Silver nanoparticles misting - an innovative method of archaeological object disinfection

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Modern approach for biodeterioration assessment and disinfection of historical book collections

Silver nanoparticles application

- filters for air purification
- filters for water purification
- stomatology
- dietary supplements
- implants
- textiles
- cosmetics, detergents
- home appliance
- computer hardware
- coatings, grouts, adhesives
- food packaging
- papermaking
- disinfection of historical object





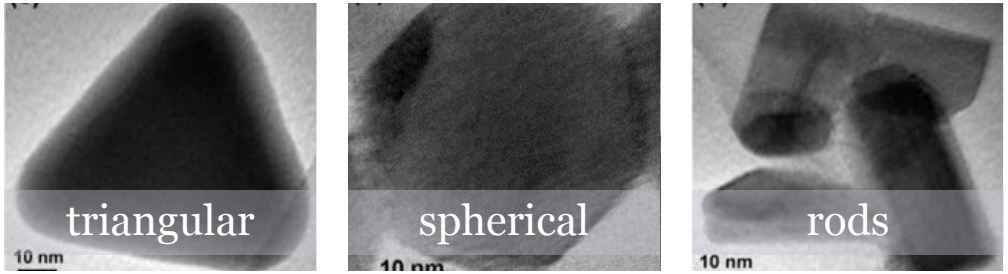


Chaloupka et al., 2010; Gutarowska et al., 2012

Silver nanoparticles (AgNPs)

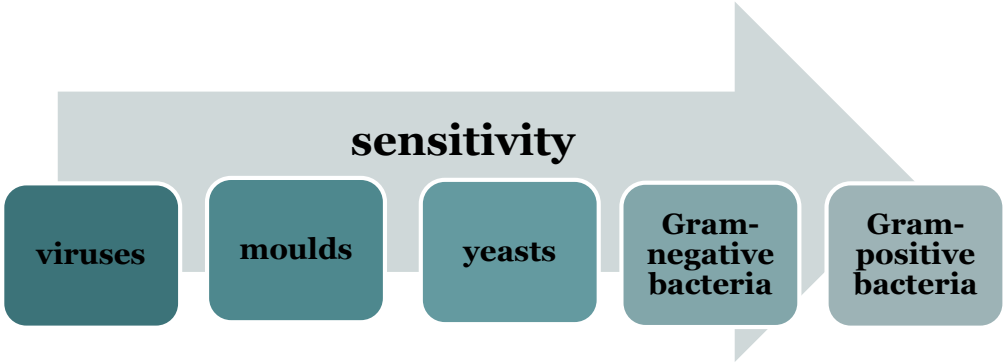
Synthesis

- chemical
- physical
- biological

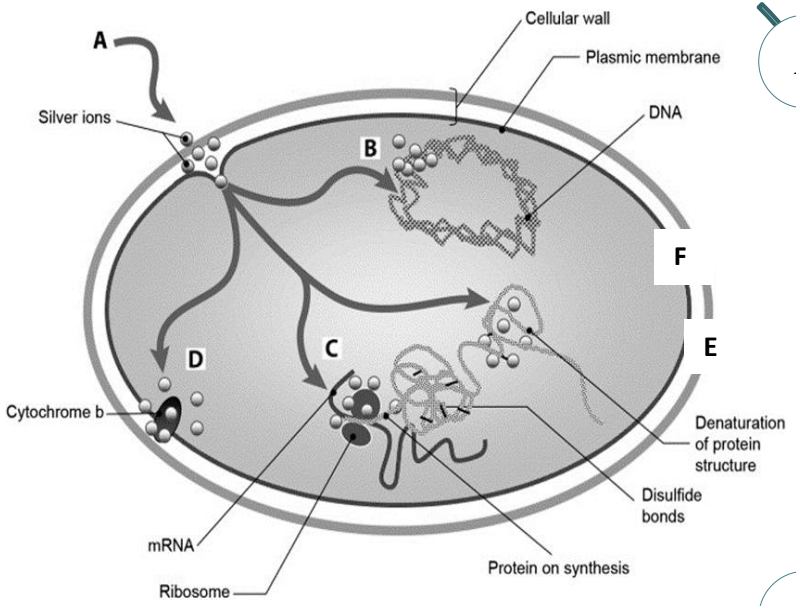


Effectiveness

- shape
- form
- size
- concentration
- microorganisms



Mechanism of antibacterial action of AgNPs



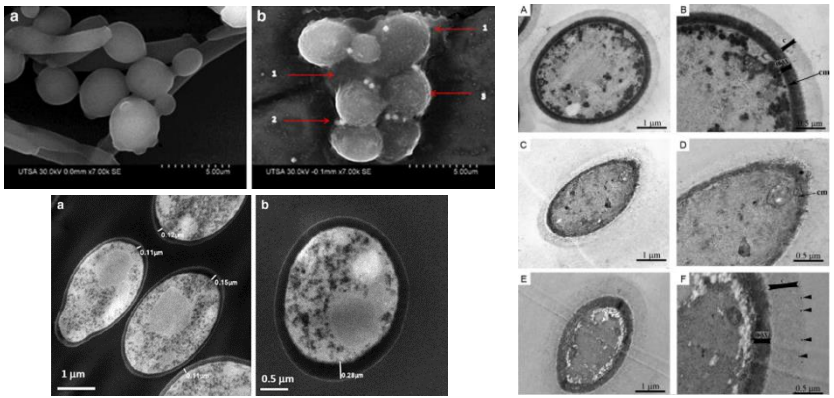
- **A** — disfunction of transport to the cell
- **B** — inhibition of DNA replication
- **C** — loss of the biological activity of the amino acid
- **D** — structural and functional changes in the cell membrane
- **E** — disturbance of the cell membrane electric potential
- **F** — outflow of protons and certain metabolites through the cytoplasmic membrane

Wzorek, Konopka, 2007; Feng et al., 2000

Mechanism of antifungal action of AgNPs

Yeasts

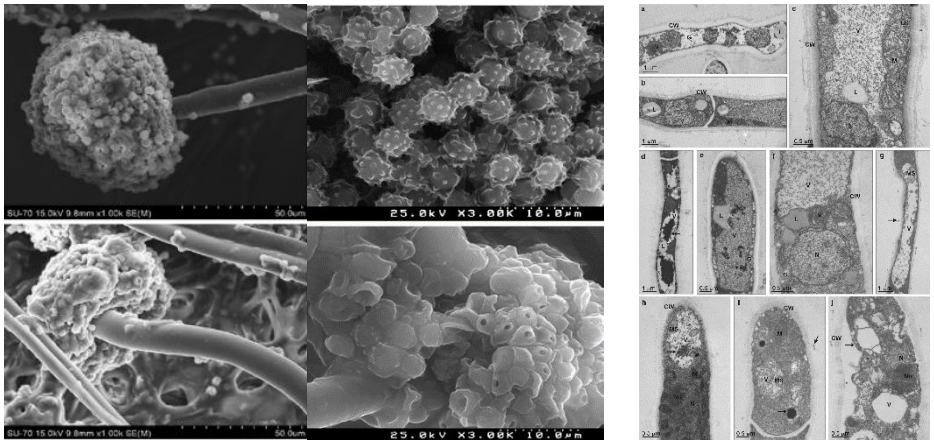
- changes of cell wall functions and structure
- creation of "pits" in the cell membrane
- disturbances of electric potential
- inhibition of budding process



Candida albicans and *Cryptococcus neoformans* after AgNPs treatment

Moulds

- inhibition of sporulation process
- changes in cell ultrastructure
- changes in metabolome (acids, enzymes, proteins)



Aspergillus niger after AgNPs treatment

Pinto et al., 2013: Ishida et al., 2014; Lara et al., 2015; Pietrzak et al., 2016

Aim and scope

Optimalization of AgNPs misting disinfection and its influence on microorganisms and disinfected materials.

1. Determination of the sensitivity of pure culture collection microorganisms and isolates from historical objects surfaces on the silver nanoparticles preparation.

2. Parameters optimization of the of effective disinfection of technical and historical materials with silver nanoparticles misting.

3. Assessment of the effectiveness and durability of disinfection depending on the microorganism, technical or historical material.

4. Analysis of the disinfection influence on mechanical and optical parameters of technical materials before and after artificial ageing.

Materials

Technical:

- paper* (Sa, Hmp, Sy, CTMP, GW)
- leather (cowhide, dyed)
- textiles (wool, silk, cotton, linen)
- wood (beech, oak, pine)

Historical:

- canvas (historical painting)
- parchment (conservation materials)
- wood (church floor; 17th c.)
- paper (map; 18th - 19th c.)
- textiles (wool, cotton, sisal; 13th – 15th c.)
- ceramics (6th – 13th c.)

Wool

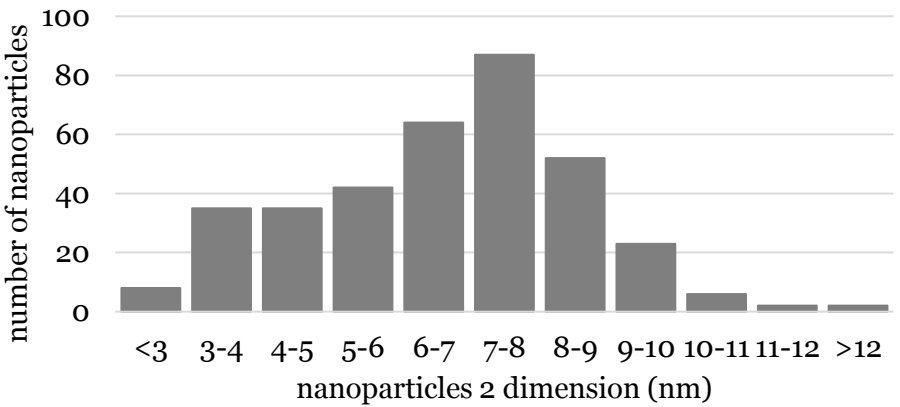
Ceramics

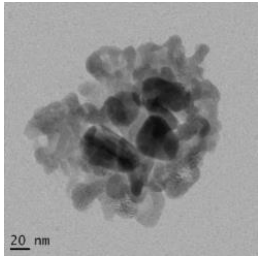
Cotton

*Sa - bleached pine kraft pulp; Hmp - bleached hemp kraft pulp; Sy - bleached spruce sulphite pulp; CTMP - chemi–thermomechanical pulp; GW - bleached groundwood from spruce (GW)

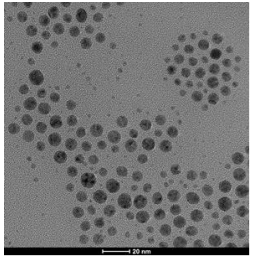
Silver nanoparticles

AgNPs 1	AgNPs 2
chemical reduction $AgNO_3$; reductor: sodium citrate; stabilizer: PVP	thermal decomposition of silver compounds; stabilizer: paraffins
concentration: 90 ppm	concentration: 1000 ppm
particle size: 10-80 nm (10-15 nm – 60-70%; 50-80 nm – 30-40%)	particle size: 3-8 nm
Mennica Polska S.A.	Amepox sp. z o.o.





SEM image of nanosilver 1



TEM image of nanosilver 2

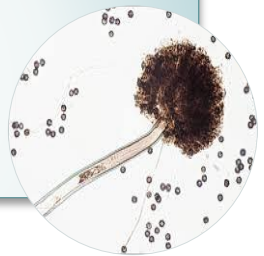
Microorganisms

BACTERIA

- *Aneurinibacillus aneurinilyticus*
- *Brevibacillus laterosporus*
- *Bacillus subtilis*
- *Bacillus megaterium*
- *Bacillus pumilus*
- *Bacillus licheniformis*
- *Sphingomonas paucimobilis*
- *Micrococcus* sp.
- *Micrococcus flavus*
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Staphylococcus lentus*
- *Staphylococcus xylosus*
- *Nocardia* sp.
- *Escherichia coli* (ATCC 10536)
- *Staphylococcus aureus* (ATCC 6538)
- *Bacillus subtilis* (NCAIM 01644)

FUNGI

- *Alternaria alternata*
- *Aspergillus versicolor*
- *Aspergillus niger*
- *Cladosporium cladosporioides*
- *Cladosporium herbarum*
- *Cladosporium macrocarpum*
- *Mucor racemosus*
- *Penicillium digitatum*
- *Penicillium carneum*
- *Penicillium crustosum*
- *Penicillium radicola*
- *Rhizopus nigricans*
- *Candida sphaerica*
- *Rhodotorula* sp.
- *Rhodotorula mucilaginosa*
- *Aspergillus niger* (ATCC 16404)
- *Penicillium chrysogenum* (ŁOCK 0531)
- *Candida albicans* (ATCC 10231)



ATCC – American Type Culture Collection; NCAIM - National Collection of Agricultural and Industrial Microorganisms; ŁOCK – Łódzki Ośrodek Czystych Kultur (Pure Culture Collection at Institute of Fermentation Technology and Microbiology, Lodz University of Technology)

Methods

Analysis	Method / Standard
Silver nanoparticles	
Characterization of preparation	SEM; TEM
Determination of AgNPs content	FAAS; LA-ICP-TOF-MS
Microorganisms	
Determination of microbial sensitivity (MIC and MBC)	ASTM E2149 – 01
Choosing the conditions for disinfection Evaluation of the effectiveness and durability of the disinfection technical materials and historical objects	AATCC Test Method 100-2012
Materials	
Assessment of mechanical parameters (breaking, elongation, tear, compression)	ISO 13934-1:1999 (textiles); ISO 3376:2005 (leather); ISO 20187:1993 (paper); ISO 1924-1:1998 (paper); BS EN 21974:2002 (paper); BS EN 384:2004 (wood)
Estimation of colour parameters	Metoda Tappi T 524 om-94 (paper); ISO 2470:1999 (paper); ISO 105-J01:2002 (leather, textiles); Metoda SCI (wood)

SEM – Scaning Electron Microscopy; TEM – TransmissionElectron Microscopy; AATCC - American Association of Textile Chemists and Colorists; ASTM - American Society for Testing and Materials; FAAS – Flame Atomic Absorbance Spectrometry; LA-ICP-TOF-MS - Laser Ablation Inductively Coupled Plasma Time-of- Flight Mass Spectrometry; MIC – Minimnal Inhibitory Concentration; MBC – Minimal biocidal Concentration; SCI - Specular Component Included

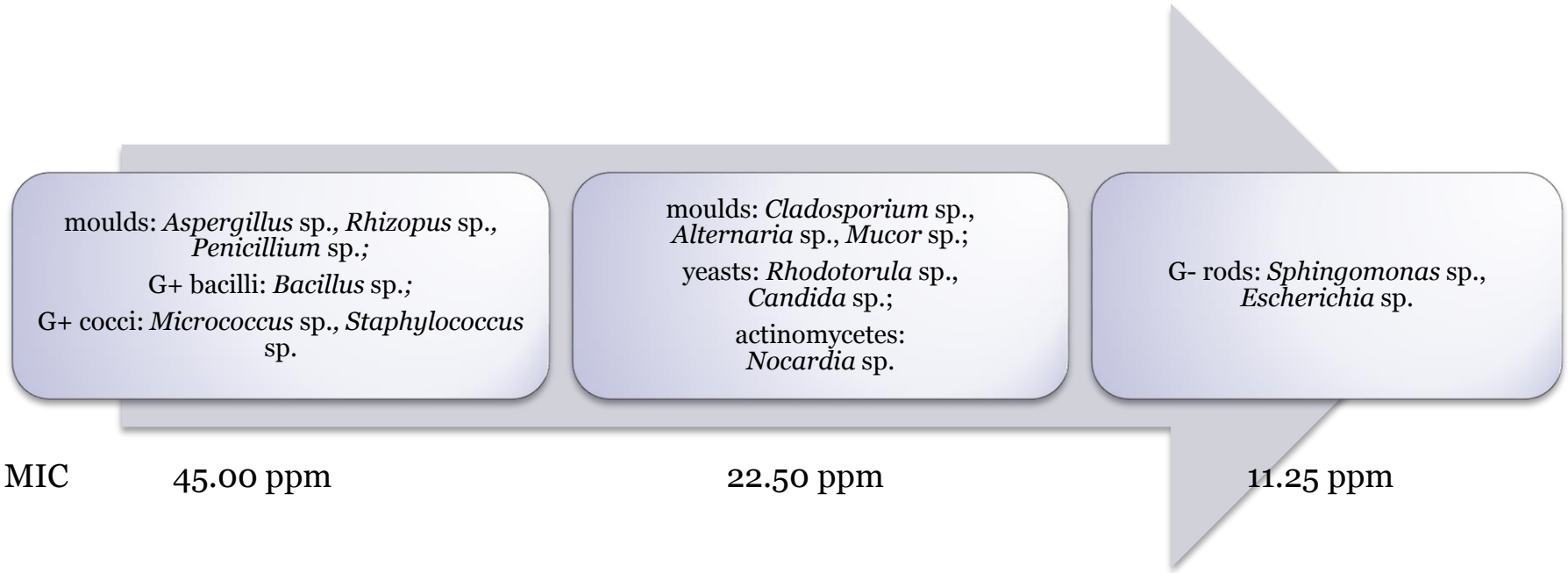
Microbial sensitivity to AgNPs

Microorganisms	Origin	MIC (ppm)	MBC (ppm)
Staphylococcus aureus	ATCC	11.25	22.50
	Museum 1	22.50	45.00
Bacillus subtilis	ATCC	22.50	> 45.00
	Archives	22.50	> 45.00
	Museum 2	45.00	> 45.00
Escherichia coli	ATCC	11.25	22.50
Nocardia sp.	Library	11.25	22.50
Aspergillus niger	ATCC	22.50	45.00
	Museum 2	45.00	45.00
Candida albicans	ATCC	11.25	22.50
Candida sphaerica	Library	22.50	22.50

MIC – Minimal Inhibitory Concentration; MBC - Minimal Biocidal Concentration; ATCC – American Type Culture Collection

Gutarowska et al., 2012

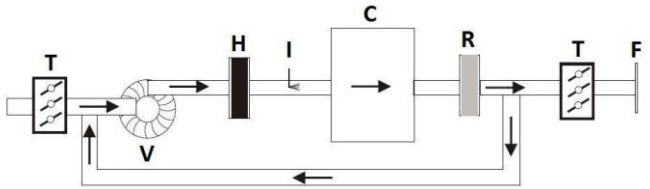
Microbial sensitivity to AgNPs



Gutarowska et al., 2012

AgNPs misting chamber

Capacity: 1.73 m³ → 36-42 books A4



T – throttling valve; V – ventilator; H – heater;
I – silver colloid injection; C – chamber; R – radiator; F - filter

Wasiak R., Laskowski Z., Czyżyk J. The microbiological protection method of archive and museum objects and installation for the microbiological protection of archive and museum objects. Patent PL399507, Poland 2012

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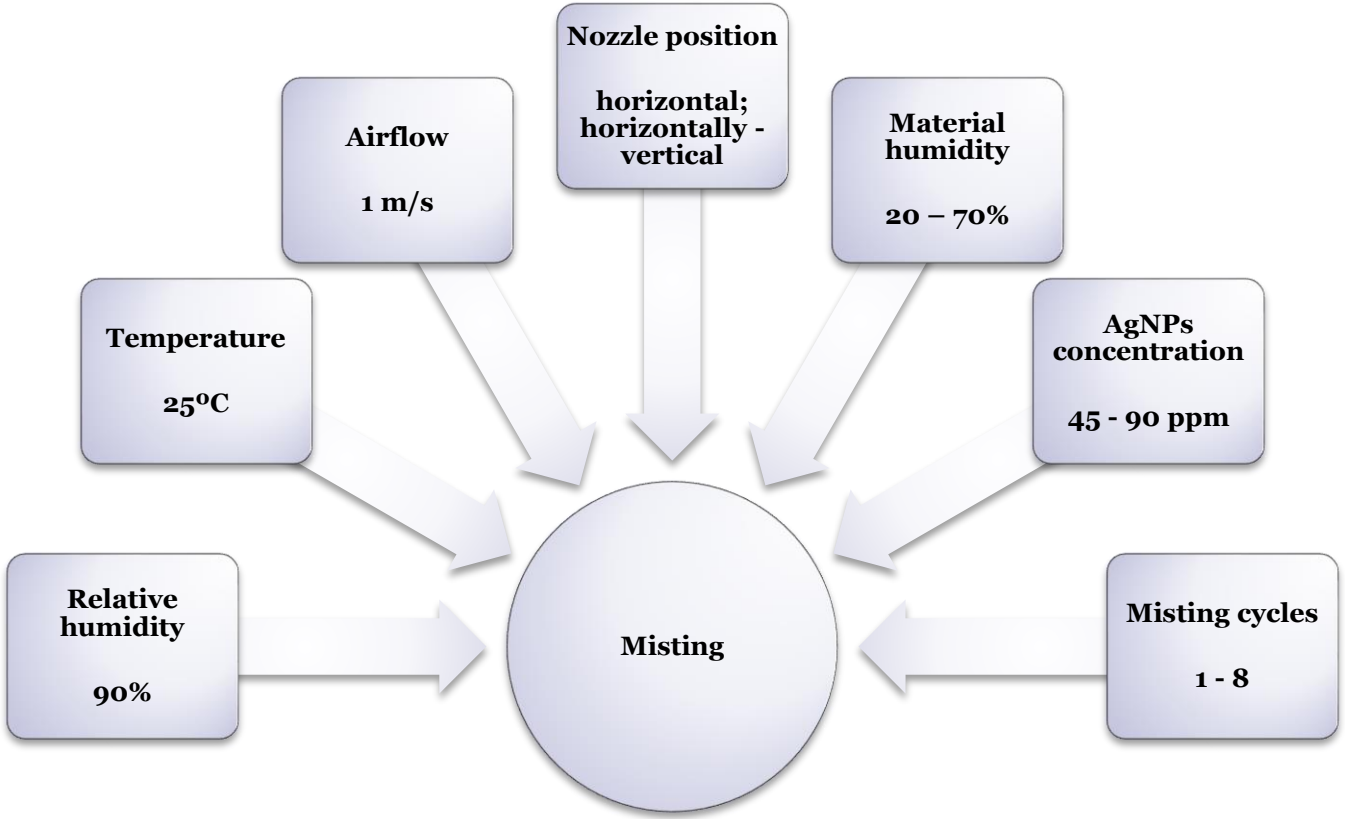
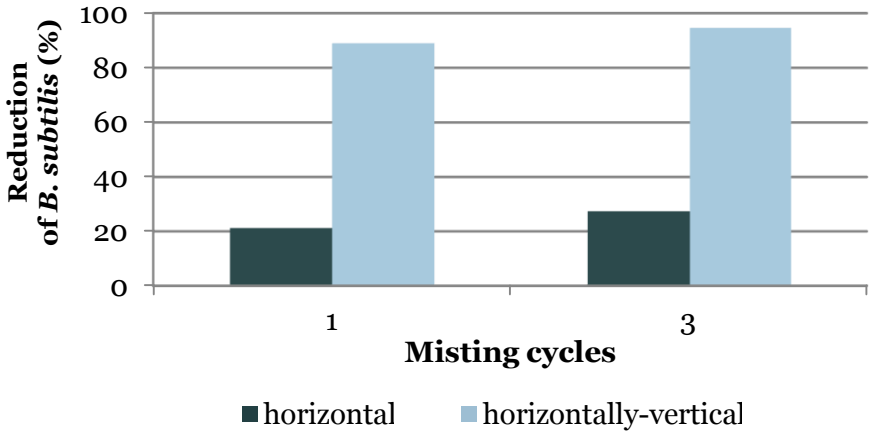
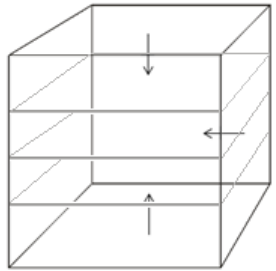
Choosing the conditions for AgNPs misting



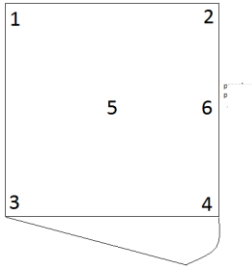
Gutarowska et al., 2012

Nozzle position and AgNPs distribution





Nozzle position (horizontally – vertical)



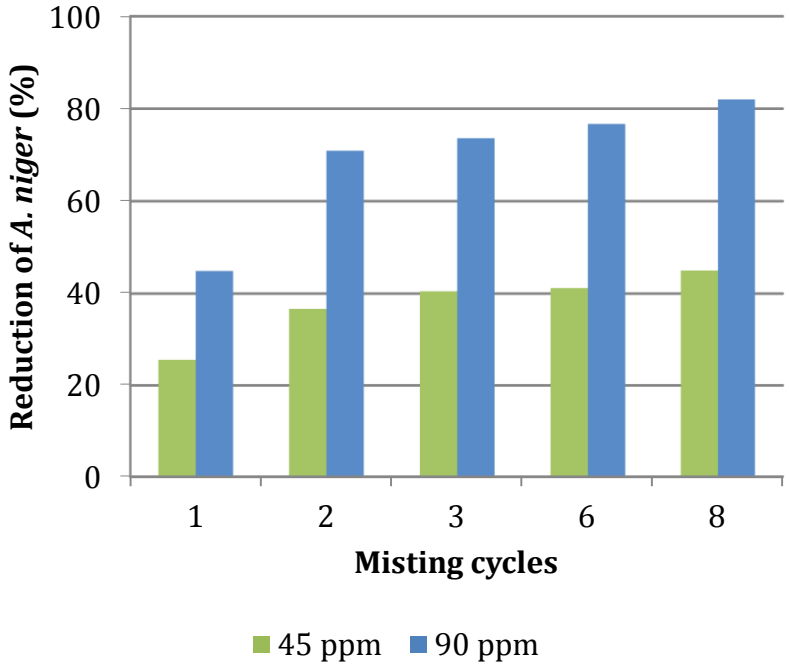
Sample points distribution

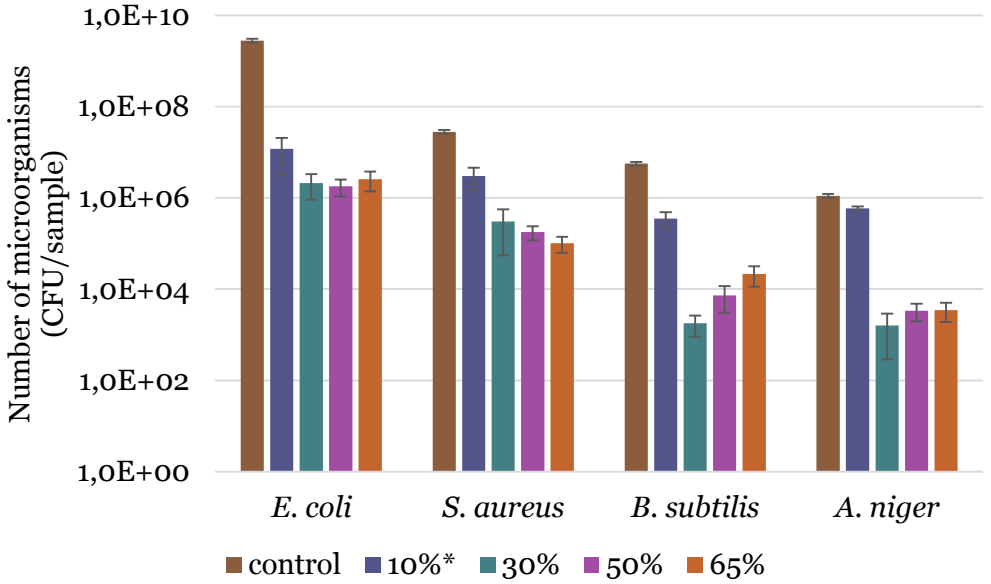
Sampling point	AgNPs amount (ppm/g)*
1	2.8
2	2.2
3	3.3
4	2.7
5	2.9
6	2.3

* FAAS – Flame Atomic Absorbance Spectrometry

Gutarowska et al., 2012

Misting cycles, AgNPs concentration, material humidity

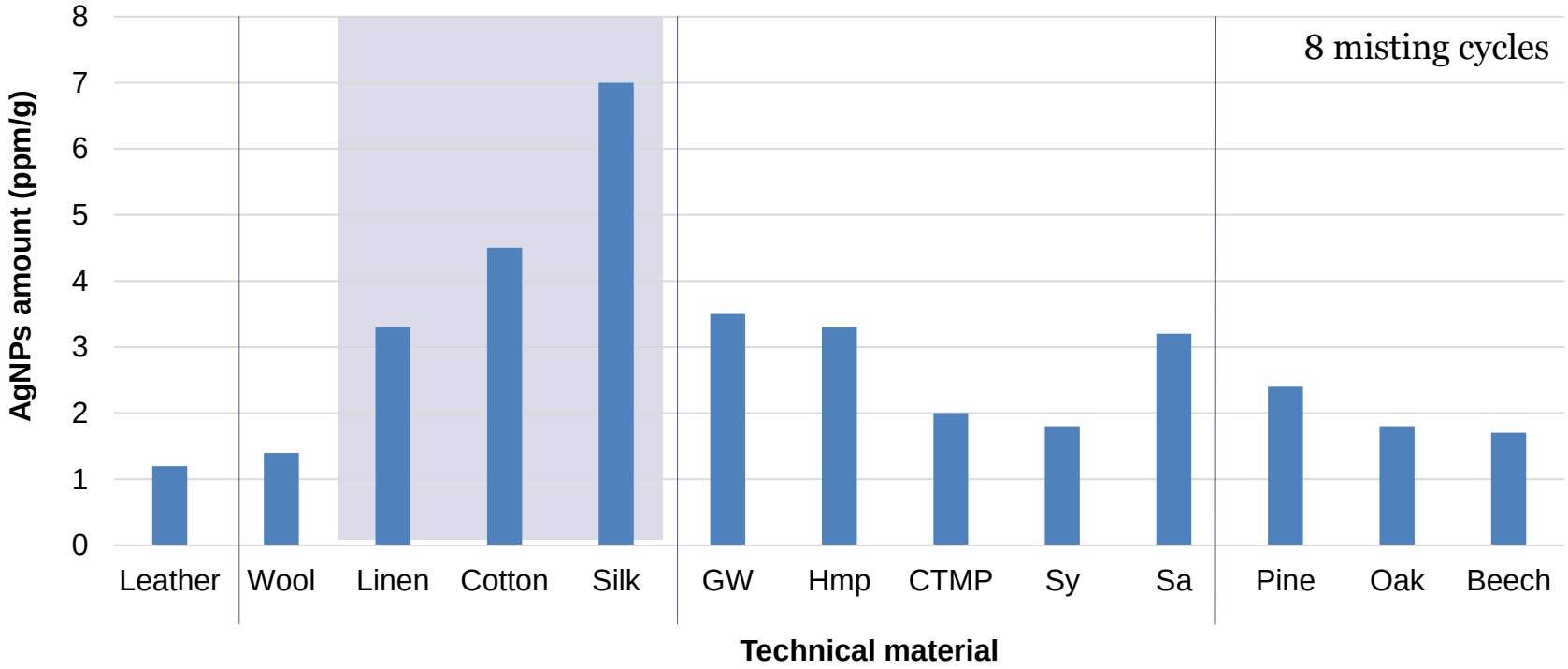




10% - dry cotton

Gutarowska et al., 2012; 2014

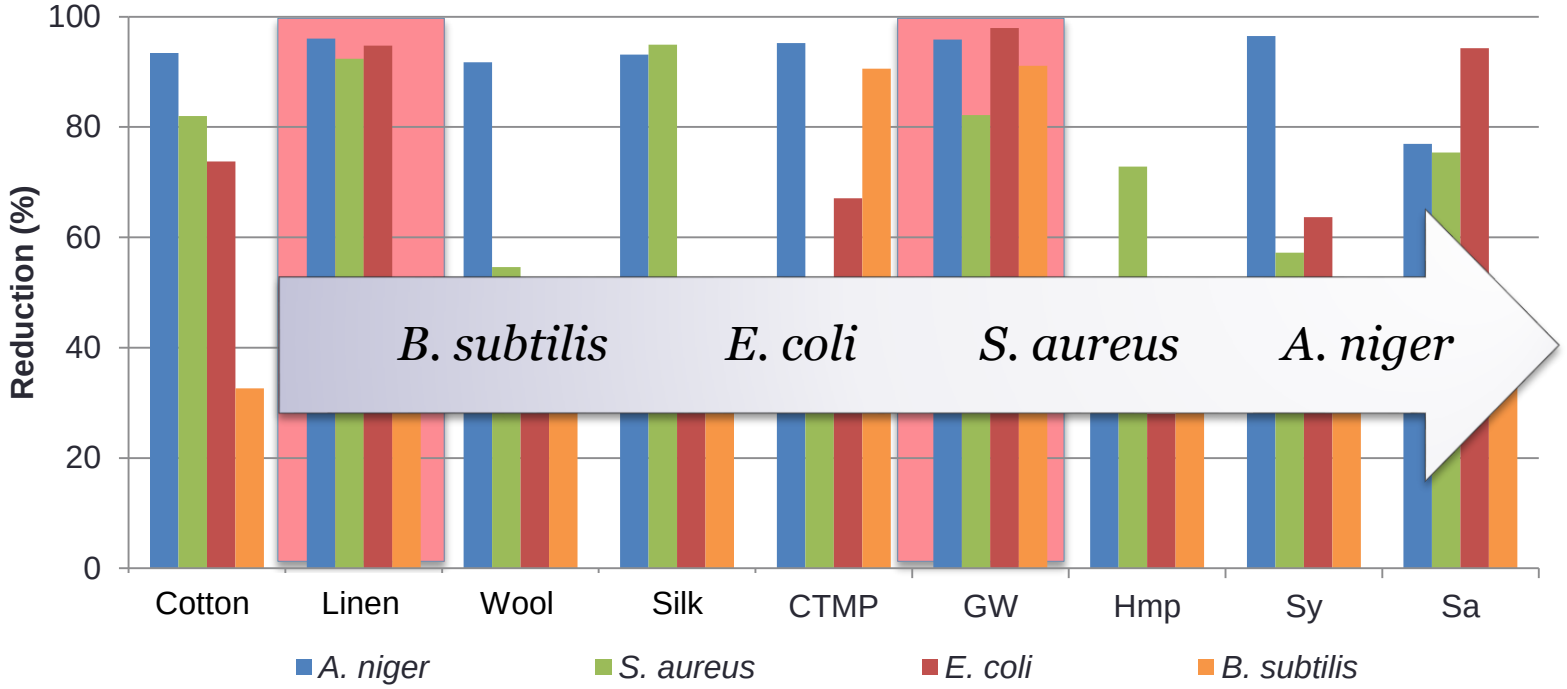
Silver nanoparticles amount by FAAS



FAAS – Flame Atomic Absorbance Spectrometry; Sa - bleached pine kraft pulp; Hmp - bleached hemp kraft pulp; Sy - bleached spruce sulphite pulp; CTMP - chemi–thermomechanical pulp; GW - bleached groundwood from spruce (GW)

Gutarowska et al., 2014

Disinfection effectiveness on technical materials



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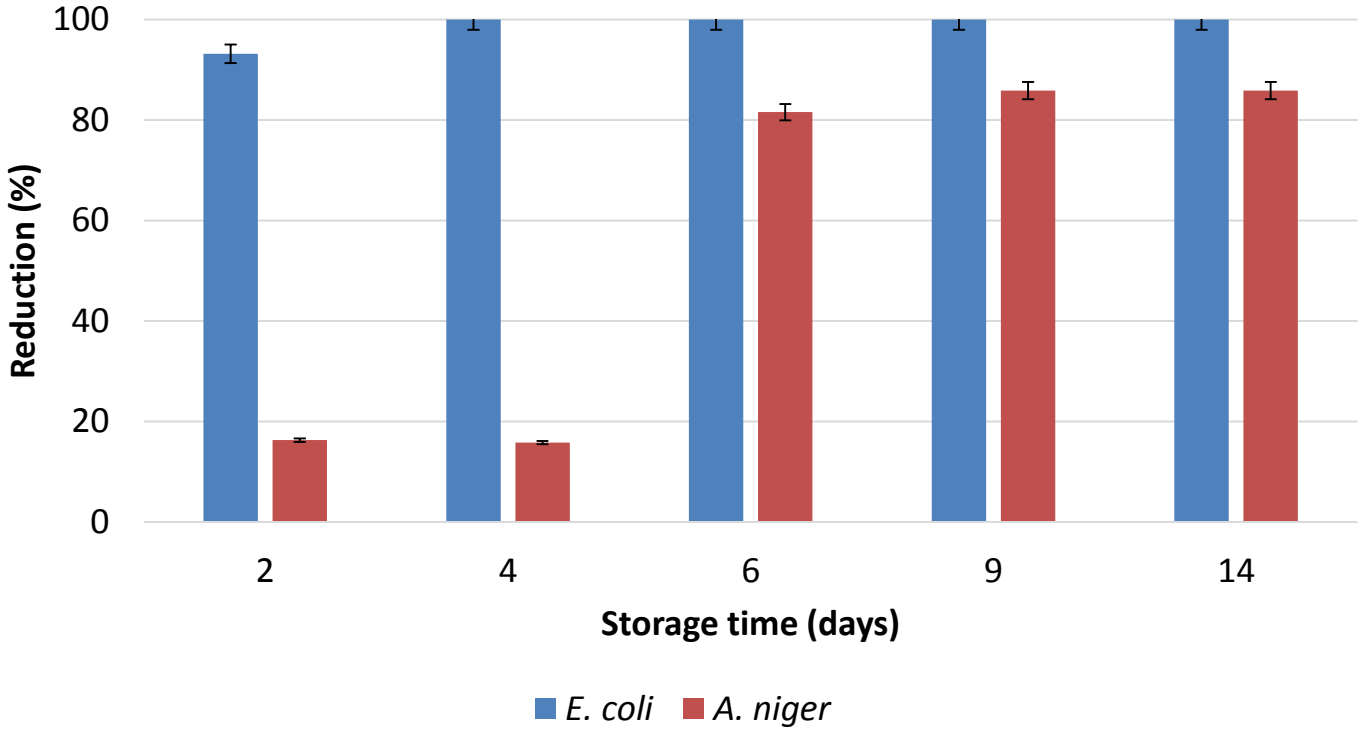
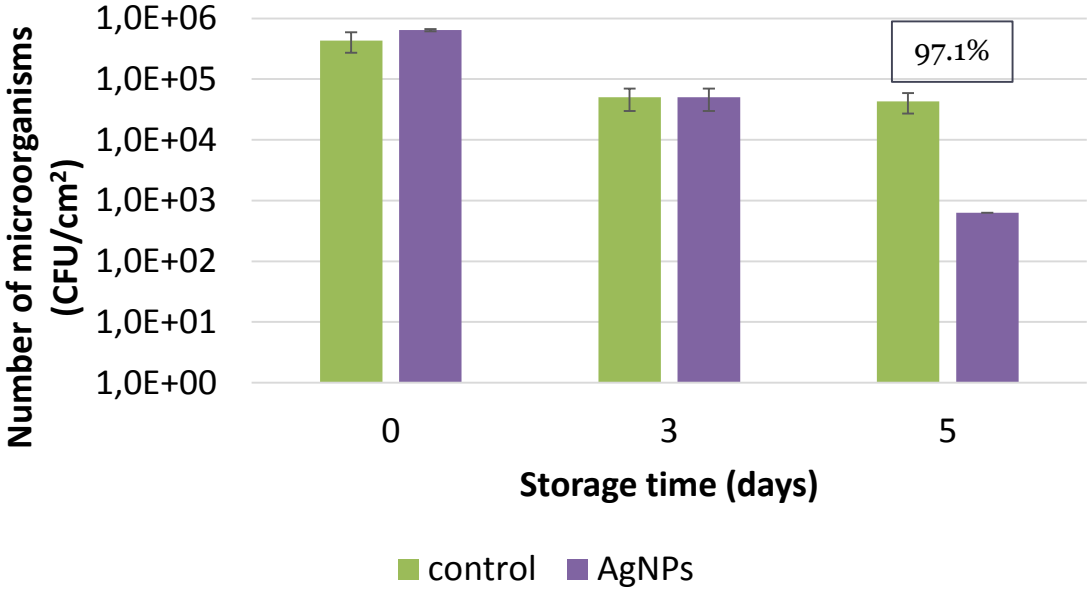
Gutarowska et al., 2014

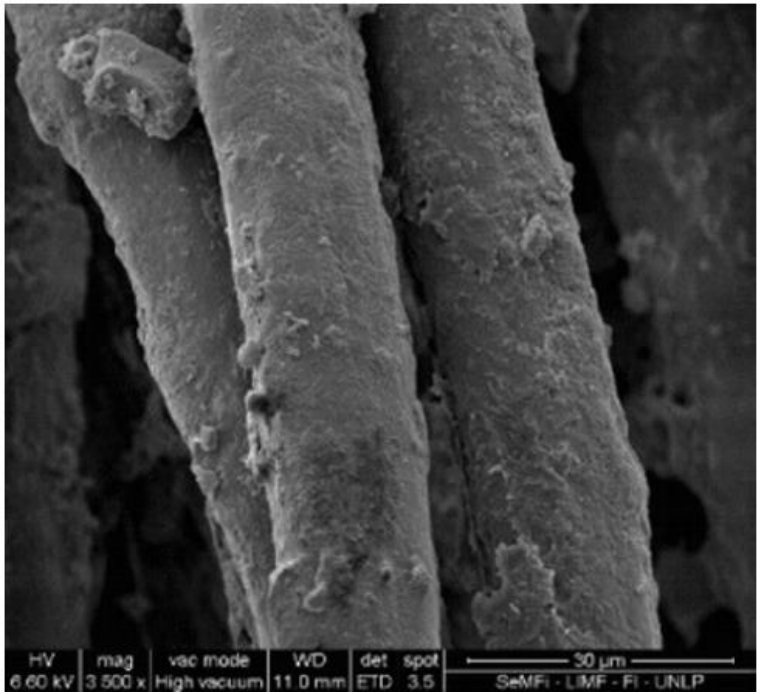
AgNPs misting durability



Storage conditions: 28°C, RH 80%

Inhibition of *Pseudomonas aeruginosa* growth



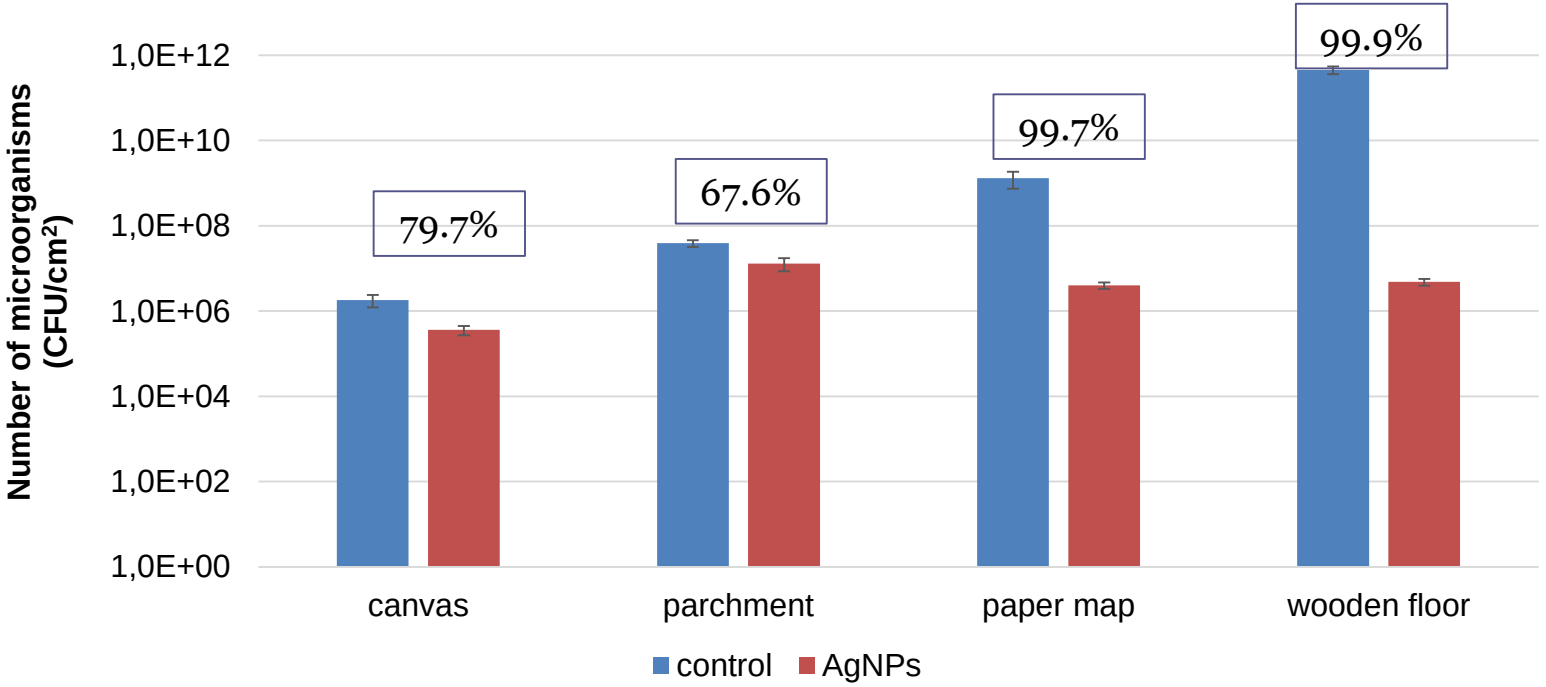


Pseudomonas aeruginosa on historical textile

Storage conditions: 28°C, RH 80%

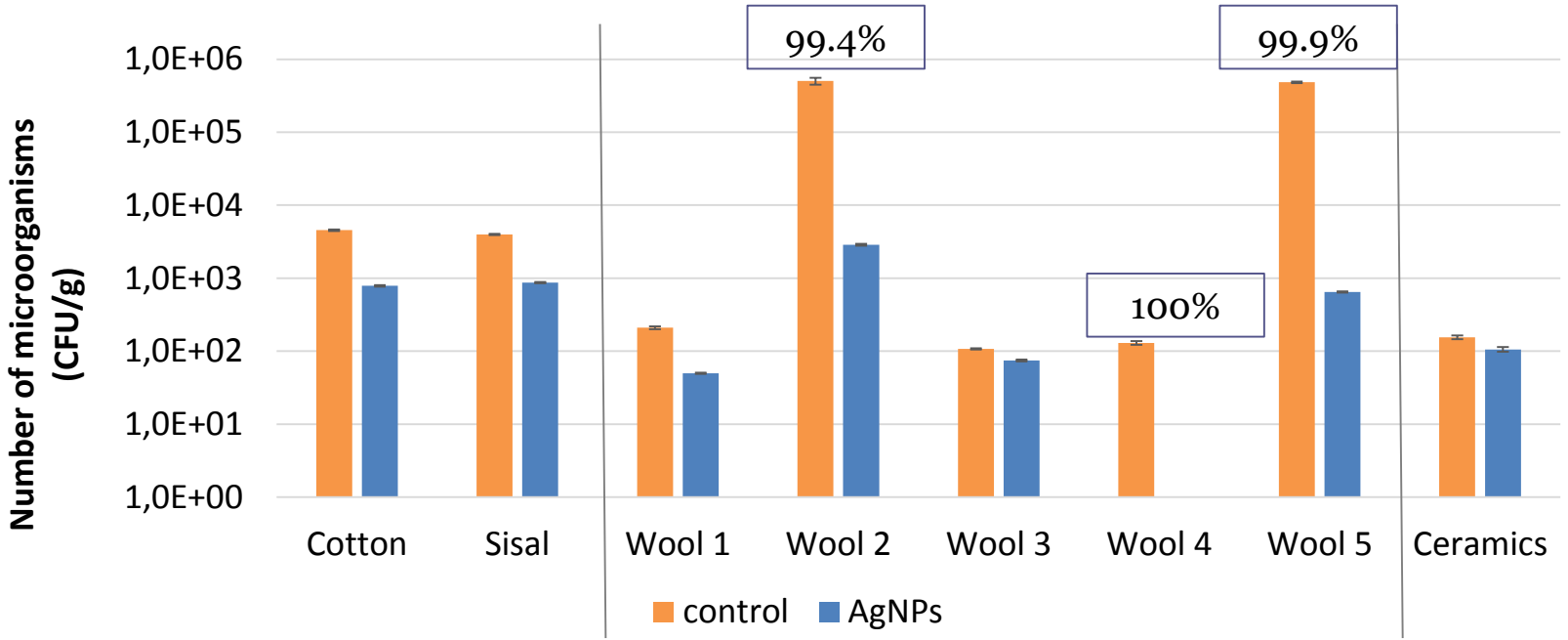
Pietrzak et al., 2016

Disinfection effectiveness on historical materials



Gutarowska et al., 2012

Disinfection effectiveness on archaeological textiles



Pietrzak et al., 2016

Mechanical parameters of paper

Paper	Control		AgNPs		Aged control		Aged AgNPs	
	Elongation (%)	Tear index (mN×m²/g)	Elongation (%)	Tear index (mN×m²/g)	Elongation (%)	Tear index (mN×m²/g)	Elongation (%)	Tear index (mN×m²/g)
GW	2.4±0.2	8.1±0.3	2.8±0.2	8.1±0.5	2.2±0.1	7.5±0.2	2.1±0.2	7.2±0.8
Sy	2.0±0.1	9.3±0.8	2.4±0.2	8.6±0.4	1.9±2.2	7.6±0.4	1.8±0.2	7.2±0.3
Sa	3.0±0.2	13.5±0.9	3.1±0.2	13.9±0.4	2.6±0.1	12.9±0.3	2.6±0.1	12.8±0.4

Sa - bleached pine kraft pulp; Sy - bleached spruce sulphite pulp; GW - bleached groundwood from spruce (GW)

Optical parameters of paper

Paper	Control	AgNPs		Aged control		Aged AgNPs	
	R457	R457	ΔE	R457	ΔE	R457	ΔE
GW	82.9±0.1	82.1±0.1	0.41	78.6±0.5	1.02	78.5±0.1	0.94
Sy	89.8±0.5	89.4±0.3	1.06	85.4±0.6	0.37	85.5±0.7	1.27
Sa	90.5±0.3	89.6±0.1	0.91	87.7±0.2	0.57	87.7±0.1	0.61

R457 – ISO Brightness; Sa - bleached pine kraft pulp; Sy - bleached spruce sulphite pulp; GW - bleached groundwood from spruce

0 < ΔE < 1 - observer does not notice the difference
1 < ΔE < 2 - only experienced observer can notice the difference
2 < ΔE < 3.5 - unexperienced observer also notices the difference
3.5 < ΔE < 5 - clear difference in colour is noticed
5 < ΔE - observer notices two different colours

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Disinfection of historical book collections

2 books

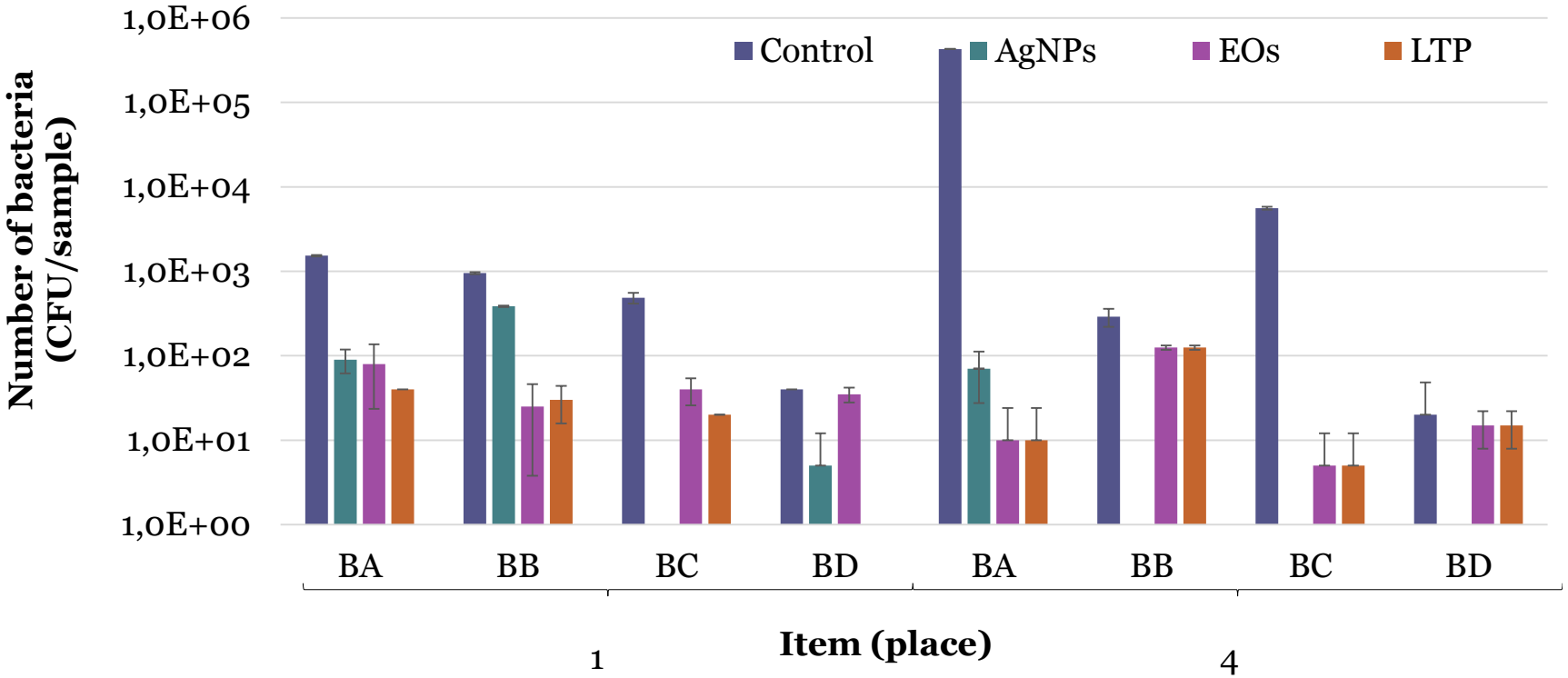
Disinfection

- Low temperature plasma
- Thyme essential oil
- AgNPs misting

AgNPs misting

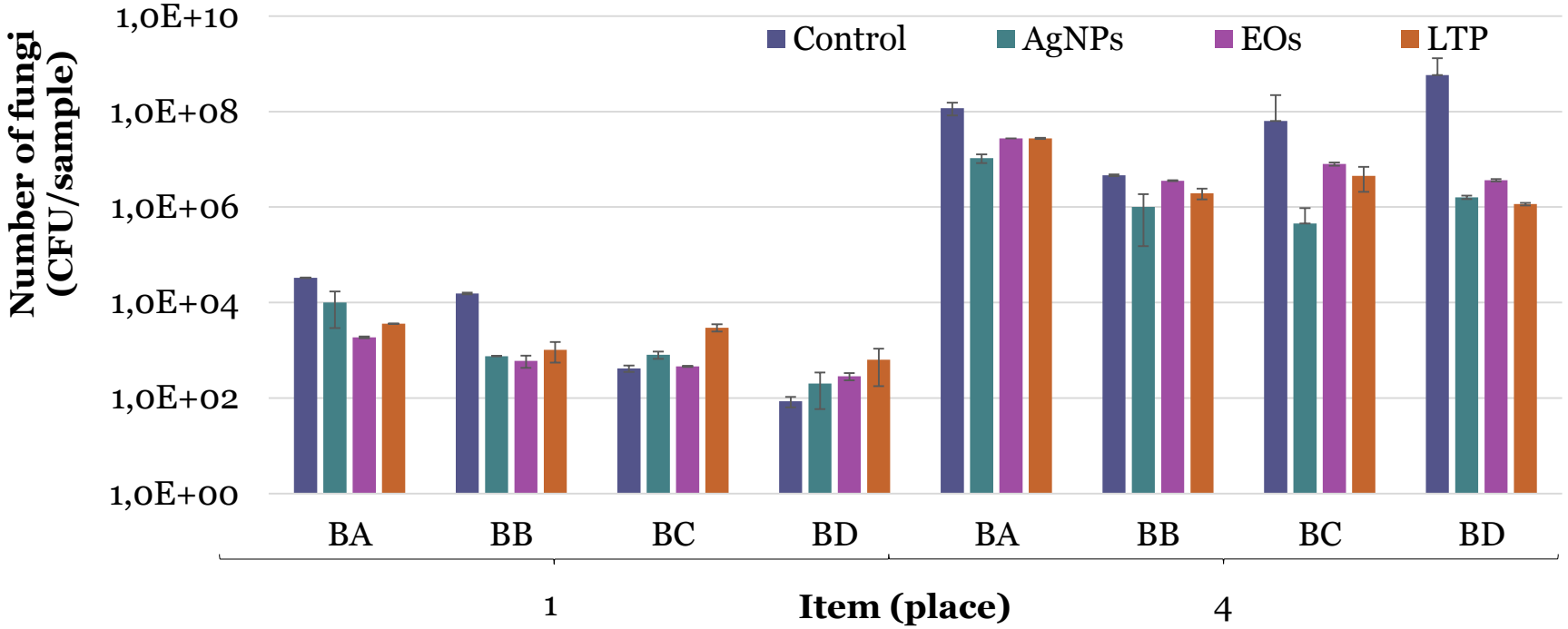
Nanosilver concentration	Misting cycles	Airflow	Temperature	RH	Duration
90 ppm	8	1 m/s	25°C	90%	520 min

Disinfection effectiveness: culture-dependent analysis



BA – front cover; BB - front free endpaper; BC – fore edge; BD – page inside the book; sample = 25 cm²

Disinfection effectiveness: culture-dependent analysis



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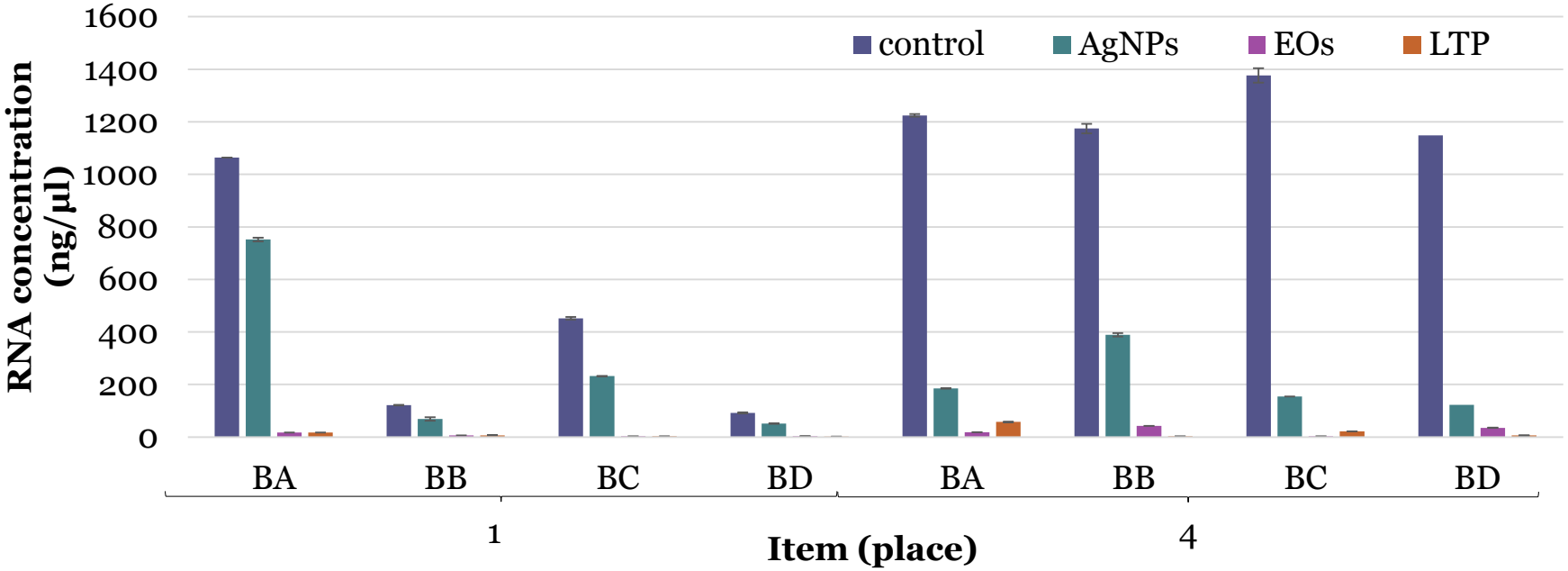
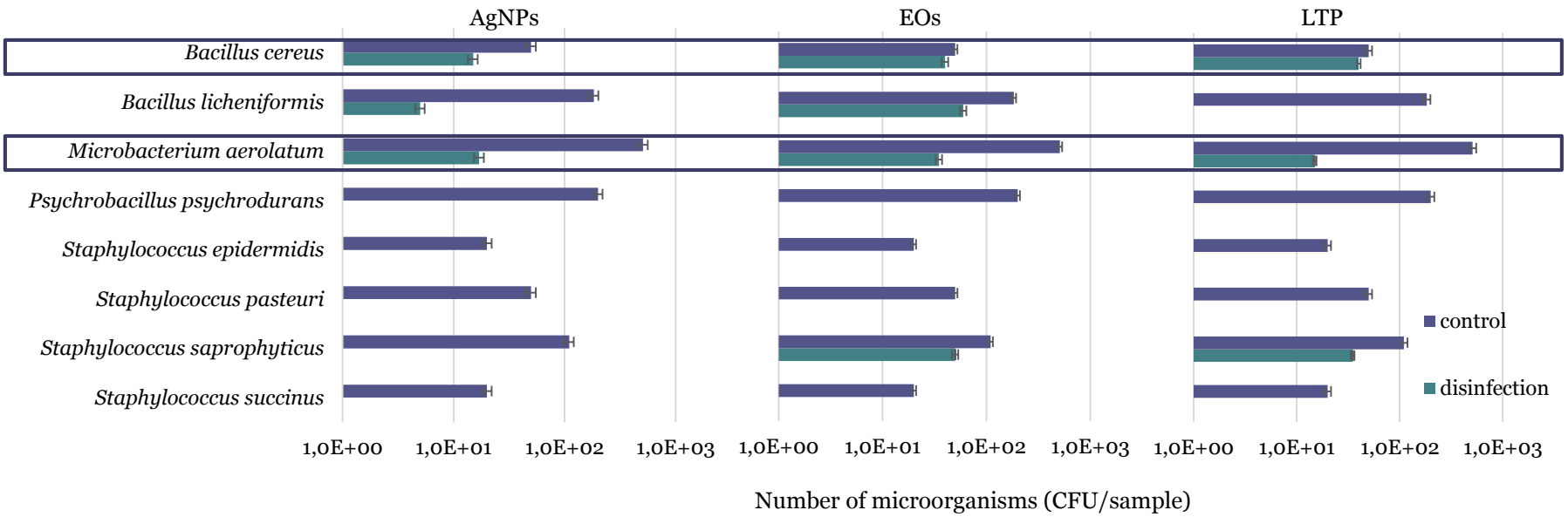
Disinfection effectiveness: culture-independent analysis



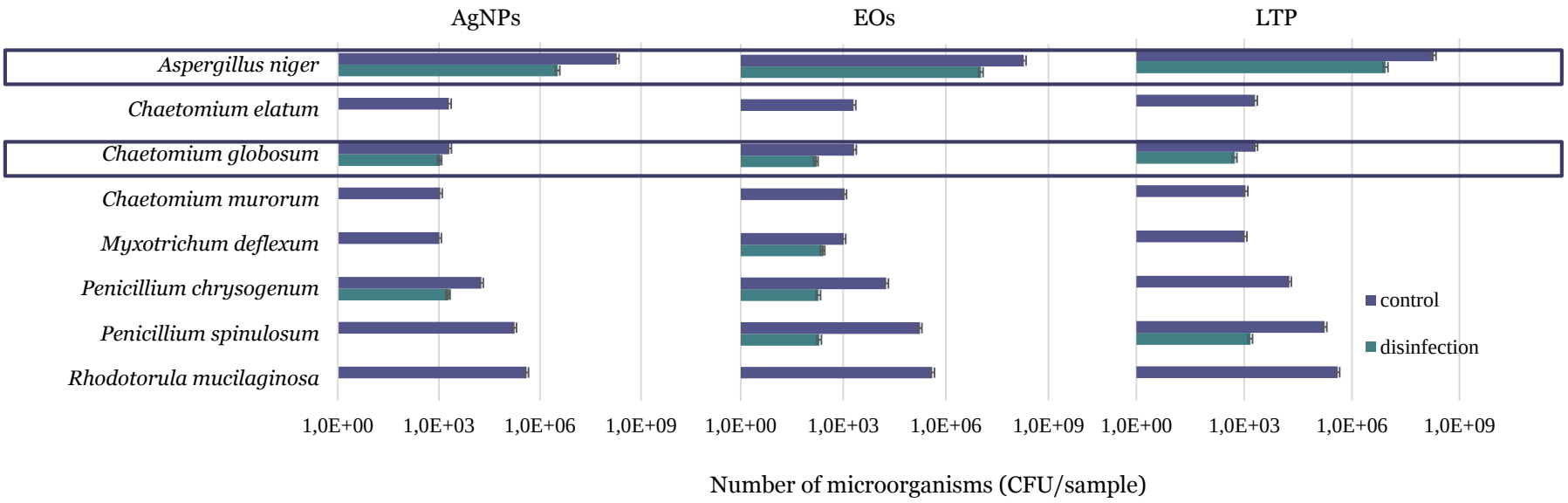
BA – front cover; BB - front free endpaper; BC – fore edge; BD – page inside the book; sample = 25 cm²

Bacterial sensitivity



sample = 25 cm²

Fungal sensitivity



sample = 25 cm²

Conclusions

1. Silver nanoparticles misting, low temperature plasma and thyme essential oil microatmosphere were efficient methods of growth inhibition of bacteria and fungi.
2. The disinfection effectiveness depended on the inhabiting microbiota, disinfected book and the sampling place.
3. The reduction of microorganism numer equalled on Book no. 1 to: AgNPs and LTP (0–100%), EOs (0–97 %); and on Book no. 4 to: AgNPs 78–100%, LTP (25–100%) EOs 23–100%).
4. RNA concentration is a good marker to assess the disinfection effectiveness.
5. *Aspergillus niger*, *Chaetomium globosum*, *Bacillus cereus* and *Microbacterium aerolatum* were insensitive to all disinfection methods.