Journal of Applied Science and Environmental Studies
JASES
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Lepidium sativum L. HORMESIS INDUCED BY HEAVY METAL STRESS FOR SEED GERMINATION AND SEEDLING GROWTH

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Received 02 Sept 2020; Revised 10 Oct 2020, Accepted 20 Oct 2020

Keywords
Germination
Heavy metal
Hormesis
Lepidium sativum

Abstract

The effect of numerous heavy metals such as Co (as $CoCl_2$), Cu (as $CuSO_4$), Zn (as $ZnSO_4$) and As (as Na_2HAsO_4) concentrations on garden cress *(L. sativum* L.) was tested. During this experiment, the measurement of several parameters such as root length (RL), plumule length (PL), germination percentage (GP), germination index (GI), germination rate index (GRI), vigor index (VI), coefficient of velocity of germination (CVG) and mean germination time (MGT) were done. The results confirmed that various treatments of heavy metals possessed statistically significant impacts on the GRI, GI, MGT, CVG and VI. However, at different metal concentrations indicated that GP did not differ significantly for Co, Cu and Zn and differ significantly for As denoting an inverted threshold dose-response model. The results showed that the trend of GI, VI, CVG of As, RL and PL of As and Cu reduced with increasing metal doses indicating an inverted threshold model. The GRI decreased with mounting metal concentrations implying an inverted U-shaped germination curve of *L. sativum* L. seeds in the experiment, and the same for PL of Co and Zn. However, the results of MGT and CVG (of Co, Cu and Zn) denote no hermetic effect. In addition, the results indicated that root length was decreased more than shoot length and was expressed as the sensitivity index. Generally, it concluded that the toxicity of the examined metals diminishes by the following succession: As > Cu > Co > Zn.

1. Introduction

Metal pollution has become a global problem and significant environmental menace, while these heavy metals accumulate in vegetation in surplus, and enter in the food chain [1, 2]. Heavy metals are significant for their impact on growth and progress of vegetation [1, 3]. However, ecosystems are polluted with metals by human actions [4]. For best progress and growth, seventeen important

substances are needed by vegetation. These elements, when needed in moderately high quantities, are identified macronutrients or, in low quantities, micronutrients. Although micronutrients are wanted in relatively minor amounts for vegetation growth, they are as vital as macronutrients. Micronutrients often act as cofactors in enzyme systems and contribute in redox reactions, furthermore to obtaining numerous other fundamental acts in vegetation. Essentially, micronutrients are implicated in the vital physiological activities of respiration and photosynthesis [5, 6] and their deficit can inhibit these essential physiological processes thus reducing yield benefit.

Copper is indispensable for vegetation development, is among the essential micronutrient. It is an important element of various enzymes, and is implicated in lignification [3]. Copper is implicated in nitrogen metabolism and carbon assimilation; its insufficiency affects in severe development retardation. Copper is moreover implicated in biosynthesis of lignin [7]. Cobalt affects plant growth, metabolism and is an important element for activities of numerous enzymes [8] and confers tolerance versus biotic and abiotic stresses [9]. Furthermore, [10] reported that addition of 8 mg cobalt to groundnut (*Arachis hypogaea* L.) plants significantly enhanced nitrogenase activity and subsequently increased growth and yield. Uptake of arsenic by vegetation is related with the mechanism of phosphate uptake, where seemingly arsenate is taken up as a phosphate analogue [11, 12]. Zinc is a non-redox micronutrient component, which has main structural and catalytic functions in various proteins and enzymes implicated in energy metabolism [3]. Nevertheless, accumulation of the metals at upper amount can be lethal for growth of plant due to their opposing effects on vegetation growth and development [13, 14].

L. sativum L. has been considered as important medicinal plant since Vedic era. In numerous countries seedlings of *L. sativum* L. are utilized in salads because of their pungent taste. *L. sativum* L. is a speedy developing annual herb. Seeds are utilized, dried or fresh, as a seasoning with a spicy flavor. Boiled seeds are used in drinks by Arabs, moreover milled in honey or as an infusion in heated milk. The seed is able to be used for soap preparing [15]. *L. sativum* L. is a rapid developing plant that is characterize by little nutrition necessity. As showed by OECD, this plant exposed to metals during germination, under standardized conditions, is an appropriate model of environmental stress [16].

The main objectives of the current investigation were to describe the response of Garden cress (*L. sativum* L.) in presence of four metals at different levels of toxicity, and to investigate hormesis effect and metal stress in *L. sativum* L. seeds germination and seedlings grown.

2. Experimantal details

2.1. Seed treatments

Garden cress seeds (*L. sativum* L.) obtained from a commercial supplier in Beni Mellal. Seeds rinsed with distilled water, then they were sterilized in 40% sodium hypochlorite for 10 min, then 70% alcohol for 60 s, then rinsed carefully with sterile distilled water and therefore seeds were germinated in different metal concentrations.

2.2. Seed germination

In order to detect the potential adverse results of individual metal on seed germination, development properties and tolerance of seeds germination were determined to selected metals. Twenty-five seeds were deposited on filter paper (Whatman No.1) at each 9 cm diameter Petri dish. Then, 5 ml of designed metal solution was added to the correspondent Petri dishes at concentrations of 0, 15, 25, 50, 150 and 250 mg L^{-1} $CoCl_2$; 0, 5, 10, 20, 50 and 100 mg L^{-1} Na_2HAsO_4; 0, 20, 50, 100, 250 and 500 mg L^{-1} $CuSO_4$ and 0, 100, 200, 400, 800 and 1000 mg L^{-1} $ZnSO_4$. Later Petri dishes were transferred into a dark germinator with 23 ± 2 °C in temperature. Seed germination was verified quotidian at a certain time. Of course, seeds were considered normally-germinated when their radicle had developed by about 2 mm in length [17]. After the 6th day, germination percentage, germination rate index, radicle and plumule length (shoot height), radicle length/plumule length ratio (R/P ratio) and vigor index (VI) were noted to assess germination performance. Data were exposed to statistical analysis.

The germination index (GI) was evaluated using the formula (Equation 1) [18]:

$$GI = (\%\ \text{germination} \times \%\ \text{root length})/\ 100) \quad \text{(Equation 1)}$$

A GI greater > 70% is considered non-phytotoxic, while < 70% is phytotoxic [19].

Germination percentage (GP) is determined as follows (Equation 2) [20]:

$$GP = (\text{Total seeds germination after day 6/Total number of planted seeds}) \times 100 \quad \text{(Equation 2)}$$

Germination rate index (GRI) is calculated using the formula (Equation 3) [21]:

$$GRI = \Sigma\ (Gt / Dt) \quad \text{(Equation 3)}$$

where Gt is the number of germinated seeds on day t and Dt is the number of days after planting when the germinated seeds were counted.

Mean germination time (MGT) was determined using the formula (Equation 4) of [22]:

$$MGT = \frac{\sum D*n}{\sum n} \quad \text{(Equation 4)}$$

where n is the number of seeds germinated on day D and D is the number of days considered from the starting of the test.

Coefficient of velocity of germination (CVG) is evaluated using the following equation 5 [23]:

$$CVG = \frac{N}{\sum_{i=1}^{k} ni*ti} * 100 \quad \text{(Equation 5)}$$

In this regard, CVG, N and ni are, the velocity of germination, total germination at the finish of the test and germinated seeds at time ti, respectively.

The vigor index (VI) was assessed as the result of root and shoot length by germination percentage [24]. Seed vigor index was determined by the formula (Equation 6) [25].

$$VI = GP\ (\%) \times RL\ (cm) \quad \text{(Equation 6)}$$

VI = vigor index.

GP = standard germination (%).
RL = radicle elongation (cm).

2.3. Data analysis

The data was assembled to obtain an average value (three replications n = 3) and analyzed statistically using SPSS software (Version 17). The data were examined employing one-way ANOVA and mean comparison was performed by the least significant difference (LSD).

3. Results and discussion

To describe the toxic effects a two-step methodology was exercised: initially we verified for a statistical difference between treated and controls, subsequently the toxicity of significant different treatments was classed [26, 27]. Analysis of variance for different parameters is shown in Table 1. The evaluation of variance of the data revealed significant influence of metal stress on germination characters of *L. sativum* L. seeds. The findings gotten by *L. sativum* L. reveal that the sensibility of this plant is linked to the metal tested used and concentrations used. Arsenic was observed to have the greatest toxic effect while the lowest effect on this plant was recorded when zinc was applied.

The results obtained showed that no effect was observed on germination when the Co, Cu and Zn were used even at high levels. The germination percentage of *L. sativum* L. was clearly influenced by arsenic treatments. Augmented arsenic concentration produced a reduction in germination percentage especially higher than 10 mg L^{-1} (Table 1), indicating an inverted threshold model.

Analysis of variance for germination index showed significant differences between metal treated and untreated seeds (Data not shown). Co and Zn treatments had almost the same effect on germination index. However, augmented concentrations of arsenic and copper produced greater diminutions in germination index values compared to the control. Among these results, the concentrations that were phytotoxic (GI < 70%) to germinating seedlings under the conditions of this study were for Co ≥ 25 mg L^{-1}, for As ≥ 10 mg L^{-1}, for Cu ≥ 20 mg L^{-1} and for Zn ≥ 200 mg L^{-1}.

Table 1: Mean comparisons of germination characters of *L. sativum* L. seeds treated and untreated. Averages with the same letter in each column, control and each metal are not significantly different ($p \leq 0.05$) based on LSD test.

	Parameters*									
Metals	**[C]** (mg/L)	**GP** (%)	**GI** (%)	**GRI** (%)	**MGT** (day)	**CVG**	**RL** (cm)	**PL** (cm)	**R/P Ratio**	**VI**
Control	0	100^{a}	100.00	30.28^{ac}	15.72^{a}	53.04^{a}	4.21	4.49^{a}	0.95	420.89
$CoCl_2$	15	100^{a}	75.00^{a}	26.00^{b}	19.78^{b}	42.18^{b}	3.13	$4.68^{a\,b}$	0.68	312.89^{a}
	25	100^{a}	61.77^{ab}	27.75^{ab}	19.67^{b}	42.38^{b}	2.61^{a}	4.55^{a}	0.57^{a}	261.56^{ab}
	50	100^{a}	58.90^{b}	31.28^{c}	16.05^{a}	52.12^{a}	2.46^{a}	4.87^{b}	0.50^{b}	245.78^{bc}
	150	100^{a}	61.33^{ab}	26.83^{b}	19.72^{b}	42.42^{b}	2.56^{a}	4.35^{a}	0.60^{a}	256.22^{abc}
	250	100^{a}	60.82^{ab}	22.54	23.83	34.99	2.53^{a}	4.64^{a}	0.55^{ab}	252.89^{bc}
Na_2HAsO_4	5	100^{a}	77.30	36.58	14.39^{a}	58.49^{a}	3.22	4.10	0.78	321.78
	10	47.33	21.01	16.06	6.94	126.11	1.86	3.79	0.50	186.22
	20	12	0.57^{a}	2.97^{b}	2.39^{b}	235.75	0.12^{a}	0.70	0.18	12.44^{a}
	50	0	0.00^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{a}	0.00^{b}	0.00^{a}	0.00^{a}
	100	0	0.00^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{a}	0.00^{b}	0.00^{a}	0.00^{a}
$CuSO_4$	20	100^{a}	63.05	30.75^{a}	17.89^{a}	$47.25^{a\,b}$	2.66	$4.24^{a\,b}$	0.64	265.56
	50	100^{a}	49.94	21.72^{b}	23.00^{b}	36.33^{c}	2.10	4.46^{a}	0.47	208.67
	100	100^{a}	18.26^{a}	21.58^{b}	23.55^{b}	35.48^{c}	0.77^{a}	4.01^{b}	0.19^{a}	76.67^{a}
	250	100^{a}	19.98^{a}	21.58^{b}	23.39^{b}	35.83^{c}	0.83^{a}	3.07	0.28	83.11^{a}
	500	100^{a}	11.72^{a}	29.31^{a}	18.44^{a}	45.66^{b}	0.49	2.35	0.21^{a}	48.89^{a}
$ZnSO_4$	100	100^{a}	74.19	32.72^{a}	15.72^{a}	53.20^{a}	3.11	4.72^{ab}	0.67	311.11
	200	100^{a}	50.37^{a}	30.92^{a}	17.22^{a}	48.47^{a}	2.12^{a}	4.47^{a}	0.48^{a}	212.22^{abc}
	400	100^{a}	62.02	31.42^{a}	16.39^{a}	51.66^{a}	2.58	4.76^{b}	0.55	258.44^{ab}
	800	100^{a}	39.01	20.20^{b}	24.17^{b}	34.68^{b}	1.63	5.35	0.31	162.67^{ac}
	1000	100^{a}	50.32^{a}	20.70^{b}	26.33^{b}	31.75^{b}	2.12^{a}	$4.70^{a\,b}$	0.46^{a}	212.22^{abc}

* [C]: concentration; GP: germination percentage; GI: germination index; GRI: germination rate index; MGT: Mean germination time; CVG: Coefficient of velocity of germination; RL: radicle length; PL: plumule length; R/P Ratio: radicle length/plumule length; VI: vigor index.

The germination index was greatest at the control treatment (0 mg L^{-1}). This parameter diminished significantly with augmenting As and Cu concentration treatment. Regarding As, the germination index was zero starting from 20 mg As L^{-1}. The Zn and Co had almost no significant effect on germination index. These findings denote an inverted threshold model (Fig. 1) and are analogous in line with [28]. They revealed that increasing Cu significantly inhibited the germination of barley, rice and wheat seeds, but Zn concentration did not influence significantly seed germination. Our results showed that the greatest germination index values were revealed with cobalt followed by Zn. In addition, the relationship between germination index (GI) and each of

metal concentrations showed a negative interaction (R^2 = 0.64, 0.82, 0.89 and 0.70 for Co, As, Cu and Zn respectively).

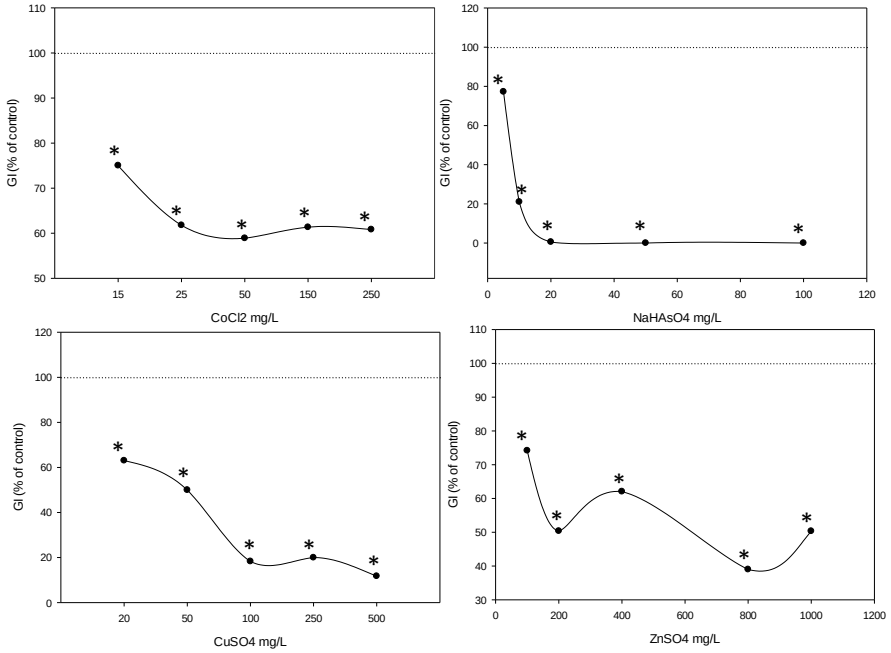

Fig. 1. Dose-response curves for effects of four heavy metals on GI of *L. sativum* L. seeds. Values are denoted as mean (n = 3). Significances from the controls are indicated as follows: *p < 0.05.

Regarding the GRI, the results are presented in the table 1. The increase of Co, Cu and Zn cause a slight effect of this parameter. However, a clear decrease of GRI was observed when 20 mg As L^{-1} was applied (2.97%). In addition, the increase of As concentration (≥ 50 mg As/L) inhibit totally the GRI (0.00%). It is worth noting that the low concentrations of As (5 mg As L^{-1}), Cu (20 mg Cu L^{-1}) and Zn (from 100 to 400 mg Zn L^{-1}) increased the GRI in comparison to the control (30.28%), displaying an inverted U-shaped curve and significant reduction was observed when the metal concentration was at higher doses (Fig. 2). The increase in GRI at low concentrations is possibly due to the hormesis effect. Hormesis phenomenon, is a dose–response relationship that is characterized by high-dose inhibition and low-dose stimulation, has been remarked before in plants developing on presence of metals [26, 27, 29-31] or other residues [32]. The phytotoxicity of different heavy metals and metalloid (As, Cr, Cd, Pb, Ni, Hg and Zn) used separately and in mixtures utilizing *L. sativum*, had a clear hormesis dose–effect relationship [26]. Moreover, [26] reported that this bio-stimulation detected at low doses could be the initial trigger of hormesis, because it could be the first adaptive response to low concentrations of one or numerous toxicants, where the first stimulation could transform into strong toxicity at longer exposure times or higher toxicant concentrations.

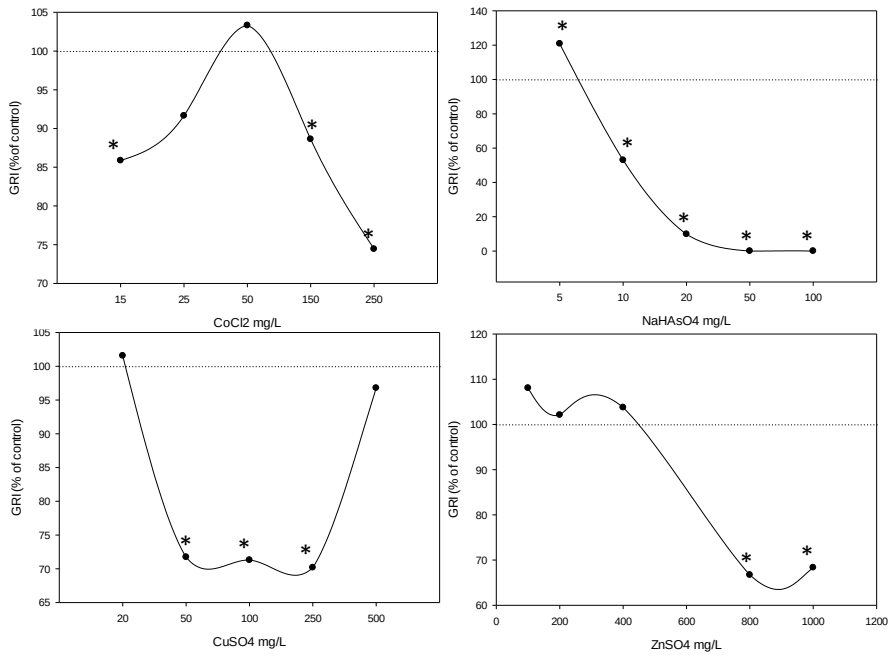

Fig. 2. Dose-response curves for effects of four heavy metals on GRI of *L. sativum* L. seeds. Values are denoted as mean (n = 3). Significances from the controls are indicated as follows: *p < 0.05.

The increase of Co, Cu and Zn levels had no effect on Coefficient of velocity of germination (CVG) of *L. sativum* with a low value comparing to the control (53.04) ranged between 31.75 and 51.66. The only remark observed regarding these three metals that the lowest concentration of Zn (100 mg Zn L^{-1}) induced a CVG almost similar to the control (53.20). These no statistically significant differences signified bio-stimulating effects (hormesis phenomenon) [26]. The results obtained for As showed a clear increase of CVG with increasing of toxicity levels with no CVG in the two highest concentrations (50 and 100 mg As L^{-1}).

The results obtained for the VI showed that Co and Zn had almost no significant effect on this parameter. However, the increase of As and Cu levels reduced significantly the VI of *L.sativum* L. The impacts of As and Cu on VI showed an inverted threshold model (Fig. 3) and As on CVG revealed an inverted U-shaped curve (Fig. 4). Meanwhile, the results of other metals on these parameters denoted no hormetic effects (Fig. 4).

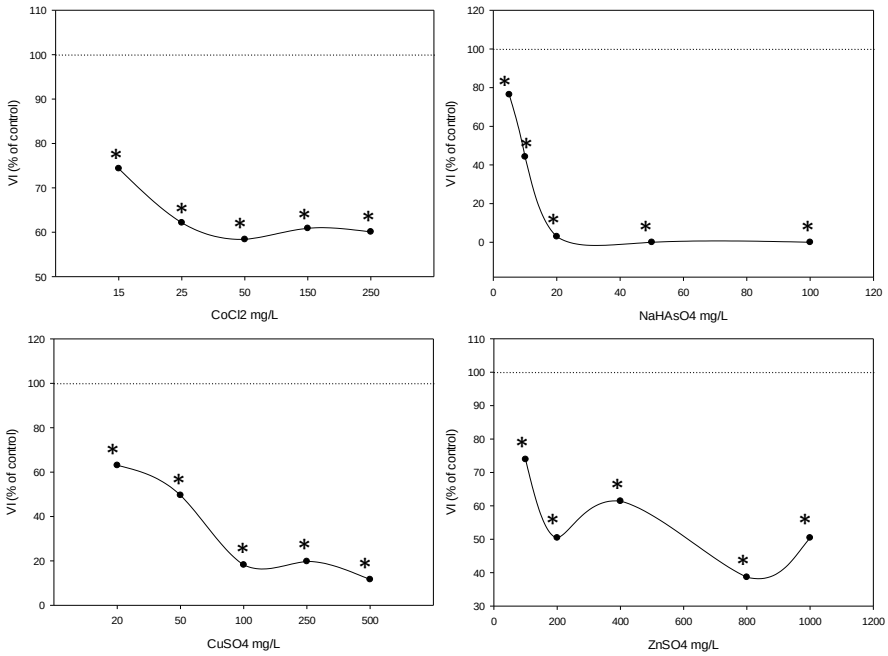

Fig. 3. Dose-response curves for effects of four heavy metals on VI of *L. sativum* L. seeds. Values are denoted as mean (n = 3). Significances from the controls are indicated as follows: *p < 0.05.

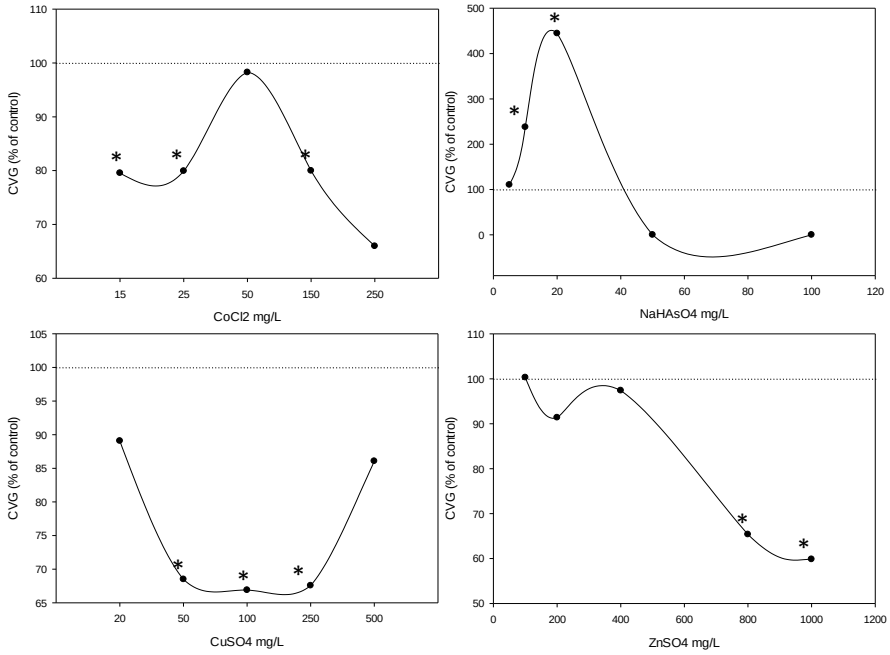

Fig. 4. Dose-response curves for effects of four heavy metals on CVG of *L. sativum* L. seeds. Values are denoted as mean (n = 3). Significances from the controls are indicated as follows: *p < 0.05.

It was obtained in this study that metal stress had a significant effect on mean germination time of garden cress seeds. Moreover, MGT considerably augmented mainly with mounting the arsenic concentration to more than 10 mg L^{-1}. The influence of metals concentrations on MGT of garden cress seed was found variable (Table 1). All the metal treatments behaved increase MGT except treatment with As behaved decrease MGT, nonetheless, untreated seeds reduced MGT (15.72 days) that was statistically similar with low concentrations of Zn, Cu and As (Table 1). Regarding these findings, it is so difficult to classify the result of metals concentrations on MGT of Garden cress seed according to the nature of the hormetic dose–response concept (Fig. 5).

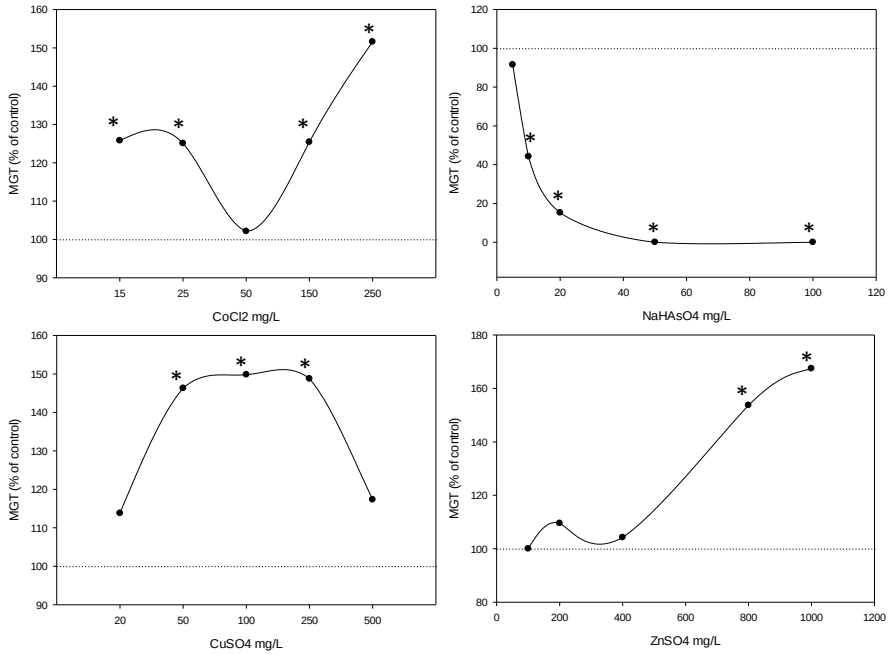

Fig. 5. Dose-response curves for effects of four heavy metals on MGT of *L. sativum* L. seeds. Values are denoted as mean (n = 3). Significances from the controls are indicated as follows: *p < 0.05.

The metal treatments used in this study affected the shoot and radicle length of Garden cress specie. Both Co and Zn induced a clear increase of the garden cress shoots, demonstrating an inverted U-shaped dose response curve (Fig. 6). However, significant inhibition was observed when As and Cu treatments were applied, comparing to the control, with the major effect was induced by As. These results showed an inverted threshold dose response model (Fig. 6). The effect of metal treatments on radicle length of *L. sativum* revealed a significant inhibition. These results showed an inverted threshold dose response model (Fig. 7).

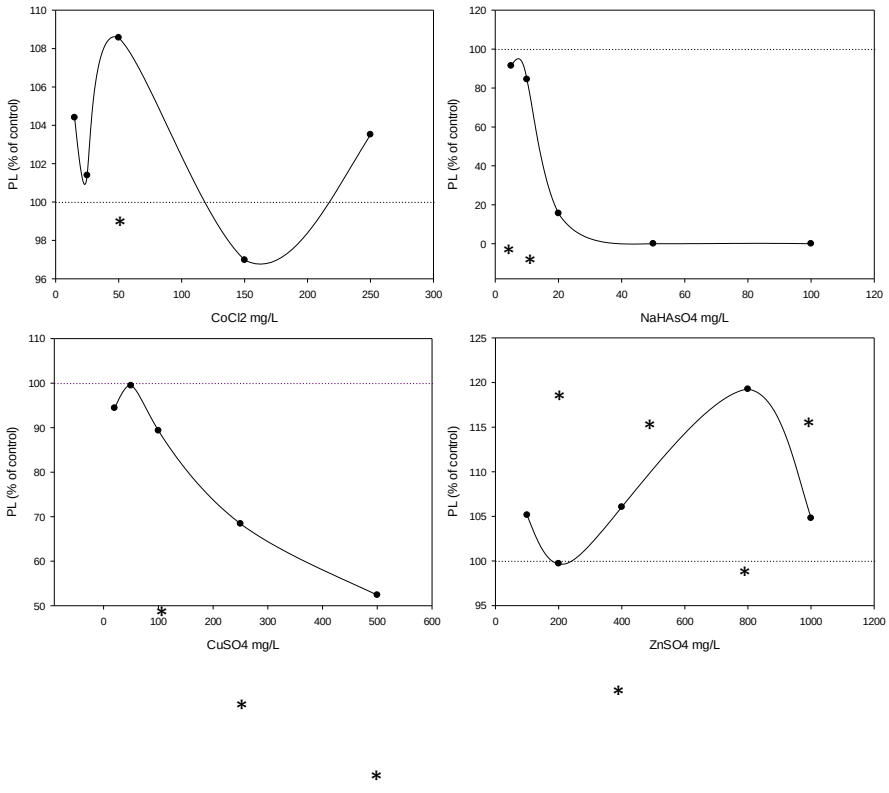

Fig. 6. Dose-response curves for effects of four heavy metals on PL of *L. sativum* L. seeds. Values are denoted as mean (n = 3). Significances from the controls are indicated as follows: *p < 0.05.

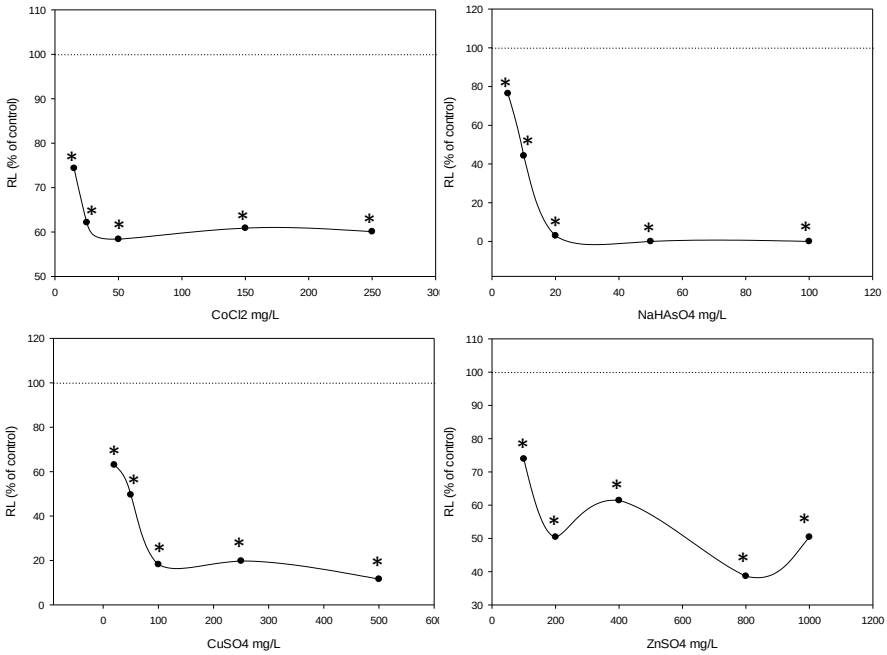

Fig. 7. Dose-response curves for effects of four heavy metals on RL of *L. sativum* L. seeds. Values are denoted as mean (n = 3). Significances from the controls are indicated as follows: *p < 0.05.

The toxicity of the tested elements on shoot and radical length can be ordered as: As > Cu > Zn > Co. Our findings are similar with [26], [28] and [33]. For *Cajanuscajan*L., seed priming with cobalt nitrate significantly increased plant height, dry matter accumulation, branch and leaf numbers, and grain yield [34]. Similarly, in peanut, seed priming with cobalt nitrate considerably augmented shelling percentage, pod yield, growth, harvest index and seed weight [34].

The variation in root development properties is possibly due to the effects of the favored accumulation of heavy metals in the developing roots and direct contact of the radical to heavy metal toxicity pursued by little mobility to the vegetation plumules [35]. In addition, an influence can be explicated as the involved roots may affect a lengthier movement of heavy metals to the shoots [35]. The variation in the L. sativum L root/shoot quotient in reaction to heavy metals, particularly As, Cu and Zn, is greatest possibly correlated to more inhibition of roots by heavy metal toxicity than plumules. Heavy metal caused modifications in the morphology and structure of the roots, may be responsible to produce a reduced root/shoot quotient of the plants [28].

The consistent inhibition of seed germination under augmenting $CuSO_4$ concentration is probably due to results of higher absorption of Cu by the seeds through imbibition and consequent toxicity [36]. The Cu-induced oxidative stress has been detected in severe diminution of enzyme activities implicated in the seed metabolic procedures allied to germination [37, 38]. A lack of consistent adverse consequences exerted by Co and Zn on seed germination is most possibly allied to interspecies differences in seed coat structures for controlling heavy metal absorption. It is reported that vegetation seeds have essential competence for selective absorption of heavy metals in nature [39].

The opposing consequences of heavy metals have apparently affected morphological and structural alterations of radicles among reticence of root hair development [40, 41] and destruct several vital cellular functions [42]. As can enter plant cells by canals of essential substances and cause negative consequences in numerous metabolic functions, causing in decreased germination and growth of certain plants [43-45]. [33] reported that As reduced radicle growing relative to concentration, the reduction being temporary at small doses (1 ppm) and permanent with greater doses. Opposing impacts of Cu on radicles are correlated to severe diminution in the growing of the longest radicle in addition to permeability of root membrane of the plants was reported [36, 46]. The toxic influences of Cu frequently altered cell division and mitotic activity of roots [3]. Moreover, Excess of Cu and Zn changed the morphology of root system, alter photosynthetic pigment and reduced dry matter yield of young grapevines [47].

Conclusion

Increasing concentration of heavy metals significantly inhibited germination rate index, germination index and vigor index. Seed germination, radicle length, plumule height and root/shoot quotient of the *L. sativum* were highly influenced by the As. The total inhibitory consequences of heavy metals determined as percent phytotoxicity were more announced on *L. sativum* L. with As and Cu. The inhibitory result on garden cress was more marked by As and Cu followed by Co and Zn. Generally, the heavy metals can be ordered of highest inhibitory influences on garden cress plants as follows: As > Cu > Co > Zn. Among these results, the concentrations that were phytotoxic to germinating seedlings under the conditions of this study were for Co ≥ 25 mg L^{-1}, for As ≥ 10 mg L^{-1}, for Cu ≥ 20 mg L^{-1} and for Zn ≥ 200 mg L^{-1}. In addition, the hermetic effect was clearly defined for GRI and PL of Co and Zn. In contrast, CVG (of Co, Cu and Zn) and MTG denote no hermetic effect. Finally, the inverted threshold dose-response model was marked, generally, the As effect on seed germination and seedling growth parameters.

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