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A STUDY ON INHABITANT MICROORGANISMS ISOLATED FROM WASTEWATER IRRIGATED SOILS OF KARACHI, PAKISTAN

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Abstract

Increasing population and urbanization results in greater production of wastewaters through industrial, commercial, domestic or agricultural activities. Discharges of these resources contain several elements including nutrients and heavy metals. Therefore, utilization of wastewater for crop irrigation alters the soil property of agricultural land by accumulating nutrients and heavy metals. Present study revealed the occurrence of elements and the existence of microorganisms in wastewater irrigated soils of two different locations adjacent to the Malir river and Lyari river. MWIS (Malir wastewater irrigated soil) and LWIS (Lyari wastewater irrigated soil) contained a greater amount of organic matter (3.13 ± 0.10 %, 3.10 ± 0.10 %) as compared to NWIS (normal water irrigated soil). Physicochemical measures such as EC (1774 ± 5.49 µS/cm), salinity (0.89 ± 0.02 %) and TDS (887 ± 5.19 ppm) and heavy metals like iron (29.02 ± 1.72 ppm), copper (0.36 ± 0.02ppm), lead (0.82 ± 0.02 ppm) and nickel (0.94 ± 0.01 ppm) were recorded higher in LWIS as compared to MWIS and NWIS. However, EC (425 ± 1.45 µS/cm) and TDS (213 ± 1.73 ppm) were found lowest in MWIS than LWIS in contrast to NWIS. Nutrients like nitrogen, potassium and heavy metal such as cadmium were approximately equal in all tested soil samples. Numerous fungal species have been sequestered from wastewater irrigated soils in which thirteen genera and twenty-seven species of fungi were isolated. Out of all fungal species, *Aspergillus flavus*, *Curvularia intermedia*, *Fusarium solani* and *Rhizopus* sp. were found dominant (6.67×10^3 cfu/g soil) in MWIS, while LWIS comprised the highest cfu (1×10^4 cfu/g soil) of *Trichoderma viride* and *A. parasiticus*. In addition, bacterial species like *Ralstonia pickettii* was found superior in MWIS with 3.24×10^7cfu/g soil. Whereas, five different strains of *Acinetobacter lwoffii / jansani* were isolated from LWIS along with *Pseudomonas aeruginosa* (4×10^5 cfu/g soil) and *Shigella dysenteriae* (1×105 cfu/g soil). The current study revealed the existence of microorganisms in different qualities of soils having complex nature that were irrigated by wastewater. It was evaluated that sequestered fungal and bacterial species were able to tolerate the complex nature of wastewater irrigated soils that have an adequate environment for the survival of fungi and bacteria.

Key words: Wastewater irrigated soils, Physicochemical measures, Heavy metals, Nutrients, Fungi, Bacteria.

Introduction

Large scale expansion in urbanization and industrialization has resulted in the production of higher quantities of wastewater. This is an important source of essential nutrients for enhancing the soil quality and productivity level (Weber *et al*., 1996). Domestic water contains a high amount of organic matter with macro and micro-nutrients (Shah *et al*., 2022; Brar *et al*., 2000). Its continuous irrigation can improve the quality level of soil (Narwal *et al*., 1993; Brar *et al*., 2000). But long-term use of wastewater for agriculture purposes directly affects the physicochemical properties of soils, however, it also causes phytotoxicity that leads to the destruction of the economy (Minhas and Gupta, 1992). Because continuous irrigation with wastewater results in retaining excessive amounts of nutrients and toxic metals in the soil that negatively affect the nature of soil and crop productivity (Vazquezmontiel *et al*., 1996). According to various researchers, irrigation with wastewater increases the level of essential nutrients i.e., nitrogen (N), phosphorus (P) and potassium (K) in the soil but on the other hand, heavy metals tend to increase with continuous irrigation.

Wastewater irrigation, applications of sludge and industrial wastes are the chief sources to contaminate the soil by adding toxic heavy metals. These heavy metals become detrimental for the agriculture field (Chen *et al*., 2004; Singh *et al*., 2004). Wastewater irrigation without proper management destroys the ecosystem by creating various environmental hazards (Mohammad & Ayadi, 2004). Consequently, the fertility of soil and crop nutrients should be maintained by proper management of wastewater irrigation (Mohammad & Mazahreh, 2003).

Microorganisms possess the intrinsic capability to survive in different types of habitats including polluted soil to absorb heavy metals as well as nutrients. Therefore, microbes play a vital role in cation exchange capability to make nutrients available for plants (Birch & Bachofen, 1990). Various microbial species have been well demonstrated for tolerating a variety of heavy metals (Hashem & Bahkali, 1994; Prasenjit & Sumathi, 2005). This tolerant quality of microorganisms towards adverse Physicochemicals and heavy metals properties of habitats substantiate them to utilize in reclaiming the contaminated soils (Gadd & Griffiths, 1977).

Microbes comprise significant tolerant characteristics of pollutants that make them dominant organisms in diversities of polluted habitats. Some strains of microorganisms produce extracellular/intracellular enzymes for resisting various metal concentrations. Likewise, certain species, comprise processes of valence transformation, active uptake, crystallization, complexation, and biosorption of heavy metals in the cell walls (Zafar *et al*., 2007). Similarly, some fungal species of *Aspergillus*, *Penicillium*, *Phanerochaete,* etc., and bacterial strains like *Bacillus*, *Pseudomonas*, *Sporophyticus*, etc. have been revealed for the elimination of heavy metals like chromium and nickel (Gopalan & Veeramani, 1994; Yan & Viraraghavan, 2003; Congeevaram *et al*., 2007). Correspondingly, various species of *Aspergillus* are found to be useful in tolerating adverse physicochemical measures and heavy metals (Congeevaram *et al*., 2007). In this case, *A. niger* is documented to significantly eliminate uranium ions, whereas, *A. oryzae* is documented for removing cadmium and copper ions from aqueous solutions (Kapoor *et al*., 1999). Likewise, various other researchers have also reported several fungal species e.g. *A. flavus*, *A. niger*, *A. versicolor*, *Curvularia* sp., *Fusarium* sp., *Helminthosporium* sp., *Humicola grisea* sp., *Nannizzia* sp. and *Scopulariopsis* sp. from heavy metals-contaminated peri-urban agricultural soils (Iram *et al*., 2012).

The main purpose of this study is to evaluate the quality of wastewater irrigated soils from different localities of Karachi and to reveal the survival ability of microorganisms in diverse nature of soils.

Material and Methods

In the present study, survey of the agricultural fields had been carried out which were irrigated with wastewaters of nearby rivers and located at Quaidabad (Malir river) and Gulshan Chowrangi (Lyari river). Various crops such as *Brassica oleraceavar. capitata* (cabbage), *Brassica oleracea* var. botrytis (cauliflower), *Lycopersicon esculentum* (tomato), *Capsicum frutescens* (chili), *C. annuum* (capsicum), *Luffa aegyptiaca* (sponge gourd), *Momordica charantia* (bitter gourd), *Lagenaria siceraria* (bottle gourd), *Spinacia oleracea* (spinach), *Abelmoschus esculentus* (okra), *Vigna unguiculata* (lobia) and *Rosa indica* (rose) plant were cultivated by the application of wastewater adjacent to Malir river. Whereas, wastewater of Lyari river was used for the plantation of some medicinal important grasses including *Alternanthera ficoidea* (Joseph's Coat), *Aerva javanica* (desert cotton/kapok bush), *Cyperus cyperoides* (flatsedge), *Eragrostis cilianensis* (candy grass) and *E. japonica* (Japanese love-grass) at the field nearby Lyari river.

Physicochemical and heavy metals analysis of soil samples: Soil samples from rhizospheres of wastewater irrigated plants were collected and analyzed with the comparison of normal water irrigated soil (NWIS). Physicochemical properties (electric conductivity, pH, total dissolved solids, salinity and oxidation-reduction potential) of soil samples were estimated by making filtrate of 20g oven-dried sieved soil with deionized water. The estimation was carried out by using Hanna Multi parameter meter (Model HI9828). The organic matter content of the soil samples was determined by Loss-on-Ignition method (Schulte & Hopkins, 1996). Whereas, alkalinity was measured by titration (Anon., 1998). Total nitrogen from 1g of each soil sample was determined by Kjeldahl method (Anon., 2000). Whereas, total phosphorus was estimated in 0.5g soil samples by the method of Olsen & Sommers (1982). Metals like cadmium, copper, iron, lead, zinc, nickel, arsenic and potassium were analyzed by Flame Atomic Absorption Spectroscopy (FAAS) PE-AAnalyst 700. Tested soil samples (10g) were digested in HNO_3 on hot plate. The solution was filtered, and the volume was raised to 50mL. Using appropriate drift blanks the amount of metals were determined and external calibration was used for quantitative analysis of metals (García & Báez, 2012).

Isolation of fungi and bacteria from soil samples: Microorganisms (Fungi and Bacteria) were isolated from the collected rhizosphere soil samples of the growing region of plants. Fungi were isolated by serial dilution and baiting techniques using potato dextrose agar (PDA) medium. Sample-poured PDA plates were incubated at 28°C for 6 days. Isolated fungal colonies were identified with the help of standard references (Wilhelm, 1955; Kenneth B. Raper, 1965; Barnet, 1969; Papavizas *et al*., 1975; Ellis, 1977; Windham & Lucas, 1987). Whereas, isolation of bacteria was carried out by serial dilution technique (1/100,000 dilution of sample) using King's B medium. Tested samples added on media plates were kept in incubator at 37°C for 24 hours and aimed for the growth of bacterial colonies. Isolated bacteria were identified by using bio-chemical analysis kits (QTS-24, DESTO).

Results

Soil is a chief source of nutrients required for plant growth. In the present study, almost equal amount of N (%) was present in all tested soil samples, although slightly high amount was found in MWIS (4.31 % ± 0.01). Similarly, the highest rate of P was detected in MWIS (2.99 ± 0.00 mg/g) whereas the lowest rate found in NWIS (0.72 ± 0.00 mg/g) and LWIS (0.30 ± 0.00 mg/g). In the case of K, almost the same amount was recorded in all soil samples. The highest rate of Mg was detected in NWIS (291.2 ± 1.73 ppm) while lower amount was recorded in LWIS (5.033 ± 0.01 ppm) and MWIS (4.972 ± 0.01 ppm). However, a higher amount of calcium was observed in MWIS (95.18 ± 1.77 mg/g) than NWIS (90.22 ppm) and LWIS (90.34 ± 0.07 ppm).

Higher measures of Physicochemical parameters like electric conductivity, salinity percentage and total dissolved solids have been obtained in LWIS as compare to the MWIS and NWIS (Table 1). However, MWIS and NWIS were found neutral in pH level, whereas LWIS was found slightly acidic (6.28 ± 0.058). A greater rate of dissolved oxygen was found in MWIS (1.05 ± 0.02 ppm) and LWIS (0.74 ± 0.01 ppm), although ORP was recorded higher in NWIS (47.76 ± 0.72 mV). The alkalinity of both wastewater irrigated soils was comparatively less (133 ppm) than the NWIS (141 ± 8.33 mg/L). Organic matter was found 3.13 ± 0.10 % and 3.10 ± 0.10 % in Malir and Lyari soils which were irrigated with wastewater. These amounts of organic matter were higher than NWIS (2.17 ±

0.03%) which was irrigated with tap water. Higher rate of iron was found in both wastewater irrigated soil as compared to the NWIS (0.29 ± 0.07 ppm). Correspondingly, the concentration of Pb was found highest in LWIS (0.82 ± 0.02 ppm) and MWIS (0.73 ± 0.01 ppm) but the lowest amount was observed in NWIS (0.32 ± 0.01 ppm). In the case of Cu, the higher rate was recorded in both wastewater irrigated soil with a slight difference as shown in table 1 and the minimum amount was found in NWIS that is 0.06 ± 0.01 ppm. The same amount of zinc was recorded in both wastewater irrigated soil (2.82 ± 0.02 ppm, 2.82 ± 0.04 ppm) but higher than NWIS (0.23 ± 0.01 ppm). While the concentration of arsenic was greater in MWIS (3.68 ± 0.04 ppm) as compared to the LWIS (3.00 ± 0.00 ppm) and NWIS (1.07 ± 0.08 ppm). A higher rate of nickel was recorded in LWIS (0.94 ± 0.01 ppm) and MWIS (0.89 ± 0.03 ppm) than NWIS (0.55 ± 0.03 ppm). Although an equal concentration of cadmium was observed in all tested soil samples that is 0.03 ± 0.00 ppm.

A total of 27 species belonging to 13 genera of fungi were isolated from wastewater irrigated soils. Species of *Aspergillus*, *Alternaria alternata*, *Blastomyces* sp., *Cunninghamella* spp., *Curvularia intermedia*, *Drechslera biseptata*, *Fusarium* spp., *Humicola* spp., *Paecelomyces lilacinus*, *Penicillium* spp., *Rhizoctonia solani*, *Rhizopus* sp. and *Trichoderma* spp. were recorded as most prevalent fungi (Fig. 1). Among all species, *A. flavus*, *C. intermedia*, *F. solani* and *Rhizopus* sp. were found dominant (6.67×10^3 ± 333.33 cfu/g soil) in MWIS while, LWIS showed the highest cfu value (1.00×10^4 ± 0.00 cfu/g soil) of *T. viride* and *A. parasiticus* as compared to other fungal species (Table 3.). On the basis of selective media and biochemical analysis, several bacterial strains have also been isolated from wastewater irrigated soil such as *Acinetobacter lwoffii / jansani*, *Pseudomonas aeruginosa*, *Shigella dysenteriae, Serratia marcescens, Ralstoniapickettii* and *Burkholderia mallei*. Amongst all isolates, *R. pickettii* were found more in MWIS with 3.24×10^7 cfu/g soil. Moreover, *P. aeruginosa* (1×10^6 cfu/g soil), *B. mallei* (3×10^5 cfu/g soil) and *S. marcescens* (1×10^5 cfu/g soil) were also observed in MWIS. Five different strains of *A. lwoffii / jansani* were isolated from LWIS. In addition, *P. aeruginosa* (4×10^5 cfu/g soil) and *S. dysenteriae* (1×10^5 cfu/g soil) were also reported in LWIS (Table 4.).

Discussion

Microorganisms are present everywhere either saprophytes or pathogens as they are soil-borne, water-borne and air-borne (Shah *et al*., 2020, Basheer *et al*., 2023). In the present research, microorganisms like fungi and bacteria have been isolated from different nature of soils that were irrigated with wastewater. From this study, 13 different genera of fungi with 27 species were reported (Table 2). Most of them were plant pathogens such as *Alternaria alternata*, *Curvularia intermedia*, *Drechslera biseptata*, *Fusarium oxysporum*, *F. solani*, *Penicillium digitatum*, *P. italicum* and *Rhizoctonia solani*. These pathogens cause various diseases in plants showing symptoms like leaf spot, blight, wilting, chlorosis, necrosis, premature leaf drop, stunted growth, damping-off, root rot, fruit rot and stem rot (Shah *et al*., 2020, Basheer *et al*., 2023). Whereas, saprophytic fungal species of genus *Aspergillus*, *Cunninghamella*, *Rhizopus* sp. and antagonists like *Paecelomyces lilacinus* and *Trichoderma viride* have also been isolated. In addition, various bacterial strains were also isolated from wastewater irrigated soil including *Acinetobacter lwoffii / jansani*, *Pseudomonas aeruginosa*, *Shigella dysenteriae, Serratia marcescens, Ralstonia pickettii* and *Burkholderia mallei*. For the survival of microbes under any medium, pH plays an important role. According to Dix & Webster (1995), fungal species usually grow in acidic medium, but some required neutral to mild alkaline nature. Yamanaka (2003) reported optimum growth of ectomycorrhizal species under pH 5 to 6 whereas various saprotrophic species tolerated 7 ≈ 8 pH. Zablotowicz *et al*., (2007) found the highest populations of *A. flavus* and *Fusarium* sp. in soil with pH ranged of 5.2 to 7.5. The extreme pH level or highly acidic medium have a negative influence on microbes. According to Davies (1993), survival of microorganisms become obstruct at below the pH range of 4.0 or increased upto 9.0. In present research, soil samples had slightly acidic to neutral pH value that comprises variety of bacterial and fungal species with dominants of *A. flavus*, *A. parasiticus, C. intermedia* and *F. solani* and *T. viride*. Similarly, Rousk *et al*., (2009) found lower fungal growth as the range of pH increased from 4 to 8 whereas bacterial growth increased by means of higher pH range. According to Fierer & Jackson (2006) pH is a significant factor to express the diversity and extent of bacteria. Similarly, Baath & Arnebrant (1994) also reported increased growth of bacteria by increasing pH from 4 to 7.

Variation in salinity level causes stress to the survival of microorganisms by osmotic potential that ultimately affects the decomposition of organic matter (Pankhurst *et al*., 2001). However, several microbes can tolerate higher salinity levels by the accumulation of organic osmolytes in their body (Baumann & Marschner, 2013). According to various researchers, fungal growth was found more sensitive to higher salinity whereas bacterial growth did not affect salt stress (Sardinha *et al*., 2003; Chowdhury *et al*., 2011). The highest population of fungal species in medium having electric conductivity of 550 $\mu S\ cm^{-1}$ was reported by Zablotowicz *et al*., (2007) but also found ceased population of fungi under the lowest EC level. In the present research, it was revealed that both types of soils contain populace of fungal and bacterial species with different EC levels. TDS are filterable and some are non-filterable gradients in wastewater according to their particle size. Present studies showed higher concentrations of TDS in LWIS with several bacterial strains and fungal isolates. Similarly, Ali *et al*., (2009) reported ten strains of bacteria such as *Aeromonas* spp., *Acinetobactor* spp., *Bacillus megaterium*, *B. subtilis*, *Bacillus* spp., *Escherichia coli*, *Lactobacillus* spp., *Micrococcus* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* from effluent having TDS of 2512 mg L^{-1}. According to Shehzadi *et al*., (2014), some bacteria like *Microbacterium arborescens* and *Bacillus pumilus* are resistant to contaminates of industrial effluent having TDS 4834 mg/L as consisting pollutant degrading properties.

Table 1. Quality parameters of wastewater irrigated soils from different localities.

Parameters	Unit	NWIS	MWIS	LWIS	F-value
Organic matter	%	2.17 ± 0.03	3.13 ± 0.10	3.10 ± 0.10	41.97***
Physicochemical measures					
pH	-	7.70 ± 0.05	7.08 ± 0.01	6.28 ± 0.05	223.59***
EC	µS/cm	736 ± 3.46	425 ± 1.45	1774 ± 5.49	33845.90***
Salinity	%	0.35 ± 0.02	0.2 ± 0.00	0.89 ± 0.02	477.14***
ORP	mV	47.76 ± 0.72	43.60 ± 0.12	41.10 ± 0.64	36.29***
DO	ppm	0.39 ± 0.01	1.05 ± 0.02	0.74 ± 0.01	700.93***
Alkalinity	ppm	141 ± 8.33	133 ± 16.67	133 ± 8.33	0.17^{ns}
TDS	ppm	368 ± 4.61	213 ± 1.73	887 ± 5.19	7282.42***
Nutrients					
N	%	4.13 ± 0.01	4.31 ± 0.01	4.01 ± 0.02	183.50***
P	mg/g	0.72 ± 0.00	2.99 ± 0.00	0.30 ± 0.00	385109.14***
K	ppm	18.02 ± 0.02	18.05 ± 0.02	18.06 ± 0.02	1.03 ^{ns}
Mg	ppm	291.2 ± 1.73	4.972 ± 0.01	5.033 ± 0.01	27277.46***
Ca	ppm	90.22 ± 0.02	95.18 ± 1.77	90.34 ± 0.07	7.68*
Heavy metals					
Fe	ppm	0.29 ± 0.07	24.08 ± 1.70	29.02 ± 1.72	121.09***
Cu	ppm	0.06 ± 0.01	0.31 ± 0.01	0.36 ± 0.02	166.07***
Pb	ppm	0.32 ± 0.01	0.73 ± 0.01	0.82 ± 0.02	456.64***
Zn	ppm	0.23 ± 0.01	2.82 ± 0.02	2.82 ± 0.04	4374.85***
As	ppm	1.07 ± 0.08	3.68 ± 0.04	3.00 ± 0.00	749.96***
Ni	ppm	0.55 ± 0.03	0.89 ± 0.03	0.94 ± 0.01	88.11***
Cd	ppm	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ^{ns}

Note: NWIS = Normal water irrigated soil, **MWIS** = Malir wastewater irrigated soil, **LWIS** = Lyari wastewater irrigated soil, **EC** = Electric conductivity, **ORP** = Oxidation-reduction potential, **TDS** = Total dissolved solids, **N** = Nitrogen, **P** = Phosphorus, **K** = Potassium, **Mg** = Magnesium, **Ca** = Calcium, **Fe** = Iron, **Cu** = Copper, **Pb** = Lead, **Zn** = Zinc, **As** = Arsenic, **Ni** = Nickel, **Cd** = Cadmium

Table 2. Presence of fungal species in different soil samples from different localities.

S. No.	Fungal species	Soil samples	
		MWIS	LWIS
1.	*Aspergillus flavus*	Blue	Grey
2.	*A. fumigatus*	Grey	Blue
3.	*A. niger*	Blue	Grey
4.	*A. parasiticus*	Grey	Blue
5.	*A. ochraceus*	Blue	Grey
6.	*A. terreus*	Blue	Blue
7.	*A. versicolor*	Grey	Blue
8.	*A. wentii*	Blue	Grey
9.	*Alternaria alternata*	Blue	Grey
10.	*Blastomyces sp.*	Grey	Blue
11.	*Cunninghamela sp. (Light Brown Colonies)*	Grey	Blue
12.	*Cunninghamela sp. (Orange Colonies)*	Blue	Grey
13.	*Cunninghamela sp. (White Colonies)*	Blue	Grey
14.	*Curvularia intermedia*	Blue	Grey
15.	*Drechslera biseptata*	Grey	Blue
16.	*Fusarium oxysporum*	Blue	Grey
17.	*F. solani*	Blue	Blue
18.	*Humicola sp. (Grey+Yellow Colonies)*	Grey	Blue
19.	*Humicola sp. (Light Brown Colonies)*	Grey	Blue
20.	*Paecelomyces lilacinus*	Grey	Blue
21.	*Penicillium digitatum*	Blue	Grey
22.	*P. italicum*	Blue	Grey
23.	*P. purpurogenum*	Grey	Blue
24.	*Rhizoctonia solani*	Blue	Blue
25.	*Rhizopus sp.*	Blue	Grey
26.	*Trichoderma harzianum*	Blue	Grey
27.	*T. viride*	Grey	Blue

Note: Blue = Present, Grey = Absent, **MWIS** = Malir wastewater irrigated soil, **LWIS** = Lyari wastewater irrigated soil

Table 3. cfu / g (soil) of Fungal Species in different soil samples from different localities.

S. No.	Fungal species	Soil samples	
		MWIS	LWIS
1.	*Aspergillus flavus*	$6.67\times10^3 \pm 333.33$	0.00
2.	*A. fumigatus*	0.00	$1.67\times10^3 \pm 333.33$
3.	*A. niger*	$4.00\times10^3 \pm 1154.70$	0.00
4.	*A. parasiticus*	0.00	$1.00\times10^4 \pm 0.00$
5.	*A. ochraceus*	$5.00\times10^3 \pm 0.00$	0.00
6.	*A. terreus*	$1.33\times10^3 \pm 333.33$	$1.67\times10^3 \pm 333.33$
7.	*A. versicolor*	0.00	$1.67\times10^3 \pm 333.33$
8.	*A. wentii*	$4.00\times10^3 \pm 1154.70$	0.00
9.	*Alternaria alternata*	$5.00\times10^3 \pm 0.00$	0.00
10.	*Blastomyces* sp.	0.00	$5.00\times10^3 \pm 0.00$
11.	*Cunninghamela* sp. (Light Brown Colonies)	0.00	$5.00\times10^3 \pm 0.00$
12.	*Cunninghamela* sp. (Orange Colonies)	$1.50\times10^3 \pm 333.33$	0.00
13.	*Cunninghamela* sp. (White Colonies)	$3.33\times10^2 \pm 333.33$	0.00
14.	*Curvularia intermedia*	$6.67\times10^3 \pm 333.33$	0.00
15.	*Drechslera biseptata*	0.00	$3.33\times10^3 \pm 666.67$
16.	*Fusarium oxysporum*	$5.00\times10^3 \pm 0.00$	0.00
17.	*F. solani*	$6.67\times10^3 \pm 333.33$	$1.00\times10^3 \pm 0.00$
18.	*Humicola* sp. (Grey+Yellow Colonies)	0.00	$5.00\times10^3 \pm 0.00$
19.	*Humicola* sp. (Light Brown Colonies)	0.00	$5.00\times10^3 \pm 0.00$
20.	*Paecelomyces lilacinus*	0.00	$3.00\times10^3 \pm 0.00$
21.	*Penicillium digitatum*	$5.00\times10^3 \pm 0.00$	0.00
22.	*P. italicum*	$1.67\times10^3 \pm 333.33$	0.00
23.	*P. purpurogenum*	0.00	$5.00\times10^3 \pm 0.00$
24.	*Rhizoctonia solani*	$1.00\times10^3 \pm 0.00$	$1.67\times10^3 \pm 333.33$
25.	*Rhizopus sp.*	$6.67\times10^3 \pm 333.33$	0.00
26.	*Trichoderma harzianum*	0.00	$1.67\times10^3 \pm 333.33$
27.	*Trichoderma viride*	0.00	$1.00\times10^4 \pm 0.00$
	F-value	**21.96*****	**122.83*****

Note: **MWIS** = Malir wastewater irrigated soil, **LWIS** = Lyari wastewater irrigated soil

In soil, particularly wastewater irrigated soils possesses variety of dead organic material from plants, animals and high C:N ratios. These organic materials are decomposed by variety of microbes including fungi and bacteria. Increased amount of organic matter improves the water-holding-capacity in soil (Zablotowicz *et al*., 2007), thus moisture allows to enhance the population rate of microbes, with particular reference to saprophytes. Therefore, organic matter progresses the fungal population (Bouwman *et al*., 1994; Kasuga & Honda, 2006). In the current study, wastewater irrigated soil also had the highest organic matter with variety of fungal population as well as bacterial populace. Noorjahan (2014) reported increased population of fungal diversity is effluent with a greater amount of organic matter such as *A. fumigatus*, *A. niger*, *A. sydowii*, *A. terreus*, *A. versicolor*, *F. moniliformis*, *Paecilomyces variotii*, *Penicillium citrinum*, *P. frequentos* and *T. harzianum*. Zablotowicz *et al*., (2007) also found large number of *A. flavus* and *Fusarium* sp. by increasing organic matter in soil from 0.7 – 4.8 %.

Microorganisms are present in every habitat due to the instinctive property of consuming contaminants for the source of nutrients. Whereas, some microbes grow under impurities because of their physicological adaptation (Gadd, 1993) and this adaptation makes their association with impurities. Various species of microbes generate certain extracellular/intracellular enzymes to tolerate heavy metals or comprise active uptake, biosorption, complexation, crystallization and valence transformation to cell walls (Zafar *et al*., 2007). Microorganisms play a vital role in mobility of metal cations by ingesting in their body that consequently altering the accessibility for plants (Birch & Bachofen, 1990). But, it depends on the tolerance of microbes that vary from species to species to take up different metals and concentration of metals in a medium. According to Hashem & Bahkali (1994) and Wainwright & Gadd (1997), several fungi possess tolerant properties to absorb heavy metals. Zafar *et al*., (2007) isolated various species of fungi including genera *Fusarium* in polluted soils that contain heavy metals. Ezzouhri *et al*., (2009) also found *Fusarium* sp. that resist heavy metals. Present research reported that both wastewater irrigated soils contain variety of fungi and bacteria. Likewise, Babich & Stozky (1985) isolated several fungal species including the genera of *Fusarium*, *Curvularia*, *Nannizzia* as well as *Helminthosporium*, and *Humicolagriseai* from contaminated soil by a variety of heavy metals like Cd, Cr, Pb, Zn etc. Similarly, bacterium *Burkholderia* sp., was isolated by Jiang *et al*., (2008) from heavy metal contaminated soil. In the same way, Filali *et al*., (2000) also reported various bacterial strains such as *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *P. fluorescens* and *Staphylococcus* sp. from wastewater medium that contained heavy metals including Cd, Hg etc.

Table 4. Biochemical tests, properties and cfu of bacterial species present in different soil samples isolated from different localities.

Tests	Bacterial strains										
	MWIS				LWIS						
	Burk	Pseu1	Rals	Serr	Acin1	Acin2	Acin3	Acin4	Acin5	Pseu2	Shig
ONPG	-	-	-	-	+	-	-	-	-	-	-
CIT	+	+	-	+	-	-	-	-	-	+	-
MALO	+	-	-	-	+	-	-	-	-	+	-
LDC	-	-	-	+	-	-	-	-	-	-	-
ADH	+	-	-	-	-	-	-	-	-	+	-
ODC	-	-	-	+	-	-	-	-	-	-	-
H_2S	-	-	-	-	-	-	-	-	-	-	-
UREA	+	-	-	-	-	-	-	-	+	+	-
TDA	-	-	-	-	-	-	-	-	-	-	-
IND	-	-	-	-	-	-	-	-	-	-	-
VP	-	-	-	-	+	-	-	-	-	-	-
GEL	+	-	-	+	-	-	-	-	-	-	+
GLU	-	-	-	-	-	-	-	-	-	-	+
NO_3/N_2	+	+	-	-	+	-	+	-	-	+	-
MALT	+	-	-	-	-	-	-	-	-	-	-
SUC	-	-	-	+	-	-	-	-	-	-	-
MANN	-	-	-	-	-	-	-	-	-	-	-
ARAB	-	-	-	-	-	-	-	-	-	-	-
RHAM	-	-	-	-	-	-	-	-	-	-	-
SORB	-	-	-	-	-	-	-	-	-	-	-
INOS	-	-	-	-	-	-	-	-	-	-	-
ADO	-	-	-	-	-	-	-	-	-	-	-
MEL	-	-	-	-	-	-	-	-	-	-	-
RAF	-	-	-	-	-	-	-	-	-	-	-
MOT	-	+	+	+	+	-	-	-	-	+	-
CO	+	+	+	-	-	-	-	-	-	+	-
Gram Stain	-	-	-	-	-	-	-	-	-	-	-
Shape	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods
Cfu/g soil	**3×10^5**	**1×10^6**	**3.24×10^7**	**1×10^5**	**1×10^5**	**6×10^5**	**6×10^5**	**1×10^6**	**1×10^5**	**4×10^5**	**1×10^5**

Note: Burk = *Burkholderia mallei*, **Pseu** = *Pseudomonas aeruginosa*, **Rals** =*Ralstoniapickettii*, **Serr** = *Serratia marcescens*, **Acin** = *Acinetobacter lwoffii / jansani*, **Shig** = *Shigella dysenteriae*

Tests: ONPG =O-nitrophenyl-beta-D-galactopyranoside, **CIT** = sodium citrate,**MALO** = sodium malonate, **LDC** = lysine decarboxylase, **ADH** = arginine dihydrolase, **ODC** = ornithine decarboxylase, **H_2S** = H_2S production, **UREA** = urea hydrolysis, **TDA** = tryptophane deaminase, **IND** = Indole, **VP** = Voges-Proskauer Acetion, **GEL** = Gelatin Hydrolysis, **GLU** = Acid from Glucose, **NO_3/N_2** = Nitrate Reduction and N_2Gas,**MALT** = Acid from Maltose, **SUC** = Acid from Sucrose, **MANN** = Acid from Mannitol, **ARAB** = Acid from Arabinose, **RHAM** = Acid from Rhamnose, **SORB** = Acid from Sorbitol, **INOS** = Acid from Inositole, **ADO** = Acid from Adonitol, **MEL** = Acid from Melibiose, **RAF** = Acid from Raffinose, **MOT** = Motility, **CO** = Cytochrome Oxidase.

Wastewater is a rich source of essential nutrients (Shah *et al*., 2022). Its application on agricultural lands can provide macro and micro-nutrients. The presence of these nutrients assures microbes to survive in the nutrient-rich medium. Zablotowicz *et al*., (2007) reported greater population of microbes in soil containing a higher amount of nutrients such as nitrate (144 mg kg^{-1}), phosphorus (233 mg kg^{-1}), potassium (1051 mg kg^{-1}) etc. However, in this study, it was observed that fungal and bacterial population was abundant in both wastewater irrigated soil. Microbes also play a vital role to enhance the fertility of soil by decomposing organic matter. Consequently, they populate and provide conditioning to soil. According to the scientists, soil amendments, saprophytic fungal and bacterial species are responsible to manage nutrient cycle in soil for plants (Medina *et al*., 2010; Chaer *et al*., 2011, Basheer *et al*., 2022). These species stimulate nutrients bioavailability through mineralization, mobilization and translocation of nitrogen, phosphorus, potassium etc. reserves in soil (Figueiredo *et al*., 2010; Ahemad & Kibret, 2014; Nguyen & Bruns, 2015).

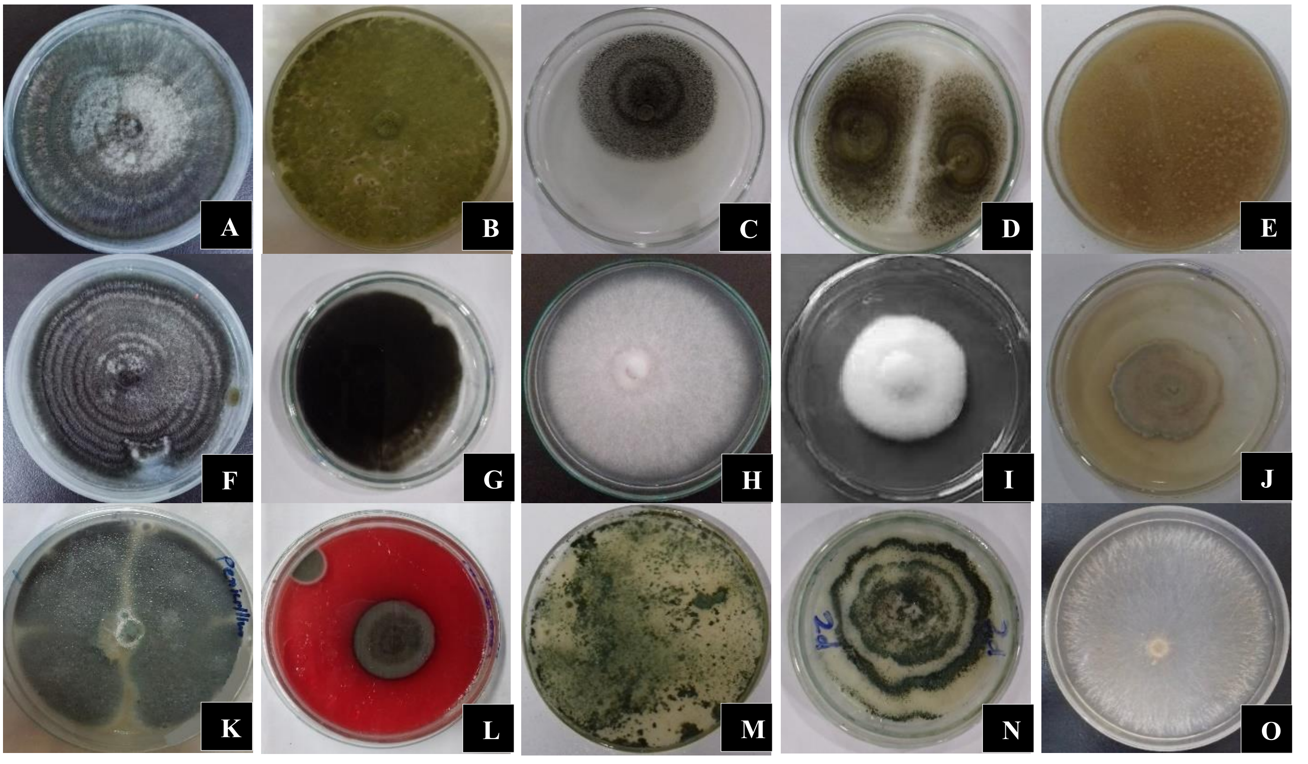

Fig 1. *Alternaria alternata* (A), *Aspergillus flavus* (B), *A. niger* (C) *A. parasiticus* (D), *A. terreus* (E), *Curvularia* sp. (F), *Drechslera biseptata* (G), *Fusarium oxysporum* (H), *F. solani* (I), *Paeciliomyces lilacinus* (J), *Penicillium digitatum* (K), *P. purpurogenum* (L), *Trichoderma harzianum* (M) *T. viride* (N), and *Rhizoctonia solani* (O).

Conclusion

This study provided basic information about the existence of microorganisms (fungi and bacteria) in different types of soils having diverse qualities that were irrigated by wastewaters. Through this study, it was evaluated that isolated fungal and bacterial species may tolerate the nature of wastewater irrigated soils as they have suitable environment for the survival of fungi as well as bacteria. Therefore, several species of both types of microorganisms have been found in soils. This study assessed the required evidence about the saprophytic and pathogenic microorganisms in wastewater irrigated soils. It can help in irrigation of agricultural land, as wastewater supplies essential nutrients and beneficial microorganisms to the land.

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