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MICROFLORA INHABITING RAW SEWAGE, SECONDARY EFFLUENT AND DEWATERED SLUDGE IN IBB, YEMEN REPUBLIC

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ABSTRACT :

The microflora of raw sewage, secondary effluent and dewatered sludge (manure) were investigated. Microbial total counts were relatively higher in raw sewage than in secondary effluent and dewatered sludge. A mongst the bacterial groups recorded in the present investigation, *faecal Streptococcus, Streptococcus pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus* and *Escherechia Coli* were found in the three substrates at 37 ^{o}C. On the other hand, *Salmonella spp.*were isolated from raw sewage and secondary effluent, but *Shigella spp* were isolated only from raw sewage. Some of these bacterial species can produce toxins and cause infections directly or indirectly through contact with sewage sludge. The most common fungal species in the tested substrates on Sabouraud's agar, without cyclohexamide at 28^{o}C were: *Aspergillus flavus, A. fumigatus, A. niger, Acremonium strictum, Aspergillus. terreus, A. versicolor, Cladosporium cladosporioides, C. herbarum, C. oxysporum, Gibberella fujikuroi, Cohliobolules hawaiiencsis, Fusarium solani, F. oxysporum, Penicillium chrysogenum*, *Geotrichium candidum* and *Scopulariopsis brevecaulis*. On Sabouraoud's dextrose agar with cycloheximide the most frequently isolated species were: *Aspergillus flavus, A. fumigatus, A. niger, Gibberella fujikuroi and Geotrichium candidum*. Some pathogenic fungi were also,isolated, but in various incidences and numbers such as *Chrysosporium tropicium C. indicum, C. parvum, Geotrichum candidum, Histoplasma capsulatum, Microsporum canis, M. gypseum* and *M. manginii*.

INTRODUCTION:

Wastewater generated from urban and rural areas after domestic use is a large source of water. It is mainly comprised of water (99,9%) together with relatively small concentrations of suspended and dissolved organic and inorganic solids (Mara and Cairncross, 1989 and UN Department of technical cooperation for development, 1985).

Among the organic substances present in sewage are carbohydrates, lignin, fats, soaps, synthetic detergents, proteins and their decomposition products, as well as various natural and synthetic organic chemicals from process industries.

Sewage sludge (wastewater) is an important source of organic matter (Stranchan., *et al.*,1983) and plant nutrients. Halderson and Zonz, (1978); Nell *et al*., (1983) found that the application of sewage sludge increased the nutrient status of the soil. It may increase agricultural production. Sewage sludge also contains pathogenic macro and microorganisms,

which can give rise to potential hazard (Abderrahman and Shahlam, 1991) to the health of humans, animals and plants. The health risk associated with wastewater is a major deterrent in wastewater reuse for irrigation. Health risk are associated with pathogens, which may spread diseases through being directly or indirectly ingested into the human body (Dudley *et al.*, 1980; WHO, 1981; 1989; FAO, 1992; Feachem *et al.*, 1983; Shuval, 1991; and Shuval *et al.*, 1986) and fungi (Velez and Diaz, 1985; and Bunes and Merk, 1992).

Pathogens pose the greatest threat to public health; especially when the receiving water is used for domestic recreation on agricultural purpose (Tchobanogeuos, 1979).

The agricultural value of sludge mainly derives from its nutrient content. Sludge, like other organic fertilizers, has long-term beneficial effects on the soil: organic matter contained in sewage sludge improves the physical properties of soil such as aggregate stability, water retention and infiltration, and reduce soil compactibility (Stone *et al.* 1998). In addition to nutritious content, the organic matter and the C/N ratio are important parameters of the sludge fertilizing potential.

There are many conditions, which may increase the health risk of wastewater reuse in agriculture. The first of these conditions is survival time of pathogenic microorganisms. The natural survival time of pathogenic organisms depends on the carrying medium and the environment. The survival time is a time during which pathogens are capable of causing diseases if they came into contact with a host under favorable condition.

The second of these conditions are pathogenic bacteria, viruses, protozoa, nematodes and fungi capable of causing diseases which can be found in foods contaminated with sewage water(Bryan,1977; Kowal *et al.*, 1980, and Rosas, 1984). They also can be found harmful to the soil, crops and grazing animals.

On the other hand Pathogenic microorganisms can be transferred from raw sewage and secondary effluent during the irrigation process, directly or in directly to the plants, animal and human, also make various infectious diseases.

Different authors have proved that 5 vegetables are contaminated with microorganisms, when they are irrigated with sewage water and when the soil is fertilized with manure because both usually contain great amounts of pathogenic organisms (Epstein *et al.*, 1982 and Larkin *et al.*, 1978), and when these vegetables are consumed, they could produce diarrhea, salmonellosis, Shigellosis, etc.(Dunlop and Wang, 1961; Kowal *et al.*, 1980 and Rosas *et al.*, 1984).

During the last three decades wastewater reclamation, recycling and reuse in agriculture have received much attention around the world, especially in the arid and semi-arid regions (Neis, 1984; Bouwer, 1982; Emeral and Kayser, 1984; and Madancy, 1981).

Yemen like many other countries in arid and semi-arid regions suffers; from shortage of water resources, so that reuse treated sewage in agriculture is an important question. That is because agriculture seems to be the greatest consumer of water. Annual water consumption has increased dramatically in the last twenty years due to significant social, industrial and agricultural developments. More than 90% of the current water demand is coming from non-renewable groundwater resources in the country.

Farmers in Yemen, living near the disposal sites of urban wastewater, especially in some of the large cities such as Sana'a, Taiz, Aden, and

Ibb are already practicing the reuse of non-treated or partially treated wastewater. (El-Zaemey, 1992). Several countries have produced guidelines, which regulate sewage sludge reuse on the basis of risk to the public health and the environment, however, in Yemen; such guidelines are not established yet.

In Yemen no investigations have been carried out on the microflora of the sewage and knowledge on the distribution of pathogenic bacteria and fungi in sewage and sludge is absent. Thus, the present study is conducted on the composition, numbers and incidence of various species of bacteria and fungi inhabiting sewage before and after purification.

MATERIALS AND METHODS:

Collection of Sewage Samples:

Thirty samples of each of raw sewage, secondary effluent (500 ml each) and manure (dewatered sludge) (250 gr) were collected from Ibb sewage treatment plant. Each sample was placed in a clean bottle, which was capped tightly and transferred to the laboratory for immediatly bacteriological and mycological analyses.

Five bacterial isolation media were used namely: Nutrient agar for plate count analysis, MF, M-endobroth, MFC agar, MacKonky agar and SS agar. 0.1 ml of each of secondary effluent and raw sewage dilution was used per plate. Three plates of each medium were used for each sewage samples. The counts were calculated per 1ml of sewage, for raw and secondary effluent, and per g dry weight for manure.

Isolation and Identification of bacteria:

Bacteria were encountered using the plate count on nutrient agar.

Total Coliform (TCF) were analyzed using the membrane filtration procedure as described by the APHA (1989) and they were cultured on M-Endo broth (APHA, 1989).

Faecal Coliforms (FC) and faecal Streptococcus (FS) were analyzed using the membrane filtration procedure described by the APHA, (1989). Faecal Coliforms were cultured on M-FC agar (Difco) while faecal *Streptococci* were grown on m-enterococcus agar (Difco).

***Salmonella* concentration was determined using a five tube most probable number (MPN) procedure. Four dilution containing 10^{-1}/ ml, 10^{-2}/ml, 10^{-3}/ml and 10^{-4}/ ml of raw sludge and Five other tubes for effluent with four dilution containing 10^{-1}/ml, 10^{-2}/ml, 10^{-3} /ml and 10^{-4} ml of secondary effluent were used. Samples were per-enriched in buffered peptone water (BPW) at 37^{o}C overnight after which 10^{-1}/ml of per-enrich culture was transferred to Rappaport-Vassilladis broth (RV). Enrichment cultures were incubated at 43^{o}C and were subcultured to xylose – deoxycolate agar(XLD) after 24 and 48 h. Presumptive *Salmonella* were purified on MacConky agar without salt and were screened using biochemical and serological tests.**

Isolation and Identification of fungi:

Two isolation media were used for isolation of fungi. Sabouraoud's dextrose agar (Moss and Mcquown, 1969) containing 40 g/l dextrose, 10 g/l peptone, 20 g agar/l, 40 mg/l Streptomycin, 20 units of Penicillin /ml and 0.05% cycloheximide (Actidione) and Sabouraoud's dextrose agar containing 40 mg/l Streptomycin and 0.003% rose-Bengal. One ml of the appropriate of each of secondary effluent and raw sewage and manure was used per plate. Three plates were used for each sewage sample. The plates were incubated at 28^{o}C for 7 days. The counts were calculated per 1ml of sewage.

Identification was carried out by using the taxonomic references of Raper and Fennell (1965), Domsch *et al.* (1980), Raper and Thom (1949); Ellis (1976); and Moubasher (1993).

RESULTS AND DISCUSSION:

Bacteria recovered from raw sewage, secondary effluent and manure:

The total count of bacteria in the raw sewage, secondary effluent and dewatered sludge were 8.2x 10^{10} C/ml, 6.7x 10^{6} C/ml and 5.3x10^{6} C/g respectively (Table 1). The most common bacteria in the above substrates was faecal coliform. It was isolated samples constituting 9.4x10^{8} C/ml, 5.2x10^{3} C/ml and 4.2x10^{2} C/g respectively. The results in Table (1) show also that the most common bacteria was *Escherichia. Coli*. It was isolated from all samples of the three substrates constituting 7.6x10^{6}, 2.8.x10^{3} C/ml and 1.2x10^{2} C/g respectively.

***Salmonella spp*. were isolated from 9 and 3 samples of raw sewage and secondary effluent (2.1x10^{2}, 1.3x10 C/ml respectively).**

Some authors reported that the *Salmonella spp.* can infect or contaminate nearly all living vectors from insects to mammals. (WHO, 1981). Human *Salmonella* infections and other bacterial infections can be caused from the direct or indirect contact with sewage sludge (Pennsylvania Environmental Network, 2002, WHO, 1981 and Doyle *et al.*, 1997). Most serotypes of *Salmonella* are pathogenic to humans. A common route of infection for humans is through ingestion of products contaminated with animal faeces (Woolcock, 1991).

***Shigella spp* were encountered only from raw sewage (1.1x10 C/ml.). Some bacterial species were also isolated in this study from raw sewage, secondary effluent and dewatered sludge (manure). Some of them can be caused infections directly or indirectly contact with sewage sludge.**

Faecal *Streptococcus, Streptococcus pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus cereus* were also isolated in the present study from the three substrates. High bacterial count were detected in all samples of the three substrates investigated (Table 1). Faecal coliform bacteria were also detected in high numbers in tested substrates.

Some of these bacteria can produce toxins and cause infections directly or indirectly to human. The great numbers of bacterial colonies were isolated from sewage sludge (raw sewage, secondary effluent and manure, 30 samples of each) at 37°C. The most common bacteria were faecal coliform (Table 1). Simpson (1982) reported that Sewage contain the wide spectrum of Bacteria. The most common bacteria in sewage sludge are the enteric bacteria (Coliforms, *Shigellae, Salmonella*, etc.). Coliforme bacteria can be contain a rare strain of *E.coli* that is pathogenic to humans (Kirk, 2003). The typical concentration of E. coli found in untreated sewage sludge is 1000,000 wet weight/g of total solids (Smith, 2003).

The results in this study are analogous to those obtained by several workers in many parts of the world (Kirk, 2003, Smith, 2003 and Simpson, 1982). Results revealed also that the bacterial concentration is high and many of them are Pathogens. Our results in this aspect correspond with those of other authors (Smith, 2003 and WHO, 1981).

Table (1) : Total counts (TC) and number of positive samples (PS) for Bacteria isolated from 30 samples of each raw sewage (colony/ml), secondary effluent (colony/ml) and manure (colony/g).

Bacteria	Raw sewage		Secondary effluent		Manure	
	PS	TC	PS	TC	PS	TC
Total colony count	30	$8.2x10^{10}$	30	$6.7x10^{6}$	30	$5.3x10^{6}$
Faecal coliforme	30	$9.4x10^{8}$	30	$5.2x10^{3}$	30	$4.2x10^{2}$
Faecal streptococcus	30	$7.8x10^{4}$	30	$6.5x10^{3}$	26	$3.2x10^{2}$
Salmonella spp	9	$2.1x10^{2}$	3	$1.3x10^{1}$	0	0
Shigella spp	2	$1.1x10^{1}$	0	0	0	0
Streptococcus pneumonia	11	$6.9x10^{2}$	8	$4.2x10^{2}$	10	$5.2x10^{1}$
Staphylococcus aureus	9	$8.7x10^{3}$	6	$6.3x10^{3}$	8	$9x10^{2}$
Pseudomonas aeruginosa	11	$3.4x10^{4}$	7	$3.2x10^{2}$	10	$2.1x10^{2}$
Bacillus cereus	10	$2.3x10^{2}$	4	$7.3x10^{1}$	8	$5.1x10^{1}$
Escherechia. Coli	30	$7.6x10^{6}$	30	$2.8.x10^{3}$	30	$1.2x10^{2}$

Fungi recovered from raw sewage:

Sixty five species belonging to twenty three genera were isolated from 30 samples of raw sewage on Sabouraoud's dextrose agar without (23 genera and 60 species) or with (21 genera and 39 species) cycloheximide at 28 ºC (Table 1). The total number of fungal propagules encountered on both media were 3309.3 and 1404.44 colony per ml.

The most prevalent genera, species on those media were *Aspergillus* (15), *Penicillium* (9), *Fusarium* (5), *Cladosporium*, (4) *Alternaria*(4), *Cochliobolus* (3) *Trichoderma*(3), *Scopulariopsis* (2) *Mucor* (2),*Geotrichium* (1) and *Gibberella* (1). They recovered from 66.6 -100% of the samples, constituting 2.17-28.75 % of total fungi respectively. Of the above genera the most frequently encountered species were: *Aspergillus flavus, A.fumigatus, A.niger, Acremonium stictum, Aspergillus terreus, A. versicolor, Cladosporium cladosporioides, C. herbarum, C. oxysporum, Gibberella fujikuroi, Cohliobolulus hawaiiensis, Fusarium solani, F. oxysporum, Penicillium chrysogenum, Geotrichum candidum* and *Scopulariopsis brevecaulis.* The above fungi were recovered previously, but with different numbers and frequencies, from sewage and sludge or soil receiving City sewage effluent (Abdel-Hafez and EL-Sharouny, 1987; Abdel-Mallek *et al.*, 1988; Gray, 1982; Niebl, *et al.*, 1982 and Ismail and Abel- Sater 1994).

On Sabouraoud's dextrose agar with cycloheximide, thirty nine species belonging to sixteen genera were isolated from 30 samples of raw sewage (Table 1).

The total number of fungal propagules encountered on this media was lower compared to those encountered on Sabouraoud's dextrose agar without cycloheximide (Table 2). The prevalent genera on this media were *Aspergillus* (9 species), *Penicillium* (6), *Cladosporium*, (3), *Fusarium* (2), *Alternaria* (2), C*ochliobolus* (2) *Chrysosporium* (3), *Gibberella* (1) and *Geotrichum* (1). They were isolated from 50-100 of the tested samples. The most common species were: *Aspergillus flavus, A. fumigatus, A. niger, Gibberella fujikuroi, Geotrichum* and *Cohliobolules hawaiiensis* were recovered in high frequency of occurrence and constituted 4.60, 3.36, 3.34, 3.35, 3.18 and 2.63 % of total fungi respectively. *A, vercicolor, A. ochraceus, Alternaria alternata, A.phragmospora, Acremonium strictum, Cladosporium cladosporioides, C. oxysporum, Fusarium solani, F. pallidoroseum, F. semitectum, Penicillium chrysogenum, P. funiculosum, P. spinolosum, Cochliobolus lunatus, Trichoderma hamatum, T. longibarchiatum, Scopulariopsis brevecaulis* and *Scopulariopsis brumptii* were recovered from

26.6-43.3% of the samples and constituted 1.43-2.93% of total fungi respectively.

***Mucor, Rhizopus* and *Aspergillus tamarrii* were not rcovered on this medium, but they encountered on On Sabouraoud's dextrose agar without cycloheximide in different frequencies. The remaining genera and species were isolated in low or rare frequency of occurrence.**

Table (2): Total counts (TC), number of isolated cases of (NCI), occurrence remarks (OR) and percentage of frequencies (F%) of fungal genera and species recorded from raw sewage on Sabouraud's agar, with and without cycloheximide at 28 °C.

Genera & Species	Sabouraud's agar Without cycloheximide			Sabouraud's agar with cycloheximide		
	TC	NCI&OR	F%	TC	NCI& OR	F%
Absidia corymbifera (Cohn) Sacc.& Trotter	62.7	9M	30	0	0	0
Acremonium Strictum W.Games	91.4	14M	46.6	52.1	10M	33.3
Alternaria	201.2	30H	100	61.1	11M	36.6
*A. alternata (*Fries) keissler	57.7	15H	50	32.3	8M	26.6
A. chlamydospora Mouchacca	37.7	6L	20	0	0	0
A. Phragmospora Van Emden	61.4	13M	43.3	0	0	0
A. tenuissima (Kunze) Wiltshire	44.4	9M	30	28.8	6L	20
Aspergillus	927.57	30H	100	413.6	30H	100
A. aureolatus Munt., Cvet. & Bata	37.9	6L	20	0	0	0
A. clavatus Desm.	28.3	8M	26.6	0	0	0
A. flavus Link	145.1	27H	90	96.4	19H	63.3
A. fumigatus Freserius	129.6	25H	83.3	70.4	15H	50
A. glaucus Link	37.7	6L	20	0	0	0
A. melleus Yukawa	57.0	12M	40	0	0	0
A. niger Van Tieghem	124.8	20H	66.6	69.9	16H	53.3
A. ochraceus Welhelm	62.7	9M	30	58.6	8M	26.6
A. sydowii (Bin. & Sart.) Thom& Church	36.1	7L	23.3	31.1	6L	20
A. tamarii Kita	37.7	6L	20	0	0	0
A. carncus (v.Tiegh) Blochwis	32.7	7L	23.3	0	0	0
A. resttrictus Smith	45.5	8M	26.6	13.3	2R	6.6
A. terreus Thom	74.3	16H	53.3	56.6	10M	33.3
A. ustus (Bain.) Thom & Church	61.4	10M	33.3	17.3	4L	13.3
A. versicolor (Vuill.) Tiraboschi	72.2	15H	50	56.6	11M	36.6
Blastomyces dermatitides Gilchrist et Stokes	57.7	9M	30	36.6	7L	23.3
Chrysosporium	35.2	6L	46.6	86.5	16H	56.6
C. tropicum Carmichael	35.2	6L	46.6	36.6	7L	23.3
C. indicum (Randhawa & Sandhu) Gary	0	0	0	31.1	6L	20
C. parvum (Emmonsia & Ashburn) Carmichael	0	0	0	28.3	4L	26.6
Cladosporium	239.6	29H	96.6	144.6	22H	73.3
C. cladosporioides (Fries)de vries	74.3	17H	56	56.6	13M	43.3
C. herbarum (Pers.) Link ex Gray	72.2	15H	50	13.3	3R	10

C. axysporium Ber. & Curt.	70.4	16H	53.3	61.4	13M	43.3
C. sphaerospermum Penzig	13.3	3R	10	0	0	0
Cylindrocarpon Congoense. Meyer	31.3	7L	23.3	35.5	12M	40
Cochliobolus	155.9	27H	90	66.3	18H	60
C. hawaiiensis Alcorn	68.4	15H	50	0	0	0
C. lunatus Nelson & Haasis	56.0	10M	33.3	43.3	11M	36.6
C. spicifer Nelson	31.5	9M	30.0	23.0	12M	40
Cunninghmella elagans Lendner	56.6	11M	36.6	0	0	0
Fusarium	292.5	30H	100	62.9	15H	50
F. solani (Mart) Sacc	64.0	20H	66.6	36.3	11M	36.6
F. oxysporum Schlecht	64.0	15H	50	26.6	5L	16.6
F. pallidoroseum (Cooke) Sacc.	52.1	12M	40	0	0	0
F. semitectum Berk & Rav.	59.1	14M	46.6	0	0	0
F. dimerum Penzig	53.3	13M	43.3	0	0	0

Table (2) : Continued

Genera &Species	Raw sewage					
	Sabouraud's agar Without cycloheximide			Sabouraud's agar with cycloheximide		
	TC	NCI&OR	F%	TC	NCI&OR	F%
Gibberella fujikuroi (Sawada) Ito	79.8	22H	73.3	70.2	16H	53.3
Geotrichum candidum Link	69.9	16H	53.3	66.6	15H	50
Graphium sp.	51.5	9M	30	0	0	0
Histoplasma capculatum Darling	0	0	0	16.6	4L	13.3
Microsporum	36.6	8M	26.6	63.2	11M	36.6
M. canis Bodin	0	0	0	13.3	3R	10
M. gypseum (Bodin) Guiart et Grigorakis	36.6	8M	26.6	33.3	7L	23.3
M. manginii (Loubiere) Curzi	0	0	0	16.6	4L	13.3
Mucor	107.6	20H	66.6	0	0	0
M. circinelloides Van Tieghem	64.0	13M	43.3	0	0	0
M. rasemosus Fresenius	43.6	8M	26.6	0	0	0
Penicillium	415.3	30H	100	83.44	20H	66.6
P. chrysogenum Thom	69.5	23H	76.6	53.3	12M	40
P. raistrickii G. Smith	47.7	9M	30.0	0	0	0
P. brevicompactum Dierckx	53.3	13M	43.3	33.3	6L	20
P. citrinum Thom	32.3	7L	23.3	24.0	4L	13.3
P. funiculosum Thom	64.0	14M	46.6	36.3	11M	36.6
P. verruculosum Peyronel	20.0	4L	13.3	13.3	2R	6.6
P. expansum Link	20.0	6L	20	0	0	0
P. spinolosum Thom	59.1	10M	33.3	0	0	0
P. rubrum Stoll	36.1	8M	26.6	30	4L	13.3
Pestalotia pezizoides de Notaris	36.1	8M	26.6	0	0	0
Rhizopus stolonifer(Ehrenb)Lindt	37.7	5L	16.6	0	0	0
Scopulariopsis	112.1	21H	83.3	56.1	14M	46.4
S. bervicaulis (Sacc.) Bain.	71.1	15H	50	56.1	14M	46.6
S. brumptii Salvanet-Duval	41.0	7L	23.3	0	0	0
Sordaria fumicola (Roberge) Cesati & de Notaris	51.1	9M	30.0	0	0	0
Sterile mycelia (white, yellow, dark)	32.3	7L	23.3	25.3	5L	16.6
Trichoderma	151.0	22H	73.3	54.8	9M	30
T. hamatum (Bon.) Bain.	47.5	12M	40.0	0	0	0
T. viride Persoon	35.1	8M	26.6	0	0	0
*T.*longibarchiatumi Rifai	68.4	14M	46.6	54.8	9M	30
Yeasts	126.3	30H	100	83.3	18H	60
Gross total count	3309.3			1404.44		
Number of genera = 23	23			21		
Number of species =65	60			39		

Occurrence remarks (OR), H= high occurrence, from 15-30 cases; M= moderate occurrence, from 8-14 cases; L= low occurrence, from 4-7 cases; R= rare occurrence, from1-3 cases (out of 30 cases).

Fungi recovered from secondary effluent :

Twenty five species belonging to twelve genera were isolated from 30 samples of secondary effluent on Sabouraoud's dextrose agar with /or without cycloheximide and at 28 ºC (Table 3).

The total numbers of fungal propagules encountered in all samples on both media were 418.6 and 773.3 colony per ml. The prevalent genera on Sabouraoud's dextrose agar without cycloheximide were *Aspergillus* (10 species), *Fusarium* (3), *Cladosporium* (2) and *Penicillium* (3) and they were isolated from 100, 66.6, 56.6 and 50% of the samples, constituting 37.5, 12.19, 11.04 and 10.7% of total fungi respectively. The most common species were *Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Cladosporium cladosporioides, Gibberella fujikuroi, Fusarium solani, F. oxysporum, Penicillium chrysogenum*, and *Scopulariopsis brevecaulis.* They were recovered from 40-26.6 % of the samples and constituted 6.89- 26.6 % of total fungi. These species were previously recovered but with different incidences from soil receiving city sewage effluent in Egypt (Abdel-Hafez and EL-Sharouny, 1987). The remaining species were collected in low and rare frequency of occurrence. *Chrysosporium* was not encountered on this media.

The most dominant fungus on Sabouraoud's dextrose agar containing cycloheximide was *Aspergillus* (10 species). It was isolated from 100% of the tested samples, constituted 38.17 of total fungi. The most dominant species was *A. flavus*. It was encountered in moderate frequency of occurrence. The remaining species were isolated in low and rare frequency of occurrence. *Cladosporium* (2 species), *Penicillium* (3), *Fusarium* (3) and *Gibbbrella* (1). They were isolated in moderate frequency of occurrence and encountered from 43.3, 43.3, 40, and 26.6 % of the total samples, representing 14.2, 13.6, 13.9 and 7.16 of the total fungi respectively.

The remaining genera and species were collected in low or rare frequency of occurrence.

Fungi recovered from dewatered sludge (manure):

Sixty species belonging to 25 genera were isolated from 30 samples dewatered sludge (manure) of secondary effluent on Sabouraoud's dextrose agar with and without cycloheximide at 28 ºC (Table 4).

The prevalent genera on cycloheximide free medium were *Aspergillus* (11 species), *Penicillium* (7), *Fusarium* (4), *Alternaria* (3), *Cladosporium* (3), *Trichoderma*(3), *Cochliobolus* (3), *Scopulariopsis* (3) *Mucor* (2) and *Gibberella* (1). They were isolated in high frequency of occurrence, constituting 3.03-28.87 % of total fungi respectively. The most common species were: *Aspergillus flavus, A.fumigatus, A.niger, Aspergillus Terreus, Gibberella fujikuroi, Fusarium solani and Penicillium funiculosum.* They were recovered from 50-60% of the samples. On the other hand *Aspergillus ochraceus, A.versicolor, C. cladosporioides, C. oxysporum, Alternaria alternata, A. tenuissima, Chaetamium globosum, Cohliobolus hawaiiencsis C.lunatus, C. spicifer, Geotrichum candidum, Fusarium oxysporum, Mucor circinoeloides, M. racemosus, Penicillium chrysogenum, Penicillium brevicompactum P. citrinum, Rhizopus oryzae, Trichoderma viride*, and *Scopulariopsis brevecaulis* were collected in

moderate frequency of occurrence. They were encountered from 26.6- 46.6% of the total tested samples, constituting 1.42-3.04 % of the total count of fungi. The remaining species were collected in low or rare frequency of occurrence.

The prevalent genera recovered on Sabouraoud's dextrose agar with cycloheximide were: *Aspergillus* (8 species), *Penicillium* (4), *Cladosporium*, (2), *Fusarium* (2), *Alternaria*(2) and *Cochliobolus* (2). They were isolated from 50-100 of the tested samples accounting to 30.6-6.99. % of total fungi respectively. The most common species was: *Aspergillus flavus.* It was the only species recovered in high frequency of occurrence, constituting 5.26 % of the total fungi.

***Aspergillus fumigatus, A. niger, A. terreus, A. ochraceus, Alternaria alternata, Cladospoium cladosporioides, Fusarium solani, Penicillium chrysogenum, P. funiculosum, Cochliobolus lunatus, Scopulariopsis brevecaulis* and *Gibberella Fujikuroi* were recovered in moderate frequency of occurrence (Table 4).**

Table (3) : Total counts (TC), number of cases of isolation (NCI), occurrence remarks (OR) and percentage of frequencies (F%) of fungal genera and species recorded from secondary effluent on Sabouraud's agar, with and without cyclohexamide at 28 ^{o}C.

Genera & Species	Sabouraud's agar without cyclohexamide			Sabouraud's agar with cyclohexamide		
	TC	NCI&OR	F%	TC	NCI&OR	F%
Alternaria	42.6	6L	20			
*A. alternata (*Fries) keissler	29.3	5L	16.6	13.3	3R	10
A. tenuissima (Kunze) Wiltshire	13.3	2R	6.6	13.3	1R	3.3
Aspergillus	290.1	30H	100	146.5	21H	50
A. flavus Link	53.3	12M	40	31.6	8M	26.6
A. fumigatus Freserius	50.0	12M	40	30.4	7L	23.3
A. niger Van Tieghem	36.0	10M	33.3	25.0	7L	23.3
A. ochraceus Welhelm	34.2	7L	23.3	17.7	3R	10
A. tamarii Kita	20.0	2R	6.6	0	0	0
A. terreus Thom	43.6	11M	36.6	28.5	7L	23.3
*A. ustus (*Bain.) Thom & Church	23.3	4L	13.3	13.3	2R	6.6
A. versicolor(Vuill.) Tiraboschi	30.0	4L	13.3	0	0	0
Chrysosporium. Tropicum Carmichael	0	0	0	22.2	3R	10
Cladosporium	85.4	17H	56.6	59.5	8M	26.6
C. cladosporioides (Fries)de vries	48.8	12M	40	26.6	5L	16.6
C. herbarum (Pers.) Link ex Gray	34.6	5L	16.6	22.2	3R	10
Fusarium	94.3	20H	66.6	45.2	12M	40
F. dimerum Penzig	22.2	3R	10	0	0	0
F. oxysporum Schlecht	26.6	8M	26.6	18.6	5L	16.6
F. solani (Mart) Sacc.	45.5	12M	40	26.6	9M	30
Gibberella fujikuroi (Sawada) Ito	43.0	13M	43.3	30.0	8M	26.6
Geotrichum candidum Link	24.7	7L	23.3	21.3	5L	16.6
Penicillium	82.8	15H	50	57.0	13M	43.3
P. chrysogenum Thom	30.0	8M	26.6	20.9	7L	23.3
P. citrinum Thom	18.6	5L	16.6	13.3	3R	10
P. funiculosum Thom	34.2	7L	23.3	22.8	7L	23.3
Rhizopus stolinefer (Ehrenb) Lindt	26.6	6L	20	0	0	0

Scopulariopsis	**34.3**	**9M**	**30**	**16.0**	**5L**	**16.6**
***S. bervicaulis* (Sacc.) Bain.**	**18.3**	**8M**	**26.6**	**16.0**	**5L**	**16.6**
***S. brumptii* Salvanet-Duval**	**16.0**	**5L**	**16.6**	**0**	**0**	**0**
***Trichoderma viride* Persoon**	**13.3**	**7L**	**23.3**	**0**	**0**	**0**
Yeasts	**36.2**	**14M**	**46.6**	**24.0**	**10M**	**33.3**
Gross total count	**733.3**			**436.8**		
Number of genera = 12	**11**			**10**		
Number of species = 25	**24**			**23**		

Occurrence remarks (OR), H= high occurrence, from 15-30 cases; M= moderate occurrence, from 8-14 cases; L= low occurrence, from 4-7 cases; R= rare occurrence, from1-3 cases (out of 30 cases).

Table (4) : Total counts (TC), number of cases of isolation (NCI), occurrence remarks (OR) and percentage of frequencies (F%) of fungal genera and species recorded from dewatered sewage (manure) on Sabouraud's agar, with and without cyclohexamide at 28 ^{o}C.

Genera &Species	Sabouraoud's agar Without cyclohexamide			Sabouraoud agar with cyclohexamide		
	TC	NCI&OR	F%	TC	NCI&OR	F%
Alternaria	**151.4**	**20H**	**66.6**	**72**	**18H**	**60.0**
***A. alternata* (Fries) keissler**	**56.0**	**10M**	**33.3**	**41.6**	**8M**	**26.6**
***A. chlamydospora* Mouchacca**	**49.5**	**7L**	**23.3**	**0**	**0**	**0**
***A. tenuissima* (Kunze) Wiltshire**	**45.9**	**9M**	**30**	**30.4**	**7L**	**23.3**
Aspergillus	**530.4**	**30H**	**100**	**315.7**	**30H**	**100**
***A. flavus* Link**	**85.1**	**18H**	**60**	**54.2**	**15H**	**50**
***A. fumigatus* Freserius**	**80.7**	**18H**	**60**	**49.5**	**14M**	**46.6**
***A. glaucus* Stoll**	**20.0**	**4L**	**13.3**	**0**	**0**	**0**
***A. niger* Van Tieghem**	**74.1**	**16H**	**53.3**	**51.1**	**12M**	**40**
***A. ochraceus* Welhelm**	**55.3**	**13M**	**43.3**	**38.0**	**7L**	**23.3**
***A .prasiticus* Speare**	**23.3**	**4L**	**13.3**	**13.3**	**2R**	**6.6**
***A. sydowii* (Bin. & Sart.) Thom & Church**	**29.3**	**5L**	**16.6**	**26.6**	**4L**	**13.3**
***A. tamari* Kita**	**24.4**	**6L**	**20**	**0**	**0**	**0**
***A. terreus* Thom**	**69.3**	**15H**	**50**	**45.3**	**10M**	**33.3**
***A. ustus* (Bain.) Thom & Church**	**32.3**	**7L**	**23.3**	**0**	**0**	**0**
***A.versicolor*(Vuill.)Tirabosci**	**36.6**	**8M**	**26.6**	**37.7**	**6L**	**20**
***Chaetomium globosum* Kunze**	**35.0**	**8M**	**26.6**	**33.3**	**6L**	**20**
Chrysosporium	**13.3**	**3R**	**10**	**37.3**	**6L**	**20**
***C. tropicum* Carmichael**	**13.3**	**3R**	**10**	**24.0**	**5L**	**16.6**
***C. parvum* (Emmonsia & Ashburn) Carmichael**	**0**	**0**	**0**	**13.3**	**1R**	**3.3**
Cladosporium	**133.4**	**26H**	**86.6**	**80.6**	**18H**	**60**
***C. cladosporioides* (Fries)de vries**	**50.7**	**14M**	**46.6**	**38.7**	**11M**	**36.6**
C. herbarum* (Pers.) Link ex *Gray	**36.1**	**7L**	**23.3**	**0**	**0**	**0**
***C. oxysporium* Ber. & Curt.**	**46.6**	**10M**	**33.3**	**41.9**	**7L**	**23.3**
Cochliobolus	**101.1**	**27H**	**90**	**66.0**	**15H**	**50**
***C. hawaiiensis* Alcorn**	**32.5**	**9M**	**30**	**0**	**0**	**0**
***C. lunatus* Nelson & Haasis**	**36.6**	**12M**	**40**	**41.3**	**10M**	**33.3**
***C. spicifer* Nelson**	**32.0**	**10M**	**33.3**	**24.7**	**7L**	**23.3**
***Doratomyces stimonitis* Smith**	**32.5**	**7L**	**23.3**	**0**	**0**	**0**
Fusarium	**181.7**	**19H**	**63.3**	**73.7**	**18H**	**60**
***F. dimerum* Penzig**	**31.1**	**3R**	**10**	**0**	**0**	**0**
***F. oxysporum* Schlecht**	**46.6**	**10M**	**33.3**	**30.4**	**7L**	**23.3**
***F. semitectum* Berk. & Rav.**	**28.8**	**6L**	**20**	**0**	**0**	**0**
***F. solani* (Mart) Sacc.**	**58.6**	**15H**	**50**	**43.3**	**12M**	**40**
***Gibberella fujikuroi* (Sawada) Ito**	**55.8**	**16H**	**53.3**	**43.8**	**13M**	**43.3**
***Geotrichum candidum* Link**	**43.3**	**8M**	**26.6**	**13.3**	**7L**	**23.3**
Graphium sp	**57.7**	**13M**	**43.3**	**0**	**0**	**0**
***Histoplasma capculatum* Darling**	**0**	**0**	**0**	**13.3**	**1R**	**3.3**
***Macrophomina phaseolina* (Tassi) Goidanich**	**24.7**	**7L**	**23.3**	**0**	**0**	**0**
Microsporum	**16.0**	**5L**	**16.6**	**28.8**	**7L**	**23.3**

M. cansi Bodin	0	0	0	13.3	3R	10
M. gypseum Bodin	16.0	5L	16.6	15.5	6L	20
Mucor	66.9	16H	53.3	0	0	0
M. cercinoloides Van Tieghem	36.9	14M	46.6	0	0	0
M. rasemosus Fresenius	30.0	8M	26.6	0	0	0
Penicillium	264.6	30H	100	121	22H	66.6
P. chrysogenum Thom	42.0	13M	43.3	32.5	8M	20
P. roistrickii G. Smith	22.2	3R	10	0	0	0
P albidum Sopp	26.6	4L	13.3	0	0	0
P. brevicompactum Dierckx	36.0	10M	33.3	0	0	0
P. citrinum Thom	34.6	10M	33.3	34.2	7L	13.3
P. funiculosum Thom	54.6	15H	50	41.0	13M	23.3
P. rugulosum Thom	32.0	5L	16.6	0	0	0
P. rubrum Stoll	16.6	4L	13.3	13.3	3R	10

Table (4) : Continued

Genera &Species		Sabouraoud's agar			Sabouraoud agar with cyclohexamide		
		TC	NCI&OR	F%	TC	NCI&OR	F%
Phialophora repens (Davidson) Conant	13.3	1R	3.3	13.3	1R	3.3	
Rhizopus	46.4	12M	40	0	0	0	
R. nigricans (Ehrenb) Lindt	29.3	10M	33.3	0	0	0	
R. oryzae Went & Prinsen	17.1	7L	23.3	0	0	0	
Scopulariopsis	95.7	18H	60	32.0	10M	33.3	
S. bervicaulis (Sacc.) Bain.	35.8	13M	43.3	32.0	10M	33.3	
S. brumptii Salvanet – Duval	28.8	6L	20	0	0	0	
S. candida (Gueguen) Vuillemin	31.1	6L	20	0	0	0	
Sordaria fumicola (Roberge) Cesati & de Notaris	20.9	5L	16.6	0	0	0	
Setosphaeria rostrata Leonard	29.3	7L	23.3	0	0	0	
Trichoderma	104.3	16H	53.3	22.2	3R	10	
T. hamatum (Bon.) Bain.	33.3	6L	20	0	0	0	
T. viride Persoon	47.7	12M	40	0	0	0	
T.longibarchiatum Rifai	23.3	4L	13.3	22.2	3R	10	
Trichothicium roseum (Pers.) Link ex gray	34.2	7L	23.3	26.6	4L	13.3	
Verticillium spp..	28.5	7L	23.3	0	0	0	
Yeasts	54.1	16H	53.3	50.4	14M	46.6	
Gross total count		2110.92			988.1		
Number of genera = 25		24			16		
Number of species =60		55			31		

Occurrence remarks (OR), H= high occurrence, from 15-30 cases; M= moderate occurrence, from 8-14 cases; L= low occurrence, from 4-7 cases; R= rare occurrence, from1-3 cases (out of 30 cases).

The remaining genera and species were isolated in low or rare frequency of occurrence.

Fungi recovered from the present study have been found in large numbers in sewage (Gray, 1982; Niebl, *et al.*, 1982; and Ismail and Abel- Sater 1994; Diener., *et al.*, 1976; Elland 1981; Larry and Wanger, 1982; Abdel-Hafez and EL-Sharouny, 1987; and Abdel-Mallek *et al.*, 1988).

Also numerous fungi recovered in our study are well known as mycotoxin producing fungi (Tseng, *et al.*, 1995; Aleksandrowies, and Smyk, 1973; Enomoto and Saito, 1972; Pitt, 1994; Rippon, 1982; Sutic, *et al.*, 1979 and Scudamore 1993).

A certain number of the fungus species recovered in this study are pathogenic (Austwick, 1983; Wadhwani and Srivastava 1985 and Pitt 1994).They are potential facultative causative agents of different mycotic infection (Velzer and Diaz, 1985; Bunse and

Merk, 1992, Sutton, *et al.*, 1998, Hoog, *et al.*, 2000).

CONCLUSION:

The workers in the sewage treatment plant most have a health risk during the treatment processes. Health risk are associated with the pathogens, which may spread through being directly, or indirectly ingested into the human body. Pathogens and toxic compounds may be disseminated through Sludge and Sewage, as well as through aerosols (Hickey and Reist, 1975; Bausum *et al.*, 1978 and Bausum *et al.*, 1982). The windy weather raises the question of potential human health hazard passed by pathogen-containing aerosols, in the sewage treatment plant and human communities in the surrounding areas.

The same problem regarding the health of agricultural workers occurs when spray irrigation of sewage effluent is used. Aerosol droplets containing pathogens have been reported to travel up to 1-2 Km (Adams and Spendlove, 1970). Pathogens are more effective when inhaled than when ingested (Melnick, *et al.*, 1978).

Two sewage workers in the Ibb sewage treatment plant suffer from an allergic skin disease (Al-Zubeiry and Al-Shargaby, 1997). But in general sewage workers suffer from an increased incidence of infection or other diseases (Pahren, and Jakubowski,1980; and SWaWWA, 1978). It is important for these workers to have suitable protective clothing, shoes and gloves. Ventilation should be satisfactory, and treatment processed should be automated to the fullest extent possible.

Perhaps the most important single factor is to make sure that sewage workers know how to avoid infection and that they are aware of and use protective measures in their daily work. However, the one of the most important questions, it is the position of sewage treatment plant. Sewage treatment plant must be far away from cities and human communities and must be built in the suitable place from a public health point of view.

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الأحياء المجهرية التي تعيش في مياه المجاري والمخلفات الثانوية السائلة والوحل الجاف في محطة التنقية في إب- الجمهورية اليمنية

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تم عزل الفطريات والبكتيريا من عينات مياه المجاري الخام والمخرجات الثانوية والمواد الصلبة (الوحل الجاف) بعد التجفيف المستخدمة في هذه الدراسة من بيئات غذائية مختلفة عند درجة تحضين ٣٧°م للبكتيريا الممرضة و٢٨° م. وقد تم عزل عدد من البكتيريا الممرضة والمحتملة، وكذلك عدد من الفطريات الممرضة أو التي يحتمل أن تسبب أمراض في ظروف خاصة.

وقد تم عزل البكتيريا *faecal Streptococcus, Streptococcus pneumonia, Staphylococcus aureus,* , *Pseudomonas aeruginosa, Bacillus cereus, Escherechia Coli* ، كما تم عزل *Salmonella spp.* من مياه المجاري ومن المخلفات السائلة. كما أن *Shigella spp* قد عزلت من مياه المجاري فقط. عدد من البكتيريا المعزولة مسببة للأمراض ومكونة للسموم وتشكل خطورة على حياة الحيوان والإنسان، كما تم عزل عدد كبير من الفطريات من مياه الجاري ومخلفاتها السائلة والصلبة على بيئة السبرود بدون السيكلوهيسامايد عند درجة حرارة تحضين ٢٨°م منها:

Aspergillus flavus, A. fumigatus, A.niger, Acremonium stictum, terreus, A. versicolor, Cladosporium cladosporoides, C. herbarum, C. oxysporum, Gibberella fujikuroi, Cohliobolules hawaiiensis, Fusarium solani, F. oxysporum, Penicillium chrysogenum, Geotrichium candidum* and *Scopulariopsis brevecaulis.

وعلى بيئة السبرود مع السيكوهيكسامايد عزلت الأنواع الفطرية الآتية :

Aspergillus flavus, A. fumigatus, A. niger, Gibberella fujikuroi* and *Geotrichium candidum,

وقد عزلت فطريات ممرضة بأعداد مختلفة على هذه البيئة، وهي:

***Chrysosporium tropicium C. indicum, C. parvum, Geotrichum candidum, Histoplasma capculatum Microsporum cansi, M. gypseum* and *M. manginii*.**