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EFFECT OF WASTEWATER IRRIGATION ON SOIL ALONG WITH ITS MICRO AND MACRO FLORA

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Abstract

Agricultural irrigation with wastewater is common in arid areas but has possible public health and environmental side effects, as effluent may contain pathogens, high level of salts, detergents and toxic metals. In order to ascertain effects of a local textile mill's wastewater irrigation on soil and subsequently the micro and macroflora, a series of analysis were conducted on soil collected from different sites with regard to pH, EC, organic matter, SO_4^{-2}, NO_3^{-1} and exchangeable cations. Soil samples were also determined for total and bio-available metal ions such as Cr^{+6}, Zn^{+2}, Cu^{+2} and Ni^{+2}. Macroflora of the mill contained high concentration of metal ions. Microbial community identified in the soil samples was different from reference soil. Vesicular arbuscular mycorrhizae (VAM) population associated with *Zea mays* irrigated by effluent contained 3 ecotypes of VAM, viz., *Glomus mossea*, *Glomus* spp., and *Acualospora* spp. Out of 34 bacterial strains isolated and characterized, dominant genera were *Bacillus*, *Micrococcus* and *Listeria*. Endurance of Cr^{+6} by *Bacillus fumus* RH109 was recorded upto 1000 mg/L, Zn^{+2} upto 325 mg/L by *Pseudomonas stutzeri* RH71 and *Alcaligenes* spp. RH88, while *Agrobacterium* spp., RH102, *Bacillus subtilis* RH 96, *Bacillus pumilus* RH84 and *Lactobacillus* spp. RH66 tolerated 150 mg/L Ni^{+2} and 18 bacterial isolates were able to grow un upto 100 mg/L Cu^{2+}. The findings suggest that irrigation with local textile wastewater not only alters the soil chemistry, but also changes bacterial and VAM population in addition to enhancing the intrinsic endurance of these microbes to different metal ions present in their microenvironment. In view of these findings, we recommend monitoring of toxic effects of wastewaters and conclude that such irrigation practices should be carried out only after treatment of wastewater.

Introduction

Soil is a heterogeneous environment, in both time and space, and microbial activity is concentrated at localized sites on and around organic residues. The decomposer communities undergo succession as organic and inorganic residues are changed. Agricultural irrigation with industrial wastewater is a common practice in arid and semi-arid regions and it is used as a readily available and inexpensive option to fresh water. Addition of such a mixed bag of compounds causes significant shift in the structure and function of a microbial community, which in turn may influence viability of soil for agriculture. The structural organization of soil particles provides a spatially heterogeneous habitat for microorganisms characterized by different substrates, nutrients, oxygen concentrations and water contents as well as variable pH values (Sessitsch *et al*., 2001). Soil bacteria degrade organic matter and promote soil moisture retention and fertility, which are important for arid ecosystem productivity and stability (Kuske *et al*., 2002). A change in soil microbial diversity, or a shift from bacterial to fungal population

has also been reported in metal contaminated soils. Oved *et al*., (2001) reported that irrigation with wastewater altered ammonia oxidizing bacterial (AOB) population in soil and *Nitrosospira* and *Nitrosomonas* species became dominant. Sarnaik & Kanekar (1995) reported alteration and reduction in number of *Pseudomonas* species from soil samples collected from the premises of a dye factory in India. Excess of soluble salts in water result in low crop yields, and if sodium is in excess, soil deterioration occurs as well. Baath (1989) reported that soils with high cation exchange capacity and more organic matter are known to bind metals and make them less available to microorganisms.

Aquatic mosses from Ave river (Portugal), near a textile industrial area (Gonclaves *et al*., 1994), have exhibited an increased concentration (µg/g dry weight) of Cr (30-46), Cu (49-56) and Zn (61-112). Heavy metals in soil samples and in washed and unwashed samples of *Telfaria occidentalis* (ugwu) and *Talinum triangulare* (waterleaf) cultivated on the bank of river Ribila in Odo-nla village (Nigeria), were determined (Akinola & Ekiyoyo, 2006). The soil was moderately polluted with cadmium, which was found in the tissue of the leafy vegetables cultivated on the river bank over time. Qadir *et al.* (1998) indicated accumulation of Pb^{+2} above recommended maximum levels in plants and soil being irrigated with city industrial effluent in Faisalabad, Pakistan.

Pakistan is an agricultural country, where industrialization is taking place in a gradually increasing phase. In the absence of adequate supply of water in the arid area surrounding the KTM, irrigation by wastewater is a welcome opportunity. Bearing this fact, the present study was conducted with an aim to assess the changes in the soil as well its macro and microflora due to irrigation by wastewater. Physiochemical parameters, such as texture, organic matter field capacity, electric conductivity, pH, P, K, sulphates, exchangeable cations and, total and bio-available metals in soil were analyzed. Determination of maximum resistivity limits (MRL) of indigenous bacterial isolates towards various heavy metals was investigated. In addition to identification of existing plant species, analysis of trace metal concentrations in the dominant plants, as well as a detailed VAM characterization in the rhizosphere of plants was carried out.

Materials and Methods

The study area characterized in this research paper is a local textile mill (KTM), located in a populated peri-urban out-skirt of Rawalpindi ($33^{o}36$'N, $73^{o}5$' E). This area is characterized by having loamy soil, mean annual rainfall 500-1000 mm, with average temperatures during the month of June to August being 30^{o}C. Coloured effluent discharged from the industry flows across the vast land within the compound of the mill, which is used to cultivate and grow fodder and cereals such as corn and wheat etc., depending upon the season. Reference site was an uncultivated land, 5Km away from the mill. Soil samples were collected from the upper 10 cm of the topsoil in sterilized plastic bags and taken to the laboratory for analysis.

Physiochemical characterization of soil: Samples were analyzed for various physical (texture, organic matter, water holding capacity), chemical (pH, electric conductivity, exchangeable cations, phosphorous, potassium, plant available and total trace metal) and microbial (bacterial and vesicular arbuscular mycorrhizae) parameters. Soil texture was determined following the method of Bouyoucos (1962) using a hydrometer. Electrical conductivity (EC) and pH were analyzed by Jeneway EC meter and Orabeco portable pH meter respectively, using soil dissolved in distilled water in a 1:2 ratio. Organic matter

was determined by the loss on ignition method (Gallardo *et al.*, 1987). Water holding capacity was analyzed by saturating 100g soil with water, followed by oven drying at 105^{o}C till a constant weight was achieved, and the difference was expressed as a percentage of original weight. AB-DTPA method for alkaline soil developed by Lindsay & Norvell (1978) was used for analysis of P, K, Zn^{+2},Cu^{+2}, Ni^{+2} and Cr^{+6}. Sulphates were determined by barium chloride procedure. For total metal content, extraction was carried out using 55% HNO_3, and analyzed following the method of Clesceri *et al.*, (1989). All metal ions were analyzed by Solar Unicam atomic absorption spectrophotometer. Exchangeable cations (carbonates, bicarbonates, chlorides, Ca^{+2}, Mg^{+2}, Na^{+1} and P), in saturation paste of soil were analyzed by titration (Raymet & Higginson, 1992). Nitrates-nitrogen estimation was done by Kjeldahl digestion method on auto-analyzer (Bremner & Mulvaney, 1982).

Identification and chemical characterization of the vegetation: Vegetation samples consisting of whole plants and their rhizospheres were collected. Plants were washed with NaEDTA and identified (Quaid-i-Azam University, Herbarium). Plants were air dried and 0.5g powdered plant material was digested in 5 mL HNO_3: $HClO_4$ (2:1v/v) until transparent. Metal ions were determined by air acetylene flame atomic absorption spectrophotometer (Solar Unicam). The analytical limits were 0.03 mg/L for Cu^{+2}, 0.02 mg/L for Zn^{+2}, 0.1 mg/L for Cr^{+6} and 0.03 mg/L for Ni^{+2}. Total nitrogen was analyzed by Kjeldahl method

Isolation and characterization of VAM: Rhizospheres were analyzed for the number of VAM spores per 50g by wet sieving and decanting technique (Gerdemann & Nicolson, 1963). The percentage of mycorrhizal colonization of roots preserved in 70% ethanol was determined using grid-line intersect method by evaluating 30 intersects of each root sample under stereomicroscope (Giovannetti & Mosse, 1980).

Isolation and characterization of bacterial isolates: Bacterial population were determined by serial dilution and plating of soil suspension on differential culture media. Bacterial isolates were identified and biochemically characterized following the methods described in Bergey's Manual of Systematic Bacteriology (Kreig & Holt, 1984). In order to assess effect of metal containing dye-wastewater on soil bacterial isolates, their maximum resistivity limits against Zn^{+2}, Cu^{+2}, Cr^{+6} and Ni^{+2} ions were analyzed by using agar amended with salts of metals ($NiCl.6H_2O$, $CuSo_4$, $ZnCl_2$ and $K_2Cr_2O_7$) in nutrient agar (Price *et al.*, 2001).

Statistical analysis: Data were analyzed statistically, applying student's 't' test, for all the studied parameters.

Results

Physiochemical characterization of soil: Soil samples collected from various sites within the factory area were analyzed for physical characters, including temperature, colour, texture, field capacity and moisture content. All soil samples belonged to loamy class of soil texture, with different temperatures (28-34°C). Low field capacity (Table 1), as compared to the reference soil (31.96±0.41), was seen, which was significantly different ($p<0.05$).

pH of the soil samples was typical of arid area soil (slightly alkaline), with non-significant differences among various sites. Lower EC of all the soil samples, as compared to the reference soil was observed. Similar pattern of total soluble salt concentration (10.73±0.12 to 35.53±0.80 meq/L) was seen with respect to the reference soil (112.80±2.04 meq/L). Organic matter was significantly different ($p<0.05$) amongst soil samples tested from various sites. Percentage organic carbon and sulphate content also showed minor variation at different sites ($p<0.05$). Significant differences were also present in all the soil samples with regard to Nitrates-N, P and K levels (Table 1).

Table 2 shows the state of various exchangeable cations in the soil samples, and also in the reference site. Sodium, bicarbonates, chlorides, Ca+Mg, K and SAR, showed significant differences ($p<0.05$) among all the samples, with minor exceptions, which were, sodium (site 2 and 3), bicarbonates (site 4 and reference), chlorides (site 2 and 4) and potassium (site 1 and 5). Carbonate content significantly differed at site 5 and 6.

To determine the impact of excess metal ions present in the effluent on surrounding area's soil, where effluent is disposed off, metal ions in total as well as bioavailable form (the form of metal which can be taken up by plants), were analyzed. Total Zn and Cu concentrations were higher at site 4 and 5 as compared to the other sites, while Ni was high at site 3 and 4, and Cr at site 4 and 6. Total Zn and Cu concentrations were very high, ranging from 21.67±0.88 mg/L to 244.33±2.33 mg/L and 59.33±0.88-265.33±0.88 respectively (Table 3). In case of bioavailable form, site 4 and 5 yielded highest levels for Zn and Cu, while highest concentrations of Ni and Cr were recorded from sites 5 and 6. Each metal ion analyzed was present in excess at all the six sites, as compared to the background values from the reference soil, except for bioavailable Zn and Cu, which were having levels for site 6 even lower than the reference soil.

Identification and chemical characterization of the vegetation: Plants collected from all the sites were identified upto species level. Maize (*Zea mays*), growing at site 1, was irrigated by dye loaded effluent, and this plant upon analysis showed highest contents of Zn (84.33±1.45 mg/kg) and Cu (22.33±1.20 mg/kg) metal ions. Dominant plants from other sites also showed presence of high contents of Zn and Cu (Table 4).

Isolation and characterization of VAM: Rhizospheric soil of various plant samples contained a large number of VAM spores. Incidence of VAM spores varied with plant type. VAM genera identified in all the soil samples as a result of wet sieving and decanting, indicated high spore incidence of *Glomus*. Spores of two morphotypes of *Glomus*, *Glomus* spp. I and II were high (181 to 447 spores/50 g soil). *Glomus mosseae* spores were found in the rhizosphere of *Zea mays* and *Bromus sativa*, having an incidence rate of 12 and 3 spores/50 g soil, respectively. *Acualospora scrobiculata* spores were found in the rhizosphere of *Zea mays*, having a low incidence (4 spores/50 g soil). Rhizospheric soil of *Ricinus communis* contained 447.33±2.40 spores/50 g soil, whereas least number of spores were found in the rhizosphere of *Zea mays* (205.33±2.60 spores/50 g soil). Root VAM colonization percentage was also calculated, and significantly greater number of external hyphae ($p<0.05$) were found in *Zea mays* (96%), as compared to all other plants. Similarly, the number of vesicles, as compared to arbuscles, were also significantly higher ($p<0.05$), in all the plants studied (Table 5).

Table 1. Physiochemical characterization of soil samples from various sites (mean of 3 analysis).

Parameter	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Reference Soil
pH	7.86 ± 0.05 a	8.24 ± 0.04 b	7.83 ± 0.03 ac	7.97 ± 0.05 acd	7.95 ± 0.02 ade	7.87 ± 0.03 acdef	7.86 ± 0.03 acdefg
Organic Carbon (%)	0.24 ± 0.01 a	0.21 ± 0.01 b	0.42 ± 0.01 c	0.10 ± 0.00 d	0.23 ± 0.01 abe	0.41 ± 0.01 cf	0.26 ± 0.08 abcdefg
EC (mS/m)	2.57 ± 0.03 a	2.86 ± 0.01 b	1.07 ± 0.01 c	3.55 ± 0.08 d	2.95 ± 0.03 be	1.13 ± 0.02 cf	11.28 ± 0.20 g
T. S. Salts (meq/L)	25.67 ± 0.33 a	28.57 ± 0.15 b	10.73 ± 0.12 c	35.53 ± 0.80 d	29.53 ± 0.32 be	11.30 ± 0.21 cf	112.8 ± 2.04 g
Organic Matter (%)	0.42 ± 0.02 a	0.36 ± 0.01 b	0.72 ± 0.02 c	0.17 ± 0.01 d	0.39 ± 0.01 abe	0.70 ± 0.01 cf	0.59 ± 0.01 g
Sulphates (mg/kg)	19.96 ± 0.23 a	17.36 ± 0.20 b	3.08 ± 0.16 c	24.82 ± 0.77 d	12.68 ± 0.27 e	7.19 ± 0.08 f	91.80 ± 1.93 g
Nitrate-N (mg/kg)	0.57 ± 0.00 a	0.41 ± 0.01 b	0.11 ± 0.00 c	0.51 ± 0.00 d	0.67 ± 0.01 e	0.17 ± 0.00 f	1.83 ± 0.03 g
P (mg/kg)	31.34 ± .26 a	22.69 ± 0.32 b	5.80 ± 0.10 c	32.42 ± 0.59 ad	40.14 ± 0.68 e	1.27 ± 0.02 f	37.04 ± 0.27 g
K (mg/kg)	228.00 ± 1.73 a	221.33 ± 1.45 b	133.67 ± 2.60 c	202.67 ± 1.86 d	208.33 ± 3.76 de	85.00 ± 1.00 f	279.00 ± 1.53 g
Field Capacity	12.37 ± 0.20 a	14.44 ± 0.11 b	18.87 ± 0.20 c	31.00 ± 0.40 d	28.91 ± 0.08 e	19.99 ± 0.12 f	31.96 ± 0.41 dg

Means sharing a common letter do not differ significantly, others differ significantly ($p<0.05$).

Table 2. Exchangeable cations (meq/L) in soil samples from various sites (mean of 3 analysis).

Site	Carbonates	Bicarbonates	Sodium	Potassium	Ca + Mg	Chlorides	SAR
1	0.57 ± 0.03 a	3.70 ± 0.06 a	1.48 ± 0.01 a	0.85 ± 0.00 a	13.27 ± 0.20 a	1.44 ± 0.03 a	0.58 ± 0.01 a
2	1.47 ± 0.07 b	6.77 ± 0.03 b	6.48 ± 0.02 b	0.34 ± 0.01 b	11.37 ± 0.23 b	2.97 ± 0.09 b	2.72 ± 0.03 b
3	0.63 ± 0.03 ac	3.47 ± 0.03 c	6.33 ± 0.05 bc	0.17 ± 0.00 c	4.01 ± 0.04 c	3.55 ± 0.04 c	4.47 ± 0.03 c
4	1.43 ± 0.03 bd	6.07 ± 0.09 d	8.25 ± 0.05 d	0.54 ± 0.01 d	12.07 ± 0.07 d	3.21 ± 0.02 bd	3.36 ± 0.01 d
5	2.17 ± 0.03 e	7.17 ± 0.09 e	3.39 ± 0.04 e	0.83 ± 0.01 ae	14.34 ± 0.18 e	7.52 ± 0.18 e	1.26 ± 0.02 e
6	0.23 ± 0.03 f	2.97 ± 0.09 f	0.60 ± 0.02 f	0.15 ± 0.00 f	7.94 ± 0.05 f	0.91 ± 0.02 f	0.30 ± 0.01 f
R	1.57 ± 0.03 bg	5.80 ± 0.06 dg	13.03 ± 0.04 g	3.41 ± 0.02 g	35.15 ± 0.13 g	13.63 ± 0.03 g	3.11 ± 0.01 g

Means sharing a common letter do not differ significantly, others differ significantly ($p<0.05$).

Table 3. Total and bioavailable metal ions (mg/kg) present in the soil samples from various sites (mean of 3 analysis).

	Zn		Cu		Ni		Cr	
Site	Total	Bioavailable	Total	Bioavailable	Total	Bioavailable	Total	Bioavailable
1	129.33 ± 0.88 a	9.20 ± 0.35 a	107.00 ± 0.58 a	12.07 ± 0.09 a	41.67 ± 0.88 a	1.88 ± 0.05 a	25.50 ± 0.17 a	2.74 ± 0.06 a
2	110.00 ± 0.58 b	10.13 ± 0.18 ab	66.67 ± 0.88 b	3.21 ± 0.03 b	37.67 ± 0.88 b	8.41 ± 0.11 b	25.67 ± 0.12 ab	1.97 ± 0.08 b
3	21.67 ± 0.88 c	2.83 ± 0.09 c	59.33 ± 0.88 c	2.65 ± 0.01 c	174.33 ± 2.33 c	3.57 ± 0.21 c	27.83 ± 0.18 c	3.20 ± 0.12 c
4	192.67 ± 1.45 d	21.30 ± 0.26 d	166.00 ± 0.58 d	19.22 ± 0.05 d	204.33 ± 1.76 d	11.36 ± 0.18 d	72.10 ± 0.57 d	1.75 ± 0.07 bd
5	244.33 ± 2.33 e	33.50 ± 0.38 e	265.33 ± 0.88 e	20.27 ± 0.04 e	61.67 ± 0.88 e	23.83 ± 0.14 e	6.13 ± 0.15 e	7.56 ± 0.15 e
6	65.00 ± 0.58 f	1.64 ± 0.01 f	73.00 ± 1.15 f	1.35 ± 0.04 f	29.33 ± 0.33 f	17.66 ± 0.25 f	50.37 ± 1.08 f	4.40 ± 0.14 f
R	41.67 ± 0.88 g	9.50 ± 0.06 a	43.33 ± 0.88 g	3.20 ± 0.04 b	0.97 ± 0.09 g	0.15 ± 0.01 g	2.83 ± 0.09 g	0.68 ± 0.02 g

Means sharing a common letter do not differ significantly, others differ significantly ($p< 0.05$)

Table 4. Metal ions (mg/kg) present in dominant plant species growing on various sites (mean of 3 analysis).

Dominant plant species/site		Zn	Cu	Ni	Cr
Zea mays	1	84.33 ± 1.45 a	22.33 ± 1.20 a	2.55 ± 0.17 a	0.95 ± 0.01 a
Bromus sativa	2	72.33 ± 1.45 b	19.67 ± 1.45 ab	2.18 ± 0.08 ab	0.73 ± 0.01 b
Canabus sativa	3	43.67 ± 1.45 c	3.67 ± 0.33 c	2.82 ± 0.09 ac	0.81 ± 0.01 c
Ricinus communis	4	64.00 ± 1.15 d	6.67 ± 0.67 d	5.58 ± 0.14 d	0.95 ± 0.01 ad
Xanthrin stromarium	5	39.67 ± 0.88 ce	19.67 ± 0.88 abe	2.53 ± 0.05 ace	0.39 ± 0.01 e
Dichanthium annulaticum	6	41.67 ± 1.20 ce	14.33 ± 0.88 f	4.85 ± 0.06 f	0.18 ± 0.01 f

Means sharing a common letter do not differ significantly, others differ significantly ($p<0.05$).

Table 5. VAM colonization in dominant plant species growing on various sites (mean of 3 analysis).

Dominant plant species/site		Number of total spores/50 g	Arbuscules (%)	Vesicles (%)	External Hyphae (%)	Internal Hyphae (%)	Identified VAM Genera
Zea mays	1	205.33 ± 2.60 a	21.33 ± 0.88 a	31.00 ± 0.58 a	96.00 ± 3.51 a	49.00 ± 1.53 a	*Glomus* spp. I, *Glomus* spp. II, *Glomus mosseae*, *Acualospora scrobiculata.*
Bromus sativa	2	412.33 ± 3.76 b	0.00 ± 0.00 b	21.33 ± 0.88 b	59.67 ± 0.88 b	10.33 ± 0.88 b	*Glomus* spp. I, *Glomus* spp. II, *Glomus mosseae*, *Aculaospora* spp.
Canabus sativa	3	266.00 ± 7.64 c	0.00 ± 0.00 b	22.00 ± 1.53 bc	49.00 ± 0.58 c	30.33 ± 0.88 c	*Glomus* spp. I, *Glomus* spp. II.
Ricinus communis	4	447.33 ± 2.40 d	0.00 ± 0.00 b	13.00 ± 0.58 d	30.33 ± 2.60 d	12.67 ± 0.67 bd	*Glomus* spp. I.
Xanthrin stromarium	5	352.33 ± 4.33 e	0.00 ± 0.00 b	1.33 ± 0.88 e	45.00 ± 2.89 ce	25.33 ± 0.88 e	*Glomus* spp. I.
Dichanthium annulaticum	6	217.00 ± 4.36 af	0.00 ± 0.00 b	1.33 ± 0.88 e	24.00 ± 0.58 df	11.00 ± 0.58 bdf	*Glomus* spp. I.
Grass	R	375.33 ± 3.28 g	4.33 ± 0.88 c	18.33 ± 0.88 bcg	69.67 ± 0.33 g	22.33 ± 0.88 eg	*Glomus* spp. I, *Glomus* spp. II.

Means sharing a common letter do not differ significantly, others differ significantly ($p<0.05$).

Table 6. Resistance pattern exhibited by the bacterial isolates from soil (mg/L).

Sr. No.	Site No.	Identification	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cr^{6+}
1.	1	*Lactobacillus* spp. RH66	150	0	65	800
2.	1	*Bacillus* spp. RH67	100	0	65	500
3.	1	*Bacillus* spp. RH70	100	50	65	900
4.	1	*Pseudomonas stutzeri* RH71	100	100	325	100
5.	2	*Bacillus* spp. RH73	100	50	0	300
6.	2	*Listeria* spp. RH77	100	0	0	100
7.	2	*Aerococcus* spp. RH78	100	100	0	0
8.	2	*Brochothrix* spp. RH83	100	0	0	500
9.	3	*Bacillus pumilus* RH84	150	50	65	800
10.	3	*Listeria* spp. RH85	100	100	65	100
11.	3	*Hydrogenophaga* spp. RH86	100	0	65	0
12.	3	*Alcaligenes* spp. RH88	100	100	325	100
13.	4	*Micrococus kristenea* RH72	100	100	65	500
14.	4	*Bacillus* spp. RH90	100	50	0	0
15.	4	*Micrococcus roseus* RH91	100	50	65	100
16.	4	*Lactobacillus* spp. RH92	100	100	65	0
17.	4	*Staphylococcus saprophyticus* RH93	100	100	130	100
18.	4	*Erysipelophrix* spp. RH94	100	100	65	800
19.	4	*Bacillus subtilis* RH 96	150	0	65	600
20.	4	*Bacillus pumilus* RH97	100	100	65	0
21.	4	*Bacillus* spp. RH98	100	100	0	0
22.	4	*Micrococcus* spp. RH99	0	50	0	0
23.	4	*Listeria* spp. RH101	100	100	65	300
24.	4	*Agrobacterium* spp. RH102	150	0	0	0
25.	4	*Rhizobacter* spp. RH103	100	100	65	600
26.	5	*Brochothrix* spp. RH104	100	50	65	100
27.	5	*Acinetobacter* spp. RH105	100	0	0	0
28.	5	*Enterococcus* spp. RH115	0	100	0	0
29.	6	*Bacillus fumus* RH109	100	100	65	1000
30.	6	*Bacillus subtilis* RH110	100	100	0	100
31.	6	*Bacillus pumilus* RH111	100	100	65	0
32.	6	*Micrococcus luteus* RH112	100	100	0	100
33.	6	*Listeria murrayi* RH113	100	50	0	0
34.	6	*Lactobacillus* spp. RH114	0	100	0	700
35.	R	*Moraxella* spp. RH116	0	20	10	0
36.	R	*Bacillus subtilis* FH05	0	0	1900	325

Isolation and characterization of bacterial isolates: All soil samples yielded a high number of colony forming units/mL (cfu/mL). Thirty four bacterial strains were isolated, identified and characterized, which belonged to different bacterial genera, dominant being *Bacillus* (11), *Micrococus* (4), *Listeria* (4) and *Lactobacillus* (3). In the rhizosphere of *Zea mays, Ricinus communis* and *Xanthrin stromarium*, cfu/mL were very high, being $16x10^6$, $12x10^6$ and $10x10^6$ respectively, as compared to the reference soil ($2x10^6$). In the soil samples, Gram positive bacteria showed a high incidence, in comparison with Gram negative bacteria (Table 6).

For comparison of metal tolerance, reference soil isolate *Moraxella* spp. RH116 and *Bacillus subtilis* FH05 from tannery effluent contaminated soil were used. Many bacterial isolates from the soil were multi-metal resistant. *Bacillus fumus* RH109 showed maximum resistance level (mg/L) against Cr^{6+} (1000), in addition to Cu^{2+} (100). *Pseudomonas stutzeri* RH71, grew in high concentration of metal amended media containing 325 mg/L Zn^{2+} and 100 mg/L Cu^{2+}, separately. *Bacillus subtilis* FH05 from tannery effluent, however, resisted Zn^{2+} upto 1900 mg/L and 325 mg/L Cr^{6+}. *Agrobacterium* spp. RH102, *Bacillus subtilis* RH 96, *Bacillus pumilus* RH84 and *Lactobacillus* spp. RH66 tolerated 150 mg/L Ni^{+2}. Endurance of Cu^{2+} upto 100 mg/L was observed in 18 isolates.

Discussion

The major objective of the study was to ascertain the impact of wastewater irrigation upon the physical and chemical characteristics of soil, as well as upon the number and composition of bacteria along with change in the plant chemical constituents. Inter-site differences revealed that soil samples from the six sites, affected by the KTM effluent, were quite distinct in terms of their physiochemical composition. However, major differences were observed, particularly in comparison with the reference site soil. Analysis of soil samples in the present study showed that they were silt to sandy loam, being low in organic matter, organic carbon, EC, sulphates, nitrates and total soluble salts, but rich in P and K.

With regards to field capacity of the soil samples, a marked difference with soil type was observed. In the present study, sandy loam soil samples demonstrated a comparatively higher content of carbonate and field capacity, but low organic carbon. Alkaline soils tend to have Ca, Mg and K in high concentrations (Kim, 1994). Soil samples from KTM showed a similar pattern with regard to Ca, Mg and K.

Site 5 soil contained highest content of both, total as well as bioavailable, Zn and Cu. Total Ni and Cr were very high in site 4. All these results demonstrated metal contamination of soil surrounding the KTM, caused by the effluent. Each metal ion analyzed was present in excess at all the six sites, as compared to the background values from the reference soil. Total and bioavailable forms of Cu and Pb have been reported to show elevated levels in soil of industrial Wuxian county of southern Jiangsu, China (Pan *et al.*, 2000).

This study demonstrates clearly that soil adjacent to the flowing textile effluent experiences change in physiochemical parameters. These elevated concentrations can be attributed to high content of metal ions in dye and mordants such as HRB 38, nickle-phthalocyanine complex, HRV5 copper containing azo dye, zinc yellow pigment, iron blue pigment, chrome yellow and green pigment, discharged into the wastewater and thus accumulated in the soil. There are no guidelines recommended for soil, with regard to allowable concentrations of heavy metal ions in Pakistan. Comparison of these metal ion contents with those laid by the Australian and New Zealand environment conservation council in 1992 (Fuentes *et al.*, 2000), clearly demonstrates that Cu in all soil samples exceed their recommended limit (60 ppm), while Zn concentration in soil of site 5 at KTM was above their recommended value (200 ppm). Ni and Cr permissible levels, under the Australian and New Zealand environmental laws were, however, more than those in the background values shown by the reference soil in the present study, which

contained low amounts of Zn, Cu, Ni and Cr in both DTPA extractable and total forms, reflecting that the reference soil is also somewhat contaminated. Considering the fact that Rawalpindi is a heavily urbanized and industrialized area, elevated levels can be expected in local soil. Much of this metal load would be from atmospheric aerosols.

Analysis of plant tissues revealed high concentrations of Zn and Cu as compared to Cr and Ni. At site 1, *Zea mays* (maize) was irrigated by waste water directly without any kind of treatment for removal of pollutants. Due to irrigation by such a mixed bag of contaminants, including heavy metals, mordants, metal salts and dyes, *Zea mays* contained high concentrations of Zn (84.33±1.45 mg/kg). *Ricinus communis* dried biomass contained elevated level of Zn, which was well correlated to excess concentrations of total and bioavailable Zn content in respective soil samples. The Ni^{2+} toxicity decreased with increasing pH, indicating a protective effect of protons (Rooney *et al*., 2007). Liu *et al*., (2007) reported that deciduous street trees in Beijing (*Populus tomentosa*, *Sophora japonica* and *Catalpa speciosa*), accumulated Cd in their tissue.

No visible differences in the morphology of all plants growing in the KTM soil were detected. Soil metal contents increased in both total and bioavailable form, but due to low concentrations of DTPA extractable metal ions, they are not readily available to the plant at the actual level of accumulation. The latter can be explained by the high buffer capacities of the soil in the area which shows neutral to alkaline soil pH, and loamy soil textures, and thus high cation exchange capacities. Despite low organic matter and organic carbon content in all soil samples, metal ions were not readily available to plants due to alkaline pH of the soil. Organic matter and organic carbon are the factors in soil that reduce the bioavailability of heavy metals. Heavy metal ions from textile effluent have bioaccumulated in these existing macroflora of KTM. In the present work, *Dicanthium annulaticum*, *Bromus sativa*, and even *Zea mays* have demonstrated a potential to be studied further for use in phytoremediation of metal ions.

Presence of mycorrhizae (*Glomus* and *Acaulospora*) and their colonization with all plants was consistent with earlier studies (Pawlowska *et al.*, 2000). *Zea mays* from KTM was heavily colonized, not only externally, but also had arbuscules and vesicles, with different VAM species (*Glomus mosseae*, *Glomus* I, *Glomus* II and *Acualospora scrobiculata*).

Glomus mosseae and *Acualospora scrobiculata* were not present in the reference soil, which shows that these strains have somehow developed metal resistance. Abou-Gazi *et al*., (1996), reported the infectivity by *Glomus* spp. and *Acaulospora* spp., in conditions similar to the present study, being dominant in soil with pH 8.1, EC 3.41 mS/m, but with high levels of Na^+ (52.75 meq/L) and Cl^- (59.32 meq/L), and irrigated with sewage water in the city of Burg El-Arab, Egypt. In the study under discussion, addition of metal complexed dye effluent to the soil did not affect VAM development under field conditions. It could be due to the fact that VAM ecotypes are already known to have different degrees of metal tolerance.

Soil samples exhibited high incidence of total cultureable bacteria, since toxic pollutants get adsorbed to the clay particles, therefore, they are not available for interactions with the microbes (Malik & Ahmed, 2002). These differences in CFU/g were mainly due to the reason that in arid landscapes, plants are considered to be “islands of fertility” with total nutrients. Not only total bacterial counts were different, but rhizospheric bacterial community composition was also distinguishable among the six dominant plants found in the KTM area, due to specific plant species having variable type of root growth, soil chemistry, and metal concentration (Girvan *et al.*, 2003).

At site 4 and 5, although soil texture was sandy loam, these samples showed highest culturable bacteria as compared to samples which were silt loam. High pH and high cation exchange capacities of soil samples could have been main factors which effected metal availability and hence elevated the number of cultureable bacteria. Data of low CFU/g, in silt loam soil samples with low organic matter, confirms the reduction in biomass carbon with metal inputs to loam (Sessitsch *et al.*, 2001). Effect of metal pollution from KTM textile effluent on soil microorganisms can be seen as a complex and an interactive combination of soil properties like texture, pH, organic matter and other nutrients.

Bacillus was the dominant genus found in the soil samples from KTM. High incidence of metal resistance and the multiple nature of resistance have been attributed as the result of continuous exposure of these strains to heavy metals. Among the isolates, *Lactobacillus* spp. RH66, *Bacillus pumilus* RH84, *Bacillus subtilis* RH96 and *Agrobacterium* spp. RH102 were Ni resistant. Earlier studies also reported development of metal resistance with continuous exposure to metal ions due to release of untreated effluent (Schmidt *et al*., 1991; McLean & Beveridge, 2001;Vainshtein *et al.*, 2003). *Bacillus* species resisted high level of Cr^{6+}, which is not only the toxic, but also the carcinogenic form of chromium. *Bacillus fumus* RH109 from KTM soil sample showed two folds more resistance to Cr^{6+}, as compared to an earlier reported *Pseudomonas* spp. (CRB5), isolated from chromate contaminated soil (McLean *et al.*, 2000).

In terms of the physiochemical and microbial parameters investigated, all sites were different from the reference site. Application of wastewater for irrigation altered chemistry of the soil, decreased concentrations of sulphates, nitrates, phosphorus, potassium, and brought a change in exchangeable cations. Electric conductivity at all sites was lower as compared to reference soil, therefore, total soluble salts (TSS) concentration was also reduced. Bacterial and VAM communities varied as soil chemistry changed. Bacterial strains (*Bacillus* spp., *Pseudomonas* spp. and *Lactobacillus* spp.) showed increased resistance to different heavy metal ions, which reflects emergence of metal resistance in bacteria due to exposure to elevated levels of metals in soil. VAM colonization with various plant species not only increased, but VAM diversity also altered, reflecting selection pressure on the VAM species under metal contamination. Metal concentration was high, but not phyto-toxic, because at alkaline pH metals are not mobile and bio-available. Hence there was change in soil fertility (less organic matter content), which can effect viability of such soil for agricultural purposes. Presence of various organic compounds and metal ions in the vegetation over a time period can pass in the food webs and chains. Therefore, before irrigation with such wastewater, effluent should be treated to remove metal contaminants. In Pakistan, very few studies are carried to assess the wastewater irrigation's hazardous effects on plants and its rhizosphere. This study clearly concludes that such practices lead to bioaccumulation of toxic metals in edible crops.

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