity stress in Pusa Ruby and Pusa Sadabahar. Cadaverine supplementation effectively reduced Na^+ contents in Pusa Rohini and Pusa Sadabahar in response to salinity stress and in a few cases in response to heavy metal stress. Therefore, it appears that the ameliorative effects of cadaverine on the shift in Na^+/K^+ brought on by stress are very tissue- or species-specific. In Zhao et al.'s 2007 study, putrescine significantly decreased Na^+ levels in the roots of barley plants, and supplemental spermidine significantly decreased Na+ levels in the leaves and stalks of rice under salt stress [71].

Elevated concentrations of heavy metals such as Cd and Pb, which cause heavy metal stress, typically prevent seed development, cell and whole-plant growth, nutrient absorption and dispersal, and respiration. Many reports discuss the effect of polyamines on plants under metal stress[72, 73]. In the present study, Cad supplementation could not reduce the endogenous accumulation of Cd and Pb in Pusa Ruby under multiple stress conditions and the heavy metal content was found to be increased by Cad except for a few cases. Whereas in Pusa Rohini and Pusa Sadabahar endogenous cd and Pb contents were reduced to significant levels even under multiple stress conditions which shows that cadaverine can overcome cadmium stress in tomato plants but its effect varies based on the different species of a plant and of course other stress conditions. In this study, Pusa Sadabahar seems to have a better stress tolerance ability under different types of stress when compared to other varieties.

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