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Assessment of Microbiological Quality of Agricultural Surface Water Sources in Muş Plain

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ABSTRACT

Agricultural waters may be a source of produce contamination in the fields. The purpose of this study was to determine the microbiological quality of agricultural surface water sources in Muş plain. Water samples were examined for four different agricultural surface water resources, including two ponds and two streams, during the agricultural season. The population of total coliform and fecal coliform were enumerated with Most Probable Number in 100 ml water samples (MPN 100 ml). Generic *Escherichia coli* populations were determined as MPN and colony forming unit (CFU) isolates in 100 ml water samples. Generic *E. coli* isolates were recovered for antibiotic susceptibility tests and genetic relationships analyses with RAPD PCR. The presence of *Salmonella* was also determined in 100 ml water samples with confirmation of *InvA* gene presence after isolation of presumptive colonies. Total coliform population reached >2.96 log MPN 100 ml^{-1}. The *E. coli* population in all surface water sources ranged from 0 to >2.96 log MPN 100 ml^{-1} and 0 to 2.78 log CFU 100 ml^{-1}. The presence of *Salmonella* was found to be positive in a total of three water samples in two sources (5%). *E. coli* isolates were found to be resistant to rifampicin (100%), erythromycin (100%), and vancomycin (97.05%). Generic *E. coli* populations isolated from streams were not grouped together in similar clusters. Results of this study indicate that agricultural surface water sources in Muş Plain may contain a contamination source when in contact with the produce.

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Muş Ovası Tarımsal Yüzey Su Kaynaklarının Mikrobiyolojik Kalitesinin Değerlendirilmesi

ÖZET

Tarımsal sular, tarlalardaki mahsül kontaminasyonunun bir kaynağı olabilir. Bu çalışmanın amacı Muş ovasındaki tarımsal amaçlarla kullanılan yüzey su kaynaklarının mikrobiyolojik kalitesini belirlemektir. Tarım sezonu boyunca iki gölet ve iki akarsu olmak üzere dört farklı yüzey su kaynağından su örnekleri alınmıştır. Toplam koliform ve fekal koliform popülasyonları 100 ml su örneğinde En Muhtemel Sayı (EMS 100 ml $^{-1}$) ile sayılmıştır. Jenerik *Escherichia coli* popülasyonları 100 ml su örneklerinde EMS ve KOB olarak belirlenmiştir. Su örneklerinden Jenerik *E. coli* izolasyonları yapılmış ve izolatların antibiyotik duyarlılık testi sonrası genetik akrabalıkları RAPD PCR ile belirlenmiştir. *Salmonella* varlığı da 100 ml su örneklerinde, olası kolonilerin izolasyonundan sonra *InvA* geninin doğrulanmasıyla belirlenmiştir. Toplam koliform popülasyonu> 2.96 log EMS 100 ml^{-1}'ye ulaşmıştır. Tüm yüzey suyu kaynaklarındaki *E. coli* popülasyonu 0 ile> 2.96 log EMS 100 ml $^{-1}$ ve 0 ile 2.78 log CFU 100 ml $^{-1}$ arasında değişmektedir. *Salmonella* varlığı iki kaynakta toplam üç su örneğinde (%5) pozitif bulunmuştur. Bütün *E. coli* izolatları rifampisin (%100), eritromisin (%100) ve vankomisine (%97.05) dirençli bulunmuştur. Akarsulardan izole edilen jenerik *E. coli* izolatları RAPD

Gıda Bilimi

Araştırma Makalesi

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Generic *Escherichia coli*
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Mahsül güvenliği
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Kontaminasyon

filogenetik analizine göre benzer kümelerde bir araya gelmemiştir. Bu çalışmanın sonuçları, Muş Ovası'ndaki tarımsal yüzey su kaynaklarının mahsülle teması sonucunda kontaminasyon riski taşıdığını göstermektedir.]

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INTRODUCTION

During the last two decades, several produce-associated foodborne outbreaks have been linked to the use of contaminated agricultural water sources, causing hundreds of illnesses (Bottichio et al., 2020; Greene et al., 2008; Pachepsky & Shelton, 2011; Team, 2007). Agricultural surface water sources, including ponds, lakes, rivers, and streams, are used by farmers depending on factors such as source availability, distance to water source, and the presence of water canals. Contamination of surface water sources with foodborne pathogens, including *Salmonella* may occur due to the introduction of fecal matter from domesticated animals and wildlife, runoff water, agricultural activities in adjacent fields, and human activities (Topalcengiz et al., 2017). Transfer of foodborne pathogens to the edible parts of produce through agricultural waters is likely expected when contaminated agricultural waters are in direct contact with the crops (USFDA, 2022). In most cases, contaminated fresh produce ends up in consumption with no further processing and a killing step directly after harvest or postharvest application.

Prevention of fresh produce from pathogen contamination starts in the fields to supply safe food. When fresh produce is contaminated in the field and indoor agriculture environments or during and after harvest, removal of pathogens with sanitizers is not considered effective due to limited reduction (Chinchkar et al., 2022; Maimaitiyiming et al., 2024; Olaimat & Holley, 2012; Topalcengiz et al., 2024; Wang et al., 2024). To assess the risk of produce contamination from agricultural waters, populations of fecal indicator microorganisms, widely generic *Escherichia coli*, are monitored at required frequencies by local and international authorities (Boyacı et al., 2023; Buyrukoğlu et al., 2021; Uyttendaele et al., 2015; Widmer et al., 2025). Weak to moderate and temporal correlations have been reported between populations of generic *Escherichia coli* and foodborne pathogens (Pachepsky & Shelton, 2011; Topalcengiz et al., 2019; Topalcengiz et al., 2017); however, testing the population of generic *E. coli* is required as the indicator of fecal contamination in most cases and recommended by the World Health Organization (WHO, 2011).

The traditional techniques used to enumerate indicator microorganisms include membrane filtration techniques and multiple tube fermentation methods based on statistical approaches (Kämpfer et al., 2008). Besides these traditional tests, alternative approaches have been developed to overcome some procedural shortcomings such as speed, specificity, and sensitivity for the detection and enumeration of *E. coli* in water systems (Alegbeleye & Sant'Ana, 2023). Alternative tests are mostly microbial metabolic, PCR-based molecular, and biochemical tests based on enzymatic reactions. One of the PCR-based molecular techniques is performed by designing *uid-A* gene-specific primers (Genter et al., 2019). The *uidA* gene encoding b-D-glucuronidase is the most commonly used fluorogenic substrate for the detection of generic *E. coli*. Although genes for the enzyme are present in these isolates, some *E. coli* fecal isolates have been shown to be negative for this activity (Dhital et al., 2024). The purpose of this study is to determine the microbiological quality of agricultural surface water sources by monitoring the generic *E. coli* population and *Salmonella* presence in Muş plain. Further, PCR confirmed generic *E. coli* strains were isolated for antibiotic testing and assessment of genetic diversity among isolates.

MATERIAL and METHOD

Water samples

Agricultural water sources, two ponds and two streams, were selected for sampling in Muş province of Türkiye (Figure 1). Ponds were fed with a creek and spring water. Surface water sources (1000 ml) were sampled with sterile glass water. A total of sixty samples (15 samples for each water source) were collected in three months, close to harvesting. pH, conductivity (HACH, HQ30d Portable Multi Meter, CO, USA), temperature of water (°C), and air were measured in triplicate. Solar radiation and relative humidity values were obtained from https://power.larc.nasa.gov/data-access-viewer/. Animal activities around and in water sources were observed.

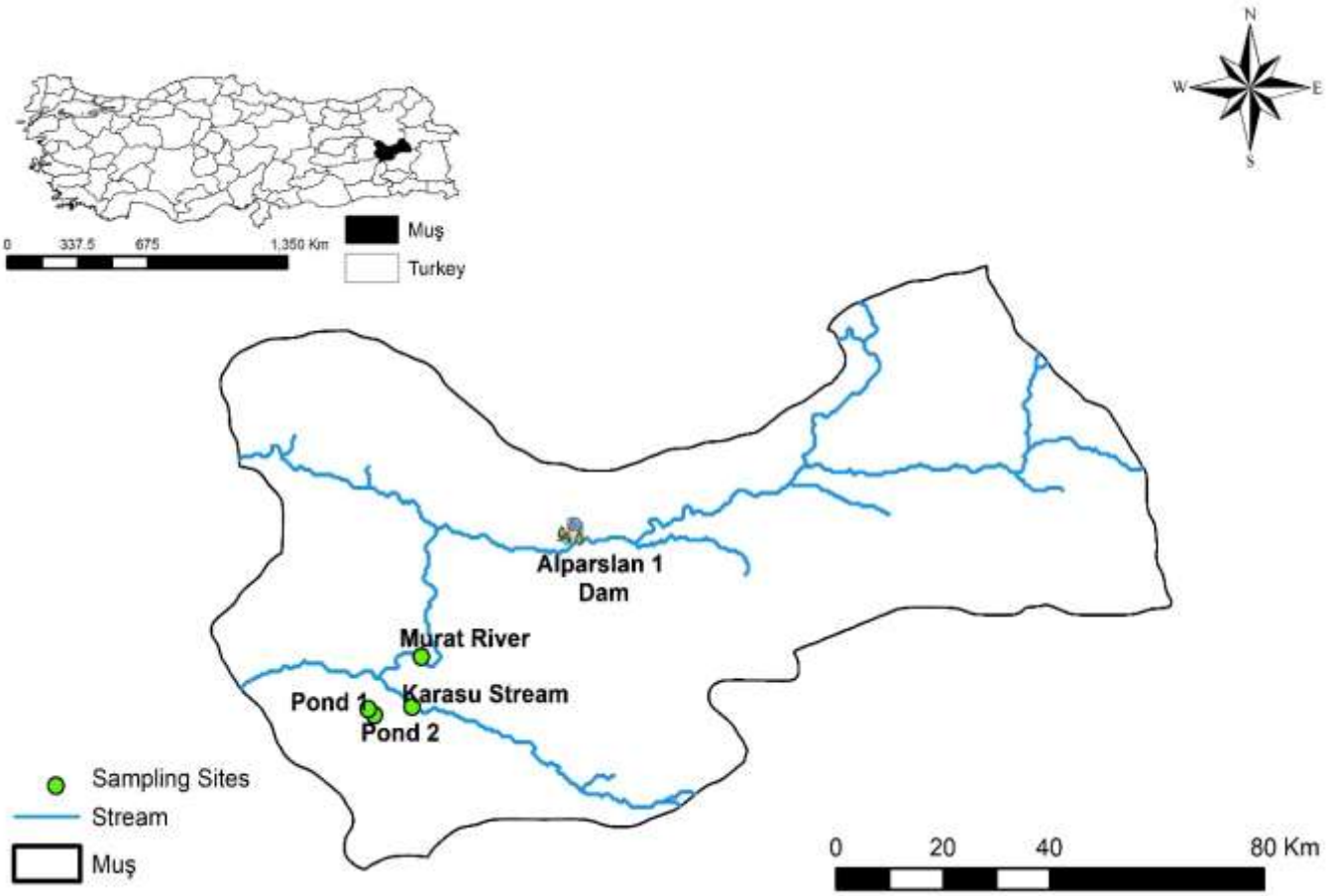

Figure 1. Location of sampling sites in Muş province of Türkiye.
Şekil 1. Türkiye'nin Muş ilindeki örnekleme alanlarının konumu.

Enumeration of indicator microorganisms

Surface water samples were brought on ice and processed in two hours. The population of indicator microorganisms was enumerated with 5 x 3 most probable number (MPN) methodology. Generic *E. coli* populations were enumerated with both the MPN and CFU by following membrane filter methodology. In MPN, water quality guidelines by World Health Organization (WHO, 1996) was followed with some modifications, Briefly, five 10 ml, 1 ml, and 0.1 ml volume of samples were placed in 10 double strength, 10 ml single strength, and 10 ml single strength Lauryl tryptose (lactose) broth with durham tubes (LTB: Biolife; Milan, Italy), in order. After incubation for 24 h at 36 ± 1°C, 0.1 ml of samples with gas production was transferred to a set of 10 ml Brilliant green lactose broth (BGLB: Biolife; Milan, Italy). All tubes were incubated for 24 h at 36 ± 1°C. 0.1 ml of samples from tubes with gas production was inoculated into *Escherichia coli* medium (EC Medium: Biolife; Milan, Italy) for incubation at 44 ± 1°C for 24 h. Final confirmation was achieved by streaking 1 loopful of positive tubes on Sorbitol MacConkey (SMAC: Biolife; Milan, Italy) and Eosin Methylene Blue (EMB: Biolife; Milan, Italy) agar. Typical colonies were noted as positive. In membrane filter enumeration, instructions by the United States-Bacteriological Analytical Manual (Blodgett, 2020a, 2020b) were followed with some modifications. 100 ml of water sample was filtered through a sterile 0.45 µm pore-size filter (Millipore, Billerica, MA, USA). Filters were placed on CHROMagar ECC (DRG, Mountainside, NJ, USA) plates. After incubation for 24 h at 36 ± 1°C, mauve colonies were counted to determine the population of generic *E. coli*. Also, two colonies from each sample were saved for molecular confirmation and tests on isolated strains.

Isolation of *Salmonella* strains

Salmonella presence in agricultural surface water samples was determined by following the modified Bacteriological Analytical Manual by the United States Food and Drug Administration (FDA, 2007). Briefly, 100 ml of double-strength lactose broth (LB: Biolife; Milan, Italy) was added to the same volume of water samples. After incubation at 36 ± 1°C for 24 h, 1 ml of broth culture was inoculated into 9 ml of tetrathionate (TT) broth (Biolife; Milan, Italy) for another round of incubation at the same conditions. A loopful of TT broth culture was

streaked on xylose-lysine-tergitol agar (XLD: Biolife; Milan, Italy) and incubated at 36 ± 1°C for 24 h. Selected typical colonies for *Salmonella* were inoculated in triple sugar iron agar (TSI: Biolife; Milan, Italy) and lysine iron agar (LIA: Biolife; Milan, Italy). Following incubation at 36 ± 1°C for 24 hours, presumptive colonies were frozen as stock culture for PCR analysis.

Colony PCR confirmation of generic *E. coli* isolates and *Salmonella*

The isolated *E. coli* and *Salmonella* strains were streaked on tryptic soy agar (TSA, Biolife; Milan, Italy) and incubated at 35˚C for 24 hours. One colony selected from the single colonies formed after incubation was suspended in 50 µl of distilled water. 3 µl of these suspensions were used as template DNA in PCR studies (Bergkessel & Guthrie, 2013).

The *uidA* gene amplification was done using a specific primer set. The UAL-1939 (5'-TATGGAATTTCGCCGATTTT-3') and UAR-2105 (5'-TGTTTGCCTCCCTGCTGCGG-3') primers were amplified from a 166 bp region of the *uidA* gene (Bej et al., 1991). PCR was carried out in a 20 µl reaction mix using Biorad master mix (Bio-Rad Laboratories, Inc., CA). A master mix that contains 2 µl of 10x PCR buffer, 2 µL of 10 mM dNTP, 1.3 µl of 25 mM $MgCl_2$, 2 µl of 100 ng l^{-1} DNA, 0.6 µl of 25 pmol primer, and 0.1 µl of 5 Unit l^{-1} Taq DNA polymerase was prepared for each sample. The volume was completed to 20 µl with distilled water. The following PCR program was used: 95 °C for 5 min pre-denaturation cycle, 95 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min for 25 cycles. After an extra extension step at 72 °C for 10 min, PCR was terminated and thereafter cooled to 4°C (Bej et al., 1991).

The PCR confirmation of *Salmonella* isolates was done by following the modified protocol by Rahn et al. (1992) to determine the presence of the *invA* gene. *S. enterica*. ser. Typhimurium (ATCC 14028) and shiga toxin-producing *E. coli* O157:H7 (ATCC 35150) were used as positive and negative controls, respectively. *invA* primer sequences were GAATCCTCAGTTTTTCAACGTTTC for forward and TAGCCGTAACAACCAATACAAATG for reverse. PCR reagent concentrations for the 50 µl reaction were as follows: 31 µl of water, 10 µl of reaction buffer, 4 µl of 25 mM $MgCl_2$, 0.4 µl of 10 mM dNTP (dATP, dCTP, dGTP, dTTP), 0.075 µl of Go Taq DNA polymerase, and 2 µl of each 10 µM Primer and 1 µl of DNA template. In practice, the PCR reaction mixture was 20 µl of water containing all reaction ingredients listed above except primers, 25 µl of Master Mix for PCR (Bio-Rad Laboratories, Inc., CA), and 2 µl of 10 µM of each primer and 1 µl of DNA template. The ideal concentration for each primer pair was 200 nmol l^{-1} for *invA*F/*invA*R. PCR conditions were: 94°C for 10 min for melting, followed by 94°C for 30 s, 60°C for 30 s, 72°C for 1 min, and a final extension of 72°C for 5 min. Gel electrophoresis was performed with 1X TBE buffer (Bio-Rad Laboratories, Inc., CA) for 60 min at 120 °C on a 50 ml 2.0% agarose gel containing 2 µl of ethidium bromide (10 mg ml^{-1}) for staining.

RAPD PCR

The following protocol was applied for the RAPD-PCR. A master mix that contains 3 µl of 10× PCR buffer, 1.2 µl of 10 mM dNTP, 1.2 µl of 25 mM MgCl2, 3 µl of 100 ng l^{-1} DNA, 1.2 µl of 25 pmol primer, and 0.4 µl of 5 Unit l Taq DNA polymerase was prepared for each sample. The volume was completed to 30 µl with distilled water. Nineteen RAPD primers were tested, and four showed amplification against the tested bacteria. The thermal cycler was arranged as specified by Bozarı (2016). The sequences (5'–3') of the primers were P8 (5'-CCTGGGTGGA-3'), P29 (5'-AGGGCGTAAG-3'), P30 (5'-AAAACCGGGC-3'), and P1247 (5'-AAGAGCCCGT-3'). Generic *E. coli* ATCC 25922 used as reference strain. The amplification was performed in a NyxTechnik thermal cycler (NyxTechnik, USA) as follows: The first step was 4 min at 95°C for initial denaturation; the second step was 41 cycles at 94°C for 45 sec, 37°C for 45 sec, and 72°C for 1 min. The final extension was set at 72°C for 4 min, then reached 4°C (Bozari, 2016; Nielsen et al., 2014). RAPD-PCR fragments were separated by 1% agarose gel electrophoresis (90 V for 40 min) using 1X TBE buffer and ethidium bromide dye. Gel images were visualized under UV light (Gökdemir et al., 2024).

Antibiotic susceptibility tests

All *E. coli* isolates were tested for antibiotic susceptibility towards different antibiotics on TSA agar. *E. coli* ATCC 25922 used as reference strain. Antibiotic susceptibility test was designed by Kirby–Bauer disc diffusion assay based on Clinical Laboratory Standard Institute guidelines (Clinical & Institute, 2020). The results were evaluated according to the values specified in EUCAST (EUCAST, 2015). Antibiotic disk included kanamycin (K) 30 µg, chloramphenicol (C) 30 µg, erythromycin (E) 15 µg, vancomycin (VA) 30 µg, gentamicin (CN) 10 µg, tetracycline (TE) 30 µg, ampicillin (AM) 10 µg, ciprofloxacin (CIP) 5 µg, rifampicin (RA) 5 µg, and sulfamethoxazole (SMZ) 300 µg. *E. coli* isolates were grown at 37 °C for 24 hours for the development of inhibition zones measured in millimeters after placement of antibiotic discs on TSA inoculated with individual strains at a concentration of ~1 x 10^8.

Statistical analysis

The polymorphic bands of all samples obtained from the RAPD technique were analyzed with the Total LAB TL 120 (Nonlinear Dynamics, 2009) programme. A UPGMA (unweighted pair group method with arithmetic mean) and a Neighbor-joining cluster dendrogram were obtained. All experiments were conducted in groups of three, and the findings were reported as means ± SD. To evaluate the significance of each experiment, Student's t-test was utilized through Microsoft Excel. The significance level of $p < 0.05$ was deemed statistically significant. To examine the correlation among environmental factors, microbiological parameters, and physicochemical water attributes, Pearson product-moment correlation coefficients (r) were performed at the significance level of $p < 0.05$ by pooling all data for each measured factor obtained from all agricultural water sources.

RESULTS and DISCUSSION

Microbiological quality of water sources

Microbiological water quality of surface water sources was monitored in two ponds and two streams for three months in Muş plain, Türkiye. All microbiological indicators followed similar trends in population fluctuations over sampling periods (Figure 2). Since the highest population that can be measured with MPN due to the chosen methodology was 3.25 log MPN 100 ml^{-1} water samples, extreme changes in population of indicator microorganisms over 3 logs cannot be observed. In both ponds, the population of total and fecal coliforms remained <2 log MPN100 ml^{-1} over 70% (11 out of 15) of sampling times where generic *E. coli* populations were enumerated <1 log CFU/100 ml at all samples (Figure 2C and 2D). In Murat River, the population of total coliform ranged between 0.8 and 3.2 log MPN 100 $ml^{-1,}$ where populations of fecal coliform and log MPN 100 ml^{-1} stayed <2 log MPN 100 ml^{-1} (Figure 2A). In the Karasu stream, the total coliform population had a population of over 2 log MPN 100 ml^{-1} in all the samples (Figure 2B). Relatively, generic *E. coli* populations were between > 2 log as in the unit of MPN 100 ml^{-1} and CFU 100 ml^{-1} in almost half of the samples.

The *uidA* primers used to investigate generic *E. coli* presence yielded 34 out of 41 isolates as positive. *Salmonella* (*invA* gene) was detected in 3/60 (5%) of the surface water samples. Generic *E. coli* is considered the indication of fecal contamination by local and international authorities in common. (USFDA, 2022; WHO, 2011), however, the population of total coliforms, fecal coliforms, enterococci, and fecal streptococci is also evaluated to assess the microbiological quality of agricultural water sources (Demir et al., 2024; Polat et al., 2020; Steele & Odumeru, 2004; Topalcengiz et al., 2017; Truitt et al., 2018). Similar to previous studies in different parts of the world, indicator microorganisms tested in this study showed similar trends (Draper et al., 2016; Montiel et al., 2023; Topalcengiz et al., 2017; Truitt et al., 2018; Won et al., 2013). In the present study, the populations of indicator microorganisms stayed below the allowed standards defined for agricultural and recreational waters by the local and international authorities (USFDA, 2022; WHO, 2011) except for the Karasu stream. Higher indicator microorganisms' population can be explained by intense human activity and animal husbandry around the Karasu stream. In previous studies, it is stated that both bovine and ovine wastes can cause soil and water pollution (Mousapour et al., 2024; Rakonjac et al., 2024). In addition, poultry facilities next to the same stream represent the logical explanation for water samples with higher *Salmonella* positives compared to other water sources (Figure 2B).

In the present study, the population of generic *E. coli* was enumerated in the unit of MPN 100 ml^{-1} and CFU 100 ml^{-1}. The linear correlation between the statistical approach and direct count of indicator microorganisms was moderate ($R^2 = 0.44$). In the United States, the Food and Drug Administration requires the monitoring of generic *E. coli* population in the unit of CFU 100 ml^{-1} rather than MPN in agricultural water sources in the Food Safety Modernization Act under the Produce Safety Rule (USFDA, 2022). The weak to moderate correlation reported in the present study supports the decision about the direct count of generic *E. coli* population in agricultural water sources by following membrane filtration as described.

The water temperature in agricultural surface water sources ranged from 14.1 to 17.6°C in Murat River, from 11.8 to 26°C.3 in Karasu stream, from 15.3 to 25.2°C in pond 1, and from 15.1 to 22.5°C in pond 2 on sampling days during harvest season in Muş plain. All water sources had a pH (between 6.60 and 8.27) and conductivity (between 10.1 and 75.5 μs cm^{-1}) within narrow ranges. The solar radiation, air temperature, and relative humidity ranged from 6.11 to 8.23 kWh m^{-2} Day^{-1}, 15.3 to 29°C, and 12.3 to 49.7% at sampling days, respectively. No rainfall measurement was obtained two days earlier before and during the sampling days. Animals observed around and in the agricultural water sources included frogs, various types of birds, and domesticated animals as sheep and water buffalo. Pearson product-moment correlation coefficients (r) and p-values for all correlations among environmental factors, microbiological parameters, and physicochemical attributes of water sources are shown in Table 1. Stronger correlation coefficients are determined with values closer to 1 and -1. The correlation coefficients between microbiological indicators were moderate and ranged from 0.46 to 0.97 (<0.001), similar to the findings

reported for surface agricultural waters in Florida and Pennsylvania in the United States (Draper et al., 2016; Topalcengiz et al., 2017). Except for a correlation between solar radiation and the population of total coliform, no significant relationships were calculated between microbiological indicators and weather parameters and physicochemical water attributes, similar to previously reported studies conducted at different regions in the United States and South Africa (Draper et al., 2016; Ijabadeniyi et al., 2011; Topalcengiz et al., 2017).

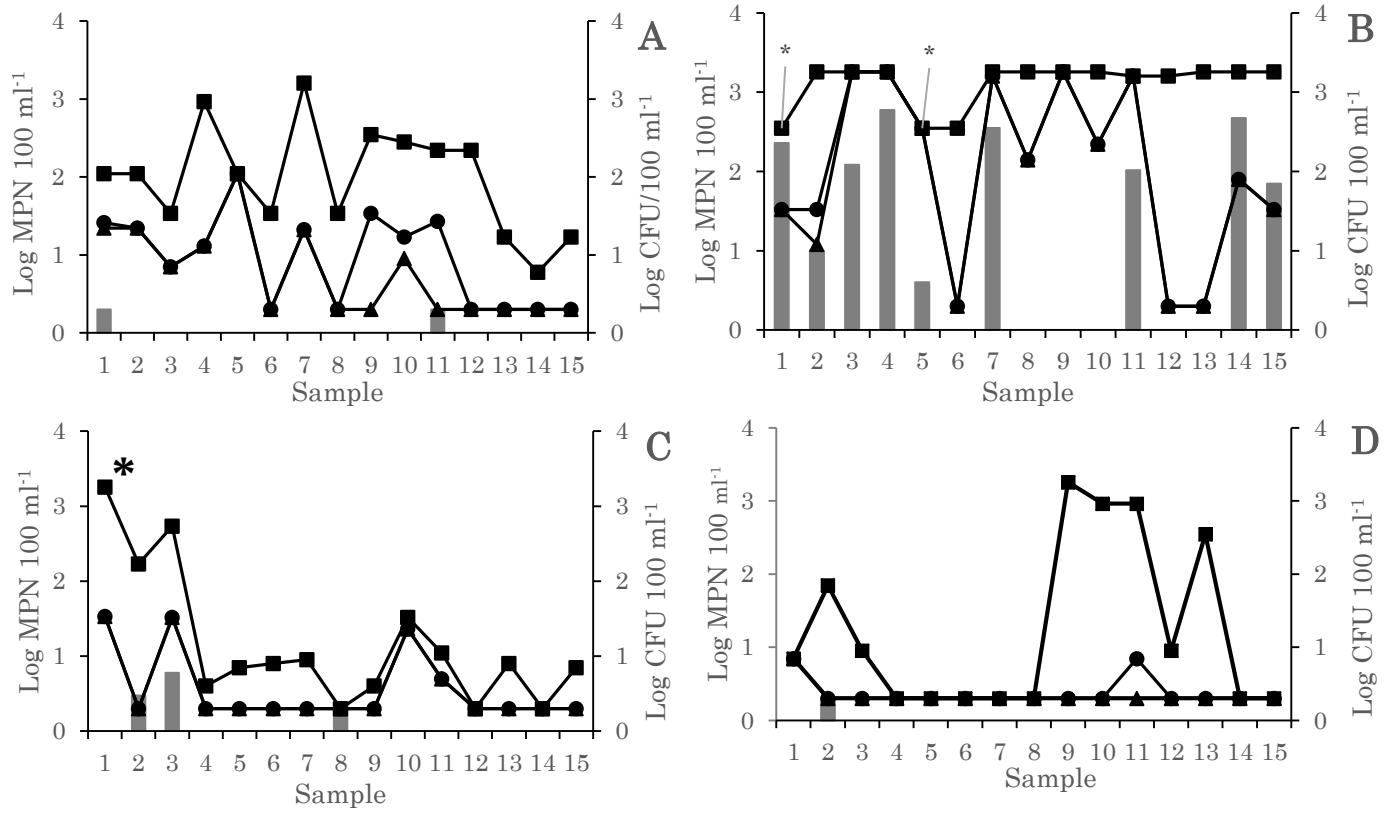

Figure 2. Population (log MPN 100 ml^{-1}) of total coliform (■), fecal coliform (●), and generic E. coli (▲; ■: log CFU 100 ml^{-1}) in sampling sites of Murat river (A), Karasu stream (B), Pond 1 (C), and Pond 2 (D) in Muş province of Türkiye. "*" indicates Salmonella (invA gene) positive in 100 ml of water sample on the related sampling day. The highest detection limit is 3.25 log MPN 100 ml^{-1}.

Şekil 2. Türkiye'nin Muş ilindeki Murat Nehri (A), Karasu Çayı (B), Gölet 1 (C) ve Gölet 2 (D) örnekleme alanlarındaki toplam koliform (■), fekal koliform (●) ve jenerik E. coli (▲; ■: log CFU 100 ml^{-1}) popülasyonu (log MPN 100 ml^{-1}). "" ilgili örnekleme gününde 100 ml su örneğinde Salmonella (invA geni) pozitifliğini göstermektedir. En yüksek tespit limiti 3,25 log MPN 100 ml^{-1}'dir.*

RAPD PCR

A total of 34 isolates were analyzed by RAPD PCR by using the neighbor-joining method. The amplifications of the *E. coli* isolate against four different primers are presented in Figure 3. All four primers generated RAPD bands; however, the most suitable similarity relationships were obtained in primers C and D. When individuals not producing bands were excluded, primer essentially divided into two clusters. In primer C, one of these clusters contained 14 individuals from the Karasu population. The remaining individuals were grouped in the second cluster. Upon examining the sub-clusters of the second cluster, they were further divided into two sub-clusters. One of the sub-clusters contained seven individuals from Pond 1, one from Pond 2, one from Karasu, and two from Murat River populations. Three Karasu, two Pond, and one Murat individual were clustered in the second sub-cluster. In the RAPD profiles of primer D, similarly, two main clusters were identified. The first cluster included 16 Karasu and three Pond 1 individuals, while the second cluster comprised six Pond 1, two Pond 2, three Karasu, and three Murat population individuals. Karasu stream and Murat River had overlapping populations, but not grouped together in similar clusters. The Karasu population was predominantly grouped within itself for each primer. Although each water source is within a 10 km diameter of the sampling area and geographically distant from flowing water populations, the pond populations sometimes clustered in the same groups (e.g., in the first cluster of primer D and the second sub-cluster of primer C).

Table 1. Pearson product-moment correlation coefficients (r) with statistical significances determined between environmental factors and each of the microbiological parameters and physicochemical water attributes for all agricultural water sources combined.

Çizelge 1. Tarımsal su kaynakları için birleştirilmiş verinin çevresel faktörler ile mikrobiyolojik parametrelerin ve fizikokimyasal su özelliklerinin her biri arasında belirlenen istatistiksel anlamlılıklara sahip Pearson çarpım moment korelasyon katsayıları (r).

	Fecal coliform (MPN 100 ml^{-1})	Generic *E. coli* (MPN 100 ml^{-1})	Generic *E. coli* (CFU 100 ml^{-1})	pH	Conductivity (µs cm^{-2})	Water temperature (°C)	Air temperature (°C)	Solar radiation (kWh m^{-2} Day^{-1})	Relative humidity (%)
Total coliform (MPN 100 ml^{-1})	0.65* (<0.001)	0.61* (<0.001a)	0.46* (<0.001)	-0.09 (0.48)	0.09 (0.50)	-0.22 (0.09)	-0.03 (0.84)	0.25* (0.05)	0.23 (0.08)
Fecal coliform (MPN 100 ml^{-1})		0.97* (<0.001)	0.65* (<0.001)	0.03 (0.84)	-0.08 (0.53)	-0.10 (0.45)	0.15 (0.26)	0.20 (0.12)	0.21 (0.11)
Generic *E. coli* (MPN 100 ml^{-1})			0.66* (<0.001)	0.06 (0.63)	-0.14 (0.29)	-0.07 (0.60)	0.17 (0.20)	0.13 (0.32)	0.13 (0.31)
Generic *E. coli* (CFU 100 ml^{-1})				-0.02 (0.90)	-0.06 (0.65)	0.068 (0.61)	0.16 (0.21)	-0.04 (0.74)	-0.14 (0.29)
pH					-0.90* (<0.001)	-0.25* (0.05)	-0.25 (0.06)	-0.23 (0.08)	-0.31* (0.02)
Conductivity (µs cm^{-2})						0.22 (0.10)	0.08 (0.57)	0.16 (0.21)	0.30* (0.02)
Water temperature (°C)							0.60* (<0.001)	0.22 (0.10)	0.21 (0.11)
Air temperature (°C)								0.31* (0.02)	0.25* (0.05)
Solar radiation (kWh m^{-2} Day^{-1})									0.59* (<0.001)

*Correlation coefficients are statistically significant ($p < 0.05$).

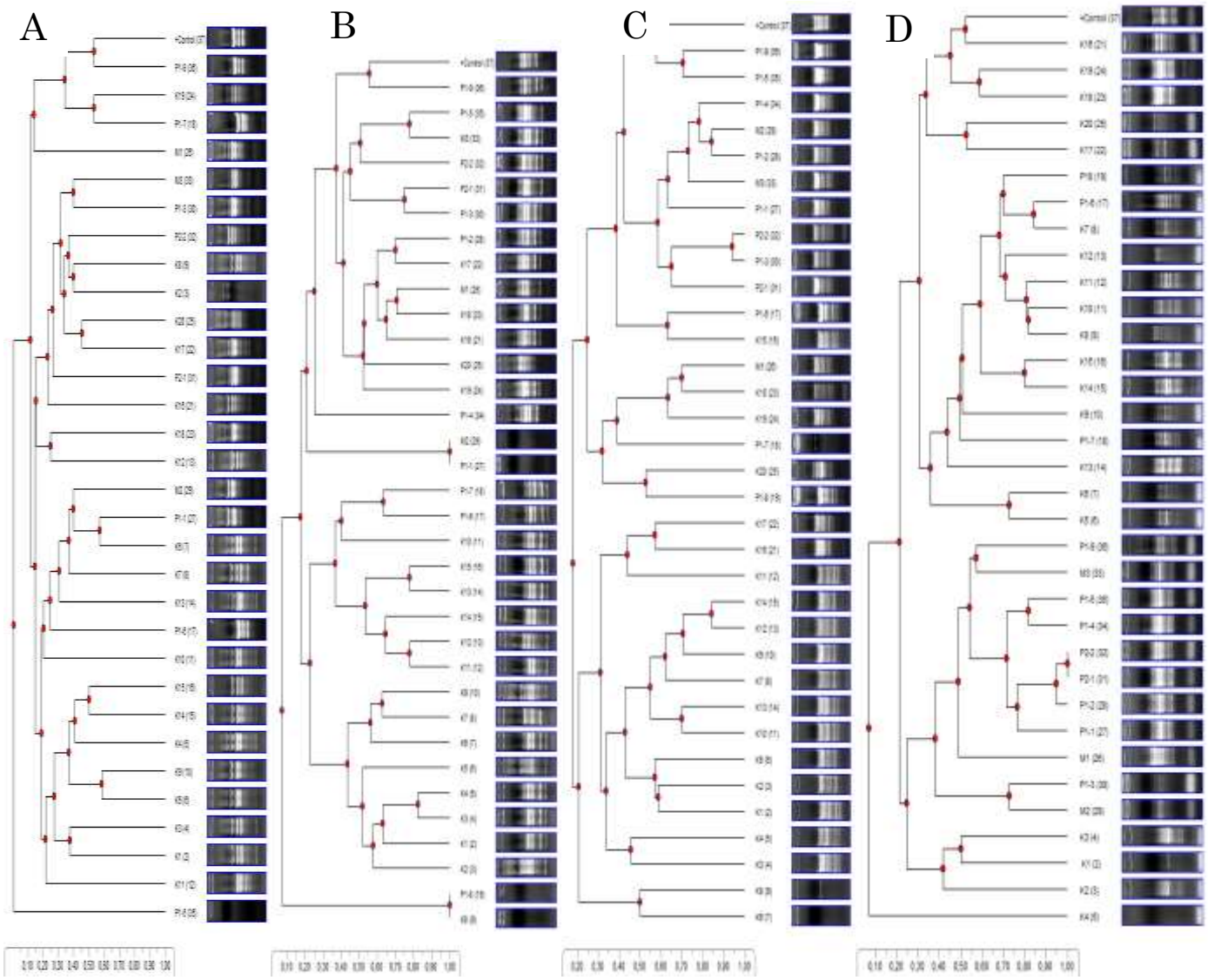

Figure 3. UPGMA cluster analysis for thirty-four E. coli isolates based on RAPD-PCR marker of 1247 (A), 8 (B), 29 (C), and 30 (D) primers.

Şekil 3. Otuz dört E. coli izolatı için 1247 (A), 8 (B), 29 (C) ve 30 (D) primerlerinin RAPD-PCR işaretleyicisine dayalı UPGMA küme analizi.

E. coli populations belonging to different phylogenetic groups occupy distinct niches in their respective ecosystems due to partial genetic differences. These genetic variations may manifest as resistance genes, novel genes associated with resistance, or new traits with functions that are not yet fully understood (Mahfouz et al., 2018). PCR-based techniques can be utilized to determine such genetic diversity among *E. coli* isolates (Alshrif & Buazzi, 2021). Particularly, antibiotic resistance genes are critical for the persistence of strains within populations. In the present study, it was observed that many of the strains were predominantly resistant to the nine different tested antibiotics. This finding suggests that, despite being isolated from different sources, the strains may be phylogenetically related. Previous studies have indicated that the genomic diversity of *E. coli* sequence types in different aquatic environments may be similar (Delgado-Blas et al., 2021).

Antibiotic susceptibility tests

The inhibition zone diameters were analyzed based on the break-point guidelines set forth by the Clinical and Laboratory Standards Institute (CLSI): isolates exhibiting an inhibition zone of 14 mm or less were classified as resistant (R), those with a diameter exceeding 20 mm were identified as susceptible (S), and isolates with a zone diameter falling between 15 and 20 mm were categorized as intermediate (I) (Onur & Önlü, 2024). All 34 *E. coli* isolates from different agricultural water sources were resistant to rifampicin and erythromycin (100%). After these antibiotics, the highest resistance was found against vancomycin (97.05%), tetracycline and gentamicin

85.29%, sulphamethoxazole 70.58%, kanamycin 47.05%, ampicillin 35.29%, ciprofloxacin 23.52% and chloramphenicol 5.8% (Table 2). Similar to the present study, 95%, 14.28% and 8.50% of *E. coli* strains isolated from water samples taken from Atatürk Dam Lake were resistant to erythromycin, tetracycline, and chloramphenicol in order, in the downstream water basin and the closest region of Türkiye (Kayış, 2022). Previously, *E. coli* strains had the highest and lowest resistance to ampicillin and chloramphenicol in studies where strains were isolated from different water surface sources in Türkiye, respectively (Karaca, Hüner, & Mercimek Takcı, 2023; Karaca, Hüner, & Takcı, 2023; Kayış, 2022; Takcı et al., 2021). The use of chloramphenicol in food-producing animals in animal husbandry was banned by the European Union in 1994, which prevented the development of resistance (Ghimpeţeanu et al., 2022). Antibiotics such as β-lactams, tetracyclines, aminoglycosides, aminoglycosides, macrolides and sulphonamides are used not only to treat bacterial infections but also as food additives to increase the growth rate of animals used for food (Mithuna et al., 2024; Shahid et al., 2021). Ampicillin, as the most widely used antibiotic in medicine and animal husbandry in Türkiye, is thought to have a great effect on this situation.

The widespread misuse of antibiotics causes the entry of these substances into agricultural waters through wastewater. Antibiotics contaminate the sewage system and other water sources through the urine and feces of animals using antibiotics, improper disposal of expired antibiotics, and hospital wastes (Anwar et al., 2020; Phan et al., 2024; Wang et al., 2020). Due to a lack of antimicrobial removal systems in use, antibiotic exposure of bacteria in these environments can create selective pressure, driving the development and proliferation of antibiotic-resistant strains (Kulik et al., 2023). Inadequate wastewater facilities, careless disposal of used antibiotics into nature, and the urine and feces of animals interacting with agricultural waters because of irrational antibiotic use by livestock breeders can be considered as the sources of antibiotic resistance in the analyzed waters in Muş province.

Table 2. Antibiotic susceptibility of generic E. coli strains isolated by different agricultural surface water sources in Muş province of Türkiye ($p<0.05$).

Çizelge 2. Türkiye'nin Muş ilinde farklı tarımsal yüzey suyu kaynaklarından izole edilen jenerik E. coli suşlarının antibiyotik duyarlılığı ($p<0.05$).

		% of generic E. coli isolates		
Antibiotic	*Disc content*	*Resistant*	*Intermediate*	*Susceptible*
Chloramphenicol	30 µg	5.8	0	94.11
Erythromycin	15 µg	100	0	0
Tetracyclines	30 µg	85.29	14.7	0
Gentamicin	10 µg	85.29	0	14.7
Vancomycin	30 µg	97.05	2.94	0
Kanamycin	30 µg	47.05	52.94	52.94
Ampicillin	10 µg	35.29	0	64.70
Ciprofloxacin	5 µg	23.52	20.58	55.88
Rifampicin	5 µg	100	0	0
Sulfamethoxazole	300 µg	70.58	17.64	11.76

CONCLUSION

Monitoring of agricultural water quality is a commonly applied method to ensure produce safety. To date, limited regions in Türkiye have been investigated for the microbiological quality of agricultural water sources. In the present study, a high population of microbiological indicator and the presence of *Salmonella* in agricultural surface waters in Muş plain indicates the risk of contamination in produce fields. Although the studied region is not considered as a main produce-growing area, locally grown produce is consumed in the region and transported to close provinces, which may increase the risk of foodborne outbreaks. In Europe and the US, provincial public health authorities (e.g. British Columbia Centre for Disease Control, BCCDC) compile and analyse antibiotic resistance trends in all clinical human *E. coli* isolates from different studies through surveillance programs Since there is no such reporting process in the studied region of Türkiye studied in the present study, it is important to carry out such studies from waters used for agricultural use to determine public health risks.

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Contribution Rate Statement Summary of Researchers

Tuba KARAKOYUN, and Didem TAŞDELEN KARASLAN have designed the study and collected the data. Muş Alparslan University, Department of Scientific Research Project Coordination provided the funding support (BAP-21-TBMYO-4901-05 ve BAP-20-FEF-4902-05). Tuba KARAKOYUN, Didem TAŞDELEN KARASLAN, Sedat BOZARI, Harun ÖNLÜ, and Zeynal TOPALCENGİZ executed the experiment with the help of Prof.Dr. Murat Aydın ŞANDA. Zeynal TOPALCENGİZ and Harun ÖNLÜ wrote the article, and critically reviewed by Prof.Dr. Sedat BOZARI.

Conflict of Interest

The authors declare they have no conflict of interest.

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