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EVALUATION OF THE EFFECTS OF Cu, Zn, AND Hg METALS ON WHEAT

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Abstract

This study was carried out to evaluate the effects of the heavy metal (HM) compounds $CuSO_4$, $HgCl_2$, and $ZnSO_4$ on wheatgrass, *Triticum aestivum*. Juice was extracted from treated leaves after 14 days of treatment for analysis of biochemical parameters including antioxidant activity, levels of protein and chlorophyll, and peroxidase (POX) enzyme activity. Seed germination was also examined, with decreased root length and paler leaves observed in treated samples, compared with control samples. The HM compounds caused a significant reduction in total chlorophyll levels and decreased the chlorophyll a/b ratio to 0.54–1.36 (compared with 1.63 in the control). Treated samples also showed significantly reduced content of total protein and phenolic compounds. Additionally, 2,2-diphenylpicrylhydrazyl (DPPH) scavenging activity was increased slightly, while peroxidase (POX) enzyme activity was reduced, compared with untreated controls. Furthermore, kinetic analysis using a Lineweaver–Burk plot showed that that the kinetic parameters K_m and V_{max} were altered in the treated samples. Cu inhibited POX uncompetitively, reducing both K_m and V_{max} without changing the V_{max}/K_m ratio, while Zn and Hg showed mixed inhibition. Overall, irrigation of wheatgrass with HM solutions affected seed germination, leaf color, antioxidant activity, and levels of chlorophyll, protein, and phenol. Furthermore, POX activity was inhibited.

Key words: Heavy metals, Antioxidants, Total phenols, Total proteins, Chlorophyll content, Peroxidase activity.

Introduction

Wheat is a type of grass widely cultivated for its seed (cereal grain), which is a major source of food worldwide. Multiple species of wheat make up the *Triticum* genus, while the most widely grown wheat species is *T. aestivum*. Wheatgrass also comes from this species, being grown to reach its maximum size 7–10 days after sprouting. Wheatgrass is high in nutrients, including vitamins and minerals, which contribute to the anti-cancer properties of this herbal product. Furthermore, wheatgrass is known as "green blood" due to its high levels of chlorophyll, which is structurally similar to hemoglobin, the supplier of oxygen to body tissues (Diwakar & Sangeeta, 2017).

Chlorophyll, which plays a beneficial role in inhibiting the metabolic production of carcinogens, has been shown to be the most effective ingredient in wheatgrass (Aydos *et al*., 2011). Wheatgrass is recognized as a potent source of antioxidants as well as minerals (iron, calcium, magnesium, selenium, manganese, phosphorus, copper), folate, bioavailable benzo(a)pyrene, vitamins (A, C, E, thiamine, niacin, B6), calcium, and amino acids (Aydos *et al*., 2011). Furthermore, antioxidant functions are conferred by multiple phenolic compounds including ferulic acid, gallic acid, caffeic acid, syringic acid, and p-coumaric acid (Kardas & Durucasu, 2014; Benincasa *et al*., 2015).

In plants, the enzymatic defense system includes catalase, peroxidase, ascorbate peroxidase, superoxide dismutase, and glutathione reductase. Plants also contain several non-enzymatic antioxidant compounds such as ascorbate, glutathione, flavonoids, phenolic compounds, tocopherol, and carotenoids (Schutzendubel & Polle, 2002; Kisa *et al*., 2016). The ability of phenolic compounds in plants to chelate metals helps to protect the plants against the damaging effects of oxidative stress (Tomas-Berberan *et al*., 2001; Cheynier, 2012).

Phenolic compounds are of great importance in the physiological and morphological systems of plants, playing key roles in growth and reproduction, and furthermore exhibiting antimicrobial activities (Balasundram *et al*., 2006). In humans, oxidative stress is an important indicator in the pathophysiology of multiple disorders, including cancers. Generation of reactive oxygen species (ROS) is inhibited by antioxidant enzymes (catalase, superoxide dismutase) and additional antioxidant compounds (Aydos *et al*., 2011; Stanner & Weichselbaum, 2013; Mulgund *et al*., 2015).

Peroxidase (POX) is an enzyme found in a wide variety of organisms from plants to humans, as well as bacteria. Its function is to break down hydrogen peroxide (H_2O_2), one of the toxins produced as a by-product of using oxygen for cellular respiration. Plant peroxidases have been reported to play diverse functions in the life cycle of plants, for example in cell wall metabolism, lignification, metabolism of ROS, wound healing, fruit development and ripening, and germination of seeds.

Heavy metals (HMs) are a significant issue in both agricultural and environmental settings, being produced by a number of activities such as mining, smelting, and the use of agrochemicals and found in urban, industrial, and agricultural wastes (Hemdane *et al*., 2016). Natural activities, including human activities, also have the potential to produce HMs as side-effects. Migration of these contaminants into uncontaminated areas as dust or leachates through the soil and spreading of heavy metals containing sewage sludge are examples of events contributing to the contamination of ecosystems (Ghosh *et al*., 2005; Abdulateef *et al*., 2014; Gupta *et al*., 2016). However, some HMs are also micronutrients, i.e., essential elements that play a crucial role as a major component of metalloproteins, cofactors in enzymatic catalysis, and manifold other cellular processes. When levels of micronutrients are suboptimal, plants develop deficiency symptoms, generally characterized by growth inhibition

and leaf bleaching or browning. Similar effects are seen at supraoptimal levels of micronutrients (Rout *et al*., 2003). Essential elements include copper (Cu), cobalt (Co), manganese (Mn), zinc (Zn), and molybdenum (Mo). Non-essential HMs or "metalloids", such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are not required by organisms; however, these HMs interfere with physiological enzymatic reactions due to their reactivity with thiols or other groups (Tchounwon *et al*., 2012; Rahman *et al*., 2012).

Mercury is a toxic element that bioaccumulates to high levels and has a great environmental impact. Methyl mercury (the most toxic form of mercury) plays a key role in damaging tertiary and quaternary protein structures by attaching to selenohydryl and sulfhydryl groups in biological molecules, which adversely affects cellular structure and function (Jaishankar *et al*., 2014; Bernohoft, 2011). The toxic effects of Hg in plants are manifested by reduced growth and lower levels of chlorophyll and protein (Chen *et al*., 2012; Siddiqui *et al*., 2018).

Zinc, an essential micronutrient with a long biological half-life, affects metabolic processes in plants. If the concentration of Zn in affected soil reaches high levels, phytotoxicity can be induced. Higher concentrations of zinc affect many plants metabolic functions, resulting in retarded growth and senescence, and may cause both limited growth of roots and shoot chlorosis in younger leaves. The phytotoxicity of Zn is indicated by decreased growth, effects on metabolic processes, and induction of oxidative damage in various tissues (Roohani *et al*., 2013).

Copper is another micronutrient that is required by plant organisms and also plays crucial roles in chlorophyll production, photosynthesis, respiratory electron transport chains, and oxidative stress protection, as well as protein, carbohydrate, and cell wall metabolism. Furthermore, Cu deficiency affects various aspects of plant metabolism and causes phototoxicity through the overproduction of ROS, which in turn cause damage to carbohydrate, lipid, protein, and DNA molecules (Fabrizio *et al*., 2019).

As a result of increased pollution in the soil and water used for irrigation, we have therefore studied the effects of some of the most prevalent contaminants in the region on the wheat plant most widely used in Jordan and the world. Therefore, the aims and objectives of this study were to evaluate the effects (*In vivo*) of specific HMs (Hg, Zn, and Cu) on *T. aestivum*, namely the effects on seed germination and levels of chlorophyll, phenols, and protein. In addition, we wished to investigate the effects on the antioxidant system.

Material and Methods

Material: Fresh wheat seeds were collected from Alqaser city, Al-Karak region, between May and June, 2020.

Preparation of crude plant extracts: Crude wheat extract (wheat juice) was obtained from the fresh leaves of the common wheat plant (*T. aestivum*). Initially, the wheat grains were sterilized by soaking in distilled water at a temperature of 50–52°C for 2 hours, followed by washing and soaking for 24 hours. The next day, the samples were filtered and placed in a damp cotton cloth for another 24 hours at room temperature. The following day, the wheat was distributed on agricultural trays sterilized with 97% ethanol and the wheat grains were sprayed at a rate of 3–5 times per day with solutions containing the HMs (Hg, Zn, Cu) or water. After that, the agricultural trays containing the wheat seeds were divided into control (absence of HMs) and treated (presence of HMs). The samples were treated with solutions containing the HMs (Hg, Zn, Cu) at concentrations of 0.1 M and 0.3 M until completion of the germination process. After the fourth day, during the germination process, the number and length of roots were measured daily for all samples.

Finally, after completion of the germination process (9–10 days), the leaves of the plants were cut and used to prepare crude plant extracts. For this, 200 g of fresh wheatgrass leaves (treated or untreated) were homogenized with distilled water in a blender for 5 min. The homogenate was filtered using a cloth sheet followed by 0.1 mm filter paper and then centrifuged at 13,000 rpm for 20 min at room temperature. The supernatant was collected and used as fresh crude extract (wheatgrass juice); the extract was kept at 4°C.

Analysis of chlorophyll content: Extracts were prepared from 50 mg of treated or untreated wheatgrass leaves using the Arnon method (Arnon, 1949), in order to determine chlorophyll levels. The extracted content was homogenized with 10 ml of ice-cold of 80% acetone. Then, $CaCO_3$ was added to the pre-processed extracts to avoid destruction of chlorophylls and other pigments; extraction was also carried out under dim light to avoid photo-oxidation of the pigments. The extracts were centrifuged at a low speed (5000rpm) for approximately 20 min to pellet the cell wall debris, and the supernatant was removed and made up to 10 ml with ice-cold 80% acetone. Absorbance (A) was recorded using a spectrophotometer at wavelengths of 480, 510, 645, and 663 nm immediately after extraction. The total concentration of chlorophylls was estimated by the Arnon formulas as follows:

Chlorophyll a (µg/ml) = (12.7 x A663) – (2.69 x A645)

Chlorophyll b (µg/ml) = (22.9 x A645) – (4.68 x A663)

Total chlorophyll (µg/ml) = (20.2 x A645) + (8.02 x A663)

Protein content in wheatgrass juice: Total protein concentration was determined by the Lowry method, using bovine serum albumin as a standard (Lowry *et al*., 1951). The concentration of soluble protein was calculated from the standard curve as mg protein per ml test sample.

Determination of total phenolic content (TPC): Total phenolic content (TPC) was determined according to the Folin-Ciocalteu procedure (Tayybe *et al*., 2014). To begin with, 1.0 ml of Folin-Ciocalteu's reagent and 0.8ml of sodium carbonate (7.5%) were added to 0.2 ml of plant extract. The tubes were then shaken and allowed to stand for 30 min before absorption was measured at 760 nm. To calculate TPC, absorbance was compared with a standard curve using different concentrations of gallic acid; TPC was expressed as gallic acid equivalents (GAE) in mg per g plant extract.

Antioxidant activity: The 2,2-diphenylpicrylhydrazyl (DPPH) free radical scavenging assay was performed by spectrophotometry, as described by Pyrzynska & Pękal (2013). Aliquots of the extracts (50µl) were added to 5 ml of a methanolic solution of DPPH (60 µM in methanol). After incubation for 30 min at room temperature, the absorbance (A) was measured at 520 nm using methanol solution as blank and Gallic acid as a control. All measurements were performed in triplicate. Inhibition of free radical scavenging activity was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{A control - A sample}}{\text{A control}} \times 100$$

Enzyme assay and kinetics: POX activity was estimated according to Kumar and Khan method (Kumar & Khan, 1982). Briefly, 1.0 mL of pyrogallol, 1.0 mL of H_2O_2 (0.05M), and 0.5 mL of wheatgrass extract were added to 2.0 mL of phosphate buffer (0.1 M, pH 6.8). After mixing and incubation for 5 min at room temperature, the reaction was terminated by the addition of 1.0 mL of H_2SO_4 (2.5 M). The absorbance was measured at 420 nm using H_2SO_4 as blank. The POX activity was expressed in Unit (U) /mg of protein, where one unit is defined as the absorbance change per min per mg of protein, Lineweaver – Burk plot was used to calculate the V_{max} and K_m values.

Statistical analysis

All analysis was carried out in triplicate using Microsoft Excel 2016.The numerical data were expressed as mean ± standard deviation (SD).

Results

Figs. 1 and 2 show the results of seed germination in control and treated samples, with time. Figs. 3–5 and Table 1 summarize the effects of HMs Cu, Zn, and Hg on total phenolic compounds, total protein, and antioxidant activity. Tables 2 and 3 show the effects of these HMs on enzyme peroxidase activity and kinetic parameters. Significant effects were found for all the above parameters.

Seed germination: Seeds of *T. aestivum* were grown in seven separate agricultural trays for 7–14 days; six trays were irrigated daily with solutions containing the HM compounds $ZnSO_4$, $CuSO_4$, and $HgCl_2$ at two concentrations (0.1 M and 0.3 M), and one was irrigated with distilled water as a control. Analysis of the seed germination process showed that the number, and length of roots were reduced and the leaves color were changed in treated samples, in comparison with the untreated controls (Figs. 1 and 2).

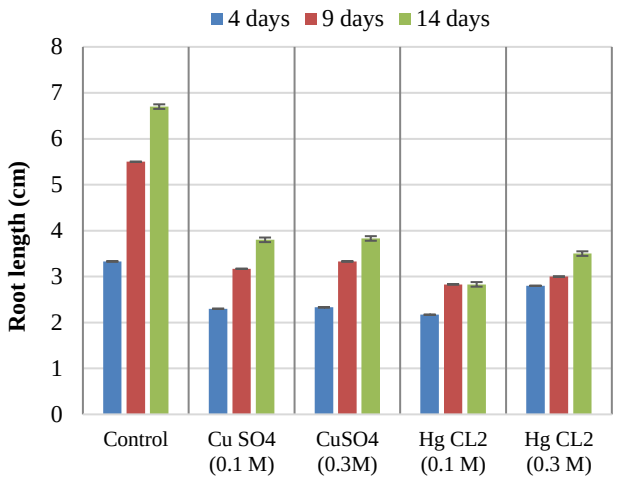

Fig. 1. Root length (cm) of the germinated seeds of wheatgrass of the control and treated samples (days). Values are mean ± SD of triplicate.

Table 1. The Chlorophylls content (mg/mL) of control and treated samples.

	Chl-a	Chl-b	T. Chl.	Chl-a/b
Control	26.04	15.97	42.0	1.63
Hg (0.1M)	25.30	22.76	48.04	1.11
Hg (0.3M)	22.73	22.17	44.87	1.03
$CuSO_4$ (0.1M)	17.93	16.81	34.73	1.07
$CuSO_4$ (0.3M)	24.66	16.43	40.01	1.6
Zn (0.1M)	15.13	28.17	40.6	0.54
Zn (0.3M)	23.23	25.22	40.65	0.92

Chl-a: Chlorophyll a; Chl-b: Chlorophyll b; T.Chl.: Total chlorophyll; Chl-a/b: Ratio of chlorophyll a/chlorophyll b. Values are means of triplicate.

For both concentrations (0.1 M and 0.3 M) of Cu and Zn compounds, levels of chlorophyll a (17.926 - 24.66mg/mL for Cu and 15.13–23.23 mg/mL for Zn) and total chlorophyll (34.73–40.01 mg/mL for Cu and 40.6 – 40.65 mg/mL for Zn) in treated samples were significantly lower, compared with the untreated control (26.041 and 42.0 mg/mL, respectively) (Table1), while the level of chlorophyll b was increased (16.43–25.22 mg/mL in the treated samples, compared with 15.97 mg/mL in the control (Table 1). The chlorophyll a/b ratio was lower in the treated samples (0.54–1.6) than the control (1.63). For Hg treatment, there was a slight reduction in chlorophyll a (22.73–25.297 mg/mL) and an increase in total chlorophyll (44.87 mg/mL at 0.1 M concentration and 48.04 mg/mL at 0.3 M).

Table 2. The protein content and activity of peroxidase enzyme in the crude wheatgrass juice of control and treated samples with HMs.

Heavy metals	Protein amount mg/mL	Total protein mg	Activity U/mL	Total activity U
Control	0.66	1.98	3.08	17.0
$CuSO_4$ (0.1M)	0.17	0.50	2.83	15.54
$CuSO_4$ (0.3M)	0.06	0.18	2.77	15.25
$ZnSO_4$ (0.1M)	0.10	0.30	2.98	16.39
$ZnSO_4$ (0.3M)	0.30	0.90	2.83	15.57
$HgCl_2$ (0.1M)	0.13	0.39	3.00	16.51
$HgCl_2$ (0.3M)	0.27	0.80	2.97	16.36

Values are means of triplicate

Fig. 2. Photograph slides of aerial parts (leaves) of wheatgrass of the control and treated samples after 10 days of germination by using an optical microscope. A. Control. B. $CuSO_4$ 0.1 M. C. $CuSO_4$ 0.3M. D. $ZnSO_4$ 0.1 M. E. $ZnSO_4$ 0.3 M. F. Hg CL_2 0.1 M. G. Hg CL_2 0.3 M.

Table 3. Kinetic parameters of POX in the crude wheatgrass juice of control and treated samples with HMs.

Heavy metals	$K_{m\ (mM)}$	$V_{max\ (\mu mol/min)}$	V_{max}/ k_m	Specific activity (unit/mg)	Effect
Control	0.014	3.08	216.6	4.7	Normal
$CuSO_4$(0.1M)	0.007	2.83	434.6	17.02	Uncompetitive
$CuSO_4$(0.3M)	0.006	2.71	430.2	25.6	Uncompetitive
$ZnSO_4$(0.1M)	0.005	2.85	527.8	28.5	Mixed inhibition
$ZnSO_4$(0.3M)	0.005	2.83	615.2	9.4	Mixed inhibition
$HgCl_2$(0.1M)	0.005	2.89	567.0	21.74	Mixed inhibition
$HgCl_2$(0.3M)	0.006	2.97	531.1	11.2	Mixed inhibition

Values are means of triplicate

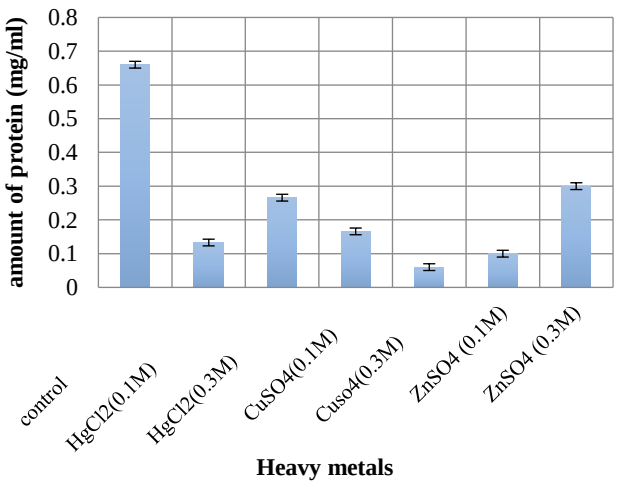

Fig. 3. The amount of protein (mg/ml) in wheatgrass juice in the absence and presence of HMs. Values are mean ± SD of triplicate.

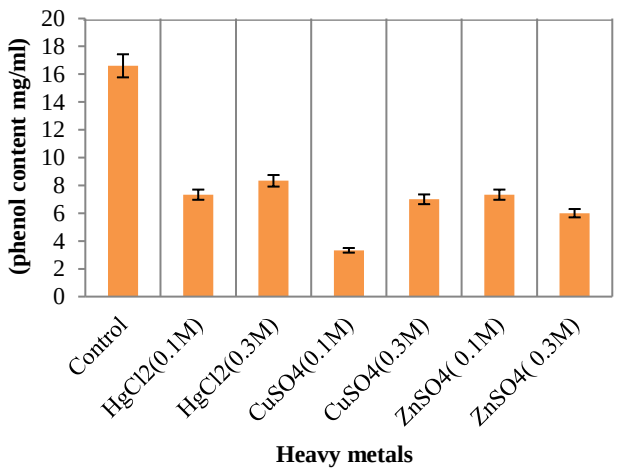

Fig. 4. The amount of total phenols in control and treated samples of wheatgrass juice. Values are mean ± SD of triplicate.

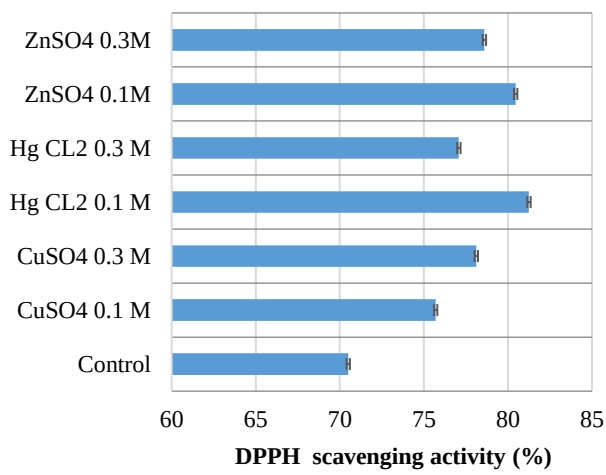

Fig. 5. DPPH scavenging activity (%) of wheatgrass juice in the presence and absence of HMs. Values are mean ± SD of triplicate.

An initial reaction was performed at different concentrations of HMs, and 1/V values were plotted against 1/[s] values. Kinetic parameters were calculated from the graph; resulting K_m and V_{max} values are summarized in (Table 3).

Total protein concentration: Lowry method was used to determination of total protein content. Fig. 3 shows the total protein content in treated and untreated samples. Both concentrations of the selected HMs significantly affected protein content in the treated samples, reducing protein levels to 54.5%–91.0%, compared with untreated samples [100%]; see Fig. 3).

Total phenolic content: TPC was calculated from the Gallic acid standard curve. Fig. 4 shows the effects of the selected HMs on TPC. As illustrated in (Fig. 4) all HMs exerted a significant effect on the treated wheatgrass samples, reducing TPC levels to 50.2%–80%, compared with untreated samples (100%).

Antioxidant activity: The effects of Cu, Hg, and Zn on the antioxidant activity of the treated samples were studied by the DPPH-radical scavenging method. When DPPH radicals encounter a proton-donating substance such as an antioxidant, free radicals are scavenged; this can be measured as a reduction in absorption using a spectrophotometric assay. Thus, DPPH radicals are widely used to investigate the scavenging activity of some natural compounds. The results showed that DPPH-scavenging activity was slightly higher in treated samples than in control samples (Fig. 5). Scavenging activity was higher in the treated samples (75.78%–81.25%), while treatment with Hg (0.1M) resulted in the highest overall DPPH activity (81.25%), compared with the control (70.50%).

Enzyme activity and kinetics: Enzyme activity was determined in control and treated wheatgrass samples. (Table 2) shows POX activity in the control and HM-

treated wheatgrass. Peroxidase activity was lower in all studied samples (2.77–3.00 U/mL), compared with the control (3.08 U/mL); this equated to a 2.9%–10.3% reduction in total activity. A Lineweaver–Burk plot was used to determine the effects of HMs on the kinetic parameters (K_m and V_{max}) of POX.

Discussion

Daily irrigation of wheatgrass plants with solutions containing the HM compounds $ZnSO_4$, $CuSO_4$, and $HgCl_2$ resulted in adverse effects on seed germination and leaf color. These results are consistent with a previous study conducted on wheat plants, in which it was shown that increasing the concentration of HMs reduced growth and affected root length, seed color, and leaf color (Shaikh *et al*., 2013). In our study, the selected HMs stimulated growth and induced a change in the color of the leaves from dark green (as seen in the control) to light green or greenish brown; in addition, decreased levels of total chlorophyll, total protein, and total phenolic compounds were observed.

Based on the Arnon method, analysis of chlorophyll levels showed that the total concentration of chlorophyll was lower in the treated samples, compared with the control extracts, while the level of chlorophyll b was higher. Furthermore, although treatment with Cu and Zn resulted in reduced chlorophyll content overall, treatment with Hg resulted in higher levels of chlorophyll, compared with the untreated control. These effects on chlorophyll a, b, and total chlorophyll concentration could be explained by the inhibition of chlorophyll biosynthesis (Dhir *et al*., 2009). For example, reasons for the decreased chlorophyll content could include lower Fe content, reduced efficiency of enzymes involved in chlorophyll biosynthesis, or replacement of the central Mg^{+2} ions in chlorophylls by HMs. These results correspond with those obtained by several other studies examining the effect of HMs on the chlorophyll content of wheat seedlings (Datta *et al*., 2011; Parlak, 2016; Siddique *et al*., 2017). Furthermore, we showed that the chlorophyll a/b ratio was significantly reduced by $CuSO_4$, $HgCl_2$, and $ZnSO_4$. These findings are in agreement with previous studies, which showed that the chlorophyll a/b ratio decreased significantly as a result of treatment with a HM (Cr), as Cr treatment affected chlorophyll a more than chlorophyll b. However, treatment of *T. aestivum* seeding with Cr has also been shown to lead to lower levels of both chlorophylls a and chlorophyll b (Ahmet *et al*., 2013). According to Rai *et al.* (2016), chlorosis and plant retardation are observed in polluted soil, and may be due to deficiencies in pigment synthesis, photosynthetic efficiency, and general metabolism. It was found that a high concentration of Zn led to a decrease in total chlorophyll and inhibited the assimilation of CO_2, while high levels of Cu affected the thylakoid membrane and chloroplast ultrastructure and inhibited photosynthetic pathways. On the other hand, Hg was found to affect both the light and dark reactions of photosynthesis by substituting for the Mg^{+2} ion *In vivo* (Amin & Latif, 2015).

Protein content was lower in wheatgrass treated with $CuSO_4$, $HgCl_2$, or $ZnSO_4$, compared with untreated wheatgrass. This decrease is due to binding of the HMs to the sulfhydryl group in proteins, which damages the normal structure. These results are consistent with a previous study conducted on arsenic-treated wheat plants, which found that the protein content was 12.4 mg/ml in control samples and 7.2 mg/ml in treated samples (Sindhu *et al*., 2015). Our results are also in line with those of Vinod *et al*., (2012), who found lower protein content in wheat treated with Cu and Zn.

Phenolic compounds are found in plants and are considered secondary metabolites; they play a crucial role in the antioxidant system and stress responses (Anagnostopoulou *et al*., 2006). As illustrated in Figure 3, there was a decrease in the level of phenolic compounds in wheat treated with HMs, compared with the untreated control. This is in agreement with previous studies; phenolic content tends to be reduced in the presence of HMs, as has been demonstrated in tomato leaves. High levels of Cd have also been found to decrease phenolic content in *Lepidium sativum* (Kisa *et al*., 2019).

In the present study, DPPH-scavenging activity in treated samples was slightly higher than in control samples. Results revealed an increase in the scavenging of DPPH radicals in plants treated with $CuSO_4$, $ZnSO_4$, and $HgCl_2$, in comparison to the control; maximal enhancement of DPPH inhibition was observed with 0.1M Hg. These results agree with those reported previously by Kapoor (2014) on *Brassica juncea* plants under Cd-induced stress showing an increase in the scavenging of DPPH radicals in Cd-treated plants (76.55%), in comparison with the control (60.69%).

The effects of HMs on peroxidase (POX) activity and kinetics in crude wheatgrass juice were analyzed according to the type of inhibition: competitive, non-competitive, or mixed. In the case of uncompetitive inhibition, the inhibitor binds only to the complex enzyme-substrate and not to the free enzyme, while in the case of mixed inhibition it may bind to either the free enzyme or the enzyme–substrate complex. In competitive inhibition, the inhibitor binds to the active site of the enzyme; this may be due to a conformational change in the enzyme caused by substrate binding, revealing an inhibitor binding site, or, alternatively, the inhibitor may bind directly to the enzyme-bound substrate and cause changes in V_{max}, K_m, or both. The V_{max}/k_m ratio is known as ″catalytic power″ and is a useful parameter for identifying which heavy metals have the greatest effects. At both concentrations tested, $CuSO_4$ was found to inhibit POX activity in crude wheatgrass juice uncompetitively, by decreasing both K_m and V_{max} values. Comparing the total activity of POX in control and treated samples with $ZnSO_4$, and $HgCl_2$ revealed that it was affected by mixed inhibition. These results agree with those reported by Lih *et al.* (2006), POX activity tends to be reduced in the presence of HMs. Results are summarized in Table 3 and analyzed according to the effects of uncompetitive inhibition and mixed inhibition.

Conclusion

The results of these experiments showed the effects of three HM compounds, $CuSO_4$, $ZnSO_4$, and $HgCl_2$ at two different concentrations (0.1 M and 0.3 M) on

wheatgrass (*In vivo*). The effects of the studied metals on wheatgrass illustrated that irrigation of wheatgrass with solutions containing these metals affected seed germination, leaf color, antioxidant activity, chlorophyll content, protein content, and levels of phenolic compounds. Furthermore, HMs exerted a slight inhibitory effect on POX activity. Cu inhibited POX activity though uncompetitive inhibition, while Zn and Hg acted via mixed inhibition. It is evident from the results of this study that the selected HMs affected several vital parameters in wheatgrass, including POX enzyme activity. Therefore, we recommend further studies to analyze the effects of other HMs on biochemical parameters and antioxidant enzyme activity on wheatgrass plants *In vivo*.

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