Journal of Basic and Environmental Sciences

ISSN
Online:2356-6388
Print:2536-9202

Research Paper Open Access

Wastewater Treatment by Biological Filtration Technique Improves Biochemical and Microbiological Parameters in Nile tilapia (*Oreochromis niloticus*)

Shireen Ashmawy[1,2], Mohamed O. Abdel-Monem[1], Salwa M. Abou El Ella[3], Elsayed E. Elsayed[2], Ghada E. Dawwam[1*]

[1]Botany and Microbiology Department, Faculty of Science, Benha,13518, Egypt.
[2]Central Laboratory for Environmental Quality Monitoring, National Water Research Center, ELkanatir 13621, Egypt.
[3]Regional Training Center for Water Resources and Irrigation.

Abstract:

Polluted water from drains outfalls in fish farms has a dangerous environmental effect. This study assesses the impact of the quality of treated wastewater by sand filter system in three different types of water as drainage water (DW), treated water (TWW), and River Nile water at El-Kanater El-Khyria (RNW) as control. The suitability of water quality for reuse in Nile tilapia farming has been examined. Based on bacteriological results, treated water has significantly reduced pathogenic bacterial diversity. The results of the fish examination indicated that changes in water quality variables have a significant impact on the blood profile of fish. The obtained data showed poor water quality in DW compared with TWW and RNW. Fish from DW had high levels of amino-aspartate activity transferase (AST) and alanine aminotransferase (ALT), increased values of creatine and urea, and decreased antioxidant enzyme activity compared to control. Thus, it could be concluded that the treatment of drainage water using a sand filter is efficient in producing high water quality for fish farming.

Keywords: Nile tilapia (*Oreochromis niloticus)*; Sand filter, microbiological parameters, biochemical parameters.

1. Introduction

Water is a vital and fateful natural resource for human beings' survival and sustenance; it is also a fundamental resource for agricultural and economic activities **(Elbahnasawy et al., 2021 and Elkorashey, 2022)**. However, population growth and subsequent economic and urban evolution have increased the demand for water throughout many countries around the world. In addition, climate change, land degradation, unregulated water withdrawal, and agricultural, and industrial pollution, have contributed to water scarcity and deterioration. The use of low-quality water leads to various environmental impacts **(Fattah and Helmy, 2015; Azzam et al., 2017)**.

Fish production is high and about 30% of Egypt's total catch once supplied. These aquatic systems receive many pollutants, such as ammonia, anions, cations, and heavy metals **(Raslan et al., 2020)** due to wastewater from the industrial activities in the region including metal, food processing, detergents and soaps manufacturing, and textile and paper production are discharged into the drain. In Egypt, Nile tilapia (*Oreochromis niloticus*) is a staple cuisine that is high in protein. For the impoverished and middle class, it is the most affordable and plentiful source of protein. The quality of the water used for tilapia farming determines the species' viability **(Ibrahim et al., 2013)**. Supplemental feeding is crucial for the feeding system to function well **(El-Sayed, 1999)**. In addition, enough supply of water with the right physiochemical quality is necessary for aquatic systems to succeed.

Wastewater treatment is important to our health as well as to keep the environment healthy and clean. The lack of proper treatment of wastewater will cause significant damage to the ecosystems when the treated wastewater is recharged to the environment. Slow sand filtration (SSF) is a cost-effective and less energy-intensive technology **(Visscher et al., 1987)**. With lower dependency on chemicals and skilled labor **(Truesdale et al., 1964)**, SSF appears as a sustainable wastewater treatment process. It integrates physical, chemical, and biological processes for contaminant removal from water **(WHO, 2011)**, characteristics of source water, temperature, filtration rate, surface ripening **(Amy et al., 2006)**, hydraulic retention time, and surface area of the biofilm are important factors for designing and evaluating the performance of SSF. The efficiency of the filter is determined by the grain size, while media depth impacts the treatment.

Filtration duration and sand type also affect the pathogen removal. Microorganisms are removed more efficiently when filters are operated in series mode. Also, air-saturated flow increases the pathogen removal efficiency by enhancing the air-water interface throughout the column **(Mahmood et al.,2011& Palmateer et al.,1999)**.

The purpose of drainage and wastewater treatment is to remove solids (suspended,

colloidal and floated), biodegradable organic matters, nutrients and elimination of pathogenic microorganisms. It is important to reuse both drainage and treated wastewater in order to blocking the gap in water needs **(Aboulfotoh , 2021)**.

The aim of this study is to explore the efficiency of a slow sand filter in purifying wastewater and determine different physical, chemical, and microbiological parameters on collected water samples before and after the filtration process. These parameters guard the percent efficiency of the used filter on the aquaculture system.

2. Materials and methods

2.1. Collected water samples

Three different water sources were used in the current study. The first water source is River Nile water (RNW) which is fresh water (control) and obtained from El-Kanater El-Khyria, Qalubia, Egypt (30° 18' 47.88" N: 31° 19' 17.04" E). The second water source is drainage water (DW) from Bahr Elbakr drain, Port Said Governorate, Egypt. The third source is the treated wastewater (TWW) using sand filters in fish farming.

Water samples pass through a system that composed of coarse and fine sand (filtration sand) occupied in the channel (20 m in length and 1 m in width). Before the treatment channel, there is a pond square-like shape (2m in length) as a sedimentation pond.

2.2. water sampling

The drainage water (DW) was collected from drainage wastewater. During the experiment water samples were collected from each basin from the three different sources of water. TWW was collected from the storage reservoir and transferred to the laboratory, stocked in the experiment basin. Surface water samples were gathered in polyethylene (PE) bottles, while random fish samples from each lake were collected in aerated doubled PE bags. Water samples were kept in ice boxes at 4 ± 0.4 ^{0}C before being immediately transferred to the laboratory for analysis. Feed was applied four times a day during the study.100 g of commercial dry food was applied for each basin per day. Feeding was gradually increased from week 6 and onwards by 1% per week, until reaching 2% BW/day by week 13.

2.3. Fish sampling

Fifty Tilapia fingerlings weighing 10 ± 5 g were obtained from the Elabasa fish farm, Abu Hammad, Sharqia Governorate, Egypt (N 30° 34' 2.1684", E 32° 2' 48.3792"). They were maintained in a 150 L acclimation tank for 10 days. The acclimation tank and experimental aquaria were aerated and maintained in a temperature-controlled room at 24± 1.5˚C. On day 10, a fish weight of 35 ± 10 g was stocked into the experimental basin1000 L aquaria at a density of 50 fish per aquarium, for 180 days (6 months).Ten fish of Nile tilapia (*Oreochromis niloticus*) were caught at the end of the period

from each basin ranging between 200 ± 20 g in weight and 20 cm in length. Fish were collected using gill nets with the help of professional local fishermen in each location.

2.4. Biochemical parameters

Fish blood plasma was spun for 15 minutes at 3500 RPM in tiny plastic vials containing whole blood and EDTA as an anticoagulant. Alanine aminotransferase (ALT) and aspartate activity transferase (AST), hematocrit parameters, and the amounts of creatine **(Tietz, 1986)** and urea **(Patton and Crouch, 1977)** were all estimated using plasma samples.

Within 35 to 40 seconds, the remaining blood samples were assembled in 1-mL disposable heparinized syringes from the fish caudal vein. Until every blood parameter was properly examined, the blood samples were stored at 4 ˚C. Instantaneous values were estimated for the hemoglobin (Hb) concentration, platelet count (PLT), hematocrit (HCT), hemoglobin (RBC) total count, and white blood cell (WBC) total count. Once diluted using Hendrick's dilution solution, total RBCs and WBCs were counted using an optical microscope and a hemocytometer (Germany). The cyan-methemoglobin approach (Asan Pharm. Co., ltd.) was utilized to determine the concentration of hemoglobin. For determining the HCT value, the microhematocrit centrifugation method was used. Erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were computed using equations (1), (2), and 3.

$$\text{MCV(f1)}= \frac{HCT(\%)X\ 10}{RBC(\frac{106}{\mu L})} \quad (1)$$

$$\text{MCH (µµg)} = \frac{Hb\left(\frac{g}{dL}\right)x10}{RBC(\frac{106}{\mu L})} \quad (2)$$

$$\text{MCHC (\%)} = \frac{Hb\left(\frac{g}{dL}\right)x\ 100}{HCT\ (\%)} \quad (3)$$

After centrifuging the leftover blood, plasma was separated. After hemolyzing the erythrocytes, they were centrifuged at 4 oC. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities of the antioxidant enzymes in the supernatant aliquots were evaluated at 25 ^{o}C using a Biochrom Libra S32 Spectrophotometer.

2.5. Microbiological analysis

All water samples were examined according to Water and Wastewater Examination Standards **(APHA and AWWA, 2017)** within 6 hours after collection. The standard plate count (SPC) of bacteria was determined by applying the pour plate method at 22°C and 37°C. Fecal coliforms (FC), Total coliforms (TC), and fecal streptococci (FS) counts were carried out using M-FC agar, M-Endo agar LES, and M-Enterococcus agar media, respectively as a dehydrated form (Difco-USA). All water samples used for bacteriological analysis were using sterile surface gridded Sartorius'' membrane filter (pore size, 0.45µm, and diameter, 47mm) coupled with stainless steel autoclavable manifold and oil-free ''Millipore'' vacuum/pressure pump. Results were recorded

as colony forming unit (CFU $100mL^{-1}$) using the following equation:

CFU⁄100mL=CFU×100ml of filtered sample

2.5.1. Isolation and Identification of Aquatic Bacteria

Some aquatic bacteria that have a potentially significant health risk to humans were given prime concern for investigation as follows **(APHA and AWWA ,2017)**:

A. *Escherichia Coli:*

E. coli detection was done according to standard method No. 9213 D using m-TEC agar medium. After incubation at 44.5°C ± 0.2°C for 24h, yellow or yellow-brown colonies are developed. These colonies were confirmed by streaking on Eosin Methylene Blue agar plates showing pink growth with a golden metallic sheen.

B. ***Pseudomonas Aeruginosa:***

Standard method No. 9213 E was applied using M-PA-C agar medium. After incubation at 41.5 ± 0.5°C for 72 h, colonies (0.8 to 2.2mm in diameter) showing a flat appearance with light outer rims and brownish to greenish black centers were selected and isolated as *P. aeruginosa*. These colonies were confirmed by streaking on cetrimide agar plates, a selective medium that inhibits other bacterial growth and enhances fluorescein and pyocyanin "Blue green" pigment production.

C. *Staphylococcus aureus:*

According to standard method No. 9213 B, Baird-Parker agar medium was used. After incubation at 35°C for 48 h, black shiny colonies surrounded by a narrow clear zone were picked up, and confirmed by streaking on Mannitol salt agar plates to give golden yellow colonies.

D. *Enterococcus faecalis:*

Using M-Enterococcus agar medium (standard method No. 9230C), colonies showing red to pink color were isolated as presumptive ***E. faecalis.***

2.6. Statistical analysis

To perform better data interpretation, The mean ± standard deviation was calculated for each parameter of water and fish tissue collected from the three different water qualities. Analysis of variance (ANOVA – one way) was carried out according to IBM SPSS Statistics 28.0.1.0.

3. Results and discussion

3.1. Fish Biochemical Parameters

Fish health and immunological potential, illness, and other parameters are evaluated by haematological examination and chemical indicators as determined by **Docan et al. (2018); Fazio et al. (2019), and Witeska et al. (2022)**. *O. niloticus's* hematological parameters, including RBCs, WBCs, Hb, HCT, MCV, MCH, MCHC, Plat, ALT, AST, urea, creatine, SOD, CAT, and GPs, are displayed in **Table 1.** Fish blood parameters are not constant; they change. Due to the lack of reference ranges for the fish blood measures, the obtained fish blood data were interpreted by contrasting the data with fish from the Nile River and the sample sites.

Table 1. Fish blood parameters for a subset of the Nile River, DWW, and TWW examined samples, where n = 10 for each site.

Parameters Mean ± SD	El Kanater El-Khyria Control (RNW)	Drainage Waste water (DWW)	Treated Waste water (TWW)
WBCs ($x10^3$ cells/mm^3)	$178.6±6.9^b$	$239.28±12.9^a$	$182.11±4.24^b$
RED Blood Cells (RBCs) $x10^6$ cells/mm^3	$1.73±0.16^b$	$2.84±0.19^a$	$1.85±0.82^b$
Hemoglobin(Hb) g/dl	$5.14±0.16^b$	$12.61±0.56^a$	$5.51±0.21^b$
Hematocrit (Hct) %	$12.60±0.55^b$	$21.34±1.77^a$	$13.87±0.73^b$
Mean Corpuscular Volume (MCVμ^3)	$90.83±0.83^b$	$58.06±6.53^a$	$91.58±1.21^b$
Mean Corpuscular Hemoglobin (MCH) (pg)	$31.32±0.63^b$	$45.36±2.86^a$	$33.72±1.45^b$
Mean Corpuscular Hemoglobin Conc. (MCHC) g/dl	$23.20±1.32^b$	$61.52±2.91^a$	$26.16±1.93^b$
Platelets Count	$120.43±2.89^b$	$198.40±14.6^a$	$132.63±3.05^b$
ALT(µ/l)	$36.64±1.54^b$	$117.48±3.7^a$	$38.08±2.66^b$
AST(µ/l)	$12.68±1.38^b$	$42.48±1.94^a$	$13.20±1.05^b$
Urea (mg/dl)	$11.47±0.58^b$	$31.60±2.42^a$	$13.14±0.51^b$
Creatine (mg/dl)	$0.40±0.030^b$	$0.90±0.043^a$	$0.43±0.022^b$
SOD u/ml	$9.73±0.38^a$	$4.76±0.48^b$	$8.99±1.31^a$
CAT mu/ml	$22.18±0.97^a$	$12.22±1.47^b$	$20.72±1.25^a$
GPX u/ml	$63.97±2.15^a$	$41.31±3.11^b$	$60.83±0.29^a$

Mean values with different letters a, b, c, and d in the same row are significant ($P < 0.05$).

Fish blood taken from DWW samples had greater quantities of WBCs, RBCs, Hb, HCT, MCV, MCH, MCHC, Plat, ALT, AST, urea, and creatine, as **Table 1** illustrates. Elevated levels of creatine and urea indicate nephrotoxicity, whereas increased levels of AST and ALT indicate hepatotoxicity. According to **Svobodová et al. (1994); Mwita and Nyalusi (2015),** the process by which the fish body produces more hemoglobin to replace oxidized or denatured RBCs, Hb, HCT, MCV, MCH, MCHC, Plat, ALT, AST, urea, and creatine variables as a result of heavy metal exposure is the explanation for the increase in hematological parameters.

Abdelhamid et al. (2019) provided support for the WBCs results in fish taken from the Nile River for *O. niloticus*. In addition, **Weinert et al. (2015)** stated that the WBC count in fish taken from DWW in comparison to the control sample increased when compared to the Nile and that this is a strong indicator of stress brought on by toxicants in aquatic

environments. It is also a reliable predictor of pathological conditions in fish.

WBCs are primarily involved in the production and distribution of antibodies, the defense against infections, and the defense against external invaders **(Ajima et al., 2015)**. Chemicals directly interact with immune cell survival and proliferation to impact the immune system. This may lead to immunosuppression, a condition that compromises immunological competence and increases the risk of infections and malignancies **(Segner et al., 2022)**. The present study's rise in WBC count suggests that the immune system may have been triggered in response to tissue damage brought on by heavy metals and ammonia. This result was supported by **Ibrahim ElSayed (2023)** and **Elarabany et al., (2019).** Additionally, **Ates et al. (2007)** discovered that exposure to $Pb2^{+}$ and Cu^{2+} pollution resulted in a large increase in WBC, MCV, and MCH in fish blood, but a decrease in SOD. This proved that the large concentrations of white blood cells in drains indicate that chemical pollutants were acting as antigens and encouraging the growth of nonspecific defense cells in these locations. These results are comparable to those of **Ibrahim ElSayed (2023)** and **Pereira et al. (2011)**, who demonstrated that changes were found in fish exposed to pollutants through a biochemical and blood survey. These variations included an increase in WBCs in fish species exposed to polluted environments, as well as pathogens **(Harikrishnan et al., 2010).**

WBCs, RBCs, Hb, HCT, MCV, MCH, MCHC, Plat, ALT, AST, urea, and creatine all rose substantially in response to rising bicarbonate ions, TSS, turbidity, ammonia, TN, and BOD. Data demonstrated a substantial rise ($P < 0.01$) in the hematological parameters and chemical indices, including RBCs, HCT, MCH, MCHC, Plat, and creatine, when Pb2+ and nitrate levels were raised.

Compared to samples from TWW and the Nile River (control), the parameters of WBCs, RBCs, Hb, HCT, MCH, MCHC, Plat, urea, and creatine exhibited considerable change in DWW. MCV, ALT, and AST were identified in high amounts in the following sequence: The Nile River (control) > DWW > TWW, and they demonstrated considerable variance in DWW and TWW when compared to the control. In comparison to the Nile River (control) samples, the antioxidant parameters SOD, CAT, and GPs demonstrated non-significant variance in TWW but substantial variation in DWW and TWW.

Fish are shielded by the antioxidant activity from free radicals produced by elevated heavy metal concentrations **(Kadar et al., 2005)**, which causes a drop in blood levels and a subsequent rise in pollution and mortality. Thus, the rise in ammonia and all the variables studied in the water, together with the fall in DO levels, caused the enzymatic activity to

drop and the hematological parameters and chemical indices to rise.

3.2. Bacteriological analysis:

Bacterial standard plate count (SPC):

Bacteriological characteristics are still the primary issue in any water quality assessment program, especially those used for irrigation and agricultural purposes. Standard plate count (SPC) was used to indicate the total number of bacteria and the microbial status of water. Results presented in **Table 2** showed obvious detectable differences in SPC levels among different sites. SPC at RNW and TWW showed minimum value (**$82x10^3$** and **$267x10^3$)** cfu ml^{-1} at 22°C and (**$29x10^3$** and **$90x10^3$**cfu ml^{-1}) at 35°C respectively. On the other hand, SPC along DWW ranged between **$400x10^4$** cfu ml^{-1} at 22°C and **$150x10^4$**cfu ml^{-1} at 35°C. **Azzam & Ibrahim (2021)** showed that SPC count is useful to evaluate the efficiency of treatment processes as well as monitor the bacterial re-growth potential and biofilm development within the wastewater treatment plants.

Table 2. Bacteriological parameters of water samples at various sampling sites.

Parameters	Sites			Guidelines***
	El- Kanater El-Khyria Control (RNW)	**Drainage water (DW)**	**Treated water (TWW)**	
SPC** (22°C) (cfuml^{-1})	**$82x10^3$**	**$400x10^4$**	**$267x10^3$**	**** -
SPC** (35°C) (cfuml^{-1})	**$29x10^3$**	**$150x10^4$**	**$90x10^3$**	-
TC** (35°C) (cfu100ml^{-1})	**$17x10^2$**	**$302x10^3$**	**$108x10^2$**	Not to exceed 5000 Tebbutt (1998)
FC** (44.5°C) (cfu100ml^{-1})	**$5x10^2$**	**$116x10^3$**	**$45x10^2$**	Not to exceed 2000 (1998) Tebbutt
FS** (35°C) (cfu100ml^{-1})	**240**	**$79x10^3$**	**$13x10^2$**	Not to exceed 35 (APHA, 2017)
E. coli**(44^0C) (cfu100ml^{-1})	**320**	**$109x10^3$**	**$28x10^2$**	**** -
PS(41^0C) (cfu100ml^{-1})	**10**	**$33x10^2$**	**$16x10^2$**	**** -
S.aureus	**25**	**$21x10^2$**	**$9x10^2$**	**** -

** SPC: standard plate count; TC: total coliforms; FC: fecal coliforms; FS: fecal streptococci. *Escherichia coli; Pseudomonas aeruginosa; Staphylococcus aureus*

*** Guidelines: Restricted limits according to Tebbutt (1998) and American Public Health Association (APHA, 2017).

**** - : No available guidelines.

Total coliforms (TC):

Total coliforms are commonly used as bacterial indicators of the sanitary quality of water since they belong to family Enterobacteriaceae. TC count in the area of study depending on site location from pollution sources. **Table 2** showed that a maximum TC of **$302x10^3$**cfu 100 ml^{-1} at DWW and a minimum TC of **$17x10^2$ and $108x10^2$** cfu 100 ml^{-1} **at RNW and TWW respectivly** . It is worth to mention that, all monitored points exceeded than the international standard limits (5000 cfu 100ml^{-1}) recommended by **Tebbutt (1998)**. Much more restricted limits have been reported by **Cabelli (1978)**, who recommended a maximum total coliforms count of 1000 cfu 100ml^{-1}.

Fecal coliforms (FC):

Fecal pollution is a major issue for surface water, drains outfalls, and rivers all over the world. Human fecal material is generally considered a great risk to human health, as it is more likely to contain human enteric pathogens **(Bhadra et al.,2003)**. Throughout this study, collected samples were contaminated with highly undesirable levels of fecal coliforms (FC). Data presented in **Table 2** showed that FC count in our area of study depending on site location from pollution sources around where a maximum of **$116x10^3$** cfu 100ml^{-1} at DWW and a minimum of **$5x10^2$** and **$45x10^2$**cfu 100ml^{-1} at RNW ,TWW respectively. According to previous results, it seems that all monitored points exceeded the international standard limits of **Tebbutt (1998)**, (FC count did not exceed 2000 cfu 100 ml^{-1}). Restricted limits (200cfu 100 ml^{-1}) for surface water intended for use as drinking water supply indicate unsafe water from a bacteriological point of view **Cabelli (1978)**. **El-Dougdoug et al. (2020)** recorded variations of FC count ranging between 112-275x103 CFU100 mL^{-1} in five drain outlets at Giza Governorate, Egypt during 2018-2019.

Fecal streptococci (FS):

Fecal streptococci comprise bacteria that are normally present in feces and gut of warm-blooded animals. The ratio FC/FS has been suggested in several reports as a method for tracing whether fecal pollution is from human or animal sources. A ratio greater than 4 indicates human fecal contamination, whereas a ratio of less than 0.7 suggests contamination by non-human sources. However, some investigators have questioned the usefulness of this ratio since it is valid only for recent (24 h) fecal pollution and FS count should not be less than 100 cfu 100 ml^{-1} **(APHA and AWWA, 2017; Mishra et al., 2018)**. As given from the data in **Table 2**, the FS counts in our area of study fluctuated around a maximum of **$79x10^3$**cfu100 ml^{-1} at DWW and a minimum of **240 and $13x10^2$** cfu100 ml^{-1} at RNA and TWW respectively, All recorded values in all locations were exceeding the standard limits (33–35 cfu 100 ml^{-1}) as given by **APHA and AWWA (2017**). **El-Hamid et al. (2021)** found a fluctuated range of FS (100 - $550x10^3$ CFU100mL^{-1}) for outfalls of River Nile drains in Egypt.

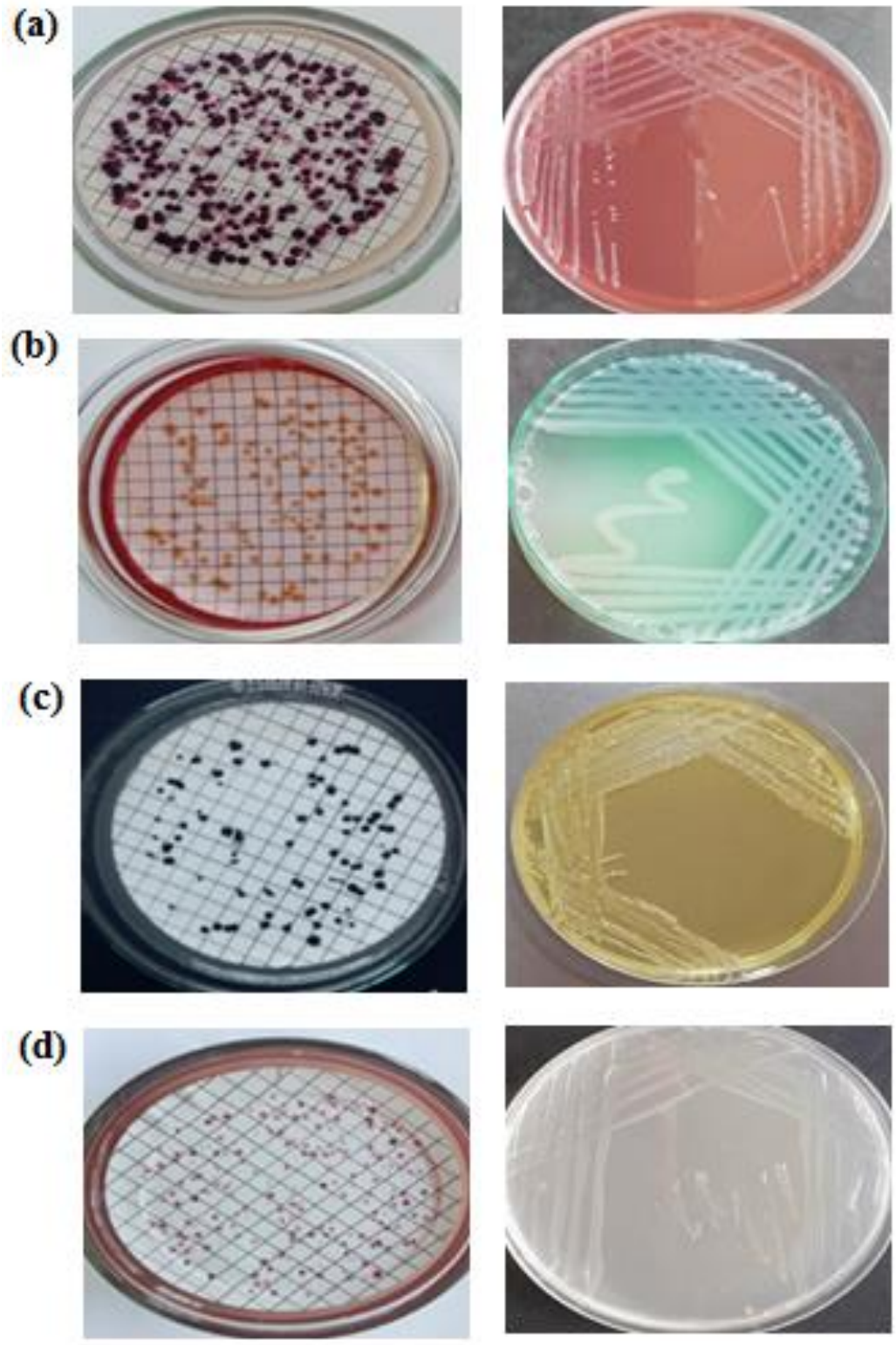

Figure 1: Growth of isolated and identified bacterial isolates on specific cultural media.

- ***a.*** *E. coli* colonies on (modified mTEC) and MacConkey agar medium, respectively.
- ***b.*** *P. aeruginosa* colonies on M-PA-C and cetrimide agar medium, respectively.
- ***c.*** *S. aureus* colonies on Baird-parker and Mannitol salt agar medium, respectively.
- ***d.*** *E. faecalis* Colonies on M-Enterococcus and nutrient agar medium, respectively.

The most widespread bacteria obtained were *E. coli* and *E. faecalis*, which indicates that the water is subjected mainly to sewage pollution **(Figure 1)**. The high incidence of *E. coli* correlated with fecal coliforms supports such findings (**Henry et al.,2016)**. On the other hand, the presence of *P. aeruginosa* and *S. aureus* in considerable densities in water resources is a matter of concern since these organisms cause a wide range of infections including skin, urinary, and respiratory tract infections. Body contact increases the chance of infections through nose, mouth, ears and cuts in the skin (**Tsoraeva and Martinez, 2000)**.

4. Conclusion

In conclusion, DWW water was the highest polluted and had the maximum levels of bacteriological characteristics, thus this was an increase in health risks to human health and aquatic living organisms. There was also an increase in the hematological parameters of WBCs, RBCs, Hb, HCT, MCV, MCH, MCHC, Plat, ALT, AST, urea, and creatine. As contamination increased in the waters, the levels of the antioxidants SOD, CAT, and GPx in the blood samples of the fish decreased. As a result, wastewater must be treated before use in fish farming. So it could be concluded that the treatment of drainage water using a sand filter is efficient in producing high water quality for fish farming to remove organic materials in water samples and fish tissue.

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