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Ameliorative effects of *Sargassum stolonifolium* amendment on physiological and biochemical parameters in *Brassica chinensis* L. under cadmium contaminated soil

Efeitos remediadores do uso de *Sargassum stolonifolium* sobre parâmetros fisiológicos e bioquímicos de *Brassica chinensis* L. sob solo contaminado por cádmio

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Highlights

Cadmium negatively affects *B. chinensis* L. physiological and biochemical parameters.
MDA, H_2O_2, and PC levels decrease in soil amended with *S. stolonifolium.*
Physio-biochemical activities were improved in *S. stolonifolium* amended group.
S. stolonifolium has the ability to reduce cadmium toxicity in *B. chinensis* plants.

Abstract

This research was carried out to examine the effects of *Sargassum stolonifolium* on reducing cadmium in *Brassica chinensis* L. tissue, its influential roles on physiological parameters and antioxidant mechanism in B. chinensis exposed to cadmium stress. Different levels of Cd (50 mg and 100 mg) with and without *S. stolonifolium* (25g, 50g and 100g) under five replications were explored in this study. Biomass, photosynthetic pigment, relative water content (RWC), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), 2,2-diphenyl-1-picrylhydrazyl (DPPH), total antioxidant activity (TAA), non-protein thiol (NPT), protein thiol

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(PT), protein bound thiol, glutathione (GSH), phytochelatins, ascorbate peroxidase (APX), Catalase (CAT), superoxide dismutase (SOD) and guaiacol peroxidase (POD) were determined. The results revealed that Cd stress significantly ($P < 0.05$) reduced plant biomass and physiological attributes, and accumulated higher Cd concentrations in plant tissues with the increasing rate of Cd concentration in the soil. However, incorporation of *S. stolonifolium* at 100 g rate in 50 mg Cd (T4) spiked soil increased the FW (40.6%) and DW (72.2%) relative to the respective treatment without *S. stolonifolium.* Similarly, Cd accumulation in roots, stem and leaves was decreased by 90.25%, 82.93% and 84.6% respectively compared to T1 (50 mg Cd) and thereby reducing leaf MDA and H_2O_2 contents by 40.1% and 68.8%, respectively, at 50 mg Cd kg^{-1} spiked soil relative to T1. An increase was noticed in the chlorophyll a, b, carotenoid, SPAD and RWC with a value of 114.6%, 20.7%, 73.7%, 44.8%, and 6.3%, respectively, over the control (T0). DPPH scavenging activity and TAA increased 119.8 and 81.5% percent respectively over the T0. Concentration increment of NPT, TT, GSH and PCs by 66.7%, 49.1%, 60.1%, 96.1% and 3.4% respectively, was noticed in T4 compared to T0. Antioxidant enzymes activities increased by APX (92.8%), CAT (73.1%), SOD (20.9%) and POD (88.9%) for T4 compared to the control. *S. stolonifolium* has the potential to improve growth and increase the defensive system of *B. chinensis* and ameliorate cadmium phytotoxicity as well as immobilization.
Key words: Biosorbent. *Brassica chinensis.* Cadmium. Phytotoxicity *Sargassum stolonifolium.*

Resumo

Esta pesquisa foi realizada para examinar os efeitos de *Sargassum stolonifolium* na biossorção de cádmio em tecido de *Brassica chinensis* L., e a influência em parâmetros fisiológicos e antioxidantes em *B. chinensis* exposta ao estresse por cádmio. Foram avaliados níveis de Cd (50 mg e 100 mg), com ou sem *S. stolonifolium* (25g, 50g e 100g), em cinco repetições. Biomassa, pigmento fotossintético, teor relativo de água (RWC), malondialdeído (MDA), peróxido de hidrogênio (H2O2), 2,2-difenil-1-picrilhidrazil (DPPH), atividade antioxidante total (TAA), tiol não proteico (NPT), tiol proteico (PT), tiol ligado às proteínas, glutationa (GSH), fitoquelatinas, ascorbato peroxidase (APX), Catalase (CAT), superóxido dismutase (SOD) e guaiacol peroxidase (POD) foram determinados. Os resultados revelaram que o estresse por Cd reduziu significativamente ($P < 0,05$) a biomassa vegetal e os atributos fisiológicos, e acumulou maiores concentrações de Cd nos tecidos vegetais com o aumento da taxa de concentração de Cd no solo. No entanto, a incorporação de *S. stolonifolium* na dose de 100 g em solo aumentou a AF (40,6%) PD (72,2%) em relação ao respectivo tratamento sem *S. stolonifolium.* Da mesma forma, o acúmulo de Cd nas raízes, caule e folhas foi reduzido em 90,25%, 82,93% e 84,6%, respectivamente, comparando T1 e, assim, reduzindo os teores de MDA e H_2O_2 nas folhas em 40,1% e 68,8%, respectivamente, a 50 mg Cd kg^{-1} de solo enriquecido em relação a T0 e T1. Um aumento foi observado na clorofila *a*, *b*, carotenoide, SPAD e RWC de 114,6%, 20,7%, 73,7%, 44,8% e 6,3%, respectivamente, em relação ao controle. Aumento da concentração de NPT, TT, GSH e PCs em 66,7%, 49,1%, 60,1%, 96,1% e 3,4%, respectivamente, foi observado em T4 em relação a T0. As atividades das enzimas antioxidantes APX (92,8%), CAT (73,1%), SOD (20,9%) e POD (88,9%) aumentam em T4, em relação ao controle. *S. stolonifolium* tem potencial para promover o crescimento e aumentar o sistema de defesa de *B. chinensis* e reduzir os efeitos negativos da fitotoxicidade do cádmio, bem como a sua imobilização.
Palavras-chave: Biossorvente. *Brassica chinensis.* Cádmio. Fitotoxicidade. *Sargassum stolonifolium.*

Introduction

The most usual sink for wastes containing heavy metals has been soil (Jadia & Fulekar, 2008). In both developing and developed regions of the world, anthropogenic activities such as the application of synthetic phosphate fertilizers, metal manufacturing, urban waste disposal, mining, smelting as well as weathering of parent material, volcanic eruptions and atmospheric deposition, are important sources of soil heavy metal pollution (Fu & Wang, 2011; Haider et al., 2021; Kamran et al., 2019). One of the most non-essential, toxic, and carcinogenic heavy metals is cadmium (Ehsan et al., 2014). Cadmium is a non-biodegradable element that has deleterious effects on the ecosystem, limits the uptake of essential plant elements, degrades soil quality, and hence reduces crop productivity (Murtaza et al., 2015; Rizwan et al., 2016a; Shaari et al., 2024).

Cadmium is very mobile and fast assimilating. It enters the plants through the roots and is then translocated through the shoots in ionic form into the vascular bundles via ascending sap and/or transporters (Dong et al., 2019). Abbas et al. (2017); Nahar et al. (2016) and Rochayati et al. (2011) reported that accumulation of Cd in plant tissues evoked physico-chemical anomalies including necrosis and ion homeostasis, chlorosis, limited micro and macronutrients uptake, net photosynthetic activity and ultimate retardation of growth. Higher cadmium content may entreat excessive production of reactive oxygen species with concomitant destruction of biomembranes by oxidation of lipids and proteins and biomolecules, as well as severe effects on the antioxidant defense system (Nagajyoti et al., 2010; Gallego et al., 2012). The production of leafy vegetables such as *Brassica chinensis* L. in soils contaminated with cadmium (Cd) poses a severe threat to human health and food safety worldwide (Kamran et al., 2019; Abdullahi et al., 2021). It is widely known that the majority of Cd in the human body originates from edible plants, particularly leafy greens (L. Huang et al., 2020).

A number of remediation technics had been put forward previously to restore soil qualities. However, the most recognized method is *in situ* immobilization which decreases soil cadmium bioavailability because of its efficacy and cost- effectiveness (Hamid et al., 2019). Nowadays, the commonly used amendments include; biochar, metal oxides, phosphate compounds, alumino-silicate minerals (zeolite), lime, and organic composts to ameliorate the toxicity of cadmium in extremely polluted soil (Rizwan et al., 2018; Saeed et al., 2019; Xiang et al., 2019). Plant hormones such as Jasmonic and Acid Salicylic Acid (Chakraborty et al., 2020), eggshell (Ok et al., 2011), hydrogel-mycorrhiza (Beniwal et al., 2011) amongst others are used for amendment.

Algae are autophytes that have chlorophyll and perform oxygenic photosynthesis. They are diverse and widely distributed. Algae are not a formal taxonomic group of organisms but rather a highly sorted assembly of organisms with sundry evolutionary ancestors and high broadly diversified genetic makeup, as evidenced by the enormous varieties that algae exhibit with regards to ultrastructure, morphological, biochemical, ecological, and physiological characteristics (Rindi et al., 2012).

The application of dead seaweed biomass as an adsorbent and immobilizer

for trace metals in soil is of specific interest. Various investigations have demonstrated that the polysaccharides found in their cell walls have high adsorption properties and selectivity for several metal cations (Jalali et al., 2002; Murphy et al., 2007; Ortiz-Calderon et al., 2017). A cellulose-based fibril skeleton and a nebulous matrix of galactans containing sulfate consisting of agar and carrageenans are found in the cell wall of red algae, while for brown algae, fucoidan and alginates constitute their cell walls (Ortiz-Calderon et al., 2017; Stiger-Pouvreau et al., 2016). The major cause of their high capacity for immobilising metal cations has been credited to the constituents of their cell walls and reserve polymers (Michel et al., 2010).

Dry algins weighing 10-40% and fucoidan 5-20% are two kinds of anionic polysaccharides that give the cell wall strength and flexibility, while mannitol and laminarans supply and store energy when needed. Other components contributing to biosorption are phlorotannins and proteins, although to a smaller extent, accounting for around 5% of the biomass dry weight (Zayed, 2018). The functional groups acetamide, carboxyl, amide, amine, sulfhydryl, and sulphate in seaweed biomass can bind and sequester the metals (Davis et al., 2003). Electrostatic attraction, van der Waals attraction, covalent bonding, complexation, ion exchange, microprecipitation, and adsorption are some of the methods used to capture metals (Raize et al., 2004).

Brassica chinensis L. potential for phytoremediation of harmful heavy metals has been the subject of several studies (Corley & Mutiti, 2017). Nevertheless, very few if any studies have probed the capacity of *Sargassum stolonifolium* biomass to remediate soil contaminated with Cd, ameliorate Cd phytotoxicity, and enhance the *B. chinensis* growth by boosting the biochemical and physiological characteristics under Cd stress. The oxidative stress, production of free radicals, and *Brassica chinensis* response after *Sargassum stolonifolium* biomass amendment to polluted cadmium soil have not been investigated. Therefore, the indispensable goals of our research are; to evaluate the effective application level of *Sargassum stolonifolium* biomass (0 g, 25 g, 50 g and 100 g) for Cd immobilization in contaminated soil and minimize its accumulation in edible plant parts and to evaluate the *Sargassum stolonifolium* biomass influence on physiological parameters and antioxidative defense mechanisms of *Brassica chinensis* L. under Cd stress. *S. stolonifolium* may have the ability to confer resistance to *B. chinensis* exposed to cadmium contamination.

Materials and Methods

Experimental design and treatment plan

Brassica chinensis (Pak Choi) L. seeds were sown on germination tray and kept at growth chamber for two weeks.

Media preparation; about 25 g, 50 g and 100 g ground SBM were measured and mixed with 1 kg soil. Cd (50 mg and 100 mg) were measured and first dissolved in 100 ml of deionized water and then added to 1 kg soil in the pot. The soil was turned 1 day after spiking to ensure even distribution of Cd. Two days later, the four-leaved healthy seedlings with uniform height were transplanted into the already contaminated soil in the pots, one seedling per pot, and moved to the Universiti

Sultan Zainal Abidin (UniSZA) greenhouse, located at 5° 45′ 4″ N and 102° 38′ 11″ E, Terengganu, Malaysia and kept for 6 weeks. Using Randomize Complete Block Design (RCBD), different treatments were organized in pentaplicate with the treatments shown in table 1 below. Forty-five (45) pots were used, each containing 1 kg of soil.

Table 1
Treatment plan for cadmium (50 and 100 mg) and *Sargassum stolonifolium* biomass (25, 50 and 100 g)

Treatment	Pot content
T0	Without Cd (control)
T1	50 mg Cd
T2	50 mg Cd + 25 g SBM
T3	50 mg Cd + 50 g SBM
T4	50 mg Cd + 100 g SBM
T5	100 mg Cd
T6	100 mg Cd + 25 g SBM
T7	100 mg Cd + 50 g SBM
T8	100 mg Cd + 100 g SBM

*SBM; Sargassum stolonifolium biomass, T; treatment.

The seedlings were irrigated with 100 ml of water once every morning. Weeds were mechanically controlled and no insecticide was used throughout the experiment. After 6 weeks in the greenhouse, the seedlings were harvested.

Biomass measurement

Plants materials were washed with metal-free de-ionized water after harvesting to eliminate dust particles from shoots and an ice-cold 5 mM CaCl2 solution was used for the roots to remove adherent metal ions from the root surface. Following that, the fresh biomass of plant parts was measured as soon as they were harvested. Brassica chinensis plants had their root-to-shoot length measured, and the roots and shoots were separated with a sterilised sharp knife. After that, they were put into the oven for 3 days at 60°C to dry. Then, the dry biomass of the roots and shoots were measured.

Determination of cadmium content in plant tissue

Cadmium levels in root, stem and leaf samples were determined, as described by Rauser (1987) with minor modifications. Dried leaf, stem, and root samples were crushed to a fine powder, sized using 0.5 mm mesh, and digested in a microwave digester with concentrated HNO3. An AAS was used to assess the quantity of cadmium.

Stress Biomarker

Malondialdehyde (MDA)

MDA was quantified using a Heath and Packer (1968) method with minor alteration. About 0.2 g fresh leaves in 2 mL 5% (w/v) TCA were homogenized in a mortar and pestle and then spun for 20 min at 13,000 g. A reaction mixture containing 0.5 ml supernatant and 1 mL TCA 20% (w/v) and TBA (0.5%) was incubated at 95°C in a water bath for 25 min. Optical density of the mixture was read at 450, 532 and 600 nm, respectively.

The amount of MDA was estimated using the following formula:

$$C(\mu m/l) = 6.45(A532 - A600) - 0.56\ A450.$$

Determination of hydrogen peroxide concentration

With few modifications, determination of H_2O_2 was performed as stated by Velikova et al. (2000). In an ice bath, the leaf (150 mg) was homogenised in 2.5 ml 0.1% TCA. The homogenized mixture was spun for 15 mins at 12,000 g. The absorbance of the reaction mixture containing 0.5 ml supernatant, 0.5 ml 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI was recorded at 390 nm. The H_2O_2 concentration was estimated from a standard Curve.

Physiological Parameters

Photosynthetic parameters

Chlorophyll (a and b) content and carotenoid were measured as described by Pompelli et al. (2013) with some adjustments. The leaves (0.2 g) were ground in 2 mL of 80% acetone containing and 0.1% $CaCO_3$ to prevent chlorophyllase activities. The homogenate was filtered and was read at 663nm, 646nm and 470nm spectrophotometrically.

After clearing surface dust from the fully grown leaves, the relative chlorophyll content (SPAD chlorophyll) was measured. On either side of the midrib, SPAD readings were collected as suggested by Ruiz-Espinoza et al. (2010).

Measurement of the relative water content (RWC)

RWC measurement was performed as conducted by Wilson et al. (2009). Briefly, the fresh weight of individual leaves was recorded immediately after being plucked and then sucked in distilled water and kept at 4 °C in the dark for 4h. immediately after, the leaves were blotted and weight (turgid) was recorded. The leaves were dried for 48 h in the oven at 80ºC for two days, dry weight was also measured. RWC was then calculated with the following formula as described by Jones (2007).

$$RWC\ (\%) = [(FW - DW)/ (TW - DW)] * 100$$

Defence system (Biochemical)

Free radical Scavenging and Antioxidant activity of extract

The extraction was performed as described by Nobossé et al. (2018). To determine the antioxidant and scavenging activities, fresh leaves were first collected and crushed in a pestle and mortar. A gram of the crushed sample was measured and

mixed with methanol (20 ml) in a conical flask, sealed with parafilm, and stirred at room temperature for 2 h. Then, the mixture was centrifuged (2500 g) for 30 min at 4°C. The supernatant was separated from the pellet. The procedure was repeated with the pellet in 10 ml methanol. The two (supernatants) were combined and adjusted to 40 ml with solvent and stored at 4°C.

DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

The technique developed by Bhagyawant et al. (2019) was adopted to measure the radical (DPPH) scavenging activity. To 3 ml of 0.1 mM DPPH, 1 ml of extract (50 mg in 1 ml methanol) was added, vortexed, and left for 10 minutes at room temperature in the dark. Optical density was recorded at 517 nm and the radical scavenging activity was calculated by the following relation:

$$\text{Percent (\%) scavenging activity} = (Ac - As)/Ac) \times 100$$

Total antioxidant assay (TAA) by phosphomolybdate method

TAA was evaluated based on the method used by Jayaprakasha et al. (2002) with some alteration. Extract (1 ml) was added to 1.5 ml of reagent (28 mM sodium phosphate, 0.6 M sulfuric acid, and 4 mM ammonium molybdate). The tubes were covered and incubated for 90 minutes in a boiling water bath at 95°C. Using a UV spectrophotometer, the optical density of the samples was read at 695 nm against a blank after they had cooled to room temperature. Butylated hydroxytoluene (BHT) as the standard, TAA was expressed as mg equivalents of BHT by using the standard BHT graph.

Non-enzymatic Antioxidants (sulfhydryl) and Enzymatic activities

Quantification of non-protein thiols (NPT) Total Thiols (TT) and Protein thiols (PT)

With modest adjustments, the contents of TT and PT were estimated as per the Sedlak and Lindsay (1968) method. Fresh leaves (250 mg) were crushed in 0.2M Tris-HCl (pH 7.4) (5 ml) and spun for 20 minutes at 4°C at 10 000g. The supernatant of which was separated for NTP and TT determination. Supernatant (0.5 ml) combined with of 0.2 mM Tris-HCl (pH 8.2) (1.5 ml), absolute methanol (7.9 ml), and 0.1 ml of 0.01 M DTNB, for an estimation of TT. After 15 minutes, the developing yellow colour was detected at 415 nm against a blank vial containing 0.5 ml distilled water instead of supernatant. With an extinction coefficient of 13,600, total sulfhydryl groups were determined and reported in µmol g^{-1} FW. NTP concentration was estimated using the reaction mixture containing 5 ml supernatant, 50% TCA (1ml) and 4 ml of distilled water and allowed to stand for 15 minutes and spun for 15 minutes at 10,000 g. Using a 2 ml deproteinized supernatant, NTP was quantified in a method similar to TT (Sedlak & Lindsay, 1968). PT was calculated by taking the difference between total thiol content and NPT content (Lukasik et al., 2019).

Glutathione

Glutathione level was estimated according to Tukendorf and Rauser (1990) and Pal et al. (2019) using Ellman's reagent. One gram of fresh leaves was extracted in 5 ml of chilled buffer containing: Tris-HCl (0.1 M, pH 8.0), 3 mM EDTA and 10 mM

$MgCl_2$ and homogenized using mortar and pestle. In a refrigerated centrifuge at 4 C, the homogenized sample was spun for 30 min at 15 000 g. Supernatant 1 ml mixed with 50 µL 10 mM 5,5´-ditiobis (2-nitrobenzoic acid) (DTNB) and 2 ml 0.4 M Tris-HCl buffer (pH 8.9, 2 ml), was incubated at 37 0C for 1 h. The reading was recorded at 412 nm after 2 min (ε = 13.6 mM^{-1} cm^{-1}).

Phytochelatin (PC)

PC was determined according the following formula;

PC = Total nonprotein thiol - Reduced glutathione (Hou et al., 2019).

Antioxidant enzymes activity

To obtain the enzyme extract (EE), 500 mg fresh leaf tissues were homogenized in chilled 50 mM K-P buffer (4 mL), having pH 7, containing 100 mmol L^{-1} EDTA with a pre-chilled pestle and mortar. After spinning at 11,500g (4 °C) for 20 min, the EE was collected in Eppendorf tubes. The aliquot of EE was used to measure soluble proteins and enzymatic antioxidant activities by maintaining the temperature at 4 °C as a whole. All readings were observed spectrophotometrically.

1. *Ascorbate peroxidase assay* (APX; E.C.1.11.1.11)- The APX activity was measured by determining the ascorbate oxidation rate using a technique adapted by Soares (2011). The reaction mixture (2 mL) containing 100 mmol L^{-1} potassium phosphate buffer (pH 7.0), 0.5 mmol L^{-1} L-ascorbic acid, 0.1 mmol L^{-1} hydrogen peroxide (H_2O_2), and 30 µL of the extract was incubated at 28ºC. The ascorbate oxidation rate was monitored at 290 nm for 3 min and enzyme activity was expressed as µmolmin^{-1} mg^{-1} protein (Extinction coefficient of 2.8 mmol L^{-1} cm^{-1}).

2. *Catalase assay* (CAT; E.C. 1.11.1.6) - CAT activity was measured by spectrophotometry at 28ºC in a final reaction mixture of 2 mL comprising 100 mmol L^{-1} potassium phosphate buffer (pH 7.0), 12.5 mmol L^{-1} hydrogen peroxide (H_2O_2), and water, as reported by Azevedo et al. (1998) with minor modifications. The activity was evaluated after the decomposition of H_2O_2, by monitoring changes in absorbance at 240 nm, with a molar extinction value of 0.0394 mmol L^{-1} cm^{-1}. The results were represented in µmol of H_2O_2 consumed per minute per mg^{-1} protein.

3. *Superoxide dismutase* (SOD, EC 1.15.1.1)- The capacity of the enzyme extract to prevent the photochemical reduction of nitroblue tetrazolium (NBT) was measured at 560 nm to quantify the activity of superoxide dismutase. An assay mixture (3 ml) containing 100 mM of phosphate buffer (pH 7.4), 1.0 mM of EDTA, 50 mM of riboflavin, 10 mM of methionine, 75 mM of NBT, and 100 mL of enzyme extract was incubated for 15 minutes under fluorescent light as described by Ahanger et al. (2020). The absorbance was measured at 560 nm and the activity was reported as µmol min^{-1} mg^{-1} protein.

4. *Guaiacol peroxidase activity (POD, EC 1.11.1.7)* - was measured by mixing 2 ml of reaction buffer (50 mM potassium phosphate buffer pH 6.8, 20 mM guaiacol, and 20 mM H_2O_2 with crude extract (25 µl) and incubated at 30°C for 10 minutes, as employed by Cavalcanti et al. (2004). The reaction was halted by the addition of 0.5 mL of 5% (v/v)

H_2SO_4 and optical density was read at 480 nm. POD activity was expressed as µmol of H_2O_2 consumed per minute per mg^{-1} protein.

Statistical analysis

All of the samples were examined in pentaplicate, and the mean values and standard errors are shown in the figures as bars. The statistically significance difference between treatment means were determined using single-factor analysis of variance (ANOVA). The SPSS 26.0 programme was used to construct ANOVAs. To determine which mean pairings were substantially different, the Tukey's HSD test (p 0.05) was used.

Results and Discussion

B. chinensis biomass

The Cd toxicity significantly ($p < 0.05$) inhibit the biomass accumulation of *B. chinensis* L. plants with the increasing rate of Cd in the soil (Figure 1). However, T1 and T5 biomass accumulation was inhibited by 17.4% FW (Figure 1A) and 51.0% DW (Figure 1B) for T1 and 50.7% FW (Figure 1C) and 76.0% DW (Figure 1D) in T5 compared to the control (T0) respectively. An increase in biomass was observed in *S. stolonifolium* treated groups. The highest biomass accumulation was recorded at T4 and T8 under 50 mg and 100 mg Cd respectively, combined with biomass (Figure 1). T4 increased by 40.6% (Figure 1A) and 72.2% (Figure 1B) while T8 increased by 54.1% (Figure 1C) and 60.4% (Figure 1D) fresh and dry weight biomass, respectively, relative to the cadmium only treated group (T1 and T5). However, a statistically significant increase and decrease in biomass accumulation was recorded when comparing T0 (control) with *S. stolonifolium* treated groups. T2, T3 and T4 increased by 3.5%, 10.4%, and 34.9% and 63.5%, 40.6% and 72.2% fresh and dry weight respectively over the control. For T6 and T7, the fresh weight decreased by 21.8% and 0.2% and increased in T8 by 7.3% over the control while the dry weight decreased by 32.3% and 8.8% for T6 and T7 and increased in T8 by 1.7% respectively compared to the control. Cadmium (Cd) is a trace element nonessential to plants that is pervasively found in the environment. Accretion of Cd in plant tissues slows the growth and leads to various symptoms of toxicity. The very foremost Cd accumulation symptom is a reduction of growth and development (El Rasafi et al., 2021; Montiel-Rozas et al., 2016). Recently, many soil immobilizing amendments like alumino-silicate minerals (zeolite), metal oxides, lime, phosphate compounds, organic composts and biochar have been used to alleviate Cd toxicity in highly polluted soils (Kamran et al., 2019; Saeed et al., 2019; Xiang et al., 2019) and use of seaweed dry biomass has not been exploited in soil amendment. Our findings demonstrate that cadmium retards *B. chinensis* L. growth hence, decreases fresh and dry weights. This finding corresponds to Perveen et al. (2015) where a significant reduction in biomass accumulation was recorded in rice. Kamran et al. (2019) reported a decrease in Pak choi biomass exposed to cadmium under pot conditions. *S. stolonifolium* enhanced fresh and dry biomass accumulation at all cadmium levels (Figure 1). Sofy et al. (2017) reported the ability of *Sargassum* spp. to stimulate growth and biomass accumulation of Pak choi under cadmium stress. Several studies have shown that amino acids, carbohydrates, phenolics,

phytohormones, vitamins, betaines, polyamines, and carotenoids, among other signaling molecules and metabolites, in seaweed (brown algae), may be responsible for enhancing plant development (Khan et al., 2019). Cytokinin is a plant growth regulator which can regulate the postharvest behaviour of plant parts (Moneruzzaman et al., 2010). However, the presence of these phytochemicals in seaweed which are released into the soil as nutrients may be taken up by *B. chinensis* to boost their growth, while some have metal chelating abilities like phenolics and amino acids thereby reducing availability of Cd for uptake. Earlier studies in horticultural and agricultural crops such as *Malus domestica* (Basak, 2008), tomato (R. Kumari et al., 2011b), wheat (G. Kumar & Sahoo, 2011a), okra (Zodape et al., 2011), broccoli (Mattner et al., 2013), strawberry (Alam et al., 2013), spinach (Fan et al., 2013), *Brassica napus* (Jannin et al., 2013), and *Triticum aestivum* L. (Ismail, 2016), have shown that seaweed triggers yield and growth.

Cadmium concentration in B. chinensis tissues

Cd concentration was determined to assess the level of Cd in various parts of *B. chinensis* at 50 mg (Figure 2) and 100 mg Cd (Figure 3) with or without *S. stolonifolium* amendment. Cd concentration was found to be statistically higher in the root followed by the leaf and stem, respectively, at all cadmium exposure levels. However, a significant decrease in Cd concentration in *B. chinensis* was recorded in *S. stolonifolium* amended soil. Cd decrease by 67.43%, 82.37% 90.25% in the root (Figure 2A), 40.24%, 72.68% and 82.93% for the stem (Figure 2B) and 49.46%, 70.74% and 84.57% in the leaves (Figure 2C) at T2, T3 and T4 groups respectively, relative to absolute Cd treated group (T1) (Figure 2). Relative to T5 (100 mg) (Figure 3), Cd decreased significantly ($p < 0.05$) in the root by 77.04%, 87.04% and 92.11% (Figure 3A); the stem 51.82%, 77.27% and 78.18% (Figure 3B) and the leaves 68%, 81.23% and 88% (Figure 3C), respectively, for T6, T7 and T8. Cd concentration increased in plant tissues as its concentration increased (Figure 2 & 3). The amount of work done to investigate the effects of cadmium in *B. chinensis* shows that cadmium in tissues of the plant increase with increasing its concentration includes Sulaiman el al. (2020); Yan et al. (2009); X. Chen et al. (2011); Rafiq et al. (2014); Huang et al. (2021). However, soil integrated with S. stolonifolium showed a significant decrease in Cd in plant parts. Various investigations have demonstrated that the polysaccharides contained in their cell walls have high adsorption properties and selectivity for several metal cations (Jalali et al., 2002; Murphy et al., 2007; Ortiz-Calderon et al., 2017; Romera et al., 2007). Alginate residues of the *Sargassum* spp. for adsorption of cadmium metal ion was studied (Nishikawa et al., 2018; Trica et al., 2019), which might have been involved in immobilization. The occurrence of functional groups like thiol, carboxyl, hydroxyl, amine, and carbonyl and other usual compounds that are supportive to the binding of metal like hydrocarbons, lipids lignins, proteins, cellulose and tannins (Znad et al., 2022) are among the components reducing Cd phytoavailability.

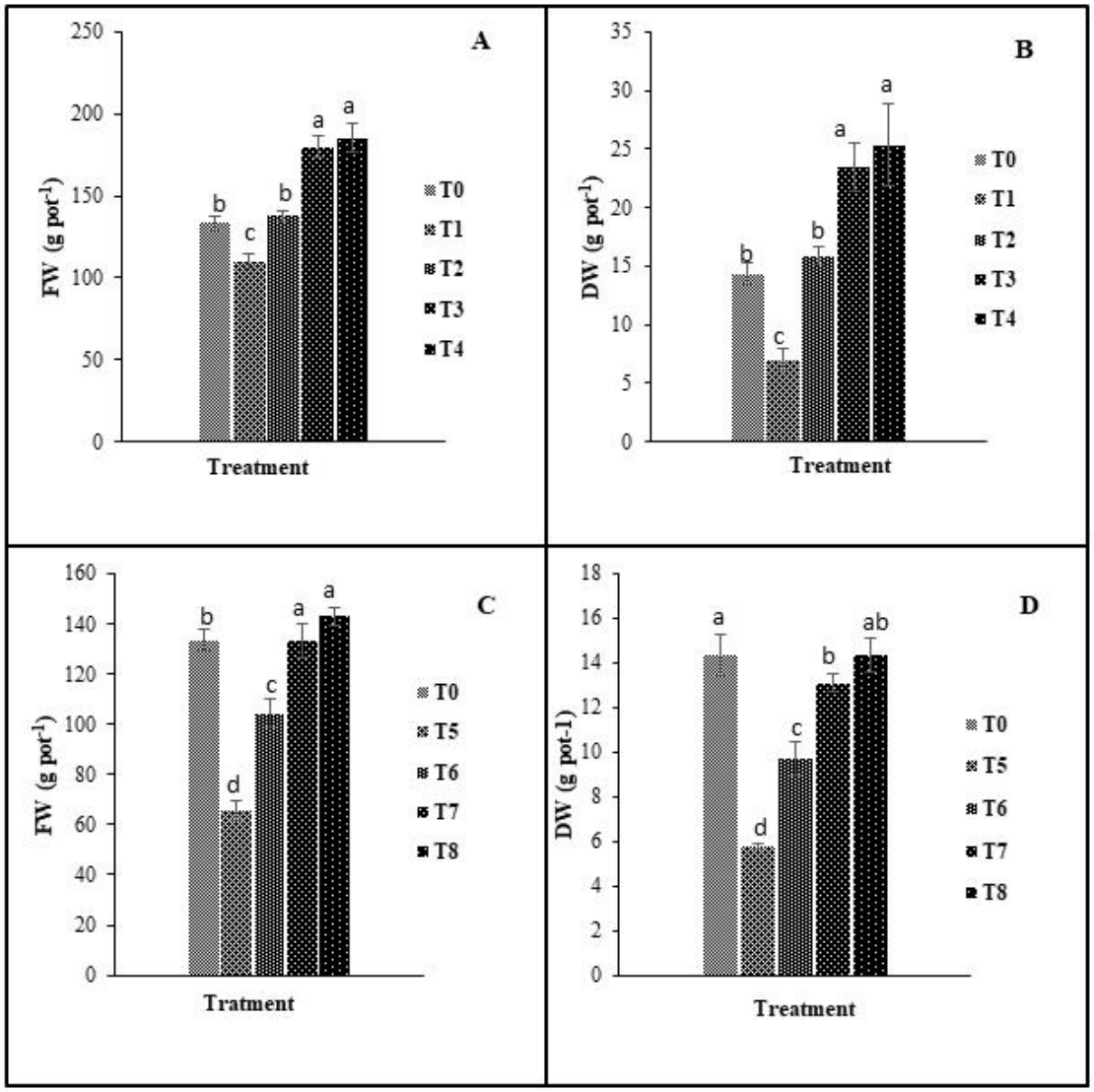

Figure 1. Effects of 50 mg and 100 mg cadmium exposure on *B. chinensis* biomass and ameliorative role of *Sargassum stolonifolium* on the biomass. A and B; fresh weight and dry weight at 50 mg Cd, C and D fresh weight and dry weight at 100 mg Cd. **T0**, control; **T1**, 50 mg Cd, **T2**, 25 g biomass + 50 mg Cd; **T3**, 50 g biomass + 50 mg Cd; **T4**, 100 g biomass + 50 mg Cd; **T5**, 100 mg Cd; **T6**, 25g biomass + 100 mg Cd; **T7**, 50g biomass + 100 mg Cd and **T8**, 100 g biomass + 100 mg Cd. Bars bearing dissimilar letter(s) at $P < 0.05$ for each group symbolize substantial difference, Tukey's HSD test. Data is the pentaplicate average (n=5). Error bars signify standard error (SE) of five replicates.

Stress biomarker

The concentrations of malondialdehyde (MDA) and H_2O_2 under different Cd stress levels are illustrated in figure 4. Cd stress resulted in a significant increase in MDA contents in *B. chinensis* leaves by, 138.3%, 27.7%, 173.6%, 128.4% and 19.1% at T1, T2, T5, T6, and T7 (Figures 4A & B), respectively, compared to the absolute

control. The same is true for H_2O_2, which increased in the plants' leaves as Cd supply increased in the soil, indicating oxidative stress in plants (Figures 4B & C). H_2O_2 increased in T1, T2, T5 and T6 by 109.8%, 21.1%, 178.9% and 31.6%, accordingly over T0 (Figures 4C & D).

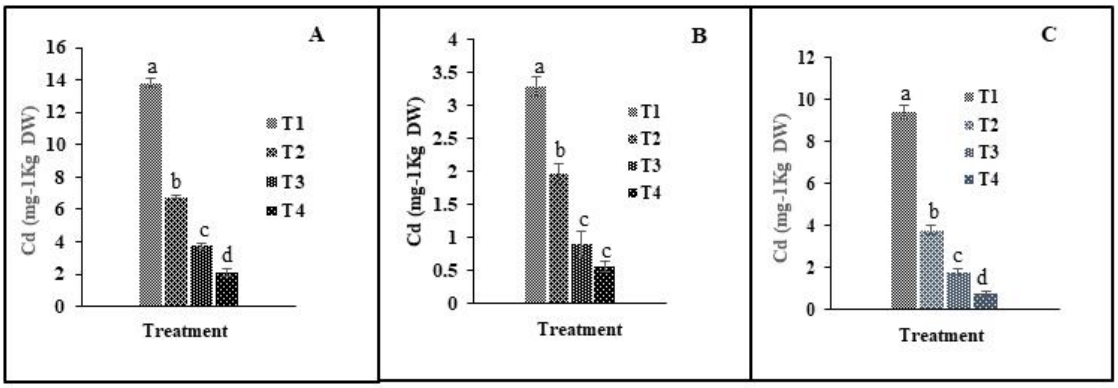

Figure 2. Cadmium absorption and accumulation at 50 mg level in (A) root, (B) stem, and (C) leaves and ameliorative effect of *S. stolonifolium* on its absorption and accumulation. **T1**, 50 mg Cd, **T2**, 25 g biomass + 50 mg Cd; **T3**, 50 g biomass + 50 mg Cd; **T4**, 100 g biomass + 50 mg Cd. Bars bearing dissimilar letter(s) at $P < 0.05$ for each group symbolize significant difference, Tukey's HSD test. Data is the pentaplicate average (n=5). Error bars signify standard error (SE) of five replicates.

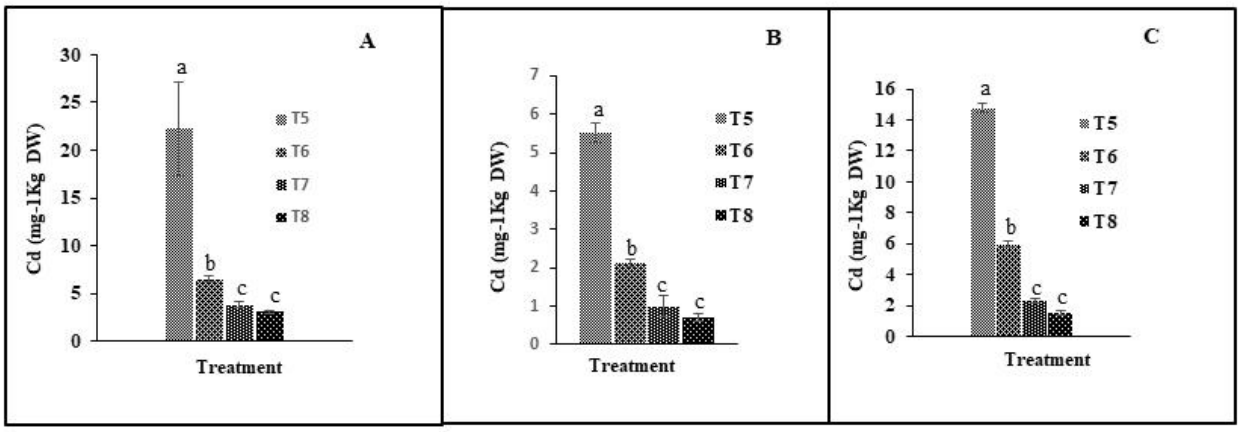

Figure 3. Cadmium absorption and accumulation at 100 mg level in (A) root, (B) stem, and (C) leaves and ameliorative effect of *S. stolonifolium* on its absorption and accumulation. **T1**, 50 mg Cd, **T2**, 25 g biomass + 50 mg Cd; **T3**, 50 g biomass + 50 mg Cd; **T4**, 100 g biomass + 50 mg Cd. Bars bearing dissimilar letter(s) at $P < 0.05$ for each group symbolize significant difference, Tukey's HSD test. Data is the pentaplicate average (n=5). Error bars signify standard error (SE) of five replicates.

However, the application of *S. stolonifolium* biomass decreases the concentration of MDA by 46.4%, 62.4%, 74.9% for T2, T3 and T4 (Figure 4A) and 16.5%, 56.5%, and 66.6% for T6, T7 and T8 (Figure 4B), accordingly, in comparison with

the corresponding Cd only treatments (T1 and T5). Comparing with the control, MDA decreased in T3, T4 and T8 by 10.4%, 40.1%, and 8.6%, respectively (Figures 4A and B). Equally, H_2O_2, decreased by 42.3%, 68.8%, and 75.3% for T2, T3, and T4 (Figure 4C) compared to T1 and 52.8%, 71.7%, and 77.1% at T6, T7, and T8 (Figure 4D) relative to T5, accordingly. Relative to the control, H_2O_2 decreased by 34.6%, 48.2%, 21.1% and 36.1% in T3, T4, T7 and T8, respectively (Figures 4C &D).

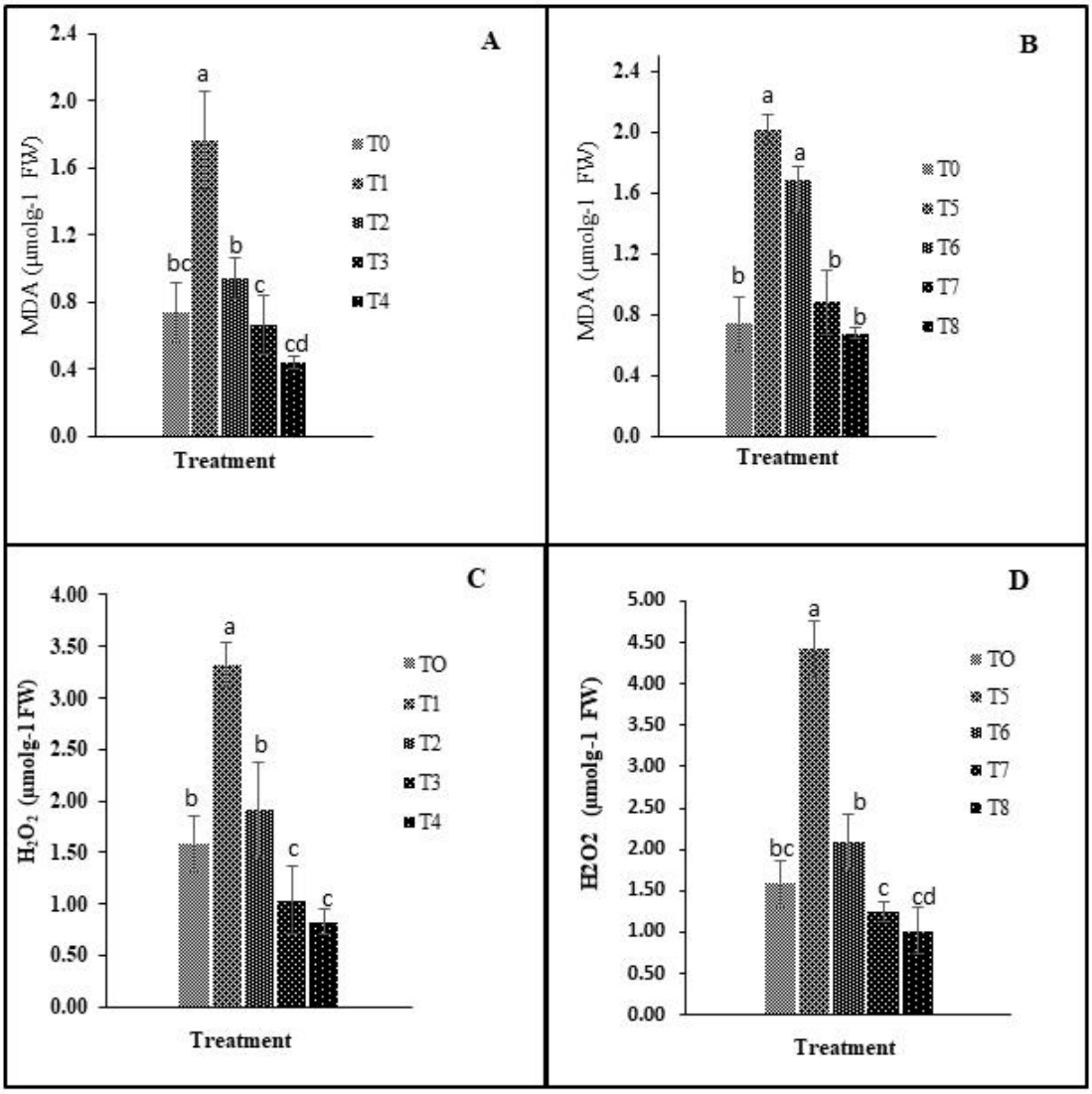

Figure 4. Cadmium induces membrane lipid peroxidation to produce MDA and generation of H_2O_2 at 50 mg and 100 mg Cd^{+2} respectively. MDA, **A** and **B**; and hydrogen peroxide **C** and **D**. T0, control; **T1**, 50 mg Cd, **T2**, 25 g biomass + 50 mg Cd; **T3**, 50 g biomass + 50 mg Cd; **T4**, 100 g biomass + 50 mg Cd; **T5**, 100 mg Cd; **T6**, 25g biomass + 100 mg Cd; **T7**, 50g biomass + 100 mg Cd and **T8**, 100 g biomass + 100 mg Cd. Bars bearing dissimilar letter(s) at $P < 0.05$ for each group symbolize substantial difference, Tukey's HSD test. Data is the pentaplicate average (n=5). Error bars signify standard error (SE) of five replicates.

Under Cd stress, oxidative damage caused by Cd may cause cell membrane rupture (electrolyte leakage), nutritional inhibition, and increased lipid peroxidation, as seen by raised levels of oxidative stress markers (MDA and H_2O_2) in figure 4. These results were in agreement with previous research where Wu et al. (2015), Sofy et al. (2017), Kamran et al. (2019) and Zhu et al. (2019) reported significant increase in leaf MDA and H_2O_2 content in Pak choi. However, a significant reduction in the concentration of MDA and H_2O_2 were recorded upon integration of *S. stolonifolium* biomass, which might be due to the reduction of root exposure to Cd and its bioavailability for uptake (Figure 4). These results agree with W. A. Kasim et al. (2015) who reported that Pretreatment with a seaweed extract of *Sargassum* spp. resulted in lower MDA levels in abiotically stressed wheat plants. Sofy et al. (2017) reported a decrease in stress biomarkers upon treating Pak choi under cadmium stress with *Sargassum* spp. By directly scavenging free radicals and limiting ROS generation through blocking xanthine oxidation, the cytokinin content of seaweed helps to reduce stress-induced free radicals (Fike et al., 2001).

Photosynthetic pigment

Both chlorophyll *a*, *b*, carotenoid, SPAD, and relative water content are affected by cadmium exposure at different levels of treatments (50 and 100 mg), as illustrated in tables 2 and 3. Chlorophyll *a*, *b*, carotenoid, SPAD and relative water content (RWC) decreased substantially in the absolute cadmium treated groups (T1 and T5). For T1 (50 mg Cd), chlorophyll *a* decreased by 45.9%, chlorophyll *b* by 57. 9%, carotenoid by 34.2%, SPAD by 21.5% and RWC by 29.7% (table 2), whereas chlorophyll a and b, carotenoid, SPAD and RWC decreased by 58.4%, 59.3%, 91.9%, 35.4% and 34.4% accordingly, at T5 (100 mg Cd), over the absolute control (table 3).

Table 2
Effects of 50 mg cadmium exposure and role of *S. stolonifolium* on photosynthetic pigments and relative water content in *Brassica chinensis* leaves 6 weeks after treatment

Treatment	Parameter				
	Chl *a*	Chl *b*	Car.	SPAD	RWC (%)
T0	13.05±1.18c	10.82±1.43bc	4.07±0.45bc	37.30±1.06c	82.01±5.27a
T1	7.06±1.11d	4.56±0.91c	2.68±0.66c	29.28±1.18d	57.69±5.77b
T2	18.72±0.62b	13.90±1.43ab	5.41±0.57ab	42.10±1.36bc	83.52±9.46a
T3	24.91±2.57a	18.76±2.06a	6.24±0.74ab	47.34±1.09b	86.94±3.77a
T4	28.01±0.90a	20.36±2.88a	7.07±1.06a	54.00±3.08a	87.15±2.31a

T0, control; **T1**, 50 mg Cd, **T2**, 25 g biomass + 50 mg Cd; **T3**, 50 g biomass + 50 mg Cd; **T4**, 100 g biomass + 50 mg Cd. Means bearing dissimilar letter(s) within a column for each treatment denote significant differ at $P < 0.05$, Tukey's HSD test. Data is the pentaplicate average ± SE. **Chl *a***, chlorophyll *a*; **Chl *b***, chlorophyll *b*; **Car.**, carotenoid; **RWC**, relative water content.

Table 3
Effects of 100 mg cadmium exposure and role of *S. stolonifolium* on photosynthetic pigments and relative water content in *Brassica chinensis* leaves 6 weeks after treatment

Treatment	Parameter				
	Chl *a*	Chl *b*	Car.	SPAD	RWC (%)
T0	13.05±1.18b	10.82±1.43a	4.07±0.45c	37.30±1.06bc	82.01±5.27a
T5	5.43±0.78c	4.40±0.79b	2.094±0.09cd	24.08±2.85d	53.76±3.76b
T6	12.52±1.21b	11.47±1.13a	4.26±0.53bc	40.98±0.35ab	72.85±5.45a
T7	14.78±1.29ab	11.02±0.83a	6.75±0.54a	44.22±0.48a	75.01±2.20a
T8	17.39±1.26a	13.06±2.39a	7.15±0.80a	46.52±1.93a	81.10±3.56a

T0, control; **T5**, 100 mg Cd; **T6**, 25g biomass + 100 mg Cd; **T7**, 50g biomass + 100 mg Cd and **T8**, 100 g biomass + 100 mg Cd. Means bearing dissimilar letter(s) within a column for each treatment denote significant difference at $P < 0.05$, Tukey's HSD test. Data is the pentaplicate average ± SE. **Chl *a***, chlorophyll *a*; **Chl *b***, chlorophyll *b*; **Car.**, carotenoid; **RWC**, relative water content.

Photosynthetic pigments and RWC significantly increased with increasing *S. stolonifolium* biomass in both treatments compared to the absolute control (tables 2 & 3). Chlorophyll *a* increased by 43.4%, 90.9%, 114.6% for T2, T3 and T4 compared to the control (Table 2) whereas T6, T7 and T8 increased by 4.1%, 13.2% and 33.3% respectively (Table 3). Chlorophyll *b* increased by 28.5%, 73.4%, 88.2%, 6.1%, 1.2%, and 20.7% respectively, for T2, T3, T4, T6, T7 and T8 over the control. Carotenoid and SPAD increased under T2 by 32.8% and 12.9%, T3 by 53. 3% and 26.9%, T4 by 73.7% and 44.8%, T6 by 4.7% and 9.9%, T7 by 65.9% and 18.6%, 75.6% and 24.7%, relative to the control (tables 2 and 3). Compared to the control (T0), RWC in T2, T3, and T4 increased by 1.8%, 6.0%, and 6.3% (table 2) and decreased in T6, T7 and T8 by 11.2%, 8.5% and 1.1%, respectively (tables 2 & 3).

Cd excess in the environment decreases chlorophyll content (X. Chen et al., 2011), and growth (Zhou & Qiu, 2005) and affects chloroplast function (X. Chen et al., 2011). Cadmium's main targets include photosynthetic machinery and pigments; carotenoid synthesis, and chlorophyll synthesis (Rafiq et al., 2014). In oilseed crops like *Brassica napus,* the coupling between Cd and chlorophyll formation reduces the density of chloroplasts, resulting in chlorosis (Baryla et al., 2001). We find that photosynthetic pigments and their activities were suppressed by cadmium (tables 2 & 3). The repressive consequences of Cd on chlorophyll a, b and carotenoid correspond with previous investigations (Jiang et al., 2007; Rafiq et al., 2014; Thapar et al., 2008; Xiong & Qiu, 2007). A substantial rise in photosynthetic pigments and activity were increased with an increase in *S. stolonifolium* biomass integration (tables 2 & 3). Previous reports show that chlorophyll content (SPAD) increased when seaweed extract was applied at a high dose (Al-Hamzawi, 2019). R. Kumari et al. (2011b) documented an increase in photosynthetic

pigment in *Lycopersicon esculentum* using extract of *Sargassum johnstonii.* Both W. A. E.-A. Kasim et al. (2016), and Mahmoud et al. (2019) reported an increase in photosynthetic pigments when *Raphanus sativus* was treated with *Sargassum* Spp. while Jafarlou et al. (2022), reported similar results in eggplant.

Leaf relative water content of *B. chinensis* drastically decreased in both the 50 (T1) and 100 mg (T5) groups (tables 2 & 3). This is due to Cd ion aggregation in tissues, which affects the absorption of soil water leading to the reduced water content in plant tissues (C.-T. Chen et al., 2004). Cd in the soil causes osmotic stress in plants by reducing leaf RWC, transpiration, and conductance of stomata, causing severe damage to the plants' physiology (Rizwan et al., 2016b). Our result conforms with earlier research that reported a decrease in relative water content, such as Singh and Tewari (2003) (*Brassica juncea* L.), Shah et al. (2011), (*Brassica napus* and *juncea*), and Ahmad et al. (2011) in (Brassica juncea). An increase in RWC was noticed in those groups treated with *S. stolonifolium* biomass. Several other previous studies, including Xu and Leskovar (2015) reported that seaweed increased the RWC of treated seedlings of spinach. Jafarlou et al. (2022) documented an increase in RWC in *Calotropis procera* (Aiton).

Free radical scavenging activity

DPPH radical scavenging activity increased across all treatments significantly compared to the absolute control (Figures 5A and B). However, *S. stolonifolium* biomass amended soil showed significant radical scavenging activity with an increase in *S. stolonifolium* biomass when compared to the absolute Cd treated groups. T1, T3, T4, T5, T6, T7 and T8 radical scavenging activity increased by 144.2%, 112.6%, 115.3%, 119.78%, 1552%, 155.2%, 162.6%, 170.6%, and 174.2%, respectively, compared to the absolute control (T0) (Figures 5A & B). T2, T3 and T4 scavenging activities increased significantly by 112.62%, 115.33% and 119.78% accordingly, relative to T1 (Figure 5A), and 104.74%, 109.88%, and 112.20%, under T6, T7, and T8, accordingly, over T5 (Figure 5B). Figure 5C & D illustrate the total antioxidant activity of *B. chinensis* extract subjected to cadmium at different levels of treatments with or without *S. stolonifolium* amendment. There is a statistically significant increase in the reducing power of the extract with or without *S. stolonifolium,* compared to the absolute control (T0) at $p < 0.05$ (Figures 5C & D). T1, T2, T3, T4, T5, T6, T7 and T8 shows 26.2%, 55.8%, 68.2%, 81.5%, 71.5%, 89.2%, 96.1%, and 136.1% significant increase, compared to the absolute control (T0) (Figure 5C & D). However, T2, T3, and T4 total antioxidant activity increased by 18.98%, 24.96%, and 30.47% compared to T1 (Figure 5C) and 9.32%, 12.53% and 27.35% for T6, T7, and T8 compared to T5 (Figure 5D). DPPH and TAA increase progressively with cadmium concentration in T1 and T5 (Figure 5A-D). Our result is consistent with earlier results. Jibril et al. (2017) reported an increase in antioxidant activities as cadmium concentration increased in *Lactuca sativa* L. A similar result was reported by Ulusu et al. (2017) using Parsley. Water and salt stress greatly raised the TAA of barley leaves, according to Fayez and Bazaid (2014), which increased with increasing the stress. It was noted in our findings that *S. stolonifolium* biomass increased scavenging activities in all

treatments subjected to it (Figure 5). According to Khan et al. (2009) seaweed extracts affect plant metabolism in a variety of ways, when spinach was treated with brown algal extract, antioxidant capacity, flavonoid, phenolics and, total soluble protein content all increased (Fan et al., 2013). And these compounds may be associated with scavenging and antioxidant activities. *Sargassum* spp. extracts were able to substantially upregulate genes involved in Jasmonic acid, Ethylene, and salicylic acid, mediating resistance signaling like PR1-a, ETR-1, and PinII, respectively, and increase in defense enzyme activity. The high antioxidant content of seaweed (such as brown algae) extract may operate to diminish oxidative damage and boost plants' tolerance to abiotic stress (Guinan et al., 2012).

Sulfhydryl containing compounds

Brassica *chinensis* exposed to cadmium at 50 mg and 100 mg Kg^{-1} soil with or without *S. stolonifolium* amendment alters the concentration of NTP, PBT, TT, GSH and PC. Cd induces production of NTP, PBT, TT, GSH and PC at 50 mg and 100 mg (tables 4 and 5) in both amended or unamended Cd spiked soil relative to absolute control. NTP, PBT, TT, GSH. NTP increased in T1, T2, T3, T4, T5, T6, T7 and T8 by 44.1%, 50.5%, 65.1%, 66.7%, 55.4%, 70.4%, 80.1% and 81.2%, respectively, compared to the absolute control. PBT insignificantly increased with increasing S. stolonifolium biomass by 11.8%, 14.6%, 25.5%, 49.1%, 7.3%, 5.3%, 6.4%, and 41.8% for T1, T2, T3, T4, T5, T6, T7 and T8, accordingly, over the control. The total thiol component was raised by 32.1%, 37.2%, 50.3%, 60.1%, 69.1%, 37.5%, 40.11%, 52.7%, and 66.6% for T1, T2, T3, T4, T5, T6, T7 and T8, respectively, over the control. GSH for T1, T2, T3, T4, T5, T6, T7 increased by 51.2%, 66.9%, 91.3%, 96.1%, 62.2%, 70.4%, 92.1%, 111.8%, and 116.5%, accordingly, relative to the absolute control. PCs increased across all treatments with the highest concentration in T1 (table 4) and T5 (table 5) compared to the absolute control and S. stolonifolium amended groups. PCs insignificantly increased by 28.8%, 15.3%, 8.5%, and 3.4% for T1, T2, T3 and T4 (table 4) whereas, for T5, T6, T7 and T8, it increased by 40.7%, 23.7%, 11.7%, and 5.1% (table 5) compared to the control.

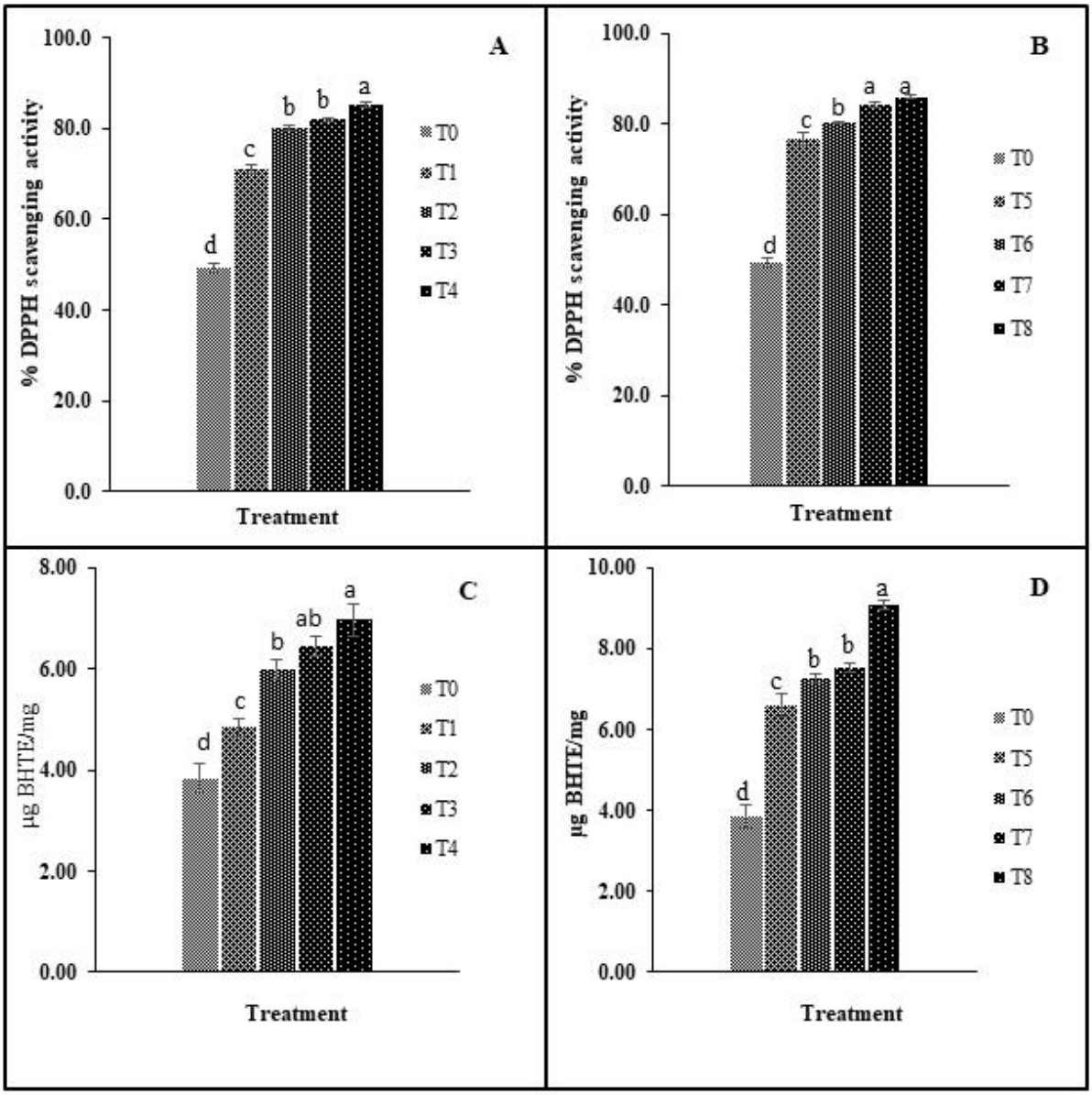

Figure 5. Effect of cadmium toxicity at 50 and 100mg Cd and *S. stolonifolium* biomass in *B. chinensis* leaf extract scavenging activity and total antioxidant activity. DPPH scavenging activity at 50 and 100 mg Cd (A) and (B) and TAA at 50 and 100 mg Cd (C & D) respectively. **T0**, control; **T1**, 50 mg Cd, **T2**, 25 g biomass + 50 mg Cd; **T3**, 50 g biomass + 50 mg Cd; **T4**, 100 g biomass + 50 mg Cd; **T5**, 100 mg Cd; **T6**, 25g biomass + 100 mg Cd; **T7**, 50g biomass + 100 mg Cd and **T8**, 100 g biomass + 100 mg Cd. Bars bearing dissimilar letter(s) at $P < 0.05$ for each group symbolize substantial difference, Tukey's HSD test. Data is the pentaplicate average (n=5). Error bars signify standard error (SE) of five replicates.

Table 4
Effects of 50 mg cadmium exposure on thiols containing compounds in *Brassica chinensis* 6 weeks after treatment and role of *S. stolonifolium* in stress alleviation

Parameter (μmol g^{-1} FW)	T0	T1	T2	T3	T4
NTP	2.74±0.11b	3.94±0.07ab	4.12±0.19a	4.51±0.09a	4.56±0.14a
PBT	1.62±0.13a	1.81±0.13a	1.85±0.21a	2.03±0.39a	2.41±0.24a
TT	4.35±0.16e	5.75±0.12cd	5.97±0.11bc	6.54±0.32ba	6.97±0.19a
GSH	1.87±0.11d	2.82±0.00c	3.12±0.00b	3.57±0.00a	3.66±0.00a
PCs	0.87±0.13a	1.12±0.011a	1.00±0.11a	0.94±0.01a	0.90±0.12a

NTP, non-protein thiol; **PBT**, protein bound thiol; **TT**, total thiol; **GSH**, glutathione; **PCs**, phytochelatins. **T0**, control; **T1**, 50 mg Cd, **T2**, 25 g biomass + 50 mg Cd; **T3**, 50 g biomass + 50 mg Cd; **T4**, 100 g biomass + 50 mg Cd; Means bearing similar letter(s) within a row for each treatment do not differ significantly at $P < 0.05$, Tukey's HSD test. Data is the pentaplicate average ± SE.

Table 5
Effects of 100 mg cadmium exposure on thiols containing compounds in *Brassica chinensis* 6 weeks after treatment and role of *S. stolonifolium* in stress alleviation

Parameter (μmol g^{-1} FW)	T0	T5	T6	T7	T8
NTP	2.74±0.11c	3.9±0.009b	4.41±0.11b	4.56±0.12a	4.96±0.12a
PBT	1.62±0.13a	1.74±0.12a	1.71±0.16a	1.72±0.12a	2.29±0.27a
TT	4.35±0.17e	5.99±0.15cd	6.13±0.64bc	6.65±0.13ab	7.25±0.27a
GSH	1.8±0.11d	3.03±0.00c	3.59±0.00b	3.96±0.01a	4.04±0.01a
PCs	0.87±0.13a	1.22±0.07a	1.07±0.11a	0.97±0.14a	0.91±0.07a

NTP, non-protein thiol; **PBT**, protein bound thiol; **TT**, total thiol; **GSH**, glutathione; **PCs**, phytochelatins. **T0**, control; **T5**, 100 mg Cd; **T6**, 25g biomass + 100 mg Cd; **T7**, 50g biomass + 100 mg Cd and **T8**, 100 g biomass + 100 mg Cd. Means bearing similar letter(s) within a row for each treatment do not differ significantly at $P < 0.05$, Tukey's HSD test. Data is the pentaplicate average ± SE.

Past research has looked at the impact of overexpressing -glutamyl-cysteine synthetase or transgenic plants expressing bacterial -glutamyl-cysteine synthetase on metal tolerance, assuming that increased GSH and PC levels would give rise to more effective sequestration of metal (Zagorchev et al., 2013). The phytochelatin combines with ions and are trapped in the vacuole, which reduces metal toxicity. Plants have defensive systems to deal with various metals. Glutathione plays a main role as a chelating agent, signaling component and antioxidant (Jozefczak et al., 2012). Tripathi et al. (1996) reported that metal exposure increased the level of NP-SH in plants. The present study revealed that NPT, PBT, TT, GSH and PC increased in T1 and T5 significantly with a Cd increment relative to the absolute control (T0) (table 4 & 5). This is consistent with other investigations

that show increased content of NP-SH upon exposure to Cd (Gonçalves et al., 2007; Mishra et al., 2006; Tiryakioglu et al., 2006). Pal et al. (2019) reported an increase in NPT, GSH and PC as cadmium concentration increased in *Eichhornia crassipes.* Cadmium treatment led to a detrimental level of non-protein thiols in *B. oleracea var. acephala* (Demir & Ozdener, 2015). Drążkiewicz et al. (2003) also reported a high effect of cadmium on GSH and PCs in maize. However, integrating cadmium contaminated soil with *S. stolonifolium* confers more resistance and an increment of antioxidants (NPT, PBT, TT and GSH). In contrast, PC decreased in the absolute control and *S. stolonifolium* amended soil compared to the absolute cadmium treated groups (T1 and T5). Our finding is in line with previous research conducted on antioxidant stimulation of seaweed on wheat (Latique et al., 2021). Increased NPT, PBT, TT and GSH in *S. stolonifolium* amended soil may be due to the enormous number of bioactive molecules from *S. stolonifolium* that stimulates key enzymes for the biosynthesis of thiol containing molecules.

Enzymatic activities

There were variations in the enzymatic antioxidant system activity when *B. chinensis* L. was exposed to different Cd concentrations with or without *S. stolonifolium* biomass. The activities indicated that Cd contaminated soil amended with *S. stolonifolium* biomass have significant antioxidant enzymes activities compared to unamended contaminated soil; T0, T1, and T5 (Figure 6 & 7). T1 and T5 have a significant increase in APX, CAT, POD, and SOD activities compared to the absolute control (T0) (Figures 6 & 7). APX increased by 31.6%, 40.0%, 73.1%, and 92.8% under T1, T2, T3 and T4 (Figure 6A) and 45.3%, 47.8%, 71.4%, and 98.5% at T5, T6, T7, and T8 (Figure 7A), respectively, in comparison with the absolute control. CAT activity increased in all treatments by 53.8%, 67.7%, 60.2%, 73.1%, 78.5%, 87.1%, 91.4%, and 95.7% for T1, T2, T3, T4, T5, T6, T7, and T8, respectively, over the control (Figures 6B & 7B). SOD was improved by 9.6%, 19.5%, 20.7%, 20.9%, 22.4%, 25.8%,27.4% and 29.4% for T1, T2, T3, T4, T5, T6, T7, and T8, respectively, over the control (Figures 6C and D). POD was raised by 44.4%, 66.0%, 85.4%, 88.9% at T1, T2, T3, and T4, (Figure 6D) and 71.5%, 81.9%. 89.56%, and 101.4% for T5, T6, T7, and T8, respectively, compared to the control (Figure 7D). APX activity increased by 6.3%, 31.5%, and 46.5% for T2, T3 and T4 relative to the absolute Cd treated group (T1) (Figure 6A), whereas there was a 1.7%, 18.0%, and 36.6%, increase for T6, T7 and T8, respectively, compared to T5 (Figure. 7A). CAT activity was pointedly enhanced by 9.1%, 4.2%, and 12.6%, for T2, T3 and T4, accordingly, compared to T1 (Figure 6B). T6, T7 and T8 increased by 4.9%, 7.2% and 9.6% respectively in over T5 (Figure 7B). A significant ($p < 0.05$) increase in SOD activity of 9.1%, 10.1%, and 10.4% at T2, T3 and T4 was recorded compared to T1 (Figure 6C) whereas T6, T7 and T8 significantly improved by 2.3%, 4.1%, and 5.73% (Figure 7C) relative to T5. The percent increase in POD activity of T2, T3 and T4 are 14.9%, 28.4%, and 30.8% related to T1 (Figure 6D), while that of T6, T7, and T8 (Figure 7D) compared to T5 increased by 6.1%, 10.5%, and 17.4%, accordingly.

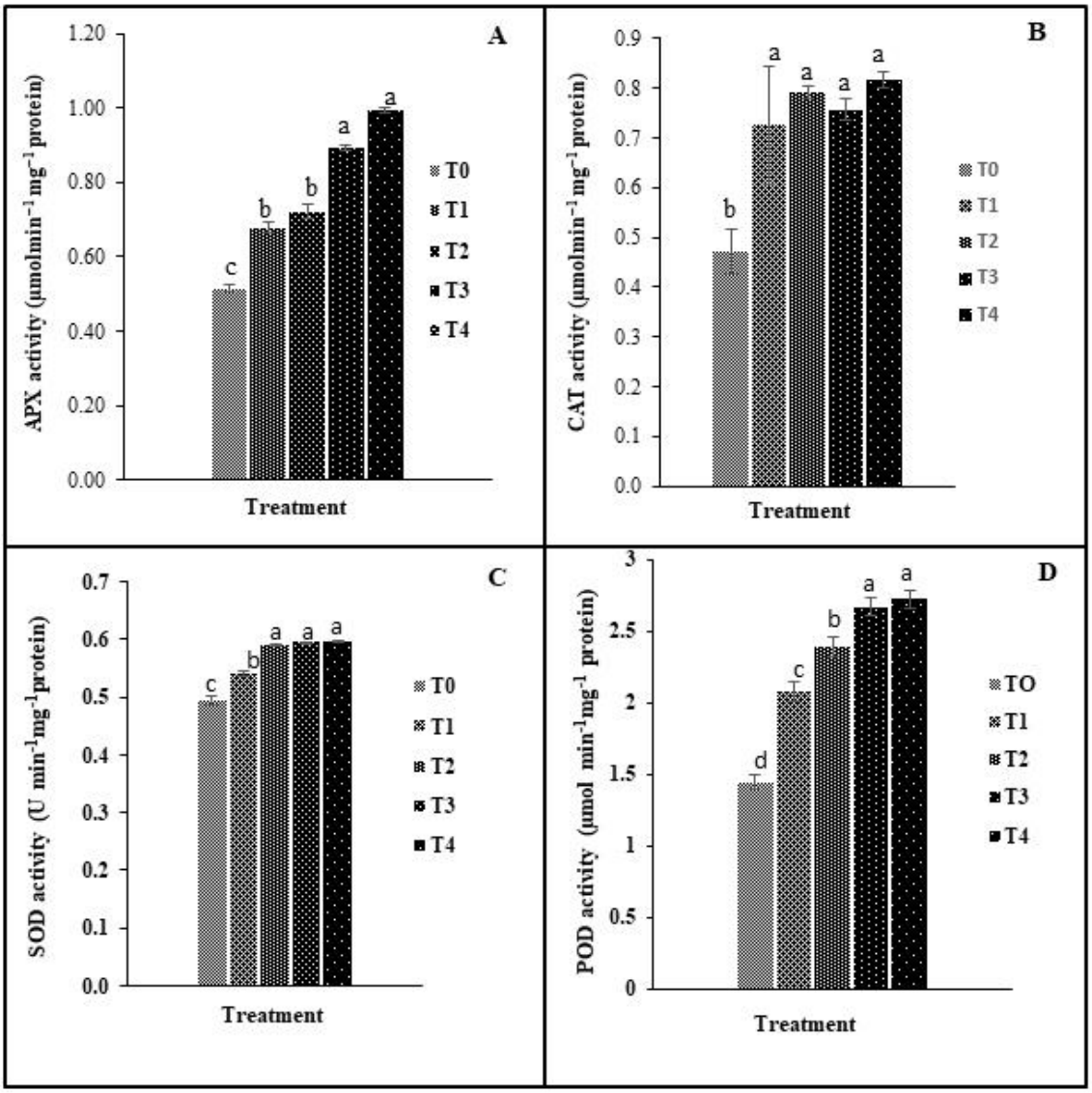

Figure 6. Effects of Cd cytotoxicity on ascorbate peroxidase (A), catalase (B), superoxide dismutase (C) and guaiacol peroxidase (D) activities in leaves of *B. chinensis* exposed to concentration (50 mg kg^{-1} soil) of Cd^{2+} with or without *S. stolonifolium* biomass. **T0**, control; **T1**, 50 mg Cd, **T2**, 25 g biomass + 50 mg Cd; **T3**, 50 g biomass + 50 mg Cd; **T4**, 100 g biomass + 50 mg Cd. Bars bearing dissimilar letter(s) at $P < 0.05$ for each group symbolize significant difference, Tukey's HSD test. Data is the pentaplicate average (n=5). Error bars signify standard error (SE) of five replicates.

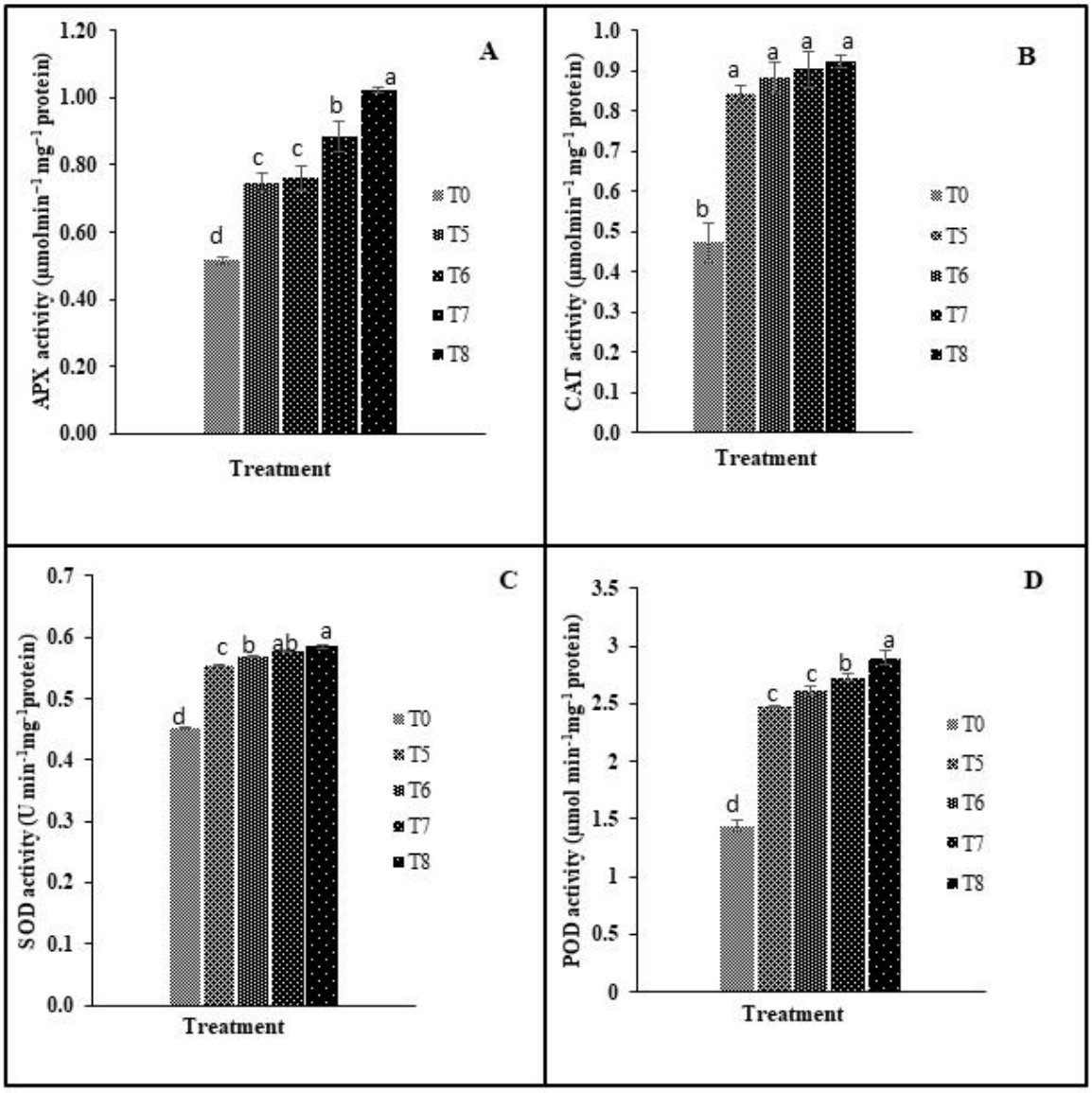

Figure 7. Effects of Cd^{2+} cytotoxicity on ascorbate peroxidase (A), catalase (B), superoxide dismutase (C) and guaiacol peroxidase (D) activities in leaves of *B. chinensis* exposed to concentration (100 mg g^{-1}soil) of Cd^{2+} with or without *S. stolonifolium* biomass. **T0**, control; **T1**, 50 mg Cd, **T2**, 25 g biomass + 50 mg Cd; **T3**, 50 g biomass + 50 mg Cd; **T4**, 100 g biomass + 50 mg Cd; **T5**, 100 mg Cd; **T6**, 25g biomass + 100 mg Cd; **T7**, 50g biomass + 100 mg Cd and **T8**, 100 g biomass + 100 mg Cd. Bars bearing dissimilar letter(s) at $P < 0.05$ for each group symbolize substantial difference, Tukey's HSD test. Data is the pentaplicate average (n=5). Error bars signify standard error (SE) of five replicates.

Plants have their own oxidative defense systems to detoxify ROS species and minimize ROS-induced structural damage when exposed to metals. Plants scavenge ROS via activating antioxidant enzymes (SOD, POD, CAT, and APX) as well as non-enzymatic antioxidants (GSH and GR) (Cvetanovska et al., 2010). The present study exhibited that SOD, POD, CAT, and APX activities were high under Cd toxicity in a dose depending pattern T1 (50 mg) and T5 (100 mg) (Figures 6 & 7), respectively, relative to the absolute control (T0). It has been previously reported that APX and CAT activities increase as cadmium concentration increases in strawberries (Muradoglu et al., 2015). Also, Li et al. (2013) demonstrated increased activities in SOD, CAT and POD in *Hibiscus cannabinus* L. However, incorporation of *S. stolonifolium* into the cadmium contaminated soil increased the antioxidant enzymes activities. W. A. E.-A. Kasim et al. (2016) disclosed that Sargassum extract counteracts the oxidative damage caused by abiotic stress not only directly, but also indirectly, by activating antioxidant enzymes like catalase and peroxidase. Furthermore, the use of seaweed resulted in an increase in antioxidant enzymes, which defend plants against toxic environmental factors (Schmidt, 2005). Earlier studies reported increased antioxidant enzyme activities when *Sargassum* spp. was supplemented. Hemida et al. (2014) abstracted increased antioxidant enzyme activities in wheat upon treatment with brown alga under salt stress. The same was reported in wheat by Ibrahim et al. (2014). Sofy et al. (2017) which also disclosed high activity of SOD, CAT and POD in *Hordeum vulgare* L. treated with *Sargassum* spp. under cadmium stress.

Conclusions

It was found that cadmium toxicity significantly affects *B. chinensis* physiological and even biochemical processes. Incorporating *S. stolonifolium* biomass has lessened the toxicity and improved the growth and development of *B. chinensis* seedlings. *S. stolonifolium* biomass it however, confers resistance to Cd^{+2} stress in seedlings. A great immobilization of Cd^{+2} was also observed at all levels of treatments by chelating with *S. stolonifolium* cell functional groups as a highly decreased cadmium concentration was detected in plant tissues. Hence, it is very important to carry out long-term evaluation of *S. stolonifolium* application in restoration of contaminated soil.

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