# The role of iron on the phytoplankton growth in heavily polluted Izmir Bay, Aegean sea.

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## ABSTRACT

In order to elucidate the role of iron in phytoplankton bloom, total and reactive iron concentration were monitored in Izmir Bay before, during and after the bloom periods in between 1993-1994.

Izmir Bay is divided into three sections; heavily polluted Inner Bay, moderately polluted Middle Bay and relatively unpolluted Outer bay. Thus, the probable effect of iron on phytoplankton growth has been observed for three different levels of pollution throughout the Bay. Izmir Bay has been suffering from anthropogenic pollution and associated water quality problems, such as hypoxia-anoxia.

Preliminary results have revealed a dynamic relation between iron and the size of phytoplankton population throughout the Bay. These results suggest that the reactive iron may be an important factor for phytoplankton bloom and its distribution in coastal waters. Additionally, PCA (Principal Component Analysis) has shown that 35% of the variation in the data collected during the surveys was related to reactive iron, phosphorus forms (dissolved, particulate and total) and ammonium, 14% of the variation was related to chl-a, phytoplankton cell numbers, DO and pH, and finally 11% of the variation was related to nitrite, nitrate, and temperature.

## INTRODUCTION

There are evidences that iron is a limiting nutrient in some oligotropic open ocean waters [1,2,3]. The recent large scale *in situ* iron-fertilization experiment (IronEx II) in the equatorial Pacific Ocean demonstrated that iron limitation of phytoplankton is a fact in regions of the ocean known as high nitrate, low chlorophyll waters (HNLC) [4,5]. Despite a significant increase of knowledge on the biogeochemistry of iron in seawater, the degree of control of the distribution of

iron in seawater by biological activities, the role of iron in algal growth, productivity and eutrophication in coastal waters are still not known very well.

In phyto-plankton, iron is essential to the biosynthesis of chlorophyll and is the central component of iron-sulfur proteins (e.g., ferredoxin; which facilitates the intercellular transfer and storage of photosynthetically energy). Iron is a key nutrient in nitrogen cycling because it is present in the enzymes nitrogenase (for nitrogen fixation) and nitrate-nitrite reductase [6, 7]. Different ratios of iron to macronutrients in seawater may regulate phytoplankton species composition and succession or may modify the grazing activity of zooplankton by creating suitable conditions for some phytoplankton species [8,9].

Despite its abundance in the Earth's crust, iron is elusive for phytoplankton because of the limited solubility of Fe(III), the most dominant form of iron in seawater [6]. However, field measurements show that more dissolved iron in various forms is present in seawater than would be expected from thermodynamic calculations [10].

Anthropogenic influences on trace metal distribution and their effect on marine ecology are more pronounced in coastal waters. The atmospheric deposition of acidifying substances (acid rain) onto land increases the leaching of Fe from the soil and sediments [11], and thereby may alter the balance between phytoplankton species in natural waters [12]. Iron enrichment affects the species composition of the phytoplankton by selectively promoting either diatom growth or the growth of coccolithophorides depending on the water mass under investigation [1]. On the other hand, researches in recent years have indicated that toxic algae proliferation is probably one of the consequences of land and waterway acidification. Fe may be important as a triggering factor [13,14], or at least it has been considered 'potentially' important [15] for the development of toxic phytoplankton blooms in natural waters. Increase of Fe in seawater may also have a direct toxic effect on grazers [8].

In this paper variations in reactive iron concentrations in Izmir Bay in relation to phytoplankton biomass based on the data collected between November'93 and July'94 are presented. Izmir Bay is one of the most polluted coastal zones in the Mediterranean Sea. This preliminary is to initiate a study in order to understand the effects of iron together with other nutrients on algal growth and productivity/eutrophication in Izmir Bay.

## METHODS

Monthly surveys of Izmir Bay were conducted over the period from May 1993 to July 1994. The surveys were done with a 8m wooden skiff. The location of each sampling station was chosen by considering hydrodynamical features of the Bay. Because of its special water characteristics, Izmir Bay will be discussed by dividing it into three sections; Inner, Middle and Outer Bay (Figure 1). Samples were taken at the surface (about 30 cm), 2m depth, 5m depth and bottom water. Samples taken by polycarbonate sampling bottles were placed in polyethylene bottles which had been soaked in 4M HCl and rinsed 6 times with distilled water. Everything which could potentially have come into contact with

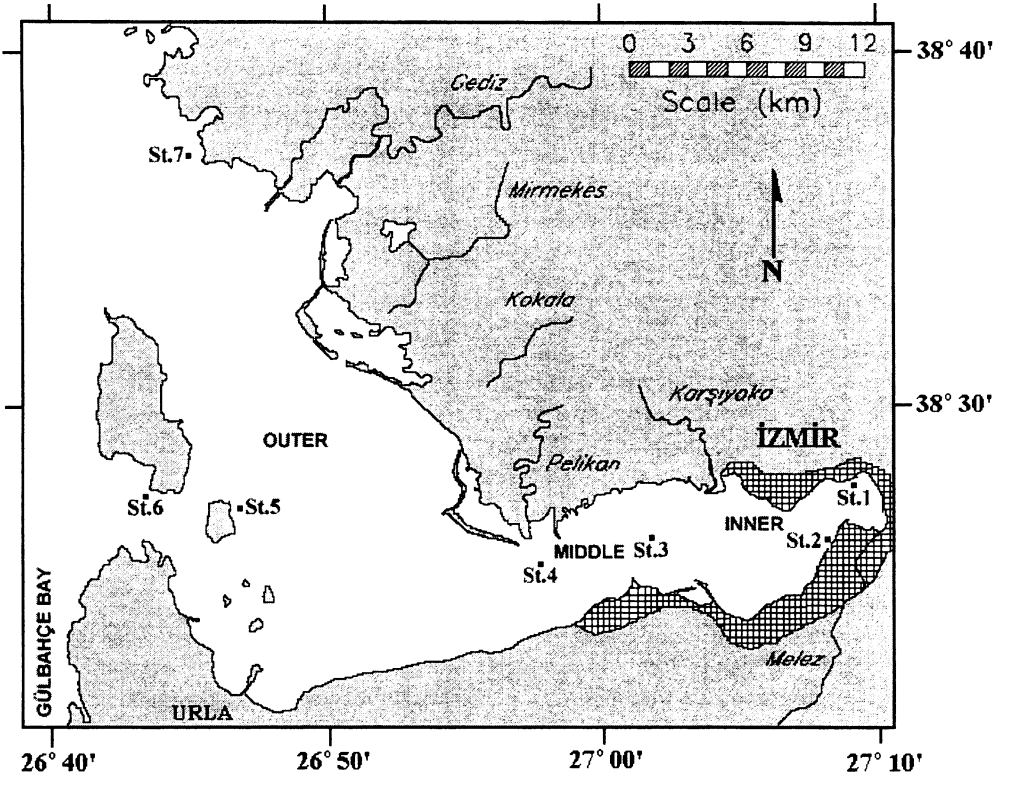



Figure 1: Locations of Sampling Stations in Izmir Bay.

sample water was religiously acid washed. Samples were taken at each sampling depth after the bottles were rinsed with sample water. The dissolved oxygen (DO) was determined using the Winkler method. Reactive iron (RFe) was measured spectrophotometrically. RFe samples were analyzed after sampling by using ascorbic acid for reduction and 2,4,6-tripyridyl,1,3,5-triazine (TPTZ) for a violet complex formation. This method given by Koroleff [16] was chosen for two reasons; i) It is known that Fe concentration is extremely high in Izmir Bay, and ii) in this method, ascorbic acid reduced the labile portion of the total iron. This probably indicates that a significant portion of the reduced iron exists as a labile iron for bioavailability in the natural environment.

The analysis of macronutrient samples were based on the methods of Murphy and Riley [17] for phosphate, Brewer and Riley [18 for nitrate and nitrite, Reusch Berg and Abdullah [19] for ammonia, and Brewer and Riley [20] for silicate.

Principal component analysis (PCA) is preferred when large matrix of correlation coefficients is available [21]. The correlation matrix is used as an input to calculate the eigen values and vectors. The eigen values are the variances of the principal components. PCA was applied to the data by using NTSYS (Version1.70) [22]. The object of the analysis is to take p variables $X_1$,

$X_2$,.....,$X_p$ and to find combinations of these producing indices $Z_1$, $Z_2$,.....,$Z_p$, which are uncorrelated. The indices are also ordered so that $Z_1$ displays the largest amount of variation, and so on. That is var($Z_1$)>var($Z_2$)>.....>($Z_p$), where var($Z_i$) denotes the variance of $Z_i$ in the data set under consideration. The $Z_i$'s are called the principal components. When doing a PCA there is always the hope that the variances of most of the indices will be so low as to be negligible. In that case the variation in the data set can be adequately described by the few Z variables [23].

## RESULTS AND DISCUSSION

In this work the reactive iron concentrations were used as a qualitative index for labile iron. Reactive iron (RFe) concentration is not quantitatively equivalent to bioavailable iron concentration, but it can be used as a qualitative indicator to understand the role of iron in algal growth. Since availability of iron differs among algae, and varies by changing the chemical conditions of seawater, it may not be possible to obtain an absolute measurement of bioavailable iron concentration [10].

Due to anoxic conditons in deep water and sediment, dredging activities in Inner Bay, and high anthropogenic inputs, RFe concentrations were quite high throughout the Bay. High concentration of dissolved organic compounds may also have an important effect on RFe distribution. For instance, humic and fulvic acids enhance dissolution of Fe, and slow down the oxidation/precipitation rate by complexing with Fe [24]. High concentrations of total Fe do not necessarily correspond with the existence of high concentrations of bioavailable iron. On the contrary, in coastal waters, there is a strong negative relationship between the total iron concentrations and the proportion of labile iron (by inference, bioavailable iron) [10]. That is, a lower proportion of total iron appears to be bioavailable when total iron concentration is high.

Table 1. The annual range and means of selective oceanographic parameters at seven stations in Izmir Bay, between May 1993 and July 1994. Mean values are indicated in bold (*BDL: Below analytical detection limit)

| Parameters | Stations | | | | | | |
|---|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | S4 | S5 | S6 | S7 |
| Temperature (°C) | 13.3-28.3 | 13.0-27.6 | 11.5-25.7 | 12.5-25.5 | 14.2-25.8 | 14.4-25.7 | 14.2-24.0 |
| | **19.88** | **19.19** | **18.23** | **19.99** | **19.46** | **19.04** | **18.29** |
| Salinity (‰) | 34.7-38.0 | 33.0-38.1 | 34.2-38.5 | 37.3-39.6 | 38.0-39.8 | 38.0-39.3 | 36.0-38.8 |
| | **36.34** | **36.02** | **36.94** | **38.19** | **38.62** | **38.56** | **37.99** |
| pH | 7.66-8.95 | 7.70-8.35 | 7.73-8.45 | 7.74-8.39 | 8.06-8.39 | 7.98-8.43 | 8.15-8.45 |
| | **8.05** | **8.11** | **8.20** | **8.11** | **8.25** | **8.23** | **8.30** |
| Secchi disc depth (cm) | 30-100 | 100-190 | 110-300 | 100-300 | 650-1150 | 650-1600 | 650-800 |
| | **57** | **133** | **179** | **193** | **833** | **983** | **725** |

At Station 1, which is heavily polluted, the Diatom population primarily consisted of *Skeletonema costatum* (about 54% and 93% of the total phytoplankton population in July'93 and April'94, respectively). However, the Dinoflagellates *Scripsiella trochoidea* was a dominant species in July'93 at Station 2 (about 73% of the total phytoplankton population) while *Prorocentrum micans* was the dominant species in April'94 (about 42% of the total phytoplankton population ).

In the Inner Bay, the reactive iron concentrations were often low when the blooms of Diatoms and Dinoflagellates occured in July 1993 and in April 1994 (Fig. 2). This relationship suggests a higher relative availability of iron by Diatoms and Dinoflagelates. While the Diatoms and Dinoflagellate populations increased during the spring bloom, the RFe concentration decreased (Fig. 2). With the population crash and decomposition of organisms, a portion of the Fe might be relased to the water as an organic complex form. However, when Euglenophycea populations were increasing during the bloom of Euglenophycea in June'94, the increase in RFe concentration continued until the period of rapid population crash. The increase in RFe with the increase in phytoplankton population suggests Euglenophycea populations may relase organic ligands, which keep more iron in reactive form. The studies on organic ligands which are possibly released by phytoplankton during blooms and slow down the oxidation rate by complexing Fe were reported by Kuma *et al.*, [25,26]. The response of Fe to the events of the bloom and crash depends upon the phytoplankton species and chemical nature of the iron. Thus, in one case (Diatoms and Dinoflagelates) Fe was removed efficiently from water during the bloom; in the other case (Euglenophycea ) Fe was retained in a labile form, with probably the relase of Fe-complexing ligands from Euglenophycea.

Another possibility is that Euglenophycea may have a mixotropic character, Due probably to this mixotropic character of Euglenophycea or due to other grazing activities, iron may be kept in reactive forms in the water column. The increase in chl-a concentration in July'94, despite the decrease in phytoplankton populations at the same time, supports grazing activities. During grazing, through ingestion, release of RFe forms are likely to occur. Nevertheless, it must be noticed that the most important source of iron along with terrestrial sources is resuspention of anoxic sediments for Izmir Bay. The extreme increase in the concentration of some variables; particularly phosphorus, ammonium and iron have been attributed to the dredging activities in the vicinity of station 1 in the inner bay in November 1993 [27].

Despite the fact that the quantity of phytoplankton bloom and RFe concentrations in the Outer Bay were lower than that of Inner Bay, there was a harmony between the distribution of RFe and phytoplankton population found in the Inner and Outer Bay (Fig.2, 4). However, the conditions in the Middle Bay were rather complex; the bloom of Diatoms , Dinoflagellates and Euglenophycea occured simultaneously in June 1994 (Fig 3) with corresponding increase in RFe concentrations at the stations. It is not clear the reason of this coexistence.

During the spring bloom, the decrease in RFe in the surface layer at all stations suggests that the phytoplankton bloom has important control over iron

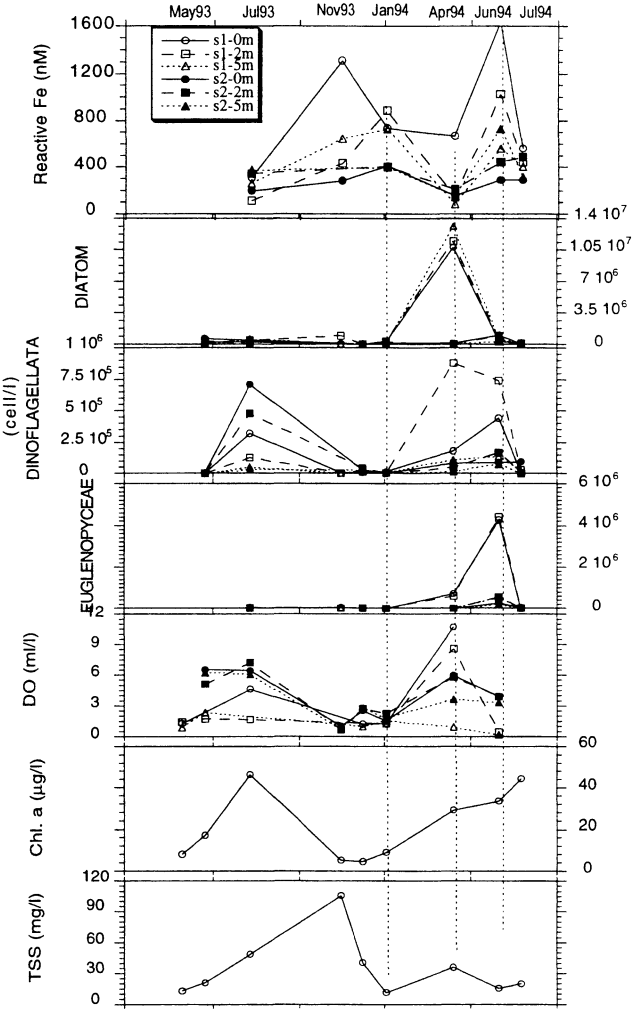



Figure 2: The montly surface (0-5m) distributions of RFe, Diatom, Dinoflagelates, Euglenophycea, DO, average Chl-a and average TSS in the Inner Bay.

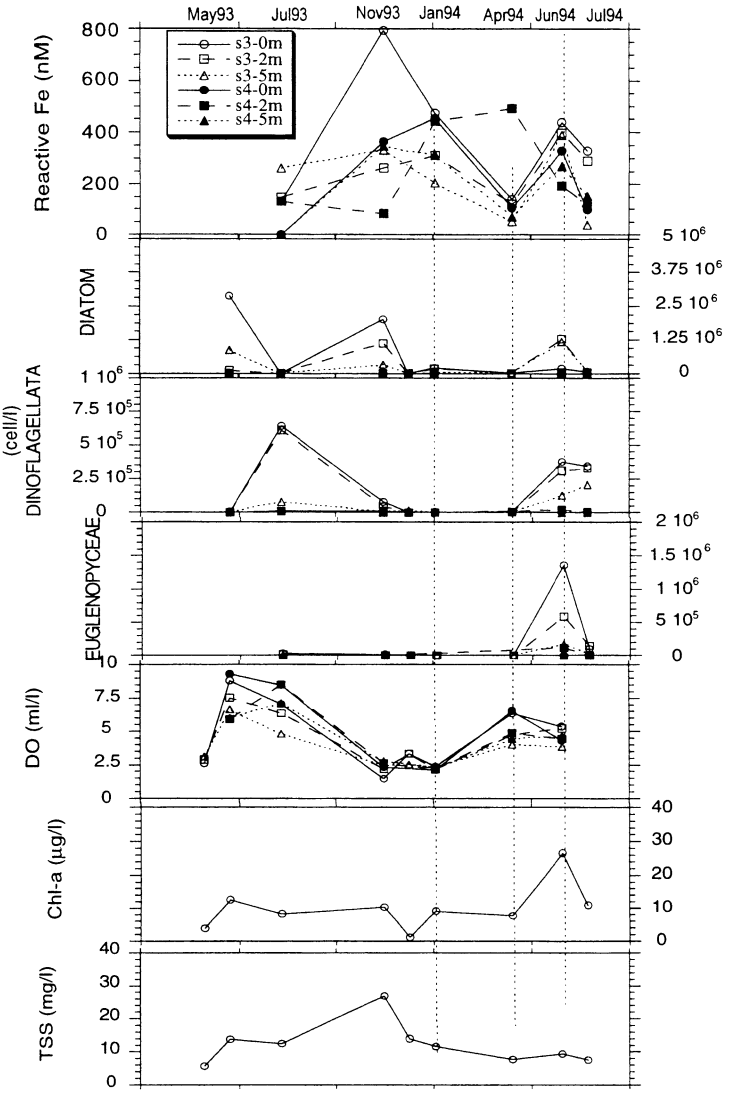



Figure 3: The montly surface (0-5m) distributions of RFe, Diatom, Dinoflagelates, Euglenophycea, DO, average Chl-a and average TSS in the Middle Bay.

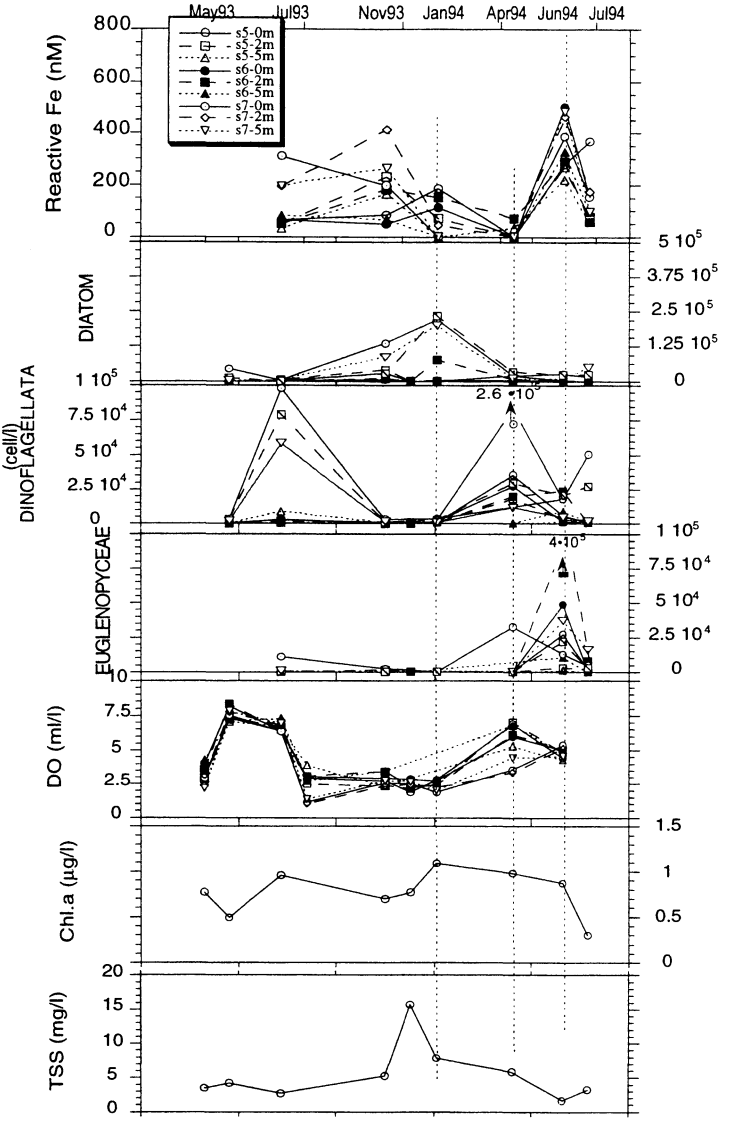



Figure 4: The montly surface (0-5m) distributions of RFe, Diatom, Dinoflagelates, Euglenophycea, DO, average Chl-a and average TSS in the Outer Bay.

distribution. RFe increased at the surface and deep water in November 1993 at all stations, this was clearly due to the dredging activity in the Bay. A corresponding TSS increase and DO decrease in November 1993 (Fig. 2, 3,4 ) also indicates this input. DO concentrations in the surface layer were high during bloom period (in July'93 and April'94) and decresed in the autumn and winter periods in three sections of the Bay.

These data shows that the fate of Fe in the seawater depends not only upon the chemical and physical conditions, but also phytoplankton blooms. In addition different species appeared also as an important factor to control the distribution of iron in the euphotic layer in Izmir Bay. The nature of the organics and type of phytoplankton and their physiological state are as important as their quantity. The control of Fe in seawater, either by chemical and physical factors or biotic factors including iron uptake by phytoplankton, zooplankton grazing and bacterial activities could not be distinguised from using these data. Despite this data implies that the decrease in RFe concentrations probably occured during the period of Diatom and Dinoflagelates growth i.e. April'94, abiotic removal of RFe from the water column might also be important during that time when DO concentrations were high in surface layer. In general, in the euphotic zone of Izmir Bay, we found an apparent harmony between phytoplankton blooms and iron distributions.

In future investigations of the specific interactions of phytoplankton species with iron, the following three points should be focused on:

1- Different roles of iron in each algae species and the effects of the different algal species on iron chemistry. This includes iron speciation, and study of the physiological state of algae and the kinetics of mechanisms,

2- the effects of other biological factors such as grazing and bacterial activity on iron-algae interactions, and

3- the effects of physical and chemical conditions of water column such as light, mixing, salinity, temperature and other environmental factors on iron-phytoplankton interactions.

## Principal Component Analysis

PCA show that about 35% of the variation in the data were related to RFe, phosphorus forms (dissolved, particulate and total), and ammonium, as an enrichment parameter, 14% is related to chl-a, phytoplankton cell numbers, DO and pH and 11% nitrite, nitrate and temperature.

The plots of Z1 and Z2 values are shown in Figure 5 by tagging each plot so that they represent their location, namely sections of the bay; Inner, Middle and Outer. The distributions of plots show the expected situation clearly. The Middle Bay shows similar characteristics sometimes to the Inner and sometimes to the Outer Bay. So the Middle Bay can be defined as the "transition zone" between heavily polluted and relatively unpolluted section. The same figure also shows that the condition in the Middle Bay may sometimes be as critical as the Inner Bay. The other remarkable point is the relative accumulation of the Outer Bay's plot in the upper right part of the figure which has less

variation, more stability, whilst totally opposite conditions were observed in the Inner Bay.

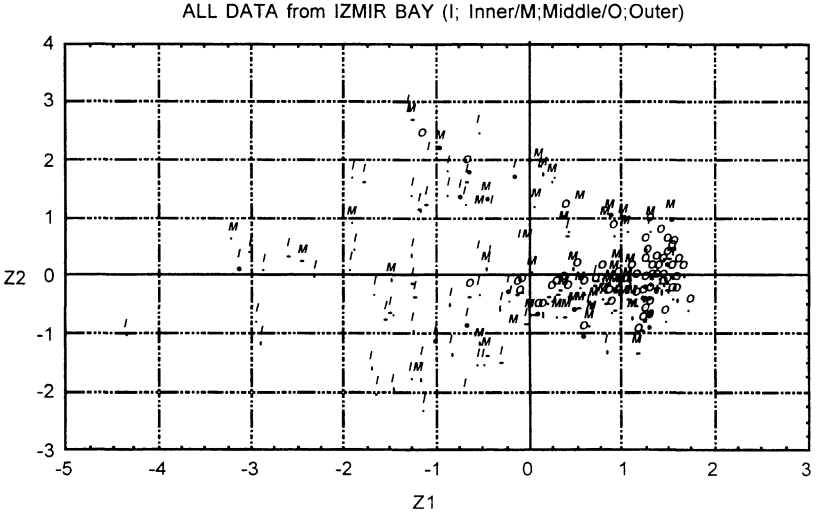



Figure 5: Plot of samples taken throughout the Bay during the survey against values for the first two principal components, $Z_1$ and $Z_2$ for ecological variables.

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