

EFFECTS OF SEAWATER INTRUSION ON SOIL SALINITY, CHANGES
OF SOIL CHEMICAL PROPERTIES AND RICE CROP YIELD IN
AGRICULTURAL SOIL



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Thesis	Effects of seawater intrusion on soil salinity, changes of soil chemical properties and rice crop yield in agricultural soil
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ABSTRACT

In the first experiment, from November 2021 to April 2022, the Bang Pakong River's water quality was investigated. Thirteen sampling sites provided the water samples, which were then subjected to analyses for pH, electrical conductivity (EC), salinity, calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), chloride (Cl^-), sulfate (SO_4^{2-}) and sodium adsorption ratio (SAR). The concentration of chloride in the river and sea was used to compute the seawater mixing ratio. Among the collection months, February 2022 recorded the highest pH value (7.56) and December 2021 was the lowest value (6.53). February was the month with the highest salinity and EC. The Na^+ had the largest concentration of cations in the water sample, whereas Ca^{2+} had the lowest concentration. From February to April, the Mg^{2+} concentration was greater than the Ca^{2+} concentration. Major anions in water were arranged as $\text{Cl}^- > \text{SO}_4^{2-}$. The greatest seawater mixing ratio (25.40%) was recorded in February, followed by 3.87 % in March and 1.27 % in April. The EC (14 mS cm^{-1}), SAR (23), and Cl^- (117 meq L^{-1}) levels above the permitted irrigation water limit, the soil and plants had several problems and became unusable in February. As a result, it was determined that the Bang Pakong River's water qualities varied each month based on the amount of precipitation and fresh water flow in the area.

The second experiment was carried out to investigate the capacity of sodium (Na) sorption in acid and acid sulfate soil namely Chachoengsao and Rangsit soils, respectively.

The laboratory experiment was conducted using Chachoengsao soil and Rangsit soil, and six different seawater mixing ratios e.g. 0%, 5%, 10%, 15%, 20% and 25% (v/v). The mixture of soil and different seawater mixing ratios was shaken for 24 hr to determine the sodium sorption capacity. According to the results, the sodium adsorption ($796.18 \text{ mg kg}^{-1}$) was increased in Chachoengsao soil compared to Rangsit soil ($212.93 \text{ mg kg}^{-1}$). Moreover, the maximum sodium sorption (1087 mg kg^{-1}) was observed in 20% seawater mixing. Based on this study, it was concluded that higher adsorption of Na was found in Chachoengsao soil compared to Rangsit soil. It might be due to the high clay content and CEC in Chachoengsao soil and the strong affinity of acidic cations in Rangsit soil. The high Na adsorption capacity in the soil as a result of the high accumulation of Na can cause nutrient imbalance and ion toxicity in plants, which will inhibit plant growth, development, and yield.

In the third experiment, pot experiments were conducted for two seasons to evaluate the response of the growth, biochemical synthesis, yield, and nutrient uptake of two different rice varieties. The experiment was conducted with 2 factors and 3 replications. The first factor was two rice varieties such as Pathumthani 1 (PT1) and RD43 which were salt-sensitive and moderately salt-tolerant varieties. The second factor was six different seawater mixing ratios; 0% (control), 2%, 4%, 6%, 8%, and 10% (v/v). According to the findings, PT1 showed higher plant growth parameters such as plant height, number of tillers hill⁻¹ and shoot dry weight because of high Chl a and Chl b contents with lower amounts of H₂O₂ compared to RD43. The RD43 maintained high relative water content (RWC) and produced more total phenol, and proline contents than that of PT1 which indicated the resistance to high salinity in the second season. Seawater mixing > 6% declined plant growth by decreasing RWC, Chl a, and Chl b contents and produced large amounts of H₂O₂, total phenol, and proline contents. The higher grain yield was observed in PT1 in the major season because PT1 had greater uptakes of K, Mg and Ca, and lower uptakes of S compared to RD43 although the yield in the second season was not different between two varieties. The lower grain yield with increased uptake of Na, Cl and S and decreased K uptake was shown in >6% seawater mixing ratio. Moreover, the higher grain yield was observed in PT1 in the first season because PT1 had greater uptakes

of K, Mg and Ca, and lower uptake of S compared with RD43 under different salinity stress. Although the plant could survive under the low to moderate salinity, lower grain yield with increased uptake of Na, Cl, and S and decreased K and Mg uptake was observed in $\geq 6\%$ seawater mixing ratios whereas the larger grain yield was observed in 0-4% seawater mixing ratio. The higher seawater mixing ratio significantly increased E_{Ce}, soluble Cl⁻, exchangeable Na⁺, SAR and ESP in soil for both seasons. Based on the results, the grain yield of PT1 could not reduce at 2% seawater mixing ratio compared to control in both seasons. However, the grain yield of RD43 decreased in all seawater mixing ratios compared with control.

In the fourth experiment, the effects of adding rice straw biochar were assessed, and the right amount of biochar was added to saline soils that are impacted by salinity in order to decrease their toxicity, sodicity, acidity, and salinity. The six salt-affected soils were treated with rice straw biochar at four different doses such as 0% (control), 1%, 3%, and 5% (w/w). The S1, S2 and S3, and S4, S5 and S6 were non-saline, low-saline and moderately-saline, respectively. The six ratios of seawater mixing were noted as six salt-affected soils. The mixture of soil and biochar was continually shaken with distilled water for seven days. In comparison to the control, the biochar treatment significantly increased the pH and E_{Ce} of the soil as the rate of biochar increased. Significant drops in the ESP and SAR values below the standard value of sodicity were noted above the 1% biochar treatment. Above the 1% biochar application rate, there was a significant drop in the concentrations of soluble and exchangeable Na⁺ and soluble Cl⁻. The application of biochar ($\geq 1\%$) resulted in a considerable decrease in soluble and exchangeable Ca²⁺ and Mg²⁺, and increase in soluble and exchangeable K⁺. The study's findings demonstrated that 1% biochar application rate was appropriate for bringing the soil's acidity down to a level that was safe for rice plants. Applying rice straw biochar reduced soluble Cl⁻ and soluble and exchangeable Na⁺, which decreased SAR and ESP and increased soil toxicity and sodicity. The available K⁺ content of salt-affected soils was greatly raised by adding biochar, which is important for rice plant growth and development.

Keywords: Seawater Intrusion, Biochemical synthesis, Salinity, Sodicity, Electrical conductivity, Chloride toxicity, Sodium toxicity, Sodium adsorption ratio

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CHAPTER 1

INTRODUCTION

1.1 Research background and motivation

The sea level rise and seawater intrusion caused by increasing temperature and atmospheric greenhouse gas concentration have occurred in rice cultivation countries such as Bangladesh, Indonesia, Vietnam, and Thailand (Sudratt and Faiyue, 2023). It is estimated that salinity will cause more than 50 % of arable land in 2050 resulting in decreased soil fertility, vegetation cover, biodiversity, and ecological functions of the soil leading to its degradation (Pessoa *et al.*, 2022).

Thailand's total salt-affected soil area is around 2.302 million hectares, which may be divided into three primary areas: the northeast plateau basin, the coastal region, and the central plain. Each of these locations has a different setting (Arunin and Pongwichian, 2015). Additionally, the majority of Thailand's major rivers, including the Pasak, Ratchaburi, Petchaburi, and Bang Pakong, directly flow into the Gulf of Thailand. These rivers are essential to the freshwater supply of the Great Central Plane, which supports agriculture, industry, fisheries, cattle, and human activities (Shwe *et al.*, 2022). The Bang Pakong River's low river plain, which is widely utilized for rice farming (Pruksanubal, 2016). The Bang Pakong River typically experiences seawater intrusion six months of the year.

The use of seawater containing a high amount of salt for irrigation decreases crop production and alters the physicochemical properties of soil (Phankamolsil *et al.*, 2021). Because the seawater contains a high salt, mainly sodium chloride (NaCl), the combination of the base cations such as potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and sulfate (SO_4^{2-}), bicarbonate (HCO_3^-), chloride (Cl^-) (Erfandi and Rachman, 2011). When the Na level is increased in the soil, it has the chance to exchange Na^+ with Ca^{2+} on the surface of clay minerals (Rahimi *et al.*, 2019). Furthermore, the high concentration of Na increases the sodium adsorption ratio (SAR) more than the standard value, especially in fine-textured soils. Increasing the SAR of water increases the absorption of water, leading to a reduction of the water flow rate in soil (Poolsab *et al.*, 2017). The study found that the exchangeable

sodium percentage (ESP) value was less than 15% when the soil was immersed at 2% salinity for two weeks, but the electrical conductivity of the saturated soil extract (ECe) was marginally higher than 4 dS m^{-1} , reaching the threshold of saline soils. When salinity above 6%, the pH of the soil fell below 8.5, the ECe beyond 4 dS m^{-1} , and the ESP (%) exceeded 15, all of which indicated that the soil had become saline-sodic (Tan and Thanh, 2021).

High salinity in soil and plant tissue enhanced osmotic stress which decreases the water absorption capacity of roots and loss of water from the leaves, nutrients imbalance, changes in the antioxidant enzymes and a decrease in photosynthetic activity (Shahid *et al.*, 2020). The rice plant growth is disturbed due to lower relative water content (RWC) in the plant which may be the decrease in cytoplasmic volume and the loss of cell turgor because of the osmotic outflow of intracellular water (Jumpa *et al.*, 2023).

Moreover, the salinity stress shows significant changes in leaf chlorophyll content, reactive oxygen species (ROS) accumulation, proline accumulation, and antioxidant activities in the plant. Chlorophyll is the most essential component in the photosynthesis process and the rate of photosynthesis depends on the level of chlorophyll content in the leaves (Kibria *et al.*, 2017). Salt stress increases the formation of ROS within plant cells and leads to damage to membrane lipids, proteins, and nucleic acids (Sahin *et al.*, 2018). Under saline stress, proline accumulation appears in different plant species (Kibria and Hoque, 2019) and it can protect membranes, proteins, and enzymes from damaging various stresses (Hossain *et al.*, 2014). Besides, the plant enhances enzymatic and non-enzymatic antioxidant activities to reduce ROS under salinity (Bayat and Moghadam, 2019). The phenolic compounds have potent antioxidant properties and free radical scavenging capabilities (Joseph *et al.*, 2015).

The plant growth and development were inhibited by salinity due to increasing osmotic potential of soil solution, upsetting nutrient balance, and ion toxicity because of Na^+ and Cl^- accumulation (Rehman *et al.*, 2019). Under salt stress, higher Na^+ uptake competes with other nutrient ions uptake especially K^+ and it causes K^+ deficiency that leads to lower K^+/Na^+ ratio in plants (Kibria *et al.*, 2017). Rice (*Oryza sativa* L.) is one of the major food sources for the increasing population worldwide, therefore, the demand

for rice needs to grow faster than other cereal crops (Zhang *et al.*, 2022). Salinity is one of the main abiotic stresses for rice in all stages according to global warming and sea level rise that affects rice production, particularly in coastal areas (Hakim *et al.*, 2013). Rice is the most susceptible to salinity among cereal crops and some rice varieties can tolerate at 3 dS m⁻¹. However, the rice yield decreased by 10% at 3.5 dS m⁻¹ and 50 % yield decreased at 7.2 dS m⁻¹ (Taratima *et al.*, 2022).

Many researchers attempted to explore how to improve rice grown in various environments, particularly in brackish water regions, seashores, and salinity problem areas (Zinnah *et al.*, 2013). In that situation, organic amendments such as biochar are used to improve the physicochemical properties of soils (Shakoor *et al.*, 2021). The biochar application in saline soils increased organic matter content and nutritional status, and improved soil physicochemical properties and structure in saline soils. It supplied Ca²⁺ to replace Na⁺ from exchange sites (Mohanavelu *et al.*, 2021) leading to lower ESP or SAR of saline soil (Yue *et al.*, 2016). Moreover, the biochar amendment in salt-affected soil reduced H⁺ ions while increasing soil organic carbon and cation exchange capacity (Wu *et al.*, 2017).

However, there has been limited information on seawater mixing ratio and water quality in the Bang Pakhong River. Moreover, the lack of information on specific seawater concentration ratios has negatively influenced rice production and soil properties in agricultural land near coastal areas of Thailand making it difficult to water and soil management in areas under seawater intrusion. The biochar application to salt-affected acidic soil may have a positive or negative effect on acidic soil reclamation along the Bang Pakong River. Therefore, the objectives of this study were as follows:

1.2 Objectives of the study

- (1) To study the quality of water and seawater mixing ratio in the Bang Pakong River, Chachoengsao Province at different times
- (2) To investigate the capacity of sodium sorption in Chachoengsao and Rangsit soils
- (3) To explore the specific seawater mixing ratios that impact on biochemical synthesis, growth, and yield of two rice varieties and soil properties

(4) To determine the positive and negative effects of biochar application on soil properties in salt-affected soil

1.3 Scope(s) of the study

This study was conducted at the Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok from August 2021 to May 2024. This study focused on seawater intrusion impact on soil and yield of rice crop by changing soil properties and biochemical synthesis of plant near coastal areas. This experiment was divided into 4 parts as follows:

1.3.1 Assessment of water quality and seawater mixing ratio in the Bang Pakong River, Chachoengsao Province

1.3.2 Influence of seawater mixing ratios on sodium sorption capacity in soil

1.3.3 Effects of seawater mixing ratios on biochemical synthesis, growth and yield of two rice varieties, and soil properties

1.3.4 Impact of rice straw biochar application on soil properties related to salinity in salt-affected soil

1.4 Benefits of the study

1.4.1 The information about the range of seawater mixing ratio from Bang Pakong River in different collection times

1.4.2 The capacity of sodium sorption in Chachoengsao soil and Rangsit soil

1.4.3 The different seawater mixing ratios influenced on the biochemical synthesis, growth, and yield of Pathumthani 1 and RD43 rice varieties and soil properties

1.4.4 The positive or negative effects of rice straw biochar application on soil properties related to salinity in salt-affected soil

CHAPTER 2

THEORY AND LITERATURE REVIEW

2.1 Important of rice

Rice (*Oryza sativa* L.) belongs to the family Oryzeae (Wasim *et al.*, 2021). It is the second most cultivated cereal in the world and the main source of food for millions of people (Santos *et al.*, 2021). With the rapid growth in population consuming rice and the deteriorating soil and water quality around the world, there is an urgent need to understand the response of this important crop towards these environmental issues. Physiological, biochemical, and molecular methods are being used in extensive efforts around the world to develop rice plants that are more suited to changing environmental inputs. The primary factor adversely influencing crop growth and yield globally is abiotic stress (Amirjani, 2010).

The foreign exchange was earned by growing rice as a valued cash crop. Based on the climate condition, the life span of rice is from 3 months to 7 months. It is one of the major carbohydrate food crops for the world population. It feeds over three million people in the world calorie consumption from 50 to 80 % every day (Wasim *et al.*, 2021). While China, India, and Pakistan produce 30%, 21%, and 18% of total world output, and 30% of total rice cultivation are Thailand, Indonesia, Myanmar, and Japan (Taratima *et al.*, 2022).

Since rice is a salt-sensitive crop, soil salinity plays a significant role in limiting output throughout a large portion of South and Southeast Asia as well as Africa. Elevated salinity has been found to have an impact on several physiological processes, including photosynthesis, respiration, nitrogen fixation, and carbohydrate metabolism. Globally, one of the biggest obstacles to cereal production is salinity stress. Rice is a salt-sensitive crop, but it is the only cereal that has been proposed as a desalination crop because it can survive flooding and because standing water in rice fields can assist leach salts from the topsoil to lower levels. For thousands of years, farmers have been growing salt-tolerant rice varieties in saline regions of India, Myanmar, Thailand, Indonesia, and the Philippines. But rice yields were only about 1 t ha⁻¹. Although rice is susceptible to salt, it

is the only grain that has been suggested for desalination because it can thrive in floods and because the standing water in rice fields can aid in the leaching of salts from the topsoil to lower levels. For generations, farmers in saline regions of India, Myanmar, Thailand, Indonesia, and the Philippines have cultivated salt-tolerant rice cultivars. However, yields of rice were only roughly 1 t ha^{-1} . An international endeavor to breed salt-tolerant rice varieties with disease and pest resistance and high yield potential has been spurred by the realization of the potential of saline areas for rice cultivation (Chandramohan *et al.*, 2014).

Thailand is one of the major exporters of rice in the world and has a vital role in food security (Pame *et al.*, 2023). Thailand has approximately 10 million hectares of rice plantations and 3.7 million farmer households. It produces more than 30 million tonnes of paddy from the wet season and dry season annually (Open Access Government, 2020). Although many rice varieties are grown in Thailand, the aromatic rice variety such as Pathumthani 1 (Salt-sensitive variety) is popular. Because it has a distinctive aroma, delicate flavor, high cooking quality, long grains, high amylose content, and a soft texture (Chaum *et al.*, 2007). Chachoengsao Province has the largest area of cultivated rice with about 65,600 ha of rice paddies in 2015 of both seasons. The river basin is an agricultural area used for the commercial production of rice, fruit, and vegetables. The famous rice variety such as jasmine rice, originate from this province (Chaiyarak *et al.*, 2019). This area receives sediments from the Bang Pakong River which is connected to the Gulf of Thailand. Therefore, the improvement of salt-tolerant rice variety is important to sustain or increase rice productivity.

2.2 Salinity problem

In coastal areas, the salinity problem is increasing day by day due to global warming with consequently sea level rise which affected more than 800 million hectares of land all over the world and caused 6% of total land area nowadays (Polash *et al.*, 2018). Soil salinity is one of the most important limiting factors for agricultural production in arid and semi-arid regions (Turhan *et al.*, 2014). Furthermore, irrigation with high amounts of salt contained in groundwater and seawater in coastal areas is

the main cause of soil salinity (Razzaq *et al.*, 2020). The brackish irrigation water may lead to the accumulation of salt in soil that declines soil fertility and crop growth (Wei *et al.*, 2019).

Worldwide, a large amount of sodium chloride is present in most agricultural soils, which causes the salinization of soil and limited productivity and quality of agricultural products. The Food and Agriculture Organization of the United Nations (FAO) estimates that the impact of salinity on agricultural land is more than 33% (Singh *et al.*, 2022). Soil salinization is one of the environmental hazards that affect plant growth because of high concentrations of salt. The soil salinity affects about 7% of the world's land, 20% of the world's cultivated land, and nearly half of the irrigated land (Shrivastava and Kumar, 2015). By the year 2050, more than 50 % of the arable land will be salinized because salt-affected areas are increasing annually by 10 % every year (Jamil *et al.*, 2011).

More salinity problems will be developed because of expanding irrigated agriculture (Kumar *et al.*, 2017). The plant responses to salinity were affected by many factors. The osmotic stress can also be observed in different physiological and biochemical parameters of plants (Kibria and Hoque, 2019). Many research reported that salinity severely affected plant growth and development (Kumar *et al.*, 2017). Consequently, soil salinity is one of the crucial environmental problems that severely impacts food security and sustainability in the world (Sudratt and Faiyue, 2023).

2.3 Types of salt-affected soils

Generally, salinity as an abiotic stress severely limits crop production. A saline soil is usually the reservoir of several soluble salts such as Ca^{2+} , Mg^{2+} , Na^+ , SO_4^{2-} , Cl^- , and HCO_3^- with exceptional amounts of K^+ , CO_3^{2-} , and NO_3^- (Ghosh *et al.*, 2016). Salinity is the condition when the electrical conductivity (EC) is sufficient to cause yield reduction of most crops. The pH of saline soils generally ranges from 7-8.5. The salt-affected soils include sodic and saline soils. They possess high levels of pH usually more than 7.8. Both cause a problem in the proper growth of plants and hinder some essential physiological processes, which leads to a reduction in the yield of crops. There is also quite a difference

in these types of soil. The pH of sodic soil is over 8.5 while the saline soil is less than 8.5. The presence of a high amount of soluble salt ions will be more EC. Therefore, the saline soil has $EC > 4 \text{ mmhos/cm}$ whereas sodic soil has $EC < 4 \text{ mmhos/cm}$. In sodic soil, the exchangeable Na % is more than 15%, however, less than 15 % is in saline soil (Ayub *et al.*, 2020). The salt-affected soils are classified according to (ANR, 2015) (Table 2.1).

Table 2.1 Classification of salt-affected soil (ANR, 2015).

Classification	Electricity Conductivity (EC) (dS m^{-1})	Soil pH	Exchangeable Sodium Percentage (ESP)	Sodium Adsorption Ratio (SAR)	Soil Physical Condition
Typical agricultural soil	< 4.0	< 8.0	< 15	< 13	Good
Saline	> 4.0	< 8.5	< 15	< 13	Good
Sodic (alkali)	< 4.0	> 8.5	> 15	> 13	Poor
Saline-sodic	> 4.0	< 8.5	> 15	> 13	Poor to Good

$>$ = greater than, $<$ = less than

2.4 Effect of salinity on physicochemical properties of soil

Salts alter some of the soil's chemical and physical characteristics, which changes the soil's suitability as a growing medium for plants. Because the pH level and concentration of salts, such as Na^+ , Cl^- , and other dissolved salts in the soil, have a direct impact on plant growth. Soil salinity tends to impede the absorption of water and nutrients, leading to a decrease in absorption of K^+ , Ca^{2+} , NO_3^- , and phosphate and an increase in intercellular ion concentration and osmotic tension. As a result, it imposes some constraints on crops and is reported as a major problem for irrigated agriculture (Heribert and Shinozaki, 2004).

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The predominant salts that accumulate in soils are Ca^{2+} , Mg^{2+} , Na^+ , K^+ , SO_4^{2-} , Cl^- , CO_3^{2-} , and HCO_3^- (Horneck *et al.*, 2007). When the Na^+ is high in the soil, it will replace Ca^{2+} and Mg^{2+} adsorbed on the soil exchange, which causes the dispersion of soil particles. The destruction of soil structure is accompanied by an increase in soil compaction, a decrease in infiltration, and hydraulic conductivity, and a reduction of oxygen availability in the root zone. Moreover, the excess amount of Na^+ in the soil can compete with Ca^{2+} , K^+ , and other cations to decrease their availability to plants (Machado and Serralheiro, 2017).

Any overabundance of salt in the soil can prevent plants from growing. Too much salt causes problems for the soil's structure. As the amount of sodium increases, so does the risk of soil aggregate dispersion. High concentrations of some salts in the soil can also poison plants (Horneck *et al.*, 2007). Besides, if the soil contains abundant toxic elements, the plants can be affected by these elements at relatively low salt concentrations. Because many salts are also plant nutrients and saline stress affected interactions among morphological, physiological, and biochemical processes, water and nutrient uptake, and plant growth and development (Shrivastava and Kumar, 2015).

2.5 Effect of salinity stress on physiology

2.5.1 Relative water content (RWC)

The leaf relative water (RWC) content is used to describe the water content of the plants. The high relative water content has a more stable osmotic balance. The RWC decreases under salinity stress (Akca and Samsunlu, 2012). Many studies stated that RWC is reduced when the salinity increases and the tolerant variety exhibited less reduction in RWC (Hussein *et al.*, 2017). When plants are subjected to salinity, firstly they face an osmotic stress that reduces water uptake by roots (Polash *et al.*, 2018).

High concentrations of salts in the soil profile may cause a reduction in water uptake due to salt accumulation in the root zone (Munns, 2005), a reduction in the osmotic potential of plants, and disruption of cell metabolic functions due to toxicity (James *et al.*, 2011). Generally, salt stress is affected on crops by osmotic, ionic imbalance, and oxidative damage (Parida and Das, 2005). Excess amounts of Na^+ in plants damage

cell membranes and organelles of plants, consequently a reduction in plant physiological mechanisms leading to plant cell death (Siringam *et al.*, 2011).

2.5.2 Chlorophyll content

The main harmful effect of salt stress is the accumulation of Na^+ and Cl^- in soil and plant tissues (Nishimura *et al.*, 2011). The entry of Na^+ and Cl^- into plant cells causes ion imbalance in plants and soil, and excessive plant uptake of these ions can create major physiological issues for plants (James *et al.*, 2011).

These physiological changes in plants include membrane disruption, inability to detoxify ROS, reduced photosynthetic rate, and transformations of the antioxidant enzymes (James *et al.*, 2011). The chlorophyll is the most important constituent in the photosynthesis process and the rate of photosynthesis varied to the level of chlorophyll content in plant leaves. The reduction of productivity in many plant species was associated with a decrease in leaf photosynthetic capacity (Kibria *et al.*, 2017).

One of the most important metabolic processes in plants, photosynthesis, is greatly impacted by stress (Mehta *et al.*, 2011). Chlorophyll monitoring is a relatively recent technique used to examine how various environmental stresses, including drought, salt, and low or high temperatures, affect the photosynthetic efficiency of leaves in greenhouse and field conditions (Zobayed *et al.*, 2005). Several studies increasing salinity reduced photosynthesis in plants (Raza *et al.*, 2006). The mechanism of photosynthesis involves several components and damage by a stress factor may reduce the overall photosynthetic capacity of the plant (Ashraf and Harris, 2013). The salinity stress leads to closure of the stomata which acts to limit photosynthesis (Hnilickova *et al.*, 2017).

2.5.3 Reactive oxygen species

These oxidative systems can interrupt the routine functions of various plant cellular components such as proteins, DNA, and lipids, interfering with dynamic cellular functions in plants (Demiral *et al.*, 2005). Reactive oxygen species including singlet oxygen ($^1\text{O}_2$), hydroxyl radical (OH^*), superoxide radical ($\text{O}_2^{\cdot-}$), and hydrogen peroxide (H_2O_2) were all strongly oxidizing compounds that were potentially harmful to cell integrity (Santos *et*

al., 2018). The increased ROS levels can affect enzyme activity and damage important cellular components that inhibit plant growth and ultimately cause plant death (Arora *et al.*, 2001).

The ROS refers to any oxygen derivative that is more reactive than an oxygen molecule (O₂) itself (Mittler, 2017). Every type of ROS has unique and distinct chemical properties (Waszczak *et al.*, 2018). Among them, hydrogen peroxide (H₂O₂) is fairly stable and, therefore, is considered the predominant ROS involved in cellular signaling (Dixon and Edwards, 2010). The ROS are generated during basal metabolism in plants in a number of subcellular locations, such as apoplastic NADPH oxidases and other oxidases, mitochondrial respiration, chloroplast photosynthesis, and peroxisome localized photorespiratory responses (Noctor and Foyer, 2016).

2.5.4 Proline

One of the cellular responses to saline situations is the alteration of metabolism and production of compatible solutes, which are distributed among different organisms (Hasegawa *et al.*, 2000). Among different compatible solutes such as proline, glycine betaine, b-alaninebetaine, and proline betaine is found in various plants under stress conditions (Nahar *et al.*, 2016). Among amino acids, glycinebetaine and proline are the most common solutes produced in plants under various stress conditions (Mansour and Ali, 2017).

The accumulation of proline is a common physiological response that is found in many plants grown in different abiotic stresses and proline has been suggested to serve as an index of stress resistance (Vicente *et al.*, 2016). Since osmotic homeostasis has been shown to correlate with salt stress tolerance, osmotic adjustment is mostly attributed to proline accumulation in response to salt stress (Hasegawa *et al.*, 2000). According to Dar *et al.* (2016), proline accumulation is often higher in salt-tolerant plant species than in sensitive ones, including rice cultivars (Choudhury *et al.*, 2007) and sunflower (Heidari *et al.*, 2011).

The proline accumulation under abiotic stress occurred in plants and increased proline accumulation in plants was correlated with increased salt tolerance. Increased proline content in plants showed higher salt tolerance in several plants such as rice, barley,

pea, and soybean under salt stress. It was evident that the leaf proline had a strong positive relationship with plant yield potential (Kibria and Hoque, 2019).

2.5.5 Antioxidant

The defense mechanisms against free radical-induced oxidative stress involve (i) preventative mechanisms, (ii) repair mechanisms, (iii) physical defenses, and (iv) antioxidant defenses. The plants defend against these reactive oxygen species by induction of activities of certain antioxidative enzymes such as catalase, peroxidase, glutathione reductase, and superoxide dismutase, which scavenge ROS (Mittova *et al.*, 2003). The non-enzymatic antioxidants such as phenol, vitamin C, vitamin E, carotenoids, lipoic acid, and others in the protection against oxidative stress were also reported (Kojo, 2004).

Phenols are abundant in plant tissues and comprise a range of secondary metabolites, such as lignin, flavonoids, tannins, and hydroxycinnamate esters. Because polyphenols have the ideal structural chemistry for scavenging free radicals, they are more effective antioxidants *in vitro* than ascorbate and tocopherols. Human nutrition depends on polyphenolic compounds, one of the most common and widespread families of plant metabolites (Ahmad *et al.*, 2010).

Phenolics represent a chemically diverse group of compounds produced by a large number of metabolic pathways. A phenolic is characterized by the presence of an aromatic ring bearing one or more hydroxyls. The ecological and physiological activities of phenolic compounds in plants are diverse and highly variable (Roitto *et al.*, 2005). Phenols and polyphenolic compounds such as flavonoids, act as antioxidants, and cytotoxic effects of oxygen radicals in plant cells are removed (Lavid *et al.*, 2001). The high amounts of phenols and flavonoids in extracts may explain their high antioxidative activities (Roitto *et al.*, 2005). It directly scavenges $O_2^{\cdot -}$, OH^{\cdot} , O_2 and can reduce H_2O_2 to H_2O via the ascorbate peroxidase reaction (Akram *et al.*, 2017).

2.6 Effect of salinity stress on rice growth

The salinity negatively impacts biochemical and physiological changes that cause plant growth inhibition and yield loss (Ghosh *et al.*, 2016). Salinity is one of the main

abiotic stresses for rice in all stages according to global warming and sea level rise that affects rice production, particularly in coastal areas (Hakim *et al.*, 2013). The increasing salinity reduces nutrient uptake by rice plants (Kordrostami *et al.*, 2017).

Compared to other significant cereal crops like wheat and maize, rice is considered a salt-sensitive crop. Salt stress has a significant impact on the morphological traits of rice plants. According to the taxonomy of crop tolerance to salt stress, the rice crop is classified as sensitive, falling between 0 and 8 dS m⁻¹. While the threshold level for salt tolerance is 3 dS m⁻¹ in rice with increasing 1 unit of electrical conductivity, it occurs 12% yield reduction. It is a moderately salt-sensitive crop and its sensitivity or susceptibility varies from species to species and growth stages (Hussain *et al.*, 2018).

Salinity has been found to impede plant growth mostly due to the osmotic effect, ion toxicity, and nutritional imbalance that lowers photosynthetic activity and causes other physiological disorders. The Na⁺ and Cl⁻ are well-known as toxic ions to damage plant cells at both ionic and osmotic levels. The salt stress in the root zone caused the development of osmotic stress, which disrupts cell ion homeostasis by including inhibition in the uptake of essential nutrients such as increased accumulation of Na⁺ and Cl⁻ (Paranychianakis and Chartzoulakis, 2005). Under salt stress, the higher uptake of Na⁺ competes with the uptake of other nutrient ions especially K⁺, which causes K⁺ deficiency and a low K⁺/Na⁺ ratio in plants (Kibria *et al.*, 2017).

Plant growth and development are directly inhibited, leading to low yield before plant death (Hakim *et al.*, 2014). The Na stress is one of the negative factors that damaged crop growth, development, and yield. It adversely affected on vegetative, reproductive, and grain-filling stages in rice plants, which decreased productivity (Rodríguez *et al.*, 2019).

2.7 Effect of salinity stress on rice yield

Rice is a crop with great economic importance (Harkamal *et al.*, 2007) and is cultivated across 114 countries globally (FAO, 2004). However, the abiotic and biotic stresses can reduce its yield. This problem will be worse in attention to the increase of global population and food sources deficiency (Kumar *et al.*, 2007).

Rice genotypes differ significantly in their ability to withstand salinity due to additive gene effects (Sahi *et al.*, 2006). According to Ali *et al.* (2014), salinity has several effects on rice, such as inhibiting germination, making it more difficult to establish crop and leaf areas, decreasing the amount of dry matter generated, delaying seed set, and even resulting in sterility. It has been well documented that the effect of salinity on seedling growth, seedling establishment, and grain yield components such as spikelet number, and tiller number has successively led to a reduction in grain yield (Zeng *et al.*, 2003). Moreover, it caused a significant reduction in the number of stems per plant, the number of panicles, length, and the number of spikelets per panicle (Rodríguez *et al.*, 2019). The salt stress negatively affects rice growth and yield depending on developmental stages, variety, salt stress level, and duration (Taratima *et al.*, 2022).

2.8 Biochar

Generally, a huge amount of organic residue and waste are produced all over the world and it causes environmental pollution and nutrient losses because of improper removal of these organic residues every year (Hazrati *et al.*, 2020). Recycling of these organic residues has been a common agricultural practice for increasing soil fertility and agricultural productivity in the farmland for decades (Wu *et al.*, 2021). Organic amendments such as livestock manure, plant residue and waste, and bio-organic fertilizer are used to alleviate soil salinity, improve soil fertility, and promote crop growth in saline soils (Chen *et al.*, 2021).

Agricultural residues are provided as an easily available material and a substance for the removal of contaminants in the environment (Lee and Park, 2020). The biochar made from agricultural waste had a high efficiency for the adsorption of different inorganic pollutants in soil and liquid aqueous solutions (Medyn'ska-Juraszek *et al.*, 2020). Biochar was generally made from the residues under limited oxygen or lack of oxygen at temperatures ranging from 300°C to 1000 °C (Yaashikaa *et al.*, 2020).

2.8.1 Effects of biochar on salt affected soils

Biochar is derived from the burning of plant materials at high temperatures in the absence of oxygen. Biochar is a main source of essential nutrients for plant growth and it improves soil structure (Guo *et al.*, 2020) and soil physicochemical properties (Niazi *et al.*, 2016). Several studies reported that biochar improved the properties of salt-affected soils, enhanced crop growth, increased the uptake of K^+ , and reduced the uptake of Na^+ (Farhangi-Abriz and Torabian, 2017). Biochar increased water desalination and removal of excessive amounts of Na^+ from solution by exchanging it with Ca^{2+} , Mg^{2+} , and K^+ cations present on biochar surface in large amounts (Gunarathne *et al.*, 2020).

The addition of biochar improved plant growth in saline soil by balancing the decreased uptake of excess ions by plants. Moreover, it also enhanced the growth and development of soil organisms by increasing aggregate formation, improving water retention, releasing nutrients, and providing a carbon source in salt-affected soils (Poonia and Parihar, 2020).

Many studies reported that the application of biochar had a positive effect on decreasing the SAR/ ESP of saline-sodic and sodic soils (Luo *et al.*, 2017). The biochar addition had many mechanisms to reduce ESP depending on the soil-plant-biochar properties. It provided directly exchangeable Ca^{2+} to replace Na^+ on the soil colloids (Chaganti *et al.*, 2015). It also increased surface charge density due to organic carbon resulting in more Ca^{2+} than Na^+ to adsorb on the soil colloids which led to lower ESP (Zheng *et al.*, 2017). Moreover, it improved soil porosity to facilitate Na leaching from the soil profile leading to reduced SAR and ESP (Yue *et al.*, 2016). The impact of biochar on SAR in salt-affected soil varied with biochar types with different contents of Ca^{2+} and Na^+ (Luo *et al.*, 2017).

CHAPTER 3

RESEARCH METHODOLOGY

There were four experiments in this study to determine the effects of seawater intrusion on soil, growth and yield of rice.

First experiment - Assessment of water quality variation for agriculture in Bang Pakong River of Thailand

- To investigate the water quality related to salinity and seawater mixing ratio in the Bang Pakong River under different collections time

Second experiment - Impact of different seawater mixing ratios on sodium sorption capacity in soil

- To investigate the capacity of sodium sorption in Chachoengsao and Rangsit soil

Third experiment- Seawater intrusion effects on growth and yield of rice by changing of biochemical synthesis and soil properties in Chachoengsao Soil

- To examine the effect of different seawater mixing ratios on growth, biochemical synthesis, yield and nutrient uptakes of Pathumthani 1 and RD43 rice varieties

- To assess the changes of soil chemical properties by different seawater mixing ratios

- To explore the safe seawater mixing ratios for growth and yield of two different rice varieties.

Fourth experiment - Influence of rice straw biochar amendment on salinity in salt-affected Soils

- To investigate the effect of different doses rice straw biochar applications on soil properties related to salinity in salt-affected soils
- To classify the suitable rate of biochar application to decline, salinity and sodicity in salt-affected soils

3.1 First experiment- Assessment of water quality variation for agriculture in Bang Pakong River of Thailand

3.1.1 Water sampling area

For this investigation, a water sample region along the Bang Pakong River in Khlong Khuean District, Chachoengsao Province, was chosen (Figure 3.1). As it flows through the province of Chachoengsao, it is roughly 230 kilometers long and 100 meters wide. The primary plantation in Chachoengsao Province, paddy fields and orchards, were the site of the sample station, which was situated above the Bang Pakong Dam, a dam designed to prevent seawater intrusion. The 13 locations (R1 through R13) were chosen so that water samples could be taken. Additionally, a sample of water was taken from the Gulf of Thailand in order to determine the ratio of seawater mixing. The Bang Pakong River's salinity during dry spells was the main focus of this experiment. During the dry season, farmers in this region used irrigation water from the Bang Pakong River for their fields. Typically, water scarcity can be an issue during the dry season in this area, the use of saline water for irrigation might be considered as an alternative of farmer. Therefore, the water sample was taken between November 2021 and April 2022, from the end of the rainy season to its beginning.

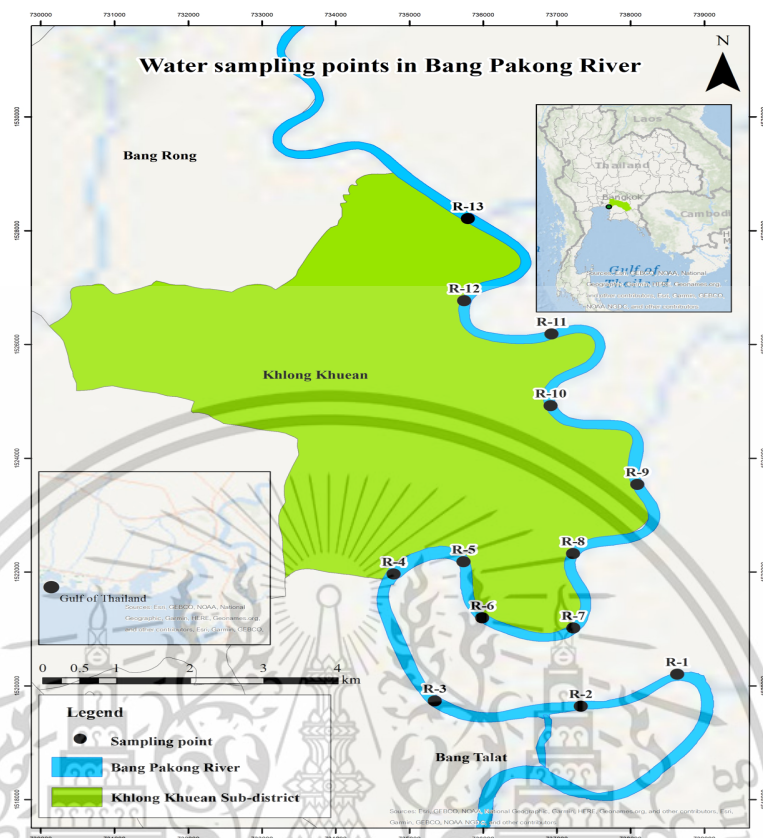


Figure 3.1 Thirteen sites for water sampling along the Bang Pakong River.

3.1.2 Water sampling

Two replications of the water sample were taken from the Bang Pakong River and the Gulf of Thailand, at a depth of one meter below the water's surface.

3.1.3 Sampling tools and containers

The water samples were collected using hygienic sampling equipment and containers. Water samples from the river were gathered using the grab sampler. A plastic bottle was filled with the water sample. The sample was kept at 4°C from collection until analysis.

3.1.4 Preparation of water sample and analysis

After filtering the water sample through a Whatman No. 1 filter, the water quality was examined. Meters were used to test the pH and EC. With the use of an inductively

coupled plasma-optical emission spectrometer (ICP-OES), the K^+ , Ca^{2+} , Mg^{2+} , Na^+ , and SO_4^{2-} were examined. Using a standard silver nitrate (0.01N) titration method and 5% potassium chromate as an indicator, the Cl^- concentration was measured (Richards, 1954). The seawater mixing ratio (F) was calculated using Cl^- content from river and sea in November 2021. The following formulas were used to determine sodium adsorption ratio (SAR) and the seawater mixing ratio.

$$SAR = \frac{[Na^+]}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$$

(Olorunfemi and Fasinmirin, 2017)

$$F = \frac{[Cl_{Sample}] - [Cl_{Fresh}]}{[Cl_{Sea}] - [Cl_{Fresh}]} \times 100$$

(Trabelsi *et al.*, 2011)

F = Seawater mixing ratio (%)

Cl_{Sample} = Chloride concentration of the sample (meq L^{-1})

Cl_{Fresh} = Chloride concentration of fresh water (meq L^{-1})

Cl_{Sea} = Chloride concentration in seawater (meq L^{-1})

3.1.5 Statistical analysis

The SPSS software performed statistical analysis on the data. The Duncan's Multiple Range Test (DMRT) at $P < 0.05$ was used for the mean comparison after analysis of variance (ANOVA) was completed.

3.2 Second experiment- Impact of different seawater mixing ratios on sodium sorption capacity in Soil

3.2.1 Study site

The laboratory experiment was carried out at the Soil Science Laboratory, Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok, Thailand.

3.2.2 Soil collection and preparation

The soil samples were collected from Chachoengsao Province. It was located at 13.7872° N latitude and 101.1504° E longitude. The total area of Chachoengsao province is approximately 5,351 km². There were 9 soil series such as Bangkok (Bk), Bang Nam Priao (Bp), Bang Pakong (Bpg), Chachoengsao (Cc), Don Mueang (Dm), Maha Phot (Ma), Ongkharak (Ok), Rangsit (Rs) and Samut Prakan (Sm) along the Bang Pakong River in Chachoengsao Province (Figure 3.2). All these soils were formed by mixing of marine sediments and riverine alluvium under influence of brackish water. Most of soil series in this area are acid to strongly acid soils (pH 4.0-6.5). Moreover, these soils were regularly flooded by seawater and irrigation in dry season from the Bang Pakong River. The salinity problems in acid or acid sulphate soil near coastal area result from the seawater intrusion particularly in dry season. Under this condition, the soil became surface crusts because of salinity. Among these soil series, Chachoengsao (pH 5.5) (acid soil) and Rangsit (pH <4.5) (acid sulfate soil) soil series were collected to use for this study. The sampling point of each location is 10 km far from the riverside of Bang Pakong River.

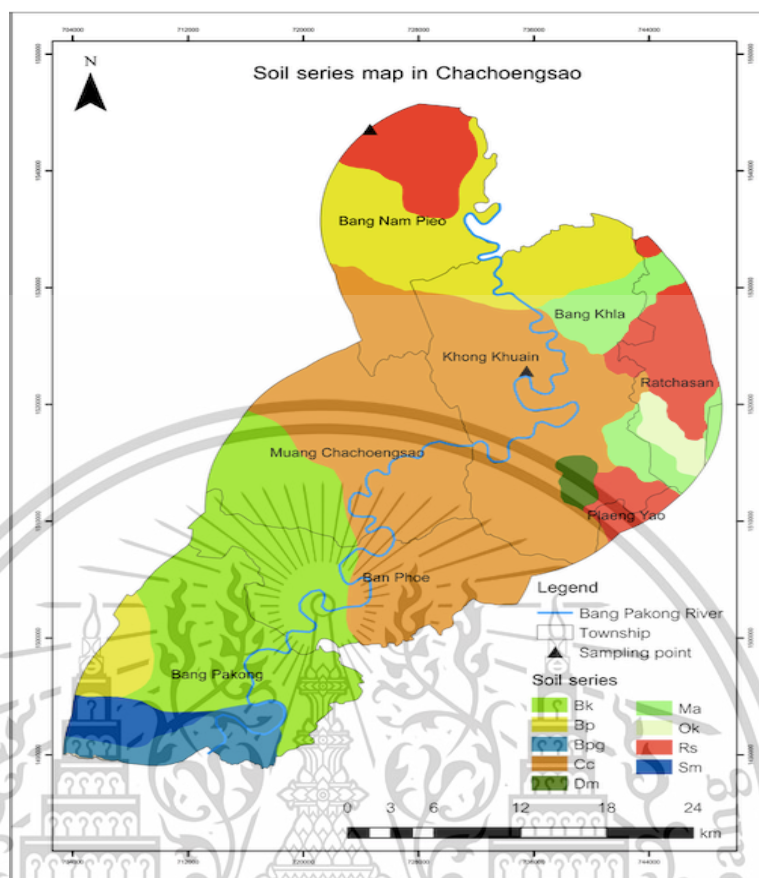


Figure 3.2 The soil series and sampling points in Chachoengsao Province.

After collecting the soil samples, the soil sample was air-dried for 14 days followed by grinding and mixing thoroughly. Then, the soil was passed through a 2 mm sieve for analysis of physicochemical properties. After that, the soil samples were analyzed for soil texture, pH, EC, soil organic matter (SOM), available phosphorus (P), total carbon (C), nitrogen (N), sulfur (S), exchangeable K, Na, Ca, and Mg, available iron (Fe), available manganese (Mn), available zinc (Zn), available copper (Cu), soluble Cl^- and sodium adsorption ratio (SAR) and exchangeable sodium percentage (ESP). The initial properties of Chachoengsao soil and Rangsit soil were presented in Table 3.1.

Table 3.1 Initial properties of Chachoengsao soil and Rangsit soil.

Parameter	Unit	Chachoengsao soil	Rangsit soil
Soil texture		Clay	Silty clay loam
Sand	(%)	2.57	0.71
Silt	(%)	35.32	69.65
Clay	(%)	62.11	29.64
pH _{soil:water; 1:1}		4.38	3.88
ECe	($\mu\text{S cm}^{-1}$)	357	512
Organic matter	(%)	2.09	3.64
Available P	(mg kg^{-1})	12.83	10.22
Soluble Cl	(mg kg^{-1})	397	585
Exchangeable K	(mg kg^{-1})	99.60	228.65
Exchangeable Ca	(mg kg^{-1})	1228	1245
Exchangeable Mg	(mg kg^{-1})	1010	635
Exchangeable Na	(mg kg^{-1})	498	465
Available Fe	(mg kg^{-1})	229	310
Available Mn	(mg kg^{-1})	26.94	18.63
Available Zn	(mg kg^{-1})	0.86	1.07
Available Cu	(mg kg^{-1})	2.43	0.89
Total C	(%)	1.44	2.13
Total N	(%)	0.13	0.18
Total S	(%)	0.16	0.17
CEC	(cmol kg^{-1})	35.16	24.15
ESP	(%)	5.05	8.38
SAR		2.09	6.11

Notes: ECe, saturated electrical conductivity; P, phosphorus; Cl, chloride; K, potassium, Ca, calcium; Mg, magnesium; Na, sodium; Fe, iron; Mn, manganese; Zn, zinc; Cu, copper; C, carbon; N, nitrogen; S, sulfur; CEC, cation exchange capacity; ESP, exchangeable sodium percentage; SAR, sodium adsorption ratio.

3.2.3 Method of soil analysis

The soil texture was determined by the pipette method (FAO, 2019a). Soil pH was measured using meters (CONSORT C830) in soil water ratios of 1:1 after 30 min of stirring. The E_c was determined by using the method of USDA (Wang *et al.*, 2023). The organic matter (OM) was determined by dichromate oxidation method (FAO, 2019b). The available phosphorus (P) was extracted using Bray II solution and determined by the Molybdenum blue method (FAO, 2021). Exchangeable basic cations (Ca²⁺, Mg²⁺, K⁺, and Na⁺) were extracted by 1N ammonium acetate solution at a pH of 7 (FAO, 2018) and determined by using an Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES). The available Zn, Cu, Mn and Fe were assessed by extracting a portion of 10 g of each soil with 20 ml of diethylene-triamine penta-acetic acid (DTPA) using standard method (Dhaliwal *et al.*, 2022) and analyzed by ICP-OES. The total sulfur (S) was measured by a CNS analyzer (LECO Corporation, 2016). The Cl⁻ content was assessed by the titration method using standard silver nitrate (0.01 N) with a 5 % potassium chromate indicator (Iqbal *et al.*, 2018). The cation exchange capacity (CEC) was estimated by 1N ammonium acetate, pH 7.0 method (FAO, 2018). The SAR and ESP were calculated as expressed in equations (1) and (2).

Calculations

The SAR and ESP were calculated as expressed in equations (1) and (2) (Olorunfemi and Fasimirin, 2017).

$$\text{SAR} = \frac{[\text{Na}^+]}{\sqrt{\frac{\text{Ca}^{2+} + \text{Mg}^{2+}}{2}}} \quad (1)$$

$$\text{ESP} = \frac{\text{Exchnageable sodium}}{\text{Soil CEC}} \times 100 \quad (2)$$

3.2.4 Sodium sorption experiment

For this experiment, 2 g of each soil was weighed and put into a centrifuge tube. The seawater was made from artificial sea salt which was prepared according to American Society for Testing and Materials (ASTM) standard D1144-9 (ASTM, 2019) (Table 3.2). The K, Ca, Mg and Na contents in different seawater mixing ratios were shown in Table 3.3. Then, the desired different seawater solutions were made by dilution of stock solution. Then, 20 ml of 0%, 5 %, 10 %, 15%, 20%, and 25 % of seawater solutions were added to the tubes. The soil samples were shaken for 24 hours to reach equilibrium. After shaking, the suspensions were centrifuged at 3000 rpm for 10 min and filtered through the No.1 filter. The equilibrium sodium concentration (C_t) of the supernatant was measured using ICP- OES. The difference between the added amount of sodium (C_0) in the seawater and the measured amount in the equilibrium solution (C_t) was determined as the net amount of sodium adsorbed (Q) by the soil (Jalali and Peikam, 2013).

$$Q = \frac{(C_0 - C_t) \times V}{W}$$

Where Q was the amount of sodium adsorbed (mg kg^{-1}); C_0 is the initial sodium concentration (mg L^{-1}); C_t was the equilibrium sodium concentration (mg L^{-1}); W was the sample weight (g); and V was the solution volume (mL) of seawater.

Table 3.2 Composition of artificial seawater (ASTM, 2019).

No.	Name		Amount (g L ⁻¹)
1	Sodium chloride	(NaCl)	24.53
2	Magnesium chloride	(MgCl ₂)	5.20
3	Sodium sulphate	(Na ₂ SO ₄)	4.09
4	Calcium chloride	(CaCl ₂)	1.16
5	Potassium chloride	(KCl)	0.695
6	Sodium bicarbonate	(NaHCO ₃)	0.201
7	Potassium bromide	(KBr)	0.101
8	Boric acid	(H ₃ BO ₃)	0.027
9	Strontium chloride	(SrCl ₂)	0.025
10	Sodium fluoride	(NaF)	0.003

Table 3.3 The K, Ca, Mg and Na composition in different seawater ratios.

Seawater (%)	K (mg L ⁻¹)	Ca (mg L ⁻¹)	Mg (mg L ⁻¹)	Na (mg L ⁻¹)
5%	23.07	15.50	31.96	562.53
10%	40.92	30.45	63.96	1132.12
15%	64.20	48.43	99.18	1690.15
20%	81.60	60.00	126.04	2213.01
25%	97.36	71.45	150.40	2677.64

3.2.5 Statistical analysis

The data were analyzed statistically by IBM SPSS Statistics version 28. Analysis of variance (ANOVA) was performed and mean comparison was done using Duncan's Multiple Range Test (DMRT) at a probability level of $P < 0.05$.

3.3 Third experiment- Seawater intrusion effects on growth and yield of rice by changing of biochemical synthesis and soil properties in Chachoengsao soil

3.3.1 Experimental site

The two seasons of pot experiments were conducted under the greenhouse at the Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL) from April to July 2022 (Major rice season) and October 2022 to January 2023 (Second rice season). KMITL is located at 13.7299° N and 100.7782° E, Ladkrabang District, Bangkok, Thailand (Figure 3.3). The monthly temperature throughout the experimental period was shown in Figure 3.4 (Power Data Access from NASA, <https://power.larc.nasa.gov/data-access-viewer/>). The average temperature in the major rice season and second rice season were 28.71 °C and 25.53 °C, respectively. The minimum and maximum temperatures were 22.40 °C and 31.52 °C in the major rice season and 20.19 °C and 29 °C in the second rice season.

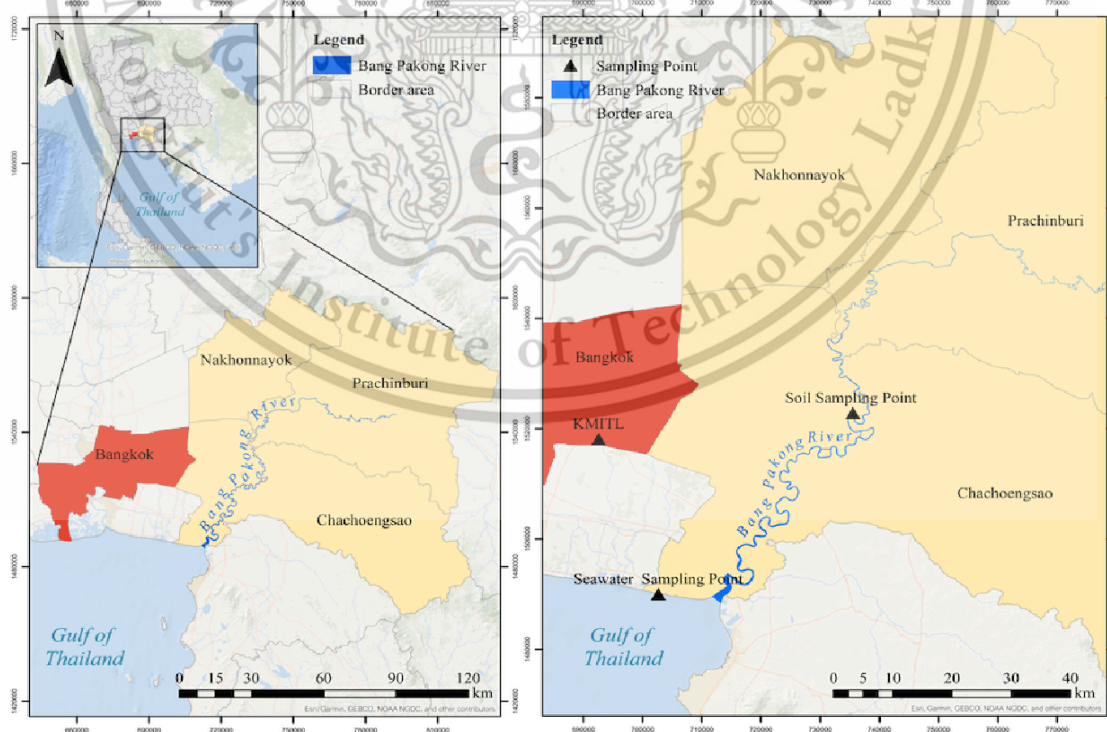


Figure 3.3 The soil collection area and experimental site.

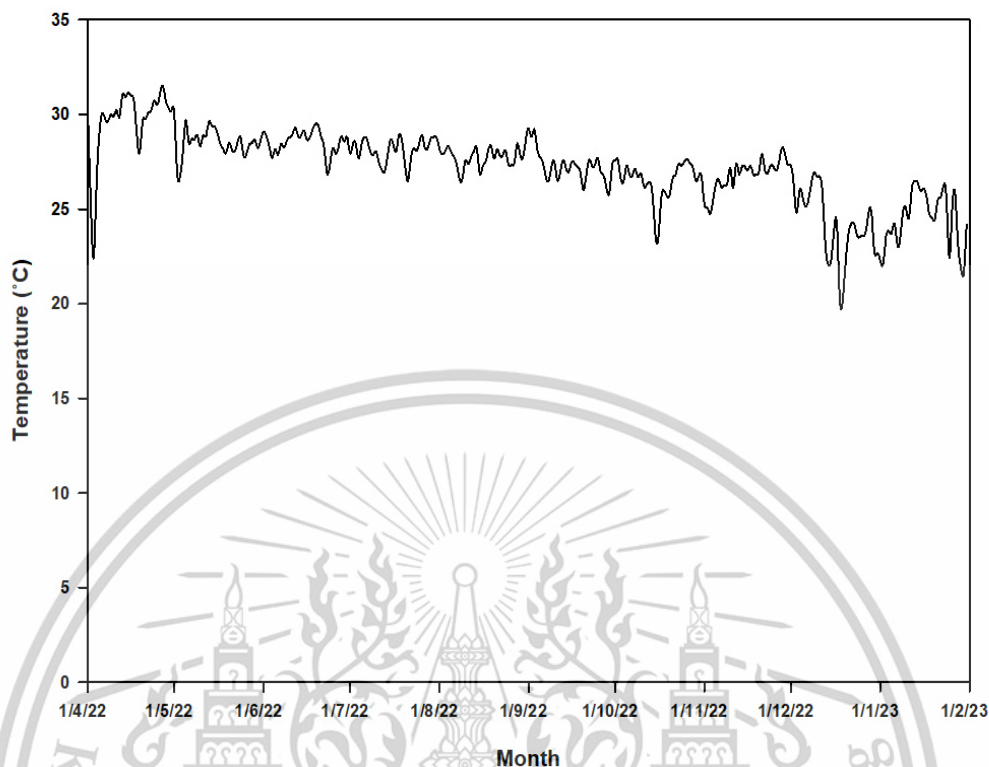


Figure 3.4 Monthly temperature data during experimental period.

(Power Data Access from NASA, <https://power.larc.nasa.gov/data-access-viewer/>)

3.3.2 Soil collection and preparation

The Chachoengsao soil series was collected from Khlong Khuean District, Chachoengsao Province located at 13.7872° N latitude and 101.1504° E longitude (Figure 3.3). The total area of Chachoengsao province is approximately 5,351 km². The western part of the province is the low river plain of the Bang Pakong River, which is used extensively for rice production by people (Pruksanubal, 2016). The watershed of the Bang Pakong River is about 17,000 km². Chachoengsao soils are formed from the mixing of marine sediments and riverine alluvium under the influence of brackish water. Tidal flats or alluvium plains, which grade down to marine deposits, occurred previously but are now free of tidal flooding. They are very deep, medium acid to neutral soils (Udomsri, 2004). The initial properties of the experimental soil were described in Table 3.1.

3.3.3 Experimental design and treatment

The two factors experiment was laid out in a 2 × 6 factorial arrangement in a randomized complete block (RCB) design with three replications. Factor A was two rice varieties (V) such as V1 = Pathumthani 1 (PT1) (Salt-sensitive variety) and V2 = RD43 (Moderately salt-tolerant variety), which are popular in Thailand. Factor B was six ratios of seawater solutions such as 0% (control) (tap water), 2 %, 4 %, 6%, 8%, and 10 % (v/v).

3.3.4 Preparation of pot and saline water

Earthen pots (36 cm top diameter and 35.5 cm height) were used, and 20 kilograms of soil sample was weighed and put into each pot. Saline water was made from artificial sea salt which was presented in Table 3.2. Then, the desired different seawater solutions were made by dilution of stock solution. The chemical composition of different seawater ratios was presented in Table 3.4. Subsequently, the soil was submerged with different seawater solutions to a depth of 5-10 cm from the surface as respective treatments. The seawater was applied to the pots two weeks before transplanting. The seawater was added once in each season as farmers used irrigation under seawater intrusion from Chachoengsao Province.

3.3.5 Crop management

The rice varieties such as Pathumthani 1 (PT1) (Salt-sensitive variety) (Pongprayoon *et al.*, 2019) and RD43 (Moderately salt-tolerant variety) (Pongprayoon *et al.*, 2019) were used as tested varieties because these two varieties are popular in Thailand. Besides, most of the farmers in Chachoengsao Province have used PT1 in their fields. Because it is a new fragrant rice and it can be grown 2-3 crops a year. Moreover, the price is higher than other rice varieties and it is a short-duration variety. The rice seeds were soaked in water for 24 hours and incubated in wet tissue for 48 hours. The germinated rice seeds were grown in a plastic bucket filled with experimental soil to get seedlings. The 21-day-old rice seedlings were transplanted into pots of submerged soil with different seawater mixing ratios for two weeks in April 2022 and October 2022 for the major and second rice seasons, respectively. For each pot, the rice plants were

planted 6 hills, and 3 seedlings in each hill. Water was applied twice a week using tap water. The water depth in the pots was controlled at 5-10 cm until two weeks before harvesting to maintain salinity. The urea fertilizer was applied in three equal splits at basal, maximum tillering stage, and panicle initiation stage with each treatment at the rate of 37.50 kg N ha⁻¹ according to initial soil analysis. The plant protection was done according to the requirements of the plant. The rice plants PT1 at 115 days and 110 days, and RD43 at 103 days and 100 days for the major and second rice seasons, respectively were harvested at full maturity stage when 90 % grains became golden yellow.

Table 3.4 Chemical composition in different seawater ratios.

Parameters	Seawater mixing ratios				
	2%	4%	6%	8%	10%
pH	7.35	6.92	6.76	6.58	6.50
EC (dS m ⁻¹)	1.28	2.43	3.54	4.58	5.60
Soluble Cl (mg L ⁻¹)	372.75	781.00	1118.25	1473.25	1846.00
Soluble P (mg L ⁻¹)	0.01	0.01	0.01	0.01	0.01
Soluble K (mg L ⁻¹)	13.01	20.41	27.31	37.16	42.86
Soluble Ca (mg L ⁻¹)	43.29	47.69	49.50	58.92	61.32
Soluble Mg (mg L ⁻¹)	25.52	51.04	76.56	102.08	127.60
Soluble Na (mg L ⁻¹)	243.69	446.24	622.34	837.30	1025.12
SAR	7.28	10.68	12.90	15.16	17.13
SSP (%)	78.95	86.59	89.84	91.13	92.49
Soluble S (mg L ⁻¹)	49.52	76.53	103.54	140.00	169.92
Soluble Fe (mg L ⁻¹)	0.01	0.01	0.01	0.01	0.01
Soluble Mn (mg L ⁻¹)	26.33	52.34	70.40	89.53	106.08
Soluble Zn (mg L ⁻¹)	0.53	0.57	0.58	0.48	0.46
Soluble Cu (mg L ⁻¹)	0.01	0.02	0.02	0.01	0.01

Notes: EC, electrical conductivity; Cl, chloride; P, phosphorus; K, potassium, Ca, calcium; Mg, magnesium; Na, sodium; SAR, sodium adsorption ratio; SSP, soluble sodium percentage; S, sulfur; Fe, iron; Mn, manganese; Zn, zinc; Cu, copper.

3.3.6 Data collection

The water pH and EC were measured starting from 7 days after transplanting (DAT) to 77 DAT (maturity stage) at 7-day intervals. The rice plant height and number of tillers were recorded at maximum tillering (MT), panicle initiation (PI), flowering (F), and ripening (R) stages. The plant height was measured from the ground level to the uppermost growing point of the plant. The number of tillers hill⁻¹ was counted in each pot. The SPAD value was measured on the last fully expanded 6 leaves per pot using the chlorophyll meter at PI, F, and R stages (SPAD-502 Plus Chlorophyll Meter, Spectrum Technologies Inc.). The shoot dry weight (SD) was recorded at the PI, F, and R stages. In each stage, the rice two hills pot⁻¹ from all treatments were collected and put into the oven at 70 °C for 72 hours after harvesting to get dry weight.

The fresh leaf samples were collected to analyze relative water content (RWC), chlorophyll content (Chl), hydrogen peroxide (H₂O₂), total phenol, and proline contents at the panicle initiation stage and flowering stage. To analyze H₂O₂, Chl a, Chl b, total phenol, and proline, the leaf samples were treated with liquid nitrogen and the samples were kept in a freezer at -80°C.

The yield and yield components were recorded from two hills pot⁻¹ after harvesting. The number of panicles pot⁻¹ (NP), number of spikelet panicle⁻¹ (SP), and panicle length (PL) were recorded. The filled grain (%) (FGP) was calculated as the ratio of the number of filled grains to the total number of grains. The 1000-grain weight (TGW) was determined by randomly selecting 200 seeds and converted to the weight of 1000 grains (Sakhare *et al.*, 2015). The rice grains were adjusted the moisture content to 14%, and the total grain weight per pot was recorded as grain yield (GY).

3.3.7 Relative water content (RWC)

The 10 youngest fully expanded leaves from each pot were collected and cut about 5 cm in length in the middle of the leaves. After that, the samples were weighed to get fresh weight and then put into a centrifuge tube. Then, the leaf samples were incubated in deionized water for 4 hours and weighed the leaf samples to record turgid weight. Later, the leaf samples were put into a brown paper bag and oven-dried at 70°C

for 48 hours and measured dry weight. The RWC was estimated following by Sairam *et al.* (2002).

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

3.3.8 Chlorophyll a and Chlorophyll b

Fresh leaf tissues were cut into small pieces by a scissor and 0.1 g of leaf tissues was homogenized using a mortar and pestle with 10 ml of 80% acetone. Then homogenized tissue sample was added to the test tube and the test tube was wrapped with aluminum foil and incubated at room temperature in the dark overnight. The next day, samples were vortexed and waited until the particulates were fallen to the bottom. The extract absorbance was measured at 646.8 nm and 663.2 nm using 80% acetone as a blank on a spectrophotometer. Chlorophyll a and b (mg g^{-1} fresh weight) were determined by Solangi *et al.* (2016).

Calculations

$$\text{Chlorophyll a:} = (12.25 * A_{663.2\text{nm}} - 2.79 * A_{646.8\text{nm}}) * \text{SW}$$

$$\text{Chlorophyll b:} = (21.5 * A_{663.2\text{nm}} - 5.1 * A_{646.8\text{nm}}) * \text{SW}$$

Where:

$$A_{648.6} = \text{Absorbance at 648.6 nm}$$

$$A_{664.2} = \text{Absorbance at 664.2 nm}$$

$$\text{SW} = \text{Sample Weight (fresh weight)}$$

3.3.9 Proline content

Free proline content was measured using method acid-ninhydrin method. Acid-ninhydrin was prepared by warming 1.25 g ninhydrin in 20 ml glacial acetic acid and 30 ml 6 M phosphoric acid with agitation until dissolved. It was kept cool, the reagent is stable for 24 hours. The plant material was weighed approximately 0.25g and homogenized in 10 ml of 3% aqueous sulfosalicylic acid. Then, the homogenate was filtered through filter paper (Wattman filter paper# 2). Next, the 2 ml of filtrate was reacted with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube, kept in a water bath (100°C) for 1 hour and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, and mixed vigorously with a test tube stirrer for 20-25 seconds. The chromophore containing toluene was extracted from the aqueous phase, warmed to room temperature, and the absorbance was measured at 520 nm using toluene as blank (Solangi *et al.*, 2016).

3.3.10 Determination of total phenolic compound

The total phenol content of the leaf was determined using the Folin-Ciocalteu (F-C) reagent. The fresh leaf samples (0.1 g) were weighed separately, homogenized in 95 % methanol at room temperature using a clean mortar and pestle, centrifuged in a refrigerated centrifuge at 4°C at a speed of 10,000×g for 20 min and the supernatant was saved. The methanolic extract at the sample size of 0.5 ml was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent. After 5 min, 2.5 ml of sodium carbonate (7.5%, w/v) was added, mixed, and incubated at 45°C for 45 min. The absorbance was measured using a spectrophotometer at 765 nm using the blank solution containing 0.5 ml 95% methanol, 2.5 ml 10% F-C reagent, and 2.5 ml 7.5% of sodium carbonate and gallic acid as standard (Stanković, 2011).

3.3.11 Determination of hydrogen peroxide (H₂O₂)

Hydrogen peroxide content (H₂O₂) was determined according to Velikova *et al.* (2000). Leaf tissues (500 mg) were homogenized in an ice bath with 5 ml 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12000×g for 15 min. Then,

0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide. The absorbance of the supernatant was read at 390 nm. The content of H_2O_2 was calculated using a standard curve.

3.3.12 Plant analysis

After harvesting, the total Cl content in plant was measured using the method of Mahouachi (2018). The total content of Na and K from the shoot and grain samples from each treatment were digested with aqua-regia (Bryson and Mills, 2014) and analyzed by ICP-OES. The total S contents in shoot and grain were determined (LECO Corporation, 2016). Then, the shoot and grain uptakes for each nutrient were calculated by multiplying the nutrient concentration with dry matter and grain yield, respectively. The total uptake was calculated by the combination of shoot and grain uptakes of each nutrient for all treatments and each nutrient uptake was expressed as mg plant^{-1} .

3.3.13 Soil sample collection and analysis after harvesting

The soil samples were collected from each pot after harvesting in each season and the soil samples were air-dried for 2 weeks and analyzed parameter as the same procedure as initial soil analysis.

3.3.14 Statistical analysis

The data were analyzed by a two-way ANOVA using IBM SPSS Statistics version 28. The means were compared by Duncan's Multiple Range Test (DMRT) at a probability level of $P < 0.05$.

3.4 Fourth experiment- Influence of rice straw biochar amendment on salinity in salt-affected soils

3.4.1 Experimental site

The experiment was carried out at the Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang District, Bangkok, Thailand (13°43'35.55"N, 100°46'50.79"E) in November 2023.

3.4.2 Collection of salt-affected soil

Following the third experiment, the salt-affected soils were collected from the pots. Six distinct soil types, namely S1 (0%), S2 (2%), S3 (4%), S4 (6%), S5 (8%) and S6 (10%), were identified based on their varying ratios of seawater mixing. In order to measure EC_e, the soil samples were crushed and passed through a 2 mm sieve and incubation experiment was carried out. The salt-affected soils in S1, S2, S3, S4, S5, and S6 had EC_e values of 1.91, 2.53, 4.02, 4.37, 4.99, and 5.93 dS m⁻¹, respectively. The soil EC_e < 2, 2-4, and 4-8 dS m⁻¹ were classified as non-saline, low-saline, and moderate-saline, respectively, by the US Salinity Laboratory staff (Yang *et al.*, 2022). As a result, S2 and S3 were low saline, S4 through S6 were moderate saline, and S1 was non-saline.

3.4.3 Collection of biochar

Using a modified small-scale biochar kiln, biochar was produced from rice straws for two hours at a pyrolysis temperature of roughly 500 °C (Petchaihan *et al.*, 2020; Yaashikaa *et al.*, 2020). 30–50% biochar was produced on average from rice straw. To mix with the soils, the biochar was sieved through 0.1 mm. The chemical composition of rice straw biochar was demonstrated in Table 3.5.

Table 3.5 The chemical compositions of rice straw biochar.

Name	pH	EC (dS m ⁻¹)	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	Total K (g kg ⁻¹)	Total Ca (g kg ⁻¹)	Total Mg (g kg ⁻¹)	Total Na (g kg ⁻¹)	CEC (cmol _c kg ⁻¹)
Rice straw biochar	7.98	1.98	533	9.32	28.47	5.38	2.71	0.63	21.20

3.4.4 Methods of biochar analysis

The pH and EC were measured at 1:5 (biochar: water) by pH and EC meter (CONSORT C830). The total sulfur (S) was measured by a CNS analyzer (LECO Corporation, 2016). The total Na, K, Ca, and Mg were analyzed by an inductively coupled plasma optical emission spectrometer (ICP-OES) after digestion with aqua regia (Chen *et al.*, 2023). The cation exchange capacity (CEC) was estimated by 1N ammonium acetate, pH 7.0 method (Saidi, 2012).

3.4.5 Scanning electron microscope (SEM), Energy-dispersive X-ray (EDX), and Fourier transform infrared spectroscopy (FT-IR) analysis

According to the Brunauer–Emmett–Teller (BET) method, the specific surface area and total porosity of rice straw biochar were assessed using the scanning electron microscopy (SEM) image and X-ray spectroscopy (EDX) spectra (Brunauer *et al.*, 1938). Fourier transform infrared spectroscopy (FT-IR) was used to determine the functional group of the rice straw biochar (model: Perkin Elmer Spectrum One). All of the spectra were obtained within the 500–4000 cm⁻¹ wavenumber range.

The elemental distribution and morphological data are provided by the EDX and SEM (Afroze *et al.*, 2020). The study's SEM picture of the rice straw biochar was displayed in Figure 3.5 (A). Carbon and oxygen were the main components of the biochar made from rice straw. Among the other components, the biochar's K content was the greatest (Figure 3.5 B). According to Liu *et al.* (2017), the specific surface area and total pores volume of the rice straw biochar were 3.10 m² g⁻¹ and 0.0025 cm³ g⁻¹, respectively, within the typical range. The functional groups of the rice straw biochar were determined by the FT-IR

spectra (Figure 3.6). Table 3.6 provided a description of the literature and functional groupings.

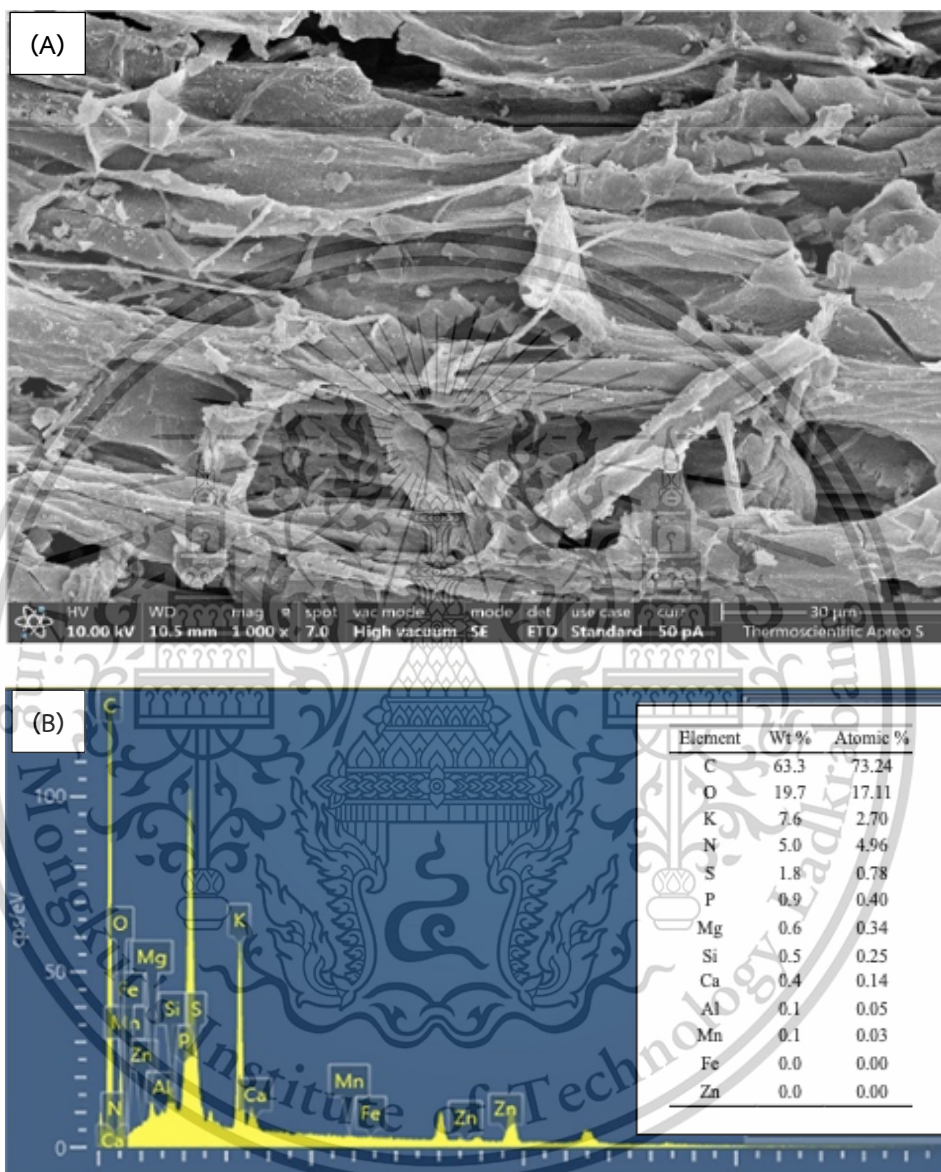


Figure 3.5 Scanning electron microscopy (SEM) image (1000x magnification) (A) and X-ray spectroscopy (EDX) spectra (B) of rice straw biochar.

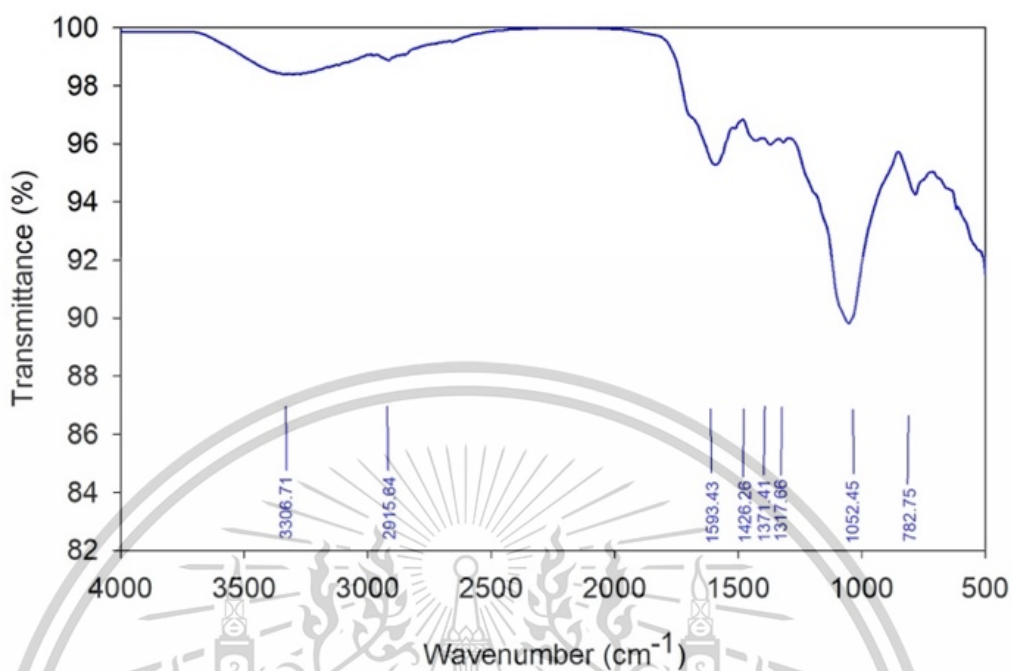


Figure 3.6 Fourier Transform Infrared (FT-IR) spectra of rice straw biochar at 500°C.

Table 3.6 Functional groups of rice straw biochar and literature data by Fourier Transform Infrared spectroscopy (FT-IR) analysis.

Functional group	Class	Intensity	Wavenumber (cm ⁻¹)	
			In Literature (Nandiyanto <i>et al.</i> , 2023)	Rice straw biochar
O-H stretching	alcohol	Strong	3200-3550	3306.71
C-H stretching	alkane	Medium	2840-3000	2915.64
C=C stretching	cyclic alkene	Medium	1566-1650	1593.43
O-H bending	carboxylic acid	Medium	1395-1440	1426.26
O-H bending	alcohol	Medium	1330-1420	1371.41
S=O stretching	sulfone	Strong	1300-1350	1317.66
C-O stretching	primary alcohol	Strong	1050-1085	1052.45
C-H bending	1,2,3- trisubstituted	Strong	760-800	782.75

3.4.6 Incubation experiment

The incubation experiment was designed using a 4 × 6 factorial structure with two components and three replications (factor B: six acid soils impacted by salt, and factor A: four doses of biochar application). Six salt-affected acid soil samples were weighed to four grams, which were then combined with the sieved biochar at 1%, 3%, and 5% (w/w) ratios. As control treatments, six salt-affected acid soils without biochar (0%) were measured. The 30 ml centrifuge tube was filled with 20 milliliters of distilled water and shaken continuously at 150 rpm/min for seven days. Based on the soil to water ratio of the incubation condition, the soil suspension was analyzed for soil pH and EC at the 1:5 soil water ratio. The soil suspension was filtered through a 0.45 µm membrane after being centrifuged for 10 minutes at a speed of x10000 g. Then, the soluble Cl^- , soluble K^+ , Ca^{2+} , Mg^{2+} , and Na^+ in the soil-extracted solution were measured. The soil and biochar combination was extracted using 1M ammonium acetate at pH 7, and the exchangeable bases K^+ , Ca^{2+} , Mg^{2+} , and Na^+ were measured.

3.4.7 Methods of soil analysis

The soil pH and EC were measured using a pH and EC meter (CONSORT C830). The soil Ece for incubation experiment was calculated from EC 1:5 solution by conversion factor (Gharaibeh *et al.*, 2021). Thus, Ece was calculated from the EC of soil water extracts (1:5). Using standard silver nitrate (0.01 N) with a 5 % potassium chromate indicator, the Cl^- content was measured by the titration method (Richards, 1954). The soluble cations, such as Na^+ , K^+ , Ca^{2+} , and Mg^{2+} in the soil-extracted solution, were analyzed by ICP-OES. Exchangeable basic cations (Ca^{2+} , Mg^{2+} , K^+ , and Na^+) were extracted by 1N ammonium acetate solution at a pH of 7 (Pansu and Gautheyrou, 2006) and determined by using an Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES). The SAR and ESP were calculated as expressed in equations (1) and (2), respectively.

3.4.8 Statistical analysis

Two-way analysis of variance (ANOVA) was performed to analyze the effect of different biochar application rates on six salt-affected soils and their interactions according to factorial laid out. One-way ANOVA was used to analyze the mean-variance in each

biochar application rate. Means were compared by Duncan's Multiple Range Test (DMRT) at $P < 0.05$ level. All statistical analysis used IBM SPSS Statistics version 28.



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CHAPTER 4

RESULTS AND DISCUSSION

4.1 First experiment- Assessment of water quality variation for agriculture in the Bang Pakong River of Thailand

4.1.1 pH

From November 2021 to April 2022, the pH value was determined at 13 Bang Pakong River sample locations (Table 4.1). The pH readings in November, February, and March did not change significantly from one another. In contrast, there were notable variations in pH at every test location in December, January, and April. This finding indicates that, in comparison to January, the pH value was lower in November, December, February, March, and April. The pH ranges with the highest averages were 6.89 to 7.11, 6.89 to 7.32, 6.22 to 6.92, 7.12 to 7.56, 6.94 to 7.16, and 7.07 to 7.26, in that order. With the exception of November, R1 consistently had the lowest pH value. Among the sampling points in each month, R11, R14, R8, R2, and R7 showed the highest values. In this investigation, the pH value was less than 7.00 in other months but over 7.00 in January and April. In surface water systems, the pH typically ranged from 6.5 to 8.5. The higher pH value indicated that the water samples had high levels of bicarbonate, carbonate, and chloride, indicating that the water was alkaline (Maraz *et al.*, 2021).

4.1.2 Electrical conductivity

From November 2021 to April 2022, the EC value was determined at 13 sampling locations along the Bang Pakong River (Figure 4.1). With the exception of November, the EC value varied significantly across all sampling points for every month of collection. Out of all the collecting months, November had the lowest EC value, while February had the greatest value. From 6.8 to 13.9 mS cm⁻¹, 0.3 to 0.4 mS cm⁻¹, 0.2 to 0.5 mS cm⁻¹, and 0.3 to 2.5 mS cm⁻¹, 0.4 to 0.8 mS cm⁻¹, were the range of average EC values. For every month, but November, R13 had the minimum values among the sampling points. With the exception of March and April, all months had maximum EC values in R1.

One important criterion for determining whether water was suitable for irrigation was the EC, or the concentration of salt content in the water (Haritash *et al.*, 2016). Water was categorized as non-saline ($<0.7 \text{ mS cm}^{-1}$), somewhat saline ($0.7\text{-}2.0 \text{ mS cm}^{-1}$), moderately saline ($2\text{-}10 \text{ mS cm}^{-1}$), extremely saline ($10\text{-}25 \text{ mS cm}^{-1}$) and very highly saline ($25\text{-}45 \text{ mS cm}^{-1}$) based on its EC value. Water having an EC of less than 0.7 mS cm^{-1} is suitable for irrigation without any limitations. EC more than 3.0 mS cm^{-1} showed severe restrictions on usage for irrigation, while EC within the range of $0.7\text{-}3.0 \text{ mS cm}^{-1}$ had modest to moderate restrictions (Roy *et al.*, 2020). Among the collection months in this investigation, February produced the highest EC value (13.90 mS cm^{-1}), and the water at all places is both moderately and extremely salinized. But from November to January, the EC value was less than 0.7 mS cm^{-1} . During the rainy season, the precipitation seeped into the ground and leached lower to replenish groundwater, which might refresh and dilute the salt of the soil water or groundwater (Wang *et al.*, 2012). In addition, R1 had the highest value when compared to the other points. Given that the R1 was situated close to the coast, this could be caused by seawater from the Gulf of Thailand.

4.1.3 Salinity

With the exception of November, every month saw a considerable increase in the salinity value across sample stations (Figure 4.1). The 13 water samples from the Bang Pakong River had varying salinity contents over a period of six months: 0.09 to 0.14 g L^{-1} , 0.16 to 0.23 g L^{-1} , 0.15 to 0.30 g L^{-1} , 4.35 to 8.89 g L^{-1} , 0.20 to 1.15 g L^{-1} , and 0.28 to 0.50 g L^{-1} . Among the collecting times, February had the highest salinity. With the exception of November, all collections showed R13 to have the lowest values. R1 displayed the highest values in every month but April. Among the collecting months in this investigation, February had the highest salinity. Due to precipitation in this area, the lower figures were displayed in November, December, January, March, and April. When there was no precipitation in the area during the dry season, the salinity of the groundwater rose dramatically. The groundwater's dilution of salt was affected by the precipitation. When there was enough rainfall, the salinity of the groundwater was reduced and the salt content was diffused (Yan *et al.*, 2015). Because of the diluting effect of the water's ionic

composition from monsoon-season precipitation, the prior study's results showed lower levels of salinity and EC (Huang *et al.*, 2014).

Table 4.1 The changes of pH value in water sample from the Bang Pakong River in different collections time, 2021-2022.

Sample	pH					
	November	December	January	February	March	April
	2021	2021	2022	2022	2022	2022
R1	7.32a	6.22b	7.12c	6.89a	6.94a	7.07b
R2	6.9a	6.70ab	7.36abc	6.94a	7.16a	7.16ab
R3	7.07a	6.78ab	7.37abc	6.98a	7.09a	7.23ab
R4	6.92a	6.81a	7.56a	6.97a	7.02a	7.25ab
R5	6.88a	6.91a	7.42ab	7.04a	7.13a	7.23ab
R6	6.79a	6.87a	7.44ab	7.04a	7.13a	7.22ab
R7	6.63a	6.86a	7.38abc	7.02a	6.98a	7.26a
R8	6.55a	6.60ab	7.21bc	7.11a	7.05a	7.21ab
R9	6.54a	6.91a	7.37abc	7.05a	7.09a	7.22ab
R10	6.57a	6.88a	7.39abc	7.01a	7.15a	7.21ab
R11	6.57a	6.92a	7.42ab	7.01a	6.99a	7.22ab
R12	6.56a	6.78ab	7.52a	7.05a	7.02a	7.13ab
R13	6.53a	6.61ab	7.41abc	7.06a	7.08a	7.14ab
F test	ns	**	**	ns	ns	*
CV %	5.46	3.21	1.70	1.01	1.23	0.85

Notes: R1 to R13 referred to water sampling points in the river, numbered from 1 to 13. The letters in same column indicated no significant difference. ns- no significant difference, * - significant at 0.05 level, ** - significant at 0.01 level by DMRT.

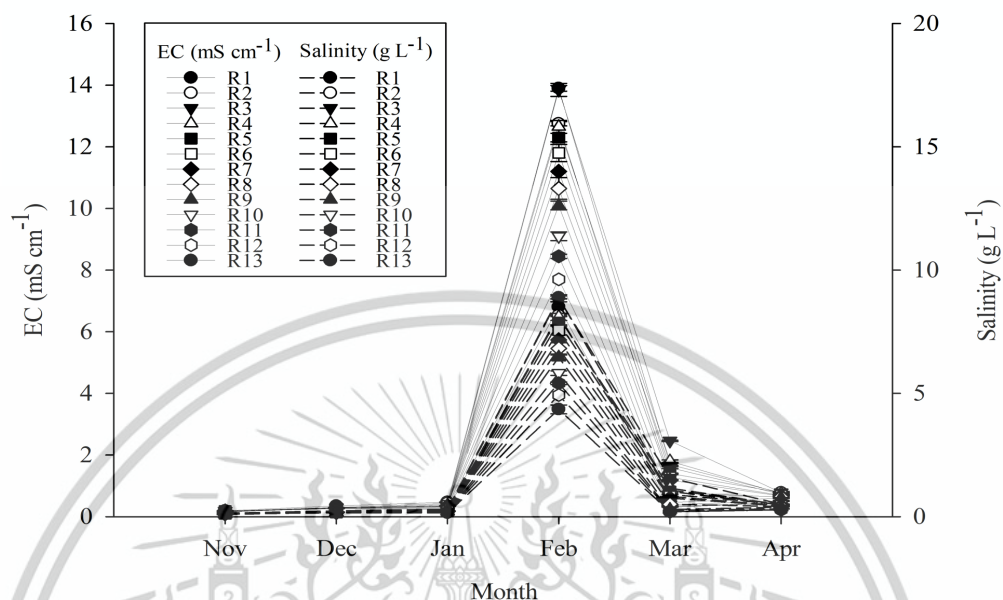


Figure 4.1 The monthly variations in the Bang Pakong River water sample's EC and salinity values in 2021-2022.

Notes: R1 to R13 referred to water sampling points in the river, numbered from 1 to 13. EC, electrical conductivity; Nov, November; Dec, December; Jan, January; Feb, February; Mar, March; Apr, April.

4.1.4 Calcium

From November 2021 to April 2022, 13 sampling sites in the Bang Pakong River were used to measure the calcium value (Figure 4.2). In November, December, and April, the Ca^{2+} value did not alter statistically from sample to sampling point; in January, February, and March, there was a highly significant variation. Among the collection months, November typically had the lowest Ca^{2+} value and February had the highest Ca^{2+} value. Monthly variations in the Ca^{2+} concentration was observed, with values ranging from 0.3 to 0.5 meq L^{-1} , 0.6 to 0.7 meq L^{-1} , 0.5 to 0.7 meq L^{-1} , 2.9 to 5.2 meq L^{-1} , 0.6 to 1.4 meq L^{-1} , and 0.7 to 0.8 meq L^{-1} . R1 provided the maximum Ca^{2+} values from December to February, while R3 provided the values for November, March, and April. All save November and April had the lowest value for the R13.

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4.1.5 Magnesium

From November 2021 to April 2022, 13 sampling stations in the Bang Pakong River were used to measure the magnesium value (Figure 4.2). Except for November, there was a highly significant variation in the Mg^{2+} level at every sampling location for every month. Among the collection months, November had the lowest Mg^{2+} value and February had the highest value. In each month, the Mg^{2+} content varied from 0.2 to 0.4 meq L^{-1} , 0.5 to 0.7 meq L^{-1} , 0.5 to 0.8 meq L^{-1} , 10.7 to 22.2 meq L^{-1} , 0.6 to 3.7 meq L^{-1} , and 0.7 to 1.2 meq L^{-1} . R1 provided the maximum Mg^{2+} values from December to March, while R3 provided the values in November, March, and April. Out of all the collection months, the Mg^{2+} value in the R13 was the lowest. In this investigation, the Mg^{2+} concentration in the water sample was lower than the Ca^{2+} concentration in the first three months, but it was higher than the Ca^{2+} concentration from February to April. In the freshwater body, Ca^{2+} and Mg^{2+} naturally maintain an equilibrium state. A high Mg^{2+} level in water caused the water to become alkaline, which has a negative impact on crop output and soil quality (Kumar *et al.*, 2007).

4.1.6 Sodium

From November 2021 to April 2022, 13 sampling stations along the Bang Pakong River were used to measure the sodium value (Figure 4.3). While it was very substantially different in other months, the Na^+ content in November was not significantly different at any stages. Among the collecting months, November had the lowest Na^+ value and February had the highest value. Monthly variations in the Na^+ content was observed: 0.4 to 0.7 meq L^{-1} , 1.2 to 1.6 meq L^{-1} , 1.1 to 2.4 meq L^{-1} , 40.8 to 84.4 meq L^{-1} , 1.4 to 13.9 meq L^{-1} , and 1.7 to 3.5 meq L^{-1} . Furthermore, among the sampling points, R1 provided the largest Na^+ values from December to February, R5 in November, R3 in March, and R2 in April. Among all the months, the R13 had the lowest Na^+ value. River water had a higher Na^+ level than Mg^{2+} and Ca^{2+} . High Na^+ irrigation water has the potential to displace exchangeable cations from the soil's clay minerals, such as Ca^{2+} and Mg^{2+} , and then replace the cations with Na^+ . Soil that was saturated with sodium lowers its permeability, which reduced its productivity for farming (Islam and Shamsad, 2009).

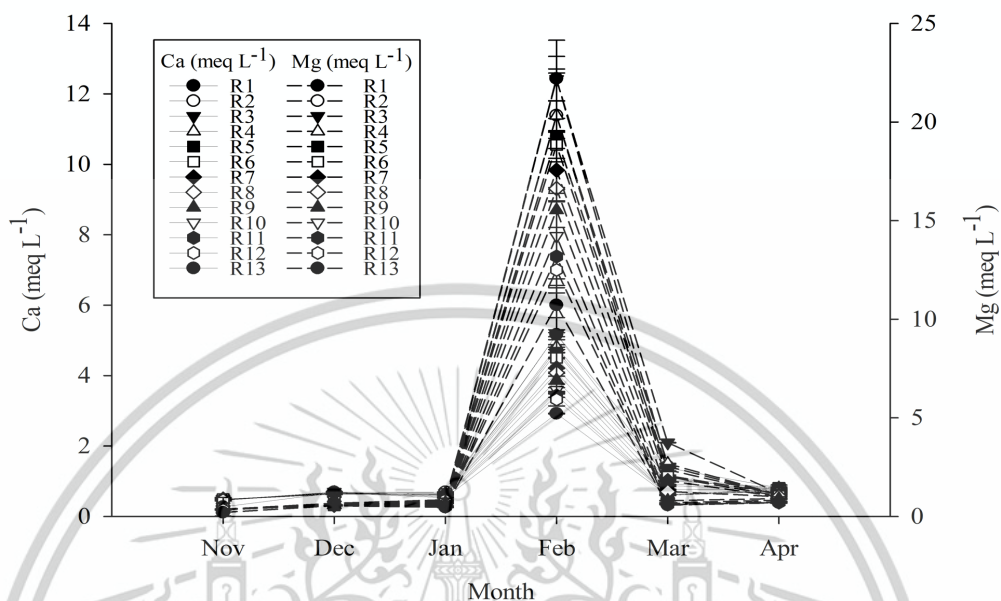


Figure 4.2 The monthly variations in the Bang Pakong River water sample's Ca^{2+} and Mg^{2+} values in 2021-2022.

Notes: R1 to R13 referred to water sampling points in the river, numbered from 1 to 13. Ca, calcium; Mg, Magnesium; Nov, November; Dec, December; Jan, January; Feb, February; Mar, March; Apr, April.

4.1.7 Sodium adsorption ratio (SAR)

From November 2021 to April 2022, 13 sampling stations in the Bang Pakong River were used to calculate the SAR value (Figure 4.3). With the exception of November, the SAR value differed highly considerably across all sampling points for every month. Generally, among the collecting months, the SAR value was highest in February and lowest in November. From 0.7 to 1.1, 1.5 to 2.0, 1.6 to 2.7, 15.6 to 22.8, 1.8 to 7.2, and 2.0 to 3.6, respectively, were the average SAR values. R13 was the minimum value over all months' sampling points. R1 experienced the highest SAR values from November to February, followed by R4 in March and R2 in April. Of the collection points, R1 had the highest Na^+ content (Figure 4.3). The R1's proximity to the estuary suggests that the seawater originating from the Gulf of Thailand may be the cause of this. As a result, R1

had a larger SAR value than the other sites. High soil acidity ratio (SAR) in irrigation water indicates that there was a risk that Na^+ may replace Ca^{2+} and Mg^{2+} in the soil through the cation exchange process. This will harm the soil's structure over time, lowering its fertility status and decreasing crop output (Gupta, 2005). Plants have very little access to water when the SAR was high since there was a noticeable decrease in the infiltration rate (Saleh, 2016).

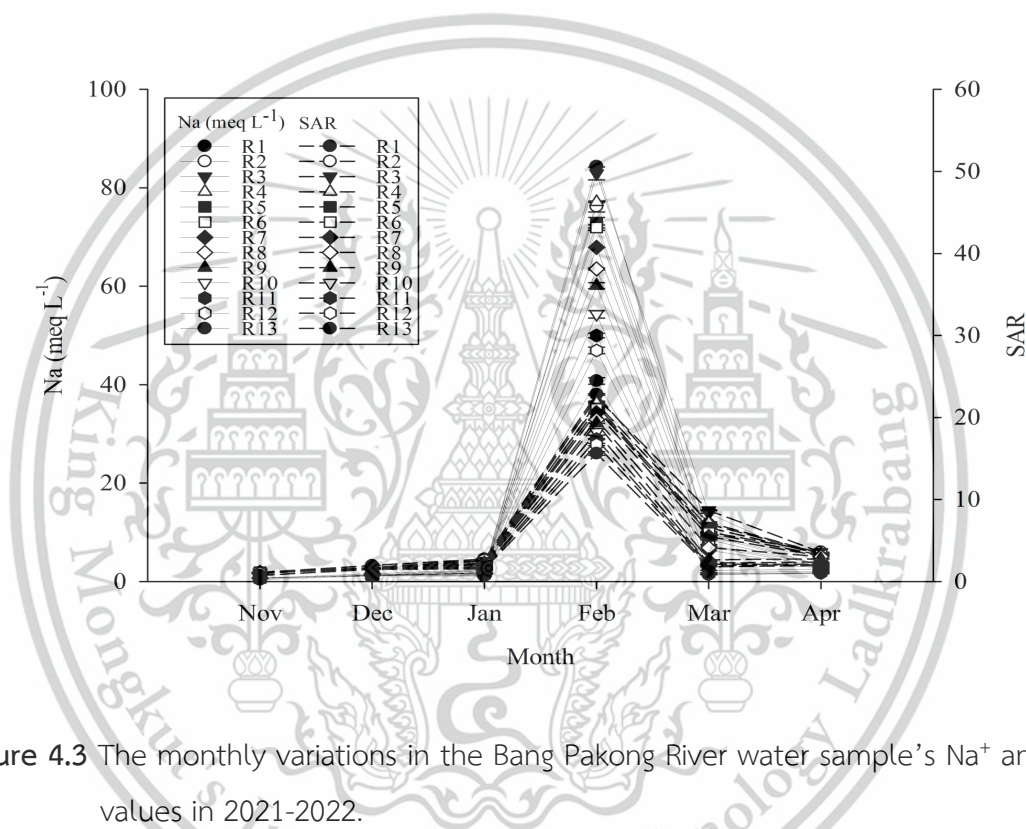


Figure 4.3 The monthly variations in the Bang Pakong River water sample's Na^+ and SAR values in 2021-2022.

Notes: R1 to R13 referred to water sampling points in the river, numbered from 1 to 13. Na, sodium; SAR, sodium adsorption ratio; Nov, November; Dec, December; Jan, January; Feb, February; Mar, March; Apr, April.

4.1.8 Chloride

There were notable variations in the chloride content between the sampling locations in January, February, March, and April (Figure 4.4). That was not appreciably different in November or December, though. The thirteen water samples taken from the

Bang Pakong River had varying Cl^- contents over a six-month period: 0.0 to 1.1 meq L^{-1} , 1.0 to 1.6 meq L^{-1} , 1.1 to 2.7 meq L^{-1} , 55.3 to 117.0 meq L^{-1} , 1.6 to 15.5 meq L^{-1} , and 1.8 to 4.4 meq L^{-1} . It was discovered that February had the highest Cl^- values among the collecting times. November's sample points displayed the highest Cl^- value in R13. However, from December to February, the greatest Cl^- value produced R1. The highest Cl^- content was reported in March by R2, while the lowest value was noted in R13. In March and April, the R3 and R2 provided the highest Cl^- content of any sites. Crops benefited from the minimal amount of Cl^- in irrigation water, but plant viability was impacted by the high Cl^- content. According to Hamza (2012), the typical limit of Cl^- was 10 meq L^{-1} , or 300 mg L^{-1} . Nevertheless, the plant leaves were adversely impacted by the Cl^- concentration of 4 meq L^{-1} (140 mg L^{-1}) (Al-Shammiri *et al.*, 2005). According to the findings, the study's maximum Cl^- (117.00 meq L^{-1}) content happened in February. In February, it exceeded the permissible limit. Additionally, in several collection points in March and April, the Cl^- value was higher.

4.1.9 Sulfate

From November 2021 to April 2022, the Bang Pakong River's 13 sampling locations had their sulfate value measured (Figure 4.4). With the exception of November, there were notable differences in the SO_4^{2-} content between sampling points in every month. In November, it was noted that the water sample from the River did not include SO_4^{2-} . Among the collecting months, December had the lowest SO_4^{2-} value while February had the highest value. The monthly variations in SO_4^{2-} content were as follows: 6.88 to 8.32 mg L^{-1} , 6.09 to 9.14 mg L^{-1} , 101.36 to 211.54 mg L^{-1} , 7.17 to 31.26 mg L^{-1} , and 11.63 to 13.07 mg L^{-1} . Additionally, R1 provided the maximum SO_4^{2-} values from December to March while R3 provided the values in April. All months but December had the lowest sulfate value according to the R13. Despite being a significant cause of salinity, SO_4^{2-} toxicity was only an issue in very small amounts, when it might potentially obstruct the absorption of other nutrients (Ibrahim, 2014). It was found in nature in large quantities, and concentrations ranging from a few to several thousand milligrams per liter may be found in natural waterways. According to the Iowa Department of Natural Resources

(2009), 250 mg L^{-1} was the permissible maximum. February saw the highest SO_4^{2-} value, which was followed by March and April. In all cases, though, the value in February was below the allowable level.

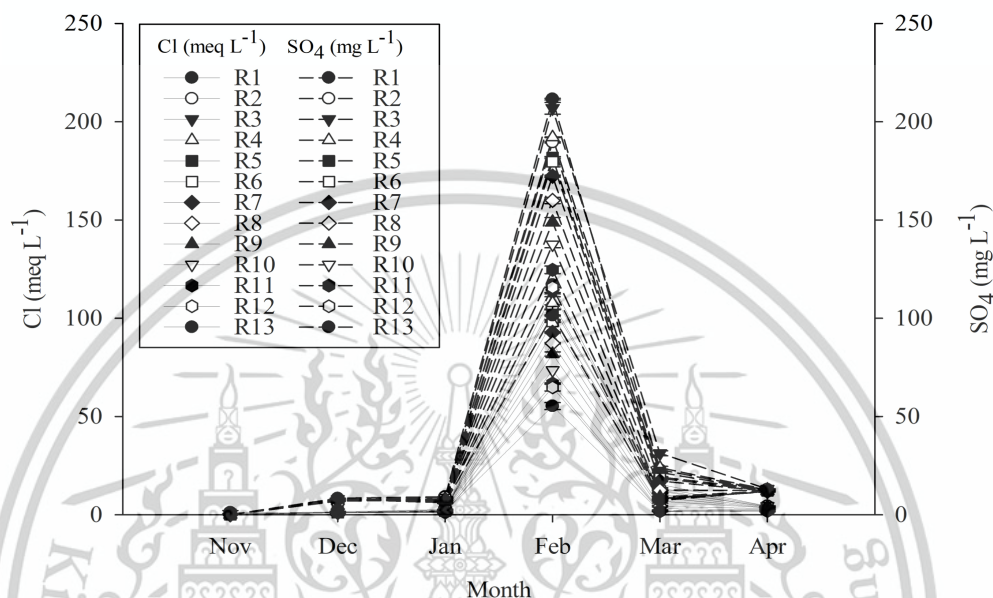


Figure 4.4 The monthly variations in the Bang Pakong River water sample's Cl^- and SO_4^{2-} values in 2021-2022.

Notes: R1 to R13 referred to water sampling points in the river, numbered from 1 to 13. Cl, chloride; SO_4 , sulfate; Nov, November; Dec, December; Jan, January; Feb, February; Mar, March; Apr, April.

4.1.10 Seawater mixing ratio

The lowest chlorine concentration of the water sample was taken as the chloride content of fresh water. The Bang Pakong River's seawater mixing ratio was altered once a month (Figure 4.5). While there was no significant difference in the seawater mixing ratio in December, there were substantial differences in other months. In December, it was -0.02 to 0.23 %; in January, it was 0.08 to 0.45 %; in February, it was 11.82 to 25.40 %; in March, it was -0.04 to 3.87 %; and in April, it was 0.11 to 3.31 %. For every month but December, there was a noticeable variation in the seawater mixing ratio at the

sampling locations. Among the collecting months, R2 had the greatest values in January and March, R1 in February, and R3 in April; R13 had the lowest mixing ratio. Among the sampling stations, R1, R2, and R3 produced the highest seawater mixing ratio. This could be because the Gulf of Thailand is close to these sampling locations. The lowest value, however, was found in R13. In places that were farther from the sea, there was less mixed seawater.

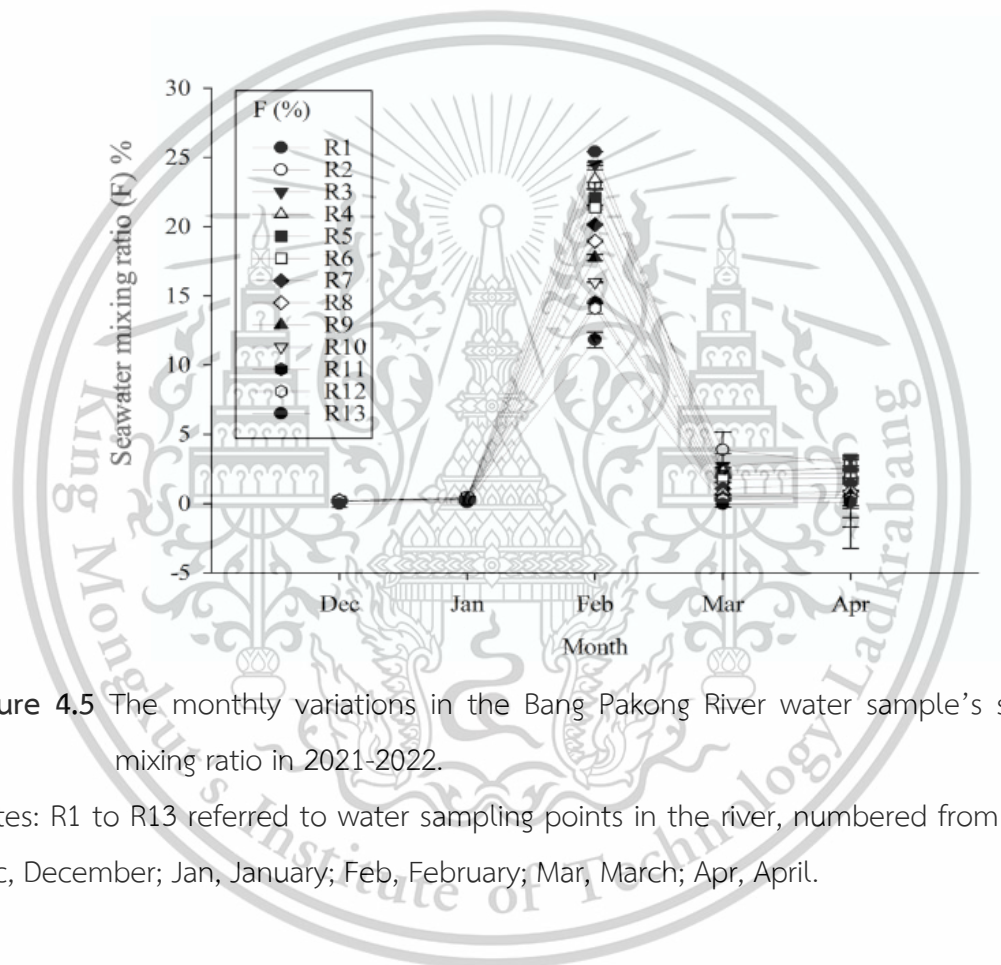


Figure 4.5 The monthly variations in the Bang Pakong River water sample's seawater mixing ratio in 2021-2022.

Notes: R1 to R13 referred to water sampling points in the river, numbered from 1 to 13.

Dec, December; Jan, January; Feb, February; Mar, March; Apr, April.

4.2 Second experiment- Impact of different seawater mixing ratios on sodium sorption capacity in soil

4.2.1 Sodium adsorption

The soil, seawater mixing ratios and the interaction between soil and seawater mixing ratios had significant effects on sodium adsorption (Table 4.2). The Na adsorption in Chachoengsao soil was significantly higher ($P < 0.01$) than that of Rangsit soil. The Na adsorption value was significantly increased ($P < 0.01$) in 20% seawater mixing ratios which was not significantly varied from 15% and 25% seawater mixing ratios, however, it was significantly different from other treatments. The higher equilibrium Na increased the Na adsorption in two different soils (Figure 4.6). In Chachoengsao soil, the maximum value of Na adsorption found in equilibrium Na was 2000 mg L⁻¹ and it was decreased over 2500 mg L⁻¹ of equilibrium Na. However, the highest Na adsorption in Rangsit soil was observed in the maximum equilibrium Na which was over 2500 mg L⁻¹. This might be because the different soils responded to different seawater mixing ratios. The excess amount of Na in the soil competed with K⁺ and Ca²⁺, that reduced their ability to crops (Machado and Serralheiro, 2017); it caused K deficiency (Kibria and and Hoque, 2019) and Ca deficiency in plant (Rodríguez Coca *et al.*, 2023). Therefore, the soils with high concentration of exchangeable Na negatively affected on plant growth by dispersion of soil particles, and nutrient deficiencies and imbalances (Machado and Serralheiro, 2017). Then, it caused water infiltration, air movement, water holding capacity, root penetration, and seedling emergence problems (Worku and Bedadi, 2016). The Na ion in the soil might be toxic to the crops (Ogunfowokan *et al.*, 2013). The entry of Na⁺ into the plant cells from the soil disturbed the ion balance in plant and appeared many injuries concerning the physiology of plant tissues like root, leaf and grain (Hussain *et al.*, 2019).

Table 4.2 Effects of various seawater mixing ratios on sodium adsorption in two different soils.

Treatment	Adsorption Na (mg kg ⁻¹)
Soil	
Chachoengsao (Cc)	796.18a
Rangsit (Rs)	212.93b
Seawater mixing ratio (%)	
0% (control)	0
5%	0
10%	577.90b
15%	991.40a
20%	1087.00a
25%	884.30ab
F test	
Soil	**
Seawater mixing ratio	**
Soil × Seawater mixing ratio	**
CV %	4.67

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

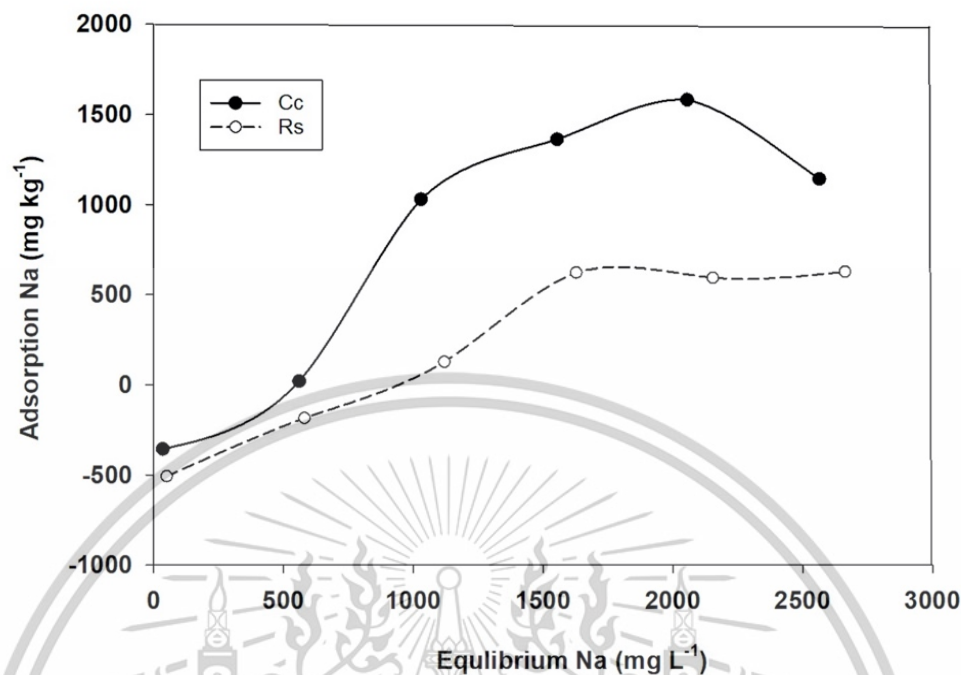


Figure 4.6 The changes of maximum amount of adsorbed Na depending on equilibrium Na concentration in two soils.

4.3 Third experiment- Seawater intrusion effects on growth and yield of rice by changing of biochemical synthesis and soil properties in Chachoengsao soil

4.3.1 Salinity effects on water pH

The water pH value was significantly affected by seawater intrusion in the major and second rice seasons (Table 4.3 and 4.4). The data indicated that the water pH was not significantly different between PT1 and RD43 rice varieties for two seasons. The water pH value was significantly varied among different seawater mixing ratios at all growth stages in both seasons except 7DAT, 14DAT, 21DAT, and 63DAT in the second rice season. The highest pH was found in 0% and the lowest value occurred in 10% seawater applications. The pH value was between 4.30- 7.0 in the major rice season, however, the value was between 5- 7 in the second rice season. The seawater treatments 6% and higher caused a reduction in pH value. According to this study, the water pH was under

7.0 in both seasons. The pH between 5.5 and 6.5 was suitable for rice cultivation. The previous study reported that the saline irrigation water did not affect soil pH during the growing period (Abedinpour, 2016). Furthermore, the previous author described that the saline soils have a pH that does not rise above 8.2. The accumulation of soluble salts reduces the pH of saline soils (Dhouha, 2015). In addition, soil pH was the most important factor in the nutrient availability of soils. In most cases, the range of pH 6.0 – 7.5 was optimum for the sufficient availability of nutrients in the soil. There was no interaction between variety and seawater treatments in this study.

4.3.2 Salinity effects on water EC

The seawater intrusion significantly affected on water EC in the major and second rice seasons (Table 4.5 and 4.6). The water EC did not vary significantly between PT1 and RD43 rice varieties at all stages except 77 DAT in both rice seasons. The water EC was significantly different ($P < 0.01$) among seawater applications. The higher seawater ratios increased water EC. The highest value was observed in 10% whereas the lowest value occurred in 0%. When seawater ratio increased to 8% and 10%, the EC value was between 4 dS m^{-1} and 5 dS m^{-1} . Additionally, it was observed that the EC value was decreasing with increasing plant growth during the growing season. Because EC was the total amount of ions in the water that was a mixture of salt and nutrients, therefore, these nutrients might be taken up by the plants. The soil EC value increased with increasing salinity of irrigation water and it is directly proportional to the salt concentration in the irrigation water (Sheferia *et al.*, 2021). The previous study stated that the threshold limit of EC for irrigation water was 3 dS m^{-1} (Adeyemi *et al.*, 2019). In this study, the EC value was more than 3.0 dS m^{-1} in seawater ratios $> 6 \%$. Hence, seawater intrusion ratios $> 6 \%$ were not suitable for irrigation to the crops. The available water for plants in the soil solution decreases drastically as EC increases. The effect of high EC on crop productivity was the inability of plants for water to compete with ions in the soil solution. The higher the EC, the less water was available to the plant although the soil appeared wet (Udom *et al.*, 2019).

Table 4.3 Effects of rice variety and seawater intrusion on water pH in rice crop in the major rice season.

Treatment	pH at DAT in the major rice season										
	7	14	21	28	35	42	49	56	63	70	77
Variety											
Pathumthani 1	4.76	6.59	5.87	5.81	6.41a	6.49	6.34	6.53	6.66	6.83	6.77
RD43	4.80	6.30	5.80	5.82	6.17b	6.47	6.33	6.47	6.59	6.68	6.75
Seawater											
0%	5.27a	7.32a	6.75a	6.62a	6.56a	6.78a	6.70a	6.81a	6.85a	7.00a	6.98
2%	4.99ab	7.37a	6.43ab	6.35a	6.50ab	6.58ab	6.52ab	6.75ab	6.80a	7.02a	6.93
4%	4.43b	6.55ab	5.96bc	6.14ab	6.42abc	6.62ab	6.46abc	6.57abc	6.79a	6.91a	6.82
6%	4.30b	6.34abc	5.70bc	5.60bc	6.19bcd	6.38ab	6.26abc	6.39abc	6.62ab	6.66ab	6.69
8%	4.64ab	5.94bc	5.23cd	5.21c	6.04cd	6.46ab	6.16bc	6.24c	6.48ab	6.45b	6.49
10%	5.06ab	5.19c	4.93d	4.99c	5.95d	6.06b	6.02c	6.27bc	6.21b	6.52b	6.64
F test											
Variety	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns
Seawater	**	**	**	**	**	*	**	**	**	**	ns
Variety X Seawater	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV%	9.56	10.70	7.11	7.14	3.82	5.29	4.20	4.31	3.60	3.17	4.70

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

Table 4.4 Effects of rice variety and seawater intrusion on water pH in rice crop in the second rice season.

Treatment	pH at DAT in the second rice season										
	7	14	21	28	35	42	49	56	63	70	77
Variety											
Pathumthani 1	5.71	5.54a	5.42a	6.00a	5.95	6.07	6.17	6.39	6.54	6.48	6.37
RD 43	5.51	5.17b	5.09b	5.56b	5.85	5.98	6.11	6.41	6.52	6.50	6.27
Seawater											
0%	5.82	5.47	5.44	6.14a	6.17a	6.26a	6.36a	6.65a	6.76	6.72a	6.54a
2%	5.63	5.40	5.42	6.02ab	6.06ab	6.09ab	6.21ab	6.51ab	6.63	6.66a	6.39ab
4%	5.92	5.36	5.35	5.63ab	5.99ab	6.04ab	6.21ab	6.52ab	6.61	6.59ab	6.38ab
6%	5.32	5.49	5.15	5.74ab	5.79ab	5.92b	6.06ab	6.35ab	6.45	6.38ab	6.30ab
8%	5.33	5.14	5.11	5.66ab	5.69ab	5.96b	6.02b	6.25b	6.48	6.38ab	6.26ab
10%	5.66	5.27	5.06	5.50b	5.67b	5.88b	5.98b	6.14b	6.24	6.21b	6.03b
F test											
Variety	ns	**	**	**	ns	ns	ns	ns	ns	ns	ns
Seawater	ns	ns	ns	*	**	**	**	**	ns	*	*
Variety x Seawater	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV %	6.32	7.03	6.22	5.48	4.13	2.60	2.87	3.43	4.57	3.86	3.68

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

Table 4.5 Effects of rice variety and seawater intrusion on water EC in rice crop in the major rice season.

Treatment	EC (dS m ⁻¹) at DAT in the major rice season										
	7	14	21	28	35	42	49	56	63	70	77
Variety											
Pathumthani 1	5.98	3.54	3.73	3.95	3.15	3.11	3.21	2.79	2.72	2.71	2.66b
RD 43	5.83	3.43	3.43	3.80	3.00	2.64	3.04	2.66	2.61	2.91	3.82a
Seawater											
0%	3.28c	2.06e	2.18d	2.24e	1.73d	1.60c	1.67d	1.46d	1.36d	1.43d	1.75
2%	4.55bc	2.59de	2.65cd	2.81de	2.07d	2.01bc	2.06cd	1.68d	1.62d	1.75d	2.09
4%	5.17b	3.16cd	3.24bcd	3.31cd	2.61cd	2.54bc	2.56c	2.18cd	2.15cd	2.29cd	2.63
6%	6.79a	3.80bc	4.00abc	4.18bc	3.44bc	3.26ab	3.48b	2.89bc	2.90bc	3.10bc	3.60
8%	7.40a	4.42ab	4.42ab	5.12ab	4.18ab	3.59ab	4.20ab	3.80ab	3.61ab	3.83ab	4.22
10%	8.23a	4.91a	5.00a	5.58a	4.45a	4.24a	4.80a	4.33a	4.35a	4.45a	5.14
F test											
Variety	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
Seawater	**	**	**	**	**	**	**	**	**	**	**
Variety × Seawater	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV%	14.26	17.24	22.46	13.71	16.40	31.87	14.53	20.09	19.71	20.03	17.81

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

Table 4.6 Effects of rice variety and seawater intrusion on water EC in rice crop in the second rice season.

Treatment	EC (dS m ⁻¹) at DAT in the second rice season										
	7	14	21	28	35	42	49	56	63	70	77
Variety											
Pathumthani 1	4.33	3.40	3.26	2.79	3.04	3.41	2.76	2.51	2.64	2.56	2.53b
RD 43	4.58	3.59	3.44	2.96	2.92	3.15	2.89	2.51	2.70	2.61	3.41a
Seawater											
0%	1.81d	1.42d	1.40d	1.22d	1.25d	1.27c	1.12d	0.96d	1.03c	1.04	1.17e
2%	2.91cd	2.23cd	2.21cd	1.76d	1.85cd	2.64bc	1.53d	1.31d	1.39c	1.34	1.56de
4%	3.64c	2.96c	2.83c	2.52c	2.62bc	2.75bc	2.41c	2.11c	2.47b	2.31	2.54cd
6%	5.27b	4.13b	3.77b	3.27b	3.40b	3.60ab	3.11b	2.84b	2.93b	2.83	3.47bc
8%	6.03ab	4.58b	4.41b	4.11a	3.79b	4.41a	4.19a	3.69a	3.93a	3.93	4.25ab
10%	7.06a	5.64a	5.47a	4.39a	4.98a	4.97a	4.60a	4.15a	4.26a	4.08	4.82a
F test											
Variety	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
Seawater	**	**	**	**	**	**	**	**	**	**	**
Variety x Seawater	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV%	16.26	15.69	14.54	11.27	21.87	25.98	13.88	13.47	18.82	12.94	23.41

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

4.3.3 Salinity effects on rice plant growth

4.3.3.1 Plant height

In both seasons, the plant height PT1 was significantly taller ($P < 0.01$) than RD43 at all stages except the panicle initiation stage in the second rice season (Table 4.7 and 4.8). The plant height decreased with increasing salinity, and it was significantly reduced ($P < 0.01$) when the seawater mixing ratios were 8% and 10% among seawater applications at all growth stages except the ripening stage in two seasons (Table 4.7 and 4.8). Thus, the seawater mixing 2% and 4% did not significantly decline plant height compared with 0% (control) at all growth stages in both seasons. The significant interaction of variety and seawater mixing ratio was observed at panicle initiation and flowering stages in both seasons (Table 4.7 and 4.8).

4.3.3.2 Tiller number

The number of tillers hill⁻¹ was significantly increased ($P < 0.01$) in PT1 than that of RD43 at all growth stages except flowering and ripening stages in the second rice season (Table 4.7 and 4.8). There was a significant difference ($P < 0.01$), ($P < 0.05$) in tillers number among seawater mixing ratios at all stages except in the ripening stage in the major rice season while it was statistically different at panicle initiation and flowering stages in the second rice season (Table 4.7 and 4.8). In the major rice season (Table 4.7), it was observed that seawater mixing ratios 2% and 4% were not significantly varied in several tillers compared to control at all growth stages. However, the seawater 6%, 8%, and 10% were significantly decreased tillers at all growth stages except seawater 6% at flowering and ripening stages compared with control. For the second rice season (Table 4.8), it was found that the higher seawater ratios reduced tillers. The lowest value was observed in the 10% seawater mixing ratio followed by 8%, 6%, and 4%. However, the number of tillers was not significantly different in 0% and 2% seawater. The interaction of variety and seawater mixing ratio was significant at panicle initiation stage in the major rice season and panicle initiation and flowering stages in the second rice season (Table 4.7 and 4.8).

4.3.3.3 Shoot dry weight

The variety and seawater mixing had a significant effect on dry weight at different growth stages in both seasons except the effects of variety at the ripening stage (Table 4.9). The value of dry weight was significantly greater ($P < 0.01$) in PT1 than that of RD43 at different growth stages except for the ripening stage in the second rice season (Table 4.9). There were significant variations ($P < 0.01$), ($P < 0.05$) in shoot dry weight among seawater mixing ratios in both seasons (Table 4.9). The shoot dry weight values were decreased with increasing salinity ratio. For the major rice season, the smallest shoot dry weight occurred in seawater mixing 10% followed by 8%, 6%, and 4% except shoot dry weight at panicle initiation and ripening stages. The highest shoot dry weight occurred in 0% and 2% seawater mixing which were not significantly different from each other. In the second rice season (Table 4.9), the largest value of shoot dry weight was observed in control which was significantly different from other treatments at the panicle initiation stage. Significant reductions in shoot dry weight were recorded in 8% and 10% seawater mixing ratios at all growth stages. The significant interaction of variety and seawater mixing ratio on shoot dry weight was observed in both seasons except at ripening stage in the major rice season and flowering stage in the second rice season (Table 4.9).

The PT1 had an improvement of growth such as plant height, tillers and shoot dry weight compared to RD43 (Table 4.7-4.9), which might be due to the different varieties of rice which had various responses under salt stress. A similar finding was reported by Theerawitaya *et al.* (2022) who found that the shoot dry weight was higher in PT1 than of RD43 (Table 4.9). The previous study reported that the biomass accumulation was lower in short growth duration than that of the long growth duration cultivars, which might be the length of the utilization of nutrients. The rice cultivars with different growth durations, which may cause the difference in biomass accumulation (Table 4.9) and yield (Table 4.14) (Chen *et al.*, 2022).

In this study, the higher seawater mixing ratios of 8% and 10% (E_{ce} at 3-4 $dS\ m^{-1}$) (Table 4.19) significantly reduced plant growth parameters (Table 4.7-4.9). A similar study also stated that increased soil salinity ($E_{ce} > 4\ dS\ m^{-1}$) caused severe reductions in plant growth (Radanielson *et al.*, 2018). This might be due to the salt

accumulation in soil reduced cell turgor and cell elongation leading to decreasing plant growth, development, and dry matter accumulation (Table 4.9) (Etesami and Glick, 2020). The previous study stated that the threshold level of ECe for rice was 3 dS m⁻¹ (Shereen *et al.*, 2022). The high salt stress adversely affected cell division and cell elongation resulting in reductions in plant growth (Table 4.7-4.9) and yield (Table 4.14 and 4.15) (Jahan *et al.*, 2023). The other mechanism might be due to the decreased water potential in cells (Coca *et al.*, 2023) that inhibited the uptake of water by plant roots and caused lower water content (Table 4.10 and 4.11) in plants under salt stress (Reddy *et al.*, 2017). Then, the plant decreased photosynthesis because of lower Chl contents (Table 4.10 and 4.11) under high salinity stress that led to retarding plant growth (Table 4.7 and 4.8) (Kamran *et al.*, 2019). A positive correlation between RWC and plant height, tillers, and shoot dry weight was observed in this study. The Chl a at the panicle initiation stage showed a positive relation with plant growth. However, the H₂O₂ was negatively correlated with shoot fresh weight and shoot dry weight (Figure 4.10 and 4.11). Furthermore, plant growth reduction was inhibited by salinity due to nutrient imbalance and ion toxicity because of the high accumulation of Na (Figure 4.7) and Cl (Figure 4.10) (Rehman *et al.*, 2019). The high concentration of salts in soil solution (ECe at 3-4 dS m⁻¹) (Table 4.19) disturbed the balance absorption of essential nutrients by rice plant (Figure 4.7-4.9), which affected the growth of the plant (Looi and Lum, 2020).

Table 4.7 Effects of rice variety and seawater intrusion on plant height and number of tillers hill⁻¹ at different growth stages of rice crop in the major rice season.

Treatment	Major rice season							
	Plant height (cm)				Tillers hill ⁻¹			
	Maximum tillering	Panicle initiation	Flowering	Ripening	Maximum tillering	Panicle initiation	Flowering	Ripening
Variety								
Pathumthani 1	51.91a	62.16a	88.02a	106.42a	3.21a	4.38a	7.70a	5.29
RD43	42.37b	56.02b	69.40b	97.10b	3.03b	3.57b	4.54b	5.13
Seawater								
0%	53.89a	66.79a	87.86a	108.19a	3.19	4.97a	6.95a	5.75ab
2%	51.98ab	64.42ab	87.70a	106.10ab	3.13	4.77a	6.55ab	6.50a
4%	51.17ab	62.79ab	85.26ab	105.85ab	3.19	4.71a	6.46ab	5.30bc
6%	44.51bc	56.92bc	75.37bc	100.73ab	3.08	3.08b	6.08ab	5.13bc
8%	41.59c	53.70c	73.68bc	98.33ab	3.08	3.07b	5.44bc	4.40c
10%	39.70c	49.92c	65.39c	91.34b	3.00	3.06b	3.75c	4.38c
F test								
Variety	**	**	**	**	**	**	**	ns
Seawater	**	**	**	*	ns	**	*	**
Variety × Seawater	ns	*	*	ns	ns	*	ns	ns
CV %	9.34	7.49	8.01	8.09	4.11	6.56	14.15	9.63

Notes: ± standard error of means. The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

Table 4.8 Effects of rice variety and seawater intrusion on plant height and number of tillers hill⁻¹ at different growth stages of rice crop in the second rice season.

Treatment	Second rice season							
	Plant height (cm)				Tillers hill ⁻¹			
	Maximum tillering	Panicle initiation	Flowering	Ripening	Maximum tillering	Panicle initiation	Flowering	Ripening
Variety								
Pathumthani 1	61.39a	69.51	97.41a	109.86a	3.21a	4.41a	5.26	5.29
RD43	57.90b	69.90	80.93b	93.22b	3.03b	3.26b	5.27	5.13
Seawater								
0%	66.08a	76.84a	95.94a	104.38ab	3.19	4.33a	5.85ab	5.75ab
2%	63.16ab	74.87ab	94.19a	107.75a	3.13	4.50a	6.56a	6.50a
4%	59.61bc	69.42bc	87.63bc	99.25bc	3.19	4.06ab	5.54b	5.30bc
6%	58.48bc	68.50bcd	90.77ab	100.63abc	3.08	3.50ab	5.25bc	5.13bc
8%	55.53c	65.76cd	84.37c	101.17abc	3.08	3.47b	4.25bcd	4.40c
10%	55.12c	62.86d	82.12c	96.08c	3.00	3.20b	3.94d	4.38c
F test								
Variety	**	ns	**	**	**	**	ns	ns
Seawater	**	**	**	**	ns	*	**	**
Variety × Seawater	ns	*	*	ns	ns	*	*	ns
CV %	5.48	5.15	3.48	4.52	4.11	11.78	8.36	9.63

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

Table 4.9 Effects of rice variety and seawater intrusion on shoot dry weight at different growth stages of rice in the major and second rice seasons.

Treatment	Shoot dry weight (g pot ⁻¹)					
	Major rice season			Second rice season		
	Panicle initiation	Flowering	Ripening	Panicle initiation	Flowering	Ripening
Variety						
Pathumthani 1	11.45a	44.30a	30.64a	4.70a	21.01a	8.56
RD43	3.79b	19.56b	13.75b	4.00b	15.77b	7.95
Seawater						
0%	12.40a	42.62a	24.79ab	6.68a	21.52a	9.63a
2%	10.36ab	46.20a	26.59a	4.83b	23.31a	8.71ab
4%	6.44c	29.17bc	24.50ab	3.39bc	20.81a	8.84ab
6%	4.92c	31.53b	20.71bc	4.49bc	19.77a	9.76a
8%	4.33c	22.18bc	21.09bc	3.10c	17.47a	7.22b
10%	7.26bc	19.76c	15.51c	3.61bc	7.67b	5.38c
F test						
Variety	**	**	**	**	**	ns
Seawater	*	**	*	**	**	**
Variety × Seawater	**	*	ns	*	ns	**
CV %	17.21	13.97	10.96	15.22	15.14	8.53

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

4.3.4 Salinity effects on plant biochemical synthesis

4.3.4.1 Relative water content (RWC)

The significantly higher ($P < 0.05$) content of RWC resulted in PT1 in the major rice season whereas the RD43 was significantly greater ($P < 0.05$) than PT1 in second the rice season at the flowering stage (Table 4.10 and 4.11). There was a significant difference ($P < 0.05$) in RWC value among seawater mixing ratios at the flowering stage in the major rice season and panicle initiation stage in the second rice season (Table 4.10 and 4.11). It was found that the higher salinity caused a decrease in RWC content compared to control in both seasons. After the application of seawater $> 6\%$ reduced RWC compared to 0% which showed the maximum value among seawater ratios. Although there was no significant interaction between variety and seawater mixing ratio on relative water content in the major rice season, the significant interaction was found at panicle initiation stage in the second rice season (Table 4.10 and 4.11). The previous study reported that the tolerance variety had ability to maintain RWC while the reduction level occurred in sensitive variety (Jumpa *et al.*, 2023), that was similar to our findings and showed that higher RWC was observed in RD43 (Table 4.10 and 4.11). The decreased RWC in the plant due to the lower osmotic potential of soil that reduced the availability of water from the soil solution to the plant caused by salt stress (irrigation water EC > 3 dS m^{-1}) with toxic effects of Na^+ and Cl^- (> 9.8 dS m^{-1}) (Bayat *et al.*, 2022). The osmotic stress caused by salinity, which increased water potential and decreased water uptake by plants resulted in reduced RWC (Table 4.10 and 4.11) that led to cell dehydration (Herawati *et al.*, 2024). When the lower RWC in the plant, it inhibits all metabolic activities of the plant because water is essential (Polash *et al.*, 2018) which supports almost all physiological and biochemical processes contributing to plant growth and development (Table 4.9) (Shahid *et al.*, 2020). The decreased RWC in the plant enhanced the production of H_2O_2 (Table 4.10) under saline condition.

4.3.4.2 Hydrogen peroxide (H_2O_2)

The RD43 produced significantly greater ($P < 0.01$), ($P < 0.05$) amount of H_2O_2 than PT1 in both seasons whereas the amount of H_2O_2 was larger in PT1 at the

panicle initiation stage in the second rice season (Table 4.10 and 4.11). Although the various seawater mixing ratios did not affect H₂O₂ content in the major rice season, it was significantly increased ($P < 0.01$) at both stages in the second rice season (Table 4.10 and 4.11). It meant that the long-time influences of salinity enhanced more production of H₂O₂. The highest value of H₂O₂ was shown in higher salinity 10% seawater mixing at the panicle initiation stage and 8% at the flowering stage. The lowest value was observed in the control which was statistically similar in 2% and 4% seawater mixing ratios at the flowering stage. The significant interaction between variety and seawater mixing ratio on H₂O₂ was occurred in both stages in two seasons except PI stage in the major rice season (Table 4.10 and 4.11). The H₂O₂ content was increased in RD43 at the flowering stage in both seasons (Table 4.10 and 4.11), which might cause lower Chl contents in plants under salinity stress from different seawater mixing ratios (Table 4.10-4.12). Under salinity stress, the rice plant produced antioxidants such as phenol to avoid excessive accumulation of ROS (Li *et al.*, 2017). The high accumulation of H₂O₂ inhibited plant growth and development (Table 4.7 and 4.8) (Chutimanukul *et al.*, 2019) because it disturbed cell membranes, lipids, and photosynthetic pigments (Table 4.12) (Kamran *et al.*, 2019), DNA (Arif *et al.*, 2020) and protein (El Ghazali, 2020).

4.3.4.3 Chlorophyll a and chlorophyll b (Chl a and Chl b)

The Chl a and Chl b were significantly higher ($P < 0.01$) in PT1 than that of RD43 at both stages except Chl a at the flowering stage in the major rice season and Chl a and Chl b at panicle initiation stage in second rice season (Table 4.10 and 4.11). The SPAD values of PT1 also demonstrated a larger value than RD43 at all growth stages in both seasons except the maximum tillering stage in the second rice season (Table 4.12). The various seawater mixing ratios were significantly different ($P < 0.01$) in Chl a and Chl b at both stages except Chl a and Chl b at the panicle initiation stage in the second rice season (Table 4.10 and 4.11). The SPAD value was significantly varied at maximum tillering, panicle initiation, and flowering stages in the major rice season and ripening stage in the second rice season (Table 4.12). The greatest contents of Chl a and Chl b occurred in control which was significantly increased among other treatments at the panicle

initiation stage whereas it was not significantly varied from 2% and 4% seawater mixing ratios at the flowering stage in the major rice season (Table 4.10). It was also shown that the maximum SPAD value was found in control at maximum tillering, panicle initiation, and flowering stages in the major rice season (Table 4.12). In the second rice season, there was no significant difference in Chl a and Chl b values at the panicle initiation stage while a significant difference was observed in the flowering stage (Table 4.11). The maximum values resulted in 10% seawater mixing and the minimum value of Chl a occurred in 6% and Chl b was in 8% seawater. The SPAD value was also not significantly different among seawater treatments at all stages except the ripening stage. The significant interaction of variety and seawater mixing ratio on Chl a and Chl b were found at both stages in two seasons (Table 4.10 and 4.11). Due to H₂O₂ toxicity in the leaf (Shakri *et al.*, 2022), the Chl a and Chl b contents were decreased (Table 4.10 and 4.11) and the finding of this study was similar to Hussain *et al.* (2019) who reported that Chl a and Chl b in rice leaves declined with higher saline stress (EC > 4 dS m⁻¹). The reduction of Chl content under an increased ratio of salinity diminished photosynthesis by decreasing CO₂ availability (Machado and Serralheiro, 2017) because of the closing stomata of the leaf (Bayat *et al.*, 2022).

4.3.4.4 Total phenol

The total phenol content in RD43 was significantly increased ($P < 0.01$) compared with PT1 in both seasons except the flowering stage in the major rice season (Table 4.13). The different seawater mixing ratios were significantly varied ($P < 0.01$) in total phenol content at the panicle initiation stage in both seasons (Table 4.13). The maximum amount of total phenol was observed in 2% seawater mixing which was significantly different from 8% and 10% seawater in the major rice season. In the second rice season, the highest value resulted in 10% seawater which was significantly increased from other treatments at the panicle initiation stage. Although there was no significant variation in total phenol among seawater mixing ratios, the higher salinity showed a higher value at the flowering stage in the second rice season. The significant interaction of variety and seawater mixing ratio on total phenol content at panicle initiation stage in both seasons.

(Table 4.13). Under salinity stress, the rice plant produced antioxidants such as phenol to avoid excessive accumulation of ROS (Li *et al.*, 2017). In this study, a larger amount of total phenol occurred in RD43 (Table 4.13) and the findings of this study were in line with the previous study stated that the salt-tolerant variety increased the contents of antioxidants (Bhowmik *et al.*, 2021). The plant produced high concentrations of phenols to survive and neutralize ROS under salt stress, which may lead to morphological changes and increase plant resistance (Sharma *et al.*, 2019). Therefore, the total phenol and H₂O₂ were negatively correlated in two rice varieties at the flowering stage in the major rice season and the panicle initiation stage in the second rice season. The higher antioxidants coupled with higher proline accumulation might play an important role in salinity tolerance (Bhowmik *et al.*, 2021), therefore, the increased contents of total phenol and proline were found in RD43 (Table 4.13). The increase or decrease of phenolic compounds in plants under salt stress was reported by various studies (Ahmed *et al.*, 2019). In this study, the plant produced more total phenol content and it increased with increasing salinity 6%, 8% and 10% seawater mixing ratio in the second rice season (Table 4.13). The high antioxidants in plants had a positive relation to tolerance of plants by scavenging the toxic levels of H₂O₂ in cells (Jumpa *et al.*, 2023). The phenolic compounds enhanced the detoxification of H₂O₂ resulting in increased resistance to salinity (Pungin *et al.*, 2023).

4.3.4.5 Proline

The proline content was significantly greater ($P < 0.01$) in PT1 than that of RD43 in the major rice season however RD43 showed higher value in the major season at both stages (Table 4.13). Although the different seawater mixing ratios did not affect on proline content in the major rice season, the increased proline content with higher salinity in the second rice season at both stages (Table 4.13). The largest value was shown in 8% and 10% seawater whereas the lowest value occurred in 2% seawater at both stages among seawater ratios. It was indicated that the seawater mixing of $> 4\%$ increased the production of proline except in the flowering stage in the major rice season. The significant interaction of variety and seawater mixing ratio on proline was shown at both stages in two seasons (Table 4.13). The result of this study showed that two rice varieties had

various responses of proline to salinity stress at different growth stages (Table 4.13). The plant produced compatible solutes such as proline which is one of the important plant defense mechanisms (Polash *et al.*, 2018). The proline production of rice plants was slightly higher in the panicle initiation stage compared to the flowering stage. According to Gerona *et al.* (2019) who stated that the amount of proline was higher in rice-tolerant varieties at the vegetative stage. The PT1 produced increased proline to response salinity stress in major rice season. The previous studies demonstrated that the highly susceptible cultivars had the highest level of proline compared to tolerant cultivars under salinity stress (Chunthaburee *et al.*, 2016). The high amount of proline accumulation in salt-sensitive cultivars might be a response to leaf damage or a symptom of stress (Figure 4.13). Moreover, the increased content of proline was not related to improved salt tolerance in rice (Datir *et al.*, 2018). In the second rice season, the greater proline value in RD43 might be the resistant variety had higher proline production than the sensitive variety (Çirka *et al.*, 2022; Alkahtani and Dwiningsih, 2023) with a long duration of salinity. The result of this study in the second rice season was in line with (Kordrostami *et al.*, 2017) who reported that the proline accumulation was higher in salt-tolerant varieties than salt-sensitive varieties. In a previous report, the proline accumulation in plants was linearly related to salt stress tolerance in plants. The higher content of proline was demonstrated in plants especially salt-tolerant plants to make ionic adjustments and scavenge H_2O_2 by enhancing antioxidants under salinity (Shahid *et al.*, 2020). The previous study demonstrated that the maximum amount of proline might be occurred in higher salinity stress (9.8 dS m^{-1}) (Polash *et al.*, 2018), this finding was similar to our study that seawater mixing ratios $> 4\%$ in the second rice season showed an increase in proline content (Table 4.13). In that case, the proline adjusted osmotic stress in the plant to maintain RWC (Ali *et al.*, 2014, Jumpa *et al.*, 2023) and improved water uptake and scavenged H_2O_2 (Hasanuzzaman *et al.*, 2021). Therefore, proline at the panicle initiation stage was negatively correlated with H_2O_2 and positively related with Chl a (Figure 4.11 and 4.12). Consequently, the osmotic regulation can resist water deficit in plants, and sustain leaf enlargement and stomata conductance (Acosta-Motos *et al.*, 2017). There were many studies reported that the rice plant indicated a positive correlation between proline accumulation and adaptation to

salt stress (Polash *et al.*, 2018) by improving the water flow into the plant cell to maintain cellular water status and plant cell turgidity (Nguyen *et al.*, 2021). According to this study, the proline at the flowering stage had a positive correlation with RWC and Chl contents in rice plants (Figure 4.11). Kordrostami *et al.* (2017) indicated that the proline accumulation was positively related to the degree of salt tolerance in rice and the high level of proline contents (Table 4.13) plant could maintain the higher green leaf area under salt stress (Table 4.12). Thus, it may seem that the plant can regulate photosynthesis which will sustain plant growth (Table 4.7-4.19) under high salinity stress.



Table 4.10 Effects of rice variety and seawater intrusion on RWC, H₂O₂, Chl a and Chl b contents at different growth stages of rice in the major rice season.

Treatment	Major rice season							
	RWC (%)		H ₂ O ₂ (mg g ⁻¹ FW)		Chl a (mg g ⁻¹ FW)		Chl b (mg g ⁻¹ FW)	
	Panicle initiation	Flowering	Panicle initiation	Flowering	Panicle initiation	Flowering	Panicle initiation	Flowering
Variety								
Pathumthani 1	75.51	86.76a	0.18	0.17b	5.82a	4.23	108.61a	99.66a
RD43	77.94	83.68b	0.18	0.18a	3.88b	4.58	79.85b	80.56b
Seawater								
0%	80.87	90.53a	0.18	0.18	6.26a	5.29a	120.94a	106.33ab
2%	78.86	85.69ab	0.19	0.17	4.16de	4.47abc	115.90b	91.10abc
4%	75.63	86.18ab	0.19	0.18	3.61e	5.10ab	96.90b	107.39a
6%	72.49	84.20ab	0.19	0.18	4.56cd	3.96c	82.85b	69.25bc
8%	75.78	81.91b	0.19	0.17	5.08bc	3.79c	78.26b	78.64c
10%	75.71	82.81b	0.18	0.17	5.43b	3.99bc	70.54b	87.95bc
F test								
Variety	ns	*	ns	**	**	ns	**	**
Seawater	ns	**	ns	ns	**	**	**	**
Variety × Seawater	ns	ns	ns	**	*	**	**	**
CV %	8.58	4.15	7.18	6.13	6.80	11.39	9.10	12.31

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

Table 4.11 Effects of rice variety and seawater intrusion on RWC, H₂O₂, Chl a and Chl b contents at different growth stages of rice in the second rice season.

Treatment	Second rice season							
	RWC (%)		H ₂ O ₂ (mg g ⁻¹ FW)		Chl a (mg g ⁻¹ FW)		Chl b (mg g ⁻¹ FW)	
	Panicle initiation	Flowering	Panicle initiation	Flowering	Panicle initiation	Flowering	Panicle initiation	Flowering
Variety								
Pathumthani 1	81.36	86.97b	0.44a	0.33b	5.83	5.96a	124.53	128.83a
RD43	83.17	89.15a	0.25b	0.37a	5.98	4.75b	119.89	95.52b
Seawater								
0%	89.96a	91.97	0.32b	0.26d	5.91	4.88b	124.14	94.24bc
2%	83.61ab	87.57	0.35ab	0.26d	5.80	4.86b	126.04	109.20bc
4%	81.86ab	88.15	0.33b	0.28d	6.06	5.88ab	121.81	120.69ab
6%	82.14ab	87.53	0.34b	0.35c	6.23	4.81b	121.29	113.51bc
8%	78.98b	86.62	0.35ab	0.54a	5.74	5.01b	121.47	90.42c
10%	77.06b	86.54	0.38a	0.42b	5.68	6.70a	118.51	145.01a
F test								
Variety	ns	*	**	*	ns	**	ns	**
Seawater	**	ns	**	**	ns	**	ns	**
Variety × Seawater	*	ns	*	**	**	**	**	**
CV %	5.60	3.44	6.70	7.10	8.47	9.98	7.34	10.67

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

Table 4.12 Effects of rice variety and seawater intrusion on SPAD at different growth stages of rice in the major and second rice seasons.

Treatment	SPAD							
	Major rice season				Second rice season			
	Maximum tillering	Panicle initiation	Flowering	Ripening	Maximum tillering	Panicle initiation	Flowering	Ripening
Variety								
Pathumthani 1	37.27a	37.60a	41.94a	42.83a	39.67	41.45a	44.16a	42.76a
RD43	33.09b	36.33b	39.58b	40.26b	39.12	38.80b	42.80b	39.44b
Seawater								
0%	37.03a	38.73a	41.72a	43.89	39.71	41.00	43.22	37.89b
2%	36.68a	38.59a	41.19ab	41.96	40.34	39.23	42.90	39.72ab
4%	36.02ab	37.71a	41.08ab	41.88	39.43	40.77	44.55	41.80ab
6%	35.16ab	36.74a	41.02ab	41.13	40.00	40.38	43.74	43.33a
8%	33.82ab	36.64a	40.47ab	40.23	36.50	39.91	42.76	43.44a
10%	32.35b	33.40b	39.08b	40.19	38.40	39.46	43.70	41.92ab
F test								
Variety	**	*	**	**	ns	**	**	**
Seawater	**	**	*	ns	ns	ns	ns	**
Variety × Seawater	ns	ns	ns	ns	ns	ns	*	ns
CV %	5.93	4.65	3.26	6.55	2.99	3.51	2.73	6.04

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

Table 4.13 Effects of rice variety and seawater intrusion on total phenol and proline contents at different growth stages of rice in the major and second rice seasons.

Treatment	Major rice season				Second rice season			
	Total phenol (mg GAE g ⁻¹ sample)		Proline (μ g g ⁻¹ FW)		Total phenol (mg GAE g ⁻¹ sample)		Proline (μ g g ⁻¹ FW)	
	Panicle initiation	Flowering	Panicle initiation	Flowering	Panicle initiation	Flowering	Panicle initiation	Flowering
Variety								
Pathumthani 1	22.86b	14.82a	89.77a	25.07a	35.04b	44.43	64.92b	37.12b
RD43	27.16a	9.29b	74.83b	22.40b	59.95a	47.71	106.23a	122.05a
Seawater								
0%	24.49ab	12.07	83.79	23.51	40.57bc	44.92	66.46cd	71.23b
2%	26.96a	12.06	70.18	24.58	36.51c	40.68	58.16d	57.21b
4%	26.15a	12.02	69.54	23.35	46.31bc	47.50	90.35ab	70.20b
6%	25.70ab	12.07	98.29	24.84	43.63bc	48.17	82.27bc	67.94b
8%	23.38b	12.09	97.31	23.83	51.68b	44.16	107.09a	88.00ab
10%	23.36b	12.03	74.78	22.31	66.30a	50.99	105.72ab	115.84a
F test								
Variety	**	**	*	**	**	ns	**	**
Seawater	**	ns	ns	ns	**	ns	*	*
Variety \times Seawater	**	ns	**	*	**	ns	**	**
CV %	5.77	0.52	16.05	7.53	12.30	15.33	11.37	15.40

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

4.3.5 Salinity effects on yield and yield components

4.3.5.1 Number of panicles pot⁻¹

The number of panicles pot⁻¹ was not significantly different between PT1 and RD43 in both seasons (Table 4.14 and 4.15). The various seawater mixing ratios significantly influenced ($P < 0.01$) on number of panicles pot⁻¹ in both seasons (Table 4.14 and 4.15). The maximum value of number of panicles pot⁻¹ was recorded in 2% seawater mixing which was significantly increased from 8% and 10% seawater in both seasons whereas it was not significantly varied from other treatments. It was observed that the higher seawater mixing ratios with a decreased number of panicles pot⁻¹ in both seasons. The interaction of variety and seawater mixing ratio was significant in panicles pot⁻¹ in both seasons.

4.3.5.2 Number of spikelets panicle⁻¹

The PT1 produced a significantly higher ($P < 0.01$) number of spikelets panicle⁻¹ compared to RD43 in both seasons (Table 4.14 and 4.15). The number of spikelets panicle⁻¹ was significantly different ($P < 0.01$) among seawater mixing ratios in both seasons (Table 4.14 and 4.15). The control produced the highest value of number of spikelets panicle⁻¹ in both seasons and it had significant variation from other treatments in both seasons. The seawater mixing ratios 2%, 4% and 6% were significantly differed in number of spikelets panicle⁻¹ value with each other in the major rice season whereas there was no significant variation in the second rice season. It was found that the increased salinity decreased number of spikelets panicle⁻¹ among seawater mixing ratios. The 10% seawater showed the lowest value of number of spikelets panicle⁻¹ and 8% seawater demonstrated the second lowest value in both seasons. The significant interaction of variety and seawater mixing ratio on spikelets panicle⁻¹ was observed in both seasons.

4.3.5.3 Panicle length

The value of panicle length was significantly higher ($P < 0.01$) in PT1 than that of RD43 in both seasons (Table 4.14 and 4.15). Although there was no significant difference in panicle length among seawater mixing ratios in the major rice season, the

panicle length was significantly varied ($P < 0.01$) in the second rice season (Table 4.14 and 4.15). The longest panicle length resulted in 2% seawater in the second rice season which was not significantly different from 0%, 4% and 6% seawater mixing while it was significantly varied from 8% and 10% seawater. It was indicated that the increase in seawater mixing ratios reduced panicle length in both seasons. The interaction of variety and seawater mixing ratio was significant in panicle length in both seasons.

4.3.5.4 Filled grain (%)

The PT1 had a significant effect ($P < 0.01$) on filled grain (%) compared with RD43 in the major rice season, however, RD43 was higher in filled grain (%) in the second rice season (Table 4.14 and 4.15). The filled grain (%) was significantly varied ($P < 0.01$) among seawater mixing ratios in both seasons (Table 4.14 and 4.15). The control gave the maximum value of filled grain (%) and it had significant variation from 4%, 8% and 10% seawater mixing in the major rice season and 6%, 8% and 10% seawater in the second rice season. The significant interaction between variety and seawater mixing ratio was found in filled grain (%) for both seasons.

4.3.5.5 1000-grain weight

The 1000-grain weight (wt) was significantly higher ($P < 0.05$) in PT1 compared to RD43 in the major rice season, however, it was not significantly different between the two rice varieties in the second rice season (Table 4.14 and 4.15). The various seawater mixing ratios significantly affected ($P < 0.01$) 1000-grain wt in both seasons (Table 4.14 and 4.15). The largest 1000-grain wt value was demonstrated in the seawater mixing ratio 2% shown in the major rice season and control in the second rice season. The smallest values were observed in 8% seawater in the major rice season and 10% seawater in the second rice season. The interaction of variety and seawater mixing ratio was significant in 1000-grain wt in both seasons.

4.3.5.6 Grain yield

The grain yield was significantly increased ($P < 0.01$) in PT1 compared to RD43 in the major rice season, however, there was no significant difference in grain yield between the two rice varieties in the second rice season (Table 4.14 and 4.15). The different seawater mixing ratios had significant effects ($P < 0.01$) on grain yield in both seasons (Table 4.14 and 4.15). The highest grain yield was recorded in 2% seawater mixing which was a significant variation from 4%-10% seawater mixing ratios in the major rice season and 6%-10% seawater in the second rice season. It was stated that the grain yield in 2% seawater mixing was not significantly different from control in both seasons. The significant interaction between variety and seawater mixing ratio on grain yield was found for both seasons (Table 4.14 and 4.15). In the major rice season, compared with the control, the grain yield of PT1 decreased by 12, 19%, 59%, and 56% at seawater mixing ratios of 4%, 6%, 8%, and 10%, respectively. There was no significant difference between the control and 6% seawater mixing ratio (Figure 4.7). The grain yield of RD43 decreased by 47%, 38%, 72%, and 89% at 4%, 6%, 8%, and 10% seawater mixing ratios, respectively, while it increased by 18% at 2% seawater mixing ratio compared with the control (Figure 4.7A). In the second rice season, the grain yield of PT1 decreased by 18%, 20% and 33% at 6%, 8%, and 10% seawater mixing ratios, respectively, compared with the control. The grain yield of PT1 increased by 49% at 2% seawater mixing ratio compared with the control; however, it was not significantly different (Figure 4.7B). The grain yield of RD43 decreased by 28%, 23%, 39%, 61%, and 86% at seawater mixing ratios of 2%, 4%, 6%, 8%, and 10%, respectively, compared with the control. However, the grain yield of RD43 was not significantly different between the control, 2%, 4%, and 6% (Figure 4.7B).

According to this study, number of spikelets panicle⁻¹, panicle length and filled grain (%) were significantly higher in PT1 compared to RD43 in both seasons except the number of panicles pot⁻¹, grain yield, and 1000-grain wt under salinity stress (Table 4.14 and 4.15). It was recorded that although grain yield was not statistically different between the two rice varieties in the second rice season, the grain yield of PT1 was higher than that of RD43 in the major rice season. According to a previous study, the difference in number of spikelets panicle⁻¹ might be the varietal characteristics of rice (Jahan *et al.*,

2023). The higher grain yield of PT1 in the major rice season might be the higher number of productive tillers (Table 4.7 and 4.8) which gave the high yield (Ologundudu and Kekere, 2016). The other possible mechanism that the higher grain yield in PT1 (Table 4.14 and 4.15) might be mainly due to the higher uptake of K nutrient (Figure 4.7), and the uptake of any nutrient by crop was directly proportional to dry matter production (Table 4.9) and grain yield (Mohan *et al.*, 2023).

Irrespective of rice variety, it was observed that the higher salinity 6 %, 8% and 10% seawater ratios caused reductions in grain yield and yield components such as number of panicles pot^{-1} , number of spikelets panicle^{-1} , panicle length, filled grain (%) except 1000-grain wt. The seawater ratios 0% -4% had larger rice grain yield and yield components in both seasons (Table 4.14 and 4.15). The number of panicles pot^{-1} was reduced with higher seawater ratios might be the high salinity level limited the distribution of carbohydrates to the developing panicles which reduced the pollen viability leading to decreased seed setting (Jahan *et al.*, 2023). The reduced number of spikelets panicle^{-1} under salinity stress (Table 4.14 and 4.15) might be due to the shortage of carbohydrate supply during grain filling and developing panicle which adversely affected the grain number and yield. The number of spikelets panicle^{-1} fertility was diminished with increased salinity levels (Ologundudu and Kekere, 2016). The result of this study was in line with according to the previous study of Jahan *et al.* (2023) who reported that the panicle length (Table 4.14 and 4.15) was decreased with higher levels of salinity (EC at 4-8 dS m^{-1}). The reduction of filled grain (%) (Table 4.14 and 4.15) might be due to the pollen sterility with increased salinity level (Jahan *et al.*, 2023). The sterility and seed setting reduction might be due to declined translocation of soluble carbohydrates to primary and secondary spikelets and disruption of starch synthesis in developing grains caused by high uptake of Na and less uptake of K in plants. Many authors reported that there was a relationship between yield reduction and Na/K ratio in plants (Mel *et al.*, 2019). Another study also stated that the high salinity decreased number of spikelets panicle^{-1} might be due to the pollen sterility, reduced 1000-grain wt (Jahan *et al.*, 2023), panicle length and filled grain (%) leading to decreased grain yield (Reshna and Beena, 2021) due to increasing panicle sterility and limiting of carbohydrates transformation (Li *et al.*, 2023). The finding of this

study was in accordance with the finding of Alkahtani and Dwiningsih (2023) who demonstrated that the rice yield was significantly under salinity stress (water EC at 3.5 dS m^{-1}). Furthermore, there was a negative correlation between grain yield and H_2O_2 contents in rice plants (Figure 4.11).

Based on the findings, the PT1 (Salt-sensitive) can be used under different seawater mixing ratios of 0%-10% compared to RD43 (Moderately salt-tolerant) because the grain yield of PT1 was higher in the first season and the second season although there was no statistically different between PT1 and RD43 in the second season (Figure 4.7).



Table 4.14 Effects of rice variety and seawater intrusion on yield and yield components of rice in the major rice season.

Major rice season						
Treatment	Number of panicles pot ⁻¹	Number of spikelets panicle ⁻¹	Panicle length (cm)	Filled grain (%)	1000-grain wt (g)	Grain weight (g pot ⁻¹)
Variety						
Pathumthani 1	15.12	50.21a	30.00a	80.93a	20.85a	21.68a
RD43	14.01	38.05b	22.54b	61.58b	19.29b	17.36b
Seawater						
0%	17.67ab	79.17a	27.13	85.81a	20.60ab	24.99ab
2%	20.45a	42.12c	27.00	78.11ab	23.33a	31.19a
4%	13.91bc	35.19d	26.90	60.26c	22.67abc	20.50bc
6%	14.63bc	49.90b	25.75	72.52abc	25.24a	19.81bc
8%	12.88c	34.77d	25.58	66.05bc	13.66c	13.20cd
10%	7.86d	23.62e	25.20	64.79bc	14.93bc	7.42d
F test						
Variety	ns	**	**	**	*	*
Seawater	**	**	ns	**	**	**
Variety X Seawater	*	**	*	*	**	*
CV %	14.32	6.17	4.16	16.71	9.07	17.17

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

Table 4.15 Effects of rice variety and seawater intrusion on yield and yield components of rice in the second rice season.

Treatment	Second rice season					
	Number of panicles pot ⁻¹	Number of spikelets panicle ⁻¹	Panicle length (cm)	Filled grain (%)	1000-grain wt (g)	Grain weight (g pot ⁻¹)
Variety						
Pathumthani 1	10.01	78.94a	31.09a	68.69b	26.72	11.79
RD43	9.50	53.44b	22.39b	79.17a	25.91	11.12
Seawater						
0%	11.17a	80.44a	27.33ab	80.43a	28.28a	15.56a
2%	11.76a	71.88b	28.08a	79.23ab	27.35ab	15.81a
4%	10.15ab	65.38b	26.68ab	80.43a	26.92ab	13.31ab
6%	10.40a	69.22b	27.10ab	69.39bc	25.96ab	10.58bc
8%	8.42bc	55.60c	25.50b	64.73c	25.32ab	8.28cd
10%	6.60c	54.63c	25.75b	69.36bc	19.04b	5.19d
F test						
Variety	ns	**	**	**	ns	ns
Seawater	**	**	**	**	**	**
Variety × Seawater	*	**	*	*	**	**
CV %	9.80	6.24	3.82	6.76	5.23	13.11

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

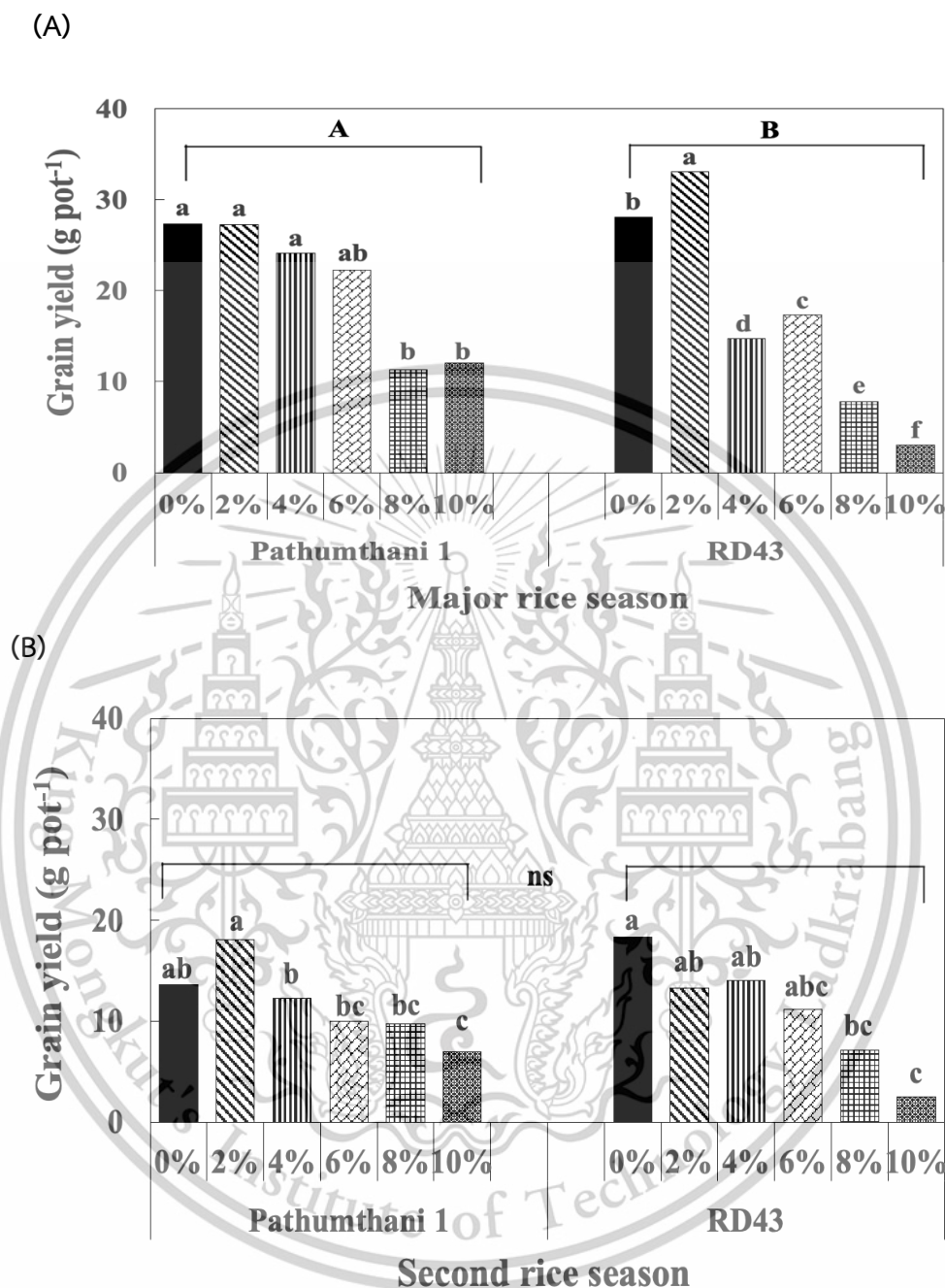


Figure 4.7 Effects of rice variety and seawater intrusion on grain yield in the (A) major rice season and (B) second rice season, respectively. Bars with the same uppercase letters are not significantly different between two rice varieties and, bars with the same lowercase letters are not significantly different among seawater mixing ratios by DMRT at $P < 0.05$.

4.3.6 Salinity effects on plant nutrient uptakes

4.3.6.1 Total sodium uptake

The highest total Na uptake in PT1 was recorded in the 10% seawater mixing ratio in the major rice season and 4% and 6% seawater which were not significantly different from 2%, 8%, and 10% seawater in the second rice season whereas the lowest value was observed in control (Figure 4.8 A and B). The total Na uptake in RD43 was not significantly different among seawater mixing ratios in the major rice season and 8% seawater mixing showed the largest value in the second rice season and it was significantly from 0% (control) and 2% seawater (Figure 4.8 A and B). There was no significant interaction between variety and seawater mixing ratio in total Na uptake for both seasons (Table 4.16 and 4.17). The previous study reported that the salt-sensitive rice plant had the higher Na uptake compared to the salt-tolerant plant (Korres *et al.*, 2022). In this study, the PT1 showed an increase Na uptake than RD43 in the second rice season, which might be due to the long duration of seawater application (Table 4.19). The salt-tolerant rice varieties showed a high K uptake and a low Na uptake in the shoot (Kordrostami *et al.*, 2017; Alkahtani and Dwiningsih, 2023). In general, the salt-tolerant plant had low uptake of Na in plant (Mel *et al.*, 2019), showed a lower Na/K in plant (RD43 in second season, Table 4.18) compared to salt-sensitive genotypes (Akter and Oue, 2018). The salt-tolerant rice varieties had a high maintenance of ion homeostasis such as low Na/K or high K/Na in the shoot compared to salt-sensitive varieties (Chunthaburee *et al.*, 2016). The excess amount of Na and Cl accumulation in plants caused the leaf senescence and death especially in older leaves resulting in decreasing plant growth (Hussain *et al.*, 2017). The higher seawater ratios 4%-10% increased the total Na uptake and total Cl uptake, and 6%-10% seawater ratios increased total Mg uptake, total Ca uptake and total S uptake in two rice varieties among various seawater ratios in both seasons.

4.3.6.2 Total potassium uptake

The total K uptake of PT1 was significantly increased ($P < 0.01$) in control and 2% seawater which had significant variation from 10% in the major rice season whereas the value in control was statistically similar to 2% and 4% seawater and the

lowest value was found in 10% seawater in the second rice season (Figure 4.8 C and D). The 2% seawater mixing ratio was significantly increased ($P < 0.01$) in total K uptake of RD43 in both seasons and it was significantly different from the 10% seawater mixing ratio in both seasons (Figure 4.8 C and D). The interaction of variety and seawater mixing ratio on total K uptake was not observed in both seasons (Table 4.16 and 4.17). The 6%, 8% and 10% seawater ratios decreased the total K uptake in the second rice season and total P uptake in the major rice season were decreased under higher salinity 6%, 8% and 10% seawater ratios in both rice varieties. The results of this study are in agreement with the findings of (Zhang *et al.*, 2018) who reported that the high salinity stress increased Na uptake and decreased K uptake. Under salinity stress, a high content of Na/K disrupted ion homeostasis and decreased uptakes of other nutrients by plants (Rahman *et al.*, 2016). The ions homeostasis was important to maintain normal cell physiological functions required for assimilation because the plants converted CO₂ and organic matters into biomass by photosynthesis. Therefore, maintaining ion homeostasis by reducing Na uptake and improving K uptake is regarded as a key strategy to adapt to salt stress (Zhao *et al.*, 2021). In addition, there was a significantly negative relationship between yield reduction (Table 4.14 and 4.15) and Na/K ratio (Table 4.18) in plant tissue under high salt stress (Mel *et al.*, 2019). This might be K was a key role in plant growth and development because it adjusted osmotic stress by maintain cell turgor and membrane potential. It also activated enzymes for metabolism, protein and carbohydrate synthesis, and regulated stomata movement (Paudel and Sun, 2023). The plant absorbed high concentrations of salt such as Cl⁻ (1672 mg kg⁻¹, 1918 mg kg⁻¹) and Na⁺ (949 mg kg⁻¹, 1303 mg kg⁻¹) (Table 4.20 and 4.21), which disturbed the K and Ca uptakes and caused ionic imbalance (Arif *et al.*, 2020). The plant growth and development were closely related to K⁺ which is required in protein synthesis, carbohydrate metabolism and enzyme activation. The K also involved in flowering, pollen germination and seed development, which eventually increased crop yield and quality (Hasanuzzaman *et al.*, 2018). Therefore, the rice yield (Table 4.14 and 4.15) was reduced in higher salinity because of low uptake K (Figure 4.8 D) in this study.

4.3.6.3 Total magnesium uptake

Although the total Mg uptake of PT1 was not significantly different among seawater mixing ratios, the highest value was observed in 4% and the smallest value was found in 10% seawater in both seasons (Figure 4.9 A and B). There was no significant difference in total Mg uptake of RD43 among seawater mixing ratios in the major season, however, total Mg uptake was significantly increased by 2% which varied from 10% seawater in the second rice season (Figure 4.9 A and B). The interaction between variety and seawater mixing ratio on total Mg uptake was not observed in both seasons (Table 4.16 and 4.17). The decreased Mg content in plants reduced growth (Table 4.7- 4.9) and grain yield (Table 4.14 and 4.15) under high salinity stress because Mg played an important role in photosynthesis as partitioning of carbohydrates and dry matter production between roots and shoots and higher Mg level was the ability to enhance photosynthesis rate (Zhao *et al.*, 2021). In our study, the reduction of plant growth and yield might be the decrease in other nutrients such as K, Ca, and Mg uptakes under salinity stress.

4.3.6.4 Total calcium uptake

Although PT1 had no significant increase in total Ca uptake among various seawater mixing ratios in both seasons, the maximum values occurred in 8% seawater in the major rice season and 6% in the second rice season (Figure 4.9 C and D). There was no significant difference in total Ca uptake among seawater in both seasons, however, 8% and 2% seawater mixing ratios gave the greatest values in the major rice season and second rice season, respectively (Figure 4.9 C and D). There was no significant interaction between variety and seawater mixing ratio in total Ca uptake for both seasons (Table 4.16 and 4.17). Furthermore, the Ca uptake and Mg uptake were lower because of the high accumulation of Na under salinity in this study. The low Ca content was one of the limiting of plant growth under salt stress (Zhao *et al.*, 2021) because the Ca is crucial for the preservation of cell membrane integrity and it is also an important key in the synthesis of new cell walls (Hakim *et al.*, 2014).

4.3.6.5 Total chloride uptake

The total Cl uptake of PT1 was significantly increased ($P < 0.01$) in the 8% seawater mixing ratio in the major rice season and 6% seawater in the second rice season, which were significantly different from control in both seasons (Figure 4.10 A and B). There was no significant difference in total Cl uptake of RD43 among seawater mixing ratios in both seasons (Figure 4.10 A and B). The maximum values were shown in 8% seawater mixing while the minimum values resulted in control in both seasons. The interaction of variety and seawater mixing ratio on total Cl uptake was not observed in both seasons (Table 4.16 and 4.17). Furthermore, in this study, the higher salinity 4%- 10 % seawater ratios increased the uptake of Cl (Figure 4.10 A and B) over sufficiency range in both seasons (Bryson and Mills, 2014). Thus, the high accumulation of Cl in plant declined normal growth (Table 4.7- 4.9) and yield of plant (Table 4.14 and 4.15) (Ishrat *et al.*, 2022) by reducing of photosynthesis due to degradation of chlorophyll (Table 4.10 and 4.11) (Hniličková *et al.*, 2019). High salinity ratios with Na^+ (Figure 4.8 A and B) and Cl^- toxicity (Figure 4.10 A and B) reduced photosynthesis pigments (Figure 4.14) formation which hindered photosynthesis leading to inhibition of plant growth (Bayat *et al.*, 2022). Furthermore, high accumulation of Cl^- in leaf tissue altered plant growth hormones, enzyme activity, photosynthetic activity due to low assimilation of CO_2 leading to reductions in plant height (Table 4.7 and 4.8) and dry weight (Table 4.9) (Hasanuzzaman *et al.*, 2021).

4.3.6.6 Total sulfur uptake

The seawater mixing ratios 8% and 10% demonstrated the significantly larger ($P < 0.01$) total S uptake of PT1 in the major season, which was significantly varied from other treatments, however, no significant variation was observed in the second rice season (Figure 4.10 C and D). The total S uptake of RD43 was ($P < 0.01$) significantly different among seawater mixing ratios in the major season and the 8% seawater mixing ratio showed the highest value and it was significantly increased compared to control (Figure 4.10 C and D). However, the various seawater mixing ratios did not significantly affect total S uptake in the second rice season. There was no significant interaction between variety

and seawater mixing ratio in total S uptake for both seasons (Table 4.16 and 4.17). The high S content in plant (Figure 4.10 C and D) was observed under high salinity stress 6 %-10% seawater mixing ratios with longer period, which caused imbalance nutrient uptake and damage to the plant cells and tissues (Balasubramaniam *et al.*, 2023).

4.3.6.7 Total nitrogen uptake

The total N uptake of PT1 was significantly different ($P < 0.01$) among seawater mixing ratios in the major rice season and there was no significant variation among seawater in the second rice season (Figure 4.11 A and B). It was observed that the maximum value was observed in 4% seawater which was significantly different from 0%, 8%, and 10% seawater mixing ratios in the major season, and 2% seawater in the second season. The different seawater mixing ratios had a significant effect ($P < 0.01$) on total-N uptake of RD43 in both seasons (Figure 4.11 A and B). The highest values occurred in 2% and 0% seawater mixing in the major rice season and second rice season, respectively and these were significantly varied from other treatments. The interaction between variety and seawater mixing ratio on total N uptake was observed in both seasons (Table 4.16 and 4.17). The higher seawater mixing ratios showed a decrease total N uptake in the major rice season, however, it did not affect the second rice season (Figure 4.11 A and B). Li *et al.* (2023) reported that the salinity decreased N uptake leading to reducing plant growth, tillers, yield and yield components. According to this study, the total uptakes of Na, Cl and S were negatively correlated with plant growth and grain yield and yield components (Figure 4.12).

4.3.6.8 Total phosphorus uptake

The PT1 indicated a significantly improved ($P < 0.01$) in total P uptake in 2% seawater mixing ratio which was significantly different from 8% and 10% in the major rice season, however, there was no significant variation in total P uptake among seawater mixing ratios in the second rice season (Figure 4.11 C and D). The different sweater mixing ratios had a significant effect ($P < 0.01$) on total P uptake of RD43 in both seasons (Figure 4.11 C and D). The 2% seawater showed the greatest values and it was significantly

different from the 8% and 10% seawater mixing ratios. The interaction of variety and seawater mixing ratio on total P uptake was not observed in both seasons (Table 4.16 and 4.17). The previous study reported that the response P uptake under salinity stress was different (Korres *et al.*, 2022), the P uptake was decreased with increased salinity in this study (Figure 4.11 A and B). In a previous study, (Balasubramaniam *et al.*, 2023) reported that when the soil had excessive amount of Na^+ , Cl^- and SO_4^{2-} (Table 4.19 and 4.20) reduced P uptake (Figure 4.11 C and D) which was required for photosynthesis and decreased water potential around the root zone and reduced water content in plant cells that led to inhibiting plant growth (Table 4.7- 4.9).

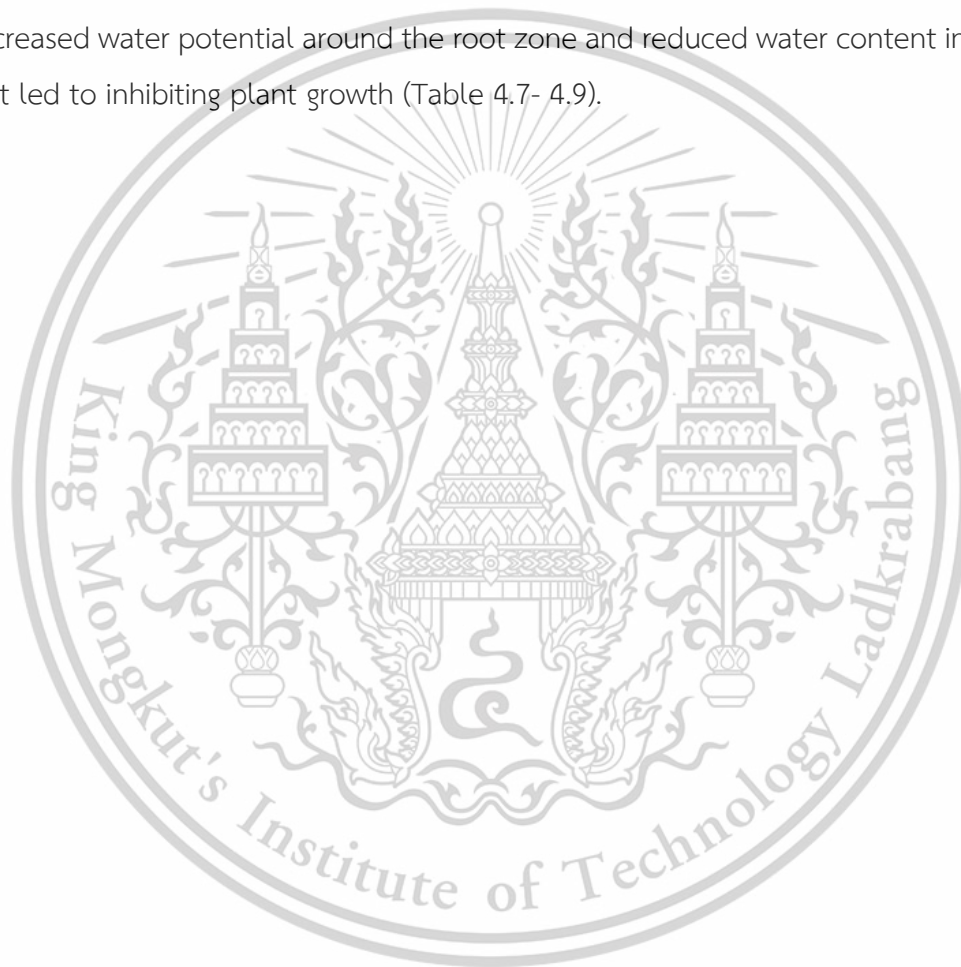


Table 4.16 Summary of the analyses of variance of nutrient uptakes in two rice varieties and six seawater mixing ratios in the major rice season.

Nutrient uptakes	Major rice season														
	Variety					Seawater mixing ratios					Variety × Seawater mixing ratios interaction				
	df	SS	MS	F	p-value	df	SS	MS	F	p-value	df	SS	MS	F	p-value
Total-Na uptake (mg plant ⁻¹)	1	0.06502	0.06502	17.36	0.0004	5	0.03451	0.00690	1.84	0.01459	5	0.00656	0.00131	0.35	0.8766
Total-K uptake (mg plant ⁻¹)	1	0.02890	0.02890	69.91	<0.0001	5	0.01696	0.00339	8.20	0.0002	5	0.00013	0.00003	0.06	0.9968
Total-Mg uptake (mg plant ⁻¹)	1	0.00840	0.00840	47.27	<0.0001	5	0.00181	0.00036	2.04	0.1122	5	0.00041	0.00008	0.47	0.7976
Total-Ca uptake (mg plant ⁻¹)	1	0.00360	0.00360	33.70	<0.0001	5	0.00107	0.00021	2.00	0.1189	5	0.00087	0.00017	1.62	0.1957
Total-N uptake (mg plant ⁻¹)	1	0.00472	0.00472	1.68	0.2220	5	0.17103	0.03421	12.14	0.0004	5	0.10858	0.02172	7.71	0.0025
Total-S uptake (mg plant ⁻¹)	1	0.01247	0.01247	11.28	0.0028	5	0.05611	0.01122	10.15	<0.0001	5	0.00831	0.00166	1.50	0.2292
Total-P uptake (mg plant ⁻¹)	1	0.00218	0.00218	9.13	0.0063	5	0.01247	0.00249	10.45	<0.0000	5	0.00099	0.00020	0.83	0.5429
Total-Cl uptake (mg plant ⁻¹)	1	0.00002	0.00002	11.88	0.0023	5	0.00001	0.000003	1.67	0.01832	5	0.000002	0.000005	0.26	0.9279

Table 4.17 Summary of the analyses of variance of nutrient uptakes in two rice varieties and six seawater mixing ratios in the second rice season.

Nutrient uptakes	Second rice season														
	Variety					Seawater mixing ratios					Variety × Seawater mixing ratios interaction				
	df	SS	MS	F	p-value	df	SS	MS	F	p-value	df	SS	MS	F	p-value
Total-Na uptake (mg plant ⁻¹)	1	0.01106	0.01106	20.12	0.0002	5	0.02660	0.00532	9.68	0.0001	5	0.00471	0.00094	1.71	0.1730
Total-K uptake (mg plant ⁻¹)	1	0.00031	0.00031	1.91	0.1811	5	0.01234	0.00247	15.09	0.0000	5	0.00204	0.00041	2.49	0.0625
Total-Mg uptake (mg plant ⁻¹)	1	0.00011	0.00001	6.34	0.0196	5	0.00037	0.000007	4.27	0.0073	5	0.00013	0.000002	1.49	0.2324
Total-Ca uptake (mg plant ⁻¹)	1	0.0000006	0.0000006	0.35	0.5587	5	0.00001	0.000002	1.62	0.1957	5	0.000007	0.000001	0.89	0.5043
Total-N uptake (mg plant ⁻¹)	1	0.00482	0.00482	10.88	0.0080	5	0.03373	0.00675	15.23	0.0002	5	0.03421	0.00684	15.45	0.0002
Total-S uptake (mg plant ⁻¹)	1	0.00130	0.00130	7.84	0.0104	5	0.00076	0.00015	0.91	0.4897	5	0.00057	0.00011	0.69	0.635
Total-P uptake (mg plant ⁻¹)	1	0.00013	0.00001	2.67	0.1165	5	0.00280	0.00005	11.29	0.0000	5	0.00050	0.00001	2.02	0.1151
Total-Cl uptake (mg plant ⁻¹)	1	0.000002	0.000002	11.57	0.0026	5	0.000007	0.000001	7.34	0.0004	5	0.000009	0.000001	0.94	0.4731

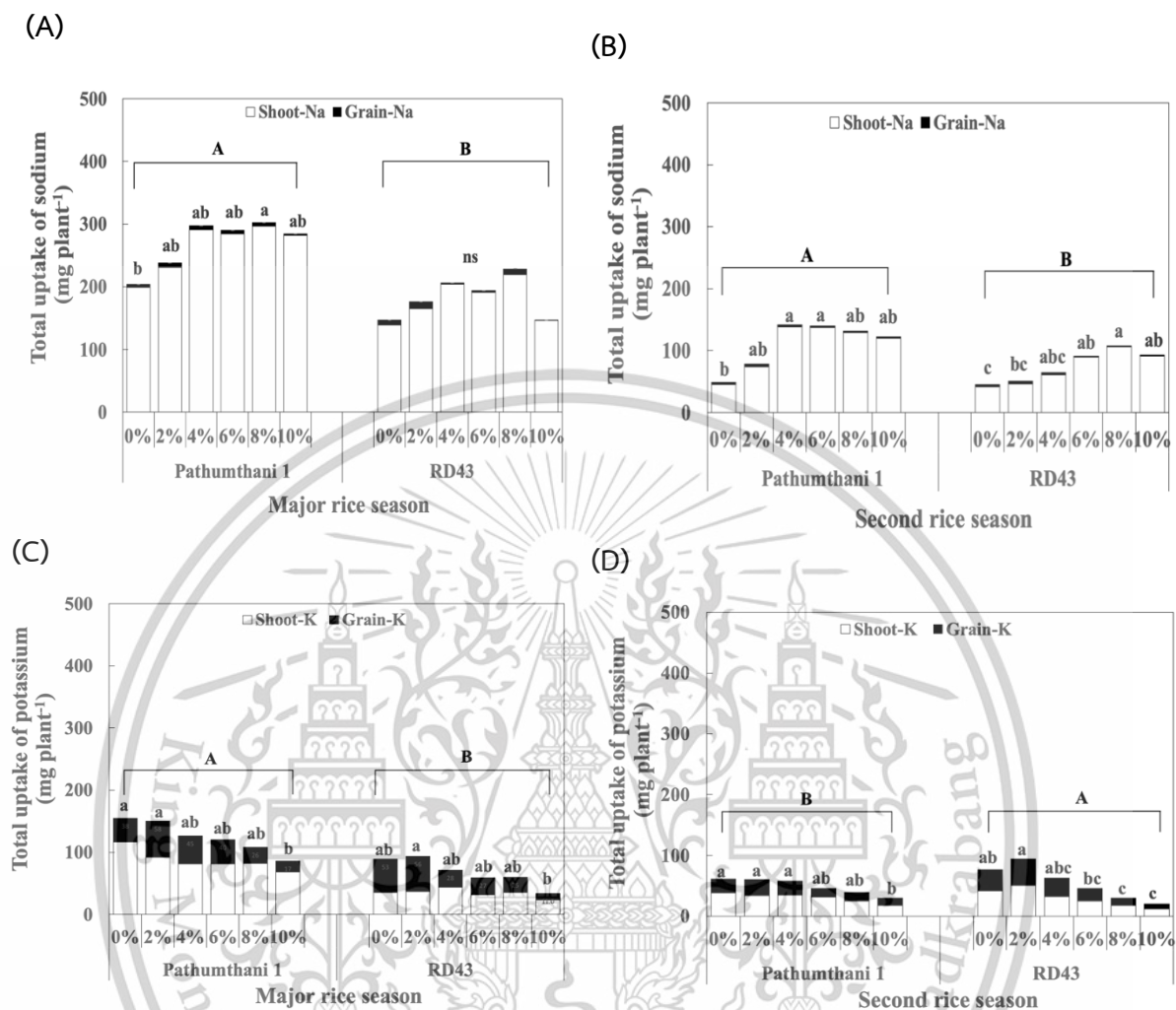


Figure 4.8 Effects of seawater intrusion on (A, B) total uptake of sodium and (C, D) total uptake of potassium of rice plant in the major rice season and second rice season, respectively. Bars with the same uppercase letters are not significantly different between two rice varieties and, bars with the same lowercase letters are not significantly different among seawater mixing ratios by DMRT at $P < 0.05$.

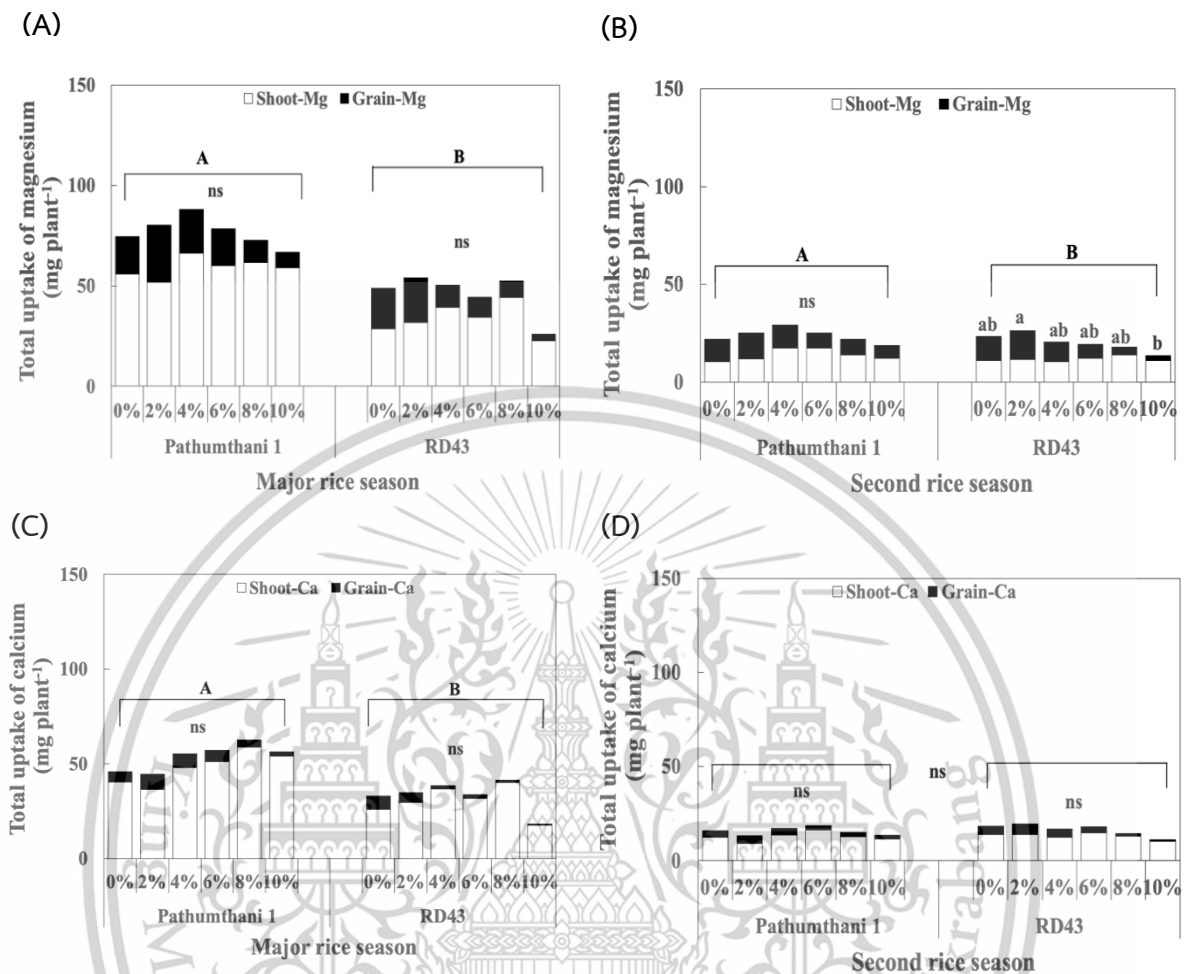


Figure 4.9 Effects of seawater intrusion on (A, B) total uptake of magnesium and (C, D) total uptake of calcium of rice plant in the major rice season and second rice season, respectively. Bars with the same uppercase letters are not significantly different between two rice varieties and, bars with the same lowercase letters are not significantly different among seawater mixing ratios by DMRT at $P < 0.05$.

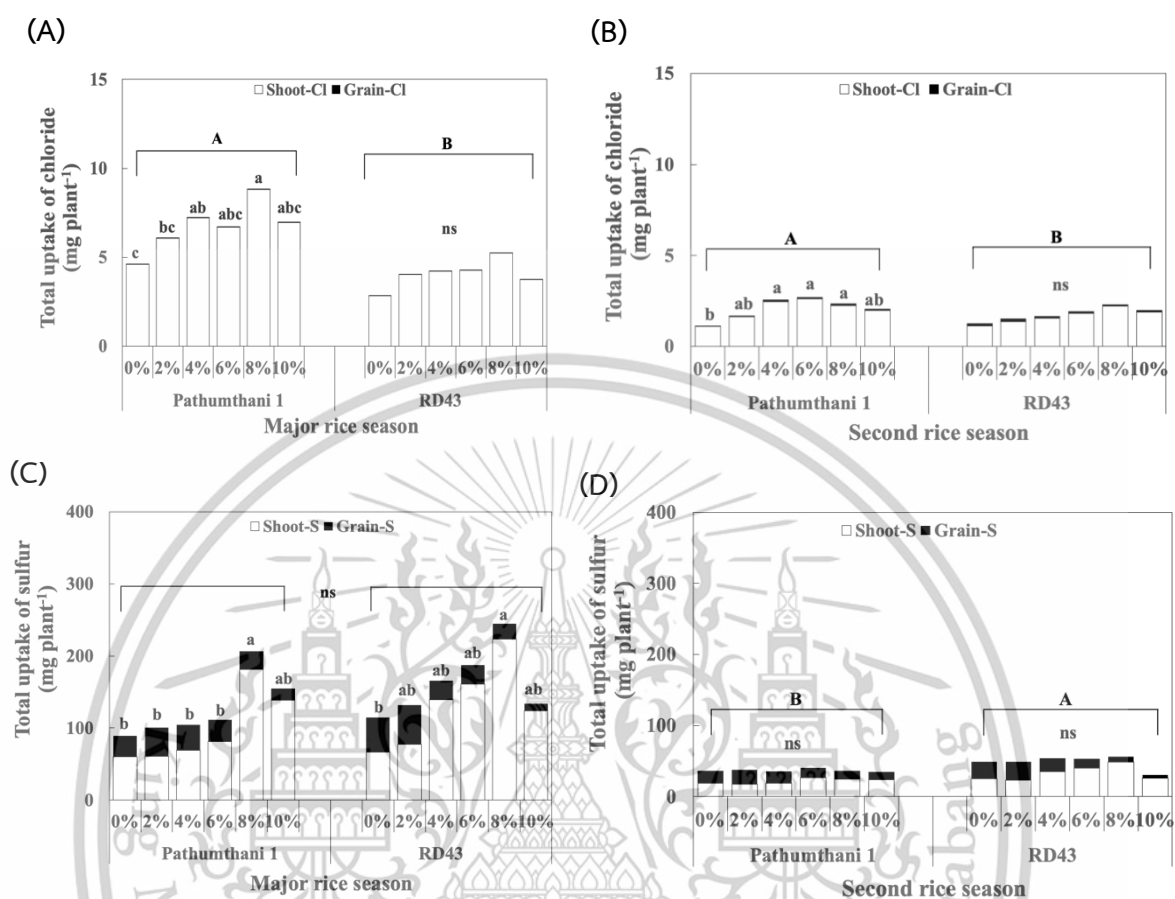


Figure 4.10 Effects of seawater intrusion on (A, B) total uptake of chloride and (C, D) total uptake of sulfur of rice plant in the major rice season and second rice season, respectively. Bars with the same uppercase letters are not significantly different between two rice varieties and, bars with the same lowercase letters are not significantly different among seawater mixing ratios by DMRT at $P < 0.05$.

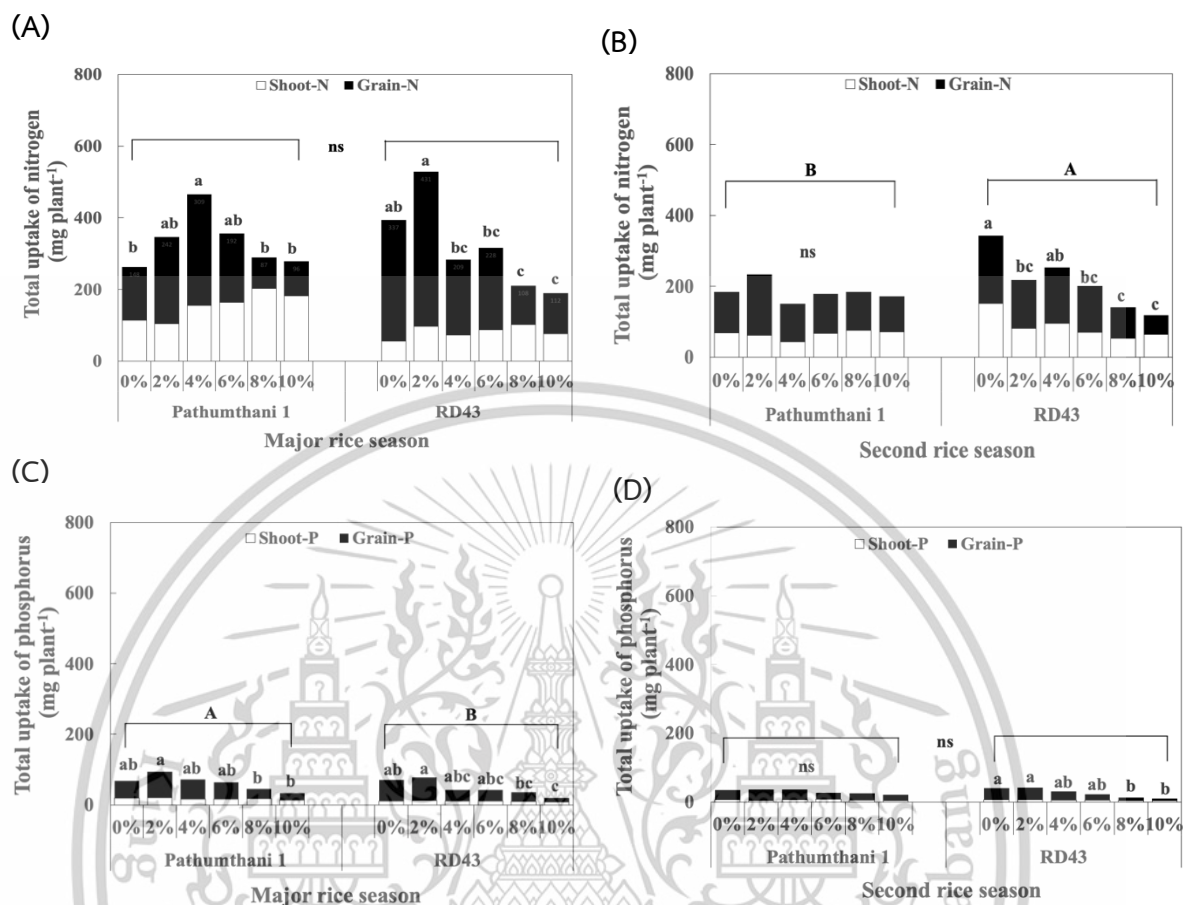


Figure 4.11 Effects of seawater intrusion on (A, B) total uptake of nitrogen and (C, D) total uptake of phosphorus of rice plant in the major rice season and second rice season, respectively. Bars with the same uppercase letters are not significantly different between two rice varieties and, bars with the same lowercase letters are not significantly different among seawater mixing ratios by DMRT at $P < 0.05$.

4.3.7 Relationship between growth, yield, biochemical synthesis, and nutrient concentrations

In the PCA model, the first of two principal component axes accounted for 91.5% and 94.6% of the total data variance in the first and second rice seasons, respectively (Figure 4.12 and 4.13). In the major rice season, the first component (PC1) accounts for 85.2% of the total variance in the dataset, indicating that it captures the most significant variation among the variables (Figure 4.12). The main trend expressed on the PC1 axis was the highly positive loadings of the grain yield, growth parameters (height, tiller number, shoot, and straw dry weight), nutrient concentrations in grain (K, Ca, Mg), biochemical synthesis (proline, total phenol, chlorophyll content), and water-related parameters, such as RWC. Moreover, the concentrations of Na, Mg, Ca, Cl, and S in straw were highly negative with grain yield and growth parameters (Figure 4.12). The results suggest that healthy growth, efficient K, Ca, and Mg translocation and partitioning to grains, high biochemical synthesis, and optimal water content increase grain yield, whereas high Na, Mg, Ca, Cl, and S accumulation in rice straw reduces growth and yield.

In the second rice season, the first and second component accounted for 77% and 17.6% of the variance in the data, respectively (Figure 4.13). The variables strongly associated with PC1 were plant growth parameters (height, tiller number, shoot, and straw dry weight), K concentration in straw Ca concentration in grain, and RWC which there was a positive correlation with grain yield. Moreover, the concentration of Na, Cl, Mg, Ca, and S in straw contributed significantly to PC1 and PC2 and had a negative correlation with GY and growth parameters (Figure 4.13). Biochemical properties, such as proline and total phenol, are positively correlated with K, Ca, and S concentrations in grain, whereas chlorophyll content has a strong positive correlation with H_2O_2 . Biochemical synthesis in the second season, such as proline and total phenol, was associated with high K, Ca, and S concentrations. In both seasons, all nutrient concentrations in straw positively correlated with the concentration in grain, except mobile elements, such as K and Mg (Figure 4.12 and 4.13), indicating less translocation of two nutrients from the vegetative part to the reproductive part.

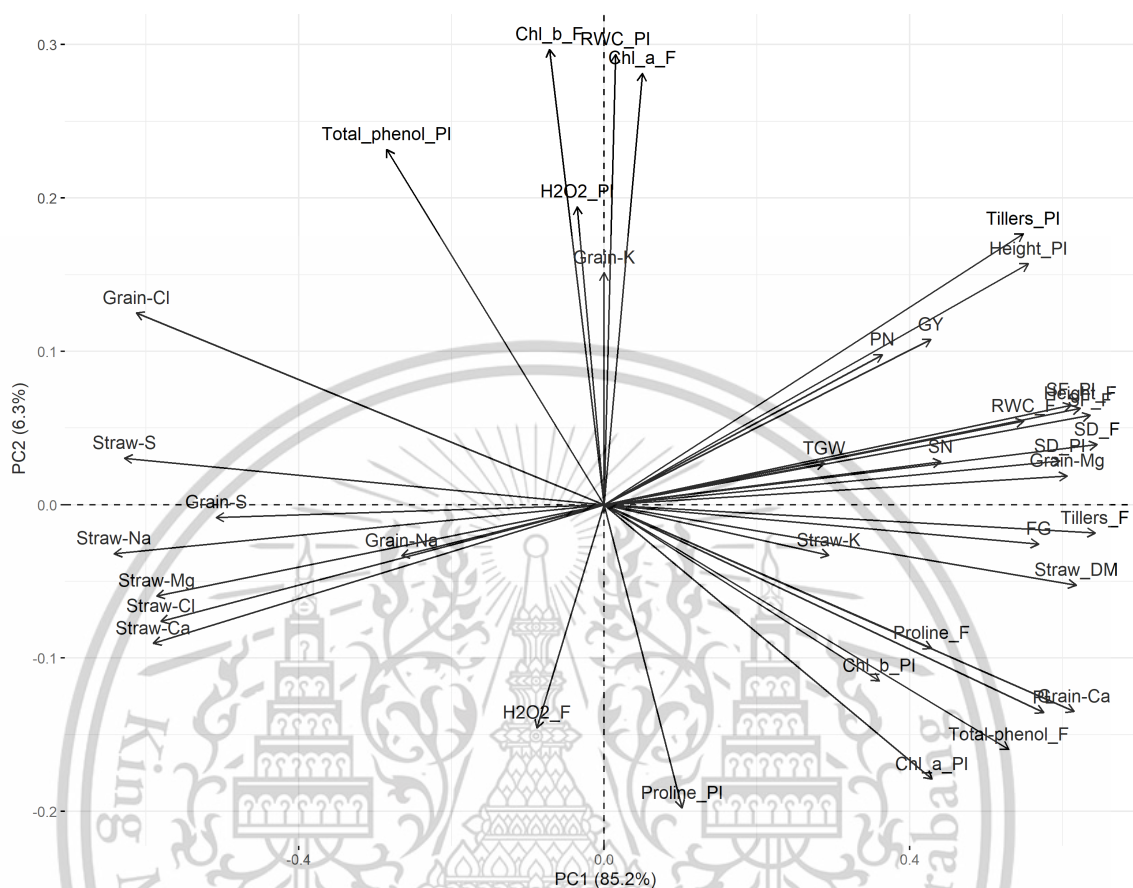


Figure 4.12 Principal component analysis (PCA) of the total data variance in the major rice season.

Notes: Height, plant height; tillers, number of tillers; SF, shoot fresh weight; SD, shoot dry weight; PN, panicle pot^{-1} ; (SN), spikelet panicle $^{-1}$; PL, panicle length; FG, filled grain; TGW, 1000-grain weight; DM, dry matter; RWC, relative water content; H_2O_2 , hydrogen peroxide; Chl a, chlorophyll a; Chl b, chlorophyll b; Na, sodium; K, potassium; Ca, calcium; Mg, Magnesium; Cl, chloride; S, sulfur; PI, panicle initiation stage; F, flowering stage.

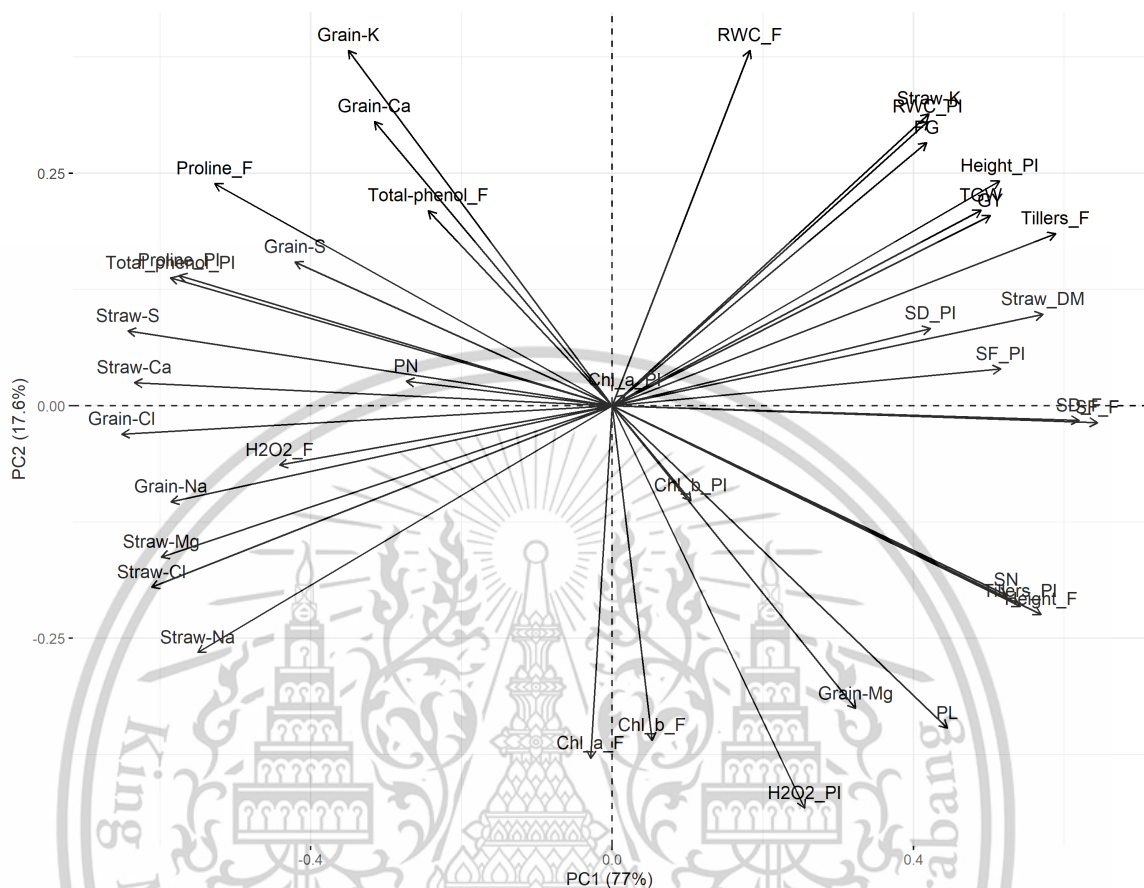


Figure 4.13 Principal component analysis (PCA) of the total data variance in the second rice season.

Notes: Height, plant height; tillers, number of tillers; SF, shoot fresh weight; SD, shoot dry weight; PN, panicle pot⁻¹; SN, spikelet panicle⁻¹; PL, panicle length; FG, filled grain; TGW, 1000-grain weight; DM, dry matter; RWC, relative water content; H₂O₂, hydrogen peroxide; Chl a, chlorophyll a; Chl b, chlorophyll b; Na, sodium; K, potassium; Ca, calcium; Mg, Magnesium; Cl, chloride; S, sulfur; PI, panicle initiation stage; F, flowering stage.

Table 4.18 Effects of variety and seawater intrusion on uptake of Na/K in plant for the major and second rice seasons.

Treatment	Major rice season		Second rice season	
	Straw	Grain	Straw	Grain
Variety				
Pathumthani 1	3.16b	0.15b	3.97a	0.17a
RD43	5.29a	0.17a	3.56b	0.14b
Seawater				
0%	2.89c	0.14b	1.12c	0.13
2%	3.65bc	0.16b	1.58c	0.14
4%	4.05abc	0.12c	3.03bc	0.14
6%	4.64ab	0.13bc	4.27ab	0.14
8%	4.93ab	0.30a	5.58ab	0.19
10%	5.19a	0.13bc	7.06a	0.20
F test				
Variety	**	**	*	*
Seawater	**	**	**	ns
Variety × Seawater	*	**	**	**
CV %	19.1	14.32	16.72	20.09

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

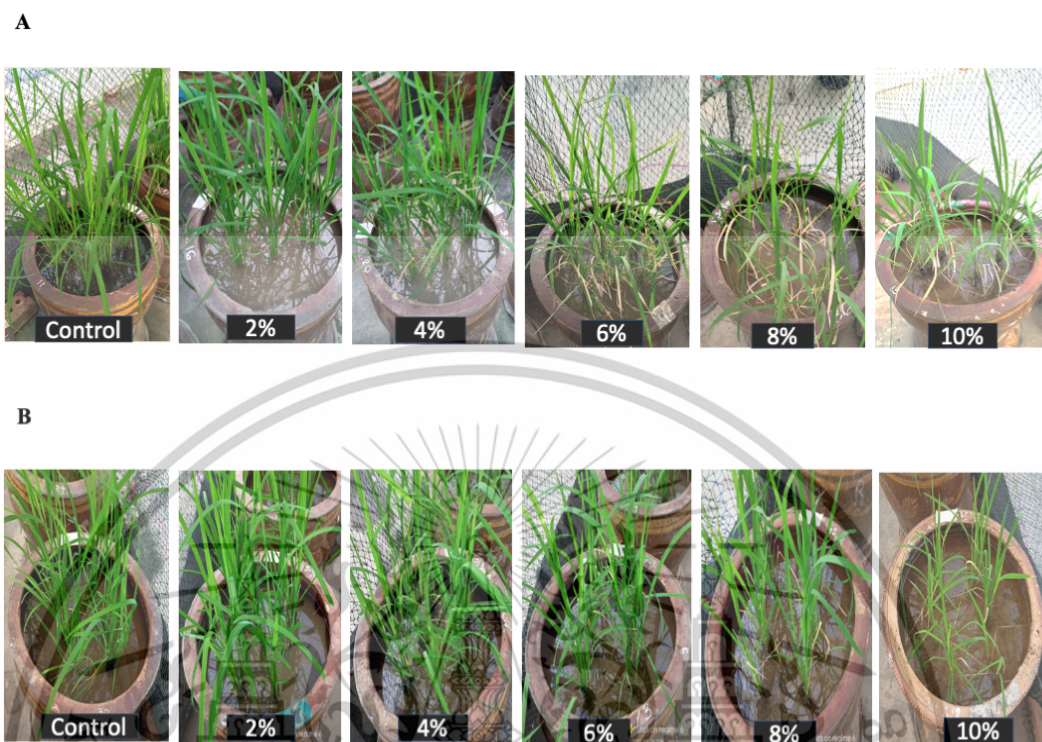


Figure 4.14 Effects of various seawater mixing ratios on rice plant growth of (A) PT1 and (B) RD43.

4.3.8 Salinity effects on soil properties

4.3.8.1 Salinity effects on pH, EC, ECe and soluble Cl^- and total S of soil

The two rice varieties had significant effects ($P < 0.01$) on pH, ECe, soluble Cl^- and total S values in both seasons except pH in the second season (Table 4.20).

The values were highest in PT1 compared with RD 43 in both seasons except pH in the major season. The ECe, soluble Cl^- and total S were highly significantly different ($P < 0.01$) among seawater intrusion ratios in both seasons whereas there was no significant difference in pH in both season (Table 4.19). The ECe, Cl^- and S were higher with higher seawater applications. The greatest values were observed in 8% and 10% seawater applications while the smallest values resulted in control. The pH value was slightly increased in the second rice season. In this study, the pH value was around 5.2. The pH between 5.5 and 6.5 was suitable for rice cultivation. The previous study reported that the saline irrigation water did not affect soil pH during the growing period (Abedinpour, 2016). Furthermore, the previous author described that the saline soils have a pH that did not rise above 8.2. The accumulation of soluble salts reduces the pH of saline soils (Dhouha, 2015). In addition, soil pH was the most important factor in the nutrient availability of soils. In most cases, the range of pH 6.0 – 7.5 was optimum for the sufficient availability of nutrients in the soil. The ECe value was increased and caused saline soil after 4% of seawater application in both seasons according to Yang *et al.* (2022). The soil ECe value increased with increasing salinity of irrigation water and it is directly proportional to the salt concentration in the irrigation water (Sheferia *et al.*, 2021). The ECe value of 2.96 dS m^{-1} entirely inhibited the growth of rice under field conditions (Nijimbere, 2014). In addition, the high ECe in soil decreased concentrations of K, Ca, and Mg in rice shoots (Farooq *et al.*, 2022). The Cl^- concentration in soil was classified as low ($<175 \text{ mg kg}^{-1}$), medium ($175\text{-}700 \text{ mg kg}^{-1}$), and high ($>700 \text{ mg kg}^{-1}$) (Re *et al.*, 2022). In this study, the 2% seawater application increased Cl^- content $>700 \text{ mg kg}^{-1}$ causing toxicity effects on most of the plants. Moreover, the Cl^- ion can move in the soil by water flowing. The more Cl^- concentration in the soil solution, the more uptake of Cl^- by the plant. The soil salinity is related to the accumulation of soluble salts especially the main portion was Cl^- . The

increasing salinity severely affected plant growth through impact on plant-water relation. Thus, the plant wilted because of less ability to absorb water from the soil (Bryson and Mills, 2014). The high amount of S had a negative impact on plants, which damaged root systems and reduced plant growth (Likus-Cieřlik *et al.*, 2018). There was no significant interaction between rice variety and seawater intrusion was observed in this study.

4.3.8.2 Salinity effects on exchangeable K^+ , Ca^{2+} , Mg^{2+} , Na^+ , SAR and ESP of soil

The two rice varieties had significant effects ($P < 0.01$) on K^+ , Ca^{2+} , Mg^{2+} , and Na^+ in both seasons (Table 4.20). In the major rice season, the RD43 significantly increased exchangeable K^+ , Ca^{2+} , Mg^{2+} however the values were significantly increased in PT1 in the second rice season. The exchangeable Na^+ was significantly increased in PT1 at the major rice season and RD43 was higher than PT1 in the second rice season. There was a significant difference ($P < 0.01$) in exchangeable K^+ , Ca^{2+} , Mg^{2+} and Na^+ among seawater mixing ratios in both seasons except exchangeable Ca^{2+} in the major rice season (Table 4.20). The highest values of exchangeable K^+ and Na^+ were recorded in 10% seawater application while exchangeable Ca^{2+} and Mg^{2+} were greater in control in both seasons. The exchangeable Na^+ was increased with higher levels of seawater applications. The SAR and ESP were not significantly different between two rice varieties in both seasons (Table 4.21). There was a significant difference ($P < 0.01$) in SAR and ESP among various seawater mixing ratios (Table 4.21). The highest SAR and ESP values were observed in seawater mixing 10% followed by 8%, 6%, 4%, 2%, and the lowest values occurred in control. When the saline water was used for irrigation, Na^+ replaced Ca^{2+} and Mg^{2+} in the soil leading to the soil became sodicity if the saline water applied continuously. When Na^+ replaced Ca^{2+} and Mg^{2+} , it damaged soil structure as a result of the formation of crusts, water logging, decreasing aeration, and infiltration. Besides, Na^+ in the soil might be toxic to the crops (Ogunfowokan *et al.*, 2013). The excess amount of Na changed the physical properties of the soil due to swelling and dispersion of soil particles. Then, it caused problems with air movement, water holding capacity, water infiltration, root penetration, and seedling emergence (Worku and Bedadi, 2016). The sodicity level was determined as ESP and SAR

values using amount of exchangeable Na^+ (Fornoda and Colinet, 2023). The high concentration of soluble salts in the soil can reduce the uptake of water by plants which causes physiological drought to plants because of salt accumulation in the root zone. The entry of Na^+ into the plant cells from the soil disturbed the ion balance in plant and appeared many injuries concerning the physiology of plant tissues like root, leaf and grain (Hussain *et al.*, 2019).



Table 4.19 Effects of rice variety and seawater intrusion on pH, ECe, soluble Cl⁻ and total S of soil in the major and second rice seasons.

Treatment	Major rice season				Second rice season			
	pH (1:1)	ECe (dS m ⁻¹)	Soluble Cl ⁻ (mg kg ⁻¹)	Total S (g kg ⁻¹)	pH (1:1)	ECe (dS m ⁻¹)	Soluble Cl ⁻ (mg kg ⁻¹)	Total S (g kg ⁻¹)
Variety								
Pathumthani 1	4.83b	2.75a	1225a	3.8a	5.16	2.94a	1285a	0.9a
RD43	5.20a	2.31b	972b	1.5b	5.24	2.13b	930b	0.7b
Seawater								
0%	5.14	1.46c	538c	2.5b	5.29	0.96c	346d	0.7
2%	5.02	1.92bc	792c	2.4b	5.22	1.36bc	488d	0.7
4%	4.99	2.24b	854c	2.5b	5.12	2.21b	994c	0.8
6%	5.01	2.90a	1234b	2.0b	5.10	3.31a	1355bc	0.9
8%	4.94	3.11a	1512ab	2.6b	5.24	3.38a	1544ab	0.9
10%	4.99	3.53a	1672a	3.6a	5.22	3.98a	1918a	0.9
F test								
Variety	**	**	**	**	ns	**	**	**
Seawater	ns	**	**	**	ns	**	**	ns
Variety × Seawater	ns	ns	ns	ns	ns	ns	ns	ns
CV%	2.70	13.81	17.93	16.69	2.29	19.13	19.39	13.45

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

Table 4.20 Effects of rice variety and seawater intrusion on exchangeable K^+ , Ca^{2+} and Mg^{2+} of soil in the major and second rice seasons.

Treatment	Major rice season			Second rice season		
	Exchangeable K^+ (mg kg ⁻¹)	Exchangeable Ca^{2+} (mg kg ⁻¹)	Exchangeable Mg^{2+} (mg kg ⁻¹)	Exchangeable K^+ (mg kg ⁻¹)	Exchangeable Ca^{2+} (mg kg ⁻¹)	Exchangeable Mg^{2+} (mg kg ⁻¹)
Variety						
Pathumthani 1	98b	1501b	1014b	128a	1607a	1126a
RD43	111a	1600a	1048a	112b	1551b	1091b
Seawater						
0%	90d	1569	1059a	95d	1609a	1146a
2%	94cd	1577	1046ab	100d	1629a	1130a
4%	102bc	1556	1030abc	113c	1580ab	1104ab
6%	110ab	1549	1035abc	125bc	1597ab	1132a
8%	110ab	1524	1017bc	134b	1520b	1084ab
10%	121a	1527	998c	152a	1542ab	1054b
F test						
Variety	**	**	**	**	**	**
Seawater	**	ns	**	**	**	**
Variety × Seawater	ns	ns	ns	ns	**	**
CV%	6.39	3.45	2.14	5.67	3.09	3.16

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

Table 4.21 Effects of rice variety and seawater intrusion on exchangeable Na⁺, SAR and ESP of soil in the major and second rice seasons.

Treatment	Major rice season			Second rice season		
	Exchangeable Na ⁺ (mg kg ⁻¹)	SAR	ESP (%)	Exchangeable Na ⁺ (mg kg ⁻¹)	SAR	ESP (%)
Variety						
Pathumthani 1	731a	8.61	12.67	885b	9.26	15.66
RD43	674b	8.26	12.61	982a	9.72	16.99
Seawater						
0%	453f	5.67d	8.20d	566d	5.25e	9.97d
2%	546e	7.24c	9.89c	637cd	7.32d	11.00cd
4%	631d	7.76c	11.34c	835c	9.27c	14.61c
6%	778c	9.05b	13.97b	1090b	10.58bc	19.40b
8%	860b	9.96ab	15.48a	1180ab	11.62ab	19.66ab
10%	949a	10.81a	16.97a	1303a	12.90a	23.39a
F test						
Variety	**	ns	ns	*	ns	ns
Seawater	**	**	**	**	**	**
Variety × Seawater	ns	ns	ns	ns	ns	ns
CV%	6.37	7.21	6.62	11.80	7.84	12.36

Notes: The mean followed by same letter in each column indicated no significant difference, ns- no significant, * and ** significant at $P < 0.05$ and $P < 0.01$, respectively by DMRT.

4.4 Fourth experiment- Influence of Rice Straw Biochar Amendment on Salinity in Salt-affected Soils

4.4.1 Soil pH

Although there was no interaction between the salt-affected soil and the biochar application, the ANOVA showed that both biochar application and the salt-affected soil had substantial ($P < 0.01$) effects on soil pH (Table 4.22). The pH of the soil varied significantly ($P < 0.01$) depending on which biochar application was used (Figure 4.15). For 0%, 1%, 3%, and 5% applications, the average soil pH values were 5.29, 5.30, 5.56, and 5.78, respectively. The applications with the greatest value were 5%, 3%, 1%, and 0%. Only in the control did the soil pH values differ significantly ($P < 0.01$) from S1 to S6, but not at other biochar application rates (Figure 4.15). While there was no statistically significant difference in soil pH values between the 0%, 1%, and 3% application rates, the addition of biochar over 1% decreased the acidity of salt-affected soils. According to a prior study, the addition of biochar raised the pH of the soil, which enhanced soil quality (Dai *et al.*, 2017). The pH difference between the salt-affected soil and biochar is the primary cause of the elevated pH of the salt-affected soil (Nath *et al.*, 2022). Thus, according to Tan *et al.* (2021) the initial pH of biochar might have a significant impact on the pH of saline soil. According to Omara *et al.* (2023), the release of base cations may have replaced exchangeable acidity on the soil surface, lowering soil acidity and contributing to the soil pH increase following biochar application. Since rice was often produced in soil that was submerged, a pH of >5.2 , or 1% biochar application, was an acceptable dose and safe level for rice.

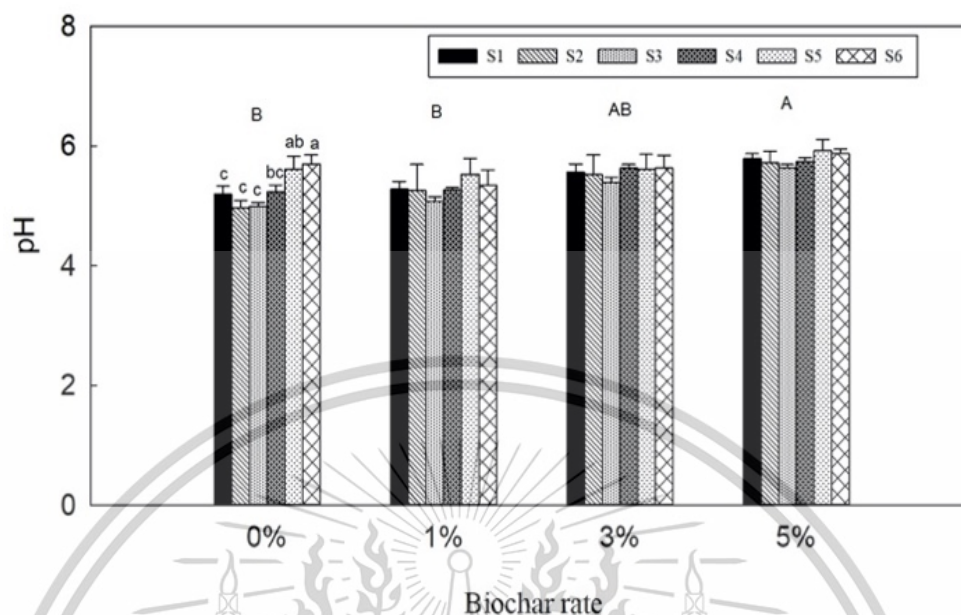


Figure 4.15 The impact of different applications of rice straw biochar on the pH of soil in soils affected by salinity. Significant variations were indicated by different capital letters for each biochar application rate and lowercase letters for each soil type at $P < 0.05$. Error bar = standard error.

4.4.2 Soil ECe

Table 4.22 showed that there was no interaction between the application of biochar and salt-affected soil, although the ANOVA did show that both factors had substantial effects ($P < 0.01$) on ECe. Among the various biochar treatments, the ECe values varied highly substantially ($P < 0.01$) (Figure 4.16). In 0%, 1%, 3%, and 5% applications, the average values of ECe were 4, 6, 8, and 9 dS m^{-1} , respectively. The ECe value rose as the amount of biochar application increased. The applications with the greatest value were 5%, 3%, 1%, and 0%. Regardless of the rates at which biochar was applied, the ECe values among the salt-affected soils increased significantly ($P < 0.01$) (Figure 4.16). S6 displayed the greatest value, while S1 had the lowest value. Furthermore, in every salt-affected soil, the ECe rose as biochar treatments increased. The biochar applications with the greatest ECe value were 5%, 3%, 1%, and 0%, respectively. According to the earlier research, adding biochar to soils impacted by salt raised the EC (Zheng *et al.*, 2022). Because the biochar

contained a lot of base ions (Li *et al.*, 2018), like K^+ in our study, which was good for rice growth in salt-affected soil, the increase rate of biochar application led to higher salinity (ECe), which was greater than the standard value (4 dS m^{-1}) (Foronda and Colinet, 2023) in soil.

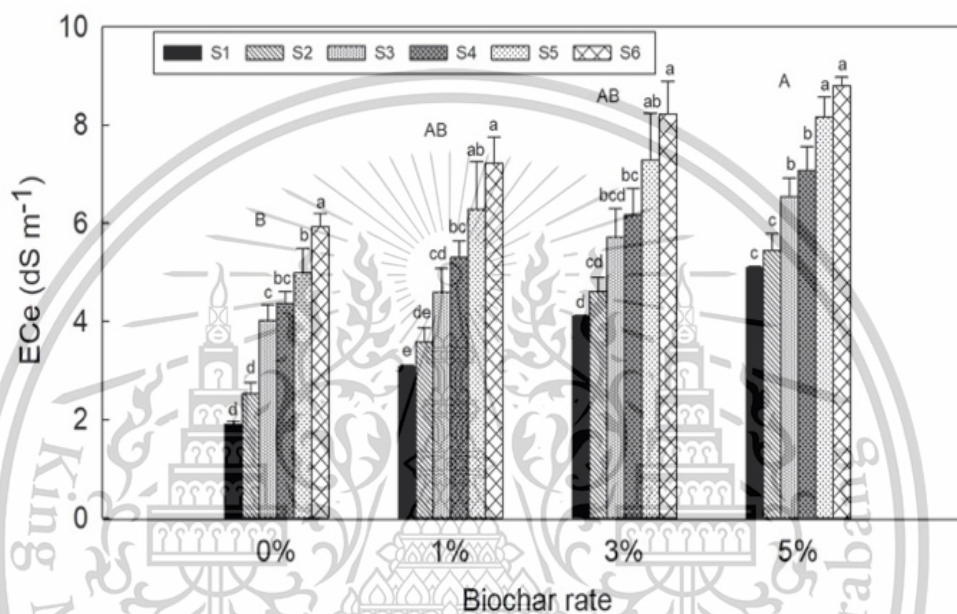


Figure 4.16 The impact of different applications of rice straw biochar on the ECe of soil in soils affected by salinity. Significant variations were indicated by different capital letters for each biochar application rate and lowercase letters for each soil type at $P < 0.05$. Error bar = standard error.

Table 4.22 Analyses of variance of soil chemical properties in four biochar application rates and six salt-affected soils.

Soil chemical properties	Biochar					Salt-affected soil					Biochar × Salt-affected soil interaction				
	df	SS	MS	F	p-value	df	SS	MS	F	p-value	df	SS	MS	F	p-value
pH	3	3.03	1.01	30.91	<0.0001	5	1.34	0.27	8.19	<0.0001	15	0.03	0.04	1.32	0.23
ECe (dS m ⁻¹)	3	84.51	28.17	115.80	<0.0001	5	140.14	28.03	115.22	<0.0001	15	0.89	0.06	00.24	0.99
SAR	3	468.59	156.20	1242.14	<0.0001	5	289.95	57.99	461.15	<0.0001	15	47.50	3.17	25.18	<0.0001
ESP (%)	3	1852.01	617.34	1625.20	<0.0001	5	761.98	152.40	401.20	<0.0001	15	275.25	18.35	48.31	<0.0001
Soluble Cl ⁻ (mg L ⁻¹)	3	2.05	6857950	1256.15	<0.0001	5	6127904	1225581	224.49	<0.0001	15	6620988	441399	80.85	<0.0001
Soluble Na ⁺ (mg L ⁻¹)	3	6172080	2057360	1463.29	<0.0001	5	1226370	245274	174.45	<0.0001	15	945445	63030	44.83	<0.0001
Exchangeable Na ⁺ (mg kg ⁻¹)	3	5937557	1979186	1944.95	<0.0001	5	2433508	486702	478.28	<0.0001	15	887221	59148	58.12	<0.0001
Soluble K ⁺ (mg L ⁻¹)	3	128434	42811.7	58.4.98	<0.0001	5	1711	342.1	46.39	<0.0001	15	134	8.9	1.21	0.2961
Exchangeable K ⁺ (mg kg ⁻¹)	3	8446447	2815482	21771.8	<0.0001	5	33599	6720	51.96	<0.0001	15	4958	331	2.56	0.0070
Soluble Ca ²⁺ (mg L ⁻¹)	3	343051	114350	2970.20	<0.0001	5	11523	2305	58.59	<0.0001	15	17712	1181	30.02	<0.0001
Exchangeable Ca ²⁺ (mg kg ⁻¹)	3	296858	98952.5	10.32	<0.0001	5	175998	35199.6	3.67	0.0068	15	42819	2854.6	0.30	0.9935
Soluble Mg ²⁺ (mg L ⁻¹)	3	189057	63019.0	2343.14	<0.0001	5	7302	1460.4	54.30	<0.0001	15	11979	798.6	29.69	<0.0001
Exchangeable Mg ²⁺ (mg kg ⁻¹)	3	978412	326137	462.88	<0.0001	5	24701	4940	7.01	0.0001	15	2532	169	0.24	0.9980

4.4.3 Soil SAR and ESP

Table 4.22 showed that the ANOVA revealed significant effects ($P < 0.01$) on SAR and ESP for the application of biochar, salt-affected soil, and the combination of biochar application and salt-affected soil. The SAR and ESP values varied significantly ($P < 0.01$) depending on the rate at which biochar was applied (Figure 4.17 A and B). For SAR in 0%, 1%, 3%, and 5% applications, the average values were 10.70, 5.04, 4.76, and 4.75; for ESP, they were 18.17%, 6.80%, 6.24%, and 6.38%. As biochar was applied, the SAR and ESP values were substantially lower (about 50%) as compared to the control (Figure 4.17 A and B). In biochar applications, the SAR and ESP values with 1%, 3%, and 5% did not show any noticeable differences from one another. When applying biochar at 0%, 1%, 3%, and 5% rates to salt-affected soils (S1 to S6), the SAR and ESP showed significant differences ($P < 0.01$) (Figure 4.17 A and B). S6 showed the highest SAR and ESP values, whereas S1 showed the lowest values. The influence of seawater application ratios was seen in S1, where the values of SAR and ESP were lowest, and highest in S6. The application of biochar resulted in a significant reduction in the SAR and ESP values (Figure 4.17 A and B). This could be because applying biochar to the soil solution caused the base cations (Ca^{2+} , Mg^{2+} , and Na^+) to drop. Anwari *et al.* (2020) found that in all salt-affected soils, there was a decrease in soluble and exchangeable Na^+ , which led to a decrease in SAR and ESP as the amount of biochar applied increased. With varying biochar applications, the SAR values fell below the critical value of 13 (Foronda and Colinet, 2023) (Figure 4.17 A). Furthermore, the use of biochar lowered ESP values (Figure 4.17 B) below the 15% threshold (Foronda and Colinet, 2023). In biochar applications, the SAR and ESP values at 1%, 3%, and 5% did not differ considerably from one another. Thus, in soils affected by salinity, the 1% biochar application rate can be utilized to lessen sodicity. This could be as a result of the high total porosity and surface area of 1% biochar application, which allows it to adsorb and hold exchangeable cations like Na (Amesalu *et al.*, 2020). Rekaby *et al.* (2021) also reported that the biochar application was an effective way to decrease soil salinity by improving soil properties by removing Na^+ .

4.4.4 Soluble chloride

The ANOVA demonstrated significant effects ($P < 0.01$) on the soluble Cl^- content for salt-affected soil, biochar application, and the combination of salt-affected soil and biochar application (Table 4.22). The amount of soluble Cl^- varied considerably ($P < 0.01$) among the different rates of biochar application (Figure 4.18). For 0%, 1%, 3%, and 5% applications, the average values of soluble Cl^- were 1535.88, 254.42, 303.72, and 354.01 mg L^{-1} , respectively. While the soluble Cl^- content of the 1%, 3%, and 5% application rates did not differ significantly from one another, the greatest value was recorded in 0%, which was significantly different from other biochar treatments. The soluble Cl^- value of the salt-affected soils (S1 to S6) varied significantly ($P < 0.01$) for each biochar application rate (Figure 4.18). S6 had the greatest values, whereas S1 had the lowest values. S6 had the highest soluble Cl^- values among the salt-affected soils in all biochar treatments, while S1 had the lowest values because the higher salinity soils had more soluble Cl^- than S1 (non-saline, control). In all salt-affected soils, the biochar treatments reduced the soluble Cl^- level in comparison to the control. Additionally, prior studies have demonstrated that adding biochar significantly decreased the soluble Cl^- level in salt-stressed soil (Huang *et al.*, 2022) and coastal saline-alkali soil (Zhang *et al.*, 2022). In salt-affected soils in this investigation, the soluble Cl^- was less than 673 mg L^{-1} at biochar treatment rates of 1%, 3%, and 5%, according to Bryson and Mills (2014). For the majority of plants, this level was safe and did not harm them. This may be as a result of the functional groups in biochar, such as C-H, C-O, and C=C stretching, retaining a partial positive charge that enabled them to adsorb various salt ions from the soil, such as Cl^- , lowering the salinity of the soil (Mao *et al.*, 2022). Therefore, the application of 1% biochar decreased soluble Cl^- in salt-affected soils in this investigation. Additionally, it will change some of the soil's physicochemical properties, which can improve crop quality, yield, and plant growth particularly in saline-alkaline soils (Yuan *et al.*, 2019).

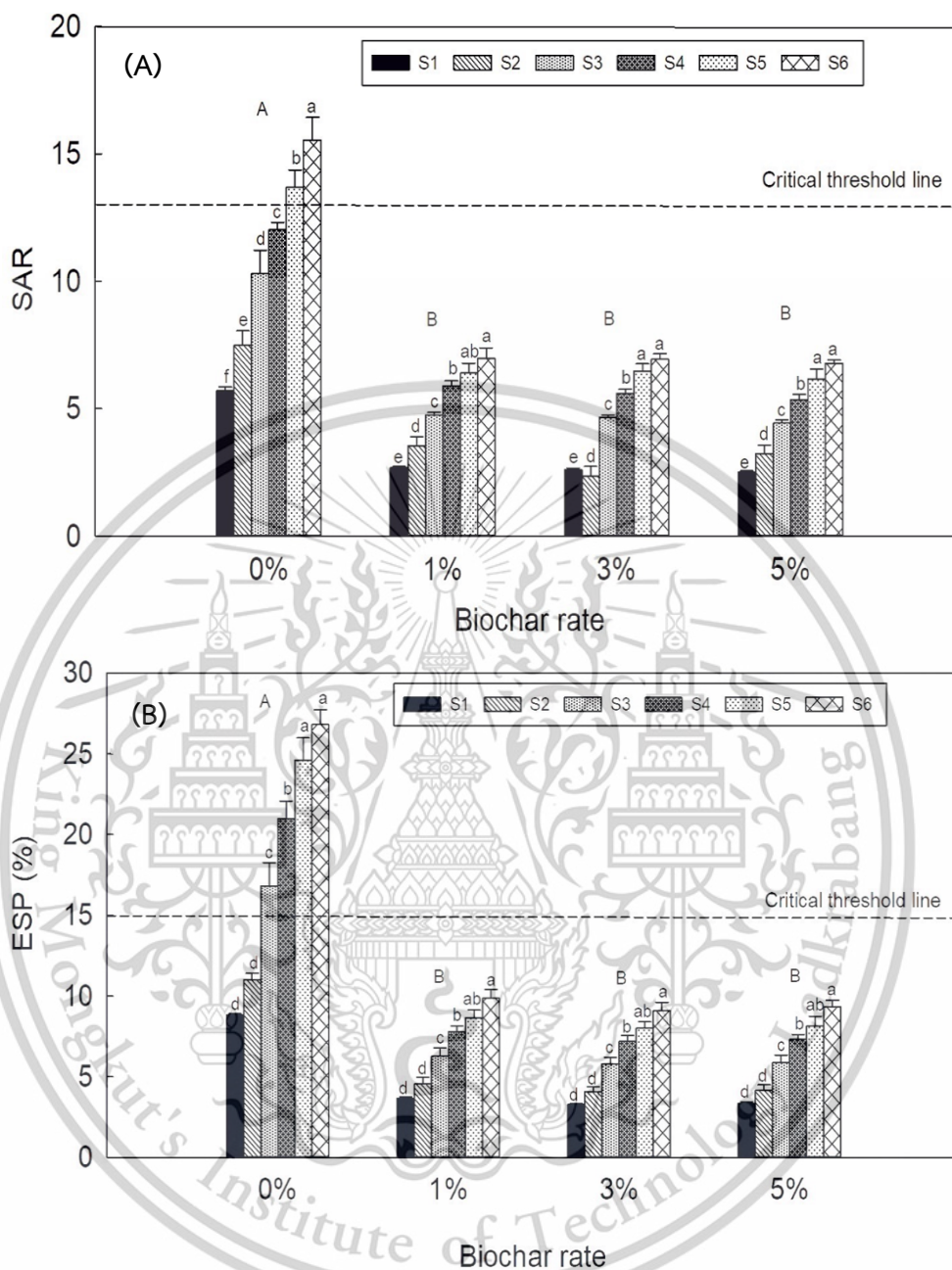


Figure 4.17 The impact of different applications of rice straw biochar on SAR (A) and ESP (B) in salt-affected soils. Significant variations were indicated by different capital letters for each biochar application rate and lowercase letters for each soil type at $P < 0.05$. Error bar = standard error.

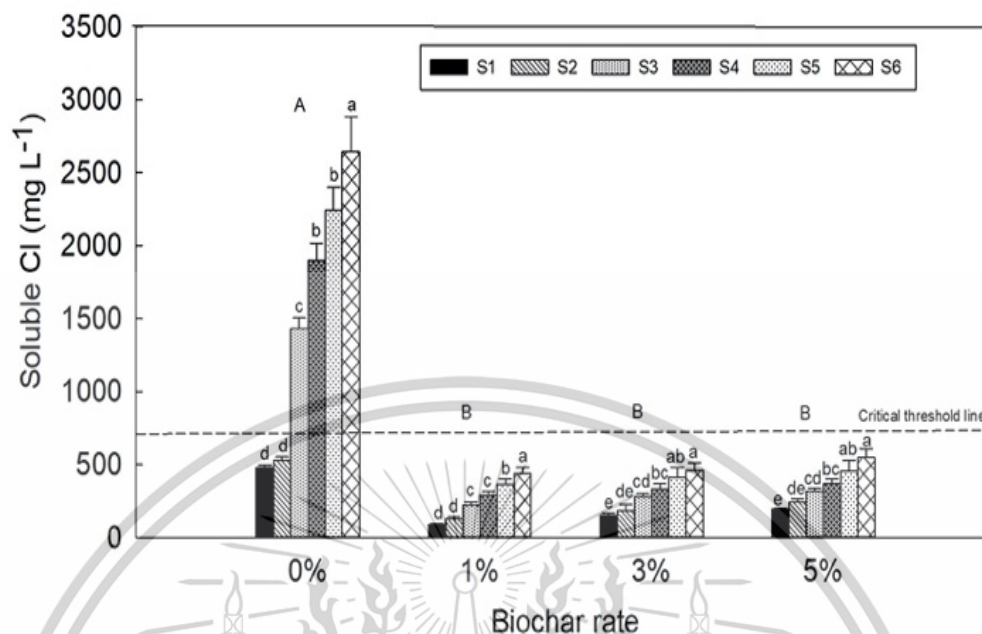


Figure 4.18 The impact of different applications of rice straw biochar on soluble Cl^- in salt-affected soils. Significant variations were indicated by different capital letters for each biochar application rate and lowercase letters for each soil type at $P < 0.05$. Error bar = standard error.

4.4.5 Soluble and exchangeable sodium

Table 4.22 showed that the ANOVA revealed significant effects ($P < 0.01$) on soluble and exchangeable Na^+ for biochar application, salt-affected soil, and the combination of biochar application and salt-affected soil. Figure 4.19 A and B showed that there were significant differences ($P < 0.01$) in the soluble and exchangeable Na^+ values amongst the various biochar application rates. In 0%, 1%, 3%, and 5% applications, the average values for soluble Na^+ were 854, 167, 182, and 186 mg L^{-1} , and for exchangeable Na^+ , they were 1029, 385, 353, and 361 mg kg^{-1} . The control (0%), which had the greatest values of soluble and exchangeable Na^+ , was found to be significantly greater than the other biochar application rates; however, there was no significant difference between the 1%, 3%, and 5% biochar application rates. With 1%, 3%, and 5% doses of biochar applications, the soluble Na^+ was reduced by 80.11%, 78.26%, and 77.62%, while the exchangeable Na^+ was lowered by 61.81%, 65.09%, and 64.34% in comparison to the

control. For each biochar application rate, there was a significant difference ($P < 0.01$) in the values of exchangeable and soluble Na^+ among the salt-affected soils (S1 to S6) (Figure 4.19 A and B). The highest amounts of soluble and exchangeable Na^+ were discovered in S6, whereas the lowest values were observed in S1. In comparison to the control, the 1% biochar application rate greatly reduced the amount of soluble and exchangeable Na^+ ions (Figure 4.19 A and B). This may indicate that the biochar has the ability to bind Na^+ in the soil solution, gradually lowering the amount of soluble and total Na^+ (Prasertsuk and Wijitkosum, 2021). One potential mechanism could be that the salt-affected soils' K^+ was swapped for Na^+ by the K^+ -rich biochar used in this investigation. The surface area, pore volume, and functional groups of biochars can all affect their ability to sorb Na^+ (Sudratt and Faiyue, 2023). The biochar's FT-IR analysis revealed that its primary functional groups were carboxy and hydroxy groups (O-H bending and stretching). These groups carried negative charges and were highly effective at adsorbing cations from the soil, such as Na^+ (Amesalu *et al.*, 2020). Because of the high porosity and surface area of the study biochar, the 1% biochar application decreased exchangeable Na^+ . This may help enhance soil structure and water and nutrient retention in salt-affected soil. Therefore, adding 1% of biochar to salt-affected soils is beneficial because it enhances the soil's physicochemical characteristics by lowering salinity or sodicity through the adsorption of Na^+ (Zaib *et al.*, 2022).

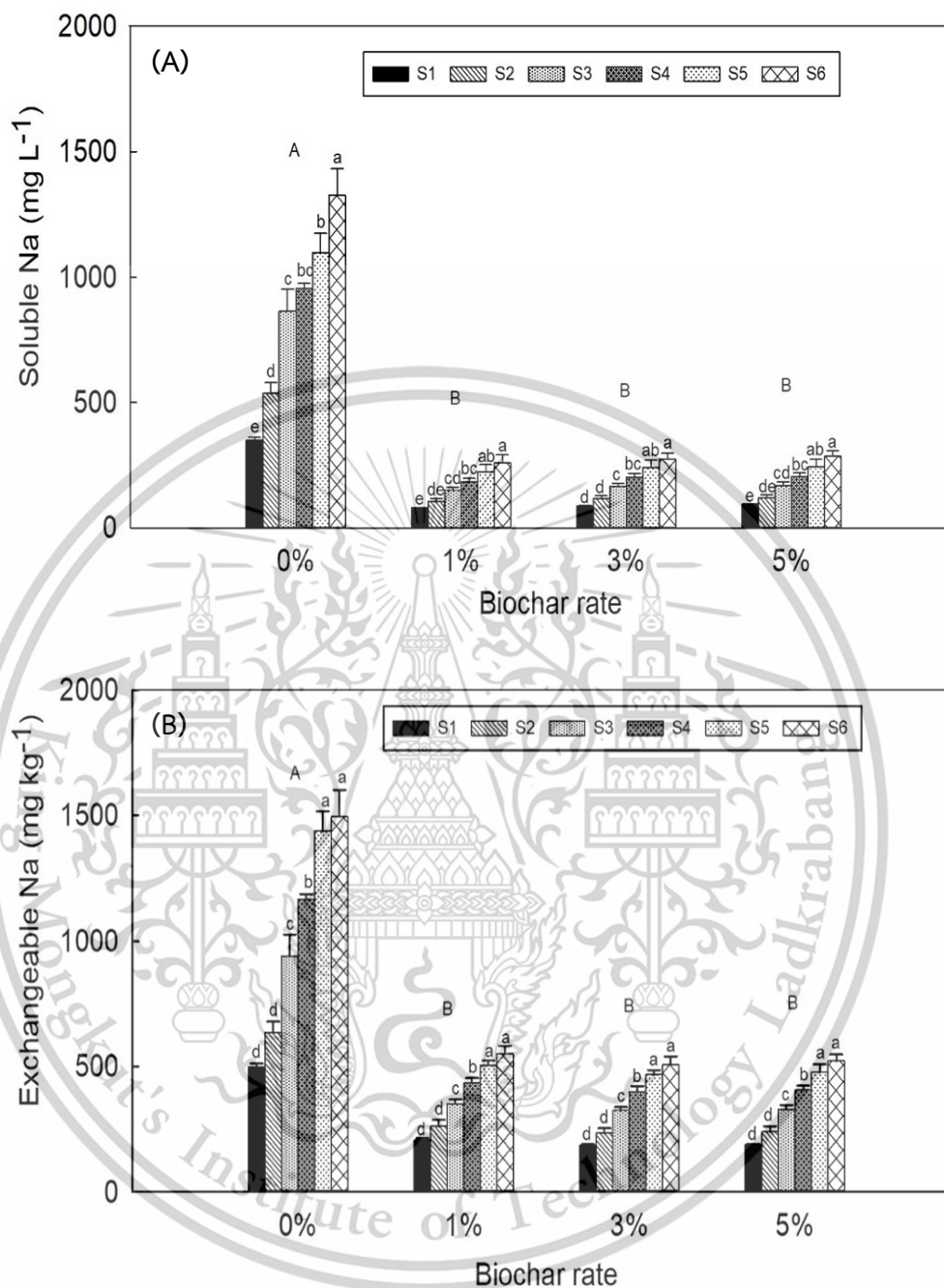


Figure 4.19 The impact of different applications of rice straw biochar on soluble Na⁺ (A) and exchangeable Na⁺ (B) in salt-affected soils. Significant variations were indicated by different capital letters for each biochar application rate and lowercase letters for each soil type at $P < 0.05$. Error bar = standard error.

4.4.6 Soluble and exchangeable potassium, calcium, and magnesium

The ANOVA indicated that the application of biochar, salt-affected soil, and the combination of biochar and salt-affected soil had significant effects ($P < 0.01$) on soluble and exchangeable K^+ , with the exception of the interaction effects on soluble K^+ (Table 4.22). The soluble and exchangeable K^+ values varied significantly ($P < 0.01$) depending on the rate at which biochar was applied (Figure 4.20 A and B). The concentrations of exchangeable and soluble K^+ that were highest in 5% were found to be 3%, 1%, and 0%. Comparing the 1%, 3%, and 5% doses of biochar treatments to the control, the soluble and exchangeable K^+ increased by 126.86% and 239.00%, 677.70% and 603.34%, and 1333.98% and 974.66%, respectively. For every biochar application rate, there were significant differences ($P < 0.01$) in the soluble and exchangeable K^+ values across the soil-affected soils (S1 to S6) (Figure 4.20 A and B). Among salt-affected soils, S6 had the highest soluble and exchangeable K^+ values, whereas S1 had the lowest values for all biochar application rates, with the exception of the exchangeable K^+ at the 3% application rate. It proved that higher salinity soils contained more K^+ than non-saline soils.

The ANOVA revealed that the application of biochar, the presence of salt in the soil, and the combination of the two had significant effects ($P < 0.01$) on soluble and exchangeable Ca^{2+} and Mg^{2+} , with the exception of salt-affected soils and the interaction effects on exchangeable Ca^{2+} and Mg^{2+} (Table 4.22). Figures 4.20 C - F demonstrated that the soluble and exchangeable Ca^{2+} and Mg^{2+} values among the various rates of biochar application differed significantly ($P < 0.01$). The soluble Ca^{2+} and Mg^{2+} among S1 to S6 in the control and the 1% biochar application rate varied significantly ($P < 0.01$), whereas the values among the different salt-affected soils at 3% and 5% application rates were not significantly different (Figure 4.20 C and E). The soluble and exchangeable Ca^{2+} were lowered by 81.09% and 19.17%, 76.71% and 9.25%, and 73.66% and 2.92% when 1%, 3%, and 5% of biochar were applied. The percentages of exchangeable and soluble Mg^{2+} that were decreased by the biochar application rates of 1%, 3%, and 5% were, respectively, 79.98% and 19.47%, 77.41% and 26.06%, and 73.85% and 24.90%. The highest quantities of soluble Mg^{2+} and Ca^{2+} among salt-affected soils in each biochar treatment were detected in S6, with the exception of soluble Ca^{2+} in the control. The exchangeable Ca^{2+}

and Mg^{2+} for each biochar application rate did not significantly differ across the different salt-affected soils (Figure 4.20 D and F). The results demonstrated that the amount of soluble and exchangeable Ca^{2+} and Mg^{2+} in the salt-affected soil was decreased by the addition of biochar.

The concentrations of exchangeable and soluble K^+ that were highest in 5% were found to be 3%, 1%, and 0%. Biochar treatment enhanced accessible K^+ , according to Wang *et al.* (2019), which was similar to increased soluble and exchangeable K^+ in salt-affected soils in this investigation (Figure 4.20 A and B). Given that K^+ was easily soluble in water and that it was competing with other cations from the biochar's exchange sites for ions, this could be a plausible process. Because K^+ is a necessary nutrient for plant growth, the K^+ -rich biochar used in this work may considerably raise the K^+ content of the extracts and saline soils (Wang *et al.*, 2019).

This study demonstrated that adding biochar to salt-affected soils reduced the amount of soluble and exchangeable Ca^{2+} and Mg^{2+} . This could be because basic ions in the biochar, like K^+ , Ca^{2+} , Mg^{2+} , and Na^+ , can modify the composition of the solute through ion exchange and dissolution (Wang *et al.*, 2019). Soluble and exchangeable Ca^{2+} and Mg^{2+} decreased in soils following the application of biochar, in line with the findings of Miranda *et al.* (2017) (Figure 4.20 C - F). This could be because of the high CEC and large surface area of the biochar, which provide it a high adsorption capacity for these ions (Gunaratne *et al.*, 2020). The alternative possibility was that the functional groups on the surface of the biochar, like the O-H bending group (carboxylic acid), the S=O (sulfone) and O-H stretching group (alcohol), carried negative charges and were able to significantly adsorb cations like Ca^{2+} and Mg^{2+} . Depending on the type of biochar, aging period, and adsorption strength, these tightly bound cations may release gradually and the biochar may improve soil fertility (Haowei *et al.*, 2019).

Following the application of biochar, this study found that the amounts of soluble and exchangeable K^+ contents increased while the amounts of Ca^{2+} and Mg^{2+} dropped (Figure 4.20 A-F). K^+ readily forms weak complexes that can be traded. Consequently, K^+ did not significantly compete with Ca^{2+} and Mg^{2+} for the biochar's binding

sites (Rengel *et al.*, 2022). Consequently, following the application of biochar at a rate that exceeded the ideal range for rice crops, the K^+ concentrations rose in salt-affected soils.



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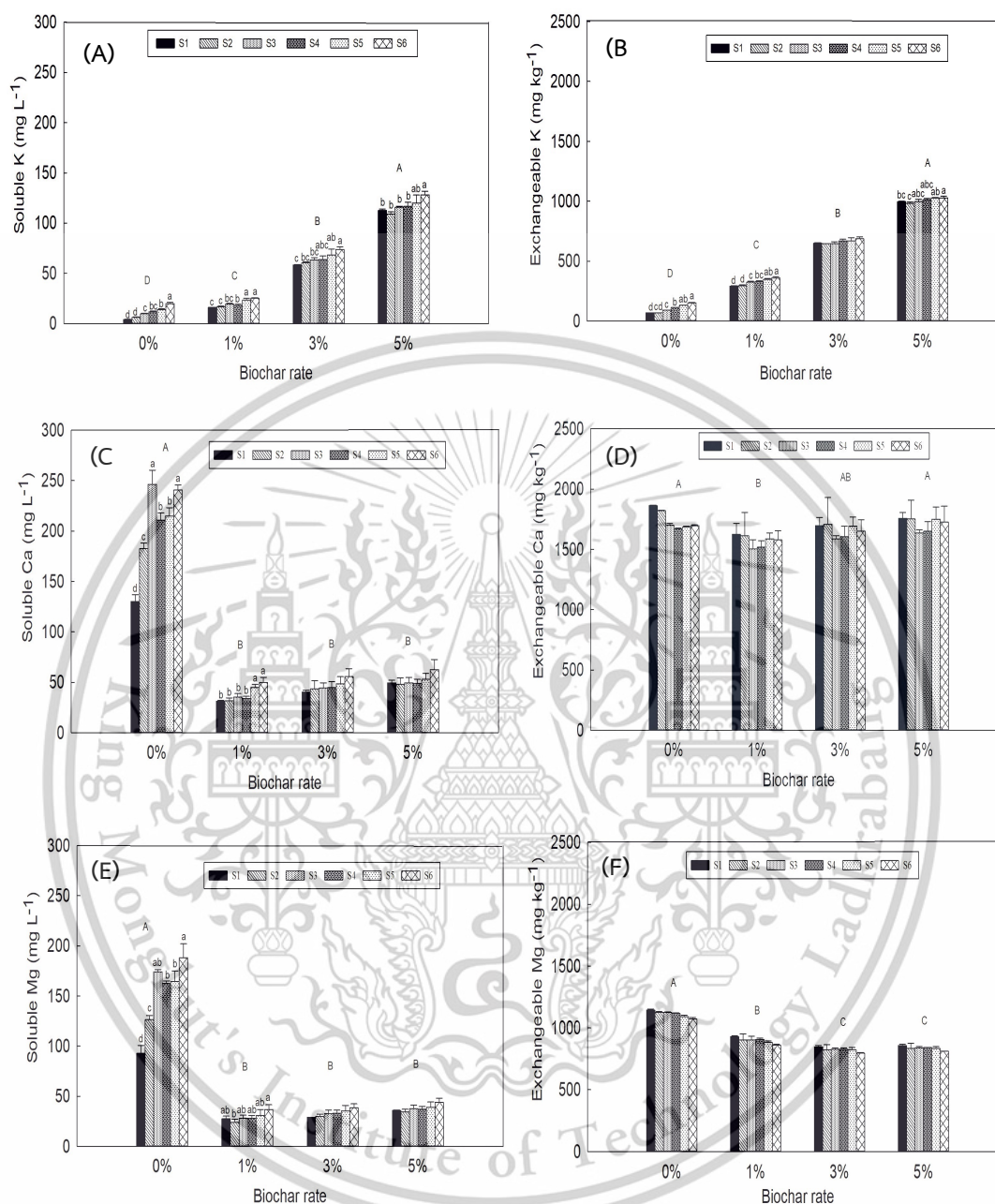


Figure 4.20 The impact of different applications of rice straw biochar on soluble K⁺, Ca²⁺, and Mg²⁺ (A, C, E) exchangeable K⁺, Ca²⁺, and Mg²⁺ (B, D, F) in salt-affected soils. Significant variations were indicated by different capital letters for each biochar application rate and lowercase letters for each soil type at $P < 0.05$. Error bar = standard error.

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

In the first experiment, it was found that the concentration of Na^+ was greater than that of other cations, and the value of Cl^- was higher than that of other anions in a water sample. When comparing February to other collection months during the study period, all metrics were higher in that month. Furthermore, February had the highest seawater mixing ratio, followed by March and April. February's river water's EC, SAR, and Cl^- values made it inappropriate for irrigation, which led to numerous issues with the soil and plants. Furthermore, the excessive salinity and sodicity of irrigation water as opposed to rainwater can harm crop productivity and impair the soil qualities of priceless agricultural property. Consequently, it was determined that the Bang Pakong River's water qualities varied each month based on the amount of precipitation and freshwater flow in the area. Additionally, the findings showed that, in order to boost agricultural output in this region, river water might be used for irrigation in fields during particular months.

Based on the results of the second experiment, the sodium adsorption was increased in Chachoengsao soil compared to Rangsit soil. Moreover, the maximum value of Na sorption was observed in 20% seawater mixing ratio among different seawater treatments. Based on the findings, it was concluded that the Chachoengsao soil demonstrated a higher Na adsorption. Additionally, there was an increase in the Na adsorption in soil in parallel with increasing seawater mixing ratios. The high amount of Na in soil as a result of seawater can cause nutrient imbalance and ion toxicity in plant, which will inhibit plant growth, development and yield.

The study of the third experiment demonstrated that the PT1 (Salt-sensitive) had higher plant height, number of tillers hill⁻¹, shoot dry weight compared to RD43 (Moderately salt-tolerant) under different seawater mixing ratios. This might be due to PT1 resulting in greater Chl a and Chl b contents than RD43 and salinity stress with a lower content of H_2O_2 . The RD43 maintained high RWC and produced more total phenol, and

proline contents (in the second season) than that of PT1. Furthermore, total phenol and H_2O_2 were negatively correlated in two rice varieties for both seasons. Among seawater mixing ratios, the higher salinity 6%, 8%, and 10% declined plant height, number of tillers hill⁻¹, shoot dry weight by decreasing RWC, Chl a, and Chl b contents. Under this condition, a large amount of H_2O_2 , total phenol content, and proline contents was observed, therefore, the high production of phenol and proline could sustain the plant growth due to the improvement of photosynthesis by adjusting water content and Chl in the plant. Moreover, the higher grain yield was observed in PT1 in the first season because PT1 had greater uptakes of K, Mg and Ca, and lower uptake of S compared with RD43 under different salinity stress. Although the plant could survive under salt stress, lower grain yield with increased uptake of Na, Cl, and S and decreased K and Mg uptake was observed in $\geq 6\%$ seawater mixing ratios whereas the larger grain yield was observed in 0-4% seawater mixing ratio. The higher seawater mixing ratio significantly increased ECe, soluble Cl^- , exchangeable Na^+ , SAR and ESP in soil for both seasons. Based on the results, the grain yield of PT1 could not reduce at 2% seawater mixing ratio compared to control in both seasons. However, the grain yield of RD43 decreased in all seawater mixing ratios compared with control.

In the fourth experiment, the various rates of applying biochar 1%, 3%, and 5% had an impact on the chemical properties of the salt-affected soils. When applying biochar at a higher rate than the control on salt-affected soils, the soil's pH and ECe increased dramatically. When comparing salt-affected soils at the 1% biochar application rate to the control, significant drops in SAR and ESP below the threshold values were noted. Toxic components including soluble Cl^- and soluble and exchangeable Na^+ showed a significant decrease at a biochar application rate of more than 1%. After applying biochar at a rate higher than 1%, there were notable increases in soluble and exchangeable K^+ and decreases in soluble and exchangeable Ca^{2+} and Mg^{2+} when compared to the control. This investigation showed that the biochar application dose of 1% was appropriate for decreasing soil acidity to the safe level for rice crops and sodicity by lowering the concentrations of soluble Cl^- , soluble, and exchangeable Na^+ that decreased SAR and ESP in salt-affected soils. Furthermore, it increased the available K^+ , which was necessary for

rice plants to develop and improved in damage soils by salinity. According to the study's findings, biochar can be added to soil to reduce factors including acidity, toxicity, and sodicity that are detrimental to rice yield and growth in salt-affected soil.

Based on the findings of four experiments, it was concluded that the higher amount of EC, Cl^- , Na^+ , SAR and seawater mixing ratio of water in the Bang Pakong River were observed in dry season. The higher seawater mixing ratio with containing high amount of salinity and sodicity increased sodium sorption in soil. Moreover, the increasing seawater mixing ratio negatively affected on plant growth, biochemical properties, nutrient uptakes and yield due to high amount of Na^+ and Cl^- in two rice varieties. When the seawater mixing ratios are $\geq 6\%$, PT1 should be used compared to RD43 in order to avoid reducing yield. Because decreasing yield with increasing seawater mixing ratio was observed in RD43 in both seasons. Besides, the higher seawater mixing increased the amount of ECE, soluble Cl^- , SAR and ESP in soil more than critical values and caused moderately saline soil, which were harmful to the crops. According to laboratory experiment, it was observed that the different rates of biochar applications to these salt-affected soils reduced the salinity and sodicity, and increased potassium in the soil that was important for rice growth and yield.

5.2 Suggestions

This study suggested that the field experiments should be conducted to investigate the response of two rice varieties to salinity from seawater mixing ratio particularly in seawater intrusion areas. Furthermore, the pot and field experiments should be carried out to know the effects of biochar amendments in soil, that reduced salinity and sodicity in soil and improved rice yield.

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