Sandra G Gómez de Saravia and *Silvia E Rastelli*, Facultad de Ciencias Naturales y Museo (UNLP) and *Marisa Viera*, Facultad de Ciencias Exactas (UNLP), have conducted research into the use of natural compounds in paints to restrict algae growth. Here, *Sandra G Gómez* explains more

Natural compounds as additives in paints for controlling algal growth

Algae are a diverse group of photosynthetic organisms, ranging from microscopic single-cell micro-organisms to very large organisms, such as seaweed. Microalgae belonging to Chlorophyta (green algae) and Cyanophyta (blue-green algae) commonly occur in biofilms. In actual fact, these phototrophic biofilms are complex microbial communities formed by cyanobacteria, microalgae and heterotrophs[1] all embedded in a mucilaginous matrix of exopolymeric substances (EPS), mainly composed of polysaccharides ranging between 50–90%[2].

Phototrophic biofilms can produce an aesthetic effect and deterioration of a building's painted surfaces[3]. It is a common practice to treat these surfaces with mechanical brushes and/or biocides in order to eradicate the micro-organisms present. Several chemicals have been used for this purpose, such as acids, pyridines[4], quaternary ammonium salts[5] and organometallic compounds[6]. However, some of these products have been banned over time due to their associated environmental and health hazards[7].

An alternative to those compounds, is the use of ecofriendly natural substances with known biocidal properties. The approach of using natural substances and herbs has been gaining prominence in the field of cultural heritage and conservation sciences since the 2000s[8]. Here we present the evaluation of the algaecide properties of isoeugenol, vanillic acid and carvacrol incorporated in an acrylic waterborne paint formulation.

BIOASSAYS

Phototrophic biofilm developed on a painted external wall was scrapped **(Figure 1)**. The sample was transported to the laboratory in a sterile condition and transferred to BG11 broth medium for maintenance of the algae community. Identification of the taxa to the genus level was based on the morphology of the individual cells, following microscopic examination and the use of literature data[9,10].

The algaecide properties of three pure organic compounds (Sigma-Aldrich, USA) present in diverse plants were evaluated. **Table 1** shows some of their characteristics. The micro-atmosphere test was used to evaluate the algaecide activity of isoeugenol, vanillic acid and carvacrol[11]. For this, 200μl of a seven day old algae culture containing 3.3 10^5 algae ml^{-1} was inoculated uniformly in a petri dish with BG11 agar. A sterile disk of filter paper (13mm) was placed on the centre of the inverted Petri dish lid and loaded with 50μl of the pure tested compound, or a dilution in DMSO from 125–1000mM (millimolar). The petri dishes were incubated under controlled photo period conditions (16/8hr light/darkness) and 25ºC for 28 days. Growth control plates were made without filter paper and with filter paper moistened with DMSO to discard inhibition of the algal growth due to the solvent. All the tests were done in duplicate.

An exterior waterborne paint was formulated[12] and the compounds that exhibited algaecide activity (isoeugenol and carvacrol) were incorporated separately into the base paint (2%w/w). The presence of the different compounds in the formulation was monitored by ATR FTIR using a Spectrum One spectrometer (Perkin Elmer, USA). The paints were applied by brushing on specimens of filter paper.

After applying three coats of paint, painted specimens were left to dry for seven days in a laboratory environment. Once dried, the samples were cut into squares (2.8cm side) for bioassays. Samples of painted filter paper were inoculated in spray form ,with the phototrophic community given an initial number of approximately 1.4 10^5 algae ml^{-1}. Then, three papers painted with the same type of paint were placed in petri dishes with BG11 agar and incubated under controlled conditions (16/8hr

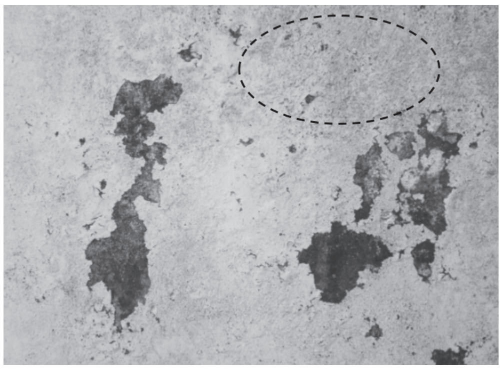


Figure 1. Painted wall with biodeterioration signs. The circle encloses the sampled zone

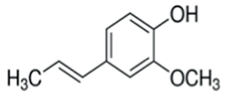
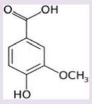
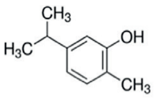
Compound and chemical structure	Characteristics
Isoeugenol 	It is a phenylpropene, a propenyl-substituted guaiacol. It is produced in the essential oils of plants, such as ylang-ylang (*Canangaodorata*). It can be synthesised from eugenol.
Vanillic acid 	It is an oxidised form of vanillin. It is also an intermediate in the production of vanillin from feluric acid.
Carvacrol 	It is a monoterpene phenol. It is found in essential oils, such as oregano (*Origanummajorana*) and thyme (*Thymus vulgaris*).

Table 1. Characteristics of the natural organic compounds tested

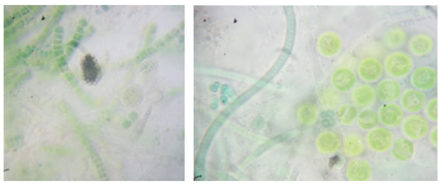


Figure 2. Coccoids and filamentous microalgae present in the phototrophic biofilm

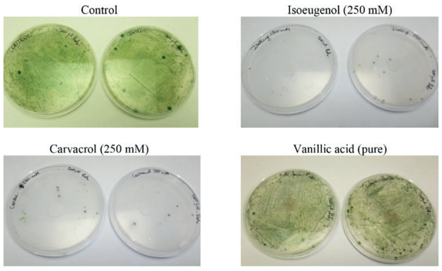


Figure 3. Results of the micro atmosphere tests after 28 days of exposure to different concentrations of the natural compounds

light/darkness, 25ºC) for 28 days. The test was performed in duplicate.

ALGAECIDE ACTIVITY

Observations with an optic microscope revealed that the biofilm is constituted by diverse genera of phototrophic organisms. The taxa determined belong to the Division Cyanophyta (*Xenococcaceae* spp., *Chroococcussp*.aff. *C. varius, Aphanocapsa* sp., *Pseudocapsasp*.aff. *P. dubia, Aphanothece* sp., *Rhabdogloea* sp., *Leptolyngbya* sp. aff. *L. compacta*); Division Chlorophyta (*Chlorococcum* sp. aff. *Chlorella* sp., *Apatococcus lobatus*); and Division Streptophyta (*Klebsormidium* sp. aff. *K. fluitans*).

Organisms belonging to these taxa were reported in buildings in Latin America and in Europe[3]. Some of the phototrophic micro-organisms found in the sample are shown in **Figure 2**.

Figure 3 shows the results of the micro-atmosphere test at the end of experiment.

As it can be seen, carvacrol (250mM) and isoeugenol (250mM) showed an almost complete inhibition of the growth, while vanillic acid (even pure) was not effective in preventing the algal growth.

Taking into account the results of the micro-atmosphere tests, isoeugenol and carvacrol were incorporated into the waterborne paint. As it can be seen in **Figure 4**, the development of algal biomass only occurred in the control samples (paint without biocides). The growth of microalgae was completely inhibited by the addition of carvacrol or isoeugenol at 2%w/w to the paint.

REMARKS

Carvacrol and isoeugenol showed good algaecide properties when incorporated into a paint formulation. In this way, they could be used in the formulation of environmental friendly exterior waterborne paints to avoid paint deterioration by algae.

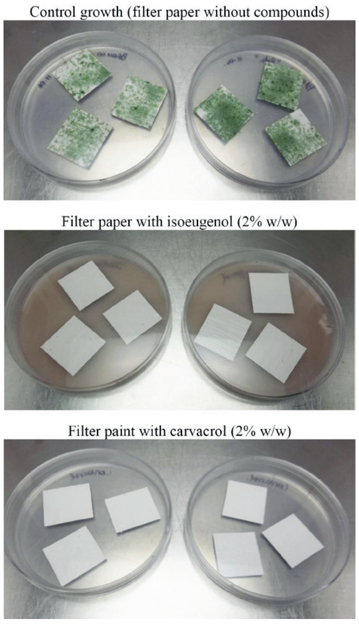


Figure 4. Results of the incubation of the filter papers painted with the control paint or paints with 2% of the natural compounds

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