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Physiological Studies on the Influence of Silicon in the Alleviation the Toxic Effects of Heavy Metals in Sunflower Plant

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Abstract

The present study was conducted at the Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt during the growth period, from April to August (2021) to evaluate the efficiency of silicon application in improving some physiological characteristics including the photosynthetic pigments, total carbohydrate, proline and total soluble protein contents in shoots of sunflower plants grown under Pb (200 and 400 ppm) and Cd (20 and 40 ppm). Furthermore, the oil productivity and the accumulation of heavy metals in the seeds of sunflower plants were detected. In general, Pb and Cd resulted in reduced photosynthetic pigments and total soluble proteins content and this reduction was increased with increasing heavy metal concentrations in the soil. On the other hand, heavy metals application leads to a significant increment in total carbohydrate and proline contents. Data also showed a negative and depressive effect of heavy metals on the oil productivity of the seeds. Accumulation of Pb and Cd had a pronounced increase in seeds by increasing the Pb and Cd concentrations in the growth media. Foliar application of potassium silicate (100 and 200 ppm) on plants grown under normal condition or exposed to heavy metals stress observed a significant increment in all measured physiological characters. Moreover, the application of silica decreased Pb and Cd contents in seeds. It could be concluded that silicon application especially the high concentration (200 ppm) was more effective to ameliorate and mitigate the depressive effect of heavy metals contamination and caused significant increments in the oil productivity of sunflower seeds.

Keywords: Cadmium, Heavy metals, Lead, Oil content, Silicon, Sunflower

1. Introduction

Most of the heavy metals, including nickel (Ni), copper (Cd), zinc (Zn), iron (Fe), zinc (Zn), cobalt (Co), arsenic (As), chromium (Cr), silver (Ag), and platinum (Pt), do not have a vital function in plants. These heavy metals are naturally occurring in the soil, however, they may accumulate in cropland soils through several different processes, including as wastewater, sludge, solid waste and chemical inputs [1]. Their contents inhibit photosynthesis, alter nutritional balances, disturb the water balance, and marginalize the biological functions of proteins, lipids, and essential components of thylakoid membranes, all of which contribute to the retardation of plant growth [2]. Furthermore, because heavy metals can transport to both humans and animals through the food chain, their existence represents a serious risk to human health [3].

Among strategies that mitigate the toxicity of heavy metals in agricultural production, silicon (Si) is frequently reported for its ability to decrease their negative effects [4]. Biologically, Si serves a number of purposes in plants, including morphological changes, improved photosynthetic capacity through an increase in grana count, chloroplast size, and chlorophyll contents, all of which lead to an increase in dry matter [5]. Moreover, Si creates a 'cuticle-

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double Si layer' in the leaves that minimizes water transpiration and protects against insect and disease attack [6]. Si generally reduced the uptake of heavy metals by chelation or arresting the heavy metals in the soil, which reduced the heavy metals' bioavailability and may have limited their movement from roots to shoots [7].

Sunflowers (*Helianthus annuus* L., Asteraceae) are the most important crop and decorative plant. It is the second-most important crop that produces edible oil after soybeans and is used as animal feed [8]. Although sunflower is usually regarded as a highly tolerant crops, that can withstand high levels of heavy metals in the soil, however growth impairment during the early phases of plant development may result in a poor crop establishment [9]. Previous research has shown that abiotic stresses such as salt, UV-B rays and metals alter the antioxidant defense system and produce oxidative damage in sunflower plants [10].

The objective of the present study is to investigate the role of silicon for increasing the tolerance of sunflower plant grown under soil polluted with heavy metals concentration (Pb and Cd). This is demonstrated by determining photosynthetic pigments, carbohydrates, total soluble proteins and proline contents during two stages of the growth period as well as detection of oil productivity and accumulation of heavy metals in harvested seeds.

2. Materials and methods

A pot experiment was carried out at a fenced area at Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt during the growth period (2021), from April to August (30−38 °C) to investigate the effect of heavy metals application, silicon treatments as well as their interaction treatments on some physiological characters of sunflower plants and oil productivity as well as the accumulation of Pb and Cd in harvested seeds.

Planting was done in porcelain pots (37 cm diameter and 39 cm in height), each pot was filled with 20 kg of sandy clay soil.

The soil were fertilized with NPK at the rate of 80 kgN, 30 kg P_2O_5 and 30 kg K_2O/feddan, which was added in the form of ammonium nitrate (33.5%), superphosphate (15.5%) and potassium sulphate (20.5%), respectively. The dose of the fertilizer was adjusted to the area of the pots.

The physical and chemical properties of the soil are presented in Tables 1 and 2.

The used seeds of sunflower plants (*Helianthus annus* L.) cultivar (Sakha 53) were obtained from Crop Field Institute, Agricultural Research Center.

Twenty seeds of sunflower were sown at 11th of April 2021 at equal distance from the soil surface. The different treatments, heavy metal, Si and the combinations of them were arranged in a complete randomized block designed in five replicates. The pots were irrigated with tap water till complete germination (10 days) then the plants were thinned to identical 10 plants/pot. Both treated as well as untreated (control) plants were irrigated with tap water to keep the soil at the level of 70% of field capacity.

Lead was added separately as lead acetate solution at the rate of 200 and 400 mg/kg soil and cadmium was added separately as cadmium sulphate solution at a rate of 20 and 40 mg/kg soil, in addition to the control (without adding heavy metals). The tested heavy metals treatments were added to the soil after 24 and 54 days from sowing.

Silicon was added as a foliar spray to the plants with water containing the concentrations of potassium silicate as following:

(a) Control (spraying with H_2O).
(b) Potassium silicate at the rate (100 ppm solution).
(c) Potassium silicate at the rate (200 ppm solution).

The first application was carried out after 25 days from sowing and the second application was carried out after 55 days from sowing.

For each heavy metal concentration, the plants were subjected to the effect of potassium silicate as (foliar application) at [100 and 200 ppm] during the

Table 1. Physical properties of the used soil.

Particles size %							Texture Class
Gravels	Fine gravels	Coarse Sand	Medium Sand	Fine Sand	Silt	Clay	
1.3	3.57	5.82	66.84	24.11	2.34	18.7	Sandy-clay soil

Table 2. Chemical properties of used soil.

PH	E.C. Mm hos/cm	Soil saturation extract							
		Cations meq/l				Anion meq/L		Heavy metals ion conc. (μg/g)	
		Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	$So4^-$	Pb	Cd
7.82	2.6	10.35	2.51	14.63	5.20	7.18	1.84	16.74	5.86

two growth stages to study its ability to ameliorate the adverse effect of heavy metal conditions.

Two samples were taken which represent the vegetative and the bud formation stages, after 35 and 65 days, respectively, from sowing. The plants were harvested after 95 days and the harvested seeds were collected. For each taken stage and seeds, some biochemical constituents were studied as follows:

2.1. Photosynthetic pigments

The photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) were calorimetrically determined at two stages 35 days (vegetative stage) and 65 days (bud formation stage) on fresh weight of plants. The concentration of pigment fractions (chlorophyll a, chlorophyll b, and carotenoids) was measured as mg/g fresh weight of plant material [11].

2.2. Total carbohydrates content

Total carbohydrates content in shoots of sunflower plants were determined and estimated colorimetrically by phenol-sulphuric acid method. A standard curve was prepared using a known concentration of glucose. Blank were prepared by using 1 ml of water instead of a plant sample. Total carbohydrate content were expressed as g/100 g dry weight [12,13].

2.3. Determination of proline content

Proline content was determined in shoots of plants and the proline concentration was determined from a standard curve and calculated on a dry weight basis as (mg/g dry wt) [14].

2.4. Determination of total soluble proteins

Total soluble protein content were determined in shoots of sunflower plants by using serum albumin as a standard protein. The concentration of soluble proteins was calculated from the standard curve and expressed as g/100 g dry weight [15].

2.5. Determination of cadmium and lead contents

Concentrations of cadmium and lead were determined in seeds and expressed as μg/g dry weight [16].

2.6. Determination of oil content

A known weight of sunflower seeds was ground in a mortar and then transferred quantitatively to a fat-free extraction thimble. The mortar was washed several times with petroleum ether (40−60 °C) and the washings were added to the extraction thimble. Extraction with petroleum ether in a soxhlet apparatus was continued for 18 h. This time was found to be satisfactory for complete extraction of oils. The extract was then quantitatively transferred to a weighted flask and the solvent was evaporated using an electric fan. The last traces of solvent and moisture were removed by heating the flask at 100 °C under reduced pressure. The flask was then allowed to cool in a desiccator and reweighing to the nearest mg. The increase in weight was equivalent to the weight of oil in the sample and the result was expressed as (% of oil) [17].

2.7. Statistical analysis

Data were statistically analyzed according to the procedure outlined by Steel and Torrie (1984) [18]. The means of the treatments were compared using least significant difference (LSD) at a 5% level of probability.

3. Results and discussions

3.1. Photosynthetic pigments

Results presented in Table 3 show the effect of heavy metal concentrations on photosynthetic pigments including chlorophyll a, chlorophyll b, and carotenoids in leaves of sunflower plants during the two stages of growth; 35 days (vegetative) and 65 days (bud formation) after sowing. There was a consistent and gradual reduction in chlorophyll a, chlorophyll b, and carotenoids contents as the concentrations of heavy metals increased in the tested soil, especially the high levels (400 ppm Pb and 40 ppm Cd). Exposure of sunflower plants to high concentration of Pb(400 ppm) decreased chlorophyll a, chlorophyll b and carotenoids contents by 50.23, 46.41, and 20.51%, respectively, at the vegetative stage compared with control plants. In addition, the high concentration of Cd (40 ppm) decreased chlorophyll a, chlorophyll b, and carotenoids contents by 75.73, 79.11, and 69.87%, respectively, at the vegetative stage compared with control plants. The same manner was found during the bud formation stage of sunflower plants. The high levels of Pb and Cd in tobacco plants may inhibit the essential enzymes for chlorophyll synthesis, which could

Table 3. Effect of heavy metals, potassium silicate applications and the interaction between them on photosynthetic pigments (mg/g fresh wt) in leaves of sunflower plants during the two studied stages (vegetative and bud formation stages).

Parameters	Chlorophyll a		Chlorophyll b		Carotenoids	
Stages Treatments	Vegetative Stage	bud formation stage	Vegetative Stage	Bud formation stage	Vegetative stage	Bud formation stage
Control	6.43	8.53	4.74	6.73	1.56	1.70
Pb200	5.32	7.62	3.41	4.63	1.43	1.52
Pb400	3.20	6.23	2.54	3.55	1.24	1.38
Cd20	2.36	5.74	1.41	2.41	1.12	1.26
Cd40	1.56	5.01	0.99	1.69	0.47	0.56
LSD at 5%	0.90	0.71	0.95	1.1	0.12	0.14
Control	6.50	8.84	4.62	6.52	1.63	1.82
S100	12.51	16.36	8.60	12.36	3.26	3.64
S200	14.34	18.08	10.55	14.41	5.14	5.56
LSD at 5%	0.51	0.43	0.70	0.73	0.63	0.68
Pb200 + s100	6.43	9.68	5.10	6.52	2.05	2.42
Pb200 + s200	7.95	10.46	6.21	7.69	2.63	3.32
Pb400 + s100	4.55	7.30	4.60	5.69	1.86	2.28
Pb400 + s200	6.14	8.84	5.49	6.61	2.44	3.18
Cd20 + s100	4.11	6.85	3.47	5.13	1.74	2.16
Cd20 + s200	5.24	7.48	4.53	5.90	2.32	3.06
Cd40 + s100	1.76	6.07	1.70	3.62	0.94	1.12
Cd40 + s200	2.91	6.88	2.16	4.55	1.09	1.57
LSD at 5%	1.0	0.9	0.62	0.69	0.39	0.42

Pb200 = 200 ppm lead acetate Pb400 = 400 ppm lead acetate.
Cd20 = 20 ppm cadmium sulphateCd40 = 40 ppm cadmium sulphate.
S100 = 100 ppm potassium silicate S200 = 200 ppm potassium silicate.

decrease the rate of photosynthetic activity and contribute to the decline in chlorophyll concentrations. The capacity of tobacco plants for photosynthetic processes may be affected by the toxicity of Cd and Pb through inhibiting electron transport [4]. The synthesis and accumulation of photosynthetic pigments were significantly decreased resulting from Pb and Cd applications in squash plants. The reduction may be due to an increase the osmotic stress, reduced leaf stomatal conductance, transpiration, and relative water content (RWC). Plant photosynthetic processes are adversely affected by lead (Pb) because it degrades the chloroplast ultrastructure and prevents the synthesis of important pigments such as plastoquinones, carotenoids, and chlorophyll. By sealing stomatal pores, it also reduces CO_2 and interferes with the electron transport chain and the Calvin cycle [2].

Concerning the effect of silicon applications on photosynthetic pigments, results show a significant increase in chlorophyll a, chlorophyll b, and carotenoids in leaves of sunflower plants was detected during the two stages of growth compared with control. Table 3 showed that the maximum values of chlorophyll a, chlorophyll b and carotenoids contents were scored for plants treated with the high concentration of silicon at the two studied stages compared with the low concentration of silicon during the growth period. Potassium silicate at 200 ppm increased chlorophyll a, chlorophyll b, and carotenoids content by 120.61, 128.35, and 215.33%, respectively at the vegetative stage relative to control plants. The same results were observed for the bud formation stage. In this connection, it was reported that silicon stabilizes proteins and membranes that protect internal organelle components and preserve chloroplast ultrastructure, it increases the pigments content in wheat plants by reducing the accumulation of hazardous ions and reactive oxygen species (ROS) [19]. Application of Si leads to increase Si content in cells wall of tobacco plants which may facilitate the absorption of light energy. Such conditions promote a greater absorption of CO_2, inhibit excessive transpiration, and facilitate light interception—all of which raise photosynthetic rates and chlorophyll contents [4].

The addition of 100 and 200 ppm silicon caused an increment in chlorophyll a, chlorophyll b, and carotenoids contents for plants grown under heavy metal concentrations. Moreover, the high level of silicon seemed to be more effective for improving photosynthetic pigments for plants grown under metal stress conditions than the low one. The increment percentage of chlorophyll a, chlorophyll b, and carotenoids contents for plants grown under high concentration of Pb(400 ppm) and treated with high concentrations of Si (200 ppm) were 91.87,116.14, and 96.77%, respectively, at the vegetative stage compared with control plants. Moreover, plants grown under Cd at 40 ppm and treated with high concentration of Si (200 ppm) showed an increment in chlorophyll a, chlorophyll b, and carotenoids contents represented by 86.53, 118.18, and 131.91%, respectively, at the same stage compared with control plants. The same trend of these results was observed at the bud formation stage. The exogenous application of Si greatly increased plant biomass, leaf area, and nutritional status of pigeonpea genotypes grown under Pb and Cd stress. Si may have positive benefits directly through the adsorption or chelation of heavy metals, or indirectly through enhanced nutrient absorption [20]. Furthermore, it was reported that adding Si as potassium silicate improved the concentration of the pigment. This improvement may be related to the biological mechanisms that rice plants can mitigate damages caused by metal stress. The oxidative damage of enzymes caused by heavy metal applications which inhibit plant photosynthesis, has been ameliorated by the increase in antioxidant defenses' capacity [21]. Application of silicon to Pb and Cd in tobacco plants causing significantly increased in photosynthetic pigments compared with heavy metal plants untreated with Si. The authors added that silicon alleviated the damage effects of metals stress on photosynthetic pigments by increasing the membrane stability index. Moreover, the chlorophylls content may be protected probably because of the high antioxidant enzymes activity that increased with silicon and prevented the degradation of leaf chlorophylls [4].

3.2. Total carbohydrates content

Total carbohydrate content in shoot system of sunflower plants were significantly increased due to the presence of heavy metals in the tested soil during the two studied stages. The increment of the total carbohydrates content was directly proportional to the concentration of heavy metals. Data presented in Table 4 clear that total carbohydrates content in sunflower plants grown under lead and cadmium conditions at (400 and 40 ppm) were increased by 13.64 and 28.47%, respectively, at the bud formation stage compared with uncontaminated plants. The same trend was also detected for the vegetative stage. The accumulation of carbohydrates under Pb stress in maize plants was related to the lack of utilization resulted in reduced growth. Moreover, osmotic regulation may play a major role in the increase in carbohydrate concentration under heavy metal conditions [22]. The toxicity of heavy metals on squash plants (*Cucurbita pepo* L.) could affect the metabolism of carbohydrates. The increment of carbohydrate content induced by Cd and Pb may suggest that squash plants operate a metabolic mechanism for channeling heavy metals with their

Table 4. Effect of heavy metals, potassium silicate applications and the interaction between them on total carbohydrates content (g/100 g dry wt), proline content (mg/g dry weight), and total soluble proteins content (g/100 g dry wt) in shoots of sunflower plants during the two studied stages (vegetative and bud formation stages).

Parameters	Total carbohydrates content		Proline content		Total soluble proteins content	
Stages Treatments	Vegetative Stage	Bud formation Stage	Vegetative Stage	Bud formation Stage	Vegetative Stage	Bud formation Stage
Control	8.06	12.75	2.20	3.22	11.35	14.06
Pb200	8.82	13.62	2.53	3.63	10.47	13.66
Pb400	9.48	14.49	2.94	3.95	7.33	10.30
Cd20	10.47	15.2	3.21	4.13	5.65	8.02
Cd40	11.12	16.38	3.65	4.56	3.57	6.63
LSD at 5%	0.61	0.82	0.16	0.20	0.74	0.39
Control	7.85	13.07	2.24	3.36	11.62	14.50
S100	12.10	21.90	4.40	5.37	13.32	16.95
S200	14.39	26.81	5.31	6.12	15.60	18.27
LSD at 5%	2.1	3.4	1.53	1.86	0.85	1.2
Pb200 + s100	9.81	15.73	2.95	3.93	11.71	15.34
Pb200 + s200	10.79	17.04	3.13	4.08	13.06	16.36
Pb400 + s100	10.68	16.98	3.42	4.39	8.57	11.98
Pb400 + s200	11.77	18.62	3.63	4.56	9.93	13.26
Cd20 + s100	11.12	17.63	3.84	4.85	6.89	9.74
Cd20 + s200	13.08	19.25	4.03	5.01	8.24	10.72
Cd40 + s100	11.77	18.27	4.26	5.22	4.81	8.31
Cd40 + s200	13.74	20.87	4.45	5.62	6.16	9.33
LSD at 5%	0.53	0.81	0.41	0.20	0.62	0.84

Pb200 = 200 ppm lead acetate Pb400 = 400 ppm lead acetate.
Cd20 = 20 ppm cadmium sulphate Cd40 = 40 ppm cadmium sulphate S100 = 100 ppm potassium silicate S200 = 200 ppm potassium silicate.

cells. Also, this increment may be due to the inhibitory effect of such metals on phloem translocation [2].

The results in Table 4 also indicated a pronounced and significant increase in total carbohydrates content in the shoot system of sunflower plants treated with silicon. A low concentration of Si (100 ppm) increased the total carbohydrate content in shoots by 54.14 and 67.55%, respectively, at the vegetative and bud formation stages relative to control plants. Moreover, the high concentration of Si (200 ppm) increased the total carbohydrate content in shoots by 83.31 and 105.12%, respectively, at the two mentioned stages relative to untreated plants. The high concentration of Si seemed to be more effective treatment for increasing carbohydrate contents than the other one at the two growth stages. The formation of carbohydrates in rice plants treated with silicon is correlated with an increase in total chlorophyll and chlorophyll a. Additionally, carbohydrates can act as osmolytes to preserve cell turgor, stabilize cell membranes, and prevent protein deterioration [23]. The increase in chlorophyll provided by Si promotes light absorption through the leaves, which raises the amount of carbohydrates in pepper plants as well as their photosynthetic activity [24]. Also, it is possible that silicon promoted the enzymatic action in rice plants, thereby activating the photosynthetic activities [25].

Interestingly, results in Table 4 illustrate that foliar application of silicon (100 and 200 ppm) especially the high concentration significantly increased the total carbohydrate content in shoot system of sunflower plants grown under heavy metal concentrations compared with control plants. The increment percentage in total carbohydrate content in shoots of sunflower plants grown under Pb and Cd at (400 and 40 ppm) and treated with (200 ppm) Si was 24.15 and 23.56%, respectively, at the vegetative stage compared with check plants. The same trend of these results was observed at the bud formation stage. Moreover data showed that the high level of Si seemed to be more effective for improving total carbohydrate content of sunflower plants grown under heavy metals application than the other one. In this respect, the higher carbohydrate depositions due to Si resulted in forming a cell wall and consequentially inhibit Pb and Cd absorption in rice plants. The most crucial component that helps rice plants to Pb and Cd stress conditions is carbohydrates, which give energy to quickly developing cells and it's the carbon skeletons required to manufacture organic compounds [6]. Additionally, it was mentioned that Si treatment may improve the growth performance of peas under cadmium stress by promoting photosynthesis, as demonstrated by higher levels of chlorophyll and the amount of carbohydrate content. Their findings shown that Si raises the amounts of cellulose, hemicellulose, and lignin in plant cells, resulting in accumulation of carbohydrates in shoots of plant. Carbohydrates are the most important soluble constituent that helps the plant to osmotic adjustments. Moreover, the higher depositions of cellulose, hemicellulose, and lignin result in forming a thick cell wall and consequently inhibit cadmium absorption [26].

3.3. Proline content

Under heavy metals stress conditions, results in Table 4 investigated that proline content in shoot system of sunflower plants was significantly increased as a result of heavy metals application during the studied stages. Exposure of sunflower plants to Pb at 400 ppm caused an increment in proline content in shoots represented by 33.63% at the vegetative stage compared with unstressed plants. In addition, Cd at 40 ppm caused an increment in proline content represented by 65.90% at the same mentioned stage compared with control plants. The same trend of these results was true at the bud formation stage. Generally, it could be observed from the results that the highest accumulation of proline content in shoots of sunflower plants was observed by a high concentration of cadmium treatments. It was reported that when wheat plants are cultivated under conditions of Cd stress, the accumulation and synthesis of different suitable solutes and osmolytes are thought to be an essential defensive strategy. Also, proline and sugar accumulation under metal stress are examples of osmoprotectants that protect wheat plant cells by adjusting vacuolar balance and cytosolic acidity [25]. Moreover, Lead-stressed sunflower plants may have higher proline concentration because of increased proline biosynthesis or decreased proline degradation [27].

Proline content in shoots of sunflower plants was significantly increased by applying potassium silicate during the studied stages. From results in (Table 4) it could be stated that applications of Si especially the high concentration seemed to be more pronounced for increasing proline content compared with a low concentration of silicon. The high concentration of Si (200 ppm) caused an increment in proline content represented by 137.0 and 82.14%, respectively, at the vegetative and bud formation stages over the control plants. In agreement with our results, it was showed that potato plants treated with Si had higher proline concentrations in their leaves, which may have been caused by more effective osmotic adjustment [28]. Moreover, it was found that the application of 4.50 mM Si increased the accumulation of sugars and proline in the leaves of wheat plants in comparison to control plants, and that these substances function as osmoprotectants [25].

Table 4 showed that proline content in shoots of the tested plant was significantly and markedly increased in heavy metals stress plants treated with the concentrations of Si compared with metal stressed unsprayed plants. This increment was more pronounced at high concentration of Si during the two studied stages. The increment of proline content for plants grown under low concentrations of lead (200 ppm) and sprayed with 100 and 200 ppm Si was 16.60 and 23.71%, respectively, at the vegetative stage compared with control plants. About the effect of cadmium, the increment of proline content for plants grown under low concentration of cadmium (20 ppm) and sprayed with 100 and 200 ppm Si was 19.62 and 25.54%, respectively, at the same mentioned stage compared with metal treated plants. The same results were true for the other stage of growth. Application of silicon causes an increment of proline content in leaves of coriander plants exposed to Pb stress that function as osmolytes. Increment of this osmolytes under Pb stress can indirectly increase the amount of reactive water content and support the appropriate function of the vital enzymes. In addition, proline may also help eliminate hydroxyl radicals, preserve protein levels and membrane stability, provide carbon and nitrogen during stressful situations, and occasionally function as a chelator [29]. Proline accumulation in wheat plants may act as an indicator for Cd^{+2} stress because it may have scavenged H_2O_2, preserving the integrity of cell membranes [25].

3.4. Total soluble proteins content

Results in Table 4 reveal that total soluble proteins content in shoots of sunflower plants were significantly depressed by increasing the concentration of heavy metals in tested soil. This was true for the two taken samples. For instance, the reduction in total soluble proteins content in shoots of sunflower plants treated with Pb at 400 ppm represented by 35.41 and 26.74%, respectively, at the vegetative and bud formation stages relative to control plants. Moreover, Cd at 40 ppm caused a marked reduction of total soluble protein contents detected by 68.54 and 52.84%, respectively, at the same mentioned stages compared with control plants. Such reduction in soluble protein content in rice plants might result from accelerating the breakdown process of proteins due to the increased protease activity that occurs under Pb situations [30]. Additionally, they observed that the reduction in soluble proteins content may caused by the toxic effects of reactive oxygen species that cause protein disintegration. It was found that the amount of soluble proteins in pea plants reduced, as the concentration of cadmium increased. According to their suggestions, cadmium may have an impact on soluble protein content because of the following: (i) increased hydrolysis of proteins, which lowers their concentration; (ii) cadmium is considered as catalytic activity; and (iii) reduction of protein synthesis was detected under cadmium stress conditions [26].

Table 4 declared a pronounced and significant increment of total soluble proteins was achieved in shoot system of sunflower plants as result of foliar applications of Si treatments. This was more obvious by high concentration of Si (200 ppm) which attained the best results for increasing total soluble protein contents at the two growth stages compared with the another one (100 ppm Si). Application of Si at (200 ppm)caused an increment of total soluble proteins in shoot system represented by 26.0% at the bud formation stage compared with the corresponding check plants. These results were also achieved during the vegetative stage of growth. In this context, It was found that, in cotton plants cultivated under typical conditions, Si plays an important role for lowering protein degradation, enhancing protein synthesis, and raising protein metabolism [31]. The increase in total soluble proteins in response to silicon may be explained by the important function of silicon in binding amino acids to generate particular proteins. The author proposed that silicon might potentially influence protein activity and/or syntheses by attaching hydroxyl groups to amino acid residues, which would in turn control the phosphorylation state of signaling proteins [19].

It is clearly observed that silicon treatments improved total soluble proteins content in shoot system of sunflower plants grown under heavy metal applications compared with metals unsprayed plants. Plants grown under (400 ppm) Pb and treated with silicon at (100 and 200 ppm) recorded an increment of total soluble protein contents represented by 16.91 and 35.47%, respectively, at the vegetative stage compared with check plants. Moreover, the increment of total soluble proteins in plants grown under (40 ppm) Cd and treated with silicon at (100 and 200 ppm) was 34.73 and 72.54%, respectively, at the same mentioned stage compared with metal treated un-sprayed plants. These results were also detected in plants during the bud formation stage.

In this regard, it was cited that application of silicon mitigated the suppressive effect of cadmium and caused a remarkable increment in soluble proteins content in leaves of cotton plants. Since antioxidant enzymes activity are increased and Cd uptake and accumulation are decreased, there is a decrease in the damage caused by ROS [32]. Also, application of Si reduced the adverse effects of Cd and increased the content of soluble proteins in the leaves of maize plants. This could be explained by the potential role of proteins in Si deposition in cell walls, although the precise nature of Si's response to cell wall constituents are yet unclear [33]. Furthermore, Si achieved a significant increment in soluble proteins content such as methionine and proline in alfalfa plants under Cd-stress. Methionine eliminates ROS oxidative damage. Additionally, it serves as a reservoir for antioxidant proteins and a substrate for the production of certain polyamines corresponding to stress tolerance, including as putrescine, spermidine, and spermine. Free radicals are scavenged by proline, which also maintains proteins and subcellular integrity under stress. Moreover, proline synthesis promotes the redox cycling and NADP + maintenance, which is crucial for plants' antioxidant defense systems under stress conditions [34].

3.5. Oil content

Data declare a significant and progressive reduction in oil production of harvested seeds with increasing heavy metals application in the tested soil comparing to control seeds. Reduction of oil in seeds of sunflower plants grown under 400 ppm Pb was 12.87% compared with control value. Moreover, 40 ppm Cd caused a great reduction in oil production represented by 26.72% compared with control seeds. Accordingly, the application of Pb resulted in a sharp decline in the concentration of chlorophylls in leaves and phenols, which considerably reduced the yield of mint oil. This decrease may be connected to how metallic elements affect carbon metabolism and enzymatic function [35]. It was revealed that metals (Pb and Cd) in mint plants had a negative influence on their essential oil productivity. Decreased biomass, lesser leaves and total leaf area contribute to lower oil yields when heavy metals are applied. In addition, the inactivation of particular enzymes involved in the metabolic pathways of secondary metabolites is the primary cause of the decline in oil output [36].

The results declare a pronounced and significant increment of oil content in yielded seeds of sunflower plants as result of Si treatments. For instance, Si at 100 and 200 ppm caused an increment of oil production in yielded seeds represented by 4.82 and 9.93%, respectively, compared with control seeds. The above results demonstrated that the application of potassium silicate especially the high concentration, seemed to be more effective for increasing oil production in harvested seeds comparing to the low concentration of potassium silicate (100 ppm). The increase in cycle growth, nutrient uptake or modifications to the population of leaf oil glands may be responsible for increasing the oil yield in sweet basil plants under Si application [37]. Applying Si to safflower plants may increase their oil content by improving their ability to aggregate oil or by reducing the oxidation of certain polyunsaturated fatty acids in the seeds. The increment in oil synthesis in the examined plants may possibly be related to an increase in seed production and the accumulation of carbohydrates [38].

Table 5 reveal that oil content in the yielded seeds was significantly increased in metaled plants treated with Si compared with metaled unsprayed plants. Plants grown under 400 ppm Pb and treated with Si at 100 and 200 ppm recorded an increment in oil percent in seeds represented by 9.67 and 16.70%, respectively, compared with control value. Meanwhile, the increments in oil seeds represented by 6.56 and 13.29%, respectively, for plants grown under Cd at 40 ppm and treated with 100 and 200 ppm Si compared with corresponding value.

Table 5. Effect of heavy metals, potassium silicate applications and the interaction between them on oil content (%)in seeds of sunflower plants during the harvest time.

Treatments	Oil content (%)
Control	42.81
Pb200	40.07
Pb400	37.30
Cd20	34.39
Cd40	31.37
LSD at 5%	1.37
Control	43.97
S100	46.09
S200	48.34
LSD at 5%	2.11
Pb200 + s100	42.65
Pb200 + s200	45.27
Pb400 + s100	40.91
Pb400 + s200	43.53
Cd20 + s100	36.99
Cd20 + s200	39.58
Cd40 + s100	33.43
Cd40 + s200	35.54
LSD at 5%	1.93

Interestingly, it can be concluded that the application of potassium silicate especially the high concentration achieved the best results for increasing oil production in harvested seeds than the low one under heavy metals conditions. Silica could improve oil yield in summer savory plants under cadmium stress events, by increasing biomass in the aerial parts, promoting cell development, improving ion uptake, and augmenting the density, size, and quantity of oil glands in the leaves of the tested plants [39].

It's worthy to mention here that, applying silicon to the tested plants is considered to reduce the harmful effects of heavy metals which reduces vegetation and reproductive growth and therefore the yield of the tested plants. Moreover, application of Si to sunflower plants grown under heavy metals (Pb and Cd) significantly increased the oil content by relieving the damage in leaf area and shoot dry matter.

3.6. Lead and cadmium accumulation in harvested seeds

Figures 1 and 2 show that treatments of Pb and Cd caused a significant and marked accumulation of their contents in harvested seeds comparing to control seeds. The increment in Pb content in seeds as result of Pb application in tested soil represented by 1.61 and 2.15 μg/g D.wt, respectively, at 200 and 400 ppm Pb compared with control seeds which was 0.99 μg/g D.wt. while, the increment of Cd content in seeds of sunflower plants as result of Cd application to soil represented by 1.20 and 1.94 μg/g D.wt,

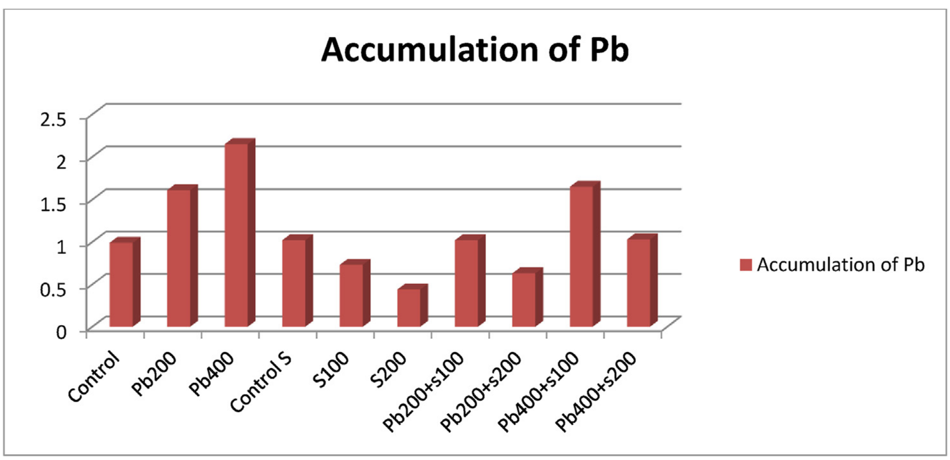

Fig. 1. Effect of Pb, potassium silicate applications and the interaction between them on Pb content (μg/g D.wt) in seeds of sunflower plants during the harvest time (95 days).

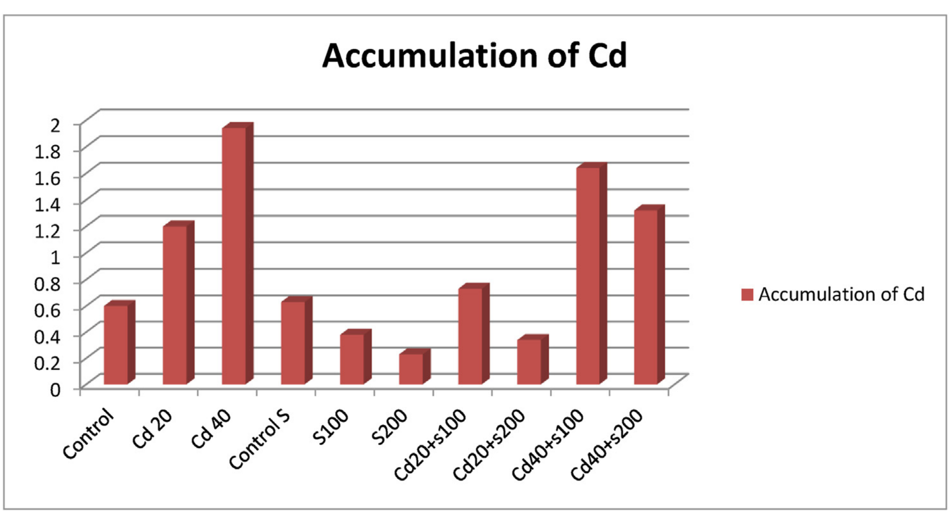

Fig. 2. Effect of Cd, potassium silicate applications and the interaction between them on Cd content (μg/g D.wt) in seeds of sunflower plants during the harvest time (95 days).

respectively, at 20 and 40 ppm Cd compared with control seeds which was 0.60 μg/g D.wt. In this connection, it was reported that, increasing Pb concentration in culture media led to a considerable rise in lead content in cotton plant roots, stems, leaves, and seeds. The growth of cotton plants was inhibited by an increase in lead (Pb) uptake and accumulation due to a decrease in biomass, pigment content, photosynthetic properties, protein, and antioxidant enzymes [31]. Certain growth symptoms were significantly reduced as a result of cadmium accumulation in grains and shoots of wheat plants. The great mobility of Cd in the phloem and its ability to accumulate in any plant organ resulted in a decrement of several growth parameters. The elevated levels of Cd in the grains and shoot may reduce the quantity of the harvested grains [40].

Generally, results showed that spraying of sunflower plants with silicon significantly decreased Pb and Cd concentrations in seeds of sunflower plants compared with control values. Application of Si at 200 ppm markedly decreased Pb and Cd accumulation in seeds by 56.86 and 63.49%, respectively, compared with control seeds. The same result was true for plants sprayed with 100 ppm Si. Data reveal that Si application especially the high concentration showed the best results for decreasing the accumulation of heavy metals within the harvested seeds. Applying 150 mg of Si/kg of soil was found to be an efficient way to immobilize cadmium in the soil, reduce cadmium absorption by wheat roots, and stop cadmium translocation from roots to shoots and grains. Plants cultivated in soil treated with Si showed lower levels of Cd in their wheat grains as compared with the control treatment. The application of Si reduced the amount of Cd that plants absorbed, which could promote the development of antioxidants that support the integrity of the plasma membrane by inhibiting the uptake of Cd. Moreover, silicon enhances the Ca status of plant tissue, which is important not only for preserving the integrity of the plasma membrane but also for competing with Cd for uptake and translocation [41]. It is well known that Si raises the amounts of cellulose, hemicellulose, and lignin in rice plant cell walls. Increased Si-induced cellulose and hemicellulose depositions contribute to form a cell wall and hence limit the absorption of heavy metals [6].

It was observed that silicon treatments had a reducing effect on the uptake and accumulation of heavy metals in seeds of sunflower plants grown under heavy metals application during the harvested time. The deficiency rate in the accumulation of Pb concentration in the harvested seeds for plants grown under 200 and 400 ppm pb and treated with 200 ppm Si represented by 0.63 and 1.03 μg/g D.wt, respectively, relative to corresponding control. Additionally, the reduction in the accumulation of Cd in harvested seeds for plants grown under 20 and 40 ppm Cd and treated with 200 ppm Si was 0.34 and 1.32 μg/g D.wt, respectively, relative to control value. It is interesting to mention here that application of silicon specially the high concentration showed the best results for reducing the uptake and the accumulation of heavy metals (Pb and Cd) in the harvested seeds of stressed sunflower plants. In comparison to control plants, exogenous application of silicon reduced the absorption and translocation of Pb in all cotton plant parts, including the roots, stem, leaves, and seeds. The two main strategies by which silicon (Si) could limit metal transport in plants are as follows: (1) Si may be able to form a complex with Pb or cause Pb to accumulate in the cell walls. Additionally, it has been observed that adding Si to lead-contaminated soils can reduce the accumulation of Pb, which in turn helps to lower the level of Pb contamination in cotton seeds [31]. Moreover, Si improves the plants' ability to withstand the harmful effects of Cd by reducing Cd absorption and translocation in wheat plants. Because Si has positive effects on mineral nutrition, its foliar application is essential for plant growth and development. The authors also added that, a sufficient amount of Si enhances the dry matter of the plant through creating nutritional balance and minimizes the toxicity of Cd by limiting Cd moving from shoots to grains [42].

3.7. Conclusions

It could be concluded that application of Si has beneficial regulatory role in sunflower plants grown under lead and cadmium pollution. This was associated by the reduction of Pb and Cd uptake and their translocation in stressed sunflower plants. Also, Si increased the content of photosynthetic pigments, carbohydrates, proline, total soluble proteins content as well as the oil productivity. Thus, it could be mentioned that Si application increased the tolerability of sunflower plants towards the toxic effects of heavy metals.Ethical

Ethical statement

Not applicable.

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Author contributions

Samar Zain el-abdeen Haggag: Visualization and draft writing.

Hanaa Mohamed Salem: Conceptualization and supervisor.

Abeer Esmail Mustafa: Project manager and supervisor.

Mohamed Abdelhameid Ismail: Revision and supervisor.

Conflict of interest

There are no conflicts of interest.

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