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A Novel Approach of Using Green Synthesized Tellurium Nanoparticles to Protect Historical Oil Paintings from Bacterial Degradation

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ABSTRACT
A novel approach was developed to investigate the applicability of green-synthesised tellurium nanoparticles (Te-NPs) for the conservation of historical oil paintings from the effects of bacteria. The Te-NPs were produced using two eco-friendly methods. The Te-NPs prepared with polyvinylpyrrolidone (PVP) and ascorbic acid (AP-Te-NPs) and the Te-NPs synthesised with *Pluchea dioscoridis* extract (PE-Te-NPs) were characterised using transmission electron microscopy (TEM) and X-ray diffraction (XRD) to confirm the formation of the Te-NPs. The antibacterial activities of Te-NPs, blank mock-up painting models (MPMs), and Te-NPs treated MPMs (0.1-5 mM) were evaluated against Gram-positive and Gram-negative bacteria. Scanning electron microscopy (SEM) was used to examine the MPMs that were degraded by the tested bacteria. The cytotoxicity of the Te-NPs was evaluated. In addition, the colour properties of the MPMs were determined to evaluate the effects of the produced Te-NPs on the MPMs. The Te nanorods had lengths ranging from 5-102 nm, and diameters between 1 and 11.5 nm. In addition, Te-NPs with spherical and flower-like shapes ranged from 3.4 to 17.9 nm. Te-NPs showed excellent antibacterial activity, especially against Gram-negative bacteria. PE-Te-NPs showed higher antibacterial activity against the tested bacteria than AP-Te-NPs. SEM micrographs and colour measurements revealed that the use of Te-NPs significantly minimised the harmful effects of the tested bacteria on MPMs surfaces. The cytotoxicity test also confirmed the potential applicability of Te-NPs (1 mM) as a sustainable agent for the conservation of historical oil paintings.

INTRODUCTION

Historical oil paintings have a complex composition with a mixture of inorganic and organic materials (Ashraf et al. 2014, 388-403). Biodeteriogens (bacteria, archaea, fungi, lichens, and insects) are a serious threat to the conservation of cultural heritage (Walter and de Viguerie 2018, 106-109). Biological attack on oil painting only occurs under poor conservation conditions such as high humidity, soil contact, poor ventilation, and infrequent maintenance (Tiano 2002, 7-12). The biodegradation of paintings is the result of interactions between living

organisms, painting materials and environmental conditions (Rosado et al. 2017, 478-486). Bacteria are the main biological agents associated with the alteration of artworks, and their development and metabolic activity are closely related to the state of their alteration (Salvador et al. 2016, 119-124). Bacteria play an important role in the mineralisation processes and the degradation of complex organic molecules (carbohydrates, proteins, lipids and cellulose) which lead to the completion of the organic matter cycle (Geweely 2023, 1-12). One of the main challenges in combating biodeterioration is therefore the control of microbial growth. Many researchers have reported different bacteria isolated from oil-based paints that are capable of biodegradation, especially *Bacillus* sp. and *Pseudomonas* sp. due to their various survival strategies due to their different survival strategies (Elsayed 2019, 238-256). Previous studies have reported that the main bacteria species of attacking oil canvas paintings are *Pseudomonas aeruginosa* (*P. aeruginosa*)*, Escherichia coli* (*E. coli*), *Bacillus cereus* (*B. cereus*)*,* and *Staphylococcus aureus* (*S. aureus*) (Ogbulie and Obiajuru 2004, 485-490); (Pangallo et al. 2009, 277-287); (Darwish 2010, 102-128); (López-Miras et al. 2013, 301-314); (Caldeira 2021, 137-154); (Torralba et al. 2021, 1098-1105). SEM examination, as well as various examinations and culture-dependent techniques, revealed the presence of *Staphylococcus* spp. on the recto of the painting and *Bacillus* spp. on the verso (Caselli et al. 2018). Some specific bacteria are known to degrade hydrocarbons from the environment, commonly found in oil-based paints and produce various acids as metabolic end products that cause rapid deterioration of the artwork (Torralba et al. 2021, 1098-1105).

The moisture content of these paintings and works of art is relatively high enough to promote the growth of bacteria that cannot utilise condensation water. In general, the microbial infestation of canvas paintings starts from the back due to the variety of organic components. The proliferation and growth of bacteria on and in the paint layer can cause it to flake, crack and peel (Tiano 2002, 7-12). These bacteria can alter the surface of paintings through staining and discolouration (Ciferri 1999, 879-885). Bacteria from the groups *Bacillus* sp. and *Pseudomonas* sp. adhere to the surface of the paint and cause its decomposition ((Kurowski, Vogt, and Ogonowski 2017, 81-92). According to a literature review, bacteria of the genera *Bacillus* and *Pseudomonas* can grow on and in paint coatings (Obidi et al. 2009, 835-840); (Kurowski, Vogt, and Ogonowski 2017, 81-92). In addition, pigments such as red and yellow ochre may also be subject to biodegradation as has been reported for some species of *Staphylococcus* and *Bacillus,* possibly leading to pigment fading (Pavić et al. 2015, 40-50); (Ciferri 1999, 879-885). Some common pigments, namely red and yellow earths, can be utilised by paint-associated microorganisms as a source of nutrients, as has been reported in the literature. They may contain minerals and/or clay that are favourable for microbial growth. Natural pigments can be the only source of essential nutrients, namely phosphate and iron, *i.e*. natural pigments can support the growth of bacteria and their metabolic activities ; (Pavić et al. 2015, 40-50); (Salvador et al. 2022, 1-19). Bacteria of the genus *Staphylococcus* could pose a higher risk for the pigment red ochre than bacteria of the genus *Bacillus* (González et al. 1999, 123-127).

Choosing the best method to control the biological deterioration is crucial for the successful treatment of contaminated artworks. One of the main known drawbacks is the difficulty of applying antimicrobial compounds without damaging the painting layers and its impact the health of conservators (Salvador et al. 2022, 1-19) (Kakakhel et al. 2019). Due to the extensive damage caused by these antimicrobial compounds (biocides), EU Regulation U Directive 98/8/EC recommends that biocides that are harmful to humans be withdrawn from the market for a certain period of time, as these chemicals have strong negative effects on human health (Gatti et al. 2021, 1-12). Due to the exponential growth of bacterial populations, mutations

often occur, allowing bacteria to evolve extremely rapidly, and the toxic nature of some commercial compounds makes their use unfeasible. For these reasons, sanitation methods based on biocides were used many decades ago to find effective solutions to control microbiological proliferation in cultural heritage (Ranalli et al. 2005, 73-83); (Ranalli and Zanardini 2021, 583-603).

Many decades ago, conservators utilised modern technologies and tried many physical methods, such as infrared(Zhang et al. 2018, 15485-15495), ultraviolet (Tiano 2002, 7-12), X-ray and γ-rays irradiation (McEvoy et al. 2023), cold atmospheric plasma (Văcar et al. 2023, 198-205) and many ancient and modern chemical biocides (Koestler et al. 1993, 265-273); (Ashraf et al. 2014, 388-403); (da Silva 2017, 5-17) to disinfect microorganisms contaminating historical materials. These physical and chemical methods used so far are temporarily efficient; but all are either extremely expensive or adversely affect the cultural property and/or museum staff and visitors due to their toxicity (Katušin-Ražem, Ražem, and Braun 2009, 729-731); (Sílvia O. Sequeira, Cabrita, and Macedo 2014, 181-199), especially chemical biocides, which are gradually unable to prevent the growth of organisms, because they do not last long , as they can be used as a source of nutrients by the indigenous microflora, whereupon this microflora develops resistance to the same biocides (Kakakhel et al. 2019). Therefore, the growing concern for the environment and human health has led to the exploration of new antimicrobial alternatives, with lower toxicity. Green materials such as essential oils (Elsayed and Shabana 2018, 71-87), (Elsayed, Shabana, et al. 2023, 113-125); (Mabrouk et al. 2023, 101-112), microbial culture extracts (Elsayed, El-Kadi, et al. 2023, 127-143) and nanomaterials such as Ag_2O, TiO_2, ZnO, etc. have a better chance of effectively protecting cultural heritage against microorganisms. These new nanoparticles of biocides are very small and can damage DNA or RNA more efficiently(Ashraf et al. 2014, 388-403); (Silvia Oliveira Sequeira et al. 2017, 45-52); (Maltman and Yurkov 2019).

Nanotechnology is an emerging field of science that deals with the design and development of innovative materials at the nanoscale range between 1 and 100 nm with unique properties for a wide range of applications in most scientific fields (Vahidi et al. 2021). It requires multidisciplinary knowledge that can be used in multiple fields and different applications (Tiwari et al. 2018, 4773-4783). Heritage preservation has also tapped into its potential, as it offers a range of opportunities for the development of nanoscale materials and methods for the conservation and restoration of cultural artefacts (Kanth and Soni 2023, 120-130). Tellurium (Te) is a rare element that occurs in minerals with a brownish-black or silvery colour. It belongs to the metalloids and its properties lie between metals and non-metals (Castro et al. 2020). Te compounds have traditionally been used as antimicrobial agents since1932 and tellurite ions have long been recognised for their antimicrobial properties (Cruz et al. 2019, 1982-1998). It has significant antibacterial properties against both Gram-negative and Gram-positive bacteria, such as *E. coli, K. pneumoniae, S. aureus, S. epidermidis, C. albicans*, *S. cerevisiae*, etc. (Cruz et al. 2019, 1982-1998); (Vahidi et al. 2021).

There are various chemical, physical and biological approaches for the production of NPs. However, the green synthesis of NPs using environmentally friendly chemicals has attracted the interest of researchers, especially when the produced NPs with excellent biological activities and low cytotoxicity are also used in contact with humans (Vahidi et al. 2021); (Sadek et al. 2022). Some methods for the production of Te-NPs may threaten the environment and minimise the sustainability and applicability of the produced Te-NPs due to the high toxicity of the chemicals used in their synthesis. For example, Te nanotubes have been synthesised using formamide as a reducing agent, which is a carcinogenic and toxic by skin contact or

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injection (Xi et al. 2005, 325-328). Tellurium nanowires have also been synthesised using concentrated hydrazine, which has acute oral and dermal toxicity as well as inhalation toxicity(Lin, Yang, and Chang 2008, 351-357).

Te-NPs are used in medicine due to their excellent antimicrobial activities against infectious diseases. They showed promising antibacterial activity against various bacterial strains such as *Staphylococcus aureus* (Abed et al. 2023, 400-412), *E. coli*, *P. aeruginosa*, and *S. aureus* (Morena et al. 2021, 14885-14893). In general, metallic nanoparticles show remarkable antimicrobial activities due to the release of ions that increase oxidative stress, resulting in the killing or inhibition of bacterial growth (Chang et al. 2014, 8305-8312). As far as we know, Te-NPs have never been used in the field of art conservation. The development a reliable, non-toxic and environmentally friendly process for the synthesis of NPs is an imperative step in the green conservation of cultural heritage. Even a small change in the synthesis route of NPs can lead to a significant change in the physicochemical and biological properties of the NPs, hence a green synthesis approach is required to produce eco-friendly, sustainable and applicable Te-NPs (Kubavat et al. 2019, 2249-4820); (Rana, Yadav, and Jagadevan 2020). Therefore, the present study reports the green synthesis of AP-Te-NPs and PE-Te-NPs for use as eco-friendly and sustainable antibacterial agents against the degradable and harmful effects of the tested bacterial strains on historical oil paintings. In this study, the effects of the produced Te-NPs on the colour properties, cytotoxicity and surface structure of MPMs were also investigated to evaluate the potential applicability of AP-Te-NPs and PE-Te-NPs in archaeology, particularly in the conservation of historical oil paintings.

1. MATERIALS AND METHODS

1.1. Materials

The leaves of *Pluchea dioscoridis* were cultivated and harvested in the Faculty of Agriculture at Damietta University in Egypt. The collection of the plant leaves was done in accordance with the relevant institutional, national and international guidelines and laws. In this regard, the minimum number of leaves required for this study was collected without jeopardising plant growth. To remove impurities, the leaves were washed with distilled water and then dried in the shade at room temperature (25 ±2 °C) for 1 week. All chemicals used in this study were purchased from Sigma Aldrich. Oil paint tubes and refined linseed oil were purchased from Winsor and Newton Ltd, London, UK. The linen fabric used as a canvas was purchased from the Egyptian Company for Textile Industry, Egypt. Powder of zinc white (zinc oxide) and animal glue (5% solid granules in hot water) were purchased from the local market.

1.2. Procedures

1.2.1. Green synthesis of Te-NPs

Potassium tellurite (K_2TeO_3) at a concentration of 10 mM was used as the precursor for Te-NPs. The Te-NPs were produced by two improved, eco-friendly methods using ascorbic acid or PE as reducing agent. In the first method, PVP was dissolved in a 10 mM potassium tellurite solution at a concentration of 6 g/100 ml. This mixture was then mixed with 20 mM ascorbic acid at a volume ratio of 1:1, resulting in a light yellow turbidity within 10 min as an indicator of AP-Te-NPs formation (Liu et al. 2016, 167-170). The second method was used to synthesise PE-Te-NPs using the aqueous extract of *Pluchea dioscoridis* (PE), which was prepared by boiling 5 g of leaves in 200 ml of deionised water for 15 min. The mixture was then filtered, and the filtrate of the aqueous extract was used for the synthesis of PE-Te-NPs. Potassium tellurite (10 mM) was added to PE in a volume ratio of 1:1 and incubated at room temperature for 24 hours. The obtained black colloidal solution confirmed the complete formation of the prepared PE-Te-NPs, and the black colour increased with increasing reduction time up to 96 hours. (Liu et al. 2016, 167-170); (Shakibaie et al. 2017, 268-276).

1.2.2. Transmission electron microscopy (TEM) and XRD analyses

The morphology and size of the Te-NPs were characterised using transmission electron microscope (JEOL, JEM 2100F, Tokyo, Japan) at 200 kV. A drop of the colloidal solution was placed on a 400-mesh copper grid coated with an amorphous carbon film, and the solvent was evaporated in air at room temperature. In addition, an X-ray diffractometer (Bruker D8 ADVANCE, Karlsruhe, Germany) was used to determine the crystalline nature of the synthesised Te-NPs.

1.2.3. Cytotoxicity test

The cytotoxicity of AP-Te-NPs and PE-Te-NPs at different concentrations from 0.125 to 2.5 mM was investigated in both WI-38 and HFB_4 cells using the assay of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye (Mosmann 1983, 55-63). WI-38 cells are human cells isolated from lung tissue. These diploid cells consist of fibroblasts from a 3-month-old female foetus. In addition, HFB_4 cells were used to study the effect of the produced NPs on human skin cells (Song et al. 2008, 1902-1908). The Te-NPs were introduced into six-well tissue culture plates containing WI-38 or HFB_4 cells. These plates were then kept at 37°C for 24 hours. In addition, the growth medium was decanted and then the wash medium was used to wash the cell monolayer twice. The change in physical signs observed in the tested cells compared to the blank experiment indicated the degree of cytotoxicity. The tissue was then picked up, and aliquot 20 µL of MTT (5 mg/mL) dissolved in phosphate buffer saline was added, followed by incubation at 5% CO_2 and 37°C for 5 hours. The MTT metabolite formazan was then resuspended in dimethyl sulfoxide (200 µL) with continuous shaking for 5 minutes. The MTT metabolite was read at 560 nm, while the background was measured at 620 nm and then subtracted.

1.2.4. Preparation of the mock-up painting models (MPMs)

The MPMs were made on canvas (linen) according to the most common and traditional recipes for ancient oil paintings described in some literatures (Luke 1988, 90-91); (Walter and de Viguerie 2018, 106-109) (Elsayed 2019, 238-256); (Fiorillo et al. 2019). The white base layer consisted of animal glue and ZnO. In addition, five pigments (common colours), namely red ochre ($\propto$-Fe_2O_3), lemon yellow ($BaCrO_4$.), blue (ultramarine, $Na_7Al_6Si_6O_{24}S_3$), green (viridian, $Cr_2O_3 . 2\ H_2O$), and brown (burnt umber, mixture of Fe_2O_3, SiO_2, and MnO_2) were used for the experimental tests and selected according to the literature data on historical painting in general (Luke 1988, 90-91); (Fuster-López et al. 2019, 225-234). After drying, the MPMs were thermally aged for 357 hours in a laboratory oven (Binder ED115, Tuttlingen, Germany) at 105 ±1 °C in the absence of light in the experimental laboratory of the Faculty of Archaeology of the University of Damietta and then kept at room temperature (25 ±1 °C) for 72 hours. After ageing, a 28 µL/cm^2 of the prepared Te-NPs were sprayed onto the MPMs at different concentrations of 0.5, 1, and 2.5 mM. After treatment with Te-NPS, the MPMs were inoculated with the tested bacteria to investigate the effect of Te-NPs on the bacterial growth of the tested bacteria.

1.2.5. Bacterial strains and cultivation methods

Gram-positive bacteria (*B. cereus* and *S. aureus*) and Gram-negative bacteria (*P. aeruginosa* and *E. coli*) were selected in this study based on literature on the biological deterioration of oil paintings ; (Sterflinger 2010, 47-55); (López-Miras et al. 2013, 301-314); (Salvador et al. 2016, 119-124); (Ciferri 1999, 879-885); (Koul and Upadhyay 2018, 597-615), and were adopted from the Department of Agricultural Biotechnology, Faculty of Agriculture, Damietta University. Nutrient agar (NA) and potato dextrose agar (PDA) were used to maintain Gram-positive and Gram-negative bacteria, respectively, at 5°C until further use. Five millilitres of a sterilised NaCl saline solution at a concentration of 0.09% was added to each slant. The tested

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bacterial cells were then loosened with a sterile inoculation loop. The bacterial cells were then removed from the slants using a vortex mixer for one minute. The plates were then inoculated with the tested bacteria using a sterile cotton swab. In addition, small wells with a diameter of approximately 7 mm were formed using a sterilised cork borer inoculated with 100 µL of the sample and incubated at 37°C (El-Kadi et al. 2018, 5-11); (Elsayed, El-Kadi, et al. 2023, 127-143).

1.2.6. Testing of MPMs treated with Te-NPs

1.2.6.1. SEM examination

The surface morphology of the MPMs, including the blank samples and the samples treated with Te-NPs and inoculated with bacterial strains, was examined using a scanning electron microscope (JEOL JSM-6510LB, Tokyo, Japan). The MPMs surfaces inoculated with *B. cereus* and *P. aeruginosa* were examined to study the degradation caused by these bacterial strains in comparison with blank samples. In addition, the surfaces of MPMs loaded with AP-Te-NPs or PE-Te-NPs at different concentrations (1 and 2.5 mM) were observed prior to inoculation with the tested bacterial strains to investigate the potential applicability of the prepared Te-NPs as antimicrobial agents against the growth and potentially harmful effects of these bacteria on MPMs surfaces.

1.2.6.2. Evaluation of the antibacterial activities

The antibacterial activities of the prepared Te-NPs, the blank MPMs and the MPMs treated with Te-NPs were evaluated according to the American Type Culture Collection (AATCC) test method (147-1988) against Gram-negative bacteria such as *P. aeruginosa* and *E. coli* and against Gram-positive bacteria, including *B. cereus* and *S. aureus*. For the antibacterial activity test 25 mm wide and 50 mm long MPM samples were used, which were placed on the medium after inoculation with the tested bacteria. The inhibition zones were carefully measured after 24 hours of incubation**.** Antibacterial activity was recorded as the average diameter of bacterial growth inhibition (mm) (Pinho et al. 2011, 493-498).

1.2.6.3. Colour measurements

The colour measurements of the tested MPMs were recorded to investigate the potential effects of bacterial growth on the colour properties of the coloured surfaces of the MPMs. In addition, the effect of the prepared AP-Te-NPs and PE-Te-NPs on the colour properties of the MPMs was investigated to determine the optimal concentration and type of Te-NPs for the conservation of historical oil paintings without significantly altering the original colours of the treated samples. The changes in the red, blue, yellow, green and brown colours of the MPMs surfaces were measured using a colorimeter (PCE-CSM 2, PCE, Germany). The colour measurements carried out with an illuminate of D65, an additional standard observer of 10° and the SCI measurement mode. The colour coordinates L*, a*, b* were determined as a function of the CIEL*a*b 1976 colour space. All measurements were performed in triplicate, 72 hours after the end of treatment with NPs and bacterial inoculation. The colour differences ($\Delta E*$) were calculated starting from the L*, a* and b* values according to the following equation:

$$\Delta E* = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{1/2}$$

Where ΔE values from 0 to 1 indicate that chromatic differences cannot be detected by the human eye, while ΔE in the range of 1–3 is considered a minor chromatic difference. Furthermore, ΔE values from 3 to 6 refer to remarkable differences, and values above 6 are considered large variations in the tested coloured surfaces (Schanda 2007, 79-91), (Marchiafava et al. 2014, 36-42).

1.2.7. Statistical analysis

The statistical analysis was carried out using the Costat version 6.311 programme (CoHort Software, Monterey, USA) in order to identify significant differences between the results obtained. In addition, statistical analysis of variance (ANOVA) was useful to compare all treatments. The standard deviation (SD) of the means was calculated, while Duncan's new range test at $p = 0.05$ was used to analyse the significant deviations (Gomez and Gomez 1984); (CoStat 2005). In this test, each sample was analysed with three replicates.

2. RESULTS AND DISCUSSION

2.1. Characterisation of the Te-NPs

The prepared Te-NPs were characterised by TEM to confirm the formation of tellurium particles at the nanoscale and to investigate their morphology. The TEM micrographs showed that the PE-Te-NPs had the shape of nanorods, while the AP-Te-NPs had three morphological shapes, including nanorods, spherical and flower-shaped, as shown in Fig. 1. However, in the TEM micrographs of AP-Te-NPs, the nanorods were the most abundant NPs. AP-Te-NPs and PE-Te-NPs nanorods exhibited lengths in the range of 11-80 nm and 5-102 nm, and the diameters ranged from 1 to 11.5 nm and from 1.9 to 10.6 nm, respectively. AP-Te-NPs with spherical and flower-like shape ranged from 3.4 to 17.9 nm. TEM analysis showed that the Te-NPs in colloidal solutions of AP-Te-NPs and PE-Te-NPs were well dispersed and monodisperse. Two types of Te-NPs, amorphous and crystalline, are formed in the colloidal solution. Then, amorphous Te-NPs are deposited on the crystalline Te-NPs as seeds for growth by a solid-solution-solid transformation mechanism. Since crystalline Te-NPs usually have an anisotropic structure, the most favourable direction for their growth is <001>; therefore, Te-NPs tend to form nanorods (Lin, Yang, and Chang 2008, 351-357). (Krug et al. 2019) reported that Te-nanoflowers with a diameter of about 112 nm can also be produced from sodium tellurite using a mixture of ascorbic acid and sulforaphane.

To confirm the successful synthesis of AP-Te-NPs and PE-Te-NPs, XRD spectra of the produced Te-NPs were recorded. As shown in Fig. 1e, the synthesised PE-Te-NPs were more crystalline than the AP-Te-NPs, which showed a broad peak ranging from18.2° to 30.9°. The spectrum of PE-Te-NPs showed sharp and strong peaks, indicating their high crystallinity. The diffraction peaks of PE-Te-NPs were attributed to crystal planes 100, 101, 102, 110, 111, 201, 202, 113, 211, 212 and 114 as reported in the international database JCPDS, 36-1452 for the hexagonal phase of Te (Song et al. 2008, 1902-1908); (Manikandan et al. 2017, 40-48). The XRD spectrum of AP-Te-NPs showed weak peaks corresponding to crystal planes 201, 202, 113 and 114, in addition to an obvious broad peak, indicating the low crystallinity of AP-Te-NPs due to the variation of the synthesis method, compared to that used in the preparation of PE-Te-NPs. The XRD spectrum of AP-Te-NPs is consistent with JCPDS Card No. 36-1452 and is in agreement with the literature (Medina-Cruz et al. 2021); (Hosseini, Lashani, and Moghimi 2023). The mixture of a very broad hump and weak and sharp peaks could be due to the different morphological shapes of the AP-Te-NPs with different crystallinity, as seen in the TEM micrographs in Fig. 1a, b.

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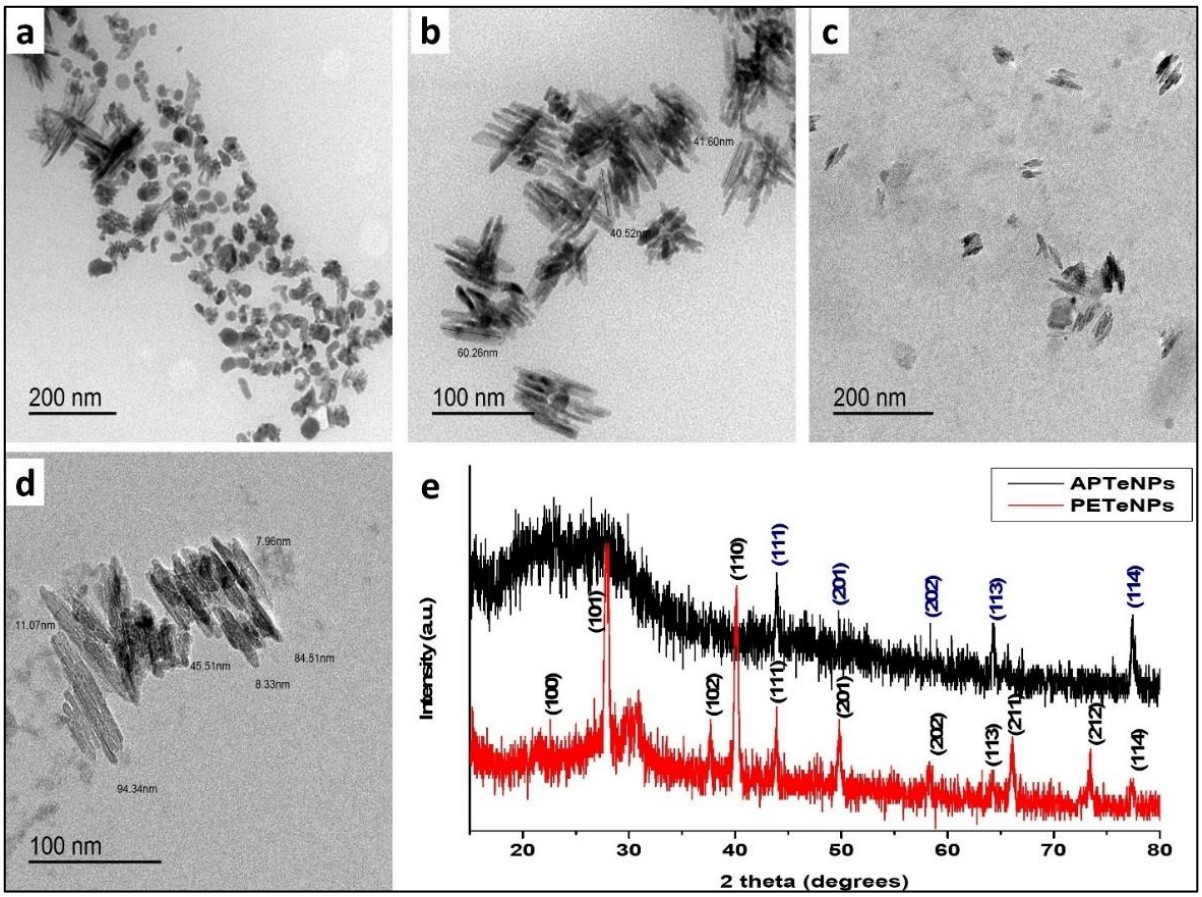

Fig. 1 TEM micrographs of the produced a,b) AP-Te-NPs, and c,d) PE-Te-NPs, and e) XRD patterns for the produced Te-NPs.

2.2. Examination of the MPMs treated with Te-NPs

2.2.1. SEM examination

In this context, the effects of the produced Te-NPs on the bacterial growth of *B. cereus* and *P. aeruginosa in vitro* and on the surfaces of the MPMs were examined. Fig. 2 and Fig. 3 show the SEM micrographs of the surfaces of blank MPMs, MPMs inoculated with *B. cereus* or *P. aeruginosa*, and MPMs treated with AP-Te-NPs or PE-Te-NPs at concentrations of 1 and 2.5 mM and then inoculated with the tested bacteria. The micrographs of the blank MPMs showed a clear surface without bacterial growth. On the other hand, the SEM images of MPMs inoculated with *B. cereus* without Te-NPs treatment showed obvious rod-shaped bacterial growth on the surface, indicating the reason for the surface degradation of these samples (Fig. 2). In addition, the SEM micrographs of the MPMs surfaces inoculated with *P. aeruginosa* showed the presence of bacterial cells on the surface of the MPMs in the form of rod-shaped bacteria, as shown in Fig. 3.

In addition, SEM micrographs of MPMs surfaces treated with AP-Te-NPs or PE-Te-NPs at concentrations of 1 and 2.5 mM and subsequently inoculated with *B. cereus* showed no bacterial growth, as shown in Fig. 2. PE-Te-NPs at both concentrations completely inhibited the growth of *P. aeruginosa*. AP-Te-NPs at a concentration of 1 mM significantly minimised the number of *P. aeruginosa* cells on the surface of the MPMs, while at a higher concentration (2.5 mM) they completely inhibited the growth of *P. aeruginosa* (Fig. 3).

The obtained micrographs confirmed the applicability of the prepared Te-NPs as a protective layer against harmful bacterial influences on MPMs surfaces, such as biodegradation. In addition, there were no significant changes in the morphology and microstructure of the MPMs after treatment with Te-NPs.

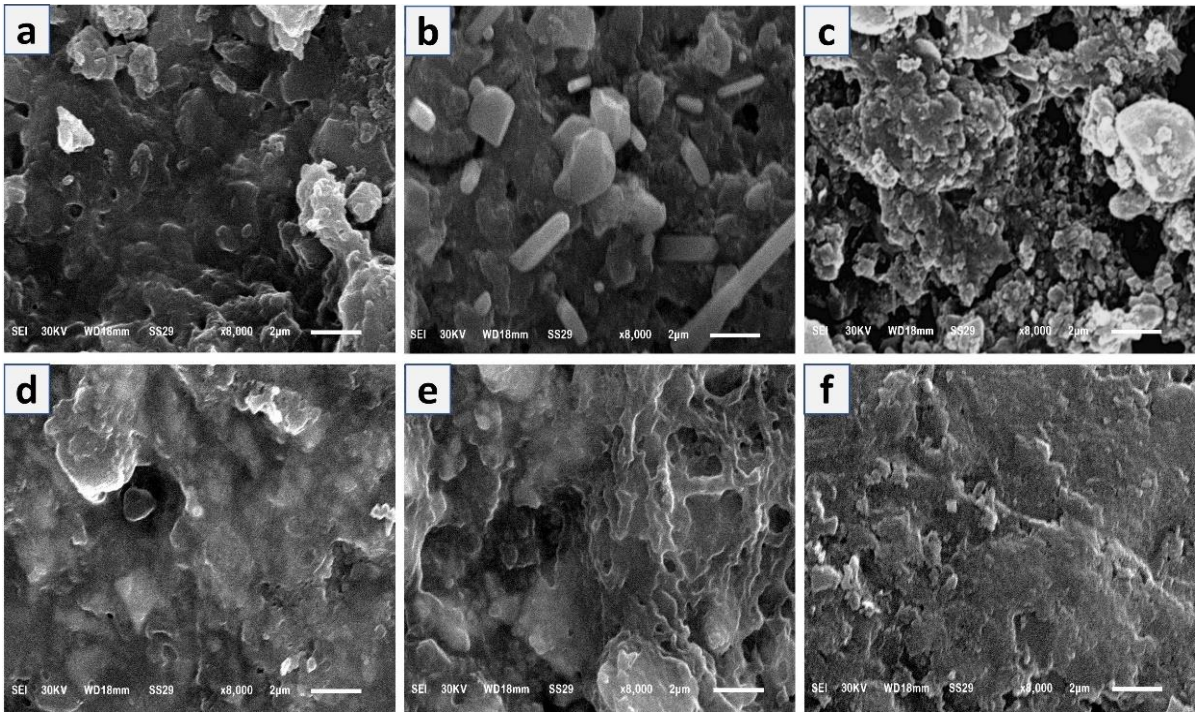

Fig. 2 SEM micrographs of a) blank MPMs, b) MPMs inoculated with *B. cereus* only, c), d) MPMs treated with AP-Te-NPs at concentrations of 1 mM, and 2.5 mM, respectively, and then inoculated with *B. cereus*, e), f) MPMs treated with PE-Te-NPs at concentrations of 1 mM, and 2.5 mM respectively, and then inoculated with *B. cereus*

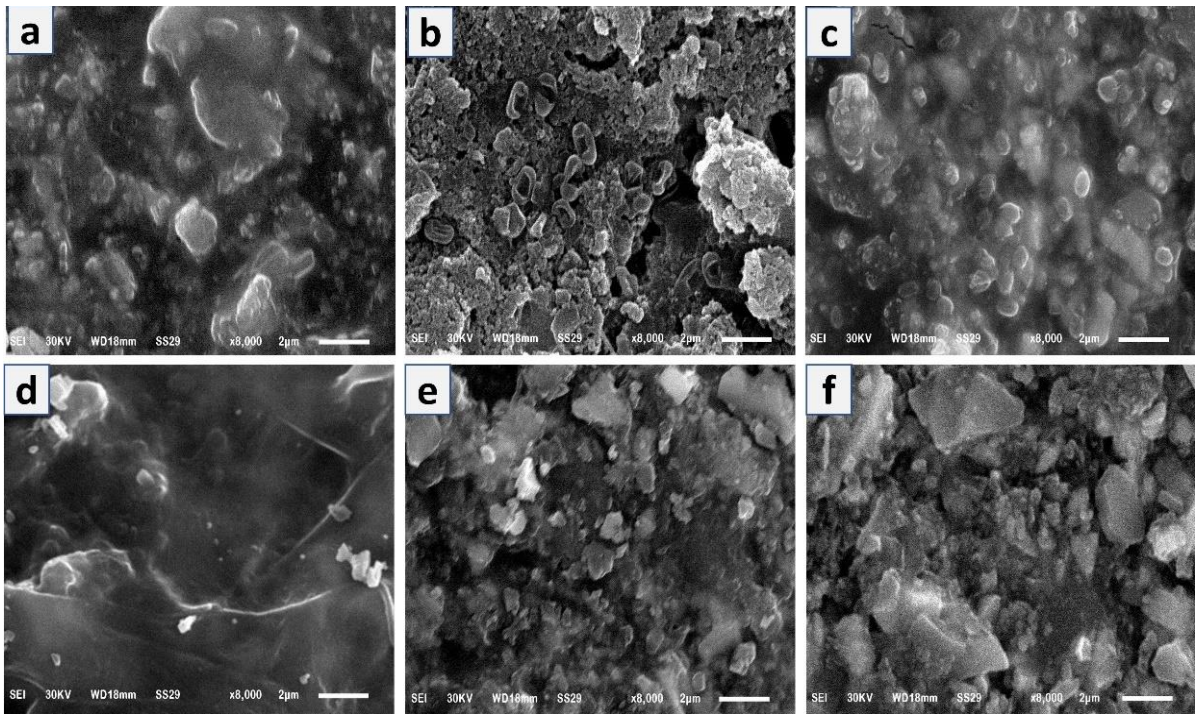

Fig. 3 SEM micrographs of a) blank MPMs, b) MPMs inoculated with *P. aeruginosa* only, c, d) MPMs treated with AP-Te-NPs at concentrations of 1 mM, and 2.5 mM, respectively, and then inoculated with *P. aeruginosa*, e, f) MPMs treated with PE-Te-NPs at concentrations of 1 mM, and 2.5 mM, respectively, and then inoculated with *P. aeruginosa*.

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2.2.2. The evaluation of antibacterial activity

2.2.2.1. Antibacterial in vitro activities

The antibacterial activities of the prepared Te-NPs (0.1-5 mM), blank MPMs, and MPMs treated with different concentrations of Te-NPs ranging from 0.5 to 2.5 mM were estimated using the inhibition zone method (see Tables 1 and 2). Their antibacterial activities were tested against *B. cereus* and *S. aureus*, as Gram-positive bacteria, and *E. coli* and *P. aeruginosa*, as Gram-negative bacteria. Initially, the antibacterial activities of AP-Te-NPs and PE-Te-NPs were evaluated at different concentrations (o.1 mM to 5 mM) against the tested bacteria to investigate the effects of Te-NPs concentration, preparation method and morphology on their antibacterial activities and to select the effective concentration range of Te-NPs for the treatment of MPMs. PE-Te-NPs showed higher antibacterial activities against the tested bacteria than AP-Te-NPs, which might be due to the rod-shaped antibacterial mode of action of the green-synthesised PE-Te-NPs, which were completely rod-shaped. On the other hand, the AP-Te-NPs exhibited different morphological shapes, including nanorod, sphere and flower shapes, as shown in the TEM micrographs in Fig. 1. Rod-shaped Te-NPs can penetrate the cell membrane by electrostatic attraction. They then penetrate it with their sharp ends (Tang et al. 2022). In addition, the antibacterial activity gradually increased with increasing concentration of Te-NPs. At higher concentrations (2.5 and 5 mM), significant differences were observed between the antibacterial activities of PE-Te-NPs and those of AP-Te-NPs. The antibacterial activities of PE-Te-NPs at concentrations of 0.5 and 1 mM increased significantly compared to AP-Te-NPs at the same concentrations, except against *E. coli*, although no significant differences were observed between the effects of PE-Te-NPs and AP-Te-NPs on its growth. However, at a low concentration (0.1 mM), no significant differences were observed in the antibacterial activities of both PE-Te-NPs and AP-Te-NPs against all tested bacteria, as shown in Table 1. The tested Gram-positive bacteria, including *B. cereus* and *S. aureus*, were more resistant to the prepared Te-NPs than Gram-negative bacteria (*E. coli* and *P. aeruginosa*), which showed a higher inhibition area of up to 23.46 cm^2 in the case of *E. coli* inhibited by PE-Te-NPs at a concentration of 5 mM. Based on the results of the antibacterial activity test of the prepared Te-NPs, the low concentration (0.1 mM) of Te-NPs showed weak antibacterial activity, while Te-NPs at a concentration of 2.5 mM showed antibacterial activity against all tested bacterial strains. In this context, a test of the antibacterial activity of MPMs treated with Te-NPs at concentrations of 0.5 to 2.5 mM was performed. Several studies have investigated the antibacterial activities of Te-NPs prepared by various biological and chemical methods against the tested bacteria and confirmed the excellent antibacterial activity of Te-NPs, which is consistent with the results of this study. For instance, (Abed et al. 2023, 400-412) tested the *in vitro* and *in vivo* antibacterial activity of Te-NPs prepared using actinomycete medium against the growth of methicillin-resistant *S. aureus* and reported that Te-NPs (2 mM) showed a promising zone of inhibition of about 24 mm (4.52 cm^2). In addition, Morena et al. (Morena et al. 2021, 14885-14893) mentioned excellent antibacterial effects for Te-NPs synthesised sonochemically using lignin as a reductant and a structural component against the growth of *E. coli*, *P. aeruginosa* and *S. aureus*, with minimum inhibitory concentrations of 0.07, 3.39, and approximately 2.39 µg/mL, respectively. In addition, a previous study found that PVP at a concentration of 8% showed no antibacterial activity against *Bacillus cereus* and *Escherichia coli*. While the PVP concentration used in the synthesis of Te-NPs did not exceed 3% in this study, suggesting that the antibacterial activity is mainly corresponds to that of Te-NPs (Adomavičiūtė et al. 2016).

Table 1 The antibacterial activities of AP-Te-NPs and PE-Te-NPs at different concentrations. a-h mean values with the same letter(s) within the same column do not differ significantly according to Duncan's multiple range test (p = 0.05)

Te-NPs	Conc.	Area of inhibition zone (cm^2)			
		P. aeruginosa	*E. coli*	*B. cereus*	*S. aureus*
AP-Te-NPs	0.1 mM	1.10 ±0.05 g	1.69 ±0.13 h	0.00 ±0.00 f	0.00 ±0.00 f
	0.5 mM	2.27 ±0.13 g	2.64 ±0.17 f	0.00 ±0.00 f	0.00 ±0.00 f
	1 mM	5.87 ±0.33 f	3.41 ±0.25 e	0.76 ±0.04 e	0.00 ±0.00 f
	2.5 mM	10.00 ±0.85 d	7.96 ±0.63 c	1.07 ±0.10 e	0.96 ±0.17 e
	5 mM	14.08 ±1.02 c	11.24 ±0.75 b	1.81 ±0.30 d	1.33 ±0.10 de
PE-Te-NPs	0.1 mM	1.30 ±0.15 g	1.89 ±0.12 gh	0.00 ±0.00 f	0.00 ±0.00 f
	0.5 mM	8.64 ±0.54 e	2.45 ±0.08 fg	1.73 ±0.18 d	1.81 ±0.18 d
	1 mM	11.04 ±0.29 d	3.63 ±0.17 e	3.20 ±0.24 c	2.59 ±0.22 c
	2.5 mM	15.79 ±1.23 b	7.15 ±0.36 d	5.24 ±0.12 b	6.61 ±0.46 b
	5 mM	20.43 ±1.20 a	23.46 ±0.66 a	7.31 ±0.42 a	9.53 ±0.69 a

2.2.2.2. Antibacterial activities of MPMs treated with Te-NPs

The MPMs treated with AP-Te-NPs (MPMs/AP-Te-NPs), which were subsequently inoculated with the tested bacteria, showed lower antibacterial activities at the same concentrations than the MPMs treated with PE-Te-NPs (MPMs/PE-Te-NPs), which were subsequently inoculated with the tested bacterial strains, except for the MPMs treated with AP-Te-NPs at a concentration of 2.5 mM. They showed higher antibacterial activity against *P. aeruginosa* than those treated with PE-Te-NPs at the same concentration. Moreover, the antibacterial activities of the MPMs treated with the prepared Te-NPs and inoculated with the tested bacteria decreased significantly with decreasing concentration of the Te-NPs, which was consistent with the results of the antibacterial test of the colloidal solution of the prepared Te-NPs. At a low concentration (0.5 mM) of Te-NPs, the MPMs showed no antibacterial activity against *B. cereus* and *S. aureus*. On the other hand, *E. coli* and *P. aeruginosa* were more sensitive to MPMs treated with a low concentration (0.5 mM) than the other bacteria tested, as shown in Table 2. The results presented in Table 2 show that Te-NPs are considered as potential antimicrobial agents to protect historical paintings from degradation by bacteria.

Table 2 The antibacterial activities of MPMs treated with different concentrations of AP-Te-NPs or PE-Te-NPs and then inoculated with the tested bacterial strains. a-g mean values with the same letter(s) within the same column are not significantly different according to Duncan's Multiple Range Test (p = 0.05).

MPMs samples	Te-NPs conc.	Area of inhibition zone (cm^2)			
		P. aeruginosa	*E. coli*	*B. cereus*	*S. aureus*
Blank	-	0.00 ±0.00 g	0.00 ±0.00 g	0.00 ±0.00 e	0.00 ±0.00 e
MPMs/AP-Te-NPs	0.5 mM	16.24 ±0.34 f	8.95 ±0.63 f	0.00 ±0.00 e	0.00 ±0.00 e
	1 mM	21.11 ±0.74 d	15.53 ±0.67 d	15.61 ±0.25 d	11.32 ±0.45 d
	2.5 mM	39.79 ±1.32 a	16.29 ±0.21 c	18.89 ±1.46 c	15.30 ±0.22 b
MPMs/PE-Te-NPs	0.5 mM	19.70 ±0.63 e	10.62 ±0.08 e	0.00 ±0.00 e	0.00 ±0.00 e
	1 mM	24.09 ±0.27 c	18.68 ±0.34 b	21.50 ±0.23 b	13.59 ±0.31 c
	2.5 mM	35.94 ±0.12 b	19.99 ±0.16 a	25.57 ±0.18 a	25.20 ±0.34 a

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2.2.3. Colour characteristics

The change in colours red, blue, yellow, green and brown in different MPMs areas after treatment with Te-NPs and inoculation with *B. cereus* and *P. aeruginosa* was recorded to study the effects of bacterial growth on the different coloured surfaces of the MPMs. In addition, the potential applicability of AP-Te-NPs and PE-Te-NPs as protective coatings against the degradative effect of the tested bacterial strains was evaluated. MPM treated with distilled water served as a negative control to separately investigate the effects of the solvent used in Te-NPs synthesis on the coloured MPMs surfaces. The most observed colour change was found in the red zones inoculated with *B. cereus*. Moreover, the yellowish surface, which had low ΔE values of 7.46 and 8.07, respectively, due to inoculation with *B. cereus* and *P. aeruginosa*, was the most resistant coloured surface to the degradative effect of the tested bacteria. The PE-Te-NPs were more effective against the tested bacteria and reduced the degradation of the different colorants used in the production of the MPMs than the AP-Te-NPs, as shown in Table 3 and Fig. 4. Treatment with PE-Te-NPs at different concentrations (1 and 2.5 mM) resulted in a significant reduction in colour change compared to the positive controls inoculated with the tested bacterial strains without NPs treatment. In the MPMs inoculated with *B. cereus*, PE-Te-NPs at a low concentration (1 mM) showed better effect on the colours tested than those at a high concentration of 2.5 mM. In contrast, the blue and brown coloured surfaces of MPMs inoculated with *P. aeruginosa* were improved with increasing PE-Te-NPs concentration. The least colour changes of the tested AP-Te-NPs treated MPMs were observed on brown coloured surfaces treated with 2.5 mM AP-Te-NPs either before inoculation with *B. cereus* or *P. aeruginosa*. In addition, the increase in AP-Te-NPs led to a significant increase in ΔE values of red and blue surfaces inoculated with *B. cereus*.

Table 3 ΔE values of blank MPMs treated with distilled water only, and MPMs inoculated with *B. cereus* and *P. aeruginosa* and MPMs treated with different concentrations of AP-Te-NPs or PE-Te-NPs prior to inoculation with the tested bacteria. a-h mean values with the same letter(s) within the same column are not significantly different according to Duncan's Multiple Range Test (p = 0.05).

MPMs	Te-NPs Conc.	Bacterial strain of inoculation	ΔE values of MPMs colours				
			Red	Blue	Yellow	Green	Brown
MPMs/water	-	-	1.12 0.110 g	0.95 0.111 j	0.98 0.065 g	1.07 0.050 g	0.39 0.035 g
MPMs inoculated with the tested	-	*B. cereus*	38.84 2.377 a	18.08 0.870 a	7.46 0.379 d	13.85 0.396 d	17.10 0.520 a
	-	*P. aeruginosa*	13.89 0.360 b	16.46 0.340 b	8.07 0.121 c	20.96 0.608 a	16.57 0.557 b
MPMs/AP-Te-NPs	1 mM	*B. cereus*	11.08 0.272 c	6.35 0.065 f	9.93 0.071 b	10.54 0.191 e	11.72 0.310 c
	2.5 mM		9.34 0.347 d	11.72 0.240 d	10.06 0.340 b	10.43 0.315 e	1.55 0.121 de
MPMs/PE-Te-NPs	1 mM		1.49 0.095 fg	1.68 0.135 hi	1.42 0.035 g	1.39 0.035 g	0.83 0.060 fg
	2.5 mM		3.60 0.198 e	2.24 0.106 gh	4.56 0.340 e	2.47 0.105 f	1.31 0.060 def
MPMs/AP-Te-NPs	1 mM	*P. aeruginosa*	10.65 0.313 cd	8.22 0.250 e	7.84 0.205 cd	15.23 0.345 c	16.16 0.495 b
	2.5 mM		12.66 0.611 b	13.71 0.550 c	13.14 0.510 a	17.65 0.295 b	0.35 0.040 g
MPMs/PE-Te-NPs	1 mM		2.46 0.436 efg	2.55 0.339 g	3.06 0.278 f	2.31 0.335 f	1.76 0.075 d
	2.5 mM		2.63 0.291 ef	1.36 0.045 ij	3.04 0.215 f	2.83 0.116 f	1.11 0.065 ef

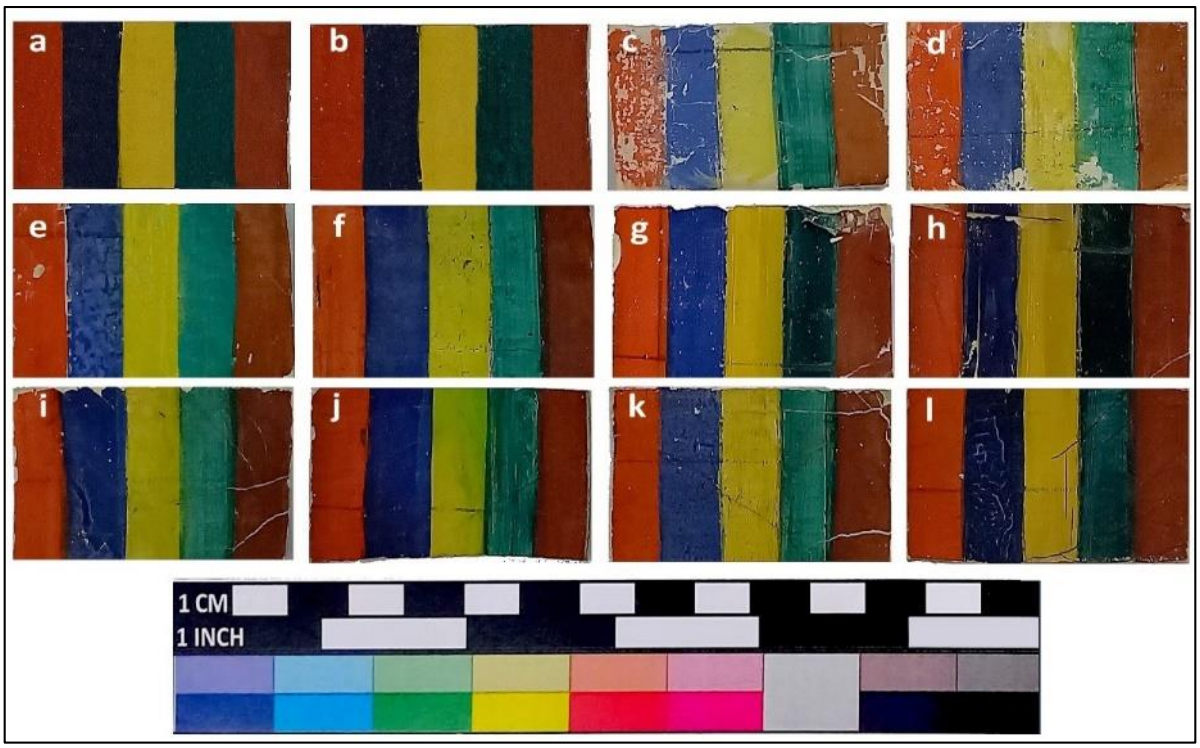

Fig. 4 Images of MPMs from a) blank sample (no inoculation, no treatment), b) negative control (water treatment), and positive controls: c) MPMs inoculated with *B. cereus*, d) MPMs inoculated with *P. aeruginosa*, MPMs treated with AP-Te-NPs at the following concentrations : e) 1 mM and f) 2.5 mM then with *B. cereus*, MPMs treated with PE-Te-NPs in concentrations of: g) 1 mM and h) 2.5 mM, then inoculated with *B. cereus*, MPMs treated with AP-Te-NPs in concentrations of: i) 1 mM and j) 2.5 mM, then inoculated with *P. aeruginosa*, MPMs treated with PE-Te-NPs in concentrations of: k) 1 mM and l) 2.5 mM, then inoculated with *P. aeruginosa*

2.2.4. Cytotoxicity of the produced Te-NPs

The cytotoxicity of the synthesised AP-Te-NPs and PE-Te-NPs was investigated using the MTT assay in the two cell lines HFB4 and WI-38 (see Table 4). Active HFB4 or WI-38 cells can reduce MTT to formazan by specific enzymes secreted by these viable cells (Sabela et al. 2018, 560-567). The cytotoxicity of the prepared AP-Te-NPs (0.5-2.5 mM) on WI-38 cells was higher than on HFB_4 cells, suggesting that the mixture of spherical, rod-shaped and flower-shaped Te-NPs is more toxic to lung cells than fully rod-shaped Te-NPs. On the other hand, PE-Te-NPs (0.25-2.5 mM) showed higher cytotoxicity on HFB_4 cells than on WI-38 cells, suggesting that the deleterious effects of PE-Te-NPs on skin were greater than their effects on lung tissue. In addition, the synthesised Te-NPs at concentrations of 0.125 to 1 mM exhibited less than 30% cytotoxicity on the cell lines tested, except for PE at a concentration of 1 mM, which had a percentage cytotoxicity value of 33.36. The average acceptable relative cell viability was above 70% (Kangwansupamonkon et al. 2009, 240-249), indicating that the prepared AP-Te-NPs and PE-Te-NPs can be safely used for the treatment of MPMs at concentrations up to 1 mM. Therefore, the results of the cytotoxicity assay confirmed the applicability of the green-synthesised AP-Te-NPs and PE-Te-NPs as environmentally friendly agents against the harmful effects of various bacterial strains on MPMs. It was found that the cytotoxicity of the prepared PE-Te-NPs against both HFB_4 and WI-38 cells increased significantly with increasing Te-NPs concentration. Corresponding results were observed for AP-Te-NPs, whose cytotoxicity gradually increased with increasing concentration in the tested cell lines. The IC_{50} values of AP-Te-NPs were 2.30 and 2.06 mM for HFB_4 and WI-38 cells, respectively, while PE-Te-NPs had lower IC_{50} values than AP-Te-NPs at 1.84 and 1.90 mM for HFB_4 and WI-38 cells, respectively. The commonly known mechanism for the cytotoxicity

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of NPs is the production of reactive oxygen species, which increase oxidative stress and lead to DNA damage and remarkable changes in cell motility (Fu et al. 2014, 64-75). Shrinkage of tested cells, fragmentation, and nuclear condensation are the most common morphological signs of cytotoxicity (Firdhouse and Lalitha 2015, 113-121).

Table 4 Cytotoxicity of Te-NPS on WI-38 and HFB_4 cells. a-g mean values with the same letter(s) within the same column are not differ significantly according to Duncan's Multiple Range Test ($p = 0.05$).

Tested NPs		HFB4		WI 38 Cells	
NPs type	Conc. (mM)	Cytotoxicity (%)	IC_{50} (mM)	Cytotoxicity (%)	IC_{50} (mM)
AP-Te-NPs	0.125	0.62 ±0.031 f	2.30 ±0.073	0.20 ±0.015 g	2.06 ±0.027
	0.25	1.05 ±0.061 f		0.93 ±0.043 g	
	0.5	2.73 ±0.099 e		4.03 ±0.286 f	
	1	3.00 ±0.194 e		18.21 ±0.484 d	
	2.5	57.71 ±1.672 b		66.19 ±1.889 b	
PE-Te-NPs	0.125	0.41 ±0.006 f	1.84 ±0.058	1.09 ±0.057 g	1.90 ±0.007
	0.25	3.72 ±0.106 e		2.70 ±0.134 f	
	0.5	11.10 ±0.297 d		9.05 ±0.212 e	
	1	33.36 ±1.172 c		22.77 ±0.868 c	
	2.5	72.71 ±1.983 a		72.07 ±1.835 a	

3. CONCLUSIONS

Green-synthesised AP-Te-NPs and PE-Te-NPs were prepared to investigate their potential applicability as antimicrobial agents on the surfaces of MPMs against the degradative effects of *P. aeruginosa, E. coli, B. cereus,* and *S. aureus.* In addition, the effects of Te-NPs and bacterial growth on the colour properties of the tested MPMs were investigated. TEM micrographs confirmed the formation of tellurium at the nanoscale in the case of AP-Te-NPs or PE-Te-NPs. Three morphological forms were observed in AP-Te-NPs, which exhibited nanorod, sphere, and flower shapes, while PE-Te-NPs were completely rod-shaped. PE-Te-NPs showed higher antimicrobial activity against the tested bacteria and better colour properties on the surfaces of MPMs than AP-Te-NPs. In contrast, they caused higher cytotoxicity than AP-Te-NPs in the HFB4 and WI-38 cell lines. In general, Te-NPs at concentrations of up to 1 mM resulted in cell viability of about or more than 70%. Based on the results of this study, PE-Te-NPs are therefore the most suitable and sustainable antimicrobial agents for the conservation of historical oil paintings.

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Author contributions

YE: Conceived the original idea and design of this study, examined the surfaces of the MPMs by SEM analysis and measured the colour properties of the tested samples. KS: Prepared the NPs in addition to their characterisation, interpreted the results of the cytotoxicity and antimicrobial tests and statistical analysis. Both authors were involved in writing and drafting the manuscript.

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أسلوب جديد لاستخدام جسيمات التيلوريوم النانوية الخضراء المصنعة لحماية اللوحات الزيتية التاريخية من التلف البكتيري

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بيانات المقال

تاريخ المقال

تم الاستلام في ١٢ فبراير ٢٠٢٤
تم استلام النسخة المنقحة في ٥ أبريل ٢٠٢٤
تم قبول البحث في ١٧ أبريل ٢٠٢٤
متاح على الانترنت في ١٧ أبريل ٢٠٢٤

الكلمات الدالة

جسيمات التيلوريوم النانوية؛
النشاط المضاد للبكتريا؛
اختبار السمية؛
الصور الزيتية؛
صيانة الآثار؛
خصائص اللون.

الملخص

تم تطوير أسلوب جديد لدراسة إمكانية تطبيق جسيمات التيلوريوم النانوية الخضراء **(Te-NPs)** للحفاظ على الصور الزيتية التاريخية من تأثيرات البكتيريا. تم إعداد **Te-NPs** بطريقتين صديقتين للبيئة. **Te-NPs** المحضرة باستخدام البولي فينيل بيروليدون **(PVP)** وحمض الأسكوربيك **(AP-Te-NPs)** وتلك المحضرة باستخدام مستخلص ***Pluchea dioscoridis*** **(PE-Te-NPs)** تمت دراسة خصائصهما باستخدام الميكروسكوب الإلكتروني النافذ **(TEM)** وحيود الأشعة السينية **(XRD)** لتأكيد تكوّن **Te-NPs**. تم تقييم نشاط **Te-NPs** المضاد للبكتيريا، وعلى نماذج اللوحات الزيتية المجهزة **(MPMs)** والتي تمت معالجتها باستخدام **Te-NPs**(٠,١-٥ مليمولر) في المعمل ضد البكتيريا الموجبة لصبغة جرام والبكتيريا السالبة لصبغة جرام. تم استخدام الميكروسكوب الإلكتروني الماسح **(SEM)** لفحص عينات **MPMs**الملقحة بالبكتيريا الجاري دراستها مقارنة بالعينات المرجعية وتلك العينات المعالجة بـ **Te-NPs** قبل التلقيح بالبكتيريا، ثم تقييم سمية **Te-NPs**. بالإضافة إلى ذلك، تم تحديد خصائص الألوان لنماذج **MPMs**لتقييم تأثير **Te-NPs** المحضرة على أسطح هذه النماذج. أظهرت **Te nanorods** أطوالًا تتراوح بين ٥-١٠٢ نانومتر، وتراوحت أقطارها من ١ - ١١,٥ نانومتر. بالإضافة إلى ذلك، تراوحت أقطار **Te-NPs**ذات الأشكال الكروية والشبيهة بالزهرة من ٣,٤ - ١٧,٩ نانومتر. كما أظهرت **Te-NPs** نشاطًا مضادًا للبكتيريا بشكل مميز، خاصة ضد البكتيريا سالبة الجرام. وقد أظهرت **PE-Te-NPs** نشاطًا مضادًا للبكتيريا أكثر تميزاً مقارنةً بـ**AP-Te-NPs** . وقد بيّنت صور **SEM** وقياسات الألوان أن استخدام **Te-NPs** قلل بشكل كبير من التأثيرات الضارة للبكتيريا التي تمت دراستها على أسطح **MPMs**، كما أكد اختبار السمية إمكانية تطبيق **Te-NPs** (١ مليمولر) كمواد مستدامة للحفاظ على الصور الزيتية التاريخية.