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Scope of phycoremediation of Arsenic using *Phormidium tenue* with special reference to modulation in cellular biochemistry

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Abstract

Phormidium tenue (Meneghini) Gomont , a microscopic filamentous cyanobacterium was exposed to 5ppm Na-arsenate (sub lethal dose) for different time intervals (1h, 3h and 24h) at a pH variation of 5.5, 7.0 and 8.5 to study the accumulation pattern of toxic metalloid arsenic. It was observed that arsenic accumulation of *P. tenue* increased with time and maximum accumulation was recorded in pH-7 after 24h exposure (80.51 mg. g^{1}). In another experiment *P. tenue* was treated with different concentrations of Sodium arsenate (0.1, 1, 5, 10, 25, 50 and 100ppm) in pH-7 to study the growth pattern and changes in biochemical parameters like, chlorophyll a , carotenoids, phycobiliproteins, total protein and carbohydrate content in control and arsenic exposed biomass. This study was done to assess primarily the ability of the cyanobacteria for arsenic removal and to study the cellular biochemistry of arsenic exposed biomass for a longer period of time. From the study it was evident that *P. tenue* responded differently in different doses of arsenic and showed optimum growth even when exposed to 50ppm arsenic for 60 days indicating arsenic resistance.

Key words: *Phormidium tenue*, Arsenic, Chlorophyll a, Carotenoids, Phycobiliproteins, Protein, Carbohydrate

Introduction

Arsenic, one of the most toxic metalloid, ranking 28th in abundance on the earth's crust is widely encountered in the environment and organisms. Arsenic can exist in four valence states viz., –3, 0, +3 and +5. Under reducing conditions, Arsenite (AsIII) is the dominant form whereas Arsenate (AsV) is generally the stable form in oxygenated environments. Ground water contamination of As have been reported for many countries with the most severe problems occurring in Asia, mainly Bangladesh (Dhar *et al.*, 1997, Biswas *et al.*, 1999), West Bengal, India (Mandal *et al.*, 1996, 1997), China (Liangfang and Jianghong, 1994) and Taiwan (Chen *et al.*, 1995). In some areas of Bangladesh, As concentration of ground water reached up to 2 mg. L^{-1} (Tondel *et al.*,, 1999), much above the WHO's (World health Organization) permissible level of 0.01 mg. L^{-1} for drinking water and the national standard level of 0.05 mg. L^{-1} for Bangladesh and India.

In general, plants employ several extracellular and intracellular mechanisms to detoxify toxic metals and metalloids. External mechanisms include exudation of extracellular ligands, whereas, internally, the plants alternate the influx/efflux of metal ions to reduce metal concentration in cell and bind it in a non- toxic form. In recent studies, it has been observed that several algae can accumulate As from water (Imamul Huq *et al.*,. 2005; Shamsuddoha *et al.*,. 2006). There are several reports regarding arsenic induced changes in growth performances of different algae including cellular biochemistry (Maeda *et al.*,. 1985, 1992a). Accumulation of inorganic As increased the b-carotene and fatty acids level (C18:1 and C18:3) and water extractable carbohydrate content in the cells of *D. salina* (Yamaoka *et al.*,., 1992). Cyanobacteria are also known to be resistant to arsenic. Methylation and excretion of As by arsenic resistant genus *Phormidium* has been reported by Maeda *et al.*,., (2004). They also reported increased growth rate of algal biomass up to 100 mg. g^{-1} As in growth medium.

In the present study, *Phormidium tenue* was treated with different concentrations of Na arsenate to ascertain its growth potential together with variation in proteins, carbohydrate and phycobiliproteins in arsenic exposed biomass. This might be essential to further assess its credibility as resistant species and would be a useful means for bioremediation of Arsenic.

Material and methods:

The marine cyanobacteria, *Phormidium tenue* was collected from National Facility for Marine Cyanobacteria (NFMC), Trichy, Tamil Nadu, India. Cultures were grown and maintained at 20˚C in a 16:8 h light/dark cycle under cool fluorescent light with an intensity of 20–30 l Em^{-2} s^{-1} in Artificial Sea Nutrient III medium (Ott, 1965). *P. tenue* was exposed to 5ppm Na arsenate spiked in ASNIII media at pH 5.5, 7.0 and 8.5 for 1h, 3h and 24hrs. In each case, before digestion, the samples were divided into two parts; one was washed with water, while the other half was washed with 5 mM Na_2EDTA. The cyanobacterial

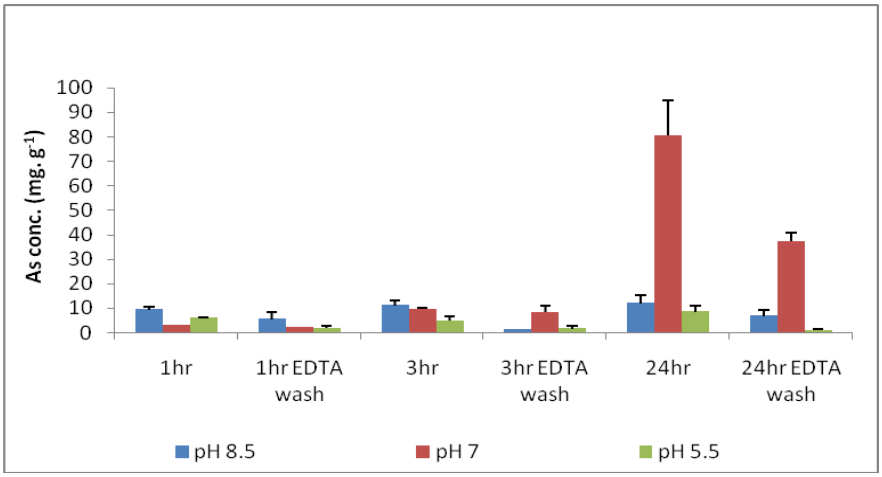
samples were digested with conc. HCl, 30% H_2O_2 and conc. HNO_3 and then filtered through No. 1 Whatman filter paper. Volumes of the samples were made up to 25 mL. These samples were then analyzed for arsenic content (APHA 1998) by atomic absorption spectrophotometry (Vapour type Varian 240 AAS).

For other experiment *P. tenue* was cultured in 250mL conical flasks spiked with Na-arsenate concentrations of 0.1, 1, 5, 10, 25, 50 and 100ppm along with a control set. At the intervals of 1, 3, 7, 10, 14, 21, 28, 35, 42, 49, 56 and 63days, biomass from these sets were taken and analyzed for chlorophyll a and carotenoid (Arnon 1949), phycobiliproteins (phycocyanin, phycoerythrin and allophycocyanin) (Siegelman and Kycia, 1978), protein (Lowry *et al.*,. 1951) and carbohydrate (Hodge and Hofreiter 1962) content.

Results:

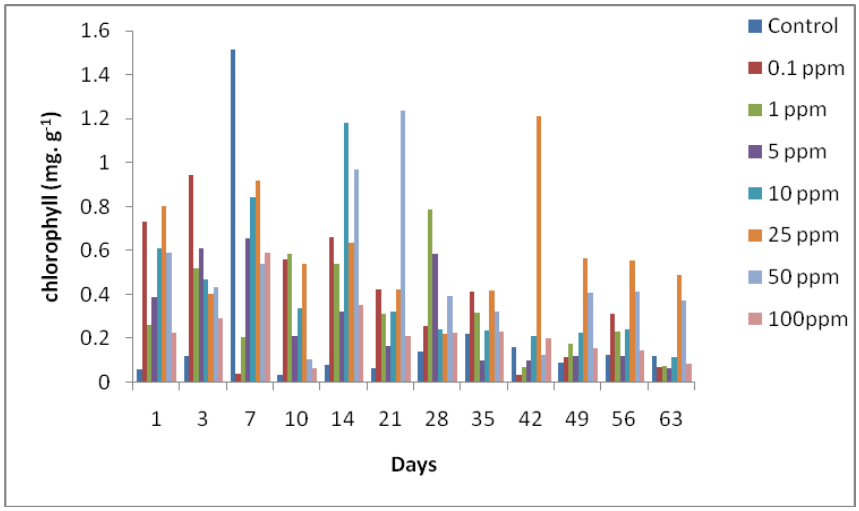
Arsenic contents were measured from cyanobacterial biomass exposed to 5ppm Na- arsenate for 1, 3 and 24hrs' exposure at pH 5.5, 7 and 8.5 (Fig-1). It was observed that at pH 7.0, *P. tenue* accumulated maximum amount of arsenic (80.51 mg. g^1) after 24 hrs' exposure. After washing the sample with 5 mM Na-EDTA the arsenic content of exposed biomass was reduced to 37.17 mg. g^1, suggesting almost 54% removal of arsenic by EDTA wash. This revealed the fact that there is a partitioning in arsenic accumulation by *Phormidium tenue*, where 54% was surface adsorption and the rest was actively accumulated by the algal biomass (Fig -1).

Fig-1: Accumulation of Arsenic in *P. tenue* at different pH level for 1, 3 and 24 h.



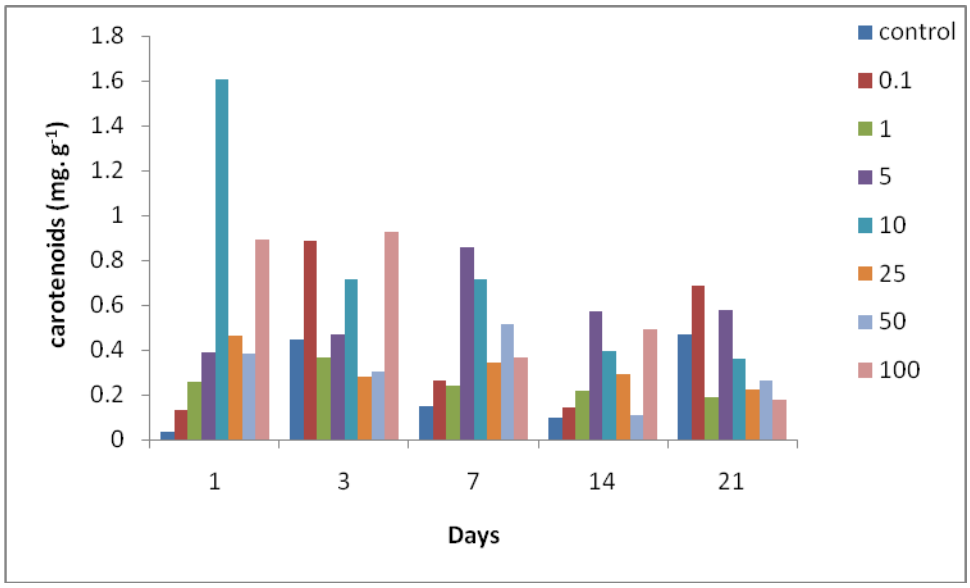
P. tenue exposed to 0.1, 1, 5, 10, 25, 50 and 100ppm of Na-arsenate showed varied results in chlorophyll content (Fig-2).Overall arsenic induced higher growth rate, showing more chlorophyll in arsenic exposed biomass. Arsenic seemed to have little effect on growth of the organism even at high concentration of As (100ppm). After 63 days of exposure, chlorophyll content of *P. tenue* exposed to 25 and 100ppm As were more than that of control set and maximum chlorophyll content was observed in algae exposed to 50ppm As (0.524 mg. g^{-1}), indicating unaffected algal growth. In case of 0.1ppm exposure, drastic reduction in chlorophyll content was noticed, where induction of hormogone development was observed together with death of mother filament. In course of time, these hormogones germinated producing new growing filament showing more chlorophyll a.

Fig-2: Variation in Chlorophyll a content in *P. tenue* under different concentration of As at different time of exposure.



Carotenoid content of treated sets at 1 day was significantly higher than control set (Fig-3). As induced changes in carotenoid content was more prominent up to 7 days of treatment showing significant increase in 0.1 to 100ppm concentrations. After that there was no significant change in carotenoid content, and a slight decrease in carotenoid content was observed in comparison to control. Thereafter, further treatment was discontinued. Highest carotenoid content was observed in 10ppm treated set, exposed for 1 day. Even at a high stress of 25, 50 and 100ppm, the carotenoid content decreased, showing resistant nature of *P. tenue*.

Fig-2: Variation in Carotenoid content in *P. tenue* under different concentration of As at different time of exposure.



Among phycobiliprotein, c-phycocyanin, c-phycoerythrin and allophycocyanin were measured. An overall increase in phycocyanin content was observed up to 14days of exposure. After that, significant rise in phycocyanin content were noticed in 0.1, 1, 25 and 50ppm exposed biomass at different time period (Fig-4). Similar results wre observed for phycoerythrin (Fig-5). But for allophycocyanin, sharp increase in this pigment content were noticed for 1 and

25ppm treatment after 21 nad 42 days of exposure respectively.

Fig-4: Variation in Phycocyanin content in *P. tenue* under different concentration of As for 63 days.

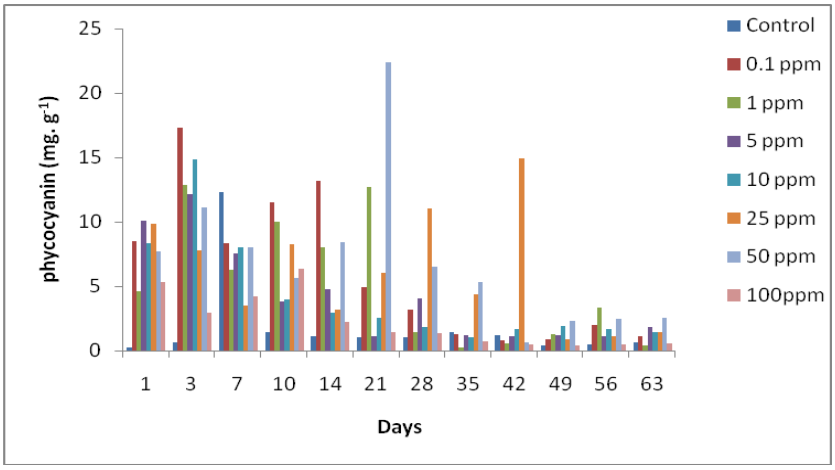


Fig-5: Variation in Phycoerythrin content in *P. tenue* under different concentration of As at different time intervals.

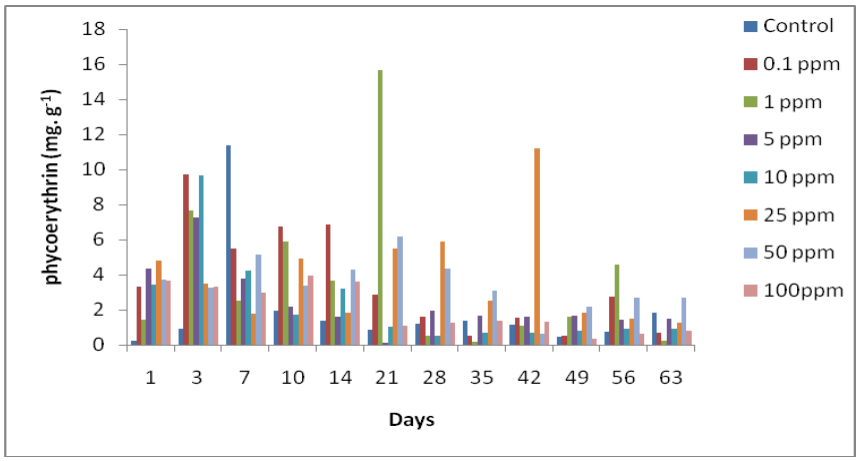
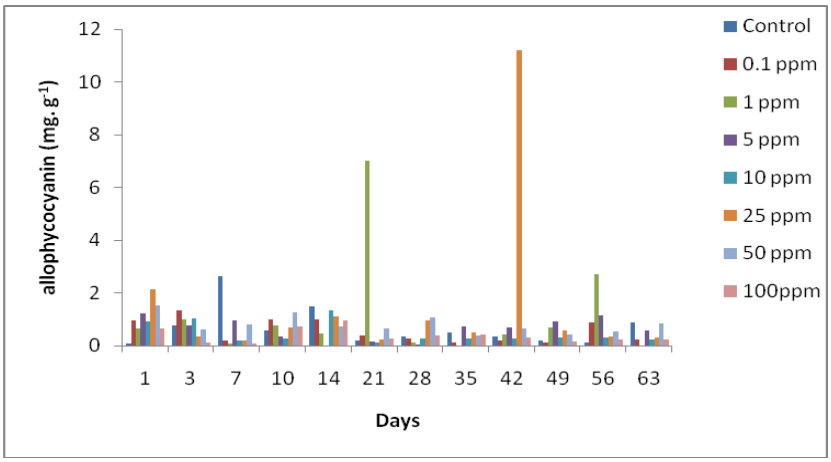
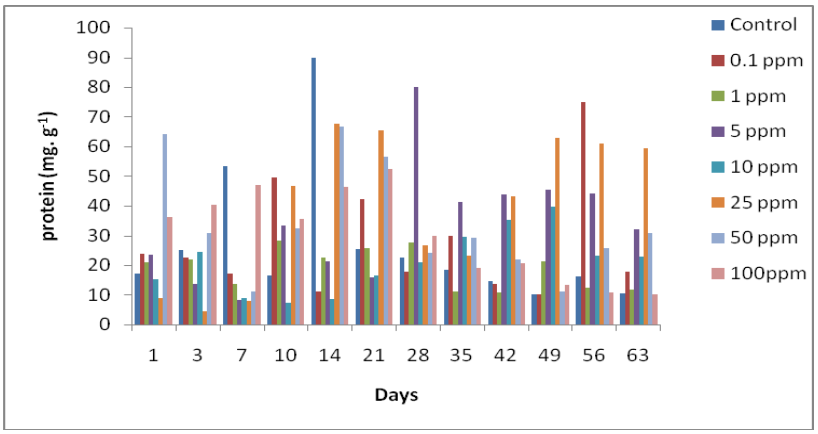


Fig-6: Variation in Allophycocyanin content in *P. tenue* under different concentration of As at different time intervals.



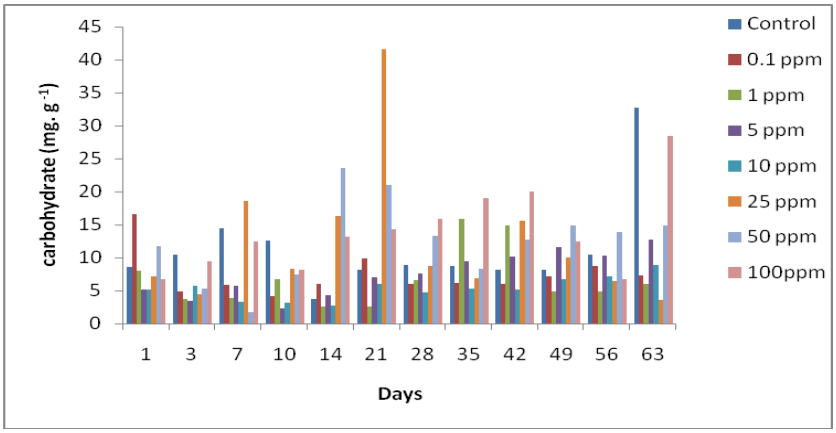
The protein content of treated biomass was significantly higher than that of control biomass exposed to different doses at different time intervals (Fig-7). Metabolic activity in control set was optimum after 14 days of growth, represented by maximum protein content followed by a gradual decline up to 63 days of culture. In case of treated sets, maximum protein content was observed in algae exposed to 5ppm As for 28days. After 63 days of exposure, maximum protein content was observed in *P. tenue* treated with 25ppm As.

Fig-7: Variation in Protein content in *P. tenue* under different concentration of As at different time intervals



In control set as well as 100ppm As spiked set, maximum carbohydrate was produced after 63 days of experimentation (Fig-8). Highest level of carbohydrate content was observed in *P. tenue* exposed to 25ppm As after 21 days of exposure. In case of 0.1ppm As exposure, carbohydrate content was recorded maximum after 24hrs.treatment. In most cases, carbohydrate amount increased after 14 days, thereafter the levels were either maintained or decreased, except in control and 100ppm of Arsenic.

Fig-8: Variation in Carbohydrate content in *P. tenue* under different concentration of As for 63 days.



Discussion

Though some heavy metals and metalloids are needed by living organisms for various metabolic processes, the physiological and/or metabolic requirements of metalloid Arsenic is not properly understood. In recent studies, it has been observed that algae can hyperaccumulate As from water (Imamul Huq *et al.*,. 2005; Shamsuddoha *et al.*,. 2006). Cyanobacterial species are able to grow in the presence of high concentrations of As(V) (up to 100

mM) and low-millimolar concentrations of As(III). This is also due to the fact that arsenate is a phosphate analogue and competes with phosphate to enter the cell. From our study, it was observed that when exposed to Arsenate stress, *Phormidium tenue* accumulated high amount of As. The resistant nature of *Phormidium* to arsenic was also reported by Maeda *et al.,*. (2004) showing increased growth rate of the genus up to the uptake level of 100 mg g^{-1}. In marine environment, the periphytic algal population showed 0.4 μm of arsenic as 20% EC value (effective concentration) in 1 h exposure (Blanck and Wangberg 1988). On the other hand, at a concentration of 80 μg/L of arsenic, the seaweed *Macrocystis pyrifera* showed minimum level of effective concentration for germ tube growth, nuclear migration and altered lifecycle pattern (Garman *et al.,*. 1994).

Generally, the immediate effect of an increased concentration of the heavy metals seems to result in inhibition of growth of any organism. The observed changes in pigment contents of the cyanobacterium suggest a different picture, as growth was not hampered due to arsenate stress and even in increasing concentrations of Na- arsenate, chlorophyll content did not change. In case of treated *P. tenue* biomass higher level of chlorophyll was recorded, when exposed to 50ppm arsenate than in control set. This result was corroborated with our previous finding in *P. laminosum*, where arsenate concentration did not affect chlorophyll concentration (Bhattacharya and Pal, 2011).

Inhibited biosynthesis of chlorophyll and carotenoids and reduced phosphorylation are most frequently observed symptoms of metal toxicity (Poskuta *et al.,*.1996; Prasad 2004; Smirnoff 1995). Increase in the levels of low molecular weight antioxidants such as carotenoids, and antioxidant enzymes such as APX are among protective mechanisms that serve to remove ROS (Pinto *et al.,*. 2003; Devi and Prasad, 1998). Approximately 1.2 and 1.8 fold increase in carotenoid content was noticed in cells treated with 15 mM As(III) and 110 mM As(V) for 72 h, respectively over their controls (Srivastava, 2009). But in our study upregulation in carotenoids synthesis was observed up to 3days of arsenic exposure after which there was a gradual decline in carotenoids content. This suggests that carotenoids biosynthesis is affected in long exposure of toxic metalloid arsenic in *P. tenue*. This result is supported by Bhattacharya and Pal (2011) as well as Chaneva *et al.,*. (2009) where, carotenoids were almost unaffected at both arsenic and copper exposure.

In the present study it was observed that, there was an increase in the overall phycobliprotein content in the arsenic treated biomass up to 21 days of treatment. Similar results were observed by Zhao *et al.,*. (2011) for *Anabaena flos-aque* in algae lytic stress. In this study, the level of phycocyanin, however, declined markedly with increasing exposure time. Srivastav *et al.,*. (2009) reported a sharp decline in phycocyanin content due to As(V) and As(III) stress, which may be due to lysis of the cell wall and disruption of the thylakoid membrane. But in our study increase in phycobiliproteins up to 21 days suggested resistant nature of *P. tenue* to As(V) stress.

Shah and Dubey (1997) reported that heavy metal stress has been shown to induce a variety of proteins resulting in an overall increase in protein content. Arsenic exposure induced protein synthesis related to oxidative stress in plants have been reported by Requejo and Tena (2005). Increased protein content due to arsenate stress in *P. laminosum* has also been reported by Bhattacharya and Pal (2011). In the present study also protein content increased indicating the role of different proteins including stress enzymes and phytochelatins in arsenic resistance.

Production of carbohydrate may be considered as stress response in form of exopolysaccharides in different environmental conditions (Sinniah *et al.,*., 1998). Increased levels of soluble sugar have been reported to be increased under salinity (Dubey, 1999), water-stress (Foyer *et al.,*., 1998) and chilling effect (Hurry *et al.,*., 1995). Choudhury *et al.,*., (2010) reported an increase in soluble reducing sugar and decrease of non reducing sugar content in arsenic stressed rice seedlings. The present results indicate the increase of carbohydrate up to 21days, thereafter a slight decrease and a constant level in the As(V) exposed sets.

Overall it was found that *P.tenue* was well adapted to arsenic stress with a prominent biochemical modulation of vegetative cell and is a highly potential genus for phycoremediation of arsenic.

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