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Effects of Cadmium on Biochemical Parameters, Viability and Bioaccumulation of *Kappaphycus alvarezii*

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ABSTRACT

The Rhodophyta, *Kappaphycus alvarezii*, is widely cultivated for its highly valuable kappa carrageenan. *K. alvarezii* has also been known for its capacity to absorb and accumulate excess heavy metal from its surrounding. The study was undertaken to unravel the effect of different cadmium treatments towards *K. alvarezii* at different exposure times by determining the biomass change percentage, algal cell viability and the changes in chlorophyll and protein content at Cd concentrations ranging from 50 to 500 µM for 5, 10 and 15 days. While there was no significant change in the percentage of the biomass change, the viability of the algal cells increased significantly with increasing Cd concentration and exposure time. This was accompanied by significant increase in protein content following similar pattern as shown by the viability of the cells. In contrast, chlorophylls a and b content decreased significantly with increasing Cd concentration. The ability of the alga to uptake Cd was also increased with increasing Cd concentrations possibly indicating that the ability of *K. alvarezii* to survive high level of Cd might be due to its ability to accumulate heavy metal. The results from this research revealed the unique manner in which *K. alvarezii* responds to Cd stress which is unlike any other algae. Thus the findings obtained from this study may contribute important information on how macroalgae adapt to heavy metal pollution.

Keywords: Cadmium, chlorophyll content, *Kappaphycus alvarezii*, protein content, uptake.

INTRODUCTION

Heavy metal is an element that occurs naturally in nature but becomes very detrimental to living organism when the level increases. The increased level of heavy metal in the environment may be attributed to anthropogenic activities including industrial, agricultural, mining, domestic effluents and atmospheric sources (Ali et al. 2019; Wang et al. 2022). Cadmium (Cd) is one of those heavy metals that are released to the environment in quite a high amount due to industrial wastes such as from Cd-nickel (Ni) battery production (Di Toppi and Gabbrielli, 2009; Gallego et al. 2012) and wastewater discharge (Abid et al., 2021). Almost 30,000 tons of Cd have been reported to be released to the atmosphere every year with approximately 4,000 to 13,000 tons being contributed by industrial activities (Faroon et al., 2012).

Cd cannot be biodegraded, and it can be taken up by the plant system through transmembrane carriers involved in the import of essential macronutrients such as calcium (Ca), iron (Fe), magnesium (Mg), copper (Cu) and zinc (Zn) (Clemens, 2006). Similar to plants, high levels of Cd may cause toxicity in algae. Cd was reported to suppress the growth rate, decrease the photosynthetic performance and relative electron transport rate, decrease the content of the photosynthetic pigments, negatively affect the ultrastructure of chloroplasts, change antioxidant enzyme activities, cause lipid peroxidation, and reduce antibiofilm activity (dos Santos et al., 2012; Wickramasinghe et al., 2017; Al-Khaldi et al., 2021). Algae

possess several mechanisms to manage heavy metal toxicity in order to preserve their metabolic activities. Heavy metals are absorbed by algae from the environment onto the algal surface through major functional groups, including hydroxyl, carboxylate, sulfate, and phosphate groups, which then pass through the algal cell and accumulate in the algal body (Monteiro et al., 2011). Proteins and glycoproteins such as alginic acid in the algal cell wall, which has a high absorption capacity of heavy metals facilitate the bioaccumulation and bioabsorption in algae (Romera et al., 2007). As in plants, heavy metal chelation in algae is carried out by metal binding peptides such as metalothioneins and phytochelatins (Balzano et al., 2020; Othman and Mohd Nasir, 2020).

Kappaphycus alvarezii is a red marine alga that is widely cultivated on the coastal area of Sabah, Malaysia for the commercially valuable carrageenan (Nor et al. 2020). The global market value for carrageenan was USD 825.0 million in 2021 with the revenue forecast of USD 1,248.6 million in 2028 (Grand View Research, 2022). Carrageenan is a phycocolloid which is widely used as gelling agent in the pharmaceuticals and cosmetics productions (Porse and Rudolph 2017; Nor et al. 2020). It has been reported previously that *K. alvarezii* possesses high biosorption capacity and was able to remove 90% of Cu, Ni, Cd and lead (Pb) from aqueous solutions in 45 minutes (Praveen and Vijayaraghavan, 2015). More recently, another study has also reported that it has great potential as bioindicators for marine heavy metal pollution because it can accumulate higher concentrations of Cu, Cd, and Pb than the metal ion concentrations in ambient sea water (Tresnati et al., 2021). However, the preceding research mainly focused on the biosorption capability of *K. alvarezii*, the biochemical changes that occur in response to Cd stress remains unknown. Since *K. alvarezii* has great potential in bioaccumulation, information on the biochemical changes is essential.

In this study, the toxic effect of Cd treatment on *K. alvarezii* was ascertained through the evaluation of changes in biomass, viability, and protein and chlorophyll contents. To evaluate the ability of *K. alvarezii* to withstand increasing Cd level, measurement of Cd uptake was also undertaken. The impact of Cd stress on *K. alvarezii* studied in this research was aimed to further explore the response and resistance of macroalgae to heavy metals.

MATERIALS AND METHODS

Plant Acclimatisation

Fresh *K. alvarezii* var. *tambalang* Doty obtained from Semporna, (4.4794° N, 118.6115° E) in Sabah, Malaysia was cleaned and maintained in a custom-made tank filled with artificial seawater consisting of sterile water and sea salt. The salinity and the temperature were kept at 28 ppt and 26 °C, respectively. Guillard's F/2 nutritional medium (Sigma Aldrich) was added into the tank and water was pumped and filtered constantly to ensure adequate aeration and maintain the cleanliness. The algal sample was maintained under 12 h light: 12 h dark (L: D) cycle and at an illumination of 20 µmol photon/m^2/s. The samples were acclimatised for at least 24 h before the experiments were performed.

Cd Treatment

Fresh algal thalli (5 g) were placed in 100 mL conical flask with 50 mL of seawater nourished with F/2 medium. Cadmium chloride ($CdCl_2$) (Sigma Aldrich) was added to each conical flask at various final concentrations (0, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µM). Three replicates were prepared for each concentration. The conical flasks were incubated at 26 °C with shaking at 150 rpm with 12 h light: 12 h dark (L: D) cycle. The media were changed every 2 days.

Biomass Change

To study changes in algal biomass exposed to $CdCl_2$, the algal thalli were cut using a sterile knife. The algal pieces were then weighed before being placed into a 50 mL conical flask filled with 25 mL of seawater enriched with Guillard's F/2 nutrient medium. $CdCl_2$ was added to each conical flask to final concentrations of 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µM with nine replications for each concentration. The control sample without Cd was also prepared. Three samples for each concentration were harvested on days 5, 10 and 15. The final weight of each sample was calculated as percentage change in biomass.

Viability Test

Algal thalli at a diameter of ~2 mm were cut using a clean and sterile razor blade into discs of less than 3 mm in thickness. Thirty algal discs were placed in 50 mL conical flask filled with 25 mL seawater nourished with f/2 medium. $CdCl_2$ was added to each conical flask at various final concentrations (0, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µM) in triplicates. The conical flasks were then incubated at 26 °C with shaking at 150 rpm under 12 h light: 12 h dark (L: D) cycle. The media were changed every 2 days. The number of living algal discs was recorded every 2 days until day 16.

Protein Estimation

Treated sample (500 mg) from each treatment was homogenised in 1 mL phosphate buffer (pH 7.0) before being centrifuged at 8000 × g at 4 °C for 15 min. The pellet was then dissolved in 1 mL of 0.1 M NaOH. Total protein amount was obtained using Bradford method (Bradford, 1976), where bovine serum albumin was used as standard. One millilitre supernatants were added into Coomassie Brilliant Blue G-250 (5 mL) and mixed thoroughly. After 10 min, absorbance of 2 mL sample was measured at 595 nm. Each treatment was performed in triplicate.

Determination of Pigment Content

The pigment content in the treated algal sample was determined using previously published method (Hiscox and Israelstam, 1979). Treated sample (100 mg) from each treatment was incubated in pre-warmed DMSO (65 °C) for 45 min or until the algal thalli became colourless. The mixture (1 mL) was then mixed with 2 mL DMSO. Absorbance readings were recorded at 480, 510, 645 and 663 nm. The chlorophyll content was determined using the following equations:

$$\text{Chlorophyll a} = (0.0127 \times A_{663}) + (0.00269 \times A_{645})$$

$$\text{Chlorophyll b} = (0.0229 \times A_{645}) + (0.00468 \times A_{663})$$

$$\text{Total Chlorophyll} = (0.202 \times A_{645}) + (0.00802 \times A_{663})$$

Analysis of Cadmium Uptake

Cadmium in the growth media that has been absorbed by the algal samples was analysed using an Atomic Absorption Spectrometer (AAS). Samples harvested for biomass change analysis were used for Cd uptake analysis. The algal samples were frozen in liquid nitrogen before being sent for Cd content analysis using an AAS at the Molecular Structure Characterisation Laboratory, Center for Research and Instrumentation Management (CRIM), UKM.

Statistical Analysis

Each experiment was performed in triplicate, and the data were expressed as means ± the standard deviation (SD). The data were analysed in RStudio version 2022.07.2+576 using two-way analysis of variance

(ANOVA) with interaction. The significant level was set at $p<0.05$. Tukey's HSD was used for multiple comparison tests of the effects of the concentration of cadmium chloride on each parameter.

RESULTS AND DISCUSSION

Change in biomass and viability assessment

K. alvarezii thalli for the control sample without Cd had a biomass change percentage of 39.08% after 5 days that decreased to 36.42% after 10 days of exposure but increased after 15 days of exposure at 41.62% (Figure 1). Samples treated with Cd also showed varied patterns in biomass change percentage at different Cd concentrations and after different days of exposure (Figure 1). A concentration-dependent slight decrease in percent biomass change was observed in samples treated with Cd until 200 µM Cd followed by slight increase until 500 µM Cd, however, this was not significant. The biomass change percentage for 500 µM Cd for 15 days (41.46%) was similar to control sample without Cd (41.62%) hence Cd concentration has no significant effect on growth. The lowest biomass change percentage (32.11%) was determined in *K. alvarezii* samples treated with 200 µM Cd for 10 days (Figure 1). There were significant increases in biomass change percentage compared to control samples shown for algal samples treated with 200 µM Cd for 15 days (41.87%) and 350 µM Cd for 10 days (43.49%). The two-way ANOVA results indicated that the interaction between the effects of concentration and exposure day was not significant for biomass change percentage. The main effects analysis showed that there was no significant difference in biomass change percentage for algal samples with increasing Cd concentration, nevertheless, the exposure day had significant influence on the growth measurement (Table 1).

K. alvarezii treated with cadmium did not show any significant change in biomass change percentage indicating that cadmium stress is not a key factor limiting growth. The results obtained in this study differed from previous results exhibited by other macroalgae, the agarophytes, *Gracilaria tenuistipitata* (Huang et al., 2010) and *G. domingensis* (dos Santos et al., 2012). *G. tenuistipitata* showed a significant decrease in growth rates between control algae without Cd and those grown with increasing Cd concentrations until 200 µg/L (1.79 µM) for 14 days (Huang et al., 2010), while the latter macroalga had declining growth rates until 300 µM for a period of 16 days (dos Santos et al., 2012). The effect of Cd treatment on the growth of *K. alvarezii* was also different from the microalgae, the freshwater unicellular green algae *Chlorella vulgaris* (Cheng et al., 2016) and *C. sorokiniana* (León-Vaz et al., 2021). The former microalga showed inhibited growth at a much lower Cd concentration at 7 mg/L Cd (62.5 µM) whereas *C. sorokiniana* exhibited decreased growth when exposed to 250 µM Cd (Cheng et al., 2016). These results indicated that *K. alvarezii* possesses a much higher tolerant to high level of Cd compared to other macroalgae and microalgae.

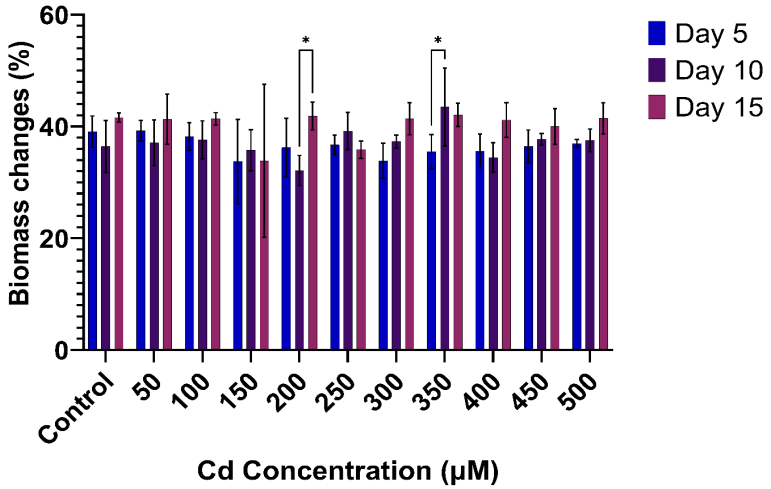


Figure 1. *K. alvarezii* growth expressed as biomass change percentage after treatment with different concentrations of Cd compared to day 0. Data are means ± SE (n = 3). Asterisks (*) indicate that mean values are significantly different between the treatments and the corresponding control. *** Significant at $p<0.001$; ** Significant at $p<0.01$; * Significant at $p<0.05$.

Table 1. Two-way ANOVA results showing the percentage of biomass change, cell viability and protein content for *K. alvarezii* treated with Cd where treatment concentration and duration were used as factors ($p<0.05$ = significant).

Source of variation	% Biomass change		Cell viability		Protein content	
	DF	Mean square	DF	Mean square	DF	Mean square
Concentration (C)	10	22.88ns	10	1545.51***	11	1696667***
Exposure (E)	2	126.4***	7	2830.01***	3	17611134***
C × E	20	16.83ns	70	143.05***	33	3620282***
Error	66	16.52	264	44.88	362	575636
Total	98		351		409	

*** Significant at $p<0.001$; ns Not-significant

The effect of Cd exposure on the viability of *K. alvarezii* showed that the algal viability increased with increasing Cd concentrations (Figure 2). Treatment of *K. alvarezii* with Cd of up to 100 µM impacted cell viability where the normal cells without Cd died on day 8, and the cells containing 50 and 100 µM died on days 10 and 14, respectively (Figure 2a). No viable cell was detected by day 16. Algal cells treated with 150 µM and above, all survived even until 16 days of treatment. Cell viability showed slight increase at 23 to 43 % when exposed to Cd at 150 to 400 µM, which dramatically increased above 80 % as the concentrations increased from 450 µM upwards (Figure 2b). These three levels of cell viability were represented by no significant difference in the percentage of living algal discs in the ranges of 0 to 100 µM, 150 to 400 µM, 450 to 500 µM while significant differences were observed between 100 and 150 µM, and 400 and 450 µM. Two-way ANOVA results showed that the viability of *K. alvarezii* was significantly influenced by the interactions between the effects of concentration and exposure duration, which indicated that different concentrations of cadmium at different exposure times affect the viability of the alga (Table 1). The results also showed that the effects of both main effects were significant.

The increased cell viability for *K. alvarezii* grown in increasing Cd observed in this study was unlike any previously published research for all types of algae. Previous research on the green microalga, *Selenastrum capricornutum*, treated with 5 ppm (5 mg/L; 44.6 µM) Cd for 6 days showed that the cellular

viability of normal algal culture at 85.35% was reduced to 34.35% (Choi et al., 2003). In a study on another microalga, *Scendesmus obliquus*, cell viability was measured as a function of Cd removal where living algal cells significantly outperformed non-living ones in cultures containing increasing Cd until 25 mg/L (223 µM) Cd for 24 h (El-Shimy, 2005). In another study on the viability of *Chlorella* sp., the colour of the culture was still green (living cells) when exposed to 20 ppm (20 mg/L; 179 µM) Cd for 60 min but changed to brown (dead cells) at 100 ppm (100 mg/L; 893 µM) Cd for the same duration of exposure (Valdez et al., 2018). More recently, the impact of Cd on the viability of the macroalga, *G. bailiniae*, showed that 5 mg/L (44.6 µM) Cd treatment for 7 days caused significant discolouration to the *G. bailiniae* thalli compared to the control algal samples (Huang et al., 2022). This discolouration was accompanied by the relative growth rate that was decreased by 52.1%.

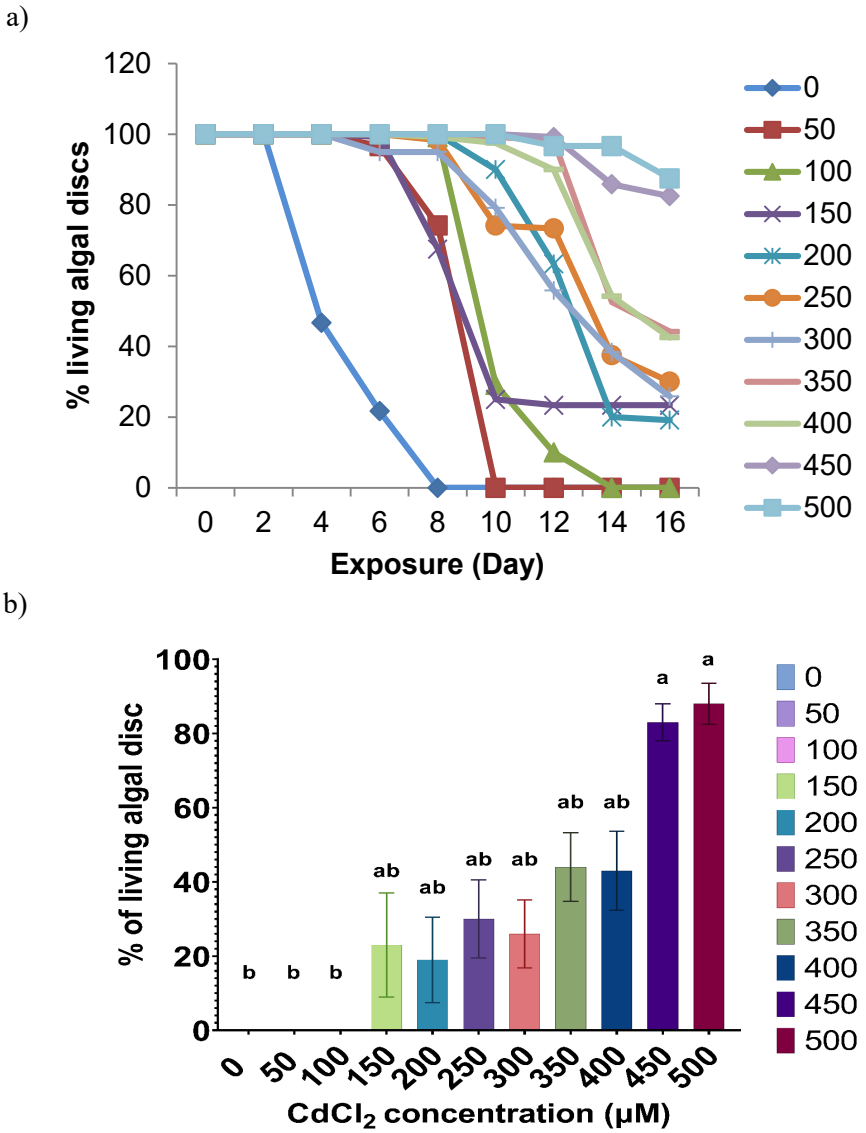


Figure 2. Effect of different Cd concentrations on the percentage of living algal discs in *K. alvarezii* after different days (a) and 15 days (b) of exposure. Data represent mean values ± SE (n = 3). Columns labeled with different letters indicate statistically significant differences (p<0.05).

Protein Content Estimation

In Figure 3, the controls are the protein content determined on Day 0 for all treatments which showed that the protein contents were the same at the start of the treatment. Two-way ANOVA results showed that the interaction effect was significant statistically. The results also showed a significant difference in total protein content in algal samples with increasing days and concentrations (Table 1). As shown in Figure 3, the effect of the concentration of Cd varied with different exposure days. The effect of Cd on soluble protein content of *K. alvarezii* showed that after 15 days of exposure, the total protein content was significantly altered for control sample without Cd, and treatments with 50, 150, 200, 250, 300, 400 and 500 µM (Figure 3; Table 1). Cd stress had a significantly increasing trend on the protein content of *K. alvarezii* under the concentrations of 50 and 400 µM throughout 15 days of treatment.

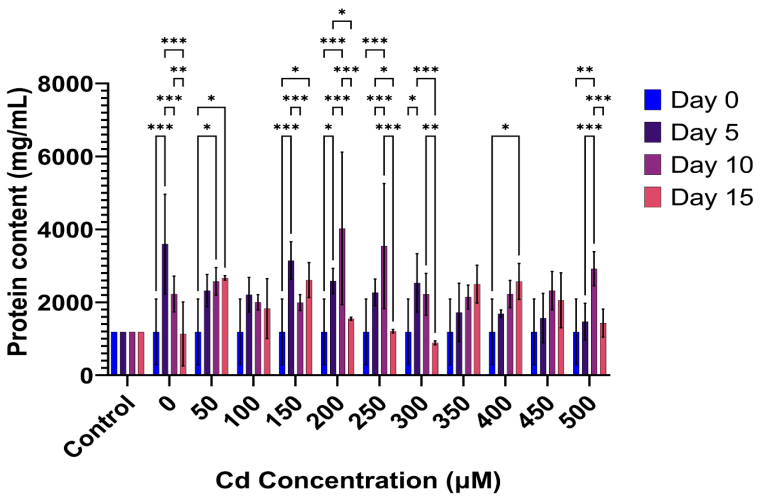


Figure 3. Effect of different concentrations of Cd on total protein content in *K. alvarezii* after different days of exposure. Data are means ± SE (n = 3). Asterisks (*) indicate that mean values are significantly different between the treatments and the corresponding control. *** Significant at $p<0.001$; ** Significant at $p<0.01$; * Significant at $p<0.05$.

Similar increase in total protein content had been reported for *C. vulgaris* treated with 0 to 7 mg/L (62.5 µM) Cd (Cheng et al., 2016). On the other hand, significant decrease in total protein content was observed for control sample and algae treated with 150, 200, 250, 300 and 500 µM Cd after 15 days of treatment. This decreasing trend was similar to previous study where total protein content for *C. sorokiniana* was very low, at only 48% of the control algal cells, upon exposure to 250 µM Cd for 2 h (Carfagna et al., 2013). The contrasting results reported for *C. vulgaris* and *C. sorokiniana* were probably because the former microalga was exposed to a much lower concentration of Cd and was tolerant to the heavy metal ion while *C. sorokiniana* was experiencing Cd toxicity effect at the higher Cd concentration (Carfagna et al., 2013). The results of the current study suggested that *K. alvarezii* possesses a much higher tolerant to high level of Cd compared to other algae probably through production of certain proteins.

Chlorophyll Content Analysis

Cd had an adverse effect on chlorophyll (Chl) (a, b and total) production by *K. alvarezii* (Figure 4). The Chl a, Chl b and Chl total (Chl t) contents after 5, 10 and 15 days exposure showed abrupt decrease for all Cd treatments. Significant differences were observed for a few Cd concentrations at 5 (50, 300, 350, 400 and 500 µM) and 10 (250 and 300 µM) days in Chl a, and 5 (50, 100, 150, 250 and 400 µM) and 15 (Control, 100, 450 and 500 µM) days for Chl b. Meanwhile, the decrease in Chl t content was not significantly different from the control samples. The highest drop in Chl a and Chl b concentrations occurred after 5 days

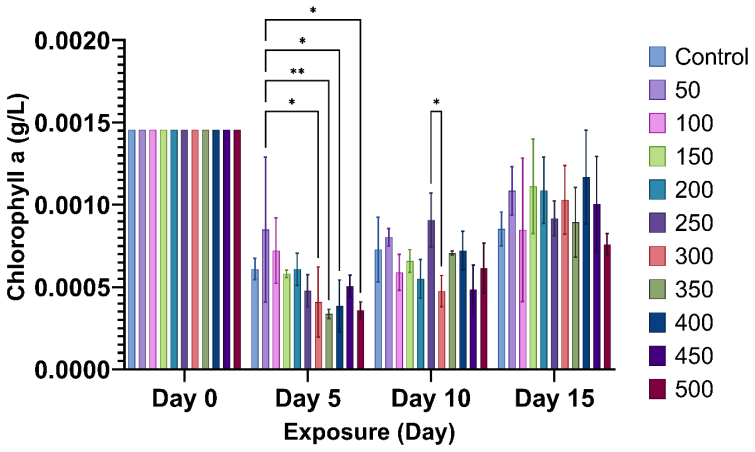
of exposure to Cd. Two-way ANOVA showed that the interaction of the effects of Cd concentration and days of exposure as well as the main effects had a significant influence on Chl a and Chl b contents in algal samples (Table 2). Meanwhile for Chl t content, exposure days had a significant influence (p<0.001) but not Cd concentration and their interaction. Similar decreasing trends in chlorophyll contents have been documented in many previous studies including in Cd-exposed macroalgae, *G. domingensis* (dos Santos et al., 2012), *Sarcodia suiae* (Han et al., 2020) and *Ulva reticulata* (Al-Khaldi et al., 2021) as well as in microalgae, *C. vulgaris* (Cheng et al., 2016) and *C. sorokiniana* (Carfagna et al., 2013).

Table 2. Two-way ANOVA results showing the chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Chl t) contents of *K. alvarezii* treated with Cd where treatment concentration and duration were used as factors (p<0.05 = significant).

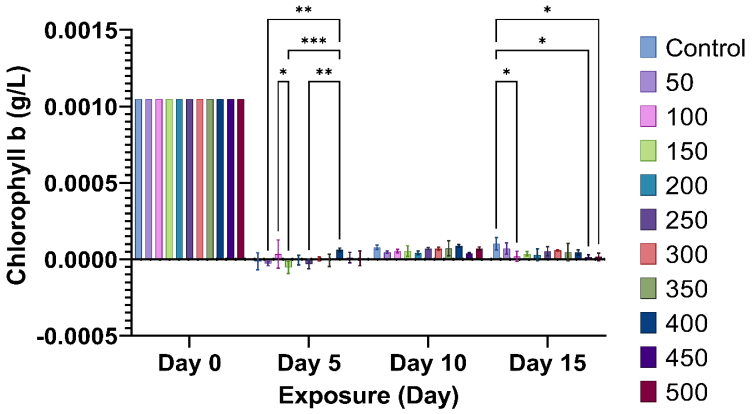
Source of variation	DF	Mean square		
		Chl a	Chl b	Chl t
Concentration (C)	10	0.00000005476*	0.000000001599*	0.0000007116ns
Exposure (E)	3	0.000005613***	0.00000849***	0.001521***
C × E	30	0.00000004235*	0.000000001416**	0.000000541ns
Error	88	0.00000002539	0.0000000008317	0.0000004666
Total	131			

*** Significant at p<0.001; ** Significant at p<0.01; * Significant at p<0.05; ns Not-significant

(a)



(b)



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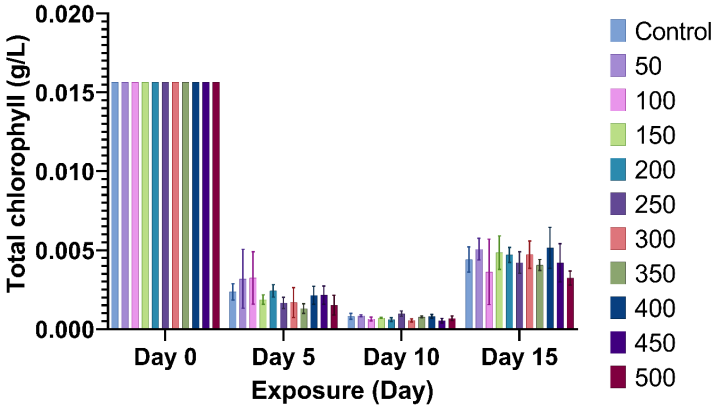

Figure 4. Effect of different concentrations of Cd on chlorophyll a (a), chlorophyll b (b) and total chlorophyll (c) contents in *K. alvarezii* after different days of exposure. Data represent mean values ± SE (n = 3). Asterisks (*) indicate that mean values are significantly different between the treatments and the corresponding control. *** Significant at $p<0.001$; ** Significant at $p<0.01$; * Significant at $p<0.05$.

When exposed to Cd concentrations similar to the current study but for a longer duration, ranging from 100 to 300 µM, the agarophyte *G. domingensis* had decreased Chl a levels after being exposed for 16 days (dos Santos et al., 2012). In other study with lower Cd concentration and short exposure time of 5 mg/L (44.6 µM) Cd for up to 24 h, the Chl a content in the red alga *S. suiae* was found to be decreased compared to ambient cadmium levels (Han et al. 2020). In a similar study but at longer duration, exposure of *U. reticulata* to 5 mg/L (44.6 µM) Cd for 2 days exhibited a decrease in Chl a content (Al-Khaldi et al., 2021). Nevertheless, none of these studies on macroalgae were statistically significant compared to control algal samples, which are unlike the current study.

Meanwhile for the microalgae, the Chl a and Chl t contents in *C. sorokiniana* exposed to 5 mg/L (44.6 µM) Cd for 24 h exhibited decreasing trends (Carfagna et al., 2013). Similarly, Cd had an adverse effect on Chl a and Chl b production by *C. vulgaris* exposed to 7 mg/L (62.5 µM) for 18 days (Cheng et al., 2016). Both studies on microalgae showed significant decrease in chlorophyll content similar to the current study. It seems that *K. alvarezii* behaved in a similar manner to microalgae for this parameter exhibiting decrease in photosynthetic pigments.

Cd Uptake

The content of cadmium in algal samples treated with different concentrations of Cd showed increasing Cd accumulation for all durations, with a maximum of 69 µg/g upon treatment with 500 µM for 5 days (Figure 5). However, significant difference was exhibited for treatment with 50 µM from 5 to 15 days. The interaction between the effects of concentration and exposure day was not significant on Cd uptake (Table 3). Furthermore, the main effects analysis showed a significant increase in Cd accumulation in algal samples with increasing treatment concentrations but not with exposure duration (Table 3).

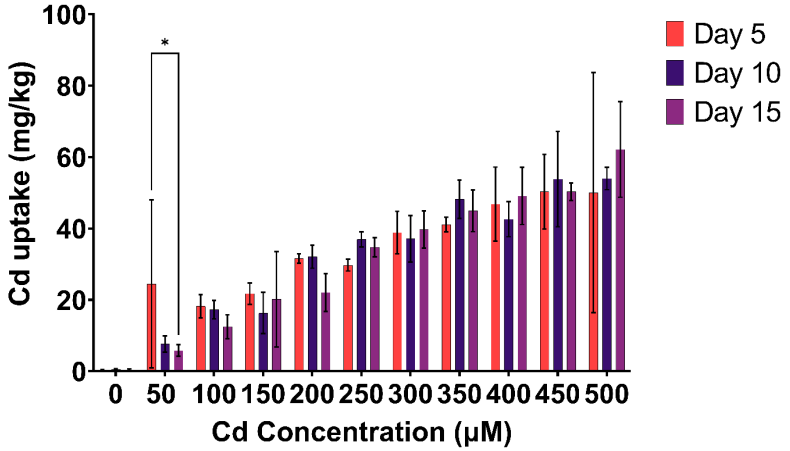

Figure 5. Effect of different concentrations of Cd on uptake of Cd in *K. alvarezii* after different days of exposure. Data represent mean values ± SE (n = 3). Asterisks (*) indicate that mean values are significantly different between the treatments and the corresponding control. * Significant at $p<0.05$.

Table 3. Two-way ANOVA results showing the Cd uptake of *K. alvarezii* treated with Cd where treatment concentration and duration were used as factors ($p<0.05$ = significant).

Source of variation	Cd Uptake	
	DF	Mean square
Concentration (C)	10	2820***
Exposure (E)	2	8.963ns
C × E	20	70ns
Error	66	86.42
Total	98	

*** Significant at $p<0.001$; ns Not-significant

The study by Han et al. (2020) on *S. suiae* following Cd exposure of 5 mg/L (44.6 µM) within 24 h showed increased bioaccumulation which was significantly higher in light environments than in dark environments ($p<0.05$). In another study using the same concentration of Cd but much longer exposure of 7 days, *U. reticulata* exhibited similar increasing trend of Cd uptake. A significant increase in metal content was observed with increasing treatment concentrations and exposure duration (Al-Khaldi et al., 2021). A study on the effect of Cd exposure to a much lower concentration (10 µM) on tissue accumulation in two marine macroalgae, *Fucus vesiculosus* and *U. lactuca*, revealed that the metal accumulation was significantly higher for both species. However, the levels of metal accumulations at long exposure time of 21 days were not significantly different from shorter durations of 48 and 96 h (Wickramasinghe et al.,

2017). In this regard, *K. alvarezii* showed similar response as *F. vesiculosus* and *U. lactuca* where increasing Cd uptake was significantly influenced by concentration and not exposure time.

The toxic effects of Cd in algae have impacted the growth rate, cell viability, and the content of the photosynthetic pigments and proteins (Choi et al., 2003; Huang et al., 2010; dos Santos et al., 2012; Cheng et al., 2016; Valdez et al., 2018; León-Vaz et al., 2021). The results reported in those studies were influenced by increasing Cd content and not duration of exposure. Furthermore, all algae could not survive high Cd concentration showing loss of viability at a low concentration 44.6 μM Cd for both microalga (*S. capricornutum*) (Choi et al., 2003) and macroalga (*G. bailiniae*) (Huang et al., 2022), and at the highest Cd concentration of 223 μM Cd for microalga (*S. obliquus*) (El-Shimy, 2005).

The effects of Cd exposure on *K. alvarezii* are very unique and have never been reported for any algal species. The only similarity between *K. alvarezii* and other algae in their response to Cd stress is the decreased photosynthetic pigments which might indicate negative impact on the photosynthesis of this alga. However, the viability of *K. alvarezii* was not affected by the decreased photosynthetic pigments and showed three phases of responses at low (0 to 100 μM), intermediate (150 to 300 μM) and high (400 to 500 μM) Cd concentrations. This pattern of viability correlated with the protein content of this alga which also showed significant differences at low (0 to 50 μM), intermediate (150 to 400 μM) and high (450 to 500 μM) Cd concentrations. The *K. alvarezii* cells were able to thrive in increasing Cd concentrations through bioaccumulation as evidenced by the increasing Cd uptake which could occur possibly through metal chelation by certain proteins as depicted by the increasing pattern of protein contents. The cysteine-rich metallothioneins and phytochelatins may play important roles in the detoxification and tolerance of metals in *K. alvarezii* similar to those algae that thrive in environments polluted by heavy metals (Pawlik-Skowronska et al., 2007). While metallothioneins have not been reported to be present in *K. alvarezii*, phytochelatin-mediated Cd sequestration in this alga had been published much earlier (Hu and Wu, 1998). The presence of Cd seems to have triggered some mechanisms that facilitate the alga to survive the stress rather than killing it. Further investigations are required to reveal the mechanisms involved in the tolerance of *K. alvarezii* towards Cd stress. The findings revealed the unique characteristics of *K. alvarezii* that can withstand and accumulate excess amount of heavy metals hence may be explored further in future phytoremediation practices.

CONCLUSIONS

The present study provided information on the toxicity of Cd on the red macroalga *K. alvarezii*. Our data suggested that the exposure of the algae to Cd did not compromise the biomass change percentage. This heavy metal caused a significant enhancement of cellular viability and protein content, as well as a strong reduction in the content of the chlorophylls. The increased capability for Cd uptake in Cd-treated cells suggests a link between the tolerance to heavy metal contamination and increased viability of the cells and protein content. *K. alvarezii* cells seem to better tolerate high Cd concentrations while appearing to be more sensitive to low Cd concentrations. These results provide new information on the effects of heavy metal contamination in macroalgae.

AUTHORS CONTRIBUTION

NI and RO conceived and designed the analysis. NI performed the experiment. NI and MIA performed the analysis. RO and MIA wrote the paper and approved the submission.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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