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Laiba Waqar
University of Sialkot
Nida Mehboob
University of Sialkot
Maqsood Ahmed
Pest Warning and Quality Control of Pesticides
Shahid Masood Shah
COMSATS University Islamabad
Adnan Arshad
Lanzhou University
Amer Rasul
Pest Warning and Quality Control of Pesticides
Rana Qaiser Saleem
Pest Warning and Quality Control of Pesticides
Muhammad Musaddiq Shah
University of Sialkot
Abdul Mateen
dr.abdulmateen@uskt.edu.pk

University of Sialkot

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Food Safety Risks from Heavy Metals and Pesticide Residues in Cauliflower (*Brassica oleracea*) from a Subtropical Region of Pakistan

Laiba Waqar[1], Nida Mehboob[1**], Maqsood Ahmed[2], Shahid Masood Shah[3], Adnan Arshad[4], Amer Rasul[5], Rana Qaiser Saleem[6], Muhammad Musaddiq Shah[1*], Abdul Mateen[1*]**

[1]Department of Biological Sciences, Faculty of Sciences, University of Sialkot, Sialkot, 51310, Pakistan
[2]Department of Agriculture, Pest Warning and Quality Control of Pesticides, Sialkot, 51300, Pakistan
[3]Department of Biotechnology, COMSATS University Islamabad, Abbottabad, 22060 Pakistan
[4]College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China
[5]Directorate General, Pest Warning and Quality Control of Pesticides, Lahore, 54000, Pakistan.
[6]Department of Agriculture, Pest Warning and Quality Control of Pesticides, Gujranwala, 52200, Pakistan.

Laiba Waqar (laibaw468@gmail.com), Nida Mehboob (nidamehboob922@gmail.com), Maqsood Ahmed (maqsoodahmed200@hotmail.com), Shahid Masood Shah (smasood@cuiatd.edu.pk), Adnan Arshad (arshad@lzu.edu.cn), Amer Rasul (amerrasul11212@gmail.com), Qaiser Saleem (qaiserrana68@gmail.com), Muhammad Musaddiq Shah (muhammad.musaddiq@uskt.edu.pk), Abdul Mateen (dr.abdulmateen@uskt.edu.pk/amateen1987@gmail.com)

[1*]**Corresponding authors:** muhammad.musaddiq@uskt.edu.pk; dr.abdulmateen@uskt.edu.pk

[**]**Authors:** Equally Contributed.

Abstract

Heavy metal contamination and pesticide residues in vegetables irrigated with wastewater pose serious risks to food safety and human health. This study evaluated the concentrations of selected heavy metals, including copper (Cu), lead (Pb), chromium (Cr), cadmium (Cd), and cobalt (Co), along with pesticide residues of chlorpyrifos, imidacloprid, carbofuran, profenofos, and lambda-cyhalothrin in cauliflower (Brassica oleracea) cultivated under wastewater irrigation in Gujranwala, Pakistan. Heavy metal concentrations in cauliflower, soil, and wastewater were determined using Flame Atomic Absorption Spectroscopy (FAAS), while pesticide residues were analyzed through High Performance Thin Layer Chromatography (HPTLC). The results revealed elevated concentrations of Cd (0.253 mg/kg), Pb (0.350 mg/kg), and Co (0.197 mg/kg) in cauliflower curds, exceeding permissible limits. Soil samples also showed higher Cd accumulation (0.403 mg/kg), whereas wastewater samples contained elevated levels of Pb, Co, Cr, and Cd beyond recommended standards. Statistical analysis indicated significant variations among heavy metals, particularly Cr and Co. Bio-concentration ratio analysis demonstrated greater accumulation of Cd and Co in curds and Cu in leaves. Chlorpyrifos residue (0.0634 mg/kg)

exceeded the maximum residue limit, highlighting potential health hazards and the need for continuous monitoring of wastewater-irrigated vegetables.

Keywords: *Cauliflower, Wastewater, Heavy Metals, AAS, Pesticide residues, HPTLC.*

1. Introduction

Cauliflower (*Brassica oleracea)* is an altered form of cabbage belonging to the mustard family *Brassicaceae* (Petruzzello et al., 2024). A rich, semi-moist and a soil exhibiting high nutrient content is best for the growth of cauliflower, requiring large quantities of fertilizers as well. Best optimum conditions for growth is slightly acidic soil with pH around 6-6.5 and temperature ranging between 15-21°C (Rafique, 2012). It has been grown in constant lower temperatures which aids in developing heads known as curd which is usually the white edible and nutritious part of this vegetable surrounded by large green leaves (Petruzzello et al., 2024). Being rich in proteins, vitamins B5, B6, manganese and fatty acids, it is good for reducing risks of cancer and works wonders for diabetes (Peterson et al., 2020). Thus, cauliflower is a nutritious and economically valuable vegetable whose cultivation and health benefits are well-supported by agronomic and biomedical research.

Cauliflower can range in colors like purple, orange and green but white cauliflowers having white curds are commonly used. The orange mutation in cauliflower was first discovered in Canadian cauliflower fields. Broccoflower is another name of green cauliflower which is usually marketed in US and Europe since 1990s. Green cauliflower comes in typical curd head shape and also in spiral forms. Anthocyanin also found in red wine and red cabbage is a water soluble pigment present in purple cauliflower that gives this cauliflower a purplish hue (Alappat & Alappat, 2020). In 2021, the global production of broccoli and cauliflower amounted to about 25.5 million tons (Seymen et al., 2024). According to FAO report 2023-2024, China is the largest producer of cauliflower in the world with 9.6-9.8 million tons of cauliflower production followed by India with 9.2-9.5 million tons' production which is more than 70% of world cauliflower production when combined. Pakistan ranked as one of the top ten producers of cauliflower in the world in 2021 and 2022 with a production volume reaching 0.3-0.35 million tons in 2024 (FAO, 2024), with varieties cultivated from May to October especially in Punjab and Sindh (Baloch et al., 2015). Cauliflower is a widely cultivated and consumed crop worldwide including Pakistan.

Ensuring a secure and sufficient food supply for a rapidly expanding global population while safeguarding human health and environmental integrity remains one of the most pressing challenges facing modern agriculture. The demand for food increases the necessity for raising agricultural production in order to meet the growing population's requirements projected to reach 9.6 billion by 2050 the pressure on agricultural production intensifies significantly (Amiri et al., 2022). Pesticides from insecticides to fungicides are the chemical shields we use to protect crops from weeds, bugs, and blight, both in the field and after harvest (Ahmed et al., 2024). Pesticides play a very significant role in agriculture to balance the increase in population and to protect the agricultural products from pests and diseases as every year, 45% of the entire agricultural production is lost globally due to pests and diseases (Kolani et al., 2016; Rani et al., 2021). The use of certain carbamates and organochlorides are prohibited in several countries (Jayarai et al., 2016), some of these compounds have been detected in the environment worldwide due to their persistent nature or illegal use of the banned chemical pesticides (Bayoumi, 2022). However, these pesticides are harmful and pose a serious danger to the environment as well as human health.

Excessive usage and lack of precaution while using these pesticides can prove to pose a severe health risk to individuals and might cause health risks like diabetes, reproductive issues, neurological disorders, cancer, and respiratory diseases (Rani et al., 2021). Excessive use of pesticides causes these chemicals and metabolites to remain in the environment and food, causing serious problems in the ecosystem and public health (Li et al., 2015). Fruits and vegetables are essential for human nutrition containing functional compounds such as carotenoids, phenolics, trace minerals, vitamins and fiber (Shashirekha et al., 2015). However, they may contain toxic residual pesticides due to the use of pesticides during the production process of agricultural products (Mebdoua et al., 2017). Pesticide residues in agricultural products are usually monitored with reference to maximum residue limits (MRLs), which represent the highest concentration of pesticide residues that is legally permitted or accepted in food commodities after the use of pesticides (Duan et al., 2016). Several studies reveal that 30% to 70% of tested food products, including fruits, vegetables, and animal-derived products, especially in Punjab and Sindh, exceed established MRLs (Abbas et al., 2024). To sustainably meet the growing global food demand, agriculture must balance increased production through essential tools like pesticides with rigorous safeguards to protect public and environmental health from their potential hazards.

Heavy metals pollution of the soil is a significant environmental issue on a worldwide level due to the swift rise of industrialization (Hu et al., 2019). Usually fresh water reservoirs and ground water is used for irrigation of crops but in developing countries like Pakistan facing water scarcity (Hussain et al., 2017), ground water is used for irrigation which is of poor quality consisting heavy metals from various anthropogenic activities which are polluting these ground water resources (Khalid, 2019). Agriculture sector uses 70% of the total waste water being used (Miller-Robbie et al., 2017). Because heavy metals (HMs) are incapable of decomposing themselves, they are toxic even at lower concentrations (Ferrey et al., 2018). Heavy metals pollution from the industrial sector has damaging impacts on life on the earth, and heavy metals buildup in vegetables and crops caused by polluted water supplies is generating serious health problems (Jacob et al., 2018). High quantities of heavy metals have been found in urban areas of developing nations like Pakistan (Alam et al., 2018). Mining activities, the use of chemical fertilizer, pest and weed killers, as well as the irrigation of agricultural land with wastewater, are examples of man-made sources of heavy metals pollution (Sarkar et al., 2018). Pollution of heavy metals (HMs) is caused by improper handling of waste from industries, road pollution, spray cans, metalworking, manufacturing, and wastewater disposal. These toxic heavy metals can easily accumulate in edible parts of plants grown in such soil due to their inability to degrade and staying persistent in soil reducing the bioavailability of macro nutrients for plants effecting plant growth (Sutkowska et al., 2020). Vegetables, being rich in proteins, carbohydrates and minerals are considered a major part of diet especially in developing countries. Serious quantities of heavy metals can be ingested in these vegetables through roots and can accumulate in edible portion of plants and vegetables due to irrigation with untreated wastewater (Jabeen et al., 2016). Consuming vegetables that are polluted with high levels of lead, copper and mercury might result in disorders with the heart, kidneys, neurological system, bones and the breakdown of cellular membranes (Balali-Mood et al., 2021). The toxicity due to accumulation of heavy metals, decreased macronutrient bioavailability, and possible neurological abnormalities are the main health problems caused by heavy metal pollution from industrial operations.

The presence of metallic compounds in fertilizers, pesticides and irrigation water adds an additional risk of metal contamination for crops. Fertilizers and pesticides contribute towards heavy metal contamination in soil and vegetables that can disrupt normal functioning of organs (Rashid et al. 2023). It is of the utmost importance to fully understand the heavy metals and pesticides contamination in cauliflower that is grown in Gujranwala district, Pakistan. The objective of this study is to thoroughly evaluate the levels of heavy metals and pesticide residues in cauliflower to offer important insights about the security regarding consuming cauliflower. These insights are crucial for preserving public health and making sure that the intake of this common vegetable continues to improve the diet and nutrition of the regional population.

2. Materials and Methods

2.1 Sample Collection

Cauliflower samples were collected from several spots along Mandiala Road, Gujranwala (see figure-2.1). To keep things clean, gloves wore, gathered both the edible curds and leaves from three different sites with three replications each, and stored everything in labeled plastic bags for later testing of heavy metals and pesticides. Gujranwala, the capital of the Gujranwala division with an area of 3622 km² and geographical coordinates of 32°9'58.8636" N and 74°11'45.2400" E (Minallah, 2016), is a significant economic center, contributing approximately 5% to Pakistan's GDP and serving as the country's third-largest industrial hub (Naz & Zaidi, 2013).

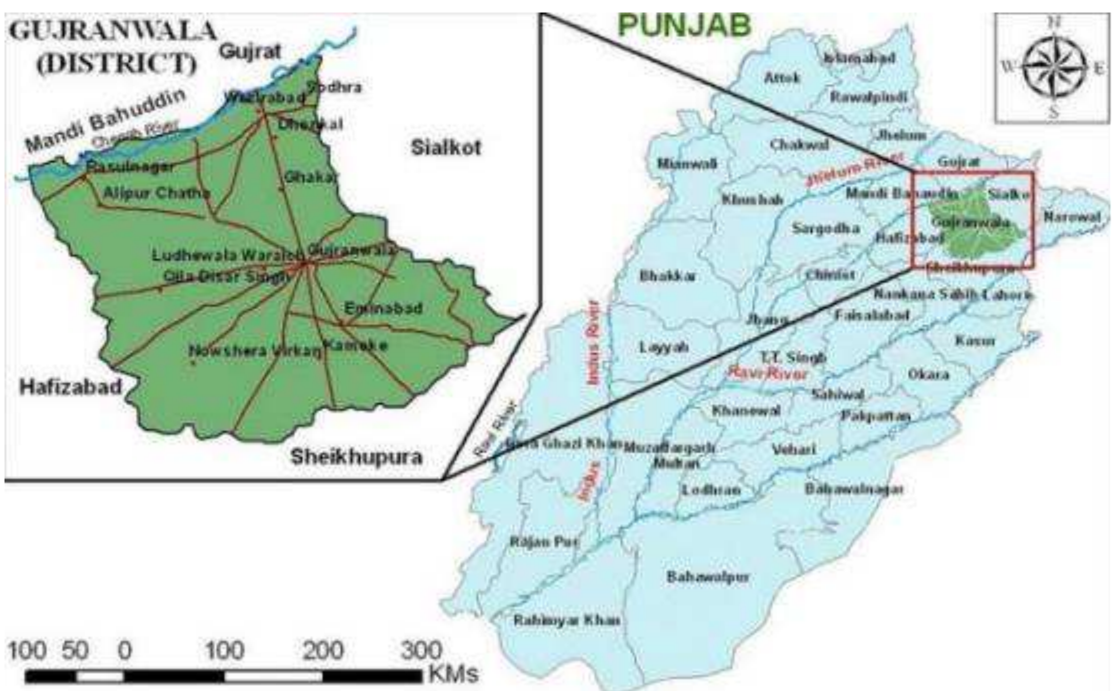

Figure 2.1. Map of Gujranwala District.

2.2 Heavy Metals analysis using AAS

2.2.1 Sample Preparation

To assess heavy metal contamination, soil (100 g from three zones) and irrigation wastewater (100 mL) were collected from the cauliflower field (Amir et al., 2017). Cauliflower samples were gently washed, cut into uniform pieces, and dried at 60°C for 24 hours to remove moisture. The dried plant material was ground into a fine powder, and along with soil samples, was sieved and stored in clean, labeled containers for homogenization (Aftab et al., 2023).

2.2.2 Sample Digestion

Homogenized samples (1 g each) were precisely weighed and gently digested in a mixture of concentrated HNO_3, HCl, and $HClO_4$ at 80°C until the solution turned colorless, releasing the heavy metals from organic matter (Ahmad et al., 2018). After cooling, the digest was diluted to 50 mL with distilled water and filtered through Whatman No. 1 paper to remove any remaining particulates (Amir et al., 2017).

2.2.3 AAS Determination

To determine heavy metal concentrations, cauliflower, soil, and water samples were analyzed using Flame Atomic Absorption Spectroscopy (FAAS) after constructing calibration curves for Copper, Lead, Chromium, Cadmium, and Cobalt at their respective wavelengths (Copper (Cu) 324.8, Lead (Pb) 283.3, Chromium (Cr) 357.9, Cadmium (Cd) 228.8, and Cobalt (Co 240.7) (Abrham & Gholap, 2021). The instrument was carefully calibrated with standard solutions before sample measurement, and the resulting data were compared against established safety limits to assess the edibility of the cauliflower (Amir et al., 2017).

2.3 Pesticide residue analysis using HPLTC

2.3.1 Sample Preparation for Pesticides Residues Analysis

Fresh cauliflower samples were finely powdered and turned into a slurry paste to ensure even mixing, then 50 g of each sample was extracted with 70-75 mL of ethyl acetate (Hanif et al., 2024). The extract was filtered, cleaned using heat-activated charcoal to remove chlorophyll and impurities, and finally concentrated on a rotary evaporator followed by a gentle nitrogen stream to obtain a purified residue for analysis (Hussain et al., 2020).

2.3.2 Steps of Pesticide Residues by HPTLC

To identify pesticide residues, vegetable samples were analyzed using High Performance Thin-Layer Chromatography (HPTLC), a reliable and straightforward method known for its efficiency and matrix tolerance (Bai et al., 2020; Sobstyl et al., 2020). Silica gel 60 plates were activated, spotted with samples and standards, then developed in ethyl acetate within a pre-saturated tank. After visualization with bromine vapor and acetylcholine esterase staining, pesticide presence was confirmed by blue spots on a white background, and quantification was achieved by comparing spot areas or diameters with standard values (Hussain et al., 2020).

2.4 Data Analysis

To compare heavy metal levels between edible and non-edible cauliflower parts, along with soil and water, we ran a t-test (Kumar et al., 2019). Before diving into the analysis, we checked that our data met the test's assumptions using a Shapiro test for normality and homogeneity of variance. A one-sample t-test then allowed us to compare our measured concentrations against WHO safety standards (Kasozi et al., 2021). The results showed significant differences ($p < 0.05$), revealing that heavy metals build up differently across the plant and its surrounding environment. The bio concentration ratio (BCR) of the samples was calculated in order to evaluate the heavy metals transfer capabilities and vegetable production depending on soil metal contents. The following formula was used to accomplish the computation;

Bio-concentration factor BCF = C_v/C_s, where C_s is the metal concentration in the soil and C_v is the metal concentration in the vegetables (Ahmed et al., 2018).

3. Results and Discussion

3.1 Heavy metals concentrations detected in samples

The Heavy metals selected included Copper (Cu), Lead (Pb), Chromium (Cr), Cadmium (Cd), and Cobalt (Co). These heavy metals were chosen based on their common presence in contaminated environments. The concentrations of heavy metals in Cauliflower leaves, curd and soil were measured in mg/kg and mg/L for wastewater samples, respectively. The concentration of heavy metals is given in table-3.1.

Concentrations of heavy metals in, cauliflower leaves, curds and soil samples showed their distribution and possible health effects. There was a considerable amount of cadmium (Cd) present, with soil having the highest concentration. The main quantities of lead (Pb) were found in the soil, which indicated soil pollution. The largest concentrations of chromium (Cr) were also found in soil, which indicated how soil pH affects its absorption. Elevated copper (Cu) levels in leaves were a sign of bioaccumulation brought on by the physiology of the plant and the soil. The uniformity of cobalt (Co) concentrations in leaves, curds, and soil suggested that cobalt plays a crucial function as a micronutrient with balanced intake and dispersion. For agricultural operations to maintain environmental health and food safety, heavy metal levels in soil and plant tissues must be closely monitored.

Table-3.1: Concentrations of Heavy Metals detected in different Samples

Sample Names	Heavy metal concentrations (ppm)				
	Cu	**Pb**	**Cr**	**Cd**	**Co**
Cauliflower Curd 1	0.3946	0.3141	0.1027	0.2173	0.1610
Cauliflower Curd 2	0.4262	0.3552	0.1937	0.3022	0.2013
Cauliflower Curd 3	0.4631	0.3991	0.1154	0.3249	0.2319
Leaves Sample 1	4.2903	0.3212	0.1515	0.2605	0.1742
Leaves Sample 2	4.563	0.3428	0.2184	0.3428	0.2158
Leaves Sample 3	4.772	0.3786	0.2399	0.3551	0.2522
Soil Sample 1	1.1913	0.3654	0.7772	0.3102	0.2524
Soil Sample 2	1.2468	0.6288	0.9248	0.3215	0.3824
Soil Sample 3	1.2896	0.7214	1.224	0.3613	0.4211
Wastewater Sample 1	0.6655	0.1426	0.2053	0.0489	0.1082
Wastewater Sample 2	0.6950	0.1629	0.2135	0.0429	0.1127
Wastewater Sample 3	0.7155	0.1554	0.2104	0.0419	0.1070

Copper (Cu); Cadmium (Cd); Chromium (Cr); Lead (Pb) and Cobalt (Co).

3.2 Cauliflower Curd Samples Descriptive Statistics

Descriptive data on cauliflower curd samples, assessing heavy metal concentrations are compared with World Health Organization (WHO) recommend limits. The average cobalt (Co) concentration 0.197 mg/kg, was found above the WHO's 0.1 mg/kg limit. The average copper (Cu) content was observed as 0.423 mg/kg, which was below the WHO's 73.3 mg/kg limit. The cadmium (Cd) content was 0.253 mg/kg, above the WHO limit. The average chromium (Cr) content was 0.177 mg/kg, which was within the WHO limits. The mean lead (Pb) concentration was 0.35 mg/kg, above the WHO's 0.3 mg/kg limit for food products. Lead exposure can be harmful, even at levels marginally above the WHO guideline as shown in figure-3.1.

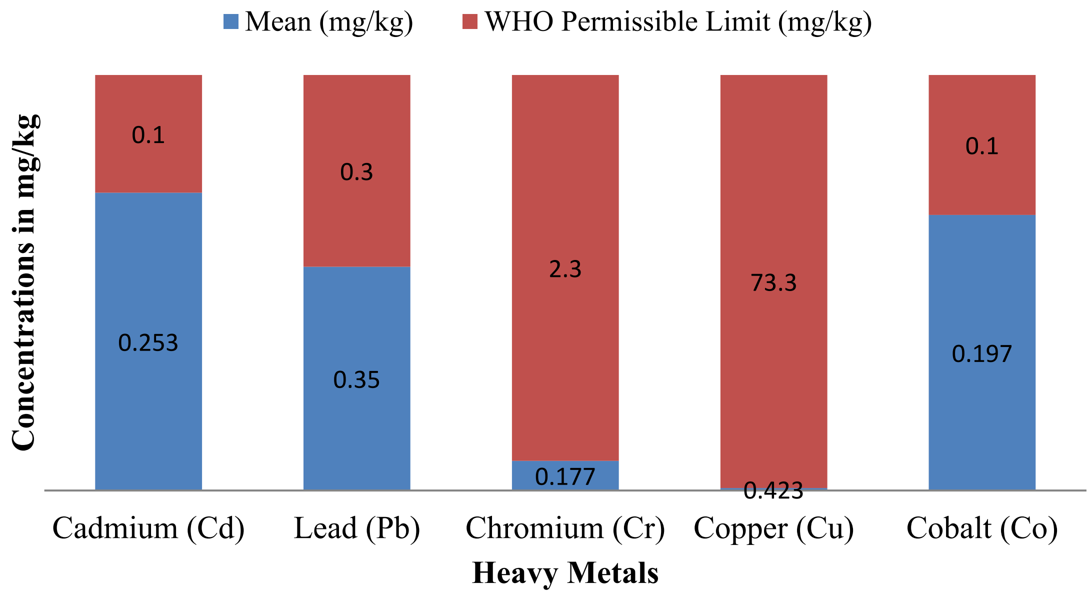

Figure-3.1: Mean concentration of heavy metals in cauliflower curd compared with WHO permissible limits.

3.3. Cauliflower Leaves Descriptive Samples Statistics

Descriptive data on cauliflower leaves samples, assessing heavy metal concentrations are compared with World Health Organization (WHO) recommended limits (figure-3.2). The average chromium (Cr) content was 0.197 mg/kg, which was within the WHO prescribed limits. The average cobalt (Co) concentration 0.21 mg/kg, above the WHO's 0.1 mg/kg limit. Similarly, cadmium (Cd) content was 0.31 mg/kg, also above the WHO limit of 0.1 mg/kg which could pose serious health hazards for consumers. The average copper (Cu) content was 4.467 mg/kg, below the WHO's 73.3 mg/kg standards. The mean lead (Pb) concentration was 0.343 mg/kg, above the WHO's 0.3 mg/kg limit for food products. The data is crucial for evaluating potential health risks associated with heavy metal exposure.

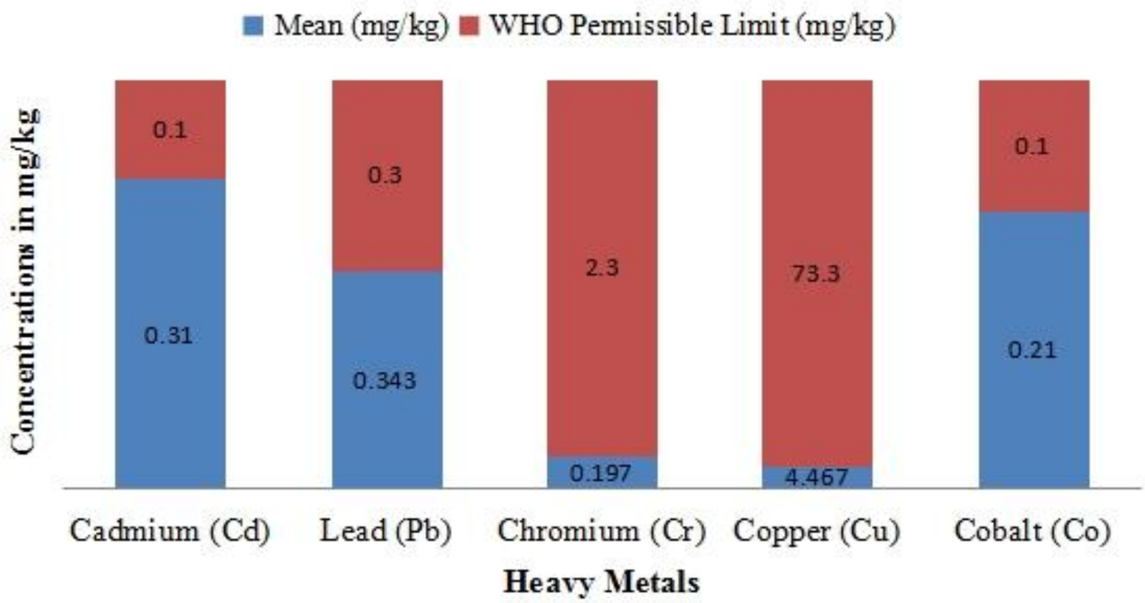

Figure-3.2: Mean concentration of heavy metals in leaves samples compared with WHO permissible limits.

3.4 Soil Samples Descriptive Statistics

The mean concentrations of heavy metals in the soil samples, including lead, chromium, cobalt, copper and chromium were compared with WHO permissible limits (figure-3.3). The figure showed that the mean concentration of cadmium was above WHO permitted levels, while all other elements, like copper, lead, chromium and cobalt, were well within safe ranges. In particular, for metals that exceed WHO permissible levels, ongoing monitoring and perhaps remedial operations are essential to protect soil quality and guarantee food safety for agricultural products.

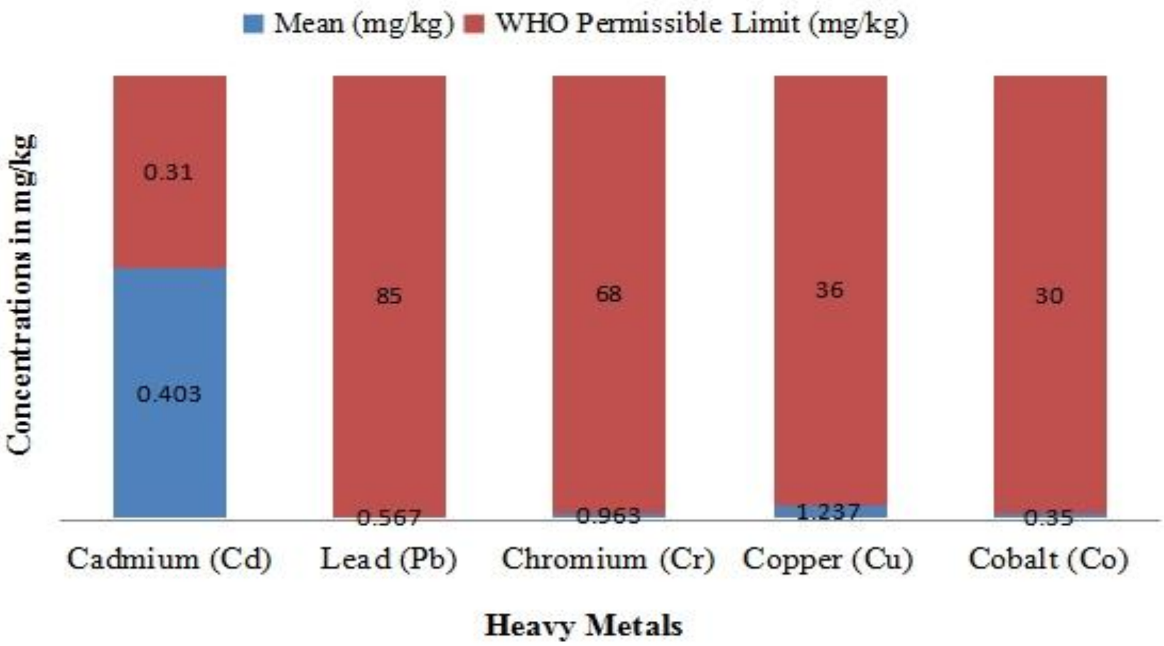

Figure-3.3: Mean concentration of heavy metals in soil samples compared with WHO permissible limits.

3.5 Water Samples Statistics

The results for wastewater showed varying levels of cobalt, chromium, lead, and cadmium that were higher than those recommended by the WHO (figure-3.4). The cobalt (Co) content was higher than what was recommended by the WHO. While, cobalt (Co) is beneficial to health in minor doses, whereas, greater quantities can cause thyroid damage, cardiovascular difficulties, and respiratory problems. The wastewater's increased cobalt (Co) levels suggest that treatment is necessary to guarantee the water's safety. The effluent contains more than four times the WHO-permitted maximum amount of chromium (Cr) that is 0.05 mg/L. The lead content (Pb) was also higher (0.1426 mg/L than what the WHO considers to be safe (0.1 mg/L). Lead (Pb) is a hazardous metal that can result in a number of health concerns, especially in young children. The wastewater sample's cadmium (Cd) content (0.0489 mg/L) was also far greater than the WHO's allowable limit that is 0.01 mg/L. Because of its high degree of toxicity, cadmium (Cd) presents significant health hazards. The only metal in this study that was within allowable limits was copper. Even if the measured copper concentration was well below the WHO guideline, it is still crucial to keep an eye. These results highlight the necessity of strict wastewater treatment and monitoring procedures in order to lower heavy metal concentrations to manageable levels and safeguard the environment and human health.

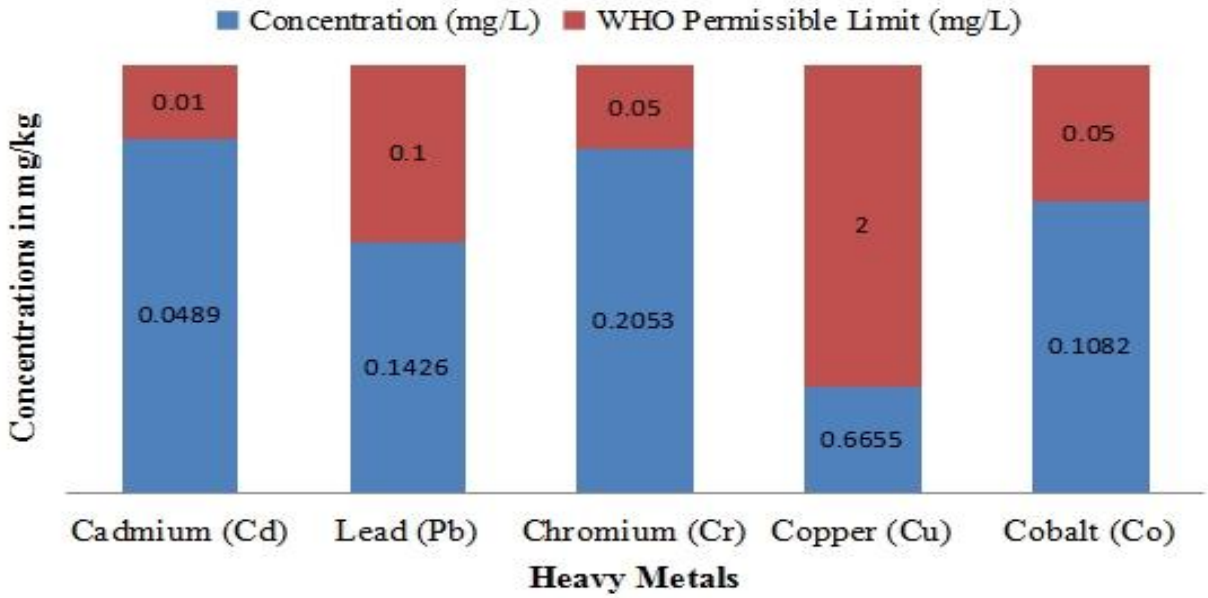

Figure-3.4: Concentration of heavy metals in wastewater compared with WHO permissible limits.

3.6 One Sample T-Test Statistical Analysis for Curd samples

The five heavy metals (cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), and cobalt (Co) were analyzed for the T test of heavy metal concentrations in cauliflower curd samples. In cauliflower curds, copper (Cu), cobalt (Co), chromium (Cr), and cadmium (Cd) showed significant differences as their *p* values were less than 0.05 which showed that concentration of these heavy metals were different than WHO limits as shown in table-3.2. On the other hand, lead (Pb) with *p* value of 0.1628 showed insignificant results meant that concentration of lead (Pb) in samples were within WHO permissible limits.

Table-3.2: One Sample T test results for heavy metals in cauliflower curds.

Heavy Metal	t Value	df	P-Value
Cu	-3594.3	2	0.00000007741*
Pb	2.1651	2	0.1628
Cr	-71.668	2	0.0001946*
Cd	6.5714	2	0.02238*
Co	4.7676	2	0.04129*

(*) indicates significant differences (p-value < 0.05). Copper (Cu); Cadmium (Cd); Chromium (Cr); Lead (Pb) and Cobalt (Co).

3.7 One Sample T-Test Statistical Analysis for Leaves samples

Five heavy metals cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), and cobalt (Co) were analyzed for the T test of heavy metal concentrations in cauliflower leaves samples. Similarly, as in curds, cauliflower leaves also showed significant differences in copper (Cu), cobalt (Co), chromium (Cr), and cadmium (Cd) as their *p* values were less than 0.05 as shown in (table-3.3) which showed that concentration of these heavy metals were at variance with WHO limits. On the other hand, lead (Pb) with p value of 0.09644 showed insignificant results meant that concentration of lead (Pb) in samples were within WHO permissible limits.

Table-3.3: One Sample T test results for heavy metals in cauliflower leaves.

Heavy Metal	t Value	df	p Value
Cu	-473.74	2	0.000004456*
Pb	2.9824	2	0.09644
Cr	-87.504	2	0.0001306*
Cd	7.9373	2	0.0155*
Co	4.7631	2	0.04136*

(*) indicates significant differences (p-value < 0.05). Copper (Cu); Cadmium (Cd); Chromium (Cr); Lead (Pb) and Cobalt (Co)

3.8. One Sample T-Test Statistical Analysis for Soil samples

In soil samples, all the concentrations of heavy metals including copper (Cu), cobalt (Co), chromium (Cr), lead (Pb) and cadmium (Cd) showed significant differences as all their *p* values were less than 0.05 as shown in (table-3.4). This states that the concentration of these heavy metals were significantly different than WHO permissible limits.

Table-3.4: One Sample T-Test results for heavy metals in soil.

Heavy Metal	t Value	df	*p* Value
Cu	-1335.3	2	0.0000005608*
Pb	-786.96	2	0.000001615*
Cd	6.4236	2	0.02339*
Cr	-532.01	2	0.000003533*
Co	-577.79	2	0.000002995*

(*) indicates significant differences (p-value < 0.05), df; degree of freedom. Copper (Cu); Cadmium (Cd); Chromium (Cr); Lead (Pb) and Cobalt (Co)

3.9 Pesticide Residues concentrations detected in samples

The pesticide residues selected included Chlorpyrifos, Imidacloprid, Carbofuran. Profenofos and Lembdacyhalothrin. Cauliflower curd sample data assessing pesticides residues concentrations were compared with their Maximum Residual Limits (MRL). The average Chlorpyrifos concentration 0.0634 mg/kg, was above the MRL 0.01 mg/kg limit. The average Imidacloprid content was 0.2221 mg/kg, below the MRL 0.5 mg/kg limit. The Carbofuran content was 0.0577 mg/kg, also below the MRL limit. The mean Profenofos and Lembdacyhalothrin concentration was also below their respective MRLs of 0.5 mg/kg, as shown in (figure-3.5). The data is crucial for evaluating potential health risks associated with Pesticide residue exposure.

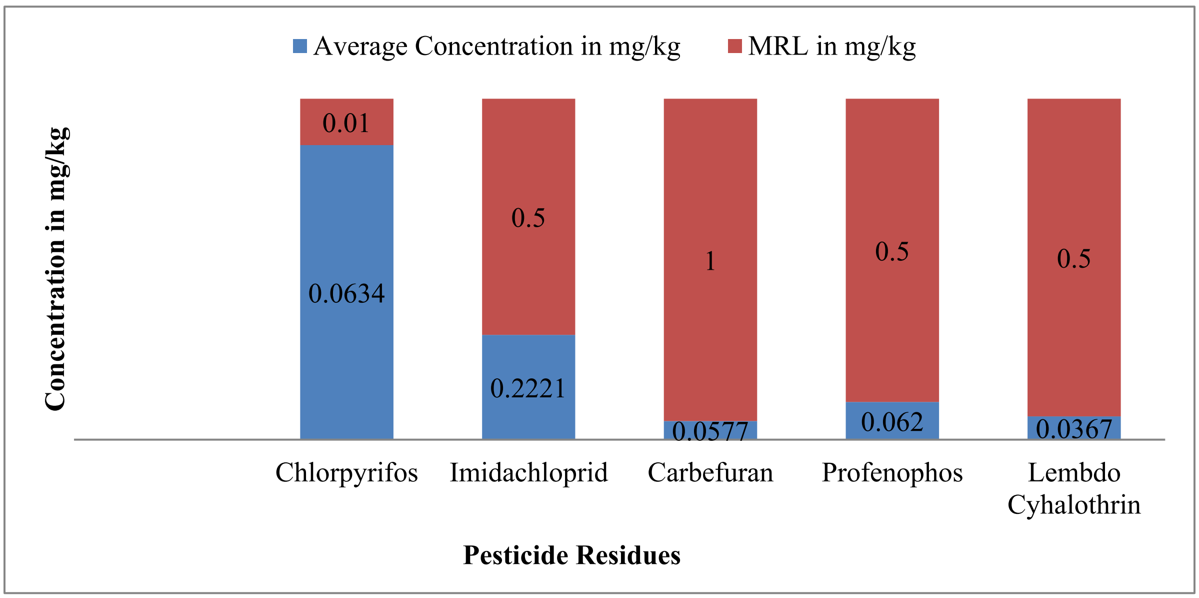

Figure-3.5: Concentration of pesticide residues in cauliflower compared with MRL permissible limits.

The Levene's test findings on variance homogeneity for five pesticides. Chlorpyrifos, Imidacloprid, Carbofuran, Profenofos, and Lembdacyhalothrin, show no huge differences in variances across groups. All *p*-values are above the 0.05 mark for each method used (based on mean, median, median with adjusted degrees of freedom, and trimmed mean) as shown in table-3.5 and graphically represents in figure-3.6. For Chlorpyrifos, *p*-values ranged from 0.638 to 0.857, for Imidacloprid from 0.123 to 0.874, for Carbofuran from 0.085 to 0.819, for Profenophos from 0.241 to 0.690, and for Lambdacyhalothrin from 0.453 to 0.904. These results prove that the variances are even across groups for all pesticides. This meets the equal variances assumption needed for later statistical tests like ANOVA.

Table-3.5: Test of Homogeneity of Variances results for pesticide residues; Mean (±SD) Pesticide residues (mg/kg) in cauliflower collected from 6 sampling sites (G1–G6)

Samples	Chlorpyrifos	Imidacloprid	Carbofuran	Profenofos	Lambda
G1	0.069 ± 0.010^{b}	0.244 ± 0.016^{c}	0.067 ± 0.012^{b}	0.109 ± 0.014^{b}	0.067 ± 0.012^{a}
G2	0.072 ± 0.012^{b}	0.252 ± 0.018^{c}	0.047 ± 0.008^{a}	0.049 ± 0.008^{a}	0.040 ± 0.008^{b}
G3	0.061 ± 0.008^{ab}	0.216 ± 0.014^{b}	0.056 ± 0.008^{b}	0.062 ± 0.012^{a}	0.040 ± 0.008^{b}
G4	0.052±0.008a	0.222 ± 0.010^{b}	0.066 ± 0.012^{b}	0.047 ± 0.008^{a}	0.020 ± 0.004^{a}
G5	0.068 ± 0.008^{ab}	0.193 ± 0.010^{a}	0.056 ± 0.008^{b}	0.054 ± 0.010^{a}	0.023 ± 0.006^{a}
G6	0.058 ± 0.010^{ab}	0.204 ± 0.012^{a}	0.047 ± 0.006^{a}	0.049 ± 0.008^{a}	0.023 ± 0.006^{a}

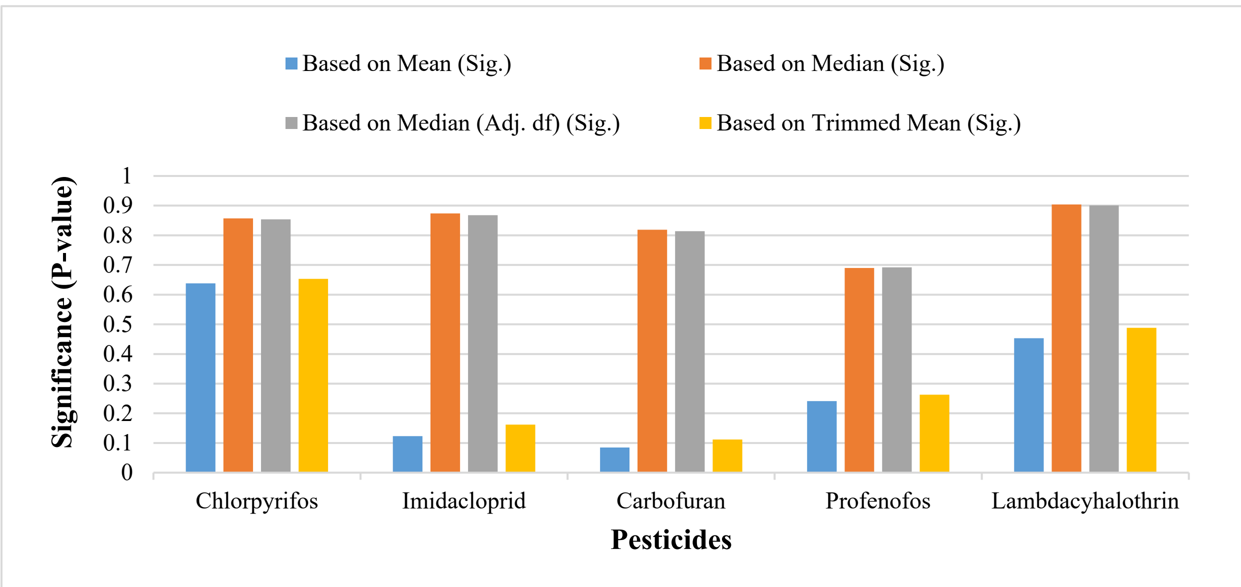

Figure-3.6: Comparison of P-values for six selected pesticides (Chlorpyrifos, Imidacloprid, Carbofuran, Profenofos, Lambdacyhalothrin, and a general 'Pesticides' group) calculated from statistical tests based on the mean and the median.

The ANOVA shown in table-3.6 and graphically shown in figure-3.7, breaks down the analysis of variance for five pesticides: Chlorpyrifos, Imidacloprid, Carbofuran, Profenophos, and Lambdacyhalothrin. It looks at the differences between groups based on their linear and deviation terms. Chlorpyrifos shows no big differences between groups ($p = 0.104$), which points to uniformity. Imidacloprid has clear differences between groups ($p < 0.001$) and a strong linear trend ($p < 0.001$), with deviations also showing importance ($p = 0.045$). Carbofuran has overall group differences ($p = 0.015$) due to deviations ($p = 0.011$), while its linear trend isn't significant ($p =$

0.234). Profenophos shows clear importance across all terms, including the combined group effect ($p < 0.001$) linear trend ($p < 0.001$), and deviations ($p = 0.001$), which suggests a lot of variability. Lambdacyhalothrin doesn't have significant overall group differences ($p = 0.068$), but its linear trend is important ($p = 0.007$), while deviations aren't ($p = 0.507$). These findings show different patterns of variance among the pesticides, with some having significant trends or deviations while others stay consistent across groups.

Table-3.6: Mean pesticide residues (mg/kg) by site with statistical significance (ANOVA and Duncan's test).

DF	5	5	5	5	5
S.S	0.001	0.008	0.001	0.009	0005
M.S	0.000	0.002	0.000	0.002	0.001
P	0.104	0.001	0.015	0.001	0.068
f	n.s	**	*	**	n.s

Means followed by the same letter are not significantly different according to Duncan's multiple range test at $\alpha = 0.05$. Different letters indicate significant differences between sites. The letters (a, b, c) are assigned in increasing order of mean residue concentration, where "a" represents the lowest mean group and "c" the highest. ns = not significant; * $p < 0.05$; ** $p < 0.01$.

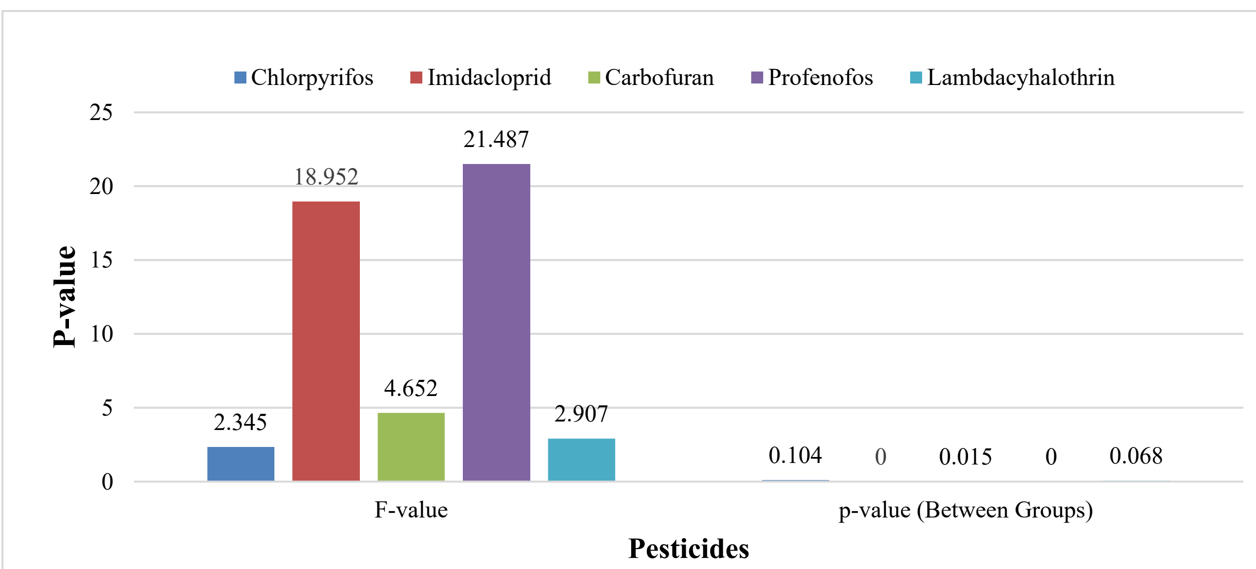

Figure-3.7: Results of a one-way ANOVA showing the F-values and P-values for the concentration of five pesticide residues across sampling sites.

3.10 Bio concentration Ratios (BCR)

Understanding the hazards to human health and environmental sustainability requires an understanding of the efficiency of heavy metal absorption by plants, which was provided by the BCRs from soil to cauliflower leaves and soil to cauliflower curds given in table-3.7.

Table-3.7: Bio-Concentration Ratio for each heavy metals.

Heavy Metals	Average Conc. in curds (mg/kg)	Average Conc. in leaves (mg/kg)	Average Conc. in soil (mg/kg)	BCR of soil to leaves	BCR of soil to curds
Copper	0.423	4.467	01.237	3.6022	0.3415
Lead	0.350	0.343	0.567	0.6025	0.6140
Chromium	0.177	0.197	0.963	0.2049	0.1841
Cadmium	0.253	0.310	0.403	0.9394	0.7673
Cobalt	0.197	0.210	0.350	0.6	0.5626

Copper (Cu); Cadmium (Cd); Chromium (Cr); Lead (Pb) and Cobalt (Co)

3.11 Bio concentration Ratios (BCR) from Soil to Leaves

With a high BCR of 0.9394, cadmium (Cd) was efficiently absorbed and accumulated in leaves. Toxic heavy metal cadmium easily moves from roots to leaves, causing serious problems for food safety when consumed through plants. The elevated BCR emphasizes the bioavailability of cadmium in soil, underscoring the necessity of rigorous monitoring and remediation strategies to reduce exposure hazards. The BCR for lead (Pb) was 0.6025, suggesting a modest level of absorption efficiency from the soil to the leaves. Concerns regarding food safety are raised by the buildup of lead near the edible plant components, such as cauliflower leaves, underscoring the need of soil management techniques in reducing the possibility of contamination.

Compared to cadmium and lead, chromium (Cr) has a poorer uptake efficiency with a BCR of 0.2049. Chemical speciation of chromium in soil affects plant uptake; trivalent chromium was less mobile and poisonous than hexavalent forms. The decreased BCR indicates that chromium translocation to cauliflower leaves was restricted, which lowers the hazards associated with food exposure but makes careful monitoring necessary because of its persistence.

With a high BCR of 3.6022, copper (Cu) was efficiently absorbed and accumulated in leaves. Disquiets about food safety are highlighted by an inconsistency between copper's potential toxicity at excessive levels and its crucial role in plant metabolism. Since of the high BCR, it is important to monitor soil copper levels and guarantee safe farming methods since copper can accumulate in edible plant tissues. With a BCR of 0.6000, cobalt (Co) has a modest uptake efficiency that was comparable to lead. The buildup of cobalt in cauliflower leaves highlights the element's potential impact on environmental health and food safety. Cobalt's mobility and absorption by plants are reflected in its moderate BCR, that initiates regarding more study into toxicity limits and regulatory requirements for agricultural usage.

3.12 Bio concentration Ratios (BCR) from Soil to Curds

In order to evaluate food safety and agricultural sustainability, it is essential to understand how heavy metals accumulate in edible plant components. The BCRs from soil to cauliflower curds showed that the BCR for cadmium (Cd) was 0.7673, suggesting a notable build-up in curds. The movement of cadmium from the soil into the cauliflower curds demonstrates the metal's bioavailability and its health hazards when consumed. The elevated BCR highlights the necessity of strict regulations on cadmium concentrations in farming soils to protect food quality and human health. With a BCR of 0.6140, lead (Pb) accumulates moderately in curds. Compared to lead and cadmium, chromium (Cr) has a lesser accumulation potential, as indicated by its BCR of 0.1841. The reduced efficiency of chromium absorption from soil was reflected in the amount of chromium in cauliflower curds, which affects evaluations of food safety and environmental sustainability. Because chromium was persistent in soil conditions, therefore, the lower BCR indicates that chromium transfers confined to edible plant components, minimizing possible dietary exposure hazards but requiring continuing monitoring. With a BCR of 0.3415, copper (Cu) shows considerable accumulation in curds. With a BCR of 0.5626, cobalt (Co) has a moderate accumulation potential that was comparable to lead's. The fact that cobalt was present in cauliflower curds emphasizes how potentially harmful it may be to both environmental and food safety. Due to the mobility and absorption of cobalt by plants, the moderate BCR indicates a need for study into toxicity limits and regulatory requirements in order to reduce the hazards connected with agricultural methods. The BCR of soil to curds for Cobalt, Copper, Cadmium, Chromium, and Lead are 0.5626, 0.3415, 0.7673, 0.1841, and 0.6140, respectively. This means that the curds contain 56.26% of the cobalt, 34.15% of the copper, 76.73% of the cadmium, 18.41% of the chromium, and 61.40% of the lead concentrations found in the soil. Comparatively, the BCR of soil to leaves for the same metals are 0.6000, 3.6022, 0.9394, 0.2049, and 0.6025, respectively. This indicates that the leaves contain 60% of the cobalt, 360.22% of the copper, 93.94% of the cadmium, 20.49% of the chromium, and 60.25% of the lead concentrations found in the soil. Notably, the leaves absorb significantly more copper from the soil than the curds, with a BCR of 3.6022, suggesting a high uptake of copper by the leaves. For the other metals, the absorption levels are somewhat similar between the curds and leaves, but the leaves still show higher absorption for cadmium and copper. These precise bio concentration ratios offer vital information on the intricate relationships that exist in agricultural ecosystems between heavy metals, soil dynamics, and plant uptake processes. It is crucial to comprehend these linkages in order to put into practice efficient soil management techniques, guarantee food safety, and safeguard the health of the ecosystem. To protect the public health from possible dangers associated with heavy metal exposure via food consumption, advance sustainable farming practices, and deepen our understanding of the processes leading to heavy metal pollution, more study and monitoring are required.

Discussion

Producing safe food ensuring public health and food quality is a cruci9al task. Usually fresh water reservoirs and ground water is used for irrigation of crops however, in developing countries like Pakistan facing water scarcity (Hussain et al., 2017), ground water is used for irrigation which is of poor quality consisting heavy metals from various anthropogenic activities which are polluting these ground water resources (Khalid, 2019). The research was conducted on wastewater which was being used for irrigation of cauliflower in Gujranwala to determine the heavy metal concentration. Likewise, commonly used pesticides were also analyzed from cauliflower for pesticides residues. The results analyzed using Atomic Absorption Spectrophotometer showed that the wastewater sample had levels of lead (0.1426 mg/L), cobalt (0.1082 mg/L), chromium (0.2053 mg/L), and cadmium (0.0489 mg/L) that are higher than the WHO's recommended limits (0.01, 0.1, 0.05, and 0.05 mg/L, respectively), posing serious health concerns. Monitoring is still necessary even when copper is under the acceptable level (0.6655 mg/L vs. 2.0 mg/L). These results underline how critical it is to treat wastewater effectively and maintain tight control in order to decrease heavy metal concentrations and safeguard public and environmental concerns.

Communities that depend on contaminated water supplies could encounter severe health effects if these problems are not resolved. Another study by (Ur Rehman et al., 2019) in Sahiwal reported high concentration of manganese and lead in wastewater. Heavy metal concentrations in soil samples were also higher than recommended limits proposed by WHO. The substantial quantity of cadmium (Cd) in the soil suggests extensive pollution, most likely from wastewater used for irrigation. Soil had the greatest concentration of lead (Pb), indicating heavy metals contaminations contamination. Because the pH of the soil affects chromium's absorption, chromium (Cr) was also prevalent. The amounts of cobalt (Co) were consistent in the soil, curds, and leaves, demonstrating its function as an essential micronutrient with balanced intake and distribution. Samples of cauliflower curd exhibited mean heavy metal concentrations with minimal change, suggesting steady absorption. There is a risk of damage to the kidneys and bone demineralization due to the average measurement of cadmium (Cd) of 0.253 mg/kg, which is more than double the WHO acceptable limit of 0.1 mg/kg. Over the WHO limit of 0.3 mg/kg, lead (Pb) averaged 0.350 mg/kg, posing risks to neurological and developmental functions. Although, the level of chromium (Cr) was well below the WHO standard of 2.3 mg/kg, its presence still has to be monitored because it can be carcinogenic. The level of copper (Cu) was 0.423 mg/kg, which is well below the WHO standard of 73.3 mg/kg. This indicates that there is no immediate risk, but long-term exposure may still be hazardous. Cobalt (Co) was detected at 0.197 mg/kg, which is more than the 0.1 mg/kg WHO standard. (Khan et al., 2019) also reported high concentrations of zinc, nickel, and chromium in vegetables. Another report by (Wadood et al., 2021) showed similar findings of elevated levels of Pb, Ni and Cd in vegetables in Rawalpindi and Multan.

A study by (Galal et al., 2023) found Pb, Zn, Fe, Cu, and Cd contamination in Soil and cauliflower. One sample T test used for statistical analysis analyzed the concentrations of five heavy metals in cauliflower curd, leaves, and soil samples. The findings showed significant differences in copper, cobalt, chromium, and cadmium concentrations with their *p* values less than 0.05, which were higher than WHO limits. Our results showed that lead concentrations were within WHO permissible limits as the *p* value of lead was higher than 0.05. similarly, in soil samples, all heavy metal concentrations showed significant differences, with *p* values less than 0.05. The results suggest that these heavy metals are not safe for consumption. T test was also previously performed by (Rafiee, 2018) for heavy metal analysis in vegetables supported out findings. Another similar research was previously performed by (Kasozi et al., 2021) in which One Sample T test was used to analyze heavy metal levels in eggplants, cabbage and tomatoes. The findings indicate that although the concentrations of cadmium, lead, and copper are generally constant, the concentrations of chromium and cobalt vary greatly, highlighting the necessity of more research and suitable management techniques to guarantee the safety of food and the health of the environment. While the levels of pesticides in cauliflower samples do not surpass permissible limits except for Chlorpyrifos whose average concentration (0.0634 mg/kg) was exceeding its MRL of (0.01 mg/kg), variations have been noticed regarding imidacloprid, Carbofuran, and profenofos. Imidacloprid is labeled as a potential hotspot for contamination, having the highest mean concentration of 0.2221 mg/kg. Variability also exists in profenofos, particularly at site 1 where it was greatly high. The lower levels of these residues would imply that the herbicides have better adherence to application guidelines. This, therefore, lays the requirement for enhanced policies on the use of pesticides and regional monitoring for reduced variability in consumption and consumer safety. A study by (Hanif et al., 2024) also assessed pesticide residues using HPTLC technique in cauliflower grown in Multan.

The way these metals accumulated was demonstrated by the bio concentration ratios (BCRs) between soil and vegetable components including leaves and curds. Curds had 61.40% of lead from soil concentrations, 56.26% cobalt, 34.15% copper, 76.73% cadmium, and 18.41% chromium. On the other hand, the concentrations of cobalt, copper, cadmium, lead, and chromium in leaves are 60%, 360.22%, 93.94%, and 60.25%, respectively. A study by An-nori et al. (2023) reported high bio concentration factor of heavy metals such as Chromium, Nickel, Copper, and lead from soil to plant tissues. In leaves, cadmium had the lowest Bio concentration ratio. Copper had the highest Bio concentration ratio, the heavy concentration of copper in the leaves of cauliflower (360.22%) correlates with An-nori et al. (2023), who reported a high bio concentration factor for metals like copper in plant tissue. The lower bio concentration factor for cadmium in leaves, relative to its Bio concentration ratio in curds, is also in agreement with An-nori et al. who noted some selective uptake of metals in edible plant tissues. Both reports show that while some metals such as copper would contribute significantly adsorbed in leaves, others like cadmium and cobalt preferentially adsorb in edible plant tissues.

Compared to curds, leaves absorb a substantially higher amount of copper from the soil, indicating a strong absorption of these metals. On the other hand, cadmium and cobalt displayed larger Bio concentration ratios in curds than in other metals, indicating a greater tendency for these metals to concentrate in the cauliflower's edible sections. The results emphasize the need for strict wastewater treatment and monitoring in order to bring down heavy metal concentrations to acceptable levels for humans and ecological concerns. To guarantee the safety of food and the health of the environment, heavy metal levels in soil and plant tissues must be regularly monitored. The study emphasizes how important it is to implement efficient management strategies in order to reduce heavy metal contamination and protect people who depend on polluted water supplies from negative health effects. Similarly, pesticides use regulatory must be employed for the pesticides having long residual effects.

Conclusion

Heavy metals contamination and excessive pesticide use in agricultural systems, particularly in cauliflower, pose significant health concerns. Results showed heavy metals contaminations in cauliflower and soil samples exceeded WHO recommended limits, indicating widespread pollution from industrial activities and untreated wastewater. Wastewater samples also exhibited higher levels of heavy metals, particularly lead and cadmium, which could have harmful effects on human health. Although pesticide residues were within permissible limits except chlorpyrifos, targeted monitoring deserves attention because of fairly high variability in some pesticides as most of the pesticides have long residual effects. Our study highlights the necessity of strict regulations and oversight of pesticide use to reduce health. Public awareness programs about proper pesticide use and clean water irrigation are also necessary. Further research should concentrate on identifying ways to reduce contamination, thereby promoting consumer safety.

Declarations

Ethics and Consent to Participate

This study did not involve human participants, animal subjects, or personal data requiring ethical approval. Therefore, no ethics statement was required. We confirm that the collection of plants complied with all relevant institutional, national, and international guidelines and legislation.

Conflict of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical responsibilities of Authors: Authors must ensure the originality of their work, avoid plagiarism and data fabrication, and accurately cite sources. They are also responsible for obtaining necessary permissions and disclosing any conflicts of interest to maintain research integrity.

Availability of data and material: All the data and material is available in the manuscript file.

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Credit authorship contribution statement

LW, and NM: Experimentation, Data analysis and manuscript writing; MA, and MMS: Data analysis and Reviewing and editing; AM, and MA: Supervision; AR, SMS, and RQS: Visualization; MMS, and MA: Conceptualization and resources, AM, MA and MMS: Analysis, reviewing, revising and editing; All authors have read and approved the final manuscript.

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