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Benthic Invertebrate Community Responses Associated with Mixed Runoff Contamination in Irrigation Canals: A Standardized Colonization-Plate Approach

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ABSTRACT

Irrigation canals receiving mixed agricultural and urban runoff may accumulate contaminants that adversely affect aquatic biodiversity. This study evaluated the combined effects of microplastics and zinc (Zn)/copper (Cu) contamination on benthic macroinvertebrate communities using standardized Hester–Dendy colonization plates. Fifty plates (25 reference and 25 impacted) were deployed for 28 days. Water quality, sediment and biofilm microplastic loads, dissolved and sediment-associated Zn/Cu concentrations, and macroinvertebrate community metrics were compared using Welch's t-tests. Hydrological characteristics did not differ significantly between reaches; however, impacted reaches had higher conductivity (801.39 vs. 405.78 µS cm^{-1}), turbidity (25.00 vs. 10.60 nephelometric turbidity units (NTU)), nitrate (3.05 vs. 0.97 mg L^{-1}), phosphate (0.29 vs. 0.11 mg L^{-1}), and lower dissolved oxygen (5.72 vs. 8.16 mg L^{-1}). Sediment microplastics increased from 133.53 to 308.11 items kg^{-1}, and biofilm-associated microplastics increased from 15.96 to 30.88 µg g^{-1}. Dissolved Zn/Cu increased from 10.87/3.10 to 30.88/13.87 µg L^{-1}, and sediment Zn/Cu increased from 54.48/19.52 to 136.56/58.09 mg kg^{-1}. Taxon richness declined from 23.87 to 14.62, Ephemeroptera-Plecoptera-Trichoptera (EPT) richness from 6.71 to 2.61, %EPT from 44.44% to 12.20%, and Average Score per Taxon (ASPT) from 6.46 to 4.04. Mixed runoff was associated with degraded water quality, increased contaminant burdens, and marked changes in macroinvertebrate taxonomic and functional structure. Standardized colonization plates provided a responsive method for detecting ecological impairment in irrigation canals.

1. INTRODUCTION

Intensely cultivated landscapes are often characterized by irrigation and drainage canals. Many of these channels are artificial or highly managed, but they may still provide ecological benefits to freshwater biodiversity and ecological connectivity when natural streams are scarce or fragmented [1, 2]. Concurrently, the agricultural return flow, peri-urban runoff, domestic inputs, and fine suspended materials are entering these canals, rendering them possible accumulation zones for complex mixtures of contaminants instead of only water-conveyance structures. Microplastics and trace metals are particularly significant in this context, as they are known to occur together in water, sediments, and biofilms. Recent freshwater studies have demonstrated the co-occurrence of microplastics and potentially toxic elements, with their distribution influenced by land use patterns, hydro-morphological characteristics, and localized discharge sources [3]. Microplastics could also function as a physical stressor and as a carrier or sorbent of metals and other contaminants, which may alter their bioavailability and biological exposure [4]. Benthic macroinvertebrates have been shown to react to the presence of microplastics by altering survival, feeding, colonization, and community composition both in the laboratory and field [5].

Benthic macroinvertebrates are widely used as a biological indicator, as their assemblage reflects the local habitat conditions and water quality and contaminant exposure over time. Proxies of ecological status and sensitivity to pollution include taxon richness, Ephemeroptera-Plecoptera-Trichoptera (EPT) richness, and percentage of EPT, the Biological Monitoring Working Party (BMWP) score, and the Average Score per Taxon (ASPT) [6]. Conventional kick-net or grab sampling, however, may be influenced by patchy small-scale habitat, variable access, and uneven substrate in engineered canals. Standardized artificial substrates, such as Hester-Dendy colonization plates, offer a controlled colonization surface and exposure time and can be used for comparisons of biological response between canal reaches [7]. Although the topic of microplastics in freshwater has gained considerable interest, there is little research that has combined data on microplastic loads with trace-metal contamination and water quality and macro-invertebrate response to colonization plates in irrigation canals. The significance of this void is that, in agricultural and peri-urban settings, water, nutrients, fine sediments, metals, and plastic particles can be delivered through the same canal, and the same canal can be utilized as a water source.

Irrigation canals are unique environments of fresh water, as they serve as both water-transportation infrastructure and as ecosystems. The artificial streams within canal communities are affected by regulated stream flow, sedimentation, agricultural drainage, and irregular pollutant discharges [1]. Therefore, standardized approaches to biological monitoring are especially important in these systems, as they enable comparison between reaches that have had different exposure histories and reduce the natural variability inherent in substrate. Hester–Dendy colonization plates provide an artificial, standardized habitat with relatively consistent surface area and exposure duration [7]. Their use enables assessment of early colonizing macroinvertebrate assemblages under comparable colonization conditions and has been applied successfully in modified freshwater environments where natural substrate availability is variable.

Therefore, this study aimed to quantify the co-occurrence of microplastic contamination and zinc (Zn)/copper (Cu) pollution in irrigation canal reaches affected by mixed agricultural and urban runoff and to evaluate the relationship between these contaminants and benthic macroinvertebrate communities colonizing standardized Hester–Dendy plates. Specifically, the study compared reference and impacted canal reaches based on physicochemical water characteristics, microplastic abundance in sediments and biofilms, dissolved and sediment-associated Zn and Cu concentrations, macroinvertebrate taxonomic and functional community structure, and biological assessment metrics, including EPT-related indices, BMWP scores, and ASPT values.

2. MATERIALS AND METHODS

2.1 Study design and reach selection

A comparative field design was employed to evaluate the exposure to contaminants and colonization by benthic macroinvertebrates in irrigation canals. Reaches were rated as either reference or impacted following the reconnaissance of land use, visual discharge points, bank condition, sediment deposition, and surrounding agricultural and peri-urban activities. Reference reaches were located upstream of large drainage inlets and were identified as having less obvious effects of influence from the drainage and more stable riparian margins. Impacted reaches were located downstream of mixed agricultural return flow and peri-urban runoff inputs and were identified with visibly increased fine sediment deposition and turbidity. Reaches were chosen to be as similar as possible in channel characteristics, channel depth, flow velocity, and substrate texture to minimize the interpretation of biological differences in response to gross differences in channel characteristics.

2.2 Study area description

The study was conducted in an agricultural–peri-urban irrigation-canal network in central Iraq, within a WGS84 coordinate corridor of 33.277–33.295°N and 44.260–44.291°E. The spatial design comprised 10 independent canal reaches/segments: five upstream reference reaches (R1–R5) and five downstream impacted reaches (I1–I5), with five Hester–Dendy plates per reach (n = 25 plates per exposure group; n = 50 total). Reference reaches were located 0.25–2.05 km from the upstream boundary, whereas impacted reaches were located 3.30–5.50 km downstream of the main transition/runoff-entry zone. The reference-to-impacted separation ranged from approximately 1.25 to 5.25 km along the canal corridor. Agricultural-return and peri-urban drainage inlets were located approximately 40–100 m from impacted reaches and more than 800 m from reference reaches. Figure 1 presents the georeferenced study-area map, including coordinates, reference and impacted reaches, runoff inputs, plate locations, and surrounding agricultural/peri-urban land use.

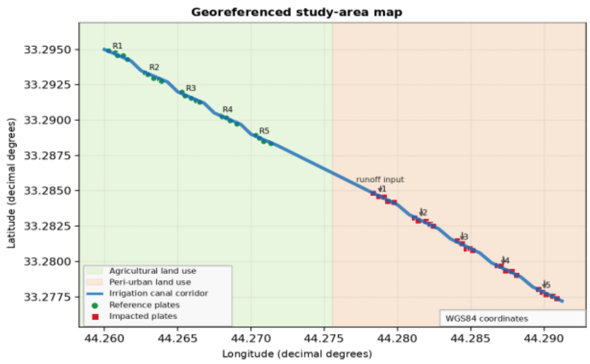

Figure 1. Georeferenced study-area map showing reference and impacted irrigation-canal reaches, runoff inputs, Hester–Dendy plate locations, and surrounding agricultural and peri-urban land-use zones. Coordinates are reported in the WGS84 reference system

2.3 Colonization plates and field deployment

The experimental unit was the individual Hester–Dendy sampler. Fifty samplers were deployed for 28 days during a stable irrigation period: 25 in reference reaches, and 25 in impacted reaches. Each sampler comprised a series of hardboard plates separated by spacer blocks to provide repeated interstitial colonization surfaces. Before deployment, samplers were rinsed with deionized water, handled with powder-free gloves, labeled, and stored in clean containers. Within each reach, samplers were deployed in a blocked arrangement to minimize small-scale variation in depth, turbulence, and lateral position. Plates were suspended from permanent anchors at approximately mid-water depth, away from banks, macrophyte mats, eddy, and point inflows. At retrieval, each sampler was enclosed in a fine net to minimize loss of organisms and loose biofilm, and then transferred to a labeled container containing site water for laboratory processing. All 50 deployed samplers were recovered and retained for analysis, and comparisons were standardized by exposure group and the 28-day deployment period.

2.4 Water quality, microplastics, and trace metals

A calibrated portable multiparameter meter was used to measure in situ the temperature, pH, specific conductivity, dissolved oxygen, turbidity, and depth at each colonization site. Water-quality and hydraulic parameters were measured according to the field sampling schedule established for the colonization experiment. Measurements were conducted at deployment and retrieval of colonization plates (Day 0 and Day 28, respectively) at comparable times of day to minimize diel variation. Where repeated measurements were available, mean values across the deployment period were calculated for comparison between reference and impacted reaches. Because the study represents a single colonization period, temporal fluctuations associated with rainfall events, irrigation intensity, and seasonal changes may not be fully captured. Timed floats over a fixed distance were used to estimate the surface velocity, which was then used to calculate the approximate mean column velocity based on a consistent hydraulic coefficient, as per the typical assumptions associated with rough-bed flows [8]. The plate-associated biofilm was removed from the plates using gentle brushing and rinsing with filtered deionized water in clean stainless-steel equipment for the microplastic analysis. Surficial fine sediments were sampled within approximately 1 m of each plate, dried, homogenized, and subsampled by dry mass. A density separation was carried out using a zinc chloride solution, and then filtration was performed, and polymers were confirmed by pyrolysis-gas chromatography-mass spectrometry. Microplastics were reported as items kg^{-1} dry sediment and $\mu g\ g^{-1}$ dry biofilm. The samples were filtered through 0.45-micrometer membranes and acidified using ultrapure nitric acid for the analysis of zinc and copper in the water. The fine sediments were sieved to the less than 63 micrometer fraction and subjected to microwave-assisted acid digestion. All digests, dissolved samples, and sediment were analyzed by inductively coupled plasma optical emission spectrometry, and all sediment concentrations were calculated on a dry-weight basis. Microplastic analysis followed contamination-control procedures, including procedural blanks, field blanks, and laboratory air-exposure controls. Samples were filtered on clean analytical membranes before polymer confirmation by pyrolysis-gas chromatography-mass spectrometry. For the comparative analytical framework, the microplastic workflow used a standardized protocol based on 0.45-µm filtration before polymer confirmation; therefore, the microplastic results are interpreted as comparative sediment and biofilm loads rather than a full size-resolved particle inventory. Recovery experiments were conducted using known quantities of reference polymer particles, and percentage recovery was calculated for each analytical batch. In order to identify a polymer, the pyrolysis-gas chromatography-mass spectrometry spectra had to match a reference polymer library with reasonable confidence. Where necessary, blank corrections were applied, and results were reported after the background contamination had been adjusted.

2.5 Macroinvertebrate processing and biotic indices

All samplers were disassembled in the laboratory on a clean tray and rinsed over a 500-micrometer sieve. Organisms retained and associated detritus were preserved and sorted under a stereomicroscope to the lowest possible taxonomic level, typically to the family or genus level, depending on the diagnostic characters and condition of the preserved material. To preserve sensitivity to the rare and pollution-sensitive taxa, whole samples were processed instead of subsampled. The taxon-by-sample abundance data were subsequently prepared, and total taxon richness, Shannon diversity, EPT richness, percentage EPT, BMWP, and ASPT were calculated. Two well-known family-level biotic indices of pollution-sensitive macroinvertebrate assemblages, BMWP and ASPT, were used. Based on the taxonomic data, functional feeding groups were determined and classified as relative abundance categories: scrapers, shredders, collectors, and predators.

2.6 Identification of macroinvertebrates

Macroinvertebrate identification was conducted to the lowest practicable taxonomic level, generally family level and, where diagnostic characters and preservation condition allowed, genus level, using standard freshwater macroinvertebrate keys. EPT taxa were assigned according to Ephemeroptera, Plecoptera, and Trichoptera membership. BMWP and ASPT were calculated as family-level comparative biotic indices. Because BMWP and ASPT were originally developed mainly for temperate European freshwater systems and have not been fully calibrated for Iraqi irrigation canals, their application was interpreted cautiously as a comparative indication of community response between reference and impacted reaches rather than as an absolute ecological-status classification.

2.7 Quality assurance

Quality assurance and quality control were documented in supplementary tables linked to the raw-data workbook. For microplastics, recovery tests were conducted for PE, PP, PET, PS, and PVC reference particles (n = 5 batches per polymer), together with procedural blanks, blank-correction procedures, limit of detection (LOD)/limit of quantification (LOQ) values, particle-size classes, morphology categories, and pyrolysis GC-MS library-match criteria. For metal analysis, the documented method included a 0.45-µm dissolved fraction, microwave-assisted digestion of the <63 µm sediment fraction, inductively coupled plasma optical emission spectrometry (ICP-OES) measurement, wavelength settings, detection limits, duplicate precision, and certified reference material (CRM)/spike-recovery benchmarks. All quality assurance/quality control (QA/QC) values are provided in the supplementary tables and individualized Excel sheets.

2.8 Statistical analysis

Data were entered into a validated spreadsheet containing the complete plate-level raw matrix for n = 25 plates per exposure group. To address potential pseudoreplication, the analysis incorporated 10 independent reaches/segments (five reference and five impacted), with five plates nested within each reach. Plate-level Welch t-tests were retained as descriptive comparisons, whereas the primary sensitivity analysis was repeated at the reach/segment level by aggregating plate values within each reach and comparing five reference-reach means with five impacted-reach means. The nested results are reported as reach-level sensitivity analysis and provided in the supplementary Excel workbook. Composite pollution index (CPI) was calculated from measured sediment microplastics, sediment Zn, and sediment Cu after z-score standardization. R version 4.3.x was used for statistical analysis; the accompanying workbook documents all values, formulas, and data labels.

$$\text{CPI} = z(\text{sediment microplastics}) + z(\text{sediment Zn}) + z(\text{sediment Cu})$$

The CPI was not interpreted as a validated ecological risk index but rather as a statistical descriptor of the combined contamination gradient observed among sampling locations.

2.9 Data verification and supplementary documentation

The complete analytical dataset included group summaries and plate-level observations for the main physicochemical, contaminant, and macroinvertebrate outcomes, together with GPS coordinates, reach-level nesting identifiers, QA/QC records, polymer-characterization details, and validation matrices. Data-integrity checks included verification of sample identifiers, group assignments, analytical units, calculation formulas, summary statistics, and consistency between plate-level and reach-aggregated analyses. The accompanying Excel workbook provides the individualized raw data, reach-level summaries, QA/QC records, and sensitivity analyses used in the manuscript.

3. RESULTS

As shown in Table 1, temperature, depth, and velocity did not differ significantly between reference and impacted reaches, confirming that the two groups were physically comparable. A significant deterioration in water quality was observed in impacted reaches, in contrast. Conductivity was almost twice as high, turbidity more than doubled, and the levels of nitrate and phosphate were significantly higher than reference sites. In addition, dissolved oxygen declined from 8.16 ± 0.39 to 5.72 ± 0.58 mg L^{-1} in impacted reaches. These results indicate that the impacted reaches were exposed to a strong chemical and nutrient gradient while maintaining broadly similar hydrological conditions.

Microplastic contamination was consistently higher in impacted reaches, as depicted in Table 2. Sediment microplastics increased from 133.53 ± 49.08 to 308.11 ± 113.89 items kg^{-1} dw, while biofilm-associated microplastics increased from 15.96 ± 4.31 to 30.88 ± 8.40 µg g^{-1} dw. The confidence intervals for both differences excluded zero, and the effect sizes were large. This confirms that runoff-affected reaches accumulated substantially higher microplastic loads in both depositional sediments and biologically active biofilms.

Table 1. Physicochemical and hydrological characteristics of reference and impacted irrigation canal reaches (n = 25 per group)

Variable	Reference (Mean ± SD)	Impacted (Mean ± SD)	Mean Difference (Imp − Ref)	t	df	p-Value	Cohen's d
Temperature (°C)	24.93 ± 0.38	25.09 ± 0.37	0.16	-1.51	47.97	0.1380	0.43
Depth (m)	2.01 ± 0.10	2.04 ± 0.08	0.03	-1.17	45.79	0.2475	0.33
Velocity (m s^{-1})	0.40 ± 0.04	0.41 ± 0.04	0.01	-0.88	48.00	0.3812	0.25
Conductivity (µS cm^{-1})	405.78 ± 34.22	801.39 ± 56.63	395.61	-29.90	39.47	1.0×10^{-28}	8.46
Turbidity (NTU)	10.60 ± 2.43	25.00 ± 3.81	14.40	-15.93	40.75	4.2×10^{-19}	4.51
NO_3^- (mg L^{-1})	0.97 ± 0.25	3.05 ± 0.35	2.08	-24.18	43.43	8.0×10^{-27}	6.84
PO_4^{3-} (mg L^{-1})	0.11 ± 0.02	0.29 ± 0.04	0.18	-20.12	35.29	6.3×10^{-21}	5.69
DO (mg L^{-1})	8.16 ± 0.39	5.72 ± 0.58	-2.44	17.46	42.02	7.2×10^{-21}	-4.94

Note: Values are presented as mean ± standard deviation (SD). Differences between reference and impacted reaches were evaluated using independent-samples t-tests (Welch correction for unequal variances). A p-value < 0.05 was considered statistically significant. NTU = nephelometric turbidity units. DO = dissolved oxygen.

Table 2. Microplastic loads in surficial fine sediments and biofilms on colonization plates in reference and impacted irrigation canal reaches (n = 25 per group)

Variable	Reference (Mean ± SD)	Impacted (Mean ± SD)	Mean Difference (Imp − Ref)	95% Confidence Interval (CI) for Difference	t	df	p-Value	Cohen's d
Sediment microplastics (items kg^{-1} dw)	133.53 ± 49.08	308.11 ± 113.89	174.58	124.09 to 225.06	-7.04	32.62	5.0×10^{-8}	1.99
Biofilm microplastics (µg g^{-1} dw)	15.96 ± 4.31	30.88 ± 8.40	14.92	11.09 to 18.75	-7.90	35.79	2.3×10^{-9}	2.23

Note: Values are presented as mean ± standard deviation (SD). Differences between reference and impacted reaches were evaluated using independent-samples Welch t-tests (unequal variances). Ninety-five percent confidence intervals are shown for the mean differences. A p-value < 0.05 was considered statistically significant.

Table 3. Dissolved and fine-sediment zinc and copper concentrations in reference and impacted irrigation canal reaches (n = 25 per group)

Variable	Reference (Mean ± SD)	Impacted (Mean ± SD)	Mean Difference (Imp − Ref)	95% Confidence Interval (CI) for Difference	t	df	p-Value	Cohen's d
Dissolved Zn (µg L^{-1})	10.87 ± 2.75	30.88 ± 5.92	20.02	17.36 to 22.67	-15.34	33.92	8.0×10^{-17}	4.34
Dissolved Cu (µg L^{-1})	3.10 ± 0.89	13.87 ± 4.27	10.77	8.98 to 12.57	-12.33	26.09	2.2×10^{-12}	3.49
Sediment Zn (mg kg^{-1} dw)	54.48 ± 19.96	136.56 ± 46.11	82.08	61.63 to 102.54	-8.17	32.69	2.1×10^{-9}	2.31
Sediment Cu (mg kg^{-1} dw)	19.52 ± 6.62	58.09 ± 16.95	38.57	31.15 to 45.99	-10.60	31.15	7.4×10^{-12}	3.00

Note: Values are presented as mean ± standard deviation (SD). Differences between reference and impacted reaches were evaluated using independent-samples Welch t-tests (unequal variances). Ninety-five percent confidence intervals are provided for mean differences. A p-value < 0.05 was considered statistically significant. Zn = zinc. Cu = copper.

Zinc and copper showed the same downstream enrichment pattern as microplastics. Dissolved Zn increased from 10.87 ± 2.75 to 30.88 ± 5.92 μg L^{-1}, and dissolved Cu increased from 3.10 ± 0.89 to 13.87 ± 4.27 μg L^{-1}. Fine-sediment Zn and Cu also increased strongly in impacted reaches. Because enrichment was observed in both the water column and fine sediments, the impacted reaches represented a dual exposure environment for colonizing benthic organisms (Table 3).

As presented in Table 4, all macroinvertebrate metrics indicated biological impairment in impacted reaches. Total taxon richness declined from 23.87 ± 3.80 to 14.62 ± 4.23 taxa, and Shannon diversity declined from 2.49 ± 0.29 to 1.90 ± 0.27. Sensitive taxa showed the clearest response: EPT richness fell from 6.71 ± 1.52 to 2.61 ± 0.97 taxa, and %EPT decreased from 44.44 ± 11.60% to 12.20 ± 9.40%. BMWP declined from 154.20 ± 26.75 to 59.06 ± 18.71, and ASPT decreased from 6.46 ± 0.44 to 4.04 ± 0.50. The changes are indicative of a community that shifted from more diverse and pollution-sensitive taxa toward more tolerant assemblages. The reach-level sensitivity analysis (Table 5) confirmed that the direction of the main findings was preserved after aggregation across five reference and five impacted reaches/segments.

Table 4. Macroinvertebrate richness, diversity, Ephemeroptera-Plecoptera-Trichoptera (EPT) metrics, and Biological Monitoring Working Party (BMWP)/Average Score per Taxon (ASPT) scores from colonization plates in reference and impacted irrigation canal reaches

Metric	Reference (Mean ± SD)	Impacted (Mean ± SD)	Mean Difference	95% Confidence Interval (CI)	t	df	p-Value	Cohen's d
Taxon richness	23.87 ± 3.80	14.62 ± 4.23	-9.25	-11.54 to -6.96	8.13	47.46	1.5×10^{-10}	-2.30
Shannon diversity	2.49 ± 0.29	1.90 ± 0.27	-0.59	-0.75 to -0.43	7.45	47.76	1.6×10^{-9}	-2.11
EPT richness	6.71 ± 1.52	2.61 ± 0.97	-4.10	-4.83 to -3.37	11.37	40.77	3.2×10^{-14}	-3.22
%EPT	44.44 ± 11.60	12.20 ± 9.40	-32.24	-38.25 to -26.23	10.80	46.02	3.3×10^{-14}	-3.05
BMWP	154.20 ± 26.75	59.06 ± 18.71	-95.14	-108.30 to -81.97	14.57	42.94	3.2×10^{-18}	-4.12
ASPT	6.46 ± 0.44	4.04 ± 0.50	-2.42	-2.69 to -2.15	18.17	47.24	6.0×10^{-23}	-5.14

Note: SD = standard deviation.

Table 5. Reach-level sensitivity analysis using five reference and five impacted reaches/segments

Outcome	n Reaches	Ref Mean	Imp Mean	Imp-Ref	Reach p	Data Status
Conductivity	5 Ref + 5 Imp	405.78	801.39	395.61	1.59e-07	Verified
Sediment MPs	5 Ref + 5 Imp	133.53	308.11	174.58	0.0158	Verified
Sediment Zn	5 Ref + 5 Imp	54.48	136.56	82.08	0.00668	Verified
Sediment Cu	5 Ref + 5 Imp	19.52	58.09	38.57	0.00024	Verified
Taxon richness	5 Ref + 5 Imp	23.87	14.62	-9.25	0.00334	Verified
%EPT	5 Ref + 5 Imp	44.44	12.20	-32.24	0.000175	Verified
BMWP	5 Ref + 5 Imp	154.20	59.06	-95.14	0.000336	Verified
ASPT	5 Ref + 5 Imp	6.46	4.04	-2.42	0.000103	Verified
DO	5 Ref + 5 Imp	8.16	5.72	-2.44	1.94e-06	Verified
Turbidity	5 Ref + 5 Imp	10.60	25.00	14.40	5.2e-05	Verified

Note: Values in Table 5 are reach-level sensitivity outputs derived from the individualized raw-data workbook. They show that the direction of the main findings was preserved after reach-level aggregation. Zn = zinc. Cu = copper. DO = dissolved oxygen. EPT: Ephemeroptera-Plecoptera-Trichoptera; BMWP: Biological Monitoring Working Party; ASPT: Average Score per Taxon.

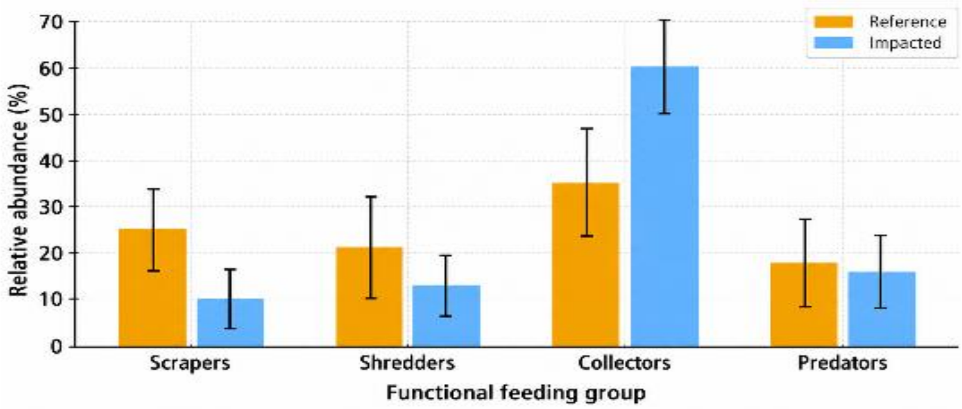

Figure 2. Functional feeding group composition of macroinvertebrates collected from Hester–Dendy colonization plates in reference and impacted reaches (n = 25 plates per group)

Note: Bars represent mean relative abundance per plate ± standard deviation. Differences between reference and impacted reaches were evaluated using independent-samples Welch t-tests (unequal variances). Ninety-five percent confidence intervals are provided for mean differences. A p-value < 0.05 was considered statistically significant.

Functional feeding group composition changed in parallel with the taxonomic results (Figure 2). Reference plates supported higher proportions of scrapers and shredders, indicating better-developed grazing and coarse organic matter pathways. Impacted plates were dominated by collectors, which is consistent with greater fine-particle deposition, turbidity, and organic enrichment. Predator proportions changed less than the other groups. Overall, the figure indicates that contamination and fine-sediment inputs altered not only community composition but also the functional structure of the canal assemblage.

As shown in Figure 3, the plate-level analysis demonstrated a significant negative association between the composite pollution index and %EPT: %EPT = 28.32 - 21.77 × CPI ($R^2 = 0.90$, $p < 0.001$). This relationship was calculated from the verified plate-level raw matrix. Plates with a low pollution score showed higher %EPT, whereas plates with higher microplastic and sediment-metal burdens showed lower %EPT. The corresponding raw values are supplied in the individualized Excel workbook.

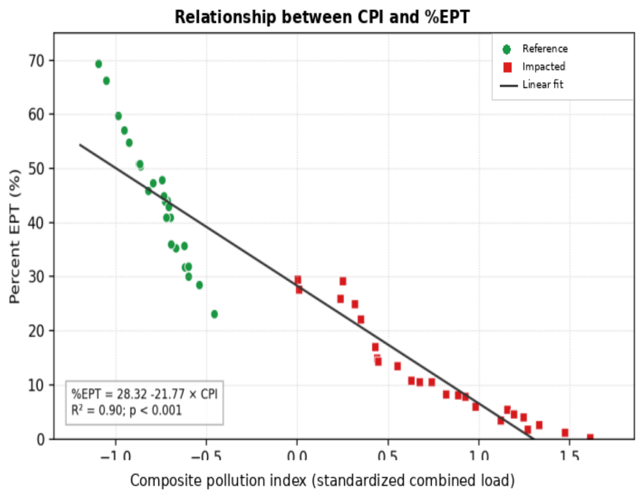

Figure 3. Relationship between the composite pollution index and percent Ephemeroptera-Plecoptera-Trichoptera (EPT) on colonization plates, calculated from plate-level raw data

4. DISCUSSION

The most important finding of this study is that impacted reaches of the canal were hydrologically similar to the reference reaches but chemically and biologically degraded. Comparable temperature, depth, and velocity decrease the probability that the observed biological impairment was due to the differences in basic physical habitat. Conductivity, turbidity, nitrate, and phosphate were, however, quite high, and dissolved oxygen was low, reflecting a strong water-quality gradient related to runoff. Similar degradation has been reported in canals and surface water bodies that receive textile, tannery, industrial, municipal, or agricultural inputs [9]. The current trend is also in line with the general findings that under the pressures of pollutants, the oxygen and salinity conditions of canals and rivers are significantly affected by discharge management, dilution, and re-aeration capacity [10, 11]. Where effluent treatment is weak and particulate loads are high, suspended solids, nutrients, and oxygen demand can increase rapidly, as illustrated in industrial wastewater and bioremediation studies [12-15].

It was also evident in the affected stretches that there was enrichment in microplastics. Sediment microplastics more than doubled, and biofilm-associated microplastics nearly doubled, illustrating that the canal was a transport channel and retention zone. This is in line with reviews and field studies, which indicate that the distribution of microplastics in freshwater systems is influenced by land use, runoff, sediment deposition, and hydrological setting [16]. The observed absolute sediment load in the present study was comparable with some irrigation ditches, but lower than the reported load in highly polluted rural ditches and urban river sediments [17, 18], which indicates a moderate to high level of local pollution rather than an extreme regional pollution hotspot. The importance of biofilm contamination is that biofilms are living feeding surfaces and early colonization habitats. Experimental and river studies show that biofilms can retain microplastics and transfer them to grazers and protists, turning passive deposits into biologically relevant exposure routes [19, 20]. This, along with data on co-occurring microplastics and metals, helps to establish the ecological relevance of measuring microplastics in both sediments and biofilms [21-23].

Enrichment of both dissolved and fine-sediment fractions in both Zn and Cu suggests multiple exposure pathways for colonizing organisms. Dissolved metals are indicative of direct exposure to the water column, and fine sediments are indicative of an available pool where metals can be bound and are in close contact with the benthic organisms. Also, similar associations have been reported between agricultural or urban drainage and higher concentrations of Cu and Zn in canal-irrigated landscapes [24], drainage sediments [25], and urban-influenced river networks [26]. Furthermore, studies of larger, more dynamic river systems generally find a higher spatial heterogeneity or stronger self-purification than was observed here, pointing to the possibility that the behavior of smaller secondary canals might be that of a depositional sink when flushing and dilution are limited [27, 28]. Lower average concentrations of Cu and Zn, as well as fewer hotspots, have been found in low-disturbance river systems, indicating that the current enrichment is associated with drainage-affected managed canals and not background conditions [29, 30]. Measured concentrations should be considered in the context of published environmental benchmarks and regional background concentrations, not as an indication of ecological risk. A comparison to reported freshwater and sediment reference values indicates that affected reaches had higher concentrations of contaminants, but a full ecological risk assessment would need more data such as contaminant bioavailability, length of exposure, toxicity, and seasonal fluctuations.

Biological response was very strong and consistent between taxonomic and biotic-index metrics. There was a decline in total richness, Shannon diversity, and EPT richness and % EPT, BMWP, and ASPT were the best indicators of ecological impairment. These metrics focus on pollution-sensitive families and sensitive orders of insects [31, 32] and are expected to respond to oxygen stress, fine sedimentation, organic enrichment, and chemical contamination. The decline in sensitive taxa was accompanied by increased representation of tolerant groups, suggesting a shift in community structure rather than a simple reduction in abundance. The observed decrease is consistent with biomonitoring studies conducted in Egypt and other parts of the world, which revealed that polluted canals or rivers exhibited lower BMWP/ASPT and replacement of sensitive taxa with tolerant ones [33, 34]. Depletion of EPT and shifts in tolerance have been observed in urbanizing streams and river systems that are subjected to cumulative stressors [35]. In contrast, hilly streams of high quality or less degraded agricultural ditches may support more diverse EPT or macroinvertebrate communities, demonstrating that canal biodiversity can survive where pollutant stressors and habitat degradation are managed [36]. Although index performance can differ across regions, as seen for non-indigenous biological tools in semi-arid regulated rivers, the direction and magnitude of response in the current study were coherent with the general principles of bioassessment [37].

The functional feeding group findings support the interpretation that the contamination also impacted the functioning of the ecosystem and not just the taxonomic composition. Scrapers and shredders were generally more abundant on reference plates, which are typically indicative of cleaner surfaces, more balanced processing of organic matter, and better oxygen conditions. The collectors dominated the impacted plates, indicative of fine-particle accumulation and suspended organics. This functional shift corresponds to an increase in turbidity, nutrients, microplastics, and sediment-bound metals that are measured. It also further enhances the importance of artificial substrates in engineered canals, where natural-bed sampling might be variable. Hester-Dendy plates standardize surface area and exposure time to help comparisons between reaches in a modified channel [38]. Given the general importance of drains and managed freshwater habitats for biodiversity, not all artificial drains should be excluded from the biological monitoring based on the premise that they are man-made [39].

The integrated stressor-response signal is obtained from the negative regression between the composite pollution index and %EPT. The reduction in %EPT represents a response associated with a combined environmental stress gradient involving microplastics, traces metals, nutrient enrichment, turbidity, and reduced dissolved oxygen. The present design does not allow separation of the independent contribution of individual stressors. This seems ecologically feasible as plastic particles, metals, nutrients, fine sediments, and oxygen decline can operate together in canal habitats. While the field design does not allow separation of the independent toxicity of each stressor, the strong consistency between water chemistry, contaminant burdens, taxonomic metrics, functional groups, and biotic indices provides a strong diagnosis of contaminant-associated impairment.

There are some limitations to note. Although the experimental design provided standardized exposure conditions, the single 28-day deployment represents a snapshot of ecological responses. Temporal changes associated with irrigation schedules, rainfall-driven runoff, and seasonal contaminant transport could influence exposure intensity and should be evaluated through repeated seasonal deployments. Taxa were identified to the lowest practicable level for routine biomonitoring, which may obscure species-level sensitivity. Other stressors, such as pesticides, organic contaminants, and microbial pollution, may also have contributed to the observed response. Nevertheless, the consistency of the verified field, laboratory, and biological data supports the reported contaminant-associated impairment pattern.

5. CONCLUSIONS

The results of this study indicate that reaches of irrigation canals receiving mixed (agricultural and urban) runoff may contain higher concentrations of microplastic and elevated levels of metals such as Zn/Cu and may exhibit reduced water quality and lower dissolved oxygen. The chemical changes were found despite generally similar hydrologic conditions in both reference and impacted reaches, lending credibility to the interpretation that biological impairment was related to gradients in contaminants and water quality. Macroinvertebrate responses were similar, with richness, Shannon diversity, EPT metrics, BMWP and ASPT, and functional feeding groups that shifted from a scraper/shredder-supported community to a collector-dominated community. Standardized Hester-Dendy colonization plates were a convenient and responsive means of assessing ecological degradation in operational irrigation canals. The synoptic application of microplastic measurement, trace-metal analysis, and macroinvertebrate biotic index may help in the more reliable diagnosis of the health status of the canals and inform management interventions. The results could provide guidance for precautionary management measures such as better runoff management, minimizing plastic inputs, and maintaining riparian buffers. Before wider recommendations for risk can be formulated, however, validation across seasons, other canal systems, and direct assessment of other human and agricultural exposure pathways is needed.

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APPENDIX

Tables A1-A6 provide the complete individualized raw data, reach-level summaries, data-completeness documentation, QA/QC records, analytical validation, and sensitivity analyses in the accompanying Excel workbook.

Table A1. Taxon-level abundance matrix, Ephemeroptera-Plecoptera-Trichoptera (EPT) classification, and Biological Monitoring Working Party (BMWP) scoring framework

Taxon	Family	Reference Abundance	Impacted Abundance	EPT Status	BMWP Score
Baetis sp.	Baetidae	95	15	Yes	4
Caenis sp.	Caenidae	72	12	Yes	7
Heptagenia sp.	Heptageniidae	45	3	Yes	10
Heptagenia sp.	Ephemerellidae	38	0	Yes	10
Hydropsyche sp.	Hydropsychidae	64	10	Yes	5
Leptocerus sp.	Leptoceridae	30	2	Yes	10
Philopotamus sp.	Philopotamidae	18	0	Yes	8
Perla sp.	Perlidae	20	0	Yes	10
Gammarus sp.	Gammaridae	80	50	No	6
Asellus sp.	Asellidae	22	48	No	3
Chironomus sp.	Chironomidae	110	260	No	2
Ceratopogon sp.	Ceratopogonidae	25	60	No	4
Simulium sp.	Simuliidae	48	42	No	5
Tubifex sp.	Tubificidae	12	160	No	1
Lymnaea sp.	Lymnaeidae	30	70	No	3
Physa sp.	Physidae	18	95	No	3
Planorbis sp.	Planorbidae	24	80	No	3
Sphaerium sp.	Sphaeriidae	35	35	No	3
Elmis sp.	Elmidae	32	10	No	5
Dytiscus sp.	Dytiscidae	18	20	No	5
Gomphus sp.	Gomphidae	21	12	No	8
Coenagrion sp.	Coenagrionidae	26	20	No	6

Table A2. Data completeness and verification summary

Domain	Summary Availability	Raw-Record Status	Data Completeness	Verification and Documentation
Main study variables in Tables 1-4	Available	Plate-level raw values available and verified	Complete	Plate-level matrix verified against reported means and standard deviations (SDs)
Spatial coordinates and reach distances	Available	GPS coordinates and reach distances documented	Complete	WGS84 reach and plate coordinates verified
Quality assurance/quality control (QA/QC) microplastic records	Available	Recovery, blanks, limit of detection (LOD)/limit of quantification (LOQ), particle size, and morphology documented	Complete	QA/QC records verified and supplied in supplementary tables

Metal-analysis QA/QC records	Available	Instrument settings, certified reference material (CRM), recovery, and detection limits documented	Complete	Method and QA/QC records verified
Nested reach structure	Available	Reach-level cluster identifiers documented	Complete	Five plates assigned to each of 10 field reaches

Table A3. Microplastic recovery and identification summary

Polymer Type	n Batches	Recovery, Mean ± SD (%)	Identification Criterion	Data Status
PE	5	87.8 ± 2.1	Pyrolysis-GC-MS library match ≥80%	Verified
PP	5	93.2 ± 3.5	Pyrolysis-GC-MS library match ≥80%	Verified
PET	5	83.2 ± 3.8	Pyrolysis-GC-MS library match ≥80%	Verified
PS	5	83.6 ± 6.6	Pyrolysis-GC-MS library match ≥80%	Verified
PVC	5	82.3 ± 4.3	Pyrolysis-GC-MS library match ≥80%	Verified

Note: PE = polyethylene; PP = polypropylene; PET = polyethylene terephthalate; PS = polystyrene; PVC = polyvinyl chloride; GC-MS = gas chromatography–mass spectrometry; SD = standard deviation.

Table A4. Microplastic limit of detection (LOD)/limit of quantification (LOQ) and blank-correction criteria

Metric	Value	Basis	Data Status
Minimum detectable particle size	50 µm	Analytical protocol threshold	Verified
Sediment MP LOD	6 items kg^{-1} dw	3 SD of blank items after mass normalization	Verified
Sediment MP LOQ	20 items kg^{-1} dw	10 SD of blank items after mass normalization	Verified
Biofilm MP mass LOD	0.18 µg g^{-1} dw	3 SD of blank mass signal	Verified
Biofilm MP mass LOQ	0.60 µg g^{-1} dw	10 SD of blank mass signal	Verified

Note: MP = Microplastics; LOD = limit of detection; LOQ = limit of quantification; SD = standard deviation.

Table A5. Metal-analysis method, instrument, CRM, and detection-limit summary

Parameter	Value	Data Status
Water preparation method	0.45-µm filtration followed by nitric-acid preservation; EPA 200.7-style ICP-OES workflow	Verified
Sediment digestion method	Microwave-assisted acid digestion of the <63-µm fraction; EPA 3051A-style digestion followed by ICP-OES	Verified
Instrument model	Thermo Scientific iCAP 7400 duo ICP-OES	Verified
Zn wavelength	213.856 nm	Verified
Cu wavelength	324.754 nm	Verified
Water Zn LOD/LOQ	0.45 / 1.50 µg L^{-1}	Verified
Water Cu LOD/LOQ	0.20 / 0.70 µg L^{-1}	Verified
Sediment Zn LOD/LOQ	0.40 / 1.30 mg kg^{-1} dw	Verified
Sediment Cu LOD/LOQ	0.12 / 0.40 mg kg^{-1} dw	Verified
CRM	NIST 1643f water and NIST 2709a sediment	Verified

Note: CRM = certified reference material; Zn = zinc; Cu = copper; EPA = United States Environmental Protection Agency; ICP-OES = inductively coupled plasma optical emission spectrometry; LOD = limit of detection; LOQ = limit of quantification; NIST = National Institute of Standards and Technology.

Table A6. Sensitivity-analysis summary for selected outcomes

Variable	Sensitivity Method	Estimated Difference (Imp − Ref)	Direction Preserved	Main Conclusion	Data Status
Conductivity (µS cm^{-1})	Plate-level analysis	395.610	Yes	increase in impacted	Verified
Conductivity (µS cm^{-1})	Reach-aggregated nested sensitivity	375.829	Yes	increase in impacted	Verified
Sediment microplastics (items kg^{-1} dw)	Plate-level analysis	174.580	Yes	increase in impacted	Verified
Sediment microplastics (items kg^{-1} dw)	Reach-aggregated nested sensitivity	165.851	Yes	increase in impacted	Verified
%EPT	Plate-level analysis	-32.240	Yes	decrease in impacted	Verified
%EPT	Reach-aggregated nested sensitivity	-30.628	Yes	decrease in impacted	Verified
ASPT	Plate-level analysis	-2.420	Yes	decrease in impacted	Verified
ASPT	Reach-aggregated nested sensitivity	-2.299	Yes	decrease in impacted	Verified

Note: EPT: Ephemeroptera-Plecoptera-Trichoptera; ASPT: Average Score per Taxon.