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The antimicrobial effect of metal substrates on food pathogens

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ABSTRACT

The development of surfaces as antimicrobial materials is important to the food industry. This study investigated the antimicrobial potential of a range of metal coated surfaces including silver, titanium, copper, iron, molybdenum, zinc and silicon (control) against *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*. The leaching potential of the metals were measured by inductively coupled plasma-atomic adsorption spectroscopy and were compared to the antibacterial activity of the metals using a nitroblue tetrazolium assay and an adapted BS ISO 22196:2011 standard. Leaching into solution from the coatings alone was not related to the antimicrobial activity of the coatings. Copper and zinc showed the greatest propensity to leach from the coatings; silver, titanium, iron and molybdenum leached at lower rates and silicon showed no leaching. Copper demonstrated the greatest antimicrobial potential followed by silver and zinc. Titanium displayed the least antimicrobial potential, however using the standard method in humid conditions resulted in increased growth of *Listeria*. This study provides evidence of the efficacy of copper and silver as effective antimicrobial metal surface coatings, however use of titanium under humid conditions suggest that surfaces for use in the food industry needs to be given careful consideration before application.

1. Introduction

The Food Standard Agency (FSA) estimates that foodborne illnesses in the UK result in a financial loss of £1.5 billion annually, while in the USA, the Centre for Disease Control (CDC) estimates that 1 in 6 (or 48 million) people become ill annually, leading to 3000 fatalities from foodborne diseases (FSA, 2011; CDC, 2016). Central to the FSA's strategy to reduce foodborne diseases in the UK is the reduction and prevention of cross-contamination between food, surfaces or equipment. This informs the FSA's recommendation that food producers and processors adopt improved practices to reduce or prevent the contamination of foods at source and minimise the spread or growth of pathogens at different production stages before consumption. Yasuyuki et al. (2010) described the prevention of bacterial attachment to material surfaces by incorporating antibacterial materials in the production of the surfaces as a potential strategy to stop bacterial contamination and transmission, highlighting the significance of development of antibacterial materials to the food industry.

The development of alternative surfaces and surface coatings as antimicrobial materials or agents for the reduction

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of microbial contamination and transmission has been suggested for use in industries (Jaiswal et al., 2012; Bastarrachea et al., 2015; Moerman, 2014). Antibacterial materials are also reported to be less harmful to the environment as they release less pollutants compared to other common antibacterial agents (Yasuyuki et al., 2010). The development of antibacterial materials is therefore important to the food industry.

Some common metals possessing antimicrobial potential have been used as surface coatings including silver (Ag), which is reported to have antimicrobial activity at low concentrations (Lemire et al., 2013; Bastarrachea et al., 2015). Silver nanoparticles have recently been widely used in innovative packaging materials and silver-based salts incorporated into biopolymer films have been suggested for development of food packaging materials (Rhim et al., 2013). Titanium (Ti) has been incorporated in stainless steel alloys in the food industry and is a biocompatible material widely used in dental implants (Ferriera Ribeiro et al., 2016). Titanium is also used as a photocatalytic surface in the form of titanium oxide (TiO_2) (Cabellero et al. 2009). Copper is an essential trace element but also antimicrobial and is used as a hygienic surface material in hospitals due to its ability to achieve contact kills (Grass et al., 2011). The antimicrobial activity of copper has been applied in medicine for centuries and has recently been shown to be effective in the control of pathogens such as *Escherichia coli* in the food industry (Noyce et al., 2006). Iron is used in its alloy form in stainless steel and is highly regarded for its mechanical properties in the food industry (Whitehead and Verran, 2007; Whitehead et al., 2015). Molybdenum is also an alloy constituent of stainless steel and is important for its contribution to corrosion resistance and mechanical shelf life of 316 stainless steel for example (Dewangan et al., 2015). Zinc has been shown to be antimicrobial, showing selective toxicity to both Gram positive and Gram negative bacteria (Reddy et al., 2007; Hernández-Sierra et al., 2008).

This study investigated the antimicrobial potential of a range of metal-coated surfaces including silver, titanium, copper, iron, molybdenum, zinc and also silicon (used as control) on three potentially pathogenic foodborne bacteria, *Staphylococcus aureus, E. coli* and *Listeria monocytogenes*. The World Health Organisation (WHO, 2015) lists *E. coli*, *S. aureus* and *Listeria* sp. amongst the foodborne pathogens of public health importance. *E. coli* is often associated with unpasteurized milk, undercooked meat and fresh fruits and vegetables (Alum et al., 2016). *S. aureus*, although a common commensal microorganism found on the skin, and sometimes in the gut microbiota, of healthy people, is a common source of food-borne diseases, causing Staphylococcal food poisoning by its enterotoxin strains when transmitted onto food products (Hennekinne et al., 2011; Dong et al., 2018). *Listeria* sp. is found in unpasteurised dairy products and various ready-to-eat foods and can grow at (FAO/WHO, 2004; Hennekinne et al., 2011; Sillankorva et al., 2012).

2. Experimental

2.1. Coating production

All the metal coatings used in this study were produced on silicon substrates, which were deposited with pure metal coatings. Using methods described by Whitehead et al. (2015), pure metal coatings (99.9% silver target, 99.5% titanium target, 99.5% copper target, 99.5% molybdenum target and 99.5% iron target (Teer coatings, UK) were deposited onto 10 × 10 mm silicon substrates using a DC mode magnetron sputtering coating technique (Advanced Energy, MDX). The silicon surface used was an uncoated silicon wafer (Montco Technologies, USA).

2.2. Microbiology

Stock cultures of the potentially pathogenic food borne microorganisms, *S. aureus* (NCIMB 9518, Campden BRI (UK)), *E. coli* CCL410 (L'Agence française de sécurité sanitaire des aliments (AFSSA, France)) and *L. monocytogenes* (EGDe ATCC culture collection) were used in this study (adapted from Whitehead et al., 2015). All microbiological agar and broths used throughout this work were obtained from Oxoid (UK).

2.3. Maintenance of cultures

A freezing mix was made of two solutions; solution A (di-potassium hydrogen phosphate (K_2HPO_4) 12.6 g L^{-1}, potassium phosphate monobasic (KH_2PO_4) 3.6 g L^{-1}, tri-sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$) 0.9 g L^{-1}, ammonium sulphate $(NH_4)_2SO_4$ 1.8 g L^{-1}, and glycerol 300 g L^{-1}, autoclaved at 121 °C for 15 min) and Solution B (magnesium sulphate ($MgSO_4{\cdot}7H_2O$) 1.8 g L^{-1}). Solution B was filter sterilised using a 10 mL Luer-LokTM syringe (BDH, UK) and an Acrodisc® filter (32 mm with 0.2 μm non-pyrogenic supar® membrane, Pall Corporation, UK). One millilitre of solution B was added to 100 mL of solution A to produce the final freezer mix. All chemicals used in the freezing mix were obtained from BDH (UK).

Stock cultures were prepared adding equal amounts of culture and freezing mix and then incubated at the appropriate temperature for 1 h. Samples were dispensed into 1.5 mL sterile plastic screw capped tubes, and frozen at −80 °C. This procedure provided stock cultures that were not attenuated or otherwise altered by successive sub-culturing. For use, the culture was defrosted at room temperature and a loop full of liquid was inoculated into broth and incubated at the appropriate temperature for 18 h. The cultures were then transferred onto agar and incubated at the appropriate temperature for 18 h and the remainder of the culture returned to the freezer for future use.

2.4. Working cultures

Once obtained, *S. aureus* or *E. coli* was streaked onto Brain Heart Infusion Agar (BHIA), and incubated at 37 °C for 24 h. *L. monocytogenes* was streaked onto Tryptone Soya Agar (TSA), and incubated at 30 °C for 24 h. The working cultures were used and stored at 4 °C for four weeks after which they were replaced. Broth media were inoculated from these plates. Brain Heart Infusion broth (BHIB) inoculated with *S. aureus* or *E. coli* was incubated for 24 h at 37 °C. Tryptone Soya Broth (TSB) inoculated with *L. monocytogenes* was incubated for 24 h at 30 °C.

2.5. Bacterial assays

A single colony of *S. aureus* and *E. coli* was inoculated into 10 mL of BHIB and incubated at 37 °C for 24 h, with shaking at 200 rpm. The resulting broth suspension was centrifuged (567 × *g* for 10 min) and the bacterial cells were harvested and washed by re-suspension into 10 mL of sterile distilled water. Following a second centrifugation (567 × *g* for 10 min), the bacterial cells were re-suspended and diluted using ster-

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ile distilled water to an OD of 1.0 at 540 nm corresponding to $1.31 \pm 0.84 \times 10^9$ colony forming units per mL (cfu mL^{-1}) for *S. aureus* and $1.27 \pm 0.77 \times 10^8$ cfu mL^{-1} for *E. coli*. The culture of *L. monocytogenes* was prepared using the same method outlined above, except that cells were incubated in TSB at 30 °C and were re-suspended to an OD of 1.0 at 540 nm in sterile distilled water corresponding to $1.54 \pm 0.23 \times 10^8$ cfu mL^{-1}.

2.6. Antibacterial effect of thin film coatings in solutions

Overnight bacterial suspensions of the test microorganisms were inoculated into the appropriate broth and were mixed with sterile broth to attain a cell density of 5×10^5 cfu mL^{-1} (determined by serial dilutions and OD readings). Substrata (metal coated silicon wafers and uncoated silicon wafers) were immersed into the broth suspensions and incubated at the appropriate temperatures with shaking at 200 rpm. The number of viable cells remaining in the culture over time (0 h, 1 h, 3 h, 5 h and 24 h) was determined by extracting 100 μL of cell suspension and performing plate counts on the appropriate agar medium. As this investigation combined both the ability of metals to leach into media and their antimicrobial potential, a reduction in the viable counts obtained from the broth solutions was taken as an indication of the antimicrobial activity of the metals immersed in the solutions.

2.7. Inductively coupled plasma-atomic emission spectroscopy (ICP-AES)

The stability of the metal substrates in solution was tested by immersing the coated substrates into 10 mL of BHIB or TSB for 24 h at 37 °C or 30 °C with shaking at 150 rpm for 30 min. Samples of the broth media were further diluted 50% (v/v) with sterile distilled water. The concentration of metal ions leached into broth media from the coatings were measured using inductively coupled plasma-atomic emission spectroscopy (ICP-MS, Agilent 7900, UK). A baseline concentration of the metal ions in the agar was determined and used as a control.

2.8. Nitroblue tetrazolium (NBT) assay

The antimicrobial activity of the metal coatings in direct contact with bacterial cells were investigated using nitroblue tetrazolium chloride (NBT, Sigma, UK), a redox dye which assists in the visualization of colonies (Wickens et al., 2014). For this assay, 10 μL of washed cells (as described previously) were immobilized on the different metal coatings at cell concentrations of approximately 10^5 cfu mL^{-1} (*S. aureus* were $2.22 \pm 1.87 \times 10^5$ cfu mL^{-1}, *E. coli* $2.63 \pm 0.27 \times 10^5$ cfu mL^{-1} and *L. monocytogenes* $1.78 \pm 0.21 \times 10^5$ cfu mL^{-1}). The cell suspensions were pipetted onto the metal surface and dried in a microbiological class 2 laminar flow cabinet. The substrata with immobilized cells were placed into sterile plastic Petri dishes and 25 mL of cooled (50 °C) TSA was poured gently over the top of the sample and set at room temperature then incubated overnight at 37 °C. The surface of the agar was flooded with 2 mL of 1 g L^{-1} of the NBT which was left to adsorb into the media.

2.9. BS ISO 22196:2011 standard method

The antimicrobial activity of the coatings was tested against the bacteria using an adapted version of the BS ISO 22196:2011. This method involved the determination of the antimicrobial activity of the coatings in the presence and absence of humidity. Substrata (10 mm × 10 mm) were placed in small sterile Petri dishes (90 mm) and 16 μL of standardized cell suspension (5×10^5 cells mL^{-1}) was pipetted onto the middle of the substrate. The coatings were covered with a piece of sterile parafilm (also 10 mm × 10 mm) ensuring that the parafilm touched the applied cell suspension. The substrates in the small Petri dishes were placed into a larger square Petri dish (24 cm × 24 cm) before incubation for 24 h on the work bench (dry conditions) and humid conditions. A humid microenvironment was created by placing tissue paper moistened with sterile distilled water into large square Petri dishes in which smaller round Petri dishes containing the substrates were placed.

Bacterial cells were recovered from the coatings post incubation by separating the parafilm from the substratum and placing each substratum and the corresponding parafilm into 10 mL of appropriate broth. The samples in the broth were vortexed for 0.5 min before colony counts were performed using 100 μL of broth suspension onto the appropriate agar. These were incubated overnight at the appropriate temperatures for 24 h.

2.10. Antimicrobial activity of titanium ions against L. monocytogenes

A colony of *L. monocytogenes* was inoculated in 5 mL of TSB and incubated at 37 °C overnight, at 200 rpm. The bacterial suspension was diluted with TSB to reach an OD of 0.05 at 540 nm. The bacterial suspension was mixed with titanium (Titanium Standard for AAS, Fluka Analytical, UK) to reach the following titanium concentrations: 10 ppm, 30 ppm and 50 ppm. Phosphate-buffered saline (PBS) was used as a blank. The OD was read after 0 h, 1 h, 2 h, 4 h, 6 h, and 24 h. As the titanium solution contained nitric acid, the bacterial suspensions containing 10 ppm, 30 ppm and 50 ppm of titanium, contained also 0.02%, 0.06%, and 0.1% of nitric acid, respectively. The experiment was therefore repeated with nitric acid only, at these concentrations. All the conditions were tested in triplicate.

2.11. Statistics

All statistical analysis undertaken in this study were performed using SPSS 2.1 (Mann–Whitney U test).

3. Results

The residual ion concentration detected by the ICP-AES analysis indicated no significant difference in the metal ion concentrations of the control constituent media (BHIB and TSB) prior to incubation with metal coating ($p = 0.87$) (Fig. 1A). For both control media, zinc and iron were present in the greatest concentrations (0.6 and 0.3 ppm respectively), while the other metal components demonstrated concentrations of less than 0.1 ppm.

There was evidence of leaching of the metal ions into the broth solutions when the metal coated silicon substrates were immersed in the media, as there was a significant difference

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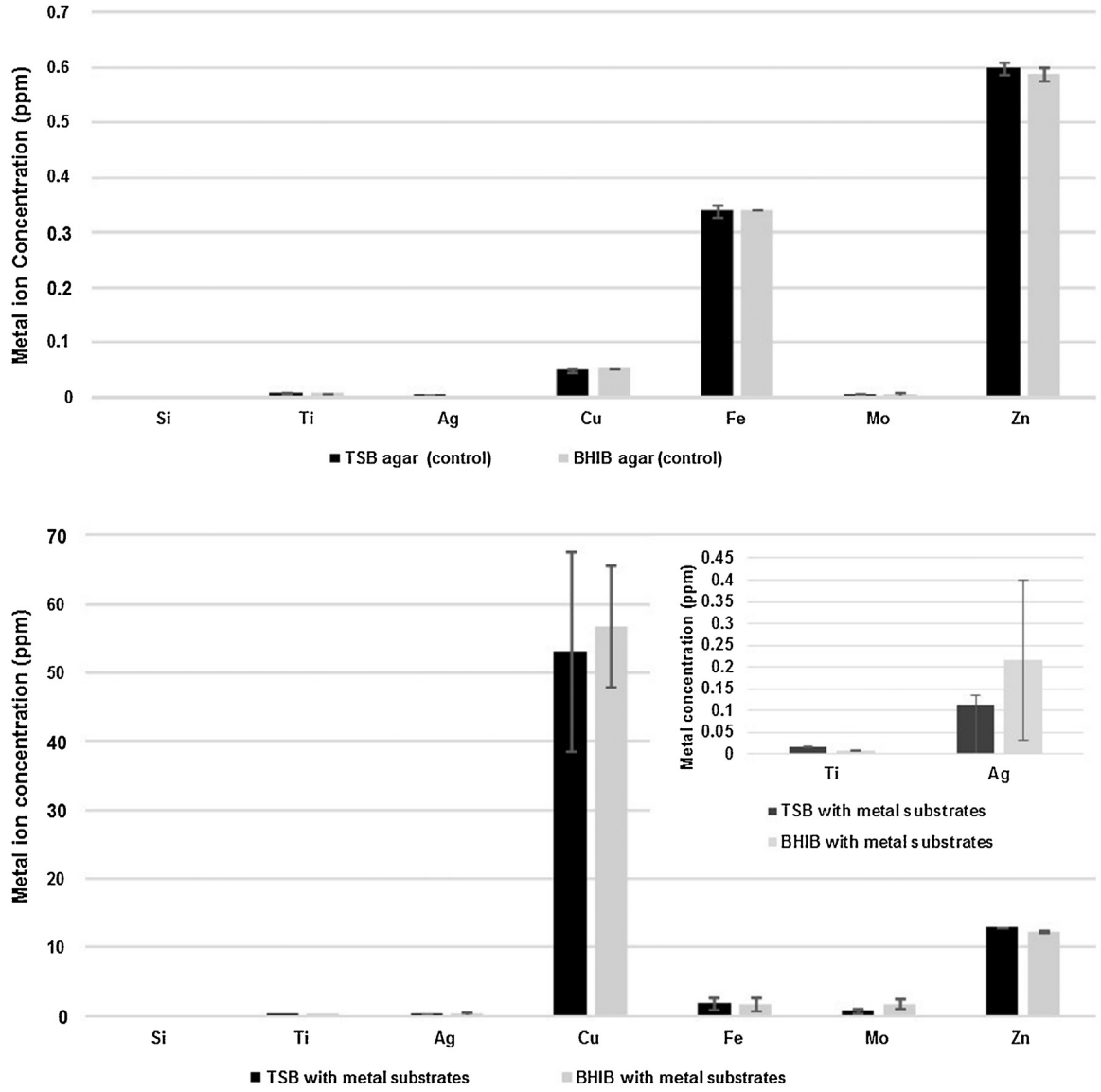

Fig. 1 – Metal ion concentrations in (A) TSB and BHIB control media prior to incubation with substrata, and (B) in TSB and BHIB media post incubation with coated substrates demonstrating Cu displayed the greatest leaching rate when incubated in the media (data is presented as mean ± SD). Note the difference on the y axis in A and B.

between the concentrations of the metal ions found in the control media and media containing metal substrates ($p = 0.04$) (Fig. 1B). However, the results obtained also showed that there was no significant difference between the concentrations of the individual ions leached in when immersed in BHIB or TSB ($p = 1.0$).

All the metals tested showed evidence of leaching when placed into broth media with the exception of silicon. Silicon was the only substrate not detected in both the control media and the media with the immersed metals, thus the silicon was not predisposed to leaching (Fig. 1A and B). In contrast, copper achieved the greatest rate of leaching. Copper concentrations of 56 ppm and 53 ppm were detected in the BHIB and TSB broths, which was significant when compared to the less than 0.05 ppm in the controls for both media (Fig. 1A and B). Zinc also showed a propensity to leaching, achieving above 10 ppm in both BHIB and TSB when compared to concentrations of less than 0.6 ppm in their equivalent control samples. Apart from silicon, titanium and silver also showed the least potential to leach into media, achieving less than 1.0 ppm ion concentrations when immersed in both broths.

When immersed in broth suspensions, the copper coating displayed the greatest antibacterial potential as it achieved a 99% cell kill for all three bacterial species after one hour incubation and no bacteria were recoverable from the broth suspension three hours after incubation (Fig. 2). Silver showed the second greatest antimicrobial potential and achieved a 46%, 99% and 34% cell kill for *S. aureus* and *E. coli* and *L. monocytogenes* respectively after one hour incubation. Silver, unlike copper, achieved complete cell death for *S. aureus* after 5 h, for *E. coli* after 3 h incubation, and a 99% reduction of cell concentration for *L. monocytogenes*. However, the average leaching potential of copper was 350 times that of silver (Fig. 1B).

Overall, copper and silver showed the most antimicrobial effect against all three bacterial strains. Molybdenum demonstrated complete bacterial kills for the *L. monocytogenes* and *S. aureus* after 24 h. Zinc demonstrated greater antimicrobial activity than molybdenum as it consistently achieved lower viable bacterial cell counts three hours after incubation for all bacterial species. Titanium and silicon achieved complete cell death only for *S. aureus* after 24 h, while iron achieved

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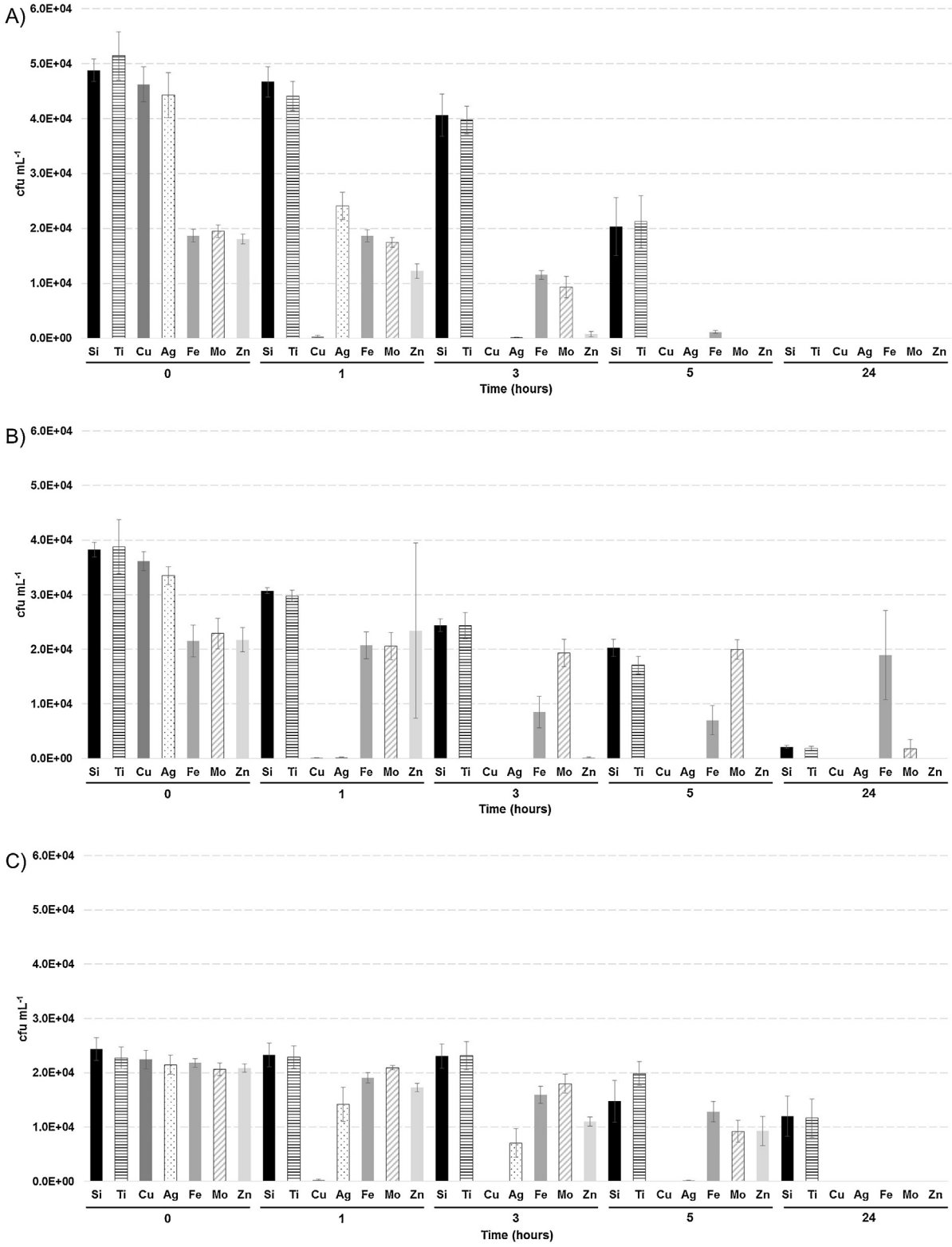

Fig. 2 – Viable bacterial counts of (A) *S. aureus* (B) *E. coli* and (C) *L. monocytogenes* recovered from BHIB and TSB suspensions, when the metal coatings were incubated for 24 h, demonstrating the antimicrobial potential of the different metals on the microorganisms with Cu (attaining 99% cell death 1 h after incubation) and Ag to be the most effective antimicrobial metals (data is presented as mean ± SE).

complete cell death for *S. aureus* and *L. monocytogenes* after 24 h.

Investigations using the NBT method also demonstrated that copper, silver and zinc had the greatest antimicrobial activities when using this assay (Fig. 3). Indeed, all coatings were inoculated with the following amount of microorganisms: $2.22 \pm 1.87 \times 10^3$ cfu cm^{-2} for *S. aureus*, $2.63 \pm 0.27 \times 10^3$ cfu cm^{-2} for *E. coli* and $1.78 \pm 0.21 \times 10^3$ cfu cm^{-2} for *L. monocytogenes*. Copper, silver and zinc achieved complete bacterial kills for all three microorganisms tested upon overnight incubation, while the other metals (molybdenum, iron, silicon and titanium) showed different degrees of antimicrobial activity against the bacteria but did not achieve complete kills.

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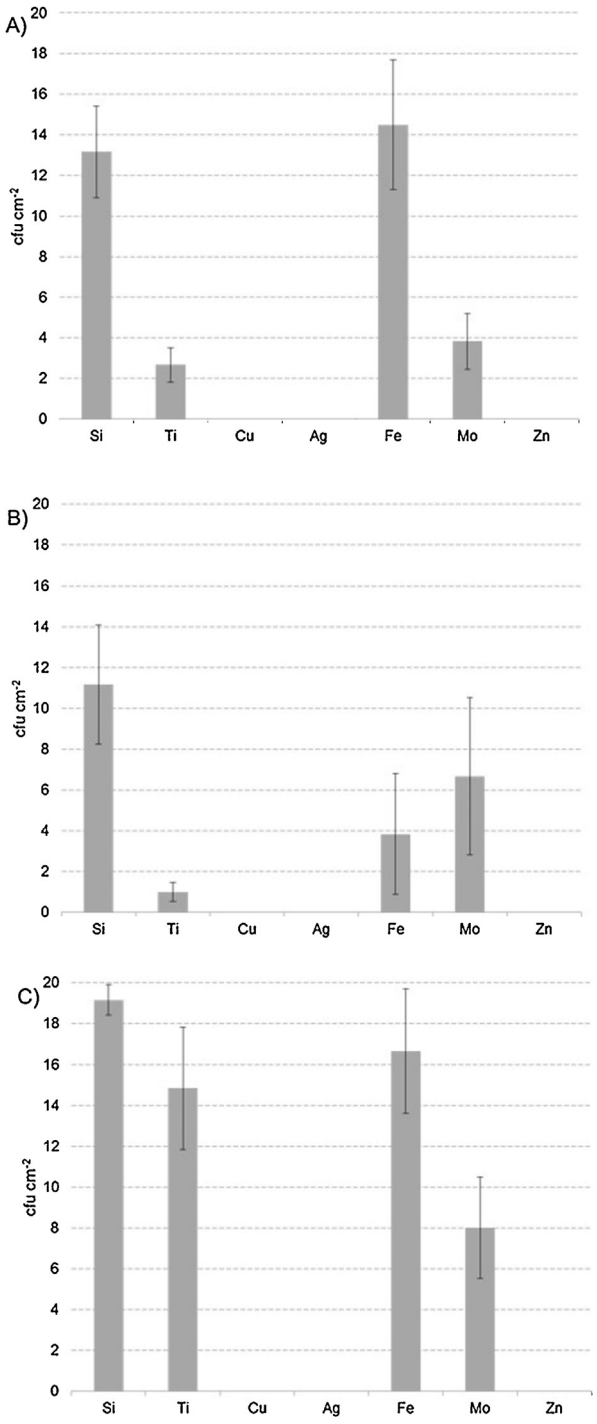

Fig. 3 – Colony count from NBT viability tests on metal coatings for (A) *S. aureus* (B) *E. coli* and (C) *L. monocytogenes* demonstrating Cu, Ag and zinc was the most effective antimicrobial metal coatings (data is presented as mean ± SE).

Results from the adapted BS ISO 22196:2011 method to determine the antimicrobial activity of the metal coatings revealed that following swabbing, 10^3–10^4 cfu mL^{-1} were not recovered, suggesting that these bacteria were either retained on the surfaces or within the swab (Fig. 4A–E). Bacteria were almost never recoverable from coatings incubated in dry conditions and only retained at best <1% of inoculum concentration at t_0 (Fig. 4D–F). There was evidence of the influence of the incubation conditions on the viability of the cells as the humid conditions allowed for the bacteria to remain viable and recoverable from some of the metal coatings (Fig. 4G–I). Only copper was able to achieve complete bacterial kills on all the coatings in both dry and humid conditions (Fig. 4D–I). Silver and zinc displayed the next greatest antimicrobial activity, achieving greater than 98% bacterial kills for all the pathogenic bacteria tested.

The effect of surface humidity on the viability of the bacteria was evident on the growth of *E. coli* and *L. monocytogenes* on coatings post incubation. *E. coli* maintained 50% viability for silicon and titanium coatings when incubated in humid conditions, while *S. aureus* retained <3% viability at best on some coatings. Although *L. monocytogenes* were not recoverable upon incubation in dry conditions, the bacteria on the silicon and titanium coatings under humid conditions achieving 45% viability on silicon and a 100% increase in recoverable cell numbers after overnight incubation on the titanium coatings.

Following the increased growth demonstrated under humid conditions, *L. monocytogenes* solutions were incubated for 24 h with different concentrations of titanium ion and nitric acid solutions (Fig. 5). At 10 ppm and 30 ppm of titanium ion solution resulted in a decrease in the growth rate of *L. monocytogenes*. A reduction on the final concentration of microorganisms after 24 h was also demonstrated (Fig. 5A). In addition, 50 ppm of titanium inhibited the growth of *L. monocytogenes*. However, it has to be noted that the media containing 10 ppm, 30 ppm, and 50 ppm of titanium, also contained 0.02%, 0.06%, and 0.1% of nitric acid, respectively. Thus the effect of the nitric acids solutions alone on cell viability, at these concentrations were carried out as a control (Fig. 5B). When 0.02% and 0.06% of nitric acid was tested against *L. monocytogenes*, there was no significant impact on the growth of *L. monocytogenes*. On the contrary, 0.1% of nitric acid reduced the growth rate and the final microorganism concentration, but did not inhibit the growth of *L. monocytogenes*. These results showed that when the concentration of titanium ions was between 10 ppm and 50 ppm there was a decrease in the growth rate and the final concentration of *L. monocytogenes* in TSB solutions.

4. Discussion

In this study, antimicrobial interactions of metals and microorganisms was investigated using a range of methods. The results indicated that all the metal coatings used in this investigation (with the exception of the control silicon), leached when placed in the bacterial media. Copper showed the greatest propensity to leach in solution, achieving five times the leaching concentration of zinc. This is in contrast to the results reported by Yasuyuki et al. (2010), where zinc achieved four times the concentration of copper when metal coupons were shaken in flasks of nutrient broth for 72 h. However, this may be explained since Yasuyuki et al. (2010) used pure metal coupons (>99.5%) and not metal coated surfaces as were used in this study.

Copper also displayed the greatest antimicrobial potential activity. Several studies have shown copper to have high contact kills on bacterial cells, including *E. coli*. Santo et al. (2011) demonstrated that bacterial cells exposed to copper coatings were killed since they accumulated copper ions and exhibited membrane and cell envelope damage when in contact with the metallic copper surfaces. They also reported that copper uptake was faster from dry copper than from moist copper surfaces. Weaver et al. (2010) also reported that copper killed off actively respiring bacterial cells after 1 h of exposure in dry

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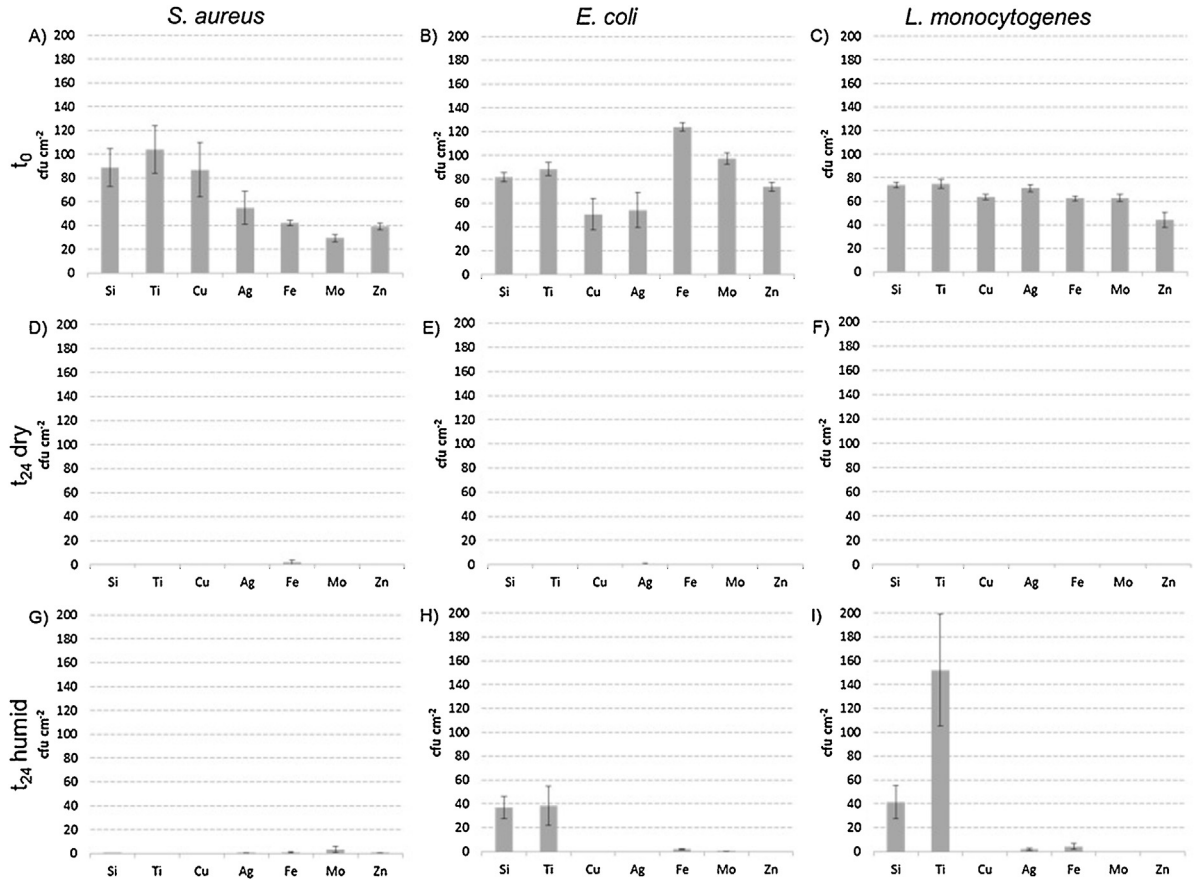

Fig. 4 – Viable bacterial counts recovered from the coatings following incubation in dry and humid conditions for overnight incubation demonstrating Cu, Ag and zinc as the most effective antimicrobial metals. (A) 0 h *S. aureus*, (B) 0 h *E. coli*, (C) 0 h *L. monocytogenes*, (D) 24 h dry *S. aureus*, (E) 24 h dry *E. coli*, (F) 24 h dry *L. monocytogenes*, (G) 24 h wet *S. aureus*, (H) 24 h wet *E. coli* and (I) 24 h wet *L. monocytogenes* (Data is represented as mean ± SE).

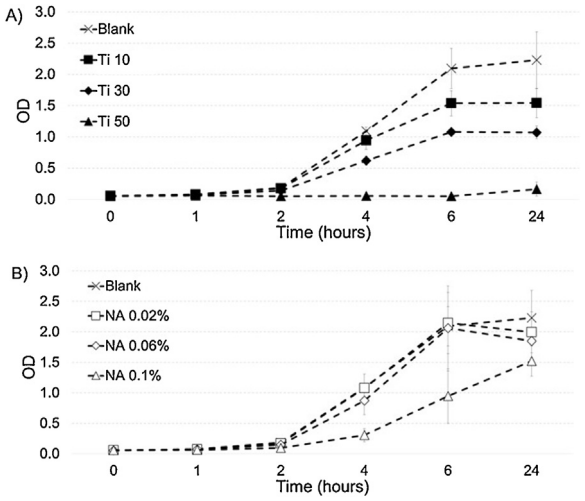

Fig. 5 – Growth of *L. monocytogenes* in TSB supplemented with different concentrations of (A) titanium (0 ppm, 10 ppm, 30 ppm, and 50 ppm) and (B) nitric acid (0%, 0.02%, 0.06%, and 0.1%).

contact investigations, but concluded that copper had little effect on membrane integrity.

In this study, silver achieved the second greatest antimicrobial effect on the pathogenic bacteria but showed low propensity to leach in solution (350 times lower than copper and 85 times lower than zinc). It has been previously reported that the antimicrobial property of silver was related to the amount and rate of silver released from the metal (Rai et al., 2009; Slate et al., 2018). This study also showed molybdenum to have a low leaching potential in the media. Kawakami et al. (2008) reported similar results using molybdenum flakes as substrata rather than metal coatings as used in this study. Molybdenum in this study displayed limited antibacterial effects in all the methods used. Zinc on the other hand displayed a high leaching rate in this study and has been reported to be antibacterial, although the exact mechanism of action have not been well elucidated (Davis et al., 2009). This study showed zinc to be effective against all bacterial strains tested but it was less effective than copper and silver. Following the viable count assays, results obtained with the zinc surface for the *E. coli* after longer periods of time, demonstrated that no cells could be recovered. This might have occurred if the zinc ions had a slower antimicrobial effect on *E. coli* since the cells may have initially have initiated a stress response to overcome the antimicrobial effect of the zinc. However, there may have been a critical threshold whereby the amount of zinc ions leached had a bactericidal effect on the bacteria.

The iron coatings also displayed a low leaching potential in the media similar to the molybdenum coatings. The iron surfaces also consistently displayed a lower antimicrobial potential than the zinc coatings. Following the viable count assays there was also a decrease in the number of bacterial recovered from the iron, until 5 h with *E. coli* and then there was an apparent increase in the number of cells recovered. We hypothesise that this effect may be due to the presence of the

Fe surface slowing bacterial growth whilst the cells bind to the surface, following which they recover and begin to reproduce again.

In the viable count assays whereby the bacteria where incubated with the surfaces and also following recovery of the bacteria from the surfaces using the ISO method, it was demonstrated that although this work used the same bacterial suspension to perform all the incubation tests that the CFU/mL values of *S. aureus* and *E. coli* for the test with Fe, Mo and Zn were lower than with the other surfaces. This may be a condition of the bacterial species, the surface and the swabbing method used. Previous work in our laboratories has demonstrated that there can be inconsistencies in the number of bacteria recovered from different surfaces using such techniques as the swabbing method (Verran et al., 2010a,b,c).

A major finding in this study revealed that the titanium coating enhanced the growth of *L. monocytogenes* under humid conditions, using the BS ISO 22196:2011 standard method. This high affinity of the facultative anaerobic *L. monocytogenes* for titanium coatings may indicate that *L. monocytogenes* could survive on titanium coatings under particular conditions. To determine the effect that the titanium was having on the *Listeria* species, cells were incubated in TSB with 10 ppm, or 30 ppm of titanium ion solution. In conditions whereby the bacteria had the nutrients to grow, a significant increase of the bacterial concentration was demonstrated. However, at 50 ppm, the titanium ions had an inhibitory effect. It may be that under conditions of growth, the titanium metal ions are not inhibitory to the bacteria at low concentrations, whereas at greater concentrations of titanium, the metal may have an inhibitory or bactericidal effect. This would go some way to explain the contradictory explanations regarding the effect of titanium on bacterial growth whereby it has been demonstrated that titanium does have antimicrobial effects (Yoshinari et al., 2000, 2001; Shibata et al., 2004) and also that titanium was not antibacterial (Kawakami et al., 2008). An alternative explanation may be that the increase in bacterial numbers enabled the cells to deal with the antimicrobial effect of the ions. Since the leaching on the surfaces was low (1 ppm), it might be suggested that above 50 ppm the titanium does have an antimicrobial effect on the cells. Further, although dry conditions lower the survival rates of bacteria on surfaces (Kusumaningrum et al., 2003; Santo et al., 2011), the food industry often operates under humid conditions.

This affinity of *L. monocytogenes* for titanium surfaces under particular conditions will be the subject of further studies. Moreover, due to this finding, it might be suggested that surfaces should be thoroughly tested before use in the food industry.

5. Conclusions

The copper followed by the silver coatings demonstrated the greatest antimicrobial activity. However, under humid conditions, when the cells were incubated on the surface for 24 h, the titanium coating resulted in increased numbers of *L. monocytogenes*. This effect may be due to an optimum leaching rate (>30 ppm) being required before titanium inactivates the growth of bacterial cells.

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