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Full Length Research Paper

Influence of different concentration of heavy metals on the seed germination and growth of tomato

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Some heavy metals in higher doses may cause metabolic disorders and growth inhibition for most of the plant species. This study was performed in order to evaluate two tomato varieties (Barakat and Local tomato) response to ordinary Heavy Metals (Fe, Pb and Cu) in northern of Iran. Five doses (0, 0.001, 0.01, 0.1 and 1%) of lead acetate, cupric carbonate and ferric chloride were investigated. The experiment was conducted under 12±2h photoperiodic laboratory condition in a laboratory germinator. Results showed that the reaction of varieties to different heavy metal compounds and those doses is varied and Barakat variety has greater resistance in more indices. Also, heavy metals kind and different doses of these compounds affected the germination and some growth indices. In this experiment the highest inhibition on shoot length 2.66 cm, root/ shoot ratio 0.87, moisture content 0.31 g, fresh weight 0.32 g and dry weight 0.059 g were obtained from Fe compound, however; had not significance different with other heavy metal compounds in case of root length 3.39 cm and coefficient of germination velocity 0.27% ($P \leq 0.05$). Also, dose of 1% appeared highest undesirable effect on all evaluated indexes.

Key words: Tomato, toxic metal, dose, coefficient of germination velocity.

INTRODUCTION

Heavy metals have recently received the attention of researchers all over the world, mainly due to their harmful effects on plant. Heavy metals are defined as metals with a density higher than 5 g cm^{-3}. 53 of the 90 naturally occurring elements are heavy metals (Weast, 1984), but not all of them are of biological importance. Based on their solubility under physiological conditions, 17 heavy metals may be available for living cells and of importance for organism and ecosystems (Weast, 1984). Among these metals, Fe, Mo and Mn are important as micro-nutrients. Zn, Ni, Cu, V, Co, W, and Cr are toxic elements with high or low importance as trace elements. As, Hg, Ag, Sb, Cd, Pb, and U have no known function as nutrients and seem to be more or less toxic to plants and micro-organisms (Breckle, 1991; Nies, 1999).Excess concentrations of some heavy metals in soils such as Cd (II), Cr (VI), Cu (II), Ni (II), and Zn (II) have caused the disruption of natural aquatic and terrestrial ecosystems (Gardea-Torresdey et al., 1996; Meagher, 2000).

Heavy metals decrease growth rate of plants by affecting various parts of root metabolism such as water and mineral uptake membrane function inhibition of enzyme activities oxidation and cross-linking of proteins inhibition of cell division induction of DNA damage and cell death but mainly and primarily disturb cellular redox environ-ment causing oxidative stress both in roots and leaves and subsequently induces some of these above mentioned toxic symptoms (Tamas et al., 2008).The toxic effects of metals have also been intensively studied at the level of biochemical - physiological process such as photosynthesis (Kupper et al., 2002), transpiration (Pandey and Sharma, 2002), enzyme activity (Astolfi et al., 2005) or metal accumulation in tissue (Palmieri et al., 2005). Some heavy metals at low doses are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition for most of the plants species (Fernandes and Henriques, 1991; Claire et al., 1991). The toxic effects of heavy metals in different crops may differ significantly (León et al., 2002). Researchers have observed that some plants species are endemic to metalliferous soils and can tolerate greater than usual amounts of heavy metals or other toxic

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Table 1. ANOVA effects due to heavy metal compounds on measured variables.

		Mean squares						
Source	df	CGV	Root length (cm)	Shoot length (cm)	Root/ shoot ratio	Moisture content (g)	Fresh weight (g)	Dry weight (g)
A	1	0.008*	17.47**	1.67**	0.17^{ns}	0.017^{ns}	0.022^{ns}	0.0005*
B	2	0.05**	1.64^{ns}	9.01**	0.029^{ns}	0.03*	0.03*	0.001**
C	4	0.46**	13.7.9**	28.96**	3.31**	0.051**	0.51**	0.008**
A*B	2	0.0001^{ns}	2.69*	0.09^{ns}	0.14^{ns}	0.003^{ns}	0.003^{ns}	0.0001^{ns}
A*C	4	0.033**	3.13**	1.04**	0.02^{ns}	0.11**	0.107**	0.0004**
B*C	8	0.108**	12.67**	5.96**	0.47**	0.06**	0.069**	0.0026**
A*B*C	8	0.0002^{ns}	0.59^{ns}	0.05^{ns}	0.03^{ns}	0.006^{ns}	0.005^{ns}	0.0001^{ns}
C.V	-	15.18	23.04	14.36	26.14	23.66	22.67	15.07

ns, ** not significant or significant at P≤0.01, ANOVA.

compounds. Heavy metal toxicity comprises inactivation of biomolecules by either blocking essential functional groups or by displacement of essential metal ions (Goyer, 1997).In addition, autoxidation of redox-active heavy metals and production of reactive oxygen species (ROS) by the Fenton reaction causes cellular injury (Stohs and Bagchi, 1995). The importance of tomato fruits as good sources of ascorbic acid (Vitamin C), β-Carotene and mineral elements has been acknowledged (Tindal, 1992). Several studies have been conducted in order to evaluate the effects of different heavy metal concentrations on seedlings or adult plants (Raskin and Ensley, 2000).In a few studies, the seeds have been exposed to the contaminants (Xiong, 1998) whereas, in this study seeds subjected to metals contaminant . Therefore, the aim of this study is to evaluate the reaction of two growing tomato seed varieties to different concentrations of ordinary heavy metal in northern of Iran.

MATERIALS AND METHODS

The experiments were conducted in a horticultural laboratory of the agricultural faculty of Guilan University, Rasht, Iran in the summer of 2010. The Barakat and Local tomato (*Lycopersicon esculentum,* Mill) seed varieties were prepared by the Horticulture Department. Three hundred viable seeds of the tomato varieties each were surface sterilized with 0.1% mercuric chloride for 30 s and rinsed several times with distilled water. Sterilized glass Petri dishes of approximately 9 cm in diameter, each containing 2 Whatman No. 1 filter papers were used as sowing container and media. Twenty seeds of each tomato variety were sown in each of the Petri dishes for different metallic compound treatment levels. Pollution was established by adding 6ml of the respective metallic compound solutions [Lead acetate ($CuC0_3$), Cupric carbonate ((CH3COO) $_2$Pb) and Ferric chloride (Fe_2Cl_3)] at the various concentrations into the Petri dishes before sowing. The following concentrations of each of the salts were 0, 0.001, 0.01, 0.1 and 1% prepared using serial dilution method. Distilled water was used as the control treatment. The Petri dishes were kept under 12±2h photoperiodic condition in a laboratory germinator at 26±100°C for germination study and the seedling growth performances observed for 21 days post germination. Each treatment was replicated three times and the solutions were replenished every other day. The criterion used for seed germination was taken as emergence of 2 mm radicle at the time of observation (Odoemena, 1988). Germination counts were recorded at 2 days intervals for 12 days after sowing and the seedlings were allowed to grow. The germination percentage of the seeds was finally determined for each of the treatments. The data were expressed as aggregate germination percentage (AGP) and coefficient of germination velocity (CGV) according to the formula of Hartmann and Kester, (1964).

$$CGV = \frac{\text{Total number of Seedling}}{A1T1 + A2T2 + AxTx}$$

Where A = the number of seedlings emerging on a particular number of days (T), and subscripts 1, 2 … x are respective number of germinated seeds per respective number of days after sowing of the seeds. After 21 days, the experiment was terminated, and the growth parameters estimated after uprooting and cleaning the seedlings. The root and shoot lengths were measured in centimeter using a meter rule and the root/shoot ratio was calculated by dividing the root length with that of the shoot. The leaf area was determined by multiplying the length and width of the leaf by a correction factor (0.75) according to the method of Verma and Poonia (1983). The fresh weight was measured with a weighing balance. Thereafter, the Petri-dishes containing the fresh seedlings were placed in desiccators which contained a Petri dish of activated silica gel for 48 h and sun dried to a constant weight. The moisture content was obtained by the difference between the fresh weight and dry weight. Data were subjected to Analysis of variance in SAS (SAS, Inc., Cary, N.C.) and means separated using the Tukey test.

RESULTS AND DISCUSSION

Our study indicated that heavy metals kind and different doses of these compounds affected the germination and some growth indices in varieties (Table 1).

The lowest value of CGV and indices were observed in dose of 1% for all compounds in both varieties. The Barakat variety showed better results in more cases compared to local variety, however, there were not significance different between varieties in relation of moisture content (MC), root to shoot ratio and fresh weight (*P*≤0.05) (Table 2). Fe-compound had highest inhibition on indices, except to CGV and root length. The inhibitory

Table 2. Affect of heavy metal compounds on measured variables.

	CGV (%)	Root length (cm)	Shoot length (cm)	Root/ shoot ratio	Moisture content (g)	Fresh weight (g)	Dry weight (g)
Dose							
0	0.27 [ab]	6.64 [a]	4.32 [a]	1.23 [a]	0.55 [a]	0.56 [a]	0.08 [a]
0.001	0.29 [ab]	5.87 [b]	3.7 [b]	1.24 [a]	0.39 [b]	0.4 [b]	0.072 [ab]
0.01	0.3 [a]	3.44 [c]	3.5 [b]	0.92 [b]	0.25 [c]	0.42 [b]	0.071 [b]
0.1	0.26 [b]	1.13 [d]	2.3 [c]	0.75 [b]	0.41 [b]	0.27 [c]	0.071 [b]
1	0.11 [c]	0.41 [d]	1.14 [d]	0.19 [c]	0.11 [d]	0.11 [d]	0.025 [c]
Variety							
B	0.26 [a]	3.944 [a]	3.14 [a]	0.91 [a]	0.36 [a]	0.37 [a]	0.067 [a]
F	0.24 [b]	3.0631 [b]	2.87 [b]	0.82 [a]	0.33 [a]	0.34 [a]	0.062 [b]
Heavy metal compound							
cupric carbonate	0.26 [a]	3.34 [a]					
lead acetate							
ferric chloride							

[z] values in a column followed by the same letter are not significantly different, P≤0.05, according to Tukey.

effect on germination of tomato seeds treated with different heavy metals was arranged in the following order: $Cu^{+2} > Fe^{+2} > Pb^{+2}$ and in relation of growth indices was: $Cu^{+2} > Pb^{+2} > Fe^{+2}$ ($P \leq 0.05$) (Table 2).

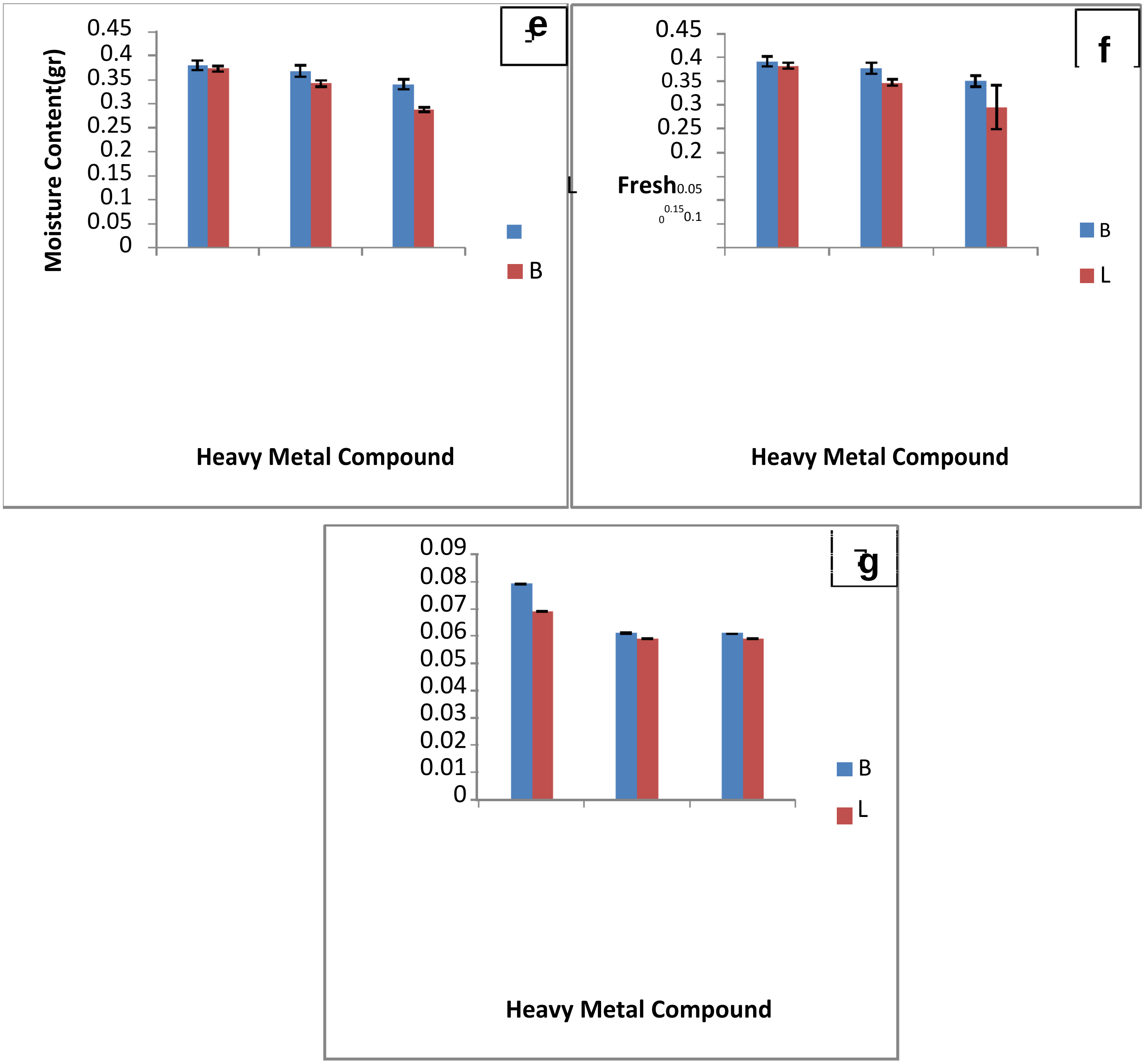
The investigation of interaction between the varieties and elements indicated the variable effects on germination and growth indices. The highest CGV was obtained when ferric chloride compound applied on Barakat variety and lowest value was related to local variety if lead acetate used as tension creator. According to interaction results of elements and varieties can be said that Barakat variety has greater resistance to elements (Fe (II), Pb (II) and Cu (II)) stress for CGV after applying them compared to local variety. Also, the Barakat variety showed better result in case of other traits after tested with heavy metal compounds (Figures 1). In other hand, must noted that a high resistance observed as significantly when cupric carbonate used with barakat variety and high sensitivity appeared after using the ferric chloride with local variety (Figures 1).

The result of interaction between doses of heavy metals and varieties showed in Figures 2. In the present study increasing of doses decreased indices value in both varieties, nevertheless, elements inhibitive charac-teristic would be increased with increasing of the doses. However, Barakat variety showed better resistance to different doses.

Indeed, as increased the doses of heavy metals, all indices showed the decreasing state. However, low sensitivity was observed relation to high doses of cu compound compared to other compounds (Figures 3).

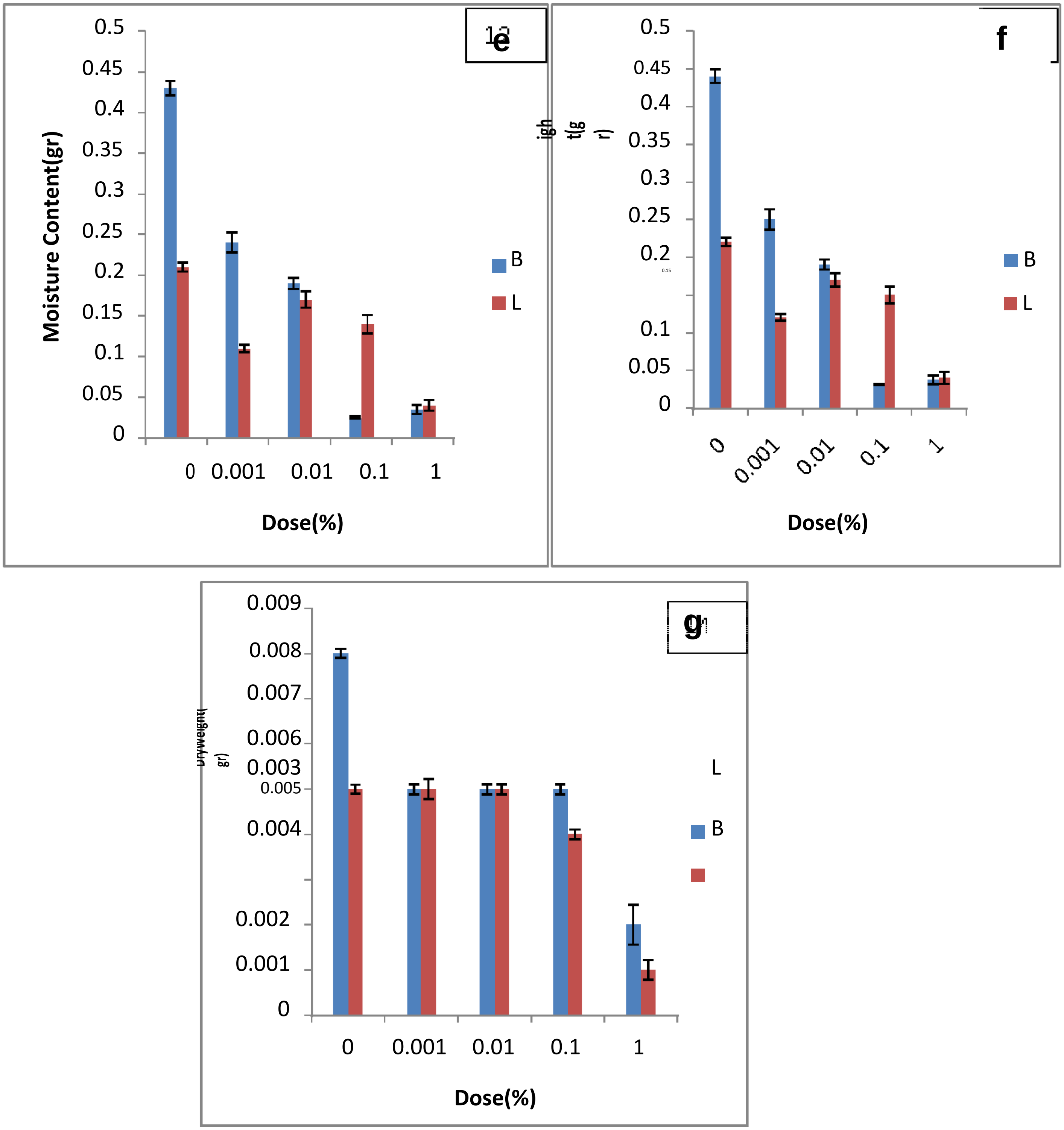
Regression model indicated that dry weight index (y) as dependent trait in this study had highest variation with root length (x_2), shoot length (x_3) and root/shoot ratio (x_4) as independent traits, respectively ($Y = 0.000581X_2 + 0.00123X_3 + 0.0017x_4$).The result of correlation between evaluated indices showed that CGV have highest correlation with stem length and dry weight, respectively ($r^2 = 0.79$). Indeed, stem length and dry weight have highest correlation together and when compared with other traits (Table 3).

Heavy metals are potentially highly toxic to all organisms including animals and plants. Suppression of seed germination and plant growth responses have been attributed to the establishment of higher toxic effect syndrome (HTES) due to high accumulation of the metallic salts within the plant body biomass (Singh and Singh, 1981). There was a significant interaction between the effects of varieties with heavy metal compound, dose with varieties and dose with metal compound. Both metals are transition elements, and their resulting redox properties have been used during evolution in the development of oxidative energy generation. Copper and iron are essential but also can be toxic to aquatic plants when presenting high concentration. Both contribute to the production of excess damaging oxidant radicals (Brewer, 2010). As observed earlier, *Synechocystis aquatilis* showed a decrease in growth with increasing metal ion concentration at initial exposure of copper (Shavyrina et al., 2001). It was reported that toxic effect of Cu and other heavy metals on wheat growth was as follow: Cd>Cu>Ni>Zn>Pb>Cr (Athar and Masood, 2002). Another study demonstrated that Cu was the second most effective metal (within Hg, Cd, Co, Pb and Zn) on the seed germination, root elongation and coleoptile and hypocotyls growth in *Triticumaestivum* and *Cucumissativus* (Munzuroglu and Geckil, 2002). Our observations on tomato are not in accordance with the



Figures 1. Interaction between tomato varieties and heavy metal compound on CGV, root length, shoot length, root/shoot ratio, moisture content, fresh weight and dry weight.

reported results. The reduction in growth could be due to inhibition of normal cell division by the metal as has been reported for *Dunaliellatertiolecta* exposed to mercury (Davies, 1976) and *Chlorella vulgaris* exposed to copper, mercury, and cadmium (Rosko and Rachlin, 1977). The decrease in the rate of cell division caused by metals is primarily attributed to their binding to sulfhydryl groups which are important for regulating the plant cell division (Fisher et al., 1981; Visviki and Rachlin, 1991). Lead (Pb) is one of the ubiquitously distributed most abundant toxic elements in the soil. The toxic level of Pb in soil results from disposal of municipal sewage sludge, mining and smelting activities, Pb containing paints, paper and pulp, gasoline and explosives. It exerts adverse effect on morphology, growth and photosynthetic processes of plants. High level of Pb also causes inhibition of enzyme activities, water imbalance, alterations in membrane permeability and disturbs mineral nutrition (Sharma and Dubey, 2005). Pb inhibits the activity of enzymes at cellular level by reacting with their sulfhydril groups.

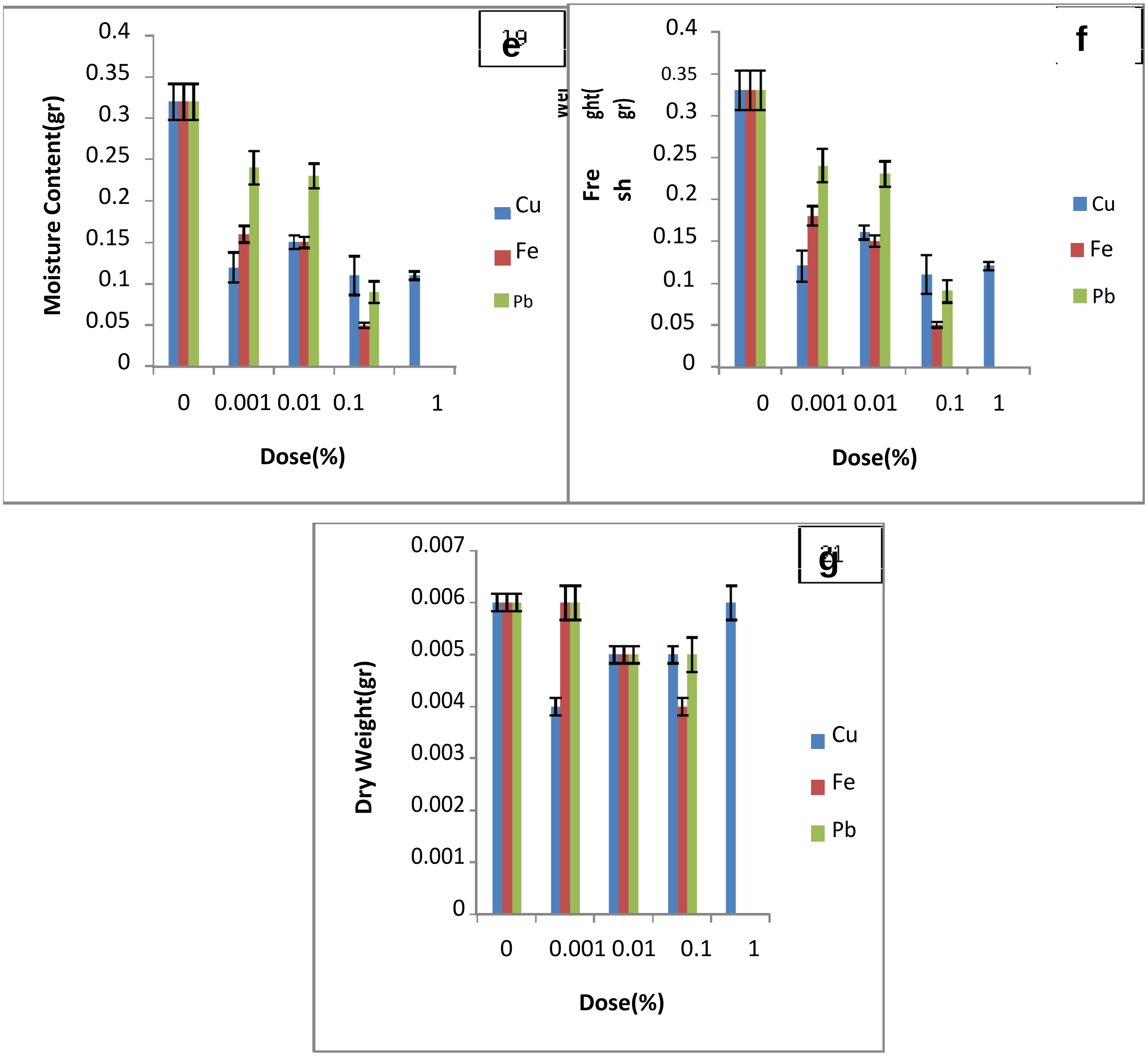


Figures 2. Interaction between tomato varieties and dose of heavy metal compound on CGV, root length, shoot length, root/shoot ratio, moisture content, fresh weight and dry weight.

High Pb concentration also induces oxidative stress by increasing the production of ROS inplants (Reddy et al., 2005).

Conclusions

We found that varieties response to heavy metal stress



Figures 3. Interaction between dose and heavy metal compound on shoot length, root/ shoot ratio, moisture content fresh weight and dry weight.

Table 3. The correlation value between indices.

	Root length (cm)	**Shoot length (cm)**	**Fresh weight (g)**	**Dry weight (g)**	**Root/ shoot ratio**	**CGV (%)**	**Moisture content (g)**
Root length (cm)	1	0.762**	0.747**	0.585**	0.914**	0.603**	0.745**
Shoot length (cm)		1	0.720**	0.813**	0.659**	0.765**	0.711**
Fresh weight (g)			1	0.616**	0.631**	0.547**	1.00**
Dry weight (g)				1	0.615**	0.750**	0.606**
Root/ shoot ratio					1	0.680**	0.627**
CGV (%)						1	0.537**
Moisture content (g)							1

** Correlation is significant at P≤0.01.

were different. In the other hand, Cupper carbonate had inhibitive less effect on germination and growth indices compared to lead acetate and ferric chloride on both varieties. Also, dose of these heavy metals must not greater than 0.01 in substrates.

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