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Influence of Amazon Sailfin Catfish, *Pterygoplichthys pardalis* on the Chemical Characteristics of Dairy Effluent

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Abstract: *Water quality of aquatic systems is much affected by the discharge of industrial effluents. Dairy industries release huge volumes of wastewater which can deteriorate water quality. Hence in the present work, the sailfin catfish, Pterygoplichthys pardalis was tested for its efficiency to improve the quality of dairy effluent in 10, 20, 30 and 40 % concentrations for five days. The fish was able to reduce the levels of chlorides, nitrates, sulphates, ammonia and total phosphorous. The differences between control and experimental sets, were evaluated for the above parameters using students' 't' test.*

Keywords: *Dairy Effluent, Pterygoplichthys Pardalis, Chemical Characteristics, Biotreatment, Fish*

INTRODUCTION

Industrial growth and development were given much importance in the earlier periods. Novel ways are now explored for solving the issues related to rapid industrialization. Industries have not only increased our economic growth but also resulted in the contamination of our environment, causing health hazards. It has also damaged our habitats and organisms (Spina et al., 2012). Among the natural resources, water is the most essential but it is being misused and abused in several ways. In many countries the availability of water is a serious issue, and pollution of water resources further worsen the situation. Human population explosion and advancements in medicine and agriculture have led to deterioration in environmental quality (Shah, 2014).

It is observed that 5-20% of total available water is used by industry (Modak et al., 1990). Industrial effluents contain many chemicals which are hazardous to living organisms (Kansal et al., 2011). Food-processing industries use huge volumes of water as an ingredient, cleaning source, and means of transport. The need for water and its dwindling supply has led to the treatment and reuse of waste water as an attractive option (Saranraj and Stella, 2012).

Dairy industries are one of the major food industries and cause water pollution. They are present all over the world (Verheijen, 1996). The dairy industry effluents contain high quantities of nutrients, organic compounds and pathogens. They have high Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) (Orhon et al., 1993). The dairy effluent has proteins, salts, fatty substances,

and lactose (Thassitou et al., 2001).

Various physico-chemical methods are employed in waste water treatment. They include sedimentation, screening, aeration, filtration, flotation, degasification, chlorination, ozonation, neutralization, coagulation, sorption and ion exchange. But they have several limitations like partial treatment, higher cost, and generation of secondary pollutants, and higher quantity solids. Hence the biological methods become a favorable alternative. Dairy effluents are treated using biological methods such as activated sludge process, aerated lagoons, trickling filters, Sequencing Batch Reactor (SBR), Upflow Anaerobic Sludge Blanket (UASB) reactor and anaerobic filters (Lokhande et al., 2011; Deepali, 2012). Even though bioremediation is confined to microorganisms or plants, the use of animals for bioremediation of waste water has invited recent attention. The ability of animals to act in a bioremediative capacity is not widely known. Animals are rarely used in bioremediation initiatives owing to ethical or human health concerns (Gifford et al., 2006). In this context, the present study has been designed to analyse the biotreatment efficiency of *P.pardalis o*n the chemical characteristics of dairy industry effluent.

Materials and Methods

Sample collection

Dairy effluent was obtained from Aavin milk factory, Madurai, Tamil Nadu, India and stored in refrigerator for further analysis. Chemical parameters such as chlorides, nitrates, sulphates, ammonia, total phosphorus and BOD were analysed based on standard methods (Rice *et al.*, 2017). The fish were brought from Aquagardens fish farm, Madurai, Tamil Nadu, India. Their mean weight was 16.87 ± 1.61g. They were acclimatized to laboratory conditions for one week. Later they were exposed to different concentrations of dairy effluent to determine the tolerance level and finally the working concentrations were chosen as 10, 20, 30 and 40%. Students' 't' test was applied to analyse the level of significance for the deviation between control and experimental sets for all the parameters tested.

Chlorides

10ml of effluent was taken in a flask and 5-6 drops of potassium chromate indicator were added. The colour of the sample became yellow and it was titrated against silver nitrate solution until a persistent brick red colour appeared. The chlorides were then calculated by the given formula.

Chlorides (mg/L) =V x N x 35.457 x 1000 / S

Where, V=volume of titrant; N= normality of titrant (0.02N); and S= volume of sample.

Nitrates

25ml of effluent was taken in a glass beaker and evaporated on a hot water bath. 0.5ml of phenol disulphonic acid was mixed with the residue and dissolved with the help of a glass spatula. 5ml of distilled water and 1.5ml of potassium hydroxide solution were added. It was subjected to thorough mixing. The supernatant with yellow colour was taken and its absorbance was read on a spectrophotometer at 410 nm by using distilled water as blank. The standard nitrate solutions were processed similarly and the absorbance was noted for each. The level of nitrates in the sample was deduced by comparing the optical density of the sample with the standard curve and the result was expressed in mg NO_3/L.

Sulphates

The effluent was filtered through filter paper and 50 ml of filtrate was taken in a conical flask. To this, 10ml of NaCl-HCl solution and 10ml of glycerol–ethanol solution were added. The sample was stirred in a magnetic stirrer for about an hour. Then the absorbance was measured at 420nm using spectrophotometer. The sulphate content of the sample was deduced in mg/l by comparing the optical

density of the sample with standard curve.

Ammonia

20ml of effluent was taken in a 25 ml conical flask. To this, 2ml of phenol-nitroprusside solution and 2ml of alkaline hypochlorite solution were added. It was made up to 25ml by adding ammonia-free distilled water and kept in dark place at 25°C for one hour. The absorbance was recorded in a spectrophotometer at 635nm using distilled water as blank. The level of ammonium ions in sample was deduced in mg NH_4 -N/L by comparing the absorbance of sample with the standard curve.

Total phosphorus

25ml of effluent was taken in a conical flask and evaporated. After cooling the residue was dissolved in one ml of perchloric acid. The flask was heated gently so that the contents become colourless. It was cooled and 10ml of distilled water and two drops of phenolphthalein indicator were added to the contents. It was titrated against sodium hydroxide solution until the appearance of slight pink colour and made up to 25ml by adding distilled water. One ml of ammonium molybdate solution and three drops of stannous chloride solution were added. After the appearance of blue colour, the absorbance was recorded in a spectrophotometer at 690 nm. The total phosphorus content of the sample was deduced by comparing its absorbance with standard curve.

Biochemical oxygen demand

Dilution water was prepared by aerating the BOD-free distilled water in a glass container for about half an hour. To one litre of this water, one ml each of phosphate buffer solution, magnesium sulphate solution, calcium chloride solution, and ferric chloride solution were added. The pH of the sample was adjusted to neutral (7.0) using 1N sulphuric acid or 1N sodium hydroxide solution. To ensure that not all the oxygen of sample is exhausted during incubation, the sample was diluted 100 times. Two sets of BOD bottles were filled with this sample and one ml of allyl thiourea solution was added to each bottle. The dissolved oxygen was estimated in one set immediately following Winkler's method of oxygen estimation. The other set of BOD bottles were incubated at 20°C for five days in a BOD incubator. The bottles were taken after five days and their dissolved oxygen content was determined.

BOD_5 20°C (mg/L) = (D_0 - D_5) x dilution factor

Where, D_0 = initial dissolved oxygen in the sample; and D_5 = dissolved oxygen left out in the sample after five days of incubation.

Results

Table 1 describes the characteristics of effluent collected from dairy industry. The chemical parameters analysed were chlorides, nitrates, sulphates, ammonia total phosphorus and BOD.

The concentrations of chlorides are exhibited in Fig. 1. Chlorides were found to be reduced after treatment with *P. pardalis*. The maximum value was observed as 396.92 mg/L in 40% concentration. It was later reduced to 276.43 mg/L after five days of treatment. The lowest value of 148.84 mg/L was recorded after biotreatment in 10% effluent concentration. The levels of chlorides after treatment with *P. pardalis* were found to decrease more than that of control.

Figure 2 divulges the concentration of nitrates in dairy effluent after treatment with *P. pardalis*. The highest concentration of nitrates was 0.77 mg/L in 40% control after one day of treatment. The lowest value recorded was 0.01 in 10% treated effluent. In general, the nitrates were greatly reduced after the treatment from the maximum of 0.75 mg/L which was recorded in 40% effluent.

Alterations in the levels of sulphates in dairy effluent after treatment with *P. pardalis* are shown in Fig. 3. The values of suphates ranged from the minimum of 0.08 mg/L to the maximum of 5.7 mg/L. Sulphate level seems to increase with the increase in concentration and found to reduce greatly from

the first day to fifth day of treatment period whereas such reduction was not observed in the control. The levels of ammonia were highly fluctuating in dairy effluent after treatment with *P. pardalis* as shown in Fig.4. Ammonia level was generally observed to be more after the treatment than that of control. The maximum and the minimum values recorded were 0.37 and 0.03 mg/L respectively.
Figure 5 illustrates the changes in total phosphorus after treatment with *P. pardalis*. The highest value was observed in 40% concentration after one day of treatment as 200 mg/L and the minimum value recorded was 40 mg/L. Figure 6 divulges the levels of biological oxygen demand after treatment with *P. pardalis*. The highest BOD value of 187.86 mg/L was found to decrease to 125.24 mg/L. The lowest value observed after the treatment period was 98.98 mg/L in 10% effluent.
Table 2 divulges the results of Students't' test for all the parameters examined for dairy effluent comparing treated and untreated sets. Significant result was obtained for ammonia and 50% significance was found in chlorides. More significant results were observed in 10% dairy effluent.

Discussion

Bioremediation is considered as one of the powerful tools in the treatment of industrial effluents. Animals can remove or reduce the level of toxic components present in wastewater to desirable level. This can be done by selecting the efficient candidate from the wild animal population. Fish can do zooextraction, zoostabilisation and zoodegradation (Mangunwardoyo et al., 2013). *Anadonta woodiana* filters water and absorbs elements or compounds through mouth, and gills. Some are accumulated in the body and partly excreted. *Limnodrilus hoffmeistri* remodel organic pollutants by releasing enzymes. Amazon sailfin catfish, *P. pardalis* is one such animal that can be used for this purpose. It is not an important commercial fish (Chavez et al., 2006; Lam and Su, 2009; Jumawan et al., 2010). The hardy nature (German et al., 2010), and its tolerance to poor water quality enabled this fish to invade (Armbruster, 2006).
Chlorides are found in water combined with calcium, magnesium, or sodium. Small quantities of chlorides are needed for normal cell functions in plants and animals. Salty whey and brine contribute to higher chloride content in dairy waste. The levels exceeding 400 mg/L are toxic to aquatic organisms. In the present work, chloride content was in the range of 148.84 – 396.92 mg/L. Removal of chloride content in sugar mill effluent was reported by Saranraj and Stella (2012) using bacteria like, *Bacillus subtilis*, *Serratia marcescens, Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli* and *Brevibacterium halotolerance*.
High level of sulphates in water causes laxative effect along with calcium and magnesium. Water with high amounts of sulphates, form scales in boilers and heat exchangers. Sulphate also causes odour and corrosion problems of sewer as it gets converted to hydrogen sulphide (Esteban, 1997). Reduction in sulphate of sugar mill effluent was reported by Saranraj and Stella (2012) after using various bacterial cultures. From the results obtained, it is inferred that gradual reduction in sulphate level had occurred due to the efficiency of *P. pardalis*.
Raw milk has ammonia, and nitrogen. 50 mg/L of nitrogen in wastewater is due to 1% loss of milk (Soederhjelm et al., 1980). In the present work, there were significant changes in ammonia in the treated effluent during the treatment period of five days, because ammonia is the excretory product of fish. Nitrates can cause methemoglobinemia if converted to nitrites and contaminate groundwater (Kushwaha et al., 2011). Nitrogen as nitrate, nitrite, or ammonia affects health. Nitrates in dairy effluent may be attributed to milk having 3.7% fat content (Kumar and Desai, 2011). In the present work, nitrate value in the treated effluent was between 0.01and 0.75 mg/L. Phosphate is the soluble form of phosphorus and is mainly through detergents and soaps used in cleaning milk processing unit. Levels of phosphates in the treated effluent were in the range of 40 – 140 mg/L.

Like most other agro-industries, dairy industries generate effluents with high BOD. BOD of effluent was 236 - 289 mg/L (Orhon, 1993). BOD reduction was also reported by Das and Santra (2010) and Gaikwad et al. (2014) from effluents after using bacterial isolates. Bioremediation studies using the aquatic plant, *Eichhornia crassipes* in domestic wastewater treatment reported reduction by 91% of biochemical oxygen demand, 16% of total dissolved solids, 70% of total suspended solids, 4% chlorides, 74% ammonium-nitrogen and 41% phosphates (Mangunwardoyo, 2013). Likewise, considerable reduction of BOD was noticed in the treated effluent. From the results of students' 't' test, it can be suggested that biotreatment of dairy effluent using *P. pardalis* can be more efficient in diluted effluent.

Conclusion

The analysis of chemical properties in the dairy effluent shows that *P. pardalis* has the capacity to remediate dairy effluent. Therefore it can be employed as an agent in biotreatment of dairy effluent.

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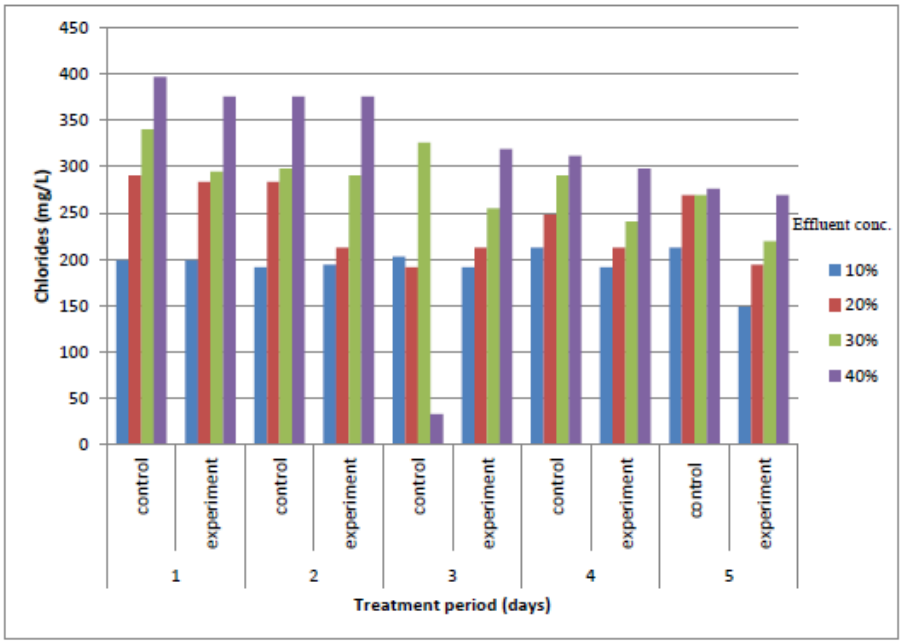

Figure 1. Changes in the levels of Chlorides (mg/L) in dairy effluent after treatment with *P. pardalis*

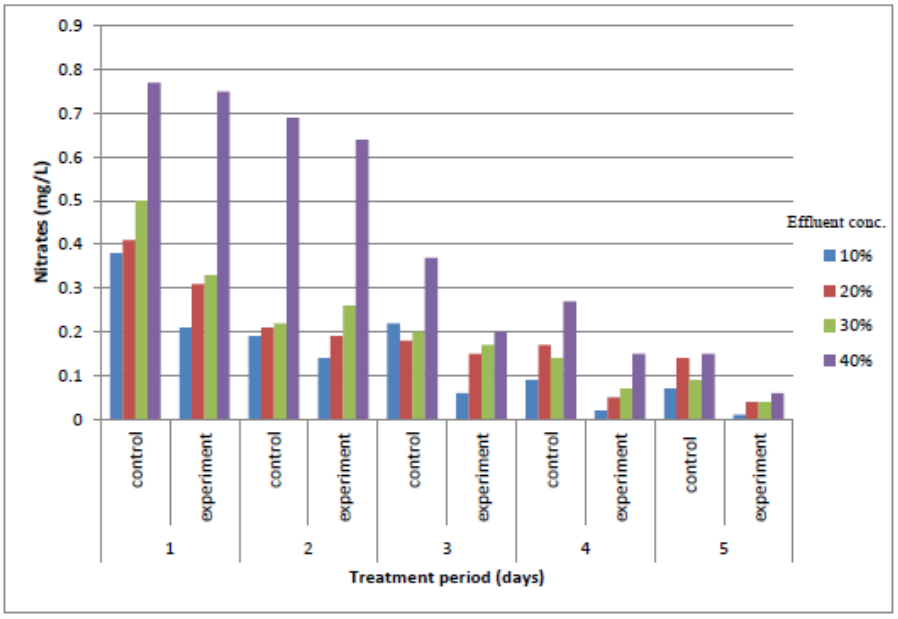

Figure 2. Changes in the levels of Nitrates (mg/L) in dairy effluent after treatment with *P. pardalis*

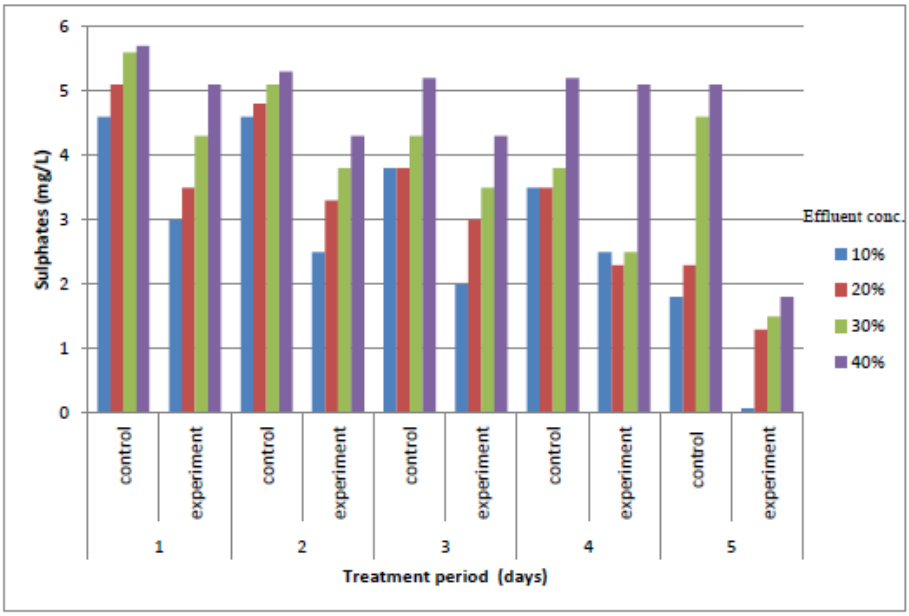

Figure 3. Changes in the levels of Sulphates (mg/L) in dairy effluent after treatment with *P. pardalis*

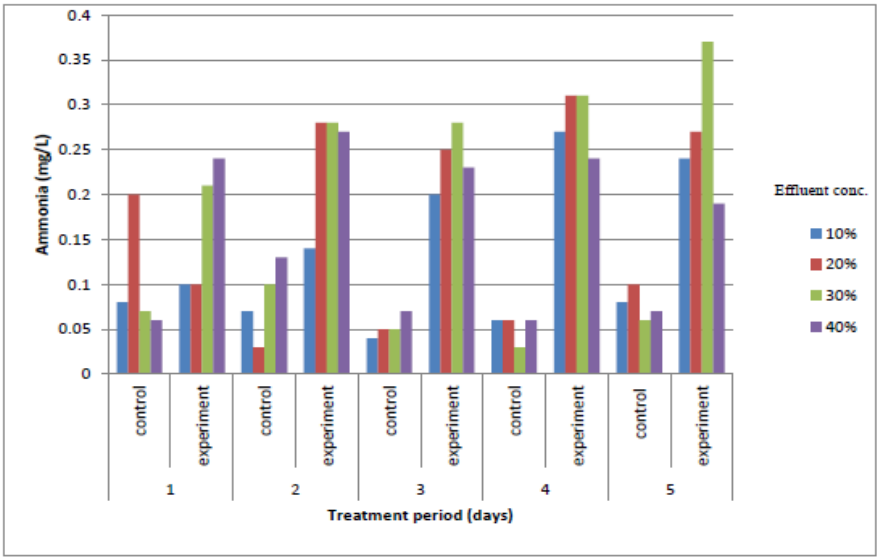

Figure 4. Changes in the levels of Ammonia (mg/L) in dairy effluent after treatment with *P. pardalis*

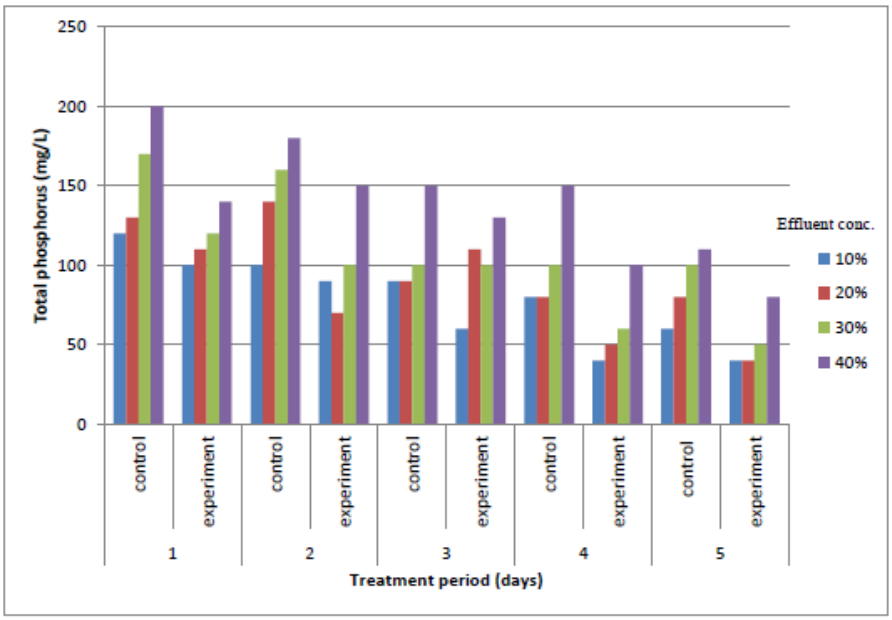

Figure 5. Changes in the levels of Total phosphorus (mg/L) in dairy effluent after treatment with *P. pardalis*

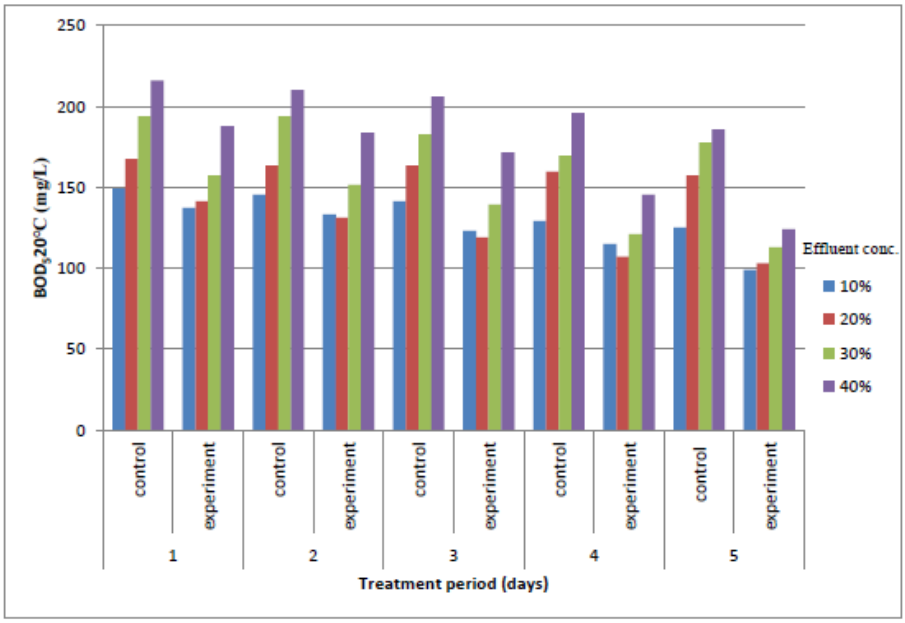

Figure 6. Changes in the levels of BOD_5 20° C (mg/L) in dairy effluent after treatment with *P. pardalis*

Table 1. Chemical parameters of dairy effluent

S.No	Parameters (unit)	Values
1.	Chlorides (mg/L)	304.93
2.	Nitrates (mg/L)	0.88
3.	Sulphates (mg/L)	7.20
4.	Ammonia (mg/L)	0.30
5.	Total phosphorus (mg/L)	210.0
6.	Biological Oxygen Demand5 20°C (mg/L)	1160.0

Table 2. Results of Students' 't' test for the various chemical parameters of dairy effluent comparing treated and untreated samples

S.No	Parameters	Level of significance at 5% level			
		Dairy effluent concentration (%)			
		10	20	30	40
1.	Chlorides	S	S	NS	NS
2.	Nitrates	NS	NS	S	NS
3.	Sulphates	NS	NS	S	NS
4.	Ammonia	S	S	S	S
5.	Total phosphorus	NS	S	NS	NS
6.	Biological Oxygen Demand5 20°C	NS	NS	NS	Ns

S – Significant; NS – Not significant