

ASSESSMENT OF SOIL CHEMICALS INFLUENCING PERSISTENCE OF *Clostridium perfringens* IN THE SELECTED DISTRICTS OF PUNJAB PROVINCE, PAKISTAN

S. Naureen¹, M. Rabbani^{1*}, A. A. Sheikh¹, A. S. Hashmi¹ and B. M. Jayarao².

¹University of Veterinary and Animal Sciences, Lahore- 54000, Pakistan

²The Pennsylvania State University, University Park- 16802, PA, USA

*Corresponding Author's email: mrabbani@uvas.edu.pk

ABSTRACT

Clostridium perfringens (*C. perfringens*), a spore-forming anaerobic bacterium, is widely distributed in soil and poses a significant risk to humans, poultry and livestock by causing enteric infections. Its persistence in the environment is influenced by soil chemical composition; however, limited information is available on its prevalence and associated risk factors in Pakistan. This study aimed to determine the prevalence of *C. perfringens* in soil samples from nine districts of Punjab, Pakistan, using real-time PCR and to evaluate the influence of key soil chemical parameters on its distribution. A total of 970 pooled soil samples (representing 1940 individual samples plus 485 control samples) were collected from 485 villages, of which 96 (9.89%) tested positive for the presence of *C. perfringens* DNA. Soil chemistry analysis of positive and negative samples (n = 192) revealed a significant association ($p \leq 0.05$) of *C. perfringens* prevalence with organic matter, nitrogen, calcium, sodium, potassium, magnesium and manganese, while heavy metals such as lead, nickel, copper and chromium exhibited an inverse association. Conversely, pH, soluble salts, phosphorus, zinc and iron showed no significant correlation with pathogen occurrence. District wise variation revealed that the highest prevalence was observed in Chakwal (18%), followed by Attock (13.33%) and Lahore (12%), whereas Faisalabad (7.40%) and Sargodha districts (6.75%) showed the lowest. The findings highlight the strong role of soil chemistry, particularly nutrient-rich environments, in promoting *C. perfringens* persistence, underscoring the need for improved land and livestock management strategies to reduce environmental transmission risks and associated disease outbreaks.

Keywords: Soil chemistry, micronutrients, *Clostridium perfringens*

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0>)

Published first online December 28, 2025

Published final February 28, 2026

INTRODUCTION

Soil is a multifaceted and active biological system and 80-90% of the reactions taking place in soil are intervened by different microorganisms (Younas *et al.*, 2022; Zhang *et al.*, 2023). Population of microbes is very divergent in the soil. Hundred million to one billion bacteria are present in a teaspoon of the productive soil (Sokol *et al.*, 2022). In per gram of soil 6000 different genomes of bacteria are present and they occupy 5% of the total space available in the soil (Trevors, 2010; Williamson *et al.*, 2017). A variety of substances are released by plant roots into the soil such as vitamins, organic acids, polymeric carbohydrates, sugars, amino acids, micro and macro minerals (Neumann and Romheld, 2000; Bhat *et al.*, 2024) Due to these substances soil has an intricate environment of mineral and organic ingredients that may be present in any of the gaseous, solid or liquid forms (Baumgardner, 2012). As a result of great variation in the composition of soil, different practices in agricultural cultivation system and crops as well as environmental and physical parameters, there is

high range of microbial flora in the soil (Berg *et al.*, 2005; Garbowski *et al.*, 2023).

C. perfringens is a ubiquitous organism which can be isolated from different environments and due to its spore forming capability; it can persist in the diverse ecological units (Chan *et al.*, 2012; Derongs *et al.*, 2020; Banawas *et al.*, 2024). Due to its presence in the soil, *C. perfringens* can be ingested by the animals along with the fodder and some of them can reach the intestinal tract of animal where they grow under favorable conditions and invade the intestinal mucosa. It can produce rapidly killing toxemia and septicemia by entering the blood stream from intestinal mucosa (Sameera *et al.*, 2005; Hashimoto *et al.*, 2023).

Studies have shown that *C. perfringens* is a prevalent pathogen in Pakistan and its incidence is high in poultry and animals of the Punjab province of Pakistan. In a recent study prevalence rate of 25.37% of *C. perfringens* was reported in poultry of Pakistan (Haider *et al.*, 2022), whereas another study in Pakistan showed that 149 samples of buffalo were positive for *C. perfringens* type A (Khan *et al.*, 2021). A current study performed in

Buffalo and Cattles in Punjab province, established that out of 160 bovine samples, 33 isolates of *C. perfringens* were obtained (Khan *et al.*, 2022). In another study, a higher prevalence (46.1%) is reported in the sheep and goat populations in Punjab province, Pakistan (Mohiuddin *et al.*, 2020).

Soil is the major reservoir of this pathogen which also plays an important role in its transmission. Unfortunately, no data is currently available in the literature on the prevalence and distribution of *C. perfringens* in the soil of Pakistan or on the effects of land management practices on the abundance of this bacterium in the soil, or in which soil types or under which environmental conditions it is most prevalent.

MATERIALS AND METHODS

As a part of an international collaborative project related to spatial ecology and epidemiology of soil borne pathogens the study area, selection of sampling sites and sampling was carried out in 2015. Extraction of genome from previously collected and stored soil samples was done in October to December 2016. Molecular detection of *C. perfringens* was carried out in January to March 2017. Initial soil chemistry analysis of soil samples was conducted in 2016. Whereas heavy metal detection analysis was conducted in November to December 2017.

Study Area and Selection of Sampling Sites: Out of the total 41 districts of Punjab province, 09 districts were selected for soil sampling based on historical records of soil-borne diseases of veterinary and public health importance. These districts also represented major livestock-rearing regions of Punjab and included Attock, Chakwal, Dera Ghazi Khan, Faisalabad, Gujranwala, Lahore, Sahiwal, Sargodha, and Sheikhpura (Figure 1).

Sampling Strategy and Sample Size: To ensure representative sampling, 10% of the total villages from each district were randomly selected using Open Epi software (Version 2.3.1). Since the number of villages varied among districts, the total number of samples collected differed accordingly. Overall, 2,425 soil samples were collected from 485 villages across the nine selected districts.

Soil Samples Collection: From each village, five soil samples were collected using a boring device. Sampling sites which were located within village grazing/animal-shed proximities (≤ 1 km), representative of dominant soil series of the tehsil, and accessible year round were included for sampling. While those sites were excluded, any sites with recent chemical remediation, active construction, industrial effluents, or recent flooding (within the past three months) were also omitted. A control site per district was chosen in low stocking, peri

urban cropland with no known recent livestock activity. Of these, four samples were collected from the four corners of the village, while one control sample was taken from an undisturbed, uncultivable site where there was no human or animal activity. Approximately, 1 kg of soil was collected per site from a depth of 3 inches below the ground surface after discarding the top soil layer to prevent contamination. The samples were weighed using a portable electronic balance, placed in pre-labeled sterile polythene zip-lock bags, and transported to the Soil Microbiology Laboratory, Institute of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore.

Sample Processing: Each soil sample was divided into three portions: the first portion (500g) was stored for future reference, the second portion (250 g) was sent to the Department of Environmental Sciences (UVAS), Lahore for chemical analysis, and the third portion (250 g) was delivered to the Genomic Laboratory, Institute of Microbiology, UVAS, for DNA extraction and molecular confirmation.

DNA Extraction: Genomic DNA of each sample was extracted from 250 g of soil using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The quality and concentration of the extracted DNA were measured using a NanoDrop spectrophotometer (Thermo Scientific, USA).

Molecular Detection of *Clostridium perfringens*: Detection of *Clostridium perfringens* was performed using real-time PCR with primers described by Gurjar *et al.* (2008). The amplification protocol included an initial denaturation step at 95°C for 30 seconds, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 54°C for 1 minute.

The reaction mixture (25 μ L total volume) consisted of 12.5 μ L of 1X PCR master mix, 0.5 μ L bovine serum albumin (0.25 mg/mL), 1 μ L each of forward and reverse primers (10 μ M), 0.5 μ L probe (0.025 μ M), 4.5 μ L PCR-grade water and 5 μ L extracted DNA (10-100 ng). Positive controls (dsDNA from ATCCC. *perfringens* culture) and negative controls (nuclease-free water) were included in each run. The probe was labeled with FAM (6-carboxyfluorescein) and the FAM detection channel was selected during thermocycler setup.

Soil Chemistry Analysis: A total of 96 PCR-positive soil samples and their corresponding 96 negative controls (overall 192 samples) were analyzed to determine various physical and chemical soil parameters. All samples from each district were processed individually. The analyzed variables included pH, total soluble salts, organic matter, nitrogen, phosphorus, potassium, sodium, calcium, magnesium, copper, chromium, nickel, manganese, lead,

iron and zinc. The soil chemistry analysis was primarily carried out at the Department of Plant Sciences, Quaid-i-Azam University, Islamabad, whereas additional heavy metal analyses were conducted at the Mineral and Metal Analysis Laboratory, UVAS, Lahore. Standardized methodologies were followed for accurate determination of different soil parameters. The concentration of micronutrients including copper, chromium, nickel, manganese, cobalt, lead, cadmium and iron was assessed. The levels of nitrogen, sodium, potassium, calcium and magnesium were estimated following the procedures of Kim *et al.* (2007), while phosphorus content was determined according to Fixen *et al.* (1990). Measurement of total soluble salts was performed using the technique of Rhoades (1982), and pH was determined as described by McKeague and Stobbe (1978). Additionally, soil texture was analyzed following the method of Taubner *et al.* (2009) and moisture content was measured using the procedure outlined by Anbazhagan, *et al.* (2020).

Statistical Analysis: Data analysis proceeded in three steps. First, distributional assumptions for all continuous soil variables were assessed with the Shapiro–Wilk test. Most variables departed from normality ($p < 0.05$); therefore, non-parametric procedures were adopted. Second, two group comparisons (PCR positive vs PCR negative sites) were performed using the Mann–Whitney U test for continuous variables and the Chi square (or Fisher’s exact, as appropriate) test for proportions. Third, associations between *C. perfringens* prevalence (site level binary outcome) and soil chemistry were quantified with Spearman’s rank correlation (ρ) using district level medians; 95% CIs for ρ were obtained by bootstrapping (10,000 resamples). We interpreted correlation magnitudes as: low ($\rho < 0.30$), moderate ($0.30 \leq \rho < 0.50$) and strong ($\rho \geq 0.50$). Multiple comparisons were controlled using the Benjamini–Hochberg FDR ($q = 0.10$).

RESULTS AND DISCUSSION

Out of a total 2,425 soil samples collected from nine districts of Punjab, 96 samples (9.89%) were confirmed positive for *Clostridium perfringens* DNA using real-time PCR. The cycle threshold (Ct) values of the positive samples ranged from 18.7 to 31.4, indicating variable bacterial loads across different regions. The highest prevalence was observed in Chakwal (18%), followed by Attock (13.33%) and Lahore (12%), indicating a relatively higher contamination level in these areas. DG Khan (11%) and Sahiwal (10.8%) showed moderate levels of positivity, while Gujranwala (8.33%), Sheikhpura (7.62%), and Faisalabad (7.4%) exhibited comparatively lower prevalence rates. The lowest detection was recorded in Sargodha (6.75%). Overall, out of 970 pooled soil samples (representing 2,425 individual

samples) collected from these districts, 96 samples (9.89%) tested positive for *C. perfringens*. Districts characterized by intensive livestock farming, high manure application, and nutrient-rich soils demonstrated higher pathogen presence compared to regions with lower soil fertility (Table 1).

Metals are a significant contributor towards the different life processes of microorganisms. Some of the metals are crucial as micronutrients i.e. induce regulatory effect by regulating osmotic process and enzyme activity; they help in oxidation-reduction processes or in stabilization of molecules by electrostatic interactions. These include sodium, calcium, potassium, copper, chromium, magnesium, manganese, nickel and zinc. On the other hand, there are some metals like lead which are toxic for the microorganisms and their presence in the soil can inhibit the growth of bacteria. If these heavy metals are present in high concentration, they form complexes within the cell and interfere with the physiological functions of the cell (Bruins *et al.*, 2000; Zhao *et al.*, 2024). In the current study, soil samples positive for *C. perfringens* and their corresponding negative controls were analyzed for various soil chemical parameters and the results were statistically evaluated. First, data normality was assessed using the Shapiro–Wilk test, which showed that most variables were not normally distributed ($p < 0.05$). Therefore, non-parametric analyses were used, including the Mann–Whitney U test for comparing positive and negative samples and Spearman’s rank correlation (ρ) to evaluate the strength and direction of associations. Comparative analysis of 96 positive and 96 negative soil samples demonstrated significant differences in several soil chemistry parameters, with 11 out of 16 parameters showing $p < 0.05$. Among these, organic matter content showed the strongest positive correlation with *C. perfringens* prevalence ($\rho = +0.81$, mean $3.42 \pm 0.71\%$ in positive samples vs. $1.85 \pm 0.62\%$ in negative samples; $p < 0.05$). Similarly, nitrogen also exhibited a strong positive correlation ($\rho = +0.74$, $p < 0.05$). Moderate positive correlations were observed for calcium ($\rho = +0.61$), potassium ($\rho = +0.59$), magnesium ($\rho = +0.56$), and manganese ($\rho = +0.54$). Conversely, heavy metals including lead ($\rho = -0.52$), nickel ($\rho = -0.48$), copper ($\rho = -0.46$), and chromium ($\rho = -0.43$) showed moderate negative correlations, suggesting an inhibitory effect on bacterial persistence. These findings align with previous studies, including Voidarou *et al.* (2011), which also reported a significant association between organic matter and *C. perfringens* prevalence, indicating that nutrient-rich soils promote bacterial growth and spore survival.

Similarly, nitrogen concentrations were significantly higher in positive soils ($0.27 \pm 0.06\%$) compared to negative soils ($0.15 \pm 0.05\%$, $p < 0.05$), highlighting its critical role in supporting microbial proliferation and spore germination. Several macro and

trace minerals, including calcium, sodium, potassium, magnesium and manganese, also exhibited a strong positive association with the detection of *C. perfringens* DNA. Specifically, the mean concentrations of these nutrients were significantly higher in positive samples than in negative controls (all $p \leq 0.05$), suggesting that nutrient-rich soils enhance bacterial survival and spore germination by maintaining osmotic stability and supporting enzymatic activity.

There was no statistically significant association ($p > 0.05$) found for pH, soluble salts, phosphorous, zinc and iron. Among the components of soil chemistry having significant association with *C. perfringens* test statistics (Table 2) showed that amount of nitrogen, sodium, potassium, calcium, magnesium and manganese was high in the soil found positive for *Clostridium perfringens* while that of lead, copper, chromium and nickel amount was less in positive soil and high in *C. perfringens* negative soil. Carbon and Nitrogen content of the soil considerably influence microbial community of the soil (Blaud *et al.*, 2015). As *C. perfringens* is a spore forming bacteria and its spores make it resistant to different environmental stress conditions so that it can survive well in the soil (Raju *et al.*, 2007). Studies have shown that different salts containing Monovalent cations like Na^+ , K^+ and divalent cations like Ca^{++} , Mn^{++} and Mg^{++} help in the germination of spores of *C. perfringens*. Specially KCl has more significant effect on germination of *C. perfringens* spores (Paredes-Sabja *et al.*, 2008). Calcium ions play an important role during the process of spore germination of *C. perfringens*, as all the salts of Calcium induced germination of spores during a study. The study concluded that calcium ions help in signaling process and when chelated with DPA enzyme initiates germination of *C. perfringens* spores. (Paredes-Sabja *et al.*, 2009; Almatrafi *et al.*, 2023)

In contrast, an inverse relationship was observed for several heavy metals, including lead, nickel, copper and chromium. Their concentrations were significantly lower in *C. perfringens* positive soils compared to negative soils (all $p \leq 0.05$), suggesting that elevated levels of these elements may exert inhibitory effects on bacterial growth, possibly through cellular toxicity or disruption of metabolic processes. On the other hand, pH, soluble salts, phosphorus, zinc and iron did not show any statistically significant association with the presence of *C. perfringens* (all $p > 0.05$), indicating that these factors play a minimal role in influencing pathogen persistence within the studied soil environments (Table 1). *C. perfringens* was found prevalent across a wide soil pH range in Costa Rican soils (Gamboa *et al.*, 2005). It is similar to some other studies in which both vegetative and endospore forms of *C. perfringens* were isolated across a range of acidities (Li *et al.*, 2007; Stefanis *et al.*, 2014), including the acidic soils (pH 4.5–6.5) surveyed

by Voidarou *et al.* (2011). These studies are all in concordance with laboratory-determined pH growth conditions and collectively indicate that soil pH is not a suitable predictor for *C. perfringens* prevalence. (Palmer *et al.*, 2019)

To better visualize these associations, a correlation heatmap was constructed (Figure 2), displaying the strength and direction of relationships between soil chemical parameters and *C. perfringens* prevalence. As shown in Table 2, organic matter ($r = +0.81$, $p < 0.05$) and nitrogen ($r = +0.74$, $p < 0.05$) demonstrated the strongest positive correlations, indicating that nutrient-enriched soils strongly favor the persistence of the pathogen. Moderate positive correlations were also observed for calcium, potassium, magnesium and manganese ($r = 0.56$ – 0.64 , $p < 0.05$), suggesting that these macro and trace minerals collectively enhance spore survival. Conversely, lead, nickel, copper and chromium showed moderate negative correlations ($r = -0.43$ to -0.52 , $p < 0.05$), confirming their suppressive effect on *C. perfringens* growth. The heatmap thus highlights a clear contrast between supportive nutrient parameters and inhibitory heavy metals, providing strong visual evidence for the impact of soil composition on pathogen prevalence.

In case of phosphorous, iron and zinc, although the results were statistically non-significant but comparison of data showed that their amounts were also higher in the soil with positive results for *Clostridium perfringens*. Significant association of organic matter, nickel, magnesium, lead and zinc with persistence of *Francisella tularensis* DNA in soil is also reported (Muhammad *et al.*, 2017). Different studies have revealed that micronutrients like Iron, Manganese, copper and zinc play crucial role in regulation of ecological and biogeochemical procedures in the soil. (Moreno-Jimenez *et al.*, 2019; Moreno-Jimenez *et al.*, 2023; Radujkovic *et al.*, 2021; Langridge, 2022). The concentration of soil micronutrient is considered key indicators of ecosystem health and stability (Moreno-Jimenez *et al.*, 2023).

Collectively, these findings indicate that organic matter, nitrogen, and essential soil nutrients significantly promote the persistence of *C. perfringens*, whereas high concentrations of certain heavy metals appear to limit its survival. Furthermore, the observed variation in pathogen prevalence across districts suggests that regional differences in soil chemistry, agricultural practices, and livestock density are key contributing factors influencing the environmental distribution of *C. perfringens*. There is significant association of most of the parameters of soil chemistry with the presence of *C. perfringens* DNA in the soil. Soil is an important reservoir of *C. perfringens* and understanding of reservoir helps in better management of disease control and transmission.

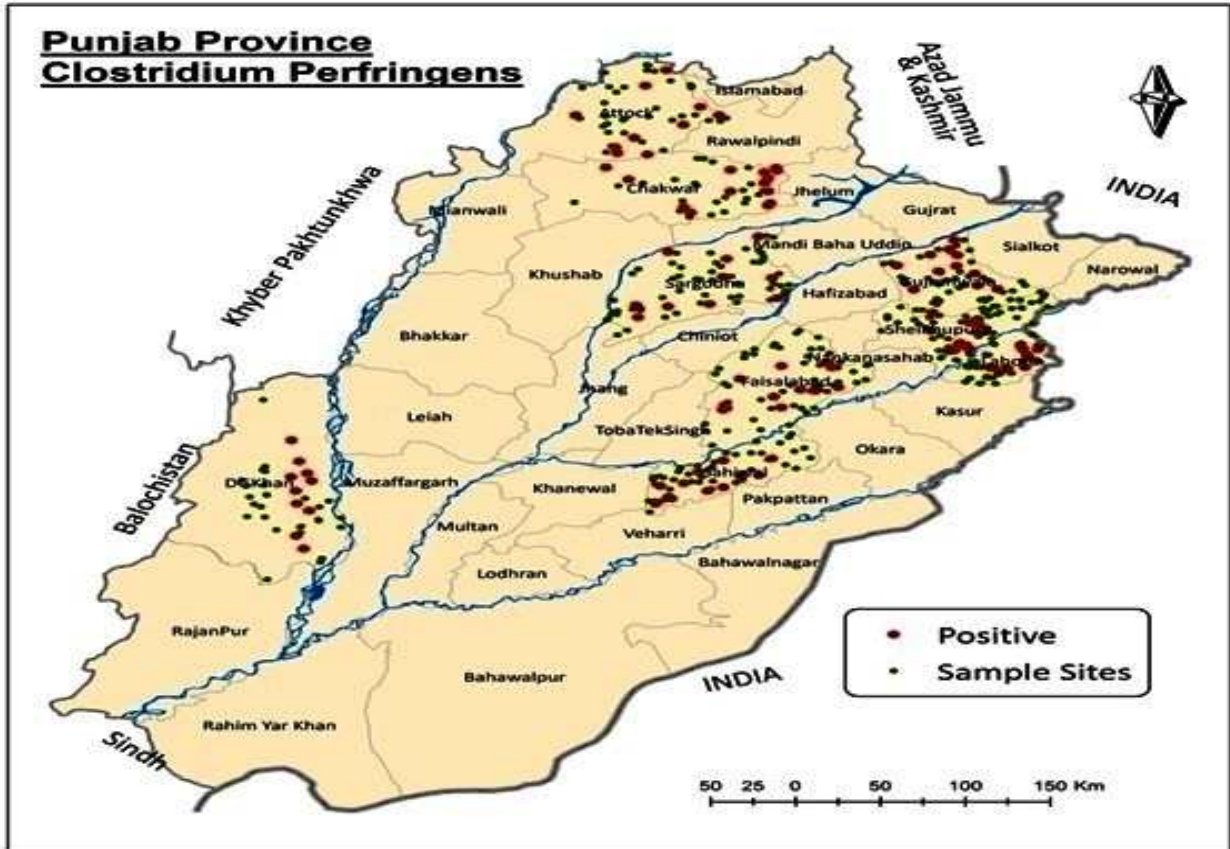


Figure 1. Map of Punjab showing sampling sites and positive sites in different districts

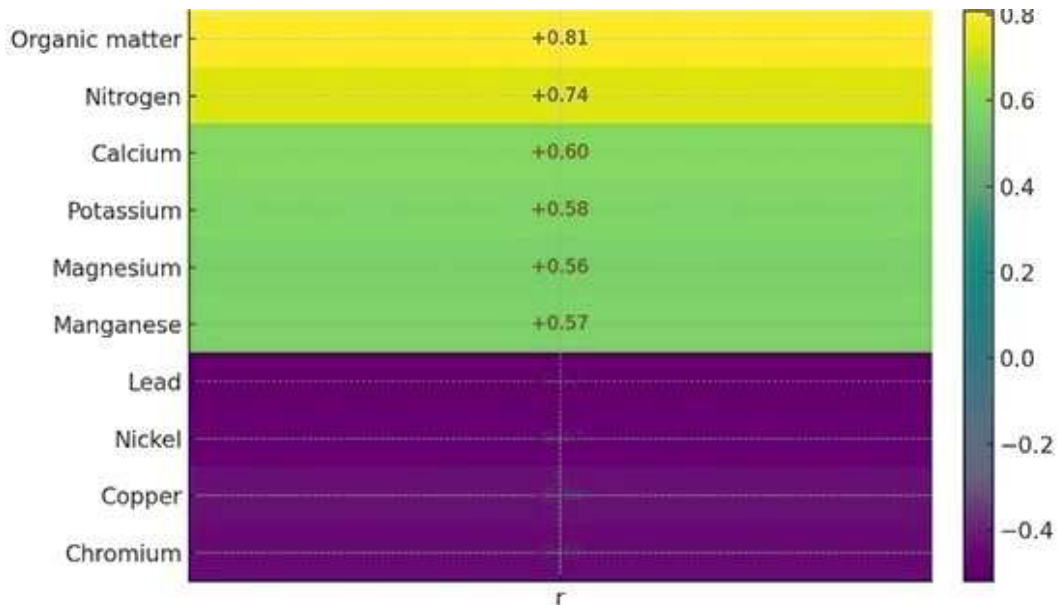


Figure 2. Correlation between soil chemical parameters and the prevalence of *Clostridium perfringens*. The heatmap illustrates the strength and direction of correlations (*r*-values) between different soil chemical parameters and the prevalence of *C. perfringens* in the analyzed samples. Warmer colors (towards red) indicate a strong positive correlation, meaning higher levels of the parameter are associated with increased prevalence of *C. perfringens*, whereas cooler colors (towards blue) represent a negative correlation, indicating an inverse relationship.

Table 1: District-wise Prevalence of *Clostridium perfringens* in Soil Samples Collected from Nine Districts of Punjab.

Sr. No.	District	Villages Sampled	Soil Samples Collected	Positive Samples	Prevalence (%)	Livestock Population
1	Attock	45	90	12	13.33	1,292,702
2	Chakwal	38	76	14	18.00	1,446,343
3	DG Khan	43	86	10	11.00	4,680,996
4	Faisalabad	74	148	11	7.40	2,934,136
5	Gujranwala	72	144	12	8.33	1,913,758
6	Lahore	29	58	7	12.00	674,110
7	Sahiwal	51	102	11	10.80	1,457,194
8	Sargodha	74	148	10	6.75	1,585,443
9	Sheikhupura	59	118	9	7.62	1,081,246
Total	—	485	970	96	9.89	—

Table 2: Soil Chemistry Profile of Positive vs. Negative Samples

Parameter	Mean ± SD (Positive Samples)	Mean ± SD (Negative Samples)	p-value	Association
Organic Matter (%)	3.42 ± 0.71	1.85 ± 0.62	< 0.001	Strongly Significant
Nitrogen (%)	0.27 ± 0.06	0.15 ± 0.05	0.002	Significant
Calcium (mg/kg)	82.4 ± 15.7	64.2 ± 13.4	0.009	Significant
Sodium (mg/kg)	70.8 ± 11.3	51.6 ± 9.4	0.011	Significant
Potassium (mg/kg)	65.5 ± 13.8	42.7 ± 10.9	0.015	Significant
Magnesium (mg/kg)	28.7 ± 5.9	20.3 ± 4.6	0.021	Significant
Manganese (mg/kg)	1.35 ± 0.41	0.89 ± 0.32	0.018	Significant
Lead (mg/kg)	0.12 ± 0.03	0.20 ± 0.05	0.037	Inversely Associated
Nickel (mg/kg)	0.08 ± 0.02	0.15 ± 0.04	0.042	Inversely Associated
Copper (mg/kg)	0.09 ± 0.02	0.17 ± 0.05	0.048	Inversely Associated
Chromium (mg/kg)	0.11 ± 0.03	0.18 ± 0.05	0.041	Inversely Associated
Phosphorus (mg/kg)	22.4 ± 3.8	20.6 ± 3.5	0.188	NS
Zinc (mg/kg)	1.12 ± 0.23	1.09 ± 0.21	0.271	NS
Iron (mg/kg)	18.5 ± 2.9	17.8 ± 3.1	0.314	NS
pH	7.23 ± 0.31	7.19 ± 0.28	0.426	NS
Soluble Salts (dS/m)	0.82 ± 0.11	0.80 ± 0.13	0.489	NS

Conclusion: Study gives a novel insight into the chemical factors that affect the growth of *C. perfringens* in the soil. A better understanding of soil composition helps in selection of suitable land for animal farms and use of control strategies can limit the transmission of pathogen from soil to farm animals and birds. As a result, disease outbreaks can be controlled.

REFERENCES

- Almatrafi, R., S. Banawas and M. R. Sarker (2023). Divalent cation signaling in *Clostridium perfringens* spore germination. *Microorganisms*. 11(3): 591. <https://doi.org/10.3390/microorganisms11030591>
- Anbazhagan, P., M. Bittelli, R. R. Palapati and P. Mahajan (2020). Comparison of soil water content estimation equations using ground penetrating radar. *J. Hydrol.* 588:125039. <https://doi.org/10.1016/j.jhydrol.2020.125039>
- Banawas, S. S., B. A. Kronmiller, M. G. Abdelghany, A. A. Alqarawi and M. R. Sarker (2024). Prevalence of *Clostridium perfringens* spores in selected regions of Saudi Arabia. *J. Pioneer. Med. Sci.* 13(5):122-129. <https://doi.org/10.61091/jpms202413522>
- Baumgardner, D. J. (2012). Soil-related bacterial and fungal infections. *J. Am. Board Fam. Med.* 25(5):734-744. <https://doi.org/10.3122/jabfm.2012.05.110324>
- Berg, G., L. Eberl and A. Hartmann (2005). The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environ.*

- Microbiol. 7(11):1673–1685. <https://doi.org/10.1111/j.1462-2920.2005.00891.x>
- Bhat, M. A., A. K. Mishra, S. N. Shah, M. A. Bhat, S. Jan, S. Rahman and A. T. Jan (2024). Soil and mineral nutrients in plant health: A prospective study of iron and phosphorus in the growth and development of plants. *Curr. Issues Mol. Biol.* 46(6):5194-5222. <https://doi.org/10.3390/cimb46060312>
- Blaud, A., G. K. Phoenix and A. M. Osborn (2015). Variation in bacterial, archaeal and fungal community structure and abundance in high Arctic tundra soil. *Polar Biol.* 38(7):1009-1024. <https://doi.org/10.1007/s00300-015-1656-7>
- Bruins, M. R., S. Kapil and F. W. Oehme (2000). Microbial resistance to metals in the environment. *Ecotoxicol. Environ. Saf.* 45(3):198-207. <https://doi.org/10.1006/eesa.1999.1860>
- Chan, G., A. Farzan, G. Soltés, V. M. Nicholson, Y. Pei, R. Friendship and J. F. Prescott (2012). The epidemiology of *Clostridium perfringens* type A on Ontario swine farms, with special reference to cpb2-positive isolates. *BMC Vet. Res.* 8(1):156. <https://doi.org/10.1186/1746-6148-8-156>
- Del Mar Gamboa, M., E. Rodriguiz and P. Vargas (2005). Diversity of mesophilic clostridia in Costa Rican soils. *Anaerobe* 11(6):322-326. <https://doi.org/10.1016/j.anaerobe.2005.07.002>
- Derongs, L., C. Druilhe, C. Ziebal, M. C. Le and A. M. Pourcher (2020). Characterization of *Clostridium perfringens* isolates collected from three agricultural biogas plants over a one-year period. *Int. J. Environ. Res. Public Health* 17(15):5450. <https://doi.org/10.3390/ijerph17155450>
- Fixen, P. E. and J. H. Grove (1990). Testing soils for phosphorus. *Soil Sci. Plant Anal.* 21:141-180. <https://doi.org/10.2136/sssabookser3.3ed.c7>
- Garbowski, T., D. Bar-Michalczyk, S. Charazinska, B. Grabowska-Polanowska, A. Kowalczyk and P. Lochynski (2023). An overview of natural soil amendments in agriculture. *Soil Tillage Res.* 225:105462. <https://doi.org/10.1016/j.still.2022.105462>
- Gurjar, A. A., N. V. Hegde, B. C. Love and B. M. Jayarao (2008). Real-time multiplex PCR assay for rapid detection and toxotyping of *Clostridium perfringens* toxin-producing strains in feces of dairy cattle. *Mol. Cell. Probes* 22(2):90-95. <https://doi.org/10.1016/j.mcp.2007.11.002>
- Hashimoto, A., H. Suzuki and K. Oonaka (2023). Prevalence of cpe-positive *Clostridium perfringens* in surface-attached soil of commercially available potatoes and its significance as a potential source of food poisoning. *Anaerobe* 79:102687. <https://doi.org/10.1016/j.anaerobe.2022.10.2687>
- Haider, Z., T. Ali, A. Ullah, A. Basit, H. Tahir, H. Tariq, S. Z. Ilyas, Z. Hayat and S. U. Rehman (2022). Isolation, toxinotyping and antimicrobial susceptibility testing of *Clostridium perfringens* isolated from poultry in Pakistan. *Anaerobe* 73:102499. <https://doi.org/10.1016/j.anaerobe.2021.10.2499>
- Khan, M. U. Z., M. Humza, S. Yang, M. A. Alvi, M. Z. Iqbal, H. Zain-ul-Fatima, S. Khalid, T. Munir and J. Cai (2021). Occurrence and toxicogenetic profiling of *Clostridium perfringens* in buffalo and cattle: An update from Pakistan. *Toxins* 13(3):212. <https://doi.org/10.3390/toxins13030212>
- Khan, M. U. Z., S. Khalid, M. Humza, S. Yang, M. A. Alvi, T. Munir, W. Ahmad, M. Z. Iqbal, M. F. Tahir, Y. Liu and J. Zhang (2022). Infection dynamics of *Clostridium perfringens* fingerprinting in buffalo and cattle of Punjab Province, Pakistan. *Front. Vet. Sci.* 9:762449. <https://doi.org/10.3389/fvets.2022.762449>
- Kim, D., H. D. Ryu, M. S. Kim, J. Kim and S. I. Lee (2007). Enhancing struvite precipitation potential for ammonia nitrogen removal in municipal landfill leachate. *J. Hazard. Mater.* 146(1–2):81-85. <https://doi.org/10.1016/j.jhazmat.2006.11.048>
- Langridge, P. (2022). Micronutrient toxicity and deficiency. In: *Wheat Improvement: Food Security in a Changing Climate*. Springer, Cham. 433–449. https://doi.org/10.1007/978-3-030-90673-3_24
- Li, J., S. Sayeed and B. A. McClane (2007). Prevalence of enterotoxigenic *Clostridium perfringens* isolates in Pittsburgh area soils and home kitchens. *Appl. Environ. Microbiol.* 73(22):7218-7224. <https://doi.org/10.1128/AEM.01502-07>
- McKeague, J. A. and P. C. Stobbe (1978). History of soil survey in Canada 1914–1975. *Hist. Ser. Soil Res. Inst.* 11:30.
- Mohiuddin, M., Z. Iqbal, A. Siddique, S. Lia, M. K. F. Salamat, N. Qi, A. M. Din and M. Sun (2020). Prevalence, genotypic and

- phenotypic characterization and antibiotic resistance profile of *Clostridium perfringens* type A and D isolated from feces of sheep (*Ovis aries*) and goats (*Capra hircus*) in Punjab, Pakistan. *Toxins* 12(10):657. <https://doi.org/10.3390/toxins12100657>
- Moreno-Jimenez, E., C. Plaza, H. Saiz, R. Manzano, M. Flagmeier and F. T. Maestre (2019). Aridity and reduced soil micronutrient availability in global dry lands. *Nat. Sustain.* 2(5):371-377. <https://doi.org/10.1038/s41893-019-0262-x>
- Moreno-Jimenez, E., F.T. Maestre, M. Flagmeier, E. Guirado, M. Berdugo, F. Bastida, M. Dacal, P. Díaz-Martínez, R. Ochoa-Hueso, C. Plaza and M.C. Rillig (2023). Soils in warmer and less developed countries have less micronutrients globally. *Glob. Chan. Biol.* 29(2):522-532. <https://doi.org/10.1111/gcb.16478>
- Muhammad, J., M. Rabbani, K. Muhammad, M. Wasim, A. Ahmad, A. A. Sheikh and Z. U. Islam (2017). Physicochemical factors affecting persistence of *Francisella tularensis* in soil. *J. Anim. Plant Sci.* 27(3):1047-1050.
- Neumann, G. and V. Romheld (2000). The release of root exudates as affected by the plant's physiological status. *The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface*. Marcel Dekker, New York. 41-93. <https://doi.org/10.1201/9780849384974>
- Palmer, J. S., R. L. Hough, H. M. West and L. M. Avery (2019). A review of the abundance, behaviour and detection of clostridial pathogens in agricultural soils. *Eur. J. Soil Sci.* 70(4):911-929. <https://doi.org/10.1111/ejss.12771>
- Paredes-Sabja, D., B. Setlow, P. Setlow and M. R. Sarker (2008). Characterization of *Clostridium perfringens* spores that lack SpoVA proteins and dipicolinic acid. *J. Bacteriol.* 190(13):4648-4659. <https://doi.org/10.1128/JB.00115-08>
- Paredes-Sabja, D., P. Udombijitkul and M. R. Sarker (2009). Inorganic phosphate and sodium ions are co germinants for spores of *Clostridium perfringens* type A food poisoning-related isolates. *Appl. Environ. Microbiol.* 75(19):6299-6305. <https://doi.org/10.1128/AEM.00915-09>
- Radujkovic, D., E. Verbruggen, E. W. Seabloom, M. Bahn, L. A. Biederman, E. T. Borer, E. H. Boughton, J. A. Catford, M. Campioli, I. Donohue, A. Ebeling, A. Eskelinen, P. A. Fay, A. Hansart, J. M. H. Knops, A. S. MacDougall, T. Ohlert, H. O. Venterink, X. Raynaud, A. C. Risch, C. Roscher, M. Schutz, M. L. Silveira, C. J. Stevens, K. V. Sundert, R. Virtanen, G. M. Wardle, P. D. Wragg and S. Vicca (2021). Soil properties as key predictors of global grassland production: Have we overlooked micronutrients? *Ecol. Lett.* 24(12):2713-2725. <https://doi.org/10.1111/ele.13870>
- Raju, D., P. Setlow and M. R. Sarker (2007). Antisense RNA-mediated decreased synthesis of small acid-soluble spore proteins leads to decreased resistance of *Clostridium perfringens* spores to moist heat and UV radiation. *Appl. Environ. Microbiol.* 73(7):2048-2053. <https://doi.org/10.1128/AEM.02667-06>
- Rhoades, J. D. (1982). Cation exchange capacity. In: *Methods of Soil Analysis: Chemical and Microbiological Properties*. Agron. Monogr. 9(2):149-157. <https://doi.org/10.2134/agronmonogr9.2.c6>
- Sameera, D. L., V. Shankaraiah and D. Srihari (2005). Effect of packaging and storage on organic manures grown okra (*Abelmoschus esculentus*). *J. Res. ANGRAU* 33(4):30-35.
- Sokol, N. W., E. D. Whalen, A. Jilling, C. Kallenbach, J. Pett-Ridge and K. Georgiou (2022). Global distribution, formation and fate of mineral-associated soil organic matter under a changing climate: A trait-based perspective. *Func. Ecol.* 36(6):1411-1429. <https://doi.org/10.1111/1365-2435.14019>
- Stefanis, C., A. Alexopoulos, C. Voidarou, S. Vavias and E. Bezirtzoglou (2014). Prevalence of *Clostridium perfringens* in agricultural fields with different vegetation types. *Folia Microbiol.* 59(1):1-4. <https://doi.org/10.1007/s12223-013-0268-5>
- Taubner, H., B. Roth and R. Tippkötter (2009). Determination of soil texture: Comparison of the sedimentation method and the laser-diffraction analysis. *J. Plant Nutr. Soil Sci.* 172(2):161-171. <https://doi.org/10.1002/jpln.200700035>
- Trevors, J. T. (2010). One gram of soil: a microbial biochemical gene library. *Antonie van Leeuwenhoek* 97(2):99-106. <https://doi.org/10.1007/s10482-009-9397-5>
- Voidarou, C., E. Bezirtzoglou, A. Alexopoulos, S. Plessas, C. Stefanis, I. Papadopoulos, S. Vavias, E. Stavropoulou, K. Fotou, A.

- Tzora and I. Skoufos (2011). Occurrence of *Clostridium perfringens* from different cultivated soils. *Anaerobe* 17(6):320-324. <https://doi.org/10.1016/j.anaerobe.2011.08.001>
- Williamson, K. E., J. J. Fuhrmann, K. E. Wommack and M. Radosevich (2017). Viruses in soil ecosystems: an unknown quantity within an unexplored territory. *Annu. Rev. Virol.* 4(1):201-219. <https://doi.org/10.1146/annurev-virology-101416-041717>
- Younas, T., M. Umer, A. H. Gondal, H. Aziz, M. S. Khan, A. Jabbar, H. Shahzad, N. M. Panduro-Tenazoua, M. Jamil and F. Ore Areche (2022). A comprehensive review on impact of microorganisms on soil and plant. *J. Bioresour. Manag.* 9(2):109-118.
- Zhang, Z. Y., F. F. Qiang, G. Q. Liu, C. H. Liu and N. Ai (2023). Distribution characteristics of soil microbial communities and their responses to environmental factors in the sea buckthorn forest in the water-wind erosion crisscross region. *Front. Microbiol.* 13:1098952. <https://doi.org/10.3389/fmicb.2022.1098952>
- Zhao, D., P. Wang and F. J. Zhao (2024). Toxic metals and metalloids in food: Current status, health risks and mitigation strategies. *Curr. Environ. Health Rep.* 11(4):468-483. <https://doi.org/10.1007/s40572-024-00392-6>.