Silver and zinc oxide nanoparticle disinfection in water treatment applications: synergy and water quality influences

Robbie A. Venis and Onita D. Basu
Department of Civil and Environmental Engineering, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada K1S 5B6
*Corresponding author. E-mail: robbie.venis@carleton.ca

ABSTRACT

The synergistic potential of silver and zinc oxide nanoparticles for water disinfection was investigated herein. By causing cell death through membrane interactions, oxidative killing, and DNA deactivation, metallic nanoparticles may be integrated with point-of-use water treatment systems for applications in rural and remote geographies. Disinfection efficacy was evaluated in batch-phase experiments under both synthetic and real water conditions, where synthetic water was varied by pH and dissolved oxygen levels. Ceramic pot filters with comparative nanoparticle concentrations were also investigated. In all cases, combinations of silver and zinc nanoparticles resulted in improved disinfection in comparison to either metal in isolation. In batch experiments, dissolved oxygen proved to be particularly impactful, with kinetic rates reducing approximately 45% when in low oxygen environment (<3 mg/L) versus high oxygen (>8 mg/L). Log removal values (LRVs) were further, on average, 31% lower in real water than synthetic water after 300 min, though silver–zinc combinations were still superior to either metal alone. In filters, those impregnated with 67% silver and 33% zinc achieved average LRVs of 2.7 and 2.9 after 60 min of filtration and 24 h of storage, respectively, while those with only silver achieved average LRVs of 2.0 and 3.1 at those same times.

Key words: ceramic water filters, drinking water, low-cost disinfection, metallic nanoparticles, rural water supply

HIGHLIGHTS

• Combining silver–zinc oxide nanoparticles improves disinfection in batch-phase and ceramic pot filter experiments.
• Nanoparticle disinfection is vulnerable to changing water quality and particularly dissolved oxygen.
• Zinc oxide supplementation of silver simultaneously improves effectiveness and reduces costs of water treatment solutions.
INTRODUCTION

Nearly 1,500 children under 5 years young died from diarrheal diseases every day of 2017, due in large part to drinking water that is microbially contaminated (WHO 2017; WHO & UNICEF 2019). These deaths, however, were not spread evenly across nations but were concentrated in those with the world’s poorest and most vulnerable populations. Specifically, 96% occurred in UN-defined Developing nations, compared with 0.03% in those defined as Developed, illustrating the clear relationship between health and economic status (UNICEF 2015). Furthermore, infant mortality, and diarrhea particularly, impacts rural communities in much greater numbers than their urban counterparts (Poel et al. 2009; Yaya et al. 2019), exacerbating the social and economic inequities that maintain a system of marginalization (World Bank Group 2016; UNECOSOC 2017).

Point-of-use water treatment systems (POUWTS) have, moreover, been highlighted as a solution to address concerns with safe water access in rural contexts, with a diversity of options available on the international market (Santos et al. 2016). In addition, while chlorine remains the most popular choice in developed systems for disinfection, access in rural areas is often more challenging. Furthermore, there are persistent complaints from individuals who find POU chlorination products unappealing due to the residual taste they leave in water, which then leads to disuse; in addition, the necessitated recurring cost of single-use chlorine products (approximately $0.10 USD/week) have also proved problematic (Sobsey et al. 2008; Luoto et al. 2012; Santos et al. 2016).

One set of alternatives that have garnered notable attention for enhanced disinfection is metallic nanoparticles, due in large part to the weight to volume ratios achieved at such a small scale (Qu et al. 2013). As such, research into nanoparticle disinfection has included applications in conventional centralized drinking water and wastewater treatment processes, as well as home-based, point-of-use water treatment technologies (Qu et al. 2013; Slavin et al. 2017; Prathna et al. 2018). Silver nanoparticles (AgNPs) are the most commonly investigated metal species due to their long history as an applicant for medical treatment, as well as their observed superiority to other metals in terms of bacterial disinfection (Rai et al. 2009; Malachova et al. 2011; Al-Issai et al. 2019). For example, Al-Issai et al. (2019) investigated silver (Ag), zinc oxide (ZnO), magnesium oxide (MgO), and copper (Cu) for disinfection in reverse osmosis and multistage filtration treated water and showed that AgNPs achieved a log removal value (LRV) of 2.43 ± 0.03, whereas ZnO, MgO, and Cu achieved LRVs of 1.80 ± 0.25, 0.77 ± 0.04, and 0.37 ± 0.08, respectively (Al-Issai et al. 2019).
Concerns, however, remain regarding silver’s health, economic, and technical impacts, hindering its applicability to POUWTS. Specifically, over-consumption of silver can lead to negative health outcomes such as Argyria or even DNA damage (Fewtrell et al. 2017), leading the World Health Organization (WHO) to recommend a 0.1 mg/L limit on silver concentration in drinking water; this concentration ensures less than 10 g of silver be consumed over a 70-year lifespan (WHO 2011). Silver is also a notably expensive material, with a cost of approximately $3 USD per gram (Argenol Laboratories, personal communication, 2017). Though variability exists depending on nanoparticle formulation (e.g. solid or aqueous), as well as by providers and location, its inclusion in water treatment technologies intended for individual use can nonetheless contribute to such products being unaffordable for low-income consumers, in particular, further limiting their widespread applicability as an antimicrobial solution to those most in need (Burt et al. 2017). Investigations of alternative metal species that can contribute to disinfection efficacy with decreased long-term potential health impacts are thus required.

ZnO has demonstrated promise as a potential alternative due to its own antimicrobial properties under diverse conditions (Sirelkhatim et al. 2015; Dimapilis et al. 2018). Furthermore, research has shown that zinc can substantially reduce the incidence and severity of diarrheal episodes when ingested, having a doubly positive effect as a water purification solution (Gitanjali & Weerasuriya 2011; Malik et al. 2013). Finally, zinc oxide not only has a cost of approximately $0.09 USD per gram, more than 30 times less than that of silver (Sigma-Aldrich 2021), but may be commonly found locally and is thus often more accessible than silver; zinc accessibility should, however, be evaluated on a case-by-case basis.

While silver has been shown to exhibit greater disinfection efficacy than zinc alone (Malachova et al. 2011; Al-Issai et al. 2019), investigation into the potential replacement or supplementation of silver with co-nanoparticle application is understudied. Of the limited research available to date in this area, most findings are intended for medical applications, whereas water treatment applications, and particularly applications within low-cost water treatment technologies, remain largely unelucidated (Motshekga et al. 2015; Garza-Cervantes et al. 2017). For example, Garza-Cervantes et al. (2017) found 32.7 mg/L of Zn^{2+} combined with 3.21 mg/L Ag^{+} achieved 100% E. coli growth inhibition after 1 h, compared with approximately 30% E. coli growth inhibition with Ag^{+} alone. The focus of this work on bacteriostasis within culture media for medical purposes does, however, lend itself to questions regarding how co-metal disinfection may be applied in an aquatic environment for bactericidal water treatment. Similarly, Motshekga et al. (2015) examined bacteria removal based on a combined solid-phase adsorption and disinfection and reported a silver–zinc disinfection synergy, but their work did not examine direct nanoparticle interventions under drinking water applications, or if such findings may be translated to technology. Finally, some recent research has also highlighted the potential of co-metal disinfection within technological applications; however, the limited literature in this domain has focused particularly on silver–copper and silver–iron combinations with variable results (Brown & Sobsey 2009; Guerrero-Latorre et al. 2015; Lucier et al. 2017; Ehdaei et al. 2020). An important research gap thus remains in terms of how combined metal nanoparticles, and silver–zinc combinations specifically, may synergistically disinfect bacteria within a water treatment application, and particularly within point-of-use water treatment technology.

In this research, Ag and ZnO nanoparticle disinfection of E. coli is investigated in isolation and in combination to evaluate their respective and joint antibacterial efficacies for water treatment applications. Nanoparticles are characterized, and bacterial destruction is observed by scanning electron microscopy (SEM). Antibacterial performance in batch-phase experiments is evaluated against several water quality parameters, which is lacking in the literature. Specifically, disinfection efficacy and kinetics with co-metal addition of silver and zinc nanoparticles under varying pH and DO conditions are studied. Furthermore, full-scale ceramic pot filters with corresponding silver–zinc nanoparticle ratios are investigated for bacteria removal using a real water source to determine technological applicability. Moreover, the research into synergistic silver–zinc metal nanoparticle disinfection for water treatment applications highlights a novel methodology that has the capacity to be easily incorporated into point-of-use technologies, improving water treatment options for lower-income and remote consumers in terms of both effectiveness and affordability.

**MATERIALS AND METHODS**

**Scanning electron microscopy**

SEM (Nano Imaging Facility, Carleton University, ON, Canada) was used to determine the size range for silver and zinc nanoparticles in both solid and aqueous phases. 1 mg/mL suspensions of each species were created and...
mixed gently for 5 min, and subsequently allowed to sit in an aqueous phase for 5 h. 1 mL of solutions were then pipetted atop a 0.45 μm filter paper and left to dry inside a desiccator overnight. A small piece of the paper was cut and adhered to a sample mount with double-sided adhesive tape and sputter-coated with gold before entrance into the SEM imaging hood. Energy dispersive spectroscopy (EDS) was used to determine which parts of the sample contained the specific nanoparticle material, as well as when both elements were present if the sample was a mixture.

Bacterial imaging under four specific challenge conditions was also conducted using SEM. Namely, no metals, 1 mg/L Ag, 1 mg/L ZnO, and 0.67 mg/L Ag and 0.33 mg/L ZnO together were added to 10^5 CFU/mL solutions, which were agitated at 50 RPM overnight. Dehydration and fixation then followed a modified procedure described by Iqbal et al. (2012). Briefly, after agitation, samples were gently mixed by hand and poured over 25 mm diameter of 0.2 μm nylon filter papers (Millipore). The filter papers were then placed within marked aluminum dishes and suspended within a 4% glutaraldehyde mixture within a 0.1 M phosphate buffer solution (75% Na₂HPO₄, 25% NaH₂PO₄) for 60 min. After 60 min, the glutaraldehyde solution was filtered again atop the papers, which were then twice resuspended in a 0.1 M PBS wash for 10 min. After washing, a 1% tannic acid solution within a 0.1 M PBS buffer was added and papers were suspended within the solution for 1 h. After said time, the tannic acid solution was filtered through the papers, which were then washed twice with distilled water for 10 min. The dehydration process commenced thereafter: papers were sequentially suspended within ethanol (EtOH) solutions of the following concentrations: (1) 35% EtOH, (2) 50% EtOH, (3) 75% EtOH, (4) 95% EtOH (×2), (5) 100% EtOH (×2). After dehydration, the aluminum dishes containing the papers were placed in a desiccator and allowed to dry overnight. A small piece of the filter papers was then cut with a specialized hole puncher and mounted on an SEM stub with double-sided tape. The samples were then sputter-coated with gold and loaded into the SEM viewing cabinet.

Nanoparticle characterization

Before suspension in water, silver nanoparticles have sharp edges and rigid bodies, ranging from approximately 100 to 600 nm in size. As shown in Figure 1(a), after suspension for 5 h, AgNPs dissolved into smaller particles ranging from 34 to 58 nm or agglomerated to form larger particles up to approximately 2 μm in diameter. ZnO nanoparticles are box-shaped rods before suspension in water, with diameters ranging from approximately 40 to 440 nm. After suspension, the shape of the particles does not change significantly, though agglomeration and dissolution occur and particle sizes change slightly to be between 30 and 680 nm (see Figure 1(b)). These results align with previous observations from the literature that noted ZnO is relatively stable in water and thus does not rapidly dissolve (Sirelkhatim et al. 2015).

When silver and zinc are added to water simultaneously, ZnO appears to agglomerate around the AgNPs and form large mixed particles (Figure 1(c)), as confirmed by EDS analysis (data not shown). Specifically, silver was only detected in the presence of zinc, and in a concentration of approximately 5% of the spectra, compared with approximately 70% for zinc.

Figure 1 | Scanning electron microscopy images of (a) silver nanoparticles after suspension in water, (b) zinc oxide nanoparticles after suspension in water, and (c) both zinc oxide and silver nanoparticles suspended in water in even molar proportions.
Challenge water solutions

Both synthetic and natural waters were examined in this research. Synthetic challenge water was created as per Table 7 of USEPA ‘Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms’ (USEPA 2002), intended to match the alkalinity of the batch-tested natural water source. Natural water for batch testing was taken from Ottawa River at the Britannia Water Treatment Plant intake (Ottawa, Canada), while ceramic pots were challenged by natural water drawn from a nearby stream to the Nelson Mandela African Institute of Science and Technology (NM-AIST) in Checkereni, Arusha, Tanzania. A summary of the synthetic water and the natural water quality conditions are presented in Table 1.

pH was adjusted before testing to approximately 6.4, 7.4, and 8.4 using 1N HCl and NaOH solutions as needed, described as Low pH, Mid pH, and High pH in Table 1, respectively. Low DO testing water was deoxygenated with compressed N₂ gas sparged into the water to maintain oxygen levels just above hypoxic conditions. These specific water quality parameters were chosen to align with the theoretical basis by which metallic ions and reactive oxygen species (ROS) are released by nanoparticles, as per either of Reaction (1) or (2).

\[
\text{NP} + \text{O}_2 \rightarrow \text{ion} + \cdot \text{O}_2 \tag{1}
\]
\[
\text{NP} + \frac{1}{2} \text{O}_2 + 2\text{H}^+ \rightarrow 2\text{ion} + \text{H}_2\text{O} \tag{2}
\]

where NP represents either AgNP or ZnO and ion is the ionic version of the associated metal in reacting (either Ag⁺ or Zn²⁺).

Bacterial cultivation and enumeration

K-12 (ATCC® 29947) *Escherichia coli* (Migula) Castellani and Chalmers were purchased from Cedarlane laboratories as a dry pellet. The pellet was hydrated with 1 mL of No. 3 Nutrient Broth and centrifuged for 10 s. 200 μL of *E. coli* stock was added to 9,800 μL of nutrient broth and incubated at 37 °C for 1 h. 1 mL of sterile, pure glycerol was added to the stock solution, after which the stock was incubated again for 3 h and subsequently divided into 200 μL aliquots, which were stored in 2 mL sterile tubes at −20 °C.

For testing purposes in batch experiments, the frozen stock was thawed at room temperature for 5–10 min, and 100 μL was added to 9,900 μL of No. 3 Nutrient Broth and incubated at 37 °C for 18–19 h, the beginning of the stationary growth phase. 1 mL of this final culture was then added to 1 L of challenge water, yielding approximately 10⁷ CFU/mL initial bacterial concentration. For pot experiments, fecal droppings from goats shepherded near NM-AIST were collected and submerged in water from the stream for 1–2 h before testing to achieve elevated levels of *E. coli* contamination that was present immediately after extraction.

*E. coli* concentrations during testing were enumerated as per EPA Method 1604 (United States Environmental Protection Agency 2002). 100 μL was taken from the experimental vessel at each measurement point and added to 900 μL of dilution water (0.5% MgCl₂, 0.125% KH₂PO₄) in a sterile, 2 mL conical vial. The sample was then diluted serially up to four steps, and samples were filtered through a 47 mm diameter, 0.45 μm pore membrane filter paper. The paper was then placed atop MI Agar Media (Thomas Scientific) and incubated for 24 h. Colonies were counted under black light by hand.

Disinfection performance of metal nanoparticle species

Disinfection efficacy and kinetics of silver and zinc nanoparticles in batch experiments were evaluated in both synthetic and natural water. The pH and DO levels were varied within the synthetic water matrix as illustrated in Table 1. The natural water was not adjusted. Prior to testing, 1 mg/mL solutions of AgNPs (Argenol Laboratories, Spain) and ZnO (Puriss, >99% (KT), Sigma-Aldrich) were created, which were subsequently added to 1 L of challenge water in the appropriate volumes to yield concentrations ranging from 0 to 1 mg/L (see Table 2). *E. coli* samples were collected at seven time points: 0, 10, 20, 30, 60, 120, and 300 min after metals were added to the challenge water. All samples were taken in duplicate. All water chemistry conditions and metal combinations were also tested twice (i.e., n = 4 per sample). Challenge water was constantly mixed at 50 RPM with two sterilized flat-blade propellers. Before experimentation, beakers were sterilized in an autoclave and wrapped on the outside with aluminum foil to prevent light penetration and ensure dark conditions throughout testing.

As demonstrated herein, a log-linear relationship between disinfection and time was observed for all metal nanoparticles evaluated. As such, the Chick-Watson Model for disinfection shown in the following equation
### Table 1 | Water quality characteristics of experimental conditions evaluated

<table>
<thead>
<tr>
<th>Water quality characteristics</th>
<th>Synthetic water</th>
<th>Natural water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ottawa River Water (Canada)</td>
<td>Checkereni Stream Water (Tanzania)</td>
</tr>
<tr>
<td>pH</td>
<td>6.40 ± 0.03</td>
<td>7.32 ± 0.10</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>2.51 ± 0.40</td>
<td>10.34 ± 0.89</td>
</tr>
<tr>
<td>ORP (mV)</td>
<td>15 ± 2</td>
<td>13 + 2</td>
</tr>
<tr>
<td>Alkalinity (mg/L CaCO₃)</td>
<td>23.7 ± 6.7</td>
<td>30.2 ± 5.8</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20.5 ± 0.5</td>
<td>20.8 ± 1</td>
</tr>
<tr>
<td>Initial E. coli concentration (10⁵ CFU/mL)</td>
<td>8.61 ± 1.11</td>
<td>8.89 ± 2.76</td>
</tr>
</tbody>
</table>

DO, dissolved oxygen; ORP, oxidation-reduction potential; CFU, colony forming units.
could be applied:

$$\ln \left( \frac{N_t}{N_0} \right) = -kCt$$  (3)

where $N_t$ is the E. coli concentration (CFU/mL) at time $t$, $N_0$ is the initial E. coli concentration, $k$ is the reaction rate constant (h⁻¹), $C$ is the concentration of disinfectant (mg/L), and $t$ is time in hours. Furthermore, because all of these experiments were conducted with 1 mg/L of disinfectant, Equation (3) may be rewritten as Equation (4), allowing for the enumeration of a kinetic rate. Both LRVs and kinetic rates are thus used for evaluation.

$$k = -\left( \frac{1}{t} \right) 2.303 \log \left( \frac{N_t}{N_0} \right)$$  (4)

**Ceramic pot filters**

**Fabrication**

Pot filters were fabricated at the Wine to Water East African (W2WEA) facility in Arusha, Tanzania. Clay and sawdust (pine wood) were first mixed in 5:4 (C:SD) volumetric ratio until homogeneous. Silver, zinc, or a mixture of the two was then impregnated within the filters via the co-firing method, where nanoparticles were dissolved into water and added to the dry ingredients to achieve a total metal concentration of 7.5 mg/kg filter. The wet mixture was then kneaded, shaped using a manual press, and labeled. Filters were thereafter left to air dry for 3 weeks, after which they were fired in a kiln up to 960 °C over 12 h. Each filter then passed 20 L of clean water through the matrix before bacterial testing.

**Experimentation**

In addition to no metal controls, filters were produced with metal mass ratios of (1) 100% Ag, (2) 67% Ag and 33% ZnO, (3) 33% Ag and 67% ZnO, and (4) 100% ZnO. Three filters were produced in each metal ratio, and each filter was tested twice (i.e. $n = 6$). Any filter with a measured flowrate greater than 4 L/h was, however, excluded, as per the exclusion criteria for sale used by W2WEA. During testing, filters were filled to achieve a hydraulic head of 25 cm above the frustum (pot bottom), which was maintained for 60 min. Filtered samples were collected from the storage container after 30 and 60 min of filtration, as well as after 24 h of storage in a darkened space. Influent water quality was evaluated before challenging the filters, whereas the filtered water quality was evaluated only after 60 min of filtration and after storage. Figure 2 illustrates a schematic of the experimental setup.

**Statistical analysis**

**Nanoparticle disinfection**

The significance of each of the treatments described was evaluated through multiple two-way ANOVAs. For experiments conducted in synthetic water, differences in LRV and kinetic rates achieved by the metal nanoparticle combinations were compared across differing pH and DO conditions at each individual measurement time. The impact of natural water on these same response variables was evaluated similarly, where nanoparticle concentrations and naming convention

<table>
<thead>
<tr>
<th>Metal nanoparticle combinations and naming convention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Massive concentrations</td>
</tr>
<tr>
<td>No metal control</td>
</tr>
<tr>
<td>1 mg/L ZnO</td>
</tr>
<tr>
<td>1 mg/L Ag</td>
</tr>
<tr>
<td>0.67 mg/L Ag, 0.33 mg/L ZnO</td>
</tr>
<tr>
<td>0.33 mg/L Ag, 0.67 mg/L ZnO</td>
</tr>
<tr>
<td>0.14 mg/L Ag, 0.86 mg/L ZnO</td>
</tr>
</tbody>
</table>
performance was compared against that in synthetic water of the same approximate pH level (pH = 7.4). Post hoc analysis using Tukey’s Honest Significant Difference (HSD) was used thereafter to determine specific differences between treatments. All statistical analysis was conducted in Rstudio (stats and base packages).

Ceramic pot performance

Differences between filters were also evaluated using two-way ANOVAs, where time and metal concentrations were used as treatment variables and LRV and percent bacteria removal were each used as measured responses. This was chosen to account for the influence of variability in the initial bacterial concentration of the natural challenge water, as highlighted in Table 1. Post hoc analysis using Tukey’s HSD, as well as one-tailed t-testing, was used for the evaluation of individual effects for the pots.

RESULTS AND DISCUSSION

Disinfection in synthetic water

Comparison of silver and zinc oxide individually

Initial studies examined silver and zinc nanoparticle disinfection efficacy when used individually under varying pH and DO conditions, and as can be observed in Figure 3, silver performance is significantly superior to zinc when water quality conditions are equivalent. For example, at the low pH, high DO level, silver achieves a disinfection kinetic rate of $-0.110 \text{ h}^{-1}$, while zinc achieves a rate of $-0.046 \text{ h}^{-1}$. Meanwhile, at the high pH level, both disinfection kinetic rates decrease slightly: in high DO water, silver and zinc achieved rates of $-0.069$ and $-0.036 \text{ h}^{-1}$, respectively, and in low DO water, they achieved $-0.057$ and $-0.027 \text{ h}^{-1}$, respectively. Moreover, when evaluated individually, it is clear that silver outperforms zinc and would be the preferred disinfectant to improve the removal of *E. coli* for water treatment.

Comparison of combined silver–zinc disinfection

Investigations into the combinatorial effect of silver and zinc nanoparticle application in challenge waters varied by pH and DO highlight both a marked difference with the disinfection of each metal when applied individually, as well as a nanoparticle sensitivity to key water quality parameters more generally. Specifically, as shown in Figure 4, two clear trends are immediately observed. First, under the High DO conditions, silver–zinc combinations outperform either species in isolation. Furthermore, there is a clear trend where $\text{Ag}_{67}\text{ZnO}_{33}$ continually results in the best outcomes, with LRVs of $3.18 \pm 0.23$, $3.00 \pm 0.22$, and $2.91 \pm 0.09$ after 300 min of mixing at pH levels of 6.4, 7.4, and 8.4, respectively.

Secondly, all metallic nanoparticles exhibited a clear and immediate suppression of LRV when high DO conditions are compared with low DO conditions. For example, as shown in Table 3, $\text{Ag}_{33}\text{ZnO}_{67}$ achieved a kinetic rate of $-0.193 \text{ h}^{-1}$ in the high DO, low pH condition, dropping to $-0.072 \text{ h}^{-1}$ in the low DO, low pH condition. With that said, silver–zinc combinations still outperformed either species in isolation ($\alpha = 0.05$), albeit to a reduced degree than observed in the high DO condition. Specifically, as illustrated in Figure 5 and Table 3,
Ag₆₇ZnO₃₃ achieved a kinetic rate of 670.0% higher than zinc alone and 173.3% higher than silver alone in high DO, mid pH challenge water, but only 102.8 and 101.8% higher than the two species, respectively, when DO was decreased. Dissolved oxygen was therefore demonstrated to have a profound influence on the disinfection performance of all metals tested, which has particularly important implications for field-based applications of nanoparticles for disinfection. As algal blooms, high fecal matter concentrations, agriculture runoff, and other environmental factors can lead source waters to hover around or fall into hypoxia, the value of the metals may be drastically reduced if exposed to such conditions.

Overall, pH did not significantly impact LRV, indicating some robustness to changing pH levels in a local water source. Others have made a similar observation when examining silver only (Fauss et al. 2014), though mechanistic investigation of DO and pH impacts on silver–zinc combined performance remains largely unelucidated.

Garza-Cervantes et al. (2017) hypothesize that the mechanism behind the observed synergistic disinfection results from an increased cell membrane permeability observed when nanoparticles are added in combination, in comparison to when added individually (Garza-Cervantes et al. 2017). Yamamoto & Ishihama (2005) further reported when cells were suddenly ‘shocked’ by an influx of zinc in concentrations higher than 0.065 mg/L (1 μM), zinc-binding proteins began rapid gene transcription to maintain intracellular zinc homeostasis, which subsequently resulted in the synthesis of intracellular cysteine, a highly reactive thiolated amino acid. Cysteine production and the complex and numerous reduction-oxidation (redox) reactions that resulting ensue are further known to both increase cell membrane permeability through increasing the active sites available for cationic metal binding (Giles et al. 2003) and lead to cell degradation directly through transient trapping of metals (Yamamoto & Ishihama 2005). Additionally, McDevitt et al. (2011) found that in Streptococcus pneumoniae, extracellular zinc in concentrations higher than 1 μM inhibited cells from absorbing other necessary nutrients, weakening the cells and increasing vulnerability to oxidative killing or bactericide by other present disinfectants, if applicable (McDevitt et al. 2011).

As zinc concentrations in this research were above 4 μM under all experimental conditions, it is hypothesized that E. coli cells were sufficiently ‘shocked’ by the zinc, leading to transient trapping of zinc within the cell, an
increase in active sites along the cell membrane for bonding with thiolated groups (i.e. active sites for cationic metal binding), and a reduction in nutrient intake that weakened the cell. Comparatively, when zinc is in isolation, these effects thus weaken the cells, but the rate of cell degradation may not be as high as when facilitated with the presence of other metals, as was found in this research. When metal combinations were added, cells became more vulnerable to attack from the silver present within the mixture, which may have (1) caused cell lysis directly by bonding with the cell membrane and causing pits, (2) penetrated the cell membrane and bonded directly with DNA in the cytoplasm, leading to degradation, and/or (3) reacted to create ROS, as shown in Reaction (1), which degraded the cells through oxidative killing (Venis & Basu 2020). As such, the improved disinfection observed with Ag67ZnO33 in comparison to the other metal mixtures thus likely resulted from the higher concentration of silver present. With that said, further research into synergistic mechanics between these two nanoparticle species is needed.

**Figure 4** | LRVs achieved after 300 min of mixing under each of the synthetic water quality conditions evaluated and detailed in Table 1; pH values of 6.4 (low pH), 7.4 (mid pH), and 8.6 (high pH) with high (>8.4 mg/L) and low (<2.5 mg/L) DO.

**Table 3** | Kinetic rates achieved by each metal species and combination under each of the water quality conditions evaluated (bold values indicates the highest rate in each experimental condition)

<table>
<thead>
<tr>
<th>Metal concentration (1 mg/L, Total)</th>
<th>Mass ratio (% (Ag:ZnO))</th>
<th>$k$ (1 h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low pH, low DO</td>
</tr>
<tr>
<td>100:0</td>
<td></td>
<td>−0.035</td>
</tr>
<tr>
<td>67:33</td>
<td></td>
<td>−0.081</td>
</tr>
<tr>
<td>33:67</td>
<td></td>
<td>−0.072</td>
</tr>
<tr>
<td>14:86</td>
<td></td>
<td>−0.070</td>
</tr>
<tr>
<td>0:100</td>
<td></td>
<td>−0.025</td>
</tr>
</tbody>
</table>
SEM imaging
SEM images were collected from E. coli cells without exposure to nanoparticles as well as after exposure to silver, zinc, and a combined solution (Supplementary Material, Figure S1). Furthermore, all imaged cells that were not treated by any nanoparticles remained entirely intact and presented strong visible contrast with the background surface (Supplementary Material, Figure S1A). In Supplementary Material, Figures S1B, C, and D, however, several observations can be made. The cells are elongated, twisted, and appear to have lower contrast with the background surface, indicating that the SEM electrons were actually able to penetrate cellular membrane due to increased permeability from lysis. Pits are also observed at edges on the E. coli cells, indicating further damage to the bacteria. A notably greater number of pits were also observed in cells that were treated with the metallic mixture than those treated by either metal in isolation (see Supplementary Material, Figure S1C, inset), with more pits observed in cells treated by silver than by zinc, as expected.

Real water analysis
Nanoparticle disinfection in natural waters
Though experimentation using synthetic water offers omniscient control of the parameters of interest and therefore allows for improved elucidation of relationships between treatment variables, understanding how those same treatments translate under real water conditions with multi-modal and complex matrices is crucial. As such, nanoparticle disinfection in natural water drawn from the Ottawa River (Ontario, Canada) was also evaluated as a comparison to the synthetic water for the pH 7.4 condition. And as shown in Figure 6, all metallic

![Figure 5](image)

**Figure 5** | Percent difference of kinetic rates from those of 100% ZnO under different pH and DO conditions.

![Figure 6](image)

**Figure 6** | Disinfection achieved by each metal species and combination under synthetic (a) or natural (b) water conditions.
nanoparticle bactericidal efficacy decreased when challenged by natural water compared to the synthetic water. It is important to note, however, that the silver–zinc combinations still outperformed the single component disinfection results. For instance, Ag1, ZnO1, and Ag67ZnO33 achieved LRVs after 300 min of $-1.14 \pm 0.04$, $-0.47 \pm 0.06$, and $-3.01 \pm 0.2$ with the synthetic water conditions versus $-0.55 \pm 0.08$, $-0.29 \pm 0.03$, and $-2.24 \pm 0.23$ in the natural water, respectively ($p < 0.01$). Note again that the Ag67ZnO33 combination in natural waters still outperformed silver alone in the synthetic water.

The observed reductions in the natural water may be attributed in significant part to the impact of natural organic matter (NOM) presence, which was approximately 8 mg/L as dissolved organic carbon in this study. Previous research has demonstrated that NOM can coat the outside of nanoparticles and create a barrier between it and cell, which inhibits interaction and reduces disinfection efficacy (Fabrega et al. 2009; Fauss et al. 2014). Attention to such water chemistry conditions is therefore important for application purposes when metals will commonly be exposed to organic matter. Nevertheless, these results importantly demonstrate that even in a complex (natural) water matrix, the combination of silver with zinc resulted in better disinfection than either metal species alone.

**Ceramic pot filter challenge test**

Results presented above clearly demonstrate that combined silver–zinc nanoparticle disinfection is robustly superior to that achieved by either single metal species across diverse water quality conditions. The investigation of how such observations translate to point-of-use water treatment applications is thus critically important for evaluating their implications in real-world applications. In these tests, samples were collected from the pot filtrate, meaning that observed removals result from both filtration (size exclusion) and nanoparticle disinfection. As such, the control pots (with no metal nanoparticle addition) demonstrate that, as expected, the size exclusion is a key component to improving the water quality in this technology. As shown in Figure 7, however, results are far more variable with these filters than those with metals, and they straddle the WHO guideline of 2 log removal. This observation, therefore, suggests that the nanoparticles support immediate disinfection in the filtrate, as observed by a decreased variance in results; more testing is, however, recommended. Bacterial removals in the control filters also decreased slightly after 24 h of storage when compared to immediately after filtration ($p = 0.13$), indicating a lack of continuous disinfection during storage. Conversely, all filters impregnated with nanoparticles achieved improved bacteria removal levels after 24 h when compared with immediately after filtration,

![Figure 7](https://iwaponline.com/h2open/article-pdf/4/1/114/908747/h2oj0040114.pdf)
as well as when compared to the control (p < 0.01). These results highlight that the nanoparticles predominantly impact bacteria removal after filtration once eluted into the receptacle, and that they importantly add value by helping maintain safe water quality in storage.

The filters containing 67% Ag and 33% ZnO were the only ones to significantly differ (p < 0.01) from the no metal control filters after 60 min, achieving an LRV of 2.60 ± 0.6 compared with 2.10 ± 0.97 for the control filters. Furthermore, more than 25% of evaluated samples for these pots achieved 100% removal after 60 min, increasing to more than 50% after 24 h, meaning that the achievable LRV in these filters was constrained by the influent E. coli concentration. As such, this trial supports the batch-phase experimentation on utilizing a combined silver–zinc matrix to maintain and/or enhance the disinfection of E. coli by ceramic filters. More experiments are therefore recommended on this topic, and specifically in terms of varying metallic concentrations and methods of application (i.e. painting and submergence versus co-firing).

CONCLUSION

Advancements in water treatment practices are necessary if the world is to achieve the UN SDG 6 of providing safe drinking water for all people, and metallic nanoparticle disinfection offers an important opportunity for innovation. In the present work, the disinfection efficacy of combined and isolated zinc oxide and silver nanoparticles were evaluated in (1) batch experiments in synthetic and natural water and in (2) ceramic pot filters challenged by natural water. This research resulted in multiple significant findings:

• AgNP and ZnO demonstrated a synergistic relationship between the two species across pH conditions ranging from 6.4 to 8.4, as well as variable DO conditions.
• E. coli removal was fairly robust to changing pH over the respective nanoparticle solutions tested.
• DO drastically impacted both the achieved disinfection and the associated kinetic rates, proving its significance as a factor in nanoparticle disinfection and highlighting a limitation of this water treatment solution if exposed to low DO waters.
• The best performing Ag/ZnO combo was a 67/33 mass ratio, which demonstrated a maximum average LRV of 3.18 compared to 1.4 for silver and 0.66 for zinc alone.
• Ceramic filters with silver–zinc combinations performed as well as, or in the case of filters with Ag/ZnO ratio of 67/33, superior to, filters with only silver, suggesting that batch-scale experimental results translate to technology application. The inclusion of nanoparticles clearly indicated a beneficial impact on maintaining and improving water quality under storage conditions of 24 h.

The clear improvement in bactericidal efficacy of silver and zinc nanoparticles when added in combination compared with when used in isolation, both in batch and pot phases, highlights a tremendous opportunity for innovative technological advancement in the field where technologies may be improved in terms of both effectiveness and affordability.

ACKNOWLEDGEMENTS

We thank Argenol Laboratories for freely providing the silver nanoparticles in this research. We would also like to thank Wine to Water (nongovernmental organization) for their support and collaboration, as well as all members of the Basu Research Group. This work was supported by the Natural Sciences and Engineering Research Council (NSERC), Canada, and Carleton University. This article is dedicated to Messiaki ‘Kim’ Kimrei (d. 2020), former operator and owner of Safe Water Ceramics of East Africa (now W2WEA). His enthusiasm to explore change and improve the filters was a testament to his desire to bring safe water to his fellow Tanzanians.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES


First received 30 April 2021; accepted in revised form 7 June 2021. Available online 23 June 2021