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RESEARCH ARTICLE

Effect of distillery spentwash fertigation on crop growth, yield, and accumulation of potentially toxic elements in rice

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Abstract

The safe disposal of industrial effluents always remained a challenging process because of their high level of nutrients, toxic elements, and salts. A pot experiment was conducted to investigate the effects of various concentrations (5%, 10%, 15%, and 20%) of sugar industry effluent spentwash (SW) fertigated with tab water (TW), on soil properties, crop growth, physiological parameters, yield components, and accumulation of potentially toxic elements (PTEs) in rice (*Oryza sativa* L.) grains and straw. The results showed that soil physico-chemical properties were modified with rise in SW concentration. Application of 5% SW significantly enhanced the plant growth, and yield components. Photosynthesis rate, transpiration rate, and stomatal conductance were significantly higher under 5% SW concentration in comparison with control. However, SW concentrations of > 5% showed inhibitory effects for all growth, physiological, and yield components. Accumulation of PTEs showed increasing trend with rise in SW concentration. However, under 5% SW concentration, all the PTEs in rice grain and straw were within the permissible limits (PLs) recommended by FAO/WHO and no health hazards were detected by health risk assessment. Based on the study results, 5% SW fertigation with TW can be applied as fertilizer for enhancing the growth and productivity of rice.

Keywords Environmental pollution · Physiological attributes · Rice · Spentwash · Toxic elements

Highlights
- We applied different concentrations of spentwash to rice crop
- Low levels of spentwash enhanced rice growth, physiology, and yield
- Higher concentrations of spentwash reduced the crop production and increased the accumulation of micronutrients and heavy metals in plant tissues and grain
- Spentwash could be applied at lower concentration for rice production

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Introduction

Industrialization has not only limited the water resources, but also increased the volume of waste generation and most of the wastes are discharged into agro-ecosystem without proper treatment, which raise serious environmental pollution concerns (Thapliyal et al. 2011; Sarwar et al. 2017; Khan et al. 2018). Among various industries, sugar industry occupies a dominant place in Pakistan economy. There are approximately 88 sugar mills in Pakistan, releasing 3.48 million tonnes of spentwash (SW) per annum (PSMA 2014; Kaloi et al. 2017). In India, more than 300 sugar industries are generating about 3.5×10^{15} l SW per annum (Chandra et al. 2004). SW is characterized by high soluble salts, potentially toxic elements (PTEs) including, (Zn, Cu, Fe, Mn, Cd, Pb, Hg, and As), cations and anions, high EC, organic matter, low pH, and plant nutrients (Monni et al. 2000; Wintz et al. 2002; Kumari and Phogat 2009; Agale et al. 2013; Purushotham et al. 2013; Das 2014; Qureshi et al. 2015; Asati et al. 2016). Since, the direct discharge of SW into water bodies or arable lands may not only prove harmful for crop productivity but also can lead to the accumulation of PTEs in food chain and can spoil soil microbiota as well as aquatic life, owing to the leaching of soluble salts (Kumar et al. 1997; Pant and Adholeya 2007; Murtaza et al. 2008). One of the dominant environmental risks associated with SW irrigation is the accumulation of PTEs in agricultural crops as well as food chain transfer (Zheng et al. 2007). Excessive phytoavailable concentration of PTEs cause physiological constrains that reduce plant root and shoot growth and put negative impact on overall crop growth and yield (Fuentes et al. 2004). Accumulation of PTEs in agricultural soils through irrigational water not only contaminates soil, but also results in food contamination and depletion of some essential elements in the body causing serious diseases (Iyengar and Nair 2000; Khan et al. 2008).

According to Dikinya and Areola (2011), use of industrial effluents for agricultural crops has several advantages as it contains appreciable amount of plant nutrients, which play a significant role in plant growth, development, and metabolic process by enhancing the soil fertility (Rath et al. 2010; Jiang et al. 2012). In this context, several studies stated that the irrigation of agricultural crops with diluted SW can significantly enhance the crop productivity and soil fertility (Ramana et al. 2002; Kaushik et al. 2005; Chandraju and Basavaraju 2007; Chandraju et al. 2008; Suganaya and Rajannan 2009; Chidankumar et al. 2009; Srivastava and Jain 2010; Sukanya and Meli 2010; Gahlot et al. 2011; Rath et al. 2013; Kaloi et al. 2017).

Rice (*Oryza sativa* L.) was selected as a test crop keeping in view its, nutritious value for human being and its growth challenges, particularly nutrients and hydrological needs. In case of nutritious significance, rice is the second major staple food of Pakistan and 60% world's population (Khaliq et al. 2015; Govt Pak 2017). However, high yielding varieties of rice also facing problem of nutrient deficiencies (Timsina et al. 2010; Khan et al. 2018). Its grains are the major source of food for human beings while its straw and remaining parts (leaves/shoot) are used as animal fodder (Sarwar et al. 2011; Kumar et al. 2014). In case of its hydrological and nutrients needs, typical 100-day rice crop needs about 5300–7000 mm water and high level of available nutrients depending on the climate, soil characteristics, and hydrological conditions (Tuong et al. 2003). In Asia, among 80–90% fresh water used for agricultural practices, 50% is required only for rice production (IRRI 2001; Liu et al. 2014) indicating dire need of water for rice. Thus, growing rice through fertigation of SW with irrigational water have two major advantages over other crops: (i) consumption of large quantity of industrial effluent in an environmental-friendly way than any other crop and (ii) fertigation of nutrient-enriched SW with TW, which can meet the nutrient requirement of the nutrient deficiency sensitive verities, that is usually hard for a local farmer in developing countries including Pakistan.

The use of industrial effluents in diluted (fertigation) form with irrigational water for agricultural crops productivity is increasing because of increasing worldwide water shortage, inappropriate disposal, and food and environmental insecurity and increasing fertilizer costs (Raverkar et al. 2000; Hati et al. 2004; Chidankumar et al. 2009). It has been estimated, that approximately 200 million hectares in 50 countries and 1/10th of the world's population is eating food produced by wastewater-irrigated crops (United Nations 2003; Kauser 2007). In Pakistan, about 80% people are using untreated wastewaters for irrigational purpose due to the fact that there are no alternate sources of irrigation, inadequate planning for disposal of industrial wastes, and unavailability of funds for wastewater collection and treatments (Khalil 2011). The potential way to combat food chain contamination, fertilizer insufficiency, and disposing of industrial effluents concerns is the fertigation of industrial effluents with irrigational water (Raverkar et al. 2000; Hati et al. 2004; Chidankumar et al. 2009). Despite the widespread use of untreated industrial effluents in Pakistan, there are no recommended doses or guidelines as suggested by USEPS and EU for application of industrial effluents for agricultural crop production (CEC 1986; Harrison et al. 1999; Fytili and Zabaniotou 2008). The crops grown on naturally contaminated sites or irrigated with various wastewaters are consumed by local residents, which may contain higher concentration of PTEs; therefore, assessment of PTEs exposure to particular organism is essential (Khan et al. 2008). In addition, presence of PTEs in SW and their effects on metal accumulation and health risks posed through consumption of rice irrigated with SW have not been assessed, although health risks assessments has been recommended by Chandra et al. (2008, 2009). Therefore, it is necessary to optimize the SW usage for production of commonly growing crops.

Keeping in view the above scenario, we planned a pot study to select the suitable concentration of SW for rice (*Oryza sativa* L. cv. Basamti-2000) crop in order to mitigate the related issues of food contamination associated with PTEs in SW, plant growth, physiological characteristics, and variations in yield and uptake of PTEs by rice various parts were quantified at various concentrations of SW fertigation with TW. This may help to reuse the SW for crop irrigation and contribute to mitigate fertilizer shortage, support the agriculture sector, and protect the agro-ecosystem.

Materials and methods

Experimental design, treatments and crop husbandry

The SW was collected from Tandilianwali-II (Younus Rehman Hajra) Sugar Mills Limited, Shah Jamal Road, District Muzaffargarh, Punjab, Pakistan. The pot experiment was conducted at the glass house of the Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan (30.259211° N, 71.514278° E, 125.26 m elevation above sea level) during 2015–2016. The climate is arid to semi-arid and total rainfall (January to December) is about 373 mm that is mostly during monsoon season.

Rice (*Oryza sativa* L.) variety super basmati-2000 was used as test crop in the experiment. Air-dried 8.5-kg soil was placed in each pot and recommended doses of NPK were applied as 0.7 g P_2O_5 pot^{-1} (46% P) as triple superphosphate, 1.0 g K_2O pot^{-1} (60% K) as potassium chloride, and 2/3 of total 1.0 g urea-N pot^{-1} (46% N) as a basal fertilizer were mixed before sowing and the remaining N was applied at the tillering stage. The experiment was conducted in completely randomized design (CRD) with five treatments [control (tap water; TW), 5% SW, 10% SW, 15% SW, and 20% SW] with three replications and SW, dilutions were made using TW.

Analysis of experimental soil and spentwash physico-chemical properties

The physico-chemical characteristics of soil and SW were determined in triplicate according to the standard methods of (Gee and Bauder 1986; Nelson and Sommers 1996; Kettler et al. 2001). Organic matter was determined by Walkey-Black method (Nelson and Sommers 1996). Nitrogen was analyzed by Kjeldhal distillation method (Keeney and Nelson 1982), while K and P were determined using the methods of Ryan et al. (2001). Soluble cations (Ca, Mg, and Na) were analyzed according to USSL given methods (USSL 1954). The soil and SW samples for PTEs were prepared according to the methods described (Jones Jr et al. 1990; Hussain et al. 2013). PTEs in soil, TW, SW, and plant-digested samples were quantified using atomic adsorption spectrophotometer (FAAS, Hitachi Z-8000, Hitachi Ltd., Tokyo, Japan). The standards were prepared from Perkin Elmer standard solution (1000 ppm) using dilution method according to the metal. In order to ensure the analytical accuracy, the results were subtracted from value of blank. All the values were recorded in triplicate. Arsenic in SW, soil, and rice digested samples was determined according to the method described (Nickson et al. 2005).

Determination growth, physiological, and yield parameters

Leaf photosynthesis (μmol m^{-2} s^{-1}) rate, leaf stomatal conductance (mmol m^{-2} s^{-1}), and transpiration rate (mmol m^{-2} s^{-1}) were measured after 40 days of transplanting the nursery using a portable Infra-Red Gas Analyzer (IRGA Ci-340, CID, USA) and after 90 days of sowing, growth parameters were determined, and after harvesting yield components were determined accordingly. Briefly, after 90 days of sowing, plant height, panicle length, number of tillers plant^{-1}, and number of ears plant^{-1} were determined at standing crop before harvest. After 115 days of sowing, plants were harvested manually and yield parameters such as grain yield, straw yield, 1000-grain weight, number of grains plant^{-1}, and grain weight plant^{-1}were analyzed accordingly.

Health risk assessment

Soil to plant transfer factor

Metal transfer factor or bioaccumulation of metal is the ratio of metals concentration in dry weight of plant tissue (grain, leaves, stem roots etc.) and concentration in soil. It is calculated as follows:

$$\mathrm{PTF} = \frac{C_{\mathrm{plant}}}{C_{\mathrm{soil}}}$$

where C_{plant} represents the concentration of metal in plant and C_{soil} represents the concentration of metals in soil on dry basis (mg kg^{-1}; Cui et al. 2005).

Daily intake of PTEs (DIM)

The daily intake of PTEs was described as the equation of DIM;

$$\mathrm{DIM} = \frac{C_{\mathrm{metal}} \times C_{\mathrm{factor}} \times D_{\mathrm{food\ intake}}}{\mathrm{BW}_{\mathrm{average\ weight}}}$$

where C_{metal} is the metal concentration in plant (mg kg^{-1}), C_{factor} represents conversion factor fresh weight into dry weight while D_{food} is for daily intake of food, and BW shows average body weight per person. Conversion factor was 0.085; average daily intake was considered 0.345 for adults and 0.232 for children kg per day per person. While, average body weight of adults was taken 73 kg and 32.7 kg for children (Ge 1992; FAO 2000; Wang et al. 2005).

Health risk index (HRI)

The health risk index was assessed on the base of oral dose reference (RfD) for each metal. The HRI was calculated according to (US-EPA 2002).

$$\mathrm{HRI} = \frac{\mathrm{DIM}}{\mathrm{RfD}}$$

HRI < 1 is considered safe for inhabitants. The reference oral dose of PTEs was 0.3, 0.3, 0.04, 0.014, 0.0005, 0.035, 0.007, and 0.0003 for Zn, Fe, Cu, Mn, Cd, Pb, Hg, and As mg kg^{-1} day^{-1}, respectively (USEPA 2002).

Statistical analysis

The data regarding all the parameters was statistically analyzed using software "SPSS 17.0®American" version. A general linear model was used to compare results of the study, and analysis of variance technique (ANOVA) was used to analyze data gathered from the experiment. Treatment differences were compared by Turkey's HSD test. The computer package software Sigma plot 12.5 was used for the preparation of graphs.

Results

SW characteristics

The SW used in this experiment had high EC, OM, and total contents of N, P, K, Ca, Mg, Na, and low pH (Table 1). The pH values for TW, 5% SW, 10% SW, 15% SW, 20% SW, and 100% SW concentrations were 6.57, 6.49, 5.8, 5.8, 5.1, 4.8, and 4.2, respectively. N varied from 2.55 to 2424.65, P ranged from 0.05 to 180.66, and K ranged from 3.59 to 8441 mg L^{-1} while the concentration of Zn, Fe, Cu, Mn, Cd, Pb, Hg, and As ranged from 0.35 to 0.87, 0.43 to 13.11, 0.37 to 9.69, 0.66 to 365.14, 0.004 to 4.26, 0.07 to 4.25, 0.007 to 3.67, and 0.02 to 2.23 mg L^{-1} respectively, while Mn concentration was the highest under 100% SW among all the PTEs tested followed by Fe, Cu, Zn, Pb, Cd, As, and Hg with respect to the various concentrations of SW tested (Table 1).

Soil characteristics

The experimental soil was loam in texture, slightly alkaline, and low in N, P, K, Ca, Mg, Na, and organic matter (Table 2). Under application of 5%, 10%, 15%, and 20% SW concentrations increase in soil pH was 0.54%, 1.77%, 2.45%, and 4.23% respectively in comparison to ((TW) tab water treated soil). Soil EC ranged from 1.68 to 1.98, while Ca, Mg, and Na varied from 623.41 to 688, 212.76 to 267.32, and 110.06 to 146.11 respectively (Table 2). Concentration of NPK showed increasing trend with rise in SW concentration and organic matter contents ranged 0.62–1.18% (Table 2). The Zn, Fe, Cu, Mn, Cd, Pb, Hg, and As concentrations in soil ranged between 17.2 and 59.12, 1.59 and 88, 9.61 and 42.84, 0.44 and 20.31, 0.23 and 6.2, 0.22 and 4.42, 0.84 and 6.54, and 0.28 and 8.87 (mg kg^{-1}) respectively and concentration of Fe was the maximum in all treatments followed by Zn, Cu, Mn, As, Hg, Cd, and Pb (Table 2).

Growth and yield components

Plant morphological characteristics such as leaf chlorosis was observed after 50 days of sowing under > 10% SW concentration. Similarly, plant height increased significantly ($p \leq 0.05$) under 5%, 10%, and 15% SW concentration and decreased at 20% SW concentration as compared with TW (Table 3). The maximum increase in plant height of 22%, and panicle length of 98%, was under 5% SW concentration in comparison to their respective controls while slight reduction in panicle length was recorded at 20% SW concentration (Table 3). Maximum number of tillers $plant^{-1}$ and number of ears $plant^{-1}$ were at 5% SW concentration, followed by 10% SW and 15% SW, while minimum at 20% SW concentration (Table 3).

Number of grains $plant^{-1}$ was significantly ($p \leq 0.05$) higher under 5%, 10%, 15% SW concentrations while under 20% SW concentration number of grains $plant^{-1}$ was found lower as compared to TW (Table 3). The maximum increase in number of grains $plant^{-1}$of 207% was observed at 5% SW concentration followed by 10% SW (128%), 15% SW (67%), and 20% SW (29%) in comparison with TW. The maximum grain weight of 12.14 g $plant^{-1}$ was recorded under 5% SW concentration followed by 10% SW 10.31 g $plant^{-1}$, SW 15% 9.30 g $plant^{-1}$, and minimum under 20% SW rate 7.03 g $plant^{-1}$ as compared to the control (Table 3). Under 5% SW concentration 1000-grain weight was 31.03 g maximum while 12.16 g was minimum under application of 20% SW concentration. The maximum increase in rice grain yield was 86% under 5% SW concentration followed by 40% under 10% SW concentration while 7% and 14% decrease in grain yield was observed under 15% and 20% SW concentration respectively as compared to the control (Table 3). Straw yield was increased by 53.46%, 13% under 5% and 10% SW concentrations respectively, while 17% and 44% decrease in straw yield was recorded under 15% and 20% SW concentration (Table 3).

Physiological parameters

The maximum leaf photosynthesis of 25.5 μmol m^{-2} s^{-1} was recorded under 5% SW concentration followed by 20.43 μmol m^{-2} s^{-1}, 15.88 μmol m^{-2} s^{-1}, under 10% and 15% SW concentrations respectively, while a minimum of 11.19 μmol m^{-2} s^{-1} under 20% SW concentration was recorded (Fig. 1a). Application of 5%, 10%, and 15% SW significantly improved stomatal conductance (opening) as compared with sole application of TW. Maximum stomatal conductance of 5.1 mmol m^{-2} s^{-1} was observed under 5% SW concentration followed by 3.44 mmol m^{-2} s^{-1}, 2.90 mmol m^{-2} s^{-1} under 10% SW concentration and TW treated rice plants respectively. The minimum stomatal conductance of 2.48 mmol m^{-2} s^{-1} was recorded under 20% SW followed by 15% SW concentration (Fig. 1a). Addition of SW decreased the transpiration rate (Fig. 1b), which reached 2.68 mmol m^{-2} s^{-1} under 20% SW concentration. A gradual decrease in transpiration was observed with increasing concentration of SW. However,

Table 1 Physico-chemical properties and PTEs concentration in SW and TW (mgL^{-1})

Property	TW	5% SW	10% SW	15% SW	20% SW	100% SW
Color	No color					Dark brown
pH	6.57 ± 0.05 b	6.49 ± 0.08 a	5.8 ± 0.23 c	5.10 ± 0.06 d	4.80 ± 0.07 e	4.2 ± 0.01f
EC (dSm-1)	0.47 ± 0.03 f	3.2 ± 0.25 e	13.47 ± 0.31 d	17.96 ± 0.17 c	21.42 ± 0.38 b	28.42 ± 0.38 a
Total Ca *	27.91 ± 0.78 f	63.3 ± 0.97 e	124.95 ± 1.83 d	174.86 ± 1.87 c	265.92 ± 2.38 b	637.12 ± 3.97 a
Total Mg *	12.13 ± 0.97 f	32.08 ± 2.22 e	64.91 ± 3.47 d	86.25 ± 5.39 c	114.37 ± 392	305.08 ± 9.19 a
Total Na *	39.62 ± 1.25 f	112.57 ± 1.82 e	183.85 ± 3.66 d	225.67 ± 2.90 c	317.24 ± 3.19 b	1159 ± 18.82 a
Total N *	2.55 ± 0.42 f	195.9 ± 6.40 e	377.99 ± 10.63 d	652.32 ± 13.77 c	844.92 ± 20.76 b	2424.65 ± 22.96 a
Total P *	0.05 ± 0.015 e	30.87 ± 5.39 d	49.57 ± 6.98 cd	65.9 ± 6.36 bc	80.07 ± 6.68 b	180.66 ± 12.00 a
Total K *	3.59 ± 0.37 f	982.45 ± 15.01 e	1660.08 ± 19.41 d	2930.83 ± 20.72 c	4148.67 ± 23.71 b	8441.34 ± 37.08 a
OM (%)	0	0.3 ± 0.02 b	0.51 ± 0.03 b	0.88 ± 0.02 b	1.11 ± 0.07 b	4.19 ± 0.23 a
Zn	0.35 ± 0.41 bc	0.35 ± 0.05 c	0.59 ± 0.16 abc	0.74 ± 0.19 ab	0.81 ± 0.16 abc	0.87 ± 0.06 a
Fe	.43 ± 0.46 e	1.19 ± 0.19 d	6.69 ± 0.49 c	9.56 ± 0.21 b	10.29 ± 0.30 b	13.11 ± 0.08 a
Cu	0.37 ± 0.21 e	1.12 ± 0.20 e	2.15 ± 0.32 d	4.35 ± 0.43 c	6.36 ± 0.48 b	9.69 ± 0.13 a
Mn	0.66 ± 0.45 e	1.25 ± 0.42 e	175.78 ± 0.29 d	198 ± 0.53 c	211.22 ± 0.26 b	365.14 ± 3.52 a
Cd	0.004 ± 0.06 c	0.07 ± 0.15 c	1.05 ± 0.16 c	2.91 ± 0.08b	3.11 ± 0.17 b	4.26 ± 0.52 a
Pb	0.07 ± 0.01 d	0.12 ± 0.35 d	2.18 ± 0.29 c	2. 82 ± 0.18 b	3.27 ± 0.38 b	4.25 ± 0.25 a
Hg	0.007 ± 0. 33 d	0.002 ± 0.16 d	1.76 ± 0.21 c	2.43 ± 0.25 b	2.87 ± 0.08 b	3.67 ± 0.79 a
As	0.02 ± 0.04 d	0.05 ± 0.01 d	0.63 ± 0.07 c	0.71 ± 0.18 c	1.29 ± 0.11b	2.23 ± 0.01 a

*mgL^{-1} , all the values are mean of three replicates ($n = 3$ ± standard deviation); different letters in each group shows significant difference at $p \leq 0.05$

maximum transpiration was recorded under 5% SW concentration compared to control and other treatments (Fig. 1b). The transpiration value in the control was 4.34 mmol m^{-2} s^{-1} which was significantly ($p \leq 0.05$) higher than under 20% SW concentration.

PTEs accumulation in rice plants

Concentration of all PTEs tested in rice grains gradually increased with increasing concentration of SW (Table 4). Maximum concentrations of PTEs were recorded under 20% SW concentration. However, concentrations of Cd, Pb, Hg, and As in grains did not show significant differences under 5% SW and sole application of TW, and the overall concentrations of PTEs in rice grains showed a trend of Zn > Fe > Mn > Cu > As > Hg > Cd > Pb (Table 4). Addition of SW with TW significantly ($p \leq 0.05$) increased the concentration of PTEs in rice straw compared to the control (Table 4). The highest concentration of all tested PTEs in rice straw was recorded under 20% SW concentration and concentration of As, Hg, and Pb did not show significant differences among 5% SW concentration and TW sole application (Table 4). The concentration of PTEs in rice straw was higher than rice grains showing an overall trend of Zn > Fe > Mn > Cu > Cd > Pb > As > Hg in rice straw (Table 4).

Plant transfer factor (PTF)

Zn PTF was highest under TW followed by 5%, 10%, 15%, and 20% SW concentrations. Similarly, the maximum PTF was found for Cu in TW and 5% SW concentration, while for Fe and Mn, it was found in TW and 10% SW concentration respectively (Table 5). The highest mean value of PTF for Cd was recorded in TW and for Pb, it was observed under 5% SW concentration. Similarly, maximum PTF for Hg was observed under 15 and 20% SW concentration while for As, it was found under TW sole application followed by 5% SW concentration (Table 5). However, the average trend of PTEs PTF was in the order of Fe > Mn > Zn > Cu > Pb > Cd > As > Hg (Table 5).

DIM and HRI of PTEs

The observed mean values of DIM for adults and children based on their average dietary intake of rice are given in Table 6. The mean values of DIM for adults and children were significantly increased with increasing concentration of SW in irrigational water (Table 6). However, all the means were lower than one. The highest values of DIM were observed for Zn followed by Fe and Mn while the lowest were observed for As and Hg, respectively (Table 6).

The noted values of HRI for PTEs for children and adults via daily dietary intake of rice are given in Table 6. The HRI of

Table 2 Selected physico-chemical properties of experimental soil and PTEs (mg kg^{-1}) concentration in soil before and after different concentrations of SW application

Soil property	TW	5% SW	10% SW	15% SW	20% SW
Sand (%)	41				
Silt (%)	38				
Clay (%)	21				
Textural class	Loam				
pH	7.32 ± 0.05 bc	7.36 ± 0.05 bc	7.45 ± 0.02 b	7.50 ± 0.08 ab	7.63 ± 0.05 a
EC (dSm^{-1})	1.68 ± 0.06 c	1.81 ± 0.05 b	1.91 ± 0.03 ab	1.93 ± 0.05 ab	1.98 ± 0.052 a
Ca (mg kg^{-1})	623.41 ± 1.57 d	637.15 ± 2.23 c	644.67 ± 4.04 c	661.00 ± 6.00 b	688 ± 3.6 a
Mg (mg kg^{-1})	212.76 ± 1.56 e	223.4 ± 1.35 d	233.52 ± 0.95 c	262.55 ± 0.5 b	267.32 ± 0.51 a
Na (mg kg^{-1})	110.06 ± 0.016 e	124.15 ± 0.81 d	132.33 ± 0.72 c	139.62 ± 1.52 b	146.11 ± 0.06 a
AB-DTPA P (mg kg^{-1})	3.8 ± 0.06 e	5.72 ± 0.17 d	8.48 ± 0.07 c	10.5 ± 0.27 b	13.43 ± 0.28 a
AB-DTPA K (mg kg^{-1})	41.83 ± 0.28 e	111.66 ± 1.23 d	128.35 ± 3.48 c	155.45 ± 336 b	172.7 ± 4.03 a
Nitrogen (%)	0.017 ± 0.003	0.02 ± 0.001	0.09 ± 0.13	0.27 ± 0.01	0.3 ± 0.001
Organic matter (%)	0.62 ± 0.03 d	0.76 ± 0.08 c	0.92 ± 0.08 bc	1.23 ± 0.15 a	1.18 ± 0.15 ab
Zn	17.2 ± 0.12 e	21.82 ± 0.23 d	33.93 ± 0.13 c	46.97 ± 0.19 b	59.12 ± 0.23 a
Cu	9.61 ± 0.47 e	12.8 ± 0.22 d	24.96 ± 0.85 c	29.41 ± 0.39 b	42.84 ± 0.69 a
Mn	0.44 ± 0.43 e	0.49 ± 0.06 d	04.46 ± 0.57 c	13.04 ± 0.78 b	20.31 ± 0.59 a
Fe	1.59 ± 0.61 e	4.01 ± 0.87 d	33.27 ± 1.03 c	55.74 ± 0.28 b	88 ± 1.06 a
Cd	0.23 ± 0.23 e	0.61 ± 0.46 d	2.24 ± 0.4 c	4.96 ± 0.18 b	6.4 ± 0.53 a
Pb	0.22 ± 0.04 e	0.34 ± 0.04 d	1.57 ± 0.33 c	2.78 ± 0.39 b	4.42 ± 0.47 a
Hg	0.84 ± 0.3 e	1.78 ± 0.21 d	3.59 ± 0.37 c	4.85 ± 0.27 b	6.54 ± 0.28 a
As	0.28 ± 0.56 e	0.47 ± 0.46 d	5.64 ± 0.4 c	7.12 ± 0.71 b	8.87 ± 0.36 a

All the values are mean of three replicates ($n = 3$ ± standard deviation); different letters in each group shows significant difference at $p \leq 0.05$

PTEs in children varied at different rates of SW and ranged as Zn 4.8×10^{-2}–7.9×10^{-2}, Fe 2.7×10^{-2}–9.2×10^{-2}, Cu 1.4×10^{-1}–2.9×10^{-1}, Mn5.8×10^{0}–1.5×10^{0}, Cd 1.5×10^{-1}–1.6×10^{0}, Pb 1.7×10^{-3}–1.8×10^{-2}, Hg 1.7×10^{-3}–1.9×10^{-1}, and As 2.2×10^{-1}–4.9×10^{0} while for adults, PTEs HRI ranged Zn 3.2×10^{-2}–5.2×10^{-2}, Fe 1.8×10^{-2}–6.1×10^{-2}, Cu 9.7×10^{-2}–1.9×10^{-1}, Mn 3.9×10^{-1}–1.0×10^{0}, Cd 1.0×10^{-1}–1.0×10^{0}, Pb 1.1×10^{-3}–1.2×10^{-2}, Hg 1.1×10^{-3}–1.3×10^{-1}, and As 1.4×10^{-1}–3.3×10^{0} (Table 6).

Discussion

The TW was neutral in physico-chemical properties, while SW had high EC, OM, NPK, and low pH as shown in (Table 1). The concentration of all the PTEs tested in 5% SW was within the PLs of (NEQS 1999 and USEPA 2002) except As (arsenic), while in all other concentrations of SW, PTEs concentration was higher than the PLs of NEQS and USEPA (Tables 1 and 7) except (Zn). The study results

Table 3 Effect of SW on rice growth and yield

Parameters	TW	5% SW	10% SW	15% SW	20% SW
Plant height (cm)	119.66 ± 3.35 c	147.06 ± 4.33 a	135.49 ± 4.19 b	132.27 ± 2.79 b	112.48 ± 2.32 c
Panicle length (cm)	16.12 ± 2.24 cd	31.89 ± 2.69 a	26.8 ± 2.45 ab	22.16 ± 2.56 bc	15.8 ± 1.38 c
Numbers of tillers (plant^{-1})	16.67 ± 1.52 c	29.00 ± 2.00 a	23.33 ± 2.51 b	17.00 ± 1.73 c	14.00 ± 1.00 c
Numbers of ears (plant^{-1})	7.00 ± 1.00 c	13.00 ± 2.00 a	11.00 ± 2.00 bc	9.00 ± 2.00 bc	7.33 ± 2.00 c
Numbers of grains (plant^{-1})	219.66 ± 11.59 e	676 ± 9.53 a	502.33 ± 2.51 b	368 ± 10.54 c	284 ± 7.15 d
1000-grain weight (g)	17.23 ± 1.33 c	31.03 ± 1.34 a	24.73 ± 1.20 b	17.6 ± 1.73 c	12.16 ± 1.13 d
Grain weight (plant^{-1})	08.39 ± 0.34e	12.14 ± 0.32 a	10.31 ± 0.5 b	9.30 ± 0.29 c	7.03 ± 0.10 d
Grain yield (ton ha^{-1})	3.98 ± 0.96 bc	7.41 ± 0.38 a	5.58 ± 0.82 b	3.68 ± 0.57 c	3.24 ± 0.42 c
Straw yield (ton ha^{-1})	7.93 ± 1.04 bc	12.17 ± 0.61 a	8.96 ± 0.79 b	6.56 ± 0.26 c	4.42 ± 0.36 d

All the values are mean of three replicates ($n = 3$ ± standard deviation); different letters in each group shows significant difference at $p \leq 0.05$

suggest that high EC and low pH would be due to the presence of soluble salts while high PTEs and salts in SW might be accumulated during the condensation process of alcohol (Chandra et al. 2008). Experimental soil was neutral in physico-chemical characteristics; rise in SW concentration significantly increased soil EC, pH, OM, Ca, Mg, Na, NPK, and PTEs concentration as shown in (Table 2) indicating that SW could act as a buffer medium for agricultural lands. These results are consistent with the previous reported studies (Ramana et al. 2002; Chandra et al. 2009; Rath et al. 2010; Narain et al. 2012; Kaloi et al. 2017) suggesting that direct application of SW is not fit for agricultural lands. However, under 5% SW concentration, soil EC, pH was neutral, and all the tested PTEs were within the PLs of (FAO 2004) for agricultural lands (Tables 2 and 7) while > 5% SW concentrations all the PTEs except (Zn, Cu) were higher than PLs of (FAO 2004).

Increase in leaf photosynthetic activity, stomatal opening, and transpiration rate under lower concentration of SW (Fig. 1a, b) indicates adequate availability of plant nutrients. Our findings are in full agreement to the previous studies by (Bellore and Mall 1975; Alia and Saradhi 1995; Swarup and Yaduvansiii 2000). According to Yadana et al. (2009), adequate amount of plant available nutrients can assimilate plant leaf area leading to better crop physiology. Improvement in rice physiology under low level of spentwash may be attributed to the plenty of required nutrients for plant uptake and presence of lower concentrations of salts, heavy metals, organic, and inorganic pollutants (Krupa and Baszynski 1995;

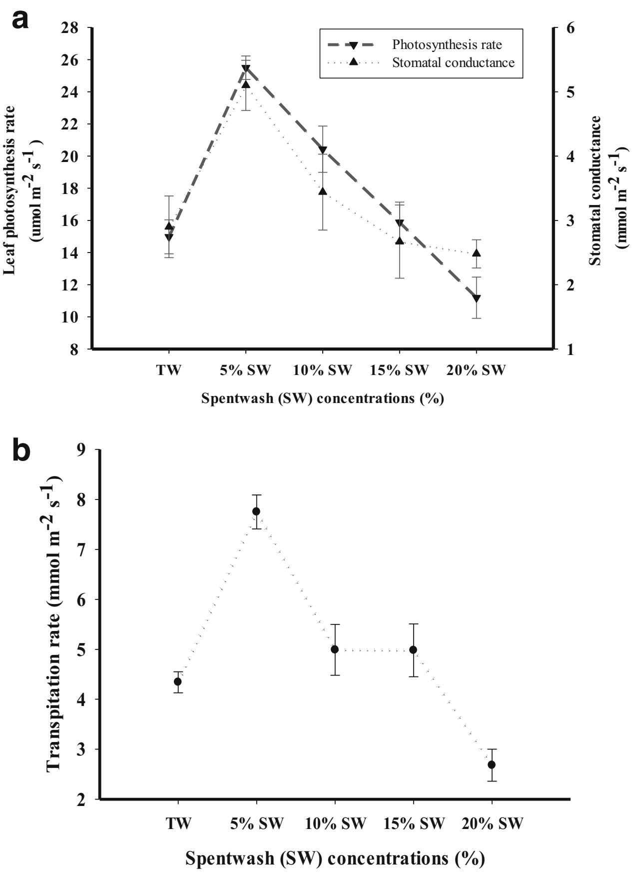

Fig. 1 Leaf photosynthesis (**a**) and transportation rate (**b**) under different spentwash concentrations

Table 4 PTEs concentrations in rice grains and straw (mg kg^{-1})

PTEs	TW	5% SW	10% SW	15% SW	20% SW
Zn	24.30 ± 1.2 d	29.24 ± 1.02 c	31.46 ± 0.53 c	34.18 ± 0.68 b	39.32 ± 0.53 a
Fe	13.73 ± 0.66 e	19.68 ± 0.54 d	35.57 ± 0.71 c	39.67 ± 0.51 b	45.8 ± 0.69 a
Cu	09.74 ± 0.54 e	11.7 ± 0.69 d	13.58 ± 0.41 c	16.75 ± 0.59 b	19.54 ± 0.42 a
Mn	1.36 ± 0.31 d	1.96 ± 0.24d	29.39 ± 1.00 c	33.56 ± 0.39 b	35.61 ± 0.94 a
Cd	0.13 ± 0.10 c	0.18 ± 0.03 c	0.35 ± 0.04 c	0.88 ± 0.11 b	1.34 ± 0.24 a
Pb	0.10 ± 0.06 c	0.21 ± 0.09 c	0.62 ± 0.31 b	0.71 ± 0.22 ab	1.05 ± 0.06 a
Hg	0.02 ± 0.01 d	0.02 ± 0.01 d	0.82 ± 0.08 c	1.69 ± 0.23 b	2.27 ± 0.0.36 a
As	0.11 ± 0.02 d	0.15 ± 0.03 d	0.82 ± 0.09 c	1.46 ± 0.10 b	2.47 ± 0.16 a
PTEs concentrations in rice straw					
Zn	32.81 ± 0.48 e	45.52 ± 1.78 d	53.48 ± 0.26 c	62.63 ± 0.27 b	67.62 ± 0.13 a
Fe	24.41 ± 0.15 e	33.09 ± 0.32 d	39.34 ± 0.33 c	41.99 ± 1.11 b	44.18 ± 0.04 a
Cu	2.01 ± 0.14 e	5.42 ± 0.40 d	12.46 ± 0.24 c	17.67 ± 0.22 b	31.30 ± 0.52 a
Mn	27.30 ± 0.42 e	31.51 ± 0.47 d	34.55 ± 0.18 c	37.26 ± 0.23 b	43.00 ± 1.16 a
Cd	0.27 ± 0.15 d	0.41 ± 0.20 c	0.81 ± 0.19 b	1.49 ± 0.39 a	3.00 ± 0.17 a
Pb	0.48 ± 0.08 c	0.85 ± 0.18 c	1.54 ± 0.21 b	1.86 ± 0.07 b	3.16 ± 0.02 a
Hg	0.10 ± 0.07 c	0.20 ± 0.03 c	0.67 ± 0.09 b	0.97 ± 0.09 ab	1.31 ± 0.27 a
As	0.67 ± 0.02 d	0.82 ± 0.12 d	1.65 ± 0.09 c	1.93 ± 0.04 b	2.76 ± 1.56 a

All the values are mean of three replicates ($n = 3$ ± standard deviation); different letters in each group shows significant difference at $p \leq 0.05$

Table 5 PTEs bioaccumulation in rice grains (mean ± standard deviation)

Element	TW	5% SW	10% SW	15% SW	20% SW
Zn	1.42 ± 0.11	1.34 ± .03	0.92 ± 0.03	0.72 ± 0.02	0.66 ± .0.01
Cu	1.01 ± 0.02	0.91 ± 0.06	0.54 ± 0.01	0.56 ± 0.02	0.45 ± 0.01
Fe	8.81 ± 1.55	4.90 ± 0.20	1.06 ± 0.02	0.71 ± 0.00	0.52 ± 0.01
Mn	3.08 ± 0.66	3.97 ± 0.33	6.59 ± 0.2	2.57 ± 0.02	1.75 ± 0.02
Cd	0.6 ± 0. 49	0.29 ± 0.01	0.15 ± 0.03	0.17 ± 0.02	0.21 ± 0.04
Pb	0.46 ± 0.29	0.64 ± 0.21	0.4 ± 0.07	0.26 ± 0.11	0.23 ± 0.03
Hg	0.02 ± 0.01	0.01 ± 0.00	0.23 ± 0.04	0.34 ± 0.03	0.34 ± 0.04
As	0.39 ± 0.07	0.32 ± 0.12	0.14 ± 0.00	0.2 ± 0.02	0.27 ± 0.02

Sinha et al. 1997; Mysliwa-Kurdziel and Strzałka 2002; Mahesh et al. 2013). However, reduction in physiological parameters with increasing concentration of SW (Fig. 1a, b) indicates interference of heavy metals, which might directly inhibit the chlorophyll synthesis and decrease the photosynthetic activity (Sinha et al. 1997; Assche and Clijsters 2010; Oncel et al. 2000). Inhibition of plant metabolic and enzymatic reactions due to high levels of heavy metals have been reported (Zeng et al. 2007; Anjum et al. 2016; Riaz et al. 2018) particularly; Cd has been reported for reduction in chlorophyll biosynthesis via reducing the complex formation between aminolevulinic acid and chlorophyll synthesizing enzyme (photoactive protochlorophyllide reductase) through blockage of acid-active thiol groups by cadmium (Stobart et al. 2010; Oncel et al. 2000).

Rice plant did not show any morphological changes initially; however, after 50 days, application of SW > 10% showed leaf chlorosis, plant height, and panicle length retardation (Table 3). Addition of 5% SW rapidly increased the plant growth components, while > 5% SW concentration showed inhibitory effects on plant growth (Table 3). Visible changes in morphological characteristics might be due to shortage of

Table 6 Daily intake of metals (DIM) and HRI (health risk index) of PTEs caused by the consumption of rice grown at different concentrations of spentwash

Treatments	Individual	Zn	Fe	Cu	Mn	Cd	Pb	Hg	As
	DIM								
TW	Adult	9.7×10^{-3}	5.5×10^{-3}	3.9×10^{-3}	5.4×10^{-4}	5.0×10^{-5}	4.0×10^{-5}	8.0×10^{-5}	4.4×10^{-5}
	Child	1.4×10^{-2}	8.2×10^{-3}	5.8×10^{-3}	8.2×10^{-4}	7.8×10^{-4}	6.0×10^{-5}	1.2×10^{-5}	6.6×10^{-5}
5% SW	Adult	1.1×10^{-2}	7.9×10^{-3}	4.7×10^{-3}	7.8×10^{-4}	7.2×10^{-5}	8.4×10^{-5}	8.0×10^{-5}	6.0×10^{-5}
	Child	1.7×10^{-2}	1.1×10^{-2}	7.0×10^{-3}	1.1×10^{-3}	1.0×10^{-4}	1.2×10^{-4}	1.2×10^{-5}	9×10^{-5}
10% SW	Adult	1.2×10^{-2}	1.4×10^{-2}	5.4×10^{-3}	1.1×10^{-2}	1.4×10^{-4}	2.4×10^{-4}	3.2×10^{-4}	3.2×10^{-4}
	Child	1.8×10^{-2}	2.1×10^{-2}	8.1×10^{-3}	1.7×10^{-2}	2.1×10^{-4}	3.7×10^{-4}	4.9×10^{-5}	4.9×10^{-4}
15% SW	Adult	1.3×10^{-2}	1.5×10^{-2}	6.7×10^{-3}	1.3×10^{-2}	3.5×10^{-4}	2.8×10^{-4}	6.7×10^{-4}	5.8×10^{-4}
	Child	2.0×10^{-2}	2.3×10^{-2}	1.0×10^{-2}	2.0×10^{-2}	5.3×10^{-4}	4.2×10^{-4}	1.3×10^{-3}	8.8×10^{-4}
20% SW	Adult	1.5×10^{-2}	1.8×10^{-2}	7.8×10^{-3}	1.4×10^{-2}	5.3×10^{-4}	4.2×10^{-4}	9.0×10^{-4}	9.9×10^{-4}
	Child	2.3×10^{-2}	2.7×10^{-2}	1.0×10^{-2}	2.1×10^{-2}	8.0×10^{-4}	6.3×10^{-4}	1.3×10^{-3}	1.4×10^{-3}
HRI									
TW	Adult	3.2×10^{-2}	1.8×10^{-2}	9.7×10^{-2}	3.9×10^{-2}	1.0×10^{-1}	1.1×10^{-3}	1.1×10^{-3}	1.4×10^{-1}
	Child	4.8×10^{-2}	2.1×10^{-2}	1.4×10^{-1}	5.8×10^{-2}	1.5×10^{-1}	1.7×10^{-3}	1.7×10^{-3}	2.2×10^{-1}
5% SW	Adult	3.9×10^{-2}	2.6×10^{-2}	1.1×10^{-1}	5.6×10^{-2}	1.4×10^{-1}	2.4×10^{-3}	1.1×10^{-3}	2.0×10^{-1}
	Child	5.8×10^{-2}	3.9×10^{-2}	1.7×10^{-1}	8.4×10^{-2}	2.1×10^{-1}	3.6×10^{-3}	1.7×10^{-3}	3.0×10^{-1}
10% SW	Adult	4.2×10^{-2}	4.7×10^{-2}	1.3×10^{-1}	8.4×10^{-1}	2.8×10^{-1}	7.1×10^{-3}	4.7×10^{-2}	1.0×10^{0}
	Child	6.3×10^{-2}	7.1×10^{-2}	2.0×10^{-1}	1.2×10^{0}	4.2×10^{-1}	1.0×10^{-2}	7.0×10^{-2}	1.6×10^{0}
15% SW	Adult	4.5×10^{-2}	5.3×10^{-2}	1.6×10^{-1}	9.6×10^{-1}	7.0×10^{-1}	8.1×10^{-3}	9.6×10^{-2}	1.9×10^{0}
	Child	6.8×10^{-2}	7.9×10^{-2}	2.5×10^{-2}	1.4×10^{0}	1.0×10^{0}	1.2×10^{-2}	1.4×10^{-1}	2.9×10^{0}
20% SW	Adult	5.2×10^{-2}	6.1×10^{-2}	1.9×10^{-1}	1.0×10^{0}	1.0×10^{0}	1.2×10^{-2}	1.3×10^{-1}	3.3×10^{0}
	Child	7.9×10^{-2}	9.2×10^{-2}	2.9×10^{-1}	1.5×10^{0}	1.6×10^{0}	1.8×10^{-2}	1.9×10^{-1}	4.9×10^{0}

Table 7 Permissible limits (PLs) of PTEs in irrigational water, soil, rice grains, and straw suggested by various agencies

Names of the PLs suggesting agencies	Zn	Fe	Cu	Mn	Cd	Pb	Hg	As
PLs for irrigational water (mgL^{-1})								
National Environmental Quality Standards for irrigational water	5	2	1	1.5	0.1	0.5		
United States Environmental Protection Agency (USEPA 2002)	5	0.3	1	0.05	0.01	0.015	0.002	0.01
PLs for soil (mg kg^{-1})								
Food and Agriculture Organization (FAO 2004)	50	5	36	0.20	0.8	85	2	0.5
PLs for rice grains (mg kg^{-1})								
World Health Organization (WHO 1993)	60	20	40	2	0.2	5	0.02	0.15
PLs for animals fodder (mg kg^{-1})								
United States of America (Cang 2004)					0.5	5	0.2	2
Russia (Cang 2004	100		8					

National Environmental Quality Standards for irrigational water (NEQS 1999)

available micronutrients and heavy metals stress (Zn, Fe) (Alfaraas et al. 2016). The increase in plant growth under lower concentration of SW may be attributed to the proper concentration of required nutrients that accelerated better functions of plant growth hormones axin and gibberline (Misra and Behera 1991). Rise in crop growth under lower concentration of SW and other effluents also have been reported (Belefant-Miller 2007; Hasanuzzaman et al. 2010; Rath et al. 2013). Decrease in plant growth under higher concentration of SW (Table 3) indicates excessive phytoavailability of heavy metals and salts in SW, which could disrupt essential nutrient uptake and ATP synthesizing enzymes (Pandey et al. 2008; Kaloi et al. 2015). The decrease in plant growth under higher concentrations of SW could be attributed, to the loss of essential plant growth intermediate metabolites due to entrance of metals into protoplasm (Subramani et al. 1997).

In present investigation, significant increase in grain and straw yield under 5% SW suggests that increase in growth rate, number of tillers, number of grain plant^{-1}, 1000-grain weight, and number of ears plant^{-1} is added to the total increase in grain and straw yield (Table 3). These finding demonstrates that at lower concentration of SW, plants attained maximum nutrients for better growth. This increase might be due to presence of considerable amount of NPK in SW (Table 1) and increased microbial activity due to added organic matter which in turn increased the availability of micronutrients (Chandraju and Basavaraju 2007; Chandraju et al. 2008; Das et al. 2010; Rath et al. 2013; Naveed et al. 2018). Higher photosynthetic activity may also lead to the production of higher dry matter (Bellore and Mall 1975; Alia and Saradhi 1995). However, reduction in 1000-grain weight and number of grain plant^{-1} suggests that at higher concentration of SW, availability of major nutrients may would be reduced for growth and development of plants. The other possible reason of grains and straw yield reduction could be the decrease in physiological activities due to higher availability of PTEs and salts (Wahid et al. 2007).

PTE accumulation in rice grains and straw SW showed gradual increase along with increase in SW concentrations (Table 4). The gradual increase of PTEs in rice grains and straw might be attributed to the higher concentration of available metals. Reduction in plant physiological activities could also be the major cause of metal accumulation in plant above ground parts, such as decrease in transpiration rate at higher concentration of SW could accumulate more metals in shoots and reduce the metal exclusion (Bose and Bhattacharyya 2008; Quartacci et al. 2006). These results are in conformity with the results of Chandraju and Basavaraju (2007), Chandraju et al. (2008), Das et al. (2010), and Rath et al. (2013). PTE concentration in rice straw was higher than grains (Table 5) indicating higher potential of straw for metal accumulation in comparison with grains; it might be due to the second crop organ after roots in contact with soil and available metals (Singh and Agrawal 2009). However, under 5% SW concentration, all the PTEs in rice grains were within the PLs of WHO (Tables 4 and 7) and concentration of PTEs was also within the PLs of PTEs in animal fodder and livestock feeds recommended by America and Russia (Table 7). It indicates that under 5% SW concentration, adequate amount of essential nutrient (Zn, Fe, Cu, Mn) is also available which might be helpful to recover the nutrient deficiency.

The higher values of PTF (bioaccumulation) under TW sole application and lower concentration of SW indicates that soil transfer and plant uptake capacity of PTEs was higher than the higher rate of SW; it may be attributed to better crop activity and plant metabolic performance under lower SW concentration cause of adequate nutrient availability and less PTE stress (Bose and Bhattacharyya 2008).

The HRI data of the present study indicated that at 5% SW rate, all the PTEs were < 1 but higher rate of SW concentration led to HRI of > 1 for As, Cd, and Mn (Table 6).

The HRI values are dependent on DIM and RfD suggested by various authorities/agencies while DIM calculated from the data. Owing to the higher value of DIM calculated (Table 6), concentration of HRI was greater. The daily dietary intake PTEs in rice grains for consumption of children and adults was within the safe limit suggested by USEPA (2002). The results of our study suggest that rice grown at lower concentration (5%) of SW would be safe for human consumption while higher rate of SW application could produce health risks for human beings and animals and lead to the contamination of agricultural lands.

Conclusions

Analysis of SW reveled that it contains abundant toxic as well as essential elements; direct application of SW in agricultural lands could cause heavy metal accumulation in soil and food. Our study results indicate that 5% SW concentration contained adequate amount of essential and nonessential plant nutrients; therefore, plant growth, physiological parameters, and yield were enhanced while, > 5% SW concentrations seem not suitable for irrigation of rice crop, as high PTE and salt concentration may directly or indirectly can reduce the plant growth and yield. Our results revealed that the rice plants irrigated with > 5% SW concentration accumulated PTEs in rice grains and straw beyond the PLs as recommended by FAO/WHO (Table 7) for human beings and animals. However, PTEs concentration in rice grains and straw was within the PLs under 5% SW concentration. Further, health risk assessment confirmed that the rice grown under 5% SW concentration is not toxic for human consumption. However, it is important that small-scale sugar industries and farmers should not put SW directly into water bodies or agricultural lands without proper treatment or dilution.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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