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The Toxic Effects of Strong Chlorin Disinfectant on Mangroves and Emission Thresholds

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Abstract

In this study, based on the simulated discharge results of chemical disinfectants, hypocotyl germination concentration gradient pre-test and concentration gradient determination experiment were set up respectively. Laboratory cultivation was conducted to compare and analyze the root germination and germination indexes, three mangrove hypocotyls of *Kandelia candel* (*Linn.*) *Druce*, *Ceriopstagal C.B. Rob.* and *Bruguiera sexangula var. Rhynchopetalas*' efficiency of cumulative root germination, cumulative germination and the cumulative expansion of the second pair of leaves, one-way analysis of variance was used to obtain the tolerance threshold of three mangrove hypocotyls to strong chlorin disinfectant. The study determined that the by-products of strong chlorin disinfectant, the toxic threshold concentrations of *Kandelia candel* (*Linn.*) *Druce*, *Ceriopstagal C.B. Rob.* and *Bruguiera sexangula var. rhynchopetala* are close to 0.55 mg/L, 0.55 mg/L and 0.25 mg/L, respectively. This concentration range is lower than the average concentration of 1.183 mg/L of active chlorine emitted from strong chlorine concentrate during pond clearing in high-level shrimp ponds, indicating that transient emissions of strong chlorine concentrate during pond clearing can have a toxic effect on mangrove plants. The strength of tolerance of the embryonic axes of the three mangrove species to effective chlorine contamination was, *Ceriopstagal C.B. Rob.* stronger than *Bruguiera sexangula var. rhynchopetala*, and *Kandelia candel* (*Linn.*) *Druce* is the weakest.

Keywords

Strong Chlorin, Toxicity Threshold, Germination of Mangrove Hypocotyls, One-Way Analysis of Variance

1. Introduction

Mangrove ecosystems are characterized by high biodiversity, high productivity,

and host thousands of various marine and terrestrial taxa [1] [2] [3], which have unique ecological, social and economic benefits [2] [3]. In recent years, mangrove ecosystems have been severely damaged, for example, in Dongzhai Harbor, Hainan, until 2016. The mangrove forests in the protected area had declined by about 100 hm^2 (accounting for 19% of the total area of mangrove forests in Dongzhai Harbor), and more than 80% of the existing mangrove forests were inefficient degraded secondary forests, the health index of the mangrove ecosystems had been decreasing. There are about 28,000 mu of shrimp ponds around the Dongzhai Harbor Reserve, and shrimp farming is dominated by high-level ponds. The terrain of high-level shrimp ponds is higher than the sea level and the breeding density is high, the sewage discharge is convenient and the amount of sewage discharge is large. Various kinds of strong chlorine essence will be used during the pond cleaning and breeding, such as calcium hypochlorite, strong chlorin, sodium dichloroisocyanurate and so on. A large number of studies have shown that these strong chlorine essences have a certain toxic effect on plant tissue cells, seed germination, and plant root, stem and leaf growth when they reach a certain concentration [4] [5] [6] [7].

Mangrove plants are typical foetal sprouting plants, the seeds in the fruits will germinate into embryonic axes on the parent tree. When the embryonic axes mature and fall into the soil, the embryonic roots within the embryonic axes develop into roots and the embryonic buds develop into shoots. The quality of the embryonic axes sprouting roots and buds in mangrove plants plays a decisive role in the survival of the embryonic axes [8]. Strong chloroalkaloid enters into mangrove forests with shrimp pond effluent discharge, however, the hazard and extent of damage to mangrove plant growth are not known. Therefore, in this study, we established a research methodology to study the toxic effects of strong chloroalkali disinfectants discharged from shrimp ponds in high ponds on mangroves and the threshold of discharges. The embryonic axes of three typical mangrove plants, namely, *Kandeliacandel* (*Linn.*) *Druce*, *Ceriopstagal C.B. Rob.*, and *Bruguiera sexangula var. rhynchopetala*, were used as the subjects of the study, and the exposure experiments to strong chloroalkali were carried out by artificial sand cultivation in the laboratory conditions. Through continuous monitoring and observation of seed germination and monitoring of germination and root sprouting of the embryonic axes, the effects of strong chlorin on the germination and growth of seedlings of different mangrove plants were investigated, with a view to obtaining the toxicity thresholds of strong chlorin on mangrove plants. Such experiments can provide theoretical basis for the protection of mangrove wetlands.

2. Research Methods

2.1. Cultivation of Embryonic Axes

Mature and healthy embryonic axes of *Kandeliacandel* (*Linn.*) *Druce*, *Ceriopstagal C.B. Rob.*, and *Bruguiera sexangula var. rhynchopetala* were collected in

Dongzhai Harbor Mangrove Nature Reserve in Hainan. Embryonic axes of similar size and length, well-developed, free of pests and mechanical damage, and close to the same maturity level were collected. The number of each embryonic axis collected at each time was not less than 200. The picked embryonic axes were wiped dry and put into ziplock bags and brought back to the laboratory with ice packs for freshness [9].

The experiment was carried out by sand cultivation, artificial seawater was used with a concentration of 1% configured with strong chlorophyll. For the three mangrove embryonic axes, five concentration gradients of strong chlorine concentrate were set up, totaling 15 pots. 13 - 15 embryonic axes were inserted in each pot, totaling more than 210 embryonic axes. The depth of insertion was 1/3 of the length of the embryonic axes, and the pots were placed on the sunny side of the laboratory for cultivation. The indoor air conditioner was adjusted to keep the indoor cultivation temperature at about 28˚C - 32˚C. The indoor humidity was 60% - 70%. The light oblique illumination was guaranteed for about 6 h per day, and the cultivation was carried out for 30 days [10]. Watering corresponding to the number of plastic pots until the water surface just submerged substrate. Small holes were cut in the bottom of the pots and connect with latex tubes, so that the water in the pots can slowly flow out and recycling cycle, so that the daily flooding time of about 8 hours, so that every day at a fixed time alternately.

2.2. Determination of the Concentration Gradient of Strong Chlorophyll

2.2.1. Pre-Test of the Concentration Gradient of Embryonic Axis Germination

According to the results of the simulation experiment on the disinfection of high initial concentration of chlorine concentrate in the laboratory, the maximum concentration of free chlorine can be reached is 1.497 mg/L, the lowest value of pH is 6.03. Thus, the concentration of chlorine concentrates, the theoretical concentration of free chlorine, and the corresponding pH value are set as shown in Table 1.

2.2.2. Determination of Embryonic Axis Sprouting Inhibition Threshold Experiment

Comprehensively analyzing the results of the three mangrove embryonic axes sprouting and germination under the contamination of strong chlorine in the pre-test, it can be seen that the free chlorine produced by strong chlorine to

Table 1. Concentration of chlorine concentrate, theoretical free chlorine concentration gradients and corresponding pH values.

Clusters	1	2	3	4	5
Strong chlorine concentration mg/L	0	3.0	5.0	6.5	7.5
Free chlorine concentration mg/L	0	0.2	0.5	1.0	1.5
pH	7.25	7.03	7.01	6.90	6.84

Kandeliacandel (*Linn.*) *Druce* embryonic axes, *Ceriopstagal C.B. Rob.* embryonic axes, and *Bruguiera sexangula var. rhynchopetala* embryonic axes in the threshold of toxicity concentration were in the range of 0.5 mg/L - 1.0 mg/L, 0.5 mg/L - 1.0 mg/L, and 0.2 mg/L - 0.5 mg/L, respectively. Similarly, taking into account the fact that individual germination indexes in free chlorine at concentrations of 0.5 mg/L, 0.5 mg/L, and 0.2 mg/L for *Kandeliacandel* (*Linn.*) *Druce*, *Ceriopstagal C.B. Rob.*, and *Bruguiera sexangula var. rhynchopetala* were somewhat different from those of the control group, further concentration gradients were designed around the three concentrations mentioned above, respectively. The concentration gradient of the inhibition threshold determination experiment was designed as follows in Table 2.

2.3. Embryonic Axis Indicators and Methods

Before cultivation, the embryonic axes of *Kandeliacandel* (*Linn.*) *Druce*, *Ceriopstagal C.B. Rob.* and *Bruguiera sexangula var. rhynchopetala* were numbered and labeled. The length, maximum diameter, and fresh weight of each embryonic axis were determined and recorded, as well as the morphological characteristics of the embryonic axes were compared. Samples were taken at 48-hour intervals after the embryonic axes were inserted for observation. With reference to the indicators of seed germination ability in the forestry industry, taking into account the special morphology of the embryonic axes of mangrove plants, the shoot and root ends were monitored separately. The cumulative length of the radicle reaches 3 mm for sprouting, and the first pair of leaves out of the terminal bud was the germination [11]. The day of the beginning of sprouting refers to the number of days required for the first sprouting of all the embryonic axes, and the day of sprouting needs to pull out the embryonic axes for observation. It should be noted that the embryonic axes are no longer pulled out for observation after the recording of the sprouting of the embryonic axes. The monitoring indexes are shown in Table 3, in which the shoot length and root length were measured using a straightedge with an accuracy of 0.1 cm, and the rest of the indexes were observed statistical indexes.

The final experimental results were statistically analyzed by one-way analysis of variance (ANOVA) using SPSS (Statistical Package for the Social Science) software for the rooting and sprouting indicators of the results of the inhibition threshold experiment [12].

Table 2. Gradient of strong chlorine concentration for inhibition threshold determination experiments.

Plant species	Theoretical free chlorine concentration mg/L				
	1	2	3	4	5
Kandeliacandel (*Linn.*) *Druce*	0.00	0.45	0.50	0.55	0.60
Ceriopstagal C.B. Rob.	0.00	0.45	0.50	0.55	0.60
Bruguiera sexangula var. rhynchopetala	0.00	0.15	0.20	0.25	0.30

Table 3. Indicators of germination and rooting of embryonic axes.

Germination indicators	Root sprouting index
germination time	root initiation date
germination rate	rooting rate
bud growth rate	root growth
Second pair of leaf unfolding time	Maximum root length

3. Discussion

3.1. Effect of Strong Chlorine Concentrate on Germination of Embryonic axes

As can be seen from **Figure 1**, in strong chlorine concentrate of 0.60 mg/L, the root sprouting day, germination time and the second pair of leaves unfolding time of the embryonic axes were delayed compared with the control group, and the rates of root sprouting and germination were also significantly reduced compared with the control group, and the period of germination was prolonged. It can be seen in **Figure 2** that the three germination rates of the embryonic axes of *Ceriopstagal C.B. Rob.* were significantly reduced and the germination cycle was prolonged compared with that of the control group in strong chlorine at a theoretical free chlorine concentration of 0.60 mg/L. In the 0.25 mg/L concentration group, the germination rate of the embryonic axes of the *Bruguiera sexangula var. rhynchopetala* was reduced and the period of germination and sprouting was prolonged, but the trends of the cumulative germination rate and the cumulative unfolding rate of the second pair of leaves were not significantly different from those of the control group (**Figure 3**). In the strong chlorine concentration of 0.30 mg/L, the rooting rate, sprouting rate and the second pair of leaf unfolding rate of the embryonic axes of *Bruguiera sexangula var. rhynchopetala* were significantly reduced compared with those of the control group, and the rooting rate, sprouting rate and the second pair of leaf unfolding rate were lagging behind those of the control group, and the sprouting cycle was prolonged.

The cumulative rooting efficiency, the cumulative sprouting efficiency and the cumulative development efficiency of the second pair of leaves showed that the inhibitory limits of the disinfection by-products of strong chlorine were 0.55 mg/L - 0.60 mg/L, 0.55 mg/L - 0.60 mg/L, and 0.25 mg/L - 0.30 mg/L for *Kandeliacandel* (*Linn.*) *Druce*, *Ceriopstagal C.B. Rob.* and *Bruguiera sexangula var. rhynchopetala*, respectively.

In summary, in the inhibition threshold test of strong chlorine disinfectant poisoning, the analytical results of sprouting and rooting indexes, cumulative rooting and sprouting efficiency and cumulative unfolding efficiency of the second pair of leaves were consistent for *Kandeliacandel* (*Linn.*) *Druce* and *Bruguiera sexangula var. rhynchopetala*, so the range of inhibition threshold concentration for *Kandeliacandel* (*Linn.*) *Druce* and *Bruguiera sexangula var. rhynchopetala* can be narrowed down to 0.55 mg/L - 0.60 mg/L and 0.25 mg/L -

0.30 mg/L, respectively. The sprouting indexes of *Ceriopstagal C.B. Rob.* were inconsistent with the others, so the range of their inhibition threshold concentration was considered to be 0.50 mg/L - 0.60 mg/L, respectively. Indicators were analyzed inconsistently with the other ones, so the range of inhibition threshold concentration was considered to be 0.50 mg/L - 0.60 mg/L.

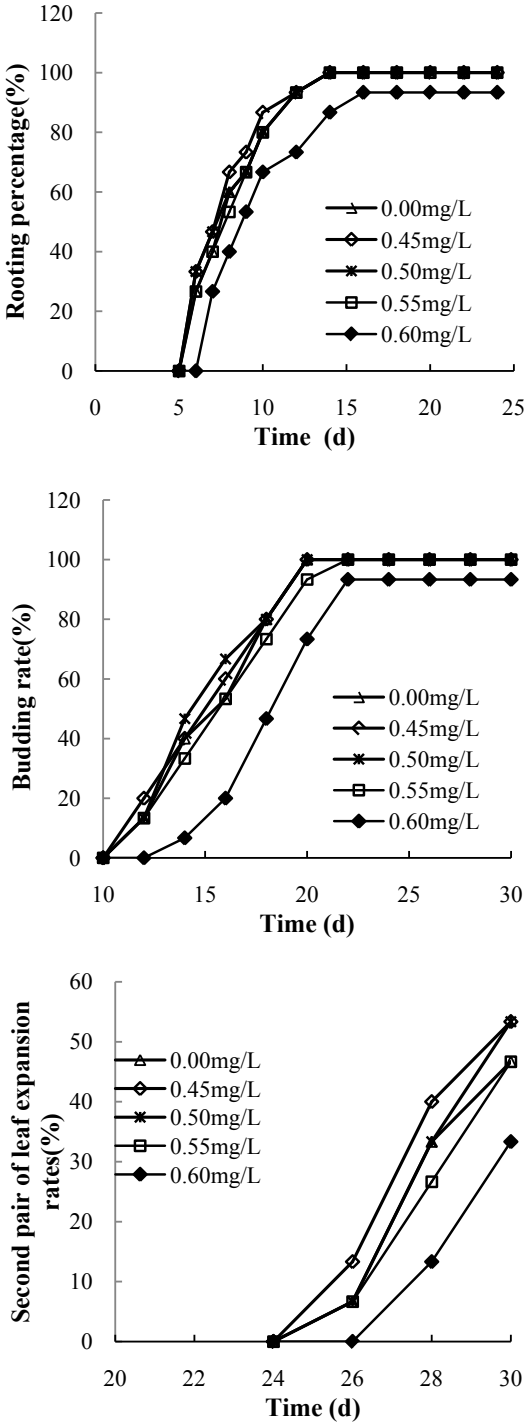

Figure 1. Statistical graph of growth indexes of *Kandeliacandel* (*Linn.*) *Druce* in strong chlorine concentrate.

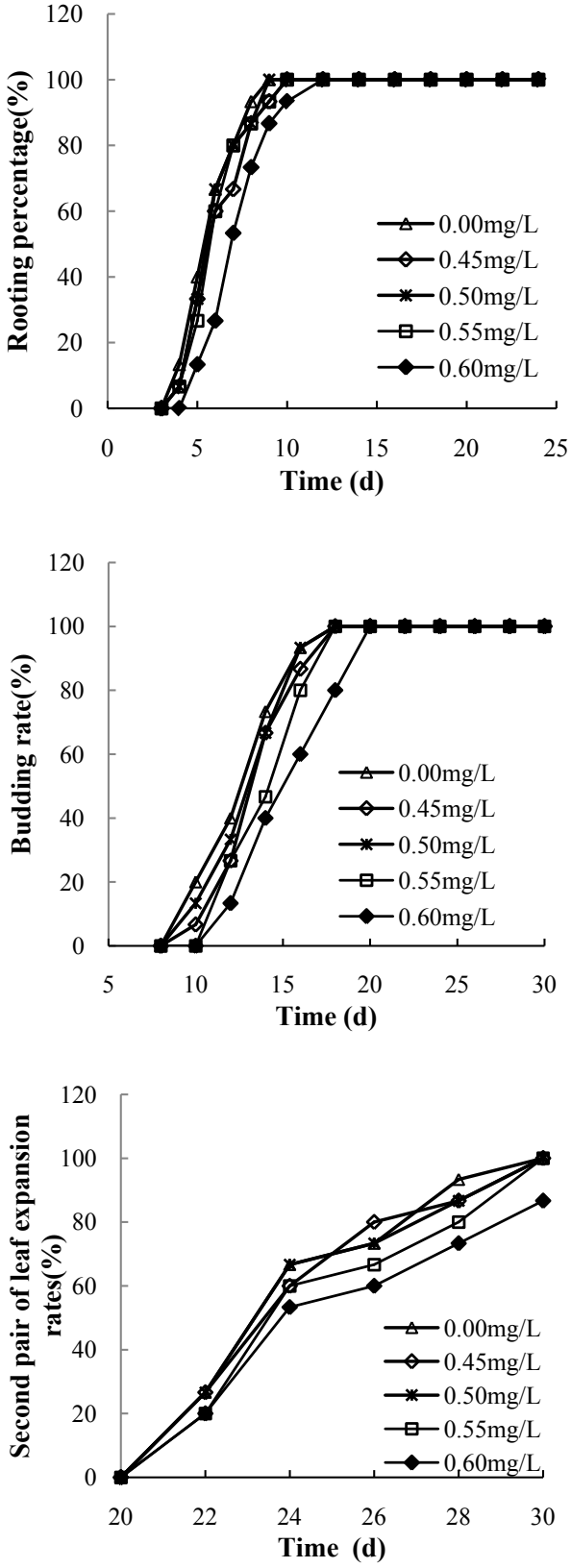

Figure 2. Statistical graph of growth indices of *Ceriopstagal C.B. Rob.* in strong chloroform.

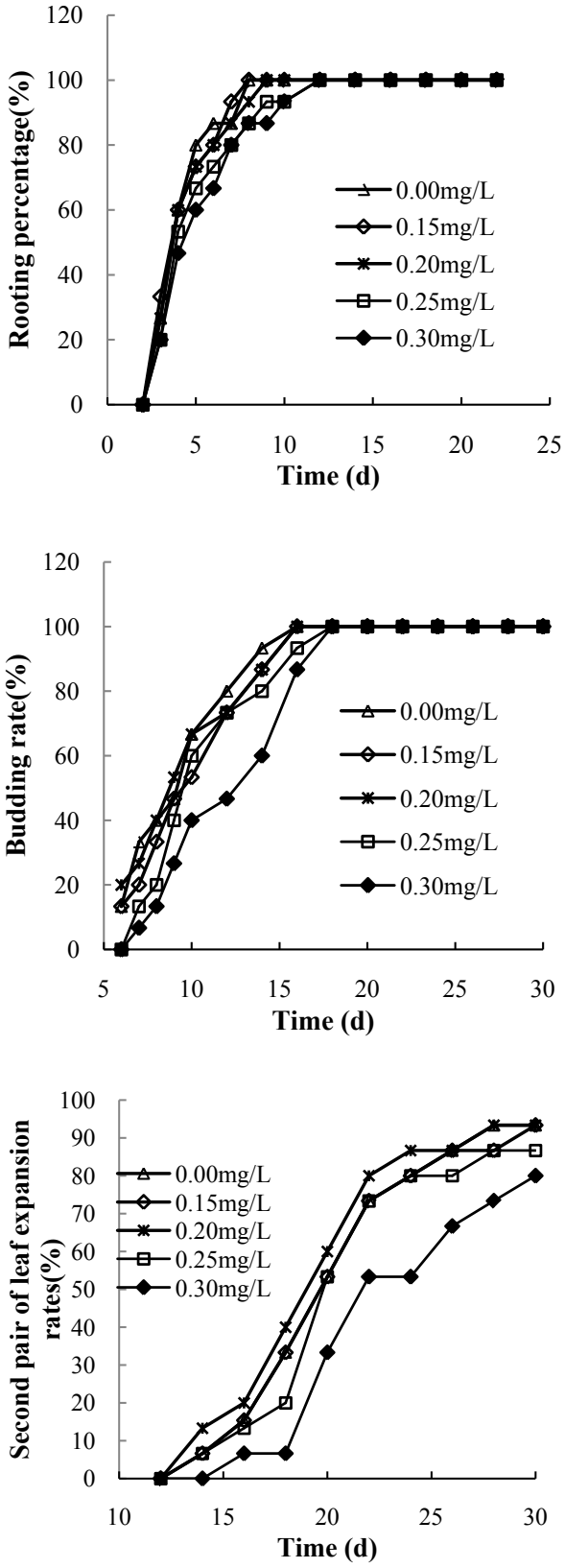

Figure 3. Statistical graph of growth indices of *Bruguiera sexangula var. rhynchopetala* in strong chlorine concentrate.

3.2. Results of One-Way Analysis of Variance (ANOVA) under Diclofenac Stress

From the results of the multiple comparison test (e.g., Table 4), it can be seen that in the strong chlorine concentrate of 0.60 mg/L, the germination time, root number and maximum root length of the embryonic axis of *Kandeliacandel* (*Linn.*) *Druce* differed significantly from those of the control group, with the significant Ps of 0.016, 0.022 and 0.015, respectively. The embryonic axis of *Ceriopstagal C.B. Rob.* in the strong chlorine concentrate of 0.60 mg/L had significant Ps of less than 0.05 for all five indicators, namely, root initiation date, root number, maximum root length and shoot growth length. In the theoretical free chlorine concentration of 0.60 mg/L in strong chlorine concentrate, the P of the five indexes, namely, the day of root sprouting, the time of germination, the number of roots sprouted, the maximum root length, and the length of shoot length growth, were less than 0.05, which were significantly different from those

Table 4. Multiple comparison test results of germination indexes of embryonic axes of three mangrove trees under strong chlorophyll stress (I indicates control group).

implicit variable	*Kandeliacandel* (*Linn.*) *Druce*			*Ceriopstagal C.B. Rob.*			*Bruguiera sexangula var. rhynchopetala*		
	(J) Theoretical free chlorine concentration (mg/L)	average difference (I1-J)	significance	(J) Theoretical free chlorine concentration (mg/L)	average difference (I2-J)	significance	(J) Theoretical free chlorine concentration (mg/L)	average difference (I3-J)	significance
root initiation date	0.450	0.267	0.774	0.450	−0.467	0.442	0.150	0.000	1.000
	0.500	0.000	1.000	0.500	−0.200	0.741	0.200	−0.200	0.799
	0.550	−0.200	0.830	0.550	−0.467	0.442	0.250	−0.800	0.311
	0.600	−1.667	0.076	0.600	−1.533*	0.013	0.300	−1.067	0.178
germination time	0.450	0.267	0.795	0.450	−0.800	0.368	0.150	−1.333	0.292
	0.500	0.400	0.697	0.500	−0.400	0.652	0.200	−0.800	0.526
	0.550	−0.400	0.697	0.550	−1.467	0.101	0.250	−2.133	0.094
	0.600	−2.533*	0.016	0.600	−2.667*	0.004	0.300	−4.333*	0.001
root growth	0.450	−0.067	0.912	0.450	−0.267	0.524	0.150	0.933	0.172
	0.500	−0.200	0.740	0.500	−0.333	0.426	0.200	1.333	0.052
	0.550	0.133	0.825	0.550	0.133	0.750	0.250	1.80000*	0.010
	0.600	1.40000*	0.022	0.600	1.13333*	0.008	0.300	2.06667*	0.003
maximum root length	0.450	−0.140	0.767	0.450	0.060	0.801	0.150	0.280	0.673
	0.500	−0.020	0.966	0.500	−0.207	0.385	0.200	0.367	0.580
	0.550	−0.007	0.989	0.550	−0.167	0.484	0.250	0.413	0.533
	0.600	1.17333*	0.015	0.600	0.65333*	0.007	0.300	1.58000*	0.019
bud length growth length	0.450	−0.033	0.677	0.450	0.080	0.412	0.150	0.307	0.607
	0.500	−0.023	0.770	0.500	0.107	0.275	0.200	−0.867	0.149
	0.550	−0.010	0.900	0.550	0.180	0.068	0.250	−0.780	0.193
	0.600	0.017	0.835	0.600	0.21333*	0.031	0.300	0.553	0.354

*Mean differences are significant at the P < 0.05 level.

of the control group. In the embryonic axes of *Bruguiera sexangula var. rhynchopetala*, in the theoretical free chlorine concentration of 0.25 mg/L of strong chlorine essence, the significance P = 0.01 for the number of roots sprouted was less than the level of significance, which was significantly different from that of the control group. While in the concentration group of 0.30 mg/L, the germination time, number of roots and maximum root length of embryonic axes of *Bruguiera sexangula var. rhynchopetala* were significantly different from the control group.

Moreover, if two or more indicators showed significant differences, it was determined that the concentration had a significant effect on embryonic axis germination. Therefore, the toxicity threshold concentrations of the by-products of the strong chlorine disinfectant were close to 0.55 mg/L, 0.55 mg/L and 0.25 mg/L for A. australis, A. angustifolia and A. aculeata, respectively.

4. Conclusion

The integration of land and sea needs to be effectively strengthened. Moreover, strictly controlling the discharge of pollutants from land-based sources, especially disinfectant pharmaceuticals into the sea, is the key issues that need to be solved urgently for the protection of mangrove forests in China nowadays. In this study, we clarified the effects of different concentrations of strong chloramines on the germination of embryonic axes of three mangrove plants through two laboratory cultivations. The results of this study showed that the inhibition threshold concentrations of the disinfectant by-products of strong chlorine concentrate ranged from 0.55 mg/L to 0.60 mg/L, from 0.50 mg/L to 0.60 mg/L and from 0.25 mg/L to 0.30 mg/L, respectively, and the toxicity thresholds of the disinfectant by-products of strong chlorine concentrate were close to 0.55 mg/L, 0.55 mg/L and 0.25 mg/L, respectively, which was lower than the average concentration of 1.183 mg/L of effective chlorine discharged from strong chlorine concentrate during the dredging of shrimp ponds in high ponds, suggesting that transient discharges of strong chlorine concentrate during dredging can have toxic effects on mangrove plants. The strength of tolerance to effective chlorine contamination varied among the three mangrove embryonic axes, specifically, *Ceriopstagal C.B. Rob.* was stronger than *Kandeliacandel* (*Linn.*) *Druce*, and *Bruguiera sexangula var. rhynchopetala* was the weakest.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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