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EFFECT OF CALCIUM AND MAGNESIUM INDUCED HARDNESS ON THE TOXICITY OF LEAD TO MICROORGANISM IN AQUATIC ENVIRONMENT AS MEASURED BY BIO-CHEMICAL OXYGEN DEMAND

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ABSTRACT

An experimental study was carried out to observe the effects of water hardness based on calcium and magnesium salt as sulphate at different concentrations ranging from 0 to 400 mg/L as $CaCO_3$ to Pb toxicity for nitrifying (azobactor) at 20°C and 30°C. The rate constant (k) and ultimate biochemical oxygen demand (L) have been calculated from BOD data taken for 1 to 15 days using Thomas graphical method. Glucose was used as the source of carbon for microorganism.

It was observed that the toxicity of Pb to azobactor decreased with increasing calcium as well as magnesium hardness at both the temperatures. The percentage reduction of BOD (over control as without hardness and Pb) was found to decrease from 51.99 to 18.83 and 54.52 to 19.45 for Ca hardness at 20°C and 30°C, respectively. Similarly, for Mg hardness at 20°C and 30°C, the percentage reduction of BOD was decreased from 51.99 to 14.85 and 51.94 to 15.25, respectively. Rate constant (k) values were found to follow the decreasing order as Mg hardness at 30°C > Mg hardness at 20°C > Ca hardness at 30°C > Ca hardness at 20°C.

Key words: Microorganism, Nitrifying bacteria, BOD, Lead toxicity, Ca hardness, Mg hardness.

INTRODUCTION

Among various non-essential elements, lead is one of the heavy metals, which is considered to be toxic to a number of biotic ecological elements. The metal is released into different environmental segments through miscellaneous point and non-point sources such as transportation, agricultural, industrial, socio-religious, and other developmental activities. In aquatic ecosystem, microbial population plays an important role in regulating the levels of the dissolved oxygen (D.O.) in the water bodies viz. rivers, lakes, ponds etc. however, the

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presence of heavy metals like Pb, Cd, and Hg etc. disturb the bio-physicochemical balance in these water bodies[1-3].

Toxic behavior of these heavy metals in aquatic system have been studied by various workers[4-6] and it has been shown that the extent of toxicity is greatly affected by pH, alkalinity, hardness and other parameters of water bodies. Microorganisms usually encounter various types of interactions with metal and metalloids in the aquatic environment. Out of those, toxicity of heavy metals (nonessential) occur through the displacement of nutrient (essential) metals from their binding sites or through legend interactions. For example, Cd tends to bind with thiol groups, and thus, inhibit the activity of sensitive enzymes[7]. In addition, both the metals i.e. essential and nonessential can damage cell membranes at their high levels to disrupt cellular functions and damage the structure of DNA[8].

Bio-chemical oxygen demand (BOD), which is a measure of the amount of oxygen used by microorganism (e.g. aerobic bacteria) while decomposing organic matter under aerobic conditions. Heavy metals have been found to influence the biochemical oxygen demand for various organic wastes. In general, it was theorized[9] that accumulation of certain heavy metals in the concentration range of < 0.1 mg/L^{-1} could reduce the observed biochemical oxygen demand by 20 to 30%. Mittal and Ratra[10] have correlated these reductions with the electrochemical potential of the metal ions. The toxicity of various transition metal ions for the microbes has been observed[11] and found that the BOD inhibition in presence of Pb and Cd is relatively large in comparison to the others like Cr, Co, Ni and Cu etc. at different temperatures.

Since a very little information is available on the effect of specific hardness e.g. in terms of calcium and magnesium hardness to reduce the toxicity of Pb to specific microbes like azobacter. The present study reports the observations found on the effect of Pb on the rate of survival of microorganism (Nitrifying bacteria) at different temperatures.

EXPERIMENTAL

Materials and methods

Preparation of stock solutions

The stock solutions of hard water with the concentration of 5 g/L^{-1} as calcium carbonate equivalent ($CaCO_3$ equivalent) were obtained by adding the appropriate amount of calcium chloride (as $CaCl_2.2H_2O$) and magnesium chloride (as $MgCl_2.6H_2O$) to de-mineralized water. The sample water solutions of different hardness varying from 0 to 350 mg/L^{-1} (as $CaCO_3$ equivalent with respect to Ca and Mg) were obtained by diluting appropriate volumes of stock solutions with demineralized water.

A stock solution of glucose (10 g/L^{-1}) was also prepared in demineralized water to obtain the concentration of 100 mg/L^{-1} in each experimental set of BOD determinations. The culture of microorganism as nitrifying bacteria (azobacter) was obtained from the microbiology research laboratory, G. B. Pant University of Agriculture and Technology, Pantnagar, India. The stock solution of lead (II) sulphate was prepared by taking appropriate amount of $PbSO_4.5H_2O$ to obtain the concentration of 5 mg/L^{-1} as Pb (II).

The composition of BOD experimental set ups

The composition of experimental set up was prepared by taking BOD bottle of 300 mL capacity containing 100 mg/L^{-1} glucose, 2 mL of nitrifying bacteria (azobacter), 5 mg/L^{-1} lead (II) metal as sulphate and hard water of varying strengths. Blank sets without Pb and hard water were also run simultaneously under identical conditions for the measurement of BOD load of glucose. The values of BOD in each experimental set were determined by standard method for various time intervals from 1 to 15 days at 20^{o}C and 30^{o}C.

Calculation of rate constant (k) and ultimate biochemical oxygen demand (L)

The rate constant (k) and ultimate biochemical oxygen demand (L) was determined by a simple graphical method developed by Thomas[12]. For this, the values of $(t/y)^{1/3}$ vs. t (where t is time of BOD incubation and y is the BOD values at the corresponding time of BOD incubation) was plotted, which gives straight lines. The intercept (A) and slope (B) of these straight lines was related to rate constant (k) and ultimate BOD (L) as follows:

$$k = 2.61\ (B/A) \text{ and} \qquad \ldots(1)$$

$$L = 1/\ (6A^2B) \qquad \ldots(2)$$

The sample plot is shown as Fig. 5 and the values calculated for rate constant and ultimate BOD are given in Table 1.

Table 1: Influence of Ca and Mg induced hardness on ultimate BOD and rate constant at 20^{o}C and 30^{o}C

Hardness as $CaCO_3$ Equivalent	Ultimate BOD (mg/L^{-1})				Rate constant ($days^{-1}$)			
	Ca at 20^{o}C	Ca at 30^{o}C	Mg at 20^{o}C	Mg at 30^{o}C	Ca at 20^{o}C	Ca at 30^{o}C	Mg at 20^{o}C	Mg at 30^{o}C
*Control-1	424.76	438.37	424.76	438.08	0.1483	0.0403	0.1483	0.1513

Cont…

Hardness as $CaCO_3$ Equivalent	Ultimate BOD (mg/L^{-1})				Rate constant ($days^{-1}$)			
	Ca at 20°C	Ca at 30°C	Mg at 20°C	Mg at 30°C	Ca at 20°C	Ca at 30°C	Mg at 20°C	Mg at 30°C
*Control-2	202.07	210.21	202.07	210.21	0.1387	0.0126	0.1387	0.1404
50	229.14	235.16	247.33	262.30	0.1341	0.1361	0.1487	0.1569
75	248.12	253.40	266.18	283.29	0.1467	0.1476	0.1600	0.1673
100	261.78	269.80	283.77	299.80	0.1490	0.1521	0.1615	0.1688
150	277.90	287.54	297.73	313.05	0.1511	0.1553	0.1632	0.1704
200	297.25	309.19	317.84	330.99	0.1454	0.1494	0.1561	0.1658
250	315.59	327.46	335.83	352.03	0.1433	0.1471	0.1528	0.1578
300	329.01	343.57	348.15	364.71	0.1447	0.1491	0.1551	0.1619
350	350.19	360.84	374.45	390.59	0.1479	0.1509	0.1556	0.1635

*Control-1: Sample water without Pb and hardness;

*Control-2: Sample water with 5 mg/L^{-1} Pb and without hardness

RESULTS AND DISCUSSION

Variation of BOD and ultimate BOD

The influence of calcium and magnesium induced hardness on the BOD variation in presence of Pb is shown in Figs. 1 to 4, while the Fig. 5 has been shown as sample curves for calculating the ultimate BOD and rate constant. These curves show that the BOD value decreases in presence of Pb without hardness. However, an addition of Ca and Mg hardness enhances the BOD values to show the decrease in toxicity of Pb for microbial. The results are in agreement with the other work reported in literature[9-11] where it was reported that the BOD is reduced in presence of heavy metals like Pb, Cd, Ni and Cr etc. The maximum values of BOD and ultimate BOD (Table 1) for nitrifying bacteria were found to be 377.0 and 424.76 mg/L^{-1}, respectively at 20°C, which was further increased to 385.00 and 438.37 mg/L^{-1}, respectively at 30°C in the blank sets without Pb and hardness.

While the minimum values of BOD and ultimate BOD were 181.0 and 202.07 mg/L^{-1}, respectively at 20°C and increased up to 186.0 and 210.21 mg/L^{-1}, respectively at 30^0C in the control set lacking hardness but having 5 mg/L^{-1} Pb (II) metal as sulphate. These values were found to be 51.99% less than the normal values found without any amendments.

Fig. 1 to 4 shows that the BOD values increased gradually with the increase of hardness of water and the maximum value was obtained at 350 mg/L^{-1} of $CaCO_3$ equivalent hardness at both the temperatures. It was also observed that the increase in BOD values follows the order as: Ca induced hardness at 20^{o}C < Ca induced hardness at 30^{o}C < Mg induced hardness at 20^{o}C < Mg induced hardness at 30^{o}C. It shows that the toxic effects of Pb to microorganism were reduced to minimum at 350 mg/L^{-1} level.

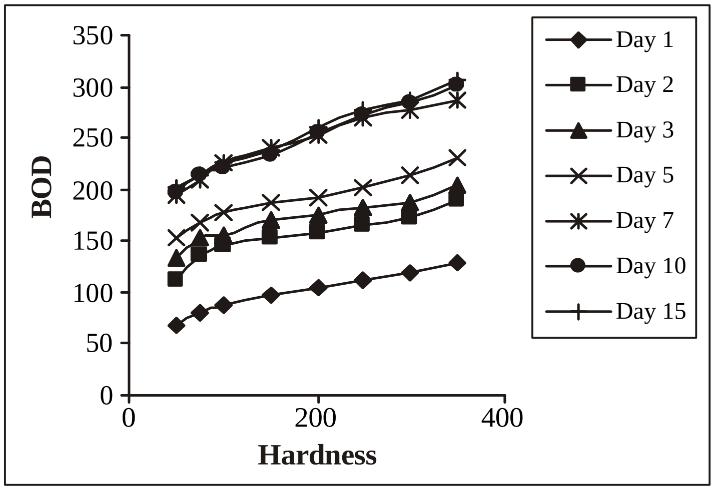

Fig. 1: Influence of Ca induced hardness on BOD at 20^{o}C

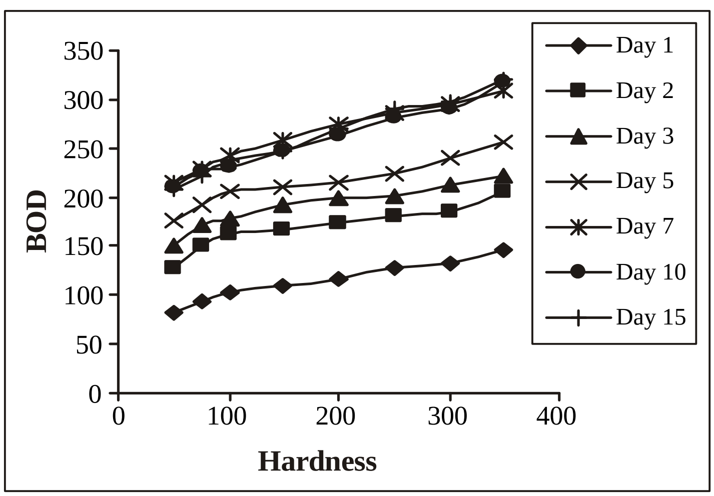

Fig. 2: Influence of Mg induced hardness on BOD at 20^{o}C

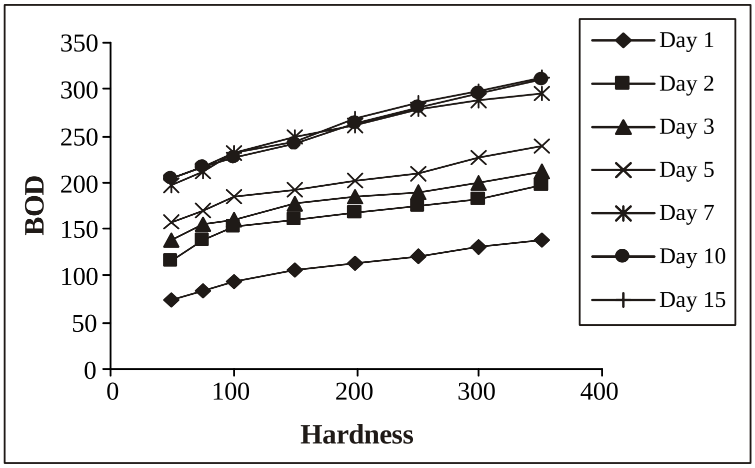

Fig. 3: Influence of Ca induced hardness on BOD at 30°C

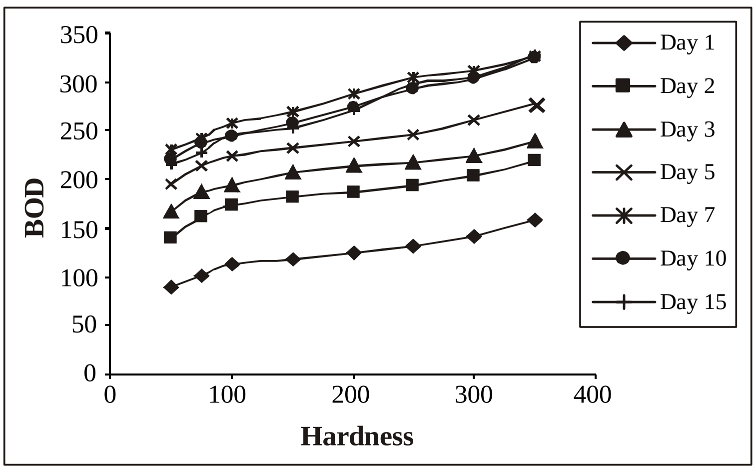

Fig. 4: Influence of Mg induced hardness on BOD at 30°C

Hardness effect on rate constant

A plot of rate constant vs. hardness (Fig. 6) showed a trend of gradual increase at initial level and then some drops in the curve have been observed.

A comparison of results (Table 1) indicate a sharp increase in the rate constant of the system with increase in hardness of water, which reached a maximum at low hardness level showing thereby that nitrifying bacteria retained their normal activity and growth at lower level (150 mg/L^{-1} of $CaCO_3$); after which the activity was again depressed by further increase in the hardness of the system. It may be due to the fact that the microbe has different kind of defense systems as the modification or elimination of membrane transport systems into the cell for the harmful metal species or efflux system (molecular pumps) for their removal from the cell interior[13].

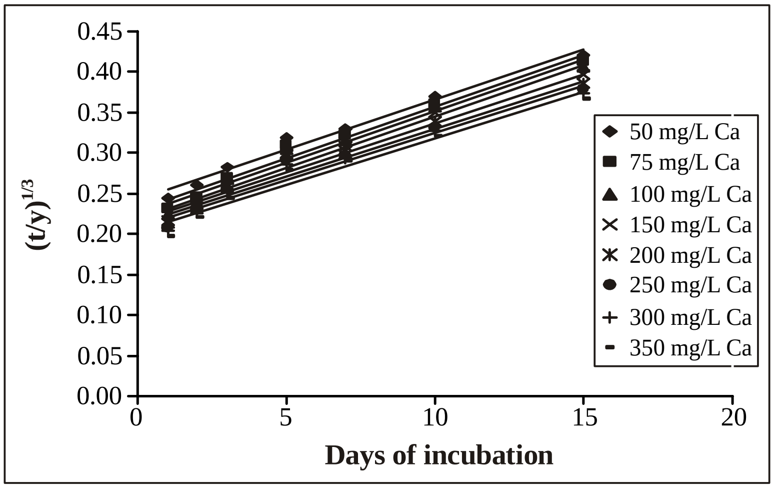

Fig. 5: Effect of Ca induced hardness at 20°C on the variation of $(t/y)^{1/3}$ vs. t

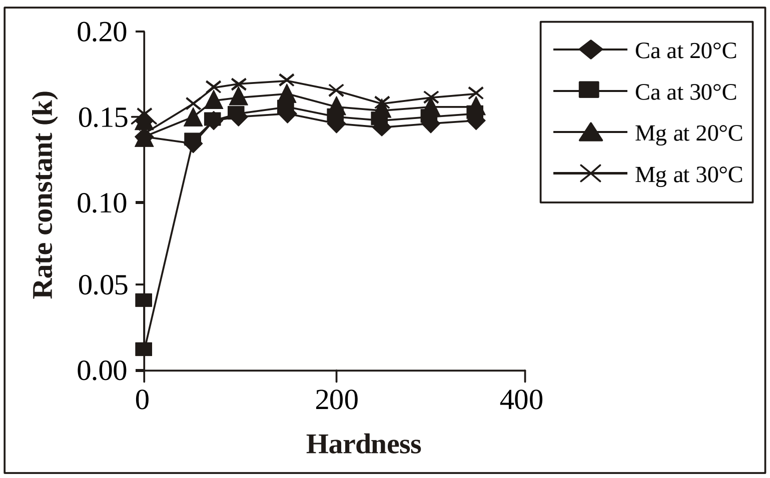

Fig. 6: Plot of rate constant vs. hardness

Percent reduction of BOD

Table 2 shows the percent reduction of BOD values as influenced by the presence of Ca and Mg induced hardness in the test water. It shows that the reduction extent follows the order: Mg at 20°C > Mg at 30°C > Ca at 20°C > Ca at 30°C. This trend may be explained on the basis of the fact that Pb causes an increase in intercellular pools of Ca and a decrease in ability of cells to extrude Ca^{2+} to lower the effective intercellular free Ca^{2+} concentration.

Table 2: Influence of Ca and Mg induced hardness on the reduction of BOD on 15th day of incubation at 20°C and 30°C

Hardness as $CaCO_3$ Equivalent	Reduction of BOD (%)			
	Ca at 20°C	Ca at 30°C	Mg at 20°C	Mg at 30°C
*Control	51.99	54.52	51.99	51.94
50	46.68	49.32	44.56	44.19
75	43.50	45.75	41.38	41.09
100	40.32	42.19	36.87	36.69
150	36.87	38.36	34.75	34.63
200	31.30	32.05	28.12	29.97
250	26.79	27.40	23.08	23.26
300	23.87	23.84	20.95	21.19
350	18.83	19.45	14.85	15.25

* Sample water with 5 mg/L^{-1} Pb and without hardness

The variation in the Pb toxicity in the water samples of different hardness could be due to the heavy metal binding with the inorganic anions, the concentration of which varies directly with hardness of natural waters[14]. Because free unbound ionic Pb is considered as toxic species in the biological systems. Miller and Mackay[15] have shown that the calcium hardness is much more effective than carbonate-bicarbonate alkalinity in reducing the heavy metal toxicity. In the present study, the calcium and magnesium both have been found effective in reducing the Pb toxicity at both the temperatures. The results also show that calcium hardness is more effective than Mg hardness.

CONCLUSION

The study conducted in these experiments suggests that the Pb is more toxic to nitrifying bacteria at 20°C than 30°C (Fig. 1 to 4). The microbes also show a quick response to the Ca and Mg induced hardness of water in resuming their normal functions in presence of lead. Therefore, it is recommended that the industrial wastes containing toxic metals like Pb at their 5 mg/L^{-1} concentration can be discharged safely in the water streams having hardness around 350 mg/L^{-1} as $CaCO_3$ equivalent without affecting nitrifying bacteria. The factors like pH, alkalinity, temperature, organic matter, and dissolved organics also play an important role in the toxic behavior of a metal to biotic segment of stream eco-system apart from hardness of water.

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