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Isolation, Identification, and Efficiency Testing of Some Fungi in the Decomposition of Calcium from Soil Contaminated with Cement around El-Mergib Cement Factory, Al-Khums, Libya

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ABSTRACT

This study was conducted to isolate, identify, and test the efficiency of some fungi in the decomposition of calcium from cement-contaminated soil around the area of El-Mergib Cement Factory, Al-Khums, Libya. Soil samples were collected from three different spots (inside the factory wall, 150 meters, and 300 meters away from the factory) with four replicates for each spot. Control samples from uncontaminated soil were also collected and replicated to compare the results. Twelve species from four fungal genera (Rhizopus, Penicillium, Fusarium, and Aspergillus) were isolated and identified utilizing Sabouraud Dextrose Agar (SDA) medium. The decomposition efficiency of calcium from the contaminated soil samples was also examined through calcium source utilization ability in the biological processes of growth and nutrition. Results have revealed that the presence and biodiversity of Rhizopus sp were significantly higher than the genus of Aspergillus in the order of (Rhizopus, Penicillium, Fusarium, and Aspergillus) respectively. The high growth rates of the Fusarium sp isolates on soil samples contaminated with CaO indicate the possibility of using these fungi in the bioremediation of soil contaminated with cement as a cheap and eco-friendly technology.

Keywords: *Fungi, decomposition efficiency, cement-contaminated soil, calcium, El-Mergib Cement Factory*

INTRODUCTION

Due to the accelerating growth the world witnessing lately, the use of cement, the most essential building material, has tremendously increased. However, the challenges facing the cement industry in particular to reach the minimum of its negative effects on the environment, which was classified as one of the most important industries that expected a negative impact on the environment surrounding the factory, which may extend to vast distances due to wind. It is customary that cement factories are established near the sources of raw materials, which are usually populated to reduce production and transportation costs. The raw material on which the cement industry is based is limestone. According to findings reported by [1] cement is mainly composed of three compounds: calcium (45-55%), silica, and aluminum.

Negative impacts of cement on human health include respiratory diseases and exposure to high noises. On the other hand, the cement industry may change the proportions of gases that make up the atmosphere, as well as the emission of carbon dioxide CO_2, which is responsible for exacerbating the problem of global warming and the consumption of large quantities of natural resources [2]. The negative effects of laminate extend to include the effect of cement on the plant life forms, as it leads to fluctuation in the size and density of the vegetation cover. It also affects growth rates,

productivity, and gas exchange resulting in clogging the stomata openings [3]. Based on [4] bioremediation is considered one of the safest ways to get rid of pollutants due to being environmentally friendly and economically inexpensive, especially using fungi, which is scientifically known as mycoremediation. The superiority of fungi in this area comes since fungi have a very complex enzymatic system that increases their ability to break down these pollutants [5]. In this study, the researcher focused on the efficiency of fungal isolates in the decomposition of calcium pollutants (CaO).

Study Area

El-Mergib Cement Factory is located on the highway in the western part of the Libyan State about 4.7 km from the city center of Al-Khums (32.38101° N, 14.13231° E) with a total area of approximately 10.3 ha and about 120 km to the east of the Libyan capital of Tripoli. El-Mergib Cement Factory is considered the first cement factory in Libya as it started production in 1968 with a production capacity of 100 tons/year. However, the production capacity has been upgraded to 330 tons/year.

Figure 1: El-Mergib Cement Factory

METHODOLOGY

Soil samples were collected in the year 2021 in four replicates from three different spots (inside the factory wall, 150 meters, and 300 meters away from the factory) in 5 -10 cm depth after scraping the upper layer of the soil (about 5 cm). Samples were also collected from unpolluted areas in the city of Al-Khums as a control to compare the results. The samples were collected using a PVC pipe with a diameter of 10 cm. 500 gm of each replicate was placed in a plastic bag, labeled, and transferred to the laboratory for further investigation [6]. Fungi were isolated and identified utilizing the medium of Sabouraud Dextrose Agar (DSA) after adding (10mg/L) of the bacterial antibiotic tetracycline, which inhibits the growth of a wide range of gram-positive and gram-negative bacteria. 1 gm of the soil samples was added to 9 ml of sterilized distilled water, shaken, and spread on the ready medium via an inoculating loop [7].The Petri dishes were incubated at 25±3 °C for 10 days in four replicates then the fungi were isolated and identified based on the morphological and microscopic features [8].

The amount of calcium (mg/L) in the soil samples extractions (1:3 ratio) was estimated by adding 100 gm of the soil sample to a flask of 300 ml of distilled water. The suspension was stirred through the magnetic stirrer for 5 minutes, filtered, and the amount of calcium was estimated in the filtrate via Atomic absorption spectroscopy (AAS). The calcium decomposition efficiency of the isolated fungi was tested by adding known concentrations of calcium oxide CaO (5% - 10%) to the medium through calcium source utilization ability in the biological processes of growth and nutrition. The isolated fungi were inoculated employing the agar well diffusion method. Three 6 mm holes were made in each plate at equal distances utilizing a sterilized cork borer and the Known CaO concentrations were added successively [9]. Dishes were incubated at 25±3 °C. A week later, the diameter of the fungal colonies was measured using the measuring tape and compared to the control.

RESULTS AND DISCUSSION

Twelve species from four fungal genera (*Rhizopus, Penicillium, Fusarium,* and *Aspergillus*) from the three selected sites (inside the factory wall, 150 meters, and 300 meters away from the factory), and the control were isolated and identified utilizing the medium of Sabouraud Dextrose Agar (SDA) as illustrated in table 1. The number of colonies was counted using the application of CFU Scope offered by iPhone. As noted from the table below that the control samples recorded the highest number of fungal colonies and the lowest number of fungal genera at 417 colonies and 4 fungal genera respectively. According to this study, we note that the genus of *Rhizopus* is the most common, whilst the genus of *Aspergillus* is the less common genus.

Based on a study conducted by [10], it was proven that the presence of fungal species in the polluted areas,

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including the fungi that were isolated in this study, makes them classified as pollution-resistant fungi. The result of this study was similar to many studies conducted in many countries including Mexico, Egypt, and Turkey on soils contaminated with cement dust in terms of the isolated fungi genera.

Table 1: Average Number of Colonies, Isolated Fungal Genera, and Species in Soil Samples.

Fungi	Inside the factory wall	150 m	300 m	Control
Rhizopus arrhizus	5	-	26	70
Rhizopus circinans	7	-	33	18
Rhizopus lyococcus	-	46	8	-
Rhizopus microspores	17	59	-	-
Rhizopus oligosporus	26	8	3	-
Rhizopus oryzae	-	38	55	60
Penicillium chrysogenum	52	31	77	91
Penicillium expansum	22	18	6	1
Penicillium rubens	23	34	-	99
Fusarium acaciae	-	55	-	78
Fusarium oxysporum	-	9	28	-
Aspergillus niger	78	-	12	-
Number of colonies	230	298	248	417
Number of genera	3	3	4	2
Number of species	8	9	9	7

*Number of colonies was counted using the CFU Scope application offered by iPhone

Table 2 illustrates the amount of calcium in studied soil samples expressed in (mg/L). The results showed that the amount of calcium in the soil decreased as we moved away from the study area, as soil samples collected from inside the factory walls recorded about 4 times the amount of calcium in the control samples, which is similar to findings reported by [11].

Table 2: Average Amount of Calcium in Soil Samples Based on Site

Site	Inside the factory wall	150 m	300 m	Control
Amount of Calcium (mg/L)	190.3	155.6	89.7	49.2

Decomposition efficiency of the isolated fungal genera was tested by adding a known concentration of calcium oxide CaO the main component of cement at (5 - 10%) to the medium of Sabouraud Dextrose Agar (SDA) employing the agar well diffusion method by making three 6 mm holes in each plate. The known concentrations of CaO were placed in the holes. Ready medium plates were incubated and the diameters of the fungal colonies were measured and reported as in table 3. Results showed that *Fusarium oxysporum* at the concentration of 5% of CaO has recorded the highest diameter reading at 7.1 cm while the lowest reading scored was at 1.7 cm for *Penicillium rubens* at the concentration of 10%. Findings directly reflect the efficiency of the *Fusarium oxysporum* and *Fusarium acaciae* in exploiting calcium and reducing its contamination of the soil.

Table 3: Average Growth Diameter at Different Concentrations of CaO

Fungi	CaO Concentration	Average Diameter
Rhizopus arrhizus	5%	4.1
	10%	3.2
	Control	6.7
Rhizopus circinans	5%	4.7
	10%	3.6
	Control	7.1
Rhizopus lyococcus	5%	4.3
	10%	3.6
	Control	6.8
Rhizopus microspores	5%	4.1
	10%	2.9
	Control	6.6
Rhizopus oligosporus	5%	4.9
	10%	3.7
	Control	7.1
Rhizopus oryzae	5%	5.1
	10%	3.9
	Control	7.5

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Penicillium chrysogenum	5%	3.6
	10%	2.7
	Control	5.8
Penicillium expansum	5%	4.0
	10%	3.3
	Control	6.1
Penicillium rubens	5%	2.3
	10%	1.7
	Control	4.9
Fusarium acaciae	5%	6.6
	10%	5.1
	Control	8.9
Fusarium oxysporum	5%	7.1
	10%	6.3
	Control	9.2
Aspergillus niger	5%	4.3
	10%	3.5
	Control	6.2

CONCLUSION

In the current study, the presence of (*Rhizopus, Penicillium, Fusarium,* and *Aspergillus*) was reported in the cement-contaminated soil samples, which were able to decompose the main component of cement (CaO) from the contaminated soil. *Fusarium* was the most efficient genera in terms of CaO removal from the soil that can be effectively used as a cheap and eco-friendly technology in the mycoremediation of contaminated soil.

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Recommendations

The author of this work would highly recommend his colleagues and other researchers pursue similar studies to confirm the findings.

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