

VITAMIN B₁₂ CONCENTRATIONS IN MANDOVI AND ZUARI ESTUARIES

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ABSTRACT

Bioassay method using *Cyclotella nana* (clone 13) was employed for the estimation of Vitamin B₁₂ in Mandovi and Zuari estuaries. At the mouth of the Mandovi estuary, B₁₂ concentration varied between 0.38 and 7.0 ng/l in surface water and between 0.12 and 0.49 ng/l in bottom samples. Near the head of the estuary, the variation in B₁₂ concentration was from nil to 2.47 ng/l. Relatively lower concentration was observed (0.20 to 1.06 ng/l) at surface at the mouth of Zuari estuary. A wide variation was observed (0.44 to 6.48 ng/l) at the head of this estuary. Various probable factors that influence the concentration of vitamin B₁₂ are discussed.

Key-words : Vitamin B₁₂, estuaries, bioassay.

INTRODUCTION

Organic carbon is associated with and derived from all living systems. In its various forms it may function as a nutrient or energy source, growth stimulator, growth inhibitor, chemical cue and so on. Thus, organic carbon may be of general interest simply as a source of nutrient or energy or it may be of special interest as a result of its specific biological activity. Its role depends upon its chemical and biological state. Organic substances, in most marine environments, occur in low concentrations but are among the most important factors which control the metabolism, abundance and distribution of micro-organisms. In this connection, much attention has been focused on vitamin B₁₂ (cyanocobalamin).

The possible role of vitamin B₁₂ as a factor controlling the rate of primary production in the oceans has received increasing attention in the past few years, since it was observed that about 70% of the phytoplankton species in culture required an exogenous supply of this vitamin (Droop, Mc Laughlin, Pintner and Provasoli, 1959). For growth of marine microorganisms, the pseudovitamin B₁₂ and related factors may prove to be as important as cyanocobalamin. Droop (1955) showed that the cyanocobalamin required by the pelagic diatom *Skeletonema costatum* was spared by pseudovitamin B₁₂, Factor A and Factor B. These findings on the lack of specificity of the form of vitamin B₁₂ preferred by diatoms showed the probable importance of 'cobalamins' (member of vitamin B₁₂ family of compounds in addition to cyanocobalamin) in the biological economy of the sea.

A number of microbiological assay methods for vitamin B₁₂ have been reported and the review of earlier methods has been given by Belser (1963) and Riley (1965). Most of the early assay methods needed pretreatment of

the samples because of lack of the suitable salt tolerant organisms. Precise results on the concentration of vitamin B₁₂ in seawater were obtained due to direct measurement using salt tolerant marine organisms. The sensitivity of the bioassay using *Cyclotella nana* Hustedt (Clone 13-1) was found to be satisfactory (Carlucci and Silbernagel, 1966).

There is no earlier report on vitamin B₁₂ in Indian waters. This paper presents the preliminary observation on the distribution of dissolved vitamin B₁₂ in Mandovi and Zuari estuaries.

MATERIALS AND METHODS

Water samples were collected using Van Dorn samplers that were sterilized with methanol before use. Samplings were done monthly from February to July, 1980 at 4 stations each in Mandovi and Zuari estuaries (Fig. 1).

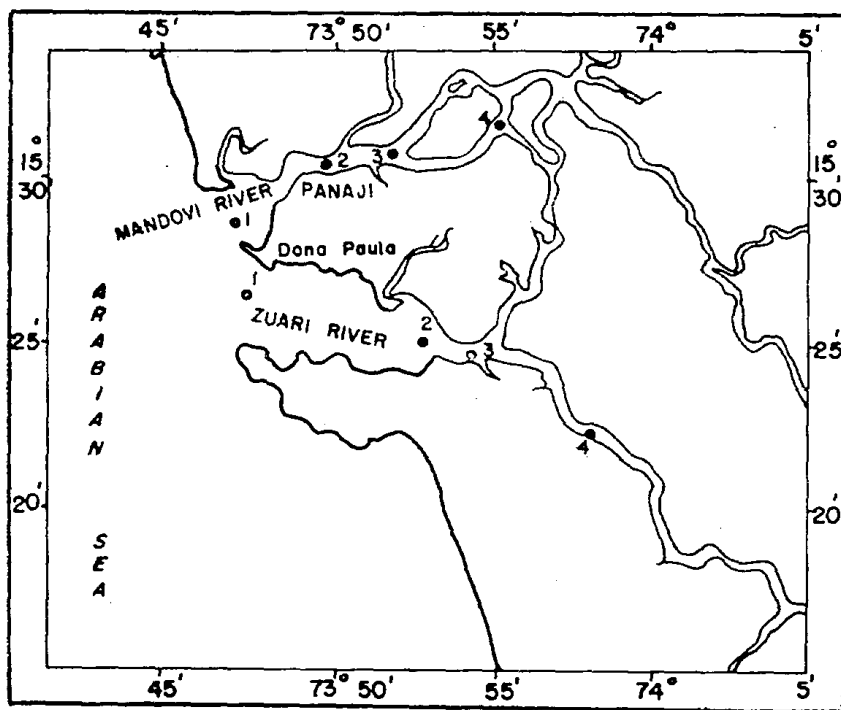


Fig. 1. Map of Mandovi-Zuari estuarine system.

Approximately 50 ml of unfiltered seawater was frozen in sterile polypropylene bottles immediately after its removal from the sampler. These samples were filtered through sterile Millipore filters (0.2 μ m pore size) just after thawing in shore laboratory.

The B₁₂ bioassay method described by Carlucci and Silbernagel (1966) was employed in this investigation. Bacteria free culture of the marine centric diatom *Cyclotella nana* (clone 13-1), used for the bioassay was obtained from the University of California, San Diego. The culture was maintained

in medium f/2, which is the standard enrichment at half strength (Guillard and Ryther, 1962). The alga was cultured in 15 ml of medium in 50 ml conical flasks under a light intensity of 4000-5000 lux and with a day/night cycle of 14/10 hours at a temperature of 20 °C.

Filter sterilized sea water samples were supplemented with sterile constituents of the medium and were inoculated with B₁₂ starved cells of *Cyclotella nana*. These were incubated for 48 hrs under constant light intensity and temperature. C¹⁴ labelled sodium carbonate was then added and the uptake of radiocarbon for 2 hrs incubation period was measured by liquid scintillation system. Suitable precautions were taken by means of internal standardization to allow for inhibitors in sea water samples and the rate of C¹⁴ uptake is proportional to B₁₂ concentration. Chlorophyll *a* was measured spectrophotometrically using the method given by Strickland and Parsons (1968).

The estimation of B₁₂ were carried out in the water samples collected from all stations given in the Fig. 1. But the results of stations 2 and 4 in Mandovi estuary and stations 1 and 4 in Zuari estuary only are presented here.

RESULTS AND DISCUSSION

The surface water at station 2 in Mandovi estuary showed variation of vitamin B₁₂ from 0.38 to 7.00 ng/l (Fig. 2) and bottom water showed lower values (0.12 to 0.49 ng/l). Higher values in surface were observed in the months of March and April, when the water had higher chlorophyll *a* (5.8 – 6.3 mg/m³). However, in May the lowest Vitamin B₁₂ value was observed when Chl *a* value was the highest. The vitamin B₁₂ concentration in surface waters at station 4 varied from non-detectable amount to 2.47 ng/l and higher values (0.20 – 2.72 ng/l) were observed in the bottom water.

Relatively lower values of vitamin B₁₂ were observed in Zuari estuary, where data for surface waters only are available. The samples from station 1 showed a variation between 0.20 and 1.06 ng/l and at station 4, it varied from 0.44 to 6.48 ng/l (Fig. 3). Higher values were observed from February to April.

The concentrations of vitamin B₁₂ in different oceanic waters are mostly lower than those in Mandovi and Zuari estuaries which otherwise agree well with the data reported by earlier workers for coastal waters. Cowey (1956) reported a value of 0.01 ng/l in open Atlantic waters and B₁₂ values ranged from 0 to 2.6 ng/l in Pacific (Kashiwada, Kakimoto and Kanazawa, 1959). Menzel and Spaeth (1962) observed that values in Sargasso Sea above 100 m are generally low ranging from 0.01 to 0.1 ng/l. The coastal waters of California showed an average value of vitamin B₁₂, 2.9 ng/l and it was 0.1 ng/l in the Central Pacific Ocean (Carlucci, 1970). Ohwada and Taga (1972) re-

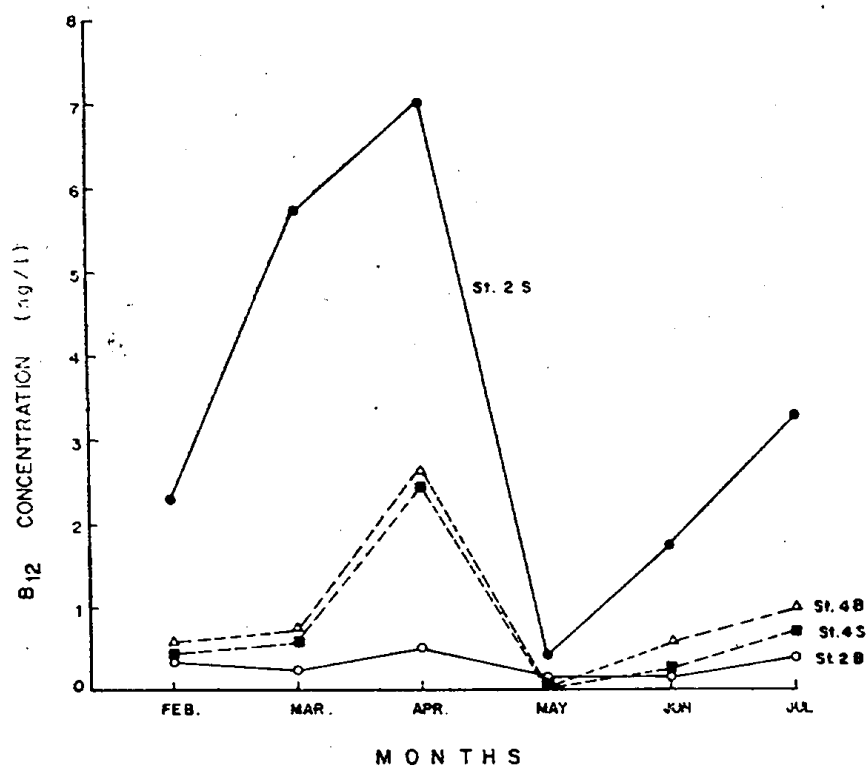


Fig. 2. Distribution of vitamin B₁₂ in Mandovi estuary.

ported the higher concentrations of the dissolved vitamin B₁₂ (upto 14.3 ng/l) in the surface coastal waters in Sagami Bay where maximum chlorophyll *a* was also observed.

An attempt was made to see any significant correlation between chlorophyll *a* and vitamin B₁₂ existed, using the data collected from both estuaries (Fig. 4). There was no definite correlation between these two parameters. But the monthly data on the distribution of chlorophyll *a* and B₁₂ (Fig. 2), showed a general positive trend. Vitamin B₁₂ was higher in Mandovi estuary during March–May when the chlorophyll *a* values were high (5.8–7.2 mg/m³), whereas it was relatively low in other months. This positive trend especially during March–April in Mandovi estuary may be due to the secretion of vitamins by phytoplankton as indicated by Carlucci (1970). In general, B₁₂ concentration in the surface water of Mandovi Estuary was higher than that of the bottom during high phytoplankton production except in May when an opposite trend was noticed. This may be due to the variations in the occurrence of dominant phytoplankton species and the variations in the requirement of B₁₂ from species to species and also from season to season (Swift, 1981). Present observations showed that (i) variations in the absolute requirement of phytoplankton for B₁₂ were reflected in the concentration of vitamin B₁₂ at a particular station (Fig. 2) and (ii) there was no statistical evidence to show that the

phytoplankton production is limited by the concentration of vitamin B₁₂ (Fig. 4).

Menzel and Spaeth (1962) noticed that a seasonal cycle in vitamin B₁₂ in the euphotic zone corresponded to the cycle of primary production. They postulated that while B₁₂ may not control production rates *per se*, it probably influences the species composition of the phytoplankton population. Moreover, it was shown by Droop (1970) through the studies on vitamin B₁₂ uptake by *Monochrysis lutheri* that the specific growth rate depends on the cell vitamin quota and the vitamin B₁₂ binders, rather than directly on concentration of vitamin in the medium.

The dominant species of diatoms at station 1 in Zuari estuary during the period of study are shown in Table I. From February to April, when lower concentration of vitamin B₁₂ was observed at this station (Fig. 3), the dominant diatom species were *Nitzschia seriata*, *Bacillaria paradoxa* and *Skeletonema costatum*. It is interesting to note that these diatom species are found to require vitamin B₁₂ (Provasoli and Carlucci, 1974). But in May when higher concentration of vitamin B₁₂ was observed, the dominant species was *Pleurosigma* sp. for which the requirement of B₁₂ is not known. Similar observation was made earlier by Menzel and Spaeth (1962) who found the occurrence of higher concentration of vitamin B₁₂ coincided with the bloom of *Rhizosolenia stolterfothii* and *Bacteriastrium* sp.

There is some uncertainty whether the amount of vitamin B₁₂ measured in the sea are low enough to be of general ecological interest. Droop (1962)

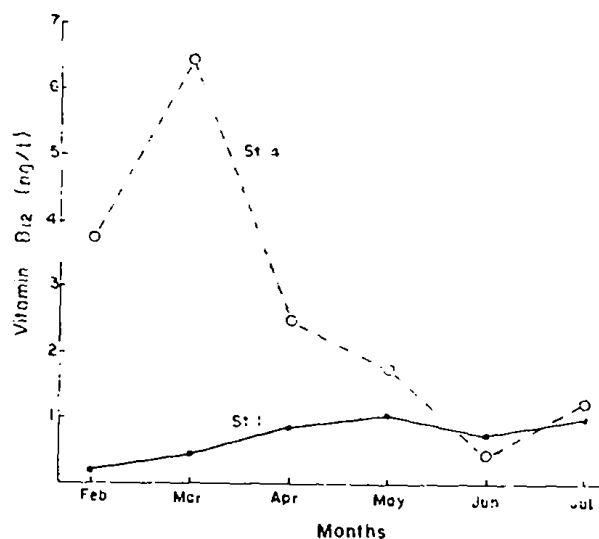


Fig. 3. Relation between vitamin B₁₂ and Zuari estuary.

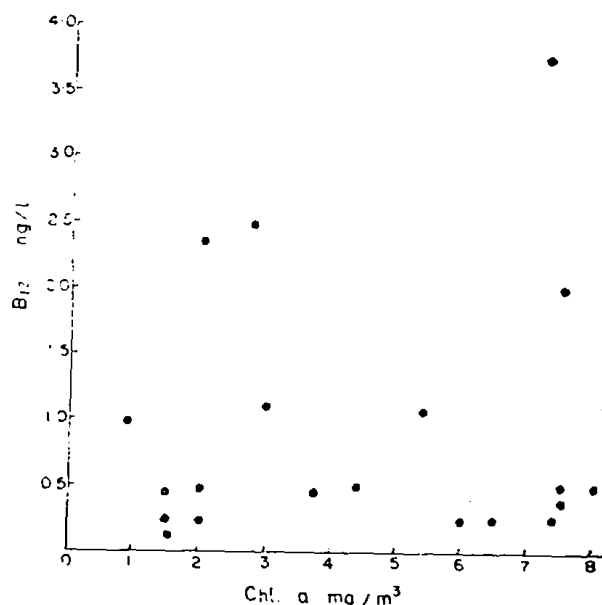


Fig. 4. Relation between vitamin B₁₂ and chlorophyll a.

Table 1. Dominant species of diatoms in Zuari estuary from February to July, 1980

Month	Species	Month	Species
Feb.	<i>Nitzschia seriata</i> <i>Hemiaulus sinensis</i> <i>Coscinodiscus</i> sp.	May	<i>Pleurosigma</i> sp. <i>Pleurosigma angulatum</i> <i>Pleurosigma elongatum</i>
Mar.	<i>Bacillaria paradoxa</i> <i>Coscinodiscus</i> sp. <i>Nitzschia seriata</i>	June	<i>Bacillaria paradoxa</i> <i>Pinnularia alpina</i> <i>Nitzschia</i> sp.
Apr.	<i>Coscinodiscus</i> sp. <i>Nitzschia seriata</i> <i>Skeletonema costatum</i>	July	<i>Skeletonema costatum</i> <i>Coscinodiscus excentricus</i> <i>Bellerophon</i> sp.

suggested that low concentration such as 0.1 ng/l might control the species composition of the plankton if the relative growth rate assumed overriding importance. Furthermore, it is uncertain how much of the vitamin B₁₂ measured by bioassay is available to the algae in question. The organism used in this study *Cyclotella nana* (clone 13-1) is not only assay sensitive to cyanocobalamin, but the vitamin B₁₂ analogues are also assayed. Since specificities of organisms to different B₁₂ analogues varies with each of the organisms, they have an ecological importance (Provasoli, 1963). Swift and Guillard (1978) and Swift (1981) have shown that vitamin B₁₂ is of ecological importance, though the requirement for it is not absolute. Further studies on vitamin B₁₂ and other ecological parameters along with the specificity of tropical phytoplankton to vitamin B₁₂ would throw more light on the effect of vitamin B₁₂ on the ecophysiology of plant production in a tropical estuary.

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