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# Antimicrobial Response of Biologically Synthesized Nanocopper against Water Borne Bacterial Strains Isolated From Yamuna River in Agra

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**ABSTRACT:** The requirement for biosynthesis of nanoparticles rises in light of the fact that the physical and chemical procedures are expensive, unsafe, take more time, and are a dreary procedure to confine nanoparticles. To deliver nanoparticles, microorganism cells or plant concentrates can be used for biosynthetic techniques. The use of plant concentrates for their biosynthetic response is one of the imperative branches of biosynthesis of nanoparticles. CuNPs additionally have a high surface-territory-to-volume proportion, low generation cost, antibacterial strength, reactant movement, and optical and attractive properties when contrasted with other metals, for example, gold, silver, or palladium. The primary trouble lies in their arrangement and conservation as they oxidize quickly when uncovered in air. Researchers are utilizing diverse idle media, for example, argon and nitrogen, to defeat this oxidation issue, additionally utilizing reducing, capping, or protecting agents to reduce the amount of copper salt utilized. Antibacterial and antifungal activity of copper nanoparticles and the impact of copper nanoparticles is utilized in various experiments of present era. Bacterial species of Yamuna river were detected. Total 36 samples of water were used for isolation purpose. *Salmonella* were detected in 5 samples with highest ranking (13.9%). *Salmonella* spp. isolates belonged to the species *S. typhi* (two isolates), *S. enterica*, *S. choleraesuis*, *S. gallinarum* while *Vibrio* were detected in 4 samples (11.2%). *Vibrio* spp. isolates belonged to the following species *V. cholera* (two isolates), *V. parahaemolyticus*, *V. navarrensis*. The presence of bacteria species indicates the pollution status, due to the sewage, human activities and industrial activities etc, the quality of river water has deteriorated which affects human as well as aquatic life.

## I. INTRODUCTION

Yamuna is one of the most polluted rivers in India, because of the large amount of the wastewater discharge. According to the Centre for Science and Environment, approximately 75 to 80 percent of the river's pollution is the result of raw sewage, industrial runoff and the garbage thrown into the river and it totals over 3 billion litres of waste per day (Mishra, 2010 and Martínez et al., 2009). Today water resources have become the most exploited natural system since man strode the earth. According to united nation report, freshwater is gradually becoming a matter of concern with nearly 900 million people affected by diarrhoea each year and an equal number suffering from disease caused by various worm. Unclean water ranks at top of the world population problem (Goel and Grad, 2008). The industrial pollutants associated with organic matter, inorganic dissolved solids and other unwanted chemicals cause serious problems in the water quality (Radha et al., 2007). Water quality can also be evaluated through chemical and physical parameters including heavy metals, trace metals, total suspended solid and turbidity. These trace elements present in virtually potable water, some of which play a role in metabolism.[1]

A copper nanoparticle is a copper based particle 1 to 100 nm in size. Like many other forms of nanoparticles, a copper nanoparticle can be formed by natural processes or through chemical synthesis. These nanoparticles are of particular interest due to their historical application as coloring agents and their modern-day biomedical ones. Copper nanoparticles display unique characteristics including catalytic and antifungal/antibacterial activities that are not observed in commercial copper. First of all, copper nanoparticles demonstrate a very strong catalytic activity, a property that can be attributed to their large catalytic surface area. With the small size and great porosity, the nanoparticles are able to achieve a higher reaction yield and a shorter reaction time when utilized as reagents in organic and organometallic synthesis.

## II. METHODOLOGY

### Synthesis of Copper nanoparticles

A 500 mL of 0.01 molar Copper (II) Chloride ( $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ) solution was prepared by dissolving that copper salt in de-ionized water. Solutions of 0.25M, 0.5M, 0.75M and 1.0M L-Ascorbic Acid were prepared in de-ionized water. Four air tight flasks, each having 50 mL of  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  solution were heated continuously at 90°C in water bath shaker (electrical/mechanical heated).

The solution of 0.25M, 0.5M, 0.75M and 1.0M L-Ascorbic Acid were added drop wise to each flask respectively. The heating and mixing continued till the color changed from no color to yellow, orange, brown and finally dark brown-black as shown in Figure 1. The whole process was completed within 17 hours. The product was kept for 12 weeks, no sedimentation or dispersion was observed with no magnification.[2]



**Figure 1:** Synthesis steps of Cu NP.

### Characterization

The concentration of synthesized Cu NP was evaluated using Atomic Absorption Spectrometer (AA6800, Shimadzu, Japan). FT-IR spectrum were accomplished and recorded with Fourier-Transform infrared spectrophotometer (Bruker, Alpha ATR) between 4000 and 375  $\text{cm}^{-1}$ , with resolution of 4  $\text{cm}^{-1}$ . The morphology and size of produced nanoparticles were characterized by X-Ray Diffraction (XRD), PANalytical, X'Pert PRO XRD system.

### STABILITY OF Cu NANOPARTICLES

The main factors that affect the use of Cu NP are the stability in the dispersion. Many capping agents such as Polyvinyl Pyrrolidone (PVP) and Polyethylene glycol are used to prevent agglomeration. L-Ascorbic acid was used as reducing as well as capping agent in this work to avoid contamination of other organic compounds.

The prepared Cu NP suspensions were placed without any further mixing or treatment for 12 weeks, no sedimentation was seen till that period. This indicates that the L-Ascorbic Acid highly stabilized the Cu nanoparticles due to its high capping power.

### COLLECTION OF WATER SAMPLES

The Yamuna river water samples were collected for the assessment of physico-chemical factors. River water Samples were collected (3 Times) of every month in BOD bottles and sterile one litter politeness bottles. The closed bottles were immersed in the river at the depth of 0.5 to 0.7 m and the stopper was opened in bottom of river and was closed again to the river water. After sample collection, the bottles were kept in ice box and transferred immediately to the laboratory for further analyses. Total 36 water samples were used for *Salmonella* spp. and *Vibrio* spp. isolation and identification purpose.[3]



#### Isolation of *Salmonella*

1ml of water sample was inoculated in 9 ml of buffered peptone water and incubated at 37°C for 18 hrs for pre-enrichment. After enrichment, a loopful of inoculums was then streaked on xylose lysine desoxycholate (XLD) agar and incubated at 37°C for 24 h. The presumptive *Salmonella* colonies appearing slightly transparent red halo with a black centre surrounded by a pink-red zone on XLD agar and were sub-cultured and for each sample, a representative pure colony was selected and stored on sterile nutrient agar, colonies were confirmed by gram staining and biochemical characterization.

#### Isolation of *Vibrio*

1ml of water sample was inoculated in 9 ml of alkaline peptone water and incubated at 37°C for 8 h for pre-enrichment. After enrichment, a loopful of inoculums was then streaked on thiosulphate citrate bile salt sucrose (TCBS) agar and incubated at 37°C for 24 h. Yellow smooth and slightly flattened colonies with opaque centers and translucent peripheries appearing after 24h of incubation were presumably considered as *Vibrio* species and were sub-cultured and for each sample, a representative pure colony was selected and stored on sterile nutrient agar, colonies were confirmed by gram staining and biochemical characterization.[4]

#### Morphological analysis

The pure culture obtained was then observed for colony and other morphological characteristics, the culture was gram stained.

#### Biochemical analysis

Gram Staining, Motility, Urease, ONPG, Catalase, Oxidase, Indole production, Voges Proskauer, Nitrate Reduction, Citrate Utilization, String (for *Vibrio*), H<sub>2</sub>S, Methyl Red test was used for both colonies of *Salmonella* spp and *Vibrio* spp as described Bergey's Manual of Systematic Bacteriology.

### III. DISCUSSION

Major ions in drinking water are correlated with palatable mineralization that affects the quality of drinking water. Water of good quality is of basic importance to human physiology and man's continued existence depends very much on its availability. Pathogens such as *Salmonella* spp, *Vibrio cholera* and *E.coli* that are shed into water body through faecal contamination perpetuate many diseases. These cause typhoid fever and dysentery. Other agents of water borne diseases are protozoan that cause diarrhoea; *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli* and *Cryptococcus parvum*. *Salmonella* is a ubiquitous enteric pathogen with a worldwide distribution that comprises a large number of serovars characterized by different host specificity and distribution. *Salmonella* is spread by the fecal-oral route of contamination. This microorganism can enter the aquatic environment directly with feces of infected humans or animals or indirectly, e.g., via sewage discharge or agricultural land run off. Salmonellae are frequently found in environmental samples. They (10<sup>3</sup> –10<sup>4</sup>) are usually present in large numbers in raw sewage CFU/L) and can still be present in wastewater effluent after advanced secondary treatment including coagulation, filtration and disinfection. *Vibrios* are primarily aquatic bacteria. Species distribution depends on sodium concentration and water temperature.[5] *Salmonella* were detected in 5 samples (13.9%) and *Vibrio* were detected in 4 samples (11.2%). Results for *Salmonella* spp. motility, catalase, nitrate MR, H<sub>2</sub>S reaction are found positive. Results for *Vibrio* spp. motility, string, citrate, indole, oxidase, ONPG, nitrate reaction are found positive the negative results of MR, H<sub>2</sub>S and urease. The carbohydrate profile of the bacteria under investigation depicts that it could utilize glucose, maltose and mannitol with weak production of acid and little or no gas production; but could not utilize xylose, lactose, sucrose and arabinose. The biochemical properties were estimated using Bergey's Manual of Systematic Bacteriology. Results for *Vibrio* spp. motility, string, citrate, indole, oxidase, ONPG, nitrate reaction are found positive the negative results of MR, H<sub>2</sub>S and urease. The carbohydrate profile of the bacteria under investigation depicts that it could utilize glucose, maltose and mannitol with weak production of acid and little or no gas production; but could not utilize xylose, lactose, sucrose and arabinose.[6]

### IV. CONCLUSION

In conclusion, present study confirmed the presence of *Escherichia coli*, *Salmonella* spp, and *Vibrio* spp. as water-borne pathogens in Yamuna water of Agra, India. It also showed the important public health problem in of Agra, Uttar Pradesh, India. The result of this study revealed that the water sources in these communities are moderate polluted and is not safe for use by the communities for drinking or other domestic needs without prior treatment. There is need to monitor regularly and mitigate the effects of community behaviour on surface waters.



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