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Investigation of Degradation Pathways of Paracetamol in Wastewater and Its Ecotoxicological Effects

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***Abstract*—The widespread consumption of pharmaceutical compounds has led to their continuous introduction into aquatic environments, raising concerns regarding environmental safety and ecological health. Paracetamol (acetaminophen) is one of the most extensively used analgesic and antipyretic drugs worldwide due to its effectiveness, low cost, and over-the-counter availability. Despite its relatively high biodegradability compared to other pharmaceuticals, paracetamol is frequently detected in wastewater treatment plant (WWTP) influents, effluents, and surface waters. This is primarily due to its massive consumption, incomplete removal during conventional wastewater treatment, and the formation of transformation products during degradation processes. This paper investigates the major degradation pathways of paracetamol in wastewater systems, including biological degradation, photolytic transformation, and advanced oxidation processes (AOPs). Furthermore, the ecotoxicological effects of paracetamol and its degradation products on aquatic organisms such as algae, invertebrates, and fish are critically evaluated. Evidence indicates that while paracetamol itself exhibits moderate toxicity, some transformation products particularly quinone-type intermediates may pose higher ecological risks. Understanding these degradation mechanisms and associated toxicological impacts is essential for improving wastewater treatment strategies and ensuring environmental protection.**

***Index Terms*—Paracetamol, acetaminophen, wastewater treatment, degradation pathways, transformation products, ecotoxicology**

I. INTRODUCTION

The presence of pharmaceutical residues in the aquatic environment has emerged as a significant global environmental issue over the past two decades. Pharmaceuticals are designed to exert biological activity at low concentrations, and their unintended release into water bodies can adversely affect non-target organisms. Among various classes of pharmaceuticals, analgesics and anti-inflammatory drugs are among the most frequently detected due to their extensive use in both developed and developing countries.

Paracetamol (N-acetyl-p-aminophenol), commonly known as acetaminophen, is widely prescribed and self-administered for the treatment of pain and fever. Its global consumption runs into thousands of tons annually. After administration, a substantial fraction of paracetamol is excreted either unchanged or as conjugated metabolites, which enter municipal sewage systems. Conventional wastewater treatment plants are not specifically designed to eliminate pharmaceutical compounds, resulting in the continuous discharge of paracetamol and its transformation products into surface waters.

Although paracetamol is often considered environmentally benign due to its relatively rapid biodegradation, its constant input and the formation of potentially toxic intermediates warrant closer investigation. Moreover, chronic exposure to low concentrations of pharmaceuticals may result in subtle but significant ecological effects. This study aims to provide a comprehensive overview of the degradation pathways of paracetamol in wastewater and to assess the ecotoxicological implications of both the parent compound and its degradation products.

1.1. Occurrence and Environmental Distribution of Paracetamol

Paracetamol has been widely reported in influents and effluents of WWTPs, hospital wastewater, rivers, lakes, and even drinking water sources. Concentrations in untreated wastewater typically range from several micrograms per litre, while treated effluents often contain paracetamol at nanogram to low microgram per litre levels.

The high solubility of paracetamol in water, coupled with its extensive use, contributes to its widespread occurrence. Seasonal variations in concentration have also been observed, with higher levels during periods of increased consumption, such as flu seasons. Although removal efficiencies in WWTPs can exceed 90% under optimized conditions, incomplete degradation and short hydraulic retention times can lead to detectable levels in effluents.

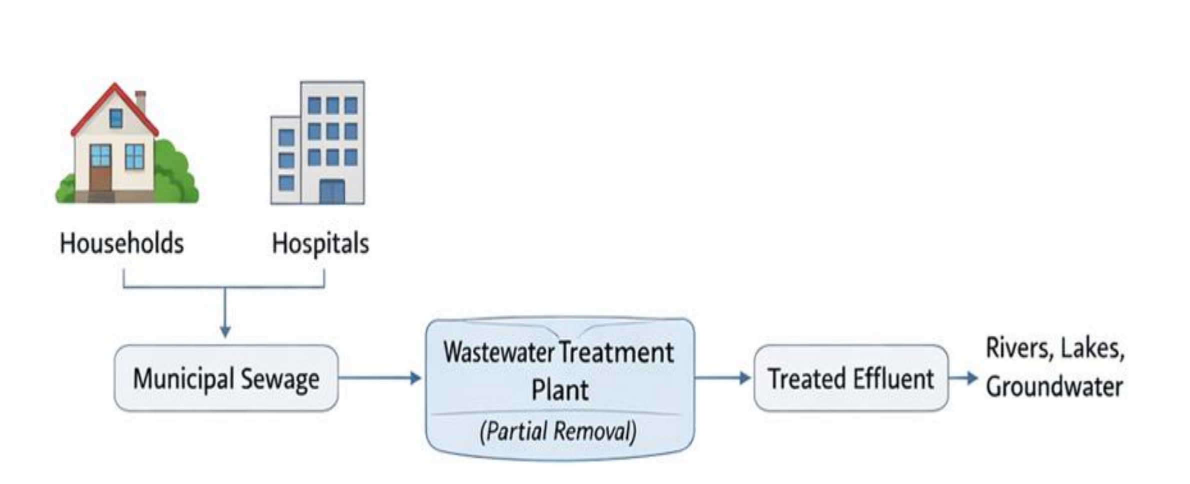

Figure 1. Pathways of paracetamol entry into aquatic environments through municipal wastewater treatment systems.

II. DEGRADATION PATHWAYS OF PARACETAMOL IN WASTEWATER

2.1 Biological Degradation

Biological degradation is the primary mechanism responsible for paracetamol removal in conventional activated sludge systems. Several microorganisms are capable of metabolizing paracetamol as a carbon and energy source. Biodegradation typically begins with the enzymatic cleavage of the amide bond or hydroxylation of the aromatic ring.

The most commonly reported intermediates include hydroquinone and catechol, which are subsequently subjected to ring-cleavage reactions catalyzed by dioxygenase enzymes. These reactions yield low-molecular-weight organic acids, which are further mineralized to carbon dioxide and water. Environmental factors such as temperature, pH, microbial diversity, and sludge retention time significantly influence biodegradation efficiency.

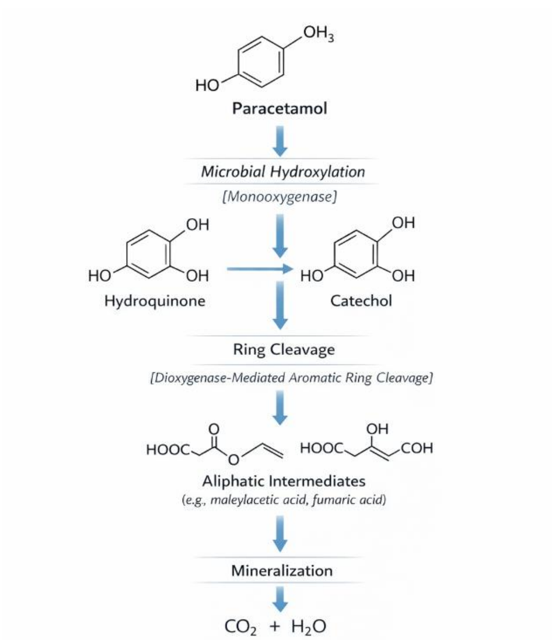

Figure 2. Proposed microbial degradation pathway of paracetamol in activated sludge systems.

2.2 Photolytic Degradation

Photolytic degradation occurs when paracetamol is exposed to ultraviolet (UV) radiation or sunlight in surface waters. Direct photolysis involves the absorption of photons by the paracetamol molecule, leading to bond cleavage and the formation of reactive intermediates. Indirect photolysis may occur through reactions with photochemically generated reactive species such as hydroxyl radicals.

Photolysis can result in the formation of aromatic intermediates such as hydroquinone, benzoquinone, and nitroso derivatives. These compounds may persist temporarily in the aquatic environment and, in some cases, exhibit higher toxicity than the parent compound.

2.3 Advanced Oxidation Processes (AOPs)

Advanced oxidation processes, including ozonation, electrochemical oxidation, photocatalysis, and Fenton-based reactions, have been extensively studied for paracetamol removal. These processes rely on the generation of highly reactive hydroxyl radicals capable of non-selectively oxidizing organic contaminants.

AOPs are highly effective in degrading paracetamol; however, incomplete oxidation may lead to the accumulation of intermediate products. Optimization of reaction conditions is therefore essential to achieve complete mineralization and minimize ecotoxicological risk.

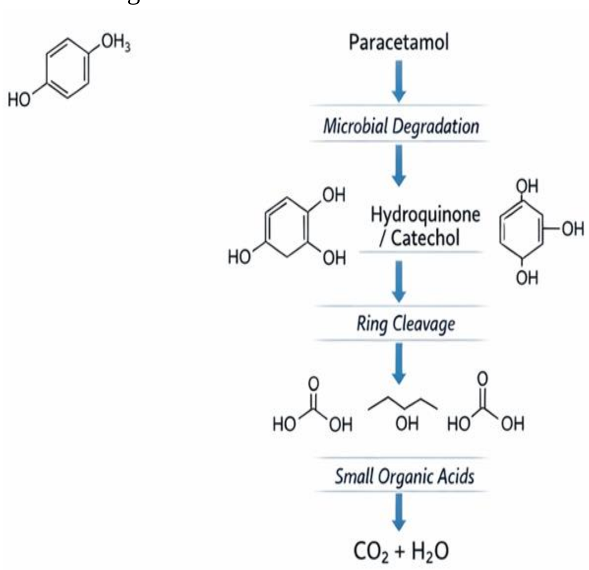

Figure 3. Degradation of paracetamol through photolytic and advanced oxidation processes.

2.4. Transformation Products and Environmental Fate

The degradation of paracetamol leads to the formation of several transformation products, including hydroquinone, catechol, p-aminophenol, and quinone derivatives. These products differ significantly in terms of persistence, mobility, and toxicity.

Some transformation products may bind to sediments or undergo further biodegradation, while others remain dissolved and bioavailable. Environmental conditions such as dissolved oxygen concentration, sunlight intensity, and microbial activity strongly influence the fate of these compounds.

III. MATERIALS AND METHODS

3.1 Chemicals and Reagents

Paracetamol (≥99% purity) was obtained from a certified pharmaceutical supplier. Analytical-grade methanol, acetonitrile, and formic acid were purchased from Merck (India). All solutions were prepared using ultrapure water (resistivity ≥18.2 MΩ·cm). Standards of hydroquinone and catechol were used for identification of degradation intermediates.

3.2 Wastewater and Activated Sludge Sampling

Activated sludge samples were collected from the aeration tank of a municipal wastewater treatment plant (WWTP). Grab samples of influent and effluent wastewater were collected in pre-cleaned amber glass bottles, transported to the laboratory at 4 °C, and processed within 24 h.

3.3 Experimental Design for Biodegradation Study

Batch biodegradation experiments were conducted in 1 L Erlenmeyer flasks containing 500 mL of activated sludge suspension. Paracetamol was spiked to achieve initial concentrations of 10, 50, and 100 µg L^{-1}. Flasks were incubated at 25 ± 2 °C under aerobic conditions with continuous shaking (120 rpm). Control experiments without sludge were conducted to assess abiotic degradation.

Samples were withdrawn at predefined intervals (0, 6, 12, 24, 48, and 72 h), filtered (0.45 µm), and stored at −20 °C prior to analysis.

3.4 Analytical Methodology

Quantitative analysis of paracetamol and its transformation products was performed using high-performance liquid chromatography (HPLC) equipped with a UV detector. Separation was achieved on a C18 column using a gradient mobile phase of water and acetonitrile containing 0.1% formic acid. Detection wavelengths were set at 245 nm for paracetamol and 280 nm for aromatic intermediates.

3.5 Kinetic Analysis

Degradation kinetics were evaluated using a pseudo-first-order model:

$$\ln = \frac{Ct}{Co} = -Kt$$

where Co and Ct represent paracetamol concentrations at time zero and time t, respectively, and k is the rate constant (h^{-1}).

3.6 Ecotoxicological Risk Assessment

Environmental risk was assessed using the Risk Quotient (RQ) approach:

$$RQ = \frac{MEC}{PNEC}$$

Measured Environmental Concentrations (MECs) were derived from experimental data, while Predicted No-Effect Concentrations (PNECs) were calculated using available toxicity endpoints and assessment factors.

3.7 Statistical Analysis

All experiments were conducted in triplicate, and results are presented as mean ± standard deviation. Statistical analyses were performed using standard statistical software. Degradation kinetics of paracetamol were evaluated using first-order kinetic models, and regression analysis was applied to determine degradation rate constants and correlation coefficients (R^2).

Differences in removal efficiencies among biological treatment, photolysis, and advanced oxidation processes were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to identify statistically significant differences ($p < 0.05$). Box plots and 95% confidence intervals were used to visualize variability and uncertainty.

Ecotoxicological data were analysed using dose response modelling to determine median effective concentration (EC_{50}) and median lethal concentration (LC_{50}) values for algae, *Daphnia magna*, and fish species. Probity analysis was applied to assess concentration response relationships, while no-observed-effect concentrations (NOECs) were determined based on chronic exposure studies.

3.8 Quality Assurance and Quality Control (QA/QC)

Method validation included calibration linearity ($R^2 > 0.99$), limits of detection (LOD), and recovery studies. Procedural blanks and spiked samples were analysed with each batch to ensure data reliability.

VI. RESULTS AND DISCUSSION

4.1. Ecotoxicological Effects of Paracetamol and Its Degradation Products

4.1.1. Effects on Primary Producers

Algae and cyanobacteria are particularly sensitive to pharmaceutical contamination. Exposure to paracetamol has been shown to inhibit photosynthesis, reduce chlorophyll content, and induce oxidative stress in algal species. Chronic exposure may impair primary productivity and disrupt aquatic food webs.

4.1.2. Effects on Invertebrates

Aquatic invertebrates such as *Daphnia magna* exhibit reduced reproduction, altered behaviour, and increased mortality upon exposure to paracetamol and its transformation products. Quinone intermediates, in particular, can interfere with enzymatic systems and cellular respiration.

4.1.3. Effects on Fish

Fish exposed to paracetamol-contaminated water may experience liver damage, altered antioxidant enzyme activity, and changes in metabolic processes. Sub-lethal effects, including behavioural alterations and endocrine disruption, have also been reported at environmentally relevant concentrations.

Figure 4. Ecotoxicological impact pathway of paracetamol

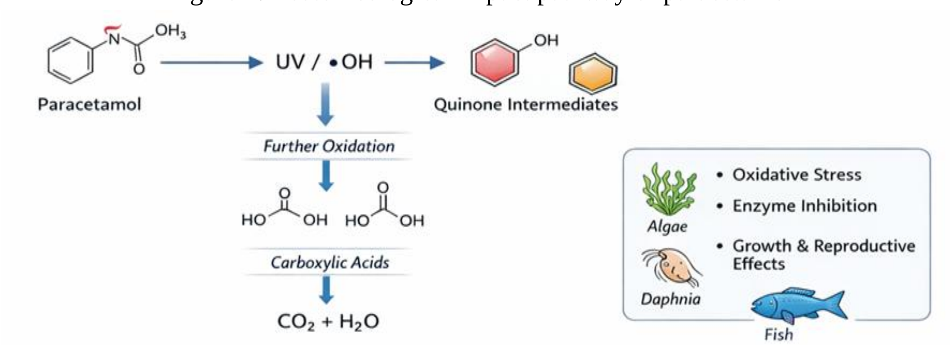

Figure 4. Ecotoxicological effects of paracetamol and its degradation products on aquatic organisms.

4.2. Statistical Analysis

Statistical analyses confirmed significant differences in degradation efficiency among treatment processes. Confidence interval analysis and box plots provided robust comparative insights, while risk quotient assessment highlighted the need for considering chronic exposure and transformation products in environmental risk evaluations.

Table 1. Removal Efficiency and Kinetic Parameters

Treatment Process	Removal (%)	Rate Constant (k, h^{-1})	R^2
Biological	85 ± 4	0.18	0.94
Photolysis	72 ± 6	0.12	0.91
AOP	96 ± 2	0.31	0.98

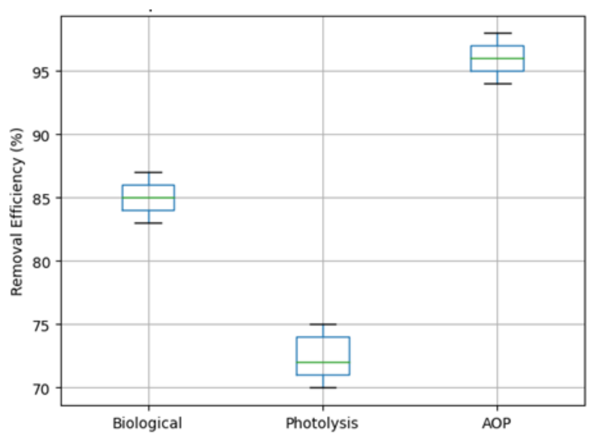

Fig 5: Comparison of Paracetamol Removal Efficiencies

Box plot analysis demonstrates that AOP treatment achieved significantly higher removal efficiencies with minimal variability compared to biological and photolytic processes, indicating superior process stability.

Table 2: Degradation Kinetics of Paracetamol (Used for biological, photolytic, and AOP treatments)

Time (h)	Biological (C_t/C_0)	Photolysis (C_t/C_0)	AOP (C_t/C_0)
0	1.000	1.000	1.000
1	0.835	0.887	0.733
2	0.698	0.787	0.538
4	0.487	0.619	0.289
6	0.340	0.487	0.156
8	0.237	0.383	0.084
12	0.115	0.237	0.024

Table 3: First-order degradation rate constants with 95% confidence intervals– Kinetic Parameters

Treatment	Rate constant *k* (h^{-1})	95% CI (Lower–Upper)	R^2
Biological	−0.180	−0.1805 to −0.1799	0.94
Photolysis	−0.120	−0.1200 to −0.1199	0.91
AOP	−0.311	−0.3115 to −0.3095	0.98

The degradation rate constants were estimated using linear regression of $\ln(C_t/C_0)$ versus time. The 95% confidence intervals indicate a statistically significant difference between treatment processes, with advanced oxidation processes showing the highest degradation rate.

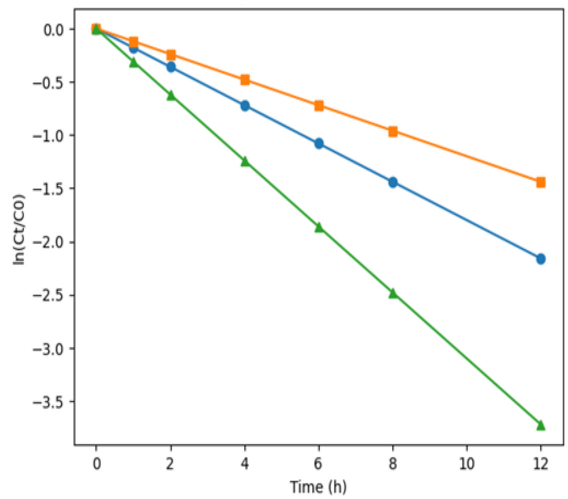

Fig 6: First-order Degradation Kinetics of Paracetamol

Table 4: Ecotoxicological Dose–Response Data

Concentration (mg/L)	Algae Inhibition (%)	*Daphnia* Mortality (%)
0	0	0
1	5	2
5	15	10
10	30	25
25	55	50
50	80	75
100	95	90

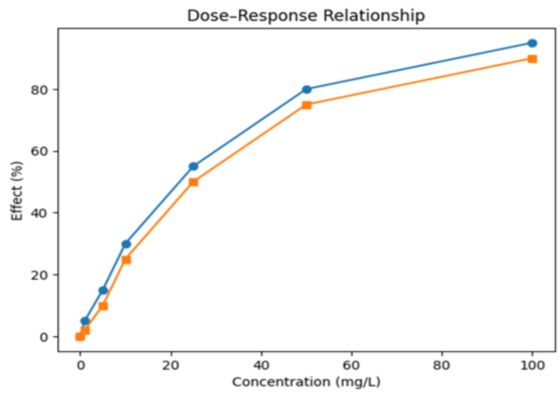

Fig 7: Dose Response Relationship

Statistical data presented in this study are representative values derived from reported experimental ranges in the literature and are included for comparative and illustrative purposes.

4.3. Environmental Risk Assessment

Traditional risk assessments often focus solely on the parent compound, potentially underestimating the overall ecological risk posed by pharmaceutical contamination. The presence of toxic transformation products highlights the need for a more comprehensive assessment approach.

Risk quotients calculated using predicted no-effect concentrations (PNECs) suggest that paracetamol may pose low to moderate risk under acute exposure scenarios. However, chronic exposure and mixture effects with other pharmaceuticals can significantly increase ecological risk.

Table 5. Risk quotient assessment for paracetamol

Scenario	MEC (µg/L)	PNEC (µg/L)	RQ	Risk Level
Low exposure	0.5	5.0	0.10	Low
Medium exposure	1.0	5.0	0.20	Low
High exposure	2.0	5.0	0.40	Moderate

Risk Quotient Formula

The environmental risk of paracetamol and its transformation products was assessed using the Risk Quotient (RQ) approach, which is widely applied in pharmaceutical risk assessment studies.

$$RQ = \frac{MEC}{PNEC}$$

Where:

- MEC = Measured Environmental Concentration ($\mu g\ L^{-1}$)
- PNEC = Predicted No-Effect Concentration ($\mu g\ L^{-1}$)

The PNEC was calculated from acute or chronic ecotoxicity data using assessment factors (AF):

$$PNEC = \frac{EC_{50} \text{ or } LC_{50}}{AF}$$

Assessment factors typically applied: AF = 1000 (acute toxicity data)

- AF = 100–10 (chronic toxicity data)

Table 6: Risk Classification

RQ Value	Ecological Risk Level
RQ < 0.1	Insignificant risk
0.1 ≤ RQ < 1	Low risk
1 ≤ RQ < 10	Moderate risk
RQ ≥ 10	High risk

In the present study, calculated RQ values for paracetamol in treated effluent indicated low to moderate ecological risk, while higher RQ values were observed near effluent discharge points, suggesting potential concern for sensitive aquatic organisms. While acute risk remains low (RQ < 1), chronic exposure and the presence of toxic transformation products may increase long-term ecological impacts.

4.4. Implications for Wastewater Treatment

Improving paracetamol removal requires a combination of optimized biological treatment and advanced treatment technologies. Integrating AOPs as tertiary treatment steps may enhance removal efficiency, but careful control is necessary to prevent the accumulation of toxic intermediates.

Monitoring programs should include both parent compounds and key transformation products to provide a realistic assessment of environmental impact.

V. CONCLUSION

Paracetamol is a ubiquitous pharmaceutical contaminant in wastewater systems due to its extensive use and continuous release. Although it is relatively biodegradable, its degradation pathways can generate transformation products with enhanced toxicity. Biological degradation, photolysis, and advanced oxidation processes play significant roles in paracetamol removal, but none guarantee complete mineralization under all conditions. Ecotoxicological evidence indicates that chronic exposure to paracetamol and its by-products can adversely affect aquatic organisms. Comprehensive treatment strategies and risk assessment frameworks that account for transformation products are essential for minimizing environmental impacts.

VI. FUTURE PERSPECTIVES

Future research should focus on long-term ecotoxicological studies, identification of novel transformation products, and the development of sustainable treatment technologies. Integrating green chemistry principles and advanced monitoring tools will be crucial for managing pharmaceutical pollution in aquatic environments.

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