Trace Metals and ^{210}Po Activity Concentrations in Macroalgae (*Cystoseira Crinita* and *Halopteris Scoparia*) and Seagrass (*Cymodocea Nodosa*) in Izmir Bay, Aegean Turkish Coast

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Abstract: Two types of macroalgae (*Cystoseira crinita* and *Halopteris scoparia*) and seagrass (*Cymodocea nodosa*) have been evaluated for their bioavailability as biomonitors of trace metals and ^{210}Po in Izmir Bay during the period of October 2017-July 2018. The levels of Al, Fe, Zn, Mn and Pb in the samples were determined using energy dispersive x-ray fluorescence spectrometry. The activity concentrations of ^{210}Po were measured by alpha spectrometry and the results have shown that *C. nodosa* were found to accumulate the maximum ^{210}Po concentration in the winter. Besides, macroalgae (*C.crinita* and *H. scoparia*) were also found to accumulate ^{210}Po at different levels in the same area. Additionaly, the investigated trace metals were determined in *H. scoparia* and *C. nodosa* in the winter. A seasonal fluctuations in trace metals and ^{210}Po concentrations were observed in the study.

Keywords: ^{210}Po, Macroalgae, Seagrass, Marine pollution.

INTRODUCTION

Recent studies in marine environment have been interested in radionuclide and heavy metal concentrations in marine organisms which effect ecological equilibrium. Marine ecosystem produce almost half of the world's oxygen and enable the home to marine species. Radionuclide and trace element concentrations in seawater, increases the pollutant risk factor for marine species. Also, marine pollution is determined with using some marine organism (macroalgae, seagrass etc.) as bioindicator.

Macroalgae are preferred in bio-kinetic studies as bioindicator for radionuclide accumulation in which responded to aquatic pollution (Nonova and Tosheva, 2016). Because marine algae are consumed as food in some cultures and moreover used in cosmetic, medical, chemical and agriculture industries (Al-Masri, Mamish and Budier, 2003), it is important to evaluate the radionuclide and trace metal accumulation abilities of these aquatic organisms. *Halopteris scoparia* (L.) Sauvageau (Phaeophyta, Sphacelariales) is one of the widespread brown macroalgae on the Mediterranean and Atlantic coasts. This species are common in cold and warm waters and distributed on rocky to sand bottom from midlittoral to the infralittoral zones. *H. scoparia* creates most abundant of microhabitats and are known good trap of sediment and also epihytets. (Sánchez-Moyano, *et al*., 2000, 2002; Patarra *et al.*, 2017). *Cystoseira crinita* Duby is a characteristic brown algae species in Mediterranean basin (Montesanto and Panayoditis, 2001) and is indicated by Huve (1972) as significant organism of infralittoral benthic vegetation in Aegean Sea and recommended by the EU Commission as an indicator species of water quality (EC, 1994). Additionally, in the framework of the Habitat Directive (92/43/EEC), it has been proposed to identify *Cystoseira* species on the Mediterranean coast for the definition of NATURA 2000 habitat code 1170 (Montesanto and Panayoditis, 2001).

Seagrass are very productive marine ecosystems providing physical structure, enhancing biomass, production diversity and substratum for other aquatic organisms (Duffy, 2006). They are perennial and prevalent in marine habitats also have sensitivity to natural and anthropogenic pressures (Sidi *et al.*, 2018). Therefore, seagrasses are favored in monitoring aquatic systems as bioindicator species (Bonanno and Di Martino, 2016). Especially, determination of marine pollution is provided by way of choosing some characteristic organisms as bioindicator for monitoring contaminant in coastal areas. *Cymodocea nodosa* (Ucria) Ascherson species is one of the five marine flowering plants exist in Turkish costs also with distribution area in the whole Mediterranean (mainly in the Eastern Mediterranean), the Atlantic Ocean. The species generally spreads in sheltered bays and lagoons and prefers fine sand, mud substrate as the

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ground. Also, they can form meadows with other sea meadows and algae species. *C. nodosa* is included in Annex I of the Bern Convention in the Mediterranean (EU, 1981). It is also included in the list of endangered species under Annex II of the Barcelona Convention (Pergent-Martini *et al*., 2015).

Polonium is a sulphur -group element and a strong affinity for biogenic particulate matter in marine system and contributes the largest radiation dose to marine biota (Karanukara, 2013). Marine organisms (plants and animals) can concentrate trace metals and radionuclides to a relatively high degree in their tissues. Macroalgae have been used in many studies relevant marine pollution because they are abundant and respond rapidly to changes in the ecosystem and cost-effective (Bonanno and Martino, 2016, Nonova and Tosheva 2016). Seawater has approximately 35 grams of dissolved salt in 1 L (Henderson, 2016). The main concentration of these salts are Na and Cl while more than 82 other elements contributing the seawater content. The elements with the concentration less than 1 mg/kg are called trace elements. These elements are important for biological activities such as P, N, Fe and Cu. But some are known with their toxic effects like Cu and Hg.

Studies on the content of radionuclides and trace metals in macroalgae and seagrass distributed along the Aegean coast are deficient but their classification and distribution are well constituted. Izmir Bay is one of the most polluted estuaries in the Mediterranean Sea (Bizsel and Uslu, 2000). The aim of the present study is to seasonally determine the accumulation of ^{210}Po and trace elements (Fe, Al, Zn, Mn and Pb) by macroalgae (*C. crinita and H. scoparia*) and seagrass (*C. nodosa*) found in Izmir Bay.

MATERIAL AND METHODS

Sampling

Izmir Bay was choosen as the sampling area (38˚ 21.921' N- 26˚ 46.915' E) which is one of the largest natural gulfs of the Mediterranean with a total area of 200 km^2 and a water capacity of 11.5 billion m^3. Izmir, which gives its name to the bay and is an important trade, industrial and cultural city, is the largest settlement area around the bay with an area of approximately 88000 ha (izsu.gov.tr). Figure **1** shows the sampling location.

Macroalgae (*C. crinita* and *H. scoparia*) and seagrass (*C. nodosa*) were collected in 2017 and 2018. The samples were carefully cleaned from epiphytes, rinsed with distilled water than dried to constant weight at 55-60°C during 24 h and their weights were determined and then mixed thoroughly, then they were ground and passed through a 2 mm mesh and homogenized.

^{210}Po Determinations in Macroalgae (*Cystoseira Crinita* and *Halopteris Scoparia*) and Seagrass (*Cymodocea Nodosa*)

Specific alpha activities were measured by alpha spectrometry system. ^{209}Po (4.88 MeV alpha emission, $t_{1/2}$=103 years) was used as the internal tracer (Standard Reference Material 4326). The chemical yields using the ^{209}Po tracer ranged between 85 and 90%. The detection limit of the alpha spectrometry system is 0.0003 Bq. Each sample was completely dissolved with 15 ml Aqua Regia in a microwave oven at 100°C for an hour and polonium was spontaneously plated onto a cupper discs in 0.5M HCl in the presence of ascorbic acid to reduce Fe^{+3} to Fe^{+2}. In order to find

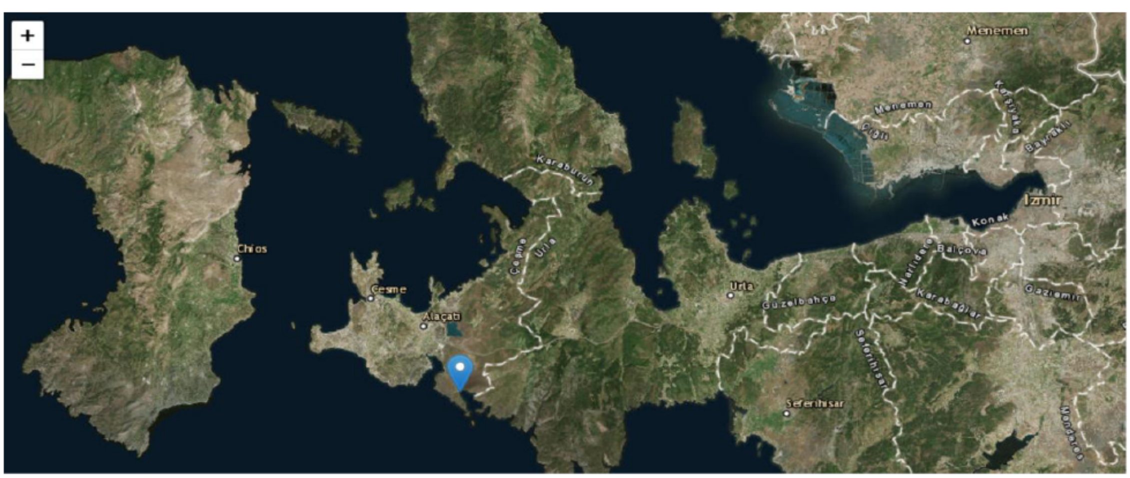

Figure 1: Map of the sampling location.

the optimum conditions for plating, the standard technique given by Flynn (1968) was modified.

Trace elements Analysis

The heavy metal content of the milled plant samples were determined by energy dispersive x-ray fluoresence spectrometry (EDXRF, Rigaku Nex CG). MCA calibration was performed before analysis. Prolene film was used in sample cups. Approximately 3 grams of plant sample was taken in to the sample cup, placed in the instrument and analyzed.

RESULTS AND DISCUSSION

In our study we determined the concentrations of 5 trace elements (Fe, Al, Zn, Mn, Pb) found in seawater composition in algae and seagrass. Also the ^{210}Po activity concentrations seasonally determined from two brown macroalgae and one seagrass species collected from Izmir Bay.

Figure **2** shows the ^{210}Po activity concentrations for the algae (*Cystoseira crinita, Halopteris scoparia*) and seagrass (*Cymodocea nodosa*) samples in each season.

The average dry weight ^{210}Po activity concentration values in the algae (*C. crinita, H. scoparia*) and in seagrass (*C. nodosa*) samples are 34.7 Bq kg^{-1}, 47.4 Bq kg^{-1} and 54.0 Bq kg^{-1} respectively. The highest ^{210}Po activity concentration (100.94 Bq kg^{-1} dw) was observed during winter in the *C. nodosa*. The minimum ^{210}Po activity concentration (7.2 Bq kg^{-1} dw) was determined in the *H. scoparia* of autumn samples. The seagrass (*C. nodosa*) samples has a higher mean ^{210}Po concentration compared to the algae (*C. crinita, H. scoparia*) samples. The activity concentration of ^{210}Po in *H. scoparia* and *C. nodosa* is higher during the winter time compared to the other seasons. The algae species (*C. crinita* and *H. scoparia*) shows the opposite trend seasonally in this study (Figure **2**). Uddin *et al*. (2015) indicated that the observed lower ^{210}Po concentration in seawater might be a result of higher winter time productivity and abundance of seaweeds in the Gulf. Although seagrass are accepted the marine forest (Waycott *et al*., 2006), investigation of ^{210}Po concentrations in seagrass are very limited in the world (Sam *et al*. 1998; Skwarzec *et al*. 2003; Suriyanarayanan *et al*., 2010) (Table **2**). The data obtained in the present study compared with the activity reported from around the globe (Table **1**). Among previous studies our results showed the highest ^{210}Po concentrations. Uddin *et al*. (2015) reported that seaweeds concentrations of ^{210}Po in the range of 16.2–19.22 Bq kg^{-1} from samples collected from the Kuwait marine environment. ^{210}Po activity concentrations in the *Cystoseira sp.* published in the literature are in the range of 4.60–32.60 Bq kg^{-1} dry weight. Suriyanarayanan *et al*. (2010) reported that the concentrations of ^{210}Po in seaweeds (*Sargassum wightii* and *Grateloupia filicina)* collected from the Mallipattinam ecosystem were 26 and 10 Bq kg^{-1}(fresh weight), respectively. Skwarzec *et al.* (2003) indicated that the lowest polonium concentration was found in *Cladophora rupestris* (0.12 Bq kg^{-1} wet weight) and the highest in *Chara crinita* (1.12 Bq kg^{-1}wet weight) collected in February 1988 from Puck Bay. Nonova and Tosheva (2016) reported lower ^{210}Po values (3.5-5.8 Bq kg^{-1} dry weight) for *C. crinita* distributed along the Bulgarian Black Sea. Sam *et al*. (1998) found that ^{210}Po

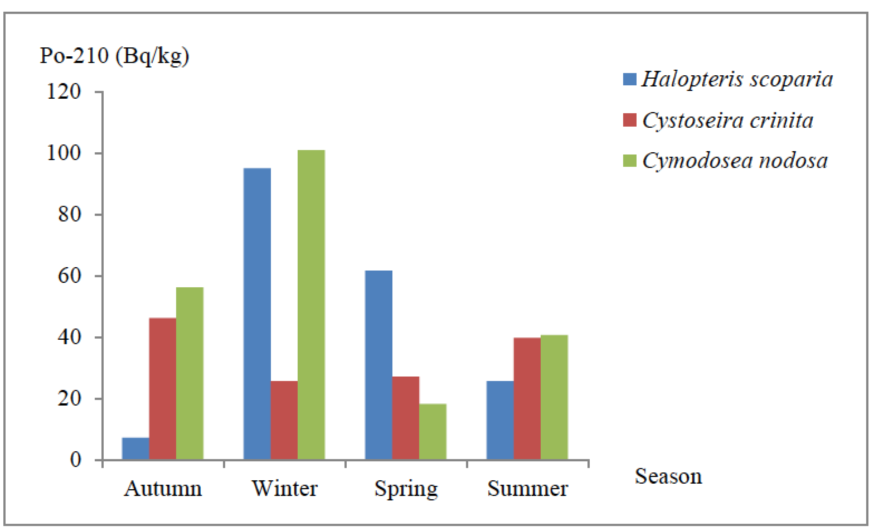

Figure 2: Concentration of ^{210}Po in macroalgae (*Cystoseira crinita* and *Halopteris scoparia*) and seagrass (*Cymodocea nodosa*) in Izmir Bay.

Table 1: Concentration of ^{210}Po in Marine Macroalgae from Various Marine Area (Bq kg^{-1})

Algae pecies	^{210}Po (Bq/kg)	Location	Reference
Turbinaria sp.	1.60	Sudan (Red Sea)	(Sirelkhatim, Sam and Hassona, 2008)
Sargassum sp.	12.60	Sudan (Red Sea)	
Padina sp.	15.00	Sudan (Red Sea)	
Cyctoseria sp.	32.20	Sudan (Red Sea)	
Stypopodium zonale	23.84	Syria (Tartous)	(Al-Masri, Mamish and Budier, 2003)
Stypopodium zonale	15.17	Syria (Ras İbn Hani)	
Sargassum vulgare	23.31	Syria (Tartous)	
Padina Pavonia	24.43	Syria (Tartous)	
Cystoseira sp.	8.08	Syria (Tartous)	
Cystoseira sp.	26.40	Syria (Lattakia)	
Cystoseira Barbata	23.66	Syria (Tartous)	
Cystoseira ercogovici	12.32	Syria (Ras Samara)	
Ulva rigida	2.70	Bulgaria (Tuzlata)	(Nonova and Tosheva, 2016)
Enteromorpha intestinalis	5.20	Bulgaria (Tuzlata)	
Cladophora vagabunda	5.50	Bulgaria (Tuzlata)	
Ceramium rubrum	6.20	Bulgaria (Tuzlata)	
Cystoseira crinita	4.60	Bulgaria (Tuzlata)	
Ulva rigida	3.40	Bulgaria (Burgas)	
Enteromorpha intestinalis	5.80	Bulgaria (Burgas)	
Ceramium rubrum	5.70	Bulgaria (Burgas)	
Fucus serratus	7.80	France (Barfleur)	(Connan *et al.*, 2007)
Fucus serratus	12.00	France (Granville)	
Fucus vesiculosus	4.50	France (Barfleur)	(Germain, Leclerc and Simon, 1995)
Fucus vesiculosus	13.70	France (Priou)	
Halimeda sp.	13.70	Sudan (Port Sudan)	(Sam *et al.*, 1998)
Cystoseira sp.	32.60	Sudan (Port Sudan)	
Padina sp.	15.00	Sudan (Port Sudan)	
Sargassum sp.	36.40	Sudan (Port Sudan)	
Ulva lactuca	2.70	Portugal (Cascais)	(Carvalho, 2011)
Codium tomentosum	2.00	Portugal (Cascais)	
Ploccamium cartilagineum	5.20	Portugal (Cascais)	
Gelidium sesquipedale	8.60	Portugal (Cascais)	
Fucus vesiculosus	9.10	Portugal (Cascais)	
Sacchoriza polyschides	1.60	Portugal (Cascais)	
Sargassum boveanum	22.50-25.60	Kuwait	(Uddin, Aba and Bebhehani, 2015)
Sargassum oligocystum	20.20-22.50	Kuwait	
Seaweeds	16.50	India (Parangipettai)	(Raja and Hameed, 2010)
Cystoseira crinita	34.70	Turkey (Izmir Bay)	This study
Halopteris scoparia	47.40	Turkey (Izmir Bay)	

concentration in algae collected from the fringing reef area at Port Sudan ranged from 13.7 Bq kg^{-1} green algae (*Halimeda* sp.) to 36.4 Bq kg^{-1} brown algae (*Sargassum* sp.).

Metal concentrations in macroalgae species are shown in Figure **3**, **4** and **5**. Our heavy metal concentration results in the samples are compared with different locations. In the study, the maximum value of

Table 2: Concentration of ^{210}Po in Marine Seagrasses from Various Marine Area (Bq kg^{-1})

Species of Seagrass	^{210}Po (Bq/kg)	Location	Reference
Cymadocea serrulata	11.00	India (Tamil Nadu)	(Suriyanarayanan *et al*., 2010)
Seagrass	22.70	Sudan (Port Sudan)	(Sam *et al*. 1998)
Cladophora rupestris	0.12	(Puck Bay) S. Baltic	(Skwarzec *et al*., 2003)
Cymodocea nodosa	54.00	Turkey (Izmir Bay)	This study

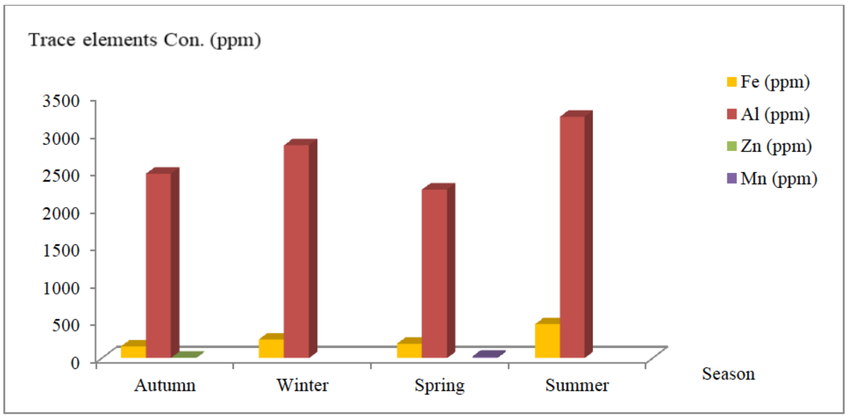

Figure 3: Concentrations of some trace metals (ppm) in *Cystoseira crinita.*

zinc is found in *C. nodosa* (8.32 ppm) in the spring and the minimum in *C. crinita* (3.28 ppm) in the autumn. The maximum of manganese is detected in *H. scoparia* (75.9 ppm) and the minimum in *C. crinita* (20 ppm) in the spring. In this study, the maximum concentration of lead is found in Halopteris scoparia in the winter, with a value of 3.91 ppm and a minimum of *C. nodosa* (2.55 ppm) in the winter. The levels of Fe and Al in the samples are remarkably higher than those of the other metals studied. But Fe and Al occur naturally in the environment. Aluminum is found in structure of the earth crust as third most abundant element (~ 8 %) (Zeraatkar *et al*., 2016; Dominguez-Renedo *et al*., 2019). Fe, Zn and Mn are necessary usual metabolic functions and generally used as cofactors of enzymes and/or as a component for constitution of cell structure (Burins *et al*., 2000). Statistical analyses (One-Sample Kolmogorov-Smirnov statistical test) were performed to check the normality of the raw data for each algal group. All the data followed a normal distribution ($p>0.05$). There is a stronger positive correlation between Fe and Zn concentrations in *H. scoparia* ($r = 0.992$, $p<0.01$). Pb is dangerous toxic heavy metal at even low level in biological system (Haferburg and Kothe, 2007). In the winter, in *H. scoparia* and *C. nodosa* metal concentrations decrease in the following order: Al>Fe>Mn>Zn>Pb, while in *C. crinita* the sequence is Al>Fe.

The results in the study for metal concentrations in *C. crinita* of the investigated location is compared with the findings of Kucuksezgin and Akcal (2009) from the same station and the mean concentration of Fe is comparable with their study but our Zn concentration in *Cystoseira crinita* is lower and Mn concentration is higher than their study. Table **3** shows the concentrations of trace elements in the macroalgae (*C. Crinita)* from various marine area. Storelli *et al*. (2001) pointed out that in benthic macrophytes, Zn levels not exceeding 100 ppm are suggested as background for nonpolluted areas. Kravtsova *et al*. (2015) reported that Al, Mn, Fe, and Zn in the *C. crinita* collected from marine protected areas of Crimea (Black Sea) are 180, 34.1, 148, 31.2 ppm, respectively. As indicated by Topcuoglu *et al*. (2003), it is not easy to compare the heavy metal concentrations in macroalgae reported from other marine environment with present results due to wide variations of the environmental parameters (salinity, temperature, pH, light, oxygen, nutrient concentrations, complexing agents) and systematic position of the algae.

CONCLUSION

In the study, ^{210}Po and trace metals are seasonally quantified in the macroalgae (*C. crinita, H. scoparia*) and in seagrass (*C. nodosa*) samples found in Izmir

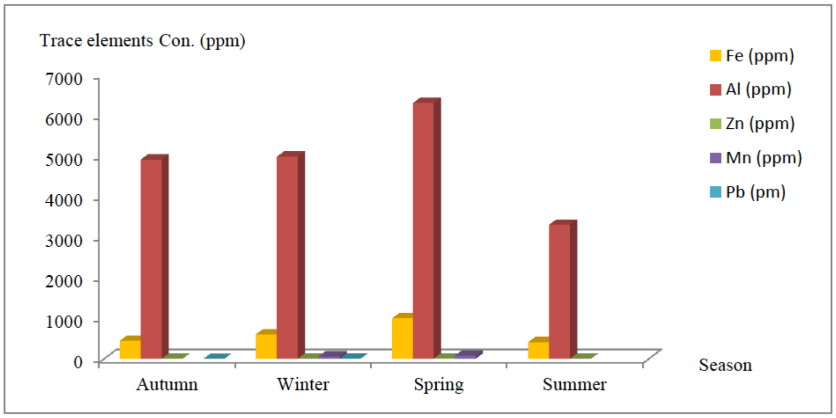

Figure 4: Concentrations of trace metals in *Halopteris scoparia.*

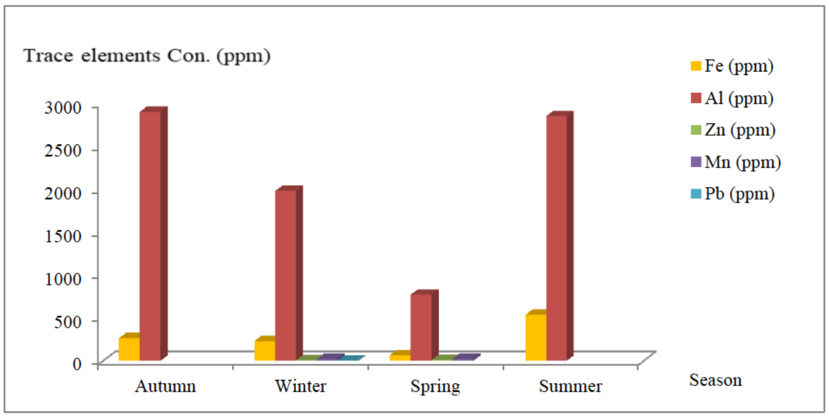

Figure 5: Concentrations of trace metals in *Cymodocea nodosa.*

Table 3: Concentrations of Trace Elements in *Cystoseira Crinita* from Various Marine Area (ppm)

Location	Al (ppm)	Mn (ppm)	Fe (ppm)	Pb (ppm)	Zn (ppm)	References
Crimea, Black Sea	180	34.10	148	-	31.20	Kravtsova *et al.* (2015)
Karantinnaya Bay (Crimea)	833	37.40	457	-	34.50	Kravtsova *et al.* (2014b)
Yalta beach (Crimea)	1458	71	650	-	54	Kravtsova *et al.* (2014a)
Batiliman tract	350	124	1400	-	85.40	Molchanov *et al.* (1988)
Cape Martyan Nature Reserve	1300	298	1100	-	46.40	Molchanov *et al.* (1988)
Sinop	4500	73	3414	-	85.80	Guven *et al.* (1992)
Eastern part of the Black Sea (Turkey)	-	22.20	-	-	61.20	Topcuoglu *et al.* (2003)
Caucasus coast	-	41.70	140	-	-	Saburin,M.Y. (2004)
Urla, Izmir	-	8.43	212.14	0.0083	27.17	Akcal and Kucuksezgin (2009)
Izmir Bay	2692.50	20	262.25	-	3.28	This study

Bay. It can be concluded that *C. nodosa* and *H. scoparia* are suitable to be used as a biomonitor for ^{210}Po especially in winter and both of them also could be used as biomonitors for trace metal pollution but further studies are required to provide comparative data on ^{210}Po and trace metal levels in macroalgae (*H. scoparia)* and seagrass (*C. nodosa*).

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