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Biocleaning of Paraloid B72 and B82 acrylic resins: an exploratory study on increasing their bioavailability

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Abstract: Exploratory assays aimed at enhancing the susceptibility of acrylic resins to microbial attack (their bioavailability) and thus facilitating xenobiotic biocleaning were conducted. The capacity of *Comamonas testosteroni, Enterobacter aerogenes* and *Rhodococcus erythropolis* to degrade Paraloid B72 and B82 acrylic resins with different particle sizes was tested in liquid media. Tween® 80, Tween®20 and DMSO were also tested as pretreatment agents with *R. erythropolis* and the largest resin particles. Paraloid degradation was measured by weight loss, and bacterial growth, redox potential and carbon content were also measured. The smaller resin particles appeared more susceptible to microbial attack. *R. erythropolis* was the most promising bacterium for biocleaning. Although Paraloid B72 and B82 have a very similar chemical structure, the latter could be easier to clean than the former. Tween® 80 and to a lesser extent Tween® 20 appeared to increase the susceptibility of the resins to microbial attack.

Keywords: bacteria, biodegradation, bioremoval, cleaning, innovative restoration methods, investigative assays, microbial degradation, synthetic polymers

Biolimpieza de resinas acrílicas Paraloid B72 y B82: un estudio exploratorio sobre el aumento de su biodisponibilidad

Resumen: Se realizaron ensayos exploratorios destinados a incrementar la biodisponibilidad y facilitar así la biolimpieza de xenobióticos. Se evaluó la capacidad de *Comamonas testosteroni, Enterobacter aerogenes* y *Rhodococcus erythropolis* para degradar las resinas acrílicas Paraloid B72 y B82 con diferentes tamaños de partículas en medios líquidos. También se probaron Tween® 80, Tween® 20 y DMSO como agentes de pretratamiento con *R. erythropolis* y las partículas de resina más grandes. La degradación del Paraloid se midió por pérdida de peso, crecimiento bacteriano, potencial redox y contenido de carbono. Las partículas de resina más pequeñas parecían más susceptibles al ataque microbiano. *R. erythropolis* fue la bacteria más prometedora para la biolimpieza. Aunque el Paraloid B72 y el B82 tienen una estructura química muy similar, el segundo podría ser más fácil de eliminar que el primero. Tween® 80 y después, Tween® 20 parecieron aumentar la susceptibilidad de las resinas al ataque microbiano.

Palabras clave: bacteria, biodegradación, biorremediación, limpieza, métodos innovadores de restauración, ensayos de investigación, degradación microbiana, polímeros sintéticos

Introduction

Before the 1960s, the restoration of monuments and works of art was not regulated by technical or legal standards. Due to the destruction of a large part of the cultural heritage in Europe during the Second World War, designing ways of protecting heritage assets became more important in the following thirty years (1946-1976). Thus, the Venice Charter was established in 1964 (see e.g. Atalan 2018), while in 1963, the Italian art theorist Cesare Brandi published *Teoria del Restauro* (Theory of Restoration), a book that changed the way the restoration is understood. This work laid the foundations for modern restoration, leading, in 1972, to the Italian Charter of Restoration and also adoption of the World Heritage Convention at UNESCO, a set of rules that remain in force (see e.g. Santabárbara 2017). At the same time, as noted by Cappitelli *et al.* (2021), the use of synthetic materials (especially vinyl and acrylic resins) in heritage conservation-restoration was almost standard practice. As these materials were considered to have suitable physical properties and are stable, transparent and resistant to (bio)degradation, they gradually replaced the traditional protective materials such as animal

glue, vegetable oils, casein and egg (see e.g. Fort 2007). However, it became apparent that many of the materials were not compatible with the treated substrate and caused changes in appearance, such as colour changes and fractures; the materials were also found to be difficult to remove (Troiano *et al.* 2014, Andreotti *et al.* 2018, Cappitelli *et al.* 2021, Bosch-Roig *et al.* 2023). According to Troiano *et al.* (2014) polymeric films or coatings in heritage works are usually removed by using surfactants or organic solvents such as acetone, xylenes, toluene and alcohols. However, these products are generally toxic, and thus potentially harmful to workers, in addition to being costly and not environmentally sustainable. Biocleaning has been used an alternative treatment in the last few decades. Biocleaning is defined as the use of living organisms and/or their enzymes as cleaning agents by controlled reproduction of their metabolic processes (Weyer *et al.* 2015). In a recent review study, Ranalli and Zanardini (2021) reported that the first research on biocleaning applied to cultural heritage assets dated back to 1970. Thus, Moncrieff and Hempel (1970) used a poultice (probably including the bacteria *Desulfovibrio desulfuricans* and *Desulfovibrio vulgaris*) to remove black crusts (sulphates) and other salts, such as nitrates, from a stone surface. Although substances of natural origin (mainly sulphate and nitrate salts) have since been effectively removed by biocleaning (see e.g. Cappitelli *et al.* 2007, Gioventù *et al.* 2011, Bosch-Roig *et al.* 2013), polymeric coatings of synthetic origin are difficult to remove by biological methods (see e.g. Sanmartín et al. 2014, Bosch-Roig *et al*. 2023). However, some important progress has been made, such as the development of methods to determine the ability of bacterial strains to remove synthetic resins such as Paraloid B72 (Troiano *et al.* 2014), and the search for aerobic bacteria potentially capable of degrading spray paint graffiti (Sanmartín *et al.* 2015, Bosch-Roig *et al.* 2021, Cattò *et al.* 2021) and dammar varnish used in easel paintings (Avogaro *et al.* 2025). Nonetheless, successful removal of synthetic polymers by biocleaning remains challenging, and further efforts are required to enhance the effectiveness of the process.

A key factor in the biological degradation of a compound is its bioavailability (understood here as the accessibility of synthetic polymers or xenobiotics - synthetic chemical substances barely degradable - to the microbial attack). *A priori*, a xenobiotic will be more susceptible to attack if it is in a physical and/or chemical state that allows it to be more readily assimilated and subsequently degraded by microorganisms (Semple *et al.* 2004). It is therefore feasible that compounds with smaller particle sizes and more chemically reactive functional groups will be more susceptible to microbial attack. Microorganisms themselves also influence this susceptibility, as they can produce biosurfactants or extracellular enzymes that improve the solubility of some xenobiotics, such as polycyclic aromatic hydrocarbons (PHAs), crude oils, dyes and pesticides, making them more receptive to biodegradation (Busi and Rajkumari 2017, Ortega-Calvo *et al.* 2020). In bioremediation studies involving contaminated soils, such as those carried out by Benyahia *et al.* (2016) and Gharibzadeh *et al.* (2016), elimination of these types of substances has also been enhanced by using surfactants, detergents and solvents. Use of these compounds has also been extended to the field of cultural heritage, in biocleaning methodologies combined with chemical treatments for removal of unwanted substances. Troiano *et al.* (2013) tested the performance of a nonionic surfactant, Tween® 20, as a pretreatment agent by applying it to the surface of a marble statue (by Lina Arpesani in 1921, which had stood in the courtyard of the *Cimitero Monumentale* in Milan, Italy) prior to biocleaning the statue with the bacterial strain *Desulfovibrio vulgaris* subsp. *vulgaris* ATCC 29579. More recently, Bosch-Roig *et al.* (2021) used Tween® 20 together with two microbial strains isolated from naturally aged graffiti in immersion assays aimed at evaluating graffiti biocleaning, obtaining different results: thus, while the bacterium *Rhodococcus erythropolis* performed best alone, addition of the surfactant to the yeast *Candida parapsilosis* enhanced oxidative degradation of the graffiti.

The Paraloid thermoplastic acrylic resins group (also known as Acryloid in USA) has been and still is extensively used in various applications in the conservation-restoration of rock, metal, glass and ceramics, mainly as an adhesive and consolidant (Vinçotte *et al.* 2019). Some of the most common resins include Paraloid B44, B66, B67, B72 and B82. The differences in chemical composition confer characteristics that enable optimal use of each type of resin with different materials and/or in different roles. Paraloid B44 is a copolymer of ethyl acrylate and methyl methacrylate (EA/MMA) containing 28.0% EA and 70.3% MMA, and a very small amount, around 1% of butyl methacrylate (BMA) (see e.g. Lazzari and Chiantore 2000). It is characterized by a high degree of hardness, making it popular for preserving artworks made of metals such as bronze (Molina *et al.* 2023). Paraloid B66 is a copolymer of around 50% methyl methacrylate and around 50% butyl methacrylate (MMA/BMA), and Paraloid B67 is a homopolymer of poly(isobutyl methacrylate) (PiBMA) (see e.g. Lazzari and Chiantore 2000). Paraloid B66 is primarily used as a maintenance coating on artworks, while Paraloid B67 is used as a varnish on paintings because it is the most water-resistant of these compounds, dries quickly and conserves the original colour of the paint (Chapman and Mason 2003). Paraloid B72 is composed of methyl acrylate and ethyl methacrylate (MA/EMA) and a small amount of 2.2% butyl methacrylate (BMA). Paraloid B72 is the polymer most widely used as a consolidant, adhesive and coating in the field of conservation-restoration, as it is the most thermostable (Molina *et al.* 2023). Paraloid B82 is a copolymer of ethyl acrylate and methyl methacrylate (EA/MMA), with proportions of 43.0% EA and 56.1% MMA, which makes it different from B44 (see

e.g. Lazzari and Chiantore 2000). It is also contained a very small amount, around 1% of butyl methacrylate (BMA). It is distinguished by a lower degree of hardness (softer) and greater flexibility than the other Paraloid resins, and it is the most widely used protective product in the conservation of historic ceramics (Petrėnas *et al.* 2015).

The aim of this preliminary study was to explore ways of enhancing the susceptibility of xenobiotics to microbial attack (the bioavailability), with the aim of improving their removal by biocleaning, which has clear advantages over traditional physical and chemical removal methods (see e.g. Sanmartín *et al.* 2014). In the first assay, the capacity of the bacteria *Comamonas testosteroni*, *Enterobacter aerogenes* and *Rhodococcus erythropolis* (previously shown to be suitable candidates for bioremoval of xenobiotics) to degrade Paraloid B72 and B82 acrylic resins with different particle sizes (length 0.25 mm, 1 mm and 4 mm) was tested. The hypothesis here was that xenobiotics with smaller particle sizes would be more susceptible to bacterial attack (more bioavailable) than bigger counterparts, since smaller particles have a larger surface-to-volume ratio, which promotes bacterial colonisation, forming a biofilm around the Paraloid particle, as well as bio-fragmentation, assimilation, and mineralisation by the bacterium, steps that complete the biodegradation process by a microorganism. Two nonionic surfactants used as detergent additives (Tween® 80 and Tween® 20) and an organic solvent (dimethyl sulfoxide: DMSO) were then each tested as pretreatment agents in a similar experiment but using only *R. erythropolis* and resins with the largest particle size (4 mm). The hypothesis here was that applying a chemical pretreatment would increase the susceptibility of the xenobiotic to bacterial attack (their bioavailability) for a similar reason to the previous assay, as the tested pretreatments reduce the surface tension of hydrophobic Paraloid, increasing its wettability and faciliting the biofilm formation around the Paraloid particle, first step of the biodegradation process. Degradation of the Paraloid resins was measured by weight loss, and bacterial growth, redox potential and carbon content were also measured in the liquid media.

The Figure 1 displays a schematic diagram of the experiments carried out in the present study.

Methodology

— *Xenobiotic materials: Paraloid B72 and B82*

Both bioavailability assays were carried out with the acrylic resins Paraloid B72 and Paraloid B82 [see Figure 2 for their chemical structures] supplied by CTS Spain (https://shop-espana.ctseurope.com/) as small elongated resin droplets about 4 mm long. Particle fractions of length 0.25 mm, 1 mm and 4 mm were prepared, taking as a reference 8 units of 4 mm long (as marketed) corresponding to a weight of 0.700±0.054 g (analytical balance Denver Instruments, Colorado, USA). The 1 mm and 0.25 mm fractions were prepared by grinding in a Goldenwall HC-300 high speed multifunction grinder, and the fractions were separated in sieves of mesh sizes 0.25 mm, 0.5 mm 1 mm and 1.25 mm. All fractions were sterilised by exposure to 37% formaldehyde (Vorquímica S.L., Spain) for 48 hours, according to the protocol described by Sanmartín *et al.* (2015).

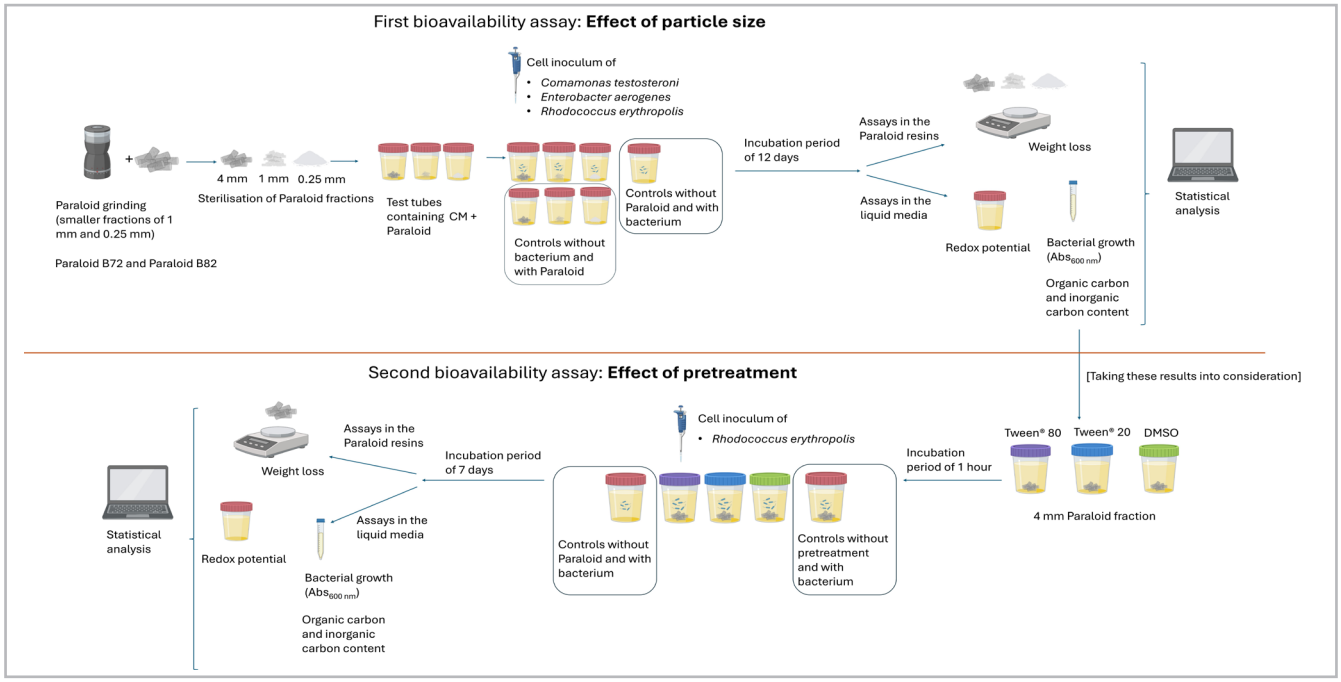

Figure 1.- Scheme of the experimental set-up.

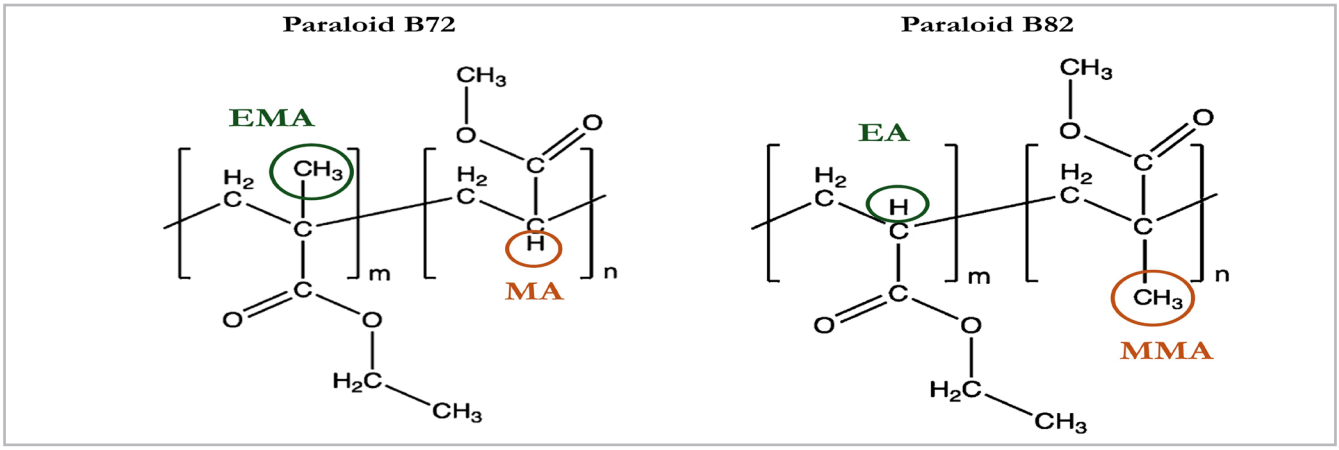

Figure 2.- Chemical structure of Paraloid B72 and B82, where B72 is a copolymer of ethyl methacrylate (EMA) and methyl acrylate (MA) and B82 is a copolymer of ethyl acrylate (EA) and methyl methacrylate (MMA).

— Bacteria selected for immersion assays

The bacteria selected were *Comamonas* (previously *Pseudomonas*) *testosteroni*, *Enterobacter* (previously *Aerobacter*; currently *Klebsiella*) *aerogenes* and *Rhodococcus erythropolis*, which has been demonstrated to be capable of degrading spray paint graffiti (Bosch-Roig *et al.* 2021, Cattò *et al.* 2021). All of the bacteria were identified by 16S rRNA sequences in the EzBioCloud database, before the start of the experiments. They were stored at -80°C in Tryptic Soy Broth (TSB) medium with 25% glycerol, before being reactivated for the experiments, carried out in Petri dishes containing Tryptic Soy Agar (TSA) medium. After growth in the Petri dishes, one colony per bacterium was inoculated in low nutrient (oligotrophic), Complete Mineral (CM) liquid medium (Cattò *et al.* 2021), slightly modified by replacing the small carbon source of sodium citrate with yeast extract, as follows: 10.5 g/L K_2HPO_4, 1.13 g/L KH_2PO_4, 1.0 g/L $(NH_4)_2SO_4$, 0.5 g/L yeast extract, 0.25 g/L $MgSO_4 \cdot 7H_2O$, and 0.015 g/L $CaCl_2 \cdot H_2O$. Both bioavailability assays were carried out in sterile 50 mL test tubes containing 25 mL of CM liquid medium. In the test tubes with bacteria, 2.5 mL (10% of the total volume) of pure CM liquid culture of each bacterium, with an absorbance of 1.0 r.u. at a wavelength of 600 nm (Wichatham *et al.* 2024), was added.

— First bioavailability assay: Effect of particle size

Comparative tests varying the particle size were carried out with Paraloid B72 and B82, of length 0.25 mm, 1 mm and 4 mm. Immersion assays were carried out in CM liquid medium for a total of 12 days under aerobic conditions, darkness and gentle agitation at 30 °C. The assays were performed in triplicate, and included controls without the bacterium and with Paraloid, and controls without Paraloid and with the bacterium.

— Second bioavailability assay: Effect of pretreatment

Comparative tests were carried using only *R. erythropolis* and the Paraloid resins of the largest particle size (4 mm long), along with the surfactants Tween® 20 (Panreac, Spain) and Tween® 80 (Panreac-AppliChem, Spain) and the solvent dimethyl sulfoxide (DMSO) (Panreac-AppliChem, Spain). The pretreatments were carried out in aqueous solutions (25 mL) containing 1.5% of DMSO as this is the amount recommended for removal of Paraloid, according to the GE-IIC (https://www.ge-iic.com), and the same amount of Tween® 20 and Tween® 80, for 1 hour, darkness and agitation at 30 °C (Furukawa *et al.* 2018). The Paraloid particles were then washed with sterile distilled water until complete removal of residues of the pretreatment agents, and inoculated in CM liquid medium for a total of 7 days under aerobic conditions, darkness and gentle agitation at 30 °C in an assay similar to the first. Tests were performed in triplicate, and included controls without pretreatment and with bacterium, and controls without Paraloid and with bacterium.

— Weight loss of treated Paraloid resins

At the end of both assays, the percentage weight loss was calculated as follows: [(wo-wf)/wo]x100, where wo is the weight at the beginning of the assay and wf is the weight at the end of assay. At the end of the assays, the Paraloid particles were first treated to remove any bacterial biofilm or other adhered compounds before being weighed. Thus, the liquid medium was removed and benzalkonium chloride (3%, 20 mL) was added to the Paraloid particles before incubation for 24-hours with shaking. The wash was then removed, sodium dodecyl sulphate (3%, 20 mL) was added, and the samples were incubated for another 24 hours under the same conditions. A final wash with distilled water was carried out under the same conditions as before, and the samples were rinsed with distilled water until complete removal of residues. The Paraloid particles were dried at 50°C for two days.

— Bacterial growth, redox potential and carbon content in the liquid media

Bacterial growth, measured as absorbance at 600 nm, was determined in a UV-VIS T8DCS spectrophotometer (Persee, China). The redox potential (ORP) was measured with a HI98194 multiparameter device (Hanna Instruments). Dissolved organic

carbon (DOC) and dissolved inorganic carbon (DIC) were measured in a TOC-5000 meter (Shimadzu, Japan).

— *Statistical analysis*

All data were analysed using R. Studio software (version 2024.12.0 + 467). The Shapiro-Wilk and Levene tests were used to verify data normality and homogeneity of variances, respectively. As some parameters did not meet the requirements for data normality or homogeneity of variance, non-parametric statistics were used. The Kruskal-Wallis test and the Conover post-hoc test were used for between group comparisons.

Results and discussion

—*First assay: Effect of particle size*

• *Weight loss*

Weight loss is probably the most reliable parameter for determining the biodegradation of plastic materials (see e.g. Nanthini Devi *et al.* 2021). Comparison of material of the same particle size did not reveal any significant differences between the samples of both Paraloid resins and the controls without bacteria [Figure 3]. However, for both resins, the weight loss of the 0.25 mm fraction was significantly higher than for the 4 mm and 1 mm fractions in all cases. Compared to the controls and the other two bacteria, *R. erythropolis* appeared to cause greatest weight loss in the smallest fraction of Paraloid B72 (0.25 mm), although overall Paraloid B82 had greater weight losses.

• *Bacterial growth*

The absorbance values at 600 nm [Figure 4] were significantly higher in the presence than in the absence of bacteria, and they were also higher in the presence than in the absence of Paraloid resin.

• *Redox potential*

The ORP values [Figure 5] decreased significantly in the samples with *R. erythropolis*. The lower ORP values may be

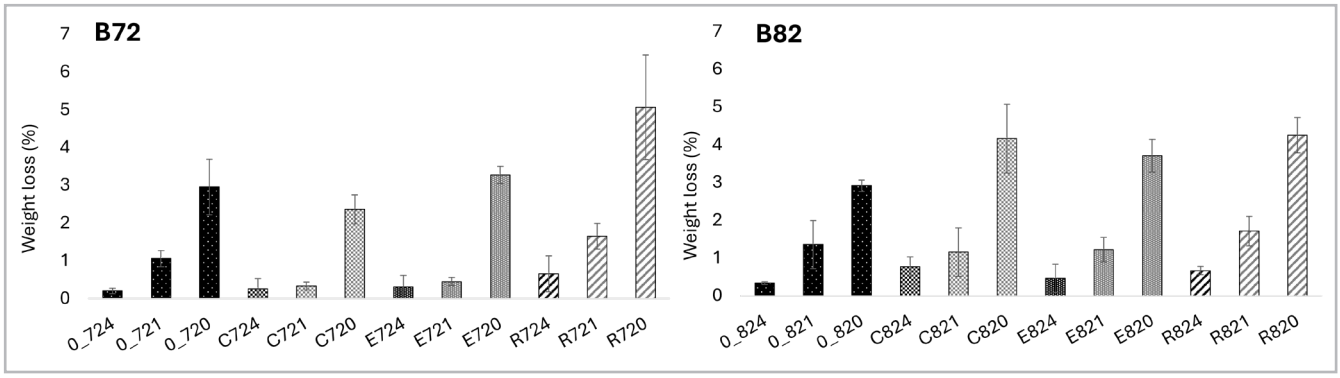

Figure 3.- Weight loss (%) of Paraloid B72 (B72) and Paraloid B82 (B82) at the end of the first bioavailability assay. Controls without bacterium and with Paraloid (0_), samples with *C. testosteroni* (C), samples with *E. aerogenes* (E), samples with *R. erythropolis* (R). Fraction sizes: 0.25 mm (0), 1 mm (1) and 4 mm (4). Histograms represent mean values and lines represent the standard deviation. No significant differences were found compared to controls.

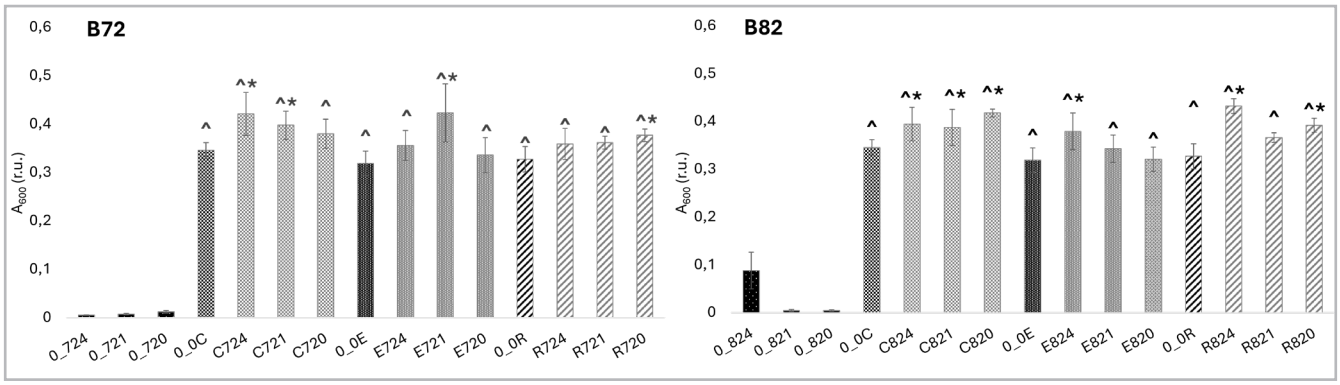

Figure 4.- Absorbance at 600 nm (r.u.) of the liquid media with Paraloid B72 (B72) and Paraloid B82 (B82) at the end of the first bioavailability assay. Controls without bacterium and with Paraloid (0_), controls without Paraloid and with bacterium (0_0), samples with *C. testosteroni (C), samples* with *E. aerogenes (E), samples* with *R. erythropolis (R)*. Fraction sizes: 0.25 mm (0), 1 mm (1) and 4 mm (4). Histograms represent mean values and lines represent the standard deviation. The asterisks (*) indicate significant differences relative to controls 0_0 ($p \leq 0.05$). The arrows (^) indicate significant differences relative to controls 0_ ($p \leq 0.05$).

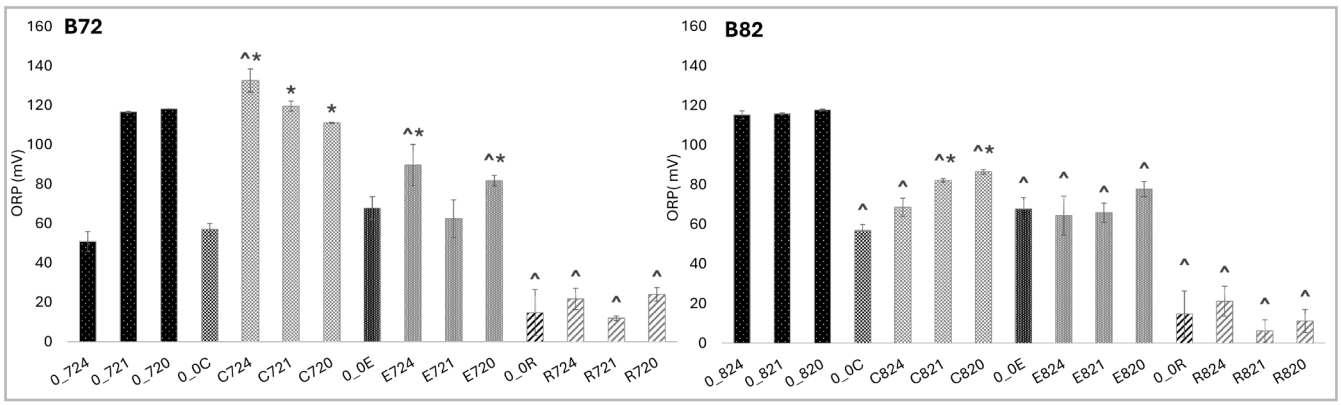

Figure 5.- Oxidation-reduction potential (mV) of the liquid media with Paraloid B72 (B72) and Paraloid B82 (B82) at the end of the first bioavailability assay. For explanation of types of controls and samples, abbreviations and significant differences relative to the controls and significance level, see legend of Figure 4.

related to the higher metabolic activity of *R. erythropolis* (Reichart *et al.* 2007). In samples with *C. testosteroni* and Paraloid B72, the ORP values increased significantly relative to both controls, without bacterium (in the 4 mm fraction) and without Paraloid (all fractions). In samples with Paraloid B82, the ORP value was significantly lower in all liquid media with bacteria than in the controls.

•*Organic carbon and inorganic carbon content*

For Paraloid B72, there was little variation in the DOC in the samples with bacterium relative to the control without bacterium [Figure 6], except for *R. erythropolis*, with significantly lower DOC values, presumably related to the biodegradation of Paraloid (Cattò *et al*. 2021).

The DIC values were significantly higher in the samples with bacterium than in the control without bacterium [Figure 6]. This result is related to the absorbance at 600 nm value [Figure 4], and it may be indicative of bacterial growth and metabolic activity. For both Paraloid resins, significant differences relative to the control without Paraloid were found in *C. testosteroni* and *R. erythropolis*, with the latter causing higher DIC values in Paraloid B72. *Rhodococcus* sp. ZCONT (KY697119) was recently used to remove Primal resin from frescoes in the Carracci Gallery at the Palazzo Farnese in Rome and traces of Paraloid

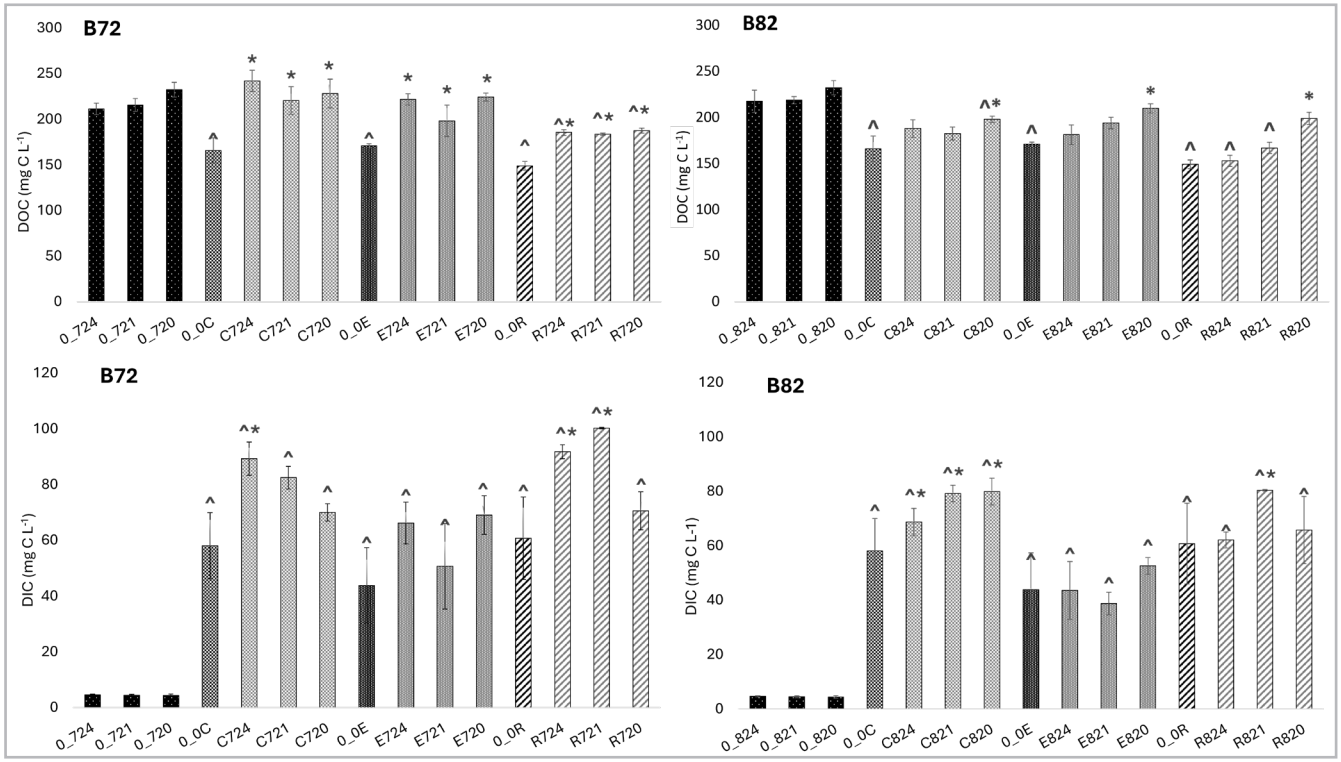

Figure 6.- Dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) (mg C L^{-1}) in the liquid media with Paraloid B72 (B72) and Paraloid B82 (B82) at the end of the first bioavailability assay. For explanation of types of controls and samples, abbreviations and significant differences relative to the controls and significance level, see legend of Figure 4.

B72 from the "Bacchus with basket" artwork, a Roman statue in Greek marble, property of the Turin Museum of Antiquities (Sprocati *et al.* 2021).

—*Second assay: Effect of pretreatment*

• *Weight loss*

There were no significant differences between the samples or the controls regarding the pretreatment [Figure 7], perhaps due to an insufficient incubation period of 7 days. However, Tween® 80 appeared to increase the susceptibility of Paraloid B72 to degradation by *R. erythropolis* to the greatest extent. A similar effect was observed in Paraloid B82 after pretreatment with Tween® 20.

• *Bacterial growth*

Regarding absorbance values at 600 nm [Figure 8], absorbance was significantly higher in the culture with Paraloid B72 but without any pretreatment than in controls without Paraloid and with bacterium. For Paraloid B82, the highest absorbance values were obtained with the Tween® 80 pretreatment, being together with the *R. erythropolis* without pretreatment significantly higher than controls without Paraloid and with bacterium.

• *Redox potential*

For the ORP measurement [Figure 9], no significant differences were found between samples and controls. However, when

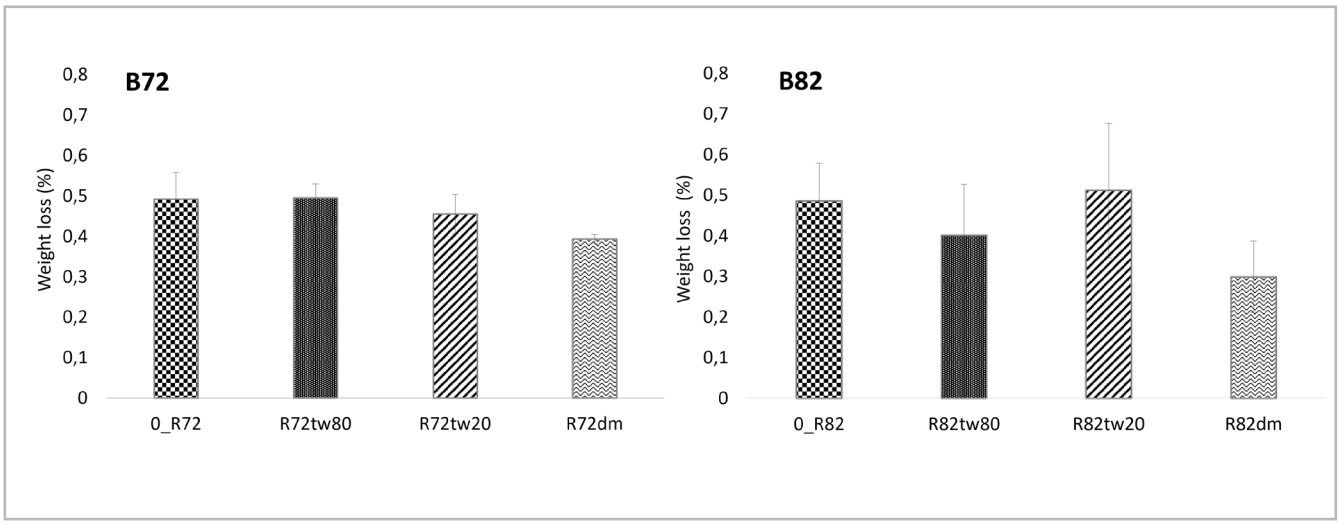

Figure 7.- Weight loss (%) of Paraloid B72 (B72) and Paraloid B82 (B82) of length 4 mm with and without R. erythropolis (R) at the end of the second bioavailability assay. Controls without pretreatment and with bacterium (0_), pretreatment with Tween® 80 (tw80), pretreatment with Tween® 20 (tw20), pretreatment with DMSO (dm). Histograms represent mean values and lines represent the standard deviation. No statistically significant differences were found relative to controls or between treatments.

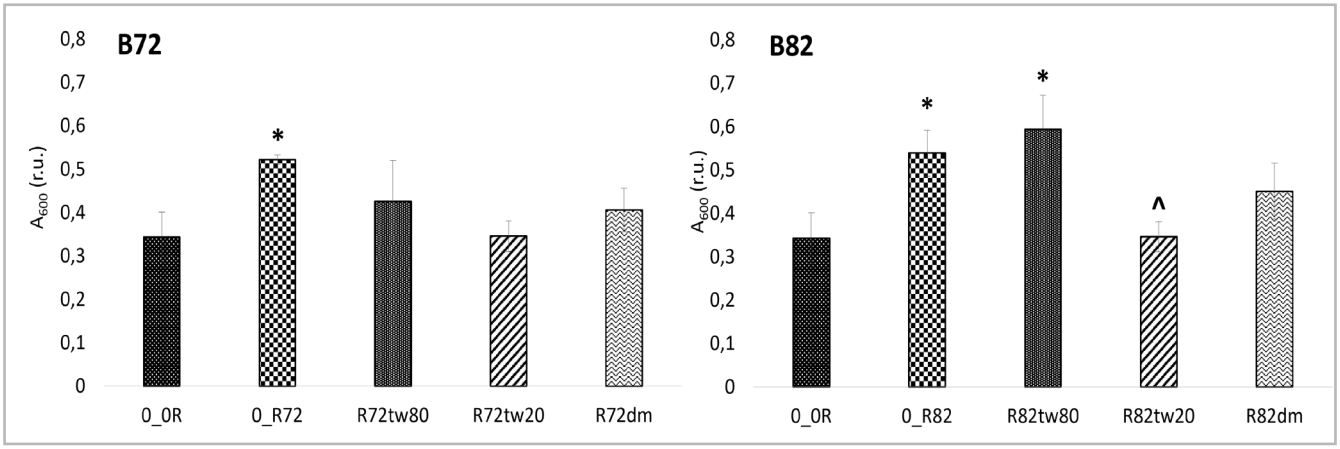

Figure 8.-Absorbance at 600 nm (r.u.) of the liquid media with Paraloid B72 (B72) and Paraloid B82 (B82) of 4 mm long and with *R. erythropolis* (R) at the end of the second bioavailability assay. Controls without Paraloid and with bacterium (0_0), controls without pretreatment and with bacterium (0_), pretreatment with Tween® 80 (tw80), pretreatment with Tween® 20 (tw20), pretreatment with DMSO (dm). Histograms represent mean values and lines represent the standard deviation. The asterisks (*) indicate significant differences relative to controls 0_0R ($p \leq 0.05$). The arrows (^) indicate significant differences compared to controls 0_R ($p \leq 0.05$).

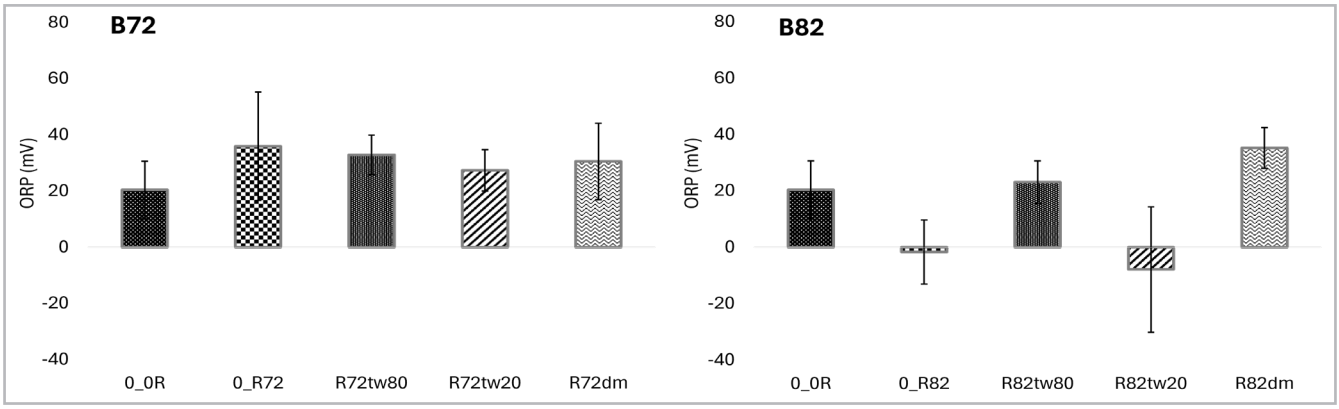

Figure 9.-Oxidation-reduction potential (mV) of the liquid media with Paraloid B72 (B72) and Paraloid B82 (B82) of particle size 4 mm and with *R. erythropolis* (R) at the end of the second bioavailability assay. For explanation of types of controls and samples, abbreviations and significant differences compared to the controls and significance level, see legend of Figure 8.

compared with the ORP values from the first assay [Figure 5], they remained low and, in this case, negative for the culture without pretreatment and for Tween® 20 in Paraloid B82. This may indicate that R. erythropolis reaches ORP values between 35 mV and -7 mV when grown in oligotrophic CM medium with or without the presence of Paraloid. [Figure 9].

• *Organic carbon and inorganic carbon content*

The DOC measurements for Paraloid B72 reached significantly higher values than controls without Paraloid and with bacterium [Figure 10]. For Paraloid B82, only the pretreatment with Tween® 20 was significantly higher than controls without Paraloid and with bacterium. The DOC was higher in this assay than in the first assay [Figure 6], where the values reached were around 250 mg C L^{-1} in the first and 800 mg C L^{-1} in the second assay. As the incubation period was shorter, it is possible that the DOC in the medium was not metabolized by the bacteria or that the DOC was not completely bioavailable.

By contrast, the DIC values were similar to those obtained in the first assay [Figure 6], fluctuating around 100 mg C L^{-1},

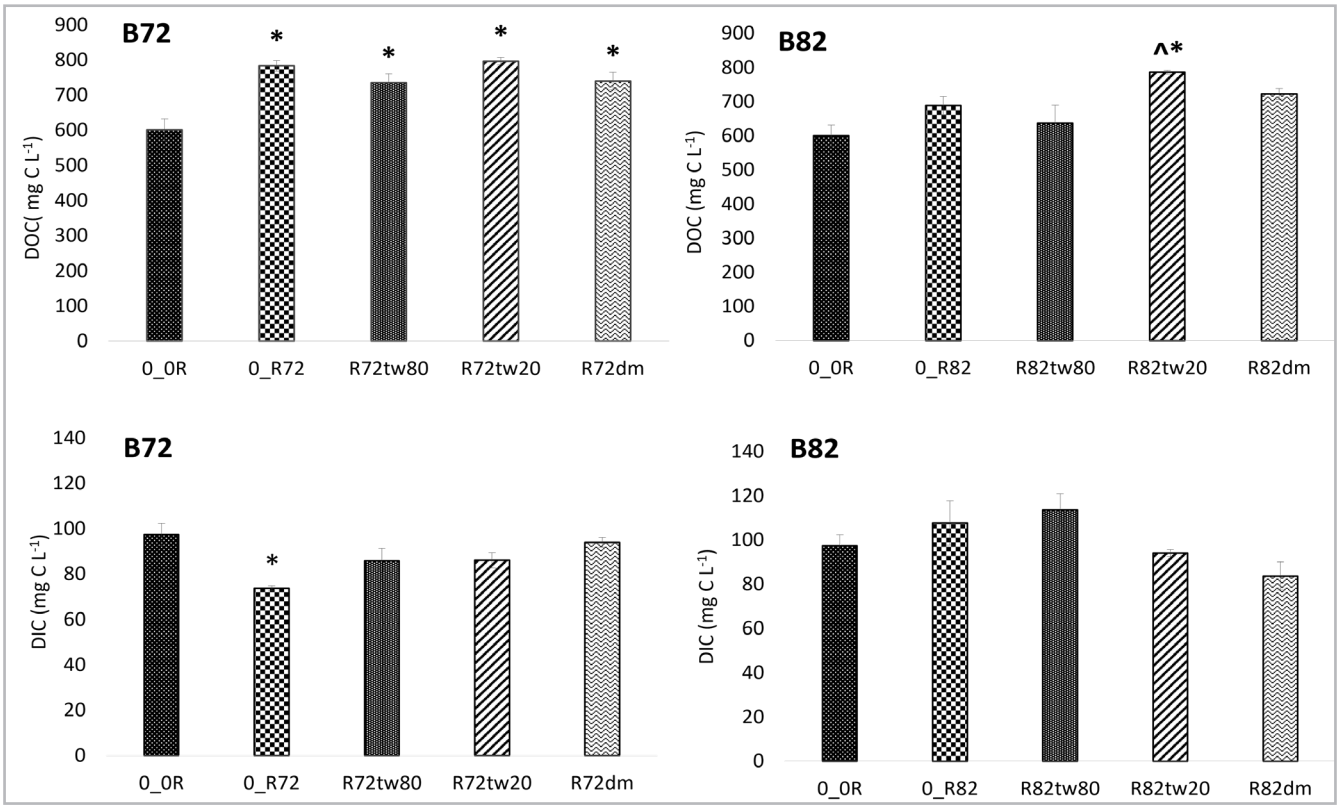

Figure 10.- Dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) (mg C L^{-1}) in the liquid media with Paraloid B72 (B72) and Paraloid B82 (B82) of length 4 mm and with *R. erythropolis* (R) at the end of the second bioavailability assay. For explanation of types of controls and samples, abbreviations and significant differences relative to the controls and significance level, see legend of Figure 8.

which indicates similar metabolic activity in these cultures. The highest DIC values for Paraloid B72 were obtained after pretreatment with DMSO, while the highest values for Paraloid B82 were obtained after pretreatment with Tween® 80 [Figure 10]. However, the only significant difference was a decrease in DIC for the Paraloid B72 with *R. erythropolis* culture without pretreatment.

In this test, Tween® 80 appeared to facilitate the susceptibility of the resins to microbial attack to a greater extent than the other pretreatments. Regarding the pretreatment agents, the results suggest that they act differently. This applies to weight loss [Figure 7], as pretreatment with Tween® 20 yielded a higher value in Paraloid B82 and Tween® 80 pretreatment yielded a higher value in Paraloid B72. However, in the biocleaning with *R. erythropolis* the absorbance values [Figure 8] and DOC [Figure 10], were scarcely affected.

Conclusions

1. Small particles of Paraloid B72 and B82 resins appear to be more susceptible to microbial attack than larger particles. However, the use of particles smaller than 1 mm increases the possibility of error in the tests, as well as not accurately representing the real conditions found in interventions on heritage assets, where the coatings are not as fragmented. Bacterial growth was higher in the presence than in the absence of Paraloid resin. The lower redox potential values, related to a higher metabolic activity, were found in the samples with *R. erythropolis*. Only *R. erythropolis* showed significantly lower DOC values, presumably related to the biodegradation of Paraloid. So, among the bacteria tested, *Rhodococcus erythropolis* showed the greatest potential for removal by biocleaning of both Paraloid resins. Paraloid B82 may be easier to removal than Paraloid B72 despite both having a very similar chemical structure.

2. Among the three pretreatment agents evaluated, Tween® 80 appeared to perform best for enhancing the biocleaning process, followed by Tween® 20. In addition, Tween® 80 appeared to work better with Paraloid B72 and Tween® 20 with Paraloid B82.

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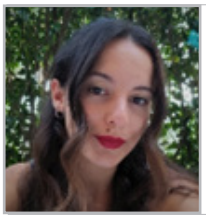

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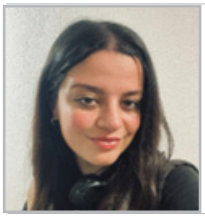

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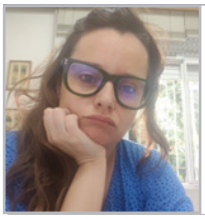

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