



## Research article

# Health risk assessment of heavy metals in saffron (*Crocus sativus* L.) cultivated in domestic wastewater and lake water irrigated soils

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## ABSTRACT

Irrigation of crops with domestic wastewater (DW) is a common practice in developing countries like India. However, domestic wastewater irrigation poses a risk of migration of toxic heavy metals to edible parts of crops, which requires serious measures to prevent their uptake. In this study, the effect of DW irrigation in comparison with Sarbal Lake water (SLW) and borewell water (BW) on soil characteristics and cultivated saffron (*Crocus sativus* L.) was investigated. For this purpose, samples of water, soil, and saffron (corm, petal, and stigma) were collected from the suburban area of Pampore, Srinagar district, Jammu and Kashmir, India. The results showed that DW irrigation had the maximum significant ( $p < 0.05$ ) influence on the physico-chemical and nutrient characteristics of the soil, followed by SLW and BW irrigation, respectively. The growth and yield parameters of saffron were also significantly ( $p < 0.05$ ) increased in the case of DW irrigation as compared to SLW and BW. The quality ranking of the cultivated saffron was found to be in accordance with the ISO standard (III: BW and II: DW and SLW). On the other hand, DW irrigation showed a significant increase in heavy metal contents (mg/kg) of saffron plant parts such as As (0.21–0.40), Cd (0.04–0.09), Cr (0.16–0.41), Cu (7.31–14.75), Fe (142.38–303.15), Pb

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(0.18–0.31), Mn (15.26–22.81), Hg (0.18–0.25), Ni (0.74–1.18), Se (0.13–0.22), and Zn (3.44–4.59), followed by SLW and BW. However, the levels of heavy metals did not exceed the FAO/WHO safe limits. Bioaccumulation factor (BAF), dietary intake modeling (DIM<0.006496), health risk assessment (HRI<0.028571), and target hazard quotient (THQ<1) analyses showed no potential health hazard associated with the consumption of saffron irrigated with DW and SLW. Therefore, the results of this study provide valuable insights into the optimization of irrigation sources for saffron cultivation.

## 1. Introduction

Wastewater liberation is one of many inducers of carbon emissions that promote global warming [1,2]. Taking into consideration soil ecological stoichiometry can allow using wastewater in agricultural practices [3]. However, irrigation times and wastewater impact on soil texture and microbial population and diversity shall be accounted [4,5]. Moreover, wastewater is the main source of pollutants that can leachate into the soil [6] but fortunately bioaccumulated by plant materials [7]. Domestic wastewater (DW) originates from domestic activities like bathing, dishwashing, laundry, and toilet flushing, among others. It contains large amounts of organic and inorganic compounds i.e., ammonium, nitrates, phosphates, heavy metals (HMs), and other chemical compounds besides millions of microbes (e.g., *Escherichia coli*) [8]. Domestic wastewater contributes to 6 and 50% of total nitrogen and total phosphorus flux, respectively around the globe [9]. Furthermore, rapid population growth and increased urbanization are the two main factors exacerbating DW loadings. The treatment of such wastewater varies significantly between developed and developing countries. As per the United Nations Economic Commission for Europe (UNECE), the percentage of treated DW was superior to 69% and as high as 100% in many developed countries, whereas such percentages were never reached in developing countries with often no treatment occurrence [10]. Various methods are used for DW treatment starting from conventional methods e.g., biodegradation, flocculation/coagulation, and precipitation, to established methods e.g., membrane separation, oxidation, and solvent extraction, and landing to highly developed methods e.g., biosorption, biofiltration, and nanofiltration [11]. Unfortunately, around 78.7% of DW are untreated in India [12]. Moreover, among the treated proportion, only the organic pollutants are given focus while the monitoring of chemical pollutants (mainly detergents) is very limited. This naturally accentuates waterborne diseases and degradation of drinking and underground water quality when disposed of. The discharge of DW in water canals results in high eutrophication rates, loss of biodiversity, and changes in species composition and aquatic ecosystems [13,14].

Domestic wastewater contains high levels of HMs i.e., cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) that can bioaccumulate in irrigated soils [15]. Increased HMs in soil naturally impact soil microbial populations [16]. However, the selection of suitable and tolerant crops can help mitigate this issue and reduce potential health risks [17]. Soil irrigation with DW resulted in increased organic matter and HMs by 20–30% and 100%, respectively, and pH value by 2–3 points [18]. Increased HM accumulation in irrigated soils is associated with a decrease in soil particle size [19,20]. It is also worth noting that soil type plays a crucial role in increasing/decreasing HM bioaccumulation [21]. Manjunatha et al. [22] found that soil irrigation with untreated DW resulted in non-significant changes in pH and electrical conductivity (EC), significant improvements in soil calcium (Ca), magnesium (Mg), nitrogen (N), and potassium (K) contents, and higher yield of cluster bean compared to control (fresh water-irrigated plants). Baniani et al. [23] mentioned that untreated DW resulted in higher cotton boll number and yield compared to plants irrigated with BW. On the other hand, Hassanpour Darvishi et al. [24] mentioned that soils irrigated with untreated DW had a high potential to reduce biochemical oxygen demand (BOD<sub>5</sub>) and chemical oxygen demand (COD) with increased nutrient availability. Although irrigation with untreated wastewater has shown increased antimicrobial resistance in soil [25], the study of the long-term effects of DW on soil and growing crops is still in its elementary state with not much reliable data.

Saffron (*Crocus sativus* L.) is one of the most expensive and rare agricultural commodities worldwide. It is well appreciated and frequently used in many Asian (Iranian, Indian, Pakistani, and Middle Eastern) cuisines. Global saffron production has been estimated at around 500 tons in 2019, with Iran as the leading producer followed by India [26]. The global saffron market has been valued at US\$ 1.07 billion in 2022 and is forecasted to reach a value of US\$ 1.77 billion between 2023 and 2029 with a 7.3% CAGR [27]. Saffron is rich in carotenoids (crocin and crocetin) (color), picrocrocin (taste), safranal (aroma), and terpenes [28]. Carotenoids and terpenes possess hepatoprotective, anti-viral, anti-inflammatory, and analgesic properties, and help reduce the risk of cancer and cardiovascular diseases [29,30]. Saffron is also a good source of flavonoids, phenolic acids, and phytosterols, and has learning and memory-enhancing effects [31]. Therefore, it can be considered a highly nutritious and medicinal food product.

A temperature of 23–27 °C for 50 days is needed for optimal flower production and a 600 mm seasonal rainfall is sufficient for adequate saffron cultivation [32]. A high humidity level can lead to corm rot or, at best, to retarded flowering [33]. Positive correlations were found between high-altitude growing sites in Western Himalaya, sandy-loam soil texture, and less rainfall and relative humidity on the one hand, and improved yield attributes, and crocin, picrocrocin, total phenolics, and total flavonoids contents on the other hand [32]. Moreover, loose and well-drained Kashmiri soil with a 6.3–8.3 pH and an EC of 0.09–0.30 dS. m<sup>-1</sup> makes it well-suitable for saffron cultivation [34]. However, reduced rainfall levels during the last few years in the Kashmir region have led to reduced saffron yields. This has pushed farmers to adopt alternative irrigation sources mainly based on agro-industrial effluents, and municipal sewage and domestic wastewaters. Such alternatives were chosen based on their low cost, high availability, and richness in nutrients which reduces reliance on chemical fertilizers. However, such a strategy cannot reduce nitrogen leaching resulting from traditional fertilization techniques [35]. Moreover, its high enclosure of HMs has been reported to induce depletion of basic soil

components, i.e., soil moisture, organic matter, and available phosphorus contents, along with reduced saffron growth [36]. Although a transition from chemical fertilization to organic farming was proposed [37], the use of effluents highly rich in organic matter, HMs, BOD<sub>5</sub>, and COD contents for saffron irrigation is a matter of deep discussion and the unraveling of its long-term effect on soil, plant, and human health is still unclear.

From an agronomic perspective, Choopan et al. [38] used municipal wastewater (MW) to irrigate saffron and found positive impacts on yield and qualitative traits. More precisely, the number of flowers per corm, stigma dry weight, and crocin and safranal contents increased in MW-irrigated plants compared to control ones (BW-irrigated plants). However, HM bioaccumulation in saffron plants as a result of MW irrigation was not investigated by these authors with no assessment of health risk. Due to increased diseases associated with food consumption, especially heavy metal-rich products, the assessment of potential health risks was found to be crucial for the well-being of society. Several studies assessed the health risks associated with the consumption of crops irrigated with wastewater [39,40]. Such types of investigations can help reduce statistical uncertainties besides other modeling methods [41,42]. Although no potential health risk was observed, continuous assessment was recommended [43,44]. This helps alarm the population from any short-, mid-, and long-term impacts from the consumption of main vegetables and fruits [45]. All these investigations concluded the safe consumption of mushrooms, mangoes, rice, and lychee collected from studied sites. However, to our knowledge, there is no previous research that assessed the health risk of heavy metals in saffron grown in wastewater-irrigated soils.

Although the World Health Organization (WHO) asked to avoid untreated wastewater in agricultural production, it depicted on the other hand that continuous health risk assessments and follow-ups could be effective though they are expensive and not easily affordable in developing countries. However, the adoption of such practices is limited by the extent to which WHO can help support and sustain farmers' behavioral change through training [46]. The present study aimed for the first time to assess the health risk of eleven heavy metals in saffron (*Crocus sativus* L.) cultivated in domestic wastewater- and lake water-irrigated soils. Besides, the effects of such irrigation sources on the physicochemical properties of soil and quantitative and qualitative traits of the saffron plant were investigated.

## 2. Materials and methods

### 2.1. Study area description

The present study was carried out in the suburban vicinity of Pampore, Srinagar district, Jammu and Kashmir, India. The study sites were close to the Sarbal Lake's bank and in the vicinity of agricultural land establishments (34°0'36.06" N and 74°55'25.68" E). In this region, saffron (*Crocus sativus* L.) and rice (*Oryza sativa* L.) are the main grown crops. The lack of DW treatment facilities in Pampore results in large inundation in private and public properties, thus resulting in severe diarrhea among residents. Although the Indian Government and local authorities have banned Pampore residents from disposing of untreated DW into water sources, such pollutants are still liberated into Sarbal Lake. This may lead to the presence of pathogenic *Escherichia coli* in liberated wastewater. On the other hand, some saffron farmers started using untreated DW and Lake's water for irrigation in the last five years to overcome the increased drought and erratic rainfall situation in Jammu and Kashmir. A total of nine sampling sites were selected for the present investigation i. e., three BW-irrigated sites (control), three DW-irrigated sites, and three SLW-irrigated sites. It is worth mentioning that this is the first study to analyze the potential accumulation of heavy metals in the soil of investigated saffron sites as well as used irrigation sources especially DW and SLW.

### 2.2. Studied saffron variety description

The famous saffron variety i.e., Kashmiri was selected for this investigation; this variety is native to the Kashmir region. It is historically known as red gold, has exceptional marketability, and is globally considered the purest and most expensive saffron variety. Kashmiri saffron is known for Lacha (or Lachha) and Mongra. The former consists of the stigmas (red) along with parts of the style (yellow), while the latter consists of the stigma alone (red) [47]. This variety can grow at high altitudes (1600–1800 m above sea level (a.s.l.)), which suits well Pampore soils (1500–1600 m a.s.l.) and is therefore widely grown by local farmers. Kashmiri saffron plants have generally medium height (23–27 cm), style length (2.8–3.0 cm), leaf length (15–21 cm), leaf width (2.0–2.3 cm), leaf (mother corm) number (10–12), flowers per plant (2–4), corm weight (16–23 g), and fresh and dry saffron weights. 39–42 days anthesis and 18–20-day flowering period are most attributed to the Kashmiri saffron variety [48,49]. Therefore, the present study aimed to assess the impact of DM and SLW used for the irrigation of saffron grown in the vicinity of Pampore and Sarbal Lake.

### 2.3. Soil and water samples collection, and biochemical analysis

Soil and water samples were collected from late August to November 2022. Water samples, i.e., BW, DW, and SLW were collected in 20 L capacity plastic containers, while soil samples were collected in zip-locked polyethylene bags. Soil and water samplings were performed twice, i.e., sprouting (4–5 weeks after transplantation) and flowering (3–4 weeks). Water and soil samples were analyzed for several physicochemical and HM properties. Purposely, the standard protocols of AOAC [50] and APHA [51] were adopted for pH, electrical conductivity (EC), and total dissolved solids (TDS) using a microprocessor-based multi-meter (1611, ESICO, Parwanoo, India). A digital dissolved oxygen meter (1801, ESICO, Parwanoo, India) was used for biochemical oxygen demand (BOD<sub>5</sub>) estimation. An open-reflux digester (Borosil 8 Position COD Digester Open Reflux, Mumbai, India) was used for chemical oxygen demand (COD) estimation. Total nitrogen (TKN) content was estimated by Kjeldahl's method and soil organic matter (OM) was determined by the

Walkley and Black method. Total potassium (TK) and total phosphorus (TP) contents were determined as per AL-Huqail et al. [39] using a flame photometer (Cole-Parmer FF-200D-I-120 Economical Flame Photometer, Vernon Hills, IL, USA) and UV-vis spectroscopy (Cary 60, Agilent Technologies, Santa Clara, CA, USA), respectively. Atomic absorption spectroscopy (Analyst 800, PerkinElmer, Waltham, MA, USA) was used for HM contents (As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn) estimation in soil and water samples [40]. Analytical conditions and certified reagent materials were previously described by Kumar et al. [52]. Casting-off of hollow cathode lamps, setting of slit width, AAS running, and calibration curves adjustment followed the methodology of AL-Huqail et al. [39]. To ensure accurate HM content results, a maximum recovery percentage superior to 98% was adopted. Limits for surface disposal and inland irrigation set by the Central Pollution Control Board of India (CPCB) and Bureau of Indian Standards (BIS), respectively were adopted for comparison.

#### 2.4. Saffron sample collection, physical measurements, biochemical analysis, and quality determination

From the same sites, saffron samples (whole plants) were collected in zip-locked polyethylene bags twice: at harvest and after drying stages. Heavy metal determination in saffron corms, petals, and stigmas (20 mg/sample) followed the same methods adopted for soil and water samples, and standard safe limits set by FAO/WHO [53,54]. Also, the USDA [55] standards were adopted for comparison. Physical parameters, i.e., days to flowering (DF; d), flowering interval (FI; d) plant height (PH; cm), style length (SL; mm), style fresh weight (SFW; g/m<sup>2</sup>), style dry weight (SDW; g/m<sup>2</sup>), leaf length (LL; cm), leaf width (LW; cm), leaf number/plant (LNP; n/plant), leaf area (LA; cm<sup>2</sup>/plant), leaf fresh weight (LFW; g), leaf dry weight (LDW; g), flower number (FN; n/m<sup>2</sup>), flower number/plant (FN; n/plant), flower fresh weight (FFW; g/m<sup>2</sup>), flower dry weight (FDW; g/m<sup>2</sup>), stigma length (SL; mm), stigma fresh weight (SFW; g), stigma dry weight (SDW; g), and stigma yield (SY; g/m<sup>2</sup>) were determined.

Furthermore, the qualitative parameters of dried saffron stigmas were evaluated. More specifically, moisture was determined according to ISO 3632. The determination of crocin (color strength), picrocrocin (bitterness), and safranal (aroma) contents were carried out at 440 nm, 257 nm, and 330 nm, respectively with a 1 cm pathway cell on a UV-visible spectrophotometer [56]. Moreover, the color measurement of dry stigma (L\*, a\*, and b\*) followed the methodology of Cuko et al. [57] where L\* refers to lightness (0 = black and 100 = white), a\* corresponds to green-red intensity (a < 0: green and a > 0: red), and b\* describes the blue-yellow intensity (b < 0: blue and b > 0: yellow). ISO 3632 standard limits were adopted to assess saffron quality. Analyses were carried out in pentaplicate for more accuracy.

#### 2.5. Statistical analysis

Although HMs are minor elements found in saffron [58], their contents can vary depending on several parameters like soil, water, and human activities, among others, and can thus affect food quality, traceability, and further human health. Therefore, it is of great importance to evaluate the bioaccumulation factor (BF) of saffron. As the HM profile of saffron has been barely outlined, the assessment of BF has been very scantily investigated to date. This indicator helps in understanding how crops react toward pollutants in contaminated soils. In this regard, the present study aimed to evaluate the BF of saffron irrigated with three different water sources. The calculation of BF was done as per the following equation (Eq. (1)):

$$BF = \frac{HMsa}{HMso} \quad (1)$$

where, *HMsa* is the HM concentration in saffron (corms, petals, and stigmas) and *HMso* represents the HM concentration in soil.

In order to understand the health risks associated with the consumption of saffron contaminated with HMs, three different non-carcinogenic risk indices including health risk index (HRI), dietary intake modeling (DIM), and target hazard quotient (THQ) were assessed. In this, Eqs. (2) and (3) were used to compute HRI and DIM values as given below [44]:

$$HRI = \frac{DIM}{RfD} \quad (2)$$

$$DIM = SS \times \frac{HMc}{Bw} \quad (3)$$

where, RfD, SS, HMc, and Bw represent oral reference dose, serving of saffron (dried weight), HM concentration, and body weight of consumer (70 kg), respectively. Additionally, THQ was used to evaluate the health risk of HM accumulation in saffron as per the following equation (Eq. (4)) [43]:

$$THQ = 10^{-3} \times \frac{Ef \times Ed \times Ir \times HMc}{Bw \times Cp \times RfD} \quad (4)$$

where 10<sup>-3</sup> is the conversion factor, Ef is the exposure frequency, Ed is the exposure duration (365 days), Ir represents the saffron ingestion rate (1.5 g/day or 0.0015 kg/day), HMc is the HM concentration in the saffron sample (mg/kg), Bw corresponds to the average body weight (70 kg and 16 kg for adult and child groups, respectively), Cp is the consumption period (25550 and 5840 days for adult and child groups, respectively), and Rd is the reference dose (As: 3.0 × 10<sup>-4</sup>, Cd: 1.0 × 10<sup>-3</sup>, Cr: 5.0 × 10<sup>-3</sup>, Cu: 4.0 × 10<sup>-2</sup>, Fe: 7.0 × 10<sup>-1</sup>, Hg: 3.6 × 10<sup>-4</sup>, Mn: 1.4 × 10<sup>-2</sup>, Ni: 2.0 × 10<sup>-2</sup>, Pb: 4.0 × 10<sup>-4</sup>, Se: 5.0 × 10<sup>-3</sup>, and Zn: 3.0 × 10<sup>-1</sup>). Further, the combined

toxicity of HM intake from saffron was calculated as per the following equation (Eq. (5)):

$$\sum THQ = THQ(As + Cd + Cr + Cu + Fe + Hg + Mn + Ni + Pb + Se + Zn) \quad (5)$$

SPSS 23.0 (IBM, NY, USA) and OriginPro 2022b (OriginLab, Northampton, MA, USA) software packages were used for data analysis and visualization. One-way analysis of variance (ANOVA) was used to detect the significant difference between water sources, irrigated soils, and saffron plant growth, yield, and compositional parameters.  $p$ -value  $< 0.05$  corresponded to statistical significance. Moreover, principal component analysis (PCA) and heatmap-based hierarchical clustering were performed to understand the relationships of HM accumulation as impacted by different irrigation sources.

### 3. Results and discussion

#### 3.1. Characteristics of domestic wastewater and agricultural soils

The biochemical properties of irrigation sources in the studied sites are shown in Table 1. Results depicted the suitability of BW for both surface disposal and inland irrigation owing to its biochemical values falling below the safe limits set by the Central Pollution Control Board of India (CPCB) and Bureau of Indian Standards (BIS), respectively. Approximately neutral pHs were attributed to BW and SLW, while DW was slightly alkaline. Nevertheless, all irrigation water sources fell within the safe limits set for surface disposal and inland irrigation. EC level was significantly higher ( $p < 0.05$ ) by 9.6 and 6.6-fold in DW and SLW than BW. TDS level was significantly increased ( $p < 0.05$ ) by 16.5- and 8.9-fold in DW and SLW compared to BW. However, the TDS level in all irrigation sources was below the limit set by CPCB for surface disposal. As per Ayers and Westcot [59], a TDS level ranging between 700 and 2000 mg/L can be slightly to moderately restricted for use for crop irrigation. The biochemical oxygen demand's levels observed in DW and SLW were significantly higher ( $p < 0.05$ ) than in BW. Biochemical oxygen demand's level in the former sources exceeded by 11.1- and 4.2-fold, and 37.2- and 14.1-fold the safe limits set for inland irrigation and surface disposal, respectively. Similarly, the COD level was significantly higher ( $p < 0.05$ ) in DW and SLW in comparison with BW. Furthermore, these irrigation water sources surpassed the safe limits for surface disposal and inland irrigation by 7.4- and 3.4-fold, respectively. It is worth noting that very high BOD and COD levels can result in decreased soil fertility and oxygen availability, and the promotion of abiotic stress leading to decreased plant growth and development [15]. In the same vein, the TKN level was significantly higher ( $p < 0.05$ ) by 21.4- and 12.8-fold in DW and SLW, than in BW. It also exceeded the safe limits for surface disposal and inland irrigation by 46.1 and 9.9%, respectively. Total potassium and TP levels were significantly higher ( $p < 0.05$ ) in DW and SLW than in BW. The former sources exceeded the safe limit for inland irrigation by 23.1- and 9.1-folds, respectively. Increased TK levels can cause the dispersal of soil clay particles, thus resulting in pore space clogging and potentially reducing magnesium (Mg) uptake by plants [60,61].

The heavy metal profiles of all three irrigation water sources depicted the following decreasing order: DW > SLW > BW, except for iron (Fe), lead (Pb), and selenium (Se), where SLW > DW > BW. Heavy metals found in water irrigation sources were in the following decreasing order: Fe > Ni > Mn > Zn > Cr > Cd > Cu > As > Pb=Hg=Se (BW), Zn > Mn > Cr > Fe > Ni > Cu > Cd > As > Pb=Hg=Se (DW), and Fe > Zn > Mn > Ni > Cu > Cr > Cd > As > Pb > Se > Hg (SLW). Lead, Hg, and Se levels were very low in BW, which explains

**Table 1**

Biochemical properties of irrigation water sources (borewell water (BW), domestic wastewater (DW), and Sarbal Lake water (SLW)) in the studied sites.

Parameters	Irrigation Water Sources			Limit for Surface Disposal <sup>a</sup>	Limit for Inland Irrigation <sup>b</sup>
	BW	DW	SLW		
pH	7.34 ± 0.16 a	8.05 ± 0.32 c	7.78 ± 0.15 b	5.50–9.00	5.50–9.00
Electricity Conductivity (EC; dS/m)	0.70 ± 0.22 a	6.70 ± 0.45 c	4.64 ± 0.34 b	Na	Na
Total Dissolved Solids (TDS; mg/L)	102.44 ± 7.39 a	1695.08 ± 7.13 c	910.12 ± 18.24 b	1900.00	Na
Biochemical Oxygen Demand (BOD; mg/L)	3.25 ± 0.44 a	1115.08 ± 9.65 c	421.97 ± 6.18 b	100.00	30.00
Chemical Oxygen Demand (COD; mg/L)	7.43 ± 0.14 a	1845.14 ± 20.47 c	842.23 ± 17.24 b	250.00	250.00
Total Kjeldahl's Nitrogen (TKN; mg/L)	8.66 ± 0.33 a	185.39 ± 2.24 c	110.94 ± 4.10 b	100.00	100.00
Total Potassium (TK; mg/L)	7.23 ± 1.18 a	144.61 ± 2.80 c	94.02 ± 5.19 b	Na	Na
Total Phosphorus (TP; mg/L)	4.51 ± 0.47 a	115.34 ± 2.88 c	45.53 ± 2.11 b	Na	5.00
Arsenic (As; mg/L)	0.05 ± 0.01 a	0.18 ± 0.01 c	0.10 ± 0.01 b	0.20	0.20
Cadmium (Cd; mg/L)	0.23 ± 0.08 a	4.40 ± 0.84 c	1.17 ± 0.13 b	2.00	2.00
Chromium (Cr; mg/L)	0.34 ± 0.09 a	6.15 ± 0.47 c	1.64 ± 0.34 b	2.00	Na
Copper (Cu; mg/L)	0.06 ± 0.01 a	4.87 ± 0.73 c	2.15 ± 0.19 b	3.00	3.00
Iron (Fe; mg/L)	0.81 ± 0.05 a	5.19 ± 0.42 b	14.23 ± 0.08 c	1.00	3.00
Lead (Pb; mg/L)	Nd	0.04 ± 0.01 a	0.09 ± 0.01 b	0.10	0.10
Manganese (Mn; mg/L)	0.49 ± 0.03 a	6.45 ± 0.33 c	3.14 ± 0.17 b	1.00	2.00
Mercury (Hg; mg/L)	Nd	0.04 ± 0.00 b	0.01 ± 0.00 a	0.01	0.01
Nickel (Ni; mg/L)	0.75 ± 0.10 a	5.13 ± 0.22 c	2.16 ± 0.04 b	3.00	3.00
Selenium (Se; mg/L)	Nd	0.04 ± 0.00 b	0.08 ± 0.01 c	0.05	0.05
Zinc (Zn; mg/L)	0.39 ± 0.08 a	10.87 ± 0.32 c	6.25 ± 0.10 b	15.00	5.00

Values are means (±SD). Different statistical letters within the same row indicate significant differences ( $p < 0.05$ ) between irrigation water sources; Na: not available; Nd: not detected; <sup>a</sup>: Central Pollution Control Board of India (CPCB); <sup>b</sup>: Bureau of Indian Standards (BIS).

their undetectability. Increased Fe, Pb, and Se levels in SLW may have been caused by both legal and illegal sand and limestone mining activities in Pampore City and its suburbs. High Se levels in irrigation water can lead to hyperaccumulation in soil and plant roots and therefore, reduced biomass [62] and change in structure and function of plant protein [63]. Besides, many health risks like immune deficiency and impaired fertility can be also associated with hyperaccumulation of Se [64]. Arsenic, Pb, and Zn contents of all three irrigation water sources were below the safe limits for surface disposal and inland irrigation of CPCB and BIS. Cadmium, Cr, Cu, Hg, and Ni contents of DW exceeded by 1.6–4.0-fold the safe limits of CPCB and BIS. Increases in such HMs can have profound and dangerous effects such as the inhibition of plant photosynthetic pigments and the induction of oxidative stress and ROS production [65, 66]. Other effects include human neurological and renal dysfunction [67,68]. Furthermore, in some cases, several types of cancer can proliferate [69]. Whereas, both Fe and Mn exceeded the safe limits for surface disposal and inland irrigation by 1.7–14.2-folds. High Fe and Mn contents in irrigation water can lead to stunting and discolored bronzing foliage [70], and impose toxicity stress on plants [71], respectively. If FAO and WHO standards are considered, As, Cd, Cr, Mn, and Ni contents exceeded the safe limits (0.03, 0.05, 0.01, 0.20, and 0.20 mg/L) in all irrigation sources. Whereas, Cu, Fe, Hg, Pb, Se, and Zn contents exceeded these limits (0.20, 5.0, 0.001, 0.003, 0.02, and 3.0 mg/L) in both DW and SLW. However, the consideration of local standard limits is adopted as almost all Kashmiri saffron's production is destined for local consumption.

Table 2 outlines the biochemical properties of soils irrigated with BW, DW, and SLW. Results showed that all parameters' values were significantly higher ( $p < 0.05$ ) in soils irrigated with DW and SLW in comparison with BW. All studied soils had even neutral (BW and SLW-irrigated soils) or slightly alkaline (DW-irrigated soils) pH. As per Zarghani et al. [72], soils with a pH range of 6.8–7.8 are the most suitable for saffron growing. A 1.3–1.6-fold increase in EC was observed in soils irrigated with DW and SLW compared to BW ones. Pirasteh-Anosheh et al. [73] outlined that saffron can grow successfully under high ECs (up to 6.0 dS/m). 43.4–80.8% increases in OM were observed in soils irrigated with DW and SLW compared to BW-irrigated ones. Zouahri et al. [74] mentioned that high organic matter content improves the absorbance potential of soil nutrients (mainly potassium and phosphorus) by saffron plants. Similarly, TKN, TK, and TP contents were significantly increased ( $p < 0.05$ ) by 1.6–2.2-folds in soils irrigated with DW and SLW in comparison with BW-irrigated ones. Although it helps in the saffron plant's growth and development, high TKN content may result in nitrogen leaching and groundwater contamination [35]. On the other hand, Chaouqi et al. [75] outlined an improved saffron quality as a result of higher availability of potassium and phosphorus in soil. HMs were significantly increased ( $p < 0.05$ ) in soils irrigated with DW and SLW compared to BW-irrigated ones. It is noteworthy that the former had higher HM contents (except Fe, Pb, and Se) than SLW-irrigated soils. The high contents of Fe, Pb, and Se in the latter may originate from the irrigation source (SLW) as depicted in Table 1. The heavy metal contents of BW-, DW-, and SLW-irrigated soils followed the respective decreasing trends: Fe > Cu > Zn > Mn > Pb > Cr > Hg > Ni > Se > Cd > As, Fe > Cu > Zn > Mn > Cr > Cd > As > Pb > Hg > Ni > Se, and Fe > Cu > Zn > Mn > Cr > Cd > Pb > As > Se > Hg > Ni. Increased HMs may result in decreased growth and yield of saffron plants along with reduced quality of the final product [36].

### 3.2. Growth, yield, color, and quality of saffron

Table 3 shows the impact of different irrigation sources on the growth and yield traits of saffron. Results depicted a significant earliness ( $p < 0.05$ ) in flowering (2.9–6.0%) of saffron plants irrigated with DW and SLW in comparison with BW-irrigated ones. Similarly, the flowering interval was significantly reduced ( $p < 0.05$ ) by 13.3–22.4%. Such earliness can be explained by the possible promotion of certain hormones (mostly IAA) responsible for flowering induction in saffron plants [76]. Higher organic matter and nutrient contents in soil can also play a crucial role in hastening the flowering period [77]. Earliness in flowering signifies a shorter

**Table 2**

Biochemical properties of soils irrigated with borewell water (BW), domestic wastewater (DW), and Sarbal Lake water (SLW).

Parameters	Irrigated Soils		
	BW	DW	SLW
pH	7.15 ± 0.11 a	7.84 ± 0.14 c	7.48 ± 0.15 b
Electricity Conductivity (EC; dS/m)	2.51 ± 0.08 a	4.10 ± 0.33 c	3.28 ± 0.12 b
Organic Matter (OM; %)	3.02 ± 0.17 a	5.46 ± 0.27 c	4.33 ± 0.21 b
Total Kjeldahl's Nitrogen (TKN; mg/kg)	137.22 ± 2.47 a	303.14 ± 15.07 c	215.60 ± 6.34 b
Total Potassium (TK; mg/kg)	71.12 ± 4.13 a	245.53 ± 5.29 c	151.04 ± 3.41 b
Total Phosphorus (TP; mg/kg)	46.69 ± 4.55 a	168.44 ± 5.18 c	85.62 ± 4.12 b
Arsenic (As; mg/kg)	0.12 ± 0.03 a	4.34 ± 0.14 c	2.61 ± 0.09 b
Cadmium (Cd; mg/kg)	0.19 ± 0.04 a	6.29 ± 0.10 c	3.73 ± 0.08 b
Chromium (Cr; mg/kg)	0.94 ± 0.11 a	8.42 ± 0.56 c	5.33 ± 0.25 b
Copper (Cu; mg/kg)	4.03 ± 0.09 a	35.11 ± 0.98 c	24.92 ± 0.84 b
Iron (Fe; mg/kg)	19.21 ± 0.33 a	41.01 ± 3.99 b	68.12 ± 0.87 c
Lead (Pb; mg/kg)	1.21 ± 0.16 a	2.18 ± 0.14 b	3.34 ± 0.26 c
Manganese (Mn; mg/kg)	1.77 ± 0.12 a	11.18 ± 1.23 c	7.59 ± 0.78 b
Mercury (Hg; mg/kg)	0.37 ± 0.04 a	0.94 ± 0.08 c	0.65 ± 0.03 b
Nickel (Ni; mg/kg)	0.32 ± 0.06 a	0.85 ± 0.05 c	0.52 ± 0.04 b
Selenium (Se; mg/kg)	0.24 ± 0.03 a	0.41 ± 0.03 b	0.66 ± 0.04 c
Zinc (Zn; mg/kg)	3.15 ± 0.16 a	17.21 ± 1.78 c	11.30 ± 1.05 b

Values are means (±SD). Different statistical letters within the same row indicate significant differences ( $p < 0.05$ ) between irrigation water sources.

**Table 3**

Impact of different irrigation water sources (borewell water (BW), domestic wastewater (DW), and Sarbal Lake water (SLW)) on growth and yield of saffron.

Parameters	Irrigated Soils		
	BW	DW	SLW
Days to Flowering (DF; d)	61.10 ± 1.20 c	57.40 ± 0.90 a	59.30 ± 0.80 b
Flowering Interval (FI; d)	16.50 ± 1.10 c	12.80 ± 0.70 a	14.30 ± 1.20 b
Plant Height (PH; cm)	24.50 ± 0.80 a	29.10 ± 0.50 c	27.20 ± 0.60 b
Style Length (SL; mm)	28.50 ± 0.30 a	31.20 ± 0.40 c	29.90 ± 0.20 b
Style Fresh Weight (SFW; g/m <sup>2</sup> )	0.2592 ± 0.001 a	0.2749 ± 0.001 c	0.2659 ± 0.002 b
Style Dry Weight (SDW; g/m <sup>2</sup> )	0.1369 ± 0.002 a	0.1527 ± 0.001 c	0.1477 ± 0.002 b
Leaf Length (LL; cm)	43.60 ± 1.20 a	50.30 ± 1.30 c	46.40 ± 1.10 b
Leaf Width (LW; cm)	3.30 ± 0.30 a	3.90 ± 0.10 c	3.60 ± 0.10 b
Leaf Number/Plant (LNP)	24.60 ± 1.20 a	28.10 ± 1.10 c	26.70 ± 1.40 b
Leaf Area (LA; cm <sup>2</sup> /plant)	153.78 ± 0.36 a	196.17 ± 0.13 c	167.04 ± 0.10 b
Leaf Fresh Weight (LFW; g)	0.462 ± 0.02 a	0.591 ± 0.02 c	0.544 ± 0.03 b
Leaf Dry Weight (LDW; g)	0.140 ± 0.05 a	0.232 ± 0.04 c	0.214 ± 0.05 b
Flower Number (FN; n/m <sup>2</sup> )	52.00 ± 1.00 a	60.00 ± 2.00 c	56.00 ± 2.00 b
Flower Number per Plant (FNP; n/plant)	2.30 ± 0.10 a	2.70 ± 0.10 c	2.50 ± 0.10 b
Flower Fresh Weight (FFW; g/m <sup>2</sup> )	36.25 ± 1.64 a	41.73 ± 1.33 c	39.44 ± 1.21 b
Flower Dry Weight (FDW; g/m <sup>2</sup> )	7.25 ± 0.33 a	8.35 ± 0.27 c	7.89 ± 0.24 b
Stigma Length (SL; mm)	35.60 ± 1.24 a	40.15 ± 1.55 c	37.80 ± 1.36 b
Stigma Fresh Weight (SFW; g)	0.0386 ± 0.001 a	0.0512 ± 0.001 c	0.0448 ± 0.001 b
Stigma Dry Weight (SDW; g)	0.0065 ± 0.0003 a	0.0081 ± 0.0003 c	0.0074 ± 0.0002 b
Stigma Yield (SY; g/m <sup>2</sup> )	1.81 ± 0.02 a	2.12 ± 0.03 c	1.95 ± 0.03 b

Values are means (±SD). Different statistical letters within the same row indicate significant differences ( $p < 0.05$ ) between irrigation water sources.

period to harvest, which can be very promising in terms of marketability. Such earliness also reduces the risk of flower damage by frost [78]. All following growth and yield traits were in the next decreasing trend: DW > SLW > BW. Plant height, SL, SFW, and SDW were significantly improved ( $p < 0.05$ ) by 11.0–15.8, 4.9–9.5, 2.6–6.1, and 7.9–11.5% in saffron plants irrigated with DW and SLW, respectively compared to BW-irrigated ones. As per Singh et al. [48], saffron plants with a height ranging between 23 and 27 cm can be considered of medium length (BW-irrigated saffron plants), while those exceeding 27 cm are considered of high length (DW- and SLW-irrigated saffron plants). The style length of saffron plants irrigated with BW and SLW can be considered medium, while that of plants irrigated with DW can be considered high [48]. The style fresh weight and SDW may have been increased due to higher nutrient uptake from irrigated soils. Leaf length, LW, LNP, LA, LFW, and LDW significantly increased ( $p < 0.05$ ) by 6.4–15.4, 9.1–18.2, 8.5–14.2, 8.6–27.6, 17.7–27.9, and 52.9–65.7%, respectively in saffron plants irrigated with DW and SLW in comparison with BW-irrigated ones. The leaf length was comparable to or slightly higher than previously denoted by Cardone et al. [79] (26.9–49.8 cm). Leaves of all saffron plants in this investigation can be considered long and wide [48]. The leaf number per plant, LA, LFW, and LDW fell within or were higher than the ranges reported by Cardone et al. [79] (20.7–27.6 leaves/plant, 74.4–192.4 cm<sup>2</sup>/plant, 0.234–0.557 g, and 0.066–0.152 g, respectively). Higher PH, LL, LW, LNP, and LA were associated with increased light intensity and cell numbers within plants [80,81]. An increase in LW can be attributed to a higher accumulation of non-structural carbohydrates, which can help plants mitigate climate change (expressed by higher CO<sub>2</sub> and greenhouse gas liberation) [82]. The flower number, FNP, FFW, and FDW were significantly increased ( $p < 0.05$ ) by 16.7–41.3, 8.7–17.4, 8.8–15.1, and 8.8–15.1%, respectively in plants irrigated with DW and SLW compared to those irrigated with BW. Our values are comparable to or higher than those reported by Sahabi et al. [83] and Cardone et al. [79] (43.7–48.6 flowers/m<sup>2</sup>, 51.0 flowers/m<sup>2</sup>, and 0.9–1.5 flowers/plant, respectively). The

**Table 4**

Impact of different irrigation water sources (borewell water (BW), domestic wastewater (DW), and Sarbal Lake water (SLW)) on quality of saffron stigmas.

Parameters	Irrigated Soils			ISO 3632 Standard Limits
	BW	DW	SLW	
Moisture (%)	10.00 ± 0.20 b	9.20 ± 0.40 a	9.50 ± 0.30 ab	12.00
Crocin (λ 440) ISO category	115.00 ± 1.00 a III	160.00 ± 1.00 b II	155.00 ± 1.00 b II	212.02
Picrocrocin (λ 257) ISO category	45.00 ± 1.00 a III	59.00 ± 1.00 b II	57.00 ± 1.00 b II	82.00
Safranal (λ 330) ISO category	25.50 ± 1.00 a Min	25.20 ± 1.00 a Min	25.30 ± 1.00 a Min	35.40
Lightness (L*)	36.80 ± 0.90 a	39.90 ± 0.30 c	38.40 ± 0.40 b	–
Green-red intensity (a*)	38.80 ± 1.10 b	37.10 ± 0.90 a	37.50 ± 0.50 a	–
Blue-yellow intensity (b*)	37.20 ± a	40.90 ± 0.40 c	39.20 ± 0.30 b	–

Values are means (±SD). Different statistical letters within the same row indicate significant differences ( $p < 0.05$ ) between irrigation water sources.

flower number of saffron plants in this investigation can be considered very high, while FNP was found medium [48]. Soils richer in nitrogen and phosphorus contents can result in the formation of a higher number of flowers [79,84]. Such finding also corroborates with those of Mollafilabi and Khorramdel [85] who mentioned increases in flower number and weight as a result of soil fertilization. The flower fresh weight of all saffron plants in this investigation was more than 2-fold higher than reported by Sahabi et al. [83] (21.8–24.3 g/m<sup>2</sup>), which outlines the effect of fertilization type on the growth and development of saffron. Also, increased flower weight was previously correlated with higher leaf sugar content [86]. The style length, SFW, SDW, and SY were significantly increased ( $p < 0.05$ ) by 6.2–12.8, 16.1–32.6, 13.8–24.6, and 7.7–17.1%, respectively in plants irrigated with DW and SLW in comparison with BW-irrigated ones. Our values are comparable or higher than the ranges depicted by Cardone et al. [79] (28.8–36.2 mm, 0.0234–0.0426 g, 0.0039–0.0071 g, and 0.8–2.0 g/m<sup>2</sup>, respectively). The stigma dry weight was doubled in fertilized soils compared to non-fertilized ones [85]. Chooan et al. [38] depicted a 0.79 g/m<sup>2</sup> of SY when plants were irrigated with municipal wastewater. Whereas, using domestic wastewater in the present investigation showed 2.7-fold higher SY than in the aforementioned study.

The impacts of different irrigation water sources on the quality of saffron stigmas are reported in Table 4. Results showed comparable ( $p > 0.05$ ) moisture content in saffron stigmas collected from BW- and SLW-irrigated soils. Whereas, saffron stigmas collected from DW-irrigated soils showed significantly ( $p < 0.05$ ) lower content (9.20%) in comparison with BW samples. Nevertheless, all collected stigmas had a moisture content below 12%, which is the safe limit set by ISO 3632. Kothari et al. [32] reported that a lower moisture content in saffron stigmas not only improves their quality but also results in a higher spice yield. Crocin significantly increased ( $p < 0.05$ ) by 39.1 and 34.8% in saffron stigmas collected from DW- and SLW-irrigated soils, respectively compared to BW ones. In addition, crocin quality moved from category III to category II, which means an increase in the color intensity of saffron stigmas. The same trend was observed for picrocrocin which showed significant increases ( $p < 0.05$ ) by 31.1 and 26.7% in saffron stigmas collected from soils irrigated with DW and SLW, respectively in comparison with BW-irrigated ones. Also, picrocrocin quality moved from category III to category II, which signifies a higher bitterness. Safranal in all saffron stigmas was comparable ( $p > 0.05$ ) with BW samples, which had the highest adsorption value (25.50 > 25.30 > 25.00). Therefore, safranal was ranked minimum as per ISO 3632 standard limits with approximately stable aroma. Crocin, picrocrocin, and safranal in all analyzed saffron stigmas were comparable to or slightly higher than those outlined by Cardone et al. [79]. Moreover, their adsorption values were all below the safe

**Table 5**

Impact of different irrigation water sources (borewell water (BW), domestic wastewater (DW), and Sarbal Lake water (SLW)) on heavy metal accumulation in saffron stigmas.

Saffron Parts	Heavy metals	Irrigated Soils			Safe Limits (FAO/WHO, USDA)
		BW	DW	SLW	
<b>Corm</b>	Arsenic (As; mg/kg)	0.21 ± 0.03 a	0.40 ± 0.02 c	0.32 ± 0.02 b	0.50
	Cadmium (Cd; mg/kg)	0.04 ± 0.005 a	0.09 ± 0.002 c	0.06 ± 0.004 b	0.10
	Chromium (Cr; mg/kg)	0.16 ± 0.01 a	0.41 ± 0.05 c	0.28 ± 0.04 b	1.00
	Copper (Cu; mg/kg)	7.31 ± 1.12 a	14.74 ± 2.24 c	11.83 ± 1.42 b	20.00
	Iron (Fe; mg/kg)	142.38 ± 3.27 a	303.15 ± 2.57 c	211.67 ± 4.58 b	425.50
	Lead (Pb; mg/kg)	0.18 ± 0.03 a	0.31 ± 0.03 c	0.23 ± 0.02 b	0.30
	Manganese (Mn; mg/kg)	15.26 ± 1.42 a	22.81 ± 1.17 c	18.13 ± 1.20 b	30.00
	Mercury (Hg; mg/kg)	0.18 ± 0.01 a	0.25 ± 0.01 c	0.22 ± 0.01 b	0.50
	Nickel (Ni; mg/kg)	0.74 ± 0.04 a	1.18 ± 0.06 c	0.95 ± 0.03 b	1.50
	Selenium (Se; mg/kg)	0.13 ± 0.02 a	0.22 ± 0.02 c	0.18 ± 0.03 b	0.40
	Zinc (Zn; mg/kg)	3.44 ± 0.36 a	4.59 ± 0.34 c	4.06 ± 0.48 b	5.00
	<b>Petal</b>	Arsenic (As; mg/kg)	0.17 ± 0.01 a	0.31 ± 0.01 c	0.24 ± 0.02 b
Cadmium (Cd; mg/kg)		0.03 ± 0.003 a	0.06 ± 0.004 c	0.04 ± 0.003 b	0.10
Chromium (Cr; mg/kg)		0.12 ± 0.01 a	0.35 ± 0.04 c	0.26 ± 0.03 b	1.00
Copper (Cu; mg/kg)		5.24 ± 1.04 a	11.38 ± 1.61 c	9.17 ± 1.52 b	20.00
Iron (Fe; mg/kg)		118.37 ± 2.67 a	240.49 ± 3.98 c	155.26 ± 3.55 b	425.50
Lead (Pb; mg/kg)		0.15 ± 0.02 a	0.27 ± 0.02 c	0.18 ± 0.03 b	0.30
Manganese (Mn; mg/kg)		12.84 ± 1.19 a	19.88 ± 1.64 c	15.79 ± 1.33 b	30.00
Mercury (Hg; mg/kg)		0.16 ± 0.01 a	0.22 ± 0.01 c	0.19 ± 0.01 b	0.50
Nickel (Ni; mg/kg)		0.55 ± 0.03 a	0.94 ± 0.05 c	0.78 ± 0.02 b	1.50
Selenium (Se; mg/kg)		0.10 ± 0.01 a	0.19 ± 0.02 c	0.15 ± 0.02 b	0.40
Zinc (Zn; mg/kg)		2.86 ± 0.57 a	4.15 ± 0.35 c	3.67 ± 0.43 b	5.00
<b>Stigma</b>		Arsenic (As; mg/kg)	0.12 ± 0.02 a	0.24 ± 0.02 c	0.18 ± 0.01 b
	Cadmium (Cd; mg/kg)	0.01 ± 0.005 a	0.04 ± 0.005 c	0.02 ± 0.005 b	0.10
	Chromium (Cr; mg/kg)	0.09 ± 0.01 a	0.27 ± 0.02 c	0.19 ± 0.02 b	1.00
	Copper (Cu; mg/kg)	4.16 ± 0.91 a	9.57 ± 0.78 c	7.44 ± 0.62 b	20.00
	Iron (Fe; mg/kg)	105.04 ± 2.44 a	210.67 ± 2.65 c	132.52 ± 2.95 b	425.50
	Lead (Pb; mg/kg)	0.11 ± 0.07 a	0.24 ± 0.05 c	0.16 ± 0.03 b	0.30
	Manganese (Mn; mg/kg)	10.38 ± 0.94 a	17.22 ± 1.13 c	13.23 ± 1.05 b	30.00
	Mercury (Hg; mg/kg)	0.12 ± 0.01 a	0.20 ± 0.01 c	0.16 ± 0.01 b	0.50
	Nickel (Ni; mg/kg)	0.41 ± 0.02 a	0.75 ± 0.03 c	0.66 ± 0.01 b	1.50
	Selenium (Se; mg/kg)	0.07 ± 0.01 a	0.15 ± 0.01 c	0.11 ± 0.01 b	0.40
	Zinc (Zn; mg/kg)	2.33 ± 0.46 a	3.78 ± 0.40 c	3.25 ± 0.37 b	5.00

Values are means (±SD). Different statistical letters within the same row indicate significant differences ( $p < 0.05$ ) between irrigation water sources.

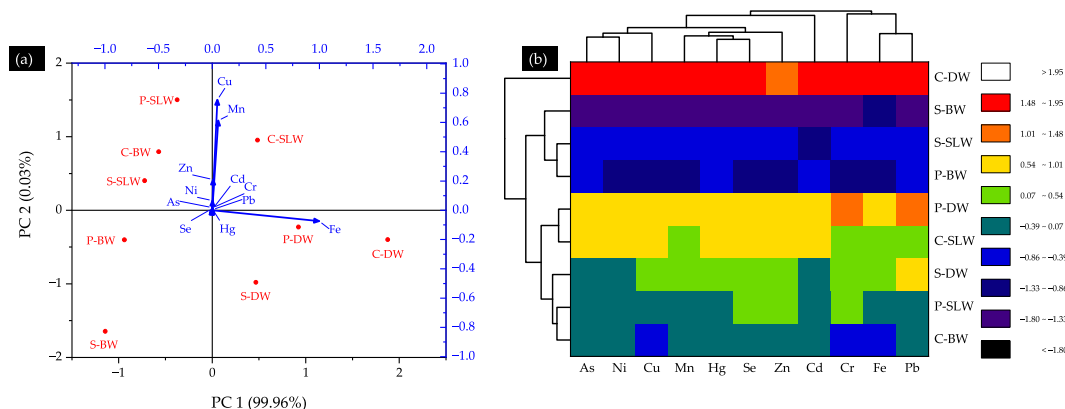


limits set by ISO 3632. It was reported that these values vary in saffron stigmas depending on soil characteristics [79,87]. Chaoqi et al. [75] linked increases in crocin and picrocrocin in saffron with increased organic matter, potassium, and phosphorus contents in growing soils. Such a result corroborates with our study's findings where soils irrigated with both DW and SLW had higher OM, TK, and TP contents in comparison with BW-irrigated ones along with improved crocin and picrocrocin adsorption values. Increased crocin and picrocrocin in saffron stigmas result in a higher quality product (and thus a higher price) with improved hepatoprotective and anti-cancer activities [88,89]. Moreover, Chooan et al. [38] found out higher crocin and safranal adsorption values (261 and 32.6, respectively) in saffron stigmas collected from plants irrigated with municipal wastewater. However, the crocin adsorption value reported in the aforementioned study exceeded the safe limit set by ISO 3632 (212.02 adsorption value).  $L^*$  and  $b^*$  significantly increased ( $p < 0.05$ ) in saffron stigmas collected from DW- and SLW-irrigated soils compared to BW-irrigated ones. Whereas,  $a^*$  was significantly higher ( $p < 0.05$ ) in the latter than in the former. Lightness,  $a^*$ , and  $b^*$  values were comparable with those reported by Cardone et al. [79] on saffron grown in Southern Italy. Also, a similar increase in  $L^*$  and decrease in  $a^*$  were observed by the same authors suggesting a certain negative correlation between them. Furthermore, it should be noted that increased  $L^*$  and decreased  $a^*$  are associated with higher saffron quality, price, and marketability [90].

### 3.3. Heavy metal accumulation in saffron

Table 5 shows the contents of HMs accumulated in the corm, petal, and stigma parts of saffron grown in soil irrigated with different water sources. The results depicted that the contents of all HMs in saffron were found significantly ( $p < 0.05$ ) the highest in the case of irrigation with DW followed by SLW and BW. Here, the levels of HMs were found the highest in corms followed by petals and stigmas. However, the contents of HMs in all plant parts did not exceed the safe limits suggested by FAO/WHO, except for Hg content in the corm part of saffron irrigated with DW. For the corm part, the concentrations (mg/kg) of selected HMs were such as: As (0.21–0.40), Cd (0.04–0.09), Cr (0.16–0.41), Cu (7.31–14.75), Fe (142.38–303.15), Pb (0.18–0.31), Mn (15.26–22.81), Hg (0.18–0.25), Ni (0.74–1.18), Se (0.13–0.22), and Zn (3.44–4.59), respectively. Corms generally act as storage organs for the plant, storing nutrients and other compounds [91]. This might be the reason behind the high occurrence of HMs in the corm part of saffron plants observed in this study. On the other hand, the contents of HMs (mg/kg) in petal part were relatively lower than in corm part i.e., As (0.17–0.31), Cd (0.03–0.06), Cr (0.12–0.35), Cu (5.24–11.38), Fe (118.37–240.49), Pb (0.15–0.27), Mn (12.84–19.88), Hg (0.16–0.22), Ni (0.55–0.94), Se (0.10–0.19), and Zn (2.86–4.15). Flower petals are primarily involved in reproductive processes, such as attracting pollinators and protecting reproductive structures. They usually store nectar which might contain several HMs as reported by Borsuk et al. [92]. Comparatively, the stigma part was characterized by the lowest levels of HMs (mg/kg) i.e., As (0.12–0.24), Cd (0.01–0.04), Cr (0.09–0.27), Cu (4.16–9.57), Fe (105.04–210.67), Pb (0.11–0.24), Mn (10.38–17.22), Hg (0.12–0.20), Ni (0.41–0.75), Se (0.07–0.15), and Zn (2.33–3.78).

As depicted in Fig. 1a, the PCA biplot showed that the type of irrigation water had a significant impact on HM availability in different parts of the saffron. In particular, the data was categorized into two major components namely PC1 (variance: 99.96%; eigenvalues: 4333.15) and PC2 (variance: 0.03%; eigenvalues: 1.37). The vector length of different HMs showed that Fe was the most dominant for corm, petal, and stigma parts irrigated with DW which is in line with the results presented in Table 5. Other HMs also showed a significant association with the type of irrigation water. Similarly, the clustered heatmap and dendrogram shown in Fig. 1b demonstrated that there were significant similarities between the HM concentrations in saffron parts (corms, petals, and stigmas) irrigated with selected water supplies (DW, SLW, and BW). The corm part of saffron irrigated with BW and the petal part of saffron irrigated with SLW showed the highest similarities for HM concentration. Also, the petal part of saffron irrigated with DW showed significant similarities with the corm part of saffron irrigated with SLW. On the other hand, the stigma part of saffron irrigated with SLW showed similarities with the petal parts of saffron irrigated with BW supplies. Moreover, the bioaccumulation factor (BAF) showed that saffron irrigated with BW had the highest values followed by SLW and DW as given in Fig. 2(a–c). The maximum BAF



**Fig. 1.** a) PCA biplot and b) clustered heatmap with dendrogram for effect and similarities of heavy metals in corm (C), petal (P), and stigma (S) parts of saffron irrigated with borewell water (BW), Sarbal Lake water (SLW), and domestic wastewater (DW).

values were reported for Mn and Fe (>5.50), whereas the lowest BAF values were reported for Cd, Cr, and Pb, respectively.

Several reports showed that saffron contains numerous HMs [58]. Out of them, Chichiriccò et al. [93] reported the availability of 21 metal elements in pollen Kitt and anther wall of saffron crocus collected from fields affected by rural activities. These authors outlined that saffron crocus could accumulate alarming levels of Cd and As. Similarly, Malakootian and HaratiNezhadTorbaty [94] reported that saffron leaves can bioaccumulate significant levels of Cu, Cd, and Pb. They suggested that leaf parts of the saffron plant have a greater ability to absorb HMs as compared to other parts. Moreover, Behdani et al. [95] investigated the levels of micro and macro elements in saffron cultivated in the soil of perennial farms in Khorasan province of Iran. The results showed that the levels of Fe, Zn, Mn, Co, Cr, and Cd were significantly impacted by the age of the saffron field i.e., annual, triennial, quinquennial, and planting region. Thus, these reports support the bioaccumulation and occurrence of HMs in different parts of saffron.

### 3.4. Health risk assessment of heavy metals accumulation

Considering the dietary importance of saffron in various cuisines, it is essential to determine whether the occurrence of certain HMs could bring any adverse health impact on consumers [96]. For this, DIM, HRI, and THQ modeling approaches were adopted to understand the potential health hazard associated with the consumption of saffron grown using selected irrigation sources. Table 6 demonstrates the DIM and HRI values of HMs in the corm, petal, and stigma parts of saffron irrigated with BW, SLW, and DW. It was observed that the values of DIM and HRI correspond to the net HM availability in saffron parts i.e., the highest for DW-irrigated saffron and the lowest for the BW-irrigated saffron. Similarly, DIM and HRI values were found to be the highest in the case of the corm part followed by the petal and stigma parts, respectively. Since the daily intake amount of saffron is relatively low (only 1.5–2.0 g per day) [97] as compared to those for fruits and vegetables (30–50 g per day) [98], the net exposure of HMs is also significantly low as indicated by the DIM values (less than 0.005153). Herein, the maximal DIM value was recorded for Fe while the minimal one was attributed to Cd. Similarly, the HRI values were also recorded to be less than 0.028571 which is significantly lower (<1) than those reported for other fruits and vegetables by other authors [43,99]. On the other hand, the THQ computations given in Fig. 3 showed that the levels of HMs in saffron parts did not exceed the threshold level of 1. This suggests that consumption of saffron grown under all types of irrigation water sources (BW, SLW, and DW) was safe from an edible point of view. Although DIM, HRI, and THQ levels suggested that there was no significant health risk associated with the consumption of saffron grown in selected irrigation water sources, though, it is crucial to monitor their levels regularly as cumulative discharge of DW and SLW in fields could result in excessive accumulation of HMs, which could later migrate into saffron plants at higher rates and lead to increased risk of contamination.

Chronic exposure to some HMs, like Cd, Pb, and Hg, has been linked to an increased risk of cancer and other health issues [100, 101]. Although saffron is generally safe in culinary amounts, there are possible chances of accumulation of HMs from the irrigated soil in which it grows. Thus, consuming saffron with elevated HM levels may contribute to the development of several diseases over time. To date, very limited studies are available on assessing the health risks of HMs in saffron plants, particularly those irrigated with different irrigation supplies. A study by Mohammadi et al. [97] evaluated the health risks of HMs in Iranian-grown saffron (known as Sohan). They found out that there was no significant health risk associated with the consumption of such a grown saffron. Recently,

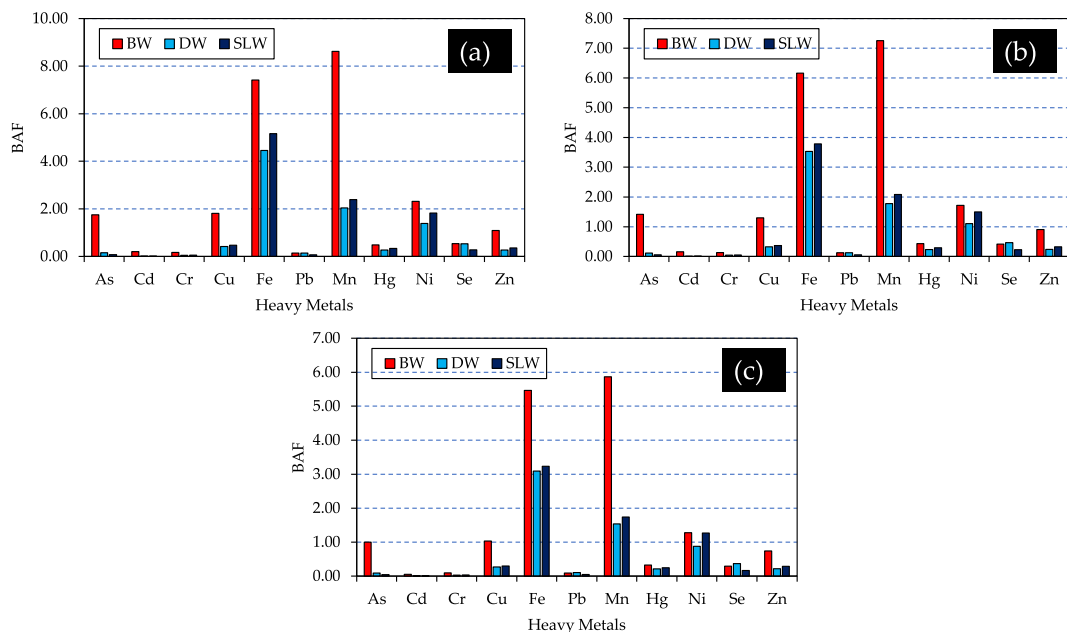
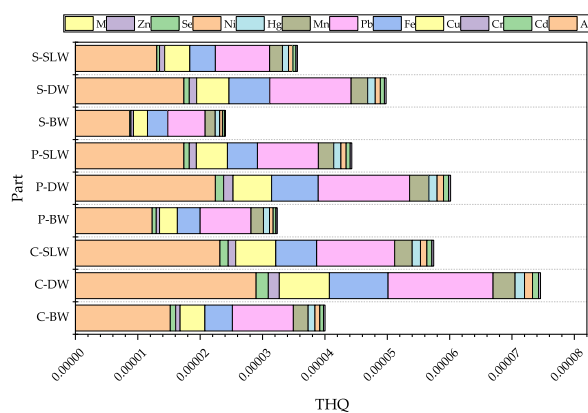


Fig. 2. Bioaccumulation factor (BAF) of heavy metals in corm (a), petal (b), and stigma (c) parts of saffron irrigated with borewell water (BW), Sarbal Lake water (SLW), and domestic wastewater (DW).

**Table 6**

DIM and HRI values of heavy metals in the corm, petal, and stigma parts of saffron irrigated with borewell water (BW), Sarbal Lake water (SLW), and domestic wastewater (DM).

Saffron Part		Heavy Metals										
		As	Cd	Cr	Cu	Fe	Pb	Mn	Hg	Ni	Se	Zn
<b>DIM</b>												
Corm	BW	0.000005	0.000001	0.000003	0.000157	0.003051	0.000004	0.000327	0.000004	0.000016	0.000003	0.000074
	DW	0.000009	0.000002	0.000009	0.000316	0.006496	0.000007	0.000489	0.000005	0.000025	0.000005	0.000098
	SLW	0.000007	0.000001	0.000006	0.000254	0.004536	0.000005	0.000389	0.000005	0.000020	0.000004	0.000087
Petal	BW	0.000004	0.000001	0.000003	0.000112	0.002537	0.000003	0.000275	0.000003	0.000012	0.000002	0.000061
	DW	0.000007	0.000001	0.000008	0.000244	0.005153	0.000006	0.000426	0.000005	0.000020	0.000004	0.000089
	SLW	0.000005	0.000001	0.000006	0.000197	0.003327	0.000004	0.000338	0.000004	0.000017	0.000003	0.000079
Stigma	BW	0.000003	0.000000	0.000002	0.000089	0.002251	0.000002	0.000222	0.000003	0.000009	0.000002	0.000050
	DW	0.000005	0.000001	0.000006	0.000205	0.004514	0.000005	0.000369	0.000004	0.000016	0.000003	0.000081
	SLW	0.000004	0.000000	0.000004	0.000159	0.002840	0.000003	0.000284	0.000003	0.000014	0.000002	0.000070
<b>HRI</b>												
Corm	BW	0.015000	0.000857	0.000686	0.003916	0.004359	0.009643	0.002336	0.001071	0.000793	0.000557	0.000246
	DW	0.028571	0.001929	0.001757	0.007896	0.009280	0.016607	0.003491	0.001488	0.001264	0.000943	0.000328
	SLW	0.022857	0.001286	0.001200	0.006338	0.006480	0.012321	0.002775	0.001310	0.001018	0.000771	0.000290
Petal	BW	0.012143	0.000643	0.000514	0.002807	0.003624	0.008036	0.001965	0.000952	0.000589	0.000429	0.000204
	DW	0.022143	0.001286	0.001500	0.006096	0.007362	0.014464	0.003043	0.001310	0.001007	0.000814	0.000296
	SLW	0.017143	0.000857	0.001114	0.004913	0.004753	0.009643	0.002417	0.001131	0.000836	0.000643	0.000262
Stigma	BW	0.008571	0.000214	0.000386	0.002229	0.003216	0.005893	0.001589	0.000714	0.000439	0.000300	0.000166
	DW	0.017143	0.000857	0.001157	0.005127	0.006449	0.012857	0.002636	0.001190	0.000804	0.000643	0.000270
	SLW	0.012857	0.000429	0.000814	0.003986	0.004057	0.008571	0.002025	0.000952	0.000707	0.000471	0.000232



**Fig. 3.** Individual and cumulative THQ values of heavy metals in the corm, petal, and stigma parts of saffron irrigated with borewell water (BW), Sarbal Lake water (SLW), and domestic wastewater (DM).

Taghavi et al. [102] mentioned that soils used for saffron cultivation could be impacted by several pollution sources which can lead to an increased risk of HM migration to saffron plants, and thereby higher risk of human exposure. Therefore, the health risks of HMs in saffron plants grown using DW and SLW supplies should be regularly monitored to avoid any potential health risks.

#### 4. Conclusions

The present study assessed the use of domestic wastewater and Sarbal Lake water as saffron crop irrigation sources. Results showed improved growth, yield, and qualitative traits of saffron plants in soils irrigated with domestic wastewater and Lake water compared to borewell-irrigated ones. Moreover, all plant parts (corm, petal, and stigma) showed heavy metal contents below thresholds. Moreover, bioaccumulation factor, and non-carcinogenic risk indices i.e., health risk index, dietary intake modeling, and target hazard quotient were all below the standard safe limits. Therefore, irrigation of saffron plants with domestic wastewater and Lake water was found safe with promising quantitative and qualitative findings. The main study's limitations can be summarized by: 1) the need to investigate saffron irrigation with domestic wastewater and Sarbal Lake water over two or more growing seasons; 2) the need to evaluate the presence of pathogenic microbes (e.g., *Escherichia coli*) in the soil, water, and plant tissues that can affect the produced crop. Moreover, it is suggested to build a model aiming at the safe use of domestic wastewater and Lake water in saffron irrigated based on geographic information system (GIS) and multiple criteria decision making (MCDM). Also, continuous assessment of health risks associated with the use of such irrigation sources on saffron is needed to unravel any potential threat to human health in both medium- and long-term. Furthermore, additional studies are needed to investigate possible health risks associated with the use of other types of wastewaters in saffron plant irrigation.

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#### Data availability statement

All data related to this study are included in the article.

#### CRedit authorship contribution statement

**Sami Abou Fayssal:** Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Pankaj Kumar:** Writing – original draft, Visualization, Validation, Software, Methodology, Data curation. **Simona M. Popescu:** Writing – review & editing, Visualization, Validation, Software, Data curation. **Mehraj ud-din Khanday:** Visualization, Software, Resources, Investigation. **Hasan Sardar:** Writing – review & editing, Visualization, Validation, Software. **Riaz Ahmad:** Writing – review & editing, Visualization, Validation, Software. **Deep Gupta:** Writing – review & editing, Visualization, Validation, Software. **Sudhir Kumar Gaur:** Writing – review & editing, Visualization, Validation, Software. **Hesham F. Alharby:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Funding acquisition. **Abdullah G. Al-Ghamdi:** Writing – review & editing, Visualization, Validation, Software, Resources.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sami Abou Fayssal is an Advisory Board Member of Heliyon journal.

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