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# EFFECT OF HEAVY METALS (COPPER AND ZINC) ON PROLINE, POLYPHENOLS AND FLAVONOIDS CONTENT OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

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## Abstract

Current research on heavy metal toxicity in the agricultural field is focused on the toxic effects of copper and zinc on the most important crop which is tomato (*Lycopersicon esculentum* Mill.). To test the responses of tomato crop to heavy metal stress, leaves and roots were exposed to different concentrations of copper and zinc (0, 200, 300, 400 and 500ppm). All comparisons to the control indicate that the stress application induces the accumulation of proline content and promotes the synthesis of antioxidants such as polyphenols and flavonoids. This effect was directly proportional to the concentrations. Furthermore, these results suggest that the highest accumulation was occurred in leaves compared to the roots with all parameters studied. Additionally, the highest effect was marked with 500 ppm in both parts of the plant. The statistical analysis shows a highly significant increase in all biochemical parameters. A positive correlation was obtained between the osmolytes and the antioxidants. This study revealed that copper and zinc are essential elements for the plant. However, these metals at high concentrations become toxic for tomato by affecting and triggering the tolerance system in response to this stress.

***Key words***: *Lycopersicon esculentum* Mill., copper, zinc, proline, polyphenols, flavonoids, phytotoxicity.

## Introduction

Plants confront multifarious environmental stresses widely divided into abiotic and biotic stresses, of which heavy metal stress represents one of the most damaging abiotic stresses (Jalmi *et al.*, 2018). Large amounts of heavy metals end up in the environment as a result of ever-increasing anthropogenic activities and economic development (Jiang *et al.*, 2019). Anthropogenic activities such as agriculture, industry and mining have contributed significantly to the accumulation of heavy metals in the soil (Correia *et al.*, 2018). Heavy metal pollution of agricultural soils is one of main concerns causing some of the different ecological and environmental problems. Excess accumulation of these metals in soil has deteriorated soil, decreased the growth and yield of plant and entered into the food chain. (Etesami, 2018). Heavy metals contaminated plants cause losses in crop production and risks for human health (Gratão *et al.*, 2019). Heavy metals cause toxicity by targeting crucial molecules and vital processes in the plant cell (Jalmi *et al.*, 2018).

Manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn), due to their relatively low levels in the cell compared to abundant metal ions such as potassium and magnesium, transition metals are often considered micronutrients and referred to as trace elements (Li *et al.*, 2018). Essential micronutrients, such as zinc (Zn) and copper (Cu) have direct roles in plant metabolism (Pandey, 2018), they are essential for a variety of functions in the living cell (Lüthje *et al.*, 2018). Soils are mainly contaminated by copper and zinc (Salducci *et al.*, 2019).

Zinc (Zn) is one of the essential plant micronutrients and is involved in several physiological functions in plants (Rizwan *et al.*, 2019) for plant growth and development (Moreira *et al.*, 2018). Zinc (Zn), an essential metal, is required by plants as they form important components of

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zinc finger proteins and also aid in synthesis of photosynthetic pigments such as chlorophyll. However, in excess amount Zn causes chlorosis of leaf and shoots tissues and generates reactive oxygen species (Pramanick *et al*., 2017). Its deficiency in plants has been widely reported in many regions of the world (Moreira *et al*., 2018).

Copper is an essential element in plants (Andrés-Colás *et al*., 2018 ; Shams *et al*., 2019), It acts as an essential cofactor of numerous proteins that play key functions in plant cell metabolism, such as the transport of electrons in mitochondria and chloroplast, the regulation of the cellular redox state (Migocka and Malas, 2018). Copper (Cu) contamination has been increasing in land ecosystems due to economic development activities. Excessive amount of Cu in soils is toxic to both plants and microorganisms (Meier *et al*., 2017). Its higher concentration can make disruption in plant growth (Shams *et al*., 2019) and may cause adverse effects on plant physiology (Chrysargyris *et al*., 2019). Copper (Cu) is among the main contaminant of agricultural soil (Napoli *et al*., 2019).

Deprivation of these elements (copper and zinc) is causing symptoms of deficiency, whereas excess can be toxic due to the production of reactive oxygen species and an imbalance of the cellular redox state. Both deficiency and toxicity are the cause of reduced growth and crop yields (Lüthje *et al*., 2018). These elements cause an increase in phytotoxicity effects in higher concentration (Wolf *et al*., 2017). The toxicity of increasing heavy metal ion in soil has been threatening the food security and environments (Ruan *et al*., 2019).

Tomato (*Lycopersicon esculentum* Mill.), this crop is of the Solanaceae family. The tomato is one of the most important crops worldwide (Marti *et al*., 2018), However, its productivity is impaired by a wide range of abiotic stresses such as the heavy metals (Gerszberg and Hnatuszko-Konka, 2017) and biotic stress (Bouzroud *et al*., 2018; Quiterio-Guitierrez *et al*., 2019; Cumplido-Najera *et al*., 2019). Tomato is an agronomically valuable crop in many countries as in Algeria and specifically in Mostaganem region, either grown in fields or greenhouses and therefore has been bred and genetically improved for centuries. Nonetheless, it remains vulnerable to diseases, consequently requiring the use of chemical pesticides, mainly in greenhouses (Ines and Bernacchia, 2018), but the overuse of these products contributes to soil contamination and has harmful effects on the microfauna, microflora of cultivated soils and on the quality of the tomato crop. In other side there is a wide range of plant protection products applied to tomatoes containing some heavy metals such as zinc and copper. In horticulture copper sulphate is utilized for the inhibition of phytopathogenic fungi. However, copper tends to accumulate in soil with a concomitant effect on soil quality and microbial diversity. The effect of the metal highlights the importance of the analysis of the consequence of copper utilization as fungicide on microbial activities (Carolina *et al*., 2019).

Plants must adapt themselves to the prevailing conditions for their survival, resulting in the acquisition of a wide range of metal tolerance mechanisms (Gratão *et al*., 2019). Heavy metals have restricted the plant regular life cycles affecting the plant primer and secondary metabolites by biochemical and physiological pathways (Kisa *et al*., 2019). The past recent decades had witnessed renewed interest to study abiotic factors that influence secondary metabolism during in vitro and in vivo growth of plants (Isah, 2019).

For instance, the antioxydants compounds such as polyphenol and flavonoids (Giordano *et al*., 2019; Sarker *et al*., 2019; Aryal *et al*., 2019). Flavonoids, a class of polyphenol secondary metabolites, are presented broadly in plants and diets (Wang *et al*., 2018).

Plant phenolics or polyphenols, the aromatic compounds with one or more hydroxyl groups, are produced by plants mainly for protection against stresses. Plants accumulate phenolic compounds in their tissues as an adaptive response to adverse environmental conditions and have a key role in the regulation of various environmental stresses (Naikoo *et al*., 2019). Furthermore, multivariate analyses, in which information about the antioxidant machinery was also included, were performed in order to identify the set of parameters related to plant tolerance (Borges *et al*., 2019).

The increased occurrence of phenols was recorded as a response to abiotic stress (Piccolella *et al*., 2018**)** and biotic stress (Eitle *et al*., 2019). According to Marti *et al.*, (2018) stress deficit irrigation can increase the amount of polyphenols and flavonoids in tomato.

Proline is an important amino acid that accumulates in plants in response to different environmental constraints (Ashraf *et al*., 2018; Jogawat, 2019). According to Li *et al*., (2018) under stress proline was accumulated in tomato**.** Plants' tolerance to heavy metal stress needs to be improved in order to allow the growth of crops with minimum or no accumulation of heavy metals in edible parts of the plant that satisfy safe food demands for the world's rapidly increasing population (Etesami, 2018). This is why this study was undertaken to evaluate the toxic effects of copper and zinc on biochemical parameters (proline, total polyphenols and flavonoids) in roots and leaves of tomato.

## Material and Methods

### Material and plant growth conditions

The seeds of tomato (*Lycopersicon esculentum* Mill.; Sain pierre variety) were chosen in this study as they are the one of the major variety planted in Mostaganem region by tomato farmers. The tomato seeds are disinfected in sodium hypochlorite solution (5%) for 5 min and rinsed thoroughly with sterile water and then, the seeds were sown in seedling trays containing compost for a period of 20 days at 25°C.

### Sowing

The experiment was carried out in a greenhouse. The tomato seedlings were transplanted into cylinders (h=50 cm, d=20 cm) at the rate of 1 plant per cylinder. Each cylinder is lined at the bottom with a grave followed by filling a substrate consisting of a mixture of sand and potting compost (2V/V) respectively. The sand was sieved and treated successively by hydrochloric acid and water. A nutrient solution of Hoagland and Arnon, (1950) was used to maintain the development of the tomato plant.

### Stress conditions

The copper stress was given as ($CuSO_4$, $5H_2O$) and zinc stress as ($ZnSO_4$, $7H_2O$). At the seedling stage, the solutions were prepared and applied separately twice during the experiment. The stress treatment was performed according to the experiment design which is consisted of a control (plants are not stressed) and five treatments (100, 200, 300, 400 and 500 ppm). Five biological independent replicates were carried out. The plants were kept in the greenhouse until the end of the experiment.

### Parameters studied

• Estimation of proline content:

Proline content in roots and leaves of the tomato plant was achieved according to the method of Batles *et al.*, (1973). The absorbance was measured at 528 nm. The proline content calculated using a calibration curve was plotted and prepared with proline, this content was expressed as milligrams per gram of dry weight (mg $g^{-1}$ DW).

• Estimation of polyphenols content:

Polyphenol content in roots and leaves of the tomato plant was estimated according to the method of Folin-Ciocalteau phenol reagent described by Singleton *et al.*, (1999). The concentration of polyphenol was calculated using a calibration curve prepared with standard gallic acid. The data were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

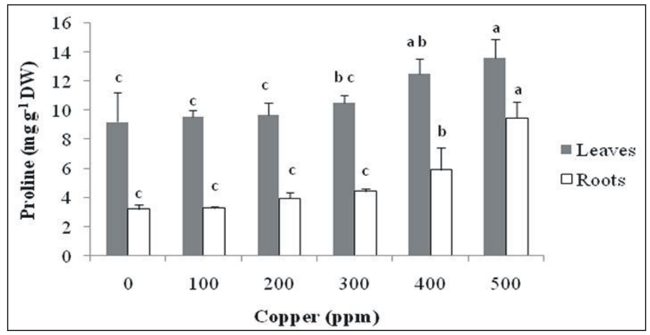


**Fig. 1:** Proline content in roots and leaves of tomato plants exposed to different concentrations of copper.

• Estimation of flavonoids content

The amount of flavonoids in roots and leaves of the tomato plant was determined through the method indicated by Zhishen *et al.*, (1999). The concentration of flavonoids was calculated using a calibration curve prepared and plotted with standard quercetin. The data were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE /g DW).

### Statistical analysis

All analyses were performed through the use of STAT BOX software. The data were calculated by variance analysis (ANOVA). The significance of differences between control and treatment was determined at the 0.05 level of probability. Data presented in this study were expressed as mean values ± standard deviation (SD). The averages are compared according to the Newman- Keuls test. Each treatment was carried out with five replicates. The correlation between proline, flavonoids and polyphenols was performed by Pearson's correlation.

## Results

### Proline content

• Proline content in leaves and roots under copper stress: The results outlined in fig. 1, revealed that the proline content was increased by copper treatment in leaves and roots. This increase was directly proportional

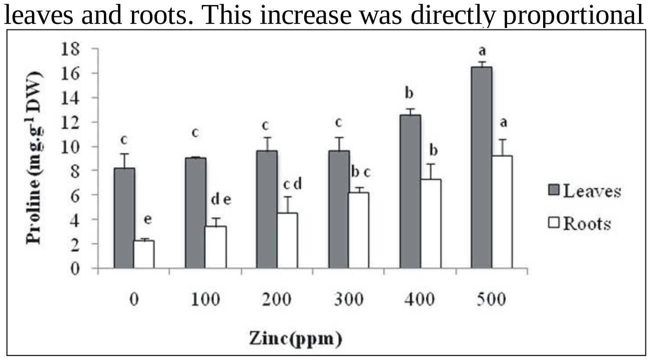


**Fig. 2:** Proline content in roots and leaves of tomato plants exposed to different concentrations of zinc.

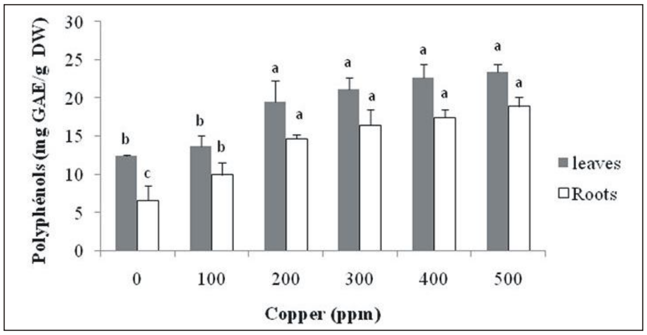


**Fig. 3:** Polyphenols content in roots and leaves of tomato plants exposed to different concentrations of copper.

to the different concentrations of copper stress. As is clearly observed in figure below there are no differences between the control and doses of 100, 200 ppm (9.21, 9.5, 9.6 mg $g^{-1}$ DW) for leaves and (3.22, 3.29, 3.9 mg $g^{-1}$ D.W) for roots respectively. This effect was more pronounced by 500 ppm with 13.62 in leaves and 9.47 mg $g^{-1}$ DW in roots. The statistical analysis showed that the effect of copper treatment was highly significant in both leaves and roots compared to the control plants.

• Proline content in leaves and roots under zinc stress: fig. 2, illustrates the accumulation level of proline in roots and leaves exposed to different concentrations of zinc (0, 200, 300, 400 and 500 ppm). As it is given below the higher effect was reported with 500 ppm in leaves and roots (16.45, 9.21 mg.$g^{-1}$ DW respectively) compared to the control that present values of (8.16, 2.23 mg.$g^{-1}$ DW) in the same parts respectively. These results suggest that the highest accumulation occurred in leaves compared to roots. Compared to the unstressed plants, the variance analysis indicated that zinc stress increased the proline content in leaves and roots; this effect was highly significant in both parts of the tomato plant.

## Polyphenols content

• Polyphenols content in leaves and roots under copper stress: In this test, an increase in polyphenols content was recorded in leaves and roots as a consequence of copper stress exposure. The results depicted in fig. 3, show that the best effect was obtained

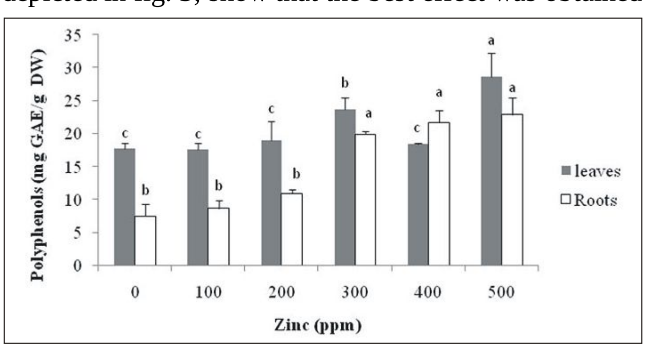


**Fig. 4:** Polyphenols content in roots and leaves of tomato plants exposed to different concentrations of zinc.

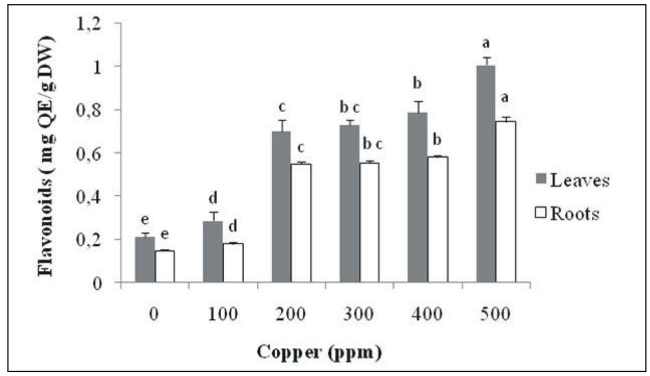


**Fig. 5:** Flavonoids content in roots and leaves of tomato plants exposed to different concentrations of copper.

at 500 ppm. The differences between 200, 300, 400 and 500 ppm were not dramatic (19.5, 21.1, 22.6 and 23.4 mg GAE/g DW). However, these values remain superior to the control and those treated by 100 ppm (12.37, 13.7 mg GAE/g DW). This test highlighted that there is a positive correlation between concentrations and the accumulation level of polyphenols. The statistical analysis plays a crucial role to explain all the data presented in figure below. As it was found, the effect of copper treatment on polyphenols content was highly significant in roots and leaves compared to the unstressed plants. Overall, these results indicate that the high content in the leaves was not much greater than that contained in the roots.

• Polyphenols content in leaves and roots under zinc stress: The response pattern was different depending on the treatment concentration and the organ exposed to stress (Fig. 4). The synthesis of polyphenols compounds, under zinc exposure, continued to increase and reached a maximum level at 500 ppm of treatment. As is shown, a decrease of this parameter was clearly detectable with 400 ppm for leaves. Only In this particular dose, the accumulation was important in roots than leaves with values of (21.68, 18.33 mg GAE/g DW respectively). However, the effect remains to be superior to the control as observed. Compared to the untreated plants, the

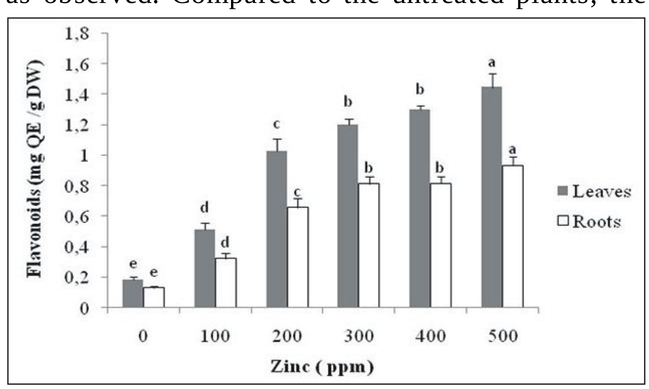


**Fig. 6:** Flavonoids content in roots and leaves of tomato plants exposed to different concentrations of zinc.

**Table 1:** Pearson correlation between proline, polyphenols and flavonoids in leaves and roots stressed by copper.

| | Leaf proline | Root proline | Leaf polypenol | Root polypenol | Leaf flavonoids | Root flavonoids |
|---|---|---|---|---|---|---|
| Leaf proline | 1.00 | 0.953** | 0.824* | 0.817* | 0.836* | 0.798 |
| Root proline | | 1.00 | 0.758 | 0.757 | 0.827* | 0.793 |
| Leaf polypenol | | | 1.00 | 0.99** | 0.981** | 0.978** |
| Root polypenol | | | | 1.00 | 0.977** | 0.968** |
| Leaf flavonoids | | | | | 1.00 | 0.997** |
| Root flavonoids | | | | | | 1.00 |

*Correlation is significant at the 0.05 level (2-tailed);
**Correlation is significant at the 0.01 level (2-tailed)

statistical test indicated a highly significant effect of zinc on the polyphenols profile for leaves and roots parts of the tomato plant.

### Flavonoids content

• Flavonoids content in leaves and roots under copper stress: Under copper stress, an increasing trend of flavonoids content was detected in roots and leaves. Slightly superior results to the control are achieved with 100 ppm. However the effect becomes constant at 200 and 300 ppm, after that it continued to increase up from 400 ppm whereas the peak of the data occurs at 500 ppm. The simulation results indicate that flavonoids were accumulated in larger amounts in leaves compared to roots (Fig. 5). In comparison with control, the statistical analysis pointed out that copper stress causes a highly significant increase in flavonoids amount in leaves and roots.

• Flavonoids content in leaves and roots under zinc stress: As can be seen from fig. 6, there is a clear trend of increasing in flavonoids content in roots and leaves after zinc application. The great effect was clearly observed at 500 ppm (1.44, 0.93 mg QE /g DW) compared to the control (0.18, 0.13 mg QE /g DW) in roots and leaves respectively. On the other hand, the leaves exposed to zinc exhibited higher accumulation than roots along the treatments. Also there is an evident relationship between the doses and flavonoids content. A highly significant increase in flavonoids content was noted in leaves and roots as compared with control plants.

### Correlation analysis

Results presented in tables 1 and 2, showed a strong positive correlation between the accumulation of proline, polyphenols and flavonoids in leave and that in roots under copper and zinc stress. It is also indicated the presence of a high positive correlation between proline and polyphenols and flavonoids, also between polyphenols and flavonoids under both heavy metals.

## Discussion

### Proline

Accumulation of proline under stress in many plant species has been correlated with stress tolerance and its concentration has been shown to be generally higher in stress tolerant as *Atriplex lentiformis* than in stress-sensitive plants (Goni *et al*., 2018; Eissa and Abeed, 2019). The accumulation was marked under heavy metals, in *Cinnamomum camphora* in response to Cu and Cd (Zhou *et al*., 2019a), in spinach under copper (Gong *et al*., 2019), in wheat under copper and pb (Jiang *et al*., 2019), in *Astragalus tragacantha* under copper, zinc and arsenic (Salducci *et al*., 2019). The accumulation of proline was also marked in tomato under different stresses, under heavy metals as Cd (Lima *et al*., 2019), Salt stress (Siddiqui *et al*., 2019 ; Poór *et al*., 2019), drought stress (Chen *et al*., 2018; Olivier and Nunes-nesi, 2018), Chilling tolerance (Aghdam *et al*., 2018; Ghanbari and Sayyari, 2018), heat and drought (Zhou *et al*., 2019b).

In the present study, the accumulation of proline was depending on heavy metal (copper or zinc) and its concentration and organ (leaves and roots). Various investigations have been conducted to identify the role of a particular amino acid(s) during Cu heavy metal stress (Kang *et al*., 2017). The current study showed the accumulation of proline in leaves and roots of tomato under copper. Our results are consistent with the findings of Nazir e*t al*., (2019), who suggested that Cu stress increased the level of proline in leaves of tomato in comparison to the control. Similar results were obtained in tomato leaves under copper stress by Kisa, (2019). Moreovre, in term of dose depending, our results concurred with those of

**Table 2:** Pearson correlation between proline, polyphenols and flavonoids in leaves and roots stressed by zinc.

| | Leaf proline | Root proline | Leaf polypenol | Root polypenol | Leaf flavonoids | Root flavonoids |
|---|---|---|---|---|---|---|
| Leaf proline | 1.00 | 0.919* | 0.767 | 0.795 | 0.762 | 0.747 |
| Root proline | | 1.00 | 0.786 | 0.956* | 0.938** | 0.934** |
| Leaf polypenol | | | 1.00 | 0.711 | 0.673 | 0.699 |
| Root polypenol | | | | 1.00 | 0.911* | 0.916* |
| Leaf flavonoids | | | | | 1.00 | 0.998** |
| Root flavonoids | | | | | | 1.00 |

*Correlation is significant at the 0.05 level (2-tailed);
**Correlation is significant at the 0.01 level (2-tailed)

Hanafy *et al*., (2017), who indicated that proline content of tomato plants significantly increased gradually by increasing the Cu levels as compared with control plants, also that of Rizvi and Khan, (2018) in *Zea mays* under Cu and Pb, of Kalaikandhan *et al*., (2018) in *Sesuvium portulacastrum* under Cu and zinc, of Nannda and Agrawal, (2018) in *Cassia angustifolia* under copper. It has been reported that the content of proline in *Raphanus sativus* was elevated in plants treated with an excess of copper (100-250 mg $kg^{-1}$), whereas the lower level of copper treatment was (50 mg $kg^{-1}$) (Chrithuuthayam *et al*., 2018). In contrast to our results, Cu treatment caused a significant decrease in the proline content in *Egeria densa* and did not change its amount in *Ceratophyllum demersum* (Maleva *et al*., 2018).

On the other hand, this study indicated the accumulation of proline in leaves and roots of tomato under zinc. Our results are in agreement with the studies of Salimi *et al*., (2019), who showed that the increases in proline contents were provoked by applying the treatments of zinc (0, 50 and 100 mg $L^{-1}$) and obtained the content of (0.45, 0.54, 0.6, mg $g^{-1}$ FW) respectively. The proline content of *Sesuvium portulacastrum* in shoot increased with increased concentration of zinc level (Kalaikandhan *et al*., 2018), these results collaborated with our findings. In addition, It is evident from the results that exposure to either metal (Co/Zn/Pb) in concentration resulted in a sharp rise in the proline content in the test plants (Menon *et al*., 2018). Subsequently there was a progressive increase in the proline content up to the $28^{th}$ day in the order 50 ppm <100ppm < 300 ppm <500pm, these data are in line with our results and also has been confirmed by the findings of (Menon *et al*., 2018), who proposed that proline accumulation was of greater magnitude in the test plants especially at 500ppm concentration in all salt. Another study conducted by Kalaikandhan *et al*., (2018) demonstrate that minimum and maximum proline accumulation were recorded at 100 mg $kg^{-1}$ and 600 mg $kg^{-1}$ respectively, for zinc and copper level in *S. portulacastrum*. Similar observations were noted in our study with lower content at 100ppm and higher content at 500ppm for copper and zinc.

According to the current study, the accumulation of proline under copper and zinc stress was higher in leaves than roots. These results are in accordance with earlier studies conducted by Girilal *et al*., (2018), who proved that proline accumulated in all vegetative organs and in fruits when plants were subjected to stress, the highest concentration was found in growing leaves. Similar results were indicated by Alves *et al*., (2018) under salinity in tomato. It was reported by Saif and Khan, (2018) that proline accumulation followed the order: leaves > roots > shoots, these results differ from some published studies which suggested that proline content is higher in roots than leaves of tomato in control and salt stress (Horchani *et al*., 2010), similar results were noted by Ullah *et al*., (2019), also higher in roots than shoots in tomato plants (Natarajan *et al*., 2018). Root proline showed a strong linear relationship with endogenous Cu accumulated after exposure to the metal (Kebert *et al*., 2017). It has been reported that application of proline significantly increased the number of roots and root fresh weight (The *et al*., 2016).

Many studies have shown the reasons behind the accumulation of proline under heavy metals and environmental stress. Chandrakar *et al*., (2018) argued that proline has a role as protective agents, hence caused enhanced growth. Also, well documented in the literature that compatible osmolytes such as proline regulate the osmotic potential of cells exposed to abiotic stresses (Yadu *et al*., 2016; Chandrakar *et al*., 2017; Wiesenthal *et al*., 2019; Alyemeni *et al*., 2018). It has been observed that the net photosynthesis and transpiration were also decreased by the application of proline in both control and salt stressed plants (Orsini *et al*., 2018). On the other hand, proline mediates the elimination of ROS (Alyemeni *et al*., 2018; Alves *et al*., 2018) and directly scavenges OH radicals (Chandrakar *et al*., 2018; Per *et al*., 2017). It also plays important roles during stress as a metal chelator (Aslam *et al*., 2017). Proline protects folded protein structures against denaturation, stabilizes cell membranes by interacting with phospholipids, or serves as an energy and nitrogen source (Per *et al*., 2017; Arroussi *et al*., 2018) and protects the plant cells from the lipid peroxidation damage (Alves *et al*., 2018).

The exact mechanism of how proline accumulation helps the plant to cope up with heavy metal stress is difficult to elucidate. However, the available evidences suggest that proline acts by protecting the key enzymes from being inactivated by toxic metal ions (Menon *et al*., 2018). There was a direct correlation between enhanced proline content and activities of anabolic enzymes namely P5CS Pyrroline-5-carboxylate synthetase and GDH involved in its biosynthesis (Garg and Singh, 2018; Aswani *et al*., 2018). Proline dehydrogenase ProDH activity was induced by water deficit in both root types, exhibiting a higher activity in the primary or taproot tapR than in the fibR lateral or fibrous roots (fibR) (Castaneda *et al*., 2018). From our results, Aswani *et al*., (2018) suggested that proline metabolism can help to mediate inter-organelle interactions. Furthermore, proline-treated rice roots showed up-regulation and down-regulation of nine and eight proteins, respectively, when compared to those in the control (The *et al*., 2019).

Recent approaches have been used to regulate and enhance the accumulation of proline content under heavy metals, in order to improve plant tolerance. For example, Inoculation with plant growth promoting rhizobacteria (PGPR) (Bindu *et al*., 2018), inoculation with *Piriformospora indica* too significantly enhanced proline content as compared to Cu alone (Nanda and Agrawal, 2018), other applications; such as, exogenous glutathione (Hasanuzzaman *et al*., 2018), exogenous melatonin (Siddiqui *et al*., 2019), application of exopolysaccharide (Arroussi *et al*., 2018). Enhancement by phytohormones such as jasmonic acid and nitric oxide (Ahmad *et al*., 2018a), with *Pseudomonas aeruginosa* in tomato under heavy metal stress (Khanna *et al*., 2019).

**Polypenols and flavonoids**

The accumulation of total phenols and flavonoids content in different organs of tomato plant under heavy metal stress has been reported by many studies, in seedlings under Cd ( Khanna *et al*., 2019), in fruits under selinium (Andrejiová *et al*., 2019), in roots and leaves under Cr and Pb (Ullah *et al*., 2019).

Focus on the results obtained in our study; we observed the accumulation of polyphenols and flavonoids in tissues of leaves and roots under copper and zinc treatment. This accumulation of antioxidant was depending on heavy metal (copper or zinc) and their concentration and organ (leaves and roots).

Copper stress induced the accumulation of polyphenols and flavonoids content in leaves and roots, these results corroborated the findings of Hanafy *et al*., (2017), who demonstrated that Cu stress caused a significant increase in phenols contents of tomato leaves under different concentration of Cu. Similar results were also obtained by Chrysargyris *et al*., (2019) in *Mentha spicata* under copper. According to Singh *et al*., (2018) and Chung *et al*., (2018), the application of CuO nanoparticles significantly enhanced the polyphenols and flavonoids content, these findings were also confirmed by (Pérez-Labrada *et al*., 2019) who found an increased the phenols (16%) in the leaves and phenols (7.8%) in the fruit compared with the control of tomato. Particularly relevant was the observation that flavonoids increased substantially in roots of *Solanum cheesmaniae* in response to excess Cu and decreased in shoots (Branco-Neves *et al*., 2017). Whereas, our findings contrast with (Kisa *et al*., 2019), who found that the applications of Cu, Cd and Pb significantly reduced the total phenolic content in tomato leaves..

Another effect in the present study was obtained by zinc application; the accumulation of polyphenols and flavonoids was also increased by zinc treatments in both organs. This is in good agreement with Ibiang *et al*., (2018), who reported that total polyphenols in fruits and shoot of tomato were significantly increased due to excess Zn. Furthermore, total phenolic compounds in roots and leaves were improved by the increasing gradient of Cd or Zn concentrations; total phenolic compounds significantly increased by 3.6-44.6% in the roots and by 0.4-126.6% in the leaves (Chen *et al*., 2019). In contrast to our results, the synthesis of antioxidants such as phenolic compounds declines as Cd and Zn leaf concentrations increase. These phenomena might be related to stress or the manifestation of a mechanism for tolerance to Cd and Zn accumulation (Sakurai *et al*., 2019). Also, it has been found that the contents of total phenol and flavonoids in the mature green fruits of tomato were reduced compared to control, following the n-ZnO nanoparticles treatment (Akanbi-Gada *et al*., 2019).

From this study, we can suggest that the accumulation of polyphenols and flavonoids content was higher in leaves than roots. These results were confirmed by (Ullah *et al*., 2019). Whereas, this level was higher in leaves than fruit (Pérez-Labrada *et al*., 2019) in tomato, in roots than shoot (Natarajan *et al*., 2018) in tomato, in fruits than shoots (Ibiang *et al*., 2018) in tomato treated by zinc. Singh *et al*., (2018) noted the correlation between these accumulations in different tissues; he demonstrated that the total phenol content in shoots showed a significant positive correlation with total phenol content in roots. It also showed a positive correlation with flavonoid content in shoots. Furthermore, the total phenol content in roots showed a positive correlation with flavonoid content in shoots. On the other hand, flavonoid content in roots and shoots also showed a positive correlation. (Singh *et al*., 2018). These findings confirm those obtained in this study.

Several researches have explained the cause and the main role of the elevated of these compounds under heavy metal stress, where they found that phenolic compounds became involved in one of the defensive systems that the plants used against Cd and Zn stress (Chen *et al*., 2019). Furthermore, these compounds provide defence against oxidative stress by acting as metal chelators and quenching of ROS (Ullah *et al*., 2019). Flavonoid and other phenolic compounds of plant origin have been reported as free radical scavengers (Singh *et al*., 2018), this is justified by total antioxidant activity in roots that showed significant positive correlation with total phenol, flavonoid (Singh *et al*., 2018). It has been indicated that Zn is a part of the antioxidant enzyme SOD (ZnSOD) thus enhances the activity of quenching the

ROS (Ahmad *et al*., 2018c). However, Zn concentrations had a moderate negative correlation with both polyphenol levels and radical scavenging activity (Sakurai *et al*., 2019). Phenolics possess hydroxyl and carboxyl groups and can bind to the metals. This may be the reason for the elevated level of secretion total phenolics in plants treated with silver nitrate (Girilal *et al*., 2018). An Other reason, An increase in soluble phenolic compounds such as intermediates in lignin biosynthesis increase cell wall endurance by the creation of physical barriers that protect cells against the harmful action of heavy metals, as well as influence the transition of metal ions within plant tissues since the lignification probably retains a substantial portion of metals into the cell wall fraction (Chen *et al*., 2019).

Regarding the effects obtained by heavy metals on the metabolism of the plant in this study, It has been reported that Plant phenolics are biosynthesized in plants from a biosynthetic intermediate, phenylalanine and shikimic acid through the shikimic acid pathway (Naikoo *et al*., 2019). Under stressful conditions, our findings were confirmed by Zaho *et al*., (2018), who indicated that Shikimate phenylpropanoid biosynthesis was perturbed by excess copper. Therefore, Cd and Zn treatments affected phenolic compounds metabolism in *Kandelia. obovata* (Chen *et al*., 2019). According to Soleimani *et al*., ( 2019), increasing phenols and flavonoids in tomatoes may arise from increasing PAL (phenylalanine ammonia-lyase) enzyme activity. This confirmed by (Aghdam *et al*., 2018). The decreases in the phenolics should be results of the decline in the activity of crucial enzymes involved in the biosynthesis of phenolic compounds under the heavy metal stress (Kisa *et al*., 2019). So, according to earlier strategies in term of plant tolerance against heavy metals, exogenous applications are used to improve antioxidant metabolism; such as, nitric oxide NO that increased the flavonoid and total phenol content in Cd-stressed tomato plants (Ahmad *et al*., 2018b). The same effect was observed under the application of jasmonic acid (Ahmad *et al*., 2018a) and exopolysaccharide treatment in tomato (Arroussi *et al*., 2018).

## Conclusion

The application of copper and zinc on the tomato plant enhanced the accumulation of osmolytes such as proline and the antioxidants compound such as polyphenols and flavonoids. This accumulation increased significantly by the increase of doses for both heavy metals. The level of proline, polyphenol and flavonoids was higher in leaves than roots; this may be due to the phytoavailability of these heavy metals by the plant. A positive correlation was recorded between all these parameters. In conclusion, to cope against heavy metal stress, tomato induces the plant metabolism involved in abiotic stress tolerance. So, molecular researches are needed to better understand the heavy metal tolerance mechanism.

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