Journal of Animal & Plant Sciences, 35(3): 2025, Page: 855-865
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2025.3.0074

QUANTIFICATION OF SELECTED ANTIBIOTICS IN HUMAN URINE AND EXPOSURE-RESPONSE OF WHEAT TO ENVIRONMENTALLY RELEVANT CONCENTRATIONS

A. N. Barnett[1], R. Zafar[2], S. Akhtar[1], D. Nabi[3] and M. Arshad[1,*]

[1]Institute of Environmental Sciences and Engineering, School of Civil and Environmental Engineering, National University of Sciences and Technology (NUST), Islamabad, Pakistan
[2]Department of Nutritional Sciences and Environmental Design, Faculty of Sciences, Allama Iqbal Open University, Islamabad, Pakistan
[3]GEOMAR Helmholtz Centre for Ocean Research Kiel Wischhofstr. Kiel, Germany
*Corresponding author's email: marshad@iese.nust.edu.pk

ABSTRACT

Soil health is critical for food security and safety, but different sources of pollutants permanently threaten it. Eco-sanitation products like human urine can contribute to the antibiotic burden of soil and consequently affect plant growth. This study aimed to assess the prevalence of five commonly used antibiotics in human urine and the effects of ciprofloxacin on wheat upon exposure to environmentally relevant concentrations. Antibiotics were extracted from the eco-sanitation product (urine) using a liquid-liquid extraction process and analyzed using high-performance liquid chromatography (HPLC). Wheat seeds (n = 15) were treated with ciprofloxacin concentrations (1-50 µg/mL) at an exposure time of 24, 48, and 72 h to assess the effect on seed germination, biomass, root, and shoot lengths. The highest mean concentration in urine was observed for ciprofloxacin (21.24 ± 9.50 µg/mL), followed by sulfamethoxazole (6.47 ± 0.48 µg/mL), levofloxacin (2.91 ± 0.23 µg/mL), and oxytetracycline (1.86 ± 0.26 µg/mL), while the lowest was observed for ofloxacin (1.23 ± 0.45 µg/mL). A significant difference was observed among the means at $P \leq 0.05$. Wheat germination did not show any significant effect upon exposure. The results also showed time and dose-dependent decreases in wheat biomass, root, and shoot lengths. At 48 h and 72 h exposures, the biomass decreased from 0.90 g to 0.40 g and from 1.30 g to 0.70 g with an increase in concentrations from 1 µg/mL to 50 µg/mL, respectively. The root length showed a decrease of 14% to 45% (24 h), 18% to 60% (48 h), and 16% to 63% (72 h) at ciprofloxacin concentrations of 1 to 50 µg/mL, respectively. The shoot length also decreased from 0% to 33% in 24 h, 0% to 14% in 48 h, and 3.0% to 22% in 72 h at a concentration range of 1 to 50 µg/mL, respectively. Antibiotic residues in humans and the phytotoxic effect on wheat can potentially harm plant growth and introduce risks to human health; therefore, public awareness is essential to promote responsible antibiotic use to safeguard human health and the environment.

Keywords: Antibiotics, wheat, phytotoxicity, liquid-liquid extraction.

Published first online May 24, 2025 Published final June 26, 2025

INTRODUCTION

Eco-sanitation products are organic and environmentally friendly substances or materials, also called "wastes" or "resources," that promote sustainable agriculture, improve soil health, and enhance plant growth and productivity (Tilley *et al.*, 2008). Some are directly generated by humans (e.g., urine and feces). These products are typically derived from natural sources, including compost, biofertilizers, and wastewater. Eco-sanitation products can provide various benefits, such as improving soil fertility, increasing nutrient availability, reducing greenhouse gas discharges, minimizing chemical fertilizers and pesticides, enhancing crop yield and quality, and improving soil health and biodiversity. However, these may also contain many pollutants (Barnett *et al.,* 2025), including antibiotics. Antibiotics are a class of drugs particularly used for anti-pathogenic and other activities. They work either as bactericides by killing bacteria or as bacteriostatics by inhibiting the growth and replication of bacterial cells to help the immune system eliminate infection in humans and other animals (Mirzaei *et al*., 2018). Antibiotics function through different mechanisms, including interrupting bacterial cell wall synthesis, preventing protein synthesis, or interfering with DNA replication and metabolic pathways. Antibiotics have transformed modern medicine by providing effective therapies for gram-positive and gram-negative bacteria and other infections. However, the increasing use of antibiotics, either indiscriminately or in a predominant manner, and their unexpected presence in the urine of healthy individuals and wastewaters (Bekoe et al., 2020; Wang et al., 2017, 2018; Zhou et al., 2021) is a global concern. This unfortunate situation suggests that the origin, prevalence, and consequences of the drugs in the urine of people without any apparent infections need further investigation. All of these unmetabolized antibiotics in urine finally enter the environment, as soil and other water bodies act like a sink (Amin et al., 2023; Fiaz et al., 2023).

Yet, antibiotics like ciprofloxacin have become widespread today, where they are given for many medical disorders, including treatment of gram-positive and gram-negative bacteria and other infections (Fakhri Ravari et al., 2022) and even for growth promotion in livestock (Caneschi et al., 2023). Environmental risk management measures such as prohibition, limitation, and emission restriction have been carried out in recent years, and antibiotics have been included in the main control scope as a new emerging environmental contaminant (Liu and Wong, 2013). The release of antibiotics and their metabolites into the surrounding environment due to this abuse has led to the development of antibiotic resistance in the ecosystem (Martínez and Baquero, 2023; Mlugu et al., 2023). The World Health Organization report forecasts that antibiotic resistance will become responsible for more than a million annual deaths by 2050 (Liu et al., 2017), requiring novel solutions to combat this problem (Awad et al., 2025).

The primary pathways by which antibiotics enter the environment are largely influenced by human actions. These include oral and intravenous administration, the release of untreated wastewater from pharmaceutical industries, metabolic processes within the human body, animal manure (Janusch et al., 2014; Yang et al., 2020), and wastewater treatment plants (Mahmood et al., 2019), leading to the contamination of plants and the environment. Research findings indicate that animals take in minimal amounts of antibiotics from their diets because most drug substances end up as unprocessed residues or chemical breakdown products in urine (Levison and Levison, 2009; Kumar et al., 2005).

Usage of urine from wastewater separation plants and sources – separated facilities during agriculture practices carries substantial hazards because of micropollutants like hormones, microplastics, and antibiotics found in urine (Jahedi et al., 2025; Zhou et al., 2021). Human urine, as the main biomarker in drug elimination, contains multiple complex substances which include heavy metals and antibiotics. The human body has become a primary research object to gain knowledge about antibiotic and other micropollutant concentrations in humans. Recent investigations in Ghana have reported the unpredicted detection of antibiotics in the urine of individuals not currently undergoing antibiotic treatment (Bekoe et al., 2020). The prevalence of antibiotics in the urine of healthy participants triggers concerns about their initial introduction into the body. Excessive antibiotic levels found in human specimens likely resulted from leftover drugs in previous treatments, together with animal food consumption because of livestock antibiotic utilization in farming practices and contamination from chemical waste and sewage that pollute the environment (Mithuna et al., 2024; Muurinen et al., 2021).

The complexity of the problem becomes evident from multiple exposure sources, which demand a comprehensive solution approach. The antibiotic Ciprofloxacin, which belongs to the fluoroquinolone class, has been observed with growing frequency in agricultural soil because of its presence in wastewater, human urine, pharmaceutical industry waste, and animal waste. The environmental build-up of ciprofloxacin triggers concerns about its toxic influence on wheat and other staple crops globally. Wheat (*Triticum aestivum* L.) functions as the primary energy food in Asian countries while serving as one of the primary cereal crops for half of worldwide population groups. Farm animals depend on wheat as a major component of their daily diet consumption. The study of antibiotic effects on wheat remains essential because it directly affects plant development therefore further investigation is necessary. Research shows that oxytetracycline antibiotics create severe physical effects on wheat seed germination along with biomass development and root and shoot length measurements (Guo et al., 2021). In Pakistan, ciprofloxacin stands as the primary antibiotic drug available over the counter as well as for veterinary applications (Shams et al., 2024). Research demonstrates that significant levels of this drug appear in chicken meat along with poultry food, according to Jammoul and Darra (2019) and Yang et al. (2020). Research conducted in Pakistan shows different concentrations of ciprofloxacin together with other antibiotic substances discovered in wastewater, rivers, hospital waste, and groundwater (Zafar et al., 2021; Shams et al., 2024; Ashfaq et al., 2016). Soil microorganisms face inhibitory and toxic developmental effects from antibiotics, which deterred seed germination and seed growth (Bao et al., 2022).

Studies have also shown that soil organisms significantly decreased by 22.2% with 10 mg/kg oxytetracycline, and the inhibiting effect increased with an increase in concentrations, drastically affecting the plant's growth (Yang *et al*., 2008). However, the urinary concentrations of antibiotics in humans and the effect on the physiological characteristics of wheat have been scarcely demonstrated, with the fact that urine could be used as liquid fertilizer and a major component of wastewater used in crop irrigation.

Understanding the presence of antibiotics in healthy urine is critical for various reasons. To start with, it sheds light on the persistence of these medications in the human body, posing questions regarding the duration of their effects and potential long-term implications on the environment. Furthermore, it helps to reduce the impact of practices such as agricultural antibiotic use and irrigation with effluent. The main objectives of the present study were; 1) quantification of concentrations of five selected frequently used antibiotics in the urine, an eco-sanitation product, and 2) Evaluation of the effects of different concentrations of ciprofloxacin, which is the most predominant with the highest urinary concentrations in the present study, on the physiological characteristics such as seed germination, biomass, root and shoot length of wheat (*Triticum aestivum* L.). The novelty of this work lies in the fact that it provides insights into the human antibiotic excretion, even in the absence of direct medical treatment, and the effects of ciprofloxacin on key physiological parameters of wheat by offering a comprehensive perspective on how antibiotic residues in urine may contribute to environmental contamination and phytotoxicity.

MATERIALS AND METHODS

Chemical Reagents and Materials: Chemicals and reagents utilized in this work were of analytical grade. Ethyl acetate and methanol were used as solvents for extraction of antibiotics in urine. Pure salt of ciprofloxacin (98% purity) and hydrogen peroxide (3%) were purchased from Sigma Aldrich. For the wheat exposure response, a standard solution of 100 µg/mL of ciprofloxacin was prepared. Concentrations of 1, 10, 20, 30, 40, and 50 µg/mL were obtained by the serial dilution method. Disposable syringe filters with a pore size of 0.45 µm and syringes of 3 mL capacity were used for filtration of urine samples. Ciprofloxacin was selected for its effect on wheat because of its higher urinary concentrations in this study and was the most detected drug in environmental samples in Pakistan (Zafar *et al*., 2021).

Ethical Approval: Informed consent was acquired from every participant prior to sample collection. The decision to participate was fully optional. An ethical clearance was given under reference number (IRB No. 06-2023-ASAB-01/01) to carry out this investigation after the Institutional Review Board (IRB) of the National University of Sciences and Technology (NUST), Islamabad, Pakistan, examined the entire procedure.

Sample Collection and Storage: Five hundred milliliters (500 mL) of fresh morning urine samples were collected randomly from each participant within two days in a prewashed and autoclaved amber bottle from the H-12 NUST community in Islamabad, Pakistan. The samples were collected from eighteen (18) healthy adult male volunteers aged 20 – 45 years who had not been on antibiotics treatment for three (03) months before sample collection. The samples were covered with aluminum foil and stored in an ice box. Subsequently, the samples were transported to the biotechnology laboratory and stored at -50°C according to the protocol described below for further processing and analysis. The Inqalaab-91 variety wheat seeds were purchased from the National Agricultural Research Centre (NARC) of Pakistan and stored in a desiccator.

Extraction and Analysis of Antibiotics from Human Urine: Urine samples were extracted using a liquid-liquid extraction method. During the process, 250 mL of real urine was mixed with 100 mL of ethyl acetate and shaken for 30 minutes in a shaking incubator at ambient temperature (Choi and Dong, 2005). The resulting mixture was placed in a separating funnel. After clearly partitioning the two immiscible liquids, the higher-density liquid, containing urine and antibiotics, was separated, and the extractants were collected in a beaker. The extraction process was repeated twice using 25 mL of ethyl acetate. The total solvent (150 mL) containing ethyl acetate and antibiotics was then evaporated to dryness at 70°C using a rotary evaporator. After evaporation, 3 mL of methanol was added to the residue and shaken for 5 minutes. The resulting extractant was filtered through a 3 mL sterilized, disposable syringe filter with a 0.45 µm pore size and injected into a sample vial for HPLC analysis. The method was optimized ($r^2 >$ 0.969) and a working concentration ranging from 10 to 1000 µg/mL. The limits of detection and quantification were determined to be within the ranges of 0.162–0.958 µg/mL and 0.490–1.811 µg/mL, respectively (Zafar *et al*., 2021).

Quantification of Antibiotics in Human Urine: All five selected antibiotics were detected using Agilent 1260 Infinity (HPLC) equipped with quaternary pump, diode array detector (DAD), Eclipse (4.6 × 250mm) C18, 5µm analytical column and autosampler for auto injection (Zafar et al., 2021). The mobile phase of 1% formic acid and acetonitrile in 84:16 ratio was used to prepare stock solutions of the five antibiotic standards at 1000 µm/mL. Standard stock solutions of each standard were subsequently generated at five concentrations, 10 to 1000 µm/mL. The different concentrations were plotted versus peak area and calibration curves developed. Calibration curves of the slope and the standard deviation of the response were also used to determine the limit of detection (LOD) and the limit of quantification (LOQ). LOD is calculated as 3.3σ/S and LOQ as 10σ/S. Antibiotic quantification was done by comparing the peak areas of the urine samples to the peak areas of the standard solution as in the following equations.

$$\text{Concentration of antibiotics} = \frac{Peak\ Area\ of\ urine\ sample}{Response\ Factor}$$

$$Response\ Factor = \frac{Peak\ Area\ of\ Standard}{Amount\ of\ Standards}$$

Effects of Antibiotic on Seed Germination and Seedling Growth: Wheat was selected for exposure-response studies. Before the seeds were exposed to antibiotics, healthy wheat seeds were treated with 3% hydrogen peroxide for about 30 minutes. Following this, a thorough washing process was undertaken, with the seeds being washed three times with double-distilled water. The seeds were then soaked in distilled water for approximately 12 hours in a Petri dish. For seed germination, 15 healthy wheat seeds were collected and wrapped in moist filter paper with different concentrations of 0, 1, 10, 20, 30, 40, and 50 µg /mL ciprofloxacin, and placed in a Petri dish with a light to dark cycle of 12:12 h at room temperature in an incubator until embryonic roots appeared (Bao *et al*., 2022). Triplicates of each concentration were used with double-distilled water as the controls. Roots with about 2 mm in length after 24 hours were considered germinated wheat. The antibiotic concentrations on the filter paper were optimized each day with 3 ml to enhance germination. After 24 hours, the percentage of germinated wheat seeds in each Petri dish at different CIP concentrations was calculated using the equation below (Bao *et al*., 2022).

$$\%\ Seed\ Germination = \frac{No.of\ Seeds\ Germinated}{Total\ No.of\ Seeds} \times 100$$

To evaluate the effect of ciprofloxacin concentrations on biomass, roots, shoots, and total plant length growth, the seedlings were exposed to different CIP concentrations for 24, 48, and 72 h. Measurements of root, shoot, and total plant lengths were recorded in centimeters (cm) and presented as the mean ± standard deviation from three replicate values. Fresh biomass was measured in grams (g/plant). The percentage decrease in length with respect to the control was evaluated using the formula below.

$$\%\ Decrease\ in\ length = \frac{Initial\ length - Final\ length}{Final\ length} X100$$

For the effect of ciprofloxacin concentrations on wheat biomass (root + shoot), the roots and shoots were sampled exclusively, the fresh biomass was then weighed using a digital chemical balance and the percentage decreased in respect to the original length of the control were calculated.

Statistical Analysis: Descriptive statistics and one-way ANOVA were applied. Mean and percentages were used for analytical measurements. GraphPad Prism 8.0.1 (244) was used for diagrams and analytical analysis.

RESULTS AND DISCUSSION

Concentration of Antibiotics in Urine: Table 1 presents the mean concentrations of five commonly used antibiotics from 18 healthy male volunteers aged 20 to 45 years. The detection rates of antibiotics varied between 50% and 72%. Sulfamethoxazole exhibited the highest occurrence, detected in 72% of the samples with an average mean concentration (AMC) of 6.47±0.31 µg/mL; the highest and lowest mean concentrations (28.6 ± 0.83 and 1.16 ± 0.87 µg/mL) were observed in samples 6 and 2, respectively (Table 1). Subsequently, ciprofloxacin and ofloxacin were detected in 60% of the samples with an average mean concentration of 12.11±0.64 and 1.23±0.35 µg/mL, respectively. The highest concentrations of ciprofloxacin (46.8±0.08 µg/mL) and ofloxacin (4.60±0.88 µg/mL) were observed in samples 13 and 4. In contrast, the lowest were identified in samples 15 and 16 (1.79±0.93 and 0.25±0.17 µg/mL), respectively (Table 1). Additionally, the detection rate of levofloxacin was 61%, with an overall concentration of 2.91±0.21 µg/mL. The maximum and minimum concentrations (15.85±0.05 and 0.10±0.70 µg/mL) were observed in samples 11 and 10, respectively. Furthermore, oxytetracycline had the lowest detection rate at 50%, with a cumulative concentration of 1.86±0.26 µg/mL. The highest and lowest concentrations (15.12±0.63 and 0.60±0.02 µg/mL) were displayed by samples 5 and 7, respectively. The concentration ranges across all samples were as follows: ciprofloxacin (0–47 µg/mL), levofloxacin (0–16 µg/mL), ofloxacin (0–5 µg/mL), oxytetracycline (0–15 µg/mL), and sulfamethoxazole (0–29 µg/mL). A statistically significant difference ($P = 0.0005$ and $F = 5.538$) was observed among the means at $P \leq 0.05$.

In this investigation, five antibiotics identified were from three different groups, namely sulfonamide, tetracycline, and fluoroquinolones, in the urine of healthy adult males, aged 20 to 45 years. The percentage detection and concentrations in this study were measured and compared to those of a similar previous study (Zhou *et al*., 2021). Results indicated a detection rate of 59% generally across the data. The detection percentage exhibited an ascending pattern with SMX at the highest levels followed by LEV then CIP and OFC and lastly OTC. The antibiotic detection analysis indicated that SMX appeared as the most prevalent substance while OTC registered the lowest detection rates at 72% and 50% respectively. Research by Zhou et al. (2021) detected lower OTC levels in Jiangsu, China among children and pregnant women than this study revealed. Study variations in human urine antibiotic detection and exposure rates could be resulted from multiple factors that include human and animal antibiotic overuse along with antibiotic policy and environmental food safety conditions, and participant dietary habits (Huang et al., 2013). The average concentrations of the different antibiotics in the urine samples followed the order: ciprofloxacin > sulfamethoxazole > levofloxacin > oxytetracycline > ofloxacin. A significant difference ($P_= 0.0005$) was observed in the mean concentrations of antibiotics. The relatively higher concentrations could be attributed to their frequently high concentrations found in most environmental samples, foodstuffs and the extensive usage as veterinary substitutes in animal breeding (Azanu *et al*., 2018; Kong *et al*., 2021; Mahmood *et al*., 2019; Yang *et al*., 2020). However, the antibiotic concentrations in this study were relatively higher compared to those reported for healthy individuals in Ghana (Bekoe *et al*., 2020). The variation in the concentrations could be attributed to the concentration of antibiotics in foods, pharmacokinetic properties of the antibiotics, age, type of antibiotics, hepatic functioning (body ability to metabolize antibiotics), and renal clearances by the kidney (Levison and Levison, 2009). For instance, high concentrations of antibiotics are generally recovered in the urine of individuals with kidney challenges. A study showed that after an oral dose of 500 mg of levofloxacin, healthy individuals recovered 343 mg/mL of it in the urine within 12 to 24 h (Rodvold and Neuhauser, 2001). However, most antibiotics have a recovery percentage in human urine, ranging from 11% to 76% (Rodvold and Neuhauser, 2001). Antibiotic levels in human urine could also depend on the drugs' bioavailability in the body. Antibiotics like fluoroquinolones, tetracycline, and sulfamethoxazole have excellent bioavailability after oral and intravenous administration. For instance, penicillin G, which is unstable in gastric acid during oral administration, has a bioavailability of < 30%, while penicillin v, which is more acid-stable, has a bioavailability ranging from 60% to 70%. On the other hand, ampicillin has a higher oral bioavailability of 74% to 92%, while ampicillin has a bioavailability of as low as 30% to 55% (Levison and Levison, 2009). Therefore, a higher antibiotic concentration in healthy individuals who have not taken any antibiotics for more than one month could be attributed to the cumulative concentrations in the various foods consumed. Notably, previous studies have identified antibiotics in wastewater (Zafar *et al*., 2021; Mirzaei *et al*., 2018), chicken meat (Jammoul and Darra, 2019), milk, fish, drinking water (Li *et al*., 2017) and vegetables (Tadić *et al*., 2021). However, these exposures raise concerns about the potential risk of developing antibiotic resistance among participants (Caneschi *et al*., 2023). Similar investigations have also reported the presence of antibiotics in various populations, including healthy individuals, children, and pregnant women (Bekoe *et al*., 2020; Wang *et al*., 2017, 2018; Zhou *et al*., 2021).

Table 1: Concentrations (µg mL^{-1}) and standard deviation (SD) of ciprofloxacin (CIP), levofloxacin (LEV), ofloxacin (OFC), oxytetracycline (OTC), and sulfamethoxazole (SMX), in eighteen (18) human urine samples. S1-S18 indicate the sample identification numbers. AMC: Average mean concentration.

Sample ID	**Concentration (µg/mL) (Mean ± SD)**				
	Ciprofloxacin	Levofloxacin	Ofloxacin	Sulfamethoxazole	Oxytetracycline
S1	0.00 ± 0.00	1.59 ±0.19	0.00 ±0.00	13.38 ±0.21	0.00 ±0.00
S2	24.28 ± 1.01	0.00 ±0.00	1.25 ±0.51	1.16 ±0.87	0.00 ±0.00
S3	20.27 ± 0.69	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	3.14 ±1.26
S4	0.00±0.00	1.66 ±0.29	4.60 ±0.88	1.45 ±0.05	4.60 ±0.88
S5	0.00 ± 0.00	0.00 ±0.00	0.00 ±0.00	4.19 ±0.08	15.12 ±0.63
S6	0.00 ± 0.00	0.00 ±0.00	0.00 ±0.00	28 63 ±0.83	1.34 ±0.88
S7	23.50 ± 1.05	0.00 ±0.00	0.59 ±0.11	16.11 ±0.20	0.60 ±0.01
S8	0.00 ± 0.00	1.25 ±0.20	0.40 ±0.21	8 23 ±0.15	0.00 ±0.00
S9	11.5 ± 4.41	3.76 ±1.39	1.87 ±1.77	0.00 ±0.00	0.00 ±0.00
S10	13.45 ± 1.85	0.90 ±0.70	0.00 ±0.00	0.00 ±0.00	1.64 ±0.61
S11	0.00 ± 0.00	15.85 ±0.05	4.03 ±0.04	12.32 ±0.17	0.00 ±0.00
S12	0.00 ± 0.00	3.95 ±0.01	1.53 ±1.28	3.16 ±0.41	0.00 ± 0.00
S13	46.82 ± 0.08	14. 65 ±0.16	2.90 ±0.06	0.00 ±0.00	0.00 ± 0.00
S14	9.04 ± 1.08	4.90 ±0.07	0.89 ±0.03	0.00 ±0.00	0.00 ± 0.00
S15	1.79 ± 0.93	0.00 ±0.00	1.89 ±1.11	17.91 ±0.16	5.09 ±0.09
S16	42.33 ± 0.15	1.35 ±1.04	0.25 ±0.17	2.23 ±1.93	0.00 ±0.00
S17	24.93 ± 0.35	0.00 ±0.04	0.00 ±0.00	4.88 ±0.34	0.60 ±0.02
S18	0.00 ± 0.00	2.27 ±0.04	2.00 ±0.04	2.75 ±0.14	1.09 ±0.34
TAM	**12.11 ±0.26**	**2.91 ±0.23**	**1.23 ±0.35**	**6.47 ±0.31**	**1.86 ±0.26**

Effect of Ciprofloxacin on Wheat Germination: Figure 1 illustrates the effect of ciprofloxacin exposure on wheat seed germination. The percentage of germinated wheat seeds varied between 70% and 74% across different ciprofloxacin concentrations. The germination percentage indicated no significant effect upon exposure to different concentrations compared with the control, even at higher concentrations. The insignificant changes in percentage germination compared with the control indicated that ciprofloxacin concentrations had very small or no phytotoxic effects on wheat seed germination. The results of these findings are aligned with a study conducted in the Shanxi province in China, in which wheat seeds were exposed to oxytetracycline and enrofloxacin, showing limited effect at concentrations greater than 40 µg/mL (Li *et al*., 2023). Similar findings were also observed with different concentrations of tetracycline exposure ranging from 1-10 µg/mL in a study conducted in Moscow (Shorina *et al*., 2023). However, the percentage reduction in seed germination was not noticed because studies have shown that the most noticeable percentage decrease in seed germination occurs at antibiotic concentrations ranging from 50 to 300 µg/mL (Shorina *et al*., 2023). On the other hand, this finding contrasts a study conducted in China by Bao *et al*. (2022), which showed that oxytetracycline at higher concentrations of 50–150 µg/mL induced phytotoxic effects on wheat seeds during germination (Bao *et al*., 2022). Similar results were also observed by Minden *et al*. (2017). The variation in these results underscored that different antibiotic concentrations affected wheat seeds differently during germination, sparking further interest and engagement in this area of research.

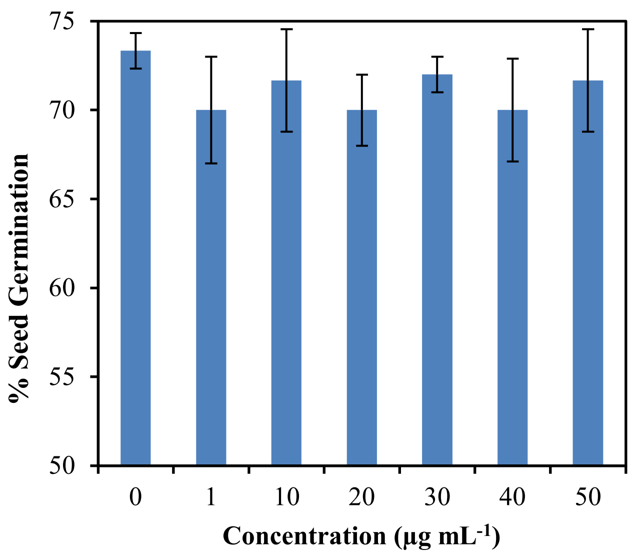

Figure 1: Effects of different concentrations of ciprofloxacin on seed germination percentage. The control is depicted with zero conc.

Effects of Ciprofloxacin on Seedling Growth Parameters: Figures 2, 3, and 4 illustrate the effect of varying ciprofloxacin concentrations on the physical growth parameters of wheat seedlings over different exposure durations. Measurements of root, shoot, and total plant lengths were recorded in centimeters (cm) and presented as the mean ± standard deviation from three replicates, as shown in Figures 2, 3, and 4. Fresh biomass (figure 5) was measured in grams (g/plant). However, it was observed that as the concentrations of ciprofloxacin increased, the plant growth parameters were negatively affected compared to the control. Growth parameters were expressed as a percentage decrease relative to the controls for 24, 48, and 72 h of exposure at concentrations ranging from 1 to 50 µg/mL. The percentage decrease in root lengths varied with both ciprofloxacin concentration and exposure time.

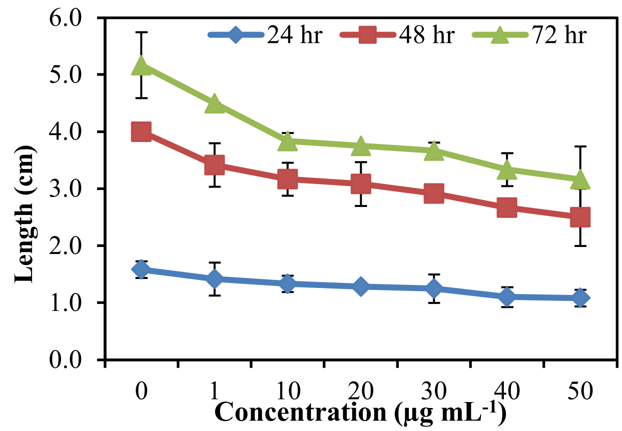

Figure 2: Effects of CIP concentrations on wheat root length after an exposure timeline of 24, 48, and 72 h. Zero (0 µg mL^{-1}) represents the control.

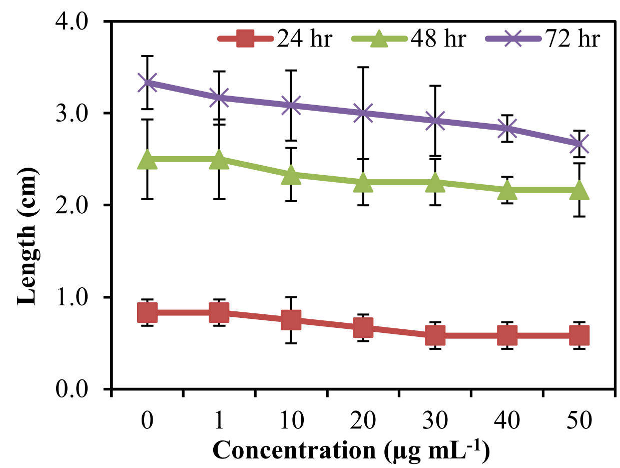

Figure 3: Effects of CIP concentrations on wheat shoot length after an exposure timeline of 24, 48 and 72 h. Zero (0 µg mL^{-1}) represents the control.

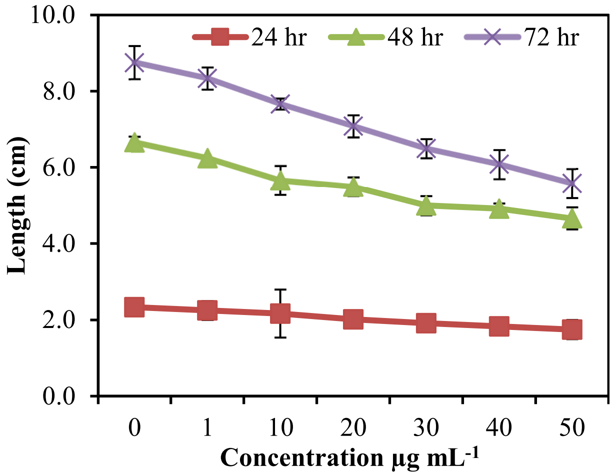

Figure 4: Effects of CIP concentrations on plant length after an exposure timeline of 24, 48, and 72 hours. The zero (0 µg mL^{-1}) represents the control.

A 24-h exposure showed a decrease of 14%, 23%, 23%, 23%, 45%, and 45% in root length at ciprofloxacin concentrations of 1, 10, 20, 30, 40, and 50 µg/mL, respectively. For 48-h exposure, the root length decreased by 18%, 25%, 29%, 38%, 48%, and 60% at the same concentrations. In 72-h exposure, a further reduction of 16%, 37%, 37%, 41%, 58%, and 63% was recorded.

The results demonstrated dose- and time-dependent inhibitory effects of ciprofloxacin on root growth. Similar trends were observed for the shoot and total plant lengths. On the other hand, fresh biomass was not significantly affected during 24-h exposure at the different antibiotic concentrations. However, at 48 and 72-h exposures, the biomass fresh weight decreased from 0.90 g to 0.40 g at 1 µg/mL and from 1.30 g to 0.70 g at 50 µg/mL compared to the control, respectively (Figure 5).

The highest decrease in the root (3.2 cm, 63%) and shoot lengths (2.7 cm, 22%) compared to the control (5.2 cm and 3.3cm), respectively, were observed at 50 µg/mL during 72-h exposure. However, the reduction of root lengths was more sensitive to ciprofloxacin exposure compared to shoots. This indicated that exposure of wheat seedlings to elevated concentrations of ciprofloxacin may have a toxic effect on wheat growth parameters, thereby inhibiting the process of cell division. Studies have revealed that antibiotics accumulated in soil may significantly influence the early seedling development of wheat by interfering with the activities of soil microorganisms (Cycoń and Mrozik, 2019), and cellular and physiological processes (Bao *et al*., 2022). Similar work by Khan *et al*. (2021) had also shown that *B. rapa ssp. chinensis* growth was inhibited in the soil contaminated with antibiotics. Their results also revealed that plant length decreased by 12% to 20%, and fresh biomass significantly decreased by 7.0% to 12% compared to the control plants at a concentration of 100 mg/kg for 24 h (Khan *et al*., 2021). In this study, the percentage reduction in wheat fresh biomass was also observed at higher concentrations and exposure times of 48 and 72 h, as presented in Figure 5. The percentage decrease in length and biomass is dependent on the type of plant, exposure time, and antibiotics used. The decrease in seedling biomass is directly related to the reduction in root and shoot lengths. The findings of this study aligned with reported work in China with wheat and maize seedlings, in which a significant decrease in biomass on exposure to individual antibiotics was recorded at 5 mg/kg (Zhang *et al*., 2024). Similar studies were also conducted with vegetables in China using three different antibiotics. The findings showed a decrease in fresh weight of shoots and roots of lettuce and Chinese cabbage (Chi *et al*., 2018).

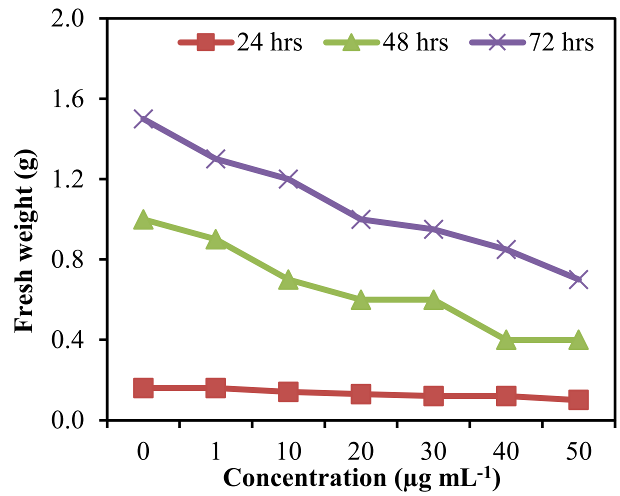

Figure 5: Effects of CIP concentrations on wheat biomass after an exposure timeline of 24, 48, and 72 hours. The zero (0 µg mL^{-1}) represents the controls

Environmental Implications: The implication of this investigation has serious environmental and agricultural consequences. While urine and wastewater use for irrigation can contribute to sustainable nutrient recycling, introducing antibiotics into agricultural systems poses risks to human and plant health and food safety. Specifically, ciprofloxacin in agricultural environments poses substantial risks to crop health and productivity. Ciprofloxacin and many other antibiotics have serious phytotoxic effects on wheat germination and early development. High concentrations greater than 50 µg/mL can cause significant reductions in germination rates, yields, root and shoot lengths, and overall biomass. Understanding these effects is of utmost importance for developing sustainable agricultural practices and minimizing antibiotic contamination by developing stringent regulations on antibiotic release into the environment. Since antibiotic pollution in food, water, and the environment is a serious global concern for environmental and health authorities, controlling antibiotic pollution in the environment is key in preventing antibiotic contamination. Some of the techniques used in controlling antibiotic pollution include biodegradation where bacteria and fungi work to break down antibiotics into non-toxic compounds. Plant-based biochar appears to be another option, involving the use of plant materials to produce biochar. Similarly, the magnetic biochar method developed by some researchers (Oladipo and Ifebajo, 2018) by mixing metals with biochar through high energy milling ball with iron oxide, potentially enables the quick and effective magnetic separation of biochar from antibiotic pollutants and wastewater. Adsorptive removal has also been reported to address antibiotic pollution (Zafar *et al*., 2023; 2024). As the problem is recent, all the methods to address the issue are also emergent and developing quickly. Techniques such as bioremediation and improved wastewater treatment and management systems could lessen these impacts, but further research and development in this field are needed to develop an integrated approach.

Conclusions: Overall, the concentration of five commonly used antibiotics and the effect of ciprofloxacin on the physical characteristics of wheat were investigated. Elevated concentrations were detected in some urine samples. The findings of this study revealed high detection rates ranging from 50% to 72% in urine samples, with sulfamethoxazole and ciprofloxacin having the highest detection rates of 72% and 60%, respectively. Ciprofloxacin negatively affected the growth parameters of wheat. Antibiotics in human urine and their phytotoxic effect on wheat cultivation have the potential to contaminate the environment, harm plant growth, disrupt ecosystems, and introduce risks to human health. Such a situation poses a significant challenge to global health, potentially compromising the efforts to combat antibiotic resistance in the developing countries context.

Declaration of Interest statement: The authors declare no conflict of interest that may influence the work reported in this manuscript.

Acknowledgements: The authors thank the Organization of Islamic Countries (OIC) through the Higher Commission Education of Pakistan in collaboration with the government of Sierra Leone, for providing the scholarship to Mr. Alfred N. Barnett.

REFERENCES

Amin, A., M. Manzoor, M.W. Ramay, A. Hassan, K. Hina, A. Syed, A.H. Bahkali and M. Arshad (2023). Metallic nanoparticles photodegraded antibiotics and co-application improved wheat growth and nutritional quality through stress alleviation. Chemosphere 323: 138189. https://doi.org/10.1016/j. chemosphere.2023.138189

Ashfaq, M., K.N. Khan, S. Rasool, G. Mustafa, M. Saif-Ur-Rehman, M.F. Nazar, Q. Sun and C. Yu (2016). Occurrence and ecological risk assessment of fluoroquinolone antibiotics in hospital waste of Lahore, Pakistan. Environmental Toxicology and Pharmacology 42: 16-22. https://doi.org/10.1016/j.etap.2015.12.015

Awad, M.A., K.M.O. Ortashi, A. Hagmusa, B. Hagmusa, E.M. Ibrahim, G. Al-Sowygh, H. Al-Shehri and R. Ramadan (2025). Comparative analysis of Adansonia digitata nanoparticle and encapsulation: synthesis, characterization, antimicrobial, and anticancer assessment. J. Anim. and Plant Sciences, 35(1): 49-57. https://doi.org/10.36899/JAPS.2025.1.0003

Azanu, D., B. Styrishave, G. Darko, J.J. Weisser and R.C. Abaidoo (2018). Occurrence and risk assessment of antibiotics in water and lettuce in Ghana. Science of the Total Environment 622–623: 293–305. https://doi.org/10.1016/j.scitotenv.2017.11.287

Bao, Y., C. Pan, D. Li, A. Guo and F. Dai (2022). Stress response to oxytetracycline and microplastic-polyethylene in wheat (Triticum aestivum L.) during seed germination and seedling growth stages. Science of the Total Environment 806: 150553. https://doi.org/10.1016/j.scitotenv.2021.150553

Barnett, A.N., A. Irum, D. Nabi, M.I. Khawar and M. Arshad M (2025). Occurrence of some heavy metals in drinking water, vegetables, and urine in Rawalpindi and Islamabad, Pakistan-Human health risk assessment. International J. Environmental Research 19:10. https://doi.org/10.1007/s41742-024-00686-3.

Bekoe, S.O., E. Orman, S. Asare-Nkansah, A.M.L. Sørensen, E. Björklund, R.K. Adosraku, M. Hansen and B. Styrishave (2020). Detection and quantification of antibiotic residues in urine samples of healthy individuals from rural and urban communities in Ghana using a validated SPE-LC-MS/MS method. SN Applied Sciences, 2(11): 1–11. https://doi.org/10.1007/s42452-020-03742-7

Caneschi, A., A. Bardhi, A. Barbarossa and A. Zaghini (2023). The use of antibiotics and antimicrobial resistance in veterinary medicine, a complex phenomenon: A narrative review. Antibiotics, 12(3). https://doi.org/10.3390/antibiotics12030487

Chi S.L., W.Z. Wang, W.H. Xu, T. Li, Y.H. Li and C.L. Zhang (2018). Effects of tetracycline antibiotics on growth and characteristics of enrichment and transformation in two vegetables. Huan Jing Ke Xue 39(2): 935-943. Chinese. doi: 10.13227/j.hjkx.201706250.

Choi, C.K. and M.W. Dong (2005). Sample preparation for HPLC analysis of drug products. Separation Science and Technology 6(C): 123–144. https://doi.org/10.1016/S0149-6395(05)80049-2

Cycoń M. and A. Mrozik (2019). Antibiotics in the Soil environment—degradation and their impact on microbial activity and diversity. Frontiers in Microbiology 10: 412419. https://doi.org/10.3389/fmicb.2019.00338

FakhriRavari A., B. Simiyu, T. Morrisette, Y. Dayo and J.C. Abdul-Mutakabbir (2022). Infectious disease: How to manage Gram-positive and Gram-negative pathogen conundrums with dual beta-lactam therapy? Drugs in Context 11: 1–18. https://doi.org/10.7573/dic.2021-8-9

Fiaz, M., I. Ahmed, S.M. Hassan, A.K. Niazi, M.F. Khokhar, Zeshan, M.A. Farooq and M. Arshad (2023). Antibiotic induced changes in nitrogen metabolism and antioxidative enzymes in mung bean (Vigna radiata). Science of the Total Environment 873: 162449. https://doi.org/10.1016/j.scitotenv.2023.162449

Guo H., J. Gu, X. Wang, Z. Song, X. Qian, W. Sun, M. Nasir and J. Yu (2021). Negative effects of oxytetracycline and copper on nitrogen metabolism in an aerobic fermentation system: Characteristics and mechanisms. J. Hazardous Materials 403: 123890. https://doi.org/10.1016/j.jhazmat.2020.123890

Guo, A., C. Pan, X. Su, X. Zhou and Y. Bao (2022). Combined effects of oxytetracycline and microplastic on wheat seedling growth and associated rhizosphere bacterial communities and soil metabolite profiles. Environmental Pollution 302: 119046. https://doi.org/10.1016/j.envpol.2022.119046

Huang, Y., J. Gu, M. Zhang, Z. Ren, W. Yang, Y. Chen, Y. Fu, X. Chen, J.W.L. Cals and F. Zhang (2013). Knowledge, attitude, and practice of antibiotics: a questionnaire study among 2500 Chinese students. BMC Medical Education 13: 163. https://doi.org/ 10.1186/1472-6920-13-163.

Jahedi, F., N.J. Haghighi Fard, N. J., Ahmadi, M., A. Takdastan, , M.H. Shoushtari, R. Dehbandi and A. Turner (2025). Microplastics in urine, sputum and lung lavage fluid from patients with respiratory illnesses. Environmental Research, 274, 121278. https://doi.org/10.1016/j.envres.2025.121278.

Jammoul, A. and N. El Darra (2019). Evaluation of antibiotics residues in chicken meat samples in Lebanon. Antibiotics 8(2): 69. https://doi.org/10.3390/antibiotics8020069.

Janusch, F., G. Scherz, S.A.I. Mohring and G. Hamscher (2014). Determination of fluoroquinolones in chicken feces - A new liquid-liquid extraction method combined with LC-MS/MS. Environmental Toxicology and Pharmacology 38(3): 792–799. https://doi.org/10.1016/j.etap.2014.09.011.

Khan K.Y., B. Ali, S. Zhang, P.J. Stoffella, S. Yuan, Q. Xia, H. Qu, Y. Shi, X. Cui and Y. Guo (2021). Effects of antibiotics stress on growth variables, ultrastructure, and metabolite pattern of Brassica rapa ssp. Chinensis. Science of the Total Environment 778: 146333. https://doi.org/10.1016/j.scitotenv.2021.146333

Kong, M., Y.Q. Bu, Q. Zhang, S.H. Zhang, L.Q. Xing, Z.Q. Gao, F.Z. Bi and G.J. Hu (2021). Distribution, abundance, and risk assessment of selected antibiotics in a shallow freshwater body used for drinking water, China. J. Environmental Management 280: 111738. https://doi.org/10.1016/j.jenvman.2020.111738

Kumar, K., S.C. Gupta, S.K. Baidoo, Y. Chander and C.J. Rosen (2005). Antibiotic uptake by plants from soil fertilized with animal manure. J. Environmental Quality, 34(6): 2082-2085. https://doi.org/10.2134/jeq2005.0026

Levison, M.E. and J.H. Levison (2009). Pharmacokinetics and Pharmacodynamics of antibacterial agents. Infectious Disease Clinics of North America 23(4): 791–815. https://doi.org/10.1016/j.idc.2009.06.008

Li, N., K.W.K. Ho, G.G. Ying and W.J. Deng (2017). Veterinary antibiotics in food, drinking water, and the urine of preschool children in Hong Kong. Environment International 108: 246–252. https://doi.org/10.1016/j.envint.2017.08.014

Li, L., T. Li, Y. Liu, L. Li, X. Huang and J. Xie (2023). Effects of antibiotics stress on root development, seedling growth, antioxidant status and abscisic acid level in wheat (Triticum aestivum L.). Ecotoxicology and Environmental Safety 252: 114621. https://doi.org/10.1016/j.ecoenv.2023.114621

Liu, S., G. Zhao, H. Zhao, G. Zhai, J. Chen and H. Zhao (2017). Antibiotics in a general population: relations with gender, body mass index (BMI), and age and their human health risks. Science of the Total Environment 599-600: 298-304. https://doi.org/10.1016/j.scitotenv.2017.04.216

Liu, J. and M. Wong (2013). Pharmaceuticals and personal care products (PPCPs): A review on environmental contamination in China. Environment International 59: 208-224. https://doi.org/10.1016/j.envint.2013.06.012

Mahmood, A.R., H.H. Al-Haideri and F.M. Hassan (2019). Detection of antibiotics in drinking water treatment plants in Baghdad City, Iraq. Advances in Public Health 2019: https://doi.org/10.1155/2019/7851354

Martínez, J.L. and F. Baquero (2023). What are the missing pieces needed to stop antibiotic resistance? Microbial Biotechnology 16(10): 1900–1923. https://doi.org/10.1111/1751-7915.14310

Minden, V., A. Deloy, A.M. Volkert, S.D. Leonhardt and G. Pufal (2017). Antibiotics impact plant traits, even at small concentrations. AoB Plants 9(2): plx010. https://doi.org/10.1093/aobpla/plx010

Mirzaei, R., M. Yunesian, S. Nasseri, M. Gholami, E. Jalilzadeh, S. Shoeibi and A. Mesdaghinia (2018). Occurrence and fate of most prescribed antibiotics in different water environments of Tehran, Iran. Science of the Total Environment 619-620: 446-459. https://doi.org/10.1016/j.scitotenv.2017.07.272

Mithuna, R., R. Tharanyalakshmi, I. Jain, S. Singhal, D. Sikarwar, S. Das, J. Ranjitha, D. Ghosh, M.M. Rahman and B. Das (2024). Emergence of antibiotic resistance due to the excessive use of antibiotics in medicines and feed additives: A global scenario with emphasis on the Indian perspective. Emerging Contaminants, 10(4): 100389. https://doi.org/10.1016/j.emcon.2024.100389

Muurinen, J., J. Richert, C.L. Wickware, B. Richert and T.A. Johnson (2021). Swine growth promotion with antibiotics or alternatives can increase antibiotic resistance gene mobility potential. Scientific Reports, 11(1), 1-13. https://doi.org/10.1038/s41598-021-84759-9

Mlugu, E.M., J.A. Mohamedi, R.Z. Sangeda and K.D. Mwambete (2023). Prevalence of urinary tract infection and antimicrobial resistance patterns of uropathogens with biofilm forming capacity among outpatients in Morogoro, Tanzania: a cross-sectional study. BMC Infectious Diseases 23(1): 1–9. https://doi.org/10.1186/s12879-023-08641-x

Oladipo, A.A. and A.O. Ifebajo (2018). Highly efficient magnetic chicken bone biochar for removal of tetracycline and fluorescent dye from wastewater: two-stage adsorber analysis. J. Environmental Management 209: 9-16. https://doi.org/10.1016/j.jenvman.2017.12.030.

Rodvold, K.A. and M. Neuhauser (2001). Pharmacokinetics and pharmacodynamics of fluoroquinolones. Pharmacotherapy 21. https://doi.org/10.1592/phco.21.16.233s.33992

Shams, D.F., M. Izaz, W. Khan, S. Nayab, A. Tawab and S.A. Baig (2024). Occurrence of selected antibiotics in urban rivers in northwest Pakistan and assessment of ecotoxicological and antimicrobial resistance risks. Chemosphere 352: 141357. https://doi.org/10.1016/j.chemosphere.2024.141357

Shorina, D., A. Philippova, A. Kazak and N. Pronkin (2023). Physiological and biochemical changes induced by tetracycline in wheat. Polish J. Environmental Studies 32(1): 241–247. https://doi.org/10.15244/pjoes/152240

Tadić, Đ., M.J. Bleda Hernandez, F. Cerqueira, V. Matamoros, B. Piña and J.M. Bayona (2021). Occurrence and human health risk assessment of antibiotics and their metabolites in vegetables grown in field-scale agricultural systems. J. Hazardous Materials 401: 123424. https://doi.org/10.1016/j.jhazmat.2020.123424

Tilley, E., C. Lüthi, A. Morel, C. Zurbrügg and R. Schertenleib (2008). Compendium of Sanitation Systems and Technologies. Development, 158. http://www.eawag.ch/organisation/abteilungen/sandec/publikationen/publications_sesp/downloads_sesp/compendium high.pdf.

Wang, H., C. Tang, J. Yang, N. Wang, F. Jiang, Q. Xia, G. He, Y. Chen and Q. Jiang (2018). Predictors of urinary antibiotics in children of Shanghai and health risk assessment. Environment International 121: 507–514. https://doi.org/10.1016/j.envint.2018.09.032

Wang, H., N. Wang, J. Qian, L. Hu, P. Huang, M. Su, X. Yu, C. Fu, F. Jiang, Q. Zhao, Y. Zhou, H. Lin, G. He, Y. Chen and Q. Jiang (2017). Urinary antibiotics of pregnant women in eastern China and cumulative health risk assessment. Environmental Science and Technology 51(6): 3518–3525. https://doi.org/10.1021/acs.est.6b06474

Yang, Q., J. Zhang, K. Zhu and H. Zhang (2008). Influence of oxytetracycline on the structure and activity of microbial community in wheat rhizosphere soil. J. Environmental Sciences 21(7): 954-959. https://doi.org/10.1016/S1001-0742(08)62367-0

Yang, Y., W. Qiu, Y. Li and L. Liu (2020). Antibiotic residues in poultry food in Fujian Province of China. Food Additives and Contaminants: Part B Surveillance 13(3): 177–184. https://doi.org/10.1080/19393210.2020.1751309

Zafar, R., D. Nabi, A.A. Al-Huqail, U. Jamil, S.J. Khan, Z. Ahmed and M. Arshad (2023). Efficient and simultaneous removal of four antibiotics with silicone polymer adsorbent from aqueous solution. Emerging Contaminants 100258. https://doi.org/10.1016/j.emcon.2023.100258

Zafar, R., R.Z. Paracha, A.F. Alrefaei, M.F. Albeshr, M.Y.J. Baloch, J. Kallerhoff and M. Arshad (2024). Removal of four antibiotics from the aqueous phase using Polyethylene adsorbent: Mechanistic understanding and reusability potential. Environmental Technology and Innovation 36: 103831. https://doi.org/10.1016/j.eti.2024.103831.

Zafar, R., S. Bashir, D. Nabi and M. Arshad (2021). Occurrence and quantification of prevalent antibiotics in wastewater samples from Rawalpindi and Islamabad, Pakistan. Science of the Total Environment 764: 142596. https://doi.org/10.1016/j.scitotenv.2020.142596.

Zhou, Y., Zhu, F., Zheng, D., Gao, M., Guo, B., Zhang, N., Meng, Y., Wu, G., Zhou, Y., and Huo, X. (2021). Detection of antibiotics in the urine of children and pregnant women in Jiangsu, China. Environmental Research, 196, 110945. https://doi.org/10.1016/j.envres.2021.110945

Zhang, Z., H. He, T. Han, X. Tian, J. Pang and H. Lambers (2023). Soil oxytetracycline alters the effects of phosphate fertilisation and Bacillus amyloliquefaciens on the bacterial community of Medicago sativa rhizosphere. Applied Soil Ecology 187: 104861. https://doi.org/10.1016/j.apsoil.2023.104861.

Zhang, Z., L. Zhao, Q. Jin, Q. Luo and H. He (2024). Combined contamination of microplastic and antibiotic alters the composition of microbial community and metabolism in wheat and maize rhizosphere soil. J. Hazardous Materials 473: 134618. https://doi.org/10.1016/j.jhazmat.2024.134618.