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Synthesis and Characterization of Activated Carbon/ZnO Nanocomposite from Water Hyacinth for Heavy Metals Adsorption and its Antimicrobial Activity

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In present work, the activated carbon was prepared from water hyacinth (*Eichhornia crassipes*) and ZnO nanoparticles synthesized by chemical process. Nanocomposites were formed using activated carbon and ZnO nanoparticles to get improved electrostatic interactions for the successful adsorption of heavy metal ions such as nickel, cadmium, lead and chromium from aqueous solution. The prepared nanocomposites were further characterized using XRD, SEM and EDAX techniques. The heavy metal ions removal efficacy was evaluated through ICP-OES. The result showed that the prepared nanocomposite efficiently adsorbed 76.75-92.40% nickel, 75.80-92.25% cadmium, 86.25-96.30% lead and 76.37-95.26% chromium. Nanocomposites showed a significant antimicrobial activity against *Streptococci* and *B. subtilis*.

Keywords: Water hyacinth, ZnO/Activated carbon, Waste water effluent, adsorption, Heavy metals, Antimicrobial.

INTRODUCTION

Water pollution has become one of the major problems and imposes serious concern for today's civilization [1]. Heavy metals contamination in water have become an environmental concern around the world [2]. Remediation of heavy metals from the soil, water and air is difficult [3]. Several conventional methods are available for treatment of chromium industrial effluents like membrane filtration [4], precipitation [5], nano filtration [6], ion exchange [7], electrocoagulation, flotation [8] and adsorption [9]. These processes have significant disadvantages such as incomplete removal, high energy requirements and production of toxic sludge. Hyper accumulator plants are used for phytoremediation location of high levels of certain heavy metals that would be toxic-location high levels of certain heavy metals that would be toxic to most organisms [10].

To overcome the negative environmental effects of heavy metals, lot of research is in progress for using aquatic macrophytes for pollution or even as bio-indicators for heavy metals in ecosystem. *Eichhornia crassipes* is a perennial free floating aquatic plant belonging to the family Pontederiaceae [11]. There are seven species of water hyacinth; the best known being the common water hyacinth (*Eichhornia crassipes*), a fast growing, free-floating aquatic weed. It is capable of assimilating large quantities of trace elements and heavy metals, some of which are essential plant growth [12]. Water hyacinth, which is the world's most toxic aquatic plant, has a high growth rate and thus the potential to remove most of the heavy metals including Cr(VI) [13]. Carbonaceous materials like activated carbon does play a major role as adsorbent in the water treatment process for the removal of some noxious metals [14]. The activated carbon with large suface area, porosity and specific functional groups like carboxyl and hydroxyl groups aid in adsorption of heavy metals [15]. Hence, the carbonized plant materials, with inherent adsorption properties may enhance the attraction forces towards heavy metals.

Nanomaterials have gained lots of interest over several decades for their applications in various fields, from medicine to energy devices. Owing to their small size and larger surface to volume ratio, these nanomaterials are now applicable in the area of environmental protection. These properties will contribute to enhanced absorptivity and reactivity towards the removal of heavy metal ions [16]. Over past decade, significant contribution in terms of application of nanomaterials in water treatment is observed and emerged as promising alternative for the removal of heavy metal ions [16]. Zinc oxide is a unique nanostructure and it exhibits photocatalytic properties [17]. The larger surface area provides of ZnO plays major role as adsorbent for the removal of heavy metal ions. Furthermore, the mechanism of removal of heavy metals by ZnO is not fully understood, it is way beyond the accepted mechanism *i.e. via* reduction of metal cations [18]. A recent study shows that reduction/oxidation by electron-hole pairs and physical adsorption are the two possible mechanisms by which the heavy metal ions can be removed by ZnO nanomaterials [19].

The present study was performed to harness the four heavy metals such as lead, nickel, cadmium and chromium removal capacities of selected water hyacinth plant (*Eichhorina crassipes*) to determine the adsorption of metals using nanotechnology methods [20]. A cost effectiveness experiment has been performed to understand the adsorption of heavy metals such as nickel, cadmium, lead and chromium using water hyacinth (*Eichhorina crassipes*).

EXPERIMENTAL

The raw materials for preparing activated carbon are the processed water hyacinth (*Eichhorina crassipes*) obtained from the basin of Anchepalya lake, Bangalore, India. Water Hyacinth sample was brought to the laboratory and authenticated from NADRI, Bengaluru, India. Its shoots were segregated and washed ten times with deionized water, cut into small pieces and dried at 70 ºC for 12 h. The dried materials were further ground well into powder form and sieved through 60 mesh sieve. All the chemicals and reagents used in this experiment were of analytical grade and used without any further purification.

Preparation of standard solutions: Certified reference standard material (NIST, Gaithersburg, USA) from Merck KGaA, Lot no.: HC90061092 was used for the preparation of standard concentration of 5 mg/L.

Preparation of activated carbon: Water hyacinth sample weighed about 6 g was added into 100 mL of phosphoric acid, stirred and soaked for 24 h. Then the sample was filtered and carbonized at 300 ºC in a nitrogen environment (@0.2 mL/min) for 30 min. The cooled solid sample was taken out and washed with 10% HCl and deionized water successively. Then the activated carbon sample was dried at 120 ºC for 24 h.

Preparation of ZnO/activated carbon nanocomposites: Zinc acetate dihydrate (0.2 M) was mixed with 40 mL of 5 % PEG and stirred in a magnetic stirrer. Activated carbon (1 g) (obtained from water hyacinth) was then added in the above mixture and stirred well for 20 min. The pH of the reaction mixture was increased to pH 9 by adding 6 M NaOH under continuous stirring with constant heating at 80 ºC for 3 h. The coloured precipitate obtained was collected through centrifugation (8000rpm), followed by washing five times with deionized water. The final product was dried at 80 ºC for 2 h. The dried sample was further grinded well using pestle mortar and collected for further characterization.

Characterization: X-Ray diffraction analysis was performed by XRD; Bruker, D8 Focus instrument to characterize the structural behavior of the adsorbent samples. Optical property of the sample was analyzed using UV-visible spectroscopy (UV-2450, SHIMADZU). SEM analysis was carried out by scanning electron microscope (SEM; Zeiss EVO 50) coupled with a Bruker energy dispersive X-ray (EDX) analyzer to examine surface morphology and elemental composition of the samples. The heavy metal ions removal efficacy was evaluated through ICP-OES (Make: Thermo-Fisher Scientific, Model: iCAP 7400).

Adsorption studies: Batch mode laboratory experiment was performed for adsorptive studies of the samples. The pH of the sample solution was adjusted using NaOH and HCl.

Effect of contact time using activated carbon: A quantity of 500 mg of activated carbon was added into four different conical flasks containing 500 mL of heavy metal solution. The different flasks were stirred well for 30, 60, 120 and 180 min, respectively. content of each flask was filtered using Whatmann filter paper No. 41. The filtrate was taken and two drops of HNO_3 and HCl were added and analyzed the samples using ICP-OES.

Effect of contact time using activated carbon/ZnO nanocomposites: The procedure was repeated in a similar manner for activated carbon/ZnO as mentioned above. The amount of heavy metals adsorbed (q_e) per unit mass of activated carbon and activated carbon/ZnO would be determined using eqn. 1 and the removal efficiency (E%) of the sorbent would be calculated using eqn. 2:

$$q_e = \frac{(C_o - C_a)}{m} \cdot \frac{V}{1000} \quad (1)$$

$$E\ (\%) = \frac{(C_o - C_a)}{C_o} \times 100 \quad (2)$$

where C_o is the initial concentration of stock sample (mg/L); C_a is the final concentration of stock sample after adsorption (mg/L); q_e is the amount of heavy metal adsorbed into the activated carbon (mg/g); V is the volume of the medium (mL); m is the amount of the activated carbon (g).

Antimicrobial activity: The prepared activated carbon and activated carbon/ZnO nanocomposite were evauated for antibacterial activity against Gram-positive bacterial *B. subtilis* and *Streptococci* strains using standard antibiotic streptomycin by disc diffusion method. Test bacteria were seeded evenly onto the surface of nutrient agar media maintaining a concentration of 1.5×10^8 CFU mL^{-1} using a sterile glass spreader. The sterile discs (6 mm) were loaded with 50 µL of pure charcoal and prepared nanocomposite in 8 and 5 mM to each disc and placed on agar plates along with standard streptomycin drug. The inoculated plates were incubated for 24 h at 37 ± 2 ºC and the zone of inhibition were measured around the discs. The experiment was repeated in triplicates along with standard.

Anti-biofilm formation activity of prepared activated carbon and activated carbon/ZnO nanocomposite: The quantitative estimation of biofilm formation was done using 96 well microtiter plate. Plates were inoculated with 180 µL of Luria-Bertani (LB) broth, 10 µL of overnight grown culture concentration to 5×10^5 CFU/mL added to each well. 10 µL of activated carbon and activated carbon/ZnO nanocomposite at different percentage were used (12.5, 25, 50 and 100%) and kept for incubation at 37 ºC for 24 h. After incubation content of the wells was removed gently and washed twice with PBS to remove bacterial cells. Bacterial biofilms were fixed with sodium acetate followed by staining with 0.1% crystal violet dye for 10 min. The stained cells which were attached to wells were washed with distilled water and air dried. 200 µL of ethanol (95%) was added in each well to elute the attached cells, and absorbance was noted at 620 nm on multimode plate reader. Sterile growth medium was the negative control and cells with no treatment served as positive controls, respectively. The percentage of biofilm inhibition was calculated using eqn. 3:

$$\text{Biofilm inhibition (\%)} = 1 - \frac{OD_{620}\text{ of cells treated with AgNPs}}{OD_{620}\text{ of positive control}} \times 100 \quad (3)$$

RESULTS AND DISCUSSION

X-Ray diffraction studies: The powder XRD patterns of activated carbon prepared from water hyacinth and activated carbon/ZnO nanocomposite are shown in Fig. 1. It is observed from Fig. 1a, two broad diffraction peaks at 2θ angles of 24º and 44º are indexed to (002) and (100) diffraction, which indicates graphitic carbons matched well with standard (ICDD 89137). The XRD pattern of the prepared nanocomposite (Fig. 1b) exhibited the peaks at 31.75º, 34.25º, 36.32º, 47.52º, 56.60º, 62.85º, 66.45º, 67.95º and 69.15º and assigned to the planes (100), (002), (101), (102), (110), (103), (200), (112) and (201). The XRD data matched with standard ICDD 891397, which attributed to the hexagonal wurtzite structure of ZnO. The crystallite size was calculated using Debye-Scherrer′s equation $D = K\lambda/\beta\cos\theta$, where the size of activated carbon and nanocomposite size was found to be 42.6 and 14.4 nm, respectively.

SEM-EDX analysis: The SEM micrographs of activated carbon particles prepared from water hyacinth are shown in Fig. 2a. The activated carbon exhibited a flake shape and has

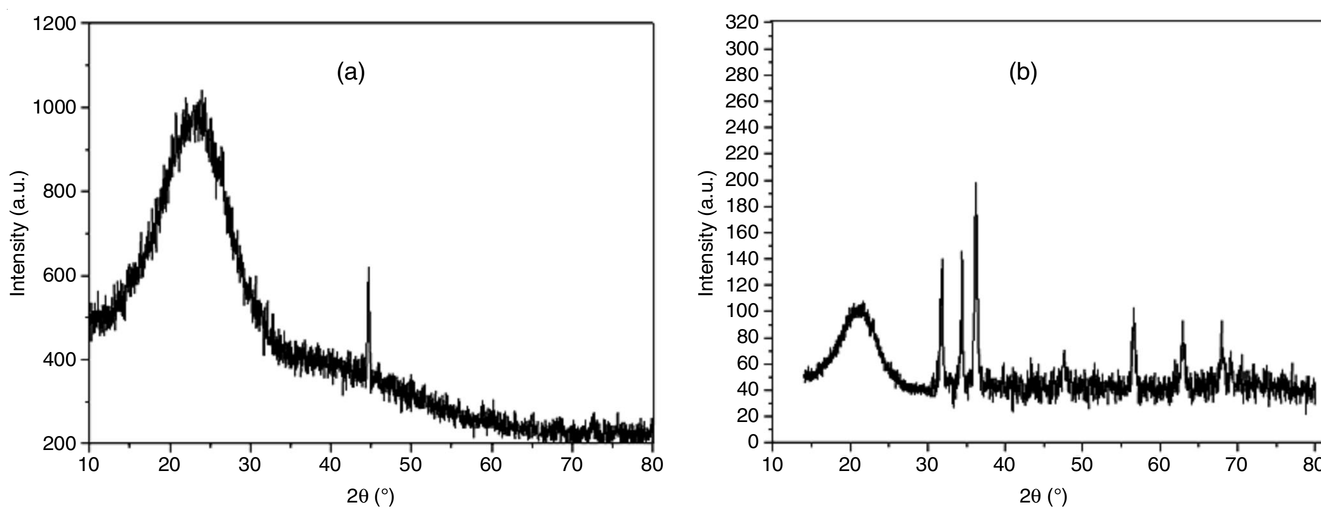

Fig. 1. XRD spectra of activated carbon obtained from water hyacinth (a); and AC/ZnO nanocomposite (b)

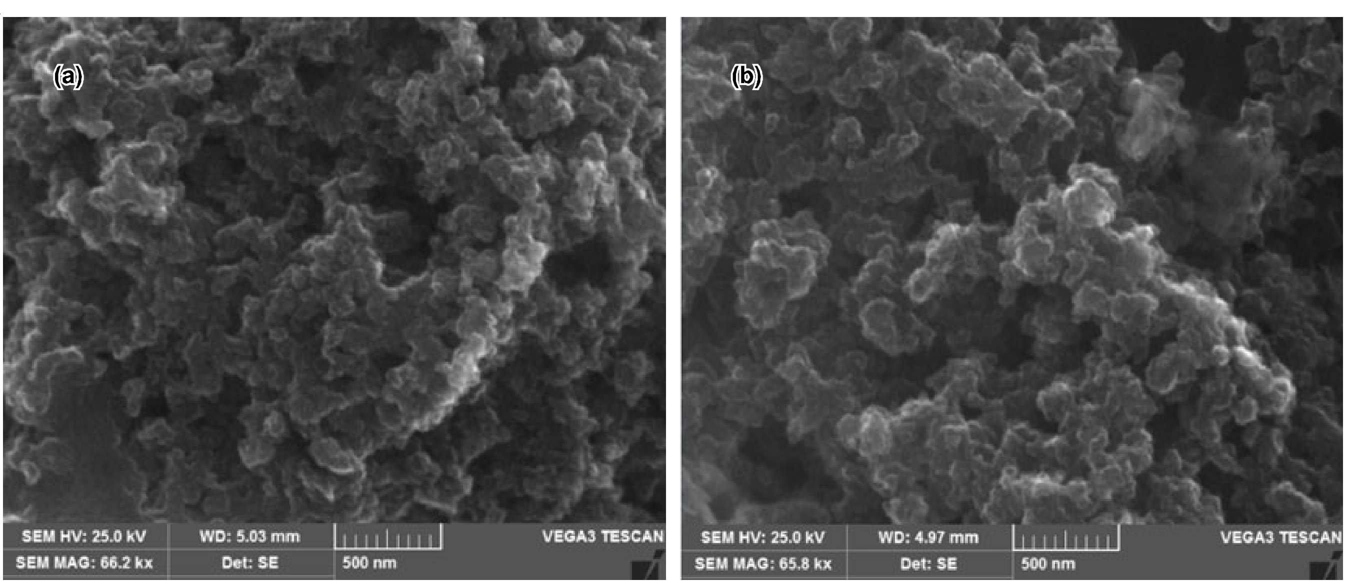

Fig. 2. SEM images of activated carbon obtained from water hyacinth (a); and AC/ZnO nanocomposite (b)

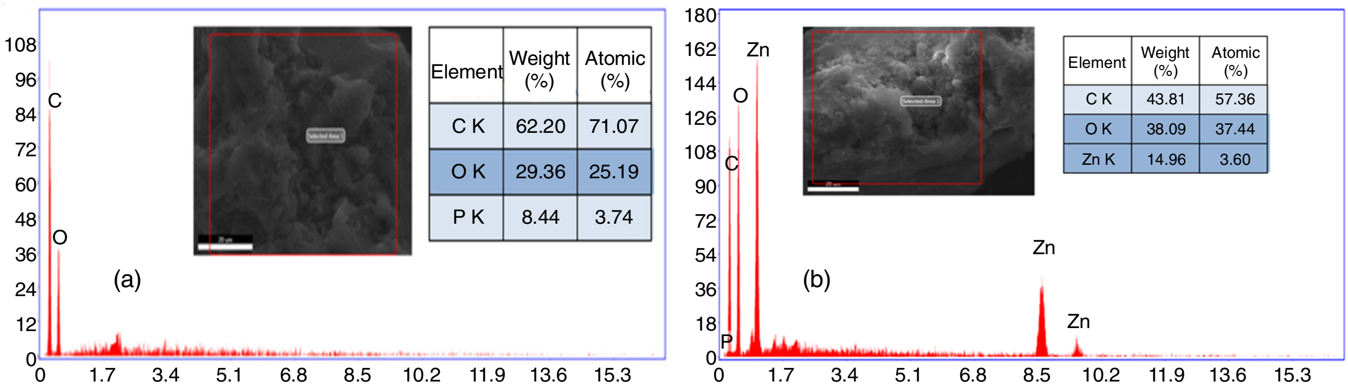

Fig. 3. EDS spectra of activated carbon obtained from water hyacinth (a); and AC/ZnO nanocomposite (b)

a porous character. The SEM image of activated carbon/ZnO nanocomposite is shown in Fig. 2b. The ZnO nanoparticles are impregnated on activated carbon partially and slightly block the porosity of the activated carbon surface. The size of the preparared nanocomposite was found to be 52 nm and also observed that the composite still displays porous nature.

The activated carbon and activated carbon/ZnO nanocomposite were further characterized by EDAX to identify the elements present in the materials. It is observed that C and O elements present in activated carbon (Fig. 3a) and Zn, O and C present in the prepared nanocomposite (Fig. 3b), further indicate that the prepared materials were free from impurities, which is in good agreement with XRD analysis.

UV-visible analysis: The UV-visible spectra of activated carbon prepared from water hyacinth and activated carbon/ZnO nanocomposite are shown in Fig. 4. Activated carbon obtained from water hyacinth shows absorption peak at 280 nm and the nanocomposite absorption peak at 375 nm due to the quantum confinement of the exactions present in the prepared sample.

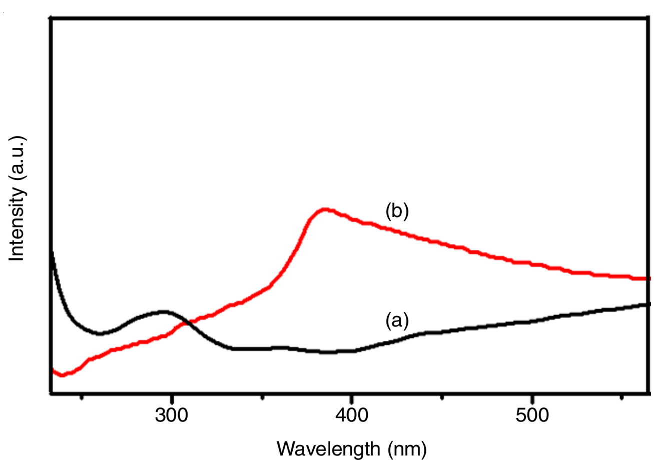

Fig. 4. UV-visible spectra of activated carbon obtained from water hyacinth (a); and AC/ZnO nanocomposite (b)

Adsorption studies: The particle size plays a significant role in the tuning of adsorption capacity in order to remediate the heavy metals from their aliquots. The experimental results show that the adsorption of heavy metals on to the activated carbon/ZnO nanocomposites is appreciable compared to the other related literature [21]. The smaller size the prepared nanocomposite (52 nm) shows a corresponding increase in the surface area available for the adsorbate-adsorbent interaction [22]. The larger surface area decreases the path inside the micropores of the activated carbon for the heavy metal ions. The functional groups present in the activated carbon also helps to bind with the divalent metal ions for their selective removal [23].

Effect of contact time: The effect of contact time on the adsorption of Ni, Cd, Pb and Cr ions on activated carbon and activated carbon/ZnO nanocomposite was studied. Activated carbon (500 mg) was added to the 500 mL metal ions solution in three different flasks. The adsorption equilibrium was studied within the range of 30 min to 180 min. Table-1 shows that the ratio of adsorption of heavy metals on to activated carbon is static. There are no significant changes in the adsorption capacity from the interval of 30 min to 60 min indicated that the equilibrium reached very fast. However, a slight increase in the adsorption at 120 min indicated that the equilibrium state is reached within the 120 min and consistent with the reported studies [24]. For cadmium, the optimum contact time for high adsorption was 120 min, there after the adsorption decreases, may due to desorption, the result matched with the reported literature [25]. The fast adsorption kinetics for the metal ions under study serves an advantage in designing the chemical water treatment systems.

The similar experiments were performed for the adsorption of same metal ions using same aliquots containing activated

TABLE-1
EFFECT OF CONTACT TIME USING ACTIVATED CARBON OBTAINED FROM WATER HYACINTH AND AC/ZnO NANOCOMPOSITE (b)

Parameter	30 min		60 min		120 min		180 min	
	AC	AC/ZnO	AC	AC/ZnO	AC	AC/ZnO	AC	AC/ZnO
Nickel (mg/L)	5.225	1.215	5.306	0.411	5.321	0.409	5.327	0.405
Cadmium (mg/L)	5.459	1.321	5.552	0.445	5.561	0.431	5.552	0.438
Lead (mg/L)	5.706	0.785	5.711	0.226	5.719	0.215	5.729	0.212
Chromium (mg/L)	5.510	1.302	5.562	0.276	5.573	0.266	5.459	0.259

carbon/ZnO nanocomposite. The values from the adsorption study are given in Table-1 indicating an increase in the adsorption compared to activated carbon alone.

Amount of heavy metal adsorption: It was observed that adsorption of heavy metals (Ni^{2+}, Cd^{2+}, Pb^{2+} and Cr^{6+}) in aqueous sample with activated carbon/ZnO nanocomposite exhibit high sensitivity compared to other nanoparticles (Table-2). The different adsorption amount (q_e) of nickel at 30, 60, 120 and 180 min of treatment results in the removal efficiency ranges at 76.75, 92.25, 92.31 and 92.40, respectively. Similarly, the removal efficiency of other metals ions *i.e.* cadmium (75.80-92.25%), lead (86.25-96.30%) and chromium (76.37-95.26%) also showed that prepared nanocomposite is effectively fissible for the removal of other heavy metal ions. The biocompatible of naturally occurring water hyacinth (*Eichhornia crassipes*) and ZnO based activated carbon nanocomposite can become strong alternative adsorbent for the removal of heavy metal ions.

Antimicrobial analysis: The bacterial growth inhibitory efficacy of activated carbon and activated carbon/ZnO nanocomposite against *B. subtilis* and *Streptococci* is shown in Table-3. Among test bacteria, susceptibility was found higher in *Streptococci* with activated carbon/ZnO nanocomposite in comparison of activated carbon alone. Inhibition of the studied bacteria by standard antibiotics was higher when compared to activated carbon and activated carbon /ZnO nanocomposite. The antimicrobial activity of crude extracts of water hyacinth against some bacterial strains were attributed due to the presence of alkaloids and its derivatives [26]. The variation in the efficacy of plant extract against different microorganisms depends upon the chemical composition of the extracts and membrane permeability of the microbes for the chemicals and their metabolism [27].

Minimum inhibitory concentration (MIC) assay: The MIC values for both activated carbon and activated carbon/ZnO nanocomposite were evaluated by serial dilution method. Activated carbon/ZnO nanocomposite (8 mM) showed highest inhibition when compared to pure activated carbon. The synthesized nanocomposite exihibited concentration dependent inhibitory activity against *B. subtilis*, whereas pure activated carbon didn't showed a promising inhibitory activity against *B. subtilis*. Similarly, both activated carbon and activated carbon/ZnO nanocomposite also exhibited activity against *Streptococci*, but at 8 mM of ZnO doped nanocomposite showed the maximum inhibitory activity (Fig. 5).

Biofilm formation inhibitory assay: The bacteria which are forming the biofilm may have the ability to cause numerous diseases, and according to the reports of the National Institutes of Health and Centre of Disease Control, 65-80% infections

TABLE-2
EFFECT OF AMOUNT OF HEAVY METAL ADSORPTION (q_e) AND ITS REMOVAL EFFICACY OF SORBENT (E%) OF ACTIVATED CARBON AND ACTIVATED CARBON/ZnO

Parameter	30 min		60 min		120 min		180 min	
	q_e	E%	q_e	E%	q_e	E%	q_e	E%
Nickel	4.01	76.75	4.89	92.25	4.90	92.31	4.92	92.40
Cadmium	4.13	75.80	5.10	91.18	5.12	92.25	5.11	92.11
Lead	4.92	86.25	5. 48	96.04	5.50	96.24	5.51	96.30
Chromium	4.20	76.37	5.25	95.04	5.30	95.23	5.19	95.26

TABLE-3
ZONE OF INHIBITION OF ACTIVATED CARBON AND ACTIVATED CARBON/ZnO AGAINST *Streptococci* AND *B. subtilis*

Organism	Zone of inhibition (mm) at 250 µg/disc					
	Pure carbon			Zn doped		
	Std (mm)	8 mM (mm)	5 mM (mm)	Std (mm)	8 mM (mm)	5 mM (mm)
Streptococci	34 ± 0.5	26.5 ± 0.8*	13.6 ± 1.5*	33 ± 0.7	25.3 ± 0.6*	14.6 ± 0.6*
B. subtilis	30 ± 1.7	23.1 ± 0.5	14.3 ± 0.6*	31 ± 0.5	23.6 ± 1.5*	15.3 ± 0.6*

All the values are represented in Mean ± SEM, n = 2, *$p > 0.05$ is compared to standard.

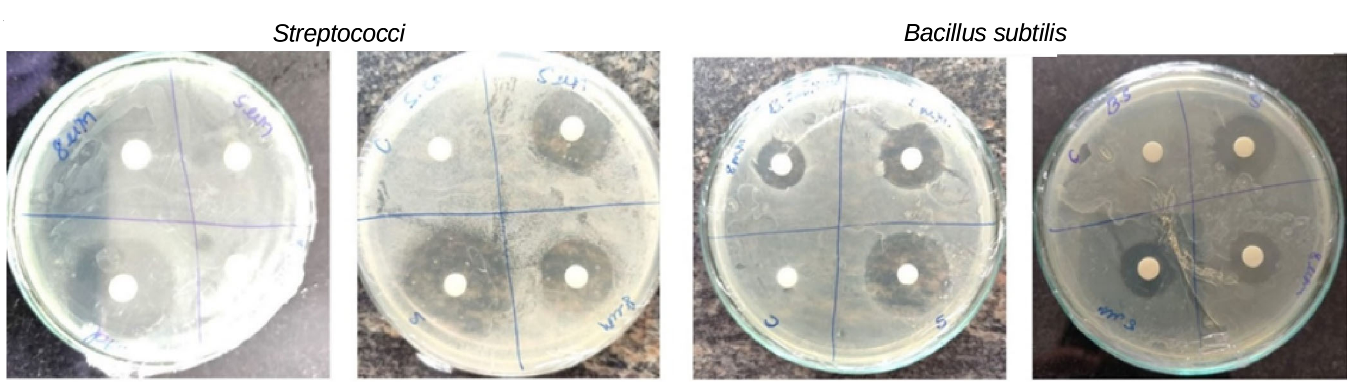

Fig. 5. Antimicrobial activity of activated carbon (sample 1) and the prepared AC/ZnO nanocomposite (sample 2) against, *Streptococci* and *Bacillus subtilis* by disk diffusion method

are due to biofilm-forming microbes. Thus, the best approach to avoid the infections by these microbes is, using the biofilm inhibitors. Many studies have reported the effective role of nanoparticles as biofilm inhibitors against target bacteria [28].

The microtiter plate method was used to quantify the biofilm formation and the % inhibition of biofilms in *Streptococci* and *Bacillus subtilis* after treatment with different concentrations of neat activated carbon and activated carbon/ZnO nanocomposite. The results showed that in most of the cases, cells treated with subinhibitory concentrations of nanocomposite significantly ($p < 0.05$) reduced the biofilm formation in a dose-dependent manner. As shown in Fig. 6, the percentage of inhibition of biofilm formation was high in activated carbon/ZnO nanocomposite against *B. subtilis* and *Streptococci*. A biofilm formation (1.5-2.5 mm) inhibited in *B. subtilis* were reported in the presence of different concentrations of activated carbon and activated carbon/ZnO nanocomposite.

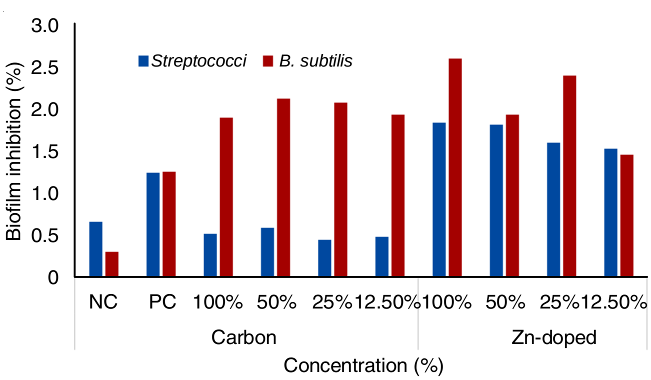

Fig. 6. Biofilm inhibitory assay of activated carbon and activated carbon/ZnO against *Streptococci* and *B. subtilis*

Conclusion

Activated carbon prepared from water hyacinth (*Eichhornia crassipes*) and incorporated with ZnO nanocomposite showed significant ability in removing heavy metals in aqueous solution. The ICP-OES results showed that the adsorption of 76.25-92.40% nickel, 75.80-92.25% cadmium, 86.25-96.30% lead and 76.37-95.26% chromium. Thus, study suggests that activated carbon from water hyacinth plants with embedded ZnO nanoparticles can be utilized for mitigating environmental pollution. Activated carbon prepared water hyacinth doped with ZnO also showed significant antimicrobial effect against *Streptococci* and *B. subtilis*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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