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Dedicated to Professor Dumitru Oancea
on the occasion on his 75th anniversary

DECONTAMINATION EFFECTS OF RADIO FREQUENCY LOW-TEMPERATURE PLASMA ON PAPER-BASED MATERIALS

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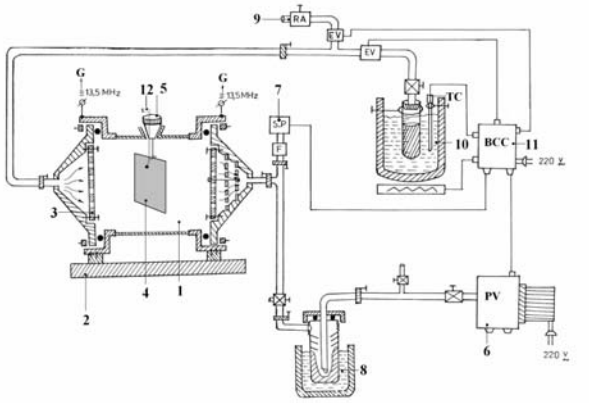
The paper presents the results of the investigations concerning the application possibilities of RF low-temperature plasma, operating in nitrogen or argon - oxygen gaseous mixture, in conservation treatments of paper-based objects - historical paper (Religious book, 1835) and industrial paper (Algebra book, 1870). The microbiological analysis revealed that N_2 plasma have a powerful fungicide effect after 10 minutes treatment time, while the Ar/O_2 plasma produced a complete inhibition of bacterial growth. SEM-EDX analyses did not show any significant modification of paper surfaces. The colorimetric analysis revealed an increasing tendency of whiteness degree, at the same time with the decrease of yellowness index, in nitrogen plasma treatment, result that can be due to the cleaning of the surface. Treatments times as long as 10 minutes or more in the Ar/O_2 plasma produce a decrease of whiteness degree, concomitantly with an increase of the yellowness index, suggesting the existence of oxidative processes of cellulose.



INTRODUCTION

Decontamination represents a priority of the preservation activities in cultural heritage domain. Frequently used decontamination procedures employ non-selective chemicals, which remanence and noxious effects on the substrates and the persons implied in preservation activities are not enough studied at this moment. In this respect, the electric discharges in gases at low pressure and the generated plasma represent an ecological, non-contact, less used alternative, with an important potential.

The utilization of low-temperature plasma is especially convenient for temperature sensitive supports, such as organic materials. The possibility of cold plasma application in the preservation of mobile cultural heritage has been investigated, aiming at decontamination of items based on organic materials.[1-4]

This paper is focused on the efficiency evaluation of RF cold plasma, working in nitrogen or argon

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oxygen gaseous mixture, in the decontamination of documents on paper support and the assessment of treatment impact on the sample, revealed by changes in the surface characteristics.

EXPERIMENTAL

1. Materials

The study has been carried out on two books printed in the 19th century, from private owners. The selection of materials was made taking into account the most representative paper supports for cultural heritage collections – handmade and industrial paper.

The two types of paper have been labeled as follows:

P1 – gelatin – sized handmade paper of cotton fibers – Religious book, printed in 1835;

P2 – alum-sized industrial paper of sulphite cellulose and mechanical pulp – Algebra book, printed in 1870, Paris.

2. Plasma treatments

The samples have been exposed to cold RF plasma, using two types of working gases: nitrogen or oxygen-argon gaseous mixture (10/90) and treatment times of 5, 10, 15 and 20 minutes.

A multi-functional RF cold plasma unit (Fig. 1) for conservation treatments of organic cultural items, patented by the authors,[5] has been designed for successive application of treatments like biologic decontamination, cleaning and protective coating with adequate polymers.

The equipment consists in a cylindrical Pyrex reactor (1), mounted on a stainless steel support (2). An electrical discharge is initiated between two inner plan-parallel electrodes (3), capacitively coupled to RF generator (G). The RF generator is working in the frequency range 1.2- 2 MHz, at a power under 100W, and has low output impedance in order to support great variations of load impedance.

The object (4), mounted on a sustaining system (5), is suspended in the central zone of the discharge. The vacuuming of the reactor is performed by a rotary vacuum pump (6) and the inner pressure is measured by an electronic vacuum meter (7). A cooling liquid-nitrogen trap (8) is inserted between the reaction vessel and the vacuum pump. The admission of the operating gas is gradually permitted by the pin valve (9).

For polymer protective coating procedure a monomer vessel (10), immersed in a thermostatic oil bath, which constant temperature is equal to the value of monomer evaporation point, is integrated in the plasma unit. The admission of monomer vapors in the reaction vessel is controlled by an electro valve.

The command block (11) monitors the operating parameters and controls the function of electric components of the unit.

By preliminary tests on minute fragments of paper the appropriate operating parameters have been established: temperature – up to 40^0C, measured with a thermocouple (12) (the temperature value has been read after stopping the RF discharge), electric field intensity in the discharge – 20÷30 V/cm, discharge power– 30W, current intensity in the discharge – 12mA and voltage between the electrodes – 280V. The current intensity in the discharge was measured by RF milliammeter (RO patent 122568/2009)[6] and the voltage between electrodes was determined with an industrial electrostatic HF kilovoltmeter C-96, URSS.

The reactor has been vacuumed to $8x10^{-2}$ mbar, then, after the introduction of the operating gas, the pressure reached $5.5x10^{-1}$ mbar, the optimum value established in the preliminary experiments.

3. Microbiological analyses

The microbiological research aimed at determining the initial degree of bacterial and fungal contamination of paper supports and evaluating the decontaminant effect of cold RF plasma treatment.

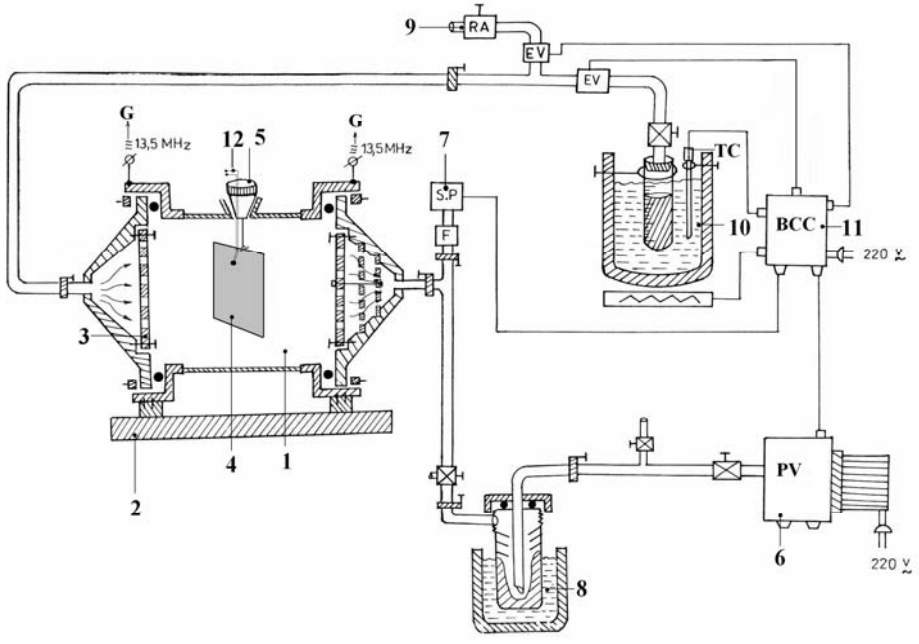


Fig. 1 – Schematic presentation of the RF plasma unit.

In order to identify the contaminant microbiota, different areas of the two books were chosen based on visible attack of microorganisms and sampled by the finger-printing method,[7] then the samples have been applied on specific culture media – nutrient agar for bacteria and Sabouraud medium for fungi. After an incubation of 24-48 hours, at 37ºC, for bacteria and 7 days, at 28°C, for fungi, the microbiota development on the surface and around the samples was studied, based on the fact that samples might contain microorganisms on the surface or at the edge, and, if they are viable, will develop on this region.

For fungal species identification, the fungi were isolated on nutrient media and the colonies have been studied by characteristic methods, involving the macro- and microscopical analysis of the colony, morphology of the mycelium, the structures, sizes, and shapes of conidiophores and conidia.

For the quantitative determination of microbiota on initial samples, as well as on the plasma treated samples, the treated samples and a reference sample were immersed in sterile distilled water (5ml) for several seconds, and then the obtained suspension was used to inoculate culture media. The nutrient media and the incubation conditions used in the determination of the microorganisms have been the same as previously. The quantitative determination was performed by counting the number of colonies developed on the surface or in depth of nutritive media, expressed in terms of CFU/ml (colony forming units/ml).

4. Physical-chemical investigations

4.1. SEM/EDX analysis

The plasma action on the paper surface was evaluated using Quanta 200 scanning electron microscope in the secondary electron mode, at 20kV accelerating voltage, with LFD detector for Low Vacuum Mode and ETD detector for High Vacuum Mode. The local elemental micro-composition within the areas under investigation was determined by energy-dispersive X-ray microanalysis (EDAX) on the Quanta 200 SEM, equipped with GENESIS XM 2i EDAX system, working at 20kV voltage.

4.2. Determination of the polymerization degree

It is known that the increase of viscosity is directly proportional to the polymerization degree (DP) of cellulose. The DP of cellulose materials was determined by the viscosimetric method according to ISO 5351 standard.[8]

A defined quantity (0.25 g) of dried cellulose (24 h at a temperature of 70°C) was dissolved in a 0.5 M cupri-ethylenediamine (CED) solution into a polyethylene bottle (50 mL) by stirring with copper balls. The determination method of viscosity is based on the measurement of the flowing time of a cellulose solution in a capillary viscosimeter (Ø=0.8 mm) at 25 °C. Dividing the measured flow time of cellulose solution (t) to the blank value of the flow time (t_o), the relative viscosity is obtained: $\eta_{rel} = k(t/t_o)$, where k apparatus constant. For each sample this method was applied twice.

The intrinsic viscosity [η] was determined by interpolation using the intrinsic viscosity table - [η] c -, where c is cellulose solution concentration. The average polymerization degree DP values (DP_n) are calculated by applying the following equation:

$$DP = \frac{95[\eta]c}{m\left[(100-b)x10^{-2}\right]} \quad (1)$$

where: m is the material mass (g) and b is the loss on drying (%).[9]

4.3. Color analysis

The evaluation of color changes was carried out with POCKETSPECT COLOR QA™ by a CIELAB system. The system is based on tristimulus values (X, Y, Z) represented in Cartesian$\cdot 10^{-1}$ or cylindrical coordinates. In this case, the Cartesian representation with the determination of the values of L, a* and b* parameters was employed, where: L* is luminosity; a* is the color change index red to green; b* is the color change index yellow to blue.

After the evaluation of parameters L, a*, b* employing Easy RGB program, the whiteness degree is calculated from the following equation:

$$W = L^*-3b^* \quad (2)$$

The yellowness index is given by the relation:

$$YI = [100\ (1.3013 \cdot X - 1.1498Z]/Y \quad (3)$$

where X, Y and Z are the trichromatic coordinates values.

RESULTS AND DISCUSSION

1. Microbiological evaluation

The microbiological analysis revealed the presence of fungi and bacteria on the two books, microorganisms observed also by SEM (Fig. 2).

Two fungal species have been identified on samples taken from the handmade paper (P_1) - *Penicillium notatum* and *Penicillium chrysogenum,* and also two species of fungi were found on the industrial paper (P_2) - *Alernaria alternata* and *Penicillium notatum*, with a relatively high load, producing numerous colonies on the studied samples.

Concerning the bacterial contamination *Gram positive rods* as well as Gram *negative rods* have been found on both types of paper. On the industrial paper (P_2) some *Gram positive cocci* were also found.

1.1. Treatment in N_2 atmosphere

The results of N_2 plasma treatment are shown in Table 1. The treatment efficiency in the inhibition of fungal development is different for the two books: for P_1, after 10 minutes of treatment only one small colony developed and 15 minutes of treatment completely inhibited the fungal growth, while for P_2 a drastic reduction of colonies number was observed – from 47 CFU/mL to 0, after only 5 minutes of treatment.

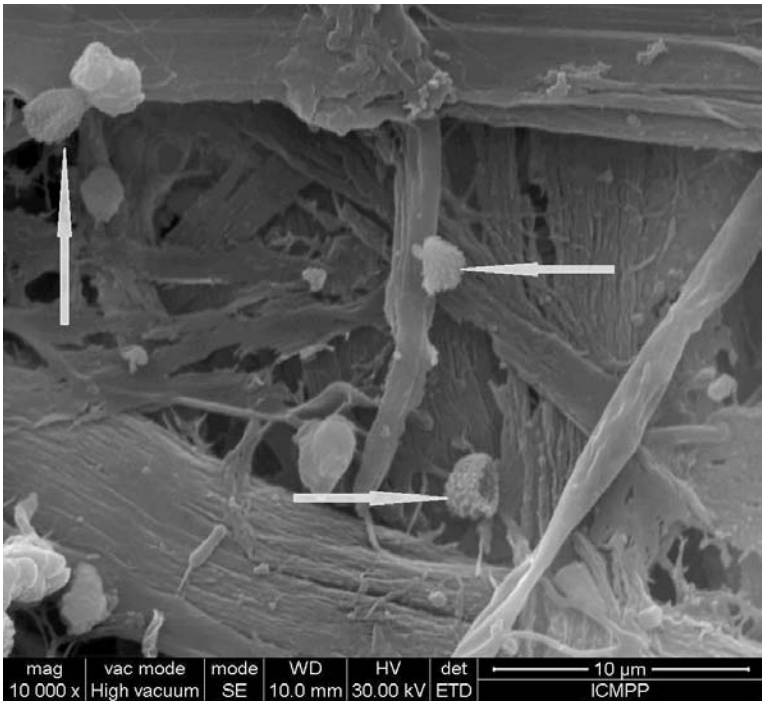


Fig. 2 – SEM image showing microorganisms presence on paper fibers (the contaminating microorganisms are indicated by arrows).

Table 1

Quantitative analysis of the microbiota on samples treated in N_2 atmosphere

Sample	Fungi (CFU/mL)					Bacteria (CFU/mL)				
	Reference	Treatment duration [min.]				Reference	Treatment duration [min.]			
		5	10	15	20		5	10	15	20
P_1	4	4	1	0	0	3	3	3	1	0
P_2	47	0	0	0	0	7	1	0	0	0

As for the bacterial attack, the colonies number remained constant (3 CFU/mL) for P_1, for treatment times up to 10 minutes, but the colonies sizes progressively diminished and their morphology was modified. After 15 minutes of treatment only one colony developed and 20 minutes treatment time completely inhibited the microorganisms' growth.

For P_2 the treatment led to a significant reduction in the number of colonies – from 7 CFU/mL to 1 CFU/mL after 5 minutes of treatment, decreasing to 0 after 10 minutes treatment time.

1.2. Treatment in Ar/O_2 *gaseous mixture*

Decontamination treatments in Ar/O_2 gaseous mixture (Table 2) led to the conclusion that the inhibition of fungal growth takes place after 20 minutes of treatment, for P_1, or after 15 minutes, for P_2. The quantitative analysis of microbiota revealed a significant decrease in CFU/mL after 5 minutes of treatment, from 4 to 2 CFU/mL for P_1, and from 47 CFU/mL to 1 CFU/mL for P_2.

Regarding the bacterial load it was found that 5 minutes of treatment for P_2 sample, and 10 minutes for P_1 sample, respectively, were successful in producing a complete decontamination (Table 2).

Comparing the results of the two treatment procedures it can be concluded that the decontamination efficiency is dependent on the nature of contaminants, on the infestation degree, as well as on the characteristics of plasma treatment – gas feed and treatment duration.

The bacterial decontamination depends on the type of colonies the two bacteria develop. The bacteria identified on P_1 sample develop under the form of R (rough) type colonies, strongly adherent to the support, while contaminant microorganism found on P_2 sample forms S (smooth) colonies, poorly adherent to the support, much easier to destroy in a short treatment time.

Table 2

Quantitative analysis of microbiota on samples treated in Ar/O_2

Sample	Fungi (CFU/mL)					Bacteria (CFU/mL)				
	Reference	Treatment duration [min.]				Reference	Treatment duration [min.]			
		5	10	15	20		5	10	15	20
P_1	4	2	1	1	0	3	2	0	0	0
P_2	47	1	1	0	0	7	0	0	0	0

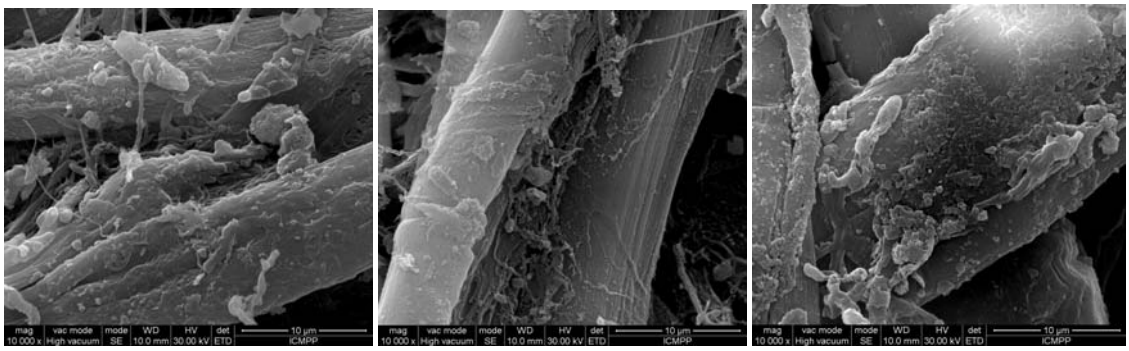
The nature of cellulose substrate also plays a role in the decontamination efficiency, as the porous surface of handmade paper needs longer duration of treatment comparing with the industrial paper, whose surface is more compact and smooth.

2. SEM/EDX analysis

The SEM images, taken before and after 20 minutes treatment in N_2 atmosphere, or in Ar/O_2 gaseous mixture, respectively, are shown in Fig. 3 – sample P_1 and Fig. 4 – sample P_2.

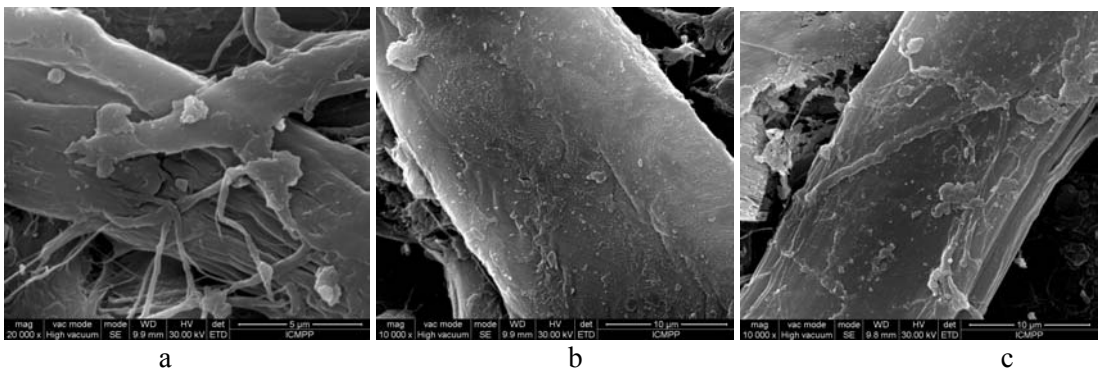
The initial aspect of the handmade paper structure – Fig. 3 a, is characteristic for cotton cellulose fibers, embedded in a binder. The initial SEM images of P_2 sample show a structure with large diameter fibers, of smooth surface, specific of industrially made paper of mechanical wood pulp.[10]

The particles visible on fibers for both types of papers may originate in the calcium carbonate filler, assumption sustained by the identification of calcium by EDX analysis (Table 3). A number of filaments cracks and fissures, as well as voids, are also visible, as a result of fibers degradation by natural aging or due to functional wearing.



a b c

Fig. 3 – SEM micrographs- the superficial aspect of handmade paper - sample P_1; a – initial aspect of the cellulose fiber structure, b – cellulose fiber structure after 20 min treatment in N_2, c – cellulose fiber structure after 20 min treatment in Ar/O_2.



a b c

Fig. 4 – SEM micrographs – the superficial aspect of industrial paper – sample P_2. a – initial aspect of the cellulose fiber structure, b – cellulose fiber structure after 20 min treatment in N_2 , c – cellulose fiber structure after 20 min treatment in Ar/O_2.

Table 3

Major elements of the surface determined by EDX

Sample	Element [% w/w]	N_2		Ar/ O_2	
		Reference	20 min	Reference	20 min
P_1	C	51.76	50.75	51.23	52.17
	O	33.34	32.73	32.20	32.09
	N	1.67	2.11	2.63	-
	Si	0.98	0.45	0.55	0.65
	Ca	2.32	2.48	2.93	1.64
P_2	C	51.43	47.79	58.37	47.98
	O	33.00	31.68	33.07	30.35
	N	-	1.39	-	-
	Si	2.97	4.25	3.04	4.37
	Ca	0.55	0.58	0.57	0.56

After plasma exposure SEM images Fig. 3, b, c and Fig. 4, b, c, do not show any damage of fibers topography or additional deterioration, regardless of the operating gas used.

Table 3 presents the results of compositional analysis performed by SEM-EDX. It can be observed that, regardless of the operating gas used, an important reduction of C concentration takes place on both types of samples. This feature could be due to the process of removing greasy organic dirt by etching and inorganic deposits (dust, ash) by mechanical erosion of the surface.

Variation of Ca percentage within measuring error limits leads to the conclusion that the plasma treatment does not affect the filling material in the paper.

3. Determination of the polymerization degree (DP)

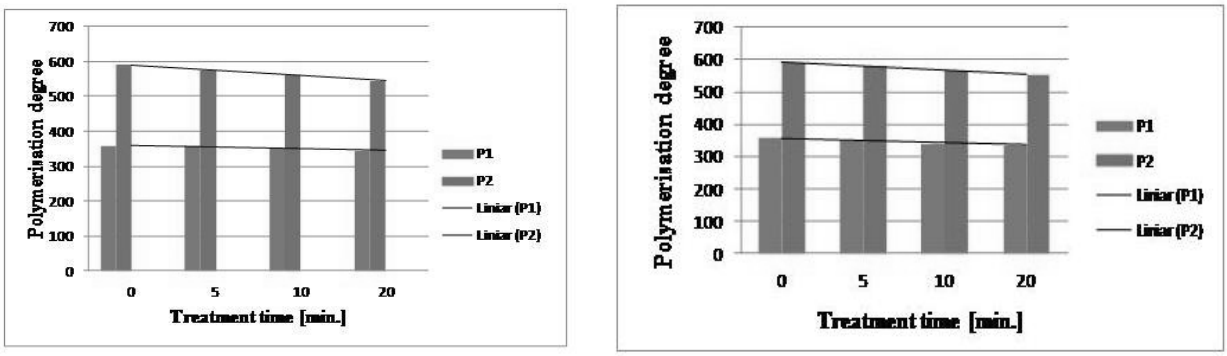
Fig. 5 presents DP behavior for nitrogen and gaseous mixture Ar/O_2 plasma treatment: DP shows a decreasing trend with the increase of treatment duration. For both operating gases this tendency is more evident for P_2 sample – the thinner industrial paper, behavior which may be explained by the better quality of raw materials of the handmade paper comparing with the mechanical wood pulp the industrial paper is made of.

4. Determination of optical characteristics

The variation of optical characteristics – whiteness degree and yellowness index – determined for the studied samples are presented in Fig.s 6 and 7.

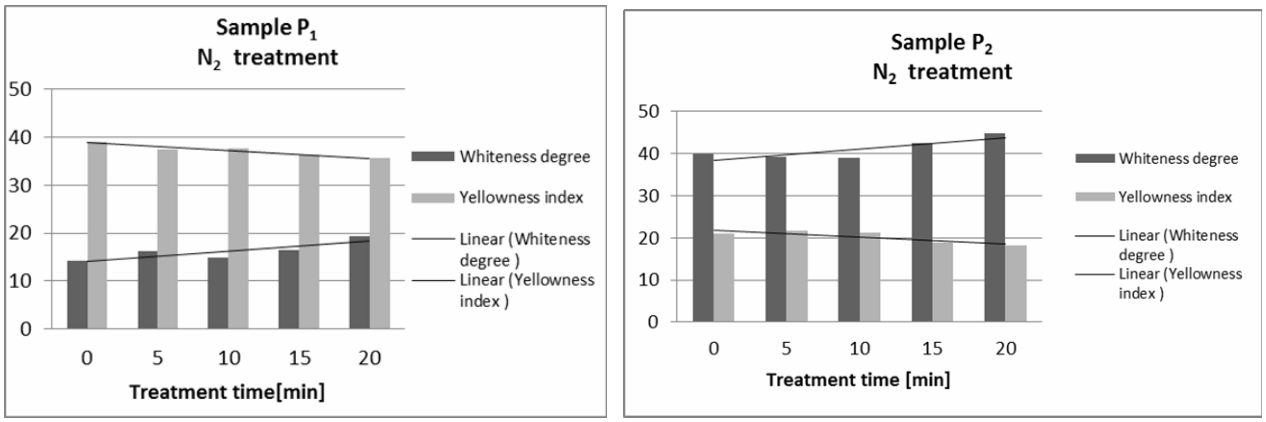
Both types of paper treated in nitrogen plasma show an increase tendency of whiteness degree, at the same time with a decrease of yellowness index, result that may be explained by surface cleaning.

On the contrary, in the Ar/O_2 plasma treatment, there is a decrease of whiteness degree, concomitantly with an increase of yellowness index, suggesting an oxidative process of cellulose support.[11]



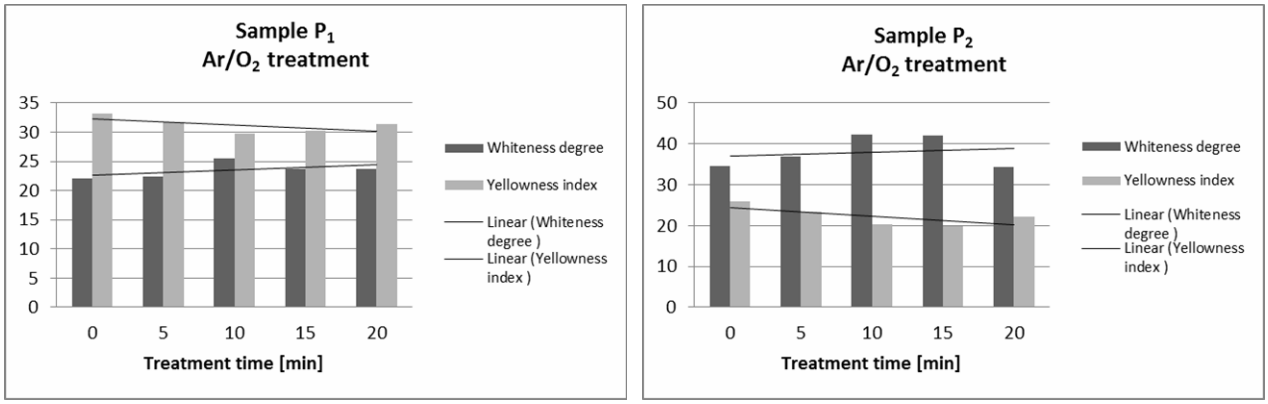
a b

Fig. 5 – DP behavior for: a- nitrogen plasma treatment b- Ar/O_2 plasma treatment.



a b

Fig. 6 – Variation of whiteness degree and yellowness index for N_2 plasma treatment.
a – P_1 sample treated in nitrogen plasma, b – P_2 sample treated in nitrogen plasma.



a b

Fig. 7 – Variation of whiteness degree and yellowness index for Ar/O_2 plasma treatment.
a – P_1 sample treated in Ar/O_2 plasma, b – P_2 sample treated in Ar/O_2 plasma.

CONCLUSIONS

The nitrogen RF plasma inhibits the fungal growth and multiplication after 10–15 minutes of treatment, depending on the type of material, while for complete bacterial decontamination the treatment duration varies from 10 minutes for P_2 sample to 20 minutes for P_1.

The exposure to Ar/O_2 plasma led to the inhibition of fungal growth and development after 10 – 15 minutes of treatment, while the bacterial decontamination takes 5 minutes treatment time for P_2 sample to 10 minutes, for P_1 sample.

The type of cellulose support plays also a role in the process efficiency, thus a porous surface – the handmade paper, needs longer treatment duration comparing to the industrial paper, which surface is more compact and smooth.

SEM/EDX and optical characteristics analysis showed only a minor superficial modification of the studied papers. The analysis of the polymerization degree presented an insignificant alteration of cellulose structural characteristics.

The results of microbiological analysis confirmed an important reduction of microorganisms number (CFU/ml) on the studied papers as a result of plasma treatment, the nitrogen plasma treatment being more efficient in fungal decontamination, while the bactericidal effect is more important in Ar/O_2 mixture plasma.

The utilization of RF low-temperature plasma in conservation treatments of cultural values on paper support has the advantage of an ecological, non-toxic, non-invasive procedure, which can be efficiently applied on very fragile objects and can be rigorously controlled.

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