



Microbial Population Dynamics of Paracetamol and Ibuprofen Impacted Effluent

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Abstract

Pharmaceutical contaminants such as paracetamol and ibuprofen are emerging environmental pollutants of concern due to their persistence in aquatic environments and potential to disrupt microbial communities. This study examined the microbial population dynamics in four pharmaceutical effluents impacted by these drugs. The microbial populations enumerated were total culturable heterotrophic bacterial counts (TCHBC), total culturable fungal counts (TCFC), total culturable paracetamol utilizing bacterial counts (TCPUBC), total culturable Ibuprofen utilizing bacterial counts (TCIUBC), and total culturable paracetamol and ibuprofen utilizing bacterial counts (TCPIUBC). Results revealed that the TCHBC ranged from $7.9 \times 10^6 \pm 2.824$ CfU/ml – $2.1 \times 10^8 \pm 1.333$ CfU/ml, TCFC ranged from $3.2 \times 10^6 \pm 1.331$ CfU/ml – $2.17 \times 10^7 \pm 2.663$ CfU/ml, TCPUBC ranged from $1.4 \times 10^7 \pm 2.306$ CfU/ml – $8.0 \times 10^8 \pm 1.331$ CfU/ml, TCIUBC ranged from $2.25 \times 10^6 \pm 1.331$ CfU/ml – $1.7 \times 10^8 \pm 2.306$ CfU/ml while TCPIUBC ranged from $1.7 \times 10^7 \pm 1.331$ CfU/ml – $1.0 \times 10^8 \pm 2.303$ CfU/ml. The elevated abundance of pharmaceutical-utilizing bacteria suggests adaptive responses within the microbial community, enabling the metabolism of paracetamol and ibuprofen as carbon sources. Such adaptation highlights the potential role of indigenous microbial populations in the natural attenuation of pharmaceutical contaminants. These findings underscore the ecological significance of microbial community dynamics in contaminated effluents and support the development of enriched bacterial consortia for targeted bioremediation in pharmaceutical wastewater treatment systems.

Keywords: Pharmaceuticals, Effluents, Paracetamol, Ibuprofen, Population Dynamics

Introduction

Pharmaceutical compounds—including widely used analgesics such as paracetamol (acetaminophen) and ibuprofen—are increasingly recognized as environmental contaminants of emerging concern, owing to their extensive use and persistence in aquatic ecosystems (Kümmerer, 2009; aus der Beek et al., 2016). These medications are frequently detected in surface waters, groundwater, and even treated drinking water, often at low concentrations (ng/L to µg/L), yet their ecological impact can be disproportionate due to bioaccumulation and chronic exposure effects (Patel et al., 2019; Al Aukidy et al., 2012). Their entry into the environment arises not only from excretion and improper disposal but also from pharmaceutical manufacturing and hospital effluents (Larsson, 2014; Verlicchi & Zambello, 2015). The analgesic paracetamol (acetaminophen) and the nonsteroidal anti-inflammatory ibuprofen are among the most commonly used drugs, and these compounds often evade full removal in conventional wastewater treatment plants, leading to their presence in effluents entering natural water bodies. Microbial communities in effluent-impacted waters may adapt to these anthropogenic pressures. Certain bacteria and fungi have demonstrated the ability to utilize paracetamol and ibuprofen as carbon sources, indicating an intrinsic potential for natural attenuation and bioremediation. For instance, specialized strains such as *Bacillus thuringiensis* and *Pseudomonas moorei* have been successfully deployed in defined bacterial consortia to decompose non-steroidal anti-inflammatory drugs (NSAIDs) including paracetamol and ibuprofen in wastewater treatment systems (Žur et al., 2018a; Žur et al., 2023a). Similarly, white-rot fungi, known for their ligninolytic enzyme systems, have shown promising capacity to degrade these persistent pharmaceuticals in aquatic environments (Tran et al., 2023a).

Emerging research continues to uncover novel microbial taxa with enhanced biodegradation capabilities. Recent investigations have highlighted *Bacillus licheniformis* PPY-2 and diverse environmental isolates capable of breaking down paracetamol, while genera such as *Variovorax*, *Rhizobium*, and *Paraburkholderia* have also shown

potential for pharmaceutical biodegradation in different settings (Priya et al., 2023a; González-García et al., 2022a). These findings collectively underscore the adaptive resilience of microbial communities to pharmaceutical pollution and suggest the feasibility of harnessing indigenous populations in effluent treatment. Against this background, this study examines the microbial population dynamics in pharmaceutical effluents impacted by paracetamol and ibuprofen, focusing on both general microbial abundance and the capacity of microbes to utilize these analgesics.

Aim

To investigate the microbial population dynamics in pharmaceutical effluents impacted by paracetamol and ibuprofen, with emphasis on microbial abundance, and the capacity of indigenous microorganisms to utilize these pharmaceuticals.

Objectives

1. To determine the total culturable heterotrophic bacterial and fungal counts in effluents impacted by paracetamol and ibuprofen.
2. To isolate and enumerate analgesic-utilizing bacteria capable of metabolizing paracetamol and ibuprofen as carbon sources.

Materials and Methods

Study Area

Pharmaceutical effluents were collected from the point of discharge of four pharmaceutical manufacturing companies located in south East Geopolitical zone of Nigeria. The individual coordinates were determined using the global positioning system (GPS). The coordinates of the sampling sites are as follows. Sample **A** (5° 28' 35.6''N, 7° 04' 54.3'' E), Sample **B** (5° 26' 30.7''N, 7° 02' 38.0''E), Sample **C** (6° 09' 54.7''N, 6° 50' 23.7''E) and

sample **D** (6° 07' 57.0''N 6° 46' 21.9''E).

Sample collection

Pharmaceutical effluent samples were collected into sterile wide mouthed sample glass bottle with screw caps from the point of discharge after the production of paracetamol and ibuprofen using standard procedures. Samples were placed in an ice chest and transported immediately to the laboratory for analysis.

Chemicals and cultivating medium

The standard paracetamol and ibuprofen were purchased from May and Bakers pharmaceutical Nigeria PLC, and Ranbaxy Nigeria limited respectively at 98% purity. All other chemicals were of highest purity commercially available. Other culture media includes Nutrient Agar, Bushnell Haas Agar, Sabouraud Dextrose Agar were all prepared according to manufacturer's instruction. All glassware and instruments used in the preparation were sterilized appropriately before use.

Serial dilution

1ml of the effluent samples, where appropriate, were measured and introduced into 9 ml sterile normal saline as a diluent (0.85 % NaCl w/v in distilled water) in glass test tubes. The samples were corked and swirled to mix properly. This represented 10^{-1} dilution. Using a new pipette in each case, 1ml of the homogenate was transferred into another test tube containing 9ml sterile diluents to make 10^{-2} dilutions. The procedure was repeated to achieve the dilution of up to 10^{-6} for each of the samples analyzed.

Preparation of Nutrient Agar

Nutrient agar was prepared using the standard formulation as described in the guidelines of the American Public Health Association (APHA, 2015). 28grams of the agar medium was accurately weighed using an electronic balance and transferred into a beaker containing approximately 900 mL of distilled water. The mixture was stirred using a magnetic stirrer until all components were completely dissolved. The final volume was then made up to 1000 mL with distilled water and autoclaved at 121°C for 15 minutes under 15 psi pressure to ensure sterilization.

Preparation of Bushnell-Haas Agar Medium

Bushnell-Haas agar was prepared according to the standard formulation described by Bushnell and Haas (1941) with slight modifications. The pH of the medium was adjusted to 7.0 ± 0.2 using 1N sodium hydroxide (NaOH). The final volume was then made up to 1000 mL with distilled water. The medium was sterilized by autoclaving at 121°C for 15 minutes at a pressure of 15 psi. After autoclaving, the medium was allowed to cool to approximately 50°C and then poured into sterile Petri dishes and allowed to solidify.

Preparation of Sabouraud Dextrose Agar (SDA) Supplemented with Chloramphenicol

Sabouraud Dextrose Agar (SDA) was prepared according to the manufacturer's instructions and standard microbiological guidelines (APHA, 2017). 56 g of commercially prepared SDA powder was weighed and suspended in 1000 mL of distilled water in a conical flask. The mixture was stirred thoroughly until completely dissolved. The prepared medium was then sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure. After sterilization, the medium was allowed to cool to approximately 45–50°C. A solution of chloramphenicol was prepared by dissolving 0.5 g of chloramphenicol powder in 10 mL of 70% ethanol. This solution was added aseptically to the cooled medium poured into sterile Petri dishes.

Microbial population and enumeration studies

Total Culturable Heterotrophic Bacterial Counts (TCHBC)

The total culturable heterotrophic bacterial count was determined by spread plate technique on nutrient agar (Accumedia, Sweden). Tenfold serial dilutions (10^{-3} – 10^{-6}) of the effluent samples were prepared in sterile saline (Chikere & Ekwuabu, 2014). 0.1 mL of the dilutions were inoculated onto nutrient agar plates, and plates incubated at 37°C for 24 h, and colonies within 30–300 (APHA, 2005) were counted to estimate total viable cells in CFU/mL.

Total culturable fungal counts (TCFC)

The total culturable fungal counts of the individual samples were enumerated on Sabouraud dextrose agar (Accumedia, Sweden) medium supplemented with 0.1% w/v chloramphenicol. Ten-fold serial dilutions of the effluent samples were made by transferring 1 mL of the effluent sample into a 9 mL of sterile saline solution in a test tube and shaken vigorously. 0.1 mL of the dilutions were inoculated onto SDA plate. The inoculated plates were then incubated at 30°C for 48 h for TCFC.

Total culturable Ibuprofen utilizing bacterial counts (TCIPUBC)

The total culturable ibuprofen utilizing bacterial counts were enumerated using Bushnell Haas Agar incorporated 2000 mg of ibuprofen amended with 0.01 % w/v nystatin in 1 liter. This was serially diluted from 10^{-1} – 10^{-5} and 0.1 mL of the dilutions plated on the Bushnell Haas agar medium and incubated at 37°C for 24 h, and colonies within 30–300 (APHA, 2005) were counted to estimate total viable cells in CFU/mL.

Total culturable paracetamol utilizing bacterial counts (TCIPUBC)

The total culturable ibuprofen utilizing bacterial counts were enumerated using Bushnell Haas Agar incorporated 2000 mg of paracetamol amended with 0.01 % w/v nystatin in 1 liter. This was serially diluted from 10^{-1} – 10^{-5} and 0.1 mL of the dilutions plated on the Bushnell Haas agar medium and incubated at 37°C for 24 h, and colonies within 30–300 (APHA, 2005) were counted to estimate total viable cells in CFU/mL.

Total culturable Ibuprofen and Paracetamol utilizing bacterial counts (TCIPUBC)

The total culturable paracetamol and ibuprofen utilizing bacterial count were enumerated using Bushnell Haas Agar incorporated 2000 mg of paracetamol and 2000 mg of ibuprofen amended with 0.01 % w/v nystatin in 1 liter. This was serially diluted from 10^{-1} – 10^{-5} and 0.1 mL of the dilutions plated on the Bushnell Haas agar medium. The inoculated plates were then incubated at 30°C for 24 hours. The resulting viable discrete colonies were enumerated.

Results

The total culturable heterotrophic bacterial counts of the individual effluent samples revealed that Sample B has the highest total culturable bacterial count (TCHBC) of 2.10×10^8 cfu/mL, followed by sample A 1.23×10^8 cfu/mL, Sample C 2.9×10^7 cfu/mL and Sample D is the least at 7.9×10^6 cfu/mL. This is presented in Figure 1.

The total culturable fungal counts of the individual samples revealed that sample C has the highest fungal count of $2.1 \times 10^7 \pm 2.663$ cfu/mL, followed by sample A $4.5 \times 10^6 \pm 2.306$ cfu/mL, and sample B $4.2 \times 10^6 \pm 3.523$ cfu/mL, while the least was recorded in sample D $3.2 \times 10^6 \pm 1.331$ cfu/mL. This is presented in Figure 2.

The total culturable paracetamol utilizing bacterial count of the individual samples revealed that that Sample B with $8.0 \times 10^8 \pm 1.331 \text{ cfu/mL}$ has the highest count followed by sample A $7.2 \times 10^7 \pm 1.331 \text{ cfu/mL}$, sample D $2.25 \times 10^7 \pm 1.331 \text{ cfu/mL}$ while sample C recorded the lowest count of $1.4 \times 10^7 \pm 2.306 \text{ cfu/mL}$. This is presented in Figure 3.

The total culturable ibuprofen utilizing bacterial count revealed that Sample A with a total count of $1.7 \times 10^8 \pm 2.306 \text{ cfu/mL}$ has the highest IUBC followed by Sample B $1.05 \times 10^8 \pm 2.303 \text{ cfu/mL}$, Sample C $9.3 \times 10^6 \pm 2.663 \text{ cfu/mL}$ and Sample D $2.25 \times 10^6 \pm 1.331 \text{ cfu/mL}$. This is presented in Figure 4.

The total culturable paracetamol and ibuprofen utilizing bacterial counts (TCPIUBC) of the individual samples revealed a TCPIUBC of $1.1 \times 10^8 \pm 1.331 \text{ cfu/mL}$ for Sample A, $1.0 \times 10^8 \pm 2.303 \text{ cfu/mL}$, for Sample B, $1.12 \times 10^7 \pm 2.303 \text{ cfu/mL}$ for sample C and $1.7 \times 10^7 \pm 1.331 \text{ cfu/mL}$ for sample D. This is presented in Figure 5.

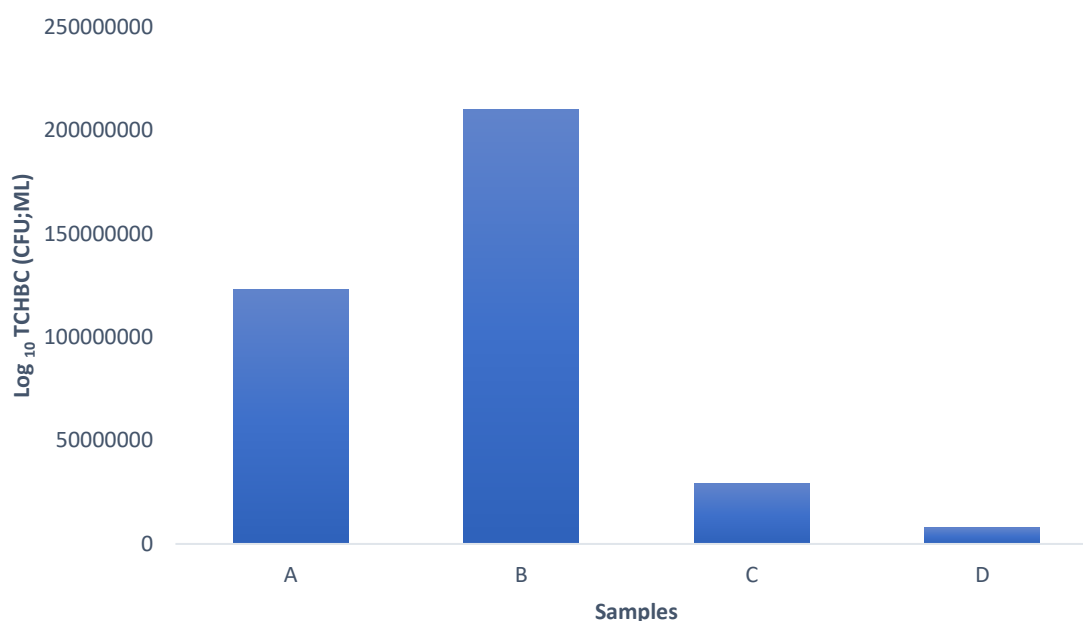


Figure 1: Total culturable heterotrophic bacterial counts.

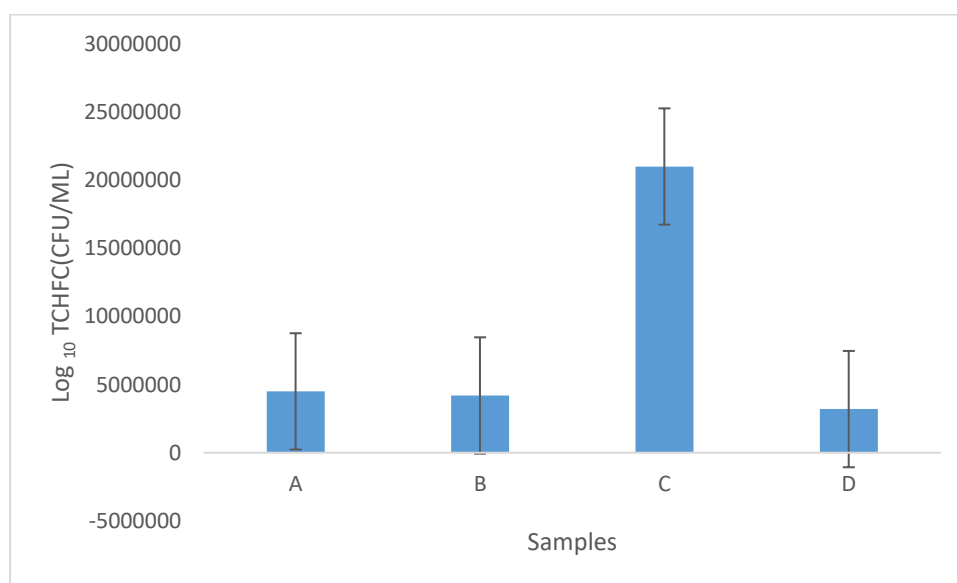
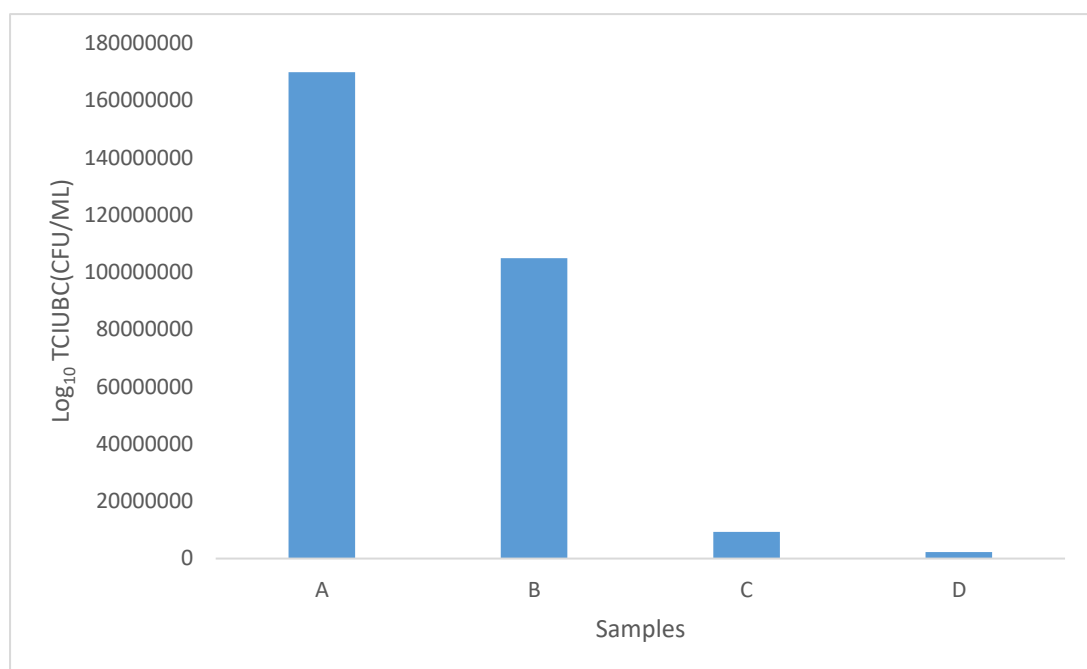
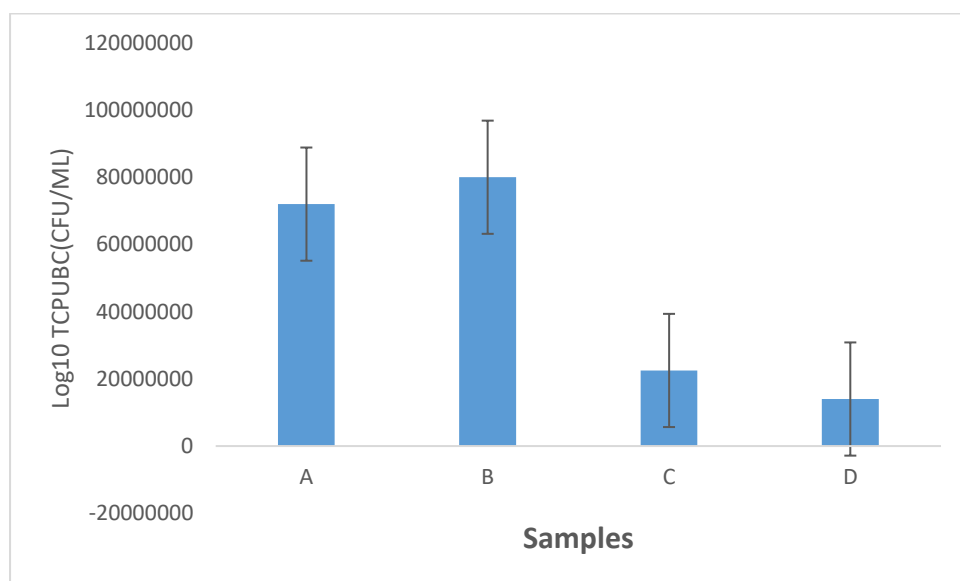


Figure 2: Total culturable fungal counts.**Figure 3:** Total culturable ibuprofen-utilizing bacterial counts**Figure 4:** Total culturable paracetamol-utilizing bacterial counts.

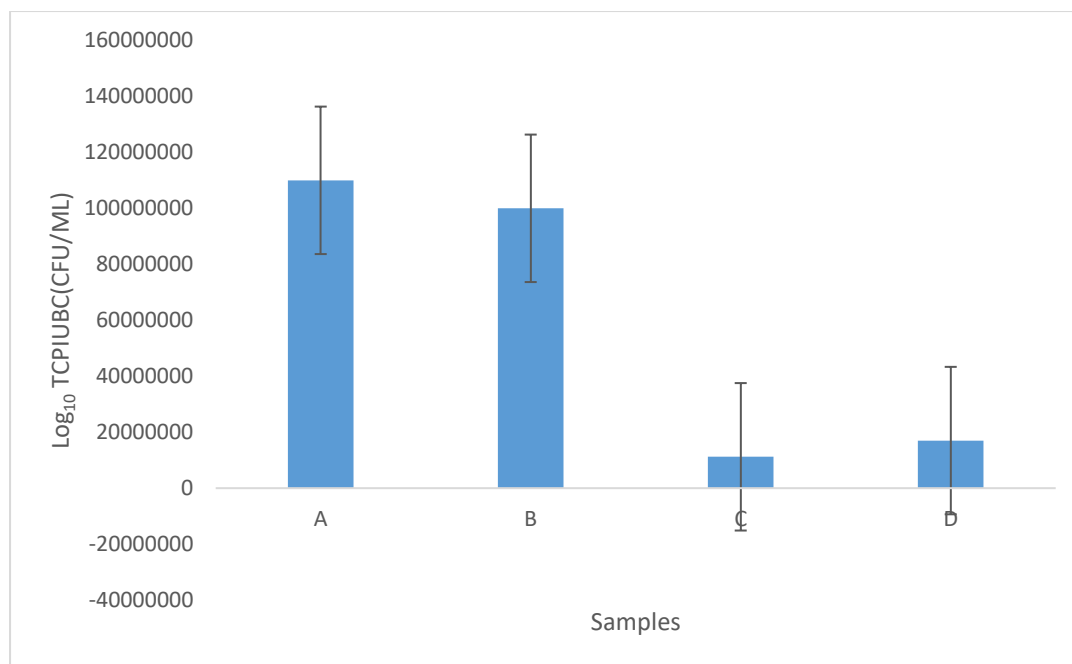


Figure 5: Total culturable paracetamol and ibuprofen utilizing bacterial counts.

Discussion

The microbial population dynamics observed in the pharmaceutical effluents examined in this study indicate a high level of microbial adaptation to pharmaceutical contamination. The total culturable heterotrophic bacterial counts (TCHBC) ranged from $7.9 \times 10^6 \pm 2.824$ CFU/mL to $2.1 \times 10^8 \pm 1.333$ CFU/mL, while total culturable fungal counts (TCFC) varied between $3.2 \times 10^6 \pm 1.331$ CFU/mL and $2.17 \times 10^7 \pm 2.663$ CFU/mL. The high counts of total culturable heterotrophic bacteria (TCHBC) and fungi (TCFC) across the four effluent samples indicate that pharmaceutical effluents can sustain dense and active microbial populations despite the presence of biologically active contaminants. These elevated heterotrophic counts are consistent with other effluent and wastewater studies showing that complex effluent matrices (organic matter, nutrients, and trace pharmaceuticals) often support large microbial biomass even when selective pressures alter community composition. (Ferreira et al., 2023). These elevated counts suggest that pharmaceutical effluents are rich in organic and nutrient loads, creating conditions favorable for microbial proliferation. Similar high heterotrophic counts have been reported in pharmaceutical effluents in Lagos and Ogun States, Nigeria (Obayiwana & Ibekwe, 2020; Obasi et al., 2019). The presence of abundant fungal populations further supports the notion that both bacteria and fungi play key roles in the degradation and transformation of xenobiotics in contaminated environments. The relatively high fungal counts observed is of high significance. Fungi, particularly white-rot species, produce ligninolytic enzymes such as laccases and peroxidases that are effective in degrading recalcitrant pharmaceuticals (Tran et al., 2023a). Their presence in the effluents suggests a potential natural attenuation pathway that complements bacterial degradation.

The high counts of paracetamol-utilizing bacteria (TCPUBC, 1.4×10^7 – 8.0×10^8 CFU/mL) and ibuprofen-utilizing bacteria (TCIUBC, 2.25×10^6 – 1.7×10^8 CFU/mL) demonstrate selection, enrichment and the ability of indigenous microbial communities to metabolize these pharmaceuticals as carbon sources. Enrichment of drug-utilizing bacteria in contaminated waters sometimes to levels higher than background heterotrophs has been reported following chronic exposure, consistent with adaptive responses (e.g., bioaugmentation and reactor studies showing enrichment of paracetamol- and ibuprofen-degraders). This pattern reflects ecological selection (substrate-driven niche expansion) and has implications for natural attenuation and engineered bioremediation. (Lara-moreno et al., 2024) The elevated TCPUBC compared to TCIUBC suggests that paracetamol may be more readily degraded than ibuprofen, a trend consistent with earlier studies showing faster microbial degradation of paracetamol due to its simpler aromatic structure (Žur et al., 2018b; Priya et al., 2023b). In contrast, ibuprofen, with its more complex and hydrophobic structure, is often less bioavailable and more resistant to microbial breakdown (Tran et al., 2023b). The presence of microbial populations capable of utilizing both drugs simultaneously (TCPIUBC, 1.7×10^7 – 1.0×10^8 CFU/mL) is of particular ecological significance, as it indicates metabolic versatility and possible synergistic degradation pathways within bacterial consortia. Enrichment

of paracetamol- and ibuprofen-utilizing bacteria follows two linked processes: (1) genetic/enzymatic capability to transform the compound (for APAP, amidases; for ibuprofen, oxygenases/hydroxylases), and (2) ecological selection where organisms with these capabilities outcompete non-degraders when the drug is present at usable concentrations (Rios-Miguel et al., 2022). Metagenomic surveys indicate a wide diversity of amidase homologs associated with APAP hydrolysis (i.e., many potential APAP-hydrolyzing enzymes spread across taxa), which explains why many indigenous bacteria can rapidly initiate APAP breakdown and thus become enriched in contaminated effluents (Rios-Miguel et al., 2022). These findings highlight the adaptive responses of effluent-associated microbial communities to pharmaceutical pollutants. Continuous exposure to pharmaceuticals likely exerts selective pressure that favors resistant and metabolically versatile microbes (González-García et al., 2022b). This has dual implications: on one hand, such microbes hold promise for bioremediation applications, since enriched consortia could be developed into treatment systems for pharmaceutical wastewater. On the other hand, the same selective pressure may drive the spread of antibiotic resistance genes (ARGs), as reported in several Nigerian pharmaceutical effluent studies (Obayiuwana & Ibekwe, 2020; Obasi et al., 2019).

Overall, the elevated microbial counts in all categories suggest that pharmaceutical effluents not only alter microbial community structures but also enrich for specialized populations with unique metabolic capacities. The presence of abundant pharmaceutical-utilizing bacteria suggests feasibility for bioremediation strategies (bioaugmentation, bio stimulation, or engineered consortia) using indigenous or cultured degrader strains. Recent experimental studies demonstrate successful bioaugmentation for both APAP and ibuprofen removal (e.g., *Pseudomonas* and *Stutzerimonas* strains for APAP (Vargas-Ordóñez et al., 2023). *Achromobacter* and other strains for ibuprofen (Fetyan et al., 2024), achieving rapid removal under lab and pilot conditions. While this adaptive potential represents a natural attenuation mechanism, it also underscores the urgent need for proper effluent treatment. Unregulated discharge of pharmaceutical wastewater into the environment risks ecological imbalance, ARG dissemination, and contamination of water resources.

Conclusion

The microbial population dynamics observed in this study demonstrate clear evidence of microbial adaptation to pharmaceutical effluent contamination. Elevated counts of heterotrophic bacteria, fungi, and drug-utilizing microbial populations indicate that effluent environments are both nutrient-rich and selective, favoring organisms with the metabolic potential to degrade pharmaceuticals such as paracetamol and ibuprofen. The higher prevalence of paracetamol-utilizing bacteria compared to ibuprofen-degraders suggests differences in biodegradability linked to structural complexity, while the detection of dual drug-utilizing populations highlights metabolic versatility and potential synergistic pathways. These findings confirm that indigenous microbial communities are actively engaged in the natural attenuation of pharmaceutical pollutants. However, continuous exposure poses ecological risks, to both aquatic organism and human health.

Recommendations

1. It is therefore recommended that further studies be conducted to identify and characterize the indigenous paracetamol and ibuprofen-degrading strains from pharmaceutical effluents and develop microbial consortia for bioremediation applications.
2. Pharmaceutical industries should also adopt advanced wastewater treatment systems (e.g., membrane bioreactors, bioaugmentation, or constructed wetlands) that enhance microbial degradation while minimizing environmental risks. Future studies should explore synergistic bacterial–fungal systems, leveraging bacterial enzymatic specificity and fungal ligninolytic activity for more effective degradation of recalcitrant pharmaceuticals.
3. Strict enforcement of effluent discharge standards, combined with incentives for industries adopting eco-friendly treatment technologies, will reduce pharmaceutical pollution burdens in aquatic environments.

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