Heavy Metal Bioaccumulation of *Nostoc carneum* (C.Agardh ex Bornet & Flahault, 1886) in Vitro Conditions

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Abstract-Today, environmental pollution due to technological development is a big problem. The remove of all kinds of toxic substances and heavy metal that cause pollution has a great importance from the environment and industrial wastes. On the other hand, the presence of heavy metals in the ecosystem can cause toxic and harmful effects, which can pose a huge risk for our health.

For this reason, biosorption of heavy metal ions by algae has emerged as a promising and potentially valuable method for industrial use. The use of microorganisms for the absorption of heavy metals presents an alternative method for the detoxification and recovery of toxic or precious metals from industrial wastes.

In order to be an alternative solution, N. carneum collected from natural habitat in Ayazma National Park (Çanakkale, Turkey) was exposured by heavy metal solution in vitro conditions for bioaccumulation ability. It is thought that the intake of heavy metal toxicity by microorganisms will contribute to an alternative method for new technological developments.

Keywords: *Nostoc carneum, heavy metals, bioaccumulation.*

I. INTRODUCTION

Increasing industrialization and world population since the beginning of the last century has brought along the concern of nutrition. With the partial impact of modern agriculture, natural resources are seriously damaged and seen. Soil is one of our most important natural resources. However, the use of soil for non-agricultural purposes, pollution by heavy metal and organic pollutants such as pollution and erosion, resulting in the loss of productivity. In this context, pollution affects all living organisms and threatens the human, which is the last link in thefood chain. In combating pollution, it is very important to know the conditions of pollution in the soil, the pollutants that cause it, the risks it creates and the conditions necessary for the proper cleaning of the soil. A large amount of time and large costs are required for the determination and improvement of the pollution level of habitat. Heavy metal pollution is a significant environmental problem with a negative potential impacton agriculture and human health. Accumulation of pollutants in the soil has important effects not only on soil productivity and ecosystem functions but also on human and animal health via food chain. Physical remediation technologies are in use to remove and destroy toxic elements. However, phytoremediation is widely viewed as an alternative to the environmentally destructive physical remediation methods. Phytoremediation, the use of plants to remove heavy metals, is an effective, environmentally friendly and cheap method. Further studies on molecular, biochemical and physiological aspects of phytoremediation should be done in order to highlight this technique for the removal of heavy metals. Plants take organic and inorganic pollutants into the body, accumulate, store or disassemble the contaminated areas. In this way, it is aimed to regulate or stabilize the contaminated land. Phytoremediation is important because it is a sustainable, cost-effective and environment-friendly technique according to other breeding methods.

Cyanobacteria called 'Blue-Green Algae' are ancient and simple life forms of earth related to bacteria. Blue green algae have wide ecological tolerance and distributed over almost a large variety habitat. Their diversity and abundance is variable to the different environmental conditions. Due to their ubiquitous nature, they can be found in any environment conditions. They can bear and abide by all environmental extreme conditions and changes. They overcome this with their extensive ecological tolerance.

Nostoc is a genus of Cyanobacteria composed of filaments of moniliform cells in a gelatinous sheath cells barrel-shaped, end-cell conical with a rounded apex, heterocyst barrel-shaped or cylindrical with rounded flat ends[1]. Nostoc can be found in soil, on moist rocks, at the bottom of lakes and springs (both fresh- and saltwater), and rarely in marine habitats. It may also grow symbiotically within the tissues of plants, such as ancient angiosperm Gunnera and the hornworts (a group of bryophytes), providing nitrogen to its host through the action of terminally differentiated cells known as heterocyst.

These bacteria contain photosynthetic pigments in their cytoplasm to perform photosynthesis. Their role as a nitrogen fixer in terrestrial ecosystems allows them to maintain symbiotic interactions with organisms including fungi, lichen, mosses, and ferns. They are largely protected from predation by their outer sheath covering and the large size of their colonies, which make them difficult for some algivores to ingest.

Nostoc, first discovered in the 19th century, is one of the most widespread phototrophic bacteria in the world. As a nitrogen fixer, these bacteria may provide plants with important nutrients and therefore can be used agriculturally. In 1988, terrestrial species Nostoc are very useful in agricultural applications because of their N_2 fixation activity, extracellular polysaccharide, photosynthetic system, and desiccation tolerance. These contribute to improving the quality of nutrient-poor soils [1].

Metals are natural constituents of soils and water and occur naturally in the environment. These metals are directly or indirectly concerned in all phases of microbial growth and enter in higher level of food chains. Many cyanobacterial species are reported remarkable affinity for heavy metals [2]. These cells have developed natural methods of responding to metals such as As, Cd, Cu, Hg and Pb through passive accumulation in cells and through surface binding to various functional groups. They have also been found to remove harmful metals from the environment [3].

Once the metals have been released into the environment, they are difficult to be removed by physical or chemical means and most of them exhibit toxic effects on organisms. Conventional physico-chemical means for removing heavy metals from wastes are generally very expensive [4]. The biological removal of heavy metals has received attention increasingly in recent years because of its potential in environment protection. Algal biomass can be utilized economically for the removal of heavy metals from industrial wastewater or other sources[5].

Accordingly, a great deal of interest has recently been generated in using microbes as biosorbents for metal removal. Algae represent the best biological treatment for wastewater because they increase O_2 content of waters via photosynthesis and sorption of some heavy metals contaminated waters. Sorption of heavy metals on phytoplankton cell surfaces is dependent on a number of factors ranging from the concentration of inorganic ions, dissolved organic matter, pH and the nature of particulates [6]. The extent of sorption and uptake of trace metals is expected to vary in algal cell surface characteristics and in the physiological state of the algae [7].

The aim of the present work was to study the ability of the taxon of Cyanobacteria *N. carneum* in bioaccumulation of some heavy metals under in vitro conditions.

II. MATERIALS AND METHODS

The blue-green algae *N. carneum* were collected fromfrom a channel supplying water to the fish breeding facility from Ida Mountain in Bayramiç-Çanakkale. The samples were dried at room temperature before dried in the oven at 70 ºC for 8 hours.

The study was carried out in laboratory. For experimental analyses, the dried samples were converted into flour by a grinder. Samples (0.5 g) were immersed in 250 ml 0.02 M metal solutions for 72 hours. These metal solutions were 0.02 M $CuCl_2$, $ZnCl_2$ and $Pb(NO_3)_2$. Also 0.5 g samples the species were immersed deionized water for 72 hours for control group.

0.5 g was weighed for each sample group into a 100 ml beaker. Than 10 ml of concentrated HNO_3 was added and allowed to stand overnight. Then, on the hotplate, , samples were heated carefully until the red NO_2 smoke had run out. On cooling samples, 3 ml of 70% $HClO_4$ acid was added and heated to boiling point. The samples were then filtered using a vacuum pump with cellulose filter papers. The solution was transferred to a 50 ml brown, capped flask and diluted with deionized water until 50 ml was completed. At the same time, each bottle was carefully labeled and the mineral and heavy metal concentrations in the solution were determined by Inductive Coupled Plasma-Mass Spectrometry (ICP-MS) using the standard preparation and calibration graph method.

III. RESULTS

The results for each group of the samples can be seen from Table 1.

As can be seen from Table 1, for control group metal concentrations were found at lower than metal solution treatments. For $CuCl_2$ treatment, the quantity of Cu was detected at very high level (≥ 10000). Also for $Pb(NO_3)_2$ group, the quantity of Pb was determined at very high level (≥ 10000). Finally for ZnCl2 treatment, the Zn level was found too high (≥ 10000).

At the end of the study, both metal concentrations have increased apparently after the treatment of 0.2 M metal solutions.

IV. DISCUSSION

Biosorption can be an alternative method to eliminate heavy metals due to its low cost, high efficiency, and minimization of secondary wastes and environmentally friendly. Green algae can be used as natural biosorbent to reduce chemical usage in waste treatment process which indirectly reduces the cost of the treatment plant [8]. Microorganisms exhibit a number of metabolism dependent and independent processes of the uptake and accumulation of heavy metals [9]. Microalgae are efficient absorbers of heavy metals [10, 11, 12, 13, 14].

The metal absorption efficiency depended on the type of biosorbent, the physiological state of the cells, and availability of heavy metal, concentration of heavy metal and chemical composition of the environment. A few heavy metals at certain concentration act as essential nutrients critically involved in the functional activities of proteins. Also they involved in supporting growth and development of living organism. However, at excess concentration of these metal ions can become harmful of living organism like Cyanobacteria. Plant species may have evolved different physiological and molecular mechanism to tolerate heavy metal damage [15]. Abd El-Hameed et al., studied two cyanobacteria sp. (*N. muscorum* and *A. variabilis*) to test to remove lead at four concentrations (0, 15, 30 and 50 mg/L). *N. muscorum* was found to be more effective than *A. variabilis* for bioremoval of lead from contaminated water. Based on these results, biomass of *N. muscorum* and *A. variabilis* can be used as an efficient low cost biomass for the removal of lead from wastewater [16].

Arun et al., examined Cu (II) removal by *N. muscorum* in a batch operated photobioreactor and its effect on biomass growth and nitrate uptake by the cyanobacterium. They found that Complete removal of Cu (II) was achieved at all the four different initial Cu (II) concentration [17].

Hazarika et al., investigated that metal bioremoval study conducted using *N. muscorum*, more than 60 % removal was achieved for all the heavy metals tested. It has also been reported that cyanobacteria tolerate and remove Pb (II) ions from solution by transforming it to insoluble sulphide or phosphate forms [18].

Hudek et al., provided that *N. punctiforme* discriminates between multiple metals with uptake of one achieved at the exclusion of another, possibly more toxic metal that can also influence the homeostasis of each other. The uptake rate of individual or multiple metals, provides insight as to the ability of N. punctiforme cells to effectively discriminate and regulate between metals [19].

Roy et al., investigated that the metal removal by *N. muscorum* in this study involves initially quick sorption onto the cell surface followed by a successive slow uptake of the metals inside the cells. Both biosorption and bioaccumulation seem to play a role on metal bioremoval by *N. muscorum* [20].

Heavy metals such as As, Pb, Cu and Hg are reported to involve for proper functioning of living organisms but also these metals can promote severe toxic effects depends on the type of organism and the nature [21].

Algae based system for the removal of toxic minerals such as lead, cadmium, mercury, scandium, tin, arsenic and bromine are also being developed [22]. Some researchers studied the effects of heavy metals on Cyanobacteria. They determined the pigment and protein content decreasing with increase of the concentration of heavy metals in the species of marine Cyanobacteria. They tested *Nostoc sp*. for their ability to tolerate different concentration of Cr [23]. Researcher indicated that Cyanobacteria have capacity to accumulate metal ions very effectively [24].

In this study,the results indicated that all heavy metal (Cu, Pb, Zn) concentrations were permissible for the species *N. carneum*. According to the study, the different metals uptake depended upon the type of biosorbent, which has different accumulation affinities towards the tested elements and this results are in agreement with the results of our investigation [25]. In another study, Brady et al., found that *Scenedesmus*, *Selenastrum and Chlorella* spp. were capable of accumulating metals such as Cu and Pb with high efficiency [26].

Heavy metal toxicity is one of the major abiotic stress factors and different species may have different mechanism to tolerate heavy metal toxicity. Cyanobacterial species are excellent indicators for the toxic metabolic levels of heavy metals. They have ability for to incorporate these metals [27]. Also these cells have developed natural methods of responding to metals through passive accumulation in cells various functional groups. It was observed that cyanobacterial cells entreated in with high metal concentration solutions accumulated higher metal contents (≥ 10000). This observation has been also investigated by Wong and Tam cultivated algal cells in with very high metal contents, also accumulated higher metal contents [28].

Figure 1. Sample N. carneum in natural habitat

Table 1. The results of determined values for metal content of N. carneum before and after treated at 0.02 M CuCl2, ZnCl2 and Pb (NO3)2 (mg/g).

Analyte (Unit)	Cu	Pb	Zn
Sample groups			
Control group	8.32±0.2	3.33±0.4	16.5±0.1
$CuCl_2$	≥10000	263.84±0.2	38.2±0.7
$Pb(NO_3)_2$	12.26±0.4	≥10000	18.1±0.5
$ZnCl_2$	227.49±0.2	31.32±0.1	≥10000

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