

STUDIES ON THE BIOFILM FORMATION BASED ON NANOPARTICLE BASED ANTIFOULING COATING

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in BIOTECHNOLOGY.

By

D.VIJAY (Register No.38230040)



**DEPARTMENT OF BIOTECHNOLOGY SCHOOL OF BIO
AND CHEMICAL ENGINEERING**

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 60119

MAY - 2022

DECLARATION

I D.VIJAY hereby declare that the Project Report entitled STUDIES ON THE BIOFILM FORMATION BASED ON NANOPARTICLE BASED ANTIFOULING done by me under the guidance of Dr.VALLI NACHIYAR (Internal) and Dr.INBAKANDAN (External) at COL.Dr.JEPPIAR RESEARCH PARK,SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in BIOTECHNOLOGY.

DATE:

PLACE:

SIGNATURE OF THE CANDIDATE



SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE
www.sathyabama.ac.in

DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **D.VIJAY (38230040)** who carried out the project entitled **Studies on the biofilm formation based on nanoparticle based antifouling coating** under my / our supervision from **20/12/2021** to **20/04/2022**.

Internal Guide
Dr.VALLI NACHIYAR
Associate professor

External Guide (if Applicable)
Dr.D.INBAKANDAN
Head of Centre for
Ocean research

Head of the Department (Name in Capital letters with Seal & Signature)
Dr.V.RAMESH KUMAR

Submitted for Viva voce Examination held on _____

INTERNAL EXAMINER

EXTERNAL EXAMINER

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr.V.RAMESH KUMAR** Head of the Department, Dept. of BIOTECHNOLOGY for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide **Dr.VALLI NACHIYAR** Associate professor, Sathyabama institute of science and technology, **Dr.INBAKANDAN** Scientist – E/Head of Centre for Ocean research **Col Dr Jeppiar Research Park Sathyabama Institute of Science and Technology** for his valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I wish to express my thanks to **Dr. P.Prakash**, Year Coordinator, all Teaching and Non-teaching staff members of the Department of BIOTECHNOLOGY who were helpful in many ways for the completion of the project.

ABSTRACT

Marine fouling is the major problem for the application of materials in marine environment. These man-made objects placed in the marine environment encounter fouling by marine organisms and biofilm formation on surfaces. On method of combating bio-fouling with the help of copper and Tri n butyltin (TBT) or by the use of biocides by using toxic antifouling coatings containing metal compound silver or surfactants. However, these coatings are not recommended for use by virtue of their potential damage to marine organisms causing environmental pollution problem. The use of nanoparticle-based metal oxide coatings represents a promising approach for development of non-toxic control technologies for macro fouling organisms by engineering the surface with either low surface energy coatings that minimize biological adhesion strength.

Ships and boats exposed to marine environment for a longer time develops biofilms on the surface and tend to corrode the surface. This will eventually affect the mobility of the ship. Painting the surface with anti-fouling coating based on nanoparticle will resist the formation of biofilms.

KEYWORDS: Biofouling, Antifouling, Macrofouling, Nanoparticles, Metal oxide coating

CONTENT

CHAPTER	PAGE NO	CONTENT
1	1	Introduction
2	3	Review of literature
3	7	Aim and Scope of the Project
4		EXPERIMENTS AND METHODS USED
	8	4.1 Exposure of metal coupons to sea water
		4.1.1 Tank setup
	9	4.2 Colony counting (small metal coupons)
	10	4.2.1 Colony counting (metal plates)
		4.2.2 Tank setup
	11	4.3 Biofilm sample collection
	11	4.4 Colony counting
	12	4.5 Total organic carbon
		4.5.1 Measurement of total organic carbon
	13	4.5.2 Total organic compound analysis
	14	4.6 Chlorophyll analysis
		4.6.1 Microplate measurement of Chlorophyll
	15	4.6.2 Microplate and Spectrophotometer measurements
		4.6.3 Calculation of chlorophyll concentration
	16	4.7 Nanoparticle based antifouling coating on metal surface
	17	4.7.1 Nanoparticle mixed with Epoxy and Pure Polyester powder coating
	21	4.7.2 Nanoparticle mixed with paint as solvent
	22	4.8 Contact angle measurement
	23	4.8.1 Surface tension
		4.8.2 Contact angle measurement
	26	4.9 Nanoparticle characterisation
	28	4.9.2 Ti (IV) Oxide powder

	28	4.9.3 Cupric oxide nanopowder
5	29	RESULTS AND DISCUSSIONS, PERFORMANCE ANALYSIS
	30	5.1 Cfu result and table
	31	5.2 TOC result
	32	5.3 Chlorophyll analysis
	34	5.4 Contact angle measurement
		5.5 Exposure of metal plates incorporated with nanoparticles
6	35	SUMMARY AND CONCLUSION

LIST OF FIGURES

FIGURE NO	TITLE	PAGE NO
1.1	Biofouling on hull part of the ship	2
1.2	Biofouling on sensor part of the ship	2
4.1	Metal coupons immersed in sea water	8
4.2	Metal coupons sonicated and water samples are inoculated	9
4.3	Metal plates immersed in sea water	10
4.4	Colonies are observation	11
4.5	Total organic carbon analyser	13
4.6	Microplate reader	14
4.7	96 Well plate	15
4.8	Berger antifouling paint	16
4.9	Epoxy powder	18
4.10	ZnO mixed with epoxy	18
4.11	CuO mixed with epoxy	18
4.12	TiO mixed with epoxy	18
4.13	Mixed nanoparticle with epoxy	18
4.14	Powder coating of plates	19
4.15	TiO coated mild steel plates	20
4.16	ZnO coated mild steel plates	20
4.17	CuO coated mild steel plates	20
4.18	Varying contact angle	22
4.19	Cross section image of the surface	22
4.20	Surface tension of droplet	23
4.21	Contact angle goniometer	25
4.22	Water droplet on hydrophobic Cu surface	25
4.23	ZnO nanopowder	26
4.24	Ti (IV) oxide nanopowder	27

4.25	CuO nanopowder	28
5.1	Water droplets on surface of different metals	33
5.2	Metals incorporated with nanoparticles	34

LIST OF TABLES

TAB NO	TITLE	PAGE NO
4.1	Nanocomposite coating with epoxy powder	17
4.2	Nanoparticle mixed with paint as solvent	21
5.1	Cfu table	29
5.2	Chlorophyll	31
5.3	Contact angle measurement	32

INTRODUCTION

Marine fouling is the major problem for the application of materials in marine environment. These man-made objects placed in the marine environment encounter fouling by marine organisms and biofilm formation on surfaces. One method of combating biofouling with the help of copper, zinc, titanium and other metal oxide nanoparticles and tri-n-butyltin (TBT) or by the use of biocides by using toxic antifouling coatings containing metal compound silver or surfactants. However, these coatings are not recommended for use by virtue of their potential damage to marine organisms causing environmental pollution problem.

The use of nanoparticle-based metal oxide coatings represents a promising approach for development of non-toxic control technologies for macrofouling organisms by engineering the surface with either low surface energy coatings that minimize biological adhesion strength.

Marine fouling is the major problem for the application of materials in marine environment. Studies on the environmental changes in the coastal ocean on timescales from minutes to decades can be achieved by the use of moorings deployed at multiple depths. These man-made objects placed in the marine environment encounter fouling by marine organisms and biofilm formation on surfaces. Progress in coastal ocean research will be enhanced with optical instrumentation that provides valuable tools for understanding the physical, chemical, biological and geological process in the coastal ocean. Most of the in situ water quality monitoring instruments and sensors made of glass need proper maintenance to ensure continued accuracy. The greatest disadvantage of these platforms is biofouling of the sensors. These moored buoy instruments are susceptible to fouling in the form of microbial and algal films that can cover optical windows and degrade data quality. The settlement of larvae of sessile invertebrates on the optical windows and their subsequent growth is also a common problem. Organisms such as barnacles, algae and bacteria that foul surfaces in aquatic environments are determined by interfacial interactions within the outermost, few nanometers of the surface and involve a wide range of bonding mechanisms.

Fouling in one form or other can occur in most locations, during all seasons, and the use of antifouling methods is essential to reduce the consequences for offshore installations.

One method of combating biofouling is by using toxic antifouling coatings containing metal compounds such as copper and tri-n-butyltin (TBT) or by the use of biocides like silver or surfactants. However, these coatings are not recommended for use by virtue of their potential damage to marine organisms causing environmental pollution problem. Even though organotin-based coatings are most efficient for long-life, due to the increased risks in the application of these toxic coatings, International Maritime Organization aims to completely remove tin-based paints from all ships around the world by 2008 in an effort to limit their use.



Fig: 1.1: Biofouling on hull part of the ship



Fig: 1.2: Biofouling on sensor part of the ship

LITERATURE SURVEY

Biofilm prevention and control are needed for spacecraft water storage and distribution systems for long duration exploration missions. One way of preventing biofilm formation involves the use of surface treatments. However, antifouling surfaces have a limited lifespan because microbes alter the surface properties and reduce their antifouling function over time. Surface treatment requires a maintenance strategy to extend the lifespan of the antifouling surface. This study aims to give an overview of state-of-the-art technologies for antifouling and maintenance methods that can be considered when developing Synergistic Biofilm Control (SyBiCo) method for spacecraft water systems.

Research Scientist, Amentum, Mail Code: LASSO-001, Kennedy Space Centre, FL, 32899.

Research Scientist, Amentum, Mail Code: LASSO-001, Kennedy Space Centre, FL, 32899.

R&D Engineer, The Bionetics Corporation, Mail Code: LASSO-008, Kennedy Space Centre, FL, 32899.

NASA, Senior Research Scientist, , Exploration Systems and Development Office, Mail Code: UB-E, Kennedy Space Centre, FL, 32899.

NASA, Water Technology Lead, Crew and Thermal Systems Division, 2101 NASA Parkway/EC3, Houston, Texas, 77058.

Biofouling is a major concern to the maritime industry. Biofouling increases fuel consumption, accelerates corrosion, clogs membranes and pipes, and reduces the buoyancy of marine installations, such as ships, platforms, and nets. While traditionally marine installations are protected by toxic biocide coatings, due to recent environmental concerns and legislation, novel nanomaterial-based anti-fouling coatings are being developed. Hybrid nanocomposites of organic-inorganic materials give a possibility to combine the characteristics of both groups of material generating opportunities to prevent biofouling.

¹Functional Materials, Department of Applied Physics, School of Engineering Sciences, KTH Royal Institute of Technology, Stockholm, Sweden

²Department of Food Engineering and Technology, Central Institute of Technology Kokrajhar, BTR, Assam, India

Shark skin surfaces show non-smoothness characteristics due to the presence of a riblet structure. In this study, biomimetic shark skin was prepared by using the polydimethylsiloxane (PDMS)-embedded elastomeric stamping (PEES) method. Scanning electron microscopy (SEM) was used to examine the surface microstructure and fine structure of shark skin and biomimetic shark skin. To analyze the hydrophobic mechanism of the shark skin surface microstructure, the effect of biomimetic shark skin surface microstructure on surface wettability was evaluated by recording water contact angle. Additionally, protein adhesion experiments and anti-algae adhesion performance testing experiments were used to investigate and evaluate the anti-biofouling properties of the surface microstructure of biomimetic shark skin. The recorded values of the water contact angle of differently micro structured surfaces revealed that specific microstructures have certain effects on surface wettability. The anti-biofouling properties of the biomimetic shark skin surface with microstructures were superior to a smooth surface using the same polymers as substrates. Moreover, the air layer fixed on the surface of the biomimetic shark skin was found to play a key role in their antibiont adhesion property. An experiment into drag reduction was also conducted. Based on the experimental results, the micro structured surface of the prepared biomimetic shark skin played a significant role in reducing drag. The maximum of drag reduction rate is 12.5%, which is higher than the corresponding maximum drag reduction rate of membrane material with a smooth surface.

We propose a new superhydrophobic surface that contains a carbon nanotube (CNT)-implanted poly (dimethylsiloxane) (PDMS)/adhesive multilayer. The adhesive provides very strong adhesion between the CNT-implanted PDMS layer and the substrate, and the CNTs on the surface exhibit superhydrophobicity. Therefore, the CNT-implanted PDMS/adhesive (CIPA) layer provides a highly reliable surface for superhydrophobicity. The fabricated CIPA surface performs far better than previously reported surfaces in terms of stability tests, such as contamination and solvent tests, and physical contact, including thermal pressure, bending, adhesion, and water jet tests. If a portion of the CIPA surface is destroyed, the surface is immediately restored because the material can regenerate the surface to its initial state. The surface can therefore maintain its superhydrophobicity even when damaged in rough environments, without self-healing or additional repair. Furthermore, because the adhesive is sprayed and coated on the surface of the substrate, a CIPA surface can be formed on three-dimensional shapes, including curved surfaces, and on various substrates.

Highly Reliable Superhydrophobic Surface with Carbon Nanotubes Immobilized on a PDMS/Adhesive Multilayer

Kyung Kuk, Jung Young, Jung Chang Jun Choi, and Jong Soo Ko

Bactericidal Activity of Superhydrophobic and Superhydrophilic Copper in Bacterial Dispersions

A method based on nanosecond laser processing was used to design superhydrophilic and superhydrophobic copper substrates. Three different protocols were used to analyze the evolution of the bactericidal activity of the copper substrates with different wettability. Scanning electron microscopy was used to study the variation of cell morphology after the attachment to superhydrophilic and superhydrophobic surfaces. The dispersions of *Escherichia coli* K12C600 and *Klebsiella pneumoniae* 811 in Luria Bertani broth in contact with the superhydrophilic copper surface showed enhanced bacterial inactivation, associated with toxic action of both hierarchically textured copper surface and high content of Cu^{2+} ions in the dispersion medium. In contrast, the bacterial dispersions in contact with the superhydrophobic copper substrates demonstrated an increase in cell concentration with time until the development of corrosion processes. The resistance of bacterial cells to Contact the copper substrates is discussed on the basis of surface forces, determining the primary adhesion and of the protective action of a superhydrophobic.

A method based on nanosecond laser treatment was used to design superhydrophobic and superhydrophilic aluminum alloy substrates showing enhanced cytotoxic activity with respect to *Escherichia coli* K12 C600 strain. It was shown that the survival of cells adhered to the superhydrophobic substrates was significantly affected by the presence of organic contaminants, which are ubiquitous in hospital practice and the food industry. The peculiarities of the texture also played a notable role in antibactericidal activity. It was found that the superhydrophilic surfaces had much higher toxicity than the superhydrophobic ones, which was explained by the mechanisms of adhesion of cells to the surface. Scanning electron microscopy and tomographic reconstruction of the adhered cells were used to study the variation of cell morphology after attachment to surfaces with different wettability. It was shown that the cytotoxicity of superhydrophobic surfaces could be significantly enhanced by using the combined antimicrobial action of bacteriophages and the superhydrophobicity of the objects.

The bactericidal activity of copper and copper alloys is well appreciated and was already exploited in medical practice in 19th century. However, despite of being an essential nutrient required by organisms to perform life functions, excess copper is extremely toxic and detrimental to health. Recent studies have shown that super hydrophobic surfaces have a significant antibacterial potential for reduction of nosocomial infections. At the same time, the prolonged contact with biological liquids may cause a degradation of the superhydrophobic copper surface and corrosion with increasing egress of toxic copper ions. These aspects are poorly studied so far. In this paper, we analyse the evolution of the properties of both the superhydrophobic copper surface and the suspension of *Escherichia coli* bacteria during their prolonged contact and study the impact of such contact on the bactericidal activity of the surface. It is shown that by controlling the corrosion resistance and the wettability of the superhydrophobic copper substrate, it becomes possible to sustain the bactericidal action of copper substrates for a long time, simultaneously avoiding the excessive corrosive degradation and release of copper ions in the environment.

AIM AND SCOPE OF THE PROJECT

AIM:

Development of new techniques like a metal oxide coating seems to be an effective method for combating fouling on sensor parts. Due to the increasing emphasis and importance of use of non-toxic materials as antifoulants, the main objective of the study is to monitor the antifouling properties of metal oxide nanoparticle coated metal coupons derived using commercial nanoparticle dispersions/sols, in a natural marine environment and to investigate the fouling organisms attached to these coated coupons submerged within the eutrophic zone where photosynthesis takes place.

OBJECTIVE:

Ships and boats exposed to marine environment for a longer time develops biofilms on the surface and tend to corrode the surface. This will eventually affect the mobility of the ship. Painting the surface with anti-fouling coating based on nanoparticle will resist the formation of biofilms and helps in mobility and low consumption of fuels which helps in depletion of greenhouse gas.

EXPERIMENTS AND METHODS USED

4.1 EXPOSURE OF METAL COUPONS TO SEA WATER:

Stainless steel, copper coupons and glass slide (used as control) are exposed to sea water and taken out on day 2, 3, 7, 9, 10 and samples are inoculated and colonies are observed.

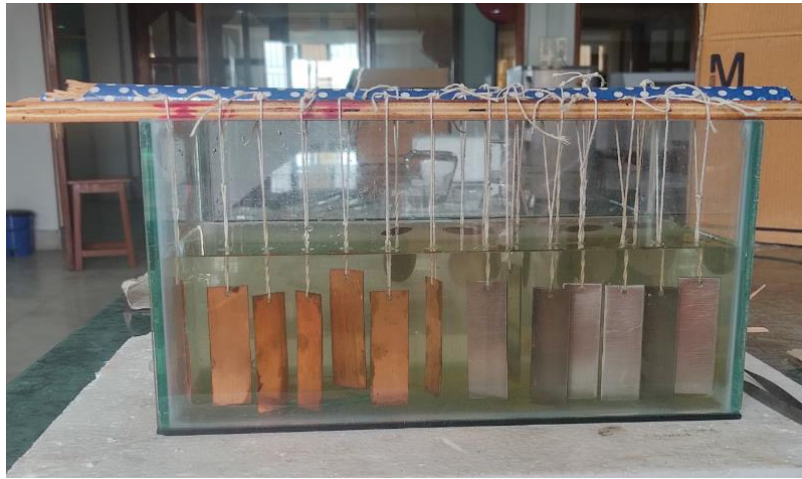


Fig: 4.1: Copper, titanium, stainless steel, glass slides (control) are immersed in sea water

4.1.1 TANK SETUP:

Medium size fish tank is washed properly and filled with sea water. Copper, titanium, stainless steel and glass slides (control) are hung with the help of thread and aeration is given via aerator. Sea water is changed for every 2 days with the help of outlet and inlet pipes without disturbing the setup.

4.2 COLONY COUNTING (SMALL METAL COUPONS):

- Samples are taken out and placed in petriplate containing sterile water.
- Sonicate the plate for same frequency and time.
- Collect the water sample and serial dilute the sample (10^{-3} to 10^{-6})
- Inoculate and count colonies on next day.
- Same procedure is followed for day 2, 3, 7, 9, 10.
- 9 plates are inoculated 5 plates + 1 control
- Zobell marine agar and agar powder are used in media.
- Inoculation is done in sterile laminar air flow chamber.
- Incubated in 34 degree Celsius and taken out on next day and colonies are counted.

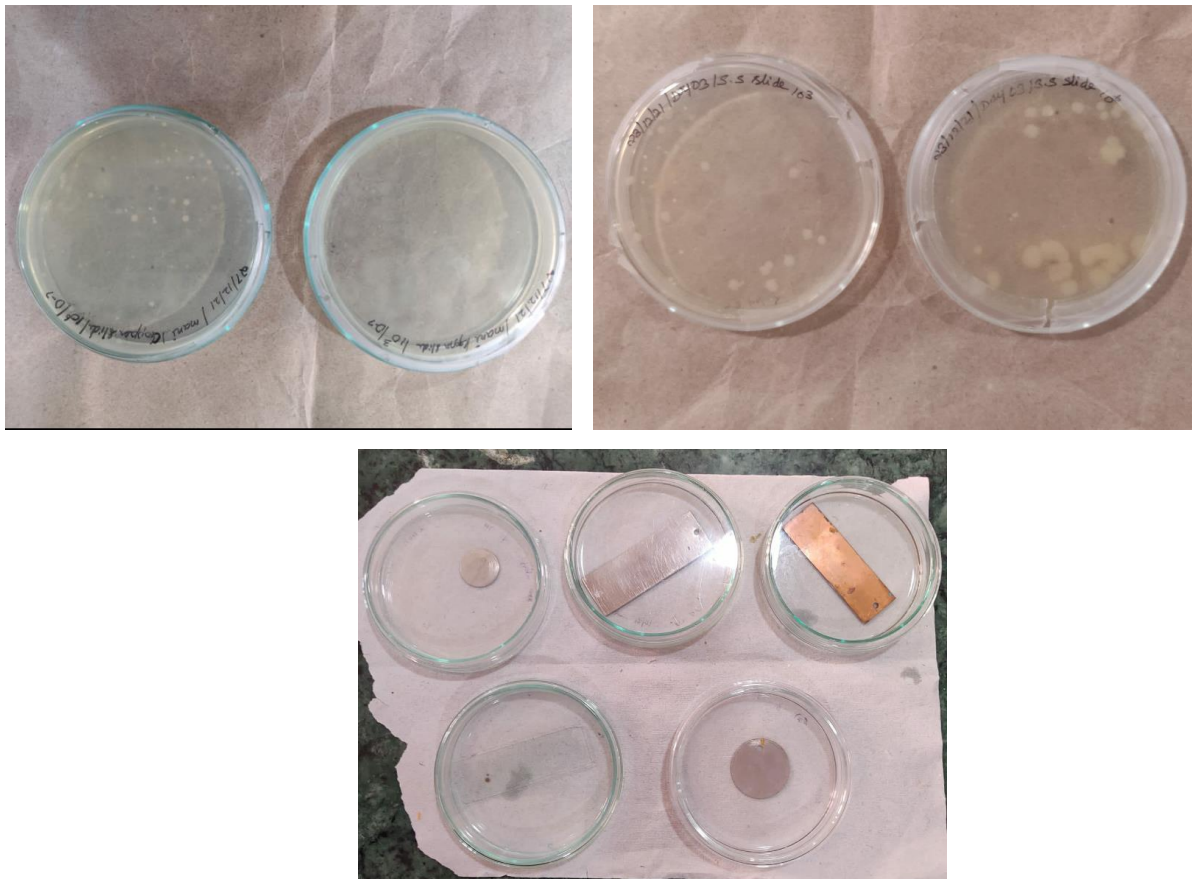


Fig: 4.2: Metal coupons are sonicated in sterile water and the water sample is collected and inoculated using spread plate method.

4.2.1 COLONY COUNTING (METAL PLATES):

Stainless steel, copper and titanium, each metal with 5 pieces are exposed to sea water and taken out on day 2, 3, 7, 9, 10 and samples are inoculated and colonies are observed.

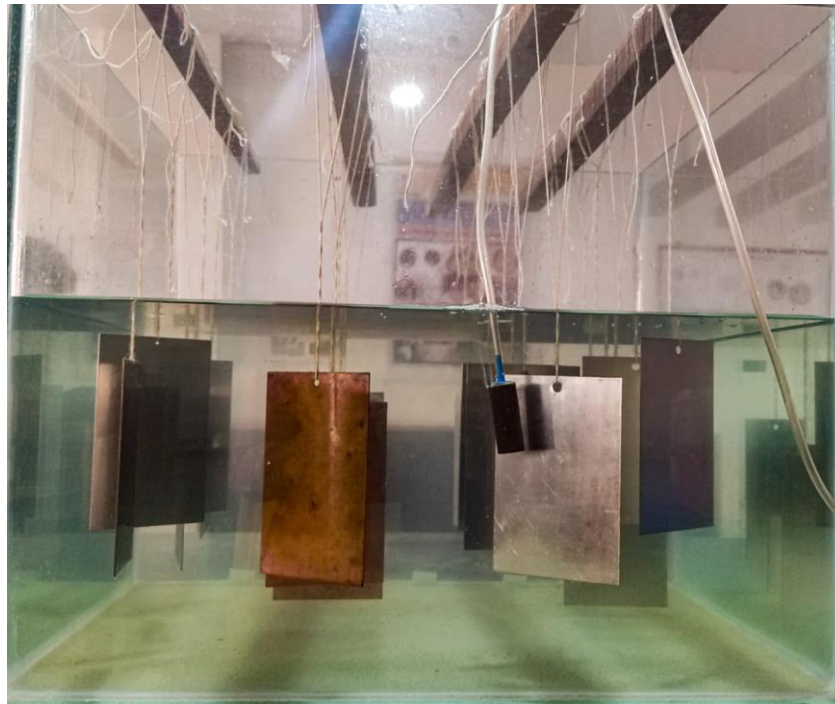


Fig: 4.3: Copper, Stainless steel, Titanium plates are immersed in sea water

4.2.2 TANK SETUP:

- A large fish tank is filled with sea water.
- Aeration facility should be provided with the help of aerator.
- Al, Cu, Ti, Stainless steel plates(each 5 plates) are immersed fully in sea water and observed 10 days for activity.
- Each plates should not be on contact and water should be changed for every 2 days.
- Plates are taken out on day 2, 4, 6, 8, 10 for CFU, TOC, Chlorophyll analysis tests.

4.3 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.

4.4 COLONY COUNTING:

- Collected water sample is diluted and transferred in 1ml eppendorf tube.
- 100 micro liter 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.

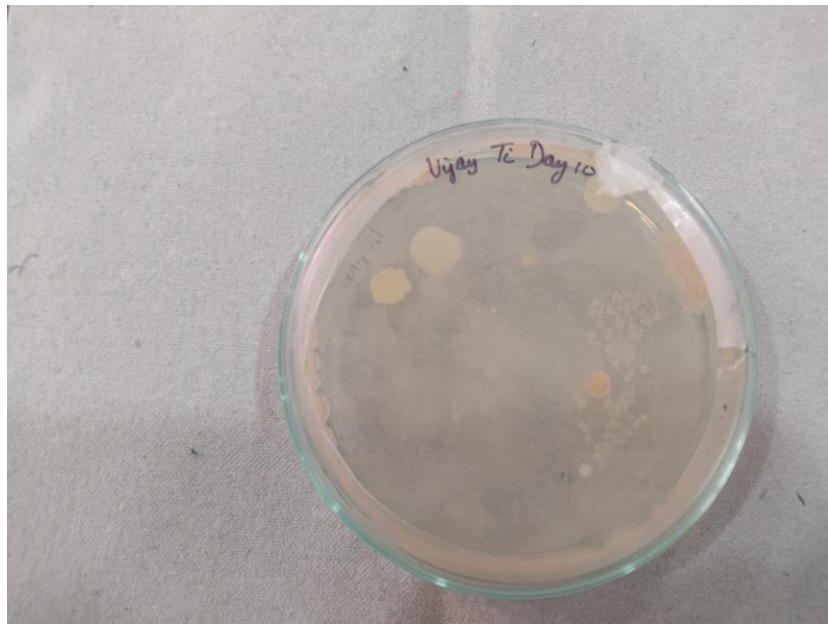


Fig: 4.4: Colonies are observed on day 10

4.5 TOTAL ORGANIC CARBON:

Total Organic Carbon (TOC) is a measure of the total amount of carbon in organic compounds in pure water and aqueous systems. TOC is a valued, analytical technique that is applied by organizations and labs to determine how suitable a solution is for their processes.

TOC has become an important parameter used to monitor overall levels of organic compounds present. This has happened despite the lack of any direct quantitative correlation between total organic carbon and the total concentration of organic compounds present and reflects the importance of having an easy-to-measure, general indicator of the approximate level of organic contamination.

It also reflects the appeal of a parameter which has a name which sounds more fundamental than it is! In many cases, the TOC is used as an on-going monitor of change or lack of change in organic content.

4.5.1 MEASUREMENT OF TOTAL ORGANIC CARBON:

TOC is measured at very different concentrations in a very wide range of systems. The table below gives an indication of Total Organic Carbon levels in various types of water. Levels vary widely within each type, but, broadly, they range from sub-ppb levels in ultra-pure water for laboratory and microelectronic applications up to hundreds of ppm in effluents and process streams.

For many of these systems, the TOC alone does not provide enough information. The carbon-containing compounds can be present in different forms and the proportions of each can be critical.

Dissolved organic carbon (DOC) is generally taken as that which will pass through a 0.45µm filter. Large particle size TOC is classified as particulate or non-dissolved (NDOC). About 50 to 75% of DOC in natural waters is in the form of polymeric organic acids - fulvic and humic acids.

4.5.2 TOTAL ORGANIC COMPOUND ANALYSIS:

- Water sample collected by scrubbing the metal plates exposed to sea water are transferred in a 15ml falcon tube.
- Before running TOC water wash is done with the help of filtered water via inlet hose available in TOC.
- For each samples the inlet hose is placed inside the tube and the sample is transferred for analysis.



Fig: 4.5: Total organic carbon analyser

4.6 CHLOROPHYLL ANALYSIS:

- Water sample is collected in a 1ml eppendorf tube and centrifuged at 5000rpm for 3 minutes.
- Later supernatant is discarded and washed with methanol and undergone water bath for 10 minutes.
- Again centrifuged at 5000rpm for 5 minutes.
- Sample is observed for UV absorbance in microplate reader.

4.6.1 MICROPLATE MEASUREMENT OF CHLOROPHYLLS:

Path length in spectrophotometers has been standardised to 1 cm and thus spectrophotometric equations for estimating chlorophyll implicitly assume a 1 cm path length. If absorbance of a chlorophyll solution is measured in a device with a path length different to 1 cm (e.g., in a microplate reader), then the standard spectrophotometric equations can only be used if they are corrected to 1 cm. The procedure is based on correcting for differences in path length so as to directly translate any published equation developed on a spectrophotometer to any format microplate with any sample volume.



Fig: 4.6: Microplate reader

4.6.2 Microplate and Spectrophotometer Measurements:

Microplate measurements were made by pipetting 200 μ L of sample (or blank) into a 96-well flat bottom polystyrene plate, which was then read with a monochromator-based microplate reader with 2.4 nm bandwidth. Microplate measurements were generally made with the lid on the microplate so as to reduce evaporation. Spectrophotometer measurements were made by transferring 200 μ L of sample (or blank) into a 1-cm path length quartz cell and reading absorbance in a spectrophotometer with 1 nm bandwidth.

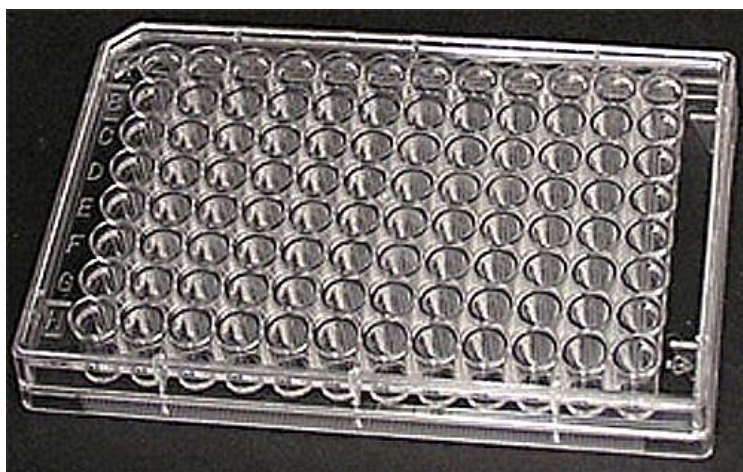


Fig: 4.7: 96 well plate

4.6.3 Calculation of Chlorophyll Concentration:

The absorbance of 200 μ L of sample in a microplate (A₆₅₂, microplate A₆₆₅, microplate) was converted into a 1-cm path length corrected absorbance using the measured path length:

$$A_{652, 1 \text{ cm}} = (A_{652, \text{microplate}} - \text{blank}) / \text{path length}$$

$$A_{665, 1 \text{ cm}} = (A_{665, \text{microplate}} - \text{blank}) / \text{path length}$$

Chlorophyll concentration was calculated from 1-cm corrected path length:

$$\text{Chl a } (\mu\text{g/ml}) = -8.0962 A_{652, 1 \text{ cm}} + 16.5169 A_{665, 1 \text{ cm}}.$$

$$\text{Chl b } (\mu\text{g/ml}) = 27.4405 A_{652, 1 \text{ cm}} - 12.1688 A_{665, 1 \text{ cm}}.$$

4.7 Ti, Zn, CU NANOPARTICLE BASED ANTIFOULING COATING ON MILD STEEL, STAINLESS STEEL, TITANIUM PLATES:

Stainless steel, Titanium, Mild steel plates are coated with Zinc, Copper, Titanium and mixed equal concentration of Zn, Ti, Cu nanoparticles with paint as solvent and epoxy and pure polyester powder coating.

140 mild steel plates are powder coated with Zn, Ti, Cu and mixed equal concentration of Zn, Ti, Cu nanoparticles(three different concentration for each a set of 16 plates) incorporated in epoxy powder and sprayed over the surface with high pressure spraying gun.

Another set of 140 mild steel plates are powder coated with Zn, Ti, Cu and mixed equal concentration of Zn, Ti, Cu nanoparticles(three different concentration for each a set of 16 plates) incorporated in pure polyester powder and sprayed over the surface with high pressure spraying gun.

48 titanium plates, 48 stainless steel plates are coated with Zn, Ti, Cu nanoparticles mixed in Asian apoclite paints with three different concentration.

Berger marine antifouling paint is used as positive control and for comparative studies.29 mild steel plates are coated with Berger marine antifouling paint and 4 titanium plates and 4 stainless steel plates are painted with Berger antifouling paint for paint based nanoparticle incorporated antifouling paint.



Fig: 4.8: Berger antifouling paint

4.7.1 NANOPARTICLE MIXED WITH EPOXY AND PURE POLYESTER POWDER COATING:

Tab: 4.1: Nanocomposite coating with epoxy powder

	Quantity of Epoxy resin	Quantity of Nanoparticle	Conc.
Control	-	-	-
Positive control	50g	50g of epoxy resin	-
Negative control	50ml	50ml of Antifouling paint	-
Experiment			
Cu	50g	0.5g of NP/50g of epoxy resin	10mg/g
	50g	1g of NP/50g of epoxy resin	20mg/g
	50g	1.5g of NP/50g of epoxy resin	30mg/g
Zn	50g	0.5g of NP/50g of epoxy resin	10mg/g
	50g	1g of NP/50g of epoxy resin	20mg/g
	50g	1.5g of NP/50g of epoxy resin	30mg/g
Ti	50g	0.5g of NP/50g of epoxy resin	10mg/g
	50g	1g of NP/50g of epoxy resin	20mg/g
	50g	1.5g of NP/50g of epoxy resin	30mg/g
Cu+ Zn+ Ti	50g	0.5g of NP/50g of epoxy resin	10mg/g
	50g	1g of NP/50g of epoxy resin	20mg/g
	50g	1.5g of NP/50g of epoxy resin	30mg/g

140 mild steel plates are powder coated with Zn, Ti, Cu and mixed equal concentration of Zn, Ti, Cu nanoparticles (three different concentration for each a set of 16 plates) incorporated in epoxy powder and sprayed over the surface with high pressure spraying gun.



Fig: 4.9: Epoxy powder



Fig: 4.10: ZnO mixed with epoxy



Fig: 4.11: CuO mixed with epoxy



Fig: 4.12: TiO mixed with epoxy



Fig: 4.13: TiO+ZnO+CuO mixed with epoxy



Fig: 4.14: Powder coating of nanoparticles mixed with epoxy on mild steel surface with high pressure spray gun

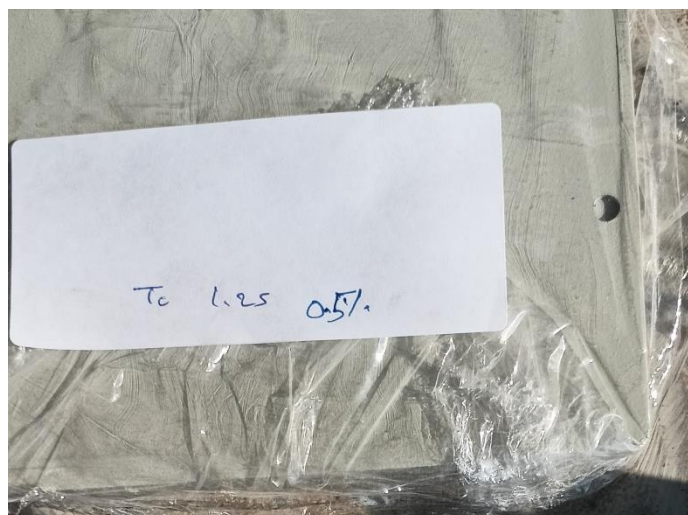


Fig: 4.15: TiO coated mild steel plate

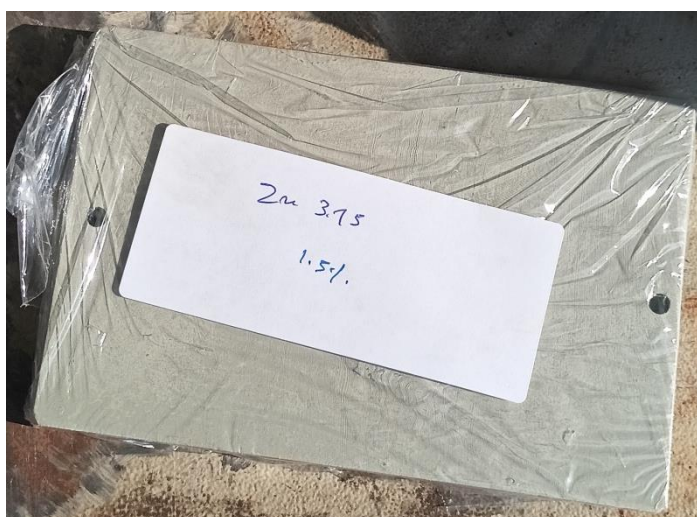


Fig: 4.16: ZnO coated mild steel plate

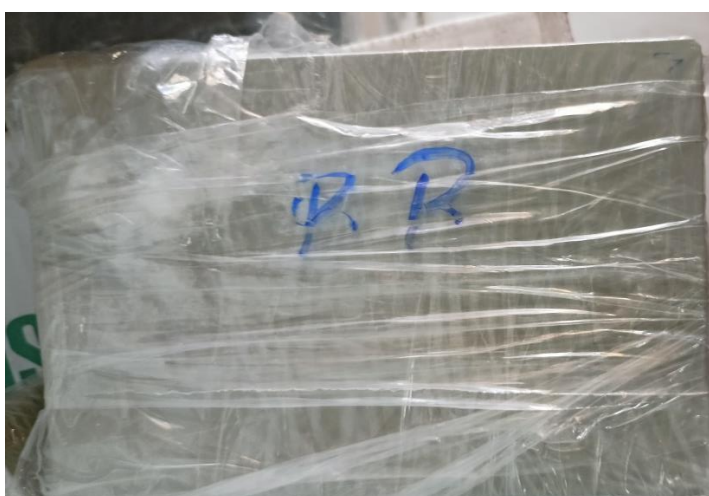


Fig: 4.17: CuO coated mild steel plate

4.7.2 NANOPARTICLE MIXED WITH PAINT AS SOLVENT:

	Quantity of paint	Quantity of Np	Conc.
Control	-	-	-
Positive control	50ml	50ml of paint	-
Negative control	50ml	50ml of Antifouling paint	-
Experiment			
Cu	50 ml	0.5g of NP/50ml of paint	10mg/ml
	50 ml	1g of NP/50ml of paint	20mg/ml
	50 ml	1.5g of NP/50ml of paint	30mg/ml
Zn	50 ml	0.5g of NP/50ml of paint	10mg/ml
	50 ml	1g of NP/50ml of paint	20mg/ml
	50 ml	1.5g of NP/50ml of paint	30mg/ml
Ti	50 ml	0.5g of NP/50ml of paint	10mg/ml
	50 ml	1g of NP/50ml of paint	20mg/ml
	50 ml	1.5g of NP/50ml of paint	30mg/ml
Cu+Zn+Ti	50 ml	0.5g of NP/50ml of paint	10mg/ml
	50 ml	1g of NP/50ml of paint	20mg/ml
	50 ml	1.5g of NP/50ml of paint	30mg/ml

48 titanium plates, 48 stainless steel plates are coated with Zn, Ti, Cu nanoparticles mixed in Asian apoclite paints with three different concentration.

4.8 CONTACT ANGLE MEASUREMENT:

A contact angle goniometer is an instrument that measures the contact angle of a droplet on a surface. This is a useful, indirect measurement of surface wetting. When a droplet starts to spread out over a surface, its contact angle becomes $<90^\circ$. Complete wetting occurs when the droplet is flat, or in other words, when the droplet has a contact angle of 0° . By suspending a droplet from the tip of a needle and comparing droplet models to the image captured by the system, contact angle goniometers can also be used to perform optical tensiometry measurements.

A contact angle gives us an indication of how well (or how poorly) a liquid will spread over a surface. While formulating an ink, contact angles provide a useful indicator of how a modification to the ink will affect its spreading.

A contact angle can be large or small, depending on the physical properties of the materials being investigated. Figure 1 shows three different droplets on a surface. The left-most droplet has a large contact angle, as it does not spread over the solid surface. The right-most droplet has a low contact angle, as it has spread well. This spreading is known as ‘wetting’, and a droplet either ‘wets’ or ‘dewets’ when deposited on a surface.



Fig: 4.18: A varying contact angle

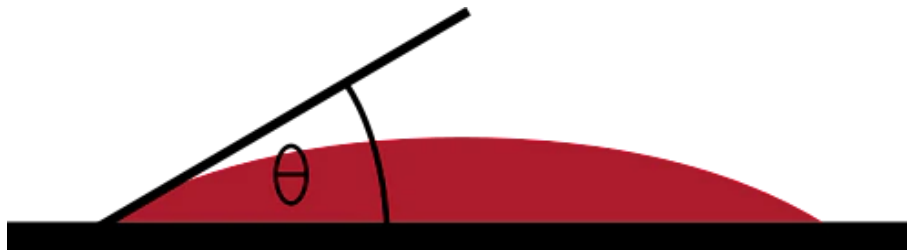


Fig: 4.19: 2D cross-section of a droplet on a solid surface. Locate the point at which the droplet outline intersects the solid surface. The angle between the droplet outline and the solid surface is the contact angle.

4.8.1 Surface Tension:

The surface tension of a droplet is determined by the interactions between its constituent molecules. In the bulk of the droplet, intermolecular forces act upon a molecule from all sides equally. However, at the surface of the droplet, there is an absence of liquid molecules on the external side.

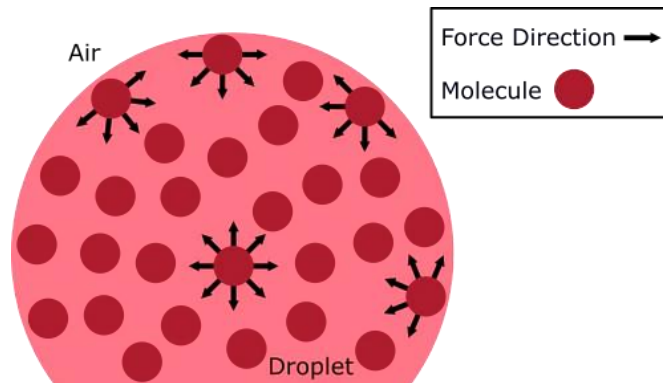


Fig: 4.20: Surface tension of a droplet

The molecules at the surface are more strongly bound to each other than the molecules in the bulk, as they are not being pulled from all sides. This means that it is more difficult for an object to penetrate the surface than it is for an object to move within the bulk once submerged.

4.8.2 MEASUREMENT:

The measurement process is as follows:

- The stage is flattened, so that the droplet does not move during deposition.
- A droplet of liquid is deposited on to the stage.
- The droplet is illuminated from behind, and an image is recorded by the camera.
- The image is analysed using code or software, and a contact angle measurement is determined.

Image Size

Importantly, the image must contain the entire droplet; you can't measure the contact angle if you can't see the edge.

Clarity

You need to be able to see the droplet clearly. The better the clarity of the edges, the more accurately you will be able to measure the contact angle. A 'fuzzy' droplet edge will make it harder to see both the slope of the droplet and the place at which it meets the solid surface.

Clarity can be affected by factors such as camera resolution, image magnification, light levels, and contrast with the background.

Camera resolution

Camera resolution determines how many pixels represent the droplet edge. If an image is highly pixelated, it is hard to determine where the edge of the droplet is.

Magnification

Image magnification has an impact on resolution. The larger an optical image is, the more pixels it will cover and the higher resolution the image will be.

Contrast

Contrast is important for finding the droplet edge. If there is low contrast, then it will be hard to separate the droplet from its background. If you are using an edge-detection technique, contrast will determine detection accuracy.

Lighting

Light levels can also affect the measurement. You must be able to see the droplet to take a measurement. If the lighting is too light or too dark, the contrast of the droplet and background can be affected.

Depth of Field

You want the entire depth of the droplet to be in focus, otherwise it will be difficult to find the baseline through the blurry front of the droplet. This can be achieved by altering the 'depth of field' of the imaging system. The actual focal point of a lens is a specific distance from it. However, there is an area either side of the focal point which you consider to be in focus. This is known as the 'depth of field'. The depth of field can be altered by using an aperture.



Fig: 4.21: Contact angle goniometer

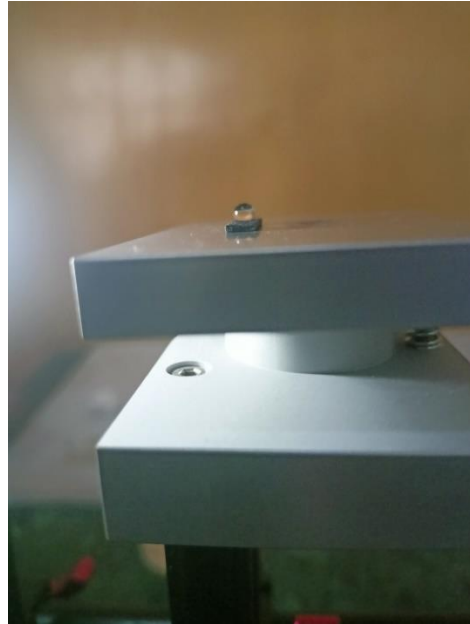


Fig: 4.22: Water droplet on hydrophobic Cu surface

4.9 Nanoparticle characterization:

4.9.1 ZnO:

Zinc oxide nanoparticles (ZnO-NPs) were synthesized via a solvothermal method in triethanolamine (TEA) media. TEA was utilized as a polymer agent to terminate the growth of ZnO-NPs. The ZnO-NPs were characterized by a number of techniques, including X-ray diffraction analysis, transition electron microscopy, and field emission electron microscopy. The ZnO-NPs prepared by the solvothermal process at 150°C for 18 hours exhibited a hexagonal (wurtzite) structure, with a crystalline size of 33 ± 2 nm, and particle size of 48 ± 7 nm. The results confirm that TEA is a suitable polymer agent to prepare homogenous ZnO-NPs.

Zinc oxide (ZnO) nanopowder is a metal oxide that is an n-type direct band semiconducting material with a band gap of 3.37 eV. Its properties include high chemical stability, photo stability, and high electrochemical coupling coefficient. It is classified as a semiconductor in group II-VI with bond energy of 60 meV, and high thermo-mechanical stability at ambient temperatures.



Fig: 4.23: Zinc oxide nanopowder <100nm particle size

4.9.2 Ti(IV) Oxide nanopowder:

Quality level:100

Assay: $\leq 99.95\%$ trace metal basis

Form: Nanopowder

Primary particle size: 21nm (TEM)

Surface area: 35-65 m²/g (BET)

Density: 4.26 g/mL at 25 °C



Fig: 4.24: Titanium(IV) oxide nanopowder

4.9.3 Cupric oxide nanopowder:

Copper oxides (Cu_2O , CuO) are p-type semiconductor materials with small band gap energy. High physical and chemical stability of metal oxide nanoparticles renders them extremely useful in catalytic applications. The structures of the compounds are monoclinic. Nanoscaled copper oxide compounds can be prepared by thermal plasma technology. A study reports its antimicrobial properties.

Quality level:100

Appearance: Black

Form: Powder

Particle size: <50 nm(TEM)

Surface area: $29 \text{ m}^2/\text{g}$

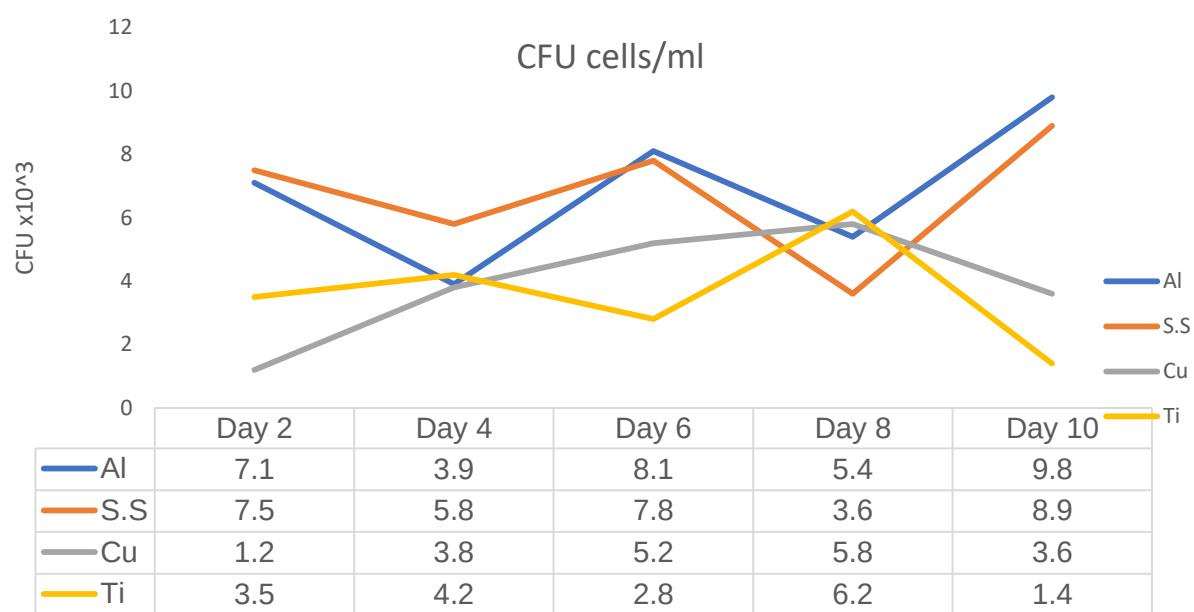


Fig: 4.25: Copper (II) oxide Nano powder

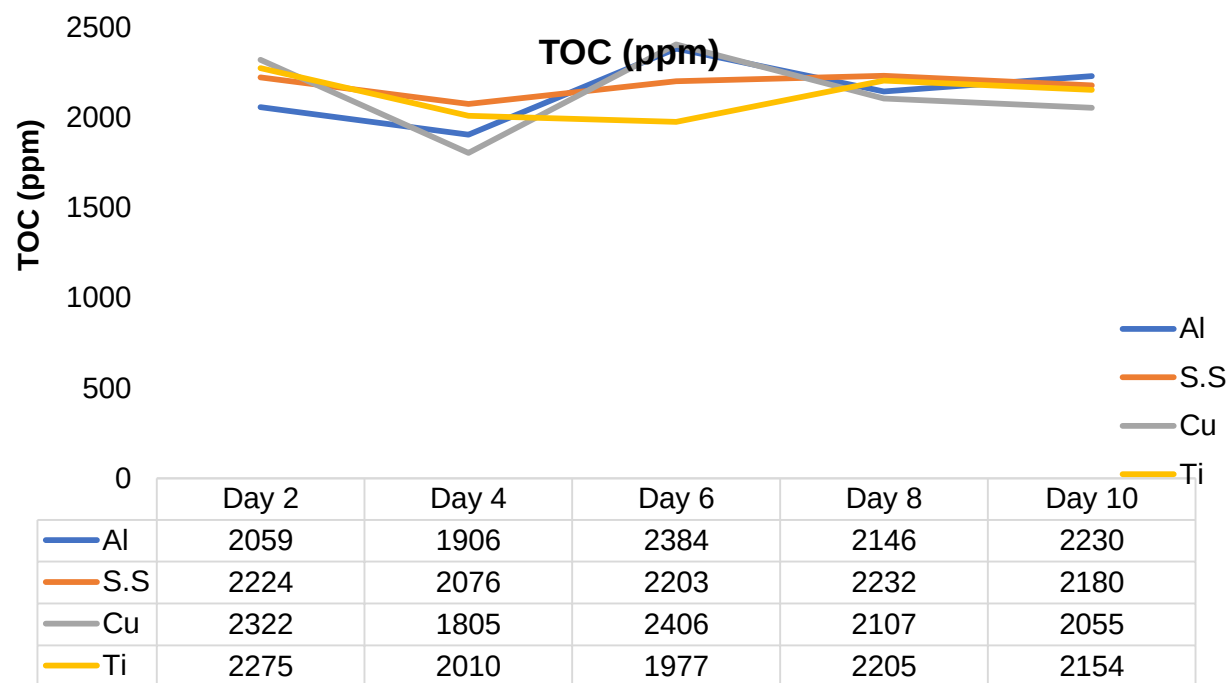
RESULTS AND DISCUSSIONS, PERFORMANCE ANALYSIS

5.1 CFU RESULT AND TABLE:

METAL	DAY 2 cfu/ml	DAY 4 Cfu/ml	DAY 6 Cfu/ml	DAY 8 Cfu/ml	DAY 10 Cfu/ml
Al	7.1×10^{-3}	3.9×10^{-2}	8.1×10^{-2}	5.4×10^{-2}	9.8×10^{-2}
Stainless Steel	7.5×10^{-3}	5.8×10^{-2}	7.8×10^{-2}	3.6×10^{-2}	8.9×10^{-2}
Ti	3.5×10^{-3}	4.2×10^{-2}	2.8×10^{-2}	6.2×10^{-2}	1.4×10^{-2}
Cu	1.2×10^{-3}	3.8×10^{-2}	5.2×10^{-2}	5.5×10^{-2}	3.6×10^{-2}

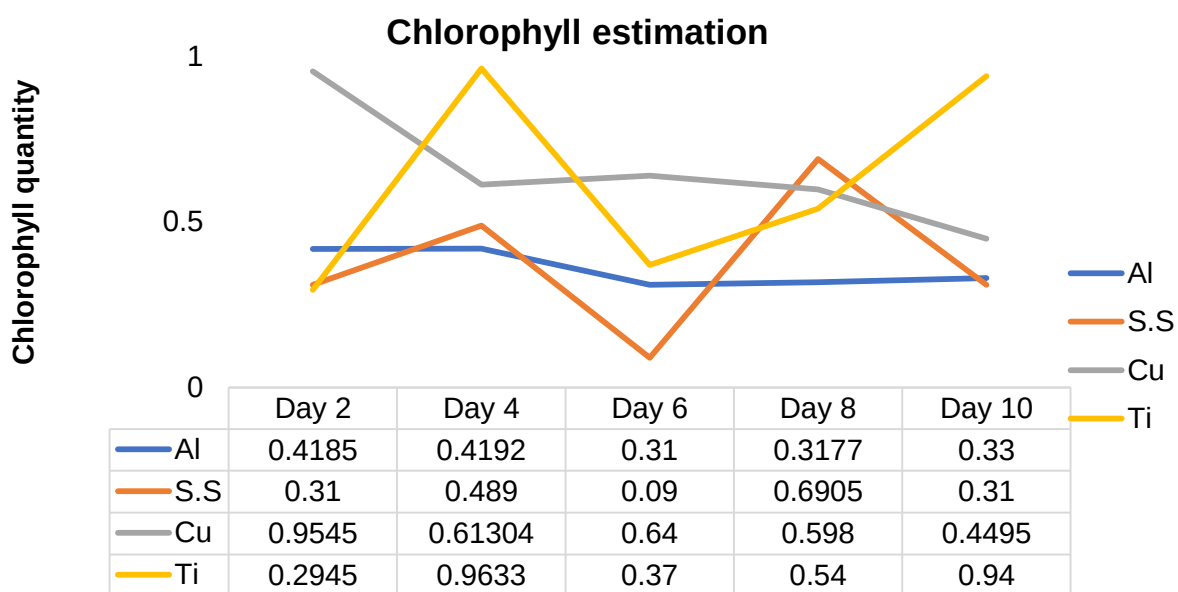


5.2 TOC RESULT:



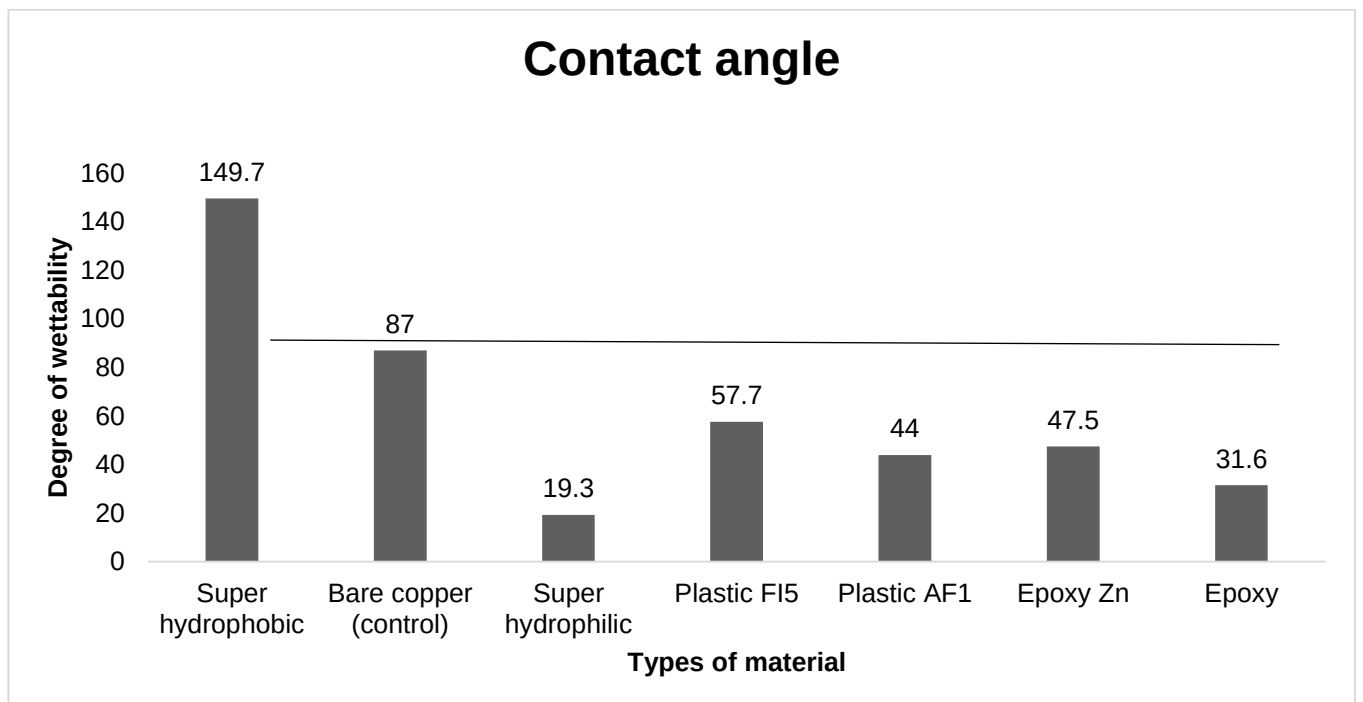
5.3 CHLOROPHYLL ANALYSIS:

METAL	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10
Al	0.4185	0.41929	0.31	0.31775	0.3325
Stainless steel	0.31	0.48904	0.09	0.69054	0.31
Cu	0.9545	0.61304	0.64	3.26685	0.4495
Ti	0.2945	0.96337	0.37	0.5425	3.8054



5.4 CONTACT ANGLE MEASUREMENT:

Type of Material	Degree of wettability
Stage surface	59.1
Bare copper	87
Super hydrophobic copper	149.7
Super hydrophilic copper	19.3
Plastic FI5	57.7
Plastic AF1	44
MS Epoxy Zn	47.5
MS Epoxy	31.6



Graph depicting the contact angle as degree of wettability of the water droplet (y axis) on the surface of different materials (x axis).

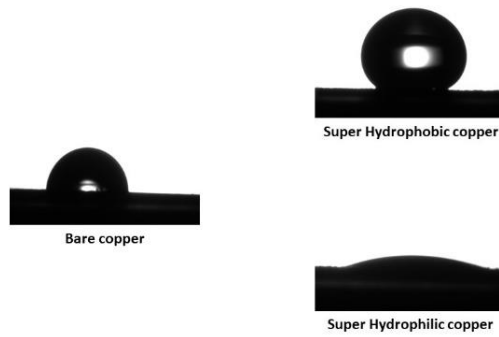
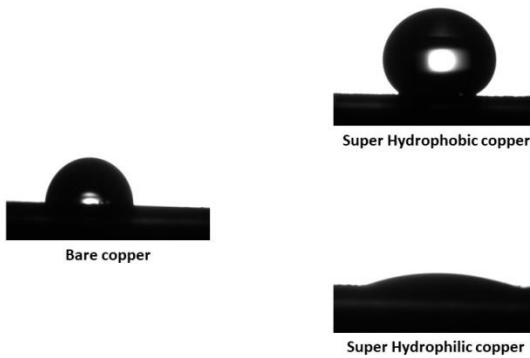


Fig: 5.1: Water droplets on surface of different materials



Super hydrophobic copper exhibits excellent resistance against water adhesion when compared to normal bare copper surface.

5.5 EXPOSURE OF METALS INCORPORATED WITH NANOPARTICLES:

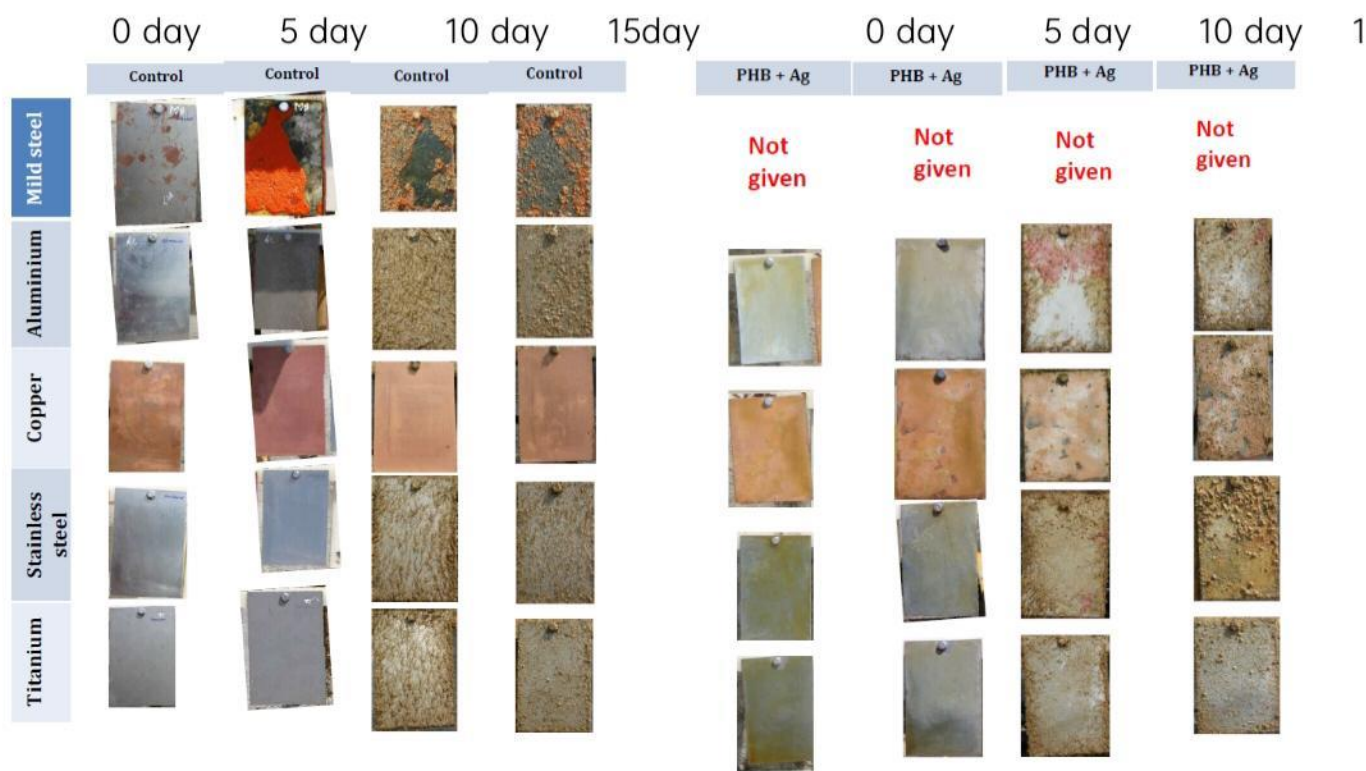


Fig: 5.2: Metals incorporated with nanoparticles

Copper and titanium nanoparticle incorporated plates shows excellent biofouling resistance.

6. SUMMARY AND CONCLUSION:

Biofouling is a major problem for maritime industries constantly affecting marine installations. Traditional antifouling methods are mainly based on the applications of biocide agents that detract or kill organisms close to vicinity, which is apparently not environment friendly. This review summarized polymeric and polymer-inorganic composite alternatives to toxic antifouling paints that show marine antifouling effects via fouling resistant, release, or degrading mechanisms. For polymer and hydrogel antifouling coatings, surface chemistry and coating techniques often determine the antifouling performance. For inorganic-polymer nanocomposite, the intrinsic characteristics of inorganic nanoparticles are responsible for fouling degradation. In photocatalytic nanostructure-based antifouling applications, ROS generated by photocatalytic nanostructures in presence of light can kill /inactivate micro- and macro-organisms. Compared to toxic biocides, the generated ROS are short-lived and only affect organisms that are in close proximity of the coatings. Thus, photocatalytic nanocoatings are less toxic to aquatic organisms compared to traditional antifouling coatings. Metal oxide nanostructure-based coatings are being considered as one of the promising environment-friendly antifouling technologies strategies that are suitable for marine applications. In contrast, only a few studies that reported inhibition of macro-fouling in the field conditions have been conducted. This could be due to the deteriorated efficiency of nanocomposites antifouling coatings in the field or the difficulties in scaling up such coatings.

An ideal antifouling coating is expected to be durable, reliable, easily applicable, cost-effective, eco-friendly, and substrate-independent. It remains very challenging to realize all the desirable requirements simultaneously. No single chemistry/strategy has yet been identified as the universal antifouling method. Instead, one multifunctional coating with synergetic strengths by combining several antifouling strategies is probably the correct way reaching the goal of a successful antifouling coating. With the large amount of high-quality and sophisticated research that is being performed, zwitterionic polymers attract much attention in the development of next-generation antifouling materials, due to the simplicity of synthesis, ease of applicability, abundancy of raw materials and availability of functional groups. At the same time, inorganic nanomaterials possess great advantage in preparation and application for antifouling. Therefore, the inorganic-polymer composite coatings represent another potentially commercial solution, especially considering their effectiveness and low cost. However, it is still necessary to develop

new polymers or composite to improve the antifouling performance, especially focusing on long-term durability when used in both static and dynamic environments. Apart from the efficiency enhancement, coating techniques also need to be developed in order to successfully translate these coatings toward large-scale applications to find their way outside the lab. Hence, the frequently used chemical grafting approaches need to be simplified, in order to cope with the physisorption techniques for coating, often by layer-by-layer or spray-painting. Combining simple coating techniques with a self-replenishing antifouling character to enhance antifouling durability and crosslinkers or catch-bonds to enhance mechanical stability, could result in technologically mature antifouling coatings with large-scale applicability in real maritime environmental conditions and on different materials such as vessels, fishing nets, and pipes. Finally, the impacts of environmental toxicity of such coatings should be more thoroughly investigated.

REFERENCE

- [1] C.G Munger, Corrosion prevention by Protective Coatings, National Association of corrosion Engineers, Houston TX, 1984.
- [2] Pachareentavita Muhammed, Nalini manju, Impact assessment of biofouling resistant nano copper oxide- polyaniline coating on aquaculture cage nets
- [3] Marie, Erik, Antifouling paints leach copper in excess – study of metal release rates and efficacy along salinity gradient.
- [4] Yebra and weinell, Key issues in the formulation of marine antifouling paints.
- [5] Jaleh, Tim and Vicki, Biofouling performance of silver based PES ultrafiltration membranes.
- [6] Krzyztof and Tadeusz, Epoxy coatings with modified montmorillonites.
- [7] QI Zhang, Coatings for the prevention of marine foulings.
- [8] Comparison of toxicity and release rates of Cu and Zn from anti-fouling paints leached in natural and artificial brackish seawater.
- [9] Bostwick, John and Alfred, Evaluation of Antifouling paints by leaching rate determination.
- [10] Efficacy and toxicity of self-polishing biocide free antifouling paints.
- [11] Diego, Soren and Cluase, Dissolution rate measurements of seawater soluble pigments for antifouling paints: ZnO.
- [12] A. Usuki, M. Kawasami, Y. Kojami, Y. Fukushima, A. Okada, T. Karauchi, O. Kamigaito, J. Mater. Res. 8 (1993) 1179. United Catalysts Inc., US Patent 4,677,158 (1987).
- [14] J. Mater. Res. 8 (1993) 1179. United Catalysts Inc., US Patent 4,677,158 (1987).
- [15] T. Spychaj, S. Spychaj, Water-thinnable Paints and Adhesives, WNT, Warsaw, 1996.
- [16] R. Pilawka, T. Spychaj, Kompozyty (Composites) 4 (2004) 33.
- [17] K. Kowalczyk, T. Spychaj, Kompozyty (Composites) 2 (2005) 3.
- [18] K. Kowalczyk, T. Spychaj, Przemysl Chemiczny (Chem. Ind.) 85 (2006) 927.
- [19] B. Gornicka, L. Gorecki, B. Czolowska, J. Zawadzka, Conference Proc. Pomerania-
- [20] Plast, Międzysdroje, Poland, 2004, p. 114.

- [21] Szczecin University of Technology, Polish Patent Applic. 376 291 (2005).
- [22] Sⁿⁱud-Chemie, Eur. Patent 1 560 879 (2006).
- [23] K. Kowalczyk, T. Spychaj, unpublished results, 2006
- [24] Luciano G, Brinkmann A, Mahanty S, Echeverría M (2017) Development and evaluation of an eco-friendly hybrid epoxysilicon coating for the corrosion protection of aluminium alloys. *Prog Org Coat* 110:78–85
- [25] Pourhashem S, Vaezi MR, Rashidi A, Bagherzadeh MR (2017) Exploring corrosion protection properties of solvent based epoxy-graphene oxide nanocomposite coatings on mild steel. *Corros Sci* 115:78–92
- [26] Mahidashti Z, Ramezanzadeh B, Bahlakeh G (2018) Screening the effect of chemical treatment of steel substrate by a composite cerium-lanthanum nanofilm on the adhesion and corrosion protection properties of a polyamide-cured epoxy coating; experimental and molecular dynamic simulations. *Prog Org Coat* 114:188–200
- Xiong Y, Jiang Z, Xie Y, Zhang X, Xu W (2013) Development of a DOPO-containing melamine epoxy hardeners and its thermal and flame-retardant properties of cured products. *J Appl Polym Sci* 127(6):4352–4358
- [27]. Naderi R, Attar M (2008) Electrochemical assessing corrosion inhibiting effects of zinc aluminum polyphosphate (ZAPP) as a modified zinc phosphate pigment. *Electrochim Acta* 53(18):5692–5696
- [28] Carboneras M, Hernández L, Del Valle J, García-Alonso M, Escudero M (2010) Corrosion protection of different environmentally friendly coatings on powder metallurgy magnesium. *J Alloy*
- [29] Lagerström Maria, Ytreberg Erik, Quantification of Cu and Zn in antifouling paint films by XRF
- [30] Franck Casse´ Geoffrey W. Swain, The development of microfouling on four commercial antifouling coatings under static and dynamic immersion
- [31] Christiane Barranguet , Evangelia Charantoni, Short-term response of monospecific and natural algal biofilms to copper exposure.
- [32] Maria Alexandra Bighiu, Biofouling of leisure boats as a source of metal pollution

[33] Patrick J. Earley, Brandon L. Swope, Marienne A. Colvin, Estimates of environmental loading from copper alloy materials

[34] Kenneth Schiff ,Dario Diehl, Copper emissions from antifouling paint on recreational vessels