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Comparative study of biochemical characteristics of halophytes from two different habitats

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Abstract

The worldwide problem of soil salinization may be solved by amelioration of saline soils, or by cultivation and selection of salt resistant plants. That's why investigation of wild halophytes gains popularity. One of the approaches in salt-resistance studies is investigation of morphological, physiological and biochemical mechanisms of adaptation to salt stress. From this point of view, halophytes growing in Georgia are practically unexplored. The purpose of the study was to investigate the activity of enzymes (peroxidase and nitrate reductase), and content of low molecular antioxidants (proline, soluble phenols, anthocyanins, carotinoides and ascorbic acid), as well as nitrates, soluble carbohydrates and total proteins in leaves of salt resistant species growing in different salty habitats of Georgia - the costal zone and near-by territory of Kumisi (east Georgia, kvemo Kartli) and Sakhare-Kapanadze lakes (east Georgia, gare Kakheti). Titration (ascorbic acid) and spectrophotometric methods were applied for investigations. There may be distinguished some biochemical indices, in spite of the salt exchange mechanism type of tested species, which changed by the same regularity following habitats: content of ascorbic acid (1.3-2.5 times, $p<0.05$), total phenols (1.2-3.6 times, $p<0.05$) and soluble carbohydrates (2-times and more, $p<0.05$) was higher in species of Sakhare-Kapsanadze lakes, compared to Kumisi same species; while in all species of Kumisi habitat content of total proteins was higher (2.7-5 times, $p<0.05$). Generally, adaptation of the tested species to studied habitats was much or less of individual character.

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Introduction

Soil salinization is the concominant result of man's agricultural activity since ancient times. According to archeological data soil salinization became even the reason for vanishement and replacement of some civilizations (Gulie *et al.,* 2014). Following the UN Food and Agricultural Organization (FAO), more than 20% of the world's agricultural area and half of the irrigated territory are saline (Gogue, 2014). Problem of soil salinization is popular in Georgia as well. The total area of saline soils is about 1.6% here (Urushadze and Blum, 2014).

The problem may be solved by amelioration of saline soils, or by cultivation and selection of salt resistant plants. That's why investigation of wild halophytes, which made only 2% of the earth' total flora, gains popularity (Dajic, 2006). Halophytes are cultivated as food and for phytoremediation purposes of heavy metal-polluted soils (Manousaki and Kalogerakis, 2011; Ventura and Sagi, 2013).

One of the approaches in salt-resistance studies is investigation of morphological, physiological and biochemical mechanisms of adaptation to salt stress. From this point of view, halophytes growing in Georgia are practically unexplored.

Accordingly, the purpose of the study was the investigation of the peculiarities of adaptation of different salt resistant species, growing on the costal zone and near-by territory of Kumisi (east Georgia, kvemo Kartli) and Sakhare-Kapanadze lakes (east Georgia, gare Kakheti), by exploration of enzymes (peroxidase and nitrate reductase) activity, and low molecular antioxidant content (proline, soluble phenols, anthocyanins, carotinoides and ascorbic acid), as well as nitrates, soluble carbohydrates and total proteins, in leaves of total antioxidant activity of leaves has been investigated as well.

Material and methods

Plant material

Plants species: *Suaeda altissima* (L.) Pall.- sea-blite, *Petrosimonia brachiata* (Pall.) Bunge (Salt-accumulating – euhalophytes), *Chenopodium album* L. – goosefoot (salt-releasing – crynohalophyte), *Artemisia lerchiana* (Web.) – sagebrash, and *Adonis bienertii* (Butkov ex Riedl.) – peasant's eye (salt-unpermeaple glycohalophytes) were used as test-objects. Material was picked on the costal zone (15-20m width line) of Kumisi (east Georgia, kvemo Kartli), Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes, at the end of August and beginning of September.The total content of salts near the costal zone of Kumisi lake is about 4.8%, and 4.5% - near Sakhare and Kapanadze lakes.

Sites of plants sampling

Sakhare and Kapanadze lakes are situated near the village Udabno, on the Iori plateau, at 822m above sea level. The climate here is dry subtropical, steppe type, with moderately cold winter and hot summer. The average annual temperature is +10.4^{o}C. The coldest month is January (with absolute minimum - 12^{o} - -14^{o}C), and the warmest are July and August (+22.2^{o}C, maximal - +37^{o}-+39^{o}C). The annual amount of precipitations is 434mm, which is not enough for agricultural crops. Black-alkaline and saline soils of meadow are characteristic for semi desert and dry valleys of the Iori plateau. Sakhare and Kapanadze lakes contain high amount of Glauber's salt (sodium sulphate) (Apkhazava, 1975; Saakashvili *et al.*, 2011).

Kumisi lake is situated between Iagludgi upland and Tsalaskure lowland, on the sauth-east of village Kumisi, at 475m a.s.l. It is the artificial salty lake in east Georgia (kvemo Kartli). The climate here is moderately humid, subtropical, with cold winter and hot summer. The average annual temperature varies between 13^{o}- 6^{o}C, while the average temperature of January (the coldest month of a year) is 0.3^{o} - -5^{o}C, and of the hottest month – July –11^{o}-25^{o}C. The mean annual amount of precipitations makes about 420mm. Soils of the lake's surrounding are gray-brown alkaline, with chloride-sulphate and sulphate-chloride-sodium salinity (Apkhazava, 1975).

Peroxidase

Activity of peroxidase was determined spectrophotometrically. The method is based on the determination of optical density of guaiacol oxidation productes at 470nm (SPEKOL 11, KARL ZEISS, Germany) and is expressed in conditional units per one gramm of fresh weight (Ermakov, 1987).

Nitrate reductase

Determination of nitrate reductase activity is based on determination of nitrites amount, obtained because of nitrate reductase reaction with infiltrated nitrates (Ermakov, 1987).

Nitrates

After the water-extraction of 500g of plant material (homogenized for 30min at room temperature), it was filtered. Hydrogene peroxide was added to 10ml of the filtrate and evaporated. disulphophenolic acid was added to the obtained sediment and optical density was determined at 410nm (SPEKOL 11, KARL ZEISS, Germany) (Danilova, 1963; Pleshkov, 1985).

Proline and soluble carbohydrates

0.5 g of dry leaves were mashed in 10ml of 3% sulphosalicylic acid and filtered. 2 ml of the filtrate was added to 2 ml of acid ninhydrin and 2 ml of ice acetic acid. After 1 h exposition on a water bath, the extract was cooled and added with 4 ml of toluene and divided in a separating funnel. Optical density of upper layer was measured on a spectrophotometer at 520 nm (SPEKOL 11, KARL ZEISS, Germany) (Bates *et al.,* 1973).

Anthrone reagent was used for determining the content of soluble carbohydrates (Turkina and Sokolova, 1971). To 100 mg of air-dry leaf material was added 96° alcohol for extraction (3-fold). The total amount of the obtained extract was evaporated on a water bath and dissolved in 5 ml of distilled water. To 0.5 ml of the tested water extract was added 2ml of anthrone reagent and heated in a water bath for 10 min. After this procedure, the test-tubes were placed in a cold water bath and 15 min later the optical density of the solution was measured at 620 nm with a spectrophotometer (SPEKOL 11, KARL ZEISS, Germany).

Soluble phenols

A 0.5 g of fresh leaves was boiled in 80% ethanol for 15 min. After centrifugation the supernatant was saved, and residues of leaves were mashed in 60% ethanol and boiled for 10 min. Obtained extract was added to the first supernatant and evaporated. The sediment was dissolved in distilled water. One ml of the received solution was added with the Folin-Ciocalteu reagent and optical density was measured at 765 nm. The chlorogenic acid served as control (Ferraris *et al.,* 1987).

Anthocyanins

20ml of ethanol, acidified with 1% HCl (99:1) was added to 100g of cut leaves and after retention in dark for 24h, the optical density of the extract was measured at 540nm (SPEKOL 11, KARL ZEISS, Germany) (Ermakov, 1987).

Chlorophylls and carotenoids

Fresh leaves of tested plants (100-200mg) were mashed with quartz sand and calcium caebonate. The mashed material was washed with ethanol. The optical density of the obtained extract was measured on spectrophotometer (SPEKOL 11, KARL ZEISS, Germany). Concentration of pigments was calculater by Wintermanns's formula (Gavrilenko *et al.,* 1975).

Total proteins

Content of total proteins was determioned after Lowry (1951).

Ascorbic acid

A titration method was used to measure the content of ascorbic acid. 2 g of fresh leaf material was mashed in 15 ml of 2% hydrochloric acid and 10 ml of 2% metaphosphoric acid, and filtered. One ml of the filtrate was added to 25 ml of distilled water and titrated with a 0.001 M solution of dichlorphenolindophenole (Ermakov, 1987).

Total antioxidant activity

This index was measured by modified method using diphenyl-picryl-hydrazyl (DPPH) (Koleva *et al.* 2002). 200 mg of experimental powder was extracted two times with 96° ethanol. Obtained extract was evaporated on a water bath and the remained sediment was dissolved in 10ml of water-alcohol mixture. 0.01 ml of the obtained solution was added with 4ml of 40 µM DPPH solution and after 30 minutes of incubation in the dark the optical density was measured at 515 nm by the spectrophotometer (SPEKOL 11, KARL ZEISS, Germany). The percent of inhibition was calculated.

Statistical analysis

Fully expanded leaves from 5-7 plants were picked for analysis. Analyses were made in three biological replicates. One way ANOVA and Tukey's multiple comparison tests were used to analyse differences between the means. All calculations were performed using statistical software Sigma Plot 12.5.

Results and discussion

Nitrates, nitrate reductase and total proteins

Low content of nitrates was revealed in all tested plants of both habitats (Fig.1).

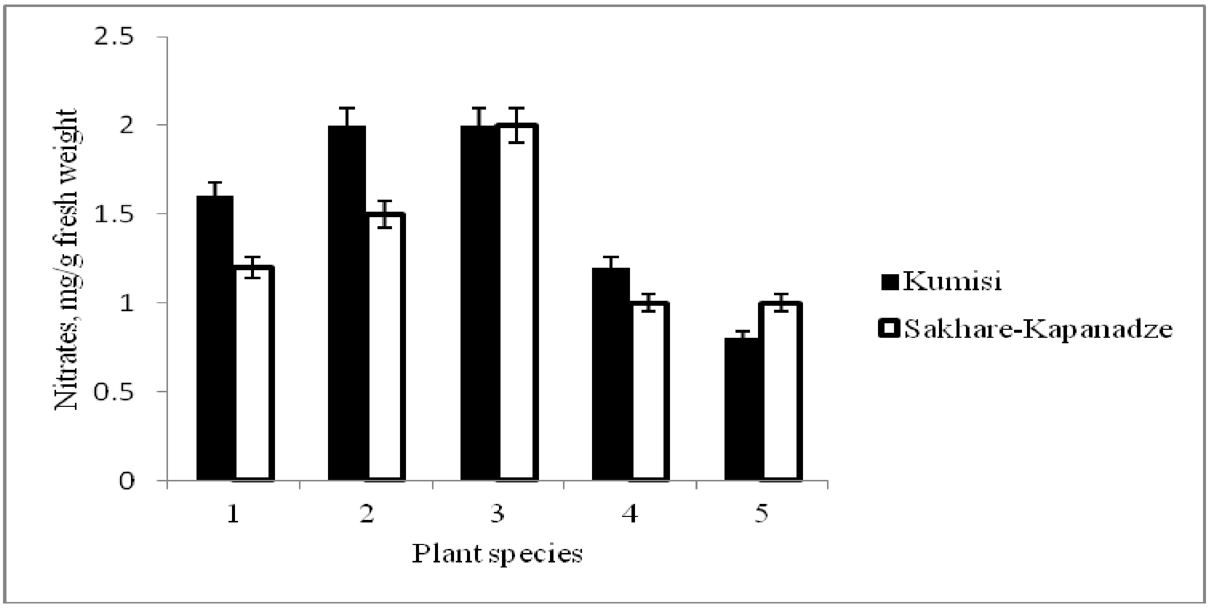

Fig. 1. Content of nitrates in leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite ($p>0.05$) 2. Petrosimonia brachiata (Pall.) ($p>0.05$) 3. *Chenopodium album* L. – goosefoot ($p>0.05$) 4. *Artemisia lerchiana* (Web.) – sagebrush ($p>0.05$) 5. *Adonis bienertii* (Butkov ex Riedl.)-peasant's eye ($p>0.05$).

Statistical difference by this index between one and the same species of studied habitats was not established ($p>0.05$).

Associated with high salinity, osmotic stress is the reason for ion toxicity and nutritional misbalance in plant, due to disorder in nitrates uptake or retention of their reduction, and is connected with nitrate reductase activity as well (Sergeichik and Sergeichik, 1988).

This situation negatively reflects on the metabolism of nitrate-containing substances. However, it has been established that high concentration of salts stimulates nitrates uptake by halophytes, and is associated with nitrate reductase activation (Marschner, 1995).

Low content of nitrates in leaves of studied halophytes may be associated with salt stress. However, according to nitrate reductase activity it may be concluded that the stress was not so severe to affect growth and development of experimental plants.

While comparing the nitrate reductase activity of same species from different habitats, no statistical differences were revealed in euhalophytes – sea-blite and petrosimonia, and glycohalophyte sagebrush ($p>0.05$) (Fig.2). Differences following habitats by this index were revealed in goosefoot and peasant's eye.

Moreover, in case of peasant's eye index of Kumisi individuals prevailed over the results of Sakhare-Kapanadze lakes (1.2times, $p<0.05$); While in case of goosefoot, in contrary, activity of the enzyme was higher in Sakhare-Kapanadze lakes individuals (1.7times, $p<0.05$) (Fig. 2).

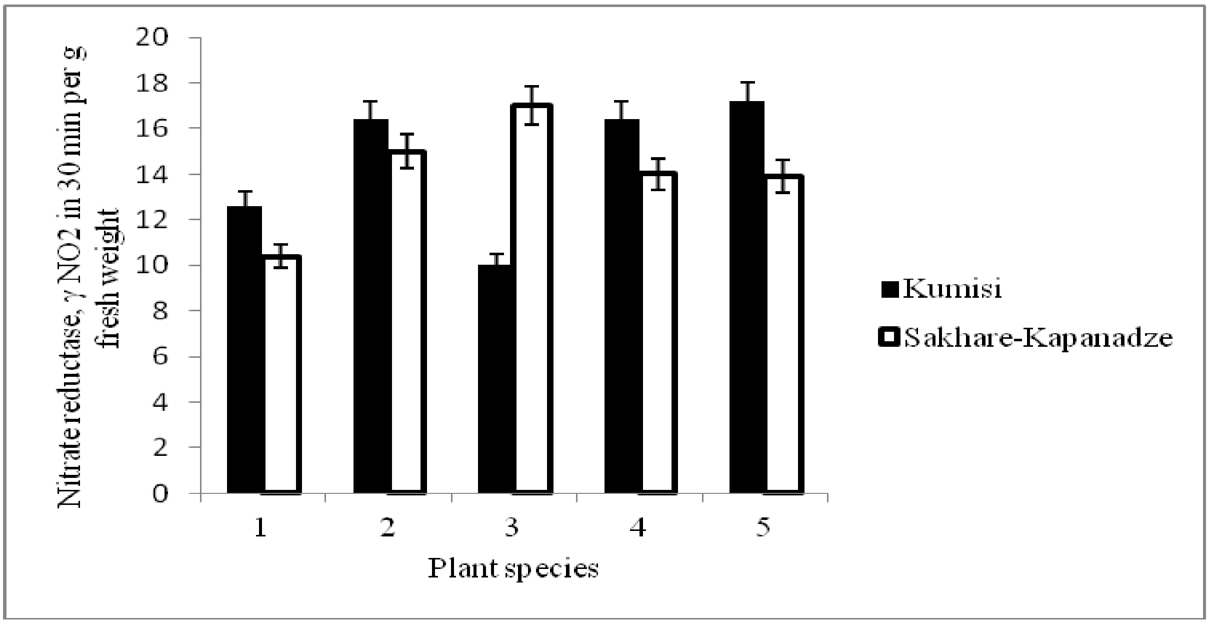

Fig. 2. Nitrate reductase activity in leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite ($p>0.05$) 2. *Petrosimonia brachiata* (Pall.) ($p>0.05$) 3. *Chenopodium album* L. – goosefoot ($p<0.05$) 4. *Artemisia lerchiana* (Web.) – sagebrush ($p>0.05$) 5. *Adonis bienertii* (Butkov ex Riedl.)-peasant's eye ($p<0.05$).

Nitrate reductase (NR, E.C. 1.6.6.1) plays an essential role in nitrogen metabolism and general development of plant. Thus, its activity gives significant information on plant's growth and development and productivity (Garg and Singla, 2005). Inhibitory effect of salinity on nitrate reductase activity has been established for many plant species (Katiyar and Dubey, 1992; Khan, 1996; Garg *et al.,* 1997).

Generally, nitrate reductase functioning in all studied species of both habitats was enough for plants further growth and development, which was reflected on nitrates effective uptake and their further metabolism as well. Amount of total proteins is one of the significant characteristics of nitrate metabolism.

Among the tested species content of total proteins was higher in leaves of glycohalophytes (sagebrush, peasant's eye), compared to other halophytes (Fig. 3). Moreover, Kumisi species significantly prevailed by this index representatives of the same species growing on Sakhare-Kapanadze lakes (2.7-5 times, $p<0.05$).

According to literary data in stress-tolerant and -adapted plants content of total proteins increases under the influence of different stresses (Kosakivska *et al.*, 2008; Kamal *et al.,* 2010), due to synthesis of various type (regulatory, protective, distcutors of damaged macromolecules hydrolases) proteins in cell. Among them are shock proteins (Munns and Tester, 2008), which take an active part in cell protection against the oxidative stress (Shao *et al.,* 2008; Gill and Tuteja, 2010).

Experimental results make possible to suppose that in Kumisi plants most part of the uptaken nitrogen was used for proteins synthesis. Moreover, proteins synthesis was more intensive in glycohalophytes (sagebrush, peasant's eye) of both habitats, compared to other species.

Different intensity of proteins synthesis in same plant species occupying different habitats with the same salinity may be considered as one of the demonstrations of flexibility and variability of adaptive reactions. Presumably, high intensity of proteins synthesis in Kumisi individuals is one of the manifestations of adaptation to the stress conditions of Kumisi.

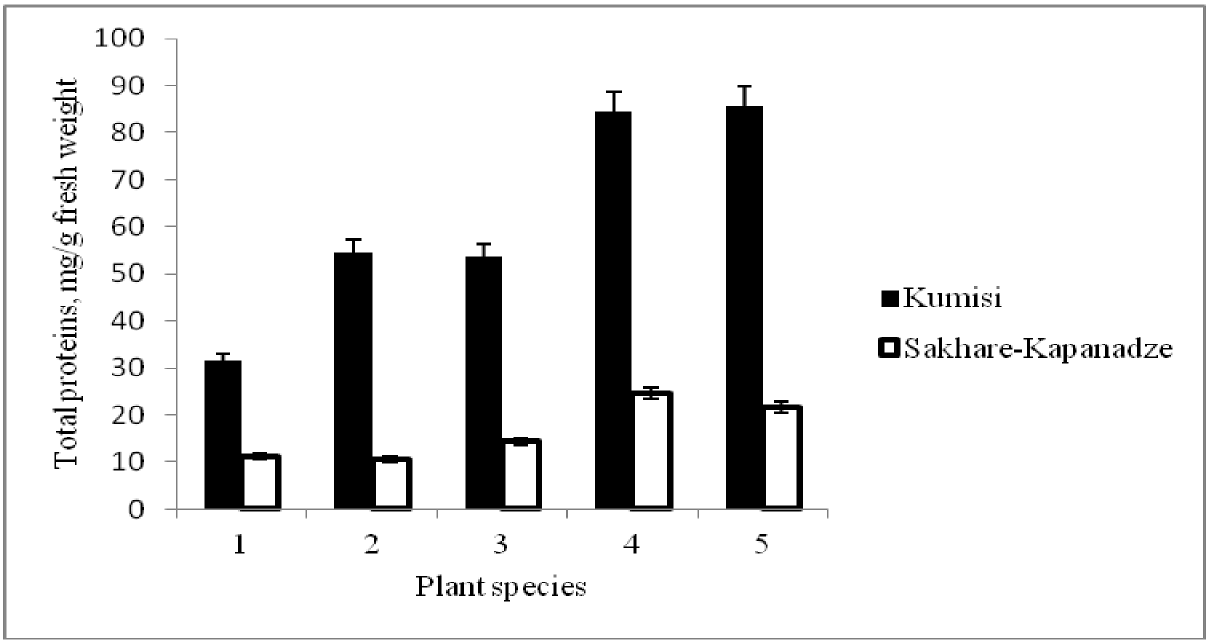

Fig. 3. Content of total proteins in leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite ($p<0.05$) 2. *Petrosimonia brachiata* (Pall.) ($p<0.05$) 3. *Chenopodium album* L. – goosefoot ($p<0.05$) 4. *Artemisia lerchiana* (Web.) – sagebrush ($p<0.05$) 5. *Adonis bienertii* (Butkov ex Riedl.)- peasant's eye ($p<0.05$).

Water-soluble proteins rise resistance of the protoplasm and thanks to hydrophylity, are responsible for water retention and water-binding ability of the cell (Orlova *et al.*, 2007).

The mechanism of adaptation to the stress conditions of Sakhare-Kapanadze lakes of the same plant species seems different.

Low intensity of proteins synthesis here may be explained by involvement of the up taken nitrogen in non-protein nitrogen-containing substances, possessing the osmoprotective role. It is known that such types of substances in plants of chenopodiaceae family are glycine-betains (Mc Neil *et al.*, 1999).

Peroxidase

Clear regularity of peroxidase activity following the habitats was not revealed in our observations. Among the tested plants of both habitats the high activity of the enzyme was revealed in leaves of goosefoot and sagebrush growing in Kumisi (Fig. 4). By peroxidase activity petrosimonia growing here prevailed the same species of Sakhare-Kapanadze lakes as well (3.7-times, $p<0.05$).

As for sea-blite and peasant's eye, in contrary, results of peroxidase activity were higher in Sakhare-Kapanadze lakes samples, compared to Kumisi results (1.5 and 1.2-times respectively, $p<0.05$).

Peroxidases (EC 1.11.1) are representatives of the big family of enzymes, which are met everywhere in the cell and have diverse functions in plant metabolism (*Passardi et al.,* 2005). In particular, they play an active role in adaptation to unfavorable conditions, plant growth and development, regulation of auxins and phenolics content, etc. (Andreeva, 1988; Cevahir, 2004; Graskova *et al.,* 2010).

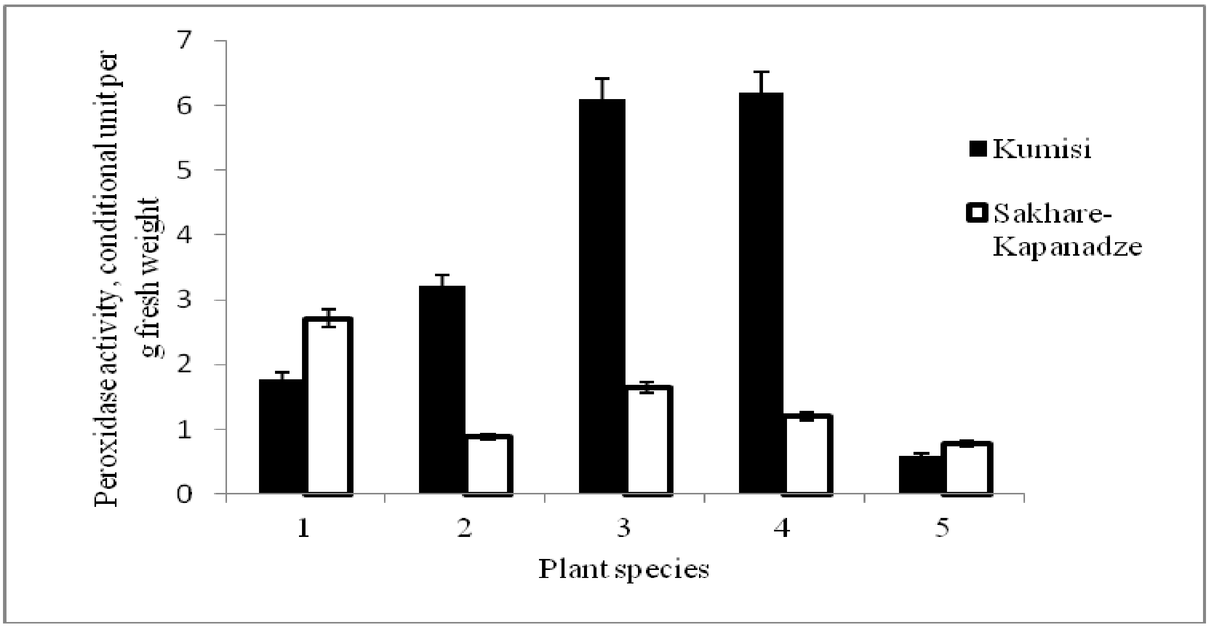

Fig. 4. Activity of peroxidase in leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite ($p<0.05$) 2. *Petrosimonia brachiata* (Pall.) ($p<0.05$) 3. *Chenopodium album* L. – goosefoot ($p<0.05$) 4. *Artemisia lerchiana* (Web.) – sagebrush ($p<0.05$) 5. *Adonis bienertii* (Butkov ex Riedl.)-peasant's eye ($p<0.05$).

Increase of the activity of peroxidase under high salinity conditions, as one of the manifestations of adapting to stress, has been proved by many authors (Bhutta, 2011; Zhang *et al.,* 2013; Bagheri, 2014).

According to experimental results it may be supposed that in studied habitats salinization is not a limiting factor, which demonstrates a clear impact on peroxidase activity. On the background of other abiotic stressors (solar ultraviolet irradiation, high temperature), which affected the studied species in combination with the salt stress, variation of peroxidase activity was of individual character.

Ascorbic acid, chlorophylls and carotenoids

Functional condition of the photosynthesis apparatus may be evaluated by the content of chlorophylls and carotenoids (Lichtenthaller, Buschmann, 2001). Opinions on the salinity influence on chlorophylls content are contradictious (Boestfleisch *et al.,* 2014; Munns and Tester, 2008).

In our early experiments increase of salinity caused enhancement of chlorophylls content in crynohalophytes and its decrease - in glycohalophytes. Generally under the same salinity conditions, content of chlorophylls was lower in glycohalophytes, compared with eu- and crynohalophytes (Chkhubianishvili *et al.,* 2016).

Our experimental results demonstrate high content of chlorophylls in euhalophytes (sea-blite, petrosimonia) and glycohalphyte peasant's eye of Sakhare-Kapanadze lakes habitat, compared to the same species of Kumisi (2, 1.3 and 1.2-times respectively, $p<0.05$) (Fig.5). While in goosefoot and sagebrush in contrary, Kumisi individuals prevailed by this index (1.4-times, $p<0.05$). Diverse content of chlorophylls in one and the same species, growing in different habitats with the same salinity in recent observations, once again proves the fact that organisms adapt to a complex of factors and not to a particular stressor. In spite of similarity, the studied habitats presumably possess many microclimatic peculiarities, which are responsible for diverse adaptive reactions of the same species at the given moment.

Content of carotenoids by habitats in tested plants changed by the same regularity as chlorophylls did (exception was goosefoot. Here the indices of both habitats were similar) (Fig.6). Increase of carotenoids in the pigment complex must play chlorophylls protecting role against photooxidation and free radicals damaging effect, formed as a result of salinity stress (Fiedor and Burda, 2014).

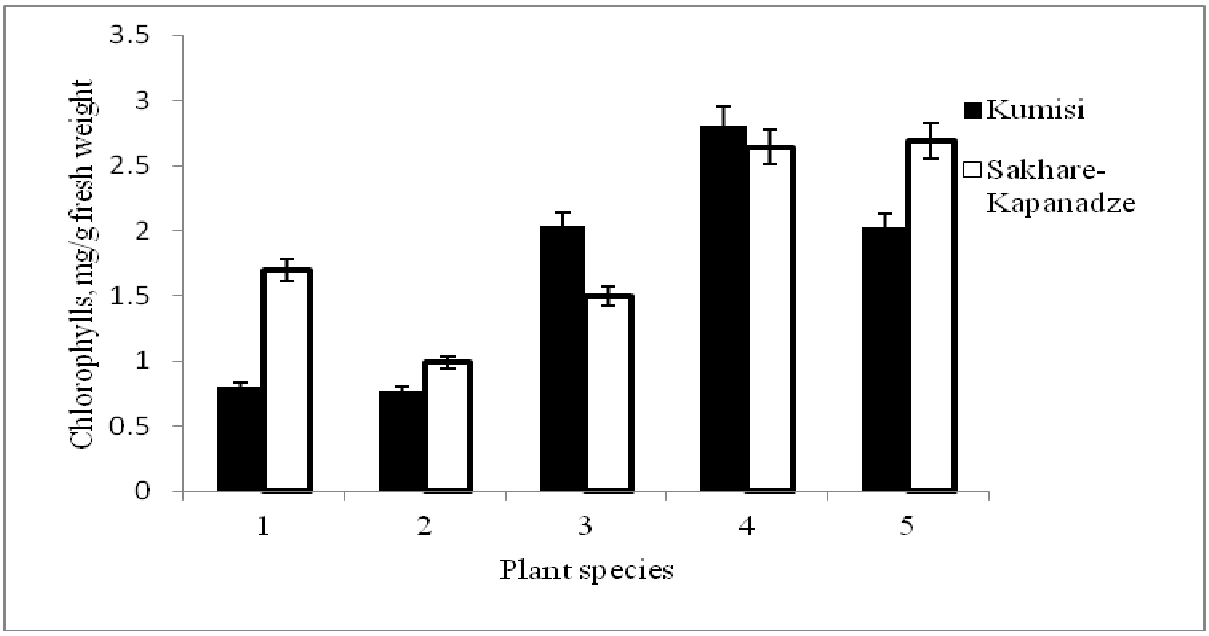

Fig. 5. Content of chlorophylls in leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite ($p<0.05$) 2. *Petrosimonia brachiata* (Pall.) ($p<0.05$) 3. *Chenopodium album* L. – goosefoot ($p<0.05$) 4. *Artemisia lerchiana* (Web.) – sagebrush ($p<0.05$) 5. *Adonis bienertii* (Butkov ex Riedl.)-peasant's eye ($p<0.05$).

Not only is the total amount of photosynthetic pigments significant for the functional activity of photosynthetic apparatus, but carotenoids to chlorophylls ratio as well. This index shows the cross-ratio of the reaction center of photosynthetic apparatus and chloroplasts' light harvesting antenna (Rozentsvet *et al.,* 2013; Kuznetsova *et al.*, 2014). In our observations, the index of carotene to chlorophyll ratio by habitats changed following the same regularity as the content of these pigments did (Fig.7). According to these data it may be supposed that the photosynthetic apparatus of euhalophytes of Sakhare-Kapanadze lakes is functionally more effective, compared to the same species of Kumisi. While in case of glycohalophytes, the picture is controversial.

It is well-established that the main neutralizer of the active forms of oxygen in plant chloroplasts, and not only there, is the ascorbate-glutathione cycle.

The key enzyme of the cycle is ascorbate perozidase. The latter's activity is greatly dependent on the quantity of ascorbic acid (Caverzan *et al.,* 2012).

Existing data prove the significance of ascorbic acid as of polyfunctional metabolite, which besides the signaling function, is responsible for plant protection against different stresses (heavy metals, salinization, temperature, UV-irradiation, etc.) by switching on the corresponding genes (Vwioko *et al.,* 2008; Zhang *et al.,* 2012).

According to literary data increase of ascorbic acid content raises resistance to salinity stress (Hemavathi *et al.,* 2010; Zhang *et al.,* 2012).

Among the tested species, the highest quantity of ascorbic acid was discovered in leaves of peasant's eye (Fig. 8).

Moreover, comparison of the obtained results by habitats did not reveal any statistical difference ($p>0.05$). In other experimental plants content of ascorbic acid was higher in Sakhare-Kapanadze lakes individuals, compared to Kumisi same species (1.3-2.5 times, $p<0.05$).

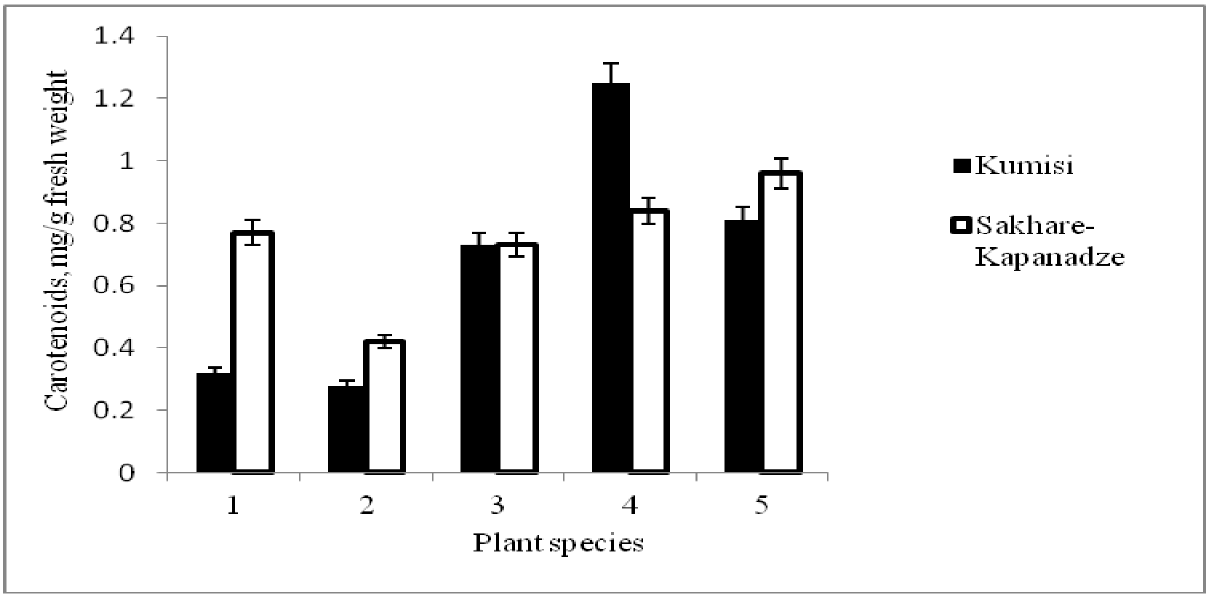

Fig. 6. Content of carotenoids in leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite ($p<0.05$) 2. *Petrosimonia brachiata* (Pall.) ($p<0.05$) 3. *Chenopodium album* L. – goosefoot ($p>0.05$) 4. *Artemisia lerchiana* (Web.) – sagebrush ($p<0.05$) 5. *Adonis bienertii* (Butkov ex Riedl.)-peasant's eye ($p<0.05$).

If we generalize the results obtained for peroxidase, ascorbic acid and chlorophylls, it may be supposed that high activity of peroxidase in goosefoot and sagebrush growing in Kumisi is responsible for better protection of the photosynthetic apparatus from stress conditions. This situation was reflected on the increased content of chlorophylls. In other experimental species growing in Sakhare-Kapanadze lakes the same role possessed ascorbic acid, proved by increased amount of chlorophylls in these plants.

However, finally, thanks to effective functioning of glutathione-ascorbate cycle and enzymatic antioxidative system growth-and development of the experimental plants of both habitats was proceeding successfully.

Diversely from chloride-sulphate and sulphate-chloride salinity of Kumisi lake, Sakhare-Kapanadze lakes are distinguished by the excess of sodium sulphate, i.e sulpate-ions. Excess of sulphate-ions presumably support increase of glutathione content in plants, as of significant antioxidant (Gallardo *et al*, 2014).

This will positively affect activity of ascorbate-glutathione cycle and consequently, plant's salt resistant ability. This may serve as explanation for increased content of ascorbate in plants, growing at Sakhare-Kapanadze lakes, which from its side positively influenced the functioning of their photosynthetic apparatus.

Proline and soluble carbohydrates

Accumulation of proline and soluble carbohydrates is one of the effective mechanisms of physiological adaptation to salinity (Kafi *et al.,* 2003).

Ptoline and soluble sugars, which generally accumulate in cell under the influence of different unfavorable factors, are universal osmolites. In case of desiccation, they protect the protein-lipid components of the membrane from denaturation (Franco and Melo, 2000; Szabados and Savoure, 2010).

The polyfunctional role of proline under stress conditions comprises osmoregulation, antioxidant, energetic, protein stabilizing and other functions, responsible for retention of cell's homeostasis (Kuznetsov *et al.*, 1999; Kartashov, 2013).

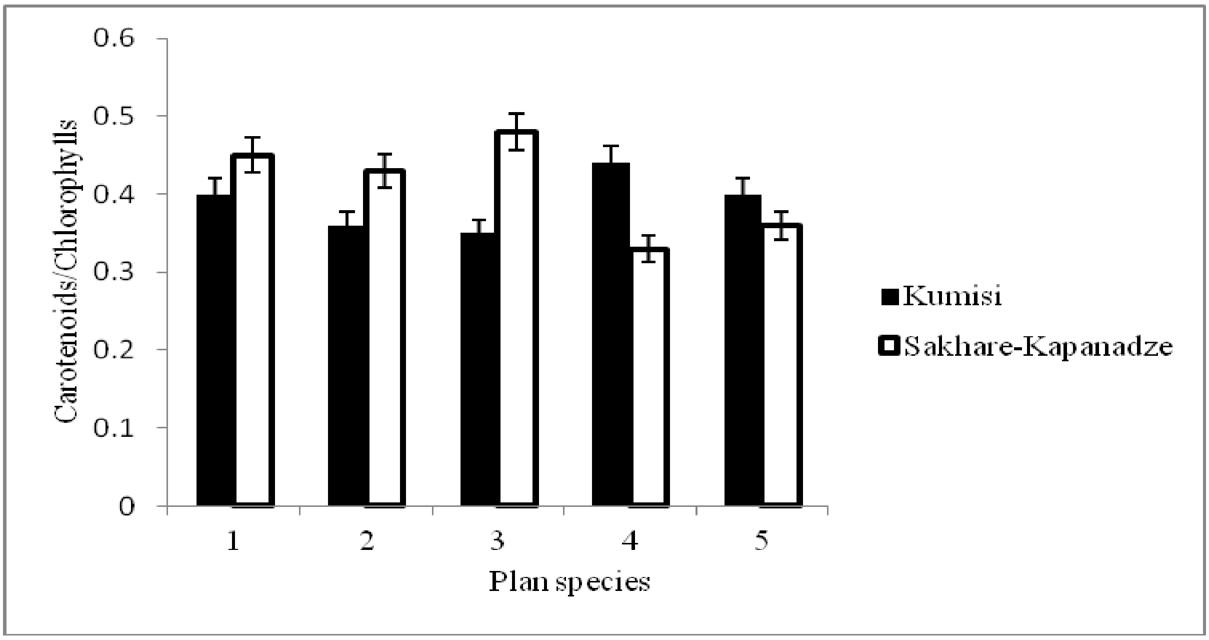

Fig.7 . Carotenoids to chlorophylls ratio in leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite ($p<0.05$) 2. *Petrosimonia brachiata* (Pall.) ($p<0.05$) 3. *Chenopodium album* L. – goosefoot ($p<0.05$) 4. *Artemisia lerchiana* (Web.) – sagebrush ($p<0.05$) 5. *Adonis bienertii* (Butkov ex Riedl.)-peasant's eye ($p<0.05$).

Carbohydrates create a low water potential in the cell, which is necessary for water uptake from highly mineralized soil solution (Eriomchenko *et al.,* 2013). Soluble carbohydrates are associated with those metabolic pathways, which cause formation of the active oxygen species (AOS) as well.

That's why they play significant role in neutralization of AOS (Couee *et al.*, 2006). Some authors point to accumulation of soluble carbohydrates under different stresses in various parts of a plant (Prado *et al.,* 2000; Mohammadkhani and Heidari, 2008).

Among the tested plants euhalophytes (seablite, petrosimonia) growing in Kumisi accumulated more soluble sugars, compared to other species (Fig. 9).

In Sakhare-Kapanadze lakes species high content of sugars was established in sagebrush, together with euhalophytes. While comparing same species of the two habitats, it becomes clear that Sakhare-Kapanadze lakes plants significantly prevailed their analogue species by content of soluble carbohydrates (2-times and more, $p<0.05$) (Fig. 9).

Content of proline was low in euhalophytes of both habitats, compared to glycohalophytes (Fig. 10). Among the tested species especially high was content of proline in Kumisi goosefoot, sagebrush and peasant's eye.

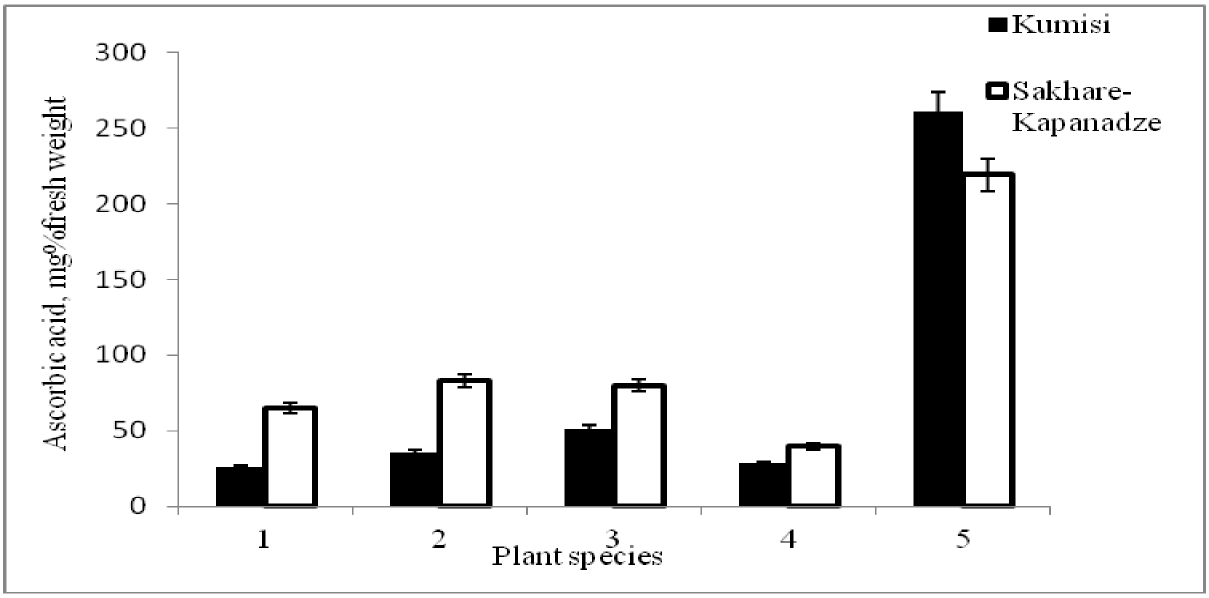

Fig. 8. Content of ascorbic acid in leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite ($p<0.05$) 2. *Petrosimonia brachiata* (Pall.) ($p<0.05$) 3. *Chenopodium album* L. – goosefoot ($p<0.05$) 4. *Artemisia lerchiana* (Web.) – sagebrush ($p<0.05$) 5. *Adonis bienertii* (Butkov ex Riedl.)-peasant's eye ($p>0.05$).

High content of proline in Kumisi goosefoot, sagebrash and peasant's eye may be indication to main osmoregulatory role of proline in these species under Kumisi conditions. While osmoregulatory function in euhalophytes (seablite, petrosimonia) here play soluble carbohydrates. As for Sakhare-Kapanadze lakes species, according to obtained results it may be concluded, that soluble carbohydrates play the leading osmoregulating function in all studied plants here.

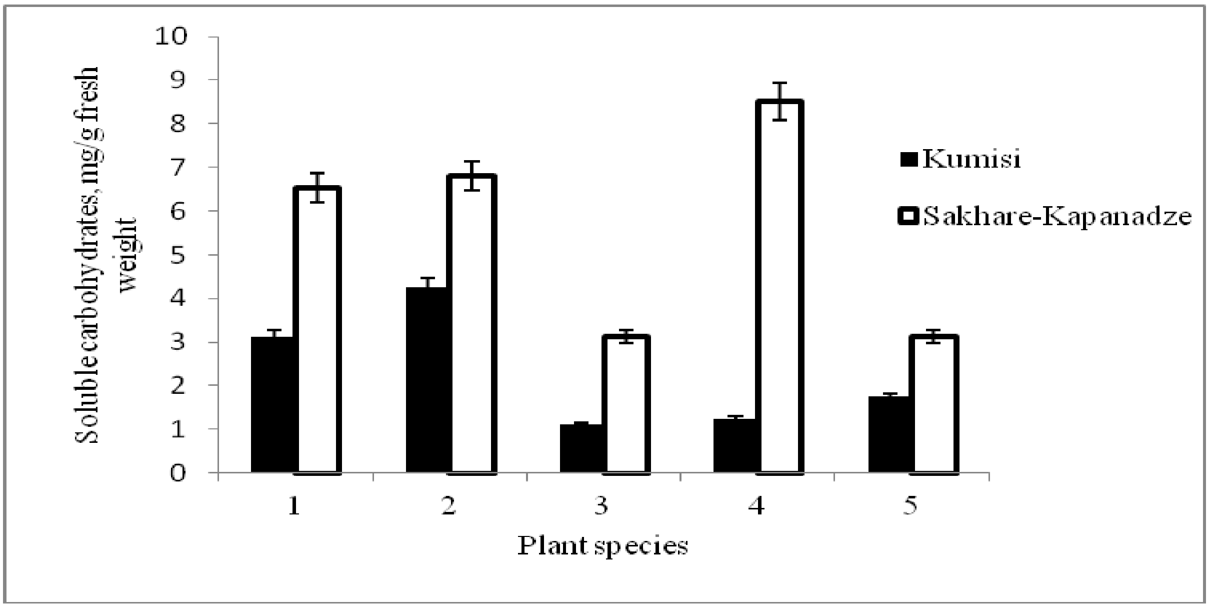

Fig. 9. Content of soluble carbohydrates in leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite ($p<0.05$) 2. *Petrosimonia brachiata* (Pall.) ($p<0.05$) 3. *Chenopodium album* L. – goosefoot ($p<0.05$) 4. *Artemisia lerchiana* (Web.) – sagebrush ($p<0.05$) 5. *Adonis bienertii* (Butkov ex Riedl.)-peasant's eye ($p<0.05$).

Soluble phenols

The highest content of phenolic substances was discovered in leaves of seablite and the lowest – in leaves of petrosimonia and peasant's eye (Fig.11). Generally species of Sakhare and Kapanadze lakes prevailed the same species of Kumisi by this index (1.2-3.6 times, $p<0.05$).

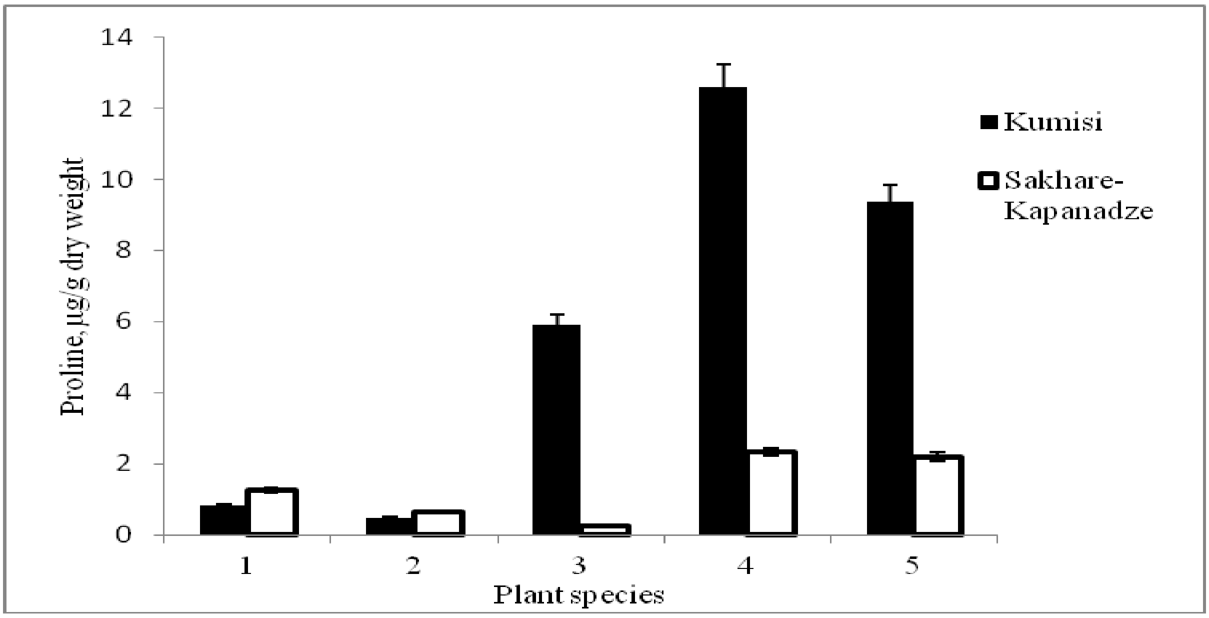

Fig. 10. Content of proline in leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite ($p<0.05$) 2. *Petrosimonia brachiata* (Pall.) ($p<0.05$) 3. *Chenopodium album* L. – goosefoot ($p<0.05$) 4. *Artemisia lerchiana* (Web.) – sagebrush ($p<0.05$) 5. *Adonis bienertii* (Butkov ex Riedl.)-peasant's eye ($p<0.05$).

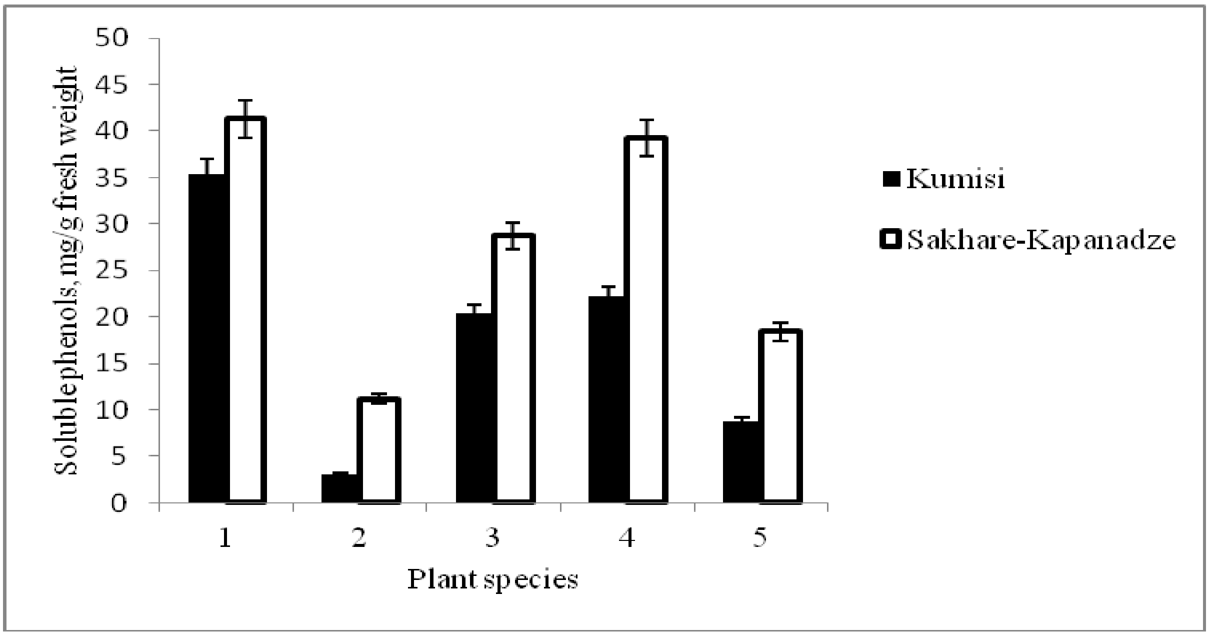

Fig. 11. Content of soluble phenols in leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite ($p<0.05$) 2. *Petrosimonia brachiata* (Pall.) ($p<0.05$) 3. *Chenopodium album* L. – goosefoot ($p<0.05$) 4. *Artemisia lerchiana* (Web.) – sagebrush ($p<0.05$) 5. *Adonis bienertii* (Butkov ex Riedl.)-peasant's eye ($p<0.05$).

Phenols are considered the most active secondary metabolites and essential antioxidants in plant (Cesar and Fraga, 2010). Significant increase of phenolic substances under different biotic and abiotic stresses, among them under salinity stress, has been established (Rezazadeh *et al.,* 2012; Ksouri *et al.,* 2007). They affect membrane permeability, ions uptake and transport, synthesis of proteins and DNA. Moreover, phenolic substances protect membrane lipids from oxidation, i.e. play protective role (Kusakina *et al.,* 2011).

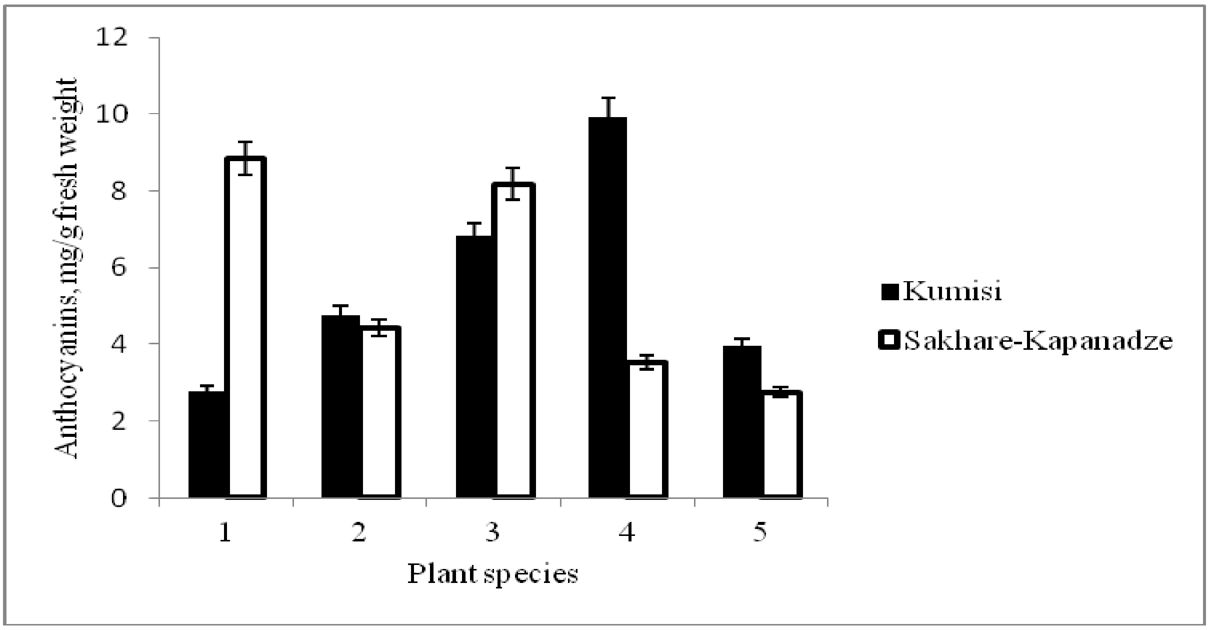

Fig. 12. Content of anthocyanins in leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite ($p<0.05$) 2. *Petrosimonia brachiata* (Pall.) ($p<0.05$) 3. *Chenopodium album* L. – goosefoot ($p<0.05$) 4. *Artemisia lerchiana* (Web.) – sagebrush ($p<0.05$) 5. *Adonis bienertii* (Butkov ex Riedl.)-peasant's eye ($p<0.05$).

High content of total phenols in Sakhare-Kapanadze lakes species may be indication to leading antioxidant function of these substances among the components of antioxidant system.

Anthocyanins

These are flavonoid group substances, concentrated in vacuole and possessing strong antioxidant properties (Kahkonen and Heinonen, 2003). According to literary data, content of anthocyanins increases under the influence of different stresses (Mobin and Khan, 2007). The protective role of anthocyanins in adaptation of plants to salinity stress, by neutralization of free radicals, has been experimentaly established (Gould, 2004). As most of stresses are linked with water deficiency, some authors speak about the osmoregulatory function of anthocyanins in plant cell (Chalker-Scott, 2002).

Comparison of the same plant species of the two habitats, cleared high content of anthocyanins in sea-blite and goosefoot from Sakhare-Kapanadze lakes compared to Kumisi individuals (3.2 and 1.2 times respectively. $p<0.05$). While in leaves of sagebrush and peasant's eye (both are glycohalophytes) growing in Kumisi this index was higher, compared to Sakhare-Kapanadze lakes samples (2.8 and 1.4 times respectively. $p<0.05$) (Fig. 12).

According to obtained results it may be supposed that in glicohalophytes, which are generally more sensitive to salinization (compared to eu- and crynohalophytes), anthocyanins play additional osmoregulative function, together with proline, under Kumisi conditions. This may be caused by different ion composition of the soils of these two habitats.

Total antioxidant activity

This is a significant, integrated index, which gaves information about the total antioxidant activity of the tested material, without specification of substances type. Some literary data demonstrate a correlation between the content of hydrophilic antioxidants and total antioxidant activity (Wu *et al.,* 2004).

There has not revealed any clear regularity in total antioxidant activity of experimental plants by habitats. The highest index was revealed in peasant's eye, growing in Sakhare-Kapanadze lakes and sagebrash, growing in Kumisi (Fig. 13).

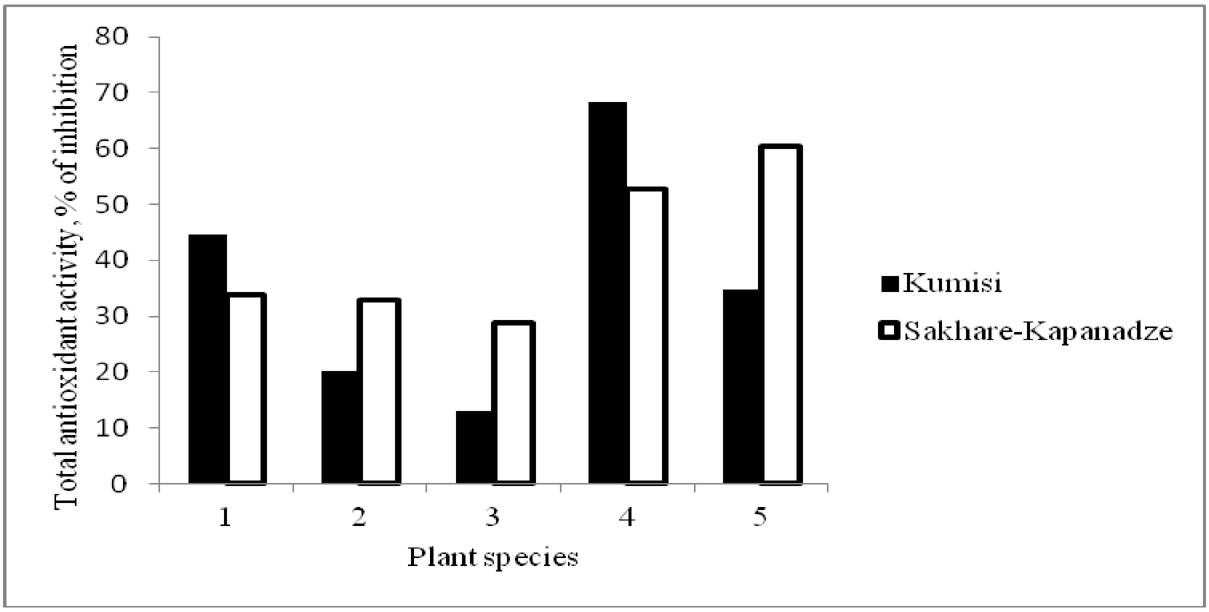

Fig. 13. Total antioxidant activity of leaves of halophytes growing on Kumisi (east Georgia, kvemo Kartli, and Sakhare and Kapanadze (east Georgia, gare Kakheti) lakes. 1. *Suaeda altissima* (L.) Pall.- sea-blite 2. *Petrosimonia brachiata* (Pall.) 3. *Chenopodium album* L. – goosefoot 4. *Artemisia lerchiana* (Web.) – sagebrush 5. *Adonis bienertii* (Butkov ex Riedl.)-peasant's eye.

As most of studied parameters represent the elements of antioxidant system, their quantity would influence the index of total antioxidant activity, both qualitatively and quantitatively. In some species of Sakhare-Kapanadze lakes high total antioxidant activity is conditioned by increased content of ascorbic acid, total phenols and anthocyanins, compared to the same species of Kumisi (goosefoot). In others together with ascorbic acid and total phenols, carotenoids prevailed (petrosimonia), or together with phenols and carotenoids, peroxidaze activity was high (peasant's eye).

It is difficult to explain higher antioxidant activity of seablite, growing in Kumisi, compared to Sakhare-Kapanadze lakes' individuals, because almost all studied antioxidants of the last habitat quantitatively prevailed Kumisi individuals.

It is difficult to explain different responses of the elements of antioxidant system of the same plant species, growing in similar by environmental conditions habitats, which are located at different places. Presumably, this may be associated with the microclimatic peculiarities of the studied habitats. Climate of Kumisi environs is of humid subtropical type, while at Iori plateau, where Sakhare-Kapanadze lakes are situated, the climate is of dry subtropical type. However, salinity of both habitats, which may be considered as one of the limiting factors for plant growth and development here, is almost the same (4.8- 4.5%).

The ion composition of salts, responsible for salinity, is to be taken into account as well. The limiting effect of salinity generally is due to sodium ions. Therefore, some authors speak about the increase of sodium's toxic effect with co-existence of anions. Moreover, sulphate anions have more toxic effect on many plant species, than chloride anions (Datta *et al.,* 1995; Renault *et al.,* 2001; Martin *et al.,* 2015). While others prove chloride salinity to be more toxic, compared to sulphate (Magistad *et al.,* 1943; Munns *et al.,* 2002).

Conclusions

From experimental results it is clear that studied biochemical indices are variable and very sensitive to environmental conditions. Since it is impossible to find identical habitats, in spite of similarity, the biochemical characteristics of same plant species, growing in similar habitats, presumably would not be identical as well.

According to our observations it is difficult to judge which type of salinity of the studied habitats was more depressive for plants. Adaptation of the tested species to studied habitats was much or less of individual character.

There may be distinguished some biochemical indices, in spite of the salt exchange mechanism type of tested species, which changed by the same regularity following habitats: content of ascorbic acid, total phenols and soluble carbohydrates was higher in species of Sakhare-Kapsanadze lakes, compared to Kumisi same species. While in all species of Kumisi habitat content of total proteins was higher.

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