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Original Article

Treatment of industrial wastewater of alcohol factories using a particle trap system and their potential for aquaculture using Daphnia (*Daphnia pulex*) and Zebrafish (*Danio rerio*) as model bioindicators

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Abstract: Considering the drought and lack of water in recent decades, effluent reuse has been suggested as an efficient way to save water sources. Also, reducing the amount of sewage before releasing it into the environment is necessary to decrease threats to human and aquatic organisms. In recent years, due to the emergence of the COVID-19 epidemic and following the massive production of alcohol, the discharge of wastewater from these factories has increased. In this research, the performance of a particle trap system (PTS) was examined in treating the effluents of an alcohol factory as vinasse and stillage and the treated water with different concentrations of the wastewater was used for Daphnia (*Daphnia pulex*) and Zebrafish (*Danio rerio*) as model bioindicators. The performance of PTS in reducing COD, BOD, and TSS for vinasse was 96.90, 97.44, and 88.43% and for stillage was 95.69, 96.77, and 90.15%, respectively. Based on the results, the LC_{50} of vinasse for zebrafish and daphnia was 0.63 and 0.76%, and the LC_{50} of stillage for zebrafish and daphnia was 0.6 and 0.65%, respectively. The mortality rate of daphnia and zebrafish was different based on wastewater concentration and duration of exposure. In high exposure concentrations, which were usually above 3%, death occurred in a shorter period of time. In conclusion, the PTS is an efficient and inexpensive system for the purification and recycling of effluent from alcohol factories.

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Introduction

Aquaculture strongly relies on the availability of adequate water sources. Therefore, the use of reused water helps to save water and reduce water contaminants (El-Gohary et al., 1995; Ng et al., 2018). Owing to the lack of water in arid and semi-arid regions, the development of water treatment systems and the use of returned water is crucial. In addition, the excessive discharge of industrial wastewater into surface water imposes harmful effects on the environment and living organisms, especially those living in aquatic environments (Matta et al., 2014; Bukola et al., 2015; Garg et al., 2022). Domestic wastewater and more importantly industrial wastewater cause a wide range of biological and ecological problems and thus the treatment of wastewater before releasing it into the natural waters could be an efficient way to reduce adverse impacts. Industrial effluents include complex combinations of organic components that increase biological oxygen demand (BOD) or chemical oxygen demand (COD) upon bacterial and non-bacterial decomposition processes, respectively (Kushwaha, 2015; Jindal et al., 2019).

Numerous researches have been done to remove or change and transform the contaminants in water (Bolong et al., 2009; Garcia-Rodríguez et al., 2014; Chen et al., 2022). Biological methods are often simple and inexpensive and thus are more efficient in removing color and organic materials from wastewater compared to other treatments (Ishak et al., 2012; Gunatilake, 2015; Barbusiński and Kalemba, 2016). However, these methods have limitations in cleaning the sewages containing toxic and non-biodegradable compounds since these compounds need some physical and chemical treatments (Crini

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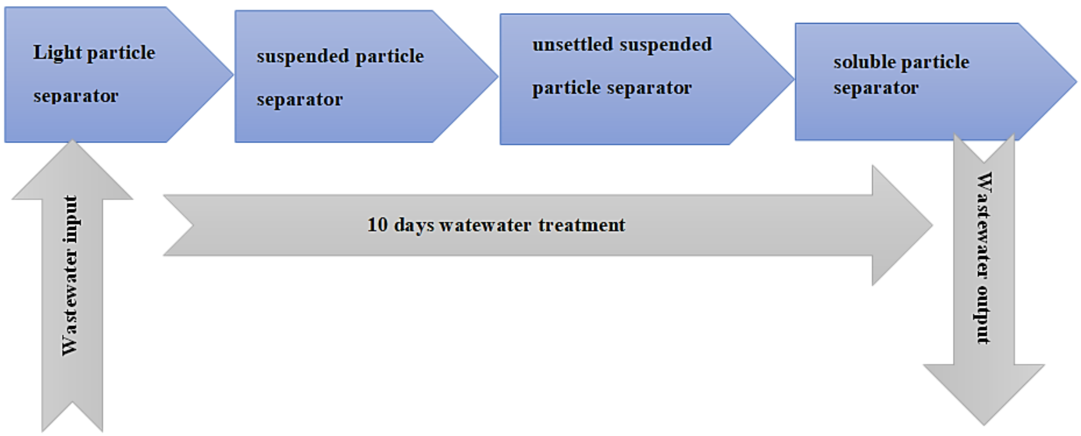

Figure 1. The stages of the particle trap system.

and Lichtfouse, 2019). Effluent from alcohol production factories is one of the major environmental problems due to the need for high BOD and COD upon degradation and the presence of toxic substances such as phenolic compounds and low pH (Satyawali and Balakrishnan, 2008).

Many biological, chemical, and physical methods have been proposed to treat the wastewater of alcohol factories (Pant and Adholeya, 2007; Kharayat, 2012; Shivajirao, 2012). Anaerobic biological methods are economical compared to aerobic ones and have been greatly developed in recent years (Pant and Adholeya, 2007). Normally, for every liter of alcohol produced, between 10-15 liters of wastewater are produced (Guruswami, 1998). Due to the increase in the production of alcohol and related products during the COVID-19 pandemic, the discharge of wastewater from alcohol factories has elevated and caused environmental problems (Havryshko et al., 2020). This wastewater has a low pH and a very dark color due to the high content of organic matter (Satyawali and Blackrishnan, 2008). Conventional treatment systems, including activated sludge, aeration lagoons, anaerobic ponds, etc., are not effective due to the very high concentration of salts in this wastewater (Sirianuntapiboon et al., 2004 a, b). Therefore, it is necessary to find an efficient and cost-effective method to treat this type of wastewater. In this research, a particle trap system (PTS) (Fig. 1) was used to treat two kinds of alcoholic distillation residue including vinasse and stillage. An oxygen injection step was done before the particle trap, and then the purification process was carried out in three stages, including the separation of surface materials, the separation of heavy particles, and the separation of fine suspended particles. At the end, as model aquatic organisms viz. zebrafish, *Danio rerio,* and daphnia, *Daphnia pulex,* were exposed to different concentrations of wastewater to determine the possibility of using treated water for aquaculture.

Materials and Methods

Preparation of wastewater: Wastewater samples (i.e. vinasse and stillage) were taken from the outlet of Jahan alcohol factory, Manjil, Guilan Province, Iran. This factory uses two fermentation processes to produce alcohol from beet molasses and wheat starch. In both cases, sugar is converted into alcohol and the rest of the materials enter the Manjil Dam without any treatment. The wastewater samples were taken in a 60 ml glass bottle. The samples were first studied in terms of the amount of suspended matter, dry matter, ammonia, phosphate, and dissolved oxygen and then used for the treatment process by PTS.

Particle trap system: A particle trap system (PTS) (Fig. 2) was used in this research including different parts to remove wastes in a glass tank with a volume of 120 liters. The wastewater is passed through continuously by a water pump (2000 L per h). The different parts of the system are shown in Figure 1, including (a) light particle separator: These materials are mainly decomposing bubble parts or those

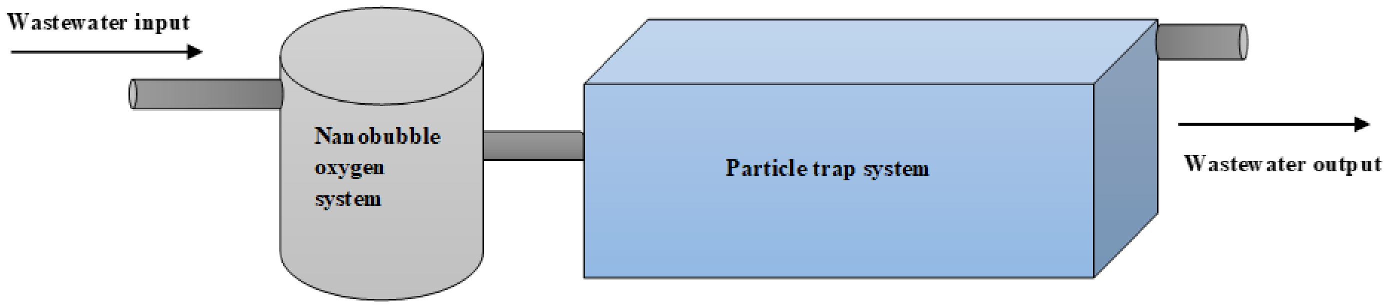

Figure 2. The stages applied for the treatment of alcoholic wastewaters.

containing fat. The structure of this part is somewhat similar to the grease trap in sewage treatment lagoons. The fat must be separated first because it causes many problems in purification, (b) the suspended particle separator: These materials are not settled due to turbulence. In this part, minerals or colloids formed will be deposited, (c) in this part the unsettled suspended materials were removed by converting the turbulent flow of water into a blade flow and (d) in this part, the soluble materials were biologically removed by a biological filter structure. This part is not discussed in the present study, because there is a pre-assumption that the soluble materials will be very small over the previous three stages. As mentioned, due to the smallness of the filters in this laboratory model, a volume of wastewater has passed through different sections many times to provide the possibility of long-term sampling. Before PTS, the wastewater in two types, including stillage and vinasse enters the compressed air tank and oxygen is injected for 3 h. This stage was designed and implemented based on the results obtained from a pre-test stage. Then the prepared wastewater was pumped into PTS. Wastewater discharged from PTS was taken every 24 h and the BOD, COD, TSS, pH, total nitrogen, and total phosphorus were determined. Sampling was done randomly within ten days and each experiment was done with three replicates.

Assays: pH was determined by a Microprocessor pH meter (HANNA Instrument company). BOD was determined according to Servais et al. (1999) by incubating a sealed sample of water for five days at 20˚C. Since industrial wastewaters have high BOD and have no microorganisms, they are diluted to a great extent. In this method, the bottle is filled with nutrient-rich and oxygen-saturated diluting water (5%) for biological growth. BOD was determined according to the following formula (Servais et al., 1999): $BOD_{(mg/L)} = [(D_1-D_2) - (B_1-B_2)]\ f / P$, where, D1 = Dissolved oxygen of original dilution water, D2 = Dissolved oxygen of diluted water after 5 days of incubation, B1 = Dissolved oxygen of dilution of seed control before incubation, B2 = Dissolved oxygen of dilution of seed control after incubation, F = % seed in D1/ % seed in B1, and P = Decimal fraction of sample use.

To determine COD, 10 mg of concentrated potassium dichromate solution was diluted in water and then was volumed to 100 ml. 30 ml of concentrated sulfuric acid was added to this solution. After cooling, 3 drops of ferroin indicator were added and the resulting solution was titrated with ferroammonium sulfate. Finally, the COD was calculated from the following equation (Afsar et al., 2023): $COD\ (mg/L) = 200 \times (a-b) \times f / V$, where, a is the volume of ferro-ammonium sulfate consumed for the test sample, b is the volume of ferroammonium sulfate consumed for the control sample, c is the titration factor of ferrous ammonium sulfate solution, and V is the sample volume.

To determine TSS, first, the dried filter (2 µm) was weighed and then the water sample was filtered. Then the used filter was dried at 105˚C and weighed again. The difference in filter weight, before and after filtering, represented the weight of suspended solids in the water sample and was calculated according to the following formula: $C_{TSS} = (m_{f+s} - m_f)/V$

The total nitrogen of the samples was determined

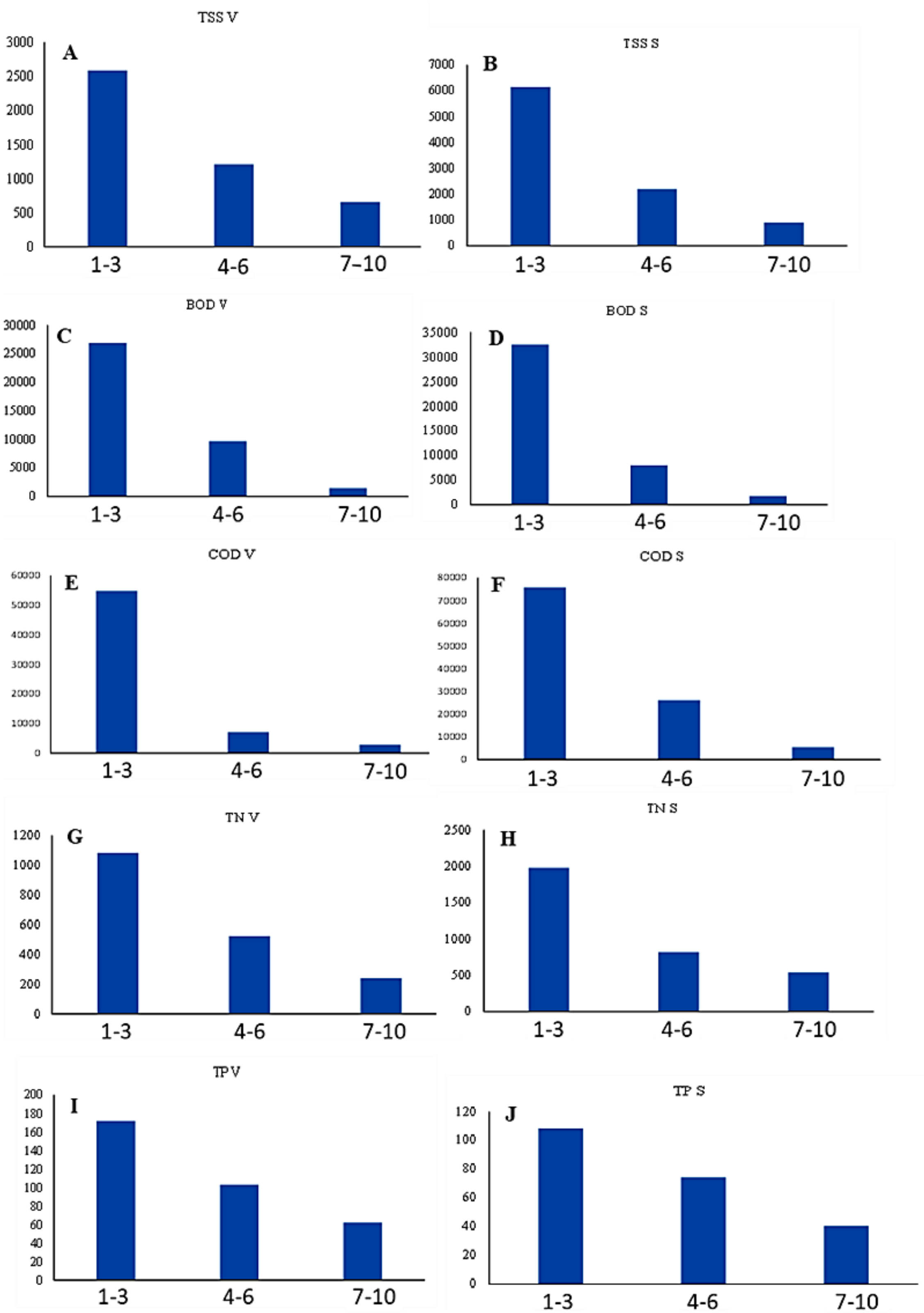

Figure 3. Changes in the chemical parameters of the wastewaters [Vinass (V) and Stillage (S)] during treatment by particle trap treatment system (Abbreviations: BOD: biological oxygen demands, COD: chemical oxygen demands, TSS: Total suspended solids, TN: total nitrogen, and TP: total phosphorous).

by Kjeldahl analysis (Lynch and Barbano, 1999). In this method, the samples were first digested by sulfuric acid, and the existing nitrogen was converted into ammonium sulfate. Then, the nitrogen in the

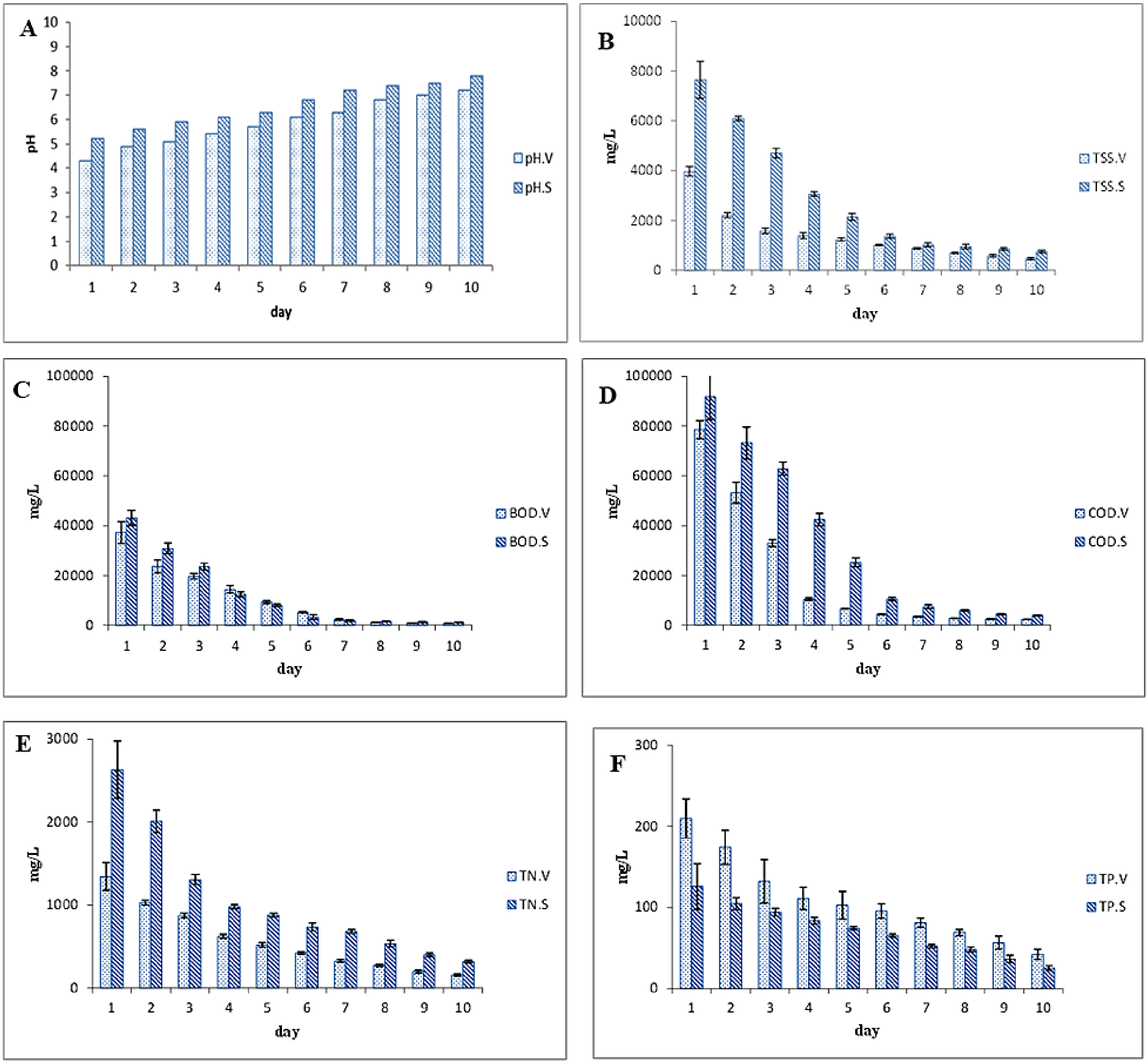

Figure 4. Changes in the chemical parameters of the wastewaters [Vinass (V) and Stillage (S)] during treatment by particle trap treatment system (BOD: biological oxygen demands, COD: chemical oxygen demands, TSS: Total suspended solids, TN: total nitrogen, and TP: total phosphorous).

ammonium sulfate is converted into free ammonia and ammonium borate by boric acid and titred using 0.1 normal sulfuric acid. To determine the total phosphorus (TP) content of the samples, one drop of phenolphthalein was added to the sample diluted in 100 ml distilled water. When the color of the solution turned pink, sulfuric acid was added drop by drop until it became colorless. Then, 4 ml of molybdate reagent and 0.5 ml of stannous chloride were added to each standard and sample and mixed. The amount and intensity of the color depends on the temperature of the solution (each degree of increase has a 1% increase in color). Therefore, the samples, standards, and reagents were kept at 25°C. After 10 min, the absorbance was read at 690 nm (Deka and Goswami, 2007).

Exposure experiment: Two model aquatic animals, daphnia and zebrafish at a stocking rate of 20 /400 L

Table 1. The daily efficiency percentage of the particle trap system.

	Chemical parameters					
Day post-treatment	pH	TSS	BOD	COD	TN	TP
2	12.24	44.17	36.14	32.27	23.42	16.85
3	3.92	28.55	17.21	37.9	14.9	24.09
4	5.56	12.12	26.52	68.54	28.76	15.87
5	5.26	11.43	34.54	35.17	16.19	7.78
6	6.56	17.69	45.41	35.8	19.2	6.82
7	3.17	13.11	53.12	19.14	22.42	14.98
8	7.35	20.3	46.16	17.44	16.89	14.75
9	2.86	16.37	20.39	11.48	26.07	18.27
10	2.78	21.84	8.19	4.67	21.69	25.29
			Stillage			
2	7.4	20.29	28.44	20.22	23.6	16.75
3	5.08	22.81	23.69	14.12	34.99	10.07
4	3.28	34.66	47.03	32.41	25.03	11.31
5	3.17	30.14	35.06	40.49	9.9	10.76
6	7.35	36.12	59.55	58	16.4	12.5
7	5.56	25.59	37.68	29.49	7.23	19.39
8	2.7	6.63	10.26	20.24	21.48	8.23
9	1.33	1031	19.59	23.89	25.25	24.14
10	3.85	11.91	5.6	12.99	19.92	30.91

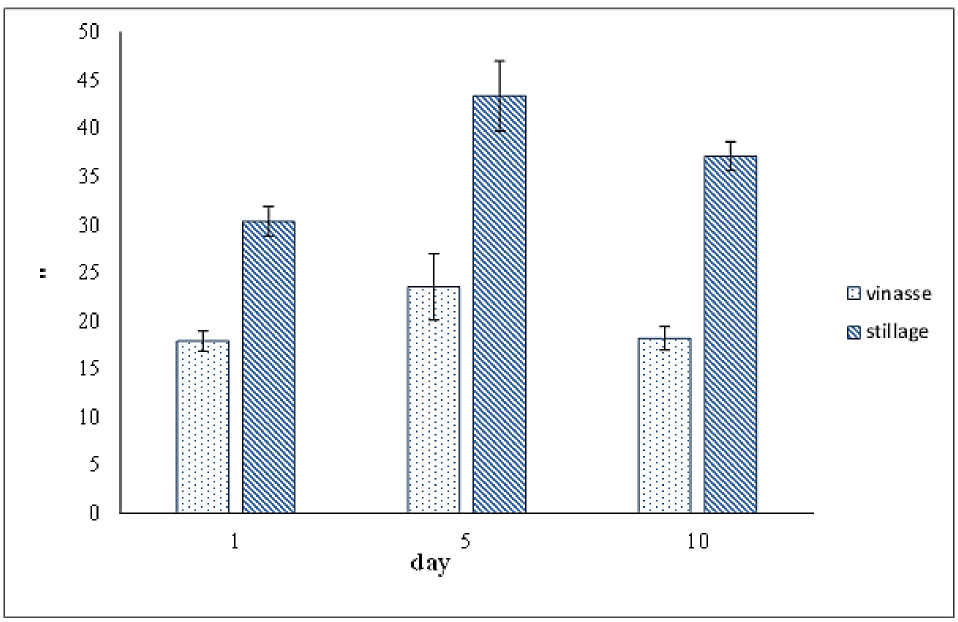

Figure 5. Comparison of the protein content of Vinass (V) and Stillage (S) wastewaters during treatment by particle trap treatment system.

tank were exposed to different concentrations of wastewater (0.35, 0.75, 1.5, 3, 6.25, 12.5, 25, 50, and 100 %) for 10 days to determine the toxicity of wastewaters with different degrees of the treatment. The animal mortality at day 1 post-exposure was recorded at 4:00, 8:00, and 24:00. After that, the mortality was recorded every 24 hours. The aeration in each tank was conducted for 5 min per h to prevent evaporation.

Data analysis: Significant differences between treatments were determined using a One-Way analysis of variance. To compare the means before and after treatment, an independent samples t-test was used. The data were analyzed by SPSS software.

Results

Chemical assays in wastewater during the treatment period: Means of chemical parameters showed significant differences between the beginning (day 1-3), middle (day 4-6), and end (day 7-10) of the experiment periods (Fig. 3, P<0.05). The means of

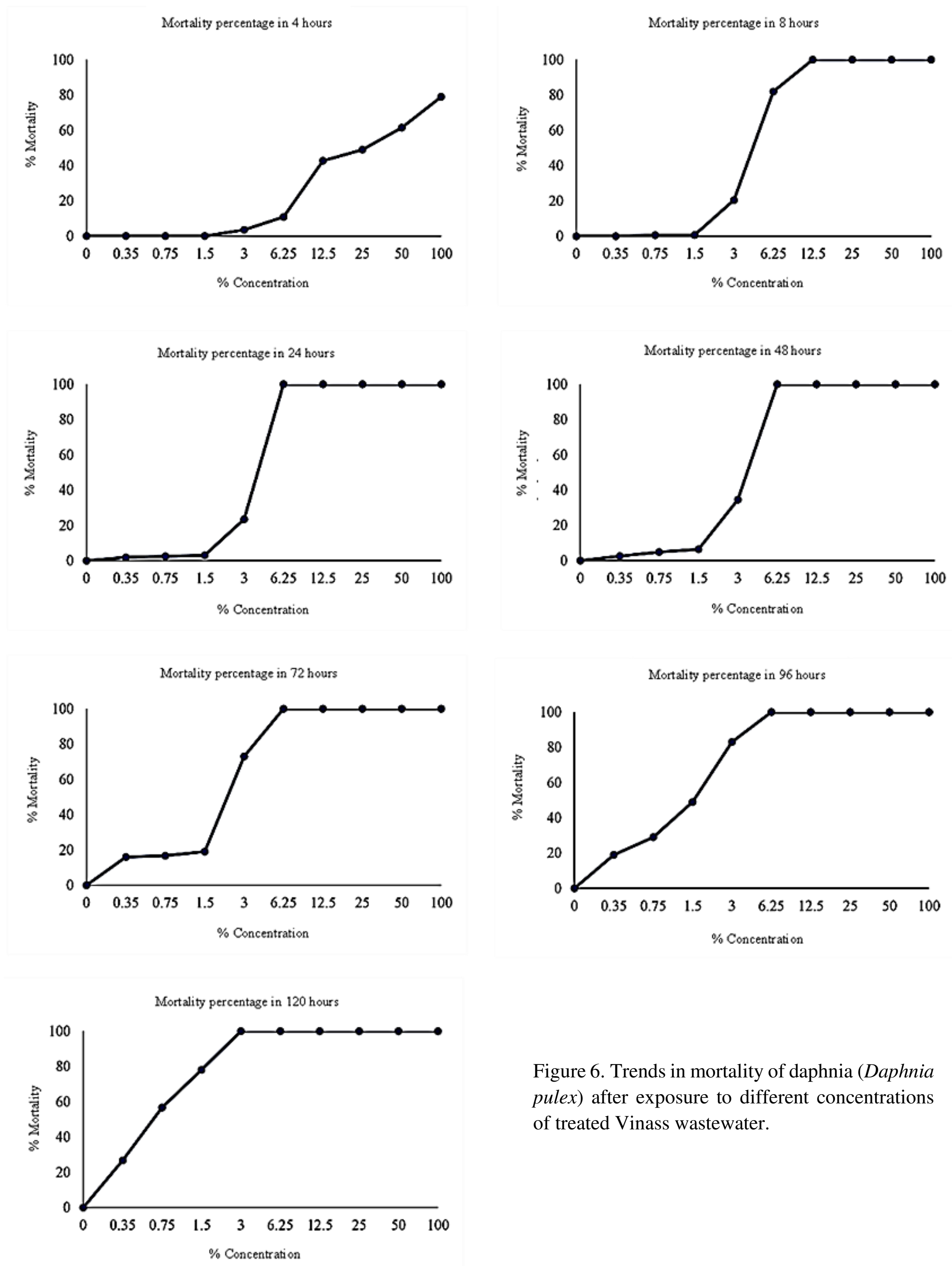

Figure 6. Trends in mortality of daphnia (*Daphnia pulex*) after exposure to different concentrations of treated Vinass wastewater.

these parameters significantly decreased during the experiment. The highest and lowest means were observed at the beginning and end of the experiment.

pH: The trend of pH changes during the period of both experiments is shown in Figure 4A. In the vinasse wastewater treatment process, the maximum and minimum pH were 7.2 on day 10 and 4.30 on day 1, respectively. In the process of stillage wastewater, the maximum and minimum pH were 7.8 and 5.2 on days 10 and 1, respectively ($P<0.05$). Taking into account the daily efficiency of the particle trap treatment system, in vinasse wastewater, the highest changes at 12.24% on day 2 and the least changes at 2.78% on day 10 were observed (Table 1, $P<0.05$). In stillage

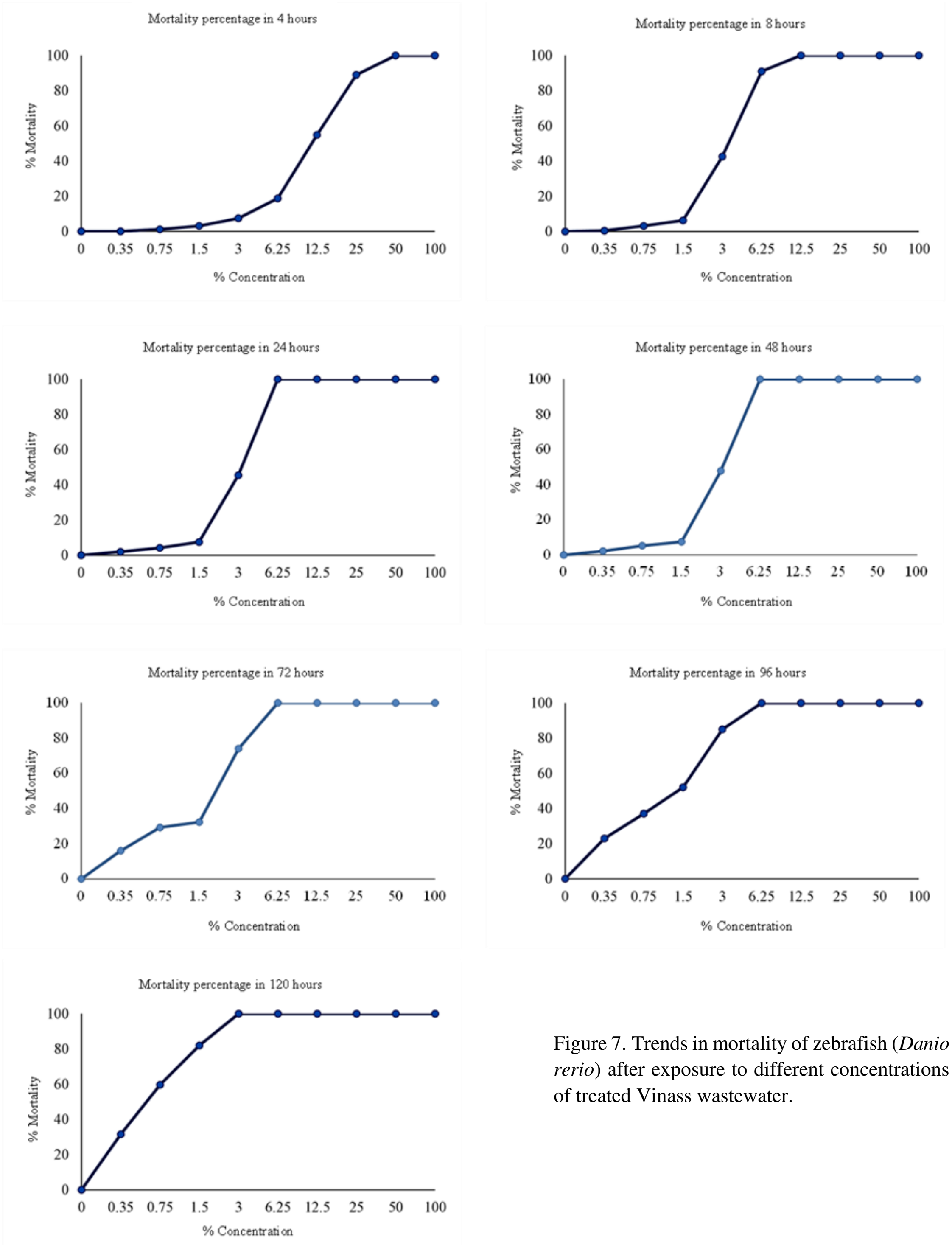

Figure 7. Trends in mortality of zebrafish (*Danio rerio*) after exposure to different concentrations of treated Vinass wastewater.

wastewater, the most changes at 7.35% were observed on day 6, and the least changes at 1.33% on day 10 (Table 1, *P*<0.05).

Total suspended solids (TSS): The trends of TSS changes during the period of both experiments are shown in Fig. 4B. In the vinasse wastewater treatment process, the maximum and minimum of TSS were 3969.67±179.62 and 459.33±42.50, which were observed on day 1 and 10, respectively (*P*<0.05). Similarly, for the stillage wastewater treatment process, the maximum (7642.67±16.742) and minimum (752.49±67.72) were found on days 1 and 10, respectively (*P*<0.05). For the vinasse wastewater, the highest and lowest daily efficiency of the particle trap treatment system, were 44.17 and 11.43% on days 2 and 5, respectively (Table 1, *P*<0.05). For stillage

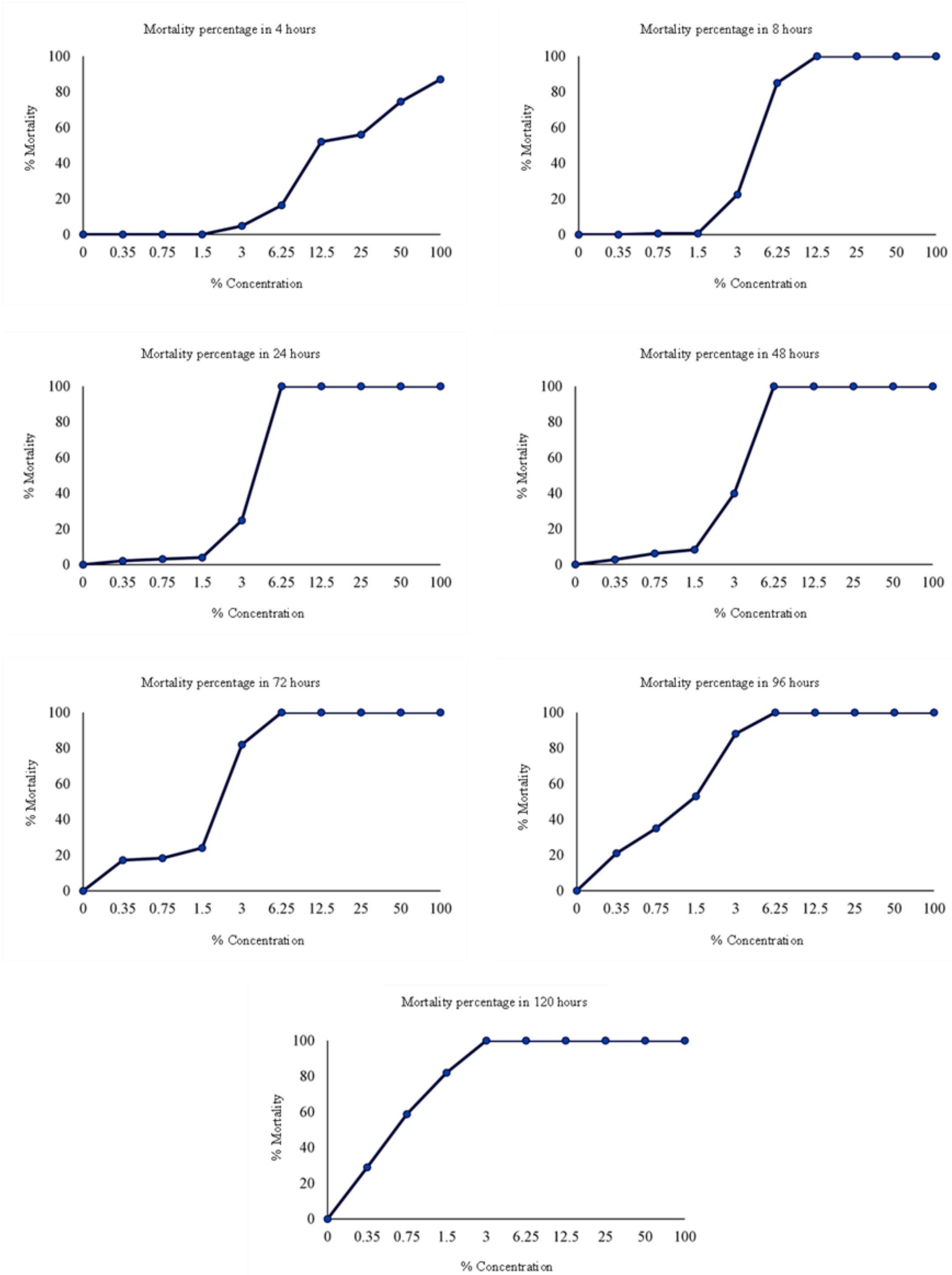

Figure 8. Trends in mortality of daphnia (*Daphnia pulex*) after exposure to different concentrations of treated Stillage wastewater.

wastewater, maximum and minimum values of 12. 36% at day 6 and 6.63% at day 8 were observed (Table 1, $P<0.05$).

Biological oxygen demand (BOD): The trend of BOD changes during the period of both experiments is shown in Figure 4C. In the vinasse wastewater treatment process, the BOD showed a higher value (37216.67±4348.08) on day 1 ($P<0.05$). On day 10, lower BOD (953.33±50.36) was recorded compared to other sampling days ($P<0.05$). Similarly, in the stillage wastewater treatment, BOD (43166.67± 3003.89) had a maximum level (1392.93±67.66) on day 1, while it showed minimum levels on day 10 ($P<0.05$). For the vinasse wastewater, the highest and

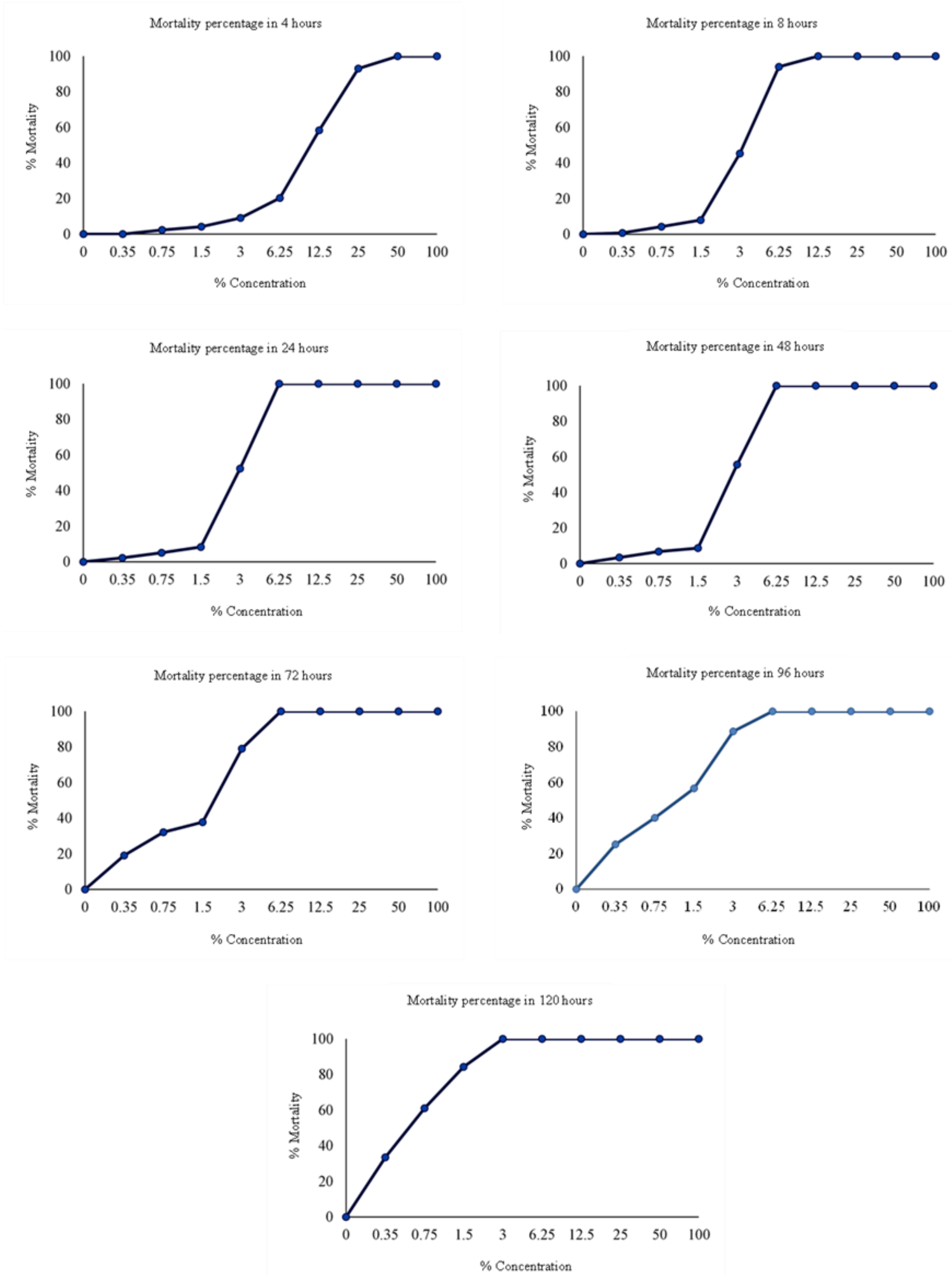

Figure 9. Trends in mortality of zebrafish (*Danio rerio*) after exposure to different concentrations of treated Stillage wastewater.

lowest efficiency of 53.12 and 8.19% were found on days 7 and 10, respectively (Table 1, *P*<0.05). For the stillage, the highest (59.55%) and lowest (5.60%) efficiency were observed on days 6 and 10, respectively (Table 1, *P*<0.05).

Chemical oxygen demand (COD): The changes in COD during treatment are shown in Figure 4D. In the vinasse wastewater treatment process, the COD showed a higher value (3629.78566±67.51) on day 1. On day 10, lower COD (2437.33±107.58) was observed compared to other sampling days (*P*<0.05). Similarly, in the stillage wastewater treatment, COD had a maximum level (9106.9106±00.04) on day 1, while it showed minimum levels (33.33±174.16) on day 10 (*P*<0.05). For the vinasse wastewater, the highest and lowest efficiency of 68.54 and 4.67% were found on days 4 and 10, respectively (Table 1, *P*<0.05). For the stillage, the highest (58%) and lowest (12.99%) efficiency were observed on days 6 and 10 respectively, (Table 1, *P*<0.05).

Total nitrogen (TN): The changes in TN during treatment are shown in Figure 4E. In the vinasse wastewater treatment process, the TN showed a higher value (343.33±166.23) on day 1 (*P*<0.05). On day 10, lower TN (157.67±13.32) was observed compared to other sampling days (*P*<0.05). Similarly, in the stillage wastewater treatment, TN had a maximum level (2630.00±348.28) on day 1, while it showed minimum levels (321.67±18.58) on day 10 (*P*<0.05). For the vinasse wastewater, the highest and lowest efficiency of 28.76 and 14.90% were found on days 4 and 3, respectively (Table 1, *P*<0.05). For the stillage, the highest (34.99 %) and lowest (23.7%) efficiency were observed on days 3 and 7, respectively (Table 1, *P*<0.05).

Total phosphorus (TP): TP changes during treatment are illustrated in Figure 4F. In the vinasse wastewater, the TN showed a higher value (209.67±24.11) on day 1 (*P*<0.05). TN showed lower values (42.33±6.11) on day 10 (*P*<0.05). Similarly, in the stillage wastewater, TN had a maximum level (126.00±28.00) on day 1, while it had a minimum level (25.33±2.89) on day 10 (*P*<0.05). For the vinasse wastewater, the highest and lowest efficiency of 25.29 and 6.82% were observed on days 10 and 6, respectively (Table 1, *P*<0.05). For the stillage, the highest (30.91%) and lowest (8.23%) efficiency were observed on days 10 and 8, respectively (Table 1, *P*<0.05).

Protein content of wastewater: The dry sludge obtained from vinasse wastewater had a protein content of 17.17±90.06, 23.3±53.46 and 18.20± 1.21%, respectively at the beginning, middle, and end of the treatment process (Fig. 5, *P*<0.05). The dry sludge resulting from the stillage wastewater had a protein percentage of 30±1.53, 43±37.65, and 37± 13.51% at the beginning, middle, and end of the treatment process, respectively (Fig. 5, *P*<0.05). The protein obtained from stillage wastewater was more than that in vinasse wastewater (Fig. 5, *P*<0.05). Also, in stillage wastewater, a significant difference was observed in protein content (Fig. 5, *P*<0.05) between all three sample times. Samples taken in the middle of the treatment process had higher protein content compared to other sampling times in both types of wastewater (Fig. 5, *P*<0.05).

Exposure experiment: Daphnia and zebrafish were exposed to different percentages of vinasse and stillage wastewaters. Exposure of daphnia and zebrafish to 0.35% vinasse resulted in a higher survival percentage compared to other concentrations. In daphnia exposed to vinasse, with the increase in wastewater concentration, the amount of Daphnia mortality increased significantly over 4-72 h, which was mostly related to concentrations above 3%, while at 96 and 120 h post-exposure, losses observed at lower wastewater concentrations (Fig. 6, *P*<0.05).

In zebrafish exposed to vinasse, fish mortality significantly increased in response to 6.25% wastewater at 4 h post-exposure (Fig 7, *P*<0.05). During the 4-48 h exposure period, fish mortality was observed at an exposure concentration of 3%. After 48 h exposure, fish mortality occurred at lower concentrations (0.35%) (Fig. 7, *P*<0.05). In daphnia exposed to stillage, with the increase in wastewater concentration from 3-6.25%, the mortality increased significantly over 4-48 h, while mortality occurred in lower concentrations 48-120 post-exposure (Fig. 8, *P*<0.05). In zebrafish, mortality significantly increased in response to 6.25% stillage during the first 4 h of exposure (Fig. 9, *P*<0.05). During 4 to 48 h exposure, the mortality increased at concentrations above 3% stillage. During 48-120 h, the mortality occurred at lower concentrations i.e. 0.35% (Fig. 9, *P*<0.05).

Discussions

A particle trap system was used to treat two types of wastewater of a distillery factory, i.e. vinasse and stillage. The process of wastewater treatment was conducted over 10 days by analyzing chemical parameters. The wastewater of alcohol production industries is acidic in nature. As a result, an increase in pH was observed after treatment, which may be due to the oxidation of organic matter upon injection of oxygen during the treatment (Sundarapandiyan et al., 2018; Yazdanbakhsh et al., 2018). The elevated levels of pH have a positive effect on the purification performance and improve the color removal process (Yazdanbakhsh et al., 2018). Oxygen attacks certain parts of organic compounds that have aromatic rings or carbon double bonds and turns them into final products such as aldehydes and carboxylic acids. Under normal conditions, the rate of these reactions is slow, and only the compounds whose tendency to react with free oxygen is more than OH radical are decomposed. As a result, this reaction directs the pH conditions towards alkalinity, resulting in better performance of the wastewater treatment. On the other hand, increases in the concentration of OH creates secondary oxidants that are stronger and more active (Beltrán et al., 2001). The pH changes in the final days of the treatment were lower than those in the first days, which may be a result of the greater decomposition rate at the beginning of the treatment process.

One of the most important physical parameters of wastewater is TSS, which includes floating materials, settleable materials, and colloids (Lepot et al., 2016; Khairun et al., 2012; Biswas, 2020). These materials act as a surface for the absorption and accumulation of chemical and biological substances, and in this way, they can create more unpleasant substances under chemical and biological reactions (Rezaei, 2014; Zhao et al., 2020). In previous studies on particle trap systems, TSS reduction was estimated for cattle wastewater by 90% (Mani Varnosfadrani, 2014), and for fish wastewater in recirculation systems by 44.12% (Rezaei, 2015). Similar results were obtained in the current study. The lowest amount of TSS was obtained at the end of the treatment period for both types of wastewater, indicating acceptable efficiency of PTS in removing TSS. Nevertheless, the system showed a higher TSS-removal efficiency for vinasse compared to stillage.

The BOD content of wastewater is a main indicator of the activity of aerobic microorganisms and is also one of the most important problems with wastewater treatment processes (Mocuba, 2010; Liu et al., 2021). The wastewater of alcohol production industries has usually high BOD content, which creates an unpleasant smell under anaerobic conditions (Mandal et al., 2003; Sankaran et al., 2014). In the present study, the PTS effectively reduced the BOD content of vinasse and stillage, because the lowest BOD was measured at the end of the treatment period. Such decrease in BOD content may be due to the oxygen injection and increasing the oxygen capacity of wastewater as well as the performance of the PTS in removing particles and coagulated organic materials and bioflocs (Sumathi and Sundaram, 2009; Ayyoub et al., 2023). The efficiency of PTS in reducing BOD was also higher in the early stages of the treatment period than in the final stages, because an efficiency of 53.12% at day 7 and 59.55% at day 6 were observed for vinasse and stillage, respectively. Overall, the TPS system showed a 97% efficiency in reducing BOD, which is a high efficiency compared to the other systems used for wastewater treatment so far. For example, 50% by biological filtration, 95% by trickle filter (Sawyer and Anderson, 1949), 80% by activated sludge and anaerobic treatment (Chen and Gibson, 1980), and 90% by activated carbon (Chandra and Pandey, 2001).

The chemical oxygen demand (COD) is an indicator, representing the chemical oxidation rate of organic matter of wastewater (Bertanza et al., 2001; Ksibi, 2006). The amount of COD in the effluent of alcohol production industries is very high causing serious environmental problems and also causes disruptions in treatment processes (Strong and Burgess, 2008; Ha et al., 2012). In the current study, COD was high in the early stages of the treatment, while significant decreases were observed in COD at the end of the treatment period, which may show the high performance of PTS in reducing the organic matter content of the wastewater. The performance of PTS in the oxidation of organic matter and the following declines in COD may be due to the oxygen injection (Skouteris et al., 2020) and the high performance of PTS in the physical filtration of organic matter. In addition, the PTS system exhibited a 97% efficiency in reducing COD, which was an appropriate performance compared to other wastewater treatment processes. For example, 80% by submerged bed reactor (Reis and Sant'Anna, 1985), 40% by trickle filter (Burnett, 1973), 60% by activated sludge and anaerobic treatment (Chen and Gibson, 1980; Chiesa and Manning, 1986) and 90% by activated carbon (Chandra and Pandey, 2001). The efficiency of PTS in reducing COD was also higher in the early stages of the treatment period than in the final stages because an efficiency of 68.54% on day 4 and 58% on day 6 was observed for vinasse and stillage, respectively, which could be due to the decreases of organic matter contents in the wastewaters with time.

During the process of oxidation and mineralization of organic matter, nitrogen is released from wastewater in the form of N_2 (Goto et al., 1998: Fdz-Polanco et al., 2001). Thus, a significant decline in TN occurs as the treatment process proceeds. Oxidation and mineralization also precipitate the phosphorus content of wastewater in the sludge (Ramasahayam et al., 2014). Similarly, TN and TP decreased both in vinasse and stillage wastewaters with time upon treatment by PTS in this study. The efficiency of PTS in removing TN and TP was higher in the initial stages of the treatment than in the final stages, which could be due to the higher concentration of organic matter at the beginning of the wastewater treatment process.

The dry sludge obtained from vinasse wastewater had a protein content of 17.9±1.06, 23.53±3.46, and 18.20±1.21%, respectively at the beginning, middle, and end of the treatment process. The dry sludge resulting from the stillage wastewater treatment was 30.37±1.53, 43.37±3.65, and 37.1±13.51, respectively for the beginning, middle, and end of the treatment process. Sludge protein percentage increased in the middle of the treatment period and then decreased in both types of wastewaters and this decrease could be due to the destruction and decomposition of proteins over time (Satyawali and Balakrishnan, 2009).

TSS includes all organic and non-organic suspended particles in the water (Clinton and Vose, 2003). In the present study, the TSS concentrations at the end of the treatment were above 500 mg/l, which is higher than the acceptable level (namely <50 mg/l) for aquaculture (Arief et al., 2022). Therefore, it is necessary to treat the wastewater several times by PTS system or to use complementary methods for treatment.

In the present study, the exposure of zebrafish and daphnia to wastewater increased the mortality, with the highest values mostly at concentrations above 3% and 4-48 h exposure duration, while with the decrease in wastewater concentration, deaths occurred in longer times, mostly above 72 h. The lowest mortality was observed at 0.35% vinasse and stillage wastewaters. These results clearly show that by treating the wastewater using PTS, the survival of the target aquatic species can be increased, which strengthens the hypothesis of the possibility of using treated water for aquaculture.

Conclusion

In conclusion, the particle trap system improved BOD, COD, TSS, TN, and TP in wastewaters with performance of 97.44, 96.90, 88.43, 88.26, and 79.81% for vinasse and 96.77, 95.69, 90.15, 77.87 and 79.89% for stillage, respectively. In addition, pH increased by 40.28 and 33.33 in vinasse and stillage wastewaters, respectively. The exposure of the fish and daphnia to different concentrations of both types of wastewaters showed that the PTS system reduces the amount of waste materials/pollutants to below 3% in order to be suitable for aquaculture.

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