

## Crossways Farm Village: Terugvoer verslag – Rioolwater toetse

Op 28 November 2024 het ek Pieter Lourens (Projekbestuur) en Johann van Zyl, (Direkteur van Viprobac), Crossways Farm Village naby Port Elizabeth (Gqeberha) in die Oos Kaap besoek.

Die doel van die besoek was om te kyk na die watersuiwering aanleg van Crossways en om water monsters te verkry op verskillende plekke in hulle suiweringsproses.

Ons vergadering was met die ontwikkelaar en hoof Dr Chris Mulder, Gerhard die Ingenieur, en Greg die plaas en aanleg bestuurder.

### A. Agtergrond Crossways Village:

Crossways die plaas is aanvanklik bekom in 2013 en die amptelike ontwikkeling daarvan het in 2019 begin. Die plaas is 600 hektaar groot. Die ontwikkeling is daarop gemik om totaal en al selfstandig te funksioneer.

Daar is tans 75 huise gebou en van hulle word permanent bewoon en baie meer word beplan vir die toekoms. Elektrisiteit word voorsien vanaf die plaaslike munisipaliteit, maar elke huis het ook 'n opsie vir gebruik van Solar krag.

Water voorsiening vir die ontwikkeling kom uit 'n dam van ongeveer 111 000 m<sup>3</sup> op die plaas. Die dam word gevoed deur verskeie invloei strome en ook 'n rivier.

Die ontwikkeling het sy eie watersuiwering aanleg en krag vir die aanleg word voorsien deur Solar energie.

### B. Die watersuiwering proses op Crossways is as volg:

(Sien meegaande vloei diagram – begin by die dam water)

1. Die water vanuit die hoof dam op die plaas word gepomp na Jojo tenks.
2. Van die tenks word die water gepomp na 'n staal struktuur waar 4 verskillende suiwering prosesse plaas vind.
  - Stollings middel vir afval stowwe
  - Minerale byvoeging
  - Chloor word gebruik om bakterie dood te maak
3. Van hier word gesuiwerde water na 'n opgaartenk gepomp en dan geberg, vanwaar dit afgevoer en gepomp word na die huise en ander gebou op die plaas.
4. Gebruikte water (drink-, was-, en riool-) word terug gepomp na die septiese tenks bo by die suiwering aanleg. Hierdie gebruikte- en riool water gaan deur 'n draaiende wiel (wat lyk soos 'n kar was masjien) waar vaste stowwe geskei word.
5. Die riool water word nou uitgelaat om deur 'n vleiland te loop, wat lei na die hoof dam.
7. Volgens terugvoer is die riool invloei ongeveer tans .1% van die dam se volume.
8. Nou word die damwater weer op gepomp na die Jojo tenks vir suiwering. **(Sien B1)**

### C. Areas van kommer:

1. Die gekontamineerde water word in 'n **vleiland** gelaat om deur die natuur gesuiwer te word en loop dan in die hoof dam vanwaar die verbruiker water bekom word. **(Sien B6)**
2. Op hierdie stadiums is daar min huise wat bewoon word en dus min riool. *Meer huise, meer verbruikers, meer riool, groter kontaminasie.* Dit kan die water suiwering negatief inpakkeer oor die langtermyn.

3. **Chloor** wat gebruik word vir die huidige suiweringsproses en vernietiging van bakterie en alge is skadelik vir die natuur, diere en mense. **(B2.d)**

#### D. Viprobac se voordele

1. In teenstelling met Chloor het slaktoetse gevind dat daar geen slaksterftes was gedurende die suiweringsproses waar Viprobac gebruik word.
2. Die toetse bevestig die veiligheid van die produk vir diere, plante en mense, terwyl dit in gekontroleerde aanbevole dosisse aangewend word.
3. 'n Byvoordeel van Viprobac (se koper/sink konsentraat) is dat dit ook aangewend word as bemestingmiddel vir allerlei gewasse en plante, terwyl dit bakterie, protosoe en bakterie verniet.

#### E. Viprobac as oplossing

Daar is twee plekke in die watersuiweringsproses op Crossways Farm Village, waar Viprobac aangewend kan word:

1. By die watersuiweringsproses waar water van die dam gesuiwer word.  
***Die aanbeveling hier is om chloor te vervang met ons Viprobac se koper konsentraat.***
2. Dan kan Viprodac watersuiwing ook aangewend word ***voordat die rioolwater in die vleiland gelaat word, indien dit ooit nodig sou wees.***

#### F. Sien die volgende aanhangsels:

1. 'n Vloeiagram van die huidige watersuiwingstelsel op Crossways.
2. Opsomming van toetse deur die Universiteit van Stellenbosch
3. Toetse gedoen met Viprodac by die Universiteit van Stellenbosch onder leiding van ***Prof T E Cloete. MSc (UFS), DSc (UP), DSc (US).***



**Pieter Lourens**  
202/01/22

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## Crossways Farm Village: Feedback Report – Wate water test

On November 28, 2024, We visited Crossways Farm Village near Port Elizabeth (Gqeberha) in the Eastern Cape with me Pieter Lourens (Project Management) and Johann van Zyl (Director of Viprobac).

The purpose of the visit was to inspect Crossways' wastewater treatment facility and to collect water samples from different points in their purification process.

Our meeting included the developer and owner, Dr. Chris Mulder, Gerhard the Engineer, and Greg the farm and plant manager.

### A. Background of Crossways Village:

Crossways Farm was initially acquired in 2013, with official development beginning in 2019. The farm spans 600 hectares, and the development aims to operate entirely independently.

Currently, 75 homes have been built, some of which are permanently inhabited, with many more planned for the future. Electricity is supplied by the local municipality, but each house also has the option to use solar power.

Water for the development comes from a dam on the farm, with a capacity of approximately 111,000 m<sup>3</sup>. The dam is fed by several inflowing streams and a river.

The development has its own water treatment plant, and power for the plant is supplied by solar energy.

### B. The Water Purification Process at Crossways:

*(See attached flow diagram – starting from the dam water)*

1. Water from the main dam on the farm is pumped to Jojo tanks.
2. From the tanks, the water flows into a steel structure where four different purification processes take place.
  - Coagulation concentrate for waste material
  - Mineral addition
  - Chlorine is used to kill bacteria
3. From here, the purified water is pumped into a storage tank and then stored, from where it is distributed to the homes and other buildings on the farm.
4. Used water (drinking, washing, and sewage) is pumped back to septic tanks at the treatment plant. This wastewater then goes through a rotating wheel (which looks like a car wash machine) where solids are removed.
5. The sewage water is released to flow through a wetland that leads to the main dam.
7. According to feedback, the sewage inflow is currently about 0.1% of the dam's volume.
8. The dam water is then pumped back into the Jojo tanks for purification

### C. Areas of Concern:

1. The contaminated water is left in a wetland to be naturally purified before flowing into the main dam, from where water is extracted and purified for consumer use
2. Currently, there are few homes inhabited, resulting in limited sewage. As more homes are built, there will be more consumers, more sewage, and greater contamination. This could negatively impact the water purification process in the long term.
3. **Chlorine used in the current purification process and for killing bacteria and algae is harmful to nature, animals, and humans..**

#### D. Viprobac's Benefits:

1. In contrast to chlorine, testing has found no snail deaths during purification tests where Viprobac was used.
2. The tests confirm the safety of the product for animals, plants, and humans when used in controlled recommended doses.
3. An added benefit of Viprobac (its copper/zinc concentrate) is that it can also be used as a fertilizer for various crops and plants while killing bacteria, protozoa, and other pathogens.

#### E. Viprobac as a Solution

There are two points in the water purification process at Crossways Farm Village where Viprobac can be applied:

1. In the water purification process where water from the dam is treated.  
The recommendation here is to replace chlorine with Viprobac's copper/zinc concentrate.
2. Viprobac water treatment can also be used before the sewage water is released into the wetland, should this ever become necessary.

#### F. See the following attachments:

1. A flow diagram of the current water treatment system at Crossways.
2. A summary of tests conducted by Stellenbosch University.  
Tests conducted with Viprobac at Stellenbosch University under the leadership of **Prof T E Cloete. MSc (UFS), DSc (UP), DSc (US).**



**Pieter Lourens**

202/01/22





# Crossways Farm Village - Watersuiwering Vloeiagram

**Viprobac**  
Water purification & disinfectant



Gesuiwerde  
drink water in huis



Gesuiwerde water  
(in hou dam vir huislike gebruik)



Water gaan deur suiweringsproses  
Chloor skadelik vir die natuur en mens



Water na hou tenks



Dam  
(111 000 m<sup>3</sup> lit)



Vleiland



Riool water vir Vleiland



Riool (was wiel)



Riool/Afvalwater



**Viprobac**  
Water purification & disinfectant

Environmental friendly: Contains Copper (Cu<sub>2</sub>), Zinc (Zn) & Water solution (H<sub>2</sub>O)  
Effective against common viruses, bacteria, and protozoa  
(plus growth stimulant for plants, vegetables and crops)



# Minimum concentration determination of a VIPROBAC (copper/zinc biocide) on two supplied samples.

## VIPROBAC as a Disinfectant for Treated Wastewater

**VIPROBAC** (Copper/Zinc biocide) has garnered attention as a sustainable and effective disinfectant for treated wastewater, offering antimicrobial properties and minimal chemical by-product formation. This review examines 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 ( $\text{Cu}^{2+}$ ) 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.

## Advantages

1. **Broad-Spectrum Antimicrobial Efficacy:** Copper is effective against a wide range of pathogens, including chlorine-resistant microorganisms such as *Cryptosporidium*.
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.
3. **Chemical Stability:** Copper does not react with organic matter to form harmful disinfection by-products (DBP's) like trihalomethanes (THM's) or haloacetic acids (HAA's).
4. **Sustainability:** Copper is naturally occurring and reusable, aligning with circular economic principles.

## Applications for Wastewater Treatment

**VIPROBAC** is particularly suited for tertiary wastewater treatment, where it can serve as a disinfectant for reclaimed water used in irrigation, industrial processes, or urban reuse. It is also used as a secondary disinfectant to maintain residual antimicrobial activity in distribution systems.

## 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.

## Comparative Analysis

Compared to traditional disinfectants like chlorine, copper offers the benefit of reduced DBP's 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.

**VIPROBAC** is a promising alternative disinfectant for treating 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.

*The objective of this study was to determine the minimum effective concentration.*

## Materials and methods

### •Samples received

VIPROBAC sample

Raw Dam Water Sample

Before Wetland Sample

### •Microbiological Analysis

The total number of bacteria was determined using a standard dilution series and plating out on Nutrient Agar. To determine the bacterial number the dilution yielding between 30 and 300 colonies was used for accuracy, as is standard practice.

The biocide effectivity was tested by adding a 1/10 (1ml/10 ml of sample) and at 1/100 up to 1/1 000 000 dilution. The contact time was 5 minutes per dilution. Each dilution was plated out to determine the number of bacteria in each dilution using a standard dilution series and plating out on Nutrient Agar.

### •Kill percentage

The following formula was used to determine the kill percentage:

**Total number of bacteria – surviving number of bacteria/ total number of bacteria x 100.**

## Results and discussion

The results in Table 1 indicate that the biocide was very effective in the 1/10 dilution killing

100% of all the bacteria in both samples. The 1/100 dilution resulted in a 95,7 % kill percentage and the 1/1000 dilution resulted in a kill percentage of 88,8% in the Raw Dam Water sample

(Table 1). The 1/10 000 dilution was ineffective, and this was also the case for the 1/100 000 dilution and the 1/1 000 000 dilution in the Raw Dam Water sample (Table 1).

The 1/100 dilution resulted in a 98,3% kill rate and the 1/1000 dilution in a 97,1 % kill rate in the Before Wetland sample (Table 1). The 1/10 000 dilution was ineffective, and this was also the case for the 1/100 000 dilution and the 1/1 000 000 dilution in the Before Wetland sample (Table 1).

Table 1. Bacterial numbers per ml after adding different biocide concentrations						
Sample	1/10 dilution	1/100 dilution	1/1000 dilution	1/10000 dilution	1/100000 dilution	1/1000000 dilution
159726_2 Raw Dam Water	ND	218	568	>1000	>1000	>1000
159727_2 Before Wetland	ND	407	712	>1000	>1000	>1000

ND = not detected

Table 2. Total number of bacteria per ml in the supplied samples without biocide added.						
Sample	10 <sup>-1</sup> dilution	10 <sup>-2</sup> dilution	10 <sup>-3</sup> dilution	10 <sup>-4</sup> dilution	10 <sup>-5</sup> dilution	10 <sup>-6</sup> dilution
159726_2 Raw Dam Water	449	51	3	ND	ND	ND
159727_2 Before Wetland	>1000	248	23	1	ND	ND

ND = not detected

The total number of bacteria in the Raw Dam Water was 5100 bacteria/ml (**10<sup>-2</sup> dilution**), and in the Before Wetland Sample the count was 24 800 bacteria/ml (**10<sup>-2</sup> dilution**) (Table 2). The Before Wetland sample had a much higher bacterial load than the Raw Dam Water sample.

### Conclusions and recommendations

- The total number of bacteria in the Before Wetland sample was significantly higher than in the Raw Dam Water sample. This suggests that the Wetland is effective in reducing the number of bacteria.
- The biocide was effective in reducing the bacterial numbers in both samples up to a dilution of 1/1000 after a contact time of 5 minutes.

It is important to note that this was a once of test. It will be necessary to conduct more tests to optimize the concentration of the biocide (**VIPROBAC**) and contact time.

A longer contact time will significantly improve the kill percentage.

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## HERE FOLLOWS AN EXTENDED CONTACT TIME TEST

### **Title: Efficacy test of a Copper/zinc biocide (VIPROBAC)**

**Prof T E Cloete. MSc (UFS), DSc (UP), DSc (US).**

### Abstract

**VIPROBAC** (Copper/zink biocide), an emerging alternative, exhibits potent antimicrobial effects due to its ionization and disruption of microbial membranes. An experiment 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. 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<sup>2+</sup>) 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).

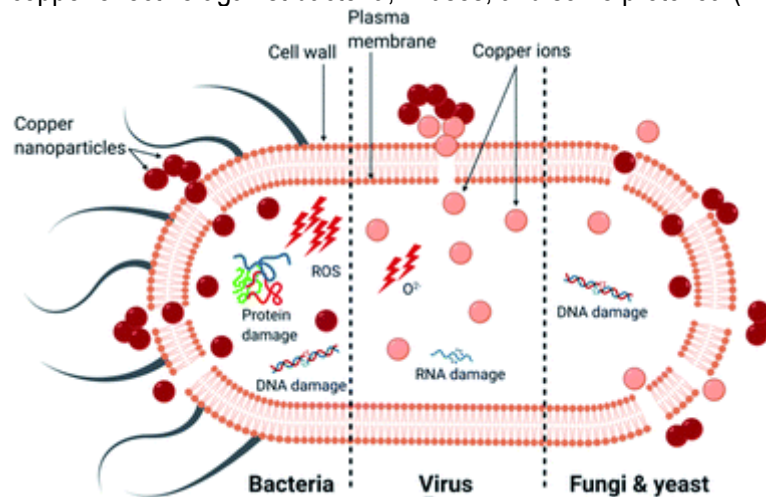


Diagram 1. The primary mechanism of death in different microorganisms by copper nanoparticles (After: Intisar et al., 2021).

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,  $\text{Cu}^+$  and  $\text{Cu}^{2+}$ , 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 (THM's) or haloacetic acids (HAAs) (Gadi, 2012).
4. **Sustainability:** Copper is naturally occurring and reusable, aligning with circular economy principles (Gadi, 2012).

### 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 used copper as disinfectant for final treated sewerage effluent. The efficacy tests assessed disinfection efficiency by measuring reductions in coliforms, *Escherichia coli*, and heterotrophic bacteria.

## Results and discussions

Table 1. Once of treatment at 150ppm

150 ppm treatment			Reduction %	Contact time hour
HPC/ml	5 050	460	90.9%	1,5h
Coliforms/100ml	50 000	450	99.1%	1,5h
<i>E coli</i> /100ml	22 000	220	99.0%	1,5h

**Table 1** 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

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**DATE:**

**7 January 2025**

**Minimum concentration determination of a copper/zinc biocide on two supplied samples.**

**Copper as a Disinfectant for Treated Wastewater**

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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 ( $\text{Cu}^{2+}$ ) 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.

**Advantages**

1. **Broad-Spectrum Antimicrobial Efficacy:** Copper is effective against a wide range of pathogens, including chlorine-resistant microorganisms such as *Cryptosporidium*.
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.

3. **Chemical Stability:** Copper does not react with organic matter to form harmful disinfection by-products (DBPs) like trihalomethanes (THMs) or haloacetic acids (HAAs).
4. **Sustainability:** Copper is naturally occurring and reusable, aligning with circular economy principles.

### 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.

### Applications in Wastewater Treatment

Copper is particularly suited for tertiary wastewater treatment, where it can serve as a disinfectant for reclaimed water used in irrigation, industrial processes, or urban reuse. It is also used as a secondary disinfectant to maintain residual antimicrobial activity in distribution systems.

### 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.

### Comparative Analysis

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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.

The objective of this study was to determine the minimum effective concentration.

## **Materials and methods**

- **Samples received**

Biocide sample

Raw Dam Water Sample

Before Wetland Sample

- **Microbiological Analysis**

The total number of bacteria was determined using a standard dilution series and plating out on Nutrient Agar. To determine the bacterial number the dilution yielding between 30 and 300 colonies was used for accuracy, as is standard practice.

The biocide effectivity was tested by adding a 1/10 (1ml/10 ml of sample) and at 1/100 up to 1/1 000 000 dilution. The contact time was 5 minutes per dilution. Each dilution was plated out to determine the number of bacteria in each dilution using a standard dilution series and plating out on Nutrient Agar.

- **Kill percentage**

The following formula was used to determine the kill percentage:

**Total number of bacteria – surviving number of bacteria/ total number of bacteria x 100.**

## **Results and discussion**

The results in Table 1 indicate that the biocide was very effective in the 1/10 dilution killing 100% of all the bacteria in both samples. The 1/100 dilution resulted in a 95,7 % kill percentage and the 1/1000 dilution resulted in a kill percentage of 88,8% in the Raw Dam Water sample



(Table 1). The 1/10 000 dilution was ineffective, and this was also the case for the 1/100 000 dilution and the 1/1 000 000 dilution in the Raw Dam Water sample (Table 1).

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<b>Table 1. Bacterial numbers per ml after adding different biocide concentrations</b>						
<b>Sample</b>	<b>1/10 dilution</b>	<b>1/100 dilution</b>	<b>1/1000 dilution</b>	<b>1/10000 dilution</b>	<b>1/100000 dilution</b>	<b>1/1000000 dilution</b>
<b>159726_2 Raw Dam Water</b>	ND	218	568	>1000	>1000	>1000
<b>159727_2 Before Wetland</b>	ND	407	712	>1000	>1000	>1000

ND = not detected

<b>Table 2. Total number of bacteria per ml in the supplied samples without biocide added.</b>						
<b>Sample</b>	<b>10<sup>-1</sup> dilution</b>	<b>10<sup>-2</sup> dilution</b>	<b>10<sup>-3</sup> dilution</b>	<b>10<sup>-4</sup> dilution</b>	<b>10<sup>-5</sup> dilution</b>	<b>10<sup>-6</sup> dilution</b>
<b>159726_2 Raw Dam Water</b>	449	51	3	ND	ND	ND
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The total number of bacteria in the Raw Dam Water was 5100 bacteria/ml (**10<sup>-2</sup> dilution**), and in the Before Wetland Sample the count was 24 800 bacteria/ml (**10<sup>-2</sup> dilution**) (Table 2). The Before Wetland sample had a much higher bacterial load than the Raw Dam Water sample.

## Conclusions and recommendations

- The total number of bacteria in the Before Wetland sample was significantly higher than in the Raw Dam Water sample. This suggests that the Wetland is effective in reducing the number of bacteria.
- The biocide was effective in reducing the bacterial numbers in both samples up to a dilution of 1/1000 after a contact time of 5 minutes.

It is important to note that this was a once of test. It will be necessary to conduct more tests to optimize the dosing concentration of the biocide and contact time. A longer contact time will significantly improve the kill percentage.

A handwritten signature in black ink, appearing to read 'C. Cloete', written in a cursive style.

Prof C Cloete

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

**Prof T E Cloete. MSc (UFS), DSc (UP), DSc (US).**

## **Abstract**

Copper, an emerging alternative, exhibits potent antimicrobial effects due to its ionization and disruption of microbial membranes. An experiment 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. 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 ( $\text{Cu}^{2+}$ ) 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).

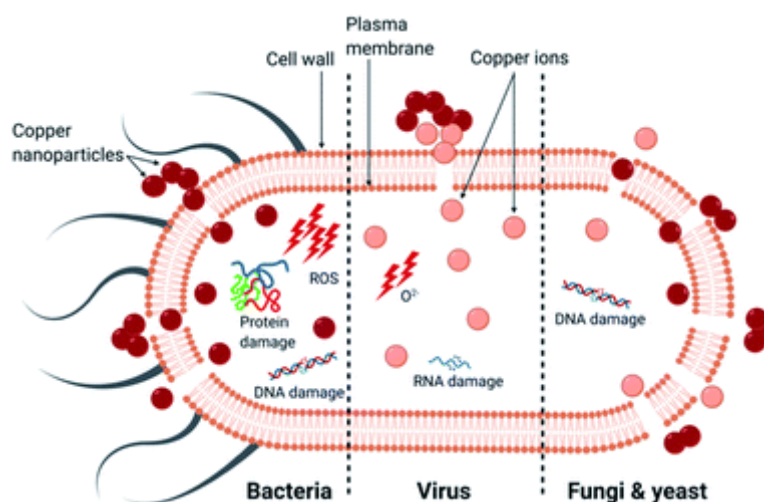


Diagram 1. The primary mechanism of death in different microorganisms by copper nanoparticles (After: Intisar et al., 2021).

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.<sup>18–20</sup> The final mechanism is the release of copper ions,  $\text{Cu}^+$  and  $\text{Cu}^{2+}$ , 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 used copper as disinfectant for final treated sewerage effluent. The efficacy tests assessed disinfection efficiency by measuring reductions in coliforms, *Escherichia coli*, and heterotrophic bacteria.



## Results and discussion

**Table 1. Once of treatment at 150ppm**

150 ppm treatment			Reduction %	Contact time h
HPC/ml	5 050	460	90.9%	1,5h
Coliforms/100ml	50 000	450	99.1%	1,5h
<i>E coli</i> /100ml	22 000	220	99.0%	1,5h

**Table 1** 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 reduction. 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