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RESEARCH ARTICLE

**Optimization of Quick Lime (CaO) and pH Application for Nursery Management of Indian Major Carps
Catla catla (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton)**

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Abstract

Static bioassays of 96 h duration were conducted in the laboratory using fry of Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*), adult tubificid worm (*Branchiura sp.*) and copepod plankton (*Cyclops sp.*) to determine lethal values (LC₅ to LC₉₅) of quicklime (CaO), alkaline pH and acidic pH to these organisms and their interactions with certain physico-chemical parameters of water. The outcome of the study indicated that the concentration of CaO below 359.77 mg/l did not produce any lethality to the fry of IMC whereas 618.51 mg/l of CaO was highly toxic (>95% mortality) to the fry of IMC. The pH values above 5.8 and below 8.5 were non-toxic to IMC fry. But, pH 4.4 and 10.22 were highly toxic (>95% mortality) to the fry of IMC. The CaO below 12.85 mg/l and pH 8.0 were non-lethal to copepod plankton. Its lethality was 95-100% above 65.09 mg/l of CaO and above pH 8.6. Likewise, 20.34 mg/l of CaO and pH 8.1 did not show any lethality to tubificid worm. But its lethality was 95-100% above 176.05 mg/l of CaO and pH 8.4. The ninety-six hours LC₅₀ values of acidic pH for copepod plankton and tubificid worm were 6.4 and 4.6 respectively. The pH values above 7.1 and 5.6 did not act as lethal to copepod plankton and tubificid worm respectively. The 95-100% lethality was recorded at a pH value of 6.5 or below in copepod plankton and at pH 3.4 or below in tubificid worm. A significant decrease (P<0.05) of hardness, alkalinity and conductivity were observed after the treatment of CaO in between 300 mg/l and 500 mg/l. In contrast, the hardness and conductivity increased significantly (P<0.05) but, the alkalinity decreased to zero with the decrease of pH from 7.6 to 4.7. The results indicated that the application of CaO quickly increased the pH values of water, which affected the IMC fry and food organisms and some physico-chemical parameters of water. On the other hand acidic ranges of pH were toxic to both fry and fish food organisms. Therefore, ideal pH of the nursery pond for IMC fry should be maintained in between 7.1 and 8.0. The application of CaO during culture period of IMC fry should be avoided and would be recommended for its application during pond preparation to avoid any threat.

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Introduction

Environmental constraints in fisheries management in India are vast (Jhingran, 1991), and several considerations are necessary to protect the potentiality of these waters. Increasing population

and higher level of human activities, including effluent disposals to surface and ground water sources, have made a very complex task throughout the world for sustainable management of water resources. The most important factors influencing global water quality are natural and anthropogenic

inputs of contaminants into the aquatic environment through disposal of wastes. These contaminants directly cause toxicity to the aquatic organisms and/or deteriorate water quality through changing the ionic balance. It results in depletion of dissolved oxygen (DO), changing the pH, alkalinity, hardness of water and in turn affects the total aquatic ecosystem. The most important characteristic feature of any aquaculture system is the pH of water. Acidity or acidic pH occurs where appreciable quantities of exchangeable base forming cations (Na^+ , K^+ , Ca^{2+} , and Mg^{2+}) are leached from the pond mud or accumulating through anthropogenic sources. In many situations, it is so widespread and the effects on aquatic life are so acute that acidity has become an important feature of any fish culture system. Pollution of aquatic ecosystem by acid drainage is considered as dangerous to aquatic life. Acid rain and acidic effluent are important pollutants of aquatic habitats all over the world (Ramesh and Ranavalaramanujam, 1996).

The aquatic resources are limited and the ecosystem health is deteriorating day by day. Aquatic ecosystems have been recognized, not as users of water in competition with other users, but rather as the base of the resources itself that needs to be actively cared for its sustainable development. 'Ecologically Sustainable Development' of aquatic environment is possible only when the physico-chemical parameters of water are managed properly. It is directly related to the augmented production. The setting of water quality guidelines or the use of water quality criteria has proven to be an important tool in supporting resource management decision (Roux et al., 1996). Therefore, water quality guidelines and its maintenance is paramount important for balancing the aquatic ecosystem. Although there are thousands of chemicals, which could find their way into water, only a few have occurred in sufficient concentration to form a continuous threat to aquatic organisms. The chemicals like acids, lime and minerals assimilate to the aquatic ecosystem through anthropogenic activities, industrial disposal, and agricultural runoff which are affecting the different characteristics of water. Among those, pH is an important characteristic for any aquatic ecosystem. The survival and reproduction of aquatic organisms depend mostly on pH and temperature. Therefore, the main objective of the study is to develop a guideline for the lime treatment and optimum alkaline and acidic pH levels to maintain the ecosystem balance in freshwater aquaculture particularly for the nursery management of Indian Major Carps (IMC).

Materials and Methods

Static bioassays of 96 h duration were conducted in the laboratory using fry of Indian Major Carps Catla (*Catla catla* Hamilton 1822), Rohu (*Labeo rohita* Hamilton 1822) and Mrigal (*Cirrhinus mrigala* Hamilton 1822), adult tubificid worm (*Branchiura* sp. Beddard 1892) and adult copepod plankton (*Cyclops* sp. Jurine 1820) following the procedure of APHA (1995). Ten days old IMC fry (size range from 1.5 to 2.0 cm), procured from a local hatchery, were stocked into glass aquaria (100 l) containing deep tube well water (pH 7.4-7.8, temperature 30.5-31.2 °C, conductivity 3.7-3.8 mS, DO 6.4-7.2 mg/l, total alkalinity 220-280 mg/l as CaCO_3 , and total hardness 890-900 mg/l as CaCO_3). The fry were fed *ad libitum* with prepared practical diet containing 30% protein, 60% carbohydrate and 5% lipid. An adult population of tubificid worms was collected from a local field site and maintained in the flat trays under flow-through condition. A stock of plankton population was collected from nearby pond using plankton net (#63 μ). The copepod *Cyclops* sp. was segregated from the stock in the laboratory and placed in 10 l aquarium to maintain a stock culture of the species for the experiment. Three fish species were chosen because they are inhabiting in three distinct hypothetical strata of water body as surface feeder (*Catla catla*), column feeder (*Labeo rohita*) and bottom feeder (*Cirrhinus mrigala*) in the aquatic ecosystems and they are the most cultivable and marketable fish for polyculture in India. The copepod zooplankton *Cyclops* sp. and benthic tubificid worm *Branchiura* sp. were chosen for their major role as live food for tropical freshwater aquaculture in India and predominantly present in these aquatic ecosystems. In addition, the chosen test organisms have good response to contaminants, easily available of genetically pure stock, quick capacity to acclimatize in laboratory conditions and easy to handle for conducting bioassays.

Short-term bioassays (96 h) were conducted to determine lethal concentrations of CaO and the lethal values of alkaline and acidic pH to IMC, worm and copepod. Bioassays on fish were carried out in 3 liters glass aquaria each holding 2 l of the same deep tube well water. Bioassays on worm and copepod were carried out in 250 ml glass beaker each holding 200 ml of water. Ten fish fry per aquarium and ten each of the worm and copepod per beaker were used. All the organisms were acclimatized in the test condition for 48 h before starting of experiment. No food was provided to the test organisms during the period of acclimatization and experiment. Treatment of CaO and H_2SO_4 (98%) were made from the stock solutions of CaO and H_2SO_4 prepared by dissolving

analytical grade CaO (Qualigens, Glaxo India Ltd.) and analytical grade H₂SO₄ (Mecrk India, Ltd.) respectively. The nominal concentrations of CaO and H₂SO₄ used in the bioassays are given in Table 1. Bioassays were conducted under a complete randomized design (Gomez and Gomez, 1984) with three replicates per treatment.

Mortality of the test organisms was recorded after every 24 h and the dead organisms from each treatment were counted and removed from the container immediately to avoid any contamination due to rotting. Lethal concentrations/values at which the test organisms died was calculated from the 96 h mean mortality and the concentration of CaO and values of alkaline pH or acidic pH by Probit Analysis Program used for calculating LC values (Probit Analysis Program, Version 1.5, EPA, USA). The pH, temperature, conductivity, DO, alkalinity and hardness of the test water were estimated following the standard methods (APHA, 1995) at the end of each bioassay (after 96 h) and their correlation with the concentrations of CaO and values of alkaline or acidic pH were also tested (Gomez and Gomez, 1984). Single factor analysis of variance (ANOVA) of the water quality parameters of the test water was done with the help of Microsoft Excel-2003 (Statistical software) to evaluate the significance of variations ($P < 0.05$) between the different treatment groups.

Results

Bioassays of CaO and alkaline pH:

Ninety-six hour's lethal CaO concentrations and lethal alkaline pH values (LC₅ to LC₉₅) with 95% confidence limit for Catla, Rohu and Mrigal fry have been presented in Figures 1 and 2. No mortality was found in control. The LC₅₀ values of CaO for Catla, Rohu and Mrigal fry were 420.21 mg/l (406.48-497.61 mg/l), 418.44 mg/l (405.2-434.37 mg/l) and 409.72 mg/l (397.18-422.36 mg/l) respectively. Its corresponding pH values were 8.9 (8.8-9.1), 9.0 (8.9-9.1) and 8.9 (8.8-9.0) respectively. The CaO concentration below 359.77 mg/l and its corresponding pH value 8.5 did not produce any mortality to the fry of IMC. But 618.51 mg/l of CaO and pH 10.22 were found to be lethal (> 95% mortality) to the fry of IMC. The pH above 8.5 was affecting the fry of IMC and its lethality was 95-100% at pH 10.22.

Ninety-six hour LC₅₀ values of CaO to copepod plankton and tubificid worm were found to be 22.02 mg/l (18.77-24.6 mg/l) and 45.09 mg/l (40.53-49.88 mg/l) respectively (Fig 3) and LC₅₀ values of pH values were 8.05 (8.0-8.1) and 8.09 (8.0-8.1) respectively (Fig 4). The CaO below 12.85 mg/l with

its corresponding pH 8.0 was non-lethal to copepod plankton. But its lethality to copepod plankton was 95-100% at above 65.09 mg/l of CaO and above pH 8.6. The pH above 8.0 was affecting copepod plankton and the lethality was 95-100% at pH 8.6. As above, 20.34 mg/l of CaO and pH 8.1 did not produce mortality to tubificid worm. But its mortality was 95-100% above 176.05 mg/l of CaO and pH 8.4.

Bioassays of acidic pH:

Ninety-six hours lethal acidic pH values (LC₅ to LC₉₅) with 95% confidence limit to Catla, Rohu and Mrigal fry have been presented in Figure 5. The LC₅₀ values of pH to Catla, Rohu and Mrigal fry were 5.3 (5.2-5.5), 5.3 (5.3-5.5) and 5.3 (5.2-5.5) respectively. The pH above 5.8 was non-lethal to IMC fry. But, pH 4.4 was lethal (>95% mortality) to the fry of IMC. The pH below 5.8 was affecting the fry of IMC. But its lethality was 95-100% at pH 4.4 or below.

The ninety-six hour's LC₅₀ values of acidic pH for copepod plankton and tubificid worm were 6.4 (6.2-6.8) and 4.6 (4.5-4.7) respectively (Fig 6). The pH values above 7.1 were non-lethal to copepod plankton. Its lethality increased as the pH value decreases from 7.1 and below the pH value of 6.0, its lethality was 95-100%. The pH value above 5.6 did not show any mortality to tubificid worm, but its mortality increased with the corresponding decrease in pH. The 95-00% mortality was recorded at pH value 3.4 or below.

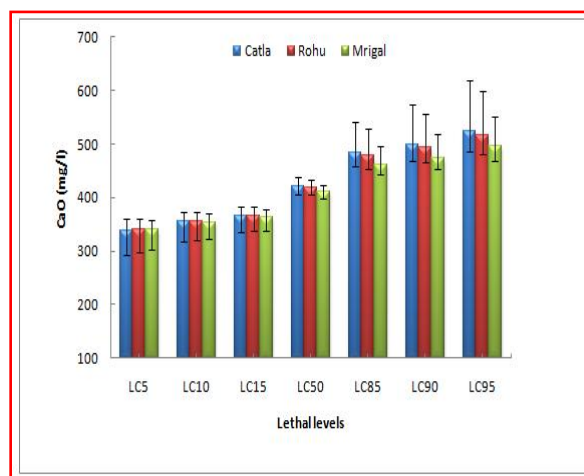


Fig 1. Ninety-six hour's lethal concentrations of CaO on Catla, Rohu and Mrigal fry

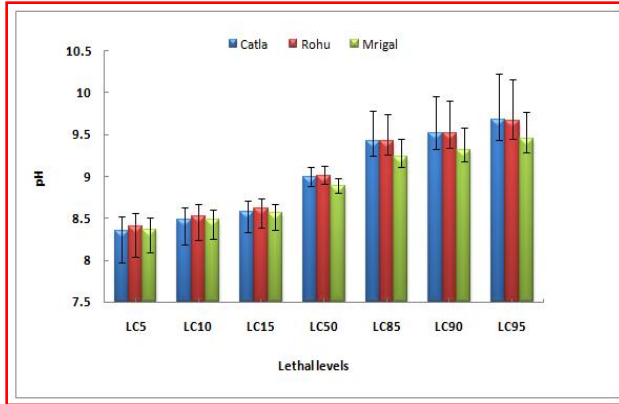


Fig 2. Ninety-six hour's lethal values of alkaline pH on Catla, Rohu and Mrigal fry.

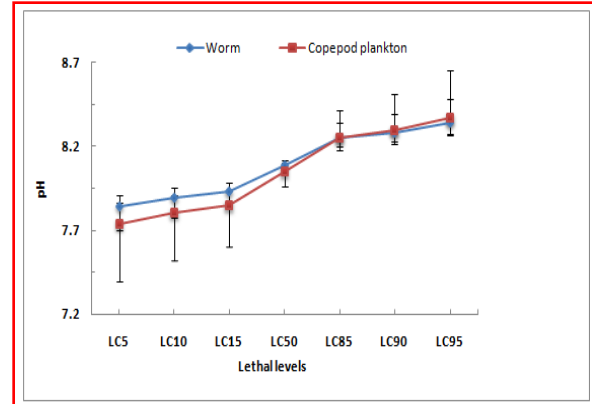


Fig 5. Ninety-six hour's lethal values of alkaline pH on copepod plankton and tubificid worm

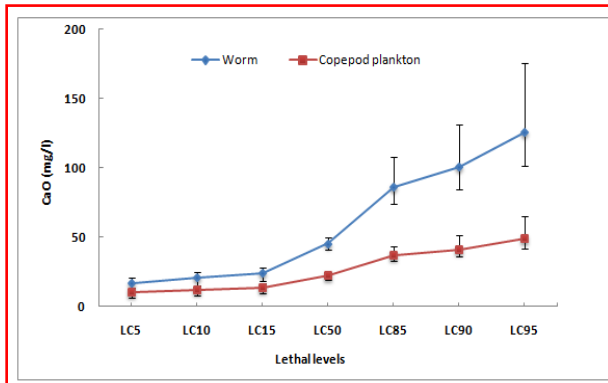


Fig 3. Ninety-six hour's lethal concentrations of CaO on copepod plankton and tubificid worm.

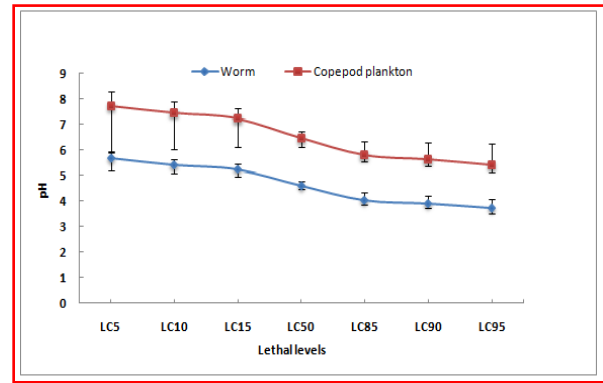


Fig 6. Ninety-six hour's lethal values of acidic pH on copepod plankton and tubificid worm.

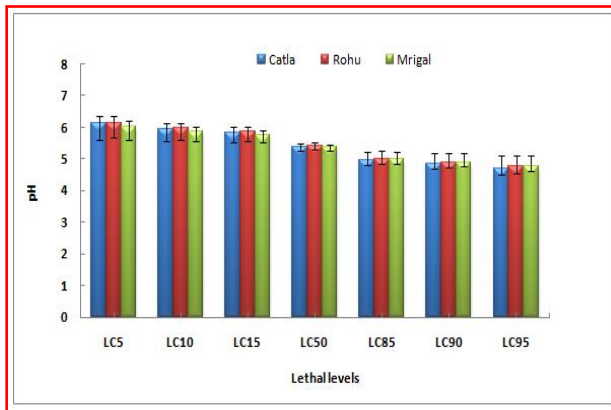


Fig 4. Ninety-six hour's lethal values of acidic pH on Catla, Rohu and Mrigal fry.

Physico-chemical properties of test water:

The alkalinity of the control water used for the bioassays was 226.67 mg/l. But the alkalinity of the water was drastically reduced to 65.00 ± 5.00 mg/l at the treatment of 300 mg/l of CaO after 96 h. Afterwards it was significantly decreased (P<0.05) to 30.33 ± 2.87 mg/l with the corresponding increase of CaO up to 500 mg/l. Significant decrease (P<0.05) of the hardness and conductivity and significant (P<0.05) increase of DO in the test medium was also observed after 96 h treatment of CaO in between 300 mg/l and 500 mg/l (Table 2).

The alkalinity significantly decreased (p<0.05) from 300 mg/l to zero with the corresponding decrease of pH from 7.6 (control) to 4.7 respectively. In contrast, the hardness of the test water significantly increased (P<0.05) from 830 mg/l to 1020 mg/l as CaCO₃ with the corresponding decrease of pH from 7.6 to 4.7. The conductivity of the test medium also increased significantly from 3.76 mS to 3.93 mS with the decreasing pH (Table 3).

Table 1. Nominal concentrations of quick lime (CaO) and sulphuric acid (H₂SO₄) used in 96 h bioassays for IMC fry, *Branchiura* sp. and *Cyclops* sp.

IMC fry		<i>Branchiura</i> sp.		<i>Cyclops</i> sp.	
CaO (mg/l)	H ₂ SO ₄ (mg/l)	CaO (mg/l)	H ₂ SO ₄ (mg/l)	CaO (mg/l)	H ₂ SO ₄ (mg/l)
300	0.0055	10	0.0050	10	0.0020
350	0.0060	20	0.0055	13	0.0025
400	0.0065	30	0.0060	16	0.0030
425	0.0067	40	0.0065	17	0.0035
450	0.0070	50	0.0077	22	0.0040
475	0.0073	60	0.0071	25	0.0045
500	0.0075	70	0.0072	28	0.0050
		80	0.0073	31	0.0055
		90	0.0074	34	0.0060
		100	0.0075	37	0.0065

Table 2. Impacts of quick lime (CaO) on the pH, DO, conductivity, hardness and alkalinity of the test medium during 96 h bioassays for IMC fry.

CaO (mg/l)	pH	DO (mg/l)	Conductivity (mS)	Hardness (mg/l)	Alkalinity (mg/l)
0	7.72 ± 0.15	6.40 ± 0.00	3.72 ± 0.04	853.33 ± 5.77	226.67 ± 5.77
300	8.17 ± 0.20	6.93 ± 0.23	3.43 ± 0.08	693.33 ± 5.77	65.00 ± 5.00
350	8.48 ± 0.39	7.33 ± 0.12	3.39 ± 0.08	660.00 ± 0.00	55.00 ± 5.00
400	8.82 ± 0.46	7.80 ± 0.00	3.37 ± 0.09	640.00 ± 5.00	51.67 ± 2.89
425	9.01 ± 0.48	7.93 ± 0.12	3.36 ± 0.09	628.33 ± 2.89	45.00 ± 0.00
450	9.23 ± 0.57	8.53 ± 0.23	3.35 ± 0.09	623.33 ± 2.89	41.67 ± 2.89
475	9.42 ± 0.6	8.60 ± 0.00	3.33 ± 0.09	618.33 ± 2.89	36.67 ± 2.89
500	9.59 ± 0.67	8.73 ± 0.12	3.32 ± 0.09	615.00 ± 0.00	30.33 ± 2.87

Table 3. Impacts of pH on the DO, conductivity, hardness and alkalinity of the test medium during 96 h bioassays for IMC fry.

pH	DO (mg/l)	Conductivity (mS)	Hardness (mg/l)	Alkalinity (mg/l)
7.65	6.53 ± 0.12	3.76±0.08	830.00±0.00	300.00±0.00
6.46	6.27± 0.12	3.81±0.07	978.33±2.89	141.67±2.89
5.95	6.20 ± 0.20	3.83±0.07	991.67±2.89	110.00±0.00
5.52	6.13 ± 0.12	3.85±0.07	1000.00±0.00	75.00±5.00
5.23	6.13 ± 0.12	3.87±0.07	1003.33±2.89	53.33±2.89
5.12	6.13 ± 0.23	3.89±0.07	1010.00±0.00	28.33±2.89
4.89	6.00 ± 0.00	3.91±0.07	1015.00±0.00	10.00±0.00
4.74	6.00 ± 0.00	3.93 ± 0.06	1020.00±0.00	0.00±0.00

Discussion

In the present investigation, the 96 h LC₅₀ values of CaO for Catla, Rohu and Mrigal were 420.21 mg/l, 418.44 mg/l and 409.72 respectively. But in case of copepod plankton and tubificid worm, the concentrations were 22.02 mg/l and 45.09 mg/l respectively. Das and Das (2005) reported that there was no mortality of common carp fry up to 500 mg/l of CaO but the 96 h LC₅₀ values of it for tubificid worm was 83.00 mg/l and for copepod plankton was 27.80 mg/l. It indicates that the IMC fry are more susceptible to CaO than common carp fry. In addition, variation in the water quality criteria of the present investigation also influenced the toxicity of CaO to fish and fish food organisms.

The tolerance limit of pH by Catla, Rohu and Mrigal was 5.8-8.5. Basu (1950) opined that the larvae of Catla could tolerate a pH range of 6.0-9.0, which is closely similar to the present study. But, Das et al. (1995) reported that the non-lethal range of pH was 5.0-9.0 for common carp fry. The survival percentage of the IMC fry decreased from pH 8.5 and its lethality was 95-100% above pH 10.22. Saha and Chowdhury (1956) also reported that survival period of Catla fry decreased at the water pH 8.8 and above. That result strongly supports the present observation. In the present investigation, the non-lethal pH of copepod plankton was 7.1-8.0. But in case of Daphnia, the non-lethal range of pH was 6.5-9.5 (Clare, 2002). The variations of results are due to the variation of test organisms used in the two different experiments and interaction of pH with the different water quality parameters.

Acidic ponds are not desirable for fish culture. When a high degree of saturation with base-forming cations takes place soil alkalinity occurs. Concentration of calcium, magnesium, and sodium carbonate can result in a preponderance of hydroxyl ions (OH⁻) over hydrogen ions (H⁺). Under this condition, the soil will be alkaline. The water borne metals generally showed their greatest toxicity to aquatic organisms in soft water of low alkalinity, low pH and low dissolved organic carbon (Flemming and Trevers, 1989; Spry and Wiener, 1991). The acid and alkaline death points for fish are about pH 4 and 11 respectively. However, if waters are more acidic than

pH 6.5 or more alkaline than pH 9-9.5 for long periods, reproduction and growth will diminish (Swingle, 1961; Mount, 1973). A change in water pH, either acidic or alkaline condition, exerted stress in fish characterized by the haematological disorders in Catla, Rohu and Mrigal (Das et al., 2006) which resulted the mortality of fish. Problems with pH are not uncommon. In mining areas, acid-mine seepage may acidify lakes and streams because of acid precipitation, which causes disastrous effects on fish population. Boyd (1979) reported that pH of pond waters could be reduced by the treatment of ammonium sulfate, which releases hydrogen ions upon nitrification; filter alum [Al₂(SO₄)₃. 14H₂O], which hydrolyzes to release hydrogen ions; agricultural gypsum (CaSO₄. 2H₂O), which increase calcium hardness resulting in precipitation of calcium carbonate. Of course, H₂SO₄, or some other strong mineral acids, can also reduce the pH of water. The application of lime in aquatic ecosystem increased the pH, alkalinity and Ca level in water (Raddum et al., 1986; Boyd and Tucker, 1998). The pH value is commonly regarded as a sensitive functional response parameter (Giddings, 1983; Stay et al., 1989). The application of burnt lime (CaO) and hydrated lime [Ca(OH)₂] increases water pH up to 10 or more and causes toxicity in aquatic organisms.

It was recorded that the alkalinity and hardness of the test water decreased with the increase of CaO concentrations. But, the dissolved oxygen (DO) of the test water increased with the increasing concentrations of CaO. The main chemical species that contribute to alkalinity are bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) ions. The relative contribution of [HCO₃⁻] and [CO₃²⁻] ions to alkalinity is 89.8% and 6.7% respectively but hydroxide (OH⁻) ions contribute to alkalinity only 0.1% (Millero et al., 1998). After the treatment of lime as CaO, the available bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) ions present in the aquatic medium were chemically bounded by the Ca²⁺ of CaO to produce CaCO₃ and it was precipitated on the bottom (Eqs 1 and 2). As a result, the [HCO₃⁻] and [CO₃²⁻] ions were decreasing with the consequent rise of [OH⁻] ions. Therefore, the alkalinity of the test medium decreased and pH of the test medium increased.



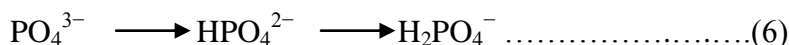
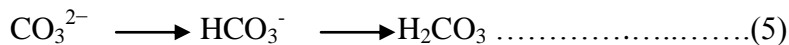
Similarly, available free cations (like Ca^{2+} , Mg^{2+} , Fe^{2+} etc.) in the system were also bounded and precipitated as oxides or carbonates of the metallic ions at high pH, which resulted in the decrease of hardness (Eqs 3 and 4). As the cations and anions

precipitated through chemical bonding, the conductivity of the test water reduced. During the process, some amount of oxygen evolved, that also increased the dissolved oxygen contents of the test water.



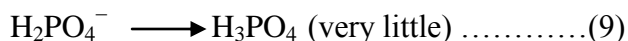
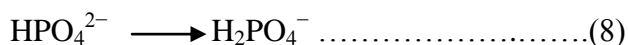
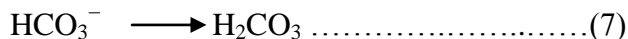
The quantity of base present in water defines what is known as total alkalinity. Common bases found in fish ponds include carbonates (CO_3^{2-}), bicarbonates (HCO_3^-), hydroxides (OH^-) and phosphates (PO_4^{3-}). Carbonates and bicarbonates are the most common and most important components of alkalinity (Wurts and Durborow, 1992). The alkalinity of test water decreased but, the hardness and conductivity of water

increased with the decreasing of pH of the test water. The discharge of acid drainage to surface waters changes the water quality by reducing the total alkalinity and pH and increasing the total hardness. As the pH drops to about 6, the bicarbonate (HCO_3^-) is getting converted into carbonic acid (H_2CO_3), leading to the decrease in alkalinity of water (Eq 5). Phosphate continues to take up protons (Eq 6).



As the pH drops to about 4, the bicarbonate becomes fully converted into carbonic acid (Eq 7). In this range, phosphate continues to take up

protons and ends up mostly H_2PO_4^- (Eq 8) and very little phosphoric acid (Eq 9). At this stage alkalinity of the water will tend to be zero (0).



The acidity of water is an overall impact of all dissolved ions on the electro-negativity of water. Acidic water tends to take free electrons whereas basic water tends to give electrons. In case of H^+ and OH^- ions, H^+ tends to take electron and OH^- tends to give electron. There will always be some bicarbonate and carbonate present in aquatic environment, but at some pH there are enough protons (H^+) in solution and if they are to be combined with the bicarbonate and carbonate present, they would all be converted to carbonic acid.

Conclusion

The study clearly indicated that both IMC fry (Catla, Rahu and Mrigal) and their food organisms (copepod plankton and tubificid worm) did not show any lethality at the ranges of pH 7.1 to 8.0. Therefore, pH in between 7.1 and 8.0 would be recommended as “optimum level” for the sustainable nursery management of IMC fry. The copepod plankton, the most susceptible organisms, can tolerate maximum 12.85 mg/l of CaO, whereas the IMC fry can tolerate maximum 359.77 mg/l of CaO during culture. Considering the sensitivity of copepod plankton, CaO may be applied as low as around 12.85 mg/l for the

management of water quality during culture. The different types of liming materials are available for pond aquaculture management. These are CaCO_3 (calcium carbonate or calcite), CaO (calcium oxide or quick lime or burnt lime), $\text{Ca}(\text{OH})_2$ (calcium hydroxide or hydrated lime or slaked lime), $\text{CaMg}(\text{CO}_3)_2$ (dolomite) etc. The application of CaO is promptly changing the pH and alkalinity of the water, which may adversely affect the fish fry and fish food organisms during culture. In addition, it is more expensive than each of the other liming materials. Wurts and Masser (2004) also opined that the use of quick lime (CaO) is not recommended because it is more expensive and can cause pH to rise rapidly to levels that can harm to aquatic life. Therefore, the application of CaO during culture period of IMC fry should be avoided and would be recommended for its application only during pond preparation to avoid any threat.

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