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RESEARCH PAPER

Ecological State of the Water Area in the Fresh Water – Saline Water Mixing Zone in Spring (the River Chernaya Estuary – Sevastopol Bay, Black Sea)

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ABSTRACT

The paper provides data on spatial distribution of petroleum hydrocarbons, heterotrophic and hydrocarbon-oxidizing bacteria in water and bottom sediments as well as on heavy metals in bottom sediments at different sites in the zone of fresh and saline water mixing, with the salinity range 1–18 ‰ during spring low water and high water. Physical and chemical characteristics of the bottom sediments are given. The highest hydrocarbon-oxidizing bacteria percentage of the heterotrophic bacterioplankton count in the indicated areas was found in April, with the maximum of 55 % determined in the transition zone waters. The share of hydrocarbon-oxidizing bacteria in the heterotrophic bacteria abundance in the water was larger than that in the bottom sediments. The highest concentrations of chloroform-extractable substances and petroleum hydrocarbons were detected in the marine zone bottom sediments, and their lowest concentrations were found in the river zone. During the period under study, the petroleum hydrocarbon input (in almost equal volumes) was constant, which is indicated by the hydrocarbon percentage of chloroform-extractable substances, which on average was 31 % for the marine zone, 29 % for the transitional zone, and 32 % for the river zone. In contrast to the river and transition zones, the concentration of chloroform-extractable substances observed in the marine zone bottom sediments was constant.

Keywords: marginal filter, bacteria, petroleum hydrocarbons, heavy metals

INTRODUCTION

Fresh water – saline water mixing zones are considered the most productive areas of the world's oceans (Telesh et al., 2013) and at the same time are the areas of transport of chemical pollutants and natural materials from terrestrial ecosystems to the open ocean (McLusky and Eliott, 2004). The important role of such zones for marine ecosystems functioning is related to the fact that they are areas of marginal filters, where at a fresh water – saline water mixing zone organic matter accumulates to high concentrations and becomes transformed (Lisitsyn, 1995). The anthropogenic component of organic matter at a fresh water – saline water mixing zone is often represented by petroleum hydrocarbons (Nemirovskaya, 2004) and trace elements (Malakhova et al., 2020b). Apart from organic compounds, the list of primary pollutants also includes heavy metals (Belenikina and Kapkov, 2008).

Heterotrophic microbiota is an influential biocenosis component of any aquatic ecosystem. Its main function is assimilation and transformation of organic compounds, including

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anthropogenic pollutants. Hydrocarbon-oxidizing bacteria (HOB) transform petroleum hydrocarbons (PHC) at various depths (Wang et al., 2020). This group of bacteria is an indicator of petroleum hydrocarbon pollution of the environment (Polyak et al., 2020). It should be noted that one of the main sources of PHCs in the Black Sea is river runoff. It accounts for about 60 % of the petroleum hydrocarbon budget input (Minkovskaya, 2017).

The Chernaya River is one of the most important rivers in the Sevastopol region in terms of length and water content. The Chernaya River mouth has a complex structure including a simple mouth reach and complex estuarine front comprising a steep enclosed part (the Inkerman estuary and the Sevastopol Bay) and an open part of the estuarine front (Minkovskaya and Demidov 2016). Within these boundaries, there are typical estuarine processes of water and substances mixing, and the estuarine water circulation generates there (Minkovskaya and Demidov 2016). Originally, the Chernaya River estuary was classified as an estuary of the liman-bay type. As a result of numerous anthropogenic transformations, the natural processes of formation of the Chernaya River mouth have been disturbed. In particular, one should note the construction of a boat basin in the Sevastopol seaport in Inkerman; opening and dredging of the river bed during creation of the "Inkerman estuary"; construction of breakwaters, which limited the water exchange with the bay, and construction of the Black River navigable canal. Moreover, this includes construction of the Chernaya River reservoir in 1954, which influenced the river water regime. Today, the mouth is considered a non-typical, anthropogenically modified area (Minkovskaya and Demidov 2016).

The Chernaya River generally floods in winter – spring, from December to April, due to the passage of Mediterranean cyclones (Sovga and Khmara, 2020). After construction of the reservoir, the river does not dry up during the low water period (as opposed to the period of natural runoff regime of rivers). Therefore, the intra-annual runoff distribution has changed: the runoff of the low water period has increased, while that of the high water period has decreased. The high water period of the Chernaya River usually lasts from November to April (with a maximum in April), the low water period lasts from May to September (with a minimum in September) (Minkovskaya, 2017). Therefore, March and April are usually full water months, and the low water season begins in May. An increasing number of researchers are getting interested in the state of estuarine ecosystems (Sovga and Mezentseva, 2019; Malakhova et al., 2020a; Sovga and Khmara, 2020; Paraskiv et al., 2021), but influence of low water and high water periods on the ultimate water quality in the fresh water – saline water mixing zone during this period has not been sufficiently studied yet.

The paper aims at identifying features of water and bottom sediment quality in the Chernaya River estuary in spring based on hydrophysical, hydrochemical and microbiological indicators.

MATERIAL AND METHODS

The study area was chosen to trace changes in the ecosystem during the transition from one type of water mass to another (Fig. 1). The marine zone (St. 1) is located in the apex of the Sevastopol Bay. The latter is the estuarine front of the Chernaya River, i. e. a closed water area of estuary type with a limited water exchange and is under anthropogenic impact (navigation, shipyards, hydraulic engineering works, domestic urban and storm water runoff) (Sovga and Mezentseva, 2019). The zone of water mass mixing is the river – sea transition zone (St. 2). The river zone proper (St. 3).

Fig. 1. Map of sampling stations at the River Chernaya estuary – Sevastopol Bay

Sampling was performed once a month during the spring of 2019. Water samples were taken from surface and bottom horizons at St. 1 (up to 4 m deep) and from the surface horizon at St. 2 (1.5–2 m deep) and St. 3 (0.5 m deep) with a sterile bathometer, bottom sediments were sampled using a Peterson grab with 0.038 m^2 sample area and at shallow depths a hand-held sampler was used.

In freshly collected bottom sediment samples, pH and Eh were determined using Nitron-pH pH-meter-thermometer. The natural water content was determined by the weight method in a laboratory environment. In prepared air-dried samples of bottom sediments, the amount of chloroform-extractable substances (CES) was determined by the weight method, and PHC content was determined by the infrared spectrometry method using FSM-1201 Spectrophotometer (Oradovsky, 1977). Concentrations of PHCs in water and bottom sediments were measured using FSM-1201 Fourier-spectrophotometer. Total heavy metal (HM) content was determined using the X-ray fluorescence method (Technique for…, 2002).

Abundance of heterotrophic (HB) and hydrocarbon-oxidizing (HOB) groups of bacteria was determined in the surface water and bottom sediments by the limiting decimal dilution method using liquid media. For HB, peptone water was used (Mironov et al., 2003). For HOB, Voroshilova-Dianova medium was used (Netrusova, 2005). As the only source of carbon and energy, 1% of sterile oil was added to each test tube after inoculation. When preparing the media, the salinity of seawater was taken into account. The most probable number of microorganisms per unit of volume was calculated using the McCrady table (in triplicate) based on a method of variation statistics (Netrusova, 2005). The samples were processed within two hours of collection.

RESULTS AND DISCUSSION

The bottom sediment samples differed in size distribution and physical and chemical characteristics. At St. 1, bottom sediments were represented by grey silt with a negative Eh value (–31 mV), at St. 2, those were silt with admixture of sand (Eh = +10 mV), and at St. 3, those were fine sand with admixture of clay and vegetation residues (Eh = +58 mV). The pH value of the studied soil samples ranged from 7.44 (St. 1) to 7.94 (St. 3). The natural water content of sediments in the marine zone (St. 1) and the fresh water – saline water mixing zone

(St. 2) was equal (64 %), while in the river zone it was slightly higher (78 %), which is generally consistent with these types of sediments. The values of hydrogen potential (rH_2) calculated for each area by Clark formula (rH_2 = Eh/29 + 2rH) were less than 20 (rH2 < 20), which according to (Ganzhara, 2001) indicates the predominance of reduction processes in bottom sediments of the studied water area. Salinity is the main indicator determining intensity of physical-chemical and biochemical processes and also one of the factors determining diversity of habitats for hydrobionts in estuarine systems (Lisitsyn, 1995). Salinity of the surface horizon water varied along the river – sea section from 1 ‰ (St. 3) to 18 ‰ (St. 1). In the bottom layer of the marine zone, the salinity was 17 ‰. The CES content varied from 460 to 520 mg/100 g air-dry in the marine zone, from 200 to 420 mg/100 g in the transitional zone, and from 100 to 250 mg/100 g in the river zone. The highest CES concentrations were observed in the marine zone (Fig. 2). The lowest CES concentrations were determined in the river sediments. In the bottom sediments of St. 1, the CES concentration was constant.

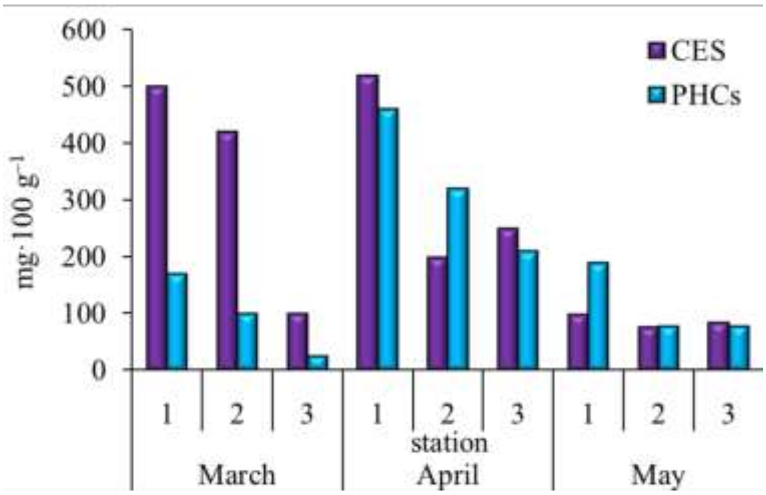

Fig. 2. Concentrations of chloroform-extractable substances (CES) and petroleum hydrocarbons (PHC) in bottom sediments of the surveyed stations

The maximum PHC concentrations (up to 190 mg/100 g) were observed in the marine zone, whereas the minimum ones (up to 26 mg/100 g) were in the river zone. In the transition zone, the values of PHC ranged from 77 to 100 mg/100 g (Fig. 2). Concentrations of heavy metals in the bottom sediment samples are presented in Table 1.

Table 1. Concentration (mg/kg) of heavy metals in bottom sediment samples of the surveyed stations

Heavy metals	St. 1	St. 2	St. 3	Clarkes as per (Dobrovolsky, 2003)
Zn	150	113	46	58
Ni	63	50	15	17
Cr	110	94	64	40
Pb	68	28	14	16
Cu	100	35	BDL*	-
Sr	237	202	275	-

* BDL – below detection limit of the X-ray fluorescence method.

The highest content of Zn, Cu, Ni, Cr, Sr and Pb (Fig. 3) was determined in the marine zone silts (St. 1).

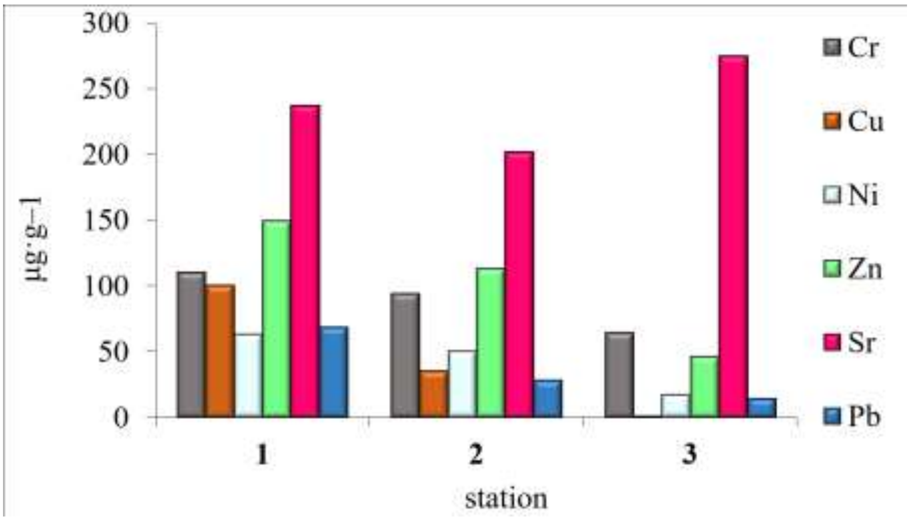

Fig. 3. Content (µg/g) of heavy metals in bottom sediments of the surveyed stations

HB abundance in the river bottom sediments (St. 3) varied from 25,000 to 95,000 bacterial cells per gram of sediments. The maximum values at St. 3 were recorded in March (95,000 cells/g). In the same month, the HB abundance values at Sts. 1 and 2 were 75,000 and 45,000 per gram respectively. In April, the number of HB at St. 1 decreased by an order of magnitude, and at St. 3 it decreased to 45,000 cells/g. In the May samples, the HB count in the river and marine zones numbered in tens of thousands. HOB were cultured from all the sediment samples. The HOB abundance in the river sediments (St. 3) was quite high in the March and April samples, amounting to 4,500 cells/g. The HOB count at the marine station during the same months was an order of magnitude lower. At both stations, the number of HOB in the May samples decreased and did not exceed tens (45 and 25 cells/g, respectively). The HOB abundance in March at St. 2 was equal to that at St. 1. The highest HOB percentage of the HB count was found at all stations in April (13 % for St. 1 and 10 % for St. 3).

The PHC concentration in the water samples ranged from 0.024 mg/L to 0.081 mg/L (Fig. 4). The PHC maximum concentration was observed in the May samples of St. 1. The oil concentration in the surface water layer was higher than that in the bottom water layer. In the river zone (St. 3), the minimum values of PHC content were determined. The threshold limit value (TLV) for PHC (0.05 mg/l) was exceeded in 33% of the water samples taken at Sts. 1 and 2. The low PHC concentrations in the waters of the surveyed stations were observed in March.

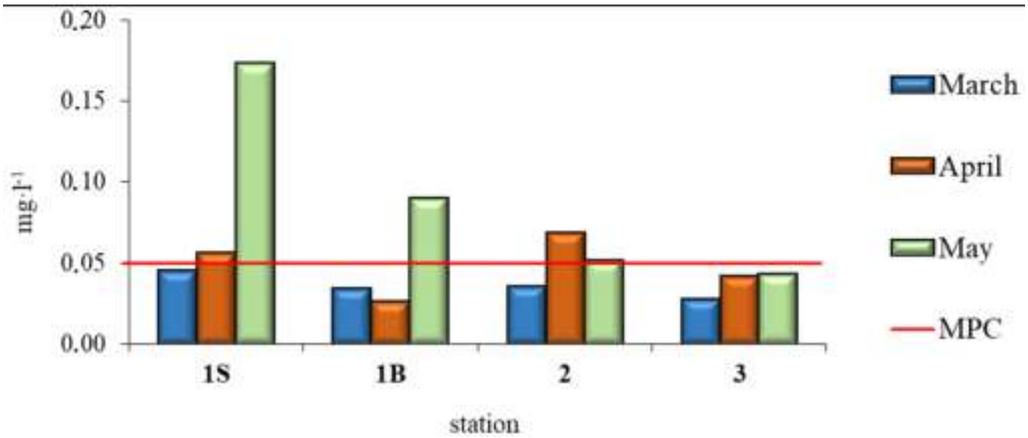

Fig. 4. Concentration (mg/l) of petroleum hydrocarbons (PHCs) in the surface (1S, 2 and 3) and bottom (1B) water layers

The minimum number of HB (1500 cells/mL) at St. 1 was recorded in the March sample, and in the subsequent samples the number of HB at this station varied from 75,000 to 95,000 cells/mL). At St. 2, the number of HB in the April and May samples was equal to that of St. 1 (4,500 and 25,000 cells/mL, respectively). At St. 3 (river zone), equal values of 25,000 cells/mL were determined in the March and May samples. In April, one order of magnitude decrease in the number of HB was noted in the water of this station. HOB were isolated from all the water samples. The highest abundance of HOB at St. 1 was found in the April sample (200 cells/mL), the lowest one was in the May sample (8 cells/mL). In March, the HOB values at St. 1 numbered in tens. At St. 2 in April, a high abundance of HOB was noted (25,000 cells/mL). Moreover, one should note exceedance of TLV for oil in this sample. In May, the number of HOB decreased to 95 cells/mL. In the fresh water of St. 3 in March, the number of HOB did not exceed 5 bacterial cells per milliliter of water. The April and May HOB values at this station were 950 cells/mL and 450 cells/mL, respectively.

Bottom sediments accumulate various types of pollutants including oil and petroleum products, therefore they are a more reliable indicative medium than mobile water layers. In turn, the physical and chemical characteristics of bottom sediments influence the intensity of processing of incoming organic substances of different nature and are an important factor in the formation of benthic communities. The highest concentrations of CESs, which corresponded to pollution levels III–IV (out of V) according to the regional classification (Mironov et al., 1986), were found in the bottom sediments of the marine zone (St. 1). At this pollution level, there are negative changes in the species composition and quantitative characteristics of macrozoobenthos in the benthic community. In particular, it is dominated by highly pollution-tolerant species, e.g. *T. reticulata*, *P. ciliata*, *C. capitata* (Mironov and Alyomov, 2018). Similar CES concentrations were determined in other parts of the apex and estuarial parts of the Sevastopol Bay (Mironov et al., 2003). In contrast to the river and transition zones, the soils of St. 1 are characterized by a constant CES concentration. These constant CES concentrations in the bay are associated with chronic pollution of the water area under anthropogenic press, while instability of CES values in the bottom sediments of Sts. 2 and 3 may be attributed to the mobility of riverbed soils during high water.

The petroleum hydrocarbon input (in almost equal amounts) during the study period was permanent, as evidenced by the PHC percentage of CES, which on average was 31 % for the marine zone, 29 % for the transitional zone, and 32 % for the river zone. Similar values were recorded previously in the estuary of the Sevastopol Bay, where the average was 30 % (Mironov et al., 2003). It was studied (Brendel and Luther, 1995; Hyacinthe et al., 2001; Belan and Moshchenko, 2009) that soils with signs of oil pollution are characterized by poverty of species along with a high abundance and biomass of pollution-tolerant forms, and with severe chronic pollution there is suppression of the entire community, including tolerant forms.

The resulting CES and PHC values are comparable with data for biogeochemical barriers in other regions. For example, PHC concentrations in the Severnaya Dvina delta during high water appeared to be comparable with those in the sediments of areas with permanent oil inputs (Nemirovskaya et al., 2015). In the sediments of the Caspian Sea northern shelf represented by sandy sediments with broken shells and algae and rather low C_{org} content (0.197–0.582 %), PHC concentrations varied in the range 7–455.7 mg/100 g (Nemirovskaya, 2013). On the western coast of Taiwan Island, in the delta of the Kaoping and Tungkam Rivers, PHC concentrations in the sediments were even higher: 86.9–1030 mg/100 g (Jeng, 2006). On the contrary, in estuarine areas of the northwest part of the Black Sea, the PHC content was lower and varied from 0.5 to 40.2 mg/100 g (Nemirovskaya, 2013); according to

(Readman et al., 2002) in the Danube delta, the PHC content was within 4.9–22 mg/100 g.

The US Environmental Protection Agency (EPA) (US EPA, 1993) has developed and proposed a Sediment Quality Criteria (SQC) for assessing the quality of natural sediments (Table 2). The introduction of SQC had two aims: first, to be able to assess the safety of bottom sediments; second, to predict potential pressure on them without causing adverse consequences for the ecosystem as a whole.

Table 2. Classification of bottom sediments of natural waters according to Sediment Quality Criteria (mg/kg)

Bottom sediments	Cd	Cr	Cu	Pb
Not polluted	-	<25	<25	<40
Moderately polluted	-	25–70	40–60	-
Highly polluted	>6	>75	>50	>60

Table 2 shows that in terms of the average content of chromium and copper (Fig. 3 and Table 2) the bottom sediments of the study area are classified as moderately polluted. The average content of nickel, zinc and lead in soil samples indicates an excessive geochemical background, which is quite likely to be related to an anthropogenic factor.

Contamination of bottom sediments with lead and nickel does not depend on the organic content of the sediments. The elevated nickel content in bottom sediments was already observed in the apex part of the Sevastopol Bay previously (Gurov et al., 2019). The zinc content was 150 mg/kg, which is consistent with the data (Malakhova et al., 2020b). Increased concentrations of zinc, nickel, and chromium were also noted previously in the apex part of the Balaklava Bay (Sevastopol region), which, as with the Sevastopol Bay, is a water area with a limited water exchange (Kotelyanets et al., 2019).

The highest HOB percentage of the HB count in bottom sediments as well as in water samples was found at all stations in April (St. 1 – 13 %, St. 3 – 10 %), however it should be noted that this percentage was lower than that in similar water samples. During the observed months, the abundance of HB in the bottom sediments at St. 3 was more stable as compared with the seaward part. It is obvious that the river runoff makes organic compounds of various etiologies available to bacteria, which confirms the river runoff influence on the quantitative indicators of heterotrophic bacteriobenthos. The high abundance of HOB at St. 3 may indicate an increase in concentrations of PHCs (Roy et al., 2002) during and after the March high water, which is confirmed by the chemical analysis of sediments from St. 3.

The low concentrations of PHCs in the waters of the surveyed stations determined in March may be due to the maximum river water runoff diluting seawater, which has already accumulated toxicants. Moreover, there is no increase of microalgae amount in March as compared with a warmer May, when elevated PHC contents in water may be the result of an increased biogenic background. The minimum PHC content in the river zone waters suggests an irregular input of pollutants into the bay with the river runoff. At the same time, exceedance of TLV for PHCs in the marine zone waters in May indicates input of fresh petroleum products coming not from the river runoff.

The HB abundance in the Chernaya River mouth is similar to that in river mouths in general (Hewson and Fuhrman, 2004). The HB abundance in the water at St. 3 in March exceeded that at the seaward St. 1, which may indicate the presence of highly digestible organics in the river runoff. It was shown (Crump et al., 2004; Hewson and Fuhrman, 2004; Mosharova et al., 2016) that heterotrophic bacterioplankton abundance is higher at the stations with the most desalinated water than in the linked ecosystems. The HB count at all stations in May numbered in tens of thousands. The HB abundance in water is similar to that in bottom sediments, apparently the fresh water and saline water mixing zone contained large amounts of suspended organic matter, which is a living spot for bacteria. In contrast, the HB abundance in the water of internal estuaries of small rivers (Tatar Strait) is much lower than that in bottom sediments (Garetova et al., 2020).

The HOB largest share in the heterotroph composition at the surveyed stations was observed in the April samples, in particular the highest value 55 % was determined at St. 2, in river water (St. 3) and sea water (St. 1) these were 21% and 26%, respectively. However, it should be noted that the destructive potential of HOB depends on many environmental factors (Rubtsova and Egorov, 2004; Xu et al., 2018). In the remaining months, the HOB percentage of the total HB count was insignificant (1–2 %), which is similar to the research results of (Hewson and Fuhrman, 2004; Alyomov et al., 2018).

CONCLUSIONS

The comparative assessment of quantitative indicators of heterotrophic and hydrocarbon-oxidizing bacteria in spring showed that at stations with different salinity levels, the abundance of heterotrophic bacterioplankton varies within equal limits. The HB abundance in the studied water area is similar to that in the bottom sediments. The highest HOB percentage of the heterotrophic bacterioplankton count at all stations was found in April, with a maximum of 55 % found in the transition zone waters. The HOB percentage of the HB count in the water was higher than that in the bottom sediments. The highest concentrations of CESs and PHCs were found in the marine bottom sediments while the lowest concentrations thereof were found in the river zone. In contrast to the river and transition zones, concentrations of CESs observed in the marine zone bottom sediments were constant. The low PHC concentration in the waters of the stations under study, which may apparently be associated with the maximum river runoff, was obtained in March. In the river zone waters, the lowest PHC concentrations were determined, as compared to the study sites. At the same time, 33 % of water samples taken at Sts. 1 and 2 exceeded TLV for PHC (0.05 mg/l). During the period under study, the PHC input (in almost equal volumes) was constant, which is indicated by the hydrocarbon percentage of CESs, which on average was 31 % for the marine zone, 29 % for the transitional zone, and 32 % for the river zone. The minimum PHC content in the river zone waters indicates nonregular input of pollutants to the bay with the river runoff. At the same time, TLV exceedance for PHC in the marine zone waters in May indicates input of fresh petroleum products coming not from the river runoff.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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