Cuprous Oxide- or Copper-Coated Jute Stick Pieces at an Air–Water Interface for Prevention of Aerial Contamination in Potable Water

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Supporting Information

ABSTRACT: Cuprous oxide and copper have been synthesized via the solvothermal process using basic copper carbonate as the source of copper. Pure Cu$_2$O or Cu could be afforded by simply varying the solvent while keeping the temperature and time constant. In this study, copper-based materials were coated on jute stick pieces (JSP) in situ. Cu$_2$O-coated JSP (Cu$_2$O-JSP) and Cu-coated JSP (Cu-JSP) were characterized by powder X-ray diffraction (PXRD) and scanning electron microscopy (SEM). Cu$_2$O-JSP and Cu-JSP were found to be active against Escherichia coli NCIM 2931 (Gram-negative bacteria) and Staphylococcus aureus (Gram-positive bacteria). The antibacterial nature of the material and the buoyant nature of Cu$_2$O-JSP and Cu-JSP were exploited to develop beaded necklace-like strands that could be floated on potable water to effectively prevent aerial contamination. Leaching of copper from both Cu$_2$O-JSP and Cu-JSP into water was found to be below the permissible limit for copper in drinking water.

1. INTRODUCTION

Readily accessible water is often contaminated due to environmental pollution, thereby causing serious health issues when consumed as such. Consumption of purified water is essential for healthy living; however, owning a personal water purification unit is not usual for many families in underdeveloped and developing countries. Community water purification plants are the go-to points for fetching purified water by many people. Storage of water is a common practice for eventual uses. However, microbial growth in stored water is inevitable, especially in an unhygienic environment. Thus, safe storage of the fetched water until the time of actual consumption should not be neglected. Global loss of 2.2 million human lives per annum in connection with unsafe drinking water and hygiene-related diseases is a disturbing statistic.

The use of copper metal and its ions as disinfecting materials has been well known since ancient time and practiced by many. Grass et al. reviewed some works related to the contact killing and self-disinfection abilities of copper surfaces. Schmidt et al. reported that the use of copper alloys on stethoscope surfaces can minimize the risk of bacteria transfer from patient to patient. Storage of water in a copper pot is reported to be beneficial, and a claim of Sudha et al. suggests that diarrheagenic bacteria are killed in this manner. Jia et al. demonstrated antibacterial properties of cellulose films coated with copper. Dankovich and Smith coated copper nanoparticles on paper filter and used the coated filter as an efficient material for water purification. Electrochemically synthesized copper oxide was employed against waterborne bacteria, as described by Pandey et al. Abou Neel et al. incorporated copper oxide into degradable phosphate-based glass fibers and demonstrated its potential for healing of wounds. In addition to the antibacterial properties, copper is also known to be effective against various viruses and fungi. Copper is an essential micronutrient, hence beneficial when present at low concentrations; however, it has some toxicity when over-consumed. On the basis of this literature survey, we considered to develop a copper-based material that could prevent aerial contamination and facilitate safe storage of water.

In this work, we developed easy methods to coat cuprous oxide or copper on buoyant jute stick pieces (agricultural waste). The antibacterial potential of copper and the floating nature of the selected variety of wood were found to be an effective combination for safe storage of purified water with respect to prevention of aerial contamination. With the material developed being inexpensive and the leaching being below the permissible level, we anticipate ample practical use of this finding. To the best of our knowledge, there is no study on prevention of aerial contamination in water reported so far.

2. RESULTS AND DISCUSSION

2.1. Selection of Support Material. We reasoned to coat copper on support materials such as buoyant wood pieces and float those on potable water to prevent aerial contamination.

Received: September 27, 2019
Accepted: November 29, 2019
Published: December 17, 2019
Jute sticks were considered as appropriate eco-friendly support materials due to the lightweight and porous nature of their wood. Jute sticks are available in plenty amount as agricultural waste; hence, it has an added economic advantage.

2.2. Cu$_2$O-JSP and Cu-JSP. Synthesis of Cu$_2$O and Cu under solvothermal conditions by refluxing a mixture of Cu(NO$_3$)$_2$ and NaOH at 1:2 ratio in glycerol medium at 140 °C is known in the literature. We wanted to follow this method to prepare copper-JSP by immersing JSP in the reaction medium during the synthesis. However, the method proved unsuitable with respect to our requirement. The JSP lost its hardness due to the delignification in the wood caused by the presence of NaOH. Further, the softened wood shrank when separated, washed, and air-dried. In this way, the coating on JSP got washed out (Scheme S1 and Figures S1 and S3).

To avoid the decomposition of lignocelluloses, it was necessary to avoid NaOH. The use of basic copper carbonate (CuCO$_3$·Cu(OH)$_2$) as the source of copper provided a satisfactory result. Optimized reaction conditions for the synthesis of Cu$_2$O- and Cu-coated JSP are schematically represented (Scheme 1). The use of glycerol–water (1:1) as a solvent favored the synthesis of Cu$_2$O, whereas the use of glycerol as a solvent favored Cu. The synthesized metal components were coated on the JSP. The hardness of JSP was not lost, and the coating on JSP was also retained after washing. It is assumed that the mild nature of copper carbonate made the difference.

The color of JSP is off-white, and upon coating, it changed to orange and reddish brown for Cu$_2$O-JSP and Cu-JSP, respectively (Figure 1). For the characterization and comparative study of JSP, Cu$_2$O-JSP, and Cu-JSP, we have recorded powder X-ray diffraction (PXRD) data (Figure 2). PXRD patterns show characteristic 2θ peak positions for Miller planes corresponding to Cu$_2$O (for Cu$_2$O-JSP) and Cu (for Cu-JSP), consistent with the X-ray diffraction pattern of Cu$_2$O (JCPDS 05-0667) and Cu (JCPDS 85-1326). The peaks at 2θ = 14°, 16°, and 22° were assigned to (110), (110), and (200) planes of cellulose crystalline form, which relatively match with 2θ peaks of other plant fibers. Scanning electron microscopy (SEM) images revealed the surface feature of uncoated JSP, Cu$_2$O-JSP, and Cu-JSP (Figure 3), and it was found that the sizes of Cu$_2$O and Cu particles that are coated on JSP are in the range of 400 to 600 nm (microparticles).

Synthesis of Cu$_2$O and Cu was also performed in the absence of JSP (Scheme S2), and the material so formed was characterized by PXRD (Figure S4) and SEM (Figure S5a,b). The particle sizes when coated on JSP or in the absence of JSP are found to be comparable for Cu$_2$O and somewhat bigger for Cu. Copper content in Cu$_2$O-JSP and Cu-JSP was analyzed by the inductively coupled plasma–optical emission spectrometry (ICP–OES) technique after performing acid hydrolysis. One piece of Cu$_2$O-JSP was suspended in 10 mL of 1 M H$_2$SO$_4$(aq) and stirred for 12 h to leach out the copper content. The JSP was lifted and washed with 2 × 5 mL of distilled water to afford adsorbed copper, if any. The washing was combined with the acidic solution containing leached-out copper. The resulting solution was used to determine copper content in Cu$_2$O-JSP. The same procedure was carried out to analyze the copper content in Cu-JSP. The copper content values per piece of Cu$_2$O-JSP and Cu-JSP were found to be 3.238 ± 0.457 and 3.802 ± 0.220 mg, respectively.

2.3. Antibacterial Activity of Cu$_2$O-JSP and Cu-JSP. After incubation of inoculated agar-nutrient plates, stacked with sterile JSP and test samples (Cu$_2$O-JSP and Cu-JSP) at 37 °C for 24 h, zones of inhibition were observed only around the test samples (Figure 4), and hence, antibacterial activity of Cu$_2$O-JSP and Cu-JSP was claimed. This may be due to the fact that, when test samples were stacked on the surface of the inoculated agar-nutrient Petri plates, the bacterial cells around the test samples were killed and there were no subsequent growth around them.

Inactivation of the bacterial cells may be due to interaction of copper with the thiol groups of the respiratory enzyme associated with the cell membrane and other membrane proteins, which leads to loss of their physiological function. On the other hand, no such destruction of the physiological functions of any membrane-bound proteins takes places around the blank samples (JSP), and bacteria remain alive and multiply with time.

2.4. Water Disinfection by Cu$_2$O-JSP and Cu-JSP. To ensure contact of bacterial cells with Cu$_2$O-JSP and Cu-JSP, all the four experimental flasks were kept in a rotary incubator shaker (100 rpm) at 37 °C. Viable bacteria in the test sample appeared zero or below the detectable limit after 4 h (Figure 5a). As JSP holds no antimicrobial properties, it did not show water disinfection activity as a result of which the number of viable bacteria in both control and blank samples remained at initial levels (Figure 5b). In the test samples, water was exposed to Cu$_2$O-JSP and Cu-JSP, and with first-hour

![ACS Omega](http://dx.doi.org/10.1021/acsomega.9b03184)

ACS Omega 2019, 4, 22514–22520

DOI: 10.1021/acsomega.9b03184

Figure 1. Images of (a) jute stick pieces (JSP), (b) Cu$_2$O coated on JSP (Cu$_2$O-JSP), and (c) Cu coated on JSP (Cu-JSP).

Figure 2. Powder X-ray diffraction of JSP, Cu$_2$O-JSP, and Cu-JSP.
exposure, approximately 0.5-log reduction in viable bacteria was observed. In the next successive hours, approximately 1-log reduction of viable bacteria continued, and the active bacterial content in the test samples was completely destroyed or reached below the detectable limit after 4 h (Figure 5).

Although copper is an essential trace element for human health, ingesting an amount with a concentration higher than 3 ppm leads to nausea, vomiting, and abdominal pain. Consistent consumption of copper at a high concentration for a week or more also leads to gastrointestinal irritation. Consistent consumption of copper at a high concentration for a week or more also leads to gastrointestinal irritation. Thus, after test experiments, leaching of copper content from CuO-JSP and Cu-JSP into water was tested. Copper leaching concentrations from CuO-JSP and Cu-JSP were found to be 0.87 ± 0.16 and 0.30 ± 10 ppm, respectively (Table 1). The leaching was found well below the permissible levels (1.3 ppm), as prescribed by the U.S. Environmental Protection Agency (EPA).

### Table 1. Copper Leaching from Test Samples after Water Disinfection Test Experiments

| experiment no. | flask-III (CuO-JSP) | flask-IV (Cu-JSP) |
|----------------|---------------------|-------------------|
| 1              | 0.722               | 0.418             |
| 2              | 1.046               | 0.264             |
| 3              | 0.849               | 0.219             |
| average copper leaching | 0.87 ± 0.16 | 0.30 ± 0.10 |

#### 2.5. Prevention of Aerial Contamination in Water by CuO-JSP and Cu-JSP.

Four beakers filled with water and containing respective buoyant materials (Figure 6a) was kept for 5 days in open air. After day 1, the microbial contamination values in control and blank water samples were found to be 80 ± 10 and 60 ± 20 CFU/mL, respectively. These results clearly showed that the viable microbial count in the blank sample was lesser as compared to the control sample. This difference may be due to adsorption of microbes on the surface of the JSP. Microbial count in the test water sample was found to be zero or below the detectable limit, which indicated that microbes were destroyed at the air–water interface after interacting with CuO-JSP or Cu-JSP. Microbial content in control and blank samples increased exponentially per day upon days of aerial exposure. From day 2 onward, the microbial content in blank water samples reached the limit of detection, indicating that CuO-JSP and Cu-JSP were effective in preventing aerial contamination.

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**Figure 3.** SEM images of (a) JSP, (b) CuO-JSP, and (c) Cu-JSP.

**Figure 4.** Antibacterial activity of CuO-JSP and Cu-JSP.

**Figure 5.** Deactivation of bacteria by CuO-JSP and Cu-JSP during water disinfection test experiments. (a) Nutrient agar plate showing microbial content in 0.1 mL of each water sample at a time interval of 1 h. (b) Variation in CFU/mL with time with standard error bars.
samples was found to be always higher than the contamination in the control samples. At the end of day 5, average microbial content in control samples was found to be $3.8 \times 10^6$ CFU/mL, and in blank samples, it was found to be $1 \times 10^7$ CFU/mL. This difference in contamination was possibly due to the substrate nature of JSP.23,24 It was possible that the adsorbed microbes on JSP started feeding on the lignocellulosic matter and multiplied, leading to more contamination. In the test water samples, it was only $600 \pm 100$ CFU/mL, which was significantly very low compared to those of control and blank samples (Figure 6b).

At the air–water interface, the materials acted as a barrier against microbes and prevented water samples from aerial microbial contamination. Copper leaching from Cu$_2$O-JSP and Cu-JSP was found to be below the action limit (Figure 7), as prescribed by the U.S. Environmental Protection Agency (EPA).25 After day 1, leaching concentrations of copper from Cu$_2$O-JSP and Cu-JSP were $0.39 \pm 10$ and $0.19 \pm 07$ ppm, respectively. With an increase in number of days of contact of test materials with water, an increase in leaching was observed, but it remained below permissible limits at the end of day 5.

2.6. Proposed Overview for the Mechanism of Action. Accumulations and activities of microbes at the air–water interface are well documented.25,26 When water gets contaminated by microbes from air, the contaminants float on the water surface due to low weight and Brownian motion. We propose that the contact of aerial microbes with water is facilitated by the Brownian motion of the microbes. Thus, the microbes interact with materials at the air–water interface. When the microbes interact with the test sample, Cu$_2$O-JSP or Cu-JSP, copper shows its action and deactivation of microbes takes place (Scheme 2). The interaction of membrane proteins of microbes with the Cu$_2$O or Cu coating of our materials might trigger the release of Cu$^+$ as well as Cu$^{2+}$ ions at the microbes. The affinity of thiol (i.e., present in microbes) toward Cu$^+$ and the ability of Cu$^{2+}$ to generate reactive oxygen species (ROS) are well-known phenomena that promote biocidal properties of these ions.28,29 Thus, the samples employed in our work are proposed to cause denaturation of membrane proteins of microbes, leading to cell death. A similar mechanism was proposed earlier by Swarnkar et al. to explain the biocidal effect of Cu nanowires.30 The actual mechanism of copper actions on microorganisms is a complex subject and beyond the scope of this paper. A few out of many proposed mechanisms are described below.

First and foremost, interaction between copper and microbes is necessary. Copper interacts with thiol groups of respiratory enzymes associated with the cell membrane and inhibits their functions.20 It also interacts with other vital enzymes involved in glycolysis and Krebs cycles, causing inhibition of these important physiological processes.21 Meghana et al. reported the Cu(I)-induced inactivation of fumarase A enzyme.22 These associations of enzymes and proteins with copper disrupt their structure and functions and cause cell death or viral inactivation. Copper-based compounds are also known to generate reactive oxygen species (ROS), creating oxidative stress that kills bacterial cells and also inactivates viruses.26,29,32,33 Antimicrobial actions of copper also include degradation of nucleic acids.34–39 Since there is no study on prevention of aerial contamination in water by any method, a comparison could not be
made. The results also suggest that such buoyant biocide can have a very huge potential to be applied in rural communities for cost-effective prevention of communicable microbial diseases.

3. CONCLUSIONS
Selective syntheses of Cu$_2$O and Cu via the solvothermal process were achieved by using basic copper carbonate where the selectivity was controlled by simple variation of the solvent of choice. The syntheses were performed in the presence of buoyant agricultural waste (jute stick pieces, JSP), which allowed in situ coating of the copper-based material on the JSP. These hybrid wood materials (Cu$_2$O-coated JSP and Cu-coated JSP) exhibited remarkable antibacterial properties. The buoyant nature of the coated JSP was explored for the prevention of aerial contamination in potable water as a proof of a new concept. Beaded necklace-like strands of the coated JSP were floated on the surface of water stored in a container. This finding is likely to find application if storage of potable water until actual consumption is a requirement.

4. MATERIALS AND METHODS
Common chemicals such as CuCO$_3$·Cu(OH)$_2$, glycerol, and sulfuric acid were purchased from Fisher Scientific, India. Nutrient broth, agar, and disposable Petri plates were acquired from HiMedia, India. Jute sticks (agricultural waste) were collected from agriculture fields (West Bengal, India). Jute sticks were transversally cut into cylindrical shell-shaped pieces (height = 1.0 ± 0.1 cm, diameter = 1 ± 0.1 cm, weight ≈ 0.2 g, and density ≈ 0.25 g/cm$^3$), washed with distilled water, then separated, and air-dried at room temperature. Purified water was sterilized prior to contamination studies. Cuprous oxide and copper were synthesized from CuCO$_3$·Cu(OH)$_2$ and coated on jute stick pieces (JSP) in situ. Standard methods were followed to study antibacterial activity. The water disinfection property and prevention of contamination of water were studied using the coated JSP.

4.1. Optimized Method for the Coating of Cu$_2$O on JSP. A round-bottom flask was charged with jute stick pieces (5 g), CuCO$_3$·Cu(OH)$_2$ (0.5527 g, 2.5 mmol), and 100 mL of glycerol–water (1:1). The mixture was then heated under reflux at 130 °C with continuous stirring for 24 h. The sample was cooled down to room temperature, and the Cu$_2$O-coated jute stick pieces were separated from the solvent. The materials so obtained were immersed in 100 mL of distilled water in a conical flask. The flask was allowed to stand for 12 h; however, it was shaken gently at an interval of 1 h to remove absorbed/adsorbed glycerol. The washing procedure was repeated at least twice to remove all absorbed/adsorbed glycerol. The Cu$_2$O-coated JSP so obtained (Cu$_2$O-JSP) was air-dried at room temperature. The surface color of the JSP turned from off-white to light orange.

4.2. Optimized Method for the Coating of Cu on JSP. The coating of Cu was carried out in a similar manner as described above, except that the reflux was carried out in 100 mL of glycerol at 130 °C to afford Cu-coated JSP (Cu-JSP). The surface color of the JSP turned from off-white to reddish brown.

4.3. Preparation of Liquid Nutrient Medium. Nutrient broth (1.3 g) was taken in a 200 mL beaker, and 100 mL of reverse osmosis (RO) water was added to it. The mixture was stirred for a few minutes to dissolve the medium completely. Twenty clean and dry test tubes of 20 mL volume each were taken, 5 mL of the dissolved medium was transferred to each test tube, and the mouths of the test tubes were closed with cotton plugs. The test tubes were sterilized by autoclaving.
them at 121 °C for 15 min, followed by cooling them to room temperature and then storing them for further use.

4.4. Preparation of Nutrient Agar Medium. Nutrient broth (6.5 g) was taken in a 1 L conical flask, and 500 mL of RO water was added to it. The mixture was stirred for a few minutes to dissolve the medium. To this liquid nutrient medium, 7.5 g of agar powder was added, and the mouth of the flask was closed with a cotton plug. It was then sterilized by autoclaving it at 121 °C for 15 min. It was then cooled to 60–70 °C, and then approximately 15–20 mL of media was poured into sterile disposable 90 mm Petri dishes. Once the media were solidified, the plates were sealed with a paraffin and stored upside down in a refrigerator (5 °C) for further use.

4.5. Antibacterial Tests of Cu₂O-JSP and Cu-JSP. Escherichia coli NCIM 2931 and Staphylococcus aureus were chosen as test bacterial species. Colonies of E. coli NCIM 2931 and S. aureus were picked up from the nutrient agar plate with a sterile inoculating loop and inoculated separately in 5 mL of sterile liquid nutrient medium. These inoculated liquid media were incubated in a rotary incubator shaker at 180 rpm and 37 °C overnight. One hundred microliters of freshly cultured bacterial suspension (at mid-log phase) was poured on the surface of the nutrient agar plate and spread uniformly using a sterilized glass rod (L-Rod). Sterile JSP as a sterile liquid nutrient medium. These inoculated liquid media were solidified, the plates were sealed with a paraffin and stored upside down in a refrigerator (5 °C) for further use.

4.6. Water Disinfection Experiments Using Cu₂O-JSP and Cu–JSP. Four 1 L conical flasks (I–IV) were taken, 500 mL of RO water was added to each flask, and their mouths were closed with cotton plugs. All the four flasks containing water were sterilized by autoclaving them at 121 °C for 15 min and cooled them to room temperature. Uncoated jute stick pieces were packed in an autoclave bag and also sterilized by autoclaving them at the same temperature for the same time. Each conical flask containing 500 mL of sterile water was contaminated with 100 μL of freshly cultured E. coli NCIM 2931 (~10⁵ CFU/mL). Flask-I was used for the control experiment, and flask-II was used for the blank experiment in which one sterile JSP was added. The remaining two flasks were used for test experiments. One piece of Cu₂O-JSP was added in flask-III, and one piece of Cu-JSP was added in flask-IV. All the flasks were kept in a rotary incubator shaker (100 rpm) at 37 °C. At a time interval of 1 h, 0.1 mL of contaminated water sample from each flask was inoculated over a nutrient agar plate and incubated at 37 °C for 24 h. After 4 h, in test samples, the active bacterial content reaches below the detectable limit or zero. Variations in CFU/mL were analyzed via the serial dilution method, as described in the previous section. After each day, 0.1 mL of the test water sample was taken in a glass vial, and copper contamination was analyzed by ICP–OES.

4.7. Prevention of Aerial Contamination of Water by Cu₂O-JSP and Cu-JSP. Three units of beaded necklace-like strands were prepared by tethering jute stick pieces using nylon thread. For the making of each necklace strand, 20 jute stick pieces, that is, (i) JSP, (ii) Cu₂O-JSP, or (iii) Cu-JSP, were employed. Four 5 L beakers were taken, and each was filled with sterile water, close to the brim. The sample in beaker-I was used for the control experiment. The sample present in beaker-II was used for the blank experiment, and a strand of sterile JSPs was allowed to float on the surface of water. The samples in the remaining two beakers were used for test experiments. Strands of Cu₂O-JSP and Cu-JSP were kept on the water surface of beaker-III and beaker-IV, respectively. A set of these four beakers was kept in open air in building premises for 5 days. At a time interval of 24 h, 0.1 mL of water samples was poured on nutrient agar and spread uniformly across its surface using a sterile glass rod (L-Rod) and allowed to dry. Three nutrient agar plates were used for each water sample. The inoculated plates were incubated at 37 °C for 24 h, and depending on microbial contamination in water, the intensity of microbial growth was observed. Variations in CFU/mL were analyzed via the serial dilution method, as described in the previous section. After each day, 0.1 mL of the test water sample was taken in a glass vial, and copper contamination was analyzed by ICP–OES. The copper content is reported for three replicates with a standard error.

## ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03184.

Processing of jute sticks, synthesis of Cu₂O and Cu from Cu₂CO₃·Cu(OH)₂, PXRD and SEM of Cu₂O and Cu, ICP–OES to find copper content in Cu₂O-JSP and Cu-JSP, and water disinfection activity and copper leaching studies (PDF)

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The authors acknowledge IIT Madras for fellowship and facilities and CFI IIT Madras for funding this research under the Innovative Project scheme no. ICSR/087/2016.

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