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Fungal and Bacterial Populations in Cement-Incorporated Soil

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ABSTRACT

Effects of Portland cement when incorporated in soil samples with three doses (0.5, 2.0 and 8.0 %, w/w) on glucophilic-, cellulose decomposing- and thermophilic-fungi; counts of bacteria; ammonifying-, aerobic non-symbiotic N_2 fixing-; thiosulphate oxidising- and thermophilic-bacteria were evaluated over 1, 2, 4 and 8 weeks of incubation. Generally, counts of all the previous mentioned active groups of microorganisms were decreased especially at the higher doses.

Key Words: Portland cement; Soil fungi; Bacteria

INTRODUCTION

Cement withdraws an attention as a pollutant. The raw materials of the cement have mainly the following constituents: 75% limestone ($CaCO_3$); 20-25% clay (AL_2O_2 + Fe_2O_3); 5% sand (SiO_2) and 2% Ferric oxide (Fe_2O_3). In fact cements are mineral materials, basically hydrated silicate and portlandite [$Ca(OH_2)_2$] for the ordinary Portland cement (Perfettini *et al*., 1991). Cement adversely affects the populations of microorganisms. In Egypt, cement-contaminated areas around cement companies were extensively studied with special reference to fungi (Abdel-Rahman *et al*., 1986; Ali *et al*., 1989; Bagy, 1991; Hemida, 1992; Bagy & Hemida, 1992; Hemida *et al*., 1993). Soil fungi and bacteria and rate of amonification, nitrification, respiration and enzyme activity were also investigated in cement-polluted areas in Poland and Turkey (Zwolinski *et al*., 1988; Hasenekoglu & Sulun, 1991).

The present study was undertaken to evaluate the cement effect on microorganisms when it mixed with soil under laboratorial conditions.

MATERIALS AND METHODS

Soil treatment with cement. A clay soil was collected from the botanical Garden, Assiut, Egypt, which found to contain: 0.8% organic matter content, 0.1% total soluble salts, 7% water content and with pH 7.9. Aliquots of 500g each of soil were placed in polyethylene bags and thoroughly mixed with 0, 0.5, 2.0 and 8.0% (w/w) Portland cement. The water content of soil was then adjusted to 12%. Treatments were set up in duplicates. Bags were incubated at 28 °C±2 for 8 weeks and at intervals, the water content was adjusted and the bags were stirred by sterilized glass rods to permit good aeration. After 1, 2, 4 and 8 weeks, soil samples were taken and assayed for isolating of fungi, and counting of bacteria; pH values of these samples were also measured.

Determination of fungi. The dilution plate method was applied as described by Johnson and Curl (1972). Modified Czapek's Dox agar medium in which glucose (10 g/L) or cellulose (20 g/L) was used for isolation of glucophilic-and cellulose-decomposing fungi, respectively. Yeast starch agar (15 g/L) was used for isolation of thermophilic or thermotolerant fungi. Rose-bengal (1/15000) was employed for all above media as a bacteriostatic agent (Smith & Dawson, 1944). Fifteen plates were used for each sample (5 plates per medium), incubated for 7-10 days at 28 °C±2 (mesophilic fungi) or at 45 °C (thermophilic fungi). Recovered fungi were identified and counted. The following references were used for identification of fungal genera and species. Raper and Fennell (1965), Rifai (1968), Ellis (1976), Booth (1977), Pitt (1979), Domsch *et al*. (1980), Sivanesan (1984) and Moubasher (1993).

Enumeration of bacterial population. The following media were used for counting of several bacterial groups: Thornton's agar medium (Thornton, 1922) for total bacterial counts, Remy's nutrient agar medium (Allen, 1957) for ammonifying bacteria, Rennie's agar medium (Rennie, 1981) for aerobic non-symbiotic N_2 fixing bacteria and Bejerinck's thiosulphate agar medium (Allen, 1957) for thiosulphate-oxidizing bacteria. Yeast starch agr (15 g/L) was also used for counting of thermophilic and thermotolerant bacteria. The plates (30 per sample; 6 per medium) were incubated at 28 °C±2 (for all groups) or 45 °C (thermophilic bacteria), for 3-7 days and the developing colonies were counted.

Statistical analysis. One way analysis of variance (PC-State Computer program) was used to analyze all data obtained. Means were separated by using Duncan's multiple range test (Duncan, 1965).

RESULTS AND DISCUSSION

Fungi in cement-incorporated soil. Tables I, II and III revealed that, twenty one species and two varieties belonging to sixteen fungal genera were isolated on glucose- (6 genera + 13 species), cellulose- (6 genera + 10 species) Czapek's agar and yeast starch agar (10 genera + 13 species + 2 varieties) media. *Aspergillus terreus, A. niger* were the most frequent species recovered on glucose- and cellulose agar media whereas *A. flavus, A. sydowii* and *A. versicolor* ranked in the second place (Tables I, II). *Aspergillus* species were also isolated frequently on yeast starch agar medium at 45 °C (*A. fumigatus, A. niger, A. terreus*) in addition to *Emericella nidulans* and *E. nidulans* var. *latus* (Table III).

Doses of 2.0 and 8.0% (w/w) of cement showed a significant inhibitory effect on the total counts of fungi recovered on glucose- and cellulose Czapek's agar, after all the incubation periods (Tables I, II). Whereas, no significant change in count has been recorded with the low dose (0.5%) except however, a significant stimulatory effect observed after 2 weeks (cellulose-decomposing fungi, Table, II) or 4 weeks (glucophilic fungi, Table I). This increase in total count was due to a significant enhancing count of *Aspergillus terreus*. However, the high concentration of cement (8.0%) lowered significantly and consistently counts of *A. niger* and *A. terreus* (Tables I, II). Furthermore, in two weeks time of incubation, counts of these two species in 2.0% cement-mixed soil were significantly decreased till the end of the experiment (Tables I, II).

Hasenekoglu and Sulun (1991) reported that, the cement-polluted soils in Turkey, contained less fungal populations than the unpolluted soils. This is nearly in agreement with the presented data. The differences in the fungal populations specially at higher doses of cement were attributed as Hasenekoglu and Sulun (1991) reported to the greater lime content. Zwolinski *et al.* (1988) showed that, the high levels of Al and Fe were behind the deleterious effect of cement on the number of soil fungi in Poland.

Table (IV) showed that incorporation of cement in soil increased the pH value specially with the highest cement concentration used.

Babich and Stotzky (1974, cited in Singh & Bharatrai,

Table I. Fungal flora (calculated per mg dry soil) of cement-incorporated soil, isolated on glucose-Czapek's agar medium at 28 °C±2

Incubation period	1 week				2 week				4 week				8 week			
% of cement (w/w)	0.0	0.5	2.0	8.0	0.0	0.5	2.0	8.0	0.0	0.5	2.0	8.0	0.0	0.5	2.0	8.0
Alternaria alternata FR. KEISSLER	0.0	0.0	0.2*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aspergillus flavus LINK	0.0	0.2	0.2	0.2	0.5a	0.7a	2.5a	0.2a	0.2a	0.2a	1.7a	0.0a	0.2	0.9	0.0	0.0
A. fumigatus FRESENIUS	0.2	0.0	0.5	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. japonicus SAITO	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0	0.2
A. niger VAN TIEGHEM	20.0a	21.7a	20.2a	3.0b	28.5a	21.5a	24.2a	1.7b	37.5a	37.2a	22.0a	0.5b	17.8a	16.8a	5.8b	0.0c
A. sydowii (BAIN. et SART.) THOM et CHURCH	0.0	0.0	0.0	0.5	1.5a	2.5a	2.7a	3.5a	0.2	0.2	0.0	0.5	0.3	0.3	0.0	0.0
A. terreus THOM	21.2ab	34.2a	15.0bc	3.5c	22.5b	34.5a	14.0b	2.5c	32.7b	64.0a	12.5c	2.7c	45.3a	45.5a	2.3b	0.2b
A. versicolor (VUILL.) TIRAB.	2.5a	1.7a	1.5a	1.7a	0.0	0.0	0.0	0.0	1.0a	1.2a	3.5a	1.5a	3.5a	1.1a	0.0b	0.7b
Emericella nidulans (EIDAM) VUILL.	0.5a	1.0a	2.0a	1.5a	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gibberella fujikuroi (SAWADO) ITO	0.0	0.0	0.0	0.0	0.0	0.5	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Penicillium corylophilum DIERCKX	0.0	0.0	0.0	0.0	1.0a	1.2a	0a	0a	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
P. funiculosum THOM	0.5	0.0	0.0	0.0	3.2a	0.5b	0.2b	0.2b	1.2a	0.0a	0.0a	0.0a	0.2	0.0	0.0	0.0
Rhizopus stolonifer (EHRENB.) LINDT	4.2a	3.0a	0.7b	0.0b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Total count	49.2ab	62.2a	41.2b	11.2c	58.2a	61.5a	44.5b	8.5c	73.0b	103.2a	40.2c	5.7d	67.9a	64.9a	8.2b	0.9c
Total number of species	7	6	8	7	6	7	6	5	6	5	4	5	8	5	2	3
Total number of genera	4	3	4	2	2	3	3	2	2	1	1	2	2	1	1	1

* Values marked with the same letter are not significantly different at 5 % comparable with the control (0.0 % cement).

Table II. Fungal flora (calculated per mg dry soil) of cement-incorporated soil, isolated on cellulose-Czapek's agar medium at 28°C±2

Incubation period	1 week				2 week				4 week				8 week			
% of cement (w/w)	0.0	0.5	2.0	8.0	0.0	0.5	2.0	8.0	0.0	0.5	2.0	8.0	0.0	0.5	2.0	8.0
Aspergillus flavus	2.0a*	3.0a	0.5b	0.5b	0.2a	1.2a	0.5a	0.2a	2.0a	1.2a	2.2a	0.0a	0.7	0.5	0.0	0.0
A. fumigatus	0.2	0.0	0.0	0.0	0.5a	0.2a	0.2a	0.2a	1.0	0.2	0.2	0.2	0.0	0	0.0	0.0
A. niger	40.7a	40.5a	33.7a	2.7b	30.2a	26.0a	15.5b	0.7c	36.5a	36.0a	20.7b	0.7c	20.8a	16.8a	2.3b	0c
A. terreus	41.2ab	60.5a	24.7bc	3.0c	20.7b	39.5a	17.2b	1.2c	55.0a	57.0a	14.2b	0.2b	62.8a	59.8a	3.5b	0.3c
A. versicolor	1.2b	1.2b	1.5b	3.5a	0.2	0.0	0.0	0.5	0.0	0.2	0.0	0.2	0.0	0.0	0.2	0.3
Cunninghamella echinulata (THAXT.) THAXT.	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Emericella nidulans	2.5a	3.0a	1.5a	2.5a	1.5a	0.7a	0.7a	0a	4.0a	5.0a	0.2b	0b	1.6	1.5	0.0	0.0
Penicillium funiculosum	0.0	0.0	0.2	0.0	3.7a	1.0a	0.2a	0a	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Rhizopus stolonifer	2.0a	2.0a	0.2a	0.0a	0.7a	0.2a	0.2a	0.5a	0.7a	2.7b	0.0b	0.0b	0.0	0.0	0.0	0.0
Trichoderma harzianum RIFAI	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total count	90.2a	111.0a	62.7b	12.7c	58.5b	70.2a	35.5c	4.0d	102.2a	101.2a	37.5b	1.5c	85.9a	78.7a	6.0b	1.1c
Total number of species	9	6	8	5	8	7	7	6	7	8	5	4	4	4	3	2
Total number of genera	5	3	5	2	4	4	4	2	4	4	2	1	2	2	1	1

* Values marked with the same letter are not significantly different at 5 % comparable with the control (0.0 % cement).

Table III. Fungal flora (calculated per mg dry soil) of cement-incorporated soil, isolated on yeast starch agar medium at 45°C

Incubation period	1 week				2 week				4 week				8 week			
% of cement (w/w)	0.0	0.5	2.0	8.0	0.0	0.5	2.0	8.0	0.0	0.5	2.0	8.0	0.0	0.5	2.0	8.0
Aspergillus fumigatus	0.2a*	1.5a	1.2a	1.0a	0	0.7	0.5	0.2	0.5	1.0	0.5	1.0	0.2	0.9	0	0.5
A. japonicus	0.0	0.0	0.0	0.0	0.0	0.2	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. niger	0.0a	1.2a	1.5a	0.7a	0.7a	0.0a	3.7a	0.0a	2.2a	0.7a	2.0a	0.2a	1.1a	0.2a	6.1b	0.3a
A. terreus	10.2a	11.7a	12.2a	4.0a	15.2b	27.0a	34.2a	3.5c	31.0b	45.0a	19.2b	0.5c	24.8a	27.1a	9.1b	0.0c
A. versicolor	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.2	0.0	0.0	0.0
Chaetomium thermophile LA TOUCHE	0.5	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Emericella nidulans	4.2a	5.7a	6.5a	1.0a	6.7a	7.0a	3.0b	0.0c	7.0ab	10.2a	1.0bc	0.0c	6.6a	5.8a	0.2b	0.0b
E. nidulans var. *latus* (THOM et RAPER) SUBRAM.	6.5a	5.0a	3.5a	3.2a	4.0b	8.7a	2.0b	0.0b	3.0ab	5.7a	0.0b	0.2b	4.9a	3.8a	0.0b	0.0b
Humicola insolens COONEY et EMERSON	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	1.0a	1.7a	0.2a	0.0a	0.3	0.0	0.0	0.0
Malbranchea pulchella var. *sulfurea* (MIEHE) COONEY et EMERSON	0.2	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Myriococcum albomyces COONEY et EMERSON	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhizomucor pusillus (LINDT) SCHIPPER	0.0	0.7	0.0	0.0	1.0	0.0	0.0	0.0	2.0a	1.2a	0.7a	0.0a	0.2	1.4	0.0	0.0
Talaromyces dupontii GRIFFON et HAUBLANCE	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Thermoascus aurantiacus MIEHE	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Torula thermophila COONEY et EMERSON	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Total count	22.2a	26.2a	25.0a	10.2b	30.0b	45.5a	45.5a	4.0c	48.7a	64.5a	24.2b	2.0c	43.9a	39.0a	17.1b	0.9c
Total number of species + varieties	6+1	6+1	5+1	4+1	7+1	6+1	6+1	2	7+1	8+1	6	3+1	7+1	5+1	3	2
Total number of genera	5	4	3	2	6	4	3	1	5	7	4	2	4	3	2	1

* Values marked with the same letter are not significantly different at 5 % comparable with the control (0.0 % cement).

Table IV. pH values of cement incorporated soil

Incubation period % of cement (w/w)	0	1 week	2 week	4 week	8 week
0.0	8.9*	7.5	8.8	7.4	8.7
0.5	10.1	9.0	9.4	8.6	9.1
2.0	11.3	10.5	10.9	11.0	10.9
8.0	11.7	11.3	11.7	11.7	11.6

* Means of two readings.

1990) suggested that, increase in pH might be responsible for decreased microbial growth. This could be also the case in the present study where the pH of soil amended with 8.0% cement (w/w) was nearly 11.6 all over the incubation period (Table IV).

Hemida *et al*. (1993) reported that, *A. niger* and *A. terreus* were also among the most encountered species in cement-polluted soil. On the other hand, Singh and Baharatrai (1990) showed that, cement dust exhibited either stimulatory effect on growth behaviour of some fungi such as *Alternaria alternata*, *A. flavus*, *A. niger* and *Fusarium oxysporum*, or inhibitory influence on growth of *Cladosporium cladosporioides*, *Penicillium chrysogenum* and *P. citrinum*. The increased populations of microbes at lowest dose of cement seem to be due to their ability to neutralize the toxic effect of cement and inherent resistance against low dose (Andrews, 1986, cited in Singh & Bharatrai, 1988).

Count of thermophilic fungi was affected drastically and significantly at the medium (after 4 and 8 weeks) and high (all over the incubation period) doses (Table III). Nevertheless doses of 0.5 and 2.0% of cement, increased count of such a group significantly after 2 weeks of incubation. Again counts of *A. terreus* are involved.

Hemida (1992) isolated some truly thermophilic fungi (*Malbranchea pulchella, Rhizomucor pusillus, Talaromyces thermophilus* and *Torula thermophila*) from soils exposed continuously to cement dust. These species are recovered

Table V. Total counts of bacteria, ammonifying-, aerobic non-symbiotic N_2 fixing-, thiosulphate oxidizing- and thermophilic-bacteria in cement-incorporated soil

Incubation period	1 week				2 week				4 week				8 week			
% of cement (w/w)	0.0	0.5	2.0	8.0	0.0	0.5	2.0	8.0	0.0	0.5	2.0	8.0	0.0	0.5	2.0	8.0
Bacterial group																
Total count of bacteria ($CFU^{\dagger}x10^4$ g^{-1} dry soil)	*91.6a	92.8a	75.5b	38.8c	105.7a	101.2a	63.0b	35.0c	98.6a	102.3a	48.8b	27.7c	56.4b	88.6a	22.1c	10.1d
Ammonifying bacteria (CFU x 10^3 g^{-1} dry soil)	43.7b	51.0a	36.0c	8.8d	48.3b	54.2a	25.7c	12.0d	42.0b	58.5a	36.2c	8.2d	36.2a	41.2a	25.7b	3.1c
Aerobic non-symbiotic N_2-fixing Bacteria (CFU x 10^3 g^{-1} dry soil)	52.5a	50.8a	28.8b	21.8c	58.3a	57.2a	32.2b	18.2c	50.5a	51.5a	36.0b	12.2c	33.7a	38.7a	28.2b	6.3c
Thiosulphate oxidising bacteria (CFU x 10^3 g^{-1} dry soil)	28.0b	38.0a	17.6c	14.3d	34.7b	43.7a	85.3c	12.8d	30.5b	44.2a	29.3b	9.3c	15.3b	23.6a	14.4b	4.8c
Thermophilic bacteria (CFU x 10^3 g^{-1} dry soil)	35.7a	18.7b	0.2c	0.5c	33.0a	34.7a	0.0b	0.0b	2.7a	0.7a	0.0a	0.0a	5.9a	3.1a	0.0b	0.0b

* Values marked by the same letter are not significantly different at 5 % comparable with the control.; † CFU = Colony forming unit.

too in the present study (Table III); in addition to several thermotolerant species as *Aspergillus fumigatus, A. terreus* and *Emericella nidulans*.

Bacteria in cement incorporated soil. The results in Table V showed that, the total counts of bacteria, ammonifying bacteria, aerobic non-symbiotic N_2 fixing bacteria and thiosulphate-oxidising bacteria were significantly decreased by increasing cement level above 2.0% throughout the incubation period. Low level (0.5%) of cement had no significant effect on the number of total bacteria and aerobic non-symbiotic N_2 fixing bacteria; but however, a significant increase in the number of ammonifying bacteria and thiosulphate-oxidising bacteria was observed. The significant increase in these microbes at low dose of cement could be attributed to slight change in soil pH toward alkaline side which been optimum for growth. Counts of thermophilic bacteria are drastically and significantly decreased by the three doses after one week incubation (Table V). Moreover, such count was completely eliminated by the higher doses after the later incubation periods.

Similarly, the reduction in population of microbes at moderate and higher doses of cement could reflects the dramatically increase in soil pH (Table IV). Similar results were obtained by Zwolinski *et al*. (1988) and Hasenekoglu and Sulun (1991). Number of bacteria and rates of ammonification, nitrification, respiration and enzyme activity were inhibited in cement-polluted soil (Swolinski *et al*. 1988). Furthermore, Estrela *et al*. (2000) noticed that Portland cement induced inhibitory effect on some bacterial strains such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Bacillus subtilis*.

Generally, the density of fungal and bacterial populations was decreased in soil treated with 2.0 and 8.0% of cement (w/w). However, doses beyond 8.0% induced sometimes a stimulatory effect especially at the earlier weeks of incubation.

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