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Ameliorative effect of *Halopteris filicina* extracts on growth parameters and genomic DNA template stability of tomato (*Solanum lycopersicum*) under lead chloride stress

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ABSTRACT

Lead is a toxic element that accumulates in agricultural soils through various anthropogenic sources. It inhibits the growth and development of plants and causes mutations in DNA. Macroalgae such as *Halopteris filicina* contain multifunctional components that may improve plant tolerance to lead stress. In this study, seeds of tomato (*Solanum lycopersicum*) were subjected to six treatments comprising two levels of lead exposure (60 or 120 μM $PbCl_2$) with or without *H. filicina* extract (0.5% in distilled water), a distilled water control, and a positive control (*H. filicina* extract) for 7 days. Physiological responses were investigated. Seedlings that had been treated with 60 and 120 μM $PbCl_2$ without *H. filicina* extract showed root growth reduction of 55% and 68.6%, respectively, relative to the control, whereas for 60 and 120 μM $PbCl_2$-treated seedlings with *H. filicina* extract applied, the reductions in root growth were lower, at 27.44% and 50.51%. The seedling viability index was decreased by 68.14% at 120 μM $PbCl_2$ application without *H. filicina* extract, whereas a 42.48% reduction was recorded for 120 μM $PbCl_2$-treated seedlings with *H. filicina* extract applied. Moreover, $PbCl_2$ accumulation resulted in a decrease in leaf pigment content. Leaf pigment content was high in plants receiving the *H. filicina* extract. The rate of lipid peroxidation caused by $PbCl_2$ was reduced with application of *H. filicina* extract. Genomic template stability was determined by using the inter simple sequence repeat-PCR technique, which revealed a decrease in DNA stabilisation with an increase in lead accumulation. However, this was alleviated by application of *H. filicina* extract. Our findings indicate that *H. filicina* extract both stimulates plant growth and protects from toxic effects by reducing accumulation of metals in the cell.

Keywords: chlorophyll content, DNA stability, ISSR-PCR, lead, macroalgae, metal tolerance, plant growth, tomato.

Introduction

Lead accumulates in agricultural soils through various anthropogenic sources such as additives in pigments used for various industrial purposes, smelting ores, discharges through accumulators, burning coal and automobile exhaust (Gottesfeld *et al.* 2018). Plants take up the increased lead accumulated in the soil, and the lead then accumulates in various plant tissues, leading to an imbalance in nutrient uptake, a decrease in growth, a disruption in cell division, changes in chloroplast structure, and a decrease in photosynthetic efficiency (Lamhamdi *et al.* 2011; Kushwaha *et al.* 2018). In addition, high lead concentrations cause a large amount of reactive oxygen species (ROS) in plants. As a result, various proteins can become damaged, and the activity of enzymes is inhibited, finally altering the expression of genes (Bali *et al.* 2019). Some studies have indicated that high lead accumulation in plants also reduces the formation of nucleic acids, results in changes to DNA synthesis, and decreases genome template stabilisation (Cenkci *et al.* 2010; Malar *et al.* 2014).

Plants have various defence systems to overcome the negative effects of metal stress. Detoxification of lead can occur by two different mechanisms. The first prevents lead

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ions from entering the plant system, defined as an exclusion mechanism. The second is intrinsic tolerance to lead stress. Toxicity in plants is decreased by the production of various secondary metabolites and metal-chelating compounds that can bind to and reduce the toxic effects of lead (Pourrut *et al.* 2011). Similarly, various biosorbents have metal-chelating characteristics akin to natural internal mechanisms in plants; they can reduce heavy metal uptake by removing heavy metals from the environment. The use of biomass adsorption techniques is becoming widespread for removing heavy metals from the environment. Among biosorbents, marine macroalgae are some of the most promising (Das *et al.* 2017; Nasab *et al.* 2017). The potential of seaweeds as biosorbents for heavy metal removal has been discussed by several investigators (Abd-Elhady 2015; Bădescu *et al.* 2017; Nazal 2019). The role of seaweed extracts as regulators of both plant growth and tolerance to heavy metal stress has not been fully investigated.

Macroalgae are an environmentally friendly natural resource, containing unique polysaccharides and various secondary metabolites. Therefore, marine macroalgae are considered valuable bioresources with promising multifunctional components that can be used for ecologically safe breeding practices for sustainable agriculture (Castro *et al.* 2012; Castellanos-Barriga *et al.* 2017). Previous research findings stress the fact that algal extracts promote plant growth. Some studies have indicated that macroalgae extracts are important biostimulants that improve plant growth, development, and seed germination without adverse effects on crop quality (Castellanos-Barriga *et al.* 2017; Mzibra *et al.* 2021). In particular, the treatment of seeds or seedlings with natural algae-derived extracts has proven to be a useful approach to increase plant productivity in response to environmental stresses such as salt and drought (Kasim *et al.* 2016; Rouphael *et al.* 2017; Goñi *et al.* 2018). Macroalgae also play a role in the development of tolerance to stress in plants; however, their function in connection with tolerance to metal stress is not fully understood.

This study investigated the lead stress tolerance capacity of the economically important crop plant tomato (*Solanum lycopersicum* L.) following application of extracts of *Halopteris filicina* (Grateloup) Kützing, a brown alga, to the plants. The protective effect of *H. filicina* extracts on genomic template stability was also determined.

Materials and methods

Collection of seaweed

Seaweed samples were collected by hand from 30 cm depth near the shore of Urla, Izmir, Turkey (38°22′2″N, 26°46′59″E). Samples were transferred to the laboratory in plastic ziplock bags and stored in a cool box. The samples were identified based on morphological features and determined as *H. filicina* (Grateloup) Kützing. Samples were rinsed with seawater followed by distilled water, and all of the epiphytes were cleaned following air-drying at room temperature. These seaweed samples were stored at +4°C until further analysis.

Analysis of *H. filicina* for antioxidant activity

The antioxidant (ROS scavenging) capacity of *H. filicina* was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. A dried algal sample (10 g) was powdered with mortar and pestle in liquid nitrogen and ground in 80% ethanol. After centrifugation at 9072*g* for 10 min, the supernatant (50 μL) was transferred to a new tube, and DPPH solution (1.95 mL) was added. The DPPH solution comprised 0.0012 g DPPH dissolved in 50 mL methanol. The samples were incubated for 15 min under dark conditions at room temperature and read in a UV–visible spectrophotometer (Pharo 300; Merck, Darmstadt, Germany) at 515 nm. Controls were 1.95 mL DPPH and 50 μL distilled water.

$$\%\ \text{Reactive oxygen scavengering capacity} = ((\text{AbsDPPH} - \text{Abssample})/\text{AbsDPPH}) \times 100$$

Preparation of algal extracts for application to seeds

Powdered dried algal samples (10 g) were ground and boiled with 100 mL distilled water in a water bath at 100°C for 1 h. The liquid seaweed extracts obtained were cooled and filtered using filter paper (Whatman No. 5). The extract supernatant was considered as a 10% concentration in each algal extract. Algal extracts of *H. filicina* were prepared to 0.5% with distilled water. Distilled water served as a control (0%).

Seed germination and seedling growth bioassay

Tomato seeds (Lot no. TR.16.20.1384.D005) were sterilised with 10% sodium hypochlorite for 8 min and rinsed with sterile distilled water three times before the experiment. After sterilisation, one experimental group was treated with distilled water (as control), and one group was treated with 0.5% algal extract (as positive control) in Petri dishes for 7 days. Likewise, solutions of $PbCl_2$ of concentrations 60 μM (L1) and 120 μM (L2) were applied to tomato seeds in Petri dishes for 7 days. Two experimental groups were established as L1 + 0.5% *H. filicina* extract (H + L1) and L2 + 0.5% *H. filicina* extract (H + L2) in Petri dishes for 7 days. Each experimental setup was performed in three independent trials.

Germination percentage (GP), mean daily germination, mean germination time, germination index, seed vigour index (SVI), and relative root elongation (RRE) were determined by the following formulae:

$$\text{GP} = \left(\frac{n}{N}\right) \times 100$$

$$\text{SVI} = \text{seedling length}\ (\text{shoot} + \text{root}) \times \text{GP}$$

$$RRE = \left(\frac{\text{mean root length in test solution}}{\text{mean root length in control}}\right) \times 100$$

where n is number of seeds germinated, and N is total number of seeds.

Each Petri dish included 10 seeds for each experimental group and dishes were kept in a climate room at 25°C for 7 days. At the end of the experimental period, shoot and root length were measured.

Determination of chlorophyll content

Chlorophyll content was measured following the method modified by Wellburn (1994). Leaf samples (100 mg) were cut into small pieces and placed in 10 mL absolute methanol. After extraction for 24 h in the dark at 4°C, the contents of chlorophyll (Chl *a*, Chl *b)* and carotenoids were measured by UV spectrophotometer at 666, 653 and 470 nm, respectively. Pigment contents were calculated according to experimental equations as described by Wellburn (1994).

Lipid peroxidation analysis

The rate of lipid peroxidation was measured by determining the quantity of malondialdehyde (MDA) (Karabal *et al.* 2003). In each experimental group, 532 and 600 nm absorbance values were measured following the thiobarbituric acid reactive substances (TBARS) method. The amount of MDA was determined by calculating the difference between the two absorbance values. There were three repetitions for each treatment.

Element analysis

Sample digestion was performed with a Titan MPS microwave digestion system (PerkinElmer, Waltham, MA, USA). Concentrated nitric acid (6 mL) and concentrated hydrochloric acid (2 mL) were added to each vessel containing a 150-mg root sample. After this, the samples were kept at room temperature for a short time, and the containers were closed and placed in the Titan. The program in Supplementary Material Table S1 was used for the microwave digestion process. When the digestion process was completed, samples were transferred to 100-mL metered balloons and diluted to 100 mL with deionised water. Inductively coupled plasma-atomic emission spectroscopy (ICP-MS/OES) (Avio 200; PerkinElmer) was used for lead analysis. Each trial was repeated three times.

DNA isolation and ISSR-PCR protocol

DNA isolation from tomato roots was done by the micro-preparation method (Fulton *et al.* 1995). After DNA yield and quality were measured using Nanodrop (Shimadzu Biotech, Kyoto, Japan), isolated DNA samples were visualised on 1% agarose gel. Inter simple sequence repeat (ISSR)-PCR was modified according to Çekiç *et al.* (2017). We used 15 different ISSR primers as molecular markers during the investigation (Table S2). Optimisation experiments of the 15 ISSR primers were performed in the control group, and the best amplified 13 primers were selected. Each analysis was repeated three times.

Genomic template analysis

Each change observed in ISSR profiles was given an $a + 1$ arbitrary score. The average was then calculated for each experimental group treatment to $PbCl_2$. Genomic template stability (GTS, %) was calculated as:

$$GTS = 100 - \left(100 \times \left(\frac{a}{n}\right)\right)$$

where a is the average number of the changes in DNA profiles, and n is the number of bands selected in control DNA profiles.

Statistical analyses

Pearson's correlation and one-way analysis of variance with *post hoc* Tukey HSD test were performed by IBM SPSS statistics 28 programme. The critical value for statistical significance was $P < 0.05$ and 0.01.

Results

ROS scavenging capacity of *H. filicina* extract

A ROS scavenging effect of *H. filicina* was revealed by the DPPH method (Table 1), indicating that *H. filicina* extracts have an antioxidant capacity.

Effect of *H. filicina* extract on growth parameters of tomato under lead stress

Extracts of *H. filicina* significantly increased the germination percentage of tomato seeds (Fig. 1). Treatments L1 and L2 resulted in 10% and 12.37% reduction in germination rate, respectively, compared with the control. Decreases in germination rates of 4.72% and 8.77% were noted in the H + L1 and H + L2 experimental groups.

An increase of 15% in shoot growth was detected at the end of 7 days in seedling that had been treated with 0.5% *H. filicina* extract alone (Fig. 2*a*). Shoot growth of L1 and L2 groups decreased by 10% and 17.4%, respectively. An increase in shoot growth was observed in H + L1 and H + L2 groups, and lead absorbance was significantly inhibited. Root growth was highest in the seedlings treated with *H. filicina* compared to control. In the L1 and L2 groups, root growth decreased by 55% and 68.6% compared with the control. In the H + L1 and H + L2 experimental groups, root growth was reduced by 27.44% and 50.51%, respectively (Fig. 2*b*, Table 2). The seedling vigour index decreased by 45% and 68.14% in the L1 and L2 groups compared with the control, whereas 33.89% and 42.48% reductions were observed for H + L1 and H + L2 groups (Table 2).

Table 1. Reactive oxygen species scavenger capacity of *H. filicina*.

Algal species	Systematic group of species	% Scavenging ($X \pm$ s.d.)
H. filicina	Ochrophyta	105.88 ± 0.04

Chlorophyll and carotenoid contents

Chlorophyll *a*, total chlorophyll and carotenoid contents were enhanced in tomato seedling leaves treated with *H. filicina* extracts (Table 3). Total chlorophyll decreased by 12.43% and 21.32% in the L1 and L2 groups, respectively, and carotenoid contents by 21.83% and 40.04%. Total chlorophyll and carotenoid contents were restored to levels higher than the control under H + L1 but not under H + L2.

Effect of lead chloride treatment on lipid peroxidation rate

No significant difference was recorded in the lipid peroxidation rate of the control and 0.5% *H. filicina* extract-only samples. However, a significant increase in MDA content was detected in groups L1 and L2 ($P < 0.05$; Fig. 3).

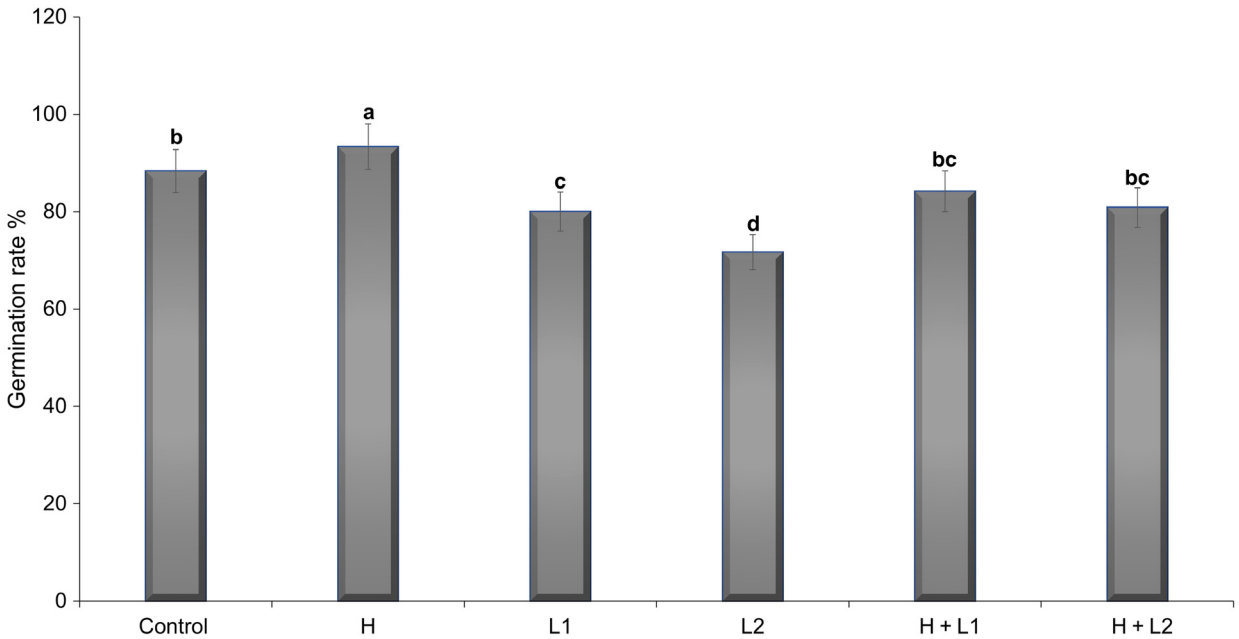

Fig. 1. Germination rate of tomato treated with 60 (L1) and 120 (L2) μM $PbCl_2$ and extract of *H. filicina* (H). Means not sharing a letter are significantly different at $P = 0.01$. Capped lines are ± s.d.

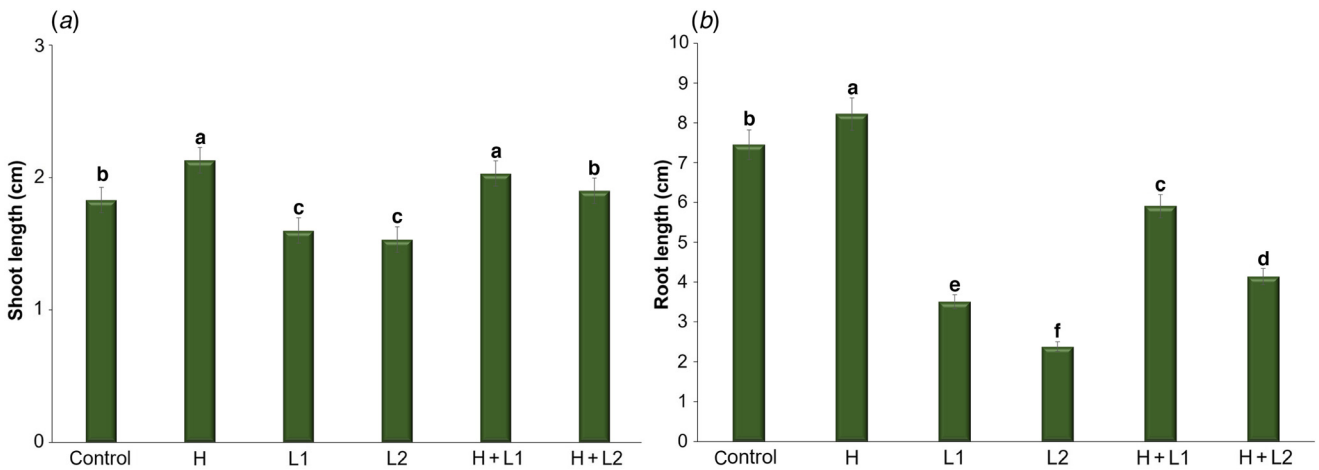

Fig. 2. (*a*) Shoot and (*b*) root length of tomato seedlings treated with 60 (L1) and 120 (L2) μM $PbCl_2$ and extract of *H. filicina* (H). Means not sharing a letter are significantly different at $P = 0.01$. Capped lines are ± s.d.

Table 2. Growth parameters of tomato seedlings treated with 60 (L1) and 120 (L2) μM $PbCl_2$ concentrations and extract of *H. filicina* (H).

Groups	Seedling vigour index	Relative root elongation
Control	839.68 ± 8.19b	100b
H	979.08 ± 12.94a	109.02 ± 0.38a
L1	421.33 ± 5.07e	46.65 ± 0.59e
L2	280.48 ± 4.79f	31.34 ± 0.73f
H + L1	677.17 ± 9.59c	78.15 ± 0.96c
H + L2	488.47 ± 2.67d	54.75 ± 0.59d

Within a parameter, means not sharing a letter are significantly different at $P = 0.01$.

The increase in MDA content due to lead stress was significantly alleviated in the H + L1 and H + L2 groups.

Lead chloride accumulation in roots

The $PbCl_2$ concentration in the roots of the L1 group was measured at 129.57 mg/kg. In the H + L1 group, accumulation in roots significantly decreased (Fig. 4). However, the highest $PbCl_2$ accumulation was found in the H + L2 group.

Effect of *H. filicina* extract on DNA template stability of tomato under lead stress

We detected 202 bands in total in the presence and absence of $PbCl_2$ and *H. filicina* extracts. Table 4 presents the observed differences in ISSR profiles as band appearance/disappearance and band-intensity decrease/increase. ISSR primers caused different DNA amplification profiles depending on the lead concentration. ISSR809 amplified DNA fragments

Table 3. Leaf chlorophyll and carotenoid content of tomato seedlings under 60 (L1) and 120 (L2) μM $PbCl_2$ and extract of *H. filicina* (H).

	Chl *a*	Chl *b*	Chl *a* + Chl *b*	Carotenoid
Control	21.07 ± 0.50abc	7.49 ± 0.21	28.56 ± 0.36ab	1764.51 ± 4.45c
H	24.79 ± 0.89a	8.42 ± 0.26	33.22 ± 0.58a	2110.40 ± 7.23b
L1	17.11 ± 0.34cd	7.90 ± 0.14	25.01 ± 0.14bc	1379.18 ± 2.02d
L2	13.32 ± 0.13d	9.15 ± 0.16	22.47 ± 0.19c	1058.75 ± 2.83f
H + L1	22.38 ± 0.24ab	8.54 ± 0.18	30.92 ± 0.13a	2215.23 ± 2.46a
H + L2	19.18 ± 0.31bc	9.45 ± 0.19	28.63 ± 0.23ab	1478.98 ± 2.78d

Within a parameter, means not sharing a letter are significantly different at $P = 0.01$.

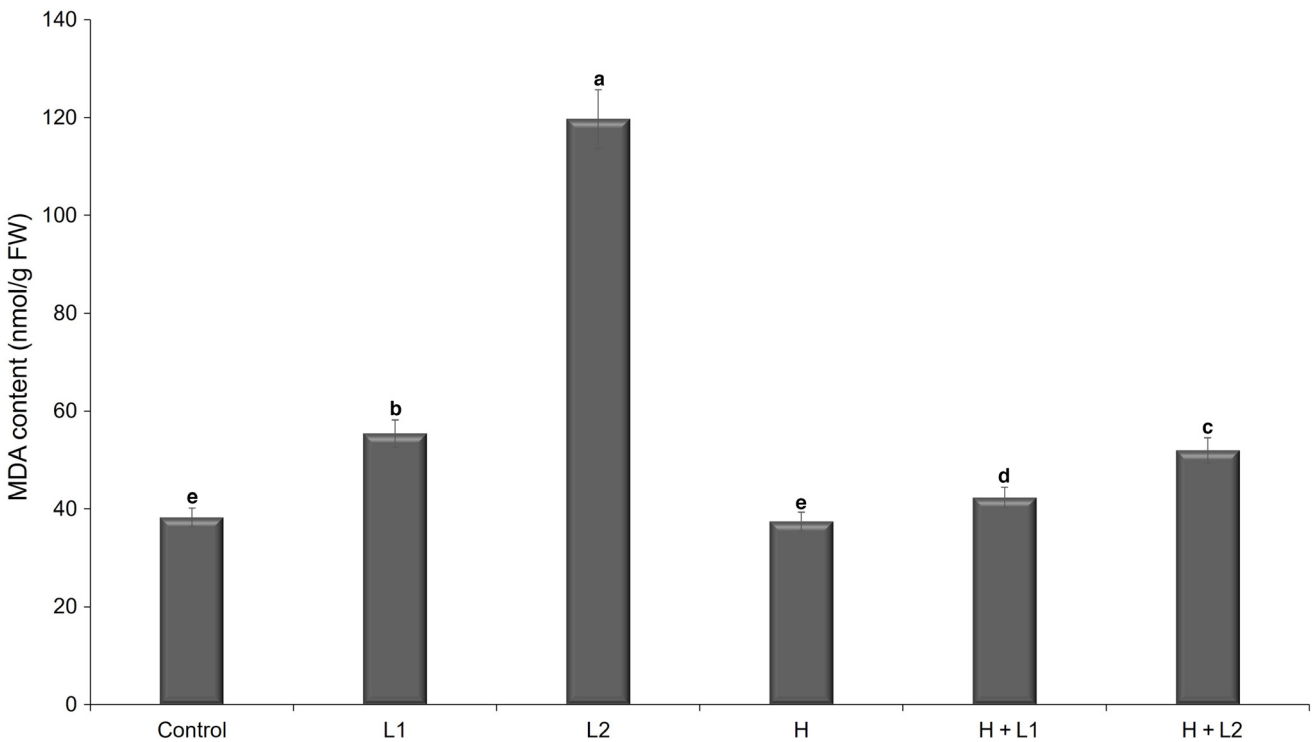

Fig. 3. Lipid peroxidation rate of tomato seedling roots treated with 60 (L1) and 120 (L2) μM $PbCl_2$ and extract of *H. filicina* (H). Means not sharing a letter are significantly different at $P = 0.01$. Capped lines are ± s.d.

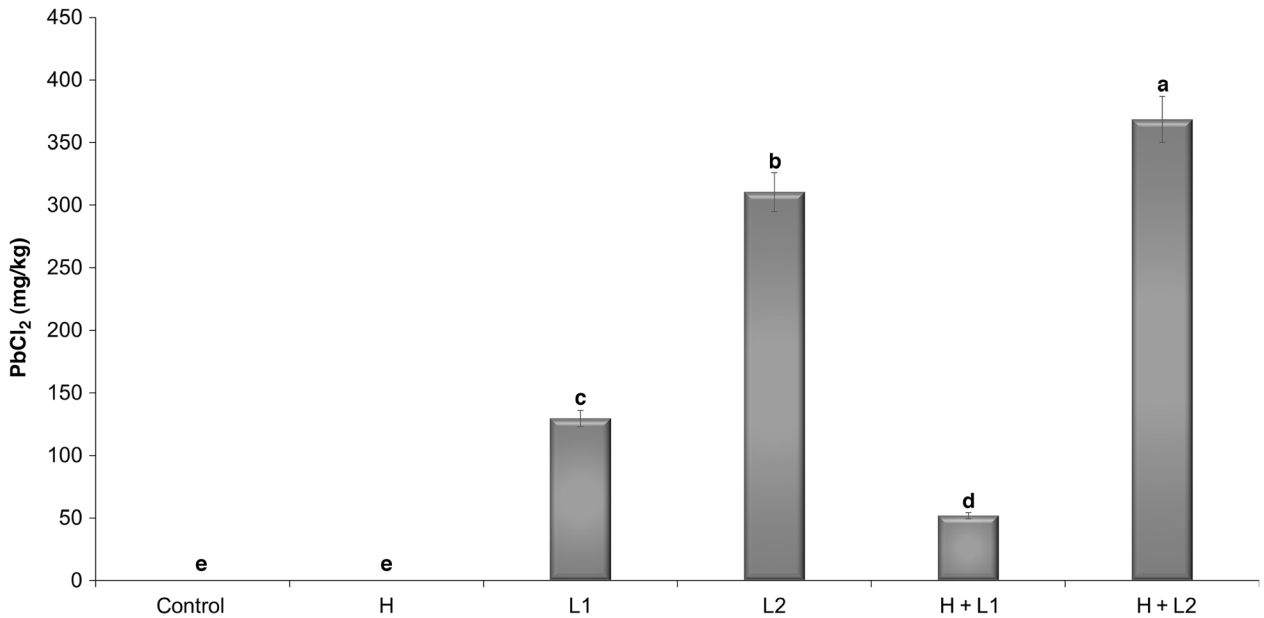

Fig. 4. Content of $PbCl_2$ in tomato seedling roots treated with 60 (L1) and 120 (L2) μM $PbCl_2$ and extract of *H. filicina* (H). Means not sharing a letter are significantly different at $P = 0.01$. Capped lines are ± s.d.

Table 4. Changes of total bands in control and polymorphic bands in treatments with 60 (L1) and 120 (L2) μM $PbCl_2$ and extract of *H. filicina* (H) for tomato roots, where a is appearance band number, b is disappearance band number, c is decreased band intensity, and d is increased band intensity.

	C	L1				L2				H				H + L1				H + L2			
		a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
ISSR809	7	0	0	0	0	0	3	1	0	0	0	0	1	0	0	0	3	0	0	0	1
ISSR810	5	0	2	0	1	3	0	1	1	0	0	0	1	1	0	0	1	1	0	0	1
ISSR818	6	3	2	0	0	1	0	0	0	0	5	0	0	1	0	0	0	1	2	0	0
ISSR842	4	5	1	0	1	7	2	0	2	0	0	0	0	5	0	0	2	4	2	0	2
ISSR828	6	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	0	1	0	2
ISSR868	2	2	0	0	0	4	0	0	0	1	0	0	0	3	0	0	0	4	0	0	0
ISSR890	4	2	1	2	0	2	0	0	3	1	1	2	1	2	1	0	2	2	1	1	1
ISSR873	8	1	1	0	2	0	2	0	1	0	0	0	0	0	0	0	0	2	0	0	2
ISSR808	12	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	8	0	0
ISSR866	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ISSR826	0	3	0	0	0	5	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
ISSR807	8	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
ISSR880	9	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	2	0	0	0	2
Total bands	75	18	9	2	4	26	9	3	7	2	6	2	4	15	1	0	12	14	14	2	11
a + b + c + d		35				45				14				28				41			

of ~1200, 1000, 850, 750, 650, 500 and 250 bp; however, amplification of 1200, 1000 and 850 bp fragments was lost in the groups treated with 120 μM $PbCl_2$. These three bands were present in the groups treated with *H. filicina* extracts, H+L1, and H+L2, depending on lead application. In addition, a significant decrease in polymorphic band formation was observed in the groups treated with *H. filicina* extracts and $PbCl_2$ (Fig. 5). The highest number of polymorphic bands was detected in the L2 group with all 13 primers used. According to the ISSR assay, GTS ratio between the control and 0.5% H treatment was ~81.08% (Table 5). The lowest GTS value (40%) was observed in the

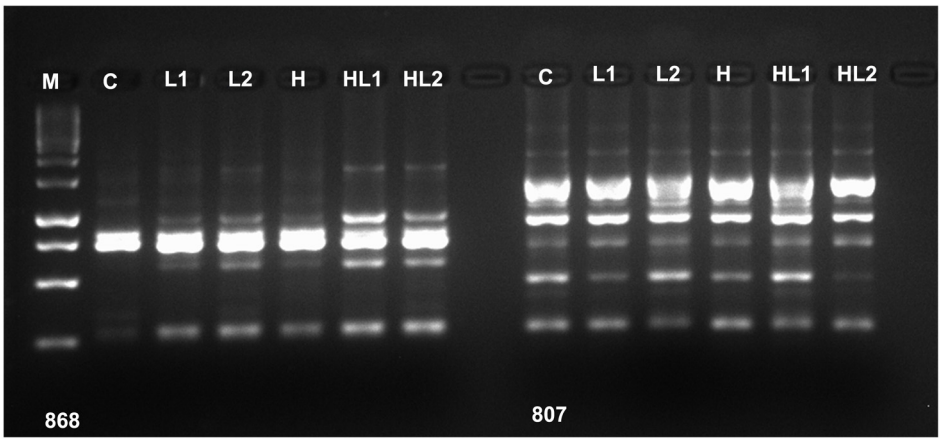

Fig. 5. ISSR-PCR results of tomato roots treated with 60 (L1) and 120 (L2) μM $PbCl_2$ and extract of *H. filicina* (H).

Table 5. Genomic template stability (GTS) ratio for 13 ISSR primers in tomato roots treated with 60 (L1) and 120 (L2) μM $PbCl_2$ and extract of *H. filicina* (H).

Treated groups	GTS ratio (%)
L1	56.0
L2	40.0
H	81.08
H + L1	62.67
H + L2	45.33

Table 6. Pearson's correlation tests of Pb uptake and lipid peroxidation rate with growth parameters and GTS ratio ($n = 9$).

	Pb uptake	Lipid peroxidation rate
GTS	**−0.8575**	−0.6714
Seedling vigour index	**−0.8083**	**−0.7761**
Relative root elongation	**−0.8810**	**−0.7768**
Pb uptake	–	0.6459

Values in bold indicate strong (negative) correlation; the other values indicate a moderate correlation.

L2 roots. Treatment with *H. filicina* extract (H + L1, H + L2) increased the GTS value compared with L1 and L2.

Pearson's correlation analysis showed that lead toxicity is related to accumulation in roots and to lipid peroxidation levels. GTS, seedling vigour index, and relative root elongation were strongly negatively correlated with lead uptake (Table 6). By contrast, lead uptake showed a strong positive correlation with the lipid peroxidation rate.

Discussion

Lead is one of the most abundant toxic elements, adversely affecting plant growth and development, cell division and photosynthetic processes, inhibiting the activities of some enzymes, and causing changes in membrane permeability. Many studies have shown that lead has a suppressive effect on seed germination (Lamhamdi *et al.* 2011; Soares *et al.* 2020). High concentrations of lead reduce germination and growth in rice seedlings (Soares *et al.* 2020). Earlier studies also indicated that inhibition of seed germination may be due to the interaction of $PbCl_2$ with amylase and protease enzymes (Lamhamdi *et al.* 2011). A decrease in seed germination percentage was observed in this study depending on the $PbCl_2$ concentration (Fig. 1). However, it has been determined that *H. filicina* extracts can reduce this inhibition. Previous studies have shown that seaweed extracts stimulate seed germination and plant growth through the action of various minerals, polysaccharides, or components such as betaines and phytohormones (e.g. IAA) (Hernández-Herrera *et al.* 2014; Michalak and Chojnacka 2015; Rouphael *et al.* 2017). Hu *et al.* (2004) also reported that the germination-enhancing effects of algal-derived polysaccharides might occur by affecting amylase and metabolic activities in seeds. In the present study, *H. filicina* extract promoted seed germination and ameliorated the inhibitory effect of $PbCl_2$, possibly via metabolites contained in the extract.

Lead is reported to have an inhibitory and concentration-dependent effect on plant growth parameters such as root/shoot length, dry/fresh weight, and vigour index (Lamhamdi *et al.* 2011; Soares *et al.* 2020). Roots, in particular, are more susceptible to $PbCl_2$ toxicity than coleoptiles because roots are in direct contact with the lead in the soil. Arias *et al.* (2010) reported that lead significantly inhibits root elongation in mesquite (*Prosopis* sp.) plants. A study on tomato seedlings determined that increased lead concentrations in the growth medium negatively affected root and shoot elongation and biomass (Akinci *et al.* 2010). The present investigation revealed a significant decrease in

root length (Fig. 2*b*), corresponding to lead accumulation of 129.57 and 310.55 mg/kg in L1 and L2 roots (Fig. 4). However, *H. filicina* significantly reduced $PbCl_2$ uptake in the L1 group and reduced the inhibitory effects on the roots (Fig. 2, Table 2).

Seaweed extracts are well known to stimulate root and shoot growth (Castro *et al.* 2012; Vinoth *et al.* 2014; Mzibra *et al.* 2021). The results obtained in our study have shown for the first time that *H. filicina* extracts can play a protective role against metal toxicity, alleviating the decrease in seedling vigour index and relative root elongation capacity expected with lead toxicity. Brown algae contain many secondary metabolites with the ability to chelate heavy metals. Our findings suggest that the toxic effect of lead can be reduced when bound to these metabolites.

Chloroplast pigments are responsible for biomass production and are immediately affected by stressful environmental conditions (Alamri *et al.* 2018). Most heavy metals inhibit chlorophyll and carotenoid biosynthesis; thus, incorporation of pigments into photosystems is delayed (Mesmar and Jaber 1991; Cenkci *et al.* 2010; Alamri *et al.* 2018). Oxidative stress also decreases chlorophyll content. Membrane lipid peroxidation is a useful biomarker of oxidative damage by metals. It has been reported that both carbonyl groups and MDA content increased in *Vicia faba* seedlings treated with 2000 mg Pb/kg (Wang *et al.* 2008, 2010). Similarly, Alamri *et al.* (2018) reported decreased biosynthesis of total chlorophyll and increased chlorophyll degradation in plants exposed to lead. The degradation of chlorophyll is associated with internal accumulation of MDA and H_2O_2 (Wang *et al.* 2008, 2010; Venkatachalam *et al.* 2017). Similarly, our study determined that lipid peroxidation increased due to increased lead accumulation, and that Chl *a* and carotenoid contents decreased (Fig. 3, Table 3); however, the MDA content was lower and total chlorophyll and carotenoid contents increased in the H + L1 group. The increase in carotenoid content and decreased degradation of chlorophyll with the administration of *H. filicina* extracts under $PbCl_2$ toxicity could be due to the effective role of the *H. filicina* extracts in reducing lipid peroxidation through their ROS scavenging activity (Table 1).

Genomic template stability is widely used as a marker for DNA damage (Sukumaran and Grant 2013). ISSR and random amplified polymorphic DNA (RAPD) profiles are used as parameters for comparison of genotoxic damage (Correia *et al.* 2014). The ISSR technique is very suitable for finding the mutational effects of toxic heavy metals; its reproducibility is higher than that of the RADP technique owing to the use of 16–25 bp primers (Bornet and Branchard 2001; Correia *et al.* 2014). We used the ISSR-PCR technique to determine the genotoxicity caused by $PbCl_2$ and the effects of *H. filicina* extracts (Table 4, Fig. 5). Some of the earlier studies revealed that lead causes changes in the RAPD profile and GTS ratio depending on concentration (Cenkci *et al.* 2010; Venkatachalam *et al.* 2017). Using the RAPD-PCR method, Venkatachalam *et al.* (2017) showed that 100 and 500 mg Pb/L resulted in DNA damage in the leaves of *Acalypha indica*. We observed that $PbCl_2$ application at different concentrations decreased the GTS. Alterations in DNA structure can increase oxidative stress depending on the applied $PbCl_2$concentration (Cenkci *et al.* 2010). Comparing our ISSR profile results with the lipid peroxidation values indicated that lead toxicity was associated with increased MDA contents in the tomato plants. On the other hand, the GTS ratio was lower in the L1 group treated with *H. filicina* extract than in the untreated samples (Table 5). These results indicate that there was less lead accumulation in the roots in the H+L1 group, which may have experienced reduced DNA damage due to oxidative stress (Fig. 3). However, our results showed that the protective effect of *H. filicina* extracts decreases as the lead concentration increases. As indicated by the results of Pearson's correlation analysis, there was a negative correlation between lead accumulation and GTS (Table 6). The increase in lead accumulation and lipid peroxidation in the H + L2 group indicates that the algal extracts are insufficient to reduce oxidative damage under high-level $PbCl_2$ application.

In conclusion, we report for the first time that extracts of the brown alga *H. filicina* reduced the possible harmful effects of lead by inhibiting its uptake in a concentration dependent-manner. The *H. filicina* extracts were determined to be good candidates for agricultural use to increase plant growth and pigment contents, and for their ROS scavenging effect. Future studies will investigate how *H. filicina* imparts tolerance to metal stress and its molecular signaling mechanisms.

Supplementary material

Supplementary material is available online.

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