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Effects of salt water intrusion on biochemical components of Alder and Ash tree fresh leaves in Karacabey Coastal Forested Wetland

Karacabey Kıyı Subasar Ormanlarında Kızılağaç ve Dişbudak yeşil yapraklarının biyokimyasal bileşimi üzerinde tuzlu su girişinin etkisi

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Abstract

Coastal forested wetlands provide substantial benefits to society, such as wave attenuation, erosion control, biodiversity support, and carbon sequestration. Many of these unique coastal ecosystems have been drained for various reasons, while those that remain are now threatened by salt water intrusion and sea level rise due to climate change. In this study, we aimed to investigate the effects of soil salinity on the biochemical components of the fresh leaves of alder (*Alnus glutinosa* L. Gaertn) and ash tree (*Fraxinus angustifolia* Vahl.) which are the dominant tree species in Karacabey coastal forested wetland next to the Sea of Marmara in Türkiye. For this purpose, fresh leaf and soil samples of alder and ash trees were collected from three zones (Z_1: 0-1 km, Z_2: 1 to 2 km and Z_3: 2 to 3 km) from the inner border (Z_3) of the forested wetland to the coastline (Z_1) of the Sea of Marmara. The fresh leaf samples were analyzed for photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids), anthocyanin, xanthophylls, free amino acids, total nitrate, proline, total polyphenols, total soluble tannins, total phenolic compounds, glucose, sucrose and total carbohydrates. The soil samples were analyzed for soil pH, electrical conductivity and soil texture. The results showed that the soil salinity decreased from the coastline (Z_1) towards the inner border (Z_3). Similarly, mean photosynthetic pigments and anthocyanin, xanthophyll also decreased from the Z_1 towards Z_3, whereas mean total polyphenols and total soluble tannin concentrations increased for the both tree species. The other biochemical compounds showed either an increase or a decrease according to the tree species. These pioneer results illustrate the important point that biotic or abiotic environment in which tree grows significantly change the specific biochemical components in the fresh leaves of alder and ash trees in the coastal forested wetlands. In turn, these changes may result in variation in nutrient cycling, carbon cycling, and organic matter turnover rates in these forest ecosystems.

Özet

Deniz kıyısı subasar ormanlar, deniz dalga etkisinin azaltılması, erozyon kontrolü, biyolojik çeşitlilik ve karbon tutma gibi önemli faydalar sağlamaktadır. Bu eşsiz kıyı ekosistemlerinin birçoğu çeşitli sebeplerle kurutulurken, geriye kalanları günümüzde iklim değişikliğinin etkisiyle denizden tuzlu su girişi ve deniz seviyesinin yükselmesi tehdidi altındadır. Bu çalışmada, Marmara Denizi kıyısında yer alan Karacabey subasar ormanlarının asli ağaç türleri olan kızılağaç (*Alnus glutinosa* L. Gaertn) ve dişbudak (*Fraxinus angustifolia* Vahl.) ağaçlarının yeşil yapraklarının biyokimyasal bileşimi üzerinde toprak tuzluluğu etkisinin araştırılması amaçlanmıştır. Bu amaçla ağaçların yaprakları subasar ormanların yayılış yaptığı deniz kıyısı ile iç kısım arasındaki üç zondan (Z_1: 0-1 km, Z_2: 1 to 2 km and Z_3: 2 to 3 km) örneklenmiştir. Yaprak örnekleri üzerinde, fotosentetik pigmentler (klorofil a, klorofil b ve karotenidler), antosiyanin, ksantofiller, serbest amino asitler, toplam nitrat, prolin, toplam polifenoller, toplam çözünür tanenler, toplam fenolik bileşikler, glikoz, sukroz ve toplam karbonhidrat analizleri gerçekleştirilmiştir. Toprak örneklerinin ise pH, elektriksel iletkenlik ve tekstürü belirlenmiştir. Sonuçlar, toprak tuzluluğunun kıyı şeridinden (Z1) iç sınıra (Z3) doğru azaldığını göstermiştir. Benzer şekilde, ortalama fotosentetik pigmentler ve antosiyanin, ksantofilin de Z1'den Z3'e doğru azalırken, toplam polifenoller ve çözünür tanen konsantrasyonları her iki ağaç türü içinde artmıştır. Diğer biyokimyasal bileşikler de ağaç türlerine göre artış veya azalma göstermiştir. Bu öncül sonuçlar, kıyı subasar ormanlarının yetişme ortamı şartlarının (abiyotik veya biyotik), Kızılağaç ve Dişbudak ağaçlarının yeşil yapraklarının biyokimyasal bileşenlerinde önemli ölçüde değişikliğe neden olabileceğini göstermektedir. Bu değişiklikler ise, zaman içinde bu subasar orman ekosistemlerinin besin döngüsü, karbon döngüsü ve organik madde dönüşüm süreçlerinin değişimine neden olabilecektir.

INTRODUCTION

Wetlands in coastal watersheds provide a wide variety of ecosystem services and mainly include ecosystems of mangroves, forested wetlands, coral reefs, salt marsh, and sea grass beds (Li et al. 2017). They support a wide range of biodiversity, purify and replenish our water, serve as a natural sponge against flooding and drought, protect our coastlines and help fight climate change (White et al. 2022, Sariyildiz et al. 2023). In addition, recent studies have shown that for per unit area, wetlands, particularly forested wetlands can accumulate more carbon compared to adjacent or upland terrestrial forest ecosystems (D'Elia et al. 2017, Sariyildiz et al. 2022).

Despite the substantial benefits that coastal forested wetlands provide to society, they are under threat due to both anthropogenic and natural factors, such as diversions and damming of river flows, disconnecting floodplain wetlands from flood flows, eutrophication, contamination, grazing, harvests of plants and animals, global climate, invasions of exotics, and the practices of filling, dyking and draining (Brinson and Malvárez 2002). Thus, more than 50% of these unique coastal ecosystems have been lost in the 20th century, while those that remain are now threatened by saltwater intrusion and sea level rise due to climate change (Li et al. 2018, White et al. 2022). As the buffer zone between land and sea, coastal wetlands are frequently threatened from both sides. In coastal wetlands, waterlogging and soil salinity appear as the main factors controlling plant community composition and structure, as well as wetland subsystems' function (Ball 1998, Santos et al. 2012). However, last three decades, the balance between water inputs resources (freshwater, groundwater and seawater inputs) that feeds the coastal forested wetlands is disturbed by climate change and this can result in an increase or decrease in the salinity concentration of those ecosystems (Li et al. 2018, Sariyildiz and Tani 2023). Freshwater input during the rainy season is not the sole factor explaining seasonal variations of groundwater salinity. Seasonal changes in the water table depend on freshwater inputs, tidal rhythm and also the loss of freshwater through evapotranspiration processes which increase landwards. Thus, the vegetation of coastal forested wetlands mainly interacts with hydrologic processes. The effects of this interaction on biochemical components of mangrove type wetlands have been subject to many researches. Particularly, salinity impacts on mangrove plant species have been studied in relation to antioxidative enzymes (Takemura et al. 2000, Parida et al. 2004a), leaf structure, rates of transpiration, stomatal conductance and rates of photosynthesis (Santiago et al. 2000, Parida et al. 2004b) and changes in chloroplast structure and function (Parida et al. 2003). Salinity induces some symptoms, including: leaf scorching, leaf shedding, twig dieback, and decreased metabolic functions leading to inhibition of vegetative growth (Paludan-Muller et al. 2002). Tolerance to salinity varies widely by tree species and genotype (Kozlowski 1997, Paludan-Muller et al. 2002).

Türkiye has also some coastal forested wetlands near to the coastline of Marmara and Black Sea. Unfortunately, not much area has left in Türkiye (only 11.400-hectare). Among them, with approximately 3800 ha areas (Akay et al. 2017), Karacabey forested wetland is formed by the accumulation of sediments deposited by creeks and streams flowing into the sea of Marmara. A number of riverine and floodplain forests in the northern part of Türkiye have already been studied in terms of ecology and biology (Ursavas and Keceli 2019). However, there has been no study available to investigate the salinity effects on biochemical components of tree species.

Exposure to salinity may cause several morphological, physiological and biochemical changes in plants, due to excess ions and water deficit. Several researchers have shown that generally the chlorophyll and total carotenoid contents of leaves decrease under salinity where the chlorosis start from oldest leaves during the salt stress (Hannachi et al. 2022). Other researches have stated that in plant, environmental stresses (biotic and abiotic) such as salinity lead to accumulation of polyphenol constituents (Dixon and Palva 1995). We therefore carried out a study in Karacabey forested wetland to understand the effects of soil salinity on the biochemical components (photosynthetic pigments, anthocyanin, xanthophylls, free amino acids, total nitrate, proline, total polyphenols, total soluble tannins, total phenolic compounds, glucose, sucrose and total carbohydrates) of the fresh leaves of Alder (*Alnus glutinosa* L. Gaertn) and Ash tree (*Fraxinus angustifolia* Vahl.).

MATERIAL and METHODS

Study Site

The study was conducted in the Karacabey forested wetlands in Bursa, Türkiye (40º23´38´´-40º21´43´´N, 28º23´02´´-28º34´21´´E) (Figure 1). Rainfall, evapotranspiration, rise and fall of groundwater mostly vary the water level in this unique floodplain ecosystem. However, intrusion of saltwater from Marmara Sea is also a major impact on the water level on the forested wetlands. Karacabey floodplain covers approximately 3800 ha. A variety of habitats can be seen in the area: sand dunes, swamp, lakes, grasslands, croplands and forested wetlands.

A semi humid climate dominates the region. The last 34-year (1988-2022) climate data shows that annual precipitation is 577.6 mm and mean annual temperature is 15.4 ^{0}C. However, annual precipitation varies according to year. For example, compared to the longer term mean annual precipitation (577.7 mm for the mean of last 34 years), the annual precipitation was very low in 2019 and 2020 (401.4 mm and 481.9 mm respectively), while it was higher (650.7 mm) in 2021. Ash tree (Fraxinus angustifolia Vahl.) and Alder (*Alnus glutinosa* L. Gaertn.) were dominant tree species in Karacabey forested wetlands (Sariyildiz et al. 2022). Karacabey plain is in the group of alluvial filled rift valley caused by tectonic movements. It was formed as a result of the collapses that took place during the Quaternary period. Limeless brown forest soils, colluvial, alluvial coastal soils and redzines can be seen throughout the Kocacay Delta (Sariyildiz et al. 2023). In the area, soil type was mainly alluvial and colluvial soils (Sariyildiz and Tani 2023).

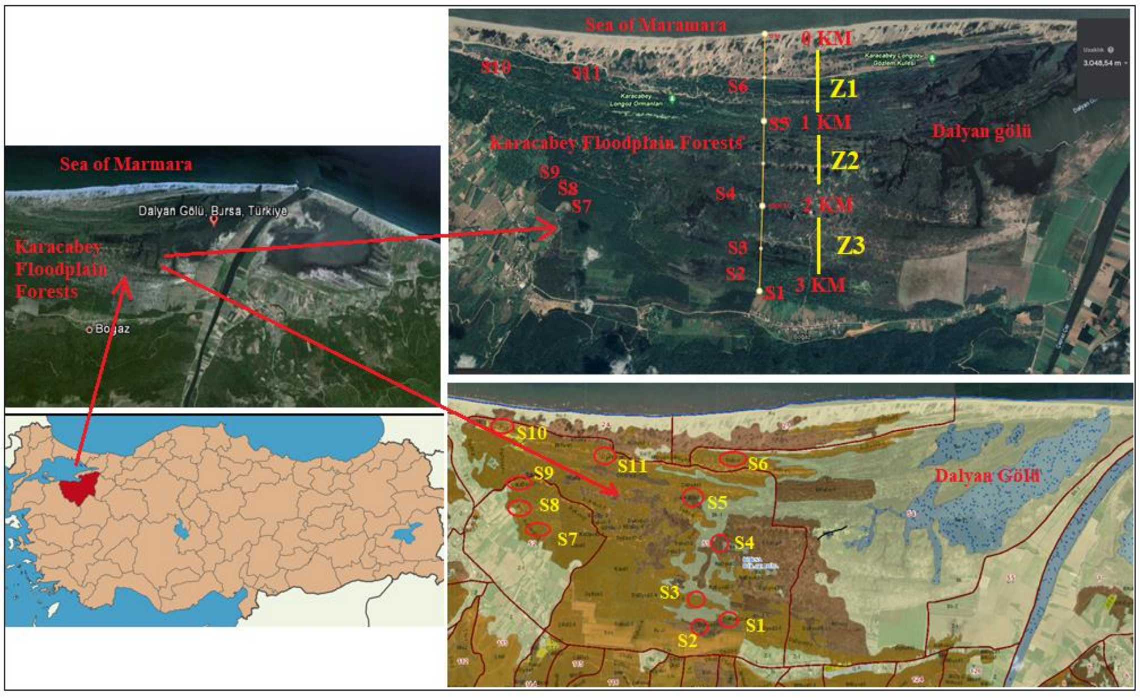

Figure 1. The location of Karacabey Floodplain Forests and sampling sites which were decided according to the distances away from the Marmara Sea as Z_1 (0 to 1 km), Z_2 (1 to 2 km) and Z_3 (2 to 3 km). S_{1-11} indicates the fresh leaf sampling sites

Study Site and Sampling

The sampling sites were decided according to the distances away from the Marmara Sea. The distance from the inner border of the floodplain forests to the Sea of Marmara was nearly 3 km. This distance was divided into 3 zones as Z_1 (0 to 1 km), Z_2 (1 to 2 km) and Z_3 (2 to 3 km) (Figure 1). Within the 3 zones, eleven sampling sites were chosen according to the distances and the distribution of the alder and ash trees in the study site. The first two sampling sites (S_1 and S_2) were the furthest away from the Marmara Sea, while the sampling sites of S_6, S_{10} and S_{11} were the nearest to the Marmara Sea. Ash tree was most dominant tree species in the study site, and so it was easy to take 3 replicate sites in each zone. However, alder was only found in the Z_2 and Z_3, but not in the Z_1. The characteristics of Ash (*Fraxinus angustifolia* Vahl.) and Alder (*Alnus glutinosa* (L.) Gaertn.) trees in the sampling sites from which the fresh leaves were collected, are given in Table 1.

Table 1. Fresh leaves collected from ash and alder dominated sites and the characteristics of the sampling trees

Distance from the coastline	Sampling sites	Age (years) Mean ± S.S	DBH (cm) Mean ± S.S.	Height (m) Mean ± S.S.
	Ash Tree			
Zone 1 (0-1 km)	S1	78	39.3	17
	S2	74	37.4	18
	S3	67	32.5	15
	Mean	**73**	**36.4**	**16.7**
Zone 2 (1-2 km)	S4	71	36.5	18
	S5	83	43.3	21
	S7	69	33.1	16
	Mean	**74**	**37.6**	**18.3**
Zone 3 (2-3 km)	S6	83	44.6	22
	S10	74	38.5	16
	S11	77	40.2	18
	Mean	**78**	**41.8**	**18.7**
Distance from the sea	Alder Tree			
Zone 2 (1-2 km)	S1	62	24.5	16
	S2	66	28.8	18
	Mean	**64**	**26.7**	**17.0**
Zone 3 (2-3 km)	S7	69	32.3	20
	S8	63	25.3	17
	S9	67	29.4	19
	Mean	**66**	**29.0**	**18.7**

Mean age of alder trees ranged from 64-year-old (Zone 2) to 66-year-old (Zone 3). Ash trees were older than alder trees ranging from 73-year-old (Zone 1) to 74-year-old (Zone 2) and 78-year-old (Zone 3). Mean DBH of ash trees was lowest in the Zone 1 (37.6 cm), and highest in the Zone 3 (41.8 cm). Similarly, mean DBH of alder trees was highest in the Zone 3 (29.0 cm) and lowest in the Zone 2 (26.7 cm). The same trend was also seen for mean height with highest height in the Zone 3 for both tree species, and lowest in the Zone 1 for ash trees and in the Zone 2 for alder trees (Table 1).

In each sampling site, three replicate subplots (20x20 m=400-m^2) located approximately 300 m. from each other were taken. The soil surface of the study site is mostly covered by water for 9 to 10 months depending on the rainy season. Thus, the best time to collect to the fresh leaf samples was in August when there was no water on the soil surface (Figure 2). To reduce the possible effect of sun and shade leaves on the biochemical constituents of alder and ash trees in our study, (1) the leaves in branches from less than 50 cm to the ground and higher than 50 cm to the terminal bud of the main stem of the tree were left out and (2) the leaves were sampled from the middle of the branch, with a minimum distance of about 15 cm to the main stem and the tip (Deepak et al. 2019). The fresh leaves were collected from 5 replicate trees, placed into the bags and brought to the laboratory (Figure 2).

The samples were then combined to form a mixed sample for each sampling site and analyzed for the leaf chemical compounds. Mean stand age, height and diameter breast height (DBH) of the sampling trees were also determined. Tree age was determined by counting each annual growth ring in the trunk of the tree. Tree height was measured with a Blume-Leiss altimeter. Diameter at the breast height (DBH) was measured using the diameter tape.

Soil samples were also collected under the sampling trees from which the fresh leaf samples were collected. The soil samples were collected in an area of 0.5x0.5 m^2 at a distance of 2 m from the base of the trunk of 5 trees. The soil samples were taken from the depth of 0-10 cm, 10-20 cm and 20-30 cm. Then, they were mixed to have a single representative sample for each sampling site and analysed for some basic soil properties.

Figure 2. The fresh leaf samples were collected in August when the lowest water on the soil surface (a), compared to the other months (b), the fresh leaves were collected from the lower parts of 5 replicate trees (c), placed into the bags and brought to the laboratory (d).

Leaf Chemical Analyses

The fresh leaf samples were brought to the laboratory, cleaned by hand and by using a brush. Fifteen leaves from each site were randomly taken and weighted for moisture content (Figure 3). The rest of the leaves were placed into plastic bags and kept into a fridge until analyses. Later for the chemical analysis, the fresh leaf samples were crushed using some solutions according to the procedure of the chemical compounds and analysed for photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids), anthocyanin, xanthophylls, free amino acids, total nitrate, proline, total polyphenols, total soluble tannins, total phenolic compounds, glucose, sucrose and total carbohydrates.

For the analysis of photosynthetic pigments, 500 mg of leaf samples were homogenized with 10 ml of 80% acetone and centrifuged at 3000 rpm for 15 minutes. The extract was utilized for chlorophyll estimation (Arnon 1949). Carotenoid amount was estimated using the Jaspars' formula according to the method of Witham et al. (1971). Anthocyanin contents were quantified by the modified method of Padmavati et al. (1997). Xantophylls were extracted with ethanol according to the methods described by Kukric et al. (2012) with some modifications. Determination of free amino acid was determined by using the method of Spies (1957). Total nitrate content was estimated according to Cataldo et al. (1975) method using rapid colorimetric method. Proline level was measured by the methods of Bates et al (1973). Total polyphenol contents were determined according to the method of (Singleton et al. 1999). Method of Schanderl (1970) was employed for determination of water-soluble tannins. Total phenolic content was determined according to a modified Folin-Ciocalteu colorimetric method. Determination of the total soluble carbohydrate, glucose and sucrose contents was carried out using the Anthrone method (Pearson et al. 1976).

Figure 3. All fresh leaf samples were cleaned in the laboratory (a), Fifteen leaves were randomly chosen and weighted for determination of moisture contents (b), The rest of the leaves were also weighed (c), and then placed into plastic bags (d) and kept in the fridge until the chemical analyses (d)

Soil Analysis

The soil samples were analyzed for soil pH, electrical conductivity and soil texture. Soil pH was determined by a combination glass electrode in H_2O (soil-solution ratio 1:2.5). Electrical conductivity (EC) was determined in 1:1 soil water extract by using conductivity meter and expressed as dS/m. Soil texture was determined by Bouyoucos' hydrometer method (Bouyoucos 1962).

Data Analysis

Analysis of variance (ANOVA) were applied for analysing the differences in the fresh leaf chemical composition and soil properties of alder and ash trees between the 3 Zones (Z1: 0-1 km, Z2: 1 to 2 km and Z3: 2 to 3 km) using the SPSS program ver. 11.0 for Windows. Following the results of ANOVAs, Tukey's honestly significance difference (HSD) test (α=0.05) was used for testing differences between group means.

RESULTS

Soil Characteristics

Soil pH, EC and soil texture of the soils of ash and alder trees collected from 3 main zones; near the sea (0 to 1 km), mid-distance (1 to 2 km) and farther from the sea (2 to 3 km) are given in Table 2. Mean soil pH, EC and sand content of ash tree soils showed a decrease from the Zone 1 (the nearest sampling sites to the Sea of Marmara) to the Zone 3 (the furthest sampling sites). Similar trends were also seen for the alder soil with the highest mean pH, EC and sand contents in the Zone 2 compared to the Zone 3. In contrary, mean silt and clay contents showed an increase from the sea sites to the inner sampling sites for soils of both tree species (Table 2).

Variation in Photosynthetic Pigments

Mean chlorophyll-a, chlorophyll-b, total chlorophyll and total carotenoids in the fresh leaves varied significantly among the zones (Zone 1, Zone 2 and Zone 3) for both tree species (Table 3). The ratio of chlorophyll a/b also varied significantly with the zones for alder trees, but not for ash trees.

In general, mean photosynthetic pigments in the fresh leaves of both tree species showed a decrease with the distances from the sea, whereas mean chlorophyll a/b ration showed an increase with the distances. For example, mean chlorophyll a and b concentrations of the fresh ash tree leaves were 0.0443 and 0.304 mg/g in the Zone 1 respectively, while in the Zone 3, they were 0.338 and 0.165 mg/g respectively. In contrast, mean ratio of chlorophyll a/b was lowest in the Zone 1 (1.75) compared to the Zone 3 (2.19).

Table 2. Characteristics (0-30 cm) of ash and alder trees' soils collected from 3 main zones; near the sea (0 to 1 km), mid-distance (1 to 2 km) and farther from the sea (2 to 3 km)

Distance from the coastline	pH	EC (dS/m)	Sand (%)	Silt (%)	Clay (%)
		Ash Tree			
Zone 1	7.28±0.12	0.573 ±0.017	76 ±1.2	10 ±0.9	14 ±1.4
Zone 2	6.80 ±0.14	0.448 ±0.011	63 ±1.5	16 ±1.3	21 ±1.7
Zone 3	6.46 ±0.08	0.360 ±0.021	59 ±1.5	18 ±1.6	23 ±2.4
		Alder Tree			
Zone 2	6.85 ±0.14	0.248 ±0.024	69 ±2.1	14 ±	17 ±2.1
Zone 3	5.50 ±0.07	0.080 ±0.014	57 ±1.8	20 ±	23 ±2.0

Variation in Anthocyanin, Xanthophyll, Free Amino Acids, Total Nitrate and Proline

Mean anthocyanin and xanthophyll concentrations in the fresh leaves of both tree species significantly decreased with the distances from the sea (Table 4). Mean anthocyanin and xanthophyll concentrations of the fresh ash tree leaves were 17.2 µmol/ml and 1.65 mg/g in the Zone 1 respectively, while in the Zone 3, they were 11.7 µmol/ml and 0.74 mg/g respectively. Similarly, mean anthocyanin and xanthophyll concentrations of the fresh alder tree leaves were higher in the Zone 2 (46.3 µmol/ml and 0.35 mg/g respectively) than in the Zone 3 (37.4 µmol/ml and 0.16 mg/g respectively).

Table 3. Mean chlorophyll-a, chlorophyll-b, total chlorophyll, total carotenoids, and the ratio of chlorophyll a/b in the fresh leaves collected from ash tree and alder trees in the floodplain forests within different distances away from the border of Marmara Sea.

Distance from the coastline	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Total carotenoids (mg/g)	Chlorophyll a/b
			Ash Tree Leaves		
Zone 1	0.443b ± 0.025	0.304b ± 0.163	0.746c± 0.188	10.3bc ± 0.376	1.75a ± 0.623
Zone 2	0.364ab± 0.048	0.177ab± 0.033	0.541ab ± 0.081	9.42abc± 0.733	2.08a± 0.101
Zone 3	0.338a± 0.112	0.165ab± 0.079	0.503a± 0.191	8.55ab± 1.76	2.19a± 0.364
F	5.175	4.706	5.926	5.475	2.639
Sig.	P< 0.05	P< 0.05	P< 0.01	P< 0.05	P> 0.05
			Alder Tree Leaves		
Zone 2	0.459b± 0.038	0.255b± 0.059	0.714bc± 0.096	10.6c± 0.214	1.85a± 0.280
Zone 3	0.316a± 0.075	0.112a± 0.027	0.428a± 0.103	8.43a± 1.96	2.83b± 0.082
F	18.228	41.24	29.336	7.116	99.555
Sig.	P< 0.001	P< 0.001	P< 0.001	P< 0.05	P< 0.001

Mean values are shown with standard deviation (±), (each zone n=9, except zone 2 for Alder n=6).

Table 4. Mean Anthocyanin, xanthophyll, free amino acids, total nitrate and proline in the fresh leaves collected from ash tree and alder trees in the floodplain forests within different distances away from the border of Marmara Sea

Distance from the coastline	Anthocyanin (µmol/ml)	Xanthophyll (mg/g)	Free amino acids (mg/g)	Total nitrate (mg/g)	Proline (µg/g)
					Ash Tree Leaves
Zone 1	17.2b ± 6.34	1.65c ± 0.630	118a ± 8.188	7.22a ± 2.96	77.7b ± 5.349
Zone 2	12.4ab ± 3.11	0.77b ± 0.253	97.7a ± 4.58	6.79a ± 3.31	76.9b ± 3.63
Zone 3	11.7a ± 1.76	0.74b ± 0.325	102a ± 17.2	5.56a ± 1.33	71.1b ± 8.10
F	4.447	12.59	7.984	0.929	3.272
Sig.	P< 0.05	P< 0.001	P< 0.01	P> 0.05	P> 0.05
					Alder Tree Leaves
Zone 2	46.3d ± 0.962	0.35a± 0.308	96.5a ± 33.8	5.39a ± 0.377	71.5b ± 5.62
Zone 3	37.4c ± 0.309	0.16a± 0.042	105a ± 15.4	4.16a ± 1.90	53.6a ± 7.90
F	676.9	9.334	0.480	2.394	22.81
Sig.	P< 0.001	P< 0.01	P> 0.05	P> 0.05	P< 0.001

Mean values are shown with standard deviation **(**±), (each zone n=9, except Zone 2 for Alder n=6)

As for mean free amino acids and proline, both tree species showed different trends. Mean free amino acids significantly decreased with the distances for the ash trees, but no significant variation with the distances was found in mean free amino acids for alder trees. Although there was a decrease in mean proline concentration with the distances for both tree leaves, statistically significant variation with the distances was not found for the ash trees, while highly significant variation was seen for the alder trees. Mean nitrate concentration did not vary significantly with the distances for both tree species.

Variation in Total Polyphenols, Total Soluble Tannin, Total Phenolic Compounds, Glucose, Sucrose and Total Carbohydrates

In contrast to photosynthetic pigments, anthocyanin and xanthophyll concentrations, mean total polyphenols and total soluble tannin concentrations in the fresh leaves of both tree species significantly increased with the distances from the sea (Table 5). Mean total polyphenols and total soluble tannin concentrations of the fresh ash tree leaves were 28.7 µg/g and 33.2 mg/g in the Zone 1 respectively, while in the Zone 3, they were 39.5 µg/g and 54.5 mg/g respectively. Similarly, mean total polyphenols and total soluble tannin concentrations of the fresh alder tree leaves were higher in the Zone 2 (42.1 µg/g and 45.3 mg/g respectively) than in the Zone 3 (54.5 µg/g and 57.6 mg/g respectively).

As for mean total phenolic compound and glucose concentrations of leaves, both species showed different trends. Although mean total phenolic compounds tended to increase (significantly) with the distances for the ash trees, statistically there was no significant variation with the distances in mean total phenolic compounds for the ash trees. However, mean total phenolic compounds significantly increased with the distances for the alder trees. Mean glucose concentration significantly increased with the distances for the ash trees, while no significant variation was seen for the alder trees.

Mean sucrose and total carbohydrate concentrations did not vary significantly with the distances for both tree species.

Table 5. Mean total polyphenols, total soluble tannin, total phenolic compounds, glucose, sucrose and total carbohydrates in leaves collected from ash and alder trees in the floodplain forests within different distances away from the border of Marmara Sea

Distance from the coastline	Total polyphenols (µg/g)	Total soluble tannin (mg/g)	Total phenolic compounds (µg/g)	Glucose (mg/g)	Sucrose (mg/g)	Total carbohydrates (%)
			Ash Tree Leaves			
Zone 1	28.7a ± 6.11	33.2a ± 2.44	49.9a ± 6.33	23.1a ± 0.991	29.0b ± 0.452	39.5ab± 4.14
Zone 2	47.7b ± 24.9	46.4ab ± 2.74	65.0ab ± 21.4	27.9b ± 3.74	29.0b ± 0.315	37.7ab ± 3.36
Zone 3	39.5ab ± 2.59	54.5b ± 23.3	59.5ab ± 2.79	25.9ab± 4.77	28.8b ± 0.616	41.2b ± 3.18
F	3.693	5.603	3.113	4.228	0.527	2.149
Sig.	P< 0.05	P< 0.05	P> 0.05	P< 0.05	P> 0.05	P> 0.05
			Alder Tree Leaves			
Zone 2	42.1ab ± 5.82	45.3ab ± 3.19	61.5ab± 5.62	32.7c ± 1.91	25.4ab ± 3.15	34.2a ± 0.95
Zone 3	54.5b ± 5.49	57.6b ± 13.4	72.3b ± 4.63	33.0c ± 1.70	21.5a ± 5.61	34.3a ± 6.43
F	17.59	4.788	16.515	0.109	2.463	0.001
Sig.	P< 0.001	P< 0.05	P< 0.001	P> 0.05	P> 0.05	P> 0.05

Mean values are shown with standard deviation **(**±), (each zone n=9, except Zone 2 for Alder n=6).

DISCUSSION

This study has shown that the chemical compositions of ash and alder fresh leaves in Karacabey floodplain forests were significantly varied with the distances from the Sea of Marmara. As much as we know, this study has released unique and pioneer results for Türkiye's coastal forested wetlands on this subject. The results indicated that both ash and alder trees near to the sea site (Zone 1) produced more photosynthetic pigments, anthocyanin and xanthophyll concentrations than those further away from the sea site (Zone 3). However, they decreased their total polyphenols, total soluble tannin concentrations compared to the trees further away from the sea site. Mean free amino acids, proline, total phenolic compound and glucose concentrations also varied with the tree species, but the trends of those chemicals were different for each tree species. Mean total nitrate, sucrose and total carbohydrate concentrations did not vary significantly with the distances for both tree species.

The amount of chlorophyll molecules in vegetative tissue may vary according to tree species, tree age, location of the leaves on the tree (shade/light), leaf age, developmental status, soil properties and climatic parameters (Li et al. 2018). Under polluted condition in terrestrial area in July, Turfan et al. (2018) showed for ash tree (*Fraxinus excelcior*) that mean chlorophyll-a varied from 0.086 to 0.113 mg/g, chlorophyll-b varied from 0.020 to 0.042 mg/g and total chlorophyll varied from 0.105 to 0.154 mg/g. However, for Ash tree species (*Fraxinus excelsior* L.) under natural conditions, Petrova et al. (2017) found much lower concentrations. They reported that mean chlorophyll-a ranged from 0.63 mg kg^{-1} to 1.29 mg kg^{-1}, chlorophyll-b varied from 0.53 to 0.94 mg kg^{-1}, and total carotenoids varied from 0.17 to 0.52 mg kg^{-1}. Parida et al. (2004c) reported for *Aegiceras corniculatum* in mangrove that chlorophyll-a varied from 0.41 to 0.55 mg/g, chlorophyll-b varied from 0.13 to 0.16 mg/g and total chlorophyll varied from 0.54 to 0.73 mg/g, total carotenoids varied from 0.11 to 0.19 mg/g and the ratio of chlorophyll a/b varied from 3.07 to 3.43. For Walnut Trees (*Juglans regia* L.) Turfan et al. (2020) found that photosynthetic pigments varied significantly with tree ages (25-, 75-, 100- and-over 400-year-old). They showed that mean total chlorophyll changed from 0.71 mg/g to 1.03 mg/g, while mean total carotenoids varied from 9.83 to 10.3 mg/g. A similar study by Turfan et al. (2019) on Oriental Beech Trees (*Fagus orientalis* L.) of different ages also showed that photosynthetic pigments increased with increasing tree ages (25-, 50-, 100-, 200- and-over 600-year-old). They showed that mean chlorophyll-a ranged from 0.147 to 0.159 mg/g, chlorophyll-b ranged from 0.128 to 0.259 mg/g, total chlorophyll ranged from 0.287 to 0.408 mg/g and total carotenoids ranged from 9.41 to 9.48 mg/g.

In this study, under water intrusion and salinity conditions, ash tree showed much higher concentrations, ranged from 0.338 to 0.443 mg/g for mean chlorophyll-a, from 0.165 to 0.304 mg/g for chlorophyll-b, from 0.503 to 0.746 mg/g for total chlorophyll and from 8.55 to 10.42 for total carotenoids. In general, the change of pigments in leaf tissue may not show a sharp decrease/increase in woody plants because the tolerance threshold of these taxa to environmental changes is generally high (Arene et al. 2020). In addition, resistant species have higher chlorophyll pigments than susceptible species (Turfan and Mese 2019). In this study, higher chlorophyll molecules in both tree species show that both species have a high tolerance to salinity. As a matter of fact, we found that the anthocyanin and xanthophyll content was also high at the nearest distance to the sea supported this result. Xanthophylls play a role in the protection of chlorophylls, especially chlorophyll-a molecule, in the chloroplast membranes. In both tree species, the chlorophyll-a/chlorophyll-b ratio was lowest at the nearest distance to the sea, but increased with distance from the sea. This indicates that the ash trees near to the sea site are under shadier area. Chlorophyll-b concentration is generally higher in shade plants or plants grown in shady conditions (Dias-Filho 2002). In our study, we noted that ash trees growing near to the sea site had higher canopy closure and also, they were protected by much taller and older oak and plane trees around the ash trees.

In addition, the decrease in chlorophyll, xanthophyll and anthocyanins in both tree species may indicate that the season progresses towards autumn and the seasonal aging of the leaves increases (Azevedo Neto et al. 2009). On the other hand, decreased chlorophyll and xanthophyll could be due to seasonal pigment degradation. So, it could be possible to say that much higher decrease in air temperatures and humidity near to the sea site may have delayed the destruction of chlorophyll or leaf senescence (Estiarte and Peñuelas 2015).

On the other hand, when it was moved away from the sea site, mean chlorophyll and anthocyanin in leaves decreased, but total phenols increased. It has been reported in the literature that total phenols accumulate more in autumn months (Xu et al 2011). We found that mean proline and amino acid concentrations in the fresh leaves were high in the environment where ash and alder trees grow. Proline controls the osmosis/turgor events by accumulating in the vegetative tissue during physiological drought conditions caused by climatic drought and soil salinity, and also preserves the integrity of cellular membranes (Hayat et al. 2012).

In our study, we found higher salinity and electrical conductivity in the soils of ash and alder trees growing near to the sea site compared to the those growing inner sites. And also, as stated previously, in the study area, annual precipitation varies according to year. Compared to the mean annual precipitation for long term (577.7 mm for the mean of last 34 years), the annual precipitation in 2019 and 2020 were lower (401.4 mm and 481.9 mm respectively), while in 2021 was higher (650.7 mm). Therefore, both higher salinity and drought conditions could be responsible for higher proline and amino acid concentrations in the leaves of the trees near to the sea site.

In fact, lower proline concentration indicates that water stress does not occur in the trees. The amount of proline was high in the fresh leaves for both tree species near to the sea site. This may have had a protective effect on the structure of the granal membranes where the pigments are localized in the chloroplasts, or it may have protected against possible damage to the salt stress of chlorophyll molecules. More accumulation of soluble amino acids in resistant species in a salty environment has been reported in detail in the literature (Azevedo Neto et al, 2009).

According to Taïbi et al. (2016), soil salinity and photosynthetic pigment levels have an inverse relationship. The amounts of chlorophyll a, b, and carotenoids are generally affected by salt levels and the interaction between salinity and different genotypes. In a study, these three photosynthetic pigments were shown to be reduced by increasing salt levels from 0 to 6 ds/m (Heidari 2012). But this was not the case in our study since mean photosynthetic pigments increased with increasing salinity, either due to ash and alder trees are more tolerant to the salinity conditions, or salt level is not enough to cause those variations in the fresh leaves.

There was no significant variation in mean total nitrate concentration, while it should decrease with salinity. This result is attributed to the fact that proximity to the sea reduces the humidity and temperature in the air. Since ash tree is a very dark green leafy species, higher nitrate levels may be expected in this species compared to alder. Nitrate is a necessary molecule in the synthesis of chlorophyll and its amount in plant tissue is reflected in the chlorophyll content (Hudson et al. 2011).

It is believed that increase in secondary metabolites synthesis in response to stressful conditions protect the cellular structures against oxidation (Chanwitheesuk et al. 2005). In general, environmental stresses (biotic and abiotic) such as salinity lead to accumulation of polyphenol constituents (Dixon and Palva 1995). Ksouri et al. (2007) showed that the polyphenols content and antioxidant activity of leaves of the halophyte Cakile maritime were increased by salinity. Parida et al. (2004c) reported the accumulation of polyphenols in moderate salinity in mangrove *for Aegiceras corniculatum*, ranged from 2.4 in control to 4.6 mg/g in 250 mM NaCl treated plants. In our study, there was no positive correlation between soil salinity (as soil pH and EC) and contents of total polyphenols or total phenolic compounds. This could be attributed to the salt concentration in the soil or water since a number of authors showed that salt concentration can have different impact on the phenolic contents in the plants. For example, Falleh et al. (2008) showed that leaf phenolic content was significantly increased at 25–50 mM NaCl, and decreased at 150 mM NaCl for *Cynara cardunculus* L. (cardoon). So, further studies are in need to investigate the relationships between the biochemical composition of leaves and salinity in Karacabey floodplain forests.

Phenolic compounds accumulate in fruits, flowers and leaves at certain wavelengths of the sun and generally in autumn. In early spring, buds are more abundant than mature leaves, so leaf buds are not green in the early stages of their development, but are of different colours. The fact that ash leaves with higher phenol concentrations could be related to the dark leaves of this species. Depending on the season and the wavelength change of sunlight, chloroplasts in leaves turn into chromoplasts and the ratio of non-photosynthetic pigments increases instead of chlorophylls in leaves. As we all know, this leads to a magnificent riot of colours in autumn.

There was no significant change in the glucose, sucrose and total carbohydrate contents of the trees with the distances from the sea site. This indicated that the metabolic reactions in the trees slowed down and the season passed into the autumn. The accumulation of assimilate in the leaves decreased and therefore the energy requirement also decreased. But there was a partial increase in total carbohydrates of the leaves of ash tree as it moved away from the sea. This shows that the respiratory rate increased in these leaves. Probably a partial lack of water may have been observed as it moved away from the sea.

It appeared that the amounts of chemical composition in the fresh leaf samples were more affected by the season. In the study sites, the direction of the wind was in general NNE, and the weather was cooler. This indicated that there was no temperature or light intensity causing the destruction of the pigment. In addition, in our study, the fresh leaf samples were collected at the end of August. This meant that the season was passing into the autumn when the metabolic reactions in the trees slowed down. In order to test the effects of the season on the amounts of chemical composition in the fresh leaf samples of alder and ash tree, we also carried out another study in Autumn (April) which supports our findings that season has significant impact on the chemical composition in the fresh leaf samples of alder and ash tree (not published data).

The fact that the soil salinity amount in our study was below the limit value (2 dS/m) that could generally harm the plant may have prevented the differences in the chemical composition of alder and ash leaves from being seen more clearly due to salinity. However, previous studies have reported that the surrounding lagoon lakes, underground waters and Marmara Sea water feeding the Karacabey Floodplain forests are quite salty (Akbulut and Tavsanoglu 2018, Sariyildiz et al. 2022). In addition, studies reported that seasonal sampling had a significant effect on soil and water salinity rates (Akbulut and Tavsanoglu 2018). Therefore, in the future, there is a need for a more detailed study on the relationship between the effects of seasons on the salinity of Karacabey Floodplain forest soils, soil surface and groundwater, and the biochemical structure of leaves.

In conclusion, the results have shown that the chemical composition of ash and alder trees vary significantly with distance away from the sea. These variations could be related to the changes in soil pH and electrical conductivity, but also to the seasonal transitions, chemical elements, tree species, regular flooding. Therefore, more detailed studies are needed to better understand the factors. The results also indicate that both tree species, especially ash trees, are salt-resistant plants that they try to adapt to the saline environment through the changes in the physiological processes that make them to some extent resist salt stress. However, with the continued intrusion of seawater and more increase in salinity, those trees could not be able to withstand under very high salt concentrations for long periods. Particularly, the irregularity in annual precipitation can change the balance between fresh water and intrusion of seawater, and thus results in more damage to the alder and ash trees in the Karacabey floodplain forests. Therefore, the necessary measures must be taken to prevent or reduce the intrusion of seawater into the Karacabey floodplain forests and so preserve this important natural landmark from perdition.

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