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	Address	Matunga, 400 019, Mumbai, India
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	Suffix	
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	Organization	Ramnarain Ruia College
	Address	Matunga, 400 019, Mumbai, India
	Email	yash.v.gupte@gmail.com
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	Particle	
	Given Name	**Seema**
	Suffix	
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	Given Name	**Vinod**
	Suffix	
	Division	Department of Life Science
	Organization	Ramnarain Ruia College
	Address	Matunga, 400 019, Mumbai, India
	Email	

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Abstract

Green algae, *Coelastrella oocystiformis* and *Chlorolobion braunii* were subjected to varying concentrations of potassium (200, 400, 600, 800, 1000 and 2000 ppm). These two algal forms were selected so as to also check if shape of algal forms plays a role in their ability to absorb potassium. The cultures were kept at 5000 lux for a period of 30 days to check their stability for potassium absorption capacity. The algal biomass was harvested, dried and converted to ash for estimation of potassium using method described by Jacobs and Hoffman (J Biol Chem 93: 685–691, 1931). *C. oocystiformis* has highest ash content of 30.5 % when exposed to 400 ppm of K^+ and at this concentration it could take up to 74.8 % of total potassium it was exposed to *C. braunii* has ash content of 37.5 % when exposed to 1000 ppm of K^+, but has potassium content of 12.35 % when exposed to 1800 ppm of K^+. *Chlorolobion braunii*, however, has greater potassium uptake of 40.66 % when exposed to 400 ppm of K^+.

Keywords (separated by '-') *Coelastrella* sp. - *Chlorolobion braunii* - Biofertilizer - Potassium

Footnote Information

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SHORT COMMUNICATION

Uptake of potassium by algae and potential use as biofertilizer

Ganesh Iyer[1] · Yash Gupte[1] · Seema Menon[1] · Palak Vaval[1] · Vinod Nagle[1]

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Abstract Green algae, *Coelastrella oocystiformis* and *Chlorolobion braunii* were subjected to varying concentrations of potassium (200, 400, 600, 800, 1000 and 2000 ppm). These two algal forms were selected so as to also check if shape of algal forms plays a role in their ability to absorb potassium. The cultures were kept at 5000 lux for a period of 30 days to check their stability for potassium absorption capacity. The algal biomass was harvested, dried and converted to ash for estimation of potassium using method described by Jacobs and Hoffman (J Biol Chem 93: 685–691, 1931). *C. oocystiformis* has highest ash content of 30.5 % when exposed to 400 ppm of K^+ and at this concentration it could take up to 74.8 % of total potassium it was exposed to *C. braunii* has ash content of 37.5 % when exposed to 1000 ppm of K^+, but has potassium content of 12.35 % when exposed to 1800 ppm of K^+. *Chlorolobion braunii*, however, has greater potassium uptake of 40.66 % when exposed to 400 ppm of K^+.

Keywords *Coelastrella* sp. · *Chlorolobion braunii* · Biofertilizer · Potassium

Potassium is found widely distributed throughout the Earth's crust. It is the seventh most abundant element in the world. It is not only found in every living plant and animal, but is also found in rocks, minerals, soils, lakes, rivers and oceans. (Armstrong 1998). Potassium is one of the four cations, viz., Ca^{2+}, Mg^{2+}, Na^{2+} and K^+ that account for most of the total cationic concentration in natural waters. Potassium contents vary from 0.8 to 5.1 mg l^{-1} in the wet seasons and 1.5–6.2 mg l^{-1} in the dry seasons in Jia-Bharali River Basin, North Brahmaputra (Khound et al. 2012). Potassium was found to range from 50.5 to 178 mg l^{-1} during a study carried out by Dubey and Ujjania (2013) in Tapi River, Gujarat.

Potassium is the predominant cation associated with cytoplasm in the cytosol. In plant material it is present in considerable concentration of 40 mmol l^{-1} (Talling 2010), and is major cation at variable concentrations in the vacuole. It is osmotically active as an osmolyte (Leigh and Sanders 1986). Its uptake is influenced by electrical properties of the cell membrane, and involves one or more ion pumps that may be sensitive to light and to the presence of other cations (Talling 2010) Potassium activates at least 60 different enzymes involved in plant growth. Potassium changes the physical shape of the enzyme molecule, exposing the appropriate chemically active sites for reaction. Potassium also neutralizes various organic anions and other compounds within the plant, which helps to stabilize pH between 7 and 8, which is optimum for most enzyme reactions. The amount of K^+ present in the cell determines how many of the enzymes can be activated and the rates at which chemical reactions can proceed. Thus, the rate of a given reaction is controlled by the rate at which K^+ enters the cell. (Armstrong 1998). When the sun's energy is used to combine CO_2 and water to form sugars, the initial high-energy product is ATP, which is then used as the energy source for many other chemical reactions. The electrical charge balance at the site of ATP production is maintained with K^+ ions. When plants are K^+ deficient, the rate of photosynthesis and the rate of ATP production are reduced, and all the processes dependent on ATP are slowed down. Conversely, it increases plant respiration, which also

✉ Ganesh Iyer
✉ Yash Gupte
yash.v.gupte@gmail.com

[1] Department of Life Science, Ramnarain Ruia College, Matunga, Mumbai 400 019, India

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contributes to slower growth and development (Armstrong 1998). Potassium also plays a major role in the transport of water and nutrients throughout the plant in the xylem. When K^+ supply is reduced, translocation of nitrates, phosphates, calcium, magnesium, and amino acids is depressed. As with phloem transport systems, the role of K^+ in xylem transport is often in conjunction with specific enzymes and plant growth hormones. An ample supply of K^+ is essential to efficient operation of these systems. (Armstrong 1998).

pH of water depends on the dissolved carbon-di-oxide (CO_2). Higher the concentration of CO_2, lower is the pH of water as CO_2 reacts with water (H_2O) to form carbonic acid (H_2CO_3) (Wurts and Durborow 1992). During photosynthesis, CO_2 and H_2CO_3 are removed as the rate of carbon fixation by algae is far more than the dissolution rate of CO_2 at air–water interface (Tundisi and Tundisi 2011). Annual import of potash by India is around 2.0 million tonne, which costs approx. US$ 275 million (Mohnot et al. 2005). Potash industry also uses huge amounts of water for potassium extraction, wastage disposal and treatment processing, with areas across the mining field attached to rivers, streams or lakes. Disposal of this water from the mining areas to environment may cause environmental damage, which in turn can cause health hazards. (UNEP and IFA 2001).

The accumulation of organic pollutants (pesticides, PCBs, DDT) and heavy metals (Cd, Pb, Se, As) in the aquatic systems can cause serious problems on environment and organisms, affecting negatively the stability of many aquatic ecosystems and can also cause difficulties for animals and human health. Algae are a group of living organisms that play an important role in controlling metal concentration in lakes and oceans and the density and absorption of pollutants such as heavy metals in their environment. The ability of algae to absorb metals has been recognized for many years (Noori et al. 2012).

Two algal species *Coelastrella oocystiformis* and *Chlorolobion braunii* were selected for the present experiment. The species were isolated at Department of Life Science, Ramnarain Ruia College, Mumbai and identified using monograph "The freshwater algal flora of the British Isles" by John et al. (2011). These species were selected as *C. oocystiformis* is spherical in shape and *C. braunii* is spindle shaped. This characteristic difference to identify the shape of algal cells leads to different osmotic and physiological responses. The two algal species were cultured in separate flasks using BG 11 media procured from HiMedia Laboratories product code M1541.

Potassium sulphate (K_2SO_4) was procured from HiMedia Laboratories (product code GRM404). A stock solution of 2000 ppm of K^+ was prepared by dissolving 446 mg of K_2SO_4 in 100 ml of distilled water. Varying concentrations of 200, 400, 600, 800, 1000, 1200, 1400, 1600 and, 1800 ppm were prepared by using the stock solution. 10 ml of cultures of *C. oocystiformis* and *C. braunii* were transferred in 10 different flasks. The flasks were labelled as 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 ppm. 15 ml of K_2SO_4 solution of the respective concentration were added to different flasks. These cultures were kept for a period of 30 days at 5000 lux of light with a cycle of 12 h light/dark. The cultures were centrifuged at 3000–4000 rpm for 5 min to obtain the algal samples as pellets. The supernatant was discarded and only the algal pellets were oven dried at 60 °C to estimate the quantity of dry biomass of algal samples subjected to different K concentration. The dry algal biomass was then converted to ash at 700 °C in a furnace for about 2 h. The ash obtained was measured gravimetrically and then dissolved in 1 ml of 5 % sulphuric acid, and 0.2 ml from this solution was used for further testing. Analysis of K content was performed using the method described by Jacobs and Hoffman (1931) and Abul-Fadl (1949). Standard graph for potassium concentrations was prepared.

The cultures were observed microscopically for any changes in cell structure after 30 days of incubation under each of the K concentrations using Lab-o-Med Lx300 microscope with camera and software for cell measurement. The pH of all the concentrations was measured using pH papers from Merck, Product Code: 109565 to observe if the incubation period with photosynthesis causes a change in pH.

The dry biomass of *C. oocystiformis* and *C. braunii* in each of the flask with varying K concentrations ranged from 25 to 40 mg. The amount of ash was recorded gravimetrically revealed that as the concentration of potassium increased from 200 to 600 ppm, the ash content also increased. Above 600 ppm, the ash content remains constant at 2.5–6 %.

The optical densities for various concentrations of AQ2 potassium treated algal ash were plotted on the standard graph to obtain estimates of potassium content (Fig. 1). It was observed that the algal cells have particular response to amount of K^+ in the water source. When exposed to a concentration of 200–800 ppm of K^+, the algal cells showed an ash content of more than 10 % (Fig. 2). Also at these concentrations, the K^+ uptake by the cells was more compared to concentrations from 1000 to 2000 ppm (Fig. 3). The trend seen for ash content and potassium uptake by both the organisms *C. oocystiformis* and *C. braunii* were identical. *C. oocystiformis* has highest ash content of 30.5 % when exposed to 400 ppm of K^+ and at this concentration it can take 74.8 % of the total potassium it was exposed to. *C. braunii* has highest ash content of 37.5 % when exposed to 1000 ppm of K^+, but has the maximum potassium content of 12.35 % when exposed to

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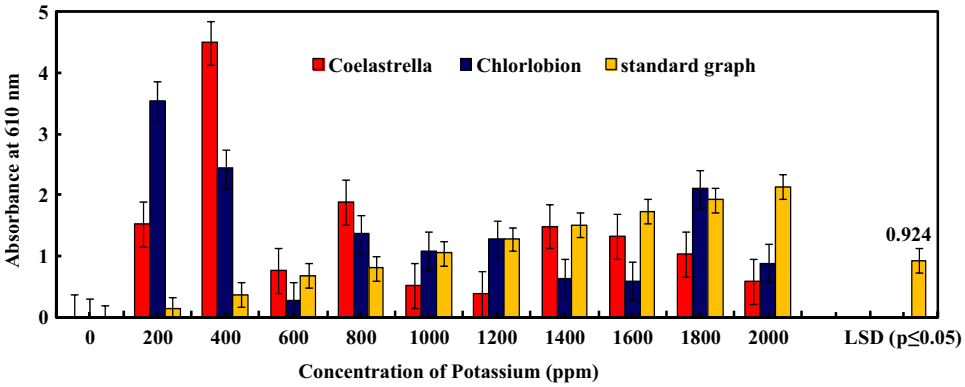


Fig. 1 Absorbance of potassium under different potassium treatments in *Coelastrella oocystiformis* and *Chlorolobion braunii* samples, and in standards. *Vertical bars* show ± SE of mean

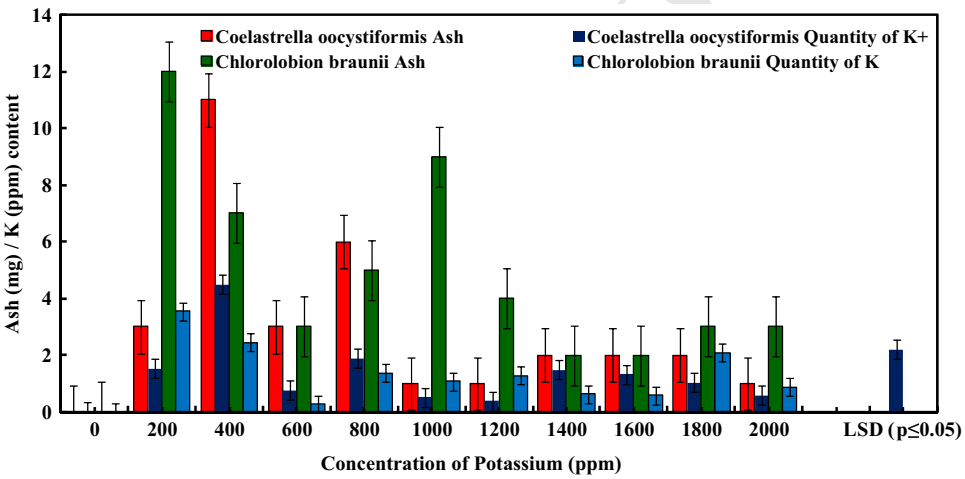


Fig. 2 Potassium (ppm) and ash (mg) content under different potassium treatments in *Coelastrella oocystiformis* and *Chlorolobion braunii*. *Vertical bars* show ± SE of mean

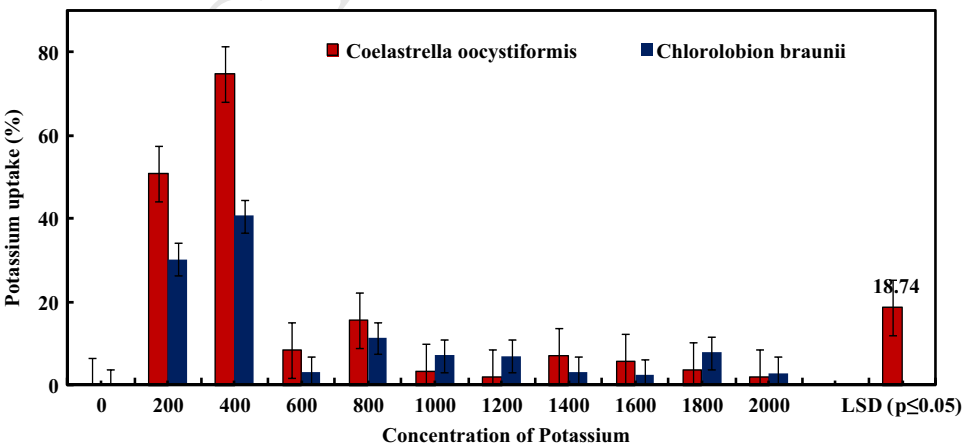


Fig. 3 Uptake of potassium in *Coelastrella oocystiformis* and *Chlorolobion braunii*. *Vertical bars* show ± SE of mean

1800 ppm of K^+. *C. braunii* however has maximum uptake of potassium of 40.66 % when exposed to 400 ppm of K^+ (Fig. 3). Thus it is clear that the basic concentration of potassium that the algal cells can tolerate remains constant at 400 ppm irrespective of the shape of cells.

Coelastrella oocystiformis is spherical in shape, whereas *C. braunii* is spindle shaped. The shape and size of cells did not have any change or deterioration after 30 days of incubation time. Some cells were seen under division as well, indicating that algal cells were healthy as under normal conditions. Although the basic concentration of potassium that both the algae could tolerate was the same, the uptake of potassium by *C. oocystiformis* was almost double at 74.8 % as compared to 40.66 % of *C. braunii*

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(Fig. 3). This difference may be due to the fact that spherical cells are better capable of tolerating osmotic pressure. pH of all the solutions prepared was measured on day 0 and 30. The pH at all varying concentrations of potassium was constant at 5.0. The pH of the varying concentrations of K mixed with 15 ml of BG11 media was 6.0. On day 0, time zero after addition of algal cells to all the final inoculum media, the pH did not change immediately. However on day 30 it was observed that the blank of *C. oocystiformis* had a pH of 11 and *C. oocystiformis* at all the varying concentrations of K had a pH of 12.0. Similarly for *C. braunii* it was observed that the blank had a pH of 8.0, whereas *C. brauni* at varying concentrations K had a pH of 9.0. This change in pH by 1 at varying concentrations of K over blank in both the organisms may be due to presence of excess of potassium, which may have led to increase in the photosynthetic rate.

AQ3 Potassium plays an important role in almost all biological processes. In the current study, we observed algal response to increasing concentrations of potassium. Although the potassium content in natural waters may vary from 1 to 200 ppm, in the present experiment algal cells were exposed from 200 to 2000 ppm of potassium. The biological responses of both algal forms *C. oocystiformis* and *C. braunii* showed maximum uptake of potassium at 400 ppm. A large amount of water is used for mining potassium, when potash is harvested by evaporating water on hectares of land open to luxurious sunlight (UNEP and IFA 2001). This area can be utilized to grow algae and mine potassium simultaneously. Potassium mining industry is a costly business with strenuous environmental effects (UNEP and IFA 2001), and algae grown at the site will help reduce pollution. As observed during this study that up to 2000 ppm K algal cells were capable of growth, but only their uptake of potassium was reduced beyond 800 ppm. It is beyond doubt that algal cells can help in mining potassium and other rare elements present with potash. Use of algal biomass as a biofertilizer for nitrogen fixation (Rippka et al. 1971) is an old practice, however use of algae for potassium can make algae a complete fertilizer. Of the basic nutrients nitrogen, phosphorus and potassium required for agricultural purposes, potassium is one of the most under-rated elements. Potassium deficiency symptoms such as thin cell walls, weakened stalks and stems, smaller and shorter roots, sugar accumulation in the leaves, and accumulation of unused nitrogen (N) encourage disease infection. Armstrong (1998) Apart from use of algae in potassium industry, it can also be used to make water potable. Algae can take up elements like potassium, calcium, sodium from water and make it clean enough to drink.

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