Heliyon 8 (2022) e12399

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Gibberellic acid improves growth and reduces heavy metal accumulation: A case study in tomato (*Solanum lycopersicum* L.) seedlings exposed to acid mine water

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ARTICLE INFO

Keywords:
Gibberellic acid
Seedling growth
Heavy metal accumulation
Tomato

ABSTRACT

This study investigated the effect of gibberellic acid (GA_3) on the growth of tomato seedlings and heavy metal accumulation within seedlings tissue irrigated with acid mine water (AMW). Three experimental treatments were administered using a completely randomized design with five replicates. The experimental treatments included were gibberellic acid + acid mine water (GA_3 + AMW), acid mine water (AMW), and tap water. Seedlings were irrigated directly in pots with 400 mL of 100% AMW at two-day intervals 21 days after planting. Drenching of the seedlings with GA_3 was done every 24 h for eight consecutive days from 28 days after planting. Results on the physicochemical analysis showed high concentrations of heavy metals (HMs) in AMW compared to tap water and the experimental treatment significantly affected the measured plant growth parameters. Tomato plants irrigated with AMW alone were shorter (4.00 cm) than plants irrigated with tap water (14.00 cm), while plants treated with AMW and GA_3 were much taller (16.50 cm) than the latter (control). Moreover, HM accumulation differed among the three treatments. Seedlings that received AMW with no GA_3 accumulated more HMs (Cd, Cr, Cu, Ni, and Zn) in their roots, stems, and leaves while plants treated with GA_3 had a decrease in the accumulation and distribution of HMs in the different plant tissues (roots, stems, and leaves) relative to AMW alone and the plants irrigated with tap water alone. The study revealed that GA_3 boosted the growth of tomato seedlings irrigated with AMW and also altered HM accumulation with the tissues of the seedlings.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is a valuable crop which constitutes about 14% of the world's annual vegetable production [1]. As food, tomato is cooked with stews and relishes, served with salads, sandwiches and can also be consumed as sauce or juice. It is rich in minerals, vitamins including vitamins A, C, K, B1, B3, B5, B6, and B7 [1]. In addition, it is a major dietary source of the antioxidant lycopene, which is linked to several health benefits such as reducing the risk of cancer. Tomato is a perennial crop although it is grown as an annual [2]. It is also a warm-season crop that does not tolerate frost and it grows optimally in the temperature range of 20–25 °C [3]. It is grown either in an open field or in controlled environments (greenhouse/tunnels). It requires sufficient water especially at critical stages, such as immediately after sowing and transplanting, and irrigation is often needed to ensure sufficient moisture during these delicate growth stages namely; vegetative growth stage, flowering and fruit set [4, 5]. In areas where heavy metal (HM) contamination of the soil and water sources, such as adjacent to mines, is prevalent, HMs pose a hazard to plant growth, development, and reproduction. HMs such as zinc (Zn), cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni), and copper (Cu) are common contaminants of vegetables such as tomato [6]. As essential nutrients, some HMs play a significant role in metabolism and plant growth and development but at quantities higher than the tolerable threshold, these HMs are serious plant growth deterrents [7]. To solve this environmental threat (HM contamination), plant growth enhancers can mitigate this environmental challenge as they have been shown to be eco-friendly and economical in tackling this problem. Plant growth regulators, such as auxins, cytokinins, and gibberellins, are well known as growth promoters whose activities regulate the ascorbate–glutathione cycle, transpiration rate, cell division, osmoregulation, nitrogen metabolism, and assimilation [1, 8], thereby countering the effects of harmful agents. Gibberellic acid (GA_3)

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https://doi.org/10.1016/j.heliyon.2022.e12399
Received 20 June 2022; Received in revised form 27 September 2022; Accepted 8 December 2022

has been shown to reverse the inhibitory effects of environmental stress such as HM contamination [9] by inducing the degradation of DELLA. In the studies of Zhu et al. [10], GA_3 improved root growth, decreased Cd content, and reduced lipid peroxidation in the roots, demonstrating that GA_3 can help to mitigate Cd toxicity. However, the effect of GA_3 on tomato seedlings irrigated with AMW is unknown. Specifically, the effect on tomato seedling growth and bioaccumulation of HM in different plant parts, namely; roots, stems, and leaves, remain unknown. The aim of this study was therefore, to evaluate whether GA_3 could enhance tomato growth and reduce HMs accumulation when subjected to AMW irrigation. A GA_3-based growth enhancer can be recommended to farmers who, without any choice, farm in areas with HM accumulation.

2. Materials and methods

Tomato plants were grown and exposed to AMW, the plants were treated with GA_3. Plant height, stem diameter, and mineral analysis of the different plant parts were analyzed.

2.1. Plants, experimental conditions, and treatments

Tomato (*Solanum lycopersicum* L.) seeds (cultivar Heinz 1370) were surface sterilized by alternate dips in ethanol (70%) for 1 min followed by sodium hypochlorite for 5 min and finally, rinsed with distilled water five times. The sterilized seeds were planted in commercial potting mix 3 Sixty using 25 cm pots. Tomato seedlings at the vegetative growth stage were selected for the study. The seedlings were maintained in the glasshouse under overhead irrigation at a day-night temperature regime of 25–28 °C. Three experimental treatments were administered using a completely randomized design with five replications. The experimental treatments included, GA_3 + AMW, AMW, and C. The first treatment was irrigation with AMW and exogenous treatment with GA_3, the second was irrigation with AMW and no GA_3, and the final treatment was irrigation with tap water. During the vegetative growth stage of the seedlings, the plants with AMW were irrigated directly in pots with 400 mL of 100% AMW at two-day intervals. One mL of 0.2 mM GA_3 double distilled water solution was sprayed on three weeks-old tomato seedlings at 24 h intervals for eight consecutive days.

Prior to administering the experimental treatments, initial plant height and stem diameter were measured, and thereafter, plant height and stem diameter measured at seven-day intervals. At termination, the plants were uprooted and each of the fifteen plants was cut into three parts (root, stem, and leaf). The experiment was repeated, and the data pooled for analysis.

2.2. Acid mine water

The acid mine water used in the study was obtained from a mine in Randfontein, Gauteng, South Africa. Prior to transporting the water to the laboratory, preliminary analysis, which included pH determination, temperature, NO_3 (nitrate), SO_4 (sulfate), dissolved oxygen (DO), total dissolved solutes (TDS), and electrical conductivity (EC) was done on-site using the Hanna HI9828 multi-parameter ion-specific meter (Hanna Instruments (Pty) Ltd, Bedfordview, South Africa). The physico-chemical characteristics of the water appear in Table 1.

2.3. Heavy metal determination in the plant tissue

From seeding to termination the experiment lasted 35 days. The chronology of events is as follows, day 1: seeding, day 21: irrigation with AMW began, day 27: GA_3 treatment began and lasted for eight days until termination of the experiment at day 35. At termination the plants were uprooted and each of the fifteen plants of the experiment was cut into three parts, the roots, the stem, and the leaves. This was also done for the second cycle of the experiment. The plant parts were individually ground, and particles were passed through a 2-mm diameter sieve. About 100 mg of root, stem, and leaf were digested with HNO_3 and HCl in a microwave oven (Milestone Ethos 1600). After mineralization, the samples were diluted, filtered, and analyzed. Metal concentrations (Cd, Cr, Cu, Ni, and Zn) of the AMW and root, stem, and leaf were measured as described for the plant samples. Three replicate analyses were performed for all analyses. HMs analyses were done using an inductively coupled Optical Emission Spectrometer (Agilent Technologies 700 series ICP-OES). Prior to the analysis, water samples were passed through Whatman filters. From the HM quantities, accumulation factor (AF) was determined using the formula [11].

$$AC = (C\ root/C\ water) \quad (1)$$

The translocation factor (TF) was also calculated to estimate the transfer of HMs from roots to shoots of the plant [11].

$$TF = (C\ shoot/C\ root) \quad (2)$$

Table 1. Physicochemical parameters and heavy metal content of tap water and acid mine water (n = 9) were sampled. The physicochemical parameters of the tap water and acid mine water were measured on the day of sampling.

			Benchmarks	
Physicochemical Parameters	Tapwater (mg/L)	AMW Water (mg/L)	South African Standard (mg/L)	WHO Standard (mg/L)
pH	7.47 ± 0.12	3.85 ± 0.14	5.0–9.7	6.5–8.5
Temperature (*C*)	21.42 ± 0.20	29 ± 0.60	-	-
EC (μS/cm)	45.98 ± 0.98	3641.33 ± 52.05	250	3000
TDS (mg/L)	128.35 ± 1.89	4874 ± 24.27	-	80
NO_3 (mg/L)	2.17 ± 0.13	6.29 ± 0.19	-	-
DO (mg/L)	16.09 ± 0.19	5.54 ± 0.18	95	150
SO_4 (mg/L)	244.55 ± 3.86	18255.33 ± 49.09	-	-
Metal	**Levels of heavy metals (mg/L)**			
Cd	0.01 ± 0.00	0.18 ± 0.01	0.01–0.05	0.01
Cr	0.04 ± 0.00	5.87 ± 0.01	0.10–1.0	0.05
Cu	0.12 ± 0.00	0.95 ± 0.10	0.1–1.0	0.005
Ni	0.04 ± 0.00	10.42 ± 0.41	0.20–2.0	0.02
Zn	0.92 ± 0.01	55.47 ± 0.69	1.0–5.0	5.00

EC-electrical conductivity, TDS-total dissolved solute, DO- Dissolved oxygen; values show the standard deviation (mean (±SE)). *Tap (Municipal water), **AMW (Acid mine water) sourced from gold mine Randfontein (water samples were analyzed in the UNISA laboratory), *** [13] standards, and **** [14] standards.

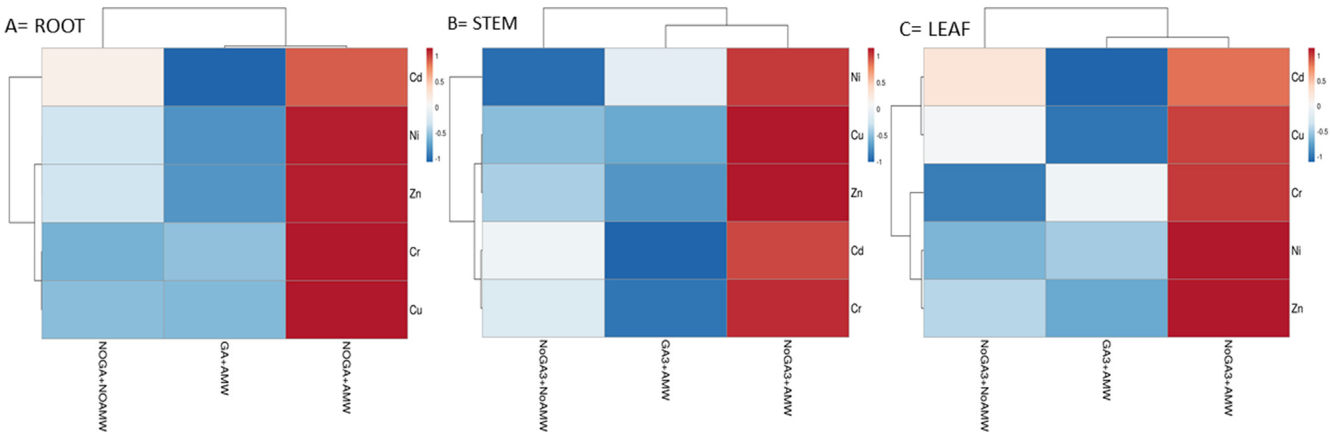

Figure 1. Heatmap of heavy metals detected on the tomato a) root, b) stem, and c) leaf. The dendrogram shows the complete linkage between the treatment (GA_3 + AMW, AMW, C) and heavy metals. The heatmap colour (red to blue) represents the row z-score of the mean relative abundance of the metal (Cu, Ni, Zn, Cd, and Cr) from high to low.

Where C is the concentration and can be expressed in mass unit per mass or volume unit. The quantities of the HMs Cd, Cr, Cu, Ni, and Zn were represented in a heatmap created with ClustVis [12]. A heatmap is a data visualization tool that shows the scale of a phenomenon as a colouring in different dimensions. To generate the dendrogram, the treatments were arranged in rows, and the values of HMs were arranged in columns using ms Excel. The values were copied and pasted on the online tool ClustVis to generate the dendrogram performed in Figure 1.

2.4. Statistical analysis

For the data analysis, since the trial cycle was repeated consecutively under similar experimental conditions, the critical growth parameters data on plant height and stem diameter ($n = 5$) per treatment and accumulation factors (root accumulation factor (RAF) Cd, RAF Cr, RAF Cu, RAF Ni, RAF Zn; stem translocation factor (STF) Cd, STF Cr, STF Cu, STF Ni, STF Zn; leave translocation factor (LTF) Cd, LTF Cr, LTF Cu, LTF Ni, LTF Zn) with three replicates ($n = 3$) were analyzed using one-way analysis of variance on Statistica version 12 (StatSoft Inc., Tulsa, OK, USA). Means were separated using the Duncan multiple range test at $P \leq 0.01$.

3. Results and discussion

3.1. Physicochemical analysis of treatment

The results of this study showed that the water sampled from the mine was confirmed to be AMW according to the benchmarks prescribed by the South African Government Department of Water Affairs and Forestry [13] and the World Health Organization [14]. The physicochemical parameters of AMW and tap water were summarized in Table 1, showing the pH, temperature, electrical conductivity (EC), total dissolved solutes (TDS), nitrate (NO_3), dissolved oxygen (DO), and sulfate (SO_4) of the AMW and indicate the permissible range given by [13, 14]. In comparing the quantities of the minerals found in the AMW and tap water, the quantities of Cd (0.18 mg/L), Cr (5.87 mg/L), Cu (0.95 mg/L), Ni (10.42 mg/L), and Zn (55.47 mg/L) in the AMW water were relatively higher when compared with the tap water (Cd (0.01 mg/L), Cr (0.04 mg/L), Cu (0.12 mg/L), Ni (0.04 mg/L), and Zn (0.92 mg/L), respectively), which is considered to be within the permissible range according to both the South African standards and the WHO standards.

3.2. Influence of GA_3 on tomato growth

The results obtained from this study showed that GA_3 boosted tomato plant height and stem diameter in plants irrigated with AMW. There was a significant ($p \leq 0.01$) difference between the different experimental treatments when compared with the GA_3, AMW, and tap water within the column (Table 2). The study examined plant height and stem diameter following irrigation with AMW; from the results obtained, GA_3 increased plant height (Table 2), whereas the stem diameter did not demonstrate any significant ($p \leq 0.01$) difference among the treatments (Table 2). All acid mine watered plants that received treatment of GA_3 were taller (16.50 cm) than plants irrigated with AMW, (4.00 cm), and tap water (14.00 cm) (Table 2). Tomato plants irrigated with AMW were able to withstand and recuperate from the abiotic stress after treatment with

Table 2. Growth response of tomato exposed to GA_3 administration and AMW irrigation as well as heavy metal accumulation factor (root).

Treatment	Plant height (cm)	Stem diameter (mm)	RAF Cd (mg/kg)	RAF Cr (mg/kg)	RAF Cu (mg/kg)	RAF Ni (mg/kg)	RAF Zn (mg/kg)
GA_3 + AMW	$16.50 \pm 0.01a^{A}$	$2.83 \pm 0.28c^{B}$	$0.02 \pm 0.00c^{E}$	$0.04 \pm 0.00c^{C}$	$0.04 \pm 0.00c^{C}$	$0.04 \pm 0.00c^{C}$	$0.05 \pm 0.00c^{D}$
AMW	$4.00 \pm 0.01b^{A}$	$4.00 \pm 0.51a^{A}$	$0.45 \pm 0.03a^{F}$	$0.72 \pm 0.02a^{E}$	$0.92 \pm 0.04a^{D}$	$1.51 \pm 0.05a^{B}$	$1.23 \pm 0.04a^{C}$
C	$14.00 \pm 0.01c^{A}$	$4.00 \pm 0.50a^{B}$	$0.06 \pm 0.01b^{F}$	$0.45 \pm 0.01b^{D}$	$0.45 \pm 0.02b^{D}$	$1.04 \pm 0.01b^{C}$	$0.25 \pm 0.02b^{E}$
F statistics	3.14**	3.10**	3.14**	1.20**	2.57**	2.57**	3.75**
Recovery percent (%)							
GA_3 + AMW			2.00	1.00	0.85	1.15	0.40
AMW			4.15	7.50	14.00	18.65	20.00
C			3.30	0.65	0.65	5.50	8.00

GA_3: Gibberellic acid, AMW: Acid mine water; RAF (root accumulation factor). Means with the same letters are not significantly different at $^{**}p \leq 0.01$. Normal letters are means within the column (A–C) while letters with superscripts are means within the rows (A–F). The recovery percent of the measured heavy metal accumulation on the root.

Table 3. Gibberellic acid reduces heavy metal translocation factors on the tomato stem.

Treatment	STF Cd (mg/kg)	STF Cr (mg/kg)	STF Cu (mg/kg)	STF Ni (mg/kg)	STF Zn (mg/kg)
GA_3 + AMW	$0.01 \pm 0.00c^{A}$	$0.01 \pm 0.00c^{B}$	$0.01 \pm 0.00c^{B}$	$0.01 \pm 0.00c^{B}$	$0.01 \pm 0.00c^{B}$
AMW	$0.41 \pm 0.01a^{C}$	$0.23 \pm 0.02a^{E}$	$0.50 \pm 0.01a^{A}$	$0.45 \pm 0.02a^{B}$	$0.33 \pm 0.02a^{D}$
C	$0.20 \pm 0.01b^{A}$	$0.04 \pm 0.00b^{C}$	$0.30 \pm 0.01b^{B}$	$0.31 \pm 0.01b^{B}$	$0.05 \pm 0.00c^{C}$
F statistics	6.10**	6.13**	3.25**	1.44**	9.00**
Recovery percent (%)					
GA_3 + AMW	0.20	0.65	0.50	0.35	0.80
AMW	1.65	2.00	2.10	7.50	5.80
C	1.15	1.15	1.65	1.65	0.15

GA_3: Gibberellic acid, AMW: Acid mine water; STF (stem translocation factor). Means with the same letters are not significantly different at $**p \leq 0.01$. Normal letters are means within the column (A–C) while letters with superscripts are means within the rows (A–F). The recovery percent of the measured heavy metal accumulation on the stem.

GA_3. The reason may be that GA_3 is involved in plant mechanisms that promote plant growth and development during stress conditions, possibly through enhanced resistance under abiotic stress [15, 16].

Taken together, the results showed that GA_3 was able to influence the growth in tomato plants irrigated with AMW. All plants that received GA_3 treatment were taller than the plants that did not receive GA_3. Tomato plants irrigated with AMW were able to withstand and recuperate from the abiotic stress after treatment with GA_3. This was probably the involvement of GA_3 in plant mechanisms associated with enhanced resistance to abiotic stress [16]. Results obtained from this study did not deviate from the established principles. The result showing the significant difference in the plant height agrees with the study of Ali et al. [17], where the application of GA_3 enhanced Mung bean plant growth under Ni stress. Studies on the effect of HMs on plants have shown that HMs are detrimental at levels higher than a certain threshold [18]. However, the plant can grow, develop, and reproduce when enhanced by a growth stimulator or any agent that improves plant fitness. To stimulate plant growth and ward off the effects of stress, the common measures are the application of plant regulators [18]. In line with these findings, an increase in plant height in mung bean, lettuce, and rocket was conceivably the most widely observed effect of GA_3 [17, 19, 20, 21]. GA_3 promotes plant development by promoting the degradation of DELLA proteins, a nuclear family of transcription factors that acts as repressors in GA_3 signaling pathways [22, 23]. The administration of GA_3 caused an increase in the number and length of cells in the epidermis, which results in an increase in the length of the petiole. The result of this study agrees with earlier submissions of Shaddad et al. [24] and Fahad et al. [25] that GA_3 improves the growth and physiological parameters of a plant, making GA_3 one of the most significant plant growth regulators used for enhancing stressed plants. GA_3 has been reported to alleviate adverse effects of stress by improving water uptake and increasing cellular membrane plasticity [26].

3.3. Effect of GA_3 on HMs accumulation

GA_3 reduced accumulation of heavy metals within tissues of tomato plant as shown (Tables 2, 3 and 4). The study showed a reduction of HMs in the root and shoot (stems and leaves) of plants treated with GA_3 compared to the AMW irrigated plants with no GA_3 and plants irrigated with tap water and received no GA_3.

The results obtained depicted GA_3 altered HM accumulation, RAF Cr, RAF Cu, RAF Ni, RAF Zn, STF Cd, STF Cu, STF Ni, STF Zn, LTF Cu, LTF Ni, and LTF Zn significantly ($p \leq 0.01$) (Table 2), however, accumulation of other HMs, RAF Cd, STF Cr, LTF Cd, and LTF Cr did not demonstrate any significant ($p \leq 0.01$) difference among the treatments. Interestingly, when compared with the GA_3 treated tomato plants within the row, RAF Cd, RAF Cr, RAF Cu, RAF Ni, RAF Zn, STF Cd, STF Cu, LTF Cd, LTF Cr, LTF Cu, and LTF Zn showed a significant difference ($p \leq 0.01$) (Table 2).

Furthermore, result of the study showed the roots accumulated more Cd, Cr, Cu, Ni, and Zn when lants were irrigated with AMW (Table 3). Moreover, results on stem and leaf translocation showed that GA_3 reduced HM translocation compared to AMW and control experimental treatments (Table 3). According to the results, the plant regulator GA_3 caused a significant reduction in the quantities of HMs accumulation and translocation in the tomatoes irrigated with AMW. Furthermore, under HM stressed conditions, GA_3 increases antioxidant levels while maintaining enzymatic activities for nitrogen assimilation, thereby counteracting toxic effects [27]. Recently, in a study by Saleem et al. [28], GA_3 alleviated Cu toxicity in white jute seedlings by increasing plant growth, biomass, photosynthetic pigments, and gaseous exchange attributes and reduction of oxidative stress in white jute seedlings by generating extra reactive oxygen species (ROS). Other plant species, such as maize, French marigold, and black nightshade, have been exogenously augmented with GA_3 to enhance plant growth and composition when cultivated in metal-contaminated soils [29, 30, 31].

Table 4. Gibberellic acid reduces translocation factors on the tomato leaf.

Treatment	LTF Cd (mg/kg)	LTF Cr (mg/kg)	LTF Cu (mg/kg)	LTF Ni (mg/kg)	LTF Zn (mg/kg)
GA_3 + AMW	$0.01 \pm 0.00c^{C}$	$0.01 \pm 0.00c^{C}$	$0.01 \pm 0.00c^{C}$	$0.04 \pm 0.00c^{A}$	$0.02 \pm 0.00c^{B}$
AMW	$0.32 \pm 0.01a^{D}$	$0.32 \pm 0.01a^{D}$	$0.54 \pm 0.01a^{C}$	$0.87 \pm 0.02a^{B}$	$0.98 \pm 0.03a^{A}$
C	$0.03 \pm 0.00b^{E}$	$0.05 \pm 0.00b^{D}$	$0.23 \pm 0.01b^{B}$	$0.86 \pm 0.02b^{A}$	$0.08 \pm 0.01b^{C}$
F statistics	9.23**	6.26**	6.32**	6.13**	6.13**
Recovery percent (%)					
GA_3 + AMW	0.65	0.15	0.50	1.50	1.30
AMW	2.15	5.30	1.50	11.65	14.80
C	1.65	1.65	1.00	0.65	3.30

GA_3: Gibberellic acid, AMW: Acid mine water; LTF (leaf translocation factor). Means with the same letters are not significantly different at $**p \leq 0.01$. Normal letters are means within the column (A–C) while letters with superscripts are means within the rows (A–F). The recovery percent of the measured heavy metal accumulation on the leaf.

The results of the root accumulation factor (RAF) showed significant differences among treatments (Table 2). The study findings revealed that plants sprayed with GA_3 had significantly lower HMs in their roots than plants irrigated with AMW. In the studies of Chauhan et al. [32], the critical enzyme activities for plant absorption interfered with the presence of HMs in the water; this interference was thwarted by the application of GA_3. It has been shown that plants accumulate metals in their roots and this is measured by the RAF, which measures the concentration of metals in the roots and water/soil (mg/kg). This shows how plants tolerate and accumulate HMs [33].

This corroborates with the earlier submission of Zhu et al. [10] that GA_3 also decreases the accumulation of HMs in rice shoots and alleviates the detrimental effect of Cd^{2+} and Pb^{2+} on broad bean and lupin plants. Bücker-Neto et al. [34] examined the involvement of plant growth regulators in signaling networks, defense mechanisms, and HM toxicity mitigation. As a result, GA_3 stimulated defense responses and promoted the production and accumulation of phytochelatins, which were involved in HM detoxification [35].

Furthermore, the application of GA_3 in our study triggered the reduction of HM accumulation in the tomato plant tissues (roots, stems and leaves). The reduction of HMs elicited by the application of GA_3 has been reported to reduce HMs such as Cd content in the plant root by reducing nitric oxide accumulation [10, 29].

Results on Accumulation Factor (AF) demonstrated a significant reduction in the accumulation of HMs in tomato plant tissues (roots, leaves, and stems) when treated with GA_3. The AF was used to describe the metabolism-mediated active transport of metals from the polluted water to the plant tissues, which is then accumulated intracellularly. AF has been classified based on hyperaccumulators and accumulators are those plants which accumulated metals >1 mg/kg whereas a value < 1 mg/kg is indicative of an excluder. Values far greater than one (>1) indicate that the plant is a potential hyperaccumulator, which can be used as a remediator to remediate water pollution [36, 37, 38]. The accumulation factor for HM in plant tissues was ascertained (Table 2), where the roots were shown to have accumulated more HMs and translocated them to the stems and leaves. In the roots, the metal ions are transported across the root cellular membrane which allows metals to enter the plant tissues [39]. Metals are first taken up in the roots by the apoplast, a free intercellular space directed towards the xylem. Because of the continuum of the root epidermis and the cortex, HMs are translocated apoplastically into plant tissue. Metals in root cells must pass through the endodermis and Casparian strip before reaching the xylem. Endodermis and Casparian strip cell walls act as a barrier to apoplastic diffusion into the vascular system [39]. As the plant grows under polluted irrigated water, a pattern of metal translocation from roots to shoots is established. These could be useful in the biological monitoring of HM contamination [40].

In this study, AF for the HM build-up in the tomato plant was ascertained (Table 3). An accumulation factor of >1 indicated that the tomato tissues (roots, leaves, and stems) had more concentration of HMs than the water sediment. Moreover, the translocation factor (TF) value influences the efficiency with which HMs are transported from the root to the shoot. When TF is greater than 1 (> 1), a plant is deemed effective in metal translocation from root to shoot; this is owing to an efficient metal transport mechanism. TF values less than 1 (< 1), on the other hand, indicate poor metal transmission, implying that these plants acquire metals more in the roots than in the shoots [36]. According to the findings of the study, < 1 values on the root tissues of HMs are in descending order Cd $>$ Cr $>$ Cu $>$ Zn in the treatments used, whereas Ni had > 1 value (1.01; 1.51; 1.04; 1.12) on the treatments GA_3+AMW. It is possible that the significance of this order could be attributed to the root being the main tissue capable of chelating HMs with phytochelatin, compartmentalizing HMs within the vacuole, and serving as an adsorbent [41]. The roots accumulated more HMs (Tables 2 and 3), which were then translocated to leaves and stems, respectively, when irrigated with AMW. Except for a few HMs, HM concentrations were in descending order of leaf $>$ stem $>$ root $>$ AMW (Tables 2, 3 and 4). Plant root (such as tomato root) is an important tissue for the chelation and adsorption of HMs. The accumulation factor (AF) and translocation factor (TF) were used to explore the HM translocation behaviours in the water-tomato plant system (TF) [36]. For most HMs, similar variable tendencies to reduce $BF_{w\text{-}r}$ (translocation from water to root) and $TF_{r\text{-}s}$ (translocation from root to stem) linked with increasing $TF_{s\text{-}l}$ (translocation from stem to leaf) were identified owing to HM detoxification and stress tolerance in tomatoes. Du et al. [41] conducted a similar study in which HMs; Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Ba, and Pb were discovered in increasing order in paddy rice root due to retention and adsorption in the root. From the results, tomato plants treated with GA_3+AMW had reduced HMs accumulation (RAF Ni 1.15 to RAF Cd 0.02, RAF Cu 0.04, RAF Ni 0.04 and RAF Zn 0.05) (Table 2); this was attributed to the presence of GA_3, which can stimulate plants to immobilize HMs [42]. The presence of GA_3 and the control had significantly lower HMs values compared to the absence of GA_3 (AMW) at $p \leq 0.01$. A value < 1 was recorded on the Ni (1.01, 1.51, 1.04, and 1.12) and Zn (1.23 and 1.20) in the roots.

This study found a reduction in the harmful effects of HMs when GA_3 was sprayed as a treatment on the plant thereby, reduced the oxidative stress and aided plant antioxidant systems. This is consistent with Sharaf et al. [42] earlier submission, in which the role of GA_3 in obliterating the negative effects of HMs such as Cd in plants was reported. Our findings also agreed with the studies of Mansour et al. [18], who found that GA_3 alleviated the detrimental effects of HMs on broad bean plants. There are different mechanisms that may prevent HMs accumulation, some mechanisms may enable detoxification of cells, or produce metabolic resistance to toxic metals [43]. Mechanisms such as cellular mechanism, which includes binding to the cell wall and extracellular exudates, reducing uptake or efflux pumping of metals at the plasma membrane, chelation of metals in the cytosol by peptides such as phytochelatins (PCs), repair of stress-damaged proteins, and compartmentation of metals in the vacuole by tonoplast-located transporters [44]. Furthermore, results using hierarchical clustering and heatmapping to show HM abundances when treated with GA_3 + AMW, AMW and control are presented (Figure 1). There was colour abundance among treatments and HMs detected ranging from red to blue colours. The authors found colour abundance patterns of HMs across the different plant tissues (roots, stems and leaves) within the treatment GA_3 + AMW, AMW and control (Figure 1).

According to previous observations, GA_3 helped reduce the toxic effects of several HMs, such as Cd in *Arabidopsis thaliana* [45]; Ni in wheat seedlings [46]; Cr in pea seedlings [47]; Cd in oilseed rape and broad bean [42, 48] as indicated (Figure 1a). High colour intensity was seen in the AMW on Cr, Cd, Cu, Ni, and Zn proving the metals' insolubility in the root. This study agrees with the earlier studies of Usman et al. [36] that HMs accumulate more in the roots of the shrub plant *Tetraena qataranse*. Likewise, Page and Feller [49, 50] observed similar retention of such HMs in the roots of wheat and lupin. Interestingly, the HMs were eliminated as GA_3 was treated (AMW) (Figure 1a). A similar pattern was seen in the roots, stem, and leaves (Figure 1a, b, and c). The HMs moved through the xylem and phloem transport networks. GA_3 can also lower oxidative stress and improve plant antioxidant systems, reducing the negative effects of HMs on plants [48]. The mobility of HMs was observed in this study, and GA_3 decreased the quantity of HMs transferred to the leaves. According to prior studies by Page and Feller [48], GA_3 reduced HM accumulation in rice seedlings and attenuated the adverse effects of HMs on broad bean and lupin plants. Metals found in the shoot (stem and leaves) were phloem-mobile when irrigated with AMW alone, and if they exceeded the limit, it will inhibit the growth of developing plant parts. HMs such as Ni has been found to be particularly phloem-mobile; similarly, Zn and Cd are mobile in the phloem and concentrate at the shoot apex (meristems) depending on the plant species and developmental stage [49, 50]. This study demonstrated the decrease of oxidative stress and increase of antioxidant systems (Figure 1a, b, and c). Several researchers have corroborated this by pointing out the GA_3's beneficial effects on HM removal [18, 29, 30, 31, 42]. Results of the clustering analysis showed substantial changes in different tomato parts (roots, stems, and leaves) with GA_3 under stressed conditions [25, 28].

4. Limitation of the study

Because the study was conducted in the glasshouse it is not known what the dynamism of a field environment would cause to the obtained results. Furthermore, only one tomato cultivar was selected for study and therefore the influence of cultivar differences on the effect of GA_3 AMW irrigated tomato is left hanging. Finally, the experiment was terminated before fruiting and therefore the effect of GA_3 on the yield and quality of the fruit is not known.

5. Conclusions

In this study, GA_3 was found to have a significant effect on tomato seedlings irrigated with AMW. The results demonstrated that GA_3 improved plant height while decreasing the quantities of HMs in tissues of AMW irrigated tomato plants. The study revealed the effect of GA_3 on HMs availability in different tomato tissues (root, stem, and leaf). Based on these results, it was therefore concluded that GA_3 can be used to alleviate the effects of HM and the associated stress on tomatoes. The study also revealed that GA_3 affected the accumulation of HMs in the roots, stems, and leaves of tomatoes. It was evident from this study that GA_3 may be used as a growth enhancer of tomato seedlings exposed to AMW and this growth regulator can reduce the accumulation of HMs in tomatoes when exposed to AMW.

For future studies, field trials in polluted areas are also encouraged to determine the effects of GA_3. Moreover, studies on effects of GA_3 on tomatoes grown to maturity for fruit analysis are recommended.

Declarations

Author contribution statement

Udoka Vitus Ogugua: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Sheku Alfred Kanu; Khayalethu Ntushelo: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Funding statement

Mr Udoka Vitus Ogugua was supported by National Research Foundation [121500].

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors would like to thank the staff of the Horticulture Centre of the University of South Africa (Unisa) for their support.

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