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Research article

Varied effects of untreated textile wastewater onto soil carbon mineralization and associated biochemical properties of a dryland agricultural soil

Mahnaz Roohi [a], Muhammad Riaz [a, *], Muhammad Saleem Arif [a], Sher Muhammad Shahzad [b], Tahira Yasmeen [a], Muhammad Atif Riaz [c], Shermeen Tahir [c], Khalid Mahmood [c]

[a] Department of Environmental Sciences & Engineering, Government College University Faisalabad, 38000, Faisalabad, Pakistan
[b] Department of Soil & Environmental Sciences, University College of Agriculture, University of Sargodha, Pakistan
[c] Soil Science Division, Nuclear Institute for Agriculture and Biology (NIAB), Jhang Road, Faisalabad, Pakistan

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ABSTRACT

Wastewater is an alternative, valuable and cost effective resource for irrigation in water-scarce arid and sami-arid regions of the world including Pakistan. Soils near urban centers are cultivated for vegetable and cash crops using untreated wastewater. Current study was performed with objectives of assessing impacts of untreated textile wastewater on some soil chemical, biological and enzymatic activities. The microcosm incubation study used a clay loam soil that received 0 (distilled-water), 25, 50 and 100% wastewater concentrations and incubated for 30 and 60 days under optimum temperature and moisture conditions. Soil respiration was measured periodically throughout the experiment over 60 days. After the incubation periods of 30- and 60-d, soils were destructively analyzed for pH, electrical conductivity (EC), water extractable organic matter (WEOM), microbial biomass carbon (MBC), microbial metabolic quotient (qCO_2) and dehydrogenase enzymatic activity. Results revealed that wastewater and incubation time significantly altered chemical, biological and enzymatic properties of soils. The observed large surge in soil respiration, at initial stage, was stimulated by dissolved organic matter in wastewater. Dehydrogenase activity increased significantly with increasing wastewater concentrations. Increase in qCO_2 with wastewater concentration and incubation time suggested more stress to microorganisms but also enhanced microbial activity under stress to synthesize biomass. We found significant positive ($R^2 = 0.64$, $p < 0.001$) relationship between soil respiration and MBC, however, correlation between WEOM and MBC was significant negative ($R^2 = 0.18$, $p < 0.01$) indicating a dynamic mismatch between carbon substrate, soil respiration and buildup of MBC pool. Wastewater concentration and incubation time interaction had significant ($p < 0.01$) effect on WEOM suggesting that WEOM accumulated over time and comparatively less utilized by microorganisms. Short- and long-term effects of untreated wastewater on soil physico-chemical and biological health should be assessed before its use for crop production.

1. Introduction

Arid and semi-arid agriculture is under pressure due to limited water availability for irrigation. In this context, wastewater has been considered a valuable, inexpensive and constantly available alternative water resource in such regions for crop production (WHO, 2006; Qadir et al., 2007). Application of wastewater is considered beneficial to the soil-plant systems due to the presence of substantial concentrations of nitrogen and phosphorus, and certain micronutrients (Filip et al., 2000; Petersen et al., 2003). However, management of the toxic substances present in wastewater pose a major challenge at the soil-microbe-plant interphase. Long-term effects of wastewater application on agroecosystems generally depend on the nature and concentrations of chemical constituents of the wastewater and physico-chemical properties of receiving soils (Lucho-Constantino et al., 2005; Ahmad et al., 2012).

* Corresponding author.
E-mail address: mr548@ymail.com (M. Riaz).

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Pakistan is an agricultural country and has started to experience acute freshwater shortage and droughts. After agriculture, the textile industry is the second largest sector and textile wastewater represents dominant part of the total industrial effluents in Pakistan, a trend similar to the global wastewater production (Faryal et al., 2007; Ghaly et al., 2014). More than 2 million tons of untreated wastewater are released into the earth's waters each day and almost 50 nations utilized this polluted water to irrigate the farming area of 20 million hectares (Hussain et al., 2002; Azizullah et al., 2011). An estimated 30% of the total 962,335 million gallons of untreated wastewater is utilized to irrigate 32,500 ha agricultural land in Pakistan on annual basis (Ensink et al., 2004; UN WWAP, 2009). Higher costs of wastewater treatment in Pakistan mean that only <8% wastewater receives primary treatment in large urban centers including Karachi, Lahore, Faisalabad, Multan and Rawalpindi (PWSS, 2002; Pak-SCEA, 2006). Higher chemical oxygen demand (COD), biochemical oxygen demand (BOD), dissolved solids, macro- and micronutrients are distinguishing properties of untreated textile wastewater (Garg and Kaushik, 2008; Arif et al., 2016).

A significant body of knowledge elaborate notable impacts on soil physical, chemical, biological and biochemical properties in response to application of treated and untreated wastewater. However, changes in such soil properties strongly depend on the time duration for which the soils have been irrigated with wastewater. Increase in soil salinity and resultant decrease in soil aggregate stability and hydraulic conductivity has been observed after irrigating the soil with treated municipal wastewater (Qian and Mecham, 2005; Rusan et al., 2007). Angin et al. (2005) found increase in soil organic matter and major cations after irrigation with untreated wastewater. In addition, numerous studies have reported positive, negative and neutral effects of wastewater on soil properties, and ultimately on the soil health, in a wide variety of agroecosystems (e.g. Schipper et al., 1996; Rutkowski et al., 2007; Chen et al., 2008; Mosse et al., 2012; Morugán-Coronado et al., 2013).

Changes in soil biological and microbiological properties including microbial biomass carbon (MBC), respiration of total microbial populations, microbial metabolic quotient (qCO_2) and enzymatic activities are often used indicators to monitor both short- and long-term effects of wastewater on soil quality (Anderson and Domsch, 1990; Brookes, 1995; Ramirez-Fuentes et al., 2002; Gianfreda et al., 2005; Adrover et al., 2012). Increase in soil respiration after application of treated wastewater has been reported earlier (Meli et al., 2002). However, in contrast, Brzezinska et al. (2006) found decrease in soil respiration rate in Eutric Histosol soils after irrigation with municipal wastewater. In agricultural soils, MBC is generally related to soil organic matter contents and any change in organic matter status of the soil could alter MBC (Houot and Chaussod, 1995). Increases in MBC in wastewater treated soil are related to carbon contents in wastewater. For example, positive effects of wastewater and sewage sludge on MBC have been observed which were dependent on the rate of application (Fernandes et al., 2005; Singh and Agrawal, 2012). Activities of a large number of soil enzymes including dehydrogenase, urease, alkaline phosphatase, catalase and glucosidase are generally measured to investigate the changes in potential activities of soil microorganisms in response to wastewater application. Dehydrogenase activity represent an overall metabolic status of the soil and is an important indicator of microbial activity (Nannipieri et al., 2002). Activity of dehydrogenase enzyme is usually higher in wastewater irrigated soil and strongly depend on concentrations of wastewater (Garcia-Gil et al., 2000; Arif et al., 2016). Moreover, activities of soil enzymes are very sensitive to the concentrations of toxic substances and salts, especially heavy metals present in wastewater. For example, Mikanova (2006) reported that higher concentrations of heavy metals in wastewater inhibited the activities of urease, dehydrogenase, arylsulfatase and invertase enzymes.

A large number of studies investigated the effects of treated and untreated wastewater on growth, development and yield of crops elsewhere (e.g. Kaushik et al., 2005; Khalid et al., 2013) and in Pakistan (Khan and Joergensen, 2009). However, very limited work is reported on how untreated textile wastewater may affect soil physico-chemical and biological properties in low fertility status agricultural soils in Pakistan. Treatment costs of wastewater are still not affordable especially in the developing countries which lead to disposal of untreated wastewater to agricultural areas (Friedel et al., 2000). A very recent study from Arif et al. (2016) found contrasting effects of untreated textile wastewater on soil nitrogen and phosphorus pools, and certain soil enzymatic activities in a 60-d incubation study. However, soil respiration, MBC and qCO_2 were not measured in their study.

We, therefore, designed a laboratory microcosm study, using untreated textile wastewater at varied application rates, with following objectives:

- how untreated textile wastewater affect soil C mineralization, MBC, qCO_2 and dehydrogenase activity at different concentration-based application rates;
- whether incubation time, in a rather short-scale study, affect soil physico-chemical and biological properties; and
- how nature and strength of relationships between soil properties are affected by untreated wastewater.

2. Materials and methods

2.1. Soil sampling, preparation and pre-experimental analysis

Soil for this study was collected from an agricultural field plot under wheat-fallow cultivation at Nuclear Institute for Agricultural and Biology Faisalabad Pakistan (31.4187° N, 73.0791° E). The field plot falls in sub-tropical monsoon climate and the average rainfall at the site is ca. 200 mm year^{-1} with high seasonal variations. At the time of sampling, soil was under fallow rotation and had not received chemical fertilizer for several weeks. Soil belonged to the Lyallpur series and classified as aridisol-fine-silty, mixed, hyperthermic ustalfic.

Soil samples were collected from the plough layer (0–15 cm depth) in April, 2012 at five points from the visibly uniform and vegetation-free field-plot with a sharp stainless steel spade. Individuals soil samples were sorted out to remove any coarse debris, living plant parts, and other visible material (roots or stones) by hand picking in the field. Soil samples were, then, air dried and sieved through a 2 mm mesh and resultant soils were mixed thoroughly to get a uniform composite sample of homogenous nature. Soil was stored in plastic bags and kept in the dark at room temperature until used in microcosm experiments. Subsamples were drawn from the composite sample for determination of pre-experimental physico-chemical parameters of soil using standard protocols described in Table 1. In addition, water-holding capacity of the soil was also measured following a method described by Jarrell et al. (1999) so that the incubation experiment could be performed at 70% of the field water capacity.

2.2. Sampling and analysis of textile wastewater

Samples of untreated textile wastewater were collected from a functional textile industry located at Khurrianwala in Faisalabad

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Table 1
Physical and chemical properties of soils used in the study.

Physical/chemical property	Units	Value	Reference
$pH_{1:5}$	–	7.92	–
Electrical conductivity ($EC_{1:5}$)	d Sm^{-1}	1.52	–
Sand	g kg $soil^{-1}$	427	Gee and Bauder (1986)
Silt	g kg $soil^{-1}$	251	
Clay	g kg $soil^{-1}$	322	
Textural class	–	Clay loam	
Total soil N	g kg $soil^{-1}$	1.61	Bremner and Tabatabai (1972)
Total organic carbon	g kg $soil^{-1}$	6.45	Walkley and Black (1934)
Soil organic matter	g kg $soil^{-1}$	11.09	
Water extractable organic matter	mg kg $soil^{-1}$	129.60	

(31.5172° N, 73.2667° E). Characteristics of textile industry from sampling area are described in detail by Arif et al. (2016). Untreated textile wastewater samples were collected in triplicate in clean plastic sampling bottles from the direct discharge point of the industrial unit. Sampling bottles were rinsed with the textile wastewater to be sampled before taking the samples. Approximately 2.5 L samples were collected for each replicate so that a sufficient volume of wastewater was present to allow all physico-chemical analysis and experimentation. Wastewater was devoid of any nitrogen or sulfur from wet deposition from the atmosphere as no rainfall had happened in the earlier weeks or on the sampling day. Samples were transported in an ice-cooler to laboratory and kept at 4 °C to stop any variation until used for further experimentation (Arif et al., 2016). Values of pH and EC were measured within 24 h of sampling whereas other analysis were performed within 3–7 days of sample collections.

2.3. Experimental

2.3.1. Microcosm fabrication, experimental design, treatment application and incubation

Microcosms were constructed using PVC pipes of 3 cm diameter and 10 cm length with a fine sealed mesh at bottom for optimum air circulation according to the method described by Riaz et al. (2012). Microcosms were filled with homogenized 100 g of soil and five microcosms were used for each treatment. Treatments consisted of wastewater application at four levels of concentration i.e. 0 (control treatment), 25, 50 and 100% and the experiment was laid out following completely randomized design (CRD) in an incubator at 24 ± 1 °C. Two sets of microcosms were prepared so that destructive soil analysis could be performed after 30- and 60-d incubation time periods. Soil filled microcosms were sprinkled with treatment solutions equivalent to 70% of field capacity and the microcosms were pre-incubated for one week before the start of the experiment. When the pre-conditioning/incubation period was over, necessary to get rid of initial large flush of microbial activity before start of the experiment, microcosms were placed in 1.5 L airtight glass jars and incubated in an incubator at 24 ± 1 °C. In order to measure the soil basil respiration and quantify soil C mineralization and microbial activity, plastic vials containing freshly prepared 20 mL 1.0 N NaOH were placed inside the incubation jars. In addition, small plastic vials of 5 mL distilled-water were also placed to prevent soil desiccation by keeping the humidity relatively constant within the incubation setup. Moreover, microcosms were reweighed at each of soil respiration measurement time or, at least, twice a week throughout the study to maintain the moisture content of each microcosm by adding an appropriate volume of respective treatment solutions. Glass jars containing an alkali trap and distilled-water plastic vials but no soil were also incubated to serve as the blanks for CO_2 evolution measurement.

2.3.2. Measurement of soil C mineralization and microbial activity

Rates of soil C mineralization and soil microbial activity were assessed by measuring soil respiration throughout the experiment by trapping CO_2 released from soil by modified method of Jaggi (1976). For this purpose, plastic vials containing 20 mL 1.0 N NaOH were placed in each incubation jar to capture the CO_2 released. Alkali traps were replaced at regular intervals with new plastic vials containing freshly prepared 20 mL 1.0 N NaOH. Excess alkali was precipitated by adding 0.5 M $BaCl_2$ solution. Concentration of CO_2 was measured by titrating the alkali solution against 0.5 M HCl using phenolphthalein, diluted into 100 mL ethanol (60%, v/v), as an indicator. Anticipating a higher level of microbial activity and C mineralization rates, soil respiration was measured more frequently in the beginning of the experiment whereas the soil respiration measurements were performed on 3–4 day interval at the later stage of the experiment. All treatment samples were corrected for the CO_2 content of blanks. Cumulative soil respiration values are presented rather than the soil respiration rates in the study. We calculated and presented the cumulative respiration for 1–30 and 31–60 days incubation periods separately so that the effects of incubation time could be investigated.

2.3.3. Destructive microcosm soil sampling

After being incubated for 30- and 60-d, soil from each microcosm was removed carefully and transferred into clean plastic bags. Soil was homogenized gently within the plastic bags and stored immediately at 4 °C until further processed for further analysis of physico-chemical, biological and biochemical properties.

2.4. Analytical protocols

2.4.1. Textile effluent analysis

Physico-chemical characteristics of untreated textile wastewater were assessed using standard protocols. Turbidity of wastewater was measured using a portable microprocessor controlled turbidity meter (Hanna, Model HI93703). pH and electrical conductivity (EC) were measured on unfiltered wastewater samples within 24 h of sampling. pH was measured with a pH meter (HANNA Model 210) fitted with a calomel and glass electrodes standardized with buffers of pH 7.0 and 10.0 respectively whereas EC was determined with a EC meter (HANNA Model HI 8733). Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were determined according to respirometric method and the method of Knechtel (1978) respectively. Other analyses of textile wastewater were performed using standard methods for examination of water and wastewater (APHA, 1998; Arif et al., 2016).

2.4.2. Soil physico-chemical analysis

Soil pH and EC was measured in soil suspension at 1:5 (soil: water w/v) with the help of pH and EC meter as described above. Moisture content (%) and field capacity of soil were calculated by gravimetric and soil saturated paste percentage (saturation percentage, SP) method respectively according to the protocols given in the Hand Book No. 60 of the U.S. Salinity Lab. Staff (1954). Soil organic matter and total organic carbon was measured by dichromate oxidation and subsequent titration with acidified ferrous ammonium sulfate using a modified Walkley and Black method (Walkley and Black, 1934; Jackson, 1962). Soil particle size analysis were performed using Bouyoucos hydrometer method (Bouyoucos, 1962) but only for the soil at the start of the experiment. Soil total N was determined by the method of Bremner and Tabatabai (1972) following Kjeldahl's digestion and distillation procedure. Values of different soil parameters were always expressed on an oven dry soil basis unless otherwise mentioned.

2.4.3. Water-extractable organic matter (WEOM), microbial biomass carbon (MBC) and microbial metabolic quotient (qCO_2)

Analysis of WEOM in moist soil samples was conducted following methods described by Ghani et al. (2003) and Zhang et al. (2006). Briefly, 5.0 g moist soil samples were weighed into 50 mL centrifuge tubes and 25 mL distilled water was added to them. The mixture was shaken on a horizontal shaker at 150 rpm for 90 min, and then centrifuged at 10,000 rpm for 5 min. The resultant supernatant was filtered through a Whatman#42 filter paper. Total organic C concentrations in the extract were quantified with a modified Walkley and Black method (Walkley and Black, 1934; Jackson, 1962).

Pool of soil MBC was determined by fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). For this, two 10-g moist soil sub-samples were taken into 50 mL centrifuge tubes. To the first set of tubes, 50 mL of 0.5 M K_2SO_4 were added and the mixture was placed on a horizontal shaker at 120 rpm for 60 min. The mixture was centrifuged at 10,000 rpm for 5 min and the supernatant was filtered through Whatman#42 filter paper and immediately frozen at −20 °C until analysis. Second set of samples was fumigated with ethanol-free chloroform in a glass desiccator for 24 h in the dark. After the fumigation and incubation process, the samples were treated a way similar to non-fumigated samples. Total organic C concentrations in fumigated and non-fumigated samples were measured using a modified Walkley and Black method (Walkley and Black, 1934; Jackson, 1962). The difference between the fumigated and non-fumigated C concentrations recorded as MBC. Since no extraction efficiency values (K_{EC}) exist for soils in Pakistan, we opted not to use K_{EC} values for MBC widely reported in literature for other soil. Microbial metabolic quotient (qCO_2) was calculated by dividing soil respiration by MBC as described by Anderson and Domsch (1990).

2.4.4. Soil dehydrogenase activity

The activity of dehydrogenase enzyme in the soil was measured for the soil incubated for 60-d with the method of Casida et al. (1964) which employs measuring reduction of 2,3,5-triphenyltetrazolium chloride (TTC) into triphenyl formazan (TPF) with spectrophotometer. Briefly, 6.0 g moist soil sub-samples were incubated with 3% TTC in a test tube for 24 h at 37 °C. After incubation, 10 mL methanol was added to extract TPF. The mixture in each test tube was mixed thoroughly and resultant supernatant was filtered by methanol washing. Absorbance of TPF was measured at 485 nm with a UV−vis spectrophotometer (Dynamica, HALO DB-20) and expressed as μg triphenyl formazan (TPF) g^{-1} soil h^{-1}.

3. Statistical analysis

Data were assessed for homogeneity and normality of distribution, and log transformed when required prior to analysis of variance tests. We performed multivariate analysis of variance (MANOVA) test to study treatment, incubation time and their interactive effects on soil variables using treatment and incubation time as independent fixed factors. Tukey's HSD posthoc test was used for multiple means comparison techniques for the soil variables when required. Effects of wastewater application on soil dehydrogenase activity were quantified using one-way analysis of variance (ANOVA) test followed by Tukey's HSD posthoc test. Linear regression curves were fitted to the exploratory scatterplots. Data were always presented as means followed by standard errors in Tables and Figures unless otherwise stated. All statistical analyses were performed using SPSS for Windows software v. 19.

4. Results

4.1. Physico-chemical properties of soil and untreated textile wastewater

Physicochemical properties of soil used in the study are presented in Table 1. Soil was alkaline clay loam and had $EC_{1:5}$ of 1.52 dS m^{-1}. Soil represented a typical Aridsol containing 6.45 and 11.09 g kg $soil^{-1}$ total organic carbon and soil organic matter contents respectively whereas the concentrations of WEOM were 129.60 mg kg $soil^{-1}$.

Characteristics of the untreated textile wastewater are shown in Table 2. The wastewater was moderately alkaline, highly turbid and greenish purple in color. Values of COD and BOD were relatively higher whereas DO concentrations were 10.7 mg L^{-1}. Total carbon (TC), total inorganic carbon (TIC) and dissolved organic carbon (DOC) contents of wastewater were 390.2, 159.9 and 230.3 mg L^{-1}, respectively. The textile wastewater was highly saline in nature and contained high concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+}.

4.2. Effects of textile wastewater on soil pH and EC

We found a highly significant wastewater application effect on soil pH and EC ($p < 0.001$; Tables 3 and 4). Values of pH increased from 8.52 ± 0.02 at 0% (distilled-water) treatment to 8.68 ± 0.02 at 100% wastewater application rate for the soil incubated for 30-d period. However, for the 60-d incubated soil, a similar increase in soil pH was observed along the wastewater concentration gradient. Incubation time has significant ($p < 0.001$) effect on soil pH which was lower in soil incubated for 60-d, however. Values of soil pH were found to be significantly higher after application of undiluted wastewater (100%) compared to the rest of the treatments at each incubation time (Table 4). Soil EC ranged from 2.15 ± 0.00 to 3.81 ± 0.00 dS m^{-1} in the 30-d and 3.10 ± 0.09 to

Table 2
Selected properties of textile wastewater.

Physical/chemical property	Unit	Value
Color	–	Greenish purple
Turbidity	FTU	91
pH	–	10.2
EC	d Sm^{-1}	7.32
Chemical oxygen demand (COD)	mg O_2L^{-1}	1129
Biological oxygen demand (BOD)	mg O_2 L^{-1}	372
Dissolved oxygen (DO)	mg L^{-1}	10.7
Total dissolved solids (TDS)	mg L^{-1}	4831
Total carbon (TC)	mg L^{-1}	390.2
Total inorganic carbon (TIC)	mg L^{-1}	159.9
Dissolved organic carbon (DOC)	mg L^{-1}	230.3
Na^+	mg L^{-1}	1300
K^+	mg L^{-1}	19.5
Ca^{2+}	mg L^{-1}	170.4
Mg^{2+}	mg L^{-1}	21.6

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Table 3
Effect of treatment, incubation time and their interaction on soil physico-chemical and biological variables.

Source	Variable	df	F-value	p-value
Treatment	pH	3	30.70	<0.001
	EC	3	53.17	<0.001
	Cumulative respiration	3	2.95	<0.05
	MBC	3	5.22	<0.01
	WEOM	3	4.39	<0.05
	qCO_2	3	5.66	<0.01
Incubation time	pH	1	592.1	<0.001
	EC	1	293.4	<0.001
	Cumulative respiration	1	147.6	<0.001
	MBC	1	34.27	<0.001
	WEOM	1	10.98	<0.01
	qCO_2		6.28	<0.05
Treatment × Incubation time	pH	3	0.95	0.429^{ns}
	EC	3	0.21	0.890^{ns}
	Cumulative respiration	3	0.63	0.602^{ns}
	MBC	3	2.09	0.121^{ns}
	WEOM	3	6.09	<0.01
	qCO_2	3	0.29	0.831^{ns}

ns = non-significant results.

treated soil. We found non-significant treatment into time interaction effect on soil respiration (Table 3).

4.4. Effects of textile wastewater on MBC, WEOM and qCO_2

Wastewater application resulted in significant changes in soil MBC ($p < 0.01$; Table 3). Increasing concentrations of wastewater reduced MBC in the soil incubated for 30- and 60-d time periods (Fig. 2a). In response to wastewater treatment, values for MBC were reduced from 370.49 ± 84.98 to 174.98 ± 19.99 mg C kg soil^{-1} in 30-d incubated soil whereas similar treatment effect trends were seen in soil incubated for 60-d. However, MBC pools in 60-d incubated soil were 1.0–1.5 folds less compared to the soil incubated for 30-d, and incubation time has significant effect on MBC ($p < 0.001$; Table 3).

Changes in WEOM concentrations after application of untreated wastewater have been shown in Fig. 2b. Treatment resulted in significant ($p < 0.05$) effect on WEOM concentrations (Table 3). For the soil incubated for 30-d time, the highest WEOM concentrations were noted at 25% wastewater application rate followed by the

Table 4
Changes in pH and EC (d S/m) of soils treated with textile wastewater and incubated for 30 and 60 days.

Wastewater concentration (%)	pH		EC (d Sm^{-1})	
	Day-30	Day-60	Day-30	Day-60
0	8.52 ± 0.02 a	8.12 ± 0.02 a	2.15 ± 0.00 a	3.10 ± 0.09 a
25	8.52 ± 0.02 a	8.16 ± 0.02 a	2.41 ± 0.00 b	3.53 ± 0.26 ab
50	8.60 ± 0.03 ab	8.20 ± 0.00 a	2.67 ± 0.09 c	3.78 ± 0.00 b
100	8.68 ± 0.02 b	8.34 ± 0.02 b	3.81 ± 0.00 d	4.21 ± 0.00 c

Values are means of five replicates followed by ± standard error of means. In a column, values with different letters differ significantly from each other for the specified period for specific parameter.

4.21 ± 0.00 dS m^{-1} in the 60-d incubated soil. Soil EC values were found to be the lowest in distilled-water treated and the highest in the wastewater treated soil at 100% concentration for soil at both incubation times. Incubation time has significant effect on soil EC values ($p < 0.001$; Table 3), however, in contrast to changes in pH, soil EC values were higher in soil incubated for 60-d compared to those incubated for the 30-d time period. Results from MANOVA revealed that soil pH and EC were not affected significantly by interactions between treatment and incubation time (Table 3).

4.3. Effects of textile wastewater on soil respiration

Changes in soil cumulative respiration in response to wastewater application over the two incubation periods are shown in Fig. 1. Treatment had significant ($p < 0.05$) effect on soil cumulative respiration (Table 3). Soil respiration responded positively to wastewater and increased sharply with the incubation time during the 1–30 day measurement period (Fig. 1a). Application of 100% wastewater resulted in consistently higher soil respiration. However, there was a dynamic shift in soil respiration in response to wastewater treatments at 25 and 50% concentrations: treatment of 50% wastewater suppressed CO_2 evolution in the beginning, however, the same treatment resulted in higher soil respiration at the later stages of the 1–30 day incubation period. Soil cumulative respiration was significantly ($p < 0.001$) lower for the measurement period spanning over 31–60 days (Table 3; Fig. 1b) and increase in soil respiration rates were also less steep. For this time period, treatment consisting of 100% wastewater produced the highest soil respiration. However, application of 25 and 50% wastewater resulted in reduced C mineralization and, hence, the soil respiration for these treatments were less compared to the distilled-water distilled-water control treatment. However, in the 60-d incubated soil, significantly higher WEOM accumulated after application of 100% concentrated wastewater (Fig. 2b). Incubation time also had a significant ($p < 0.05$) effect on WEOM concentrations. Resultantly, WEOM concentrations of 60-d incubated soil were higher in distilled-water and, 50% and 100% wastewater applied soil compared to those incubated for 30-d at the same treatment levels. We found significant ($p < 0.01$) treatment and incubation time interactive effect on WEOM concentrations (Table 3).

Microbial metabolic quotient (qCO_2; respiration to biomass ratio) increased significantly ($p < 0.01$) in response to wastewater application (Tables 3 and 5). Values of qCO_2 increased from 0.71 ± 0.11 in distilled-water treated soil to 1.68 ± 0.34 in 100% wastewater treated soil after 30-d incubation period whereas similar treatment effects were noted in soil incubated for 60-d. We also found significant ($p < 0.05$; Table 3) incubation time effect on qCO_2: values of qCO_2 were higher in 60-d incubated soil than the 30-d incubated soil (Table 5). There was non-significant treatment into incubation time effect on qCO_2 however.

4.5. Effects of textile wastewater on soil dehydrogenase activity

Changes in dehydrogenase activity in soil samples treated with wastewater and incubated for 60-d are shown in Fig. 3. Application of wastewater significantly ($p < 0.05$) increased the soil dehydrogenase activity which increased from 35.46 ± 0.58 μg triphyenyl-formazan 12 h^{-1} g soil^{-1} in distilled-water treated soil to 45.32 ± 0.74 μg triphyenyl-formazan 12 h^{-1} g soil^{-1} in wastewater treated soil at 100% concentration level. Dehydrogenase activity was also significantly higher in soil treated with 50 and 100% concentrated wastewater compared to the distilled-water

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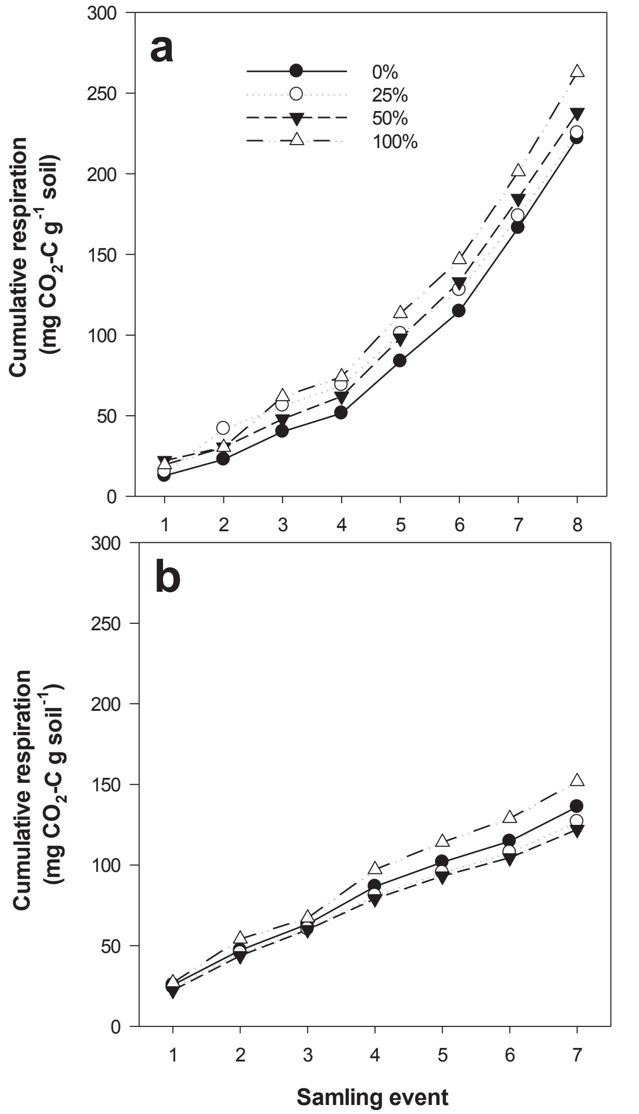

Fig. 1. Cumulative respiration (mg CO_2-C g soil^{-1}) of soils treated with textile wastewater and incubated for a) 0–30 days and b) 31–60 days. Values are means of five replicates.

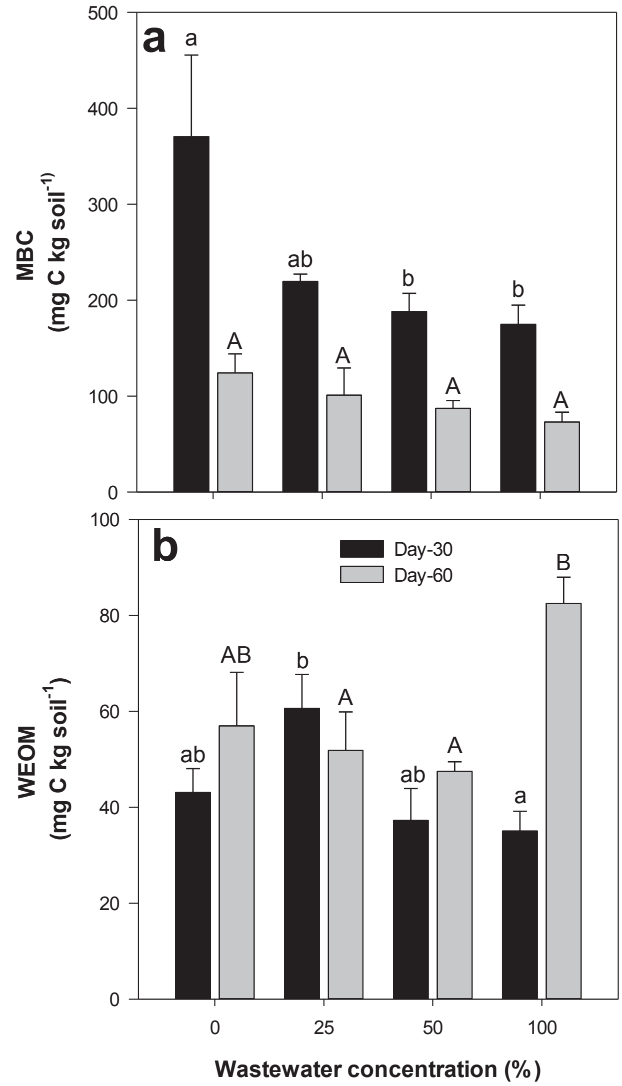

Fig. 2. Effects of textile effluent application on a) MBC (mg C kg soil^{-1}) and b) WEOM (mg C kg soil^{-1}) in soils incubated for 30 and 60 days. Values are means of five replicates and contain ± standard errors of means. Black bars with different letters indicate significant treatment effects in soils incubated for 30 days and grey bars with different letters indicate significant treatment effects in soils incubated for 60.

amended soil. Increase in soil dehydrogenase activity in response to wastewater concentration was relatively linear ($y = 3.09x+31.91$, $R^2 = 0.92$; from Fig. 3).

4.6. Relationships among the soil variables

We produced bivariate scatter plots to explore the relationships between dependent and independent soil variables, and the strength of these correlations was computed by fitting linear regression lines. There was a significant positive relationship of soil pH with soil respiration ($R^2 = 0.82$, $p < 0.001$; Fig. 4a) and MBC ($R^2 = 0.51$, $p < 0.001$; Fig. 4c). Soil EC had a significant negative correlation with soil respiration ($R^2 = 0.40$, $p < 0.001$; Fig. 4b) and MBC ($R^2 = 0.67$, $p < 0.001$; Fig. 4d). We also observed a rather weak but significantly negative correlation between WEOM and MBC ($R^2 = 0.18$, $p < 0.01$; Fig. 5a) whereas the correlation between MBC and soil respiration was significant and positive ($R^2 = 0.64$, $p < 0.001$; Fig. 5b). We also related the soil dehydrogenase activity to the soil respiration and noted a significant positive relationship between them ($R^2 = 0.21$, $p < 0.05$; Fig. 6a). Data were log transformed before drawing scatterplot between the soil dehydrogenase activity and qCO_2 values, and resultantly, a significant positive correlation was observed between these two variables ($R^2 = 0.21$, $p < 0.05$; Fig. 6b).

Table 5
Changes in microbial metabolic quotient (qCO_2; respiration to biomass ratio) of soils treated with textile wastewater and incubated for 30 and 60 days.

Wastewater concentration (%)	Day-30	Day-60
0	0.71 ± 0.11 a	1.23 ± 0.21 a
25	1.01 ± 0.12 ab	1.55 ± 0.33 a
50	1.31 ± 0.10 ab	1.46 ± 0.16 a
100	1.68 ± 0.34 b	2.18 ± 0.23 a

Values are means of five replicate followed by ± standard error of means. In a column, values with different letters differ significantly from each other for specified period for specific parameter.

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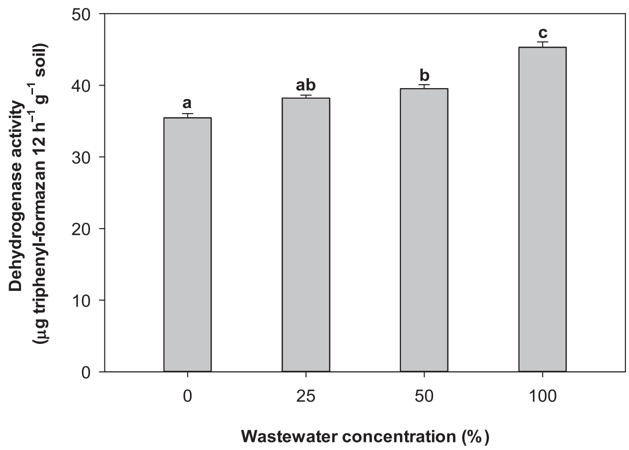

Fig. 3. Effects of textile effluent application on dehydrogenase activity (µg triphyenyl-formazan 12 h^{-1} g $soil^{-1}$) of soils incubated for 60 days. Values are means of five replicates and contain ± standard errors of means. Bars with different letters differ significantly from each other at $p < 0.05$.

5. Discussion

5.1. Changes in soil pH and EC

The results of this study revealed that the untreated textile wastewater application led to significant increase in soil pH along wastewater concentrations, however, pH values of the soil incubated for 60-d was significantly less than those from the 30-d incubated soil. Soil EC increased consistently and significantly in response to treatment and incubation time. Increase in soil pH after application of untreated wastewater could be the result of addition of exchangeable cations such as Na which was present in higher concentrations in untreated wastewater. However, the observed decrease in soil incubated for 60-d may be due to the acidifying effect from production of free hydrogen ions following decomposition of organic compounds present in wastewater as suggested by Mojiri (2011). Similar increases in pH have been reported after irrigation with treated (Qian and Mecham, 2005; Adrover et al., 2012) and untreated wastewater (Gelsomino et al., 2006). However, we argue that the decrease in soil pH in 60-d incubated soil might not exist for longer time due to the natural buffering capacity of soil. Increases in soil EC was possibly due to release of mineral nutrients following decomposition of organic matter in untreated wastewater (Anjaneyulu et al., 2011). In addition, alkaline pH and high salinity of the incubated soil corresponded to highly saline and alkaline nature of the untreated wastewater used in the study. Values of pH and EC of the untreated wastewater exceeded the National Environmental Quality Standards (NEQS) values as well as the standard limits according to the FAO Guidelines for irrigation (NEQS, 1997; Pescod, 1992). Several studies have also observed changes in soil pH and EC after irrigation with wastewater from variety of origins; for examples, Faryal et al. (2007) found decrease in soil EC after application of textile wastewater whereas increase in soil pH have been noted after irrigation with distillery wastewater (Kaushik et al., 2005), olive oil mill effluent (Sierra et al., 2007) and dye industrial wastewater (Ahmad et al., 2012). Irrigation with high pH and EC wastewater could potentially lead to entering more K^+ into soil clay exchange complex and producing alkaline hydrolysis reactions (Di Serio et al., 2008; Marsilio et al., 1990; Proietti et al., 1995). Such changes may deteriorate the soil structure or disperse the clay particles and, consequently, affect the soil biological activities (Chaerun et al., 2011).

5.2. Changes in soil C mineralization, MBC and WEOM

Treatment and incubation time had marked and significant effects on CO_2 evaluation dynamics in this study. Cumulative respiration was always higher in soil treated with 100% wastewater, however, soil respiration was less over the 31–60 day measurement compared to the first phase of measurement i.e. 1–30 day. In contrast to the soil respiration measured for 1–30 d, treatment consisting of 25 and 50% wastewater had lower CO_2 evolution compared to the distilled-water control. Initial higher C mineralization was likely the result of the rapid mineralization of organic matter (labile C pool) by microorganisms or instant biodegradation of organic compounds present in untreated wastewater (Piotrowska et al., 2006). Our results are contrary to those described by Brzezinska et al. (2006) who measured decreased CO_2 evolution following application of wastewater at higher doses. Availability of organic C generally enhance soil respiration, however, salinization from cations such as Na^+ in wastewater can disrupt this relationship negatively but this toxic ionic effect may be overcome by the dominant organic C effect on soil respiratory activity (Friedel et al., 2000). This effect could be, at least, partially responsible for the higher soil respiration in soil treated with 100% concentrated wastewater. However, the reduction in cumulative CO_2 evolution measured over 31–60 day incubation period in soil treated with 25 and 50% wastewater might reflect the dominance of salinity effect over the C availability effect.

Increase in wastewater concentrations and incubation time seemed to have negative impact on MBC contents. We found more or less similar MBC contents for soil treated with 50% and 100% wastewater concentration, and incubated for 30- and 60-d duration. Our results are in contrast to widely reported findings claiming increase in MBC after application of both treated and untreated wastewater, and attributed this increase to easily degradable organic matter and other nutrients present in wastewater (e.g. Garcia-Gil et al., 2000; Chen et al., 2008). These studies, however, were performed in the field and on much longer timescale compared to our study. We also relate this decrease in MBC to soil salinity due to highly saline nature of the textile wastewater used in this study. Decrease in MBC generally corresponded to reduced in C mineralization in 60-d incubated soil. Such an observation suggests reduced C substrate availability over the time as the experiment progressed. Consequently, low metabolic activity resulted in reduced C mineralization and MBC. This trend could also suggest steady-state microbial activity in response to wastewater application at a particular concentration level (Pascual et al., 1997; Armenta et al., 2012).

Both the treatment and incubation time factors significantly altered WEOM concentrations. Concentrations of WEOM were significantly higher for soil incubated for 60-d than 30-d at 50% and 100% wastewater application rates. Similar trends have been observed after application of municipal and sewage sludge by Mojiri (2011) and Armenta et al. (2012). In another study, Sierra et al. (2007) found that olive oil mill waste water increased the concentration of soil organic matter which stimulated the biological activity by providing easily mineralizes fractions of water soluble organic carbon. In some long-term studies, the concentrations of water soluble organic carbon have also been found to increase in soil mainly due to the high content of dissolved organic matter in wastewater (e.g. Fernandes et al., 2005; Kaushik et al., 2005; Alvarez-Bernal et al., 2006; Adrover et al., 2012). We observed a significant interaction between the treatment and incubation time which suggested buildup of soil organic matter and WEOM over the time. We applied the wastewater treatments only at the beginning of the experiment and only small fractions were added later on when required to adjust the moisture content of the

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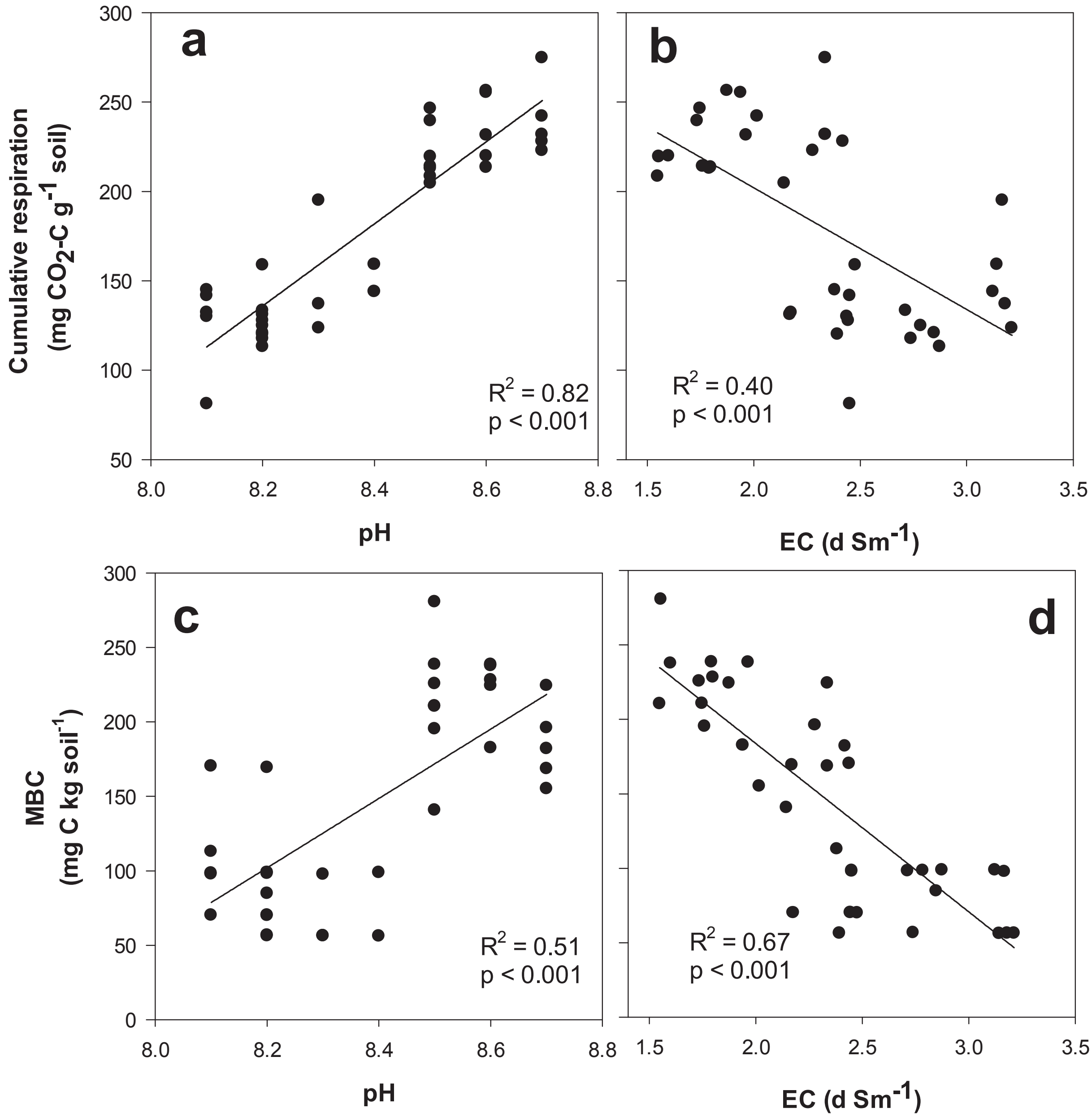

Fig. 4. Relationships of cumulative respiration (mg CO2-C g soil^{-1}) with a) pH and b) EC and MBC (mg C kg soil^{-1}) with c) pH and d) EC.

soil. However, the higher WEOM concentrations could be the result of the widely accepted phenomenon that addition of organic matter with wastewater increase soil organic matter contents (An et al., 2004).

5.3. Effects on microbial metabolic quotient (qCO_2)

Microbial metabolic quotient (qCO_2; respiration to biomass ratio) increased with wastewater treatment and incubation time. qCO_2 has been considered an important parameter to interpret microbial activity and efficiency under stressed conditions. It also reflects the energy requirement by microorganisms to maintain their metabolic activity to synthesize biomass (Bardgett and Saggar, 1994). Higher qCO_2 values generally indicate an ecological disturbance such as toxic effects and greater ecosystem productivity (Anderson and Domsch, 1993; Islan and Weil, 2000). Our results suggest that increasing the concentration of the wastewater increased the qCO_2 values and, hence, the stress to the microorganisms. However, increase in qCO_2 values could also suggest enhanced microbial activity. Such observations state that microorganisms may adopt defensive strategies under stressed conditions by increasing their respiration per unit biomass (Anderson and Domsch, 1993). In these conditions, the microorganisms produce more energy for their survival as a defensive mechanism in hostile environment but they are not actually proliferating. Results of qCO_2 reported in this study are similar to those obtained by Carmo (2001) and Fernandes et al. (2005) who found higher qCO_2 values in sewage sludge treated soil at higher application rates. Increase in qCO_2 values with treatment in this study could also be due to addition of organic matter rich wastewater as suggested by Wardle and Ghani (1995). However, increase in qCO_2 values over time may indicate higher microbial activity under more stressed conditions as labile C source either diminished or not accessible by microorganisms.

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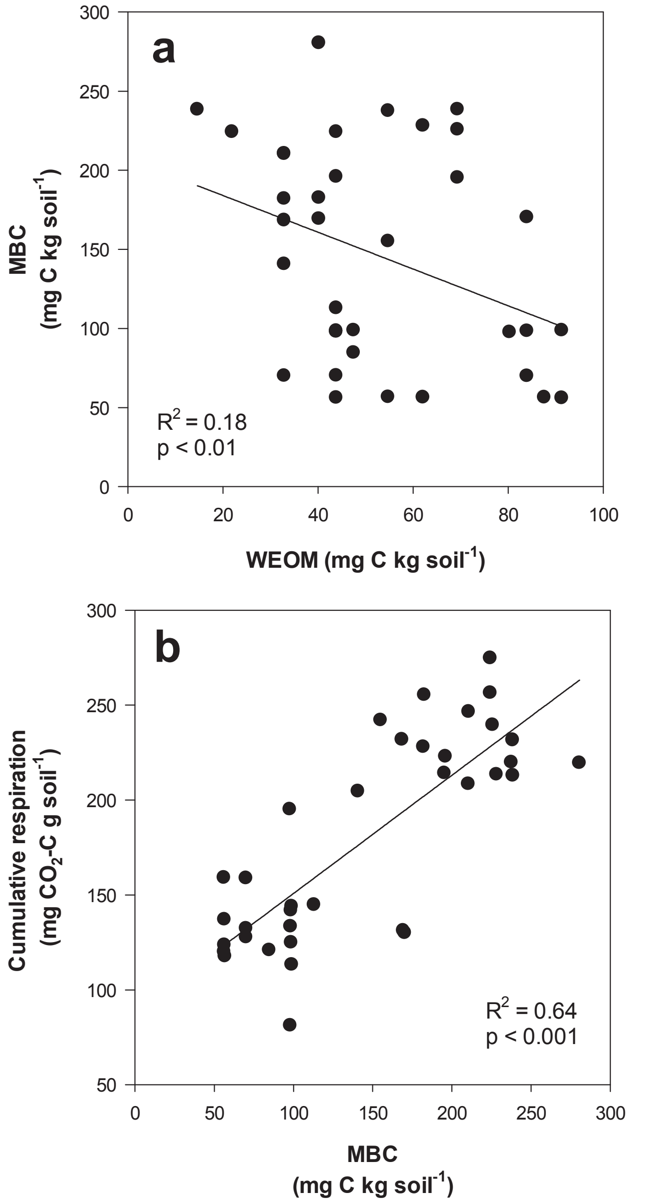

Fig. 5. Relationship of a) MBC (mg C kg soil^{-1}) with WEOM (mg C kg soil^{-1}) and b) cumulative respiration (mg CO_2-C g soil^{-1}) with MBC (mg C kg soil^{-1}).

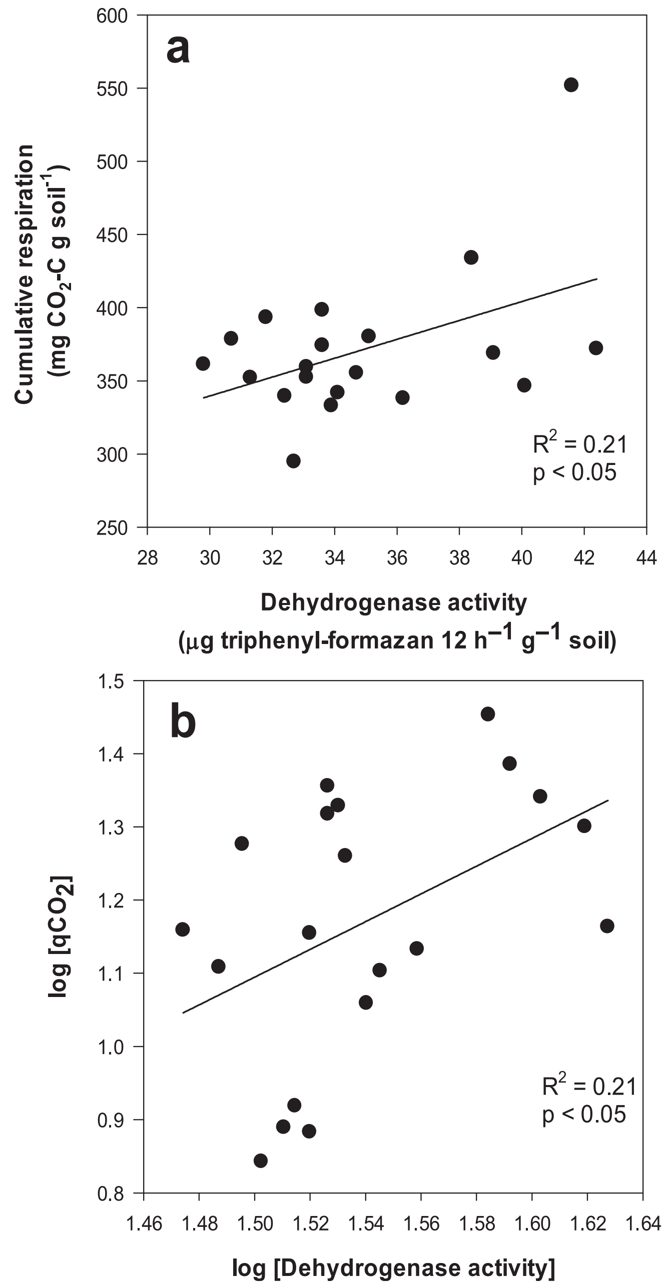

Fig. 6. Relationship between a) dehydrogenase activity (μg triphyenyl-formazan 12 h^{-1} g soil^{-1}) and cumulative respiration (mg CO_2-C g soil^{-1}), and b) dehydrogenase activity and microbial activity quotient (qCO_2). Data belongs to soil samples incubated for 60 days whereas cumulative respiration represents soil respiration measurements from 0 to 60 days.

5.4. Changes in soil dehydrogenase activity

Dehydrogenase activity increased significantly with application of wastewater for soil incubated for 60-d. Dehydrogenase is an intracellular enzyme which represent soil metabolic state and considered a compelling indicator of soil microbial activity under semiarid conditions (García et al., 1994). This consistent increase in dehydrogenase activity could be related to the high labile C contents of the textile wastewater (DOC, 234.3 mg L^{-1}). The biodegradation of organic compounds by soil enzymes and resultant high C contents in soil in terms of WEOM could have increased the dehydrogenase activity (Arif et al., 2016; Kaushik et al., 2005). The activity of dehydrogenase enzyme is generally sensitive to abiotic stress. For example, dehydrogenase activity decreased with heavy metal stress (Mikanova, 2006), slag particles (Anjaneyulu et al., 2011) and treated wastewater (Kayikcioglu, 2012) due to low or no availability of organic nutrients and/or toxicity. Friedel et al. (2000) found similar results and observed higher dehydrogenase activity in soil treated with wastewater. Our findings of increase in dehydrogenase activity are consistent with those reported by Arif et al. (2016) for the similar soil, however, values of dehydrogenase activity were slightly higher in the current study. Brzezińska et al. (2001) summarized various studies claiming varied results of sewage sludge on soil enzymatic activities and identified the lack of studies relating to wastewater.

5.5. Influence of changes in soil variables

Changes in soil pH and EC generally reflect immediate effects of wastewater application. pH and EC had contrasting but significant relationships with soil respiration and MBC. We found significant positive linear relationship of soil pH with soil respiration and MBC whereas the same correlation was significant negative for EC. Negative relationship of MBC and soil respiration with EC could be due to high concentration of salts present in untreated wastewater (Mohammad and Mazahreh, 2003; Arif et al., 2016). Significant correlation was observed between soil respiration and MBC in this study which is in accordance with some other studies reporting similar relationships (e.g. Fernandes et al., 2005; Chaerun et al., 2011). This relationship also indicates that MBC contents are parallel to soil respiration (Chaerun et al., 2011). Bene et al. (2013) reported that olive mill wastewater altered MBC and soil respiration in a short term scale, however, over the longer time period, no significant impact was found.

High solubility of WEOM in wastewater could increase rate of biodegradation in soil, however, at higher wastewater concentrations, labile C might not be available to microorganisms; the later argument is supported by negative correlation between MBC and WEOC. We found positive relationships between soil dehydrogenase activity and soil respiration and, also, between microbial qCO_2 and dehydrogenase activity. This suggests that increasing the concentrations of C substrate in wastewater enhanced growth of native soil microorganisms and resulted in higher levels of dehydrogenase synthesis (Benitez et al., 1999). We measured soil dehydrogenase activity in soil incubated for 60-d only and could potentially overlooked the changes on shorter scale. Piotrowska et al. (2006) claimed higher soil respiration and dehydrogenase activity in soil incubated for 28-d when labile C pool decomposed at faster rates, however, dehydrogenase activity decreased over time. Nevertheless, these findings suggest a dynamic mismatch between microbial activity and substrate availability at higher wastewater concentrations.

6. Conclusions, implications and further research

We conducted an incubation study to investigate effects of untreated textile wastewater on soil C mineralization, MBC, qCO_2, dehydrogenase activity and some chemical properties of Aridisol which had never received wastewater in the past. Four levels of wastewater concentrations and two incubation regimes were used in the study. The results revealed that wastewater application and incubation time significantly altered soil microbial activity and soil chemical variables. The presence of organic matter and labile C in untreated wastewater stimulated microbial activity, however, as the experiment progressed, microbial activity was reduced. Increase in qCO_2 with wastewater concentration and incubation time suggesting more stress to microorganisms, and also, enhanced microbial activity under stress to synthesize biomass. We found increased dehydrogenase activity along wastewater application rates suggesting microbial utilization of C substrate. We observed a dynamic mismatch between substrate availability, labile C pools and microbial activity which suggested evidences of inability of microorganisms to access available substrate. Results of this relatively short-term study should, however, be interpreted cautiously in low organic matter nutrient poor Aridisol used in this study as enhanced microbial activity could be short-lived due to readily available C source. Majority of the wastewater was applied only once in this study whereas repeated application could have differential effects on soil variables. Application of industrial wastewater is an alternative, valuable and cost effective source of irrigation in arid and semi-arid agriculture including Pakistan. However, both short and long-term effects of wastewater irrigation on soil physicochemical, biological and biochemical attributes as well as plant growth should be assessed mechanistically.

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