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1 Bronze degradation and conservation

Bronze conservation is a challenging task due to the complex nature of the artefacts and of their corrosion products in terms of chemical composition, metallographic structure and aesthetic results. For the development and evaluation of protective treatments, studying the composition of bronze, its processes of degradation and the ecological impacts are key issues.

A number of studies has been carried out to investigate the dynamics of bronze degradation; most of them were focused on archaeological artefacts [1, 2, 3, 4, 5], while the literature considering modern works of art is still limited [6, 7, 8].

The degradation of bronze metal surface doesn't necessarily have a negative impact on the overall preservation of the artefact. Under particular conditions, the formation of alteration products on the bronze surface induces the settling of layers known as “noble patinas”, which actually protect the substrate from further degradation. These patinas can have a variety of colours, do not alter the original shape of the object and are naturally generated by the exposure of bronze to a clean humid environment, or can be intentionally produced with particular surface treatments (e.g. waxes, oils or intentional corrosion). However, in presence of aggressive agents such as acid rains and pollutants, soluble compounds are generated, including chlorides, oxides, carbonates, hydrochlorides, nitrates, sulphides, and sulphates; which are capable of triggering massive and deeper alteration of the alloy [5, 9, 10, 11]. Pristine bronze is particularly exposed to these phenomena, but coated artefacts are also prone to an accelerated weakening of the surface protective treatment. One of the most harmful degradation processes of bronze is named “bronze disease”, and consists of a cyclic corrosion reaction involving chlorine and copper at the interface between the external patina and the surviving metal. The reaction generates cuprous chloride (nantokite) and commonly concerns objects recovered from marine environment or in sea water due to the abundance of chlorides, but can affect even artefacts subjected to the contamination of soil, water and atmosphere. When nantokite is exposed to moisture and oxygen, a self-sustaining process of copper dissolution is induced, and bronze surface is transformed into a greenish crumbly powder (atacamite and its polymorphs), which can rapidly disfigure the artefact and compromise its structural integrity and appearance [12]. Despite being observed mainly on archaeological artefacts, bronze disease is representative of the potential vulnerability of this alloy to weathering and general exposure to aggressive environments.

2 Protective treatments

Protecting treatments are increasingly developed to oppose these processes according to two main strategies. Waxes and polymeric coatings are a traditional strategy for bronze protection, based on the formation of a thick barrier layer which separates physically the substrate from the aggressive agents present into the environment [13]. Bees wax, microcrystalline wax, carnauba wax are products traditionally used for bronze conservation [14], but they produce layers with limited long-term stability and requiring a considerable maintenance to result in a long term duration [15]. As an alternative strategy, organic formulations can be applied to form chemisorbed thin films, and acts as inhibitors of the corrosion reaction by slowing down the anodic and/or cathodic corrosion reaction rates [16, 17, 18, 19]. Benzotriazole and its derivatives are the most commonly used products in cultural heritage, as they are very effective in copper protection. However, it has been demonstrated that the presence of alloying elements and multiphasic structures in bronze can decrease significantly the performances of benzotriazole-based inhibitors

[17]. This pushes conservators to identify new alternative compounds and formulations [18, 19, 20, 21]. Moreover, the well-known toxicity of benzotriazole and some of its derivatives has increased the demand of harmless products. Most widely, corrosion inhibition and barrier effect are combined into double protective coatings, consisting of an inner inhibitor film covered by a polymeric barrier layer, in order to lend an integrated protection to the bronze.

3 (Eco)toxicity of protective treatments

Developing innovative, eco-compatible and human-safe protective treatments for bronze requires comprehensive (eco)toxicological testing. Traditional approaches based on chemical determinations of hazardous compounds generally measure the concentration of a limited range of specific substances in leachates obtained from real or simulated treatment/exposure conditions. These traditional analytical methodologies are typically very sensitive and designed to be selective for specific chemical species, but are often also expensive and time-consuming. More importantly to the present interests, they base also on the reductionist assumption that the overall toxicity of a complex formulation can be inferred as the simple sum of toxic effects due to their individual components. Still, this premise is essentially wrong in realistic scenarios and can lead to underestimate the biological effects of a product.

Bioassay methods are a valuable option to understand the eco-toxicological behavior of innovative products. Bioassays assess the effect of the release of a chemical (i.e. the new developed formulation) in the environment by exposing representative organisms, chosen as bioindicators, to a range of concentration of the substance. **Table 1** reports a summary of the assays proposed in the OECD guidelines for aquatic organisms [22]. Bioindicators for aquatic risk assessment studies belong to different phila (microalgae, cyanobacteria, crustaceans, mollusks, plants and fishes) and have the advantage of being sensitive to a wide range of bio-available substances, and represent the entire trophic chain. If the assays are carried out using microorganisms, the drawback of time consumption is overcome because microbiotests are cheap, use small test volume and are available as kit; that is an advantage for toxicity screening and assessment [23]. Due to the application of control conditions, the bioassays reported in the OECD guidelines are standardized, and allow obtaining comparable data and complete toxicity curves, which are useful to evaluate end-points such as mortality, growth or reproduction.

Table 1: Summary of OECD bioassay for water organisms.

TEST NUMBER	TYPE OF TEST	TEST ANIMALS	PRINCIPLE OF THE TEST	TEST DURATION
201	Growth inhibition test	Freshwater microalgae and cyanobacteria	Determine the effects of a substance on the growth of freshwater microalgae and/or cyanobacteria. Exponentially growing test organisms are exposed to the test substance in batch cultures over a period of normally 72 hours.	72 hours
203	Acute toxicity test	Fishes	Assess the acute toxicity of chemical exposing fishes to a range of concentration of the substance preferably for a period of 96 hours	96 hours
210	Early-life Stage Toxicity Test	Fishes	Define the lethal and sub-lethal effects of chemicals on the stages and species tested.	Species-dependent (from 2 weeks to 32 days)

TEST NUMBER	TYPE OF TEST	TEST ANIMALS	PRINCIPLE OF THE TEST	TEST DURATION
211	Reproduction test	*Daphnia magna*	Assessing the effect of chemicals on the reproductive output of the target species exposing young female organism to the test substance added to water at a range of concentrations. The test duration is 21 days	21 days
212	Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages	*Oncorhynchus mykiss* *Brachydanio rerio* *Cyprinus carpio* *Oryzias latipes* *Pimephales promelas*	Assessing lethal and sub-lethal effects of the chemical exposing embryo and sac-fry stages of fish to a range of concentrations of the test substance dissolved in water.	Up to 55 days (according to the species)
215	Juvenile growth test	*Oncorhynchus mykiss*	Assessing the effects of prolonged exposure to chemicals on the growth of juvenile fishes.	28 days
221	Growth Inhibition Test	*Lemna* sp.	Quantify substance-related effects on vegetative growth over a period of 7 days based on assessments of selected measurement variables	7 days
229	Short Term Reproduction Assay	Fathead minnow Zebrafish Japanese Medaka	Screening of substances active on the endocrine system of fish species during a limited part of their life-cycle. At termination of the 21-day exposure period, two biomarker endpoints (vitellogenin and secondary sexual characteristic) are measured in males and females.	21 days
231	Amphibian metamorophosis assay (AMA)	*Xenopus laevis*	Empirically identify substances which may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis exposing tadpoles to a minimum of three different concentrations of a test chemical and a dilution water control for 21 days. This is the only existing assay that detects thyroid activity in an animal undergoing morphological development.	21 days
234	Fish Sexual Development Test (FSDT)	*Oryzias latipes* *Danio rerio* *Gasterosteus aculeatus*	Screen and test the potential endocrine disrupters. In the test, fish are exposed, from newly fertilized egg until the completion of sexual differentiation, to at least three concentrations of the test substance dissolved in water. A biological sample (blood plasma, liver or head/tail homogenate) is collected for VTG analysis from each fish and the remaining part is fixed for histological evaluation of the gonads to determine the phenotypic sex.	About 60 days post-hatch
236	Fish Embryo Acute toxicity test (FET)	*Danio rerio*	Newly fertilized zebrafish eggs are exposed to the test chemical for a period of 96 hours and every 24 hours up to four apical observations are recorded as indicators of lethality.	96 hours
239	Toxicity test in a water-sediment system	*Myriophyllum spicatum*	Assess chemical-related effects on the vegetative growth of the target species growing in standardized media (water, sediment and nutrients) over a period of 14 days.	14 days

TEST NUMBER	TYPE OF TEST	TEST ANIMALS	PRINCIPLE OF THE TEST	TEST DURATION
240	Medaka Extended One Generation Reproduction Test (MEOGRT)	Japanese Medaka (fishes)	Evaluate the potential chronic effects of chemicals, including potential endocrine disrupting chemicals, on fish. This test measures several biological endpoints (primarily: survival, gross development, growth and reproduction).	19 weeks
241	Growth and Development Assay (LAGDA)	*Xenopus laevis*	Assess early development, metamorphosis, survival, growth, and partial reproductive maturation on larval amphibian. The test also enables measurement of a suite of other endpoints that allows for diagnostic evaluation of suspected endocrine disrupting chemicals (EDCs) or other types of developmental and reproductive toxicants.	Generally 16 weeks (maximum 17 weeks)
242	Reproduction test	*P. Antipodarum*	Assess the effect of chemicals on the target species evaluating embryo numbers in the brood pouch at the end of 28 days exposure.	28 days
243	Reproduction test	*Lymnaea stagnalis*	Assess the effect of chemicals on the target species exposing reproducing adult to a concentration range of the test chemical and monitoring for 28 days for their survival and reproduction.	28 days

As a general principle, the bioassay involving different bioindicator species at different trophic levels is an efficient and essential tool for predicting environmental hazards to the aquatic ecosystem.

The most widely used microbiotest for toxicity assessment is based on the inhibition of the bioluminescence of *Vibrio fischeri* or *Photobacterium phosphoreum* [24], a luminescent marine bacterium whose light production is directly proportional to the metabolic status of the cell. The *Vibrio fischeri* test has the advantage of being sensitive to a wide range of chemicals compared to other bacterial assays, is rapid, reproducible and shows good correlations with other standard acute toxicity test [25]. Farré et al [26] mentioned that the test presents also some disadvantages because *Vibrio fischeri* is a marine bacteria, and thus can be used only in saline solution, a medium which can enhance the insolubility of some organic compounds. In addition, the test works with a maximum tolerable level of methanol of 10%.

The use of *Daphnia* and *Ceriodaphnia* as species for toxicity testing is widely documented in aquatic risk assessment studies [26, 27, 28, 29, 30, 31, 32, 33, 34, 35]. The tests are standardized and are carried out by exposing the organisms to toxic substances under controlled conditions; results are usually expressed as the 50 % equivalent concentration value (EC_{50}). The use of *Daphnia* and *Ceriodaphnia* for toxicological assessment has many advantages. They are freshwater organisms representative of an intermediate level of the trophic chain, and this allows obtaining integrated information because the presence or absence of these crustaceans in an ecosystem (i.e. water bodies or soil) influence both primary producers and secondary consumers. Moreover, *Daphnia* and *Ceriodaphnia* are suitable for laboratory-scale tests because they are small, with a short reproductive cycle and are highly sensitive to a wide range of substances.

To our knowledge, dedicated applications of bioassay methods to the specific task of bronze preservation are not available yet. Limited and often incomplete eco-toxicological data can be

found in the literature regarding individual components of the formulations, still unrelated to the cultural heritage field.

INCRALAC, composed of acrilic resins and benzotriazole, is the most used commercial product for the protection of copper alloys. In spite of its diffusion, only a few ecotoxicological information are available for some of its hazardous ingredients, particularly benzotriazole and the organic solvents (e.g. toluene), but the overall ecotoxicology of INCRALAC has not been specifically assessed.

Wu et al. [36] reported data on benzotriazole toxicity derived from tests on several plant species (alfalfa, poplar, pumpkin, corkscrew, horseradish) and all except horseradish died within 4 weeks after the *application* of the compound. Pillard et al. [37] compared the toxicity of benzotriazole, two methyl-benzotriazoles and a butyl-benzotriazole on three widely used test microorganisms (*Ceriodaphnia dubia*, *Pimephales promelas* and *Vibrio fischeri*). *Vibrio fischeri* showed to be more sensitive than the other organisms to all the tested substances, while *C. dubia* was less sensitive than *P. promelas*, except to butyl-benzotriazole. The latter induced acute toxicity at a concentration of ≤3.3 mg/l to all organisms, thus being proved as the most toxic amongst the tested compounds. Kim et al. [38] selected nine widely used benzotriazole-based UV stabilizers and tested them on *Daphnia pulex*. No acute toxic effects were observed for all compounds within the applied dosage (<10 mg/l), but the authors raised concerns about the potential bioaccumulation and hazardous chronic effects on the aquatic ecosystem.

Toluene is a well-known substance and ecological information about its interaction with fishes, aquatic invertebrates and algae are available. For example, an EC50 of 8 mg/l for test carried out on *Daphnia magna* in 24 hours has been reported [39]. However, producers state that ecological injuries are not known or expected under normal use of this solvent.

4 Conclusions

Formulations based on alkoxysilanes or fluoropolymers have been proposed as alternative products for the inhibition of corrosion of bronze and are considered safer than INCRALAC. To our knowledge, the scientific literature does not report specific studies about the ecotoxicity of these formulations, while a few data are available for the single substances, but, as stated above, the overall toxicity of a product is not expected to be simply associated to the sum of the effects due to the individual constituents.

In conclusion, it must be highlighted that the scientific literature lacks of studies and data concerning the ecotoxicological behavior of the inhibitors for the conservation of bronze, regardless the products are traditionally or newly developed.

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