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Properties of phosphatases from green alga *Scenedesmus incrassatulus* Bahlin and blue-green alga *Synechococcus aeruginosus* Nag.

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Abstract. Studies on phosphatases (acid and alkaline) in *Synechococcus aeruginosus*, a prokaryotic blue-green alga, and *Scenedesmus incrassatulus*, an eukaryotic green alga under different conditions revealed that the acid phosphatase exhibited maximum activity to pH 4·7 and 37°C in both the algae while alkaline phosphatase displayed greatest activity at 37·5°C and 10 pH in *Synechococcus aeruginosus* and at 10·6 pH and 37·5°C in *Scenedesmus incrassatulus*. The K_m values were found to be 50 and 17 mM for acid phosphatase and 4·2 and 8·3 mM for alkaline phosphatase in blue-green and green algae respectively. The inhibitory effect of metal ions (Cu^{2+}, Fe^{2+}, Hg^{2+}, Zn^{2+}) on acid phosphatase activity is similar in both the algae whereas on alkaline phosphatase, their influence varies. Sodium fluoride and EDTA inhibited both the phosphatases activity with the increased concentrations in both the algae. Benzene hexachloride (50%) retarded these enzyme's activities at higher concentrations in these algae.

Keywords. Acid and alkaline phosphatases; green alga; blue-green alga.

1. Introduction

The existence of non-specific phosphatases in micro-organisms as well as in higher plants is well known and these enzymes supply phosphates from phosphate esters under phosphate deprivation while orthophosphates suppress their activity. The contribution of algae to biogeochemical transformations of soil and enhancement of fertility of soil and aquatic habitats by nitrogen fixation has been well documented (Kumar 1985; Singh 1961; Venkataraman 1966). However, the phytoplanktonic algae of rice fields and aquatic bodies could make use of phosphatases to hydrolyse organic or inorganic phosphates either from stored cellular polyphosphates or the environment as well as aquatic rice fields where farmers very frequently apply the fertilizers and agrochemicals. The measurement of phosphatases activity in algae not only indicates the presence or absence of phosphates in the algal cells but also in the environment including aquatic bodies.

Extensive studies both on intra and extra-cellular acid phosphatases were reported in *Ochromonas danica* (Patni and Aaronson 1974), *Chlorella* sp. (Price 1962), *Euglena* sp. (Blum 1965) and alkaline phosphatase activity in *Rivularia* sp. (Livingstone and Whitton 1984). Sarma and Kanta (1982) evidenced only the activities of acid and alkaline phosphatases in vegetative cells undergoing sporulation of *Anabaena cylindrica*. Earlier studies relating to few properties and localization of phosphatases were mainly confined to *Euglena* sp., *Chlorella* sp. and a filamentous blue-green alga *Rivularia* sp. of aquatic bodies but not of rice fields. In view of lack of adequate information on the properties and kinetics of

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phosphatases (acid and alkaline) in rice field algae with or without the stress of agrochemicals and fertilizers, comparative studies on the properties of acid and alkaline phosphatases in the eukaryotic colonial alga *Scenedesmus incrassatulus* and the prokaryotic unicellular alga *Synechococcus aeruginosus*, isolated from rice fields, have been undertaken and the observations are reported in this paper. This study is also expected to give an account of eco-physiological aspects of algae in the rice fields.

2. Materials and methods

2.1 *Materials*

Axenic, clonal population of *S. incrassatulus* and *S. aeruginosus* were raised in a medium worked out by Hughes *et al* (1958) and modified by Allen (1968). The culture as well as experimental methods have been already described (Reddy *et al* 1983). The pH of the basal medium was adjusted to 7·5 for the eukaryotic alga *S. incrassatulus* and 8·5 for the prokaryotic alga *S. aeruginosus* before autoclaving. A 14 day old culture of these algae was employed for enzyme study.

2.2 *Methods*

2.2a *Preparation of phosphatases (acid and alkaline) and their assays*: Harvested algal culture of 14 days old (126×10^6 cells of blue-green alga and 816×10^5 cells of green alga) was washed and suspended in 2 ml ice-cold glass distilled water and then macerated at 0°C in an ice-cold mortar with acid washed sand (700 mg) for about 25 min to obtain aqueous extract. The homogenate was kept in ice-cold for 30 min and centrifuged at 4260 g for 10 min to remove debris and sand particles. The clear supernatant was used for phosphatases (acid and alkaline) assays.

2.2b *Assay for acid and alkaline phosphatases*: The activity of acid and alkaline phosphatases was assayed in duplicate by the methods of Patni and Aaronson (1974) and Bessy *et al* (1946) respectively. The assay mixture (1 ml) for acid phosphatase contained 0·5 ml of 15 mM *p*-nitrophenyl phosphate dissolved in 0·1 M acetate buffer (4·7 pH), 0·2 ml of 0·1 M acetate buffer (4·7 pH) and 0·3 ml of crude enzyme extract; while for alkaline phosphatase, the test solution (1 ml) consisted of 0·5 ml of 15 mM *p*-nitrophenyl phosphate dissolved in 0·1 M carbonate buffer (10·6 pH), 0·2 ml of 0·1 M carbonate buffer (10·6 pH) and 0·3 ml of enzyme extract. The total proteins of crude enzyme extracts of blue-green and green algae were recorded as 63·3 and 30 μg per ml respectively by the method of Lowry *et al* (1951). The assay mixture of acid and alkaline phosphatases were separately incubated for 30 min at 37°C and the reaction was terminated in both these tests by the addition of 5 ml of 0·1 N NaOH. The *p*-nitrophenol released by the acid and alkaline phosphatases was measured at 420 and 405 nm respectively in spectronic-20 spectrophotometer (Bausch and Lomb, USA).

For the measurement of activities of these enzymes, one unit is defined as the quantity of enzyme required to liberate 1 μmol of *p*-nitrophenol per ml per min, under standard conditions. The specific activity was expressed as enzyme units per

mg of protein. To study the substrate specificity of acid and alkaline phosphatases of these algae, different phosphate esters (*p*-nitrophenyl phosphate, sodium β-glycerophosphate, glucose-1-phosphate, glucose-6-phosphate) were selected and assayed the liberated free inorganic phosphates by the method of Fiske and Subbarow (1925). One unit of enzyme activity on different phosphate esters was defined as the quantity of enzyme required to liberate one μmol of inorganic phosphate per min.

2.2c *Chemicals*: Bovine serum albumin (Sigma Chemical Company, USA), *p*-nitrophenyl phosphate (John Baker Inc., USA) and sodium β-glycerophosphate, glucose-1-phosphate, glucose-6-phosphate (Loba-chemie Indoaustranal Co., Bombay) were employed in this study. All the other chemicals used were of highest purity obtained from British Drug House, Bombay.

3. Results and discussion

3.1 *Effect of pH on the phosphatases (acid and alkaline) activity*

Both acid and alkaline phosphatase activities in *S. aeruginosus* and *S. incrassatulus* were assayed using *p*-nitrophenyl phosphate as substrate in 0·1 M glycine-HCl buffer (2·2 – 3·6 pH), 0·1 M acetate buffer (4·2 – 5·6 pH), 0·1 M barbital buffer (6·2 – 8 pH), 0·2 M glycine-NaOH buffer (8·6 pH) and 0·1 M carbonate buffer (9–11 pH). The pH-activity profiles of acid and alkaline phosphatases of these algae is represented in figure 1. It is evident (figure 1) that optimum activity of acid phosphatase was recorded at 4·7 pH in both the algae while the alkaline phosphatase activity was optimum at 10 pH and 10·6 pH in *S. aeruginosus* and *S. incrassatulus* respectively. The above obtained results relating to optimum pH (4·7) for the activity of acid phosphatase of *S. incrassatulus* is slightly different optimum pH range than in *Euglena gracilis* (Blum 1965) and *Ochromonas danica* (Patni and Aaronson 1974) but quite different from the results of *E. gracilis* strain SML-1 (Blum and Buetow 1963). The range of optimum pH (4·7) for acid phosphatase activity was not reported in any blue-green alga, while the optimum activity of alkaline phosphatase ranges also differs in these algae from the earlier reports (Livingstone and Whitton 1984; Aaronson 1971). Thus the pH-activity

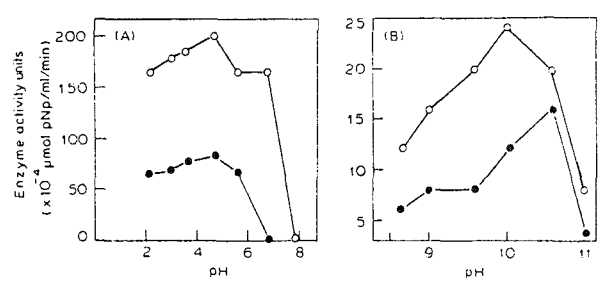


Figure 1. Effect of pH on the activity of acid phosphatase (**A**) and alkaline phosphatase (**B**). (○), *S. aeruginosus*; (●), *S. incrassatulus*.

profiles of phosphatases (acid and alkaline) reflect the pH at which the important proton-donating or proton-accepting groups in the enzyme's catalytic sites are in the required state of ionization.

3.2 *Effect of temperature on the phosphatases (acid and alkaline) enzyme*

Effect of temperature on the phosphatases (acid and alkaline) activity in *S. aeruginosus* and *S. incrassatulus* was investigated at 30, 35, 37, 37·5, 45, 55, 60 and 65°C and results recorded are represented (figure 2). It is evident (figure 2A) that the activity of acid phosphatase was optimum at 37°C in both the algae. At 65°C, green algal acid phosphatase became inactive while blue-green algal enzyme was still active. Phosphatase enzyme activity with alkaline buffer was optimum at 37·5°C in both the algae and it became inactive at 60°C (figure 2B). Earlier reports did not mention the temperature tolerance of phosphatases in any algae. The differential activity of phosphatases of green and blue-green algae at different temperatures is probably due to the variation in the inactivation of the associated proteins of the acid and alkaline phosphatases.

3.3 *Enzyme activity at different concentrations of substrate*

The enzyme activities (acid and alkaline) were gradually increased upto 15 mM of *p*-nitrophenyl phosphate in both the algae. In the graphs of double reciprocal plots, substrate concentration was expressed in molars. From the double reciprocal plots (figure 3), the K_m values of acid phosphatase were determined as 50 and 17 mM while for alkaline phosphatase 4·2 and 8·3 mM for blue-green and green algae respectively. Comparative studies indicate the affinity of acid phosphatase towards the substrate in blue-green alga is less when compared to green alga, while in the case of alkaline phosphatase, the affinity towards the substrate is in reverse.

3.4 *Substrate specificity*

Table 1 shows the specificity of acid and alkaline phosphatases towards different phosphate esters in *S. aeruginosus* and *S. incrassatulus*. In both these algae, acid and

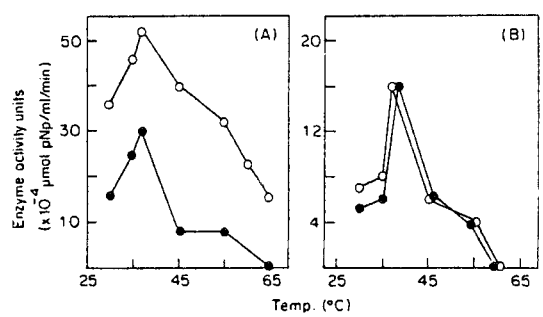


Figure 2. Effect of temperature on acid (**A**) and alkaline (**B**) phosphatases. (○), *S. aeruginosus*; (●), *S. incrassatulus*.

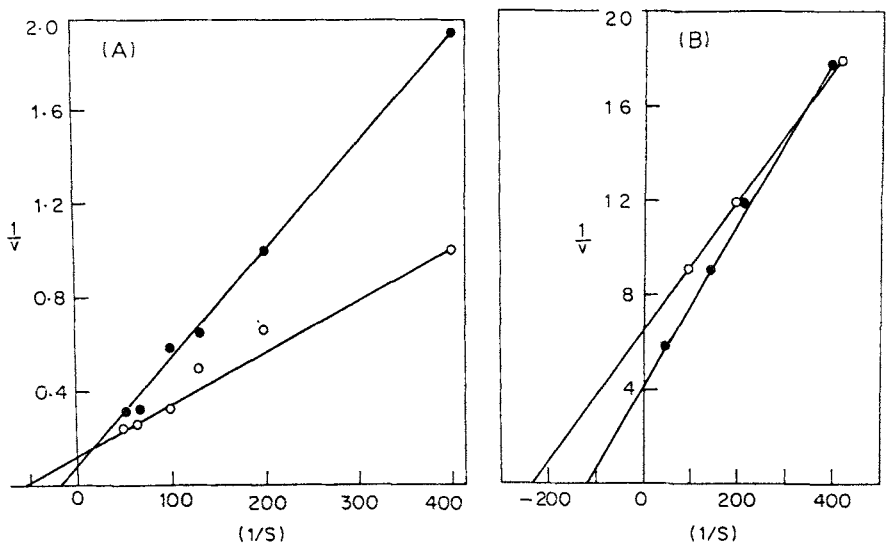


Figure 3. Lineweaver-Burk plots (double reciprocal plots) for acid phosphatase (**A**) [(●), *S. aeruginosus*; (○), *S. incrassatulus*] and alkaline phosphatase (**B**) [(○), *S. aeruginosus*; (●), *S. incrassatulus*].

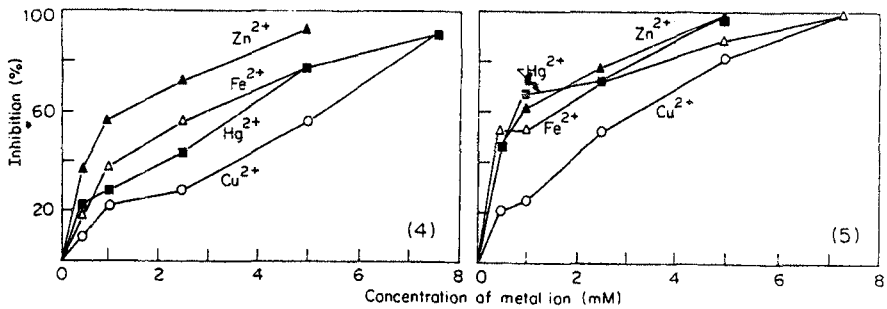
Table 1. Substrate specificity of algal phosphatases [phosphatases activity (μmol phosphate liberated per min) towards *p*-nitrophenol phosphate was taken as 100]. The liberated phosphate per min in alkaline phosphatase is 0·005 μmol (*S. aeruginosus*), 0·003 μmol (*S. incrassatulus*) and in acid phosphatase is 0·004 μmol in both the algae.

	Alkaline phosphatase (relative rate of hydrolysis)		Acid phosphatase (relative rate of hydrolysis)	
Substrate	*S. aeruginosus*	*S. incrassatulus*	*S. incrassatulus*	*S. aeruginosus*
p-Nitrophenyl phosphate	100	100	100	100
Sodium β-glycerophosphate	80	100	207	212
Glucose-1-phosphate	60	67	82	88
Glucose-6-phosphate	69	49	80	81

alkaline phosphatases were found to have high specificity towards sodium β-glycerophosphate as compared to other substrates. None of earlier studies mentioned the results relating to specificities of different substrates.

3.5 *Effect of metal ions (Zn^{2+}, Fe^{2+}, Hg^{2+}, Cu^{2+}), sodium fluoride, EDTA and benzene hexachloride 50% on the phosphatases (acid and alkaline) activity*

The activities of acid and alkaline phosphatases of these algae were assayed in different metal ions (figures 4–7) sodium fluoride, EDTA and an insecticide benzene hexachloride (BHC) 50% (table 2). The results represented (figures 4 and 5) indicate that the per cent inhibition of activity of acid phosphatase of these algae increased with the augmented concentrations of metal ions. Among the metal ions, Cu^{2+}



Figures 4 and 5. 4. Effect of metal ions on acid phosphatase activity in *S. incrassatulus*. (○), Cu^{2+}; (■), Hg^{2+}; (△), Fe; (▲), Zn. 5. Influence of metal ions on acid phosphatase in *S. aeruginosus*. (▲), Zn; (■), Hg; (△), Fe; (○), Cu.

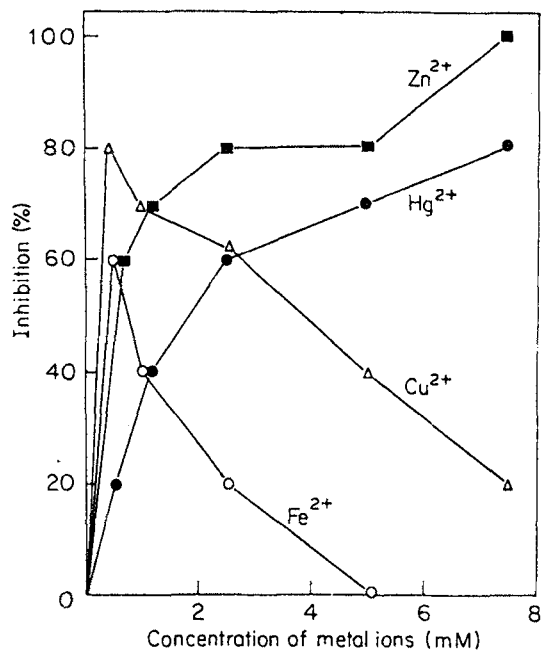


Figure 6. Effect of metal ions on alkaline phosphatase activity in *S. incrassatulus*. (△), Cu; (■), An; (●), Hg; (○), Fe.

appeared to be less inhibitory at low doses as compared to others while with the increased concentrations, Zn^{2+} and Fe^{2+} seemed to be more toxic in both the algae. The per cent inhibition of activity of alkaline phosphatase of these algae was augmented upto 0·5 mM dose of Cu^{2+} and Fe^{2+} and further increase of concentrations, per cent inhibition reduced significantly (figures 6 and 7), Hg^{2+} and Zn^{2+} enhanced the inhibitory activity of alkaline phosphatase in both the algae. It can be inferred that, probably, the low concentrations (0·5 mM) of Cu^{2+} and Fe^{2+} combine with alkaline phosphatase and substrate forms enzyme-metal-substrate complex that led to enhance the inhibition while higher doses of metal ions

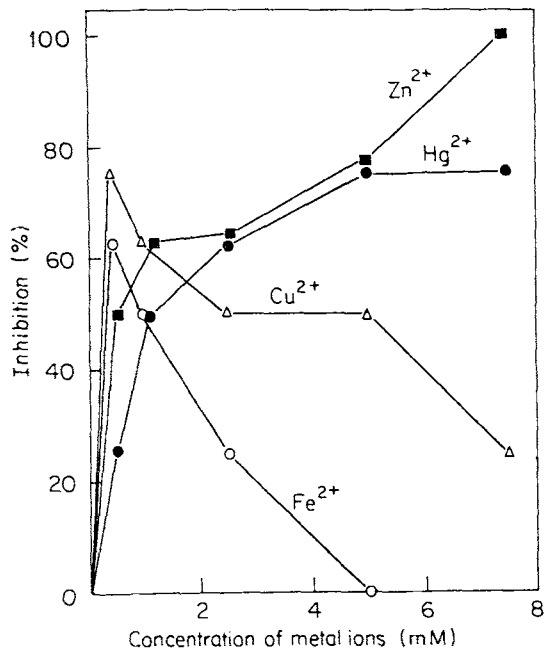


Figure 7. Influence of metal ions on alkaline phosphatase activity in *S. aeruginosus*. (△), Cu; (■), Zn; (○), Fe; (●), Hg.

Table 2. Effect of chemicals on the activity of phosphatases (enzyme specific activity units μmol of *p*-nitrophenol released/min/mg protein).

	Alkaline phosphatase		Acid phosphatase	
Conc. of chemical (mM)	*S. aeruginosus* (specific activity)	*S. incrassatulus* (specific activity)	*S. aeruginosus* (specific activity)	*S. incrassatulus* (specific activity)
Control	0·03	0·05	0·13	0·21
Sodium fluoride				
0·02	0·02(33)	0·02(60)	0·07(46)	0·12(42)
0·03	0·01(66)	0·01(80)	0·07(46)	0·08(61)
0·04	0·01(66)	0·00(100)	0·05(61)	0·03(85)
0·05	0·00(100)	0·00(100)	0·00(100)	0·00(100)
EDTA				
0·02	0·02(33)	0·04(20)	0·13(0)	0·21(0)
0·03	0·01(66)	0·02(60)	0·12(7)	0·16(23)
0·04	0·01(66)	0·01(80)	0·10(23)	0·08(61)
0·05	0·01(66)	0·01(80)	0·07(46)	0·02(90)
BHC 50%				
2·5	0·02(33)	0·02(60)	0·12(7)	0·12(42)
5·0	0·01(66)	0·01(80)	0·10(23)	0·08(61)
7·5	0·01(66)	0·01(80)	0·05(61)	0·02(90)
10·0	0·00(100)	0·00(100)	0·04(69)	0·00(100)

Numbers in parentheses indicate the per cent inhibition.

combine with enzyme that brings out the conformational changes and converting into an active form or the high doses of these metals might be precipitated with

alkaline pH range (Mildvan 1970). However, with acid phosphatase of these algae, the reduction of the activity is increased with the enhanced concentration of metal ions attributing to the formation of metalloenzyme substrate complex as mentioned in alkaline phosphatase.

The data in table 2 shows that sodium fluoride (0·05 mM) exerted total inhibition of the activity of acid and alkaline phosphatases in both the algae. Phosphatases (acid and alkaline) activities were inhibited with the enhanced concentrations of EDTA in these algae. Neither of the enzymes (acid and alkaline phosphatases) of these algae could be inhibited significantly at 7·5 mM of BHC (50%) in both the algae while at 10 mM, both the enzymes were completely inhibited except acid phosphatase in *S. aeruginosus*. The inhibitory effect of sodium fluoride on phosphatases of these algae is attributed to the formation of fluoride enzyme complex as reported in *O. danica* (Patni and Aaronson 1974) and *Anabaena* sp. (Reddy and Sarada 1983). The reduction of activity of both phosphatases in these algae with the augmented doses of EDTA indicate that the metal ions might be chelated and make the enzymes inactive. Therefore, it can be concluded that the crude phosphatases require metal ions for their activity. The data (table 2) indicating the effectiveness of BHC on the activity of phosphatases of these algae occurs at very high doses.

References

Aaronson S 1971 The synthesis of extracellular macro-molecules and membranes by a population of the phytoflagellate *Ochromonas danica*; *Limnol. Oceanogr.* **16** 1–9

Allen M M 1968 Simple conditions for growth of unicellular blue-green algae on plates; *J. Phycol.* **4** 1–4

Bessy O A, Lowry O H and Brock M M 1946 A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum; *J. Biol. Chem.* **164** 321–329

Blum J J and Buetow D E 1963 Biochemical changes during acetate deprivation and repression in *Euglena* sp.; *Exp. Cell Res.* **23** 407–421

Blum J J 1965 Observations on the acid phosphatases of *Euglena gracilis*; *J. Cell Biol.* **24** 223–234

Fiske C and Subbarow Y 1925 The colorimetric determination of phosphorus; *J. Biol. Chem.* **66** 375–400

Kumar H D 1985 General introduction; in *Algal cell biology* (New Delhi, Madras: Affiliated East-West Press) pp 5–6

Livingstone D and Whitton B A 1984 Water chemistry and phosphatase activity of the blue-green alga, *Rivularia* sp. in upper Teesdale streams; *J. Ecol.* **72** 405–421

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with the Folin phenol reagent; *J. Biol. Chem.* **193** 265–275

Mildvan A S 1970 Metals in enzyme catalysis; *Enzymes* **2** 446–536

Patni N J and Aaronson S 1974 Partial characterization of the intra and extracellular acid phosphatases of an alga, *Ochromonas danica*; *J. Gen. Microbiol.* **83** 9–20

Price C A 1962 Repression of acid phosphatase synthesis in *Euglena gracilis; Science* **135** 46

Reddy T R K, Rao J C S and Radhakrishna T M 1983 Toxicity of oil refinery effluent on physiological responses of algae; *Phykos* **22** 86–93

Reddy T R K and Sarada K 1983 Toxicity of fluoride on growth and morphogenesis of *Anabaena* sp; in *Proc. All India Appl. Phycol. Congr.*, Kanpur, pp 213–220

Sarma T A and Kanta S 1982 Variation in enzyme levels during sporulation in blue-green algae; *Biochem. Physiol. Pflanz.* **177** 278–282

Singh R N 1961 *Role of blue-green algae in nitrogen economy of Indian agriculture* (New Delhi: Indian Council of Agricultural Research)

Venkataraman G S 1966 Algalization; *Phykos* **5** 164–174