# Lipid Profiling and DNA Profiling: Biomarkers Against Metal Stress in Nostoc Muscorum

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## Abstract

The mechanisms by which different metals exert their toxicity in living organisms is very diverse, especially their involvement in oxidative biochemical reactions through the formation of ROS. The present study focuses on the molecular targets of the metal stress as high concentrations of metal ions are known to promote a variety of damages at the molecular level, including

oxidative stress induced damage to lipids of biological membranes and DNA. Metals exerted differential toxicity on different parameters of *N. muscorum*. Amongst the selected metals Cu gave contrasting results at different treatment doses whereas Al adapted well at all the chosen concentrations. Lipid chromatography of *N. muscorum* following 20 µM metal (Al and Cu) showed 7 bands of lipid. The lipid profile after treatment with Al showed negligible decrease in the intensities of the bands whereas Cu treatment showed prominent decrease in band intensities. Further DNA fragmentation analysis under metal stress is performed. In case of Cu, the fragmented DNA migrated rapidly in the agarose gel leaving a smear like pattern on the gel whereas no fragmentation was observed in Al treated *N. muscorum*. The present study for the very first time reports the unequivocally differential response exhibited by Al and Cu.

**Keywords:** *N. muscorum*, aluminum, copper, DNA fragmentation, Lipid profile

## 1. Introduction

Metal contamination of ecosystems is a serious global environmental concern. Several metals enhance the intracellular formation of reactive oxygen species (ROS) by mechanisms depending on their chemical nature which can lead to the damage of macromolecules and to a decrease of oxidant defenses in a wide range of non-target microorganisms including cyanobacteria [1]. Aquatic organisms have been considered as test species because of their sensitivity to oxidative damage from concerning chronic exposure or sublethal concentrations of metals. They can provide model systems for investigation of different mechanisms of ROS mediated damage to cellular components, how cells respond, how repair mechanisms ameliorate this damage, and how oxidative stress can lead to disease [2].

The mechanisms by which metals exert their toxicity in living organisms is very diverse, especially their involvement in oxidative biochemical reactions through the formation of ROS. Molecular mechanisms of metal cytotoxicity include damage to plasma membranes, following binding to proteins and phospholipids, inhibition of Na, K dependent ATPases, inhibition of transmembrane amino acid transport, enzyme inhibition, lipid peroxidation and oxidative DNA damage, depletion of antioxidant enzymes (such as glutathione) through the generation of ROS [3].

Metal ions can penetrate inside the cell, interrupting cellular metabolism and in some cases can enter the nucleus. Metals and their chelate complexes, such as copper, aluminum are implicated in lipid peroxidation which is initiated mainly by hydroxyl radicals, especially in transition metal-catalyzed reactions and subsequently in the promotion of cell death [4]. DNA is another

key cellular component that is particularly susceptible to oxidative damage by ROS. Metal cations can also bind to DNA through ionic and coordinated bonds in a reversible way, but cannot produce all the lesions observed in chromatin of cells. Hence, not only the direct, but mostly indirect effects of metals on nuclear chromatin must be considered more important in DNA damage. Additionally, the heterogeneity of DNA molecules allows for $HO^{\bullet}$ attacks, including the nucleobases and the sugar–phosphate backbone resulting in fragmentation of deoxyribose with single-strand breaks, and oxidation of the sugar moiety leading to programmed cell death (PCD) [5].

Copper (Cu) and Aluminum (Al) show widespread occurrence, varying toxicity and tolerance level in organisms due to the difference in mechanisms of toxicity elicited by them. Al is a major constituent of acid soils and is one of the major stresses to plants. Although most of the Al in soils is incorporated into precipitated forms, which are harmless to plants but under acid soil conditions, these minerals solubilize to a limited extent [6] and the toxic ion $Al^{3+}$ is released into the soil solution. The only oxidation state for aluminum in biological system is +III and any binding of Al will therefore mainly be electrostatic and has an especially strong affinity for sensitive binding sites of the cell wall, phosphate ions as well as for organic phosphorus compounds [7]. These rapid Al-induced changes in cell wall can lead to the inhibition of water and mineral uptake imitating drought stress [8].

There is a sizeable volume of literature available regarding Al toxicity to higher plants whereas there are also reports indicating that Al has a beneficial effect on plant growth and this seems to be especially true for native plant species that are adapted to acid soils [9]. Although there are several reports demonstrating Al tolerance nevertheless the exact mechanism underlying Al-tolerance is least understood.

Cu in the form of $Cu^{2+}$ is an essential microelement which when present in high concentration accumulates in microalgae and interferes with numerous physiological processes as a component of different enzymes, taking part in electron flow and redox reactions in the photosynthetic apparatus [10]. Latest investigations demonstrate that cyanobacteria have metal requirements resulting in $Cu^{2+}$ trafficking to the thylakoids [11]. However, it was found, that excessive $Cu^{2+}$ concentration has harmful effects on growth and photosynthesis, destroying the photosynthetic apparatus and plasma membrane permeability of plants [12] and algae.

Considerable information on metal toxicity with respect to various physiological mechanisms exist but only limited data are available regarding the toxic effect of Al and Cu on biomolecules. Thus, the aim of this study was to assess the toxicity of two chemically diverse metal (Al and Cu) on

*Nostoc muscorum*, as it has already been screened as a moderately sensitive strain for assessment of metal toxicity [13].

## 2. Material and methods

### 2.1. Organism and growth conditions

*N. muscorum,* a filamentous, heterocystous cyanobacterium was obtained from Dr. Sheo Mohan Prasad, Department of Botany, University of Allahabad, Allahabad, India. The strain was maintained in the culture room at 27 ± 2 °C. For regular experiments, cultures were grown in CHU-10 medium (pH 7.5) without nitrogen source under photosynthetic photon flux density (PPFD) of 75µmol $m^{-2}$ $s^{-1}$ and 14hrs photoperiod. The cultures were shaken manually two to three times daily.

### 2.2. Preparation of metal solution and treatment

Stock solution of $CuCl_2.2H_2O$ and $AlCl_3.6H_2O$ (1000 µM), was prepared in sterilized double distilled water and the solution was further sterilized by passing through Millipore membrane filter (0.22 µm). From the stock solution required concentration (20µM) were prepared in 100 ml of CHU-10 medium containing culture suspension equivalent to 0.1 mg $ml^{-1}$ dry weight. One set of experiment was performed for each parameter with three different measurements. Further, the cells were withdrawn from the treated and untreated cell suspensions depending upon the incubation duration and amount required for various experimental studies.

### 2.3. Extraction and estimation of total lipids

The total lipids were extracted from treated and untreated cyanobacterial biomass using modified method of [14]. The extracted total lipids were separated by thin layer chromatography (TLC) following the method of [15]. Visualization of the plate was performed by Iodine vapor method [16].

### 2.4. DNA Extraction and Analysis

A 50-mL of logarithmic phase culture of untreated and NaCl treated *N. muscorum* (optical density 0.5 at 750 nm) was taken and DNA was extracted by proteinase K digestion method [17]**.** Untreated and NaCl treated DNA were mixed with the sample buffer (0.125% bromophenol blue, 30% glycerol) in 3:1 ratio and loaded in the wells of 1% agarose gels. Electrophoresis was carried out for 2 hrs. at 50V and the gels were viewed under UV light.

## 3. Results and Discussion

### 3.1. Comparative Lipid profile using TLC

Lipid chromatography of *N. muscorum* following 20 µM metal (Al and Cu) exposure for 3 days showed 7 bands of lipid after iodine vaporization (Fig. 1 A and B) of which 3 of them were relatively more prominent. Rf value: 0.36 corresponding to DG/PG (Digalactosyldiglyceride/ phosphatidyl glycerol), Rf value: 0.47 corresponding to GL (Glycolipid) and Rf value: 0.57 corresponding to unidentified glycoside. The lipid profile after treatment with Al showed negligible decrease in the intensities of the above bands whereas Cu treatment showed prominent decrease in band intensities.

Earlier worker reported the presence of 10 lipid spots in *Anabaena ambigua* [18, 19] and 8 spots in *Mastigocladus laminosus* [20]. In present findings the Lipid spots showed similar Rf values (0.36, 0.47 and 0.57) as reported by earlier workers suggesting the presence of sulphaquionovosyl diglyceride, Glycerol and digaloctosyl diglyceride, monogalactosyl diglyceride. The loss of intensity of lipids bands by Cu be due to the destruction of lipids caused by peroxidation [21]. No change in the lipid profile in the Al-treated *N. muscorum* further strengthen our notion that the Al either produces relatively less level of deleterious ROS or they may be detoxified due to induction of various dehydrins like protein.

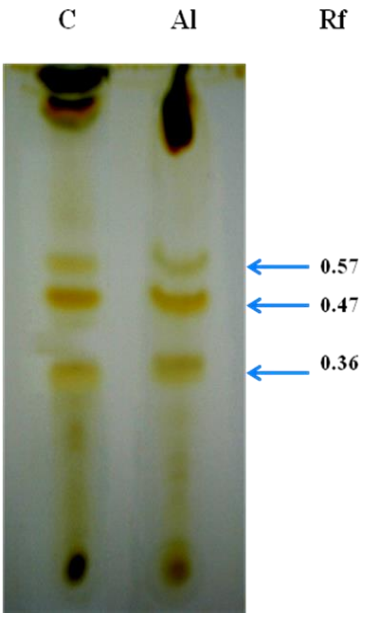


**Fig. 1. (A)**

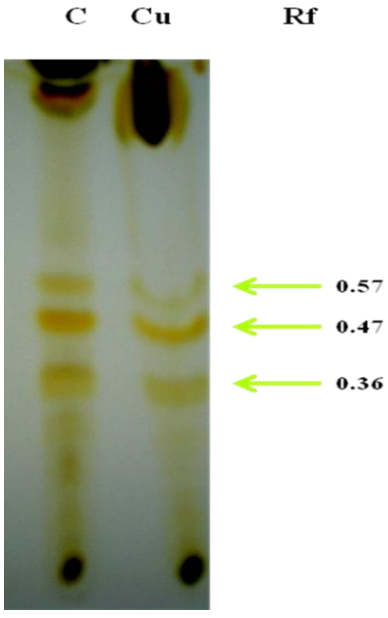


**Fig. 1. (B)**

**Fig. 1:** Thin-layer chromatogram of lipid extracts from cell preparations of *N. muscorum* (A) (Lane 1 Total lipids from untreated *N. muscorum*; Lane 2 Total lipids from 20µM Al treated

*N. muscorum*. (B) (Lane 1 Total lipids from untreated *N. muscorum* Lane 2 Total lipids from 20 µM Cu treated *N. muscorum*.

### *3.2. DNA fragmentation analysis using agarose gel electrophoresis and Fluorescence spectral studies under metal stress*

As shown in Fig. 2 (A) (Lane 1), genomic DNA was detected in the form of a single sharp band on the upper portion of the gel in the untreated sample showing no fragmentation of DNA. When *N. muscorum* was exposed to 20µM Al for 48 hours, again a single band of DNA was observed at the position where genomic DNA was detected in the untreated *N. muscorum* indicating that the DNA in 20µM Al treated *N. muscorum* was not fragmented. Fig 2 (B) (Lane 2), clearly shows the fragmentation of DNA from Cu treated *N. muscorum*. In case of Cu, the fragmented DNA migrated rapidly in the agarose gel leaving a smear like pattern on the gel.

Transition metal like Cu that possess high-binding affinity to DNA sites can catalyze the production of $OH^{\bullet}$ in close proximity to the DNA molecule, thus ensuring repeated attack upon the DNA by an efflux of hydroxyl radicals [22]. A similar pattern of DNA damage was observed by [23] in freshwater *(Nostoc spongiaeforme)* and marine *(Phormidium corium)* cyanobacteria exposed to light stress. However, further work is needed to identify the actual cause and mechanism of degradation.

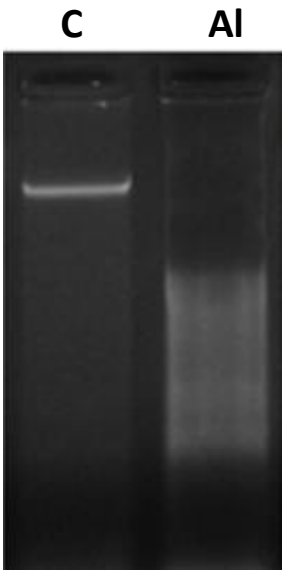


**Fig. 2. (A)**

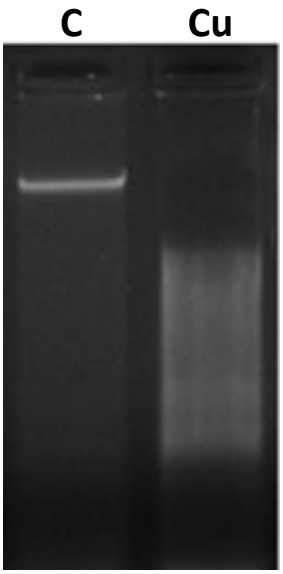


**Fig. 2. (B)**

**Fig. 2:** DNA fragmentation pattern (A) in presence of 20 µM Al (Lane- 1 DNA from untreated *N. muscorum*; Lane- 2 DNA from 20µM Al-treated *N. muscorum* (B) (Lane 1 DNA from untreated *N. muscorum*; Lane 2 DNA from 20µM Cu-treated *N. muscorum.*

### 3.3. Fluorescence spectral analysis

Fluorescence spectra of isolated DNA (5 µg/ml) of untreated and metal treated (Al and Cu) *N. muscorum* was taken using ethidium bromide as an external chromophore (Figure 5). DNA isolated from Cu treated *N. muscorum* exhibited maximum decrease in fluorescence intensity as compared to the control. However, Al treated cyanobacterium did not show any significant decrease in fluorescence intensity (7.1%).

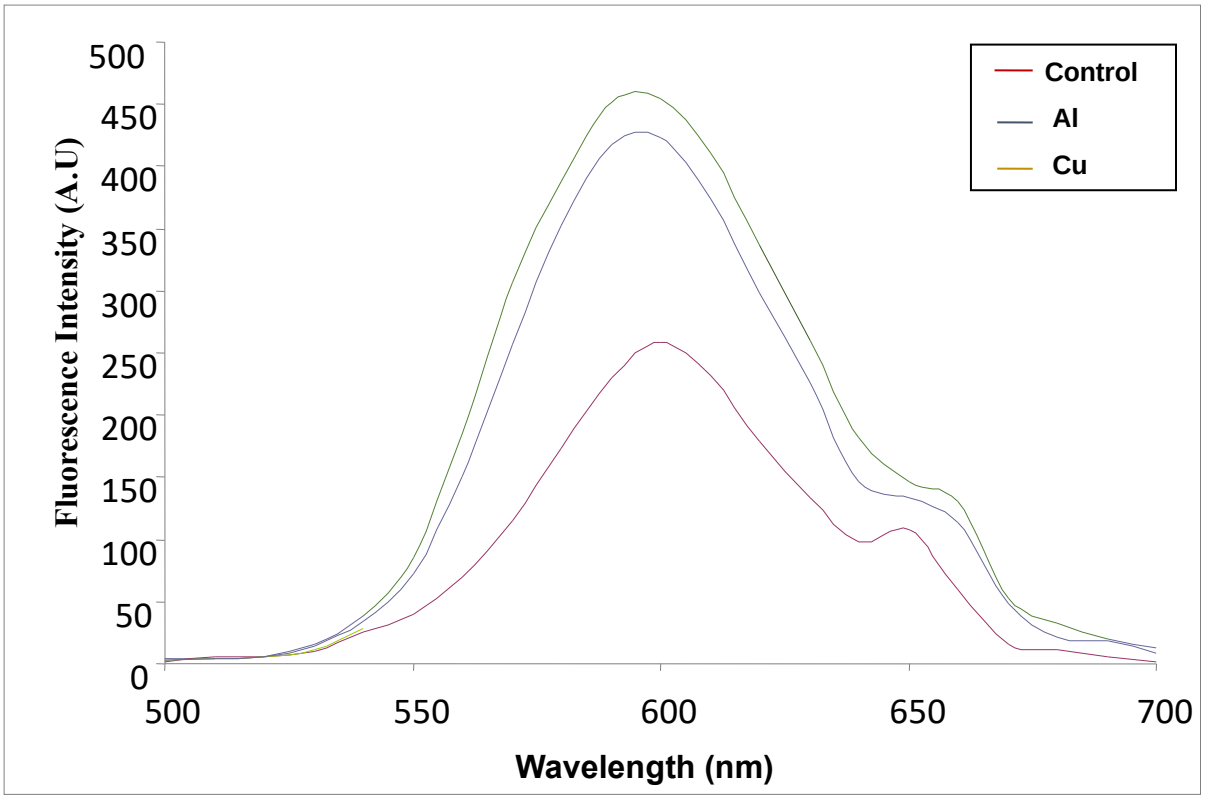


**Fig. 3:** Fluorescence emission spectra of isolated DNA from untreated and metal treated (Al and Cu) *N. muscorum.*

Further, the levels of lesions in the untreated DNA were quite similar to that obtained with the Al-treated DNA indicating no DNA damage with Al. It is a well-known fact that Al induces

dehydration stress and the relatively no DNA damage in the Al-treated *N. muscorum* could be attributed to its dehydrating ability as the DNA in dry state is less prone to the damage by ROS [24]. In another study conducted by [25] on root cells of *Allium cepa*, it was shown that low concentrations of Al (1–10µM) induced adaptive response conferring genomic protection which is again in good agreement with the induction of various proteins in the Al-treated *N. muscorum*. To our knowledge, only one publication has shown DNA degradation during KCl-induced PCD in *Anabaena* sp. [26]. In the present study, it is demonstrated that the metal stress (Cu) causes DNA fragmentation in *N. muscorum*. This is in accordance with [27] who presented evidence, that salt-induced PCD in plants and yeast is caused by ionic stress. [28] also demonstrated that salt stress-induced cell death in the unicellular green alga *Micrasterias denticulate.* Salt stress-induced DNA laddering as a hallmark of PCD has also been described in barley roots [29], rice root tips [30] or tobacco protoplasts [31].

Further the fluorescence emission spectral studies of DNA which were carried out in the presence of the two test metals showed the occurrence of metal induced strand breaks. Cu was found to be the more damaging than Al. This may be attributed to the perturbation of electronic state of the bases by interaction of $Cu^{2+}$ ions in G-C pairs finally leading to strand breaks.

**Conclusion**

It can be concluded, that Al-induced stress in *N. muscorum* is probably dealt with a different physiological and molecular mechanism(s). the absence of significant lipid degradation and DNA fragmentation in Al-treated cells suggests that Al-induced stress is either less deleterious or is mitigated by protective responses such as dehydration tolerance or the induction of stress-associated proteins. In contrast, Cu exposure led to marked lipid peroxidation and pronounced DNA fragmentation, indicating severe oxidative damage and activation of programmed cell death (PCD) pathway.

These observations underscore the chemically diverse nature of the two metals and their distinct modes of toxicity. Moreover, the observed alterations in lipid profiles and DNA integrity under metal stress highlight the utility of these parameters as reliable oxidative biomarkers for assessing environmental metal toxicity in cyanobacteria. Taken together, this study provides valuable insights into the metal-specific stress responses in *N. muscorum* and lays the groundwork for further investigations into the cellular defense mechanisms employed by cyanobacteria against abiotic stress.

**Authors' contributions:**

Adeeba Shamim: Writing – original draft, Conceptualization. Sadaf Mahfooz: Writing – review & editing. Irum: Writing – review & editing. Gyanendra Tripathi: Writing – review & editing Suhail Ahmad: Writing – review & editing Alvina Farooqui: Supervision, Conceptualization, Review & editing.

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