The aim of this study was to identify differences in the distribution of Mn (Manganese), Fe (Iron), NO$_3^-$ (Nitrates) and reactive phosphates (RP) in the water columns of the eastern and western basin of the Black Sea region. Accordingly, the selected sampling area covered offshore waters from west to east including Istanbul (station KD01), Ereğli (KD2A, 2B, and 2C), İnebolu (KD03), Sinop (KD04), Ordu (KD5A, 5B, 5C) and Trabzon (KD06). The B and C codes for stations 2 and 5 indicate that the stations are towards the basin centers of the Black Sea. At all the stations of the study area, the Mn values were below the limit of detection (5 µg/L) up to 100 m depth. The Mn concentration reached maximum at 200 m depth of stations KD2B, KD5B, KD5C and KD06. This situation could be due to mobilization of Mn from the continental slope sediments (Mn pump). Also, it was observed that relatively high values of nitrates were associated with low RP concentration in the deep waters of the eastern basin. If nitrate is present in the suboxic zone, bacteria prefers nitrate and reduce it as N$_2$ (g). Then, the FeOOH≡RP (ferric oxyhydroxides≡reactive phosphate) particulates sink into deeper layers without reduction by bacteria and cause the upper layers to become poor in phosphates. In case of low values of nitrate in suboxic zone, FeOOH≡RP particulates are reduced to dissolved Fe$^{2+}$ by the bacteria. Significantly higher levels of nitrate concentrations were observed at station KD06. In conclusion, the eastern basin center is characterized by relatively higher nitrate concentrations in water columns compared to those in the western basin center. Therefore, the eastern basin contains lower RP concentrations and this limits the phytoplankton growth. It is concluded that where nitrate concentrations are higher than 2 µM, the bacteria preferred to reduce the nitrates. On the other hand, particulate Fe can be reduced chemically with H$_2$S in the deeper layers, where there is lower nitrate concentration.

[Keywords: Iron; Manganese; Reactive phosphate; Nitrate; Black Sea]
located along the coastal zones of the sea: Ukraine, The Russian Federation, Georgia, Turkey, Bulgaria and Romania, and seventeen are EU countries including Albania, Austria, Belarus, Bosnia, and Herzegovina, Croatia, the Czech Republic, Germany, Hungary, Italy, Macedonia, Moldova, Montenegro, Poland, Slovakia, Serbia, Slovenia, and Switzerland. It is connected to the Mediterranean Sea through the Turkish Bosphorus (Istanbul and Canakkale) in the south – which is the narrowest passage in the world with an average width of 1.6 km and a total length of 41 km – and to the Azov Sea in the north through the Kerch Strait, which has a depth less than 20 m.

The most important characteristic of the Black Sea is the presence of permanently anoxic deep water due to H₂S. Therefore, the Black Sea comprises the largest anoxic body of water in the world. Hydrogen sulphide provides the basic conditions for the accumulation of abyssal sediments rich in organic matter. Abyssal sediments in the Black Sea are devoid of benthic fauna and consists of only the remains of plankton due to H₂S contamination.

Hydrogen sulphide, found under the surface water layer containing oxygen, is related to the changes in the Mediterranean and the Black Seas as a result of fresh water inflow at high flow rates. This hydrographical regime consists of deep waters originating in the Mediterranean Sea coming through the Bosphorus with high salinity, together with river-originated surface waters with low salinity. Hydrogen sulphide is the main physical obstacle for a sharp pycnocline mix centered at a depth of 50 m and it is the reason for a stable anoxic interface. Most of the factories with various facilities in the Black Sea region have no treatment plants, and this causes regional pollution. These particularly include hazelnut facilities, wood and stone flooring production and fish oil factories in the east, and iron and steel factories in the west that carry tons of heavy metal and other pollutants to the Black Sea. Additionally, metal levels in the Black Sea are increasing with each passing day due to oil and air pollution.

**Sampling and chemical analysis**

In this study, the selected water sampling area covered Ereğli (KD2A, 2B, 2C), Inebolu (KD03), Sinop (KD04), Ordu (KD5A, 5B, 5C) and Trabzon (KD06) offshore waters beginning at İstanbul (station KD01) and going from west to east. The B and C codes for stations 2 and 5 indicate that the stations are towards the basin center of the Black Sea.

Six of these stations are in the coastal regions from Bosphorus to Trabzon and four stations are towards the east and west basin centers. Water samples were taken from 0, 2.5, 5, 10, 20, 50, 80, 100, 150 and 200 m depths. Sampling trips were performed during September 2008 (KD01, KD2A, KD2B, KD2C) and May 2008 (KD03, KD04, KD5A, KD5B, KD5C) with the TCG-Çeşme boat and April 2008 (KD06) with the R/V-1 Surat Research boat (Fig. 1).

![Fig. 1 — The locations of the sampling stations using the TCG-Çeşme boat and R/V-1 Speed Research boat in the Black Sea (The map was taken from Beşiktepe with modifications).](image-url)
For the metal analysis, samples were put into 50 mL high-density plastic bottles, followed by the addition of 0.2 mL of concentrated HCl (Hydrochloric acid) to prevent metal adsorption for the conservation of water sample. Additionally, water samples were put into 1 L polyethylene containers for nutrient analyses. To remove the sulphides in the water samples taken for nitrate and phosphate analyses, 0.2 ml of 2 M zinc acetate was added to a 100 ml sample. Nitrate and RP analyses were conducted according to Strickland and Parsons16 using 10 cm light-path cuvettes in a Hach-Lange DR4000 spectrophotometer. Heavy metal analyses were performed using a Metrohm 797 VA Computrace for voltammetry according to the DPASV (differential pulse anodic stripping voltammetry) method in HMD (hanging mercury drop) mode (method 2: voltametric determination for Fe concentrations <200 µg/L. Application bulletin 317/1e voltametric determination of iron, Metrohm, DOL: 6 µgFe/L. Manganese in drinking water, application No: 90, Metrohm, DOL: 5µgMn/L). As the samples were not filtered, the measured values contain dissolved Fe and Fe_L (Leachable-iron) extracted from dissolved particulate Fe.

Criteria for interpretations of spatial distributions

The interpretation criteria adopted in the study were:

(a) As is known, nitrification of ammonium requires oxygen and therefore maximum nitrate determines ammonium nitrification. On the other hand, regions with zero oxygen doesn’t necessarily mean regions with no oxygen because oxygen supply rate to the environment can be equal to the oxygen consumption rate in the environment. Therefore, vertical nitrate measurement is sufficient for the purpose of this study.

(b) Minimum nitrate determines the lower limit of denitrification in terms of depth. In water with high H2S and no oxygen, nitrification is inhibited. Knowing the minimum nitrate in depths with depleted nitrate is sufficient because of their H2S-rich content. Moreover, FeS whose precipitation in the Black Sea waters is possible is known to have poor adsorption ability17. Therefore, within the scope of the study, sulfur cycle was not investigated.

(c) Deep Black Sea waters, which are known to have reducing properties, have high alkalinity18. Therefore, Fe^{3+} particles can be reduced and precipitate as FeCO3 or can be dissolved as Fe^{2+}.

(d) Shewanella putrefaciens is known to preferentially reduce dissolved compounds such as nitrate, and only reduce FeOOH and MnO2 in the absence of nitrate. It is estimated that, in depths with high nitrate content, FeOOH≡RP and MnO2 particles will sink until they reach depths with depleted nitrate (<0.5 µM). Based on this information, below the nitrate-containing layer in the vertical water column, Fe and Mn particles will dissolve and reactive phosphate will be released. It was assumed that phosphate release in deeper waters (150-200 m) will make its supply to oxygen-containing waters more difficult.

Statistical analysis

For the values that deviated from the normal distribution (values exceeding ± 2 for skewness) the Kruskal-Wallis test was conducted with the median values and the significance of the differences (p<0.05) were tested19.

Results and Discussion

The values of reduced density (σ_t) were: KD2B: 16.7 (for 200 m), KD2C: 16.6 (for 200 m), KD5B: 16.6 (for 200 m), KD5C: 16.6 (for 200 m), and KD06: 16.4 (for 200 m); σ_t values of the other stations were lower than 16.2. Konovalov et al.20 reported that for south-western area in Black Sea, the permanent H2S onset occurs at σ_t ~16.4 (higher than the average basin-wide σ_t of 16.2).

Manganese was at below the limit of detection in the water column at the coastal stations (KD01 and KD2A). Manganese concentration at the KD2B station was < 5 µg/L up to 150 m, while the maximum nitrate level decreased at 200 m and Mn increased at the same depth up to 15 µg/L. The decrease in nitrate levels to < 1 µg atN/L at 200 m indicates that the increase in Mn may be in the form of Mn (II) (Fig. 2). This result was in line with the inhibition of bacterial MnO2 reduction by nitrate, reported by Dollhopf et al.18 The 15 µgMn/L levels observed at station KD2B (200 m) was consistent with the explanation by Kempe et al.21 to some extent. Kempe et al.21, pointed to shallow coastal sediments as a source of Mn. But our findings indicate mobilization of Mn from the continental slope sediments (not near the coast), which may be greatly enhanced by internal waves warping the H2S-containing waters and operating a Mn pump. Manganese was at low concentration along the water column up to 200 m in the western gyre (KD2C).

Iron gets accumulated at 80-100 m in the western gyre (KD2C). The High Fe_L concentrations were
observed at the stations KD01 (0-10 m and 50-80 m), KD2A (20-50 m) and KD2B (0-20 m) indicating the Sakarya river and Bosphorus input (Fig. 2). The maximum value of nitrate concentration was observed at 150 m depth of the station KD2B. In the western basin center (KD2C), nitrate concentration reached a maximum at 100 m depth. This may be caused by patchiness in the distribution of Fe in the water column. Particulate Fe passes through the depths where high concentrations of nitrate are observed, without being reduced; but it is reduced and dissolved at 150 m where nitrate concentration is low. At station KD2C, Fe increased above and below the maximum nitrate values. Indeed, higher concentrations of RP in depths greater than 150 m (KD2C) helps to explain that Fe is reduced and dissolved. Low RP concentrations in the surface waters of the stations in Figure 2 indicate that phosphate was consumed by phytoplankton, and it left the surface waters by sinking in the form of detritus (export production) and FeOOH=RP particulates. The mineralisation of detritus and release of RP as took place between 50-150 m probably. Reduction of Fe+3 and release of RP took place at greater depths contributing RP values to some extent. The relatively high RP concentrations were seen at 100-150 m depths (KD2B) indicating detritus mineralization (eutrophication).

In Figure 3, FeL reached a maximum (360 µg/L) at a depth of 50 m at station KD5B. The RP decreased to <3 µg atP/L at the same station probably due to the adsorption on particulate Fe. The relative RP at stations KD5A (20 m) and KD5B (0 m and 80 m) were in higher concentrations; however, they significantly decreased up to 200 m (< 0.8 µg atP/L) in the eastern basin center (KD5C). In Figure 3, RP isopleths were almost vertical in the eastern basin gyre center (KD5C). This indicates that the release of RP by the reduction of particulate Fe occurred at levels deeper than 200 m. The maximum nitrate level observed at station KD5B at 80 m depth was >2.5 µg

![Fig. 2 — FeL, Mn, NO3− and RP distributions in the western basin of the Black Sea.](image-url)
However, it decreased at deeper levels. On the other hand, at station KD5C, nitrate concentrations reached maximum levels (ca. 2 µg atN/L) in depths of 10-20 m and ≥ 140 m. Nitrate concentrations were higher at the stations in the eastern basin (KD5A, KD5B and KD5C) compared with those in the western basin (KD01, KD2A, KD2B and KD2C). It was indicated that Fe was in particulate form in the eastern basin. The reduction of Fe occurred at levels deeper than 200 m in the eastern basin center.

Fig. 3 — FeL, Mn, NO$_3^-$ and RP distributions in the Ordu transect in the eastern basin of Black Sea.
It was explained that RP concentrations are lower in the eastern basin compared with those in the western basin (KD2C). Therefore, it takes a longer time for the waters in deeper levels in the eastern basin to reach the surface. The value of Mn concentration was below 5 µg/L up to 100 m depth at stations KD5A, KD5B and KD5C (Fig. 3). The maximum Mn concentration was observed in depth of 200 m at station KD5B. It should be particulate form of Fe and Mn because of the relatively high nitrate levels between 80 m and 200 m at stations KD5B and KD5C.

Manganese concentrations were below the measurement limits down to 80 m in the coastal and deeper offshore stations (Fig. 3). At deeper levels, Mn concentration exceeded 40 µg Mn/L. The maximum Mn concentration was observed at the level of 200 m, near the continental slope (KD5B). High Mn concentration at station KD5C was due to the lateral transports from station KD5B. Schnetger and Dellwig\textsuperscript{22} reported that total Mn values were found ranging LOD (limit of detection)-7 µM between 2007-2008. This range is consistent with our data.

Iron levels were high in the deep waters in some coastal stations (KD03 and KD04) and the surface water of station KD5A due to Yesilirmak River (Fig. 4). The RP values were maximum where low Fe concentrations were detected in surface waters of stations KD03 and KD04 indicating terrestrial phosphate contribution. The highest Fe concentration was observed at 200 m depth of station KD06. The RP concentrations were at the levels of < 0.5 µg atP/L down to 100 m depth at station KD06, due to high Fe concentration. The nitrate concentration at station KD06 was high throughout the water column. Thus iron throughout the water column should be in the particulate form. On the other hand, the increase in RP concentration (5.47 µg atP/L) at 200 m depth supports decomposition of organic matter. The Mn levels were below the detection limit at stations KD03, KD04, KD5A and up to 150 m at station KD06 (Fig. 4).

Fig. 5 gives the distribution of RP concentrations against the average Fe concentrations with 95% confidence limits. According to Blomqvist et al.\textsuperscript{8}, the atomic adsorption ratio is Fe/RP=2; in Figure 5, the
RP values corresponding the Fe values were lower than expected RP values. This probably indicates that the iron oxides that adsorbed RP passed through the suboxic zone (SOZ) and precipitated to bottom without being reduced by the bacteria due to the relatively high nitrate concentrations in the suboxic zone at station KD06 in the Batumi eddy and to deeper levels than 200 m at station KD5C in the eastern basin center gyre (Fig. 6). Because *Shewanella putrefaciens* primarily reduces dissolved compounds, iron oxides cannot be reduced by the bacteria if dissolved nitrate exists 18.

The RP concentrations in comparison to the average Fe concentrations at stations KD2B and KD2C were expected to be higher than the observed values. This situation can be explained by eutrophication, because the western basin center is more biologically productive (more export production) than the eastern basin. The coastal zone from İnebolu to Ordu (stations KD03, KD04, KD5A and KD5B) contain higher average RP concentrations than the average iron concentrations can bind. Therefore, the primary productivity in the coastal zone mentioned may not be limited to RP (except

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**Fig. 5** — The relationship between Leachable Fe (Feₙ) and Reactive Phosphate (RP) at all stations. Horizontal and vertical lines show the 95% confidence interval.

**Fig. 6** — The indirect effects of the NO₃⁻ concentrations on the RP concentrations in the western and eastern basins. The data used in this graph includes that from the surface to the lower boundary nitrate peak (> 0.4 µg atN/L) in the suboxic layer. Below the depth of maximum nitrate level, Fe(III) reduction with H₂S and RP releases take place. Nitrate and RP units are µg atNO₃⁻−N/L and µg atP/L respectively.
station KD06, which is thought to be located in the Batumi eddy).

The higher Fe concentrations obtained in this study compared to those reported in the previous studies\(^{23,6}\) can be associated with the dominant arid and warm climate condition up to 2007. The increased contribution of rainfall and streams along with the dominant rainier and colder climate conditions after 2007 may explain this situation. In addition, the adsorption of heavy metals — in samples from polyethylene containers — to the inner walls of the container have resulted in lower Fe concentrations, as expected, from the previous studies\(^{24}\). Also, Lippiat et al.\(^{24}\) reported that the leachable particulate iron (Fe\(_{L}\)) concentrations in Northern Alaska coastal waters were measured ranging from over 1 µM in Alsek River plume to less than 5 nM at the base of Cook Inlet via the massive input of glacial-derived particulate iron.

Accordingly, the stations are divided into two groups based on the Fe/RP plots in Figure 5. Both groups explain the declining RP concentrations because of the increased Fe concentrations. The difference between the western and the eastern basins was the lower RP concentrations in the western basin, which contained the same concentrations of Fe as the eastern basin. It was seen that stations KD03 and KD06 were included in the western group. Bacterial reduction of Fe oxyhydroxides in the suboxic zone and thus the release of absorbed phosphates was controlled by the nitrates in both stations.

Figure 6 describes the superposition of nitrates on the RP/Fe relationship. Both reactive phosphate values and the relatively high nitrates values were similar at stations KD03 and KD5B. Higher nitrate concentrations were observed at stations KD5C and KD06 in the eastern basin. However, a value of <0.5 µg at P/L was observed as a result of RP being carried down to the deep waters via FeOOH.

Different Fe/RP relationships in the western basin stations are probably associated with higher primary production in the western basin. Iron oxides and export production may have an important role as carriers in the RP transport to the deep waters. Probably, eutrophication has a greater effect on RP levels compared to that of the nitrates. On the other hand, lower nitrate concentrations at stations KD01 and KD2A may be associated with the presence of a combined nitrification and denitrification process (Fig. 6). Reactive Phosphate was released by organic matter mineralization below the 100 m depth at stations KD2B and KD2C. Therefore, the average RP values at stations KD2B and KD2C were higher than that of station KD01 and KD2A. Consequently, observation of the higher average RP values can be related with increasing eutrophication.

Figure 7 shows the median RP, Fe\(_{L}\) and NO\(_3^-\) concentrations at 10 stations in the Black Sea with a 95% confidence interval for the statistical comparisons. Median Fe\(_{L}\) concentrations at stations of KD01, KD2A, KD2B and KD2C in the west were statistically different from the stations of KD5C and

![Fig. 7 — A comparison of the median Fe (leachable and dissolved), NO\(_3^-\)-N and RP concentrations and 95% confidence intervals from the west to the east Black Sea. The Kruskal-Wallis Test statistic is 42.9284 (p<0.05). Since the P-value is less than 0.05, there is a statistically significant difference amongst the medians at the 95% confidence level.](image-url)
KD06 in the east. The $F_{FeL}$ concentrations in the eastern area were greater than those in the western area. The median nitrate concentrations in all three areas differ statistically. Nitrate concentrations from the west to east illustrated an increasing trend. This increasing trend can be resulted in preferring of the bacteria to reduce nitrate instead of $Fe^{3+}$. The phosphate concentrations at the middle and middle-east stations (KD03, KD04, KD5A and KD5B) differed statistically from the west (KD01, KD2A, KD2B and KD2C) and east stations (KD5C and KD06). The stations KD03, KD04, KD5A and KD5B are located near the Zarbana, Kızılırmak and Yeşilırmak rivers, respectively. Nitrate concentrations higher than 2 µM at stations at KD5C and KD06 cause the reduction of $FeL$ and release of phosphates at deeper depths.

The relatively high $FeL$ values at station KD5C were in line with the relatively low concentrations of RP. In the deep waters of the same station, relatively low RP values and the highest $FeL$ value show that $FeL$ was reduced and RP was released at 200 m depths. The average Fe concentration detected in surface waters at station KD06 was low in places where average RP concentration was low. This can be explained by statistically different and high nitrate concentrations that subsequently carry the release process of RP to the deeper levels (Fig. 7).

High NO$_3^-$ and low $FeL$ concentrations in the surface waters at station KD06 in the Batumi eddy may probably be associated with very low RP concentrations. High nitrate concentrations carry the reduction process of FeOOH=RP particulates to deeper levels and decrease the possibility of enrichment of the surface waters. It has been shown that $\gamma$-proteobacteria Shewanella sp. reduces nitrates rather than FeOOH particulates$^{18}$. High nitrate, low RP and high $FeL$ concentrations in the deep waters of the same station might indicate that Fe remained substantially in particulate form. The supporting fact was that low $F_{Fe}$ concentrations at the surface and high $FeL$ concentrations in the deep were associated with $FeL$ being carried in particulate form and accumulating in deep waters. On the other hand, at station KD06 – which was included in the Batumi eddy – chlorophyll-$a$ content was minimum, whereas Fe and Cu content were high because of the anthropogenic contributions from the mining areas. Therefore, due to low phytoplankton biomass, both lower phytochelatin secretion$^{25,26,27}$ and higher Fe concentration might have caused the removal of RP by precipitation with the FeOOH$^5$. As a result, primer production in the eastern basin center (KD5C) and Batumi eddy (KD06) was limited by RP, whereas RP was found in the water column in the western basin. Reactive Phosphate was carried down to deep waters in the eastern basin with FeOOH and could not be reduced in the suboxic zone due to its high nitrate concentrations. Phytoplankton uptake and sinking of the cells (export production) through eutrophication in the western basin can be an important mechanism in the transport of phosphates through the suboxic zone.

**Conclusion**

Generally, there is evidence of RP release about beginning $Fe^{3+}$ reduction at the nitrate concentration < 2 µM. The mobilization of Mn from the continental slope sediments, (not near the coast) which may be greatly enhanced by internal waves warping, lateral movements of the H$_2$S-containing waters and operating a Mn pump. At all the stations of study area, the Mn values were below the limit of detection up to 100 m depth. The relatively higher Mn amounts in the deep and shallow waters in the coastal stations show that the sediment served as a Mn source.

In the coastal stations of eastern basin, detritus mineralization at 20-80 m, in the coastal and central stations the nitrification at 80-100 m and the evidence of denitrification (<2 µM nitrate) at >200 m explain $Fe^{3+}$ reduction and RP release taking place at the depths of greater than 200 m. In the Eastern gyre characterized by weak upwelling at the centre, vertical RP transport and diffusion to euphotic zone must be difficult. On the other hand, RP has been provided to central area by the lateral transport and diffusion. Nutrient contribution of lateral transport via Kızılırmak, Yeşilırmak and other rivers were found to be important in the eastern basin.

In the gyre of western basin, detritus mineralizations begin at 50 m. The evidence of poor nitrification at 100 m and the evidence of denitrification at 120 m clarify $Fe^{3+}$ reduction and RP release taking place at 150-200 m. Mineralization and Fe reduction supply RP vertically to euphotic zone. The <2 µM nitrate levels cannot inhibit $Fe^{3+}$ reduction by bacteria (probably shawanellla sp.) and vertically RP supplies to euphotic zone were found to be important in the western basin.

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