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Full Length Article

Use of Bioremediated Sewage Effluent for Fish Survival

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Abstract

Two fresh water fish species Tilapia (*Oreochromis mossambicus*) and Common Carp (*Cyprinus carpio*) were cultured to investigate the survival rate in bioremediated sewage effluent of Shehzad town, Islamabad, Pakistan. Two earthen ponds one with fresh water and second with bioremediated sewage effluent with dimension of 20×40 m were selected at Fisheries and Aquaculture Programme, NARC. Fish survival was investigated after fortnight sampling. Physicochemical parameters of bioremediated water were within permissible limit recommended for fish. Less than one percent survival was observed in bioremdiated water pond whereas 100% fish survival was recorded in fresh water pond. Further investigation and results showed the higher level of ammonical-nitrogen (NH_4^+-N; 31.08 mg/L), nitrate-nitrogen (NO_3^--N; 18.58 mg/L) and chlorides (Cl^-; 39.61mg/L) in bioremediated sewage water that were main cause of fish mortality. Complete fish survival was recorded in bioremediated sewage effluent after phytoremediation with Coontail (*Ceratophyllum demersum*) plant that has potential of removing NH_4^+, NO_3^- and Cl^- from sewage waste water. This study showed that this treated sewage water required further treatment for removal of NH_4^+-N, NO_3^--N and Cl^- by using phytoremdiater Coontail (*C. demersum*).

Keywords: CRD; Ammonical nitrogen; Coontail

Introduction

The technological advancement, urbanization and increase in global population are continuous threat to mankind. Significant increase in population growth has been observed form last few decades in all over the world that become the major reason of urbanization, industrial activities and water scarcity (Buzina *et al.,* 1989). These activities have increased a huge amount of discharge and range of pollutants that could reach the water reservoirs and have undesirable effects on fresh water fish and fisheries (Heath, 1987; Mc Cail *et al*., 1988; Bhatnagar *et al.*, 1989; Nauman, 2002; Azmat *et al*., 2012; Jabeen *et al*., 2012; Naz and Javed, 2012).

Sewage waste water is posing devastating impact on ecosystem, certain reliable technologies are required to combat with this situation such technologies should be of simple design cost effective and it must use non sophisticated equipment (Ghosh, 1995; Aslam *et al*., 2011). Bioremediation encompasses biological methods for cleanup of contaminated soil and groundwater. It gives nature a helping hand by establishing the conditions in contaminated environment so that appropriate microorganisms flourish and carry out the metabolic activities to detoxify the contaminants (Latha, 2004).

One of the recent economical technologies for treatment of domestic waste water includes constructed wetlands. These constructed wetlands are low cost, easy to maintain and have strong potential for application in developing countries especially for rural communities as compared to conventional treatment systems which are difficult to adopt by developing nations (Kissivi, 2011).

Excreta contains rich amount of inorganic nutrients such as nitrogen, phosphorus, potassium, and organic matter which are useful for plants. Humans excrete 30 g of carbon every day (90 g of organic matter), N in amount of 10-12 g, P, 2 g and K, 3 g. Feces contain most of the organic matter, while (70-80%) of the nitrogen and potassium are contained in urine. Feces and urine contain equal amount of phosphorus (Drangert, 1998).

In stabilization ponds for treatment of domestic waste water, several transformations of organic contents produces nutrients and great amount of algae, which are beneficial to agriculture and aquaculture. Stabilization ponds use simple technology and do not need equipment or conventional energy, and are accessible to economics of developing countries. For these reasons, it is the first option among treatment technologies whenever sufficient area with little economic value is available (Juliana, 2006).

According to estimate, the country like Pakistan produces about 100,000 L waste water annually (Global Environment Program in Pakistan, 2001). National Institute of Bioremediation (NIB) at National Agricultural Research Centre (NARC) is treating 2.83 L of waste water/day from CDA sector Shehzad town, Islamabad, Pakistan. This water is treated in constructed wetlands at NARC through specific phytoremediation potential plants. This treated water is found to be fit for irrigation purpose. Present study was

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aimed to investigate the potential of this treated waste water for fish production and followed with following objectives (1) fish (Tilapia and Common Carp) survival in bioremediated sewage effluent (2) differential studies of physicochemical parameter affecting the fish survival in bioremediated water ponds and (3) identification of critical variable affecting fish survival and its management.

Materials and Methods

Experimental Details and Treatments

Study was conducted at nursery ponds of Fisheries and Aquaculture Program, National Agriculture Research Centre (NARC), Islamabad, Pakistan to investigate the fish survival rate in bioremediated sewage effluent of Shehzad Town, Islamabad being treated at constructed wetlands of Bioremediation Site II. Two earthen ponds with dimension of 20 × 40 m were selected at fisheries and aquaculture program, NARC. One pond (P1) was filled with bioremediated sewage effluent from bioremedtion orchard site II. Other pond (P2) was filled with fresh water. Two fresh water fish species Tilapia (*Oreochromis mossambicus)* and Common Carp (*Cyprinus carpio)* were selected as these are hardy fishes and can better tolerate any adverse condition (Thomas and Leonard, 1995). To investigate the household products being used in Shehzad town a survey was conducted and sewage water samples were collected in order to analyze the physicochemical parameters.

Physiochemical Parameters of Fresh Water and Bioremediated Sewage Effluent

Physical parameters include the study of temperature and turbidity. The temperature of pond water and air (atmosphere) was recorded with the help of thermometer at 0^oC. The light penetration was measured by using 'secchi's disc'.

The chemical parameters included water pH with the help of pH micrometer (Jenway 3510), alkalinity was estimated by methyl orange indicator (A.P.H.A, 1981). Total water hardness was estimated by Erich Rome black T (EBT) indicator (A.P.H.A, 1981). Electrical conductivity was measured by using water proof potable EC meter, cyber scan series 600. Total dissolved solids were measured by using water proof TDS tester, dual range (HANNA-HI 9635). Analysis of water salinity was done by using potable salinity refract meter (Lamotte. 5-0020). Free CO_2 was determined by using phenolphthalein titration method (Water Quality Criteria, 1972), while ammonical nitrogen (NH_4^+-N) with sodium phenate/hypochlorite method (Ryan *et al.,* 2001). Nitrate nitrogen of each sample was determined by using hydrazine reduction method (Kamphake *et al.,* 1967). Chloride was determined by titration with silver nitrate (Ryan *et al.*, 2001). Heavy metal analysis was done by using vacuum filtration of sample by using micro cellulose membrane followed by metal detection through Atomic absorption spectrometer (Perkin Elmer AA 700). Biological parameters included study of dissolved oxygen DO by (Standard Methods; 4500-O A, 4500-O B, 4500-O C, 4500-O G) and Biological oxygen demand BOD.

Investigation for Fish Survival

For investigation of fish survival, 50 Tilapia and 50 common carp fingerlings were stocked in both bioremediated water pond and fresh water pond. Water quality parameters were determined. Netting was done fortnightly with the help of drag and cast nets. Study in aquariums was carried out with different dilutions of 20, 40, 60 and 80% of bioremediated water to determine the survival rate of fresh water fish in different concentrations of bioremediated sewage effluent. Water quality parameters were studied for these dilutions. Experiment was conducted in two circular tanks containing 2500 mL of water in each. Two freshwater fish species Tilapia and common carp were introduced in both tubs with ratio of 1:2 potential plant Coontalil (*Ceratophyllum demersum*) was introduced in both circular tanks. One kg of Coontail was introduced in 1 circular tank and ½ kg in second. Physicochemical parameters were recorded and test for determination of NH_4^+-N concentration, NO_3^- and Cl^- was recorded after every 24 h for four days.

Statistical Analysis

The data recorded for three replicates of bioremediated sewage effluent was subjected to analyses of variances using Minitab software in Completely Randomized Design (CRD). For significance of F-value, LSD was used for mean comparison at 5% level (Steel *et al*., 1997).

Results

Fish showed 80% survival in 20% dilution that comprised of 80% fresh water and only 10% of bioremediated sewage effluent. Physicochemical parameters were recorded in all four trials.

Identification of Critical Variable in Sewage Water of Shehzad Town, Islamabad

After five trials for fish survival, survey was conducted to investigate the composition of different household products used by the community of the Town to find critical variable causing fish mortality. Survey showed that 35% people use Head and Shoulders hair shampoo, which contain active ingredient zinc sulphide. To investigate further, heavy metal analysis of bioremediated water was done. Zn levels at both the sites of bioremediated sewage fell within permissible range, which was not toxic to fish growth

Table 1: Concentration of NH_4^+-N, nitrates and Cl^- in bioremediated sewage effluent

Parameters	Control (T0) mg/L	Bioremediated sewage effluent mg/L	After Phytoremediation mg/L
NH_4^+-N (mg/L)	0.23	31.08	0
NO_3^--N(mg/L)	0.12	18.58	0
Cl^- (mg/L)	0.41	39.61	33

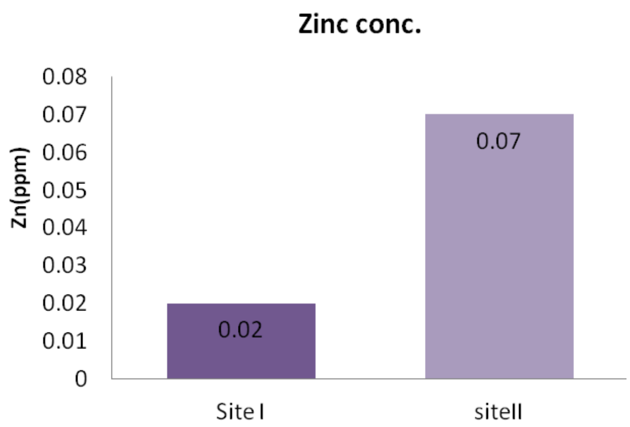

Fig. 1: Zinc concentration of Bioremediated pond site I and II

(Fig. 1). Heavy metal results showed that Zinc and all other metals were within permissible range and not toxic for fish. Further analysis was carried to explore the reason of fish mortality and found that bioremediated water contain high level of NH_4^+-N and NO_3^--N. Results showed very high level of NH_4^+-N, NO_3^--N, and Cl^-, which become the reason for fish death (Table 1).

Phytoremediation of Bioremediated Sewage Water

For remediation of such high concentration of NH_4^+-N, NO_3^--N, and Cl^-, in bioremediated sewage water, Coontail (*C. demersum)* plant known for its phytoremediation potential was used. LC50 experiment was conducted in order to check the phytoremediation efficacy of Coontail. Results showed remarkable decrease in NH_4^+-N from 31.08 to 0.31 mg/L and NO_3^--N from 18.58 to 1.5 mg/L in a four-day experiment (Figs. 2, 3).

Two circular tanks both with 2500 L of bioremediated sewage water, one contain 1 kg plant and other contain 1/2 kg plant was used to check the efficiency by different volume and mass. Results showed that both1 kg and ½ kg mass of Coontail have same potential for removal of NH_4^+-N, NO_3^--N, and Cl^-, was in the same ratio by both different volume of 1 kg and ½ kg Coontail plant (Fig. 4). So ½ kg of Coontail plant was enough for removal of these ions from 2500 L bioremediated sewage water.

Discussion

Physicochemical parameters studied during this research fall within permissible range. A highest alkalinity (125.180 mg/L) was recorded at the end of experiment. Average alkalinity remained between 155 and 180 mg/L in both ponds. Lawson (1995) studied the physicochemical parameters and recommended the alkaline level of 5-500 mg/L for fish survival in fresh water. In experimental pond 1, the values of total hardness range from 89 to 300 mg/L in all trials while in fresh water, hardness lies between 135 to 350 mg/L.

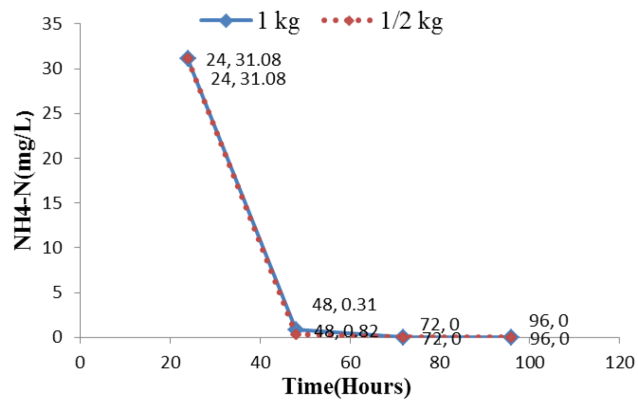

Fig. 2: Concentration of NH4$^+$-N in LC50 experiment with 1 kg and 1/2 kg plant (coontail)

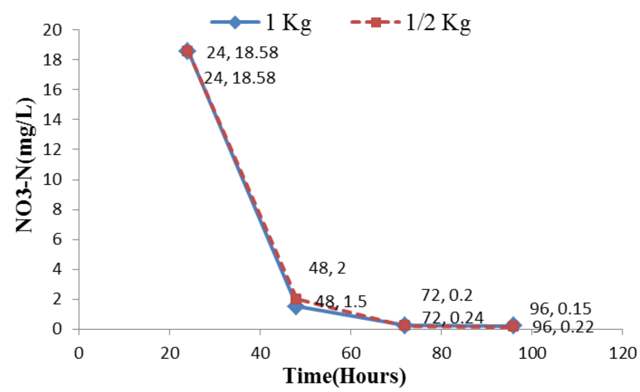

Fig. 3: Concentration of Nitrate nitrogen (NO_3^--N) in LC50 experiment with 1 and 1/2 kg plant (coontail)

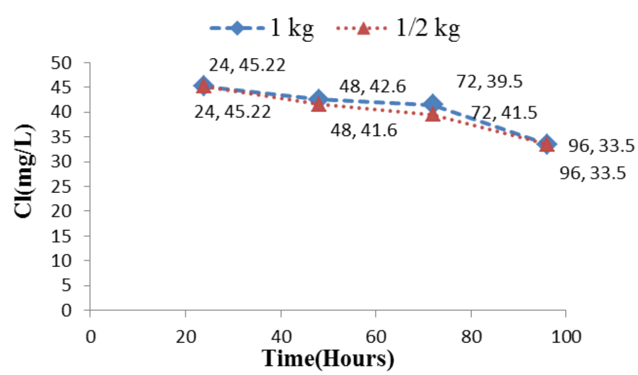

Fig. 4: Concentration of Cl^- in LC50 experiment with 1 and 1/2 kg plant (coontail)

Average pH values of both ponds (P1and P2) were 6.0 and 8.0. Lloyd (1992) studied that pH below 6.5 slow the growth rate of fish. The optimum range for best growth and survival has been found to be 6.5-9.0 (Tarazona and Munoz, 1995). Mean values of total dissolved solids remained between 360 to 370 mg/L in both ponds. Mitchell and Stapp (1992) found that high concentration of TDS effects fish growth and survival. The lowest temperature recorded was 18 at the start of experiment and it increased to 30 at the end of experiment. U.S. Environmental Protection Agency (1976) studied the water quality parameters for fish survival and recommended the optimum temperature for fish survival ranges 27.

In fresh water pond (P1) EC varies from 0.2 to 0.3 mg/L and same as in bioremediated water pond (P2) with slight difference in change of 0.1% in all trials. Lewis (1986) studied the effect of salinity on fish survival and recommended the salinity level of 0.5 mg/L for fresh water fish. The mean dissolved oxygen (DO) recorded was 4.5 in fresh water, while 9 mg/L in bioremediated water. Lloyd (1992) recommended the tolerable range of DO from 3 to5 mg/L. Mean values of DO recorded in both ponds P1 and P2 found within the recommended values. Mean CO_2 values were recorded as 25 in P1 and 30 mg/L in P2. Water Quality Criteria (1972) recommended the carbon dioxide level of 20 mg/L for fish survival and growth. All physicochemical parameters fall within permissible limit required for fish survival.

About 50% people of the area use Harpic as toilet cleaner, which contains ammonical products (Reckitt Benckiser). As survey showed that 15 % people of Shehzad town have common use of Dettol soap but Dettol concentration was found very low in waste water so it has no harmful effect on fish. Zn is a potential toxicant to fish, which causes disturbances of acid-base and ionoregulation, disruption of gill tissue and hypoxia (Everall *et al*., 1989; Hogstrand *et al.*, 1994). Malakootian *et al.* (2011) studied the effect of heavy metals on fish growth and survival in tuna fish and found that zinc concentration of 0.019 to 0.035 µg is permissible for fish and has no effect on fish survival and growth. As the survey showed that toilet cleaner and other floor, glass cleaners have active ingredient of NH_4^+-N. Ammonia is toxic to fish and aquatic organisms, even in very low concentrations. When levels reach 0.06 mg/L, fish can suffer gill damage. When levels reach 0.2 mg/L, sensitive fish like trout and salmon begin to die (U.S. Environmental Protection, 1976). Results showed very high level of NH_4^+-N, NO_3^--N and Cl^- leading to fish death. Foroughi *et al.* (2010) explored the efficacy of Coontail for removal of NH_4^+-N, NO_3^--N and Cl^- from wastewater.

This research concludes the potential of bioremediation sewage effluent of CDA sector Shehzad town for fish production. No fish survival was observed in bioremediated water but 100% fish survival was recorded when this water was further treated with phytoremediating potential plant Coontail to reduce amonical and nitrate concentration which is lethal for fish.

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