**Title: Efficacy test of a Copper/zinc biocide**

**Abstract**

Copper, an emerging alternative, exhibits potent antimicrobial effects due to its ionization and disruption of microbial membranes. An experimental was conducted to assess disinfection efficiency by measuring reductions in coliforms, *Escherichia coli*, and heterotrophic bacteria.

## Introduction

The disinfection of treated wastewater is critical to reducing public health risks and environmental contamination (Lawrence et al., 2008). There is a tremendous amount of literature on and experience with wastewater disinfection alternative. However, it is difficult to sift through all of the available information, especially for relatively newer technologies. In addition, there are many factors, some of them site-specific, that influence whether a facility changes disinfection practice, and which alternative it chooses.

Traditional disinfectants such as chlorine have been effective but are increasingly scrutinized for their potential to form harmful DBPs.

### Copper as a Disinfectant for Treated Wastewater

Copper has garnered attention as a sustainable and effective disinfectant for treated wastewater, offering antimicrobial properties and minimal chemical by-product formation. Herewith a summary of copper's mechanisms of action, advantages, limitations, and environmental impacts in the context of wastewater treatment. The potential for copper-based disinfection to serve as an alternative or complement to traditional methods is also discussed.

The disinfection of treated wastewater is crucial for protecting public health and ensuring compliance with environmental discharge standards. Copper, traditionally used in agriculture and water systems for its biocidal properties, is gaining interest as a wastewater disinfectant due to its non-toxic residues and effectiveness across various microbial strains.

### Mechanism of Action

Copper ions ($Cu^{2+}$) exhibit strong antimicrobial activity by interacting with microbial cell membranes, leading to structural damage and leakage of intracellular contents. Additionally, copper ions disrupt enzyme functions and generate reactive oxygen species, further impairing microbial viability. These mechanisms make copper effective against bacteria, viruses, and some protozoa (Intisar et al., 2021).

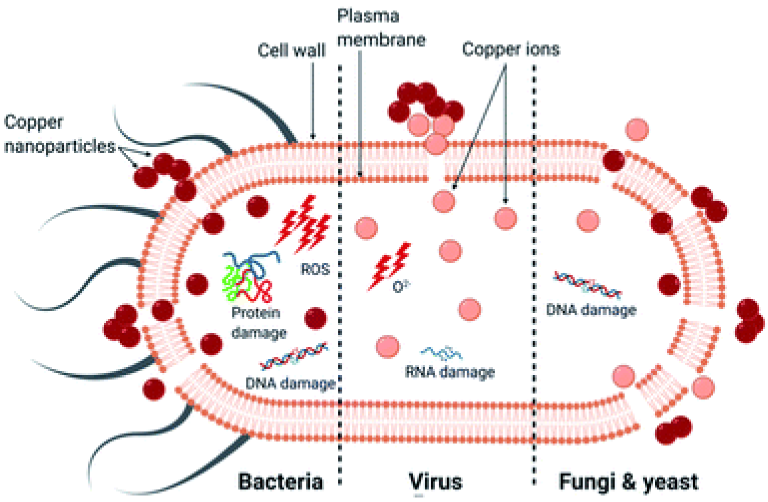


Diagram 2. The primary mechanism of death in different microorganisms by copper nanoparticles (After: Intisar et al., 2021).

The antimicrobial action of copper is the generation of reactive oxygen species (ROS) by reduction of copper through a Fenton-like reaction, leading to enzyme and non-enzyme mediated oxidative damage involving lipid peroxidation, protein oxidation and DNA damage.18–20 The final mechanism is the release of copper ions, Cu+ and Cu2+, which damage the membrane and infiltrate the cell, inducing an oxidative stress response involving endogenous ROS. The consensus view of the cause of microbial cell death due to copper is a combination of these processes with the relative importance of each dependent on the microorganism (Intisar et al., 2021).

The reason why no resistance but only tolerance to copper is found in microorganisms exposed to constant relatively high doses of copper, is probably because copper exerts its biocidal/antimicrobial activity not through one mechanism (as most antibiotics), but through several parallel non-specific mechanisms (Gadi, 2012).

**Advantages**

1. **Broad-Spectrum Antimicrobial Efficacy**: Copper is effective against a wide range of pathogens, including chlorine-resistant microorganisms such as Cryptosporidium (Gadi, 2012).
2. **Environmentally Friendly Residues**: Unlike chemical disinfectants, copper residues in treated wastewater are typically non-toxic at regulated levels and can provide a residual effect for ongoing microbial suppression (Gadi,2012).
3. **Chemical Stability**: Copper does not react with organic matter to form harmful disinfection by-products (DBPs) like trihalomethanes (THMs) or haloacetic acids (HAAs) (Gadi, 2012)..
4. **Sustainability**: Copper is naturally occurring and reusable, aligning with circular economy principles (Gadi,2012).

**Challenges**

1. **Cost of Implementation**: While copper is abundant, its use in large-scale wastewater treatment may require significant initial investment for dosing systems and monitoring equipment.
2. **Potential for Toxicity**: Excessive copper concentrations can harm aquatic ecosystems, necessitating precise dosing and compliance with environmental discharge limits.
3. **Limited Efficacy in High Organic Loads**: The presence of high organic or particulate matter can reduce copper's antimicrobial efficiency, requiring pre-treatment steps.

**Environmental and Regulatory Considerations**

Copper levels in treated effluent must adhere to stringent environmental standards, such as those set by the U.S. Environmental Protection Agency (EPA) and European Union regulations, to prevent bioaccumulation and ecological harm. Strategies such as controlled dosing and periodic monitoring can mitigate risks.

**Objectives of this study**

- This study copper as disinfectants for final treated sewerage effluent, did efficacy tests to assess disinfection efficiency by measuring reductions in coliforms, *Escherichia coli*, and heterotrophic bacteria.

## Results and discussion

- Copper/Zinc based biocide
  - **Microbiological analysis**

**Table 1. Heterotrophic plate count (cfu/ml) at 15ppm treatment at specific operating conditions.**

| Date | Treated wastewater (cfu/ml) | Disinfected final effluent (cfu/ml) | Reduction % | g/h | Flow rate dosing l/h | Retention time (h) |
|---|---|---|---|---|---|---|
| 10-Dec | 188 000 | 304 000 | -61% | 27,30 | 0,03 | 1,66 h |
| 11-Dec | 113 000 | 328 000 | -190% | 28,80 | 0,03 | 1,57 h |
| 12-Dec | 450 000 | 176 000 | 60.8% | 41,90 | 0,04 | 1,07 h |
| 13-Dec | 416 000 | 98 000 | 74.6% | 45,50 | 0,05 | 0,96 h |

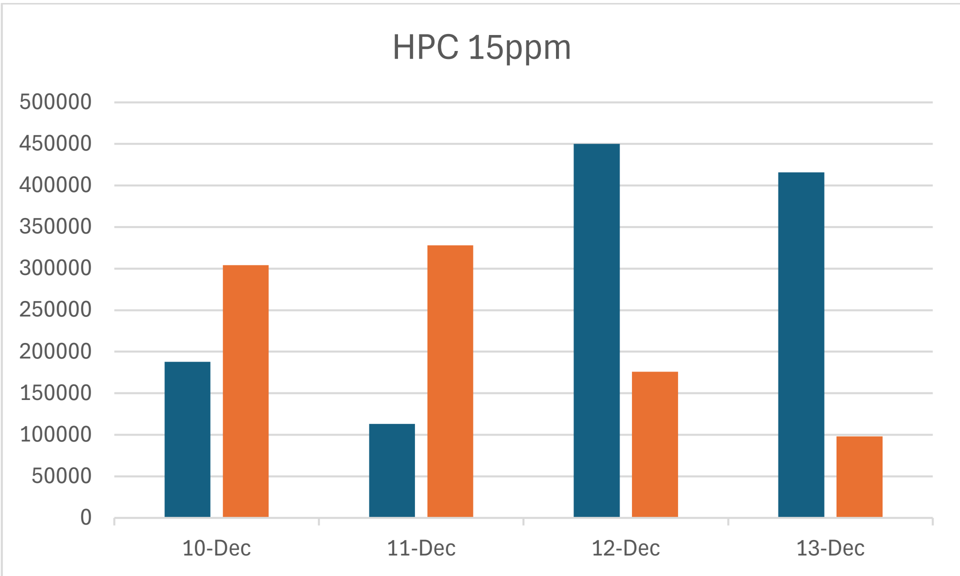


**Figure 1. Heterotrophic plate count per ml at 15ppm treatment (Series 1 = Treated effluent and Series 2 = Disinfected effluent).**

## Analysis of Heterotrophic Plate Count Results

The data in **Table 1 (Figure 1)** presents heterotrophic plate counts (HPC) per ml in final treated wastewater and disinfected final effluent at a treatment level of 15 ppm. This analysis evaluates the effectiveness of the treatment process based on the observed changes in HPC levels. On **10-Dec** and **11-Dec**, the HPC increased in the disinfected effluent compared to the final treated wastewater. This was attributed to the first two days which were used to clean the system from algae and other residual organic matter (Photograph 4).

On **12-Dec** the HPC decreased from 450,000 to 176,000 (**60.9% reduction**) and on **13-Dec** decreased 416,000 to 98,000 (**76.4% reduction**). This indicates improved disinfection performance on these days indicating that the disinfectant cleansed the system on the 10$^{th}$ and 11$^{th}$ of December. Necertheless at 15 ppm, the disinfectant concentration was insufficient to achieve consistent microbial inactivation, particularly under high organic load conditions such as was observed on the 10$^{th}$ and 11$^{th}$ of December.

Higher g/h values (41.90 and 45.50) are associated with positive reductions, indicating an improvement in the process effectiveness. The flow rate dosing increases from 0.03 to 0.05 l/h as the reductions improve. This suggests a positive correlation between dosing flow rate and performance. Retention time decreases as the reductions improve. The highest retention time (1.66 h) corresponds to a -61% reduction, while the lowest retention time (0.96 h) aligns with the highest reduction (74.6%).

**Table 2. Coliform count (cfu/100ml) at 15ppm at specific operational conditions.**

| Date | Treated wastewater (cfu/100ml) | Disinfected final effluent (cfu/100ml) | Reduction % | g/h | Flow rate dosing l/h | Retention time (h) |
|---|---|---|---|---|---|---|
| 10-Dec | 91 000 | 67 000 | 26.3% | 27,30 | 0,03 | 1,66 h |
| 11-Dec | 68 000 | 86 000 | -26,4% | 28,80 | 0,03 | 1,57 h |
| 12-Dec | 128 000 | 108 000 | 15.6% | 41,90 | 0,04 | 1,07 h |
| 13-Dec | 160 000 | 72 000 | 55.0% | 45,50 | 0,05 | 0,96 h |

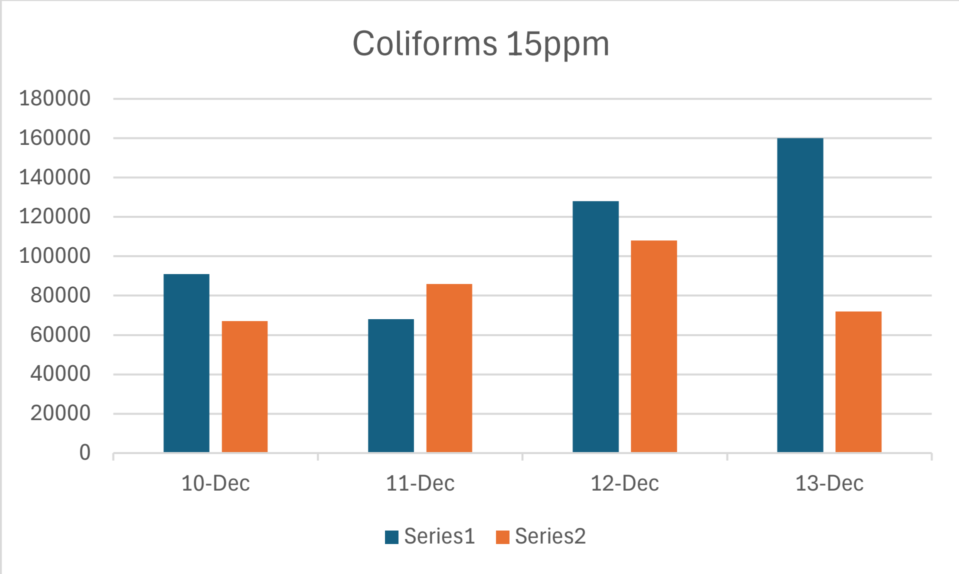


**Figure 2. Coliform count per 100ml at 15ppm (Series 1 = Treated effluent and Series 2 = Disinfected effluent).**

The data in **Table 2 (Figure 2)** shows coliform counts (per 100 ml) in final treated wastewater and disinfected final effluent at a treatment level of 15 ppm. This analysis evaluates the disinfection process's effectiveness based on coliform reduction trends. On **11-Dec**, the coliform count increased by **26.5%**, indicating system cleansing as was shown by HPC results. The disinfection process achieved reductions on **10 Dec, 12 Dec, and 13 Dec**, with the highest reduction (**55.0%**) on **13-Dec**. Even on days with reductions, the remaining coliform counts in the disinfected effluent were relatively high, suggesting that a 15ppm treatment level was insufficient to meet stringent water quality standards. While the process shows some potential, further optimization of the disinfectant concentration and operational conditions is needed to ensure consistent and effective coliform reduction.

**Table 3. *E coli* count (cfu/100ml) at 15ppm at specific operational conditions.**

| Date | Final treated wastewater | Disinfected final effluent | Reduction % | g/h | Flow rate dosing l/h | Retention time (h) |
|---|---|---|---|---|---|---|
| 10-Dec | 10 000 | 19 000 | -90.0% | 27,30 | 0,03 | 1,66 h |
| 11-Dec | 11 000 | 10 000 | 9.0% | 28,80 | 0,03 | 1,57 h |
| 12-Dec | 70 000 | 62 000 | 11.42% | 41,90 | 0,04 | 1,07 h |
| 13-Dec | 61 000 | 42 000 | 31.1% | 45,50 | 0,05 | 0,96 h |

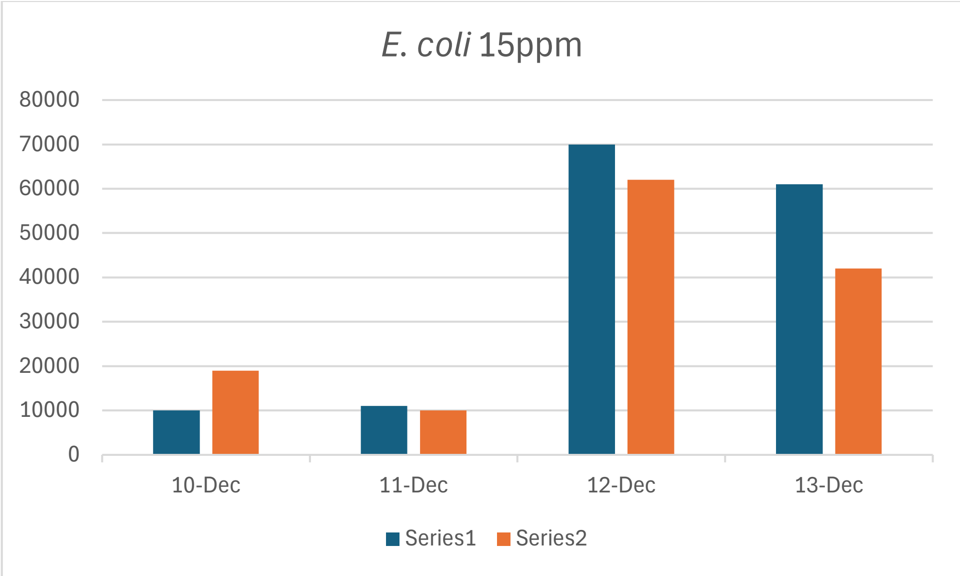


**Figure 3.** ***E coli*** **count per 100ml at 15ppm (Series 1 = Treated effluent and Series 2 = Disinfected effluent).**

## Analysis of *E. coli* Count Results

The data in **Table 3 (Figure 3)** provides *E. coli* counts (cfu/per 100 ml) for final treated wastewater and disinfected final effluent at a treatment level of 15 ppm. This analysis examines the disinfection process's effectiveness and identifies trends and potential challenges. On **10-Dec**, the *E. coli* count increased by **90.0%**, indicating cleansing of the system.The process achieved reductions on **11-Dec, 12-Dec, and 13-Dec**, with the most significant reduction (**31.1%**) occurring on **13-Dec**.The reductions achieved (ranging from **9.1% to 31.1%**) are relatively low, suggesting that a 15ppm treatment level was not be sufficient to effectively reduce *E. coli* in the treated effluent.

**Table 4. Heterotrophic plate count (cfu/ml) at 100ppm treatment at specific operational conditions.**

| Date | Treated wastewater (cfu/ml) | Disinfected final effluent (cfu/ml) | Reduction % | g/h | Flow rate dosing l/h | Retention time (h) |
|---|---|---|---|---|---|---|
| 16-Dec | 37 000 | 14 000 | 62.2% | 285 g/h | 0,28 l/h | 0,96 h |
| 17-Dec | 33 000 | 5 300 | 88.4% | 205 g/h | 0,21 l/h | 1,40 h |
| 18-Dec | 76 000 | 8 800 | 83.9% | 218 g/h | 0,22 l/h | 1,36 h |

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|---|---|---|---|---|---|---|
| 19-Dec | 33 600 | 2 900 | 91.4% | 119 g/h | 0,12 l/h | 2,50 h |

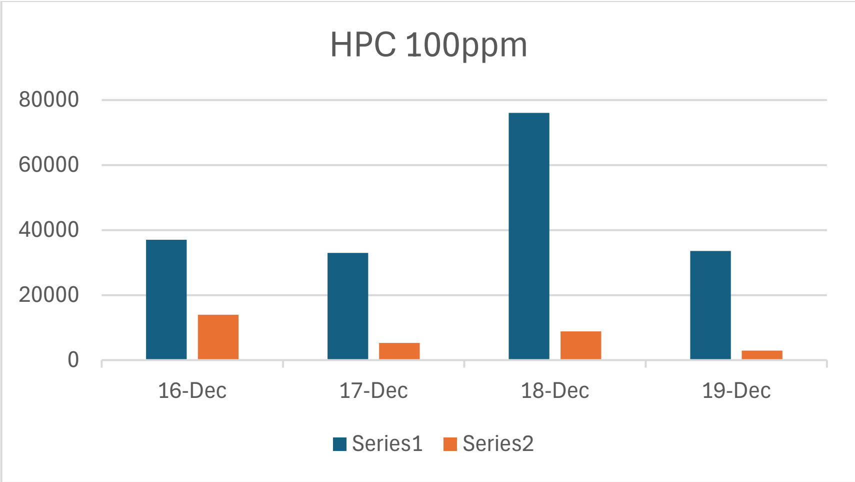


**Figure 4. Heterotrophic plate count per ml at 100ppm treatment (Series 1 = Treated effluent and Series 2 = Disinfected effluent).**

The data in **Table 4 (Figure 4)** shows heterotrophic plate counts (HPC) per ml in final treated wastewater and disinfected final effluent at a treatment concentration of 100 ppm. This analysis evaluates the disinfection process's effectiveness based on observed reductions in HPC. The treatment achieved substantial reductions in HPC on all dates, with reduction percentages ranging from **62.2% (16-Dec)** to **91.4% (19-Dec)**. This demonstrates the effectiveness of a 100ppm disinfectant concentration in reducing HPC levels in treated wastewater culminating in the highest efficiency on **19-Dec (91.4%)**. While reductions were notable, residual HPC in the disinfected effluent (ranging from **2,900 to 14,000 CFU/ml**) suggests that further optimization may be needed to meet stringent water quality standards, depending on regulatory requirements. The disinfection process at 100 ppm demonstrated effectiveness in reducing HPC, achieving up to 91.4% reduction. However, the presence of residual HPC suggests opportunities for further optimization to ensure compliance with water quality standards and consistent performance. Higher reductions (88.4% and 91.4%) correspond to lower g/h values (205 and 119 respectively). Lower reductions (62.2%) are associated with the highest g/h value (285). Reduction percentages are generally higher when the flow rate dosing is lower. For example: 62.2% reduction: 0.28 l/h and 91.4% reduction: 0.12 l/h. Longer retention times correlate with higher reductions (91.4% reduction: 2.50 h retention time and 62.2% reduction at 0.96 h retention time.

**Table 5 Coliform count (cfu/100ml) at 100ppm at specific operational conditions.**

| Date | Treated wastewater (cfu/100ml) | Disinfected final effluent (cfu/100ml) | Reduction % | g/h | Flow rate dosing l/h | Retention time (h) |
|---|---|---|---|---|---|---|
| 16-Dec | 83 000 | 71 000 | 14.5% | 285 g/h | 0,28 l/h | 0,96 h |
| 17-Dec | 76 000 | 3 900 | 94.9% | 205 g/h | 0,21 l/h | 1,40 h |
| 18-Dec | 8 000 | 4 900 | 38.8% | 218 g/h | 0,22 l/h | 1,36 h |
| 19-Dec | 92 000 | 6 400 | 93.0% | 119 g/h | 0,12 l/h | 2,50 h |

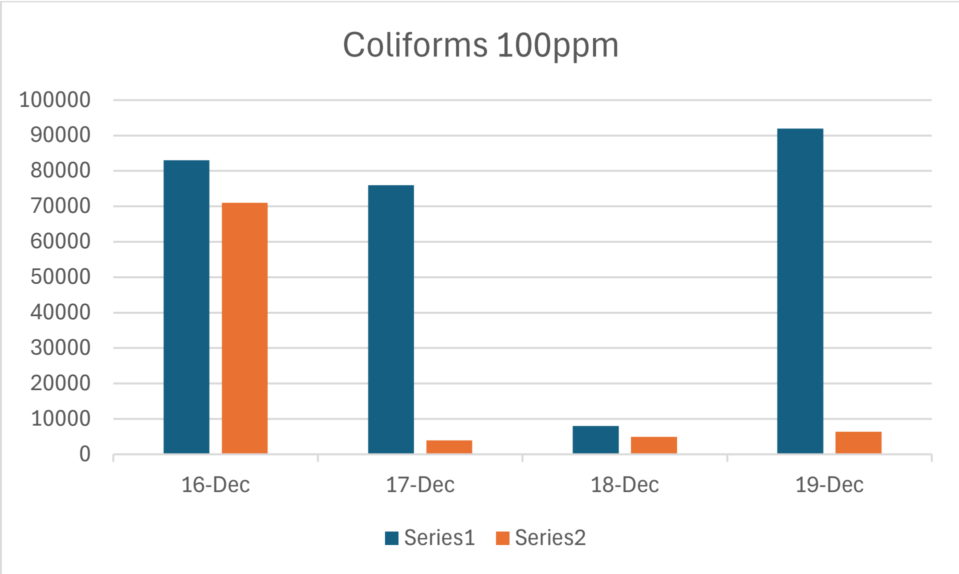


**Figure 5. Coliform count per 100ml at 100ppm (Series 1 = Treated effluent and Series 2 = Disinfected effluent).**

**Table 5 (Figure 5)** presents coliform counts (per 100 ml) in final treated wastewater and disinfected final effluent treated with a 100ppm disinfectant dose. This analysis evaluates the disinfection process's effectiveness in reducing coliform levels. On **16-Dec**, the reduction efficiency was only **14.5%**, suggesting possible inefficiencies in the disinfection process. On **17-Dec** and **19-Dec**, very high reductions were achieved (**94.9%** and **93.0%**, respectively), demonstrating the potential effectiveness of the 100ppm treatment under optimal conditions. On **18-Dec**, a moderate reduction (**38.8%**) was observed, indicating variability in performance.

Despite reductions, residual coliform counts in the disinfected effluent varied significantly, with the lowest value observed on **17-Dec (3,900 CFU/100ml)** and the highest on **16-Dec**

**(71,000 CFU/100ml)**. These results suggest inconsistent outcomes and potential operational challenges. The disinfection process at 100 ppm demonstrated potential for high coliform reduction (up to **94.9%**) on some days, but variability in performance highlights the need for process optimization. Addressing factors contributing to low reduction efficiency will help ensure consistent and effective coliform removal. Lower reductions (14.5% and 38.8%) are associated with higher g/h values (285 and 218, respectively). Higher reductions (94.9% and 93.0%) occur with lower g/h values (205 and 119, respectively). The lowest flow rate dosing (0.12 l/h) corresponds to the second-highest reduction (93.0%). Higher flow rates (0.28 l/h) correspond to lower reductions (14.5%). Longer retention times (1.40 h and 2.50 h) correlate with higher reductions (94.9% and 93.0%). Short retention times (0.96 h) correspond to the lowest reduction (14.5%).

**Table 6. *E coli* count (cfu/100ml) at 100ppm at specific operational conditions.**

| Date | Final treated wastewater | Disinfected final effluent | Reduction % | g/h | Flow rate dosing l/h | Retention time (h) |
|---|---|---|---|---|---|---|
| 16-Dec | 30 000 | 22 000 | 26.7% | 285 g/h | 0,28 l/h | 0,96 h |
| 17-Dec | 25 000 | 1 100 | 95.6% | 205 g/h | 0,21 l/h | 1,40 h |
| 18-Dec | 2 400 | 1 900 | 20.8% | 218 g/h | 0,22 l/h | 1,36 h |
| 19-Dec | 41 000 | 2 600 | 93.7% | 119 g/h | 0,12 l/h | 2,50 h |

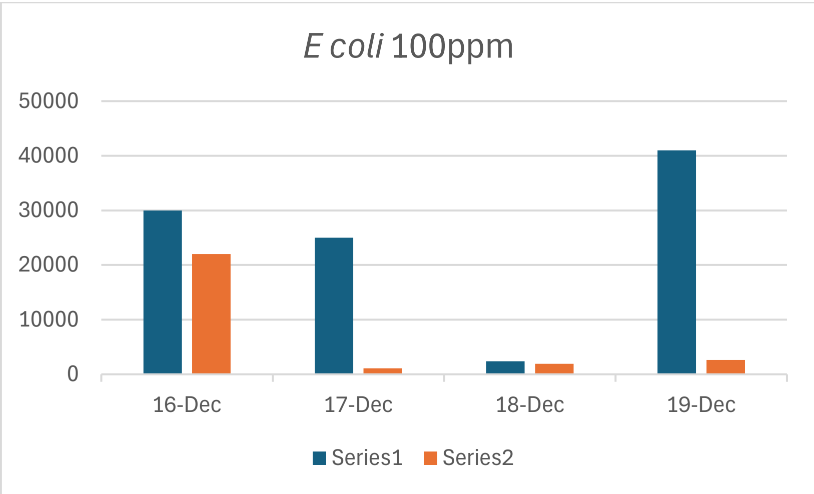


**Figure 6. *E coli* count per 100ml at 100ppm (Series 1 = Treated effluent and Series 2 = Disinfected effluent).**

The data in **Table 6 (Figure 6)** shows the *E. coli* counts (per 100 ml) in final treated wastewater and disinfected final effluent at a disinfectant concentration of 100 ppm. The reduction efficiency varies significantly across the four days, ranging from **20.8% (18-Dec)** to **95.6% (17-Dec)**. The highest initial *E. coli* count was observed on **19-Dec (41,000 per 100 ml)**, yet the disinfection process achieved a substantial reduction of **93.7%**. On **18-Dec**, despite the relatively low initial count (**2,400 per 100 ml**), the reduction was minimal (**20.8%**), indicating variability in the disinfection process's efficacy. The results indicate that while the disinfection process can achieve high *E. coli* reductions (up to **95.6%**), variability in efficiency suggests operational or environmental factors are influencing performance. Higher g/h values (285 and 218) are associated with significantly lower reduction percentages (26.7% and 20.8%). Lower g/h values (205 and 119) correspond to very high reductions (95.6% and 93.7%). Higher flow rate dosing (0.28 l/h) is linked to lower reductions (26.7%). Lower flow rates (0.12 l/h) align with the second-highest reduction (93.7%). Short retention time (0.96 h) corresponds to the lowest reduction (26.7%). Longer retention times (1.40 h and 2.50 h) result in the highest reductions (95.6% and 93.7%).

**Table 7. Once of treatment at 150ppm**

| **150 ppm treatment** | | | **Reduction %** |
|---|---|---|---|
| **HPC/ml** | **5 050** | **460** | **90.9%** |
| Coliforms/100ml | 50 000 | 450 | **99.1%** |
| *E coli*/100ml | 22 000 | 220 | **99.0%** |

**Table 7** shows the results of a once-off treatment at **150 ppm**, with the corresponding reductions in heterotrophic plate count (HPC), coliforms, and *E. coli*. The analysis evaluates the effectiveness of a higher disinfectant dose on these microbial indicators. The 150ppm treatment resulted in **exceptional reductions** across all three microbial indicators: A **90.9%** reduction in the HPC indicates substantial microbial load reductio. A **99.1%** reduction in Coliform numbers indicates nearly complete elimination of coliforms. A **99.0%** reduction in *E coli* numbers, demonstrating highly effective control of E. coli. The **150ppm** dose proved highly effective in significantly reducing microbial contamination in treated wastewater, with nearly complete removal of coliforms and *E. coli*, and a strong reduction in HPC. The 150ppm

concentration appears to be sufficiently high to achieve substantial microbial reduction, particularly for more resilient microorganisms like *E. coli* and coliforms.

**Conclusion:**

Compared to traditional disinfectants like chlorine, copper offers the benefit of reduced DBPs and a longer-lasting antimicrobial effect. However, its cost and potential for ecological impact at high concentrations require careful management. When used alongside other disinfection methods, copper can enhance overall system efficacy.

Copper is a promising alternative disinfectant for treated wastewater, combining efficacy, environmental friendliness, and sustainability. Its application is particularly advantageous for systems prioritizing reduced chemical by-products and long-term antimicrobial activity. Future research should focus on optimizing dosing strategies, developing cost-effective copper delivery systems, and exploring synergies with other disinfection technologies.

**References**

Gadi Borkow (2012). Using Copper to Fight Microorganisms. *Current Chemical Biology,* 2012*, 6,* 000-000

Intisar Salah, Ivan P. Parkin and Elaine Allan. (2021) Copper as an antimicrobial agent: recent advances. *RSC Adv*., 11, 18179