Bioprospecting of Neem and Hibiscus against Corrosion Caused By Soil Microbes on Zinc and Its Alloys Buried In Soil

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Abstract: This study concerns with the role of corrosion product deposition in microbial corrosion between zinc and its alloys buried in the soil and soil microbes such as S.aureus, Streptococcus, B. subitilis, Lactobacillus, Proteus, Corynebacterium, Pseudomonas, A.niger, Mucor and Desulphovibro sp and also about inhibition by using plant extracts of Neem and Hibiscus which have phytochemicals like flavonoids and alkaloids that detecting by using techniques such as weight loss and thermodynamic studies. Langmuir and Freundlich, Temkin and Florry-Huggins models are employed to analysis adsorption occurred in the experimental data of adsorption isotherms. The Freundlich, Langmuir and Temkin models are employed to analysis adsorption occurred in the experiment.

Keywords: Neem, Hibiscus, S.aureus, Streptococcus, B. subitilis, Lactobacillus, Proteus, Cornybacterium, Pseudomonas, A.niger, Mucor and Desulphovibro sp. Langmuir and Freundlich, Temkin and Florry-Huggins models, Zinc, Adsorption, corrosion inhibition.

INTRODUCTION

Bioprospecting – the exploration of biodiversity for commercially valuable genetic and biochemical resources – has been seen as a potentially powerful tool for conservation. Bioprospecting may not only provide raw materials for pharmaceuticals but for various other plant-based industries in the field of arts, crafts and home-made products and also cosmetics. In a biodiversity, the plants dominate because of the self-sustaining ability, where the animals and humans depend upon autotrophs for survival. The plants prepare their own food with the help of sunlight by photosynthesis for the yield of primary metabolites (i.e.) proteins, lipids, carbohydrates and nucleic acid. After the synthesis of primary metabolites, the excessive energy in the plants is stored as in the form of secondary metabolites (i.e.) photochemical, antioxidants, etc. The plants utilize these secondary metabolites to protect themselves from biotic and abiotic stress factors. Humans use the plant products for our survival, for making shelter, getting rain through transpiration process, consuming all carbon dioxide and leaving oxygen by plants through photosynthesis, thereby purify the air too. They are used for basic needs and also for remediation for many diseases to the animals, human and also for controlling the pollution. A search of such potential plant and its products to add value to the health of globe is called Bioprospecting.Even with untold centuries of human experience behind us and a movement into a medium era of misty and automa tion natural product based compounds have an immersed impact on modern medicine since about 40% of prescription drugs are bas ed on them.

Corrosion of metal is an electrochemical process that involves oxidation (anodic) and reduction (cathodic) reactions on metal surfaces. Corrosion is typically a result of contact with soluble chloride salts found in the soil or water, which requires moisture to form solutions of these salts. Several key factors that influence the severity and rate of corrosion include the amount of moisture in the soil, the conductivity of the solution, the pH of the solution, and the oxygen concentration within the soil (aeration). The organic content of the soil, soil porosity, and soil texture indirectly affect corrosion of metals in soil by influencing the key factors. The presence of high acidity ($pH \leq 5.5$) in water or soil is considered a corrosive condition for concrete and certain metals (carbon steel, zinc, aluminum, and copper).

Soil as a corrosive environment is probably of greater complexity than any other environment. The corrosion process of buried metal structures is extremely variable and can vary range from rapid to negligible. In facts pipes in soil can be perforated within one year, presenting very localized or uniform corrosion attack.

The origin of soil is in the earth's crust and four main classes of soil have been proposed.

1. Soil formed in situ from soft rocks, such as chalk and volcanic ash.
2. Soil formed in situ from hard rocks, such as graphite, limestone, and marble.
3. Soil transported from their place of origin before weathering to form soils, they include those moved by water (marine and beach sediments), wind (sand) and glacial drift.
4. Soil formed in situ from organic material such as peat.

Soil are commonly named and classified according to a size range of their particulate matter. For example, sand, silt, and clay derive their name from the predominant size range of their inorganic constituents.

The soil is the region for the proliferation of microbes with many colonies which developing around soil particles. Many microbes in soil habitats normally are much higher than those in water. Soil as a corrosive environment is probably of greater complexity than any other environment. In facts, Pipes, concrete, tube wells etc., in soil can be perforated within one or few months, presenting very localized or uniform corrosion attack. The chemical constituents of soil are Silica, Alumina and dissolved substances such as H^+, Cl^-, SO_4^-, HCO_3^- ions. Soil with the poorest drainage like clay, silt, and loam are the most corrosive than gravel and sand which is least corrosive.
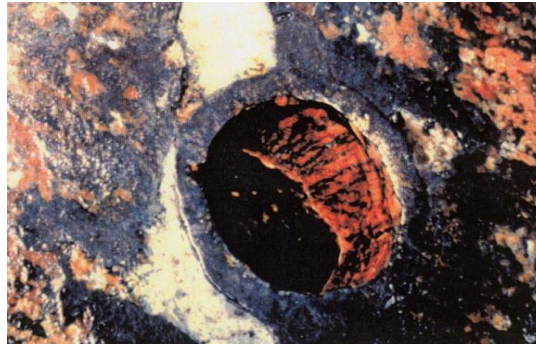
TYPES OF CORROSION
UNIFORM CORROSION
This is also called general corrosion, this is the surface effect produced by most direct chemical attacks (e.g., by an acid) resulting in a uniform etching of metal. On polished surface, this type of corrosion is first seen as a general dulling of the surface and if allowed to continue, the surface becomes rough and possibly frosted in appearance. The discolorations or general dulling of metal created by its exposure to elevated temperature is not to be considered as uniform itching corrosion.

GALVANIC CORROSION
It is an electrochemical action of two dissimilar metals in the presence of an electrolyte and an electron conductive path. It occurs when dissimilar metals are in contact.

CREVICE OR CONTACT CORROSION
It is the corrosion produced at the region of contact of metals with metals or metals with non-metals. It may occur at washes, under barnacles, under applied protective films.

CORROSION IN CONCRETE
Concrete is widely used structural material mainly frequently reinforced with carbon steel reinforcing rods, post tensioning cables or pre-stressing wires. The steel is necessary to maintain the strength of the structure, but it is subjected to corrosion.

EROSION CORROSION
It is the result of a combination of an aggressive chemical environment and high fluid surface velocities.

DEALLOYING
It is a rare form of corrosion of finding in copper alloys, gray cast iron, and some other alloys. Dealloying occurs when the alloy loses the active component of the metal and retain the more corrosion resistant component in a porous "sponges" on the metal surface.

PITTING CORROSION OR MICROBIAL CORROSION
This localized form of corrosion is characterized by the formation of irregularly shaped cavities on the surface of the metals. Microbial corrosion (also called microbiologically-influenced corrosion or MIC) is corrosion that is caused by the presence and activities of microbes. This corrosion can take many forms and can be controlled by biocides or by conventional corrosion control methods. Most MIC takes the form of pits that form underneath colonies of living organic matter and mineral and bio deposits. This biofilm creates a protective environment where conditions can become quite corrosive and corrosion is accelerated. Their diameter and depth depend on the several parameters related to the metals, medium and service condition. Rate and intensity of pitting corrosion can be assessed neither by determining the weight loss nor by measuring released hydrogen.

FACTORS AFFECTING CORROSION
The factor such as the presence of impurities in metals, electrolytes carbon dioxide in water and the protective coating itself increase the rate of corrosion.

POSITION OF METALS IN ELECTROCHEMICAL SERIES
Highly reactive metals undergo corrosion faster than least reactive metals. Reactivity of metals can be found from the electrochemical series.

$Au \rightarrow Cu \rightarrow Pb \rightarrow Fe \rightarrow Zn \rightarrow Al \rightarrow Mg \rightarrow Na \rightarrow K$

Reactivity increases with the electrochemical series.

PREVENTION OF CORROSION
Corrosion is slow but continuously eating away the metal and consequence of rusting. Rusting can be avoided by not letting moist air come in contact with the metal surface.

METHODS OF PREVENTING CORROSION AND RUSTING
The methods used to prevent corrosion by tarring, painting, enameling, galvanizing, shredding, tin plating, electroplating.
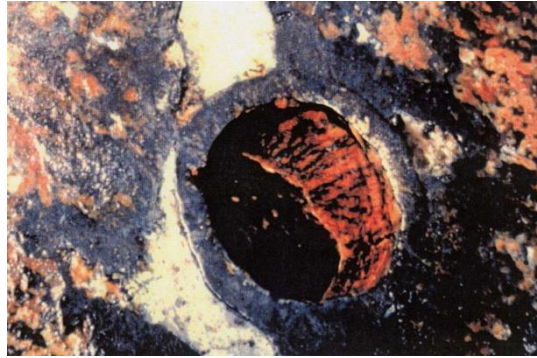
CORROSION ON SOIL
If the soil is corrosive enough it can even attack copper water lines. Typically, the surface of the water line at the bottom of the trench experiences the most corrosion. The difference in oxygen concentration between the soil which was dug up to create the trench and then replaced (thus high oxygen content) and the undisturbed soil at the bottom of the trench (low oxygen content) creates a corrosion cell

Metal buried within an aerated or disturbed soil with a particular resistivity and soluble chloride concentration generally will not experience the same amount of corrosion as a similar metal placed in the same soil in a compacted, less aerated state.

Corrosion is the deterioration of a metal as a result of chemical reactions between it and the surrounding environment. Both the types of metal and environmental conditions, mainly gasses that are in contact with the metal, determine the form and rate of deterioration and also influenced by some microbial performing different electrochemical reactions and secreting proteins and metabolites that can have secondary effects. Some microbes are able to cause and also inhibit corrosion. The metallic corrosion in soil is aqueous and its mechanism is electrochemical.

MAIN CORROSION FACTORS IN SOIL
The metal corrosion in soil is determined primarily by such factors such as Soil type, Resistivity, moisture content and its level of electrical (ionic) conductivity, aeration and oxygen content, relative acidity (P^H) or alkalinity (P^{OH}), amount of dissolved salts Chemistry (pH, Cl- , SO4 2- , alkalinity / acidity, redox), Bacteria, External factors – Differential aeration / resistivity / chemistry ("long-line" corrosion) – External stray currents (dc or ac) and microbial activity. The two conditions necessary to initiate metal corrosion in soil are water and oxygen. After these factors, a number of variables can affect the corrosion process.

MICROBES IN SOIL
Microorganisms in soil are important because they affect soil structure and fertility. Soil microorganisms can be classified as bacteria, actinomycetes, fungi, algae and protozoa. Each of these groups has characteristics that define them and their functions in soil.

One of the most distinguished features of bacteria is their biochemical versatility. A bacterial genus called *Pseudomonas* can metabolize a wide range of chemicals and fertilizers. In contrast, another genus known as *Nitrobacter* can only derive its energy by turning nitrite into nitrate, which is also known as oxidation. The genus *Clostridium* is an example of bacterial versatility because it, unlike most species, can grow in the absence of oxygen, respiring anaerobically. Several species of *Pseudomonas*, such as *Pseudomonas aeruginosa* are able to respire both aerobically and anaerobically, using nitrate as the terminal electron acceptor. Mycobacterium avium and M. paratuberculous present on pastures or barns are the most common non-tuberculosis mycobacteria detected in soil. (Eisenberg et al., 2009). Without the presence of oxygen, anaerobic bacteria, especially *Desulfovibrio* and *Desulfotomaculum*, are common. *D. africanus* is another common corrosion-causing microorganism. The *Desulfotomaculum* genus comprises sulfate-reducing spore-forming bacteria; *Dtm. orientis* and *Dtm. significant* are involved in corrosion processes.

Bacterial corrosion may appear in form of pitting corrosion, for example in pipelines of the oil and gas industry. Anaerobic corrosion is evident as layers of metal sulfides and hydrogen sulfide smell. On cast iron, a graphitic corrosion selective leaching may be the result, with iron being consumed by the bacteria, leaving graphite matrix with low mechanical strength in place.

EFFECTS OF CORROSION
Corrosion is influenced by the complex processes of different microorganisms performing different electrochemical reactions and secreting proteins and metabolites that can have secondary effects. Information on the identity and role of microbial communities that are related to corrosion and corrosion inhibition in different materials and in different environments is scarce. Some microorganisms are able to both cause and inhibit corrosion.

The metal corrosion in soil is aqueous and its mechanism is electrochemical. Three elements are necessary for the operation of the corrosion cell.

1. Galvanic corrosion cells operating on the metal surface formed by anode and cathode sites. Usually, the metal is a good conductor for electron flow. The corrosion reaction is done on the anode areas.
2. Electrolyte (moisture) on the metal surface. The moisture-containing dissolved ionic substances is a good ionic conductor.
3. An oxidizing agent (Oxygen in air).

The condition of soil can range from atmosphere to completely immersed metal depending upon the soil compactness and moisture content. The corrosiveness of soil depends upon an interaction between climate and soil reactions. The variation in soil composition and structure can create the distinctive corrosive environments, which results in different metal activities and oxygen concentration at the metal or soil interface.

CAUSES OF CORROSION
Most of the metals exist in nature in combined forms mainly in the form of ore. So extraction of metal from its ore is an unnatural process in which component of a stable system is being separated.

Ore → Extraction → Metal → Corrosion → Corrosion products
During the process of extraction, a number of steps like concentration, roasting, and melting are involved and finally, the ore is reduced into metal. Therefore metals in their final refined state are highly unstable and have a tendency to revert back to their original state that is to a stable state by the process of corrosion (Oxidation).

CORROSIVE METALS USED IN THESIS
Zinc is a transition metal with the following characteristics: period 4, group IIB, atomic number 30, atomic mass 65.4, density $7.14 \, g \, cm^{-3}$, melting point 419.5°C, and boiling point 906°C. Zinc occurs naturally in soil (about $70 \, mg \, kg^{-1}$ in crustal rocks), but Zn concentrations are rising unnaturally, due to anthropogenic additions. Most Zn is added during industrial activities, such as mining, coal, and waste combustion and steel processing. Many foodstuffs contain certain concentrations of Zn. Drinking water also contains certain amounts of Zn, which may be higher when it is stored in metal tanks. Industrial sources or toxic waste sites may cause the concentrations of Zn in drinking water to reach levels that can cause health problems. Zinc is a trace element that is essential for human health. Zinc shortages can cause birth defects. The world's Zn production is still on the rise which means that more and more Zn ends up in the environment. Water is polluted with Zn, due to the presence of large quantities present in the wastewater of industrial plants. A consequence is that Zn-polluted sludge is continually being deposited by rivers on their banks. Zinc may also increase the acidity of waters. Some fish can accumulate Zn in their bodies when they live in Zn-contaminated waterways. When Zn enters the bodies of these fish, it is able to biomagnify up the food chain. Water-soluble zinc that is located in soils can contaminate groundwater. Plants often have a Zn uptake that their systems cannot handle, due to the accumulation of Zn in soils. Finally, Zn can interrupt the activity in soils, as it negatively influences the activity of microorganisms and earthworms, thus retarding the breakdown of organic matter.

In this present study, the corrosion inhibition performance of *Azadirachta indica (Neem)* and *Hibiscus rosasinensis* leaves solution extract on the corrosion of zinc-plate test specimens in 2N HCl will be carried out at both the ambient temperature of 30˚C and elevated the temperature of 60˚C. This acid was selected for investigation because of their known and strong corrosive action. A positive result from this investigation could lead, later to the identification of the specific compound(s)/active constituents in the leaves that cause(s) corrosion inhibition. This could be economically and technologically beneficial.

This work will investigate the phytochemical constituents, corrosion inhibition and adsorption characteristics of aqueous extract of *Azadirachta indica (Neem) and Hibiscus rosasinensis* on Zinc immersed in HCl solution.

BIOLOGICAL SAMPLES USED IN THIS WORK ARE
NEEM (AZADIRACHTA INDICA)

Neem is a natural medicine since ancient times in Ayurveda even each and every part of Neem plant acts as medicinal values. Meliaceae or the Mahogany family is a flowering woody family of plants Species in the genus *Azadirachta* are closely related to and sometimes confused with species of the genus *Melia* and widely distributed throughout the tropics and subtropics, with only slight penetration into temperate zones.

Neem has an effect on degenerative diseases such as diabetes, cancer, TB, bronchitis, conjunctivitis, allergies, stress, insomnia, etc. The phytochemical substances present in Neem are alkaloids, quinones, resins, tannins, biochemical substances etc. Nimbin is the bitter compound isolated from Neem leaves. Bitterness is due to the presence of terepenes. The most important bioactive compound is Azadirachtin which is an insect repellent. The flavonoids and azadirachtin act as anti-corrosive agents and also anti-microbial agents which prevent the corrosion caused by soil microbes in acidified medium of soil on buried metal in soil and also adsorption behavior properties on metal to prevent the corrosion.

Several studies worked out on the different parts of Neem plant for the various activities such as anti-inflammatory, antipyretic, antifertility, analgesic, immune stimulant, anticancer activity, antidiabetic, antimicrobial, antimalarial, etc. Neem extract has been only involved very occasionally in environmental studies and research with the analysis of adsorption of Pb (II) from aqueous extract of Neem leaves by Bhattacharya and Sharma, Studies of Cu corrosion by Neem leaves extract in H_2SO_4 by Oguzie 2006, adsorption activity by Sanjay Sharma and Corrosion inhibitive properties of Neem in acid solution by Valek, 2007 and more.

HIBISCUS ROSASINENSIS

Hibiscus is a genus of flowering plants in the mallow family, Malvaceae. The genus is quite large, containing several hundred species that are native to warm-temperate, subtropical and tropical regions throughout the world. *Hibiscus rosa-sinensis*, known colloquially as Chinese hibiscus, China rose, Hawaiian hibiscus, and shoeblack plant, is a species of tropical hibiscus, a flowering plant in the Hibisceae tribe of the family Malvaceae, native to East Asia.

Hibiscus is used as anti-inflammatory, demulant, aphrodisiac, refrigerant, menorrhagic, anti-diarrhetic, anticomplementary activity, antifertility, etc. The phytochemical substances present in Hibiscus are steroids, flavonoids, tannins, biochemical substances, alkaloids, resins, vitamin B complex, terepenes etc. Flavonoids are present which gives a bitter taste to leaves and used as anti-corrosive and anti-microbial activities which are equal to Neem.

Several studies worked out on different species of Hibiscus plant for various activities such as anti-diabetic, anti-pyretic, anti-microbial and anti-inflammatory etc. Several workers worked on this plant to be effective in the treatment of arterial hypotension by Dwivedi, 1977; antifertility effect of Hibiscus by Sethi,1986; Singh, 1982; corrosion inhibition for metal in the acid medium by Rajendra, 2009, Anuradha,2007.
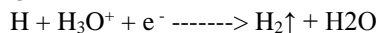
MECHANISM OF CORROSION INHIBITION
Generally, zinc dissolve in hydrochloric acid solution due to somewhat hydrogen type of attack, the reaction taking place at the microelectrodes of the corrosion cell being represented as,
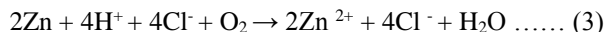
Zn $\longrightarrow$ Zn^{2+} + 2e$^-$ (anodic reaction) -------------- (1)
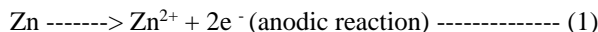
The reduction reaction is indicated by a decrease in valence or the consumption of electrodes, as shown by the following equation.

2H+ + 2e$^-$ -------> 2H $_{(ads.)}$ (Cathodic reaction) -------------- (2)

Or

H + H$_3$O$^+$ + e$^-$ -------> H$_2$↑ + H2O

Thus, the total corrosion reaction of zinc in acidic chloride solution is as following: (A. Zhang and L.Xin)

2Zn + 4H$^+$ + 4Cl$^-$ + O$_2$ → 2Zn $^{2+}$ + 4Cl $^-$ + H$_2$O …… (3)

The mechanism of an inhibitor of corrosion is believed to be due to the formation and maintenance of a protective film on the metal surface.

ADSORPTION PROPERTY

Adsorption is the adhesion of atoms, ions, or molecules from a gas, liquid, or dissolved solid to a surface. This process creates a film of the *adsorbate* on the surface of the *adsorbent*. Adsorption is present in many natural, physical, biological, and chemical systems, and is widely used in industrial applications such as activated charcoal, capturing and using waste heat to provide cold water for air conditioning and other process requirements (adsorption chillers), synthetic resins, increase storage capacity of carbide-derived carbons, and water purification. Adsorption is usually described through isotherms, that is, the amount of adsorbate on the adsorbent as a function of its pressure (if gas) or concentration (if liquid) at a constant temperature. The quantity adsorbed is nearly always normalized by the mass of the adsorbent to allow comparison of different materials. (*Foo, K.Y.; Hameed, B.H. (2010).*

Several mathematical models can be used to describe experimental data of adsorption isotherms. The Langmuir and Freundlich, Temkin and Florry-Huggins models are employed to analysis adsorption occurred in the experimental data of adsorption isotherms. The Freundlich, Langmuir and Temkin models are employed to analysis adsorption occurred in the experiment.

BIOLOGICAL SAMPLES

In the present study, Plants belonging to Neem (Meliaceae) and Hibiscus (Malvaceae) family, having the history of medicinal use in traditional medicine system. Best known species are Neem (*Azadirachta indica)* and Hibiscus (*Hibiscus rosa-sinensis*), both of which has been the subject of substantial pharmacological and clinical investigations over the decades, but few are utilizing. Several species in these two families are also important species.

PHYTOCHEMICAL STUDIES

All the solvents were used based upon their increasing polarity index. The extracts were evaporated to dryness on a water-bath. The plant extracts were distilled off with distillation apparatus and yielded quantities of (leaf) extracts in different solvents were obtained and were further taken to evaluate the phytochemical studies. The percentage yield of plant extracts is shown in Table.

To find the percentage yield of extracts

$$\text{Percentage of yield extracts} = \frac{\textbf{weight of the extract}}{\textbf{weight of raw material}}$$

WEIGHT LOSS METHOD (GRAVIMETRIC METHOD)

The weight loss method was employed for the two temperatures 30˚C and 60˚C. In this procedure, the mass loss of the metal in without *Azardirachta indica* and *Hibiscus rosasinensis* extract (uninhibited solution) and with *Azardirachta indica* and *Hibiscus rosasinensis* extract (inhibited solution) were measured and recorded.

From the data, the percentage of inhibition efficiency (% I) and degree of surface coverage (Ө) were calculated using the following equations,

Percentage of inhibition efficiency **IE = (1 – W$_2$/W$_1$) x 100**

Where W$_1$ and W$_2$ are the weight of the metal in uninhibited and inhibited solutions

Degree of surface coverage **(Ө) = (1 – W$_2$/W$_1$)**

Corrosion rate (CR) = mm /d = 87.6 x (W / DxAxT)

W = weight loss in milligrams, D = metal density in g /cm^3 , A = area of sample in cm^2, T = time of exposure of the metal sample in hours, 87.6 is a conversion factor.

CORROSION INHIBITION ACTIVITY
EFFECT OF CONCENTRATION OF HCL ON ZINC CORROSION

Zinc corrodes in different concentrations of HCl solutions since there was a decrease in the original weight of zinc. The corrosion The corrosion of the zinc in HCl increases with the concentration of the HCl acid. Similar results were obtained at 303K and 333 K. The graph shows values of corrosion rate (CR) of zinc in all the concentrations of HCl studied and it shows that corrosion rate increases with an increase in HCl acid concentration. This observation is attributed to the fact that the rate of chemical reaction increases with increasing concentration

MECHANISM OF CORROSION INHIBITION

The mechanism of an inhibitor of corrosion is believed to be due to the formation and maintenance of a protective film on the metal surface. Further, when log (θ /1-θ) is plotted against log C straight lines are obtained in the case of aqueous extract of *Azardirachta indicia and Hibiscus rosa-sinensis* studied. This suggests that the inhibitor covers both the anodic as well as cathodic regions through general adsorption following Langmuir isotherm, Frumkin, Temkin, and Flory-Huggins.

Data obtained for the degree of surface coverage were used for the evaluation of different adsorption isotherms (Langmuir, Frumkin, Temkin and Flory-Huggins).

Free Energy of Adsorption

The equilibrium constant of adsorption of aqueous extract of *Azardirachta indica* and *Hibiscus rosasinensis* leaves on the surface of Zinc is related to the free energy of adsorption (ΔGads) according to Equation.

ΔG = - 2.303 RT log (55.5K)

ΔG = - 2.303 RT [log 55.5 + log K]

Where *R* is the gas constant and *T* is the temperature. The free energy of adsorption was calculated from values of *K* obtained from Langmuir and Freundlich, Temkin and Flory-Huggins according to Equation and is recorded.

CONCLUSION

Ethanol extracts of fresh Neem and HR were more efficient than aqueous extract. This study reveals that antimicrobial effect of Neem and HR will make it easier for dosage determination and chemotherapeutic index the extract. Each extract has its MIC which is the highest dilution plant extract that still retains an inhibitory effect on the microbial growth which causes corrosion.

These extracts have to be proved that the Neem has higher antioxidant potential than HR. The antioxidant such as Phenols, Flavonoid, Tannins, Terpenoids, Alkaloids etc., have proved to be present in these extract which is more efficient for antimicrobial and anticorrosion activity.

The percentage of inhibition in the presence of these inhibitors was decreased with temperature which indicates the physical adsorption was the predominant inhibition mechanism because the quantity of adsorbed inhibitor decreased with increasing temperature.

The IE of the plant extracts increased with increasing in extract concentration and with temperature moisture and atmosphere. These results suggested that IE of secondary metabolites depends upon the factors such as their charge distribution, no. of adsorption sites, the heat of adsorption, mode of interaction with the metal surface, mode of inhibition of microbial growth in moist condition and formation of the metallic complex.

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