

## Elucidating the role of melatonin or sugar beet pulp pellet in physiological improvement characteristics and promoting the growth of *Moringa oleifera* under lead stress

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### Abstract

Pot experiments were conducted to evaluate the effect of root and foliar uptake of Pb on *Moringa oleifera* plants. The levels of Pb used in the experiments were 100 and 400 ppm Pb. The lead application affected the growth, photosynthetic pigment, carbohydrates, proline, oxidative stress biomarkers, mineral contents, and Pb accumulation in *Moringa* plants. The growth parameters, chlorophyll, Na, K, and Ca content declined in the case of soil or foliar Pb application. The foliar lead application revealed a more negative effect on the growth of moringa plants than the soil application. However, Pb-stressed moringa plants increased carbohydrates, proline, H<sub>2</sub>O<sub>2</sub>, MDA, electrolyte leakage, Pb, and Mg content. In addition, the possible role of melatonin (MEL) and sugar beet pulp pellets (SBP) in ameliorating lead toxicity and enhancement of phytoremediation was investigated. It was found that supplemental addition of MEL or SBP increases the growth parameters, photosynthetic pigments, carbohydrates, proline, and minerals compared to stressed moringa plants. Moreover, a decrease in hydrogen peroxide, lipid peroxidation, and electrolyte leakage was observed under MEL and SBP treatments. The ameliorating effect of SBP was more pronounced than that of MEL. Furthermore, MEL application enhanced the phytoremediation capacity of moringa plants.

**Keywords:** electrolyte leakage; lipid peroxidation; mineral content; phytoremediation

### Introduction

*Moringa* (*Moringa oleifera* Lam), family Moringaceae, is a fast-grown crop native to India and Pakistan. It is now indigenous to many regions in Africa, Arabia, South East Asia, the Pacific and Caribbean Islands, and South America. *Moringa* is commonly known as the miracle tree as it has a multitude of medicinal and nutritional values. *Moringa* is a multi-purpose plant that can be used as a field or fodder crop, crop growth enhancer, bio-pesticides, biogas, water purification, phyto-medical source, etc. (Nouman *et al.*, 2013; Suarez *et al.*, 2003). All plant parts of *Moringa oleifera* are traditionally used for different purposes, but leaves are generally the most used (Popoola and Obembe, 2013; Sivasankari *et al.*, 2014). *Moringa* leaves have been reported to be a rich source of protein, minerals, vitamins, and antioxidants (Fuglie, 2000; Reetu *et al.*, 2020).

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Melatonin (N-acetyl-5-methoxytryptamine) is a low molecular weight tryptophan-derived natural product produced in all living organisms from bacteria to mammals (Back *et al.*, 2016; Choi *et al.*, 2017). In higher plants, melatonin was first discovered in 1995 (Dubbels *et al.*, 1995; Hattori *et al.*, 1995). Afterward, its presence has been clearly demonstrated in all plant organs, including the root, stem, leaf, flower, fruit, and seed (Arnao, 2014; Cole *et al.*, 2008). Melatonin (MEL), a powerful antioxidant, has been shown to be important in plant stress tolerance (Cui *et al.*, 2017; Sharma and Zheng, 2019). Exogenous administration of MEL has been shown to reduce the negative impacts of several biotic and abiotic stressors by increasing antioxidant enzyme activity and non-enzymatic antioxidant production (Hasan *et al.*, 2015). Furthermore, MEL has been shown to be capable of reducing the adverse effects of heavy metals stress through activating various antioxidant mechanisms (Hasan *et al.*, 2015; Sarafi *et al.*, 2017).

Sugar beet pulp (SBP) is the fibrous, energy-rich by-product resulting from the water extraction of sugar contained in the root of the sugar beet (*Beta vulgaris* L.). It is a popular feed used by dairy farmers as a structural carbohydrate and is also widely used as an ingredient in the production of pet foods. Caravaca *et al.* (2005) demonstrated that sugar beet-residue amendment increased the total carbohydrates and soluble C-fraction (water-soluble C and water-soluble carbohydrates) in the rhizosphere of *Cistus albidus* L. and *Quercus coccifera* L. Furthermore, it was reported that there is an increase in growth and nutrient uptake of alfalfa grown in soil amended with microbial-treated sugar beet waste, according to Rodriguez *et al.* (2015).

Heavy metal pollution of air and agricultural soils is one of the most prevalent ecological problems on a global scale. Industrialization, urbanization, uncontrolled use of fossil fuel resources, pesticides, chemical fertilizers, mining, smelting activities, and improper waste management remain the major causes of elevated levels of toxic heavy metals in the environment (Shahid *et al.*, 2017; Maksoud *et al.*, 2022). According to the US Environmental Protection Agency, lead is one of the most common heavy metal contaminants in the environment threatening human health (Kushwaha *et al.*, 2018; El-Sheshtawy *et al.*, 2022). Although lead is not an essential element for plants, it is absorbed easily and accumulated in different plant parts (Sharma and Dubey, 2005). Pb not only affects plant growth and productivity but also enters the food chain, causing health hazards to humans and animals. Excess of Pb in plants can alter a series of biological mechanisms. It can affect seed germination (Nautiyal and Sinha, 2012), cause reduction in growth, promote leaf chlorosis and root system darkening (Gopal and Rizvi, 2008), reduce stomatal conductance and size of the stomata (Xiong, 1997), affect the activity of enzymes (Dawood *et al.*, 2022), suppress photosynthesis due to interruptions in the electron transfer reaction (Fouda and Sofy, 2022), reduce respiratory rate (Romanowska *et al.*, 2008), disrupt mineral nutrition and water balance, promote changes in hormonal status and alter the structure and permeability of membranes (Abu-Shahba *et al.*, 2022). Phytoremediation is a low-cost, eco-friendly, and successful treatment approach that has inspired considerable interest (El-Sheshtawy *et al.*, 2021). The mechanisms and applications of phytoremediation have been researched by several researchers (Arya *et al.*, 2013; Lamhamdi *et al.*, 2013; Kasim *et al.*, 2014; Winska-Krysiak *et al.*, 2015; Sorrentino *et al.*, 2018). It utilizes the ability of specific plants to accumulate, retain or decompose the toxic metal or organic pollutants in soil, water or air. Through the food chain, heavy metal poisoning has resulted in widespread human catastrophes. Low-accumulating vegetables and crops should be given special attention in order to reduce heavy metal contamination (Wang *et al.*, 2021).

This study was conducted to 1) evaluate the growth and Pb accumulation by *Moringa oleifera* plants exposed to various levels of Pb (supplied as lead nitrate) at vegetative growth periods, 2) determine whether *Moringa oleifera* plants can translocate high concentrations of lead to the shoots and 3) the effect of lead on ionic contents of *Moringa oleifera* either in both cases of soil or foliar lead application.

## Materials and Methods

### *Plant materials and growth conditions*

The experimental work was run with many homogeneous *Moringa oleifera*. In addition, pure seeds strains were obtained from the Ministry of Agriculture, Mansoura, Egypt.

Equal amounts (8 kg) of a homogeneous mixture of variously treated sand: clay soil (2:1, v/v) were weighed in black polythene bags; A uniform lot of *Moringa* (*Moringa oleifera*) seeds was collected and surface-sterilized for 3 minutes in a 0.01 percent HgCl<sub>2</sub> solution. The sterilized seeds were rinsed with distilled water several times.

Two experiments (A and B) were carried out. The first experiment (A) included the growth of seeds in contaminated soil with lead (100 and 400 ppm Pb(NO<sub>3</sub>)<sub>2</sub> either alone or in combination with melatonin (100 µM), as a priming solution, or sugar beet pulp pellet, as an amendment, at a ratio of 10 g/kg soil according to Ogundiran *et al.* (2018). The second experiment (B) included the spraying of plant leaves with lead (100 and 400 ppm Pb(NO<sub>3</sub>)<sub>2</sub> either alone or in combination with melatonin (100 µM) or with sugar beet pulp pellet.

These bags are divided into an appropriate replicated number of experimental groups according to the type of the experiment conducted as follows:

C; control with tap water,

L1; with amendments of 100 ppm Pb

L2; with amendments of 100 ppm Pb + melatonin (MEL).

L3; with amendments of 100 ppm Pb + sugar beet pulp pellet (SBP).

H1; with amendments of 400 ppm Pb.

H2; with amendments of 400 ppm Pb + melatonin (MEL)

H3; with amendments of 400 ppm Pb + sugar beet pulp pellet (SBP).

### *Data collection*

In both experiments, samples were taken for analysis after 36 days from the date of sowing the seeds. Sampling was carried out to include all plants allotted for each treatment. The collected samples were used to assess the growth parameters (root and shoot length; fresh and dry weights of root and shoot; water contents). Triplicate samples were taken to determine ionic contents of K, Na, Ca, and Mg. Lead concentration and bio-concentration (BCF), as well as translocation (TF) factors, were calculated. In addition, photosynthetic pigments, carbohydrates, oxidative stress markers and proline contents were determined in moringa plants.

### *Chemical analysis of soil*

After the incubation period and before cultivation, the collected soil samples were air-dried and then sieved through 2 mm sieve to remove coarse gravel. The sieved soils were then preserved in plastic bags, and these were later used for various chemical analyses. The soil solution was prepared at a ratio 1 soil: 5 waters. The solution was shaken well for one hour and then filtered. The filtrate was used in the chemical analysis of soil. Soil pH was determined with the help of a glass electrode pH meter. The electrical conductivity was measured at a soil: water ratio of 1:5 using an EC meter. Carbonates and bicarbonates were determined according to Jackson *et al.* (1973). Chloride ions were estimated according to Piper (1966). Oxidizable organic carbon (as an indication of the total organic matter) was determined as described by Piper (1966) and Jackson (1967). The total nitrogen was determined by the conventional semi-micro-propagation of the Kjeldahl method of Chibnall *et al.* (1943). Sulphate contents were determined according to Jackson (1967). The chemical and physical analyses of the tested soil are presented in Table 1.

**Table 1.** Chemical analyses for experimental soil

Parameter	Values
E.C (ds.m <sup>-1</sup> )	1.50
pH (1:2.5)	7.72
Organic carbon%	0.019
Total N %	0.12
C/N	0.158
K <sup>+</sup> (meq / L)	1.8
Na <sup>+</sup> (meq / L)	5.7
Ca <sup>++</sup> (meq / L)	4.05
Mg <sup>++</sup> (meq / L)	3.45
HCO <sub>3</sub> (meq / L)	1.08
Cl	4.17
SO <sub>4</sub> <sup>-</sup>	9.75

### *Biochemical analysis*

#### Estimation of photosynthetic pigment

The plant photosynthetic pigments, chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Car), were estimated as described by Hiscox and Israelstam (1979).

$$\text{Chlorophyll a (mg g}^{-1} \text{ F Wt)} = 12.7 (A663) - 2.69 (A645)$$

$$\text{Chlorophyll b (mg g}^{-1} \text{ F Wt)} = 22.9 (A645) - 4.68 (A663)$$

$$\text{Carotenoids (mg g}^{-1} \text{ F Wt)} = 5.02 A480$$

$$\text{Total pigments (mg g}^{-1} \text{ F Wt)} = \text{Chl a} + \text{Chl b} + \text{Car}$$

#### Estimation of carbohydrates and proline content

The different carbohydrate fractions were extracted according to the method adopted by Yemm and Willis (1954). Next, sucrose content was determined using a modification of van Handel (1968). Next, total soluble sugars (TSS) content was determined using the modification of the procedures of Yemm and Willis (1954). Finally, the amounts of total carbohydrates in the plant extracts were obtained using the standard curve of glucose according to Hedge *et al.* (1962). Finally, the method used in the present study, the proline content, according to Bates *et al.* (1973).

#### Determination of hydrogen peroxide, lipid peroxidation, and electrolyte leakage

Hydrogen peroxide was estimated according to Alexieva *et al.* (2001). Lipid peroxidation was estimated by the determination of the content of the product of unsaturated fatty acid peroxidation, malondialdehyde (MDA), following the method of Heath and Packer (1968). Finally, electrolyte leakage (E.L) was determined to assess the membrane permeability, according to Lutts *et al.* (1996).

#### Determination of ionic contents

The experimental plants were separated into shoots and roots when sampled at 36-days old. The plant samples were oven-dried at 80 °C for 48 hours. The dried matter was digested in concentrated HNO<sub>3</sub> and then made up to a known volume with deionized water as described by Motsara and Roy (2008). Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Pb<sup>2+</sup> concentrations were measured by Atomic Absorption Spectrophotometry (GBA Sens AA instrument). Data were calculated as mg g<sup>-1</sup> dry weight.

Bio-concentration (BCF) factors were calculated as in the following formulae (Li *et al.*, 2014; Usman *et al.*, 2019):

$$\text{Shoot bioconcentration factor (BCFs)} = \text{C shoot} / \text{C soil}$$

Root bioconcentration factor (BCFr) = C root /C soil

Translocation factor (TF) = BCFs/ BCFr

#### Determination of hormone levels

The determination of indoleacetic acid (IAA), abscisic acid (ABA), gibberellins (GA<sub>3</sub>) was performed on the sugar beet pulp. Samples were surface dried and cleaned with a paper towel, immediately weighed, and then extracted overnight with 30 ml 80% cold aqueous methanol (< 0 °C) in darkness at 4 °C. The extract was centrifuged at 5000 rpm and 4 °C for 15 min. The supernatant was collected. The extract was injected into a reverse-phase HPLC with a methanol gradient in 0.6 % acetic acid to detect IAA, ABA, and GA<sub>3</sub> (Yuan *et al.*, 2005). Table 2 shows the hormonal and some ionic constituents of the SB.

**Table 2.** Hormonal contents and some ionic contents of the sugar beet pulp pellet

Parameter	Values
Ionic content	
Potassium (mg g <sup>-1</sup> Dwt)	11.75
Sodium (mg g <sup>-1</sup> Dwt)	100.80
Calcium (mg g <sup>-1</sup> Dwt)	29.21
Magnesium (mg g <sup>-1</sup> Dwt)	0.11
Hormonal content	
IAA (µg ml <sup>-1</sup> )	281.27
GA <sub>3</sub> (µg ml <sup>-1</sup> )	449.82
ABA (µg ml <sup>-1</sup> )	81.90

#### *Data analysis*

The full data of the variously treated moringa plants were statistically analysed using a one-way analysis of variance (ANOVA). In addition, a comparison among means was carried out by calculating Fisher's test at 5% probability level. All the analyses were made using Minitab (version 18).

## **Results**

#### *Growth parameters*

As shown in Table 3, a significant reduction in all the measured growth parameters in Pb-treated plants was observed compared with the control values. This decrease was more severe in the plants treated with 400 ppm Pb in soil and foliar application. For 100 ppm Pb in soil application, the % decrease in length, fresh weight, and dry weight of shoot were 11.6, 30.63, and 28.77, respectively, compared to control without treatment. These parameters were decreased by 26.08, 22.60, and 37.04% in the case of foliar application, as compared with control. In response to pre-treatment with MEL or SPB, a significant increase in all the growth parameters was apparent compared to Pb-treatment. Compared with the control values, the water content markedly decreased in the stressed plants by 27.89 and 45% at 100 ppm Pb soil and foliar application and by 46.32 and 37.62% at 400 ppm Pb soil and foliar application, respectively. It was recorded that MEL and SBP treatment-induced increases in water content compared to Pb applied. However, the SPB application had the best alleviation effect in soil and foliar applications.

**Table 3.** Effect of lead stress with MEL or SBP on growth parameters of *Moringa oleifera* plants

	Treatment	Shoot			Root			W.C
		Length (cm)	F. wt. (g)	D wt. (g)	Length (cm)	F. wt. (g)	Dry wt. (g)	
Soil application	Control	27.07 ± 0.16 e	3.33 ± 0.05 c	0.73 ± 0.01 c	7.67 ± 0.18 d	1.46 ± 0.01 c	0.27 ± 0.01 c	3.80 ± 0.03 d
	100 ppm Pb	23.93 ± 0.16 g	2.31 ± 0.04 e	0.52 ± 0.02 d	5.67 ± 0.18 f	1.13 ± 0.02 d	0.17 ± 0.004 e	2.74 ± 0.03 e
	+MEL	25.47 ± 0.25 f	2.63 ± 0.02 d	0.61 ± 0.01 cd	6.50 ± 0.10 e	1.42 ± 0.02c	0.26 ± 0.01 cd	3.18 ± 0.02 d
	+Bect	32.13 ± 0.16 c	4.22 ± 0.08 b	0.91 ± 0.01 b	9.00 ± 0.16 b	2.22 ± 0.004 ab	0.48 ± 0.01 bc	5.05 ± 0.08 b
	400 ppm Pb	20.30 ± 0.36 h	1.65 ± 0.02 f	0.35 ± 0.01 e	3.37 ± 0.01 g	0.89 ± 0.02 e	0.15 ± 0.01 f	2.04 ± 0.02 ef
	+MEL	28.17 ± 0.18 d	3.58 ± 0.01 c	0.74 ± 0.02 c	7.83 ± 0.09 d	1.72 ± 0.03 c	0.30 ± 0.003 c	4.26 ± 0.04 c
	+Bect	39.80 ± 0.25 a	7.17 ± 0.10 a	1.67 ± 0.06 a	10.90 ± 0.11 a	2.93 ± 0.09 a	0.61 ± 0.03 a	7.83 ± 0.10 a
Foliar application	100 ppm Pb	21.10 ± 0.06 h	2.04 ± 0.01 d	0.45 ± 0.002 c	7.00 ± 0.16 d	0.78 ± 0.004 g	0.14 ± 0.001 f	2.09 ± 0.02 e
	+MEL	25.37 ± 0.08 f	2.05 ± 0.04 d	0.44 ± 0.005 c	8.77 ± 0.08 c	1.25 ± 0.01 c	0.26 ± 0.01 cd	2.59 ± 0.02 e
	+Bect	31.27 ± 0.053 c	3.08 ± 0.08 b	0.70 ± 0.02 b	8.50 ± 0.09 c	2.19 ± 0.06 b	0.51 ± 0.01 b	4.06 ± 0.08 c
	400 ppm Pb	20.33 ± 0.48 h	1.62 ± 0.01 e	0.36 ± 0.003 d	6.70 ± 0.08 e	0.61 ± 0.01 f	0.11 ± 0.002 h	1.90 ± 0.02 f
	+MEL	29.3 ± 0.04 d	2.36 ± 0.07 c	0.51 ± 0.02 c	7.40 ± 0.11 d	0.63 ± 0.02 fg	0.13 ± 0.01 g	2.35 ± 0.04 e
	+Bect	33.90 ± 0.52 b	4.59 ± 0.07 a	1.00 ± 0.01 a	9.50 ± 0.16 b	2.17 ± 0.01 b	0.50 ± 0.01 b	5.26 ± 0.07 b

Means ( $\pm$ standard error) followed by different letters on the same column show significant differences according to Fisher's test at  $p < 0.05$ . W.C; water content

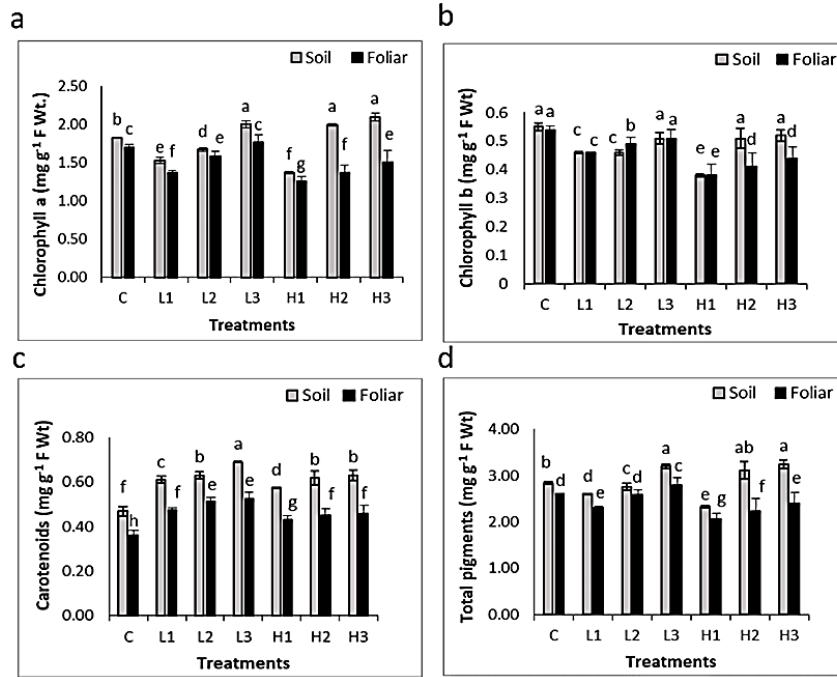
#### *Photosynthetic pigments*

The data are presented in Figure 1 (a-d) show the various photosynthetic pigments that appeared to be slightly changed from the appropriate control values in response to 100 ppm Pb soil application. In 400 ppm Pb-treated plants, an apparent significant decrease was observed in Chl a, Chl b and total pigments by 15.9, 16.36 and 8.42% for soil application and by 19.41, 14.8, and 11.42%, respectively, for foliar application compared to the control values. However, Car content increased in plants under the soil or foliar application with different concentrations of Pb compared to the control. Treatment of plants with MEL or SBP leads to a significant increase in the content of all photosynthetic pigments under Pb stress. The higher values were recorded under SBP application in case of soil and foliar Pb application.

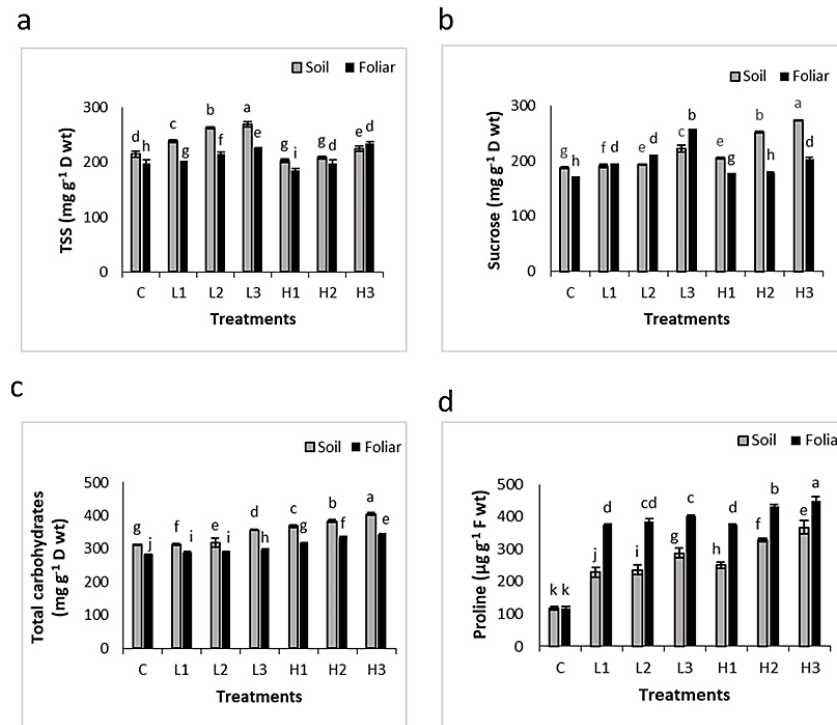
#### *Changes in osmolytes and secondary metabolites*

For the plants treated with Pb in soil, the content of total soluble sugars (TSS), sucrose, total carbohydrates, and proline content significantly increased at two concentrations of Pb, as compared with control, the increase being more pronounced with 400 ppm Pb Figure 2 (a-d).

In general, the MEL or SBP-treated plants showed significant increases in TSS, sucrose, total carbohydrates, and proline content compared to the plants exposed to Pb stress either in soil or foliar application.



**Figure 1.** Effect MEL or SBP on photosynthetic pigments (a-d) of *Moringa oleifera* plants grown under Pb stress  
 C; control, L1; 100 ppm Pb, L2; 100 ppm Pb + MEL, L3; 100 ppm Pb + SBP, H1; 400 ppm Pb, H2; 400 ppm Pb + MEL, H3; 400 ppm Pb + SBP. Means ( $\pm$ standard error) followed by different letters on the same bar show significant differences according to Fisher's test at  $p < 0.05$ .



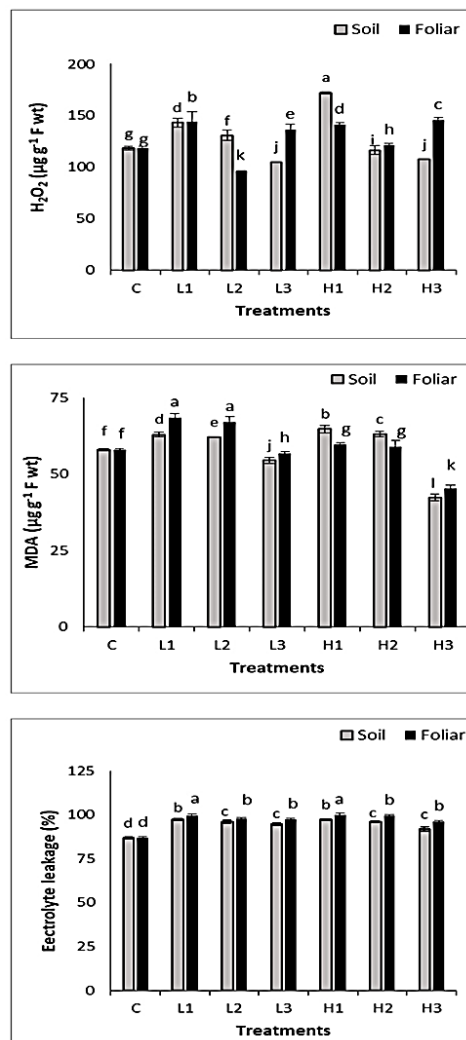
**Figure 2.** Effect MEL or SBP on a) total soluble sugars (TSS), b) sucrose, c) total carbohydrates, and d) proline content of *Moringa oleifera* plants grown under Pb stress  
 C; control, L1; 100 ppm Pb, L2; 100 ppm Pb + MEL, L3; 100 ppm Pb + SBP, H1; 400 ppm Pb, H2; 400 ppm Pb + MEL, H3; 400 ppm Pb + SBP. Means ( $\pm$ standard error) followed by different letters on the same bar show significant differences according to Fisher's test at  $p < 0.05$ .

*Changes in H<sub>2</sub>O<sub>2</sub> content, MDA and electrolyte leakage.*

It is evident in Figure 3 that hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content significantly increased under Pb stress in both soil and foliar applications compared with the control values. The % of the increase in H<sub>2</sub>O<sub>2</sub> content at 100 and 400 ppm Pb as foliar treatment was 21.73 and 23.24, respectively. The soil treatment obtained the maximum value at 400 ppm Pb at 45.48% compared to the control. Treatment with MEL or SBP reduced the accumulation of H<sub>2</sub>O<sub>2</sub> content under Pb stress.

The soil treatment of Pb induced a marked increase in the lipid peroxidation represented by malondialdehyde (MDA) at 100 and 400 ppm Pb in case of soil and foliar application compared to non-stress plants. Supplemental addition of SBP or MEL to Pb-stressed plants caused a marked MDA decline in Pb treatment; the lower values being recorded under SBP treatment.

Pb stress caused significantly increased electrolyte leakage (EL%) to *Moringa* plants compared with the control. The % of EL increase under Pb in soil application was as follows: 14.21 at 100 and 14.85% at 400 ppm Pb, respectively. At Pb foliar application, E.L increased by 12.04 and 11.75% at 100 and 400 ppm Pb. On the other hand, significant decreases were shown under MEL or SBP compared with Pb-stressed plants.



**Figure 3.** Effect MEL or SBP on H<sub>2</sub>O<sub>2</sub> content, MDA, and electrolyte leakage of *Moringa oleifera* plants grown under Pb stress  
 C; control, L1; 100 ppm Pb, L2; 100 ppm Pb + MEL, L3; 100 ppm Pb + SBP, H1; 400 ppm Pb, H2; 400 ppm Pb + MEL, H3; 400 ppm Pb + SBP. Means (±standard error) followed by different letters on the same bar show significant differences according to Fisher's test at p < 0.05.

*Changes in lead content and mineral concentration*

Lead concentration

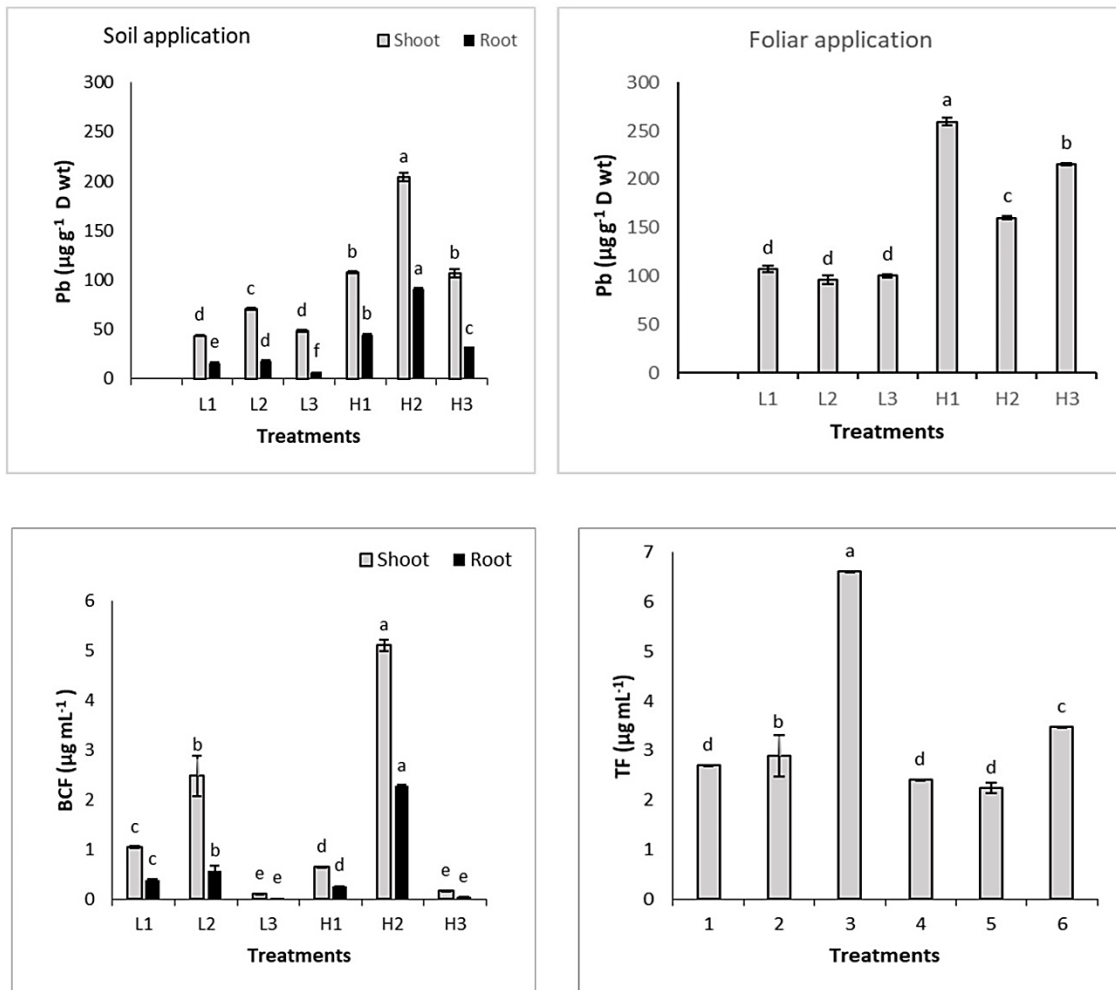
The effect of MEL and SBP on Pb content in shoots and roots of the tested *Moringa* plants was presented in the present investigation. Figure 4 data demonstrated that the Pb content in shoot and root significantly increased with increased Pb concentration. In soil application, the magnitude of accumulated Pb in root was less than that in the shoot. Application of MEL at 100 ppm Pb induced an increase in the translocation of Pb to shoot by 43.98% and the accumulation in the root by 11.20%. Also, at 400 ppm Pb, MEL induced increases in the translocation and accumulation of lead by 88.82% and 102.85%. On the other hand, SBP treatment markedly reduced the lead uptake by root by 59.20% at 100 ppm Pb and 31.58% at 400 ppm Pb, compared with the stressed plants. No Pb was detected in the root when Pb was applied by the foliar method. For foliar application, when MEL and SBP were combined with 100 ppm Pb, a non-significant reduction in Pb content was observed compared to Pb-stressed plants. However, the combination of 400 ppm Pb with MEL or SBP caused a significant decrease in Pb content, the magnitude of reduction is more pronounced with MEL.

*Bio-concentration (BCF) factors*

The measurements of BCF and TF described the Pb accumulation potential of the various treatments. Bio-concentration factor values greater than one were observed in the shoots with the application of 100 ppm Pb or 400 ppm Pb in the soil either alone or combined with MEL in shoot and root. The highest value was determined at the treatment of 400 ppm Pb with MEL. On the other hand, the stressed moringa plants showed a significant decrease in BCF value ( $BCF < 1$ ) when supplied with SBP compared to the Pb treatment alone. Transfer factor value was found to be higher than 1 in all the various treatments. Application of MEL or SBP exhibited a significant increase in TF value compared with Pb-stressed plants; the lower value was recorded at 400 ppm Pb (Figure 4).

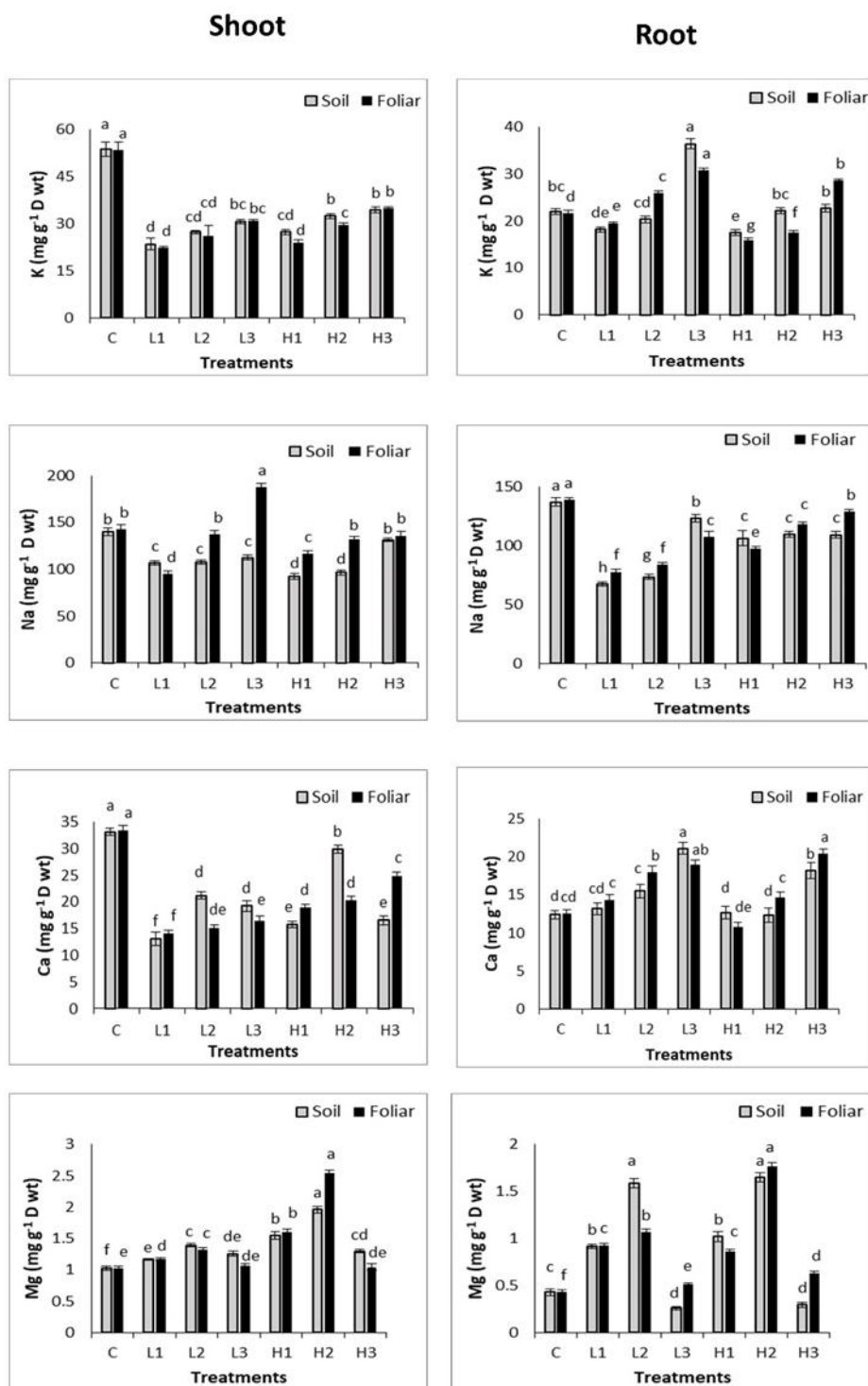
*Mineral content*

The effect of lead concentrations (100 and 400 ppm Pb) either alone or combined with MEL or SBP on the ionic contents of moringa plants is presented in Figure 5. The obtained data revealed that, in shoots and roots of the pb-treated plant, K and Na accumulation significantly reduced in case of the soil and foliar application, as compared with the control values. The % of the decrease in K and Na in roots was 17.30 and 50.99% for soil application and 9.69, 44.09% for foliar application, respectively. Moreover, K and Na in shoots were decreased by 56.35 and 23.71% for soil application and 58.43, 33.34% for foliar application compared to the control values. The plants treated with Pb by two methods, soil or foliar application, showed a non-significant change in Ca content in the roots and a significant reduction in the shoots. The combination of Pb with MEL or SBP exhibited an increase in K, Na, and Ca content in the shoots and roots, the magnitude of increase being more pronounced with SBP application. On the other hand, Mg content was observed to significantly increase in response to Pb treatment either alone or combined with MEL. However, supplemental addition of SBP showed a significant decrease in Mg content in the shoots and roots compared to the Pb group alone.



**Figure 4.** Effect MEL or SBP on lead content, BCF, and TF of *Moringa oleifera* plants grown under Pb stress

C; control, L1; 100 ppm Pb, L2; 100 ppm Pb + MEL, L3; 100 ppm Pb + SBP, H1; 400 ppm Pb, H2; 400 ppm Pb + MEL, H3; 400 ppm Pb + SBP. Means ( $\pm$ standard error) followed by different letters on the same bar show significant differences according to Fisher's test at  $p < 0.05$ .



**Figure 5.** Effect MEL or SBP on the mineral content of *Moringa oleifera* plants grown under Pb stress C; control, L1; 100 ppm Pb, L2; 100 ppm Pb + MEL, L3; 100 ppm Pb + SBP, H1; 400 ppm Pb, H2; 400 ppm Pb + MEL, H3; 400 ppm Pb + SBP. Means ( $\pm$ standard error) followed by different letters on the same bar show significant differences according to Fisher's test at  $p < 0.05$ .

## Discussion

The growth of *Moringa* (*Moringa oleifera*) plants was affected by lead as one of the serious heavy metals. Heavy metal stress-induced toxicity in living organisms is a complex phenomenon. Heavy metals have been reported to induce numerous toxic effects to plants, such as oxidative stress, osmotic disturbance, specific ion toxicity, and nutrient deficiency (Abbas *et al.*, 2018). In this way, heavy metals can seriously alter various biochemical and physiological pathways involved in plant growth, survival, and development (Kováčik *et al.*, 2018).

Toxic metals such as As, Pb, and Cu have been reported to be taken up by roots from polluted soils as well as leaves (foliar organs) from the atmosphere and can accumulate these heavy metals in several parts of plants (Xiong *et al.*, 2016; Shahid *et al.*, 2019). In the present study, the growth of moringa plants was significantly reduced in response to soil and foliar application of Pb compared to control (Table 1). Similar results have already been reported under root uptake in water hyacinths (Malar *et al.*, 2016), *Moringa oleifera* (Azeez *et al.*, 2019), and after foliar application of Pb in radish (Salim *et al.*, 1993) and *Spinacia oleracea* (Shahid and Khalid, 2020). Reduced plant growth under heavy metal toxicity had been correlated with low water potential, hampered nutrient uptake and oxidative stress (Sharma and Dubey, 2005), and this is in agreement with our results where the water content in *Moringa* stressed plants were reduced at 100 and 400 ppm Pb compared to the control.

The application of melatonin or SBP under Pb stress improved growth parameters. Our findings align with those of Wang *et al.* (2019), who found that melatonin application at 100  $\mu\text{M}$  enhanced the plant biomass and root length of *Nicotiana tabacum* L. under Cd stress. In addition, Xie *et al.* (2018) reported that the pre-treatment with melatonin at 100  $\mu\text{M}$  reduced Pb damage and resulted in longer shoot and root lengths and higher biomass accumulations compared to stressed bermudagrass plants. Such a positive effect on seedling growth is consistent with the hypothesis that melatonin may exhibit some auxin-like effects in plants (Hernandez-Ruiz *et al.*, 2004; Kolář and Macháčková, 2005; Arnao and Hernández-Ruiz, 2007). Recently, Sadak *et al.* (2020) stated that MEL is considered a master plant regulator. It promotes plant growth and development by acting as a signalling molecule linked to defence mechanisms against various biotic and abiotic stresses.

Following our results, Caravaca *et al.* (2005) discovered that the addition of sugar beet, rock phosphate, and *Aspergillus niger* directly into the soil and the mycorrhizal inoculation of seedlings could significantly enhance the growth of *Cistus albidus* L. and *Quercus coccifera* L. The improvement of growth when Pb-stressed plant supplemented with SBP in the soil may be due to the high ionic contents of SB, as well as the high content of hormonal amount, especially GA<sub>3</sub>, as presented in Table 2.

A perusal of the data presented in Figure 1 revealed that treatment with Pb induced a decrease in chl a, chl b and total pigments under soil and foliar Pb application. The magnitude of the decrease is more pronounced under 400 ppm Pb stress. Following the present results, Rasool *et al.* (2020) demonstrated considerable increase in chlorophyll in *Zea mays* L. grown under Pb stress. Similar results were also reported in previous publication studying the photosynthetic pigments under Pb stress in *Brassica napus* L. (Ali *et al.*, 2014).

Carotenoids play key roles in protecting Chl pigments under stress conditions (Choudhury and Behera, 2001). The high levels of these pigments in Pb-exposed plants, especially those with 1,000 mg kg<sup>-1</sup>, could indicate that the photosynthetic machinery is being protected from photo-oxidative damage. In this study, supplemental addition of melatonin or SBP to Pb-stressed plants caused an increase in the pigment fractions compared to Pb treatment alone. Hasan *et al.* (2015) reported similar results, stating that pre-treatment with MEL significantly boosted photosynthetic machinery, chlorophyll content, and biomass accumulation in tomato seedlings exposed to cadmium toxicity. Moreover, Ahammed *et al.* (2020) stated that pre-treatment of

cucumber seedling with MEL improved the chlorophyll a and chlorophyll b contents under low and high iron supply compared with iron treatments alone. In further support of the present results, Mohamed *et al.* (2021) stated that the rocket plants that were treated with melatonin applied either by priming or soaking significantly enhanced chl a and chl b and carotenoids to the control and Pb-stressed plants. Application of exogenous melatonin in wheat plants under Cd stress resulted in an increase in chlorophyll content, which could be linked to a decrease in H<sub>2</sub>O<sub>2</sub> concentration (Kaya *et al.*, 2019). The same finding was reported in our results, as shown in Figure 3. Melatonin can reduce chlorophyll degradation in stressed plants due to its direct antioxidant function (Park *et al.*, 2013). Also, melatonin performs as a scavenger of reactive oxygen species in the first line of defence against oxidative stress. It is evident from the present work results that the amendment of contaminated soil with SBP increased the total pigments. These results are in accordance with Karanatsidis and Berova (2009), who studied the effect of organic fertilizers on the content of chlorophyll pigments and rate of photosynthesis. Our results agreed with the previous findings obtained on other vegetable crops by Sofy *et al.* (2021).

As a dynamic process, carbohydrate metabolism changes under the influence of environmental conditions. Soluble carbohydrate has an important role in the osmotic adjustment and stability of bimolecular and membrane (Farahat *et al.*, 2007). Plants can employ total soluble sugars as a carbon skeleton or an energy source to generate additional organic molecules in normal circumstances. TSS can be used as an osmotic regulator in stressful situations and can protect essential enzymatic activity from excessive intracellular inorganic ion concentrations. In this study, TSS, sucrose, and total carbohydrates markedly increased at 100 and 400 ppm Pb in case of soil and foliar Pb application (Figure 2). In agreement with our results, Alkhatib *et al.* (2019) reported Pb stress-induced noticeable accumulation of total carbohydrates in *Leucaena* leaves. Several studies have reported this phenomenon in most plant species under various abiotic stress (Gilbert *et al.*, 1997; Balibrea *et al.*, 2000; Pattanagul and Thitisaksakul, 2008; Alkhatib *et al.*, 2016). According to Dhanapackiam and Ilyas (2010), organic solute accumulation could enhance internal osmotic pressure, allowing the plant to tolerate some abiotic stress. In concordance with the current investigation, Siddiqui *et al.* (2019) found a marked increase in total soluble carbohydrate under salt stress and MEL application. In *Arabidopsis* seedlings treated with melatonin, a higher accumulation of soluble sugars was detected in the roots than that in the leaves (Zhao *et al.*, 2015). In the present study, supplemental addition of SBP induced an increase in TSS, sucrose, and total carbohydrates content compared to Pb-treated plants. In harmony with our results, Anli *et al.* (2020) reported that the supplemental addition of biofertilizers generated a significant increase in TSS content compared to drought-stressed plants.

Proline contents in stressed *Moringa* plants were markedly increased above the control, the magnitude of increase being more pronounced in the case of Pb foliar treatment. Following our results, Sofy *et al.* (2020) reported the exposure of maize plants to Pb increased proline content compared with control. Also, the accumulation of proline in response to heavy metals of many plants was recorded by other investigators (Tripathi and Gaur, 2004; Odjegba and Fasidi, 2006; Dey and Mondal, 2016). It has been demonstrated that free proline acts as an osmoprotectant (Ashraf and Foolad, 2007; Molinari *et al.*, 2007), protein stabilizer (Sharma and Dietz, 2006), metal chelator (Sharma and Dubey, 2005), an inhibitor of lipid peroxidation (Mehta and Gaur, 1999), free radical scavenger (Ashraf and Foolad, 2007; Molinari *et al.*, 2007), prevent enzyme destruction, and reduce the toxic effects of lead (Tripathi and Gaur, 2004). Therefore, it may be concluded that the accumulation of proline could be regarded as one of the major physiological mechanisms of heavy metals stress tolerance (Chadzinikolau *et al.*, 2017; Lima *et al.*, 2019). In the present study, supplemental addition of MEL and SBP induced an increase in proline content compared to Pb-treated plants. In consistent with our findings, Zhang *et al.* (2020) observed that MEL treatment enhanced proline accumulation under Pb stress. Similar results were also reported in previous publications studying the proline accumulation under heavy metal stress (Campos *et al.*, 2019; Farouk and Al-Amri, 2019). On the other hand, it was found that

mycorrhizal plants grown in the SBP-amended soil reached the highest proline were the least damaged (in terms of plant growth) by drought (Medina and Azcón, 2010).

The present experiment showed that Pb stress caused increases in H<sub>2</sub>O<sub>2</sub>, lipid peroxidation, and electrolyte leakage compared to control (Figure 4). The accumulation of H<sub>2</sub>O<sub>2</sub> is now known to reflect the oxidative stress and the changes of antioxidants in different compartments of plants. The increase in H<sub>2</sub>O<sub>2</sub> results in enhanced lipid peroxidation and, accordingly, increases the membranes' leakage (Ashraf, 2009). Similar results have been observed by Dalyan *et al.* (2018) for *Brassica juncea*, Azeez *et al.* (2019) for *Moringa oleifera*, and Giannakoula *et al.* (2021) for *Citrus aurantium*. However, the exogenous application of MEL and SBP alleviated oxidative stress caused by Pb stress by lowering H<sub>2</sub>O<sub>2</sub> and MDA levels and decreasing EL. Ahammed *et al.* (2019) mentioned that MEL acts as a multi-regulatory molecule and a universal antioxidant, with a stimulating role for plant's antioxidant defence system under a range of environmental stress. Many researchers showed significant reductions in reactive oxygen species accumulation, MDA content, and EL level in watermelon, tomato, soybean, maize, and by MEL supplementation, under abiotic stresses (Zhang *et al.*, 2017; Nawaz *et al.*, 2018; Okant and Kaya, 2019; Jahan *et al.*, 2020). Moreover, supplemental addition of SBP to Pb-stressed plants showed a decline in H<sub>2</sub>O<sub>2</sub>, MDA, and E.L compared to stressed moringa plants. Similarly, Anli *et al.* (2020) observed that applying single or combined biofertilizers under water stress revealed reduced MDA and H<sub>2</sub>O<sub>2</sub> content compared with non-amended controls.

As shown in Figure 5, lead accumulation significantly increased with increasing lead supply by soil and foliar application. Supporting our result, it was reported that metal accumulation in plants is obviously linked to metal concentration in the growing environment (Labra *et al.*, 2006). In the current study, Pb-stressed moringa plants displayed higher Pb content in shoots than roots. The ability of plants used in phytoremediation techniques to accumulate heavy metals into harvestable plant parts such as stems and leaves is one of their most important characteristics. The BCF and TF can be used to determine whether a plant species has phytoremediation potential. Hyperaccumulators have BCF and TF values greater than one, while metal excluders have values less than one (Yoon *et al.*, 2006).

The BCF and TF values observed in moringa plants in this investigation demonstrated its Pb hyperaccumulating tendencies. A comparable report on BCF and TF value > 1 was detected in multi-tolerant hyperaccumulator species such as *Pteris vitata* and *Pityrogramma calomelanos* (Soongsombat *et al.*, 2009). The present level of accumulation of Pb in aerial parts of *Moringa* is evidence of its ability to translocate the accumulated metal in the above-ground tissues, which is a characteristic feature shown by metal accumulators. On the other hand, there was no lead detectable in the roots of moringa plants in foliar application. Also, it was found that the amount of Pb uptake was higher in the shoot in foliar application than in soil treatment. This is in accordance with those obtained by Salim *et al.* (1993), who stated that the amount of uptake for Cd, Pb, and Cu in radish plants was higher in foliar-treated plants than in root-treated plants.

The priming of seeds in MEL seems to be a useful option for cleaning toxic pollutants from the environment by improving phytoremediation processes. In the present study, MEL pre-treatment increased Pb accumulation in both shoots and roots for soil Pb application. Our results are in agreement with the work of Nazarian and Ghanati (2020). MEL treatment of As-stressed rice (*Oryza sativa*) plants resulted in increased As transport from the roots to the shoots, and therefore, a higher accumulation of As in shoots was observed. In contrast, in the foliar application, it was observed that moringa plants showed a decline in Pb content shoots and roots. In support of our results. The study of Cd and Al in several subcellular compartments revealed that MEL inhibited Al and Cd mobilization into vacuoles and the cell wall, resulting in a significant reduction in Al and Cd toxicity in rapeseed (*Brassica napus*) seedlings (Sami *et al.*, 2020). It was observed that the effect of MEL was more pronounced at 400 ppm Pb for the two ways of application. On the other hand, the combination of SBP with Pb stress revealed decreases in Pb content in both shoots and roots for soil and foliar application. Our results are in harmony with those of Yang *et al.* (2017), who stated that organic fertilizer caused a reduction in Cd uptake by rice seedlings grown in Cd-contaminated soil.

Pb is known to physically block numerous ions from reaching their absorption sites on the roots, preventing their uptake. However, the substantial reductions in ionic content seen in this study are unlikely to be due to ion absorption inhibition alone and are more likely due to extra ion leakage from the plants (Nareshkumar *et al.*, 2014). Pb soil and foliar application caused a decline in K and Na contents compared to control values in both shoots and roots. Moreover, Ca content increased in the root but decreased in the shoot. These results appear that the impacts of Pb on other nutrient elements are complex and may depend on the tissue types. In addition, Mg content elicited an increase in both shoot and root; in the case of plant shoot, the magnitude of increase was more pronounced at 400 ppm Pb. In agreement with our results, Lamhamdi *et al.* (2013) reported that Pb stress-induced reduction in Na, K, and Ca uptake in wheat plants. Yilmaz *et al.* (2009) reported that Ca levels showed significant decreases with increasing Pb applications in the shoot.

In contrast, an opposite trend was noticed for Ca content in roots at all Pb concentrations. Also, the levels of Mg showed significant decreases in roots and shoots, while an opposite trend was observed for Mg in leaves of eggplant. This suggests different mechanisms for the uptake of Ca and Mg for the different plant parts. Our findings paralleled those of Azooz *et al.* (2012), who found that excessive Cu enhanced Ca and Mg levels in wheat seedlings. Furthermore, the Ca content in *Theobroma cacao* seedlings increased in the root but decreased in the stems (Souza *et al.* 2014). On the other hand, the application of melatonin and SBP reversed the harmful impacts of Pb on Na, K, and Ca. However, SBP application elicited a decline in Mg content compared to Pb treatment in the case of soil and foliar application. Melatonin regulates the level of plant mineral nutrients in plants and relieves stress by allocating resources to maintain those elements. For example, the application of MEL substantially alleviated K content in *Malus* plants under different stress conditions (Mukhopadhyay *et al.* 2016) and improved mineral nutrition in cucumber plants under nitrate stress (Zhang *et al.* 2017).

## Conclusions

In the current study, Pb stress caused oxidative injury in plants, as evidenced by raised levels of oxidative stress markers and decreased biomass output. The adverse effect of the foliar Lead application on moringa plants was higher than the soil treatment. On the other hand, application of MEL and SBP consistently decreased H<sub>2</sub>O<sub>2</sub> accumulation, increased the chlorophyll content and carbohydrates, and subsequently relative water content, membrane integrity, and biomass accumulations. However, supplemental addition of SBP was seen to be more efficient in mitigating overall stress responses than MEL. Furthermore, MEL application enhanced the phytoremediation capacity of moringa plants.

## Authors' Contributions

Conceptualization: MFE, MEY, and WMS; Data curation; Formal analysis; Investigation; Methodology; Resources; Software: MFE, MEY, and WMS; Validation; Visualization: MFE, MEY, and WMS; Writing - original draft: MFE, MEY, and WMS; Writing - review and editing: MFE, MEY, and WMS. All authors read and approved the final manuscript

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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