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Manifestations and environmental implications of microbially-induced calcium carbonate precipitation (MICP) by the cyanobacterium *Dolichospermum flosaquae*

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Abstract:

The aim of this work is to explore the ability and magnitude of the temperate cyanobacterium *Dolichospermum flosaquae* in MICP (microbially-induced calcium carbonate precipitation). Environmentally, MICP controls the availability of calcium, carbon and phosphorus in freshwater lakes and simultaneously controls carbon exchange with the atmosphere. Cultures of *flosaquae* were grown in BG11 medium containing 0, 1, 1.5, 2 and 4 mg Ca^{2+} L^{-1}, as cardinal concentrations previously reported in freshwater lakes, in addition to a control culture (BG11 containing 13 mg Ca^{2+} L^{-1}). Growth (cell number, chlorophyll a, and protein content) of *D. flosaquae* was generally reduced by elevating calcium concentrations of the different salts used (chloride, acetate, or citrate). *D. flosaquae* seems able to perform MICP as carbonate alkalinity was sharply induced up to its highest level (six times that of the control) at a citrate concentration of 4 mg Ca^{2+} L^{-1}. Calcium carbonate was formed at a pre-precipitation stage as the minimum pH necessary for precipitation (8.7) has been scarcely approached under such conditions. MICP took place mostly relying on photosynthesis and respiration, but not on urease activity, as urea was not supplemented in the growth media. However, *D. flosaquae* exhibited strong urease specific activity in *in vitro* assays (four times that of the control at 4 mg Ca^{2+} citrate L^{-1}). Residual calcium exhibited its lowest value at 4 mg Ca^{2+} citrate L^{-1}, coinciding with the highest alkalinity level. Consumed calcium was increasing with chlorophyll a

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content, but not with increasing cell numbers. The experiments should be repeated in a future study, but in the presence of urea, to evaluate the full potential of calcium carbonate precipitation of *D. flosaquae*, its dynamics and impact on biogeochemical cycles of calcium, carbon and phosphorus in freshwater lakes.

Key Words:

Dolichospermum flosaquae – MICP - Photosynthesis – Respiration – Urease – Alkalinity – Calcium

Introduction:

Microbially-induced calcium carbonate precipitation (MICP) depicts an exogenous or endogenous microbial activity that takes place during heterotrophic growth of numerous fungi and bacteria or during photoautotrophic growth of cyanobacteria in their natural environments including water, soils, tufas, biofilms or geological formations. Furthermore, bacterial, and cyanobacterial mucilaginous sheath (capsular polysaccharides or exopolysaccharides) as well as fungal chitin act as nucleation sites for $CaCO_3$ crystallization by binding Ca^{2+} onto their carboxylic groups. MICP requires sufficient Ca^{2+}, an alkaline pH and suitable microorganisms. Availability of nucleation sites is very important for stable and continuous calcium carbonate bio-mineralization (**Phillips** et al 2013). In particular, cyanobacteria are active prokaryotes performing MICP (**Payandi**-Rolland 2019; **Xu** et al 2019). Several metabolic processes such as photosynthesis, respiration, sulfate, nitrate or sulfide reduction have been recorded as driving mechanisms for MICP (e.g. **Seifan** et al 2019). However, ureolytic activity has been also found as a major mechanism catalyzing MICP via CO_2 and ammonia production during urea hydrolysis. **Castro**-Alonso et al (2019) reported on a series of complex reactions of urease, calcium, and carbonate during MICP surrounding the cells. Ca^{2+}, beside being a component of $CaCO_3$, is inductive for urease activity resulting in a pronounced upregulation. Furthermore, morphology of the calcite crystal is strain-specific **(Hammes** et al 2003) and depends on the type of the calcium salt present (**Achal** and Pan 2014).

Subsequent to precipitation of calcium carbonate, the bioavailability of both calcium and phosphorus as well as CO_2 are lowered in lakes; which, in turn, arises as rate-limiting to growth and nutrition of aquatic microbiota, e.g. via photosynthetic activity in the case of cyanobacteria. This metabolic process is widely explored and regarded as

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a promising phenomenon for use in various industrial applications. In addition to the governmental control of acid deposition into lakes, MICP may be responsible (at least in part) for the widespread threat of calcium decline in freshwater lakes around the globe, as calcium co-precipitates simultaneously with carbonate (**Jeziorski** and Smol 2017). After studying thousands of water samples in tens of countries (spatially and temporally), **Weyhenmeyer** et al (2019) concluded that the global median calcium concentration was 4.0 mg L^{-1} with 20.7% of the water samples showing Ca^{2+} concentrations ≤ 1.5 mg L^{-1}, a threshold considered critical for the survival of many Ca^{2+} dependent organisms.

The hypothesis of this work is to explore whether *Dolichospermum flosaquae*, a major temperate cyanobacterium, is able to perform MICP in freshwater lakes. Dependence of MICP magnitude on Ca^{2+} concentration and salt type of chloride, acetate or citrate as well as the powering metabolic process are also concerned within this study. Photosynthesis, respiration, total alkalinity and urease activity of *D. flosaquae* are measurables assessed to elucidate their role in mediating MICP and to detect the effect of the applied treatments. The results obtained are discussed on the lights of their anticipated environmental impact and implications.

Materials and Methods:

Experimental Set up:

Cultures of the cyanobacterium *D. flosaquae* were incubated at different Ca^{2+} concentrations (0, 1, 1.5, 2 and 4 mg Ca^{2+} L^{-1}) of different salts (chloride “Cl”, acetate “Ac”, or citrate “Cit”) supplemented into calcium free BG11 medium specific for cyanobacteria (**Stanier** et al 1971). *D. flosaquae* was also grown in full BG11 medium (containing 13 mg Ca^{2+} L^{-1}, which is considered the control culture “Con+”) in addition to a reference calcium-deprived culture (BG11 devoid of any supplemental calcium “Con-”). Culture media were inoculated with 10 ml of 5 days old cells of *D. flosaquae* in conical flasks capped with aluminum foil. Cultures were shaken for 4 weeks at 22±1 °C and white light intensity of 25 µmole m^{-2} sec^{-1} (14h light:10h dark cycle).

Analytical Methods:

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96 At the end of the experiment, i.e. after growth for 4 weeks, the following parameters of
97 the variously treated *D. flosaquae* cultures were analyzed and assessed as follows:

98 - Cell number and chlorophyll a were simultaneously assessed using a YSI-
99 multiparameter probe.
100 - Protein contents were estimated according to the method of **Bradford** (1976). Cells
101 were extracted in boiled water, centrifuged and proteins were assessed in the
102 supernatant The binding of protein molecules with the Coomassie Brilliant Blue dye
103 under acidic conditions results in a color change from brown to blue, measured at a
104 wavelength of 595 nm using a BioTek Synergy 2, multidetector microplate reader
105 (Vermont, USA).
106
107 - Total alkalinity was assessed by titration of 50 mL algal culture media with 0.1M HCl
108 following **Choi** et al (2017) and **Xu** et al (2019), and then calculated using the
109 following equation:

110 $$CaCO_3 + 2HCl \rightarrow CaCl_2 + H_2O + CO_2$$

111 Based on the reaction stoichiometry between $CaCO_3$ and HCl, the mole ratio of $CaCO_3$
112 to HCl is 1:2; by dividing the number of moles of HCl by 2, the product is the number
113 of moles of $CaCO_3$. The number of moles of $CaCO_3$ would be multiplied with its
114 molecular weight to get the yielded respective $CaCO_3$ mass.

115 - Photosynthetic activity: The light-induced O_2 evolution by *D. flosaquae* in different
116 cultures was followed by means of an oxygen sensor (PreSens MicroXTX3O_2
117 sensor, SoftwareTx3v6O_2, Presens, Germany) at the same growth conditions (white
118 light intensity of about 25 µmole m^{-2} sec^{-1} at room temperature, i.e. 22±1ºC).
119 Respiration (O_2 uptake) was also monitored using the same oxygen sensor, but in
120 the dark.
121
122 - Assessment of residual free [Ca^{2+}] in the growth media: At the end of the experiment,
123 calcium was assayed by calcium kits (ab102505, Calcium Detection Assay Kit-
124 colorimetric, abcam) and determined at a wave length of 575 nm using a BioTek
125 Synergy 2, multidetector microplate reader (Vermont, USA). Consumed calcium was
126 then calculated by subtracting residual from total calcium.
127

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- Urease enzyme (UE) activity was assayed spectrophotometrically following the procedure of **Mobley** et al (1988) and quantified using a calibration curve of ammonia. The in vitro assay mixture of UE contained intact cells of *D. flosaquae*, urea (200 mM), phenol red (7 μg mL^{-1}) and phosphate buffer (pH 6.8). After 10 min, the developed color, as a result of liberated ammonia from urea hydrolysis, was determined at a wavelength of 500 nm using the same microplate reader (as described above).

- Ammonia accumulated in the different culture media at the end of the experimental period was assessed as mentioned above in urease-liberated ammonia.

- The pH values of the differently treated cultures were determined via a pH meter (WTW3301, Germany).

All experiments and assessments were conducted in triplicates and the mean values ± SE (standard error) are presented in the figures.

Results:

Under culture conditions, growth indices (cell number, chlorophyll - and protein contents) of *D. flosaquae* were variably affected in response to calcium concentration as well as to its counter anion (chloride, acetate, or citrate). Growth of *D. flosaquae* decreased as calcium concentrations of all salts were lowered, following a relative growth enhancement (higher than the control) at a threshold value of 1.5-2.0 mg Ca^{2+} L^{-1} of the calcium salts citrate and acetate, respectively, while continually lowered by calcium chloride. Calcium-deprived cultures (Co-) exhibited markedly lower growth rates than Ca^{2+} supplemented ones (Co+) in terms of cell number, chlorophyll a and protein contents (Fig. 1).

The pH of 7.0 was set for all *D. flosaquae* cultures at the beginning of the experiment; thereafter, it was elevated to levels ranging between pH 8.0 - 8.7, depending on calcium treatment (Fig. 2). A certain calcium concentration of each salt induced a higher pH than control or calcium-deprived cultures. The highest pH elevation (up to 8.7) occurred at calcium chloride and calcium citrate concentrations of 1 mg Ca^{2+} L^{-1}

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but decreased at higher concentrations. However, Ca^{2+} acetate resulted in the highest pH elevation of 8.7 at the highest added concentration (4 mg Ca^{2+} L^{-1}). Accordingly, the pH elevation depended on the calcium concentrations and type of Ca^{2+} salt (chloride, acetate or citrate).

Fig. (3) presents the net photosynthetic oxygen evolution (P_N) and dark respiratory oxygen uptake (R_D) of *D. flosaquae* in dependence on the imposed calcium treatments. P_N was severely inhibited in calcium deprived cultures of *D. flosaquae* relative to control cultures while R_D was enhanced. Different calcium salts exerted different impacts, but in most calcium treatments, net photosynthetic oxygen evolution was higher than in the control cultures. In calcium chloride and acetate treated cultures of *D. flosaquae*, P_N and R_D enhanced with increased concentrations of calcium while in citrate treated *D. flosaquae* cultures, both P_N and R_D decreased. Photosynthesis:respiration (P_N:R_D) ratios, which represent the net productivity of cells or cultures, were severely inhibited by calcium deprivation while calcium chloride, acetate and citrate induced inhibition or stimulation of P_N:R_D, depending on the calcium concentration.

Total alkalinity (T alkalinity), ammonia as well as corrected carbonate alkalinity values (C alkalinity), calculated by subtracting ammonia concentration from total alkalinity of the differently treated *D. flosaquae* cultures, are shown in Fig. (4). Total alkalinity exhibited its absolutely lowest amount in the control culture of *D. flosaquae*, despite it contained the highest Ca^{2+} concentration (13 mg Ca^{2+} L^{-1}) while calcium deprivation remarkably enhanced alkalinity up to three times that of the control cultures (5 to 15 mmol carbonate µg Chl^{-1}). Furthermore, alkalinity level in any of the calcium treated cultures was markedly higher than that of control or calcium-deprived cultures, with a maximum alkalinity level at calcium citrate concentration of 4 mg Ca^{2+} L^{-1}. All calcium acetate concentrations induced more or less similar alkalinity levels whereas calcium chloride induced its highest stimulation at 2 mg Ca^{2+} L^{-1}. As ammonia may interfere with carbonate alkalinity, ammonia has been assessed and detected in trace amounts not affecting total alkalinity (Fig. 4).

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Residual calcium was assessed while total and consumed fractions were calculated (per mL culture and per unit chlorophyll) and presented in Fig. (5a&b); consumed calcium means its incorporation into or precipitation as calcium carbonate. It is important to mention that in calcium-deprived cultures, i.e. without any external supplementation, calcium concentration was still 2.26 mg Ca^{2+} L^{-1}, nevertheless. This amount might have been released from cellular apoplastic regions as well as from intracellular stores. Therefore, a virtual concentration of total calcium is given to account for the externally supplemented concentration of calcium (0, 1, 1.5, 2 or 4 mg Ca^{2+} L^{-1}) and the amount of calcium found at calcium-deprivation (i.e. 2.26 mg Ca^{2+} L^{-1}), which was assumed to be equally released by each culture. Control cultures displayed the highest levels of all calcium fractions as they started at the highest total virtual concentration of 15.26 mg Ca^{2+} L^{-1} (i.e. 13 mg Ca^{2+} L^{-1} in BG11 plus 2.26 mg Ca^{2+} L^{-1} released). On the contrary, calcium-deprived cultures exhibited the lowest levels of all calcium fractions since no calcium had been added and thus the released calcium was the only calcium resource.

Residual calcium (in the culture media) and consumed calcium (per unit chlorophyll and per unit volume) increased with elevated calcium additions (Fig 5a). The lowest amounts of residual calcium were recorded in citrate treated cultures (almost equal to the consumed fraction and about 50% of total calcium). The concentration of 4 mg citrate L^{-1} enhanced the calcium consumption nearly up to that of the control despite the big difference in the externally supplemented calcium concentration (4 vs. 13 mg Ca^{2+} L^{-1}, respectively). In chloride and acetate, residual calcium was considerably higher indicating less incorporation into calcium carbonate. Consumed calcium per unit chlorophyll (C/Chl) was increasing with increasing supplemented calcium concentration; the highest enhancement was recorded at citrate (Fig 5b).

Urease enzyme (UE) activity is presented in Fig (6); specific activity “SA” represents the rate of enzyme activity as µmole ammonia released. μg^{-1} protein. min^{-1} while total activity “TA” represents the rate of enzyme activity as µmole ammonia released. mL^{-1} algal suspension. min^{-1}. Total activity is the product of specific activity per µg protein multiplied by the amount of protein per unit volume (mL) of algal cultures. Calcium deprivation inhibited UE activity; the magnitude of inhibition on a volume basis “TA”

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was more pronounced than the enzyme specific activity "SA" because enzyme (protein) contents were also lower. Calcium chloride induced the highest rates of UE, total and specific activity, at 1.5 mg Ca^{2+} L^{-1}; otherwise, it was inhibitory at lower or higher concentrations. Calcium acetate induced the highest rates of "TA" and "SA" at moderate concentrations of 1.5 and 2 mg Ca^{2+} L-1, both lowest and highest concentrations of 1 and 4 mg Ca^{2+} L^{-1} severely inhibited the enzyme activity. Calcium citrate induced a continuous increase in urease activity (SA) up to its "absolutely" highest rate at 4 mg Ca^{2+} L^{-1} among other concentrations and salts; such highest rate of urease activity was in accordance with the highest level of calcium consumption i.e. calcium may be inductive to urease activity in *D. flosaquae*. The order of UE enhancement was as follows citrate > acetate > chloride.

Discussion:

Our results indicate that *Dolichospermum flosaquae* is able to perform MICP (microbially-induced calcium carbonate precipitation). Therefore, the intensive blooms of this organism have the potential to control the overall biogeochemistry dynamics in freshwater bodies, i.e. controlling the availability of calcium, carbon, and phosphorus in addition to carbon emissions into the atmosphere. In this work, the capability of the cyanobacterium *D. flosaquae* in freshwater MICP was studied at different concentrations of three calcium salts (chloride, acetate, and citrate). It proved that different salt types and calcium concentrations exerted different impacts on *D. flosaquae* growth and metabolism. The studied concentrations (0, 1, 1.5, 2 and 4 mg Ca^{2+} L^{-1}) are critical depending on previous records in the literature. In this concert, **Weyhenmeyer** et al (2019) reported that the global median calcium concentration was 4.0 mg L^{-1} with 20.7% of the water samples showing Ca^{2+} concentrations of ≤ 1.5 mg L^{-1}, a threshold considered critical for the survival of many organisms. Growth of *D. flosaquae* in terms of cell number, protein – and chlorophyll a content, was inhibited by calcium deprivation as well as by higher concentrations of calcium. However, concentrations of only 1.5 mg Ca^{2+} L^{-1} of acetate and citrate were stimulatory for *D. flosaquae* growth.

D. flosaquae seems able to perform MICP, as inferred from alkalinity levels in the growth media, elevated pH values, and residual vs. consumed calcium levels.

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However, MICP occurred but at a pre-precipitation stage since no precipitation has been seen by naked eyes, due to the inability of the organism to surpass the minimum pH threshold under our experimental conditions of inactive urease due to absence of urea (discussed later). Therefore, ammonia concentrations were found to be marginal in the culture media. Its interference with carbonate alkalinity can be thus ruled out indicating that the assessed alkalinity levels are substantially carbonate alkalinity. Carbonate alkalinity exhibited the lowest levels at control cultures but increased via calcium deprivation. However, it was induced up to its maximum level (six times that of the control) by the highest calcium concentration of the citrate salt (4 mg Ca^{2+} L^{-1}). This notion suggests that the capacity of *D. flosaquae* for carbonate formation depends on the salt type as well as on the Ca^{2+} concentration. In this respect, calcium chloride has been recorded to be the best salt for the production of calcite by *Bacillus* sp. among several other calcium sources used (**Achal** and Pan 2014). In this work, however, calcium citrate apparently fits the studied organism more than chloride or acetate.

Alkaline pH is a prerequisite for calcium carbonate formation and stability. Most calcite precipitation occurs under alkaline conditions of pH 8.7 to 9.5 **(Ferris** et al 2003; **Dupraz** et al 2009). When pH levels decrease, carbonates tend to dissolve rather than precipitate **(Loewenthal** and Marais 1982). *D. flosaquae* exhibited a continuous ability of elevating the pH of the culture medium to high pH values, which in turn, may have caused a slow and long lag phase of growth, but favorable conditions for MICP. However, as long as the pH of the cultures did not surpass 8.7, i.e. the lowest pH for precipitation, carbonate has been formed but did not precipitate (see references above: **Loewenthal** and Marais 1982; **Ferris** et al 2003; **Dupraz** et al 2009; **Gebauer** et al 2010).

Amongst the multiple microbial metabolic activities described in the literature to support MICP (e.g. **Anbu** et al 2016), that of *D. flosaquae* mostly relied on photosynthesis and respiration under conditions of this work. Urease activity, the most universal metabolic process powering MICP, is unlikely in this case, as the growth media was not supplemented with urea. UE activity is a potentially major source of ammonia (shifting the pH around the cell to the alkaline side) and CO_2 (transforming into calcium carbonate precipitates).

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287 UE activity is a potentially major source of ammonia (shifting the pH around the cell to
288 the alkaline side); meanwhile CO_2 and Ca^{2+} transform into calcium carbonate
289 precipitates. Urea hydrolysis via UE activity is not complex (**Hammes** et al 2003;
290 **Achal** et al 2011; **Stabnikov** et al 2013). However, the high ability of *D. flosaquae* to
291 shift the pH to alkalinity, without urea being included in the culture medium, indicates
292 sources of alkalinity other than the urease-dependent ammonia production, i.e.
293 photosynthesis and respiration in our case. In this context, aerobic bacteria release
294 CO_2 via cell respiration, which is paralleled by an increase in pH due to ammonia
295 production (**Ng** et al 2012). **Hamilton** et al (2009) stated that lakes in carbonate-rich
296 watersheds commonly precipitate calcium carbonate as calcite; this is accelerated by
297 photosynthetic uptake of carbon dioxide, elevating the pH to 9–10 and reducing
298 concentrations of calcium and alkalinity by up to 60%. However, urea hydrolytic strains
299 showed higher calcite precipitation (~20–80%) in comparison with other metabolic
300 pathways (**Achal** et al 2009). **Okwadha** and Li (2010) reported that the amount of
301 $CaCO_3$ precipitation depends more on Ca_2^+ concentrations than urea concentrations.
302 The *in* vitro assay of UE (EC 3.5.1.5), per se, was also affected by calcium
303 concentration and salt type, i.e. it was inhibited by calcium deprivation while it exhibited
304 maxima at 1.5, 2 and 4 mg Ca^{2+} L^{-1} for chloride, acetate, and citrate, respectively.
305 Calcium induces UE activity; **Hammes** et al (2003) found that UE activity increased by
306 tenfold in the presence of 30 mM Ca^{2+} relative to its absence. UE activity is related to
307 cell (**Ng** et al 2012), urea and calcium concentrations (**De Muynck** et al 2010), and
308 high pH (**Jones** et al 1982)**.** In addition, UE is only active at high pH values specific
309 for urea hydrolysis. It has been reported that the optimum pH for UE is 8.0, above
310 which the enzyme activity decreases (**Stocks**-Fischer et al 1999; **Gorospe** et al 2013).
311 In this work, the results indicate that UE activity of *Dolichospermum flosaquae* was
312 enhanced due to an increase in specific activity of the enzyme rather than to higher
313 cell numbers or biomass. Urease and carbonic anhydrase expression and activities
314 are genetically and synergistically co-regulated for MICP (**Dhami** et al 2014; **Castro**-
315 Alonso et al 2019).

316 Residual calcium was the least in citrate-treated cultures, compared with other salts
317 (chloride or acetate). At 4 mg Ca^{2+} L^{-1} of citrate, in particular, the lowest residual Ca^{2+}
318 level coincided with the highest alkalinity level, indicating its transformation to calcium
319 carbonate. Consumed calcium per unit chlorophyll a was increased to its highest level

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also at 4 mg Ca^{2+} L^{-1} of citrate treated cultures. Actually, Ca^{2+} is not likely utilized by metabolic processes, but accumulates outside the cells where it is readily available for $CaCO_3$ precipitation (**Silver** et al 1975). In this work, however, the unique and superior stimulating effect of 4 mg Ca^{2+} L^{-1} calcium citrate compared with other salts (chloride or acetate) implies intracellular intervention of calcium ions as well as the accompanying anion in the intracellular metabolism. In this respect, citrate may serve as a carbon source and internal buffer.

Conclusions:

- *D. flosaquae*, a major representative of temperate freshwater cyanobacteria, contributes to the microbially-induced calcium carbonate precipitation (MICP) with pronounced consequences for Ca^{2+} availability in freshwater lakes as well as carbon emissions to the atmosphere.
- Carbonate was formed but did not precipitate, as the organism could not increase the pH of the cultures beyond 8.7, which is considered the minimum pH value for calcite precipitation. Although it is not a precipitate, the formed calcium carbonate proves CO_2 and calcium sequestration.
- The mechanism(s) powering MICP seem to be photosynthesis and respiration without the participation of urease activity (as urea was not supplemented). However, UE activity elucidated a strong activity at our in vitro assays, which might maximally operate for MICP in cases of urea supplementation.
- Calcium citrate, particularly at 4 mg Ca^{2+} L^{-1} was the most inductive for MICP. For the emerging MICP-dependent technologies, it is therefore, recommended to apply calcium citrate because it shows outstanding enhancement of the process.
- The results can be used in modelling the environmental implications of MICP for biogeochemical cycles of calcium, carbon and phosphorus in freshwater lakes.

Figure legends:

Figure (1): Growth (cell number (10^3 cells mL^{-1}), chlorophyll ($x10^{-3}$ µg mL^{-1}) and protein contents (µg mL^{-1}) of the cyanobacterium *Dolichospermum flosaquae* as influenced

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by various calcium treatments: Control (B), 0, 1, 1.5, 2 and 4 mM of calcium chloride (C), calcium acetate (A) and calcium citrate (Ct). Control cultures were grown in BG11 medium containing 13 mg Ca^{2+} L^{-1} (chloride), 0 is calcium deprived, i.e. not supplemented with any external calcium.

Figure (2): pH changes of the cyanobacterium *Dolichospermum flosaquae* as influenced by calcium treatments (as in figure 1).

Figure (3): Photosynthesis and respiration rates of the cyanobacterium *Dolichospermum flosaquae* as influenced by calcium treatments (as in figure 1).

Figure (4): Total alkalinity (carbonate and ammonia as mmol. µg Chl^{-1}) of the cyanobacterium *Dolichospermum flosaquae* as influenced by calcium treatments (as in figure 1).

Figure (5): Residual (mg L^{-1}), total (mg L^{-1}) and consumed calcium (mg L^{-1} or µg µg Chl a^{-1}) of the cyanobacterium *Dolichospermum flosaquae* as influenced by calcium treatments (as in figure 1).

Figure (6): Urease activity, T (total) and SA (Specific Activity) of the cyanobacterium *Dolichospermum flosaquae* as influenced by calcium treatments (as in figure 1).

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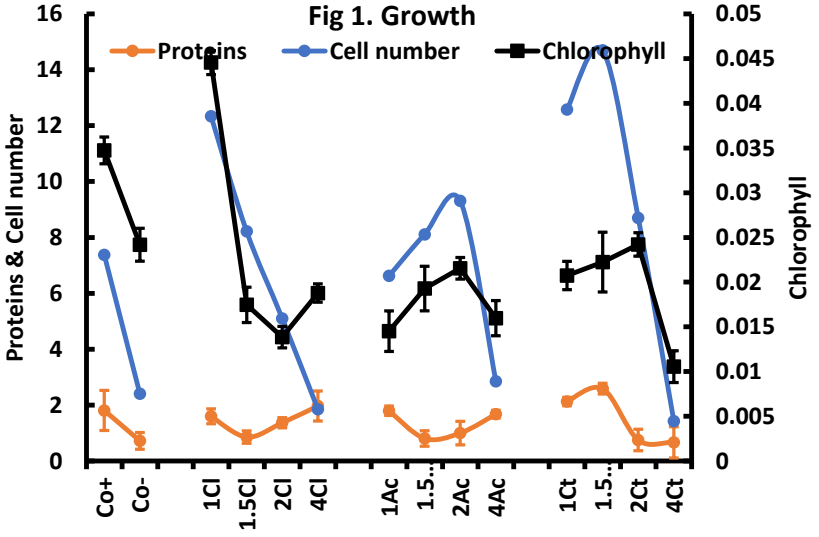
The first author (R. A.-B.), designed the work, implemented the experiments and wrote the drafts, the second author (E.A.H.), helped in the experiments and calculated the standard errors, the third author (H.P. G.) hosted the first two authors in his lab in IGB and revised the manuscript.

Competing interests

There are no competing interests among authors.



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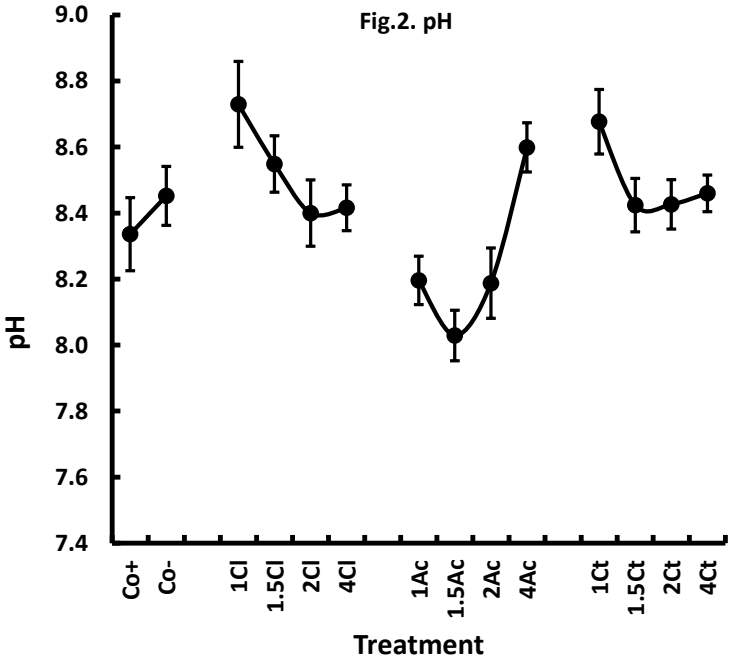

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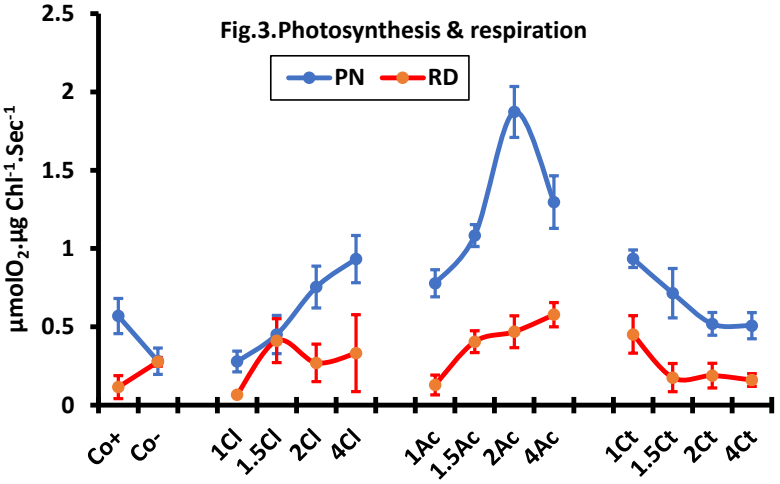

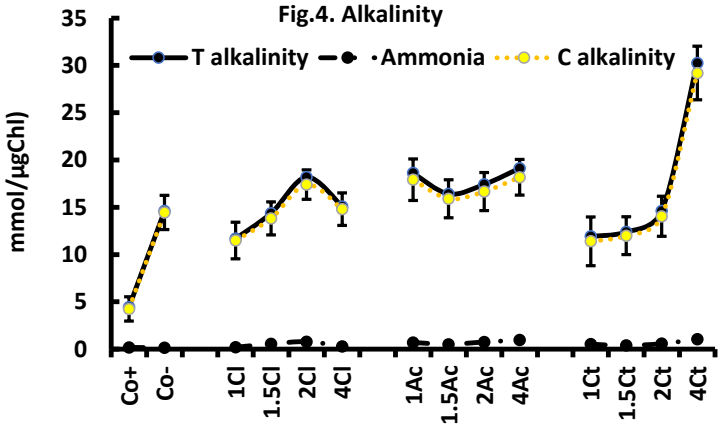

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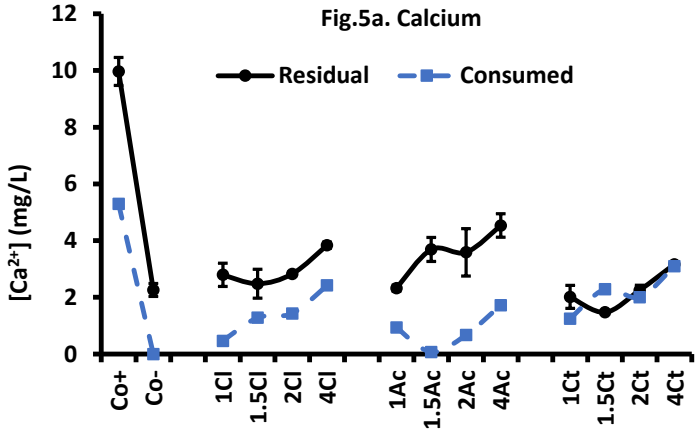

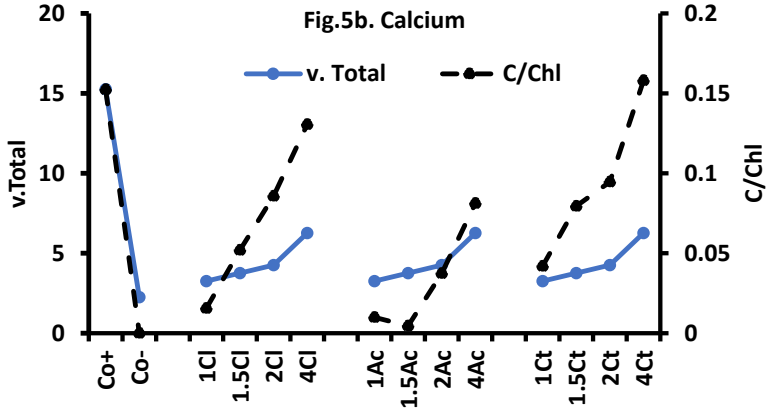

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