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Relative concentrations of Na, Ca and Mg for growth of some diatoms*

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Abstract. The two types of waters, i.e., freshwater and seawater differ in their total solids (salinity), ratio of monovalent to divalent cations, the amount of the predominant cations or anions, and the Ca/Mg ratio. The growth behaviour of four diatoms isolated from these habitats was studied in different concentrations of Na, Ca and Mg which were varied taking into account the salient features of both water types. Results indicate that the growth response of these diatoms show little relationship to the composition of either fresh or seawater. It is evident that the ecological category of a species cannot be determined merely on the basis of presence or absence of its growth in certain concentrations and ratios of major ions.

Keywords. *Amphora coffeaeformis* ; *Cyclotella meneghiniana* ; *Navicula pelliculosa* ; *Nitzschia frustulum*.

1. Introduction

The principal differences between the two main types of natural waters, i.e., freshwater and seawater (brackish water represents the intermediate type) consist in the total solids (salinity), ratio of monovalent to divalent cations, the amounts of the predominant cations or anions, and the Ca/Mg ratio. Differences in tolerance or requirement for these major inorganic ions are frequently held responsible for differences in algal flora of water bodies (Rodhe 1948; Vollenweider 1950; Provasoli *et al* 1954). Surprisingly very little work has been done with laboratory cultures to ascertain whether or not there are differences in the behaviour of algae that can clearly distinguish them as freshwater, brackish water and marine species or as steno or euryhaline species (*cf.* Provasoli *et al* 1954; Droop 1958). Experiments were planned in which diatoms isolated from different habitats were subjected to major ions such as Na^+, K^+, Mg^{++}, Cl^-, HCO_3^- and $SO_4^=$ in different ratios. In a preliminary experiment the effect of varying the concentrations of Na, Ca and Mg on growth of four diatoms was studied and the results are reported.

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* Memoir No. 347 from the centre.

2. Materials and methods

Amphora coffeaeformis (Agardh) Kütz. (A 1058)†, *Cyclotella meneghiniana* Kütz. (A 1050), *Navicula pelliculosa* (Bréb.) Hilse (A 404), and *Nitzschia frustulum* (Kütz.) Grunow (A 1098), were used. The diatoms, except *A. coffeaeformis*, were grown in modified Reimann medium (Reimann *et al* 1963; p. 76). *A. coffeaeformis* was grown in 8000 mg/l Na (as NaCl) amended Reimann medium. Experience has shown that $SO_4^{=}$ can be replaced by Cl^- provided it is not reduced to a level below the nutritional needs of the diatom. Therefore cations of Na, Ca and Mg were added as chlorides in different concentrations to the basal Reimann medium in an experimental series that gave a 5 × 5 Ca and Mg framework for each Na concentration. Reimann medium minus Na served as an additional Ca and Mg framework. Na ranged 300-24000 mg/l, Mg 0·4–4000 mg/l and Ca 0·2–2000 mg/l in 5 approximately equal logarithmic steps in this framework. One ml of uniform exponentially grown cell suspension of each diatom species, standardized on the basis of optical density of pigment extract (0·01), were inoculated into 9 ml of the respective media and incubated at 24° ± 1° C and light intensity of 2000 lux in a 12/12 light dark cycle. Growth was measured by yield at the end of exponential period (12 days) and was expressed as circles whose diameter is proportional to optical density measurements. Growth was compared with that obtained in Reimann medium (control) and NaCl amended Reimann media (without Ca and Mg additions).

3. Results

The experimental design varying the salinity, monovalent/divalent cation ratio and Mg/Ca ratio, took into account salient features that typify fresh and seawater. Results are presented in figures 1–4. The following general reading of the figures is possible. Growth along bottom left to top right diagonal will be indicative of a critical Ca/Mg ratio. Growth along the other diagonal i.e. from top left to bottom right will mean that Ca and Mg are interchangeable. If there is a critical Ca/Na or Mg/Na ratios, Ca and Mg optima will rise with increase in Na concentrations. An analysis of figures yielded data on optimum and range of ionic concentrations and ratios of these cations for growth of each species which are presented below and in table 1.

3.1. *Amphora coeffeaeformis*

This diatom shows tolerance to the widest range of NaCl concentrations. There is also a clear-cut correlation between Na and Mg. Growth occurs at both extremes of salinity i.e. unfavourable NaCl concentrations, only when Mg concentration is maintained at the 40-400 mg/l level. The diatom grows at high Mg concentration irrespective of Mg/Ca ratio. Both high Ca/Mg ratio or Mg/Ca ratio are unfavourable for growth at optimum Na concentration. Judging from NaCl tolerance studies alone, it would appear that the best growth occurred at mid

† Accession number.

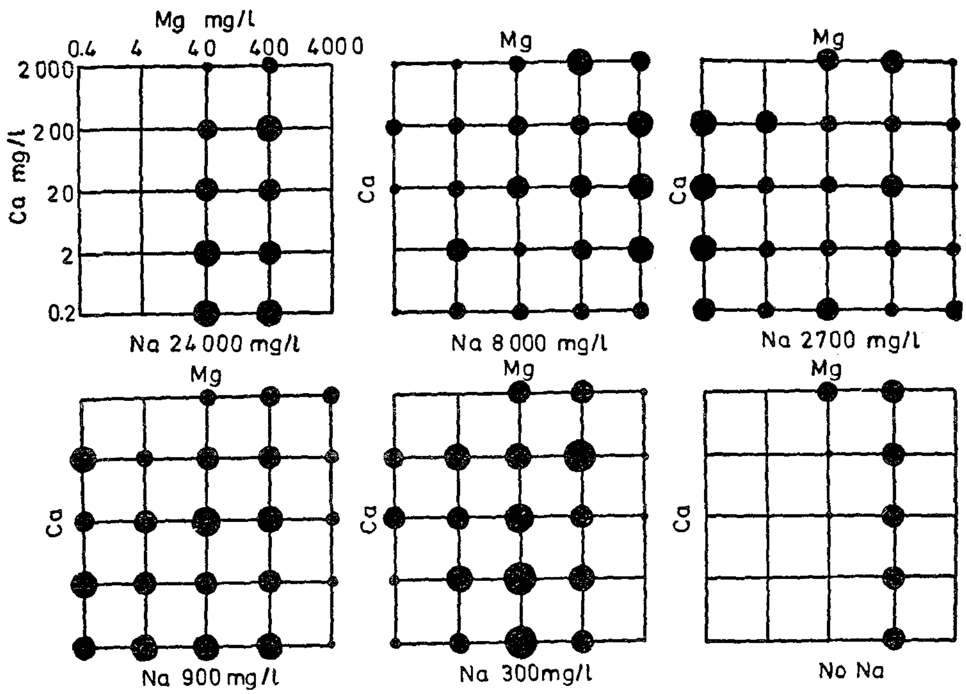

Figure 1. *Amphora coffeaeformis.*

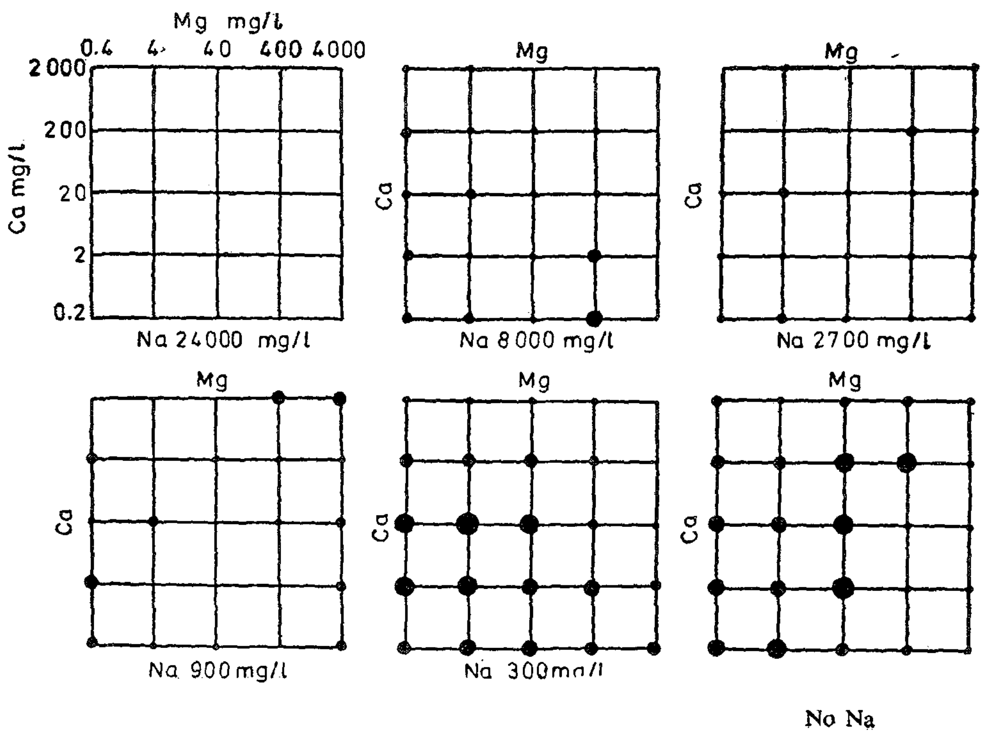

Figure 2. *Cyclotella meneghiniana.*

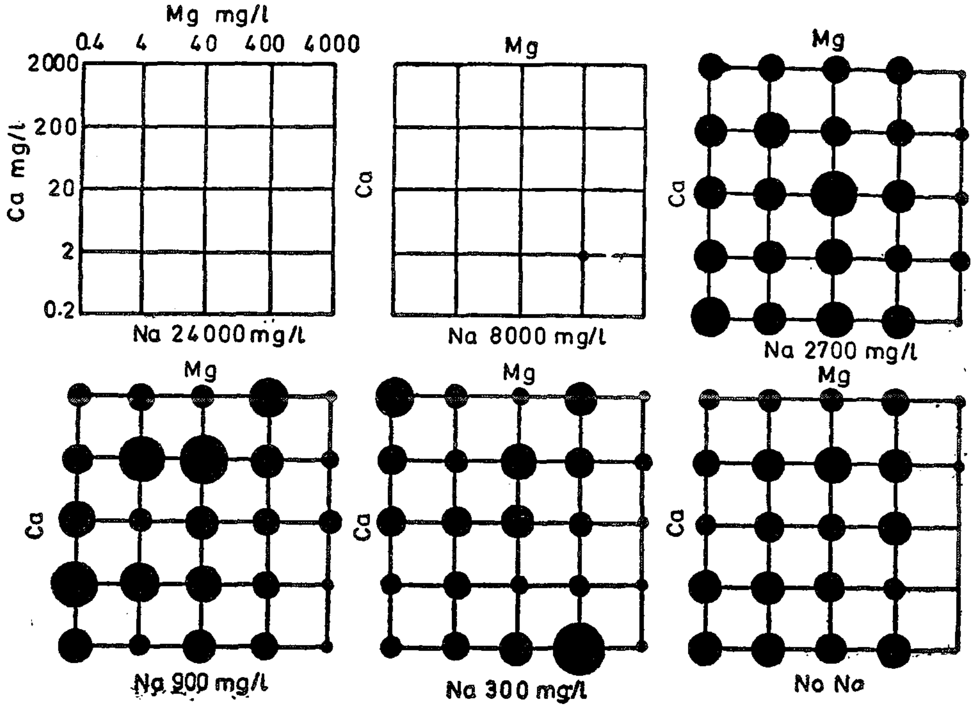

Figure 3. *Navicula pelliculosa.*

Table 1. Optimum and the range of concentrations (mg/l) and ratios of cations for growth of diatoms.

Diatoms	Na	Ca	Mg	Na/Ca	Ca/Mg
Amphora	600	10	100	6	0·1
coffeaeformis	20–24,000	0·1–2,000	0·4–4,000	15–0·1	500–0·005
Cyclotella	100	20	10	3	2·00
meneghiniana	20–300	0·2–100	0·4–200	5–0·4	50–0·005
Navicula	1,200	200	200	3	1·00
pelliculosa	20–5,000	0·1–2,000	0·4–400	50–0·025	5000–0·0005
Nitzschia	300	10	40	6	0·25
frustulum	20–6,000	0·1–400	0·4–400	25–0·5	50–0·0005

NaCl concentrations. Desikachary and Rao (1972) classified this strain of *A. coffeaeformis* as mesoeuryvalent. At favourable concentration of Mg, however, irrespective of the concentration of Ca, the diatom grows well in very low (freshwater) as well as very high (seawater) total solids.

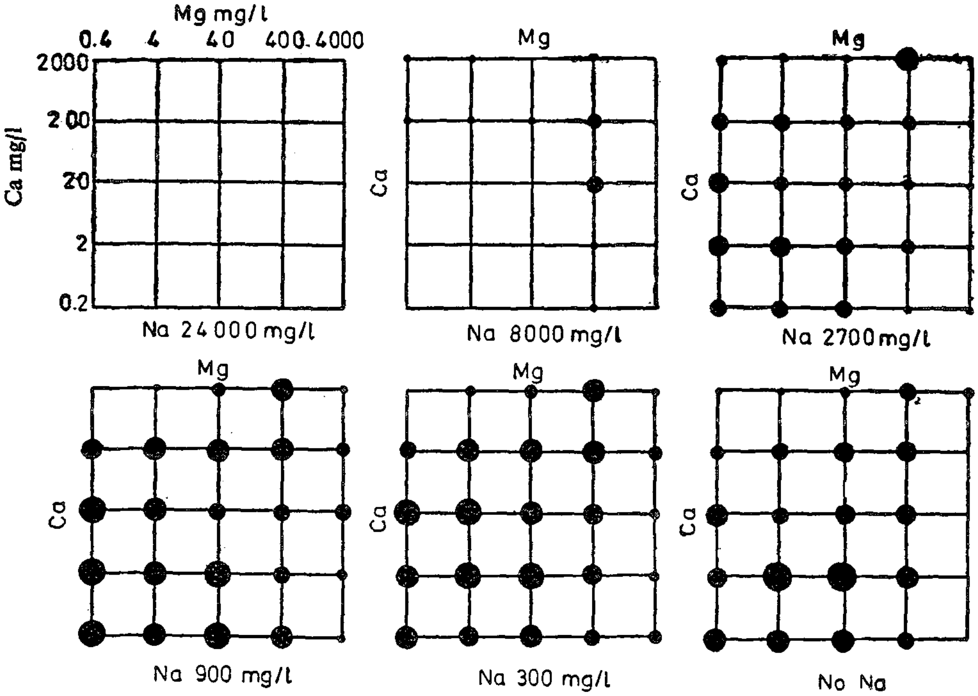

Figure 4. *Nitzschia frustulum.*

Figures 1–4. Response of diatoms to varying concentrations of Na, Ca and Mg. All frames are identical with regard to Ca and Mg and the concentrations (mg/l) are indicated in the top left frame. Na concentrations (Mg/l) are indicated below each frame. Inoculum O.D. which is 0·01 is scaled to 0·5 mm circle and yield is proportional to this scale. Yield in Reimann medium (control): *A. coffeaeformis* 0·03 ; *C. meneghiniana* 0·03 ; *N. pelliculosa* 0·06; *N. frustulum* 0·05. Yield of *A. coffeaeformis* in Reimann + Na 8000 mg/l = 0·07.

3.2. *Cyclotella meneghiniana*

Growth occurs only in low monovalent/divalent ion ratios. Absolute levels of Ca and Mg rather than the ratio play a role in tolerance of the diatom to NaCl concentrations. Mg/Ca ratio 20 to 1 and Ca/Mg ratio 5 to 0·5 seem to be favourable for growth in media with no added Na. With increase in NaCl growth is restricted only to very low Ca and Mg. Within a particular range there seems to be a critical Ca/Mg ratio as suggested by the diagnoal growth increase. Extremes of Ca/Mg and Mg/Ca ratios are not tolerated by the diatom. Desikachary and Rao (1972) studied the NaCl tolerance and concluded that *C. meneghiniana* grows equally well in all higher concentrations of NaCl-amended Reimann medium. The present study indicates that this is true only in the absence of even small amounts of Ca and Mg in the medium.

3.3. *Navicula pelliculosa*

Na above 8,000 mg/l does not support growth. Growth is about the same in other Na concentrations. Even then, it is clear from the figure that as the monovalent/divalent ratio increases growth also occurs in higher Ca/Mg ratios. In other words, ability to grow in high NaCl concentration is enhanced if there is a corresponding Ca increase in the medium. A similar relationship of Na with Mg does not exist. At favourable Na concentrations the diatom grows in a wide range of monovalent/divalent ratios. Mg concentration higher than 400 mg/l does not support growth. Within the optimum Mg levels the Ca/Mg ratio is widely interchangeable.

3.4. *Nitzschia frustulum*

The diatom generally prefers a low monovalent/divalent ratio. In favourable Na concentration high Ca/Mg ratio supports growth up to 200 mg/l Ca level. At 2000 mg/l Ca level, growth occurs only when Mg is correspondingly high at 400 mg/l. Such an interaction between Mg and Ca has been noticed by Vollenweider (1950) in his studies on *Fragilaria, Asterionella* and *Tabellaria* (see Provasoli 1958). Mg concentration higher than 400 mg/l, irrespective of the Mg/Ca ratio, supports growth only at favourable Na levels. At 900 mg/l Na level there is a definite Mg/Ca ratio within which growth occurred.

4. Discussion

The absolute concentration and the ratio of different chemical constituents, besides other factors, distinguish the two types of natural waters i.e. fresh and seawater. Total solids of freshwater are in the range of 50-500 mg/l, the monovalent/divalent ratio is usually less than 1·5 and Ca and Ca and Mg ratio is about 6 : 1. Ca and HCO_3 are the predominant cation and anion respectively. On the other hand, total solids of seawater are in the range of 30000-38000 mg/l, monovalent/divalent ratio is usually about 6·5 and Ca/Mg ratio is low at about 0·3. Cl is the predominating anion while Na and Mg are the predominating cations. Concentration of Na range from 10720 mg/l in seawater to 2 mg/l in hard and 6 mg/l in soft freshwater, Ca range from 420 mg/l (seawater) to 51 mg/l in hard water to as low as 1 mg/l in soft water and Mg range from 1520 mg/l in seawater to 27 mg/l in hard water and 1 mg/l in soft water. Table 1 summarises the optimum and the range of concentrations and ratios of cations for growth of diatoms. *N. pelliculosa* and *N. frustulum*, the two freshwater diatoms, show optimum cation requirement greater than what is normally found in typical freshwaters. It is to be expected that *C. meneghiniana* and *A. coffeaeformis* show cation optimum, between sea and freshwater as they are not typically marine but are considered estuarine and euryhaline. The euryhaline growth behaviour of *C. meneghiniana*, however, is totally changed when there is an interaction of cations in the medium. Similarly the mesoeuryvalent *A. coffeaeformis* grows very well at extremes of salinity at favourable Mg concentrations (see Desikachary and Rao 1972). *A. coffeaeformis* and *N. frustulum* show optimum Ca/Mg ratio typical of seawater. The other two diatoms show a Ca/Mg optimum which is intermediate between fresh and seawater. The optimum monovalent/divalent cation ratio for all the diatoms is nearer sea than freshwater. When absolute concentrations and ratios of cations are considered, these diatoms grow in a wide range, from less than freshwater to greater than seawater concentrations and at ratios which encompass ratios at which ions occur in both the water types.

Provasoli *et al* (1954) have shown that the ecological category of a species might be indicated by its optimal monovalent/divalent ion or Ca/Mg ratios. Results of this study, which are similar to that of Droop (1958), indicate that the growth response of these diatoms show little relationship to the composition of either fresh or seawater. The understanding of what really distinguishes a marine from a freshwater diatom will have to await more intensive studies on typical stenohaline species from both habitats.

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