

Study the corrosion effect of some fungal species from Shatt Al-Arab water against mild steel and Brass metals *In Vitro*

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Abstract

This study included isolating some species of fungi from samples of Shatt Al-Arab water and choiced five species to study their ability for the corrosion of Mild steel and Brass metals. Identified These species as *Aseprgillus flavus*, *Candida albicans*, *Cryptococcus neoformans* *Geotrichum candidum*, *Penicillium sp.* Which it caused corrosion on the metals in a semi continuous batch culture during three time intervals (28,56,84)days.

The results show the species *Aseprgillus flavus* exhibited corrosion net values after 84 days 4.5071 mg/cm²/day on mild steel. While the corrosion net values on Brass was less compared with mild steel , also the corrosion net values for five isolates on Brass was various during three time intervals , the higher corrosion net values after 84days for yeast *Cryptococcus neoformans* gave higher values 6.8373. mg/cm²/day Further more , X-Ray Diffraction technique was employed to study the topography of the corroded surfaces for mild steel and Brass and the results showed deformity in crystal plane of two metals

1-Introduction

Corrosion is decaying of metals and building materials or it losed their chemical and physical features for their reaction with exterior media whether air or soil or water , etc . many factors influence corrosion can be classified in to two groups they are: internal factors include chemical structure of metal . internal efforts, that is changes happening in the metal and its

surface. External factors include the nature of the media and its chemical components, and micro organisms like fungi which play important role in corrosion (Herrera and Videla, 2005). The term(MIC) Microbiologically Influenced Corrosion means the corrosion effects towards metals which produced by aerobic and an aerobic micro organisms (Herrera and Videla,2004) These metabolic products that

corrode the metals could be organic acid or non organic acid . A high range of micro organisms would share this type of corrosion like fungi which cause corrosion to different metals like steel, lead, copper and aluminum, this corrosion is sometimes due to organic acids which are formed by the growing molds and yeast (Al Haidri and Al Muslah, 1989)

Fungal corrosion has brought attention especially after the second world war-when noticed the appearance of great damage for metals because of the influence of fungi in/north pacific. These livings were specialized by great ability variant environmental conditions like the dry cold weather or moist hot .(EMI , 1996)

The economic damages of corrosion are large, they can be estimated by millions of dollars every year in England, America and Japan, These damages are in increase with industrial development and technological, which result it different resistance of plastics and metals against corrosion(Trethewey and Chamberlain, 1996). The damages can be divided into :-

- 1- Direct: includes the destroyed parts of machine or devices or metallic pipes or buildings or electric wires beside of the wages usage cost and devices and methods of avoiding the corrosion .
- 2- Indirect : includes the wasted time during the maintenance and repair or when having an accident which cause the lost of production or pollutions of products and exterior media or causalities (human lost), beside of increasing in the consumption of metals and other building materials (Mohammed , 1990).

Because of little studies in our country about role of the fungi in corrosion of metals which cause damage in our industrial foundations and other economic stations like oil , fertilizer and transport, petro-chemicals, air conditioners, electric wires and geometric materials , so that , this study aimed for isolation and identification of some fungal isolates from Shat AL - Arab water and testing their corrosion influence on mild steel and brass in the laboratory to measure the role played some of fungal species in corrosion.

2-Materials and Methods

During this study there had been a collection of 40 watery samples from Shat AL-Arab , they were taken by clean plastic containers . Their capacity 250 ml; they were opened under the water in depth of 20-30 cm and closed compactly when they were under water , then they were moved to the laboratory spread method was used on the surface of (agar) and using the plates of Petri dishes contained media of (Sabourauds dextrose agar) and antibiotic was added to it chloramphenicol to prevent the growth of bacteria, 0.1 ml from samples were added on the media so spread by L-Shape, then, the plates were closed and put in incubator on temperature 27 centigrade for 2-3 weeks

Solutions , stains and cultural media

Solution , stains and cultural media have been used during the study as following :-

- 1-Nigrosin staining solution used to explore *Cryptococcus neoformans* .
- 2-Lactophenol-ethylene blue is used to stain and stabilize fungi for microscope examination.

3-Standard Mc Farland (collee *et al.*, 1996) culture media.

The following culture media were used to study the isolated funguses :-

1-(SDA) Sabrauds Dextrose Agar (McGinnis ,1980)

2-Emmons Sabourauds Dextrose Agar (EADA) it is pH was arranged by using PH-meter , the type is Jenway 3320 (Germany Jenway comp)

3-Malt Extract Agar (MEA) (McGinnis , 1980)

4-Pulverized sun flower seeds (pals medium)

5-Christensens urea agar (Bucklay , 1989)

6-Fermentation broth medium ,(kreger Van Rij, 1984)

7-Yeast Nitrogen base (YNB) (Al – Zatari *et. al*, 1990)

0.05 gm from antibiotic chloramphenicol has been added to any of the mentioned media and sterilized by using Autoclave , 121C° under pressure 15 pound Inch² for 20 min.

Examination and identification of specimens

The plates have been examined three days later and they were watched every day for 2-3 weeks because they grow slowly but the plates which do not have growth were neglected after four weeks . The first examination for the plates has been done by using sterilized needles on plates contain culture media in order to purify them, These isolated things were moved on slant media in secro cap tube and kept in the fridge after growth. To study the feature of fungal

isolates they were examined under light microscope by fetching slides stabilized with lactophenol-methylene blue depending on morphological and microscopical featuresfor the colonies on culture media. The isolated fungi were identified during the study in the laboratory with assistance of the following resources:- Guarro Hoogde (1995), Ellis(1994) and McGinnis (1980)

Identification of yeasts

Yeasts were dentified on culture media by using the following tests depending on (Ellis, 1994 and McGinnis,1980) after they were purified on Sabouruads Dextrose Agar in slant :-

- 1- Growth at 37°C
- 2- Growth on cycloheximide medium
- 4- Germ tube formation test
- 5- Hyydrolysis of urea test
- 6- Sugars fermentation test .
- 7- Sugars assmiltion test .
- 8- Phenol oxidase production test .

Determiation of the percentage of the components mild steel and brass samples

Mild steel and brass were used in corrosion experiments , mild steel and bras was prepared locally by university of Basrah, collage of engineer's the percentage of the components in it is chemical structure was measured as shown in table (1).

Table (1): The percentage of elements in the chemical structure of mild steel and brass.

Mild steel		Brass	
Elements	%Percentage	Elements	%Percentage
ferrous	99.04	copper	95
manganese	0.05	Zinc	5
silicon	0.25		
Carbon	0.30		

Fetching the samples

Gutoff machine (Model type 0180) prepared by the company (Meta serve) was used for cutting the samples of mild-steel and brass like cylinder discs that have diameter and known area. Oxidated layers were removed from the samples surface by polishing by using the (polisher) prepared by the company (Bushler Ltd). The polish was done in two stages :-

First stage:- Grinding, this process was done by using Emery paper with the following grinding degrees: 220, 320, 400, 500, 600, 800, 1000.

Second stage :- polishing, this process was done by using a covered disk with a special type of cloth (Velvet), polishing for (1) degree Ap-Micro-cloth wheel with adding Alumina until the surface glinting like a mirror.

Testing the ability of fungi to corrode

Corrosion experiments were done purified isolates with a different periods of time to study the ability of fungi to cause corrosion the following. (Bill and Lim, 1981):-

Corrosion in purified isolates

The samples of mild steel and brass were cleaned with acetone to remove fates then they

are put in a flask containing (2N) HCl for 30 minutes to remove the detergent layers and to be clean and quietly ready for corrosion. Then, these samples were weighed with a sensitive balance and sterilized by burning on a flame of light. Then they were put in a glass flask containing (250) ml from sterilized culture media (Sabourauds Dextrose broth) every flask was inoculated with (3) ml from a purified refresh isolates with concentration $10^6 \times 9$ reproductive unit/ml according to McFarland standard (Collee et al., 1996) by sterilized pipette the flasks were put in a temperature (30°) the experiment lasted for (84) days distributed on period of times (28, 56, 84) day with 2 replicates for each sample at the same time a control sample were fetched by using a glass flask containing a culture media and metal only.

Removing the results of corrosion from the specimens of mild steel and brass

Bell and Lim (1981) method was followed to remove the results of corrosion. that the samples were washed completely with hot water and put in a glass flask containing a glacial acid for (30) minutes to remove all the result of corrosion, then they were washed with distill water and dried by the filter papers.

Measuring of corrosion rate

Corrosion rate has been measured by using weight loss method (Bell and Lim, 1981). The samples have been weighed before and after doing the experiment to know the lost weight and according to corrosion rate of the following equations :-

$$\text{Corrosion rate (milligram/centimeter}^2\text{/day} = \frac{\text{loss weight rate (milligram)/surface area (centimeter}^2\text{)} }{\text{period of time of corrosion (day)}}$$

Potential Hydrogen measurement (pH)

Ph has been measured for liquid culture media before and after being inoculated with isolated fungi by using PH-meter .

Chemical test for detect about some acid in liquid culture media after 84 day

1- Carboxylic acid reagent

One ml from liquid culture media inoculated with isolated fungi after 84 day added for it little amount from sodium bicarbonate when appeared air bubbles this indicator found carboxylic acid. (Kmib,1985).

2- Nitrate reagent

This reagent was prepared from 200 mg of Di Phenyl Amine to 100 ml conce H_2SO_4 thus added it for 1 ml from liquid culture media inoculated with isolated fungi after 84 day when blue color indicator of Nitrate this means Nitric Acid is found (Interkin and Keronise,1983).

X- Ray Diffraction

X- Ray Diffraction performed by using X-Ray Diffraction System (Philips.B.W.1253) for samples before and after corrosion for steel and brass metals (Culity , 1967).

Statistical Analysis

Statistical analysis for the results was done by using t-test (Al-Rawi and Khalaf Alla , 2000)

3-Results and Discussions

Corrosion is considered the problem of present time. Because of the damage to tools , Kits and foundations annually due to corrosion , this damage causes millions of dinnars. This process leads to complete damage for products, covered food and medicines which effect the health of man (Krikidgy et .al, 1990) .

Isolation of some fungi form Shat Al-Arab water was done during the present study . Five isolated fungi only were selected , the yeasts species were identified as in table (2) by then there was identification of the filamentous species depended on : McGinnis (1980) , Ellis (1994) , and , Guarro and Hoodgde (1995) .

The pH of liquid culture media which is inoculated with fungi has been measured as it is shown in table (3).

Table (2) : Typst of yeasts isolates and diagnosis tests

s	Yeasts species	Growth at 37 c'	Cycloheximide resistance	Formation germ tube	Phenol oxidase production	Urease production	Suger fermenttation		Suger assimilation										
							glucose	lactose	glucose	mallose	sucrose	galactose	trehalose	glucose	lactose	mallose	sucrose	galactose	trehalose
1	<i>Candida albicans</i>	+	+	+	-	-	-	+	+	v	+	-	+	+	+	v	+	-	+
2	<i>Cryptococcus neoformans</i>	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
3	<i>Geotrichum candidum</i>	+	+	-	-	-	-	-	+	-	-	-	+	-	+	-	-	-	+

V : Variation

Table (3) The PH of liquid culture media inoculated with the five fungal isolates after the passage of (84) day.

S	Fungal Isolates	PH before 84 day	PH after 84 day
1	<i>Aseprgillus flavus</i>	7	5
2	<i>Candida albicans</i>	7	5.5
3	<i>Cryptococcus neoformans</i>	7	5.06
4	<i>Geotrichum candidum</i>	7	5.58
5	<i>Penicillium sp.</i>	7	5.07

The pH in culture media changed from neutral to acid , the reason is the ability of fungi to produce organic acids and non-organic acids through its metabolic activity this results supporting by carboxylic acid and nitrate reagent which give us +ve for all fungal isolates,

with great role in corrosion . (Beech and Flemming, 2002).

The corrosion effect for five fungal isolates toward mild steel and brass has been also tested. As it is shown in tables (4,5,6,7,8,9) and in Figure (1,2).

Table (4) corrosion of samples of mild steel during (28) day day after day in five fungal culture .

S	Fungal isolates	Rate of weight loss (mg)	Area of metal by cm ²	Corrosion rate mg / cm ² / day	Net of fungal corrision mg / cm ² / day
1	<i>Aseprgillus flavus</i>	29.5	5.1369	0.2051	0.0606
2	<i>Candida albicans</i>	25.3	4.8934	0.1847	0.0402
3	<i>Cryptococcus neoformans</i>	31.2	5.7375	0.1942	0.0497
4	<i>Geotrichum candidum</i>	26.3	5.7375	0.1637	0.0192
5	<i>Penicillium sp.</i>	28.3	5.4782	0.1845	0.0400
6	Control	21.7	5.3644	0.1445	0.0000

Table (5) corrosion of samples of mild steel during (56) day after day in five fungal culture .

S	Fungal isolates	Rate of weight loss (mg)	Area of metal by cm ²	Corrosion rate mg / cm ² / day	Net of fungal corrision mg / cm ² / day
1	<i>Aseprgillus flavus</i>	62.9	4.8934	0.2295	0.1379
2	<i>Candida albicans</i>	62.9	5.1369	0.2187	0.1270
3	<i>Cryptococcus neoformans</i>	57.7	5.7375	0.1796	0.0879
4	<i>Geotrichum candidum</i>	49.3	5.7375	0.1534	0.0618
5	<i>Penicillium sp.</i>	59.2	5.4782	0.1930	0.1013
6	Control	25.2	4.9094	0.0917	0.0000

Table (6) corrosion of samples of mild steel during (84) day day after day in five fungal culture .

S	Fungal isolates	Rate of weight loss (mg)	Area of metal by cm ²	Corrosion rate mg/cm ² /day	Net of fungal corrsion mg/cm ² / day
1	<i>Aseprgillus flavus</i>	1971.2	5.1369	4.5683	4.5071
2	<i>Candida albicans</i>	92.4	4.8934	0.2248	0.1637
3	<i>Cryptococcus neoformans</i>	206.4	5.7375	0.4283	0.3672
4	<i>Geotrichum candidum</i>	81.8	5.7375	0.1697	0.1086
5	<i>Penicillium sp.</i>	92.6	5.4782	0.2012	0.1401
6	Control	25.2	4.9094	0.0611	0.0000

Table (7) corrosion of samples of brass during (28) day day after day in five fungal culture .

S	Fungal isolates	Rate of weight loss (mg)	Area of metal by cm ²	Corrosion rate mg / cm ² / day	Net of fungal corrsion mg / cm ² / day
1	<i>Aseprgillus flavus</i>	0.9	4.1427	0.0077	0.0065
2	<i>Candida albicans</i>	0.6	4.8934	0.0043	0.0031
3	<i>Cryptococcus neoformans</i>	0.8	4.5522	0.0062	0.0050
4	<i>Geotrichum candidum</i>	1	5.1200	0.0069	0.0057
5	<i>Penicillium sp.</i>	2	5.0072	0.0142	0.013
6	Control	0.15	4.5522	0.0012	0

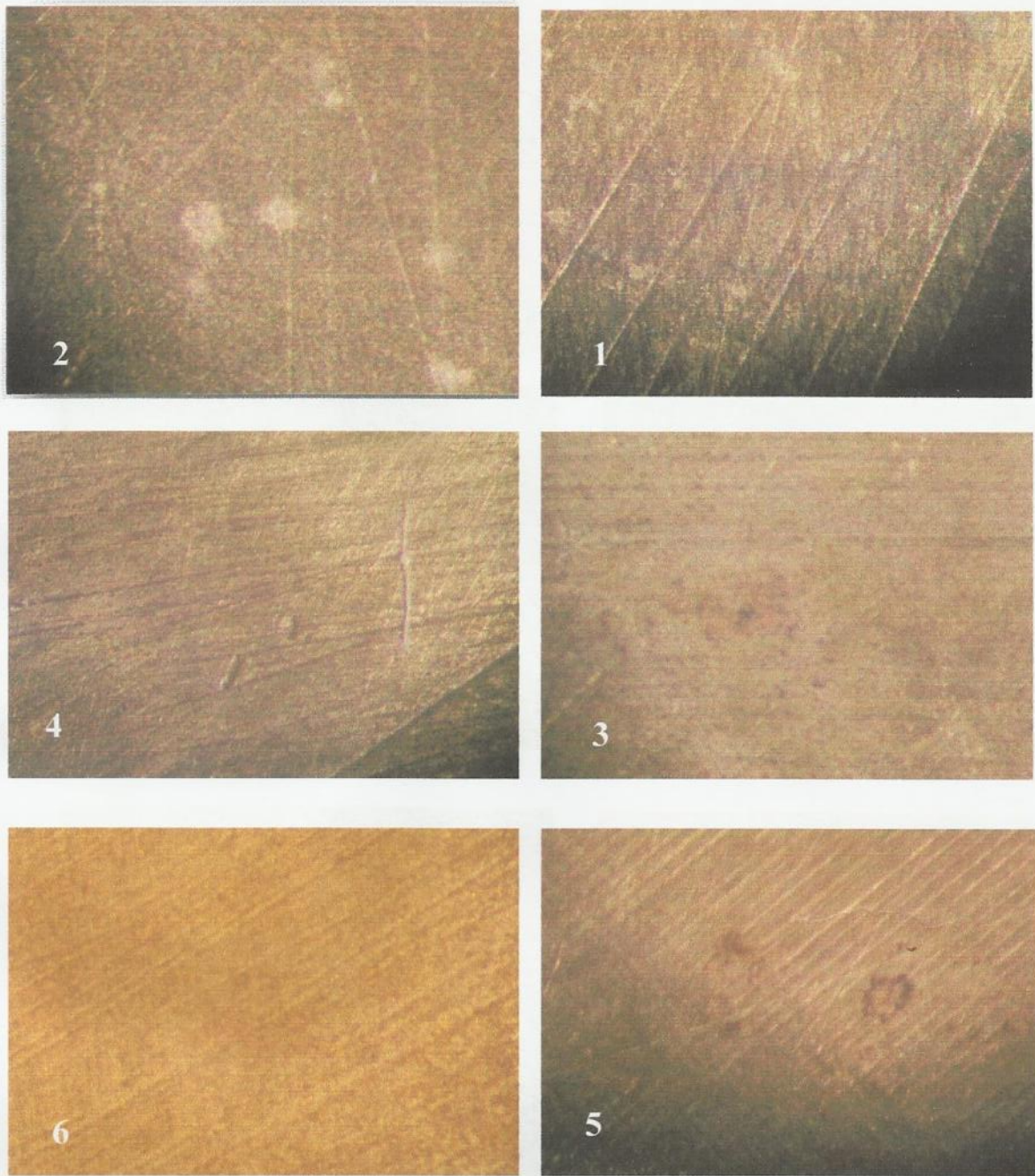


Fig. (1) : Corrosion of brass metal after 84 day in the following fungi culture
1- *Aspergillus flavus* 2- *Candida albicans* 3- *Cryptococcus neoformans*
4- *Geotrichum candidum* 5- *Penicillium sp.* 6--Control

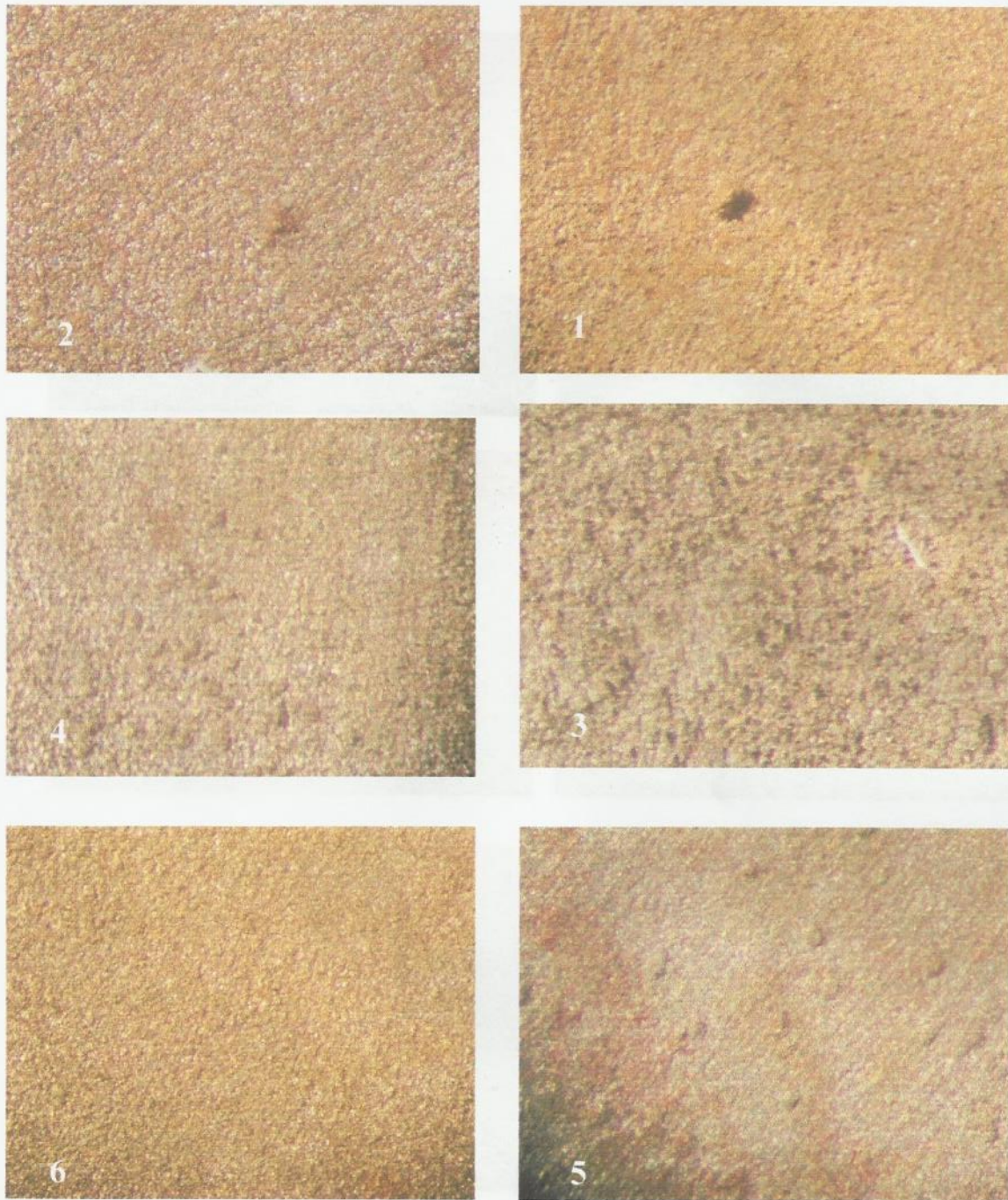


Fig. (2) : corrosion of mild steel metal after 84 day in the following fungi culture
1- *Aspergillus flavus* 2- *Candida albicans* 3- *Cryptococcus neoformans*
4- *Geotrichum candidum* 5- *Penicillium sp.* 6--Control

Table (8) corrosion of samples of brass during (56) day day after day in five fungal culture .

S	Fungal isolates	Rate of weight loss (mg)	Area of metal by cm ²	Corrosion rate mg / cm ² / day	Net of fungal corrosion mg / cm ² / day
1	<i>Aseprgillus flavus</i>	2	4.1427	0.0086	0.0042
2	<i>Candida albicans</i>	2.9	4.8934	0.0105	0.0061
3	<i>Cryptococcus neoformans</i>	2.4	4.5522	0.0094	0.0050
4	<i>Geotrichum candidum</i>	2.3	5.1209	0.0080	0.0036
5	<i>Penicillium sp.</i>	3.4	5.0072	0.0121	0.0077
6	Control	1.1	4.4384	0.0044	0.0000

Table (9) corrosion of samples of brass during (84) day day after day in five fungal culture .

S	Fungal isolates	Rate of weight loss (mg)	Area of metal by cm ²	Corrosion rate mg / cm ² / day	Net of fungal corrosion mg / cm ² / day
1	<i>Aseprgillus flavus</i>	392.6	4.1427	1.1282	1.1261
2	<i>Candida albicans</i>	5.4	4.8934	0.0133	0.0011
3	<i>Cryptococcus neoformans</i>	2615.3	4.5522	6.8394	6.8373
4	<i>Geotrichum candidum</i>	3.5	5.1209	0.0081	0.006
5	<i>Penicillium sp.</i>	6.2	5.0072	0.0147	0.0126
6	Control	0.8	4.5522	0.0021	0.0000

The results showed metals which exposure to corrosion in the surfaces of mild steel species in the pure culture of five isolates the existing of bio film on the surface of metallic species is a shape like sticky material and this film forms because of sticking the fungus in the surface of the metal that encourages the corrosion to happen because of precipitate metabolic results for stuck livings (Flemming and Beech , 2002) and these result meets with (Kobrin , 1976) that

referred that fungi are from micro organisms that cause corrosion may happen formation for bio film , as shown by (sequeira , 1988) . The corrosion may happen because of the sticky materials which is produced by fungi that metabolic results for these livings lead to the formation chemo electric cell or cells differential aeration so that a difference will happen in oxygen concentration between two different areas from the metal surface because of

the growth of colonies on metal surface which causes corrosion and this corrosion appears in different forms like pitting corrosion and crevice corrosion .

Results showed increase of corrosion rate with the passage of time to exposure. These results were compared with chemical corrosion due to control species by the effect of culture media. also during this study the highest rate of corrosion by *Aspergillus flavus* in comparison with other isolates and rising gases from the liquid culture media inoculated by *Aspergillus flavus* in high rate. this phenomenon was not noticed in the media which is inoculated with other species, the reason is that the speed of metabolic action of fungi, one of them is fermentation so that, there will be increase in the production of gas CO₂ , predicting the acid like carboxylic acid, nitric acid, etc. This will make its ability of corrosion higher than the other species like *Geotrichum candidum* that produce acids but to lesser extent. This agrees with what mentioned by (Tiller and Sequeira ,1998) who have shown that mild steel from the most metals that exposed to corrosion because of produced acids by microscopic livings like funguses and also showed that producing a big rate of gas CO₂ encourage some types of corrosion to happen like stress corrosion cracking .

The result of statistical analysis have exposed existing difference under a level of probability $P < 0.05$ in the influence of the five fungal isolates on mild steel , They are varied in there influence tables (4,5,6) that the fungus *Aspergillus flavus* gave the higher value in the corrosion, mean-while the fungus *Geotrichum*

candidum gave lesser value generally during (28,56,84) day, also acid pH for the culture media which is inoculated by the fungus (*Aspergillus flavus*) lesser value (5), meanwhile *Geotrichum candidum* higher value (5.58) due to the ability of the fungus *Aspergillus flavus* to produce different organic acids and different non- organic acids like nitric and carboxylic acid and other acids that would make it is ability of corrosion higher in comparison with *Geotrichum candidum*.

It has been also noted that the fungus *Aspergillus flavus* showed increase in corrosion of mild steel in high rate after 84 day in comparison with the two periods 56 and 28 the reason is passing of metal in an important phenomenon happens to the metals when they are emerged in specific solutions and it is negative phenomenon or inability of metal which mean decreasing in corrosion rate that the production of gas CO₂ and production of acids leads to creating chemo electrical cell or differential acidification between two different areas of the metal surface because of the formed acid from fermentation and production of gas CO₂ in a big mount which may lead to the formation of chemical compounds with to decreasing the values of potential hydrogen on the surface of the metal and then increasing in corrosion (Crolet et. al. , 1994)

Generally , the net corrosion values on the brass were lesser than mild steel of five isolated species and statistical analysis results showed existing spiritual differences and under level probability $P < 0.05$ in the influence of five isolated species on mild steel and brass .

Also it has been noticed that the higher net corrosion values during 28 days on brass were by *Aspergillus flavus*, meanwhile during 56 day were for *penecillium sp.* and during the period 84 for the yeast of *Cryptococcus neoformans* which showed highest net value of corrosion, the reason is variation in influence for the metal to have the passivity which happens because of the damage to the layer that's prevent the corrosion, that this layer is stable for specific limitation of ability of the solution, this layer stays continuous to protect the metal from corrosion until it gets smashed, Then the metal continues to corrode again (Fontona and Green, 1978) or the reason may be the copper being from the poisoning metals against fungi and this meets with (EMI, 1996) referred that the copper could be killer factor against fungi or it may abstract its growth and production, this result meets with (Sequeira, 1988) who showed that ions of copper are hard poison against fungi and yeasts more than micro organism like bacteria which shows resistance for the high concentration of copper so it is possible to replace the materials that are made from steel like electric wires which exposes to

some of fungal species made of copper to avoid it is corrosion by fungi.

X-Ray Diffraction

The result of X-Ray Diffraction to the samples (5) mild steel, (2) brass, which exposed to *Aspergillus flavus*, *Cryptococcus neoformans*, sequences after period 84 day found maximum peak for X-Ray in angle 45 compared with minimize peak of control samples (brass mild steel) in crystal plane 2.02 Angstrom as Fig. (3,4,5,6).

In the above figures showed differences in diffraction peaks, which followed for angle $2\theta = 45^\circ$ before and after corrosion; which means the peak, have high reflex intensity before corrosion because the regularity of plane crystal for metal while decrease peak diffraction intensity because deformity the plane crystal for metal result of corrosion (Cullity, 1967).

We conclude from the result up the page that the corrosion rates which showed by the tables are milligram / centimeters² / day, these values if multiplied by 360 day for corrosion for a year, we will find out that corrosion rates are high and cause economic damage for different institution.

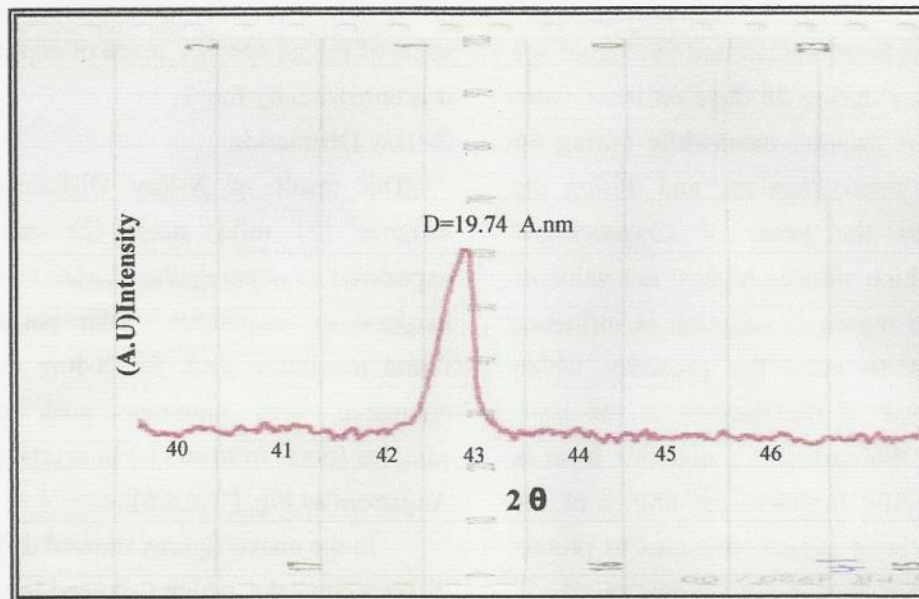


Fig. (3) sample of mild steel exposed for , *Aspergillus flavus* after 84 day
D= difference between two crystal plane

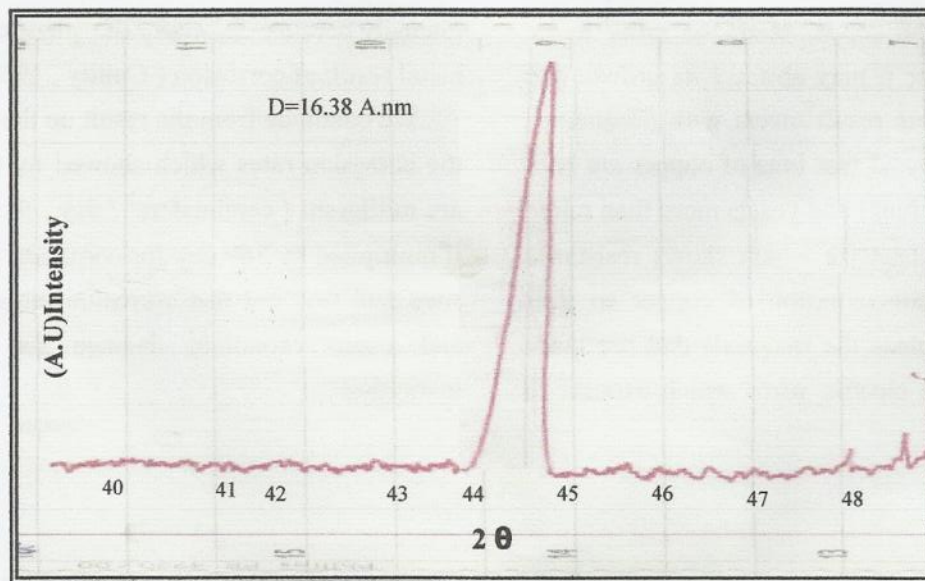


Fig. (4) sample control of mild steel after 84 day .

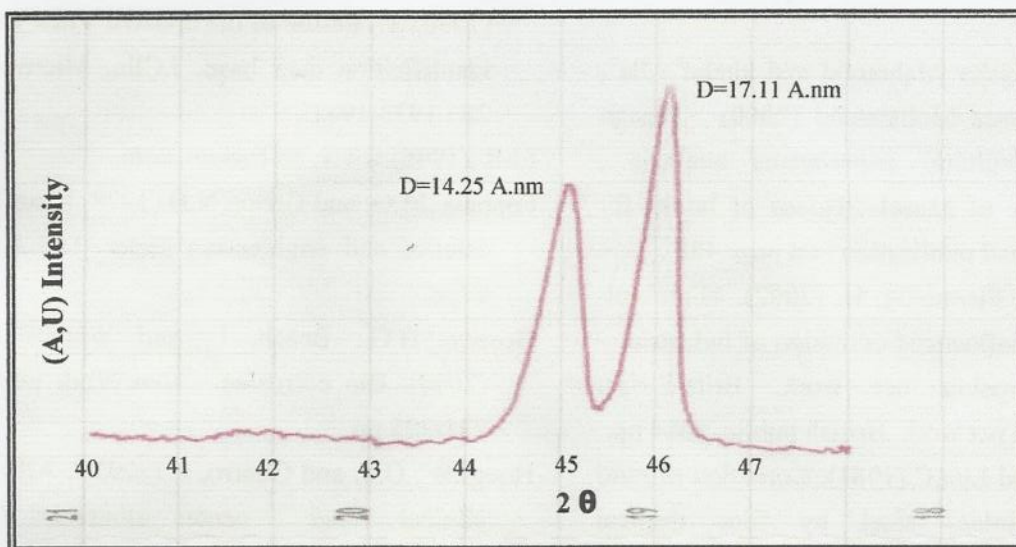


Fig. (5) sample of mild steel exposed for , *Cryptococcus neoformans* after 84 day .

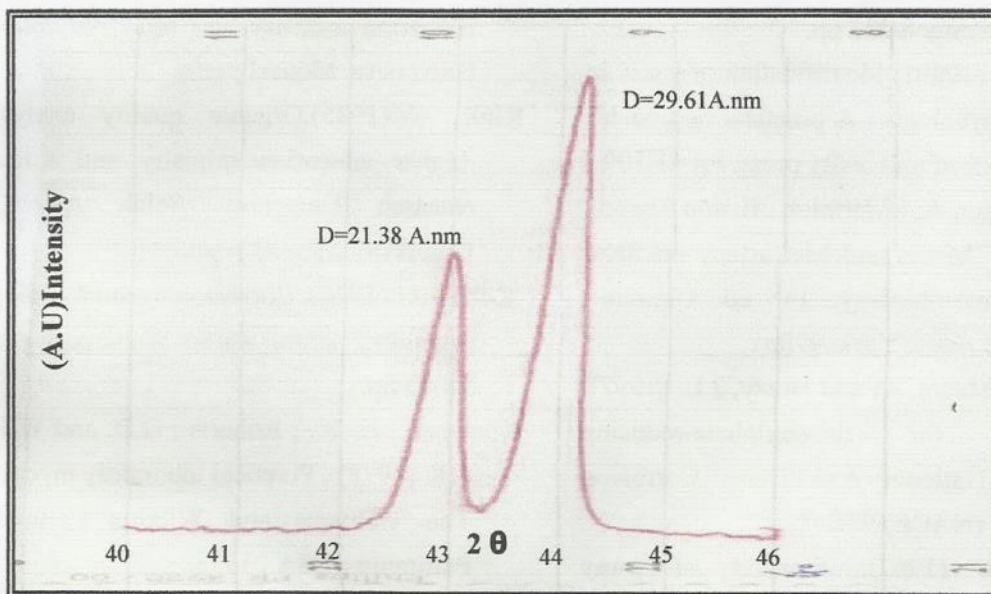


Fig. (6): sample control of brass after 84 day.

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دراسة التأثير التآكلي لبعض الأنواع الفطرية المعزولة من مياه شط العرب تجاه معدني الحديد المطاوع والبراص مختبرياً

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الملخص

تضمنت الدراسة الحالية عزل بعض الانواع الفطرية من عينات مياه شط العرب و اختيرت خمس انواع فطرية لدراسة قابليتها على تاكل معدني الحديد المطاوع والبراص وشخصت كـ *Cryptococcus neoformans*، *Candida albicans*، *Aseprgillus flavus*، *Penicillium spp.*، *Geotrichum candidum*. اذ درست قابليتها على التاكل في مزارع دفعة شبة مستمرة و لثلاث فترات زمنية (28، 56، 84) يوماً. وقد اظهرت الدراسة قابلية تلك الانواع الفطرية على تاكل عينات الحديد المطاوع في المزارع الفطرية، اذ بلغت اعلى قيمة لصافي التاكل في مزارع النوع *A. flavus* بعد مرور 84 يوم 4.5071 ملغم / سم² / يوم، في حين كانت قيمة صافي التاكل على معدن البراص اقل من قبل كل العزلات المختبرة إذ كانت بعد مرور 84 يوم 6.8373 ملغم / سم² / يوم لخميرة الـ *C. neoformans* كذلك أُجري فحص حيود الأشعة السينية لمعدني الحديد المطاوع والبراص بعد مرور 84 يوم بتأثير عزليتي . *A. flavus* و *C. neoformans* لدراسة حالة سطوح عينات المعادن قبل وبعد التآكل وأظهرت النتائج وجود تشوه في مستوى السطح البلوري للمعدن بعد التآكل .