# FUNGAL INFLUENCED CORROSION OF METALS AND THEIR INHIBITION BY PLANT EXTRACTS

**BY**
**IMO, EJEAGBA OKORIE**
**(B.Tech., M.Sc)**

REGISTRATION NUMBER: 20124771928

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# CERTIFICATION

This is to certify that this work "Fungal influenced corrosion of metals and their inhibition by plant extracts" was carried out by Imo, Ejeagba Okorie in the Department of Microbiology, School of Post Graduate Studies, Federal University of Technology, Owerri, Nigeria.

Prof. Mrs. J.C. Orji
Principal Supervisor
26/05/2021
Date

Prof. C.O. Nweke
Co-Supervisor
26/5/2021
Date

Prof. C.E. Nwanyanwu
Co-Supervisor
26/05/2024
Date

Prof. C.E. Nwanyanwu
Head of Department
26/05/2024
Date

Prof. F.O.U.Osuala
Dean, SOBS
25/06/21
Date

Prof. C.C. Eze
Dean, Post Graduate School
.....
Date

Prof. G.C. Okpokwasili
External Examiner
26/05/2021
Date



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## DEDICATION

I dedicate this work to my family for their love and care.

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## ABSTRACT

Growth of fungi on metal surfaces can initiate, facilitate and accelerate corrosion reactions, altering the composition and integrity of such metal without changing its electrochemical nature. Studies on fungal influenced corrosion of mild steel (MS) and aluminium (Al) in the presence of *Acremonium kiliense*, *Aspergillus fumigatus* and *Penicillium chrysogenum* and their inhibition by seed extracts of *Aframomum melegueta* and *Piper guineense* were carried out using gravimetric and potentiodynamic polarization techniques. MS (C-0.30%, Si-0.30 %, Mn-0.30 %, P-0.045 %, S-0.050 %, Cr-0.064 %, Cu-0.040 %, Ti-0.04 % and balance Fe) and Al (>95.5 %) plates, 2x2x0.14 cm and 3x1.5x0.1 cm in size respectively were contaminated with the mentioned fungi in Petri dishes with nutrient medium imitating organic pollution. The results revealed that the metals reacted differently to the impact of fungi. The influence depends on the capacity of fungi to develop on the metal surface and produce metabolites stimulating changes in polarization resistance and destroying the surfaces. *A. fumigatus* proved to be the most active destructor of mild steel and aluminium with cumulative corrosion rate (ΣCR) of 7.85±0.91 mpy, 7.49±0.57 mpy and corrosion current density ($I_{corr}$) of 279.4 μA/cm$^2$, 201.2 μA/cm$^2$ for mild steel and aluminium respectively. The gravimetric analysis further revealed that the corrosion rate and weight loss of the metals increased with time. Cold water extracts of *A. melegueta* and *P. guineense* were assessed for their anticorrosion and antifungal activities. The anticorrosion studies at 25mg/mL concentration showed that the inhibitors are mixed-type inhibitors inhibiting both the cathodic and anodic sites. *P. guineense* showed the highest inhibition efficiency (IE) against *A. fumigatus* (87 %IE). *A. melegueta* was more effective against *A. kiliense* (85 %IE). The study shows that the attachment and growth of fungi on the surface of metals can influence their corrosion reactions. That the phytochemical constituents of plant extracts could be exploited for use in the control of microbial influenced corrosion of metals.



**Key words**: Fungi, metals, corrosion, plant extracts, polarization, corrosion rate

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background

Microbiologically influenced corrosion is the degradation of metallic structures by the activities of microorganisms (Beech, 2013). It is an electrochemical process where the activities of microorganisms are able to initiate, facilitate or accelerate the corrosion reaction without changing its electrochemical nature (Lewandowski & Beyenal, 2008). The presence of microorganism on metal surface can modify their chemistry, often promoting the establishment and maintenance of physicochemical reactions not normally favoured under otherwise similar conditions (Beech & Sunner 2004).

Fungi play an important biogeochemical role in the biosphere and are intimately involved in the cycling of elements and transformation of both organic and inorganic substrates (Lugauskas *et al.,* 2009). They are ubiquitous members of the sub-aerial and subsoil environments, and often become a dominant group in metal-rich or metal-polluted habitats. Fungi have ability for growth under extreme environmental conditions which allows their successful colonization of metal surfaces and other metal rich-habitats. The growth of fungal mycelia on the metallic surfaces is closely related to electrochemical processes (Juzeliunas *et al.,* 2007). Studies of metal overgrowth in open natural environments shows that fungi are most intensively involved in places where the metal comes into contact with water and air. It has been reported that the electrical characteristics of steel, aluminum and titanium can be worsened by some species of fungi belonging to the genera *Aspergillus, Penicillium, Sporotrichum, Cladosporium,* and *Paecilomyces* (Crod-Ruwisch, 2000). The possibilities of some fungal species to grow on metal surfaces are determined by their secreted metabolites which enable them to adapt to new environmental and nourishment conditions. In aqueous environments, metals are corroded not only by purely chemical or electrochemical reactions

but also by metabolic activities of microorganisms (Koch, Brongers, Thomas, Virman, & Payer, 2002).

Majority of metals in nature are found in their natural state, i.e., in their stable oxidized conditions as oxides, chlorides, carbonates, sulphates, sulphide etc (Maiara, Emmanuel, Patricia & Luana, 2013). The extraction of these metals from their appropriate mineral involves a reduction process in which a great deal of energy is absorbed (Maiara *et al.*, 2013). As a consequence of this large deal of energy input, the metal is in a high energy condition and will return to its former stable oxidized low energy state as quickly as environmental conditions will allow. It is the energy difference between the pure metal and its oxidized form that is the driving force for corrosion. Therefore, electrochemical process of metal corrosion occurs due to difference in chemical potential between the metal and the environment, involving the reaction of the metal and non-metallic substances in the medium (Shi, Xie, & Gong, 2011) These reactions can be influenced at their various steps by microbial activities especially when these organisms are in close contact with the metal surface forming biofilms (Beech, 2003). The resulting metal deterioration is known as microbiologically influenced corrosion. If a metal comes into contact with water, positive metal ions are released into the solution and leave free electrons on the metal.

$$Me \leftrightarrow Me^{z+} + z\, e^{-} \qquad (1)$$

The reaction shifts to the right if the liberated electrons are continuously removed, resulting in a net dissolution of the metal. Free electrons cannot be released as such into the medium; usually they can be consumed by reactions with oxidizing substances from the aqueous phase at metal-water boundary. Such electron acceptors can be oxygen, protons, acids or water (Costa, Vaz, Oliva, & Correia, 2010) Areas on the metal where metal dissolution or electron uptake reactions occur are termed anodic and cathodic sites, respectively. The accumulation of products of cathodic and anodic reactions on the surface of metals or on metal-water

interface tends to slow down the rate of corrosion (Beech, Sunner & Hiraoka, 2005; Cord-Ruwisch, 2000; Lee & Newman, 2003). This process is termed polarization. It may be broken down if the corrosion products are removed, leading to depolarization and consequently to continuous corrosion.

Microorganisms including fungi are able to depolarize both cathodic and anodic sites either directly by their metabolic activities or indirectly by excretion of chemically reactive products (Beech & Sunner, 2004). Such microorganisms are particularly corrosive as they grow in colonies or films attached to the metal surface and thereby create local electrochemical cells with highly stimulated reactions. As a result, corrosion by microorganisms often occurs as pitting, which is usually more severe than corrosion processes that are evenly distributed over the metal surface (Crod-Ruwisch, 2000).

In air, metal corrosion is linked with an attack of oxygen which is mainly chemical (Uhlig, 2011). On the other hand, metallic iron also undergoes severe corrosion in the absence of oxygen, sometimes even at higher rate than in the presence of oxygen. Such an anaerobic corrosion is mostly due to microbial activities (Beech, 2003). The most aggressive corrosion is usually observed in oxic-anoxic environments where both aerobic and anaerobic microorganisms develop (Videla, 2001). Various mechanisms of biocorrosion, which reflect the variety of physiological activities carried out by different types of microorganisms, have been identified (Beech *et al.,* 2005). A very large variety of microorganisms can enhance the corrosion rate of metals through their metabolic processes. The direct mechanism of microbial corrosion is the utilization of metal or metabolization of an electrochemically produced component such as hydrogen from cathode by microorganisms. The physiological activity of fungi and the abundance of their metabolites allow them to attach to metals: aluminum, iron, copper, zinc and poly-aniline-modified nickel (Juzeliunas *et al.,* 2006). According to Lugauskas *et al.* (2010), metal surface is a convenient place for fungal mycelia

to attach. Acid produced by fungi are damaging to metals (Little & Ray 2001). These include formic, citric and acetic acids. Bacteria also produce a wide range of enzymes for example hydrolytic and proteolytic enzymes as well as lyases, which are able to react with substrates. Such enzymes are broadly categorized as ecto-enzymes (associated with the cell, but expressed outside the cytoplamic membrane) and extracellular enzymes i.e., present in the medium in free forms. The latter category includes polysaccharides, proteases, lipases, esterase, peptidases, glycosidase, phosphatases and oxidoreductases. The release of some of these enzymes into their external environment by microorganisms can influence corrosion (Beech & Sunner, 2004). According to Rabus, Hansen, and Widdel, (2000), the hydrogenase enzyme produced by *Escherichia coli* and sulfate-reducing bacteria (SRB) can utilize molecular hydrogen and may be associated with the cathodic hydrogen depolarization thus causing the corrosion of steel casing and pipes in oil fields. Similarly, when fungi grow on metal surfaces, they not only can consume the nitrate and sulfur accumulated on the eroded poles (such as iron) but also the hydrogen, oxygen and other gaseous products formed on the metals hence depolarizing the metals and enhancing corrosion. Therefore, microbial corrosion processes at metal surfaces are associated with microorganisms or the products of their metabolic activities (Beech *et al.,* 2005). These microbial metabolic products can affect cathodic and/ or anodic reactions thereby altering the electrochemistry at the metal solution interface (Hector & Liz, 2005).

**Statement of the Problem**

Metals are widely used in industrial applications due to their strength and structural integrity. Most metals are normally coated for corrosion protection while cathodic protection may also be used. For selected applications, galvanization (zinc coating) may be used to protect steels in atmospheric environments (Muthukumar *et al.,* 2003). Bituminous coal tar and asphalt dip coatings (Akpabio, Ekott, and Akpan, 2011) are often used on the exterior of buried pipelines

and tanks while polymeric coatings are used for atmospheric and water environments. However, biofilm tends to form at flaws in coating surfaces. Furthermore, acid producing microorganisms have been found to dissolve zinc and polymeric coatings (Mansfeld, Hsu, Oernek, Wood, & Syrett, 2001). Poor quality water systems and components within areas that accumulate stagnant water and debris are prone to microbiologically induced corrosion. This has been seen to occur in underground pipes that have been left unused for periods of time (Akpabio *et al.,* 2011). The exteriors of buried pipes (of water and wastewater) and tanks especially in the soil and wet environments are persistently being exposed to microorganisms which influence corrosion (Videla, 2002).

Microbial influenced corrosion has been linked with most of the internal corrosion problems in oil transportation pipelines, storage tanks and drainage systems including the growth of microorganisms on industrial systems and materials resulting in fouling and corrosion problems with serious attendant economic consequences (Maiara *et al.,* 2013). Corrosion of oil field equipments, piping and industrial system materials can lead to potentially hazardous system malfunctions as well as costly damage and repair cost (Akpabio *et al.,* 2011). Fungi can also affect metals and structural parts of buildings thereby accelerating the corrosion rates and deterioration of such materials and these may lead to problems even in buildings less than five years old (Muthukumur *et al.,* 2003). Most laboratory and field studies on microbial influenced corrosion have focused mostly on bacterial involvement; however, other microorganisms such as fungi can influence corrosion processes. In many environments, including humid atmospheric conditions, fuel-water interfaces, fungi tend to dominate the micro flora and hence influence corrosion (Little & Ray, 2001). Fungi are the most desiccant-resistant microorganisms and can be active at water activity value of 0.06, whereas few bacteria remain active at $a_w$ value below 0.09 (Beech 2003). This attribute makes it possible

for fungi and their mycelia to attach, survive and colonize metal surfaces even in extreme environmental conditions.

According to Maiara *et al.* (2013), the methods employed to prevent biocorrosion involve inhibiting the growth or metabolic activity of microorganisms and changing the environment in which the corrosion process occurs in order to avoid the prolifiration of these organisms. These methods tend to reduce or eliminate metal exposure to the action of biocorrosion, either by direct elimination of microorganisms or by reducing the effect of their metabolites on the metal (Videla, 2003). The use of inhibitors or biocides is one of the best ways to prevent metal and alloy from corrosion. Several inhibitors like Cinnamaldehyde, formaldehyde, nonylphenol –ethlene oxide surfactants and naphthylquinolinium in use are toxic to human and the environment. There are serious efforts to develop new inhibitors of plant origin for metals subjected to various environmental conditions. Plant represents an interesting source of compounds currently being explored for use in metal corrosion protection including the inhibition of microbial growth and biofilm formation. This is because inhibitors of plant origin are biodegradable, non toxic, environmentally friendly and cheap.

**Aim of the Study**

The aim of this research therefore is to study the influence of some fungi on the corrosion of metals and their inhibition by plant extracts.

**Objective of the Study**

**The study objectives are to:**

i. Isolate and identify fungi from corroded metals.

ii. Study the influence of the fungal isolates on the corrosion of mild steel and aluminium.

iii. Study the capacity of the fungal isolates to grow on mild steel and aluminium.

iv. Study the morphological changes on mild steel and aluminium surfaces after exposure to the fungal isolates.

v. Study the corrosion behaviuor of mild steel and aluminium after exposure to fungi by gravimetric methods.

vi. Study the effects of the seed extracts of *Piper guineense* (Uziza) and *Aframomum melegueta* (Ose oji) on the rate of fungal growth and metal corrosion ability of the fungal isolates.

vii. Determine the inhibitory efficiency (IE) of extracts of *Piper guineense* and *Aframomum melegueta* on the corrosion of mild steel and aluminum.

viii. Analyse the corrosion behaviour of the metals after exposure to fungal isolates by electrochemical techniques.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Fungal Corrosion of Metal

Fungi and their functional capacity are important ecological factors of the environment, which often determine the duration of the effective use of metal and their ware (Lugauskas, Leinartas, Grigucevičienė, Selskienė and Binkauskienė, 2008). Fungi find their way unto metal surface under the action of adhesion forces and they start to function even at the lowest moisture (Lugauskas *et al.,* 2009). In such a manner they form chemical bond with the metal. However, not all fungi can survive on metal surface. Most of them die from stress, unfavourable conditions on the metal surface, such as alternating moisture, temperature, physical, chemical and technical parameters (Juzeliunas *et al.,* 2007). Only fungi that are able to incorporate the metal as a link into their activity chain connecting them with the environment and whose functioning helps minimize the tension between the metal and their vital needs can survive.

Fungi have been implicated in the corrosion of many metals and their alloy used in fabrication and construction of buildings. Fungal influenced corrosion (FIC) has been reported for some metals and aluminum alloys exposed to hydrocarbon fuels during transport or storage (Videla, 2001). Differential aeration caused by the adherence of fungal mats can cause crevice corrosion. The reaction of aluminum and its alloys used in aircraft building to the influence of fungi and their metabolites has been studied (Lugauskas *et al.,* 2008). Changes in the composition of surface layer of metal or metal alloy are one of the evidences confirming that fungi have penetrated the metal surface and used it to satisfy their nutritional needs. For example, Lugauskas *et al.* (2008) noted that the formation of *Aspergillus niger* colonies and their colour are determined by oxygen concentration in metal oxide layer. They

further observed that the pH of the surface of copper alloys decreases with an increase in oxygen quantity which is mostly pronounced in the zone where fungi are growing. In other to reveal the response of fungi developing on metal surface rich in organic substances, Lugauskas *et al.* (2008) exposed Al, Fe, Cu and Zn plates in close contact with peat compost. The results obtained suggest that fungi developing in the environment actively participate in the process of surface damage and destruction.

Fungal corrosion via acid generation is associated with the degradation of organic materials such as lubricants (Little, Staehle and Davis, 2001) cladding, and jet fuel where there is relatively high level of organic material and some water. Fungi are able to generate a wide range of organic acids under aerobic conditions (Lugauskas *et al.,* 2008). According to Gadd (2010), these acids are generated to allow the sequestration of metals from the environment. The impact of organic acids on the corrosion of radioactive waste containers was reviewed by Rosales (1985) who indicated that both formic and acetic acid was able to promote steel corrosion particularly if present as a vapor. Numerous reports document fungal growth in passenger compartment of in-service aircraft coated with polyurethane paint. Little *et al.* (2001) documented the following eight fungal genera associated with H-3 aircraft: *Pastolotia, Trichoderma, Epicoccum, Phoma, Strephylium, Hormodendrum* (also known as *Cladosporium*), *Penicillium* and *Aureobasidium*. These organisms were cultured from virtually all interior surfaces, including primer and polyurethane –coated 2024 T-6 aluminum UNS A92024), fiberglass structures members, caulking, synthetic fabrics, wiring and air conditioning ducts. According to them the concentration of organisms depends on the availability of nutrients and water. They maintained that the fungi did not degrade the polyurethane topcoat, but they derived nutrients from hydraulic fluid, deposited on surfaces during the normal operation of the helicopter and a corrosion inhibiting compound applied to surfaces during maintenance.

Little *et al.* (2001) reported fungus influenced corrosion of undesignated carbon steel wire rope used in military applications. Laboratory data and field statistics implicated the practice of wrapping in plastic, stored carbon steel highline under humid conditions with microbial influenced corrosion. Fungal growth was observed on interiors of some wooden spools stored outdoors and corrosion was most pronounced on wraps of wire in direct contact with the wooden spool flanges. *Aspergillus niger* and *Penicillum* sp were isolated from the wooden spool flanges. Large quantities of organic acid by-products induced by these fungi selectively dissolve or chelate copper, zinc and iron forming pits which persist under anaerobic conditions established under fungal mat. Many metals are essential for fungal growth and metabolism (Gadd, 2010). Metals exact toxic effects in many ways: they can inhibit enzymes, displace or substitute essential metal ions, cause disruption of membranes and interact with the systems that normally protect against the harmful effects of free radicals.

## 2.2 Forms of Corrosion

Corrosion is often categorized by the cause of the metal's chemical deterioration (Lynes 2011). The most commonly used categories for corrosion are: uniform or general corrosion and localized corrosion including pitting, galvanic corrosion, crevice corrosion, inter-granular corrosion, stress corrosion, cracking corrosion, selective corrosion, leaching corrosion, under deposit corrosion and erosion corrosion (Winston & Uhlig, 2008).

### 2.2.1Uniform Corrosion

General or uniform corrosion is the uniform loss of metal over an entire surface (Uhlig, 2011). Uniform corrosion is relatively easy to detect and its effect are quite predictable. On the basis of tonnage wasted, this is the most important form of corrosion. As corrosion occurs uniformly over the entire surface of the metallic component, it can be practically controlled

by cathodic protection, use of coating or paint or by simply specifying a corrosion allowance (Lynes, 2011)

### 2.2.2 Localized Corrosion

Localized corrosion affects small area (Videla, 2002). The predominant form of localized types of corrosion are pitting, crevice corrosion, and under deposit corrosion (Winston & Uhlig, 2008). Surface anomalies may increase the susceptibility of localized corrosion. In addition, aggressive substances in aqueous environment for example chlorides or sulphate may induce localized corrosion of carbon steel (Lynes, 2011).

### 2.2.3 Pitting Corrosion

Pitting corrosion is a localized form of corrosion by which cavities or 'holes' are produced in the material (Uhlig, 2011). Pitting is considered to be more dangerous than uniform corrosion damage because it is more difficult to detect, predict and designed against as corrosion products often cover the pits (Lynes, 2011). According to Lynes (2011), a small narrow pit with minimal overall metal loss can lead to the failure of an entire engineering system. Pitting corrosion which for example is almost common denominator of all types of localized corrosion attack, may assume different shapes. Pitting is brought about by localized chemical or mechanical damage to the protective oxide film. Factors which can cause the breakdown of a passive film include acidity, low dissolved oxygen concentration and high concentration of chloride and localized damage or poor application of a protective coating. Pitting corrosion can produce uncovered pit or covered with a semi-permeable membrane of corrosion products. Pits can also be either hemispherical or cup shaped. Deep pits can develop with only a relatively small amount of metal loss and thus it can be missed in gravimetric analysis (Lynes, 2011).

### 2.2.4 Crevice Corrosion

Crevice corrosion is a localized form of corrosion usually associated with a stagnant solution on the micro-environment level (Biezma, 2001). Such stagnant micro environments tend to occur in crevices such as those formed under gaskets, washers, insulation materials, fasten heads, surface deposit, disbanded coatings, threads, cap joints and clamps (Uhlig, 2011). Crevices corrosion is initiated by changes in local chemistry with the crevice such as the depletion of inhibitors in the crevices, depletion of oxygen in the crevice, a shift to acidic condition in the crevice and builds-ups of aggressive ionic species like chloride.

### 2.2.5 Galvanic Corrosion

Galvanic corrosion refers to corrosion damage induced when two dissimilar materials are coupled in a corrosion electrolyte (Biezma, 2001). It occurs when two or more dissimilar metals are brought into electrical contact under aqueous medium such as water. When a galvanic couple forms, one of the metals in the couple becomes anode and corrodes faster than it would all by itself while the other becomes the cathode and corrodes slower than it would. In a bimetallic couple, the loss of noble material will become the anode of the corrosion cell and tends to corrode at an accelerated rate compared to an uncoupled condition. Galvanic corrosion can be one of the most common forms of corrosion and the most destructive.

### 2.2.6 Inter-Granular Corrosion

The microstructure of metals and alloys is made up of grains, separated by grain boundaries. Inter granular corrosion is localized attack along the grain boundaries or areas immediately adjacent to grain boundaries while the bulk of the grains remain largely unaffected (Lynes, 2011). Biezma (2001) observed that this form of corrosion is usually associated with chemical segregation effects or specific phases precipitated on the grain boundaries. These

areas of precipitation can produce zones of reduced corrosion resistance in the immediate vicinity. Inter-granular corrosion attack is usually related to the segregation of specific elements or the formation of a compound in the boundary.

### 2.2.7 Stress Corrosion Cracking

Stress corrosion cracking (SCC) is the cracking induced from the combined influence of tensile stress and a corrosion medium. The impact of SCC on a material usually falls between dry cracking and the fatigue threshold of the material. The required tensile stress may be in the form of directly applied stress or in the form of residual stress (Biezma, 2001).

## 2.3 Economic Importance of Microbial Influenced Corrosion

Corrosion of pipelines and industrial metal structures is a very wide spread problem both in Nigeria and around the world. Structures such as natural gas, crude oil and water pipelines are some of the many structures reported to have been affected by microbial corrosion all around the world (Levlin, 1992). A lot of money is lost every year due to corrosion and problems associated with corrosion. The consequences of corrosion are many and varied and the effects of these on the reliable and efficient operation of equipment or structures are often more serious than the simple loss of a mass of metal. Failures of various kinds and the need for expensive replacement may occur even though the amount of metal destroyed may be quite small. According to Maiara (2013) some of the major harmful effects of corrosion can be summarized as follows:

***i.*** Loss of mechanical strength and structural failure or breakdown as a result of reduction in metal thickness. When the metal is lost in localized zone so as to give a crack-like structure, very considerable weakening may result from quite a small amount of metal loss.

***ii.*** Hazard or injuries to people arising from structural failure or breakdown.

***iii.*** Loss of time in availability of profit-making industrial equipments.

***iv.*** Reduced value of goods due to deterioration of appearance.

***v.*** Contamination of fluids in vessels and pipes e.g., beer goes cloudy when small quantity of heavy metals is released by corrosion. Also drinking water is polluted and contaminated when pipelines corrode and sewage water passes through the pin holes on the pipes caused by microorganisms.

***vi.*** Perforation of vessels and pipes allowing the escape of their contents and possible harm to the surroundings.

***vii.*** Loss of technically important surface properties of a metallic component. These could include frictional and bearing properties, ease of fluid flow over a pipe surface reflectivity or heat transfer across a surface. Mechanical damage to valves, pumps or blockage of pipes by solid corrosion products.

According to Gerrhardus *et al.* (2002), in the United States, industries and government lose approximately 276 million dollars or 3.1percent of the GDP (Gross Domestic Product) as a result of corrosion and corrosion-related problems. It was estimated that about 25 to 30% of this total cost could be avoided if corrosion preventive technologies are put in place (Gerrhardus *et al.,* 2002). In Australia, Great Britain and Japan, for example the cost of corrosion is approximately 3 to 4 percent of the GDP (Salami, Wewe, Akinyemi & Patinovoh, 2012). According to World Corrosion Organization (World Corrosion Organization, 2012), the annual cost of corrosion is greater than 3% of global GDP; however, governments and industries pay little attention to corrosion. The wear of storage tanks and oil pipelines, drainage and water supply channels caused by corrosion may result in micro-leaks causing environmental contamination and pollution (Maiara, 2013). Interestingly, studies have shown that over 20% of all corrosion damage to metallic materials is microbiologically influenced (Beech, 2004) of which fungi contribute a significant percentage.

In August 1983, a major pipeline (Ogoda-Brass 24") failed at Oshika village in Ahoada Local Government Area of Rivers State, Nigeria, and an estimated 5,000 barrels of oil was spilled. The cost of the incident was conservatively put at $1.5 million (Rim-Rukehand & Awelefe, 2006). Soil buried pipelines of steel used for transportation of different products are highly damaged by corrosion which are caused not only by abiotic environmental factors but also influenced by microbial activities (Bhattarai, 2013). Among the various corrosion processes, microbial influenced corrosion of materials is reported to account for up to 50% of the damages (Beech & Sunner, 2004). According to them, the industries mostly affected include the nuclear and fuel electricity power generating sectors, pipelines, oil fields and offshore industries. In some municipal systems such as drinking water distribution lines, high rate of microbial corrosion not only cause significant losses to the economy, but also directly affect the public health of communities (Volk, Schiermann & Lechevallier, 2000). Filamentous fungi (*Cladosporium resinae*) with their heavily packed mycelia are detrimental to metal surfaces and can also be found in closed systems such as fuel storage tanks and pipelines (Pope & Pope, 1998). One particular area where there are a large number of reported cases of microbial influenced corrosion of metals is in the oil and gas industry. According to Gu, Xu, Zhang, Li, and Linderger, (2015) one of the main reasons for this is the use of water (often seawater) injection in enhanced oil recovery operations to increase reservoir pressure. The practice brings in nutrients and microbes (Gieg, Jack & Foght, 2011). Microbial corrosion has been associated with the rapture/ failure of pipelines used in transportation of petroleum and its products from oil and gas fields flow stations tanks farms, depots and terminals in Nigeria. Rim-rukeh and Awatefe (2006) reported the repeated failure as a result of corrosion of Obrikom-Ebocha field to Ebocha Oil Centre. According to them, 8 cases of failure along the pipeline route was reported within a space of two years.

Another area with high reported cases of microbial influenced corrosion is in fire protection systems (Pope & Pope, 2000; Gu *et al*., 2015). Most water protection systems utilize untreated water that is left stagnant for a long period of time. This creates an environment for microbial growth and biofilm formation. These microorganisms cause microbial influenced corrosion which results in pinhole leaks mostly at the weld seams and along the bottom of the pipeline. Gu *et al.* (2015), observed that these microorganisms can cause biofouling which can hinder water from flowing through the pipe in an emergency. According Pope and Pope (2000), Microbial influenced corrosion can be found in both steel and copper piping of fire protection systems. Microbial influenced corrosion can sometimes cause failures of these systems within months of initial installation. The buildup of biofilm and other corrosion products in the sprinkler heads can also cause them to fail (Gu *et al*., 2015).

Copper is a widely used material in potable water systems and heat exchangers (Teng, Guan & Zhu, 2008; Gu *et al*., 2015; Puigdomenech & Taxen, 2000). According to Kikuchi, Ozawa, Sakane, Kanamaru and Tohmoto, (1999), copper is relatively corrosion resistant and is considered toxic to many microbes in nature. Despite this, microbial influenced corrossion of copper, especially in the form of localized corrosion has been reported in many parts of the world, especially in low flow and stagnant conditions (Gu *et al*., 2015). A typical example was the failure of a domestic hot water system installed above the ceiling of a retirement home that failed after only 12 years (Labuda ,2003).

Nuclear power plants have also experienced many failures due to microbial influenced corrosion, with some plants spending upwards of $100 million on the problem (Angell, 2002). According to her, the two main areas that experience microbial influenced corrosion in nuclear power plants are heat exchangers and fire protection systems. These systems are vital to the safety of nuclear power plants and their failure could force the power plant to shut down operations (Gu *et al.*, 2015). Failure of nickel alloy heat exchanger tubes as a result of

microbial actions were reported in the late 1980s at several Ontario Power Generation CANDU power plants less than a year after installation. The power plant uses heavy water ($D_2O$) on the tube side of the heat exchanger and raw water on the shell side. These conditions created an ideal environment for microorganisms to thrive, especially in the shell side of the heat exchanger, which in addition to being difficult to clean also reduced fluid velocity (Gu *et al*., 2015). MIC can also cause problems at hydroelectric power plants (Yu, Dillon, & Henry, 2010). According to Lindardt and Nichtawitz (2003), multiple cases have been reported of microbial influenced corrosion of stainless-steel components in hydroelectric power plants. An example of this was the pitting corrosion observed after only 18 months of service on the turbine blades and discharge rings of the hydroelectric power plant on the Maas River in the Netherlands (Gu *et al*., 2015).

Besides the severe influence of biocorrosion in industrial sectors, the effect of microbes on the corrosion of medical implants has been investigated. A lot of metal implant surfaces are continually exposed to the biological environment of human body which contains water, organic compounds, microorganisms, enzymes, chlorides, and amino acids to mention but a few (Hansen, 2008). Several strains of microbes such as aerobic iron-oxidizing bacteria (IOB), fungi and SRB have been found in the surrounding physiological environment in the human body (Boopathy, Robichaux, LaFont & Howell, 2002; Manivasagam, Dhinasekaran & Rajamanickam, 2010). These microorganisms form biofilms which can cause the biocorrosion of implants and associated infections (Gino, Starosvetsky, Kurzbaum, & Aromon, 2010; Vianna, Holtgraewe, Syfart, Conrads, & Horz, 2008). Chang *et al.* (2003), observed the corrosion of dental metallic materials made of titanium alloys and stainless steel in the presence of *Streptococcus* mutants and the metabolic products such as lactic acid, carbonic acid and glucan-binding proteins. These studies on microbial influenced corrosion and electrochemical behavior of titanium alloys and stainless-steel orthodontic wires in the

presence of microorganisms reveal that the interactions between metal implant surface with microbial cells and their metabolites have important impacts on corrosion propagation and damage (Costerton, Lewwandowski, Caldwell, Korber & Lappin -Scott, 1994).

**2.4 Biofilm**

Biofilm is an assemblage of microbial cells that are irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material (Rodney, 2002). According to Larsen, Rasmussen, Pedersen, Sorensen, Lundgaard and Skovhus (2010), they are densely packed communities of microbial cells that grow on living and inert surfaces and surround themselves with secreted polymers. Videla and Herrera (2005) described it as gel containing 95% or more water and a matrix of exopolysaccharidic substances (EPS), in which microbial cells and inorganic detritus are suspended. Non-cellular materials such as mineral crystals, corrosion products, clay or silt particles, depending on the environment in which the biofilm has developed may also be found in the biofilm matrix. Biofilm can form on a wide variety of surfaces including living tissues, industrial or potable water system piping, or natural aquatic systems (Donlan & Costerton, 2002). Many microbial species form biofilms, and their study has revealed them to be complex and diverse. Biofilms affect interactions between metal surfaces and the environment, not only in biodeteroriation processes such as microbial corrosion, but also in several biotechnological processes applied in material recovery and handling. According to Videla (2003), the key to the alteration of conditions at a metal surface, and hence the enhancement or inhibition of corrosion is the formation of a biofilm. Biofilm formation on metals is the result of an accumulation process-not necessarily uniform in time and space (Videla & Herrera, 2005) that starts immediately after metal immersion in aqueous environment. A thin film (approximately 20-80 nm thick), due to the deposition of inorganic ions and organic compounds of high relative molecular weight, is formed in a first stage. This initial film can alter the electrostatic charges and

wettability of the metal surface, facilitating its further colonization by bacteria. In a short time (minutes or hours depending on the aqueous environment in which the metal is immersed), microbial growth and EPS production result in the development of a biofilm.

Videla and Herrera (2005), observed that a sequence of inorganic changes takes place at the metal surface immediately after its immersion in an aggressive medium. This sequence involved the process of metal dissolution and corrosion-product formation. According to them both biological and inorganic processes occur within the same time period, but in opposite directions at the metal solution interface. Whereas corrosion and corrosion-product accumulation occur from the metal surface towards the solution, biofilm formation is the result of accumulation processes directed from the bulk towards the metal surface. The consequent corrosion behaviour of the metal varies depending on the degree of this reciprocal interaction.

### 2.4.1 Biofilm formation

Biofilm formation consists of a sequence of steps and begins with the adsorption of macromolecules (proteins, polysaccharides and humic acid) and small molecules (fatty acids and lipids) at surfaces. Adsorbed molecules form conditioning films that alter the physiochemical characteristics of the surface including surface hydrophobicity and electrical charge (Little & Lee, 2007). The amount of adsorbed organic material is a function of ionic strength and can be enhanced on metal surface by polarization. Attachment is due to microbial transport and subsequent binding to surfaces. The extent of adhesion of microorganisms and adhesion pattern depend on microbial characteristics, including cell-surface hydrophobicity and charge, cell size, presence of flagella and pili, and properties of the substratum such as chemical composition, surface roughness, crevices, inclusions and coverage by oxide films or organic coatings, the composition and strength of the aqueous medium and hydraulic flow regime (Little & Lee, 2007).

During the initial stage of biofilm formation, the major factor controlling the rate of colonization is hydrodynamics (Beyenal & Lewandowski, 2002). Microbial colonization begins with the movement of microorganisms to surface mediated by at least three mechanisms:

*i.* Diffusion transport due to Brownian motion

*ii.* Convective movement due to the liquid flow and

*iii.* Active movement of motile microorganisms near the interface.

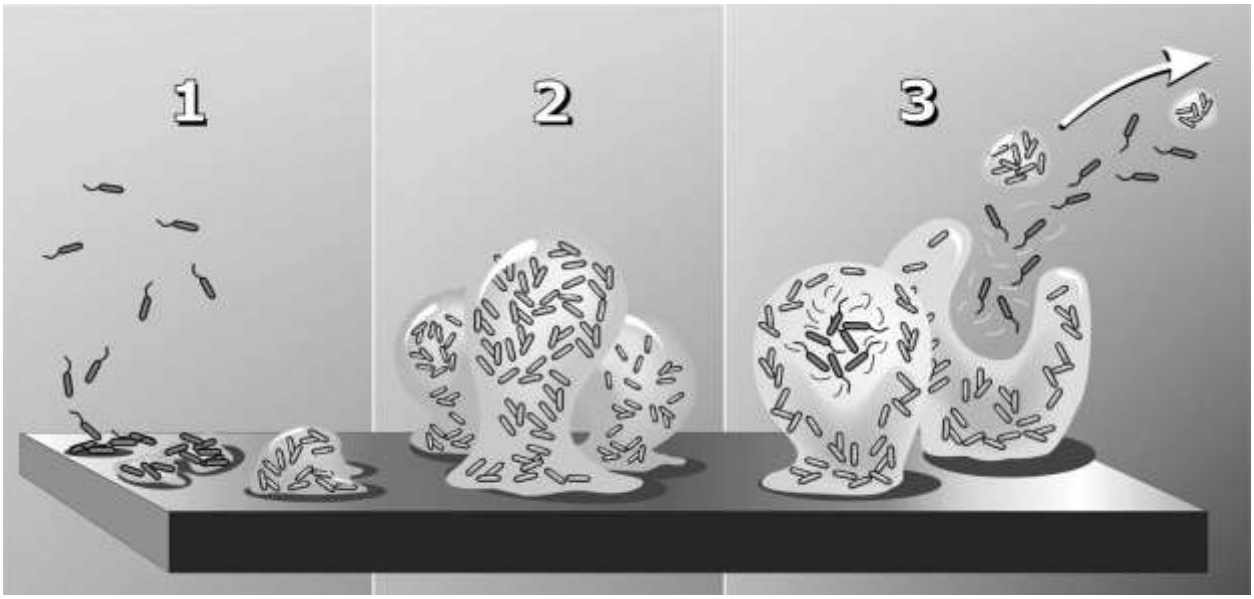



Figure 2.1: The biofilm life cycle in the three steps: 1. attachment, 2. growth of colonies and 3. detachment in clumps or ‘‘seeding dispersal’’ (adopted from Videla 2003).

Once the microbial cell is in contact with a surface, it may or may not adhere. According to Videla (2003), the ratio of a cell number adhering to a surface to the cell number transported to the surface depends on the surface properties, physiological state of the organism and hydrodynamics near the surface.

**2.5 Metal Binding by Extracellular Polymeric Substances**

The capacity of extracellular polymeric substances EPS to bind metal ions is important to microbial influenced corrosion (Kinzler, Gehrke, Telegdi & Sand, 2003; Rohwerder, Gehrke,

Kinzler & Sand, 2003). It depends on both the microorganism species and on the type of metal (Beech *et al.,* 2003). According to Beech *et al.* (2005) metal binding by EPS involves interactions between the metal ions and anionic functional groups (e.g., carboxyl, phosphate, sulfate, glycerate, pyruvate and succinate groups). These are common on the protein and carbohydrate components of exopolymers. The presence of, and affinity for metal ions in different oxidation states in the biofilm matrix can result in substantial shifts in the standard reduction potentials. For example, according to Kinzler *et al.* (2003), Fe (III/II) redox potential varies significantly with different ligands (from +1.2V to -0.4V). EPS-bound metal ions can, therefore act as electron 'shuttles' and open up novel redox reaction pathways in the biofilm/metal system, such as direct electron transfer from the metal (e.g., iron) or a biomineral (e.g., FeS).

Beech *et al*. (2005) observed that in the presence of a suitable electron acceptor (e.g., oxygen in oxic system or nitrate under anaerobic condition), such redox pathways would lead to depolarization of the cathode and thus increased corrosion. Although the presence of metal ions within the biofilm matrix has been acknowledged as pertinent to biocorrosion, the likely involvement of EPS-bound metal ions in direct electron transfer from the base metal to a suitable electron acceptor has been largely overlooked (Beech *et al.,* 2003). According to Kinzler *et al.* (2003), a recent study of iron-hydroxide-encrusted biofilm collected from a subterranean location revealed that bacterial exo-polymers and most likely acidic polysaccharide, could act as a template for the assembly of akaganeite (b-FeOOH) pseudo-single crystals. The observed mineralization was shown to result from the contact between the EPS and oxidized iron through ferric iron binding with carboxylic group on the polymer. The authors pointed out that the oxidation of ferrous ions and the subsequent precipitation of iron oxyhydroxide on the biofilm exopolymers releases protons, leading to decrease in the pH outside the cell membrane. They further maintained that the purpose of polymer production is

to localize iron oxyhydroxide mineral precipitation immediately outside the cell to increase metabolic energy generation of the cell through the enhancement of proton motive force and subsequently corrosion.

### 2.6 Role of Fungal Biofilms and Mycelia in the Corrosion of Metals

Fungi are considered the primary colonizers of surfaces in both natural and man-made environments (Beech & Sunner, 2004). Microbial influenced corrosion is attributed not to planktonic microorganisms, but instead to adherent organisms in the form of biofilms on surfaces (Larsen *et al*., 2010). Majority of biocorrosion investigations have addressed the impact of pure or mixed culture microbial biofilms on corrosion behavior of iron, copper, aluminium and their alloys (Little & Ray, 2002). These organisms typically coexist in naturally occurring biofilms, forming complex consortia on corroding metal surfaces (Baker, Ito & Watanabe, 2003; Kjellerup *et al.*, 2003).

Many fungi adhere to metals and form mat of hyphae on material surfaces, with interactions governed strongly by the material surface properties and adhesion mechanisms (Lichter *et al.*, 2008; Lichter, Van Vliet, & Rubner, 2009). Biofilm formation follows a series of critical steps: an initial rapid and reversible fungal attachment, a more stable, longer term attachment, fungal replication and matrix secretion, biofilm maturation, and finally fungal dispersal (Lichter *et al.*, 2009; Monds & O'Toole, 2009). According Vu, Chen, Crawford and Ivanova (2009), the main structural components of the fungal biofilms are produced by fungi themselves through the secretion of extracellular polymeric substances (EPS). EPS is composed chiefly of polysaccharides, proteins, nucleic acids and lipids. Biofilms can be anywhere from μm to mm in thickness (Zuo, 2007). They can develop and mature over the course of hours, days, or months, depending on the microbial species and environment (Beech & Sunner, 2004). Through these biofilms, the fungi create a unique and sustaining

microenvironment that can differ significantly-in terms of composition and distribution of solids, fluid, and gas from the overall macro environment.

The formation of biofilm itself does not necessitate an increased corrosion rate, and can actually lead to corrosion resistance in some circumstances (Videla & Herrera, 2005). According to Dubiel, Hsu, Chien, Mansfeld and Newman, (2002), some organisms within biofilms can remove corrosion-promoting agents (such as oxygen $O_2$) as part of their metabolism, and can generate physical barriers that protect surfaces from corrosion (Zuo, 2007; Avidan, Satanower, & Banin, 2010).

Biofilm is composed of four components: the surface to which the microorganisms are attached, the biofilm (the microorganisms and matrix), the solution of nutrients, and the gas phase. Each compartment consists of several components, and the number of components may vary depending on the type of study. For example, in biocorrosion studies it is convenient to distinguish four components of the surface: (1) the bulk metal, (2) the passive layers, (3) the bio-mineralized deposits on the surface, and (4) the corrosion products. Fungi and indeed microorganisms can modify each of these components in a way that enhances corrosion of metal surface (Lewandoski & Beyenal, 2008). Not all biofilms are harmful for structures they use for anchoring (Obuekwe, Westlake, Cook & Costerton 1981). Rather some members of biofilms appear to be beneficial and exhibit corrosion protective roles. Zuo *et al.* (2005) observed that aluminum (Al2024) was passive in artificial seawater (AS) in the presence of protective biofilm of *Bacillus subtilis.* After the administration of antibiotics in the AS to kill the bacteria, pitting corrosion was noticed within few hours, indicated by characteristic changes in the impedance spectra that was used (for the estimation of corrosion). Jayaraman, Earthman, and Wood, (1997) also reported that *Pseudomonas frngi* biofilms on SAE1018 steel decreased the corrosion rate compared to sterile controls by two to ten folds over a period of four weeks in batch reactors. The corresponding decrease in

continuous reactor was also reported to be 40folds (Jayaraman *et al.,* 1999c). According to Zuo *et al.* (2005), many aerobic bacteria have been shown to decrease the rate of mild steel corrosion due to biofilm formation.

When biofilm accumulate on the metal surfaces, reactants and products of fungal metabolic reactions occurring in the space occupied by the biofilm affect the solution chemistry and the surface chemistry, and both types of modification may interfere with the electrochemical processes naturally occurring at the interface between the metal and its environment (Coester & Cloete, 2005; Lewandowski & Beyenal, 2008). According to them, the reactants and products of electrochemical reactions occurring at a metal surface interact with reactants and products of microbial metabolic processes occurring in the biofilms in a complex way. Some of these interactions accelerate corrosion and some may inhibit corrosion. The interactions that accelerate corrosion, and are characteristic enough, are called mechanisms of microbial influenced corrosion. They further observed that corrosion reactions can be modified by the metabolic reactions in biofilms in different ways.

### 2.6.1 The biofilm as a chemical barrier

The existence of a biofilm can impede the diffusion of chemical species- both into and out of the biofilm thus producing localized chemical environments that are significantly different from the entire environment (Beech and Sunner, 2004). According to Beech and Sunner (2004), these localized environments can in turn, greatly affect the underlying corrosion in a number of ways. In aerobic condition, oxygen concentration may differ greatly across the biofilm due both to $O_2$ diffusion limitations as well as $O_2$ consumption by aerobic microorganisms (Rohwerder *et al.,* 2003). Beech and Sunner (2004), further observed that even in an aerated environment, anaerobic regions can develop beneath a biofilm allowing for the proliferation of SRB and other corrosion enhancing anaerobes.

By inhibiting the diffusion of corrosive chemicals away from the metal surface, biofim can effectively act as a concentration multiplier (Beech & Sunner, 2004). For example, Beech and Sunner (2004), observed that the $H_2S$ generated by SRB in anaerobic region is potentially more potent when encapsulated beneath a biofilm than otherwise. Koch *et al.* (2002), demonstrated a significant difference in the rate of corrosion between an SRB colony and a sterile medium to which an equivalent quantity of sulfide was periodically added. In the former case, the biogenic sulfide was produced beneath a biofilm and near the metal surface, while in the latter case the abiotic sulfide was added directly to the bulk solution. In addition to concentrating the organic acid produced by fungi on metal surface, the biofilm can also concentrate the corroded ferrous ions a key factor in biocorrosion (Lee & Characklis, 1993).

### 2.6.2 Physical Structure of Biofilm

The physical structure and the heterogeneous nature of the biofilm also contribute to the corrosion process (Rickard & LutherIII, 2007). Biofilms are often patchy and of varying thickness at the microscale. These create isolated pockets of oxygen depletion which can instigate localized corrosion through the establishment of differential aeration cells (Picioreanu & van Loosdrecht, 2002). Roe *et al.* (1996), demonstrated this effect by placing a µm-thick patch of agarose- a biogel with an oxygen diffusivity that is slightly lower than that of water –on to a low- carbon steel coupon, and observed localized corrosion beneath the abiotic film. Additionally, it has been proposed that enzyme embedded within the biofilm– such as hydrogenase–or other chemical properties of biofilm may also enhance corrosion (Beech & Sunner, 2004).

According to Videla and Herrera (2005), non-uniform or patchy colonization by microbial biofilms results in the formation of differential aeration cells, where areas under respiring colonies are depleted of oxygen relative to surrounding non-colonized areas. These effects

give rise to potential differences and, consequently, to corrosion currents. The areas under respiring colonies become anodic and there metal dissolution occurs (Videla, 2003).

### 2.6.3 Change in the Oxidation-Reduction condition

Biofilms can alter the oxidation-reduction conditions at the metal-solution interface (Videla & Herrera, 2005; Beech *et al.,* 2005). Diffusion of oxygen in oxic aqueous media is frequently impeded by the diffusion and reaction resulting from aerobic metabolites within the biofilm (Beech, 2004; Videla, 2000). Lewandowski and Beyenal (2008), observed that microelectrode measurements in biofilm that accumulates in a flow indicate that the dissolved oxygen can decrease to zero at a distance of only 180μm from the metal surface. According to them, SRB which need a reducing environment to grow can proliferate at the bottom of biofilms despite a measurable dissolved oxygen concentration on the surface of the metal. It therefore means that redox conditions within the biofilm and at the biofouled metal surface are closely related to the respiration and metabolic activity of sessile microorganisms (Videla & Herrera, 2005).

**Change in inorganic passive layers**

Biofillms can alter the structure of inorganic passive layers thereby increasing their dissolution and removal from the metal surface (Videla, 2000). Some metabolic activities of fungi within the biofilm may remarkably affect the passive layers on metal surfaces thereby enhancing corrosion. Another example is the reducing capacity of ferric to ferrous ions inherent to some microbial by-products (Gentil, 1988). In carbon steel surfaces immersed in saline medium, localized attack preferentially occurred (Videla, 2003), beneath microbial colonies as a consequence of differential aeration between covered and uncovered areas due to microbial reduction of insoluble ferric deposits into soluble ferrous compounds (Videla & Herrera, 2005).

### 2.6.4 Enhancement of corrosion rate

According to Beech and Coutinho (2003), biofilms enhance corrosion rate through the creation of oxygen concentration or differential aeration cells caused by the patchy distribution of microbial colonies and their products. As a result, localized electrochemical corrosion cells are established. Oxygen concentration gradient is created in the surface biofilm due to the consumption of oxygen by aerobes. Thus, deeper layers of the biofilm can turn anaerobic, which is a habitat for the growth of SRB (Beech *et al*., 2005). The EPS, which constitutes the main mass of biofilm and underpins these heterogeneities and microenvironments, can also influence corrosion more directly by binding and /or retention of corrosion products, called the chelation of metal ions by the EPS (Hamilton, 1998b). According Videla (2003), the surface of the metal where the biofilm is, may become a substrate with respect to acting as a source of metallic energy. Other ways include alteration of corrosion inhibitor's stability at the metal surface, modification of the medium's conductivity and inhibition of biocide action (Okezie *et al*. 2013).

## 2.7 Types of Microbial Corrosion

Microorganisms such as fungi, bacteria and algae under certain conditions can thrive and enhance the corrosion of metals and their alloys by their physical and direct involvement in the corrosion reactions (Lugauskas *et al.,* 2008). They also can accelerate the process by the production of extracellularly produced metabolic products resulting in the formation of acids (e.g., sulphuric, carbolic or organic acids), hydrogen sulfide and ammonia. Microorganisms can also impact corrosion process singularly or in combination with other mutualistic organisms in aerobic and anaerobic conditions.

### 2.7.1 Aerobic microbial corrosion

Aerobic microbial corrosion is the corrosion caused by microorganisms in aerobic conditions and is often as a result of the production of corrosive metabolites, usually an acid, either mineral or organic (Geesey *et al.*, 2000). In aerobic corrosion, oxygen concentration can be very low, for instance underneath microbial colonies or biofilms (De Beer & Stoodley, 2000). The anodic dissolution of Fe to $Fe^{2+}$ preferentially takes place at such micro-oxic to anoxic sites, whereas electrons flow to the other sites where they can reduce molecular oxygen (Gu & Mitchell, 2000). According to Uhlig (2011), the $Fe^{2+}$ formed may be oxidized chemically or by iron-oxidizing bacteria to hydrates of ferric oxides that are deposited as rust on the metal surface.

Fungi and some slime-producing bacteria like *Pseudomonas* sp are commonly found in connection with aerobic corrosion. They colonize the metal surface, thereby creating oxygen-free environments for anaerobic bacteria, especially SRB (Videla, 2001). Exopolymeric substances (EPS) excreted by these microorganisms may contain organic acids and salts at high concentration which may stimulate metal deterioration. According to Gu and Mitchell (2000), some groups of aerobic bacteria also produce strong inorganic acids and thus become very corrosive toward iron. The most significant group is the genus *Thiobacillus* members which produce sulfuric acid by oxidizing sulfur species (Kavita *et al*., 2011). *Thiobacillus thiooxydans* and *Thiobacillus ferrooxydans* are the most common representatives that have been reported to be involved in corrosion (Gu & Mitchell, 2000). Another group that may contribute to aerobic corrosion is the stalked bacteria of the genus *Gallionella* and filamentous bacteria of genera *Leptothrix, Clonothrix, Sphaerotilus, Crenothrix* and *Lieskeella* (Monds & O'Toole 2009). Members of this group may gain energy from the oxidation of ferrous to ferric ion, or at least stimulate such a process which results in massive deposition of ferric hydroxide. As a consequence, condensed anoxic zones are formed and the

metal surface is partitioned into small anodic sites exposing large cathode areas where electrons reduce the available oxygen (Rao, Sairam, Viswanathan & Nair, 2000; Starosvetsky, Armon, Yahalom & Starosvetsky 2001). Sulfur-oxidizing bacteria (SOB) are other group of microorganisms that can produce sulfuric acid from the oxidation of sulfur or sulfide.

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow FeSO_4 + 2H_2SO_4 \qquad (2)$$

In fuel and oil storage tanks fungal species such as *Aspergillus*, *Penicillium* and *Fusarium* may grow on fuel components and produce carboxylic acids which corrode the iron (Little *et al.*, 2001). In the presence of light algae can produce organic acids and decrease the pH in the environment, thereby favoring corrosion.

### 2.7.2 Anaerobic microbial corrosion

Metal and their alloys corrode easily in oxygen-free environments (Cord-Ruwisch, 2000). Pipelines, offshore oil platforms and underground structures have been reported to be quite vulnerable to biological corrosion which is assumed to be mediated by different groups of microorganisms respiring with oxidized compounds such as sulfate, nitrate, ferric iron or carbon dioxide. Sulfate-reducing bacteria (SRB) are chiefly responsible for anaerobic corrosion particularly in environments with high sulfate concentration such as sea water (Cord-Ruwisch, 2000). The mechanism by which sulfate reducers accelerate metal corrosion has attracted many investigations, but details of the process are still inadequately understood (Cord-Ruwisch, 2000).

According to Lee *et al.* (1995), the corrosiveness of these organisms is partly due to their metabolic product $H_2S$ and partly due to a supposed more direct electrochemical effect

termed cathodic depolarization. Hydrogen sulfide $H_2S$ accelerates metal corrosion by acting as a source of bound protons and by precipitation of $Fe^{2+}$ as FeS (Lee *et al.,* 1995).

$$Fe + H_2S \rightarrow FeS + H_2 \quad (3)$$

$$Fe_2^{+} + H_2S \rightarrow FeS + 2\,H^{+} \quad (4)$$

The formed $H_2$ may be utilized further by SRB or by other $H_2^{-}$ scavenging microorganisms.

According to Cord-Ruwisch (2000) sulfate reducers particularly species of genus *Desulfovibrio,* are able to utilize $H_2$ rapidly and with high affinity, even at an $H_2$ partial pressure down to approximately 0.02 Pa. The enzyme hydrogenase ($H_2$ase) catalyses the reversible reaction

$$H_2 \leftrightarrow 2\,H^{+} + 2e^{-} \quad (5)$$

The enzyme has been investigated biochemically as well as genetically mostly in *Desulfovibrio* sp.

**2.8 Corrosion by Cathodic Depolarization**

The most discussed mechanism of microbial corrosion is a depolarization via oxidation of cathodic hydrogen as formulated in the cathodic depolarization theory (Chris, David, & Paul, 2017). In contact with water, metal becomes polarized by losing positive metal ions (anodic reaction). In absence of oxygen, the liberated electrons reduce water-derived protons (cathodic reaction) to form hydrogen that remains on the metal surface, where a dynamic equilibrium is assumed to be established (Chris *et al.*, 2017). Fungi and sulfate –reducing bacteria are able to remove the formed hydrogen so that a net oxidation of metal takes place. According to Gu and Xu (2010), it does not become clear whether the depolarization is due to the consumption of atomic or molecular hydrogen.

The net reaction of corrosion is as follows:

$$4\,Fe + SO_4^{2-} + 3HCO_3^- + 5H^+ \rightarrow FeS + 3FeCO^3 + 4H_2O \qquad (6)$$

Experimental evidence to support the cathodic depolarization theory has been provided mostly with *Desulfovibrio* sp because they utilize $H_2$ very effectively and can be easily cultivated under laboratory conditions (Chris *et al*., 2017). Cord-Ruwisch (2000), also demonstrated the oxidation of cathodic hydrogen with sulfate in different growing cultures of hydrogenase –positive *Desulfovibrio* sp. These authors revealed that the process occurred only if an organic electron donor such as lactate was present. It was supposed that a simultaneous utilization of $H_2$ and the organic substrate took place. The rate of corrosion was found to be directly proportional to the metabolic activity of *Desulfovibrio* strains.

**2.9 Mechanism of Microbial Corrosion and Classification**

An important aspect of quantifying mechanism of microbial influenced corrosion and indeed fungal corrosion of metal is to demonstrate how the microbial reactions interfere with the corrosion processes (Lewandowski & Beyenal, 2008). There is no universal mechanism of microbial influenced corrosion (Beech & Sunner, 2004; Lewandowski & Beyenal, 2008). Instead, many mechanisms exit and some of them have been described and quantified better than others (Lewandowski & Beyenal, 2008). According to Beech (2003) it is known that mechanical and structural factors all play a role, both biotic and abiotic in nature, and these may all work in concert to cause and sustain microbial influenced corrosion. Among the first proposed mechanism for microbial influenced corrosion is the cathodic depolarization theory- also known as the classical theory – which was put forth by Kuhr (Chris *et al*. 2017) in order to explain the unexpected high rate of corrosion failure encountered on buried cast iron pipelines in the Dutch countryside. Beech and Sunner (2004) stated that in anaerobic conditions where SRB thrive, hydrogen ions typically serve as the terminal electron acceptor

at the cathode in a corrosion reaction. The reduced hydrogen would then adsorb onto the metal surface, polarizing it. According to the classical theory, the role of SRB was to consume this cathodic hydrogen by means of enzyme hydrogenase, catalyzing the recombination of the adsorbed atomic hydrogen gas, thus depolarizing the cathode. However recent studies have also reported the participation of fungi in cathiodic depolarization reaction (Videla, 2001).

According to Beech and Sunner (2000), various mechanisms of biocorrosion, which reflect the variety of physiological activities carried out by different types of microorganisms, have been identified. And the diversity of these mechanisms is such that it is difficult to expect that a single unified concept can be conceived to bring them all together (Lewandowski & Beyenal, 2008). From what has been demonstrated by numerous researchers, accelerated corrosion of metals in the presence of microorganisms stems from the microbial modifications to chemical environment near metal surfaces (Beech *et al.*, 2005; Geiser *et al.*, 2002; Lee & Newman, 2003). Such modifications depend, of course, on the properties of the corroding metal and on the microbial community structure of the biofilm deposited on the metal surface (Beech & Sunner, 2004; Olesen, Avci, & Lewandowski, 2000; Olesen, Yurt, & Lewandowski, 2001).The conclusion that there are many mechanisms of microbial influenced corrosion, rather than a single one, is generally accepted in the literature and was exemplified by the paper by Starosvetsky *et al.* (2007),who concluded that to uncover MIC in technological equipment failures require an individual approach to each case, and that an assessment of the destructive role of the microorganisms present in the surrounding medium is possible only by analyzing and stimulating the corrosion parameters found in the field (Lewandowski & Beyenal, 2008).

Quite concisely, Beech *et al*. (2005) described microbial influenced corrosion as a consequence of coupled biological and abiotic electron-trasfer reactions i.e., redox reactions

of metals enabled by microbial ecology. Emde, Smith, and Facey, (1992) isolated iron-reducing fungi from tubercles in a water distribution system suggesting another mechanism whereby corrosion may be accelerated by this group of microorganisms. According to Gentil (2007), the action of microorganisms in the processes of corrosion can occur through one or more of the following: (a) Formation of biofilms that enable the emergence of differential aeration cells, (b) Formation of corrosive media due to the acids generation, (c) Modification on the resistance of films exiting on the metal surfaces caused by products of microbial metabolism and (d) Direct influence on the speed of anodic and cathodic reactions.

### 2.9.1 Formation of biofilms that enables the emergence of aeration cells

Fungi and other microorganisms influence the corrosion of metal through the formation of differential aeration cells at different locations on the metal surface (Lewandowski & Beyenal, 2008). The effect of different concentrations of oxygen at different locations on the metal surface can be caused by the active consumption of oxygen by microorganisms in biofilms not uniformly distributed on the metal surface. It can also be caused by passive mechanism in which oxygen access to some areas is physically obstructed. According to Lewandowski and Beyenal (2008), if the oxygen concentrations at two adjacent locations on an iron surface are different, then the cell potentials at these locations are different as well. The location where the oxygen concentration is higher will have a higher potential (more cathodic) than the location where the oxygen concentration is lower (more anodic). The different in potential will give rise to current flow from the anodic locations to cathodic locations and to the establishment of corrosion cell. This is the mechanism of differential aeration cells, and the prerequisite to this mechanism is that the concentration of oxygen varies among location (Acuna, Ortega-Morales, & Valadez-Gonzalez, 2006; Dickinson & Lewandowski, 1998; Hossain & Das, 2005). According to Lewandowski and Beyenal (2007), many measurements using oxygen microsensors have demonstrated that oxygen

concentrations in biofilms can vary from one location to another. For example, fungal growth on metal surface can partially seal the surface leading to the formation of bubbles. These bubbles will isolate a certain part of metal surface, a partial electrochemical corrosion is established under the bubble and this kind of corrosion is free from cathodic protection. Under suitable conditions, the metal surface under the bubble will become lively and turn into a pole of electrochemical cell while others outside the bubble will turn to another pole of the cell thereby further enhancing corrosion.

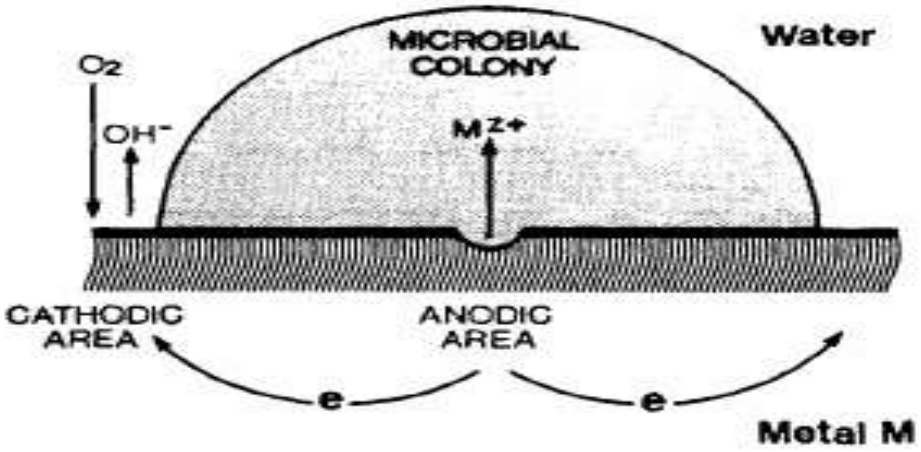



Figure 2.2 (a) Beneath a microbial colony (Videla, 2001)

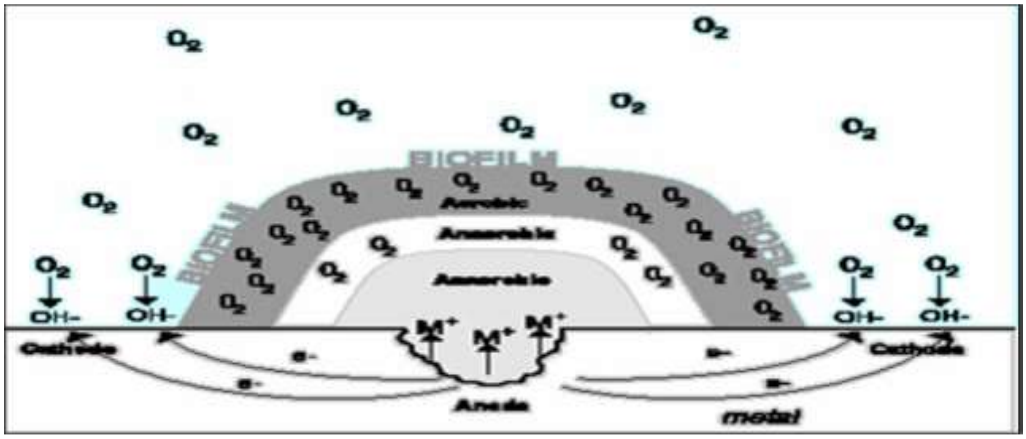



Figure 2.2 (b) Caused by heterogeneous biofilm

Diffrential aeration cells *(http://www.edstrom.com/DocLib/biofilm.pdf*)

### 2.9.2 Formation of Corrosive Media Due to Acids Generation

Fungi generally acidify their microenvironment via a number of mechanisms, which include the excretion of protons and organic acids, respiratory $CO_2$ can result in carbonic acid formation. In addition, fungi excrete a variety of other metal-complexing metabolites (e.g., siderophores, carboxylic acids, amino acids and phenolic compounds (Burgstaller & Schinner, 1993)). The acids produced by fungi are damaging to metal and their alloys (Videla, 2003). This mechanism of MIC is closely related to the type of microorganisms active in the biofilm and to their metabolic reactions (Beech 2003; Videla & Herrera, 2005; Xu *et al.,* 2007). Lugauskas & Beyenal (2008) reported the corrosion of metal surface by *Alternaria alternata, Aureobasidium pullutans* and *Fusarium proliferatum* species. They also confirmed the capacity of fungi mycelia to damage the conductive polymeric coating of polyaniline-modified nickel.

### 2.9.3 Modification on the resistance of films exiting on the metal surfaces caused by products of microbial metabolism

Biofilms facilitates the removal of protective films on the metal surface when they detach (Videla & Herrera, 2005). Videla (2001), observed that fungi grow rapidly on the polarized metal surface. Their continual outspread will generate pressure to the metal leading to the rupture of surrounding coatings. Copper-nickel alloys in seawater can be colonized by fungi and other organisms after extended periods of exposure despite their perceived anti-fouling properties. The detachment of the biofilms can cause the protective coating of metals to fall off. Videla (2003), also observed that biofilm formation on metal surfaces is mostly conditioned by the chemical nature and distribution of inorganic passive layers and by the elemental composition of the substratum (Cetin & Aksu, 2003). They further started that several months after exposure; fungal mycelia can be found entrapped between layers of corrosion products and EPS in a layered structure. Biofilm detachment might facilitate the

removal of inorganic passive layers, resulting in a patchy distribution of the biofilm which can further lead to corrosion.

Mat of fungal mycelia, observed sometimes as the predominant biofouling species, can facilitate passive layer detachment through adhesive effects developed at the fixation points and assisted by water flow velocity (Videla & Herrera, 2005) or through other physical means.

### 2.9.4 Direct Influence on the Speed of Cathodic and Anodic Reactions

The existence of a fungal biofilm can impede the diffusion of chemical species-both into and out of the biofilm-thus producing localized chemical environments that are significantly different from the bulk. These localized environments can in turn, greatly affect the underlying corrosion and also directly influence the speed of cathodic and anodic reactions (Beech & Sunner, 2004). By inhibiting the diffusion, the corrosive chemicals away from the metal surface, the biofilm can effectively act as a concentration multiplier (Rohwerder *et al.*, 2003). The organic acid generated by fungi in aerobic region is potentially made more potent when encapsulated beneath a biofilm than otherwise. In addition to concentrating the organic acids, the biofilm can also concentrate the corroded ferrous ions, a key factor in microbial influenced corrosion.

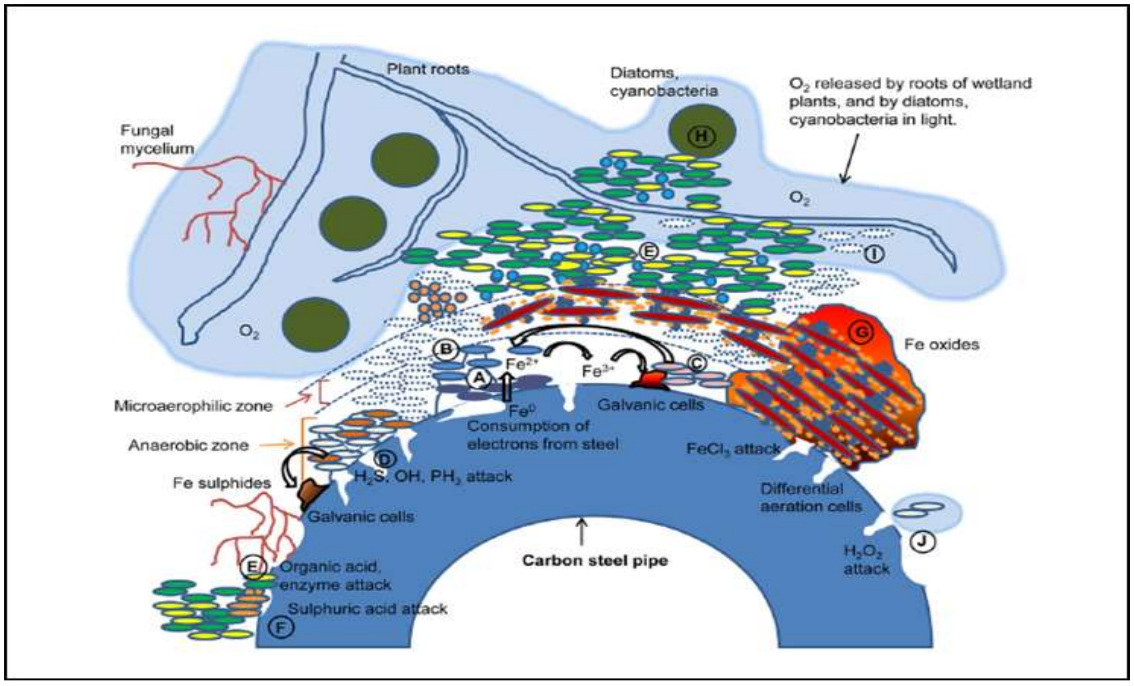



Figure 2.3 Systematic description of microbial influenced corrosion (Copied from Usher *et al.*, 2014)

### 2.9.5 Classification of Microbial Influenced Corrosion

**Type I**

Microbial influenced corrosion can be classified into three categories to distinguish the various mechanisms (Gu 2012a; Gu & Xu, 2013). According to them, type I microbial influenced corrosion is caused by electrogenic bacteria. Electrogens are microbes that are capable of cross-cell wall electron transfer (*Gu et al.*, 2015). Electrogenic bacteria can actively form pili for electron transfer and energy distribution and perform respiration metabolism (Chang *et al.*, 2003). In this class, microbial influenced corrosion is caused by the respiration of microbes using elemental iron (or other active metal) as electron donor. In other to reduce the oxidants, microbes extract electrons from elemental iron and transport them across cell wall into cytoplasm (Gu *et al.*, 2015). The SRB and NRB that attack carbon

steel belong to Type I microbial influenced corrosion metabolism (Chang *et al.,* 2003; Gu *et al.,* 2015).

**Type II**

Type II microbial influenced corrosion is defined as the corrosion caused by the metabolites secreted by microorganisms. These metabolites are oxidants such as volatile fatty acids. The microbial influenced corrosion caused by fungi and fermentative bacteria such as acid producing bacteria (APB) belongs to this category (Gu & Xu, 2012a). Here protons and undissociated organic acids are extracellularly reduced on the metal surfaces without catalysis using the electrons released by the oxidation of elemental metals (e.g., $Fe^{o}$). Fermentative microorganisms cause Type II microbial influenced corrosion by secreting organic acid such as HAc and other fatty acids. The free (i.e., undissociated) organic acids are corrosive. Corrosion by these oxidants also occurs abiotically like the same way as conventional acid corrosion (Gu & Xu, 2012a).

**Type III**

In other to have the energy and carbon sources, some bacteria attack organic polymers (Gu & Xu, 2013). Type III microbial influenced corrosion is caused by microorganism that secret enzyme that degrades the extracellular organic substances of materials and plasticizers in the polymers as nutrients. It is a non electrochemical corrosion, also known as biodegradation (Gu, 2012a). Anaerobic and aerobic microbes are able to cause Type III microbial influenced corrosion.

## 2.10 Microbiologically Influenced Corrosion and Mineral Precipitation

According to Enning and Garrelfs (2014) two microbial infulenced corrosion mechanisms electrical microbial influenced corrosion (EMIC) and chemical microbial influenced

corrosion (CMIC) are distinguished to have direct effect on mineral precipitation and these are galvanic cells and acid attack. The process of biocorrosion releases metallic ions from the metal surface and creates a concentration gradient towards the reaction region. Released metallic ions react with anions present and induce a concentration gradient of anions from the distant boundary to the reaction region. Enning and Garrelfs (2014), observed that the concentration gradients lead to molecular diffusion from both metal surface and distant boundary towards the reaction region and result in mineral precipitation as well as biomass formation.

### 2.10.1 Effects of galvanic cells on mineral precipitation

Fungi and indeed microorganisms have the ability to precipitate and adsorb a variety of dissolved metal ions into their cells (Mc Lean, Lee & Beveridge, 2002) which is favorable to the formation of galvanic cells between the metal surface and biofilm. According to Konhauser (2009), the presence of galvanic cells can alter the conductivity of low frequency currents. This effect is essential for the partial separation of oxidative (anodic) and reductive (cathodic) reactions. Javaherdashiti (2008), observed that different precipitated minerals result in different functions of formed galvanic cells. In terms of iron sulphide precipitation for example, the formed galvanic cells are actually moving the corrosion potential into negative direction and thus a higher corrosion rate. If iron carbonate is precipitated, the galvanic cells can even be protective to further corrosion due to high resistance of carbonate to the free flow of electrons (Chan, 2011). The type and magnitude of precipitated minerals are therefore critical for the unset of fungal influenced corrosion.

### 2.10.2 Effects of acid attack on mineral precipitation

With the presence of fungi on the surface of metal, various byproducts of fungal activities including organic acids are produced. The presence of acids increases the corrosion rate by

changing the local pH via hydrogen permeation (Biezma, 2001). In addition to the corrosive effects of produced acids, the acidity of local environment is also essential for the stability of minerals. With a drop in pH, the solubility of some minerals, for instance iron carbonate, increases remarkably. Huang, Bollag and Senesi, (2002) observed that organic acids can increase the mineral dissolution rate by 2-4 times compared to rain water. Once the protective barrier, such as iron carbonate is dissolved by the acid, iron will be re-exposed to corrosive agents and this will lead to the reestablishment of a high corrosion rate. There the acidity of an environment is one of the keys to understand the interaction between microbial influenced corrosion and mineral precipitation.

### 2.11 Corrosion Inhibition by Microorganisms

Corrosion inhibition is the slowing down of the corrosion reaction and is usually performed by substances (corrosion inhibitors) that when added in small quantities to a given environment, decrease the rate of attack by this environment on a metal (Videla & Herrera, 2005). Many inhibitory substances produced by microorganism are known. For example, gramicidin-S a residue cyclic peptide is a very potent antimicrobial peptide produced by bacteria isolated from soil. It is effective in killing a broad range of bacteria and fungi (Lee & Newman, 2003). Although the antimicrobial action of the peptide is not clear, it is believed that it interacts with cell membrane, resulting in the rapture and leakage of cellular contents. Videla (2003), observed that microbial inhibition of corrosion is hardly mentioned in the literature. According to him microorganisms can contribute to corrosion inhibition by different mechanisms. These include neutralizing the action of corrosive substances present in the environment, forming protective films or stabilizing a pre-existing protective film on a metal and inducing a decrease in the medium corrosiveness. Videla (2003), further observed that the general key features of microbial inhibition of corrosion shows that microbial inhibition of corrosion is rarely linked to a single mechanism of a single species of

microorganisms. He noted that the main mechanism of microbial corrosion inhibition is always linked to a marked modification of the environmental conditions at the metal-solution interface due to biological activities (Videla & Herrera, 2005; Videla, 2003).

According to Videla *et al*. (2002), microbial corrosion inhibition is frequently accomplished through a decrease in the cathodic rate by microbial consumption of a cathodic reactant (e.g., oxygen consumption by respiratory activity), decreasing of the medium aggressiveness in restricted areas of the metal solution interface (e.g., by neutralizing acidity) and providing or stabilizing protective films on metal (e.g., biofilm exoplymers with metal binding capacity). Videla (2003), observed less weight loss for carbon steel in contact with dense suspension of living bacteria than in a similar suspension of dead bacteria. They suggested that protection is the result of metabolic activity, including decreased oxygen. Attached cells may also form diffusion barrier to and from the metal surface (Beech, 2003). Little *et al*. (1992) stated that under certain conditions, SRB could initiate a passive sulfide film on metal surface. Videla (2003) reported corrosion inhibition due to bacteria films and possible role of exopolymers. Within naturally-occurring biofilms complex interactions occur. Some interactions will accelerate and others may inhibit corrosion (Little *et al*., 1992).

Researchers at University of Connecticut, University of Southern California and University of California at Irvine evaluated the concept of Corrosion Control Using Regenerative Biofilms (CCURB) for a variety of materials such Al 2024, mild steel, and cartridge brass in laboratory test (Mansfield *et al.,* 2001; Oerneck *et al.,* 2001) as well as field test (Earthman *et al.,* 2001). According to them the results showed that two strains of *Shewanella* produced microbiologically influenced corrosion inhibition (MICI) of Al 2024, brass and mild steel in artificial sea water. These results suggest that MICI is a more common phenomenon than previously assumed. Al 2024 is very susceptible to pitting corrosion in seawater; however, it

has been found that a number of microorganisms are able to prevent pitting of Al 2024 in artificial seawater (Mansfield *et al.*, 2001).

### 2.11.1 Corrosion inhibition by biofilms

Not all biofilms are harmful to structures they use for anchoring. Rather some biofilms appeared to be beneficial and exhibit corrosion protective roles. There have been a few reports of corrosion inhibition in the presence of biofilms. Zuo *et al.* (2005) observed that aluminum (Al 2024) was passive in artificial seawater (AS) in the presence of protective biofilm of *Bacillus subtilis*. After the administration of antibiotics in AS to kill those bacteria, pitting corrosion occurred within few hours, indicated by the characteristic changes in the impedence spectra used for the estimation of corrosion.

Many aerobic bacteria have been shown to decrease the rate of mild steel corrosion due to biofilm formation (Oernek *et al.,* 2001). According to Zuo *et al.* (2005), experiments employing genetically engineered bacteria capable of producing inhibitors-polyglutamate or polyasparate has been reported. Videla *et al.* (2002), speculates that under certain conditions, SRB could initiate a passive sulfide film on metal surface.

## 2.12 Control of Microbial Corrosion

One of the classic concepts for maintaining an industrial system free of the deleterious effects of MIC is ‘to keep the system clean’ (Videla, 2002). Although in practice this is a very difficult task, several general methods both physical and chemical can be used. MIC is acknowledged to occur on wide range of metals; however, most reported failure analysis has focused on iron, copper, aluminum and their alloys (Beech, 2003). There are various approaches to prevent underground and underwater corrosion, though all suffer from certain disadvantages. Videla (2003), suggested that use of non-corrodible material such as high silicon iron, austenitic chromium, nickel steels, asbestos cement and plastic (PVC and

polyethylene) which have shown high corrosion resistance. But these non-metallic materials are not without problems of their own, including microbial degradation. Application of protective coatings to prevent the growth of SRB is another way to retard corrosion. According to Videla (2002), a non-aggressive surrounding for a pipeline in natural environments can be made by completely surrounding it with at least 25 cm thick layer of chalk.

### 2.12.1 Methods involved in the control of biocorrosion

The methods employed to prevent biocorrosion act by inhibiting the growth or metabolic activity of microorganisms changing the environment in which the corrosion process occurs in order to avoid the adaptation of these bacteria (Videla, 2002). Physical (cleaning procedures) and chemical methods (sanitization through the use of biocides and antifouling coatings such as inks or corrosion inhibitors) are used generally combined to improve the procedure (Videla, 2002; Videla & Herrera, 2005).

**Physical Methods**

Microbiologically influenced corrosion is mitigated primarily using the old-fashioned "spray and scrub" strategy (Gu *et al*., 2015). Physical methods include flushing, which perhaps is the simplest, although of limited efficacy (Videla & Herrera, 2005). A special case is the use of flushing supported by cleaners or jointly with chemical agents that induce biofilm detachment. Abrasive or non-abrasive sponge balls are frequently employed in industry (Videla, 2002). However abrasive sponge balls can damage protective passive films and non-abrasive sponge balls are not very effective with thick biofilms. In pipelines, pigs can be deployed to scrub the internal pipeline surface and to spray biocides. Some pipelines especially the older ones are not designed for pigging due to their tight turns and other pig-unfriendly structures (Gu *et al*., 2015).

### *i.* Chemical Methods

The most common chemical method for controlling microbial influenced corrosion and biofouling in industrial water systems is the use of biocides (Videla & Herrera, 2005). The addition of a site-specific combination of chemicals to an operation system is known as chemical treatment, which may include agents designed to inhibit corrosion, scavenge oxygen, alter pH, control scaling and microbial growth or their activities (Beech & Sunner, 2004). Corrosion inhibitors can be either oxidizing compounds (e.g., chromate, nitrate and molybdate), which encourages the production of low solubility corrosion product films on the metal surface or non-oxidizing toxicants (carbonates, silicates and phosphates), which form protective films or adsorption type inhibitors (highly polar, anionic organic molecules) which absorb on metal (Videla, 2001). Chlorine, ozone and bromine are three typical oxidizing agents of industrial use. Non-oxidizing biocides are reported to be more effective than oxidizing biocides for overall control of algae, fungi and bacteria as they are more persistent, and many of them are pH-independent (Videla, 2002). Combinations of oxidizing and non-oxidizing biocides or of two non-oxidizing biocides are often used to optimize the microbiological control of industrial water systems.

In many cases, as in pipelines and storage systems, sessile microorganisms are the major cause of microbiologically influenced corrosion. An effective approach has been to use product, such as quaternary amines, that prevent or inhibit such fungi and bacteria from attaching to pipeline wall (Videla, 2002). According to Videla and Herrera (2004), dichloromethyl hydanton, chlorinated isocyanurates, calcium hyochlorite (oxidizing biocides) and acrolein, chlorinated phenols, quaternary ammonium compounds, methylene bisthiocyanate, hexachlorodimethyl carbonate (non-oxidizing biocides) are used to retard the growth of different types of microorganisms.

Some biocides are administered in pure forms, while others are formulated in solutions or carriers (Videla & Herrera, 2004). According to Videla (2002), prescreening of potential biocides is commonly done before implementation to ensure site-specific performance. They further observed that using combinations of biocides at a lower concentration than they would have been used individually can extend the range of treatment and reduce cost. Switching biocide products periodically can restore effective control in a system developing resistance to the continuous use of one product (Videla, 2002). According to Lee and Newman (2003), design of a biocide treatment must also include the safe disposal of used treatment fluid. These trends continue to challenge those involved in biocide treatment to come up with new products and approaches that are cost-effective and environmentally acceptable.

Lee and Newman (2003), suggested that glycol/seawater mixtures containing 50% glycol would inhibit corrosion of stainless steel. Mixture containing concentration of 50% propylene glycol-based coolant inhibits pitting corrosion. According to them, a slightly higher concentration (55%) was found for corrosion protection in ethyl glycol mixture.

### *ii.* Protective or antifouling coating

Buried structures and interior of tanks and piping are often protected from corrosion by the application of protective coating (Videla, 2003). Protective coatings can be used to prevent microbial influenced corrosion on metal surfaces exposed to soil or natural water or internal surface in contact with operating fluids (Videla, 2002). According to Videla (2002), coating buried structures with asphalt, enamel, plastic tape or concrete is frequently used to prevent contact between the steel structure and the environment. An ideal coating substance should be adherent, coherent, completely nonporous, mechanically resistant to hazards encountered during delivery, laying and backfilling and chemically resistant to prolonged contact with all kinds of natural environments (Videla, 2003).

Protective coatings are subject to abrasion and reapplying of coatings increases the protection cost and traditional coating applications use toxic volatile organic solvents, which may cause severe environmental concerns (Zuo, 2007).

### *iii.* Cathodic protection

Buried and submerged structures and some interior surfaces in processing facilities are protected by imposition of an electrical potential known as cathodic protection (CP) which prevents oxidation of metal (Uhlig, 2011). The objective with cathodic protection is to suppress the electrochemical reaction taking place at the anode. Under normal corrosive conditions, current flow from the anode results in a loss of metal at the anodic site with resultant protection of the metal at the cathodic site. By making the structure you want to protect cathodic, protection can be provided. According to Uhlig (2011), CP can prevent all kinds of corrosion; including microbial influenced corrosion, if adequate potentials are sustained. The technique has also been applied to tanks, clarifiers and heat exchanger. In the presence of anaerobic bacteria, including SRB, applied CP potentials must be more negative than usual to achieve good protection for exposed steel (Little & Wagner, 1994). They observed that whilst potential sufficiently negative to drive hydrogen evolution at the metal surface causes a significant increase in pH that may discourage bacterial attachment and activity; such high potentials are neither practical nor prudent for field study. CP seems unable to stop localized initiated corrosion when a stable biofilm of SRB had already established on stainless steel in a chloride- containing medium (Little & Lee, 2007).

## 2.12.2 Biocides

Biocides are chemical substance intended to destroy, deter, render harmless, or exert a controlling effect on any harmful organism by chemical or biological means. According to Lin and Ballim (2012), biocide is any chemical agent that is able to kill living organisms. The

control of biocorrosion entails the use of biocides (Saravia, Guiamet & Videla, 2013). They observed that it could be used in conjunction with other substances such as dispersive or penetrating agents that are able to increase the efficiency of the treatment.

Biocides can be either oxidizing or non-oxidizing agents (Videla & Herrera, 2005). Oxidizing agents such as chlorine, chlorinating compounds, choramines and bromine are commonly used in freshwater systems (Lin & Ballim, 2012). The effectiveness of chlorine compounds depends on factors such as pH, temperature as well as light. Murthy and Venkatesan (2009) observed that chlorine compounds are not very effective when used to control biofilms. Non-oxidizing biocides are more stable than oxidizing biocides (Lin & Ballim, 2012) and can be used in a variety of different environments. They have been shown to be effective against a broad range of microorganisms such as bacteria, fungi and algae as well as a greater persistence in the environment (Videla & Herrera, 2005). The choice of the biocide or inhibitor to use in the control of biocorrion is very important. A study by Rajasekar, Anandkumar, Maruthamuthu, Ting and Rahman (2010), shows that the widespread use of ester-based or toxic biocides in the petroleum industry has led to the growth and dominance of *Bacillus* species, due to their ability to form resistance spores.

### 2.12.3 Bioelectric effect with antimicrobial agents

The use of antibiotics as biocides for the prevention of metal corrosion has been difficult to predict due to frequent resistance to antibiotics as well as the drastic low growth rates of cells in biofilms (Del Pozo, Rouse & Patel, 2008). Studies have shown that microorganisms within a biofilm are 500 to 5000 times more resistant to antibiotics than plaktonic microorganisms (Costerton *et al.,* 2005). Bioelectric effect is the application of a relatively small direct current electric field in addition to biocides to increase the killing actions of biocides (Wellman, Fortun & Mc Leod,1996). Blenkinsopp, Anderson, Khoury, and Costerton, (1992) and Wellman *et al.* (1996) reported that in the presence of an electric current flowing through

the chamber in which the biofilm was present, the effectiveness of an antimicrobial agent such as glutaraldehyde was greatly enhanced up to 8 log orders. Results showed that the current alone was unable to affect the biofilm suggesting a synergistic action between the antimicrobial and the electric current field density.

Blenkinsopp *et al.* (1992), observed that electric currents are able to disrupt the organization of the biological membranes present in the cell well as membrane analogs, both prokaryotic and eukaryotic metabolic and developmental processes. According to Costerton *et al.* (2005), electrophoretic forces enable the antimicrobial agents to overcome diffusion barriers that prevent their access to biofilm cells. Low strength electric fields have been shown to disrupt the resistance mechanisms of biofilms. The results mentioned shows that the bioelectric effect has the potential to reduce the number of biocides used in the control of biocorrosion without decreasing their effectiveness and therefore may be attractive to industries (Blenkinsopp *et al.,* 1992).

### 2.12.4 Biocompetitive exclusion

Biocompetitive exclusion (BE) is becoming increasingly promising techniques in the control biocorrosion and reservoir souring by SRB (Videla & Herrera, 2007). This strategy has been successfully used in oil industries, laboratory and field scale. It involves the use of nutrients that stimulate the growth of competitive bacteria or microorganisms such as nitrate reducing bacteria (NRB) that are able to displace the SRB from the community (Tabari, Tabari & Tabari, 2011). The addition of nitrate has been shown to induce a shift in the dominant bacterial populations from the sulphate reducing bacteria to nitrate reducing bacteria, which thereafter prevents the production of hydrogen sulphide and SRB growth (Videla & Herrera, 2007). According to Videla and Herrera (2007), the actual microbiological basis is not very well understood and it is still not known whether autotrophic or heterotrophic NRB have the most significant role. However, it has been shown that the enrichment of NRB in the system

inhibits the growth of SRB due to the production of toxic by products such as nitrite as well as by increasing the redox potential (Lin & Ballim, 2012).

The effect of low nitrate concentration treatment on the microbial communities and on corrosion of oil weld at Veslefrikk and Gullfaks during a 7 to 8 years continuous nitrate injection was investigated by Lin and Ballim (2012). They discovered a rapid reduction in the number and activity of the SRB. A reduction in corrosion rate of up to 40% was also observed when compared to biocide treatment. The biofilm community during the long-term treatment remained highly diverse and relatively stable. This study has shown that nitrate injection has the potential to provide a stable and long-term method of SRB inhibition when compared to the use of biocide.

### 2.12.5 Ozone

Ozone has attracted special interest in recent years as an effective and non-polluting biocide for mostly cooling water systems (Videla, 2002). Ozone readily attacks bacterial cell walls and is more effective against the thick-walled spores of plant pathogens and animal parasites than chlorine at practical and safe concentrations (Suslow, 2003). They observed that the use ozone offers several advantages over other biocides and these include (*i.*) minimal on-site chemical inventory; (*ii.*) non-toxicant discharge; (*iii.*) potential for water conservation. Studies have shown that combination of high toxicity during treatment with non-toxicant discharge could make ozone the leading choice biocide in the near future.

Dissolved ozone is able to reduce planktonic *Pseudomonas fluorescens* bacterial numbers below detectable limits in only 15 min at 0.28 ppm and in 30min at 0.14 ppm (Videla, 2003) in agreement with previously reported results for *Escherichia coli* and *Legionella pneumophilia*. Strittmatter, Yang, and Johnson, (1992) studied the biocidal action of ozone on microbial biofilms formed on stainless steel coupons after 7 hours of incubation in a *P.*

*fluorescens* culture within the 0.2 to 0.5 ppm concentration range for several contract times. They observed that no biocidal action was found for 0.28 ppm ozone concentration at the lowest contact time assayed (5min). Conversely, for 10 min contact time and 0.5 ppm ozone concentration, the number of viable microorganisms was approximately 30 times lower than that found for both 15- and 30-min contact times at 0.5 ppm ozone concentration. They therefore deduced that biocidal action was not significantly increased when the contact time was extended. They also observed that ozone action on stainless steel biofilm was not only able to kill sessile bacteria, but to detach them (Strittmatter *et al.*, 1992).

### 2.12.6 Use of plant extracts

The known hazardous effects of most synthetic inhibitors and the need to develop cheap, nontoxic and eco-friendly process have urged researchers to focus on the use of natural products (Amitha & Bharathi, 2012). The environmental toxicity of organic corrosion inhibitors has promoted the search for green corrosion inhibitors as they are biodegradable, do not contain heavy metals or other toxic compounds. In addition to being environmentally friendly and ecologically acceptable, plant products are inexpensive, readily available and renewable (Gunasekaran & Chauhan, 2005; El-Etre, Abdallah, & El-Tantawy, (2006); Li, Zhao, Liang, & Hou, 2005).

Tannins, organic acids, alkaloids, pigments and proteins from plants are known to inhibit metal corrosion (Singh *et al.*, 2012; Merritt & Brown, 2004). Extracts of *Zenthoxylum alatum* were active on the corrosion of carbon steel in phosphoric acid (Gunasekaran & Chauhan, 2005). Li *et al.* (2005) investigated the inhibitory effect of berberine extracted from *Coptis chinensis* in soft steel which was active against corrosion in 1M sulfuric acid. Molecules present in aqueous extract for Fenugreek leaves were spontaneously adsorbed on mild steel surface and were capable of inhibiting corrosion on steel in a dose-dependent manner in the presence of HCl and $H_2SO_4$. Aqueous extract of *Rosmarinus officinalis* (Kliskic *et al.*, 2000),

*Lawsonia inermis* leaves (El-Etre, 2006), *Allium sativum* (O‟toole, Kaplan, & Kolter, 2000) and *Phaseolus vulgaris* (Abdel-Gaber, Abd-El-Nabey, Sidahmed, El- Zayady & Saadaway, 2006) inhibited metal corrosion.

The adhesive protein from the marine mussel *Mytilus edulis* and the bovine serum albumin (BSA) were both absorbed on carbon steel and were able to inhibit corrosion (Zhang *et al.,* 2011). Plant products have shown antimicrobial activity and studies in the use of extracts or isolated compounds to combat human-pathogens and phytopathogenic bacteria and microorganisms involved in corrosion are well documented (Lambert *et al.,* 2001; Albuquerque *et al.,* 2006; Costa *et al.,* 2010). The activity of an aqueous extract of *Brassica nigra* on planktonic and sessile *Pseudomonas* sp., the fungus *Aspergillus fumigatus* and a mixture of SRB revealed a promissory biocidal action against microorganisms frequently found in industrial biofilms (Videla, 2003).

***Piper guineense***

*Piper guineense* belongs to the family Piperaceae or Sapotaceae (Ekanem, Udoh & Oku, 2010). *P. guineese* is commonly referred to as Uzizi, African black pepper or Ashanti pepper (Ogbonna, Nzoaki & Yajima, 2013). *P. guineense* is a perennial woody climber that grows up to 10m or more in height (Okoye & Ebeledike, 2013). It is a plant of wet tropics and requires a well distributed rainfall and temperature to thrive. According to Okoye and Ebeledike (2013), the leaves are considered appetitive, carminative and eupeptic. He also observed that they are used for the treatment of cough and bronchitis, intestinal diseases and rheumatism (Sumathykutty, Rao, Padmakumari & Narayanan, 1999). Nwaiwu and Imo (1999) reported the antifungal properties of the essential oil of *P. guineense* on food-borne fungi.

Figure: 2.4. Sun-dried seeds of *Piper guineese* (Adopted from Echo *et al.*, 2012)

***Aframomum melegueta***

*Aframomum melegueta* belongs to the family Zingiberacea. It is a perennial herb with short stems, highly branched leaves and adventitious roots (Ogbonna *et al*., 2013). In tropical Africa and indeed Nigeria, the plant is cultivated mainly for its use in ethno-medicine than spice. *A. melegueta* is a spice that is widely used in many cultures for entertainment, religious rites, food flavor and as part of many traditional doctors' medications (Okwu, 2001). According to Ogbonna *et al*. (2013) the phytochemical screening of plant extracts as antimicrobial agents of plant diseases showed that *A. melegueta* contains high doses of tannins saponins, glycosides and polyphenols than other weed plants. Also, the fruit and seeds contain a volatile oil with a pungent, peppery taste due to the presence of aromatic ketones called paradole (Echo, Osuagwu, Agbor, Okpako & Ekanem, 2012).

Figure 2.5 Sun dry seed of *Aframomum melegueta* (Alligator pepper) (Adopted from Echo *et al.*, 2012).

## 2.13 Metal Coupons

### 2.13.1 Mild Steel

Mild steel, also known as plain-carbon steel or low carbon steel is the common form of steel because of its price. It provides material properties that are acceptable for many applications (Edwards, 2006; Park, Lee, & Shin, 2005). Mild steel contains approximately 0.05-0.15% carbon making it malleable and ductile. Mild steel has a relatively low tensile strength, cheap and easy to form and its surface hardness can be increased through carburizing.

Mild steel is often used when large quantities of steel are needed as structural steel. The density of mild steel is approximately 7.8 g/cm$^3$ (7850 kgm$^3$ or 0.284 lb/m$^3$) (Edwards, 2006). Many of the everyday objects that are created of steel are made using mild steel including automobile chasses. Because of its poor corrosion–resistance, it must be painted or protected and sealed in order to prevent rust from damaging it. A light coat of oil or grease is able to seal this steel and aid in rust control (Park *et al.*, 2005). Unlike high-carbon steel, mild steels are weldable, and are used in construction projects where a higher-carbon version could simply break. Most of the pipelines in the world are created using mild steel (Edwards, 2006).

### 2.13.2 Aluminium

Aluminium is a relatively soft, durable, lightweight, ductile and malleable metal with appearance ranging from silvery to dull grey depending on the surface roughness. It is non-magnetic and does not easily ignite (Vargel, 2004). A fresh film of aluminum serves as a good reflector (approx 92%) of visible light (Frank, 2009).

With the density of 2.70 g $cm^3$, aluminium has about one-third the density and stiffness of steel. It is mostly machined, cast, drawn and extruded. Aluminum is an excellent thermal and electrical conductor, having 59% the conductivity of copper, both thermal and electrical while having only 30% of copper's density. Aluminum is capable of superconductivity with a superconducting at critical temperature of 1.2 kelvin and critical magnetic field of about 100 guass (Vagel, 2004). The corrosion resistance of aluminum is excellent, because a thin surface layer of aluminum oxide forms when the bare metal is exposed to air effectively preventing further oxidation, in a process termed passivation (Vargel, 2004). Aluminum is almost always alloyed, which markedly improves its mechanical properties, especially when tempered. For example, the common aluminum foil and beverage can are alloys of 92% to 99% aluminum (Frank, 2009). Aluminum and its alloys are vital to the aerospace industry and important in transportation and structures, such as building facades and window frames (Totten & Mackenzie, 2003).

### 2.14 Some Fungi Involved in Corrosion

***Acremonium kiliense***

*Acremonium kiliense* is a saprophytic fungus, characterized by light microscope as a Gram-positive microorganism with septate hyphae (Das, Saha, Dar & Ramachandran, 2010). *Acremonium* species are slow growing fungi and are initially compact and moist. It is a soil-habiting imperfect fungus. *A. kiliense* grows well indoors under wet conditions (Zuccro *et al.*,

2000). When indoors, it originates either from outdoor air, contaminated crawl spaces or contaminated building materials. The spores of *A. kiliense* are formed in mass resulting in limited aerosolisation (Zuccaro, Summerbell, Gams, Schroers & Mitchel, 2000).

Because of its high affinity for water, *A. kiliense* is often isolated from cooling coils, drain pans, window seals and surfaces of pipelines that pass through damp or wet environments (Larone, 2011). In a controlled study conducted by Larone (2011), on mould growth on wet gypsum wall board in an indoor environment, *A. kiliense* was among early colonizers along with *Cladosporium* and *Penicillium*. It was detected three weeks after immersing the building materials in water.

***Aspergillus fumigatus***

*Aspergillus fumigatus* is a species of the genus *Aspergillus*. It is widespread in nature and is typically found in soil and decaying matter such as compost heaps where it plays an essential role in carbon and nitrogen recycling. The fungus is capable of growth at 37°C or 99°F and can grow at temperature up to 50°C with conidia surviving at 70°C. *A. fumigatus* grown on some building materials can produce gentotoxic and cytotoxic mycotoxins such as gliotoxin (Nieminen *et al.,* 2002). The organism mostly acquires their nutrients from external environment in order to survive. Examples of nutrient uptake include that of metals, nitrogen and macromolecules such as peptides (Dagenais & Keller, 2009). *A. fumigatus* has two mechanisms for the uptake of iron which include reductive iron acquisition and siderophore-mediated (Haas, 2003). Reductive iron acquisition includes conversion of iron from the ferric ($Fe^{+3}$) to the ferrous ($Fe^{+2}$) state and subsequent uptake via FtrA, an iron permease. The life cycle of *A. fumigatus* consists of two phases: a hyphal growth phase and a reproductive (sporulation) phase. The switch between growth and reproductive phases of this fungus is regulated in part by secondary metabolite production (Tao & Yu, 2011).

***Penicillium chrysogenum***

*Penicillium chrysogenium* was formerly known as *Penicillium notatum*. It is a species of fungi in the family of Trichocomaceae. It is common in temperate and sub-tropical regions and can be found in damp or water-damaged buildings (Andersen, Frisvad, Søndergard, Rasmussen & Larsen, 2011). The mycelium of *P. chrysogenum* typically consists of a highly branched network of multinucleated septate, usually colorless hyphae. Many branched conidiophores sprout on the mycelia bearing individually constricted conidiospres. Commonly known as molds, they are among the main causes of spoilage (Samson, Seifert, Kuipers, Houbraken & Frisvad, 2004). *P. chrysogenum* usually reproduces by forming dry chains of spores (or conidia) from branch –shaped conidiospores. The conidia are typically carried by currents to new colonization sites. In *P. chrysogenium,* the conidia are blue-green and the mold sometimes extrudes a yellow pigment. However, *P. chrysogenum* can not be identified based on colour alone. Salo (2016) observed that the production of metal ion binding siderophores is typical among *P. chrysogenum* that grows naturally at various challenging environments. These groups of iron-chelating compounds support fungal growth under iron limiting conditions by carrying $Fe^{3+}$ in cell (Boukhalfa & Crumbliss, 2002). It is the source of several β-lactam antibiotics and most significantly penicillin. Other secondary metabolites of *P. chrysogenum* include roquefortine C, meleagrin, chrysogine, xanthocillins, secalonic acids, sorrentanone, srbicillin and PR-toxin (de Hoog, Guarvo & Figueras, 2000). *P. chrysogenum* has also been linked with biogenic weathering and biodeterioration of ancient monuments and buildings (Kavita, Verma & Motolal, 2011).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Collection of Samples

#### 3.1.1 Metals coupons

The metals used in this study include (*i*) Mild steel composed of the following (wt %): Carbon (0.30%), Silicon (0.30%), Manganese (0.30%), Phosphorus (0.045%), Sulfur (0.050%), Chromium (0.064%), Copper (0.040%), Titanium (0.04%) and Fe (98.9%).

(*ii*) Aluminum (Al >99.5%). They were collected from the Material and Metallurgical Department of Federal University of Technology Owerri.

#### 3.1.2 Plant materials

The plant materials used in this study were the seeds of *Piper guineense* (Uziza) and *Aframomum meleguata* (Ose oji). They were collected from National Root Crop Research Institute Complex, Umudike, Abia State, Nigeria.

#### 3.1.3 Sample for microbial isolation

Samples for microbial isolations were corroded metal pipelines collected within the Nigerian National Petroleum Cooperation (NNPC) Depot Aba, Abia State and from aluminium roofing sheets (from roof tops of some houses) in Aba, Abia State Nigeria. Corroded parts of the metals including corrosion products and slimy coated aluminium sheets were scrapped and aseptically collected in sterile bottle using sterile spatula. The bottles were tightly capped, labeled and transported to laboratory for analysis.

## 3.2 Preparation of Plant Extracts

The plant extracts were prepared using standard procedure outlined by Echo *et al.* (2012). The dried plant seeds were washed with sterile distilled water. Two hundred grams (200g) of each seed were pulverized separately with a household blender Model QBL-18L40, (Honkoung). Hundred grams (100 g) of each powdered seed weighed into 1000mL conical flasks were soaked with 500ml of each of the solvents, namely 95% ethanol (ET), methanol (MT), cold water (CW), at room temperature (28±2ºC) and hot water (HW, 90ºC). The flasks were kept for 48 h on the laboratory bench at room temperature (28±2ºC) to allow for maximum extraction of components. Thereafter, the contents of each flask were filtered using Whatman filter paper No 1. The filtrates were then evaporated to dryness using a rotary evaporator (Stuarc Scientific England). The residues (the stocks) were then stored in sterile reagent bottles in the fridge until analysis. The various stocks were then analysed qualitatively and quantitatively.

### 3.2.1 Qualitative phytochemical screening of the seed extracts

The following phytochemicals were assayed for: alkaloids, saponins, flavonoids, terpenes, glycosides and tannins.

#### *i.* Test for alkaloids

Five hundred milligrams of each extracts were weighed into 500 mL conical flask stirred with 100 mL of 1% HCl and heated in a water bath and filtered. One milliliter (1mL) of filtrate each was treated with Dragendoff reagent (solution of Potassium Bismuth Iodine). Formation of red precipitate indicated the presence of alkaloids (Gracelin, De Britto & Kumar, 2013).

### *ii.* Test for glycosides

Five hundred milligrams of each extract were first hydrolysed with 100 mL dil. HCl in a 500 mL conical flask. The extracts were then treated with Ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was then cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicated the presence of glycosides (Gracelin *et al.,* 2013).

### *iii.* Test for phenols

Five hundred milligrams of each extract were treated 3 drops ferric chloride solution in a 500mL conical flask. Formation of bluish black colour indicated the presence of phenols (Gracelin *et al.,* 2013).

### *iv.* Test for tannins

Five hundred milligrams of each extract were weighed into 500 mL conical flask stirred with 10mL of distilled water. This was then filtered and ferric chloride reagent was added to the filtrate. A blue precipitate was taken as evidence for the presence of tannins (Trease & Evans, 2002).

### *v.* Test for terpenes

Five hundred milligrams of each extract were weighed into 500 mL conical flask and dissolved in 100 mL water and treated with 4 drops of copper acetate solution. The formation of emerald green colour indicates the presence of terpenes (Echo *et al.,* 2012).

### *vi.* Test for flavonoids

Five hundred milligrams each of the extracts was weighed in 500 mL conical flask stirred with 2 drops of magnesium strips (metal). Two drops conc. HCl was then added. The formation of red coloration indicated the presence of flavonoids (Gracelin *et al.,* 2013).

## 3.2.2 Quantitative Phytochemical Screening of the Seed Extracts

### *i.* Determination of phenols

Determination of phenols was done using spectrophotometric method as outlined by Echo *et al.* (2012). Five grams (5 g) of each of the sample was boiled for 15 minutes with 50mL of ether for the extraction of the phenolic component. Five milliliters (5mL) of the extracts were pipette into a 250mL volumetric flask and then 10mL of distilled water, two milliliters (2mL) of ammonium hydroxide solution and 5mL of conc. amylalcohol were added. The samples were made up to mark and left on the bench to react for 30 minutes to allow for colour development. The absorbance of the solution was read using a spectrophotometer at 550nm wavelengths (Gracelin *et al.,* 2013).

### *ii.* Determination of flavonoid

Two grams (2g) of each of the samples were introduced into 50mL of 50% methanol at room temperature for 30 minutes. Thereafter whole solution was filtered through with Whatman filter paper No. 1 (110nm). The filtrates were later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight and recorded (Gracelin *et al.,* 2013).

### *iii.* Determination of glycosides

One gram (1g) of each of the samples was extracted with 50mL of 50% ethanol at room temperature. The crude extracts were warmed in a water bath, cooled and 5% lead acetate

was added. The precipitate was filtered out using Whatman filter paper No. 1 (110nm). The residue on the filter paper was dried to a constant weight using an oven and the weight recorded (Gracelin *et al.,* 2013).

### *iv.* Determination of alkaloids

Determination of alkaloids was done using the method outlined by Echo *et al.* (2012). Two grams (2g) from each of the samples was weighed into a 250mL beaker and 200mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hrs. This was then filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extracts until the precipitation was complete. The whole solution was allowed to settle and the precipitates were collected and washed with dilute ammonium hydroxide and then filtered. The residue was then dried, weighed and recorded.

### *v.* Determination of tannins

Determination of tannin was done using the method outlined by Gracelin *et al.* (2013). Five hundred milligrams (500mg) of each sample were weighed into 50mLplastic bottle. Later 50 mL of distilled water was added and shaken for 1 h in a mechanical shaker. This was then filtered into a 50 mL volumetric flask and made up to the mark. Then 5mL of the filtrate was pipette out into a test tube and mixed with 2 mL of 0.1M $FeCl_3$ in 0.1N HCl and 0.008 M potassium ferrocynide. The absorbance was then measured at 120 nm within 10 minutes.

### *vi.* Determination of saponins

Twenty grams (20g) of each sample was put into a conical flask and $100cm^3$ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous

stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol (Gracelin *et al.,* 2013).

### 3.3 Antifungal Screening of the Extracts

The antifungal activities of the extracts were tested by agar well diffusion method as outlined by Mayuri *et al.* (2015) against the three fungi (*Acremonium kiliense, Aspergillus fumigatus* and *Penicillium chrysogenum*) at 50 mg/ mL and 100 mg/ mL concentrations for each.

#### 3.3.1 Isolation of fungi

The isolation of fungi from the corroded metals and aluminium roofing sheet samples was done by using modified Beech *et al*. (2000) method. A 10-fold serial dilution of the corroded metal sample suspension was prepared by weighing out 1g of corroded metal samples into 9 mL of sterile distilled water in sterile 20mL test tube. This constituted $10^{-1}$ dilution. The corroded metal suspension was vigorously shaken for 3 minutes by hand. After shaking, the $10^{-1}$ dilution was allowed to stand for 30 seconds. Then using a sterile pipette, 1mL was removed from the middle of the suspension and transferred into 9 mL sterile distilled water to achieve $10^{-2}$ dilution. The content of the $10^{-2}$ dilution test tube was shaken and dilution continued until the $10^{-7}$ dilution was obtained. After the serial dilutions, aliquots (0.1 mL) of dilutions $10^{-5}$ to $10^{-7}$ were plated in duplicate on potato dextrose agar PDA (Oxoid) plates supplemented with antibiotics streptomycin (5µg/mL) spread evenly and incubated at 28ºC for 3 – 5 days. Thereafter each morphologically discrete fungal colony was sub-cultured and purified by repeated streaking on PDA plates. Pure cultures were then preserved on PDA slants in Bijou bottles and stored at 4°C in a refrigerator for further studies.

### 3.3.2 Identification of fungal isolates

The fungal isolates were identified based on their morphological characteristics and microscopic appearances using taxonomic guides and standard procedures as outlined by Hussain *et al.* (2011). Wet mount for examination and identification of fungal isolates were employed. A drop of lactophenol cotton blue stain was placed on a clean slide. Using sterile inoculating needle, a 5-7 days cultures of the fungi grown on PDA were transferred unto clean grease free slides. The specimen were flooded with lactophenol cotton blue stain for 3 to 5 minutes. The slides were carefully covered with cover slip to avoid air bubbles and then mounted on the microscope. The following features were looked for and recorded: colony colour, type of stoma, nature of hypha, special reproductive structures, asexual spores, special vegetative structures, conidial head and vesicle shapes according to Hussian, Charles, Spear, Brajendra and David, (2011).

### 3.3.3 Preparation and standadization of inocula

The inocula used for the work were prepared according to the method outlined by Petrikkou, Juan, Manuel, Alicia, and Emilia, (2001). From the stock culture stored in the fridge, isolates were subcultured on PDA and incubated at room temperature (28±2ºC) for 7 days on the lab bench. Thereafter, the surface of the agar was flooded with 50 mL sterile distilled water and the sporulated arial mycelia were scraped with a loop. The suspensions obtained were then filtered to remove the hyphae and thereafter the filterate which contained the spores were collected in sterile tube. The spore suspensions were adjusted to spore concentration of $10^6$ spores/mL by microscopic enumeration with a cell counting hematocytometer (Neubauer Chamber; Merck, S.A., Madrid, Spain).

### 3.3.4 Methodology for antifungal screening

Tenth of a milliliter (0.1mL) of the standardized fungal suspension was evenly spread on PDA. Using a sterile cork borer of 4 mm in size, each plate was punched and 0.1mL of the extract poured in the hole. Bored hole filled with sterile distilled water was used as control. Each sample was prepared in triplicate. The plates were allowed to stand for 30 minutes and then incubated at room temperature (28±2$^{o}$C) for 7 days. The mean of zone of inhibition was used to calculate the mycelial inhibition (%) in relation to the control using the formula:

Mycelial inhibition (%) = $\left(\frac{\mathbf{A-B}}{\mathbf{A}}\right) x100$

A=Diameter of colony in the control (mm)

B=Diameter of the zone of inhibition in the treatment (mm).

## 3.4 Determination of Minimum Inhibitory Concentration (MIC) of the Extracts

The MIC of the extracts was determined according to the method outlined by (Elizabeth, Tukur, and Adeboyo,1999) using PDA. After sterilization, 20 mL of the molten agar medium in 100 mL conical flask was inoculated with 0.2 mL aliquots of standadised cultures and aseptically transferred into sterile Petri dishes. This was allowed to set at room temperature for about 10 minutes and then kept in a refregirator for 30 minutes. Using a sterile cork borer of 4mm in diameter, three uniform cups/wells were made in each Petri dish. A drop of the sterile molten agar was used to seal the base of each well. Tenth of a milliliter (0.1mL) of the extracts containing different concentrations (12.5, 25, 50 and 100 mg/mL) were added to the well of each Petri dish. The controls which contain 0.1mL of sterile water instead of plant extract were also set up without plant extracts. This was repeated for each concentration and for all the extracts in triplicate. The plates were incubated at room temperature (28±2$^{o}$C) for 7 days. The mean zones of inhibition were measured. The lowest concentration of the extracts

that showed clear visible growth inhibition was recorded as the minimum inhibitory concentration.

### 3.5 Determination of the Influence of Fungi on the Corrosion of Mild Steel and Aluminium

The determination of the influence of two fungi *Acremonium kiliens*e and *Aspergilius fumigatus* on the corrosion of mild steel and aluminium was conducted using the procedure outlined by Lugauskas *et al.* (2009). The experimental layout for this study is shown in Figure 3.1.

**3.5.1 Medium:** The medium used was malt extract agar (MEA) poor in nutritive materials. It was supplemented with streptomycin (5µg/mL).

**3.5.2 Preparation of metal coupons**: The metals were first polished with silicon carbide abrasive paper (from grade no. 400 to 1000) and cut into coupons of specific dimension 2cm x 2cm x 0.14cm for mild steel and 3cm x 1.5cm x 0.1cm for aluminum.

The coupons were then cleaned with distilled water, dried in acetone and weighed with electronic weighing balance (Nicolet Model 37500). Weighed coupons were stored in moisture-free desiccators prior to use.

**3.5.3 Preparation and standardization of inoculum:** This was done according to the method outlined by Petrikkou *et al.* (2001) as described in section 3.33.

**3.5.4 Methodology:** The prepared metal coupons were placed in Petri dishes containing 20 mL MEA medium and supplemented with streptomycin (5µg/mL). Thereafter, 0.4 mL standadised suspensions of the respective fungal isolate were spread over the plates and metal coupons.

In the control (K), metal was placed on MEA medium but not inoculated with fungi. The plates were incubated at room temperature (28±2°C). The entire experiments were uniformly prepared in triplicates, labeled accordingly and inserted on the same day for each fungus isolated. For each fungal isolate and each metal coupon, 12 plates were inoculated. For each control, a total of 12 plates were also set up. At 10 days interval, two of the plates inoculated with fungal isolates and two of each of the uninoculated control were removed and analysed for the following:

**a.** Macroscopic and microscopic examination of the metal coupons

**b.** Gravimetric corrosion measurement.

**(a) Macroscopic and Microscopic examination of the metals**

Each metal coupon retrieved at 10 days interval was carefully examined to determine the intensity of fungal growth and contact with the metals in accordance with the method outlined by Lugauskas *et al*. (2009).

The intensity of fungal growth and metal deterioration was assessed by physical observation of the coupons. The metal coupons were also observed on the light microscope to ascertain the growth of the fungi on the surface of the coupons. Morphological changes on the metals were evaluated using an optical microscope with a CCD camera at about 50X magnification and the magnitude of marker was 10μm per 1 cm of the photograph in accordance with the scheme [EM ISO 10289: 1999]. The observations were rated as follows:

- No fungal growth observed on specimens under a light microscope – 1 point
- Mycelia with branched hyhae and possibly sporulation, visible under light microscope – 2 points
- Growth of fungi, sparce but visible to the naked eye under the light microscope, sporulation clearly visible – 3 points
- Growth of fungi clearly evident but covering <25% of the tested surface -4 points

- Heavy growth of fungi visible to the naked eye and covering >25% of the surface

5 points (Lugauskas *et al.*, 2009).

**(b) Gravimetric corrosion measurement**

***i.*** **Weight loss measurement**

Weight loss of the metal coupons with respect to time was conducted using the method outlined by Oguzie *et al.* (2013). The coupons were retrieved at 10 days intervals progressively for 60 days. Thereafter the metal coupons were scraped with spatula, washed with distilled water, dried on the lab bench and weighed. The weight loss was taken to be the difference between the weight of the coupons at a given time and its initial weight. Average values for each experiment were obtained and used in subsequent calculations.

Calculations:

Weight loss ($\Delta W$) = $W_i$ - $W_f$

Where $W_i$ = Initial weight

$W_f$ = Final weight

***ii.*** **Determination of corrosion rate**

Corrosion rate (CR) is used to determine the rate at which a metal will corrode over a period of time.

$$\mathbf{CR} = \frac{\mathbf{k\Delta W}}{\mathbf{A\rho t}}$$

A= Exposed surface area =2(LW+LH+HW) $cm^2$

Where L= length of the coupon

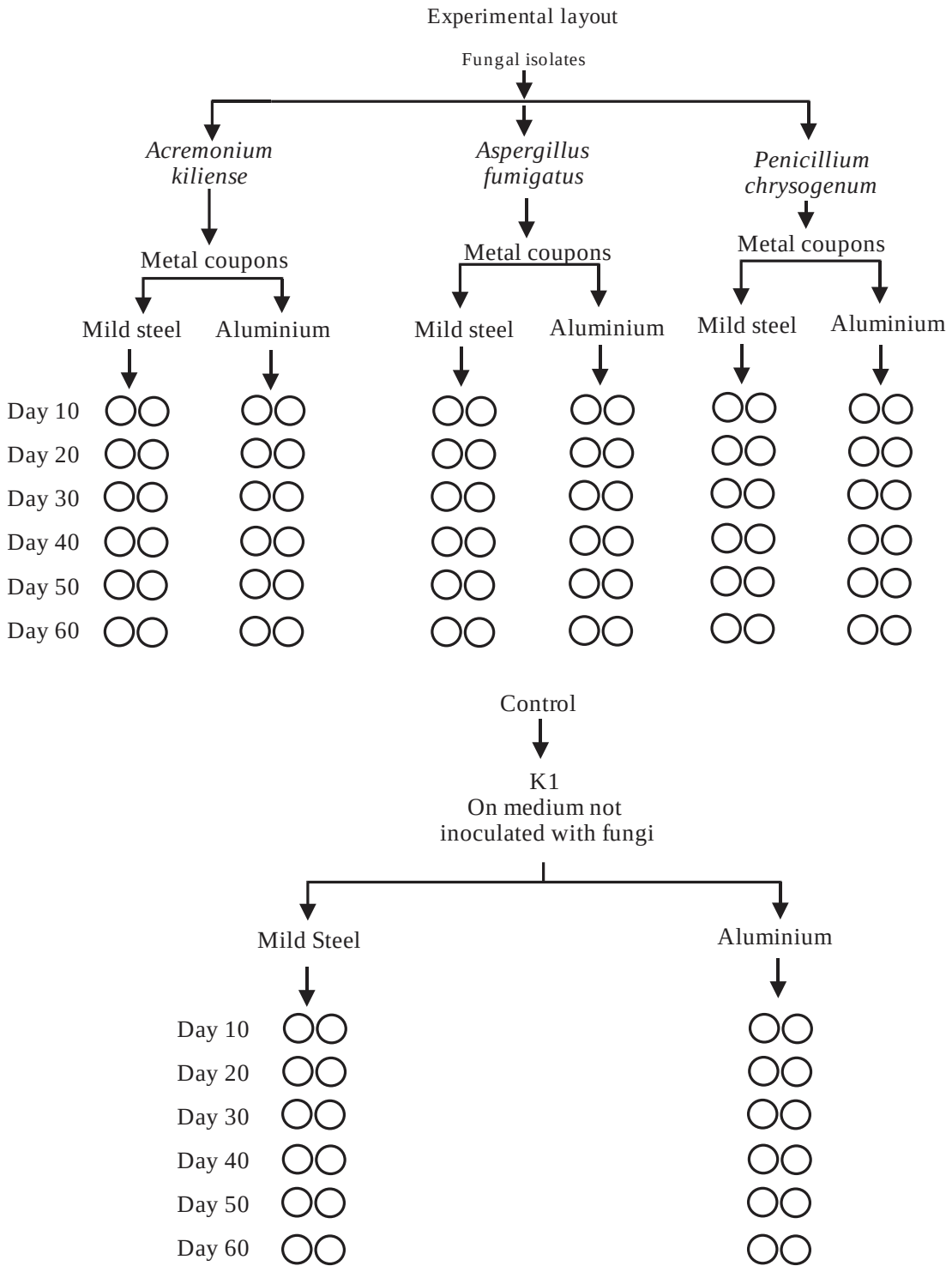
W= width of the coupon

H= height of the coupon or thickness

k = corrosion rate constant (143,700 mpy)

ρ =density of metal coupon ($g/cm^3$)

ΔW= weight loss of coupon (g); t = time (days)




**Legend:** K1 = On medium not inoculated with fungi

Fig. 3.1: The experimental layout for the determination of the influence of fungi on corrosion of mild steel and aluminium.

## 3.6 Determination of the Effect of the Plant Extracts on the Corrosive Ability of Fungal Isolates

The effect of two plant extracts (*Aframomum melegueta* and *Piper guineense*) on the corrosion ability of two fungal isolates (*Acremonium kiliense* and *Aspergillus fumigatus*) on two metals (mild steel and Aluminium) were investigated in accordance with the procedure outlined by Lugauskas *et al.* (2009). The experimental layout is shown in Figure 3.2.

**3.6.1 Medium:** The medium used was malt extract agar supplemented with streptomycin (5μg/mL).

**3.6.2 Preparation of metal coupons:** The prepared metal coupons were dipped into a conical flask containing 20mL plant extracts (25mg/mL) and allowed to dry in air at room temperature (28±2ºC).

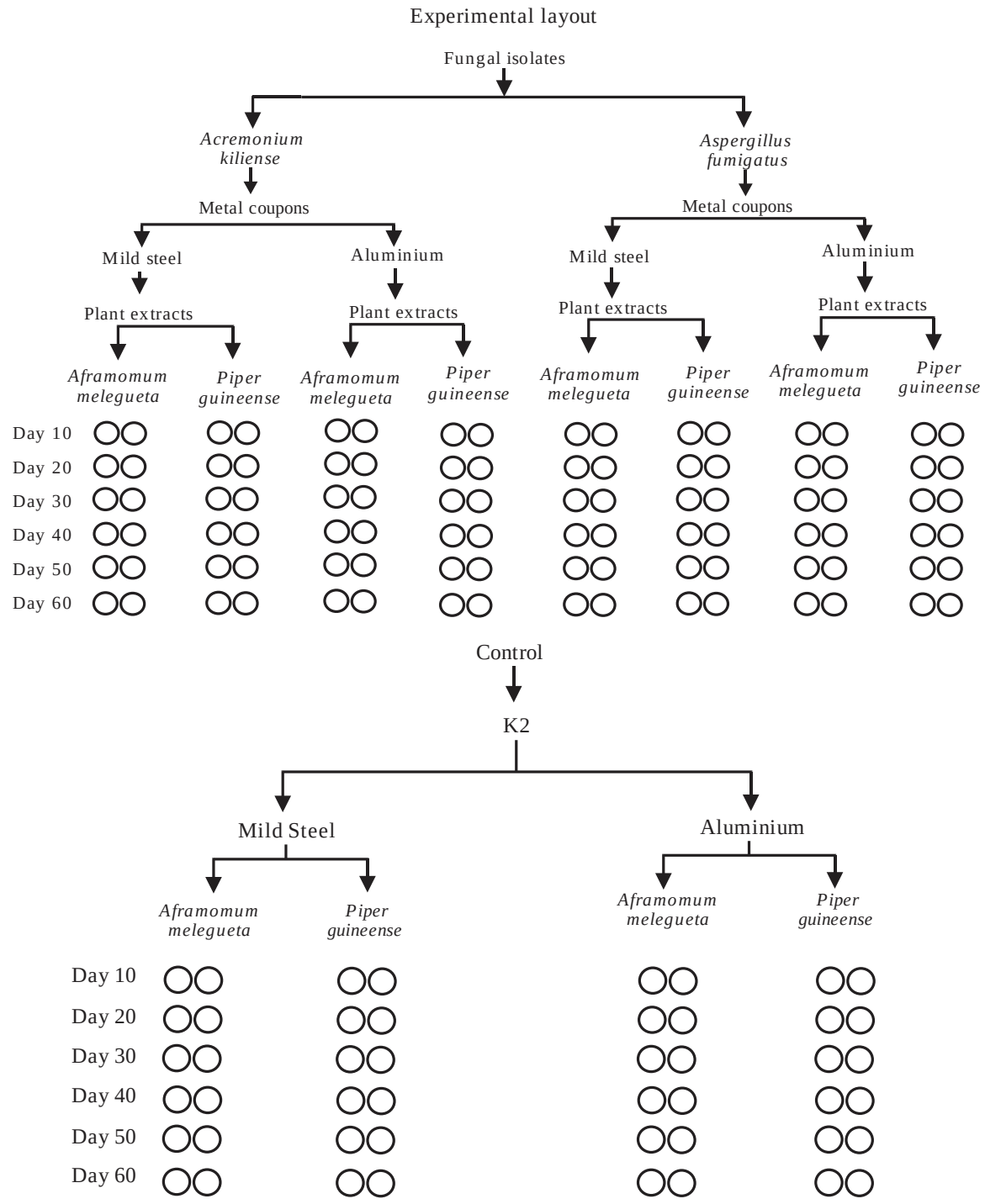
**3.6.3 Preparation and standardization of fungal inoculum:** The inoculum used was prepared according to the method outlined by Petrikkou *et al.* (2001) as described in section 3.33.

**3.6.4 Methodolgy:** The coupons were allowed to dry at room temperature and then carefully placed in sterile Petri dishes with MEA and inoculated with 0.4 mL standadised suspension of fungal isolates. In the control (K2), metal treated with extracts was placed on MEA medium but not inoculated with fungi. Medium with metals was incubated at room temperature (28±2ºC). The entire experiments were uniformly prepared in duplicates, labeled accordingly and inserted on the same day for each fungus isolated and plant extract. The experiment was observed at 10 days intervals for a period of 60 days and analysed for the gravimetric corrosion measurement as descused in section 3.6.

### 3.7 Determination of Inhibition Efficiency IE (%)

The efficiency of corrosion inhibition (IE) was quantified by comparing the corrosion rate of mild steel and aluminum specimens without extracts ($CR_{uninhibited}$) and in the presence of extracts ($CR_{inhibited}$). The percentage inhibition was calculated as follows:

$$IE\ \% = \left(\frac{\mathbf{CRuninhibited - CRinhibited}}{\mathbf{CRunihibited}}\right) x100$$




**Legend:** K2 = Metal treated with extracts and placed on MEA not inoculated with fungi

Fig. 3.2 The experimental layout for the determination of the effect of plant extracts on the corrossion of mild steel and aluminium in the presence of fungal isolates.

## 3.8 Electrochemical Measurements

The potentiodynamic polarization test was carried out in a standard three-electrode glass cell of 500 ml capacity using Electrochemical System workstation (PAR 263). A graphite rod served as counter electrode and, a saturated calomel electrode (SCE) was used as reference electrodes. A mild steel and aluminum specimen of 1 $cm^2$ dimension were used as working electrode. Electrochemical measurements were carried out at 30±1$^oC$, using standard procedures as outlined by Oguzie *et al.* (2013), in aerated solutions at the end of 1800s of immersion, which allowed the open circuit potential (OCP) values to attain steady state. The polarization (PDP) experiments were then conducted at a scan rate of 0.333 mV/s. The potential range employed was -250 mV to + 300 mV versus corrosion potential. Powersuite software was used in analyzing the polarization data.

### 3.9 Statistical Analysis

The mean and standard error (SE) values of two (n=2) or three (n=3) replicates were calculated and the difference between treatments tested by a one-way ANOVA. If the differences were significant, the student's *t*-test comparisons were carried out to determine where the difference in sample mean lie. The expression 'significant', as used in the text, refers to statistical significance at $p \leq 0.05$.

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Phytochemical Composition of the Plant Extracts

The result of the quantitative phytochemical constituent analysis of the plant materials are presented in Table 4.1. The constituents include for *P. guineense*: alkaloids 1.67±0.29 mg, flavonoids 1.92±0.04 mg, cardiac glycosides 39.31±1.30 mg, tannins 0.64±0.21 mg, saponins 0.67±0.01 mg and phenols 39.24±1.2 mg. While *A. melegueta* has the following constituents: alkanoids 2.17±0.29 mg, flavonoids 2.04±0.05 mg, cardiac glycosides 58.58±1.1 mg and phenols 36.68±1.1 mg.

Glycocides and phenols gave highest yields with 39.31±1.80 mg and 58.58±1.1 mg and 39.24±1.2 mg and 36.68±1.1 mg for *P. guineense* and *A. melegueta* respectively. Terpenes and steroids were below detectable limits in both *Aframomum melegueta* and *P. guineense*.

Table 4.1: Phytochemical constituents of extracts of *P. guineense* and *A. melegueta*.

| Parameters | *Piper guineense* (mg) | *Aframomum melegueta* (mg) |
|---|---|---|
| Alkaloids | 1.67±0.29 | 5.17±0.29 |
| Flavonoids | 1.92±0.04 | 2.04±0.05 |
| Tannins | 0.64±0.05 | 0.41±0.11 |
| Terpenes | 45.76±0.30 | — |
| Cardiac glycoside | 39.31±1.30 | 58.58±1.1 |
| Steroids | 19.33±0.10 | — |
| Saponins | 0.67±0.010 | 1.23±.03 |
| Phenols | 39.24±1.2 | 36.68±1.5 |

Legend: — Below detectable limit

## 4.2 Morphological characteristics of the Fungal Isolates

Table 4.2 shows the morphological characteristics of the fungi isolated and used in the study.

Isolate 1: Flat grayish colonies, with balls of ellipsoidal conidia accumulated at the ends of long slender philides, long straight conidiophores. The isolate was most probably *Acremonium kiliense.*

Isolate 2: Flat speading colonies, grayish near apex with white margin, powdery smooth surface. Presence of rough stalks, with conidia slightly roughened. Short stalk conidiophores with pear –shaped vesicle, crowded phialides pointing upwards. The isolate was most probably *Aspergilius fumigatus*.

Isolate 3: Blue–green colonies, flat and cottony in texture, branched conidiophores, presence of brush–like clusters. Round conidia with branching chain like at the tips of the phialides. The isolate was most probably *Penicillium chrystogenum*.

Table: 4.2 Morphological characteristics of fungal isolates

| Fungi | Colony colour Physical (morphology) | Predominate features | Conidiophores | Conidia | Most probable organism |
|---|---|---|---|---|---|
| Isolate1 | Gray to orange, reverse brown. Flat wrinked top. | Balls of ellipsoidal conidia accumulated at the ends of long slender phialides | Long, straight, slightly tapering phialides arising as side-brancheson hyphae | Ellipsoidal, accumulating in slimy balls at the ends of phialides | *Acremonium kiliense* |
| Isolate 2 | White colony with central part greenish in colour with dark pigmentation | Septate branching mycelia Conidiophores inflates to form vesicles with fan-like sterigmata | Vesiculate conidipores. Stalks short, vesicle pear shaped, pointing upwards | Round, slightly roughened | *Aspergillus fumigatus* |
| Isolate 3 | Bluish green to dark green colony. Colourless mycelium at periphery of the colony | Dense brush-like spores bearing structures with branched conidiophores terminated by cluster of flask-shaped phialides. | Small heads on short stalks. Short spreading conidiophoers, small. | Smooth, ellipsoidal, long divergent chains with prominent disjunctors between the spores. Leamon shaped conidia | *Penicillium chrysogenum* |

## 4.3 Antifungal Profiles of the Plant Extracts

The results of the antifungal screening of individual plant extract (cold water CW extract, hot water (HW) extract, ethanol (ET) extract and methanol (MT) extract at concentrations of 100 mg/mL and 50 mg/mL are shown in Table 4.3. *P.chrysogenum* was not sensitive to any of the extracts at both 100 and 50 mg/mL concentrations. *Acremonium kiliense* and *Aspergillus fumigatus* both exhibited varying degrees of sensitivity to different extracts at 100 mg/mL and 50 mg/mL.

The mean percentage mycelia inhibitions of CW extract of *P. guineense* at 100mg/mL were 63.7±1.4 and 65.0±2.0 for *Acremonium melegueta* and *Aspergillus fumigatus* respectively. Futhermore, for all the extracts, the percentage mycelia inhibitions were higher at 100 mg/mL concentration than at 50mg/mL concentration. The fungus *A. kiliense* was not sentitive to the ET and MT extracts of *Aframomum melegueta* at 50mg/mL. At 100 mg/mL, the percentage mycelial inhibitions of the fungus were 9.3±1.1 and 8.1±1.6 for the ET and MT extracts respectively.

## 4.4 Minimum Inhibitory Concentrations (MIC) of the Plant Extracts

Table 4.4 shows the minimum inhibitory concentrations of the extracts. The cold-water extract of *P. guineense* and *A. melegueta* had the lowest MIC of 25 mg/mL for *A. fumigatus* and *Acremonium kiliense*. *P. chrysogenum* did not show sensitivity to the inhibitory effects of the extracts at all the concentrations.

The highest MIC values of 100 mg/mL were observed with HW and ET extracts of *Aframomum melegueta.* Further works was done using cold water extracts at 25 mg/mL concentration.

Table 4.3: Mean % inhibition of different concentration of the extracts.

| Plant Extract | | | | | | |
|---|---|---|---|---|---|---|
| | ***Acremonium kiliense*** | | ***Aspergilius fumigatus*** | | ***Penicillium chrysogenum*** | |
| **Conc. of Extracts (mg/mL)** | **100** | **50** | **100** | **50** | **100** | **50** |
| ***Piper guineense*** | **Zones of inhibition (mm)** | | | | | |
| Cold Water (CW) | 63.7±1.4 | 42.2±1.1 | 65.0±2.0 | 25.6±1.0 | 0 | 0 |
| Hot Water (HW) | 23.1±1.6 | 12.1±1.2 | 41.6±1.1 | 28.1±2.6 | 0 | 0 |
| Ethanol (ET) | 15.1±1.2 | 8.1±1.4 | 18.6±1.3 | 5.1±1.3 | 0 | 0 |
| Methanol (MT) | 12.2±1.4 | 10.3±1.1 | 10.5±1.6 | 7.0±1.1 | 0 | 0 |
| ***Aframomum melegueta*** | **Zones of inhibition (mm)** | | | | | |
| Cold Water (CW) | 20.7±1.6 | 13.6±2.1 | 47.1±2.3 | 20.4±1.8 | 0 | 0 |
| Hot Water (HW) | 13.5±1.8 | 5.90±1.1 | 16.3±1.2 | 8.90±1.0 | 0 | 0 |
| Ethanol (ET) | 9.30±1.1 | 0 | 7.40±1.0 | 5.90±1.1 | 0 | 0 |
| Methanol (MT) | 8.10±1.6 | 0 | 7.40±1.0 | 5.90±1.1 | 0 | 0 |

Legend: CW=cold water, HW=hot waterET =ethanol, MT=methanol

0 =No inhibition

Table 4.4: The minimum inhibitory concentrations (MIC) after 7 days of incubation

| **Inhibition zone diameter* in mm with different concentration of extract** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***Acremonium kiliense*** | | | | ***Aspergillus fumigatus*** | | | |
| **Solvent/Plant extract (mg/mL)** | **100** | **50** | **25** | **12.5** | **100** | **50** | **25** | **12.5** |
| Cold Water (CW) | | | **Zones of inhibitions (mm)** | | | | | |
| *Piper guieneense* | 10.0 | 8.5 | 8.0 | 0 | 10.5 | 9.5 | 9.0 | 0 |
| *Aframomum melugueta* | 27.0 | 11.0 | 9.5 | 0 | 10 | 9.5 | 8.5 | 0 |
| Hot Water (HW) | | | **Zones of inhibition (mm)** | | | | | |
| *Piper guieneense* | 19.5 | 13.5 | 0 | 0 | 9.5 | 8.0 | 0 | 0 |
| *Aframomum melugueta* | 27.0 | 11.0 | 0 | 0 | 10 | 9.5 | 0 | 0 |
| Ethanol (ET) | | | **Zones of inhibition (mm)** | | | | | |
| *Piper guieneense* | 16.0 | 10 | 0 | 0 | 12 | 10.5 | 0 | 0 |
| *Aframomum melugueta* | 10.5 | 0 | 0 | 0 | 7.5 | 8 | 0 | 0 |
| Methanol (MT) | | | **Zones of inhibition (mm)** | | | | | |
| *Piper guieneense* | 10.5 | 8.5 | 0 | 0 | 16.0 | 13.0 | 0 | 0 |
| *Aframomum melugueta* | 12.3 | 0 | 0 | 0 | 17.0 | 11.0 | 0 | 0 |

*The diameter measurement includes that of the hole (well) 4mm in the agar
Legend: HW=hot water, CW=cold water, ET=ethanol and MT=methnol

## 4.5 Influence of Fungi on the Corrosion of Mild Steel and Aluminium

### 4.5.1 Macroscopic and microscopic examination of the metals after exposure to fungal isolates

#### (i) Capacity of fungi to grow on metal surface

The results of the macroscopic and microscopic examination of the metals after 60 days exposure to the fungal isolates is shown in Tables 4.5 and 4.6.

Table 4.5 shows that after 50 days, macroscopic examination of mild steel exposed to *A. kiliense* revealed no growth of this organism on the metal surface. However microscopic examination revealed that from 20 days exposure, fungal colonies and conidia were scattered on the edges of the metal.

The macroscopic examination of mild steel after 10 days exposure to *A. fumigatus* revealed growth on the edges of the metal. After 50 days, nets of mycelia/hyphae covered the whole metal surfaces. The microscopic examination revealed that after 10 days of exposure, fungal conidia and colonies were sparsely scattered on the mild steel surfaces.

After 20 days exposure, the macroscopic examination revealed that *P. chrysogenium* grew and covered the whole metal surfaces. The microscopic examination revealed also that thin nets of mycelia/hyphae covered the surfaces of the metal coupons.

Table 4.5 Growth of fungi on mild steel surface after days of incubation

| Fungal species | Days of incubation | | | | | |
|---|---|---|---|---|---|---|
| | **10** | **20** | **30** | **40** | **50** | **60** |
| *A. kiliense* | No growth. | No growth. | No growth.<br><br>Conidia adhered to edges of metal | No growth.<br><br>Conidia adhered to edges of metal | No growth.<br><br>Conidia adhered to edges of metal | No growth.<br><br>Conidia adhered to edges of metal |
| *A. fumigatus* | Fungi cover only the edges of metal.<br><br>Conidia scattered over metal surfaces | Net of mycelia hyphae cover metal surfaces | Fungi covers the whole surface of metals | Fungi cover metal surface.<br><br>Fungal conidia on the edged of metal | Fungi grew intensively on edges of metal.<br><br>Network of mycelia on metal surfaces | Fungi grew intensively on edges of metal.<br><br>Network of mycelia on metal surfaces |
| *P. chrysogenum* | No growth | Fungus cover metal surfaces | Fungi grew intensively on edges of metal | Fungi grew intensively on edges of metal with thin net of hyphae over metal surface | Fungi grew intensively on edges of metal with colonies sparsely scattered over the surface | Fungi grew intensively on edges of metal with colonies sparsely scattered over the surface |

Table 4.6 shows that after 50 days, the macroscopic examination of aluminium exposed to *A. kiliense* revealed no growth of this organisim on the aluminium surface. However, microscopic examination revealed that from 20 days exposure, fungal colonies and conidia were scattered on the edges of the metal.

The macroscopic examination of aluminium after 40 days exposure of *A. fumigatus* revealed that the fungus grew intensively on the edges of the metal. The microscopic examination revealed that after 10 days exposure, thin nets of mycelia/hyphae spread over metal sufaces.

After 40 days exposure, the macroscopic examination of *P. chrysogenium* revealed that the fungus grew on the edges of the metal. However microscopic examination revealed that from 30 days exposure, fungal colonies were scattered on the edges the metal.

Table 4.6 Growth of fungi on aluminium surface after days of incubation

| Fungal species | Days of Incubation | | | | | |
|---|---|---|---|---|---|---|
| | **10** | **20** | **30** | **40** | **50** | **60** |
| *A. kiliense* | No growth. | No growth.<br><br>Colonies adhered on edges | No growth.<br><br>Discrete limited colonies on edges of the metals | Discrete colonies on the edges of metal. | No growth.<br><br>Conidia adhered to edges of metal | No growth.<br><br>Conidia adhered to edges of metal |
| *A. fumigatus* | Fungi cover only the edges of metal.<br><br>Limited conidia formed on metal surfaces | Net of mycelia hyphae cover metal surfaces | Fungi cover the whole surface of metals. Mycelia spread over metal surfaces | Fungi grew intensively on edges of metal. Mycelia spread over metal surfaces | Fungi grew intensively on edges of metal. Mycelia spread over metal surfaces | Fungi grew intensively on edges of metal.<br><br>Network of mycelia on metal surfaces |
| *P. chrysogenum* | No growth.<br><br>Colonies scattered on edges of metal. | No growth | Discrete colonies on the edges of metal. | Fungi grew intensively on edges of metal | Discrete colonies on edges of metal | Discrete colonies on edges of metal |

### (ii) Morphological changes on the metal surfaces after exposure to fungi

Table 4.7 shows the morphological changes on the metal surfaces after 60 days exposure to the influence of fungi. The pictures of the metals are also shown in Figures 4.1 and 4.2 for metals exposed to *A. kiliense*, *A. fumigatus* and *P. chrysogenum* respectively. The results showed corrosion spots on the surfaces of mild steel coupons exposed to the three organisms as well as on the surfaces of aluminium exposed to *A. kiliense* and *A. fumigatus*. Visible colour changes were observed on the surfaces of aluminium exposed to *A. kiliense* and *A. fumigatus*.

Different degrees of deterioration were observed on the metals. The intensity of deterioration on points 1—5 scale shows that mild steel exposed to *A. fumigatus* has the highest intensity of deterioration point 4 on the scale.

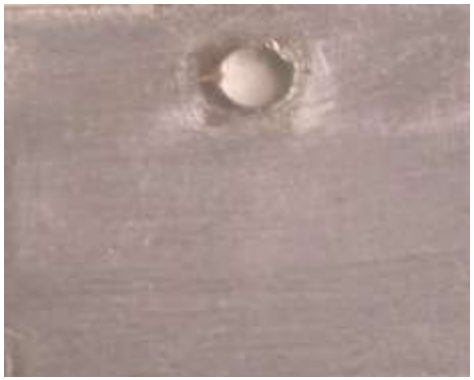
Table 4.7: Morphological changes on metal surfaces exposed to fungi

| **Fungal species** | **Changes on metal surfaces** | **Intensity of deterioration on points 1-5 scale** |
|---|---|---|
| **Mild Steel** | | |
| *Acremonium kiliense* | Prominent corrosion spots seen on the surfaces, more centered at the edges | 2 |
| *Aspergillus fumigatus* | Metal visibly and heavily corroded with coarse corrosion spots over the surfaces and edges with visible pits | 4 |
| *Penicilium chrysogenum* | Prominent blackish spots-like pits scattered over the surfaces of the metals | 3 |
| **Aluminium** | | |
| *Acremonium kiliense* | Corrosion spots seen on the edges of metals | 2 |
| *Aspergillus fumigatus* | Visible colour change on the surfaces of the metals with dark corrosion spots | 3 |
| *Penicilium chrysogenium* | Visible colour changes with patches | 2 |

MS-AK

MS-AF

MS-PC



MS K1

Figure 4.1: Microscopical view of changes in mild steel after 60 days of exposure to fungi

Legends: MS-AK =Mild steel exposed to *A. kiliense*; MS-AF = Mild steel exposed to *A. fumigatus*.MS-PC = Mild steel exposed to *P. chrysogenum*; MS K1 = Mild steel exposed to nutrient medium but not inoculated with fungi.

AL-AK

AL-AF

AL-PC

AL-K1

Figure 4.2: Microscopical view of changes in aluminium after 60 days of exposure to fungi
Legends: AL-AK = Aluminium exposed to *A. kiliense*; AL-AF = Aluminium exposed to *A. fumigatus*
AL-PC = Aluminium exposed to *P. chrysogenum*; AL K1 =Aluminium exposed to nutrient medium not inoculated with fungi.

## 4.6 Corrosion Behaviour of Metal Exposed to Fungi

### 4.61 Influence of *Acremonium kiliense* on the corrosion behaviour of mild steel and aluminum

The results of the influence of *A. kiliense* on the corrosion behavior of mild steel and aluminium after 60 days exposure is shown in Figures 4.3 and 4.4. Figure 4.1 showed that the weight losses of both mild steel and aluminium exposed to *A. kiliense* were greater than the losses observed when the metals were not exposed to the organisms. After 60 days incubation, the weight loss of mild steel exposed to *A. kiliense* was 0.05g relative to the control which was 0.02 g. Also, the weight loss of aluminium exposed to *A. kiliense* was 0.02 g relative to the control which was 0.01 g after 60 days incubation. Overall, the weight loss of mild steel exposed to A. kiliense was greater than the weight loss of aluminium exposed to the organism after 60 days incubation.

The corrosion rate of mild steel and aluminium in the presence of *A. kiliense* relative to the control are shown in Figure 4.4. After 20 days exposure, there were progressive increases in corrosion rate with time for mild steel exposed to *A. kiliense* relative to the control. After 60 days incubation, the corrosion rate of mild steel exposed to *A. kiliense* was 2.0 mpy relative to the control which was 0.6 mpy. Also, the corrosion rate of aluminium exposed to *A. kiliense* was 2.0 mpy relative to the control which was 0.5 mpy after 60 days incubation.

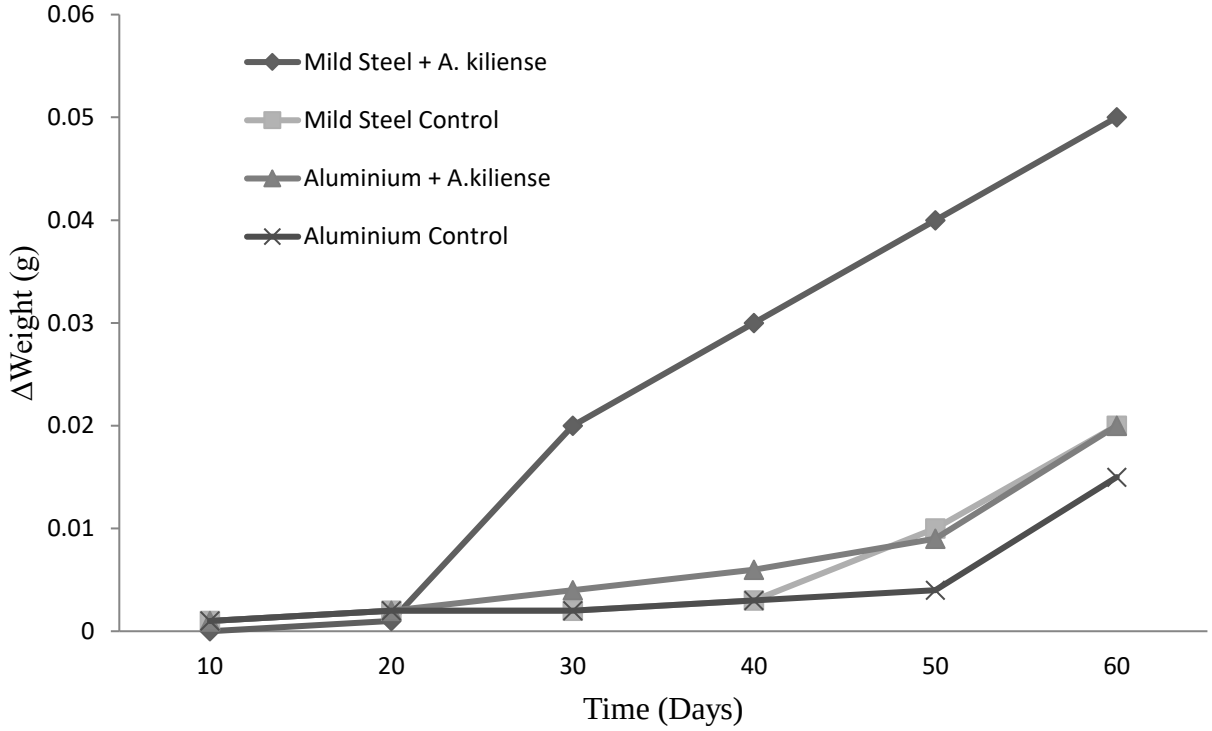



Fig.4.3 Effects of exposure of mild steel and aluminium to *Acremonium kiliense*

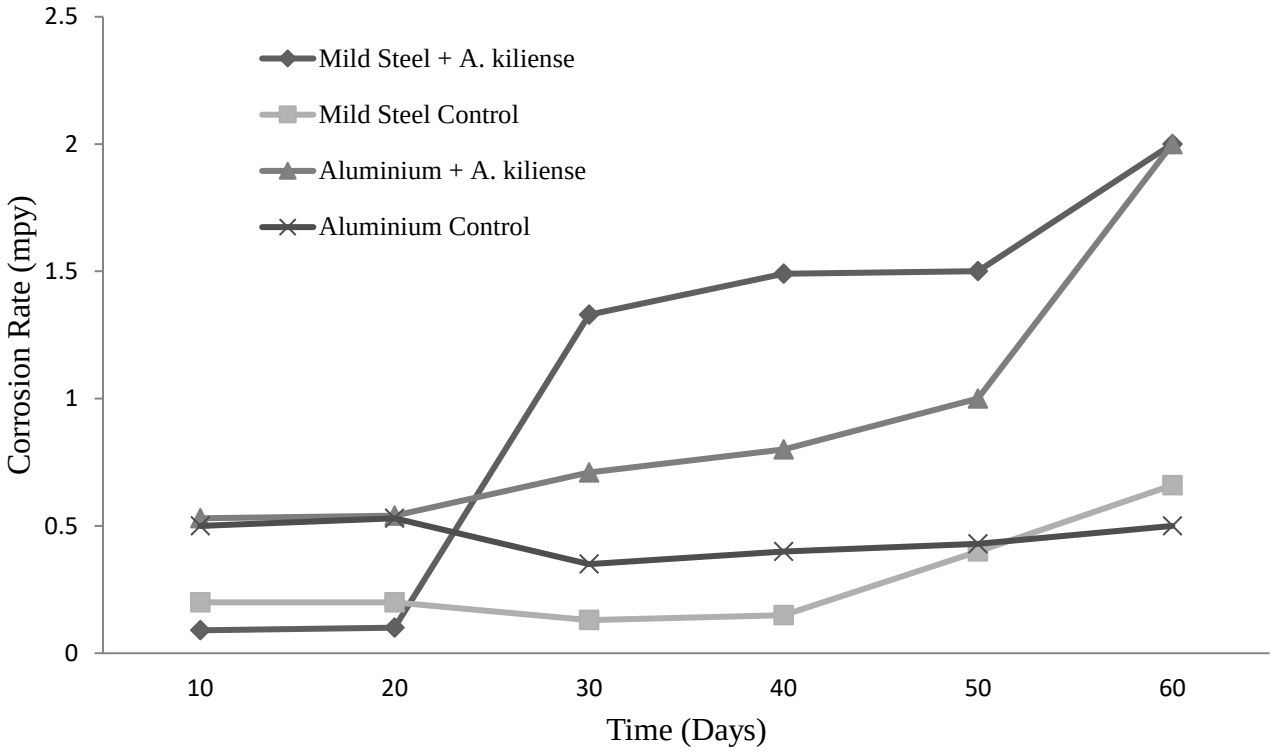



Fig.4.4 Corrosion rate of mild steel and aluminium in the presence of *Acremonium kiliense*

### 4.62 Influence of *A. fumigatus* on the corrosion behaviour of mild steel and aluminum

The results of the influence of *A. fumigatus* on the corrosion behavior of mild steel and aluminium after 60 days exposure is shown in Figures 4.5 and 4.6. Figure 4.5 showed that the weight loss of both mild steel and aluminium exposed to *A. fumigatus* were greater than the losses observed when the metals were not exposed to the organisms (control). After 60 days incubation, the weight loss of mild steel exposed to *A. fumigatus* was 0.08 g relative to the control which was 0.02 g. Also, the weight loss of aluminium exposed to *A. fumigatus* was 0.034 g relative to the control which was 0.01 g after 60 days incubation.

The corrosion rate of mild steel and aluminium in the presence of *A. fumigatus* relative to the control are shown in Figure 4.6. There was progressive increase in corrosion rate with time for metals exposed to *A. fumigatus* relative to the control. After 60 days incubation, the corrosion rate of mild steel exposed to *A. fumigatus* was 2.6 mpy relative to the control which was 0.6mpy. Also, the CR of aluminium exposed to *A. fumigatus* was 2.2 mpy relative to the control which was 0.5 mpy after 60 days of incubation.

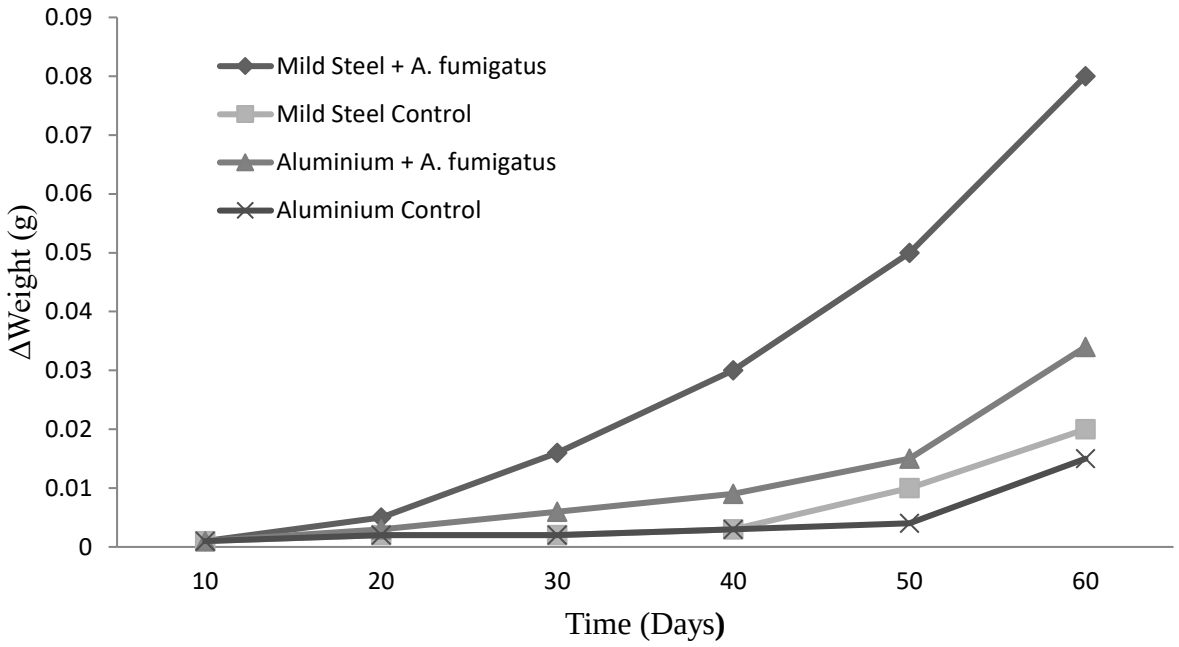



Fig. 4.5 Effects of exposure of mild steel and aluminium to *A. fumigatus*

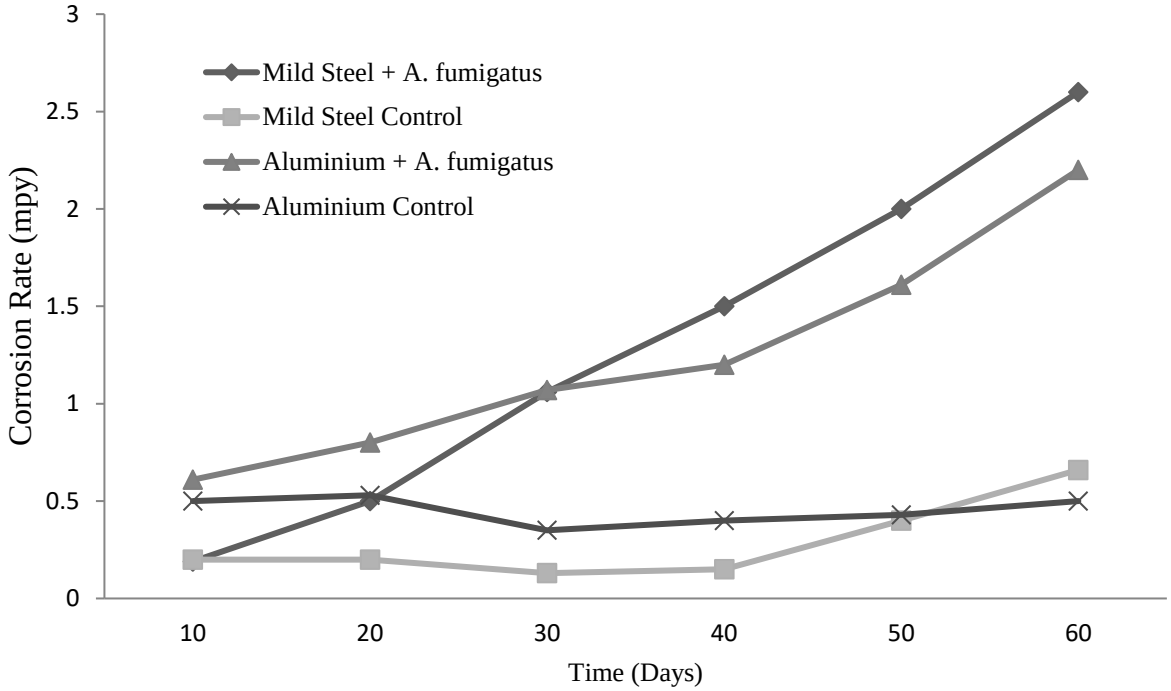



Fig.4.6 Corrosion rate of mild steel and aluminium in the presence of *A. fumigatus.*

### 4.63 Influence of *P. chrysogenum* on the corrosion behaviour of mild steel and aluminum

The results of the influence of *P. chrysogenum* on the corrosion behavior of mild steel and aluminium after 60 days exposure is shown in Figures 4.7 and 4.8. Figure 4.7 showed that the weight loss of both mild steel and aluminium exposed to *P. chrysogenium* were greater than the losses observed when the metals were not exposed to the organisms. After 60 days incubation, the weight loss of mild steel exposed to *P. chrysogenum* was 0.07g relative to the control which was 0.02g. Also, the weight loss of aluminium exposed to *P. chrysogenum* was 0.03 g relative to the control which was 0.01g after 60 days incubation.

The corrosion rate of mild steel and aluminium in the presence of *P. chrysogenum* relative to the control are shown in Figure 4.8. There was progressive increase in corrosion rate with time for metal exposed to *P. chrysogenum* relative to the control. After 60 days incubation, the corrosion rate of mild steel exposed to *P. chrysogenum* was 2.3 mpy relative to the control which was 0.6 mpy. Also, the corrosion rate of aluminium exposed to *P. chrysogenium* was 2.7 mpy relative to the control which was 0.5 mpy after 60 days of incubation.

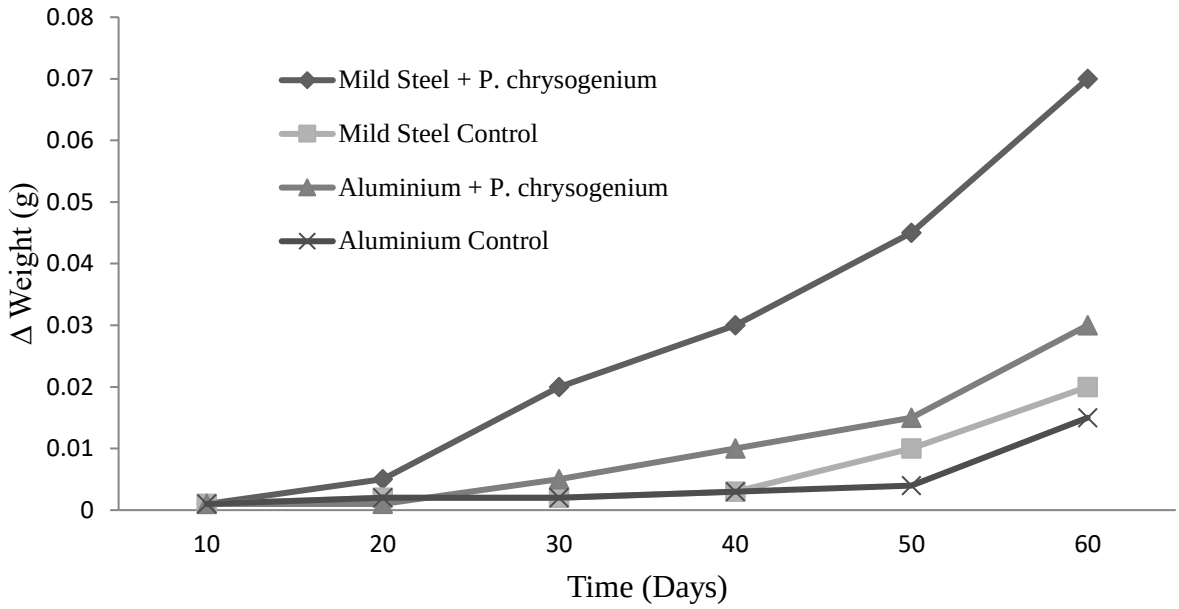



Fig.4.7 Effects of exposure of mild steel and aluminium to *Penicilium chrysogenium*

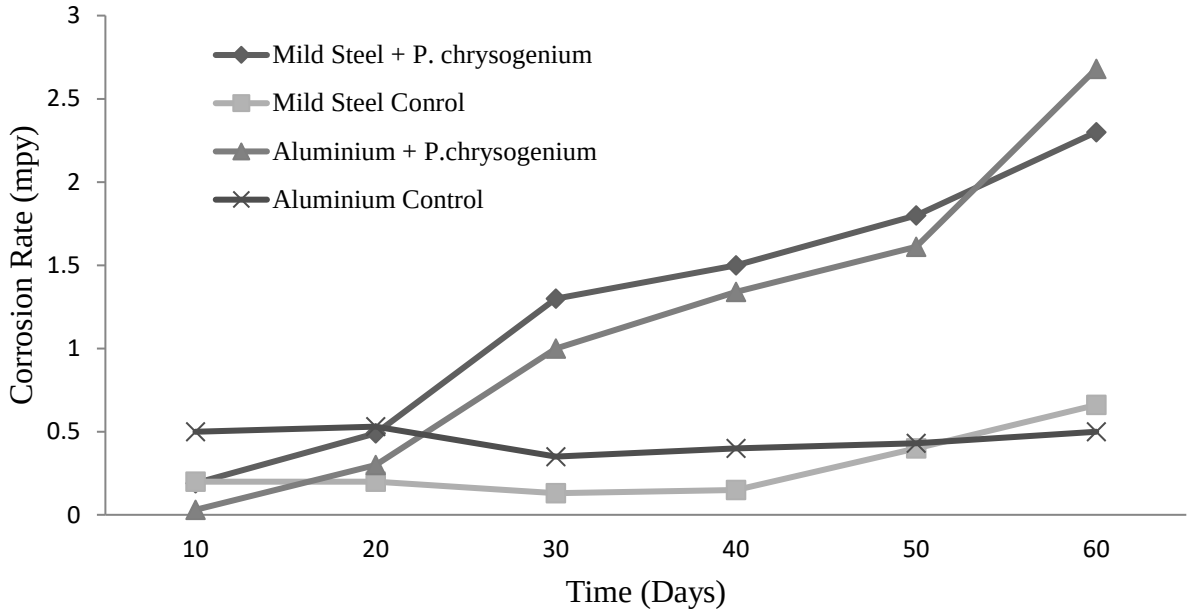



Fig. 4.8 Corrosion rate of mild steel and aluminium in the presence of *Penicilium chrysogenum.*

## 4.7 Determination of the Effects of Plant Extracts on the Corrosion Ability of Fungal Isolates

### 4.7.1 Effects of cold-water extract of *A. melegueta* on the corrosion behaviour of mild steel in the presence of *A. kiliense* and *A. fumigatus*

Figures 4.9 and 4.10 show the results of the effects of extract of *A. melegueta* on the corrosion of mild steel influenced by *A. kiliense* and *A. fumigatus*. Throughout the 60 days incubation, there was a steady increase in weight loss and corrosion rate of untreated mild steel in the presence of fungal isolates. However, the increase in weight loss and corrosion rate was more pronounced in the untreated mild steel than in the cold-water extract treated mild steel in the presence of both fungal isolates. For the untreated mild steel exposed to *A. kiliense*, the weight loss increased from 0.0005g to 0.05g and the corrosion rate increased from 0.09mpy in 10 days after incubation to 2.0 mpy in 60 days after incubation. While for the cold-water extract of *A. melegueta* treated mild steel exposed to *A. kiliense*, the weight loss increased from 0.005g to 0.007g and the CR increased from 0.039 mpy to 0.3mpy 60 days after incubation.

In the case of untreated mild steel exposed to *A. fumigatus*, the weight loss increased from 0.001g to 0.08g and the corrosion rate increased from 0.9 mpy to 2.6 mpy while weight loss increased from 0.0001g to 0.005g and corrosion rat increased from 0.01mpy to 0.2mpy in the cold-water extract of treated mild steel after 60 days of incubation.

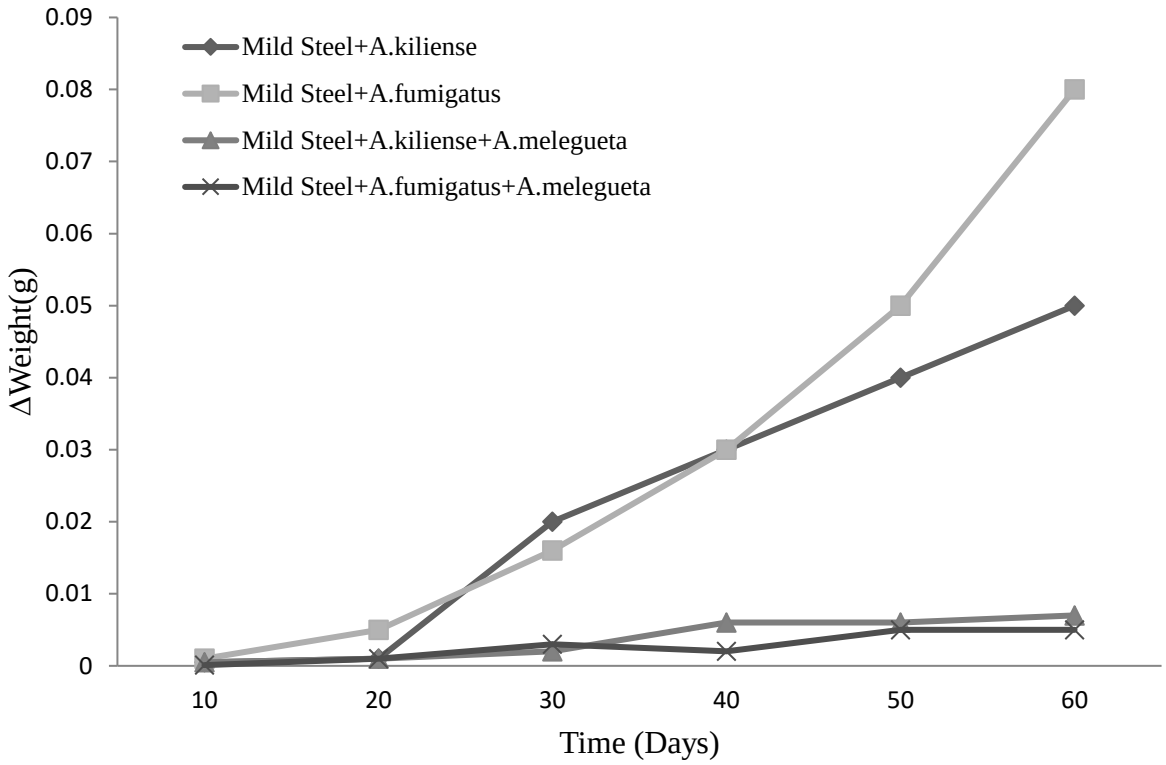



Figure 4.9 Weight loss of fungal influenced corrosion of mild steel treated with *Acremomum melegueta* extracts.

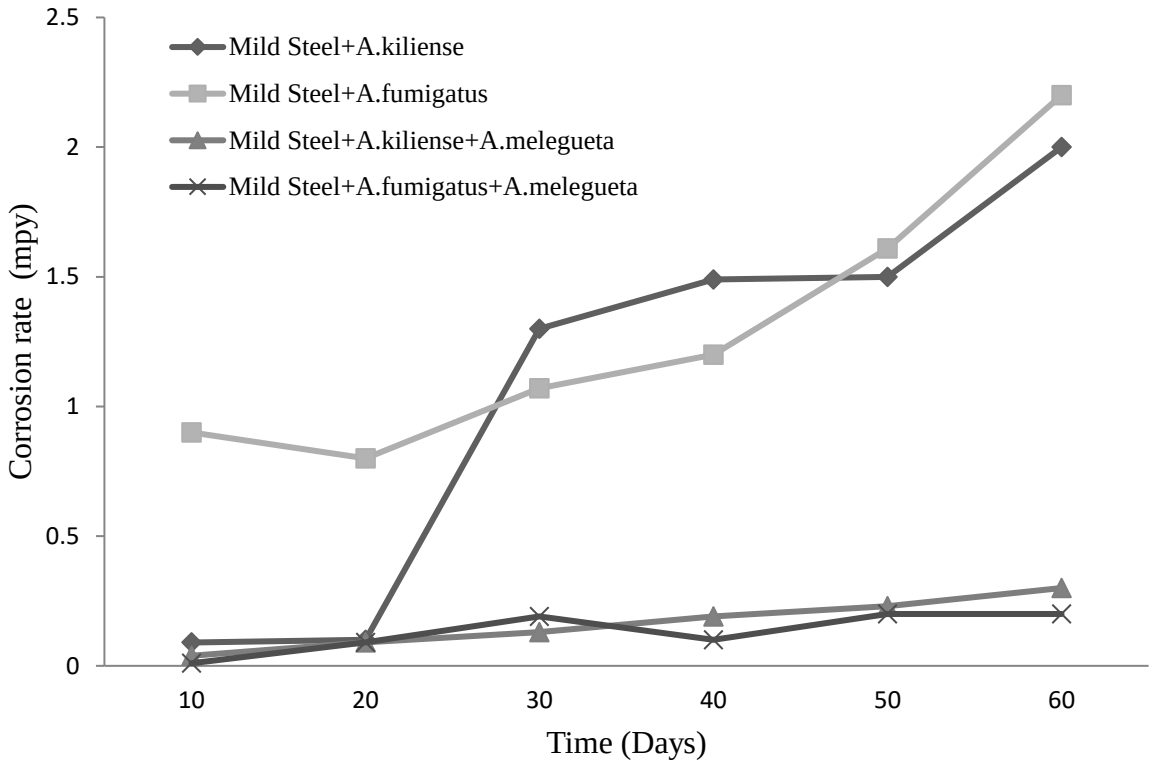



Figure 4.10: Effects of extracts of *A. melegueta* on fungal influenced corrosion of mild steel.

### 4.7.2 Effects of cold-water extract of *A. melegueta* on the corrosion behaviour of aluminium in the presence of *A. kiliense* and *A. fumigatus*

Figure 4.11 and 4.12 show the results of extract of *A. melegueta* on the corrosion of aluminium influenced by *A. kiliense* and *A. fumigatus*. For the untreated aluminium exposed to *A. kiliense*, the weight loss increased from 0.001g to 0.02g and the CR increased from 0.53mpy in 10 days after incubation to 2.0 mpy in 60 days after incubation. While for the cold-water extract of *A. melegueta* treated aluminium exposed to *A. kiliense*, the weight loss increased from 0.001g to 0.004g and the CR increased from 0.053 mpy to 0.35mpy 60 days after incubation.

In the case of untreated aluminium exposed to *A. fumigatus*, the weight loss increased from 0.0013g to 0.034g and the CR increased from 0.61 mpy to 2.2 mpy. While weight loss increased from 0.0001g to 0.007g and CR increased from 0.05mpy to 0.62mpy in the cold-water extract of *A. melegueta* treated aluminium after 60 days of incubation.

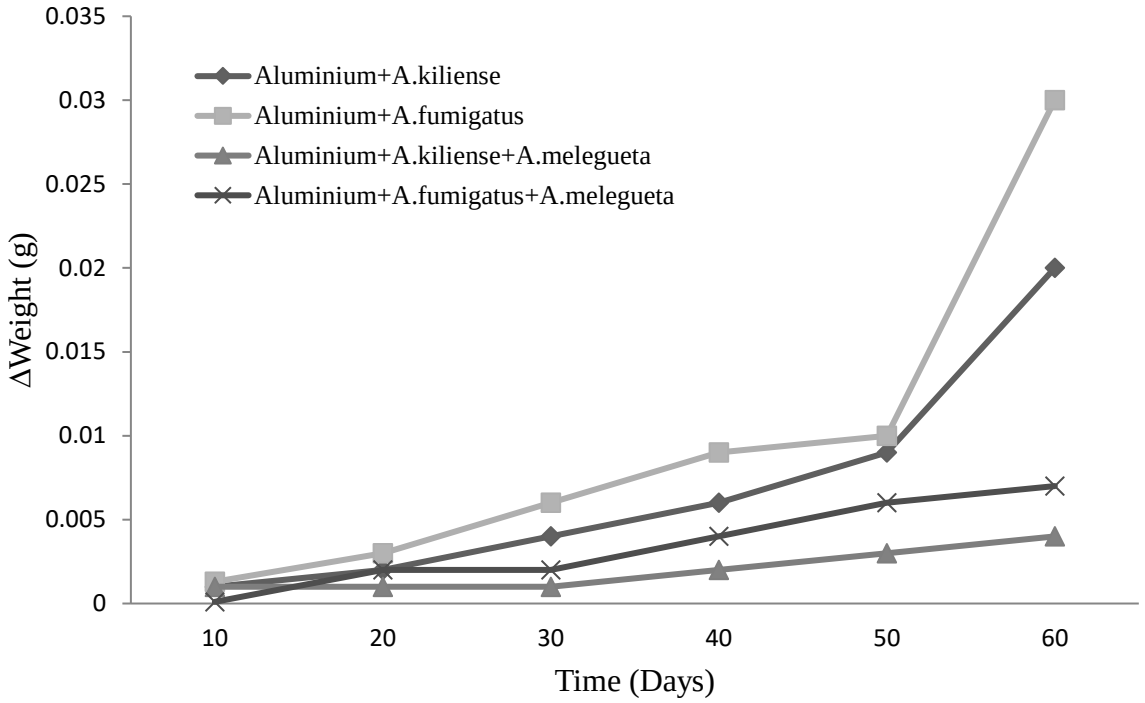



Figure 4.11 Weight loss of fungal influenced corrosion of aluminium treated with *Acremonium melegueta* extracts.

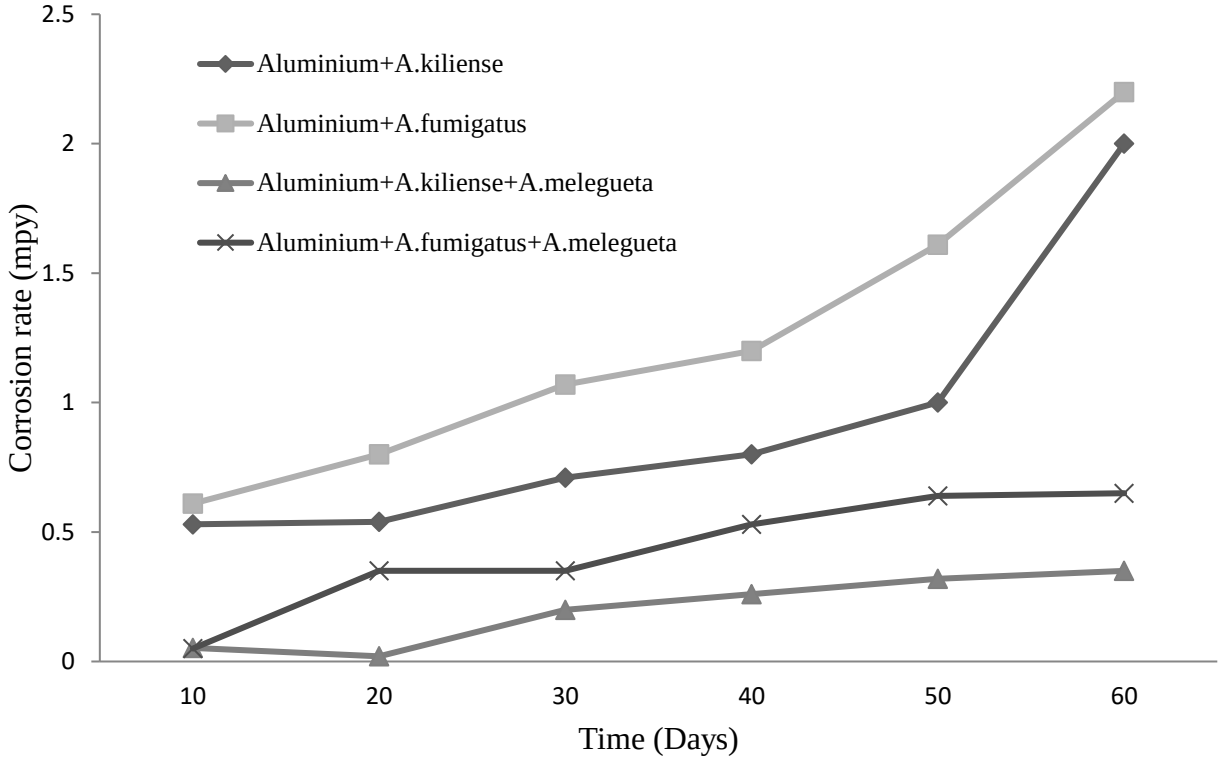



Figure 4.12: Effects of extracts of *A. melegueta* on fungal influenced corrosion of aluminium

### 4.7.3 Effects of cold-water extract of *P. guineense* on the corrosion behaviour of mild steel in the presence of *A. kiliense* and *A. fumigatus*

Figures 4.13 and 4.14 show the results of extract of *P. guineense* on the corrosion of mild steel influenced by *A. kiliense* and *A. fumigatus*. Throughout the 60 days incubation, there was a steady increase in weight loss and CR of untreated mild steel in the presence of fungal isolates. The increase in weight loss and CR was more pronounced in the untreated mild steel than in the treated mild steel. For the untreated mild steel exposed to *A. kiliense*, the weight loss increased from 0.0005g to 0.05g and the CR increased from 0.09mpy in 10 days after incubation to 2.0 mpy in 60 days after incubation. While for the cold-water extract of *P. guineense* treated mild steel exposed to *A. kiliense*, the weight loss increased from 0.0001g to 0.008g and the CR increased from 0.053 mpy to 1.39mpy 60 days after incubation.

In the case of untreated mild steel exposed to *A. fumigatus*, the weight loss increased from 0.001g to 0.08g and the CR increased from 1.9 mpy to 2.6 mpy while weight loss increased from 0.0001g to 0.004g and CR increased from 0.01mpy to 0.15mpy in the cold-water extract of *P. guineense* treated mild steel after 60 days of incubation.

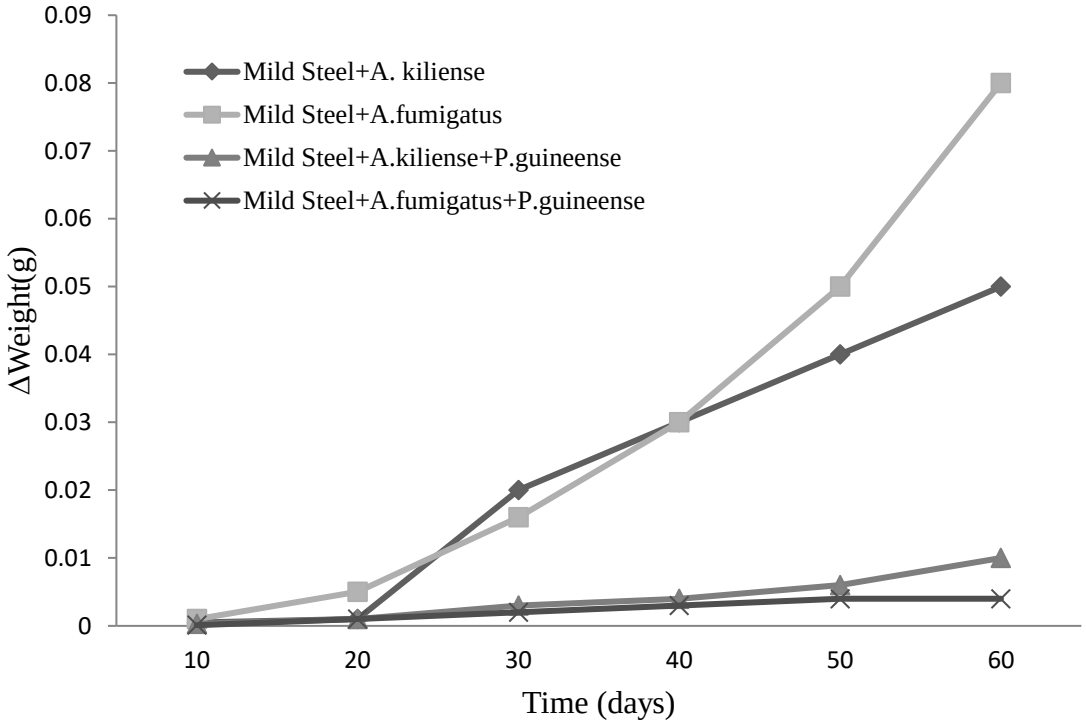



Figure 4.13 Weight loss of fungal influenced corrosion of mild steel treated with *Piper guineense* extract.

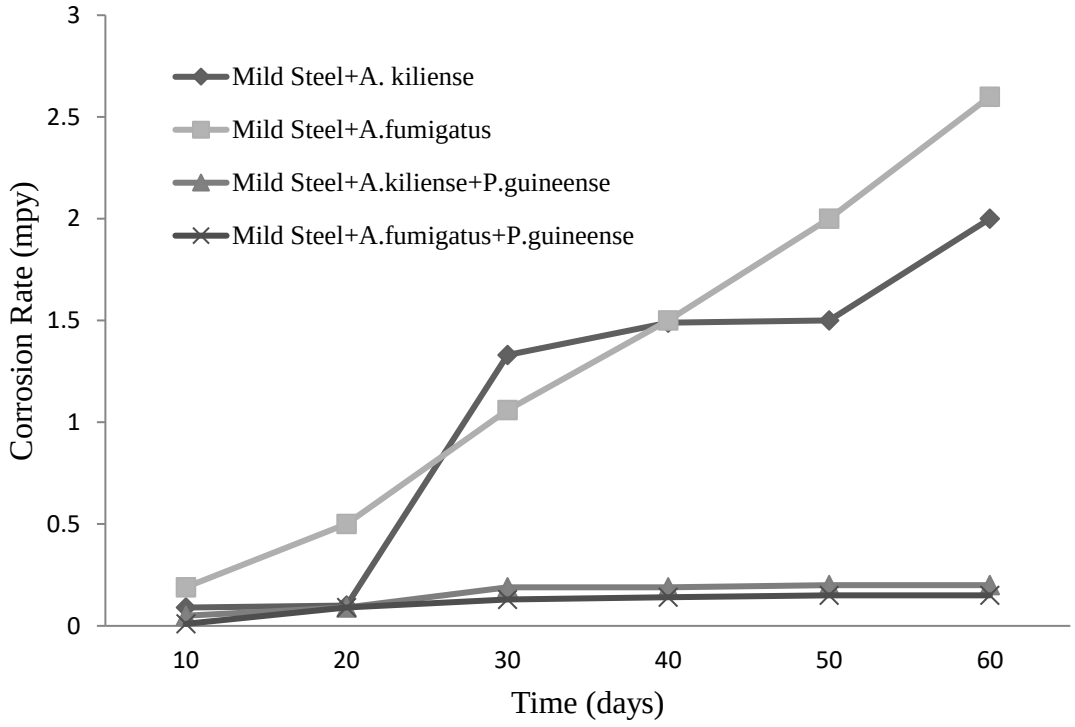



Fig.4.14 Effects of extracts of *P.guineense* on fungal influenced corrosion of mild steel

### 4.7.4 Effects of cold-water extract of *P. guineense* on the corrosion behaviour of aluminium in the presence of *A. kiliense* and *A. fumigatus*

Figures 4.15 and 4.16 show the results of extract of *P. guineense* on the corrosion of aluminium influenced by *A. kiliense* and *A. fumigatus*. For the untreated aluminium exposed to *A. kiliense*, the weight loss increased from 0.001g to 0.02g and the CR increased from 0.53 mpy in 10 days after incubation to 2.0 mpy in 60 days after incubation. While for the cold-water extract of treated aluminium exposed to *A. kiliense*, the weight loss increased from 0.001g to 0.008g and the CR increased from 0.053 mpy to 1.39 mpy 60 days after incubation.

In the case of untreated aluminium exposed to *A. fumigatus*, the weight loss increased from 0.0013g to 0.034g and the CR increased from 0.61 mpy to 2.2 mpy. While weight loss increased from 0.0001g to 0.015g and CR from 0.05 mpy to 1.34 mpy in the cold-water extract of treated aluminium after 60 days of incubation.

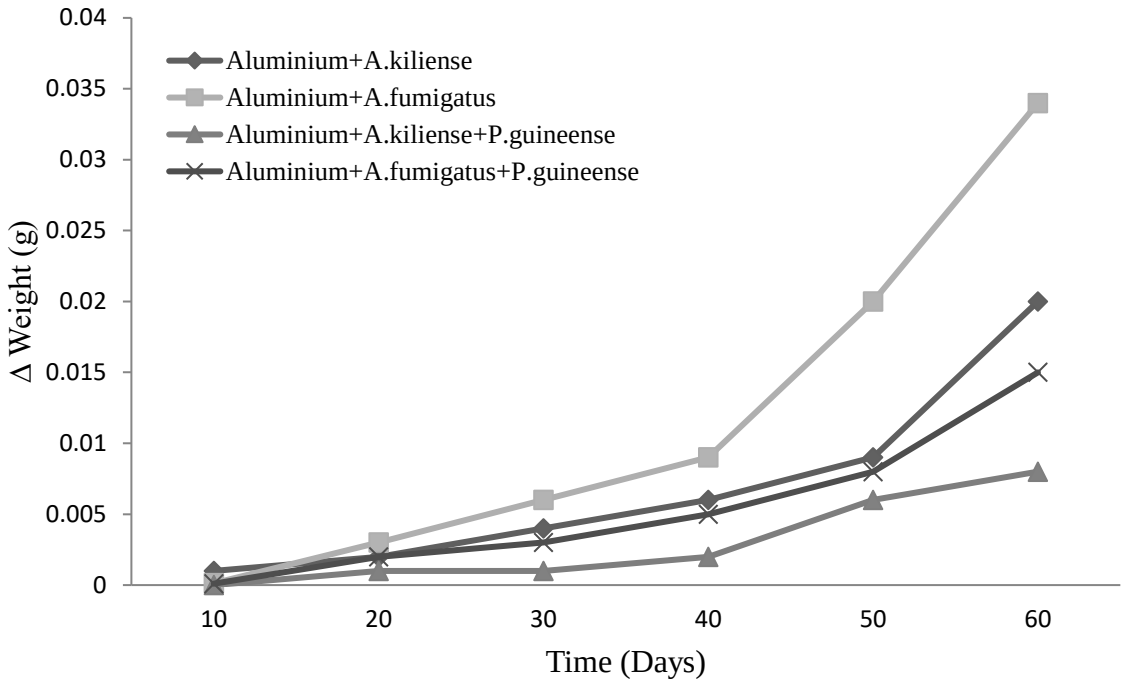



Figure 4.15 Weight loss of fungal influenced corrosion of aluminium treated with *Piper guineense* extracts.

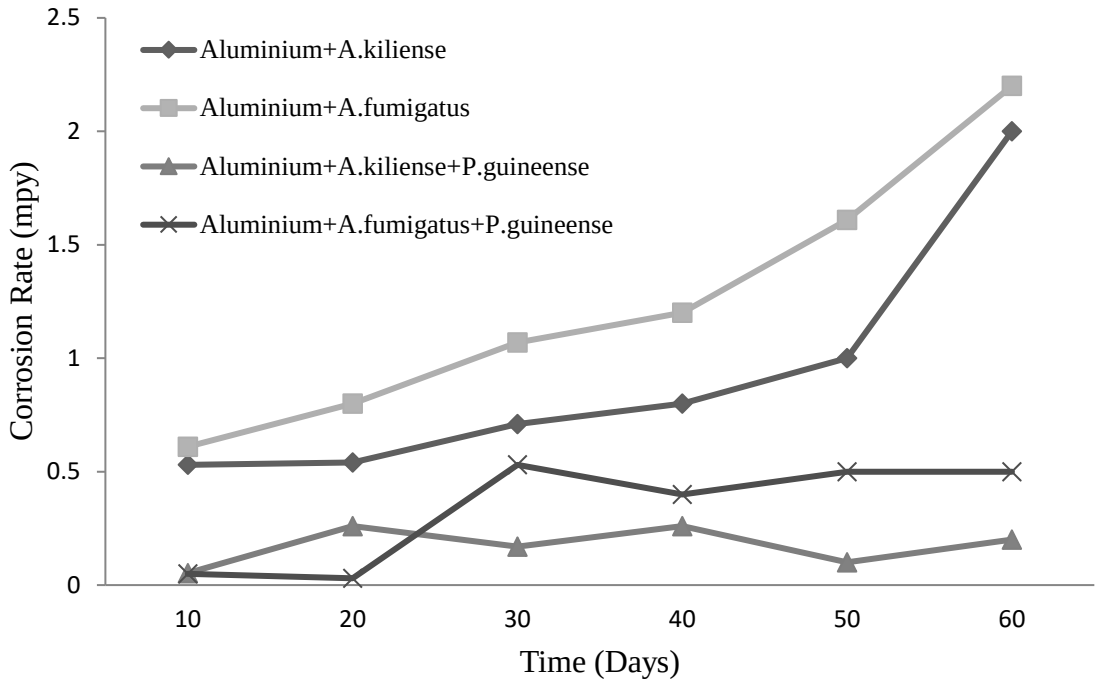



Fig. 4.16 Effects of extracts of *P. guineense* on fungal influenced corrosion of aluminium

## 4.8 Cumulative Corrosion Rate and Weight Loss of Fungal Influenced Corrosion of Mild Steel and Aluminium in the Presence and Absence of Plant Extracts

The results of the cumulative corrosion rate and weight loss of fungal influenced corrosion of mild steel and aluminium in the presence and absence of the extracts are shown in Tables 4.8 and 4.9. Table 4.8 shows that for *A. kiliense* influenced corrosion there were significant variations ($p < 0.05$) on the cumulative corrosion ($\sum$CR) of mild steel (0.98±0.10 mpy) and aluminium (1.15±0.15 mpy) obtained in the presence of cold water extract of *A.melegueta* when compared with $\sum$CR results obtained (6.51±0.19 mpy and 5.58±0.55 mpy) when the metals were exposed to *Acremonium kiliense* in the absence of the extract. A similar trend was observed when mild steel and aluminium were treated with cold water extract of *P. guineense* (Table 4.8).

Results from Table 4.9 showed that there were significant variations ($p < 0.05$) in the $\sum$CR of mild steel (0.79±0.07 mpy) and aluminium (2.54±0.15 mpy) when mild steel and aluminium were treated with the cold-water extract of *A. melegueta* compared with $\sum$CR results obtained (7.85±0.91 mpy and 7.49±0.57 mpy) when the metals were exposed to the influence of *A. fumigatus* in the absence of the extract. A similar trend was observed when *P. guineense* was applied (Table 4.9).

Table 4.8 Cumulative corrosion rate and weight loss of *A. kiliense* influenced metal corrosion in the presence and absence of plant extracts.

| **Cumulative corrosion rate and weight loss (∑CR and ∑ΔW)** | | | | | | |
|---|---|---|---|---|---|---|
| Parameters | **Mild Steel** | | | **Aluminium** | | |
| | MS | +AM | +PG | AL | +AM | +PG |
| ∑CR | 6.51±0.19 | 0.98±0.10 | 1.11±0.10 | 5.58±0.55 | 1.15±0.15 | 1.04±0.49 |
| ∑ΔW | 0.14±0.02 | 0.02±0.00 | 0.02±0.00 | 0.04±0.01 | 0.01±0.00 | 0.018±0.00 |

Legend: MS=Mild steel, AL= Aluminium AM=*Aframomum melegueta*, PG= *Piper guineense*, ∑CR= cumulative corrosion rate and ∑ΔW=cumulative weight loss.

Table 4.9 Cumulative corrosion rate and weight loss of *A. fumigatus* influenced metal corrosion in the presence and absence of plant extrats.

| **Cumulative corrosion rate and weight loss (∑CR and ∑ΔW)** | | | | | | |
|---|---|---|---|---|---|---|
| Parameters | **Mild Steel** | | | **Aluminium** | | |
| | MS | +AM | +PG | AL | +AM | +PG |
| ∑CR | 7.85±0.91 | 0.79±0.07 | 0.67±0.05 | 7.49±0.57 | 2.54±0.15 | 2.01±0.50 |
| ∑ΔW | 0.18±0.03 | 0.07±0.00 | 0.01±0.00 | 0.07±0.00 | 0.02±0.00 | 0.03±0.01 |

Legend: MS=Mild steel, AL= Aluminium AM=*Aframomum melegueta*, PG= *Piper guineense*, ∑CR= cumulative corrosion rate and ∑ΔW=cumulative weight loss.

## 4.9 Determination of the Inhibition Efficiency (%Ie) of the Extracts on Metal Corrosion

The results of the metal corrosion inhibition efficiency of the extracts on the corrosion of mild steel and aluminium are shown in Table 4.10. The results showed that for corrosion not influenced by fungi, *A. melegueta* had the highest IE of 57.5% for aluminium while *P. guineense* had the lowest inhibition efficiency of 2.8% for mild steel.

In the case of corrosion influenced by fungi, extracts of both *A. melegueta* and *P. guineense* elicited high inhibition efficiency (greater than 80%) for corrosion of mild steel influenced by *A. fumigatus* and less than 45% for corrosion of mild steel influenced by *A. kiliense*. The highest inhibition efficiency for aluminium (68%) was however obtained for corrosion influenced by *A. kiliense* and treated with *A. melegueta*.

Table 4.10 Inhibition efficiency of plant extracts on the corrosion of mild steel and aluminium in the presence and absence of *A.kiliense* and *A. fumigatus* after 60 days incubation.

| **Treatment** | **Inhibition efficiency (IE) (%)** | |
|---|---|---|
| **Inhibitor only** | Mild Steel | Aluminium |
| *Aframomum melegueta* | 34 | 57.5 |
| *Piper guineense* | 2.8 | 43.5 |
| **Fungi+ Inhibitor** | | |
| *Acremonium kiliense + Aframomum melegueta* | 40 | 68 |
| *Acremonium kiliense + Piper guineense* | 31 | 35 |
| *Aspergillus fumigatus + Aframomum melegueta* | 85 | 58 |
| *Aspergillus fumigatus + Piper guineense* | 87 | 43 |

## 4.10 Electrochemical Corrosion Measurement

Table 4.11 shows the results of the potentiodynamic polarization test and the corresponding polarization data for the corrosion behavior of mild steel and aluminium in the presence of *A. kiliense*, *A. fumigatus* and *P. chrysogenum* and the effects of the cold-water extracts of *A. melegueta* and *P. guineense* on the corrosion processes. For mild steel, *A. fumigatus* gave the highest corrosion current density ($I_{corr}$) of 279.4 μA/cm$^2$ followed by *P. chrysogenium* and then *A. kiliense* with 258.6 μA/cm$^2$ and 223 μA/cm$^2$ respectively. For aluminium, *A. fumigatus* gave the highest corrosion current density ($I_{corr}$) of 201.2 μA/cm$^2$ followed by *P. chrysogenum* and then *A. kiliense* with 184.6μA/cm$^2$ and 183.7 μA/cm$^2$ respectively.

Table 4.11 Polarization data for mild steel and aluminium in the presence and absence of fungi

| **Fungi /Fungi+Extract** | **I(µA/cm$^2$)** | | **$E_{corr}$ (mV) Vs SAC** | | **$i_a$** | | **$i_c$** | |
|---|---|---|---|---|---|---|---|---|
| | **Mild steel** | **Aluminium** | **Mild steel** | **Aluminium** | **Mild steel** | **Aluminium** | **Mild steel** | **Aluminium** |
| *Acremonium kiliense* | 223.0 | 183.7 | –417.3 | –795.0 | 122.7 | 100.3 | 100.6 | 121.4 |
| *Aspergillus fumigatus* | 279.4 | 201.2 | –485.4 | –796.4 | 115.1 | 97.6 | 75.1 | 109.8 |
| *Penicillium chrysogenum* | 258.6 | 184.6 | –490.2 | –734.5 | 107.0 | 90.6 | 69.5 | 104.6 |
| Control | 187.9 | 153.9 | -491.8 | −701.6 | 105.3 | 89.5 | 69.2 | 100.3 |
| *Acremonium kiliense* +*Aframomum melegueta* | 113.0 | 85.9 | –548.2 | –613.4 | 104.2 | 84.4 | 71.4 | 97.2 |
| *Aspergillus fumigatus* + *Piper guineense* | 102.0 | 78.6 | –509.8 | –614.5 | 102.6 | 80.7 | 73.2 | 96.6 |
| Control | 167.9 | 143.5 | –461.8 | –501.6 | 101.3 | 98.5 | 49.2 | 97.3 |

Legend: $I_{corr}$=Corrosion current density; $E_{corr}$= Corrosion potential; $i_a$ =Anodic current; $i_b$=Cathodic current

### (i) Potentiodynamic polarization result of *A. kiliense* influenced corrosion of mild steel and aluminium

Figure 4.17 shows the potentiodynamic polarization curve of *A. kiliense* influenced corrosion of mild steel. The corrosion current density ($I_{corr)}$ of mild steel in the presence of *A. kiliense* increased to 223 μA/cm$^2$ relative to the control 187.9 μA/cm$^2$ after 60 days incubation. The anodic current $i_a$ in the presence of the fungi (122.7 μA/cm$^2$) was also higher than the anodic current $i_a$ (105.3 μA/cm$^2$) in the absence of the fungi.

Figure 4.18 shows the potentiodynamic polarization curve of *A. kiliense* influenced corrosion of aluminium. The $I_{corr}$ of aluminium in the presence of *A. kiliense* increased to 183.7μA/cm$^2$ relative to the control (153μA/cm$^2$) after 60 days incubation. However, the $i_c$ (121.4) was higher than the $i_a$ (100.3).

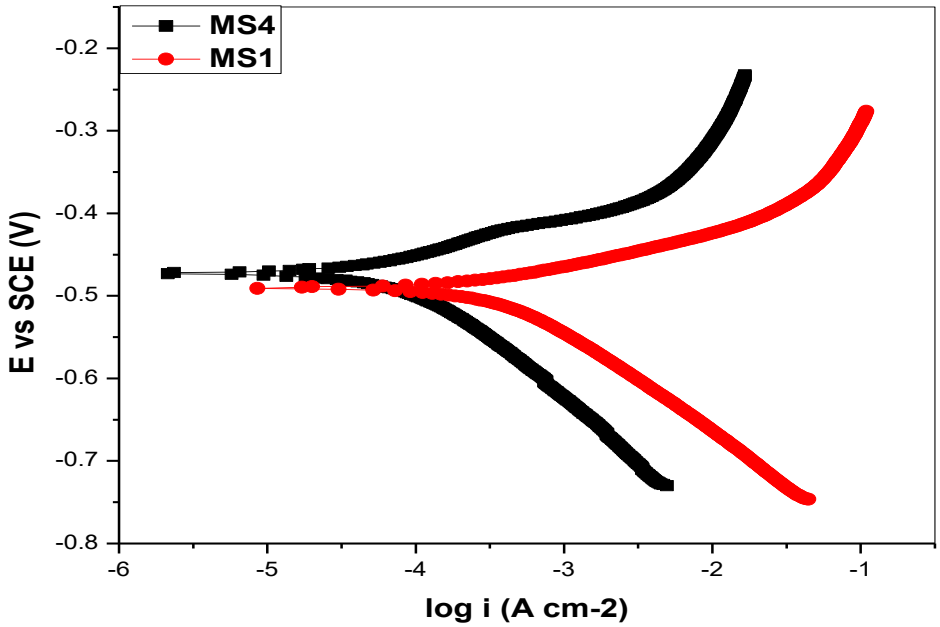



Figure 4.17: Potentiodynamic polarization curves of mild steel in the presence of *A. kiliense.*
Legend: MS1 =Mild Steel + *Acremonium kiliense*
MS4 = Control

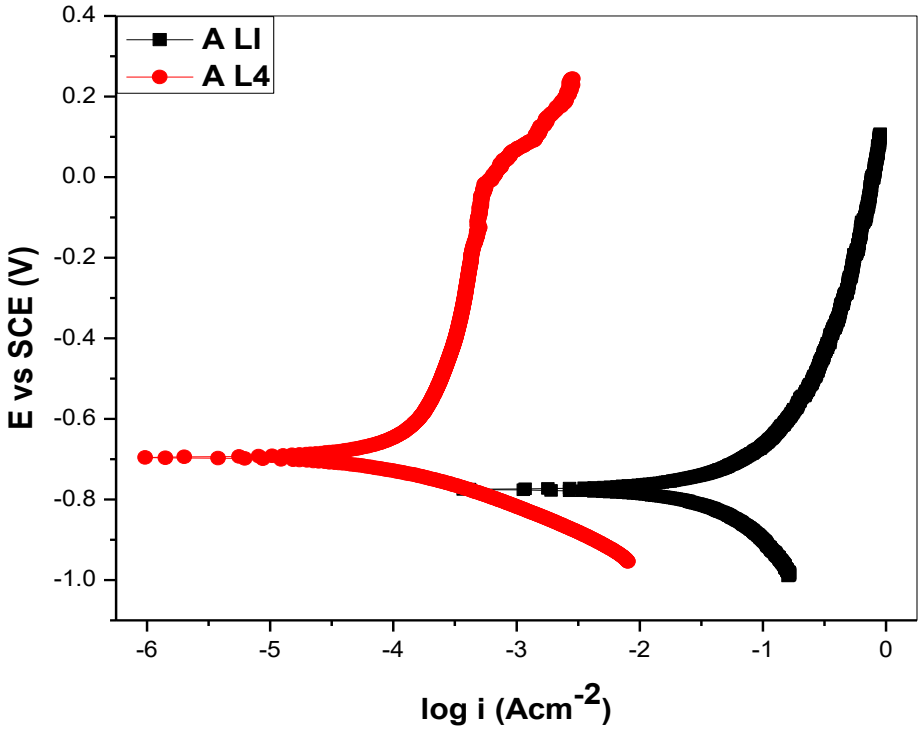



Figure 4.18: Potentiodynamic polarization curves of aluminium in the presence of *A. kiliense*.
Legend: AL1 = Aluminum+ *Acremonium kiliense*
AL4 = Control

### (ii) Potentiodynamic polarization result of *A. fumigatus* influenced corrosion of mild steel and aluminium

Figure 4.19 shows the potentiodynamic polarization curve of mild steel corrosion influenced by *A. fumigatus*. The $I_{corr}$ of mild steel in the presence of *A. fumigatus* increased to 279.4 μA/cm$^2$ relative to the control (187.9 μA/cm$^2$) after 60 days of incubation. The $i_a$ was higher in the presence of the fungi (115.1 μA/cm$^2$) compared to the $i_a$ (105.3 μA/cm$^2$) in the absence of the fungi.

Figure 4.20 shows the potentiodynamic polarization curve of *A. fumigatus* influenced corrosion of aluminium. The $I_{corr}$ of aluminium in the presence of *A. fumigatus* increased to 201.2 μA/cm$^2$ relative to the control (153.3 μA/cm$^2$) after 60 days of incubation. The $i_c$ (109.8 μA/cm$^2$) was higher than the $i_a$ (97.6).

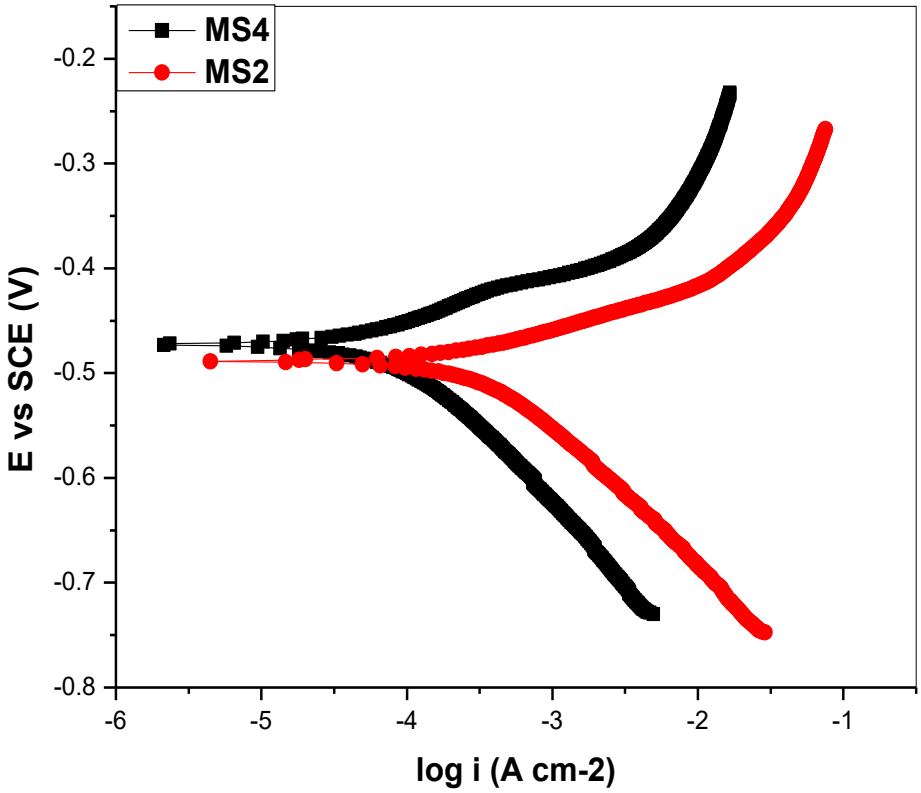



Figure 4.19: Potentiodynamic polarization curves of mild steel in the presence of *A. fumigatus*
Legend: MS2 = Mild Steel +*Aspergillus fumigatus*
MS4 = Control

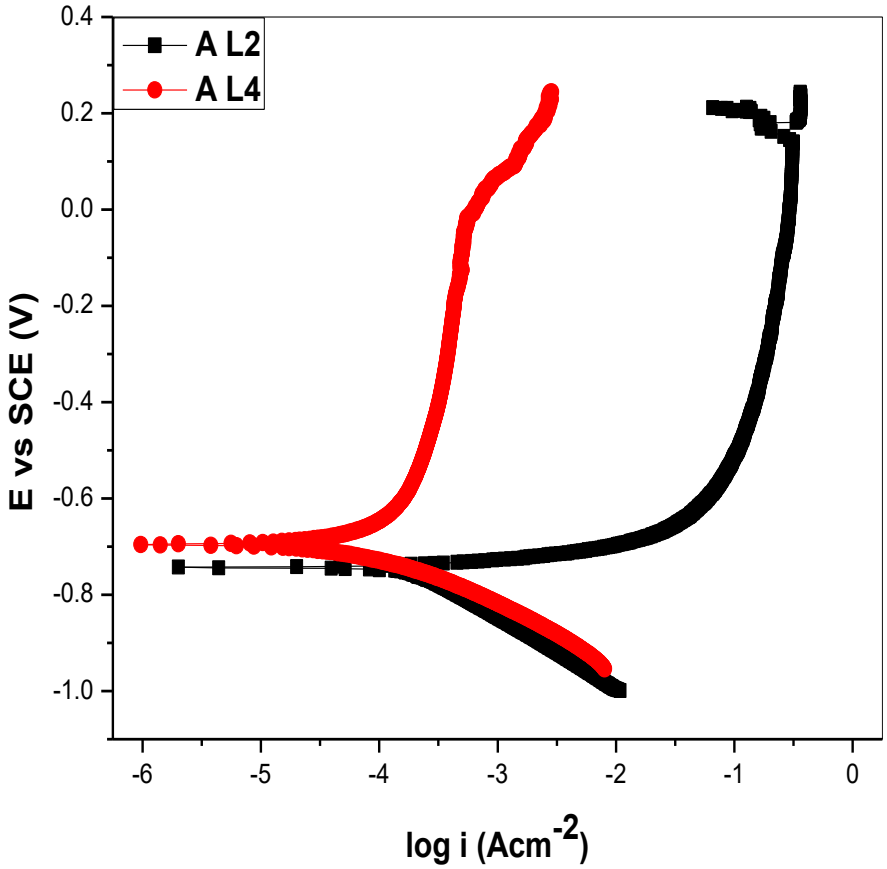



Figure 4.20: Potentiodynamic polarization curves of aluminium in the presence of *A. fumigatus*.
AL2 = Aluminium + *Aspergillus fumigatus*
AL4 = Control

### (iii) Potentiodynamic polarization result of *P.chrysogenum* influenced corrosion of mild steel and aluminium

Figure 4.21 shows the potentiodynamic polarization curve of mild steel corrosion influenced by *P. chrysogenum*. The $I_{corr}$ in the presence of *P. chrysogenum* increased to 258.6 μA/cm$^2$ relative to the control (187.9 μA/cm$^2$) in the absence of the fungi after 60 days incubation. The $i_a$ was also higher (107 μA/cm$^2$) in the presence of *P. chrysogenum* compared to the $i_a$ (105.3 μA/cm$^2$).

Figure 4.22 shows the potentiodynamic polarization curve of *P. chrysogenum* influenced corrosion of aluminium. The $I_{corr}$ of aluminium in the presence of *P. chrysogenum* increased to 184.6μA/cm$^2$ relative to the control (153.3 μA/cm$^2$) after 60 days of incubation. The $i_c$ (104.6 μA/cm$^2$) was higher than the $i_a$ (90.6 μA/cm$^2$).

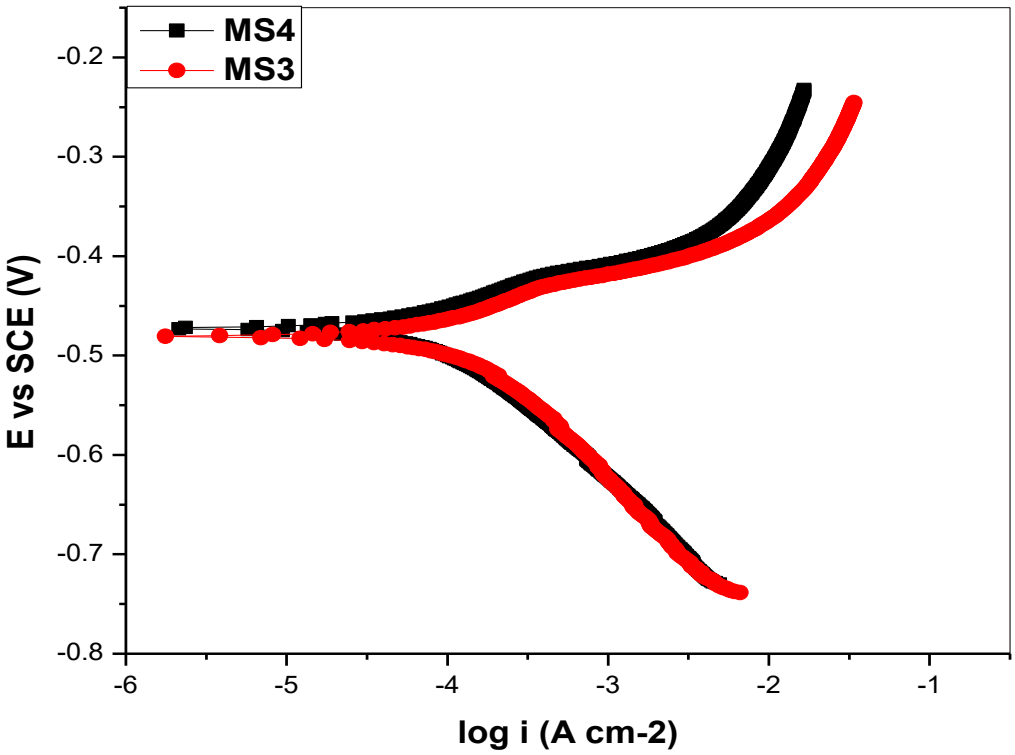



Figure 4.21: Potentiodynamic polarization curves of mild steel in the presence of *P. chrysogenum.*
Legend: MS3 = Mild Steel + *Penicillium chrysogenum*
MS4 = Control

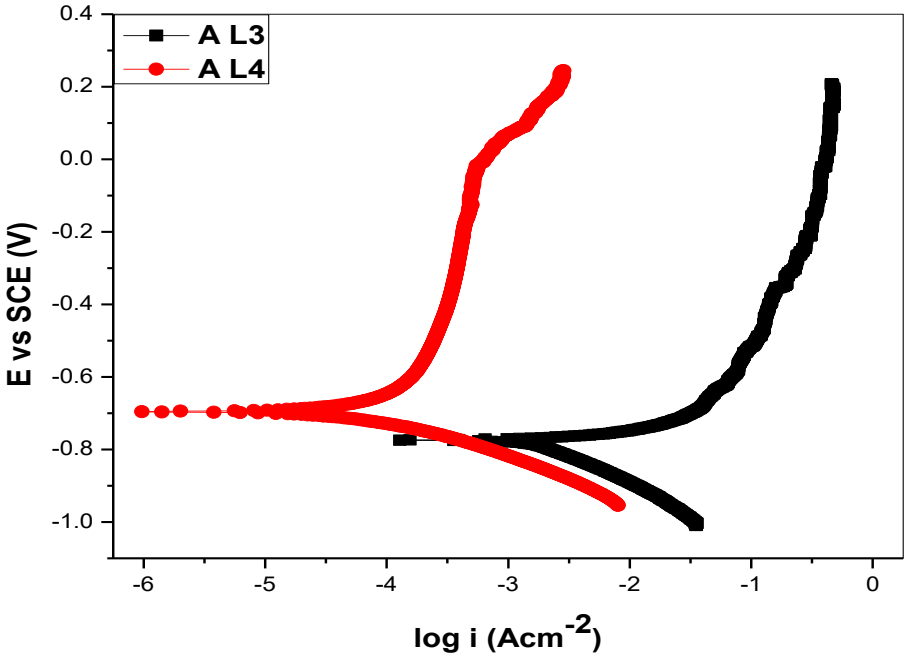



Figure 4.22: Potentiodynamic polarization curves of aluminium in the presence of *P.chrysogenum.*
Legend: AL3 = Aluminium + *Penicillium chrysogenum*
AL4 = Control

### (iv) Potentiodynamic polarization result of *A. kiliense* infuensed corrosion of mild steel and aluminium treated with cold water extract of *Aframomum melegueta*

Figure 4.23 shows the potentiodynamic polarization curve of *A. kiliense* infuensed corrosion of mild steel treated with cold water extract of *Aframomum melegueta*. The $I_{corr}$ decreased to 113.0 μA/cm$^2$ when the mild steel was treated with *Aframomum melegueta* compared with $I_{corr}$ observed with the metal it was exposed to the fungi (223.0 μA/cm$^2$) after 60 days. However, the $i_a$ (104.2 μA/cm$^2$) in the presence of the extract was higher than the $i_c$ but lower than the $i_a$ in the absence of the fungi and extract.

Figure 4.24 shows the potentiodynamic polarization curve of *A. kiliense* influenced corrosion of aluminium treated with cold water extract of *A. melegueta*. The $I_{corr}$ decreased to 85.9 μA/cm$^2$ when the aluminium was treated with the extract of *A. melegueta* relative to $I_{corr}$ observed with the metal was exposed to the fungi (183.7 μA/cm$^2$) and the control (153.5 μA/cm$^{2)}$ after 60 days incubation.

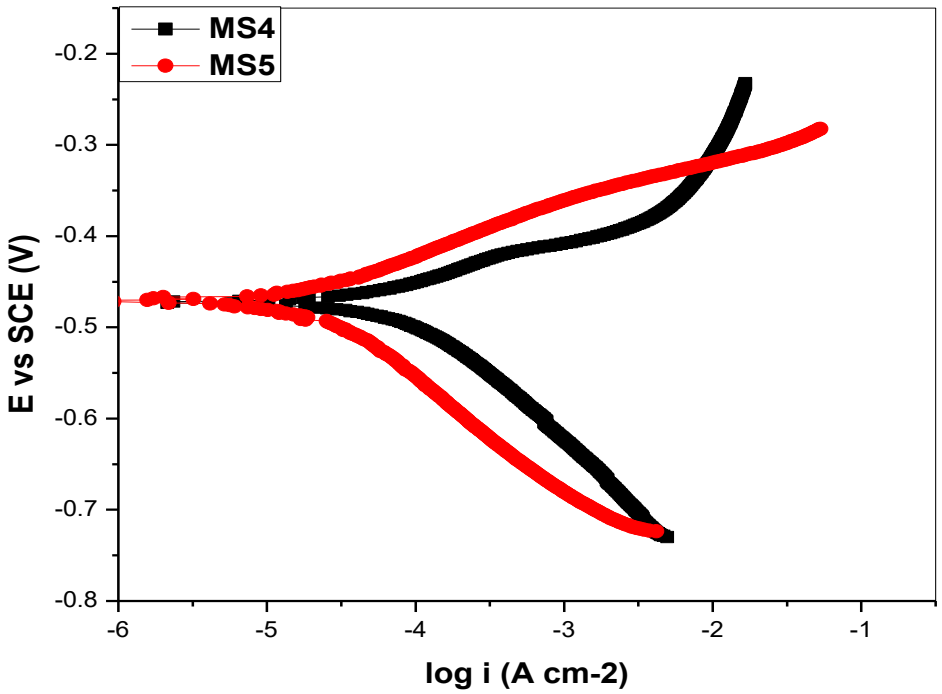



Figure 4.23: Potentiodynamic polarization curve for mild steel in the presence of *A. kiliense* and inhibitor *Aframomum melegueta.*
Legend: MS5 = Mild Steel + *A. kiliense* with extract *Aframomum melegueta*
MS4 = Control

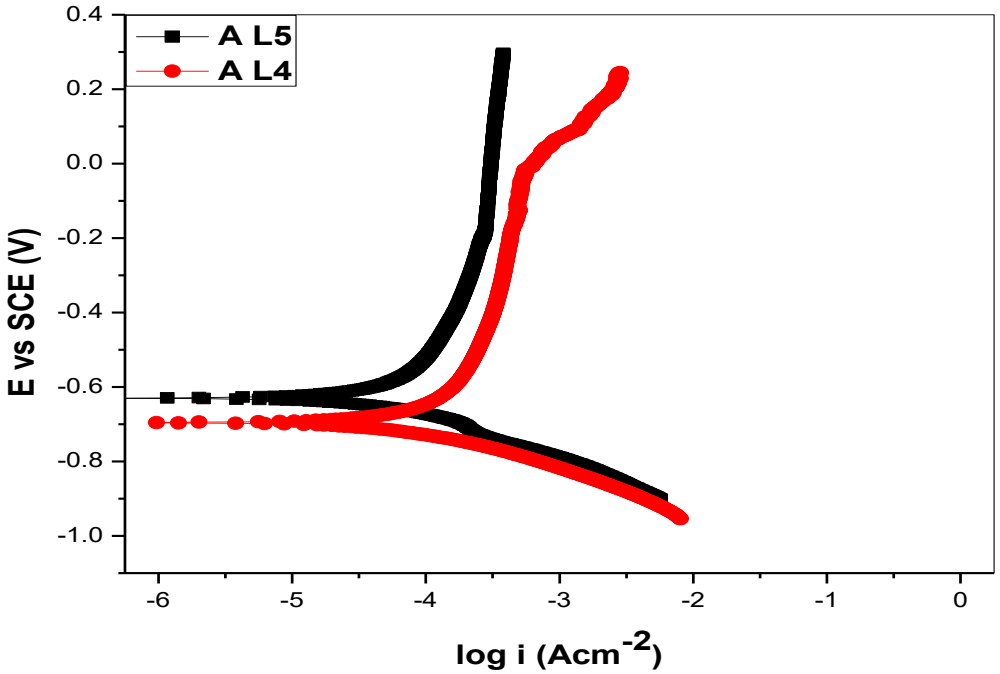



Figure 4.24: Potentiodynamic polarization curve for aluminium in the presence of *A. kiliense* and extract *A. melegueta*
Legend: AL5 = Aluminum +*A. kiliense* with extract *Aframomum melegueta*
AL4 = Control

### (v) Potentiodynamic polarizatoion result of *A.fumigatus* influenced corrosion of mild steel and aluminium treated with cold water extract of *P. guineense*.

Figure 4.25 shows the potentiodynamic polarizatoion of *A.fumigatus* influenced corrosion of mild steel treated with cold water extract of *P. guineense*. The $I_{corr}$ observed when the metal was treated with *P. guineense* extract was lower (102.0 μA/cm$^2$) compared with the value (279.4 μA/cm$^2$) observed when the metal was exposed to *A. fumigatus* after 60 days of incubation. Although there was higher $i_a$ (102.6 μA/cm$^2$) relative to the control (105.3 μA/cm$^2$), the corrosion potential ($E_{corr}$) was lower (–509.8mV) in the mild steel treated with the extract relative the metal exposed to fungi (–279.4 mV) and the control (–491.8 mV).

Figure 4.26 shows the potentiodynamic polarization curve of *A. fumigatus* influenced corrosion of aluminium treated with cold water extract of *P. guineense*. The $I_{corr}$ decreased to 78.6 μA/cm$^2$ when the aluminium was treated with the extract of *P. guineense* relative to $I_{corr}$ observed with the metal was exposed to the fungi (201.2 μA/cm$^2$) and the control (153.5 μA/cm$^2$) after 60 days incubation.

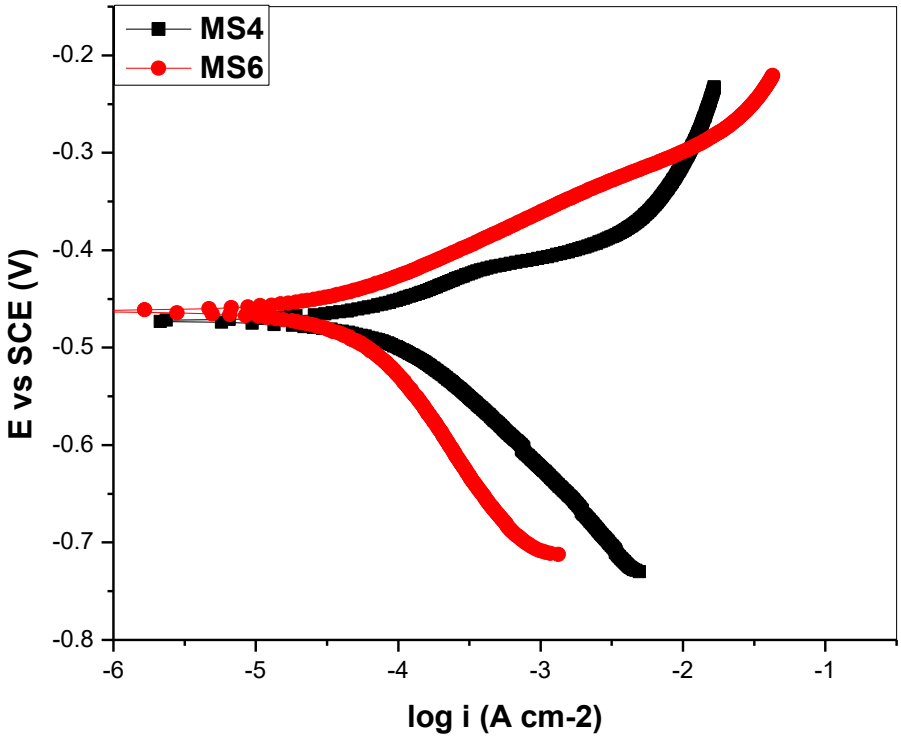



Figure 4.25: Potentiodynamic polarization curve for mild steel in the presence of *A. fumigatus* and inhibitor *P. guineense*
Legend: MS6 = Mild Steel + *A. fumigatus* with extract *Piper guineense*
MS4 = Control

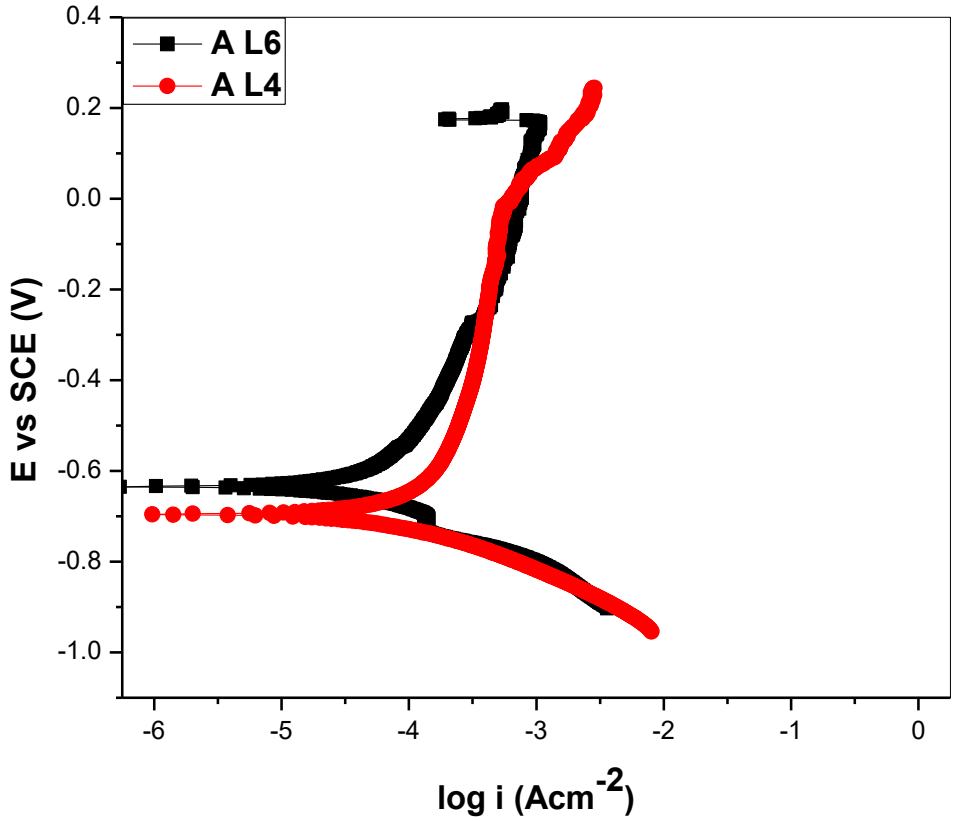



Figure 4.26: Potentiodynamic polarization curve for aluminium in the presence of *A. fumigatus* and inhibitor *P. guineense.*
Legend: AL6 = Aluminum + *A. fumigatus* with extract *Piper guineense*
AL4 = Control

### 4.11 Discussion

Fungi play an important biochemical role in the biosphere and are intimately involved in the corrosion of metals. They are ubiquitous members of sub-aerial and sub-soil environment and often become a dominante group in metal–rich or metal-polluted habitats. Fungi have ability to grow under extreme environmental conditions which allows their successful colonization of metal surfaces. Microbial corrosion and biodeterioration are directly related to the presence of biofouling deposits on metal surfaces mediated by microorganisms adhered to metal surfaces. As microorganisms like fungi attach and grow on metal surfaces by means of their physiological activities, they are able to change the electrochemical conditions on the metal in the most corrosion relevant way. Additionally, fungi produce carbon dioxide which reacts with water to form carbonic acid. Fungi cause aesthetic degradation via surface attachment in addition to loss of mechanical properties due to enzymatic activities and production of aggressive organic acids and metabolites (Little *et al.,* 2001). Unfortunately, inspections for microbial influenced corrosion generally are often overlooked until expensive problems such as damaging leaks occur or the corrosion is so prevalent that large areas of the system have to be replaced (Akpabio *et al.,* 2011). The need to study the influence of fungi on metal corrosion and fungal corrosion inhibition is the cynosure of this research.

The study was divided into three experiments. In experiment one of the studies, fungi were isolated from corroding metal pipes and aluminium sheets. The identification of the isolated fungal species showed the presence of *Acremonium kiliense*, *Aspergillus fumigatus* and *Penicillium chrysogenum*. Little *et al.* (1992), isolated *Fusarium* sp, *Penicillium* sp and *Hormoconis* sp from corroding tendons in a post-tensioned structure. Akpan and Mohammed (2015), also reported the isolation of *Aspergillus fumigatus* from a corroded pipeline. Lugauskas *et al*. (2009), reported that the most frequent saprotrophic fungi isolated from most metal surfaces are species from the genus *Aspergillius*, *Penicillium*, *Scopulariopsis,*

*Paecilomyces*, *Trichoderma*, *Fusarium*, *Rhizomucor*, *Rhizopus*, *Mucor* and *Alternaria*. Bento *et al.* (1996), also isolated *A. fumigatus* from diesel storage tank. Little *et al.* (1992), isolated *A.fumigatus* from condemned aluminium corroding aircraft component parts. They observed that *A. fumigatus* together with other fungal species isolated promoted the microbial influenced corrosion of aluminium AL2024-T3 alloy used in the construction of aircraft.

The result of the phytochemical screening of the seed extracts of *Piper guineense* and *Aframomum melegueta* shows that they contain alkaloids, flavonoids, tannins, sapronin, terpenes, steroids, cardiac glycocides and phenols. This is in line with the findings of Okoye and Ebeledike (2013) and Oguzie *et al.* (2012), who isolated alkaloids, flavonoids, saponins and tannins from the seed extracts of *P. guineense*. Also, the phytochemical screening of *P. guineense* and *A. melegueta* conducted by Echo *et al.* (2012), showed that the seeds of these plants contained alkaloids, flavonoids, tannins, saponins, steroids, terpenes, phenols and cardiac glycosides. These phytochemicals have been proved to possess biocidal and inhibitory activities against a wide range of microorganisms (Aiyegoro & Okoh, 2009).

The results of the antifungal screening of individual plant extracts (cold water CW extract, hot water HW extract, ethanol ET extract and methanol extract) at 100 mg/mL and 50 mg/mL showed that *P. chrysogenium* was not sensitive to any of the extracts at both 100 and 50mg/ml concentrations. *A. kiliense* and *A. fumigatus* both exhibited varying degrees of sensitivity to the extracts at 100 mg/mL and 50 mg/mL. The inhibitory activities against the fungi may be due to the presence of high levels of phytochemical like saponins, tannins, phenols and flavonoids among others in the extracts. Oguzie *et al.* (2013), reported that saponins present in the extracts act on the microbial cells as detergents, dissolving lipids and thus causing the loss of cellular contents. They also stated that tannin moieties, with their protein-binding abilities interacts with bacic constituents of proteins present in cell walls, cell membranes and cytoplasm with resultant inhibition of key metabolic functions of the cells.

The authors also stated that components with phenolic structures such as phenylpropenes are known to be very active against microorganisms. These could have contributed to the fungal growth inhibition observed. Echo *et al*. (2012) reported that these phytochemicals exhibit a wide range of biological effects due to their antioxidant properties. Okoye and Ebeledike (2013) also reported that flavonoids are antioxidant and equally anti-inflammatory. Kubmarawa, Ajoku, Enworem and Okorie, (2007) reported the importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains. Doss *et al.* (2009) reported that tannins extracted from the plant *Solanum trilobatum* Linn and assayed against the bacteria *Staphylococcus aureus*, *Streptococcus pyrogenes*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Escherichia coli* using agar diffusion method, exhibited antibacterial activities against the microorganisms. Kredy (2010) also studied the antimicrobial activities of sapronin extract from *Zizihus spina* and found it to be active against *E. coli, Proteus mirabilis* and *Streptococcus pneumoniae*. Cushine and Lamb (2005) reported that alkaloids are effective against most bacteria and fungal species. Nwaiwu and Imo (1999) also reported the antifungal properties of the essential oil of *P. guineense* on food-borne fungi. It is therefore possible that the antimicrobial properties of these extracts might have played some roles in inhibiting the growth of the fungi and subsequent inhibition of the corrosion processes.

The percentage mycelial inhibitions were higher at 100 mg/mL than at 50 mg/mL concentration. This showed that the antifungal actions of the extracts were dose dependent. The results of the minimum inhibitory concentration (MIC) of the plant extracts showed that the cold-water extract of *A. melegueta* and *P. guineense* had the lowest MIC of 25 mg/mL for *Aframomum kiliense* and *Aspergillus fumigatus*. The highest MIC values were observed with HW and ET extracts of *Aframomum melegueta.* Several authors reported MIC from ethanol extracts of *P. guineense* and *A.melegueta* (Oguzie *et al.,* 2012; Oguzie *et al.,* 2013; Echo *et*

*al.,* 2012; Okoye & Ebeledike 2013). The MIC from the cold-water extracts obtained in this study could be attributed to the method of extraction used. Unlike the other extraction processes that employ high temperature, the maceration process is cool and gentle and its products are not damaged by exposure to temperature in the excess of ambient. The minimum inhibitory concentration obtained from cold water extracts could have been as a result of the method of extraction used.

The results of the influence of fungi on the corrosion of mild steel and aluminium are shown in Tables 4.5 and 4.6. The result of the exposure of the fungi to mild steel and aluminium for 60 days, their macroscopic and microscopic examination and capacity to grow and adapt to the metal surfaces showed that *A. kilense* did not grow intensively on the surface of the mild steel and aluminium but on the edges of the metals close to the MEA. Zuccro *et al.* (2000) reported that *Acremonium* species are slow growing fungi. This might explain why no growth was observed on the surfaces but only distinct colonies were observed on the edges of the metal coupons. It could also be that *A. kiliense* requires organic deposits on metal surfaces to attach and grow. *A. fumigatus* grew prominently on the surface of mild steel throughout the period of exposure with conidia scattered over the whole surfaces. Several authors reported the growth of *Apergillus* species on metals (Videla, 2003; Gu *et al.* 2015; Beech, 2004; Lugauska *et al.,* 2009; Agarry and Salam, 2016). Lugauska *et al.* (2009) reported the growth of *Aspergillus* sp on metal plates with colonies formed and conidia scattered over the metal plate surfaces. *P. chrysogenium* grew on the surface of mild steel with its mycelia covering most part of the metal. However, the growth was most prominent on after 60 day of incubation (Plate PCM18). Lugauskas *et al.* (2009) reported the growth of *Penicillium* sp on the surface of metal plate in Petri dishes filled with sterile agar medium of malt extract low in nutritive value. The growth of the fungi on mild steel shows its ability to survive on the surface of the metal. Salo (2016) observed that *P. chrysogenium* could grow naturally at

various challenging environment. Little *et al.* (2001) reported fungal growth on interiors of some wooden spools stored outside from which they isolated *Apergillus* and *Penicillium* species.

The result of the exposure of *A. fumigatus* and their capacity to grow and adhere to aluminium showed that *A. fumigatus* grew on the surface of aluminium throughout the period of exposure with continuous spread of its mycelia and conidia over the surfaces and edges of the aluminium metal. Juzeliunas *et al.* (2007) reported the growth of *A. fumigatus* on aluminium parts of abounded air craft. The result of the growth of *P. chrysogenium* on aluminum was not as pronounced as its growth on mild steel. Discrete colonies were seen scattered around the edges of the metal. After 40 days, *P. chrysogenium* grew prominently covering the whole metal surfaces with the mycelia spreading over the entire metal (PCA12). Juzeliunas *et al.* (2007) observed that not all fungi can survive on metal surface. Most of them die from stress and unfavourable conditions on the metal surface, such as alternating moisture, temperature, physical, chemical and technical parameters. Only fungi that are able to incorporate metal as a link into their activity chain connecting them with the environment and and their vital needs can survive (Juzeliunas *et al.,* 2007).

The study of morphological changes on the metal surfaces after exposure to fungi for 60 days is shown on Table 4.7. Prominent corrosion spots were seen on the surfaces of the mild steel with more of these corrosion defects centered on the edges. However, these corrosion spots and visible defects were more prominent on metal coupons exposed to *A. fumigatus*. Videla *et al.* (2001) reported that fungi were responsible for the corrosion of many metals and alloys used in the fabrication and construction of buildings. It is therefore possible that the corrosion spots and changes observed on the metal coupons may have been caused as a result of the growth of the fungi. To support this, Lugauskas *et al.,* (2008) stated that changes in the

composition of surface layer of metals and alloys are some of the evidences confirming that fungi have penetrated the metal surfaces and use it to satisfy their nutritional needs.

On the surfaces of aluminum, corrosion spots were observed on the edges. Lugauskas *et al.* (2008) reported that the results of the studies performed under laboratory conditions indicated that *Acremonium* species contaminated aluminum metals. According to them, this fungus produces fusidic acid and its sodium salt fusidin which affected the metal surfaces. Videla (2003) also stated that acids produced by fungi are damaging to metals and other materials. On the surface of aluminium exposed to *A. fumigatus*, there were visible colour changes with dark corrosion spot. Fungi are able to generate a wide range of organic acids under aerobic conditions (Lugauskas *et al.,* 2008). It is therefore possible that the deteriorations and changes on the metal surfaces might have been caused by the fungi.

The results of the influence of *A. kiliense, A. fumigatus* and *P. chrysogenium* on the corrosion behavior of mild steel and aluminum after 60 days exposure have showed that the growth and attachment of these fungi on mild steel and aluminum significantly influenced their corrosion rate over the period as evident in the increase in corrosion rates and weight losses. For example, the corrosion rate of mild steel exposed to *A. kiliense*, *A. fumigatus* and *P. chrysogenium* increased from 0.09 mpy to 2.0 mpy, 0.19 mpy to 2.6 mpy and 0.19 mpy to 2.3 mpy respectively between 10 and 60 days of exposure. Similarly, there was also corresponding increase in weight loss ($\Delta W$) observed for all the fungi during the 60 days exposure. It could also be seen that corrosion rate increased with increase in time. This was very pronounced after 30 days of exposure to the fungi and then continued progressively for the rest of the period. This observation is typical of a metal that does not demonstrate passivity effects. Similar observations have been reported (Agerry & Salam, 2016). Videla (2002) reported that corrosivity increased with contact time due to accumulation of metabolites under fungal colonies attached to metal surfaces. Results of the gravimetric

analysis, showed that *A. fumigatus* was more corrosive with the cumulative corrosion rate ∑CR and ΔW of 7.85±0.9 mpy and 0.18±0.03 mpy; 7.49±0.52 g and 0.068±0.012 g followed by *P. chrysogenium* 7.58±0.79 mpy and 0.17±0.02 mpy; 6.96±0.95 g and 0.06±0.01g and *A. kiliense* 6.51±0.79 mpy and 0.14±0.02 mpy; 5.58±0.5 g and 0.042±0.007 g for mild steel and alluminium respectively. Bento *et al*. (2005) reported the corrosive activities on mild steel ASTM 283-93-C used as storage tank for urban diesel. They observed that *A. fumigatus* had the highest value for steel weight loss. The authors also stated that solid phase micro extraction (SPME) performed after 60 days incubation showed the presence of propionic acid. It is therefore possible that the high corrosion and weight loss observed in presence of *A. fumigatus* for mild steel and aluminium metals could be as a result of propionic acid. Kavita, Verma and Motolal (2011), linked *P. chrysogenum* with biogenic weathering and biodeterioration of ancient monuments and building metal materials. Salo (2016) also observed that the production of metal ion binding siderophores is typical among *P. chrysogenum* that grows naturally at various challenging environments. Akpan and Iliyasu (2015), reported that *Penicillium* and *Aspergillus* spp are organic acids producers. The organic acid (formic, citric acetic and propionic) byproducts produced by these fungi have been linked to the deterioration and corrosion of copper and zinc samples in environment polluted with organic substances (Lugauskas *et al.,* 2009). It is possible that these organic acids produced by these fungi might have contributed to the corrosion of these metals.

The gravimetric results of the corrosion behavior of mild steel and aluminum in the absence of fungi showed that the corrosion rate and weight loss data were lower compared to the values obtained when the metals were exposed to fungi. The ∑CR observed when mild steel was exposed for 60 days in the absence of any fungi was 1.74±0.02 mpy while ∑CR observed when same metal was exposed to *A. kiliense*; *A. fumigatus* and *P. chrysogenum* was 6.51±0.19 mpy, 7.85±0.91 mpy and 7.58±0.79 mpy respectively. Similarly, the ∑CR of

aluminium not exposed to fungi (2.71±0.10mpy) was significantly lower than aluminium exposed to fungi (5.58±0.55 mpy, 7.49±0.57 mpy and 6.96±0.95 mpy for *A.kiliense*, *A. fumigatus* and *P. chrysogenum* respectively). These results suggest that the corrosion rates might have been influenced by the growth of fungi on the surface of the metals. The corrosion rates may also have been enhanced due to the creation of oxygen concentration or differential aeration cell caused by the patchy growth and distribution of fungal colonies and their metabolites on the metals (Beech 2005). Juzeliunas *et al.* (2007) reported that the overgrowth of metallic surfaces with fungus mycelia was closely related to electrochemical processes. It is also possible that since corrosion is an electrochemical process, the increase in corrosion rate observed could have been due to fungal growth on the metals. To further support the results obtained from this study, Stokes and Lindsay (1979) stated that the electrical characteristics of steel and aluminium can be worsened by growth and attachment of some species of fungi belonging to the genera *Aspergillus* and *Penicillium*.

The results of the effects of cold-water extracts of *A. melegueta* and *P. guineense* on the corrosion behaviour of mild steel and aluminium in the presence of *A. kiliense* and *A. fumigatus* showed that the extracts inhibited both the growth of fungi and corrosion processes. This was evident from the gravimetric data on the corrosion rate and weight loss observed. Throughout the 60 days incubation, there were steady increase in weight loss and corrosion rates of untreated and treated mild steel and aluminiumin the presence of fungal isolates. However, the increase in weight loss and corrosion rates were more pronounced in the untreated mild steel and aluminium. The decrease in weight loss and corrosion rates observed on the treated metals could have been due to the treatment of the metals with the extracts. Oguzie *et al.* (2013) studied the anticorrosion effects of the ethanol extract of *Capsicum frutescens* on the low carbon using gravimetric, impedence and polarization techniques. They reported that *C. frutescens* effectively inhibited both corrosion and growth

of sulfate –reducing bacteria (SRB) due to the action of the phytochemical constituents present in the extract which included alkaloids, tannins and saponins (which are also present in *P. guineense* and *A. melegueta*). It is therefore possible that the inhibition of the corrosion and fungal growth observed might have been as a result of the presence of these phytochemicals in the seed extracts. The authors also stated that saponin possesses fungicidal activities against *A. fumigatus* species. Although the detailed mechanism of the fungicidal actions of *A. melegueta* and *P. guineense* were not extensively investigated, the observed growth inhibition abilities of the cold-water extracts of these plants could be attributed to the lipid dissolving ability of saponin moieties which results in loss of cellular content of fungi (Oguzie *et al.*, 2013). Oguzie *et al.* (2012) studied the anticorrosion effect of *P. guineense* leave extract on corrosion associated SRB, *Desulfotomaculum* species. *P. guineense* was found to be excellent inhibitor for both corrosion and SRB growth. They also attributed both effects to phytochemical constituents present in the extract. Manoj, Jinendra, Anita and Gupta, (2013) reported the inhibiting abilities of tannins, alkaloids, organic amino acids and organic dyes of plant origin. The authors also reported the inhibition of carbon steel corrosion by *Phllanthus amarus* extracts (PAE)-$Zn^{2+}$ system using weight loss method. Senhaji *et al.* (2013) observed that there was a significant reduction in the mass loss of steel sample with the addition of lignin extract when compared with the sample in which no lignin was added.

The values of the corrosion rate in the absence ($CR_{uninhibited}$) and presence of extracts ($CR_{inhibited}$) were used to estimate the inhibition efficiency from gravimetric data. *A. melegueta* was more effective (57.5%) in the absence of any fungi than *P. guineense* (2.8%). On the surface of aluminium, *P. guineense* showed the highest percentage inhibition efficiency (IE %) against *A. fumigatus* (87%) and *A.kiliense* (85%). *P. guineense* showed the least IE% against *A. kiliense* on both mild steel (31%) and Al (35%). Singh *et al.* (2012) reported that *Andrographis paniculata, Strychnous nuxvomica* and *Moringa oleifera* plant

extracts showed inhibition efficiency above 98%. The authors used weight loss methods to determine the inhibition efficiency of the inhibitors. Rajam *et al.* (2013) also investigated the inhibition efficiency of an aqueous extract of garlic on the corrosion of carbon steel in well water by weight loss method.

The potentiodynamic polarization results of *A.kiliense* influenced corrosion of mild steel and aluminium showed that the corrosion current density ($I_{corr}$) increased in the presence of the fungi (223.0 μA/cm$^2$) relative to the control (187.9 μA/cm$^2$) after 60 days of incubation. The anodic current $i_a$ (122.7 μA/cm$^2$) was also higher that the cathodic current $i_c$ (100.6 μA/cm$^2$). This result corresponds with previous gravimetric results. Videla, (2001) reported that any biological effect that facilitates the anodic reaction or that separates the anodic and cathodic sites, will increase corrosion. It is possible that the presence of fungi on the metal could have increase the anodic reaction leading to increase in anodic current and subsequently to the corrosion of the metal.

The potentiodynamic polarization results (Table 4.11) of *A. fumigatus* and *P. chrysogenium* influenced corrosion of mild steel and aluminium also showed that the $I_{corr}$ increased in the presence of the fungi relative to the control (in the absence of fungi). Beech (2004) reported that $I_{corr}$ is proportional to corrosion rate. And the higher the anodic curren $i_a$ the higher the anodic reaction leading to metal dissolution (Little and Lee, 2007). It can also be observed that the $I_{corr}$ increasesd in the presence of all the fungi when compared with the values observed in the absence of fungi, indicating an increase in corrosion reaction. This suggests that the presence of the fungi and their metabolites might have induced slight electrochemical activities on the mild steel. Qing *et al.* (2007) pointed out that metabolic by-products and biofilm formation accelerated pitting and corrosion rate of AZ1B magnesium alloy in artificial seawater. Mansfield *et al*. (2002) used potentiodynamic polarization technique to examine the overall corrosion behavior of a corrosion system. The authors observed that

increase in $I_{corr}$ was due to the influence of microorganisms on the rate of the anodic and cathodic reactions. Inline with the results of the gravimetric studies, the polarization curves showed that *A. fumigatus* had the highest influence on the corrosion behavior of mild steel and aluminium with highest $I_{corr}$ of 279.4 μA/cm$^2$ for mild steel and 201.2 μA/cm$^2$ for aluminium respectively. The potentiodynamic polarization curve (Fig. 4.21) of mild steel in the presence and absence of *P. chrysogenum* showed no distinct difference on the cathodic branch but the anodic branch changed quite slightly suggesting that the anodic site was more sensitive to corrosion with the resultant metal dissolution. The potentiodynamic polarization curve of alluminium in the presence and absence of *A. kiliense*, *A. fumigatus* and *P. chrysogenum* showed similar cathodic and anodic polarization behavior but the anodic branches were more sensitive to corrosion than the cathodic branches. This is also evident in the values of $I_{corr}$ observed in the presence of all the fungi. However, the $i_a$ did not follow the same part. This can be attributed the passivity of aluminium and the existence of oxidation film on the aluminium.

A substantial shift of corrosion potential ($E_{corr}$) towards noble values occured throughout the period of exposure of the mild steel (Table 4.11) to fungi. The shift to positive potential observed (–417.3 mV/SCE for *A.keliense*, –485.4 mV/SCE for *A. fumigatus* and –490.2 mV/SECfor *P.chrysogenium*) correlates with the growth of fungi on the mild steel when compared with –491.8 mV/SEC obtained in the absence of fungi. Similar observation was reported by Faisal, Rahub, John, David and Brajendra, (2013). The potential shift clearly supports the findings that activities and growth of fungi species enhanced the redox quality of the medium and accelerated the metal dissolution. The positive shifts in $E_{corr}$ may also be as a result of ennoblement which is an indication of potential corrosion. Ennoblement in microbiologically influenced corrosion has been acknowledged by different investigators as probably the most notable phenomenon in microbial influenced corrosion studies (Faisal *et*

*al.,* 2013; Little *et al.,* 2001 & Videla 2003). It has been attributed to the microbial colonization and biofilm formation which collectively result in organometallic catalysis and acidification of the metal surface which promotes pitting corrosion as was observed on the mild steel in the presence of fungi.

The potentiodynamic polarization results of *A. kiliense* influenced corrosion of mild steel and aluminium treated with cold water extract of *Aframomum melegueta* showed that $I_{corr}$ decreased to 113 μA/cm$^2$ when mild steel was treated with *Aframomum melegueta* compared to the $I_{corr}$ value observed when the metal was exposed to fungi (233.0 μA/cm$^2$) after 60 days. This is in line with earlier studies. Similar observations have been reported by Oguzie *et al.* (2012) in their study of corrosion and microbial growth inhibiting effects of *Aframomum melegueta*. However, the $i_a$ in the presence of the extract was higer than $i_c$ in the absence of extract. No explanation could be given for this. Similar trend was observed for the polarization curve of *A. kiliense* influenced corrosion of aluminium treated with cold water extract of *Aframomum melegueta*.

The potentiodynamic polarization results of *A. fumigatus* influenced corrosion of mild steel and aluminium treated with cold water extract of *P. guineense* showed that the $I_{corr}$ decreased when the metals were treated with the extracts relative to the untreated metals. The study also showed that $E_{corr}$ was lower (–509mV) in the mild stel treated with the extract relative to the mild steel exposed to fungi (–485.4 mV) and control (–491 mV). Corrosion potential shows the tendency of metal to corrode in a given environment (Videla, 2000). It therefore implies that the treatment of mild steel with the extracts lowered the corrosion processes. The study also showed that the extracts exhibited mixed inhibition of the metal corrosion process. Ahamad, Prasad and Quraishi, (2010) stated that if displacement in $E_{corr}$ is > 85mV, the inhibitor can be seen as a cathodic or anodic type inhibitor and if the displacement is < 85 mV the inhibitor can be seen as mixed type. In this study, the $E_{corr}$ values are all less than

85mV, which indicates that the inhibitors are mixed type inhibitors showing more inhibition at the cathodic than at the anodic sites.

# CHAPTER FIVE

## 5.1 Conclusion

This study shows that mild steel and aluminum metals respond to the effects of different fungi depending on the fungal ability to grow on the metal surface in extreme conditions and produce metabolites stimulating changes on the metal surface. Cold water extracts of *Aframomum melegueta* and *Piper guineense* inhibited the corrosion of mild steel and aluminum influenced by *Aspergillus fumigatus* and *Acremonium kiliense*. The ethanol, methanol, hot water and cold-water extracts of the seeds were not active against *Penicillium chrysogenum* in all the concentration tested. Polarization measurements showed that the corrosion inhibition proceeded via mixed-type mechanism. The antifungal effects of the cold-water extracts of *A. melegueta* and *P. guineense* are attributed to the phytochemical constituents of the alkaloids, tannins and saponins which disrupts the growth and essential metabolic functions of the fungi. Consequently, the results obtained in this study support the hypothesis that the attachment and growth of fungi on the surface of metal can influence their corrosion and that the phytochemical constituents of plant extracts could be exploited for use in the control of fungal influenced corrosion of metals.

## 5.2 Recommendations

Further studies should be conducted to ascertain individual fungal metabolic products associated with the corrosion of the metals. The electrochemical impedance spectra of the corrosion process should be investigated to further understand the mechanism of absorption of the organic constituents of the extracts on the surface of the metals. In this study, the extracts were tested individually. I suggest further studies on the synergistic effects of the extracts. Detailed mechanism of the biocidal action of the extracts should be extensively investigated in further studies**.**

## 5.3 Contributions to knowledge

1. Most studies on biocorrosion centers predominantly on the role of bacteria especially sulfate reducing bacteria (SRB). This research has further broadened the knowledge on biocorrosion as it relates to the role played by fungi.
2. The use of potentiodynamic polarization method in combination with gravimetric method further improved the interpretation of the corrosion data which most studies did not do.
3. This study was able to establish that *Acremonium kiliense*, *Aspergillus fumigatus* and *Penicillium chrysogenum* can influence the corrosion of mild steel and aluminium.
4. The study also established that the corrosion associated with *Acremonium kiliense* and *Aspergillus fumigatus* can be inhibited with seed extracts of *Aframomum melegueta* and *Piper guineense.*

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**PLATES**

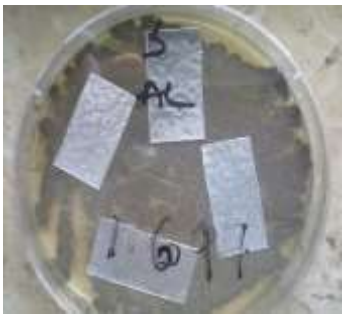
AKM1

AFM2

PCM3

AKA1

AFA2



PCA3

Figure P.1: General view of fungi growth on PDA with mild steel and aluminium after 10 days.

Legends:

AKM1=*Acremonium kiliense*, AFM1= *Aspergillus fumigatus* and PCM1= *Penicilium chrysogenium*

AKA1= *Acrmonium kiliense*, AFA= *Aspergillus fumigatus* and PCA3= *Penicilium chrysogenium*.

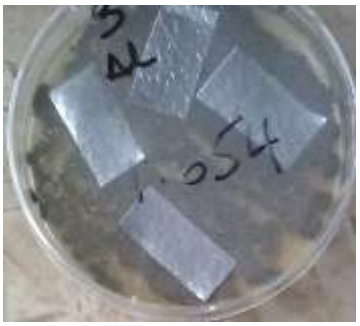
AKM4

AFM5

PCM6

AKA4

AFA5



PCA6

Figure P.2: General view of fungi growth on PDA with mild steel and aluminium after 20 days.

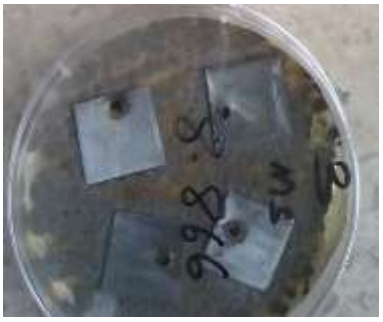
Legend:

AKM4=*Acremonium kiliense* + mild steel, APM5= *Aspergillus fumigatus* + mild steel and PCM6= *Penicilium chrysogenium* +mild steel

AKA4= *Acrmonium kiliense* + aluminium, AFA5= *Aspergillus fumigatus* + aluminum and PCA6= *Penicilium chrysogenium* + aluminium

AKM7

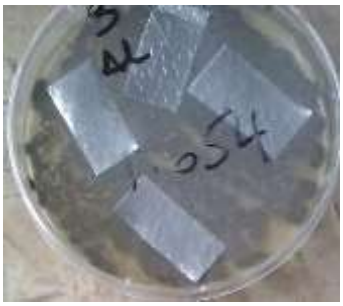
AFM8



PCM9

AKA7

AFA8



PCA9

Figure P.3: General view of fungi growth on PDA with mild steel and aluminium after 30 days.

Legend:

AKM7=*Acremonium kiliense* + mild steel, APM8= *Aspergillus fumigatus* + mild steel and PCM9= *Penicilium chrysogenium* +mild steel

AKA7= *Acrmonium kiliense* + aluminium, AFA8= *Aspergillus fumigatus* + aluminum and PCA9= *Penicilium chrysogenium* + aluminium


AKM10


AFM11


PCM12

AKA10

AFA 11

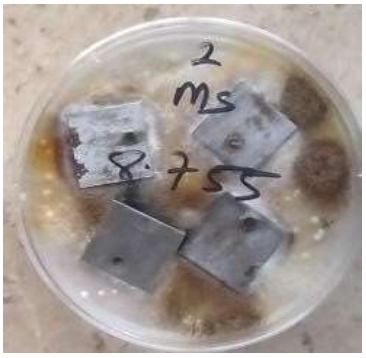
PCA12

Figure P.4: General view of fungi growth on PDA with mild steel and aluminium after 40 days.

AKM10=*Acremonium kiliense* + mild steel, APM11= *Aspergillus fumigatus* + mild steel and PCM12= *Penicilium chrysogenium* +mild steel

AKA10= *Acrmonium kiliense* + aluminium, AFA11= *Aspergillus fumigatus* + aluminum and PCA12= *Penicilium chrysogenium* + aluminium


AKM13




AFM14

PCM15


AKA13

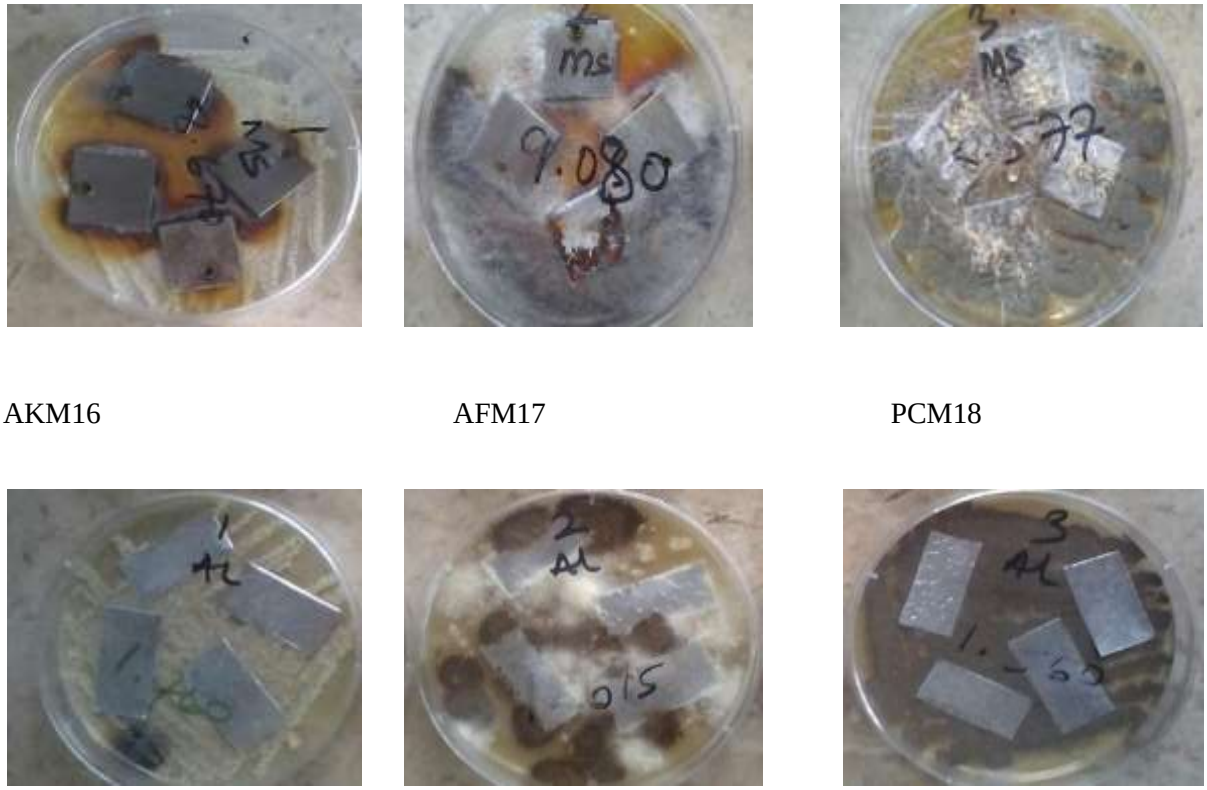

AFA 14

PCA15

Figure P.5: General view of fungi growth on PDA with mild steel and aluminium after 50 days.

AKM13=*Acremonium kiliense* + mild steel, APM14= *Aspergillus fumigatus* + mild steel and PCM15= *Penicilium chrysogenium* +mild steel

AKA13= *Acrmonium kiliense* + aluminium, AFA14= *Aspergillus fumigatus* + aluminum and PCA15= *Penicilium chrysogenium* + aluminium



AKM16 AFM17 PCM18

AKA16 AFA17 PCA18

Figure P.6: General view of fungi growth on PDA with mild steel and aluminium after 60 days.

AKM16=*Acremonium kiliense* + mild steel, APM17= *Aspergillius fumigatus* + mild steel and PCM18= *Penicilium chrysogenium* +mild steel

AKA16= *Acrmonium kiliense* + aluminium, AFA17= *Aspergillius fumigatus* + aluminum and PCA18= *Penicilium chrysogenium* + aluminium

## APPENDICES

### Preparation of Extract Dilutions

A stock solution of plant aqueous extract was prepared by dissolving 1000mg of the extract in 5mL of sterile distilled water and then made up to 10mL in 10mL volumetric flask. Thereafter, the initial concentration of the extracts (100mg/mL) was diluted using double fold serial dilution by transferring 1mL of the extracts into 1mL of distilled water to obtain 50mg/mL concentration.

The above process was repeated to obtain other dilutions respectively: 25mg/mL and 12.5mg/mL.

Table A3: The influence of *A. kiliense* on the corrosion behavior of mild Steel

| Fungi | Metal density ($gcm^{-3}$) | Initial weight (g) | Final weight (g) | Δ weight (g) | Surface area ($cm^2$) | Exposed time (Days) | Corrosion rate CR (mpy) |
|---|---|---|---|---|---|---|---|
| *A. kiliense* | 7.90 | 8.858 | 8.857 | 0.000 | 9.12 | 10 | 0.09 |
| | 7.90 | 8.800 | 8.799 | 0.001 | 9.12 | 20 | 0.10 |
| | 7.90 | 8.490 | 8.470 | 0.020 | 9.12 | 30 | 1.33 |
| | 7.90 | 8.592 | 8.562 | 0.030 | 9.12 | 40 | 1.49 |
| | 7.90 | 8.794 | 8.754 | 0.040 | 9.12 | 50 | 1.50 |
| | 7.90 | 8.670 | 8.620 | 0.050 | 9.12 | 60 | 2.00 |

Table A4: The influence of *A. kiliense* on the corrosion behavior of aluminum

| Fungi | Metal density ($gcm^{-3}$) | Initial weight (g) | Final weight (g) | Δ weight (g) | Surface area ($cm^2$) | Exposed time (Days) | Corrosion rate CR (mpy) |
|---|---|---|---|---|---|---|---|
| *A. kiliense* | 2.70 | 1.665 | 1.664 | 0.001 | 9.9 | 10 | 0.53 |
| | 2.70 | 1.574 | 1.571 | 0.002 | 9.9 | 20 | 0.54 |
| | 2.70 | 1.682 | 1.670 | 0.004 | 9.9 | 30 | 0.71 |
| | 2.70 | 1.644 | 1.630 | 0.006 | 9.9 | 40 | 0.80 |
| | 2.70 | 1.695 | 1.680 | 0.009 | 9.9 | 50 | 1.00 |
| | 2.70 | 1.760 | 1.740 | 0.020 | 9.9 | 60 | 2.00 |

Table A5: The influence of *A. fumigatus* on the corrosion behavior of mild steel

| Fungi | Metal density ($gcm^{-3}$) | Initial weight (g) | Final weight (g) | Δ weight (g) | Surface area ($cm^2$) | Exposed time (Days) | Corrosion rate CR (mpy) |
|---|---|---|---|---|---|---|---|
| *A.fumigatus* | 7.90 | 8.717 | 8.716 | 0.001 | 9.12 | 10 | 0.19 |
| | 7.90 | 9.080 | 9.075 | 0.005 | 9.12 | 20 | 0.50 |
| | 7.90 | 8.937 | 8.921 | 0.016 | 9.12 | 30 | 1.06 |
| | 7.90 | 8.464 | 8.434 | 0.030 | 9.12 | 40 | 1.50 |
| | 7.90 | 8.755 | 8.705 | 0.050 | 9.12 | 50 | 2.00 |
| | 7.90 | 8.759 | 8.679 | 0.080 | 9.12 | 60 | 2.60 |

Table A6: The influence of *A. fumigatus* on the corrosion behavior of aluminum

| Fungi | Metal density ($gcm^{-3}$) | Initial weight (g) | Final weight (g) | Δ weight (g) | Surface area ($cm^2$) | Exposed time (Days) | Corrosion rate CR (mpy) |
|---|---|---|---|---|---|---|---|
| *A. fumigatus* | 2.70 | 1.615 | 1.612 | 0.001 | 9.9 | 10 | 0.61 |
| | 2.70 | 1.578 | 1.575 | 0.003 | 9.9 | 20 | 0.80 |
| | 2.70 | 1.578 | 1.572 | 0.006 | 9.9 | 30 | 1.07 |
| | 2.70 | 1.658 | 1.649 | 0.009 | 9.9 | 40 | 1.20 |
| | 2.70 | 1.705 | 1.690 | 0.015 | 9.9 | 50 | 1.61 |
| | 2.70 | 1.615 | 1.581 | 0.034 | 9.9 | 60 | 2.20 |

Table A7: The influence of *P.chrysogenium* on the corrosion behavior of mild steel

| Fungi | Metal density ($gcm^{-3}$) | Initial weight(g) | Final weight(g) | Δ weight (g) | Surface area ($cm^2$) | Exposed time (Days) | Corrosion rate CR (mpy) |
|---|---|---|---|---|---|---|---|
| *P.chrysogenium* | 7.90 | 8.583 | 8.582 | 0.001 | 9.12 | 10 | 0.19 |
| | 7.90 | 8.577 | 8.572 | 0.005 | 9.12 | 20 | 0.49 |
| | 7.90 | 8.866 | 8.842 | 0.020 | 9.12 | 30 | 1.30 |
| | 7.90 | 8.822 | 8.792 | 0.030 | 9.12 | 40 | 1.50 |
| | 7.90 | 8.517 | 8.467 | 0.045 | 9.12 | 50 | 1.80 |
| | 7.90 | 8.475 | 8.405 | 0.070 | 9.12 | 60 | 2.30 |

Table A8: The influence of *P. chrysogenium* on the corrosion behavior of aluminum

| Fungi | Metal density ($gcm^{-3}$) | Initial weight (g) | Final weight (g) | Δ weight (g) | Surface area ($cm^2$) | Exposed time (Days) | Corrosion rate CR (mpy) |
|---|---|---|---|---|---|---|---|
| *A.chrysogenium* | 2.70 | 1.706 | 1.704 | 0.001 | 9.9 | 10 | 0.03 |
| | 2.70 | 1.697 | 1.692 | 0.001 | 9.9 | 20 | 0.30 |
| | 2.70 | 1.654 | 1.649 | 0.005 | 9.9 | 30 | 1.00 |
| | 2.70 | 1.600 | 1.590 | 0.010 | 9.9 | 40 | 1.34 |
| | 2.70 | 1.044 | 1.029 | 0.015 | 9.9 | 50 | 1.61 |
| | 2.70 | 1.560 | 1.530 | 0.030 | 9.9 | 60 | 2.68 |

Table A10. Inhibition effects of *A. melegueta* on the corrosion of mild steel in the absence of fungi.

| Exposed time (Day) | Initial weight (g) Metal +Inhibitor | Final weight(g) Metal +Inhibitor | Δweight (g) Metal +Inhibitor | Δweight (g) Metal –Inhibitor | Surface area ($cm^2$) | Corrosion rate CR (mpy) |
|---|---|---|---|---|---|---|
| 10 | 9.066 | 9.066 | 0.000 | 0.001 | 9.12 | 0.09 |
| 20 | 8.830 | 8.828 | 0.002 | 0.002 | 9.12 | 0.17 |
| 30 | 9.863 | 9.859 | 0.003 | 0.002 | 9.12 | 0.19 |
| 40 | 8.917 | 8.913 | 0.004 | 0.003 | 9.12 | 0.20 |
| 50 | 8.840 | 8.834 | 0.006 | 0.010 | 9.12 | 0.23 |
| 60 | 9.398 | 9.378 | 0.010 | 0.03 | 9.12 | 0.36 |

Table A11. Inhibition effects of *Piper guineense* on the corrosion of mild steel in the absence of fungi.

| Exposed time (Day) | Initial weight (g) Metal +Inhibitor | Final weight(g) Metal +Inhibitor | Δweight (g) Metal +Inhibitor | Δweight (g) Metal –Inhibitor | Surface area ($cm^2$) | Corrosion rate CR (mpy) |
|---|---|---|---|---|---|---|
| 10 | 8.919 | 8.919 | 0.000 | 0.001 | 9.12 | 0.09 |
| 20 | 8.859 | 8.856 | 0.003 | 0.002 | 9.12 | 0.20 |
| 30 | 8.822 | 8.817 | 0.005 | 0.002 | 9.12 | 0.30 |
| 40 | 8.801 | 8.792 | 0.007 | 0.003 | 9.12 | 0.30 |
| 50 | 8.430 | 8.410 | 0.010 | 0.010 | 9.12 | 0.40 |
| 60 | 9.314 | 9.264 | 0.010 | 0.030 | 9.12 | 0.40 |

Table A12. Inhibition effects of *A. melegueta* on the corrosion of aluminium in the absence of fungi.

| Exposed time (Day) | Initial weight (g) Metal +Inhibitor | Final weight(g) Metal +Inhibitor | Δweight (g) Metal +Inhibitor | Δweight (g) Metal –Inhibitor | Surface area ($cm^2$) | Corrosion rate CR (mpy) |
|---|---|---|---|---|---|---|
| 10 | 1.926 | 1.925 | 0.000 | 0.001 | 9.9 | 0.05 |
| 20 | 1.702 | 1.699 | 0.003 | 0.002 | 9.9 | 0.15 |
| 30 | 1.735 | 1.733 | 0.003 | 0.002 | 9.9 | 0.05 |
| 40 | 1.765 | 1.761 | 0.001 | 0.003 | 9.9 | 0.13 |
| 50 | 1.810 | 1.808 | 0.003 | 0.004 | 9.9 | 0.23 |
| 60 | 1.875 | 1.873 | 0.005 | 0.006 | 9.9 | 0.30 |

Table A13. Inhibition effects of *Piper guineense* on the corrosion of aluminium in the absence of fungi.

| Exposed time (Day) | Initial weight (g) Metal +Inhibitor | Final weight(g) Metal +Inhibitor | Δweight (g) Metal +Inhibitor | Δweight (g) Metal –Inhibitor | Surface area ($cm^2$) | Corrosion rate CR (mpy) |
|---|---|---|---|---|---|---|
| 10 | 1.582 | 1.582 | 0.000 | 0.001 | 9.9 | 0.05 |
| 20 | 1.975 | 1.974 | 0.001 | 0.002 | 9.9 | 0.02 |
| 30 | 1.832 | 1.831 | 0.001 | 0.002 | 9.9 | 0.17 |
| 40 | 1.925 | 1.923 | 0.002 | 0.003 | 9.9 | 0.26 |
| 50 | 1.772 | 1.769 | 0.003 | 0.004 | 9.9 | 0.32 |
| 60 | 1.928 | 1.920 | 0.008 | 0.006 | 9.9 | 0.40 |