ผลของความเค็มต่อค่าชลศักย์และความเข้มข้นของเกลือในไซเล็มของไม้ป่าชายเลน

นางสาวชนิตา ปาลิยะวุฒิ

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EFFECTS OF SALINITY ON WATER POTENTIAL AND SALT CONCENTRATIONS IN XYLEM OF MANGROVE TREES

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for the Degree of Master of Science in Botany

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ผลของความเค็มต่อค่าชลศักย์และความเข้มข้นของเกลือในไซเล็มของไม้ป่าชายเลน 4 ชนิด คือ แสมขาว พังกาหัวสุมดอกแดง หงอนไก่ทะเลและตะบูนขาว ที่ได้รับความเค็ม 0 10 20 30 40 และ 60 ppt ณ เรือนทดลอง ภาควิชาพฤกษศาสตร์ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย พบว่า ไม้ป่าชายเลนสามารถเจริญได้ในความเค็ม 0 10 20 30 และ 40 ppt โดยที่ แสมขาว พังกาหัวสุมดอกแดง และตะบูนขาว มีการเจริญเติบโตดีที่สุดเมื่อเจริญในความเค็ม 10 ppt มีมวลชีวภาพในเดือนที่ 11 เท่ากับ 17.7 94.8 และ 63.6 กรัม/ต้น ตามลำดับ หงอนไก่ทะเลมีการเจริญเติบโตดีที่สุดเมื่อเจริญในความเค็ม 0 ppt มีมวลชีวภาพในเดือนที่ 11 เท่ากับ 44.4 กรัม/ต้น แต่ถ้าความเค็มเพิ่มขึ้นเป็น 60 ppt ไม้ป่าชายเลนตายหมด และพบว่าความเข้มข้นของโซเดียมและคลอไรด์ในไซเล็มแซปเพิ่มขึ้นเมื่อความเค็มเพิ่มขึ้น โดยที่ แสมขาวที่เจริญในความเค็ม 40 ppt มีความเข้มข้นของโซเดียมและคลอไรด์ เท่ากับ 114.23 mmol/l และ 113.64 mmol/l ตามลำดับ ส่วนในพังกาหัวสุมดอกแดงที่เจริญในความเค็ม 40 ppt มีความเข้มข้นของโซเดียมและคลอไรด์ เท่ากับ 6.96 mmol/l และ 4.10 mmol/l ตามลำดับ ค่าชลศักย์ของไม้ป่าชายเลน 4 ชนิดลดลงเมื่อความเค็มเพิ่มขึ้น เนื่องมาจากการลดลงของค่าศักย์ออสโมซิสที่ประเมินมาจาก pressure-volume curves ซึ่งมีสาเหตุมาจากการสะสมของตัวถูกละลายในเซลล์มากขึ้นโดยส่วนใหญ่จะเป็นโซเดียมและคลอไรด์ ยกเว้นในหงอนไก่ทะเลพบว่าความเข้มข้นของโซเดียมและคลอไรด์มีปริมาณน้อย

จากการศึกษาครั้งนี้แสดงให้เห็นว่าพังกาหัวสุมดอกแดงซึ่งเป็นไม้ป่าชายเลนที่ไม่มีต่อมเกลือมีประสิทธิภาพในการกีดกันเกลือเข้าสู่ภายในต้นมากกว่าแสมขาวซึ่งเป็นไม้ป่าชายเลนที่มีต่อมเกลือ

ภาควิชา .....
สาขาวิชา .....
ปีการศึกษา.....

ลายมือชื่อนิสิต .....
ลายมือชื่ออาจารย์ที่ปรึกษา .....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

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KEYWORD : MANGROVE / SALINITY / WATER POTENTIAL / ION CONCENTRATIONS / GROWTH

CHANITA PALIYAVUTH : EFFECTS OF SALINITY ON WATER POTENTIAL AND SALT CONCENTRATIONS IN XYLEM OF MANGROVE TREES. THESIS ADVISOR : ASSIST. PROF. PIPAT PATANAPONPAIBOON, Ph.D., THESIS CO-ADVISOR : BARRY CLOUGH, Ph.D. 115 pp. ISBN 974-03-0276-9.

The effects of salinity on water potential and salt concentrations were investigated in four species of mangroves. Seedlings of *Avicennia alba, Bruguiera gymnorrhiza, Heritiera littoralis* and *Xylocarpus granatum* were grown at salinities of 0, 10, 20, 30, 40 and 60 ppt in a greenhouse at the Department of Botany, Faculty of Science, Chulalongkorn University. All four species could grow at salinities of 0, 10, 20, 30 and 40 ppt. Plant biomass was closely linked to salinity. A salinity of 10 ppt was found to be optimal for growth. After 11 months growth at this salinity biomass accumulation in *Avicennia alba*, *Bruguiera gymnorrhiza* and *Xylocarpus granatum* were 17.7, 94.8 and 63.6 g/plant, respectively. *Heritiera littoralis* grew best at 0 ppt salinity, in which biomass accumulation after 11 months of growth was 44.4 g/plant. Seedlings of all species died at a salinity of 60 ppt. Na and Cl concentrations in *Avicennia alba* and *Bruguiera gymnorrhiza* xylem sap increased with increasing salinity. Na and Cl concentrations in *Avicennia alba* xylem sap at 40 ppt salinity were 114.23 and 113.64 mmol/l, respectively. Na and Cl concentrations in *Bruguiera gymnorrhiza* xylem sap at 40 ppt salinity were 6.96 and 4.10 mmol/l, respectively. Analysis of pressure-volume curves showed that shoot water potential in all four species decreased with increasing salinity due to a reduction in osmotic potential. The decrease in osmotic potential was attributed to increasing solute concentrations, mainly Na and Cl, in the leaves of all species except *Heritiera littoralis*, which had remarkably little Na and Cl in the leaves.

The results indicated that *Bruguiera gymnorrhiza*, which does not have salt-secreting glands, was more efficient in excluding salt than *Avicennia alba*, which has salt-secreting glands.

Department ……. Student's signature ……..

Field of study …… Advisor's signature ……

Academic year ……..... Co-advisor's signature ……..

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CHAPTER 1

INTRODUCTION

Origination and Rationale

Mangroves are mainly tree species that grow in tidal saline wetlands along tropical and subtropical coasts. Mangroves ecosystems, are influenced by a range of environmental factors, including coastal physiography, climate, tides, waves and currents, water salinity, dissolved oxygen and soil nutrients (Aksornkoae, 1989). Of these environmental factors, salinity is considered to be one of the most important (Ball, 1996). Mangroves can be considered as a special group of halophyte, which despite the high water-retaining capacity of the saline substrate in which they live, can maintain a good water balance, due to diverse regulating mechanisms such as stomatal behavior, osmotic adjustment, succulence and salt excretion (Snedaker and Snedaker, 1984).

A Mangrove forest is an important natural resource. Mangroves are a group of plants composed of species belonging to several unrelated families. They are biologically and economically highly productive ecosystems and play an important economic role in traditional local communities. They are an important source of wood, protectors of shorelines and source of reduced carbon for estuarine food-webs which support the nurseries of many commercially important species of fish and crustaceans.

At present, mangroves keep decreasing. In the last survey in 1996, only 1,047,390 rai of mangroves were left in Thailand. There are in the southern (89.2%), eastern (7.55%), central or gulf of Thailand (3.3%) (Jaruppat and Jaruppat, 1997). Mangroves have been converted or destroyed for other purposes including agriculture which is mainly shrimp farms. Therefore, mangroves must be attended to, restored and grown seriously.

There are some problems about the rehabilitation and reforestation of mangroves. Rehabilitation and reforestation often do not succeed because there is little knowledge of how mangroves respond to geographic and environmental factors. Therefore, understanding of the natural ecology of mangrove species is necessary to rehabilitate them successfully.

Under natural conditions, mangroves must tolerate large ranges of salinity and water potential, but the physiological mechanisms are poorly understood and require integration of several processes. In Thailand, there have been few studies of the effect of the environmental factors on the physiology of mangrove species.

This research deals with a study on the effects of salinity on water potential in shoot, and the concentrations of sodium, potassium and chlorine in xylem sap of mangroves with and without salt secreting glands.

Objectives

1. To study the effects of salinity on water potential of mangroves.
2. To study the effects of salinity on salt concentrations in xylem sap of mangroves.

Within these objectives, the specific objective was to test 3 hypothesis relating to osmoregulation in mangroves.

Hypothesis

1. Four mangrove species exclude salt with the different efficiency.
2. That the salt concentration in xylem is proportional to the salinity of water around the roots.
3. Large changes in shoot water potential are accompanied by only a small change in tissue water content.

Experimental design

1. Study the effects of salinity on salt concentrations in xylem sap.
2. Study the effects of salinity on water potential of shoots.
3. Study the effects of salinity on growth.

Contribution

1. To provide be basic information on water relations and osmoregulation in mangroves.
2. To improve understanding of the mechanisms of salinity tolerance to salinity.
3. To enhance understanding of plant-environment interactions that may be important in rehabilitating mangroves successfully.

CHAPTER 2

LITERATURE REVIEW

High soil salinity can affect mangroves and other plants in two ways. First, excessive uptake of NaCl can have direct effect on plant metabolism, very high levels of NaCl in the cytoplasm generally being toxic (Flowers et al., 1977). Secondly, a high soil salinity makes it more difficult for the plant to take up water (Clough et al, 1982). Consequently, mangroves display many anatomical and morphological characteristics of xerophytes, such as anatomy of leaves. These features, which include thick cuticles, wax coatings, sunken or otherwise hindered stomata and the presence of various types of water storage tissue (Clough et al, 1982).

2.1 Salinity effects on plant growth

Salinity has long been recognized as an important factor regulating physiological processes, plant growth, height, survival and zonation of mangroves. High soil salinities are due to the accumulation in the soil determined by a mixture of many different ions (seawater = 3.3-3.7% salt by weight), but mainly sodium chloride. Mixtures of with differing ionic composition ions can have different effects on plant growth and metabolism (Orcutt and Nilsen, 2000).

An increase in soil salinity commonly causes a reduction in plant growth and yield. The relationship between salinity and growth reduction is not uniform among the same for all ions; some are more toxic than others. It is also true that a combination of ions has a less detrimental effect on growth than does an equimolar concentration of one ionic species (Bernstein, 1975). One immediate response of plants to elevated salinity is a decrease in the rate of leaf expansion. Consequently, whole-plant leaf area is reduced and growth is decreased. Shoot growth decreases proportionally more than root growth, causing an increasing in the root/shoot ratio, the greater the effect of salinity on productivity (Cheeseman, 1988).

Banuls and Millo (1992) studied one-year-old *Citrus sinensis* seedling grown at increasing levels of salinity, and found that the chloride salt reduced plant dry weight and decrease defoliation. Accumulation of chloride in leaf tissue caused a sharp decrease in photosynthesis and stomatal conductance.

Alarcon et al. (1993) studied *Lycopersicon esculentum* and *Lycopersicon pennillii* growing on silica sand in a growth chamber exposed to 0, 70, 140 and 210 mM NaCl nutrient solutions for 35 days, and found that high salinity reduced leaf area and leaf number together with shoot dry weights. Sibole et al. (1998) studied *Phaseolus vulgaris* L. seedlings growing in 25, 50 and 75 mM NaCl, and found that all treatment inhibited stem growth more than leaf growth. Growth was much reduced in 75 mM NaCl with the progress of time. Epron et al. (1999) studied oak seedlings grown in 50 and 250 mM NaCl, and found that root and shoot biomass were reduced and was affected root elongation. Meneuzzo et al. (2000) studied two wheat cultivars (*Triticum durum* Desf.) grown in 0, 50 and 100 mM NaCl, and

found that the highest salt concentration reduced shoot growth and shoot and root biomass production. In both cultivars water potential and osmotic potential decreased with increasing salinity. Turgor maintenance and osmotic adjustment that occurred, was associated with an accumulation of Na, and Cl and, to a lesser extent of K, in cell sap.

Halophytes, including mangroves, tolerate high soil salinity. The mechanisms of salt tolerance are complex and variable, and involve factors such as ionic potential across membranes, osmotic relationships, enzyme activation and protein synthesis (Orcutt and Nilsen, 2000). Sathe et al. (1985) found that Na and K of *Avicennia officinalis* leaves could be correlated with levels of chlorophylls of the leaves. It seemed that endogenous levels of Na play important role in chlorophyll synthesis. With increase in Na and decrease in K, total chlorophylls decrease were also found in *Aegiceras corniculatum* and *Sesuvium portulacastrum* (Shinde and Bhosale, 1985). Khan et al. (1999) studied *Halopyrum mucronatum* (L.), which is perennial grass found on the coastal dunes of Karachi, Pakistan. Plant were grown in 0, 90, 180 and 360 mol m^{-3} NaCl in sand culture found that fresh weight of shoots and roots peaked at 90 mol m^{-3} NaCl. A further increase in salinity inhibited plant growth, ultimately resulting in plant death at 360 mol m^{-3} NaCl.

Most mangrove species require some salt for growth. Downton (1982) studied *Avicennia marina* grown at different salinities for 11 months, and found that the well growth was at 10, 25 and 50% seawater. Some species such as *Sonneratia lanceolata*, growth optimum in fresh water conditions (Ball and

Pidsley, 1995) and some species, such as *Rhizophora mangle*, are unable to grow to maturity in fresh water (Werner and Stelzer, 1990). Ball and Pidsley (1995) studied *Sonneratia alba* and *Sonneratia lanceolata* grown in salinities ranging 0-50% seawater reported that *Sonneratia alba* grew well in 5-50% seawater. *Sonneratia lanceolata* grew well in 0-5% seawater. Ball (1988) found that the diminution in growth of mangrove species could be attributed entirely to a decrease in leaf area ratio until very high salinities were reached. Yasumoto et al. (1999) studied callus of *Sonneratia alba* J. Smith that were cultured on MS medium supplemented with 0 to 500 mM NaCl found that maximun growth was observed with 50 mM NaCl. At 500 mM NaCl, growth of callus was completely inhibited. Cellular Na and Cl were greatly increased by the treatment with NaCl.

2.2 Salt uptake, salt balance and osmoregulation

In mangrove, like most other plants, there are 4 primary mechanisms for managing high salt salinity:

1. Exclude NaCl selectively at the root.
2. Store excess NaCl in the cell vacuole and other organs and utilize it to generate low osmotic potentials to facilitate water uptake.
3. An increase in succulence.
4. Salt gland for elimination of salt from the leaves.

2.2.1 Salt uptake and ion accumulation

The control of salt concentration in mangrove tissue does not seem to differ significantly from that of other halophytes. Salt in high concentrations in plant tissue is seemingly toxic (although the physiological reason is not clear) and must be largely excluded (Tomlinson, 1986). The roots of all mangroves, like those of other plant, take up specific cations and anions selectively, and discriminate selectively against others, such as sodium, which could be potentially toxic if taken up in excess. Scholander (1962) demonstrated experimentally that salt separation process must occur at or near the root surface.

Moon et al. (1986) examined the pathway and mechanisms for salt uptake by mangrove (*Avicennia marina*) and concluded that salt uptake is a passive process that occurred via apoplastic transport of both salt and water through a wall of cells in the apical region of the root, where there was no secondary thickening of cell walls. They found that the main barrier to salt uptake was located in the hypodermis (at the outer surface of the root) rather than at an endodermis as in most in non-mangrove plant species. If salt uptake by mangroves is indeed a passive process (i.e. there is no transport across cell membranes in the roots) then it is likely that the salt concentration in the xylem will be proportional to the salinity of the water around the roots, and independent of the transpiration rate.

Measurement of NaCl concentration and osmotic potential of xylem sap of mangroves indicated that NaCl is largely excluded from the xylem. Scholander et al. (1962) have confirmed the almost fresh-water quality of mangrove sap by showing that in *Aegiceras*, *Aegialites* and *Avicennia marina* there is only 0.2-0.5% NaCl present, which in *Rhizophora mucronata* it is only 0.02-0.05%. This capacity

to exclude NaCl from xylem is an important factor in the maintenance of internal balance (Clough et al., 1982).

Scholander et al. (1962) suggested that mangroves could be divided into two basic groups according to the mechanism for coping with salt, those that excluded salt (salt excluders) and those that secreted salt (salt secretors). In Scholander's thinking, mangroves with salt-secreting glands in their leaves (e.g. *Avicennia*, *Aegialites* and *Aegiceras*) were considered to be salt secretors, while those without salt-secreting glands (e.g. *Rhizophora*, *Sonneratia*, *Lumnitzera* and *Bruguiera*) were considered as salt excluders. This view is still widely held today.

Accumulation is in part compensated by salt secretion via salt glands in the less efficient salt excluders (Tomlinson, 1986). Of the mineral cations essential to the nutrition of plants, potassium is required in the largest amounts. The salt exclusion mechanisms of mangroves therefore must be selective, it must have a sufficient discriminatory capacity to absorb ion in appropriate concentrations, and there must be a preferential selective of potassium in competition with high concentrations of sodium (Tomlinson, 1986). Storey and Jones (1979) studied *Atriplex spongiosa* and *Suaeda monoica* growing at different salinities, and found that both species had high affinities for Na and maintained constant but low shoot K contents with increasing salinity. These trends were more marked with *Suaeda monoica* in which Na stimulated the accumulation of K in roots. Dongre and Bhosale (1985) studies on *Clerodendrum inerme* growing at different salinities found that Na and Cl uptake in leaf increased with increasing salinity. Khan et al. (2000) studied *Suaeda fruticisa* plant grown in saline conditions (200-400 mol m^{-3})

found that water potential and osmotic potential of plant became more negative with an increase in salinity. Leaf Ca, Mg and K concentrations decreased with increasing salinity, while both Na and Cl increased and reached 1,391 and 1,673 mmol kg^{-1} dry weight, respectively. Downton (1982) studied *Avicennia marina* grown at different salinities for 11 months. Seedlings grown on nutrient alone accumulated mainly K (Na and Cl being unavailable) and probably depended upon organic substances, in particular organic anions, to generate internal osmotic potential. Plants on 0-100% seawater treatments behaves as typical halophytes accumulating increasing levels of sodium and chloride as external salinity increased, even though the leave can regulate steady-state ion concentrations by mean of salt glands. Sodium replaced potassium, but preferential potassium uptake was maintained over a wide range of external concentrations. Suarez et al. (1998) studied *Avicennia germinans* L. seedlings growing at different salinities in field. This study showed that ion concentrations were higher in the high-salinity site (22-35%). Ion concentrations explained 73 and 66% of the osmotic potential estimated by P-V curves for leaves from low and high salinity sites, respectively.

Scholander (1968) demonstrated that the root of mangroves system efficiently functions as a partial to almost complete ultrafiltration system and that the water potentials of the leaf cells are always lower than that of seawater.

2.2.2 Osmoregulation in plant tissue

The activity of enzymes in halophytes, as in most higher plant cells, is generally inhibited by high level of salt (Flowers et al., 1977). Whereas inorganic

ions, especially Na and Cl seem to be responsible for osmoregulation in the cell vacuoles of mangroves and other halophytes, osmotic adjustment in the cytoplasm appears to be maintained by accumulations of amino acid proline and quaternary ammonium compounds choline and betaine (Flowers et al., 1977).

The water relations of mangroves can be discussed in terms of water potential (Ψ) of tissues of the plant and of the environment, where in any system the water potential is the algebraic sum of the osmotic potential and turgor potential.

$$\Psi = \Psi_{\pi} + \Psi_{\rho}$$

where Ψ is water potential

Ψ_{π} is osmotic potential

Ψ_{ρ} is turgor potential

The water potential for pure water is established as zero. Water moves from the area of high water potential to the area of lower water potential. Water and minerals must enter root cells before they reach the xylem. Water enters root cells because the water potential within root cells is less than that of the soil solution (Mader, 1996). Most land plants use soil water, which contains little salt and therefore, has an osmotic potential that is close to zero. Seawater contains half-molar concentrations of sodium and chloride ions, and has an osmotic potential closed to –2.5 Mpa. (Mader, 1996). When the soil salinity increases, the plant has

to adjust water potential lower than soil salinity to take up water (Clough, 1985). Downton (1982) studied *Avicennia marina* grown at different salinities for 11 months, and found that the well growth was at 10, 25 and 50% seawater. This study demonstrated that the osmotic potentials of seedling were more negative than those of the external solutions by at least 2 MPa in all of the treatments. Turgor pressures of approximately 0.8 MPa were evident for the salt-treated plants, but were much lower (0.2-0.3 MPa) for plant receiving only nutrient. The three most common changes in plant water relations that occur under hypersaline conditions are osmotic adjustment, turgor reduction and decrease cell wall elasticity. Osmotic adjustment occurs both in the apoplast (salt accumulation) and symplast (sugar, nonprotein amino acid or quaternary amine accumulation). Osmotic adjustment in halophyte often occurs by accumulation of inorganic ions (Orcutt and Nilsen, 2000).

Balonas and Longstreth (1984) studied *Alternanthera philoxerides* (Mart.) Griseb. grown in 0 to 400 mM NaCl, and found that values of osmotic potential decreased, while the bulk elastic modulus increased with salinity. Tissue water potential was lower than rhizosphere water potential. These increases in bulk modulus of elasticity with salinity provided a mechanism by which a large difference between plant water potential and rhizosphere water potential, the driving force for water uptake, could be produced with relatively little water loss by the plant. Nabil and Coudet (1995) studied the response of *Acacia nilotica* subspecies to salt stress, and found that water potential and osmotic potential decreased with salinity, the lower osmotic potential enabling the plant to maintain

turgor. Furthermore, the point of zero turgor occurred at high relative water content under all treatments. An increase in elastic modulus was observed under stress.

Suarez et al. (1998) studied *Avicennia germinans* L. seedilngs growing at different salinities in field. This study provided evidence that leaves of *Avicennia germinans* L. seedlings adapt to hypersaline soils by increasing solute concentration by 52% and cell elasticity by 26%. Both processes allow leaf water uptake and turgor maintenance over a large range of soil water potential. Rada et al. (1989) used pressure-volume curve to estimate leaf water potential relation of three mangrove species (*Rhizophora mangle, Conocarpus erectus* and *Coccoloba uvitera)* in Venezuela. They found that leaf osmotic potential and elasticity decreased during the dry season when salinity levels tended to increase in the interstitial soil water. This osmotic adjustment was due to changes in either symplasmic water fraction, the osmotically active solutes in the cells, or both. Suarez and Sobrado (2000) used pressure-volume analysis and dew point hygrometer to determine leaf water relation parameters of *Avicennia germinans* L. seedlings grown at different salinities. Seedlings responded to an increase in salinity from 0 to 32 ppt by decrease in osmotic potential at full turgor from –2.3 to –3.5 MPa and osmotic potential zero turgor from –2.7 to –4.3 and increase in volumetric modulus of elasticity from 19-27 MPa.

2.2.3 Succulence

Mangrove leaves characteristically contain high level of Na in the leaf, most of which is probably located in cell vacuoles. Leaf succulence in mangroves

is an adaptation which allow salt that cannot be excluded by the root to be accumulated in tissues within the leaf so that it can cause little physiological damage (Clough et al., 1982). Leaf succulence in mangroves is associated with the enlargement of cells in the leaf, principally in the hypodermal and mesophyll tissues (Chapman, 1976). Jenning (1968) suggested that the high content of water might serve to dilute the salt entering the leaf with the transpirational stream, thus minimizing the concentrations of possibly toxic ions.

Suarez and Sobrado (2000) used pressure-volume analysis and dew point hygrometer to determine leaf water relation parameters of *Avicennia germinans* L. seedlings grown at different salinities. Seedlings responded to an increase in salinity from 0 to 32 ppt by an increase in leaf succulence as reflected in an increase in leaf water content per unit area from 300 to 360 g m^{-2}.

With some mangroves there must be also some significance in the increasing degree of succulence (Chapman, 1976) and greater extent of storage tissue in leaves of increasing age and in places of high salinity (Tomlinson, 1986). Shinde and Bhosale (1985) studied *Aegiceras corniculatum* and *Sesuvium portulacastrum* grown at different salinities. They found that the salinities induce succulence. The leaf thickness, water content, area, mass and volume of leaf increased and leaf density decreased along with the increasing concentration of NaCl. In *Laguncularia mangle*, there was a fourfold increase in leaf thickness from the youngest to the oldest leaves along a shoot (Biebl and Kinzel, 1965). Some results argue against the development of increasing succulence as the leaf ages. Atkinson et al. (1967) showed that the dry weight and water content of the leaves

remain nearly constant for all but the most recent unfolded, and perhaps the oldest, leaves on the shoot. Hwang and Chen (1995) studied *Kandelia candel* (L.) seedlings grown at different salinities, and found that they did not show increase succulence in tissues at high salinities.

2.2.4 Salt glands

Only a few genera of mangroves (*Avicennia*, *Aegiceras, Aegialitis* and *Acanthus*) appear to posses salt-secreting glands in their leaves. In *Avicennia* salt glands are formed only under saline conditions (Saenger, 1982) where as in *Aegiceras* they appear to be formed whether or not salt is present in the medium. They are entirely absent from *Acanthus* grown in freshwater (Saenger, 1982). Joshi et al. (1975b) concluded that among salt-extruding species, *Avicennia* is the most efficient and consequently able to grow in a high saline conditions whereas the less efficient *Aegiceras* and *Acanthus* are restricted to saline habitats.

The salt glands of mangroves secrete mainly NaCl and control their salt balance by secreting NaCl. In salt secretors the NaCl concentration of xylem sap is relatively high, but still about one-tenth of the concentration of salt in seawater. Roots only partially exclude salt. The absorbed salt is primarily excreted metabolically via salt glands. The voided salt in solution can crystallize by evaporation, can be blown away, or is otherwise washed off (Tomlinson, 1986).

Of these 4 mechanisms, only the first 2, regulation of salt uptake and osmoregulation, are common to all mangrove species. These are the main topic of the present work.

CHAPTER 3

MATERIALS AND METHODS

3.1 Plants materials and growth conditions

3.1.1 Plant species

The selected species were *Avicennia alba* Bl., *Bruguiera gymnorrhiza* Lamk., *Heritiera littoralis* Dry. and *Xylocarpus granatum* Koen. Of these four species, only *Avicennia alba* has salt glands.

Avicennia alba

This species was collected from Samutsongkarm Province in July 1999 (Figure 3.1). The propagules were selected for uniform weight (1.7 ± 0.06 g/seed).

Bruguiera gymnorrhiza

This species was collected from Rayong Province in August 1999 (Figure 3.2). The propagules were selected for uniform weight (35.9 ± 3.08 g/propagule).

Herlitiera littoralis

This species was collected from Rayong Province in August 1999 (Figure 3.3). The propagules were selected for uniform weight (16.1 ± 4.32 g/seed).

Xylocarpus granatum

This species was collected from Samutsongkarm Province in August 1999 (Figure 3.4). The propagules were selected for uniform weight (55.0 ± 16.06 g/seed).

3.1.2 Cultivation and growth conditions

Individual plants were grown in 350 grams of sand in a plastic pot 6.5 cm. in diameter and 11 cm. tall. Pot size was determined by the maximum size that could fit inside the pressure bomb. There were 3 replications for each species in each treatment. The pots were placed in a container of 45 x 60 x 32 cms. (width x length x height). One container contained 54 pots.

Seedlings were initially germinated at 0 ppt salinity to promote rapidly a uniform establishment. The water level was maintained for a week and drained once a week. After the first pair of leaves had expanded, the salinity was gradually increased stepwise until the designed salinity treatment had been imposed. Salinity prepared from water from salt farm which salinity is 120 ppt and diluted with tap water to get concentration of 0, 10, 20, 30, 40 and 60 ppt. Salinity was checked every day for 11 months by hand refractometer and water levels were maintained by daily addition of tap water. Solutions were changed every 2 weeks and added 4 liters half-strength Hoagland nutrient solution in the container every month (Patanaponpaiboon, 1989) (Figure 3.6).

All plants were grown in a greenhouse at a Department of Botany, Faculty of Science, Chulalongkorn University (Figure 3.5). The following experiments were carried out from July 1999 to June 2000.

Figure 3.1 (A) *Avicennia alba*

(B) Seeds of *Avicennia alba*

Figure 3.2 (A) *Bruguiera gymnorrhiza*

(B) Seedlings of *Bruguiera gymnorrhiza*

A

B

Figure 3.3 (A) *Heritiera littoralis*

(B) Seeds of *Heritiera littoralis*

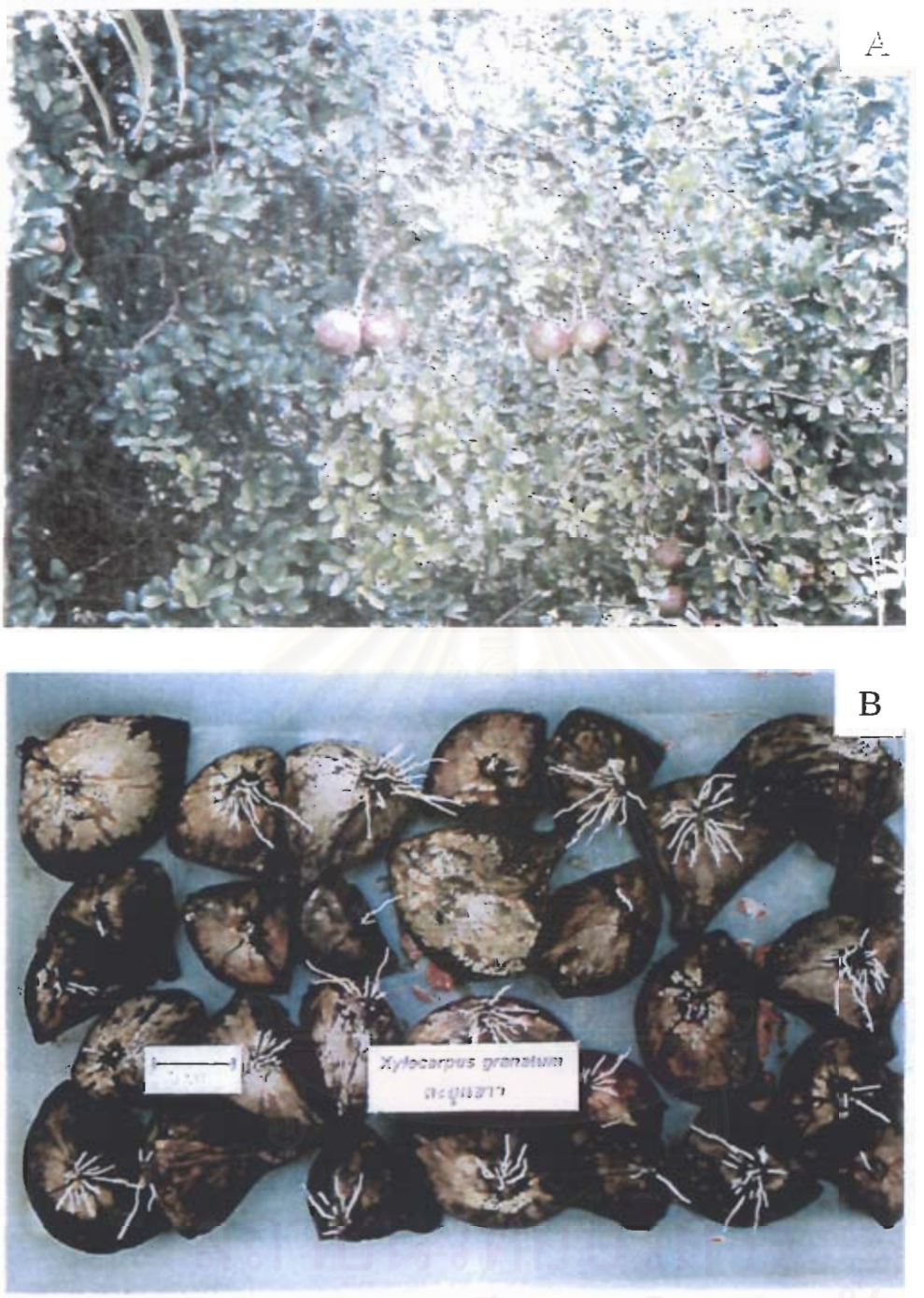

Figure 3.4 (A) *Xylocarpus granatum*

(B) Seeds of *Xylocarpus granatum*

Figure 3.5 Greenhouse for experiment at Department of Botany, Faculty of Science, Chulalongkorn University.

Figure 3.6 Seedlings of *Avicennia alba* (A), *Bruguiera gymnorrhiza* (B), *Heritiera littoralis* (C) and *Xylocarpus granatum* (D) planting in greenhouse.

3.2 Study on effects of salinity on salt concentrations in xylem sap and leaves

3.2.1 Collection of xylem sap and leaves

When the plants were 4-5 months old, a plant from each salinity was measured as follows:

1. The transpiration rate of each plant was measured for one day before collecting xylem sap. For this, the 50 ml of water of the same salinity was added to the pot, and the pot was then sealed and weighed. After 8 hrs, the plant was weighed again to calculate the flow rate through the plant.
2. The pot was transferred to a pressure bomb and the shoot was excised at the base about 15-17 cm. above the sand (root junction). To collect the sap, a plastic tube was inserted over the cut root base and sap was withdrawn from this tube with a syringe (Figure3.7). The pressure of the pressure bomb was adjusted to give a flow rate the as same as that measured in the plant before it was placed in the pressure bomb. A samples of xylem exudate was collected every 0.5 hr and its osmolality measured as soon as possible using a pressure vapor osmometer (Wescor Inc. Logan, Ut., USA). If the flow rate was slow (< 0.4 ml./hr.) samples were taken every hour. Samples were kept at -20 ^{o}C (Moon et al, 1986).
3. The leaf samples were collected from each salinity level and kept in an oven at 70 ^{o}C for 72 hours. Then the samples were digested with HNO_3 and $HCLO_4$ as described by Oweeczkin and Kerven (1980) to analyze Na, K and to analyze Cl as described by Attanun et al. (1994).

3.2.2 Chemical analyses

Na and K were measured by atomic absorption spectrometry (Model 3110). Cl from xylem sap was measured with a Radiometer CMT 10 coulometric chloride titrator. Cl from leaf was measured by Mercuric thiocyanate method (Chloride Reagent Set (HACH)) then measured with spectrophotometer as describe in Hach DR/2000 Spectrophotometer Procedures Manual (1988).

3.3 Study on effects of salinity on shoot water potential and water potential components

3.3.1 Pressure-volume analysis

Pressure-volume analyses were carried out on the shoots when the plants were 4-5 months old. The general procedure was:

To fit our pressure bomb the shoots were always between 13 and 15 cm. long. The shoot was cut from the plant, quickly weighed, enclosed in a plastic bag to reduce evaporation, and sealed in the pressure bomb with the cut end protruding from the top. The pressure in the bomb was then adjusted with Nitrogen gas until the water came out. After reading the pressure, the pressure in the pressure bomb was increased by only 0.1 MPa and left for 20 min. The sap which was exuded by shoot at its cut surface was collected in preweighed grass vials (containing tissue paper) which were weighed again to give the increase in weight and hence water volume quantitative terms (Figure 3.8). After 20 mins, the excess pressure was reduced sufficiently for the water at the cut surface to disappear to just below this surface (usually around 0.05 MPa).

The new and the lower value of water potential was then determined by increasing the pressure very slowly (0.02 MPa min^{-1}). This procedure was repeated until several points on the linear portion of the pressure-volume curve have been obtained. The data were used to plot the inverse of water potential ($1/\Psi$) as a function of relative water content (RCW) as describe by Tyree and Hammel (1982). The water potential components were determined from the pressure-volume (P-V) analysis: osmotic potential at full turgor (Ψ_π^{100}) and at zero turgor (Ψ_π^{0}), wall relative water content (wall RWC), relative water content of shoot (RWC^0) and relative water content of symplast at zero turgor (RWC_{sym}^{0}), molar concentration of solute at full turgor and at zero turgor (SD), average modulus of elasticity (ε) as describe by Schulte and Hinckley (1985). These parameters were calculated with the program which was written by Barry Clough.

3.4 Study on effects of salinity on growth

3.4.1 Allometric equation for estimation of biomass of each species

The fifteen plant samples were selected to determine plant dry weight at different sizes. The diameter of the stem and height of plant from 5 cm above the sand to the base of shoot apex was measured. The plants were separated into roots, stems and leaves, which were weighed, and then oven dried at 80 °C for 72 hours to determine dry weight (Figure 3.9). The plant dry weight was found to fit the simple allometric equation;

$$W = a\,(D^2L)^b \qquad (3.1)$$

where W is plant dry weight, D is stem diameter, L is shoot length from 5 cm above the sand to the base of shoot apex, and a and b are constants.

The regression coefficients a and b eq. (3.1) were determined by least square method. The allometric relationship between W and D^2L of *Avicennia alba* was determined as;

$$W = 1.88(D^2L)^{0.88} \qquad (3.2)$$

($r^2 = 0.99$)

Bruguiera gymnorrhiza as;

$$W = 9.18(D^2L)^{0.61} \qquad (3.3)$$

($r^2 = 0.98$)

Heritiera littoralis as;

$$W = 1.50(D^2L)^{0.76} \qquad (3.4)$$

($r^2 = 0.99$)

and *Xylocarpus granatum* as;

$$W = 3.13(D^2L)^{0.67} \qquad (3.5)$$

($r^2 = 0.96$)

where r^2 was correlation coefficient, respectively.

3.4.2 Growth

Survival rates were measured for the first 3 months after planting. For the measurement of growth, seedlings were chosen randomly at each salinity and their height, diameter and leaf number measured. The height was recorded from 5 cm above the sand (except in *Burguiera gymnorrhiza* where it was measured from the top of the hypocotyl) to the base of shoot apex. The diameter of the stem was recorded from 5 cm above the sand (or 5 cm above the top of the hypocotyl in the case of *Burguiera gymnorrhiza*). Measurements were carried out every month for 11 months.

3.5 Data analysis

A CRD (Complete Randomized Design) with 3 replications per treatments were used. An analysis of variance employing Duncan's Multiple Range Test at the 0.05 confidence level was used to compare differences in salt concentrations, water potential parameters, and height, diameter, number of leaves and biomass between treatments.

Figure 3.7 Collection of xylem sap from pressure bomb.

Figure 3.8 Pressure bomb technique to collect sap from shoots for generating data for pressure-volume curves.

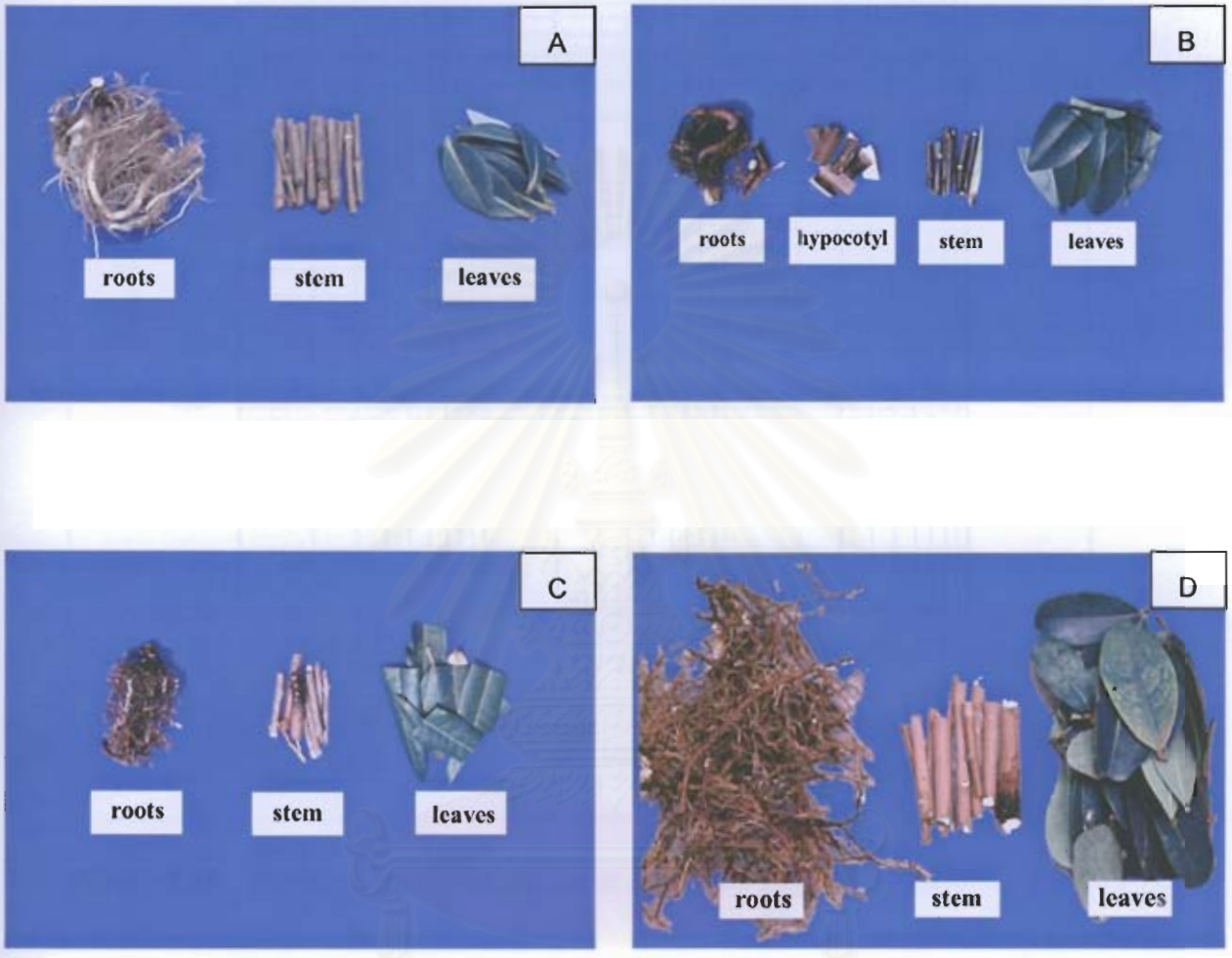

Figure 3.9 (A) Sample of *Avicennia alba* for determining biomass.

(B) Sample of *Bruguiera gymnorrhiza* for determining biomass.

(C) Sample of *Heritiera littoralis* for determining biomass.

(D) Sample of *Xylocarpus granatum* for determining biomass.

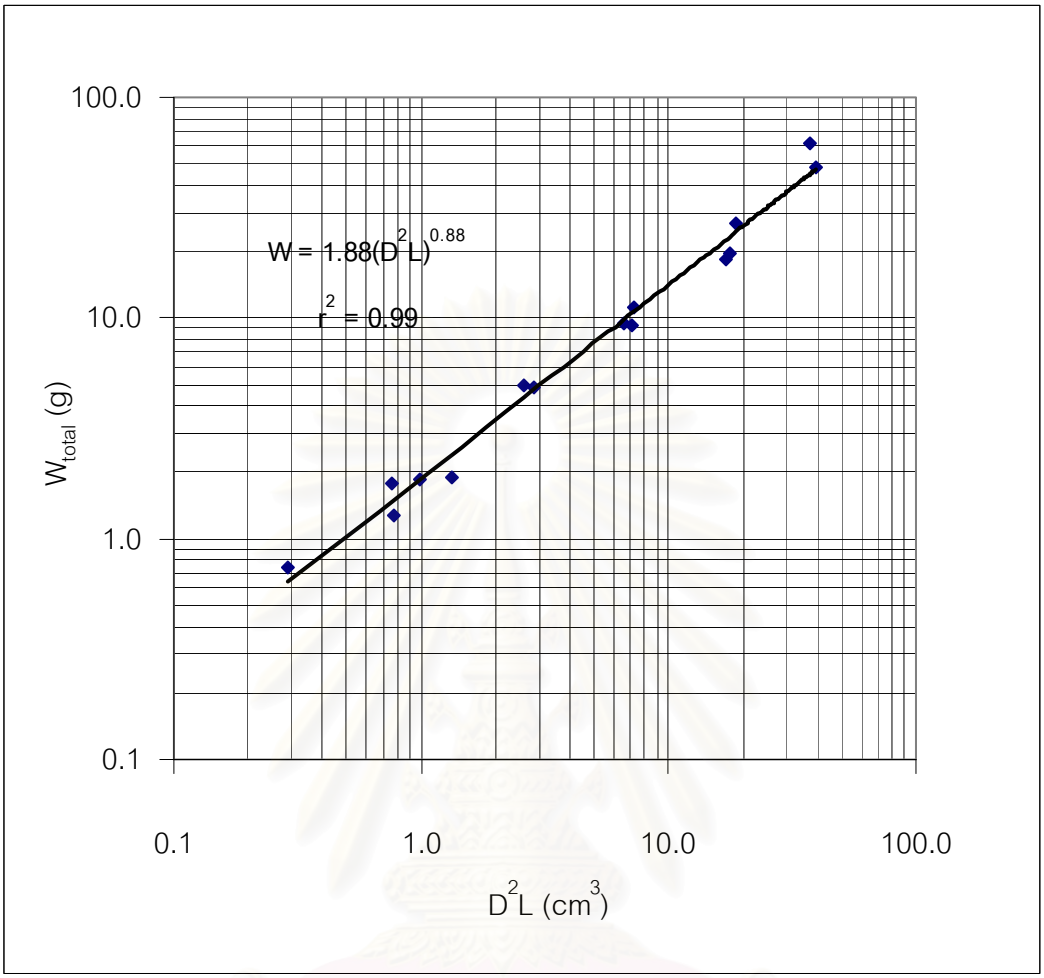

Figure 3.10 The allomertic relationship of *Avicennia alba.*

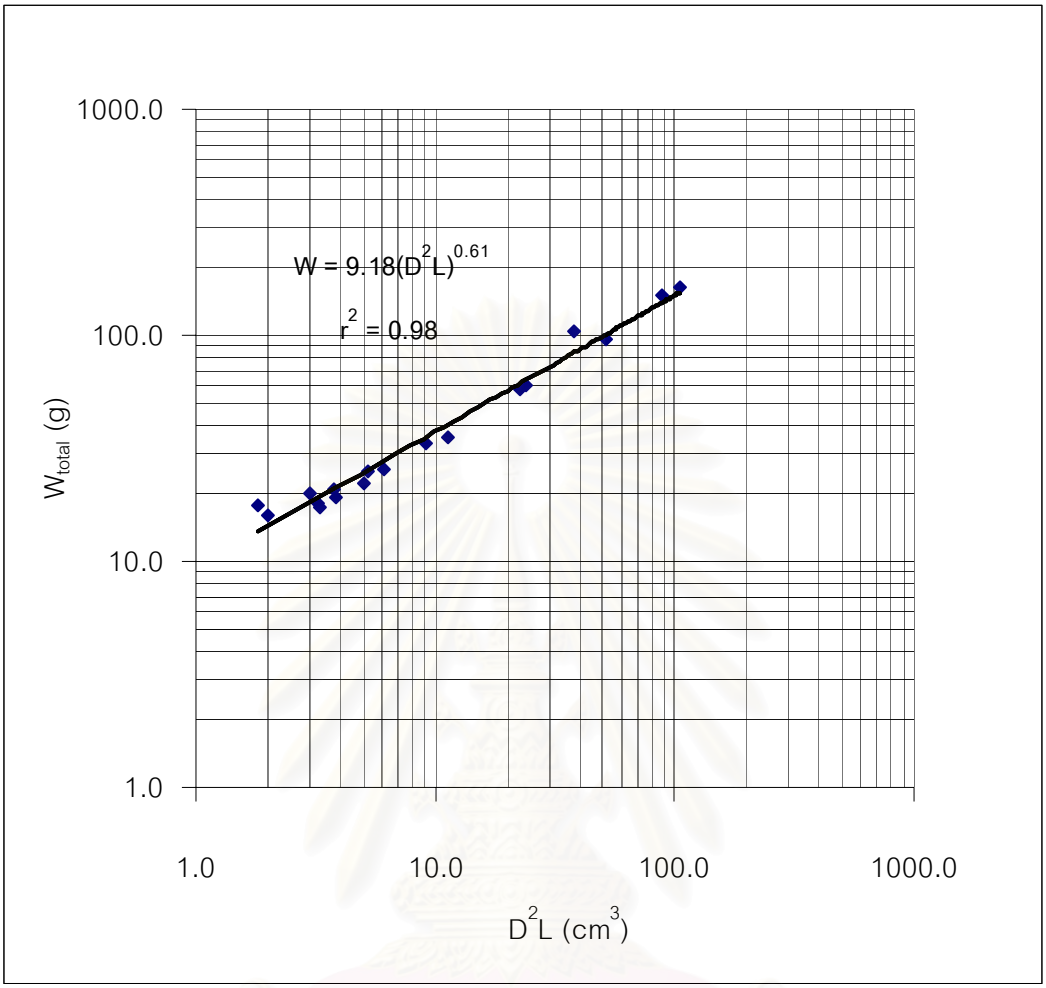

Figure 3.11 The allomertic relationship of *Bruguiera gymnorrhiza.*

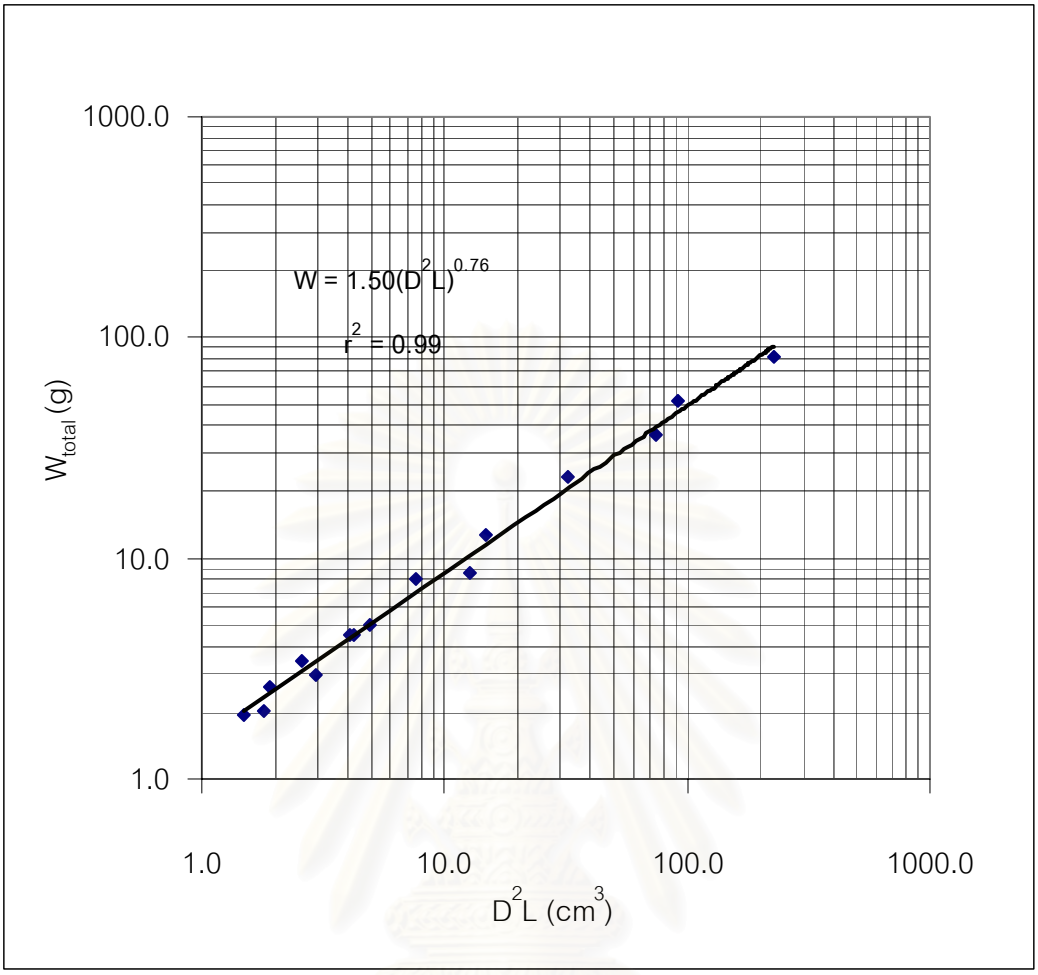

Figure 3.12 The allomertic relationship of *Heritiera littoralis.*

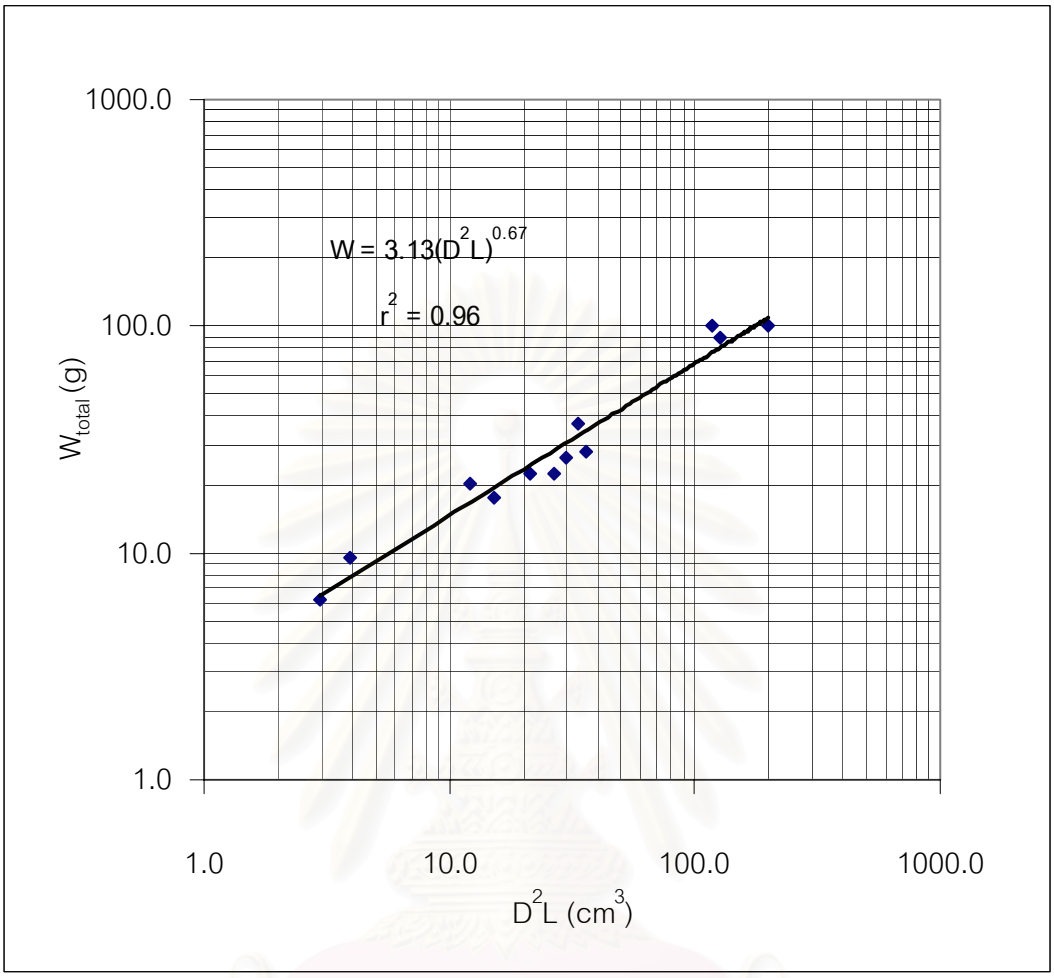

Figure 3.13 The allomertic relationship of *Xylocarpus granatum*.

CHAPTER 4

RESULTS

4.1 Effects of salinity on salt concentrations in xylem sap and leaves

4.1.1 Salt concentrations in xylem sap

Xylem sap could not be collected from *Heritiera littoralis* and *Xylocarpus granatum* using the pressure bomb, so ion concentrations could not be measured in the xylem sap of these two species. Furthermore, ion concentrations were not measured in the xylem sap *Avicennia alba* at 0 ppt and *Bruguiera gymnorrhiza* xylem sap at 0 ppt and 10 ppt because the concentrations were below the detection limit of the atomic absorption spectrometer.

In *Avicennia alba* and *Bruguiera gymnorrhiza* Na and Cl concentrations in xylem sap increased with increasing salinity (Figures 4.1-4.2 and Tables 4.1-4.2). In *Avicennia alba* Na and Cl concentrations in xylem sap were similar. The increase in osmolality with increasing salinity was accompanied by a 30-40 times rise in Na and Cl concentrations in *Avicennia alba* (Figure 4.1 and Tables 4.1, 4.6). K concentrations were always much lower than those of Na and Cl at equivalent salinities, and increased only by about 4-fold from 0 to 40 ppt.

Percentage uptake of Na and Cl (compared to the external solutions) by *Avicennia alba* increased with increasing salinity and the values of both ions were similar, whereas the percentage uptake of K was nearly constant at 40-50% at all salinities (Figure 4.3 and Table 4.3). Percentage uptake of Na and Cl at salinity 40 ppt were 14.77 and 16.31. The mean K:Na ratio in xylem sap from 10 – 40 ppt was higher than the external solutions (Table 4.5). The mean K:Na ratio decreased with increasing salinity. The mean K:Na selectivity ratio (Pitman, 1976) at a salinity of 10 ppt was about 8-fold higher than at a salinity of 40 ppt (Table 4.5).

Na and Cl concentrations of *Bruguiera gymnorrhiza* in xylem sap increased by about 5 times with increasing salinity. The K concentrations were always lower than Na and Cl (Table 4.2). However, as was the case with *Avicennia alba*, the percentage uptake of K in *Bruguiera gymnorrhiza* xylem sap compared to the external solution was higher than that of Na and Cl at every salinity (Figure 4.4 and Table 4.4). Percentage uptake of Na and Cl at 40 ppt was 1.31 and 0.60, respectively. The mean K:Na ratio in xylem sap from 10 – 40 ppt treatments was higher than the external solutions (Table 4.5). The mean K:Na ratio decreased with increasing salinity. The mean K:Na selectivity ratio (Pitman, 1976) at salinity 10 ppt was about 3.7-fold higher than salinity 40 ppt (Table 4.5).

4.1.2 Salt concentrations in leaves

Ion concentrations in *Avicennia alba*, *Bruguiera gymnorrhiza, Heritiera littoralis* and *Xylocarpus granatum* leaves at different salinities were shown in Figures 4.6-4.9 and Tables 4.7-4.10, respectively.

The general pattern of increasing Cl concentration with increasing salinity in all four species was similar to Na, although Cl concentrations were about 2-fold higher than Na concentrations (Tables 4.7-4.10).

Na concentrations in *Avicennia alba* significantly increased with increasing salinity except 10 and 20 ppt, which were not significantly different. Cl concentrations tended to increase with increasing salinity but there was no significant difference from 0 to 30 ppt (Table 4.7). K concentrations tended to decrease with increasing salinity and the values were lower than Na and Cl concentrations (Table 4.7).

Na concentrations in *Bruguiera gymnorrhiza* increased with increasing salinity. There was no significant difference in Cl concentrations between salinity treatments (Table 4.8). K concentrations tended to decrease with increasing salinity and the values were lower than Na and Cl concentrations (Table 4.8).

Na and Cl concentrations in *Heritiera littoralis* tended to increase with increasing salinity (Table 4.9). K concentrations tended to remain constant with increasing salinity. Ion concentrations in *Heritiera littoralis* lower than ion concentrations in other species.

Na concentrations in *Xylocarpus granatum* tended to increase with increasing salinity and there was significant difference among salinity. There was no significant difference in Cl concentrations among all salinity (Table 4.10).

Mg and Ca concentrations of all species showed constant or little change with increasing salinity (Tables 4.7-4.10). Mg and Ca concentrations of all species were very low when compared with Na, K and Cl concentrations.

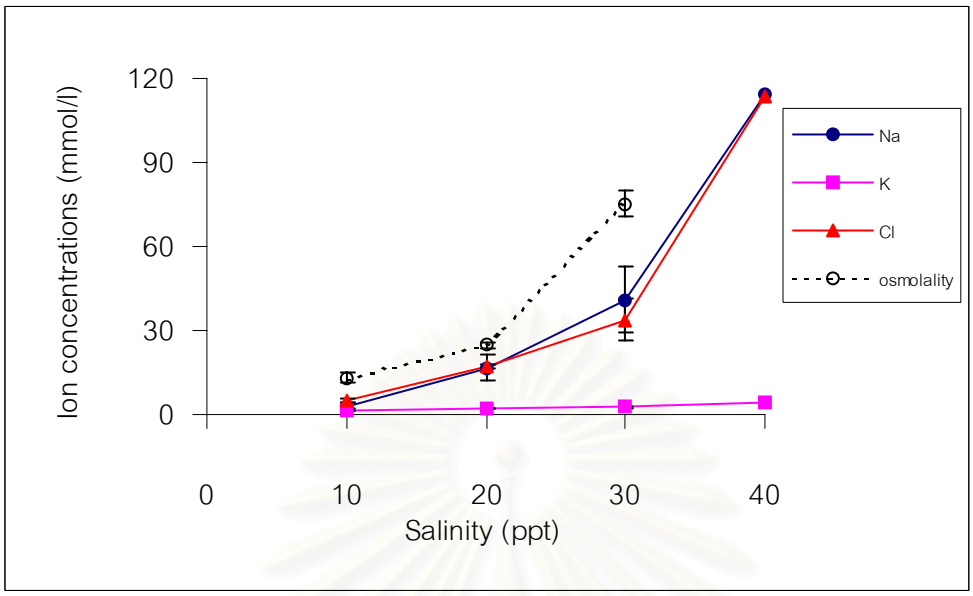

Figure 4.1 Ion concentrations in *Avicennia alba* xylem sap at different salinities.

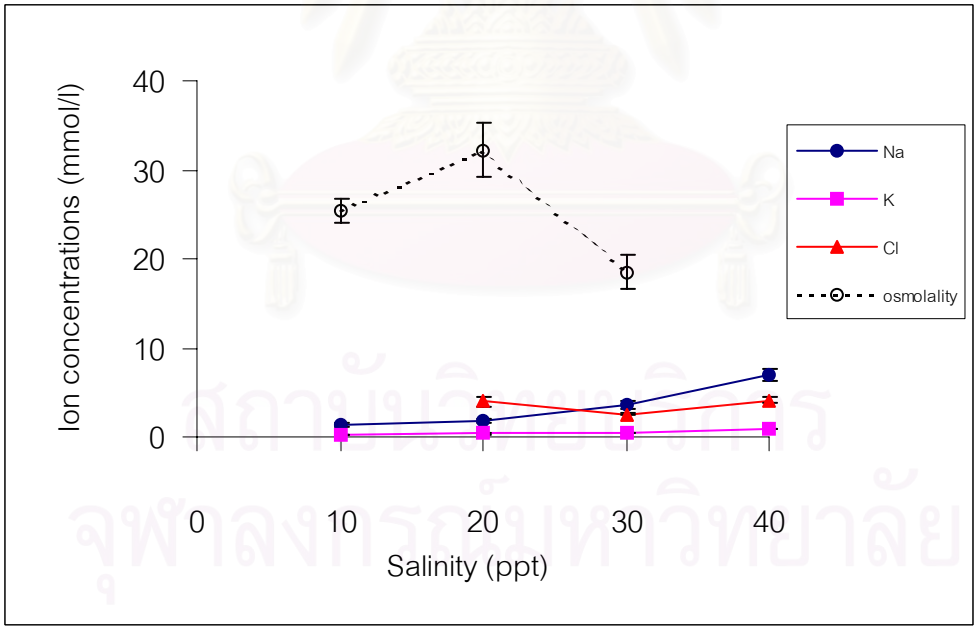

Figure 4.2 Ion concentrations in *Bruguiera gymnorrhiza* xylem sap at different salinities.

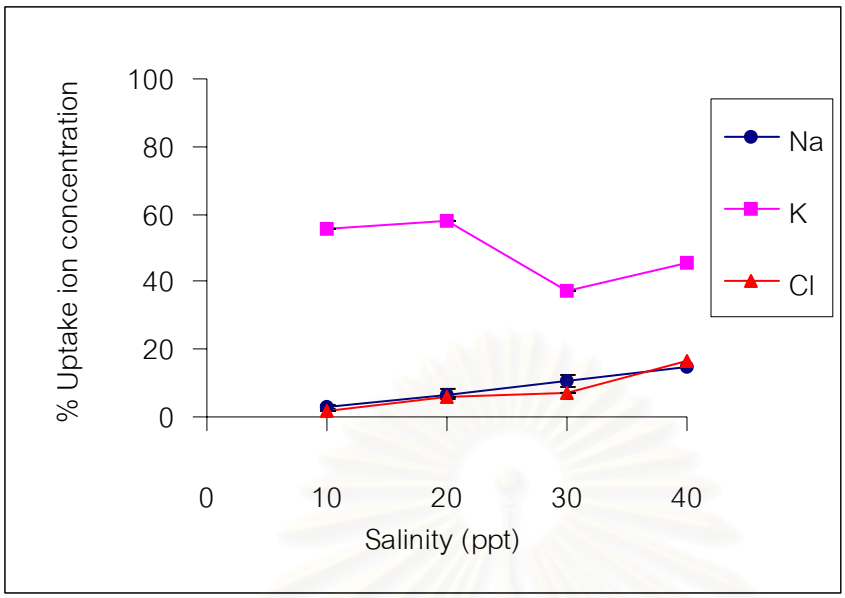

Figure 4.3 Uptake of Na, K and Cl by *Avicennia alba* at different salinities. (100 x concentration in xylem / concentration in external solution)

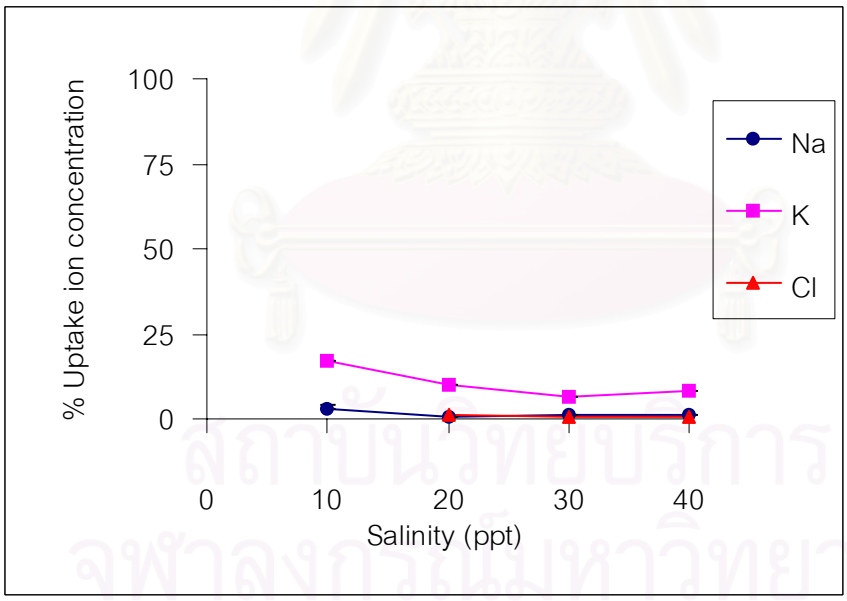

Figure 4.4 Uptake of Na, K and Cl by *Bruguiera gymnorrhiza* at different salinities. (100 x concentration in xylem / concentration in external solution)

.

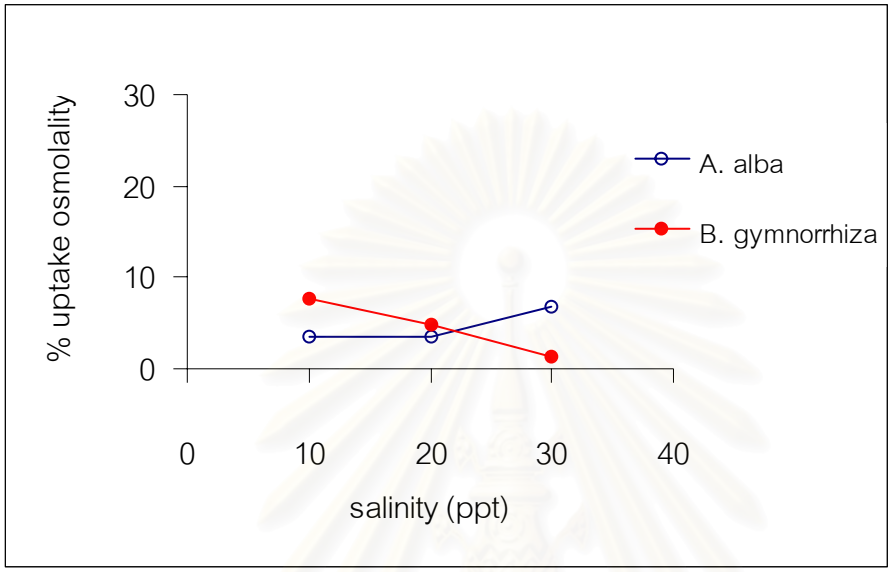

Figure 4.5 Uptake of osmolality by *Avicennia alba* and *Bruguiera gymnorrhiza* at different salinities. (100 x concentration in xylem / concentration in external solution)

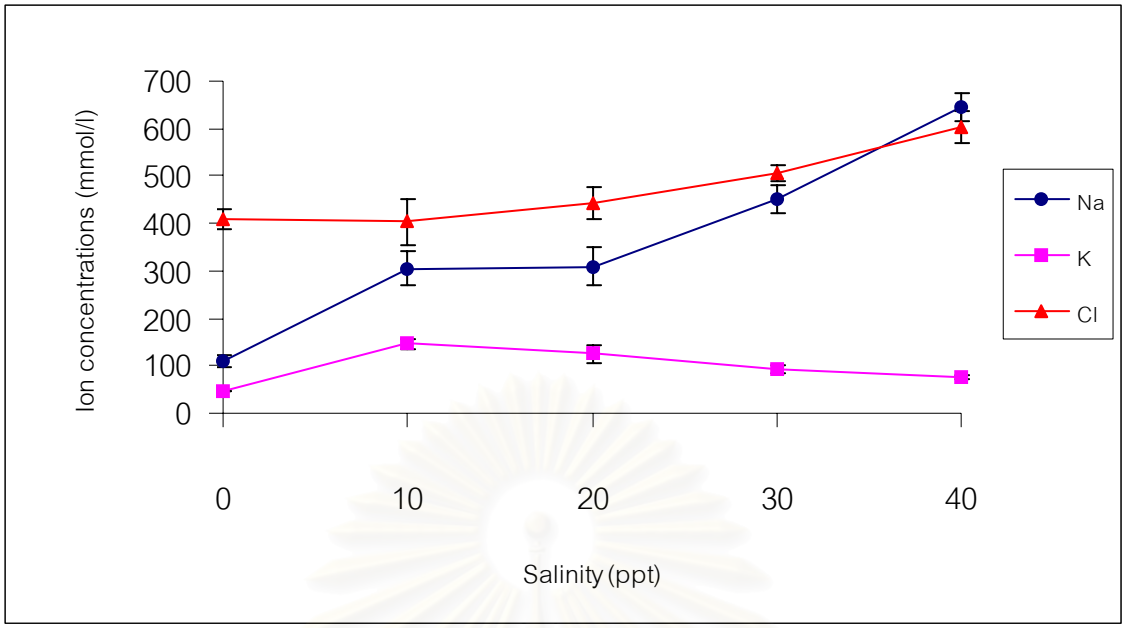

Figure 4.6 Ion concentrations (based on leaf water content) in *Avicennia alba* leaves at different salinities.

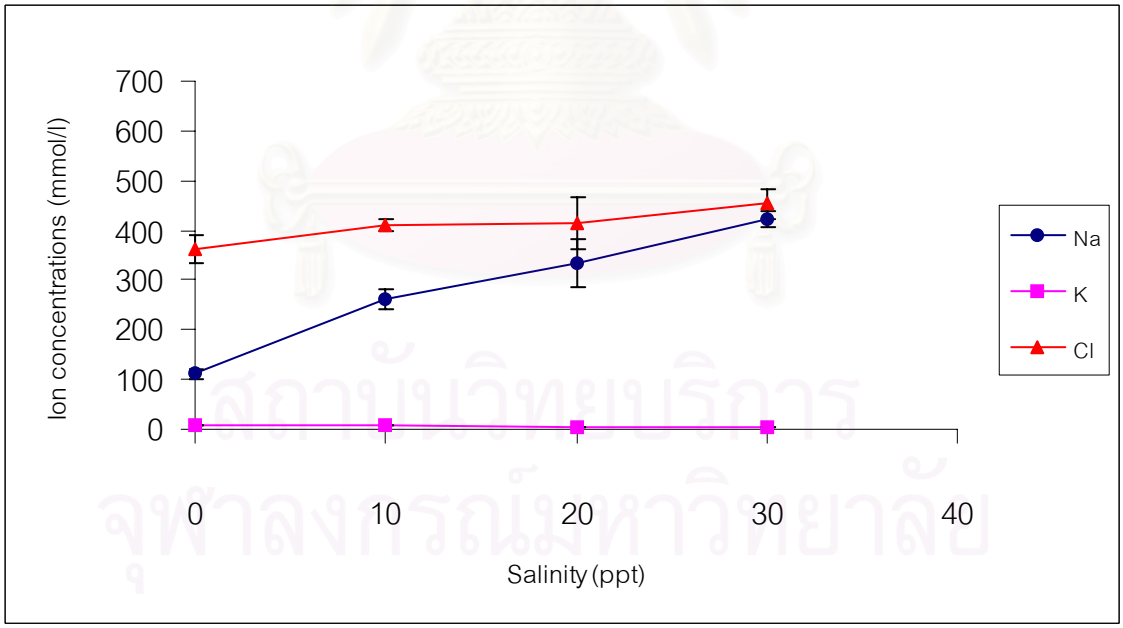

Figure 4.7 Ion concentrations (based on leaf water content) in *Bruguiera gymnorrhiza* leaves at different salinities.

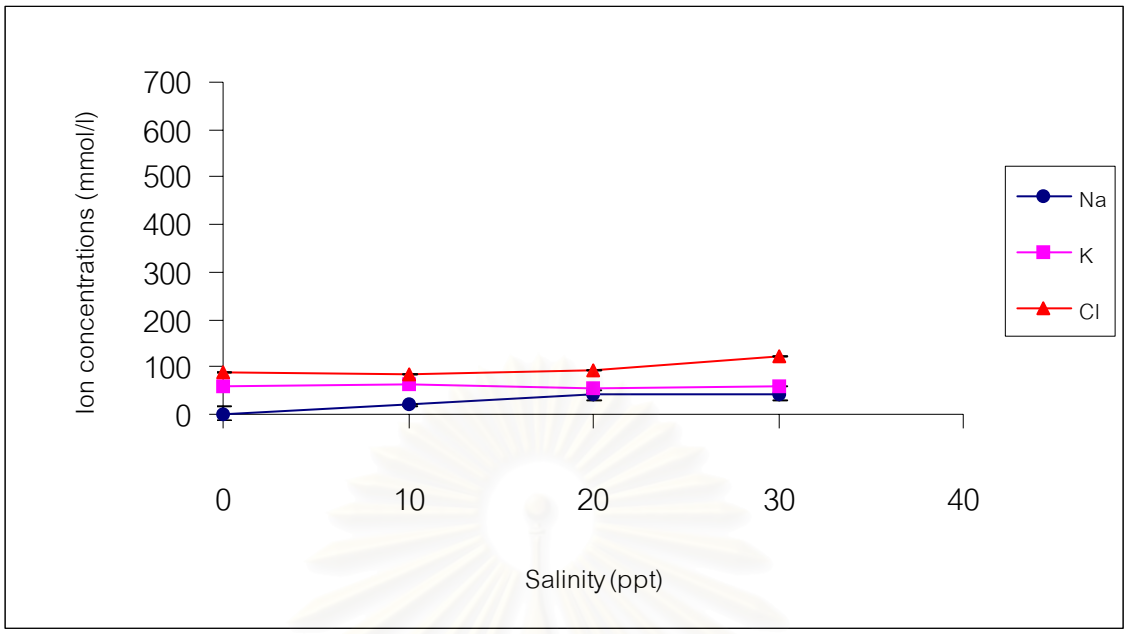

Figure 4.8 Ion concentrations (based on leaf water content) in *Heritiera littoralis* leaves at different salinities.

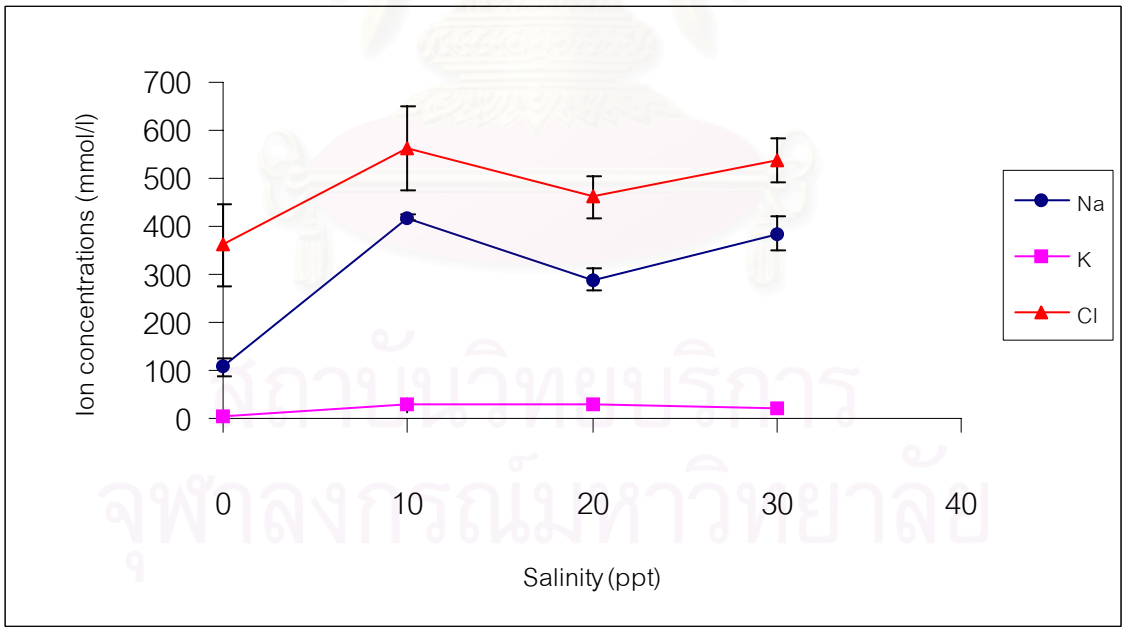

Figure 4.9 Ion concentrations (based on leaf water content) in *Xylocarpus granatum* based on leaf leaves at different salinities.

Table 4.1 Ion concentrations in *Avicennia alba* xylem sap at different salinities

Salinity (ppt)	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)
0	-	-	-
10	2.96 ± 0.33[a]	1.27 ± 0.18 [a]	5.05 ± 0.46 [a]
20	16.62 ± 0.97 [a]	2.31 ± 0.15 [a]	17.40 ± 0.45 [b]
30	41.01 ± 4.74 [b]	2.79 ± 0.17 [b]	33.88 ± 0.64 [c]
40	114.23 ± 11.69 [c]	4.47 ± 0.33 [c]	113.64 ± 7.31 [d]

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.2 Ion concentrations in *Bruguiera gymnorrhiza* xylem sap at different salinities

Salinity (ppt)	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)
0	-	-	-
10	1.34 ± 0.21 [a]	0.26 ± 0.03 [a]	-
20	1.86 ± 0.22 [a]	0.36 ± 0.04 [a]	3.95 ± 0.62 [a]
30	3.61 ± 0.50 [b]	0.50 ± 0.02 [b]	2.54 ± 0.12 [b]
40	6.96 ± 0.78 [c]	0.87 ± 0.06 [c]	4.10 ± 0.35 [a]

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.3 Uptake of Na, K and Cl by *Avicennia alba* at different salinities (100 x concentration in xylem / concentration in external solution)

Salinity (ppt)	Na^+ (%)	K^+ (%)	Cl^- (%)
0	-	-	-
10	3.01 ± 0.44 [a]	55.57 ± 1.09 [a]	1.68 ± 0.00 [a]
20	6.68 ± 0.43 [b]	58.04 ± 0.13 [a]	6.18 ± 0.00 [b]
30	10.76 ± 1.47 [c]	37.39 ± 0.12 [a]	7.09 ± 0.00 [b]
40	14.77 ± 1.95 [d]	45.30 ± 0.03 [a]	16.31 ± 0.01 [c]

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.4 Uptake of Na, K and Cl by *Bruguiera gymnorrhiza* at different salinities (100 x concentration in xylem / concentration in external solution)

Salinity (ppt)	Na^+ (%)	K^+ (%)	Cl^- (%)
0	-	-	-
10	1.17 ± 0.18^{a}	17.26 ± 0.04^{a}	-
20	0.64 ± 0.05^{b}	10.21 ± 0.01^{b}	1.07 ± 0.00^{a}
30	1.14 ± 0.06^{a}	6.41 ± 0.00^{b}	0.55 ± 0.00^{b}
40	1.31 ± 0.10^{a}	8.13 ± 0.01^{b}	0.60 ± 0.00^{b}

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.5 Effect of salinity on the K:Na selectivity of *Avicennia alba* and *Bruguiera gymnorrhiza* xylem sap

The K:Na selectivity ratio ($S_{K,Na}$) was computed from K_{plant} /Na_{plant} ÷ K_{watere}/Na_{water}(Pitman 1976).

	Avicennia alba			*Bruguiera gymnorrhiza*		
Salinity (ppt)	K:Na ratio	$S_{K:Na}$	K:Na External solution	K:Na ratio	$S_{K:Na}$	K:Na External solution
0	-	-	0.26 ± 0.09^{b}	-	-	0.00 ± 0.00^{a}
10	0.51 ± 1.21^{a}	24.11 ± 6.77^{a}	0.03 ± 0.01^{a}	0.24 ± 0.06^{a}	22.75 ± 5.40^{a}	0.02 ± 0.01^{b}
20	0.15 ± 0.02^{b}	8.19 ± 1.29^{b}	0.02 ± 0.00^{a}	0.21 ± 0.03^{ab}	18.36 ± 3.19^{a}	0.01 ± 0.00^{a}
30	0.07 ± 0.01^{b}	3.61 ± 0.78^{b}	0.02 ± 0.00^{a}	0.14 ± 0.01^{b}	5.76 ± 0.47^{b}	0.03 ± 0.00^{bc}
40	0.04 ± 0.00^{b}	3.01 ± 0.22^{b}	0.01 ± 0.00^{a}	0.12 ± 0.01^{b}	6.07 ± 4.65^{b}	0.02 ± 0.00^{c}

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.6 Osmolality in xylem sap and uptake of osmolality by *Avicennia alba* and *Bruguiera gymnorrhiza* at different salinities

Salinity (ppt)	*A.alba*		*B. gymnorrhiza*	
	Osmolality (mmol/kg)	% uptake	Osmolality (mmol/kg)	% uptake
0	-	-	-	-
10	13.14	3.43	25.39	7.68
20	24.97	3.46	32.22	4.75
30	75.17	6.71	18.53	1.27
40	-	-	-	-

Table 4.7 Ion concentrations (based on leaf water content) in *Avicennia alba* leaves at different salinities

Salinity (ppt)	Na^+ (mmol/l)	K^+ (mmol/l)	Mg^{++} (mmol/l)	Ca^{++} (mmol/l)	Cl^- (mmol/l)
0	107.91 ± 12.71 [a]	46.41 ± 0.87 [a]	0.10 ± 0.01 [bc]	0.03 ± 0.01 [a]	408.93 ± 21.57 [a]
10	304.65 ± 36.09 [b]	146.74 ± 10.19 [c]	0.07 ± 0.01 [ab]	0.01 ± 0.01 [b]	402.85 ± 46.90 [a]
20	308.77 ± 39.49 [b]	125.08 ± 18.95 [c]	0.06 ± 0.02 [a]	0.01 ± 0.02 [b]	441.41 ± 33.42 [a]
30	450.83 ± 30.34 [c]	91.58 ± 7.75 [ab]	0.11 ± 0.01 [c]	0.01 ± 0.01 [b]	504.55 ± 16.35 [ab]
40	643.50 ± 29.48 [d]	75.90 ± 4.90 [ab]	0.16 ± 0.01 [d]	0.01 ± 0.01 [b]	604.08 ± 33.86 [b]

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.8 Ion concentrations (based on leaf water content) in *Bruguiera gymnorrhiza* leaves at different salinities

Salinity (ppt)	Na^+ (mmol/l)	K^+ (mmol/l)	Mg^{++} (mmol/l)	Ca^{++} (mmol/l)	Cl^- (mmol/l)
0	110.92 ± 9.91 [a]	7.39 ± 0.11 [a]	0.01 ± 0.00 [a]	0.04 ± 0.00 [a]	362.27 ± 29.76 [a]
10	261.09 ± 21.56 [b]	6.72 ± 0.22 [a]	0.01 ± 0.00 [a]	0.02 ± 0.00 [b]	411.20 ± 12.06 [a]
20	333.46± 49.02 [bc]	3.79 ± 0.26 [b]	0.01 ± 0.00 [a]	0.02 ± 0.00 [b]	415.38 ± 51.71 [a]
30	421.57 ± 16.52 [c]	4.12 ± 0.21 [b]	0.01 ± 0.00 [a]	0.02 ± 0.00 [b]	453.72 ± 30.14 [a]
40	-	-	-	-	-

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.9 Ion concentrations (based on leaf water content) in *Heritiera littoralis* leaves at different salinities

Salinity (ppt)	Na^+ (mmol/l)	K^+ (mmol/l)	Mg^{++} (mmol/l)	Ca^{++} (mmol/l)	Cl^- (mmol/l)
0	14.79 ± 14.79 [a]	57.01 ± 5.74 [a]	0.02 ± 0.00 [a]	0.05 ± 0.01 [a]	88.54 ± 6.87 [a]
10	20.20 ± 1.96 [a]	64.25 ± 4.89 [a]	0.01 ± 0.00 [a]	0.02 ± 0.00 [b]	82.41 ± 2.10 [a]
20	40.12 ± 10.62 [a]	55.54 ± 2.64 [a]	0.02 ± 0.01 [a]	0.02 ± 0.00 [b]	93.54 ± 3.54 [a]
30	55.65 ± 14.52[a]	59.12 ± 2.17 [a]	0.02 ± 0.00 [a]	0.03 ± 0.00 [b]	123.83 ± 9.04 [b]
40	-	-	-	-	-

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.10 Ion concentrations (based on leaf water content) in *Xylocarpus granatum* leaves at different salinities

Salinity (ppt)	Na^+ (mmol/l)	K^+ (mmol/l)	Mg^{++} (mmol/l)	Ca^{++} (mmol/l)	Cl^- (mmol/l)
0	107.56 ± 18.06 [a]	2.35 ± 0.04 [a]	0.01 ± 0.00 [a]	0.05 ± 0.01 [a]	360.56 ± 83.67 [a]
10	417.34 ± 6.21 [c]	27.56 ±13.09 [b]	0.01 ± 0.00 [a]	0.04 ± 0.00 [a]	562.08 ± 87.20 [a]
20	289.20 ± 24.58 [b]	31.05 ± 2.23 [b]	0.01 ± 0.00 [a]	0.03 ± 0.00 [a]	460.85 ± 42.51 [a]
30	384.13 ± 34.92 [b]	20.60 ± 4.11 [ab]	0.01 ± 0.00 [a]	0.04 ± 0.00 [a]	537.66 ± 47.59 [a]
40	-	-	-	-	-

Means within each column followed by the same letter are not significantly different (P>0.05)

4.2 Effects of salinity on shoot water potential and water potential components

The value of water potential (Ψ), osmotic potential at full turgor (Ψ_π^{100}) and at zero turgor (Ψ_π^{0}), wall relative water content (wall RWC), relative water content of shoot (RWC^0) and relative water content of symplast at zero turgor (RWC_{sym}^{0}), molar concentration of solute at full turgor and at zero turgor (SD), average modulus of elasticity (ε) obtained from pressure-volume curves for all species were shown in Tables 4.11-4.14.

Water potential of shoots at the first balancing pressure decreased with increasing salinity in all species (Tables 4.11-4.14).

Wall relative water content, relative water content of shoot and symplastic relative water content at zero turgor of all species were nearly constant with increasing salinity, and there were no significant differences between salinity treatments (Tables 4.11-4.14).

Both the osmotic potential at full turgor and osmotic potential at zero turgor, decreased in all species with increasing salinity (Tables 4.11-4.14).

In *Avicennia alba,* the osmotic potential at full turgor was 1.49 MPa lower in plant at 40 ppt those at 0 ppt, and the osmotic potential at zero turgor decreased by 1.66 MPa from 0 ppt to 40 ppt. A similar pattern was evident in the other three species: in *Bruguiera gymnorrhiza*, the osmotic potential at full turgor and at zero turgor decreased by 1.59 MPa and 1.63 MPa, respectively, from 0 to 40 ppt; in

Heritiera littoralis the osmotic potential at full turgor and at zero turgor decreased by 0.67 MPa and 0.73 MPa, respectively, from 0 to 40 ppt; and in *Xylocarpus granatum* the osmotic potential at full turgor and at zero turgor decreased by 1.84 MPa and 2.37 MPa, respectively, from 0 to 40 ppt (Tables 4.11-4.14).

The molar concentration of solute at full turgor and molar concentration of solute at zero turgor increased with increasing salinity of all four species (Tables 4.11-4.14).

The modulus of elasticity of all species except *Heritiera littoralis* increased by about 2-fold between the 0 and 40 ppt salinity treatments (Tables 4.11-4.14).

Table 4.11 Effects of salinity on water potential (Ψ), osmotic potential at full turgor (Ψ_π^{100}) and at zero turgor (Ψ_π^{0}), wall relative water content (wall RWC), relative water content of shoot (RWC^0) and relative water content of symplast at zero turgor (RWC_{sym}^{0}), molar concentration of solute at full turgor and at zero turgor (SD), average modulus of elasticity (ε) obtained from pressure-volume curves for *Avicennia alba*.

			Full Turgor		Zero Turgor				
Salinity (ppt)	Ψ (MPa)	Wall RWC	Ψ_π^{100} (MPa)	SD (mol/L)	Ψ_π^{0} (MPa)	RWC_0	RWC sym	SD (mol/L)	ε (MPa)
0	-0.93±0.11^{a}	0.25 ±0.01^{a}	-2.49 ± 0.07^{a}	1.02 ± 0.03^{a}	-2.88 ± 0.06^{a}	0.90 ±0.01^{a}	0.86 ±0.01^{a}	1.18 ± 0.03^{a}	17.09 ±1.79^{a}
10	-0.94±0.03^{a}	0.26 ±0.04^{a}	-2.72 ±0.06^{ab}	1.12 ± 0.02^{ab}	-3.44 ±0.23^{ab}	0.85 ±0.04^{a}	0.80 ±0.06^{a}	1.41 ± 0.10^{ab}	16.33 ±6.56^{a}
20	-1.62±0.04^{b}	0.28 ±0.03^{a}	-3.14 ± 0.15^{b}	1.29 ± 0.06^{b}	-3.82 ±0.61^{ab}	0.89 ±0.06^{a}	0.85 ±0.08^{a}	1.57 ± 0.25^{ab}	28.5 ±49.00^{a}
30	-1.74±0.04^{b}	0.31 ±0.04^{a}	-3.65 ± 0.21^{c}	1.50 ± 0.09^{c}	-4.14 ±0.19^{bc}	0.92 ±0.00^{a}	0.88 ±0.03^{a}	1.70 ± 0.08^{b}	31.00 ±8.53^{a}
40	-3.16±0.09^{c}	0.34 ±0.01^{a}	-3.98 ± 0.20^{c}	1.63 ± 0.09^{c}	-4.54 ±0.24^{bc}	0.92 ±0.01^{a}	0.88 ± .03^{a}	1.86 ± 0.10^{bc}	30.61 ±1.28^{a}

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.12 Effects of salinity on water potential (Ψ), osmotic potential at full turgor (Ψ_π^{100}) and at zero turgor (Ψ_π^{0}), wall relative water content (wall RWC), relative water content of shoot (RWC^0) and relative water content of symplast at zero turgor (RWC_{sym}^{0}), molar concentration of solute at full turgor and at zero turgor (SD), average modulus of elasticity (ε) obtained from pressure-volume curves for *Bruguiera gymnorrhiza*.

			Full Turgor		Zero Turgor				
Salinity (ppt)	Ψ (MPa)	Wall RWC	Ψ_π^{100} (MPa)	SD (mol/L)	Ψ_π^{0} (MPa)	RWC_0	RWC sym	SD (mol/L)	ε (MPa)
0	-0.58± 0.02 [a]	0.54 ± 0.02 [a]	-1.81± 0.05 [a]	0.74 ± 0.02 [a]	-2.34± 0.10 [a]	0.90 ± 0.01 [a]	0.77 ± 0.01 [a]	0.96 ± 0.04 [a]	7.42 ± 0.20 [a]
10	-1.08± 0.02 [a]	0.15 ± 0.03 [b]	-2.48± 0.09 [b]	1.02 ± 0.04 [b]	-2.98± 0.11 [b]	0.86 ± 0.01 [b]	0.83 ± 0.01 [b]	1.22 ± 0.04 [b]	13.80 ± 0.81 [b]
20	-1.64± 0.02 [a]	0.14 ± 0.01 [b]	-2.68± 0.01 [b]	1.10 ± 0.01 [b]	-3.09± 0.02 [b]	0.89 ± 0.00 [a]	0.87 ± 0.00 [b]	1.27 ± 0.01 [b]	18.57 ± 0.26 [c]
30	-2.27± 0.09 [b]	0.14 ± 0.03 [b]	-3.11± 0.01 [c]	1.28 ± 0.01 [c]	-3.58± 0.01 [c]	0.89 ± 0.00 [a]	0.87 ± 0.01 [c]	1.47 ± 0.00 [c]	22.53 ± 1.06 [d]
40	-2.04± 0.15 [b]	0.21 ± 0.04 [b]	-3.40± 0.11 [d]	1.39 ± 0.04 [d]	-3.97± 0.15 [d]	0.89 ± 0.00 [a]	0.86 ± 0.01 [d]	1.63 ± 0.06 [d]	22.18 ± 0.84 [d]

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.13 Effects of salinity on water potential (Ψ), osmotic potential at full turgor (Ψ_π^{100}) and at zero turgor (Ψ_π^{0}), wall relative water content (wall RWC), relative water content of shoot (RWC^0) and relative water content of symplast at zero turgor (RWC_{sym}^{0}), molar concentration of solute at full turgor and at zero turgor (SD), average modulus of elasticity (ε) obtained from pressure-volume curves for *Heritiera littoralis.*

			Full Turgor		Zero Turgor				
Salinity (ppt)	Ψ (MPa)	Wall RWC	Ψ_π^{100} (MPa)	SD (mol/L)	Ψ_π^{0} (MPa)	RWC_0	RWC sym	SD (mol/L)	ε (MPa)
0	-0.89 ± 0.02 [a]	0.53 ± 0.03 [a]	-2.60 ± 0.11 [a]	1.10 ± 0.05 [a]	-3.65 ± 0.14 [ab]	0.86 ± 0.01 [a]	0.71 ± 0.01 [a]	1.50 ± 0.06 [ab]	8.88 ± 0.49 [a]
10	-1.66 ± 0.05 [ab]	0.27 ± 0.14 [b]	-2.45 ± 0.01 [a]	1.01 ± 0.05 [a]	-3.13 ± 0.16 [a]	0.84 ± 0.02 [a]	0.78 ± 0.01 [a]	1.29 ± 0.07 [a]	10.64 ± 0.12 [ab]
20	-1.63 ± 0.10 [ab]	0.19 ± 0.01 [b]	-3.64 ± 0.22 [c]	1.50 ± 0.09 [c]	-4.61 ± 0.21 [c]	0.83 ± 0.01 [a]	0.79 ± 0.01 [a]	1.90 ± 0.09 [c]	16.52 ± 1.83 [b]
30	-1.34 ± 0.05 [ab]	0.18 ± 0.01 [b]	-2.98 ± 0.12 [ab]	1.23 ± 0.05 [ab]	-4.23 ± 0.35 [ab]	0.76 ± 0.02 [b]	0.71 ± 0.03 [a]	1.74 ± 0.15 [bc]	9.76 ± 0.66 [a]
40	-2.57 ± 0.60 [b]	0.40± 0.03 [ab]	-3.37 ± 0.21 [bc]	1.38 ± 0.09 [bc]	-4.38 ± 0.24 [bc]	0.86 ± 0.02 [a]	0.77 ± 0.04 [a]	1.80 ± 0.10 [bc]	14.60 ± 2.25 [ab]

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.14 Effects of salinity on water potential (Ψ), osmotic potential at full turgor (Ψ_π^{100}) and at zero turgor (Ψ_π^{0}), wall relative water content (wall RWC), relative water content of shoot (RWC^0) and relative water content of symplast at zero turgor (RWC_{sym}^{0}), molar concentration of solute at full turgor and at zero turgor (SD), average modulus of elasticity (ε) obtained from pressure-volume curves for *Xylocarpus granatum*.

			Full Turgor		Zero Turgor				
Salinity (ppt)	Ψ (MPa)	Wall RWC	Ψ_π^{100} (MPa)	SD (mol/L)	Ψ_π^{0} (MPa)	RWC_0	RWC sym	SD (mol/L)	ε (MPa)
0	-0.85± 0.04 [a]	0.34 ± 0.08 [a]	-2.06 ±0.16 [a]	0.85 ± 0.06 [a]	-2.46 ±0.12 [a]	0.90 ± 0.00 [a]	0.84 ± 0.03 [a]	1.01 ± 0.05 [a]	12.20 ± 2.53 [a]
10	-1.30± 0.06 [b]	0.30 ± 0.04 [a]	-2.51 ±0.08 [a]	1.03 ± 0.03 [a]	-2.89 ±0.11 [a]	0.91 ± 0.01 [a]	0.87 ± 0.01 [a]	1.18 ± 0.04 [a]	18.01 ± 1.74 [a]
20	-1.70± 0.07 [c]	0.23 ± 0.08 [a]	-2.62 ±0.07 [a]	1.08 ± 0.03 [a]	-2.95 ±0.08 [a]	0.92 ± 0.01 [a]	0.89 ± 0.01 [a]	1.21 ± 0.03 [a]	21.65 ± 0.44 [ab]
30	-2.70± 0.21 [d]	0.22 ± 0.10 [a]	-3.90 ±0.44 [b]	1.60 ± 0.18 [b]	-4.83 ±0.41 [b]	0.91 ± 0.02 [a]	0.88 ± 0.02 [a]	1.80 ± 0.17 [b]	32.46 ± 7.09 [b]
40	-	-	-	-	-	-	-	-	-

Means within each column followed by the same letter are not significantly different (P>0.05)
Plant dead at salinity 40 ppt

4.3 Effects of salinity on growth

4.3.1 Survival rates

The germination rates of *Avicennia alba, Bruguiera gymnorrhiza, Heritiera littoralis* and *Xylocarpus granatum* were 99.4%, 99.4%, 76.2% and 57.4%, respectively (Figure 4.10).

The average percent survival rates of *Avicennia alba, Bruguiera gymnorrhiza, Heritiera littoralis* and *Xylocarpus granatum* during the first 3 months of the salinity treatments were shown in Figure 4.11 and Table 4.15. Survival rates decreased in all species with increasing salinity, all species died at a salinity of 60 ppt.

All species exposed to high salinity (30 and 40 ppt) showed symptoms of wilting after only one month, particularly during the sunny periods and the hottest hours of the days. All species except *Avicennia alba* developed more slowly at high salinity (30 and 40 ppt) than at 0 ppt.

After three months in the salinity treatments, *Avicennia alba* had the highest survival rate (100%) at salinity 0, 10 and 20 ppt, falling to 94.4%, 68.5% and 0% at salinity 30, 40 and 60 ppt, respectively. *Bruguiera gymnorrhiza* had a survival rate of 100% at 0, 10 and 20 ppt, decreasing to 98.2%, 33.3% and 0% at 30, 40 and 60 ppt, respectively. *Heritiera littoralis* had 100% survival rate only at 0 ppt, the average survival rate of *Heritiera littoralis* was 88.1%, 64.3%, 60.0%,

9.5% and 0% at 10, 20, 30, 40 and 60 ppt, respectively. *Xylocarpus granatum* had a survival rate of 100% at 0 and 10 ppt, then falling to 71.4%, 68.8%, 53.6% and 0% at 20, 30, 40 and 60 ppt, respectively. In term of survival rate, *Avicennia alba* was the most salt tolerant followed by *Bruguiera gymnorrhiza*, *Xylocarpus granatum* and *Heritiera littoralis* in that order.

4.3.2 Growth

The height of stem

All species increased in height with time (Figures 4.12-4.15.), except *Xylocarpus granatum* at 20, 30 and 40 ppt which sometimes showed a decrease in average of height because larger plants died during the experiment. The height of all species generally decreased with increasing salinity (Figures 4.12-4.15).

After 11 months height growth of *Avicennia alba* was greatest in 10, 20 and 30 ppt (51.7, 45.6 and 37.4 cm, respectively). Plants grown at 0 and 40 ppt had significantly less height growth than those at intermediate salinities (Table 4.16). *Avicennia alba* grown at 10-40 ppt accumulated salt crystals on the under sides of the leaves. Plants in the 0 ppt salinity treatments (control) developed chlorotic and necrotic lesions on the leaves several months after establishment and there was also tendency for shoot apices to blacken.

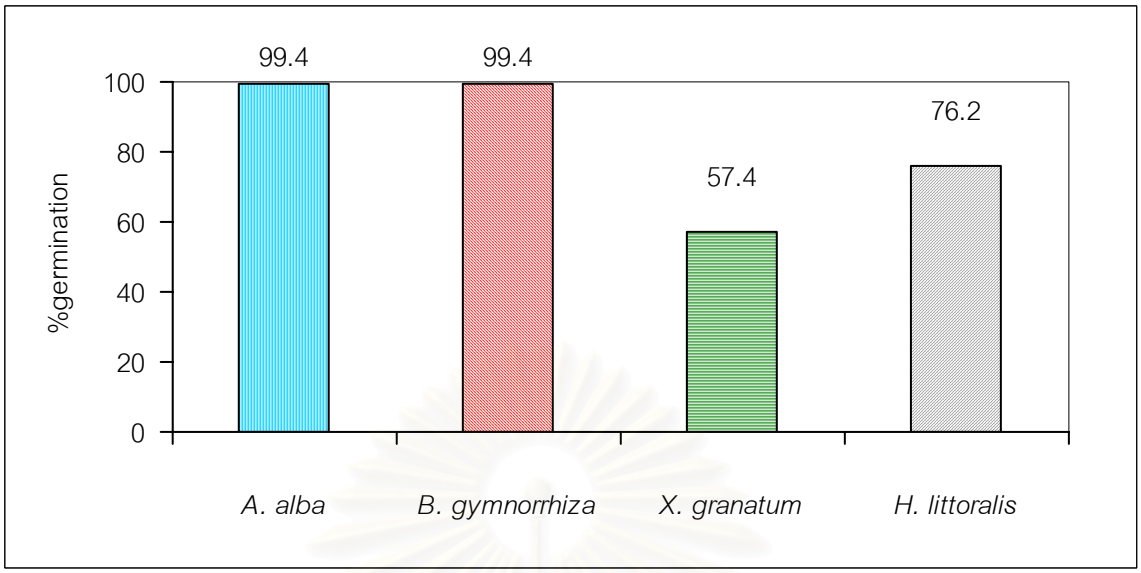

Figure 4.10 The germination rates of *Avicennia alba*, *Bruguiera gymnorrhiza*, *Heritiera littoralis* and *Xylocarpus granatum*.

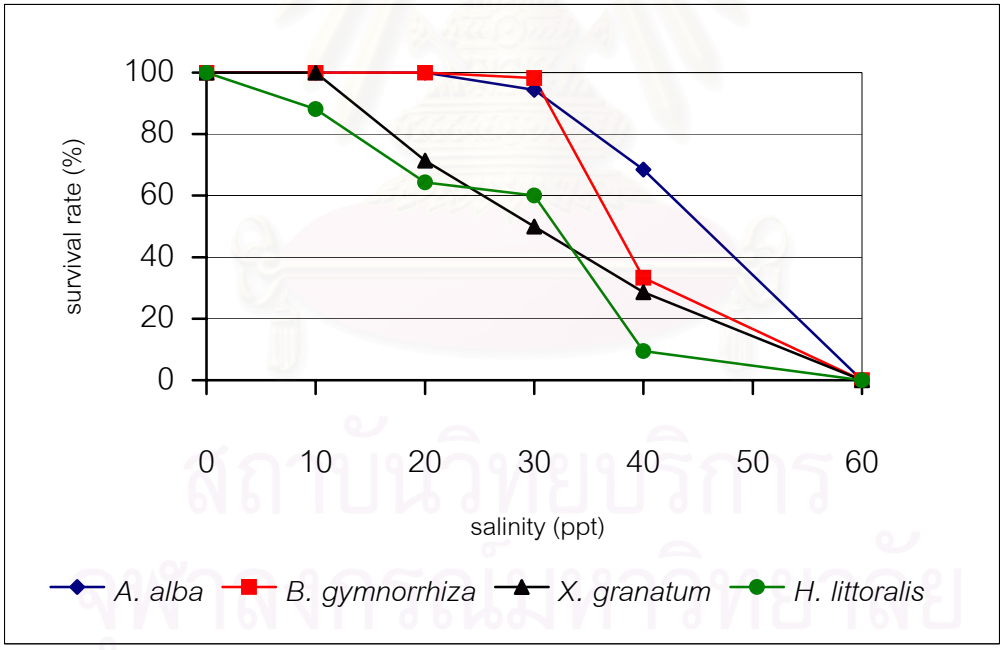

Figure 4.11 Survival rates of *Avicennia alba*, *Bruguiera gymnorrhiza*, *Heritiera littoralis* and *Xylocarpus granatum* for up to three months at different salinities.

Table 4.15 Survival rates of *Avicennia alba***,** *Bruguiera gymnorrhiza*, *Heritiera littoralis* and *Xylocarpus granatum* for up to three months at different salinities

salinity (ppt)	Percent survival rate											
	Week after treatment with salinity											
	A. alba			*B. gymnorrhiza*			*H. littoralis*			*X. granatum*		
	1	6	12	1	6	12	1	6	12	1	6	12
0	100	100	100	100	100	100	100	100	100	100	100	100
10	100	100	100	100	100	100	100	100	88.1	100	100	100
20	100	100	100	100	100	100	100	100	64.3	100	71.4	71.4
30	100	96.3	94.4	100	100	98.2	100	82.6	60.0	100	68.8	50.0
40	100	68.5	68.5	100	51.9	33.3	100	50.0	9.5	100	53.6	28.6
60	100	0	0	100	0	0	100	12.5	0	100	0	0

The mean of heights of *Bruguiera gymnorrhiza* grown in 0, 10, 20, 30 and 40 ppt salinity treatments for 11 months were 44.9, 54.9, 39.7, 34.4 and 16.2 cm, respectively. There was a significant higher rate of height growth in plants grown at 10 ppt compared with other salinities (Table 4.17). *Heritiera littoralis*, grew best in the 0 ppt treatments. The mean of heights of *Heritiera littoralis* grown at 0, 10, 20 and 30 ppt for 11 months were 57.1, 45.7, 32.7 and 30.1 cm, respectively (Table 4.18). The mean of height of *Xylocarpus granatum* grown at 0, 10, 20, 30 and 40 ppt for 11 months were 60.1, 67.2, 50.2, 35.6 and 29.4 cm, respectively (Table 4.19).

The diameter of stem

All species increased in diameter of stem with time (Figures 4.16-4.19), except *Xylocarpus granatum* at 20, 30 and 40 ppt and *Heritiera littoralis* at 40 ppt which sometimes showed a decrease in average of diameter because larger plants died during the experiment. The diameter of stem of all species generally decreased with increasing salinity (Figures 4.16-4.19).

After 11 months, the mean of diameters of *Avicennia alba* grown at 0, 10, 20, 30 and 40 ppt were 0.39, 0.49, 0.45, 0.40 and 0.30 cm, respectively. There was no significant difference in the diameter of stem between plants grown in 0 and 30 ppt salinity treatments (Table 4.16). The mean of diameters of *Bruguiera gymnorrhiza* grown in 0, 10, 20, 30 and 40 ppt salinity treatments for 11 months were 0.87, 0.91, 0.81, 0.67 and 0.50 cm, respectively (Table 4.17). The mean of diameters of *Heritiera littoralis* grown at 0, 10, 20, and 30 ppt for 11 months were

1.20, 1.22, 0.68 and 0.59 cm, respectively (Table 4.18). Plant grown at 0 and 10 ppt diameters were similar. The mean of diameters of *Xylocarpus granatum* grown at 0, 10, 20, 30 and 40 ppt for 11 months were 1.02, 1.15, 0.90, 0.50 and 0.43 cm, respectively (Table 4.19).

Number of leaves

All species tended to decrease the number of leaves with increasing salinity except *Avicennia alba* (Figures 4.20-4.23).

Avicennia alba increased the number of leaves with time (Figure 4.20) until month 7, after that the number of leaves tended to decrease. The number of leaves of *Avicennia alba* in the all salinity treatments, were more than plant grown at 0 ppt (control). The mean of number of leaves of *Avicennia alba* grown at 0, 10, 20, 30 and 40 ppt for 11 months were 5, 8, 10, 11 and 9 leaf, respectively. There was a significant of number of leaves in plants grown at 0 ppt when compared with other salinity (Table 4.16). The mean of number of leaves of *Bruguiera gymnorrhiza* grown in 0, 10, 20, 30 and 40 ppt salinity treatments for 11 months were 16, 20, 16, 15 and 12 leaf, respectively (Table 4.17). The mean of number of leaves of *Heritiera littoralis* grown at 0, 10, 20, 30 and 40 ppt for 11 months were 20, 16, 7 and 6 leaf, respectively (Table 4.18). The mean of number of leaves of *Xylocarpus granatum* grown at 0, 10, 20, 30 and 40 ppt for 11 months were 35, 45, 29, 5 and 4 leaf, respectively (Table 4.19).

Biomass

The biomass of all species decreased with increasing salinity (Figures 4.24-4.27).

The mean weights of *Avicennia alba* grown at 0, 10, 20, 30 and 40 ppt for 11 months were 7.6, 17.7, 13.3, 9.1 and 4.1 g/plant, respectively. There was no significance difference in biomass between plants grown in 0 and 30 ppt salinity treatments (Table 4.16). The mean of weights of *Bruguiera gymnorrhiza* grown in 0, 10, 20, 30 and 40 ppt salinity treatments for 11 months were 79.7, 94.8, 67.5, 48.4 and 21.5 g/plant, respectively. There was a significant difference in biomass between all salinities (Table 4.17). The mean of weights of *Heritiera littoralis* grown in 0, 10, 20 and 30 ppt for 11 months were 44.4, 37.3, 12.0 and 9.1 g/plant, respectively (Table 4.18). The mean of weights of *Xylocarpus granatum* grown in 0, 10, 20, 30 and 40 ppt for 11 months were 51.9, 63.6, 40.8, 13.5 and 10.1 g/plant, respectively (Table 4.19).

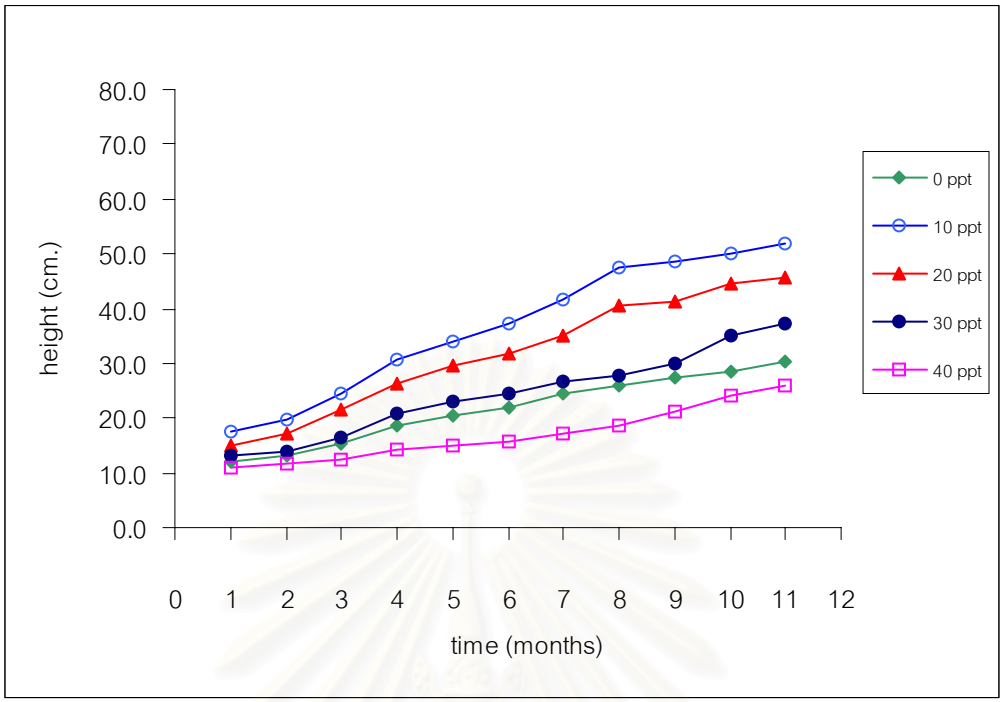

Figure 4.12 Average of height of *Avicennia alba* at different salinities.

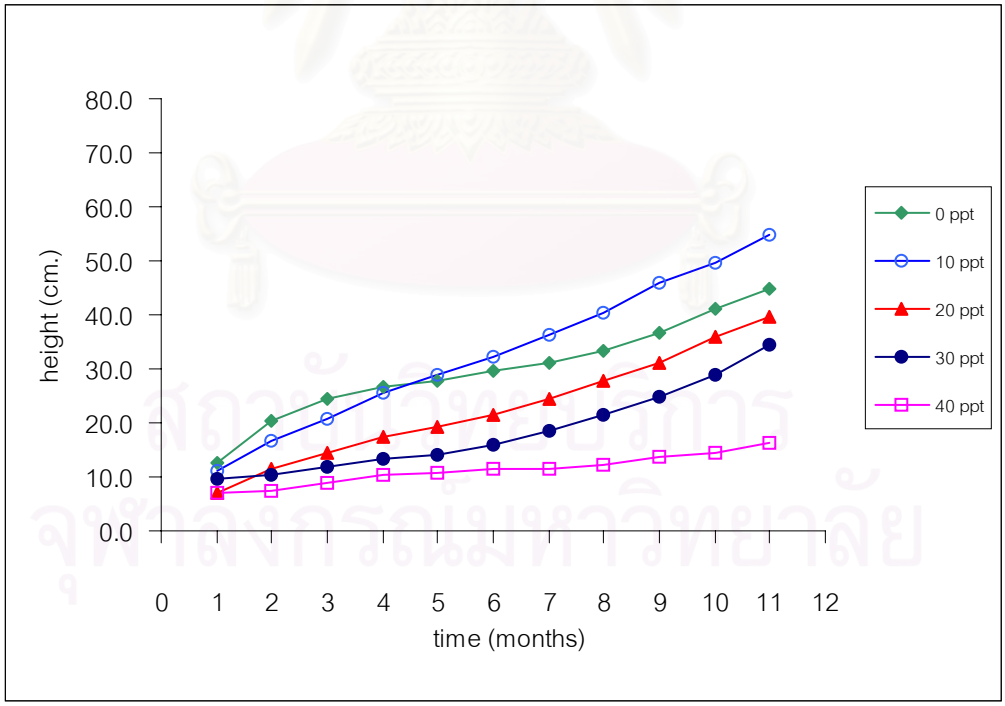

Figure 4.13 Average of height of *Bruguiera gymnorrhiza* at different salinities.

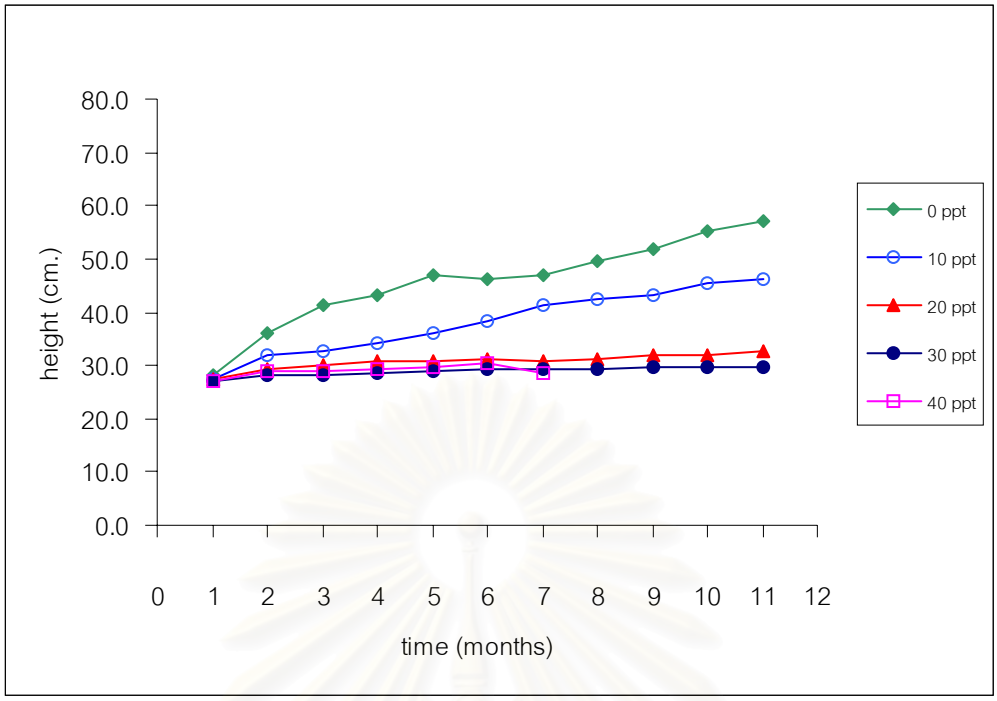

Figure 4.14 Average of height of *Heritiera littoralis* at different salinities.

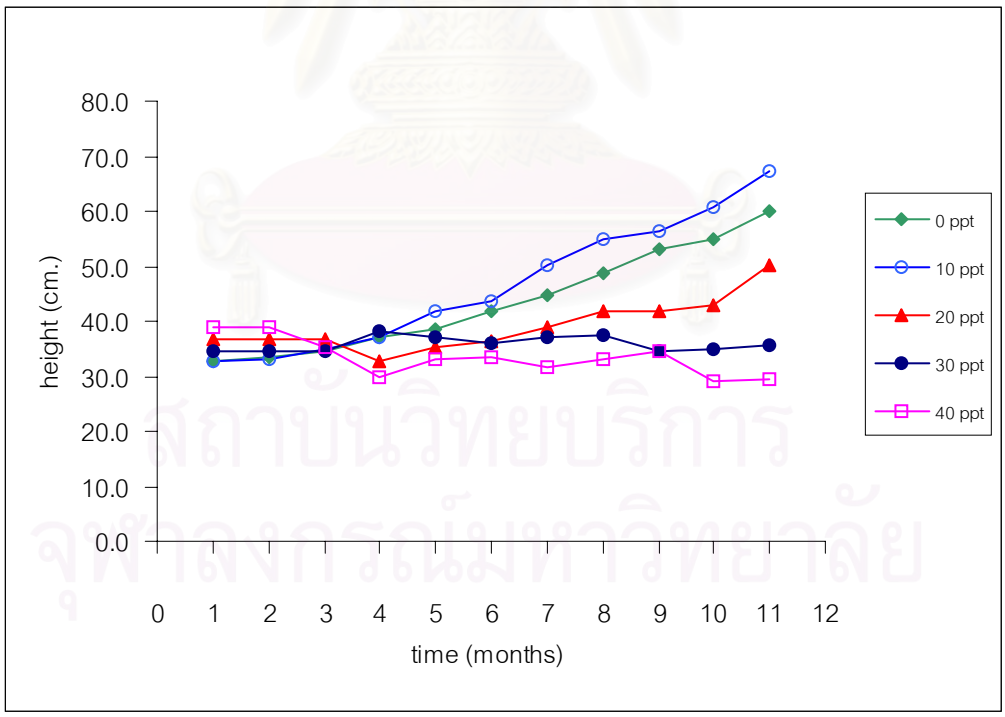

Figure 4.15 Average of height of *Xylocarpus granatum* at different salinities.

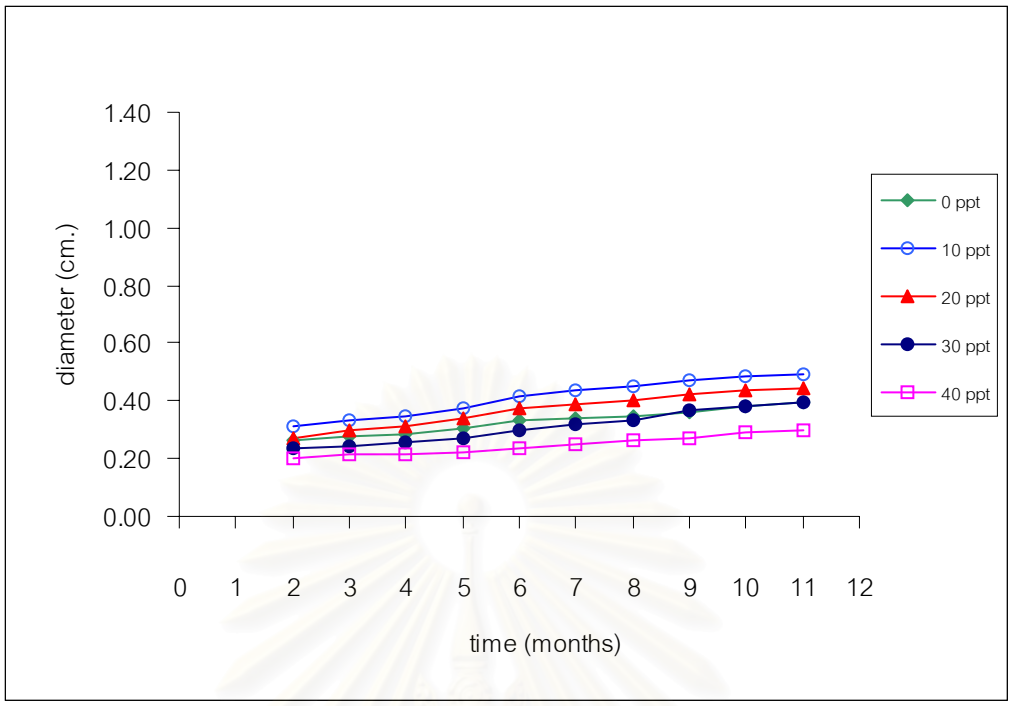

Figure 4.16 Average of diameter of *Avicennia alba* at different salinities.

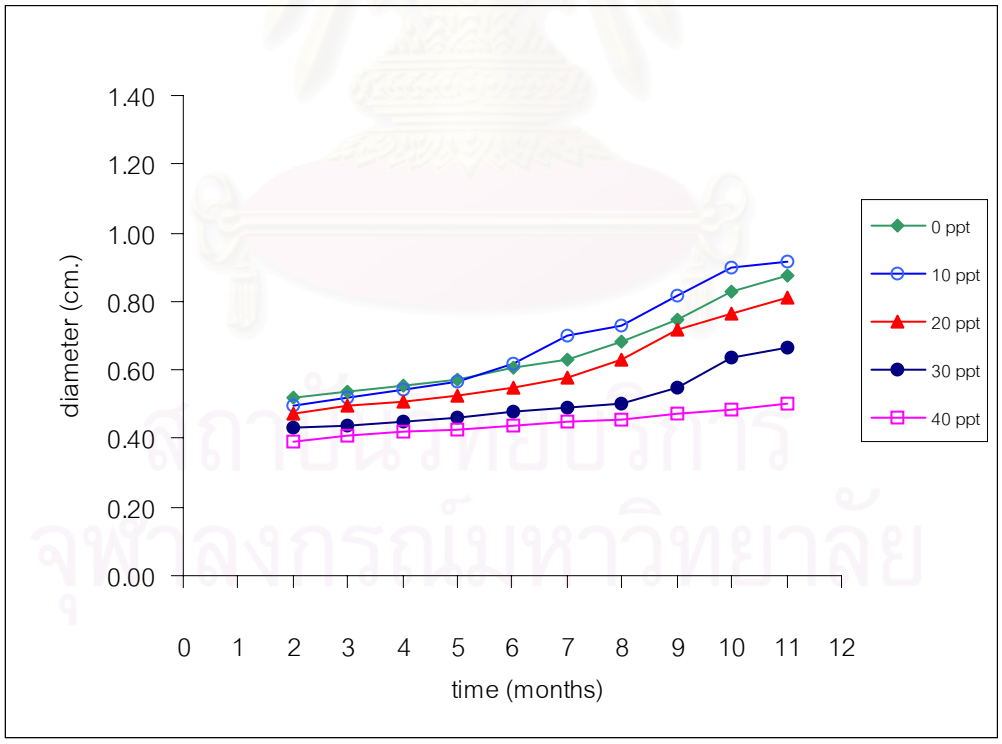

Figure 4.17 Average of diameter of *Bruguiera gymnorrhiza* at different salinities.

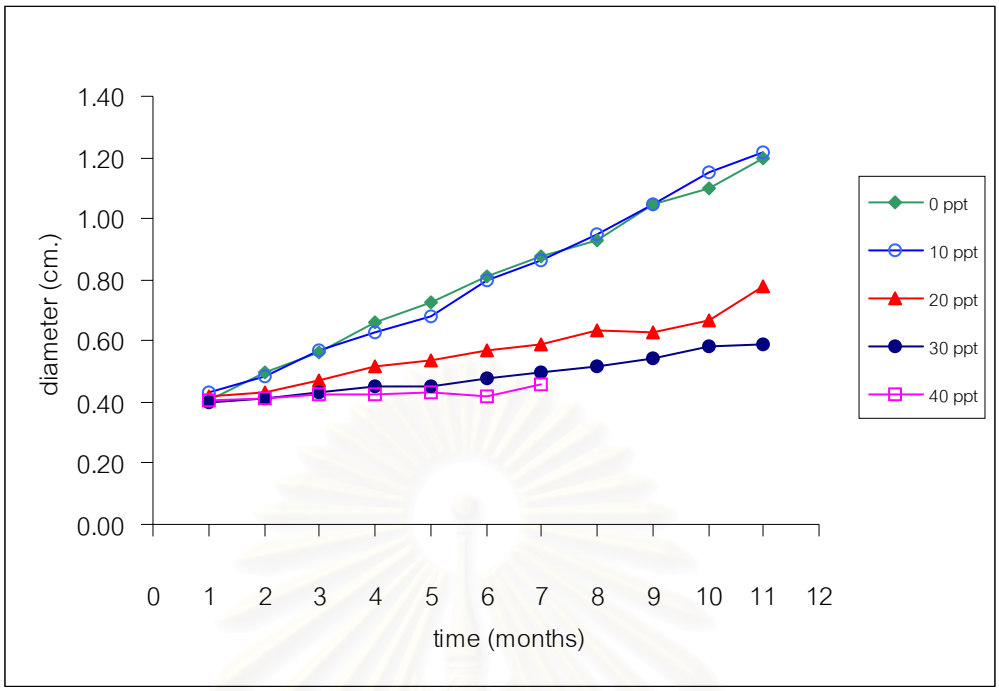

Figure 4.18 Average of diameter of *Heritiera littoralis* at different salinities.

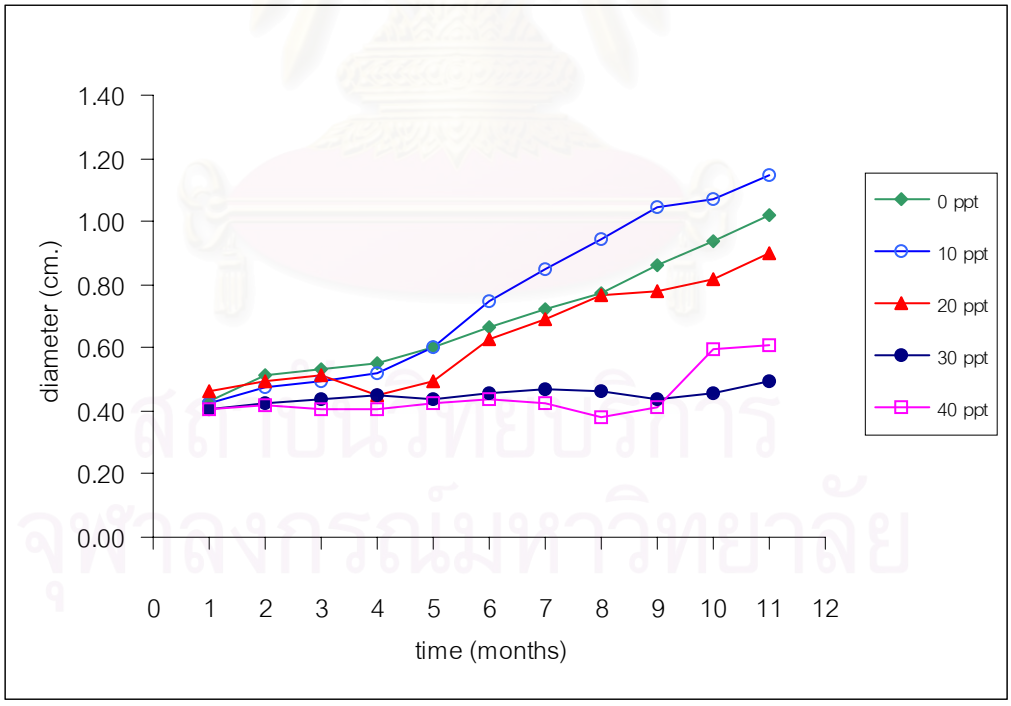

Figure 4.19 Average of diameter of *Xylocarpus granatum* at different salinities.

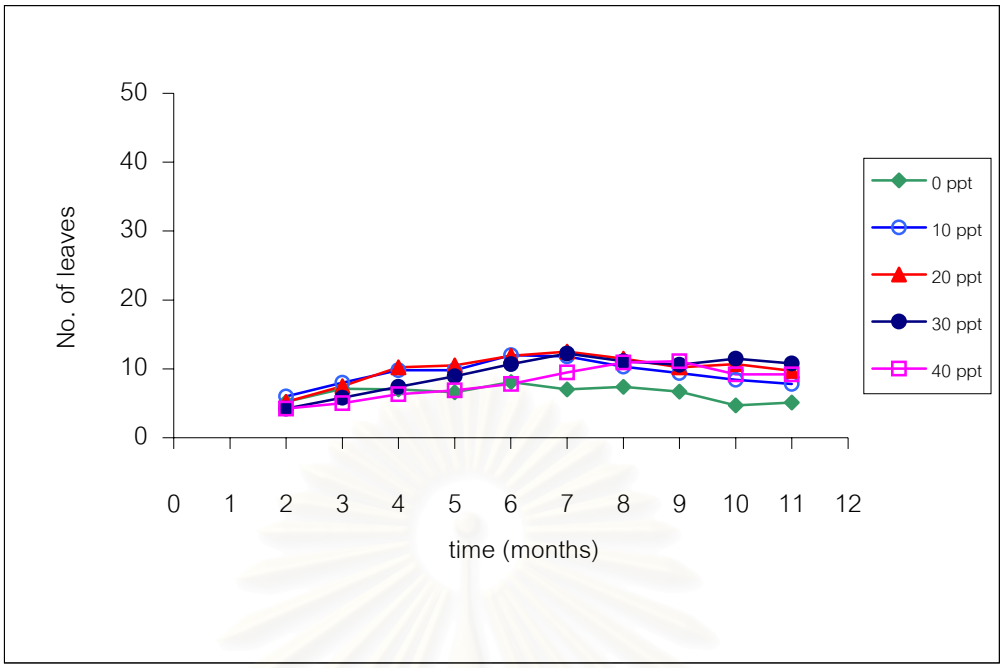

Figure 4.20 Average of number of leaves of *Avicennia alba* at different salinities.

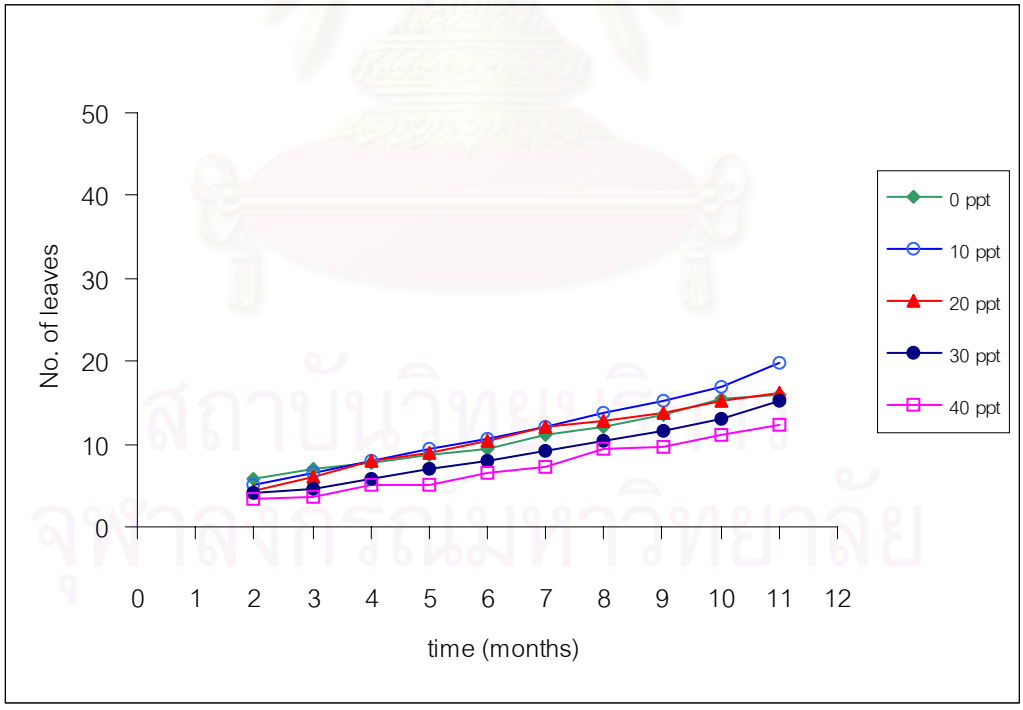

Figure 4.21 Average of number of leaves of *Bruguiera gymnorrhiza* at different salinities.

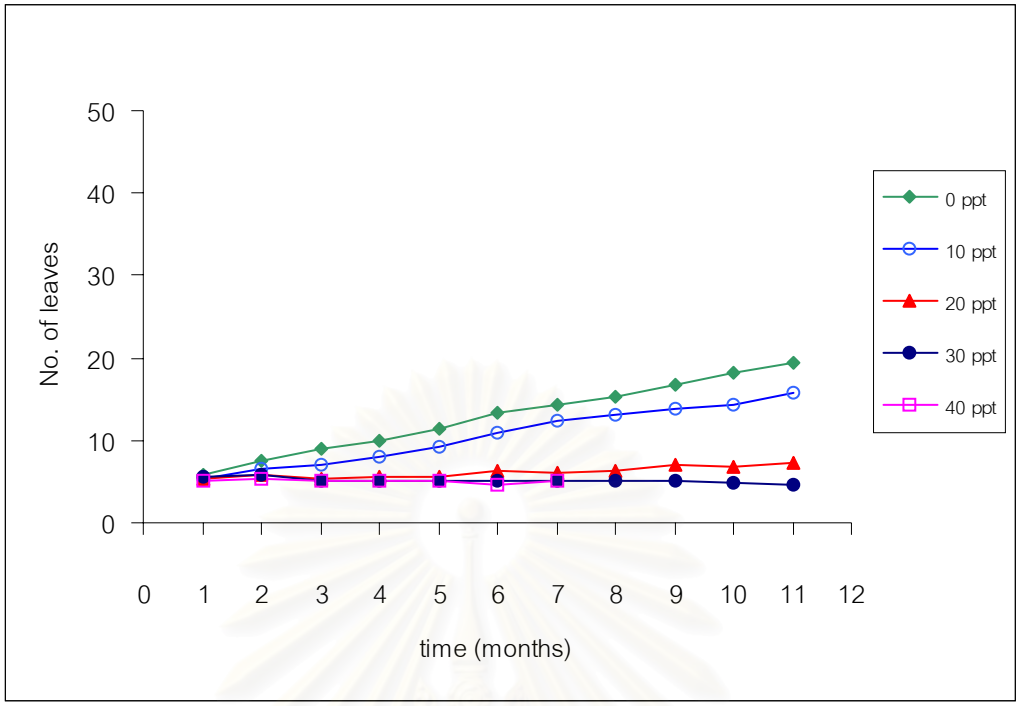

Figure 4.22 Average of number of leaves of *Heritiera littoralis* at different salinities.

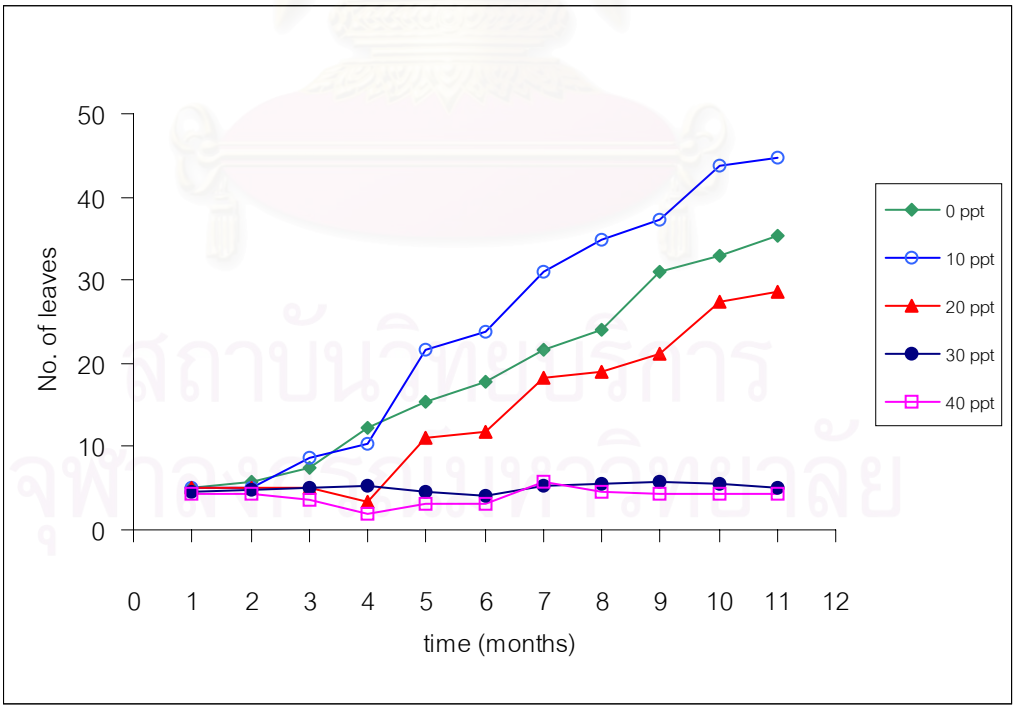

Figure 4.23 Average of number of leaves of *Xylocarpus granatum* at different salinities.

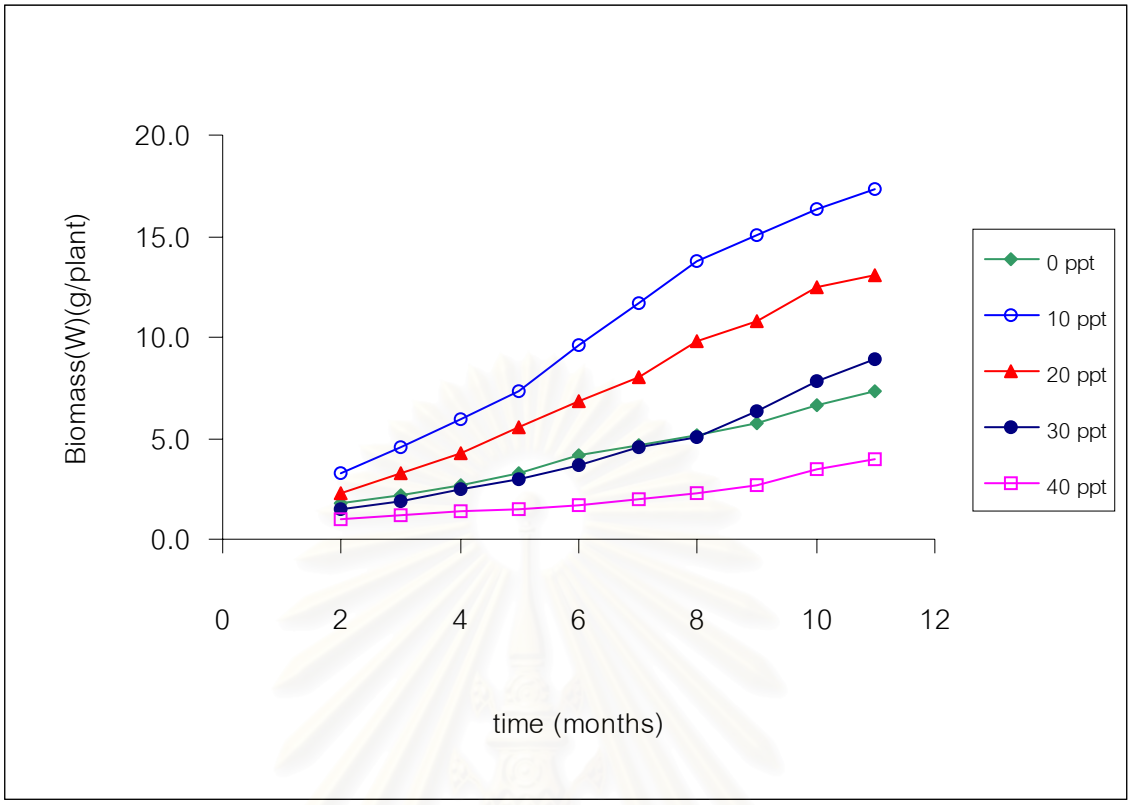

Figure 4.24 Biomass of *Avicennia alba* at different salinities.

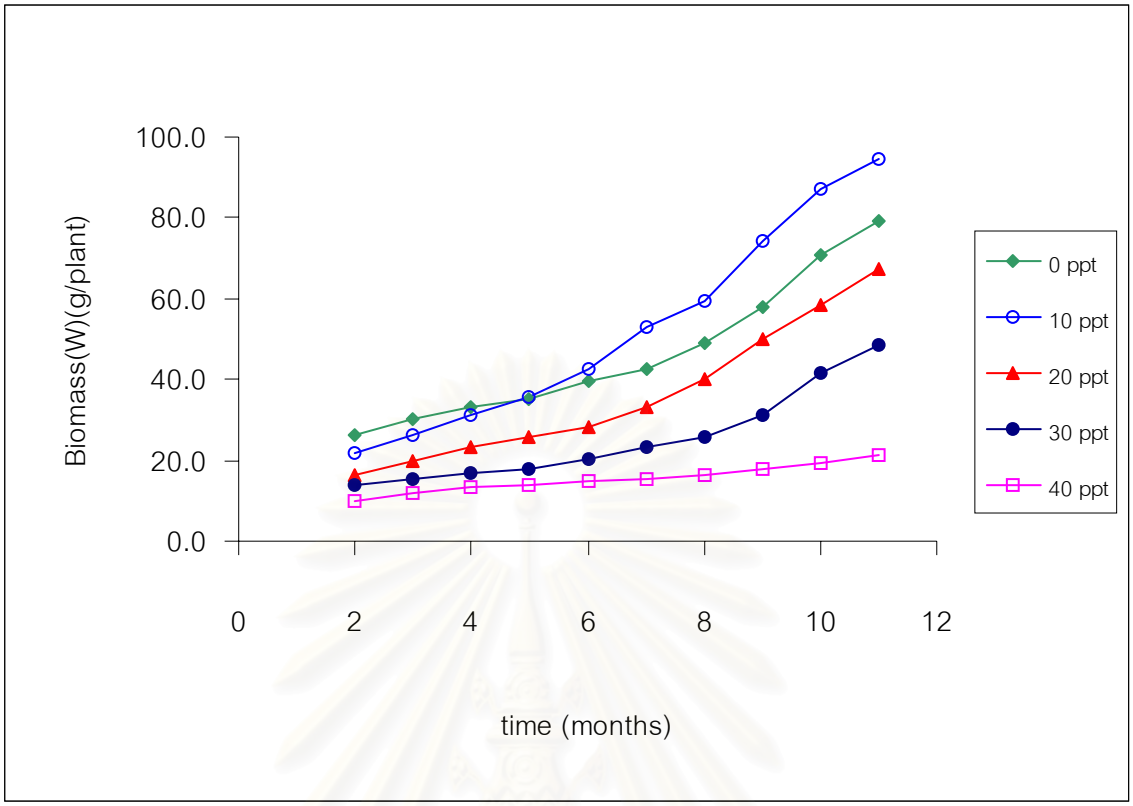

Figure 4.25 Biomass of *Bruguiera gymnorrhiza* at different salinities.

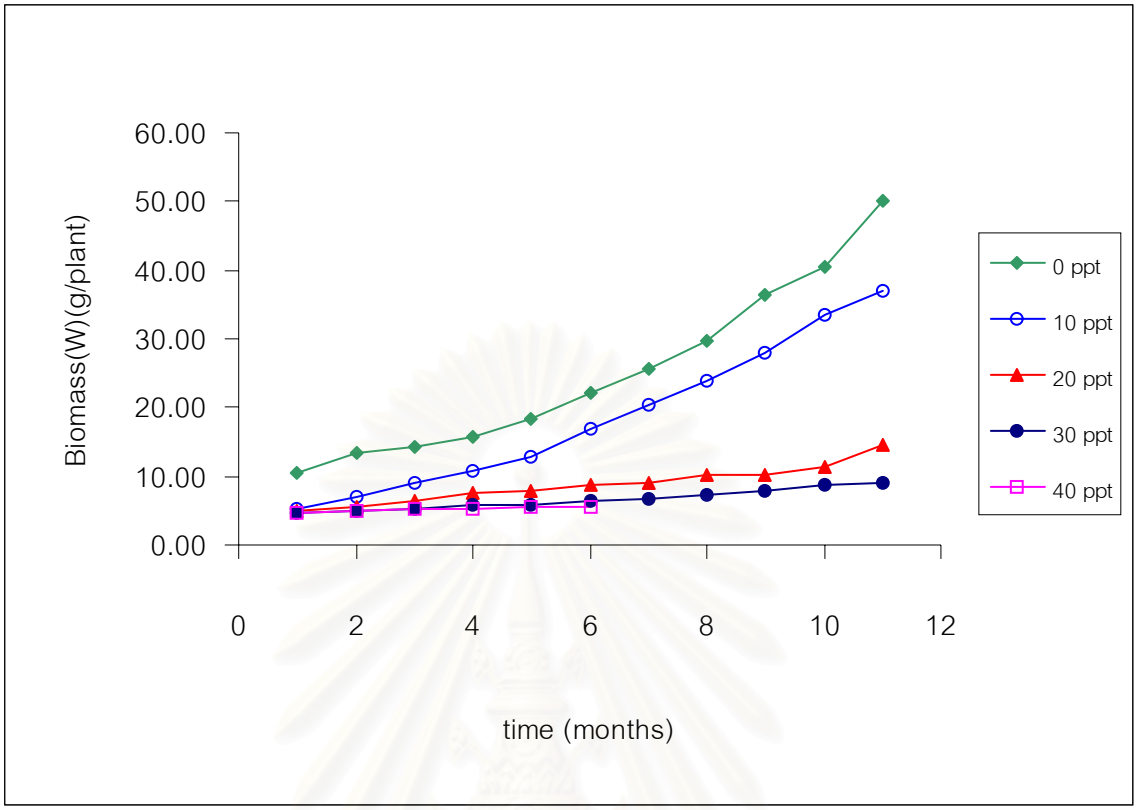

Figure 4.26 Biomass of *Heritiera littoralis* at different salinities.

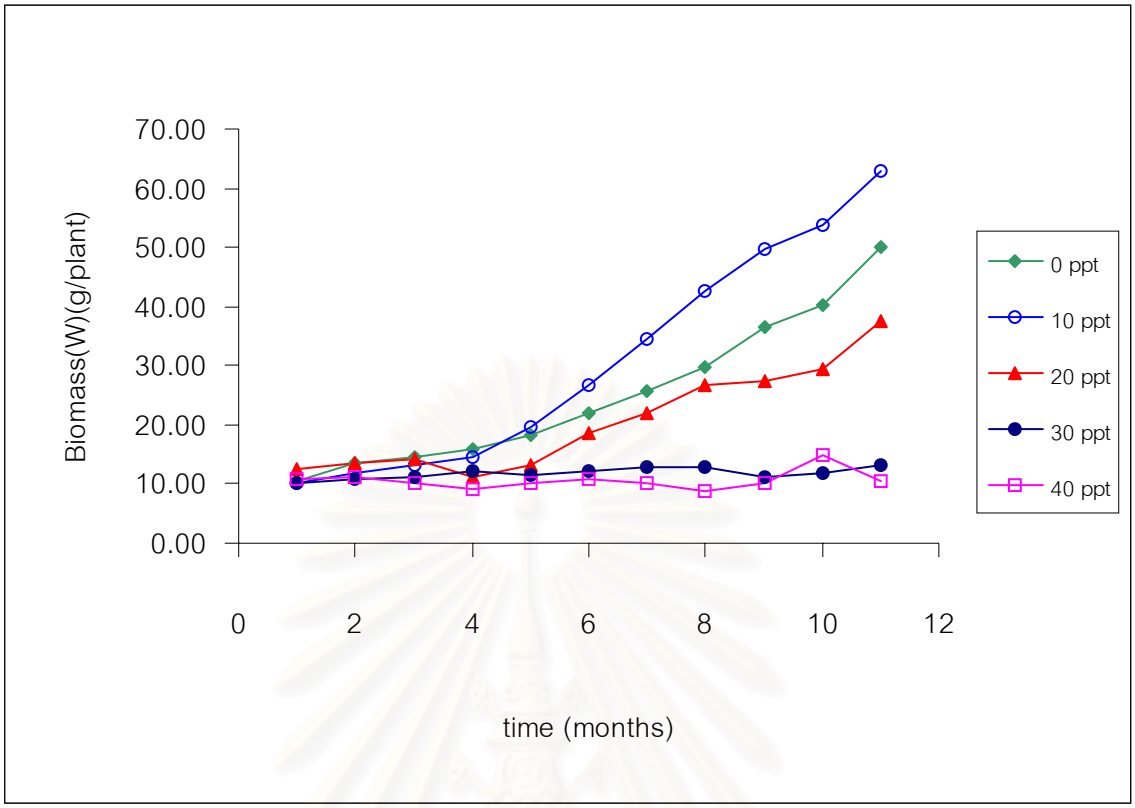

Figure 4.27 Biomass of *Xylocarpus granatum* at different salinities.

Table 4.16 Average of height, diameter and number of leaves of *Avicennia alba* at different salinities at 11 months

Salinity (ppt)	Height (cm.)	Diameter (cm.)	No. of leaves	Biomass (g/plant)
0	30.3 ± 2.00^{a}	0.39 ± 0.02^{b}	5 ± 0.48^{a}	7.6 ± 0.94^{b}
10	51.7 ± 2.36^{c}	0.49 ± 0.02^{d}	8 ± 0.90^{b}	17.7 ± 1.45^{d}
20	45.7 ± 3.33^{c}	0.45 ± 0.02^{c}	10 ± 0.52^{bc}	13.3 ± 1.37^{c}
30	37.4 ± 2.10^{b}	0.40 ± 0.02^{b}	11 ± 0.88^{c}	9.1 ± 0.96^{b}
40	26.1 ± 1.32^{a}	0.30 ± 0.01^{a}	9 ± 3.01^{bc}	4.1 ± 0.49^{a}

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.17 Average of height, diameter and number of leaves of *Bruguiera gymnorrhiza* at different salinities at 11 months

Salinity (ppt)	Height (cm)	Diameter (cm)	No. of leaves	Biomass (g/plant)
0	44.9 ± 1.95^{c}	0.87 ± 0.03^{cd}	16 ± 0.08^{b}	79.7 ± 4.62^{d}
10	54.9 ± 2.21^{d}	0.91 ± 0.02^{d}	20 ± 0.91^{c}	94.8 ± 3.81^{e}
20	39.7 ± 2.06^{bc}	0.81 ± 0.02^{c}	16 ± 0.64^{b}	67.5 ± 4.29^{c}
30	34.4 ± 0.74^{b}	0.67 ± 0.01^{b}	15 ± 0.40^{b}	48.4 ± 1.52^{b}
40	16.2 ± 0.79^{a}	0.50 ± 0.01^{a}	12 ± 0.85^{a}	21.5 ± 0.43^{a}

Means within each column followed by the same letter are not significantly different (P>0.05)

Table 4.18 Average of height, diameter and number of leaves of *Heritiera littoralis* at different salinities at 11 months

Salinity (ppt)	Height (cm.)	Diameter (cm.)	No. of leaves	Biomass (g/plant)
0	57.1 ± 3.96^{c}	1.20 ± 0.07^{b}	20 ± 2.10^{b}	44.4 ± 5.61^{b}
10	45.7 ± 2.11^{b}	1.22 ± 0.05^{b}	16 ± 0.61^{b}	37.3 ± 3.00^{b}
20	32.7 ± 1.59^{a}	0.68 ± 0.03^{a}	7 ± 0.65^{a}	12.0 ± 1.10^{a}
30	30.1 ± 1.78^{a}	0.59 ± 0.04^{a}	7 ± 0.93^{a}	9.2 ± 1.36^{a}
40	-	-	-	-

Means within each column followed by the same letter are not significantly different (P>0.05)Plant dead at salinity 40 ppt

Table 4.19 Average of height, diameter and number of leaves of *Xylocarpus granatum* at different salinities at 11 months

Salinity (ppt)	Height (cm.)	Diameter (cm.)	No. of leafves	Biomass (g/plant)
0	60.1± 4.03 ^{cd}	1.02 ± 0.09 ^{b}	35 ± 3.60 ^{bc}	51.8 ± 8.11 ^{b}
10	67.2 ± 3.08 ^{d}	1.15 ± 0.05 ^{b}	45 ± 3.28 ^{c}	63.6 ± 5.22 ^{b}
20	50.2± 7.71 ^{bc}	0.90 ± 0.10 ^{b}	29 ± 7.49 ^{b}	40.5 ± 9.78 ^{b}
30	35.6± 1.45 ^{ab}	0.50 ± 0.03 ^{a}	5 ± 0.71 ^{a}	13.5 ± 1.36 ^{a}
40	29.4 ± 6.90 ^{a}	0.43 ± 0.07 ^{a}	4 ±0.67 ^{a}	10.1 ± 3.65 ^{a}

Means within each column followed by the same letter are not significantly different (P>0.05)

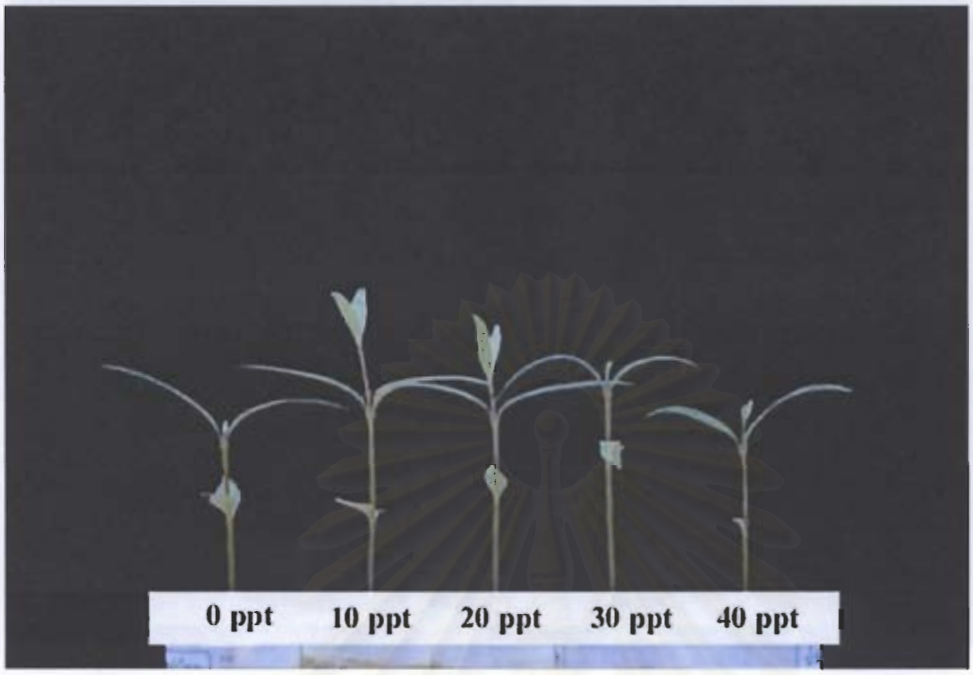

Figure 4.28 Growth of *Avicennia alba* at different salinities at 2 months.

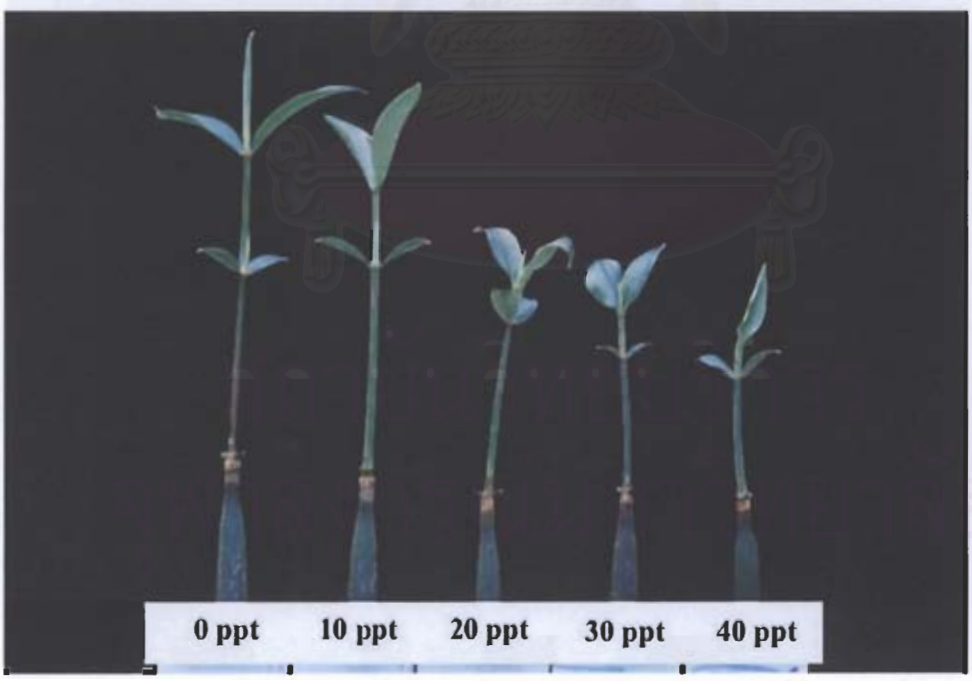

Figure 4.29 Growth of *Bruguiera gymnorrhiza* at different salinities at 2 months

Figure 4.30 Growth of *Heritiera littoralis* at different salinities at 2 months

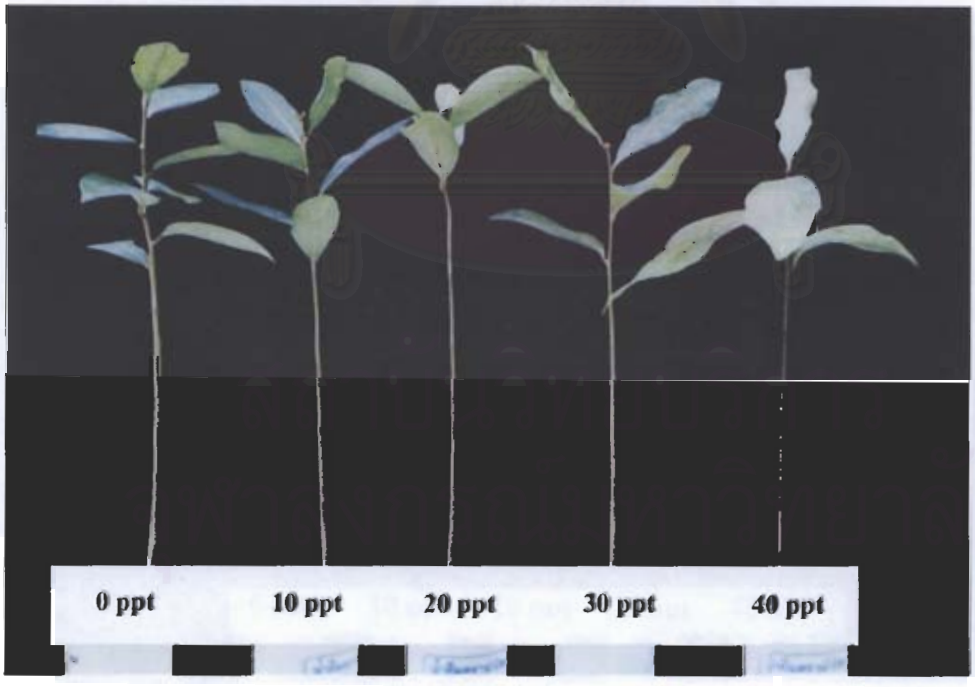

Figure 4.31 Growth of *Xylocarpus granatum* at different salinities at 2 months

Figure 4.32 Growth of *Avicennia alba* at different salinities at 4 months

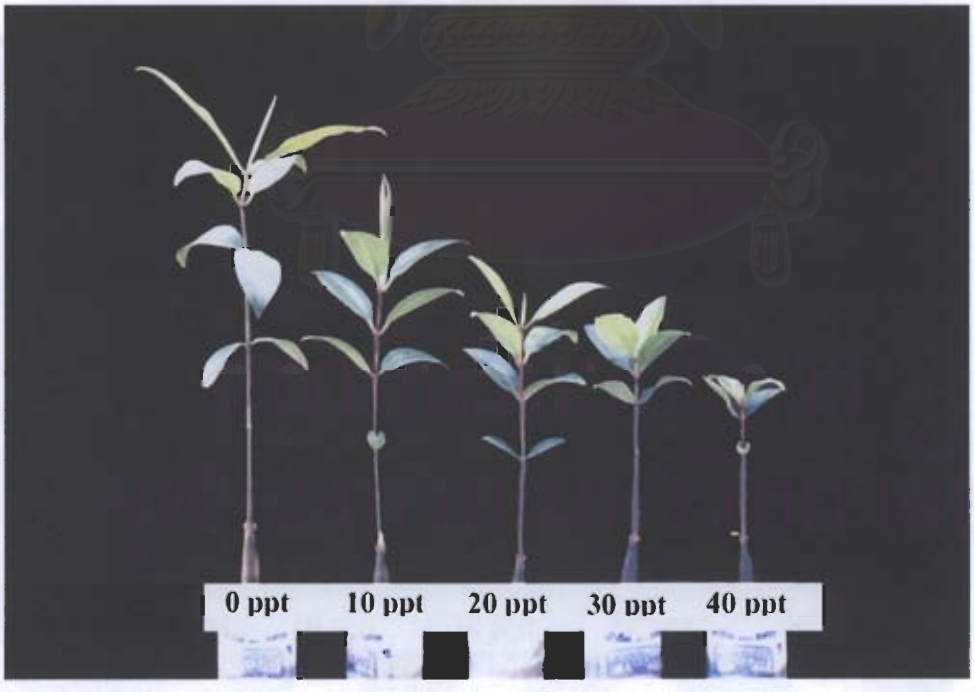

Figure 4.33 Growth of *Bruguiera gymnorrhiza* at different salinities at 4 months

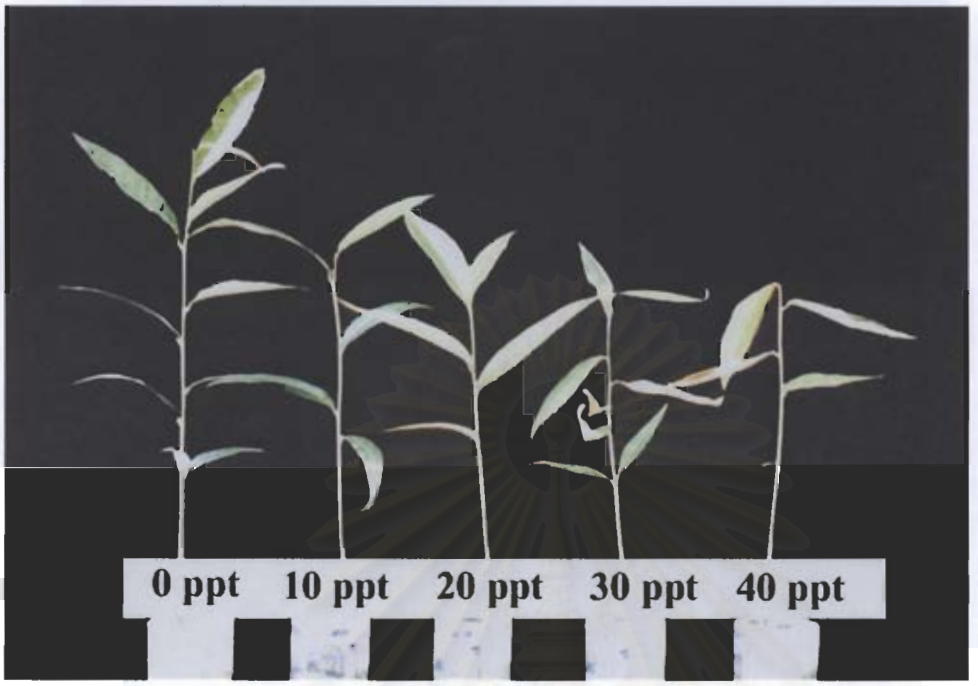

Figure 4.34 Growth of *Heritiera littoralis* at different salinities at 4 months

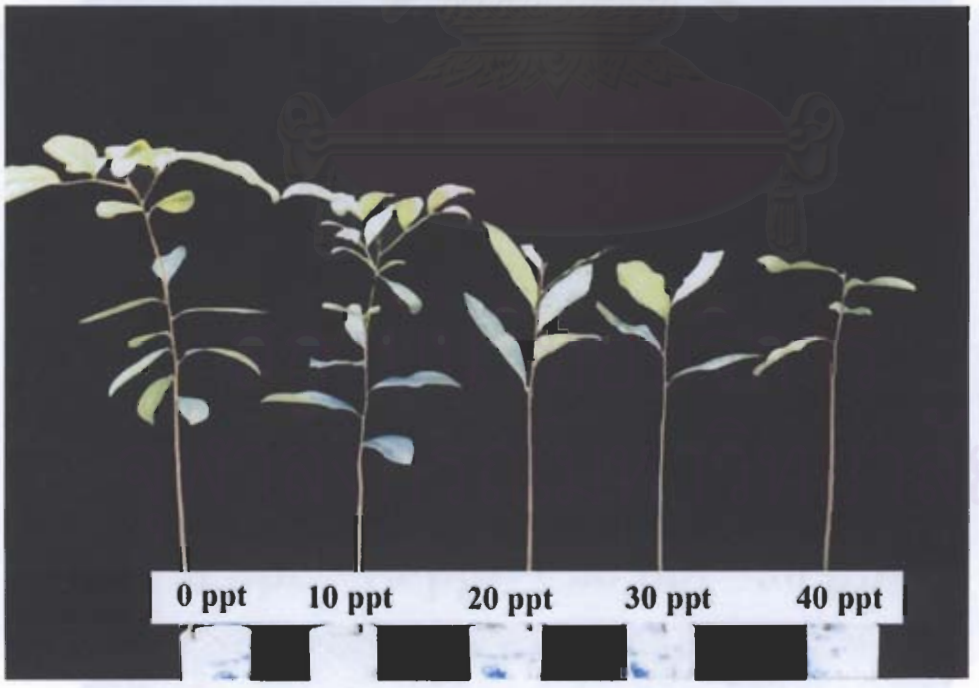

Figure 4.35 Growth of *Xylocarpus granatum* at different salinities at 4 months.

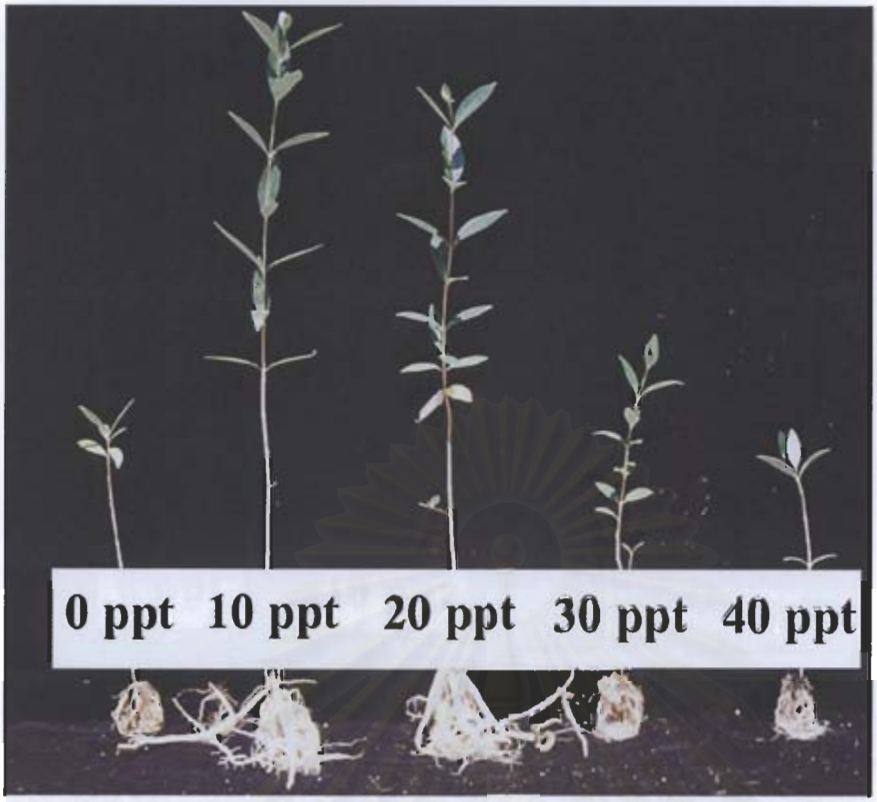

Figure 4.36 Growth of *Avicennia alba* at different salinities at 11 months

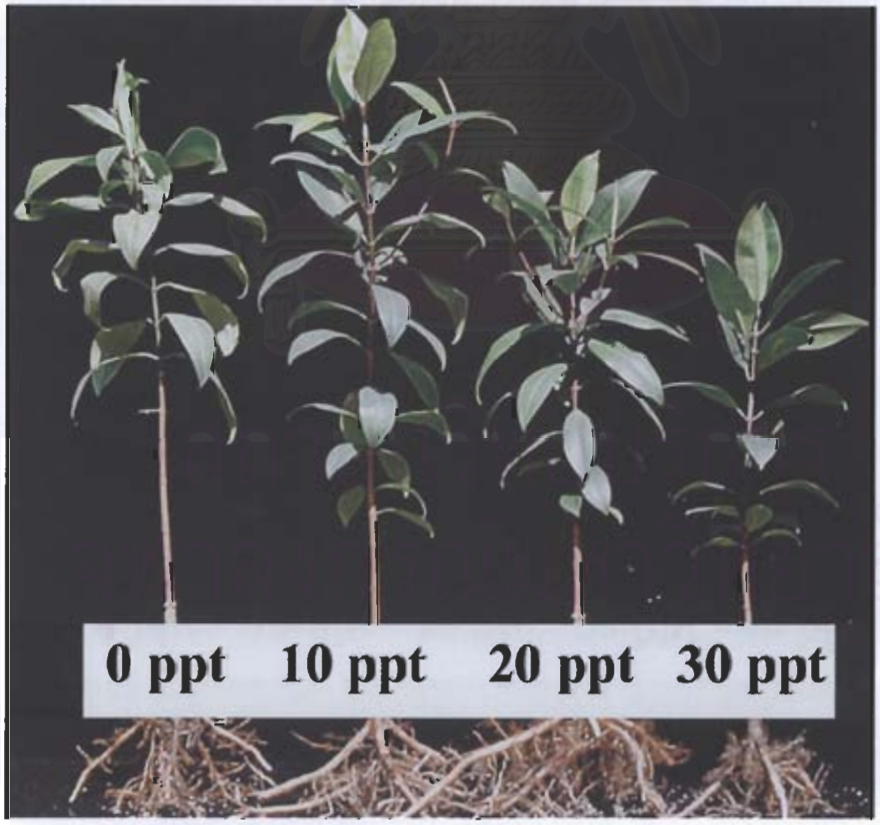

Figure 4.37 Growth of *Bruguiera gymnorrhiza* at different salinities at 11 months

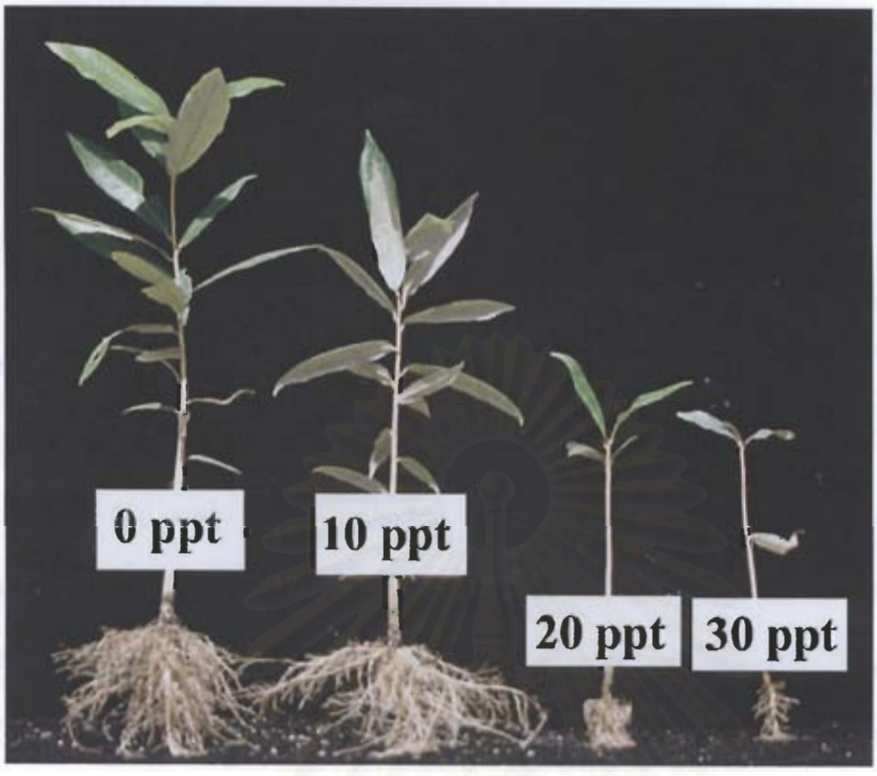

Figure 4.38 Growth of *Heritiera littoralis* at different salinities at 11 months

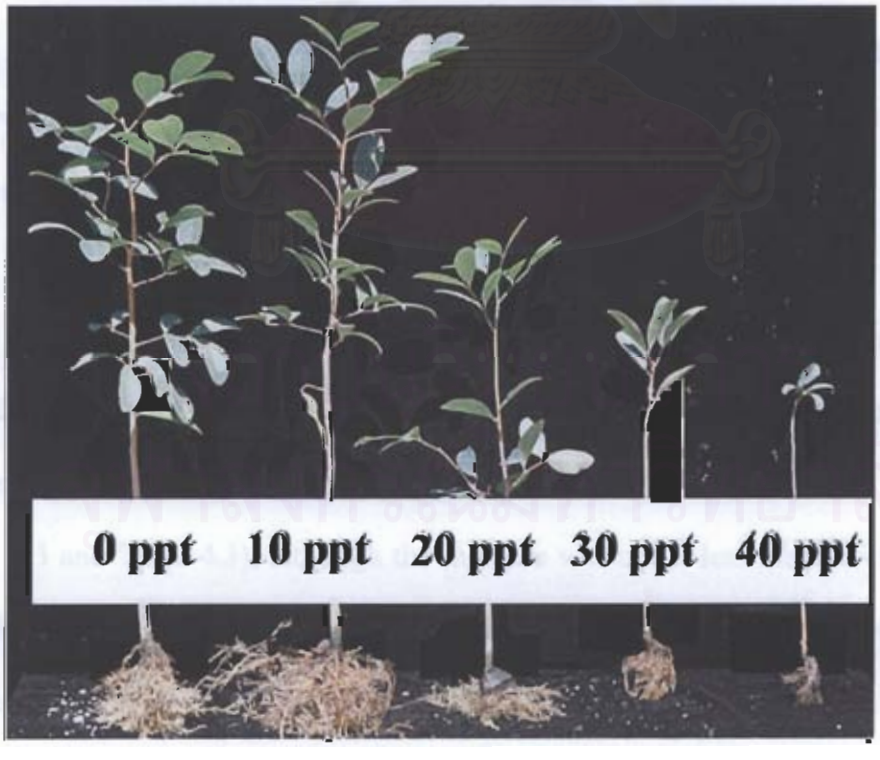

Figure 4.39 Growth of *Xylocarpus granatum* at different salinities at 11 months

CHAPTER 5

DISCUSSION

5.1 Effects of salinity on salt concentrations in xylem sap and leaves

Xylem sap could not be collected from *Heritiera littoralis* and *Xylocarpus granatum* using the pressure bomb. In both species the cotyledons are persistent at the base of the stem (epicotyl) for some time after germination. When they finally detach (or are accidentally broken off) scar tissue forms to cover the wound. It is possible that this scar tissue is leaky, thereby admitting air, or water (when the scar is submerged), making it very difficult to obtain reliable samples with young plants such as those used in this work. This is the first attempt to use this technique with *Heritiera littoralis* and *Xylocarpus granatum*, and further work is needed to resolve problems with using the pressure bomb technique with those species.

Absolute concentrations of Na and Cl in the xylem sap increased markedly with increasing salinity in *Avicennia alba* (Figure 4.1 and Table 4.1). Furthermore, the uptake ratio for both Na and Cl increased with increasing salinity (Figure 4.3 and Table 4.3), although the increase was much less marked than that shown by the absolute concentrations. Increasing uptake of NaCl in the xylem sap was accompanied by increasing NaCl concentrations in the leaves (Figures 4.3, 4.6 and Tables 4.3, 4.7). On the other hand, in *Bruguiera gymnorrhiza* xylem sap, ion concentrations increased with increasing salinity whereas the percentage uptake of

Na did not change significantly and the percentage uptake of K and Cl decreased with increasing salinity (Figures 4.3-4.4 and Tables 4.3-4.4). Ion concentrations and the percentage uptake ion of *Avicennia alba* were higher than *Bruguiera gymnorrhiza*.

The K:Na ratio, both species particularly *Avicennia alba*, had high affinities for K as shown by high K level in plant receiving low salinity (10 ppt.) When the salinity increased, the K:Na ratio and K:Na selectivity decreased (Table 4.5). They showed that K:Na ratio and K:Na selectivity particularly *Avicennia alba*, decreased due to dramatic increase Na uptake in the xylem sap.

The salt-induced increases in osmolality were accompanied by rise in Na, K and Cl concentrations in xylem sap of *Avicennia alba* (Figure 4.5 and Table 4.6). In *Bruguiera gymnorrhiza* the osmolality increased from salinity 0-10 ppt and drop at 30 ppt. This discrepancy maybe due to instrument error, because the ion concentrations of the xylem sap increased with increasing salinity. So the osmolality of xylem sap would be expected to increase with increasing salinity. The percentage uptake of osmolality in *Avicennia alba* was increased with increasing salinity (Table 4.6) where as the percentage uptake of osmolality in *Bruguiera gymnorrhiza* decreased with increasing salinity. Pardossi et al. (1998) studied *Apium graveolens* grown at 5, 50, 100 and 300 mM NaCl solution also found that osmolality increased with increasing salinity.

These results indicate that in both species, Na and Cl increased with increasing salinity and ion uptake by *Avicennia alba* was much greater than by

Bruguiera gymnorrhiza. In other words, *Bruguiera gymnorrhiza* was more efficient in excluding Na and Cl. The salt concentration in xylem is proportional to the salinity of water around the roots in *Avicennia alba* where as this was not clear for *Bruguiera gymnorrhiza*.

In all species Cl concentrations in leaves at salinity 0 ppt were higher than expected, possibly due to the tapwater being used to make up the 0 ppt salinity treatment. In all species Na and Cl concentrations in leaves tend to increase with increasing salinity. In *Heritiera littoralis* the values were lower when compared with other species. *Heritiera littoralis* had lower Na and Cl concentrations were associated with both habitat and physiological features. *Heritiera littoralis* is regarded as brackish water or a mangal associate (Chapman, 1976). Popp (1984a) studied young and old leaves from 22 mangrove species of Northern Queensland (Australia) were found that Na and Cl concentrations of *Heritiera littoralis* were lower when compared with other species. The ion concentrations of shoots are in part a function of root metabolism, particularly in a species lacking a leaf salt extrusion mechanism (Pitman, 1976). These results were agreed with other studies. Clough (1984) studied *Avicennia marina* and *Rhizophora stylosa* grown in nutrient solutions containing 0, 25, 50, 75 and 100% seawater also found that Na and Cl concentrations in leaves, stem and root increased with increasing salinity. The concentrations of Na and Cl in *Avicennia marina* were higher than *Rhizophora stylosa.* Downton (1982) studied *Avicennia marina* grown at different salinities for 11 months. Plants on 0-100% seawater treatments behaves as typical halophytes accumulating increasing levels of Na and Cl as external salinity increased, even through the leave can regulate steady-state ion concentrations by mean of salt

glands. Suarez et al. (1998) studied on *Avicennia germinans* L. seedlings growing at different salinities in field. This study showed that ion concentrations were higher in the high-salinity site.

The most important ions as far as the osmotic adjustment of halophyte are concerned with Na, K and Cl (Flowers and Yeo, 1986). The water salinity had little effect on the concentrations of Mg and Ca in all species (Table 4.7-4.10) which was the same result from *Avicennia marina* and *Rhizophora stylosa* grown in nutrient solutions containing 0, 25, 50, 75 and 100% seawater (Clough, 1984). But this study is different from Khan et al. (2000) who studied the succulent species, *Sueda fruticisa* grown in saline conditions (200-400 mol m^{-3}). They found that Ca Mg and K concentrations in leaves decreased with increasing salinity.

Bruguiera gymnorrhiza, Heritiera littoralis and *Xylocarpus granatum* are all considered to be salt-excluding species (Scholander et al., 1962) which keep the salt content of their xylem sap by ultrafiltration in the roots. Since little work has been done on mangrove roots, it is not known exactly, where this ultrafiltration system located. Ido (2000) studied *Avicennia marina* and *Rhizophora apiculata* found that Na concentration distribution in root of genus *Avicennia* increased stem-ward from root tip, while that of genus *Rhizophora* decreased hypocotyl-ward from root tip. He suggested that selective saline absorption depends on not endodermis of radial-ward but cell membrane of axial-ward. Moon et al. (1986) examined the pathway and mechanisms for salt uptake by mangrove (*Avicennia marina*). They found that the main barrier to salt uptake was located in the hypodermis (at the outer surface of the root) rather than at an endodermis as in most in non-mangrove

plant species. So *Avicennia alba* may be like *Avicennia marina* in the same way to uptake Na and Cl ions. Therefore, Na and Cl concentration in xylem sap is higher than *Bruguiera gymnorrhiza* that is maybe like *Rhizophora apiculata.*

However, final concentrations in the leaves of *Avicennia alba, Bruguiera gymnorrhiza, Heritiera littoralis* and *Xylocarpus granatum* are similar (Table 4.7-4.10). Moreover, it cannot be said that in each species there is only one mechanism responsible for regulation of ion content in the leaves (Popp, 1984a).

Both glycophytes and halophytes have been found to accumulate a number of different solutes in the cytoplasm when they experience saline conditions. Accumulation of cytoplasmically compatible solutes cannot be the only method of osmotic adjustment for plants in long-term hypersaline conditions because much of the total plant photosynthate would be required for osmotic adjustment (Wyn-Jones, Gorham and McDonnell, 1984). Therefore, both inorganic ions (Na, K, Ca) and organic solutes are involved in osmotic adjustment of halophytes. Also, in any one species, several different organic solutes may accumulate in response to salinity (Orcutt and Nilsen, 2000).

Ion concentrations in leaves of *Heritiera littoralis* are low when compared with other species but the value of osmotic potential at full turgor is close to that of other species (Table 4.9). This suggests that there is accumulation of compatible organic solutes in *Heritiera littoralis* to generate an internal osmotic potential. Popp (1984) found that in *Heritiera littoralis* leaves the cytoplasm contained a high

citrate content, and small low molecular weight carbohydrates (LMWC) and proline concentrations.

The reduced growth of *Avicennia alba, Bruguiera gymnorrhiza, Xylocarpus granatum* and *Heritiera littoralis* under high salinity are associated with Na and Cl ions in leaves by increasing solute concentration (Na and Cl), decreasing osmotic potential and cell elasticity. All processes of plant allow leaf uptake water and turgor maintenance through a large range of soil water potential.

This may explain the zonation of *Avicennia alba, Bruguiera gymnorrhiza, Heritiera littoralis* and *Xylocarpus granatum* in terms of survival and different distribution under natural conditions.

The high concentrations of Na and Cl in the xylem sap and the salt secreting glands in the leaves of *Avicennia alba* suggest the salt balance of the shoot is maintained mainly by salt secretion from the leaves. By contrast, salt balance in the shoot of *Bruguiera gymnorrhiza* appears to be regulated mainly by the low concentrations of Na and Cl.

5.2 Effects of salinity on shoot water potential and water potential components

Water potential of shoots at the first time of the balancing pressure from pressure bomb and osmotic potential at full turgor of all species decreased with increasing salinity (Tables 4.11-4.14). These results were consistent with Khan et al. (2000). They studied *Suaeda fruticisa* plant grown in saline conditions (200-400 mol m^{-3}) found that water potential and osmotic potential of plant became more negative with an increase in salinity.

It is evident from shoot water potentials and other parameters (Tables 4.11-4.14), that *Avicennia alba, Bruguiera gymnorrhiza, Heritiera littoralis* and *Xylocarpus granatum* had osmotically adjusted to the salinity imposed on them. Adaptative decrease in plant osmotic potential for maintaining turgor in response to salinity have been widely reported (Flower et al., 1977; Rada et al., 1989; Suarez et al., 1998). This phenomenon was well illustrated by the decrease in osmotic potential at full turgor with increasing salinity (Tables 4.11-4.14). The values of osmotic potential reported in this study are within the range of values reported for studies on mangroves subject to salinity, like *Avicennia germinans* (Suarez et al., 1998). Under salinity conditions the osmotic potential was adjusted as a result of solute accumulation in leaf sap. Concentration of solutes in shoot of all species increased with increasing salinity, while the symplastic water fraction remained constant (Tables 4.11-4.14). In agreement with other reports, *Avicennia germinans* decreased osmotic potential under high salinity conditions the symplastic water fraction remained constant (Suarez et al., 1998). This contrast with result reported for *Rhizophora mangle,* where osmotic potential under high salinity conditions

decreases as a result of a reduction in the symplastic water fraction (Rada et al., 1989). However, all mangrove species accumulate solutes in their vacuole under salt stress. This process increases the capability for water uptake and maintained turgor when soil water content and water potential in soil decrease (Morgan, 1984; Clough et al., 1982).

The difference between osmotic potential at full turgor and at zero turgor reflects the ability of species to continue extracting water from saline soils while maintaining turgor (Meinzer et al., 1983). Its average value of 0.57, 0.50, 0.99 and 0.51 MPa for *Avicennia alba, Bruguiera gymnorrhiza, Heritiera littoralis* and *Xylocarpus granatum,* respectively studied here in are the range with an average values of 0.8 MPa for several drought-hardy European species (Meinzer et al., 1983) and 0.5 MPa for several temperate deciduous forest species (Meinzer et al., 1983) but contrast with an average values of 0.3 MPa for several tropical savanna woody species (Meinzer et al., 1983). The value tended to increase at high salinity (40 ppt).

The relative water content at zero turgor is a measure of the ability to maintain turgor in the presence of increasing leaf water deficits (Meinzer et al., 1983). These workers reported relative water contents at zero turgor of 0.80, 0.79 and 0.85 for drought-hardy, temperate deciduous forest and tropical savanna woody species, respectively. In the present study, the relative water content at zero turgor of all species were nearly constant with increasing salinity, with values for *Avicennia alba, Bruguiera gymnorrhiza, Heritiera littoralis* and *Xylocarpus granatum* of 0.85-0.92, 0.86-0.90, 0.76-0.86 and 0.90-0.92, respectively. This

suggests that there was no morphological or anatomical adaptation to increasing salinity, notwithstanding the increase in modulus of elasticity with increasing salinity.

There were a number of reports of an increase in the modulus of elasticity in response to increasing salinity in mangroves (Rada et al., 1989) and in other halophytes (Bolanos and Longstreth, 1984; Nabil and Coudret, 1995). In the present study, the bulk modulus of elasticity also increased with increasing salinity. The modulus of elasticity is influenced mainly by two factors, the rigidity and strength of cells walls, and the turgor potential at full turgor. The latter, in turn, is influenced by the osmotic potential at full turgor. In this work, the bulk modulus of elasticity was calculated from the change of turgor potential divided by the change in relative water content, $\Delta TP/\Delta\theta$, over the full range of turgor from zero to full turgor (Tyree, 1981). However, it is important to note that the relationship between turgor potential and relative water content is not linear, and the elastic properties of cell walls are such that turgor changes progressively more than relative water content as the cell approaches full turgor (Tables 4.11-4.14). Consequently, the modulus of elasticity also increases progressively as the shoot moves from zero to full turgor. The increase in modulus of elasticity with increasing salinity in all species is attributable mainly to the corresponding increase in osmotic potential which results from greater accumulation of solutes (presumably mainly NaCl) at higher salinities. If there had been a change in the elastic properties of the cell walls with increasing salinity, then this should have been reflected in a change in the symplastic relative water content at zero turgor. However, there was no

evidence a significant change in the symplastic relative water content at zero turgor in plants grown at different salinities.

5.3 Effects of salinity on growth

The germination rates of *Avicennia alba*, *Bruguiera gymnorrhiza* and *Heritiera littoralis* were high but germination rate of *Xylocarpus granatum* was not high due to the method of cultivation. The water level was maintained for a week and drained once a week, so the *Xylocarpus granatum* seeds were in water for a week and found that they produced tannin.

Survival was much reduced in all species after 3 months at higher salinities, with *Avicennia alba* having significantly better survival than other species at salinities above 30 ppt. At 40 ppt, the sequence from highest to lowest survival was, in order, *Avicennia alba*, *Bruguiera gymnorrhiza*, *Heritiera littoralis* and *Xylocarpus granatum*. This is consistent with the generally observed zonation pattern of these species with respect to salinity.

Overall, stem height and diameter, and plant biomass decreased in all species with increasing salinity, consistent with other reports of the effect of salinity on the growth of mangroves (e.g. Downton, 1982; Clough, 1984). In addition, there was a reduction in leaf number. This reduced leaf number could be the result from excess Na and Cl ions inducing death of expanded leaves. Our result was consistent with those reported by Pardossi et al. (1998) for salt-treated

celery plant in hydroculture and Nabil and Coudret (1995) for salt-treated *Acacia nilotica*.

Avicennia alba grew much better at 10 and 20 ppt than at other salinities. This response suggested that *Avicennia alba*, like *Avicennia officinalis* (Teas, 1979) and *Avicennia marina* (Downton, 1982; Clough, 1984) is an obligate halophyte and requires sodium chloride for successful growth in the long run. Plants grown at the highest level of salinity (40 ppt), a concentration higher than that of seawater (30 ppt), looked healthy in appearance and tolerant of the conditions. *Avicennia alba* grown in 0 ppt (control) developed chlorotic and necrotic lesions on many leaves several months after establishment and there was also tendency for shoot apices to blacken, as also noted by Downton (1982) and Clough (1984) in *Avicennia marina*. It is well known the growth of many halophytes is depressed in the absence of NaCl in the culture medium (Jennings 1976; Flower et al., 1977). This response is often ascribed to the inability of halophytes to accumulate sufficient inorganic ions for osmoregulation when NaCl is not present in the substrate (Jennings, 1976; Flower et al., 1977).

In *Bruguiera gymnorrhiza*, and *Xylocarpus granatum* grown in the 10 ppt salinity treatment grew much better. In *Heritiera littoralis* grown in the 0 ppt salinity treatment grew better than plant grown in the 10 ppt salinity treatment but there was no significant difference among these salinity. These responses suggested that *Bruguiera gymnorrhiza*, *Heritiera littoralis* and *Xylocarpus granatum*, like other halophytes, required sodium chloride for growth. These results were consistent with the study from Ball and Pidsley (1995), who found that

Sonneratia alba grew well in 5-50% seawater while *Sonneratia lanceolata* grew well only in 0-5% seawater.

From our results all species tended to decrease the growth with increasing salinity and died at high salinity (60 ppt). Because under saline condition plant growth is limited by both water (osmotic) stress and salt toxicity (Munn and Termaat, 1986). The salt outside the roots reduced the availability of water to the plant. So it is difficult for the plant to take up the water. In plants exposed to high salinities, salts taken up by the plant may accumulate to toxic level in the older leaves. Finally, they show premature senescence and start to die (Pardossi et al., 1998).

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

Conclusion

1. *Avicennia alba* and *Bruguiera gymnorrhiza* differ in their efficiency to exclude salt.
2. The salt concentration in xylem of *Avicennia alba* is proportional to the salinity of water around the roots.
3. Large changes in shoot water potential of *Avicennia alba, Bruguiera gymnorrhiza, Heritiera littoralis* and *Xylocarpus granatum* are accompanied by only a small change in tissue water content.
4. Growth of *Avicennia alba, Bruguiera gymnorrhiza, Heritiera littoralis* and *Xylocarpus granatum* decrease with increasing salinity.

Recommendations

1. It would be useful to study on organic concentrations in leaves such as proline and glycinebetaine to clearly understand the osmoregulation.
2. A more detailed investigation of ion concentrations in roots, stems and leaves is needed to compare the ion concentrations in each part.
3. These results can be used to select suitable species for rehabilitation. *Avicennia alba* is suitable for planting at higher salinities, *Bruguiera gymnorrhiza* and *Xylocarpus granatum* are suitable for planting at 0 and 10 ppt salinity, and

Heritiera littoralis is suitable for planting at 0 ppt salinity. These studies should be expanded to other species such as *Avicennia marina, Avicennia officinalis* or *Rhozophora apiculata* and try to compare on each species.

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APPENDICES

APPENDIX A

Term Definitions

Apoplast Cell walls and other spaces not completely surrounded by cell membranes. Water in the apoplast is called 'Apoplastic' water.

Full turgor The Turgor potential (a positive value) equals the osmotic potential (a negative value), then the water potential is zero. The cells cannot take up more water because further expansion is prevented by the rigid cell walls.

Osmotic potential The chemical potential of water in a solution. It has the units of MPa. The osmotic potential (or chemical potential) of pure water at normal pressure and temperature is 0. Any salts or other osmotically active substances will reduce the activity of water in solution and thus lower the osmotic potential, giving a negative number. Therefore osmotic potentials are usually negative in value.

Relative water content A number between 0 (no water) and 1 (when the tissue is fully turgid and water potential = 0).

Symplast The continuum of cells and plasmodesmata that is bounded by a differentially permeable cell membrane. This includes the protoplast and organelles inside it, and the plasmodesmata that connect cells. All of the water inside the symplasm is called 'Symplastic' water.

Turgor potential The physical pressure exerted by cell walls or other physical boundaries that stop cells and other organs containing aqueous solutions from expanding indefinitely. It has units of MPa, and is positive in value.

Water potential The chemical potential of water in a solution, plant tissues and soil. It has the units of MPa.

Zero turgor The point of incipient plasmolysis, when the tissue has just lost all turgor (Turgor potential = 0) and the cells begin to behave as perfect osmometers.

APPENDIX B

1. Digestion

1.1 *Dry ashing method for analysis chloride in leaf*

1.1.1 Weigh 0.2-0.5 g of dry ground sample into an acid-washed procelain basin.

1.1.2 Add 1 ml CaO (30g/l) and swirl gently.

1.1.3 Ignite at 550 °C for 90 minutes in a muffle furnace.

1.1.4 When cool dilute with warm water 10 ml and heat on stream bath for 30 minutes.

1.1.5 Filter through a filter paper into a 50 ml volumetric flask and dilute to volume.

1.1.6 Final solution is colorless and to be analized chloride.

1.2 *Wet ashing method for analysis sodium, potassium, magnesium and calcium in leaf.*

1.2.1 Weigh 0.2-0.5 g of dry ground sample into a 50 ml Kjeldahl flask.

1.2.2 Add 10 ml HNO_3, swirl gently and digest slowly at moderate heat (50 °C) for 1 hour.

1.2.3 Increase the heat at 130 °C and digest for 90 minutes until brown fumes disappear.

1.2.4 Set aside to cool and add 4.5 ml $HClO_4$.

1.2.5 Digest at 190 °C until white fumes disappear (2 hours).

1.2.6 Filter through a filter paper into a 50 ml volumetric flask and dilute to volume.

1.2.7 Final solution is colorless and to be analized sodium, potassium, magnesium and calcium

2. Analysis chloride in leaf

2.1 Prepare standard chloride solution 0, 2, 4, 8, 10 and 12 ppm 5 ml. from standard chloride 1000 ppm.

2.2 Use 5 ml sample solutions as described prior.

2.3 Add 0.4 ml mercuric thiocyanate solution in sample aliquots and standard chloride solution and swirl to mix.

2.4 Add 0.2 ml ferric ion and swirl to mix.

2.5 After 10 minutes the solutions are brown.

2.6 Measure the absorbance at 455 nm with spectophotometer.

2.7 Prepare a calibration curve from standard solutions and use it to obtain mg Cl in the sample aliquot.

2.8 Calculation chloride obtain from the graph and apply factors for dilution or concentration and correct to dry weight where necessary.

3. Analysis sodium, potassium, magnesium and calcium in leaf and xylem sap.

3.1 Prepare standard chloride solution 0, 10, 20 and 30 ppm from standard chloride 100 ppm (by dilute with 5% $HClO_4$ for leaf).

3.2 Prepare sample solutions as described prior and dilute with 5% $HClO_4$ for leaf and for xylem sap can be used directly.

3.3 Measure with atomic absorption spectrometry (model 3110) and select the 589 nm wavelength for Na, 766.5 nm wavelength for K, 258.2 nm wavelength for Mg and 422 nm wavelength for Ca.

3.4 Prepare a calibration curve from standard solutions and use it to obtain ppm Na, K, Mg and Ca in the sample solutions.

3.5 Calculation sodium, potassium, magnesium and calcium obtain from the graph and apply factors for dilution or concentration and correct to dry weight where necessary.

Table Compositions of Hoagland nutrient solution

Compound		Molecular Weight (g/l)	Concentration
1. calcium nitrate	$Ca(NO_3)_2.4H_2O$	236.1	1 M
2. potassium nitrate	KNO_3	101.1	1 M
3. magnesium sulphate	$MgSO_4.7H_2O$	246.5	1 M
4. potassium dihydrogen phosphate	KH_2PO_4	136.09	1 M
5. micronutrient			
5.1 Boric acid	H_3BO_3	2.86	0.5 mg.B/ml
5.2 Copper chloride	$CuCl_2.2H_2O$	0.05	0.02mg.Cu/ml
5.3 Manganese chloride	$MnCl_3.2H_3O$	1.81	0.5 mg.Mn/ml
5.4 Zinc chloride	$ZnCl_2$	0.11	0.05mg.Zn/ml
5.5 Sodium molybdate	$Na_2Mo_4.2H_2O$	0.025	0.01mg.Mo/ml
6. Fe-EDTA			5 mg.Fe/ml
6.1. disodium ethylenediaminetetraacetate	$C_{10}H_{14}O_8Na_2.2H_2O$		
6.2 Ferric chloride	$FeCl_3.6H_2O$		

Table Concentration half-strength of Hoagland solution

Stock solution	Volume (ml/water 2 liters)
$Ca(NO_3)_2.4H_2O$	5
KNO_3	5
$MgSO_4.7H_2O$	2
KH_2PO_4	1
Micronutrient	1
Fe-EDTA	1

APPENDIX C

Average height of *Avicennia alba (cm)*

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	12.2	13.1	15.5	18.5	20.5	22.1	24.3	26.1	27.4	28.7	30.3
10	17.7	19.6	24.6	30.8	34.0	37.1	41.8	47.3	48.6	50.1	51.7
20	15.1	17.1	21.7	26.1	29.7	31.6	35.1	40.6	41.3	44.7	45.6
30	13.1	13.9	16.5	20.7	22.9	24.6	26.6	27.9	30.1	35.1	37.4
40	11.1	11.7	12.3	14.4	15.1	15.6	17.2	18.6	21.1	24.0	26.1

Average diameter of *Avicennia alba (cm)*

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	-	0.27	0.27	0.28	0.30	0.33	0.34	0.35	0.36	0.38	0.39
10	-	0.31	0.33	0.35	0.37	0.42	0.44	0.45	0.47	0.48	0.49
20	-	0.27	0.30	0.31	0.34	0.37	0.39	0.4	0.42	0.44	0.45
30	-	0.23	0.25	0.26	0.27	0.30	0.32	0.33	0.36	0.38	0.40
40	-	0.20	0.22	0.22	0.22	0.24	0.25	0.26	0.27	0.29	0.30

Average number of leaves of *Avicennia alba*

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	-	5	7	7	7	8	7	7	7	5	5
10	-	6	8	10	10	12	12	10	9	8	8
20	-	5	8	10	11	12	13	12	10	11	10
30	-	4	6	7	9	11	12	11	11	12	11
40	-	4	5	6	7	8	10	11	11	9	9

Average biomass of *Avicennia alba* (g/plant)

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	-	1.8	2.1	2.6	3.3	4.2	4.7	5.2	5.7	6.7	7.3
10	-	3.3	4.6	5.9	7.3	9.6	11.7	13.7	15.1	16.4	17.7
20	-	2.3	3.3	4.3	5.5	6.9	8.1	9.8	10.8	12.5	13.1
30	-	1.5	1.9	2.4	2.9	3.7	4.6	5.1	6.4	7.8	8.9
40	-	1.0	1.2	1.3	1.5	1.7	2.0	2.3	2.7	3.5	4.0

Salinity 60 ppt plant dead

Average height of *Bruguira gymnorrhiza (cm)*

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	12.8	20.4	24.5	26.8	27.9	29.7	31.2	33.3	36.7	41.2	44.9
10	11.1	16.6	20.7	25.5	28.8	32.3	36.1	40.4	46.0	49.6	54.9
20	6.9	11.5	14.5	17.6	19.4	21.4	24.4	27.9	31.1	35.9	39.7
30	9.6	10.3	11.8	13.3	14.1	16.0	18.7	21.6	24.7	29.0	34.4
40	7.0	7.3	9.1	10.5	10.7	11.4	11.6	12.3	13.7	14.3	16.2

Average diameter of *Bruguira gymnorrhiza (cm)*

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	-	0.52	0.54	0.56	0.57	0.61	0.63	0.68	0.75	0.83	0.87
10	-	0.50	0.52	0.54	0.57	0.62	0.70	0.73	0.82	0.90	0.91
20	-	0.47	0.49	0.51	0.53	0.55	0.58	0.63	0.72	0.76	0.81
30	-	0.43	0.44	0.45	0.46	0.48	0.49	0.50	0.55	0.64	0.67
40	-	0.39	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.49	0.50

Average number of leaves of *Bruguira gymnorrhiza*

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	-	6	7	8	9	9	11	12	13	15	16
10	-	5	7	8	9	11	12	14	15	17	20
20	-	4	6	8	9	10	12	13	14	15	16
30	-	4	5	6	7	8	9	11	12	13	15
40	-	3	4	5	5	7	7	9	10	11	12

Average biomass of *Bruguira gymnorrhiza* (g/plant)

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	-	26.1	30.3	33.3	35.3	39.5	42.6	48.8	58.0	70.7	79.2
10	-	21.8	26.1	31.2	35.5	42.5	52.9	59.6	74.3	86.9	94.8
20	-	16.4	19.8	23.2	25.6	28.4	33.1	39.9	49.8	58.6	67.2
30	-	13.7	15.2	16.9	18.0	20.1	23.0	25.9	31.1	41.4	48.4
40	-	9.8	11.8	13.4	13.8	14.7	15.5	16.3	18.1	19.3	21.5

Salinity 60 ppt plant dead

Average height of *Heritiera littoralis* (cm)

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	28.4	36.1	41.4	43.4	47.0	46.1	47.0	49.8	51.8	55.3	57.1
10	27.3	31.9	32.5	34.2	36.0	38.4	41.4	42.4	43.2	45.4	46.3
20	27.4	29.5	30.0	30.8	30.8	31.0	31.0	31.4	31.8	32.1	32.7
30	27.0	28.1	28.2	28.7	28.8	29.2	29.2	29.3	29.5	29.8	29.6
40	27.0	29.0	29.1	29.3	29.9	30.6	28.5	-	-	-	-

Average diameter of *Heritiera littoralis (cm)*

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	0.41	0.49	0.56	0.66	0.73	0.81	0.87	0.93	1.05	1.10	1.20
10	0.43	0.48	0.57	0.63	0.68	0.80	0.87	0.95	1.05	1.15	1.22
20	0.42	0.43	0.47	0.52	0.54	0.57	0.59	0.63	0.63	0.67	0.78
30	0.40	0.41	0.43	0.45	0.45	0.48	0.50	0.52	0.54	0.58	0.59
40	0.41	0.41	0.43	0.43	0.44	0.42	0.46	-	-	-	-

Average number of leaves of *Heritiera littoralis*

Salinity	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	6	8	9	10	12	13	14	15	17	18	20
10	5	7	7	8	9	11	12	13	14	14	16
20	5	6	5	6	6	6	6	6	7	7	7
30	6	6	5	5	5	5	5	5	5	5	5
40	5	5	5	5	5	5	5	-	-	-	-

Average biomass of *Heritiera littoralis* (g/plant)

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	4.9	7.8	10.6	14.0	17.2	20.0	22.8	26.1	32.5	36.6	44.4
10	5.1	6.9	9.0	10.7	12.8	17.0	20.4	23.8	27.9	33.5	37.1
20	5.0	5.4	6.3	7.5	7.9	8.6	9.1	10.2	10.3	11.3	14.6
30	4.6	4.9	5.3	5.8	5.8	6.3	6.7	7.2	7.7	8.7	8.9
40	4.7	5.0	5.3	5.4	5.6	5.4	-	-	-	-	-

Salinity 60 ppt plant dead

Average height of *Xylocarpus granatum (cm)*

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	32.7	33.3	34.7	37.2	38.7	41.9	44.8	48.6	53.0	54.9	60.1
10	32.7	33.0	35.0	37.1	42.0	43.6	50.0	54.9	56.4	60.6	67.2
20	36.9	36.9	36.9	32.8	35.3	36.2	38.8	41.8	42.0	42.7	50.2
30	34.6	34.6	34.6	38.3	37.2	36.0	37.1	37.4	34.4	34.8	35.6
40	38.9	38.9	35.2	29.7	33.0	33.6	31.5	33.3	34.4	29.2	29.4

Average diameter of *Xylocarpus granatum (cm)*

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	0.43	0.51	0.53	0.55	0.60	0.66	0.72	0.77	0.86	0.94	1.02
10	0.42	0.47	0.50	0.52	0.60	0.75	0.85	0.95	1.05	1.07	1.15
20	0.46	0.49	0.51	0.45	0.49	0.63	0.69	0.77	0.78	0.82	0.90
30	0.41	0.43	0.44	0.45	0.43	0.46	0.47	0.47	0.44	0.46	0.50
40	0.41	0.42	0.41	0.41	0.42	0.44	0.42	0.38	0.41	0.60	0.61

Average number of leaves of *Xylocarpus granatum*

Salinity (ppt)	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	5	6	7	12	16	18	22	24	31	33	35
10	5	5	9	10	22	24	31	35	37	44	45
20	5	5	5	3	11	12	18	19	21	27	29
30	5	5	5	5	4	4	5	6	6	6	5
40	4	4	4	2	3	3	6	5	4	4	4

Average biomass of *Xylocarpus granatum* (g/plant)

Salinity (ppt)	month1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10	month 11
0	10.5	13.4	14.4	15.8	18.3	22.1	25.7	29.7	36.5	40.4	50.2
10	10.2	12.0	13.2	14.5	19.5	26.6	34.6	42.6	49.6	53.8	63.3
20	12.4	13.6	14.2	11.0	13.2	18.7	22.1	26.8	27.4	29.6	37.5
30	10.1	10.7	11.0	12.3	11.6	12.0	12.7	12.7	11.1	11.8	13.3
40	10.8	11.3	10.1	9.1	10.2	10.9	10.0	9.0	10.1	15.0	15.5

Salinity 60 ppt plant dead

BIOGRAPHY

Miss Chanita Paliyavuth was born on June 25, 1976 in Bangkok. She graduated with Bachelor degree (2^{nd} class honors) in Botany from Department of Botany, Faculty of Science, Chulalongkorn University in 1997. She commenced the Master degree in Botany, Department of Botany, Faculty of Science, Chulalongkorn University in 1998.