

## Summary

There are many toxic pollutants that are constantly released into the environment in large quantities, including azo dyes, which have negative effects on the environment and humans. Therefore, it is necessary to dispose of them using safe and environmentally friendly methods. The current study was conducted on a wool textile factory in Thi Qar Governorate with the aim of determining the ability of microorganisms (fungi and bacteria) isolated from the water and soil of the factory to biodegrade and break down azo dyes, especially Methylene Blue and Congo Red and try to elevate the efficiency of the biodegradation using bio stimulation and bio augmentation in vitro by adding different carbon and nitrogen sources in . experiments

Collected samples monthly for the period from March 2022 to February 2023 from four different stations, three of which are within the wool textile factory, and the fourth station represents the area where liquid waste is collected from the third station to the river. The results were expressed in a monthly format. The study included measuring some physical and chemical factors of the selected stations for the study, including water temperature, pH, electrical conductivity, total dissolved solids (TDS), total suspended solids (TSS), sulfates (SO<sub>4</sub><sup>-</sup>), biological oxygen demand (BOD<sub>5</sub>), and chemical oxygen demand (COD). The results of the tests and chemical analysis of the water in the study stations showed that the temperature ranged between (12 - 49) °C of the study stations. The pH of the studied stations' water was alkaline and ranged between ( 8 - 9.5) . The electrical conductivity values ranged between (3120 – 9350 ) µs /cm. The total dissolved solids recorded values ranging between (910 – 6100) mg /L, and the biological oxygen demand values ranged between (30 – 240) mg /L . As for the nutrients, the sulfates (SO<sub>4</sub>) recorded values ranging between (326 – 2440) mg /L . The fourth station recorded the highest values in the physical and chemical tests, as it is a station for collecting liquid waste from the wool textile factory, in addition to the presence of a car wash station a few meters away from it. In addition, concentrations of six trace elements (Pb, Ni, Zn, Br, Cu, Fe) were measured in selected water and soil samples from the study stations using the flame atomic absorption spectrophotometer. The annual mean concentrations of these elements in the water were (8.45, 9.87, 20.25, 0.19, 3.77, 20.84 ) µg /L, respectively. The study showed that iron had the highest concentration in the water samples compared to the other trace elements. As for their concentrations in the soil samples, the annual mean concentrations were (6.87, 14.85, 2.71, 19.71, 18.31 µg /g for the elements (Pb, Ni, Zn, Br, Cu, Fe) ) respectively, . and copper recorded the highest concentration among the other trace elements

From the study stations, 384 samples were collected, with 8 samples collected from four stations over a period of 12 months. From these samples, 10 fungal types were

isolated, belonging to 7 fungal genera. fungi were diagnosed based on their morphological characteristics, and the diagnosis of 4 fungal types, including *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus ochraceus*, and *Penicillium funiculosum* was confirmed. The results of the current study indicate that the frequency or occurrence rate of fungal general isolated from water and soil ranged from 2.26% for Zygomycetes and Basidiomycetes fungi, which represents the lowest frequency, to 90% for Ascomycetes fungi, which represents the highest frequency among the isolated fungal communities. At the genus level, the lowest occurrence rate was for the genera *Rhizopus*, *Fusarium*, and *Rhizoctonia* each reaching ( 2.26%) , while the dominance at the genus level was for the genus *Aspergillus* with an .occurrence rate of 100%

As for bacteria, the results showed that *Pseudomonas aeruginosa* bacteria had the highest prevalence, with a percentage of (98.95%). This was followed by *Bacillus thuringiensis* bacteria with a prevalence rate of (97.65%). The other two types *E. coli* and *Klebsiella sp.*, had a lower prevalence, with percentages of (57.29, 52.08)%, respectively. The mentioned bacteria were diagnosed based on biochemical tests, and the diagnosis of two types was confirmed *Pseudomonas aeruginosa* and *Bacillus . thuringiensis*

The experiments were conducted to test the growth suitability of microorganisms on solid and liquid media containing Methylene Blue, Congo Red dye, and the addition of glucose and  $\text{NH}_4\text{Cl}$  once, and maltose and  $\text{NaNO}_2$  again, at different pH levels (4, 7, 10). The study results showed the growth suitability of fungi in PDA solid medium containing MB and CR dyes at concentrations of (50, 150, 250) ppm. In the solid medium containing MB dye, the lowest colony diameter was recorded for *Aspergillus terreus* fungus, measuring (7.52) cm, while the highest colony diameter was recorded for *Aspergillus niger* fungus, measuring (8.10) cm. In the media containing CR dye, the highest colony diameter was recorded for *Aspergillus niger*, measuring ( 8.23) cm, while the lowest colony diameter was recorded for *Aspergillus terreus* measuring (7.75) cm. The highest colony diameter was observed at a concentration of (50) ppm for both dyes, while a concentration of (250) ppm was sub lethal .concentration for both selected fungi in the study

As for the liquid mineral medium MSM, which is treated with MB and CR dyes, and supplemented with glucose,  $\text{NH}_4\text{Cl}$ , Maltose, and  $\text{NaNO}_2$ , at pH values of (4, 7, 10) the study results showed that Maltose acts as a carbon source and  $\text{NaNO}_2$  acts as a nitrogen source. At pH = 10 it was found to be the optimal condition for the growth of fungi and their biodegradation process. The highest dry weight rate of fungal mycelium was achieved in the liquid mineral medium containing Methylene blue dye, Maltose, and  $\text{NaNO}_2$  as carbon and nitrogen sources , At pH = 10, the fungal strain *Penicillium funiculosum* showed a weight of (2.50 gm) compared to the other

additives and concentrations. The lowest weight rate for fungal mycelium was recorded for the fungus *Aspergillus terreus*, which reached to (1.65) gm at pH = 4 in the presence of glucose and NH<sub>4</sub>Cl. As for the CR dye, the highest dry weigh rate of fungal mycelium was achieved by the same aforementioned additives, reaching (2.14) gm for the fungus *Aspergillus terreus* , The lowest weight rate for fungal mycelium was (1.73) gm for the fungus *Aspergillus ochraceus* at pH = 4 in the presence of glucose and NH<sub>4</sub>Cl. These results were confirmed by breaking down the two dyes by the microorganisms using FTIR device and determining the percentage of biodegradation for the two dyes by the selected microorganisms for the study using the UV-Visible spectrometer. The results showed that the highest biodegradation percentage for MB and CR dyes was (99.51% , 96.70%) respectively by the fungus *Aspergillus niger* at pH = 10 in the presence of maltose and NaNO<sub>2</sub>. The results also demonstrated the enhancing and stimulating effect of fungal interactions, indicating that glucose and NH<sub>4</sub>Cl at pH = 10 are optimal for the biodegradation process. The dry weights of fungal mycelium reached (4.30) gm in the Methylene Blue dye medium, while they reached (3.45) gm in the Congo Red dye medium. As for bacteria, they differed in their response to the azo dyes depending on the concentrations. The colony counts of bacteria in solid media containing MB dye reached (30× 10<sup>8</sup>) and (22× 10<sup>8</sup>) for *Pseudomonas aeruginosa* and *Bacillus thuringiensis* respectively. In solid media containing CR dye, the colony counts of bacteria reached (23× 10<sup>8</sup>) and (21×10<sup>8</sup>) for *Pseudomonas aeruginosa* and *Bacillus thuringiensis* respectively. As for the liquid mineral MSM media containing MB and CR dyes, supplemented with glucose, NH<sub>4</sub>Cl, maltose, and NaNO<sub>2</sub> at pH values of ( 4, 7, 10) the results showed that the optimal factors for the growth of bacterial colonies and their biodegradation process were in media containing glucose as a carbon source and NH<sub>4</sub>Cl as a nitrogen source at pH 10. The highest biodegradation percentage was achieved, reaching (97.71% , 95.14%) for *Pseudomonas aeruginosa* with MB and CR dyes, respectively. The toxicity of the two dyes was tested before and after the process of biodegradation on seeds of *Hordeum vulgare* . The toxicity results showed the biodegradation effectiveness in reducing the toxicity of two dye , as the toxicity of MB dye decreased from 77% to 30%, and the toxicity of CR dye decreased from 85% to 40% after the biodegradation of the two dyes . Also, the results showed an increase in the percentage of seed germination after the process of biodegradation of the dyes. The seed germination rate increased from 60% to 95% when using MB dye, and it increased from 75% to 93% when using CR dye after the . treatment