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Feasibility of using Diluted and Chemically Treated Wastewater for Fish Culture

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Abstract

Wastewater treatment using waste stabilization ponds is very efficient and inexpensive but requirement of large areas of land is a major constraint. An alternative is direct dilution of wastewater and treatment using floating aquatic plants. This study was undertaken to assess the effect of diluted waste water on histology of gills. The results showed severe pathological alterations in gills of *Labeo rohita* up to 1: 6 dilution and changes were less at dilution 1:8 and above. In urban areas, where both land and water availability are constraints, another option is chemical treatment. Phosphorus and chemical oxygen demand (COD) from wastewater was removed by chemical precipitation using zeolite, alum (Aluminium sulfate) and their combinations. It was observed that alum and zeolite absorb phosphorus; 85% and 78% respectively and remove COD; 67% and 57% respectively. But zeolite-alum mixture removes phosphorus (85%) and COD (68%) at much lower dose and also helps in balancing the pH.

Keywords: Wastewater, zeolite, alum, phosphorus, COD

Introduction

Wastewater normally originates from household usage and other municipal sources (Mojid et al., 2010). Wastewater from these areas contains solid wastes, fecal matter, engine oil, grease, diesel, and harmful chemicals and microbes that adversely affects the environment and people (Mbuligwe & Kaseva, 2005). As per Sato et al. (2012) on an average only 8% of wastewater generated is treated in low-income countries when compared to 70%, 38% and 28% respectively for high-income, upper, middle-income and lower middle income countries.

Availability of suitable water is one of the most important limiting factors for development of sustainable aquaculture in urban areas. As a consequence, use of wastewater effluents for aquaculture is on the rise in developing countries (Ahamdi et al., 2014). The use of sewage effluent for raising fish productivity was recognized much earlier in countries like China, Taiwan, Malaysia, Thailand, Indonesia and Germany (Kumar & Sierp, 2003). It is reported that irrigation with sewage or sewage mixed with industrial effluents results in saving of 25 to 50 percent of nitrogen (N) and phosphorous (P) fertilizer and leads to 15-27% higher crop productivity, (Anonymous, 2004). However, agricultural waste, municipal and industrial effluents discharged directly to natural water resources are found to cause fish mortality due to hypoxia, high levels of organic substances, inorganic salts and heavy metals (Zaghloul et al., 2007).

An efficient treatment option for conventional wastewater is to use primary sedimentation followed by secondary biological treatment using high-rate biological processes. Many studies conclusively show that wastewater treatment using waste stabilization ponds is very efficient, and inexpensive in terms of capital and maintenance costs. Two disadvantages of waste stabilization ponds are high land requirements, and high water loss through evaporation (Mara, 2000).

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An alternative to this is direct dilution of wastewater and treatment using floating aquatic plants (Bramwell & Prasad, 1995). These studies have shown that the system has low cost and land requirement and is well suited for small communities.

In urban areas where both land and water availability is a constraint another option is chemical treatment. Usually 65-90% of the organic matter in wastewater is colloidal or particulate, which can be reduced by chemical pretreatment of raw wastewater (Denis et al., 2008). High removal efficiencies of heavy metals and total suspended solid (TSS) as well as high reduction in biological oxygen demand (BOD) and COD were achieved by chemical treatment using ferric chloride, ferric or ferrous sulfate, alum, lime and ionic polymers (Gökhan et al., 2011; Pauline et al., 2008). Clay minerals, such as bentonite and zeolite, are some of the potential adsorbents which have large specific surface areas with net negative charge and can be electrically compensated by inorganic and organic cations from the environment (Konig et al., 2012). Compared to poly aluminum chloride; their usage for water and wastewater treatment are increasing because of their easy availability, low price, as well as ion exchange that is highly capable of adsorbing wide range of pollutants including heavy metals in waters (Wang & Peng, 2010).

Fish exposure to contaminants induce lesions in different target organs, especially gills (Winkaler et al., 2001; Tkatcheva et al., 2004; Oliveira Ribeiro et al., 2005; Benli et al., 2008). Gills are multifunctional and highly characteristic structural feature of fishes that serve a range of vital functions including osmotic and ionic regulation, acid-base regulation, gas exchange, nitrogen excretion and detoxification (Evans, 1987; Evans et al., 2005; Koca et al., 2005). Fish gills are primary site of toxic action of many waterborne pollutants and are particularly sensitive to adverse environmental conditions. This is due to their direct and continuous contact with the environment, large surface area and the small diffusion distance between the water and blood (Evans, 1987; Bhagwant & Elahee, 2002; Olson, 2002). Histopathological events are considered fast and efficient for detection of acute and chronic adverse effects in fish (Winkaler et al., 2001; Ayas et al., 2007), as they allow examining specific target organs. Furthermore, alterations found in the organs are normally easier to identify than the functional ones (Fanta et al. 2003) and serve as warning signs of damage to animal health (Marlasca et al., 1998; Tetreault et al., 2011). Thus histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies (Das & Mukherjee, 2000; Oliveira Ribeiro et al., 2005; Miranda et al., 2008; Pugazhvendan et al., 2009).

The aim of the present study was to determine the effect of wastewater through histological analysis of fish gill. The study also investigates the comparative and the combination effects of zeolite and alum as adsorbents and coagulants for wastewater treatment based on the removal efficiencies of total phosphorus and COD.

Material and Methods

Wastewater samples were collected from sewage drainage of Versova, Mumbai by using 5-liter Niskin Water Sampler and fixed. Dissolved oxygen of fixed samples was measured by Winkler's method (Winkler, 1888) and BOD was measured after 5 days of incubation at 20°C in BOD incubator. Water samples were analyzed using standard methods as per APHA (2002), where pH was determined using pH meter, turbidity by portable turbidity meter, nutrients (NH_3-N, NO_2-N, NO_3-N, total phosphorus) and COD by spectrophotometer.

Waste water after initial analysis was diluted with tap water in the ratio of 1:2, 1:4, 1:6, 1:8, 1:10 and 1:12. Each dilution was used in triplicate. Twenty fishes (*L. rohita,* weighing 3.6 ± 0.3 g) were introduced in each dilution with mild aeration. The experiment was carried out for 21 days and the mortality rate was recorded. For histological study, one fish from each dilution was taken out randomly after experimental period except from raw sewage water and 1:2 dilution, in which all the fish died within 2 and 16 days respectively.

For histological examination, gill tissue was quickly fixed by immersion in phosphate buffer formalin (100 ml formaldehyde, 37%, 4 g sodium dihydrogen orthophosphate, 6 g disodium orthophosphate and 900 ml distilled water), dehydrated in graded ethanol solution, embedded in paraffin using automated tissue processor (Thermo scientific- Shadon, Citadel 2000, USA) and automated tissue embedder (Shadon Histocentre 3, USA) as described by Roberts (2001) with some modifications. Embedded tissues were cut (5 µm sections) using rotary microtome (Leica RM2245, Germany).

After removing paraffin, tissues were rehydrated and stained with haematoxylin and eosin as described in Martoja & Martoja-Pierson (1970) and photographed using a microscope (Carl Zeiss, Germany).

Chemical treatment of municipal wastewater was undertaken using different adsorbent and coagulant. Zeolite (HiMedia, Mumbai) was sieved using vibrator sieve shaker to obtain smaller particle size so as to increase surface area and also adsorption capacity (Hussain et al., 2006). Alum was obtained from Chirag Chemicals, Mumbai.

Characterized waste water was used to run the coagulation-flocculation experiments by using zeolite and aluminium sulphate (alum) alone and in combination of the two to achieve the optimum dosage. Coagulant dosage was optimized via a series of jar tests. A conventional jar test apparatus having six beakers held together with six-spindle steel paddles was used to perform these coagulation-flocculation experiments.

From the jar tests, desirable concentrations were selected for outdoor study. Each batch of treatments was kept for 10 days in triplicate with mild aeration. After the experimental period, samples were collected and various analyses were done is triplicate. COD and total phosphorus of wastewater were determined using a spectrophotometer.

Data were analyzed by one-way analysis of variance (ANOVA) using SPSS 16 and comparison between means were made using Duncan's new multiple range tests keeping significance level at $p<0.05$.

Results and Discussion

Different chemical parameters of collected wastewater samples are listed in table 1. Analysis indicates that COD and total phosphorus were present in significant quantities; viz., 320 mg l^{-1} and 10.70 mg l^{-1} respectively and it exceeded the effluent discharged standard of EPA. All the fish exposed to undiluted sewage water died within 2 days (Table 2) and gill sections showed atrophid secondary lamellar cells, distended primary epithelial cells and curling of filaments (Fig. 1B). Navaraj & Yasmin (2012) noticed whole epithelium degeneration and rigidity loss of lamellae in *O. mossambicus* exposed to untreated paper mill wastewater. The gills of control fish showed clear unaffected gill architecture with intact gill lamellae and gill filaments (Plate 1A). When wastewater was diluted at 1:2 dilution with freshwater, the gill lesions exhibiting marked hyperplasia of secondary lamellae, lifting of lamellar and filamentary epithelium, edema were more evident (Plate 1C). Intensive filamentary epithelium proliferation, leading to almost complete lamellar fusion was noted in the gills of fish exposed to 1:4 dilution (Plate 1D). Similar observation, like cell proliferation with consequent thickening of the filament epithelium was reported by Fontaínhas-Fernandes et al., (2008) in *Oreochromis niloticus* exposed to treated sewage water. The severity of the lesions/changes observed in this study decreased with the increase of dilution factor. The results showed severe pathological alterations in gills of *L. rohita* treated with upto 1:6 dilution (Plate 1E) and changes were less at dilution 1:8 and higher (Plates 1F, 1G & 1H). Similar observations such as lamellar cell hyperplasia, epithelial lifting, lamellar fusion and edema in *O. mossambicus* treated with tannery industry wastewater was reported by Navaraj & Yasmin (2012). The histopathological changes in the present study revealed structural damages to the gills of *L. rohita* in a decreasing order (irreparable, severe, moderate and slight) with decreased concentration of waste-

Table 1. Chemical parameters of wastewater sample

Parameters	Minimum	Maximum	Average*	Effluent Discharged
pH	7.1	7.8	7.3	5-9
BOD5 at 20°C ($mg.l^{-1}$)	106	196	158	20
COD ($mg.l^{-1}$)	257	392	320	100
Total phosphorus ($mg.l^{-1}$)	8.9	13.2	10.70	1.0

* *Average values for period of collecting between May, 2015 and July, 2015*

water due to dilution. Mortality recorded was in decreasing order and fish exposed to higher dilutions showed no significant alterations in behavior compared to control (Table 2).

Reduction in COD *i.e.* the biodegradable organics is an objective of wastewater treatment (Jiang et al., 2004). Besides COD, the removal of phosphorus and nitrogen from wastewater appears to be a practical way of limiting algal blooms in the receiving waters and making it suitable for fish culture. In the study, zeolite and aluminium sulphate (alum) alone and in combination were used for removal of total phosphorus and COD from wastewater to a suitable limit for fish culture.

Zeolites have wide application as ammonia removers (Jamil et al., 2010), as adsorbent media against different suspended solids (Milan et al., 2001) and as a promising sorbent for heavy metals (Motsi et al., 2009). Based on jar tests, 9 different concentration of zeolite were used for the study and total phosphorus and COD (mg l^{-1}) of wastewater after treatment for 10 days are tabulated in table 3. In the study, percentage removal of total phosphorus and COD versus zeolite dose is plotted in fig. 2. Figure 2 shows that the optimum dose of zeolite for removal of total phosphorus was 350 mg l^{-1}, which removed up to 78% of total phosphorus. In case of COD, though highest reduction of 57% was achieved at a zeolite dose of 450 mg l^{-1}, increment reduction of COD was better up to a concentration of 300 mg l^{-1}. According to Ismail et al. (2013) zeolite can remove up to 54.5% of COD from palm oil mill effluent.

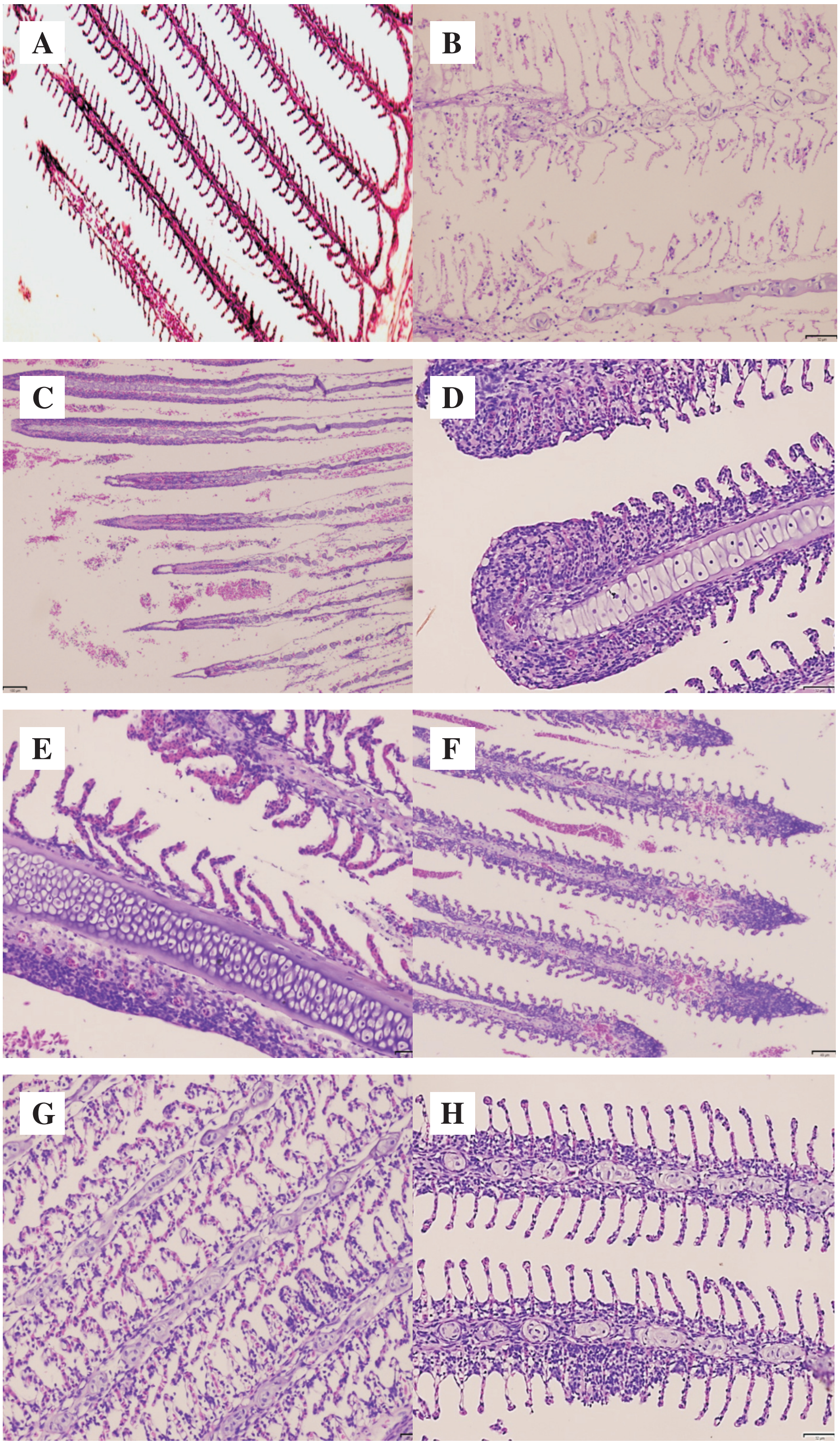
Inorganic metal salts such as aluminium sulphate (alum) are generally used in wastewater and water treatment (Ghafari et al., 2009). In the present study, total phosphorus and COD (in mg l^{-1}) of wastewater after zeolite treatment for 10 days is tabulated in table 3.84% removal of total phosphorus was recorded at a dose of 400 mgl^{-1} alum (Fig. 2). But effective coagulation was achieved (80%) with much lower dose of alum at a concentration of 250 mg l^{-1}. Highest COD removal of 67% was attained at highest alum dose used *i.e.*, 450 mg l^{-1}. From figure 2, it is clear that alum has better phosphorus and COD removal efficiency than zeolite. At a concentration of 300 mg l^{-1} 83% of total phosphorus was removed by alum, whereas at the same concentration, zeolite removed only 76% of total phosphorus. In the case of COD, alum at highest concentration could reduce 67% of COD but in case of zeolite the reduction was only 57%. Ismail et al. (2013) reported that compared with avocado seed carbon, animal horn, fly ash, activated charcoal, brick kiln, bagasse and wood ash; zeolite was least efficient in terms of COD removal capacity from palm oil mill effluent.

Total phosphorus and COD (in mg l^{-1}) content of water after treatment for 10 days using combinations of zeolite-alum at concentration of 300 mg l^{-1} are tabulated in table 4. Maximum removal of total phosphorus (85%) and COD (68%) was achieved by using zeolite and alum combination at a ratio of 50:50

Table 2. Percentage mortality of *Labeo rohita* seed exposed to different dilution of sewage

Dilution (Sewage: Freshwater)	Duration of Exposure (day)							
	1	3	6	9	12	15	18	21
1:2	3.3±1.7	23.3±8.8	36.7±6	80±5.8	90.0±5.8	96.7±3.3	100±0.0	100±0.0
1:4	0	13.3±3.3	23.3±3.3	23.3±3.3	23.3±3.3	33.3±8.8	36.7±7.3	36.7±7.3
1:6	0	0	3.3±3.3	8.3±1.7	8.3±1.7	8.33±1.7	8.3±1.7	11.7±4.4
1:8	0	0	0	0	0	3.33±1.7	5.0±0.00	5.0±0.00
1:10	0	0	0	0	0	0	0	0
1:12	0	0	0	0	0	0	1.66±1.7	1.7±1.7
Undiluted Sewage	63.3±3.3	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0
Control	0	0	0	0	0	0	0	0

The observed values were expressed as mean ± standard error



Plates 1 Gill sections of fish. A: Control fish; B: Undiluted sewage; C: 1:2 sewage (16th day); D: 1:4 sewagel; E: 1:6 sewage; F: 1:8 sewage; G: 1:10 sewage; H: 1:12 sewage

Table 3. Total phosphorus & COD (in mg l^{-1}) of wastewater after treatment for 10 days with Alum & Zeolite

Parameters	Chemical	Doses in mg l^{-1}									
		Control	50	100	150	200	250	300	350	400	450
Total Phosphorus (mg l^{-1})	Alum	10.70± 0.032^{a}	7.28± 0.072^{b}	6.30± 0.049^{c}	5.10± 0.055^{d}	4.10± 0.087^{e}	2.20± 0.036^{f}	1.80± 0.066^{g}	1.80± 0.067^{g}	1.60± 0.032^{g}	1.70± 0.071^{g}
	Zeolite	10.70± 0.032^{a}	7.80± 0.057^{b}	6.20± 0.056^{c}	4.70± 0.047^{d}	3.10± 0.073^{e}	3.00± 0.061^{e}	2.60± 0.059^{f}	2.30± 0.042^{g}	2.30± 0.063^{g}	2.30± 0.054^{g}
COD (mg l^{-1})	Alum	320± 2.333^{a}	244± 1.527^{b}	214± 1.761^{c}	198± 4.372^{d}	176± 2.598^{e}	147± 3.014^{f}	125± 1.827^{g}	122± 2.106^{g}	112± 4.651^{h}	105± 1.483^{h}
	Zeolite	320± 2.33^{a}	291± 2.96^{b}	249± 3.37^{c}	234± 2.18^{d}	211± 1.82^{e}	176± 0.98f	151± 1.32^{g}	144± 2.67^{h}	141± 2.37^{h}	139± 1.749^{h}

The observed values are expressed as mean ± standard error. Mean values with different superscripts are significantly ($P \leq 0.05$) different from each other.

Table 4. Total phosphorus & COD (in mg l^{-1}) of water after treatment for 10 days using combinations of zeolite-alum at concentration of 300 mg l^{-1}

Parameter	Control	Alum @ 300 mg l^{-1}	Zeolite @ 300mg l^{-1}	Zeolite-alum combinations at concentration of 300 mg l^{-1}								
				10:90	20:80	30:70	40:60	50:50	60:40	70:30	80:20	90:10
Total Phosphorus	10.7 ±0.03^{a}	1.8 ±0.07^{b}	2.6 ±0.06^{c}	2.00 ±0.08^{d}	2.10 ±0.06^{d}	2.00 ±0.04^{d}	1.70 ±0.04^{e}	1.60 ±0.06^{e}	1.60 ±0.08^{e}	1.90 ±0.05^{f}	2.30 ±0.07^{g}	2.40 ±0.05^{g}
COD	320 ±2.33^{a}	125 ±1.83^{b}	151 ±1.33^{c}	115 ±2.08^{d}	118 ±1.45^{d}	106 ±1.85^{e}	106 ±2.54^{e}	115 ±3.03^{d}	118 ±2.41^{d}	131 ±2.1^{f}	132 ±2.7^{f}	137 ±2.41^{g}

The observed values were expressed as mean ± standard error. Mean values with different superscripts are significantly (Pd"0.05) different from each other.

to 60:40 and concentration of 300 mg l^{-1} (Fig. 3). These combinations exhibit better removal for both parameters compared with the use of alum or zeolite alone at same concentration (Fig. 3). On the other hand, alum is acid forming and can substantially reduce total alkalinity and pH to the point where it is toxic to fish (Chatterjee et al., 2009). Therefore, by combining alum with zeolite, the negative effects associated with the use of alum can be minimised.

Total phosphorus and COD data versus exposure time are presented in figure 4. The results indicate that with increase of contact time, total phosphorus and COD level decrease. The results also indicate that the decrease in both the parameters is rapid in the initial stage but slowed gradually with time. This phenomenon is in agreement with Darus et al. (2007) who showed that after reaching equilibrium, the adsorbate normally forms a surface layer, which is only a molecule thick, on the surface of the adsorbent which prevents further attachment to it.

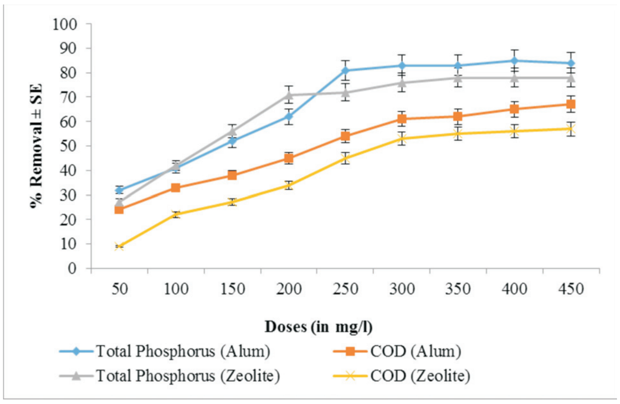


Fig. 2. Effect of alum and zeolite dosages on total phosphorus and COD removal

In conclusion, the results of the histological study indicate the probability of using diluted wastewater for fish culture, as an increase of dilution factor significantly reduced the pathology on fish gills. For

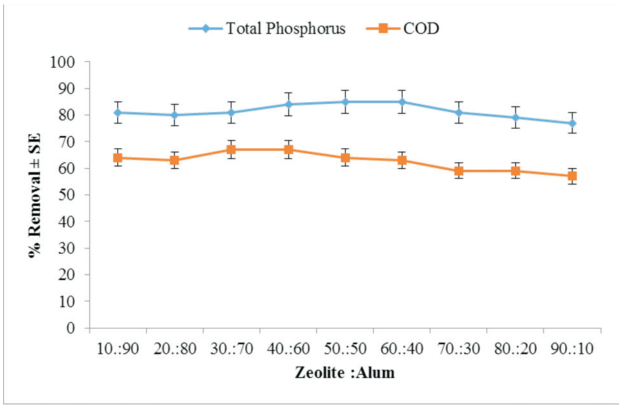


Fig. 3. Removal of total phosphorus and COD by use of different ratio of Zeoite-Alum.

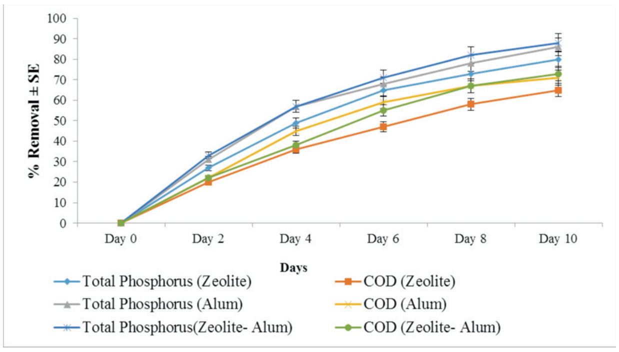


Fig. 4. Changes in total phosphorus and COD with time

chemical treatment, alum can be a good coagulant for total phosphorus because it can achieve more than 80% of removal with a dosage of 250 mg l^{-1}. The study also revealed that the best coagulant was zeolite-alum mixture with high rate of removal of total phosphorus (85%) and COD (67%) compared to zeolite and alum alone or zeolite-lime combinations. Suitable combinations of zeolite-alum also help in balancing the high pH. However microbiological and chemical quality of waste water need to be ensured before its use for fish culture.

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