

Water uptake and salt accumulation under *Rhizophora stylosa* seedling planted in controlled salinity and inundation levels

Endah D. Hastuti, Munifatul Izzati, Erma Prihastanti

Department of Biology, Faculty of Science and Mathematics, University of Diponegoro, Tembalang, Semarang 50275, Central Java, Indonesia. Corresponding author: E. D. Hastuti, endah_pdil@yahoo.com

Abstract. Salinity and inundation dynamics are two of the environmental condition frequently found in mangrove ecosystems that are suggested as the drivers for mangrove vegetation. This research aimed to study water uptake, salt accumulation and the impact of controlled salinity and inundation level on the water uptake and salt accumulation under *Rhizophora stylosa*. The research was carried out through laboratory experiments which involved five salinity settings, including 15 ppt, 20 ppt, 25 ppt, 30 ppt and 35 ppt and three inundation settings, including 10 cm, 15 cm and 20 cm. Triplication was performed to obtain the range of data deviation. The experiment was carried out for 100 days with periodic observation. Two data, including water uptake and salt accumulation, became the main focus of the research, accompanied by univariate statistics as the analysis method. The research found that water uptake fluctuates over period by an increasing trend. Inundation levels were proven to impact the differentiation of water uptake rate significantly. The highest water uptake occurs in the highest inundation. In the meantime, salt was accumulated in the planting media, which simultaneously affected differentiation by salinity setting and inundation level. The highest salt accumulation resulted from 35 ppt salinity and 15 cm inundation treatment. These findings suggest that *R. stylosa* performs physiological adaptation to cope with environmental disturbances, such as increasing water uptake and excluding salt content from the water.

Key Words: accumulation, adaptation, exclusion, physiology, salt, uptake.

Introduction. Environment-driven stress has been a mystery in relation to mangrove vegetation. Many environmental parameters are suggested as the drivers of stress in the mangrove, with the additional complexity of the interaction between parameters (Barraclough et al 2020). As a result, different effects of a parameter are often found on the same mangrove species (Cerón-Souza et al 2014; Peel et al 2019).

Mangrove plants typically grow in coastal areas where environmental quality fluctuates continuously (Seiler et al 2015; Xia & Jiang 2016). Since mangrove inhabits coastal areas, environmental quality dynamics becomes typical disturbance for mangrove vegetation (Zhang et al 2016). This disturbance is suggested to induce dynamic stress in mangrove plants (Fricke et al 2017). However, mangrove vegetations are adaptive to environmental pressure, especially coastal dynamics. Typically, mangroves would respond the environmental pressure through certain physiological responses (Lv et al 2019). In order to grow appropriately, mangrove requires optimum environmental condition. However, since the coastal area has dynamic environmental conditions, mangrove vegetations are frequently faced with unfavourable condition. There are various stress drivers in mangrove vegetation, such as temperature (cold/heat), salinity, inundation, siltation, etc. (Sarker et al 2019; Wang et al 2021). Each parameter stimulates a different response in mangrove plants.

Salinity or salt concentration in the water is one of the mangrove's most considerable stress drivers (Peters et al 2020; Rahman 2020). Mangrove is known to inhabit the saline environment, such as coastal areas and estuaries. However, some research found that mangroves have better growth in the freshwater environment. Mangrove is known to absorb fresh water while inhabiting saline environment (Ladd &

Sachs 2015). Therefore, most mangrove species are found to excrete salt content (Yan et al 2017).

Flooding is also frequent in the mangrove ecosystem (Munji et al 2014). Due to tidal activity, mangrove plants are flooded for a certain period each day (Kumbier et al 2021). However, in some sections, mangrove plants are permanently inundated (Hilmi et al 2022). Inundation could occur at various levels, depending on the season and relative position to the coastline.

Mangrove plants have the ability to cope with dynamic environmental conditions through particular physiological adaptations (Naskar & Palit 2015). Therefore, mangrove could survive despite the strong disturbances it achieves. The change in water uptake rate is considered as one of the mechanisms owned by mangrove vegetation to cope with environmental disturbance, especially related to salinity and inundation regime (Bathmann et al 2020).

Even though mangrove inhabits a saline environment, it is still unknown whether water is absorbed as a whole or filtered in advance. Logically, if the water is absorbed as a whole, there should be no salinity changes in the remaining water. Thus, the stress level undergone by mangroves should remain constant. However, if filtering is performed prior to the absorption, water salinity should increase, causing an alteration of environmental pressure. This research aimed to study the water uptake, salt accumulation, and the impact of controlled salinity and inundation level on the water uptake rate and salt accumulation under *Rhizophora stylosa* seedlings.

Material and Method

Research design. This research was designed as a laboratory experiment in a greenhouse. Mangrove *Rhizophora stylosa* was planted in drums/buckets with designed settings. Buckets with an approximate volume of 50 L were filled with soils from actual mangrove forests to a height of 30 cm. Next, water with the designed salinity was added to the designed inundation level.

Experiment design. The experiment was designed as a 5 x 3 factorial, involving water salinity and inundation level as research factors (independent variables), resulting in 15 experiment arrangements. Salinity levels applied in the treatment were 15 ppt, 20 ppt, 25 ppt, 30 ppt and 35 ppt. While inundation levels applied were 10 cm, 15 cm, and 20 cm. Each treatment was replicated as many as three times.

Data collection. Data collection was carried out between August and November 2022 for a total of 100 days. The observation was carried out periodically, with an average interval between observations of ten days. The observation was carried out consecutively for water salinity and uptake volume. Salinity was measured using Horiba Water Quality Checker. Water uptake was assessed based on the volume needed to refill the bucket to the designed volume.

Statistical analysis. Data analysis was carried out using a univariate statistic, including salinity and inundation level as the factors and water uptake rate and salt accumulation as the dependent variables.

Results. The research found that water uptake by mangroves varied by treatment and time. Fluctuation of water uptake was found in all the treatments. Based on the analysis result, the highest water uptake was observed in the combination of 20 ppt (salinity) + 20 cm (inundation). In comparison, the lowest uptake was observed in the combination of 35 ppt (salinity) + 10 cm (inundation).

The trend of water uptake by *R. stylosa* seedlings is shown in Figure 1. Water uptake by *R. stylosa* shows an identical trend for all treatments. Water uptake tends to increase in the early period, then decrease in the middle and increase again in the end.

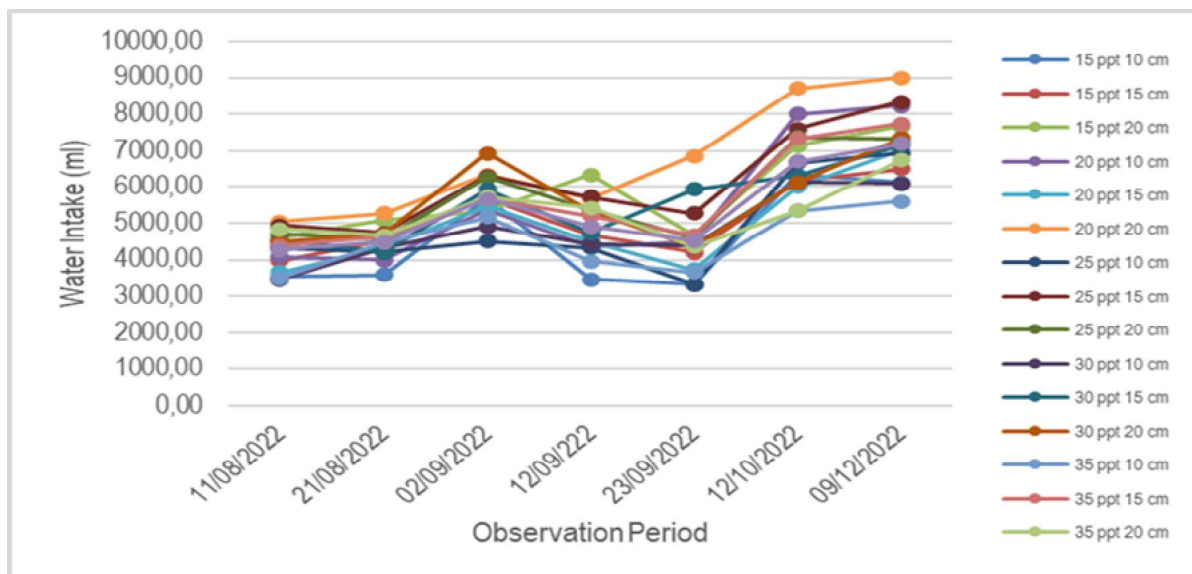


Figure 1. Trends of periodic water uptake by *R. stylosa* planted in different salinity and inundation settings.

The statistical analysis showed that salinity and inundation levels did not have significant simultaneous impact on water uptake by *R. stylosa*. The analysis showed F value of 1.049 with probability of 0.437. Significant partial effect was obtained from inundation treatment. Based on statistical analysis result, the F value was 3.540 with probability 0.042. Significant difference of water uptake was observed between treatments with inundation level 10 cm and 20 cm. Detailed comparison of total water uptake between treatments is shown in **Error! Reference source not found.**

Table 1
Total water uptake by *Rhizophora stylosa* under different salinity and inundation settings

Salinity	Inundation			Average by salinity
	10 cm	15 cm	20 cm	
15 ppt	32,073.3±8,494.5	35,713.3±7,950.0	40,806.7±9,590.1	36,197.8±8,441.9
20 ppt	38,550.0±6,620.6	34,700.0±7,777.1	46,876.7±1,149.0	40,042.2±7,447.3
25 ppt	34,300.0±5,026.9	42,910.0±2,225.8	40,193.3±10,830.6	39,134.4±7,170.2
30 ppt	33,770.0±5,551.7	38,793.3±6,985.9	39,283.3±8,399.5	37,282.2±6,672.9
35 ppt	31,713.3±8,849.4	39,616.7±1,939.3	37,103.3±5,675.3	36,144.4±6,387.5
Average by inundation	34,081.3±6,492.0	38,346.7±5,922.2	40,852.7±7,495.0	37,760.2±7,101.7

Observations of salt accumulation showed a trend of increasing salinity in all treatments. However, in-depth inundation analysis showed that the accumulated salt content fluctuated. Treatment with 15 ppt salinity showed the lowest salt accumulation over time, although the position alternated between the three inundation levels. Initially, the highest salt accumulation was observed in 35 ppt (salinity) + 20 cm (inundation). However, from the third observation to the last, it was taken over by 35 ppt (salinity) + 15 cm (inundation). Figure 2 shows the trend of time salt accumulation under *R. stylosa* of all treatments.

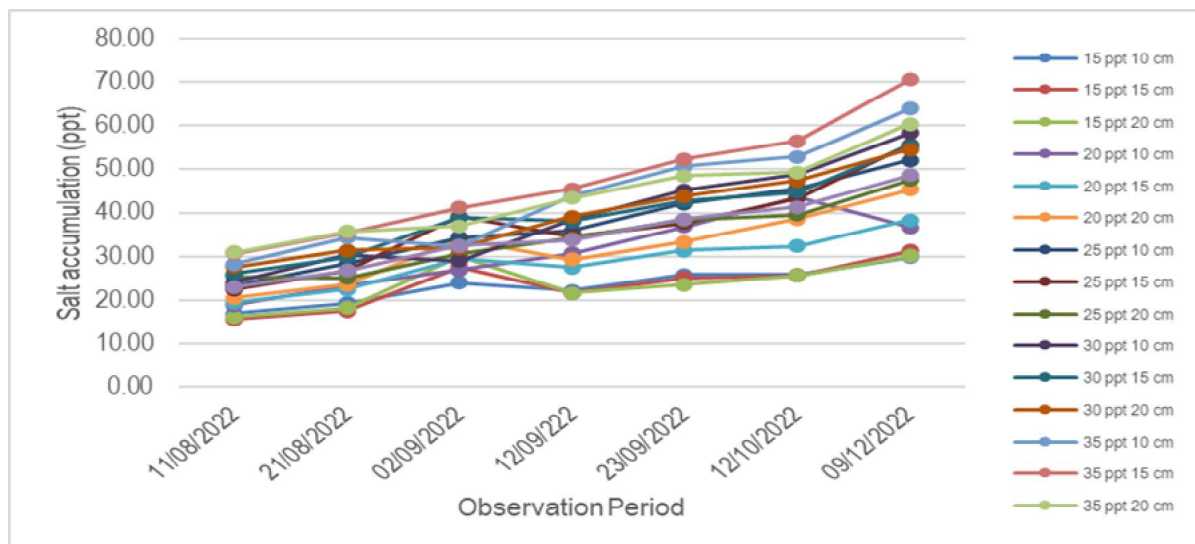


Figure 2. Trends in salt accumulation level under *R. stylosa* planted in different salinity and inundation levels.

Final salt accumulation. Figure 2 shows that the salt accumulation rate under *R. stylosa* differed. As a consequence, the final salt concentration was different between treatments. Statistical analysis was performed for the final salt concentration. The univariate test showed a significant partial effect of salinity and a simultaneous effect of salinity and inundation level on the salt accumulation under *R. stylosa* plants. The analysis resulted in an F value of 5.383 with a probability of 0.000 for simultaneous effect, while the partial effect of salinity showed an F value of 17.741 with a probability of 0.000. Meanwhile, the partial effect of inundation showed no significant difference. Detailed final salt accumulation under *R. stylosa* for each treatment, along with the post hoc analysis result, is shown in Table 2.

Based on the results shown in Table 2, salinity plays a dominant role in salt accumulation. While combined with the inundation level, the distinct difference in salt accumulation was obtained from the lowest salinity (15 ppt) and highest salinity (35 ppt) treatment. However, while observed partially, a distinct difference was observed from 25 ppt. Further analysis was carried out with correlation. The result showed a significant positive correlation between water uptake rate and salt accumulation under *R. stylosa*. Statistical analysis showed a correlation coefficient of 0.372 ($p = 0.012$), suggesting that the correlation is significant regardless the weak correlation index between both parameters. The indices suggest that the fluctuation of one parameter (either salt accumulation or water uptake) was not a random occurrence or independent from each other, but undeniably related to the other parameter's fluctuation. As the result implies, there is approximately 37.2% of salt accumulation which is due to water uptake process, while the another 62.8% is due to other drivers.

Table 2
Final salt accumulation in the water under *Rhizophora stylosa* planted in different salinity and inundation levels

Salinity	Inundation			Average by salinity
	10 cm	15 cm	20 cm	
15 ppt	29.9±15.5 ^a	31.3±7.1 ^a	30.2±6.9 ^a	30.5±9.2 ^p
20 ppt	36.7±11.2 ^{ab}	38.3±8.3 ^{ab}	45.4±2.2 ^{abc}	40.1±8.1 ^{pq}
25 ppt	52.2±7.8 ^{abc}	55.7±5.3 ^{abc}	47.6±14.5 ^{abc}	51.8±9.3 ^{qr}
30 ppt	58.3±8.4 ^{abc}	55.4±12.3 ^{abc}	54.6±7.4 ^{abc}	56.1±8.5 ^r
35 ppt	63.9±14.7 ^{bc}	70.5±5.8 ^c	60.5±5.8 ^{bc}	64.9±9.5 ^r
Average by inundation	48.2±16.7	50.2±16.0	47.6±12.7	48.7±14.9

Note: similar letter in different cells (combined treatment), rows (average by salinity), columns (average by inundation) indicates insignificant difference.

Discussion. This research found that even though the rate was different, mangrove tended to have typical trend of water uptake. Water uptake rate tended to increase over time. The variation of water uptake rate shows that mangrove experienced different level of pressure. While the similarity of periodic trend shows that mangroves in all treatments were developing.

Plant water uptake rate is determined by various factors, including internal and external. Internal factor is related to plants' condition, such as growth level and morphological features, such as the number of branch and leaf abundance (Schwendenmann et al 2014; Vetterlein & Doussan 2016). However, this research used mangrove seedlings at the early stage, where morphologic development is still similar. The external factor is related to the growth environment's condition, such as the ambient temperature, salinity and water level (Kotagiri & Kolluru 2017; Yang et al 2012). Referring to the finding of this research, several factors are suggested to affect the trend of water uptake rate by *R. stylosa* seedlings, including mangrove growth, ambient temperature dynamics and salinity-induced stress. As mangrove grows, the need for water for its metabolism is increased (Pérez-Montaña et al 2014). It is reflected by the increasing uptake of water shown in the observation. However, the difference in mangrove's growth rate could affect the later differentiation of water uptake rate. The research was applied to mangrove seedlings, expected to hinder the bias caused by the mangrove's initial condition.

Based on the results of this study, there is a possibility that there was a decreasing ambient temperature in the middle of the experiment. It is shown by the decreasing uptake rate of water by mangroves. Ambient temperature plays an important role in plant's metabolism, especially in the water uptake rate (Yang et al 2018). During the warm days, a higher evaporation rate stimulates the increase of metabolism rate, causing water uptake rate to increase (Zarebanadkouki et al 2013). Thus, an appropriate water supply is needed to keep up with the increased metabolism. On the contrary, during calm days, the metabolism rate is reduced, causing a decrease in the water uptake rate.

Referring to the result, water uptake was only affected significantly by inundation level. Higher inundation levels promoted a higher uptake rate. The high inundation level can reach stilt roots above the substrate and increase the area of water absorption. *R. stylosa* has a stilt root system that appears at the base of the stem or lower branches in a hanging position above the substrate and curves towards the substrate (Srikanth et al 2016). However, the interesting fact is the effect of salinity. Statistically, salinity has no significant effect on water uptake. These results indicate that *R. stylosa* has a wide range of salinity tolerance. According to Sivasankaramoorthy (2012) variation in the salinity required for optimal growth of *R. stylosa* varies from 10 to 50‰. Meanwhile, *R. stylosa* has a mechanism for regulating salt levels by accumulating salt in the plant environment through ultra-filtration of the roots before being absorbed, so it does not affect the water volume absorbed (Basyuni et al 2014). However, there is a tendency for the highest water absorption at a salinity of 20 ppt due to the highest growth rate of *R. stylosa* at a salinity of 20 ppt, which stimulates the highest water absorption. This is supported by the research of Aziz & Khan (2001) which states that the weight and height growth of *R. stylosa* are optimal at a salinity of 20 ppt.

Salt accumulation under *R. stylosa* suggests that water is not absorbed as a whole. Water is filtered in the roots before being absorbed and transported to the leaves (Krishnamurthy et al 2014; Noor et al 2015). Thus, it could be suggested that the salt concentration of water being absorbed is reduced. Consequently, salt is accumulated in the water, increasing its concentration in the environment.

Filtering chemical content is a mechanism available in plants as a protective mechanism from unsuitable environmental conditions (Keiluweit et al 2015). Plants' defence against environmental pressure in the root area is expressed in various anatomical and physiological adaptations. Mangrove roots are proof of anatomical adaptation in mangroves to cope with the inundated environment. However, aside from the root modification, a certain physiological process also occurs, such as the change of its pore size.

The impact of inundation level in promoting salt accumulation was insignificant. Inundation level showed its role in promoting salt accumulation, although statistical analysis did not show its significance. A non-linear trend was found, where the treatment with 15 cm inundation and salt accumulation was highest compared to the 10 cm and 20 cm. In the meantime, the impact of salinity on salt accumulation was evident, where a linear trend was found. This suggested that under 15 cm inundation, *R. stylosa* had the most efficient process of excluding salt during water uptake.

Salt accumulation was more influenced by salinity treatment. The higher the salinity, the higher the salt accumulation. This is because *R. stylosa* has the ability to filter salt water before it is absorbed into the roots so that salt accumulates in the growing environment. According to Kim et al (2016) *R. stylosa* can grow even in saline water, and the salt level in its roots is regulated within a certain threshold value through filtration. The root possesses a hierarchical, triple-layered pore structure in the epidermis, and most Na⁺ ions are filtered at first and the second sublayer of the outermost layer. The significance of the correlation between water uptake and salt concentration proves this synthesis.

Conclusions. Salinity and inundation levels were proven to significantly impact mangrove physiological processes expressed by water uptake and salt accumulation. Although the water uptake dynamics is dominantly related to mangrove growth, the inundation level was proven to affect the differentiation significantly. On the other side, salt was also accumulated in the water, which rate was significantly affected simultaneously by salinity and inundation settings, suggesting that *R. stylosa* responded to the environmental pressure by performing salt exclusion prior to the water uptake process.

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Conflict of interest. The author declares that there is no conflict of interest.

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Authors:

Endah Dwi Hastuti, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Prof. Sudarto street, No. 13, Tembalang, District of Tembalang, Semarang City, Central Java, Indonesia 50275, e-mail: endah_pdil@yahoo.com

Munifatul Izzati, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Prof. Sudarto street, No. 13, Tembalang, District of Tembalang, Semarang City, Central Java, Indonesia 50275, e-mail: Munifatul_Izzati@yahoo.com

Erma Prihastanti, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Prof. Sudarto street, No. 13, Tembalang, District of Tembalang, Semarang City, Central Java, Indonesia 50275, e-mail: eprihast@yahoo.co.id

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