Indian Journal of Biotechnology and Pharmaceutical Research
Journal homepage: www.ijbpr.in
ISSN: 2347-3266
2022, 10(2): 1 – 7

Research Article

The effect of nickel on the photosynthesis, and nitrogenase activity of Cyanobacterium *Nostoc calcicole*

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ABSTRACT

Photosynthesis was determined by uptake of $^{14}C02$ from $NaH^{14}CO_3$. Photosynthesis was significantly inhibited by nickel concentrations of 0.50 ppm and more than 0.50 ppm. It was observed that photosynthesis was inhibited 92.8% by10 ppm and 98.2% by 20 ppm Ni^{2+} concentration at 10^6 cfu per milliliter cell concentration. Nitrogenase activity was determined using the acetylene reduction technique. Acetylene reduction was also significantly inhibited by nickel concentrations of 0.50 ppm and more than 0.50 ppm. It was observed that acetylene reduction was inhibited 90.1% by10 ppm and 95.5% by 20 ppm Ni^{2+}concentration at 10^6 cfu per milliliter cell concentration. The photosynthesis and acetylene reduction sensitivity was significantly increased after 12 hours preincubation in the presence of nickel ion. Under these conditions acetylene reduction was more sensitive than photosynthesis. The photosynthesis and acetylene reduction was inhibited 81% and 98% by 0.5ppm Ni^{2+} concentration respectively.

KEY WORDS: Cynobacterium, nickel, photosynthesis and acetylene reduction

Introduction

Environmental problems are multiplying and becoming complex due to the increasing population, uncontrolled urbanization and industrialization. The primary source of pollution are mining, burning of fossil fuels, smelting of the metallicferous ores, fertilizers, pesticides, municipal waste, and sewage (Nriagu et al., 1996). The release of heavy metals from industries, agriculture and sewage into the environment has created many problems for both human health, aquatic ecosystems. (Hartmann et al., 1964, Inthorn et al., 2002, and Srinivas et al., 2013) and adverse effect on micro flora and form algal blooms (Brand 2010).

Received: 05.02.2022, Revised: 18.02.2022, Accepted: 29.02.2022

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Algal blooms cause oxygen depletion in the aquatic environment. The nutrient-enriched environments cause a dramatic increase in the cyanobacteria population and a subsequent decrease in other competing bacteria. The cyanobacteria will then become the dominant population in the aquatic environment. Many species of cyanobacteria secrete toxic metabolites (Banack 2010, Brand 2010) and cause various diseases. Heavy metal pollution is an area of constant concern for environmentalists.

Nickel is causing much conern beacause of its increasing level in the environment and its toxicity and carcinogenecity towards mammals (IARC 1976). Nickel is an essential micronutrient that plays an important role in health of eukaryotes and prokaryotes (Rodionov 2006). Nickel-associated enzymes play a crucial role in maintaining the global cycle for nitrogen, carbon, and oxygen (Zhang 2009). However, high concentrations of nickel exposure

could be potentially toxic. Nickel has been shown to cause detrimental damage to lung tissue (Tchou-Wong 2011, Zhou 2009) and is categorized as a potential carcinogen (Tchou-Wong 2011).

Most of the reports deal with the eucaryotic green algae (Greenfield 1942, Hutchinson 1973, Stokes 1975, Sakaguchi et al. 1977, Wong et al. 1978) and studies on the procaryotic cyanobacteria are rare (Fitzgerald and Faust 1963, Fitzgerald 1964, Gibson 1972, Edler and Horne 1978). Communication dealing specifically with the effect of nickel on cyanobacteria are absent.

Cyanobacteria is an ideal candidate to study heavy metal response mechanisms due to its fast growth, easy maintenance and grow in a wide range of environmental conditions. These organisms are photosynthetic and are thereby classified as primary producers. Many are also able to fix nitrogen, which is one of the basic processes in the essential nitrogen cycle. Cyanobacteria may contribute up to one-half of the annual nitrogen budget in some eutrophic lakes (Horne and Goldman 1974) and are economically significant in the cultivation of rice.

The purpose of present study was to find out effect of nickel on photosynthesis, and nitrogenase acitvity of cyanobacterium *Nostoc calcicola*.

Materials and Methods

Microorganisms

A strain of cyanobacterium *Nostoc calcicola* Breb was used in this study obtained from Algal Research Laboratory, Centre of Advance Study in Botany, Banaras Hindu University, Varanasi (Uttar Pradesh) India, in the form of mass cultured in a capacity of 500 ml Erlen-Mayer flask containing 200ml Allen – Arnons combined nitrogen free suspension medium.

Incubation parameters

The cyanobacterium were sub cultured in 500 ml Erlen-Mayer flask containing 200ml Allen-Arnons combined nitrogen free medium and incubated photoautotrophically under the air conditioned room which was illuminated with white fluorescent lights (intensity 14.4 watt m^{-2} on the surface of culture flask) with 18/6 light/dark cycle. All cultures flasks were routinely shaken with the help of rotator flask shakers at 130 rpm for 20-30 minutes to obtain homogenous suspension in liquid medium

Nickel

Nickel used in study was procured in the form of Nickel Chloride (Nickel II chloride 6-hydrate Anala R) from BDH Reagents and Chemicals UK. Nickel (Ni^{+2}) ions as working solutions (5, 10, 15 and 20µ M) were prepared by dissolved nickel chloride in deionised distilled water.

Acetylene Reduction

Nitrogenase activity was determined using the acetylene reduction technique (Hardy et at. 1973). The 50 ml tissue culture flasks were used for this study. Each flask contained 9.0mL of algal culture (10^7 cells) and 1 mL of either merlium or a nickel solution. These flasks were sealed with tight fitting rubber stoppers and a 10% volume of air removed and replaced with acetylene. Activity was monitored at every hour for 5 hours. One-millilitre gas samples from the sealed flasks were injectcd into gas chromatography for analysis of ethylene. The ethylene peaks were identified by retention time and quantitated by comparing peak heights to standard curves.

Photosynthesis

Photosynthesis was determined by the uptake of $^{14}CO_2$ from $NaH^{14}CO_3$ (Amersham Corporation, Oakville, Ontario. Canada). Each flask contained 8.9 mL of algal culture (10^7 cells), 0.1 mL of radioisotope (final HCO_3 concentration of 3.4 µM) and 1 mL of either medium or nickel solution. Activity was monitored at every 30 min for 2 h. One-millilitre of assay samples were taken and filtered through 0.2 µm membrane filters. The filtered samples were washed succesively with 5 mL of 0. 1 N HCI and 5 mL of water and then dried under an infrared lamp. The filters were placed cell side up into 20 mL scintillation vials, covered with 10 mL of a cocktail containing 5 g PPO (2.5-diphenyloxazole) and 0.3g POPOP (1.4-bis [2-(5- phenyloxi1zolyI)] benzene) per litre toluene.

The samples were analysed in a liquid scintillation counter. Counts were corrected for percentage of counting efficiency using the external standards channel-ratio method and reduced by the background level.

Results

Acetylene reduction was significantly reduced at 0.5 ppm and greater than 0.5 ppm Ni^{2+} concentrations. The 95.5% acetylene reduction inhibition was observed at 20 ppm Ni^{2+} concentrations (Table 1). The less than 0.5 ppm Ni^{2+} concentrations caused no significant inhibition of nitrogenase activity. For control the rate of ethylene production was 0.80 nmol. For 20, 16, 10, 6, and 0.5 ppm Ni2+ the rate of ethylene production was 0.05, 0.06, 0.10, 0.25, and 0.56 at , respectively (Fig. 2).

Table 1 . The effect of Ni^{2+} on acetylene reduction by *Nostoc calcicola*

Ni^{2+} concentration (ppm)	nmol C_2H_4 produced per 10^6 cells		
	Average	SD	% inhibition
0	6.942	0.321	-
0.5	5.542	0.295	20.1
1	4.13	0.311	42.8
2	2.786	0.214	59.2
4	2.096	0.186	68.4
6	1.547	0.123	74.6
8	1.123	0.156	82.6
10	0.834	0.176	90.1
14	0.764	0.121	92.8
18	0.66	0.116	94.1
20	0.606	0.093	95.5

Activity was assayed after a 5 hours incubation.

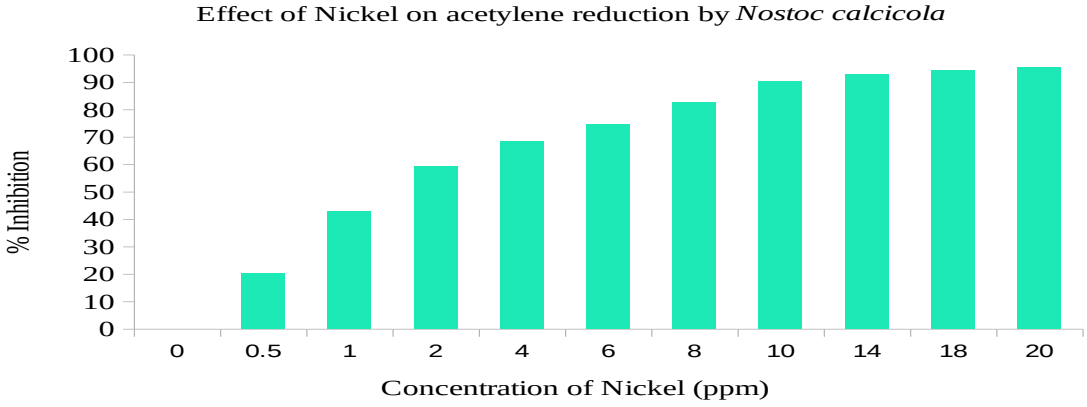

Figure 1: **Graphical representation (Effect of Ni^{2+} on acetylene reduction by *Nostoc calcicola)***

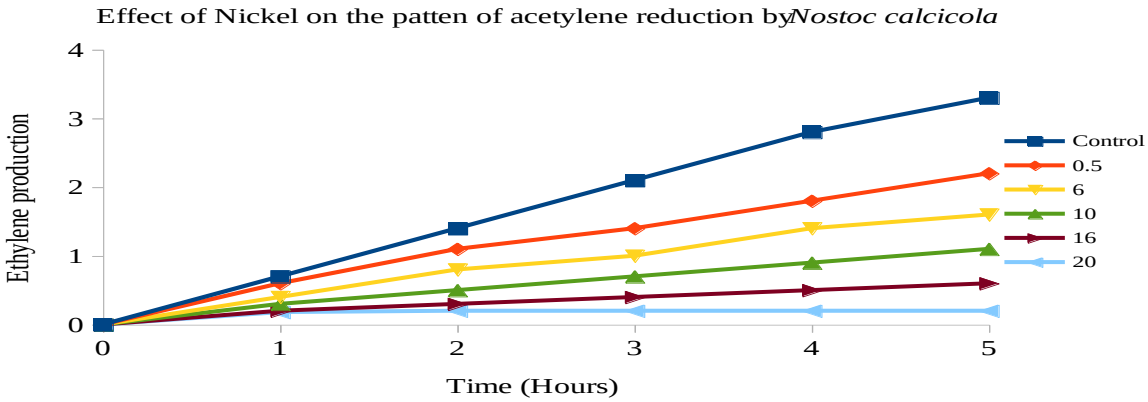

Figure 2: **Effect of Ni^{2+} on pattern of acetylene reduction by *Nostoc calcicola***

Nickel toxicity was significantly increased when the algal cells were preincubated with 0.05, 0.10, 0.25, and 0.50 ppm nickel for 12 hours in in dark such that acetylene reduction was inhibited by 42%, 88%, 94%, and 98%, respectively.

Photosynthesis was also significantly inhibited by nickel concentrations of 0.50 ppm and greater. The 58.6%, 98.2% inhibited was observed by 2 ppm and 20 ppm Ni2+ concentration respectively (Table 2).

Photosynthesis

Table 2 . The effect of Ni^{2+} on $^{14}CO_2$ by *Nostoc calcicola*

Ni^{2+} concentration (ppm)	nmol C_2H_4 produced per 10^6 cells		
	Average	SD	% inhibition
0	5.862	0.236	-
0.5	4.678	0.214	19.3
1	3.482	0.186	38.6
2	2.435	0.167	58.6
4	1.946	0.156	68.4
6	1.426	0.145	76.7
8	0.823	0.135	87.8
10	0.678	0.111	92.8
14	0.513	0.093	93.6
18	0.465	0.086	94.9
20	0.284	0.034	98.2

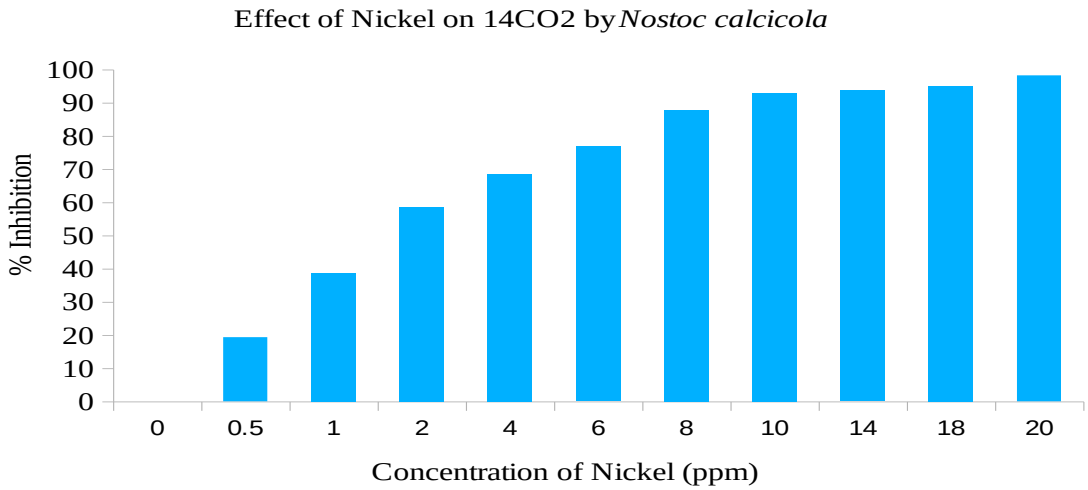

Figure 3: **Graphical representation (Effect of Ni^{2+} on $^{14}CO_2$ by *Nostoc calcicola)***

Nickel toxicity was significantly increased when preincubating the agal cells in the dark for 12 hours in the presence of 0.05, 0.10, 0.25, and 0.50 ppmNi^{2+}concentration. such that photosynthesis was inhibited by 41, 62, 79, and 81%. respectively.

Discussion

Nostoc calcicola was sensitive to nickel ion and it was decreased sensitivity of photosynthesis ($^{14}CO_2$ uptake) and acetylene reduction.The low concentration of nickel ion at <0.5 ppm was not effect photosynthesis and acetylene reduction. The low concentrations of nickel ion have not potential to access the sensitive sites within the cell. (Puckett 1976).

However, it is difficult to make comparisons between these studies because various cell concentrations were used in the methods involved in this study. The nickel toxicity towards photosynthesis and acetylene reduction in A. inaequalis was estimated during short term incubations. The photosynthesis was shown less sensitive than acetylene reduction, when preincubated agal cells for 12 hours in the presence of Ni^{2+} ion concentration. Nickel is accumulated internally by some algae (Stokes 1975) Cyanobacteria and other prokaryotes can directly be damaged by nickel as well. Nickel can target or interfere with important enzymes involved in photosynthesis (Boisvert 2007, Hemlata 2009). The mechanism of this damage is still undefined but it is thought that nickel acts as free-radical initiator which induces severe damage to the photo system (Boisvert 2007). Nickel can also bind to the cell wall of the bacteria inducing stress (Lee 1996). Nickel is also lethal to Scenedesmus in the some concentrationrange (Hutchinson 1973)

Since cyanobacteria are important components of the biosphere, the effects of pollutants on these organisms should be of primary concern.

Conclusions

In the present study, it is concluded that high concentration of nickel ions were produced toxic impact on photosynthesis and nitrogenase activity of *Nostoc calcicola* Breb. Decreased in photosynthesis and nitrogenase activity occurs as increase the nickel concentration in the medium. Therefore there is a need to implement certain rules that help in the reduction of metal level from a wide range of sources such as from the metal processing industries, fertilizers and pesticides producing industries. The vegetable crops have the ability to uptake the heavy metals through their roots and transport them to the edible portion of the plant that are consumed by people or fed to animals and their increased concentrations in human food chain over a long time can cause damage to health.

Acknowledgements

Authors are thankful to Vice Chancellor of Bundelkhand University Jhansi (U.P.) India for encouragement and for providing necessary facilities.

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How to Cite This Article:

Kumar S, and Bhargava S. The effect of nickel on the photosynthesis, and nitrogenase activity of Cyanobacterium Nostoc calcicole. Indian J. Biotech. Pharm. Res. 2022; 10(2): 1 – 7.