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Effect of Heavy Metals on the Growth of Total Phytoplankton Load

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ABSTRACT: The experiment was performed to evaluate effect of heavy metals on total phytoplankton load (TPL) using water of Turag River adjacent to Ashulia locating on the north-eastern side of Dhaka city, Bangladesh. Total phytoplankton load comprises of *Euglena sp., Borodinella sp., Pediastrum biradiatum, Pinnularia sp., Fragillaria sp., Fragillaria crotonensis, Gloeocapsa sp., Navicula sp., Cynedra sp., Crucigenia sp., Chlorella sp., Spirogyra sp., Phacus acuminatus, Phacus circulatus., Nitzschia sp*. and *Nitzschia clausii*. Phytoplankton load showed the abundances Bascillariophyceae (43.75%) > Chlorophyceae (37.50%) > Euglenophyceae (18.75%). The average maximum growth rate (log transformed) of TPL in control culture was -0.25µg/l and treated cultures using 1ppm, 3ppm, 5ppm, 7ppm concentration of heavy metals (Zn and Cu) were 0.03 µg/l, 0.03 µg/l, -0.11 µg/l and -0.26 µg/l, respectively. In treated culture using 1ppm concentration of heavy metals (Zn and Cu) the growth rate of phytoplankton load increased significantly whereas the growth rate decreased at higher concentrations (3ppm, 5ppm and 7ppm) of heavy metals. The implication of this finding can be used to monitor health of riverine ecosystems and management of river pollution.

Keywords: phytoplankton, biovolume, Zn, Cu, pollution.

INTRODUCTION

Heavy metals pollute aquatic ecosystem through the discharges from agricultural and industrial sources (Fathi et al., 2008). Inorganic pollutants enrich heavy metal presence in wastewater unchanged for long time in environment and make aquatic ecosystem toxic (Aung et al., 2013; Sari & Tuzen, 2008). Moreover, heavy metals cause serious problem of great concern for environment as well as ecosystem by toxic role (Ackova, 2018). Morphological and biochemical characteristics of phytoplankton mostly affected in presence of heavy metals in aquatic ecosystem (Afkar et al, 2010; Rocchetta et al., 2006).

In aquatic ecosystem, Phytoplankton is one of the most important primary producers (Tiwari & Chauhan, 2016) and energy flow in an aquatic ecosystem starts from them (Ferdous et al., 2012). About 50%-85% of molecular oxygen is released through photosynthesis that is performed by phytoplankton (Roach, 2004). Phytoplankton fixes and converts solar energy into chemical energy (Baruah et al., 2012). They are the major indicator of aquatic toxicity and respond directly to presence of many chemical particles available in freshwater (Dembowska & Jozefowicz, 2015; Pham, 2017) and coastal area (Karbassi et al., 2017). Thus, phytoplankton changes in water quality of aquatic ecosystem (Chopra et al., 2013).

There is a strong and intensive relationship between heavy metals and the

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growth of phytoplankton (Afkar et al., 2010). Heavy metals are treated as stress factor for the growth of phytoplankton (Fathi et al., 2010) and causes physical and morphological changes of phytoplankton in certain aquatic ecosystem (Lande et al., 2017). Continuous addition of heavy metal in aquatic ecosystem increases bioaccumulation of toxicants in food chain and gets in phytoplankton body through biochemical process (Aty et al., 2013; Aung et al., 2013; Melcakova & Ruzovic, 2010). Many heavy metals like Cd, Pb, Zn, Cu, Hg and Cr have significant effect on physical and morphological characteristics as well as growth of phytoplankton (Atici et al., 2008; Afkar et al., 2010; Jamers et al., 2013).

Turag river is located at north eastern side of Dhaka city (Mobin et al., 2014). This river is very important as it providing ecological and economical services for people of adjacent area. A few experiments have been conducted in presence of heavy metals using water of Turag river (Mohiuddin et al., 2016; Mokaddes et al., 2013). This river is ecologically critical area (DoE, 2001). It is required to find out the status of environmental health of this critical area. Thus, the experiment was conducted to evaluate effect of heavy metals on the growth of phytoplankton using water collected from Turag river.

MATERIALS & METHODS

Five water samples (1L×5= 5L) were collected from Turag river adjacent to Ashulia (latitude N= 23°86.538' and longitude E= 90°35.072') (Fig. 1). Collected water samples were mixed together and kept undisturbed for 12h. After sedimentation of phytoplankton, 4L of sample water of the bucket was sucked out carefully from upper layer keeping sediment layer of phytoplankton undisturbed and final volume was adjusted to 1L which was used as stock culture of test phytoplankton following whole-effluent algal assay approach (Wong, 1995). 5ml of randomly selected stock culture was used 5 times to count test phytoplankton load (Afkar et al., 2010) using advanced photo microscope (Olympus B×43, Japan) and to calculate bio-volume of total phytoplankton load (Lande et al., 2017). For Quality Control (QC) of the phytoplankton biovolume analysis, five randomly-selected samples were studied.

Heavy metal solution of Zn ($ZnSO_4.7H_2O$) and Cu ($CuSO_4.5H_2O$) was prepared in 1ppm, 3ppm, 5ppm and 7pmm concentrations and mixed with modified bold basal medium (Lande et al., 2017; Park et al, 2010). To set treatment culture, 100ml of phytoplankton containing stock sample water was added to 100ml of prepared growth medium and made it 250ml volume culture by addition of 50ml of distilled water in a 250ml conical flask. This treatment culture was made separately for different concentrations of heavy metals (Zn and Cu) solution. To set control culture, stock culture of test phytoplankton was grown in same condition like treatment culture without addition of heavy metal solution. Each control and treatment culture had three replications (Lande et al., 2017; Wong, 1995). The standard protocols for analytical Quality Assurance (QA), such as sampling and sample preserving etc., were performed in the designated laboratory prior to the samples' analyses. However, physical and chemical characteristics of sample water was determined and the mean±SD value of temperature, total dissolved solids (TDS), pH, Dissolved Oxygen (DO), Electric Conductivity (EC) and Biological Oxygen Demand (BOD_5) were 31.79±0.37 °C, 191.55±15.61 mg/l, 6.48±0.03, 1.31±0.34 mg/l, 475.66±69.47µS/cm and 2.61±0.24 mg/l, respectively.

All control and treatment cultures were incubated in 12:12 h light and dark cycle under white florescent lamps and temperature was maintained at 23± 2°C for 7 days (Kumar et al., 2014; Wong, 1995). To prevent clumping, cultures were shaken twice daily (Afkar et al., 2010).

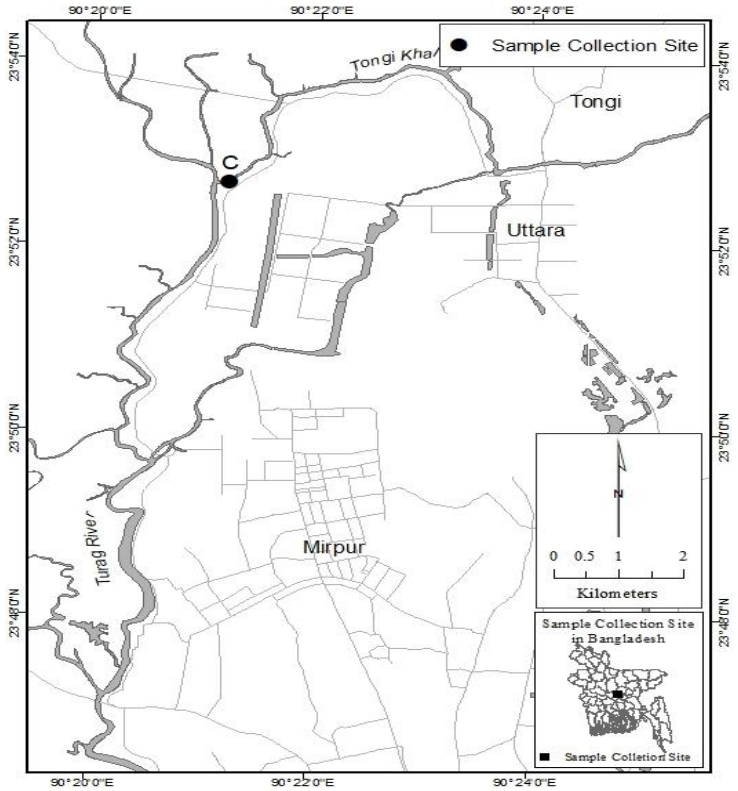

Fig. 1. Location of water sample collection site (C) in Turag river, Ashulia, Dhaka, Bangladesh.

Bio-volume was calculated at two days interval (3rd, 5th and 7th day) interval for both of treated and control cultures. However, initial bio-volume of culture of test phytoplankton was calculated just after collection using advanced photo microscope (Olympus B×43, Japan) and the following equation was used (APHA, 2005)

$$Vt = \Sigma (Ni \times Vi)$$

Where, Vt is the total cell volume μm^3/ml, Ni is the number of cells of the ith species/ml, Vi is the average cell volume of cells of ith species.

Growth rate of test species was calculated using the equation (Nichol, 1973; Wong, 1995)

$$\mu_{max} = (\ln X_2 - \ln X_1)/t_2 - t_1$$

Where, X_2 is the bio-volume concentration at end of selected time interval, X_1 is the bio-volume concentration at beginning of selected time interval, t_2-t_1 is the elapsed time (in days) between selected determination of bio-volume.

Unpaired t-test was performed to compare between growth rate of control and treated cultures with test phytoplankton load and different concentrations of heavy metals using SPSS version 22.0 for windows.

RESULTS & DISCUSSION

A total of 16 phytoplankton taxa belonging under 12 genera of Bascillariophyceae (7), Chlorophyceae (6) and Euglenophyceae (3) were recorded before starting the experiment (Fig. 2). In total test phytoplankton load *Euglena sp., Borodinella sp., Pediastrum biradiatum, Pinnularia sp., Fragillaria sp., Fragillaria crotonensis, Gloeocapsa sp., Navicula sp., Cynedra sp.,Crucigenia sp.,Chlorella sp., Chlamydomonas sp., Phacus acuminatus, Phacus circulatus, Nitzschia sp*. and *Nitzschia clausii* were found (Fig. 2). The percentage composition of TPL showed the pattern as Bascillariophyceae (43.75%) > Chlorophyceae (37.50%) > Euglenophyceae (18.75%).

In control culture, the initial bio-volume

of TPL was 4.46µg/l. In addition, 4.11µg/l in 3rd day, 2.86µg/l in 5th day and 2.11µg/l in 7th day of observation (Fig. 3). In treated culture with 1ppm of heavy metals (Zn and Cu) solution in 3rd, 5th and 7th day of observation the bio-volume were 2.82µg/l, 2.8µg/l and 3µg/l, respectively. In treated culture with 3ppm of heavy metal solution in 3rd, 5th and 7th day of observation the bio-volume were 3.17µg/l, 3.91µg/l and 3µg/l, respectively. In treated culture with 5ppm of heavy metal solution in 3rd, 5th and 7th day of observation the bio-volume were 3.17µg/l, 1.4µg/l and 1.4µg/l, respectively. In treated culture with 7ppm of heavy metal solution in 3rd, 5th and 7th day of observation the bio-volume were 1.67µg/l, 1µg/l and 1µg/l, respectively (Fig. 3a, b, c).

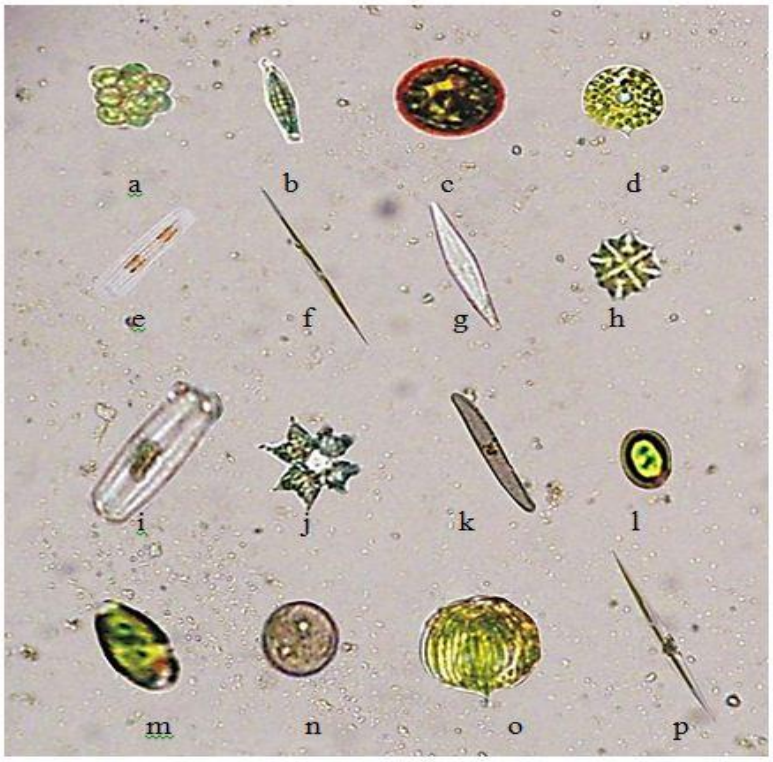

Fig. 2. Test phytoplankton taxa recorded in water of Turag river a. *Borodinella* sp., b. *Navicula* sp., c. *Chlorella* sp., d. *Phacus acuminatus,* e. *Fragillaria crotonensis,* f. *Synedra* sp., g. *Fragillaria* sp., h *Crucigenia* sp., i. *Pinnularia* sp., j. *Pediastrum biradiatum,* k. *Nitzschia clausii,* l. *Chlamydomonas* sp. m. *Euglena* sp., n. *Gloeocapsa* sp., o. *Phacus circulatus,* p. *Nitzschia* sp. (10×40).

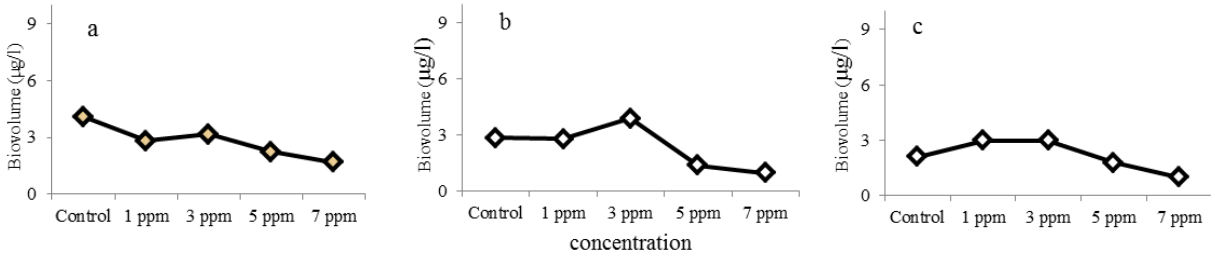

Fig. 3. Phytoplankton bio-volume of control culture (-hm= without heavy metals) and treated culture (+hm= with heavy metals) using different concentrations of heavy metals in growth media in (a) 3rd day, (b) 5th day, (c) 7th day of observation.

In control culture, the growth rate of test phytoplanktonic load was -0.25±0.15µg/l whereas in treated culture the growth rate was 0.03±0.05µg/l in 1 ppm, -0.03±0.34µg/l in 3ppm, -0.11±0.51µg/l in 5 ppm and -0.26±0.36µg/l in 7 ppm concentrations of heavy metal solution (Fig. 4).

With compared to control culture, the growth rate of total phytoplankton load increased significantly (p=0.0001*) in 1ppm treated culture (Table 1). With compared to 1ppm concentration of heavy metals treated culture the growth rate of total phytoplankton load in 3ppm (p=0.65), 5ppm (p=0.72) and 7ppm (p=0.06) treated cultures showed no significant differences. With compared to 3ppm concentration of heavy metals treated culture the growth rate of total phytoplankton load of 5ppm (p=0.71) and 7ppm (p=0.21) treated cultures showed no significant comparison. With compared to 5ppm concentration of heavy metals treated culture the growth rate of total phytoplankton load of 7ppm (p=0.5) treated cultures showed no significant comparison.

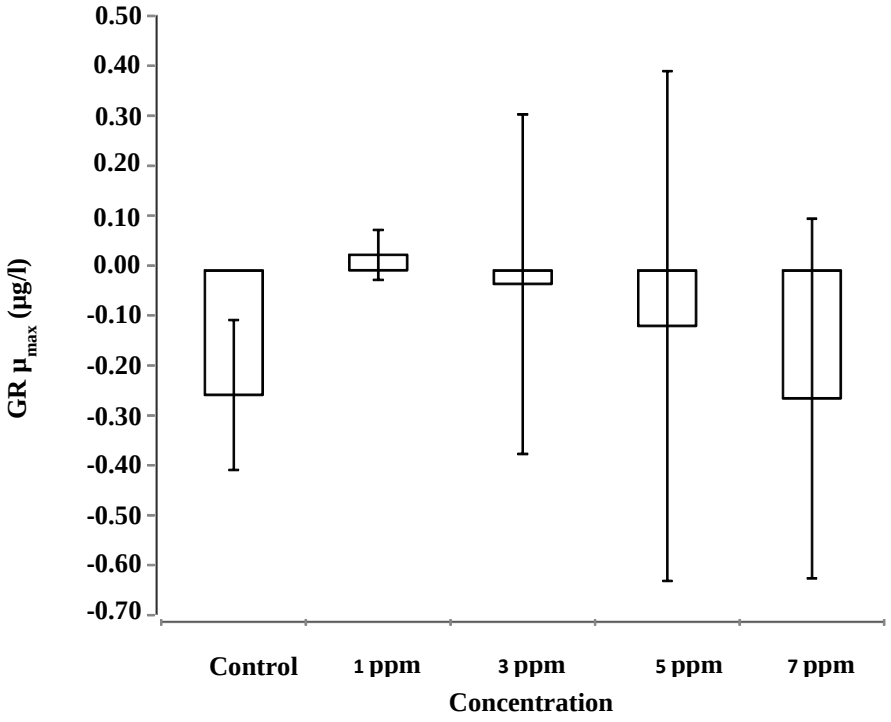

Fig. 4. Growth rate (GR) of test phytoplankton load in different concentration of heavy metals solution.

Table 1. Comparison of growth rate of phytoplankton between control culture with treated cultures using 1ppm, 3ppm, 5ppm and 7ppm of heavy metal (Zn and Cu) concentrated treated cultures in Turag river (Mean±SEM, n=3)

Culture	Growth rate (μ_{max}) Mean±SEM
Control culture (-hm)	-0.25±0.15
Treated 1ppm culture (+hm)	0.03±0.05
t/p	5.34/0.0001*
Treated 3ppm culture (+hm)	-0.03±0.34
t/p	1.89/0.07
Treated 5ppm culture (+hm)	-0.11±0.51
t/p	0.87/0.39
Treated 7ppm culture (+hm)	-0.26±0.36
t/p	0.08/0.94

*= p<0.05, -hm = without heavy metals, +hm = with heavy metals

In this study, the growth rate of control and treated cultures were determined. Result showed that the growth rate of phytoplankton load increased significantly in 1ppm heavy metal (Zn and Cu) treated culture whereas, decreased at high concentrated (3ppm, 5ppm and 7ppm) heavy metal treated cultures. Regenmortel et al. (2018) express same growth pattern of phytoplankton. Phytoplankton can abolish their toxic effects at low concentration of heavy metals and increased respiration by using heavy metals as micro nutrient for growth and photosynthesis (Lande et al., 2017; Afkar et al., 2010; Osman et al., 2004; Bilgrami & Kumar, 1997). In addition, heavy metals at low concentration can stimulate metabolic process of phytoplankton, thus decreases metal toxicity (Afkar et al., 2010). As a result, photosynthesis process leads phytoplankton community to increase their number (Kumar et al., 2014). However, in this experiment the toxic effect of high concentrations (3ppm, 5ppm and 7ppm) of heavy metal reduce growth rate in treated cultures. Fisher and Jones (1981) expressed that Zn^{2+} at low concentration assist to produce chlorophyll in the body of photosynthetic microorganisms. Prassad & Prassad (1987) reported that heavy metals at higher concentration impact production of enzyme which is involved to chlorophyll synthesis. It is also may be due to the oxidative potential of Cu that influences reduction of chlorophyll, decrease of oxygen evolution rate and deplete ATP by inhibition of enzymes which stimulate metabolism in the body of phytoplankton (Muwafq & Bernd, 2006).

CONCLUSION

The average maximum growth rate (log transformed) of TPL in control culture was -0.25µg/l and treated cultures using 1ppm, 3ppm, 5ppm, 7ppm concentration of heavy metals (Zn and Cu) were 0.03 µg/l, 0.03 µg/l, -0.11 µg/l and -0.26 µg/l, respectively. In treated culture using 1ppm concentration of heavy metals (Zn and Cu) the growth rate of phytoplankton load increased significantly whereas the growth rate decreased at higher concentrations (3ppm, 5ppm and 7ppm) of heavy metals. The implication of this finding can be used to monitor health of riverine ecosystems and management of river pollution.

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