HEALTH RISKS OF HEAVY METALS UPTAKE BY THE CURDS OF CAULIFLOWER (*BRASSICA OLERACEA* VAR. *BOTRYTIS*) GROWN IN CONTAMINATED AGRICULTURAL LANDS

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Abstract. Food safety and quality protection are a necessity and should draw considerable attention due to the great hazard of consuming food contaminated with heavy metals. In this context, the present study was conducted to evaluate the potential of uptake of heavy metals by cauliflower curds and its associated health risks. A sampling of soil and cauliflower plants was carried out on six farms, equally distributed throughout polluted and unpolluted sites south Greater Cairo, Egypt, for investigating their chemical characteristics and plant growth parameters. The pollution load index (PLI) of the estimated heavy metals (except Ni) was greater than 1. Besides the soil was highly polluted (PLI > 5) with Pb, Zn, Fe, Cu, and Cd. Cauliflower plants showed a significant reduction in plant density, stem and root length, fresh and dry biomass, and curd production as well as plant pigments under polluted conditions. The contents of N, P, carbohydrates, and proteins in the curds and roots of cauliflower plants from the unpolluted site were considerably higher than those estimated on the polluted site. The bioaccumulation factor of the investigated heavy metals, except Cd, Cu, Mn, and V on the unpolluted site, and all metals in the polluted site, was greater than one. However, the translocation factor of most investigated heavy metals, except Pb, Cu, Ni, and Fe in the unpolluted site and Cr, Cu, and Co in the polluted site, was less than one. The results showed that Pb, Cd, Mn, Fe, and Ni contaminations in wastewater-irrigated cauliflower plants have the highest potential to cause a health risk to public consumers, where their health risk index exceeded one. Based on the present investigation, it is strongly recommended to avoid vegetable cultivation in polluted areas, and if cultivated, the vegetable consumption is not recommended.

Keywords: *wastewater, polluted soil, heavy metals, health hazard, edible inflorescence*

Introduction

Food security and safety is a global public issue (Eid et al., 2020a), where there is great attention from researchers regarding this issue and the health hazards associated with the consumption of contaminated foodstuffs (Farahat et al., 2017). It was expected that the global food demand will be doubled by 2050 due to the population growth (Chen et al., 2014). Consequently, agricultural production will be rapidly developed to support food

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security (Wu et al., 2021). Food quality protection and food safety should draw considerable attention since there is a great hazard from consuming heavy metals contaminated food (Orisakwe et al., 2012). According to Jolly et al. (2013) and Yang et al. (2016), crop plants uptake heavy metals by their roots and may transfer and concentrate them in high concentrations in their edible parts even at low soil heavy metals content. The global industrial revolution is a major source of high heavy metals concentration beside the anthropogenic discharge of industrial and sewage effluents, atmospheric deposition, and fossil fuel (Galal et al., 2019b).

Wastewater is an alternative irrigation source commonly used in developing countries for high-value food crops irrigation and to increase the yield of food crops in urban areas (Wu et al., 2017). Food plants are an important source of nutrients such as minerals, carbohydrates, fibers, and vitamins, which are necessary for human health (Galal et al., 2019a). Various vegetables cultivated on industrial and sewage effluents are being consumed by the public, and consequently causes serious health hazards because of the heavy metals' accumulation via dietary intake of these contaminated food plants (Perveen et al., 2011; Galal et al., 2019b). Cauliflower (*Brassica oleracea* L. var. *botrytis*) is an important vegetable, of the Brassicaceae family, grown worldwide and has a lot of uses directly as a vegetable or as component in soups, salads, and so forth (Ma et al., 2021). Worldwide, cauliflower has an area of 8.88 million ha with about 16.40 million tons gross production (Ali, 2015). It is a rich source of minerals, vitamins, and dietary fibers, and constitutes important phytochemicals, which protect against diabetes, cancer, and cardiovascular diseases (Hegazy and Ammar, 2019). Moreover, cauliflower is cultivated, and consumed vegetable crop widely irrigated with wastewaters in some areas of the developing countries including Egypt.

Heavy metal pollution of agricultural lands is a serious environmental issue since it can deteriorate the soil (Eid et al., 2020a) and damage crop growth, yield, quality, and agricultural products safety, which threatens human health (Zu et al., 2017). Therefore, the assessment of the amounts of heavy metals in plants is significant for hazard level determination and environmental maintenance (Kumar et al., 2019b; Eid et al., 2020b). Besides, investigating plant-heavy metals content is crucial to mitigate their concentration in crop plants and avoid their toxicity (Lente et al., 2014; Galal et al., 2019a). Moreover, the health risk assessment can distinguish polluted from unpolluted sites, detect the highest risk paths for a given site, and control pollutants, which threatens human health (Shehata and Galal, 2020).

Few studies were carried out for estimating heavy metals concentration in cauliflower crop and the possible health risks due to its consumption (Khanal et al., 2014; Singh and Singh, 2014; Amir et al., 2016; Rangamani et al., 2017; Rehman et al., 2018; Galal et al., 2019b). There is a systematic assessments urgency for making critical decisions to avoid serious health problems due to heavy metals entry in the food chain with invisible toxicity. Consequently, the present study was conducted to investigate the impact of wastewater-irrigated soils on the growth and production of cauliflower plants, also, to evaluate its potential to concentrate heavy metals in the edible parts, and its associated health risks.

Materials and Methods

Study sites

Two sites were selected for the present investigation (*Fig. 1*): 1) Polluted site, which receives wastes from industrial effluents, anthropogenic activities, and agricultural drainage. It was located at Ekhsas area (29° 47' 29.31"N - 31° 18' 38.05"E), south Cairo Province, Egypt, which houses about 7.79 million of the 18.29 million consumers of Greater Cairo (Elawa, 2015). Due to the bad drainage systems, the soils of this site may suffer from rising water levels, which may end in desertification. On the other hand, the unpolluted (reference) site receives irrigation waters from the river Nile, which in turn receives no wastes discharge of industrial or municipal wastes. It was located at Marazik village (29° 52' 16.94"N - 31° 16' 14.74"E) south Giza Province. The agricultural lands of the study sites, which extend along the Nile Valley, were characterized by alluvial soils. The prevailing climate of the study area including the two sites showed that the mean annual rainfall was 1.67 - 2.13 mm year^{-1}, while the annual mean temperature was 21.08 ºC, and the annual mean relative humidity was 52.68 - 56.08%. The irrigation water of the polluted site is more saline and has higher pH as well as heavy metals concentration than the unpolluted one.

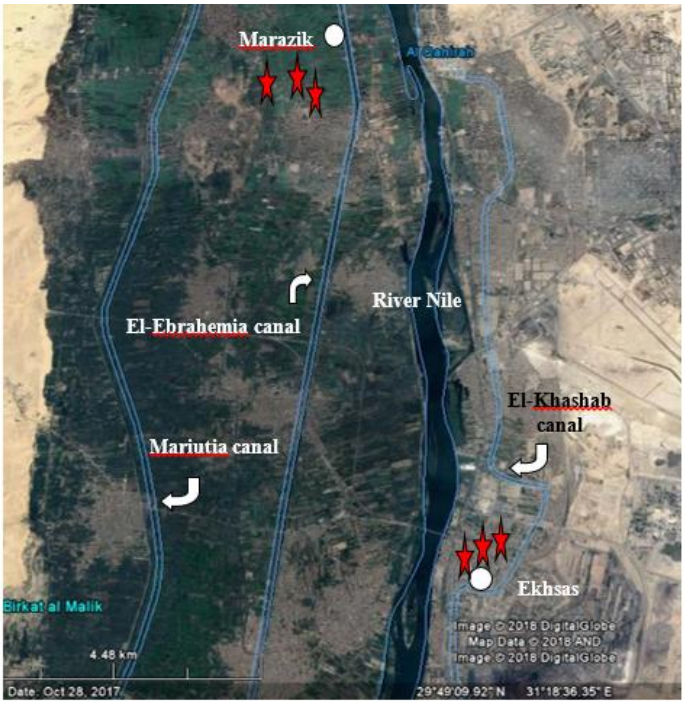

Figure 1. *Location map of the study area showing the study sites*

Sampling design

The cauliflower seeds are sown in nursery bed with a rate of 400 to 500 gm/ha during July and the crop was harvested in November. The seedlings are transplanted in main field after 3 – 4 weeks with spacing 60 × 60 cm. Irrigation may be given to the crop every 10-15 days, where at the time of head formation, there should be enough moisture in the field. A sampling of cauliflower plants was carried out from six farms (each about four acres), equally distributed in the polluted and unpolluted sites. At each cultivated farm, 10 quadrats (each 4 m^2) were randomly chosen to harvest the cauliflower plant samples. The number of individuals in each quadrat were recorded to calculate plant density (individual 100 m^{-2}) and then one individual from each qaudrat ($N = 60$) were harvested and transferred to the laboratory. The plant root was separated from the soil using soil core method. Plant samples were washed with tap water and twice with distilled water to remove any debris or dust. The stem and total root length and the number of leaves per individual were measured. Then, samples were divided into roots, leaves, and curds (edible part). The divided parts were weighed before and after (stem and leaves) oven drying at 105 ºC until constant weight, to determine their fresh and dry biomass (weight/unit area) as well as plant (curd) production.

Plant analysis

Chemical constituents

Three composite oven-dried samples of the cauliflower roots and curds (n = 36) were collected from each of the polluted and unpolluted farms and ground into a powder in a metal-free plastic mill for further analysis. The total nitrogen (N) was estimated by the Kjeldahl method, while P was determined by applying the molybdenum blue method using a spectrophotometer (CECIL CE 1021), and K was determined using a flame photometer (CORNING M410). The total soluble proteins were estimated following to Lowry et al. (1951), while carbohydrates (total soluble sugars) were estimated following to the anthrone technique (Umbriet et al., 1959). Heavy metals concentration of the different plant organs was determined using the acid digestion method. Whereas, a ground sample of 1 g was digested in a 20 ml tri-acid mixture of HNO_3:H_2SO_4:$HClO_4$ (5:1:1, *v/v/v*) till a transparent color appeared, then digested material was filtered and diluted with double distilled water to 25 ml (Allen, 1989). The concentrations of Pb, Cd, Co, Fe, Cu, Ni, Mn, Zn, Cr, and V were determined with PyeUnicamSp 1900 Recording Flame Atomic Absorption Spectrophotometer. The heavy metals' detection limits (in $\mu g\ l^{-1}$) were: 15.0 for Pb; 0.8 for Cd; 9.0 for Co; 5.0 for Fe; 1.5 for Cu, Mn, Zn; 6.0 for Ni; 3.0 for Cr and 2.0 for V. The confidence level for all detection limits was 98%. All the above-mentioned methods are gathered from Allen (1989).

Quality assurance and quality control

The accuracy of the heavy metals' determination was verified using a certified reference material (SRM 1573a, tomato leaves). The digestion and analysis of the reference material followed the same methods applied to the cauliflower tissues. Triplicate samples were used for heavy metals digestion and estimation. Measured concentration was compared with the certified value to determine the accuracy as percentage. The recovery rates for SRM 1573a ranged from 94.8 to 103.7%.

Pigment analysis

Three cauliflower plant leaves from each quadrat, in the polluted and unpolluted sites, were collected and combined to form three composite samples for each farm ($N = 36$). Chlorophyll a, b and carotenoids were prepared from 2 g fresh weight of the leaves dipped in 50% (v/v) acetone in complete darkness at 4°C overnight, which was then measured spectrophotometrically against a blank (aqueous acetone) at three wavelengths: 453, 644 and 663 nm (Allen, 1989). The content of each pigment was calculated from the following equations:

$$\text{Chl. a} = 10.3\ A_{663} - 0.918\ A_{644} \tag{Eq.1}$$

$$\text{Chl. b} = 19.7\ A_{644} - 3.87\ A_{663} \tag{Eq.2}$$

$$\text{Carotenoids} = 4.2\ A_{453} - (0.0264\ \text{chl. a} + 0.426\ \text{chl. b}) \qquad \text{(Eq.3)}$$

where A is the absorbance. The values were then expressed as (mg g^{-1} fresh wt.).

Soil analysis

For soil chemical analysis, three composite sub-surface samples from the plant rhizosphere were taken from each farm in the polluted and unpolluted sites ($N = 36$). Soil water extracts (1:5 w/v) were prepared for determining pH value using a glass electrode pH meter (Model 9107 BN, ORION type) and electrical conductivity (EC) using conductivity meter 60 Sensor Operating Instruction Corning. The inorganic nutrients (N, P, and K) were estimated using Atomic Absorption Spectrometer (Shimadzu AA-640-12). The concentration of Fe, Cd, Cu, Co, Cr, Mn, Ni, Pb, As, Ag, V, and Zn were estimated as mentioned for plant analysis (Allen, 1989). The operational conditions and instrument setting were done according to the manufacturers' specifications.

Data analysis

The pollution load index (PLI)

The pollution load index was estimated to determine soil pollution by each heavy metal as follows:

$$\text{PLI} = \text{Cp} / \text{Cu} \qquad \text{(Eq.4)}$$

where Cp and Cu are the concentration of heavy metal in both polluted and unpolluted soils, respectively (Liu et al., 2005). According to Lu et al. (2014), the soil was categorized based on the PLI as follows: unpolluted (PLI = 0-1); unpolluted-moderately polluted (PLI = 1-2); moderately polluted (PLI = 2-3); moderately-highly polluted (PLI = 3-4); highly polluted (PLI = 4-5); and very highly polluted (PLI > 5).

Bioaccumulation (BF) and Translocation (TF) factors

The bioaccumulation factor determines the plant's ability to uptake certain metal concerning its soil concentration; it was calculated as:

$$\text{BF} = C_r / C_s \qquad \text{(Eq.5)}$$

where C_r and C_s are the concentration of heavy metals in the root and soil, respectively. However, the translocation factor (TF) measures the translocation ability of heavy metal from the root to the plant edible part; it was determined as:

$$\text{TF} = C_c / C_r \qquad \text{(Eq.6)}$$

where C_c and C_r are the concentration of plant edible part (curd) and root heavy metal (Shehata and Galal, 2020).

Health risk assessment

The assessment of the health risk of a pollutant requires the determination of the level and way of exposure to a target organism. The daily intake of metals (DIM) for both adults and children was calculated as the average of their consumption of polluted plants (Khan et al., 2008).

$$DIM = (C_{hm} \times C_{fa} \times D_{fi}) / B_{aw} \qquad \text{(Eq.7)}$$

where C_{hm} is the plant heavy metal concentration (mg kg^{-1}), C_{fa} is a conversion factor, D_{fi} is the daily intake of vegetable, and B_{aw} is the average body weight. To convert fresh to dry weight, a conversion factor (0.085) was used (Rattan et al., 2005). The average body weights for children and adults are 32.7 and 55.9 kg, while the average daily intake of metal is 0.345 and 0.232 kg $person^{-1}$ day^{-1}, respectively (Asgari and Cornelis, 2015). Moreover, the health risk index (HRI) for the local inhabitants, consuming the contaminated plants, was calculated as the ratio of assessed crop exposure and the reference oral dose (Galal, 2016). The HRI value greater than one is a danger for human health and may cause a health risk for the consumers (US-EPA, 2012).

Statistical analysis

The paired-sample t-test was used to assess the differences in the soil and plant variables between the unpolluted and polluted sites. However, the significant variation in nutrients and heavy metals among the different plant parts were assessed using one-way analysis of variance (ANOVA 1) according to SPSS software (SPSS, 2012). Duncan's multiple range test at $p < 0.05$ was used to identify significant differences between means.

Results

Soil properties

The variation in soil characteristics indicated highly significant differences ($P < 0.001$) in all investigated soil variables between polluted and unpolluted farms (*Table 1*). Soil pH and EC as well as all heavy metals (except Ni) were high in the polluted compared to the unpolluted soil. Conversely, the contents of N, P, and K were significantly reduced in the polluted rather than unpolluted soils. Heavy metals concentration in the polluted soil had the sequence: Fe > Zn > Pb > Mn > Cu > Cd > Cr > Co > Ni > V. The PLI of the estimated metals (except Ni) was greater than 1 with the highest value (64.8) for Pb. Most estimated heavy metals except Cr, Cu, and Ni in the polluted site, in addition to Fe, Co, and V in the unpolluted site were above the tolerable limits.

Growth properties

The growth properties of cauliflower plants showed a significant reduction in all estimated parameters in the polluted farms (*Table 2*). The plant density was reduced from 200.00 to 100.00 individual 100 m^{-2}, while the stem length was from 37.67 to 20.00 cm, root length from 16.00 to 11.33 cm and the number of leaves per individual was from 7.67 to 5.33. Moreover, the differences in the fresh biomass, dry biomass, and production between unpolluted and polluted farms were highly significant ($P < 0.01$) (*Fig. 2*). For instance, the mean fresh biomass, dry biomass, and production in unpolluted farms were about 3.5 times higher than that in the polluted ones. The measurements of these growth parameters in the unpolluted site were 44.1±1.9, 5.5±0.8, and 42.3±4.2 t $acre^{-1}$, respectively, while in the polluted farms were 12.1±2.3, 1.6±0.7, and 11.5±1.7 t $acre^{-1}$. The reduction percentage of these parameters were 72.6, 70.9, and 72.8 %, respectively.

Table 1. *Chemical properties (Mean ±SD) of cauliflower soils (N = 18) from polluted and unpolluted farms*

Soil variable		**Unpolluted soil**	**Polluted soil**	**t-test**	**PLI**	**Tolerable limit WHO (1996)**
pH		6.81±0.01	7.47±0.04	26.56***		
EC µS cm^{-1}		2.00±0.02	7.88±0.03	333.20***		
Total N	**mg kg^{-1}**	317.33±41.21	66.17±0.76	196.20***		
Total P		23.61±0.18	6.43±0.08	153.22***		
K		420.33±22.18	31.20±0.20	322.29***		
Pb		0.89±0.01	57.67±1.53	64.38***	64.80	0.01 – 50
Cd		0.20±0.01	1.20±0.01	122.47***	6.00	0.02 – 0.7
Cr		0.42±0.00	0.85±0.01	92.43***	2.02	5 – 30
Cu		2.34±0.01	22.70±0.26	133.20***	9.70	0.27 – 100
Ni		0.34±0.00	0.23±0.02	10.66***	0.68	5.0
Fe		12.00±0.10	134.3±35.69	37.26***	11.19	0.15 – 7
Mn		16.30±0.02	51.33±2.08	29.15***	3.15	20.0
Zn		3.11±0.01	60.33±1.53	64.88***	19.40	10 – 50
Co		0.31±0.00	0.38±0.02	8.20***	1.23	0.02
V		0.08±0.00	0.22±0.03	18.45***	4.00	0.001

***: $p < 0.001$

Table 2. *Growth parameters (Mean±SD) of cauliflower (N = 60) grown in unpolluted and polluted farms*

Variable	**Farm**		**t-test**
	Unpolluted	**Polluted**	
Density (individuals 100 m^{-2})	200.00±35.42	100.00±10.61	7.81*
Stem length (cm)	37.67±2.08	20.00±1.00	8.04*
Root length (cm)	16.00±1.00	11.33±1.15	6.02*
Number of leaves individual^{-1}	7.67±0.29	5.33±0.58	6.25*

*: $p < 0.05$

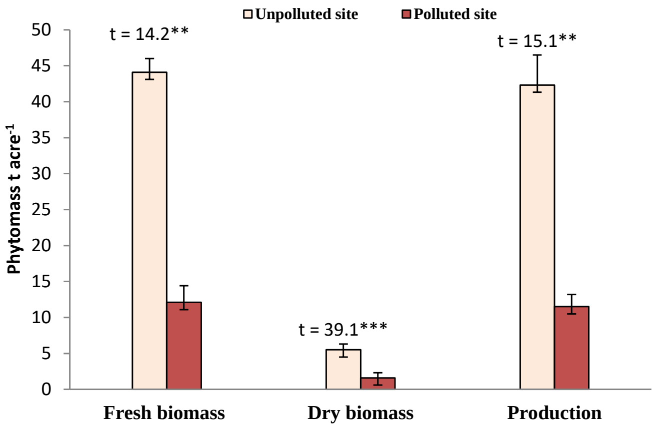

Figure 2. *Biomass and productivity of cauliflower crop cultivated in unpolluted and polluted farms. Vertical bars are standard deviation. **: p < 0.01, ***: p < 0.001*

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Plant analysis

Plant nutrients

The concentration of N, P, carbohydrates, and proteins in the curds and roots of cauliflower plants from the unpolluted site were considerably higher than their estimated relatives in the polluted site (*Table 3*). Also, the highest contents of N, carbohydrates, and proteins (1.73±0.13, 19.17±1.92, and 10.79±0.81%, respectively) were recorded in the curd tissues of the unpolluted plants, while the lowest N and proteins (1.18±0.16 and 7.40±1.01%) were recorded in the polluted plant roots and carbohydrates (11.48±0.80%) in the polluted plant curds. On the other side, the highest P and K contents (2.85±0.32 and 23.92±1.52) were recorded in the unpolluted plant roots, while the lowest (0.87±0.08 and 20.32±0.39) were recorded in the polluted plant roots and curds, respectively.

Table 3. *Inorganic and organic constituents in the roots and shoots (N = 36) of cauliflower plants grown in unpolluted and polluted soils. Maximum and minimum values are underlined*

Variable	Unpolluted farm		Polluted farm	
	Curd	**Root**	**Curd**	**Root**
Inorganic				
N (%)	1.73±0.13a	1.46±0.09ab	1.32±0.12b	1.18±0.16c
P (%)	2.05±0.28b	2.85±0.32a	1.12±0.05c	0.87±0.08c
K (mg kg^{-1})	21.24±4.46a	23.92±1.52a	20.32±0.39a	22.91±2.45a
Organic (%)				
Carbohydrates (%)	19.17±1.92a	18.39±1.32ab	11.48±0.80c	16.07±1.56b
Proteins (%)	10.79±0.81a	9.10±0.59ab	8.23±0.74b	7.40±1.01c

Means with the same letter in the same raw is not significantly differed s by Duncan’s multiple range tests at $P < 0.05$

Plant heavy metals

The analysis of heavy metals in the cauliflower plant tissues showed significant variation among the different organs with all investigated metals concentrations considerably higher in the polluted than unpolluted farms (*Table 4*). It was found that the polluted plant root accumulated the highest concentrations of all investigated heavy metals except Cr and Co (4.30±0.26 and 4.45±0.05 mg kg^{-1}), which were recorded in the curd tissues of the same plants. On the other side, the unpolluted plant curds had the lowest concentrations of Cd, Cr, Mn, Zn, Co and V (0.67±0.14, 0.58±0.14, 9.42±1.04, 6.67±0.38, 0.64±0.15 and 0.03±0.01 mg kg^{-1}), while their roots accumulated the lowest Pb, Cu, Ni, and Fe (7.00±0.50, 1.00±0.25, 1.67±0.52 and 631.75±22.34 mg kg^{-1}). In the polluted site, the heavy metals concentration fell in the order: Fe > Pb > Mn > Cd > Ni > Zn > Cu > V > Co > Cr.

Plant pigments

The pigments analysis of the cauliflower leaves showed a significant difference in chlorophyll a and b between polluted and unpolluted farms (*Fig. 3*). It was found that chlorophyll a and b were significantly reduced from 1.75 to 1.19 mg g^{-1} and from 0.90 to 0.68 mg g^{-1}, respectively in the polluted farms. However, the content of carotenoids was not significantly changed.

Table 4. *Heavy metals concentrations (Mean±SD) in the roots and shoots (N = 36) of cauliflower crops cultivated in unpolluted and polluted farms. Maximum and minimum values are underlined*

Heavy metal (mg kg^{-1})	**Unpolluted area**		**Polluted area**	
	Curd	**Root**	**Curd**	**Root**
Pb	7.50±1.39b	7.00±0.50b	531.67±7.64a	623.33±18.93a
Cd	0.67±0.14c	1.00±0.25c	86.67±12.58b	115.00±13.23a
Cr	0.58±0.14a	0.94±0.06a	4.30±0.26a	3.62±0.25a
Cu	13.33±3.82c	1.00±0.25d	43.33±2.89b	57.67±3.79a
Ni	4.08±1.66c	1.67±0.52c	41.67±7.64b	83.33±19.86a
Fe	737.33±0.52c	631.75±22.34c	2531.67±24.66b	5588.33±23.63a
Mn	9.42±1.04c	9.75±0.66c	90.00±5.0b	125.00±13.23a
Zn	6.67±0.38c	10.58±0.88c	27.33±3.01b	71.67±7.64a
Co	0.64±0.15b	0.93±0.08b	4.45±0.05a	4.25±0.35a
V	0.03±0.01c	0.04±0.01c	4.80±0.09b	16.48±20.80a

Means with the same letter in the same raw is not significantly differed s by Duncan's multiple range tests at $P < 0.05$

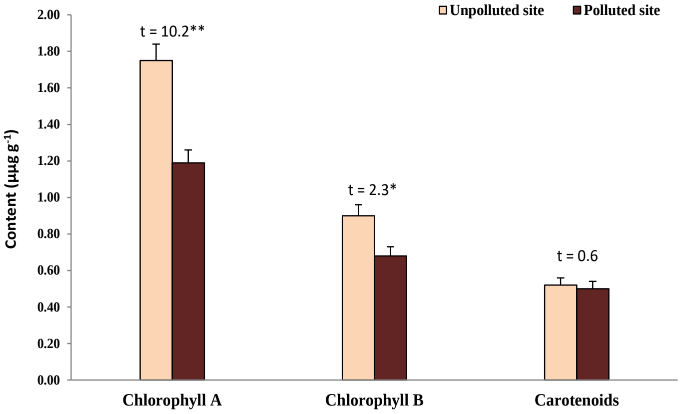

Figure 3. *Photosynthetic pigments content of cauliflower leaves cultivated in unpolluted and polluted farms. Vertical bars are standard deviation. **: $p < 0.01$*

Bioaccumulation (BF) and translocation (TF) factors

The bioaccumulation and translocation potential of cauliflower plants was high in both polluted and unpolluted sites (*Table 5*). The BF for the estimated heavy metals (except Cd, Cu, Mn, and V in the unpolluted site) was greater than one. The highest BF (575.0) was recorded for Cd in the polluted site, while the lowest (0.43) was recorded for Cu in the unpolluted site. The BF of the investigated heavy metals in the polluted farms fell in the order: Cd > Ni > V > Fe > Co > Pb > Cr > Mn > Cu > Zn. On the other side, the TF of most investigated heavy metals, except Pb, Cu, Ni, and Fe in the unpolluted site and Cr, Cu, and Co in the polluted site, was less than one. Some heavy metals were translocated from belowground root to aboveground curds in the sequence: Cr > Co > Cu > Pb in the polluted farms, while Cu > Ni > Fe > Pb > V > Mn in the unpolluted ones.

Table 5. *Bioaccumulation (BF) and translocation (TF) factors of heavy metals in cauliflower plants grown in unpolluted and polluted farms*

Heavy metal	**Unpolluted farm**		**Polluted farm**	
	BF	TF	**BF**	TF
Pb	7.87	1.07	10.81	0.85
Cd	0.83	0.67	575.00	0.75
Cr	2.23	0.62	4.24	1.19
Cu	0.43	13.33	1.85	1.03
Ni	4.94	2.45	362.30	0.50
Fe	52.65	1.17	41.60	0.45
Mn	0.60	0.97	2.44	0.72
Zn	3.40	0.63	1.19	0.38
Co	3.01	0.69	11.09	1.05
V	0.53	1.00	76.06	0.29

Daily intake of metals (DIM) and health risk assessment

The DIM for the investigated heavy metals (except Fe) was less than one for both adults and children consuming cauliflower plants grown in wastewater-irrigated soils (*Table 6*). The contribution of wastewater-irrigated crops to dietary intake of Fe, Pb, and Cd per individual per day was 1.3281, 0.2789, and 0.0455 mg day^{-1}, respectively for adults, and 1.5267, 0.3206, and 0.0523 mg day^{-1} for children. Moreover, the results showed that Pb, Cd, Mn, Fe and Ni contamination in wastewater-irrigated cauliflower plants have the greatest potential to pose a health risk to the consumers, where their health risk index (HRI: 278.913, 45.467, 3.372 1.897 and 1.093, respectively for adults, and 320.628, 52.267, 3.877, 2.181 and 1.256 for children) was greater than one (*Table 6*). Similarly, the HRI of Pb (3.9345 and 4.5229) in the unpolluted crops was greater than one for both adults and children, respectively.

Table 6. *Daily intake of metals (DIM: mg day^{-1}) and health risk index (HRI) of adults and children for individual heavy metals in the edible parts of cauliflower plants grown in unpolluted and polluted soils. Values > 1 are bold*

Heavy metal	**Polluted area**				**Unpolluted area**				**R_fD**	**References**
	DIM		**HRI**		**DIM**		**HRI**			
	A	**C**	**A**	**C**	**A**	**C**	**A**	**C**		
Pb	0.2789	0.3206	**278.913**	**320.628**	0.0039	0.0045	**3.9345**	**4.5229**	1.0×10^{-3}	(US-EPA 2013)
Cd	0.0455	0.0523	**45.467**	**52.267**	0.0004	0.0004	0.3515	0.4040	1.0×10^{-3}	(US-EPA 2013)
Cr	0.0023	0.0026	0.002	0.002	0.0003	0.0003	0.0002	0.0002	1.5×10^{0}	(US-EPA 2013)
Cu	0.0227	0.0261	0.568	0.653	0.0070	0.0080	0.1748	0.2010	4.0×10^{-2}	(FAO/WHO 2013)
Ni	0.0219	0.0251	**1.093**	**1.256**	0.0021	0.0025	0.1070	0.1230	2.0×10^{-2}	(US-EPA 2010)
Fe	**1.3281**	**1.5267**	**1.897**	**2.181**	0.3868	0.4447	0.5526	0.6352	7.0×10^{-1}	(FAO/WHO 2013)
Mn	0.0472	0.0543	**3.372**	**3.877**	0.0049	0.0057	0.3530	0.4058	1.4×10^{-2}	(FAO/WHO 2013)
Zn	0.0143	0.0165	0.048	0.055	0.0035	0.0040	0.0117	0.0134	3.0×10^{-1}	(FAO/WHO 2013)
Co	0.0023	0.0027	0.054	0.062	0.0003	0.0004	0.0078	0.0090	4.0×10^{-2}	(US-EPA 2013)
V	0.0025	0.0029	0.001	0.002	0.0000	0.0000	0.0000	0.0000	1.8×10^{0}	(FAO/WHO 2013)

Discussion

Heavy metals accumulation in the food chains from water or soil is a great concern, which and requires great attention worldwide (Rangamani et al., 2017). In the present study, the soil chemical analysis indicated high values of most investigated variables including salinity and heavy metals (except Ni) in the polluted farms of cauliflower crop. These results coincided with those of Galal et al. (2018, 2019a,b), Eid et al. (2020a), and Shehata and Galal (2020). This elevated heavy metals' concentration may be attributed to the anthropogenic and industrial activities as well as the excessive use of fertilizers in the polluted site. Besides, the high concentrations of Cd, Cu, and Cr in the soil samples may be due to the excessive usage of pesticides and fertilizers without the awareness of farmers about their application, in addition to the use of manures produced from combustion wastes or non-degradable municipal solid wastes. It is worth noting that most investigated heavy metals except Cr, Cu, and Ni in the polluted site, and Fe, Co, and V in the unpolluted site were above the permissible limits (WHO/FAO, 2013). Moreover, the PLI data indicated that the polluted farms were highly polluted (PLI > 5) with Pb, Zn, Fe, Cu, and Cd; moderately polluted (PLI = 1-4) with Cr, Mn, Co, and V; and unpolluted (PLI < 1) with Ni (Lu et al., 2014).

The growth criteria of the cauliflower plants indicated a significant reduction in all measured parameters under pollution stress, where the mean plant density, stem length, root length, and the number of leaves were about 1.5 times higher in the unpolluted than polluted sites. These results were in line with those of Kumar et al. (2019) on the same plant. This reduction may be due to the higher quantities of heavy metals, especially Pb, Ni, Fe, Mn, Cu, Co, and Cr accumulated in the different tissues of these plants. The reduction in plant growth parameter due to heavy metals was confirmed by many researchers (Hadi et al., 2010; Galal et al., 2018, 2019a; Shehata and Galal, 2020) with special reference to Pb and Cd, which considerably reduce the number of leaves, and stem and root length (Hadi et al., 2014). It was reported that Co and Cr ions inhibit plant growth in terms of shoot and root length, shoot and root fresh and dry weight, and the number of leaves, and this may be carried out by reducing photosynthetic pigments, photosynthetic activity (Farahat et al., 2017) and inhibiting cell division and elongation (Ghazi et al., 2019).

The present investigation exhibited a significant reduction in the cauliflower biomass and production in the heavy metals-polluted site. The production of cauliflower curd was 42.3 t ha^{-1} in the unpolluted farms, which decreased to 11.5 t ha^{-1} in the polluted site. Khanal et al. (2014), recorded 12.43 t ha^{-1} cauliflower curd yield in soil amended with heavy metal-rich sludge, while Tripathi et al. (2016) recorded 62.1 t ha^{-1} in a freshwater-irrigated sandy soil. Eid et al. (2021) attributed this high yield to the higher macronutrients and organic matter content in the sludge. Conversely, the reduced biomass and production of curd in the polluted farms may be attributed to the chlorophyll synthesis inhibition and leaf tissue damage due to heavy metals (Chauhan and Joshi, 2010). Subsequently, the low photosynthetic activity and diminished plant growth apparently reduced the plant biomass and yield (Nagajyoti et al., 2010). According to Chatterjee and Chatterjee (2000), heavy metal toxicity depresses biomass, decreases Fe concentration, chlorophyll a and b, and lowers catalase activity in the leaves along with the accumulation of high levels of heavy metals in the different plant parts. Furthermore, the decrease in macronutrients, especially N and P, in polluted conditions may also reduce plant biomass and yield (Batabyal et al., 2016).

Pigment paleness has been observed in the cauliflower plants exposed to soil and irrigation water pollutants. Significant decrease in the chlorophyll a and b was recognized in the plants cultivated in polluted farms; and this may be attributed to the high accumulation power of this plant to heavy metals, which destruct chloroplasts and inhibit chlorophyll synthesis (Eid et al., 2019), or decrease the number of leaves chloroplasts (Galal et al., 2019b). Toxic heavy metals enhance chlorophyll degradation and consequently inhibit its synthesis (Chauhan and Joshi, 2010). Regarding the high salinity of the polluted farms, Farooq et al. (2013) and Galal et al. (2019a) reported a reduction in cauliflower chlorophyll content, which might be due to chlorophyllase activity enhancement at higher salinity levels. Moreover, heavy metals may also activate the pigment enzyme and accelerate the pigment decomposition (Hamadouche et al., 2012).

Different sources and levels of nutrients had a remarkable effect on the biomass and yield of cauliflower (Batabyal et al., 2016). The cauliflower tissues accumulated high contents of inorganic and organic nutrients, which were greater in the unpolluted than polluted farms. These nutrients amounts were higher than those recorded for Spanish, Australian, and American cauliflowers (Cunningham et al., 2001; USDA, 2019; Collado-González et al., 2021). According to Batabyal et al. (2016), cauliflower plants accumulate a higher concentration of macronutrients (N, P, K), which induce curd formation. Besides, Pejman et al. (2015) and Kumar et al. (2019) confirmed that a higher concentration of macro- and micro-nutrients may stimulate crop productivity, but also may possess toxic and harmful impacts on the biological behaviour. Moreover, Collado-González et al. (2021) recorded 15.6% protein content in the healthy cauliflower curd, while the present study recorded 8.23 and 10.79% in the polluted and unpolluted (healthy) plants, respectively.

Long term polluted water irrigation can change the physicochemical properties of soil and leads to heavy metal uptake by vegetable crop plants. In the present investigation, the analysis of the heavy metal of cauliflower plants showed significant differences in the plant tissues between polluted and unpolluted sites. This result coincided with that of Ur Rehman et al. (2019), who reported that heavy metal concentrations were higher in wastewater-irrigated than in freshwater-irrigated food crops. The cauliflower plants accumulated most heavy metals except Cr and Co in their roots rather than the curd in line with Kumar et al. (2019) on the same plant. Besides, they accumulated higher metals concentration from polluted rather than unpolluted farms. The heavy metals accumulated in the curd in the polluted site were higher than those accumulated by the same tissue in sludge-amended soil (Khan et al., 2018) and wastewater-irrigated soils (Khan et al., 2013). Previous studies have reported that vegetable crops can show high levels of heavy metals due to their gradual accumulation if irrigated with sewage and industrial effluent (Balkhair and Ashraf, 2016). The current data showed a very high concentration of Cd, Pb, and Zn as compared to previously reported average levels in different vegetables (Zhuang et al., 2009; Singh et al., 2010; Cao et al., 2014). The continuous adding of metals by irrigation with polluted water and low leaching metals into the lower layers of soil may be a reason for the high concentration of heavy metals in cauliflower tissues. In the polluted site, the heavy metals concentration fell in the order: Fe > Pb > Mn > Cd > Ni > Zn > Cu > V > Co > Cr. Similar results were suggested by Galal (2016) on summer squash, Farahat et al. (2017) on maize and wheat, Galal et al. (2018) on cabbage, and Galal et al. (2019a) on common mallow. Jahangir et al. (2009) and Kalisz et al. (2018) confirmed the variability in the uptake and accumulation potential of elements in the cauliflower curds.

Cauliflower plants could uptake heavy metals from the soil, accumulate them in the roots, and may translocate some of them to their edible curds. The bioaccumulation potential of cauliflower plants indicated that they mainly retain heavy metals in their roots due to the decreased mobility of these metals from the below- to the above-ground parts. The BF of the investigated heavy metals in the polluted farms fell in the order: Cd > Ni > V > Fe > Co > Pb > Cr > Mn > Cu > Zn. Similar results were postulated by Shehata and Galal (2020) on cucumbers and Galal et al. (2018 and 2019a) on cabbage and common mallow, respectively. On the other side, some heavy metals such as Cr, Co, Cu, Fe, Pb, and V could translocate from the belowground roots to the aboveground curds, which indicates the possibility of cauliflower being an accumulator and phytoextractor of these metals. These results coincided with those of Eid et al. (2017) on spinach and Galal et al. (2018, 2019a). The translocation and accumulation of these metals in the edible parts of cauliflower will expose the public consumers to high health risks.

Heavy metals accumulation in the edible vegetables is a common threat to the public health (Ur Rehman et al., 2019). Vegetable nutritional quality is the major concern because it is a principal component of the human diet (Hu et al., 2020). The health risk assessment of a pollutant requires the estimation of the exposure level, and this can be achieved by determining the exposure routes (i.e., food chain) to the target organisms (Arora et al., 2008). The DIM data indicated that the dietary intake of metals from consuming plants grown in polluted soils is higher than that of the unpolluted site. In contrast with the polluted site, the DIM values from unpolluted plants were to some extent free of risks for all estimated metals (except Fe) for adults and children (WHO, 1996). Similar results were postulated by Ur Rehman et al. (2019) and Rehman et al. (2018) on wastewater-irrigated cauliflower. Whereas the HRI for these metals was < 1 indicating no consequence from plant transfer through the food chain and safe consumption (US-EPA, 2012). On the other hand, consumption of contaminated cauliflower may cause health complications due to the high dietary intake of Pb, Cd, Mn, Fe, and Ni grown in the polluted site, whereas the HRI of these metals was greater than one indicating hazards to consumers due to the presence of plants in high proportion in the diet of local populations and consequently have a higher risk to human health. According to Khanal et al. (2014), a higher concentration of Cd and Pb suggests potential risks to human health through the consumption of cauliflower. A similar result was reported by Ma et al. (2021), where the plant curd accumulated a high concentration of Cd with high human health risk.

Conclusion

The PLI of the estimated heavy metals (except Ni) exceeded one, and the polluted farms were highly polluted with Pb, Zn, Fe, Cu, and Cd; moderately polluted with Cr, Mn, Co, and V; and unpolluted with Ni. Irrigation of cauliflower with heavy metals contaminated wastewater has led to the accumulation of heavy metals such as Cr, Co, Cu, Fe, Pb, and V in its edible parts, and this exposes the public consumers to high health risks. The growth and production of cauliflower were negatively affected by the high levels of heavy metals in the polluted soil. Cauliflower is a potential plant for phytostabilization since it had high BF of most estimated metals (except Cr, Co, Cu, Fe, Pb, and V) with low TF for these metals. Consumption of polluted cauliflower may cause health complications due to the high dietary intake of Pb, Cd, Mn, Fe, and Ni grown in the polluted site. Additionally, the HRI for these metals was greater than one indicating hazards to consumers due to the presence of plants in a high amount in the diet of local populations and consequently have a higher risk to human health. To avoid the entrance of metals into the food chain, environmental protection laws must be implemented to monitor the industrial and municipal effluents in agricultural lands. Also, washing cauliflower curds with water and various household chemical solutions can remove the dirt and dust particles and significantly decrease heavy metals. Therefore, the present study strongly recommends not to grow vegetables in polluted areas and even if it is necessary, their consumption is not recommended.

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