However, reports about the application of EOs in cultural heritage conservation, as a valid alternative.1 to traditional biocides, are still very limited to in vitro applications and need an application "on-field" to test the effectiveness, the proper way of application, and a monitoring strategy to define the efficiency over time. In vitro analysis is limited as biofilms are known to have different biological properties on stone surfaces than in suspension [10]. In addition, other factors such as the surface of application, the environment, and the weather could influence the effectiveness of essential oils in the field. The aim of this study was to test a combination of treatments, especially focusing on evaluating the effectiveness of selected EOs according to their biocidal activity as defined by the literature, through the application in a real case study [1,38–40]. Pure EOs and mix of EOs were compared to a conventional product based on QACs and two commercially green products based on EOs, which were taken as references. The monitoring of their effectiveness was assessed when applied on a white and black mosaic in the XIX room of "Insula delle Muse" in the Archaeological Park of Ostia Antica (Rome).The biocidal effect of many EOs has already been tested, such as for: Origanum vulgare, Rosmarinus officinalis, Lavandula angustifolia, Thymus vulgaris, Allium sativum, Pimpinella anisum, Eugenia caryophyllata, Calamintha nepeta, Cinnamum zeylanicum, Carum copticum, S. aromaticum, Citrus sinensis, Melaleuca alternifola, Cuminum cyminum, Eucalyptus globulus etc.In addition, Tabata and colleagues [14] reported Pseudomonas species to be responsible for the degradation of chloride compounds, contained in QACs, since microor- ganism consortia can feed on carbon sources from organic residuals contained in QACs, thus encouraging recolonization.Traditionally, the biocides derive from chemical products including acids, pyridines, quaternary ammo- nium salts, and organometallic compounds, with benzalkonium chloride, permethrin, and sodium fluoride [1] that are dangerous for human health and for the environment [9].This was apparent in the reported case study, in the Cave of Lascaux (France), where the fungus Fusarium solani during repeated treatments gained resistance, hence favoring the spread of other microorganisms (e.g., Ochroconis lascauxensis, Ralstonia spp.Stupar and colleagues (2014) [37], applying the biocide activity of Origanum vulgare, Rosmarinus officinalis, and Lavandula angustifolia EOs, against fungal strains isolated from different artifacts, discovered that O. vulgare showed the strongest inhibitory response.Ketones are the main constituents of sage oil (thujone, camphor) and peppermint oils (menthone, carvone), while fennel, eucalyptus and rosemary oils are rich in ethers: an ethole and 1,8-cineole, respectively.According to the colonizing organisms and the characteristics of the inter- ested area (surfaces, cracks, or pores), the entity of the damage changes, thus varying from exterior damage to irreversible disintegration of the inner substrate [4].EO configuration depends on many factors including the genotype (species, cultivation and ecotype), the ecological factors (geographic origin, climate, and soil characteristics), and processing techniques [22].EO activity in affecting microbial growth takes place through different pathways: indeed, EOs inhibit growth by affecting cytoplasmic membrane integrity, influencing cellular metabolism, affecting enzymatic activity, and impacting protein synthesis [13,30–32].Veneranda et al. [1] analyzed 10 different EO constituents that ratified thymol (Thyme sp. EO), eugenol (Clove sp. EO) and cinnamaldehyde (Cinnamon sp. EO) as the best enduring inhibitors.Department of Biology, Ecology and Earth Science DIBEST, University of Calabria, Via Pietro Bucci, * Correspondence: info@yococu.com Methods Protoc.2022, 5,

.[37.[1,16,29,33–36].