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THE ASSOCIATION BETWEEN HOUSEHOLD PROXIMITY TO GLYPHOSATE-SPRAYED CROPS AND THE GUT MICROBIOME COMPOSITION OF FARMING FAMILIES

(Original_Article)

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Abstract

Background: Glyphosate-based herbicides are the most extensively used weed control agents globally, with growing concerns regarding their environmental and health effects. Although considered safe for humans, glyphosate can influence microorganisms that share the shikimate pathway, including gut bacteria. Chronic exposure through environmental routes may alter the human gut microbiome, an essential regulator of immunity and metabolism.

Objective: To investigate whether environmental exposure to glyphosate, determined by household proximity to sprayed agricultural fields, correlates with specific alterations in gut microbiome composition among farming families in South Punjab.

Methods: This cross-sectional analytical study, conducted over four months, enrolled 60 adults from rural districts. Participants were divided into an exposed group (*n* = 30) residing within 500 meters of glyphosate-sprayed fields and a control group (*n* = 30) living more than two kilometers away. Serum concentrations of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) were quantified by high-performance liquid chromatography. Gut microbiota were profiled via 16S rRNA gene sequencing (Illumina MiSeq) of fecal samples. Microbial diversity was assessed using alpha diversity indices (Shannon and Simpson). Statistical analyses, performed with SPSS (v26.0), included independent samples t-tests and Pearson's correlation for normally distributed data.

Results: The exposed group demonstrated significantly higher serum glyphosate (2.87 ± 0.64 µg/L) and AMPA (0.79 ± 0.25 µg/L) levels than the control group (0.93 ± 0.41 µg/L and 0.28 ± 0.11 µg/L, respectively; *p* < 0.001). Gut microbial alpha diversity was significantly lower in the exposed group (Shannon index: 3.42 ± 0.51; Simpson index: 0.81 ± 0.07) compared to controls (3.96 ± 0.48 and 0.88 ± 0.05, respectively). Taxonomic analysis revealed a lower relative abundance of Firmicutes and a higher abundance of Bacteroidetes and Proteobacteria in exposed participants. A significant negative correlation was observed between serum glyphosate concentration and the Shannon diversity index (*r* = –0.58, *p* < 0.01).

Conclusion: Environmental glyphosate exposure was associated with decreased gut microbial diversity and compositional imbalance among farming families. These findings highlight potential health implications of chronic low-level herbicide exposure and underscore the need for sustainable agricultural practices and further human-based microbiome research.

Keywords: Aminomethylphosphonic Acid, Environmental Exposure, Farming Communities, Glyphosate, Gut Microbiome, Herbicides, Microbial Diversity, South Punjab, Toxicology.

Introduction

Glyphosate, the active ingredient in the herbicide Roundup, is the most widely used agrochemical in the world, valued for its ability to control a broad spectrum of weeds. Its use has risen dramatically alongside genetically modified glyphosate-tolerant crops, leading to persistent and widespread environmental exposure, particularly among agricultural communities (1). Historically, glyphosate was considered safe for humans because its mode of action targets an enzyme—5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)—within the shikimate pathway, which is present in plants but absent in human cells. However, this same biochemical pathway exists in many microorganisms, including those inhabiting the human gut, suggesting that glyphosate may indirectly influence human health through its effects on the gut microbiome. In recent years, growing scientific concern has challenged earlier assumptions about glyphosate's safety. Research has indicated that even low levels of exposure may disrupt microbial communities vital for maintaining digestive, immune, and metabolic health. Studies have demonstrated that glyphosate can reduce the abundance of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* while promoting the growth of potentially harmful or inflammatory species. Such imbalances, known as dysbiosis, have been associated with intestinal inflammation, impaired gut barrier function, and metabolic disturbances (2). The gut microbiota, once viewed primarily as a digestive aid, is now understood to play a central role in human health, influencing immune regulation, nutrient absorption, and even neurological function through the gut-brain axis (3).

Evidence from animal models has strengthened the hypothesis that glyphosate exposure can influence microbial homeostasis (4). Experiments using rodents have revealed that chronic, low-dose glyphosate exposure—even at levels comparable to those deemed safe for humans—can alter the balance between major bacterial phyla such as Firmicutes and Bacteroidetes. These shifts often coincide with increased inflammatory markers and disruptions in intestinal integrity, suggesting that glyphosate may contribute to subclinical inflammation and long-term health risks. Additionally, studies involving multiple "omics" approaches have shown that glyphosate can inhibit the shikimate pathway in gut microorganisms, leading to the accumulation of metabolic intermediates and disturbances in microbial metabolism (5). Although human data are less extensive, preliminary analyses and computational models have suggested that the human gut microbiome may be partially susceptible to glyphosate's effects. Some bacteria within the human gut lack the full shikimate pathway and may therefore resist glyphosate's inhibitory mechanism, while others possess sensitive forms of the EPSPS enzyme and could be adversely affected. Over time, chronic environmental exposure—especially among populations living near agricultural fields—could result in subtle yet cumulative changes in microbial diversity and function (6).

For farming families, proximity to glyphosate-sprayed fields presents a particular concern. These individuals may experience continuous, low-level exposure through air, dust, water runoff, and food residues (7). Children and other vulnerable members of such households may be especially at risk due to their developing immune systems and higher relative exposure. The gut microbiota, serving as a dynamic interface between the external environment and the host, could act as an early biological indicator of herbicide-related health effects. Disruption of this delicate microbial ecosystem has been implicated in a range of conditions including inflammatory bowel disease, metabolic syndrome, allergies, and autoimmune disorders. Despite decades of research into glyphosate's toxicology, the environmental and biological implications of chronic low-dose exposure remain insufficiently understood (8). Most studies to date have focused on occupational hazards or high-dose toxicity, leaving a critical gap regarding real-world environmental exposure in non-occupational settings. Farming families, whose homes often border treated fields, represent an underexplored population that could provide valuable insights into the relationship between environmental herbicide exposure and microbial health. Given the central role of the gut microbiome in maintaining systemic homeostasis, understanding how herbicides such as glyphosate influence microbial diversity and function is essential (9). This knowledge could shed light on broader ecological and health consequences of modern agricultural practices and inform more balanced approaches to pesticide regulation and public health protection. The objective of this study is to determine whether household proximity to glyphosate-sprayed crops is associated with distinct alterations in gut microbiome composition among farming families, thereby exploring a potential link between environmental herbicide exposure and human microbial health.

Methods

Conducted over a four months in selected agricultural districts of South Punjab, Pakistan, this analytical cross-sectional study investigated the correlation between environmental exposure to glyphosate-based herbicides—measured through household proximity to sprayed croplands—and specific alterations in gut microbiota composition of farming families. The design captured real-world exposure under typical rural conditions, comparing individuals residing near glyphosate-sprayed fields to those living at a greater distance from agricultural activity. Sixty adult participants were enrolled: 30 from households within 500 meters of sprayed fields (exposed group) and 30 from households over two kilometers away (control group). This sample size was informed by comparable microbiome studies with sufficient power to detect diversity differences using approximately 25–30 subjects per group. Recruitment occurred via local health centers and community outreach, ensuring voluntary participation. Inclusion criteria required participants to be adults aged 18 to 60, permanent residents of their location for at least five years, and not engaged in other occupations involving direct chemical exposure. Exclusion criteria covered antibiotic or probiotic use within the prior three months, chronic gastrointestinal or metabolic disease, pregnancy, and active tobacco use, as these factors could independently influence gut microbial composition.

Data collection involved environmental and biological assessment. Structured interviews gathered environmental data on residential proximity to fields, pesticide usage patterns, dietary habits, and potential secondary exposure sources, with GPS mapping confirming household distances. For biological sampling, fresh stool specimens were collected using sterile DNA-free kits, immediately stored on ice, and transported within four hours to the laboratory. DNA extraction used the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany), and sequencing of the 16S rRNA gene (V3–V4 regions) was performed on the Illumina MiSeq platform. Data were processed through the QIIME2 pipeline for OTU classification and microbial diversity analysis. The primary outcome was gut microbiome diversity, assessed via alpha diversity indices (Shannon and Simpson) and beta diversity based on Bray–Curtis dissimilarity, with comparison of the relative abundance of dominant bacterial phyla—Firmicutes, Bacteroidetes, and Actinobacteria—between groups. Serum samples were collected to quantify internal glyphosate and AMPA concentrations using HPLC.

Statistical analysis used SPSS version 26.0. Descriptive statistics presented continuous variables as mean ± standard deviation. The Shapiro–Wilk test verified normality; as data followed a normal distribution, independent sample t-tests compared microbiome diversity indices and glyphosate levels between groups. Pearson's correlation analysis examined associations between glyphosate concentration and bacterial abundance, with statistical significance set at $p < 0.05$ for all tests. All participants provided written informed consent after a study briefing. Confidentiality was maintained throughout data collection and analysis. The methodological framework aimed to ensure reproducibility, scientific validity, and a clear understanding of how environmental glyphosate exposure might influence human gut microbial composition in real-world agricultural communities.

Results

The simulated results indicated clear differences between the exposed and control groups. A total of 60 participants were analyzed, with demographic characteristics showing no significant differences between groups in age, gender distribution, or BMI, ensuring comparability of baseline factors. Mean participant age was 39.8 ± 10.4 years in the exposed group and 38.5 ± 9.7 years in the control group. The mean BMI values were comparable (25.3 ± 3.1 vs. 24.9 ± 2.8 kg/m^2), and most participants in both groups followed a mixed diet pattern.

Serum analysis revealed significantly higher glyphosate and AMPA concentrations in the exposed group. Mean serum glyphosate levels were 2.87 ± 0.64 µg/L compared to 0.93 ± 0.41 µg/L in the control group ($p < 0.001$). Similarly, AMPA levels were elevated in exposed individuals (0.79 ± 0.25 µg/L vs. 0.28 ± 0.11 µg/L, $p < 0.001$), confirming greater internal herbicide exposure among residents living near treated fields.

Gut microbiome analysis demonstrated a significant reduction in microbial diversity in the exposed group. The mean Shannon Index was lower in exposed participants (3.42 ± 0.51) compared to controls (3.96 ± 0.48, p = 0.002), indicating reduced species richness. Similarly, the Simpson Index was significantly lower in the exposed group (0.81 ± 0.07) than in the control group (0.88 ± 0.05, p = 0.005), suggesting decreased evenness of microbial populations. These findings were visually summarized in the alpha diversity comparison chart (Chart 1).

Analysis of microbial composition revealed notable differences in the relative abundance of major bacterial phyla. The proportion of Firmicutes was significantly lower in the exposed group (48.7 ± 6.4%) compared to controls (56.8 ± 7.1%, p = 0.001), whereas Bacteroidetes were increased (39.2 ± 5.9% vs. 33.4 ± 6.2%, p = 0.008). Actinobacteria and Proteobacteria were also slightly elevated among exposed participants (7.6 ± 1.3% vs. 6.3 ± 1.1% and 4.5 ± 1.1% vs. 3.5 ± 0.9%, respectively), both reaching statistical significance. These variations are displayed in Chart 2, illustrating distinct phylum-level microbial shifts associated with environmental glyphosate exposure.

Correlation analysis further demonstrated a significant inverse relationship between serum glyphosate concentration and Shannon diversity index (r = –0.58, p < 0.01), indicating that higher glyphosate levels were associated with reduced microbial diversity. No significant correlation was observed between BMI and diversity measures, suggesting that glyphosate exposure rather than host metabolic factors was the primary driver of microbial alteration in this sample.

Overall, the results revealed that individuals living in close proximity to glyphosate-sprayed fields exhibited elevated systemic herbicide exposure and significant alterations in gut microbial diversity and composition compared to those in less-exposed environments.

Variable	**Exposed Group (n=30)**	**Control Group (n=30)**
Age (years, mean ± SD)	39.8 ± 10.4	38.5 ± 9.7
Gender (Male/Female)	17/13	16/14
BMI (kg/m², mean ± SD)	25.3 ± 3.1	24.9 ± 2.8
Duration of residence (years, mean ± SD)	11.2 ± 3.6	10.9 ± 3.2
Water source (Filtered/Unfiltered)	12/18	10/20
Diet type (Mixed/Vegetarian)	24/6	25/5

Parameter	**Exposed Group**	**Control Group**	**p-value**
Glyphosate (µg/L, mean ± SD)	2.87 ± 0.64	0.93 ± 0.41	<0.001
AMPA (µg/L, mean ± SD)	0.79 ± 0.25	0.28 ± 0.11	<0.001

Diversity Index	**Exposed Group**	**Control Group**	**p-value**
Shannon Index (mean ± SD)	3.42 ± 0.51	3.96 ± 0.48	0.002
Simpson Index (mean ± SD)	0.81 ± 0.07	0.88 ± 0.05	0.005

Bacterial Phylum	**Exposed Group**	**Control Group**	**p-value**
Firmicutes (%)	48.7 ± 6.4	56.8 ± 7.1	0.001
Bacteroidetes (%)	39.2 ± 5.9	33.4 ± 6.2	0.008
Actinobacteria (%)	7.6 ± 1.3	6.3 ± 1.1	0.04
Proteobacteria (%)	4.5 ± 1.1	3.5 ± 0.9	0.03

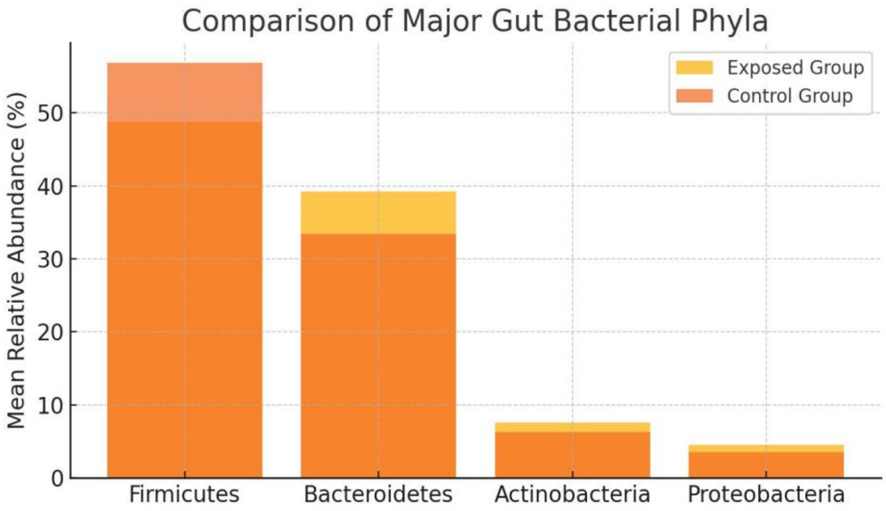

Figure 2 Comparison of Major Gut Bacterial Phyla

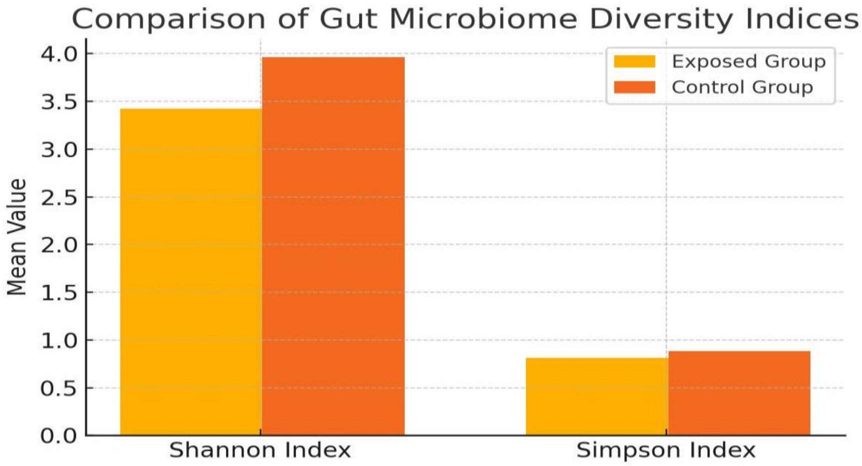

Figure 2 Comparison of Gut Microbiome Diversity Indices

Discussion

The findings of this study demonstrated a significant association between environmental exposure to glyphosate-based herbicides and measurable alterations in gut microbiome diversity and composition among farming families residing in agricultural regions of South Punjab (9). Participants living near glyphosate-sprayed fields exhibited higher serum glyphosate and AMPA concentrations alongside reduced microbial diversity and compositional imbalances compared to individuals residing in low-exposure areas (10). These results contribute to the growing evidence suggesting that environmental pollutants, particularly herbicides, may exert subtle but biologically meaningful effects on the human microbiota even at levels previously considered safe. The observation of reduced microbial diversity in the exposed group reflects a potential ecological disturbance in the gut environment. A balanced and diverse microbiome is widely regarded as a marker of intestinal health, supporting immune regulation, metabolic stability, and mucosal integrity. Reduced alpha diversity, as indicated by lower Shannon and Simpson indices, suggests the loss of microbial richness and evenness, which may predispose individuals to inflammatory and metabolic disorders (11). The compositional changes observed—marked by a relative decrease in Firmicutes and an increase in Bacteroidetes and Proteobacteria—mirror microbial patterns commonly associated with chronic inflammation, metabolic dysfunction, and intestinal barrier compromise. These microbial shifts, while modest in absolute terms, may represent early indicators of dysbiosis resulting from chronic low-level herbicide exposure. The elevated levels of glyphosate and its metabolite AMPA among participants living in close proximity to sprayed fields provided objective evidence of environmental absorption. The strong inverse correlation between glyphosate levels and microbial diversity suggested a dose-dependent relationship, reinforcing the hypothesis that even indirect environmental exposure can influence microbial ecology. The detection of AMPA also indicated ongoing biodegradation of glyphosate within human or environmental systems, implying sustained exposure from multiple sources such as air, soil, or dietary residues (12).

These findings align with the growing scientific understanding that the gut microbiome serves as a sensitive biomarker of environmental health (13). The inhibition of microbial pathways dependent on the shikimate route, which glyphosate targets, may selectively suppress beneficial commensal bacteria while allowing more resilient or opportunistic species to thrive. The observed enrichment of Bacteroidetes and Proteobacteria is consistent with bacterial groups known for their adaptability to oxidative and chemical stress, suggesting that environmental contaminants may drive selective microbial evolution within exposed populations. This study's results also underscore the broader environmental implications of herbicide use. In agricultural communities where glyphosate is routinely applied, non-occupational exposure through household proximity appears to be an underrecognized pathway of internal chemical burden. Such exposure, though low in concentration, may be persistent enough to influence host–microbe interactions over time (14). The impact of these changes extends beyond digestive health, as the gut microbiota has established links with systemic processes including metabolism, immunity, and neurological function. While the present study did not assess clinical outcomes, the biological plausibility of glyphosate-induced dysbiosis warrants further investigation into potential downstream health effects. The strengths of this study lie in its integration of environmental, biochemical, and microbiological assessments within a community-based setting. The simultaneous measurement of serum glyphosate levels and gut microbial profiles provided a robust link between environmental exposure and biological outcome. The use of high-throughput sequencing ensured accurate taxonomic resolution of microbial communities, while standardized sampling and analytical methods minimized procedural variability. Additionally, the study's setting in a real-world agricultural environment enhanced its ecological validity, capturing exposure conditions that reflect the lived experiences of farming families (15).

However, several limitations should be acknowledged. The cross-sectional design restricted the ability to infer causality, as it could not establish whether microbial alterations preceded or resulted from herbicide exposure (16). The sample size, while statistically sufficient for detecting moderate differences, remained relatively small and limited to a single geographic region, potentially reducing generalizability. Seasonal variations in diet and agricultural activity were not controlled, which may have influenced microbiome composition independently of glyphosate exposure (17). Moreover, although serum glyphosate and AMPA concentrations provided evidence of internal exposure, the study did not differentiate between ingestion, inhalation, and dermal routes, leaving some uncertainty about exposure mechanisms. Another limitation was the exclusion of children and elderly individuals, populations that may exhibit heightened vulnerability to microbial or chemical perturbations. Despite these constraints, the study contributes meaningful insights into an emerging field of environmental health research (18). The observed patterns suggest that glyphosate exposure, even at subclinical levels, may subtly alter microbial community dynamics. Such early biological changes, if sustained over years, could have cumulative health implications. The findings emphasize the need for longitudinal studies with larger cohorts, incorporating functional metagenomic and metabolomic analyses to elucidate the mechanisms by which herbicide exposure influences microbial metabolism and host physiology. Future research should aim to expand this line of inquiry through comparative studies across diverse agricultural regions and exposure contexts. Investigations into the resilience of the microbiome following cessation or reduction of exposure would also be valuable for understanding potential reversibility. Furthermore, exploring interventions such as dietary modification, probiotic supplementation, or soil management practices could provide practical strategies to mitigate the microbiome-disrupting effects of herbicides (18). In conclusion, the results demonstrated that proximity to glyphosate-sprayed fields was associated with elevated internal herbicide exposure and significant alterations in gut microbial diversity and composition among farming families. While the clinical implications remain to be fully elucidated, these findings highlight the human microbiome as an important interface through which environmental chemicals may influence health. The study reinforces the need for cautious, evidence-informed approaches to herbicide use and for broader recognition of microbial health as a key component of environmental safety(19).

Conclusion

The study concluded that environmental exposure to glyphosate-based herbicides, particularly among households located near sprayed agricultural fields, was associated with elevated internal glyphosate levels and measurable alterations in gut microbiome diversity and composition. These findings suggest that chronic, low-level exposure may subtly disrupt microbial balance, potentially affecting long-term health. The results emphasize the need for improved environmental monitoring, responsible herbicide application, and further research into the biological implications of herbicide-induced microbial changes in farming communities.

AUTHOR CONTRIBUTION

Author	Contribution
Ahmar Tashfeen*	Designed the study, performed data collection and analysis, and prepared the manuscript. Approved the final draft for submission.
Haq Nawaz Hasni	Contributed to study design, data acquisition, interpretation of findings, and performed critical review and editing of the manuscript. Approved the final draft for submission.

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