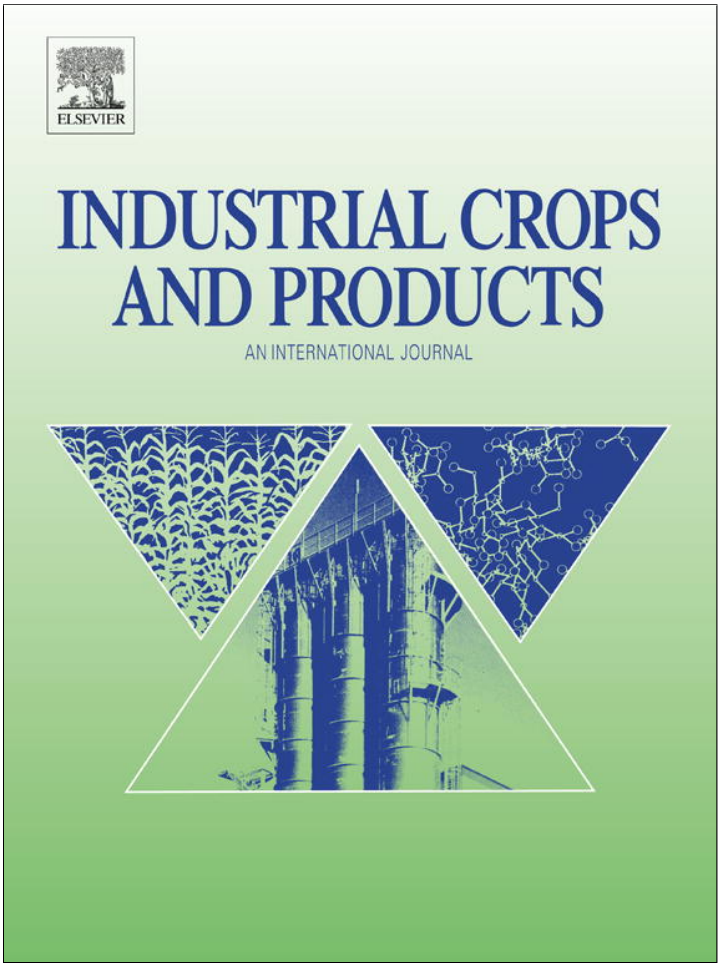
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Productivity, essential oil yield, and heavy metal accumulation in lemon grass (*Cymbopogon flexuosus*) under varied wastewater–groundwater irrigation regimes

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A B S T R A C T

Huge quantities of wastewater generated from municipalities need to be disposed off at regulated rates in non-edible crops viz. aromatic and medicinal plants to avoid food chain contamination and protecting the valuable natural resources. For finding optimum loading rates, an experiment was conducted in lysimeters during 2007–2010 on a sandy loam soil taking 5 irrigation depth:cumulative pan evaporation water regimes (irrigation at 0.6, 0.8, 1.0, 1.2 and 1.5 ID:CPE) of primary treated wastewater, groundwater and their conjunctive use and studied their effects on the herbage yield, essential oil yield, accumulation of heavy metals in lemon grass (*Cymbopogon flexuosus*). Averaged over water quality, herbage yield, dry biomass and essential oil yield varied from 10.11 to 13.68; 3.02 to 3.99 kg m^{-2} and 53.6 to 70.1 mL m^{-2} and were 43, 32 and 30% higher at 1.0 ID:CPE compared to 0.6 ID:CPE, respectively. The yields obtained at 1.0 and 1.2 ID:CPE were at par but significantly reduced with further wetter irrigation regime of 1.5 ID:CPE. Similar yields of lemon grass were obtained at various irrigation regimes of wastewater alone or in conjunction with groundwater and on an average were significantly (16%) higher than the sole use of groundwater. Concentrations of Cd, Cr, Ni and Pb in the herb ranged from 1.54 to 1.85, 3.27 to 4.04, 4.35 to 5.58 and 3.53 to 4.46 mg kg^{-1}, respectively at different irrigation regimes. The accumulation of heavy metals was the maximum in wastewater irrigated lemon grass which got reduced with conjunctive mode and the least with groundwater irrigation. However, heavy metal concentrations in essential oil were not influenced by the water application rates and water quality. In essential oil, Cd was in traces whereas average Cr, Ni and Pb concentrations were 0.14, 0.10 and 0.04 ppm, respectively. Heavy metal concentration both in herb and essential oil were well below the critical or permissible limit. With wastewater irrigation, there was a significant improvement in soil fertility status. Heavy metals started accumulating in soil but were well below the threshold level to reduce the crop growth. The results demonstrated that lemon grass could be successfully grown using primary treated municipal wastewater alone or in conjunction with groundwater at 1.0–1.2 ID:CPE for achieving higher crop productivity without contamination of the end product – the essential oils.

1. Introduction

With increasing fresh water use by the remunerative sectors like municipalities and industries, the availability of water for agriculture is projected to reduce drastically. This in turn means that larger volumes of wastewater are expected to be generated in the coming years. The total sewage generation from Class-I cities and Class-II towns of India is about 38,254 MLD, of which only 11,787 MLD (i.e. about 35%) is treated. Thus most of the sewage is largely let out untreated into either groundwater or natural drainage system thereby causing water pollution (CPCB, 2009). Land application of wastewater for irrigation is considered to be a safe and low cost wastewater disposal strategy with many profits including conservation of water, supplementing water supplies for irrigation and the use of nutrients in the wastewater for productive purposes (Lopez et al., 2006). Wastewater is source of livelihood and increasingly used for irrigating vegetables, fruits, food grains and fodder

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crops in peri-urban areas of several developing countries. However, wastewater also contains salts, pathogens, heavy metals and other pollutants that may contaminate, food chain and hence poses serious threat to human and animal health (Gupta et al., 2008; Simmons et al., 2010).

Cultivation of crops with non-edible economic parts viz. aromatic grasses, cut flowers, etc. has often been proposed as a remunerative and a viable option for preventing pollutants' entry into the food chain (Lal et al., 2008a,b). Lemon grass *(Cymbopogon flexuosus*), a perennial aromatic sedge producing huge biomass, is widely grown for essential oil (Zheljazkov et al., 2011). The oil distilled from the foliage of the lemon grass is used in soaps, detergents, insect repellents, cosmetics and perfumes. India is the largest producer (300–350 tonnes annum^{-1}) of lemon grass oil; 80% of which is exported (National Horticulture Board, Govt. of India, 2005). Medicinal and aromatic crops require substantial amounts of water throughout the growing season (Dudai, 2005). In the northern and the western parts of India, Lemon grass is cultivated as an irrigated crop. Irrigated cultivation favours its enhanced herbage and oil yields (Singh et al., 1996).

However there has been a growing gap in the global production and demand of the lemon grass oil (3900 metric tonnes; Barbosa et al., 2008). Hence, to meet the demand of this industrial crop, expansion of its production to the wastewater irrigated lands seems to be a sustainable option. However, as wastewater is often associated with a low/negligible cost, therefore there is a general tendency of the farmers to over irrigate thereby leading to its negative impacts in terms of say crop lodging, heavy metal accumulation and soil sickness (Minhas and Samra, 2004).

Thus in order to: (a) understand impacts of irrigation water quantity and quality, under different irrigation regimes, on the soil heath; biomass/oil yield and heavy metal accumulation in lemon grass (*C. flexuosus*) and (b) to propose viable wastewater irrigation strategies, a detailed (3 years long) study (from November 2007 to August 2010) was conducted at the farmlands of the Central Soil Salinity Research Institute, Karnal (Haryana), India.

2. Materials and methods

2.1. Experimental lay out and irrigation treatments

The experimental site (located at 75°57′E longitude and 29°43′N latitude) comprised of 45 – circular lysimeters (internal diameter: 0.90 m and depth: 2 m). These were packed with sandy loam (Alfisol) soil, having 60% sand; 27% silt; 13% clay; 7.4 initial pH; 0.26 dS m^{-1} electrical conductivity; 0.35% organic carbon and 0.1% calcium carbonate. On 6th November (2007), each lysimeter was planted with 3-rooted slips of lemon grass (variety OD-19) that was raised following standard agronomic practices (involving 90 kg N and 30 kg P_2O_5 ha^{-1} fertilizer dose). The crop was initially (up to March 2008) established with groundwater and thereafter irrigated with either wastewater (WW)/groundwater (GW) alone or in conjunction (in cyclic mode) at ID:CPE (i.e. irrigation depth:cumulative pan evaporation) schedules of 0.6, 0.8, 1.0, 1.2 and 1.5. The experimental design comprised of 3-replicates of 15-irrigation treatments, arranged in a factorial randomized block design. The depth of each irrigation was set at 50 mm. Amount of rain received during crop growth period was deducted from the cumulative pan evaporation and net amount of irrigation water applied in a year was calculated as (cumulative pan evaporation − rainfall received) × ID:CPE treatment. The applied (primary treated) wastewater was collected from a nearby Sewage Treatment Plant in Karnal (Haryana). Tables 1–3 illustrate the quality and quantity of irrigation waters applied during the experimental period, under varied irrigation regimes.

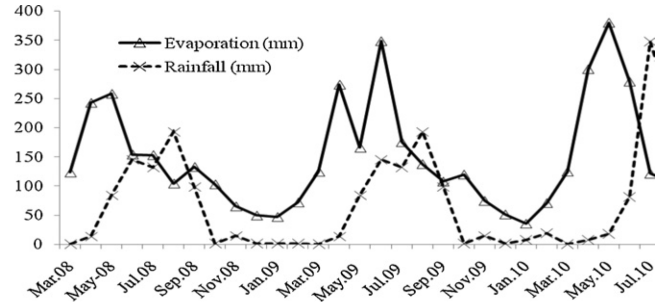

Fig. 1. Monthly pan evaporation (mm) and rainfall (mm) from March 2008 to August 2010 at the study site.

2.2. Climate

Monthly evaporation and rainfall recorded during the study period are presented in Fig. 1. The climate of the study area is semi-arid monsoonal subtropical type. More than 70% of the annual rainfall is received during the monsoon period from July to September. Evaporation rate is generally high during April to June and low during November to February. Of the total (1291 mm) rainfall, about 680 mm was received during March (2008) to December (2008); 477 mm during January (2009) to December (2009) and 133 mm during January (2010) to June (2010). The corresponding total open pan evaporation (total: 4275 mm) for the afore-mentioned periods were 1386, 1699 and 1190 mm, respectively. The amount of rain received (578 mm) in July (2010) and August (2010) was more than 2.5 times higher than the cumulative pan evaporation (227 mm), thereby requiring no additional irrigation during these 2 months.

2.3. Plant, oil, soil, water sampling and analysis

During the experimental period, the crop was harvested seven times. At each harvest, fresh biomass, dry biomass and percent dry weight (% DM) were determined following oven desiccation (at 64 °C for 48 h) of air dry biomass. Dried plant samples were finely ground, wet digested and analyzed for different elements.

To determine essential oil yield and its quality, oil from fresh leaves of lemon grass was extracted using a glass distillation assembly. Thereafter, a known amount (0.2 mL) of oil was digested in nitric acid and subjected to heavy metal analysis. Contents of heavy metals in herb and essential oil were estimated by transversely heated graphite furnace atomic absorption spectrophotometer Analytikjena ZEEnit 700 P (GFAAS) equipped Zeeman background correction. Determinations on Cd, Cr, Ni and Pb with a minimum detection limit of 0.01, 0.14, 0.45 and 0.13 ng g^{-1} were made at wavelength of 228.8, 357.9, 232.0 and 283.3 nm, respectively. Argon was used as the protective and purge gas.

Depth wise soil samples (surface 0–15 and 15–30 m) were also collected from each lysimeter, at the end of the experiment. These were air dried, passed through 2 mm sieve and analyzed for various parameters. The determinations on soil pH (1:2), electrical conductivity (EC 1:2) were made as per the Jackson (1973) methods. Organic carbon (OC) was determined by wet digestion method (Walkley and Black, 1934). The available phosphorus (P) and potassium (1 N NH_4OAc extractable – K) were on the other hand determined through Olsen et al. (1954) and Hanway and Heidel (1952) methods, respectively. DTPA extractable heavy metals (viz. Cd, Ni, Pb) concentrations were determined as per Lindsay and Norvell (1978) method. Readily available soil chromium (Cr) concentrations were determined using diphenylcarbazide method (Bartlett and James, 1979). Besides, dehydrogenase activity (DHA)

Table 1
Physico-chemical characteristics of groundwater and wastewater.

Parameter	Unit	Groundwater	Wastewater	Maximum concentration for irrigation
pH	0–14	7.7	8.2 ± 0.2	6.5–8.0 (Pescod, 1992)
EC	dS m^{-1}	0.6	1.3 ± 0.2	<3.0 (Pescod, 1992)
RSC	mequiv. L^{-1}	1.6 ± 0.2	2.8 ± 0.2	2.5 (Richards, 1954)
SAR		1.7 ± 0.2	4.6 ± 0.5	9.0 (Ayers and Westcot, 1985)
BOD_5	mg L^{-1}	Tr	62 ± 6	<100 (CPCB, 2008)
COD	mg L^{-1}	Tr	112 ± 10	
Nitrogen (N)	mg L^{-1}	Tr	18 ± 2	
Phosphorous (P)	mg L^{-1}	0.3	4.3 ± 0.4	
Potassium (K)	mg L^{-1}	3.5	17 ± 1	
Cadmium (Cd)	mg L^{-1}	Tr	0.1 ± 0.01	0.01 (Pescod, 1992)
Chromium (Cr)	mg L^{-1}	Tr	0.3 ± 0.02	0.1 (Pescod, 1992)
Nickel (Ni)	mg L^{-1}	Tr	0.3 ± 0.02	0.2 (Pescod, 1992)
Lead (Pb)	mg L^{-1}	Tr	1.2 ± 0.12	5.0 (Pescod, 1992)
Total coliforms	MPN/100 mL	Nil	$2.4 \pm 0.3 \times 10^3$	<10,000 (CPCB, 2008) No limit for crops not eaten raw

Table 2
Amounts of nutrients supplied through irrigation water from March 2008 to June 2010.

Treatments	Total irrigation water requirement during March 2008 to June 2010 (I_R, in mm)	Total irrigation water application during March 2008 to June 2010 (I_R – total rainfall of 1291, in mm)	Nutrients supplied by irrigation water (kg m^{-2})								
			N			P			K		
			GW	WW	GW:WW	GW	WW	GW:WW	GW	WW	GW:WW
ID:CPE											
0.6	2565	1274	Tr	22.9	11.5	0.4	5.5	2.9	4.5	21.7	13.1
0.8	3420	2129	Tr	38.3	19.2	0.6	9.2	4.9	7.5	36.2	21.8
1.0	4275	2984	Tr	53.7	26.9	0.9	12.8	6.9	10.4	50.7	30.6
1.2	5130	3839	Tr	69.1	34.5	1.2	16.5	8.8	13.4	65.3	39.3
1.5	6412	5121	Tr	92.2	46.1	1.5	22.0	11.8	17.9	87.0	52.5

Tr, traces; WW, wastewater; GW, groundwater, GW:WW groundwater and wastewater in cyclic mode.

was determined as per Casida et al. (1964) procedure and soil total coliform count through pour plating method (APHA, 2005).

The EC and pH of water samples was determined using standard EC and pH metres, respectively. Nitrogen content in water samples was determined through nitrogen analyzer. The P content was measured by ascorbic acid method using colorimeter, whereas K was measured using flame photometer. Besides this, total trace metal (viz. Cd, Cr, Ni and Pb) concentrations in di-acid (HNO_3 and $HClO_4$) digested water samples were estimated with atomic absorption spectrophotometer and total coliform count through multiple-tube fermentation method, using lauryl tryptose broth.

2.4. Metal transfer factor

Soil to plant metal transfer factor was computed as ratio of the concentration of metal in plants (on dry weight basis) to its DTPA-extractable or readily available metal contents in soil.

2.5. Statistical analysis

The data on each parameter were subjected to a two way ANOVA analysis (Gomez and Gomez, 1984) with separation of means by Duncan multiple range test (Duncan, 1955) using SPSS software (SAS Institute Inc., Cary, NC). All tests of significance were done at the 5% probability level.

Standard deviation (S_N) was estimated as:

$$S_N = \sqrt{\frac{1}{N}\sum_{i=1}^{N}}(w_i - \overline{w})^2$$

where N are the number of w_i observations and w is their arithmatic mean.

Table 3
Amounts of heavy metals added through irrigation water from March 2008 to June 2010.

Treatments	Total water required as per treatment (mm)	Irrigation water applied (mm)	Heavy metals added by irrigation water (g ha^{-1})											
			Cd			Cr			Ni			Pb		
			GW	WW	GW:WW	GW	WW	GW:WW	GW	WW	GW:WW	GW	WW	GW:WW
ID:CPE														
0.6	2565	1274	Tr	102	51	Tr	383	191	Tr	359	179	Tr	1529	765
0.8	3420	2129	Tr	170	85	Tr	639	320	Tr	606	303	Tr	2555	1277
1.0	4275	2984	Tr	239	119	Tr	896	447	Tr	855	428	Tr	3581	1790
1.2	5130	3839	Tr	307	154	Tr	1152	576	Tr	1104	552	Tr	4607	2303
1.5	6412	5121	Tr	410	205	Tr	1536	768	Tr	1476	738	Tr	6144	3072

Tr, traces; WW, wastewater; GW, groundwater; GW:WW groundwater and wastewater in cyclic mode.

3. Results and discussion

3.1. Nutrient and heavy metal loading

Table 1 illustrates the quality of (partially treated) wastewater and groundwater irrigations. As evident from Table 1, the groundwater had 7.7 pH and 1.6 mequiv. L^{-1} Residual Sodium Carbonate (RSC). Bio-chemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), nitrate–nitrogen, ammonical–nitrogen, heavy metals and *E. coli* were present in traces. In contrast, the primary treated – wastewaters were slightly alkaline (pH: 8.2 and RSC: 2.8 mequiv. L^{-1}) and associated with 62 mg L^{-1} BOD, 112 mg L^{-1} COD, >2.4 × 10^4 per 100 mL total coliforms, 18 mg L^{-1} nitrogen (N), 4.3 mg L^{-1} phosphorus (P), 17 mg L^{-1} potassium (K), 0.1 mg L^{-1} cadmium (Cd), 0.3 mg L^{-1} chromium (Cr), 0.3 mg L^{-1} nickel (Ni) and 1.2 mg L^{-1} lead (Pb) concentrations.

The wastewater used for irrigation was carried in 5000 L capacity tanker from the Sewage Treatment Plant, which was sufficient for 3 months. To overcome the temporal changes in quality, wastewater was collected in the months of March (spring), June (peak summer pre-monsoon), September (post monsoon) and December (winter) and analyzed once in 3 months for various parameters. The temporal variation in NPK contents in wastewater ranged from 6.9 to 10.7%, whereas it was only 5.5 to 9.9% in case heavy metal. The source of groundwater was deep submersible tube well installed at 45 m depth. No variation was recorded in the water quality of groundwater the minor changes in SAR and RSC. Therefore, for calculating the amounts of plant nutrients and heavy metals added through irrigation water, average concentration of the element was accounted. The standard deviation values for temporal changes in wastewater quality parameters are shown in Table 1.

Amount of nutrients and metals contributed through irrigation water (Tables 2 and 3) was obtained as a product of total irrigation water applied for different irrigation regime, during the study period, and its average (individual) nutrient/metal concentration. The amount of nitrogen added through wastewater (WW) alone or in conjunction with groundwater (GW:WW), having trace levels of nitrogen, was observed to be varying between 23–92 g m^{-2} and 11–44 g m^{-2}, respectively under different ID/CPE based irrigation schedules. While that of phosphorus ranged from 5.4 to 21.9 and 2.8 to 11.3 g m^{-2}, respectively. Due to higher potassium concentrations in both (partially treated) wastewater (WW) and groundwater (GW), its supply through WW, GW:WW and GW was observed to be about 4.0, 4.5 and 11.7 times higher than that for phosphorus (Table 2).

Similarly, amount of Cd, Cr, Ni and Pb additions through wastewater irrigations during the study period ranged from 102 to 410, 383 to 1536, 359 to 1476 and 1529 to 6144 mg m^{-2}, under different water application schedules (Table 3). These levels reduced to just half when applied in conjunction with groundwater, having metal concentrations in traces.

3.2. Herbage yield

Lemon grass herbage yield responded to both quantity and quality of applied irrigation waters, under varied irrigation regimes (Table 4). Averaged over water quality, total herbage yield of lemon grass in the entire experimental duration ranged from 10.11 to 13.68 kg m^{-2}. Under all three irrigation water qualities or modes (i.e. single or conjunctive use) of irrigation, frequent irrigation water applications at ID:CPE ratio of 0.8 or more resulted in significantly higher herbage yields. Total herbage yield at 1.0 ID:CPE was 34% higher than at 0.6 ID:CPE. The increase in fresh biomass obtained at 1.0 and 1.2 ID:CPE were not significant, rather total herbage yield was adversely affected at 1.5 ID:CPE. Higher production with increasing ID:CPE up to 1.0–1.2 ratio could be due to availability of adequate moisture for this shallow rooted crop and its favourable effects on biochemical and microbial processes. However, irrigation at 1.5 ID:CPE could have led to aeration stress due to excessive moisture conditions (Singh, 1999; Fatima et al., 2002).

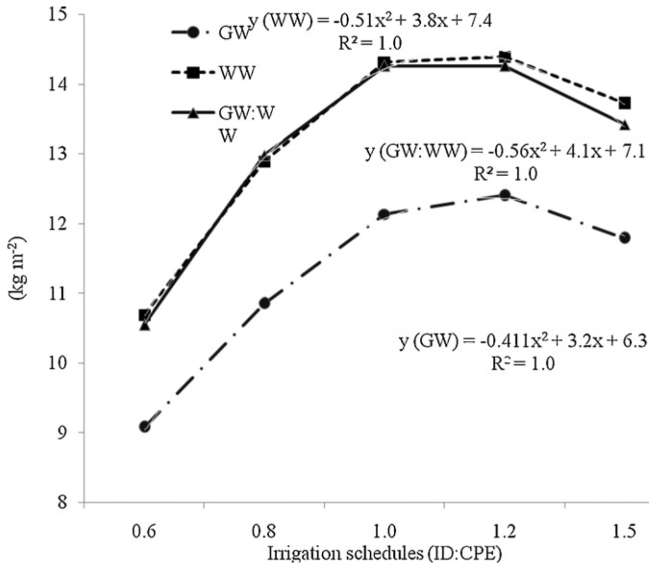

Fig. 2. Response functions of herbage yield of lemongrass to irrigation schedules and water quality.

In general, the lemon grass herbage yields from either only wastewater irrigated lysimeters (13.20 kg m^{-2}) or from those irrigated in conjunction with groundwater (13.09 kg m^{-2}) were comparable and significantly (16%) higher than those from sole groundwater use. This may be primarily attributed to an increased soil organic matter buildup (about 32%, Table 8) and nutrient supply (Table 2) in the (partially or fully) wastewater-irrigated treatments (Jimenez, 2005; Lopez et al., 2006). The interaction between water quality (WQ) and irrigation regimes (IR) had significant and positive effects on harvest wise biomass and total herbage yield. These effects could be ascribed to beneficial effects of added plant nutrients with wastewater use once the crop water requirement is fulfilled at higher ID:CPE.

The moisture content in lemon grass in various harvests varied from 27.7 to 31.9% with an average of 29.6%. The total dry biomass obtained from all seven harvests ranged from 2.72 to 4.23 kg m^{-2} with irrigation regimes and water quality treatments and followed the same trend as observed in fresh herbage yield.

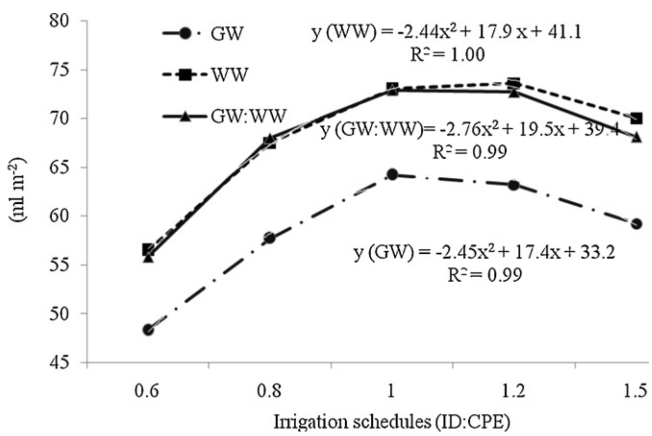

Fig. 3. Response functions of essential oil yield of lemongrass to irrigation schedules and water quality.

Table 4
Herbage yield and total dry biomass (kg m^{-2}) of lemon grass under varied water quality and irrigation schedules.

Water quality (WQ)	Irrigation schedules (IS)					
	0.6	0.8	1.0	1.2	1.5	Mean
1st harvest (16 July 2008)						
GW	1.81 ± 0.30^{a}	2.02 ± 0.22^{ab}	2.22 ± 0.33^{abc}	2.13 ± 0.17^{ab}	2.02 ± 0.18^{ab}	2.04
WW	2.36 ± 0.27^{bcd}	2.68 ± 0.28^{de}	2.88 ± 0.23^{e}	2.73 ± 0.18^{de}	2.73 ± 0.18^{de}	2.68
GW:WW	2.24 ± 0.21^{bc}	2.62 ± 0.18^{cde}	2.84 ± 0.27^{e}	2.78 ± 0.18^{de}	2.70 ± 0.17^{de}	2.64
Mean	2.14	2.44	2.65	2.55	2.48	2.45
LSD5%			WQ 0.14; IS 0.18; WQ × IS 0.31			
2nd harvest (17 November 2008)						
GW	1.45 ± 0.16^{a}	1.86 ± 0.16^{bcd}	2.11 ± 0.21^{defg}	2.20 ± 0.20^{defgh}	2.12 ± 0.15^{defg}	1.95
WW	1.55 ± 0.19^{ab}	1.90 ± 0.27^{bcde}	2.31 ± 0.18^{fgh}	2.44 ± 0.23^{gh}	2.40 ± 0.21^{gh}	2.12
GW:WW	1.58 ± 0.26^{abc}	1.93 ± 0.08^{cdef}	2.28 ± 0.25^{efgh}	2.55 ± 0.21^{h}	2.43 ± 0.26^{gh}	2.16
Mean	1.53	1.90	2.24	2.40	2.32	2.07
LSD 5%			WQ 0.14; IS 0.18; WQ × IS 0.31			
3rd harvest (13 May 2009)						
GW	0.62 ± 0.08^{a}	0.88 ± 0.17^{abc}	0.99 ± 0.14^{bcde}	1.08 ± 0.22^{bcdef}	1.04 ± 0.13^{bcdef}	0.92
WW	0.88 ± 0.14^{abc}	1.10 ± 0.21^{cdef}	1.28 ± 0.16^{ef}	1.35 ± 0.20^{f}	1.21 ± 0.26^{def}	1.17
GW:WW	0.76 ± 0.08^{ab}	0.94 ± 0.11^{bcd}	1.18 ± 0.24^{cdef}	1.26 ± 0.18^{def}	1.26 ± 0.12^{def}	1.08
Mean	0.75	0.97	1.15	1.23	1.17	1.06
LSD 5%			WQ 0.13; IS 0.17; WQ × IS 0.29			
4th harvest (13 August 2009)						
GW	1.60 ± 0.27^{a}	1.86 ± 0.37^{ab}	2.04 ± 0.15^{ab}	2.13 ± 0.24^{ab}	1.90 ± 0.14^{ab}	1.91
WW	1.78 ± 0.19^{ab}	2.13 ± 0.17^{ab}	2.17 ± 0.22^{b}	2.06 ± 0.16^{ab}	2.02 ± 0.21^{ab}	2.03
GW:WW	1.77 ± 0.27^{ab}	2.21 ± 0.39^{b}	2.22 ± 0.36^{b}	2.29 ± 0.26^{b}	2.06 ± 0.47^{ab}	2.11
Mean	1.72	2.07	2.15	2.16	1.99	2.02
LSD 5%	WQ	NS	IS	0.26	WQ × IS	0.45
5th harvest (3 November 2009)						
GW	1.16 ± 0.19^{a}	1.43 ± 0.09^{abcd}	1.62 ± 0.34^{bcd}	1.50 ± 0.19^{abcd}	1.43 ± 0.10^{abcd}	1.43
WW	1.21 ± 0.22^{ab}	1.62 ± 0.51^{bcd}	1.84 ± 0.14^{d}	1.80 ± 0.27^{d}	1.64 ± 0.13^{cd}	1.62
GW:WW	1.24 ± 0.08^{abc}	1.56 ± 0.25^{abcd}	1.70 ± 0.10^{d}	1.69 ± 0.09^{d}	1.51 ± 0.16^{abcd}	1.54
Mean	1.20	1.54	1.72	1.66	1.53	1.53
LSD 5%			WQ 0.16; IS 0.20; WQ × IS 0.35			
6th harvest (4 May 2010)						
GW	0.67 ± 0.13^{a}	0.85 ± 0.08^{ab}	0.97 ± 0.19^{bcd}	1.02 ± 0.11^{bcd}	0.94 ± 0.12^{abc}	0.89
WW	0.85 ± 0.22^{ab}	1.03 ± 0.21^{bcd}	1.25 ± 0.26^{def}	1.36 ± 0.12^{ef}	1.19 ± 0.09^{cdef}	1.13
GW:WW	0.82 ± 0.16^{ab}	1.07 ± 0.23^{bcde}	1.39 ± 0.13^{f}	1.21 ± 0.09^{cdef}	1.16 ± 0.10^{cdef}	1.13
Mean	0.78	0.98	1.20	1.20	1.10	1.05
LSD 5%			WQ 0.12; IS 0.16; WQ × IS 0.27			
7th harvest (30 August 2010)						
GW	1.77 ± 0.20^{a}	1.95 ± 0.17^{ab}	2.18 ± 0.29^{abc}	2.34 ± 0.29^{abc}	2.34 ± 0.34^{abc}	2.12
WW	2.06 ± 0.27^{abc}	2.43 ± 0.09^{bc}	2.58 ± 0.40^{c}	2.65 ± 0.43^{c}	2.53 ± 0.49^{bc}	2.45
GW:WW	2.14 ± 0.36^{abc}	2.65 ± 0.41^{c}	2.65 ± 0.22^{c}	2.48 ± 0.20^{bc}	2.3 ± 0.22^{abc}	2.44
Mean	1.99	2.34	2.47	2.49	2.39	2.34
LSD 5%			WQ 0.23; IS 0.29; WQ × IS 0.50			
Total herbage yield						
GW	9.08 ± 0.88^{a}	10.85 ± 0.75^{bc}	12.13 ± 0.46^{de}	12.40 ± 0.48^{def}	11.79 ± 0.61^{cd}	11.25
WW	10.69 ± 0.45^{bc}	12.89 ± 0.54^{defg}	14.31 ± 0.24^{h}	14.39 ± 0.12^{h}	13.72 ± 0.73^{gh}	13.20
GW:WW	10.55 ± 0.52^{b}	12.98 ± 0.87^{efg}	14.26 ± 0.43^{h}	14.26 ± 0.70^{h}	13.42 ± 0.94^{fgh}	13.09
Mean	10.11	12.24	13.57	13.68	12.98	12.51
LSD 5%			WQ 0.44; IS 0.56; WQ × IS 0.98			
Total dry biomass						
GW	2.72 ± 0.23^{a}	3.19 ± 0.41^{abc}	3.53 ± 0.33^{bcde}	3.58 ± 0.30^{bcde}	3.34 ± 0.28^{abcd}	3.25
WW	3.20 ± 0.26^{abcd}	3.82 ± 0.3.2^{bcde}	4.21 ± 0.34^{ef}	4.17 ± 0.53^{e}	4.00 ± 0.25^{de}	3.88
GW:WW	3.13 ± 0.35^{ab}	3.87 ± 0.33^{cde}	4.23 ± 0.34^{ef}	4.18 ± 0.71^{f}	3.93 ± 0.29^{de}	3.87
Mean	3.02	3.63	3.99	3.98	3.76	3.67
LSD 5%			WQ 0.14; IS 0.19; WQ × IS 0.32			

WQ, water quality; IS, irrigation schedules. Means (separated by DMRT) followed by same letters are not significantly different at ($p < 0.05$).

3.3. Essential oil yield

The oil content in fresh herb of lemongrass in different harvests varied from 0.48 to 0.57% with a mean value of 0.52% and was not significantly influenced by water application rates or irrigation water quality (data not presented).

As herbage yield, lemon grass essential oil yield also responded to both quantity and quality of applied irrigation waters (Table 5). Over all harvests and irrigation regimes, total oil yield varied from 48.3 to 73.6 mL m^{-2}. Oil yields with groundwater irrigations at 1.0 ID:CPE were comparable with those obtained through wastewater applications at 0.8 ID:CPE. However these were significantly lower than those obtained through wastewater applications at 1.2 ID:CPE ratio. Thus, as compared to the groundwater irrigation, use of wastewater alone or in conjunction with groundwater resulted in about 14–15% higher total essential oil yield. As expected due to increased moisture stress and decreased biomass, any further increase in the wastewater/groundwater application rates (beyond 1.2 – ID:CPE ratio), either alone or in conjunction, had a detrimental impact on the total herbage oil yield.

Table 5
Essential oil yield (mL m^{-2}) as influenced by water quality and irrigation schedules.

Water quality (WQ)	Irrigation schedules (IS)					
	0.6	0.8	1.0	1.2	1.5	Mean
1st harvest (7 July 2008)						
GW	9.8 ± 1.4^{a}	10.4 ± 0.6^{a}	11.8 ± 1.8^{abc}	11.0 ± 0.9^{ab}	10.1 ± 1.1^{a}	10.6
WW	12.3 ± 1.6^{abcd}	13.8 ± 1.4^{cd}	14.3 ± 0.7^{cd}	13.3 ± 0.6^{bcd}	13.5 ± 1.0^{bcd}	13.5
GW:WW	11.9 ± 2.0^{abc}	13.8 ± 1.0^{cd}	14.6 ± 2.6^{d}	13.9 ± 1.4^{cd}	13.6 ± 1.1^{bcd}	13.5
MEAN	11.3	12.7	13.6	12.7	12.4	12.5
LSD5%			WQ 1.0; IS 1.30; WQ × IS 2.2			
2nd harvest (17 November 2008)						
GW	8.0 ± 0.8^{a}	10.4 ± 1.4^{abcd}	11.2 ± 1.1^{bcde}	11.6 ± 0.8^{cde}	11.1 ± 0.9^{bcde}	10.5
WW	8.6 ± 1.1^{ab}	10.0 ± 1.3^{abcd}	12.4 ± 0.9^{de}	12.7 ± 2.1^{de}	12.4 ± 1.8^{de}	11.2
GW:WW	9.0 ± 1.6^{abc}	10.2 ± 1.3^{abcd}	11.4 ± 1.9^{cde}	13.4 ± 2.1^{e}	12.6 ± 2.4^{de}	11.3
Mean	8.5	10.2	11.7	12.6	12.0	11.0
LSD 5%			WQ NS; IS 1.6; WQ × IS 2.7			
3rd harvest (13 May 2009)						
GW	3.5 ± 0.6^{a}	4.9 ± 0.9^{abc}	5.6 ± 1.1^{bcde}	5.5 ± 0.7^{bcde}	5.1 ± 0.7^{bcd}	4.9
WW	4.8 ± 0.6^{abc}	5.7 ± 0.9^{bcde}	6.6 ± 0.4^{de}	6.9 ± 1.1^{e}	6.1 ± 0.9^{cde}	6.0
GW:WW	4.2 ± 0.4^{ab}	5.2 ± 0.9^{bcd}	6.3 ± 1.2^{cde}	6.7 ± 1.1^{de}	6.3 ± 0.6^{cde}	5.7
Mean	4.2	5.3	6.2	6.3	5.8	5.6
LSD 5%			WQ 0.7; IS 0.9; WQ × IS 1.5			
4th harvest (13 August 2009)						
GW	8.1 ± 1.3^{a}	9.6 ± 1.1^{abc}	10.4 ± 0.9^{bc}	10.2 ± 0.8^{abc}	9.0 ± 1.1^{abc}	9.5
WW	8.9 ± 0.5^{abc}	11.1 ± 0.8^{c}	10.6 ± 0.6^{bc}	10.0 ± 0.5^{abc}	10.1 ± 0.7^{abc}	10.1
GW:WW	8.8 ± 1.3^{ab}	11.0 ± 1.9^{bc}	10.6 ± 1.8^{bc}	10.8 ± 0.8^{bc}	9.6 ± 1.4^{abc}	10.2
Mean	8.6	10.6	10.5	10.3	9.6	9.9
LSD 5%			WQ NS; IS 1.2; WQ × IS 2.1			
5th harvest (3 November 2009)						
GW	6.2 ± 0.8^{a}	7.5 ± 0.8^{abcd}	8.9 ± 1.4^{bcde}	8.0 ± 0.7^{abcde}	7.4 ± 1.3^{abcd}	7.6
WW	6.7 ± 1.1^{ab}	8.8 ± 1.6^{bcde}	9.8 ± 1.6^{de}	10.2 ± 1.8^{e}	9.2 ± 1.3^{cde}	8.9
GW:WW	6.8 ± 0.7^{abc}	9.4 ± 1.2^{de}	9.1 ± 1.1^{bcde}	9.3 ± 1.7^{de}	8.7 ± 0.9^{bcde}	8.7
Mean	6.6	8.6	9.3	9.2	8.4	8.4
LSD 5%			WQ 1.1; IS 1.4; WQ × S 2.5			
6th harvest (4 May 2010)						
GW	3.7 ± 0.7^{a}	4.5 ± 0.4^{ab}	5.3 ± 0.9^{bcd}	5.4 ± 1.0^{bcd}	5.0 ± 0.9^{abc}	4.8
WW	4.6 ± 0.7^{ab}	5.8 ± 1.1^{bcd}	6.7 ± 0.9^{de}	7.4 ± 0.5^{e}	6.2 ± 0.5^{cde}	6.2
GW:WW	4.5 ± 0.7^{ab}	5.5 ± 0.9^{bcd}	7.3 ± 1.4^{e}	6.3 ± 0.5^{cde}	5.9 ± 0.5^{bcde}	5.9
Mean	4.3	5.3	6.4	6.4	5.7	5.6
LSD 5%			WQ 0.8; IS 1.0; WQ × IS 1.8			
7th harvest (30 August 2010)						
GW	9.0 ± 0.8^{a}	10.4 ± 0.8^{ab}	11.0 ± 0.8^{ab}	11.5 ± 1.9^{ab}	11.5 ± 1.5^{ab}	10.7
WW	10.6 ± 2.2^{ab}	12.3 ± 1.8^{b}	12.6 ± 1.8^{b}	13.1 ± 1.1^{b}	12.5 ± 2.3^{b}	12.2
GW:WW	10.6 ± 2.0^{ab}	12.9 ± 1.9^{b}	13.6 ± 0.9^{b}	12.3 ± 2.1^{b}	11.4 ± 1.7^{ab}	12.2
Mean	10.1	11.9	12.4	12.3	11.8	11.7
LSD 5%			WQ 1.4; IS 1.8; WQ × IS 3.0			
Total Oil yield (mL m^{-2})						
GW	48.3 ± 4.3^{a}	57.7 ± 2.7^{bcd}	64.2 ± 2.0^{de}	63.2 ± 3.4^{cde}	59.2 ± 5.4^{bcd}	58.6
WW	56.5 ± 4.5^{bc}	67.5 ± 1.0^{ef}	73.0 ± 2.7^{f}	73.6 ± 2.9^{f}	70.0 ± 5.3^{ef}	68.1
GW:WW	55.8 ± 3.1^{b}	68.0 ± 3.9^{ef}	72.9 ± 4.1^{f}	72.7 ± 4.0^{f}	68.1 ± 5.2^{ef}	67.5
Mean	53.6	64.6	70.1	69.8	65.7	64.7
LSD 5%			WQ 3.3; IS 4.1; WQ × IS 7.3			

WQ, water quality; IS, irrigation schedules. Means (separated by DMRT) followed by same letters are not significantly different at ($p < 0.05$).

This was also evident from the quadratic response functions (Figs. 2 and 3) developed for determining lemon grass herbage and oil yields, under varied irrigation schedules and water qualities. The aforementioned results were in complete conformity with the findings of Singh (1999), Chakraborty et al. (2010) and Darvishi et al. (2010).

3.4. Heavy metal accumulation

Metal contents in the dry matter (Table 6) of lemon grass, grown under varied irrigation regimes, were also estimated. Heavy metals present in the effluents used for irrigation tend to accumulate in the soils, become bio-available and eventually get translocated to plants (Toze, 2006). Concentrations of Cd, Cr, Ni and Pb in the plant under consideration varied from 1.54 to 1.85, 3.27 to 4.04, 4.35 to 5.58 and 3.53 to 4.46 mg kg^{-1}, respectively at different irrigation regimes. Due to low heavy metal concentrations in the irrigation waters, high soil pH and organic carbon contents (Mantovi et al., 2005), metal concentrations in the plant under consideration were observed to be well below their critical levels (such as Cd and Cr: 5–30 mg kg^{-1} dry matter; Ni: 10–100 mg kg^{-1} dry matter and Pb: 30–300 mg kg^{-1} dry matter; as proposed by Kabata-Pendias and Pendias (1992)) and hence did not contribute to any visual plant injury symptoms during the study period.

However, in general, dry matter produced through wastewaters (either alone or in conjunction with groundwater) were found to be associated with higher metal concentrations than those produced from groundwater alone. For instance, Cd concentrations in the wastewater produced lemongrass dry matter (either alone or in conjunction with groundwater) were 4.5 and 2.8 times higher

Table 6
Heavy metal contents in lemongrass herb.

Treatments	ID:CPE	Heavy metals (mg kg^{-1} dry weight)			
		Cd	Cr	Ni	Pb
GW	0.6	0.38 ± 0.09	2.16 ± 0.21	2.50 ± 0.60	2.12 ± 0.54
	0.8	0.38 ± 0.09	2.18 ± 0.32	2.55 ± 0.59	2.18 ± 0.43
	1.0	0.40 ± 0.11	2.14 ± 0.44	2.54 ± 0.63	2.13 ± 0.51
	1.2	0.38 ± 0.10	2.02 ± 0.38	2.42 ± 0.48	2.06 ± 0.54
	1.5	0.39 ± 0.11	2.15 ± 0.48	2.34 ± 0.55	2.07 ± 0.53
WW	0.6	1.54 ± 0.59	3.27 ± 0.62	4.35 ± 1.04	3.53 ± 0.98
	0.8	1.66 ± 0.63	3.66 ± 0.65	4.79 ± 1.08	3.87 ± 1.09
	1.0	1.77 ± 0.74	3.78 ± 0.74	5.19 ± 1.23	4.10 ± 1.15
	1.2	1.83 ± 0.70	3.86 ± 0.73	5.58 ± 1.35	4.46 ± 1.38
	1.5	1.85 ± 0.75	4.04 ± 0.82	5.45 ± 1.42	4.45 ± 1.24
GW:WW	0.6	1.05 ± 0.38	2.65 ± 0.57	3.46 ± 0.62	3.00 ± 0.60
	0.8	1.07 ± 0.40	2.83 ± 0.69	3.53 ± 0.53	3.10 ± 0.59
	1.0	1.16 ± 0.37	2.93 ± 0.62	3.77 ± 0.65	3.32 ± 0.68
	1.2	1.06 ± 0.35	3.12 ± 0.73	3.83 ± 0.54	3.41 ± 0.64
	1.5	1.14 ± 0.36	3.12 ± 0.76	3.96 ± 0.59	3.50 ± 0.71
LSD	WQ	0.05	0.09	0.11	0.11
(5%)	IR	0.06	0.11	0.14	0.15
	WQ × IR	0.10	0.19	0.24	0.25

± values indicate standard deviation, WW, wastewater; GW, groundwater; GW:WW groundwater and wastewater in cyclic mode.

than the sole groundwater irrigated (with a maximum at the sixth harvest). Similarly, Cr, Ni and Pb concentrations in the two-levels of wastewater irrigated lemongrass dry matter were about 2 and 1.5 times higher than the one produced with groundwater alone (with a maximum at the fourth harvest).

Frequent use of sole or conjunctive wastewater applications also seems to be significantly affecting the overall plant herbage metal concentrations. As compared to the wastewater irrigations schedules at ID:CPE ratio of 0.6, herbage metal concentrations were found to be about 9–19% higher when these irrigations were scheduled at ID:CPE ratio of 1.0. However, this was not observed to be the case for the groundwater irrigations, which were associated with non-significant metal concentrations at varied irrigation schedules. Thus interaction effects between water quality and irrigation schedules were positive and significant for only wastewater irrigated treatments.

However, this was not observed to be the case with the lemon grass oil (Table 7), as the metal concentrations in oil seemed to be neither influenced by the water application rates nor water quality. Also, these were observed to be in very low concentrations and well within the critical limits for even food (Cr: 1.5 ppm; Cd: 1.5 ppm, Ni: 2.5 ppm and Pb: 1.3 ppm, Lone et al., 2003; FSSAI, 2011). Average Cr, Ni and Pb concentrations in the lemongrass oil were found to be about 0.14, 0.10 and 0.04 ppm, respectively.

Transfer factors (TFs), i.e. the ratio of metal concentration in lemongrass to DTPA-extractable or readily available metal in soil differed considerably for different metals. The order of transfer of metals from soil to plant was Cr > Ni > Cd > Pb with mean TFs values of 3.06, 3.05, 1.67 and 1.30, respectively. TFs for lemon grass irrigated with wastewater were the highest followed by conjunctive water use and lowest with groundwater. However, TFs were not influenced with irrigation scheduling. In case of essential oil, TFs values were very small ranging from 0.00 to 0.17 only and not swayed with quality and scheduling of irrigation (Fig. 4). This indicates that heavy metals in essential oil do not increase with increasing concentrations of metals in herb and soil. This is in concurrence with the findings recorded by Rattan et al. (2005) in crops irrigated with municipal sewage.

The contents of heavy metals so far were below the threshold level in the herbage of lemon grass, therefore no threat is expected even if used for medical purposes. With the long term wastewater use and heavy metal build up in soil, contents of heavy metals in the herb may exceed the permissible limit. The metals were not transferred from the herbage to the essential oil as indicated by non significant relationship of metal content with water quality and application rates (Table 7). Therefore, essential oil will be safe even if used for medical purposes.

Zheljakov and Jekov (1996) also observed very low concentration of heavy metals in plant essential oil extracts. Further, Zheljazkov et al. (2005) reported that aromatic crops could be grown on heavy metal enriched soils, without causing any significant risks of metal transfer from soil to oil and alterations in essential oil composition. Deployment of steam distillation process for essential oil extraction seemed to be the main reason for their lower presence in the oil extracts (Scora and Chang, 1997; Bernstein et al., 2009). Additionally, the heat applied during oil extraction eliminates the health concerns due to human bacterial pathogens.

Table 7
Heavy metal content in lemongrass essential oil (mg L^{-1}).

Water quality	ID: CPE	Heavy metals (mg kg^{-1} dry weight)			
		Cd	Cr	Ni	Pb
GW	0.6	ND	0.12	0.09	0.05
	0.8	ND	0.17	0.10	0.04
	1.0	ND	0.15	0.07	0.04
	1.2	ND	0.12	0.11	0.05
	1.5	ND	0.13	0.09	0.03
WW	0.6	ND	0.16	0.13	0.04
	0.8	ND	0.17	0.10	0.05
	1.0	ND	0.15	0.13	0.04
	1.2	ND	0.15	0.10	0.05
	1.5	ND	0.15	0.09	0.04
GW:WW	0.6	ND	0.13	0.06	0.04
	0.8	ND	0.11	0.09	0.05
	1.0	ND	0.11	0.10	0.04
	1.2	ND	0.15	0.13	0.05
	1.5	ND	0.13	0.14	0.04
LSD	WQ	NS	NS	NS	NS
(5%)	IS	NS	NS	NS	NS
	WQ × IS	NS	NS	NS	NS

ND, not detected; NS, non-significant; WW, wastewater; GW, groundwater; GW:WW groundwater and wastewater in cyclic mode.

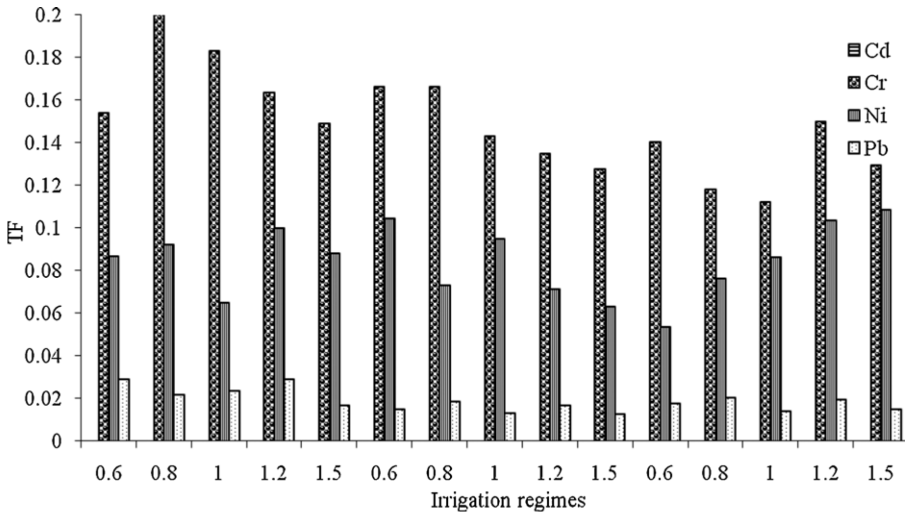

Fig. 4. Metal transfer factors for essential oil of lemon grass in relation to water quality and irrigation schedules.

Thus as lemon grass oil is primarily used for non-edible purposes like manufacturing of soaps, detergents, insect repellent preparations, cosmetics and perfumes therefore, it appears to be a feasible selection for avoiding extensive food chain (metal) contamination and sustainable wastewater use in agriculture.

3.5. Soil properties

Increasing levels of wastewater irrigations led to increased soil electrical conductivity (from 0.44 to 0.59 dS m^{-1}) and organic carbon (from 0.49 to 0.60%) levels in the top (0–15 cm) soil layer (Table 8). Due to increased organic load and nutrients in the wastewaters, the available – P, K concentrations and the dehydrogenase activity in the wastewater irrigated soil was also improved by 14, 6 and 20% respectively, over the groundwater irrigated soils. Further, compared to the groundwater irrigated soils, the surface layers (0–15 cm) of the (partially treated) wastewater irrigated soils were observed to be associated with about 17–29 times higher total *coliform* counts, suggesting that wastewater use stimulated these microfloras. An increase in metabolic activity of soil microorganisms and an improvements in the soil fertility status, with wastewater irrigation, has also been previously reported by Goyal et al. (1995), Meli et al. (2002), Minhas and Samra (2004) and Rattan et al. (2005).

Similar enhancements in heavy metal contents were also noticed (Table 8). The concentration of extractable metals in the test soils were in the order Pb > Cd > Ni > Cr. With prolonged wastewater use, heavy metal accumulation may pose problems for crop growth. However, though the concentration of the Ni, Cr and Cd in the (partially treated) wastewater irrigations were about 1.5–10 times higher than their permissible limits yet due to their high pH they did not seem to be posing any threat of soil contamination (Awashthi,

Table 8
Soil properties after the harvest of lemongrass.

Water quality	ID: CPE	pH	EC (dS m^{-1})	OC (%)	Av. P (kg ha^{-1})	Av. K (kg ha^{-1})	Cd (mg kg^{-1})	Cr (mg kg^{-1})	Ni (mg kg^{-1})	Pb (mg kg^{-1})	Dehydrogenase activity (mg TPF/kg soil/h)	Total coliforms (10^3 cfu/g)
GW	0.6	7.50	0.28	0.40	14.7	229	0.49	0.78	1.04	1.71	103	1.2
	0.8	7.60	0.35	0.41	14.7	230	0.51	0.82	1.08	1.84	108	1.5
	1.0	7.67	0.28	0.44	14.5	230	0.51	0.82	1.08	1.71	111	1.6
	1.2	7.63	0.31	0.41	14.8	235	0.44	0.73	1.10	1.71	113	1.6
	1.5	7.60	0.37	0.40	15.5	226	0.54	0.87	1.02	1.78	114	1.6
	Mean	7.60	0.32	0.41	14.9	230	0.5	0.80	1.06	1.76	110	1.5
WW	0.6	7.70	0.44	0.49	16.7	239	0.64	0.96	1.25	2.66	115	38.0
	0.8	7.63	0.44	0.54	16.9	241	0.67	1.02	1.37	2.66	128	42.7
	1.0	7.90	0.56	0.57	17.2	243	0.69	1.05	1.37	3.00	134	43.3
	1.2	7.83	0.55	0.57	16.8	249	0.71	1.11	1.40	3.02	140	49.0
	1.5	7.90	0.59	0.60	17.1	249	0.73	1.18	1.42	3.13	144	50.0
	Mean	7.79	0.52	0.55	16.9	244	0.69	1.07	1.33	2.90	132	44.6
GW:WW	0.6	7.77	0.34	0.46	16.16	235	0.58	0.93	1.12	2.26	108	22.0
	0.8	7.63	0.34	0.49	16.07	238	0.61	0.93	1.18	2.45	118	27.7
	1.0	7.83	0.37	0.52	16.63	240	0.66	0.98	1.16	2.83	125	26.7
	1.2	7.73	0.40	0.54	16.47	245	0.67	1.00	1.25	2.56	125	30.7
	1.5	7.77	0.43	0.54	16.67	245	0.69	1.00	1.29	2.64	128	31.0
	Mean	7.75	0.38	0.51	16.4	241	0.64	0.98	1.21	2.56	121	27.6
LSD (5%)	WQ	0.10	0.05	0.03	0.54	4.2	0.03	0.09	0.08	0.17	5	2.1
	IS	NS	0.06	0.05	NS	5.5	0.04	NS	0.11	0.23	7	2.7
	WQ × IS	0.22	0.11	0.08	1.20	9.5	0.07	0.22	0.17	0.38	12	4.6

WW, wastewater; GW, groundwater; GW:WW groundwater and wastewater in cyclic mode.

2000). Further, with increased water applications (particularly at ID:CPE ratios of 1.0 or 1.2, Table 8), the change in the aforementioned soil properties was not very significant thereby depicting non-significant interactions between irrigation water qualities and regimes during the experimental period.

4. Conclusions

Wastewater irrigations alone or in conjunction with groundwater produced higher lemon grass herbage and essential oil yields. Lemon grass herbage and essential oil yields were the maximum at IW:CPE ratios of 1.0–1.2. Any further increase in irrigation water applications proved to be detrimental to both herbage and oil yields. Heavy metal concentrations in the essential oil of the wastewater irrigated lemon grass were also found to be within the permissible levels thereby leading to low food chain contamination risks. Thus, lemon grass oil, having good export potential, could be a remunerative wastewater disposal option. However, even under such situations, continuous monitoring of the soils, plants and groundwater for any built-up of potentially toxic elements and pathogens is a must for protecting our valuable natural resources.

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