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Some Characteristics and Identification of Fungi Contaminated by Alkomos Cement Factory

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Abstract**—**Soil samples were collected from and around Alkomos cement factory, Alkomos town, Libya. Soil physiochemical properties were determined. In addition, olive leaves were scanned for their fungal content. This work can conclude that the results obtained for the examined physiochemical characteristics of soil in the area studied prove that cement dust from the Alkomos cement factory in Libya has had a significant impact on the soil. The affected soil properties are pH and total calcium content. These characteristics were found to be higher than those in similar soils from the same area. The increment of soil pH in the same area may be a result of precipitation of cement dust over the years. Different responses were found in each season and each site. For instance, the dominance of fungi of soil and leaves was lowest at 100 m from the factory and the evenness and diversity increased at this site compared to the control area and 250 m from the factory.

Keywords**—**Pollution, Soil Microbial, Alkomos, Libya.

I. Introduction

MONITORING the contamination of soil by different kinds of pollutants is of great importance because of their potential effects on arable land and groundwater. An example of such a pollutant is cement dust, which can affect the chemical properties of soil. The quantity of cement dust emitted from Benghazi (Libyan city) cement factory is approximately 200 g/m^2 per month, about 40 times more than the standard dust allowed (5.79 g/m^2 per month) and there was substantial accumulation of this cement dust on olive leaves [1]. Cement dust pollution has been found to damage certain physiological characters in olive trees in Libya, causing reductions in leaf total chlorophyll content and chlorophyll a/chlorophyll b ratio [2]. The same achieve has been found in Greece [3] and in Turkey [4]. Reference [1] reported that olive oil content in fruit exposed to cement dust was significantly reduced compared with control plants, from 0.206 l/kg to just 0.117 l/kg. In addition, the physicochemical properties of soil contaminated by cement were changed which made the soil unsuitable for plant growth [5]. As soon as cement contacts the soil surface its constituents enter inside the earth. Its metal constituents undergo several reactions which can affect soil including its chemical and physical characteristics [6]. The biological, physical and chemical properties of soil, such as water content, electrical conductivity, and pH, were all found to be affected when treated by raw materials of cement [7].

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II. Aims

No enough reports studied soil fungi around Alkomos cement factory in Libya. Therefore, this paper aims to investigate the effect on soil of cement dust emitted from the Alkomos cement plant in Libya. Genera of fungi which are present in soil and leaf surface microflora in the area contaminated by cement were investigated, as well as pH, moisture, and calcium content in the polluted soil, comparing the findings with those for soil samples taken from an unpolluted area.

III. Materials and Methods

A. Outline of the Cement Factory and Surrounding Area

The field research area lies near the coast of Libya at latitude 32.65 N (north) and longitude 14.26 E (east). The factory is located about 100 km east of Tripoli (Libya’s capital city). In the summer, the main direction of the wind is from the desert (south) to the sea (North). However, in the winter the wind blows from west to east.

B. Soil and Plant Sampling

In summer and winter seasons, soil samples were collected from three sampling stations (A, B and C) at each of three sites, 100 m and 250 m from the Alkomos cement factory and 100 km away in the area named Misurata for the control. Samples of olive leaves were collected in separate new clean polythene bags from olive trees from each station (A, B and C) at each site (100 m, 250 m and 100 km) in the winter season.

C. Soil Moisture and pH

A common method is used in which a soil sample is dried at 80°C to a constant weight for soil water content. In view of the high concentrations of calcium carbonate and other substances which could increase soil pH, pH was measured. The procedures outlined by [8] were used for soil moisture and pH.

(i) Soil Moisture Calculation

$$\text{Water content (\%)} = (M_w - M_d)/M_d \times 100$$

M_w = Mass of wet soil sample; M_d = Mass of dry soil sample.

D. Soil Calcium Analysis

Because of the high content of calcium in cement, the concentration of calcium was measured in soil from each station (A, B and C) at each site (100 m, 250 m and 100 km). Calcium was determined using the method of [9].

E. Fungal Isolation

The dilution plate method was used for the estimation of soil fungi, as described by [10]. However, fungi were isolated from olive leaves as described by [11] with slight changes.

(i) Data Analysis

Techniques for analysing fungal community data with the data obtained, several indices were calculated: ecological numerical techniques were used to calculate the soil and plant fungal dominance and diversity. The percent abundance was analysed by k-dominance curves [12]. Some species diversity was analysed using the Shannon diversity statistic (H') [13], which is the most commonly used diversity index in ecology, and Simpson's dominance index (D) [14].

The Shannon diversity statistic (H'):

$$H' = - \Sigma P_i \ln P_i$$

where $P_i = n_i/N$ is the proportional abundance of the species and n_i is the number of colonies of species i, N is the total number of colonies.

Simpson's dominance index (D):

$$D = \Sigma n_i (n_i - 1)/N(N - 1)$$

F. Statistical Analysis

Data were analysed using SPSS or Minitab. For pH, calcium and water content, there were three replicates per site (one replicate in one station). Three to six replicates (cultures) from each site (100 m, 250 m and 100 km) were used in the analysis of data on isolation of fungi from both soil and plant leaves. Using one-way classification, analysis of variance (ANOVA) of the polluted and control soil and plant samples was undertaken. Throughout $P \leq 0.05$ was used to define statistical significance mean separations were carried out using Duncan's test. Microsoft Excel was used to produce graphs.

IV. Results and Discussion

A. Soil pH

The results in this paper show that pH values in the summer were significantly higher in soil from the polluted sites ($P < 0.01$) than in unpolluted soil (Table I). It ranged between 8.0 and 10.0 of the contaminated summer soils, consequently were more alkaline than is typical of most soils in locations around Alkomos city in Libya (pH 7.9) [15].The increase in soil pH could be the result of continuous addition of cement dust from the cement factory over many years [6].This would be in line with the observation that pH in soils in the area studied was higher than in previous years [16].

B. Soil Moisture

Soil moisture was measured for both contaminated samples and unpolluted samples in both seasons (Fig. 1). The results show highly significant differences between groups ($P < 0.01$). The water content of soil from the polluted sites was not significantly different from that of the control soil in the summer ($P = 0.4$) (Fig. 1). However, in winter significant differences among sites were recorded ($P = 0.005$). The difference in water content between the polluted and unpolluted areas could be the widespread green cover in the unpolluted area and lack of green cover in the polluted site area. Green cover is known to diminish maximum soil temperature and decrease evaporation from the soil [17]. Less soil moisture was measured in an area polluted by cement in Turkey than in an unpolluted area [4].

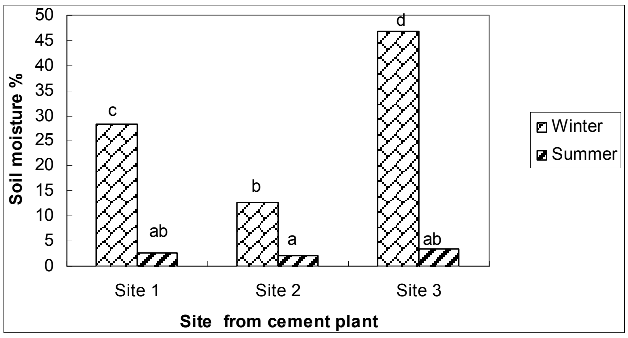

Fig. 1 Average water content of soil samples from site 1, site 2 and site

TABLE I
MEASURED pH OF SOIL SAMPLES FROM SITE 1(100 M), SITE 2 (250 M) AND SITE 3 (100 KM)

Season	Distance from cement plant		
	Site 1	Site 2	Site 3
Winter	7.9 a	7.9 a	8.0 a
Summer	10.0 b	9.8 b	8.0 a

Values within a row followed by the same letter are not significantly different at P = 0.05 according to Duncan's test.

C. Soil Calcium

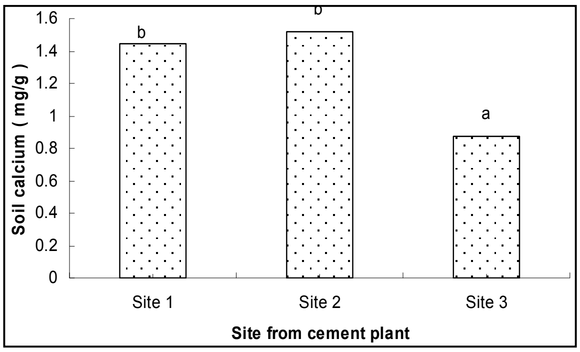

Fig. 2 Average calcium content of summer soil samples from site 1, site 2 and site 3

There were significant differences in calcium content between areas ($P < 0.001$). The calcium content in soil from the polluted areas was significantly greater than in soil from the unpolluted area, Fig. 2. This indicates that a major source of calcium ions in the study area (Alkomos) is industrial activity, in particular the cement plant. A previous investigation with Alkomos soil (including the first two distances in the study area) indicated that the calcium content ranged between 0.002 and 0.360 mg/g [18]. which means that the calcium contents in our results are about four times higher than the calcium content previously found in that area. Also in Nigeria, there was a high content of certain metals, including heavy metals and calcium, in soil surrounding two cement factories [19]. This may be attributed to the calcareous nature of cement-dust depositions on the soil surface [6].

D.Fungi Isolated From Soil

Eleven species belonging to five genera of fungi were recovered from all soils. *Aspergillus, Penicillium, Fusarium* and *Rhizopus* were the most frequently found genera in the soils studied in both polluted locations (Tables II and III). The total abundance of fungi in the polluted soil was lower than in the control, but differences were only statistically significant for some species. The diversity of fungi in soils exposed to cement dust is generally low when compared with other fungi from unpolluted soil [20].

TABLE II
SUMMARY OF FUNGAL COLONIES ISOLATED FROM SUMMER SOIL SAMPLES FROM SITE 1, SITE 2 AND SITE 3

Fungus	Distance from cement plant		
	100 m	250 m	100 km
Aspergillus flavus	4a	9a	5a
Aspergillus niger	4ab	8b	2a
Aspergillus ochraceus	0a	0a	7b
Aspergillus sp_1	10a	0a	0a
Aspergillus sp_2	2a	0a	7a
Fusarium oxysporum	0a	22a	22a
Fusarium sp	6a	0a	15a
Mucor sp	6a	0a	2a
Penicillium chrysogenum	10a	0a	24a
Penicillium sp	6a	0a	15a
Total number of species	8	2	9
Total number of genera	4	2	4
Total number of colonies	48	39	99

Numbers are the total number of colonies in 4 plates.

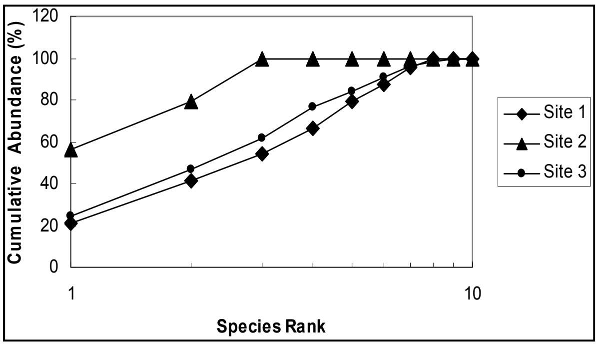

Fig. 3 Combined measures of species diversity in summer soil of site 1, site 2 and site 3 using k-dominance curves

There were no significant differences between numbers of colonies of each species isolated from the different sites in the summer, except for *Aspergillus ochraceus* and *Aspergillus niger* (Table II). However, in the winter *Aspergillus ochraceus*, *Aspergillus flavus*, and *Aspergillus* sp. showed significant differences from the control (Table III). The genus *Aspergillus* displayed numerous colonies and species, more than *Penicillium*, in all sites. References [21] and [22] observed the same trend for *Aspergillus* compared to other fungal species .The highest dominance curves were in site 2 in summer soil (Fig. 3); this was due to *Fusarium oxysporum* (Table II). The most dominant fungus in winter (Fig. 4) was also *F. oxysporum* (Table III).

TABLE III
SUMMARY OF FUNGAL COLONIES ISOLATED FROM WINTER SOIL SAMPLES FROM SITE 1, SITE 2 AND SITE 3

Fungi	Site 1	Site 2	Site 3
Aspergillus flavus	0a	0a	5b
Aspergillus niger	0a	0a	2a
Aspergillus ochraceus	2ab	0a	7b
Aspergillus sp.	0a	25b	7a
Fusarium oxysporum	53a	13a	22a
Fusarium sp	0a	0a	15a
Mucor sp	0a	0a	2a
Penicillium chrysogenum	12a	13a	24a
Penicillium sp	0a	0a	15a
Total number of species	3	3	9
Total number of genera	3	3	4
Total number of colonies	67	51	99

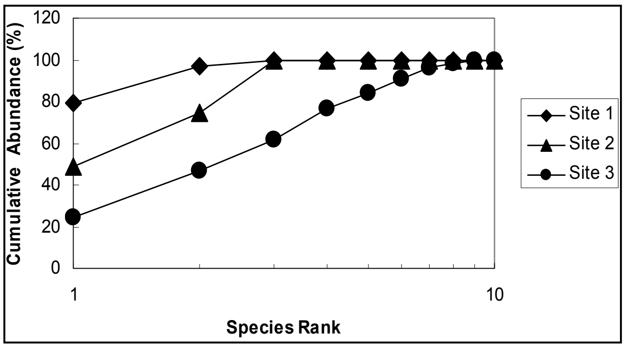

Fig. 4 Combined measures of species diversity in winter soil of site 1, site 2 and site 3 using k-dominance curves

As the degree of dominance by a few species at site 1 increases in winter soil, evenness and diversity decrease at all investigated locations. This trend has also been reported by [23], who stated that winter is the most favorable time for high quantities and species diversity.

The three sites investigated did not all have the same species richness: there were 8, 3 and 9 species found at sites 1, 2, and 3 respectively in summer time. In summer soil a higher evenness was found at site 1 (100 m) than sites 2 and 3. Since dominance is the opposite of diversity, it is clear that site 1 has greater diversity than site 2. There was a strong predominance of one species, *Fusarium oxysporum*, in site 2, with only a small number of the other two species. Heavily disturbed sites, for example those affected by pollution, tend to be dominated by a few species. The results in this paper indicate, however, that the polluted sites could have greater diversity than the unpolluted site, which implies that there were some species of fungi tolerant to this kind of pollution.

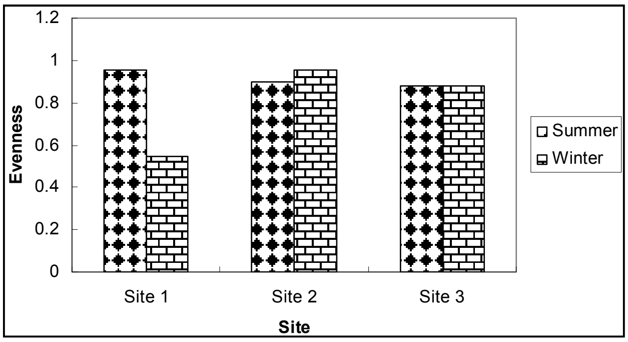

Fig. 5 Species diversity of soil samples from site 1, site 2 and site 3 in summer and winter measured using evenness (E = H′/ln[S])

A. niger has been isolated from all summer soil polluted by cement and is considered the most common genus, occurring in 100% of each sample [21].

It was expected that there would be a low number of fungi in soil and plant samples from close to the cement plant. Interestingly, in this investigation the highest total of number of colonies of fungi (up to 65%) was found in the soil samples taken 100 m away from the factory. These results are similar to the findings in soil polluted by cement factory [10]. The absence of certain fungi in soils polluted by cement dust may be a result of high calcium in cement which causes an increase in soil pH [24]. Consequently, this leads to low abundance or an absence of fungi in the soil [21].

E. Fungi Isolated from Leaves

When fungi were Isolated from olive leaves that had been exposed to cement dust for a long time (Table IV), the most common colonies isolated were from *Aspergillus*, *Fusarium*, *Mucor*, and *Penicillium* were present in the polluted area studied but completely lower colonies number appeared from the control samples (Table IV). Figs. 6 and 7 show that there was lower diversity in the control plants. This was because 75% was covered by a single species (*Aspergillus* sp.), in comparison with the two polluted sites.

TABLE IV
SUMMARY OF FUNGAL COLONIES ISOLATED FROM WINTER PLANT SAMPLES STUDIED (LEAF DISCS) FROM SITE 1, SITE 2 AND SITE 3

Fungus	Site 1	Site 2	Site 3
Aspergillus flavus	15b	11ab	0a
Aspergillus niger	10a	12a	2a
Aspergillus sp_1	42a	31a	40a
Aspergillus sp_2	6a	0a	0a
Fusarium oxysporum	17a	20a	4a
Fusarium sp	15a	12a	0a
Penicillium chrysogenum	13a	0a	8a
Total number of species	7	5	4
Total number of genera	3	2	3
Total number of colonies	118	86	54

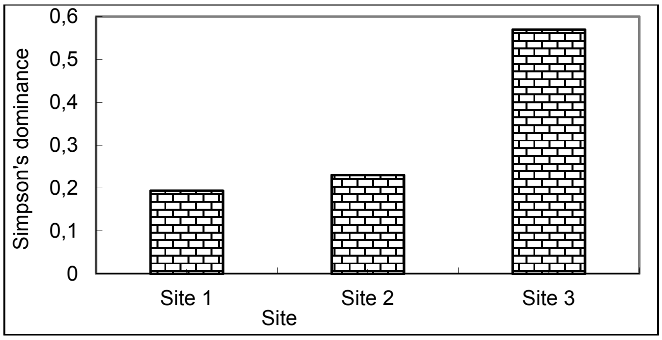

Fig. 6 Measurement of species dominance of plant samples from site 1, site 2 and site 3 in winter using Simpson's index

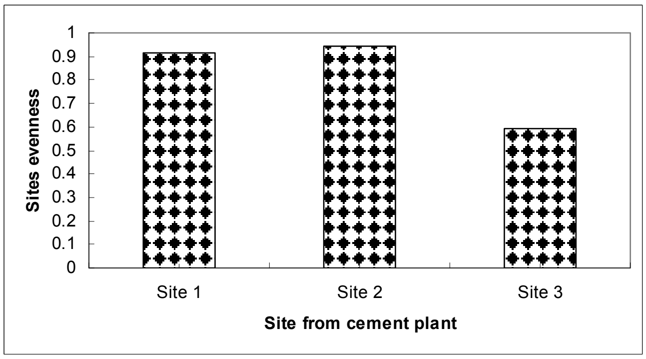

Fig. 7 Measurement of species diversity of plant samples from site 1, site 2 and site 3 in winter using the evenness (E = H′/ln[S])

V. CONCLUSION

The influence of cement dust in the area of pollution on the presence of species was investigating by studying the numbers of colonies of various fungi. Conclusively, it has been observed that human activity has a negative influence on the quality of soil around Alkomos (city in Libya). The low fungal load, as well as high densities of calcium, suggests that pollution of soil by cement dust has occurred. In addition, the quality of the air and pH values in the area studied, along with other observations, can be used to classify cement as the pollutant.

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