

3D Printed Antimicrobial Cups for Heavy Metal Chelation

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Introduction

Safe drinking water is a fundamental requirement for life. It also is a critical component for agriculture, irrigation, and commercial industries. Industrial water pollutants often include heavy metal ions such as lead, cadmium, copper, arsenic, and mercury. Exposed individuals to these suffer from numerous health issues including disorders in the renal, neurological, gastrointestinal, endocrine, immune, reproductive systems, and also skin diseases.

On the other hand, *Escherichia coli* (*E. coli*) is one of the bacterial species that are pathogenic to humans. Some of the *E. coli* serotypes, once present in water, lead to acute diarrheas and gastroenteritis. Hence, methods for the efficient removal of these types of contaminants from drinking water draw immense importance to the global community.

Most of the conventional water purification techniques (i.e. use of chemical agents like chelators, flocculants, organic dyes, and methods including filtration, reverse osmosis, and distillation) are optimized at industrial scale making them non-affordable for the general public. Moreover, when dilapidated infrastructure becomes the transportation mode of water, especially in areas of poor-living conditions, obtaining clean water becomes a major issue. With the target of addressing these challenges, we present a low-cost eco-friendly composite fabricated from chitosan and polylactic acid (PLA).

Chitosan contains lone electron pairs on the nitrogen atoms of the amine groups and oxygen atoms of the hydroxyl groups. These act as chelating sites for the heavy metal ions (Wu et al. 2001). On the other hand, electrostatic interactions between chitosan and negatively charged cell wall of *E. coli*, could disrupt the bacterial cell membrane, cause structural damage and leakage of cellular content inhibiting the bacterium. Our strategy in the present work was to combine chitosan with PLA, a biodegradable thermoplastic, forming a composite that could be 3D printed into

free standing objects. After demonstrating toxic heavy metal chelation and antimicrobial properties for the composite material, cup-like robust structures could be 3D printed proving the potential capabilities to further enhance its performance via 3D printing, a growing technology that can achieve complex, high surface areas not conducive for conventional moulding (Appuhamillage et al. 2024).

Materials and Methods

PLA (Luminy LX175) in fine resin pellet form (melt flow index-MFI at 190 °C/2.16 kg) was received from Natur Tec Lanka (Pvt) Ltd, Sapugaskanda, Sri Lanka. Chitosan flakes ($\geq 75\%$ deacetylated, molecular weight (Mw) 3,800-20,000 Da) was purchased from Sri SR Laboratory (Pvt) Ltd, Colombo 13, Sri Lanka. Lead (II) nitrate, Copper (II) nitrate trihydrate, and Cadmium (II) nitrate tetrahydrate were purchased in powder form from Athula Laboratory Equipment (Enterprises), Kaduwela, Sri Lanka. Ethylenediaminetetraacetic acid (EDTA) in powder form was purchased from Glorchem Enterprise, Colombo 11, Sri Lanka. Ultrapure water was obtained from a Nuve/NS 103 water purification system. Metal concentrations were analyzed using an Agilent 7900 inductively coupled plasma mass spectrometer (ICP-MS) under helium mode. All ICP-MS measurements were carried out using an aqueous solution containing 3% nitric acid and 2% hydrochloric acid as the matrix. Each measurement was calculated as an average of three measurements.

Preparation of composites

Neat PLA pellets and chitosan flakes were mechanically ground to 250 microns particle size. These powders were mechanically mixed homogeneously according to Table 1. Using a steel mould ((40 mm (length) $\times$ 40 mm (width) $\times$ 1 mm (thickness)), composites were prepared using a heat-press at 180 °C for 3 min under 0.3 MPa pressure.

Table 1. Composition of the composites in wt.%.

Material	PLA (wt%)	Chitosan (wt%)
Control (neat PLA)	100	0
10 C/PLA	90	10
20 C/PLA	80	20
30 C/PLA	70	30
40 C/PLA	60	40
50 C/PLA	50	50
60 C/PLA	40	60

Thermal characterization

As-prepared composites and neat PLA (the control) were subjected to TGA analysis using the TG analyzer (TGA 5500- Discovery TA) at 30-700 °C under a nitrogen atmosphere and at a 10 °C/min heating rate. DSC analysis was also performed using a DSC Q100-TA instrument in the 30-300 °C temperature range at a heating and cooling rate of 10 °C/min.

Heavy metal adsorption tests of the prepared composites

The as-prepared composite discs were completely and separately immersed in aqueous stock solutions of each metal ion in concentrations that represent moderately polluted water bodies (Das and Halder 2017, Appuhamillage et al. 2019). For the detection of Pb²⁺ levels, a 30 ppb Pb²⁺ stock solution (250 mL) was prepared using the nitrate salt in ultra-pure water, and the immersed discs were subjected to mechanically stirring at 50 rpm at pH 7.0 and room temperature for 5, 10, 15, 30, and 60 min. After each time period, the supernatant was analyzed via ICP-MS. To detect Cu²⁺ and Cd²⁺ levels, the same procedure was carried out but using 1500 ppb and 30 ppb stock solutions prepared using the corresponding metal nitrate salt respectively. Neat PLA was used as the control at each occasion.

Antimicrobial activity

As a proof of the concept, the antimicrobial activity of the composites 30 C/PLA and 60 C/PLA was investigated against Gram-positive strains such as *Staphylococcus aureus* (ATCC 6538) and *Bacillus cereus* (ATCC 14579) and Gram-negative *Escherichia coli* (ATCC 8099). The agar diffusion method was followed

(Zhang et al. 2015) with minor adjustments.

3D Printing

Fused filament fabrication (FFF) based 3D printability was evaluated for the prepared composites, starting from the lowest chitosan loaded 10 C/PLA material. Using the 10 C/PLA, 20 C/PLA and 30 C/PLA composites, 3D printable filaments having a square-shaped cross-section (dimension: 1.24 mm (W) × 1.24 mm (H) × 20 cm (L)) were prepared with a stainless steel mould of same dimensions, under the heat-press conditions mentioned before. Using these filaments, cup-like shapes of 20 mm outer diameter, 0.4 mm wall thickness, and 10 mm height were 3D printed via a FFF 3D printer (Creality-Ender 3). The 30 C/PLA was also 3D printed into a complex object with pass-through holes and estimated total surface area of ca 2,413 mm².

Mechanical properties of 3D printed cups

A universal testing machine (Testometric-M500-50CT Win-Test) was used for the compression tests that were performed on the 3D printed 30 C/PLA cups. A compression rate of 5 mm/min was used at room temperature conditions. Ultimate compressive strength, toughness, and elastic Young's modulus were determined as averages from three measurements. PLA was used as the control.

Results and Discussion

Heavy metal adsorption tests of the prepared composites

As per the results shown in Figure 1, 30 C/PLA and 60 C/PLA composites were capable of producing safe drinking water within 5 min of contact time with Pb²⁺ solution, as the remaining Pb²⁺ concentration goes below the EPA level of 15 ppb (denoted by the dotted line in Figure 1) within 5 min. Further, all the three composites show significantly higher Pb²⁺ adsorption than that of neat PLA, the control, within all the contact times tested.

Antimicrobial activity

As per the results illustrated in Figure 2, inhibition zones were observed for the Gram-negative pathogenic

strain *E. coli* (ATCC 8099) for the 30 C/PLA and 60 C/PLA composites. In fact, the circular film diameter with the inhibition zone resulted for 60 C/PLA was slightly higher (13.5 mm) than that of the 30 C/PLA (12.0 mm). No inhibition zone was observed for neat PLA, the control.

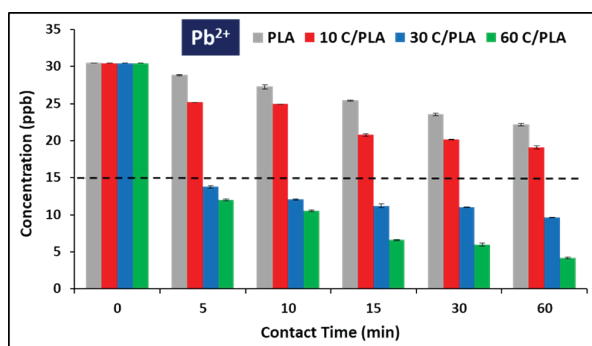


Figure 1: Comparison of the remaining Pb²⁺ concentrations in solution for 10 C/PLA, 30 C/PLA, 60 C/PLA, and neat PLA. EPA action level (15 ppb for Pb²⁺) is shown in black dotted line. A contact time of 0 min represents the initial Pb²⁺ concentration (30 ppb) in the stock solution. Adapted from Wijenayake et al. 2025.

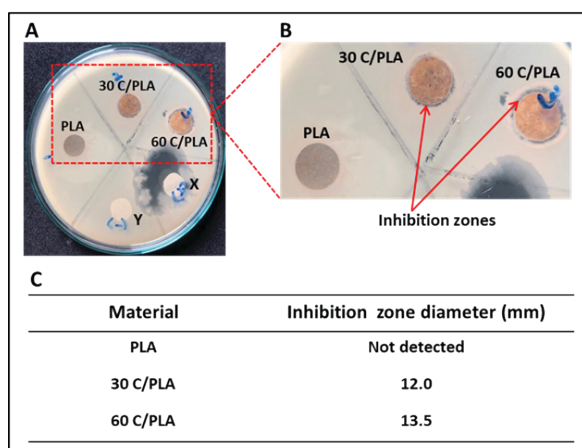


Figure 2: Antimicrobial activity tests. (A) Agar plate containing the circular film discs of neat PLA, 30 C/PLA, 60 C/PLA composites, the positive-control Amoxicillin (labeled X), and blank filter paper disc as the negative-control (labeled Y). (B) Zoomed section of the agar plate showing the inhibition zones of 30 C/PLA and 60 C/PLA against *E. coli* (ATCC 8099), and (C) resulting inhibition zone diameter (mm) vs. material used against *E. coli* (ATCC 8099). Adapted from Wijenayake et al. 2025.

3D Printing

In order to test whether we could use the C/PLA composite to fabricate a free-standing object that could be practically applied as a water filtration device, FFF-based 3D printing was carried out starting from the lowest chitosan loaded 10 C/PLA composite. According to the results depicted in Figure 3 (A), cup-like shapes could be successfully 3D printed using the composites; 10 C/PLA, 20 C/PLA, 30 C/PLA, and neat PLA. Furthermore, the 30 C/PLA material was also successfully 3D printed into a more complex free-standing object having pass-through holes and an estimated total surface area of ca 2,413 mm², as shown in Figure 3 (D). This proved that the chitosan/PLA composite, as a toxic heavy metal adsorbent, has the potential to be further developed into more complex geometries with internal cavities and high surface areas via 3D printing, which is not conducive easily by conventional moulding techniques like injection moulding.

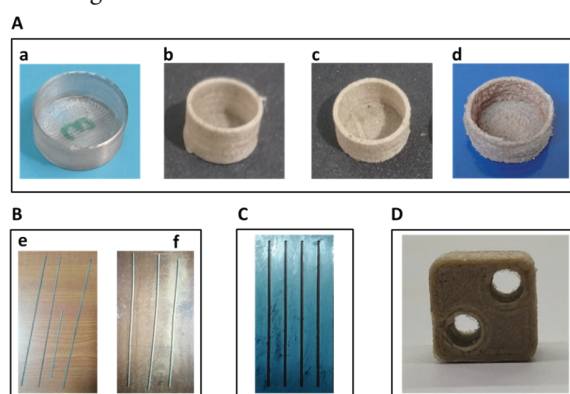


Figure 3: 3D printing of composites. (A) 3D printed cup-like shapes using neat PLA (a), 10 C/PLA (b), 20 C/PLA (c), and 30 C/PLA (d). (B) Representative filaments prepared for 3D printing using neat PLA (e) and 30 C/PLA (f). (C) A set of four steel moulds (dimension of one: 1.24 mm (W) × 1.24 mm (H) × 20 cm (L)) used to prepare the filaments. (D) 3D printed free-standing complex geometry with an estimated total surface area of ca 2,413 mm² using 30 C/PLA.

Adapted from Wijenayake et al. 2025.

Mechanical properties of 3D printed cups

As per the results shown in Figure 4, all the mechanical properties; ultimate compressive strength, toughness, and Young's elastic modulus had been

significantly improved in the 30 C/PLA composite compared with neat PLA, the control.

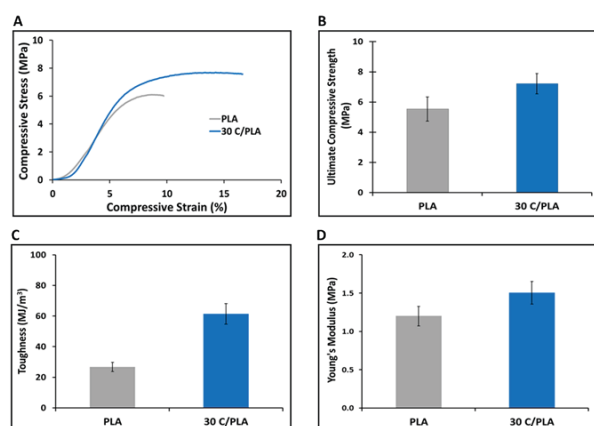


Figure 4: Resulting mechanical properties of the 3D printed 30 C/PLA cups against neat PLA. (A) Representative stress vs. strain plots of 3D printed neat PLA and 30 C/PLA cups. (B), (C), and (D) depict the comparison of ultimate compressive strength, toughness, and Young's modulus of the 3D printed 30 C/PLA cups vs. neat PLA, respectively. Adapted from Wijenayake et al. 2025.

Conclusions and future work

In conclusion, the work reported here introduced a direct fabrication of eco-friendly 3D printable antimicrobial composite that has the ability to capture toxic heavy metal ions from contaminated water. For the first time, a 3D printable biodegradable chitosan/PLA composite (30 C/PLA) has reduced toxic Pb^{2+} levels below EPA-AL before 5 min and shown reusability achieving ca 96% of the original Pb^{2+} adsorption efficiency even after the fifth regeneration cycle. This material has also shown antimicrobial properties against *Escherichia coli*, a bacterium that could live in water. The additively manufactured cups of 30 C/PLA resulted significantly improved compressive strength, toughness, and Young's modulus with respect to neat PLA, the control.

Moreover, a separate study would evaluate the effects of other parameters such as pH of the medium,

concentration of competing/coexisting ions, and temperature on the metal adsorption kinetics of the composites. The effect of adsorbent surface area on metal filtration efficiency would also be evaluated by fabricating 3D printed geometries with high surface areas and directly applying for heavy metal adsorption in water.

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