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Feasibility of hydrogen peroxide as oxygen promoterduring drastic conditions in aquaculture set up having *Ctenopharyngodon idella* as a test fish specie

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Key words: Hydrogen peroxide, Dissolved oxygen, Physico-chemical parameters

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Abstract

The main focus of present study was to evaluate the safe concentration of hydrogen peroxide and its effectiveness on the rate of increased release of dissolved oxygen in the water media in emergency Anoxiasituations. Two trials were conducted in relation to temperature factor, one in winter and the other in summer season in the presence of *Ctenopharyngodon idella* (Grass carp) for DO evaluation. The results of Trial (I) at low temperature in winter revealed that the maximum concentration of dissolved oxygen (D.O) release remained at 13.6 ppm with an applied dose of 16ml/40L of 6% H_2O_2 at 12°Cwithin 24 hours while at the same temperature, the recorded D.O release was only 3.5 ppm in the control. The same experiment was repeated as Trial (II) at high temperature in summer. It was observed that after the application 16 ml/40L of 6% H_2O_2, D.O release reached upto9.9 ppm at 32°C while the maximum concentration of D.O remained2.4ppm in the control. The present study also showed that the *Ctenopharyngodon idella* was very much sensitive to hydrogen peroxide and its excessive use caused fish mortality. Themortality rate was 20% and 40%at 12.0 ppm hydrogen peroxide concentration,50% and 60% at 14.0 ppm and 80% and 90%at 16.0 ppm during winter and summer phases, respectively. The maximum increase in Do level was achieved at 10.3 and 8.4 ppm in winter and summer phase effectively and safely without any fish mortality.

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Introduction

Dissolved oxygen is the most vital fundamental parameter in water ecosystem, lifeline for the pond environment and is a vital component for the survival of pond fish (Lewis, 1993). Frequently, it is the key substance in determining the extent and kinds of life in a pond. Maximum proportion of oxygen is obtained as a result of photosynthetic activity of phytoplankton. As the oxygen is produced during daylight hours, its level decreases overnight and is lowest just prior to sunrise (Parther, 2004). The amount of oxygen consumed by the fish is a function of its size, species, age, feeding rate and temperature. The concentration of dissolved oxygen in natural water is influenced by the relative rates of diffusion to and from the atmosphere, photosynthesis by aquatic plants and aspiration by aquatic biological community (Boyd, 1990). It is the key substance in determining the extent and kinds of life in a pond (Manahan, 2004).

Dissolved oxygen (D.O) depletion occurs directly by chemical oxidation of dissolved organic matter. The DO content in water is controlled by balancing the oxygen input with received consumptive metabolism of oxidizable matter (Wetzel, 1983).Reduced oxygen levels causes lethal and sublethal (physiological and behavioural) effects in various organisms, especially in fish. Younger fish tended to be more sensitive than older fish (Doudoroffand Shumway, 1970; Alabaster and Lloyd 1982; Crochet, 2005). It results significant abnormal development, such as deformed tails and spines, and abnormal nervous systems and brain development (Silver *et al.,* 1963). In the presence of other stressors, DO depletion results in combined hypoxia and hypercapnia (Jensen, *et al.,* 1993). Low dissolved oxygen concentration decreases the ability of water body to support aquatic life. It can also lead to slow growth, suffocation and reduce the disease resistance, eventually leading to fish mortality (Higginbotham, 1997). The depletion is the most common cause of fish mortality (Manahan, 2004). During D.O depletion fishes gulp at the surface of the water which is especially noticeable early in the morning when oxygen levels are likely to be lowest. Fish might congregate near sources of fresh incoming water. Large fish usually die first, if the problem is severe enough, smaller fishes will follow (Crochet, 2005).

Hydrogen peroxide is a strong oxidizing agent, used as a disinfectant, the raputant and improves water quality by increasing oxygen. A number of different elements, compounds, enzymes as well as light, heat and high pH, all accelerate the degradation of hydrogen peroxide. Hydrogen peroxide decomposes into oxygen and water, so as a result there is an increase in dissolved oxygen contents of water (Spain *et al.,* 1989). Decomposition of hydrogen peroxide may take minutes to a few hours, depending upon initial hydrogen peroxide concentration, the number and type of microorganisms in the soil and mineral contents. The capacity of water to hold oxygen is low, so the oxygen is far more limited in the aquatic habitat than in the air. Moreover, the solubility of oxygen in water depends on many factors i.e. temperature, salinity and wind action etc. Hydrogen peroxide is used to increase dissolved oxygen for fish fauna in emergency situation where the mechanical aeration is not possible. FDA has also proved hydrogen peroxide is the significant antibacterial and antifungal agent for aquaculture (Russo *et al.,* 2007). It controls external parasitic infestations (Rach *et al.,* 2000). It is a benign chemical to decrease cyanobacteria (Barrington *et al.,* 2013). It is effective in treating fish and fish eggs infected by fungi (Rach *et al.,* 2011). Hydrogen peroxide has been commonly used as an oxygen source because of the limited concentrations of oxygen that can be transferred into the groundwater using aeration followed by reinjection of the oxygenated groundwater into the aquifer (Zappi *et al.,* 2000).

The main focus of the present study is to evaluate the dissolved oxygen concentration with hydrogen peroxide in relation to temperature factor. This research was, therefore, conducted to determine the effectiveness of hydrogen peroxide to enhance the dissolved oxygen levels in water in emergency situations; to study the persistence of dissolved oxygen in water after the application of H_2O_2 and its effects on fish survival and growth.

Materials and methods

Experimental Site & Design

Two trials of experiment with nine sets of treatments were conducted in winter and summer season each at Chemistry Laboratory, Fisheries Research & Training Institute, Lahore. For each trial 27 glass aquaria having dimensions 1 x 2 x 1.5 feet were filled by adding pond water up to 40L in each aquarium. The fingerlings of grass carp (*Ctenopharyngedon idelia*) having average weight of 2.8 ± 0.35 g were procured from the Central Fish Seed Hatchery, Lahore. Prior to initiation of any experimental work the fish was acclimatized to laboratory environment for two weeks. During this transient period fish were kept in well aerated aquaria and fed on artificial feed (Yvonnen *et al.,* 1987). After acclimatization ten fish were stocked in each glass aquaria.

Physico-chemical parameters

The physico-chemical parameters i.e. temperature, dissolved oxygen and pH were determined with the help of available digital meters while alkalinity, hardness, T.D.S and Chlorides were analyzed titrimetrically (A.P.H.A., 2012; Waheed *et al.,* 2015; Waheed *et al.,* 2017).

Statistical Analysis

ANOVA statistical analysis on SPSS (version 17) was applied to find the significant differences for dissolved oxygen release by hydrogen peroxide (Steel *et al.,* 1996).

Results

Dissolved Oxygen

Two experimental trials i.e. winter and summer were conducted in the mid of January and in 2nd week of May, the average temperature of water was recorded at 12°C and 32°C, respectively. The results of winter and summer trials are illustrated by Figures 1&2, respectively. Prior to addition of 6% H_2O_2, the DO for all the treatments was 3.5 and 2.4 ppm in winter and summer trials, respectively.

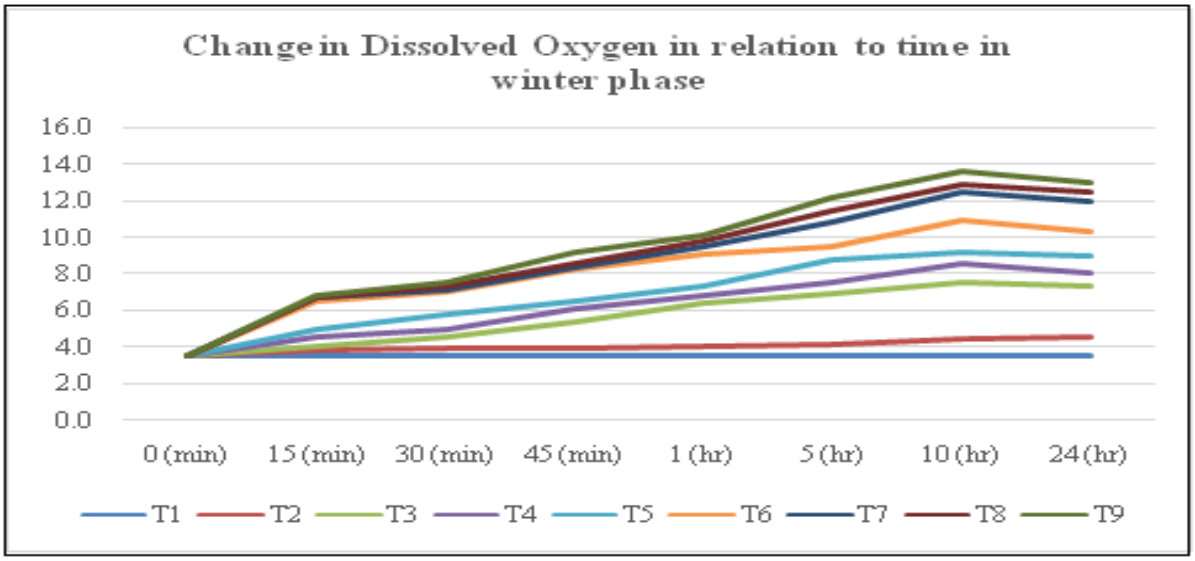

Fig. 1. Change in Dissolved Oxygen concentration (ppm) in relation to time in winter phase.

The significant differences in oxygen released were observed in nine treatments T1, T2, T3, T4, T5, T6, T7, T8 and T9 i.e. by applying eight different doses of hydrogen peroxide (6%) along with one control. The addition of 2ml/40L, 4ml/40L, 6 ml/40 L, 8 ml/40L,10 ml/40L, 12ml/40L, 14ml/40L and 16 ml/40L of 6% H_2O_2, the D.O values increased from 3.5ppm to 6.8ppm (i.e., 3.8ppm,4.0 ppm,4.5ppm,5.0 ppm, 6.5ppm, 6.7ppm, 6.7ppm, 6.8ppm, respectively) within 15 minutes in winter while in summer the values increased from 2.4ppm to 4.2ppm (i.e., 2.5ppm,2.8ppm,3.0ppm,3.3ppm,3.5ppm,3.9ppm,4.0 ppm,4.2ppm,respectively). The D.O values reached to its maximum value within 30 minutes of the dose application i.e. up to 13.0ppm and 9.9ppm after 24 hours in winter and in summer trials, respectively.

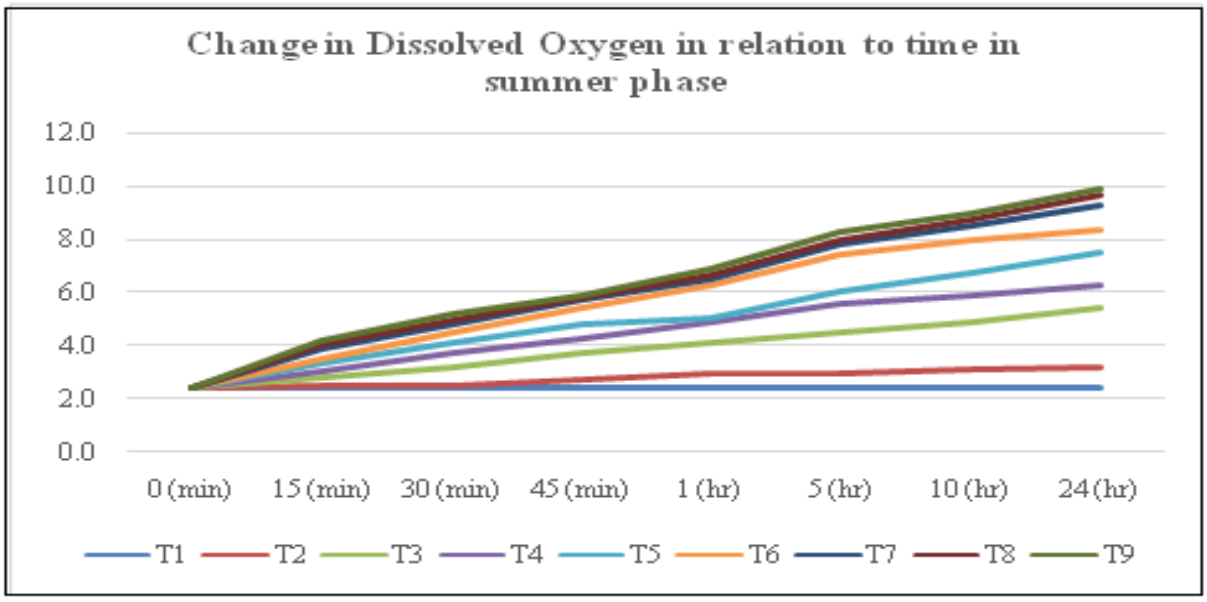

Fig. 2. Change in Dissolved Oxygen concentration (ppm) in relation to time in summer phase.

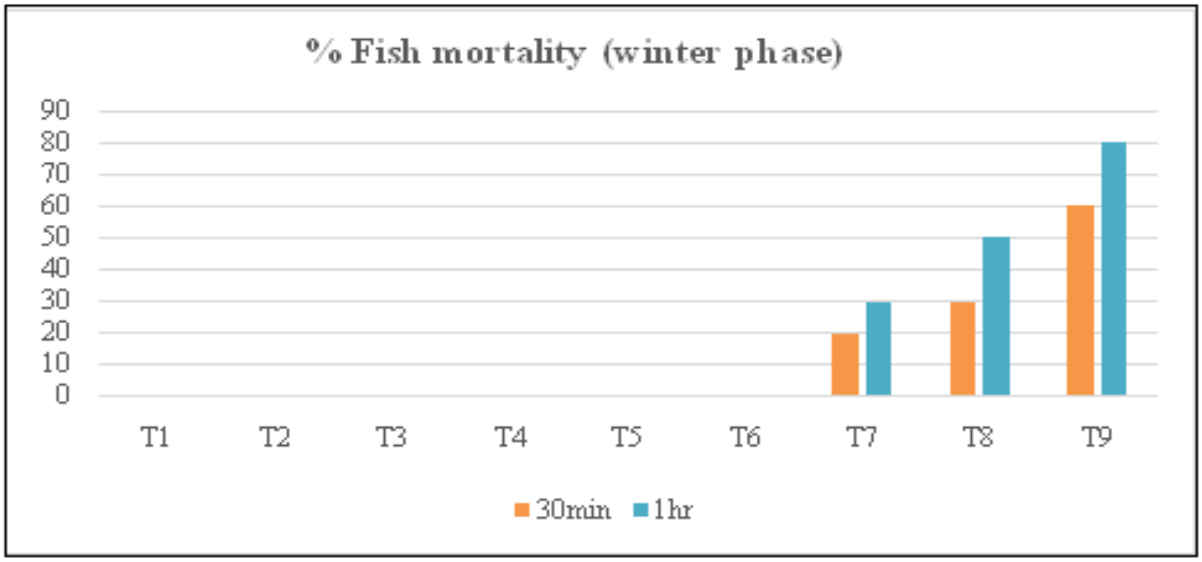

Fig. 3. Fish mortality (%age) in winter phase.

Fish Survival

As far as the mortality of fish was concerned, there was no mortality of fish observed in the treatments T1, T2, T3, T4, T5 and T6 till 24 hours of the application of 6% H_2O_2 both in winter and summer trials (Figure 3 & 4), however, the fish mortality was observed in the treatments T7, T8& T9 after 30 min of dose application. There was 20%, 30% and 60% of the mortality of fish observed after 30 min of the 6% H_2O_2 in winter and 30%, 30% and 60% summer trial respectively.

Whereas, the mortality of fish observed in the treatments T7, T8 & T9 after one hour were 30%, 50% and 80% in winter and 40%, 60% and 90% in summer trial, respectively after that no mortality was observed.

pH

Prior to addition of 6% H_2O_2, the initial reading of pH for all the treatments ranged from 7.9 to 8.0 in winter and 8.0 to 8.1 in summer trials, respectively.

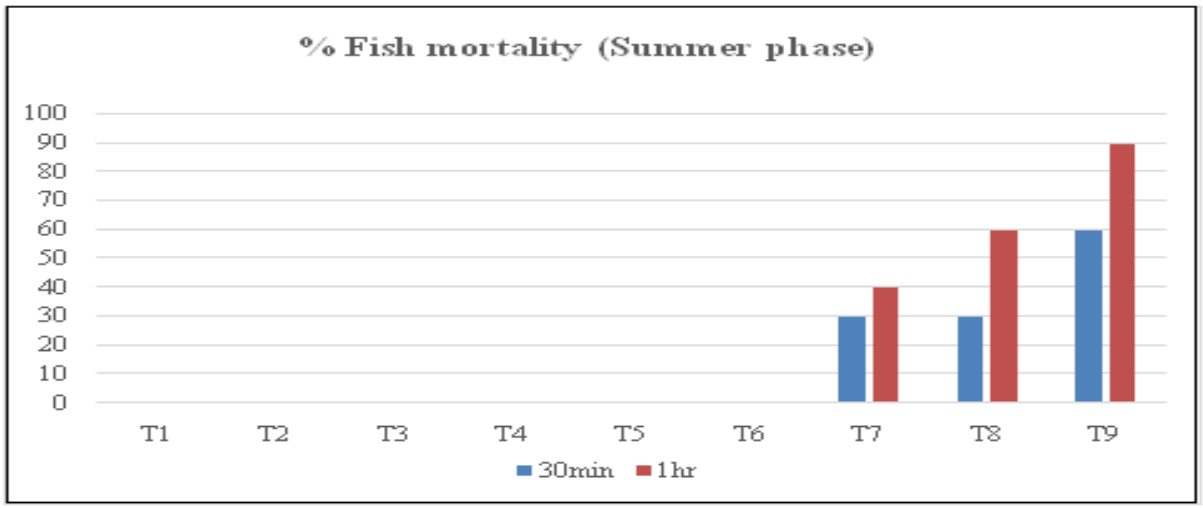

Fig. 4. Fish mortality (%age) in summer phase.

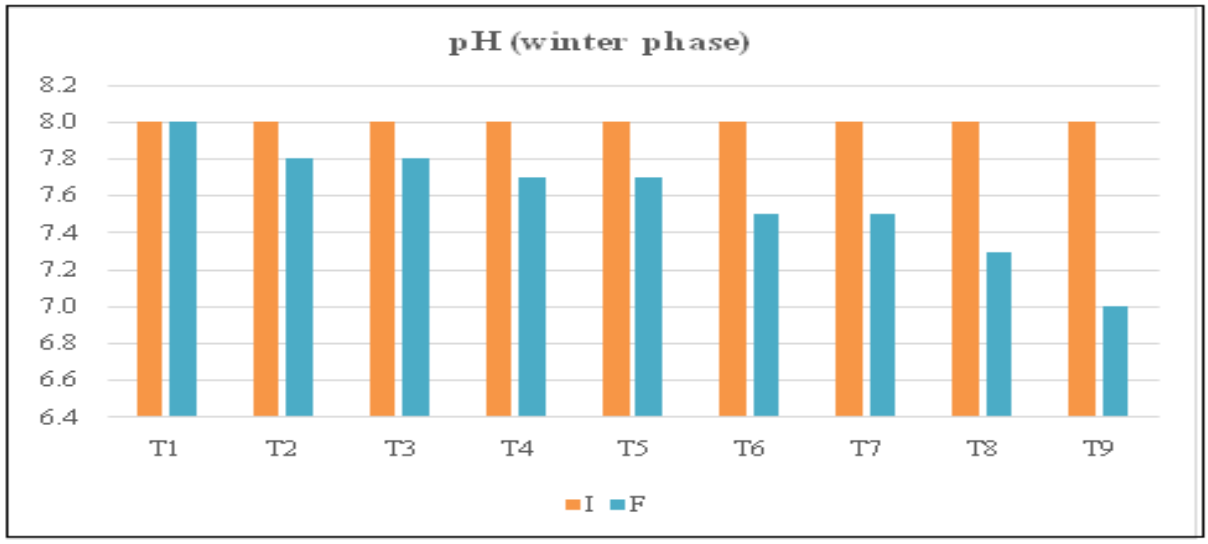

Fig. 5. Effect of pH (winter phase).

After the addition of subsequent doses of 6% hydrogen peroxide, the value of pH decreased from 8.0 to 7.0 in winter while in summer the value was decreased from 8.1 to 7.4 after 30 minutes respectively. It can be evaluated that an average decrease in pH level both in winter and in summer trial after 30 min was 1.0 and 0.7 respectively as shown in Fig 5 and 6.

Alkalinit

The initial reading of alkalinity for all the treatments was 300mgL^{-1} in winter and 320 to 325mgL^{-1}in summer trial respectively. After the addition of various doses of 6% hydrogen peroxide, the value of alkalinity decreased from 300 to 175mgL^{-1} in winter while in summer the value got decreased from 320 to 185mgL^{-1} after 30 minutes respectively.

It meant that an average decrease in alkalinity level both in winter and in summer trial after 30 min was 125mgL^{-1} and 135mgL^{-1} respectively as shown in Fig 7 and 8.

Hardness

From Fig 9 and 10, it can be seen that the initial reading of hardness for all the treatments ranged from 195mgL^{-1} to 194mgL^{-1} in winter and 195mgL^{-1} to 198 mgL^{-1} in summer trials, respectively.

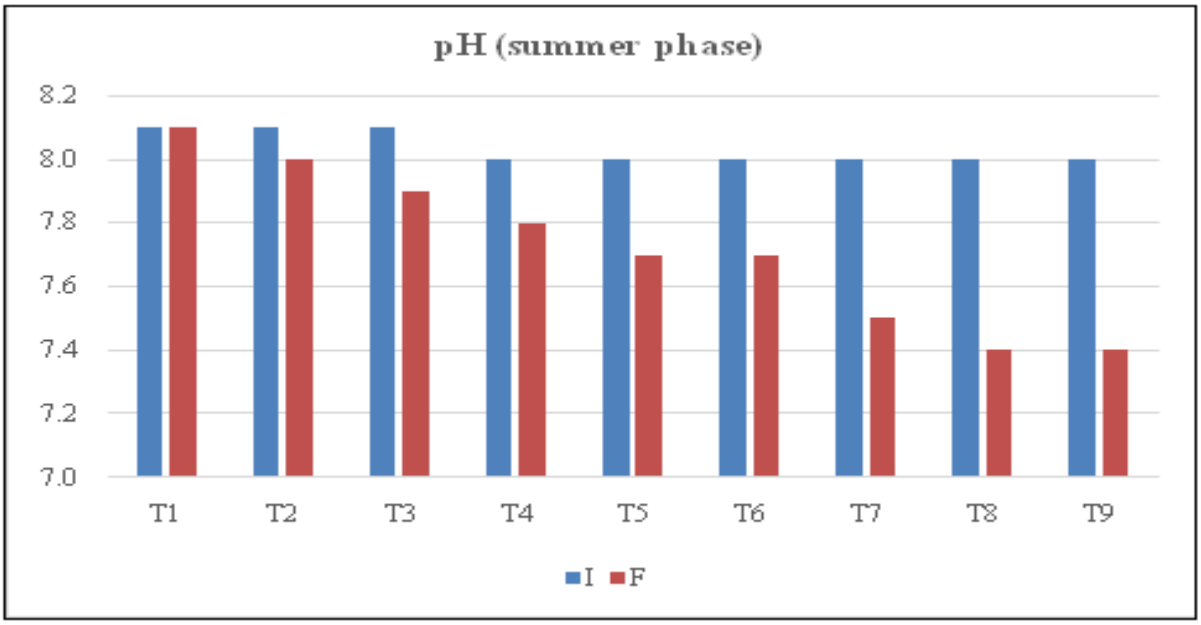

Fig. 6. Effect of pH (summer phase).

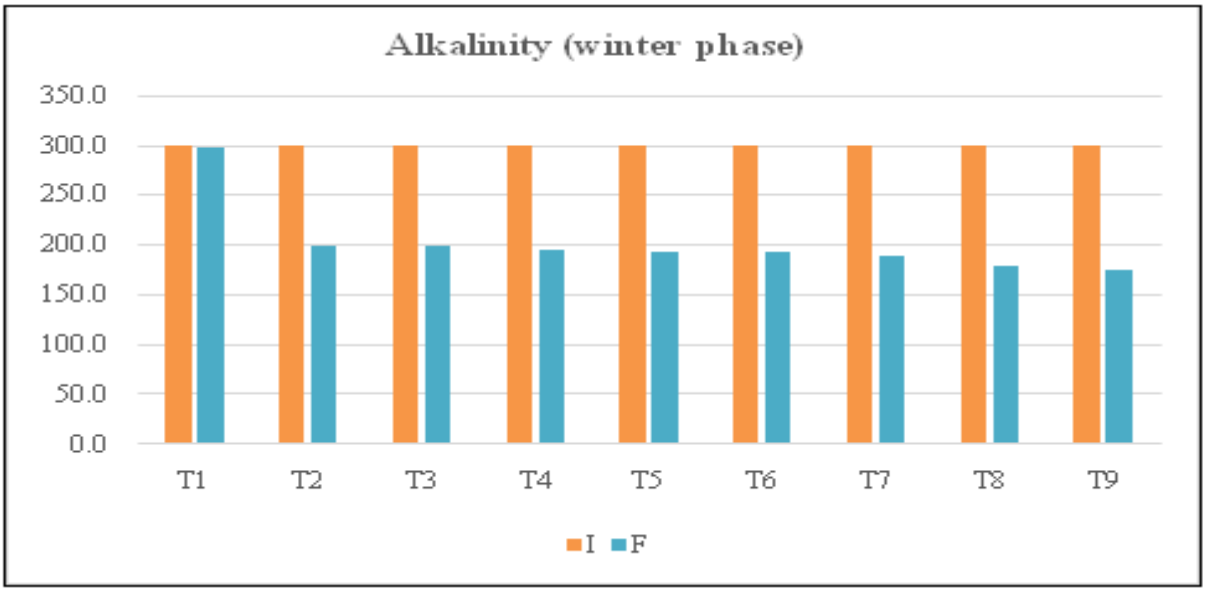

Fig. 7. Effect of Alkalinity (ppm) in winter phase.

After the addition of various doses of 6% hydrogen peroxide, the value of hardness got increased from 195 to 242mgL^{-1} in winter while in summer the value increased from 195to 245 after 30 minutes respectively. This revealed that an average increase in hardness both in winter and in summer trial after 30 min was 47 and 50mgL^{-1} respectively.

T.D.S

The initial reading of T.D.S for all the treatments ranged from 680 to 675mgL^{-1} in winter and 680 to 685 mgL^{-1} in summer trial respectively. After the addition of subsequent doses of 6% hydrogen peroxide, the value of T.D.S increased from 680 to 830 mgL^{-1} in winter while in summer the values increased from 680 to 820mgL^{-1} after 30 minutes, respectively. It meant that an average increased in T.D.S both in winter and in summer trials after 30 min was 150 and 140mgL^{-1}, respectively as shown in Fig 11 and 12.

Chlorides

The initial reading of Chlorides for all the treatments were 40mgL^{-1} in winter and in summer trial respectively. After the addition of various doses of 6% hydrogen peroxide, there was no effect on the value of Chlorides both in winter and in summer trials, respectively as shown in Fig 13 and 14.

Discussion

The results of this investigation showed that maximum concentration of dissolved oxygen was observed when the dose of 16 ml/40L ofH_2O_2 (6%) was administered for both winter and summer trials. While in control the dissolved oxygen was 3.5ppm and 2.4ppm during winter and summer, respectively. Application of a dose of 10ppm of hydrogen peroxide (6%) increased the DO level from 3.5ppm to 13.6 ppm during winter while it increased from 2.4ppm to 9.9ppm during summer.

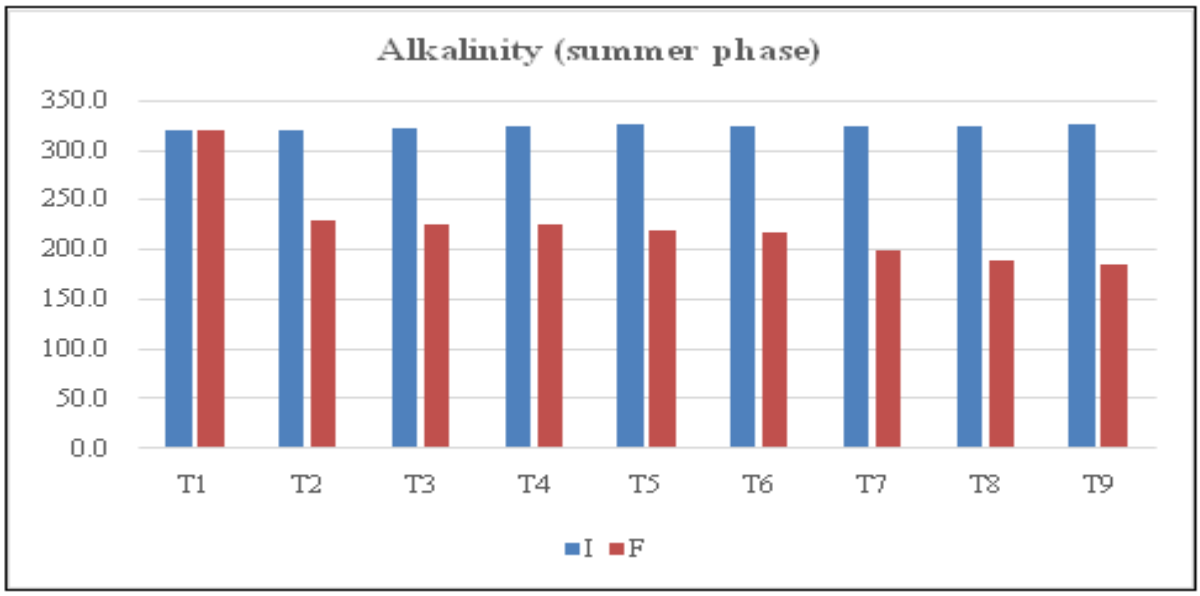

Fig. 8. Effect of Alkalinity (ppm) in summer phase.

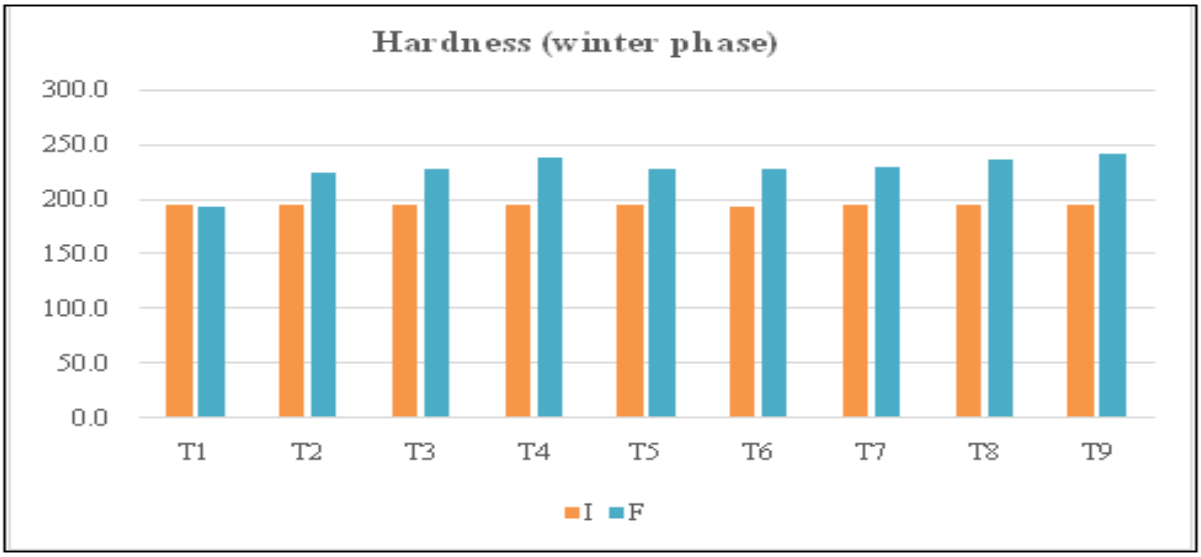

Fig. 9. Effect of Hardness (ppm) in winter phase.

It took 24 hours both in winter and summer trial to reach the maximum DO level due to the H_2O_2 (6%). The decline of DO level was observed after 24hours in winter trial only.

The present study also showed that *Ctenopharyngodon idella*are very much sensitive to hydrogen peroxide and its excessive use can cause their mortality. Fish species including the experimental *Ctenopharyngodon idella* are more vulnerable to mortality if more than 10ml/40L dose is used. During the present trials no mortality were observed even at the 10ml/40L dose of H_2O_2 (6%).The mortality started in the treatments T7, T8& T9 having 12, 14 & 16 ml/40L after 30 min and continued up to 45 min both in winter and summer trial, respectively.

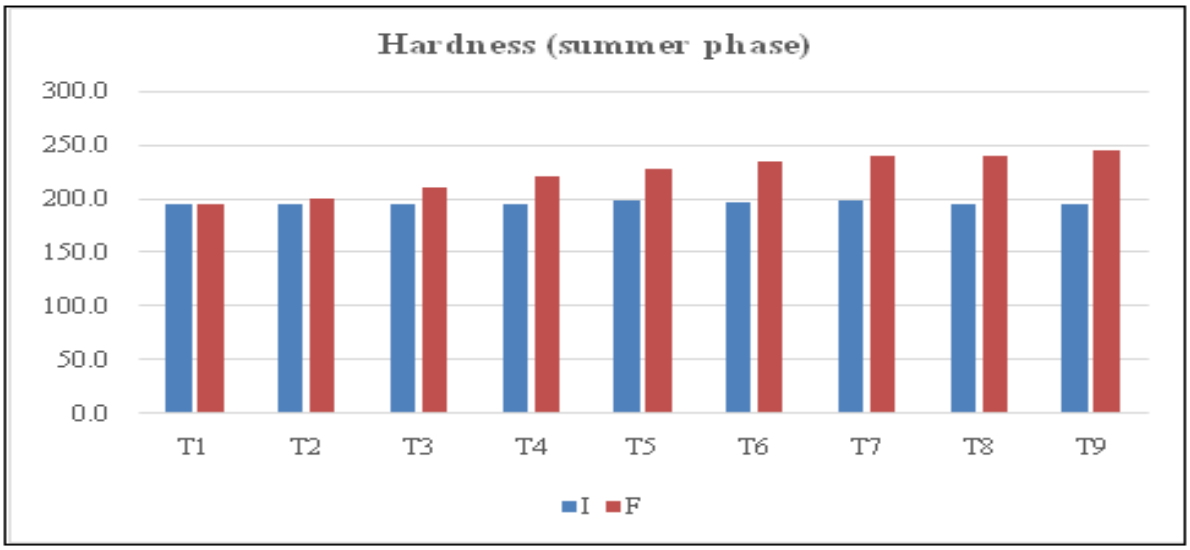

Fig. 10. Effect of Hardness (ppm) in summer phase.

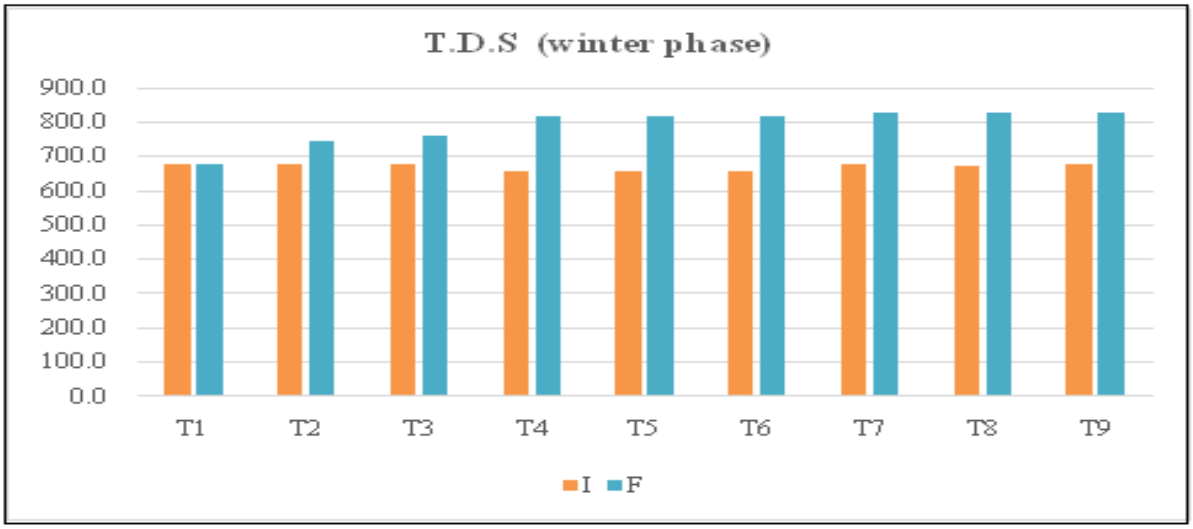

Fig. 11. Effect of T.D.S (ppm) in winter phase.

As far as physico-chemical parameters were concerned, no considerable changes were observed before and after the application of five doses of hydrogen peroxide (6%). Hydrogen peroxide has pH 6.2 which is slightly acidic.

The induction of H_2O_2 caused slight decrease in pH and alkalinity while there was an increase in hardness and T.D.S and simply no effect on the chloride level in water.

Since pond water was used in the experiment which has its own buffering system showing no considerable changes in water quality criteria. Therefore, the use of hydrogen peroxide did not affect the physico-chemical characteristics of water.

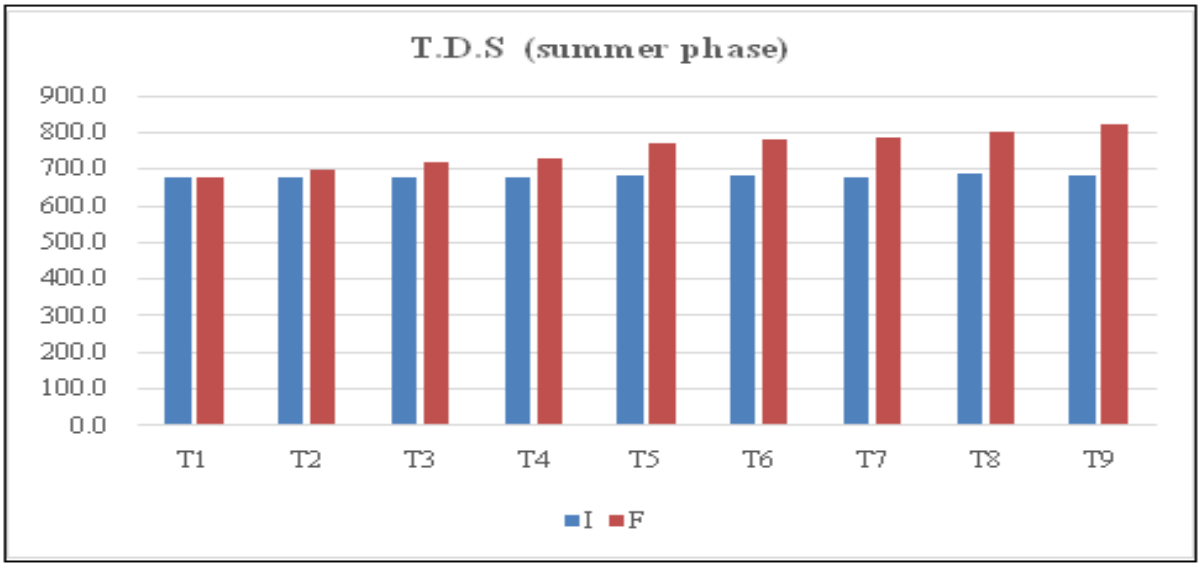

Fig. 12. Effect of T.D.S (ppm) in summer phase.

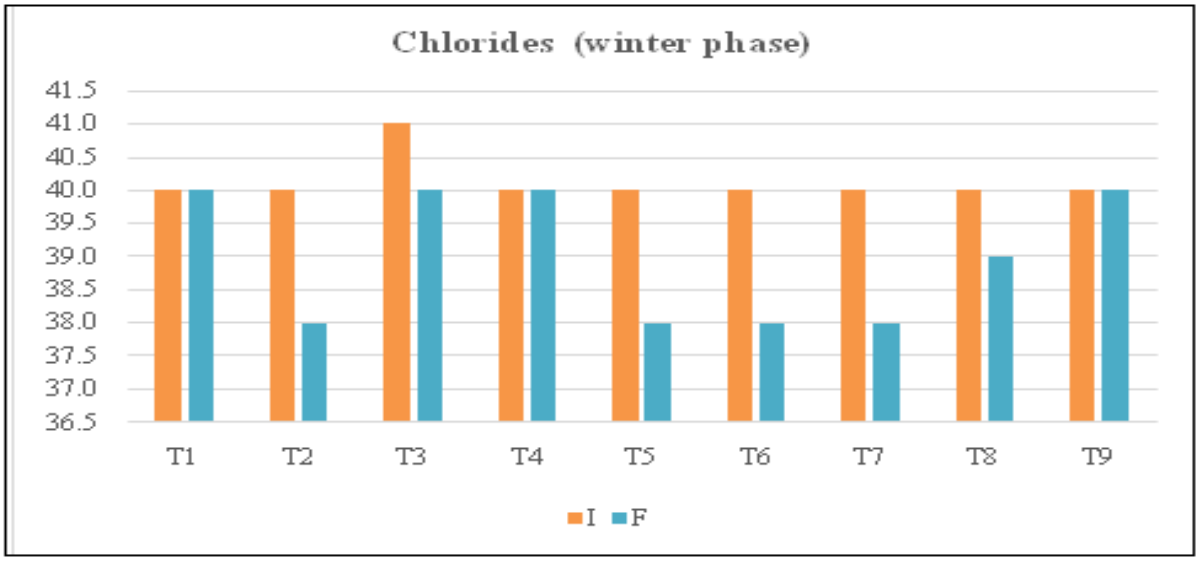

Fig. 13. Effect of Chlorides (ppm) in winter phase.

Our research has been supported by Taylor and Ross (1988) who used Hydrogen peroxide as a source of oxygen for the transportation of live fish by generating oxygen through H_2O_2 source in a container that was separate from fish holding tank; H_2O_2 was found to produce no clinical toxicity. Our research is also in agreement with the findings of Rach *et al.,* 2011who studied and found a correlation between the toxicity of hydrogen peroxide and the life stages of rainbow trout; larger fish were more sensitive, generally, the toxicity of hydrogen peroxide increased for all species as water temperature increased.

It was also found effective in treating fish and fish eggs infected by fungi. Clayton and Summerfelt1996determined that D.O concentrations increased from 0.25 to 1.16 ppm (µL/L),one hour after the addition of Hydrogen peroxide because of the dissociation of H_2O_2 ($2H_2O_2 \rightarrow 2H_2O + O_2$), which chemically raised the oxygen concentration substantially. Nykänen, *et al.,* 2012 demonstrated that granulated calcium peroxide (CaO_2) behaves as a slow oxygen releasing compound and increases necessary oxygen level of the sediment and hypolimnion of many Finnish lakes suffering from anoxia due to increasing nutrient loads.

Our studies are also in agreement of Barrington *et al.,* 2013 who assessed and found the use of H_2O_2 beneficial in rapidly suppressing the cyanobacterial and microcystin concentrations and also preventing them from entering the environment within hydrogen peroxide application days resulting in an increase in growth of eukaryote phytoplankton. The presence of organic matter serves as a catalyst to accelerate the decomposition of hydrogen peroxide and the release of dissolved oxygen.

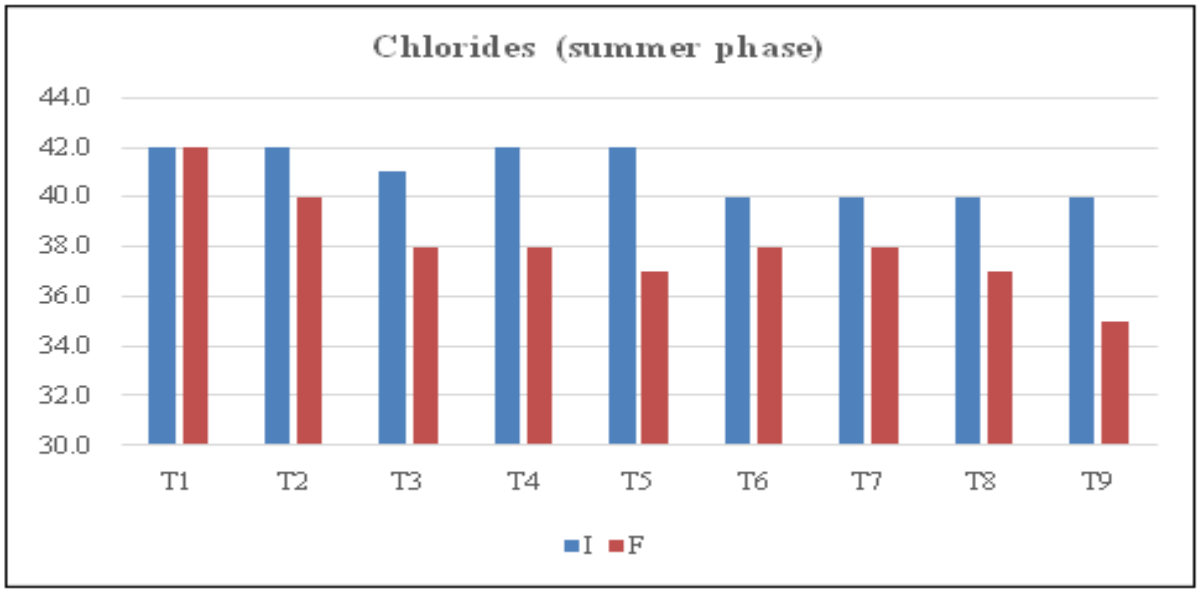

Fig. 14. Effect of Chlorides (ppm) in summer phase.

Our research has also been supported by the results of Griffiths, 2002 who introduced hydrogen peroxide which chemically raised the D.O concentration substantially of a given volume of water. He also described that by Using H_2O_2 in controlled protocols, the filter bacteria were not harmed. He recommended its emergency application only and discouraged its continual use of H_2O_2 because of its oxidisation effects on delicate gills of fish.Cooper *et al.*, 1989 also studied H_2O_2 concentration as a tracer for short term water mixing processes in Lake Waters and found it useful as a powerful oxidizing agent influencing metal speciation, degradation of some organic pollutants, as well as the survival and behavior of organisms. Rach *et al.,* 2000 concluded results of their experiments demonstrating that it is important to consider the effects of species, life stage, and water temperature when conducting hydrogen peroxide treatments. Gaikowski *et al.,* 2011observed that there was no mortality of sac fry of rainbow trout at hydrogen peroxide concentrations of 1,000 µL/L or lower, however, other species or strains may be more sensitive than rainbow trout.

They recommended that other species and strains should be initially treated with hydrogen peroxide at 500 µL/L until monitoring of egg mortality identifies the presence or absence of a sensitive period. Arndt *et al.,* , 2011 conducted two separate trials where eggs of rainbow trout *Oncorhynchus mykiss* were cultured with the use of hydrogen peroxide and formalin treatments to control fungal infections and results were significantly better for hydrogen peroxide. Marking *et al.,* 1994 subjected 21 chemicals for antifungal activities against *Saprolegnia*-infected eggs of rainbow trout (*Oncorhynchus mykiss*), a ubiquitous genus of aquatic fungi frequently found in fish hatcheries. Fourteen compounds were ineffective for control of fungus on rainbow trout eggs or were toxic to the eggs. The seven compounds that effectively controlled fungus on infected eggs and provided a reasonable margin of safety included hydrogen peroxide. Burson *et al.,* 2014 conducted the first successful field application of H_2O_2 to suppress a marine harmful algal bloom, *Alexandrium* sp.

The key advantage of this method is that the added H_2O_2 decays to water and oxygen within a few days, which enables rapid recovery of the system after the treatment. This is. The results show that H_2O_2 treatment provides an effective emergency management option to mitigate toxic *Alexandrium* blooms, especially when immediate action is required. Hans *et al.,* 2012 proposed the use of dilute H_2O_2 for the selective elimination of harmful cyanobacteria from recreational lakes and drinking water reservoirs, especially when immediate action is urgent. A key advantage of this method is that the added H_2O_2 degrades to water and oxygen within a few days and thus leaves no long-term chemical traces in the environment. Christopher *et al.,* 1999 examined the mechanism and kinetics of surface catalyzed hydrogen peroxide decomposition and degradation of contaminants in the presence of sand collected from an aquifer and a riverbed and concluded that H_2O_2 contributed to contaminant degradation.

Conclusion

The efficacy of the Anoxia treatment with H_2O_2has been found effective from 15 minutes to 24 hours and even beyond that also. It increased D.O from a minimum of 3.5 to a maximum of 13.6 ppm in winter and 2.4 to 9.9 ppm in summer. The physico-chemical parameters remained within the suitable ranges in this whole research period after application of 6% H_2O_2. Furthermore, there was no adverse effect on *Ctenopharyngodon idella* up to 10ml/40L dose of H_2O_2 (6%) above that fish mortality occurred. Hence, the use of $H_2O_2$for controlling an emergency situation and enhancing D.O levels in the pond water can be recommended up to a dose of 10ml/40L.

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