

Engineering Assessment of Antibiotic Transport, Retention, and Exposure Pathways in Wastewater impacted Rural Soil–Water Systems of Punjab, Pakistan

Abid Ali ^{a, b, *}, Audil Rashid ^{a, c}, Shahid Mahmood ^a, Tariq Mahmood ^d, Anika Batool ^a, Munnaza Khalid ^e, Ismat karim ^f and Muhammad Shafiq ^g

^a Department of Environmental Sciences, PMAS Arid Agriculture University, Rawalpindi, Pakistan

^b Department of Biological Sciences, Islamabad Model College for Boys, H-9, Islamabad, Pakistan

^c Eco-Health Research Lab, Faculty of Science, University of Gujrat, Gujrat, Pakistan

^d Department of Wildlife Management, PMAS Arid Agriculture University, Rawalpindi, Pakistan

^e Holy Family Hospital (Gynae Unit-I), Rawalpindi Medical University, Rawalpindi, Pakistan

^f Department of Chemistry, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand

^g Department of Pathology, Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan

*Corresponding author: abid.mughal@imcbh9.edu.pk

Received: 01 February 2026, Revised: 14 February 2026, Accepted: 31 March 2026, Published: 01 April 2026

KEY WORDS

Environmental Engineering
Antibiotic Transport
Soil–water Interaction
Wastewater Irrigation
Rural Exposure Pathways
HPLC Analysis

ABSTRACT

The presence of heavy metals (HMs) and antibiotic residues together in agricultural soils is a critical environmental engineering issue in rural areas where untreated wastewater irrigation, livestock manure irrigation and poor waste management practices are prevalent. These coupled systems of soil and water have the potential to increase contaminant transport, retention, and persistence and thus increase environmental exposure pathways for rural populations. This study examines the HMs role in mediating environmental behaviour and retention of antibiotics in rural soils of Punjab, Pakistan focusing on process-level interactions which are pertinent to environmental engineering systems.

Soil samples from two rural areas, Chountra and Sialkot ($n = 26$ and 38 , respectively) were sampled and analyzed for Cd, Cr, and Pb and the antibiotics that are widely used, using atomic absorption spectrophotometry and high-performance liquid chromatography (HPLC). Results revealed a higher concentration of HMs in soils of Sialkot (Cd: 3.13 , Cr: 26.78 , Pb: 21.44 mg kg⁻¹) than Chountra. The prevalent antibiotic residues in soil included erythromycin (38.76 mg kg⁻¹), doxycycline (21.56 mg kg⁻¹) and ciprofloxacin (14.32 mg kg⁻¹). Uptake by the naturally growing vegetation found *Medicago sativa* to be the most important accumulator (0.7 - 2.9 mg kg⁻¹) followed by *Euphorbia hirta* (0.8 - 2.5 mg kg⁻¹).

Correlation analyses showed complex interactions between HMs and antibiotics, especially between Cd and Pb, which contribute to the increase of adsorption and retention of antibiotics in soils. These results show that sorption processes mediated by Cd and Pb are responsible for antibiotic persistence and mobility, particularly affecting the retention of Doxycycline (DOX) and Levofloxacin (LEV) in rural soil-water systems. From an environmental engineering perspective, the findings highlight deficiencies in rural wastewater reuse and manure management systems and emphasize the need for improved decentralized treatment, controlled irrigation practices, and exposure mitigation strategies to reduce long-term contaminant loading and environmental exposure risks.

1. Introduction

Heavy metal (HM) contamination of soils is a persistent and far-reaching environmental problem with implications for ecosystem functioning, agricultural sustainability, and human exposure pathways [1]. Metals such as cadmium (Cd), chromium (Cr), lead (Pb) and mercury are widely known for their ecotoxicity and health hazards [2,3]. However, with respect to their direct toxic effects, comparatively limited attention has been given to the role of heavy metals as mediators of the environmental behaviour of other emerging contaminants, especially antibiotic residues, in soil systems. From an environmental engineering perspective, this interaction is important because it controls the transport, retention, and long-term persistence of contaminants in soil-water environments. Recent international studies [29] emphasise that the co-existence of these contaminants globally remains a challenge for environmental engineering due to site-specific geochemical variations.

Antibiotic residues in soils have become a growing concern because of the widespread use of antibiotics in human medicine, livestock production, and preventive veterinary practices. When released into the environment via wastewater irrigation, manure application, and industrial discharges, antibiotics act as mobile organic pollutants, and their fate is highly dependent on soil physicochemical properties [4]. Co-occurrence of heavy metals and antibiotics in soils leads to complex, multifaceted interactions that can alter antibiotic mobility, bioavailability, and sorption behaviour [5,6]. These coupled processes are of extreme importance to Environmental Engineering, as they are directly related to the performance and limitations of wastewater reuse systems, agricultural management practices, and pollution control infrastructure.

Globally, accumulation and toxicity of heavy metals in soils have been widely reported, especially in areas dominated by industrial and agricultural activities [7]. In rural environments near industrial areas or irrigated with untreated or partially treated wastewater, heavy metals and antibiotics commonly coexist, increasing environmental loading and persistence [8-12]. Heavy metals can alter soil microbial activity and geochemical conditions, thereby altering antibiotic degradation and increasing their retention via adsorption onto soil particles. Such interactions may prolong the time antibiotics remain in the environment, thereby increasing the likelihood of repeated environmental exposure via soil contact, crop uptake, and livestock feeding routes.

In rural agricultural systems, the intensive use of antibiotics in livestock farming further exacerbates

this problem. The application of manure and wastewater contaminates soils with both antibiotics and heavy metals, disrupting microbial communities involved in metal transformation and contaminant attenuation. As a result, soil-plant-water systems may constitute reservoirs of low levels of antibiotics with continuous presence. While potential environmental exposure risks have been associated with chronic exposure, adverse health outcomes are best interpreted as downstream effects of inefficiencies in the environmental system rather than health system phenomena.

Despite increasing concern, there remains a scarcity of integrated studies on the behaviour of heavy metals in relation to antibiotics in the rural soil-water system from an environmental engineering perspective. There is still insufficient understanding of the mechanisms underlying antibiotic endurance, retention, and bioavailability under HM-contaminated conditions. Overcoming this knowledge gap is important for designing effective wastewater reuse controls, soil remediation procedures, and exposure mitigation strategies.

Therefore, the current research aims to examine the occurrence and interactions of heavy metals and antibiotic residues in soils and naturally growing plants in rural Punjab, Pakistan. In addition, examination of livelihood-related exposure pathways is undertaken to link engineering-based contaminant transport data directly to the risk profiles of rural populations. By addressing contaminant interactions, transport, and retention processes, this study aligns with environmental engineering goals and enhances overall understanding of chronic exposure risks in rural communities. From an environmental engineering standpoint, limited attention has been given to how heavy metals mediate the transport, retention, and persistence of antibiotics within coupled soil-water systems under wastewater irrigation regimes. Understanding these interactions is essential for improving the design, performance, and management of rural wastewater reuse systems and for minimising unintended environmental exposure.

2. Materials and Methods

2.1 Materials

Analytical-grade antibiotic standards (98% purity) for the 6 antibiotics studied were used for calibration and quantification. HPLC grade acetonitrile and

methanol, analytical grade phosphoric acid, nitric acid (HNO₃) and perchloric acid (HClO₄) were used for sample extraction and digestion. Deionised water was used throughout the analytical procedures. Soil and plant samples were collected and placed in pre-cleaned containers to reduce contamination and photodegradation before laboratory analysis.

2.2 Study Site Description and Sampling

The study was conducted in two rural areas of Punjab, Pakistan, located adjacent to the Sialkot and Rawalpindi city suburbs. Sialkot is an industrial centre with a well-established, densely populated rural area and extensive natural vegetation (Figure 1), where industrial effluents often discharge through open water channels into surrounding agricultural lands. The Chountra region, close to Rawalpindi, has several villages crisscrossed by an industrial wastewater channel (Nullah Leh), which is commonly used for irrigation purposes (Figure 2).

Soil samples of Chountra (n = 26) and Sialkot (n = 38) were taken according to a previously described standardised protocol [15]. Composite soil samples were collected from the surface layer (0–20 cm) of representative agricultural sites that are affected by wastewater irrigation. Sampling was conducted during the dry season to minimise dilution effects associated with monsoon rainfall. In addition, naturally growing plant species with high grazing potential, located close to contaminated areas, were hand-collected after surface debris removal. Plant samples were taken to the laboratory for the determination of tissue contaminant concentrations [16].

2.3 Sample Preparation and Laboratory Analysis

The estimation of heavy metals and antibiotics has been conducted in a structured process that includes problem identification, data acquisition, and analytical evaluation.

For soil antibiotic analysis, 1 g of homogenised soil was transferred into a 50 mL centrifuge tube and extracted with 10 mL of phosphoric acid (0.05 M) and 10 mL of acetonitrile. The mixture was vortexed for 20 s, sonicated for 30 min in a water bath (maintained at <30°C to prevent antibiotic degradation) and centrifuged at 4000 rpm for 5 min. The supernatant was decanted, and the extraction was repeated with 5 mL of acetonitrile. Combined extracts were

concentrated under a gentle nitrogen stream, filtered, cleaned in a cartridge, eluted with methanol and subjected to the HPLC analysis [17].

Heavy metal concentrations in soil samples were determined using the USEPA Method 3050B, which involves acid digestion of the soil and subsequent quantification by atomic absorption spectrophotometry (AAS). This method was chosen because it has a short processing time, less acid consumption and high metal recovery efficiency [18].

Plant samples were thoroughly washed, air-dried at room temperature until constant weight was reached (about 24 h), and analysed from dry-weighted above-ground tissues. Wet digestion with nitric and perchloric acids was used to extract Cd, Cr, and Pb prior to AAS determination [19]. Antibiotic residues in plant tissues were quantified by high-performance liquid chromatography (HPLC) and expressed as µg of antibiotics/kg DW [20].

All samples are analysed by High-Performance Liquid Chromatography (HPLC) in isocratic mode at room temperature, using a Shimadzu system (autosampler SCL-10 AVP, pump SPD-10A, oven CTO-10AVP) with UV detection at 280 nm. UV detection at 280 nm was selected because the target antibiotics exhibit strong absorbance at this wavelength. The mobile phase was 16.5% acetonitrile in 0.05 M phosphoric acid, with a flow rate of 1 mL min⁻¹ and an injection volume of 50 µL. Separation was performed using an Agilent C18 column (250 x 4.6 mm, 5 µm).

2.4 HPLC Method Validation and Quality Assurance/Quality Control

Analytical quality assurance and control procedures were implemented to ensure data reliability. Calibration curves were prepared using multi-point external standards, and the coefficient of determination (R²) was greater than 0.99. Limits of detection (LOD) and limits of quantification (LOQ), which ranged from 0.05 to 0.15 µg kg⁻¹ and 0.16 to 0.45 µg kg⁻¹, respectively, for the six studied antibiotics, were set according to signal-to-noise ratios of 3:1 and 10:1, respectively. Recovery experiments were conducted using matrix-spiked samples, yielding acceptable recoveries ranging from 80–120%, confirming analytical accuracy and method reliability. Procedural blanks, duplicate samples, and periodic checks of standards were included to monitor analytical precision and accuracy.

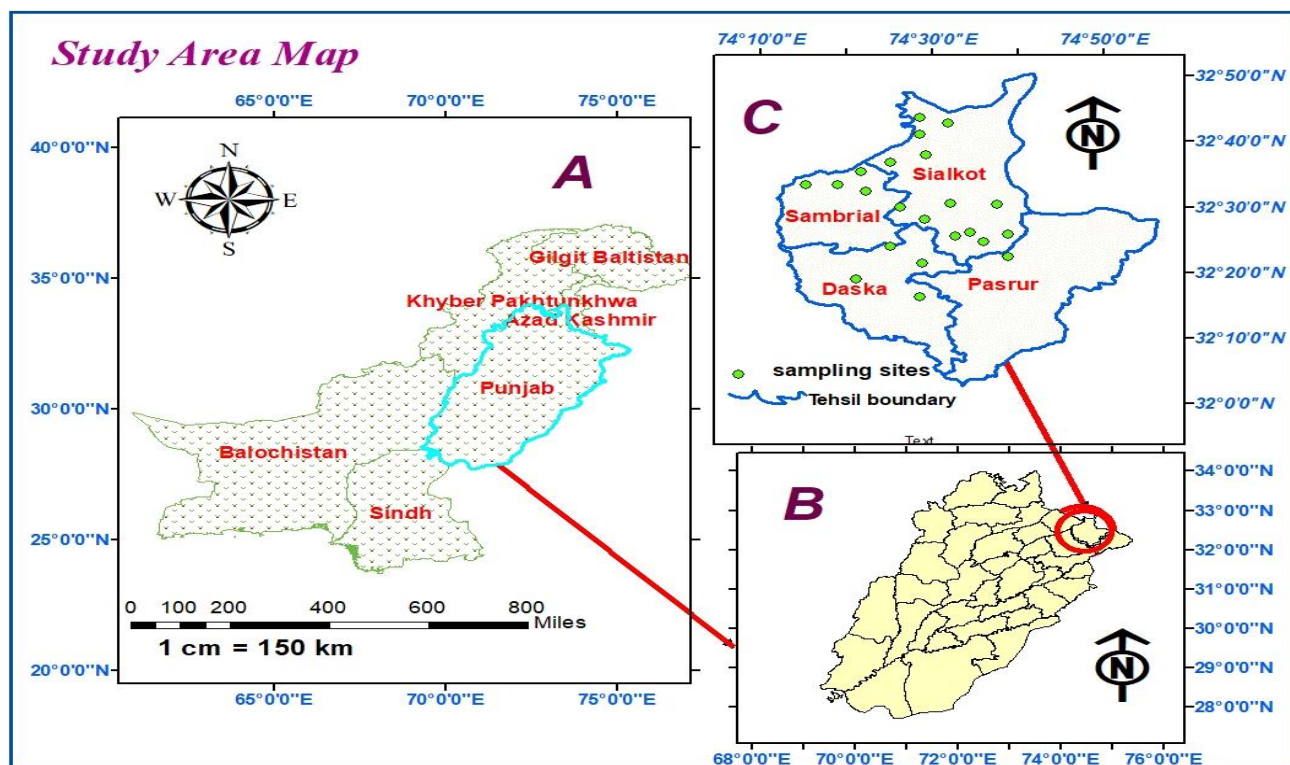


Fig 1. Location of sample sites in the Sialkot rural area.

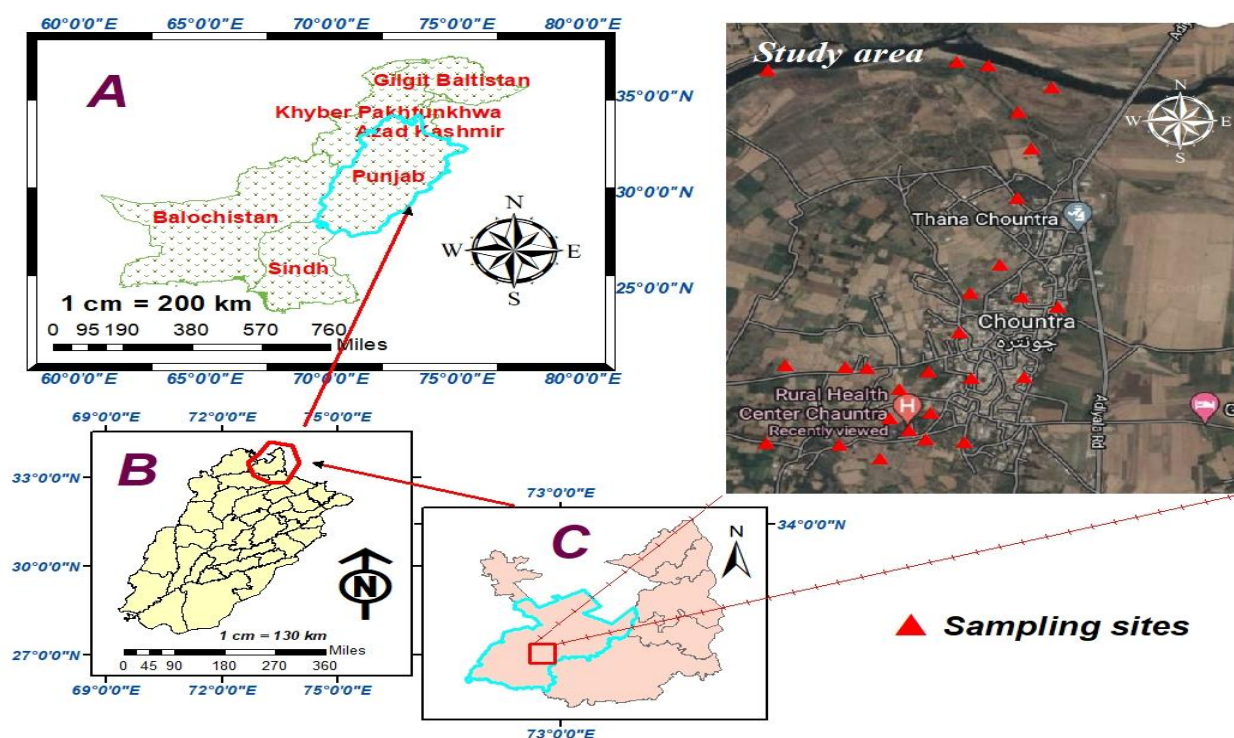


Fig. 2. Location of sample sites in the Chountra rural area.

2.5. Field survey

In rural environments, exposure routes (ERs) are pertinent for examining how cattle and other livestock can be exposed to antibiotics present in these settings. In that regard, we have determined that there are several risks of exposure to daily operations in rural communities. The risks related to exposure paths have been categorised and assigned codes for systematic analysis (Table 1). Further, the

measurements aimed to assess the rate and extent of their livestock's interactions with the environment.

ER1 also investigated how frequently surface water was used as a drinking water source for cattle per week, an essential factor for their daily hydration and nutrition. ER2 concentrated on what happens to surface water when it is used to bathe the cattle, and this illuminates how frequently these animals indulge themselves in water activities, which can influence their health too.

ER3 and ER4 examined the kind of vegetation used as cattle food, and ER3 focused on how many times *Medicago* is fed to cattle, whilst ER4 examined how many times cattle are fed *Euphorbia*. The exposure statistics were gathered on people-soil interactions related to agricultural activities, using variables ER9-ER12 as defined in Table 1. The variables to be used are the frequency with which wastewater is used as irrigation, the number of times contact with soil occurs per week with the children, the number of times contact with soil occurs per week with the women, and the number of times contact with soil occurs per week with the male farmers. The assumption is that more frequent contact is typically associated with greater interaction and exposure to soil-related elements. All these exposure routes provide an overall insight into the ecological interactions and management of cattle and are useful for securing their welfare and health, as well as streamlining agricultural activity.

2.6 Data Processing and Statistical Analysis

Descriptive and inferential statistical analyses were conducted using Microsoft Excel (Office 365) and version 16 of the software package, for Windows, called "Statistical Package for Social Sciences" or

"SPSS". Differences between variables were assessed with t-tests, ANOVA and Tukey post hoc tests. Correlation and regression analyses were carried out to determine the effects of soil pH, organic matter, and heavy metals on antibiotic concentrations. Multivariate Principal Component Analysis (PCA) was performed to identify dominant patterns of exposure and to assess associations between environmental contaminants and exposure pathways. Statistical significance cut-off was $p < 0.05$. Geographic mapping of study locations was performed in ArcGIS.

3. Results

3.1. Heavy Metals in Soil

Table 2 shows the contents of the heavy metals and other soil parameters of Chountra (n=26) and Sialkot (n=38). The percentage of organic matter (OM) is somewhat higher in Sialkot than in Chountra at 1.84 and 1.63, respectively. The pH level in Sialkot is higher (8.8), indicating an alkaline environment, and the clay content is higher (14.2) than in Chountra (11.6). The levels of the three heavy metals in the soil of rural Sialkot are higher (Table 2)

Table 1

Rural population exposure monitoring parameters used to collect the information on a seven-point Likert-scale through questionnaire-based survey.

Exposure characteristics	Code for exposure risk ¹
How is often surface water used for cattle drinking per week	ER1
How is often surface water used for cattle bathing per week	ER2
How often do you use <i>Medicago</i> as cattle food per week	ER3
How often do you use <i>Euphorbia</i> as cattle food per week	ER4
How often do you use <i>Amaranthus</i> as cattle food per week	ER5
Contact frequency of children with wastewater per week	ER6
Contact frequency of women with wastewater per week	ER7
Contact frequency of male farmers with wastewater per week	ER8
Frequency of use of wastewater for irrigation (1 low to 7 high)	ER9
Contact frequency of Children with soil per week	ER10
Contact frequency of Women with soil per week	ER11

Table 2

Characteristics of soil samples collected from two rural areas of Punjab Province, Pakistan.

Soil parameters ¹	Chountra (n=26)	Sialkot (n=38)
OM (%)	1.63 ± 0.21	1.84 ± 0.18
pH	7.6 ± 0.35	8.8 ± 0.42
Clay (%)	11.6 ± 1.4	14.2 ± 1.9
Cd (mg kg ⁻¹)	2.02 ± 0.28	3.13 ± 0.45
Cr (mg kg ⁻¹)	13.09 ± 2.15	26.78 ± 3.62
Pb (mg kg ⁻¹)	15.48 ± 2.56	21.44 ± 2.89

* Each sample is a composite of three soil samples of closest vicinity.

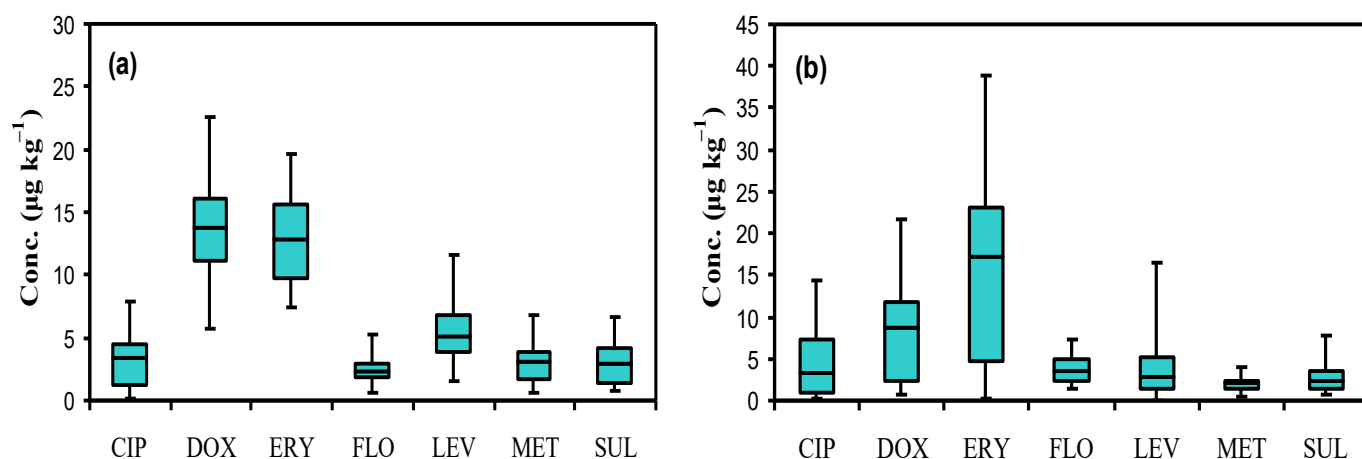


Fig. 3. Estimated values (median & interquartile ranges) of antibiotics in soil samples collected from rural areas of Punjab province: (a) Chountra rural area; (b) Sialkot rural area.

3.2. Antibiotics in Soil

Figure 3a indicated that ciprofloxacin (CIP) experienced the greatest spectrum of concentrations and the greatest median of values in Figure 3a among the examined antibiotics at Chountra. Doxycycline (DOX) and erythromycin (ERY) have higher median concentrations than the other materials. The interquartile range of ERY is slightly higher, indicating that the soil at Chountra sites is more variable. (Figure 3a). By contrast, FLO, LEV, MET and SUL were lower in concentration and more homogenous.

Conversely, the soil antibiotic analysis in the countryside Sialkot area revealed that CIP, DOX and ERY showed a wide range of concentrations, with greater variability and higher average concentrations (Figure 3b). Doxycycline has the greatest range of IRQ, and sulfamethoxazole has the lowest median and total concentration (Figure 3b). The high variability of ERY in Chountra sites suggests intermittent localised discharge of livestock waste rather than continuous wastewater irrigation.

3.3. Plant uptake of HMs and Antibiotics

The uptake of the three plant species in two sites, Sialkot and Chountra compared (Figure 4), showed that the uptake of Cr and Cd was relatively higher in the plant species compared on Pb (Figure 4). Cr values are much higher in the Sialkot region than in the Chountra region across all three plants. The highest value of *Amaranthus viridus* is $4 \mu\text{g kg}^{-1}$, which is followed by *Euphorbia hirta* and *Medicago sativa*.

When comparing sites across the plant species under analysis, a large difference is observed between the two species, except for *Euphorbia hirta*. Pb was detected in all three species of the Sialkot region, and there was no significant difference between the species (Figure 4). In the Chountra region, Pb concentration was higher in *A. viridus* and *Euphorbia hirta*, with the former containing considerably more than the latter.

3.4. Diagnostic Ratio Analysis for HMs-Antibiotic Mediation

Figure 5 compares the concentrations of antibiotics in plants from the two locations in this study. At Sialkot, *Medicago sativa* and *Euphorbia hirta* had significantly higher Ciprofloxacin levels than Chountra had ($p < 0.01$ and $p < 0.05$, respectively). DOX was uptaken by all the Sialkot plants, with the highest concentration in *Medicago sativa*. Nevertheless, the concentration of *Euphorbia hirta* was lower in Chountra. Both *Medicago sativa* and *Euphorbia hirta* in Sialkot were positive in erythromycin, although the concentration of *Medicago sativa* was higher. In Chountra, detectable levels were observed only for *Medicago sativa*. Only *Medicago sativa* of Sialkot was found with florfenicol. Higher levels of levofloxacin were observed in *Euphorbia hirta* in Chountra ($p < 0.05$), with much higher levels in *Medicago sativa* in Sialkot ($p < 0.01$). *Amaranthus viridus* did not show any detectable level of Levofloxacin. In both areas, sulfamethoxazole was detected in *Medicago sativa* and *Euphorbia hirta*, and no significant difference was observed.

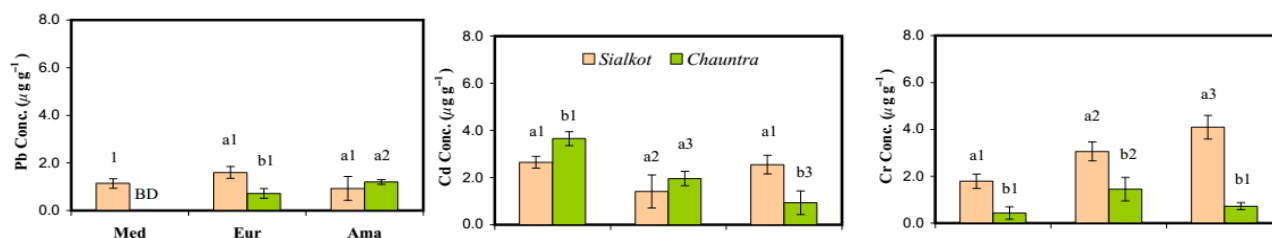


Fig. 4. Comparison of mean concentration of heavy metals in grazing plants collected from two rural sites. Bars with different letters (between sites) are significantly different, while bars with different numbers (among 3 plant species) are significantly different at the detectable limit.

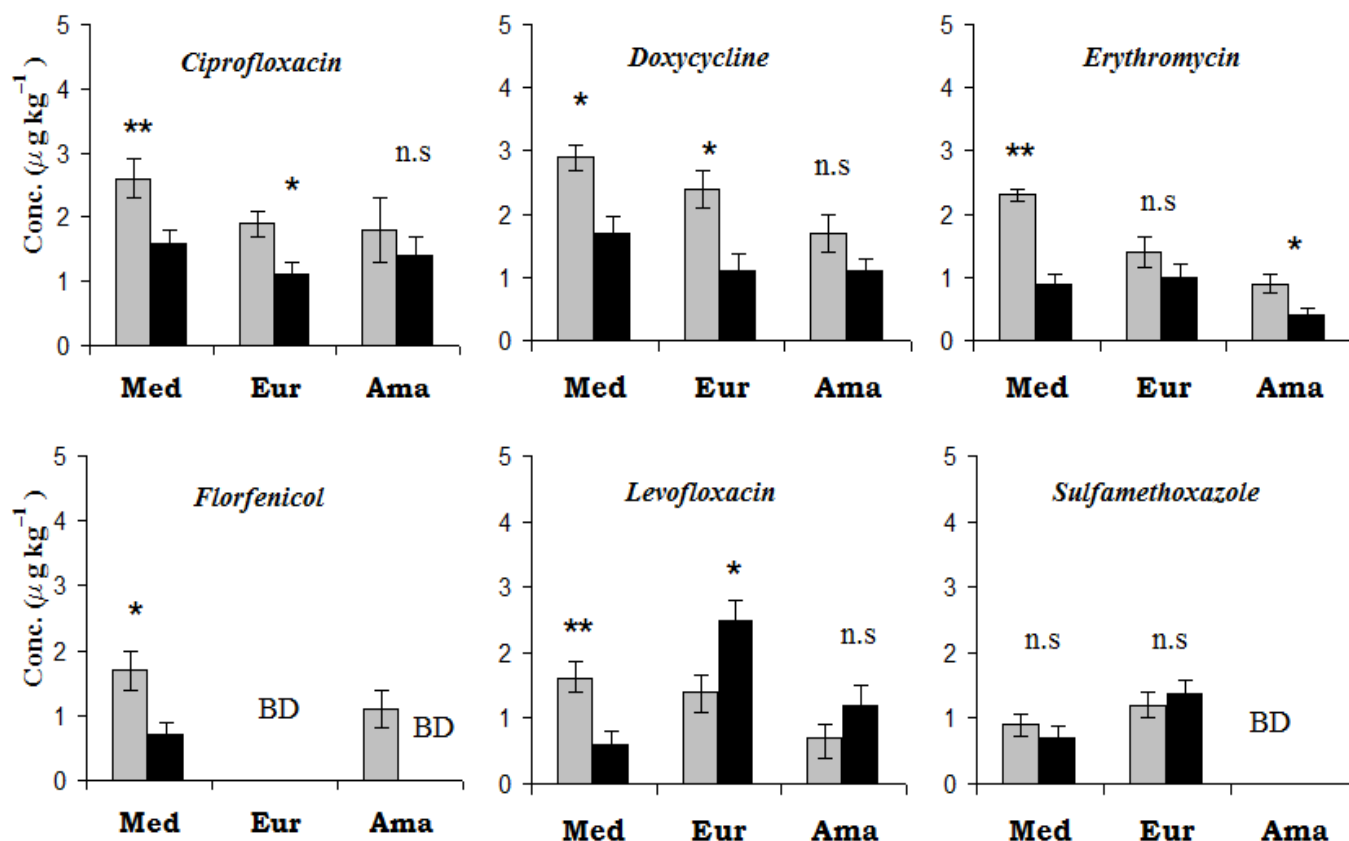


Fig. 5. Comparison of antibiotic levels detected in plants collected from two rural areas of Punjab province. Sialkot rural areas are shown in light bars (left), whereas dark bars (right) show rural areas from Chountra. All values are means ($n=3 \pm \text{SD}$).

Note: plant names are Med = *Medicago sativa*; Eur = *Euphorbia hirta*; Ama = *Amaranthus viridis*, BD = below detectable limit.

3.5. Exposure Risks in Rural Areas

The rural field survey, in questionnaire form, included analyses of demographics and livelihoods, as well as a few characteristics of the Chountra (197) and Sialkot (228) populations' exposure to the environment, using the chi-square test (Table 3). Geographical heterogeneity was evident in significant differences in rural residency ($p < .01$) and active rural living ($p < .001$). The majority of respondents reported self-prescribed antibiotics in both locations; however, use dependent on livestock, whether by self-prescription or a veterinarian's recommendation, was greater in Sialkot than in the Chountra rural area. There was also a significant difference in the

frequency of antibiotic use for human purposes between the two areas ($p < 0.001$) when categorised by specific reasons. In the rural regions of Chountra and Sialkot, the most frequent use was for the treatment of waterborne diseases, followed by chest/respiratory infections and skin allergies (Table 3). A noticeable difference was observed in the distribution of livestock antibiotic use ($p < 0.01$), with a very significant contrast in Chountra and Sialkot. The families in Chountra did not seek professional advice before treating livestock with antibiotics, whereas in Sialkot, more families consulted veterinarians and physicians or followed drug recommendations.

Table 3

Demographic and livelihood characteristics of the rural population in surveyed areas with antibiotic exposure.

Variable	Rural Area		Chi-sq (<i>p</i> -value)
	Chauntra (<i>n</i> =197)	Sialkot (<i>n</i> =228)	
Gender			
Male	105	106	n. s³
Female	92	122	
Age (years)			
< 12	33	26	0.04
12 – 40	71	85	
> 40	93	117	
Residing at rural community			
Less than 3 years	63	47	<0.01
More than 3 years	134	181	
Education			
Illiterate	41	35	0.022
Primary school	87	74	
High school	46	96	
College or above	23	23	
Livelihood			
Active rural life	94	119	<0.001
Partially active	59	87	
Completely inactive	44	22	
Antibiotic use (human)			
Personal (self-medicated)	90	82	<0.05
Administered (clinical)	71	101	
Excluded responses	36	45	
Antibiotic use (livestock)			
Veterinary (self-decided)	56	78	<0.01
Veterinary (physician or drug)	39	64	
Excluded responses	102	86	
Use purpose (human)			
Chest and/or respiratory infections	44	69	<0.001
Skin and/or allergies	47	51	
Injuries	36	29	
Water-borne diseases	70	79	
Use purpose (livestock) ¹			
Weight increase	25	34	n. s³
Milk increase	39	59	
Disease cure	31	49	
Risk factor of exposure			
Atopic dermatitis	51	90	0.024
Food allergies & related symptoms	35	28	
Allergic rhino conjunctivitis	43	44	
Asymptomatic ²	68	66	

¹ Focused exclusively on responses about antibiotic usage in livestock animals.² non-allergic respondents were used as a 'reference category' in logistic regression analysis.³ n. s = not significant (*p* > 0.05)

They were primarily aimed at boosting livestock weight and milk production, not at curing diseases. Reviewing the prevalence of allergies in the Chountra and Sialkot regions, the percentages of different allergies differ significantly. There is an increased prevalence of reported atopic dermatitis symptoms in

Sialkot (90 cases) and a lower prevalence in Chountra (*n*=51). However, food allergies are a bit more common in Chountra (*n*=35) than in Sialkot (*n*=28). The incidence of Allergic Rhinitis is also almost equal in the two regions, and the number of cases with no symptoms is also close, with 68 in Chountra and 66 in Sialkot.

Table 4

Logistic regression outcomes highlight key risk factors among the rural population of Punjab exposed to antibiotics.

Allergic Symptoms	Unexposed	Exposed	Odds Ratio	Beta	p-value	95% CI ¹
Atopic dermatitis	66	68				
No						
Yes	44	141	3.11	1.135	<0.001	1.92–5.02
Food allergies & related symptoms	68	66				
No						
Yes	42	63	1.85	0.618	0.020	1.09–3.14
Allergic rhino conjunctivitis ²	74	51				
No						
Yes	36	87	3.50	1.255	<0.001	2.06–5.94

¹ 95% Confidence Interval.

² nose and eye problems that occur at least once a week.

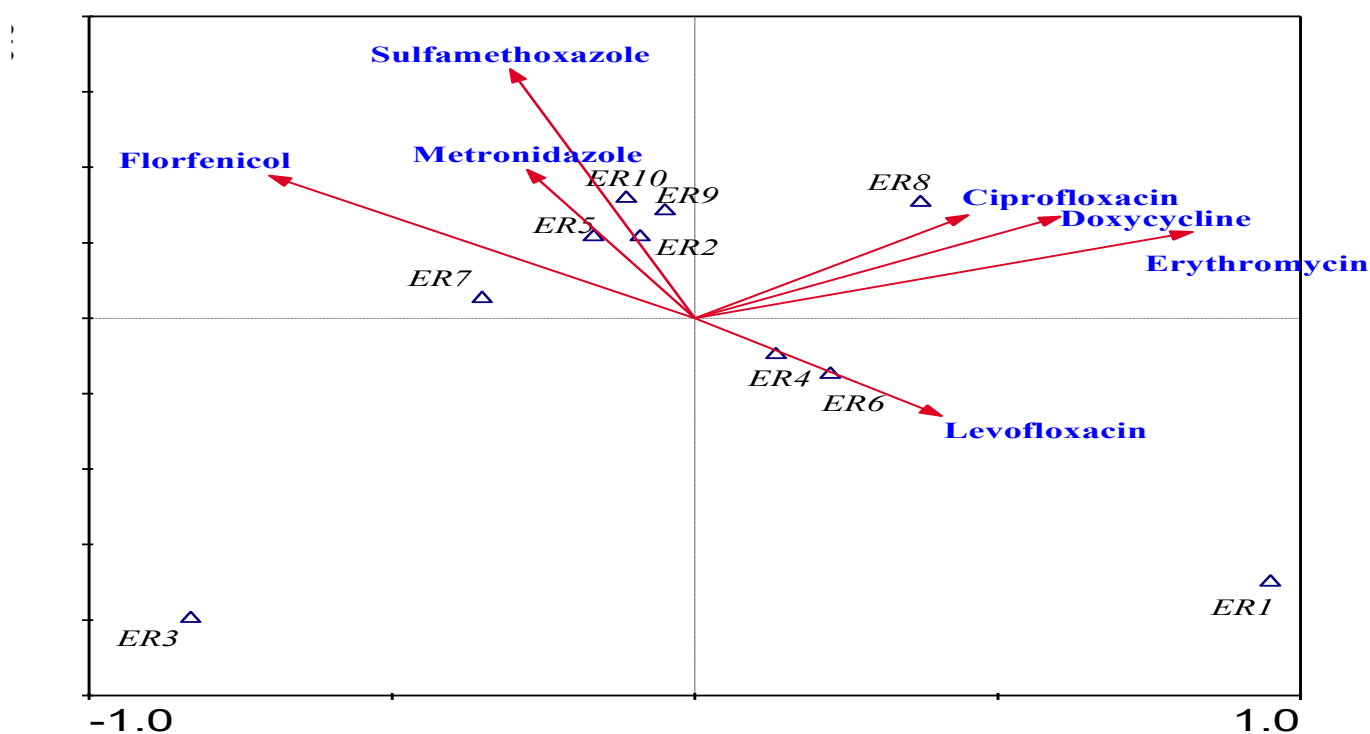


Fig. 6. PCA attributes exposure characteristics across the antibiotic concentration gradient at Chountra sites.

3.6. Logistic Regression and Principal Component Analysis (PCA)

Antibiotic exposure was a predictor variable and was examined using logistic regression, along with the allergy conditions. Principal Component Analysis (PCA) was applied to reduce data dimensionality and to identify dominant associations between environmental contaminants and exposure pathways. According to the findings, the Exposure factor is also a strong predictor of atopic dermatitis, with a large effect size. The Odds ratio (OR) of 3.1 indicates that the odds of reporting atopic dermatitis symptoms among the rural population with considerable exposure are 3.1 times higher than among those

without exposure. Food allergies and associated symptoms were the predictor variable, with the predictive value indicated by an OR of 1.855 ($p = 0.022$), meaning that individuals exposed to the antibiotic were 1.8 times more likely to have food allergies and related symptoms than those who were not

Rural livelihood activities associated with exposure and allergic rhinoconjunctivitis showed a significant correlation ($OR = 3.5$, $p < 0.001$). This indicates that elevated antibiotic exposure in soil increases the likelihood of allergic rhinoconjunctivitis by 3.5 times, with a considerable influence on the risk of other allergic diseases.

A PCA biplot in Figure 6 shows the relationship between the antibiotic concentration gradient and exposure features of Chountra sites. Eigenvectors, shown by the red arrows, indicate the contributions of each antibiotic to the two major components on the biplot. The triangles marked ER1-ER9 are the exposure routes, indicating the possibility of a relationship between the proximity of the triangles to the antibiotic vectors and the antibiotic concentrations. It emerges that the majority of the exposure routes, such as weekly contact frequency of children with soil (ER5), women with wastewater (ER7), and *Amaranthus viridus* as cattle feed (ER10), are limited to FLO, MET, and SUL antibiotic exposure. In Sialkot rural, PCA indicates two trends in exposure characteristics (Figure 7), namely, various avenues of exposure that are more likely to prevail in rural life.

We have identified three principal components (PCs) with eigenvalues greater than 1. These axes explain 23.2, 18.6, and 12.3 per cent of the variance in our data set for PC1, PC2, and PC3, respectively. PCA findings indicate that the best antibiotics, i.e., CIP and DOX, showed a strong association with the first axis, followed by MET and FLO with the second axis (Figure 7). The occurrence of DOX and ERY is evidently associated with exposure factors, such as livestock reliance on local plants (*Euphorbia hirta*) and surface waters (ER1). The exposure of children to wastewater (ER6) was also associated with regions where high concentrations of ERY and DOX were found in the soil. The exposure of females and males to FLO and MET (ER7 and ER8) differs, as it is largely explained by their engagement in cattle bathing (Figure 7).

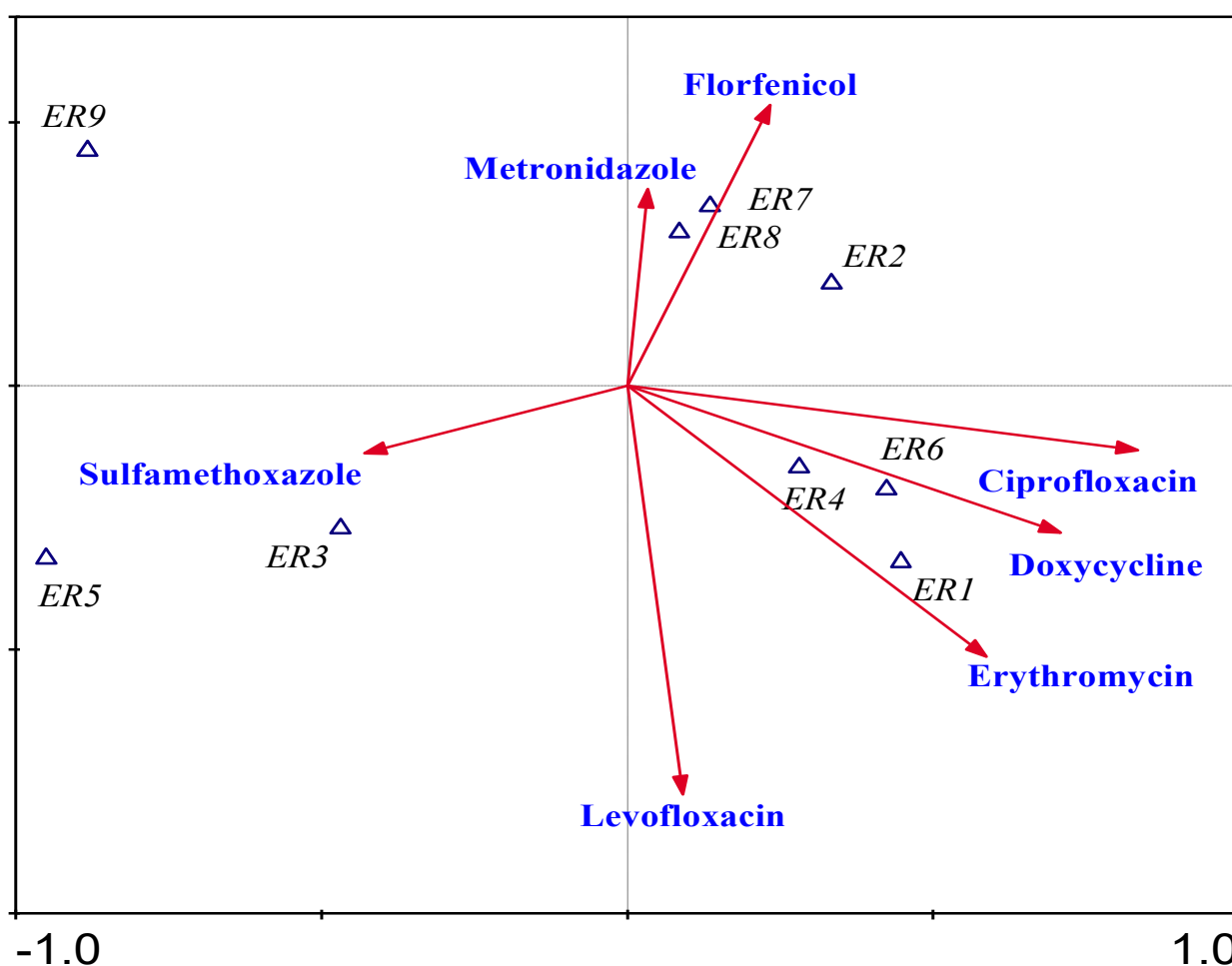


Fig. 7. PCA attributes plot of exposure characteristics along the soil antibiotic concentration gradient at Sialkot sites.

3.7. Heavy Metals – Antibiotics Interaction

This correlation study between the availability of antibiotics and heavy metals in the soil of the Chountra site (Table 5) demonstrates significant correlations between Cd and DOX ($r=0.42$; $p<0.05$) and LEV ($r=0.42$; $p<0.05$). There is a strong negative relationship between Cr and DOX ($r = -0.52$; $p < 0.05$),

associated with low levels of Cr, but a considerable positive relationship with ERY ($r = 0.38$; $p < 0.05$). Interesting positive correlations ($p<0.05$) are found between Pb and DOX, and between FLO (Table 5). Also, Pb and the LEV have a strong correlation ($p<0.05$). In addition, significant negative correlations ($p<0.01$) have been found between soil pH and Cd, and between pH and Pb (Table 5).

Table 5

Correlation analysis of heavy metals, antibiotics, and soil parameters for the Chountra area

	CIP	DOX	ERY	FLO	LEV	MET	SUL	Cd	Cr	Pb	pH	Cl
Cl	1											
D	0.227	1										
E	0.571**	0.247	1									
FL	0.615**	0.318	0.592**	1								
LE	0.354*	0.462*	0.324	0.201	1							
M	-0.136	-0.198	0.208	0.136	-0.121	1						
S	0.438*	0.539**	0.128	0.240	0.028	0.345	1					
C	0.356	0.214	0.119	0.417*	0.446*	0.298	0.366*	1				
Cr	-0.452*	0.221	0.310	-0.562**	-0.058	-0.423*	-0.565**	0.259	1			
P	0.334	0.125	0.441*	0.174	0.392*	0.054	0.403*	0.115	0.336	1		
p	0.576**	0.477*	0.653**	0.182	0.483*	0.348	0.183	-0.332	-0.218	-0.375*	1	
Cl	-0.411*	-0.397*	-0.596**	-0.628**	-0.125	-0.208	-0.325	0.139	0.042	0.343	-0.136	1
O	-0.534**	-0.303	-0.488*	-0.335	-0.417*	-0.251	-0.683**	-0.454*	-0.375*	0.105	-0.347*	0.

Note: * $p < 0.05$; ** $p < 0.01$, Ciprofloxacin=CIP, Doxycycline=DOX, Erythromycin=ERY, Florfenicol=FLO, Levofloxacin=LEV, Metronidazole= MET, Sulfamethoxazole=SUL

Table 6 indicates that CIP levels show highly significant correlations with DOX and ERY ($r=0.87$; $r=0.62$; $p < 0.01$), respectively, in soil from the Sialkot area. The correlation of doxycycline with ERY ($p < 0.01$) and FLO ($p < 0.05$) is also highly significant, and Levofloxacin (LEV) is significantly correlated with ERY ($p < 0.01$) and DOX ($p < 0.05$). The interrelation between heavy metals and antibiotics appears different. The most notable of them were Cd and Pb, then Cr. Doxycycline shows a strong relationship with the three metals, but with Cr it shows an inverse relationship. The other prominent correlation of HMs with antibiotics was that LEV

significantly correlated with Cd and Pb ($p < 0.05$) (Table 6). Cd ($r = -0.63$, $p < 0.01$) and Pb ($r = -0.55$, $p < 0.05$) have highly significant negative correlation with the pH of the soil. The significant negative correlation between soil pH and Cd/Pb indicates that lower pH values likely increase the solubility and mobility of these metals, thereby altering their interaction with organic antibiotic molecules. Conversely, soil organic matter shows a highly significant negative relationship with pH ($p < 0.01$), which is significant because it can influence the accessibility of antibiotics and metals in soil (Table 6).

Table 6

Correlation analysis of heavy metals, antibiotics, and soil parameters for the Sialkot area.

	CIP	DOX	ERY	FLO	LEV	MET	SUL	Cd	Cr	Pb	pH	Clay
Cl	1											
D	0.876**	1										
ER	0.616**	0.847**	1									
FL	0.401*	0.504**	0.361	1								
LE	0.338	0.431*	0.514**	0.142	1							
M	-0.165	-0.085	0.164	0.147	-0.006	1						
SL	0.011	0.127	0.251	0.078	0.239	0.345	1					
Cd	0.221	0.424*	0.187	0.331	0.414*	0.314	0.383*	1				
Cr	0.137	-0.524*	0.384*	0.229	0.172	-0.133	0.170	0.443*	1			
Pb	-0.074	0.408*	0.207	0.583*	0.406*	0.058	0.052	-0.247	0.116	1		
p	0.247	0.270	0.288	0.455*	0.398*	0.169	0.479*	-0.634**	-0.131	-0.552*	1	
Cl	-0.261	-0.414*	-0.409*	-0.277	-0.204	-0.208	-0.233	-0.041	0.068	-0.239	-0.310	1
O	-0.185	-0.264	-0.314	-0.275	-0.394*	-0.165	-0.284	-0.398*	-0.185	-0.376*	-0.497**	0.61

Note: * $p < 0.05$; ** $p < 0.01$, Ciprofloxacin=CIP, Doxycycline=DOX, Erythromycin=ERY, Florfenicol=FLO, Levofloxacin=LEV, Metronidazole=MET, Sulfamethoxazole=SUL

3.8. Diagnostic Ratio Analysis

The findings of the diagnostic ratio analysis (Figure 8) show a curious trend regarding the effect of heavy metal concentration on the availability of antibiotics in soil. As Cd/Pb ratios and pH values increase, the CIP/LEV ratio increases significantly. At slightly acidic conditions (Figure 8a), the antibiotic clusters exhibit a Cd/Pb ratio of 0.1-0.2. The CIP/LEV index shows an intermittent distribution across a wider range of Cd/Pb ratios (0.05-0.2), where soil pH rises

(Figure 8b). At high pH yields with soil pH greater than 8.0, it is evident that the points of the CIP/LEV ratio cluster when the heavy metal ratio is greater than 0.2 (Figure 8c). This implies that the presence of heavy metals also affects the variations of antibiotic retention in the soil. The CIP/LEV index serves as a diagnostic tool for environmental engineers; a ratio clustering above 0.2 in alkaline conditions suggests that metal-mediated sorption is the dominant factor in antibiotic retention over natural degradation.

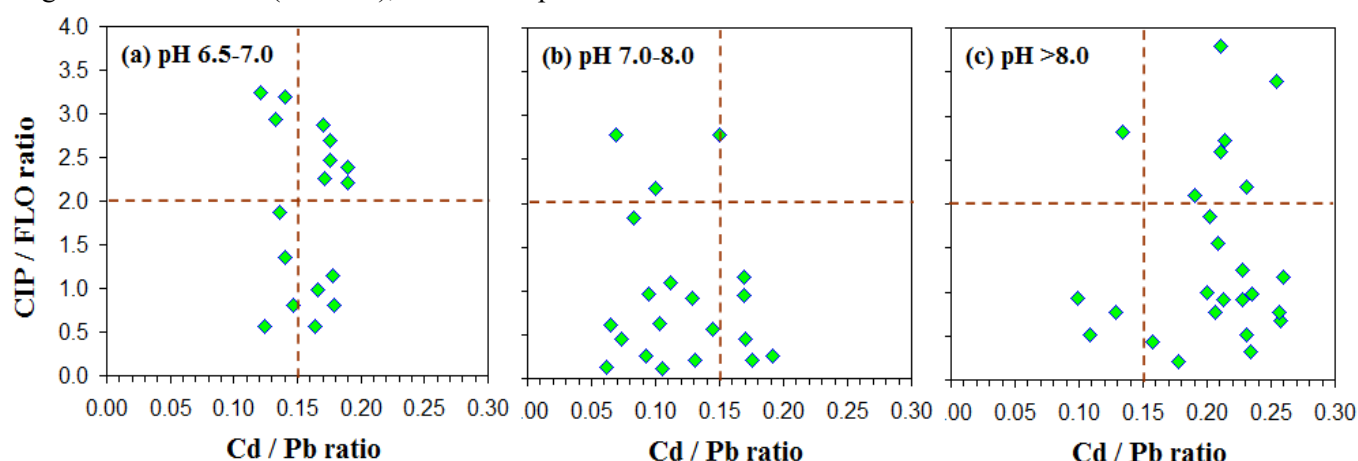


Fig. 8. Mediatory role of heavy metals in governing antibiotics availability in soil, analysed by diagnostic ratio

4. Discussion

The results related to the soil parameters, Chountra and Sialkot, indicate that the reason why the OM content at Chountra is slightly lower than that at Sialkot is the fact that the OM content has an important

effect on the decomposition of xenobiotics and nutrient cycling [21] as well as the mobility of organic contaminants like pesticides [22]. The accessibility of the HMs and antibiotics in the soil in rural areas of Chountra and Sialkot can be explained by several factors. The most likely cause of the high HM

contamination of soil in the rural Chountra area is industrial wastewater discharge near Rawalpindi. Agricultural activities prevail in both regions, which appears to be a good source, as it has been repeatedly reported that animal farming wastes, sewage sludge, and wastewater discharge from pharmaceutical industries can contaminate soils with antibiotics [23].

The results of the differences in the antibiotics taken up by plants in Sialkot and Chountra suggest that plants differ in their ability to take up soil antibiotics, especially *Medicago sativa* and *Euphorbia hirta*. Such variations may be attributed to soil type and plant growth [24], as rural areas have higher soil organic matter content, which has been very conducive to vegetation growth. Also, the herbaceous nature of plants has added to the dynamics of vegetation growth. Given that these two rural regions are likely to have livestock farming, there has been an increase in the application of organic manure to soil, thereby making the rhizosphere environment more favourable for the transfer of antibiotics between soil and plants [25]. The presence of Ciprofloxacin and Levofloxacin in high concentrations in *Medicago sativa* and *Euphorbia hirta* indicates that antibiotic residues are continuously deposited in the soil of both the Chountra and Sialkot rural regions. This may be caused by the closer proximity of cattle farms and the mass of cattle excretion, which often serves as a source of antibiotic release [26,27].

The accumulation of antibiotics in plants is greatly influenced by environmental factors, including improper disposal of sewage and animal waste, as well as the manner in which plants take up antibiotics [28,29]. Nevertheless, low-element homeostasis can also be caused by the presence of antibiotics in soil, which alters the scaling relations between roots and other plant organs, influencing metabolic processes and plant performance [30]. This is likely the reason *Medicago sativa* has become over 2.0 mg g⁻¹ of Ciprofloxacin, Doxycycline, and Erythromycin, given its low stature and high palatability as a grazing animal food. Moreover, two additional plant species with shallow root systems have been found to take up antibiotics through interaction with soil interstitial water, where most organic and inorganic contaminants are present in solution [31-33]. The rural terrain where this study is carried out, antibiotic sorption, mobility, and transport of antibiotic depends on the soil pH and clay dependent micropores that play a major role in the contaminant dynamics in soil as highlighted by the researcher [34,35] which are further affirmed by our correlation analysis especially on soil pH and heavy metals and identified them as contributors to the dynamics of availability of antibiotics in soil to be absorbed by the plants.

The outcome of our Principal Component Analysis (PCA) indicates that the populations in the rural areas of the Chountra and Sialkot sites are mainly exposed to various antibiotic pathways (ER1-ER12), with the most notable being Sulfamethoxazole, Florfenicol, and Metronidazole. The interpretation of this relationship was facilitated by exploring the causal factors and livelihood attributes of the rural population under investigation. For example, it has been reported that the use of treated wastewater for irrigation may lead to the release of antibiotics, such as Sulfamethoxazole, into soils previously subjected to untreated wastewater [36]. When we examined rural sites, we found wastewater irrigation, which could be one of the sources of the development of antibiotic gradients at our study sites. This is because long-term wastewater irrigation has led to significant accumulation of antibiotics such as Ciprofloxacin, Sulfamethoxazole, and Carbamazepine, and may also increase the risk of antibiotic exposure and resistance in the local population [37,38].

The health hazard posed to the population in the Chountra and Sialkot regions due to exposure to antibiotics is directly linked to the rural lifestyle observed in the Punjab Province. This relationship was identified through PCA analysis, which indicated a high concentration of antibiotics in the rural environment. This may pose health hazards to the local population engaged in crop farming and animal husbandry, as well as to individuals who consume their domestic farm products [48]. The research has indicated that these practices subject rural people to antibiotics and amplify the transferable antibiotic resistance genes [39,40]. This raises concerns about the risks it may pose to human and animal health.

The incidence of Sulfamethoxazole, Florfenicol, and Metronidazole in soil through frequent contamination of children's wastewater and the use of wastewater in irrigation, particularly in rural Chountra, and through livestock consumption and bathing in surface water may pose a significant risk. Probably, long-term irrigation with wastewater tends to reduce antibiotic levels in the soil, especially in the surface layer, and raises a special concern for children who regularly come into direct contact with soil [41]. In addition, surface feeders, such as herbaceous vegetation plants found in the countryside areas studied in this paper, are also sources. This suggests a significant health risk due to increased reliance on herbaceous plants as animal feed, as illustrated by the new trend in our PCA results.

The research aims to estimate exposure-based risk factors for disease symptomatology among rural residents of the Chountra and Sialkot districts. The

survey data are processed and prepared for a logistic regression analysis. The odds ratio of 3.1 indicates that the probability of atopic dermatitis is 3 times higher among people residing in rural areas who have stayed in the same community for more than 3 years and whose livelihood activities are rural.

The odds ratio (3.5) indicates that the exposed population is more likely to have allergic rhinoconjunctivitis (nose and eye problems at least once a week) than those who were either inactive or moderately active in their rural lives. As the rural population has become more exposed to antibiotics, both in veterinary and personal use, and the population has a high degree of skin contact with wastewater, the risk of food allergies and the associated symptoms is likely to increase 1.85-fold, based on a significant odds ratio compared to a no-exposure group [48].

In all cases, the regression coefficient b is positive. There is a clear correlation between antibiotic exposure levels and increased illness symptoms.

The results reported here provide a significant study of the correlation between heavy metals and antibiotics. The presence of heavy metals and antibiotics in the surrounding environment has been found to be strongly linked. Interestingly, both Chountra and Sialkot rural areas, which have high rates of antibiotic residues in soil or vegetation, have been affected by heavy metals such as Cd and Pb. It is worth investigating the intricate, multidimensional interactions between antibiotics such as Sulfamethoxazole, Doxycycline, Erythromycin, and Florfenicol in the soil environment, as well as between these antibiotics and heavy metals such as Cd and Pb in soil. Li et al. [42] recently found that the accumulation of antibiotics in soils is associated with soil heavy metals in a concerning manner. This result illustrates the risks that HMs pose to soil ecosystem health. Equally, it has been reported that the mixture of arsenic and sulfamethoxazole is associated with the increased prevalence of antibiotic resistance genes in the gut of earthworms [43] which is a sign of a complex interaction between the level of antibiotics and heavy metals in soil that may cause the impact on the microbial communities [44] and the prevalence of antibiotic resistance genes [45].

One of the livelihood practices in our study has been established in Chountra and Sialkot, where villagers heavily use livestock manure in their farms. This will be a common practice in rural areas and may contaminate the environment with heavy metals and antibiotics, potentially harming the environment. The prolonged use of livestock manure in the rural Punjab poses a potential threat to the environment and may increase population exposure, as its application in

agricultural soil can lead to environmental pollution from heavy metals and antibiotics [46]. Further, it is notable that the heavy metals studied in this paper, such as Cd, Cr, and Pb, are strongly bound to soil colloids and can persist in the environment for long periods. These can affect the flow of other pollutants in the soil, such as antibiotic residues [48]. Therefore, in this case, it is important to find effective soil remedies that will help eradicate or immobilise the heavy metal in the soil. One potential solution would be to use pyrolysis to treat soil contaminated with livestock faeces [47], which appears to be effective at removing metals but can also help remove antibiotics.

It has been identified that the antibiotic concentrations detected in this research varied from one sampling site to another due to differences in wastewater input intensity, soil properties, and irrigation methods. Increased levels in surface soils are indicative of a high retention potential, likely due to adsorption onto organic matter and fine soil fractions. On the contrary, the presence of detectable contaminants in irrigation water indicates the continued movement of contaminants through hydraulic routes.

From the view of an environmental engineer, these results point to the importance of deficient wastewater systems and uncontrolled reuse systems in the persistence of antibiotics in rural environments. The concentration gradient between the soil and water matrices observed provides insight into the transport processes, which are useful for system-scale modelling and optimisation of treatment. Rather than directly attributing effects to health outcomes, the discussion focuses on the importance of environmental exposure channels resulting from failures in engineering systems and management gaps. From a system-scale engineering perspective, the observed contaminant gradients reflect inefficiencies in wastewater conveyance, treatment, and reuse practices, emphasising the need for improved control measures within rural soil–water management frameworks.

5. Conclusions

This research shows that, in rural wastewater reuse, the introduction of antibiotics can be measured by their transport and retention in soil–water systems, highlighting an important environmental engineering problem. The continued presence of these contaminants indicates a constraint in current wastewater management and irrigation practices, rather than a single behavioural factor. The results can be used to justify engineering interventions, including enhanced decentralised treatment, controlled reuse, and soil management approaches that minimise long-

term environmental loading. These findings support the implementation of decentralised wastewater treatment, controlled irrigation strategies, and soil management interventions to reduce long-term contaminant retention and environmental exposure in rural ecosystems.

Author Contributions: Conceptualization, Abid Ali and Audil Rashid; Data curation, Abid Ali, Shahid Mahmood and Tariq Mahmood; Formal analysis, Aniqah Batool, Munnaza Khalid, Ismat Karim and Muhammad Shafiq; Investigation, Abid Ali, Aniqah Batool and Muhammad Shafiq; Methodology, Abid Ali, Audil Rashid; Resources, Abid Ali, Audil Rashid, Shahid Mahmood, Tariq Mahmood, Munnaza Khalid

5. References

- [1] Q. Mahmood, A. Rashid, S. S. Ahmad, M. R. Azim, and M. Bilal, "Current status of toxic metals addition to environment and its consequences," in *The Plant Family Brassicaceae: Contribution Towards Phytoremediation*, 2012, pp. 35–69.
- [2] E. E. Golia, "The impact of heavy metal contamination on soil quality and plant nutrition," *Sustainable Chemistry and Pharmacy*, vol. 33, Art. no. 101046, 2023.
- [3] P. Napoletano, N. Guezgouz, E. Di Iorio, C. Colombo, G. Guerriero, and A. De Marco, "Anthropic impact on soil heavy metal contamination in riparian ecosystems of northern Algeria," *Chemosphere*, vol. 313, Art. no. 137522, 2023.
- [4] J. N. Jannat et al., "Pollution trends and ecological risks of heavy metal(loid)s in coastal zones of Bangladesh: A chemometric review," *Marine Pollution Bulletin*, vol. 191, Art. no. 114960, 2023.
- [5] A. Heydari et al., "Co-selection of heavy metal and antibiotic resistance in soil bacteria from agricultural soils in New Zealand," *Sustainability*, vol. 14, no. 3, Art. no. 1790, 2022.
- [6] X. Wang, B. Lan, H. Fei, S. Wang, and G. Zhu, "Heavy metal could drive co-selection of antibiotic resistance in terrestrial subsurface soils," *Journal of Hazardous Materials*, vol. 411, Art. no. 124848, 2021.
- [7] P. J. Collignon and S. A. McEwen, "One health—Its importance in helping to better control antimicrobial resistance," *Tropical Medicine and Infectious Disease*, vol. 4, no. 1, Art. no. 22, 2019.
- [8] R. S. Singer, R. Finch, H. C. Wegener, R. and Muhammad Shafiq; Supervision, Audil Rashid; Validation, Shahid Mahmood and Tariq Mahmood; Visualization, Audil Rashid; Writing – review & editing, Abid Ali and Audil Rashid. All authors have read and agreed to the published version of the manuscript.
- [9] U. Anand et al., "Potential environmental and human health risks caused by antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs) and emerging contaminants (ECs) from municipal solid waste (MSW) landfill," *Antibiotics*, vol. 10, no. 4, Art. no. 374, 2021.
- [10] X. Pan et al., "Effects of livestock manure on distribution of heavy metals and antibiotics in soil profiles of typical vegetable fields and orchards," *Journal of Ecology and Rural Environment*, vol. 28, no. 5, pp. 518–525, 2012.
- [11] M. H. Wong, "Heavy metal contamination of soils and crops from auto traffic, sewage sludge, pig manure and chemical fertilizer," *Agriculture, Ecosystems & Environment*, vol. 13, no. 2, pp. 139–149, 1985.
- [12] J. Xue et al., "Occurrence of heavy metals, antibiotics, and antibiotic resistance genes in different kinds of land-applied manure in China," *Environmental Science and Pollution Research*, vol. 28, pp. 40011–40021, 2021.
- [13] F. Tam-tam et al., "Assessing the fate of antibiotic contaminants in metal contaminated soils four years after cessation of long-term wastewater irrigation," *Science of the Total Environment*, vol. 409, no. 3, pp. 540–547, 2011.
- [14] Y. Xu, J. Xu, D. Mao, and Y. Luo, "Effect of the selective pressure of sub-lethal level of heavy metals on the fate and distribution of ARGs in the catchment scale," *Environmental*

Pollution, vol. 220, pp. 900–908, 2017.

- [15] M. Ali, T. Ahmad, and A. Rashid, “Phytosociological synthesis as inferred from soil analysis of some industrial areas of Punjab,” *Asian Journal of Plant Sciences*, vol. 3, no. 3, pp. 320–324, 2004.
- [16] A. Rashid, N. Ayub, T. Ahmad, J. Gul, and A. G. Khan, “Phytoaccumulation prospects of cadmium and zinc by mycorrhizal plant species growing in industrially polluted soils,” *Environmental Geochemistry and Health*, vol. 31, pp. 91–98, 2009.
- [17] B. Englert, “Method 1694: Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS,” U.S. Environmental Protection Agency (EPA), 2007, pp. 1–72.
- [18] Y. J. A. B. da Silva, C. W. A. do Nascimento, and C. M. Biondi, “Comparison of USEPA digestion methods to heavy metals in soil samples,” *Environmental Monitoring and Assessment*, vol. 186, pp. 47–53, 2014.
- [19] I. O. Akinyele and O. S. Shokunbi, “Comparative analysis of dry ashing and wet digestion methods for the determination of trace and heavy metals in food samples,” *Food Chemistry*, vol. 173, pp. 682–684, 2015.
- [20] K. Bian et al., “Determination of multi-class antimicrobial residues in soil by liquid chromatography-tandem mass spectrometry,” *RSC Advances*, vol. 5, no. 35, pp. 27584–27593, 2015.
- [21] L. Zhao, Y. H. Dong, and H. Wang, “Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China,” *Science of the Total Environment*, vol. 408, pp. 1069–1075, 2010.
- [22] V. L. R. Pullagurala et al., “Plant uptake and translocation of contaminants of emerging concern in soil,” *Science of the Total Environment*, vol. 636, pp. 1585–1596, 2018.
- [23] L. Michelini et al., “Sulfadiazine uptake and effects in common hazel (*Corylus avellana* L.),” *Environmental Science and Pollution Research*, vol. 22, pp. 13362–13371, 2015.
- [24] M. Ahmad et al., “Phosphorus-loaded biochar changes soil heavy metals availability and uptake potential of maize (*Zea mays* L.) plants,” *Chemosphere*, vol. 194, pp. 327–339, 2018.
- [25] M. Conde-Cid et al., “Occurrence of tetracyclines and sulfonamides in manures, agricultural soils and crops from different areas in Galicia (NW Spain),” *Journal of Cleaner Production*, vol. 197, pp. 491–500, 2018.
- [26] J. Li, H. Zhang, Y. Chen, Y. Luo, and H. Zhang, “Sources identification of antibiotic pollution combining land use information and multivariate statistics,” *Environmental Monitoring and Assessment*, vol. 188, pp. 1–12, 2016.
- [27] L. J. Zhou et al., “Excretion masses and environmental occurrence of antibiotics in typical swine and dairy cattle farms in China,” *Science of the Total Environment*, vol. 444, pp. 183–195, 2013.
- [28] G. Di Marco et al., “Tetracycline accumulates in *Iberis sempervirens* L. through apoplastic transport inducing oxidative stress and growth inhibition,” *Plant Biology*, vol. 16, no. 4, pp. 792–800, 2014.
- [29] M. Pan et al., “Co-application of sewage sludge, Chinese medicinal herbal residue and biochar attenuated accumulation and translocation of antibiotics in soils and crops,” *Sustainability*, vol. 15, no. 8, Art. no. 6972, 2023.
- [30] V. Minden, B. Schnetger, G. Pufal, and S. D. Leonhardt, “Antibiotic-induced effects on scaling relationships and on plant element contents in herbs and grasses,” *Ecology and Evolution*, vol. 8, no. 13, pp. 6699–6713, 2018.
- [31] L. Zeng et al., “The effects and mechanisms of pH and dissolved oxygen conditions on the release of arsenic at the sediment–water interface in Taihu Lake,” *Toxics*, vol. 11, no. 11, Art. no. 890, 2023.
- [32] Q. Sun, S. Lin, G. Liu, and P. Li, “Biochar derived from post-adsorbent for immobilizing Cu and Cd in sediment: The effect on heavy metal species and the microbial community composition,” *Toxics*, vol. 11, no. 8, Art. no. 666, 2023.
- [33] P. De Luca, C. Siciliano, J. B. Nagy, and A. Macario, “Treatment of water contaminated with diesel using carbon nanotubes,” *Applied Sciences*, vol. 13, no. 10, Art. no. 6226, 2023.
- [34] S. Thiele-Bruhn, “Pharmaceutical antibiotic compounds in soils—A review,” *Journal of*

- Plant Nutrition and Soil Science, vol. 166, pp. 145–167, 2003.
- [35] U. Sen, B. Esteves, T. Aguiar, and H. Pereira, “Removal of antibiotics by biochars: A critical review,” *Applied Sciences*, vol. 13, no. 21, Art. no. 11963, 2023.
- [36] M. Carrillo et al., “Desorption of sulfamethoxazole and ciprofloxacin from long-term wastewater-irrigated soils of the Mezquital Valley as affected by water quality,” *Journal of Soils and Sediments*, vol. 16, pp. 966–975, 2016.
- [37] P. Dalkmann et al., “Long-term wastewater irrigation reduces sulfamethoxazole sorption, but not ciprofloxacin binding, in Mexican soils,” *Journal of Environmental Quality*, vol. 43, no. 3, pp. 964–970, 2014.
- [38] M. Broszat et al., “Wastewater irrigation increases the abundance of potentially harmful Gammaproteobacteria in soils in Mezquital Valley, Mexico,” *Applied and Environmental Microbiology*, vol. 80, no. 17, pp. 5282–5291, 2014.
- [39] A. Rashid and M. S. Coyne, “Chronic exposure and the risk of building antibiotic resistance in rural communities of the developing world,” *World Journal of Agriculture & Soil Science*, vol. 1, no. 5, 2019.
- [40] I. D. Kampouris et al., “Treated wastewater irrigation promotes the spread of antibiotic resistance into subsoil pore-water,” *Environment International*, vol. 146, Art. no. 106190, 2021.
- [41] P. Dalkmann, C. Siebe, W. Amelung, M. Schlöter, and J. Siemens, “Does long-term irrigation with untreated wastewater accelerate the dissipation of pharmaceuticals in soil?” *Environmental Science & Technology*, vol. 48, no. 9, pp. 4963–4970, 2014.
- [42] M. Li et al., “Occurrence, spatial distribution and ecological risks of antibiotics in soil in urban agglomeration,” *Journal of Environmental Sciences*, vol. 125, pp. 678–690, 2022.
- [43] H. Wang et al., “Arsenic and sulfamethoxazole increase the incidence of antibiotic resistance genes in the gut of earthworm,” *Environmental Science & Technology*, 2019, doi: 10.1021/acs.est.9b02277.
- [44] Y. Xu, J. Xu, D. Mao, and Y. Luo, “Effect of the selective pressure of sub-lethal level of heavy metals on the fate and distribution of ARGs in the catchment scale,” *Environmental Pollution*, vol. 220, pp. 900–908, 2017.
- [45] X. Wang, B. Lan, H. Fei, S. Wang, and G. Zhu, “Heavy metal could drive co-selection of antibiotic resistance in terrestrial subsurface soils,” *Journal of Hazardous Materials*, vol. 411, Art. no. 124848, 2021.
- [46] M. Pan et al., “Co-application of sewage sludge, Chinese medicinal herbal residue and biochar attenuated accumulation and translocation of antibiotics in soils and crops,” *Sustainability*, vol. 15, no. 8, Art. no. 6972, 2023.
- [47] R. Tian et al., “Preparation of biochar via pyrolysis at laboratory and pilot scales to remove antibiotics and immobilize heavy metals in livestock feces,” *Journal of Soils and Sediments*, vol. 19, pp. 2891–2902, 2019.
- [48] Rana, M. S., Wang, Q., Suzuki, M., Wang, W., Isobe, Y., Sultana, A., & Maduka, T. O. (2025). Distribution of Presumably Contaminating Elements (PCEs) in Roadside Agricultural Soils and Associated Health Risks Across Industrial, Peri-Urban, and Research Areas of Bangladesh. *Sustainability*, 17(21), 9885.