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Sediment-water interactions with eelgrass (*Zostera* spp.) from Sinop shores of the Black Sea

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ABSTRACT
This study is to provide information on levels of the elements in leaves and roots-rhizomes of *Zostera marina* and *Zostera noltei*, their surrounding sea water and sediments collected from Akliman and Türkeli coastal areas of Sinop city in the Black Sea four times between September 2015 and July 2016. The concentrations of Al, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Hg and Pb in digested samples were measured by Agilent Technologies, 7700X ICP-MS (inductively coupled plasma mass spectrometer). The results showed that the concentrations of metals were found in the order Hg < Cd < Co < As < Pb < Ni < Cu < Zn < Mn < Al = Fe. *Zostera* species were bio-accumulative for Al and Mn (BCF ≥ 5000); and macro-consantrator for Zn and Cd (BSAF > 2). The general capacities of element translocation were found as acropetal for Al, Mn, Ni, Cd and Hg in eelgrass. The present findings interpreted for the first time in the Turkish Black Sea waters. Both species of *Zostera* in high concentrations of these elements may be due to untreated wastes that are discharging from different sources such as harbor activities, agricultural and domestic wastes in coastal areas. In order to assess the impact of contaminants and take necessary actions, the region needs to be monitored regularly.

Key words: *Zostera marina*, *Zostera* (*Zosterella*) *noltei*, Black Sea, Sinop coasts, Contaminants.

INTRODUCTION
Eelgrasses are marine angiosperms that inhabit shallow, unconsolidated bottoms of most coastal areas. They are considered a valuable component of coastal ecosystems because of the identification of different ecological functions, services and resource (Surugiu 2008). Eelgrass biocenosis, rich in species diversity, is cornerstone and highly productive ecosystems which fulfill a key role in the world. The fate of eelgrass can provide resource managers with advance signs of deteriorating ecological conditions caused by poor water quality and pollution.
In the Black Sea, six marine phanerogams including *Zostera marina*, *Z. noltii (noltei)*, *Potamogeton pectinatus* (*Stuckenia pectinate*), *Ruppia maritima*, *R. spiralis* and *Zannichellia major* were defined by Milchakova (1999). *Zostera marina* and *Z. noltei* are eelgrass found abundantly in intertidal areas particularly in Sinop shores of the Black Sea. The coastal areas of the Black Sea are ecologically and economically important but these coastal areas are facing many types of pollution (Bat *et al.* 2018). Marine pollution either natural or anthropogenic is a big hazard to the health of people and marine biota especially benthic communities that seriously impact the whole ecosystem.
The continental shelves of Turkey include Sinop coasts with natural resources of rich flora and fauna (Bat *et al.* 2011). The chemical composition of eelgrass faces noticeable variation in changing environments i.e., the seasons, the habitats, and depth at which they grow. It is accepted fact that accumulation of contaminants in seagrass is related to their levels in seawater as well as sediment and it has been noticed that it is also influenced by changing season. The seagrass possess a great ability to accumulate heavy metals (Bat *et al.* 2016). The eelgrasses are most effectively used as indicator of pollution by certain contaminants. Previous studies on eelgrass meadows and their

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associated faunal assemblages and also their heavy metal uptakes were lacking in the Turkish Black Sea coasts (Tuncer 1985; Mutlu *et al*. 1992; Tuncer & Yaramaz 1992; Güven *et al.* 1993; Aysel *et al.* 2000, 2006; Sezgin *et al.* 2001; Gönlügür 2003; Kırkım *et al.* 2006; Bilgin *et al*. 2007; Ersoy Karaçuha *et al.* 2009; Bat *et al.* 2016). Due to anthropogenic activities resulted in the problem of pollution, especially of the coastal ecosystem of the Black Sea. Lack of any pre-existing data on accumulation of elements in marine angiosperms and in the ambient medium (water and sediment) led to this study for investigations on the ecological health of Sinop coastal waters in the Black Sea. This study was conducted to gather information that will serve as baseline values of Al, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Hg and Pb in leaves and root-rhizomes of *Zostera marina* and *Z. noltei* collected from Akliman and Türkeli coastal areas of Sinop city in the Black Sea. The relationship between these elements in eelgrasses, seawater and sediment was also studied.

MATERIALS AND METHODS

The samples used in this research were *Z. marina* and *Z. noltei* and their surrounding water and sediments were collected from Akliman and Türkeli coastal areas of Sinop city in the Black Sea (Fig. 1).

All Sinop coasts were investigated, however common eelgrass (*Z. marina*) and dwarf eelgrass (*Z. noltei*) were found in Türkeli and Akliman sandy-muddy substrate, respectively. Sampling sites had different degrees of human impacts, where pollution inputs were mainly due to untreated municipal wastewater, farming and touristic activities. Mainly, Akliman is affected by anthropogenic activities in summer season with growth tourist population; the latter site is affected by small human settlements and agricultural contaminants.

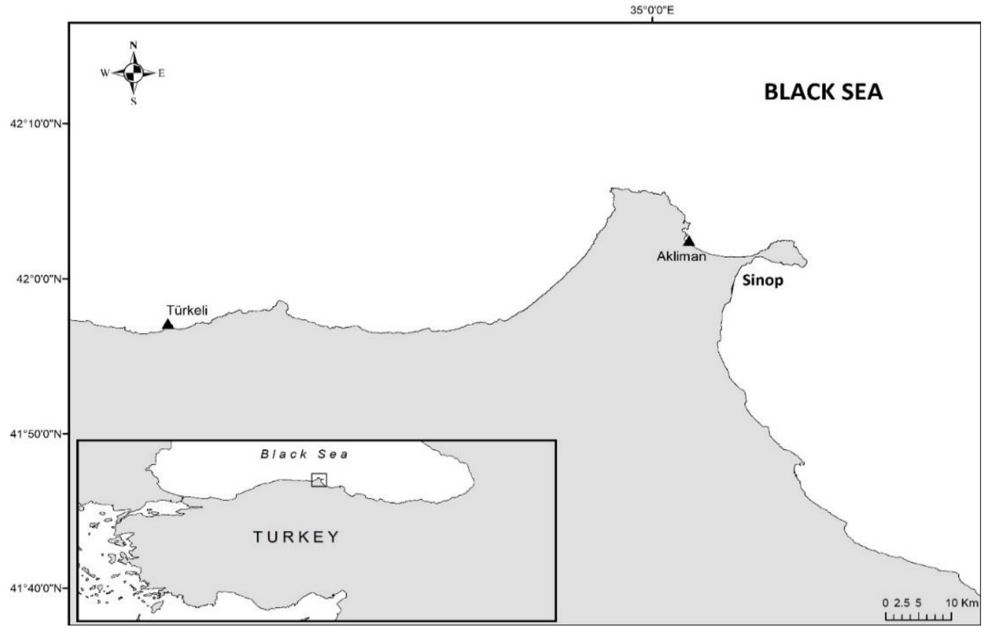

Fig. 1. Study area.

Sampling was carried out four times between September 2015 and July 2016. Eelgrasses were carefully cleaned with filtered sea water for removal of mud debris and other epiphytes, labelled, and transported to the laboratory. In the laboratory, they were washed again with double distilled water, dissected into roots-rhizomes and leaves, and dried at 70 ºC for 48 h till a constant weight was achieved. According to Aquatic plant HPR-FO-08 method (Milestone), dried samples digested with Suprapur ® HNO_3 (nitric acid) using a microwave digestion system (Milestone Systems, Start D 260) for element analyses.

At each 250 mL sea water samples, a 1 mL of concentrated ultrapure HNO_3 was dropped and stored in polyethylene bottles. Sediment samples were collected from the top 5 cm of the upper layer using a sediment core with an internal diameter of 10 cm. After transportation, they dried at 105 ºC for 24 h. The less than 63 µm (Förstner & Wittmann 1983) was digested with Seawater Sediment HPR-EN-33 methodology (Milestone Systems, Start D 260). Concentrations of Al, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg, Pb and As were determined in three replicates by ICP-MS. Results of mean levels were found as mg kg^{-1} dry wt. in eelgrass and sediments, and ppb in water samples. The methodology was validated by the Certified Reference Materials (CRMs) (BCR

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279-*Ulva lactuca*). Analytical precision results for sample replicates obtained were in agreement with the certified values at 99% confidence level.

Following analyses, values determined for biota-sediment accumulation factor (BSAF), bio-concentration factor (BCF) and translocation factors (TF) to assess mobility of elements. BSAF and BCF are parameters to describe bioaccumulation of sediment and water-associated metals into the ecological receptors (Geyer *et al*. 2000; Kleinov *et al.* 2008). TF analysis was used to measure the mobility of a given element within plants, where TF > 1 values result in a higher translocation capability (Deng *et al*. 2004).

The values are calculated according to the following formulas BSAF = $C_{biota}/C_{sediment}$; BCF = C_{biota}/C_{water}; TF = C_{leaves}/C_{roots}; where C_{biota}, $C_{sediment}$, C_{water}, C_{leaves} and C_{roots} are respectively levels (ppb for water, mg kg^{-1} dry wt. for others) of a specific element in water, sediment, as well as leaves and roots of both species.

One sample *t*-test and One-Way ANOVA were used for comparisons between metal concentrations in samples. Means were compared by Duncan multiple comparison test and the p value ≤ 0.05 was considered to indicate statistical significance. SPSS ver. 21.0 was used for statistical analyses of data.

RESULTS AND DISCUSSION

In each species concentrations of eleven elements Al, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Hg and Pb were separately determined for leaves and roots-rhizomes (Table 1). The data reveals high variability in elements concentrations in leaves and root-rhizomes of both species along the Sinop coasts of the Black Sea between sampling sites.

The results show high variability in metal concentrations between sites and between leaves and roots-rhizomes of *Z. marina* and *Z. noltei* from Sinop shores of the Black Sea. Essential metals Fe, Al, Zn and Mn were recorded at high levels.

The general metal contents increased in the order of Hg < Cd < Co < As < Pb <Ni <Cu < Zn < Mn < Al = Fe. The maximum accumulation of Al was in roots-rhizomes of *Z. noltei* (4957.4 mg kg^{-1} dry wt.). The concentrations of different metals in seagrass tissues followed the similar order: Fe > Zn > Pb > Cu (Table 2). Compared with other studies, Pb level was lower than others (1.9-3.7 mg kg^{-1} dry wt.), a trend more clear pattern in the polluted sites. Moreover, it is commendable that small variations in the Hg and Cd levels were found among species and sites, indicating that no major source of these metals occurred in Sinop coastal waters of the Black Sea. Different regions tend to respond differently to exposure to heavy metals. A wide range of metal uptake abilities among tissues of *Zostera* spp. were shown in Table 2.

The measurements of results were compared to trace elements in sea water and sediments according to proposed values by regulatory agencies EC 1998, TS-266 2005, EPA 2008, WHO 2011, CSQG 2001 and NOAA 2009 (Table 3). Pb concentrations in seawater exhibited variations (p ≤ 0.05) depending upon summer season in Akliman. Levels of Ni, Cu and Pb in sediments displayed statistical significance depending upon locations (p ≤ 0.05). In general, heavy metal concentrations were found below the threshold values for sea water and sediments.

BSAFs are in the range of 1 to 2, BSAF > 2 as macro-consantrator is named by Nenciu *et al*. (2016). Mean values of BSAF ranged from 0.08 (Pb) to 6.9 (Zn) (Table 4). *Zostera* spp. were sediment accumulators for Zn and Cd (BSAF > 2), due to their greater accumulation in sediments over time. Over 100 mg kg^{-1} Zn levels in benthic algae may be related to anthropogenic pollution (Storelli *et al*. 2001).

Table 1. Heavy metal concentrations (mg kg^{-1} dry wt.) in leaves and root-rhizomes of *Zostera* spp. from Sinop shores of the Black Sea.

Metals (mg kg^{-1})	*Zostera marina*		*Zostera noltei*	
	Leaves	**Roots rhizomes**	**Leaves**	**Roots rhizomes**
Al	1847.4	4957.4	3266.9	2055
Mn	761.8	444	459.5	339.3
Fe	1719.7	4151	2237.3	3214
Co	2	2	1.7	1.7
Ni	7.3	11.5	4.6	4.2
Cu	13.6	14	5.2	5.2
Zn	114.8	115.2	78.0	85.8
As	2	3.7	1.6	2.2
Cd	0.1	0.1	0.5	0.3
Hg	0.017	0.029	0.012	0.007
Pb	1.9	3.7	3.2	4

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Table 2. Relative concentrations of metals (pollution sources are not identified at sites).

Species	Metal concentration	References
Z. marina	Fe > Zn > Pb > Cu > Cd	Tuncer 1985
Z. marina (leaves)	Fe > Mn > Ni > Zn > Co > Cr > Pb > Cd	Tuncer & Yaramaz 1992
Z. marina (shoots)	Fe > Mn > Zn > Ni > Co = Pb > Cr > Cd	Tuncer & Yaramaz 1992
Z. marina	Fe > Zn > Pb > Cu > Ni	Güven *et al.* 1993
Z. marina	Mn > Zn > Cu > Pb > Co > Ni > Cr	Prego & Cobelo-Garcia 2003
Z. marina (leaves)	Cr < Ni < Pb < Cu < Fe	De Casabianca *et al*. 2004
Z. marina (roots-rhizomes)	Ni < Cr < Pb < Cu < Fe	De Casabianca *et al*. 2004
Z. marina	Fe > Mn > Pb > Zn > Ni > Co > Cu > Cd	Macias-Zamora *et al*. 2008
Z. marina	Fe > Mn > Zn > Ni > Pb > Cd > Cu	Riosmena-Rodriguez *et al*. 2010
Z. noltei	Fe > Al > Zn > Cu > As > Pb > Cd > Hg	Bat *et al*. 2016
Z. marina (leaves)	Al > Fe > Mn > Zn > Cu > Ni > Co > As > Pb > Cd > Hg	This study
Z. marina (roots-rhizomes)	Al > Fe > Mn > Zn > Cu > Ni > As > Pb > Co > Cd > Hg	This study
Z. noltei (leaves)	Fe > Al > Mn > Zn > Cu > Ni > Pb > As > Co > Cd > Hg	This study
Z. noltei (roots-rhizomes)	Fe > Al > Mn > Zn > Cu > Ni > Pb > As > Co > Cd > Hg	This study

Table 3. Trace elements in superficial marine waters and bottom sediments in sampling stations according to different regulations.

Metals	Seawater (ug l^{-1})						Sediment (mg kg^{-1} dry wt.)			
	Akliman	Türkeli	1	2	3	4	Akliman	Türkeli	5	6
Al	208.55	206.82			200	100	3584	4321		
Mn	12.91	33.52	50	50	50	100	333	325		460-1100
Fe	285.13	348.87	200	200	300	300	7260	7041		%2-4
Co	1.53	1.31					2.7	3.5		
Ni	4.23	5.08				20	7.5	18.2	20.9-51.6	16-75
Cu	2.88	3.72	2000		1000	2000	4.6	9	34-270	16-110
Zn	368.58	224.25					21.1	16.7	150-410	120-820
As	1.56	1.82			10	10	5.1	4.1	8.2-70	
Cd	0.46	0.32	5	5	5	3	0.1	0.05	1.2-9.6	0.6-33
Hg	0.04	0.03	1		2	1	0.01	0.02	0.15-0.71	
Pb	6.2	1.81	10	10	15	10	39.3	3		31-250

1. EC 1998, 2. TS-266, 3. EPA 2008, 4. WHO 2011, 5. NOAA 2009, 6. CSQG 2001.

Marsh area and intense agricultural activities in Türkeli station affected Zn and Cd levels in *Z. marina*. The quality of BCFs has not been explicitly developed by regulatory agencies, the values of BCFs are varied in the range between 1000 and 5000 (Arnot & Gobas 2006). The *Zostera* species were found as very bio-accumulative for elements of Al and Mn (BCF ≥ 5000). This result confirmed by Mn concentrations in leaves of *Z. marina* and *Z. noltei* (761.8 and 459.5 mg kg^{-1} dry wt.) that were much fold higher than accumulated in roots-rhizomes of *Z. marina* and *Z. noltei* (444 and 339.3 mg kg^{-1} dry wt.).

Table 4. The mean BSAF and BCF values of *Zostera marina* and *Zostera* (*Zosterella*) *noltei* from Sinop shores of the Black Sea.

Metals	*Z. marina*				*Z. noltei*			
	Leaves		Roots-rhizomes		Leaves		Roots-rhizomes	
	BSAF	BCF	BSAF	BCF	BSAF	BCF	BSAF	BCF
Al	0.4	8769.6	1.1	23532.6	0.9	16334.7	0.6	10274.1
Mn	2.3	22042.5	1.4	12858.2	1.4	45948.4	1	33931.3
Fe	0.2	4503.4	0.6	10872.7	0.3	7990.4	0.4	11479.8
Co	0.6	1304.5	0.7	1646.7	0.6	1151.1	0.6	1142.6
Ni	0.4	1226.9	0.6	1950	0.6	1094.8	0.6	996.1
Cu	1.5	4149.8	1.5	4268.6	1.1	1803.4	1.1	1777.7
Zn	6.9	536.3	6.9	538.6	3.7	211.6	4.1	232.9
As	0.5	1056.3	0.9	1971.9	0.3	976.4	0.4	1359.2
Cd	2.5	390.5	1.7	268.1	5.2	1031.5	3.1	610.8
Hg	0.9	611	1.4	1029.3	1.2	290.3	0.7	174.4
Pb	0.6	1283.7	1.2	2547.7	0.08	517.6	0.1	644.5

BSAF ≥ 2 (macro-consantrator) and BCF ≥ 5000 (very bio-accumulative) values were shown in bold.

Values of translocation from roots-rhizomes to leaves were highest for Hg (1.71) while lowest for Fe (0.01) (Fig. 2). In general, Al, Mn, Ni, Cd and Hg exhibited acropetal (below to above ground) translocated. Penello & Brinkhuis (1980) observed uptake of Cd and Mn through roots, rhizomes and leaves. This upward movement was observed in this study (TFs: 1.72 in *Z. marina* and 1.35 in *Z. noltei*). Bidirectional translocation capabilities of Hg

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and Ni were observed between species. Translocation of Co, Cu and Zn in *Zostera* tissues were found minimal. Carter & Eriksen (1992) found little Cu translocation between *Z. muelleri* tissues. In the present study, Cd was accumulated by above tissues in *Z. noltei*, suggesting a possible sink in Akliman station. Brinkhuis *et al*. (1980) observed bidirectional Cd movements, predominantly basipetally (below to above) translocated in *Z. marina*. Moreover, Faraday & Churchill (1979) established that Cd was accumulated by above and below ground tissues of *Z. marina* in direct proportion to that of the sea water. Pb appeared to be taken up from water in sampling sites. The studies of Lyngby & Brix (1982) and Bond *et al*. (1985) confirmed lead intake by leaves from water column. Data of Pb translocation between tissues is inadequate, but the upward Pb translocation in *Z. muelleri* was observed to be positively correlated with temperature while negatively with salinity (Bond *et al*. 1985).

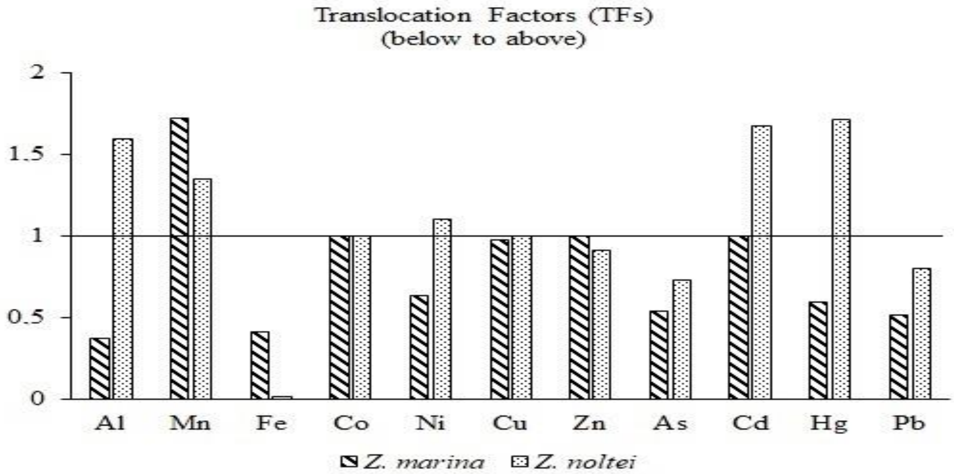

Fig. 2. Values of translocation factor ($C_{leaf}/C_{root\text{-}rhizome}$)
(>1 indicate higher translocation capability)

The causes for the variations found in metal concentration in both seagrasses sediment and seawater due to higher terrestrial inputs caused by increased rain fall with a consequent increase in suspended particles with adsorbed metals. Fe is the basic need for the normal growth of seagrasses and due to this reason these species absorbed Fe from their surrounding environment as compared to other elements except Al and Mn. The reason for bioaccumulations of Al and Mn in seagrasses is that these plants are the better representatives of Al and Mn in sediment and seawater compared to the other organisms. High Zn concentrations were also because seagrasses from the seawater readily concentrate Zn.

CONCLUSION

The current work indicates that owing to continuous exposure to pollution the accumulation of contaminants in seagrass can rise. Higher amounts of the elements accumulated by the seagrasses might be due to increase in the agricultural influx waters, domestic wastes and some anthropogenic activities. If these persist then this can be very detrimental for the coastal ecosystem in future. It is also suggested that the regular monitoring of flora in the coastal areas is essentially required and the seagrasses can be used as pollution indicators for assessment of environmental health of marine coastal areas which merit further investigation.

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Authors' contribution

EA performed experimental part and wrote the manuscript draft. LB designed the study protocol and supervised the whole project. Both of them purposed the research idea and prepared the final version of the paper.

Declarations

It is confirmed that work has not been published, not under consideration for publication elsewhere, approved by all authors and, if accepted, it will not be published elsewhere in the same form, in English or in any other language.

Conflict of Interests

Authors declare that there is no conflict of interest.

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کنش متقابل رسوب-آب با علف مارماهی (Zostera spp.) از سواحل سینوپ دریای سیاه

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چکیده

این مطالعه انجام شد تا اطلاعاتی را در خصوص سطوح عناصر در برگ‌ها و ریشه‌ها – ریزوم‌های زوسترا مارینا و زوسترا نولتئی، آب و رسوب دریای اطراف آنها ارائه کند. نمونه‌های این مطالعه از نواحی کرانه‌ای آکلیمان و تورکلی شهر سینوپ در دریای سیاه طی چهار دفعه از سپتامبر ۲۰۱۵ تا ژولای ۲۰۱۶ تهیه شد. غلظت Al, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Hg و Pb در نمونه‌های هضم شده توسط دستگاه ICP-MS (inductively coupled plasma mass spectrometer) اندازه-گیری شد (Agilent Technologies, 7700X). نتایج نشان داد که سطوح فلزات به ترتیب زیر نسبت به هم قرار داشتند: Hg < Cd < Co < As < Pb < Ni < Cu < Zn < Mn < Al = Fe. گونه‌های زوسترا برای آلومینیوم و منگنز نقش تجمع کننده زیستی را داشتند (BCF ≥ 5000) و برای روی و کادمیوم ماکروکونسانتراتور (بزرگ تغلیظ کننده) بودند (BSAF > 2). ظرفیت‌های عمومی جابه‌جایی در علف دریایی به صورت اکروپتال (از ریشه به سمت برگ) برای Al, Mn, Ni, Cd and Hg بود. نتایج این مطالعه برای اولین بار در آبهای دریای سیاه ترکیه تفسیر می‌شود. غلظت‌های بالا در هر دو گونه زوسترا ممکن است به خاطر تصفیه نشدن پساب‌هایی باشد که از منابع مختلف مانند فعالیت‌های کارگری، پساب‌های کشاورزی و خانگی به نواحی کرانه‌ای می‌ریزند. برای ارزیابی اثر آلاینده‌ها و انجام اقدامات لازم، این منطقه باید به طور منظم پایش شود.

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