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Microbial and Heavy Metals Analyses of Sewage Sludge from Saudi Arabia

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Abstract. Analyses of 25 sewage sludge samples from Riyadh region of Saudi Arabia, showed high concentrations of Al,Cd,Co,Cu,Mn,Ni,Zn and Pb. Al was very high in the tested sites and the metal content of the present study were greater than the levels of these elements in some other Saudi Arabian soils. A total of 23 microbial isolates (19 fungi and 4 bacteria) were isolated from the tested sites. High concentrations of heavy metals in the sewage sludge may lead to pollution problems due to their use as soil additive or land filler.

Introduction

The problem of disposal of sewage is considered to be one of the important problems that faces those who take care of the environment nowadays. This problem is becoming complicated with the increasing of population and the decreasing of the used ways to dispose the sewage [1]. Many countries including Saudi Arabia aimed at using the correct ways to dispose and use the sewage [2-5]. The properties of sewage sludge may vary considerably with the area and treatment process, and the agricultural benefits of sludge are well known, but increased levels of sludge in the soil will result in an increase in heavy metals [6;7].

The environmental pollution, especially by chemicals, is one of the most effective factors in the destruction of the biosphere components. Among all chemical contaminants, trace elements are believed to be of a specific ecological, biological, or health significance. Energy and mineral consumption by man is the main cause of trace element pollution in biosphere [8].

The application of sewage sludge to agricultural land adjacent to sewage works is a well established practice. In Saudi Arabia sewage sludge has become more widely used on farm. The value of sewage sludge as possible sources of N, P and K was assessed by different workers and attention was drawn to the possible dangers from accompanying heavy metals present [9].

Sewage provides a normal and good habit at for many microorganisms. For example *E. coli* is used as an index for fecal pollution. Sewage contains pathogenic and nonpathogenic microorganisms as well as a variety of other organic materials including food wastes and detergents [10; 11]. The present investigation aimed to isolate some microorganisms and to determine the heavy metal content of sludge from Riyadh region, Saudi Arabia.

Materials and Methods

Sewage sludge samples were collected in sterile 100 ml polyethylene plastic bottles with screw caps from five sites of Riyadh region, according to the method described by Black *et al.* [12]. Five samples of the disposed of sewage sludge from each site were collected. Five replicates of each sample were taken for microbial and mineral analyses.

Microbial isolation

The soil dilution plate method was used for microbial isolation [13]. Czapek's agar and nutrient agar media, were used for fungal and bacterial isolation, respectively. The dishes were incubated at 30°C for 5 days for bacterial isolation and at 27°C for one week for fungal isolation. Fungal and bacterial genera and species were identified [14-20].

Metal analysis

Dried sewage sludge (20 g) was ground for 90 minutes in mechanical agate morter. Samples were digested in concentrated analar grade nitric acid to obtain a measure of total metal content of Al,Cd,Co,Cu,Mn,Ni,Pb and Zn. The procedure was as follows; 05 g of air dried sludge was placed in a 100 ml beaker with 15 ml concentrated nitric acid, covered with a watch-glass and heated at 100°C for 15 minutes. After digestion, the digest were made up to 50 ml dionised water and analysed using absorption flame spectrophotometer (Pye Unicam Sp9 equipped with Sp9 computer) [21].

Table 1. Genera and species of microorganisms isolated from sewage sludge

General and Species	Sites					Frequency %
	1	2	3	4	5	
Alternata alternata	+	−	+	+	+	80
Aspergillus flavus	+	+	+	−	+	80
Aspergillus niger	+	+	+	+	+	100
Cephalosporium sp.	−	+	+	−	−	40
Chrysosporium sp.	−	−	−	+	−	20
Cladosporium herbarum	+	−	+	+	−	60
Coniothyrium sp.	+	+	−	−	−	40
Epicoccum sp.	+	−	+	−	−	40
Fusarium oxysporum	+	+	−	+	−	60
Gliocladium sp.	+	−	+	−	−	40
Mucor sp.	+	+	−	−	−	40
Nigrospora sp.	−	+	+	−	−	40
Paecilomyces sp.	−	−	+	−	−	20
Penicillium chrysogenum	+	+	+	+	+	100
Penicillium citrinum	+	+	+	+	+	100
Pestalotia sp.	+	−	−	−	+	40
Trichocladium sp.	−	+	+	−	−	40
Trichoderma sp.	−	−	+	−	+	40
Ulocladium atrum	+	+	+	+	+	100
Bacillus sp.	+	+	−	+	+	80
Escherichia coli	+	+	+	+	+	100
Pseudomonas sp.	−	+	+	+	+	80
Staphylococcus sp.	−	+	−	+	−	40

Results and Discussion

Nineteen fungal and 4 bacterial isolates were isolated (Table 1). *Aspergillus niger, Penicillium chrysogenum, Penicillium notatum, Ulocladium atrum* and *Escherichia coli* were the dominating microorganisms in the sewage sludge from the tested sites. *Alternaria alternata, Aspergillus flavus, Bacillus* sp. and *Pseudomonas* sp. were with 80% frequency. The fungal species, *Cladosporium herbarium,* and *Fusarium oxysporum* showed frequency of 60%, while *Cephalosporium* sp.,

Chrysosporium sp., *Coniothyrium* sp., *Epicoccum* sp., *Gliodcladium* sp., *Mucor* sp., *Nigrospora* sp., *Paecilomyces* sp., *Pestalotia* sp., *Trichocladium* sp., *Trichoderma* sp. and *Staphylococcus* sp. were with frequency less than 60%.

A. alternata, A. flavus, A. niger, C. herbarum, F. oxysporum, P. chrysogenum, P. notatum and *U. atrum* were isolated earlier from Saudi Arabian soils [22].

Bacillus sp., *E. coli, Pseudomonas* sp. and *Staphylococcus* sp. were isolated from Saudi Arabian soil [23]. They also were isolated from sewage and waste water in different places of the world [13;24].

The saprophytic fungi obtained from sewage sludge, Athens, U.S.A. were *Cephalosporium* sp., *Chrysosporium* sp., *Coniothyrium* sp., *Epicoccum* sp., *Gliocladium* sp., *Nigrospora* sp. and *Pestalotia* sp. [25].

Table 2. Total metal content (μg/g) of sewage sludge (n =5, ± standard deviation)

Metal	Sites				n = 5
	1	2	3	4	5
Al	359 ± 5.0	388 ± 6.1	365 ± 5.0	373 ± 5.0	378 ± 6.5
Cd	42 ± 1.1	36 ± 1.6	41 ± 1.5	39 ± 1.0	45 ± 1.3
Co	39 ± 1.1	40 ± 1.5	36 ± 2.1	35 ± 2.0	38 ± 1.6
Cu	45 ± 1.3	40 ± 2.1	39 ± 1.5	46 ± 1.8	41 ± 2.6
Mn	37 ± 1.0	35 ± 1.3	36 ± 1.5	36 ± 1.2	38 ± 1.1
Ni	31 ± 1.3	36 ± 1.5	33 ± 2.1	35 ± 1.0	39 ± 1.1
Zn	53 ± 2.1	48 ± 1.6	46 ± 2.1	52 ± 1.9	48 ± 1.8
Pb	30 ± 1.1	29 ± 1.0	26 ± 1.1	28 ± 1.0	31 ± 1.2

The sludges of the five sites differ slightly in their mineral composition (Table 2). The estimated concentrations of the minerals recorded here were found to be higher than that reported in some Saudi Arabian soils [26;27].

As a result of field experiment carried out in the United States, it was reported that the considerable amounts of Co,Cu and Zn that most sludge contain could in acid soils to be toxic to plants [11].

Microbial transformation and assimilation of heavy metals are well known [28;29].

Microbial growth on high metal concentrations, resulted in soil contamination with heavy metals, it can be said that the use of such materials as fertilizers is not without serious hazards particularly when used at heavy rates of application over a number of years and it is advisable that the composition of individual sludge lots should be assessed before their use as soil fertilizer.

Conclusion

Sewage sludge generally contains relatively large amounts of heavy metals. The application of sewage sludge to agricultural lands will result in high content of heavy metals which un-doubtly will affect the soil properties and plant growth. Sewage sludge disposal has become a major public health and ecological problem.

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التحليل الميكروبي والمعدني لمخلّفات الصرف الصحي في المملكة العربية السعودية

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(سُلِّمَ في ١٤١٤/٩/١٨هـ ؛ وقُبل للنشر في ١٤١٥/١/٢٦هـ).

ملخص البحث. تمّ في هذه الدراسة تحليل (٢٥) عيّنة من مخلّفات الصرف الصحي لمنطقة الرياض بالمملكة العربية السعودية، وقد أثبتت نتائج هذه الدراسة أنّها تختلف في محتواها المعدني لكل من الألومنيوم والكاديوم والكوبالت والنحاس والمنجنيز والنيكل والخارصين والرصاص، كما أن المحتوى المعدني لمخلّفات الصرف الصحي في هذه الدراسة يفوق المحتوى المعدني لبعض عيّنات التربة في المملكة العربية السعودية. أما بالنسبة للتحاليل الميكروبية فقد سجلت في العيّنات التي حُلّلت تسعة عشر جنسًا ونوعًا من الفطريات وأيضًا ٤ أجناس من البكتريا.

وقد أوضحت نتائج هذه الدراسة أنّ احتواء مخلفّات الصرف الصحي على تراكيز عالية من العناصر المعدنيّة ربما يعمل على زيادة مشكلات التلوث نتيجة لاستخدام مخلّفات الصرف الصحي كسماد بإضافته إلى التربة.