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Effect of Priming with Natural Plant Extract on the Growth, Hormonal Status, and Yield Value of *Triticum asetivum* (L.) Grown under Lead and Nickel Stress

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HEAVY metal pollution is widespread worldwide, threatening global food security. An experiment was conducted on wheat (*Triticum asetivum* L.) to evaluate the hazard impacts of lead (Pb) and nickel (Ni) stresses and the mitigating role of *Sonchus oleraceus* (S) extract as a grain priming application. It was revealed that the growth traits of wheat plants (e.g., including lengths, fresh and dry masses of root and shoot, leaf area, and yield attributes) were significantly reduced by 100mM Pb or Ni. Chlorophylls, total pigments, and photosynthetic activity (Fv/Fm) declined, although carotenoids increased significantly. Ni stress had the highest reduction compared to the control. Moreover, the endogenous status of phytohormones and the chemical composition of the yielded grains were modified by Pb and Ni in a different pattern, indicating that wheat had varying tolerance responses to different heavy metals. The beneficial components of S that support its promising alleviating role were exhibited in an analysis of extracts. Most wheat metabolic activities were restored by the S priming, with additional significant enhancements in growth criteria, photosynthetic parameters, and crop yield productivity. The accumulation of heavy metals in the produced grains was limited by priming with S extract, with an additional enhancement in their nutritional value. The phytohormonal balance was retrieved after grain priming with the S extract. Therefore, using natural S extract as a simple priming application could be a sustainable and safe method to alleviate the adverse effects of Pb and Ni stress on wheat productivity.

Keywords: Heavy metals, Minerals, Photosynthetic pigments, Phytohormones, Wheat, Yield.

Introduction

Due to anthropogenic activities, heavy metal accumulation in the environment is one of the most common contaminants affecting humans, animals, and plants worldwide (Sharma et al., 2022). Among non-essential metals, lead (Pb) is a naturally occurring non-degradable metal with a robust binding capability, resulting in high plant accumulation (Giannakoula et al., 2021). It has been reported as the second most lethal metal after arsenic, depending on its bioavailability, toxicity, and human exposure (Kasim et al., 2014). Its phytotoxicity is manifested by decreased crop growth, photosynthesis suppression, cellular structure distortion, hampered nutrient uptake, and hormonal imbalance (Dubey et al., 2018)resulting in cellular damage and altered cellular ionic homeostasis. As a consequence, plants start detoxification mechanisms. Here, we review heavy metal toxicity and impact. We discuss metabolism and detoxification strategies of heavy metals and metalloid, with emphasis on the use of transcriptomics, metabolomics, and proteomics. A section highlights microRNA (miRNA. Nickel (Ni) is an essential trace metal with diverse oxidation states, as its most stable form is Ni^{2+} (Sachan & Lal, 2017). In plants, Ni is considered a vital nutrient due to its activating role of urease as a significant enzyme in nitrogen metabolism (Eid et al., 2021). Excessive Ni accumulation in plants severely inhibits growth, photosynthesis, mineral intake, and yield productivity (Aqeel et al., 2021). Moreover, heavy metals have been demonstrated to cause various modifications in the endogenous

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phytohormones levels of plants, thus altering the regulation of different physiological processes and harming plant architecture (Sharma et al., 2022). However, the correlation between endogenous phytohormones and cell homeostasis under heavy metal stress is poorly understood. Therefore, further studies are needed.

Many mechanisms involved in detoxifying and withstanding metal toxicity are activated at the cellular level (El-Sayed, 2019; Moustafa et al., 2019; Saber et al., 2022). Exploring these coping strategies is crucial to managing and enhancing plant tolerance. In this context, natural biostimulant applications could be validated as an eco-friendly method for improving plant responses under heavy metal stress. Various natural plant extracts have been used to enhance plant productivity and defense mechanisms (Kasim et al., 2019; Sobhy et al., 2019). *Sonchus oleraceus* L. (sow thistle) is a winter weed and a traditional medicinal plant with a wide occurrence belonging to the family Asteraceae (Corrêa et al., 2020). Their edible leaves are commonly used to treat gastrointestinal and inflammatory disorders (Chen et al., 2019). Its leaves are rich in various bioactive compounds, such as phenolics, alkaloids, flavonoids, terpenes, tannins, ionone glycosides, proteins, carbohydrates, fibers, carotenoids, and vitamin C (Li & Yang, 2018).

Wheat (*Triticum asetivum* L.) is the most pivotal cereal crop globally. Nearly one-third of the world's population consumes it as a staple diet, ensuring the nutritional security of people worldwide (Nessim & El-Shenody, 2018). The over-utilization of chemical fertilizers could cause metal accumulation and high toxicity in wheat crops (Chen et al., 2020). Therefore, the primary goal of this research was to explore the physiological and hormonal imbalances induced by Pb and Ni in *T. asetivum* crop yield. Further, this investigation aimed to assess the efficacy of priming wheat grains with *Sonchus* extract as a natural biostimulant to relieve Pb or Ni phytotoxicity.

Materials and Methods

Plant material

Wheat grains (Sakha 94) were provided for experimental use by Gimmizza Agricultural Research Station, Gimmizza, Gharbia Governorate, Egypt. The grains were selected for uniform sizes and shapes.

Collection, preparation, and chemical analysis of plant extract

Fresh shoots of *S. oleraceus* were gathered from cultivated fields during the ripening period (November–December). The samples were washed, chopped into fine pieces, and mixed in an electric blender with 100mL of tap water for every 100g before being centrifugated and filtered using Whatman No. 1 filter paper. This concentration was regarded as a 100% *Sonchus* (S) extract. The extract was further subjected to quantitative analysis of various components, including minerals (e.g., N, P, K, Ca, Mg, Mn, Zn, Cu, Fe, Ni, and Pb), phytochemicals (e.g., total soluble proteins and carbohydrates, amino acids, phenolics, flavonoids, proline, glycine betaine, cysteine, and ascorbic acid), and phytohormones. Furthermore, the aqueous crude S extract was analyzed for its ascorbic acid content according to Oser (1979).

Experimental design

Throughout the growing season (December 2020), wheat grains were sterilized with immersion in 5% sodium hypochlorite solution for 5min. Then, the grains were rinsed several times with distilled water. The grains were separated into two groups for priming treatment. A 12h period was determined depending on the preliminary experiments. The first experimental group was presoaked in distilled water. The second group was soaked for 12h in 100% S extract. After that, 10 grains from each group were sown in a plastic pot (40cm diameter and 45cm depth) filled with 20kg clay-sandy soil (2:1w/w) and left to grow under natural day/night conditions while receiving tap water once weakly. On the 10th growth day, the first group (water-soaked) of seedlings was subdivided into three treatments. Seedlings in the first treatment represented the control, which was irrigated with water until the end of the experiment. As single stress treatments, seedlings in the second and third treatments were irrigated with 100mM lead acetate or 100mM nickel sulfate, respectively. The second group (S extract-soaked) was subdivided into two treatments: either irrigated with 100mM Pb as a combined Pb+S treatment or with 100mM Ni as a combined Ni+S treatment. The stress irrigation was repeated four times at 20-day intervals until the end of the growing season. Otherwise, the pots received tap water once weakly. All treatments were represented in triplicate. The harvesting was conducted at the flowering stage (66 days old) and yield stage (125 days old).

Growth parameters quantification

The harvested 66-day-old plants were separated into shoots and roots for growth criterion measurement (e.g., root and shoot lengths, fresh and dry masses of root and shoot, and leaf area). The yield parameters were measured, and the dry grains were finely ground to powder for further analysis.

Photosynthetic parameter estimation

In the flowering plants, the photosynthetic pigments were determined at the fourth leaf from the ground using the spectrophotometric method described by Arnon (1949) for chlorophylls (Chl. a and Chl. b) and Horváth et al. (1972) for carotenoids. The leaves (0.1g) were extracted using 80% acetone, and the pigments were estimated photometrically and expressed as mg/g fresh mass (f.m.). The photosynthetic activity (Fv/Fm) was measured in dark-adapted leaves, depending on the photosystem II performance, using a digital fluorometer (OS-30 P, Hudson, USA).

Endogenous phytohormone detection

Quantitative analysis of endogenous phytohormones was performed in the shoots of wheat plants (66-day-old plants) and the freshly prepared S extract. According to Shindy & Smith (1975), the samples were extracted with methanol. The content of phytohormones was determined using gas chromatography (GC, Hewlett Packer D, HP 6890 series) at the Arid Land Agriculture Research Center, Faculty of Agriculture, Ain Shams University. The estimated endogenous phytohormones were auxins, such as indole acetic acid (IAA), gibberellins, represented by gibberellic acid (GA), abscisic acid (ABA), and cytokinins, fractioned as aromatic cytokinins (kinetin-derivative), and zeatin.

Phytochemical analysis

Phytochemical analysis was applied to the S extract and harvested grains. According to Dubois et al. (1956), the phenol sulfuric acid technique was employed to quantify total soluble carbohydrates (TSC). The contents of the total soluble proteins (TSP) were determined as described by Bradford (1976). The total amino acids (TAA) were assessed in the ethanolic extracts of the dried samples using the photometric technique of Lee & Takahashi (1966). The total phenolics (TPs) levels were estimated quantitatively using the method described by Jindal & Singh (1975) in the previously prepared ethanolic extract. The colorimetric aluminum chloride technique was used for total flavonoids (TFs) estimation, according to Chang et al. (2002). The proline content was estimated according to Bates (1973). Estimation of glycine betaine (GB) was applied according to the method of Grieve & Grattan (1983), while cysteine was estimated by Gaitonde (1967).

Mineral ions quantification

The S extract sample and the root, shoot, and grains of the wheat plants were digested with a mixture of $HNO_{3:}H_2O_2$(5:2 v/v). The digested filtrate obtained was used to estimate the mineral composition (K, Ca, Mg, Mn, Zn, Cu, Fe, Pb, and Ni) using an inductively coupled plasma-optical spectrophotometer (Polyscan 61E, Thermo Jarrell-Ash Corp., Franklin, MA, USA). The colorimetric assay by Allen et al. (1974) was utilized for N and P estimation using the Rochelle reagent and molybdenum blue methods, respectively, against their standard calibration curves.

Statistical analysis

The results were analyzed statistically using one-way Analysis of Variance (ANOVA) to determine the degree of significance of the obtained results. The analysis was conducted using the COSTAT statistical program. A significance level of $P \leq 0.01$ was considered.

Results and Discussion

Chemical analysis of plant extract

It was revealed that the S extract analysis contained a wide range of beneficial constituents (e.g., minerals, phytohormones, proteins, amino acids, carbohydrates, phenolics, flavonoids, proline, cysteine, GB, and ascorbic acid), as shown in Table 1. Macronutrients (e.g., N, P, K, Ca, and Mg) and other micronutrients (e.g., Mn, Zn, Cu, Fe, and Ni) were also represented. According to the present data, K, N, and P had the highest levels, while Ni had the lowest content. Among the phytochemical constituents, ascorbic acid had a high content (9.6mg/mL). At the same time, other antioxidants, including phenolics and flavonoids, had concentrations of 6.1 and 0.159mg/mL in the extract, demonstrating its high antioxidant activity. Carbohydrates, proteins, and amino acids had elevated levels in the S extract. Moreover, quantifying the phytohormonal content of the S extract accounted for an elevated level of GA (3.51µg/100mL) and aromatic cytokinins (0.885µg/100mL).

TABLE 1. Phytochemical analysis of *Sonchus* aqueous extract

Minerals (µg/ mL)		Phytochemicals (mg/mL)		Phytohormones (µg/100mL)	
N	1015±4	Total soluble proteins	9.57±0.11	Gibberellic acid	3.51±0.18
P	140±19	Total soluble carbohydrates	14.6±0.2	Indole acetic acid	0.208±0.009
K	1277±35	Total soluble amino acids	12.3±0.4	Abscisic acid	0.018±0.003
Ca	289±7	Total phenolics	6.1±0.3	Aromatic cytokinins	0.885±0.019
Mg	99.6±8.6	Total flavonoids	0.159±0.013	Zeatin	0.104±0.009
Mn	1.54±0.30	Proline	0.277±0.001		
Zn	1.18±0.07	Glycine betaine	0.081±0.001		
Cu	0.42±0.07	Cysteine	0.013±0.002		
Fe	1.57±0.25	Ascorbic acid	9.6±2.0		
Ni	0.02±0.01				
Pb	*				

* Not detected

Growth criteria under heavy metal stress and the S extract priming

As shown in Table 2, there was a significant adverse effect on all growth parameters for plants irrigated with 100 mM Pb or Ni. Pb and Ni stress caused a highly significant decrease in root and shoot traits, including lengths and fresh and dry masses, compared to the control plants. Such growth and biomass losses have been attributed to the excessive interference of Pb or Ni with most biochemical and physiological processes relevant to normal plant growth and development (Ashraf et al., 2017). The mitotic activity of the plant cells was lowered by the high solubility and biochemical reactivity of the metals inside the cells, reducing the length and development of the roots and shoots (Kazouz et al., 2020). Similarly, heavy metal stress has been recorded to reduce growth in many plants, including faba beans, wheat, and sunflower (Kasim et al., 2014; Sobhy et al., 2019; Ragab & Saad-Allah, 2020). Also, the leaf area significantly decreased under Pb and Ni applications by 32% and 28%, respectively, relative to the control plants (Table 2). The obtained decrease in leaf area was responsible for a considerable reduction in the photosynthesis apparatus as a side effect of metal toxicity (Nas & Ali, 2018).

All the measured growth parameters of the wheat plants were positively enhanced by the priming with S extract, as recorded in Table 2. The growth reductions obtained with Pb or Ni stresses were successfully limited by *S.* extract due to its rich content of many beneficial constituents (Table 1). The relieving ability of S extract was significant under Ni stress, and the studied growth parameters mostly reached the control values. The wide range of these components could contribute to growth and development, thus boosting plant tolerance (Li & Yang, 2018; Kasim et al., 2019). The rich content of phytohormones and mineral elements, including macronutrients (e.g., N, P, K, Mg, and Ca) and micronutrients (e.g., Mn, Cu, Fe, Zn, and Ni), was another advantage that could sustainably stimulate plant growth.

Photosynthetic parameters under heavy metal stress and S extract priming

In response to Pb or Ni applications, the photosynthetic criteria were noticeably harmed, as shown in Fig 1. The levels of chl a, chl b, total pigments, and chl a/b ratio were significantly reduced by 13%, 7%, 4%, and 6%, respectively, after Pb application compared to the control. The Ni stress had a more severe reduction in chl b and total pigments, with decrease percentages of 20% and 7%, respectively. Consequently, there was an 8% rise in the chl a/b ratio relative to the control (Fig. 1). The negative impacts of Pb or Ni were attributed to the inhibition of enzymes involved in chlorophyll biosynthesis and photosynthesis, altered stomatal conductance, and disrupted chloroplast membranes (Kazouz et al., 2020). The toxic effects of Ni on chl b content, the major chlorophyll of PS II, were demonstrated by the elevation of the chl a/b ratio under Ni stress, causing a disturbance in the fluidity and composition of the oxygen-evolving complex (Beri & Sharma, 2016).

TABLE 2. Effect of grain priming in *Sonchus* aqueous extract on growth parameters of wheat plants grown in clay-sandy soil (2:1w/w) and irrigated with 100mM Pb and Ni treatments

Treatments	Length (cm/organ)		Fresh mass (g)		Dry mass (g)		Leaf area (cm^2/leaf)
	Root	Shoot	Root	Shoot	Root	Shoot	
Control	41.8±0.29a	43.2±0.58b	0.72±0.03a	2.91±0.02a	0.21±0.011a	0.59±0.006a	7.6±0.2b
Pb	34.6±0.21b	37.2±0.29d	0.58±0.02c	1.94±0.09c	0.16±0.005c	0.47±0.012c	5.2±0.2c
Ni	32.5±0.42c	37.2±0.25d	0.41±0.01d	1.32±0.09d	0.12±0.006d	0.42±0.015d	5.5±0. 1c
Pb + S	42.0±0.00a	39.9±0.36c	0.76±0.01a	2.37±0.06b	0.18±0.010b	0.50±0.006b	7.5±0.1b
Ni + S	42.0±0.20a	44.5±0.50a	0.64±0.01b	3.00±0.10a	0.18±0.006b	0.60±0.006a	8.0±0.1a
LSD	0.68	1.07	0.04	0.19	0.02	0.03	0.37
F-value	965	197	223	283	45	214	250

Values are means of the three replicates with ± SD. The different letters indicate significant differences at P< 0.01 according to the LSD test.

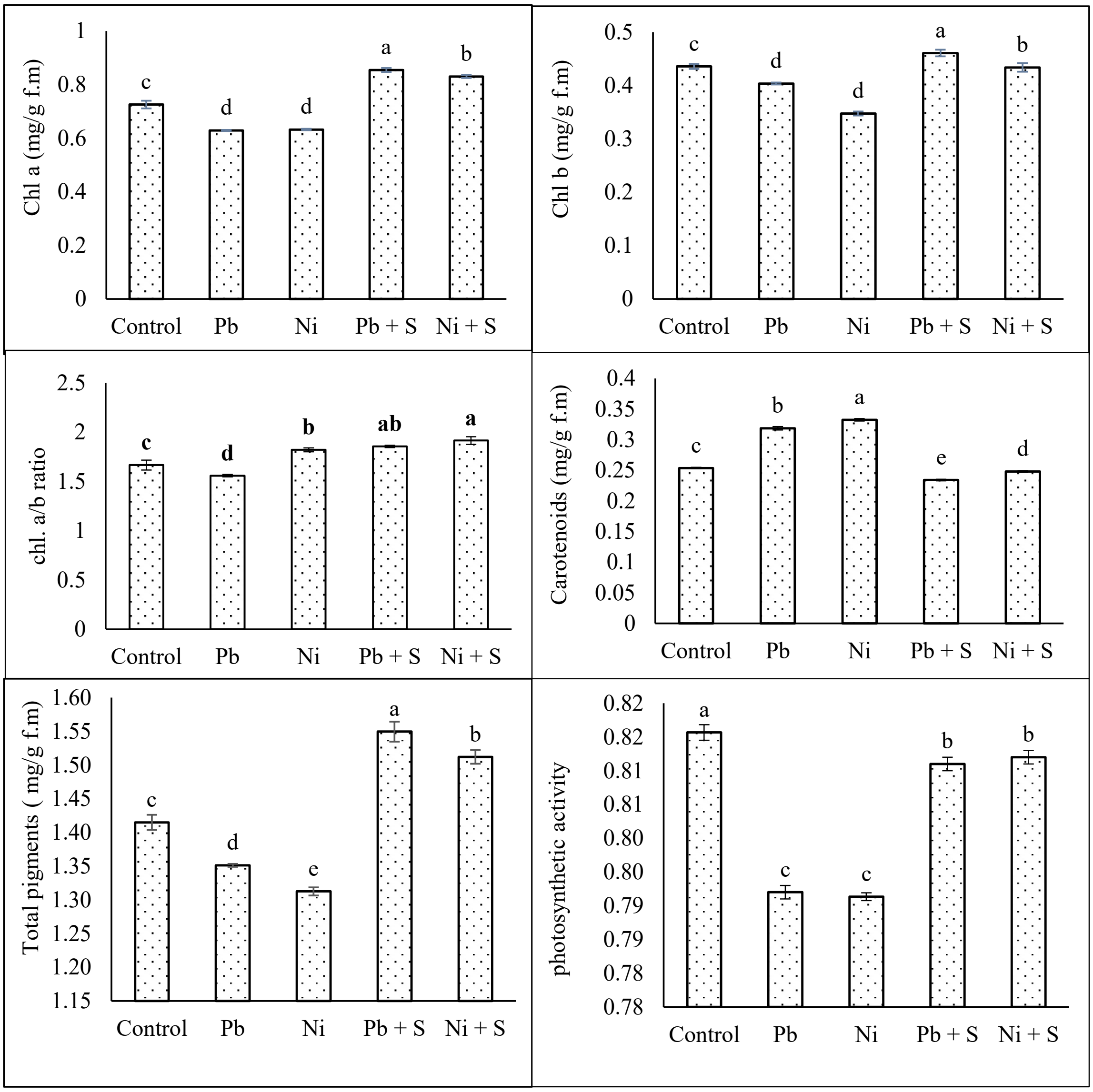

Fig. 1. Effect of grain priming in *Sonchus* aqueous extract on photosynthetic parameters of wheat plants grown in clay-sandy soil (2:1w/w) and irrigated with 100mM Pb and Ni treatments [The different letters indicate significant differences at P< 0.01 according to the LSD test]

Further, the carotenoids level showed a significant elevation in response to both heavy metal stresses. At the same time, their photosynthetic activity decreased by 3% compared to the control treatment (Fig. 1). Increasing the carotenoids content by metal stress refers to an increase in the non-enzymatic antioxidant defense, offering its free radical scavenging employment (Shabani et al., 2016). The inhibited Fv/Fm (photochemical efficiency of PS II) reflected a depletion of the photosynthesis process that positively correlated with the blockage of electron transport, reduction of Calvin cycle enzymes, and disruption of chloroplast ultrastructure (Nessim & El-Shenody, 2018).

On the other hand, the harmful impacts of Pb and Ni stress were lessened by priming wheat grains with S extract (Fig. 1). A significant elevation in the contents of chl a, chl b, total pigments, and even chl a/b were recorded under both Pb+S and Ni+S treatments. The values of these contents were triggered over the control levels. Moreover, the presoaking of wheat grains with S extract restored the photosynthetic activity after Pb or Ni stress and significantly increased by 3% compared to the single stress treatments. The overproduction of carotenoids induced by either Pb or Ni was retrieved by S extract application to reach near the control levels, indicating a mitigating effect. The results were similar to previous reports that used natural plant extracts to enhance photosynthetic pigment levels and photosynthetic efficiency against drought and heavy metals (Kasim et al., 2016; Sobhy et al., 2019). The S extract priming could supply the plant with adequate amounts of several minerals, including Mg and Fe, which may enhance chlorophyll biosynthesis. Moreover, the high ASA content in the S extract could activate plant antioxidant machinery by reducing the photodamage of thylakoid membranes induced by heavy metals (Tambussi et al., 2000).

Endogenous phytohormones under heavy metal stress and S. extract priming

Abiotic stressors, including heavy metals, have been linked to changes in phytohormones synthesis, distribution, and signal transduction in plants due to their cellular toxicity (Fahad et al., 2015). Nevertheless, the involvement of endogenous phytohormones in heavy metal stress adaption has not been fully explained (Saini et al., 2021). In this study, the estimated phytohormones had many modifications in response to heavy metal stress. Accordingly, Pb stress resulted in a significant rise in the levels of GA, ABA, and aromatic cytokinins (ArCKs) by 29%, 41%, and 6%, respectively, compared to the control. The levels of IAA and zeatin exhibited marked reductions under Pb stress by 98% and 57%, respectively, compared with the control treatment. Moreover, total cytokinins (TCKs) was increased slightly by Pb compared to the control. Alternatively, Ni application resulted in a remarkable elevation in the contents of IAA, GA, ABA, and zeatin by ratios of 29%, 42%, 464%, and 47%, respectively, compared to the control. At the same time, a significant reduction in ArCKs content and TCKs was caused by the Ni application.

The results shown here provide evidence of the contribution of phytohormones to plant responses to heavy metal stress, where they altered their endogenous levels. The GA level was elevated because Pb or Ni stress could be linked to triggering cellular processes connected to heavy metal responses and their involvement in stimulating plant development (Verslues, 2016). Similarly, exposure to Zn stress elevated the endogenous GA content, indicating a relationship between endogenous GA and heavy metal stress adaption (Atici et al., 2005). The upregulation of ABA under both metal stresses might be induced by excessive reactive oxygen species (ROS) acting as a signaling molecule, regulating stomatal closure, and preventing water loss under stress conditions (Bücker-Neto et al., 2017). In response to toxic metal exposure, the expression of genes involved in ABA biosynthesis and ABA signaling was considerably induced (Huang et al., 2012). A higher endogenous ABA concentration was previously recorded under Cd stress due to the elevated expression of heavy metal ATPase, which is responsible for root-to-shoot metal translocation via the apoplast (Lu et al., 2020).

The distinct decrease in endogenous IAA under Pb stress might be due to the suppression of IAA synthesis, transportation, and distribution, which may be accompanied by growth disruption (Jalmi et al., 2018). Moreover, higher ROS concentrations were stimulated by Pb stress that could potentially increase oxidizing activity by IAA oxidase and its catabolism (Emamverdian et al., 2020). However, the endogenous IAA triggered by Ni stress indicated the different responses of wheat plants to various heavy metal stresses. The overproduction of IAA due to Ni indicates its ability to regulate growth and development under abiotic stress situations (Siddiqui et al., 2017). It was recorded in several studies that the alteration of the endogenous levels of auxins upon heavy metal application had positive and negative relations between stress and auxin levels (Srivastava et al., 2013; Yuan & Huang, 2015). Increased expression of the auxin biosynthesis gene (YUCCA6), which encodes flavin monooxygenase-like proteins, was obtained in Arabidopsis after Cd treatment. However, arsenic stress decreased its expression and endogenous IAA content (Fattorini et al., 2017). Auxin homeostasis is controlled differently under various heavy metal stresses. Thus, IAA depletion induced by Pb has an acute impact on plant growth, leading to a decrease in meristematic activity and cell division (Saini et al., 2021).

Moreover, Pb and Ni stress had different effects on endogenous CK (Table 3). In response to Pb application, a significant decrease in zeatin content was observed, while ArCKs reached a significant rise, indicating their varied roles. Regarding Ni stress, it caused upregulation of zeatin while it significantly decreased the levels of the ArCKs and TCKs levels. The efficient role of endogenous ArCKs in abiotic stress adaptation has recently been discussed despite not being traditionally reported as a part of stress response machinery (Castander-Olarieta et al., 2021). The decreased CKs content due to heavy metals could be attributed to its degradation and the stimulation of oxidases involved in CKs catabolism (Hashem, 2014). Treatment with Pb might lessen the pool of active CKs and mediate their deactivation by N- and O-glucosylation (Piotrowska-Niczyporuk et al., 2020). Nevertheless, heavy metal stress was recorded to improve CKs levels in the shoots of Arabidopsis plants. These findings were attributed to the decreased expression of the CKs catabolic gene and cytokinin oxidase induced by Cd stress and the increased expression of isopentyl transferase (Vitti et al., 2013). The overproduction of endogenous CKs, as a response to stress, was interestingly accompanied by increased ROS, leading to less resistance and decreased chlorophyll content (Wang et al., 2015) that coincided with our results (Fig. 1).

According to the data in Table 3, the heavy metal stress impacts were modified by priming wheat grains with S extract. Under Pb stress, S extract priming restored the declined levels of IAA and zeatin, reaching their values near the control counterparts. Moreover, the Pb-induced elevations of GA, ABA, ArCKs, and TCKs contents were distinctly hindered to match the control levels with S priming. Therefore, the normal phytohormonal content was restored due to *S.* extract priming under Pb application. Under Ni application, *S.* priming caused a diverse modulation of endogenous hormones and modified the Ni-induced up- and down-regulations. Ni-induced ABA was hampered by 29% after *S.* priming compared to the single Ni treatment. Whereas the increased levels of IAA and GA decreased slightly compared to Ni stress, their values remained higher than those of the control. Compared to Ni stress, *S.* priming caused a 27% reduction in the ArCKs and a 12% increase in the level of zeatin. The substantial regulation of these endogenous phytohormones could be ascribed to the S extract content of the phytohormones (Table 1). Phytohormones can boost diverse signal transduction pathways and enhance heavy metal stress tolerance and modulation when applied exogenously at adequate levels (Sytar et al., 2019; Saini et al., 2021). For instance, the exogenous application of cytokinins was recorded to improve stress tolerance by triggering endogenous levels in wheat leaves (Veselova et al., 2005). The S extract, with its rich phytohormones content, could modulate the protein redox state by blocking the action of free radicals and replacing the defense roles of endogenous hormones (Ben Massoud et al., 2018). As previously proposed by Liu et al. (2020), the decreased CKs content in the combined Ni+S treatment may support its increased tolerance to abiotic stress. Prospectively, plants with reduced cytokinin levels could have a high tolerance for abiotic stresses due to their inhibited synthesis or stimulated degradation (Avalbaev et al., 2016; Liu et al., 2020). The homeostasis of cytokinins and various synergistic events that occur under heavy metal stress in plant cells require further study and comprehensive explanations.

Yield parameters and mineral content of harvested grains under heavy metal stress and S. extract priming

The harmful effects of irrigation with Pb or Ni stress were manifested in hindered yield productivity (Table 4). The estimated yield traits, including spike length, spike mass, spikelet number, number of grains/spike, the mass of grains/spike, the mass of 1000 grains, and the percentage of grain filling, were significantly lowered by Pb or Ni stresses. Compared to the control, Ni had more severe impacts where the spike mass, the mass of grains/spike, and the mass of 1000 grains decreased by 27%, 44%, and 40%, respectively. The reduction in yield parameters under Pb and Ni was a direct consequence of vegetative growth reduction (Table 2), nutrient disturbance (Table 5), and inhibited photosynthetic traits (Fig. 1). Similarly, a recorded decline in the photosynthesis capacity of rice leaves led to reductions in the carbohydrate requirements of pollen and spikelet tissues (Sharma et al., 2018).

Furthermore, The significant decrease in IAA content and vegetative growth under Pb stress (Table 3) may be responsible for spikelet sterility and losses at the yield drop (Sharma et al., 2018). The high content of Pb or Ni in the yielded grains (Table 5) could cause high oxidative damage and diminished transport of assimilates toward developing grains, harming plant productivity (Kasim et al., 2019). The high metal content in the grains could interfere with metal binding to the cell wall, leading to increased rigidity and reduced cell division (Kasim et al., 2014).

As shown in Table 5, Pb stress lowered N, P, and K contents of yielded grains by 18%, 27%, and 32%, respectively. At the same time, Ni stress reduced N, P, and K contents by 11%, 22%, and 16%, respectively relative to control. Moreover, the irrigation with Pb or Ni dramatically increased the levels of Pb or Ni in the obtained grains by 53% and 87%, respectively, compared to their control values. According to these results, Pb or Ni toxicities were found to be extended to the inhibited uptake of minerals, which corresponded to high levels of Pb and Ni (Kang et al., 2017). The cell metabolism can be disrupted entirely due to the ionic replacement of Pb and Ni with other divalent cations in many vital locations (Sobhy et al., 2019). The shortage of N content in the yielded grains may be elucidated by the high affinity of Pb and Ni for the active site of nitrate reductase, inhibiting protein synthesis, growth, and development of plants (Nas & Ali, 2018).

TABLE 3. Effect of grain priming in *Sonchus* aqueous extract on endogenous phytohormones of wheat plants grown in clay-sandy soil (2:1w/w) and irrigated with 100mM Pb and Ni treatments

Treatments	Endogenous phytohormones (mg/100g f.m)					
	IAA	GA	ABA	ArCKs	Zeatin	TCKs
Control	2.08±0.09b	67.9±1.7c	0.137±0.017d	11.58±0.06b	0.793±0.017b	12.36±0.03a
Pb	0.43±0.11c	87.7±0.9b	0.192±0.002c	12.23±0.10a	0.338±0.025c	12.57±0.07a
Ni	2.69±0.10a	96.1±2.1a	0.771±0.020a	8.49±0.11d	1.165±0.152a	9.66±0.11b
Pb + S	1.92±0.07b	64.8±0.8c	0.164±0.008cd	9.29±0.24c	0.657±0.052b	9.95±0.35b
Ni + S	2.53±0.03a	93.1±0.9a	0.551±0.009b	6.16±0.14e	1.309±0.021a	7.46±0.15c
LSD	0.22	3.56	0.03	0.36	0.18	0.46
F- value	337	338	1531	903	88	416

Values are means of the three replicates with ± SD. The different letters indicate significant differences at P< 0.01 according to the LSD test.

TABLE 4. Effect of grain priming in *Sonchus* aqueous extract on yield parameters of wheat plants grown in clay-sandy soil (2:1w/w) and irrigated with 100mM Pb and Ni treatments

Treatments	Spike length (cm)	Spike mass (g)	Spikelet No/spike	No of grains/ spike	Mass of grains/ spike (g)	Mass of 1000 grains (g)	Grain filling (%)
Control	9.77±0.06a	1.47±0.01b	19±1a	31±1b	1.15±0.01b	37.83±0.12b	78.7±0.32c
Pb	8.17±0.06d	1.32±0.02c	16±1b	27±1c	1.03±0.01c	35.97±0.61c	74.9±0.06d
Ni	8.57±0.06c	1.07±0.06d	16±1b	27±1c	0.64±0.01e	22.86±0.09e	72.9±0.64e
Pb + S	9.47±0.06b	2.05±0.05a	19±1a	34±1a	1.52±0.02a	39.07±0.07a	89.3±0.35a
Ni + S	9.73±0.06a	1.24±0.02c	18±1a	30±1b	0.76±0.02d	32.08±0.04d	83.3±0.06b
LSD	0.15	0.09	1	2	0.04	0.73	0.92
F- value	479	326	21	179	1741	1603	1036

Values are means of the three replicates with ± SD. The different letters indicate significant differences at P< 0.01 according to the LSD test.

TABLE 5. Effect of grain priming in *Sonchus* aqueous extract on mineral nutrients and heavy metals of wheat yielded grains grown in clay-sandy soil (2:1w/w) and irrigated with 100mM Pb and Ni treatments

Treatments	Macronutrients (mg/g d.m)			Heavy metal ions (µg/ g d.m)	
	N	P	K	Pb	Ni
Control	3.48±0.13ab	0.41±0.02a	0.38±0.01a	2.50±0.04bc	14.3±1.5c
Pb	2.87±0.07c	0.32±0.02c	0.26±0.02d	3.83±0.22a	11.5±0.8d
Ni	3.10±0.04b	0.38±0.01ab	0.32±0.01c	2.31±0.09c	26.7±0.8a
Pb + S	3.44±0.08ab	0.30±0.01a	0.31±0.02c	2.84±0.10b	13.0±1.5cd
Ni + S	3.50±0.08a	0.35±0.02bc	0.36±0.01b	2.27±0.15c	17.8±0.6b
LSD	0.30	0.03	0.02	0.37	2.54
F- value	16.32	23.66	67.78	58.74	113.98

Values are means of the three replicates with ± SD. The different letters indicate significant differences at P< 0.01 according to the LSD test.

On the other hand, grain priming with the S extract alleviated the inhibitory effects of Pb or Ni stress and caused a significant increase in all the measured yield parameters, where their values were close to the control (Table 4). The current results are in agreement with those of Yasmeen et al. (2013). These researchers recorded that moringa leaf extract improved wheat grain yield under abiotic stress due to its micro-and macro-elements, antioxidants, and cytokinins. The high GA in the S extract (Table 1) might regulate various plant processes involved in stress adaptation and enhance yield growth and grain productivity (Kosakivska et al., 2022). Additionally, priming with S extract significantly improved the N, P, and K content of the yielded grains in both Pb+S and Ni+S treatments to match the control levels (Table 5). The high accumulation of Pb or Ni was significantly lowered by S extract priming, and their levels decreased by 26% and 33% in the Pb+S and Ni+S treatments compared to the single stress treatments. Therefore, the increased wheat yield is related to enhanced mineral uptake and decreased accumulation of Ni and Pb. The S extract contained a wide range of essential minerals and nutrients (Table 1) that can intensify endogenous mineral levels and enhance crop yield. These results follow those by Sobhy et al. (2019), who used weed extract as a safe priming strategy to decrease metal accumulation in wheat. On a similar scale, exogenous GA applications increased the N content and proteins under Cr stress and modulated the activities of the enzymes incorporated in nitrogen assimilation and ROS detoxification (Gangwar et al., 2011). Applying adequate levels of CKs in conjunction with ABA alleviated Co stress and regulated its uptake, translocation, and bioaccumulation (Kamran et al., 2021).

Chemical composition of harvested grains under heavy metal stress and S extract priming

The results in Figs. 2 and 3 indicated that Pb or Ni stress applications caused an accumulation of a wide range of osmolytes and secondary metabolites in the harvested grains. TSC, TSP, TAA, proline, GB, TPs, and TFs were significantly increased by Pb stress relative to the control. Meanwhile, their contents also increased with Ni stress, except for TSP and proline. TSC accumulation in grains under Pb and Ni treatments could be an adaptive mechanism for maintaining osmotic adjustments (Khanna et al., 2019). The obtained increase in TSP with Pb stress could be attributed to the induction of stress proteins involved in the maintenance of the cellular redox state (Kasim et al., 2019). Alternatively, the reduction in protein levels under Ni stress might be due to protein degradation correlated with the increased free amino acid content (Hildebrandt, 2018). In this context, the Ni-induced TAA of grains may be related to inhibited protein aggregation (Khan et al., 2020). Moreover, the increased TAA in the yielded grains under both stresses could be attributed to the altered expression of stress-responsive genes (Khanna et al., 2019).

The accumulated proline in wheat grains under Pb stress may be linked to its role as a stress marker in plant tolerance and survival under stress. It is an osmoregulatory compound and a chaperone molecule for protein stabilization and ROS scavenging (Tousi et al., 2020). Nevertheless, Ni treatment decreases proline levels in wheat grains, which may be a result of inhibiting its biosynthetic enzymes due to accumulated Ni metals (Meena et al., 2019). Proline oxidation might be an alternative respiratory source that provides the cell with essential energy during abiotic stresses (Hildebrandt, 2018). Elevated GB levels by Pb and Ni could be related to the increased expression of genes encoding its biosynthetic enzymes (Islam et al., 2021) and its defensive effects against stress, including osmotic adjustment and macromolecular stabilization (Ahmad et al., 2013). The accumulation of phenolics and other secondary metabolites in wheat grains under Pb and Ni stress (Fig. 3) was mainly ascribed to antioxidative defense and adaptation against heavy metals (Kasim et al., 2016). Wheat grains have been reported to accumulate increased phenolic and flavonoid contents due to their radical scavenging ability (Li et al., 2015). The different defense strategies of wheat under various heavy metal stresses were indicated by these results.

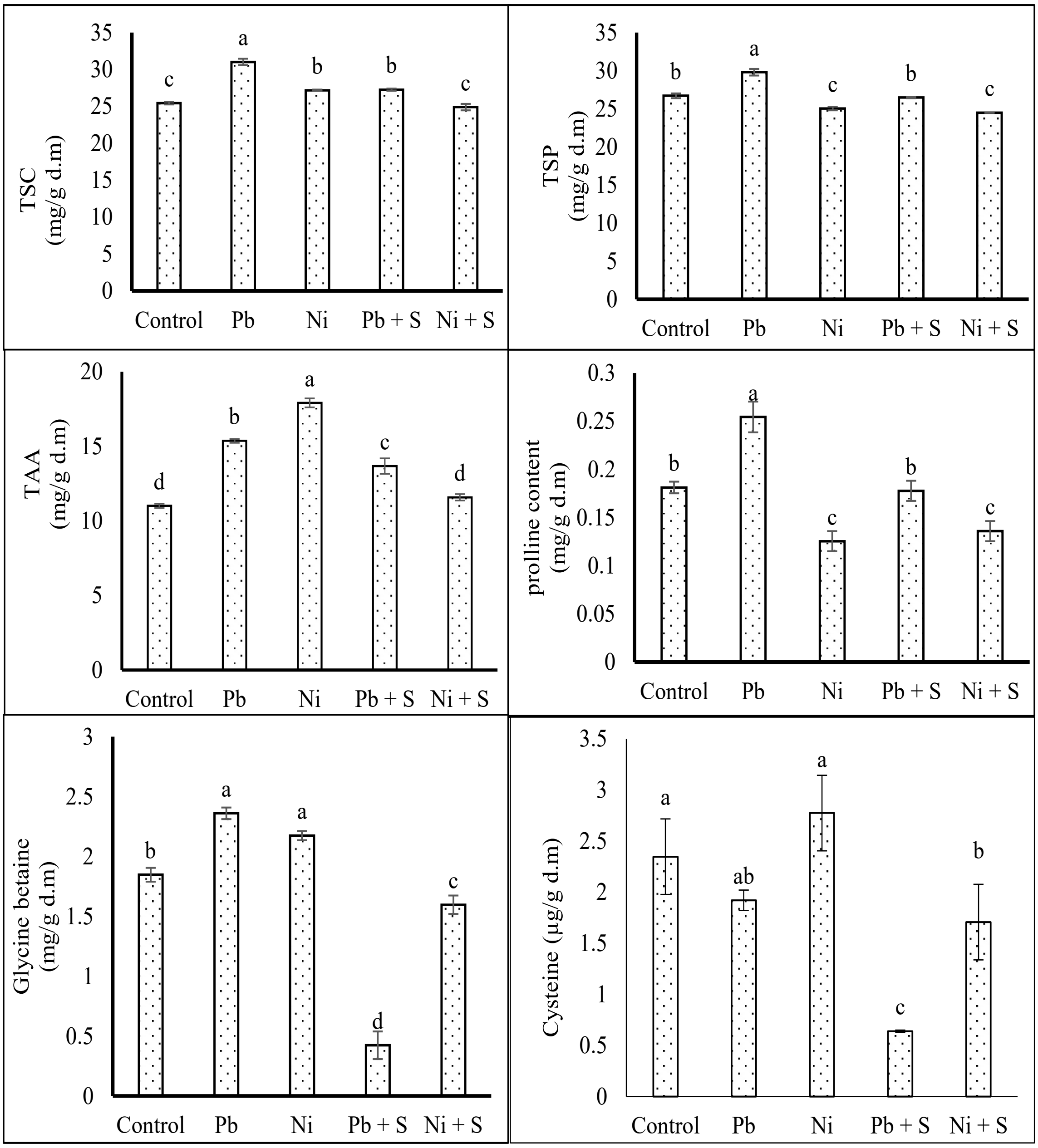

Fig. 2. Effect of grain priming in *Sonchus* aqueous extract on the chemical composition of yielded wheat seeds grown in clay-sandy soil (2:1w/w) and irrigated with 100mM Pb and Ni treatments [The different letters indicate significant differences at P< 0.01 according to the LSD test]

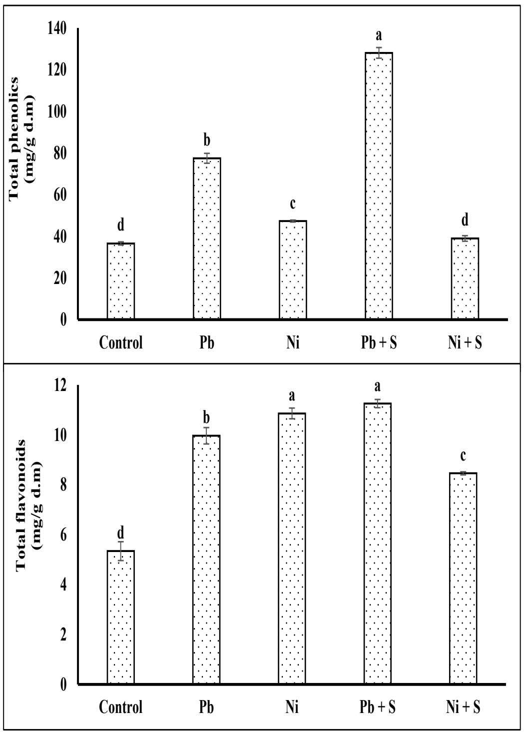

Fig. 3. Effect of grain priming in *Sonchus* aqueous extract on chemical composition yielded seed parameters of wheat plants grown in clay-sandy soil (2:1w/w) and irrigated with 100 mM Pb and Ni treatments. The different letters indicate significant differences at P< 0.01 according to the LSD test

The priming with the *S.* extract resulted in a noticeable recovery from Pb and Ni applications. At the same time, it restored the TSC, TSP, TAA, and proline levels in the yielded grains to reach the control values (Fig. 2). Similarly, the harvested Vicia faba seeds treated with heavy metals and exogenous phytohormones had an enhanced accumulation of soluble sugars and proteins (Mansour & Kamel, 2005). Under Pb stress, the priming with S extract caused a pronounced increase in TPs and TFs contents by 65% and 13% respectively; but significantly lessened GB and cysteine levels relative to Pb stress (Fig. 3). Concerning Ni stress, the contents of GB, cysteine, and TPs were retrieved to match the control values by S extract presoaking. The recorded high levels of TPs and TFs could be due to their role in ROS scavenging by neutralizing free radicals and their chelating metal properties (Jia et al., 2018). The cysteine content was pronouncedly decreased by priming with the S extract, which may be related to its involvement in non-enzymatic antioxidant biosynthesis (Saad-Allah & Ragab, 2020). The decreased accumulation of GB and proline in the grains may be illustrated by the decreased metal uptake and toxicity.

Conclusion

The current investigation could demonstrate that wheat had varying responses to Pb and Ni stressors, causing hormonal imbalance, vegetative growth reduction, and yield loss. The application of *Sonchus* (S) extract as a simple and safe grain priming method can successfully retrieve the cellular homeostasis, growth, photosynthesis, and phytohormones balance, maintaining yielded grains productivity and nutritional value. Therefore, the S extract is a potential source of highly bioactive constituents that could be recommended as a sustainable method for alleviating Pb and Ni stress in wheat.

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تأثير النقع بمستخلص نباتي طبيعي على النمو ومحتوى الهرمونات وإنتاجية القمح المزروع تحت ضغط الرصاص أو النيكل

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يعد تلوث المعادن الثقيلة مشكلة واسعة الانتشار تهدد الأمن الغذائي العالمي. في هذه الدراسة، تم إجراء تجربة على القمح لتقييم التأثيرات الخطرة لإجهاد الرصاص والنيكل والدور المعالج لمستخلص الجعضيض. أوضحت النتائج أن 100 ملي مول من الرصاص أو النيكل قلل نمو نباتات القمح بما في ذلك الأطوال والكتل الطازجة والجافة للجذور والمجموع الخضرى ومساحة الأوراق وانتاجية المحصول. وايضا انخفض الكلوروفيل، والأصباغ الكلية، ونشاط البناء الضوئي بسبب المعادن الثقيلة ولكن ازدادت الكاروتينات. بينما كان تأثير النيكل الضار أعلى مقارنة بالمعاملة القياسية. أدى كل من الرصاص أو النيكل لتغيير محتوى الهرمونات النباتية الداخلية والتركيب الكيميائي للحبوب المنتجة مما يشير إلى أن القمح لديه استجابة تحمل مختلفة للمعادن الثقيلة المختلفة. أظهر تحليل مستخلص الجعضيض مكوناته المفيدة التي تدعم دوره المعالج الواعد حيث أدى إلى استعادة معظم الأنشطة الأيضية وعزز بشكل كبير قياسات النمو، والتمثيل الضوئي، وإنتاجية المحصول. ومن المثير للاهتمام أن النقع باستخدام مستخلص الجعضيض قلل من تراكم المعادن الثقيلة في الحبوب المنتجة وعزز قيمتها الغذائية وتم استرداد التوازن الهرموني النباتى. لذلك يمكن استخدام مستخلص الجعضيض الطبيعي بالنقع كطريقة آمنة مستدامة للتخفيف من الآثار السلبية لاجهاد الرصاص أو النيكل على إنتاجية القمح.