Article Type: Research Article
J Name: Modern Phytomorphology
Short name: Modern Phytomorphology
ISSN: ISSN 2226-3063/eISSN 2227-9555
Year: 2023
Volume: 17
Page numbers: 75-78
DOI: 10.5281/zenodo.2023-17-200121
(10.5281/zenodo.Year-Volume-PDFNo.)
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RESEARCH ARTICLE

The Heavy metals effect on the physiological traits of the tomato (*Solanum lycopersicum l*)

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Received: 12.04.2023, Manuscript No.: mp-23-95243| **Editor Assigned:** 16.04.2023, Pre-QC No. mp-23-95243(PQ) | **Reviewed:** 22.04.2023, QC No. mp-23-95243(Q) | **Revised**: 28.04.2023, Manuscript No. mp-23-95243(R) | **Accepted:** 30.04.2023 | **Published:** 05.05.2023

Abstract

This study aims to investigate the agricultural lands pollution with lead and cadmium elements, in the Muhammad Sakran district/ Baquba province of the Diyala governorate, and the effect of these elements on the physiological traits of the tomato plant. Study samples were collected from three different stations; each station includes six farms three of them were irrigated with river water, while the others were irrigated with drainage water. The tomato samples were randomly collected from study sites between 25/05/2021 and 08/06/2021, and the lead and cadmium concentration in the samples were analyzed in the lab. The study also investigates the effect of the accumulated elements on the glutathione and proline content in the tomato plant. The results revealed significant differences at a probability level of 5% for the stations and water source factors in regard with the lead element concentration. Station number one recorded the highest average concentration (0.308 parts per million) of lead in the vegetative group. Though, the third station showed the lowest average of 0.15 parts per million. As for the water source factor, the river water recorded the highest lead concentration (0.275 parts per million) in the vegetative group of the plant. Also, significant differences in the glutathione and proline content, due to the station factor, were recognized. The third station recorded the highest glutathione average of (97,900 nanomol.kg^{-1}) in the plant, whereas, the first station recorded the highest proline average of 55,295 nanomol.kg^{-1}. In regard with the water source factor, the river water recorded the highest average of glutathione (75.222 nanomol kg^{-1}) and proline (54.530 nanomol kg^{-1}) compared to the drainage water.

Keywords: Heavy Metals, Tomato, Proline, Glutathione, Pollution

Introduction

Heavy metals are defined as a group of metals and semi-metals that have an atomic density greater than 4 g/cm3, which is five times the density of water (Hawkes, 1997). They are critical environmental pollutants; their toxicity is of great concern because they do not decompose in the environment and are not easily metabolized, which is the reason why they accumulate in the environment (Gjorgieva Ackova, 2018).

The heavy metals including lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), zinc (Zn), chromium (Cr), silver (Ag), and arsenic (As) affect the plants' physiological and biochemical processes. The extent to which these materials impact the plants depends on plant type, heavy metal type and concentration, and the exposure period (Ahmad & Mirza, 2018).

Water pollution with heavy metals is a major issue that must be addressed carefully. This could be a major task to reduce the heavy metals impact on the first product in the food chain (plant), and thus the subsequent consumers (animals and humans). Some of these metals, such as copper, zinc and manganese are advantages for plant's metabolism if they are in low concentrations (Rai et. al., 2021). However, other metals like the mercury, cadmium and lead have a toxic effect, it causes a growth inhibition and thus plant death (Haider et. al., 2021; Okereafor et. al., 2020).

Antioxidants play an important role in protecting plants from the oxidative activity of free radicals, which results from the exposure of plants to various abiotic stresses. Free radicals cause an oxidative damage to the important macromolecules such as proteins, fats and DNA (Chandrakar et. al., 2017). Glutathione (GSH) is an important non-enzymatic antioxidant, it is a thiol compound, soluble in water, and of low molecular weight (Rezayian et. al., 2019). The Glutathione is essential for mineral balance; it has a vital role in protecting the plants from heavy metal poisoning by forming the phytoglatin. Further, in the absence of phytoglatin, it plays an antioxidant role and detoxifies heavy metals effects (Gupta et. al., 2010; Seth et. al., 2012).

Proline is another non-enzymatic antioxidant, it is an amino acid that is produced by the protein hydrolysis process. It is an organic compound, non-toxic in its high concentrations, has a high solubility, and low molecular weight (Sofy et. al., 2020). It accumulates in the plant's tissues that is exposed to various stresses, and helps in relieving stress through its function as an osmotic regulator, a mineral chelator, and a free radical scavenger (Vladimirovna Afanasyeva & Ayushevna Ayushina, 2019). In another words, it provides a powerful mechanism to protect plant's cells from damage (Kishor et al., 2014).

Tomato plant belongs to the Solanum melongena L family; it is one of the most important vegetable crops in Iraq and the world. Its importance is signified by its nutritional value, as well as the diversity of consumption methods, such as fresh, cooked, or processed food products (Costa & Heuvelink, 2018).

Several studies have reported the medical importance of the tomato fruits, and described their role in reducing the risk of many health issues, such as the cancer and the cardiovascular diseases. This is attributed to the inncluded biologically active compounds, such as ascorbic acid, lycopene, polyphenols and tocopherols, as well as carotenoids and anthocyanins (Giovannetti et al., 2012; Vinha et al., 2014). Hence, the aim of this study is to estimate the non-enzymatic antioxidants (glutathione and proline) content in the tomato plants that were irrigated with river water and drainage water.

Materials and Methods

Samples collection

The samples were randomly collected from three stations belong to the Muhammad Sakran district/ Baquba province / Diyala governorate, during the period between 25/05/2021 and 08/06/2021. Each station has six farms, three of them are irrigated with river water, and the other three are irrigated with drainage water. The collected samples were kept in polyethylene bags, and the sample's number, the station's name and the irrigation water type were written on it.

Estimating the tomato samples content of the heavy metals

The plant samples were digested by adding 4 ml of the concentrated nitric acid (HNO3) to a 2 g of dried and crushed plant samples in a glass beaker, and covered with a watch glass for 60 minutes. It was left for 24 hours before the beaker and the glass were put on the heater at a temperature of 105°C, for an hour, until the appearance of fumes. Then the sample was cooled before a 3 ml of concentrated perchloric acid HCLO4 was added. After that, the beaker with no cover was placed on the heater and heated to dryness. The sample was then cooled, and 2 ml of concentrated acid (HCL) and 3 ml of distilled water were added before the beaker was put back on the heater at a 75° and then cooled. Then the samples were filtered and collected in a volumetric vial of 25 ml capacity. The volume was supplemented with distilled water to the mark, then the content of the lead and cadmium was measured using an atomic absorption spectrometer.

The determination of the glutathione and proline content in the tomato plant

The extraction

The amino acids were extracted according to the method presented by (Rasmus Dahl Lassen 2018). An amount of 3 g of the samples was placed in a 25 ml volumetric flask where a 25 ml of hydrochloric acid (6M) was added at 150°C for 3 hours. The samples were then dried in a rotary evaporator before a 5 ml of the sodium citrate (pH 2.2) was added. Then a 0.45um plastic filter was used to filter the samples prior to doing the injection process.

The determination of the glutathione and proline content in the plant samples

A 200 µl of Orthophthalein Aldehyde (5%) (OPA) was add to 1 ml of the extracted sample and agitated for 2 min, then 100 µl of the mixture was injected into the HPLC device.

The test was performed in the laboratories of the Ministry of Science and Technology/Department of Environment and Water. A German made HPLC device was employed in the test using the method presented by (Scriver CR, 2001). A carrier phase consists of (methanol: acetonitrile: 5% formic acid) in a proportion of (20: 60: 20) and a flow rate of 1 ml/min was employed. A separation column (ZORBAX Eclipse-AAA; 3.5 µm; L × i.d. = 150 × 4.6 mm) was used to separate the amino acids, while the fluorescence reagent was used to detect the amino acids with wavelengths of (Ex =445 nm, Em =465 nm). Finally, the Plainity 2015 software was then used to analyze the amino acids.

Preparation of the standard compound

A 0.1 g of each of the high purity amino acids (99.9%) was dissolved in non-ionic water and transferred to a 250 ml conical flask. Then the volume was filled to the mark, where the concentration became 250 parts per million. The dilution law was used to prepare the calibration curve concentrations that have been injected into the device.

Results and Discussion

The results given in (Tab. 1) reveal significant differences at the probability level of 5% for the station and the water source factors. The first station depicted the highest lead element concentration average of 0.308 parts per million, in the vegetative group, compared to 0.15 parts per million for the third station which is the lowest. However, for the water source factor, the river water recorded the highest average of 0.275 parts per million. The reason behind that is attributed to the high lead pollution of the soil and water in the first station which is probably due to the excessive use of chemical fertilizers compared to the other stations.

Table 1. The lead element concentration in the vegetative group of the tomato plant (P.P.M.)

Station	Water source		Average (P.P.M.)
	River water	Drainage water	
1st	0.466	0.15	0.308
2nd	0.21	0.11	0.16
3rd	0.15	0.15	0.15
The average	0.275	0.136	
LSD	Station: 2.904	Water source: 3.218	Interaction: N.A.

(Tab. 2) signifies no significant differences in terms of the cadmium element concentration in vegetative group between the station and the water source factors. The second station showed the highest average cadmium concentration in the vegetative group of 0.346 parts per million compared to the other stations. The results also show that the river water surpasses the drainage water in terms of giving the highest cadmium concentration average of 0.353 parts per million in the vegetative group. This indicates the contamination of the tomato plant with the cadmium element, which is due to soil contamination with cadmium, in addition to the pollution of the water sources.

Table 2. The cadmium concentration in the vegetative group of the tomato plant (P.P.M.)

Station	Water source		Average (P.P.M.)
	River water	Drainage water	
1st	0.353	0.29	0.321
2nd	0.413	0.28	0.346
3rd	0.293	0.386	0.339
The average	0.353	0.31	
LSD	Station: N.A.	Water source: N.A.	Interaction: N.A.

(Tab.3) shows significant differences in the tomato plant content of the glutathione. The results reported 41.705 for the station factor, 150,251 for the water source factor, and 42.221 for the interaction between the two factors. The third station recorded the highest glutathione concentration average of 55.295 nanomol.kg^{-1}. As for the water source factor, the river water showed the highest average of 75.222 nanomol.kg^{-1} compared to drainage water. The reason why the glutathione concentration increases in tomato plants that have been grown in a heavy metal stress conditions is attributed to the heavy metal's role in stimulating the synthesis of GSH by providing the sulfur which is a constituent of the GSH molecule. The other reason could be the increase in the synthesis and activity of the GSH1 enzymes and the glutathione synthetase (GSH2) in response to redox changes caused by the metal (Gromes et al., 2008)

Table 3. The glutathione concentration in the vegetative group of the tomato plant (nanomol.kg^{-1})

Station	Water source		Average (nanomol.kg^{-1})
	River water	Drainage water	
1st	74.866	7.05	40.958
2nd	52.9	5.25	29.075
3rd	97.9	12.69	55.295
The average	75.222	8.33	
LSD	Station: 41.705	Water source: 150.251	Interaction: 42.221

(Tab. 4) shows significant differences in the tomato plant content of the proline. The results reported 112.627 differences between the station factor, 29.272 for the water source factor, and 43,857 for the interaction between the factors. The first station recorded the highest proline concentration of 74.416 parts per million in the vegetative group of the plant compared to lowest value (3.040 parts per million) recorded by the third station. As for the water source factor, the river water showed the highest concentration of 54,530 ppm. The ability of the plant to pproduce and accumulate proline is a physiological mechanism to withstand various environmental stresses, including heavy elements (Ghosh et al., 2022). It is believed that the accumulation of proline (which is probably synthesized from the glutamic acid) results from the accumulation of the NaDH and H^+ as a consequence the electronic transport system activity reduction (Venekemp, 1989; Sawhney et al., 1990; Alia et al., 1993). The synthetic proline acts as a mechanism to reduce both of the level accumulated NADH, and the acidity ($2NADH^+2H^+$).

Table.4 The proline concentration in the vegetative group of the tomato plant (P.P.M.)

Station	Water source		Average (P.P.M.)
	River water	Drainage water	
1st	79.8	69.033	74.416
2nd	81.9	2.55	42.225
3rd	1.89	4.19	3.04
The average	54.53	25.257	
LSD	Station: 112.627	Water source: 29.272	Interaction: 43.857

Conclusions

Through the current study, we concluded, that there is a direct relationship between the accumulation of lead in the tomato plant and its content of proline as a result, as well as a direct relationship between the accumulation of cadmium in the plant and its content of glutathione as a result.

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