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Effect of nickel on certain physiological and biochemical behaviors of an acid tolerant *Chlorella vulgaris*

P. K. Rai, Nirupama Mallick & L. C. Rai

Laboratory of Algal Biology, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi, India

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This study concerns the inhibitory effects of acid pH and nickel on growth, nutrient (NO_3^- and NH_4^+) uptake, carbon fixation, O_2 evolution, electron transport chain and enzyme (nitrate reductase and ATPase) activities of acid tolerant and wild-type strains of *Chlorella vulgaris*. Though a general reduction in all these variables was noticed with decreasing pH, the tolerant strain was found to be metabolically more active than the wild-type. A reduced cation (NH_4^+, Na^+, K^+ and Ca^{2+}) uptake, coupled with a facilitated influx of anions (NH_4^+, PO_4^{3-} and HCO_3^-), suggested the development of a positive membrane potential in acid tolerant *Chlorella*. Nevertheless, a tremendous increase in ATPase activity at decreasing pH revealed the involvement of superactive ATPase in exporting H^+ ions and keeping the internal pH neutral. A difference in Na^+ and K^+ efflux of the two strains at decreasing pH suggests there is a difference in membrane permeability. The low toxicity of Ni in the acid tolerant strain may be due to the low Ni uptake brought about by a change in membrane potential as well as in permeability. Hence, the development of superactive ATPase and a change in both membrane potential and permeability not only offers protection against acidity, but also co-tolerance to metals.

Keywords: *Chorella vulgaris*, acid tolerance, ATPase, nickel toxicity, nutrient uptake, photosynthesis

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Introduction

Plasma membranes are generally known to possess negative charges due to the dissociation of the phosphate groups of phospholipids. Exposure of algae to acidic pH causes protonation of phospholipids, hence the resulting positively-charged groups determine the surface change of the plasmamembrane (Gibrat *et al.* 1985). Development of a positive membrane potential, which in turn gives rise to a positive zeta potential (as zeta potential is dependent on the surface charges; see McLaughlin 1977), is thought to be the main factor for survival of organisms in acidic environment (Gimmler *et al.* 1989, 1991).

Gimmler *et al.* (1988), while working on acid tolerant *Dunaliella acidophila*, demonstrated that the positive membrane and zeta potentials minimized the H^+ influx from the medium into the cells at a chemical gradient of 10^6 across the plasma membrane, thereby maintaining the cytoplasmic pH to neutrality (pH 7.0). In addition, the plasma membranes of plant as well as algal cells possess an active electrogenic H^+-ATPase pump parallel to the passive diffusion pathway. This creates an electrochemical gradient, which in turn regulates the flux of other ions, i.e. the efflux of cations (antiport) and the influx of anions (symport; see Hirsch *et al.* 1992). The existence of an H^+-ATPase pump in the plasma membranes of acidophilic *Cyanidium caldarium* and *D. acidophila* has also been demonstrated (Enami *et al.* 1986, Sekler *et al.* 1991).

Notwithstanding this, the surface potential also plays an important role in the uptake of cations. It has been demonstrated that a decrease in zeta potential by 8 mV caused a 20-fold increase in apparent K_m of the potassium uptake system in yeast cells (Theuvenet & Borst-Pauwels 1983). Since toxic metals are generally cations, it is presumed that the positive membrane potential of acid-tolerant organisms should play a significant role in regulating the uptake and toxicity of metals. Gimmler *et al.*

Address for correspondence: L. C. Rai, Laboratory of Algal Biology, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005, India. Fax: (+91) 542 312059.

(1991) reported that the acidophilic *D. acidophila* possessed greater resistance to Al, La, Au, Cu, Cd and W. However, nothing is known about the physiological and biochemical differences between wild-type and acid-tolerant algae when they are exposed to metals. Therefore, the objectives of this study were as follows:

- to investigate Ni toxicity on various physiological and biochemical processes of wild-type and acid-tolerant *Chlorella vulgaris*.
- to explore the mechanism(s) of acid tolerance in laboratory-grown *C. vulgaris*.

This study is of particular ecological significance because it should offer information about the physiological and biochemical basis of survival of algae in harsh environments (like acid mine drainage, tanneries, metal processing industries and others).

Materials and methods

The unicellular green alga *C. vulgaris* was grown axenically in modified Chu-10 medium (Gerloff *et al.* 1950; pH 6.8) under 72 μmol photon m^{-2} s^{-1} PAR light intensity and 14:10 LD cycle at 24 ± 2 °C. An acid-tolerant strain of this organism was isolated by successive subculturing of the wild-type in an acidic pH (pH 5.0) buffered with 10 mM MES buffer. Both the strains were subcultured twice per week into fresh medium and only cultures from the logarithmic phase of growth were used for further study.

Exponentially-grown wild and tolerant strains of *C. vulgaris* cells were transferred to media with different pHs (i.e. 6.8, 5.0, 4.0 and 3.5 pH) and buffered with 10 mM MES buffer. Stock solution of $NiCl_2 \cdot 6H_2O$ was prepared in water double distilled in glass, and sterilized by being passed through millipore membrane filters (0.45 μM), before being added to the culture medium. To study the effect of Ni, the culture media was spiked with the LC_{50} dose of Ni (2 μg ml^{-1}). Toxicities at different pHs were evaluated by comparisons with controls (untreated cultures of both strains in the respective pH).

Measurement of growth

The final growth yield in terms of Chl *a* content (Brody & Brody 1961) was measured using a spectronic-20 colorimeter after 15 days of treatment.

Photosynthesis and ATP content

Carbon fixation was measured by recording the uptake of ^{14}C from $NaH^{14}CO_3$ (specific activity 18.5 × 10^5 Bq) in a LS 7000 liquid scintillation counter, as described by Rai & Raizada (1986). Photosynthetic O_2 evolution was measured with a polarographic oxygen electrode enclosed in a 10 ml airtight reaction vessel connected to an oxygen analyzer (digital oxygen system, model 10; Rank Brothers, UK). The photosynthetic electron transport activity was measured by the method of Lien (1978). The ATP pool was measured by luciferin–luciferase assay (Larsson & Olsson 1979) using a LKB-1250 luminometer.

Uptake of ions

For measurement of Na^+, K^+ and Ca^{2+} uptakes the alga was treated with (10 mM) chlorides of each salt and incubated for 24 h. The cells were harvested by centrifugation, washed in 2 mM EDTA and digested using the method of Rai & Raizada (1987). The cation content was measured with a Perkin-Elmer 2380 atomic absorption spectrophotometer. The uptakes of Na^+, K^+ and Ca^{2+} were calculated by subtracting the ion-contents of untreated cells. The uptakes of NH_4^+, NO_3^- and PO_4^{3-} were estimated colorimetrically by measuring the depletion of these nutrients from the external medium using the Nessler's reagent method (Herbert *et al.* 1971), the brucine sulfuric acid method (Nicholas & Nason 1957), and the stannous chloride method (APHA 1985), respectively.

Enzyme activities

Nitrate reductase activity was estimated by measuring the formation of NO_2^- from NO_3^- (Camm & Stein 1974). The reagents used were sulfanilamide and α-(N-1) naphtylethylene diamine dihydrochloride. Mg^{2+}-dependent ATPase activity was measured using the method of Ohnisi *et al.* (1975). Inhibition kinetics of ATPase and nitrate reductase were studied at different substrate concentrations.

Na^+ and K^+ efflux

The loss of Na^+ and K^+ from the cells exposed to both an acidic pH and Ni was measured following the method of Rai & Raizada (1987).

Ni uptake

Ni uptake over a 2 h incubation period was estimated with an atomic absorption spectrophotometer, using the method of Martin (1979).

Statistical analysis

The results were verified by Student's '*t*' test.

Results

Growth

Table 1 presents the interactive effects of pH and Ni on growth behaviors of wild-type and acid-tolerant *C. vulgaris* after 15 days of treatment. As compared

Table 1. pH altered Ni toxicity on growth, photosynthesis (carbon fixation and O_2 evolution) and ATP content of the wild-type and acid-tolerant strain of *C. vulgaris*

pH		Final yield (μg Chl *a* ml^{-1})		$^{14}CO_2$ fixation (CMP $\times 10^2$ μg protein^{-1})		O_2 evolution (μM O_2 evolved mg protein^{-1} h^{-1})		ATP content (μg ATP μg protein^{-1})	
		wild-type	tolerant strain	wild-type	tolerant strain	wild-type	tolerant strain	wild-type	tolerant strain
6.8	C	21.47 ± 0.53	18.03 ± 0.48	3.72 ± 0.52	4.23 ± 0.22	42.33 ± 0.48	51.02 ± 0.52	1.35 ± 0.05	1.23 ± 0.03
	Ni	12.63 ± 0.40	12.33 ± 0.39	2.56 ± 0.22	3.57 ± 0.4	25.77 ± 0.21	43.82 ± 0.39	0.96 ± 0.04	0.93 ± 0.02
		(41)	(32)	(31)	(16)	(39)	(14)	(29)	(24)
5.0	C	6.10 ± 0.42	16.14 ± 0.46	1.85 ± 0.51	3.34 ± 0.21	34.96 ± 0.49	45.24 ± 0.61	0.64 ± 0.08	0.98 ± 0.05
	Ni	NG	7.08 ± 0.45	0.66 ± 0.75	2.36 ± 0.3	14.94 ± 0.36	33.94 ± 0.5	0.19 ± 0.06	0.66 ± 0.04
			(56)	(64)	(30)	(57)	(25)	(70)	(33)
4.0	C	NG	9.8 ± 0.39	1.30 ± 0.52	3.16 ± 0.3	29.9 ± 0.47	43.75 ± 0.53	0.55 ± 0.05	0.72 ± 0.05
	Ni	NG	3.35 ± 0.46	0.29 ± 0.4	1.99 ± 0.5	9.01 ± 0.28	31.15 ± 0.31	0.12 ± 0.03	0.49 ± 0.06
			(62)	(78)	(37)	(70)	(29)	(78)	(32)
3.5	C	NG	6.09 ± 0.48	1.04 ± 0.35	2.92 ± 0.5	27.17 ± 0.46	36.56 ± 0.52	0.50 ± 0.03	0.70 ± 0.02
	Ni	NG	NG	0.35 ± 0.25	1.68 ± 0.39	6.98 ± 0.25	24.51 ± 0.36	0.08 ± 0.02	0.43 ± 0.02
				(98)	(42)	(74)	(33)	(84)	(39)

All the values are mean ± SE; NG denotes no growth; data in parentheses denote percent inhibition; '*t*' significant at $P < 0.1$; C = control, Ni = nickel-treated (2 μg/ml). Chl *a* = chlorophyll *a*.

to the wild-type, where complete inhibition of growth was noticed at pH 4.0 and 3.5, the tolerant strain showed only 39 and 62% reduction in growth, respectively, at these pHs. However, the growth reduction was 72 and 11%, respectively, for the wild-type and tolerant strain at pH 5.0. Growth inhibition at LC_{50} of Ni at pH 6.8 was only 41 and 32%, respectively, for the wild-type and the tolerant strain. At pH 5.0, 4.0 and 3.5 the nickel-induced growth inhibition was, respectively, 56, 62 and 100% for the tolerant strain. However, the wild-type *C. vulgaris* could not survive in the presence of Ni at these pHs.

Photosynthesis and ATP content

The data given in Table 1 demonstrate a pH-dependent reduction in carbon fixation, O_2 evolution and ATP content for both strains. Ni toxicity was low in the tolerant strain, with inhibition percentages of 16, 30, 37 and 42% and 14, 25, 29 and 33%, respectively, for carbon fixation and O_2 evolution at pH 6.8, 5.0, 4.0 and 3.5, compared with 31, 64, 78 and 98% (carbon fixation) and 39, 57, 70 and 74% (O_2 evolution) for the wild-type. The inhibition of the electron transport chain (data not shown) and ATP pool (see Table 1) also depicted a trend similar to O_2 evolution. In the wild-type the inhibition of ATP was 53, 60 and 63%, respectively, at pH 5.0, 4.0 and 3.5. However, in the tolerant strain these percentages were 35, 41 and 43%, respectively. Nickel supplementation reduced the ATP pool by 29, 70, 78 and 84% in the wild-type, compared with 24, 32, 33 and 39% in the tolerant strain, respectively, at pH 6.8, 5.0, 4.0 and 3.5.

Uptake of NH_4^+, Na^+, K^+ and Ca^{2+}

A general reduction in the uptake of NH_4^+, Na^+, K^+ and Ca^{2+} by both the strains was noticed with decreasing pH (Table 2). Supplementation of 2 μg ml^{-1} Ni increased the inhibition of NH_4^+ uptake to 27, 61, 82 and 89% in the wild-type, respectively, at pH 6.8, 5.0, 4.0 and 3.5. However, in the tolerant strain this reduction was only 18, 28, 34 and 38% at the above pHs. Likewise, the Ni-induced inhibition of Na^+ uptake was about 36, 42, 53 and 66% and 28, 35, 42 and 48%, respectively, in the wild-type and the tolerant strain at pH 6.8, 5.0, 4.0 and 3.5. The inhibition of Na^+, K^+ and Ca^{2+} uptake was higher in the tolerant than in the wild-type strain at decreasing pH.

Uptake of PO_4^{3-} and NO_3^-

In contrast to cations, the uptake of anions increased with decreasing pH (Figure 1a and b) in the acid-tolerant strain. The uptake of both PO_4^{3-} and

Table 2. Effect of pH and Ni on uptake of cations by the wild-type and acid-tolerant strain of *C. vulgaris*

pH		NH_4^+ uptake ($\mu g\ NH_4^+\ \mu g\ protein^{-1}$)		Na^+ uptake ($\mu g\ Na^+\ mg\ protein^{-1}$)		K^+ uptake ($\mu g\ K^+\ mg\ protein^{-1}$)		Ca^{2+} uptake ($\mu g\ Ca^{2+}\ mg\ protein^{-1}$)	
		wild-type	tolerant strain	wild-type	tolerant strain	wild-type	tolerant strain	wild-type	tolerant strain
6.8	C	1.16 ± 0.04	1.20 ± 0.04	38.21 ± 0.52	27.4 ± 0.48	34.9 ± 0.60	24.9 ± 0.50	32.8 ± 0.48	29.6 ± 0.38
	Ni	0.85 ± 0.04	1.03 ± 0.04	24.37 ± 0.41	19.8 ± 0.39	24.1 ± 0.52	20.44 ± 0.36	18.95 ± 0.44	17.9 ± 0.36
		(27)	(14)	(36)	(28)	(31)	(18)	(42)	(40)
5.0	C	0.93 ± 0.03	1.11 ± 0.03	31.80 ± 0.46	24.05 ± 0.62	30.0 ± 0.39	20.57 ± 0.28	26.4 ± 0.39	18.7 ± 0.50
	Ni	0.36 ± 0.03	0.80 ± 0.03	18.47 ± 0.39	15.55 ± 0.5	18.4 ± 0.41	15.79 ± 0.40	13.4 ± 0.52	10.4 ± 0.45
		(61)	(28)	(42)	(35)	(39)	(23)	(49)	(44)
4.0	C	0.79 ± 0.03	0.99 ± 0.04	28.67 ± 0.56	17.7 ± 0.40	27.6 ± 0.50	18.0 ± 0.32	21.1 ± 0.42	13.1 ± 0.46
	Ni	0.14 ± 0.05	0.65 ± 0.05	13.45 ± 0.40	10.3 ± 0.35	14.8 ± 0.36	13.06 ± 0.50	7.34 ± 0.36	6.79 ± 0.39
		(82)	(34)	(53)	(42)	(46)	(27)	(65)	(48)
3.5	C	0.71 ± 0.04	0.8 ± 0.04	23.76 ± 0.32	13.6 ± 0.28	25.0 ± 0.31	16.2 ± 0.46	17.3 ± 0.35	6.6 ± 0.32
	Ni	0.13 ± 0.03	0.49 ± 0.03	8.13 ± 0.38	7.04 ± 0.40	12.2 ± 0.42	11.35 ± 0.32	3.98 ± 0.44	2.75 ± 0.38
		(89)	(38)	(66)	(48)	(51)	(33)	(77)	(58)

All the values are mean ± SE; data in parentheses denote percent inhibition; '*t*' significant at $P < 0.1$; C = Control, Ni = nickel-treated (2 μg/ml).

NO_3^- was approximately 2-fold higher at pH 3.5 than pH 5.0. However, the wild-type showed a reverse trend. Ni supplementation reduced the uptake of PO_4^{3-} by 30, 43, 50 and 58%, respectively, at pH 6.8, 5.0, 4.0 and 3.5 in the wild-type, whereas in the tolerant strain these reduction percentages were about 27, 38, 41 and 47% at the above pHs. However, after Ni supplementation, the reduction in NO_3^- uptake was about 39, 69, 74 and 86% in the wild-type, compared with 28, 37, 40 and 45% in the tolerant strain in the above pH order.

Nitrate reductase and ATPase

The data given in Figure 1d clearly demonstrate the complete inhibition of nitrate reductase activity of the wild-type *C. vulgaris* at pH 4.0. The tolerant strain showed 8% activity at this pH. However, complete inhibition occurred at pH 3.5. Though Ni toxicity was found to increase with decreasing pH, this was more pronounced in the wild-type. Approximately 2.5- and 8.0-fold increases in ATPase activity were noticed, respectively, at pH 5.0 and 3.5 compared with pH 6.8 in the tolerant strain (see Figure 1c). However, a reverse trend was observed for the wild-type alga.

The kinetics of nitrate reductase (data not shown) and ATPase activity (Figure 2) of both the strains showed a variation in V_{max} and a constant K_m even at varying pH and in the presence of Ni. It is interesting to note that K_m and V_{max} values of ATPase did not change in both strains at pH 6.8. However, a significant increase in reaction rate (V_{max}) was observed in the tolerant strain with decreasing pH. But the reverse was true in the case of the wild-type.

Na^+ and K^+ efflux

A significant difference in Na^+ and K^+ loss was noticed between the wild-type and the tolerant strain at decreasing pH (see Table 3). The loss of Na^+ was 21, 24 and 31% in the wild-type, and 4, 6 and 7% in the tolerant strain, respectively, at pH 5.0, 4.0 and 3.5. Ni supplementation increased the loss to 30, 39, 43 and 49%, respectively, at pH 6.8, 5.0, 4.0 and 3.5 in the tolerant strain. In the wild-type this was 39, 62, 70 and 92% at the above pHs. Efflux of K^+ also followed the similar trend, though the values were low compared with Na^+.

Ni uptake

Table 3 presents the data on Ni uptake by both strains of *C. vulgaris*. Compared with pH 6.8, the wild-type registered about 2.9-, 3.2- and 3.4-fold increases in Ni uptake respectively at pH 5.0, 4.0 and 3.5. Though a similar trend prevailed for the tolerant strain, the values were respectively 67, 58, 34 and 32% lower than the wild-type at pHs 6.8, 5.0, 4.0 and 3.5, respectively.

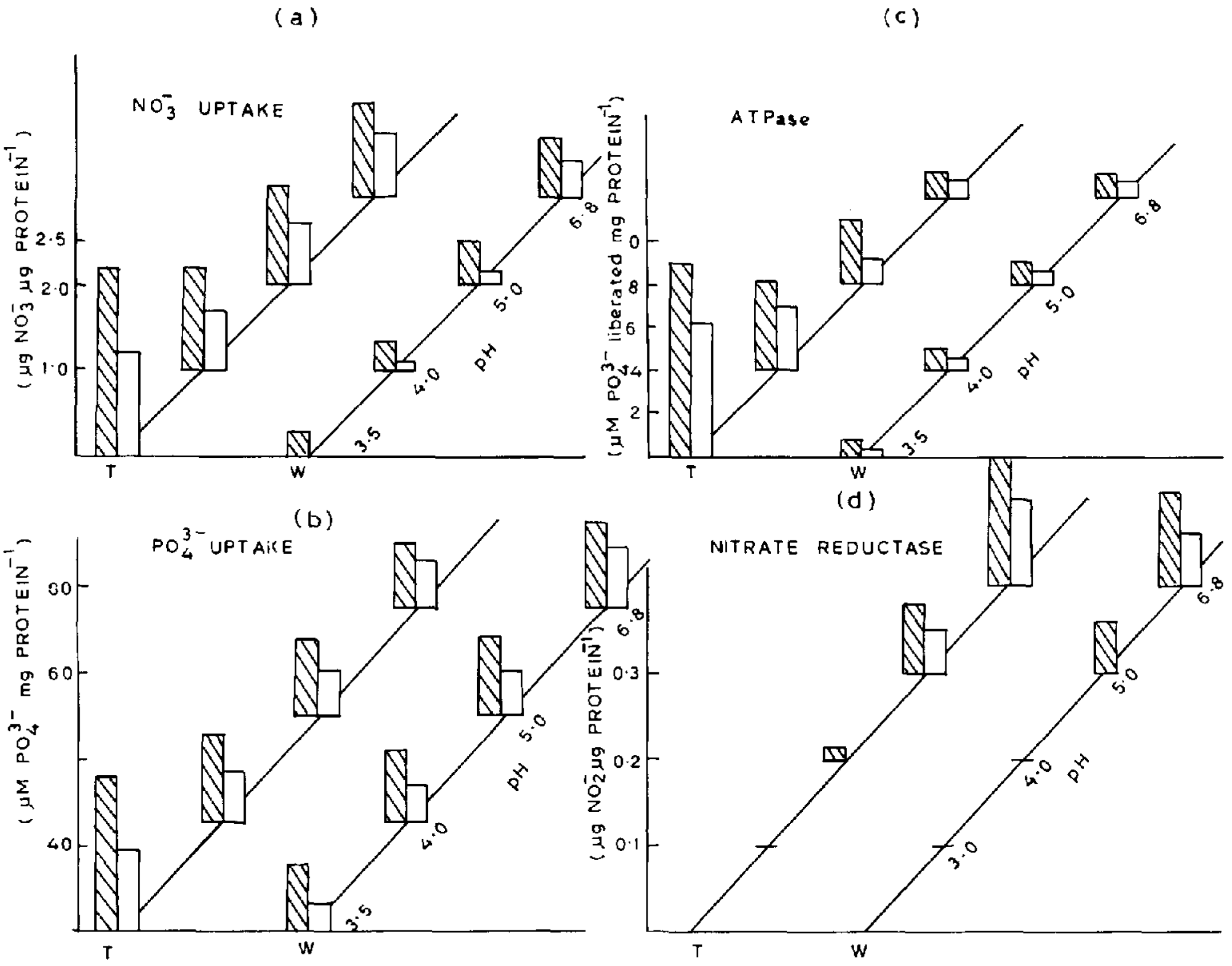

Figure 1. pH-induced effects of Ni on NO_3^- uptake (**a**), PO_4^{3-} uptake (**b**), ATPase (**c**) and nitrate reductase (**d**) activities of the wild-type and the acid-tolerant strain of *C. vulgaris*. ▨, control; ☐, Ni treated; W, wild type; T, tolerant strain.

Table 3. pH induced effect of Ni on Na^+ and K^+ efflux and Ni uptake by wild-type and tolerant strains of *C. vulgaris*

pH		Na^+ efflux (μg Na^+ μg protein^{-1})		K^- efflux (μg K^+ μg protein^{-1})		Nickel uptake (μg Ni μg protein^{-1} × 10^{-1})	
		wild-type 100% 15.2 ± 0.24	tolerant strain 100% = 16.8 ± 0.29	wild-type 100% = 12.32 ± 0.34	tolerant strain 14.4 ± 0.41	wild-type	tolerant strain
6.8	C	—	—	—	—	—	—
	Ni	5.9 ± 0.3	5.03 ± 0.2	2.36 ± 0.52	1.6 ± 0.35	0.09 ± 0.02	0.03 ± 0.02
5.0	C	3.2 ± 0.2	0.69 ± 0.3	2.48 ± 0.41	0.29 ± 0.3	—	—
	Ni	7.4 ± 0.36	6.3 ± 0.42	4.42 ± 0.29	3.53 ± 0.41	0.26 ± 0.02	0.11 ± 0.01
4.0	C	3.6 ± 0.3	1.01 ± 0.4	3.12 ± 0.30	0.29 ± 0.4	—	—
	Ni	8.1 ± 0.4	7.81 ± 0.3	4.73 ± 0.40	4.15 ± 0.38	0.39 ± 0.01	0.19 ± 0.02
3.5	C	4.7 ± 0.2	1.18 ± 0.5	3.49 ± 0.3	0.86 ± 0.5	—	—
	Ni	9.7 ± 0.2	9.31 ± 0.3	5.02 ± 0.5	4.2 ± 0.3	0.31 ± 0.02	0.21 ± 0.02

All the values are mean ± SE.
'*t*' significant at $P < 0.1$.
C – control. Ni = nickel treated (2 μg/ml).

Discussion

This study offers physiological and biochemical evidence to support the earlier observation that acidification of lakes results in decreased species richness and death of algae (Stokes 1983). Michnowicz & Weaks (1984) and Rachlin & Grosso (1991), while working on the effects of pH on metal toxicity in green algae, demonstrated that acidic pH inhibits growth. The present study not only offered support to these earlier findings (see Table 1) but also demonstrated reduction in growth, carbon fixation, O_2 evolution, the electron transport chain (data not shown), and the ATP content of the wild-type and acid-tolerant strain of *C. vulgaris*. Thus growth retardation at decreasing pH appears to be due to the overall inhibition of the metabolic variables of the test alga.

An interesting point that emerged from this study was that the tolerant strain had a better metabolic performance than the wild-type. This suggested that the tolerant strain was physiologically more efficient than the wild-type. Although the wild-type, when challenged by acid pH and metal, became metabolically less active (see Tables 1, 2, and Figures 1, 2) it still registered a higher cation (NH_4^+, Na^+, K^+, Ca^{2+} and Ni^{2+}) uptake compared with the tolerant strain. In such a low metabolic state, a high cation influx could occur due to simple diffusion, where the negatively-charged plasmamembrane of the wild-type would act as an attractive force. Nevertheless, the increased membrane permeability which occurs at decreasing pH (as demonstrated by increased Na^+ and K^+ efflux) (Table 3) could be another factor in this facilitated diffusion.

The opposite occurred in the tolerant strain. The

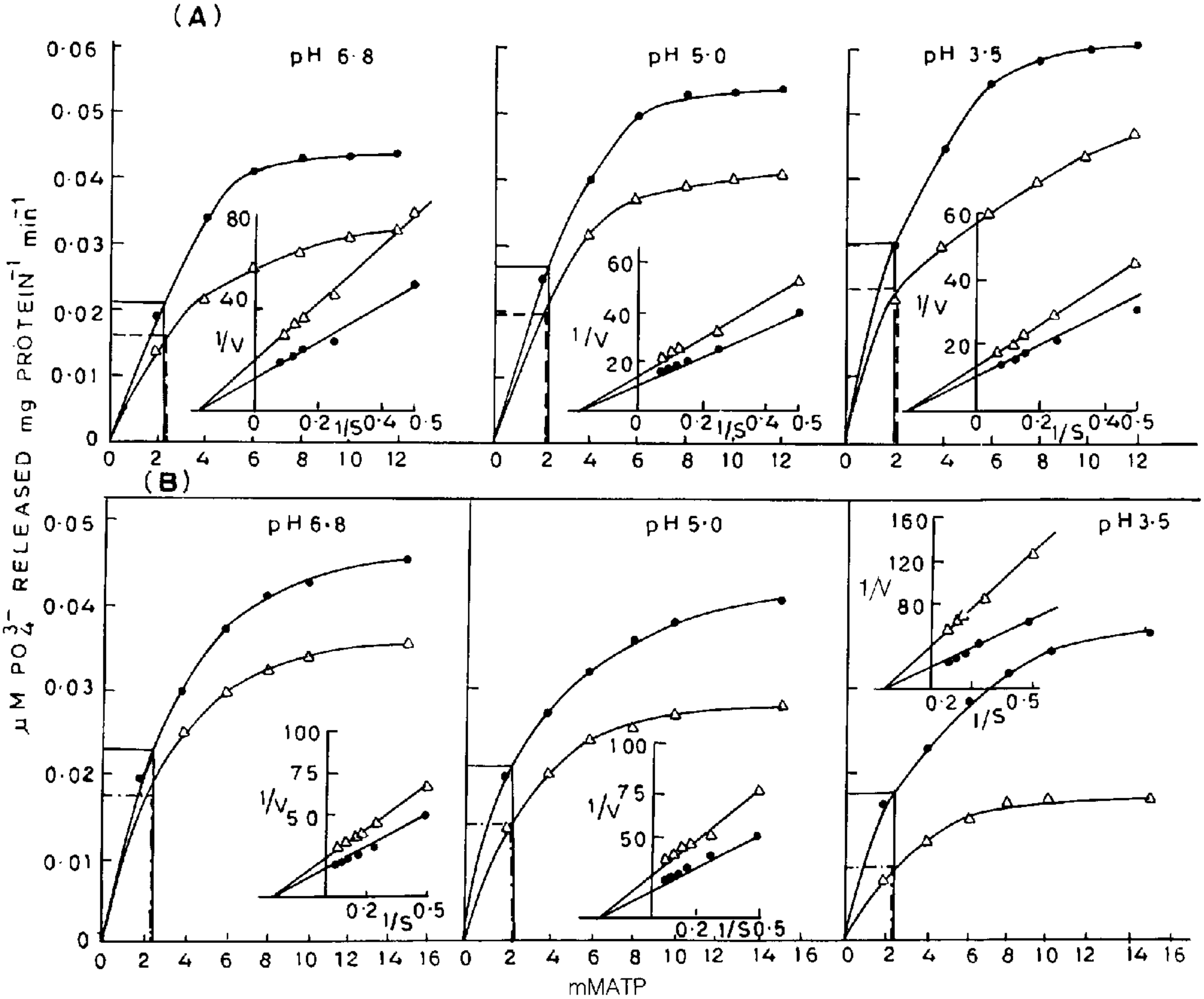

Figure 2. Interactive effect of acid pH and Ni on the kinetics of ATPase activity of acid-tolerant (**A**) and wild-type (**B**) *C. vulgaris*. ●, control; △, Ni treated; $1/S$ = mM ATP; $1/V$ = μM PO_4^{3-} mg protein^{-1} min^{-1}.

general reduction in cation uptake in the tolerant strain might indicate the development of a positive membrane potential (as known for *Dunaliella acidophila*, see Gimmler *et al.* 1989) which may act as a repelling force for cations, thus decreasing their uptake. Under such circumstances the anion uptake should be promoted. Thus the observed increase of NO_3^-, PO_4^{3-} and $H^{14}CO_3^-$ uptake (Table 1 and Figure 1) at decreasing pH not only supported the development of a positive membrane potential but prompted us to believe that anions might play a significant role in the charge balance (H^+ and anions) and in regulating the internal pH of the acid-tolerant strain. It is interesting that an approximately 2-fold increase in NO_3^- uptake was noticed at pH 3.5 when nitrate reductase activity was completely inhibited (Figure 1). This increased uptake of NO_3, even in the absence of its assimilatory enzyme, gives further support to the contention that anions play a crucial role in bringing the internal pH to neutrality.

The plasma membrane H^+-ATPase is known to play a significant role in many life functions of plant cells by mediating ATP-dependent H^+ extrusion to the cell exterior (Briskin & Hanson 1992). A tremendous increase in the ATPase activity of the acid-tolerant *C. vulgaris* (about 2.5- and 8-fold, respectively, at pH 5.0 and 3.5, compared with pH 6.8, see Figure 1) supports the findings of Enami *et al.* (1986) and Sekler *et al.* (1991), who reported that H^+ extrusion in acid-tolerant *Cyanidium caldarium* and *D. acidophila* was brought about by the plasmamembrane ATPase.

The kinetic study of ATPase (Figure 2) further demonstrated that the enhanced efficiency was due to an increased reaction velocity of ATPase in the tolerant strain at decreasing pH. Surprisingly, the wild-type and the tolerant strain did not exhibit any significant difference in ATPase activity at pH 6.8.

This suggests that the ATPase of acid-tolerant *C. vulgaris* was activated/stimulated only at acid pHs. Thus, a highly efficient ATPase together with a positive membrane potential and a facilitated anion uptake enabled the test alga to survive and grow at pH 3.5 (Table 1). The complete failure of the wild-type to survive at pHs 4.0 and 3.5 could be due to its inability to regulate ion transport and probably also the internal pH. Since a positive membrane potential hinders the entry of cations into a cell, a low cation pool will be expected in that strain. However, the high intracellular Na^+ and K^+ pool in the acid-tolerant strain indicates the existence of K^+/Na^+, H^+ co-transport, as reported for acidophilic bacteria (Bakker 1990) and the green alga (Carandang *et al.* 1992).

A comparison of the nickel toxicity in both strains demonstrated that all the physiological and biochemical processes of the acid-tolerant strain were less affected by nickel than the wild-type alga. This is not only due to the reduced uptake of nickel in the tolerant strain (see Table 3) but also to the decrease in membrane permeability, and conceivably the membrane potential. The decrease in permeability could also be due to an increased lipid content (15% in the tolerant strain and 10% in the wild-type). These observations led us to conclude that the positive membrane potential, coupled with facilitated anion influx and the superactive ATPase, are not only responsible for acid tolerance in algae but also offer co-tolerance to metals.

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