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The efficiency of some fungi species in wastewater treatment

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Abstract: Using microorganisms in eliminating or reducing the impacts of harmful remnants is very ancient manner. The current study was conducted to explore the potential of utilizing some fungi species that isolated from the main sewage treatment plant in Al-Muamirah area, Babylon-Iraq, in reducing some pollutants. Six fungi taxa *Aspergillus flavus*, *A. niger*, *A. terrues*, *Candida albicans*, *C. krusei*, and *Penicillium digitatum* were identified before any treatment process, whereas only four fungi species *A. flavus* 20%, *A. niger* 20%, *A. terrues* 10%, and *P. digitatum* 18% were recognized after completing the physical and chemical treatment stages. Only three taxa *A. niger*, *A. terrues*, and *P. digitatum* were employed to reveal their capability in treating the sewage water, which represent the biological treatment stage as the final step of the treatment processes. The results showed a considerable capability of these fungi species in decreasing many variables values such as pH, total soluble solids (*TSS*), electrical conductivity (*EC*), salinity, total alkalinity, chlorides, nitrite, and phosphate. Where, slightly low reduction was detected in *TSS* value in all experiments (1.1–5.9%), similarly, both *EC* and salinity which were decreased with low ratios (6.6%, 3.9%, respectively). Taxon *A. terrues* exhibited high ability in reducing the total alkalinity and chloride ions in the treated water (30.9%, 43%, respectively) in comparison with the other two fungi species. Furthermore, all three fungi species were posed high capability in decreasing nutrients, where both nitrite and phosphate ions were highly reduced (87–97% and 22.8–32.1%, respectively). Based on these findings, we suggest using other microorganisms and exploring their capacity in removing the pollutants, and revealing the ability of the above fungi taxa in removing other pollutants.

Keywords: bioremediation, fungi, nitrite, phosphate, wastewater

INTRODUCTION

Bioremediation is the natural use of living organisms such as bacteria and fungi or plants to reduce the toxicity of many environmental pollutants, degrade or detoxify hazardous compounds that threaten human health or environmental integrity [Leong, Chang 2020; Singh *et al.* 2020]. Bioremediation principles can be achieved by several techniques such as bio-augmentation, bioventing, bio-filters, bio-sorption, composting, bioreactors, land-farming, and bio-stimulation [Huang *et al.* 1991; Kumar *et al.* 2015; Lellis *et al.* 2019].

Bioremediation typically involves mineralization and detoxification processes by transferring the waste into inorganic compounds. In addition, some microorganisms can accumulate the waste by absorbing it and then using them as a base material for growth and reproduction [Abatenh *et al.* 2017; Demnerová *et al.* 2005; Wałowski 2019]. Bioremediation may also be accomplished by bio-absorption, a physio-chemical process that occurs naturally. The absorption of organic pollutants takes place through several mechanisms such as transformation and precipitation in and out of living cells [Kapoor, Viraraghavan 1997; Xia *et al.* 2019].

Mycoremediation is another kind of bioremediation in which some species of fungi play an important role, reducing the impact of pollutants in an environment [Asiriuwa *et al.* 2013; Prasad 2018]. This kind of bioremediation is considered a natural and effective technique that does not produce any toxic by-products or permanent hazard compounds when the environmental pollutants are removed entirely due to mineralization processes [Elshafie *et al.* 2020; Thenmozhi *et al.* 2013]. Moreover, some species of fungi can exchange soil ions, adjust their permeability, and remove toxic substances from the polluted environment. Additionally, some fungi can break down and degrade metals, chlorides, phenolic compounds, polycyclic aromatic hydrocarbons, petroleum hydrocarbons, insecticides, and biopolymers [Lloyd 2002]. One of the most important traits of fungal mycelia is having the ability to dissolve the insoluble substrates by producing different kinds of extracellular enzymes, which target the metals in industrial waste to transform them into low-grade materials or metal-bearing minerals [GADD 2001]. Furthermore, some enzymes produced by numerous species of fungi can assimilate varieties of carbohydrate complexes without the hydrolysis process, which facilitates the degradation of a wide range of pollutants [ABEYSINGHE *et al.* 2020].

Various fungal species can mineralize, release, and store multiple elements, ions and accumulate many toxic substances [IRWIN 1996] through having a rich network of filaments that occur in a large volume and occupy the upper layer of the soil. This filament network enables the fungus to collect the required minerals from the soil. Fungi also have an important role in destroying different organic compounds and recycling elements that lead to numerous vital substances as secondary metabolites such as antibiotics, mycotoxins, various organic acids, vitamins, and many other crucial compounds [MITCHELL 1998]. In addition, fungi are known to grow under a wide range of environmental circumstances, for example, growing in low pH and low nutrient concentrations [LAWTON, JONES 1995]. This study is aimed to identify the fungi species that grew successfully in industrial wastewater and reveal their efficiency in decreasing the hazardousness of the wastewater pollutants.

MATERIALS AND METHODS

SAMPLE COLLECTION

Six wastewater samples were collected during October 2018 from two stations in the main wastewater treatment plant (Al-Muamirah) in the province of Babylon-Iraq. The first three samples represented the wastewater before any treatment process, while the second three samples were taken from the final settling ponds after performing the treatment processes. This samples collection process was repeated in December 2018.

ESTIMATING AND CULTURING FUNGI SPECIES

Various types of media were used to estimate and culture the fungi species in the wastewater samples.

- **Potato dextrose agar (PDA)**

This cultural medium was prepared by following the manufacturer's instructions by dissolving 39 g from PDA powder in 250 cm^3 of distilled water, forming the final volume of 1000 cm^3. Then, the prepared culture was sterilized by autoclaving it at 121°C under 103 kPa pressure for 20 minutes. The use of PDA helps to reveal the fungi isolates that exist in the wastewater samples [SHARMA 2010].

- **Potato dextrose broth (PDB)**

This cultural medium was prepared through following the manufacturer's instruction by dissolving 24 g of PDB powder in 1000 cm^3. Then 250 $mg \cdot dm^{-3}$ of an antibiotic (Chloramphenicol) was added to the prepared medium and fully mixed. After that, 300 cm^3 of medium was transferred to 500 cm^3 glasses flasks. This step was repeated many times, and all flasks were then sterilized by autoclaving them in 121°C under 103 kPa pressure for 20 minutes [SHARMA 2010]. The prepared liquid media were ready to use for the subsequent experiments.

- **Isolate and diagnose fungi species in wastewater samples**

Detection of microorganisms was performed using the moist chamber method. In lab, one cm^3 of the transferred wastewater samples was taken each time and placed in Petri dishes containing PDA medium. This process was repeated three time for each sample (replicates), then all Petri dishes were incubated in 25 ±2°C for seven days. After incubation period, the developed fungi species were classified depending on their morphological features following the standard techniques. The appearance percentage of each fungi species was calculated according to the following equation:

$$\text{Species appearance percentage} = (x/y)\ 100$$

where: x = number of samples that have fungus species, and y = total number of isolates.

EFFECTS OF THE CRUDE FILTRATE OF FUNGI SPECIES ON WASTEWATER ELEMENTS

- **Prepare crude filtrates of fungi species**

Only three from the six identified fungi species (*Aspergillus terrues*, *A. niger*, and *Penicillium digitatum*) were diagnosed after the physical and chemical stages in the wastewater treatment plant. The other species were neglected from further experiments because it either completely absent, or occurred with very low ratios. PDB flasks that prepared in the previous steps were used to prepare filtrates as following: for each of the above fungi species, four PDB flakes were taken and inoculated with two discs (1 mm diameter) of five days old colonies of each species that cultured and isolated from PDA medium. All 12 prepared flasks were then incubated at 25 ±2°C for 14 days. Shaking samples every two days for two minutes was applied during the incubation period. The fungus mycelia that grew in that media were separated from the broth by filtering the broth medium through a filter paper (Whatman No. 1) and then through a Millipore filter (0.45 μm). The filtrates were collected in sterile flasks separately under sterilized conditions and kept in a refrigerator until use [MITCHELL 1998].

- **Injection of isolated fungi with municipal wastewater**

100 cm^3 of the filtrated crude of each fungus isolate was added to a sterile flask contained 250 cm^3 of wastewater sample. The prepared 12 flasks were used to evaluate the physical and chemical variables after treatment process.

PHYSICAL AND CHEMICAL ANALYSIS

The pH, total dissolved solids (*TDS*), electrical conductivity (*EC*), and wastewater salinity were measured using HANA\211 multi-meter equipment. Total alkalinity was determined according to the procedure described by LIND [1979], where 50 cm^3 of sample water was titrated with H_2SO_4 (0.02 N), and the results were expressed as $mg \cdot dm^{-3}$. Chlorides were estimated in a titrated manner with the standard of silver nitrate (0.0141 N) and potassium hydroxide as the indicator [APHA 2005]. According to the method described in PARSONS *et al.* [1984], nitrites were determined by adding sulfonyl amide and N-naphthyl ethylene diamine dihydrochloride solutions and then measuring the optical density at wavelength 543 nm using the spectrophotometer. Effective phosphates were synthesized according to the Murphy and Riley method [MURPHY, RILEY 1962], which SMITH

[2004] demonstrated by using a mixed reagents solution consisting of potassium antimonyl tartrate H_2SO_4, ascorbic acid, and molybdate ammonium. The optical density was measured at 885 nm using the spectrophotometer.

RESULTS AND DISCUSSION

ISOLATION AND DIAGNOSIS OF FUNGI SPECIES

Six isolates of fungi were identified and isolated from the central wastewater treatment plant (Photo 1). The highest frequency of fungi species was observed before treatment in the following order: *Aspergillus flavus* 13%, *A. niger* 19%, *A. terrues* 11%, *Candida albicans* 45%, *C. krusei* 4%, and *Penicillium digitatum* 8%. After treatment, the frequency of some of these identified fungi species was sharply decreased as the following ratios: *A. flavus* 29%, *A. niger* 29%, *A. terrues* 15%, and *P. digitatum* 27%. Whereas, both taxa *C. albicans* and *C. krusei* were completely absent after treatment, and entirely removed from further experiments (Fig. 1). Moreover, because of some technical issues, only three taxa (*A. niger*, *A. terrues*, and *P. digitatum*) were employed in our experiments.

THE PHYSICAL AND CHEMICAL CHARACTERS FOR MYCOREMEDIATION

The physical and chemical characteristics of water samples collected from the final settling ponds of the wastewater treatment plant are shown in Table 1.

As an effective parameter, the pH highly influences the organism's metabolism processes and has an essential role that controls the existence of microorganisms in the wastewater. Each microorganism has a particular range of pH by which it can successfully grow in its surrounding. Various species of fungi can change the pH in their environment to be more acidic due to the production of various organic acids and different metabolic materials [Okpokwasili, James 1995]. In this study, the pH was highly decreased during the experiment period. On day three, the highest reduction was reached when the wastewater samples were treated with *A. niger*, *P. digitatum*, *A. terrues*, respectively, in comparison with the control (Fig. 2). This reduction may be caused due to the production of different organic acids such as oxalic acid by these fungi species. This result coincided with Khalaf's study [2010], which detected such reduction in pH of water samples that treated with some fungi species.

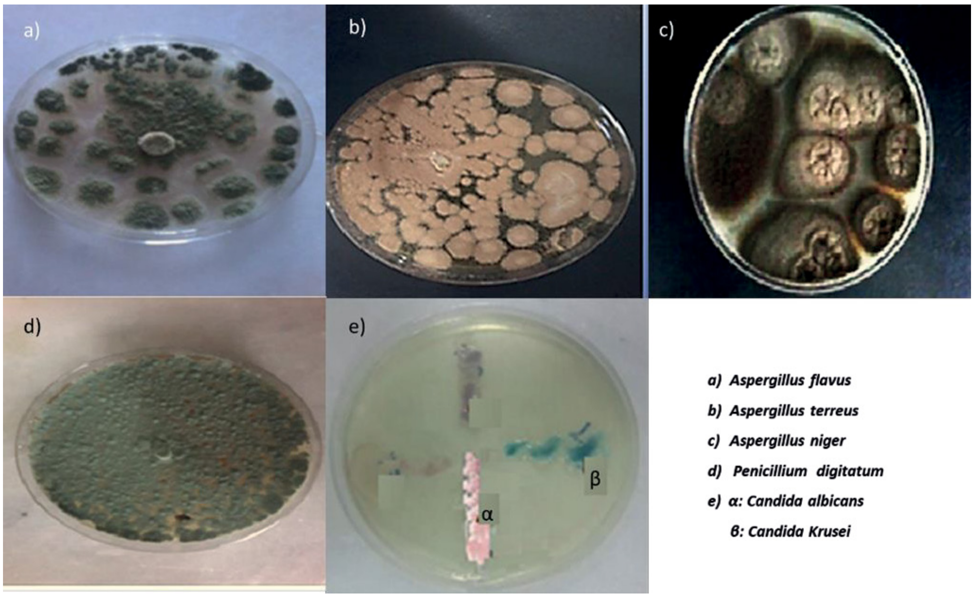

Photo 1. Fungi isolated from wastewater treatment plant: a) *Aspergillus flavus*, b) *A. terreus*, c) *A. niger*, d) *Penicillium digitatum*, e) α: *Candida albicans*, and β: *C. krusei* (phot. *I.M. Al Hussaini*)

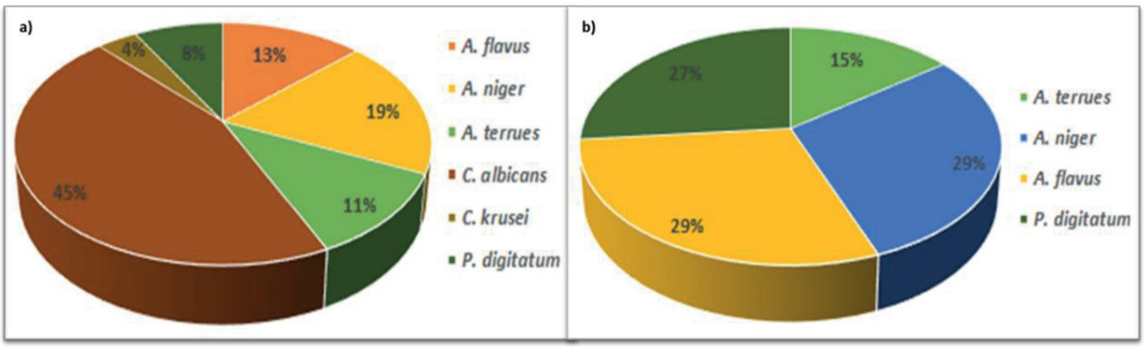

Fig. 1. Percentage of the frequency of isolated fungi: a) before wastewater plant treatment, b) after wastewater plant treatment; source: own study

Table 1. Physical and chemical properties of wastewater before biological treatment

Measured factor	Value in municipal wastewater
pH	8.6
TDS ($mg \cdot dm^{-3}$)	1680
EC ($\mu S \cdot cm^{-1}$)	2380
Salinity (‰)	2.03
Total alkalinity (mg $CaCO_3 \cdot dm^{-3}$)	420
Chloride ($mg \cdot dm^{-3}$)	487.8
Nitrite ($\mu g \cdot dm^{-3}$)	149
Phosphate ($\mu g \cdot dm^{-3}$)	62.6

Explanations: *TDS* = total dissolved solids, *EC* = electrical conductivity. Source: own study.

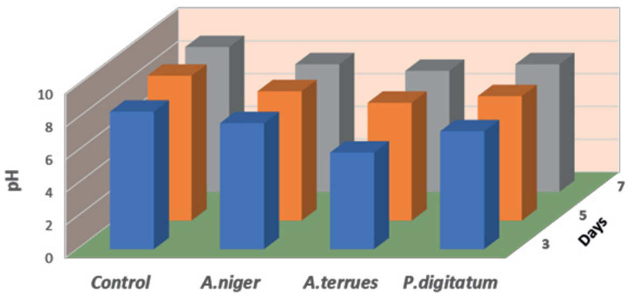

Fig. 2. pH values after treatment; source: own study

Numerous studies have confirmed that solids are the primary contaminant in the water [Smith 2004; Wetzel 2001]. Solids are usually found in two forms: soluble, consisting of negative ions combined with positive element ions, and suspended solids. The main sources of solids contaminants in the water are domestic and industrial wastewaters. In this study, the total soluble solids in wastewater samples were estimated before and after the treatment experiments.

The results shown in Figure 3 indicate the values of total soluble solids of the wastewater treated with fungi *A. niger*, *A. terrues*, and *P. digitatum* were reduced, and the reduction ratio was reached 5.3% on the third day, 5.9% on the seventh day, and 1.1% on the third and fifth day, respectively. The reduction of the total solids in the treated wastewater may resulted from the use of some ions such as chloride, potassium, sodium, and other salts, which represent the soluble solids, by fungi for growth and reproduction purposes [Alkdsawy 2013].

The other studied parameter were the electrical conductivity and salinity. The electrical conductivity is considered an indicator of dissolved salts in water and correlates highly with the total dissolved solids [APHA 1976]. The impacts of the three fungi species, which grew in the wastewater, on *EC* value is presented in Figure 4, where the highest reduction in *EC* value was happened during the third and fifth days and the reduction rate reached to 6.5%. Also, our findings displayed that 3.9% of salinity was removed when these fungi species grew in the wastewater (Fig. 5). The reduced electrical conductivity values and salinity may be explained that micronutrients and ions were used by these microorganisms for growth and reproduction purposes to increase their biomass [Venosa, Zhu 2003].

Alkalinity, as an indicator for water contents of bicarbonate, carbonate, hydroxides, and other ions [Lind 1979], was detected to explore the integration of the possible weak acids and their salts with the balancing system of carbon dioxide, and bicarbonate and carbonate ions [Hussein *et al.* 1991]. The results exposed that fungi species have an essential role in treating The total alkalinity, where an obvious reduction was occurred on the third day of the study period, and the detected reduction rates were 11.9% for both *A. niger* and *P. digitatum*, and 30.9% for *A. terrues* (Fig. 6). The decreased alkalinity values during the treatment experiment may be resulting from the formation of carbon dioxide as the final product of the biological treatment processes, which led to produce an extra carbonic acid that affected the alkalinity values negatively [Wetzel 2001].

Chloride ion was also investigated in this study, and the results expose an ability of the used fungi species in reducing the concentrations of chloride ions in the treated wastewater throughout the experiment period. The highest reduction rate 43% was reached on the third day when fungus taxon *A. terrues* was used, while only 22% and 34% reduction ratios were detected when taxa *A. niger* and *P. digitatum*, respectively, were used at the same period (Fig. 7).

These high reduced values of chloride concentration may be interpreted due to the ability of fungal cells to take the high ratio of chloride ions from their surroundings to increase the osmotic pressure, and that will help the flow of water from outside to inside the cells without damaging the protoplasm. These ions are entirely controlled by the plasma membrane, but the mechanism of this process is still unknown [Ali 1998].

Nitrogen, as an important element of growth used by fungi in building different biomolecules such as amino acids, proteins, and vitamins [Elser, Hamilton 2007] that are normally contributed to forming various cellular organelles. Both inorganic

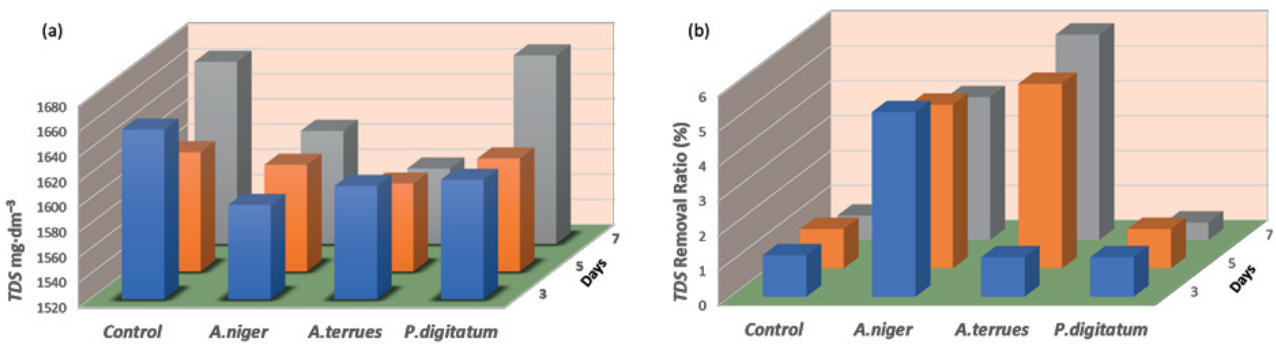

Fig. 3. Concentrations (a) and percent bio removal (b) of total dissolved solids (*TDS*); source: own study

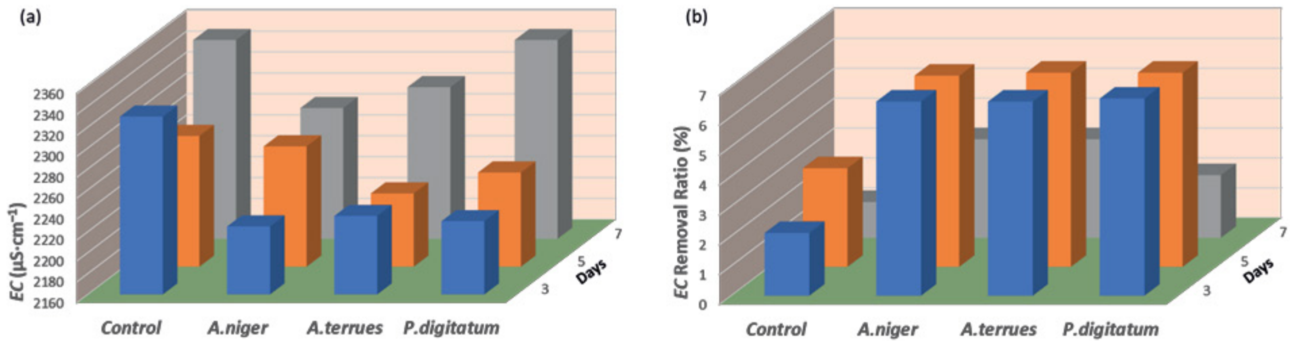

Fig. 4. Concentrations (a) and percent removal (b) of electrical conductivity (*EC*); source: own study

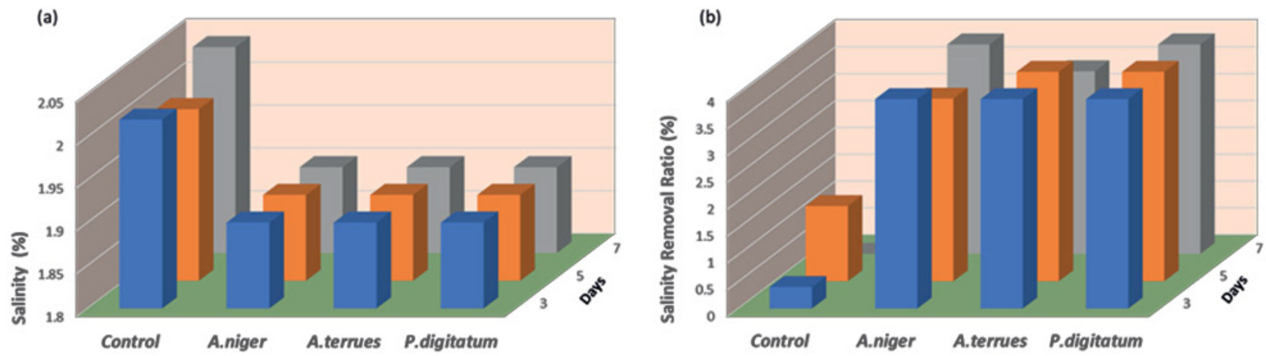

Fig. 5. Concentrations (a) and percent bio removal of salinity (b); source: own study

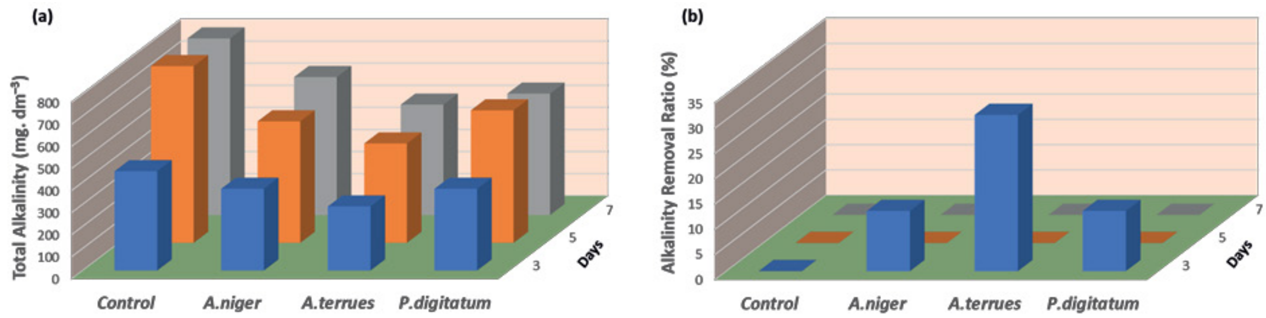

Fig. 6. Concentrations (a) and removal of total alkalinity (b); source: own study

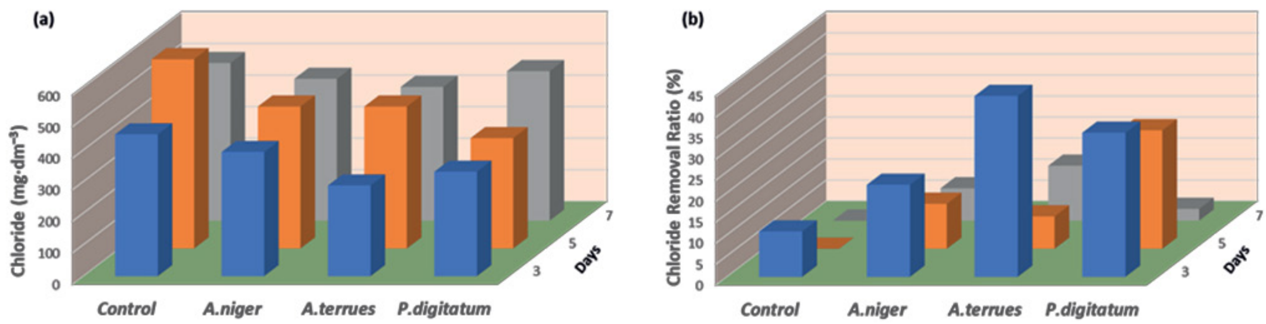

Fig. 7. Concentrations (a) and bio removal of chloride (b); source: own study

and organic nitrogen playing an important role in fungal growth and determining the mechanism of nutrients transporting through the vascular system in the cytoplasm [CAIRNEY 2005]. Figure 8 shows the values of nitrite concentrations in wastewater treated with fungi species, and a significant reduction of nitrate concentration was detected during the experiment. The highest detected reduction rates in nitrite concentration (97% on the third day) were reached when the wastewater was treated by *A. niger*, *A. terrues*. In contrast, 86% of the nitrite concentration was reduced during the same duration due to *P. digitatum*.

Phosphorus is mostly found primarily in natural water and wastewater in orthophosphate or organically bound phosphates [APHA 1999]. Fung species *A. niger* and *A. terrues* present an important role in reducing phosphate concentration during the treatment experiment, and the highest reduction rates (26.8 and 32.1%. respectively), were reached on the third day of the

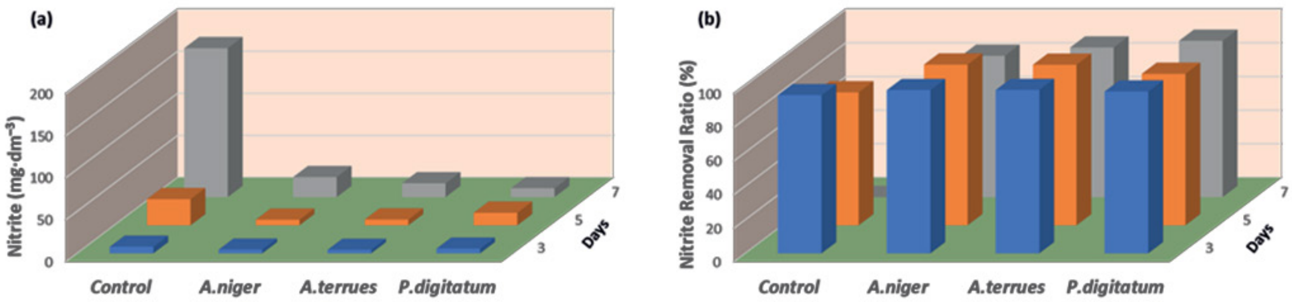

Fig. 8. Concentrations (a) and bio removal of nitrite (b); source: own study

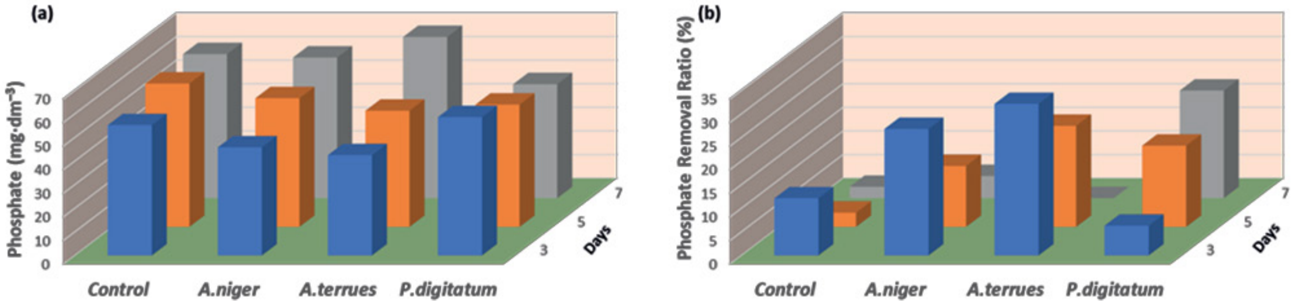

Fig. 9. Concentrations (a) and bio removal of phosphate (b); source: own study

experiment. The phosphorus ratio was reduced by 22.8% on the seventh day then *P. digitatum* was used (Fig. 9). As estimated in the current study, decreased phosphate values result from using this element in various vital processes such as growth and propagation, where it is essential to build different energy-rich compounds like phospholipids and nucleic acids [VASSILEV, VASSILEVA 1992].

CONCLUSIONS

The problems we face in today's world in clearing our harmful remnants are far greater than those previously faced. The use of microorganisms to decompose residues is not new. Humans have been using bioremediation methods to decompose municipal wastewater, refinery waste, and residues of specific chemical processes in the soil for many years. Bioremediation is the process by which the dynamics or toxicity of contaminants in a place is eliminated or reduced by using biological processes. Bioremediation is how microorganisms are used to properly manage the decomposition and transport of organic chemicals in soil or contaminated debris. This study elucidates the role of some fungi species (*A. niger*, *A. terrues*, and *P. digitatum*) in treating some pollutants in sewage water. It proved the efficiency of using these species in reducing the concentration of chlorides (22–43%), nitrites (97%), and phosphates (22.8–32.1%). It should be noted that the current study detected a slight reduction in total dissolved solids (1.1–5.9%) and electrical conductivity (6.5%). For future studies, we can mention the following two suggestions:
– use of new fungi species and other microorganisms in treatment processes;
– examine the capability of the studied species in reducing other pollutants such as heavy metals and hydrocarbon compounds.

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