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A comparative study of the effectiveness of antifouling paints for the protection of ship hulls from biofouling

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Abstract

The release of antifouling paints' biocides into natural waters is a major concern because they may be toxic to non-target marine organisms. Their widespread use over the years led to regulations, resulting to bans or restrictions of their use. Nanoparticles are a rapidly developing sector. Especially, copper-based nanoparticles can be used as biocide in antifouling paints, a very promising application of nanoscience. In this study is monitored the leaching rate of copper from two commercial copper-based antifouling paints with different percentages of Cu_2O, 25% and 35%, and from an experimental copper-based antifouling paint with copper oxide nanoparticles as biocide, in a percentage of 5%. Painted aluminum panels are submerged in sea water for 180 days. The release from the panels will be measured with ICP-MS and will be compared. The aim of this study is to propose an alternative biocide, copper oxide nanoparticles, which may be more effective to biofouling, compared to conventional copper, in less quantities. Better results, with less accumulation of copper, less possibilities of toxicity to non-target organisms, so less harm to the marine environment. This is a way to achieve SDGs, especially #14-Life Below Water, one step closer to "conserve and sustainably use the oceans, seas and marine resources for sustainable development".

Introduction

One could say that marine environment is not only the most beautiful creation of nature, but also the most valued resource of Earth. It includes the oceans, seas and all adjacent coastal areas. According to Agenda 21 from the UNCED (United Nations Conference on Environment & Development, Rio de Janeiro, Brazil, 3 to 14 June 1992), the marine environment "forms an integrated whole that is an essential component of the global life-support system and a positive asset that presents opportunities for sustainable development". However, in the past people did not always see the marine environment with concern and did not recognize its importance for an integrated whole. Only after disasters resulted in marine pollution, people started to really care and concern about the marine environment. Such a disaster was caused, for instance, by the use of tributyltin (TBT). TBT is a biocide used in antifouling paints, which after around 30 years of use, banned and subsequently copper became the dominant biocide.

According to International Maritime Organization, antifouling paints are used to coat the bottoms of ships to prevent sea life, such as algae and mollusks attaching themselves to the hull. The new Convention defines "anti-fouling systems" as "a coating, paint, surface treatment, surface or device that is used on a ship to control or prevent attachment of unwanted organisms".

The use of toxic antifouling compounds is an environmental dilemma. Their use results in adverse effects to the marine ecosystem, in which the biota is affected by the toxic compounds and the marine ecosystem can be severely disturbed. However, when no antifouling paint is applied to ships, an additional 7.4 million tons of fuel, which means increase of approximately 4 %, will be added to the global usage of 184 million tons, according to global calculations for 1989. The fuel contains 85–86 % carbon, which results in approximately 3.1 tons of CO_2 per every ton of fuel that is consumed. By using an effective antifouling paint, approximately 22.8 million tons of CO_2 can be prevented from being released into the atmosphere each year. (Andersson, Brynolf, Lindgren, & Wilewska-Bien, 2016)

According to World Paints & Coatings Market Report conducted in 2016, the global market for paints in 2016 was approximately 50 million tons per year, but less than 10% was used in specialty applications, such as antifouling paints. (The Freedonia Group, 2016) Antifouling paints represent around 1%, this means about 0.5 million tons. It is known that these paints contain up to approximately 50% copper, so through their application it is possible that up to 0.25 million tons per year of copper are released to the marine environment. Given that small vessels are anchored in marinas, harbors and shipyards for a long period of time, it is expected that the amount of copper potentially released in such enclosed areas is quite high. Because of the extreme high amount of released copper, its concentration may have a negative impact not only on the specific target organisms, but on non-target, too. (Keller, et al., 2017) Global demand for paint and coatings is forecast to increase to 63.7 million tons in 2024, worsening the present situation. (Global Paint & Coatings Study #: 4043, 2021)

There are some measures adopted by the United Nations for vessel hull coatings. These measures are part of the 2030 Agenda for Sustainable Development, adopted by all United Nations Member States in 2015. (United Nations Sustainable Development Goals, 2015) The Agenda "provides a shared blueprint for peace and prosperity for people and the planet, now and into the future". It includes the 17 Sustainable Development Goals (SDGs), "which are an urgent call for action by all countries - developed and developing - in a global partnership". The SDGs are closely integrated with each other, there should be balance and harmonization in terms of social, economic and environmental sustainability, because an action or outcome from one goal may affect the other goals. The vessel hull coatings, and especially the biocides they contain, are of high importance to achieve key topics of the SDGs shown in the following table (Table 1): (Kim H.-J. , 2021)

SDG	3 GOOD HEALTH AND WELL-BEING	13 CLIMATE ACTION	14 LIFE BELOW WATER	17 PARTNERSHIPS FOR THE GOALS
DESCRIPTION	Ensure healthy lives and promote well-being for all at all ages	Take urgent action to combat climate change and its impacts	Conserve and sustainably use the oceans, seas and marine resources for sustainable development	Strengthen the means of implementation and revitalize the Global Partnership for Sustainable Development

Table 1: SDGs in combination with vessel hull coatings

The purpose of this study is to propose an alternative solution as biocide in vessel hull coatings to achieve these key topics of the SDGs. One promising alternative solution among others is nanoparticles of copper oxide, which are used in a percentage of 5% compared to the commercial copper oxide, which is used in a percentage up to approximately 50%, so the amount of the released copper will be less and subsequently the accumulation will be less. In addition, nanoparticles of copper oxide are very effective compared to commercial copper oxide, thus microorganisms will not attach on the hull and the negative effects of biofouling will be prevented.

To meet the target of this study, initially there is a reference to the biofouling process, its impacts in the environmental and economic sector and to the need of antifouling paints. Subsequently, there is a focus on the antifouling paints and their impacts to marine environment. The use of tributyltin, which was a big chapter in antifouling development history, the use of copper for many years, even till nowadays, and the use of booster biocides are also analyzed. In addition, there is a need of reference to the antifouling paints regulation. Nanomaterials will be the next important part of this study. Definition, synthesis, applications and a focus on copper nanoparticles used in antifouling paints are necessary to be referred. Finally, there is the experimental part, where commercial antifouling paints are compared with an alternative antifouling paint containing copper oxide nanoparticles, which may be a more sustainable solution.

1. The need of antifouling paints

1.1 Biofouling process

Marine biological fouling, usually termed marine biofouling, can be defined as the undesirable accumulation of microorganisms, plants and animals on artificial surfaces immersed in sea water. (Yebra, Kiil, & Dam-Johansen, 2004) The biological process of marine biofouling takes place regardless of geographical location and climatic conditions, so any submerged and semi-submerged structure accumulates marine species that have the tendency to attach permanently, grow and multiply on its surface. (Michelis & Gougoulidis, 2015) It is estimated that in fouling are involved approximately 4000-5000 species, which can be classified into two groups based on the size of the fully grown specimen, macrofouling, which includes animals and plants, and microfouling, which includes a complex of mucilaginous mixture of bacteria and diatoms, also known as slime. (Coating and Inspection Manual, 1997) According to other sources, fouling organisms in the marine environment can be divided into two categories: hard and soft foulers. Hard foulers are organisms with solid skeletons, tubes or shells, such as barnacles, mussels, tubebuilding polychaetes, bryozoans and corals. Soft foulers are organisms that lack hard structures, such as ascidians, macroalgae, hydroids and sponges. (Andersson, Brynolf, Lindgren, & Wilewska-Bien, 2016) Some of these species swim, other are carried about by currents and many of them, in order to fulfil their life cycles, find a surface on which they attach. Regarding the present study, such surfaces are ships' bottoms. (Coating and Inspection Manual, 1997)

The biofouling process consists of four stages: primary film formation, biofilm formation, diatom and protozoan colonization and settlement of invertebrate larvae and algal spores (Figure 1).

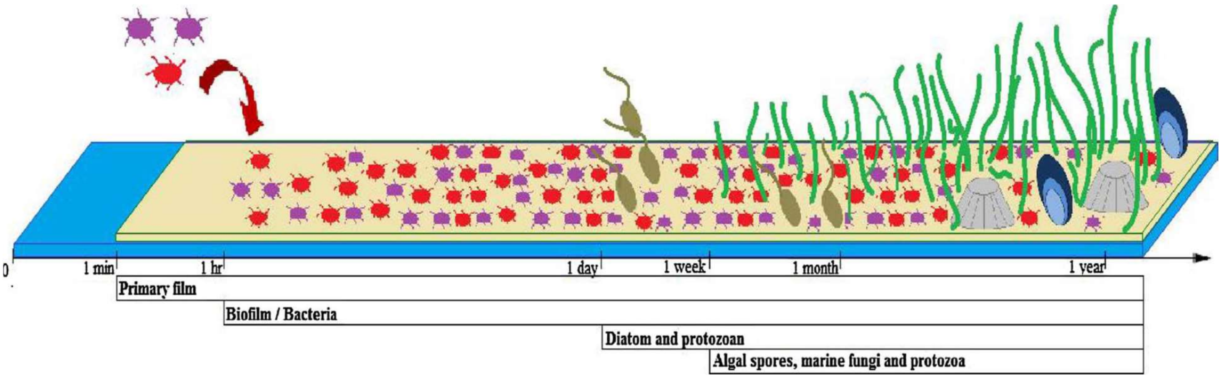


Figure 1: The four-step process of biofouling formation (Amara, Miled, Slama, & Ladhari, 2017)

Primary film formation is a process which starts with a rapid adsorption of organic molecules already present in the water, such as proteins, polysaccharides, nucleic acids, humic acids and possibly inorganic compounds onto the substrate. This film is formed within minutes of exposure of surface to seawater.

The biofilm formation takes place on several steps. Initially, bacteria are transported to the conditioned area due to factors, such as gravity, dynamics of fluids and electrostatic interactions. Subsequently, the bacteria interact weakly with the conditioned surface, in a way that they can be detached simply by rinsing or by shear conditions. This step is termed reversible adhesion and it is followed by the irreversible adhesion, where permanently attachment occurs in minutes. The roughness of irregular microbial colonies helps to trap more particles and organisms. After some days, follows the fourth step, termed maturation and dispersion and involves the settlement and the growth of larger marine invertebrates together with the growth of macroalgae.

Within some days or weeks of the immersion of a surface in seawater, begins the settlement of unicellular eukaryote, which is the third stage. Finally, regarding the final and longest stage of biofouling process, the settlement and growth of larger marine invertebrates together with the growth of macroalgae occur within several days to weeks after the immersion. (Amara, Miled, Slama, & Ladhari, 2017)

1.2 Impacts of biofouling - Need of antifouling paints

Biofouling has a major economic impact on the maritime sector. The presence of fouling increases the frictional resistance of the vessel by increasing hull roughness and wall shear stress. Fouling accumulation (Figure 2) also increases the weight of the vessel, so the propulsive power required to move the ship at the same speed increases, leading either to a loss of speed for the same propulsive power or to a rise in fuel consumption to maintain the speed profile.

There are side effects also on the environment caused by biofouling. The increase in fuel consumption leads to increase of emitted CO_2 and other greenhouse gases. Other airborne emissions, such as sulfur oxides (SO_X), nitrous oxides (NO_X) and particulate matter are also increased due to fouling.

Moreover, fouling attached to the hull can be source for translocation of invasive aquatic species, which can pose a threat to the local ecosystem. (Michelis & Gougoulidis, 2015) Together with pollutants, the overexploitation of resources and the destruction of habitats, invasive species have been identified as one of the four largest threats to the marine environment. (Andersson, Brynolf, Lindgren, & Wilewska-Bien, 2016) They can also have a negative economic and social impact, because they may be destructive for aquaculture, fishery and infrastructures. Unfortunately, the effects caused by invasive species on marine environments are almost always irreversible. (Michelis & Gougoulidis, 2015)

Figure 2: Fouling accumulation (animine.eu)

1.3 Historical development of antifouling systems

Biofouling has been recognized as problematic for more than 2000 years and many kinds of antifouling methods have been investigated. (Amara, Miled, Slama, & Ladhari, 2017) (Yebra, Kiil, & Dam-Johansen, 2004) It is said that early Phoenicians and Carthaginians have used pitch and possibly copper sheathing in ship's bottoms, while other ancient cultures have used wax, tar and asphaltum. Wax and tar were also used by the Greeks in 300 B.C., and even lead sheathing, which was secured with copper nails by both Romans and Greeks. Centuries later, from 13th to 15th century, pitch, sometimes blended with oil, resin or tallow was extensively used for the protection of ships. (Yebra, Kiil, & Dam-Johansen, 2004) In the 18th century, several countries returned to the use of copper sheathing, with copper and zinc nails, and experimented with sheathings of zinc, lead, nickel, galvanized steel and other materials, as well as copper-coated wood sheathing. After the Second World War, copper sheathing was abandoned, when it was discovered that the iron nails used had corroded due to the galvanic action of the copper sheathing, with the consequent structural risks that this implied. In 1786 nails of a copper–zinc alloy started to be used, which were sufficiently resistant to be employed in shipbuilding. In the mid of 19th century and after several attempts, a variety of paints was developed, based in the idea of dispersing a toxicant in a polymeric vehicle. These antifouling paints contained copper, arsenic or mercury oxide as toxicants dispersed in linseed oil, shellac or rosin. (Almeida, Diamantino, & Sousa, 2007) (Yebra, Kiil, & Dam-Johansen, 2004)

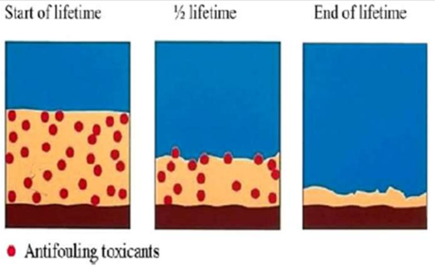
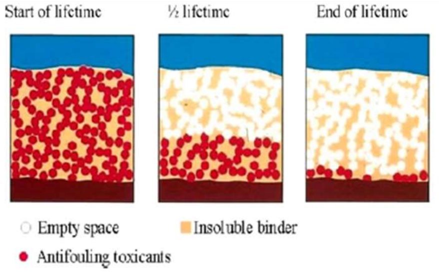
The first organometallic paints with tin, arsenic, mercury and others appeared around 1950 and gave rise, after numerous and successive developments to tributyltin (TBT)-based antifouling paints. (Almeida, Diamantino, & Sousa, 2007) Tributyltin was originally used to combat the tropical disease Bilharzia. However, the substance had also excellent antifouling properties, and it was commercialized as an antifouling biocide by incorporating it into paint. Tributyltin works as a toxicant with primary activity on shell and vegetative fouling. (Andersson, Brynolf, Lindgren, & Wilewska-Bien, 2016)

The main types of antifouling paints used on steel hulls in the second half of the 20th century can be classified into three categories, based on the chemical properties of the paint matrix and the mechanisms involved in releasing toxic compounds (Table 2).

The first category includes antifouling paints that have a water-soluble resin as matrix and the toxic compound is at the coating surface. The biocides are oxides of lead, arsenic, mercury or copper, and their lifespan is approximately 6-12 months (Amara, Miled, Slama, & Ladhari, 2017) or 12-15 months. (Almeida, Diamantino, & Sousa, 2007) The classical film-forming material in these systems contains high proportions of rosin, which is a natural resin obtained from the exudation of pine and fir trees. Natural rosin-based paints consist of some acids, which contain double bonds and a carboxyl group. These double bonds affect the stability of the rosin, because they make it oxidable when exposed to air, so the application of the paint can only be performed a short time before immersion. (Yebra, Kiil, & Dam-Johansen, 2004) One more disadvantage of antifouling paints of this category is that in stationary conditions they have relatively weak biocidal activity, something that makes them unsuitable for slow speed vessels or ships that remain idle for long periods. (Almeida, Diamantino, & Sousa, 2007)

For the second category, long-life antifouling paints, the matrix must be insoluble in water. High quantities of toxicants remain in the paint, but the concentration at the surface falls below the effective level and the coatings must be replaced after 18-24 months. (Amara, Miled, Slama, & Ladhari, 2017) The binders that are used have high molecular mass, e.g., acrylics, vinyls, chlorinated rubber, which are insoluble in sea water. The toxicants they contain are gradually released, so the sea water spreads through the pores that are left empty and go on to dissolve the next toxicant particles. The toxicant release rate decreases in time, protection becomes less efficient and the surface rougher, more liable to retain pollutants from the sea water, a fact that contributes to blocking the release of toxicants. (Almeida, Diamantino, & Sousa, 2007)

The third category includes self-polishing antifouling paints, where the biocide is bound to the polymeric matrix and is released by hydrolysis at the paint surface. The rate of release is constant but depends on water movement and its life usually ranges between 4 and 5 years. (Amara, Miled, Slama, & Ladhari, 2017)

	Main components		
Type of paint	**Binder**	**Pigment/biocide**	**Mechanisms**
Water-soluble matrix	Rosin and others	Copper, arsenic, zinc, mercury or iron oxides	
Insoluble matrix	Acrylic resins, vinyl resins or chlorinated rubber polymers	Copper and zinc oxides with or without organo-metallic compounds	

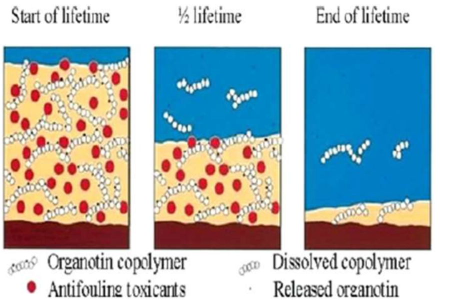
Self-polishing paints containing tin (TBT-SPC)	Acrylic polymer with TBT groups bonded to main chain by ester binders	Zinc oxide and insoluble pigments or copper oxide, tri-organo-tin and co-biocides	

Table 2: The three main categories of antifouling paints based on the chemical composition of the paint matrix, used in the second half of 20th century

1.4 Main antifouling paints - Marine pollution

1.4.1 Tributyltin

A characteristic example of this category is tributyltin self-polishing copolymers paint (TBT-SPC), compatible with both steel and aluminum hulls, which was the most successful antifouling system ever. These paints are based on acrylic copolymer, usually methyl methacrylate, with TBT groups bonded to the main polymer chain by ester bonds (Figure 3), in which the polymer is soluble in sea water. (Almeida, Diamantino, & Sousa, 2007) So, after immersion, the soluble pigment particles in contact with sea water begin to dissolve. The copolymer of TBT in the paint is hydrophobic, something that prevents sea water from penetrating the paint film, as a result sea water can only fill the pores created after the dissolution of the soluble pigment particles. The carboxyl-TBT linkage is hydrolytically unstable under slightly alkaline conditions. These are usually the conditions of marine waters and result in a slow, controlled hydrolysis reaction, where the TBT moiety is cleaved from the copolymer.

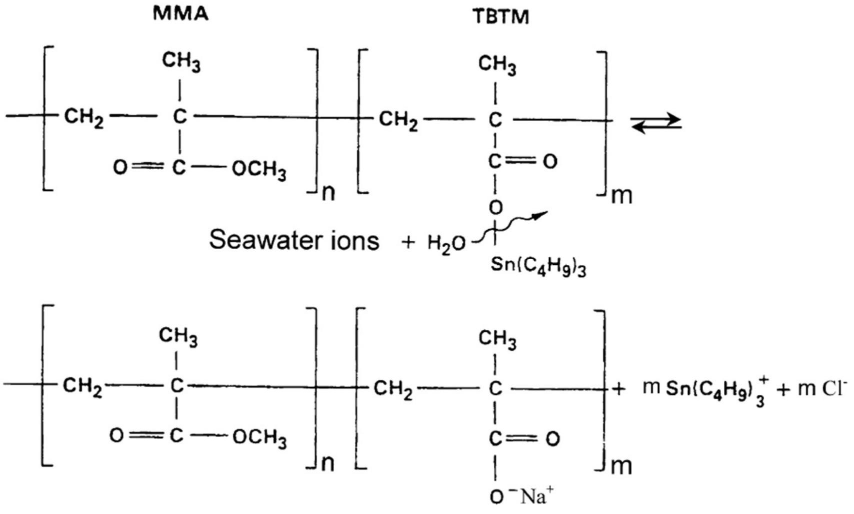


Figure 3: Controlled release mechanism of TBT copolymer by hydrolysis (Yebra, Kiil, & Dam-Johansen, 2004)

The main advantage of these paints is that it is possible for the rate of reaction to be customized. So, the paint industry could design different antifouling paints for ships with different activities. (Yebra, Kiil, & Dam-Johansen, 2004) Fast vessels require more efficient antifouling protection, because they are more sensitive to increases in fuel consumption caused by biofouling, so they need products with a low polishing rate, while slow vessels or vessels that spend long periods of time in ports require products with faster polishing rate. (Almeida, Diamantino, & Sousa, 2007) The manipulation of the rate of the reaction leads to the delay of the dry-docking period by five years. One more advantage is that when dry-docking and repainting are needed, the old paint does not require removal; repainting can be conducted directly on the old paint, saving both time and money. (Andersson, Brynolf, Lindgren, & Wilewska-Bien, 2016)

When tributyltin is released from antifouling coatings in the aquatic environment, it is quickly removed from the water column and adheres to bed sediments because TBT has a high specific gravity (near 1.2 Kg l^{-1} at 20°C). The absorption of TBT to sediments is reversible, so contaminated sediments can act as a long-term source of dissolved-phase contamination to the overlying water column. (Antizar-Ladislao, 2008) TBT can either be absorbed by organic materials or bound to suspended particles in the water. So, it is accumulated in the tissues of marine organisms through the food web. In this way, it affects the higher trophic organisms, such as birds and mammals. (Tang, et al., 2021) Indeed, TBT and its related agents were found to be highly toxic because of their lipophilicity, which allows them to penetrate the biological membranes and to disturb the function of mitochondria. (Amara, Miled, Slama, & Ladhari, 2017) (Antizar-Ladislao, 2008) It is demonstrated to cause impairments in growth, development, reproduction and survival of many marine species. TBT has proven to be extremely toxic to aquatic organisms in early life stages, e.g., fish larvae are very sensitive to TBT, and in the adult life stages, too. Studies investigated the effect of TBT toxicity on several marine organisms and resulted that species with a high rate of uptake or a low rate of metabolic conversion and elimination presents relatively high bioaccumulation ratios.

TBT was originally designed for use on the hulls of large ships, but an aggressive marketing program in the 1960s saw its fashionable use worldwide on much smaller craft in the oceans and within inland waterways. (Antizar-Ladislao, 2008) However, the impact of organotins on marine organisms induced many governments to restrict its use. Some years later, in 1982, France has banned the application of TBT-based antifouling paints on vessels less than 25 meter long, when several oyster farms experienced major declines due to reduced oyster growth, anomalies in larval development, and shell malformation affecting 80–100% of individual oysters. Another major negative impact was the identification of intersex effects (or condition of imposex, which is the superimposition of male features such as penis in females). (Janssen & Roose, 2011) In Figure 4 is obvious the development of a penis in female gastropod Ocenebra erinacea at only 1 ng/L of TBT. (Amara, Miled, Slama, & Ladhari, 2017) This was a documented case which revealed that hormonal disturbances do occur in marine animals. For this reason, TBT can be called endocrine disrupter. (Janssen & Roose, 2011)

Due to the multiple negative effects, the International Maritime Organization decided in October 2001 to place a global ban on the use of TBT. By 2003, no ship was permitted to be repainted with it, and beginning 1 January 2008, all vessels were required to be free from TBT. (Andersson, Brynolf, Lindgren, & Wilewska-Bien, 2016)

Although the use of these antifouling paints was restricted by IMO in 2008, TBT contamination is still detected worldwide, which is likely due to its persistence and illegal use. Several recent studies have also found the presence of TBT in the samples of seawater, sediments, and aquatic organisms. In particular, high concentrations of TBT have been found in areas adjacent to harbors, marinas, and fishery ports in coastal waters. (Laranjeiro, Sanchez-Marín, Oliveira, Galante-Oliveira, & Barroso, 2017) (Quintas, Allvarez, Arias, Garrido, & Marcovecchio, 2019) (Tang, et al., 2021)

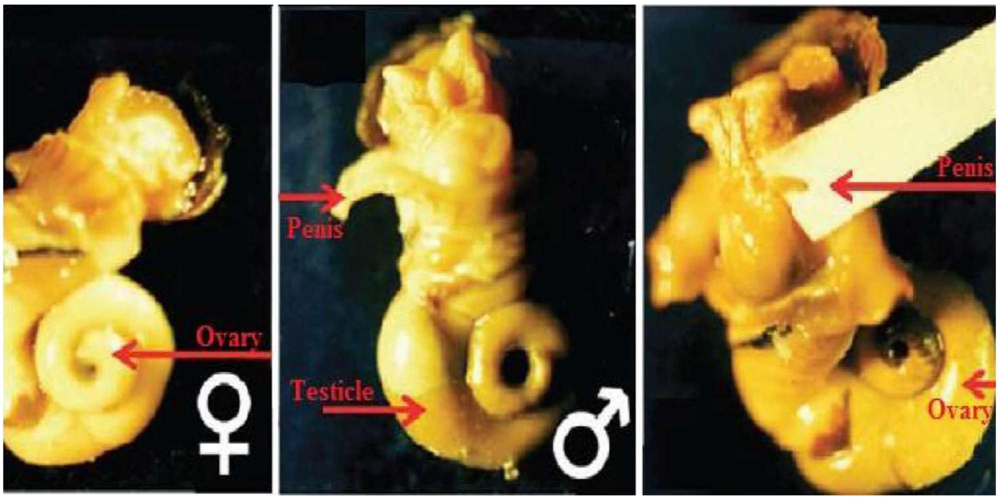


Figure 4: Penis development in female gastropod Ocenebra erinacea (Amara, Miled, Slama, & Ladhari, 2017)

1.4.2 Copper

With the gradual elimination of organotin based formulations, copper has become the principal biocide component of most antifouling paints. Copper compounds, used in antifouling paints as their pigments, are copper oxide, copper thiocyanate or metallic copper, leaking copper in ionic form. (Andersson, Brynolf, Lindgren, & Wilewska-Bien, 2016) The most usually form in antifouling paints is copper oxide (Cu_2O). (Amara, Miled, Slama, & Ladhari, 2017) The way in which copper compounds are released is described by the following reactions:

$$\frac{1}{2}Cu_2O(s) + H^+ + 2Cl^- \rightarrow CuCl_2^- + \frac{1}{2}H_2O \ (1)$$

$$CuCl_2^- + Cl^- \leftrightarrow CuCl_3^{2-} \ (2)$$

Reaction (1) is irreversible and is influenced by the kinetics, while reaction (2) is instantaneous, reversible and may be permanent equilibrium. Since sea water is an oxygenated medium, copper complexes are quickly oxidized to Cu^{2+}, which is the main biocidal and the most toxic form to living organisms, originated by cuprous oxide. (Almeida, Diamantino, & Sousa, 2007) In this ionic form, copper easily passes through cell membranes. (Andersson, Brynolf, Lindgren, & Wilewska-Bien, 2016) However, copper ions are not equally efficient against all types of fouling organisms. The sensitivity of biofouling to copper ions decreases in the following order: microorganisms, invertebrates, algae, bivalves and macrophytes.

Copper is an essential element that is required for the normal growth of all plants and animals, so it is naturally present in sea water and measures for the limitation of its use cannot be implemented, like the case of TBT. According to some sources, it is estimated that the amount of copper released by antifouling paints amounts to 3000 tons per year (Almeida, Diamantino, & Sousa, 2007), while according to other sources this amount is equal to 15000 tons per year. (Andersson, Brynolf, Lindgren, & Wilewska-Bien, 2016) In both cases, the amount of copper is insignificant compared to the 250000 tons originating from natural sources. However, copper is lipophilic and tends to bioaccumulation, fact that explains why it has remained in the formulations of antifouling paints over the years. (Almeida, Diamantino, & Sousa, 2007) Specifically, the aquatic organisms receive copper nanoparticles through the gills, digestive tract, olfactory organ and the skin. At the cellular level, nanoparticles enter the cell and then induce toxic effects on the living organisms. Copper nanoparticles can be transferred to higher trophic levels due to accumulation, so there is a growing concern about effects of copper on the body system of fish and on the trophic chain generally. (Ghais, Bhardwaj, & Kumbhar, 2020) In the food products industry, the use of copper and zinc as antifouling compounds is unwelcomed for both health and marketing perspectives. One of the main European Union laws concerning chemical safety, the Dangerous Substances Directive, listed these two metals as being toxic to aquatic organisms, with long-term adverse effects on the environment. For these reasons, their release into the environment requires control. Many other formulations have been developed to replace toxic antifouling. These new environment-friendly biocides have been developed as tributyltin free coating alternatives, such as Irgarol 1051 (which was later banned), DCOIT, diuron and zinc pyrithione. (Amara, Miled, Slama, & Ladhari, 2017)

1.4.3 Modern Biofouling - Booster Biocides

Modern antifouling coatings are divided into two main categories, biocidal and non-biocidal. Biocidal antifoulings include coatings that their mechanism is based on the release of biocides in sea water, like the cases of Cu and TBT. As it was mentioned above, after the ban of tributyltin, copper has become the predominant biocide, while additional booster biocides are utilized to target copper tolerant marine species. The three main biocidal antifouling technologies currently available are Control Depletion Polymers, Self-Polishing Copolymers (SPC), and Hybrid or Self-Polishing AF coatings. The non-biocidal group includes Fouling Release coatings and Hard Inert coatings. These coatings are regarded to be non-toxic in terms of not containing biocides to control fouling. (Michelis & Gougoulidis, 2015)

Control Depletion Polymer (CDP) coatings contain biocides which are integrated into a water-soluble rosin binder. The slow dissolution of rosin, to enable biocides to leach, is performed by hydration. (Michelis & Gougoulidis, 2015) The SPC technology with copolymer-bound was the leading antifouling type since 1950. Twenty years ago, Japanese paint companies succeeded in replacing TBT with copper or zinc-pyrithione and created the first TBT-free self-polishing paints. Self-Polishing Copolymers are insoluble metallic or organic synthetic polymers containing biocides. The surface layer of the coating contains biocides (copper, zinc, and booster biocides), which are released into the water by a chemical reaction (hydrolysis) through which the copolymer becomes soluble. This process enables continuous exposure of new active coating surface. The Hybrid antifouling coatings have the CDP features of surface tolerance and attractive volume solids, together with the SPC features of polishing rate control, biocide release control and a reduced leached layer size. The addition of a hydrolysable polymer, such as copper acrylate, to rosin to form this type of antifouling has been further enhanced using zinc-pyrithione boosting biocides. (Watermann & Eklund, 2019)

As it was mentioned above, a new generation of antifouling biocides, “booster” biocides came up as alternatives to replace the previously banned organotin base compounds. The most frequently used antifouling biocides have become cybutryne or Irgarol 1051, DCOIT, diuron and zinc pyrithione.

Several algal species have exhibited tolerance to high copper levels. To boost the effects against algal foulers, the substance Irgarol 1051 has been added to copper based antifouling paints. (Andersson, Brynolf, Lindgren, & Wilewska-Bien, 2016) Its chemical structure is based on sulfur and nitrogen ($C_{11}H_{19}N_5S$). (Amara, Miled, Slama, & Ladhari, 2017) Irgarol 1051 is an herbicide that is primarily toxic to algae and cyanobacteria. Its toxicity is derived from its ability to inhibit photosynthetic electron transport in chloroplasts and cyanobacteria. For these groups of organisms, Irgarol 1051 has a very high toxicity. (Andersson, Brynolf, Lindgren, & Wilewska-Bien, 2016) The lack of a systematic evaluation of the environmental risk of this biocide together with its worldwide use, brought a new threat to the marine environment. Banned in 2017 from the EU PT 21 list (Table 4, Chapter 1.5.2), which include all antifouling products approved in the European market, Irgarol 1051 exhibits a relative long half-life in seawater of up to 350 days and high toxicity. (de Campos, et al., 2022)

SeaNine 211 is one of the commercial antifouling agents currently in use globally. The biocidal ingredient in SeaNine 211 to yield antifouling activity is the DCOIT (4,5-dichloro-2-n-octyl-4-96 isothiazolin-3-one). Widespread pollution has been detected for DCOIT in different matrices, such as seawater, sediment and even biota. Although DCOIT is claimed to degrade fast in the aquatic sediment with half-life less than one hour, its accumulation has also been detected in sediments samples around the world. Exposure to DCOIT at very high concentrations will disrupt the endocrine system and impair the reproductive performance. Therefore, in view of the very high toxicity to non-target marine organisms, the accumulation of DCOIT in environmental matrices will endanger the health of marine organisms and thus result in non-negligible threats to the safety of marine ecosystem. (Chen & Lam, 2017)

Diuron works in the same way as Irgarol 1051. It is a photosynthetic inhibitor and primarily toxic to algae and cyanobacteria. Its chemical structure is based on chlorine and nitrogen $C_9H_{10}C_{l2}N_2O$. (Amara, Miled, Slama, & Ladhari, 2017) Diuron is relatively soluble in water and it is present at high concentrations in marine surface waters, but it has only been detected at low concentrations in sediments. Diuron exhibits limited bioaccumulation, is relatively persistent in seawater and is stable to UV radiation exposure. It can remain suspended and available for uptake by marine organisms. The degradation products of Diuron have been reported to be up to 215 times more toxic than the original substance. (Andersson, Brynolf, Lindgren, & Wilewska-Bien, 2016)

Zinc pyrithione (ZnPt) is one of the most widely used alternative antifouling biocides as a replacement for TBT. Its chemical structure is based on sulfur, zinc and nitrogen ($C_{10}H_8N_2O_2S_2Zn$). (Amara, Miled, Slama, & Ladhari, 2017) In the aquatic environment ZnPT is rapidly degraded, mostly by photolysis. However, where UV light is not able to penetrate through the water column because of turbidity or a deeper water depth, ZnPT may accumulate and persist in the sediment, continuously exerting toxic effects on the marine environment. While the photolysis and transchelation processes have been relatively well-studied, little information about the biodegradation and hydrolysis of ZnPT is available. Products formed during the processes are mostly unidentified. (Soon, et al., 2019)

1.5 Antifouling paint legislation

Legislation about antifouling products has been developed by many countries and regions in order to control the use and marketing of antifouling paints for the protection of human health, animals and the environment. Two of the most important pieces of legislation are the IMO International Convention for the Control of Harmful Antifouling Systems on Ships and the Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 (Biocidal Products Regulation) concerning the making available on the market and use of biocidal products. (Pereira & Ankjaergaard, 2009)

1.5.1 The AFS Convention

As it was mentioned above, while organotin tributylin or tributyltin (TBT) was initially designed for use on hulls in large ships, its use extended worldwide also on smaller crafts after a marketing campaign in the 1960s. It is probably the most toxic substance, whose introduction into the marine environment was intentionally. In the 1970s in France there was a high percentage of mortalities of oyster larvae, reduction of oyster growth, shell deformations in a level that make them unmarketable. But the results of the use of TBT were not observed only in France. In the 1980s in England there was a decline of the dog whelk population. The results of TBT are also worrying when studies showed that it can cross the placenta in marine mammals. TBT was detected in the blood of people who fish especially for a living and may have everyday contact with it. This fact would have serious long-term consequences because TBT is toxic after ingestion and after contact with the skin and the results were obvious not only to fishermen, but also to shipyard workers, who were exposed to dust and vapors containing TBT. TBT is called endocrine disruptor due to its intersex effects on some organisms, but in human can affect most body systems, such as endocrine, respiratory, reproductive, integumentary, cardiovascular, digestive and immune systems.

Because of the severity of impacts caused by TBT, a need of marine ecosystem and maybe human protection was born. Some regulations and laws for the restriction of TBT use were developed during 1980s and 1990s, with the France as a pioneer. Of course, at the beginning the regulations were adopted to protect oyster mariculture and subsequently the financial of the stakeholders, so there were financial interests. The regulations inhibited the application of paints containing TBT on small vessels, with length less than 25 meters, but only regionally. Recreational vessels regarded as the biggest contributor to the problem via passive leaching, because they spend a lot of time in enclosed marinas. Other countries followed with TBT restrictions, such as the UK (1987), USA (1988), Canada (1989), Australia (1989) and the EU (1989). (Dafforn, Lewis, & Johnston, 2011)

However, the pollution caused by TBT leads to a need of a global legal binding instrument. The Paris Commission asked the International Maritime Organization's Marine Environment Protection Committee (IMO MEPC) to investigate the need for measures to limit the use of TBT on seagoing ships in 1988. IMO recognizes the harmful environmental effects of TBT in 1989 and then, in 1990 IMO's Marine Environment Protection Committee (MEPC) adopted a resolution on "Measures to control potential adverse impacts associated with the use of tributyltin compounds in antifouling paints" according to which Governments-Member states of IMO were recommended to adopt measures for the elimination of the use of antifouling paints that contain TBT on non-aluminum hulled vessels of less than 25 meters length and also of the TBT-based paints with an average leaching rate of more than 4 μm of organotin per cm^2 per day.

IMO adopted an Assembly resolution in November 1999, which urged MEPC to develop a global, legally binding instrument to address the harmful effects of anti-fouling systems used on ships. This instrument developed by MEPC would ensure an international prohibition on the application of organotin compounds which act as biocides in anti-fouling systems on ships by 1 January 2003, and a complete prohibition by 1 January 2008. The instrument responsible for the application of these measurements was later adopted as the International Convention on the Control of Harmful Anti-fouling Systems on Ships.

The AFS Convention was adopted on 5 October 2001 and entered into force on 17 September 2008. The Convention, except for the prohibition of the use of organotin compounds in antifouling paints, will also establish a mechanism for the prevention of any possible future use of other harmful substances used in antifouling systems. It includes three parts, the Articles, the Annexes and Regulations and it has also Appendices. Figure 5 illustrates the important dates in the AFS Convention. (Pereira & Ankjaergaard, 2009)

According to the Annex 1 of the Convention, organotin compounds acting as biocides in antifouling paints shall not be applied or re-applied by an effective date of 1 January 2003. In addition, by an effective date of 1 January 2008 the measures are stricter, since ships shall not have applied on their hulls, external parts and surfaces organotin compounds or they shall have applied a coating, which does not let organotin compounds leach from the below antifouling system, because it does not comply with the regulation.

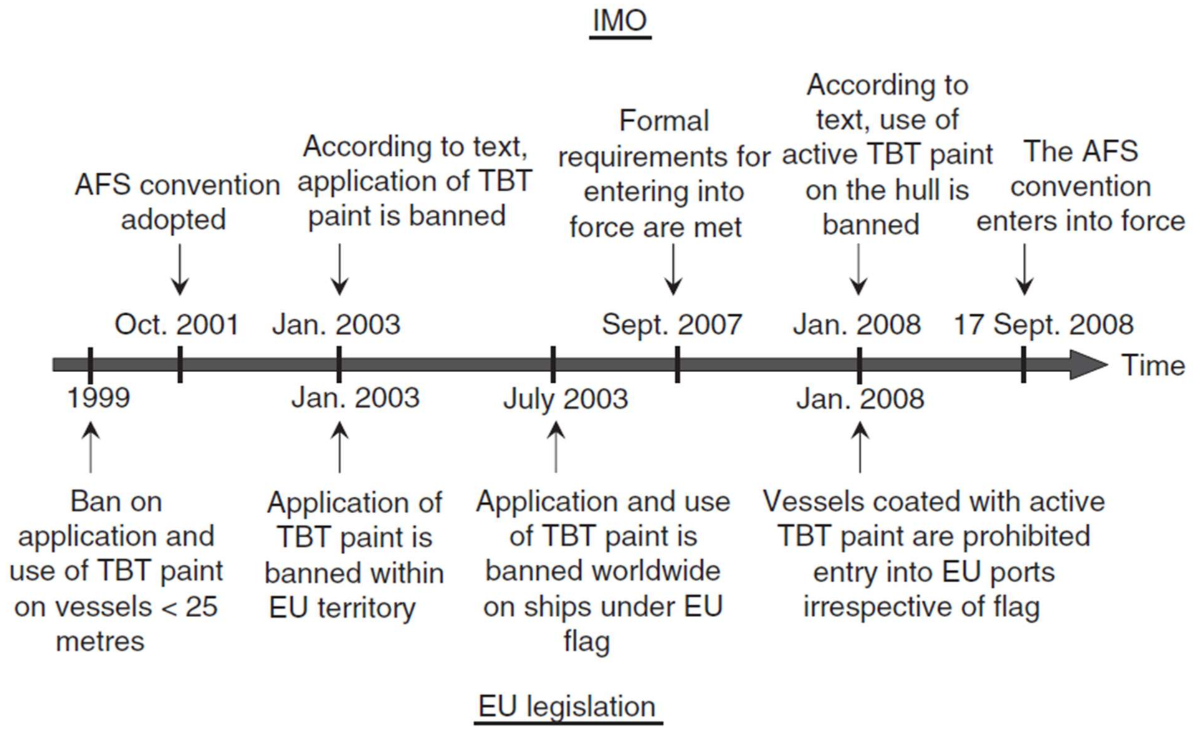


Figure 5: IMO and EU legislation regarding TBT

All these measures are applied to all ships. The only exceptions are fixed and floating platforms, floating storage units (FSUs) and floating production storage and off-loading units (FPSOs) with a construction date before 1 January 2003, without drydocking on or after 1 January 2003.

An initial survey should be conducted before ships are put into service or before the International Certificate is issued for the first time, for ships which are above 400 gross tonnage and above, except for foxed or floating platforms, FSUs and FPSOs. In addition, when there is change regarding antifouling systems, again survey should be conducted. For ships less than 400 gross tonnage, which are 24 meters or longer, should have a Declaration on Antifouling Systems, signed by the owner or authorized agent.

As it was mentioned above, Irgarol is a booster biocide with very high toxicity. Accumulation is an important drawback about Irgarol, initially in macro algae, fish and later in humans. In all European Union Member State, marketing and use of antifouling paints are prohibited. MEPC wants to include controls on cybutryne to the AFS Convention. (Johnson, 2018) There are many scientific data proving that cybutryne causes serious effects to the environment and especially to aquatic ecosystems. Thus, in 2021 MEPC adopted amendments in order to control the biocide. These amendments entered into force on 1 January 2023. According to them, ships shall not apply or reapply antifouling systems with cybutryne, remove antifouling paint with this biocide at the next renewal after 1 January 2023, but no later than 60 months from the last time that was applied antifouling containing cybutryne.

There are four Resolutions included to AFS Convention. Briefly, Resolution 1 regards the early and effective application of the Convention, Resolution 2 the future work of the organization pertaining to the Convention, Resolution 3 the approval and test methodologies for antifouling systems on ships and Resolution 4 the promotion of technical cooperation. (Johnson, 2018) (Dafforn, Lewis, & Johnston, 2011) (International Maritime Organization, n.d.)

1.5.2 The Biocidal Products Regulation

An example of legislation about antifouling products applying to a region of countries is the Biocidal Products Directive (EU, 1998). It entered into force on 14 May 2000 and intended to regulate the production, marketing and use of non-agricultural pesticides in the European Union, such as wood preservatives, drinking water disinfectants, etc. (Pereira & Ankjaergaard, 2009) In 2013, the Biocidal Products Directive was superseded by the Biocidal Products Regulation.

The purpose of this Regulation is a better functioning of the internal market, which will be achieved by the harmonization of the rules on the making available on the market and the use of biocidal products. A high level of protection of both human and animal health and the environment is also a target of this Regulation. The biocidal products, before placed on the market, require authorization, subsequently the active substances contained in that products have to be previously approved. However, this process is time-consuming, thus there are certain exceptions. When there are active substances in the Review Program, the biocidal products that contain them can be made available on the market and used, until the final decision of the approval, up to 3 years later. The authorization consists of two steps. The first step includes the evaluation of the active substance and the fulfillment of certain criteria, so that it can be approved in s specified product-type. The second step regards the authorizations of each product that consists of, contains or generates the approved active substance. Table 4 illustrates the list of substances of PT 21: Antifouling products, with a status of approved, not approved, initial application for approval in progress, approved-renewal in progress. From the table it is obvious that the use of cybutryne is banned. (European Chemichals Agency (ECHA), n.d.)

Active Substances					
Substance name	EC/List no.	CAS no.	Approval start date	Approval end date	Approval status
4,5-Dichloro-2-octylisothiazol-3(2H)-one (4,5-Dichloro-2-octyl-2H-isothiazol-3-one (DCOIT))	-; 264-843-8	64359-81-5	01/01/2016	31/12/2025	Approved
Bis(1-hydroxy-1H-pyridine-2-thionato- O,S)copper (Copper pyrithione)	-; 238-984-0	14915-37-8	01/10/2016	31/12/2025	Approved
Copper	231-159-6	7440-50-8			Initial application for approval in progress
Copper	231-159-6	7440-50-8	01/01/2018	31/12/2025	Approved
Copper thiocyanate	-; 214-183-1	1111-67-7	01/01/2018	31/12/2025	Approved
Dichloro-N-[(dimethylamino)sulphonyl] fluoro-N-(ptolyl)methanesulphenamide (Tolylfluanid)	-; 211-986-9	731-27-1	01/07/2016	31/12/2025	Approved
Dicopper oxide	-; 215-270-7	1317-39-1	01/01/2018	31/12/2025	Approved
Free radicals generated in situ from ambient air or water	-	-			Initial application for approval in progress
Medetomidine	-	86347-14-0	01/01/2016	30/06/2025	Approved-Renewal in progress
N-(Dichlorofluoromethylthio)-N',N'- dimethyl-N-phenylsulfamide (Dichlofluanid)	-; 214-118-7	1085-98-9	01/11/2018	31/12/2025	Approved
*N'-tert-butyl-N-cyclopropyl-6-(methylthio)-1,3,5-triazine-2,4-diamine (**Cybutryne**)*	-; 248-872-3	28159-98-0			**Not approved**
Pyrithione zinc (Zinc pyrithione)	-; 236-671-3	13463-41-7			Initial application for approval in progress
Tralopyril	-	122454-29-9	01/04/2015	31/03/2025	Approved
Zineb	-; 235-180-1	12122-67-7	01/01/2016	31/12/2025	Approved

Table 3: List of PT21 substances

Nanomaterials are also under Biocidal Products Regulation. Nanomaterials are contained in many everyday products that are on the European market. Some examples are batteries, antibacterial clothing, cosmetics and coatings. Despite their advantages, they may be dangerous for human health and the environment, when they are not used properly. So, European Chemicals Agency (ECHA) collaborates with Member State competent authorities, the European Commission, Non-Governmental Organizations (NGOs), industry associations and international organizations to achieve implementation of EU chemicals legislation for nanomaterials. In this way, any possible risk caused by nanomaterials will be controlled. There are specific provisions for nanomaterials defined in the Biocidal Products Regulation. Their application for active and non-active substances are according to some characteristics. The particles should have a size of 1-100 nm in at least one dimension in a percentage of 50% or more and be in an unbound state or as an aggregate or agglomerate. (European Chemichals Agency (ECHA), n.d.)

In conclusion, after all the damages caused by TBT and after TBT banned, copper become the predominant antifouling biocide. However, its use is carefully considered in several countries. There are already restrictions on copper release rates in Canada and Denmark. There are also other strategies regarding particular vessels, ecological sensitive areas and highly contaminated areas in countries and areas, like Sweden, San Diego Bay and California. In addition, in some countries there is regulation for “booster” biocides, like the aforementioned cybutryne or Irgarol. Also, diuron is banned in Denmark and in the Netherlands. There is a need of continuous studies about the use of biocides for the prevention of any harmful result caused by them and for the protection of human health and the environment. (Dafforn, Lewis, & Johnston, 2011) (Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products Text with EEA relevance, 2012)

2. Nanoparticles

2.1 Introduction - Characteristics and synthesis of Nanoparticles

The study of matter's properties at the nanoscale is known as nanoscience and it focuses on the distinct, size-dependent properties of solid-state materials. The production of nanoparticles is not necessarily conducted by modern synthesis laboratories, but it has existed in nature for a long period of time. Nanoparticles, naturally occurred, include organic, such as proteins, polysaccharides, viruses, and inorganic compounds. The naturally occurring nanoparticles are mainly produced by weathering, volcano eruptions, wildfires or microbial processes. The use of nanoparticles can be tracked to ancient times. An example is dated 4500 years ago, when clay minerals were applied as natural nanomaterials to control reinforcement of a ceramic matrix with natural asbestos nanofibers. Metal nanoparticles were also used as color pigments in luster and glass technology. The special optical properties of nanoparticles are obvious in the example of Lycurgus cup in the British Museum (Figure 6). It was probably manufactured by Romans in the 4th century and in daylight it has a green color, with changes to red when illuminated from the inside. In this cup, gold nanoparticles were used in ruby glass. (Heiligtag & Niederberger, 2013)

Figure 6: The Lycurgus Cup, left: the cup is green because of the reflected light, right: the cup is red because of the transmitted light

The term nanoscience differs from the term nanotechnology. It is known that the word nanotechnology was firstly used by Dr. Norio Taniguchi in 1974. According to Taniguchi "Nanotechnology mainly consists of the processing of separation, consolidation and deformation of materials by one atom or one molecule", or the ability to manipulate a single nanoscale object is defined as nanotechnology. In 1981 the scanning tunneling microscope and later the invention of the atomic force microscope allowed scientists the ability not only to see and observe molecules, but to move them as single building blocks. It is worth noting that nanoscale objects may have unique properties depending on their size, so the term "nanomaterials technology" may be better than nanotechnology. (Mulvaney, 2015)

As the name suggests, nanoparticles (NPs) are particles with sizes between 1 and 100 nm. In Figure 7, nanoparticles are compared with materials of different sizes, from large particles, like a tennis ball, to atomic particles, like glucose and water molecules. (Roy, 2016) These nanoparticles have unique properties that are different from those of bulk materials. There is a variety of properties depending on the size of the NPs. It is important the span of the NPs to be controlled in order to provide materials with particular properties. One of the most important reasons why nanomaterials have different properties in nanoscale compared to larger particles of the same material is the surface area to volume ratio. In nanomaterials this ratio is very large. Atoms on the surface of a material are often more reactive than those in the center, so a larger surface means that the materials are more reactive. One more fundamental reason for the diversity of NPs' properties are the new quantum impacts, which can be vital for the determining of the materials properties and characteristics. (Rafique, et al., 2016)

Except for the differences concerning the structural properties, at nanoscale optical properties can change, too. When the light is incident on the materials, it can be absorbed or scattered. If the size of a specific materials is less than 20 nm, absorption is significant and if the size is greater than 100 nm, scattering is significant. So, it can be achieved optimal amount of absorption or scattering by designing nanoparticles of different sizes and this may result in different color of particles of different sizes. For instance, copper is opaque at the bulk level, while at nano level becomes transparent.

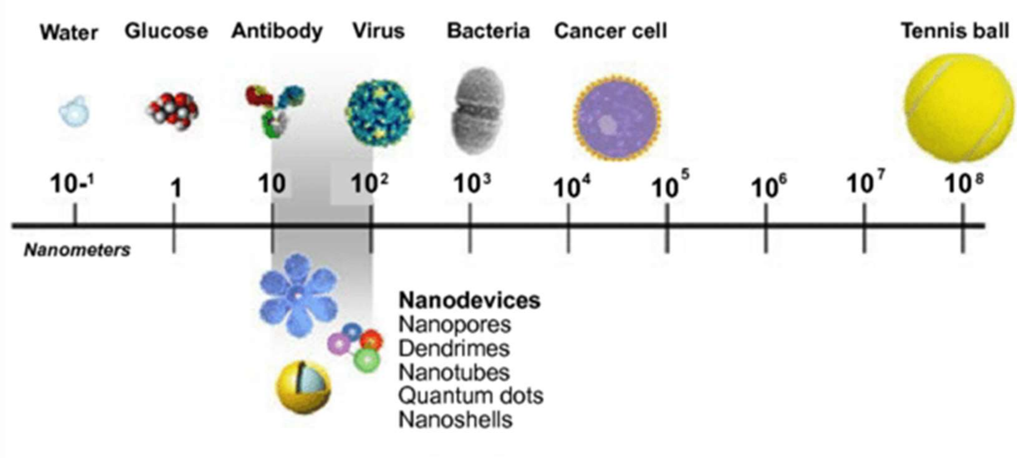


Figure 7: Size of nanoparticles

Generally, many properties of NPs differ from those of the single crystal with the same composition, like magnetic, catalytic, electrical properties, and even physical properties are improved, like mechanical hardness and thermal stability.

The division of nanoparticles can be in two groups, organic and inorganic NPs. The organic nanoparticles include carbon NPs, like fullerenes and carbon nanotubes, while inorganic NPs include magnetic NPs, noble metals NPs, like silver and gold, and semi-channel NPs, like titanium oxide and zinc oxide. Some inorganic and more particularly metallic NPs widespread used are silver, aluminum, iron and copper nanoparticles. Due to their numerous applications in diverse fields, copper nanoparticles (Cu-NPs) have recently drawn a lot of interest from scientists.

There is a variety of methods for the synthesis of nanoparticles, including physical, chemical, biological and hybrid techniques. There are two approaches for the production of nanomaterials, the top-down approach and the bottom-up approach. The top-down approach is a physical process, where bulk material is progressively reduced to nanosized dimensions with cutting, griding and scratching techniques. In other words, mechanical, thermal or chemical energy is applied to a starting bulk material to break it down into smaller fragments. A great disadvantage of this method is the wide size distribution of the creating particles. The bottom-up approach was introduced to overcome this drawback. This approach is a chemical and biological process, where the development of the structure of the NPs is conducted by atoms, molecules or clusters. (Rafique, et al., 2016) The biological synthetic strategy can also be named as “green synthesis”. Methods of this strategy are based on the biological synthesis of NPs, which means that they use plant extracts, raw materials coming from fruits and vegetables, algae, bacteria, fungi and residues. All these methods produce nanoparticles which are called biogenic NPs. (Calabrese, La Parola, Testa, & Liotta, 2021) Examples of physical methods are mechanical milling, laser ablation and mechanical griding. A few examples of chemical processes are chemical reduction and microwave method. More methods used for the synthesis of NPs are illustrated in Figure 8. (Rafique, et al., 2016)

In conventional methods, where large quantities can be achieved in a short period of time, NPs are accompanied with toxic byproducts, dangerous for the environment. To avoid these toxic wastes,

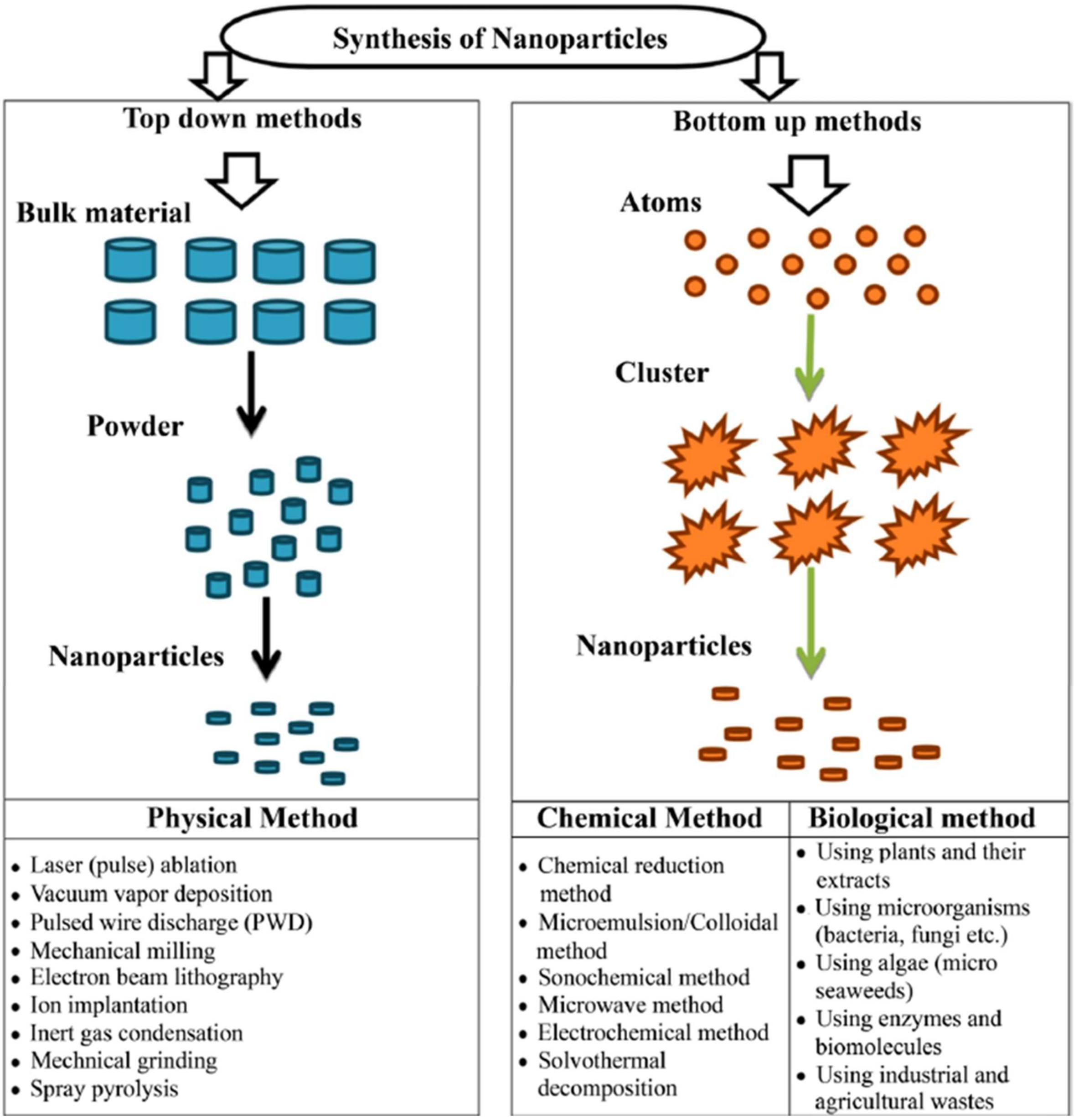


Figure 8: The top-down and bottom-up approaches of NPs' synthesis

nowadays there is a growing interest in green synthesis of NPs, in the field of green technology or green nanobiotechnology. In this field can be achieved synthesis of nanoparticles through biological routes such as bacteria, fungi, plants and enzymes or their byproducts, such as proteins, with the help of various biotechnological tools. (Rafique, et al., 2016)

2.2 Applications

Nanotechnology in combination with progress in natural sciences, like chemistry, physics, materials science and biotechnology, improve many technology and industry factors. The new, novel materials produced by this combination have unique properties, because of their structures on the nanometer scale.

Nanomedicine is the medical application of nanotechnology. It includes from medical use of nanomaterials to nanoelectronic biosensors. Particularly, nanoscale particles or molecules are developed to improve drug bioavailability, which is their presence where they are needed the most in the human body. Though nanoengineered devices molecular targeting can be achieved, which is very important in treating illnesses and diseases, such as cancer. So, a potent treatment can be delivered to cancer cells with a specific targeting, minimizing the risk to normal, healthy tissues.

Nanotechnology is increasing the development of skincare products and cosmetics. Research in the field of medicines has shown that smaller particles can easily absorbed into the skin and repair damage more efficiently. So, the ability of nanotechnology to help with the healing and repair of tissue can be used in cosmetics industry.

Nanotechnology can improve the agricultural and food industry, too. With the contribution of nanotechnology, can be achieved rapid detection of disease and can be enhanced the ability of plants to absorb nutrients. Smart sensors and delivery systems help the agricultural field fight viruses and other crop pathogens. Nanotechnology is also applied on manufacturing, processing and packaging of food.

It is noteworthy that nanotechnology can be applied for environmental protection. A major problem of recent years is the synthesis and release of highly toxic organic compounds into the environment to be used directly or indirectly over a long period of time. Some of these compounds are pesticides, fuels, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). While organic substances can easily degrade when are introduced into the environment, some combined chemical compounds show high resistance against biodegradation via native flora.

As a result, the management of contaminated soil and ground water due to dangerous chemical compounds is one of the most important environmental concerns. Nanotechnology could be a solution to this problem. Today’s technology is not adequate to solve it, but conventional treatment in combination with nanoparticles could increase the efficiency of contaminants removal, e.g., organic materials. For instance, iron nanoparticles can transform and detoxify a variety of common environmental contaminants, such as chlorinated organic solvents, organochlorine, pesticides and PCBs, and also according to studies semiconductor TiO_2 has the ability of oxidative or reductive removal of organic pollutants. Generally, application of nanotechnology in environmental science is categorized into four parts, remediation, which is the most growing part, protection and maintenance, which are the main parts in environmental science, and last, enhancement, which is the smallest of the four parts.

Nanotechnology has the potential to be the key in many fields, such as medicine, cosmetics, food, agriculture, environment. However, the safety of nanoparticles is controversial and there is a wide debate for their potential impact on environmental and health effects. (Fulekar, 2010) (Mobasser & Firoozi, 2016)

2.3 Characterization

As it was mentioned above, the range of nanoparticle applications extends to many fields, from medicine to agriculture industry and environmental protection. Depending on the nature of their applications, the characterization of these nanoparticles for several physical, chemical and biological properties is of great importance. Regarding the applications, cytotoxicity is very important to be determined, because it varies from one material to another. There are some recommended parameters that could a nanomaterial characterization includes, which are morphology, crystal structure, purity, size and size distribution, chemical composition, surface area, surface properties, the degree of aggregation under experimental conditions, stability (solubility/metal ion release under experimental conditions), surface reactivity, surface chemistry and surface charge. There are many characterization techniques for the determination of these properties, but a complete characterization of nanoparticles is still challenging, because in many circumstances the chemical and physical properties of nanoparticles are time dependent. In order to characterize nanoparticles for the aforementioned properties, there are some techniques, as the following table indicatively shows (Table 5).

Properties of nanoparticles	*Methods of characterization*
Morphology (shape, size)	Dynamic light scattering (DLS)
	Electron microscopy (scanning (SEM)/transmission (TEM))
	Atomic force microscopy (AFM)
Topography (surface)	X-ray diffraction (XRD)
	Brunaeur-Emmett-Teller (BET) analysis
Chemical	UV - visible spectroscopy
	Energy dispersive X–ray (EDX) spectroscopy
	X–ray photoelectron spectroscopy (XPS)
	Fourier transform infrared spectroscopy (FTIR)
Electrical	Electrokinetics (zeta/cyclic voltammetry studies)
Optical	Microscopy
	Double photon correlation spectroscopy

	Raman spectroscopy
	Surface plasmon resonance
Biological	*In vitro* cell viability
	In vivo
	Microbial colony viability

Table 4: Techniques for the nanoparticle characterization

Two of the most basic properties of nanoparticles collected at the beginning of characterization are the particle shape and size. Especially, the particle size is very important to determine whether nanoparticles are suitable for a specific application. One common method for the analysis of hydrodynamic particle size and distribution of the particles over a range of sizes is dynamic light scattering (DLS). With this technique is also feasible the analysis of nonspherical particles, such as rods. However, it can be used only for a certain range of nanoparticle concentrations. Electron microscopy (scanning-SEM/ transmission-TEM) is used for the details of the nanoparticle shape and surface because both are imaging methods. SEM offers high resolution and the morphology of the bacteria can be observed, but the preparation of samples is a tedious process. TEM can determine the shape, size and arrangement of particles that comprise a material. However, it requires very thin samples to be electron transparent, that means extensive preparation time and not representative results because of the small experiment area.

More commonly, for the crystal structure is used X-ray diffraction (XRD). This analysis is based on the fact that each crystalline solid has its own pattern, so from the analysis can be obtained the particular crystalline structure and the chemical composition of the material.

Regarding the composition of nanomaterials, when nanoparticles are too small for SEM to detect them, there are two other methods typically used, the energy-dispersive X-ray (EDX) spectroscopy and the X-ray photoelectron spectroscopy (XPS). The second one can be used to provide more information about oxidation state of the element in the material. A drawback of both techniques is the sensitivity of the impurity, whose levels are in the range >0.1%. (Kumar & Dixit, 2017) (Djurišić, et al., 2014)

Except for SEM and TEM, another imaging tool is the atomic force microscopy (AFM), which provides three-dimensional images at high resolution with results for the surface topography. However, AFM can also be used to measure forces between bacteria cells and surfaces. Compared to electron microscopy, AFM can be used in air and in aqueous environment, with simultaneously examinations of surface topography and interactions. So, it can be applied in studies of biofouling on surfaces in aqueous environments. With this technique can be examined the antifouling activity and the characterization of nanomaterials because the morphology and the cell interactions can be observed during the analysis. An important advantage is that it does not require sample preparation and is not a destructive method, but it is time consuming and the field of view is small. (Weir, Lawlor, Whelan, & Regan, 2008)

One more method of characterization about the surface of nanoparticles is the surface charge analysis (zeta potential analysis), which is used for the determination of their surface charge in a colloidal solution. Due to the charge on the surface of nanoparticles, a thin layer of counter ions is attracted to the surface. So, on the surface there is a double layer of ions. The electric potential at its boundary is known as the zeta potential. It is very important to a further analysis of the nanoparticles surface and to a prediction of their stability in a solution. Zeta potential measurement is also a predictor of the strength of the interaction between nanoparticles and bacteria cells. However, a disadvantage is that the measurement can be affected by the pH and the ionic strength. The ability of nanoparticles to contain their chemical and physical composition over a period of time is defined as stability of nanoparticles. Physical stability includes phenomena like agglomeration, sedimentation, change of crystallinity state and growth of crystals. (Kumar & Dixit, 2017) (Djurišić, et al., 2014) (Weir, Lawlor, Whelan, & Regan, 2008)

UV–visible spectroscopy and Fourier transform infrared spectroscopy (FTIR) can be used for the characterization of nanoparticles. Particularly, UV-visible spectroscopy is used for the confirmation of the presence of nanoparticles in a liquid. It is also a simple and quick method, which does not require time consuming preparation. The shape of nanoparticles can be indicated, but for more details on size distribution and on shape, the analysis can be followed by visualization methods, such as TEM. FTIR can be used to identify the presence of substances on nanomaterial surface, like water, and other agents like modifying/capping agents. (Djurišić, et al., 2014) (Weir, Lawlor, Whelan, & Regan, 2008)

According to Adeleye et al., there are some major physicochemical properties of Cu-based nanoparticles that are determined for their characterization. In their research, they measure initial hydrodynamic diameter and zeta potential, with SEM images for the determination of the size and the surface charge. The determination of copper content (wt%) is achieved with ICP-AES measurements. Further characterizations are done via XRD, XPS, BET and SEM. (Adeleye, Conway, Perez, Rutten, & Keller, 2014)

2.4 Copper-based Nanoparticles

As it was mentioned above, there is a need to prevent attachment of marine organisms on ship hulls. The main reasons are economic, because biofouling increases significantly fuel consumption due to the increase of drag resistance. In this way, there is also an increase of greenhouse gases, so the reasons of antifouling need are environmental, too.

The use of antifouling paints on ship hulls cannot be out of control. There are conventions to define which toxic substances can be used in antifouling paints and the quantities of them. As it was mentioned in chapter 1.5.1, on October 1, 2001, the International Maritime Organization (IMO) adopted a global mandatory legal document, the International Convention for the Control of Harmful Antifouling Bottom Systems of Ships. According to this Convention, the prohibition of antifouling paints which are more toxic to marine organisms by ships of all countries is mandatory since January 1, 2003. From then, efforts have been made for the development of a low toxic or non-toxic antifouling paint, which will be environmental and economical acceptable, no poisonous to marine species and at the same time with excellent efficiency in port area and at sea. The copper oxides have widespread use, particularly the demand of cuprous oxide antifouling paints is constantly increasing. Particularly, Cu_2O nanoparticles have major advantages compared with common cuprous oxide. Cu_2O NPs have strong chemical activity, so catalytic adsorption and antifouling performance are better than those of common cuprous oxide. This is the reason why there are so many preparation methods of Cu_2O NPs and there are many active research efforts for an improved production method of Cu_2O NPs. (Shi, et al., 2019)

Copper is one of the most widely used materials on the planet. The worldwide annual production of copper in 2015 was 18.7 million tons. It is estimated that 39% of copper is used in electrical and electronics applications, 30% in pipes and the remaining fraction on machinery, vehicles and consumer products. Of the total copper production, only a few hundred tons is used to produce copper-based nanoparticles. (Keller, et al., 2017) Synthesis and characterization of copper nanoparticles can be by different methods. The use and development of the metal cluster in new fields, in a new generation of nanoelectronic devices are hindered by the stability and reactivity of copper-based nanoparticles. (Khanna, Gaikwad, Adhyapak, Singh, & Marimuthu, 2007) The fraction of Cu-based NPs is relatively small compared to the global NPs production, approximately 200 tons per year for 2010. However, there are concerns about the environmental risk because of their toxicity and some characteristics of their life cycle. Cu-based NPs are commonly used not only in marine antifouling paints, but also in agricultural biocides as the active ingredient. The introduction of them in the environment happens intentionally in a direct way, as they are toxic substances. (Conway, Adeleye, Gardea-Torresdey, & Keller, 2015)

Copper exists as elemental copper, Cu^0, and in two oxidation states, Cu^+ and Cu^{2+}. This speciation of copper is used to develop nanomaterials with various properties. Elemental copper is highly conductive, so this ability is taken advantage for applications where electron transfer is necessary. The first oxidation state of copper, Cu^+, is typically used as Cu_2O NPs, but it is unstable and can cycle between Cu^+ and Cu^{2+}. It is used for the catalysis of many reactions and it has been studied for antimicrobial and antifouling applications. The second oxidation state of copper, Cu^{2+}, is used as CuO for energy storage and sensing applications. (Keller, et al., 2017)

2.5 Comparison between bulk materials and nanoparticles of copper

2.5.1 Characteristics and Activity

One major characteristic of metal-based NPs is their antimicrobial activity. The antimicrobial attribute of bulk metals was known from ancient times, for example copper and silver have been used for water sanitization. Nowadays, an extended use of copper is as a component in antifouling paints. Conventionally, in antifouling paints is used a variety of copper compounds that release Cu^+, such as copper (I) thiocyanate, copper oxide (I), cuprous thiocyanate etc. According to research, it is known that the settlement of macrofouling organisms can be prevented by a Cu^+ concentration of 4.68 μg cm^{-2}. However, a domination of microfouling organisms on copper based antifouling coatings has been reported with a Cu^+ ion release of 3.0-6.4 μg cm^{-2} d^{-1}. Ions Cu^+ are oxidized to more stable and toxic Cu^{2+}, which form inorganic and organic ligands. In this way, the availability of free copper ions is reduced, subsequently the toxicity is reduced. In addition, it has been found a microbial cells tension to develop tolerance upon exposure to copper. (Padmavathi, et al., 2019)

The solution to this problem comes from copper oxide nanoparticles. These NPs can control the copper tolerant strains. Comparing bulk materials to nanoparticles of the same material, nanoparticles as antimicrobial substances have many advantages. They cause damage to DNA by compromising the cell membrane. In this way, they also cause protein denaturation, inactivation of enzymes and toxic ion release. (Padmavathi, et al., 2019) They have the ability to target multiple biomolecules and microbes compromising the development of resistant strains.

Copper, compared to other metals, like silver and gold, is cheaper and more easily accessible. Subsequently, Cu-based NPs, compared to other metallic NPs are cheaper and are produced more easily. It is known that copper is necessary for human and other mammalian life because it takes part in many biological processes which are essential for living., for instance copper ion release enhances and supports generation of tissues. (Ermini, 2021) It is necessary not only for eukaryotic, but also for prokaryotic organisms, because ionic copper is involved in redox reactions, such as electron transport, oxidative respiration and denitrification. (Padmavathi, et al., 2019) However, when the concentration of copper exceeds the required level, regarding both its ionic and metallic forms, can be toxic to many organisms. For instance, copper can accumulate in the body, causing long-term toxicity and other related diseases. Compared to terrestrial organisms, copper is quite toxic to aquatic biota, because the sensitivity to copper and copper-based nanoparticles depends on many factors, like surface-area-to-volume ratio, respiratory rates, and regarding fish, their sensitivity depends on flow rate over gill surfaces, etc. So, considering ecosystem health, concentration and bioavailability of copper in natural environments are two very important factors. (Keller, et al., 2017) Even though copper participates in many biological processes, it can be toxic to cells, it can be biocidal. Depending on the chemical composition, the shape and the size of the copper-based nanomaterial, at a certain range of concentration, can cause reduction of the viability of cells. (Ermini, 2021) (Padmavathi, et al., 2019)

Generally, the antibacterial effects caused by heavy metal nanoparticles can follow four known mechanisms (Figure 9): reactive oxygen species (ROS) production, particularly in the presence of illumination, release of metal ions, accumulation of nanoparticles on cell walls and cell lysis caused by internalization of NPs into cells. (Cheng, et al., 2019)

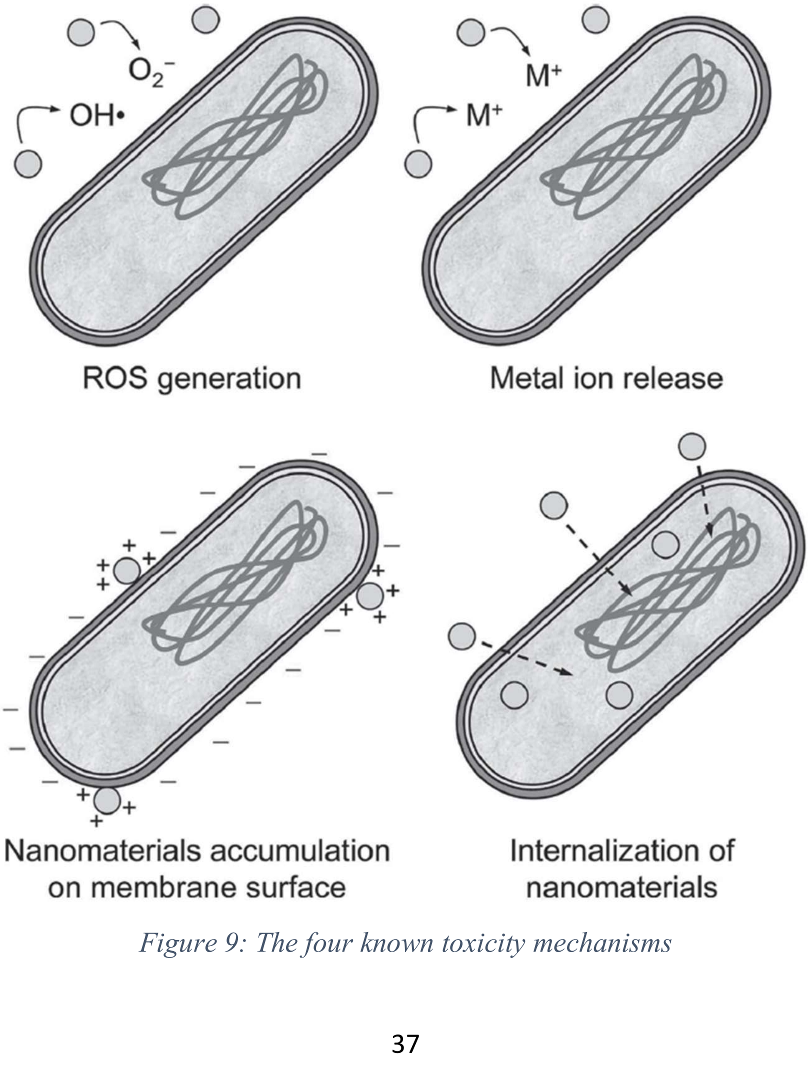


Figure 9: The four known toxicity mechanisms

There are some ROS which are responsible for bactericidal action of metal oxide nanomaterials. Some of them are OH radicals, superoxide ions and hydrogen peroxide. The production of ROS can be by both UV and visible illumination. The characterization of the damage caused to bacteria cells by nanoparticles can be done by Raman and FTIR spectroscopies, which can detect changes in phospholipids and proteins. Another use of FTIR spectroscopy related to the toxicity of nanoparticles is the characterization of the interaction between phospholipids in the cell membrane and metal oxide nanoparticles. Lastly, XPS measurements can detect any charge transfer between metal oxide nanoparticles and the bacteria cells. This charge transfer may result to change of oxidation state of the nanoparticle's metal, Cu^{2+} to Cu^{+} for example in the framework of this study. Apart from ROS generation, another toxicity mechanism is metal ion release, e.g., in aqueous solutions. The sensitivity to metal ions may differ from one organism to another. In addition, the metal ion toxicity depends on the medium where the cells and the nanoparticles are.

An additional toxicity mechanism is related to the way that the nanomaterial and the cell wall interact each other. In some cases, the toxicity of metal oxide nanoparticle requires contact with the cells. In other cases, the adsorption of the nanoparticles onto the cell surface is not required, but toxicity can be achieved even if they are separated by a membrane. The accumulation of nanomaterials on the surface membrane can be achieved due to electrostatic interaction between the nanoparticles and the cells, as it is shown in Figure 9. Nanoparticles are charged positively while bacteria membranes are charged negatively, so they are electrostatically attracted. In this way, there is adsorption of nanoparticles onto the membrane. The electrostatic interaction can be affected by many factors, like electrolytes and pH, and therefore is affected the toxicity of the nanoparticles. The adsorbed nanoparticles can result in oxidative stress to the bacteria and cytotoxicity because of the redox reaction on their surface. They can also change the membrane permeability and the transport exchanges across the membrane, facts that cause cell death. The interaction between nanoparticles and membranes are not only electrostatic, but they can also be Van der Waals forces, hydrophobic interactions, etc. After the membrane disruption, nanoparticles can be internalized and diffused inside the cytoplasm. Via electron microscopy it can be distinguished if nanoparticles are bound on the membranes or if they are internalized inside the cells. In conclusion, the toxicity of the four mechanisms is still not fully clear despite extensive studies. (Djurišić, et al., 2014)

According to various research, Cu NPs and CuO NPs show different action on microbes. It is more possible that Cu NPs interact with the membrane of bacteria compromising its integrity. In contrary, CuO NPs penetrate the membrane of bacteria and release ions within the cell. The penetration depends on the shape of the nanoparticle. According to Applerot et al., the size of NPs is also especially important for their efficacy. After comparison of CuO NPs of different sizes, 30 and 2 nm diameter, they concluded that the smaller nanoparticles are more effective against bacteria. They use transmission electron microscopy (TEM) to confirm that 2 nm NPs disrupt and penetrate the bacterial membrane more efficiently than 30 nm NPs. (Applerot, et al., 2012) (Ermini, 2021) However, according to Padmavathi et al., regarding copper ions released from copper oxide NPs, the surface contact is a very important factor that damages the cell membranes, except for the bacterial action of copper ions. (Padmavathi, et al., 2019)

It is important to mention that nanoparticles have a major disadvantage, the aggregation phenomena. The antimicrobial activity of metal-based NPs, and particularly here of Cu-based NPs, is strongly negatively affected by aggregation phenomena. The aggregation resulting to reduction of the surface interaction between NPs and the bacteria membrane wall. When there are not any stabilizing agents, soluble metal NPs have the tension to make larger aggregates due to their low thermodynamic stability. It is necessary to achieve a fine dispersion of them and avoid agglomeration. For these reasons, the use of stabilizing supports may be requirement in some cases. Such supports used for the prevention of metal nanoparticle agglomeration, here for Cu-based nanoparticles are, for example, silica or titania. (Calabrese, La Parola, Testa, & Liotta, 2021)

2.5.2 Examples of copper-based nanoparticles

Regarding the study of Padmavathi et al. that was mentioned before, the aim of the research is the synthesis of morphologically different CuO NPs from $Cu(NO_3)_2$ by varying the concentration of hexamine and the capping agent, which is cetyltrime - thylammonium bromide (CTAB), and the evaluation of their efficacy in controlling the biofilms of bacterium Staphylococcuc lentus, which is a copper tolerant marine bacterium.

Figure 10 illustrates the various morphologies of the different copper oxide nanoparticles that were synthesized. Figure 11 shows the architecture of S. lentus in the absence and in the presence of CuO NPs.

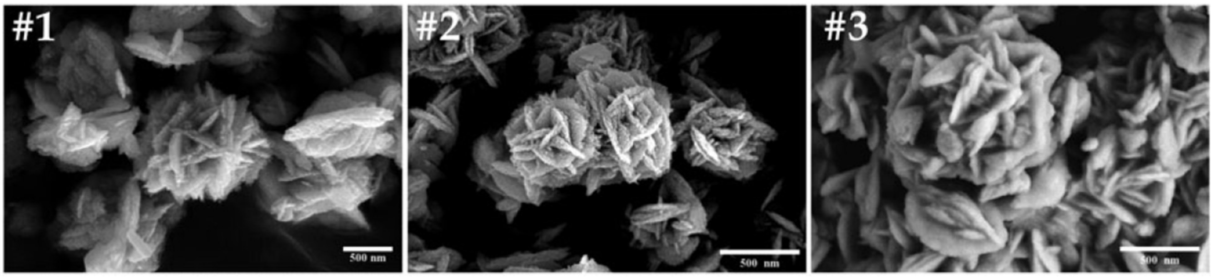


Figure 10: The morphologies are: (#1) CuO (0.5 M hexamine) – flower like, (#2) CuO (0.75 M hexamine) – flower like and (#3) CuO (1 M hexamine) – sheaf like.

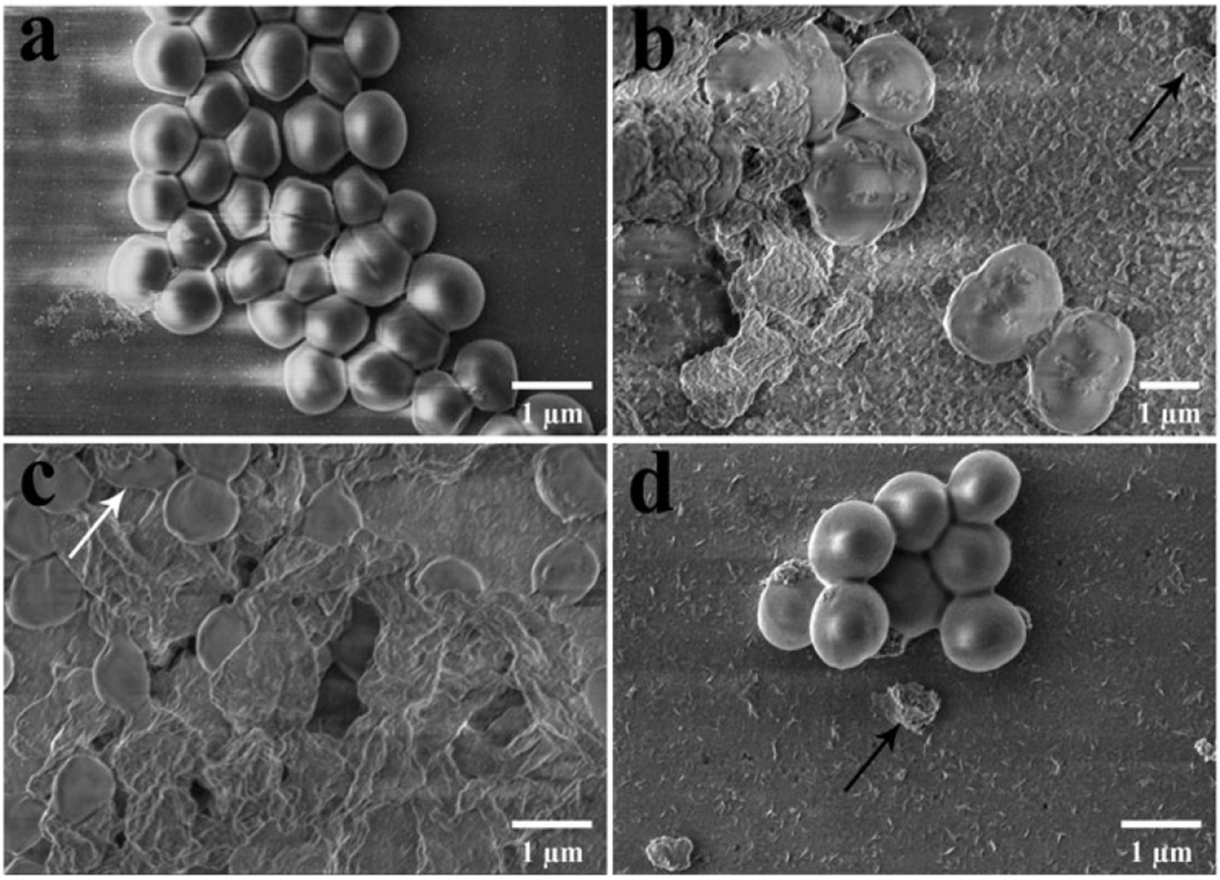


Figure 11: Figure a shows the biofilm architecture of S. lentus as cell clusters with intact cell membranes. Figures b, c and d illustrate the biofilm of S. lentus formed in the presence of CuO NPs.

The biofilm cells have damaged cell membranes, damage caused by nanoparticles by surface contact. All the three cases of CuO NPs are highly effective in removing biofilms of metal tolerant bacteria. So, the antifouling paints' properties will be improved using CuO NPs, which also will offer a controlled and sustained release of biocidal copper ions. (Padmavathi, et al., 2019)

Another example of how copper oxide nanoparticles affect the cell membrane because of surface contact is obvious in study of Zhao et al., where CuO NPs are toxic to Chlorella pyrenoidosa. These organisms excrete extracellular polymeric substances (EPS), with which interact the attached onto the surface of the algal cells CuO NPs. The EPS layer of algae is thickened, which probably is a protective mechanism of the algae. Despite the thickening, CuO NPs internalize by endocytosis and transform to Cu_2O NPs with an average size of 5 nm.

Figure 12 (A) shows an intact and well-shaped untreated cell. Exposure to Cu^{2+} causes wrinkles on the external surface of the cell and make it slightly irregular, as shown in Figure 12 (B). However, exposure to CuO NPs causes distortion of the algal cells, which possess irregular morphology, as shown in Figure 12 (C) and (E). Figures D and F illustrate the attachment of particle aggregation on the surface of cells exposed to CuO NPs. The yellow box shows that CuO NPs can be accumulated by the algal cells, as it is obvious a cluster of aggregates in a cell wall perforation. The red boxes are areas of the cells where is analyzed the mass percentage of present Cu. The result is a suggestion of the attachment of CuO NPs on the external surface of algal cells. In the same study, it is proposed that another result of CuO NPs contact is membrane damage. The damage caused by CuO NPs is time dependent and is significantly increased after two days of exposure. Comparing the membrane damage caused by CuO NPs and by Cu^{2+}, the first case is much higher than the second one, since the damage reaches a percentage of 40% of the algal cells. It was thought that membrane damage was caused by oxidative stress. However, the determination of ROS generation of algal cells after exposure to CuO was similar to Cu^{2+}. So, the membrane damage is probably caused by direct physical contact and penetration. (Zhao, et al., 2016)

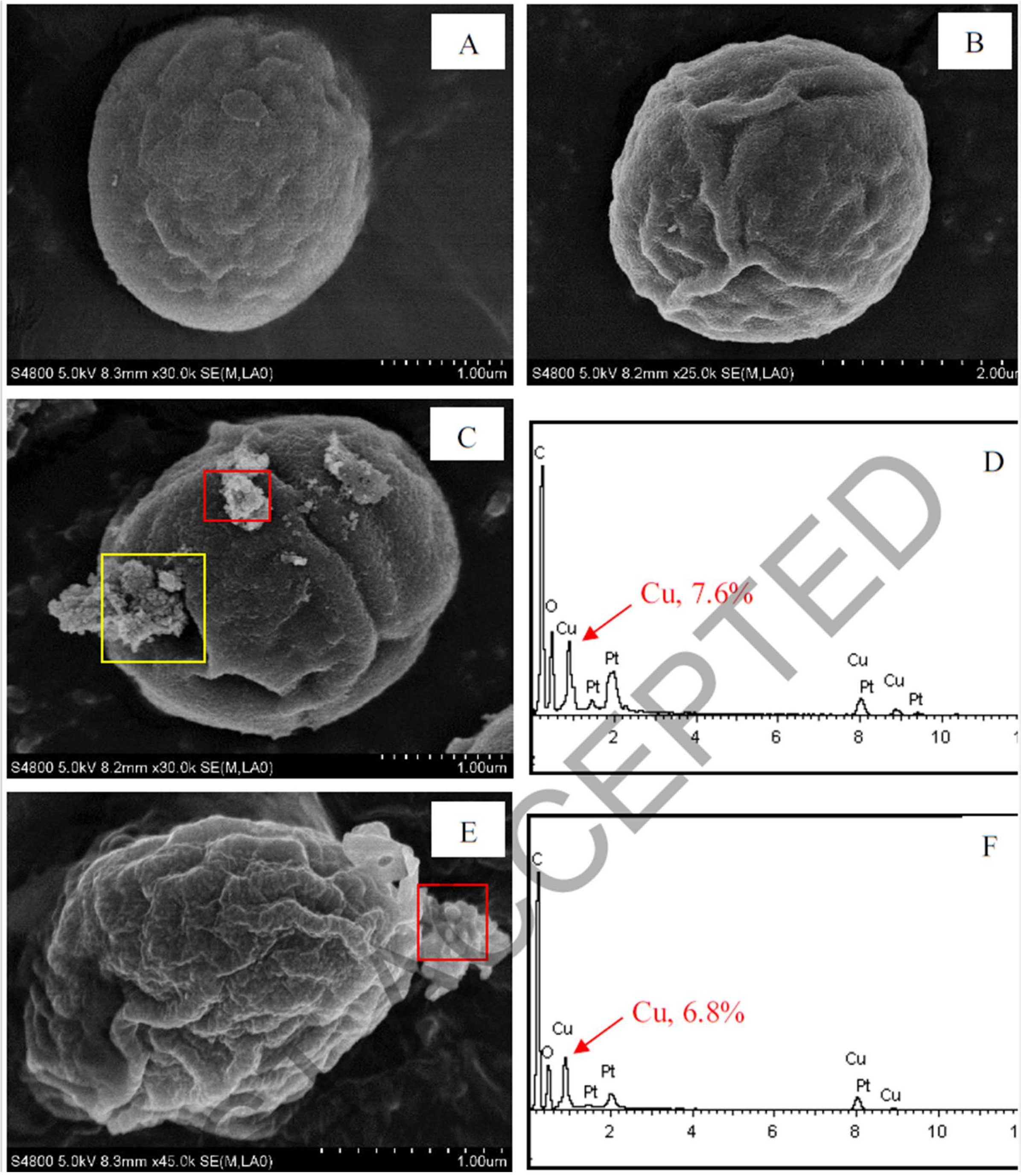


Figure 12: Comparison of exposure of cells to Cu^{2+} and CuO NPs

2.6 Environmental fate of copper-based nanoparticles

When nanoparticles are released into the environment, they begin to undergo some transformations depending on their composition and environmental parameters. The most common transformations are homoaggregation, heteroaggregation, sedimentation, dissolution, oxidation in oxic environments, reduction or sulfidation in anoxic waters, coating with natural organic matter, etc. (Figure 13)

Aggregation of copper-based nanoparticles in natural waters depends on parameters, such as their composition, aqueous media properties, natural organic matter (NOM) concentration and pH. The density of copper-based nanoparticles is high, indicatively the density of Cu NPs is 8.96 g/cm^3 and of CuO NPs is 6.31 g/cm^3. So, when Cu NPs aggregate and reach micron dimensions, they settle out rapidly in the absence and in the presence of organisms. When organisms are present, the sedimentation of the aggregated nanoparticles takes place in a slower rate, because nanoparticles are stabilized by NOM released by the organisms. Generally, NOM, surfactants and polymers stabilize copper nanoparticles due to electrostatic or steric influences. Contrary to Cu NPs, stability of CuO nanoparticles is influenced by salinity and pH. In freshwater they are relatively stable, in lagoons and seawater occur rapid aggregation and sedimentation and at lower pH occurs sedimentation in higher levels. Sedimentation is also depended on particle size. The higher the particle size is, the faster the sedimentation is. Nanoparticle stability is also influenced by adsorption of ions, such as phosphate ions, which stabilize CuO nanoparticles and prevent their sedimentation.

Dissolution of copper-based nanoparticles depends on their aggregation, oxidate state, pH and NOM. When they are highly aggregated, the surface area is reduced, so the dissolution rate is reduced. The dissolution rate is also reduced when pH increased. Particularly, the dissolution rate of CuO nanoparticles is very slow, slower in seawater compared to freshwater.

Transformation of copper in the environment is very complicated, since it is controlled by the chemistry of both the particles and the environment. Copper is partitioned into aqueous, solid and biological media. When referring to aqueous media, copper is free and in complexed ions, to solid media is particulate and to biological media is adsorbed and incorporated. Copper nanoparticles release the dissolved Cu$^+$, which subsequently are oxidized to Cu^{2+} and are complexed in the environment. So, in natural waters, there is inorganic copper as complexes of carbonate, hydroxide and NOM. When the conditions are low redox with high S^{2-}, are formed insoluble compounds of CuS.

Bioaccumulation of copper-based nanoparticles has been observed in many cases. Some of them are cell membranes, single-cell organisms, even fish, marine invertebrates and terrestrial plants. There is a continuous input of copper-based nanoparticles in the environment and given the aforementioned processes of copper transformation, there is a need of more research regarding the effect of copper-based nanoparticles from paints and the potential accumulation, due to the continuous use, at higher concentration. (Keller, et al., 2017)

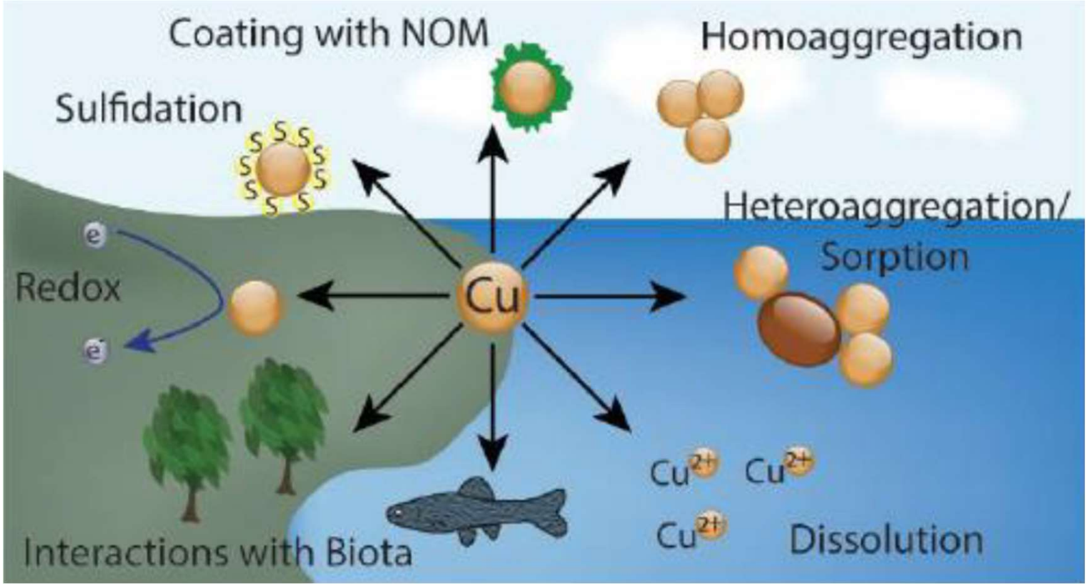


Figure 13: Environmental fate of copper-based nanoparticles

3. Experimental Section

3.1 Materials and methods

3.1.1 Antifouling Paints

In the present study are used in total three antifouling paints, a control paint and a primer. Two of the antifouling paints used are commercial, WILCKENS AF 100 RED BROWN – CKF 3559 (AF No 1) and LUSTRALAC AF 100 RED BROWN – 52320 (AF No 2). According to the technical data sheets provided by the manufacturer, the main biocidal ingredient for both antifouling paints is Cu_2O, with copper contents 35 wt % and 25 wt % respectively. For the needs of the study two more paints are used. A non-commercial antifouling paint is produced especially for the purpose of this research. In the laboratory, a matrix of antifouling paint is produced, with the appropriate materials, e.g., resin, fillers, solvents, but without biocides. In the produced free-biocides matrix are incorporated 5 wt % nanoparticles of Cu_2O (AF No 3). In addition, it is essential for a paint with no biocides to be used, which is served as control. Last, for the pretreatment of the panels an appropriate primer is also necessary. (Table 5)

Paint	*Product name*	*Manufacturer*	*Color*	*Active Substance Content*
AF No 1	Wilckens AF 100	Wilckens	Red Brown	35 wt % Cu_2O
AF No 2	Lustralac AF 100	Wilckens	Red Brown	25 wt % Cu_2O
AF No 3	AF with nanoparticles	Laboratory	Red Brown	5 wt % nanoparticles Cu_2O
Control	-	Laboratory	Red Brown	-
Primer	Wilkopox al-tie coat	Wilckens	Ivory	-

Table 5: Investigated paints

The paints are applied to panels according to the manufacturer instructions. In order to choose the appropriate panels for this experiment, research was carried out on the material used for the construction of ships and more specifically for the construction of ship hulls.

3.1.2 Panels

Egyptians are regarded to be the first ship builders. Since 4000 BC, when shipbuilding probably began and for centuries, the first and dominant material used in this industry was wood. Over the years, the use of wood has declined because of the characteristic of decay when wood is exposed to the marine environment. Continuous progression in hull construction materials is obvious. Humans started to use new materials and apply new production methods in shipbuilding. In the manufacturing process, a variety of materials is used, depending on the kind of vessel which is going to be built. By the time steel and aluminum were introduced in the industry of ship construction, the dominance of wood faltered and wood gave up its first position to the new materials. The most used materials in the hull construction are steel and aluminum alloys.

The ship's hull is not a one-piece construction, but a combination on elements. Some of them are the keel, shell, pillar, deck, and stringer, which are responsible for the ship to be durable, safe and waterproof. (Mentes & Çağlayan, 2022) (Nwokedi, et al., 2018) In addition, ship hull consists of various pieces, fitted and welded for the formation of structural parts or components of the hull. These are fitted and welded to form hull blocks, which subsequently are integrated to form large or general blocks. The last ones, together with some other small parts are assembled into a ship hull. It is necessary to highlight that welding is an important process and it is taken into consideration for the final decision of ship hull material. (Guidelines for inspection of hull welds, 2017)

3.1.2.1 *Steel*

Naval steels can be distinguished in several types and grades, depending on their mechanical properties and their chemical composition. According to Lloyd’s Register, steel having a specified minimum yield stress of 235 N/mm^2 is regarded as mild steel (Lloyd's Register, 2022) or as normal strength steel and is denoted ‘NSS’ (Bureau Veritas, 2022), while steel having a higher specified minimum yield stress is regarded as higher tensile steel (Lloyd's Register, 2022) or as higher strength steel and is denoted ‘HSS’ (Bureau Veritas, 2022). The definition of yield stress of a solid is complicated and not very easily described. Barnes after wide research at definitions of yield stress (or yield strength), he concluded that “a simplistic definition of the yield stress of a solid is essentially the point at which, when increasing the applied stress, the solid first shows liquid-like behavior, i.e., continual deformation.” (Barnes, 1999) In other words, when the hull material does not plastic deform permanently, while it is under stress or load, expresses its ability of yield strength. Another mechanical property of hull material is tensile strength, which is its ability to withstand a pulling force and cannot break under the referred stress or load. Yield stress and tensile strength are the most important properties taken into consideration when the appropriate ship hull material is needed to be chosen for the ship construction. (Nwokedi, et al., 2018)

In the following table (Table 7) are indicated the steel grades according to Lloyd’s Register and Bureau Veritas.

Grade	***Lloyd’s Register***	***Bureau Veritas***
A, B, D, E	mild steel grade	normal strength steel grade
AH, DH, EH, FH	higher tensile steel grade	higher strength steel grade

Table 6: Steel grades

The supply of steel material is made in five strength levels, which are 27S, 32, 36, 40 and 47. For the fully identification of a steel and its properties, it is essential to express it with the grade letter followed by the number of the strength level. (Lloyd's Register, 2022) In the following table (Table 8) are indicated the steel grades with their corresponding chemical composition:

	Grade						
Chemical composition %	A	B	D	E	AH, DH, EH	EH47	FH
Carbon	0.21 max	0.21 max	0.21 max	0.18 max	0.18 max	0.18 max	0.16 max
Manganese	2.5 × C% min	0.80 min	0.60 min	0.70 min	0.90-1.60	0.90-2.00	0.90-1.60
Silicon	0.50 max	0.35 max	0.10-0.35	0.10-0.35	0.50 max	0.55 max	0.50 max
Sulphur	0.035 max	0.035 max	0.035 max	0.035 max	0.035 max	0.020 max	0.025 max
Phosphorus	0.035 max	0.035 max	0.035 max	0.035 max	0.035 max	0.020 max	0.025 max
Aluminum	-	-	0.015 min	0.015 min	0.015 min	0.015 min	0.015 min
Niobium	-	-	-	-	0.02-0.05	0.02-0.05	0.02-0.05
Vanadium	-	-	-	-	0.05-0.10	0.05-0.10	0.05-0.10

Table 7: Chemical composition of steel grades

Different structures, or in this case different parts of a ship, may need different type of steel. There is a variety of steel grades used for the construction of hulls. According to Bureau Veritas, the main steel hulls accompanied by their mechanical properties are displayed by Table 9:

Steel grades for plates with $t_{as\_built} \leq 100$ mm	**R_{eH} (N/mm^2)**	**R_m (N/mm^2)**
A-B-D-E	235	400-500
AH32-DH32 EH32-FH32	315	440-570
AH36-DH36 EH36-FH36 EH36CAS1-EH36CAS2 FH36CAS1-FH36CAS2	355	490-630
AH40-DH40 EH40-FH40 EH40CAS1-EH40CAS2 FH40CAS1-FH40CAS2	390	510-660
EH47 EH47CAS1-FH47CAS2	460	570-720

Notes

1. t_{as_built}: As-built thickness taken as the actual thickness provided at the newbuilding stage

2. R_{eH}: Specified minimum yield stress

3. R_m: Specified minimum tensile strength

Table 8: Grades of steel hulls

3.1.2.2 *Stainless steel*

Ships made of steel are strong and tough, but steel as material is heavy and because of the constant exposure to water and humidity, requires corrosion protection and a lot of maintenance. A solution to these problems concerning ship hull construction is stainless steel. Stainless steel can be up to three times stronger than the carbon steel used for shipbuilding. In this way, the thickness of the ship hull could be reduced, resulted to reduction of the weight of the whole ship. This fact combined with the appropriate design, so that the ship cannot buckle under wave load, can lead to a construction strong, light and safe. (Willston, 2020)

Austenitic stainless steels are a group of steels used in seawater environments, installations in the chemical, paper and food industries and many others. Austenitic steels have a chemical composition, with a structure that provides corrosion resistance and make them stable in a wide range of temperature. The AISI 316L type steel is chromium-nickel stainless type of steel and belongs to the group of austenitic stainless steels. (Kowalski, Licznerski, Supernak, & Emilianowicz, 2020) There are also other steels that belong to this group and are used for ship hull

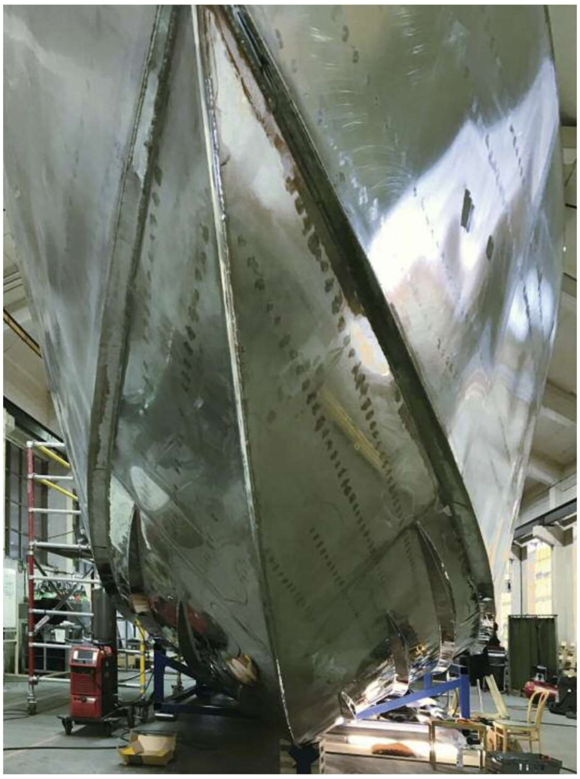


Figure 14: Stainless steel hull at the shipyard before painting

construction, like Nitronic 50, AL 6XN and German stainless steel 1.3964.9. The German Navy has used stainless steel in order to reduce its warship's magnetic signature with the use of nonmagnetic materials, so that they cannot be detected. Moreover, the use of austenitic stainless steels is enhanced, because the possible use of GRP (Glass Reinforced Plastic) as an alternative material is restricted to ship hulls 60 m or less length because of the limited stiffness of the material. AISI 316L is preferable compared to the other stainless steels, because it is more corrosion resistant, being not so costly as the others at the same time. (Sedriks & Dudt, 2001) 316 and 316L stainless steel types are similar in many ways, like cost, durability and high stress durability. The difference between them is that 316L has lower carbon content, "low" it is the word from which comes the letter "L".

In the following table (Table 10) is indicated the chemical composition of stainless steel 316 and 316L, where it is obvious that the only difference between them is the percentage of carbon, 0.08% for 316 and 0.03% for 316L.

Chemical composition %	*Type of stainless material*	
	316	**316L**
Chromium	16-18	16-18
Nickel	10-14	10-14
Molybdenum	2-3	2-3
Manganese	2	2
Silicon	0.75	0.75
Nitrogen	0.1	0.1
Phosphorus	0.045	0.045
Sulphur	0.03	0.03
Carbon	0.08	0.03

Table 9: Chemical composition of stainless steels AISI 316 and 316L

Lower carbon content makes it preferable in case of high corrosion and when there are high temperatures, especially in those situations where welding is important. As it was mentioned above, welding is a process for the formation of the structural parts of the hull and for the final assembly of them into a ship hull. (Gupta, Singh, Yadav, Jain, & Pratap, 2020)

3.1.2.3 *Aluminum*

As the years passing by, with the development of new technologies in the field of manufacturing, there is a need of new, advanced materials. Ferrous materials have been used for centuries, now it is time for non-ferrous materials, with very useful and unique properties, especially for the field of shipbuilding. Aluminum alloys have received attention as some of these new advanced products that have been developed. They are light weight, strong and corrosion resistant, which are the three most important characteristics for a material to be used in this industry. The series of aluminum 5xxx is very often used on ships. This series has many grades that can be used as hull material. Important role for the decision of the hull material plays its price. Specific aluminum alloys with very good corrosion resistance especially in marine environment are 5052 and 5083. (Arifuddin, Hidayat, Pawara, & Zulkifli, 2022)

There are different types of alloys, regarding the deformation they can stand. Wrought and cast alloys are two different types of alloys. Cast alloys are brittle and cannot be shaped of formed. On the contrary, wrought alloys accept mechanical deformation and can acquire the desired shapes and forms. There are two types of wrought aluminum alloys; alloys hardened by cold working and alloys heat treatable.

The symbolism of wrought aluminum alloys can be succeeded with four digits and a single letter of the temper designation which shows the condition of the materials annealed, fabricated cold works and heat treated. The first of the four digits defines the major alloying element, the second indicates the alloy's modifications, and the third and fourth digits can show the different alloys in the group with no numerical significance. An exception is series 1xxx where the last digits show the quantity of alloy's aluminum. So, in the cases of aluminum 5052 and 5083 the first digit is 5xxx and that defines the major alloying element which is magnesium in a percentage of 2.2-2.8% and 4.0-4.9% respectively. Magnesium gives some properties to the alloy, such as corrosion resistance, weld ability and make them non heat treatable. (Bhowmik, Srivas, & Khandelwal, 2016) As a result, it has a perfect marine application. When there is a combination of materials used for the construction of ship hull, Arrifudin et al. after their experimental test where they compare the yield stress of some combinations of alloys 5052 and 5083, they conclude that the recommended connection materials for the hull structure are variations 5083-5052 and 5083-5083. (Arifuddin, Hidayat, Pawara, & Zulkifli, 2022)

According to ASTM B209 Standards Specification for Aluminum and Aluminum - Alloy Sheet and Plate, the chemical compositions of aluminum alloys 5052 and 5083 are indicated by the following table (Table 11): (Bhowmik, Srivas, & Khandelwal, 2016)

Chemical composition %	*Alloy*	
	5052	**5083**
Magnesium	2.2-2.8	4.0-4.9
Iron	0.4	0.4
Silicon	0.25	0.4
Chromium	0.15-0.35	0.05-0.25
Manganese	0.1	0.4-1.0
Zinc	0.1	0.25
Copper	0.1	0.1
Titanium	-	0.15
Other elements (each)	0.05	0.05
Other elements (total)	0.15	0.15
Aluminum	Remainder	

Table 10: Chemical composition of aluminum alloys 5052 and 5083

Regarding the symbolism, after the four digits, there is a capital letter of the temper designation and one or more digits providing information about the process. The letter gives information about the general way the alloy has been mechanically and/or thermally treated to achieve the desired properties. There are five letters. F, as fabricated and O, annealed, are used for wrought products made by shaping process such as rolling, extrusion, forging, drawing. H, strain hardened, is used for alloys with increased strength by strain hardening, such as rolling and drawing, at room temperature. The digits following the letter H indicate the approximate amount of cold work and the nature of any thermal treatments. W, solution heat treated, is used for alloys that age naturally and spontaneously after solution heat treating. Last, T is for alloys thermally treated to produce stable tempers other than F, O or H. (Kaufman, 2000) According to ASTM B928/B928M – 15 Standard Specification for High Magnesium Aluminum-Alloy Products for Marine Service and Similar Environments, the only alloy's 5083 tempers manufactured and corrosion tests for intended use in marine hull construction are H116 and H321. For alloy 5052, the most common temper used for hull construction is H32. It has very good corrosion resistance to seawater and to marine atmosphere. Alloy 5052-H32 has very good weldability and it is a medium to high strength alloy, worked hardened by rolling and then stabilized by low temperature heat treatment to quarter hard.

Apart from the aforementioned advantages, aluminum alloys are also easily recyclable because of their low melting points and safe in case of fire because they are flame retardant. The shape of hull made of aluminum alloys can be freely designed, molds are not used for the construction. An appropriate hull shape combined with the light weight of aluminum can enhance the fuel efficiency, which means save of money, something very important in marine industry.

Government, warship, leisure vessels, small marine crafts (tinnies) are only some of the areas where these alloys are used in marine industry. (Kim, et al., 2013)

3.1.2.4 *Comparison of materials*

In conclusion, the main materials that are used in shipbuilding are wood, steel, stainless steel and aluminum. After the analysis of their characteristics and their properties, a comparison must be done, in order for the appropriate and more representative material for the panels to be chosen.

A reference to the cost of these materials is also necessary. The construction of a ship hull is cheaper when it is made of wood, followed steel and then aluminum. Aluminum has the highest cost of all. However, it is important for the life cycle cost of a vessel to be considered and not only its initial cost. In the case of the same ships with one and only difference, the kind of hull material, their lifetimes are also different. The lifetime of wood and steel hulls is approximately 20 years, when the lifetime of aluminum hull is 25 years. This is because apart from the initial manufacturing cost, there are operation and maintenance costs that are constantly created during the life of the ship. Wood has one important disadvantage; it is less durable because it is decay prone. Regarding aluminum, as it was mentioned above, one of its main benefits is its low weight. Specifically, weight of aluminum alloy is 2.723 tons/m^3 and weight of stainless steel is 7.84 tons/m^3; as a result of these values, a hull structure can save 65% of weight compared to a stainless-steel structure, ships with steel hull need more powerful machines resulting to an increased fuel consumption and high maintenance costs. Especially for cargo ships, aluminum allows the transportation of more goods, subsequently shorter payback period, lower annual average cost, higher net benefit. So,

Figure 15: The Iceberg Effect about costs

regarding costs of analyzing materials, aluminum is the best decision of ship hull material. (Figure 15) (Moraes & Wilson, 2013) (Gupta, Singh, Yadav, Jain, & Pratap, 2020)

However, comparing aluminum alloys which is the best option? Aluminum alloys 5052 and 5083 have excellent corrosion resistance, which makes them very good choices as materials used in marine environment. Alloy 5052 has magnesium content low enough that it does not suffer from stress corrosion cracking. According to ASTM B 928/B 928M-07 Standard Specification for High Magnesium Aluminum-Alloy Sheet and Plate for Marine Service and Similar Environments, stress-corrosion cracking is a cracking process that requires the simultaneous action of a corrodent and a sustained tensile. When the content of magnesium exceeds 3%, which happens with alloy 5083, and with simultaneous exposure at 50°C to 225°C, it is possible for the stress-corrosion cracking to be observed. (Crane & Gangloff, 2015)

Moreover, aluminum alloy 5083 is more expensive than aluminum alloy 5052, which in combination with the previous advantages leads to the selection of alloy 5052 as the material of the panels for the experiment of this study.

3.2 Water-Salinity

In Lagerstörm et al. (2020), an experiment is conducted where panels painted with antifouling paints of different contents of Cu_2O are immersed in different harbors located in Sweden, in waters with different salinities. The experiment's results showed that one important parameter which affects the release rate of the Cu_2O is salinity. Specifically, an analysis of the study sites salinities against the copper release rates concluded that the release rates are increased at higher salinity. (Lagerström, Ytreberg, Wiklund, & Granhag, 2020) The present study does not focus on the leaching copper in different salinities, but in the release rate of antifouling paints containing different contents of copper. The desired result cannot be succeeded with the use of freshwater, but with the use of saline water with high salinity. In this way, maximum release rates of copper in the shortest period of time can be succeeded.

The sampling of the sea water will take place at the sea surface, so there is a need for research about the sea surface salinity (SSS) to be conducted. If there was possibility of sea water sampling from all over the Greek seas, the best choice would be the northeastern region of Crete. The part of the Mediterranean where the Aegean Sea is located can be characterized by low precipitation, elevated salinities and high evaporation rates. There is a variety of SSS values, which change seasonally and increase from north to south, ranging from less than 31 to more than 39 PSU. (Dimiza, Koukousioura, Triantaphyllou, & Dermitzakis, 2016) The SSS values can change because of freshwater input from rivers, other runoff and groundwater-discharge mechanisms, precipitation, evaporation, etc. A specific example is the water exchange between the Black Sea and the Eastern Mediterranean through the Dardanelles and the Sea of Marmara, and the cyclonic movement of water. The northeastern and central Aegean Sea is influenced by upwelling and there are different sea surface temperature values, air temperature values and different wind speed. (Tukenmez & Altiok, 2022) But generally, the northern Aegean Sea, influenced by the Black Sea, is less saline than the southern part. Through the Strait of Gibraltar enter the Mediterranean Sea the Atlantic surface waters. These waters are characterized by low salinity and are marked by an initial value of about 36.2 PSU. As it is known, the Mediterranean is a semi-enclosed basin, where evaporation greatly exceeds precipitation and river runoff. Consequently, when the Atlantic surface waters enter the Mediterranean, and flowing toward the Levantine Sea, they transformed into very saline intermediate and deep waters, with deep water salinity values of about 38.4 and 38.7 in the western and eastern sub-basins, respectively. So, the southern and southeastern Aegean

Sea is influenced, resulting to more saline waters and this is the reason why the northeastern region of Crete would be the best option for sampling. (Figure 16) (Sammartino, Aronica, Santoleri, & Buongiorno Nardelli, 2022)

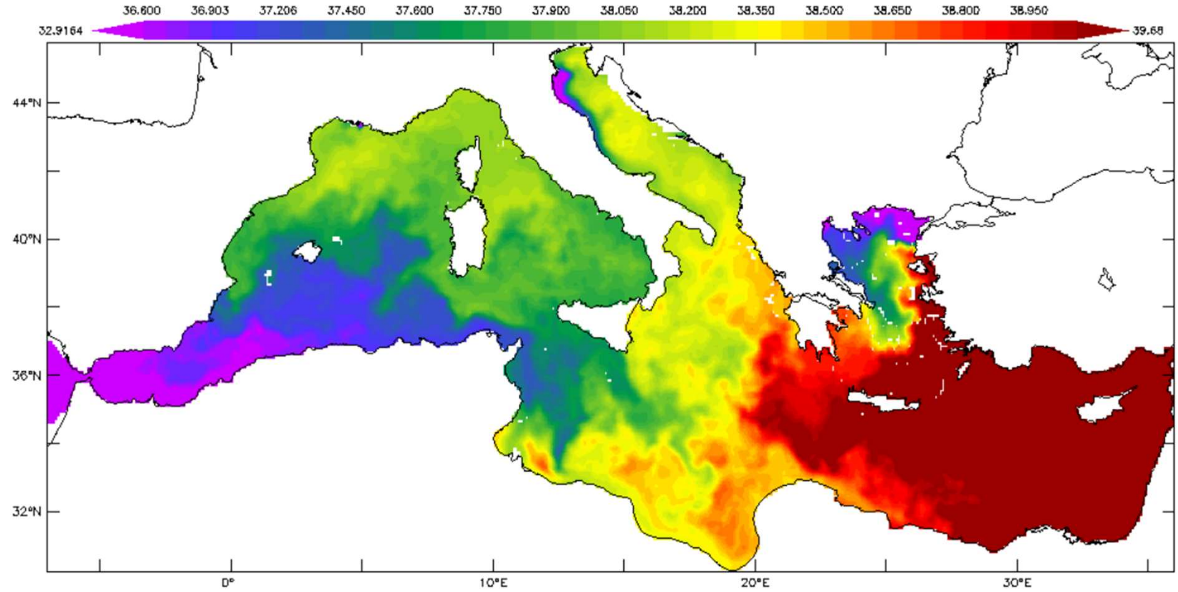


Figure 16: Salinity map of the Mediterranean Sea (https://poseidon.hcmr.gr/)

However, if the choice of seawater sampling is limited within the coastal zone of Attica region, for the present study to be more representative, again the best option to achieve the goal of the experiment is the most saline water. According to the salinity map of Greece (Figure 17), the appropriate region is the Megara Gulf, characterized with salinity value of about 38.75 PSU.

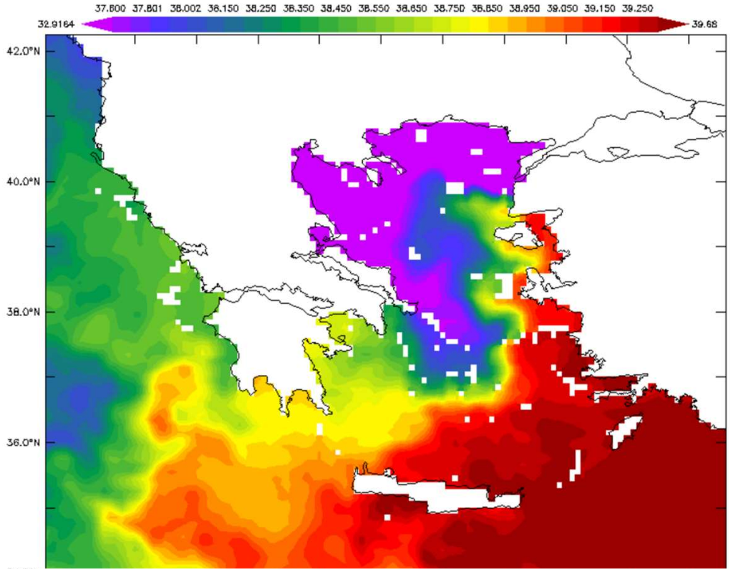


Figure 17: Salinity map of Greece (https://poseidon.hcmr.gr/)

3.3 Water-Static conditions

The evaluation of the efficacy of an antifouling paint is typically conducted through field tests. In these tests, pretreated and painted panels are exposed in seawater under static or dynamic conditions or under both of them. Static conditions are the worst-case scenario for an antifouling paint because fouling organisms are more easily settle on the ship hull. So, in the present study static conditions are intentionally chosen in order to be examined the release rates of the different antifouling paints in the worst scenario and subsequently the most effective paint of them. (Lagerström, Ytreberg, Wiklund, & Granhag, 2020) However, here the tests are not field, but laboratory tests. According to the "Guidelines on the Efficacy Data Requirements for Approval of Non-Agricultural Pesticide Products: Antifouling Products" (UK), laboratory, simulated field and field tests have their own purpose. The purpose of laboratory tests is the demonstration of the inherent biological properties of an active ingredient, e.g., as an anti-weed or an anti-animal agent, of simulated tests, the demonstration of antifouling capability of a test formulation under static conditions and last, the purpose of field tests is the demonstration of antifouling capability under in-use service conditions. (Thompson, CTI, & Lewis)

To fulfill the purposes of each kind of test, it would be worth the examination of the antifouling paints also on the field. ECHA recommends static testing in coastal waters because they are the perfect representation of the worst-case scenario. Coastal waters, near shore are usually characterized by high fouling intensity, combined with the static conditions of these waters, result to an environment which favors the settlement of fouling organisms. If an antifouling coating is effective in these waters, its use will be successful also in open sea and brackish waters.

If there was possibility of field tests, there are some indicative criteria for an acceptable static test of an antifouling product. Initially, as it was mentioned above, the location of the tests is very important. Preferable location is in coastal zones. Secondly, the duration of testing should be at least one fouling season, at least 6 months, including the period of peak fouling activity. In Greece, this period could be from April – May until October, including summer months with the highest activity of recreational and commercial vessels and with the peak of fouling activity at the same time. In addition, the number of replicates and the use of control are factors to be considered. Regarding the replicates, it is necessary to be at least three per product for the reliability and the integrity of the experiment. The control is a surface which has no antifouling effect. That means that it may be a surface coated with, e.g., only a primer or a primer and a matrix of antifouling coating but without biocides. The role of control is very important and should be included in almost every experiment because it is an indication of the degree of fouling settled on the surface under static conditions, in case of absence of biocides in the coating. After the time period of the immersion of the field tests, the percentage fouling on control and test panels should be evaluated. If the test panels are covered by macrofouling in a percentage lower than 25% while the control panel has a fouling coverage at least 75%, then the result is acceptable. In the case of the experiment in the present study, the results should be compared with the results of the laboratory tests and an appropriate evaluation should be followed. (Lagerström, Ytreberg, & Wrange, Assessment of efficacy and excesstoxicity of antifouling paints for leisure boats, 2021)

3.4 Experimental set-up

3.4.1 Painted Surfaces

For this study are used aluminum sheets of aluminum alloy 5052 non heat treatable for the reasons that were analyzed above. Aluminum sheets are approximately 2 mm thick and are cut into dimensions 3 × 1 cm. The aluminum panels are pretreated and painted according to the manufacturer's instruction. Their surface must be clean; dust, dirt, any oil or grease has to be removed with a solvent (acetone). Otherwise, there is a danger for this dirt and oil to penetrate deeper in the aluminum panels with the process of sanding which follows. Subsequently, the surface can be prepared by sanding with sandpaper to succeed adhesion of the primer. After sanding, the resulting dust must be removed again. Before painting, the surface must be completely dry.

Prior to application of each antifouling paint, a primer should be applied. WILKOPOX AL tie-coat – EPAS 1502 is a two-component epoxy primer which cures to a strong coating with excellent anti-corrosive and adhesion properties. It is the ideal primer because it is suitable for aluminum surfaces. According to the Technical Data Sheets (TDS), preferable methods of application are airless spray, brush or roller. In this case, airless spray will be used for the application. The recommended Wet Film Thickness (WFT) is 140 μm, which will be measured with a wet film thickness gauge, 25-2000 micron scale (Sheen Instruments LTD). The solids volume is about 43%, so the Dry Film Thickness (DFT) will be approximately 60 μm, which will be verified with the appropriate equipment, the Elcometer 415, Paint and Powder Gauge (for measuring on smooth surfaces).

When the primer is completely dry, each antifouling paint can be applied. Again, according to the TDS of the two commercial antifouling paints, preferable methods of application are airless spray, brush or roller. For this application airless spray will be used. The recommended WFT is 90 μm, which will be measured with the wet film thickness gauge. The DFT will be approximately 50 μm, which will be verified again with the Elcometer 415.

The same process is followed for the other two paints, the non-commercial antifouling paint, where nanoparticles of Cu_2O are incorporated in a matrix without biocides and for the paint with no biocides, which is served as control.

The number of aluminum panels painted with each antifouling paint is three for the precision and the reliability of the method. So, the total number of the panels needed are twelve.

Figure 19: Wet Film Thickness Gauge

Figure 18: Elcometer 415 for DFT measurement

After all pretreatments of all surfaces, before and after painting, all panels are weighed for the determination of mass of paint applied.

When the panels are completely dry, they are ready to be immersed into the bottles with the seawater. According to TDS of the two commercial antifouling paints, the time to reach the level of touch dry at 20°C is 20 minutes. ASTM D1640/D1640M-14(2022) Standard Test Methods for Drying, Curing, or Film Formation of Organic Coatings defines that a film of coating is considered touch dry or dry-to-touch "when it no longer adheres to the finger and does not rub up appreciably when the finger is lightly rubbed across the surface". Even though touch dry time is only 20 minutes, here the samples are allowed to dry for 24 h to assure complete drying and then submerged in water.

3.4.2 Preparation of Seawater

The sampling of seawater is conducted from coastal waters from the location discussed above. The seawater must be filtered by a 0.2 μm filter for the removal of any potential interferences. Such materials may be suspended sediments, other debris, even microorganisms, which may hinder the following process.

For the determination of the release rate of the copper leaching from antifouling paints, the initial level of copper in the seawater is needed. It is not right to regard the value of copper as zero, because there may be significant levels of it in the sample water, e.g., in marinas have detected high levels of copper because of the recreational boats that are housed there. Consequently, an initial measurement must be done by ICP-MS to determine the total amount of copper ($Cu_{total,initial}$).

3.4.3 Final Analysis

After the painting of the panels and the preparation of the water, the painted panels are immersed in the bottles filled with the seawater for 180 days. The duration of testing is selected because approximately 6 months is one fouling season, so 180 days is a representative period.

On day 180, panels are removed from the seawater. Each bottle is shaken vigorously to mix up the leachate released into water from painted panels. A sample is digested with trace-metal grade HNO_3, followed by ICP-MS analysis to determine total Cu (Cu_{total}).

For the calculation of release rate of copper, there is an initial measurement of Cu before the immersion and the final, after the immersion and the removal of the panels from the bottles. The difference in measured metal concentration represents the amount of metal lost. This amount of metal lost divided by the difference in immersion time Δt (days) results to the release rate between two time periods, especially when the measurements are conducted not only between the initial and the final measurement, but in a different immersion time, midway through the experiment: (Lagerström, Lindgren, Holmqvist, Dahlström, & Ytreberg, 2018)

$$Release\ rate = \frac{Net\ Release}{\Delta t} = \frac{Release_{t_2} - Release_{t_1}}{t_2 - t_1}$$

3.4.4 Storage of the samples

If it is not feasible for the final analysis for the determination of the total copper to be conducted, it should be examined if the bottles with the immersed panels can be stored and for how long.

When painted panels are immersed in seawater, there is an amount of copper detected in the aqueous phase after a period of time. The total amount leached out of the panels, Cu_{total}, can be distinguished into fractions, which are affected by salinity. The first hours, the most of the Cu_{total} detected is ionic Cu (Cu_{dis}), a smaller percentage is Cu_{nano} and there is no bulk Cu. With the time, Cu_{bulk} increases steadily, while Cu_{dis} decreases. In natural seawater, the dissolution of Cu_2O is controlled by the ions, mainly chloride ions (Cl^-):

$$\frac{1}{2}Cu_2O + H^+ + 2Cl^- \rightarrow CuCl_2^- + \frac{1}{2}H_2O$$

When ions Cl^- are not anymore available, the dissolution of Cu_2O may still occur, while protons (H^+) may react with the oxygen atom (O) in Cu_2O to produce cuprous ions Cu^+, with a very slow reaction rate because pH range in typical natural waters is 6 to 8. In addition, Cu_2O particles in the paint matric react with Cl^-, releasing Cu^+, which can be rapidly converted to cupric ion (Cu^{2+}) in oxygenated water:

$$CuCl_2^- + Cl^- \leftrightarrow CuCl_3^{2-}$$

In this way can be also explained the reason of the higher release rate in waters with higher salinity. In conclusion, when painted panels are immersed in seawater, there will be fractions of copper. However, the total amount of copper detected in the aqueous phase at first will be increased in a higher rate, and then in a very slower, it may be regarded zero and the amount of coper stable. So, if the bottles are sealed, the samples can be stored for a long period of time. (Adeleye, Oranu, Tao, & Keller, 2016)

4. Conclusion - Comments

The impacts from the application of antifouling paints to the marine environment are of great importance. As lesson from the incidents of organotin, there is an urgency to comprehensively evaluate the environmental risks of other antifouling paints or "booster biocides" to local systems. These new alternatives to antifouling paints were intended to cause less harm to the environment than organotin biocides. However, in some cases the accumulation levels are extreme high, despite claims of rapid degradation, resulting to be biocidal on non-target organisms.

Nowadays, there is a growing concern for environmental protection, so harmful biocides are expected to be phased out. Such serious issues should be discussed and executed with the Sustainable Development Goals (SDGs) of the United Nations. Particularly, it is essential to be taken measures to reach goal No. 3 regarding health and well-being and No. 14 regarding life below water.

An alternative solution is the use of copper-based nanoparticles as biocides in antifouling paints, which is the proposal of this study. It is expected that copper-based nanoparticles will be used in less quantities than conventional copper, being more effective at the same time.

There is a need for more reliable and sensitive analytical methods to be developed to accurately describe the environmental release and distribution of biocides to understand the environmental effects. For example, zinc pyrithione can be hydrolyzed or biodegraded, breakdown and transform into other metabolites, but in deeper waters it may be accumulated and persist in the sediment. In addition, most of the products from the above processes may be unidentified. The same situation occurs for many biocides. There is a need for assessment, evaluation, research and development.

Products and infrastructure can be changed, but not marine organisms.

Regarding the change of products, following the ban on tributyltin and other booster biocides, there has been an interest in the synthesis of effective and ecofriendly compounds from natural resources. Such ecofriendly compounds are found in seaweed, bacteria, algae, corals, sponges and terrestrial plants. (Kyei, Akaranta, & Darko, 2020)

There are studies that confirm the ability of using alternative marine natural products extracted from different marine algae as antifouling agents. Marine algae are one of the largest producers of biomass in the marine environment. They produce a wide variety of chemically active metabolites in their surroundings. These active metabolites have antibacterial, antiviral, antifungal and anti-macrofouling properties, which are effective in the prevention of biofouling process. The fatty acid and steroids extracted from different seaweeds are very promising as safe, economic additives for antifouling paint manufacture. (Ibrahim, Moawad, Attia, El-Naggar, & Tadros, 2019)

There are also some other strategies for antifouling coatings, using non-toxic, non-biocide release based principles for marine applications. The two main strategies are the following: firstly, the "detachment of biofoulants" mostly from "hydrophobic surfaces" using the hydrodynamic shear flow caused by the movement of the ship and in this way seeks to reduce the adhesion strength as much as possible for an efficient removal of the settled organisms, and secondly, the strategy "prevention of attachment" of biofoulants by applying a "hydrophilic coating" which retains a hydration layer and aims to avoid settlement. (Nurioglu, Esteves, & With, 2015)

Finally, boat owners must comply with regulations and conventions and not to use illegally antifouling paints that have been banned. They should also have the necessary knowledge and use antifouling paints right. A typical antifouling paint erodes slowly over time, giving rise to a slow but controlled release of biocides to the water. However, many boat owners choose to use paints containing higher amounts of copper than those needed, so the paint contains more copper that it is eventually depleted from the paint during a boating season. In addition, most boat owners repaint their boat hull each year, resulting in a high accumulation of copper on the hull.

In conclusion, the use of toxic antifouling compounds is no more an environmental dilemma. Their use is necessary, because without them, there will be many negative consequences at environmental and economic level. However, it is necessary more research to be carried out regarding the impacts of current biocides in use and for new developments to be used in the future.

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