Isolation and identification of some pathogenic species of Fungi from Shatt-al-Arab River

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Abstract. This study aimed to isolation and identification of pathogenic fungi from Shatt – al-Arab River in Basra city, Fourteen water samples were collected from different area from Shatt- al-Arab River (AL Ashar, AL Tnoma, AL Makal, AL Qurna, AL Karma, AL Jabiluh, AL- Hartha), from October to December in 2017, with 250 ml volume, this samples centrifuged at 5000 rpm for 10 min at room temperature, the floating was removed and then take the precipitate and pour directly into the center of the media of SDA and PDA and then incubation in a temperature range25-27c for 4 days after that the growth on the media made pure culture and each fungi species diagnosed based on the cultural and microbiological phenotypes, smear prepared with lacto phenol cotton blue stain and the results show 57.1% of growth was Aspergillus niger, 85.7% Aspergillus flavus and 42.8% was Aspergillus candidus and 14.2% was Rhizopus, while the results show 42.8% of growth was Penicillium.

1. Introduction :
The waters of Shatt al-Arab River suffers from serious pollution, which is evident in many types of fish that settled in its waters, and some of them have disappeared permanently, The causes of pollution in Shatt al-Arab River water vary, starting with wastewater, household waste, industrial and oil waste in addition to the salty tide flowing from the Arabian Gulf. Pathogenic and non-pathogenic microorganisms are commonly found in rivers. [1]. Infections acquired by recreational water interaction are normally mild, rendering routine surveillance systems difficult to detect. And if the disease is more serious, it can be difficult to connect it to the water. Targeted epidemiological studies, on the other hand, have linked faecally contaminated recreational water to a variety of negative health outcomes (including gastrointestinal and respiratory infections).Targeted epidemiological studies, on the other hand, have linked faecally contaminated recreational water to a variety of negative health outcomes (including gastrointestinal and respiratory infections). This can lead to a serious illness, burden and financial loss [2]. The pathogen, its type, and the number of microorganisms that can cause infection or disease determine the number of microorganisms that can cause infection or disease, the conditions of exposure, and the host's susceptibility and immune status. In the case of viral and parasitic protozoan infections, this dosage may be as low as a few viable infectious units [3]. Kelley et.al., 2003 provided a comprehensive investigation of filamentous fungi in US distribution systems. A.alternate, A. niger,Cladosporium sp, Epicoccum nigrum, Penicillium sp. In Norway, the Aspergillus, Penicillium and Trichoderma are detected in drinking water, surface and ground water [4]. While in Pakistan the Aspergillus niger and A. clavatus are isolated from water and fruit juice [5].In addition the Cladosporium, Penicillium, Aspergillus and Fusarium are isolated from municipal water of Australia [6]. While in Brazil the Penicillium, Aspergillus and Phoma are detected in water treatment plant and tap water [7]. As well as the Aspergillus, Cladosporium and Penicillium are isolated from surface, spring and ground water of Portugal [8,9].
When the world's population rises, so does the demand for water, placing greater pressure on these resources [10]. Previous contributions on fungi found in the surface sediments of the Shatt Al-Arab River in Iraq, on the other hand, were based on taxonomic studies that described several new and interesting species. [11, 12, 13, 14 and 15]. Studies on the occurrence of thermophilic and thermo resistant bacteria in Shatt Al-Arab River sediments, as well as the incidence of dermatophytes and other keratinophilic fungi in Shatt Al-Arab surface sediments, were also carried out [16]. One of the most significant prerequisites for a healthy life is an ample supply of clean drinking water. When a substance is introduced to our natural world, it has a detrimental impact on nature's ability to dispose of it. A pollutant is something that has a negative impact on people's health, comfort, land, or climate. The majority of contaminants are released into the atmosphere by sewage. [17]. Filamentous fungus in drinking water has become a serious issue. One of the most important issues was the identification of isolated strains to species rather than genus level, which was a direct result of the efforts of key researchers in the field. More information about changes in biodiversity within locations and over time can be gleaned when species identification is completed. Characteristics of the isolates [18].

According to the importance of Shatt-al-Arab River for Basra city and there is no study about biological pollutant from different source, therefore the study aimed to isolation and identification of some pathogenic fungi from different parts of it.

2. MATERIALS AND METHOD:
Fourteen samples are taken from various Shatt al-Arab River locations (AL Ashar, AL Tnoma, AL Makal, AL Qurna, AL Karma, AL Jabiluh, AL-Hartha), from October to December in 2017, with 250 ml volume under deep 15 cm of water then taken to the lab to investigation for the micro pollutant in water [19]. This samples were centrifuged at 5000 rpm for 10 min. at room temperature, the floating was removed and then take the precipitate and pour directly into the center of the media of SDA and PDA and then incubation in a temperature range 25-27°C for 4 days after that the growth on the media made, pure culture and each fungi species diagnosed based on the cultural and microbiological phenotypes, smear prepared made with lacto phenolcotton blue stain [20].

3. Results and Discussion:
The results show 57.1% of growth was *Aspergillus niger*, 85.7% *Aspergillus flavus* and 42.8% was *Aspergillus candidus* and 14.2% was *Rhizopus*, while the results show 42.8% of growth was *Penicillium*. Table (1) show the different species of fungi isolating from different site of Shat Al-Arab, and the table (2) show the percentages occurrence of fungi species in Shatt Al- Arab River.

| Site of collection | Type of fungi                  |
|-------------------|--------------------------------|
| Al-Ashar          | *Rhizopus, A. Flavus, A. candidus* |
| Al-Garma          | *A. niger*                     |
| Al-Jobiluh        | *A. flavus*                    |
| Al-Hartha         | *A. flavus*                    |
| Al-Qurna          | *A. niger, A. candidus, A. flavus* |
Table (2) show the percentages occurrence of fungi species in Shatt al- Arab River

| No | Fungi            | Number of appearance | Percentage of occurrence |
|----|------------------|----------------------|-------------------------|
| 1  | *A. niger*       | 8                    | 57.1%                   |
| 2  | *A. flavus*      | 12                   | 85.7%                   |
| 3  | *A. candidus*    | 6                    | 42.8%                   |
| 4  | *Rhizopus* sp.   | 2                    | 14.2%                   |
| 5  | *Penicillium* sp.| 6                    | 42.8%                   |

3.1 *Aspergillus niger*:

3.1.1 Colony morphology:

when culture on SDA the Conida produce the pigment aspergillin during maturation, Starting with a white to yellow felt-like layer of mycelia, paint it black, The Reverse is white or pale in color . [21] as appear in figure 1-A.

3.1.2 Microscopic Morphology:

The hyphae are Septet and hyaline , Conidiophores are long (450-3000 M) with spherical vesicles measuring 35-75 m at the apex. Metulae almost completely cover the entire surface from which the phialides extend in *A.niger*. Conidia have a globose shape, are brown to black in colour, have a rugged base, and have a diameter of 3.5-4.5 M. hyaline septet, [21]. As shown in Figure1 – B.
3.2 Aspergillus flavus:

3.2.1 Colony Morphology:
Growing at a breakneck pace, in just three days, it will be fully mature. The color ranges from greenish-yellow to olive, with a white line running along it. The texture is often floccose, particularly near the middle, and can range from velvety to woolly in appearance. On Sda newspapers, reverses from cream to tan to yellowish is unremarkable. [22]. As appear in Figure 2-A.

3.2.2 Morphology at the microscopic level:
The hyphae are septate have long conidiophores (420-800 X 8.5-17 M) particularly just below the vesicle, with a rough or even spiny texture. Vesicles range in size from spherical to elongate, with a width of 20 to 45 meters. Seriation in A. flavus is variable, with most strains being around 20% biseriate, but some strains being almost entirely uniseriate. Metulae (8-10 X 5-7 m) cover three-quarters of the vesicle's surface, from which the phialides (7-12 X 3-4 m) emerge. The phialides are supported by the metulae, which together form the biseriate structure. Conidia have smooth to finely roughened walls and vary in size from globose to ellipsoidal (3-6 m). [22]. Figure 2-B.

3.3 Aspergillus candidus:

3.3.1 Colony Morphology:
A. candidus is a fast-growing fungus that matures in about three days. The surface color of colonies can range from a yellowish brown to a drab olive with grays. They may have a lighter outer lip, and droplets of purple exudates may appear on the maturing colony's surface. The back of the card is a yellowish brown color. The texture was even and velvety. [23].Figure 3-A.

3.3.2 Morphology at the microscopic level:
Septate hyphae are produced by A. candidus, from which smooth-walled conidiophore stipes extend. As conidiophores mature, they can turn a brownish color. Stipes are usually small (130 m to 300 m), but the ones measured on the isolate in this study were only around 150 m long. Vesicles were subspherical in form and tiny (7 to 15 m in diameter), bisect the vesicles from the vesicles. [23]. As in Figure 3-B.
3.4 Rizopus:

3.4.1 Colony Morphology:
Rhizopus is a fast-growing fungus that can easily fill a petrie dish with soft, cotton-candy-like growth in as little as 5 days. The maturation of the sporangiospores within the sporangium causes growth to be whitish in color, which can turn brown with age [24].

3.4.2 Microscopic Morphology:
Sporangiophores on the stolons, solitary or in tufts, diverging from the point where the rhizoids form; sporangia round; The apophysis is missing or partially visible, and the sporangiophores are ovoidoid [24]. As in Figure 4

![Image](https://via.placeholder.com/150)

Figure (4) *Rhizopus* on SDA (400x)

From results above, the most fungi isolates belong to tow genera: *Aspergillus* and *Rhizopus*. During the analysis, 14 samples were collected from seven different locations in Shatt AL-Arab, and all of the samples were positive. One of the most frequently isolated genera in water is Aspergillus as found in study of [25 and 26]. Fungi, as we know, are one of the most diverse classes of living organisms on the earth, despite being understudied globally [27]. UV radiation is a common form of water treatment. In terms of fungi, [28]. Since UV exposure is related to pigmentation in fungi, fungi with pigmented spores, such as Aspergillus and Penicillium, have higher radiation tolerance and are less susceptible to UV. [29]. This was also found in a study conducted by [30], in which melanized, thick-walled organisms predominated following water treatment. The effect of UV dose on fungi in drinking water should be investigated. This treatment method could be used as an in-house treatment in hospitals, according to a new study on the impact of copper and silver ionization on fungal colonization in hospital water systems [31]. Internal water treatment in hospitals may be a useful tool in the fight against the fungus outbreak. Furthermore, since many of the fungal species found in drinking water are common contaminants in food and beverages, additional water treatment could help food processing plants and other businesses where water quality is critical. Fungi, on the other hand, will grow on their own. [32].

3.5 Statistical analyses:
The results show, Al- Ashar, Al- Qurna and Al Makael has significant differences $p > 0.05$ compare with other sites of sample collection, and Al- Qurna and Al Makael has significant differences $p > 0.05$ compare with Al- Ashar site [33].
4. Conclusions and Recommendations:

- There are many sources of pollution in Shatt al-Arab river Al-Qurna and AL-Ashar was the most pollutant which isolated many types of pathogenic fungi which is: *Aspergillus niger, Aspergillus candidus, Aspergillus flavus, Penicillium chrysogenum*

- The pathogenic fungi *A. flavus* found in highest ratio in Shatt al-Arab river reached to 85.7 %Which is isolated from six sites we collect of the samples excepting one site not isolated from.

- Conducting more studies in this field to find out the types of chemical and biological pollutants that cause pollution in the waters of the Shatt al-Arab river being a vital source for Basra residents in particular.

- Control of sewage water that extends into the Shatt al-Arab river.

- Applying environmentally-friendly biological treatments to treat pollution in Shatt al-Arab River.

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