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PHYSIOLOGICAL STUDIES ON THE HEAVY METALS RESISTANCE MECHANISMS IN TOMATO PLANTS GROWING UNDER INTERCROPPING CONDITIONS.

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ABSTRACT: Pot experiments were conducted in the greenhouse of the Faculty of Agriculture in Shebin El-Kom, Menoufia University, during 2018 and 2019 seasons, to study the cultivation of (sensitive) tomato plants in a loading system with watercress plants (resistant) in a soil contaminated with heavy metals. Three levels of lead were used, which are 0 (control), 1000 and 2000 mg / kg of soil, and three levels of cadmium metal, which are 0 (control), 100 and 300 mg / kg of soil, each alone. Use of growth inducers as nanoparticles silicon and seaweed extract and its affects on tomato or watercress plants under heavy metals stress.

The characteristics of vegetative growth and some of the physiological and chemical characteristics such as photosynthetic pigments, water relations, activity of some antioxidant enzymes, Pb and Cd accumulation and some quantitative and qualitative characteristics of the yield were studied. The soil contamination with lead and cadmium minerals at all levels led to a decrease in all studied parameters. There was an increase in the accumulation of lead and cadmium metal within watercress plants by increasing the concentrations of lead and cadmium in the contaminated soil, and there was a decrease of the heavy metals residual in the polluted soil. Spraying with silicon and seaweed extract on tomato plants increased the vegetative growth characteristics, relative water content, plant pigments, yield and its components for tomato plants. While it led to a decrease in the concentration of the enzymatic activity of peroxidase and phenoloxidase. Also, increased the efficiency of watercress plants' accumulation of heavy metals. From the above, it is cleared that the use of hyperaccumulators plants (watercress) to accumulate heavy metals led to a decrease in the residual of the two heavy metals, lead and cadmium, in the contaminated soil, and this led to a better growth conditions for tomato plants grown in the intercropping system with watercress plants in soil contaminated with lead and cadmium.

Key words: Intercropping system, Tomato, Watercress, lead, Cadmium, Si and Seaweed.

INTRODUCTION

Heavy metal contamination is one of the most serious environmental problems limiting plant productivity and threatening human health. Amongst the substances that contribute anthropogenically to pollution of the biosphere, trace elements are one of the most toxic. Lead (Pb) and cadmium (Cd) are toxic metals of increasing environmental concern as they enter the food chain in increasingly significant amounts (Luptáková *et al.*, 2002 & Verma and Dubey, 2003).

In the world, contamination of agricultural lands associated with heavy metals is widespread, and heavy metals are one of the most prevalent factors that cause public health problems, and enter the body (i.e. crops grown on soil contaminated with heavy metals). A growing body of evidence indicates that

soil organisms, critical to soil health and fertility, are sensitive to heavy metal stress (Dahlin *et al.* 1997) and that soil biodiversity is declining due to heavy metal contamination (Giller *et al.* 1998). In the future, moreover, plant extraction, which is the use of plants to extract heavy metals from contaminated soils, has emerged as a promising method for treating low to medium contamination soils (Salt *et al.* 1995). However, some ubiquitous heavy metals particularly lead; have limited plant uptake availability due to the complexity with hard soil fractions (Rieuwerts *et al.* 1998). Tomato (*Solanum lycopersicum*, family *Solanaceae*) is one of the most important vegetable crops in Egypt and other world countries for fresh consumption, industry processing and exportation.

Increasing the production and the quality of tomato fruits is very important objective to meet the higher human population demand.

Nanotechnology opens a large scope of novel application in the field of biotechnology and agricultural industries, because nanoparticles (NPs) have pore size, and particles morphology. Nanoparticles can serve as˝ magic bullets˵ containing herbicides, nano-pesticide fertilizers, or genes, which target specific cellular organelles in plant to release their content.

Nano Silicon (Si) fertilizer is known as an ecologically compatible and environmental friendly technique to stimulate plant growth. Silicon was reported to reduce the hazard effects of various abiotic and biotic stresses including salt stress, metal toxicity, drought stress, radiation damage, various pests and diseases caused by both fungi and bacteria, nutrients imbalance, high temperature and freezing (Ma, 2004; Etesamy and Jeong 2018). Silicon has emerged as an important nutrient for a range of horticultural crops i.e., cucumber, strawberry and tomato (Ma, 2004).

Nano Seaweed has been used worldwide to increase plant growth and yield. Modern agriculture is searching for new biotechnologies that would allow for a reduction in the use of chemical inputs without negatively affecting crop yield or the farmers' income. In recent years, the use of natural seaweed as fertilizer has allowed for partial substitution of conventional synthetic fertilizer (Khan et al. 2009; Zodape et al. 2010). In addition, a number of commercial seaweed extract products are available for use in agriculture and horticulture and can be used as liquid extracts applied as foliar spray, soil drench, or in granular/powder form as soil conditioners and manure (Thirumaran et al. 2009, and Hussain *et al*. 2021).

Several techniques have been using for removing heavy metal contaminants from the environmental but these techniques have limitations such as high cost, long time, logistical problems and mechanical complexity. Phytoremediation can be used as an alternative solution for heavy metal remediation process because of its advantages as a cost-effective, efficient, environment- and eco-friendly technology based on the use of metal-accumulating plants. According to previous studies, several plants have a high potential as heavy metals bioaccumulator and can be used for phytoremediation process of heavy metals.

MATERIALS AND METHODS

The current research was conducted under controlled conditions of artificial pollution with lead and cadmium (heavy metals) in the greenhouse of the Agriculture Faculty, Menoufia University during the 2018 and 2019 seasons to study the effect of the Pb and Cd

pollutants on tomato plants (sensitivity plant) which grown under intercropping system with watercress plants (hyperaccumulator plant) in soil polluted with different concentrations of the above mentioned heavy metals, and treated with foliar leaf applications as seaweeds and silicon in form of nanoparticles to investigate that, the phytoremediation (is a new method in which green plants are used to absorb or detoxify heavy metals), also study the absorption and transfer of lead and cadmium from the pollutant soil to both watercress and tomatoes, and the effect of plant sensors (foliar leaf) as seaweeds and silicon on phytoremediation method and intercropping system. Moreover, knowing the preference for cultivation on land contaminated with lead and cadmium by means of intercropping system or not.

Artificial pollution of the soil was done by adding lead salts in the form of Pb $(NO_3)_2$ at concentrations of 0, 1000 and 2000 mg (Pb) / kg soil and cadmium in the form of Cd Cl_2. $2H_2O$ at concentrations of 0, 100 and 300 mg (Cd) / kg soil (Ernst, 1996).

Foliar leaf applications as Seaweeds (0.5ml/l) nano Biostimulators contains [sea weeds 7% + salicylic acid 5% + proline 4%) was obtained from Zhengzhou Zheng Shi Chemical Co., Ltd. China.And Silicon ((Si: 2 mM), nano Silicon (SiO_2) was obtained from Bio Nano Technology Com., Egypt. Foliar leaf applications were done at three times at 30, 45and 60 days from transplanting.

The experiment treatments were designed as complete randomizes design, tow–way analysis with four replications and included 15 treatments.

The seedlings of tomato plants and the watercress seeds aforementioned were obtained from the Horticulture Research Institute Agriculture Research Center in Cairo.

Ten seeds of watercress per pot were sown at 5th February in both seasons in pots 30 cm diameter, after 15 days the watercress seedlings were thinned to five uniform seedling, and three seedlings of tomato plants were transplanting after 20 days to the same pots which planted with the watercress seeds, each pot filled with 8 kg of clay sandy soil with ratio 2:1. The physical and chemical characteristics of experimental soil are shown in Table (1) according to Page (1982).

All pots were fertilized at the recommended rates of N, K and P fertilizers according to the recommendations of agricultural ministry. After sowing, the pots were watered immediately by tap water through the experimental period whenever necessary maintain the soil water content at 65% of the water holding capacity. Weeds and best control as well as other agriculture practices were used whenever necessary.

Sampling:

Plant samples were taken at 60 days from transplanting to determine the following data:

1. Growth characters:

1.1. Root length (cm).
1.2. Plant height (cm).
1.3. Number of leaves per plant.
1.4. Roots, Stem and Leaves dry weight.
1.5. Leaf area per plant (cm^2) using the dry weight method described by A.O.A.C. (1995).

2. Water relations:

2.1. Relative water content (RWC) was determined by using the method of Barrs and Weatherley (1962).

$$RWC\ \% = \frac{F.wt. - D.wt.}{T.wt. - D.wt.} \times 100$$

Where's, F.wt. = fresh weight of leaf (g).
T.wt.= turgid (or saturated) weight (g).
D.wt. = dry weight (g).

2.2. Membrane leakage (ML) was determined as described by Sun *et al.* (2006), and calculated as: (EC1/EC2) × 100. The initial conductivity (EC1) and final electrolyte conductivity (EC2) was measured after boiling the plant samples for 15 min.

Table (1): Some physical and chemical properties of experimental soil:

Properties	Value
Physical analysis	
Sand %	34.74
Silt %	15.86
Clay %	48.96
Texture	clay sandy
Chemical analysis	
PH	7.64
O.M. %	0.68
$CaCO_3$	1.51
Ec (mmhos/cm)	1.9
Soluble ions (meq/100 g soil)	
HCO_3	0.59
Cl^-	0.47
SO_4^{-2}	0.77
Na^+	0.53
K^+	0.48
Ca^{+2} + Mg^{+2}	0.82
Total N (100)	0.26
Avail. P($Mg.g^{-1}$)	0.52
Heavy metals (mg/1000 g soil)	
Lead	0.98
Cadmium	0.09

3. Photosynthetic pigments:

According to the method described by Fadeel's, 1962 methods cited in A.O.A.C. (1995) then calculated as mg / g D.wt.

4. Determination of Antioxidant Enzymes activity:

Peroxidase and phenoloxidase activity in optical density/g (O.D./g fresh weight after 2 and 45 min), respectively were measured in the fresh leaves using the methods described by Fehrman and Dimond (1967) and Broesh (1954), respectively.

5. Heavy metals accumulation:

Accumulation of Heavy metals were determined by atomic absorption spectrophotometer (Model Perkin Elmer) and expressed as µg/g^{-1} dry weight according to Cottenie *et al*., (1982).

6. Yield and its components:

At harvest (during the last month of tomato plants age), the following data were recorded:

Fruits number per plant, fruits weight per plant (g), fruit volume (cm^3) and heavy metal concentration in fruits were

determined by using the above mentioned methods.

Vitamin C: Vitamin C content was determined in fruit juice using 2, 6-dichlorophenolindo-phenol blue dye as mg ascorbic acid per 100 ml Juice A.O.A.C. (1980), and expressed as mg/100 ml juice.

Experimental design and statistical analysis:

The experimental pots were arranged in a complete randomized block design with six replicates. Tow–way analysis of variance (ANOVA) was used to assess the significant difference in the heavy metal concentrations among different treatments, the standard statistical analysis following the proceeding described by Gomez and Gomez (1984) using the computer program of Costat Software (1985). The analyzed data then presented in tables.

RESULTS AND DISCUSSION

1. Vegetative Growth characters:

The data in Table (2) showed that, all concentrations of Pb and Cd caused a significant decrease in root length, plant height number of leaves, leaf area, of tomato plants. The concentrations of Cd were more effect on tomato than Pb concentrations. The highest value of decrease was recorded at Cd level of 300 ppm. Results presented in Table (3) showed that, dry weight of root, stem, leaves and whole plants of tomato were significantly affected by different concentration of Pb and Cd. The concentrations of Cd 100ppm were less harmful than Cd 300ppm.

From the results in Tables (2 and 3), it was observed that, the foliar application of seaweed and silicon (nanoparticles) caused a significant increase in previous growth parameters of tomato plants. The highest increases were recorded at treatment of SWE, at the first and the second seasons as compared with the untreated plants.

The interactions between heavy metals (Pb and Cd) and nanoparticles (SWE and Si) were observed in Tables (2 and 3) and citied that, all levels of Pb and caused a decrease in tomato growth parameters, but seaweed and silicon caused a recovering to a deleterious effects of heavy metals when spraying on tomato plants when compared with the control plants. These results were confirmed in the second season. These results were according to the findings by Shuyi *et al*. (2013) and who showed that, spraying silicon could promote growth of lettuce, and inhibit the absorption of Cd and Pb and decrease the content and accumulation of Cd and Pb in shoots and roots. These results are in agreement with the findings of Arun *et al*. (2014) and Hussain *et al*. (2021) who found that, the seeds treated with seaweed at different concentrations promote various parameters such as root length, shoot length and number of lateral roots of tomato plants. Sobkowiak (2016) reported that, the impact of Cd on the shoot length was not linearly related with the dose. Leaf number and leaf area had linear negative relationship with the dose of cadmium. Moreover, Cd reduced the growth of soybean in proportion to their doses. In addition, the Si is not an essential nutrient considered, but it is a beneficial element to mentian growth in stressfull environments. Facing the toxicity of Cd has also increased the resistance of various crops. In tomato, and cucumber plants, the concentration of Cd in the shoots and leaves decreased. (Zargar *et al*. 2019, Li *et al.* 2016, and Wu *et al.* 2015).

2. Water relations & Membrane integrity:

Data presented in Table (4) showed that, there was a remarkable gradual decrease in RWC in leaves of tomato plants with concentrations of Pb and Cd compared with the control which reached about 21% on RWC with 300 ppm Cd at the first season. The highest increase in MI was recorded at 300 mg/kg Cd compared with Pb concentrations and Cd, which caused a marked increase in this respect by about, 88% at the first season compared to its control.

Table (2): Effect of heavy metals, nanoparticles and their interactions on growth characters of tomato plants at 60 days from transplanting during the growing seasons of 2018 and 2019.

Characters / Treatments		Root length. (cm)		Plant height. (cm)		Leaves Number.		Leaf area. (cm^2)	
Heavy metals (ppm)	Inducers (ppm)	Season I	Season II	Season I	Season II	Season I	Season II	Season I	Season II
0	-	16.33	21.00	52.50	53.50	10.83	11.17	370.63	262.50
Pb1000	-	15.67	15.33	50.50	51.00	9.67	9.17	307.39	206.16
Pb2000	-	13.83	13.67	47.17	44.17	9.00	8.83	158.68	140.60
Cd100	-	15.17	14.17	48.83	48.33	9.17	9.00	217.32	166.00
Cd300	-	11.50	11.50	41.33	41.17	8.17	8.33	122.18	63.17
LSD at 5%		0.215	0.254	0.364	0.242	ns	ns	2.923	5.094
-	0	14.00	14.10	46.90	45.70	9.10	9.00	209.34	141.56
-	Si	14.40	14.80	48.10	47.90	9.40	9.20	221.43	169.12
-	Sw	15.10	16.50	49.20	49.30	9.60	9.70	274.94	192.37
LSD at 5%		0.124	0.117	0.248	0.179	ns	ns	0.583	0.634
0	0	16.0	19.0	51.5	51.5	10.5	10.5	319.75	230.70
	Si	16.5	20.0	52.0	52.5	11.0	10.5	327.36	249.78
	Sw	16.5	24.0	54.0	56.5	11.0	12.5	464.77	307.03
Pb1000	0	15.5	14.5	50.5	50.0	9.5	9.0	301.41	175.75
	Si	15.5	14.5	50.5	51.5	9.5	9.0	305.36	218.18
	Sw	16.0	17.0	50.5	51.5	10.0	9.5	315.40	224.54
Pb2000	0	13.0	13.5	46.0	44.0	9.0	8.5	145.56	116.61
	Si	14.0	13.5	47.5	44.0	9.0	9.0	156.48	147.73
	Sw	14.5	14.0	48.0	44.5	9.0	9.0	174.00	157.45
Cd100	0	15.0	14.0	48.0	46.0	9.0	9.0	176.50	161.67
	Si	15.0	14.0	48.0	49.0	9.0	9.0	189.67	163.73
	Sw	15.5	14.5	50.5	50.0	9.5	9.0	285.79	172.59
Cd300	0	10.5	9.5	38.5	37.0	7.5	8.0	103.50	23.06

	Si	11.0	12.0	42.5	42.5	8.5	8.5	128.28	66.20
	Sw	13.0	13.0	43.0	44.0	8.5	8.5	134.75	100.25
LSD at 5%		0.087	0.069	0.078	0.090	ns	ns	0.036	0.031

Table (3): Effect of heavy metals, nanoparticles and their interactions on dry weight of tomato plants at 60 days from transplanting during the growing seasons of 2018 and 2019.

Characters / Treatments		Dry weight (g) season I				Dry weight (g) season II			
Heavy metals (ppm)	Inducers (ppm)	Root	Stem	Leaf	Whole	Root	Stem	Leaf	Whole
0	-	2.10	1.76	4.69	8.56	2.23	1.66	4.40	8.29
Pb1000	-	1.47	1.28	3.64	6.40	1.30	1.58	3.55	6.43
Pb2000	-	0.59	1.09	2.41	4.09	0.58	0.94	2.09	3.61
Cd100	-	0.79	1.17	2.97	4.93	0.98	1.33	3.22	5.53
Cd300	-	0.52	0.90	1.88	3.30	0.39	0.71	0.94	2.05
LSD at 5%		0.069	0.079	0.526	0.784	0.147	0.062	0.256	0.698
-	0	0.94	1.16	2.82	4.92	0.87	1.20	2.48	4.55
-	Si	1.07	1.20	3.08	5.35	0.98	1.22	2.86	5.07
-	Sw	1.27	1.36	3.47	6.09	1.43	1.32	3.18	5.93
LSD at 5%		0.129	0.040	0.258	0.427	0.085	0.016	0.248	0.403
0	0	1.80	1.57	4.03	7.40	1.54	1.65	3.89	7.08
	Si	2.03	1.60	4.58	8.21	1.74	1.66	4.50	7.90
	Sw	2.47	2.12	5.47	10.06	3.41	1.67	4.80	9.88
Pb1000	0	1.29	1.24	3.42	5.95	1.13	1.55	3.44	6.12
	Si	1.45	1.24	3.67	6.36	1.29	1.59	3.52	6.40
	Sw	1.68	1.37	3.84	6.89	1.48	1.61	3.68	6.77
Pb2000	0	0.57	1.03	2.30	3.90	0.44	0.89	1.60	2.93
	Si	0.58	1.12	2.32	4.02	0.49	0.93	2.18	3.60
	Sw	0.61	1.13	2.62	4.36	0.82	1.01	2.48	4.31
Cd100	0	0.62	1.14	2.82	4.58	0.89	1.27	3.08	5.24
	Si	0.73	1.15	2.85	4.73	0.99	1.28	3.15	5.42
	Sw	1.01	1.22	3.24	5.47	1.05	1.45	3.44	5.94
Cd300	0	0.43	0.84	1.52	2.79	0.37	0.65	0.37	1.39

	Si	0.56	0.90	1.97	3.43	0.40	0.65	0.97	2.02
	Sw	0.57	0.95	2.16	3.68	0.41	0.84	1.48	2.73
LSD at 5%		0.012	0.010	0.020	0.119	0.008	0.008	0.054	0.140

Table (4): Effect of heavy metals, nanoparticles and their interactions on relative water content (RWC) and membrane integrity (MI) of tomato plants at 60 days from transplanting during the growing seasons of 2018 and 2019.

Characters		RWC (%)		MI (%)	
Heavy metals (ppm)	Inducers (ppm)	I season	II season	I season	II season
0	-	75.68	72.29	25.98	32.50
Pb1000	-	73.35	64.57	33.99	44.86
Pb2000	-	69.59	55.81	42.25	49.35
Cd100	-	72.39	61.36	38.99	48.03
Cd300	-	59.58	50.66	48.76	51.52
LSD at 5%		0.671	2.702	0.660	1.649
-	0	68.16	59.47	39.83	47.48
-	Si	70.37	61.03	38.20	45.50
-	SWE	71.82	62.32	35.96	42.78
LSD at 5%		0.438	1.146	0.876	1.493
0	0	74.45	70.09	29.15	38.58
	Si	76.03	72.41	26.84	34.93
	SWE	76.57	74.36	21.94	23.99
Pb1000	0	72.94	62.48	35.64	47.37
	Si	73.44	64.73	33.63	43.60
	SWE	73.67	66.50	32.71	43.60
Pb2000	0	68.60	54.66	43.76	49.56
	Si	68.64	55.87	42.47	49.52
	Sw	71.52	56.90	40.51	48.97
Cd100	0	72.04	60.52	39.45	48.94
	Si	72.34	61.75	39.30	47.76
	SWE	72.80	61.82	38.22	47.40

Cd300	0	52.77	49.58	51.14	52.95
	Si	61.42	50.37	48.74	51.68
	SWE	64.56	52.03	46.41	49.92
LSD at 5%		0.284	0.875	0.077	0.933

It was noticed from data presented in Table (4) that, relative water content was significantly increased with Si and SWE treatments on tomato plants as compared with the control which reached about 5% at the first season. Results in the second season are like to the first season. Meanwhile, Si and SWE extract caused a remarkable decrease in MI, the highest decrease in tomato MI was recorded with SWE treatment by about 88% at the first season as compared with the untreated plants.

As for the effect of the interaction between (Pb and Cd) and (SWE and Si) were observed in Table (4) citied that, all levels of Pb and Cd caused a decrease in relative water content and increase MI in tomato leaves, seaweed and silicon caused a recovering to a deleterious effects of heavy metals when spraying on tomato plants, grown in contaminated soil with Pb and Cd when compared with the control plants. These results were confirmed in the second season.

Similar findings have been demonstrated by Sobkowiak (2016) who mentioned that, heavy metals or toxic ions reduced root hair and had a deleterious effect on the root-absorbing area and water uptake. Moreover, metals are able to decelerate short-distance water transfer both in symplast and apoplast, which reduce the movement of water into the vascular system and affect water content, which decreased in various organs. These results are in agreement with the flinging of Khan et al. (2020) demonstrated that, nano Si application reduced the oxidative stress caused by Cd in leaves was indicated by the reduced production of hydrogen peroxide and electrolyte leakage.

3. Photosynthetic pigments:

The obtained results in Tables (5 a and b) showed that, all levels of Pb and Cd decreased the concentrations of photosynthetic pigments (i. e. chlorophyll a, b, a + b and carotenoids) in leaves of tomato plants, meanwhile the ratios of a / b and a + b / carotenoids showed an uncorrupted results at the levels of Pb an Cd.

Data illustrated that, foliar application of silicon and seaweed, had a significant increase in leaf chlorophylls and carotenoids concentrations of tomato plants. Marked effects were noticed in the ratios of chl. a / b and chl. a + b / carotenoids.

The interaction data showed that, all levels of different heavy metals caused a decrease in tomato chl. a, b, a + b and carotenoids, but silicon and seaweed caused a recovering to this decrease which caused by heavy metals when spraying on tomato plants, and grown in contaminated soil with heavy metals when compared with the untreated plants. The same results were found in the second season. These results are according to the findings by Saad-Allah *et al.* (2016) who found that, Cd stress caused a significant reduction in, photosynthetic pigments, however, presoaking in seaweed extract enhanced the photosynthetic pigments in case of control plants. Additionally, the interactive combination of cadmium stress and algal extract showed a significant amendment of cadmium stress on photosynthetic pigments. Suriyaprabha *et al.* (2012) reported that, absorption of nano silicon dioxide significant increase in organic compounds such as proteins, chlorophyell and phenols and plant dry weight.

Table (5 a): Effect of heavy metals, nanoparticles and their interactions on photosynthetic pigments in leaves of tomato plants at 60 days from transplanting during the growing season of 2018 .

Treatments / Characters		Chl.a (mg/g dwt)	Chl.b (mg /g dwt)	Caro. (mg/g dwt)	Total Chl (mg/g dwt)	Chla/b	T.Chl/Car.
Heavy metals (ppm)	Inducers (ppm)						
		Season 2018					
0	-	8.18	5.00	6.43	13.18	1.71	2.15
Pb1000	-	4.65	2.31	3.88	6.96	2.08	1.79
Pb2000	-	3.23	1.59	2.07	4.82	2.02	2.33
Cd100	-	4.13	1.74	2.98	5.87	2.37	2.00
Cd300	-	2.48	1.49	1.64	3.97	1.66	2.57
LSD at 5%		0.281	0.186	0.012	0.148	0.393	0.002
-	0	4.23	2.03	2.68	6.26	2.05	2.47
-	Si	4.55	2.35	3.37	6.90	2.02	2.07
-	SWE	4.82	2.91	4.15	7.73	1.84	1.96
LSD at 5%		0.211	0.023	1.243	0.932	0.191	0.017
0	0	7.58	3.53	4.35	11.11	2.15	2.55
	Si	8.18	4.95	6.00	13.13	1.65	2.19
	SWE	8.78	6.53	8.93	15.31	1.34	1.71
Pb1000	0	4.35	1.95	3.53	6.30	2.23	1.78
	Si	4.73	1.98	3.98	6.71	2.39	1.69
	SWE	4.88	3.00	4.13	7.88	1.63	1.91
Pb2000	0	2.85	1.58	1.98	4.43	1.80	2.24
	Si	3.38	1.60	1.99	4.98	2.11	2.50
	SWE	3.45	1.60	2.25	5.05	2.16	2.24
Cd100	0	4.05	1.65	2.48	5.70	2.45	2.30
	Si	4.05	1.75	3.00	5.80	2.31	1.93
	SWE	4.28	1.83	3.45	6.11	2.34	1.77

Cd300	0	2.33	1.43	1.08	3.76	1.63	3.48
	Si	2.40	1.47	1.88	3.87	1.63	2.06
	SWE	2.70	1.58	1.97	4.28	1.71	2.17
LSD at 5%		0.174	0.014	0.011	0.095	0.115	0.002

Table (5 b): Effect of heavy metals, nanoparticles and their interactions on photosynthetic pigments in leaves of tomato plants at 60 days from transplanting during the growing season of 2019.

		Season 2019					
0	-	5.90	2.18	3.98	8.08	2.71	2.03
Pb1000	-	4.53	1.82	3.05	6.35	2.48	2.07
Pb2000	-	2.30	1.18	2.15	3.48	1.94	1.62
Cd100	-	3.25	1.35	2.38	4.60	2.41	1.93
Cd300	-	1.45	1.08	1.45	2.53	1.34	1.82
LSD at 5%		0.135	0.325	0.821	1.524	0.045	0.027
-	0	3.11	1.46	2.42	4.56	1.99	1.87
-	Si	3.32	1.50	2.55	4.81	2.10	1.88
-	SWE	4.04	1.61	2.84	5.65	2.43	1.95
LSD at 5%		0.072	0.198	0.433	0.803	0.036	0.018
0	0	5.63	2.03	3.75	7.66	2.77	2.04
	Si	5.78	2.18	3.90	7.96	2.65	2.04
	SWE	6.30	2.33	4.28	8.63	2.70	2.02
Pb1000	0	4.05	1.73	2.93	5.78	2.34	1.97
	Si	4.13	1.75	3.08	5.88	2.36	1.91
	SWE	5.40	1.98	3.15	7.38	2.73	2.34
Pb2000	0	2.10	1.13	2.12	3.23	1.86	1.52
	Si	2.10	1.13	2.15	3.23	1.86	1.50
	SWE	2.70	1.28	2.18	3.98	2.11	1.83
Cd100	0	2.70	1.35	2.25	4.05	2.00	1.80
	Si	3.23	1.35	2.40	4.58	2.39	1.91
	SWE	3.83	1.35	2.48	5.18	2.84	2.09
Cd300	0	1.05	1.05	1.05	2.10	1.00	2.00

	Si	1.35	1.07	1.20	2.42	1.26	2.02
	SWE	1.95	1.11	2.10	3.06	1.76	1.46
LSD at 5%		0.011	0.023	0.031	0.159	0.009	0.008

4. Antioxidant Enzymes Activity:-

Results in Table (6) showed that, all concentrations of Pb and Cd significantly increased the peroxidase enzyme activity on leaves of tomato plants which recorded by 70 % with 300 ppm Cd in the first season when compared with the control. Meanwhile a great increase in the activity of phenoloxidase enzyme, this increase was more pronounced at level of Cd 300 which recorded by 83% compared with the control in the first season . The same results in the second season like the first one.

Table (6): Effect of heavy metals, nanoparticles and their interactions on antioxidant enzymes activities (OD) in leaves of tomato plants at 60 days from transplanting during the growing seasons of 2018 and 2019.

Treatments / Characters		Peroxidase (OD after 2 mint/g fw)		Phenoloxidase (OD after 45 mint/g fw)	
Heavy metals (ppm)	Inducers (ppm)	season I	season II	season I	season II
0	-	0.430	0.374	1.111	1.000
Pb1000	-	0.555	0.426	1.296	1.370
Pb2000	-	0.651	0.496	1.704	2.222
Cd100	-	0.618	0.471	1.555	2.000
Cd300	-	0.729	0.541	2.037	2.222
LSD at 5%		0.030	0.023	0.057	0.041
-	0	0.635	0.476	1.644	1.866
-	Si	0.589	0.462	1.578	1.766
-	SWE	0.566	0.447	1.400	1.655
LSD at 5%		0.021	0.013	0.611	0.038
0	0	0.478	0.389	1.222	1.222
	Si	0.411	0.367	1.222	0.944
	SWE	0.400	0.367	0.889	0.833
Pb1000	0	0.578	0.433	1.333	1.444
	Si	0.544	0.433	1.333	1.333
	SWE	0.544	0.411	1.222	1.333
Pb2000	0	0.655	0.500	1.778	2.222
	Si	0.655	0.500	1.667	2.222
	SWE	0.644	0.489	1.667	2.222
Cd100	0	0.644	0.489	1.667	2.222
	Si	0.622	0.467	1.555	2.111

	SWE	0.589	0.456	1.444	1.667
Cd300	0	0.822	0.567	2.222	2.222
	Si	0.711	0.544	2.111	2.222
	SWE	0.655	0.511	1.778	2.222
LSD at 5%		0.015	0.011	0.134	0.029

Data recorded in Table (6) cleared that, SWE and Si caused a remarkable decrease in the peroxidase enzyme activity in leaves of tomato plants. Also, it can be observed from the same table peroxidase enzyme activity in leaves was decreased under the same treatment with nanoparticles which recorded by 11% in peroxidase enzyme activity and 15% in phenoloxidase enzyme activity compared with the control plants. The second season is similar to the first one.

These results are in accordance to those recorded by Saeed *et al.* (2014) who mentioned that, application cadmium to tomato plants resulted in increasing peroxidatase activity by 72% and ascorbic acid oxidase by 12% compared to the control plants. The obtained results confirmed by those recorded by Tripathi *et al.* (2016) who said that, under Cd stress and Si application enhances the activities of antioxidant enzymes and reduces the oxidative stress by decreasing the malondialdehyde (MDA). Silicon also reduces Cd toxicity by structural alterations in plants and by regulating gene expression.

As for the effect of the interaction between (Pb and Cd) and (SWE and Si) were observed in Table (6) citied that, all levels of Pb and Cd caused an increased in oxidase enzyme activity and peroxidase enzyme activity in tomato leaves, but seaweed and silver caused a recovering to a deleterious effects of heavy metals when spraying on tomato plants, and which grown in contaminated soil with Pb and Cd when compared with the control plants. These results were confirmed in the second season. Similar findings have been demonstrated by Shuyi *et al.* (2013) showed that, spraying silicon could enhance activities of SOD and POD. Zargar et al. (2019) reported that, nano Si treatment had a stimulation of enzymatic and non-enzymatic antioxidants enzymes in rice shoots which decreased Cd accumulation. Moreover, Si supply reduces lipid peroxidation (LPO) intensity whereas increases enzymatic (superoxide dismutase, SOD, APX and glutathione reductase) and non-enzymatic (ascorbate and glutathiome) antioxidants activities in cucumber (Shi *et al.* 2005, Maksimovic *et al.* 2012 and Khan *et al.* 2020).

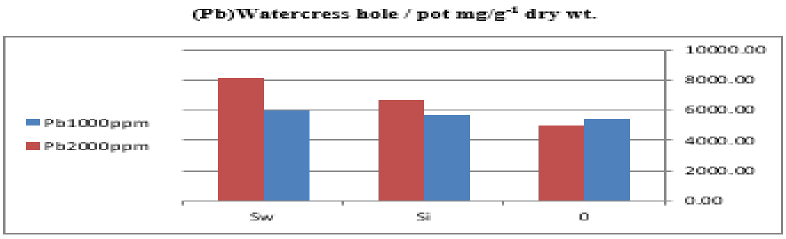
5. Accumulation of heavy metals by watercress plants grown under contaminated soil by Pb and Cd:

The obtained results in Table (7) & Fig 1 cleared that, the accumulation of lead and cadmium by watercress plants increased with increasing the concentration of Pb from 1000 to 2000ppm and Cd from 100 to 300ppm. The residual concentration of lead and cadmium in polluted soil after the harvest plants increased with increasing the level of Pb and Cd. The highest accumulation in whole plants of watercress was recorded at the Pb level of 2000ppm and Cd level of 300ppm. As well as the highest level of the soil residual of Pb and Cd were observed in the highest same levels of Pb and Cd after harvest crop, if compared with their control plants. The same trend was obtained in the second season. These results are in agreement with those obtained by Liu *et al*. (2009) on wheat plants, Fazal *et al*. (2010) on maize plants and Yongsheng *et al*. (2011) showed that, the ability of Pb accumulation of tea plant, having a positive correlation with Pb (800, 1100, 1400, 1700 and 2100 mg/kg soil) treatment concentrations, was in the sequence of root>stem>shoot. Root was the main part of tea plant to fix Pb. Yilmaz and Parlak (2011) on *Groenlandia densa* plants who found that, Cd concentrations in plants increased with increasing Cd (0-20mgL^{-1} cadmium nitrate) supply levels.

Table (7): Accumulation of heavy metals by Watercress and Tomato plants grown under contaminated soil by Pb and Cd during the growing seasons of 2018 and 2019.

Characters / Treatments	Tomato mg/g^{-1} dry wt.			Watercress whole / pot (mg/g^{-1} dry wt. heavy metals)	Soil Residual (mg / pot heavy metals)
	Root	Shoot	Fruits		
	Season I				
Control	0	0	0.00	0.48	0.00
Pb1000	84	55	0.01	5448	2408
Pb1000+si	61	40	0.00	57398	2150
Pb1000+SWE	47	31	0.00	5968	1949
Pb2000	255	168	0.02	4974	10596
Pb2000+si	175	116	0.00	6721	8982
Pb2000+SWE	147	97	0.00	8162	7571
Cd100	10	7	0.03	554	226
Cd100+si	8	5	0.00	568	219
Cd100+SWE	6	4	0.00	582	206
Cd300	48	31	0.05	788	1532
Cd300+si	33	22	0.00	876	1467
Cd300+SWE	28	18	0.00	1141	1210
	Season II				
Control	0	0	0.00	0.32	0.00
Pb1000	94	62	0.02	5172	2668
Pb1000+si	68	45	0.00	5494	2384
Pb1000+SWE	53	35	0.00	5747	2161
Pb2000	285	188	0.02	3772	11748
Pb2000+si	196	129	0.00	5705	9964
Pb2000+SWE	164	108	0.00	7308	8399
Cd100	12	8	0.03	528	251

Cd100+si	9	7	0.00	543	242
Cd100+SWE	7	4	0.00	559	228
Cd300	53	35	0.04	613	1697
Cd300+si	37	24	0.00	710	1627
Cd300+SWE	31	20	0.00	1004	1342



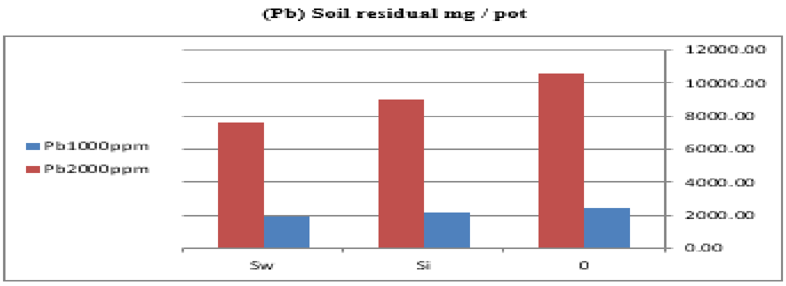


Fig 1: Effect of heavy metals, nanoparticles and on accumulation of Pb in Eruca plants and soil during the growing season of 2019.

Data in the Table (7) showed that, the ability of Pb and Cd accumulation in watercress plants, having a positive effect with seaweed and silicon treatments, as compared with the untreated plants. Meanwhile, the residual concentration of Pb and Cd in soil after the harvest crop decreased with applications of SWE and Si. The higher value of accumulation of Pb and Cd was observed at seaweed treatment. Similar findings have been demonstrated by Shuyi *et al.* (2013) and Dong *et al.* (2019) who found that, *Pennisetum glaucum* and *Pennisetum glaucum* - P. *purpureum* were used to explore the impacts of Si application on alleviating Cd toxicity and its possible mechanism. Treatments consist of four levels of Cd (0, 10, 50, and 100 mg•kg−1) with or without 2.0 mM Si amendments. Under Cd stress, Si application significantly increased Si content and reduced Cd content.

Accumulation of heavy metals by tomato plants grown under contaminated soil by Pb and Cd:

The results in Table (7) cited that, the concentrations of lead and cadmium in root and shoot tomato plants increased with increasing the concentration levels of Pb and Cd. The highest concentration of heavy metals in tomato plants was recorded at the Pb level of 2000ppm and

Cd level of 300ppm. The concentrations of Pb and Cd in tomato roots were a higher than the concentrations in tomato shoots, but in tomato fruits were had a very low concentrate, if compared with their control plants. The same results were recorded in the second season. These results are in agreement with those obtained Kisa (2018) who pointed that, accumulation of metal ions in plants causes the formation of free radicals and stimulates the antioxidative defense systems.

Data in the Table (7) observed that, the silicon and seaweed foliar leaf applications caused a decrease in heavy metal concentration in roots and shoots of tomato plants which grown in polluted soil. The seaweed extract recorded a higher decrease in heavy metals content in tomato tissues, as compared with the untreated plants. The results in season one and two are the same. Our results are accordance with the findings by Goyal *et al*. (2017) who fond that, silica application can reduced toxic effects of heavy metal on *V. radiata* and improved its quality in terms of sugar and protein content.

Lead may enter the roots through several pathways, and a particular pathway is through ionic channels. Although, lead uptake is a non-selective phenomenon, it nonetheless depends on the functioning of an H^+/ATPase pump to maintain a strong negative membrane potential in rhizoderm cells (Hirsch *et al*. 1998). Several authors have demonstrated that Ca^{2+}-permeable channels are the main pathway by which lead enters roots (Wang *et al*. 2007).

6. Yield and its components:

Data presented in Tables (8a and 8b) showed that, fruit length, fruit around, fruit volume, fruits weight per plant, fruit number per plant and vitamin C in tomato fruits were decreased at all treatments of Pb and Cd. The highest value of reduction was obtained at level of Cd 300 by about 52, 39, 63, 78 and 53%, respectively. The same results were obtained in the second season.

From The obtained results in Tables (8a and 8b) it can be said that, SI and SW levels significantly increased the fruit length, fruit around, fruit volume, fruits weight per plant, fruit number per plant and vitamin C in tomato fruit similar to those obtained in the first one.

The interactions between heavy metals and nanoparticles were illustrated in Tables (8a and 8b) and found that, all levels of Pb and Cd caused a decrease in tomato fruit length, fruit around, fruit volume, fruits weight per plant, fruit number per plant and vitamin C in tomato fruits, but silicon and seaweed caused a recovering to deleterious effects which caused by heavy metals when treated plants of tomato, when compared with the untreated plants. The same result was observed in the 2^{nd} season.

The treatment of SW recorded a higher increase in these measures by about 12, 8, 15 and 20%. The results in the second season w

Residual heavy metals (Cd, Co, Ni and Pb) were examined in the harvested fruits. The obtained results were compared with those of tomato fruits cultivated in non-contaminated soil. Results demonstrate that soil contamination with heavy metals have a negative effect on tomato fruits characteristics. These results are in agreement with those recorded by Patel *et al.* (2020) observed that, papaya plants were sprayed with different concentrations of silicon (potassium silicate and ortho silicic acid at 0.2 and 0.4 %) and seaweed extract (2 and 4 %) either alone or in combinations at 3, 4, 5 and 6 months after planting. Application of potassium silicate at 0.4% + seaweed extract at 4% proved most effective in

reducing physiological loss in weight and increasing shelf life with improved fruit firmness in papaya cv. Red Lady. Sensory parameters i.e. color, texture, flavor, taste, general appearance and overall acceptability were significantly better under foliar application of ortho silicic acid 0.2% + seaweed extract 2%.

It can be observed that, all lead and cadmium treatments inhibited plant growth, water relations and chemical constituents in tomato plants. On the other hand the treated with heavy metals (Pb and Cd) levels increased their concentration in roots and shoots of tomato led to negative effect on yield and its components. As well as plant pigments, TSS and minerals were decreased under contaminate conditions, thus the plant growth as well as the yield decreased. Similar results were obtained by Hashem *et al.* (2017) who mentioned that, on tomato plants. El-Gamal and Hammad (2003) too reported that, lead (250 and 500 mg/l) and cadmium (100 and 200 mg/l) negatively affected on fruit yield and fruit quality of tomato plants. Increase in heavy metal (Ni, Cd, Cu, Cr, Pb and Zn) concentration in foliage of plants grown in sewage sludge-amended soil caused unfavorable changes in physiological and biochemical characteristics of plants leading to reductions in yield (Singh and Agrawal, 2007).

Table (8a): Effect of heavy metals, nanoparticles and their interactions on yield and its components of tomato plants during the growing season of 2018.

Treatments \ Characters		Fruit length (cm)	Fruit around (cm)	Fruit volume (cm^3)	Fruits weight / plant (gm)	Fruit N. / plant	Vitamin C. (mg/100g f.wt. fruits)
Heavy metals (ppm)	Inducers (ppm)	season I					
0	-	4.55	3.94	12.37	430.82	14.99	11.98
Pb1000	-	4.22	3.56	10.17	340.84	12.94	11.06
Pb2000	-	3.03	2.96	7.14	227.93	10.35	9.69
Cd100	-	3.72	3.16	8.06	273.53	11.75	10.24
Cd300	-	2.18	2.39	4.63	95.41	7.08	5.37
LSD at 5%		0.640	0.186	0.853	42.301	1.299	0.052
-	0	3.32	3.10	7.95	242.98	10.42	8.98
-	Si	3.57	3.16	8.25	263.46	11.33	9.36
-	Sw	3.73	3.35	9.21	314.69	12.51	10.66
LSD at 5%		0.148	0.056	0.278	18.998	0.844	0.034
0	0	4.38	3.82	11.97	379.11	13.83	11.15
	Si	4.58	4.00	12.57	417.76	14.35	11.77
	Sw	4.70	4.00	12.57	495.60	16.80	13.02
Pb1000	0	4.10	3.50	9.63	333.36	12.10	11.37
	Si	4.25	3.50	9.63	344.49	13.08	11.31
	Sw	4.30	3.67	11.26	344.67	13.63	10.49
Pb2000	0	2.98	2.93	6.95	202.66	9.82	8.94
	Si	3.00	2.96	7.19	225.06	10.23	9.63
	Sw	3.12	3.00	7.28	256.07	10.99	10.50
Cd100	0	3.63	3.03	7.42	258.94	11.47	10.13

	Si	3.73	3.12	7.89	265.44	11.83	9.99
	Sw	3.80	3.33	8.86	296.21	11.95	10.60
Cd300	0	1.50	2.20	3.80	40.81	4.88	3.30
	Si	2.30	2.23	3.99	64.53	7.15	4.12
	Sw	2.75	2.75	6.09	180.90	9.20	8.70
LSD at 5%		0.012	0.028	0.053	0.167	0.111	0.017

Table (8b): Effect of heavy metals, nanoparticles and their interactions on yield and its components of tomato plants during the growing season of 2019.

Treatments / Characters		Fruit length (cm)	Fruit around (cm)	Fruit volume (cm^3)	Fruits weight / plant (gm)	Fruit N. / plant	Vitamin C. (mg/100g f.wt. fruits)
Heavy metals (ppm)	Inducers (ppm)	Season II					
0	-	4.44	5.07	11.37	386.05	18.11	9.88
Pb1000	-	3.93	4.73	9.32	296.58	16.61	8.58
Pb2000	-	2.95	3.83	6.53	200.02	13.88	7.34
Cd100	-	3.55	4.15	7.68	231.72	15.53	7.50
Cd300	-	1.93	2.87	4.23	87.25	9.60	4.27
LSD at 5%		0.532	0.154	0.709	35.160	1.079	0.047
-	0	3.17	3.97	7.35	212.66	13.54	6.94
-	Si	3.40	4.06	7.64	230.95	14.60	7.26
-	Sw	3.51	4.37	8.49	277.36	16.10	8.33
LSD at 5%		0.123	0.046	0.231	15.791	0.702	0.026
0	0	4.28	4.94	11.06	339.20	16.77	9.16
	Si	4.64	5.13	11.29	382.44	17.18	10.00
	Sw	4.40	5.13	11.77	436.50	20.37	10.48
Pb1000	0	3.75	4.69	8.89	283.46	15.91	8.53
	Si	3.92	4.66	8.88	299.56	16.91	8.72
	Sw	4.12	4.84	10.20	306.72	17.00	8.48
Pb2000	0	2.86	3.71	6.33	181.29	13.34	6.91
	Si	2.96	3.74	6.72	195.05	13.64	7.21
	Sw	3.04	4.05	6.53	223.71	14.67	7.91
Cd100	0	3.68	3.98	6.95	222.83	15.32	7.45
	Si	3.50	4.12	7.60	217.33	15.85	6.99
	Sw	3.46	4.34	8.48	254.99	15.41	8.05
Cd300	0	1.27	2.52	3.50	36.51	6.37	2.67
	Si	2.00	2.63	3.73	60.37	9.40	3.40
	Sw	2.52	3.47	5.47	164.87	13.03	6.73
LSD at 5%		0.018	0.023	0.069	0.139	0.093	0.021

Conclusion

It can be concluded from the present work that, lead and cadmium treatment, inhibited tomato plants growth, water relations and chemical Moreover, it was found that with increasing heavy metals concentrations, there was an increase in their concentrations in roots and shoots which reflected a negative effect on yield and its components, also increased antioxidants enzymes. The more interested obtained results that growing Eruca plants (hyperaccumulator) with tomato plants, resulted in highest accumulation of heavy metals in whole plants of watercress especially with increasing the level of heavy metal treatments. In addition, the ability of lead and cadmium accumulation in watercress plants having a positive correlation with seaweed and silicon application. The most effective treatment was seaweed which recorded a higher value of Pb and Cd accumulation. It can prove that silicon and seaweed treatments as foliar application on tomato plants enhanced plant tolerance to heavy metal toxicity. In addition, it is proved from this study that, the beneficial effect of growing hyperaccumulator plant like watercress with economic plants like tomato plants in form of intercropping system to prevent and protect tomato yield from the deleterious effects of heavy metals contamination with application silicon or seaweed in form of nanoparticls as foliar application to improve tomato tolerance to heavy metals and increasing watercress accumulation of heavy metal and reduce heavy metal uptake by tomato.

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دراسات فسيولوجية على آليات مقاومة المعادن الثقيلة في نمو نباتات الطماطم تحت ظروف التحميل

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الملخص العربى

أجريت تجارب أصص في صوبة كلية الزراعة بشبين الكوم جامعة المنوفية خلال موسمي 2018 و 2019 لدراسة زراعة نباتات طماطم (حساسة) بنظام التحميل مع نباتات الجرجير (المقاوم) في التربة الملوثة بالمعادن الثقيلة. معالجة التربة الملوثة بالمعادن الثقيلة عن طريق زراعة نباتات شرهة الامتصاص لهذه المعادن ، وهو الجرجير. دراسة معدل إمتصاص ونقل الرصاص والكادميوم من التربة إلى نباتات الجرجير بالملجم / كغم من التربة. دراسة تأثير تلك العناصر الثقيلة على النمو والعلاقات المائية والمحتوى الكيميائي وكذلك المحصول ومكوناته لنباتات الطماطم. تم إستخدام ثلاثة مستويات من الرصاص وهي صفر (كنترول) و 1000 و 2000 ملجم / كجم من التربة وثلاثة مستويات من الكادميوم وهي صفر (كنترول) و 100 و 300 ملجم / كجم من التربة كل منها على حدة. مع إستخدام محفزات النمو مثل جزيئات السيليكون النانوية وخلاصة الأعشاب البحرية وتأثيرها على نباتات الطماطم أو الجرجير تحت إجهاد المعادن الثقيلة.

تمت دراسة خصائص النمو الخضري وبعض الخصائص الفسيولوجية والكيميائية مثل أصباغ التمثيل الضوئي والعلاقات المائية ونشاط بعض الإنزيمات المضادة للأكسدة وتراكم الرصاص والكادميوم وبعض الخصائص الكمية والنوعية للمحصول. أدى تلوث التربة بمعدن الرصاص والكادميوم على جميع المستويات إلى انخفاض في جميع القياسات المدروسة ، وحدث زيادة في تراكم معدن الرصاص والكادميوم داخل نباتات الجرجير عن طريق زيادة تركيز الرصاص والكادميوم في التربة الملوثة. كما انخفض تركيز المعادن الثقيلة المتبقية في التربة الملوثة. أدى رش نباتات الطماطم بالسيليكون ومستخلص الأعشاب البحرية إلى زيادة خصائص النمو الخضري والمحتوى المائي النسبي والأصباغ النباتية والمحصول ومكوناتها لنباتات الطماطم. بينما أدى إلى انخفاض في تركيز النشاط الأنزيمي للبيروكسيديز والفينول اوكسيديز. كما زادت كفاءة نباتات الجرجير في تراكم المعادن الثقيلة ، ومما سبق يتضح أن استخدام نباتات شرهة الإمتصاص للمعادن الثقيلة (الجرجير) أدى إلى انخفاض المتبقي في التربة من المعادن الثقيلة الرصاص والكادميوم في التربة الملوثة بهما ، مما أدى إلى ظروف نمو أفضل لنباتات الطماطم المزروعة بنظام التحميل مع نباتات الجرجير في التربة الملوثة بالرصاص والكادميوم.

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