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Screening of indigenously isolated *Nostoc* sp. for chromium tolerance

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Abstract

A possible alternative for removal of heavy metal such as chromium from the polluted environment is by using cyanobacterial species. In the present work, sample was collected from the indigenous site. Isolation and purification of the culture was done by BG-11 medium. The pure culture was identified by standard microscopic methods. Based upon these observations the isolated pure culture was confirmed as *Nostoc* sp. and cultivated using the BG-11 medium. According to the dose response relationship, chromium showed stimulatory effects on various metabolic activities in photosynthetic organisms at lower concentration; including chlorophyll a, b and total protein etc., whereas increasing concentration of chromium had inhibitory effect. Hence the response of isolated pure *Nostoc* culture to different concentrations (5, 10, 15, 20 mgl^{-1}) of chromium (VI) was investigated. Growth of the *Nostoc* sp. in the presence of chromium (VI) was expressed in terms of dry weight, chlorophyll-a and protein content. Tolerance to chromium was occurred at 5 and 10 mgl^{-1} concentration of chromium (VI) as compared to control and the optimum growth was observed at 9th day of incubation.

Keywords:*Nostoc*, Chromium, Tolerance, Chlorophyll-a, Phycocyanin

Introduction:

Among all the pollutants, heavy metals are most dangerous one as these are non –biodegradable and persist in environment (Carol *et al*., 2012). These enter into the water resources through both natural and anthropogenic sources. Chromium is considered an essential nutrient for numerous organisms, but at elevated levels, it is toxic and mutagenic (Shi *et al*., 2002). Hexavalent chromium (Cr (VI)) compounds are being in trace amounts; are present as either dichromate ($Cr_2O_7^{2-}$) in acidic environments or as chromate (CrO_4^-) in alkaline environments (Srinath *et al*., 2002).

Chromium exists in several oxidation states, of which the Cr (VI) as being highly mobile and toxic. It is widely used in industry in a wide variety of commercial processes (Avudainayagam *et al*., 2003; Sathwara *et al*., 2007). The unregulated disposal of the chromium containing effluent in both developing and developed countries has led to the contamination of soil, sediment, surface and ground waters (Zayed and Terry 2003).

Several technologies have been developed for decontaminating waste water by using micro-organisms for heavy metal removal (Shukla *et al*., 2007). Amongst all, a possible alternative is the use of cyanobacteria which have their own importance in chromium tolerance, due to their simple growth requirements, nitrogen fixing capability and large biomass production (Prakasham and Ramakrishna 1998).

Cyanobacteria (also known as blue-green algae) are the largest and most diverse group of prokaryotes oxygenic photosynthetic. Their habitats vary from fresh and marine water to terrestrial environments (Chinnasamy *et al*., 2007). They respond to many extreme physic-chemical stress conditions such as heavy metals, (Duangrat *et al*., 2002) light deprivation, pH, temperature etc. (Derek *et al*., 2005; Torresdey *et al*., 1998).

In the present work indigenous nitrogen-fixing *Nostoc* sp. was isolated from freshwater samples and tested for their ability to tolerate the increasing concentrations of Cr (VI).

Material and methods:

Isolation and identification of culture:

An inoculum of freshly collected indigenous terrestrial sample was processed aseptically for isolation and purification purpose by using standard methods (Mostafa *et al*., 2006; Torrecilla *et al*., 2004). Identification of the pure culture was carried out on the basis of criteria given reference literature Prescott (1970), Anagnostidis and Komarek (1988) and Komarek and Anagnostidis (1989) and the pure *Nostoc* sp. was cultivated (Yavuz *et al*., 2006).

Metal tolerance experiment:

The *Nostoc* isolate was tested for their ability to tolerate different concentrations of Cr (VI) (5, 10, 15, 20 mgl^{-1}) incorporated into BG-11 medium. Its effect on growth of the isolate was determined by incubating with a uniform suspension of 10-day-old *Nostoc* culture in 100 ml BG-11 medium contained in 250 ml Erlenmeyer flasks. The control was maintained in which no Cr (VI) was incorporated. The culture was incubated for 12 days during which the growth of the bacterium was observed at 24 h intervals (Ezaka 2011).

Measurement of growth of *Nostoc* culture:

Tolerance to metal was decided on the basis of dry weight, chlorophyll-a and protein content.

Chlorophyll-a was estimated by following the method of McKinney (1941) and the protein content was estimated by Lowry (1951) against bovine serum albumin as a standard. Dry weight of biomass was determined at end of the experiment. Samples were taken in triplicate.

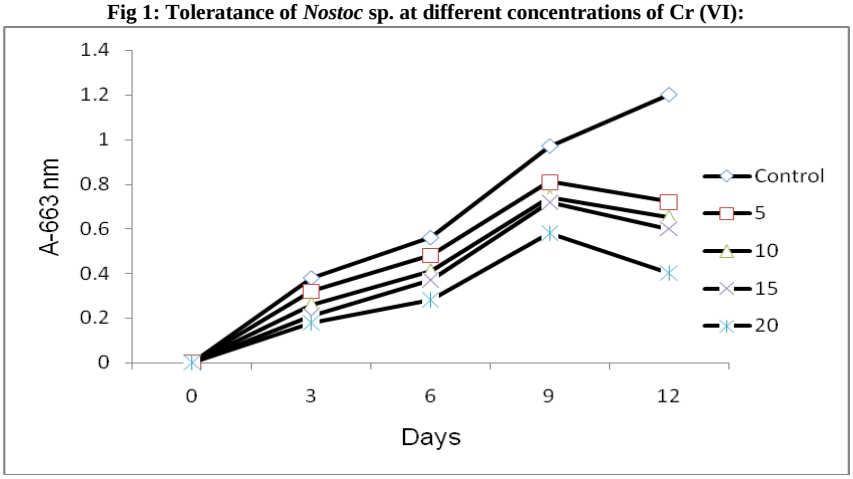
Results and discussion:

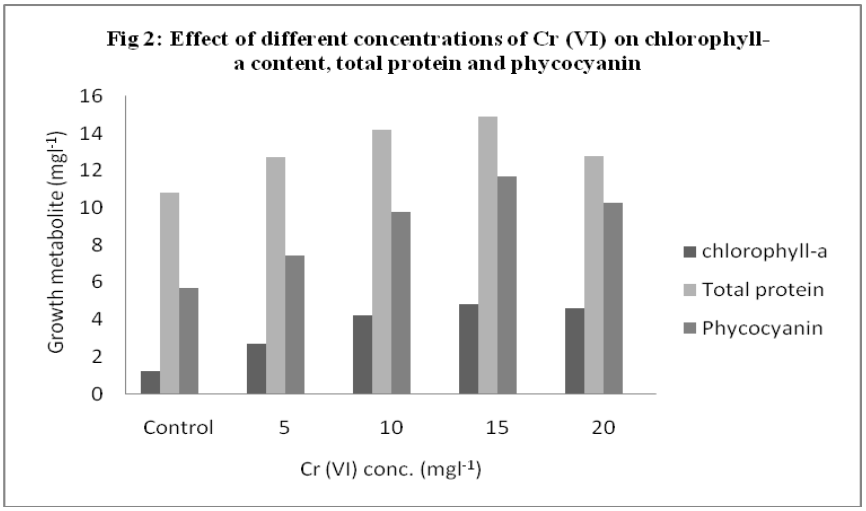
Isolation and Identification culture:

In present investigation a pure culture of cyanobacterium was obtained and the morphological characteristics were studied microscopically. These observations showed that the cells were filamentous with false branching, presence of akinetes and heterocystous thallus with mucilaginous sheath. Hence the isolated culture was identified as *Nostoc* sp.

Results showed that there was decreasing growth of isolated *Nostoc* sp. with increasing concentrations (5, 10, 15, 20 mgl^{-1}) of Cr (VI). As compared to the control better tolerance was achieved at 5 and 10 mgl^{-1} concentrations of Cr (VI). There was no appreciable growth after 10^{th} days of incubation (Fig 1). The effect of Cr (VI) tolerance of *Nostoc* sp. was observed by assessing its growth in terms of chlorophyll-a, total protein and phycocyanin concentration. Fig 2 showed that the culture was adapted to 15 mgl^{-1} Cr (VI) concentration as maximum increase in concentration of the growth metabolites at this concentration.

Fig 1: Toleratance of *Nostoc* sp. at different concentrations of Cr (VI):





Initially during treatment with increasing concentration of Cr (VI), the growth of isolated *Nostoc* sp. increases and maximum dry weight of the biomass was obtained at10 mgl^{-1} concentration. The decrease in dry weight of the biomass was observed from15 mgl^{-1} upwards (Table 1). These results indicated that between 10-15 mgl^{-1} the culture was in stationary phase and it was adapted to Cr (VI) at10 mgl^{-1}concentration. The results were compared with control.

Table 1: Effect of Cr (VI) tolerance on dry weight of *Nostoc* biomass:

Days	Dry biomass (%) control	Effect of Cr (VI) tolerance on dry weight of *Nostoc* biomass	
		Metal Concentrations (mgl^{-1})	Dry biomass (%)
3rd	52	5	148
6th	174	10	202
9th	222	15	186
12th	234	20	118

Traditionally, chemical technologies have been used for the removal of Cr (VI) from contaminated soil and groundwater (Yinhui and Dongye 2007). However, such technologies can be expensive and time consuming. A possible alternative is the use of microorganisms which have been shown to be chromium reducers (Vatsouria *et al*., 2005). Cyanobacterial genera including *Chroococcus* (Kamra *et al*., 2007), *Anabaena* (Chakraborty *et al*., 2011), *Phormidium* (Shanab and Essa 2007) were reported to reduce chromium.

The exact mechanism is not known but survey results indicated that there may be a role of some enzymes such as chromium reductases. Different chromium resistant genes have been characterized. During tolerance mechanism the *Nostoc* cells can reduce soluble Cr (VI) to insoluble Cr (III), which is less mobile and less toxic; this reduces the Cr-toxicity level. The use of cyanobacteria in heavy metal removal is a cost-effective and environment friendly method, due to very less requirements and the utilization of solar radiations by the organism.

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