Évidence of limitation by molybdenum and nitrogen on the growth of the phytoplankton community of the Lobo Reservoir (São Paulo, Brazil)

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ABSTRACT

The effects of artificial enrichment by molybdate, nitrate and phosphate on a natural phytoplankton community, obtained after a 14-day incubation "in situ" at the Lobo Reservoir (São Paulo, Brazil), were studied. The results showed that molybdenum and nitrogen are essential for algal growth and chlorophyll increase. Our experiment revealed that phosphorus does not play an important role in stimulating the development of the phytoplankton community.

KEY WORDS : Phytoplankton — Growth — Molybdenum — Nitrogen — Limitation — Tropical Reservoir.

RÉSUMÉ

ÉVIDENCE DE LA LIMITATION DU MOLYBDÈNE ET DE L'AZOTE SUR LA CROISSANCE DE LA COMMUNAUTÉ PHYTOPLANCTONIQUE DU RÉSERVOIR DU LOBO (ÉTAT DE SÃO PAULO ; BRÉSIL)

Les effets d'enrichissement par du nitrate, phosphate et molybdate sur la communauté phytoplanctonique du Réservoir du Lobo (État de São Paulo, Brésil) ont été étudiés en janvier 1980. Des échantillons d'eau, recueillis à la surface de l'écosystème, ont été distribués dans une série d'Erlenmeyers de 2 l, dans lesquels nous avions ajouté préalablement les éléments nutritifs, dans des combinaisons et des teneurs différentes (voir tabl. I). Les réponses à la fertilisation, mesurées après 14 jours d'incubation « in situ », ont été: matières en suspension, chlorophylle a, relation carotène/chlorophylle et comptage des cellules algales. Ces résultats ont été obtenus respectivement, par gravimétrie, extraction dans de l'acétone à 90 % et lecture spectrophotométrique à 665 nm, calcul de la relation entre les lectures de densité optique à 430 et 665 nm et énumération dans une chambre de Sedgwick-Rafter. Les moyennes, écart-types et coefficients de variation, calculés pour chaque traitement, sont présentés dans le but de vérifier la tendance centrale et la variabilité des données obtenues. Une analyse de variance multivariée a aussi été effectuée pour tester les différences obtenues entre les réponses à l'addition des éléments nutritifs. Les résultats ont permis de constater: 1) l'existence d'effets interactifs nitrate - molybdate en présence de phosphate, sur la chlorophylle et le nombre de cellules algales; 2) une croissance significative de la chlorophylle et du nombre de cellules algales dans les milieux contenant du phosphate et du nitrate, mais sans différence importante entre les cultures enrichies avec des basses ou hautes teneurs de nitrate; 3) aucune influence de l'addition croissante de molybdate dans les cultures contenant du phosphate et du nitrate en basse teneur; 4) une production significative de chlorophylle et de cellules algales dans

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les milieux enrichis par du phosphate et de hautes teneurs en nitrate et molybdate; 5) aucune influence du phosphate sur le développement du phytoplancton. Cette étude confirme la basse qualité nutritive des eaux de ce réservoir, en particulier en azote, et accentue l'importance du molybdène sur la croissance du phytoplancton quand il est additionné en haute teneur.

MOTS-CLÉS : Croissance du phytoplancton — Molybdène — Azote — Limitation — Réservoir tropical.

INTRODUCTION

The concentration of trace metals in ionic solution, and in particular of molybdenum, is generally very low in surface waters (WETZEL, 1975). In flowing waters these elements may be found in different forms. GIBBS (1973) reported that river water may transport these metals under ionic forms, organic complexes, and absorbed or precipitated on solids and on crystalline structures. GIBBS (1973) also reported that the solubilities of these elements are quite variable, but that in general most of then (> 70 %) are found in crystalline and absorbed solids. The other are found in organic and seston complexes and very few in solution (WETZEL, 1975). This explains the low molybdenum concentrations found in freshwater with variable contents ranging from 0.1 to 0.6 $\mu g.l^{-1}$ (BACHMAN & GOLDMAN, 1964). BRADFORD *et al.* (1968) examining 170 alpine lakes in California, found a quite similar average concentration (0.4 $\mu g.l^{-1}$) in all systems with the exception of 2 where, due to their geotermic nature, high contents were observed (100 $\mu g.l^{-1}$). In a small shallow pond in Belgium, DUMONT (1972) found comparatively high mean values (12-14 $\mu g.l^{-1}$) in the summer, quite similar to those of oceanic waters (BURTON, 1969). HEAD & BURTON (1970) observed that in an estuary the molybdenum concentrations increased towards continent-ocean and stated that dilution by freshwater was the most important factor in molybdenum distribution in the estuary.

GOLDMAN (1960) was the first to emphasis the ecological importance of molybdenum as a stimulant of the primary phytoplanktonic productivity in Castle Lake, California.

The present paper is the first record of limitation by molybdenum of the development of phytoplankton in a tropical aquatic ecosystem. The importance of molybdenum in stimulanting phytoplanktonic growth is approached through an experiment of enrichment. Its possible interactions with nitrate and phosphate are also studied.

The results presented here make part of a project for study the role of inorganic nutrients in stimulating the phytoplankton productivity of the Lobo Reservoir in the course of different seasons (HENRY, 1981).

MATERIAL AND METHODS

1. The Environment

The Lobo Reservoir (State of São Paulo, Brazil), located at 22°15′ S and 47°49′ W is formed by the damning of the Itaquari River (its main tributary) and other streams (Fig. 1). This is an environment without distinct vertical gradients in physical-chemical factors, with an optical structure without considerable variations on the seasonal cycle, a relatively homogeneous thermic structure in the water column (amplitude less than 8-10 °C) and a high oxygen content (80 to 100 % of saturation (TUNDISI *et al.*, 1972a and b). According to MORAES (1978), "reactive" silicate does not limit the growth of diatoms. However, low concentrations of organic phosphate, total phosphorus, nitrate and nitrite are characteristics of the environment (MORAES, 1978). These low concentrations of nutrients led TUNDISI *et al.* (1977) to suppose that nitrogen and heavy metals are predominant elements in limiting the phytoplankton production.

2. Experimental Approach

Samples of surface water containing natural phytoplankton collected at station I (Fig. 1), on 01/11/1980, were placed in a series of 2-liter Erlenmeyers. After adding the specific nutrient solutions for each one of the eight treatments (Table I) and 0.5 $g.l^{-1}$ of TRIS buffer (VIEIRA & TUNDISI, 1979), the flasks were incubated "in situ" (Station II, Fig. 1), for a period of 14 days. Four replicates for each one of the treatments were used.

The phosphate and nitrate levels applied were derived from concentration data on these nutrients by MORAES (1978). We used rates 100 times (for phosphate) and 10 and 100 times (for nitrate) higher than the maximum concentrations recorded during the seasonal cycle of these nutrients at the Lobo Reservoir. The same concentrations and 5 times these employed by GOLDMAN (1960) were used for molybdenum.

The responses to enrichment measured after incubation were suspended matter, determined by gravimetry (TEIXEIRA & KUTNER, 1962), chlorophyll (non-corrected for pheophytin) in extracts

TABLE I

Reagents, concentrations and experimental outline of enrichment carried out in January, 1980 at the Lobo Reservoir (São Paulo, Brazil).

Réactifs, concentrations et schéma d'expérimentation de l'enrichissement employés en janvier 1980 dans le Réservoir du Lobo (État de São Paulo, Brésil)

SYMBOL	CONCENTRATION ($\mu g.l^{-1}$)	REAGENTS	TREATMENTS (T)		
			NUMBER	ADDITION WITH	NUMBER OF REPLICATE
P_0	0		T_1	$P_0+N_0+Mo_0$	4
P_1	500	KH_2PO_4	T_2	$P_1+N_0+Mo_0$	4
N_0	0		T_3	$P_1+N_1+Mo_0$	4
N_1	350	KNO_3	T_4	$P_1+N_2+Mo_0$	4
N_2	3500		T_5	$P_1+N_1+Mo_1$	4
Mo_0	0		T_6	$P_1+N_2+Mp_1$	4
Mo_1	100	$Na_2MoO_4.2H_2O$	T_7	$P_1+N_1+Mo_2$	4
Mo_2	500		T_8	$P_1+N_2+Mo_2$	4

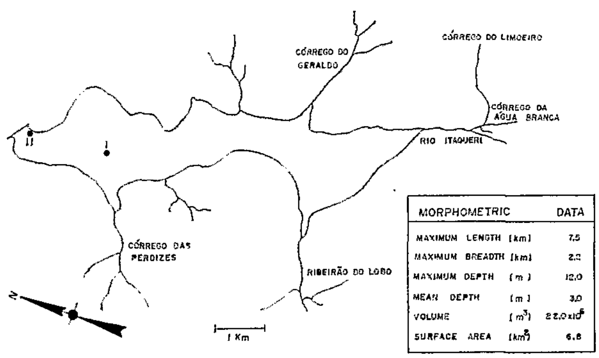

FIG. 1. — Lobo Reservoir (São Paulo) : stations of collection of water samples (I) and incubation (II)

Réservoir du Lobo (État de São Paulo, Brésil) : stations de prélèvement des échantillons d'eau (I) et d'incubation (II)

of acetone and read at 665 nm in a PM2A Zeiss spectrophotometer (GOLTERMAN & CLYMO, 1969), caroten/chlorophyll ratio according to MARGALEF (1964) and cell counts of total phytoplankton in a SEDGWICK-RAFTER cell.

3. Statistical Analysis

Values obtained for each of the 4 responses to artificial enrichment in each of the eight treatments are expressed by the mean, standard deviation and coefficient of variation. A square-root transformation was used for the total cell number (count datum) in order to adjust it to normality (COHRAN & COX, 1957).

An analysis of multivariate variance was used in order to test differences between means of phytoplankton responses to fertilization (MORRISON, 1967). It was thus possible to detect differences among treatments for the set of the 4 variables together (multivariate values of F), and for each one of them alone (univariate values of F). The interrelations among the 4 measured responses are shown through the calculation of correlations among variables taken two-by-two.

Ten analytical hypotheses were formulated in order to show the differences between means obtained from different responses to enrichment. The first (Ho_1) studies the possible effect of nitrate × molybdenum interaction (N × Mo) in the presence of phosphate (P_1). In case of rejection of Ho_1, the differences between means of each response were tested (Ho_2) comparing the low nitrogen (N_1) treatments with those with high nitrogen (N_2) concentration, all of them in presence of phosphate (P_1). Differences among means of each response in treatments with increasing molybdate concentrations (all of them in presence of phosphate-P_1) were also noticed (Ho_3). The effect of enrichment with phosphate (Ho_4); the addition of increasing levels of nitrate (N_0, N_1 and N_2) in the presence of phosphate (Ho_5); the enrichment with increasing concentrations of molybdate (Mo_0, Mo_1 and Mo_2) in the presence of phosphate (P_1) and nitrate (N_1) (Ho_6) or N_2 (Ho_7); the comparative effect among treatments with different levels of nitrate (N_1 and N_2) both in presence of phosphate (P_1) and molybdate (Mo_1), (Ho_8); the comparative effect among treatments with different levels of nitrate (N_1 and N_2), both in the presence of phosphate (P_1) and molybdate (Mo_2) (Ho_9); and the effect of nitrate and phosphate in different combinations, without molybdate (Mo_0) (Ho_{10}) were also studied.

RESULTS

Table II shows the initial experimental conditions at the water surface at station I on 01/11/1980.

TABLE II

Suspended matter, chlorophyll "a", caroten/chlorophyll ratio, nitrate, ammonia and phosphate levels in superface water at station I in the Lobo Reservoir (initial conditions of the experiment)

Matières en suspension, chlorophylle a, relation carotène/chlorophylle, nitrate, ammonium et phosphate de l'eau de surface à la station I du Réservoir du Lobo (conditions initiales de l'expérience)

Suspended matter (mg.l-1)	4.0
Chlorophyll "a" (µg.l-1)	11.608
Caroten/chlorophyll ratio	2.629
Nitrate (mg.l-1)	< 0.1
Ammonia (mg.l-1)	0.1
Phosphate (mg.l-1)	0.1

The means, standard deviations and coefficients of variation of the 4 responses of phytoplankton to enrichment are shown in Figure 2. Chlorophyll and cell counts increased significantly after adding nutrients.

The variability of suspended matter in the 8 treatments was not easily noticed, but mean values were in all cases slightly superior to initial values. As to the caroten/chlorophyll ratio, the mean values were inferior to the initial values in all treatments except 2 (T_1 and T_2).

The multivariate values of F regarding the 10 hypotheses, are shown in Table III. All were statistically significant except those regarding hypothesis Ho_4 and Ho_8. For the set of 4 variables, there were significant differences among means of treatments analysed in each of the hypothesis, except in the 2 mentioned above.

The univariate values of F concerning the possible differences among the means of the treatments formulated for each hypothesis and each response to enrichment are shown in Table IV. In contrast with their respective multivariate values (see Table III), the univariate values in hypothesis Ho_2,

TABLE III

Multivariate values of F for all of the 10 hypothesis

Valeurs multivariées de F obtenues dans les 10 hypothèses étudiées

HYPOTHESIS	F MULTIVARIATE
Ho_1	6.3168*
Ho_2	7.7009*
Ho_3	21.0140*
Ho_4	2.7221
Ho_5	25.7407*
Ho_6	13.4230*
Ho_7	18.4489*
Ho_8	1.1864
Ho_9	18.8324*
Ho_{10}	33.7560*

* $p < 0.05$

TABLE IV

Univariate values of F for each response of the phytoplanktonic community to enrichment in the 10 hypothesis

Valeurs univariées de F obtenues pour chacune des quatre réponses à l'enrichissement, dans les 10 hypothèses étudiées

VARIABLE	HYPOTHESIS									
	Ho_1	Ho_2	Ho_3	Ho_4	Ho_5	Ho_6	Ho_7	Ho_8	Ho_9	Ho_{10}
Suspended matter	1.3610	0.8208	1.0163	0.1247	0.5818	0.2819	0.0954	0.3017	3.1166	0.2493
Chlorophyll "a"	3.7118*	—	—	0.0864	10.5918*	1.2100	8.7390*	0.5474	9.4398*	9.1080*
Caroten/chlorophyll ratio	1.0020	0.4397	1.0330	1.7613	18.6828*	2.0008	0.0342	0.0035	2.3373	29.2117*
Cells number/ml	3.4326*	—	—	0.3368	12.1633*	2.3462	16.4685*	1.0017	2.7354	9.2189*

* $p < 0.05$.

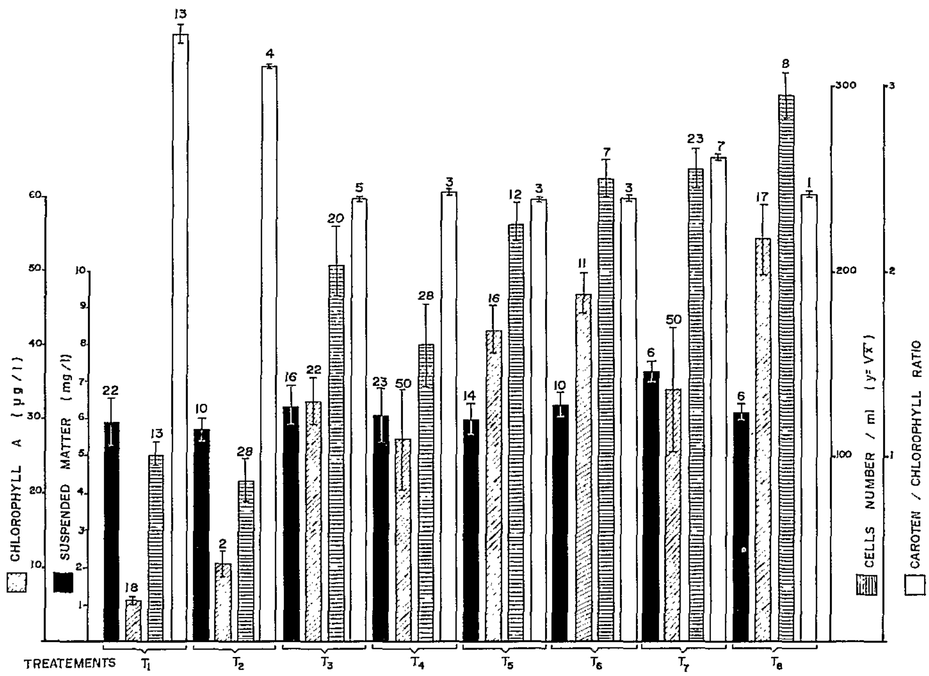

FIG. 2. — Means, standard deviations (I) and coefficients of variation (numbers above the bars) of suspended matter, chlorophyll, caroten/chlorophyll ratio and cell counts obtained in treatments (T, for individual discrimination, see Table 1) after 14-day incubation at the Lobo Reservoir

Moyennes, écart-types et coefficients de variation (nombres au-dessus des figures) du matériel en suspension, de la chlorophylle a, de la relation carotène/chlorophylle et du nombre de cellules algales obtenues dans chaque traitement (T, pour la discrimination individuelle, voir tabl. 1), après 14 jours d'incubation dans le Réservoir du Lobo

Ho_3 and Ho_6 were not significant. The univariate values of F in hypothesis Ho_4 and Ho_8, as also their respective multivariate values, were not statistically significant. Table V shows a summary of the differences among means in treatments for each response to fertilization.

The matrix of the correlations showed a single significant positive correlation (chlorophyll and cell counts) (see Table VI).

DISCUSSION

Molybdenum plays an important role in nitrate assimilation (SYRETT, 1962). It acts as a co-factor in nitrate reductase (WIESSNER, 1962). Besides, it is involved in the biological fixation of nitrogen (GOLDMAN, 1965), and it controls the supply of hydrogen donors or energy carriers for protein synthesis (WIESSNER, 1962).

Not only the light factor controls nitrate assimilation (COLE and TOETZ, 1979; NELSON and CONWAY, 1979), but also molybdenum has great influence on nitrogen metabolism. It acts indirectly on the photochemical reactions of photosynthesis of the algae (WALLEN and GARTIER, 1975).

The importance of molybdenum in phytoplankton development was well documented in the present experiment. The results show the existence of interaction nitrate × molybdenum in the presence of phosphate in chlorophyll and number of cells responses (hypothesis Ho_1, Table IV). Therefore, the presence of molybdenum and nitrate in cultures caused a significant increase of chlorophyll and cell number.

Molybdenum is present in nitrate reductase enzyme, and plays an important role in NO_3^- assimilation (VEGA *et al.*, 1971). Hence, it makes possible an increase in the amount of nitrogen necessary to chlorophyll formation and protoplasm synthesis. This fact explains the chlorophyll and cell increase resulting from molybdenum and nitrate addition.

Simple addition of phosphate and nitrate causes a significant increase in the number of cells and chlorophyll. However, the effects are identical, whatever the concentrations of nitrate (N_1) or (N_2)

TABLE V

Comments on differences among means obtained in treatments (T) for each response and in the hypothesis in which the univariate values of F were statistically significant

*T_2 refers to the mean of chlorophyll obtained in treatment (T_2) (see Fig. 2). The same goes for the other symbols on this table

Commentaires sur les différences entre les moyennes obtenues dans les traitements (T) et dans les hypothèses où les valeurs univariées de F ont été significatives

** T_2 se rapporte à la moyenne de la chlorophylle obtenue au traitement T_2 (voir fig. 2) et, de manière analogue pour les autres symboles de ce tableau*

VARIABLE	HYPOTHESIS			
	Ho_5	Ho_7	Ho_9	Ho_{10}
Suspended matter	—	—	—	—
Chlorophyll "a"	$T_2^* < (T_3 = T_4)$	$(T_4 = T_6) < T_9$	$T_7 < T_8$	$T_1 < T_3$ $T_1 < T_4$
Caroten/Cholorphyll ratio	$T_2 > (T_3 = T_4)$	—	—	$T_1 > T_3$ $T_1 > T_4$
Cells number/ml	$T_2 > (T_3 = T_4)$	$T_4 < T_6 < T_8$	---	$T_1 < T_3$ $T_1 < T_4$

TABLE VI

Matrix of calculated correlations among responses to enrichment

Matrice de corrélations calculées entre les réponses à l'enrichissement, prises deux à deux

VARIABLES	SUSPENDED MATTER	CHLOROPHYLL "a"	CAROTEN/CHLOROPHYLL RATIO
Chlorophyll "a"	0.26	—	—
Caroten/chlorophyll ratio	— 0.21	— 0.30	—
Cells number/ml	0.30	0.83*	— 0.21

r (0.05 ; 30) = 0.35

are be (hypothesis Ho_5, see Table V and Fig. 2). The addition of increasing molybdenum concentrations to cultures containing (P_1+N_1) dit not significantly alter the results (hypothesis Ho_6). However, when enriching the media (P_1+N_2) with increasing molybden contents, we noticed differences among the 3 treatments. A higher chlorophyll level and cell production was obtained in flasks with 500 $\mu g.l^{-1}$ of molybdenum (Mo_2) (hypothesis Ho_7, see Table V and Fig. 2). This observation, mainly for chlorophyll was confirmed after examination of the results of test Ho_9. Data obtained by GOLDMAN (1960) showed that the addition of 0.1 $mg.l^{-1}$ of molybdenum led to an increase in the photosynthesis rate of the lacustrine phytoplankton in June. In October 0.050 $mg.l^{-1}$ of Mo was more effective than higher concentrations. In winter, GOLDMAN (1960) considered the addition of 0.025 $mg.l^{-1}$ of Mo, an adequate concentration to increase the photosynthesis rate. DUMONT (1972) found that in Donk Lake an average concentration of 5 $\mu g.l^{-1}$ of Mo would be sufficient to fulfil the nutritive necessities of the algae. Our data, however, showed that for the Lobo Reservoir, the algal community growth (chlorophyll and number of cells) was higher with increasing contents of molybdenum, in presence of phosphate and a high nitrate level.

Phosphate was not essential to the phytoplankton growth (hypothesis Ho_4, Table III and IV), which confirms the results of other experiments (HENRY, 1981). However, occasionally phosphorus was also necessary to the increase of algae (HENRY and TUNDISI, 1981). Therefore, this experiment indicates that the waters of the Lobo Reservoir are nutritionnally poor, as well in macronutrients as in molybdenum.

In this study, the algal community increased significantly after the addition of nitrate to culture media. For this reason, nitrogen may be considered a primary limiting factor to phytoplankton development. This was also the case in complementary studies where phosphorus also played an important role and was considered as a secondary limiting element to phytoplankton development (HENRY, 1981).

This research shows the important role of nitrogen in the eutrophication of reservoirs and natural freshwater tropical lakes. To some extent, this is contradictory to reports which emphasize the effects of phosphorus as a main cause of eutrophication of lakes and reservoirs in temperate regions. The limitation of the phytoplankton growth by nitrogen and the eutrophication processes resulting from the addition of nitrogen in tropical waters seem to be characteristics of the tropical, natural or artificial freshwater ecosystem.

ACKNOWLEDGEMENTS

We are grateful to Dr. P. R. CURI for his statistical assistance; to FAPESP and CNPq for financial support; to Mrs. H. Toledo for the English translation and to N. A. Basso for typing and drawing of the illustrations.

Manuscrit reçu au Service des Éditions de l'O.R.S.T.O.M. le 7 mai 1982

REFERENCES

BACHMAN (R. W.) and GOLDMAN (C. R.), 1964. — The determination of microgram quantities of molybdenum in natural waters. *Limnol. Oceanogr.*, 9 : 143-146.

BRADFORD (G. R.), BAIR (F. I.) and HUNSAKER (V.), 1968. — Trace and major element content of 170 high Sierra lakes in California. *Limnol. Oceanogr.*, 13 : 526-530.

BURTON (J. D.), 1969. — Molybdenum in the Vellar Estuary, Southern India. *J. Cons. int. Explor. Mer.*, 33 : 103-104.

COHRAN (W. G.) and COX (G. M.), 1957. — Experimental designs. John Wiley, London, 611 p.

COLE (B.) and TOETZ (D.), 1979. — Interactions between light and nitrate concentration in controlling nitrate uptake by reservoir phytoplankton. *Arch. Hydrobiol.* 86 : 269-283.

DUMONT (H. J.), 1972. — The biological cycle of molybdenum in relation to primary production and water-bloom formation in a eutrophic pond. *Verh. int. Verein. Limnol.*, 18 : 84-92.

GIBBS (R. J.), 1973. — Mechanisms of trace metal transport in rivers. *Science, N.Y.*, 180 : 71-73.

GOLDMAN (C. R.), 1960. — Molybdenum as a factor limiting primary productivity in Castle Lake, California. *Science, N.Y.*, 132 : 1016-1017.

GOLDMAN (C. R.), 1965. — Micronutrient limiting factors and their detection in natural phytoplankton populations. *Mem. Ist. ital. Idrobiol.*, 18 (Suppl.) : 123-135.

GOLTERMAN (H. L.) and CLYMO (R. S.), 1969. — Methods for chemical analysis of freshwaters. Blackwell, Oxford, 166 p.

HEAD (P. C.) and BURTON (J. D.), 1970. — Molybdenum in some ocean and estuarine waters. *J. mar. biol. Assoc. U.K.*, 50 : 439-448.

HENRY (R.), 1981. — Efeitos da eutroficação artificial por nutrientes inorgânicos na comunidade fitoplanctônica na Represa do Lobo (Brotas-Itirapina, SP) e no Lago D. Helvécio (Parque Florestal do Rio Doce, MG). PhD., Thesis, Univer. Fed. S. Carlos, 172 p.

HENRY (R.) and TUNDISI (J. G.), 1982. — Efeitos de enriquecimento artificial por nitrato e fostato no crescimento da comunidade fitoplanctònica da Represa do Lobo (« Broa », Brotas-Itirapina, SP). *Ciência e Cultura, S. Paulo*, 34 : 518-524.

MARGALEF (R.), 1964. — Correspondence between the classic types of lakes and the structural and dynamic properties of their populations. *Verh. int. Verein. Limnol.*, 15 : 169-175.

MORAES (E. de M.), 1978. — Ciclo sazonal, distribuição horizontal e vertical e interrelações ecologicas de nutrientes na Reperesa do Lobo (Brotas-Itirapina, SP). M. Sc. Thesis, Univ. S. Paulo, 153 p.

MORRISON (D. F.), 1967. — Multivariate statistical methods. McGraw Hill, New York, 238 p.

NELSON (D. M.) and CONWAY (H. L.), 1979. — Effects of the light regime on nutrient assimilation by phytoplankton in the Baja California and the northwest Africa upwelling systems. *J. mar. Res.*, 37 : 301-308.

SYRETT (P. J.), 1962. — Nitrogen assimilation. *In* : LEWIN (R. A.). Physiology and biochemistry of algae. Academic Press, New York : 171-188.

TEIXEIRA (C.) and KUTNER (M. B.), 1962. — Plankton studies in a mangrove environment. I. First assessment of standing stock and ecological factors. *Bol. Inst. Oceanogr., S. Paulo,* 12 : 101-124.

TUNDISI (J. G.), TUNDISI (T. M.), ROCHA (O.), GENTIL (J. G.) and NAKAMOTO (N.), 1977. — Primary production, standing stock of phytoplankton and ecological factors in a shallow tropical reservoir (Represa do Broa, São Carlos, Brasil). *Sem. Medio Ambiente y Represa,* 1 : 138-172.

TUNDISI (J. G.), TUNDISI (T. M.), STRIXINO (G.), MARINS (M. A.), MATTOS (E.) and SANTOS (E. P.), 1972a. — Estudos ecológicos em ambiente lacustre. I. Resultados e análise de dados de levantamento geral de fatores ambientais (Julho, 1971). Univ. Fed. S. Carlos, 21 p.

TUNDISI (J. G.), TUNDISI (T. M.), STRIXINO (G.), MARINS (M. A.), MATTOS (E.) and SANTOS (E. P.), 1972b. — Estudos ecológicos em ambiente lacustre. II. Resultados e análise de dados de levantamento geral de fatores ambientais (Fevereiro, 1972). Univ. Fed. S. Carlos, 63 p.

VEGA (J. M.), HERRERA (J.), APARICIO (P. J.), PANEQUE (A.) and LOSADA (M.), 1971. — Role of molybdenum in nitrate reduction by *Chlorella. Pl. Physiol.*, 48 : 294-299.

VIEIRA (A. A. H.) and TUNDISI (J. G.), 1979. — Notas sobre o cultivo de algumas espécies de algas de água doce. *Rev. Brasil. Biol.*, 39 : 703-706.

WALLEN (D. C.) and CARTIER (L. D.), 1975. — Molybdebum dependence, nitrate uptake and photosynthesis of freshwater plankton algae. *J. Phycol.*, 11 : 345-349.

WETZEL (R. G.), 1975. — Limnology. W. B. Saunders, Philadelphia, 743 p.

WIESSNER (W.), 1962. — Inorganic nutrient. *In* : LEWIN (R. A.). Physiology and biochemistry of algae. Academic Press, New York, p. 267-286.