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# Development of antibacterial silver treatments on HDPE nets for agriculture

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**ABSTRACT:** The defense mechanism of crops associated with the use of polymeric nets and fabrics is only physical and, hence, ineffective against the bacterial contaminations. The presence of an antibacterial agent associated with the use of conventional agro-textiles can represent a great advantage in the prevention of plant diseases and for food safety. The aim of this work was the development of antibacterial silver-coated HDPE nets for an innovative application such as agriculture. Antibacterial coatings on high-density polyethylene nets were obtained by a patented nanosilver deposition technique based on the *in situ* photo-reduction of a silver solution. The concentration of silver deposited was defined by testing different silver solutions from a biological point of view. Moreover, in order to improve the adhesion of the silver coating to the substrate, the nets underwent low-pressure plasma treatment before the silver deposition. The materials were characterized in terms of quality of the coating through scanning electron microscopy, and in terms of antibacterial capability on Gram positive and Gram negative bacteria through qualitative and quantitative microbiological tests. The most effective process parameters were defined and the importance of performing plasma pretreatment on this specific substrate was assessed. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41623.

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## INTRODUCTION

The control of pests, insects and plant diseases is crucial in ensuring healthy crops and enhancing agricultural productivity.[1] Even if some concerns are present, the pesticides are still widely used in agriculture. Some serious concerns associated to the use of pesticide are represented by the acquisition of pest resistance to chemicals, health hazard, possibility of chemical residues in food and environmental degradation.[2] The continuous innovation of polymeric technology and manufactured plastic has promoted the diffusion of plastic materials in the agricultural field.[3] In addition to the reduction of herbicide and pesticide consumption, the use of plastic nets also offers protection from rain, frost and wind.[4,5] The most widely used material for the production of agricultural nets is high-density polyethylene (HDPE).[4] However, only a physical barrier against the microorganisms is offered to plants by polymeric envelops.

The aim of this work was the development of advanced polymeric nets with antimicrobial properties, in order to reduce the use of chemicals in agriculture and to preserve the crops from contaminations associated to vector insects. High-density polyethylene (HDPE) is an extensively studied polymer with a relatively simple chemical structure and well-known bulk properties.[6] HDPE is the most common material used for agricultural nets because it is non-toxic, recyclable, waterproof and durable, and has good mechanical properties. Permeable covers are extensively used in certain types of cultivation such as fruit-tree farming for bird and insect protection.[4] Nets with holes of variable size can prevent the passage of different parasites, but they cannot protect agricultural crops from bacterial contamination associated to vector insects.[7,8] Indeed, the transmission of *E. coli* via insect vectors such as the fruit fly and the vinegar fly has been demonstrated.[9,10] However, the defense mechanism of crops associated with the use of polymeric nets and fabrics is only physical and, hence, ineffective against the bacterial contaminations. Recent concerns about the environmental impact of pesticides and the quality of food have stimulated the interest in novel approaches to preserve crop health.[11] Moreover, most of the pesticides do not exhibit a targeted action and their indiscriminate use can induce an alteration of the ecosystem.[12] The growing interest in different applications of silver has been reported in a wide range of fields, such as the modification of different natural and synthetic materials.[13] Efforts have been recently made by the researchers of the University of Salento (Lecce, Italy) on the identification of antibacterial substances with characteristics of good adhesion to the substrate and resistance to atmospheric agents for application in agriculture.

In particular, in this work, silver has been selected as antimicrobial agent for the prevention of bacterial contamination on agriculture nets because of the well-known antimicrobial properties[14] characterized by long-term activity against a broad spectrum of microorganisms.[15] HDPE nets have been deposited with silver nanoparticles by adopting a patented technology developed at the University of Salento[16] and based on the photo-reduction of a silver precursor. The technology consisted in dipping the HDPE nets in a silver solution and, then, in exposing the substrate to an ultraviolet source in order to induce the synthesis and deposition of the silver clusters on the surface of the material. The percentage of silver deposited has been selected by evaluating the antibacterial capability of the silver-treated nets on Gram positive and Gram negative bacteria. Moreover, in order to improve the adhesion of the coating to the substrate, an additional low-pressure cold plasma treatment was performed before the silver deposition. Argon is often used for plasma pretreatments to induce grafting[17,18] or cross-linking[19,20] of molecules in a polymer surface. Other applications are the removal of contamination or surface ablation to increase the surface roughness and for improved adhesion properties.[21] The effect of the argon flow on the durability of the coating was evaluated before and after washing the nets in tap water.

## EXPERIMENTAL

### Materials

HDPE nets for agriculture used in this research were provided by SACHIM S.r.l. (Putignano, Bari - Italy). These nets are transparent white and designed with hole dimensions of 390 × 770 micron to offer protection against the whitefly. The silver solution was prepared by adopting methanol ($CH_3OH$, Sigma Aldrich) as both solvent and reducing agent in the photochemical reduction. Silver nitrate ($AgNO_3$, Sigma Aldrich) was used as precursor for silver nanoclusters.

### Silver Deposition on HDPE Nets

The silver deposition technology adopted in this work is based on the *in situ* photo-reduction of silver nitrate. First, the nets were dipped in an alcoholic solution containing the silver precursor dissolved in methanol by magnetic stirring. To define the most appropriate composition of the silver solution in terms of antibacterial capability, three silver solutions containing respectively 0.1 wt/v %, 0.5 wt/v % and 1 wt/v % of silver nitrate dissolved in methanol were prepared. The wet substrates were exposed to UV lamp ($\lambda = 365$ nm, $t = 20$ min, distance 20 cm) in order to induce the synthesis of silver clusters on the surface of the nets. All the characterizations were performed after washings in tap water to avoid the presence of any trace of unreacted silver nitrate.

The HDPE nets were also subjected to low-pressure plasma treatment in order to improve the adhesion of the coating to the polymeric substrate. Plasma treatment (Plasma Solution Instruments) was performed under argon gas flow set at 100 $cm^3$ $min^{-1}$ with an electrical power setting of 300W and a dwell time of 20 min per side.

### Characterization

The effect of the percentage of silver deposited as well as the effect of plasma treatment before silver deposition were evaluated from a microbiological point of view through agar diffusion tests on *Escherichia coli* DH5(α) and *Staphylococcus aureus S1* according to Standard ‘SNV 195920-1992’. The samples were UV sterilized for 30 min and placed in contact with bacteria on agar plates in a petri dish. Then, samples of net (about 0.8 $cm^2$) were placed over bacteria and the dishes were incubated in oven at 37°C for 24 h. After this time, the inhibition area to bacteria growth was evaluated according to the levels of antibacterial capability indicated by the Standard. Thus, if the width of the bacteria free zone on the plate resulted larger than 1 mm, the antibacterial capability of the samples was labelled as “good”. If the sample resulted totally colonized by bacteria, its capability was labeled as “insufficient”. Moreover, the samples treated by adopting the silver solution selected (1 wt/v %) were also characterized through bacterial enumeration tests in order to quantify the percentage of antibacterial efficacy (ABE %). For both *E. coli* and *S. aureus*, 4 ml of Luria–Bertani (LB) broth were inoculated into sterile tubes with 1 ml of bacterial suspension. Each sample was introduced in the sterile tube and incubated at 37°C for 24 hours. After incubation, the samples were removed and serial dilutions were performed in LB broth. From each dilution, 100 μl were plated on agar plates for *E. coli* and on nutrient agar for *S. aureus*. The plates were then incubated at 37° C for 24 h and the number of bacterial colonies was counted. The number of viable bacteria was evaluated for each sample and the percentage of antibacterial efficacy (ABE %) was calculated according to the following equation[22]:

$$\text{ABE}\% = (V_c - V_t)/V_c \times 100$$

where $V_c$ and $V_t$ indicate respectively the number of colonies for control and silver-treated sample.

Moreover, in order to evaluate the effect of plasma treatment on the adhesion of the silver coating to the substrate, the nets treated with 1 wt/v % of silver were subjected to 10 washing cycles in tap water. Then, SEM (Zeiss EVO) and TGA (Mettler) analyses were performed before and after the washings to evaluate the presence and distribution of the silver particles on the substrate and to quantify the percentage of silver deposited. In order to evaluate the effect of plasma treatment on the polymeric surface of the net, samples of HDPE nets were also analyzed at higher magnifications through a Field Emission SEM (FESEM Merlin Zeiss) before and after argon plasma.

TGA was carried out by heating the untreated and silver treated samples from room temperature to 1200°C with nitrogen flow rate of 50 mL/min. The amount of silver deposited was calculated as a difference between the residual combustion products of the silver treated net and the untreated one. Moreover, energy dispersive X-ray spectroscopy EDX (Bruker – X flash detector 5010 Ev 127) was also adopted to confirm the presence

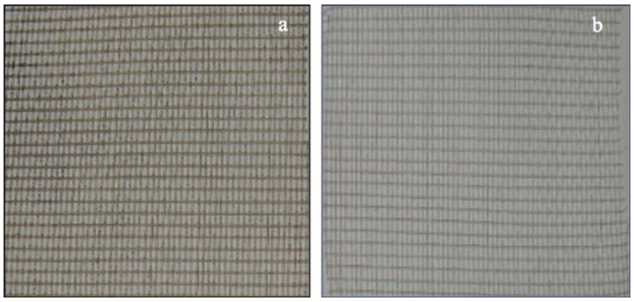


**Figure 1.** Visual comparison of silver treated HDPE net (a) and untreated HDPE net (b). The silver treated sample shows the typical darkening of the substrate due to the presence of the silver coating. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of silver on the surface of the samples treated with 1 wt/v % of silver and subjected to plasma treatment.

## RESULTS

To prevent the bacterial contamination of crops associated to insect vectors, HDPE nets were deposited with a nanometric layer of silver particles. The technology adopted for the silver treatment ensured successfully coated substrates. As visible in Figure 1, a slight darkening of the polymeric substrate is visible in comparison with the untreated sample. No discoloration area was observed on any treated sample.

Three different silver solutions containing 0.1, 0.5, and 1 wt/v % of silver nitrate were prepared and tested from a microbiological point of view. The results of agar diffusion tests on *Escherichia coli* and *Staphylococcus aureus* are reported in Figure 2(a,b), respectively. As expected, the untreated samples did not show antibacterial activity on any bacterial strain [Figure 2(a), Sample a; Figure 2(b), Sample a']. Samples treated with 0.1 wt/v % Ag [Figure 2(a), Sample b; Figure 2(b), Sample b'] and 0.5 wt/v % Ag [Figure 2(a), Sample c; Figure 2(b), Sample c'] demonstrated insufficient antibacterial activity, while 1 wt/v % Ag [Figure 2(a), Sample d; Figure 2(b), Sample d'] resulted effective in inhibiting the bacterial growth.

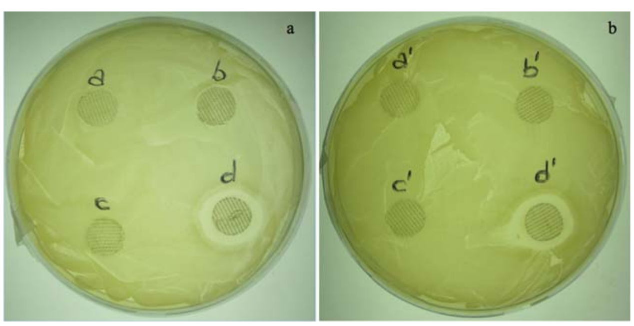


**Figure 2.** Agar diffusion tests on *E. coli* [Figure 2(a)] and *S. aureus* [Figure 2(b)]. Figure 2(a): untreated HDPE net (a); HDPE net treated with 0.1 wt/v % Ag (b); HDPE net treated with 0.5 wt/v % Ag (c); HDPE net treated with 1 wt/v % Ag (d). Figure 2(b): untreated net (a'); HDPE net treated with 0.1 wt/v % Ag (b'); HDPE net treated with 0.5 wt/v % (c'); HDPE net treated with 1 wt/v % Ag (d'). This test aimed to evaluate the effect of the percentage of silver on the antibacterial properties of the net. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

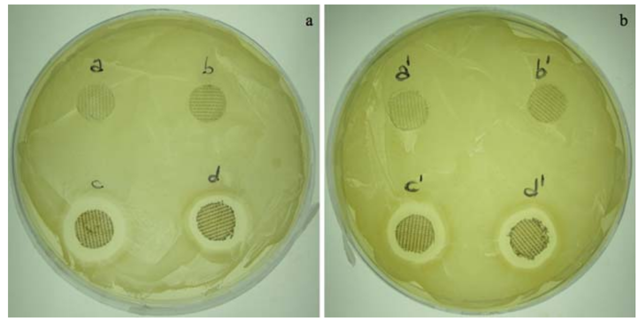


**Figure 3.** Agar diffusion tests on *E. coli* [Figure 3(a)] and *S. aureus* [Figure 3(b)]. Figure 3(a): untreated HDPE net (a); HDPE net treated with 1 wt/v % Ag and subjected to washings (b); plasma-treated HDPE net coated with 1 wt/v % Ag (c); plasma-treated HDPE net coated with 1 wt/v % and subjected to washings (d). Figure 3(b): untreated HDPE net (a'); HDPE net treated with 1 wt/v % Ag and subjected to washing (b'); plasma-treated HDPE net coated with 1 wt/v % Ag (c'); plasma-treated HDPE net coated with 1 wt/v % and subjected to washings (d'). This test aimed to evaluate the effect of plasma treatment on the adhesion of the silver coating to the substrate. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The results from agar diffusion tests performed on plasma-treated net coated with 1 wt/v % of silver are reported in Figure 3(a) for *E. coli* and in Figure 3(b) for *S. aureus*. As visible in Figure 3, an inhibition area to bacteria growth is visible only around the samples subjected to plasma treatment, before [Figure 3(a), Sample c; Figure 3(b), Sample c'] and after the washing cycles [Figure 3(a), Sample d; Figure 3(b), Sample d']. When plasma treatment was not performed, the washing cycles significantly affected the antibacterial capability of the materials [Figure 3(a), Sample b; Figure 3(b), Sample b'], thus indicating the lower adhesion of the coating to the substrate.

SEM images obtained by samples treated with 1 wt/v % of silver and subjected to the washing cycles are reported in Figure 4. When compared with the neat net [Figure 4(a)], a mild improved roughness is visible on the net after plasma treatment [Figure 4(b)]. A good distribution of silver particles is still visible on the samples subjected to plasma treatment [Figure 4(e,f)] before [Figure 4(e)] and after [Figure 4(f)] the washings. On the other hand, a reduced presence of silver particles on the net is visible after washings [Figure 4(d)] where plasma treatment was not performed [Figure 4(c,d)].

To demonstrate the effect of argon plasma on the polymeric substrate, SEM analysis was also performed on the untreated net and on plasma-treated net at higher magnifications (41KX) by using a field emission scanning electron microscope FESEM. The results are reported in Figure 5, where a different roughness is clearly visible on plasma treated sample [Figure 5(b)] when compared with the untreated one [Figure 5(a)].

Thermo-gravimetic analysis allowed the quantification of the silver amounts deposited on the substrates and also the evaluation of the durability of coating. The results of TGA analysis are collected in Table I. After the washings, the loss of silver

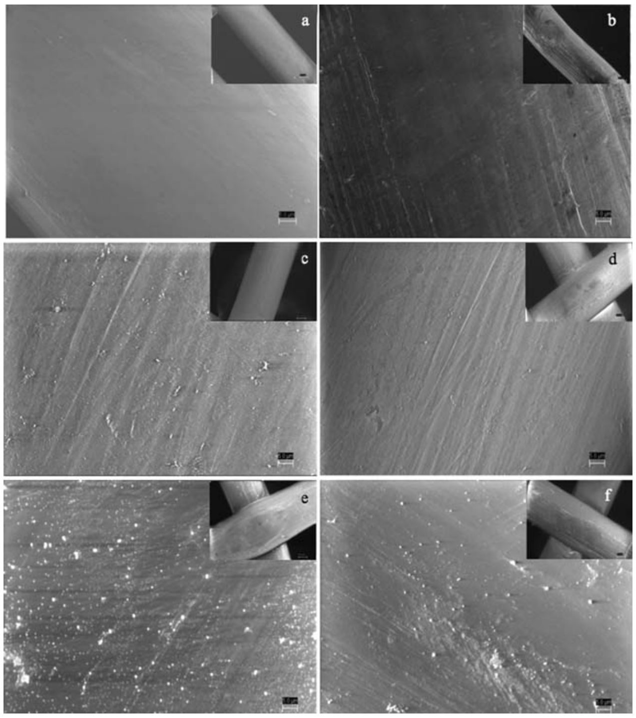


**Figure 4.** SEM analysis at 3000 magnifications on untreated HDPE net (a); HDPE net subjected to plasma treatment (b); HDPE net coated with 1 wt/v % Ag (c); HDPE net coated with 1 wt/v % Ag and subjected to washings (d); plasma-treated HDPE net coated with 1 wt/v % (e); plasma-treated HDPE net coated with 1 wt/v % Ag and subjected to washings (f). Insets of figures show the samples at lower magnifications (500×). This analysis aimed to evaluate the effect of plasma treatment on the adhesion of the silver particles to the substrate.

resulted 85.29% when plasma treatment was not performed and 5.33% when the nets were subjected to plasma treatment before the silver deposition, thus confirming again the efficacy of the argon flow in improving the stability of the silver coating on this specific substrate.

EDX analysis also confirmed the presence of silver on the treated sample subjected to argon flow and to washings, as visible in the graph reported in Figure 6(b,c) where the presence of the silver peak is visible in the expected position before and after the washings. The amount of silver deposited after photo-reduction and evaporation of solvent were calculated by EDX and resulted 1.44% before washings and 1.41 after washings. No silver peak is visible in the same position indicated in the graph reported in Figure 6(a) for the untreated net. Other peaks corresponding to the elements of the substrate such as carbon are also visible.

The antibacterial efficacy of these samples was also evaluated through quantitative antibacterial tests on *E. coli* [Figure 7(a,b)] and *S. aureus* [Figure 7(c,d)]. In Figure 7 representative pictures obtained by the bacteria enumeration tests are reported. A significant reduction in colony number is clearly visible for the silver-treated samples [Figure 7(b,d)] in comparison with the untreated samples [Figure 7(a,c)] for both the bacteria strains. The antibacterial efficacy resulted 99% and 90% respectively for *E. coli and S. aureus.*

## DISCUSSION

The broad-spectrum antimicrobial properties of silver have long been recognized, particularly in form of nanoparticles due to the high surface to volume ratio.[23]

In this work an innovative application for silver coatings on polymeric substrates has been proposed. HDPE nets are commonly used in agriculture to protect the crops from insects and atmospheric agents, but silver coatings on HDPE nets have never been proposed before as antimicrobial agent for plant protection.

The silver-coated nets were developed as an external protection from bacterial contamination associated to vector insects, without any contact between the coating and the plant. The silver deposition technology adopted in this work, extensively applied to different natural and synthetic materials,[24,25] is based on the *in situ* photo-reduction of a silver salt and provides the synthesis/deposition of silver nanoparticles.[16] This technology does not require the use of any binder and nor complex techniques of deposition or vacuum evaporation systems, thus facilitating the process in terms of cost and time. Moreover, the silver deposition process can be easily integrated in the industrial process for the production of the HDPE nets, thus reducing again the costs for the treatment. Another advantage associated to the presented technology lies in the process itself. As the silver deposition process requires the UV exposure of the material, sunny

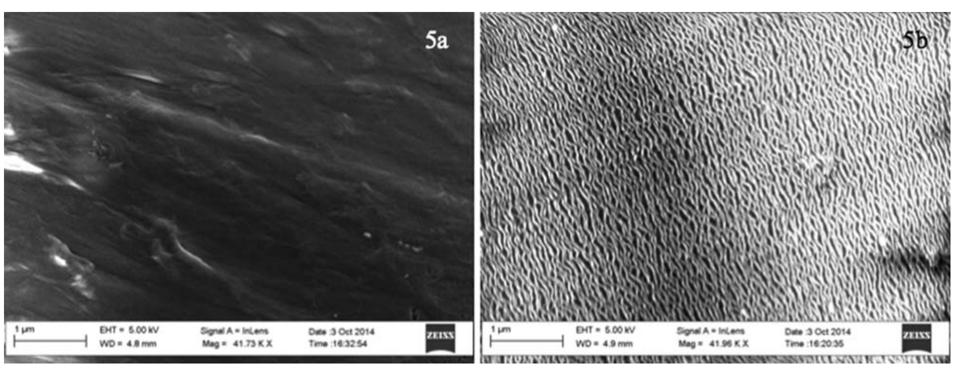


**Figure 5.** SEM analysis at 41KX on untreated net (a) and HDPE net subjected to plasma treatment (b). This analysis aimed to demonstrate the effect of plasma treatment on the surface of the polymeric substrate.

**Table I.** Evaluation of the Effect of Plasma Treatment on the Adhesion of the Coating to the Substrate

| | wt % Ag before washings | wt % Ag after washings | $\frac{\text{wt \% Ag (beforewashings)} - \text{wt \% Ag (afterwashings)}}{\text{wt \% Ag (beforewashings)}} \times 100$ |
|---|---|---|---|
| Ag/HDPE net | 0.68 | 0.10 | 85.29% |
| Plasma-treated Ag/HDPE net | 0.75 | 0.71 | 5.33% |

The table reports the mean values of silver percentage on the nets and the loss of silver calculated by TGA analysis after washings.

weather conditions cannot affect the treated materials. Even if previous studies demonstrated that this technology applied to polymeric materials resulted in the good adhesion of the silver coating to the substrate even after prolonged time in contact with flowing aqueous solutions,[26,27] in this study plasma treatment resulted necessary to ensure long lasting antibacterial properties. Indeed, plasma has been recognized as effective in modifying the chemical/physical properties without affecting the bulk characteristics of the materials, and also in modifying the wetting and adhesive properties of various polymers.[28] On the HDPE nets used in this work, improved roughness of the substrate was expected after plasma pretreatment with argon, with a consequent improvement in the adhesion of the coating to the polymer.

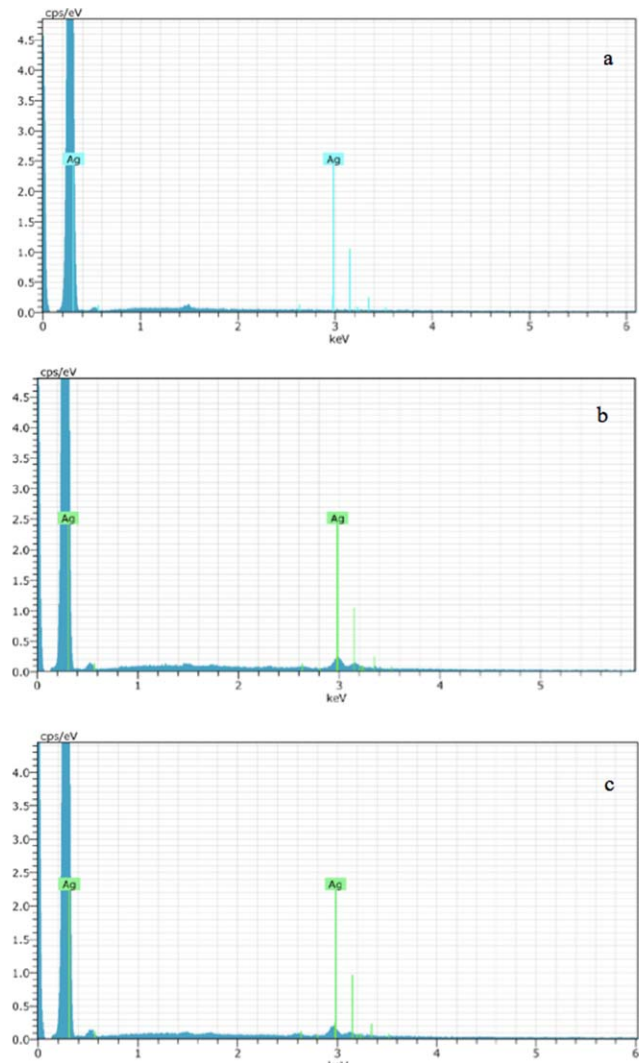


**Figure 6.** EDX analysis on the untreated HDPE net (a), plasma-treated HDPE net coated with 1 wt/v % Ag (b) and on plasma-treated HDPE net coated with 1 wt/v % Ag and subjected to washings (c). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In previous studies the dimension of the silver nanoparticles obtained by adopting the presented technology was investigated.[26] Particles with dimension about 2 nm were observed though transmission electron microscopy (TEM) on the silver solution deposited on copper grid; while particles of 20 nm locally aggregated in bigger clusters were observed on polymeric substrates through SEM analysis.[26] The research activities described in this work aimed to determine the most appropriate silver solution as well as the influence of the additional plasma treatment on the silver coating deposited. At this purpose, three concentrations of silver (namely 0.1 wt/v %, 0.5 wt/v % and 1 wt/v %) were tested from a microbiological point of view (Figure 2). Only the samples treated with the solution containing 1

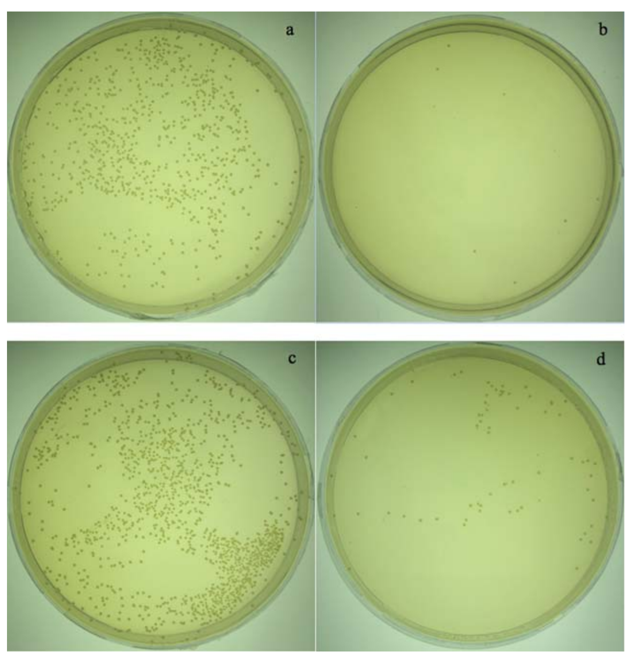


**Figure 7.** Representative pictures from bacterial enumeration test on *E. coli* (a, b) and *S. aureus* (c, d). Bacterial colonies grown in presence of untreated HDPE nets (a, c) and plasma-treated HDPE nets coated with 1 wt/v % Ag (b, d). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**Table II.** Antibacterial Efficacy (ABE %) of Plasma-Treated Samples Coated with 1 wt/v % Ag on *E. coli* and *S. aureus* Calculated Through Bacterial Enumeration Tests

| Bacterial | Materials | CFU after 24 H | ABE (%) |
|---|---|---|---|
| *E. coli* | Untreated sample | $5.7 \times 10^8$ | 99 |
| | Ag/ HDPE net | $3 \times 10^6$ | |
| *S. aureus* | Untreated sample | $7.6 \times 10^8$ | 90 |
| | Ag/ HDPE net | $7.3 \times 10^7$ | |

wt/v % of silver nitrate demonstrated good antibacterial activity on representative Gram positive and Gram negative bacteria strains [Figure 2(a), Sample d; Figure 2(b), Sample d'], a zone of inhibition of bacteria proliferation larger than 1 mm being visible around these samples. For this reason, the study on the influence of plasma on the silver coating was evaluated only on samples treated with 1 wt/v % of silver. In order to verify the effect of plasma treatment on the adhesion of the silver coating to HDPE, a set of samples was exposed to the additional argon flow before the silver deposition. Then, 10 washing cycles were performed on all the silver-coated samples. After washings, the samples were characterized in terms of antibacterial properties [Figure 3(a,b)] and morphology [Figure 4(a–e)]. As visible in Figure 3, the antibacterial capability of the silver treated samples resulted significantly reduced by washings when plasma treatment was not performed [Figure 3(a), Sample b; Figure 3(b), Sample b']. On the other hand, no difference in the width of the bacteria inhibition area around the samples exposed to plasma can be observed before [Figure 3(a,b), Samples c, c'] and after the washing cycles [Figure 3(a,b), Samples d, d'].

SEM analysis confirmed these results [Figure 4(a–f)]. Even if no macroscopic evidence of modification was observed after plasma treatment, the net subjected to argon flow [Figure 4(b)] exhibited a mild improved roughness in comparison with the neat net [Figure 4(a)]. A good distribution of the silver particles locally aggregated in large clusters can be observed on the silver-treated sample [Figure 4(c)], while a larger amount of silver particles is visible on the silver-treated net exposed to plasma [Figure 4(e)]. The washing cycles affected the silver coating on the nets not subjected to plasma treatment, as visible in Figure 4(d) where the coverage of the HDPE fibers resulted reduced. A good distribution of silver particles is still visible in Figure 4(f), thus confirming the effectiveness of plasma pretreatment on the specific substrate studied in this work. Moreover, SEM pictures reported in Figure 5 at higher magnification for the untreated net [Figure 5(a)] and plasma-treated net [Figure 5(b)] clearly demonstrate that argon plasma had a significant effect on the surface roughness of the polymer, thus improving the adhesion of the silver coating to the substrate.

As visible in Figure 6 where the position of the silver peak is indicated in the graphs, the EDX analysis confirmed the presence of silver on the plasma-treated net coated with silver, before and after washings [Figure 6(b,c)] in comparison with the untreated net [Figure 6(a)].

The loss of silver due to the washing cycles was calculated through thermogravimetric analysis (TGA) and the results are reported in Table I. The percentage of silver removed from the substrates with and without plasma treatment resulted, respectively, 5.33 wt % and 85.29 wt %.

The evaluation of the antibacterial efficacy (ABE %) performed through the serial dilution method on *E. coli* and *S. aureus* is reported in Figure 7 and Table II. A significant difference in the number of colonies was observed between the treated and the untreated samples, thus confirming the strong antibacterial activity of the treated samples [Figure 7(b,d)] against Gram-negative and Gram-positive bacteria compared to untreated samples [Figure 7(a,c)]. All the microbiological characterization demonstrated the good efficacy of the silver deposition technology adopted in this work in reducing the microbial colonization of the nets, and indicated that the silver-coated nets could be proposed as an interesting alternative to the common strategies adopted to preserve crops from contaminations. Moreover, the costs of the silver treatment can be considered low in terms of chemicals adopted and equipment required for the process.

## CONCLUSIONS

In this work an innovative application for silver has been proposed, such as the development of antimicrobial HDPE nets for agriculture as protection of crops from microorganisms associated to vector insects. The ease of the silver deposition and the good adhesion of the silver coating to the substrate are distinctive features of the technology adopted. In this work, 1 wt/v % of silver was defined as the lowest percentage necessary to inhibit bacteria proliferation and also an additional low-pressure plasma treatment was required to improve the stability of the coating on this specific substrate. The silver-coated nets demonstrated good antimicrobial activity against both Gram-positive and Gram-negative bacteria, so they can represent an interesting alternative to reduce the use of pesticides as a defense against parasites, bacteria, and insects. Moreover, many benefits in terms of food safety, water quality, worker, and environment safety could be obtained. Future works aim to compare the results obtained with experiments on commercial pesticide and herbicide.

## ACKNOWLEDGMENTS

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