

ELEMENTAL COMPOSITION OF THE NANOPARTICLE BASED ANTIFOULING COATINGS

Submitted in the partial fulfillment of the requirements for the award of

Bachelor of Technology degree in Biotechnology

By

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BONAFIDE CERTIFICATE:

This is to certify that this project is the bonafide work of R.Suvetha (38230037) who carried out the project entitled “**ELEMENTAL COMPOSITION OF THE NANOPARTICLE BASED ANTIFOULING COATING**” under my supervision from December 2021 to April 2022.

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DECLARATION:

I declared that the Project Report entitled “**ELEMENTAL COMPOSITION OF THE NANOPARTICLE BASED ANTIFOULING COATING**” done by me under the guidance of Dr. Valli Nachiyar Associate Professor, Department of Biotechnology, Sathyabama Institute of Science and Technology and Dr. Inbakandan Scientist – E/Head of Centre for Ocean Research, Col.Jeppiar Research park, Sathyabama Institute of Science and Technology is submitted in partial fulfillment of the requirement for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT:

Combinations of nanoparticles based antifouling coatings were made by Center for Ocean Research to combat the biofouling problem in the marine environment. As the TBT based antifouling paints are banned in the industry due to the TBT leaching and its adverse effect caused to marine organisms, shipping industries are searching for a new solution to solve this marine biofouling adhesion. Any coatings or product developed to solve this problem should be evaluated properly to know the concentration of the leachate, exited out from the surface. In this backdrop, the present study will evaluate the concentration of the nanoparticles leaching in a control environment using ICPMS.

Metal surfaces coated with the combination of nanoparticles will be characterized with Energy-dispersive X-ray spectroscopy to know the percentage of nanoparticles on the surface. Then the coated surfaces will be exposed to control seawater environment for 100 days with a constant temperature, salinity, light and pH. Elemental composition in the seawater will be determined with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on 0, 25, 50 and 100th day of the experiment. The elemental composition will be spastically analyzed to know the impact of leaching in a seawater environment. This will be a baseline data to know the toxicity of the nanoparticles used to design the coatings to combat marine biofouling.

Keywords: Biofouling, Nanoparticle coating, ICP-MS

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CHAPTER - 1

INTRODUCTION:

Biofouling is an accumulation of microorganisms, plants, algae, barnacles or small animals. It is unwanted on surfaces such as ships and submarine hulls, pond or river that cause degradation to primary purpose. Nanocoatings are used for preventing biofouling. They made to combat the fouling problem in marine environment. In early times, they use TBT(Tributyltin) as the material to prevent biofouling. Later TBT was banned due to their toxicity that effect the organisms which living in marine environment. It is a major problem in aquatic environment because it's extremely toxic to non-target organisms which linked

to immuno-suppression and imposex (development of male characteristics to females) in bivalves and can be persistent. The undesirable accumulation of biological matter on the surfaces of submerged objects such as ship hulls and pier pylons. It is a global problem for marine industries in both economic and environment. The control of biofouling is the complex problem, to prevent or remove the fouling organisms from the surfaces to minimise impact on non-target organisms then culture environment. Antifouling is an ability of designed materials and coatings which remove or prevent fouling of organisms on wetted surfaces. Recently, studies have began researching antifouling methods inspired to living organisms this type of design known as bio mimicry. According to several estimates nearly 1700 species comprising over 400 species organisms responsible for biofouling. It is divided into micro fouling, biofilm formation and bacterial adhesion and macro fouling attachment of longer organisms. Alternatively approach actively being sought which include more environmentally acceptable biocides or biocide delivery system non toxic fouling release coating and natural antifouling agents and processes. Microorganisms attachment to the surfaces located in the marine water has become a significant problem. Historically, the antifouling properties of the coatings were achieved by using biocides, which had a negative consequence to the marine environment. Currently, alternative environmental friendly methods are required. This thesis aimed to investigate and produce the antifouling coatings that can be used as potential candidates in the marine industry. In this study, a range of novel polymer nanocomposite coatings was fabricated via the method of solvent and tested based on the strategy of microbial adhesion. The composition of the coatings mainly contains polydimethylsiloxane (PDMS) and different nanomaterials. The coatings applied on glass substrate were characterised using X-ray spectroscopy (XRD), scanning electron microscopy (SEM), contact angle measurements, inductively coupled plasma mass spectroscopy (ICP-MS) and atomic force microscopy (AFM). In biofouling assays, attachment of bacteria *B. Subtilis* and three marine microalgae (*Skeletonema* sp., *Amphora* sp., *D. Salina*) was investigated in laboratory scale. The obtained results suggested that small amount of nanoparticles in the polymer matrix can improve the antifouling settlement behaviour of the coatings. All microalgae attached more on PDMS/SiO₂ and control surfaces (glass and PDMS) compared to the coatings containing multiwall carbon nanotubes (MWCNT) and sodium

bismuth titanate (NBT). The influence of contact time, surface roughness and surface wettability was also studied. The microbial attachment varied significantly with respect to contact time and surface properties. There was no obvious evidence showing that the wetting properties and the roughness of the coatings have an effect on growth. Finally, the physico-chemical properties of all tested microorganisms and coatings were investigated in order to examine the thermodynamic model for cell adhesion prediction. The work of attachment and its components were quantified based on contact angle measurements and calculated surface energy along with OWRK (Owens, Wendt, Rabel and Kaelble) approach. The results showed that the thermodynamic modeling did not predict microbial adhesion. However, it revealed that there is a possible link between work of attachment and the actual microbial adhesion if some coatings are tested separately.

CHAPTER - 2

REVIEW OF LITERATURE:

The fouling phenomena is common and diverse ranging from fouling ship hulls, natural surfaces in marine environment, fouling of heat transfer components through ingredients which contained in cooling of water or gas even development of plaque or calculus on teeth. In studies they made nanoparticle based coating to combat the fouling problem, to obtain leachates from biocides or free biocides of silicone fouling release coatings (FRC) in an experimental condition that was analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). In this paper commercial silicone applied then allowed to dry, wash with seawater to remove any residues. After set immersion time natural seawater leachates were collected by (Hellendhal glass staining jar) placing 6slides for each paint.

80ml of 0.22 μ m natural seawater at 37% salinity 8pH. Total painting surface = 180 cm² in 180ml water volume, water volume ratio during leaching about 4 times ratio waterman et al, (2005), more than 15 times ASTM D6442, Around 18 times Okamura et al, (2012). Hellandhal jars kept at 25 °C for 72h. Leachates were collected then kept in dark at 4 °C for 24hrs for performing toxicity bioassay. In leachate toxicity they seen under stereo microscope to observe the movement of nauplii. Chemical which was leach from plates that was analysed by ICP-MS. Antifouling activity of marine actinobacteria in titanium dioxide nanoparticle (Gaurav et al 2014) in this synthesis of titanium dioxide nanoparticle from marine actinobacteria to check their antifouling activity against isolated bacteria. They have taken five actinobacteria strains and two biofouling isolated bacteria. Spread plate method starch casein agar diluted to 50% with seawater to avoid fungal contamination then agar plates supplemented with nystatin. Actinobacteria colonies were formed like powdery and leathery form, isolated actinobacteria was incubated with SS broth(soluble starch – 25g, glucose -10g, yeast extract -2g, CaCO₃-3g, trace elements - 1ml and distilled water – 1000ml), incubate in shaker incubator for 7days at 28°C. Crude samples are centrifuged and pellets are discarded. In that supernatant TiO₂ is added and incubate for 24hrs. In culture broth 1ml supernatant added then absorbance was taken UV Visible spectrophotometer reduction is confirmed. Sample diluted with SDS(Sodium Dodecyl sulphate) analyzed under ultrasonification. For AFM samples are with glass slides then 2mg of TiO₂ NPs mixed with 200mg KBr, grounded well analyzed under FTIR the range is 4000 – 450cm⁻¹. To check the activity, biofouling organisms isolated by ZMA(Zobell Marine Agar) incubate at 28°C for 3 to 5 days. Colonies selected and purified by nutrient agar incubated for 6hrs. Grain staining and mannitol salt agar identify the biofouling bacteria, activity was done by agar well diffusion method. 100ul media poured in well and check for inhibition then incubated at 37°C for 2 hrs. Maximum zone of inhibition occur in 3 samples out of 5 they are considered as staphylococcal then in sample 2 which has more antifouling activity when compared to sample 3 therefore the combined of TiO₂ and extract is more potent which can be used for production of antifouling paints. Later shows that TiO₂ affect the degradation of positively charged dyes. Biofouling become major problem in marine microbiology, to prevent this problem by making non toxic or less toxic agents, non corrosive and cost effective remedy for

biofouling. Protective [1]organic coatings can offer these functions and consequently are largely used in the shipping industry to increase the working life of systems and improve its reliability. Leaching of fresh hydrothermal sulfide ores are used. Samples crushed with tungsten carbide mortar and pestle again with mortar under nitrogen to avoid atmosphere sample oxidation. The chemical components are degassing and duplicates are prepared, mixture stirred with PTFE at 5 to 20 degree celcius in water bath. [2] A small portion removed at 1,4,6,8 and 30hours by dispersable syringe(10ml).samples are filtered preserve in HNO₃ in polypropylene tube for ICP-MS. Rest of the sample for pH and ORP. ORP electrode with KCl Ag/AgCl system. All samples cleaned with 2NHNO₃. Desalinate with chelating resins before samples are precleaned with acetone and milli Q water. Conditoned with buffer solution. In this study, the application of copper-containing antifouling paint in freshwater was concluded to be redundant as the products contributed no antifouling effect. Any release of copper in such environments thus only pauses a risk to the environment without any beneficial gain for the boat owner. Although there is a ban in Swedish freshwater for the use of biocidal antifouling paints, that is not the case for most EU countries. These member states may gain from performing risk/benefit analysis to assess whether the continued use of biocidal antifouling paints in freshwater is indeed necessary. For marine waters, the mapping of the critical Cu release rate revealed variations in the sensitivity of the fouling community in the Baltic Sea/Kattegat, with higher release rates required to deter macrofouling at higher salinities. However, as the release rate of Cu [3] from antifouling paints also increases with increased salinity, all the eight tested paints were found to be efficient regardless of location along the salinity gradient. In fact, the Cu release rates of a majority of the paints greatly exceeded the critical release rate. For some products, a reduction of up to 80% in the release rate of Cu would be possible without any loss in efficiency. The properties of these products are thus not in alignment with the principles of the BPR which requires the release of active substances to be the minimum necessary. Even[4] though an overwhelming percent of antifouling solutions used worldwide are commercialized in the form of coatings, it is indeed likely that many readers, somehow related to antifouling (AF) research, are not familiar with basic paint technology concepts. This may become a serious problem because, at some point, the success of their research will inevitably require scaling-up their lab experiments

to an actual coating capable of withstanding extensive long-term field testing. The aim of this chapter is to provide part of the theoretical basis on paint technology to apply new AF concepts in the most efficient manner. In other words, no single promising AF concept should be abandoned because of unsatisfactory field results attributed to an improper paint formulation, fabrication or application. It has been intended as a help for antifouling researchers willing to scale-up their ideas for novel AF coatings from the lab to a panel immersed at a sea site. Once the types and amounts of controlled-release binder(s) and the active agent(s) have been chosen, the next formulation parameter to be considered is the pigment volume concentration (PVC). Such parameter, and, more specifically its ratio to the critical PVC (CPVC), is key to the final paint performance, and can determine the success of the formulation. The same applies to correct pigment dispersion procedures. The potential presence of unbroken pigment clusters, only partially wetted by the binder phase will also influence the final paint properties and potentially yield a good formulation into a failing coating. Similarly, the addition of suitable amount and types of rheological additives is a must in order to avoid heterogeneous dry film thicknesses along the test panel (because of, e.g., sagging) or heterogeneous paint composition along the paint film (due to, e.g., pigment settling during solvent evaporation). Antifouling paints are extremely sensitive paint systems, in which all the paint working mechanisms are tightly coupled and must be finely synchronized so as to develop the full potential of the formulation. Even though there is no doubt that finding a correct combination of controlled-release binder and active substances is the most difficult part when developing novel antifouling coatings, it is not difficult at all to develop failing coatings out of promising raw materials. As a clear example, paint companies spent many years of research to optimize copper-containing tributyl tin based coatings, in spite of the recognized AF efficiency of both agents. Antifouling paint research will therefore strongly benefit from correct use of coating formulation principles to fully evaluate the potential of a new concept. Research projects involving an important investment in time and resources could upgrade from promising ideas to promising products, too often the most difficult step for academic research, with the subsequent benefit to the marine environment.[5] Polyethersulfone (PES) polymer is widely used in membrane fabrication due to its good mechanical and chemical stability. Fouling and in particular biofouling leads to reduced

efficiency due to the hydrophobic characteristics of the membrane. In this work, we report on the fabrication of antimicrobial ultrafiltration membranes from PES and a range of silver additives. Commercial silver additives in the form of silver zinc zeolites and silver zinc glasses as well as some common organic silver salts were used. The effect of silver additives on membrane biofouling was extensively studied by both static and dynamic methods using bacteria culture. These tests demonstrated the anti-biofouling properties of modified membranes in comparison with control membranes. The issue of silver leaching during membrane fabrication is of critical importance from both sustainability of membranes and environmental aspects. This was studied in detail for PES–silver zinc zeolite membranes. The treatment of membrane with sodium hypochlorite had the most detrimental effect on silver retention in membrane. Leaching of silver was higher in seawater due to the ion exchange membranes. Initial tests of membranes were performed in single-cell cross-flow filtration circuits. Direct observation of the biofilm on the two membranes indicated that there was less biofilm on the blend membrane than the PES control membrane. Results from the carbohydrate assay were consistent with these observations with overall mean absorbance 30% of the PES membrane. Also it was found that for both nutrient and bacteria filtration the permeate flux was higher for the modified membrane. An alternative configuration with two cross-flow cells in parallel was designed to test two membranes with the same culture. When PESIrgaguard B5000 blend membrane was compared with the control PES membrane in the same circuit the differences between membranes were insignificant, while the amount of biofilm on the PES membrane was significantly reduced compared to the amounts of biofilm measured when the PES membrane was tested separately. The result supports the idea that the antimicrobial activity of the PES-Irgaguard B5000 blend membrane is due to leachate from the membrane. Silver concentration in the permeate lines was up to 0.01 ppb/cm² of membrane during the course of the test. The studies with ionic silver salts with organic anions as antimicrobial additives showed much lower

Table 7 Summary of leaching results from ICP analysis

Fabrication Stage	Vol. (l)	Time (min)	Ag (µg/l)	Zn (µg/l)
Non-solvent immersion	2	10	6.63 ± 0.97	162.34 ± 22.7
Hot water	1	30	6.98 ± 0.65	302 ± 33
Water (day 1)	1	1,440	0.748 ± 0.88	558.4 ± 21.31
Water (day 2)	1	2,880	1.137 ± 0.12	958 ± 53
NaOCl-1	0.5	30	26.2 ± 6.3	49.7 ± 3.44
NaOCl-2	0.5	30	19.5	30.6
NaOCl-3	0.5	30		

84.9 102 NaOCl-4 0.5 30 36.4 ± 1.13 50.6 ± 1.26 Glycerol 0.5 240 8.35 ± 1.48 377 ± 38

a Between 2 and 12 replicates were used for each test. J. Mansouri et al. / Desalination and Water Treatment 13 Downloaded by [MUSC Library] at 23:08 19 May 2016 stability of these membranes. There was significant loss of silver during membrane fabrication for these membranes with initial silver content of 2,000 ppm. On the other hand, the residual silver in membrane modified with Irgaguard B7000 was in the range of 1,000–1,400 ppm for initial loading of 2,000 ppm. One important aspect for the antimicrobial membranes is the level of leaching of active components and subsequent loss of activity during the lifetime of membrane. To study this effect, the long-term leaching of silver and zinc from PES-Irgaguard B5000 was investigated. After five months of testing, the leaching in Milli-Q water from Irgaguard blended membrane was around 0.08 and 90 ppb/cm² of membrane for silver and zinc, respectively. The silver leaching in sea water was 10 times greater due to the ion exchange of silver with the salt present in the sea water. The leaching of active components is expected to slow down when particles at the surface or pore walls are completely depleted. Static tests results showed that slow leaching rate after about 60 d of test. Antimicrobial activity assays of the PES-Irgaguard B5000 membranes aged for nine months in water and tested in bacteria filtration demonstrated the biocidal properties, which shows the long-term stability of these membranes. The release of silver ions may be further controlled by selecting the suitable copolymers to encapsulate the silver in membrane. Such copolymers may have block segments with high affinity with silver and membrane material.[6] Two types of modified layered aluminosilicates (montmorillonites) with different grain size and gallery spaces were tested as nanofillers in epoxy coating compositions for a steel substrate. Organophilic montmorillonites, in the amount of 2.5 and 5 wt% were introduced to waterborne and to solvent-type epoxy coating materials based on bisphenol-A epoxy resins and various curing agents. The results of this work indicate that processing properties of coating compositions leveling and drying time as well as mechanical properties of coatings hardness, scratch and abrasion resistance, adhesion to steel were positively affected by a layered aluminosilicate nanofiller. Enhanced water resistance in lower water absorbance of coats, especially those formed from waterborne compositions, has been found. Modified montmorillonites(mMMT) can improve some of the properties of

waterborne and benzyl alcohol-based epoxy coating composition and coats. The effectivity of modified montmorillonite (mMMT) depends on dose and type of nanofiller modification. PS-1 is perfect suitable modified montmorillonite for waterborne epoxy coating system and N5 appropriate to solvent type epoxy coating compositions. Some properties of water epoxy coats can improved by N5 vice versa, the some features of solvent type coating properties can upgraded by PS1 nanofiller too. Epoxy resin (ER) is an attractive material for metal protection against corrosion. Although nanosized particles are relatively novel fillers for polymers, they are currently used primarily in thermoplastics (i.e. PP, PA, PBT, PET) and chemoreactive cast-resin systems (epoxy and unsaturated polyester resins). aluminosilicates are often utilized as thixotropic/thickening agents in organic coating compositions , modified montmorillonites are more widely used in polymeric composites thus significantly enhancing mechanical properties, thermal stability, fire resistance and barrier properties for gases/fluids of filled nanocomposites . Layered aluminosilicates could be incorporated into coatings either in a form of powder or in water dispersion where they positively influence their mechanical and protective properties . Organic-based materials with metal adhesion properties are widely used as metal anticorrosive agents in many industries include aeronautics, automobiles, architecture, ships, and oil tanks. Epoxy resin-based materials are among the most widely organic-based materials, [7] since they have unique properties and versatility . Nanofillers with different aggregate grain size and gallery space were used for the modification of waterborne and solvent-type coating properties. [8] Biocide-containing anti-fouling paints are regulated and approved according to the added active ingredients, such as Cu. Biocide-free paints are considered to be less environmentally damaging and do not need an approval. Zn, a common ingredient in paints with the potential of causing adverse effects has received only minor attention. Laboratory experiments were conducted in artificial brackish seawater (ASW) and natural brackish seawater (NSW) to quantify release rates of Cu and Zn from biocide-containing and biocide-free labeled eroding anti-fouling paints used on commercial vessels as well as leisure boats. In addition, organisms from three trophic levels, the crustacean *Nitocra spinipes*, the macroalga *Ceramium tenuicorne* and the bacteria *Vibrio fischeri*, were exposed to Cu and Zn to determine the toxicity of these metals. The release rate of Cu in NSW was higher

from the paints for professional use ($3.2\text{--}3.6\ \mu\text{g cm}^{-2}\text{d}^{-1}$) than from the biocide leaching leisure boat paint ($1.1\ \mu\text{g cm}^{-2}\text{d}^{-1}$). Biocide-free paints did leach considerably more Zn ($4.4\text{--}8.2\ \mu\text{g cm}^{-2}\text{d}^{-1}$) than biocide-containing leisure boat paint ($3.0\ \mu\text{g cm}^{-2}\text{d}^{-1}$) and ship paints ($0.7\text{--}2.0\ \mu\text{g cm}^{-2}\text{d}^{-1}$). In ASW the release rates of both metals were notably higher than in NSW for most tested paints. The macroalga was the most sensitive species to both Cu ($\text{EC}_{50} = 6.4\ \mu\text{g l}^{-1}$) and Zn ($\text{EC}_{50} = 25\ \mu\text{g l}^{-1}$) compared to the crustacean (Cu, $\text{LC}_{50} = 2000\ \mu\text{g l}^{-1}$ Zn, $\text{LC}_{50} = 890\ \mu\text{g l}^{-1}$), and the bacteria (Cu, $\text{EC}_{50} = 800\ \mu\text{g l}^{-1}$ and Zn, $\text{EC}_{50} = 2000\ \mu\text{g l}^{-1}$). The results suggest that the amounts of Zn and Cu leached from anti-fouling paints may attain toxic concentrations in areas with high boat density. To fully account for potential ecological risk associated with anti-fouling paints, Zn as well as active ingredients should be considered in the regulatory process. This study showed that release rates of Cu from the investigated anti-fouling paints under brackish water conditions are considerably higher for ship paints compared with leisure boat paints. The coatings released substantially less Cu and Zn when leached in NSW compared with ASW, presumably due to higher TOC and pH in NSW. Therefore, using ASW-derived release rates of Cu and Zn is likely to overestimate the leaching in field. In addition, release rates of Cu under brackish water conditions ($\sim 7\text{‰}$) appear to be lower compared with leaching at a salinity of 35‰ . The release rate of Zn was notably higher for leisure boat paints; in particular, the two “biocide-free” paints released 2–12 times more Zn than the paints for professional use. The toxicity tests suggest that both Cu and Zn may be toxic to the nontarget organism *C. tenuicorne* in semi-enclosed areas with high boat activity where elevated trace metal concentrations can be expected. Based on these data, we recommend that the levels of Zn should be considered when evaluating the potential environmental impacts of anti-fouling paints.[9] It has been shown that the solution of 10 micrograms of copper per sq. cm. of paint surface per day will prevent attachment of all marine growths which normally present a serious ship-bottom fouling problem. Any of these organisms, which include barnacles, annelids, mollusks, hydroids, bryozoa, tunicates, and some species of algae, will attach to paints which consistently maintain lower copper leaching rates. The growth of many microbiological and some types of algae does not appear to be inhibited by a leaching rate of 10 micrograms per sq. cm. per day, but these organisms are seldom of practical importance in ship-bottom fouling. The value

of 10 micrograms per sq. cm. per day for the minimum adequate leaching rate is lower than that determined by LaQue (10) from weight loss measurements of nickel-copper alloy panels. Leaching rate determinations on copper alloys also indicate that some of them may require a higher leaching rate than paints to prevent fouling. Failure of ineffective copper-base ship-bottom paints to develop adequate leaching rates is usually the result of fundamental errors in formulation. The most common of these are the use of insufficient toxic, as illustrated by the data of Table V, or selection of an unsuitable vehicle. Of course the compound used as the toxic must have an appropriate solubility in sea water in order to leach. As there are practical limits to the amount of toxic which can be incorporated in a paint film, it is necessary to have an accurately controlled leaching rate to avoid premature exhaustion of the toxic reserve. Young (19) first stated the dilemma that the toxic must be soluble and, if soluble, will be washed away, which has discouraged many investigators of the problem ever since. Determination of the rate at which toxic must be liberated permits a more accurate appraisal of the situation. Thus, to maintain uniform adequate copper leaching rate for one year, a paint film must contain at least 3.6 mg. of copper per sq. cm. of surface area. At a spreading rate of 300 square feet per gallon, this corresponds to a copper content of 2.2 pounds per gallon of paint, a figure well within the limits of practical paint formulation. However, since most effective paints leach at an excessive rate when first exposed and maintain steady-state leaching rate somewhat greater than the minimum adequate value, a fairly large factor of safety as to toxic content must be allowed. The leaching rate method has proved to be a valuable tool.

[10] The ban on harmful substances in antifouling paints requires the development of new antifouling strategies. Alternatives should be as effective as conventional paints but of lower toxicity. In the present study two commercially available, self-polishing antifouling paints [11-15] were examined in order to get information on their antifouling properties and toxicological potential. Efficacy was shown in settlement assays with the marine barnacle species *Balanus amphitrite*, however, efficacy was related to toxic effects observed on target and non-target organisms. [16] Toxicity of the paint extracts was concentration-dependent and differed according to the paint and the species investigated. Toxicity could at least partially be attributed to zinc leached from the paints. Effects of a water-soluble paint were more pronounced in larvae of *B. amphitrite*, *Artemia salina* and in the green

algae *Dunaliella tertiolecta*. Embryos of the freshwater species *Danio rerio* and *Vibrio fischeri* were more affected by a paint based on organic solvent which can be used by the paint technologist in the formulation and testing of antifouling paints. By judicious use of this test a great deal of information upon the characteristics and behavior of antifouling paints can be obtained in the laboratory. It must be stated, however, that the leaching rate test is not presented as a substitute for panel exposures or fouling tests. It is a supplementary tool and has its greatest value when used in conjunction with ship or panel tests. When used in this manner, it serves to eliminate many poor paints in the initial stages, to aid in formulation, and to give insight into the reasons for that failure of various paints. The ultimate evaluation of the effectiveness of antifouling paints will continue to be based on the performance of the formulations on ships in service. [11] The dissolution of soluble pigments from both tin-based and tin-free chemically active antifouling (AF) paints is a key process influencing their polishing and biocide leaching rates. In this context, a low time- and resources-consuming method capable of screening the pigment behaviour in the search for the most promising materials or mixtures is of great interest. A preliminary attempt to develop such a method is presented in this paper based on the widely used ZnO pigments.[18-21] While highly pure, nano-polished, monocrystalline ZnO substrates yielded very low dissolution rates in the order of $17.3 \pm 3.7 \text{ g Zn}^{2+} \text{ cm}^{-2} \text{ day}^{-1}$, pellets prepared by compacting and sintering technical grade ZnO pigments dissolved about three times faster according to inductively coupled plasma mass spectrometry (ICP-MS) measurements.[22] The rougher and more porous surface exposed, together with the larger number of defects in the lattice structure, are hypothesised to be responsible for the faster sea water attack of the pellets compared to the ZnO crystals.[24] In any case, the ZnO dissolution rates reported in this paper are markedly lower than those associated with the sea water dissolution of cuprous oxide (Cu_2O) particles which are also used in AF paints. Experimental performance testing of model antifouling paints formulated with ZnO and/or Cu_2O demonstrates that the binder/pigment interaction should not be disregarded if the leaching of sea water soluble pigments from paint systems is to be determined. Methods to determine the release of biocides (e.g. copper) and substances of concern (e.g. zinc) from antifouling paints are required for both the development of efficient products and their environmental risk

assessment. To date, there are only two standardized methods available to estimate such release rates, but their reliability has been put into question. An alternative method, allowing determination of environmental release rates in the field of metallic or organometallic biocides by X-Ray Fluorescence (XRF), has been developed and applied in recent years. In this study, the potential for standardization of the XRF method is investigated through evaluation of its accuracy, precision and transferability between instruments. Accurate quantification of copper (Cu) and zinc (Zn) in $\mu\text{g cm}^{-2}$, despite differences in chemical composition, was demonstrated through comparison of calibration regression slopes for ten different antifouling paints and confirmed through the measurement of validation samples. Universal antifouling paint calibration curves are proposed for the determination of Cu and Zn in thin paint films, with a prediction uncertainty of around $\pm 130 \mu\text{g cm}^{-2}$ for both metals. The transferability of the method to another instrument was also demonstrated. [29] For both analyzers, concentrations of validation samples were within 5 % of those determined through wet chemical analysis. Pre-requisites and recommendations for the application of the method as well as its applicability to both short- and long-term release rate studies in the field are also presented and discussed. The composition and abundance of microfouling communities that develop on antifouling coatings reduce the performance and efficiency of ship operations, microfouling was investigated during static and dynamic seawater immersion of four commercial antifouling coatings, of which three were biocide based (tributyltin self-polishing, copper self-polishing, copper ablative) and one was biocide free (silicone fouling release). The total bacterial counts were similar on all coatings after static immersion, but after dynamic immersion the largest decrease in numbers was seen on the fouling release coating. The [30] bacterial population on the fouling release surface was also more heterogeneous than on the biocide-based coatings. After static immersion, diatom populations were dominated by *Amphora*, *Navicula* and *Synedra*; after dynamic immersion, no diatoms could be detected on the copper ablative coating and only *Amphora* was left on the fouling release coating. [31] The effect of copper additions (Cu ranging from 0 to $30 \mu\text{M}$) on the photosynthesis of three different microalgal biofilms was studied to identify the factors that cause sensitivity differences between benthic and pelagic algae. The response of biofilms which colonized artificial substrata in the River

Meuse was compared with those of two laboratory-grown monospecific biofilms, one consisting of the diatom *Synedra ulna*, and the other composed of a filament-forming cyanobacterium, *Oscillatoria* sp. The photosynthetic yield Φ_{II} (quantum efficiency of photosystem II) was studied with PAM (Pulse Amplitude Modulated) fluorimetry. *S. ulna* biofilms appeared to be the most sensitive to Cu, followed by the cyanobacteria, while natural biofilms, dominated by supposedly very sensitive diatom species such as *Melosira varians* and *Diatoma vulgare*, were the most resistant to Cu. In the highly productive biofilms, pH is suggested to play a role in lowering toxicity by helping the precipitation of cupric ions. Cu accumulation by the biofilms during the exposure period followed a linear relationship with Cu concentration, saturation not being observed ; natural biofilms had an accumulation factor of $1-2 \pm 5-10$ relative to the concentrations in the water, while the diatoms growing unattached to the substratum had a higher concentration factor, up to $4 \pm 9-10$. It was concluded that the physical structure of the biofilm (package of cells and thickness), and not the species composition, was the main factor regulating the sensitivity of the biofilm to Cu toxicity during short-term exposures. [32] The release of harmful metals from antifouling paints to water bodies is a well-known problem. In this study, we measured both the amount of biofouling growth on leisure boats during one season as well as the concentration of metals accumulated by the biofouling matrix. Furthermore, the efficiency of antifouling paints and mechanical boat cleaning as well as the effect of hull colour on biofouling were evaluated. Unlike paint residues, biofouling waste has never been regarded as a source of metal contamination and has previously been neglected in the scientific literature. Our results revealed that the biofouling waste contained very high concentrations of metals, up to 28,000 mg copper/kg dw and 171,000 mg zinc/kg dw, which exceeds the guidance values for least sensitive land use in Sweden by factors of 140 and 340, respectively. This observation is important because the contaminated biofouling waste is commonly disposed of in boatyard soils at the end of each season, thus increasing the levels of metal pollution. Moreover, there was no significant difference in the amount of biofouling if the boats were coated with copper or zinc containing paints or no paint at all, indicating that biocide paints might not be necessary in lower salinity areas such as the Stockholm archipelago. For boats that were not painted at all during the season, those washed on boat washers had on average half of the amount of

biofouling compared to boats that were not cleaned mechanically. The results of the study indicate the importance of proper management of biofouling waste as well as the use of more environmentally friendly removal methods for biofouling such as boat washers.[33] Metal release rates were measured from four different copper alloy-based materials used by the aquaculture industry: copper sheet machined into a diamond mesh, copper alloy mesh (CAM), silicon bronze welded wire mesh, and copper sheeting, and compared with conventional nylon aquaculture net treated with a cuprous oxide antifouling (AF) coating. Release rates were measured in situ in San Diego Bay using a Navy-developed Dome enclosure system at nine different time points over one year. As expected, copper was the predominant metal released, followed by zinc and nickel, which were fractional components of the materials tested. Release rates followed a temporal trend similar to those observed with copper AF coatings applied to vessel hulls: an initial spike in copper release was followed by a decline to an asymptotic low. Leachate toxicity was consistent with prior studies and was directly related to the metal concentrations, indicating the alloys tested had no additional toxicity above pure metals.[34] Trace metals, especially copper, are commonly occurring contaminants in harbors and marinas. One source of copper to these environs is copper-based antifouling coatings used on vessel hulls. The objective of this study was to measure dissolved copper contributions from recreational vessel antifouling coatings for both passive leaching and hull cleaning activities. To accomplish this goal, three coating formulations, including hard vinyl, modified epoxy and a biocide free bottom paint were applied on fiberglass panels and placed in a harbor environment. In situ measurements of passive leaching were made using a recirculating dome system. Monthly average flux rates of dissolved copper for the hard vinyl and modified epoxy coatings were 3.7 and 4.3 $\mu\text{g}/\text{cm}^2/\text{day}$, respectively, while flux rates for the biocide free coating was 0.2 $\mu\text{g}/\text{cm}^2/\text{day}$. The highest passive flux rates were measured initially after cleaning activities, rapidly decreasing to a baseline rate within three days, regardless of copper-based coating formulation. Hull cleaning activities generated between 8.6 and 3.8 μg dissolved copper/ cm^2/event for the modified epoxy and hard vinyl coatings, respectively. Aggressive cleaning using an abrasive product doubled the copper emissions from the modified epoxy coating, but produced virtually no change in the much tougher hard vinyl coating. When compared on a mass basis, roughly 95% of copper is

emitted during passive leaching compared to hull cleaning activities over a monthly time period for a typical 9.1 m power boat.

CHAPTER - 3

3.1 AIM:

To determine the leaching of nanoparticle antifouling coatings by ICP-MS

3.2 OBJECTIVES:

- The combination of nanoparticle based antifouling coating to combat the biofouling problem in marine environment.
- In present studies the concentration of nanoparticle leaching which was studied by using ICP-MS.
- The main problem of biofouling on marine vessels is associated with increased friction created as result of presence of organisms on vessel surface.
- The impact of biofouling reduced by coating the instrument with established antifouling substances or applying silicone coat.

3.3 RESEARCH PROBLEM:

- The main problem of biofouling on marine vessels is associated with increased friction created as result of presence of organisms on vessel surface.
- The impact of biofouling reduced by coating the instrument with established antifouling substances or applying silicone coat.

CHAPTER - 4

METHODOLOGY:

4.1 EXPOSURE OF METAL ELEMENTS IN SEAWATER:

Stainless steel, Copper coupons and glass slide are used as control which are exposed to seawater taken out day on (2,3,7,9 and 10) samples are inoculate, colonies are counted and observed.



Fig:1 Above pictures are copper slide, stainless steel slide, glass slide, small and big coupons. They are immersed in tank containing seawater. The apparatus leaves for 2-3 days for formation of biofilm.

4.2 COLONY COUNTING:

Samples are taken out then placed in petriplate containing sterile seawater and sonicate the plate for 15minutes in a same frequency. Collect the samples which was serially diluted (10^3 and 10^6) these two samples are taken counted for CFU. Same procedure followed for day (2,3,7,9 and 10).

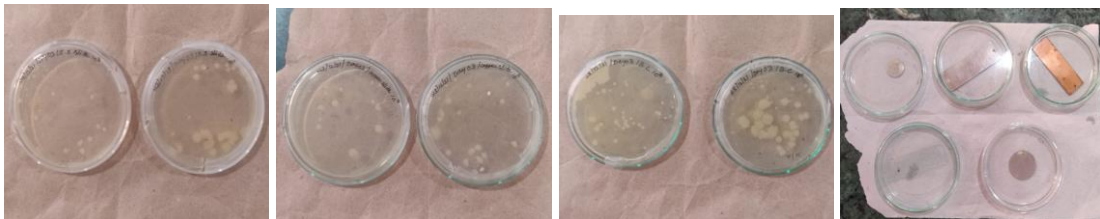


Fig:2 The above picture is about after the immersion of apparatus in seawater that was taken out from tank, each apparatus was sonicated for 15min after that serial dilution was done. After serial dilution spread plates was done.

4.3 GRAM STAINING:

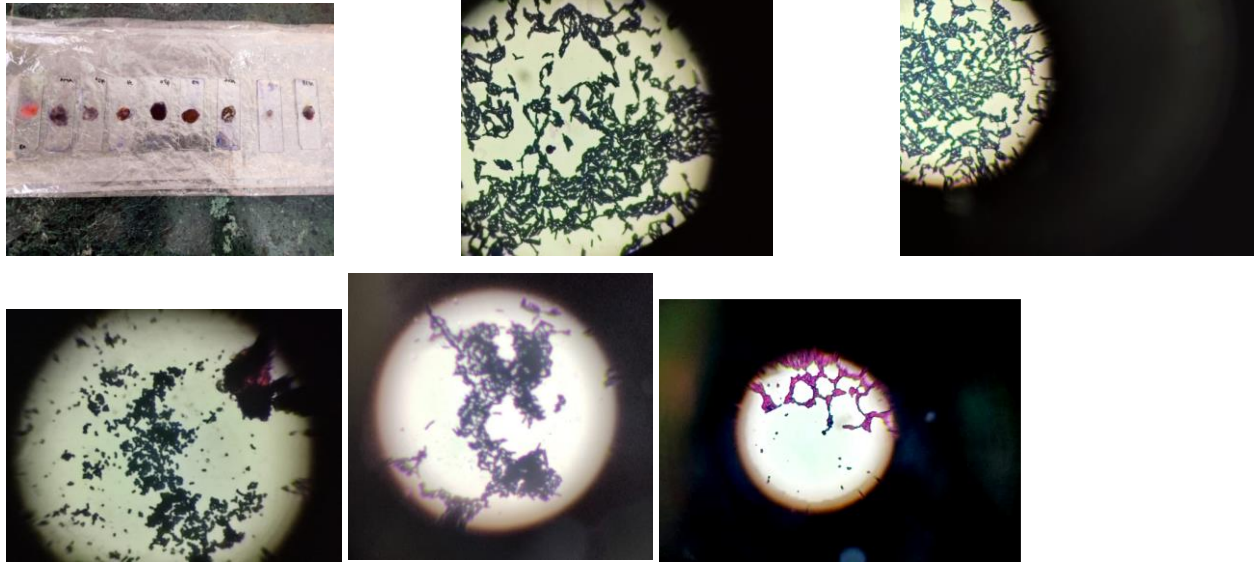


Fig3: The above picture is about whether to check our bacteria is gram positive gram negative.

4.4 INOCULATION OF SAMPLES:

- 9 plates are inoculated(5 plates + 1control)
- Zobell marine agar and agar agar used as media
- Samples are spread plated and incubate at 35 degree celcius and counted.

CHAPTER - 5

NANOPARTICLE SYNTHESIS:

Copper oxide, Zinc oxide and Titanium oxide are synthesized and mixed with paint then coated on the surface of metal.

5.1 SYNTHESIS OF COPPER NANOPARTICLE:

Synthesis of CuO nanoparticle by chemical precipitation method for the determination of antibacterial efficacy against Streptococcus and Staphylococcus species. 100M CuSo₄ dissolved in 50ml stirred by magnetic stirrer at room temperature. 0.2M of CuCl₂.H₂O prepared, 1ml of glacial acetic acid added and heated to 100°C at constant stirring. 8NaOH added till pH reaches to 7. The color of solution formed green to black, black precipitate formed. Precipitate centrifuged and washed 3 to 4 times with distilled water. An obtained precipitate dried in air flow for 24hrs. Powder is taken for characterization.

$$\text{Weight} = \text{mol.wt} \times \text{req.vol} \times \text{conc}/1000$$

$$= 134.45 \times 50\text{ml} \times 0.2\text{M}/100$$

$$= 1.3445\text{g}$$

NaOH - 8M

$$\text{Weight} = \text{mol.wt} \times \text{req.vol} \times \text{conc}/1000$$

$$= 40 \times 50\text{ml} \times 8\text{M}/1000$$

$$= 16\text{g}$$

5.2 SYNTHESIS OF ZINC NANOPARTICLE:

- 0.02M aqueous zinc acetate dihydrate dissolved in 50ml of distilled water vigorous stirring.
- At room temperature aqueous 2M NaOH added drop by drop to reach pH 12.
- Then placed in a magnetic stirrer for 2 hours.
- After completion of reaction white precipitate formed was washed thoroughly with distilled water ethanol to remove impurities.
- Precipitate dried in hot air oven for overnight at 60°C

Zinc acetate:

$$\text{Weight} = 0.02\text{M} \times 219.49 \times 50\text{ml}/1000$$

$$= 0.2194\text{g}$$

NaOH -2M

Weight= $2M \times 40 \times 50ml/1000$

= 4g

5.3 SYNTHESIS OF TITANIUM NANOPARTICLE:

- Mix 3.5ml Titanium Isopropoxide in 40ml of distilled water(99% purity).
- Add 0.2 - 0.3ml of nitric acid(HNO₃) purity 65% until it reach the pH 1.85.
- Solution was put on a magnetic stirrer for 24 hours.
- Sol was aged for 6 hours at 50°C yeilding a transparent sol.

CHAPTER - 6

6.1 TANK SETUP:

A large tank which filled with seawater. Aeration facility also provided with aerator. Aluminium, Stainless steel, Titanium and Copper plates are tied and hanged with stick immersed in seawater observe activity for 10 days. Each plates shoul not contact with one and another then every 2 days seawater must changed. Plates are taken out days on(2,4,6,8 and 10) for CFU,TOC and Chlorophyll Estimation.

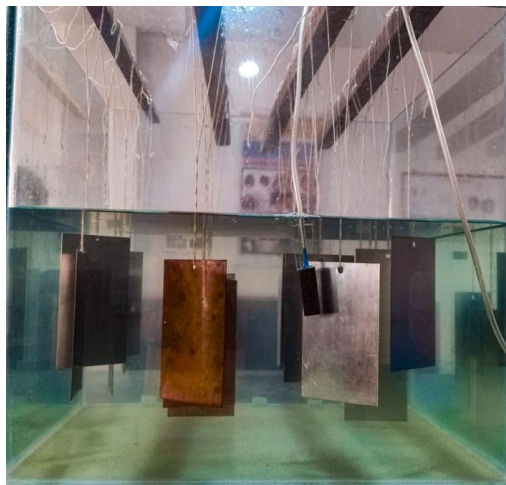


Fig4: Image represents the immersion of copper, aluminium, stainless and titanium plates in tank provided with aeration

6.2 BIOFILM SAMPLE COLLECTION:

Plates are taken out on respective days mentioned above and the plates are scrubbed with brush by pouring sterilized and filtered sea water along the side of plates and brushed simultaneously. Scrubbed water is collected in culture plate and transferred into a reagent bottle and sample names are marked.

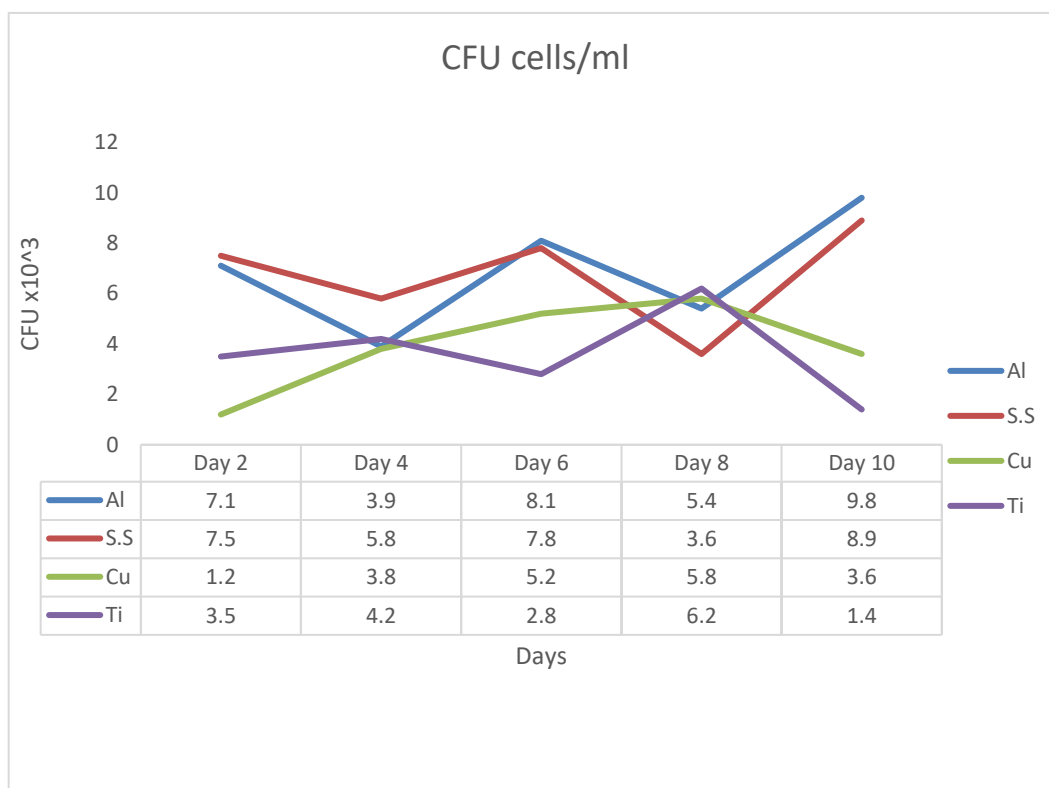


Fig:5 Graph represents the CFU of biofilm forming colonies

Collected water sample is diluted and transferred in 1ml eppendorf tube. 100 micro litre of sample is poured in zobell marine agar culture media. Spread the water sample evenly with L rod and kept in incubator for 24 hours. Colonies are observed. Repeat this procedure for respective days.

6.3 TOTAL ORGANIC COMPOUND TEST:

Total Organic Carbon (TOC) measurement is commonly used to determine the degree of organic contamination in water. Total Organic Carbon (TOC) is an indirect measure of organic molecules present in water and measured as carbon.

- Coupon sample are taken in a vial
- It was measured by oxidizing organic compounds present in the form which can be quantified.
- Open sample table file then click connect icon, to achieve ready state it takes approximately 40 min.
- Once they are in ready state the LED light on front door is green.
- Place sample solutions onto autosampler turntable, key in vial number position.
- Click start to run the process.

$$\text{TOC} = \text{TC} - \text{IC}(\text{difference})$$

TOC (ppm)

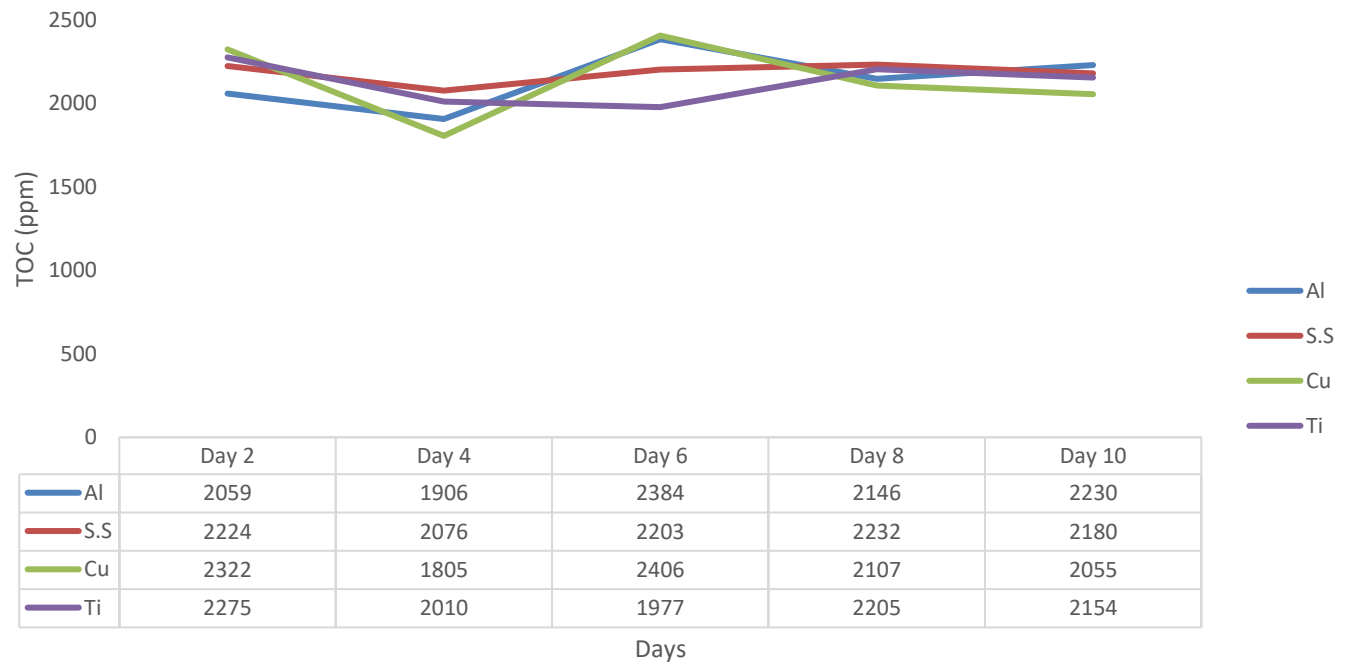


Fig:6 It represents the Total Organic Compounds in collected samples, zigzag was happened due to leaching metals in seawater

6.4 CHLOROPHYLL ESTIMATION:

- 2.5ml of sample taken
- Centrifuged at 4600rpm for 10min
- Collect pellets & discard supernatant
- To the pellet add 2.5ml of methanol

- Addition of methanol in pellet should be kept in waterbath for 5min at 60 degree celcius
- Again centrifugation done at 4600rpm for 10min
- Absorbance of sample read at 652nm and 665nm

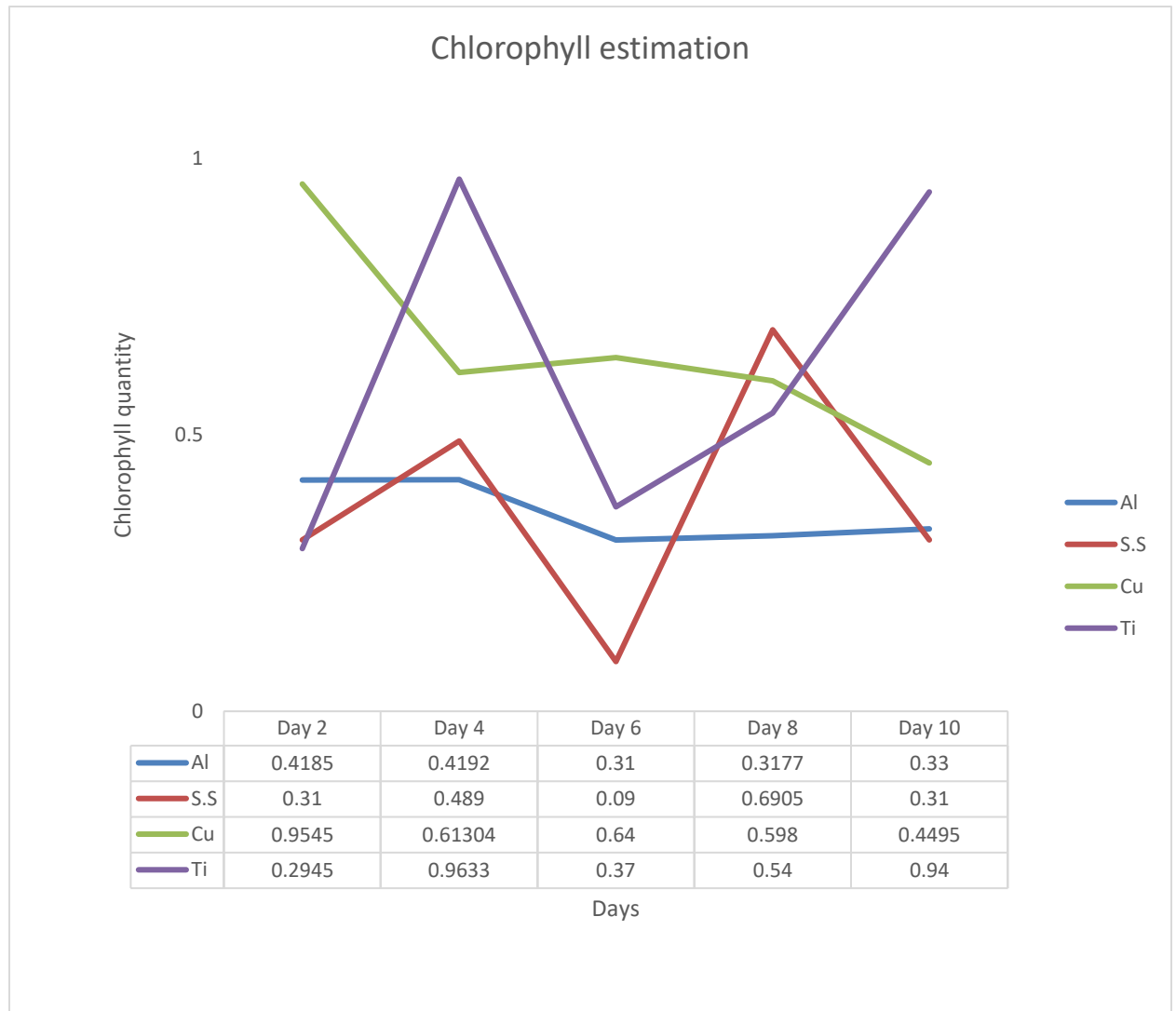


Fig7: Its represents the chlorophyll estimation of biofilm growing in waterbody

$$\text{Formula} = \text{chl}a = 16.26A_{665} - 8.54A_{652}$$

CHAPTER - 7

7.1 COATING OF METALS:

Mild steel is used here to check the leachates of nanoparticle in seawater. Totally 280 plates are coated out of that they are divided into control, positive control one (epoxy coating) and positive control two (epoxine coating) negative control (anti-fouling paint) and for experiment they are coated with nanoparticle. Here we use three different nanoparticles at three different concentrations (0.5%, 1% and 1.5%) for both Epoxy and Epoxine. Small coupons are immersed in a beaker containing seawater. They are taken out day on 10, 20, 30, 40, 50, 75 and 100. Samples are analyzed by ICP-MS.

7.2 ANALYSIS OF ICP-MS (Inductively Coupled Plasma Mass Spectrometry):

It is an elemental analysis technology capable of detecting most of the elements in the periodic table at milligram to nanogram levels per liter. ICP-MS is an analytical technique used to measure elements at trace levels in biological fluids. Three types of mass spectrometers used in commercial ICP-MS are single quadrupole, time of flight and magnetic sector. In this instrument helium and argon is the main source because Helium used as collision cell of ICP-MS system it will effectively eliminate wide range of polyatomic interferences through kinetic energy discrimination. Argon torch is used to atomize and ionize the sample which is introduced in aerosol or by laser ablation then argon gas significantly improves the plasma robustness that allows the direct analysis of high matrix samples such as undiluted seawater ICP-MS. Optimum performance for high IP analytes in some samples possible after addition of online addition of water and carbon. It detects the metals at very low concentration in high temperature source with Mass spectrometry. Argon gas has high temperature of >6000K. Plasma contains free electrons, positive ions, neutral atoms or molecules. Ions are separated by their mass/charge (m/z) signal intensities are directly proportional to concentration of elements in sample. The needed dilute acids are nitric acid, hydrochloric acid or alkali like ammonium hydroxide, Tetramethylammonium hydroxide. For solid samples the chemical

digestion is require digestion carried out by strong acids or alkali, dry heating block or block pressure microwave to assist digestion.

7.3 PREPARATION OF SAMPLE FOR ICP-MS:

- 1g of sample weighted and transferred to digestion tube(nitric acid and peroxide).
- 8millilitre of concentration nitric acid and 2millilitre of hype pure peroxide added to tubes under fume hood.
- Tubes closed tides and kept it in open for 24hours.
- Releasing pressure tgen closed tightly before digestion unit.

7.4 Digestion unit:

- Tubes are transferred to digestion unit .
- After digestion tubes removed from unit.
- One millilitre of digested samples transferred to sampling tubes by filter connected syringe.
- Digested samples are volumed upto 30millilitres with distilled water.
- Samples are placed in ICP-MS with use of blank and standard solutions.

7.5 STANDARD SOLUTION FOR ICP-MS:

- From standard solution of 10,000ppb we need 100ppb, from that 100ppb we have to made 5ppb,10ppb,15ppb,20ppb and 25ppb.
- For 100ppb sub stock, pipette 0.5ml of 10,000ppb standard solution transfer to 50ml vial.
- Fill the vial with deionized water or ultra pure water to 50ml and mark it with 5ppb sub stock(1% nitric acid).

CHAPTER - 8

RESULT AND DISUSSION:

Release rate:

8.1 Copper:

The average release rate between day 0 to 10 of exposure. For Cu it has two parameters affect the release rate: salinity and Cu content. Increased salinity also shows that increase the dissolution rate of CuO₂ particle as well as solubility and coating matrices results in increased release of copper from coating surface. No correlation between Cu content and Cu release. A recent studies found that an increased concentration of ZnO albeit when added to same based coatings formulation that increase the release of copper. Only two parameter salinity and Cu content, were found to be significant with resulting model able to account. Differences in paint matrix between the coating could account for variability.

8.2 Zinc:

The effect of salinity on the Zn release rates is not as clearcut as for Cu . Although the highest release rates are obtained at the highest salinity for some paints no significant linear regressions between salinity and Zn release rates could be established for any of the paints . For most the highest or second to highest release rates instead occur in freshwater, suggesting perhaps water parameters other than salinity may be influencing the release rate of Zn. Zn content as an explanatory variable was also not significant at

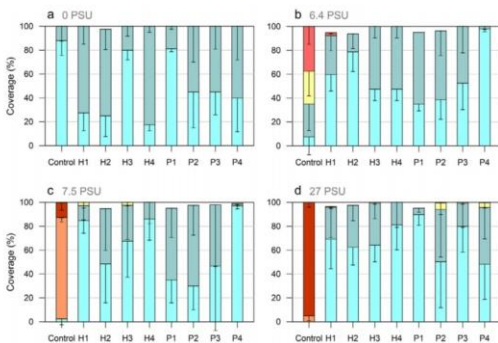
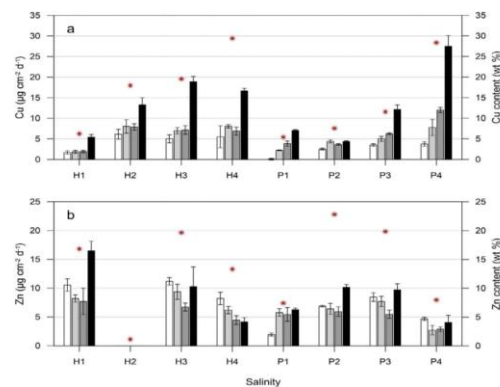


Fig: 7 Average coverage on control and coated panels with Epoxy and Epoxine coatings



any of the studied sites when considered

Fig:8 Average Cu and Zn leachates from epoxy and epoxine coated between 0 to 19 days of immersed study sites. Error bars shows that standard deviation for 3 panels. Zn release rates for panels were very low as no ZnO included in its formulation.

separately . Out of the five parameters tested for significance in a multiple regression model, three were found to be significant on the Zn release rate: paint type, Cu content and Zn content. The overall fit of the model is however poor with $r^2 = 0.540$. The prediction model of the Zn release rate is thus more uncertain than that of Cu, suggesting that unknown parameters have a greater influence on the release of Zn than that of Cu. These are most likely related to the specific composition of the paints and go beyond the simple distinction between hard or polishing.



Fig9 and 10 fig9 represents nanoparticle based antifouling coating which is Epoxy Fig10 represents without nanoparticle its only antifouling coating for control.

CHAPTER - 9

CONCLUSION:

The application of copper-containing antifouling paint in freshwater was concluded to be redundant as the products contributed no antifouling effect. Any release of copper in such environments thus only poses a risk to the environment without any beneficial gain for the boat owner. Although the use of biocidal antifouling paints, that is not the case for most EU countries. These member states may gain from performing risk/benefit analysis to assess whether the continued use of biocidal antifouling paints in freshwater is indeed necessary. For marine waters, the mapping of the critical Cu release rate revealed variations in the sensitivity of the fouling community in the Baltic Sea/Kattegat, with higher release rates required to deter macrofouling at higher salinities. However, as the release rate of Cu from antifouling paints also increases with

increased salinity, all the eight tested paints were found to be efficient regardless of location along the salinity gradient. In fact, the Cu release rates of a majority of the paints greatly exceeded the critical release rate. For some products, a reduction of up to 80% in the release rate of Cu would be possible without any loss in efficiency. The properties of these products are thus not in alignment with the principles of the BPR which requires the release of active substances to be the minimum necessary.

CHAPTER - 10

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