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A Novel Nanocomposite Cellulose Acetate Membrane using Green Synthesized Silver Nanoparticles for Bioremediation of Leachate

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ABSTRACT

Conventional remediation techniques have become outdated and insufficient to treat the influx of pollution from different fronts (air, water, and soil). Green synthesis of nanoparticles is an eco-friendly approach to remediate these contaminants and Membrane technology is increasingly becoming popular for the treatment of wastewater due to their efficiency and versatility against a wide array of contaminants. Cellulose acetate (CA) is a polymer obtained from cellulose and hence considered biodegradable, making it a more environmentally friendly option over other conventional polymers. In this present study, silver nanoparticles were synthesized using Staphylococcus aureus and characterized by UV-vis Spectrometer, Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDAX). The synthesized green silver nanoparticles were assimilated onto synthesized CA membrane films to fabricate nanocomposite membranes (CA-X, CA-X1 and CA-X2). EDAX results showed higher counts of silver at 3keV on the CA-X, confirming that silver nanoparticles were properly embedded on the membrane. Physio-chemical tests performed on the collected sewage, showed that the total dissolved solids (TDS) were found to decrease significantly during the first hour of treatment, CA-X1 showed 16.2% decrease and 21.95% decrease was observed by CA-X2. A decrease in total nitrogen content by 38.88% and 41.36% for CA-X1 and CA-X2 respectively was recorded after a week's treatment. Therefore, the work displayed the capability of cellulose acetate nanocomposite membrane for leachate treatment, since it displayed its potential in remediating leachate in a short span of time and scalability could be achieved for a larger volume of leachate with larger nanocomposite membranes.

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INTRODUCTION

Advancements in science and technology have greatly contributed to the rather luxurious daily lifestyle we now lead. But this rapid acceleration in urbanization has led to the emergence of environmental concerns on a global scale. Advanced agronomic methods and industrial expansion contaminate water and soil worldwide. Strategies such as bioremediation and physiochemical remediation have been employed for treating pollutants. Bioremediation is the removal of toxic environmental contaminants using living organisms, primarily microorganisms in most recent modules. It has shown wide public acceptance due to its eco-friendly, cost-effective, and sustainable approach towards waste treatment (Vidali., 2001).

Bio-based nanoparticles synthesis is an emerging and eco-friendly approach for the remediation of environmental pollution. These nanoparticles have shown to be effective against carcinogens, heavy metal contamination and contaminated water (Singh *et al*., 2023). Bio-nanotechnology is the combination of both biological and physio-chemical principles to produce

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nano-sized particles with specialized functions and is often referred to as green synthesis or green chemistry. (Nadaroğlu *et al*., 2017). Silver nanoparticles (AgNPs) have garnered widespread interest attributing to their diverse potentiality in fields such as biomedicine, healthcare, and environmental remediation (Agrawal et al., 2018).The cost-effective and eco-friendly nature of AgNps have greatly increased their popularity worldwide. Current trends have highlighted the straightforward, ecofriendly, and economical synthesis of AgNPs by bacteria, thus increasing the reports of AgNP synthesis by various bacterial genera (both gram-positive and gram-negative) and species(Javaid et al., 2018). Metallic silver for centuries has been known for its anti-bacterial capability which can be attributed to silver ions dissolving from the surface of bulk silver (Lv et al., 2009). This specific property of AgNPs has greatly contributed to their research and use in wastewater treatment systems in both domestic and industrial scenarios. Membrane systems have also become an increasingly popular technology for the treatment of wastewater due to their efficiency and versatility against a wide array of contaminants: organic membranes are those formed from synthetic polymers derived from petroleum, such as poly-sulfone (PS), polyether sulfone (PES), and polyvinylidene fluoride (PVDF) (Dong *et al*., 2021). Cellulose acetate (CA) is a polymer obtained from cellulose and hence considered biodegradable. Recent studies have highlighted the capability of cellulose acetate as a capping agent owing to its hydrophilicity and ability to acquire desired properties by being functionalized by different groups. This study aimed to combine the aspects of both green synthesis methods and membrane systems to fabricate a biosynthesized nanocomposite cellulose acetate (CA) membrane and to assess its potential outcomes toward the bioremediation of sewage and leachate. Silver nanoparticles were synthesized by the reduction of silver nitrate by *Staphylococcus aureus* and assimilated onto the synthesized membrane film to form a nanocomposite membrane.

The objectives of the study are:

- Green synthesis of silver nanoparticles from bacterial colonies
- Fabrication of cellulose acetate nanocomposite membrane using biosynthesized silver nanoparticles
- Assessment of nanocomposite cellulose acetate membrane for bioremediation of leachate/sewage.

MATERIALS AND METHOD

Soil sample was collected in sterile container from the landfill site at MCC campus and Kundrathur, at a depth of 15cm. The sample was then transported to the laboratory for the isolation of bacteria. The collected soil sample was brought to the laboratory and a gram of soil sample was weighed and mixed with 99ml of sterile saline solution. Serial dilution was done up to 10^{-7} in sterile saline (0.9% (w/v) NaCl) solution.

Nutrient agar (NA) was prepared and sterilized. From the serially diluted soil sample, dilutions from 10^{-4} to 10^{-7} were selected, 1ml of appropriate dilutions were poured into sterile petri plates and to this sterile nutrient agar was added at bearable warmth. The plates were incubated in incubator at 37ºC for 48 hours. Bacterial colonies were isolated and subsequently sub-cultured until pure colonies were obtained. Pure colonies were inoculated into sterile nutrient broth and kept for incubation for 48 hours. After incubation, 10ml of bacterial suspension was taken in plastic centrifuge tubes and then centrifuged at 6000rpm for 15 minutes for two cycles (Das *et al*., 2014). The resultant supernatant was then taken for silver nanoparticle synthesis.

Conical flasks were taken and to each flask 45ml of 1mM $AgNO_3$ was added. The solution was then mixed using a magnetic stirrer, 5ml of prepared bacterial suspension was then added to the $AgNO_3$ solution slowly. (Biswas *et al*., 2019). The solution was stirred for 10-15 minutes until homogeneity was obtained. The obtained mixture was sealed with aluminum foil and kept in a cool, dry place with minimal light exposure for 3-5 days (Das *et al*., 2014).

The synthesized nanoparticles were purified and concentrated by repeated centrifugation at 5000rpm for 30 minutes at 4ºC. The supernatant was discarded, and the pellet was re-dissolved in double distilled water. The concentrated nanoparticle solution was then poured into a petri plate and dried in a hot air oven at 70ºC for a duration of 24h (Wang *et al*., 2022). The resultant dry sample was scrapped off with a sterile blade or spatula. The powdered nanoparticles were stored for characterization.

The formation of silver nanoparticles was monitored by sampling an aliquot (2ml) of the solution after 15 cycles and measurement of VIS spectra was done in a range of 400-500nm (Ashraf *et al*., 2016).

The membranes used in this experiment were prepared by the non-solvent induced phase separation method (NIPS). The polymer solution was immersed in a non-solvent bath, resulting in the rapid precipitation of the polymer at the interface leading to the formation of a very thin membrane layer (Dong *et al*., 2021b). Firstly, for the preparation of cellulose acetate membrane, in a 15ml glass vial, 12.5ml of acetone was taken and 1g of cellulose acetate was added to it. The polymer was allowed to dissolve until a clear dope solution was obtained. The dope solution was then placed on a glass slide and was carefully rolled using a glass rod ensuring that the solution was evenly distributed along the glass slide. The glass slide was then placed into deionized water, the film falling off the glass slide was then washed and preserved (Wang and Ding., 2022).

Secondly, for the preparation of cellulose acetate-silver nanoparticle membrane, to the dope solution was added powdered biosynthesized nanoparticles and was recorded as CA-X1. Thirdly, the CA membrane was treated using the biosynthesized silver nanoparticle solution as the non-solvent bath and was recorded as CA-X2.

The biosynthesized silver nanoparticles were characterized by FTIR analysis. The elemental composition (purity) of nanoparticles were assessed using energy dispersive X-ray spectrometer (EDS). Nanoparticle impregnated on CA membrane was characterized by SEM.

The leachate/sewage sample collected from the MCC farm was taken in 3 sterile glass beakers, each containing 150ml of the sample. CA-X1 and CA-X2 membranes were each placed into separate beakers using sterile forceps, ensuring that the membrane was uniformly placed in the sample avoiding curling/folding. A control sample was also maintained. The beakers were stored in a cool and dry area, a cover was also placed on top of each beaker to avoid foreign contaminants.

The physio-chemical parameters of samples were assessed before and after treatment with CA-X membrane. The parameters such as pH, alkalinity, hardness, chloride, iron, total dissolved solids, fluoride, ammonia, nitrite, nitrate, total nitrogen, and phosphate were analyzed using TWAD (Tamil Nadu Water Supply and Drainage) kit.

RESULTS AND DISCUSSION

Bacterial colonies isolated from landfill and waste disposal sites were subsequently sub-cultured to obtain pure colonies. The pure culture of *Staphylococcus aureus* was obtained as a bright, yellow-colored colony on nutrient agar, and was assessed for its ability to synthesis silver nanoparticle. This was in line with the biologically produced AgNPs using culture supernatants of *Staphylococcus aureus* showing significant antimicrobial activity against methicillin-resistant S. aureus as reported by Nanda *et al* in 2009.

The reduction of $AgNO_3$ into silver nanoparticles was determined by observing the change in color of the aqueous solution over the exposure period. A color change from cloudy-white to brown was observed within 3 days of the exposure period (Fig.1). The positive result was maintained for a duration of 48 hours to further confirm nanoparticle synthesis (Das et al., 2014) The reducing agent used in this study was isolated cells of *Staphylococcus aureus*.

Fig. 1. Color change of silver nitrate solution due to biosynthesis of silver nanoparticles.

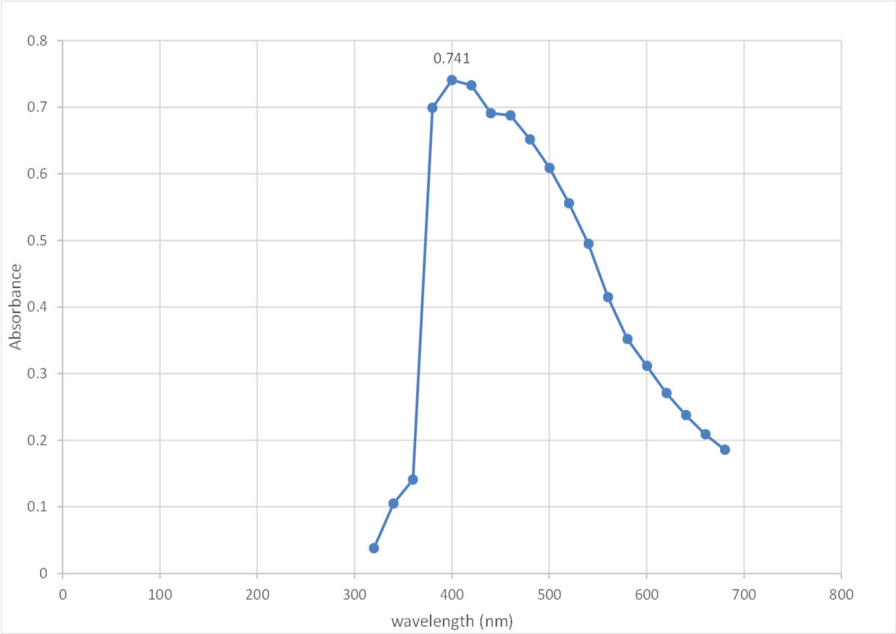


Fig. 2. Visible spectrum chart of biosynthesized silver nanoparticles from *Staphylococcus aureus* (peak at 400nm).

Biosynthesis of silver nanoparticles was confirmed by Vis-spectrophotometer. The visible spectra of the silver nanoparticle were recorded at 400nm (Fig.2).

Silver nanoparticles have been found effective for bioremediation but chemical approaches for synthesis are costly and may also lead to accumulation of toxic by-products. The study was able to demonstrate the synthesis of silver nanoparticles using *Staphylococcus aureus*, thus providing a green alternative to conventional methods. *Staphylococcus aureus* was found to be capable of reducing $AgNO_3$ into metallic silver extracellularly, which showed similar results to the study done by Otari *et al*., in 2014.

The aqueous solution of the silver nanoparticles showed a similar visible spectral peak of

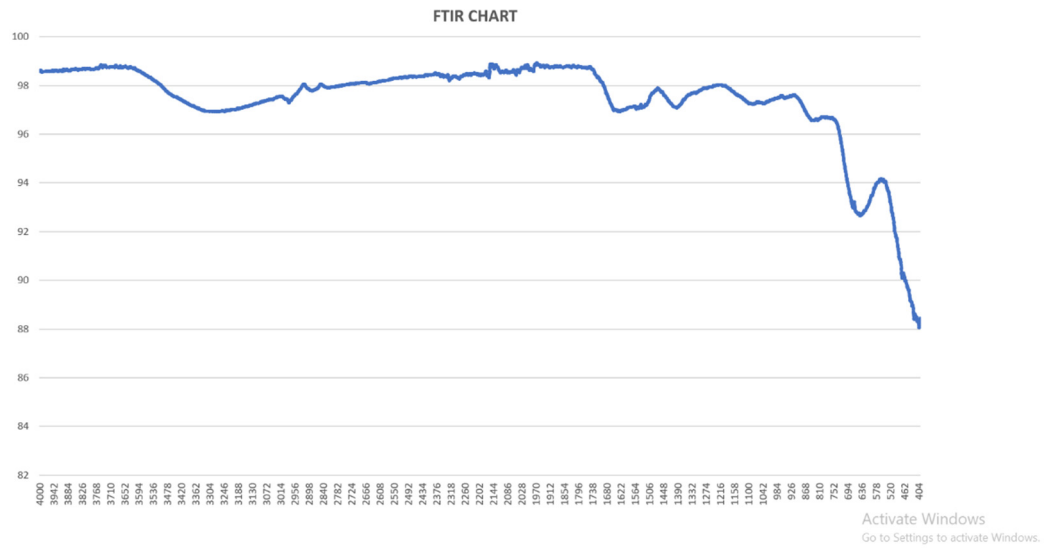


Fig. 3. FTIR spectroscopic micrograph of biosynthesized silver nanoparticles using *Staphylococcus aureus*.

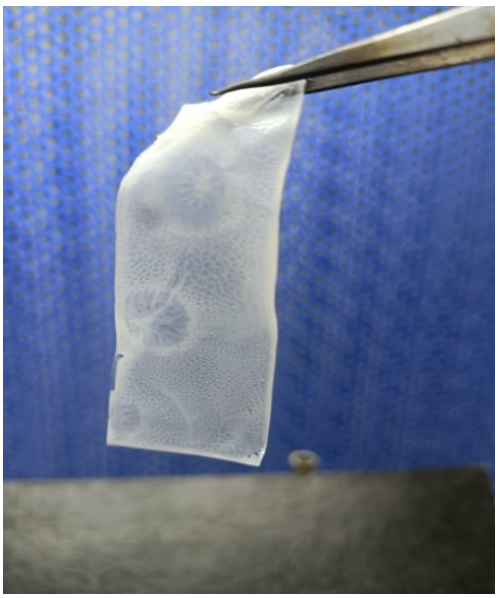


Fig. 4. Fabrication of CA-X1 membrane using NIPS method

400nm when compared with the peaks of silver nanoparticles obtained by Nanda *et al*., in 2009. The visible spectrum absorption was also in line with the spectral line for silver nanoparticles obtained by Das *et al*., in 2014, thus confirming the synthesis of silver nanoparticles.

FTIR measurements were carried out to identify potential functional groups of biomolecules in *Staphylococcus aureus* cell extract that were involved in the capping and reduction of silver ions into silver nanoparticles (Fig.3).

The FTIR results showed slightly dissimilar peaks and peak positional shift to 1600cm-1 range correlated with the results obtained by Govindaraju *et al*., in 2010, for their study using *Solanum torvum* leaf extracts which had a peak at 1450cm-1. This could be assumed that the functional groups associated with the molecular bonds had decreased or increased resulting in the possible change in hybridization and electron distribution in the associated molecular bonds.

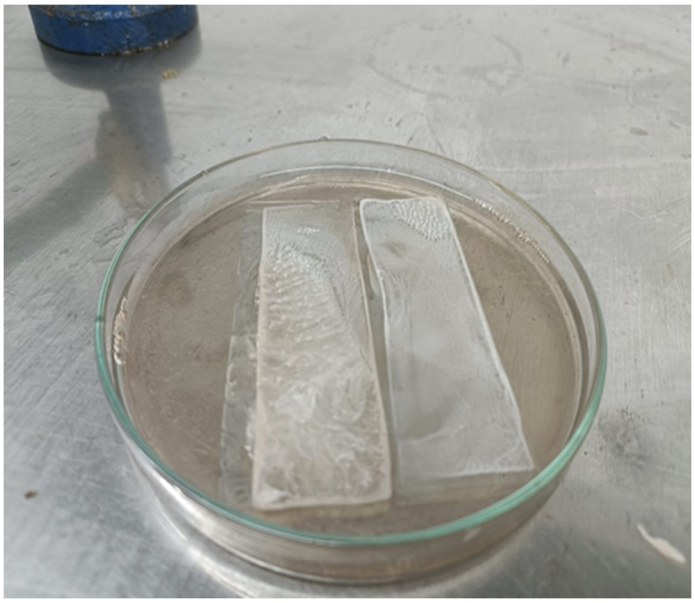


Fig. 5. Fabrication of CA-X2 membrane using NIPS method (silver nanoparticle solution being the non-solvent component)

The cellulose acetate silver nanoparticle membrane was fabricated following the non-solvent induced phase separation (NIPS) procedure (Wang and Ding., 2022) (Fig.4,5)

CA is a polymer that is frequently used in the creation of membranes and has undergone substantial investigation. CA is typically utilized as a material for dialysis applications and can be used to construct microfiltration (MF) (Chen *et al*., 2004), reverse osmosis (RO), nanofiltration (NF), and ultrafiltration (UF) (Sivakumar *et al*., 2006). High flux, moderate salt rejection, chlorine resistance, and cost-effectiveness are some characteristics of CA membranes. Additionally, non-toxicity and ease of processing are also considered (Faria et al., 2017). Therefore, cellulose acetate (CA) nanocomposite membranes were fabricated in the present study following the non-solvent induced phase separation (NIPS) to obtain nanocomposite membranes.

The surface morphology, size, and shape of biosynthesized nanoparticles on the CA-X membrane were analyzed by Scanning Electron Microscope. The SEM images displayed that the silver nanoparticles were globular with a rough surface morphology. The images from SEM showed the size of silver nanoparticles to be within the range of 200nm (Zahoor *et al*., 2021) (Fig.6).

Energy dispersive X-ray spectroscopy (EDS) study was also performed to confirm the presence of bio-synthesized silver nanoparticles on the membrane. A sharp peak for silver was observed at 3keV which confirmed the presence of silver nanoparticles due to Surface Plasmon Resonance (Jemal *et al*.,2017). Silver nanoparticles generally demonstrated a typical optical absorption peak due to Surface Plasmon Resonance at approximately 3 keV (Bindhu & Umadevi., 2013) (Fig.7)

SEM images displayed globular or spherical-shaped nanoparticles within the range of 200nm which was also shown by Jemal *et al*., in 2017. EDAX results showed higher counts of silver at 3keV on the CA-X which coincides with the results obtained by Bindhu and Umadevi., in 2013, showing that silver nanoparticles were properly embedded on the membrane.

Synthesized CA-X membranes were subjected to sewage/leachate treatment by immersing the membranes into leachate/sewage samples in a sterile container and the treatment process was monitored for 1 week. The results of the membrane treatment were assessed at the end of the

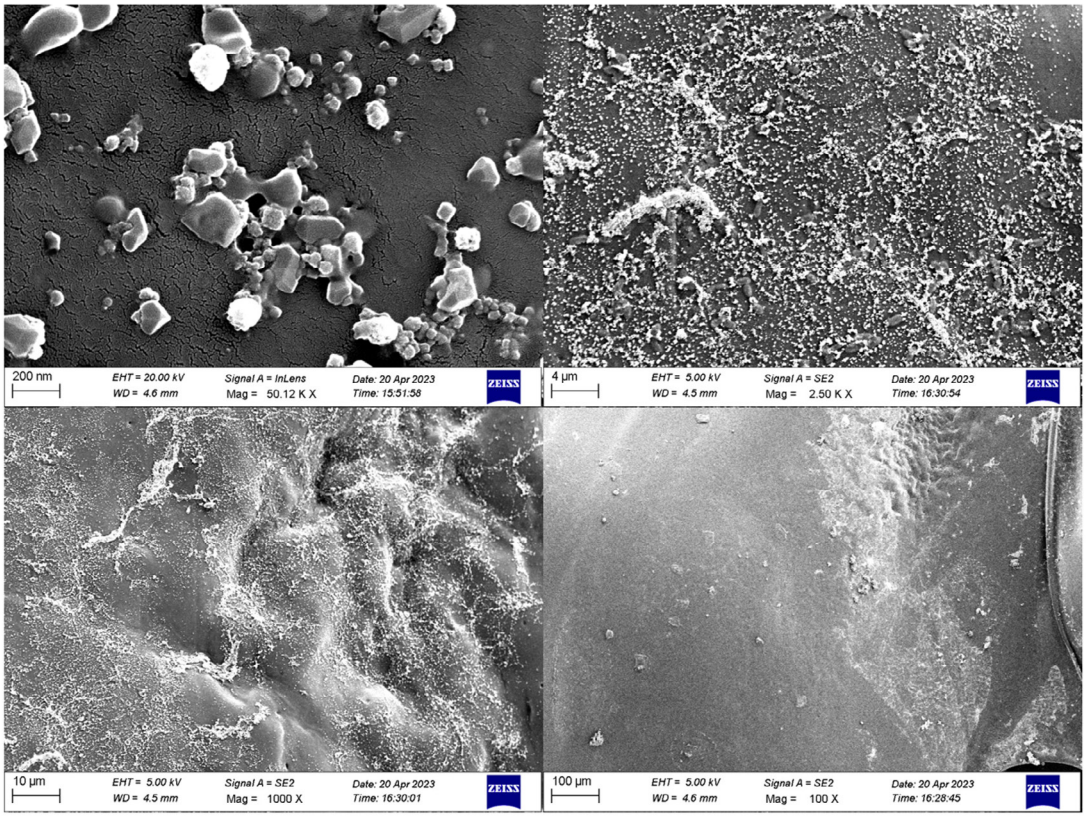


Fig. 6. Images of biosynthesized silver nanoparticles from *Staphylococcus aureus* on CA-X membrane.

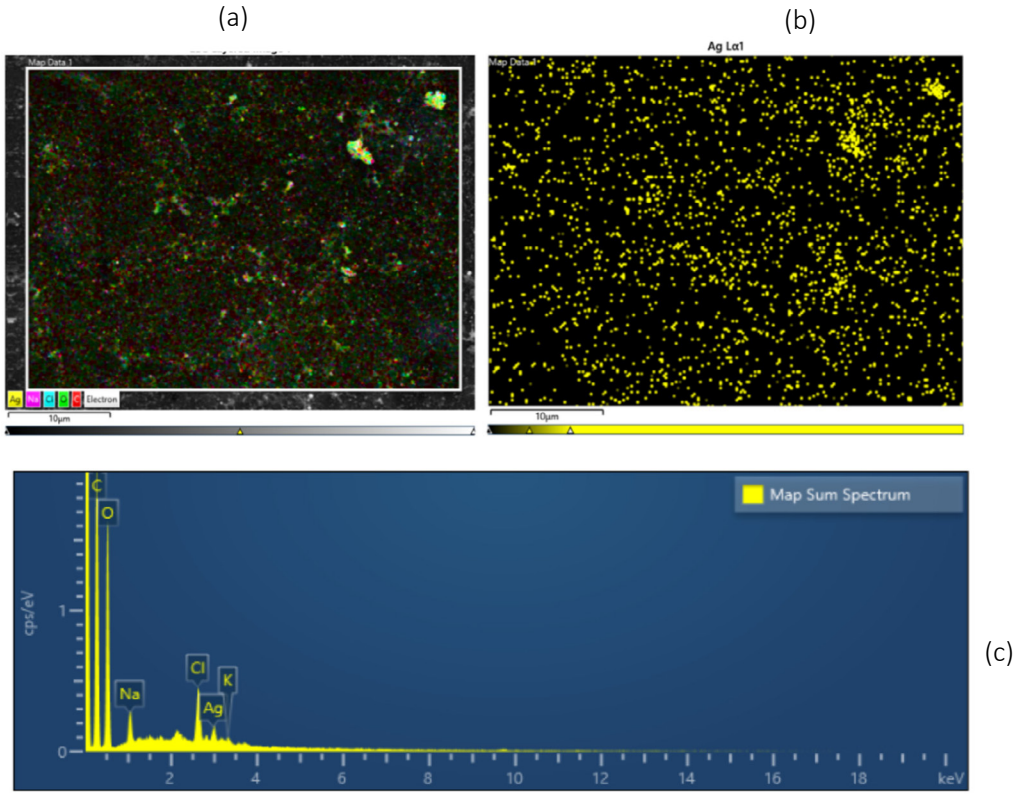


Fig. 7. (a) EDS image of biosynthesized silver nanoparticles on CA-X membrane. (c) EDS elemental analysis of silver nanoparticles on CA-X membrane (peak at 3keV).

monitoring period and compared with a control. TWAD kit was used to check the parameters of sample before and after treatment with cellulose acetate-silver nanoparticle membrane (Table 1,2).

Figures 8 to 16 depicted the various parameters of leachate/sewage after 1 hour and 1 week treatment respectively.

Figure 17 and Figure 18 compared the treated leachate/ sewage with untreated leachate/ sewage.

pH was found to show a similar increase on treatment with the CA-X membranes which may be indicative of algal growth. The total dissolved solids (TDS) were found to decrease significantly during the first hour of treatment, CA-X1 showed a 16.2% decrease and 21.95% decrease was exhibited by CA-X2. CA-X2 showed a drastic 37.6% decrease in total nitrogen levels after the initial hour of treatment while CA-X1 did not display any significant changes

Table 1. Values obtained for samples treated with CA-X1 membrane using TWAD kit.

Duration of treatment	pH	Alkalinity (mg/L)	Hardneess (mg/L)	Cl^- (mg/L)	TDS (mg/L)	Fe (mg/L)	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	P (mg/L)	F^- (mg/L)
0^{th} hour (control)	7	500	500	230	1476	1	5	0.5	75	2	1.5
1 hour	8	380	430	220	1296	1	5	0.2	75	1	1.0
1 week	9	400	400	280	1236	1	4	0.2	45	1	1.0

Table 2. Values obtained for samples treated with CA-X2 membrane using TWAD kit.

Duration of treatment	pH	Alkalinity (mg/L)	Hardneess (mg/L)	Cl^- (mg/L)	TDS (mg/L)	Fe (mg/L)	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	P (mg/L)	F^- (mg/L)
0^{th} hour (control)	7	500	500	230	1476	1	5	0.5	75	2	1.5
1 hour	8	350	420	200	1200	0.3	5	0.2	45	1	1.0
1 week	9	350	410	230	1152	1	2	0.2	45	1	0.5

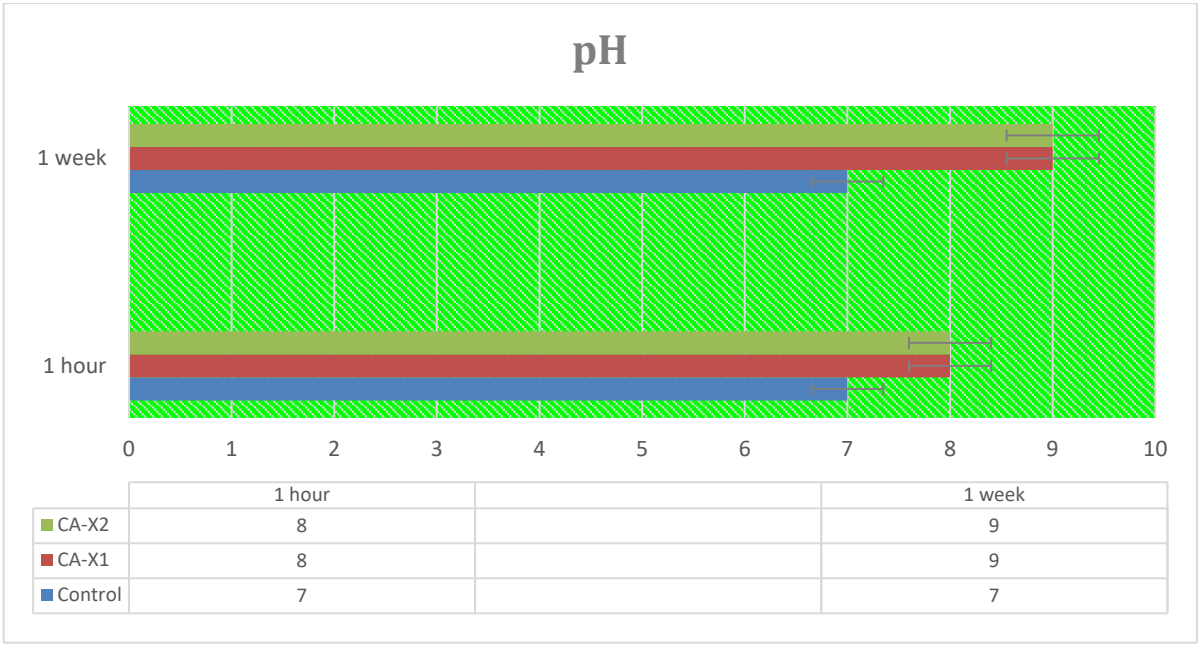


Fig. 8. Comparison of pH of the control sample, CA-X1 treated sample, and CA-X2 sample at 1 hour and 1-week intervals.

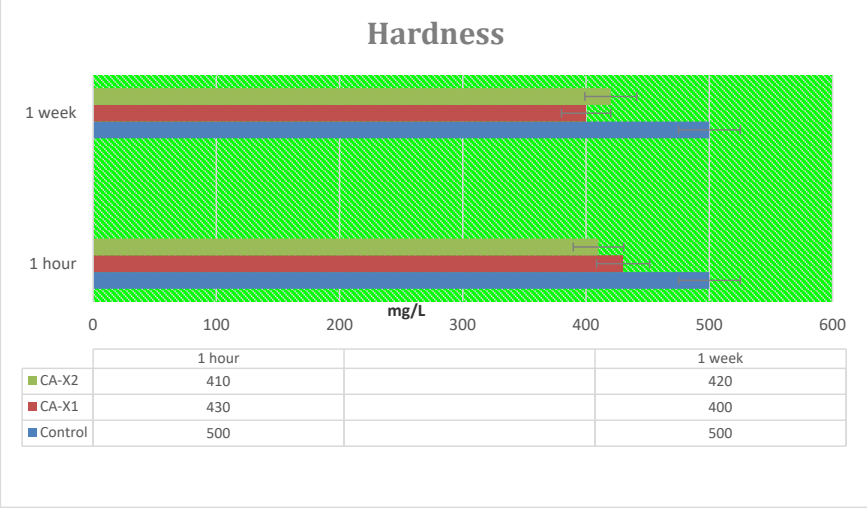


Fig. 9. Comparison of hardness of the control sample, CA-X1 treated sample, and CA-X2 sample at 1 hour and 1-week intervals.

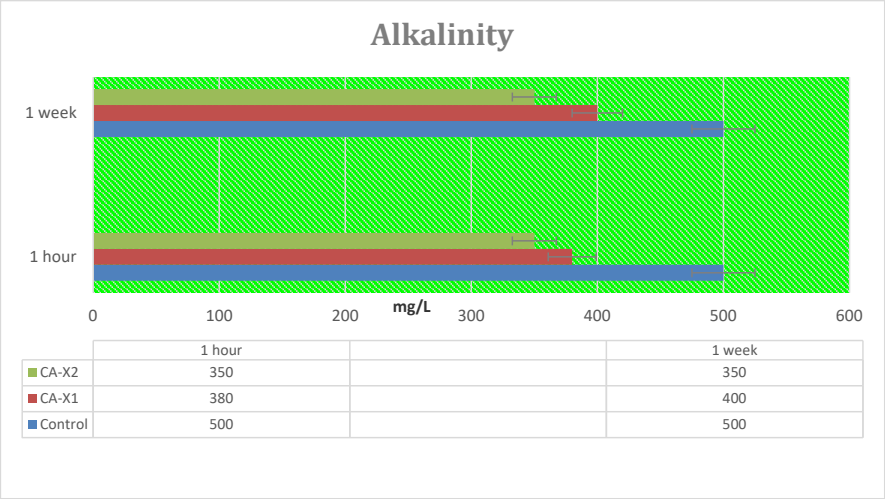


Fig. 10. Comparison of alkalinity of the control sample, CA-X1 treated sample, and CA-X2 sample at 1 hour and 1-week intervals.

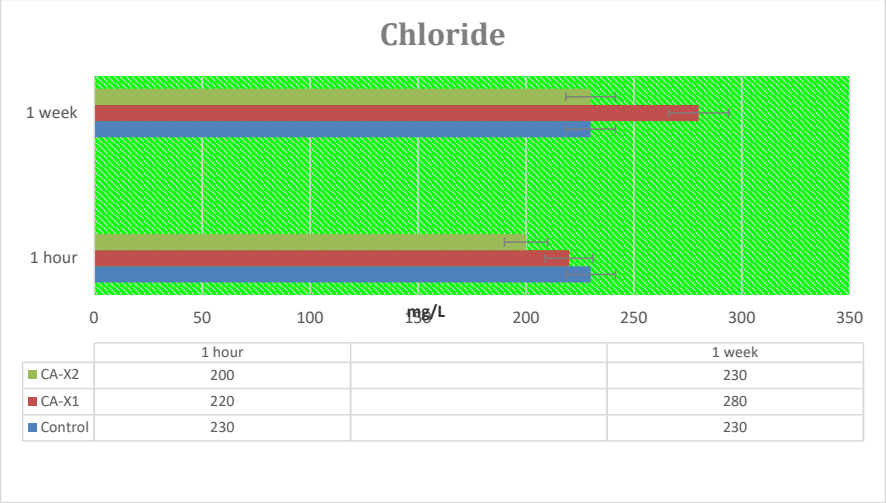
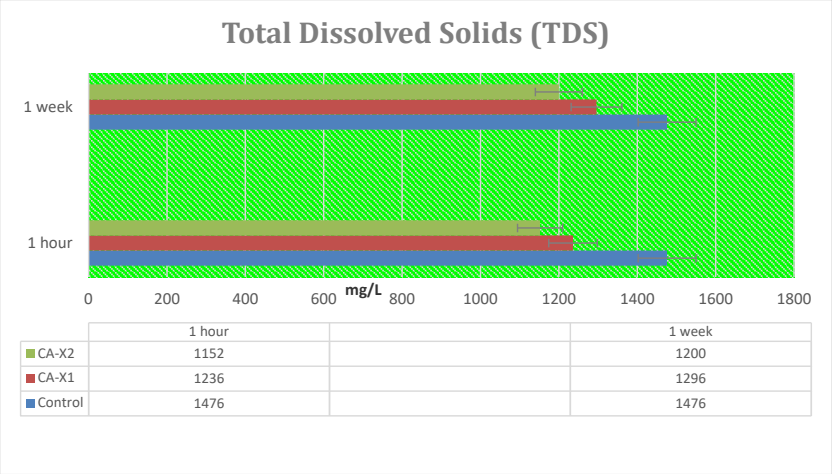
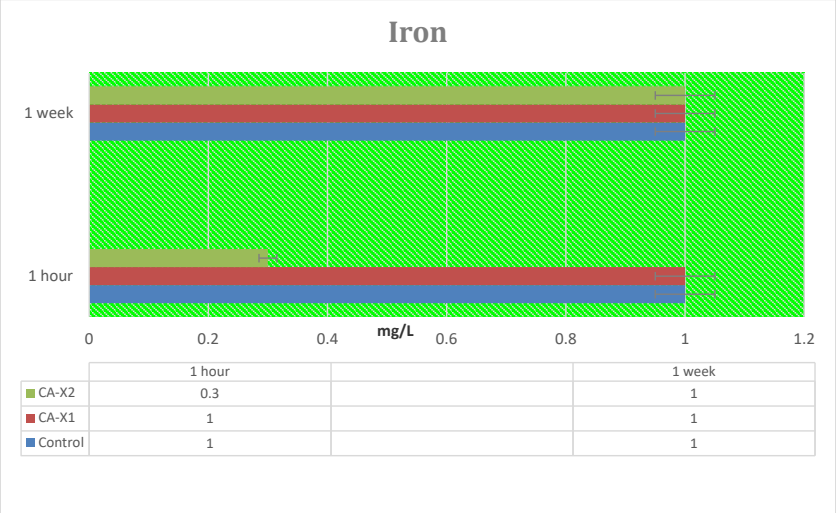


Fig. 11. Comparison of chloride level of the control sample, CA-X1 treated sample, and CA-X2 sample at 1 hour and 1-week intervals.



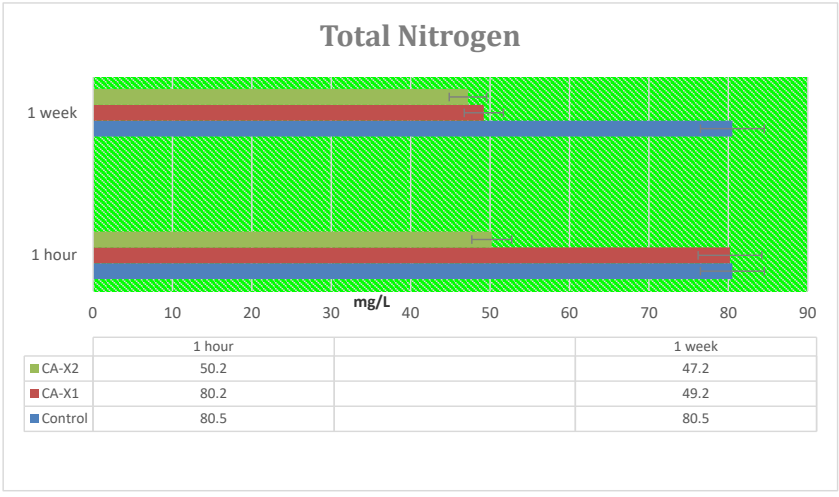
	1 hour		1 week
CA-X2	1152		1200
CA-X1	1236		1296
Control	1476		1476

Fig. 12. Comparison of total dissolved solids in the control sample, CA-X1 treated sample, and CA-X2 sample at 1 hour and 1-week intervals.



	1 hour		1 week
CA-X2	0.3		1
CA-X1	1		1
Control	1		1

Fig. 13. Comparison of iron level in the control sample, CA-X1 treated sample, and CA-X2 sample at 1 hour and 1-week intervals.



	1 hour		1 week
CA-X2	50.2		47.2
CA-X1	80.2		49.2
Control	80.5		80.5

Fig. 14. Comparison of total nitrogen level in the control sample, CA-X1 treated sample, and CA-X2 sample at 1 hour and 1-week intervals.

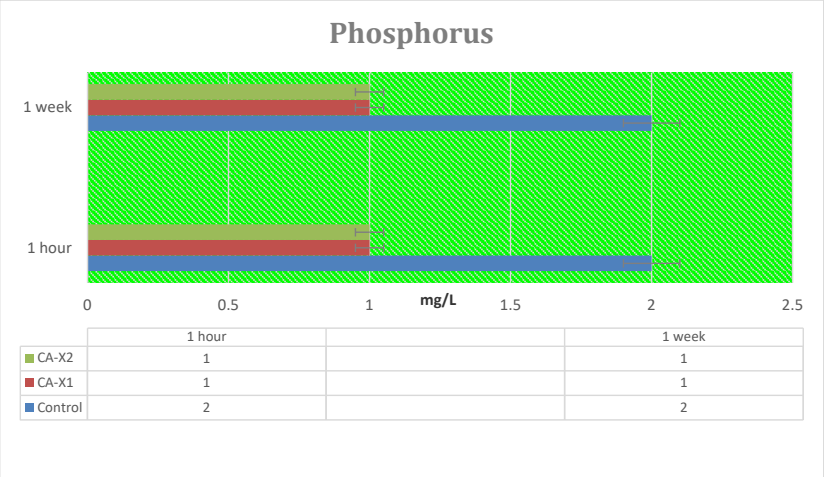


Fig. 15. Comparison of phosphorus levels of the control sample, CA-X1 treated sample, and CA-X2 sample at 1 hour and 1-week intervals.

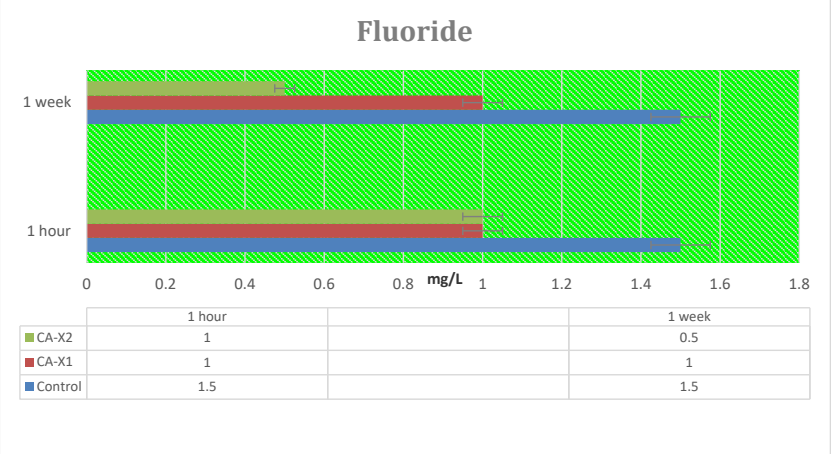


Fig. 16. Comparison of fluoride levels of the control sample, CA-X1 treated sample, and CA-X2 sample at 1 hour and 1-week intervals.

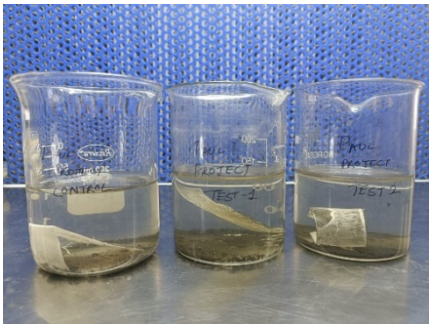


Fig. 17. Sample after 1 week of treatment with CA-X membrane

in the total nitrogen content of the sample within the first hour. The one-week treatment observations showed similar results for both membranes, samples showed a decrease in total nitrogen content by 38.88% and 41.36% for CA-X1 and CA-X2 respectively. Sachin *et al* in 2022 reported the usage of nanomaterial-based assemblies such as nanomaterials combined

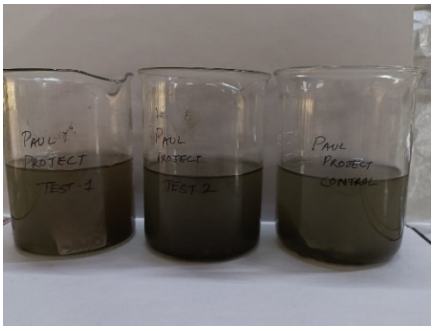


Fig. 18. Sample before treatment

with microbes, chemical catalysts, enzymes, etc., for leachate treatment. Thus, earlier literature had reported the significance of different nanomaterials using titanium nanoparticles, silver nanoparticles, and so on in treating various pollutants such as cadmium, chloride, iron, nitrates, hardness etc., in the leachate. However, the present study has an implication over the previous study in demonstrating the efficacy of nanocomposite membrane impregnated with silver nanoparticles in removing most of the pollutants in the leachate.

The Total Dissolved Solids (TDS) before and after treatment of the leachate by CA -X1 were recorded and subjected to t-test (TDS before treatment was found to be 1476 and after one week treatment was reduced to 1236 mg/L. Similarly, the two tailed t-test was done for hardness of leachate before (500mg/L) and after one week of treatment (400mg/L) by CA-X1. Since the p-value was .019974, the result was not considered statistically significant at $p < .01$.

CONCLUSION

Silver nanoparticles (AgNPs) have widespread applications in fields such as biomedicine, healthcare, and environmental remediation. Hence, in this study, silver nanoparticles were synthesized from bacteria obtained from landfill sites. *Staphylococcus aureus* was isolated and used to produce silver nanoparticles via a green synthesis pathway. Silver nitrate ($AgNO_3$) was used as the metal precursor and biosynthesis of silver nanoparticles was confirmed by the colour change (cloudy white to brown) within 3-5 days. Visible spectra and FTIR analysis of the silver nanoparticles were performed. The visible spectra of the nanoparticles showed a peak at 400nm confirming AgNP synthesis. Cellulose acetate (CA) nanocomposite membranes were prepared following the non-solvent induced phase separation (NIPS) of membrane fabrication. Cellulose acetate is a biodegradable polymer and was utilized due to it being a more environmentally friendly and economical alternative over its other conventional counterparts. Moreover, cellulose acetate's hydrophilic nature and ability to be functionalized by different groups to achieve desired properties make it a good alternative capping agent for AgNPs over other organic polymer sources. However, cellulose acetate showed lesser physical stability compared to other organic polymers, making the membranes prone to physical wear and tear on long exposure to the sample.

The membranes, CA-X1 and CA-X2 were fabricated following the NIPS methodology.

Confirmation and characterization of silver nanoparticles on nanocomposite cellulose acetate (CA) membrane was done by SEM and EDAX studies. The SEM images of the biosynthesized silver nanoparticles displayed a rough morphology, and the nanoparticles were globular in shape. The silver nanoparticles were speculated to be within the range of 200nm. EDAX results showed a sharp peak for silver nanoparticles at 3keV which was confirmation of their presence on the surface of the cellulose acetate membrane.

The bioremediation potential of CA nanocomposite membranes was assessed by checking their effects on untreated sewage sample collected from the MCC farm. Changes in the physio-chemical parameters of the sample due to nanocomposite CA membrane were studied using TWAD kit.

The initial, one-hour, and one-week physio-chemical parameters (pH, alkalinity, hardness, TDS, chloride, iron, ammonia, nitrate, nitrite, fluoride, phosphate) of the sample on treatment with CA nanocomposite membrane were assessed and results displayed the bio-remedial capability of given CA nanocomposite membrane. The treatment showed significant reduction in total dissolved solid (TDS) and total nitrogen content. Further studies assessing the biological parameters (anti-bacterial, anti-viral, anti-fungal, etc), biofouling resistance, thermal and mechanical stability as well as flow rate and integration into membrane bioreactor systems can be performed to give more solid evidence regarding the membrane potentiality.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

STATEMENTS & DECLARATION

Ethical Approval

"Not applicable"

Consent to Publish

All authors hereby declare their consent to publish the research article.

Author contributions

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