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Effects of salinity on seedling gemination and growth of early seedlings of the *Najas indica* (Willd.) Cham.

Dang Thi Le Xuan[1,*], Phan Thi Thuy Hang[2], Ton That Phap[2], Hoang Cong Tin[3], Luong Quang Doc[2]

[1]*Department of Biology, Hue University of Education, Thua Thien Hue, Vietnam*
[2]*Faculty of Biology, University of Sciences, Hue University, Thua Thien Hue, Vietnam*
[3]*Faculty of Environmental Science, University of Sciences, Hue University, Thua Thien Hue, Vietnam*
[*]E-mails: bplexuan@gmail.com

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ABSTRACT

Najas indica (Willd.) Cham. is a freshwater submerged aquatic vegetation. *N. indica* is an annual plant. Therefore, seeds in the sediment and the survival and growth of seedlings play an important role in re-establishing new populations of the species. The purpose of this study was to determine the seed density in the sediment, examine the effect of salinity on seedling germination from seeds, and assess the impact of the salinity on the growth of early seedlings of *N. indica* under the experimental condition. The seed density of *N. indica* was highest from September to November and the lowest in May to July 2018. Seedlings of the species were observed in the range of salinity from 0–15‰. No seedling was recorded at 20‰ salinity during the experimental period. The number of seedlings, growth rate, shoot length, and the number of internodes of seedlings of *N. indica*, all had maximum values at a salinity of 5‰, while leaf length tended to decrease with increasing salinity. The study showed that the optimum salinity for seedling germination from seeds and growth of seedlings of *N. indica* species from the Cau Hai lagoon was at 5‰ salinity. The study results supply the necessary information for the protection and development of meadows of the *N. indica* species in the lagoon environment of Vietnam.

Keywords: *Najas indica*, salinity, lagoon, submerged aquatic vegetation.

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INTRODUCTION

Submerged aquatic vegetations (SAVs) are flowering plants entirely immersed in the water environment; they include both freshwater and marine species [1]. The aboveground biomass of SAVs is an ideal home for many aquatic animals [2, 3]. Shoots, branches, and leaves of SAVs reduce the speed of water flow and promote sedimentation, thereby preventing erosion of the bottom [4–6]. The SAV ecosystem is one of the most productive ecosystems on the Earth [7]. SAVs meadows are enormous carbon stores. A previous study noted that the carbon storage capacity of SAV ecosystems is 35 times higher than that of tropical rainforest ecosystems [8]. Most SAVs have both asexual and sexual reproduction; asexual reproduction is through the vegetative growth of rhizomes, while sexual reproduction produces many flowers, fruits, and seeds. Physical disturbance, herbivores, eutrophication, flooding, and silt deposition are factors affecting the distribution of SAV meadows [9], in which the change in salinity of the water environment plays an important role [10, 11]. Water salinity exceeding the tolerance limit can cause adverse effects on SAVs, such as growth stops and even death [12–14].

Najas indica is a freshwater SAV species belonging to the *Najas* genus [15, 16], but it distributes in both freshwater and brackish water environment. The species has been recorded in the estuarine and lagoon environments of India, Sri Lanka, and Burma [10, 11, 15–18]. In Vietnam, *N. indica* is widely distributed in rice fields, canals, estuaries, and lagoons. In the Cau Hai lagoon, *N. indica* had a wide distribution area and was one of the dominant species, especially in areas with low salinity < 15‰ [10]. *N. indica* was also recorded as the dominant species in areas with salinity < 10‰ of the Chilika lagoon (India) [16].

In the experimental conditions, *N. indica* shoots survived and grew at salinity from 0–15‰, in which the optimum salinity for the species growth was determined to be 5–10‰. In addition, previous studies showed that *N. indica* could absorb some heavy metals (Pb, As, Cr) [19–21]. Under oxidative stress conditions, *N. indica* had biochemical responses such as increasing proline accumulation, the activity of antioxidant enzymes or carotenoid concentrations [12, 13, 19, 21, 22]. In the natural conditions of the Cau Hai lagoon, the growing season with high biomass and coverage of *N. indica* coincides with the dry season. The *N. indica's* growth season starts in the early dry season, and the species decay begins at the end of the dry season or the beginning of the rainy season, in which the salinity was noted as a factor affecting the distribution of the species [10]. *N. indica* is an annual plant; therefore, seedling germination from seed, survival, and growth of seedlings are important for re-establishing new species' new meadows. The assessment of seed density in sediments contributes to the prediction of the possibility of re-establishing new populations. However, information about seed density in the sediment, the ability to form seedlings from seeds, and the effects of salinity on the growth of *N. indica* seedlings have not been mentioned in previous studies. For the above reasons, the purpose of the study was to (1) determine the seed density of *N. Indica* species in the sediment of the Cau Hai lagoon; (2) and examine the effect of salinity on seedling germination and growth of seedlings of the *N. indica* species under culture conditions. The study results contribute to explaining the distribution of *N. indica* meadows in the lagoon environment in Vietnam.

MATERIALS AND STUDY METHODS

Collecting samples in the field

Seed samples were collected every two months in the Cau Hai lagoon (16°19'22"N, 107°50'59"E) of Tam Giang - Cau Hai lagoon system, Thua Thien Hue province (Fig. 1). The survey was conducted from May 2018 to November 2019 with 10 sampling trips at 10 sites where *N. indica* species were present. Used a boat to go to the sampling sites located and fixed during the survey period by using Garmin GPSMAP®78 (Garmin-USA, Taiwan), at each sampling site, water environment parameters such as salinity, temperature, pH, turbidity (NTU) were determined in the field by a HORYBA U-50 Series Multi-Parameter Water Quality Meter (HORIBA Advanced Techno Co., Ltd, Tokyo, Japan). The depth of the water column was measured by a hand-held depth tool, Handy Depth Sounder Hondex PS-7 (Honda Electronics Co., Ltd, Tokyo, Japan). Seeds were collected inside three standard squares (0.5 m × 0.5 m) randomly placed inside a meadow. At each standard square, we digged a layer of sediment approximately 5 cm thick, then sieved it through a sieve with a mesh of 0.2 mm; the seeds with a little sediment retained on the sieve are placed in a plastic bag with a waterproof label. Seed samples were brought to the laboratory for quantification. Seeds were manually counted through observation under a stereomicroscope (JSZ5B-Genius, China).

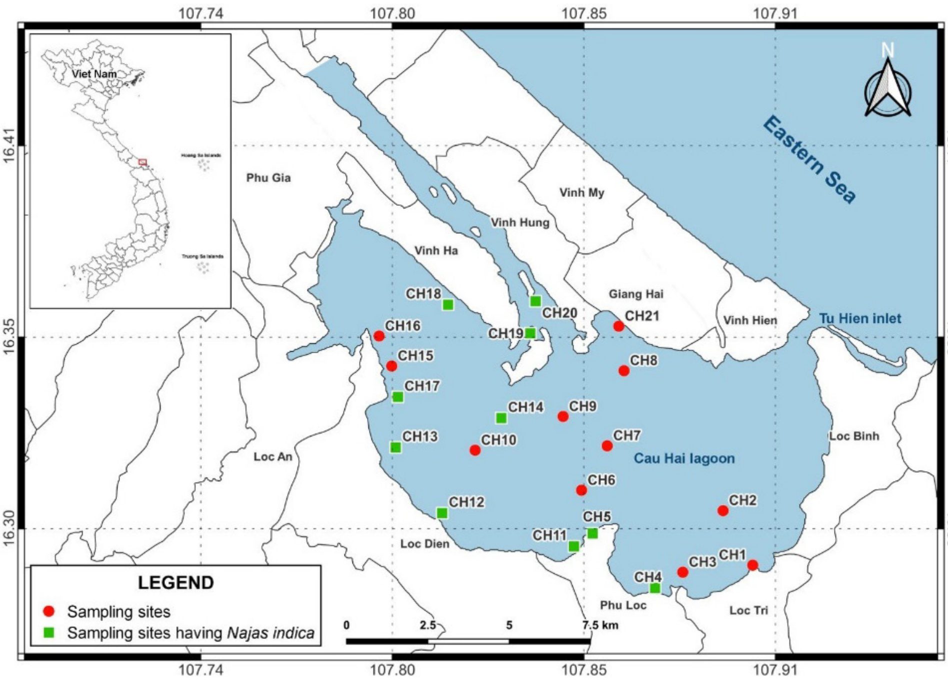

Figure 1. The map of sampling sites in the Cau Hai lagoon

The Experiment to examine the effect of salinity on seedling germination and growth of early seedlings of the *Najas indica*

Preparation of experimental materials

Seeds and sediments used for the experiment were collected at the same site (depth of 1.1 m, salinity of 8.7‰), where *N. indica* meadows densely grew in the Cau Hai lagoon. Inside the meadows, the surface sediments were sieved to collect the seeds. Seeds and some sediments used as media were brought to the laboratory. In the laboratory, seed samples were washed again with tap water, then the seed samples were placed in a pectri dish and on a stereomicroscope, observed and selected good seeds. Using tap water and sea salt [23] prepared the water environment in six glass tanks (70 cm × 40 cm × 44 cm) respectively, with six salinity levels (0‰, 5‰, 10‰, 15‰, 20‰, and 25‰). Sediments used for the experiment were sieved through a sieve with a mesh of 0.2 mm to remove seeds of *N. indica*, gravel, and plant and animal debris. Then they were placed in plastic boxes sized 26 × 20 × 18 cm.

Experiment design

The experiment was designed in an outdoor experimental area (with a transparent plastic roof) of the Faculty of Environment, University of Science, Hue University. The experimental period was conducted for 28 days (from March 20th to April 18th, 2020). In each box, three hundred seeds were sown on the sediment surface. Every three boxes (corresponding to three repetitions) were placed in a glass tank with a pre-prepared water medium. Each glass tank was fixed with a circulating water filter, and the water level was maintained at 25 cm during the experimental period.

Experimental monitor and data collection

From the 7th day of the experimental period, the shoot length of seedlings was measured at a frequency of one every three days; and the seedlings' growth rate was calculated by the formula (1):

$$G = \frac{L_{i+1} - L_i}{t} \quad (1)$$

in which: G: the growth rate of seedlings (mm/day); L_i: the shoot length of seedlings in previous measurement; L_{i+1}: the shoot length of seedlings in the next measurement (mm); *t:* number of days between each measurement (three days).

The number of seedlings and various morphological characteristics such as leaf length, internode length, internodes, and branches per seedling at different salinities were determined at the end of the experiment. Salinity and environmental factors, including pH and turbidity of the water, were checked every 3 days by HORYBA. The experiment was conducted in outdoor conditions with a transparent roof. Therefore, the air temperature and light intensity depending on nature, was from 25.5–28.2ºC, and the light intensity measured at noon reached 32.7–34.9 lux using Handy Lux Meter (Testo 540 Handy Lux meter, China).

Data analysis

Data were analyzed using IBM SPSS Statistics Version 20 software. Before analysis, all variables were examined for normal distribution by the Shapiro-Wilk Test. The significant difference of variables was tested by Friedman ANOVA and Wilcoxon matched-pairs; or Repeated Mesures ANOVA or One-Way ANOVA and post hoc test by using Duncan. The correlation between the variables tested by Spearman's or Pearman's method depends on the variables' normality.

RESULTS AND DISCUSSIONS

Seed density of *Najas indica* in Cau Hai lagoon

The survey showed that the average seed density of *N. indica* in the sediments of Cau Hai lagoon was 2,765 ± 790 seeds.m^{-2} and the seed density changed spatially and temporally. The study recorded that the seed density of *N. indica* had a significant difference in the months (*FA*, $\chi^2_{(9,\, n\, =\, 10)} = 31.9$, $p < 0.0001$). The seed density of the species was lowest in May 2018 and July 2018, respectively, 94.5 ± 21.1 seed.m^{-2} and 83.5 ± 12.7 seed.m^{-2}. The seed density of the species increased rapidly in September 2018 (7,811.6 ± 4,893.9 seed.m^{-2}) and peaked in November 2018 (7,831.2 ± 4,646.8 seed.m^{-2}. Then the seed density decreased in January 2019 (4,048.6 ± 2,646.8 seed.m^{-2}) and continued to decline in March 2019 (581.5 ± 158.2 seed.m^{-2}). The seed density of the species increased again in May 2019 (3,211.1 ± 2,213.1 seed.m^{-2}), then it gradually decreased in July 2019 (1,905.9 ± 1,262.1 seed.m^{-2}), September 2019 (1,169.3 ± 745.5 seed.m^{-2}), and November 2019 (914.9 ± 579.4 seed.m^{-2}), (mean ± SE, *n* = 10) (Fig. 2A). Thus, the seed density of *N. indica* in 2018 was higher than in 2019. The seed density of *N. indica* in the sediments began to increase when the *N. indica* population reached reproductive maturity with the appearance of decay signs. At this time, the fruits ripened and released seeds, and decay and breaking of the shoot promoted the release and storage of seeds in the sediment. Therefore, the highest seed density in the sediment was the event followed by the peak in biomass of the species. For example, the biomass of *N. indica* peaked in July 2018 [10], and the seed density in the sediments also peaked from September to November 2018. The high seed density of the species could be maintained for several months; then, the seed density rapidly decreased when the *N. indica* populations decayed completely. The reason was that during the decay period of *N. indica* populations, the "seed bank" was not provided with a source of seeds. On the other hand, the seeds were eaten by seed-eating animals, which was a common event of SAVs [24–26].

According to space, the survey results recorded that there was a significant difference in the seed density at the sampling sites (*FA*, $\chi^2_{(9,\, n\, =\, 10)} = 50.94$, $p < 0.0001$). The seed density of *N. indica* species was a maximum of CH4 (16,739.6 ± 5,662.2 seed.m^{-2}), CH11 (6,492 ± 2,687.6 seed.m^{-2}), and CH12 (1,368.5 ± 1,068.4 seed.m^{-2}). The seed density was lowest at CH19 (103.3 ± 31.7 seed.m^{-2}), (mean ± SE, *n* = 10) (Fig. 2B). The spatial change of *N. indica* seed density in the sediment also corresponded to the species' spatial change of cover and biomass. Our survey found that sites mono-species meadows of *N. indica* thrived and grew with high biomass and coverage (CH4, CH11, CH12, and CH18) [10] that had high seed density in the sediment. In contrast, the sites of *N. indica* growing together with *Halophila beccarii* formed multi-species meadows with low biomass and coverage (CH19, CH20) [10] were also sites to have low seed density (CH19, CH20) (Fig. 2B).

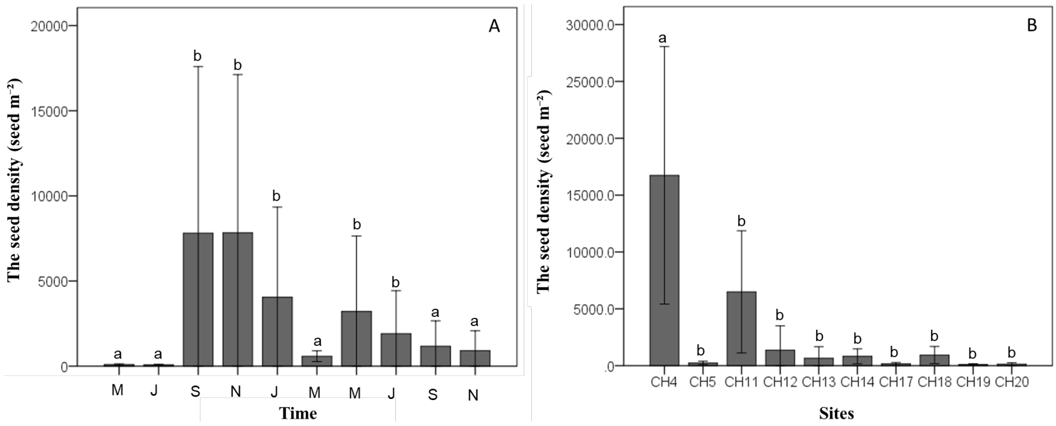

Figure 2. Seed density of *Najas indica* in the sediments in months (A); and at sites (B) in the Cau Hai lagoon. Symbols *a* and *b* represented the difference in seed density between the months and the sampling sites

Effects of the salinity on seedling germination and growth of the seedlings of *Najas indica*

The number of seedlings of Najas indica

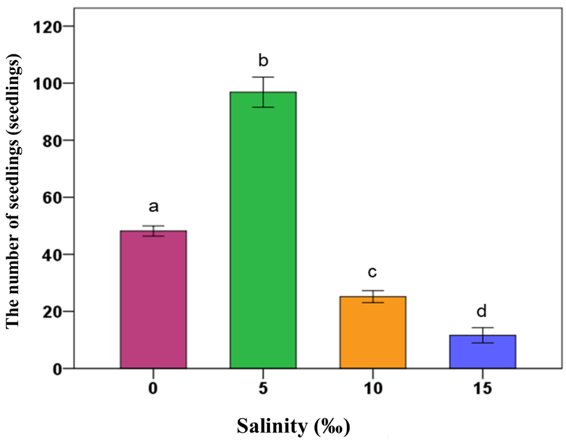

Figure 3. The number of *N. indica* seedlings (seedlings) in salinity levels after 28 days, (mean ± SE, *n* = 3)

Experimental results found that the salinity significantly affected the number of seedlings formed from seeds in the sediment of the *N. indica* species. The species' seedlings were observed at salinities of 0‰, 5‰, 10‰, and 15‰; but no seedlings were found at salinity of 20‰ during the experimental period. The number of seedlings was significantly different in salinities (AVOVA, $F = 94.9$, $p < 0.0001$) (Fig. 3).

The experimental results showed that the salinity had an apparent effect on the seedling germination from seeds in the sediments of the *N. indica*. This effect was clearly expressed through the difference in the number of seedlings at the salinity levels. The number of seedlings was highest at the salinity of 5‰ (104.7 ± 5.5 seedlings); it significantly decreased at salinity of 10‰ (32.3 ± 3.8 seedlings), and the lowest was recorded at 15‰ (13 ± 3.8 seedlings) (Fig. 3), (mean ± SE, *n* = 3). It suggested that the salinity of 5‰ was the most suitable condition for germination and seedling formation of the *N. indica* species.

The growth rate of seedlings of N. indica

One-way ANOVA statistical analysis showed that the growth rate of *N. indica* seedlings was significantly different in the experimental salinities (ANOVA, $F = 8.3$, $p < 0.0001$). The growth rate of seedlings was highest at the salinity of 5‰ (3.2 ± 0.5 $mm.day^{-1}$) and significantly decreased at salinity of 0‰ (1.9 ± 0.3 $mm.day^{-1}$) and 10‰ (1.7 ± 0.2 $mm.day^{-1}$). The lowest growth rate of seedlings was recorded at 15‰ (1.0 ± 0.2 $mm.day^{-1}$), (mean ± SE, $n = 27$) (Fig. 4). Our experimental results showed that the growth rate of seedlings of the *N. indica* species was maximal at the salinity of 5‰ and 0‰; these values were significantly lower in salinity from 10–15‰, and the lowest was at 15‰ salinity. Thus, the seedling growth of *N. indica* was suitable for common salinity conditions (0–5‰).

The salinity clearly affected the morphological characteristics of seedlings of *N. indica*, such as seedling length, the number of internodes, and leaf length (ANOVA, $p < 0.0001$). Seedling length and the number of internodes were maximal at the salinity of 5‰ (82.7 ± 2.7 mm; 5.5 ± 0.2 internodes) and lowest at the salinity of 15‰ (25.7 ± 2.6 mm; 2.6 ± 0.2 internodes) (Figs. 5A, 5B). Leaf length was longest at the salinity of 0‰ (25.4 ± 0.8 mm), leaf length tended to decrease with increasing salinity from 5‰, 10‰, and 15‰ gradually (respectively, 23.3 ± 0.7 mm; 21.5 ± 0.7 mm; 21.6 ± 0.6 mm), (Fig. 5C), (mean ± SE, $n = 30$).

Our experiments showed that the seedling of *N. indica* had a relatively low salinity requirement (5‰) (Fig. 6). In the experimental condition, the optimum salinity for growth of *N. indica* shoots was 5–10‰ [27]. Thus, compared with the adult shoots, the seedlings of the species had significantly lower salinity requirements for growth. These results were consistent with records of the species in the natural environment. In the Cau Hai lagoon, seedlings of *N. indica* were observed in the early dry season when the rainy season had just ended with water salinity still low. For example, the seedlings were observed in March at a CH4 salinity of 7.7‰. The *N. indica* populations grew abundantly at salinity from 4–15‰, and the biomass of the species tended to increase with water salinity [10].

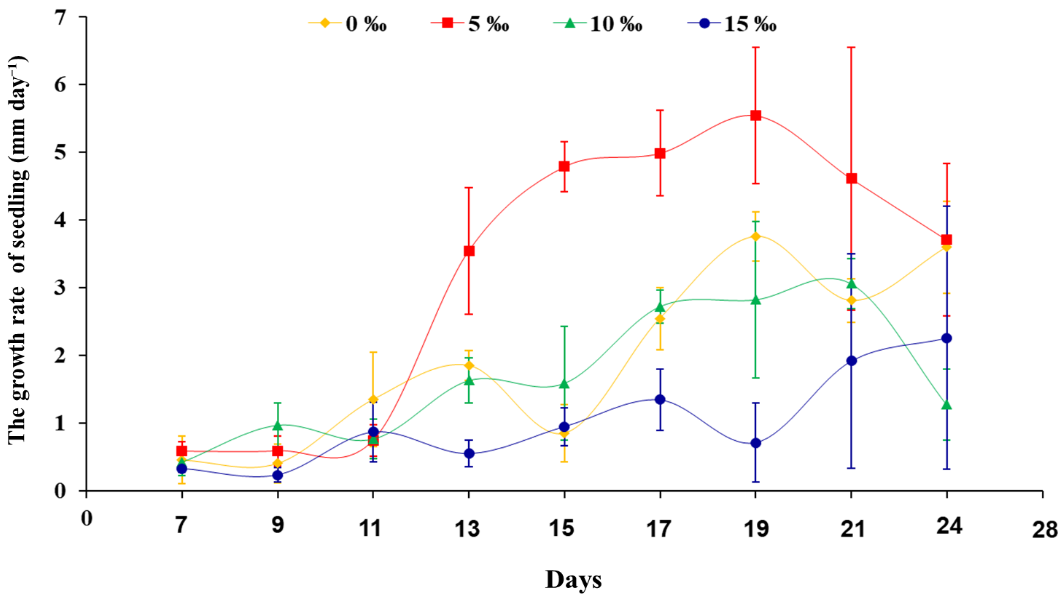

Figure 4. The growth rate of seedlings of *Najas indica* in the experimental period, (mean ± SE, $n = 27$)

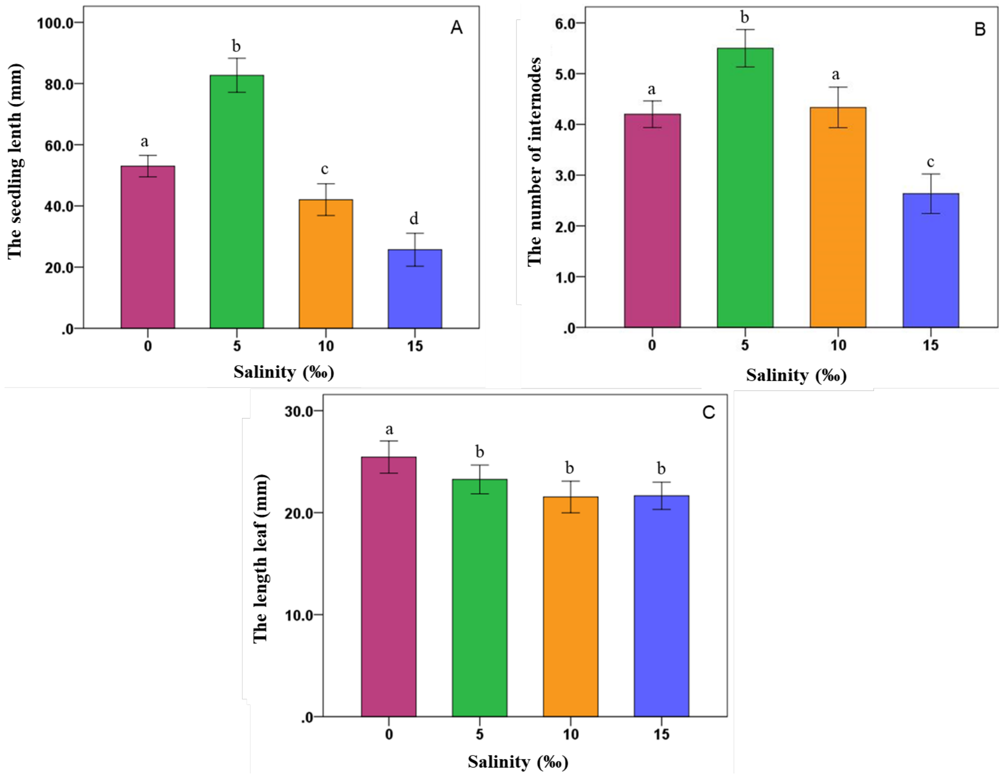

Figure 5. The morphological characteristics of seedlings of *Najas indica* in experimental salinity levels after 28 days. Seedling length (A); number of internodes (B); and leaf length (C), (mean ± SE, *n* = 30)

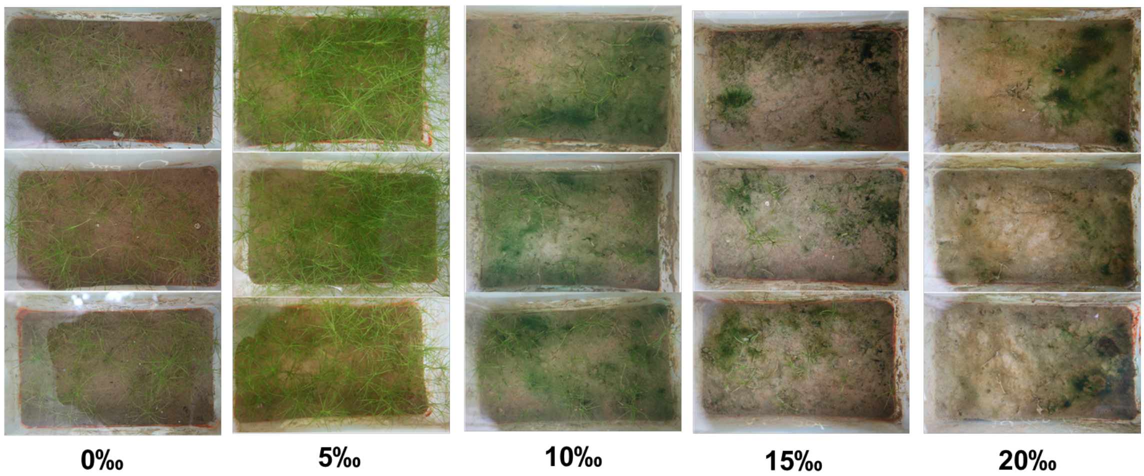

Figure 6. The seedlings of *Najas indica* in the salinity levels after 28 days

CONCLUSION

The seed density of *N. indica* in the sediments of the Cau Hai lagoon changed in space and time. The seed density of the species peaked from September to November 2018 at sites CH4 and CH11. Seedlings presented in the salinity between 0‰ and 15‰. They reached the highest at salinity of 5‰, but no seedling was observed at 20‰. The leaf length of seedlings of the *N. indica* species tended to decrease with increasing salinity, while the seedling length and number of internodes had a maximum value at salinity of 5‰. It suggested that the optimum salinity for seedling germination and the growth of seedlings of *N. indica* was 5‰. The research results complemented information about the sexual reproduction of the *N. indica* species that contributed to explaining the distribution records of the species in the natural environment.

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