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Antimicrobial and Anticancer Activities of *Ricinus Communis* Biochar and its Composites with ZnO and CuO Nanoparticles for Chromium Contaminated Water

Ahmad Hassan[1], Abrar Hussain[2], Faisal Mehtab[3], Saiqa Sarwar[4], Amna Abrar[5]. Sana Shamim[6], Ieman Tariq[7], Ayesha Tariq[8]

[1]EPA Punjab & Department of Chemistry, University of Agriculture, Faisalabad, Punjab, Pakistan.
[2]Graduate Institute of Biological Science & Technology, China Medical University, Taiwan.
[3]Department of Chemistry, Government College University Lahore, Punjab, Pakistan.
[4]Department of Environmental & Life Sciences, Women University Multan, Punjab, Pakistan.
[5]DeDepartment of Chemistry, School of Science, Tianjin Key Laboratory of Molecular Optoelectronic Science, Tianjin University, Tianjin 300072, China.
[6]Department of Pharmaceutical Chemistry, Dow University of Health Sciences, Ojha Campus, Karachi, Sindh, Pakistan.
[7]Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Punjab, Pakistan.
[8]Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Punjab, Pakistan.

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Correspondence to: Amna Abrar, Department of Chemistry, School of Science, Tianjin Key Laboratory of Molecular Optoelectronic Science, Tianjin University, Tianjin 300072, China.
Email: abraramna@tju.edu.cn

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ABSTRACT

The existence of toxic heavy metals and harmful microorganisms in water supplies presents significant dangers to both the environment and public health. In this research, biochar produced from the Ricinus communis was created and modified with zinc oxide and copper oxide nanoparticles to improve its ability to fight against microorganisms and cancer, particularly in chromium-polluted water scenarios. The composite substances were formulated and tested using the agar well diffusion technique to assess their effectiveness in inhibiting the growth bacterial culture E. coli, Aureus and fungal species A. Fumigatus, A. Flavus and A. Niger. The findings demonstrated considerable inhibition zones, with the biochar augmented with ZnO and CuO nanoparticles displaying the most substantial antimicrobial effectiveness, which is ascribed to the combined action of metal oxides and biochar in producing reactive oxygen species and damaging microbial membranes. Beyond the assessment of antimicrobial properties, the anticancer capabilities of the nanocomposites were examined via in vitro cytotoxicity tests against specified cancer cell lines. The composite showed effects on cell viability that were dependent on the dose, indicating its potential as a multifunctional material for biomedical and environmental uses. This research concludes that the biochar derived from Ricinus communis, enhanced with ZnO and CuO nanoparticles, provides a promising and sustainable method for reducing both biological and chemical pollutants in contaminated water environments, while also showing significant anticancer potential.

INTRODUCTION

Water contamination has become a major problem in the modern period. The toxicity of heavy metals for living things and marine life makes them a more well-known and serious problem among environmental contaminants. A heavy metal is defined as any naturally occurring element with an atomic number and elemental density greater than 20 and 5gcm^3 respectively. According to Kanamarlapudi et al. (2018), heavy metals are a special category of naturally occurring elements that last for a very long time in the environment and are not biodegradable. The Earth's crust naturally contains heavy metals. Intake of heavy metals into the environment might come from manmade or natural sources. There is a very thin line between these elements' toxicity and necessity for living things. Arsenic, antimony, chromium, cadmium, beryllium, lead, copper, zinc, selenium, mercury, nickel, thallium, and silver are among the 13 metals that the USEPA has identified as dangerous heavy metals (Ramos et al. 2002; Salman et al. 2015).

Water pollution caused by Cr poses a serious threat to ecological safety, attributed to its ability to move easily, its harmful effects, and its potential to cause cancer, as highlighted by the U.S. EPA and other sources (U.S. EPA, 2024). Effluents from industries such as leather production, electroplating, and textiles continuously introduce Cr into water bodies, leading to risks of bodily harm, oxidative damage, and cancer upon human interaction (Sharma et al., 2023). Traditional cleanup methods—such as chemical reduction, membrane filtration, and ion exchange prove effective but frequently struggle with issues related to expense, scalability, or causing additional environmental damage (Jagadeesh et al., 2025). Techniques based on adsorption utilizing affordable and eco-friendly materials like biochar are increasingly favored for the capture of Cr(VI) due to their beneficial surface attributes, including high porosity and active functional groups (Lehmann & Joseph, 2015).

Biochar is a substance rich in carbon and characterized by its porous nature, produced through the pyrolysis of biomass in an environment with restricted oxygen. It has the capability to eliminate heavy metals by means of processes such as electrostatic adsorption, ion exchange, surface complexation, and in some instances through redox-mediated reduction (Sekar et al., 2024). Nonetheless, natural biochar frequently do not possess enough redox-active sites to convert Cr(VI) to the less harmful Cr(III), and their ability to adsorb may be diminished in intricate wastewater environments. Consequently, efforts have been made to modify biochar through techniques such as metal impregnation or the incorporation of nanoparticle in order to improve both its adsorption ability and functional characteristics (Mongy et al., 2024). Specifically, metal oxide nanoparticles like ZnO and CuO demonstrate significant antimicrobial and anticancer effects, and when they are anchored onto biochar, they can enhance its active sites and overall multi-functionality (Bansal et al., 2022).

Biosorption has been considered a simple, easy-to-operate, cost-effective and ecofriendly technique with bio-degradable and inexpensive materials (Almomani et al. 2020). Several feedstock can be used for biochar preparation, but industrial and agricultural wastes stand out because they allow the reuse of these materials (Zhang et al. 2018). Ricinus communis, commonly known as castor, is a widely grown oilseed crop across the globe that yields agricultural by-products such as seed shells, stalks, and husk that are considered to have low economic value but possess significant potential for high-carbon biochar production. Biochar made from castor has demonstrated good production rates, advantageous surface properties, and compatibility for activation and composite development (Sekar et al., 2024). The use of biochar from R. communis not only provides a cost-effective and eco-friendly option but also contributes to the promotion of a circular economy by optimizing agricultural waste. Numerous investigations using diverse plant extracts as antimicrobial activities have been conducted to release and identify new antibacterial compounds (Bassam et al. 2006). Many nations employ various plants as a source of potent and strong medications for medical purposes (Kubmarawa et al. 2007).

The interest in this scientific study of *R. communis* is based on numerous examples of how well it works to treat a variety of ailments. The *Ricinus communis* plant's seeds or leaves was used to make extracts that was used to screen for bacterial and fungal activity Nevertheless, research into castor biochar enhanced with ZnO and CuO nanoparticles for the dual purposes of Cr removal and bioactivity specifically antimicrobial and anticancer properties has been limited thus far (Palanivel et al., 2020).Due to their beneficial characteristics and numerous uses in daily life, metal oxide nanoparticles have drawn a lot of attention in recent years. Copper oxide (CuO) and zinc oxide (ZnO) nanoparticles are two of these (Gholami et al. 2019). These two oxides are among the benign oxides that have demonstrated a wide range of utility as biological agents. Their distinctive qualities, which are attributed to their size, forms, and compositions, are responsible for their wide range of applications and the current surge in attention given to them (Dobrucka et al. 2018).

ZnO nanoparticles are widely acknowledged for their extensive antimicrobial properties, effectively combating both Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus. This is achieved through mechanisms such as the production of reactive oxygen species (ROS), the release of Zn, disruption of cell membranes, and infliction of intracellular harm. They also show harmful effects on various cancer cell lines, including those of liver carcinoma, by instigating oxidative stress, impairing mitochondrial function, and promoting apoptosis. Likewise, CuO nanoparticles demonstrate significant antibacterial and antifungal properties, acting against species like A. Niger, A. flavus, and A. fumigatus. Their effectiveness relies on the creation of ROS, protein damage, disruption of membrane integrity, and infliction of DNA injury. Additionally, CuO nanoparticles are capable of inducing toxic effects in cancerous cells through oxidative pathways.

Integrating ZnO and CuO nanoparticles with biochar could yield versatile composites: improved adsorption of Cr(VI) due to expanded active surface areas and redox catalytic properties, sustained antimicrobial capabilities achieved through the generation of reactive oxygen species on the biochar surface, and anticancer effectiveness directed at liver cancer cells measured through the MTT assay. Biochar has the ability to stabilize nanoparticles, minimize their clumping, and control their release enhancing environmental safety alongside functionality (Luyen et al., 2023)

The well diffusion technique remains a commonly employed assay for assessing the antimicrobial effectiveness of solid substances: agar plates are seeded with various microbial strain E. coli, S. aureus, A. Niger, A. flavus, A. fumigatus and wells filled with testing composites facilitate diffusion; the resulting inhibition zones reflect antimicrobial strength (Ghasemi et al., 2021). This approach provides a basic yet important comparative evaluation among different bacterial and fungal organisms. To identify anticancer properties, the MTT assay (Mosmann, 1983) measures cell survival based on the mitochondrial conversion of tetrazolium into formazan. Frequently utilized with hepatic (liver) cancer cell cultures like HepG2, it acts as a screening method to assess cytotoxic capabilities, though interference from nanoparticles and constraints of the assay need to be addressed through appropriate controls (Pravin et al., 2023).

While there has been research on metal oxide-biochar composites for the purpose of capturing heavy metals, there is a lack of studies that combine the elimination of Cr(VI) with both antimicrobial and anticancer attributes within a single R. communis biochar composite. To fill this void, it is essential to create castor biochar, apply ZnO and CuO coatings, and conduct a rigorous assessment of Cr absorption, antimicrobial properties and anticancer evaluations. Executing such comprehensive evaluations supports the objectives of a circular economy by providing a material sourced from agricultural by-products, capable of purifying water contaminated with Cr, stopping harmful microbes, and displaying initial cytotoxic effects on liver cancer cells in laboratory settings. If proven effective, this versatile material could be utilized in decentralized water purification systems in areas with limited resources, where it is essential to manage both pathogen presence and heavy metal contamination simultaneously.

METHODOLOGY

Materials and Chemicals

We purchased Potassium dichromate ($K_2Cr_2O_7$), Sodium chloride (NaCl), Potassium hydroxide (KOH), Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) and Hydrogen chloride (HCl) from Merck, Faisalabad. Cr stock solution was made using prepared distilled water.

Preparation of *Ricinus Communis* Biochar

The biomass of *Ricinus Communis* (RC) was collected from the local areas. RC was cut into small pieces by fodder making machine. The smaller RC flakes were washed with double distilled water to remove impurities adhering to the biomass surface. The air-dried material was placed in a biochar unit to prepare RC biochar at 600°C for 90 minutes. After 90 minutes, the biochar unit was turned off and the prepared biochar was allowed to cool (24h), then the biochar was crushed and sieved by fine sieve. The fine material was washed with DI water to remove the soluble components of the biochar and dried again in an oven at 85°C for 12 h. The dry RCB were kept to prepare the mixture, then for additional testing (Jameel. at el., 2024).

Preparation of RCB/ZnO/CuONPs Composite

To prepare a mixture of RCB with ZnO and CuO nanoparticles (RCB/ZnO/CuONPs), the precipitation method was used. 0.1M ZnSO4 and 0.2M KOH solutions were prepared and used for RCB/ZnO/CuO synthesis. A solution of 1000 ml $ZnSO_4$ and $CuSO_4$ were mixed in 0.5g RCB for 20 min, then titrate the mixture with 1000 ml of KOH solution. This process was repeated until 1000 ml of each solution had been titrated according to the aforementioned approach. The RCB/ZnO/CuONPs combination were filtered using what man filter paper 42, and the mixture were oven dried for 24 hours at 85°C. (Imran et al. 2021). RCB, RCB/CuO and RCB/ZnONPs were stored in a plastic container for additional testing to eliminate Cr from polluted water.

Screening of Bacterial and Fungal Activity

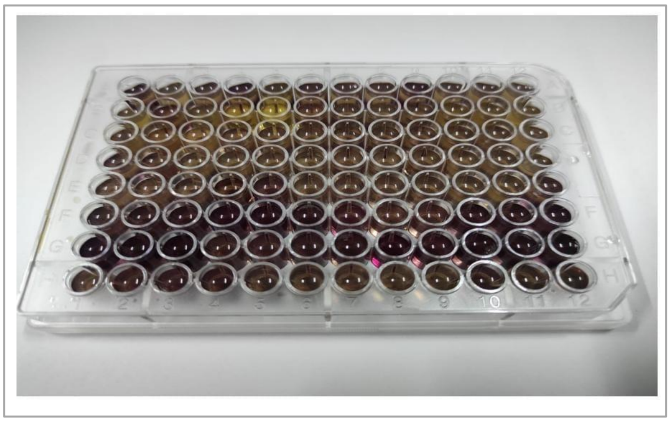
The well diffusion method, also known as the agar well diffusion method, was widely used technique for screening the antibacterial and antifungal activity of substances such as antibiotics, plant extracts, or synthetic compounds. Prepared agar plates with a suitable growth medium for the target bacteria or fungi. The medium was solidified in petri dishes. Using aseptic techniques, inoculated the agar plates with a standardized suspension of the test microorganisms (bacteria or fungi). Once the inoculated agar was solidified, used a sterile cork borer or pipette tip to create wells in the agar. Test substances were placed in these wells. Filled each well with a known quantity of *Ricinus communis* and ZnO-CuO/NPs to test. Incubated the plates at the appropriate temperature for the target microorganisms (e.g., 37°C for most bacteria). Allow time for the substances to diffuse into the agar and inhibit the growth of microorganisms. After incubation, measured the zones of inhibition, which were clear areas around the wells where microbial growth was inhibited. The diameter of these zones was used to estimate the potency of the test substances. The well diffusion method provides a simple and cost-effective way to screen for antibacterial and antifungal activity, but it's important to note that it offers only preliminary results.

Cell Line Culture

The HepG2 cell line was used to determine the assessment of the anticancer potential of RCB, RCB/CuO and RCB/ZnO NPs. Cells were taken in 75 cm^2 culture flasks in the presence of 10ml Dulbecco's modified Eagle's medium (DMEM). Then10% fetal bovine serum (FBS) and 1% 100x L-Glutamine was added to the culture flask to enhance the growth of cells. The cell lines were incubated for 24 hours at 37^oC in a humid atmosphere having 5% CO_2. The cells were replenished regularly with fresh media after 2-3 days. For this, the cells were treated with trypsin (2-5ml) which covered the cell layer present in a flask to detach the cells from the surface. When cells were detached from the surface of the flask and into single-cell suspension form, they were diluted and transferred to new culture flasks containing 10 ml of DMEM where they reattach and further divided. These cells were used for further trials and analysis.

Figure 2.5

MTT assay performed in 96-plate using different concentrations of RCB, ZnO/RCB, CuO/RCB



Potential of Cytotoxicity

The HepG2 is a human liver cancer cell line that was used to test anticancer activities. Cells were cultured in 96-well plates with 2×10^4 cells concentration in each well. Cells were treated with three distinct NPs (RCB, RCB/CuO, RCB/ZnO) prepared by chemical precipitation method with different concentrations (25 µg/mL, 50 µg/mL, 100 µg/mL) by dissolving in Phosphate buffer saline, as well as it is also kept as negative control. After a 24-hour incubation time, 10µL of (MTT) (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) at a final concentration of 5 mg/mL was added to each well, and the wells were incubated for 4 hours. The optical density at 570 nm was measured after adding 150 µL of 0. 1 dimethyl sulfoxide (DMSO). The formula $[(Ac–As) \div (Ac)] \times 100$ was used to quantify the percentage of inhibition, where Ac and As indicate control, sample absorbance, correspondingly.

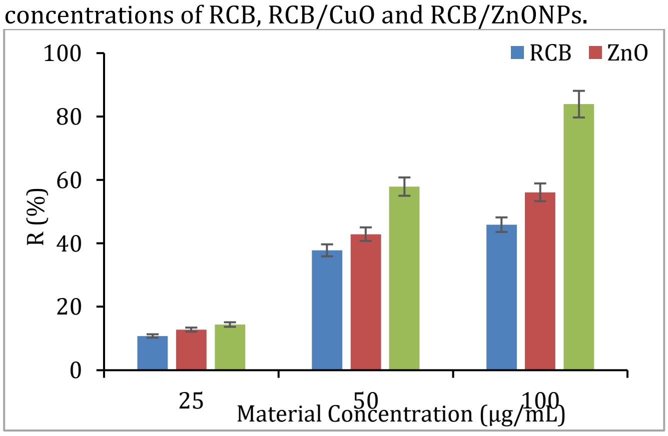
RESULTS and DISSCUSION

Growth Inhibition rate of Bacterial species

The following graph shows that there is a reduction in the growth of E. coli working with RCB, ZnO and CuO NPs. By increasing material concentration, the reduction rate also increases. The bacterial growth was reduced from 14-86% by using CuONPs ranging from 25-100ppm.While RCB and ZnO NPs shows bacterial reduction from 10-56%.

Figure 4.1

Growth reduction rate of *E. coli* by using different concentrations of RCB, RCB/CuO and RCB/ZnONPs.

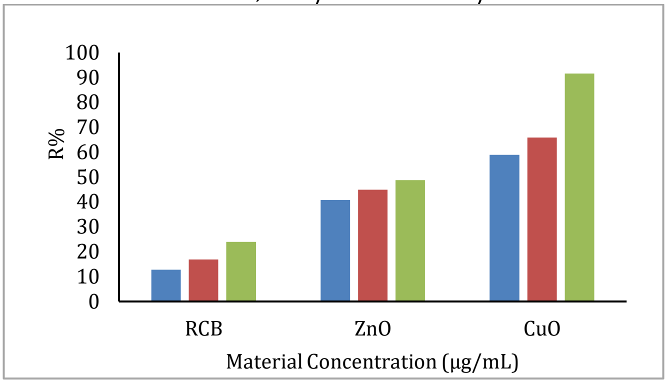


Growth Inhibition rate of S Aureus

The figure shows that when Aureus in agar plate worked with RCB, ZnO and CuONPs. The growth rate was reduced from 23.9-91.6% by using CuONPs, while the growth inhibition rate of Aureus was reduced from 16.9-65.7% with ZnONPs. By increasing material concentration 25-100ppm the growth of bacterial species reduced.

Figure 4.2

Reduction rate of S. Aureus by using different concentrations of RCB, RCB/CuO and RCB/ZnONPs.



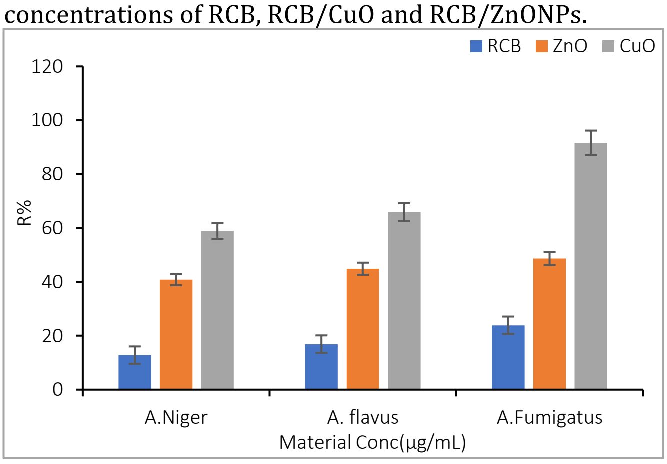
Growth reduction rate of Fungal Species

Fungal species such as A. Niger, A. flavus and A. fumigatus was observed with RCB, ZnO and CuO. Aspergilla's fumigatus shows greater reduction than A. flavus and A. Niger observed with RCB, ZnO and CuONPs. The A. Niger shows 12.8% growth reduction when treated RCB, 40.8% with ZnO and 58.9% with CuO nanoparticles. While A. Flavus shows growth reduction rate of 16.9-70% when treated with RCB, ZnO and CuO nanoparticles.

A. Fumigatus shows higher growth reduction of 91% with CuO nanoparticles, 50% with ZnO and 25% when treated with RCB. All treatments exhibited significant growth inhibition compared to the control, with A. fumigatus showing the highest percentage reduction in mycelial growth. The enhanced susceptibility of A. fumigatus may be attributed to its comparatively thinner cell wall structure and lower melanin content, which potentially increased the permeability of the fungal cell membrane to the reactive oxygen species (ROS) and metal ions released from ZnO and CuO nanoparticles.

Figure 4.3

Growth Inhibition rate of fungal species by using different concentrations of RCB, RCB/CuO and RCB/ZnONPs.



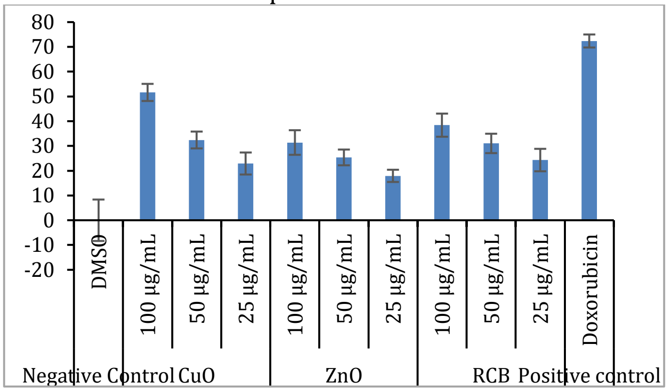
Anticancer Activity of RCB, CuO/RCB, ZnO/RCB NCs

Figure shows that by using different concentrations of Ricinus communis, ZnO and CuO nanoparticles the cell death of hepatic liver (HepG2) cell line occurs. With

100ug/L of CuONPs 51.6% cell death happens, RCB shows 38.9% cell death whereas ZnONPs shows lowest cell death of 31.2% at highest 100ug/L concentration. Among these three material used the CuO cause more cell death due to the fact that it generates ROS which leads to damage of cellular components like lipids, proteins and DNA. It is evident from the figure that toxicity of CuO depends on factors such as duration time and concentration.

Figure 4.4

Percent cell death of hepatic liver G2 cell line



DISCUSSION

The antifungal and Antibacterial activity of *Ricinus communis* biochar and its composites with ZnO and CuO nanoparticles was evaluated against Aspergillus niger, A. flavus, and A. fumigatus, E coli and S. Aureus in chromium-contaminated water. CuO and ZnO NPs show potential for antibacterial and antifungal activity. By using RCB, RCB/ZnO and RCB/CuO NPs the growth is inhibited which gain importance in the remediation of water sources from contaminants, metals and other chemicals. Literature review shows that CuO and ZnO are beneficial for antibacterial activity. Both CuO and ZnO colloids behave as toxic agents developing inhibition zones. On the other hand, ZnO nanoparticles inhibit bacteria only at sufficiently high concentrations ~0.5 mol/L while toxic behavior of the CuO nanoparticles begins at any smaller concentration. The results obviously show that all tested microorganisms possess the highest sensitivity to the most concentrated colloids. It was observed that the inhibition zone in well diffusion measurements are larger compared with the disk diffusion method which can be explained by the fact that in well diffusion, we double the volume of NPs solutions and increase the diffusion of NPs through the medium. According to Sirelkhatim et al. (2015) higher concentration and volume lead to better antibacterial activity.

ZnO and CuONPs can have different impacts on hepatic (liver) G2 cells based on concentration, duration of exposure, and nanoparticle specifics. Research examining the relationship between ZnO/CuO NPs and hepatic cells frequently concentrate on oxidative stress, Geno toxicity, and possible cytotoxicity. These nanoparticles may cause liver cells to become genotoxic. This covers chromosomal abnormalities, DNA damage, and changes in the way the cell cycle progresses. It is well known that when ZnO NPs come into contact with cells, they release reactive oxygen species, or ROS. In hepatic G2 cells, an excess of reactive oxygen species (ROS) can result in oxidative stress and damage to cellular constituents as proteins, lipids, and DNA. It's crucial to remember that different experimental circumstances may provide different results and that the biological reactions to ZnO NPs might be complex. The biological reactions to nanoparticles are usually studied using in vitro cell culture models, and the results help us comprehend the possible dangers and uses of these substances.

CONCLUSION

The current research indicates that biochar derived from Ricinus communis (RCB) and its combinations with ZnO and CuO nanoparticles demonstrate excellent antimicrobial and anticancer activities against various microbial threats and hepatic (G2) liver cancer cells in water contaminated with chromium. Utilizing the well diffusion technique, substantial inhibition zones were observed for Escherichia coli, Staphylococcus aureus, Aspergillus niger, A. fumigatus, and A. flavus, with greater effectiveness noted at elevated concentrations (25, 50, and 100 mg/L). The collaborative effect of the ZnO and CuO nanoparticles combined with RCB not only enhanced antimicrobial performance but also exhibited significant cytotoxic activity against liver cancer cell lines, indicating a promising potential for use in water purification and medical applications. These results affirm that nanocomposites based on biochar present an efficient, eco-friendly, and economical approach for addressing heavy metal pollution and simultaneously managing microbial threats while hindering the growth of cancer cells. Subsequent investigations should prioritize evaluating in vivo toxicity, refining the synthesis of nanocomposites, and increasing production for practical wastewater management and therapeutic uses.

REFERENCES

Almomani, F., Bhosale, R., Khraisheh, M., Kumar, A., & Almomani, T. (2020). Heavy metal ions removal from industrial wastewater using magnetic nanoparticles (MNP). *Applied Surface Science*, *506*, 144924. https://doi.org/10.1016/j.apsusc.2019.144924

Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon*, *6*(9), e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

Bansal, M., Garg, R., Garg, V., Garg, R., & Singh, D. (2022). Sequestration of heavy metal ions from multi-metal simulated wastewater systems using processed agricultural biomass. *Chemosphere*, *296*, 133966. https://doi.org/10.1016/j.chemosphere.2022.133966

Dobrucka, R. (2017). Antioxidant and catalytic activity of biosynthesized CuO nanoparticles using extract of Galeopsidis herba. *Journal of Inorganic and Organometallic Polymers and Materials*, *28*(3), 812-819. https://doi.org/10.1007/s10904-017-0750-2

Ghasemi, M., Turnbull, T., Sebastian, S., & Kempson, I. (2021). The MTT assay: Utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. *International Journal of Molecular Sciences*, *22*(23), 12827.

https://doi.org/10.3390/ijms222312827

Gholami, P., Dinpazhoh, L., Khataee, A., & Orooji, Y. (2019). Sonocatalytic activity of biochar-supported ZnO nanorods in degradation of gemifloxacin: Synergy study, effect of parameters and phytotoxicity evaluation. *Ultrasonics Sonochemistry*, *55*, 44-56. https://doi.org/10.1016/j.ultsonch.2019.03.001

Kanamarlapudi, S. L., Chintalpudi, V. K., & Muddada, S. (2018). Application of Biosorption for removal of heavy metals from wastewater. *Biosorption*. https://doi.org/10.5772/intechopen.77315

Kubmarawa, D., Ajoku, G. A., Enwerem, N. M., & Okorie, D. A. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *African Journal of Biotechnology*, *6*(14). https://www.ajol.info/index.php/ajb/article/view/57755/0

Lehmann, J., & Joseph, S. (2015). Biochar for environmental management: Science, technology and implementation (2nd ed.). Routledge

Metkar, S. P., Fernandes, G., Navti, P. D., Nikam, A. N., Kudarha, R., Dhas, N., Seetharam, R. N., Santhosh, K. V., Rao, B. S., & Mutalik, S. (2023). Nanoparticle drug delivery systems in hepatocellular carcinoma: A focus on targeting strategies and therapeutic applications. *OpenNano*, *12*, 100159. https://doi.org/10.1016/j.onano.2023.100159

Mongy, Y., & Shalaby, T. (2024). Green synthesis of zinc oxide nanoparticles using rhus coriaria extract and their anticancer activity against triple-negative breast cancer cells. *Scientific Reports*, *14*(1). https://doi.org/10.1038/s41598-024-63258-7

Jagadeesh, N., & Sundaram, B. (2023). Adsorption of pollutants from wastewater by Biochar: A review. *Journal of Hazardous Materials Advances*, *9*, 100226. https://doi.org/10.1016/j.hazadv.2022.100226

Palanivel, T. M., Pracejus, B., & Victor, R. (2020). Phytoremediation potential of castor (Ricinus communis L.) in the soils of the abandoned copper mine in northern Oman: Implications for arid regions. *Environmental Science and Pollution Research*, *27*(14), 17359-17369. https://doi.org/10.1007/s11356-020-08319-w

Perumal, R. S., & Muralidharan, B. (2024). Valorization of ricinus communis outer shell biomass to biochar: Impact of thermal decomposition temperature on physicochemical properties and EMI shielding performance. *Results in Engineering*, *24*, 103097. https://doi.org/10.1016/j.rineng.2024.103097

Salman, M., Athar, M., & Farooq, U. (2015). Biosorption of heavy metals from aqueous solutions using Indigenous and modified lignocellulosic materials. *Reviews in Environmental Science and Bio/Technology*, *14*(2), 211-228. https://doi.org/10.1007/s11157-015-9362-x

Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H., Ann, L. C., Bakhori, S. K., Hasan, H., & Mohamad, D. (2015). Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. *Nano-Micro Letters*, *7*(3), 219-242. https://doi.org/10.1007/s40820-015-0040-x

Sharma, A., et al. (2023). Chromium toxicity: Environmental and health impacts. *Science of the Total Environment*, 857, 160076

Shakya, A., & Agarwal, T. (2019). Removal of Cr(VI) from water using pineapple peel derived biochars: Adsorption potential and re-usability assessment. *Journal of Molecular Liquids*, *293*, 111497. https://doi.org/10.1016/j.molliq.2019.111497

Thi Luyen, N., Van Nguyen, K., Van Dang, N., Quang Huy, T., Hoai Linh, P., Thanh Trung, N., Nguyen, V., & Thanh, D. V. (2023). Facile one-step pyrolysis of ZnO/Biochar Nanocomposite for highly efficient removal of methylene blue dye from aqueous solution. *ACS Omega*, *8*(30), 26816-26827. https://doi.org/10.1021/acsomega.3c01232

U.S. Environmental Protection Agency. (2024). IRIS toxicological review of hexavalent chromium (Cr(VI)). Integrated Risk Information System (IRIS).

Zhang, X., Zhang, L., & Li, A. (2018). Eucalyptus sawdust derived biochar generated by combining the hydrothermal carbonization and low concentration KOH modification for hexavalent chromium removal. *Journal of Environmental Management*, *206*, 989-998. https://doi.org/10.1016/j.jenvman.2017.11.079