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EVALUATION OF HEAVY METALS IN VEGETABLES IRRIGATED WITH SEWAGE WATER: A CASE STUDY OF RADISH AND ZUCCHINI IN KHAN BELA

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Abstract

Toxic contaminants in wastewater pose severe hazards to both the environment and human health, emphasizing the urgent need for efficient and eco-friendly remediation technologies. Among the various treatment methods, photocatalytic degradation stands out as a sustainable and green approach for the removal of organic pollutants, particularly synthetic dyes. In this work, a $CeO_2/Zn_2V_2O_7$ heterostructured nanocatalyst was synthesized using the coprecipitation technique and assessed for its photocatalytic efficiency in degrading methylene blue.The synthesized materials were characterized for their structural, optical, and morphological properties using X-ray diffraction (XRD), UV–Visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM). The $CeO_2/Zn_2V_2O_7$ hybrid catalyst demonstrated significantly improved photocatalytic performance compared to pristine $Zn_2V_2O_7$, with the 50% CeO_2 composition achieving the highest degradation efficiency. This enhancement is attributed to more effective charge carrier separation and superior redox activity, confirming the potential of CeO_2-based heterojunctions as promising materials for advanced wastewater treatment applications.

1. INTRODUCTION

The variety of weather conditions has an impact on freshwater availability in some regions of Pakistan. Most of the country's suburban areas use sewage or dirty water for agriculture to meet their water needs. Every key metal required by plants is present in sewage water in trace amounts[1-2]. Untreated industrial sewage water contains complex organic and inorganic hazardous species, including heavy metals, pharmaceutical chemicals, and other contaminants. The soil is getting polluted due to excessive heavy metal contamination from anthropogenic and natural sources. A plethora of research has recently been conducted on heavy metals because of their toxicity, capacity for bioaccumulation, and persistence in the natural environment. Heavy metals have a specific density of at least 5 g/cm3 and an atomic mass of 20 or more[3-5]. Heavy metals are absorbed by the roots and stored in the edible portions of the plants, causing a decrease in the weight and height of the plants [6-7]. In contrast to inhalation and skin contact, around 90% of the intake of heavy metals occurs through food consumption in humans[8]. In contrast to inhalation and skin contact, around 90% of the intake of heavy metals occurs through food consumption in humans[9]. After ingestion, heavy metals are converted into their oxidized states by the acidic medium of the stomach and then attached with enzymes and proteins to produce free radicals and oxidants to trigger oxidative stress in human

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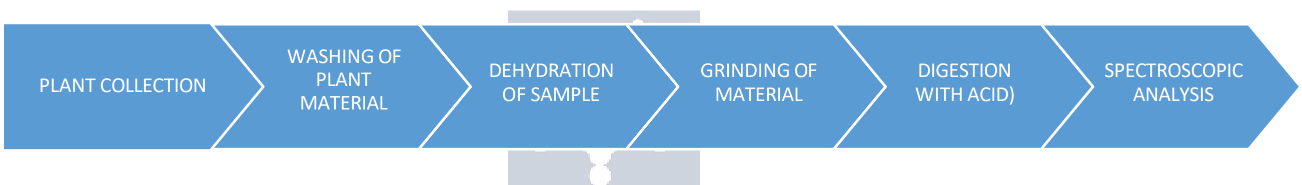
blood, depending on gender and age. The DNA is damaged by oxidative stress, which further contributes to cellular and molecular changes and many complicated disorders. Oxidative stress promotes cancer cells and contributes to numerous neurological and cardiovascular conditions[10]. The bioaccumulation of heavy metals (Cd, Zn, Pb, Cr, Ni, and Cu) has adverse effects on the human body. A heavy metal mainly associated with environmental contamination is cadmium. Symptoms of acute Cd exposure include shortness of breath, nausea, and lung edema.[11]. Zinc deficiency mainly causes low immunity, hair loss, dermal issues, emotional disorders, diarrhea, and weight loss. Zinc deficiency mainly causes low immunity, hair loss, dermal issues, emotional disorders[12], diarrhea, and weight loss[13]. Chromium compounds have a variety of harmful effects on humans. Short-term contact with chromium produces eye and skin damage. The long- term effects of cadmium exposure include joint diseases[14] (osteoarthritis), brittle bone diseases (Itai-Itai), and severe pain irritation, while long-term exposure causes kidney damage, lung cancer, and inflammation. [15-16]. Copper is also very toxic as a result of the production of free radical species, which damage DNA, proteins, and other cellular structures[17]. The focus of our work is to report the heavy metal accumulation in vegetables[18] (radish and zucchini), commonly grown in Khan Bela's pre-urban areas, by irrigation of sewage water, and to study the health effects of heavy metals.

2. MATERIALS AND METHODS

Our research methodology is based on five phases, i.e., collection, washing, dehydration, grinding, and digestion of vegetable samples, as shown in Flow chart 1.



Flow chart 1. Overall research methodology

2.1. Collection of plants

Two vegetable samples, Radish (*Raphanus sativus* var.) and Zucchini (*Cucurbita pepo*), were collected from several sites to examine the heavy metal accumulation in consumable parts of the plants. The site was also geo tagged with the help of a SpotLense camera. The plants were irrigated with either freshwater or sewage water, depending on the location. A total of five radish samples were taken, while six zucchini samples were also taken.

Radish samples preparation: Five radish (*Raphanus sativus* var) root samples were collected from five locations in Khan Bela: Basti Machii, Basti Shareef, Sultania Colony, Ghanda Nalla, and Basti Machiya. One sample was obtained from each site. The fields in these areas have been irrigated with sewage water for the past 50 years. During the early stages of crop cultivation, diammonium phosphate (DAP) and urea were applied as the main fertilizers. Urea was used to promote growth, while DAP supplied phosphate for plant development. DAP was applied at sowing, and each acre of land received half a sack of urea fertilizer. The pesticides Match® (Syngenta) and Coragen® (FMC Corporation) were also used during cultivation. Sewage water irrigation was applied at intervals of 8–10 days. The labels for sewage water samples are provided in Table 1.

Table 1. Labelling of samples, locations, geotag location and part of the plant used.

Sr. No.	Plant Name	Location	Altitude, Latitude	Part used
1	Radish	Basti machii	28.9486, 70.71767	Roots
2	Radish	Basti shareef	28.95839, 70.7448	Roots
3	Radish	Sultania Colony	28.9597, 70.74207	Roots
4	Radish	Ghanda nalla.	28.96296,70.74285	Roots
5	Radish	Basti machiya.	28.94848, 70.7178	Roots

Zucchini samples preparation: Six zucchini (*Cucurbita pepo*) samples, including fruit, net, and seed, were collected from four sites in Khan Bela: Zafar Abad, Jalilabad, Basti Balian, and Ghanda Khoooh. These fields have been continuously cultivated with vegetables for the past 50 years. Diammonium phosphate (DAP) and urea were applied as fertilizers, with two sacks of urea per acre added after crop establishment and DAP applied at planting. Imidacloprid (Syngenta®), Match® (Syngenta), and Coragen® (FMC Corporation) were used as pesticides and insecticides, applied five days after planting. Irrigation was supplied every eight days throughout the growing season. The labels for the collected samples are provided in Table 2.

Table 2. Labelling of samples, different locations, and part of the plant used.

Sr. No.	Plant Name	Location	Altitude, Latitude	Part used
1	Zucchini	Asif Naghar	28.95844, 70.74486	Fruits
2	Zucchini	Zafar Abad	28.89757, 70.66859	Net
3	Zucchini	Zafar Abad	28.89757, 70.66859	Seed
4	Zucchini	Jalilabad	28.94406, 70.73323	Fruits
5	Zucchini	Basti Balian	28.94849, 70.71784	Fruit
6	Zucchini	Ghanda Khoooh	28.95519, 70.74152	Fruit

2.2. Washing

The collected plant samples were thoroughly washed with tap water to remove dirt and residual insecticides or pesticides from the surface. The samples were then air-dried, rinsed with distilled water for maximal pollutant clearance, and air-dried again. Radish leaves were removed with a knife, and the roots were cut into small slices. For zucchini, the external skin was peeled off with a knife, and the flesh was sliced. The skin of radish roots was removed using a radish shredder and then further reduced into smaller slices with a slicer. All samples were stored separately and labeled with specific codes (1, 2, 3, 4, etc.).

2.3. Dehydration

For dehydration, the samples were first placed in sunlight for three days and then dried using a Memmert oven (Figure 1a). The plant material was spread on flat trays covered with aluminum foil and heated at 100
°C for 10 hours. The flap rate was set at 70%, which represents the ratio of evaporated gas remaining in the chamber to the total gas inside the chamber. After dehydration, the samples were completely dried, with radish slices turning dark brown and zucchini slices turning black.

Figure 1. Plant sample (a) Drying in Memmert oven (b) Grinding (c) Weighting

1.1. Grinding

The dried samples were ground into a fine powder using an ST grinder (Figure 1b). The powdered material was then passed through gauze to obtain a uniform fine fraction, which was weighed (1 g each; Figure 1c) and stored in polythene bags for further analysis. Fine powder was preferred because it is easier to digest and filter during subsequent processing. Each bag was labeled with a specific code using a permanent marker.

1.2. Digestion of samples

Two types of digestion methods are commonly used: dry digestion and wet digestion. Dry digestion, also called dry ashing, is carried out without the use of a digestion aid, and the final product is obtained in solid form. In contrast, wet digestion, or acid digestion, involves the use of a digestion aid, and the resulting product is in aqueous form. In this study, the wet digestion method was employed.

Digestion of radish sample: For digestion, 1.00 g of dried radish sample was placed in a conical flask. In a separate beaker, 13 mL of HNO_3, 3 mL of H_2SO_4, and 3 mL of $HClO_4$ were mixed, and the acid mixture was then poured onto the sample. The flask was heated on a spirit lamp at 160 °C for 3 hours. Brown fumes appeared within 5 minutes and continued for approximately 35 minutes, after which the solution became colorless and emitted smoke. After 5 minutes, the solution turned yellowish for about 15 minutes, accompanied by white fumes. No further reaction occurred for the next 2 hours. At that stage, 5 mL of additional HNO_3 was added, which caused the yellow color to disappear, indicating completion of the reaction. Heating was stopped when ~3 mL of the solution remained. The residue was diluted with 25 mL of distilled water and filtered through Whatman No. 42 filter paper. The filtrate volume was adjusted to 50 mL using distilled water and stored in a sealed, labeled plastic bottle. All samples were washed with deionized and distilled water before sealing. This procedure was repeated for each radish sample, and the prepared solutions were sent to the University of Agriculture, Faisalabad, for atomic absorption spectroscopy (AAS) analysis of heavy metal concentrations.

Digestion of for zucchini sample: For digestion, 1.00 g of dried zucchini sample was placed in a conical flask. In a separate beaker, 39 mL of HNO_3, 9 mL of H_2SO_4, and 9 mL of $HClO_4$ were mixed to prepare the acid solution. A volume of 19 mL of this acid mixture was added to the zucchini sample, and the flask was heated at 160 °C. After 4 minutes of heating, the solution turned pink, then changed to yellow after 4 minutes, dark brown after another 4 minutes, and black with white fumes after 4 more minutes. After 1 additional minute, the solution became colorless. At this stage, 3 mL of the acid mixture was added, and heating was continued; no further change was observed, indicating completion of the reaction. The mixture was filtered through Whatman No. 42 filter paper, and the filtrate was diluted to a final volume of 50 mL with distilled water. The solutions were stored in plastic bottles previously washed with deionized and distilled water, sealed with tape, and labeled with permanent markers. The complete digestion procedure required approximately 2.5 hours at 160 °C. All zucchini samples were digested using this method and sent to the University of Agriculture, Faisalabad, for heavy metal analysis by atomic absorption spectroscopy (AAS).

2. RESULTS AND DISCUSSION

Sample analysis was performed at the Central Hi-Tech Laboratory, University of Agriculture Faisalabad (UAF), using a Hitachi Polarized Zeeman Atomic Absorption Spectrometer. Atomic absorption spectroscopy (AAS) was employed to determine the concentrations of heavy metals in the digested samples. Specific wavelengths selected for the detection of individual heavy metals, are summarized in Table 3. Operating parameters of atomic absorption spectrometer are described in Table 4 and operational conditions for heavy metal analysis are mentioned in Table 5.

Table 3. Wavelengths used for analyzing heavy metals.

Radicle name	Cd	Zn	Pb	Cr	Ni	Cu
Wavelength (nm)	228.8	213.9	283.3	339.3	232.0	324.8

Table 4. Operating parameters of atomic absorption spectroscopy.

Sr no.	Parameters	Operating parameters
1	Machine	Hitachi Polarized Zeeman AAS
2	Model no.	Z-8200
3	Brand	Hitachi
4	Manufacturer	Japan
5	Year of introduction	2020

6	Gas used	Acetylene (Air-C_2H_2)

The concentration of different heavy metals (Cd, Zn, Pb, Cr, Ni, and Cu) were analyzed in both radish and zucchini samples using an atomic absorption spectrophotometer.

Standards Preparation: Calibrated standards were prepared from a commercially available stock solution (AppliChem®,1000 ppm, aqueous). For the preparation of working standards, highly purified, deionized water was used. Prior to use, all glass apparatus used in the analytical work was immersed in 8N HNO_3 overnight and rinsed several changes with de-ionized water to prevent contamination. The concentrations reported in the laboratory test report represent instrument readings; the actual concentrations were calculated using the following formula.

Actual concentration of heavy metal in the sample = Result of AAS × Final Dilution

Final concentrations were calculated by multiplying the instrument reading by the dilution factor and expressed in mg/kg, following FAO/WHO standards.

Table 5. Operational conditions applied in the determination of heavy metals by AAS.

HM	Wave length (nm)	Slit Width (nm)	Lamp Current (mA)	Burner Heights (mm)	Oxidant gas pressure Flow rate (Kpa)	Fuel gas Pressure Flow rate (KPa)
Cd	228.8	1.3	7.5	5.0	160	**6**
Cu	324.8	1.3	7.5	7.5	160	**7**
Cr	359.3	1.3	7.5	7.5	160	**12**
Pb	283.3	1.3	7.5	7.5	160	**7**
Ni	232.0	0.2	10.0	7.5	160	**7**
Zn	**213.9**	**1.3**	**10.0**	**7.5**	**160**	**6**

During this process, the burner head was standard type, and the flame was air (C_2H_2) in all cases.

Table 6. Concentration of different heavy metals in vegetable samples (in mg/kg)

Sample	Location	Cd	Zn	Pb	Cr	Ni	Cu
1	Basti machii	2	32	3	5	6	49.5
2	Basti shareef	0.5	16	0	7.5	6	26.5
3	Sultania Colony	2.5	20.5	0	14	2	18.5
4	Ghanda nalla.	0.5	30.6	1.0	6.1	4.	16.8
5	Basti machiya.	0	17.5	3	10	3	28.5
6	Asif Naghar	1	41	3	4.5	3.5	31.5
7	Zafar Abad	0.5	30.2	5.5	5.5	2.2	37.9
8	Zafar Abad	0.5	33.5	3.5	3	3	16
9	Basti Jalilabad	1.5	45.2	0	3.6	6.2	23.4
10	Basti Balian	0.5	40.7	1.6	4.9	2.7	24.2
11	Ghanda Khoooh	0.5	30	5	5.5	5.5	28

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Concentration of Cadmium: According to FAO/WHO guidelines, the permissible limit of cadmium (Cd) in vegetables is 0.2 mg/kg. In this study, all samples except sample 5 exceeded this concentration. Sample 5, collected from Basti Machiya—a rural village without a sewerage system—contained Cd within the permissible range. In contrast, sample 3 showed the highest Cd accumulation, most likely due to irrigation with untreated sewage water and elevated background Cd levels in the soil of Khan Bela. The distribution of Cd concentrations is illustrated in Figure 2.

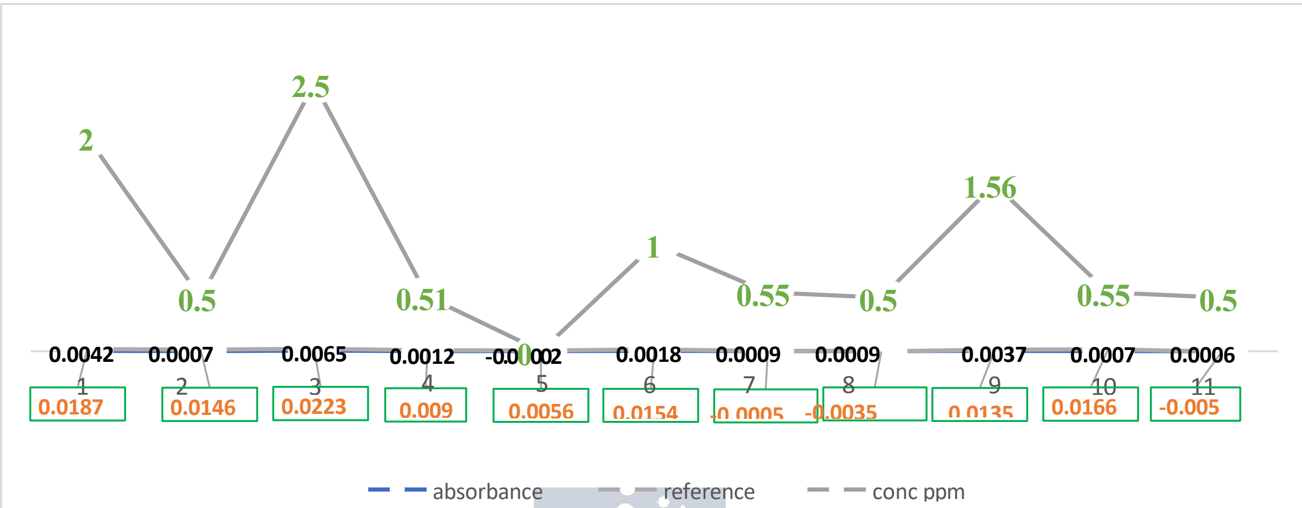

Figure 2. Concentration of Cd in samples

Concentration of Zinc: The FAO/WHO permissible limit for zinc (Zn) in vegetables is 99.4 ppm. None of the analyzed samples exceeded this threshold, indicating that Zn contamination is not a major concern for vegetables cultivated in Khan Bela. The relatively safe levels of Zn across all samples are clearly demonstrated in Figure 3.

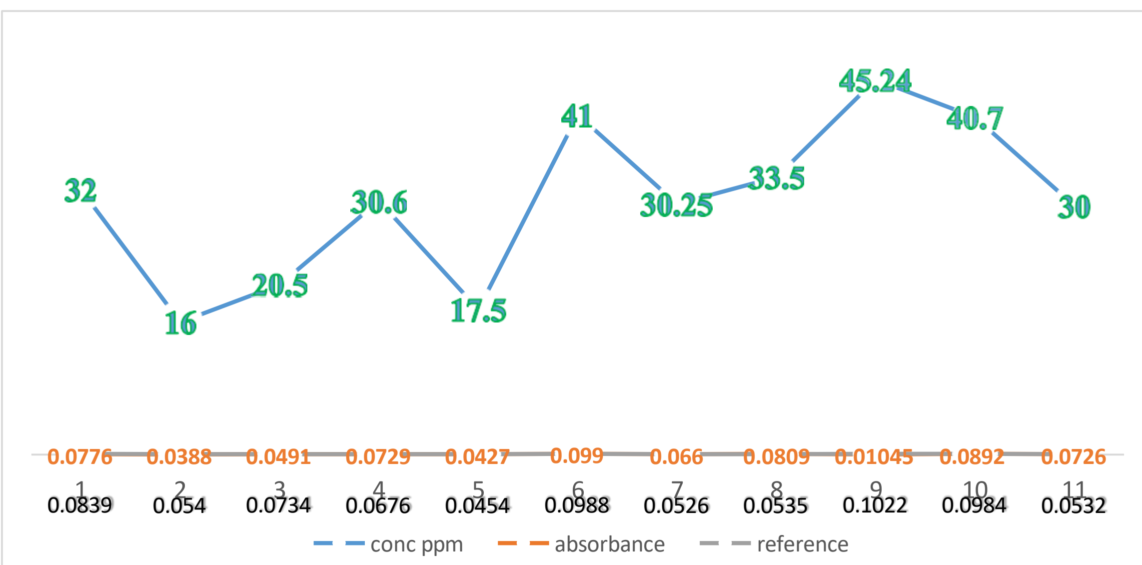

Figure 3. Concentration Zn in samples

Lead concentration: Lead (Pb) is widely recognized for its carcinogenic potential. In this study, samples 2, 3, and 9 were free of detectable Pb, while all remaining samples surpassed the WHO permissible limit of 0.3 ppm. Sample 7, obtained from ripened zucchini, contained the highest Pb concentration. The graphical representation (Figure: 4) highlights the variations in Pb levels among different samples.

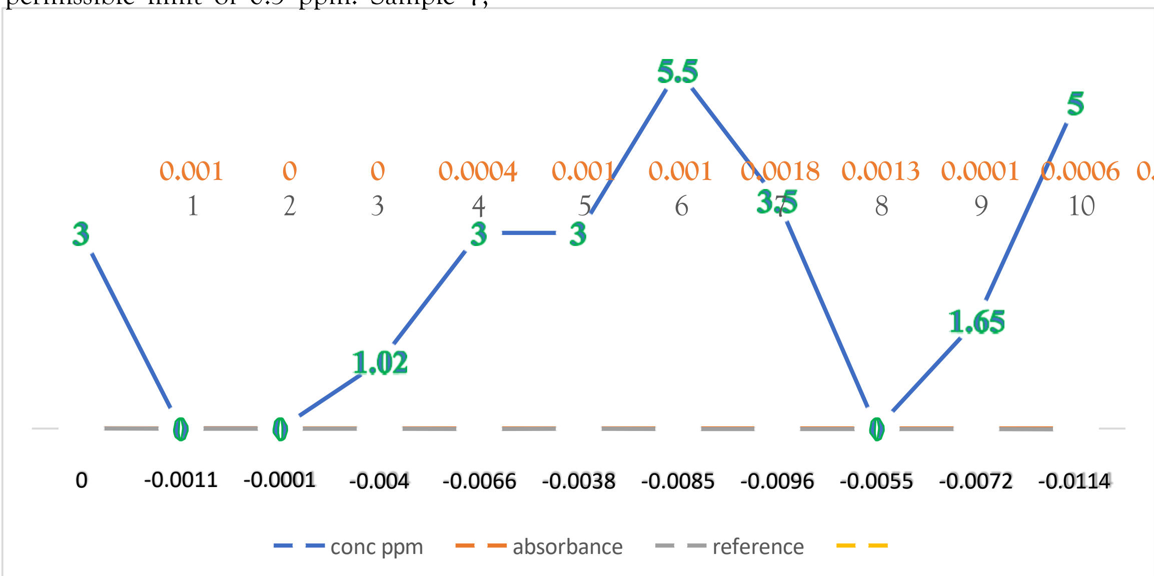

Figure 4. Concentration of Pb in samples

Chromium concentration: The WHO standard for chromium (Cr) in vegetables is 0.13 mg/kg. All eleven samples analyzed in this study were above this limit. The elevated Cr concentrations may reflect both naturally enriched soils in Khan Bela and anthropogenic inputs from local activities such as leather tanning. The extent of Cr contamination is further emphasized in Figure 5.

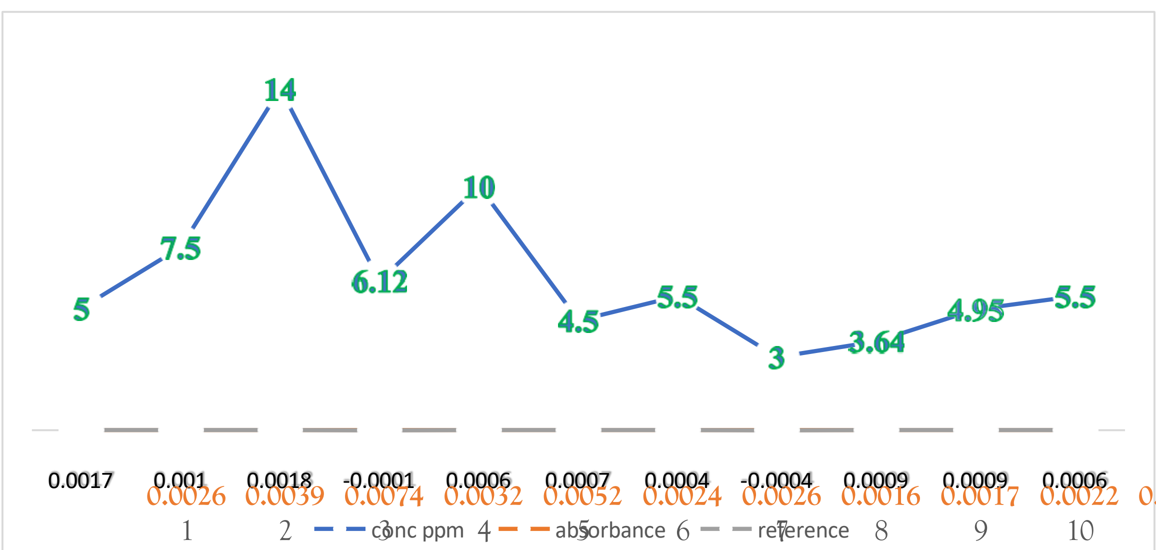

Figure 5. Concentration of Cr in samples

Nickel Concentration: According to WHO guidelines, the maximum permissible concentration of nickel (Ni) in vegetables is 67.9 ppm. In the present study, all analyzed samples were within this limit, indicating that Ni contamination is not a major concern for the vegetables of Khan Bela. The safe range of Ni concentrations is clearly presented in Figure 6.

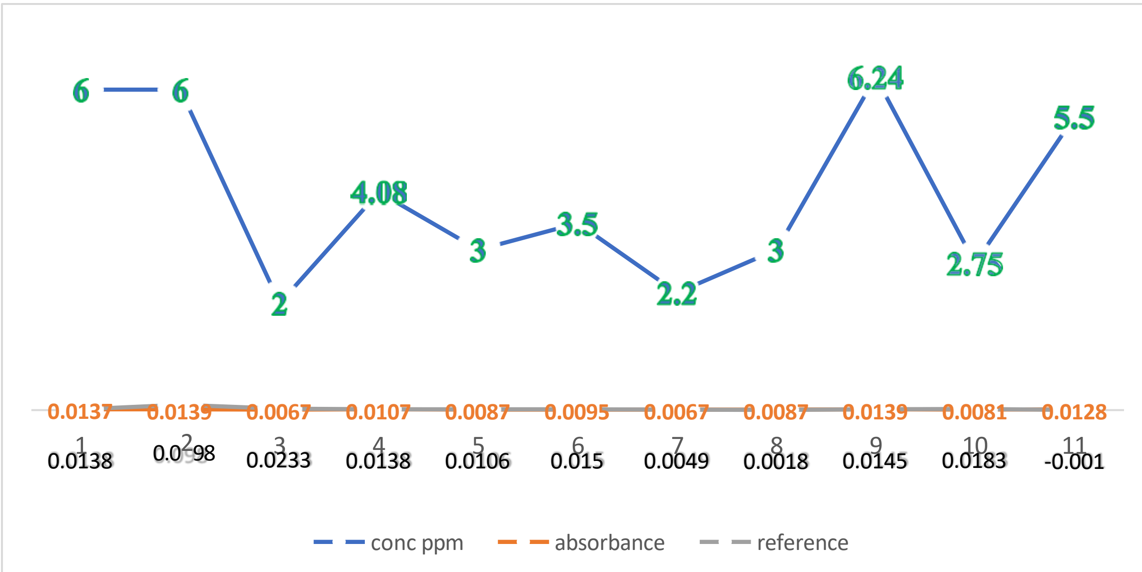

Figure 6. Concentration Ni in samples

Copper concentration: According to World Health Organization (WHO) standards, the permissible limit for copper (Cu) in vegetables is 73.3 ppm. All analyzed samples were within this limit, indicating that Cu levels in Khan Bela vegetables are acceptable. The concentrations of Cu across samples are depicted in Figure 7.

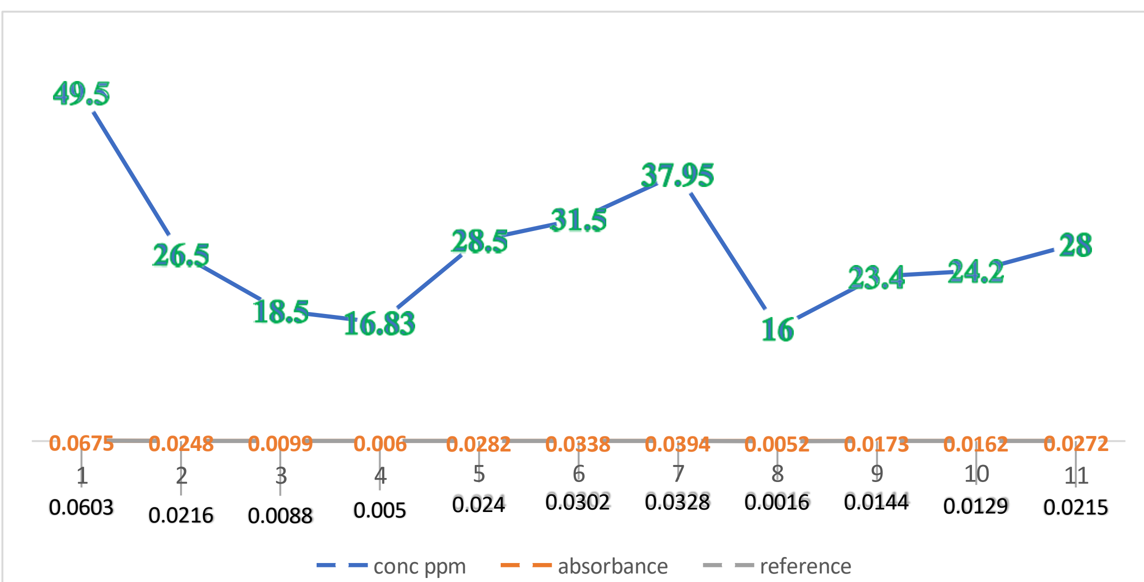

Figure 7. Concentration of Cu in samples.

3. CONCLUSION

Heavy metals in vegetables irrigated with sewage water are a major global concern due to their adverse effects on human health and the environment[19]. Both natural processes and anthropogenic activities contribute to the accumulation of these metals in soil and water. The wet digestion method proved effective for extracting and analyzing heavy metals from plant samples. A comparison of radish and zucchini irrigated with sewage water revealed that heavy metals accumulate not only in roots but also in the edible portions of vegetables. Elevated concentrations were observed due to the direct discharge of untreated industrial wastewater, which enriches soils with heavy metals. Furthermore, soil composition strongly influences the extent of metal uptake in vegetables. Farmers often prefer sewage water for irrigation because of limited freshwater availability and the reduced need for chemical fertilizers[20]. However, the results of this study demonstrate that long-term use of sewage water leads to heavy metal accumulation in both edible and non-edible plant tissues, posing serious health risks to consumers. These findings underscore the urgent need for regular monitoring of heavy metals, awareness programs for farmers, and the implementation of wastewater treatment practices to minimize risks to food safety and public health.

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