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# Prevention Microbiological Using Functionalized Materials As Ecological Additives In Hygienic Paints

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**Abstract**

At the present time one of the environmental challenges is the microbiological control in the indoor environment. The microorganisms that form biofilms on substrates located inside buildings and houses contribute to the formation of bio-aerosols when they are, partial or totally, dispersed and transported by the air. An example is the poisoning by certain fungal products as the mycotoxins (carcinogenic) that comes from fungi that can generally be in the interiors of buildings. Therefore, it is of vital importance contribute to avoid and/or minimize the microbiological growth in the indoor environments. In such sense the evaluation of new antimicrobial agents is fundamental and can be applied in diverse areas of development of protective coating and construction materials. The global efforts to reduce to dangerous remainders and technologically clean the chemical processes are being integrated with modern progresses as much in science as in the industry. In this investigation will work with remainders of seeds of sunflower functionalized with 3-aminopropyltriethoxysilane (APS), as support of additives, to a compound of Mo and K, that will act as antimicrobial, to be used in hygienic paintings. The originating solid of the sunflower seeds will be functionalized by means of reactions between organosilanes and the superficial groups of the mentioned solid. The characterization of synthesized solids will be made by means of SEM-EDS, DRX, FT-IR, textural properties, potentiometric titration, among others. These solids were microbiologically evaluated in front of fungi and bacteria, having given promissory results for novels ecological additives in hygienic paints.

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*Keywords:hygienic paints, sunflower seeds, Lindqvist phase, antimicrobial activity.*

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## 1. Introduction

In the last campaign 2012/2013, Argentina integrated the select lot the four main producers of sunflower seed, with a production of 3,100.000 tons. Our country produces about 9% of the global total of world production, and is located on the fourth place. Most domestic production is destined for industrialization to obtain oil and flour sunflower, both for export and for use in the domestic market (Calzada, 2012). The main macronutrients of sunflower seed are lipids, carbohydrates and proteins; on the shell there is a high content of lignin and cellulose-hemicellulose. For the production of oil from seeds are hulled mechanically. The shells are abundant agro-industrial waste which has been marketed for special purposes as making firewood for home and other products with high in fiber, but these markets are limited.

Also, the shells has tried to use as material fodder for ruminants such as cows and sheep, but the high lignin content makes it not suitable as animal feed. Furthermore, it has established lay to rest the shells on the floor, but this practice is dangerous for the healing of the grounds because they contain *Sclerotiniasclerotium*, a fungal pathogen to plants. Therefore, shells often are burned in processing plants (Curvetto et al. 2005).

In this work were evaluated for antimicrobial activity of the ash of sunflower seed shells functionalized with different concentrations of 3-aminopropyltriethoxysilane (APS) and, subsequently, impregnated with the Lindqvist $[V_2Mo_4O_{19}]K_4$ (KMo) type polyoxometalate, for use as antimicrobial agents or biocides in hygienic paints. In addition, the samples were characterized by different techniques as Fourier Transform Infrared (FTIR), potentiometric titration with *n*-butylamine, Scanning Electron Microscopy and Energy Dispersive (SEM-EDS), X-ray diffraction (XRD), and structural properties ($S_{BET}$).

## 2. Experimental procedure

### 2.1. Washing ash of sunflower seed shell

The outer ash sunflower seeds (CG) were used as solid to functionalize and subsequently in support of the Lindqvist-type phase $[V_2Mo_4O_{19}]$ $K_4$. These were washed with hot water to remove excess powder (earth) which might come mixed.

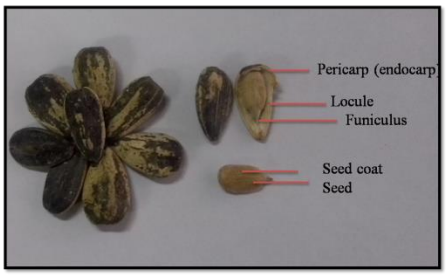


Fig. 1.Part of sunflower seed.

### 2.2. Functionalization of the CG washed

The method used for the functionalization of pure and washed CG with 3-aminopropyltriethoxysilane (APS), was to add different concentrations of the same. The solid obtained was dried at 100 °C for 2 h, obtaining the following concentrations 0.5% (w/w), 1.5% (w/w), and 3.0% (w/w) of APS, respectively.

### 2.3. Synthesis of compound type Lindqvist $[V_2Mo_4O_{19}]K_4$

For obtaining different reactive compound ($MoO_3$, K(OH), $NH_4VO_3$, HCl and potassium acetate), were used. The mixture was stirred at 50 °C, yielding an orange solution which was filtered in hot. The obtained solution was placed in a crystallizer to form crystals of theLindqvistphase. After this refer as KMo.

### 2.4. Impregnation of solid CG functionalized with APS

In Table 1 is observed the nomenclature of synthesized samples. They were synthesized as follows: 1 g of support (CG-0.5APS, CG-1.5APS, CG-3.0APS) was contacted with 4 ml solution of $[V_2Mo_4O_{19}]K_4$, during 24

h,tofunctionalize solids, in the amount of 5% (w/w), expressed in% (w/w) of K.

Table 1-Nomenclature used for samples and reagents from initial mass CG

| **Sample** | **Functionalizing APS (%(w/w))** | **Phase KMo (%w/w *K*)** |
|---|---|---|
| CG | | |
| CG-0.5APS | 0.5 | |
| CG-1.5APS | 1.5 | |
| CG-3.0APS | 3 | |
| CG-0.5APS-KMo | 0.5 | 5 |
| CG-1.5APS-KMo | 1.5 | 5 |
| CG-3.0APS-KMo | 3 | 5 |

2.5. Characterization of the synthesized samples

Absorption-Desorption N ($S_{BET}$), potentiometric titration with *n*-butylamine,ScanningElectron Microscopy and Energy Dispersive (SEM-EDS), Digital Photography, X-ray diffraction (XRD) and Infrared Fourier Transform(FTIR). The textural properties, as surface area of solid ($S_{BET}$) were determined using a Micromeritics 2100 Accusorb using $N_2$ as adsorbable gas. Acid estimating material properties was performed by potentiometric titration with *n*-butylamine. Was carried out in a pH/mV/°C based on a microprocessor 211 Hanna Instruments pH, using a combined pH electrode: 0.025 ml/min of *n*-butylamine solution in acetonitrile (0.05 N) to a known amount (0.05g) of the solid of interest, previously suspended in acetonitrile (90 ml), and stirred for a period of 3 h were added. X-ray diagrams (XRD) were performed with a Philips model PW-1390 (control channel) and PW-1394 (motor control) chart recorder with built-sweep. Cu Ka radiation was used (α=1.5417 Å), nickel filter, 20 mA and 40 kV high voltage source, scanning angle (2θ) between 5° and 60°, scanning rate of 2°/min and amplitude of the vertical scale 2000 counts/sec. Scanning Electron Microscopy and Energy Dispersive (SEM-EDS), SEM was performed to obtain solid micrographs using a Philips Model 505, working at a potential of 15 kV, on samples supporting on graphite and gold. The images were obtained with a ADDAII acquirer with Soft Imaging System. FT-IR spectra were obtained using a Bruker IFS 66 and the sample include in KBr. Measurements were made in a range between 400 and 4000 $cm^{-1}$.

2.6. Antifungal activity

Antifungal activity of the solid was measured using a variation of the agar diffusion and Kirby Bauer technique called "cut-plug" (Kenawy et al. 2006,Alamri et al. 2012). The evaluation of the antimicrobial activity through this type of procedure is determined by contacting the assessed through its inoculated with microorganisms (Morello et al. 2003) solid surface. The agent studied diffuses radially forming a concentration gradient. At the end of the incubation period the solids are surrounded by a zone of inhibition. In each case, the measured diameter of the inhibition zone, which is related to a greater or lesser susceptibility of microorganisms used for testing against the target agent.

The fungi *Alternariaalternata* and *Chaetomiumglobosum* and *Penicillium sp.* and *Aspergillusfumigatus* isolated from previously painted substrates bio-deteriorated (Prescott et al.,1999), were used as biomarkers to determine the antifungal activity of the ashes of sunflower seeds with APS and APS-KMo. Subcultures were performed mentioned above isolates in Petri dishes. The composition of the agarized culture medium (MCA) was used: 1.5 g agar, 1 g dextrose, 0.5 g proteose peptone, 0.1 g $KH_2PO_4$, 0.05 g $MgSO_4.7H_2O$ and distilled water. The plates were incubated in an oven at 25 °C between 15-25 days depending on the species used. The above fungi were used in this work, not only for their ability to grow on damaging them paintings and films, also by the negative effects that they have on human health (Vagui et al. 2005, Cooley et al. 2004). From subcultures, the inoculums were obtained by

removingspores with the aid of a loop solution and 5 ml of 0.85% (w/v) NaCland Tween 20 0.005% (w/v). The concentration of spores (0.3-0.5x$10^6$ spores/ml), in the inoculum was adjusted through a Neubauer chamber.

MCA plates inoculated with 200 µl of spore suspension were prepared. Then holes were made with 7mm diameter where 20 mg of each of the solid evaluated (CG with APS and APS-KMo) and their respective controls were introduced (CG and without solid). The procedure was performed triplicate for each solid. Finally, plates were incubated for 48 h at 25 °C. At the conclusion of the test close observation of the plates carried out and, should there be the inhibition halos produced by each solid. Photographic record was taken in each samples; was considered whether or not growth on the solid and the degree of inhibition around it. Therefore, diameters larger than 7 mm indicate a greater susceptibility of the organism against the tested product. In this regard was taken into account whether the inhibition was complete (no growth of mycelium) or had a decrease in mycelial growth around the solids studied.

### 2.7. Antibacterial activity

The bacteria *Escherichia coli* and *Staphylococcus aureus* were used as bioindicators of the antibacterial activity of CG with APS and APS-KMo with the corresponding controls. Subcultures were performed in slant agar tubesfor bacteria (BVAC). The composition of the culture medium used was: 0.1 g yeast extract, 0.1 g proteose peptone, 0.1 g starch, 0.1 g dextrose, 0.1 g casamino, 0.05 g pyruvic acid, 0.06 g $KH_2PO_4$, 0.01 g $MgSO_4$ and 1.5 g agar to 100 ml of solution. The tubes were incubated for 24 h in an oven at 37 °C. The antibacterial activity of the solid by the same method was evaluated previously detailed, "cut-plug". After 24 h cultivation, saline suspensions were obtained by adjusting the turbidity of McFarland 0.5 (1.5x$10^8$ Ufc/ml) scale. Dilution is then performed to obtain a bacterial suspension of 1.5x$10^6$. BVAC plates inoculated with 500 µl of the bacterial suspension were prepared. Then three holes weremake where 20 mg of each control studied solid was added,respectively. The plates were incubated for 24 h at 37 °C. At the end of the test the inhibition, hales were measured and results recorded by digital photographs.

## 3. Results and Discussion

With respect to the potentiometric titration, it was observed that the initialpotential ($E_i$), the initial acidity indicators solid synthesized decreased coinciding with the steep fall of the impregnated samples (Fig.2). This behavior leads to that all samples have a high basicity, the predominant property Lindqvist type phases, in this case the KMo.

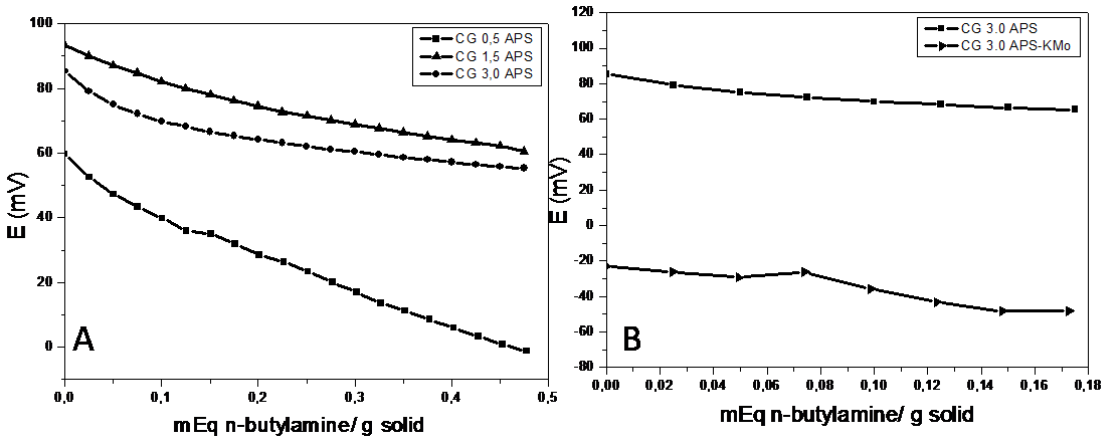


Fig. 2.Potentiometric curves samples: (A) CG-0.5APS, CG-1.5APS and CG-3.0APS;(B) CG-3.0APS and CG-3.0APS-KMo.

Lindqvist structure, published in 1950, is the most symmetrical of isopolyanions and involves the merger of six octahedra sharing a common vertex which is an oxygen atom that is bonded to the six metal centers (Fig. 3).

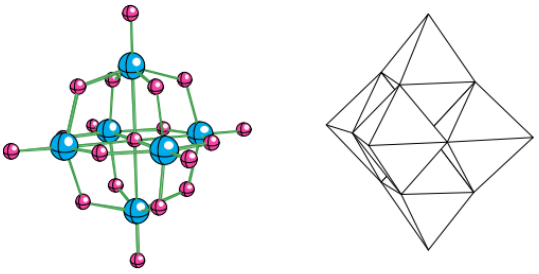

Fig. 3.Lindqvist structure.

With respect to FTIRspectra (Fig. 4) changes in the spectra of the husk ash functionalized sunflower seeds are not perceptible with respect to the ashes sunflower, but there is a variation for the three visible solids impregnated with different amounts of APS, which may be attributableto the groups containing Mo and V, in KMo phase.

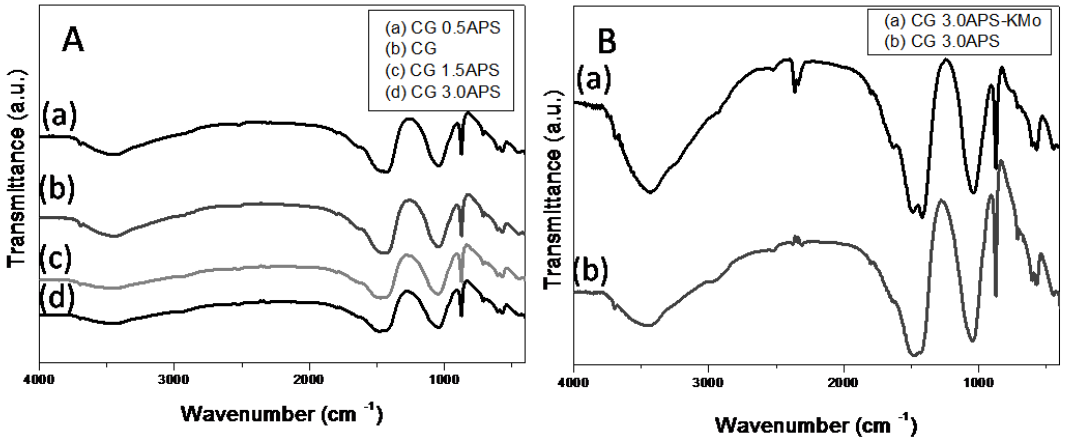


Fig.4.FT-IR of samples: (A) CG, CG-0.5APS, CG-1.5APS and CG-3.0APS;(B) CG-3.0APS and CG-3.0APS-KMo.

The presence in XRD of KMophase has a signal in accordance with 2Ɵ 28.2, observed(Fig. 5). The ashes of sunflower have a characteristic signal 30 2Ɵ and that signal mentioned previously (Fig.6 A and B).

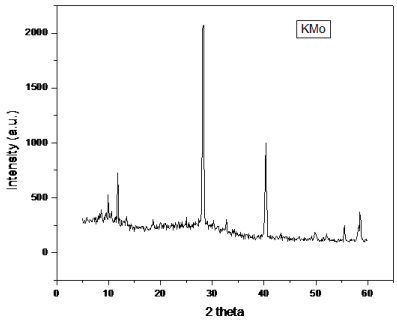


Fig. 5.Lindqvist structure pattern.

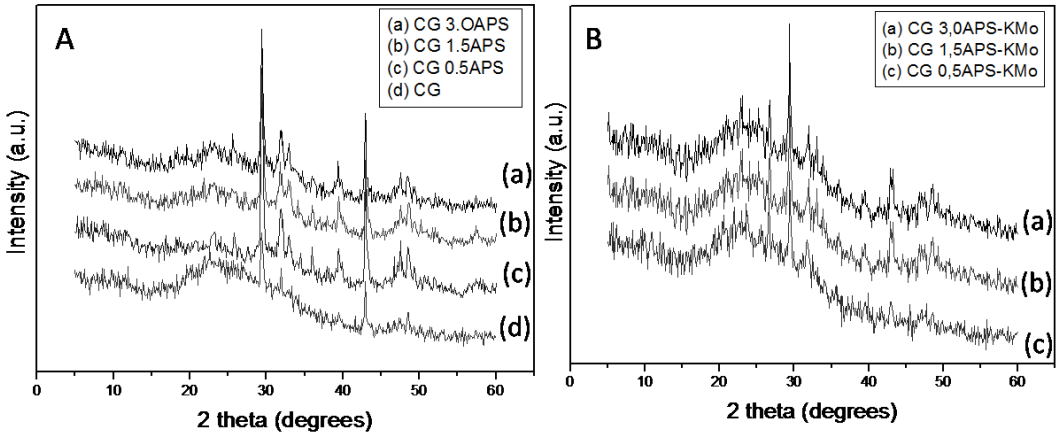


Fig.6.XRD pattern of samples: (A) CG, CG-0.5APS, CG-1.5APS y CG-3.0APS; (B) CG-0,5APS-KMo, CG-1,5APS-KMo y CG-3,0APS-KMo

Besides, it was observed the samples tested have low or no surface area. This characteristic makes the agar diffusion test is more difficult, since the average should contact the porous material so that an inhibition on the growth of microorganisms occurs. If the surface area is low there is no sufficient porosity to make it happen.

The samples were characterized by SEM-EDS and results obtained by EDS correspondingwithpotentiometric titration. Numerous cations were found and to make evident the presence of some of them, such as Ca, a comparative mapping was performed. Be concluded that the low acidity, high or sample presenting basicity is due in part to the presence of these cations on the surface in contact with the APS and in the pores of the solid (ash).

Table 2 shows the results obtained from agar diffusion assess with solid tested and corresponding controls to determine it antifungal activity thereof are presented.

Table 2 -Assess agar diffusion. Diameter of inhibition zone (mm) average, after 48 h.

| **Sample** | ***Alternaria alternata*** | ***Chaetomium globosum*** | ***Penicillium sp.*** | ***Aspergillus fumigatus*** |
|---|---|---|---|---|
| Control | -- | -- | -- | -- |
| CG | IS | 23.1* ± 3.1 | IS | 17* ± 1.2 |
| CG-0.5APS | IS | 23.4* ± 2.3 | IS | 24.5* ± 1.3 |
| CG-1.5APS | IS | 24.4* ± 1.7 | IS | 20* ± 3.3 |
| CG-3.0APS | IS | 23.3* ± 1.4 | IS | 22.0* ± 3.0 |
| CG-0.5APS-KMo | IS | 27.3* ± 0.3 | IS | 30.3* ± 2.0 |
| CG-1.5APS-KMo | IS | 32.2* ± 0.5 | IS | 29.7* ± 1.7 |
| CG-3.0APS-KMo | IS | 8.9 ± 0.8 | IS | 27.9* ± 1.5 |

[a] Average data from triplicate. * Indicates lower growth around the solid. IS: inhibition in solid (7mm in diameter). –Uninhibited.

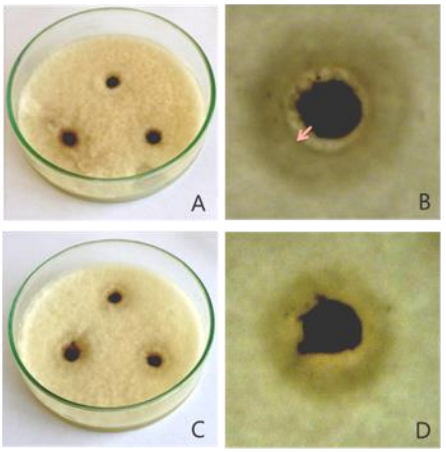


Fig. 7.Photographs of fungal culture of *C. globosum* with solid (A, B) CG-3.0APS-KMo; (C, D) CG,representing ( ➞ ) the halo of inhibition.

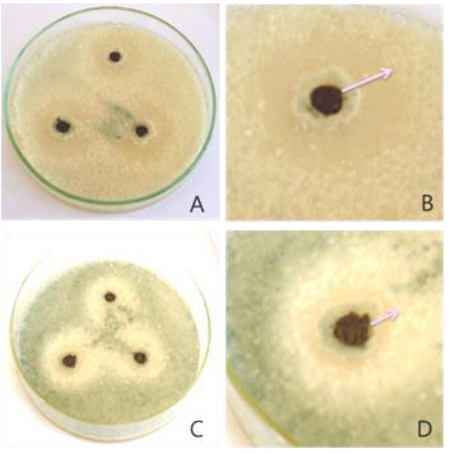


Fig. 8. Photograph of fungal culture of *A.fumigatus*with solid (A, B)CG-3.0APS-KMo; (C, D) CG. Thearrow ( ➞ ) represents the halo of lower growth.

Antifungal activity was observed in cultures of *C. globosum* (Fig. 4) and *A. fumigatus* (Fig. 5), the first showing greater susceptibility to solid-CG-3.0APS KMO presented since an inhibition average of 8.9 mm without mycelium growth (Fig. 4 A, B). Notably, among the species used for performing the assay *C. globosum* is the greater water content required for its germinating spores and mycelium can develop [10]. Therefore, increased hydrophobicity of the agent under study could further affect normal development.

Regarding culture of *A. fumigatus* shown in Fig. 5, it can be observed that there is less growth of mycelium with CG-3.0APS-KMO solid with respect to control, since the greenish hue is observed characteristic for conidia production.

In cultures of *A. alternata* and *Penicillium sp*. there was no growth on solid but no change was observed with respect to controls with CG.

In Table 3 the results obtained from the agar diffusion test with solid and corresponding controls tested for antibacterial activity thereof are presented. The results show increased susceptibility of *S. aureus* against CG-3.0APS-KMO showing a halo appreciably relative to control with the solid support alone (Fig. 6) average inhibition (9.8 mm). In the case of *E. coli* with the results obtained functionalized solid showed no difference with the controls (Fig. 7).

Table 3 - Evaluation of antibacterial activity by agar diffusion assay. Diameter of inhibition zone (mm) after 24 h[a].

| **Sample** | ***Escherichiac oli*** | ***Staphylococcusaureus*** |
|---|---|---|
| Control | -- | -- |
| CG | -- | -- |
| CG-0.5APS | -- | 7.9 ± 0.2 |
| CG-1.5APS | -- | 10.7 ± 1.5 |
| CG-3.0APS | -- | 10.7 ± 1.0 |
| CG-0.5APS-KMo | -- | -- |
| CG-1.5APS-KMo | -- | -- |
| CG-3.0APS-KMo | -- | 9.8 ± 0.7 |

[a]Average data from triplicate. –Uninhibited

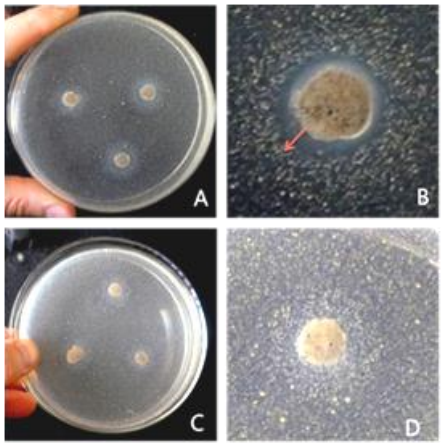


Fig. 6. Pictures of *S. aureus* bacterial culture with the solids (A, B) CG 3.0 APS- KMO ;(C, D) CG, representing ( → ) the inhibition zone.

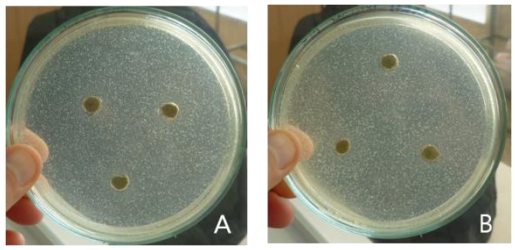


Fig. 7.Photos of *E. coli* bacterial culture whit the solids (A) CG 3.0 APS- KMO; (B) CG.

## 4. Conclusion

Tested solid CG-3,0APS-KMO has presented the highest antimicrobial activity because it proved effective against a larger number of fungal isolates and also to inhibit the growth of one of the bacterial strains used. These preliminary results allow us to think about the next stage where they could evaluate the efficiency of the integrated solid bioactive formulation paint hygienic nature. Should be continued testing new compounds related to recyclables, giving rise to eco-friendly additives.

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