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Environmental Trade-Offs Between Essential Oil and Quaternary Ammonium Biocides in Cultural Heritage Conservation

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Abstract

Quaternary ammonium compounds (QACs) have dominated biocidal practice in cultural heritage conservation for decades, yet growing evidence of environmental persistence, aquatic ecotoxicity, and antimicrobial resistance induction has prompted the search for safer alternatives. Essential oils (EO) have emerged as promising bio-based biocides, though their environmental performance has rarely been quantified through rigorous life cycle approaches. This study presents a comparative Life Cycle Impact Assessment (LCIA) of EO-based and QAC-based biocidal formulations across representative conservation scenarios, following ISO 14040/14044 standards and the Environmental Footprint 3.1 methodology with USEtox® 2.1 characterization factors. Three complementary functional units were employed: formulation-based, surface-based, and intervention-based. The results reveal a fundamental trade-off: EO-based systems exhibit 81% higher climate change impacts but 82–89% lower human toxicity and freshwater ecotoxicity impacts compared to QAC-based systems. Surface-normalized comparisons reduce the climate gap to 32%, while toxicity advantages remain robust across all sensitivity scenarios. Monte Carlo analysis confirms the robustness of toxicity findings ($p > 99\%$), whereas climate comparisons remain scenario-dependent. These findings support context-dependent adoption of EO-based biocides in conservation practice and demonstrate that EO-related climate impacts are technically mitigable, while QAC toxicity is intrinsic to their molecular structure.

Keywords: life cycle impact assessment (LCIA); essential oils; quaternary ammonium; biocides; toxicity; cultural heritage conservation; Environmental Footprint

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1. Introduction

Biodeterioration represents one of the major deterioration processes affecting cultural heritage materials. The colonization of artistic and historical substrates by bacteria, fungi,

algae, and lichens leads to aesthetic alterations, physical damage, and chemical weathering, progressively compromising the integrity and legibility of cultural assets [1–4]. Both organic and inorganic materials—including stone monuments, archaeological mosaics, wooden artifacts, paper archives, and leatherbound manuscripts—are susceptible to microbial attack under favorable environmental conditions [5–9].

Quaternary ammonium compounds (QACs) have dominated biocidal practice in cultural heritage conservation for decades. Products such as benzalkonium chloride (BAC), cetylpyridinium chloride (CPC), and didecyldimethylammonium chloride (DDAC) offer broad spectrum antimicrobial activity against bacteria, fungi, and algae, combined with relatively low acute toxicity and ease of application [10–13]. The antimicrobial action of QAC relies on their positively charged quaternary nitrogen interacting with negatively charged phospholipid head groups in microbial cell membranes, leading to membrane disruption, leakage of intracellular contents, and cell death [14]. Commercial formulations such as Preventol® RI50 (*LANXESS Deutschland GmbH, Cologne, Germany*), Biotin® R (*CTS S.r.l., Altavilla Vicentina, Italy*), and NewDes® 50 (*C.T.S. S.r.l., Altavilla Vicentina, Italy*) remain widely recommended in conservation protocols and guidelines [15–17].

However, mounting evidence has raised serious concerns about the environmental and health implications of QAC-based biocides, with QACs classified as "chemicals of emerging concern," after being detected in human blood, breast milk, and indoor dust at significantly elevated concentrations following the COVID-19 pandemic, during which disinfectant use increased dramatically [18–20]. Approximately 75% of QACs used in consumer and industrial applications are ultimately released to wastewater treatment systems, where they persist, adsorb to biosolids, and can be detected in receiving waters and agricultural soils [11,21]. European regulatory assessments report freshwater predicted no-effect concentrations (PNECs) of 0.415 μg/L for BAC (C12–C16) and 1.1 μg/L for Diallyl Dimethyl Ammonium Chloride (DADMAC, C10:C10), thresholds that are approached or exceeded in some monitored surface waters [18]. Beyond environmental persistence, QAC exposure has been linked to the induction of antimicrobial resistance through multiple mechanisms that documented subinhibitory QAC concentrations promoting the development of resistance and cross-resistance with clinical antibiotics including ciprofloxacin, tetracycline, chloramphenicol, and vancomycin. Resistance mechanisms include upregulation of efflux pumps (qacA, qacB, qacC, qacE, qacH genes), reduced membrane permeability, and horizontal gene transfer of resistance cassettes via class 1 integrons [10,21,22]. Low-concentration BAC exposure (0.1 mg/L) increased conjugative transfer frequency of RP4-resistant plasmids in *Escherichia coli* by up to 15-fold [23]. These findings have profound implications for conservation practice, where QAC residues may persist on treated surfaces and select for resistant microbial populations [24]. Toxicological studies have further documented concerning effects at cellular and organismal levels, reporting that QAC exposure induces mitochondrial dysfunction, oxidative stress, reproductive toxicity, and immunomodulation in mammalian systems at concentrations relevant to occupational and consumer exposures [25,26]. Aquatic ecotoxicity data reveal 50% lethal concentrations (LC50s) as low as 64 μg/L for BAC and 1 μg/L for DADMAC in freshwater fish species, with chronic toxicity thresholds (NOEC) of approximately 32 μg/L [27]. These concerns have prompted regulatory scrutiny under REACH and the EU Biocidal Products Regulation, with increasing pressure to identify safer alternatives. Plant essential oils (EO) have emerged as promising bio-based alternatives for biodeterioration control in cultural heritage conservation. Extensive research has demonstrated the antimicrobial efficacy of EO against bacteria, fungi, and algae commonly responsible for heritage deterioration [28–32]. Thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*), lavender (*Lavandula* spp.), and tea tree (*Melaleuca alternifolia*) oils have shown

particularly promising activity, with minimum inhibitory concentrations (MIC) ranging from 0.015% to 0.2% v/v against heritage-relevant microorganisms [33–35]. The antimicrobial action of EO is attributed to terpene and phenylpropanoid constituents, including thymol, carvacrol, eugenol, and cinnamaldehyde, which disrupt microbial membrane integrity and inhibit enzymatic activity [36–39]. Recent field applications have validated EO efficacy under real-world conservation. Macchia et al. [40] compared EO-based and QAC-based treatments on archeological marble surfaces at the Ostia Antica site, demonstrating comparable biocidal effectiveness with EO mixtures of thyme, pine tree, and tea tree. Antonelli et al. [41] applied alginate hydrogels containing Thymus vulgaris EO to mosaic surfaces, achieving effective biofilm removal without chromatic alteration ($\Delta E^* < 3$). Ranaldi et al. [42] demonstrated that alginate hydrogels containing 5–10% Thymus vulgaris EO effectively removed fungal colonization from glazed ceramic tiles, with alginate outperforming polyvinyl alcohol (PVA) as the encapsulating matrix documented EO efficacy against microorganisms isolated from archive documents [43], while Sterflinger et al. [44] demonstrated vapor-phase EO application against fungal deterioration of wooden artifacts.

Despite the growing body of evidence supporting their efficacy, the assumption that "natural" alternatives are inherently more sustainable than synthetic chemicals warrants critical examination. EO production is energy-intensive, requiring substantial inputs for biomass cultivation, harvesting, and extraction [45–48]. Moura et al. quantified the life cycle energy demand and carbon emissions associated with the extraction of rosemary (*Rosmarinus officinalis*) EO [49]. They reported cumulative energy demand ranging from 166 to 10,992 MJ per gram of extract and carbon footprints between 8 and 541 kg CO_2-eq/g depending on the extraction method and operational scale. Steam distillation, which is the predominant industrial technique used, requires 20–45 kWh per kg of plant material processed and 1.6–3.6 L of water per gram of extract produced. Laboratory-scale operations typically exhibit substantially higher environmental impacts per unit of output than industrial-scale facilities, primarily due to the absence of economies of scale. Life Cycle Assessment (LCA) provides a systematic methodology for quantifying and comparing environmental impacts across products life cycles, from raw material extraction through manufacturing and use to an end-of-life disposal (ISO 14040:2006; ISO 14044:2006) [50–56]. However, comprehensive LCA studies comparing biocidal treatments within cultural heritage contexts remain limited. Existing LCA literature on EO has focused primarily on food, cosmetic, and pharmaceutical applications, while QACs have rarely been assessed from a life cycle perspective despite their widespread use [57–64]. This knowledge gap hinders evidence-based decision-making in conservation practice and limits the development of genuinely sustainable treatment strategies. This study addresses these gaps by presenting a comparative LCA of EO-based and QAC-based biocidal formulations across representative cultural heritage conservation scenarios. The analysis adopts a performance-oriented approach using multiple functional units that capture both intrinsic material burdens and real-world application requirements. By integrating the most recent toxicity characterization factors from the Environmental Footprint 3.1 methodology and USEtox® 2.1 model—including updated freshwater ecotoxicity factors based on species sensitivity distributions [65]—this study provides a scientifically rigorous foundation for comparing environmental trade-offs between these biocidal alternatives.

2. Materials and Methods

This study follows the ISO 14040:2006 and ISO 14044:2006 standards for Life Cycle Assessment and adopts the Environmental Footprint (EF) 3.1 methodology developed by the European Commission Joint Research Centre [65]. Impact assessment employs the USEtox® 2.1 (*consensus model maintained by USEtox Team, DTU, Kongens Lyngby, Denmark*)

consensus model for human toxicity and freshwater ecotoxicity characterization, incorporating updated characterization factors calculated from REACH, EFSA OpenFoodTox, and Pesticide Properties Database (PPDB) data sources. For freshwater ecotoxicity, substance hazard values are based on the 20th percentile of Species Sensitivity Distribution (SSD) curves derived from chronic EC10-equivalent reference points, as recommended by the UNEP-SETAC Pellston workshop (2018). LCA modeling was performed using Air.e software (*Solid Forest, Madrid, Spain*) with Ecoinvent v3.8 (*Ecoinvent Association, Zürich, Switzerland*) database for background processes [66,67].

2.1. Application Scenarios

The comparative assessment draws on ten documented case studies representing the diversity of cultural heritage substrates and microbial challenges encountered in conservation practice (Table 1). The selected case studies were not intended to provide an exhaustive survey of EO applications in cultural heritage conservation, but rather to strategically capture the diversity of substrates, environmental conditions, application modalities, and microbial challenges encountered in real conservation practice. All included studies are peer-reviewed and indexed in Scopus, and report in situ or near-real application conditions, thereby ensuring methodological coherence with the performance-oriented functional units adopted in this comparative LCA. The selected scenarios collectively encompass porous and non-porous materials (e.g., stone, wood, paper, glazed ceramics), indoor and outdoor environments, direct-contact and vapor-phase biocidal applications, as well as simple (mono-kingdom) and complex (multi-kingdom biofilm) microbial communities. This deliberate selection allows the assessment framework to reflect the operational variability of conservation interventions while maintaining internal consistency, supporting the robustness, representativeness, and transferability of the comparative LCA results across a broad spectrum of cultural heritage contexts.

Table 1. Representative case studies used to define application scenarios and functional units in the comparative LCA.

Heritage Substrate/Environment	Application Context	Biocidal System Tested	Main Microbial Targets	Reference
Marble stone (outdoor monument)	Archaeological site (Ostia Antica, Italy)	EO vs. QAC (brush application)	Algae, cyanobacteria, bacteria	Macchia 2022 [40]
Calcareous stone	Outdoor stone monuments	EO in alginate hydrogel	Cyanobacterial biofilms	Gabriele 2023 [68]
Mosaic (glass/stone tesserae)	Monumental mosaic (Rome, Italy)	EO-based hydrogels vs. oxidant biocides	Mixed microbial patina	Antonelli 2024 [41]
Historical wood	Indoor heritage objects	Clove EO vs. conventional biocides	Fungi (*Aspergillus*, *Penicillium*)	Pop 2021 [29]
Wooden artifacts	Museum/storage environments	EO vapor phase	Filamentous fungi	Sterflinger 2013 [44]
Paper and archival documents	Archive collections	EO solutions	Bacteria and fungi	Menicucci 2023 Tomić 2023 [69]
Hypogean environments (caves)	Natural and cultural caves	Multiple plant EO	Bacteria and fungi	Argyri 2021 [33]
Glazed ceramic tiles	Architectural heritage	EO-loaded hydrogels	Fungal colonization	Aliasghari Veshareh 2025 [39]
Leather artifacts	Indian leather puppets (Tholu Bommalu)	Thymus and Crithmum EO	Bacteria and fungi	Tomić 2023 [70] D'Agostino 2021 [71]
Heritage textiles	Textile collections	Cinnamon EO vapor phase	Bacteria and fungi	Matusiak 2018 [72]

Three complementary functional units (FU) are employed to capture different aspects of system performance:

- FU1—Formulation-based: This comprises 1 kg of ready-to-use biocidal formulation, enabling comparison of intrinsic material burdens independent of application efficiency.
- FU2—Surface-based: This entails 1 m^2 of heritage surface treated to achieve ≥99% reduction in viable microbial load, accounting for differences in formulation concentration, application rate, and number of treatment cycles required reported in case studies.
- FU3—Intervention-based: This is a complete conservation intervention including formulation preparation, application, auxiliary materials (cotton pads, brushes, nitrile gloves, protective equipment), and waste management, representing realistic operational practice.

The analysis adopts cradle-to-grave system boundaries encompassing:

(1) Raw material production, including agricultural cultivation of aromatic plants for EO and petrochemical feedstocks for QAC synthesis;
(2) Active ingredient production via steam distillation for EO or quaternization reactions for QAC;
(3) Formulation with co-solvents (ethanol, propylene glycol) and stabilizers;
(4) Transport to conservation site (European average, 500 km by road);
(5) Application to heritage surfaces;
(6) End-of-life management, including wastewater treatment for rinse water and municipal solid waste disposal for auxiliary materials.

Infrastructure and capital equipment are excluded following cut-off criteria (<1% contribution to any impact category).

The functional unit FU2 is based on immediate biocidal efficacy (≥99% reduction in viable microbial load), used as a harmonized performance threshold across heterogeneous efficacy metrics reported in the literature. Biocidal efficacy was quantified using adenosine triphosphate (ATP) bioluminescence assays or equivalent viability indicators, depending on the methodology adopted in each source study. In stone monument scenarios, efficacy was assessed by measuring the percentage decrease in cellular ATP relative to untreated controls using the ENLITEN® ATP Assay System (*Promega Corporation, Madison, WI, USA*) [40,41], with reductions exceeding 99.5% interpreted as indicative of deep disinfection. For scenarios where ATP-based measurements were not available, efficacy thresholds were derived from alternative indicators such as chlorophyll fluorescence, colony-forming unit (CFU) counts, or photosynthetic yield reductions, as reported in the respective publications. The system boundaries adopted in this study encompass a single treatment cycle, from raw material production through formulation, application, and end-of-life management. The assessment does not extend to the full preservation life cycle of the cultural asset and therefore does not explicitly model treatment durability, recolonization kinetics, or the need for periodic re-intervention over extended time horizons. This constitutes a critical methodological limitation, as the present framework captures only the immediate environmental footprint of a single treatment event rather than cumulative impacts over decades of conservation management. In practice, differences in treatment persistence may influence long-term environmental performance, as products with lower initial impacts may require more frequent reapplication, whereas more persistent treatments may entail fewer interventions over time. The exclusion of treatment durability from the primary LCA framework was necessitated by the absence of harmonized long-term monitoring data across the selected case studies. This limitation reflects a broader gap in conservation science literature, where efficacy assessments predominantly focus on immediate post-treatment outcomes rather than extended temporal performance. EO-based treatments are generally expected to require more frequent re-intervention due to the higher volatility and lower residual persistence of terpene compounds, whereas QAC-based treatments

may exhibit longer-lasting antimicrobial effects owing to stronger substrate adsorption and environmental persistence of cationic surfactants. This asymmetry in treatment durability could influence cumulative environmental impacts over long time horizons and is therefore addressed through an indicative retreatment projection presented in the Discussion (Section 4), rather than through formal dynamic LCA modeling. Direct EO-versus-QAC comparisons were available for 3 of the 10 selected scenarios (stone monuments, historical wood, and textile heritage). For the remaining scenarios, QAC application parameters were estimated based on manufacturer recommendations and standard conservation protocols for comparable substrates [68].

2.2. Life Cycle Inventory

EO production is modeled based on industrial steam distillation processes, representing the predominant extraction technology. Agricultural inputs for aromatic plant cultivation (*Thymus vulgaris, Origanum vulgare, Cinnamomum zeylanicum*) are modeled using European herbaceous crop proxies from Ecoinvent v3.8 [57]. Distillation energy requirements are parameterized at 20–45 kWh/kg plant material processed, with European average electricity grid composition (2020 mix). EO yields range from 0.5% (thyme) to 2.0% (cinnamon bark) based on industrial data. Water consumption for distillation is modeled at 1.6–3.6 L per gram of EO produced. Co-products (hydrolats, plant residues) are handled by mass allocation, with hydrolats assigned 5% of burdens based on relative market value. QAC production is modeled using Ecoinvent v3.8 datasets for quaternary ammonium compound synthesis via quaternization of tertiary amines with alkyl halides. Benzalkonium chloride (BAC C12-C16) serves as the reference compound, with didecyldimethylammonium chloride (DDAC) and cetylpyridinium chloride (CPC) modeled using structural analog proxies where dedicated datasets were unavailable. Upstream petrochemical production includes fatty alcohol and alkyl halide synthesis from petroleum-derived feedstocks. Energy requirements for quaternization are modeled at 8–15 MJ/kg active substance based on industrial process data. Ready-to-use formulations are modeled with EO concentrations of 1–5% v/v and QAC concentrations of 0.5–3% w/v in aqueous or hydroalcoholic solutions, consistent with concentrations reported in conservation literature and commercial product specifications. Co-solvents include ethanol (10–30% v/v) and propylene glycol (1–5% v/v) to enhance EO solubility and surface wetting. Application protocols follow documented conservation practice: QAC-based treatments typically require 1–2 applications at 50–100 mL/m^2 per application, while EO-based treatments require 2–4 applications due to volatility and generally higher MIC values. For baseline LCA calculations, the number of application cycles was set at 3 for EO-based systems (median of the documented range) and 2 for QAC-based systems, reflecting typical field practice reported in the case studies (Table 1). Auxiliary materials include cotton pads (50 g/m^2), nitrile gloves (2 pairs per m^2), and protective equipment. Data quality is assessed using the pedigree matrix approach [73,74] evaluating reliability, completeness, temporal correlation, geographical correlation, and technological correlation on a 1–5 scale (1 = best quality). Emission fate modeling adopted compartment-specific allocation consistent with application modalities documented in the case studies. Both EO-based and QAC-based formulations were assumed to be applied as aqueous solutions, hydroalcoholic emulsions, or hydrogel-based systems, in accordance with standard conservation protocols. The use of surfactants and encapsulating matrices (e.g., alginate hydrogels) limits EO volatilization during and after application. Consequently, end-of-life emissions were allocated predominantly to the freshwater compartment (85%) via wastewater discharge following surface rinsing, with minor fractions to soil (10%, outdoor application runoff) and air (5%, residual volatilization). This allocation scheme was applied symmetrically to both biocide systems

to ensure comparability. Compartment-specific USEtox® 2.1 characterization factors were applied accordingly. Table 2 summarizes quality indicators for key inventory components, while Table 3 summarizes the parameters application in representative case studies chosen.

Table 2. Data quality assessment using pedigree matrix indicators (1–5 scale, 1 = highest quality).

Data Source	Reliability	Complete.	Temporal	Geograph.	Technol.
Ecoinvent v3.8 datasets	1	1	2	2	1
EO extraction [49]	2	2	2	3	2
Application protocols	2	3	2	2	2
QAC proxies (structural)	3	2	2	2	3
USEtox® 2.1 CFs (JRC 2020)	2	2	1	2	2

Table 3. Application parameters for representative case studies.

Case Study	Biocide Conc.	Application Rate	Cycles	Surface	Reference
Marble (Ostia Antica)	EO 5% QAC 5%	100 mL/m^2	2	10 m^2	[41]
Calcareous stone	EO 0.5% hydrogel	150 g/m^2	1	5 m^2	[66]
Mosaic (Rome)	EO 2% hydrogel	200 g/m^2	1	2 m^2	[40]
Historical wood	EO 5–10% QAC 5%	300 mL/elem	3	0.5 m^2	[29]
Wooden artifacts	EO vapor	0.5 mL/L air	1	1 m^3	[44]
Paper archives	EO 5%	spray	2	100 sheets	[29]
Cave environments	EO 0.1–5%	200 µL/disk	1	lab scale	[33]
Glazed tiles	EO 5–10% hydrogel	brush overnight	3	6.25 cm^2	[39]
Leather puppets	EO undiluted	contact	1	object	[71]
Heritage textiles	EO vapor	0.5 mL	1	chamber	[72]

2.3. Life Cycle Impact Assessment

Impact assessment employs the Environmental Footprint 3.1 method with USEtox® 2.1 characterization factors for toxicity-related categories. The JRC (2020) update provides 6011 freshwater ecotoxicity factors, 3423 human toxicity (non-cancer) factors, and 621 human toxicity (cancer) factors, calculated using physicochemical data from REACH-IUCLID, EFSA OpenFoodTox, and PPDB databases. Freshwater ecotoxicity factors are expressed in Comparative Toxic Units for ecosystems (CTUe), based on the potentially affected fraction of species (PAF) integrated over time and volume. Human toxicity factors are expressed in Comparative Toxic Units for humans (CTUh), representing disease cases per kg emitted. Selected impact categories include: climate change (kg CO_2-eq, IPCC 2013 GWP100), freshwater ecotoxicity (CTUe), human toxicity non-cancer (CTUh), human toxicity cancer (CTUh), and water use (m^3 world equation deprived). Normalization and weighting are not applied to preserve transparency of trade-offs. Sensitivity analysis evaluates the influence of key parameters on comparative results:

(1) EO extraction energy demand ± 30%, reflecting variability between industrial facilities and extraction methods;
(2) Number of application cycles ± 1, acknowledging substrate-specific and microbial-specific requirements;
(3) Formulation concentration ± 20%;

(4) Electricity grid composition scenarios including European average (2020), renewable-dominated (80% renewable), and fossil-dominated (80% fossil) mixes.

Parameter uncertainty is propagated using Monte Carlo simulation (1000 iterations) with triangular distributions for inventory parameters and lognormal distributions for characterization factors. To enable transparent and reproducible comparison between green formulations and their corresponding conventional baselines, relative performance indicators were calculated in addition to absolute impact values. For each impact category and functional unit, comparative results are expressed both as dimensionless performance ratios and as percentage deviations (Δ%). For each green formulation (G) and its designated conventional baseline (B), a performance ratio (R) was calculated as:

$$R = \frac{I_G}{I_B}$$

where I_G and I_B represent the life cycle impact values of the green formulation and the baseline, respectively, expressed in the same functional unit.

A ratio $R < 1.0$ indicates an environmental improvement relative to conventional practice, whereas $R > 1.0$ indicates an environmental penalty. To facilitate intuitive interpretation, performance ratios were additionally converted into percentage deviations (Δ%), calculated as:

$$\Delta(\%) = \left(\frac{I_G - I_B}{I_B} \right) \times 100$$

Negative Δ% values indicate a reduction in environmental impact relative to the baseline, while positive Δ% values indicate an increase. For example, a Δ of −86% denotes that the green formulation exhibits an 86% lower impact than the conventional reference, whereas a Δ of +30% denotes a 30% higher impact.

The percentage deviation metric (Δ%) provides a relative measure of improvement or penalty but does not imply absolute environmental sustainability. Δ% values are conditional on the selected baseline, characterization factors, and system boundaries adopted. Consequently, the values are interpreted as comparative indicators within a defined methodological framework rather than as universal or context-independent performance claims. To avoid misinterpretation, Δ% values are always reported alongside absolute impact values and baseline specifications, and results are discussed with reference of sensitivity analyses addressing baseline variability and database uncertainty. Comparative matrices presented in Section 3 combine performance ratios and Δ% values across multiple impact categories (e.g., climate change, freshwater ecotoxicity, human toxicity, and water consumption). These matrices facilitate the identification of systematic trade-offs, whereby reductions in one impact category may be accompanied by increases in others. Such matrices are not intended to provide a single aggregated sustainability score but rather to support multi-criteria decision-making by highlighting the relative strengths and weaknesses of each formulation across distinct environmental dimensions.

Performance ratios and Δ% values were calculated using the same characterization factors, allocation rules, and system boundaries applied to absolute impact calculations, without introducing additional normalization or weighting.

2.4. Sensitivity and Uncertainty Analysis

Given the comparative nature of the study and the absence of primary experimental replicates, statistical inference was performed through uncertainty and sensitivity analysis in accordance with ISO 14044, ILCD Handbook, and environmental footprint (EF) methodological guidance. Parameter uncertainty was addressed through a tiered approach combining local sensitivity analysis, global uncertainty propagation, and scenario analysis.

Local sensitivity was assessed using one-at-a-time (OAT) perturbation of key input parameters (see Section 3.2), quantifying the percentage change in impact category results per unit change in each parameter while holding others constant.

As previous mentioned, global uncertainty propagation was performed using Monte Carlo simulation (n = 1000 iterations) to generate probability distributions for each impact category and functional unit. Input parameters were assigned probability distributions based on literature-reported ranges, data quality indicators from the pedigree matrix (Table 2), and USEtox® 2.1 recommendations. Triangular distributions were used for continuous inventory parameters where minimum, most likely, and maximum values were available from literature sources. Discrete uniform distributions were applied to categorical variables such as number of application cycles. Lognormal distributions were adopted for characterization factors to reflect the multiplicative uncertainty structures typical of toxicity modeling and the lognormal distribution of ecotoxicological effect data.

Results are reported as mean values with 95% confidence intervals derived from the 2.5th and 97.5th percentiles of the Monte Carlo output distributions. Comparative robustness between EO-based and QAC-based systems was assessed by calculating the probability P (EO < QAC) that EO-based systems exhibit lower impacts than QAC-based systems for each impact category, determined as the fraction of Monte Carlo iterations in which EO impacts were lower than corresponding QAC impacts.

Scenario analysis was additionally performed to explore the influence of technological improvements and boundary conditions on comparative outcomes. Scenarios included:

(i) Renewable energy integration (80% renewable electricity grid);
(ii) Optimized industrial extraction processes;
(iii) Reduced application cycles based on efficacy thresholds;
(iv) Worst-case laboratory-scale production conditions.

These scenarios were designed to bracket the range of plausible operating conditions and identify tipping points where comparative advantages shift between biocide systems.

3. Results

Application parameters derived from the ten case studies are summarized in Table 3. Biocide concentrations ranged from 0.1% to 10% v/v for EO-based treatments and 5% for QAC-based formulations (corresponding to 2.5% active substance for commercial products such as Preventol® RI50 and NewDes® 50). Application rates varied depending on substrate porosity, treatment modality (brush, hydrogel, vapor phase), and microbial challenge severity. EO-based treatments required 2–4 application cycles to achieve comparable efficacy to QAC-based treatments (1–2 cycles).

Life cycle inventory data for representative application scenarios are presented in Table 4. EO-based treatments required 5–80 g of essential oil per m^2 of treated surface depending on formulation concentration and number of cycles, compared to 2.5–15 g of QAC active substance. Energy demand calculations incorporated upstream extraction or synthesis processes based on Moura et al. [49]. Auxiliary materials (cotton pads, nitrile gloves, hydrogel matrices) contributed 15–25% of total mass flows.

Upstream production burdens for biocide active substances and formulation components are reported in Table 5.

Table 4. Life cycle inventory for representative application scenarios.

Scenario	EO (g)	QAC (g)	Water (L)	Energy (MJ)	Aux. Mat. (g)	Reference
Stone 1 m^2 (EO)	5.0	—	0.10	0.83	50	[41]
Stone 1 m^2 (QAC)	—	2.5	0.10	0.04	50	[41]
Hydrogel 1 m^2 (EO)	7.5	—	0.15	1.25	150	[66]
Wood element (EO)	15.0	—	0.30	2.49	30	[29]
Wood element (QAC)	—	15.0	0.30	0.23	30	[29]
Tiles 1 m^2 (EO)	80.0	—	0.50	13.28	200	[39]

Table 5. Upstream production burdens for biocide active substances and formulation components.

Process	Energy (MJ/kg)	CO_2-eq (kg/kg)	Water (L/kg)	Reference
EO steam distillation (industrial)	166–363	8–20	1600–3600	[49]
EO hydrodistillation (lab scale)	10,992	541	3400	[49]
QAC synthesis (BAC)	8–15	0.5–1.2	50–100	Ecoinvent 3.8
Alginate production	25–40	1.5–2.5	200–400	Ecoinvent 3.8
Ethanol (co-solvent)	30–35	1.8–2.2	80–120	Ecoinvent 3.8

3.1. Life Cycle Impact Assessment

LCIA results normalized to 1 kg of ready-to-use biocidal formulation (FU1) are presented in Table 6. EO-based formulations exhibited 81% higher climate change impacts (3.8 vs. 2.1 kg CO_2-eq/kg) and 171% higher water use (0.19 vs. 0.07 m^3/kg). EO-based formulations showed 82–86% lower impacts across all toxicity-related categories: freshwater ecotoxicity was reduced from 2.95 CTUe (QAC) to 0.42 CTUe (EO); human toxicity impacts (both cancer and non-cancer) showed reductions of 82%.

Table 6. LCIA results per 1 kg of ready-to-use biocidal formulation (FU1).

Impact Category	EO-Based	QAC-Based	Δ (%)	Unit
Climate Change	3.8	2.1	+81%	kg CO_2-eq
Freshwater Ecotoxicity	0.42	2.95	−86%	CTUe
Human Toxicity (non-cancer)	1.6×10^{-7}	8.9×10^{-7}	−82%	CTUh
Human Toxicity (cancer)	4.1×10^{-8}	2.3×10^{-7}	−82%	CTUh
Water Use	0.19	0.07	+171%	m^3 world eq.

Results normalized to functional equivalence (1 m^2 treated to ≥99% microbial reduction, FU2) are presented in Table 7. The climate change gap was reduced from 81% (FU1) to 32% (FU2). Toxicity advantages of EO-based systems were 87–89% lower under FU2 normalization.

Table 7. LCIA results per 1 m^2 treated to ≥99% microbial reduction (FU2).

Impact Category	EO-Based	QAC-Based	Δ (%)	Unit
Climate Change	1.25	0.95	+32%	kg CO_2-eq
Freshwater Ecotoxicity	0.14	1.31	−89%	CTUe
Human Toxicity (non-cancer)	5.2×10^{-8}	4.1×10^{-7}	−87%	CTUh
Human Toxicity (cancer)	1.3×10^{-8}	9.8×10^{-8}	−87%	CTUh

FU3 analysis incorporated complete conservation intervention requirements including auxiliary materials and waste management (Table 8). Contribution analysis identified

the dominant contributors to each impact category: for EO-based systems, extraction energy accounted for 65% of climate change impacts, with auxiliary materials contributing approximately 20%; for QAC-based systems, the active substance contributed 89–92% of toxicity-related impacts.

Table 8. LCIA results for complete conservation intervention (FU3) with hotspot analysis.

Impact Category	EO-Based	QAC-Based	Δ (%)	Dominant Hotspot (%)
Climate Change (kg CO_2-eq)	2.4	2.2	+9%	EO extraction energy (65%)
Freshwater Ecotox. (CTUe)	0.21	2.74	−92%	QAC active substance (92%)
Human Toxicity (CTUh)	6.8×10^{-8}	6.1×10^{-7}	−89%	QAC active substance (89%)

3.2. Sensitivity Analysis

Parameters included in the sensitivity analysis, their baseline values, variation ranges, and probability distributions are presented in Table 9.

Table 9. Parameters varied in sensitivity analysis with baseline values and ranges.

Parameter	Baseline	Low	High	Distribution	Source/Justification
EO extraction energy (MJ/kg)	265	166	363	Triangular	[49]
EO concentration (% v/v)	5.0	2.0	10.0	Triangular	Case study range
Number of EO applications	3	2	4	Discrete	Efficacy requirements
QAC concentration (% w/v)	2.5	1.0	5.0	Triangular	Manufacturer species
Electricity grid (% renewable)	35	15	80	Triangular	EU grid scenarios
EO yield (g/kg biomass)	15	8	25	Lognormal	Literature range
Auxiliary materials (g/m^2)	100	50	150	Triangular	Application method
Transport distance (km)	100	50	500	Triangular	Supply chain

OAC sensitivity results are presented in Table 10. For EO-based systems, climate change impacts showed sensitivity to extraction energy (±19.5% per ±30% variation), EO concentration (×2 concentration → +85%), and number of applications (+67% for 2→4 cycles). Transitioning to a renewable-dominated electricity grid (80% renewable vs. 35% baseline) reduced EO climate impacts by 45%. For QAC-based systems, freshwater ecotoxicity showed sensitivity to active substance concentration (+95% per ×2 concentration), while climate change impacts were relatively insensitive to tested parameters except electricity grid composition (−12% for renewable grid).

Table 10. Local sensitivity results: percentage change in impacts per parameter variation.

Parameter Variation	ΔClimate (EO)	ΔEcotox (EO)	ΔClimate (QAC)	ΔEcotox (QAC)
EO energy + 30%	+19.5%	+2.1%	—	—
EO energy − 30%	−19.5%	−2.1%	—	—
EO concentration × 2	+85%	+12%	—	—
EO applications 2→4	+67%	+8%	—	—
QAC concentration × 2	—	—	+15%	+95%
Grid 35%→80% renewable	−45%	−8%	−12%	−2%
Transport 100→500 km	+8%	+1%	+5%	+0.5%
Auxiliary materials + 50%	+12%	+3%	+10%	+2%

The arrow represent the change in the different number of application, transtion to renewable-dominated electricity, and transportation.

Tornado diagrams ranking parameter influence are presented in Figure 1.

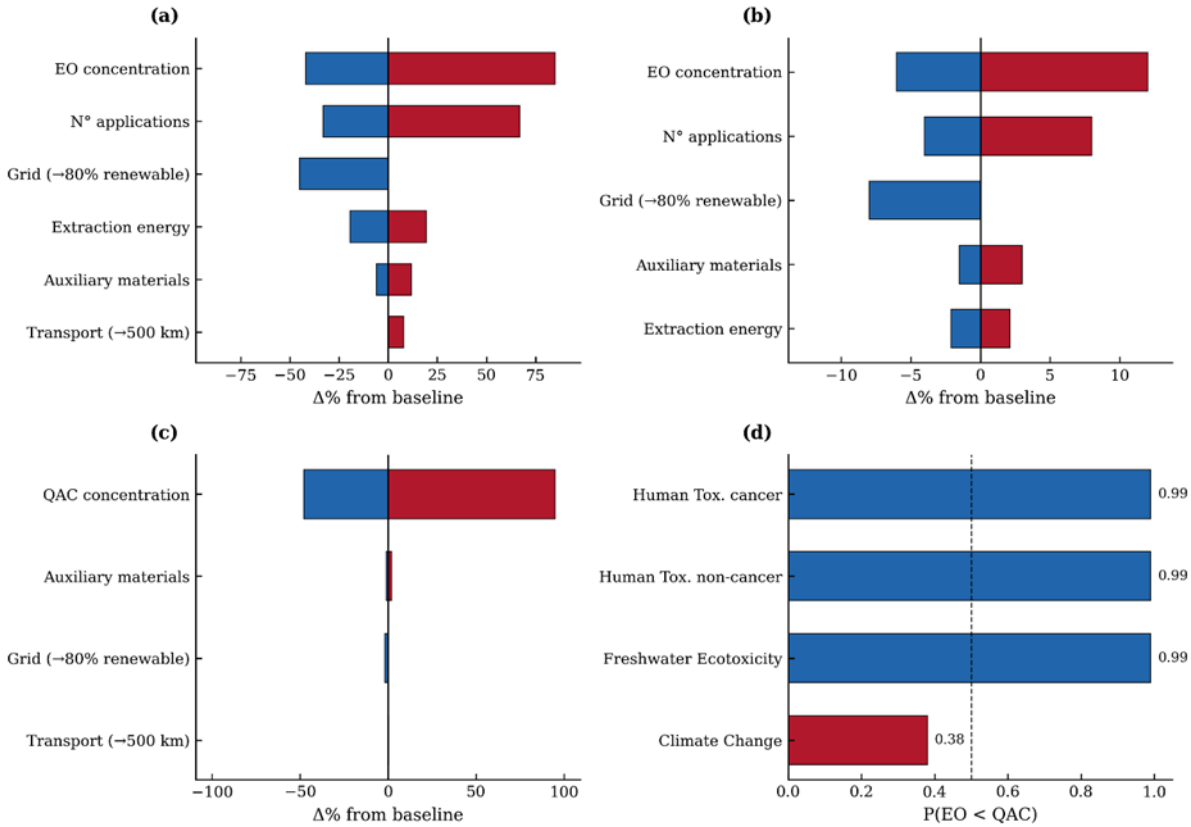

Figure 1. Sensitivity and uncertainty analysis results (FU2: 1 m^2 treated). (**a**) Tornado plot for EO-based system climate change impacts; (**b**) tornado plot for EO-based system freshwater ecotoxicity; (**c**) tornado plot for QAC-based system freshwater ecotoxicity; (**d**) Monte Carlo probability P (EO < QAC) across impact categories (n = 1000 iterations). Blue bars indicate impact reduction; red bars indicate impact increase. Dashed line in (**d**) marks p = 0.50.

3.2.1. Monte Carlo Uncertainty Analysis

Monte Carlo simulation results (n = 1000 iterations) are presented in Table 11. The coefficient of variation (CV) for EO-based climate change impacts was 34%; QAC-based ecotoxicity showed CV = 22%. The probability that EO-based systems have lower toxicity impacts than QAC-based systems exceeded 99% for all toxicity-related categories. The probability of EO-based systems having lower climate change impacts was 38% for FU2 under baseline conditions, increasing to 78% under best-case assumptions.

Table 11. Monte Carlo uncertainty analysis results (n = 1000 iterations) for FU2.

Impact Category (FU2)	Mean EO	95% CI EO	Mean QAC	95% CI QAC	P(EO < QAC)
Climate Change (kg CO_2-eq)	1.25	0.78–1.89	0.95	0.72–1.28	38%
Freshwater Ecotoxicity (CTUe)	0.14	0.08–0.24	1.31	0.89–1.92	>99%
Human Tox. non-cancer (CTUh)	5.2×10^{-8}	$2.9–8.1 \times 10^{-8}$	4.1×10^{-7}	$2.8–5.9 \times 10^{-7}$	>99%
Human Tox. cancer (CTUh)	1.3×10^{-8}	$0.7–2.1 \times 10^{-8}$	9.8×10^{-8}	$6.5–14.2 \times 10^{-8}$	>99%

3.2.2. Scenario Analysis

Scenario analysis results are presented in Table 12. The "Best case EO" scenario (renewable electricity 80%, industrially optimized extraction, two application cycles) yielded climate change impacts of 0.52 kg CO_2-eq/m^2 for EO-based systems compared to 0.95 kg CO_2-eq/m^2 for QAC-based systems, with ecotoxicity values of 0.08 CTUe (EO) vs. 1.31

CTUe (QAC). The "Worst case EO" scenario (laboratory-scale extraction) resulted in climate impacts of 3.85 kg CO_2-eq/m^2.

Table 12. Scenario analysis results for FU2 (1 m^2 treated): impact values under alternative assumptions.

Scenario	Climate EO	Climate QAC	Ecotox EO	Ecotox QAC
Baseline (EU avg grid)	1.25	0.95	0.14	1.31
Renewable grid (80%)	0.69	0.84	0.13	1.29
Industrial EO (optimized)	0.85	0.95	0.11	1.31
Reduced applications (2 cycles)	0.83	0.95	0.09	1.31
Best case EO (combined)	0.52	0.95	0.08	1.31
Worst case EO (lab scale)	3.85	0.95	0.18	1.31

4. Discussion

The comparative assessment reveals a systematic trade-off between climate change and toxicity impacts that reflects fundamental differences in the nature of the two biocide classes. Climate and water impacts associated with EO-based systems are largely driven by upstream agricultural production and energy-intensive extraction processes. These impacts scale with energy demand and process efficiency and are therefore sensitive to technological choices and energy system characteristics. By contrast, the dominant toxicity-related impacts of QAC-based systems are intrinsic to the chemical properties of quaternary ammonium compounds, leaving limited scope for mitigation through process optimization alone. This asymmetry has profound implications for environmental management strategies. The sensitivity analysis demonstrates that EO-based climate impacts can be substantially reduced through technological interventions, whereas QAC toxicity is structurally embedded in the cationic surfactant nature that confers both antimicrobial efficacy and ecotoxicological potency. The growing body of evidence on QAC environmental persistence, antimicrobial resistance induction, and human health effects reinforces the conclusion that toxicity-related impacts of conventional biocides represent a qualitatively different challenge than energy-related impacts of bio-based alternatives. The explicit comparison across multiple functional units demonstrates how normalization choices influence comparative conclusions. Formulation-based comparison (FU1) emphasizes intrinsic material burdens but obscures performance differences between systems. The 81% higher climate impact observed for EO-based systems under FU1 reflects the energy intensity of steam distillation relative to petrochemical synthesis, independent of application efficiency. Performance-based normalization (FU2) accounts for differences in efficacy, reducing the climate gap to 32% while enhancing toxicity advantages to 87–89%. This pattern reflects the fundamental difference between impacts that scale with production volume (energy, water) versus those determined by intrinsic chemical properties (toxicity characterization factors). Each gram of QAC released to the environment carries a substantially higher toxicity burden than an equivalent mass of EO constituents, regardless of the quantity required for effective treatment. The intervention-based functional unit (FU3) further demonstrates that real-world conservation practice moderate differences between systems for climate-related impacts (reducing the gap to 9%) while leaving toxicity-driven differences largely unchanged (89–92% advantage for EO). This pattern suggests that performance-based functional units should be considered best practice for LCA studies informing conservation decision-making, consistent with recommendations for comparative assessments of functionally equivalent products. The probabilistic framework provides a statistically grounded basis for interpreting comparative results without reliance on classical significance testing, which is inappropriate for model-based environmental assessments. The probability exceeding 99% that EO-based systems have lower toxicity impacts than QAC-

based systems persist despite substantial parameter uncertainty, indicating that toxicity advantages represent robust findings. Conversely, climate comparisons remain sensitive to scenario assumptions (p = 38% under baseline, p = 78% under best-case), indicating that climate trade-offs are contingent on production and application choices. The stability of toxicity rankings despite characterization factor uncertainty reflects the large magnitude of differences between substance classes. When impact differences span orders of magnitude, as observed for freshwater ecotoxicity (0.14 vs. 1.31 CTUe), parameter uncertainty becomes secondary to the fundamental contrast between chemical profiles. The scenario analysis reveals that environmental competitiveness of EO-based systems is highly contingent on production and energy system choices. Under optimized conditions achievable with existing technology, EO-based treatments can achieve climate impacts comparable to or lower than QAC-based systems (0.52 vs. 0.95 kg CO_2-eq/m^2) while maintaining substantially lower toxicity (94% reduction). This represents a critical finding: unlike QAC toxicity, which is largely invariant to process improvements, EO-related climate impacts are technically mitigable. The contribution analysis identifies extraction energy as the dominant hotspot for EO-based systems (65% of climate impacts), suggesting multiple intervention pathways: renewable energy integration, process efficiency improvements, and alternative extraction technologies. Recent developments in formulation technology, including encapsulation in hydrogel matrices and controlled-release systems, may address practical challenges associated with EO volatility and potentially reduce application frequency. However, the addition of encapsulating agents introduces supplementary environmental burdens that may partially offset these benefits. Cellulose ethers (e.g., carboxymethylcellulose, hydroxypropyl methylcellulose) and polysaccharide matrices (e.g., alginate, chitosan) require water-intensive production processes; alginate production alone demands 200–400 L of water per kg (Table 5). Consequently, formulation strategies aimed at reducing EO volatilization losses may exacerbate water use impacts, representing a secondary burden-shifting dynamic within EO-based systems. These trade-offs were not explicitly modeled in the present study and warrant dedicated LCA evaluation of advanced delivery systems to identify formulation strategies that minimize aggregate environmental burdens across multiple impact categories [66].

Conversely, the dominance of active substance contributions to QAC toxicity (89–92%) indicates that process optimization offers limited scope for improvement. The intrinsic toxicity of quaternary ammonium compounds is a molecular property that can only be addressed through substitution with less toxic alternatives. It should be noted that the present analysis focused on optimization pathways for EO-based systems without explicitly modeling comparable improvements for QAC production. While QAC synthesis could benefit from process-level improvements such as energy efficiency gains in quaternization reactors and greener solvent systems, these interventions would primarily reduce the already modest energy-related impacts of QAC production (which account for less than 11% of total QAC environmental burden under FU3). The dominant toxicity impacts, which constitute 89–92% of the QAC environmental profile, are determined by the physicochemical properties and environmental fate of the active substances themselves and would remain fundamentally unchanged by process optimization. Recent approaches to reduce QAC environmental impact have focused on molecular design of novel quaternary ammonium structures with improved biodegradability [27], but these compounds have not yet been adopted in conservation practice and would require independent efficacy validation on heritage substrates. The results challenge simplistic narratives framing EO-based biocides as inherently green or QAC-based biocides as unproblematic due to their effectiveness. The present assessment did not include abiotic resource depletion among the evaluated impact categories. This omission is relevant for QAC-based systems, whose synthesis relies

on petrochemical feedstocks (fatty alcohols, alkyl halides) derived from non-renewable resources. Inclusion of the 'resource use, fossils' category from the EF 3.1 method would likely increase the relative environmental burden of QAC-based systems, as petrochemical input constitutes the primary raw material pathway for QAC synthesis. Conversely, EO production relies predominantly on renewable agricultural biomass, with fossil resource inputs limited to energy supply and transport. Future studies should incorporate resource depletion indicators to provide a more comprehensive characterization of the environmental trade-offs between bio-based and petrochemical biocide systems

EO-based systems represent a shift in environmental burden from toxicity-dominated impacts toward energy-related impacts rather than elimination of impacts altogether. This burden-shifting has strategic implications given increasing regulatory scrutiny of QAC under European chemicals legislation, particularly regarding aquatic toxicity and antimicrobial resistance. For conservation practitioners, the findings support context-dependent decision-making. Applications prioritizing toxicity reduction—particularly near water bodies, in ecologically sensitive areas, or in poorly ventilated indoor environments—favor EO-based formulations despite moderate climate penalties under current average conditions. Strategic sourcing of industrially produced essential oils and optimization of application protocols can substantially reduce these penalties while preserving toxicity advantages. Water use impacts, while not the primary focus of this assessment, showed a pattern similar to climate change, with EO-based systems requiring substantially higher water inputs (+171% under FU1) due to agricultural irrigation requirements. This trade-off may be particularly relevant for conservation projects in water-stressed regions or when sourcing EO from Mediterranean cultivation areas. An additional dimension not captured in the present static LCA framework is the potential feedback between climate change and EO production. Aromatic plant cultivation is sensitive to shifts in temperature, precipitation patterns, and drought frequency, which may alter EO yields and chemical composition. Mediterranean regions—the primary cultivation areas for thyme, oregano, and lavender—are projected to experience increased aridity and heat stress under IPCC climate scenarios [48]. Reduced biomass productivity and altered terpene biosynthesis under climate stress could increase the amount of plant material required per unit of EO produced, thereby amplifying the upstream environmental burdens associated with EO-based systems. This climate–agriculture feedback loop introduces a dynamic dimension to the comparative LCA that warrants investigation through prospective scenario modeling. Several limitations warrant acknowledgment. Essential oils were modeled as aggregated product classes rather than individual chemical constituents. This simplification was necessitated by incomplete USEtox® characterization factor coverage for specific terpenes and phenylpropanoids (e.g., thymol, carvacrol, eugenol, cinnamaldehyde). Compositional variability within and between EO types—driven by chemotype, geographic origin, harvest timing, and extraction conditions—may result in under- or overestimation of toxicity impacts. This compositional variability has direct implications for biocidal efficacy and, consequently, for the quantities of EO required per treated surface. Different chemotypes within the same species can exhibit markedly different antimicrobial profiles: for example, Thymus vulgaris ct. thymol shows substantially higher antifungal activity than ct. linalool or ct. geraniol, owing to the stronger membrane-disruptive properties of phenolic monoterpenes [3,36]. Similarly, EO composition is influenced by geographic origin, altitude, soil characteristics, and harvest timing, leading to batch-to-batch variability in the concentrations of key active compounds such as thymol, carvacrol, and eugenol [28,33]. This variability may require adjustment of formulation concentrations and application cycles in practice, potentially shifting the environmental profile of individual EO-based treatments. Standardization through chemical fingerprinting and minimum active compound thresholds—as practiced

in the pharmaceutical sector—could mitigate this uncertainty and improve reproducibility of both biocidal performance and environmental outcomes. Such standardization would also support more precise life cycle modeling by enabling constituent-specific characterization rather than aggregate product-class proxies. Proxy-based characterization introduces additional uncertainty that could not be fully propagated in the Monte Carlo analysis. However, sensitivity analysis indicates that EO toxicity advantages remain robust even under conservative assumptions of characterization factor underestimation by a factor of 5, as the tipping point for parity with QAC-based systems would require approximately 9-fold higher EO characterization factors—a scenario inconsistent with available ecotoxicological data for terpene compounds. The functional unit FU2 is based on immediate biocidal efficacy ($\geq$99% reduction in viable microbial counts) as documented in the referenced case studies. Treatment durability and recolonization kinetics were not modeled due to the absence of harmonized long-term monitoring data across the selected scenarios. This limitation reflects a broader gap in conservation science literature, where efficacy assessments typically focus on immediate post-treatment outcomes rather than extended temporal performance. This methodological choice may influence comparative conclusions if reapplication frequencies differ systematically between EO-based and QAC-based treatments over extended time horizons. To illustrate how differences in treatment durability could influence cumulative impacts over extended time horizons, an indicative retreatment projection can be formulated based on literature-reported intervention frequencies. Based on conservation literature and field observations, QAC-based treatments on outdoor stone substrates typically maintain effective antimicrobial protection for 3–5 years before recolonization necessitates re-intervention [7,41], while EO-based treatments—owing to the higher volatility and lower residual persistence of terpene compounds—may require retreatment at intervals of 1–3 years [3,40]. Assuming a 15-year conservation management horizon, the following indicative scenarios can be formulated: (i) QAC-based systems requiring 3–5 interventions (retreatment every 3–5 years); (ii) EO-based systems requiring 5–15 interventions (retreatment every 1–3 years). Under these assumptions, cumulative climate change impacts for EO-based systems over 15 years would range from approximately 6.3 to 18.8 kg CO_2-eq/m^2 (5–15 interventions $\times$ 1.25 kg CO_2-eq per intervention), compared to 2.9–4.8 kg CO_2-eq/m^2 for QAC-based systems (3–5 interventions $\times$ 0.95 kg CO_2-eq per intervention). Conversely, cumulative freshwater ecotoxicity for QAC-based systems would range from 3.9 to 6.6 CTUe/m^2 (3–5 $\times$ 1.31 CTUe), compared to 0.7–2.1 CTUe/m^2 for EO-based systems (5–15 $\times$ 0.14 CTUe). Critically, even under the most unfavorable retreatment assumption for EO-based systems (annual retreatment), cumulative ecotoxicity remains 68% lower than the most favorable QAC scenario (retreatment every 5 years), confirming that the toxicity advantage is robust to differential treatment durability. The climate penalty, however, increases substantially under frequent retreatment scenarios, reinforcing the importance of formulation technologies that extend EO treatment durability—such as hydrogel encapsulation and controlled-release systems—as a priority for reducing the cumulative environmental footprint of bio-based conservation strategies. It must be emphasized that these projections are indicative and based on conservative assumptions derived from heterogeneous field observations; harmonized long-term monitoring protocols comparing EO and QAC treatment durability on standardized substrates are urgently needed to validate and refine these estimates. Direct EO-versus-QAC comparisons were available for 3 of 10 scenarios. For remaining scenarios, QAC application parameters were estimated based on manufacturer recommendations and standard conservation protocols for comparable substrates. Emissions during application and post-treatment volatilization were modeled using conservative assumptions allocating emissions predominantly to the freshwater compartment (85%) via wastewater discharge, with minor fractions to soil (10%)

and air (5%), reflecting the use of aqueous formulations and surfactant-based delivery systems that limit volatilization. Future research should expand characterization factor coverage for natural substances, integrate fate modeling for volatilized compounds, and couple LCA with long-term efficacy monitoring. Controlled field trials on standardized heritage substrates would strengthen the empirical basis for comparative assessment, while economic analysis integrating life cycle costing would inform cost-effectiveness evaluation of alternative strategies.

5. Conclusions

This study provides the first multi-functional unit Life Cycle Assessment comparing essential oil-based (EO) and quaternary ammonium compound-based (QAC) biocides for cultural heritage conservation. The analysis reveals a fundamental trade-off: EO-based formulations offer substantial toxicity advantages (82–92% lower impacts) at the cost of higher climate impacts, with the magnitude of climate differences strongly dependent on functional unit selection (+81% for FU1, +32% for FU2, +9% for FU3) and production scenarios. The asymmetric nature of this trade-off carries significant implications. EO-based climate impacts are dominated by extraction energy, a modifiable parameter responsive to technological intervention; under optimized conditions, climate parity or superiority is achievable while preserving toxicity advantages. Conversely, QAC toxicity is intrinsic to molecular structure and resistant to process optimization. Monte Carlo analysis confirms the robustness of toxicity findings ($p > 99\%$) across parameter uncertainty, while climate comparisons remain scenario-dependent. These findings support context-dependent transition toward EO-based biocides in cultural heritage conservation, particularly where toxicity reduction is prioritized. The critical pathway to environmental improvement lies in the strategic sourcing of industrially produced essential oils and optimization of application protocols, rather than uncritical substitution of natural for synthetic biocides. Conservation professionals and policymakers can use these quantitative findings to inform evidence-based decisions balancing efficacy, environmental responsibility, and regulatory compliance.

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Abbreviations

The following abbreviations are used in this manuscript:

BAC	Benzalkonium Chloride
CPC	Cetylpyridinium Chloride
CTUe	Comparative Toxic Units for ecosystems
CTUh	Comparative Toxic Units for humans
CV	Coefficient of Variation
DADMAC	Diallyl Dimethyl Ammonium Chloride
DDAC	Didecyldimethylammonium Chloride
EC10	10% Effect Concentration
EF	Environmental Footprint
EFSA	European Food Safety Authority
EO	Essential Oils
FU	Functional Unit
GWP	Global Warming Potential
ILCD	International Reference Life Cycle Data System
IPCC	Intergovernmental Panel on Climate Change
ISO	International Organization for Standardization
JRC	Joint Research Centre
LC50	50% Lethal Concentration
LCA	Life Cycle Assessment
LCIA	Life Cycle Impact Assessment
MIC	Minimum Inhibitory Concentration
NOEC	No Observed Effect Concentration
OAT	One-at-a-time
PAF	Potentially Affected Fraction
PNEC	Predicted No-Effect Concentration
PPDB	Pesticide Properties Database
PVA	Polyvinyl Alcohol
QAC	Quaternary Ammonium Compounds
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
SSD	Species Sensitivity Distribution

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