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Zeolite-Assisted Immobilization and Health Risks of Potentially Toxic Elements in Wastewater-Irrigated Soil under Brinjal (*Solanum melongena*) Cultivation

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Abstract: Application of wastewater to agricultural soils not only enhances economic benefits but is also considered as a safe disposal option by the administrators. Worldwide, peri-urban horticulture is a common practice for growing vegetables. When agricultural soils are irrigated with wastewater, numerous potentially toxic elements (PTEs) contained therein are bioaccumulated and pose health risks. The presented study aimed to reveal the PTEs, i.e., copper (Cu), cadmium (Cd), nickel (Ni) and lead (Pb) concentration in the agricultural soils irrigated with wastewater for longer times. Zeolite, a natural mineral was used to immobilize these in contaminated soils to reduce its availability to brinjal (*Solanum melongena* L.). During a pot study, zeolite was applied at four different levels, i.e., 0.25, 0.50, 1.00 and 2.00% in contaminated soil, keeping one control. The results revealed that growth as well as biochemical and physiological characters were found best with treatment receiving zeolite at 2.00%. In edible parts (fruit), PTE contents were found lowest in the same treatment. Relative to the control, ~121, 87, 120 and 140% less DTPA-extracted Cu, Cd, Ni and Pb in soil was found with this treatment. Based on the results, it was revealed that zeolite effectively immobilized Cu, Cd, Ni and Pb in the soil. Although all the applied levels of zeolite had positive potential to immobilize PTEs in wastewater-contaminated soil, zeolite applied at 2.00% proved most effective.

Keywords: peri-urban horticulture; irrigation water; health risks; metals remediation; toxic elements

1. Introduction

Globally, 70% of the wastewater generated is used for irrigation to agricultural crops and contains considerably higher amounts of pollutants including potentially toxic metals (PTEs) [1]. Contaminant is any unwanted material which causes unfavorable effects on quality of soil, air, and water [2,3]. The PTEs have various dangerous functions in both human and plant growth and functionality, but they can be labeled as contaminant when their concentrations are increased above a threshold point [4,5]. The industrial segment creates a lot of wastewater, which is ultimately released into fresh and irrigation water channels [6–8]. As there is no proper framework for the treatment of these effluents, farmers utilize wastewater for irrigation of agricultural fields, especially in peri-urban areas for vegetables production. An advantage of using wastewater is that the wastewater is full of many nutrients which promote plant growth and yield. However, in combination with these nutrients, wastewater also contains PTEs and other organic contaminants. These contaminants pose serious threats to plant and human life when accumulated in excess [1,9].

Wastewaters from textile industries that contain high level of PTEs must be detoxified/immobilized before their adoption in agricultural fields for irrigation, to avoid their accumulation in the soils [10,11]. Due to their human and plant health disruptions, i.e., gastrointestinal aggravation, and liver and kidney diseases [12,13], root damage, and reduced plant growth and yield, wastewater containing excessive PTEs should be considered for treatment. Numerous strategies, i.e., oxidation–reduction, flocculation, electro-coagulation, and adsorption, as well as treatment via constructed wetlands, reverse osmosis, and use of natural and artificial zeolites are used for the treatment of wastewater [14,15].

In comparisons with the other remediation technologies, zeolite might be more appropriate because of its quality to alter pH estimation of soil as it is hydrated aluminosilicate mineral, with permeable structure, showing profitable physicochemical properties such as cation exchanger atomic sieving, catalysis, and sorption [16]. Among various zeolite species, clinoptilolite is the best exchanger, having an expanded cation trade limit and security in acidic situations [17–19]. Their structure comprises a system of $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ tetrahedron connected to each other's corners by sharing oxygen particles. The substitution of silicon (Si^{4+}) by aluminum (Al^{3+}) in tetrahedral destinations brings about more negative charges and a high cation trade limit [19]. Zeolites, as common cation exchangers, are reasonable substitutes to expel dangerous cations [20,21]. As such, clinoptilolite is by all accounts the most effective particle exchanger and particle specific material [22,23] for expelling and balancing out overwhelming PTEs [24,25].

Brinjal (*Solanum melongena* L.), also known as eggplant, is a common vegetable food crop and widely used around the world. It is rich in folic acid, which is an integral part of the red blood cells synthesis. It is also used to combat anemia, a disease of blood deficiency and cancer. It supplies enough folic acid to pregnant women if daily taken. It is also low in sodium and rich in anti-oxidants, vitamins, iron, magnesium, calcium, potassium, and zinc [26,27]. Brinjal is commonly cultivated in peri-urban areas which are majorly irrigated with industrial effluents containing numerous toxic elements. These toxic elements can translocate and accumulate in the edible parts of the crops and can cause human health risks [5]; in addition, PTEs can interfere with the plant growth, biochemistry and yield by leaf chlorosis, stunted growth, and resulted in limited uptake of essential nutrients and protein synthesis [28,29]. So far, no scientific study has been reported to assess the metal-associated human health risks through metal-contaminated brinjal consumption. This study hypothesized that the application of zeolite in agricultural soils could be a suitable solution to mitigate the transfer of toxic element in vegetables and, therefore, reduce the risk associated with human health. The main objectives of this research work were to assess capability of clinoptilolite for metal remediation/ immobilization in metal-contaminated wastewater-irrigated soils in brinjal vegetable crop and associated health risks after consumption of metal-contaminated brinjal.

2. Materials and Methods

2.1. Collection of Soils and Amendment

Wastewater-irrigated PTEs-contaminated soil was collected from the Uchkera village, Faisalabad (31.2732 N, 73.020 E) (a site where a wastewater treatment facility is present for primary treatment of wastewater and has continuously irrigated the soil of the area with industrial wastewater for more than four decades). The soil samples were collected from the upper layer (0–15 cm). Collected samples were air-dried and sieved from 2 mm sieve, and then physico-chemical properties and elemental composition were determined using standard procedures before being filled in glazed pots (Table 1). Only a single artificial zeolite type was bought from scientific store, Faisalabad, Pakistan.

Table 1. Pre-experiment soil characteristics.

Properties	Unit	Value	Reference
pH_s	-	7.8	[30]
EC_e	dS m^{-1}	1.82	[31]
TSS	$mmol_c\ L^{-1}$	18.2	[30]
SAR	$(mmol\ L^{-1})^{1/2}$	9.13	
$Ca^{2+} + Mg^{2+}$	$mmol_c\ L^{-1}$	45	
Na^{+}	$mmol_c\ L^{-1}$	13.7	
$CO_3{}^{2-}$	$mmol_c\ L^{-1}$	Absent	
$HCO_3{}^{-}$	$mmol_c\ L^{-1}$	40	
Cl^{-}	$mmol_c\ L^{-1}$	75	
Texture	-	Sandy loam	[32]
OM	%	0.77	[33]
Cu	mg kg^{-1}	86	[34]
Cd	mg kg^{-1}	2.27	
Ni	mg kg^{-1}	2.32	
Pb	mg kg^{-1}	2.85	

2.2. Treatment Plan and Experimental Set-Up

After pre-experimental analysis, the PTEs-contaminated soil was mixed with zeolite (Z) at four different rates (0.25%, 0.5%, 1.0% and 2.0% w/w) and obtain five treatments. These treatments were control (Z-0%), Z-0.25%, Z-0.5%, Z-1.0% and Z-2.0% with three replicates of each treatment. The ceramics pots having 10 kg capacity were filled with the treated soil according treatment plan and were transferred with extreme care to the wire-house (having light (8–10 h), humidity (40–50%) and temperature (37 °C)). Afterwards, these pots were irrigated to attain field capacity for seed sowing. The seeds of brinjal variety "Shamli", popular for its long-fruit-shape trait, were purchased from a registered seed store named "Siddiq seed store". Prior to sowing, the seeds were placed on a moist filter paper for 8 h. Later, four seeds of brinjal were sown in each pot. After two weeks of plant emergence, the plants were thinned and maintain one plant per pot. The plants were fertilized with recommended doses of NPK nutrients at the rate of 50, 60 and 50 mg kg^{-1}, respectively, and other agronomic practices such as weeding, irrigation and plant protection measures were also applied in all experimental units. Brinjal plants were allowed to grow for a period of 100 days.

2.3. Data Collection

2.3.1. Plant Analysis

Chlorophyll contents (SPAD value) was taken after 40 days of sprouting of seedling in the morning time of three fully expanded leaves using SPAD-502 m. Gaseous exchange parameters (photosynthetic rate and stomatal conductance) of three healthy fully expended leaves per replication were determined via portable narrow chambered infrared gas analyzer (IRGA, LCA-4, Hoddesdon, UK), and the average of three was reported. The brinjal plants were harvested on the 100th day of growth by carefully clipping the plants immediate above the surface of soil using a sharp scissor and segregated into shoot, root, and fruit. The shoot, root, and fruit of brinjal were thoroughly washed with tap water for removing the adhesive dust and to wash away adhered PTEs in the free spaces of the root and length of root and shoot were recorded. The fresh biomass (shoot, root, and fruit) of brinjal was recorded and then dried (at 70 ± 10 °C, for 24 h) in an oven until the constant dry weight was obtained.

2.3.2. Enzymes Activity

The enzyme was extracted by macerating fresh fruit pulp in pre-cooled 0.2 *M* sodium phosphate buffer (pH 7.0). The extract was centrifuged at 5000 rpm for 15 min [26]. The supernatant was used for analysis of enzyme activities. Peroxidase (POD) enzyme activity was measured using guaiacol as substrate at 470 nm. A change of 0.01 unit per minute in absorbance was considered equal to one unit POD activity. Superoxide dismutase (SOD) activity was assayed according to [35]. One unit of SOD activity was measured as the amount of enzyme required to cause 50% inhibition of the nitroblue tetrazolium (NBT) reduction measured at 560 nm. Ascorbate peroxidase (APX) activity was determined in the presence of 2.0 mmol L^{-1} ascorbic acid and 2.0 mmol L^{-1} EDTA by measuring the decrease in absorbance at 290 nm [36]. Catalase (CAT) enzyme activity was assayed by following the decomposition of H_2O_2 at 240 nm with a UV spectrophotometer [37].

2.3.3. Plant PTE Contents

Dried plant samples were ground to a fine powder passed through a sieve (0.5 mm mesh size) and digested in a di-acid mixture (HNO_3: $HClO_4$, 2:1) as recommended by [38]. Digested material was filtered, and 50 mL final volume was made by adding distilled water and Cu, Cd, Ni and Pb was assessed via Atomic Absorption spectrophotometer (AAS) (Solaar S-100, CiSA, Thermo Fisher Scientific, Cramlington, UK) equipped with respective cathode lamp.

2.3.4. Soil Analysis

After harvesting of plants, the samples of soil were collected from each experimental pot with the help of soil sampler. Chemical analysis of these soil samples was performed by using protocols given in [30]. The ECe and pHs were determined using EC meter and pH meter, HCO_3^-, CO_3^{2-}, Cl^-, Ca^{2+} + Mg^{2+} were determined from the saturated soil paste extract. Ammonium bicarbonate-diethylene tri-amine penta acetic acid (AB-DTPA)-extractable concentrations were used as an availability index of the elements in our soils. The available Cu contents in the soils was extracted with 1 M ammonium bicarbonate (NH_4HCO_3) + 0.005 M diethylene tri-amine penta acetic acid (DTPA) solution according to [39], using AAS.

2.3.5. Human Health Risk Assessment

The normal body weight of children and adult (male and female) were considered for human risk assessment. Since children present higher daily intake values than the older population since ingested PTEs are distributed in a lower body mass [40]. Values of average daily intake (ADI) were calculated based on the trace element contents in vegetables, body weight, and consumption habits [41], by using Equation (1). All the parameters used in the study with their values are elaborated in Table 2.

$$\mathrm{ADI} = \frac{\mathrm{ED} \times \mathrm{C} \times \mathrm{IR} \times \mathrm{EF}}{\mathrm{AT} \times \mathrm{BW}} \quad (1)$$

Concentrations of PTEs in soil and vegetable samples from exposed and control were analyzed for the enrichment factor (EF). Enrichment factor for TEs was calculated using the following equation:

$$\mathrm{EF} = \frac{\mathrm{T_a}}{\mathrm{T_c}} \quad (2)$$

where T_a and T_c are the concentrations of PTEs (mg kg^{-1}) in exposure areas and in the control area, respectively.

Table 2. Description of parameters and values used in the study.

Factor	Description	Unit	Male	Female	Child
C_{plant}	Heavy metal in plant	mg kg^{-1}	-	-	-
EF	Exposure frequency	day year^{-1}	350	350	350
ED	Exposure duration	year	30	30	6
BW	Body weight of the exposed individual	kg	66	59	18.6
AT	Averaged time	days	365ED	365ED	365ED
IR	Vegetable intake	kg day^{-1}	0.260	0.260	0.130

Average body weight for the age group of 1–6 years children is 18.60 kg [42]. The mean weight of age group 30 y men was 66 kg while it was 59 kg in women. Mean height of men and women was 165.8 cm and 153.9 cm, respectively [43]. Average daily vegetable intakes taken for children and adults (male and female) were 130 and 260 g person^{-1} day^{-1}, respectively, as reported in the literature [44]. The oral reference dose value for Cu, Cd, Ni and Pb at 0.04, 0.001, 0.02 and 0.004 (mg kg^{-1} day^{-1}) was used, respectively [41].

2.3.6. Translocation Factor

The Cd translocation factors (TF) were calculated using the following equation [45]. As:

$$TF = \frac{C_s}{C_r} \quad (3)$$

where C_s and C_r are the metal concentrations (µg g^{-1}) in shoot and roots, respectively. Here, a TF > 1 indicates that the plant translocated Cd effectively from root to shoot.

2.3.7. Remediation Factor

The remediation factor (RF) of Cd was calculated using the following equation [45]:

$$RF\ (\%) = \frac{C_s \times SDW}{C_s \times W_s} \times 100 \quad (4)$$

where C_s is metal concentration in shoot, SDW is shoot dry weight (g), and W_s is weight of soil (kg) in pots.

2.4. Statistical Analysis

The data collected was subjected to summary statistics using Minitab v17.1.0., and treatments were compared using the least significant difference (LSD) test at $p \leq 0.05$. All the illustrations were generated using Origin 2019b v9.65.

3. Results

3.1. Physiological Parameters

Maximum chlorophyll contents were recorded with Z-2.0% (46.03 SPAD value) followed by Z-0.50% (Figure 1). Maximum stomatal conductance Z-2.0% (+39%) followed Z-0.50% (+33%), while photosynthetic rate was found maximum in Z-1.0% (+298) compared to the control (Figure 1). Applied zeolite significantly increase plant stomatal conductance and photosynthetic rate. Maximum values for stomatal conductance and photosynthetic rate were recorded with Z-2.0% (Figure 1).

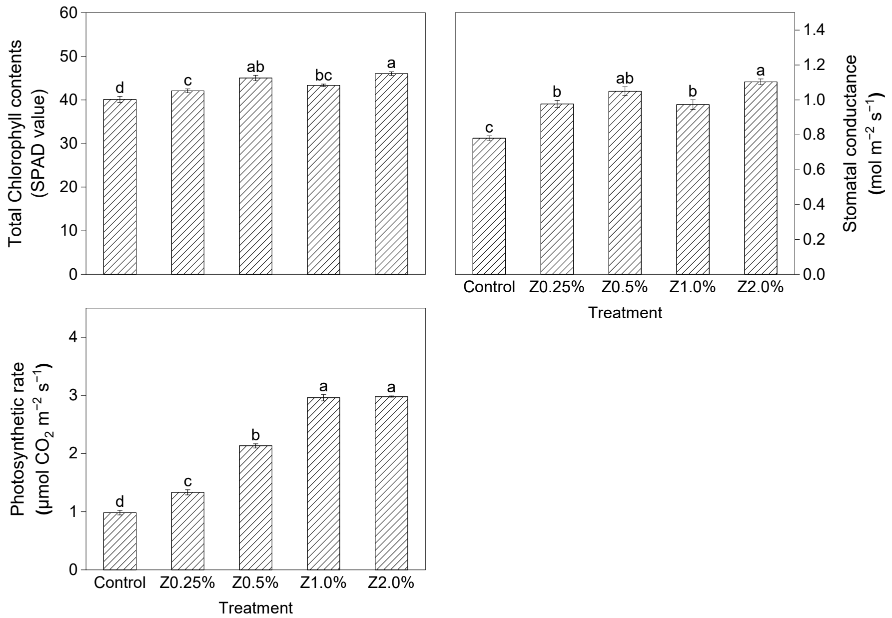

Figure 1. Effect of zeolite on plant physiological parameters (total chlorophyll contents, stomatal conductance, and photosynthetic rate). Different letters and error bars on the top of column represent significant difference $p < 0.05$ and standard error (SE), respectively.

3.2. Fruit Enzymatic Activity

Zeolite treatment significantly increases the SOD, POD, CAT and APX activity in fruit pulp (Figure 2). Maximum antioxidant enzyme (SOD) activity was observed in Z-2.0%, and it was about 47% higher with respect to the control. Similarly, a 52%, 34%, and 16% increase in POD, CAT and APX activity was observed, respectively, with the highest tested dose (Z-2.0%) in comparison to the control.

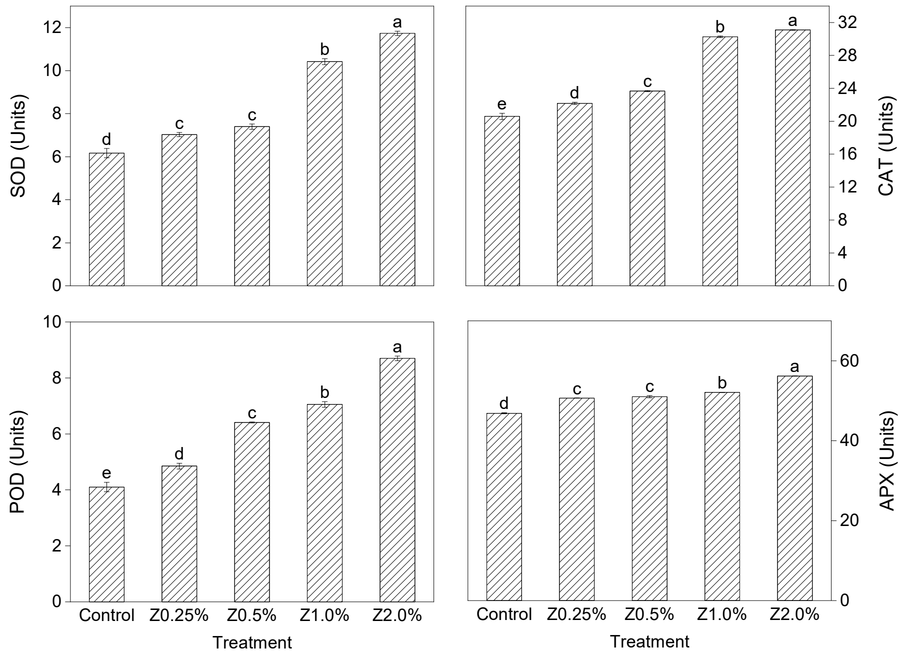

Figure 2. Effect of zeolite on brinjal fruit enzymatic activity. The SOD (superoxide dismutase), POD (peroxidase), CAT (catalase) and APX (ascorbate peroxidase). Different letters and error bars on the top of column represent significant difference $p < 0.05$ and standard error (SE), respectively.

3.3. Growth Parameters of Plants

The responses of plant growth characteristics, i.e., shoot and root length, and fresh and dry weight of shoot and root were recorded in the ranges 41.03–54.33 and 15.33–22.80 cm, 94.77–133.00 and 23.60–29.17, and 32.53–35.30 and 12.73–17.80 g pot^{-1}, respectively (Figure 3). These characteristics were significantly improved by the application of zeolite at different rates to wastewater-irrigated soil. Maximum growth improvement was observed by the application of 2% zeolite (Z-2.0%). The same treatment also increased shoot and root length significantly by 32.41 and 48.70%, respectively, as compared to the control. Similarly, the highest shoot fresh weight (40%) and shoot dry weight (23%) were observed with Z-2.0%, as compared to the control. However, the difference was statistically non-significant when compared with other treatments (Z-1.00%). The highest root fresh (35.30 g pot^{-1}) and dry (17.80 g pot^{-1}) weights were also observed with Z-2.0%. Regarding brinjal fruit weight, the maximum fruit fresh and dry weights were observed in same treatment with 19 and 16% improvement relative to the control, respectively (Figure 3).

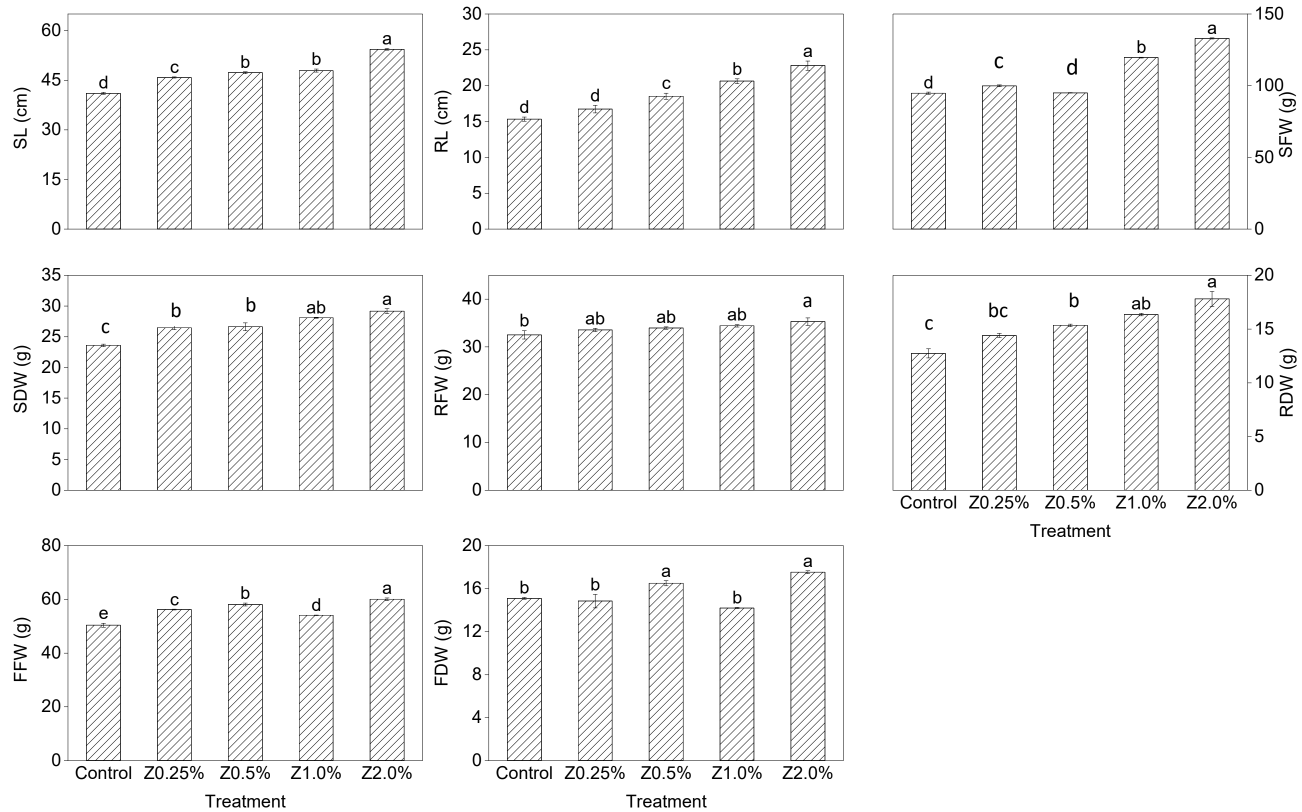

Figure 3. Effect of zeolite on plant growth parameters. Shoot length (SL), root length (RL), root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (SFW), shoot dry weight (SDW), fruit fresh weight (FFW), fruit dry weighs (FDW). Different letters and error bars on the top of column represent significant difference $p < 0.05$ and standard error (SE), respectively.

3.4. Post-Harvest Soil Characteristics

Application of zeolite to metal contaminated wastewater-irrigated soil significantly affected the soil characteristics, i.e., pHs, EC_e, HCO^{3-}, Cl^- Ca^{2+} + Mg^{2+} (Table 3). Post-harvest soil analysis revealed that pHs increased with zeolite treatment, especially in Z-2.0%, and soil pHs increased 5.72% as compared to the control treatment. The ECe values of the samples recorded were in the range of 1.1–1.7 dS m^{-1}, and the highest ECe were observed in Z-2.0%. Similarly, the soil basic ions, i.e., HCO_3^-, Cl^- and Ca^{2+} + Mg^{2+} increased significantly in the range 4.53–6.61, 32.42–44.17, 68.07–76.08 and 41.69–46.07 $mmol_c$ L^{-1}, respectively, when treated with Z-2.00%.

Table 3. Post-experiment soil characteristics.

Treatment	pH_s	EC_e	HCO_3^-	Cl^-	$Ca^{2+} + Mg^{2+}$
Control	7.52 ± 0.00	1.16 ± 0.03	32.43 ± 0.72	68.07 ± 0.67	41.69 ± 0.07
Z0.25%	7.72 ± 0.01	1.49 ± 0.09	35.20 ± 0.75	70.10 ± 0.05	44.03 ± 0.03
Z0.5%	7.72 ± 0.01	1.57 ± 0.04	40.06 ± 0.23	74.51 ± 0.53	45.37 ± 0.20
Z1.0%	7.73 ± 0.01	1.65 ± 0.03	38.09 ± 0.45	72.31 ± 0.40	45.25 ± 0.66
Z2.0%	7.95 ± 0.02	1.73 ± 0.04	44.17 ± 0.14	76.08 ± 0.15	46.07 ± 0.05
LSD value	0.0428	0.1849	2.0072	1.6506	1.1935

Values ± SE presented in table for HCO_3^-, Cl^- and $Ca^{2+} + Mg^{2+}$ are in $mmol_c\ L^{-1}$ and EC_e in $dS\ m^{-1}$.

The results clearly show that as the rate of zeolite application increased, the soil available metal content decreased (Table 4). The treatment Z-2.0% stabilized 121, 87, 120 and 140% more Cu, Cd, Ni and Pb of wastewater-irrigated soil, as compared to the control, respectively.

Table 4. Effect of zeolite treatments application on metal concentrations (mg kg^{-1}) in different parts of plant and soil.

Treatment	Fruit	Shoot	Root	Soil	TF	RF
			Cu			
Control	9.62 a	18.52 a	25.67 a	44.84 a	0.72	236
Z0.25%	9.95 a	17.31 a	22.20 b	38.19 b	0.78	265
Z0.5%	7.66 b	11.3 b	17.33 c	32.81 c	0.65	266
Z1.0%	5.34 c	10.8 b	11.82 d	26.74 d	0.91	281
Z2.0%	5.52 c	9.2 b	10.22 e	20.28 e	0.90	292
			Cd			
Control	0.36 a	0.47 a	0.59 a	0.78 a	0.80	236.0
Z0.25%	0.27 b	0.43 ab	0.43 b	0.77 a	1.00	264.7
Z0.5%	0.10 c	0.28 bc	0.37 b	0.66 b	0.76	266.3
Z1.0%	0.02 d	0.15 c	0.23 c	0.64 b	0.65	281.0
Z2.0%	0.01 d	0.15 c	0.17 c	0.41 c	0.88	291.7
			Ni			
Control	0.37 a	0.50 a	0.57 a	0.80 a	0.88	236.0
Z0.25%	0.27 b	0.42 b	0.58 a	0.80 a	0.72	264.7
Z0.5%	0.37 a	0.38bc	0.4 b	0.80 a	0.95	266.3
Z1.0%	0.18 c	0.35 c	0.41 b	0.54 b	0.85	281.0
Z2.0%	0.11 d	0.23 d	0.30 c	0.36 c	0.77	291.7
			Pb			
Control	0.47 a	0.66 a	0.83 a	0.95 a	0.79	236.0
Z0.25%	0.33 b	0.55 b	0.76 b	0.92 a	0.72	264.7
Z0.5%	0.26 c	0.48 c	0.53 c	0.71 b	0.90	266.3
Z1.0%	0.10 d	0.28 d	0.42 d	0.53 c	0.67	281.0
Z2.0%	0.00 e	0.22 e	0.20 e	0.40 d	1.10	291.7

Different letters indicate statistical difference among the treatments (LSD, $p < 0.05$).

3.5. Plant Tissue PTE Concentrations

The concentration of PTEs in roots, shoots and fruit significantly decreased with increasing zeolite dose (Table 4). The Cu contents in plant roots, shoots and fruit were in the range 25.67–10.22, 18.52–9.2, and 9.95–5.52 mg kg^{-1}, respectively. The minimum concentration was recorded with Z-2.0% treatment. With the same treatment, the maximum decrease was recorded with respect to the Cd contents (21% and 24%) in plant roots and shoots was recorded. The minimum Ni contents (0.3, 0.23 and 0.11 mg kg^{-1}) in plant roots, shoots and fruit was observed with Z-2.0%. The Pb concentration in plant roots, shoots and fruit varied in the range 0.83–0.2 mg kg^{-1}, 0.66–0.22 mg kg^{-1} and 0.47–0 mg kg^{-1}, respectively, and the minimum was observed with the highest tested zeolite dose.

The translocation and remediation factors were significantly affected by the application of increasing zeolite doses. The maximum translocation (91%) and remediation factor (292) were recorded with Z-1.00 and Z-2.00%, respectively, in the case of Cu. The detailed summary of the treatment effects on remediation and translocation factors on Cd, Pb and Ni are presented in Table 4.

3.6. Human Health Risk Assessment

Average daily intake (DI) of Cu, Cd Pb and Ni via consumption of brinjal is shown in Table 5. Our results revealed that DI decreased with an increase in the application level of zeolite to soil. The maximum DI values was observed in children in comparison with adults, whereas among adults, the intake rates in women were higher than men. The risk of non-carcinogenic toxicity to children and adult residents caused by PTEs were calculated and are shown in Table 5. The non-carcinogenic risks (HQ) decreased with the application rate of zeolite. The estimated value of HQ was less than 1 with all treatments.

Table 5. Effect of zeolite treatment on average daily intake (ADI) of trace metals and health risk index (HQ) under different treatments.

Treatment		DI				HQ			
		Cu	Cd	Pb	Ni	Cu	Cd	Pb	Ni
Children	Control	0.015	0.00056	0.00073	0.00057	0.371	0.561	0.183	0.029
	Z0.25%	0.014	0.00037	0.00045	0.00036	0.339	0.368	0.112	0.018
	Z0.5%	0.011	0.00015	0.00038	0.00052	0.280	0.146	0.095	0.026
	Z1.0%	0.007	0.00003	0.00014	0.00024	0.181	0.033	0.035	0.012
	Z2.0%	0.008	0.00002	0.00000	0.00017	0.208	0.015	0.000	0.008
Men	Control	0.008	0.00032	0.00041	0.00032	0.209	0.316	0.103	0.016
	Z0.25%	0.008	0.00021	0.00025	0.00020	0.191	0.207	0.063	0.010
	Z0.5%	0.006	0.00008	0.00021	0.00029	0.158	0.083	0.054	0.015
	Z1.0%	0.004	0.00002	0.00008	0.00014	0.102	0.018	0.020	0.007
	Z2.0%	0.005	0.00001	0.00000	0.00009	0.117	0.008	0.000	0.005
Women	Control	0.009	0.00035	0.00046	0.00036	0.234	0.354	0.115	0.018
	Z0.25%	0.009	0.00023	0.00028	0.00023	0.214	0.232	0.071	0.011
	Z0.5%	0.007	0.00009	0.00024	0.00033	0.177	0.092	0.060	0.016
	Z1.0%	0.005	0.00002	0.00009	0.00015	0.114	0.020	0.022	0.008
	Z2.0%	0.005	0.00001	0.00000	0.00010	0.131	0.009	0.000	0.005
Guideline values	Parameter	UL	TWI	TDI	TDI				
	value	10 mg day^{-1}	2.5 µg kg^{-1} bw^{-1} week^{-1}	0.3 µg kg^{-1} bw^{-1} day^{-1}	2.8 µg kg^{-1} bw^{-1} day^{-1}				
	Reference	[46]	[47]	[48]	[49]				

HQ > 1 indicates significant risk to human health, while HQ < 1 indicates that trace metals intake is not harmful. Bw: body weight, UL: upper-level intake, TDI: tolerable daily intake, TWI: tolerable weekly intake.

4. Discussion

Post-harvest analysis of wastewater-contaminated soil revealed that properties such as ECe, CO_3^{2-}, HCO^{3-} and Ca^{2+} + Mg^{2+} were significantly altered with application of zeolite (Table 3). The ECe is directly related to ion exchange. Zeolite is the negatively charged mineral having pores filled with K, Na, Ca, Mg and H_2O molecules, allowing for the exchange of ions and the release of water back and forth [50]. In this study, the applied zeolite has led to an increase in soil ECe. Other researchers have also reported the increase in ECe in zeolite amended soil [51,52]. The increase in ECe with the zeolite treatments is attributed to the presence of mineral ions in the zeolite, as well as its salt-holding capacity [53]. Similarly, high content of base cations such as Ca^{2+} + Mg^{2+} was also observed in zeolite treated soil. High concentration of base cations in acidic soil amended with zeolite was also reported [50].

Soil pH is a fundamental and essential factor that significantly influences the metal behavior in soil and affects the effectiveness of PTEs in soil; it also affects the level of toxicity the soil and plants face, both directly and/or indirectly [54]. In this experiment, increased soil pHs was observed in zeolite treated soil (Table 3). This might be possible due to the strong alkaline pH and release of base cations [55]. It was reported that the addition of zeolite resulted in a significant increase in the soil pH and promoted carbonate precipitation and oxide formation, thereby reducing the quantity of toxic elements in the soil [54]. The content of amendments added and the incubation time have a substantial effect on the immobilization of toxic elements and the increase in pH of soil [56]. Since most toxic elements have very low mobility under alkaline conditions, increasing the soil pH can often remediate the contamination in soil by PTEs [57]. There are different processes including selective adsorption, metal precipitation with oxides, hydroxides, phosphates, and carbonates to reduce the mobility of PTEs in soil solution which increase the soil fertility and promote the plant growth by providing the best conditions [29,58].

The results demonstrated the significant reduction in Cu availability in zeolite amended soil (Figure 3). This might be due to increased soil pHs, CEC and high surface area with zeolite application [59]. Similar results were also reported by [55]. The main mechanism of action of zeolite in dropping PTEs phytoavailability is a blend of the ion exchange properties of the zeolite and their capability to enhance soil pH [60], furthermore, the fixed metal contaminants are either retained in the zeolite framework or precipitated as a metal carbonate or oxide as the soil pH rises. It has been also reported by [61] that the chemical fixation by zeolite is widely assumed to decline exchangeable toxic element contents in polluted soil, because of its adsorption on the lattice of tectosilicate. The zeolite can fix PTEs in the mineral clay layer of the soil by diffusion and increase the pH of the soil for a longer time [25]. It is well documented that besides being a cation exchanger, zeolite also serves as cation absorbent, such as PTEs, so the zeolite can reduce PTEs pollution in the environment [61,62].

Brinjal is recommended crop for its safe growth on PTEs-contaminated soil based on the pattern of PTEs accumulation and their distribution in edible portion of different parts of crop plants [63]. In this experiment, the significant positive effect of zeolite, a toxic element immobilization agent, on plant growth was noticed (Figure 1). These consequences are compatible with the results of [64]. They suggested the zeolite for cultivation of brinjal after observing its dual beneficial effect, the first being in terms of increased flowering, and second possibly being to serve to hold nutrients from leaching, which may benefit farmers by saving on fertilizer cost. Contrarily, it has been also reported that zeolite decreased plant growth of *Dieffenbachia amoena* in the absence of nutrients, but increased leaf number and stem diameter in the presence of nutrients [52]. It is possible that in the absence of any other added fertilizer (chemical or otherwise), plants lacked all the nutrients necessary, and under these conditions, plants had reduced height in the presence of zeolite. However, in the present study, recommended doses of NPK were added that might be a reason for the increased plant height, biomass and fruit weight (Figure 1). In a study conducted on the effects of the integrated use of zeolite and organic manure on the yield of sunflower, increased plant height, SPAD value, seed weight, and biological yield have been observed [65]. It has been reported that the application of zeolite increased soil chemical fertility at the beginning of barley cultivation and enhanced hydro-soluble concentrations of the nutrients in soil that resulted in enhancement of barley yield [66], like the response of brinjal regarding chlorophyll contents, photosynthetic and stomatal conductance in this study (Figure 2). The addition of zeolite might reduce N loss from soil caused by leaching, since this amendment has the capability to absorb nitrogen and release it gradually during the growth season. Having access to sufficient N in soil increased N absorption and led to more photosynthesis by plants [65]. It was documented that zeolite at 5% dosage significantly enhanced chlorophyll content in the aerial surface of maize [67].

The significant decrease in the available PTEs concentration of soil, due to zeolite application (Figure 3), consequently reduced the uptake of PTEs by plant, and thus, the results presented less plant PTEs concentration in zeolite treatments, as compared to the control. Correlation analysis strengthens these findings. Similarly, a reduction in Cu phytoavailability in corn and cabbage plants with the addition of zeolite was reported by [20]. Additionally, it was also reported that a zeolite amendment significantly reduced the Cd and Cu accumulation in the shoots of pakchoi [68], while a reduction in Pb, Cd, Cu, and Zn accumulations in rice tissues, due to elevated soil pH and CEC with the application of hydroxy-histidine with zeolite, was reported by [69].

It is summarized that zeolite is a good source of PTEs immobilization in wastewater-contaminated soil. It not only reduced the uptake of Cu by plants but also increased the plant growth and yield-contributing parameters. The application of zeolite also improved the chemical characteristics of soil.

Antioxidant enzymes such as SOD, POD, CAT and APX are produced by plants under PTEs stress to protect them from the damaging effects of chain reaction of PTEs-produced ROS chain reactions [70]. Antioxidant enzymes in brinjal plants were enhanced in our experiment by addition of different zeolite concentrations, compared to the control (Figure 2). It should be noted that the positive effects were more evident in the treatment that included 2% zeolite addition. Based on previous research, these findings may be predicted from the results of [70], who found that biochar and chitosan application to Cd and Ni-contaminated soils increased SOD, CAT, and APX activities in sunflower and mung bean, respectively. The same mechanism could also be responsible in our study. Antioxidant defense mechanisms in plants have improved as a result of the capacity of applied amendments, i.e., biochar [71,72], to boost plant health [73]. Antioxidant activity is boosted by chitosan capacity to reduce the formation of damaging free radicals [74] and to promote the vital nutrients by changing cell osmotic pressure [75]. Covalent grafting of antioxidant activity on the backbone of CH is also facilitated by the reactive functional groups on its surface [73,75].

There was a considerable reduction in Ni, Cd, and Pb concentrations in brinjal roots, shoots, and fruit when zeolite was applied with increasing concentrations. Shoots, roots, and fruit treated with 2.00% zeolite showed the greatest decrease in metal concentrations. Several studies have shown that application of zeolite in the soil greatly boosted PTEs accumulation in the plant roots, as well as in the aerial body parts, i.e., leaves and stems; for example, [76,77] have shown that the application of PTEs-immobilizing agents, i.e., biochar, chitosan and zeolite, can effectively immobilize PTEs and inhibit their absorption in plants. The higher surface area, precipitation, ion exchange, complexation and compartmentalization of PTEs in non-edible plant tissues might be a plausible reason for this decline in metal concentration in different regions of brinjal plant [76,78,79]. Chelation of metallic ions through inter- or intra-molecular binding could also be a mechanism [80].

Consuming vegetables as part of one's diet may provide useful data on a person's intake of nutrients, bioactive chemicals, and food pollutants, as well as any nutritional deficiencies or contamination they may have [41]. As, Cd, and Pb EDIs were calculated based on the average metal content in brinjal fruit and the corresponding consumption rate for each food type [81]. The consumption rates of brinjal for children and adults are reported in Table 2. In terms of human exposure to PTEs, oral is by far the most common route [82]. The studied PTEs were shown to be the most significant contributors to the possible health risk posed by food consumption in the research region (Table 5) by the total EDIs of Cu, Ni, Cd, and Pb from all analyzed samples. Based on these data, we conclude that PTEs could be the major components contributing to the potential health risk via consumption of the PTEs-contaminated brinjal fruit.

Table 5 give the THQ for the non-carcinogenic risk of the PTEs investigated in brinjal when consumed by adults and children. In terms of health effects, the THQ estimation approach does not give a quantitative assessment of the likelihood that a population exposed to contaminants would suffer a negative health consequence, but it does provide an indicator of the degree of risk. The THQ values of Cu, Ni, Cd, and Pb did not surpass the threshold value of 1, and hence, brinjal crop production in PTEs-contaminated areas is deemed dangerous and not advised for frequent intake. As a result, consumers have a significant risk of non-carcinogenic hazards.

5. Conclusions

Overall, it was concluded that zeolite is a good source of Cd, Cu, Pb and Ni immobilization in wastewater-irrigated, potentially toxic elements-contaminated soils. Zeolite not only reduced the uptake of Cd, Cu, Pb and Ni from brinjal plants but also increased the growth and yield-contributing parameters. The application of zeolite also improved the chemical characteristics of soil, and the trend of treatment for improving chemical characteristics was zeolite at 2.00% > 1.00% > 0.50% > 0.25% and minimum with control. Moreover, the application of zeolite-based treatments reduced the human health risks by reducing potentially toxic elements uptake in the fruit of brinjal crops. Based on the fact that zeolite could be a good source of PTEs immobilization, it should be tested for the immobilization of a wide array of PTEs in other vegetables and dynamic environmental conditions to test its efficacy.

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