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Effect of Glucose, Glutamate, and 2-Oxoglutarate on Mercury Toxicity to *Chlorella vulgaris*

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Heavy metals are the pollutants of major concern because they concentrate within the organism to a level greater than that in the environment and remain persistent for long period. Ironically in many of the fresh water bodies especially in the rivers and lakes the human input of these unwanted elements is many times greater than the natural inflow (Nriagu et al. 1979). Among them mercury is the most important heavy metal which finds its way into aquatic habitat through domestic sewage, industrial effluents, coal mine drainage, and agricultural runoffs. The toxicological effect of mercury is intensified due to transformation of organic and inorganic compounds into a more toxic form by the microorganisms. Being cumulative in nature, mercury toxicity in course of time is likely to get biologically magnified becoming more catastrophic to the mankind and man's environment.

Numerous studies have demonstrated that the organic and inorganic constituents of a medium affect the toxicity of heavy metals both in controlled and natural conditions (Babich and Stotzky 1979; Rai et al. 1981; Rai and Raizada 1988; Sudhakar et al. 1991). Considerable variation exists among algal toxicity methods despite attempts at standardization. Experimental end points in these studies range from percent inhibition to LC50's. However, Payne and Hall (1979) suggested that the algistatic concentration of a chemical can be used to study its toxicity without a population bias. Taking this into view we observed the effects of three separate carbon sources (glucose, glutamate, and 2-oxoglutarate) on the toxicity of the algistatic dose of Hg^{2+} in case of a green alga Chlorella vulgaris.

MATERIALS AND METHODS

The axenic cultures of Chlorella vulgaris Beij. were grown at 27± 2°C in 50 mL of Chu No 10^{+} medium (Safferman and Morris 1964) with A_6 micronutrients (Gerloff et al. 1950) contained in 100-mL borosilicate glass conical flasks. Standard inoculum of mid-log

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preculture (7 d old) was 0.4 mL per 10 mL (initial density 7.2 x 10^5 cells/mL), contained in 18 x 150-mm borosilicate culture tubes for the experiments. The cultures were maintained in a condition similar to that described by Mohapatra and Mohanty (1992).

Survival test was made by inoculating the alga in nutrient agar plates (initial density 15 cells/cm^2) separately with varying concentrations of Hg^{2+} (0.0 to 0.3 mg/L) using $HgCl_2$, available from Glaxo India Limited, Bombay (99.5% pure); and by counting the colonies after 10 d with the help of a binocular microscope. Glucose, glutamate and 2-oxoglutarate (available from E.Merck India Limited, Bombay as 99%, 99.5% and 99.5% pure, respectively) were added separately at different concentrations (0.0 to 20.0 mg/L) to the culture tubes containing the static dose of Hg^{2+} (0.200 ± 0.004 mg/L at p = 0.05) at log phase to study the effect of these nutrient sources on Hg^{2+} toxicity. The cultures containing only the static dose of Hg^{2+} served as the control. The experimental sets were taken in triplicates and this was repeated three times. Analysis of growth (O.D. at 678 nm), pigment biomass (Arnon 1949), and protein contents (Lowry et al. 1951) was made at every 4 d till 12 d. The lowest significant deviations (LSDs), confidence limits (at p = 0.05) and standard errors were calculated to find out the degree of significance of the results (Snedecor and Cochran 1967). The LC50 and static concentration of the heavy metal to C. vulgaris were calculated plotting survivability against concentration on a log-log graph and included in the text.

RESULTS AND DISCUSSION

The average values of percent survival of C. vulgaris at different nominal concentrations of Hg^{2+} showed that though slight decrease in the survival rate up to 0.01 mg/L of Hg^{2+} was observed, it was not significant (LSD = 2.08, p = 0.05). On the other hand, significant reduction of the survival rate of the alga was observed with $\geq$ 0.1 mg/L of Hg^{2+}. In case of the test alga the LC50 of the heavy metal was 0.128 ± 0.009 mg/L, while the population remained static at 0.200 ± 0.004 mg/L (at 95% confidence limit) and had complete death at 0.3 mg/L.

Addition of glucose, glutamate, and 2-oxoglutarate to the Hg^{2+} - amended Chu No 10^+ medium, however, resulted in acceleration of growth of C. vulgaris (Fig. 1). The growth enhancement in presence of glucose was found significant at all the tested concentrations even at the very first observation, i.e., 4th d (Fig. 1A). On the other hand, growth acceleration was not significant with 10 mg/L of glutamate till the 8th d while on the 12th d the acceleration of growth even at the lowest tested concentration (5mg/L) was found significant (Fig. 1B). But with 2-oxoglutarate, the lowest tested concentration did not show significant enhancement of growth vis-a-vis reduction of Hg^{2+} toxicity till the end of the experiment though other tested concentrations (10-20 mg/L) caused significant growth acceleration (Fig. 1C). Among the three carbon

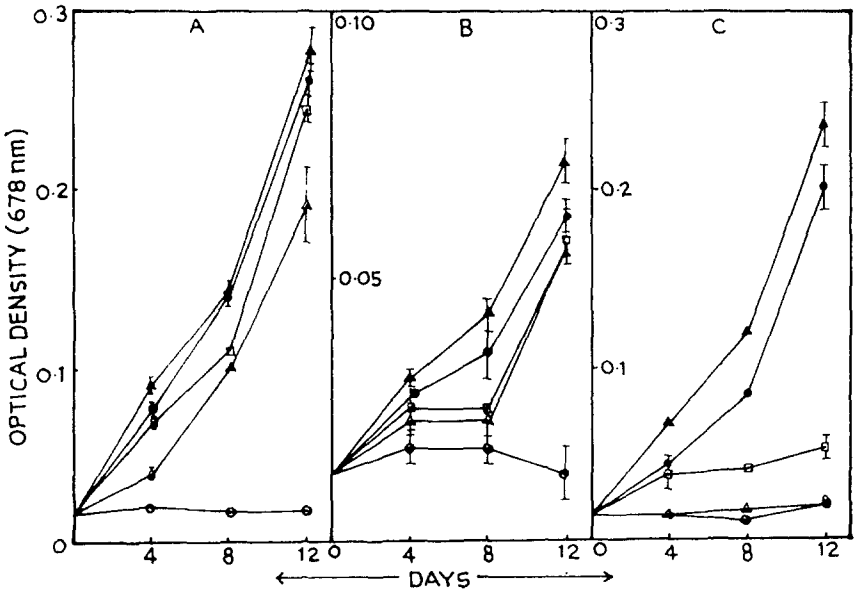

Figure 1. Effect of Hg^{2+} at the algistatic dose (0.2 mg/L) supplemented with (A) glucose, (B) glutamate, and (C) 2-oxoglutarate on growth of C. vulgaris. o——o 0, Δ——Δ 5, □——□10, ●——● 15, and ▲——▲ 20 mg/L carbon compound . Vertical lines represent the standard errors of the means.

sources, growth in the glucose enriched Hg^{2+} -amended Chu No 10^{+} medium was more accelerated whereas enrichment with glutamate showed the minimum.

The chlorophyll biomass of C. vulgaris was found to be highly influenced by the carbon sources in Hg^{2+} -amended cultures (Fig. 2). At the static dose, chlorophyll biomass of the test organism was very low and there was no significant increase in pigment content till the 12th d, though at all the tested concentrations of each carbon source the content was increased. However, the rate of acceleration and the degree of significance (LSD) varied with time, carbon source and dose. In glucose enriched cultures the increase in pigment content was significant only at concentrations $\geq$ 10 mg/L on the 4th d but at all the tested concentrations on subsequent days. Similarly in glutamate-amended cultures pigment contents varied significantly at all the tested concentrations except with 10 mg/L on the 4th d and between 5 and 10 mg/L on the 8th d (Fig. 2B). On the other hand, the pigment biomass of C. vulgaris supplemented with 2-oxoglutarate was found significant at all the tested concentrations (Fig. 2C).

Protein content of the test alga was enhanced at all the tested concentrations of the added carbon sources (Fig. 3). Glucose was found to be most effective in increasing the protein content despite the presence of static concentration of Hg^{2+} (Fig. 3A). Significant increase in protein content was observed with increase

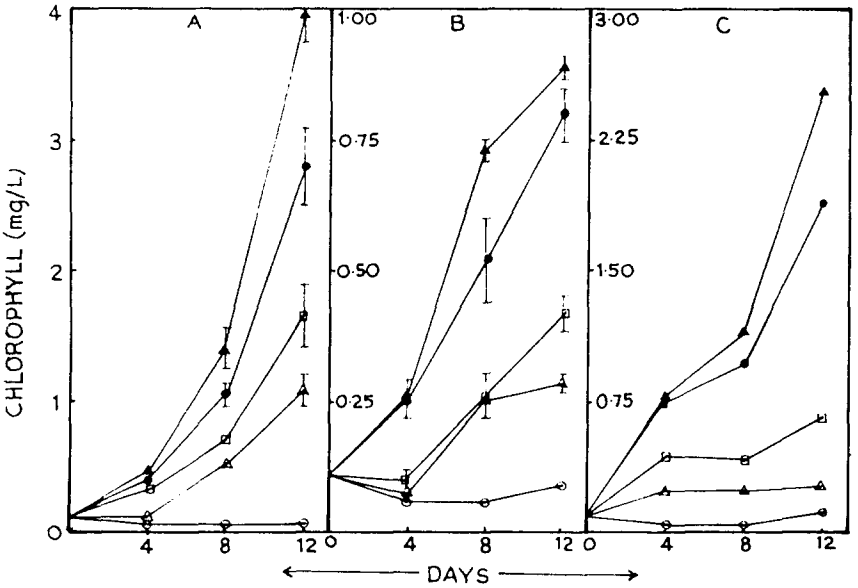

Figure 2. Effect of Hg^{2+} at the algistatic dose (0.2 mg/L) supplemented with (A) glucose, (B) glutamate, and (C) 2-oxoglutarateon chlorophyll biomass of C. vulgaris. ⊖——⊖ 0, Δ——Δ 5, □——□ 10, ●——● 15, and ▲——▲ 20 mg/L carbon compound. Vertical lines represent the standard errors of the means.

in glucose concentrations in all the three observations, exceptions being at 10 and 15 mg/L of glucose on the 4th d, and at 15 and 20 mg/L on the 8th and 12th d. Compared to the control, there was almost seven fold increase in the protein content after 11 days of incubation with 5 mg/L, i.e., the lowest tested concentration of glucose. On the other hand, no increase in protein content with different concentrations of glutamate was observed on the 4th d compared to that initially (Fig. 3B). On this day, protein was found to be lower than the initial. However, the content increased at concentrations $\geq$ 10 mg/L of glutamate on the 8th d and significantly at all the tested concentrations on the 12th d. Similarly with 2-oxoglutarate, degradation of protein was observed at $\leq$ 15 mg/L on the 4th d and at 5 mg/L on the 8th d while the content increased at other concentrations (Fig. 3C). The increase in protein content was only significant at $\geq$ 15 mg/L and at all tested concentrations of the carbon source on the 8th d and 12th d, respectively.

It is known that free, uncomplexed metal ion is most toxic form of a metal to microalgae. Thus the effects of the environmental variables on the bioavailability and the toxicity of Hg^{2+}, in case of aquatic microorganisms, are usually attributed to their impact on the availability of free mercury (Babich and Stotzky 1979; Rai et al. 1981). Studies with copper, however, have demonstrated that addition of inorganic (ammonia, and hydroxyl) and low molecular weight organic (alanine, glycine, glutamate, and

mercury ligand species (Hg^{2-zL}) and the toxicity of the Hg salts (Babich and Stotzky 1979). It is, therefore, to be expected that the relative toxicity of these complexes would be less than that of the free Hg^{2+} because of the reduced charge (net charge = 2-zL) and the effects of steric hinderance. Certain carbon sources like glutamate, glutamine, aspartate, alanine and proline form the respective neutral complexes with free Hg^{2+} (i.e.,the Hg^{2+} ion is bound through the α-amino and α-carboxyl groups and carries a net charge of +1 while the terminal carboxyl group is negatively charged, resulting in a net charge zero). Being neutral such complexes have a low affinity for the binding sites on the cell surfaces (Farrell et al. 1990). Similar role of complexes is possible in our experiment.

Measurement of chlorophyll biomass is a means to assess the photosynthetic efficiency of algae (Myers 1951). Increase in pigment biomass enhances the photosynthetic activity of the algae while decrease in their concentration retards photosynthesis. The reduction of photosynthesis and chlorophyll content was reported when Hg^{2+} was added with humic acid instead of other organic nutrients (Hongve et al. 1980). This was attributed to the acidic nature of the carbon source by Hongve et al. (1980). In the present study the low ameliorative action of glutamate in the Hg^{2+} amended system is probably due to the above fact. The increase in growth rate with increased concentration of carbon sources might also be due to acceleration of rate of photosynthetic carbon fixation by the alga.

Mercury affects the protein content of algae by influencing both synthesis and degradation process (De Filippis and Pallaghy 1976). The metal ion has a greater affinity towards sulphur containing amino acids than the other amino acids. It attacks the -SH bond causing the reduction in synthesis and acceleration in degradation of proteins (Rai and Raizada 1988). The binding preference of Hg^{2+} for sulfhydryl,thioether , and imidazole groups at catalytically active centers in enzymes provides the biochemical basis for much of its toxicity. Consequently, molecular arrangements which inhibit the ability of Hg^{2+} to combine with these enzymes will result in reduced toxicity. The ameliorative effects of the carbon sources in the present experiment vis-a-vis the direct relation between the increase in protein content and organic carbon addition might be due to such molecular arrangement of the complexes. Moreover, because the Hg^{2+} ion is bound at the center of such complexes (Babich and Stotzky 1979; Farrell et al. 1990), it is presumably shielded from direct interaction with the cells. This probably protected the protein synthesizing enzymes from direct toxic action of Hg^{2+} and resulted in synthesis of proteins.

Secondly, Chlorella has been found to grow well with addition of organic carbon sources. It can grow in the dark on added glucose, maintaining its pigment system intact (Myers 1951). One possible

generalization is that the monosaccharides are more effectively taken by the algal cells when compared with other carbon sources. Addition of such organic carbon sources provides additional energies which suppress the inhibitory action of heavy metals on metabolic activities of phytoplankton (Wu and Lorenzen 1984). In the present observation glucose was found most effective in toxicity reduction of Hg^{2+} leading to the conclusion that the ameliorative action of a carbon source on Hg^{2+} is directly related to its energy content and its ready utilization by the cell. However, such action may vary with the test organisms, culture conditions and Hg^{2+} concentrations.

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