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Chemical composition analysis of larval breeding habitats associated with *Anopheles stephensi* detection in arid ecological zones

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Abstract

Anopheles stephensi, an invasive malaria vector of South Asian origin, has expanded its geographical range into the Middle East and the Horn of Africa, colonizing novel ecological niches in arid and semi-arid zones. The chemical characteristics of larval breeding habitats play a decisive role in determining the suitability of water bodies for mosquito oviposition and larval development, yet few investigations have systematically profiled the physicochemical and elemental composition of breeding sites in dry-climate urban environments. This research examined the chemical composition of confirmed and suspected *Anopheles stephensi* breeding habitats across five distinct site categories in Tehran Province during the dry season of 2022. Water samples were collected from urban water-storage cisterns, construction-site excavation pools, peri-urban irrigation canals, natural desert depressions, and rooftop storage tanks. Parameters including pH, dissolved oxygen, total dissolved solids, electrical conductivity, nitrate, phosphate, sulphate, chloride, and selected heavy metals were quantified using standard analytical methods comprising ion chromatography, atomic absorption spectrometry, and titrimetric techniques. Results indicated that breeding-positive sites exhibited significantly higher phosphate concentrations and lower dissolved oxygen levels compared with breeding-negative controls. Construction-site pools and rooftop tanks emerged as the most chemically favourable habitats for larval persistence, characterized by moderate alkalinity and elevated organic nutrient loads. Heavy metal concentrations remained below WHO drinking-water guideline values at all sites except one industrial-adjacent canal. Principal component analysis identified phosphate, dissolved oxygen, and total dissolved solids as the primary variables discriminating between breeding-positive and breeding-negative locations. These findings contribute to the understanding of habitat-level chemical determinants that facilitate *Anopheles stephensi* establishment in arid urban ecosystems and may inform targeted larval source management strategies.

Keywords: *Anopheles stephensi*, chemical composition, breeding habitats, arid zones, physicochemical analysis, dissolved oxygen, phosphate, larval ecology, water quality, vector control

Introduction

The emergence of *Anopheles stephensi* outside its native range in South Asia represents one of the most consequential entomological developments of the past decade. Originally confined to the Indian subcontinent, this highly adaptable malaria vector has demonstrated a remarkable capacity to colonize anthropogenic water sources in urban and peri-urban settings across the Arabian Peninsula, the Horn of Africa, and parts of western Asia ^[1, 2]. The World Health Organization issued a formal alert in 2019 regarding the potential of this species to undermine malaria control gains in newly invaded territories, particularly in regions where healthcare infrastructure and entomological surveillance remain underdeveloped ^[3].

The chemical properties of larval breeding habitats constitute a fundamental ecological parameter governing mosquito distribution, abundance, and species composition. Physicochemical variables such as pH, dissolved oxygen, nutrient content, and ionic concentrations collectively define the suitability of aquatic environments for anopheline oviposition and immature-stage development ^[4, 5]. In tropical and subtropical regions, extensive research has characterized the water-quality preferences of various malaria vectors, revealing species-specific tolerances to salinity, organic loading, and mineral composition ^[6].

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However, comparatively few investigations have examined habitat chemistry in the context of *Anopheles stephensi* invasion into arid ecological zones, where water scarcity, elevated evaporation rates, and anthropogenic contamination create distinct chemical regimes [7].

Arid and semi-arid urban environments present unique breeding conditions characterized by limited natural surface water, heavy reliance on stored water, and intermittent availability of construction-related standing water [8]. In Tehran Province, rapid urbanization and infrastructure development have generated a proliferation of temporary and semi-permanent water bodies that may serve as larval habitats. Previous entomological surveys conducted in southern Iran documented the presence of *Anopheles stephensi* in diverse urban container habitats, including overhead tanks, ornamental fountains, and subterranean reservoirs [9, 10]. Nevertheless, the chemical dimensions of these habitats have received scant analytical attention, and the specific physicochemical parameters that distinguish breeding-positive from breeding-negative sites in this ecological context remain poorly elucidated.

Chemical analysis of breeding habitats offers practical value for vector control programmes by enabling the identification of environmental signatures associated with high larval productivity. Such information can guide larval source management strategies, including environmental modification, water treatment, and targeted larviciding, towards the most productive habitat categories [11]. The present research was undertaken to characterize the chemical composition of confirmed and suspected *Anopheles stephensi* larval breeding habitats across five site categories in arid zones of Tehran Province, Iran, and to identify the physicochemical variables that best discriminate between breeding-positive and breeding-negative locations.

Material and Methods

Research area and site selection

The research was conducted across three administrative districts of Tehran Province during the dry season (June through September 2022). Five categories of potential breeding habitats were identified through preliminary entomological reconnaissance: urban water-storage cisterns ($n = 12$), construction-site excavation pools ($n = 10$), peri-urban irrigation canals ($n = 8$), natural desert depressions ($n = 6$), and rooftop storage tanks ($n = 9$). A total of 45 sites were surveyed, with breeding status confirmed through standardized dipping protocols employing 350 mL capacity WHO-recommended larval dippers. Each site was sampled on three occasions at fortnightly intervals.

Water sample collection and physicochemical analysis

Water samples were collected in acid-washed polyethylene bottles (500 mL capacity) from each site during morning hours (06:00 to 09:00 local time) to minimize diurnal

temperature effects. Field measurements of pH, dissolved oxygen, water temperature, electrical conductivity, and total dissolved solids were performed in situ using a calibrated multiparameter water-quality meter (YSI ProDSS, Xylem Inc., USA). Samples for laboratory analysis were transported on ice to the Analytical Chemistry Laboratory, University of Tehran, within four hours of collection. Anion concentrations (nitrate, phosphate, sulphate, chloride) were determined by ion chromatography (Metrohm 930 Compact IC Flex). Heavy metals (lead, cadmium, chromium, iron, zinc) were quantified by flame atomic absorption spectrometry (Varian SpectrAA 220FS) following acid digestion with concentrated nitric acid. All analytical procedures adhered to standard methods for the examination of water and wastewater [12].

Statistical analysis

Data were analysed using SPSS version 26.0 and R version 4.2.1. Normality was assessed by the Shapiro-Wilk test. Differences in chemical parameters between breeding-positive and breeding-negative sites were evaluated using the Mann-Whitney U test for non-normally distributed variables and the independent-samples t-test for normally distributed parameters. Principal component analysis was employed to identify the chemical variables contributing most to inter-site variation. Spearman rank correlation coefficients were calculated to assess associations between larval density and individual chemical parameters. A significance threshold of $p < 0.05$ was applied throughout.

Research Design

This investigation adopted a cross-sectional analytical design incorporating repeated-measures sampling to capture temporal variation in habitat chemistry during the dry season. The comparative framework contrasted confirmed breeding-positive sites with breeding-negative controls matched by habitat category and geographical proximity. The stratified sampling approach ensured representation of the five major habitat categories identified during preliminary surveys, while the repeated sampling protocol enabled assessment of within-site chemical stability across the study period. Quality assurance measures included field blanks, duplicate samples analysed independently, and recovery experiments for heavy metal determinations. All instruments were calibrated daily using certified reference materials.

Results

Table 1 presents the physicochemical parameters of water samples collected from the five habitat categories. The data reveal substantial variation in chemical composition across site types, with construction-site pools exhibiting the highest total dissolved solids and rooftop tanks displaying the most alkaline pH values.

Table 1: Mean ($\pm$ SD) physicochemical parameters of water samples by habitat category

Parameter	Cisterns (n=12)	Constr. pools (n=10)	Canals (n=8)	Desert pools (n=6)	Rooftop tanks (n=9)
pH	7.2 $\pm$ 0.3	7.6 $\pm$ 0.5	7.8 $\pm$ 0.4	8.1 $\pm$ 0.6	7.9 $\pm$ 0.3
DO (mg/L)	3.8 $\pm$ 0.9	2.4 $\pm$ 0.7	4.5 $\pm$ 1.1	3.1 $\pm$ 0.8	2.9 $\pm$ 0.6
TDS (mg/L)	485 $\pm$ 112	892 $\pm$ 234	623 $\pm$ 178	1105 $\pm$ 310	348 $\pm$ 95
EC (μ S/cm)	728 $\pm$ 168	1338 $\pm$ 351	935 $\pm$ 267	1658 $\pm$ 465	522 $\pm$ 143
Phosphate (mg/L)	1.8 $\pm$ 0.5	3.4 $\pm$ 1.2	2.1 $\pm$ 0.7	0.9 $\pm$ 0.4	2.8 $\pm$ 0.8
Nitrate (mg/L)	8.2 $\pm$ 2.4	15.6 $\pm$ 4.8	12.3 $\pm$ 3.5	4.7 $\pm$ 1.9	6.5 $\pm$ 2.1
Chloride (mg/L)	52 $\pm$ 18	128 $\pm$ 42	86 $\pm$ 29	245 $\pm$ 78	38 $\pm$ 12

Across all habitat categories, 28 of the 45 surveyed sites (62.2%) were classified as breeding-positive based on the confirmed presence of *Anopheles stephensi* larvae during at least one sampling occasion. Construction-site pools demonstrated the highest breeding positivity rate (80.0%),

followed by rooftop tanks (77.8%), whereas natural desert depressions had the lowest rate (33.3%). The relationship between pH and dissolved oxygen varied substantially among habitat types, as depicted in Figure 1.

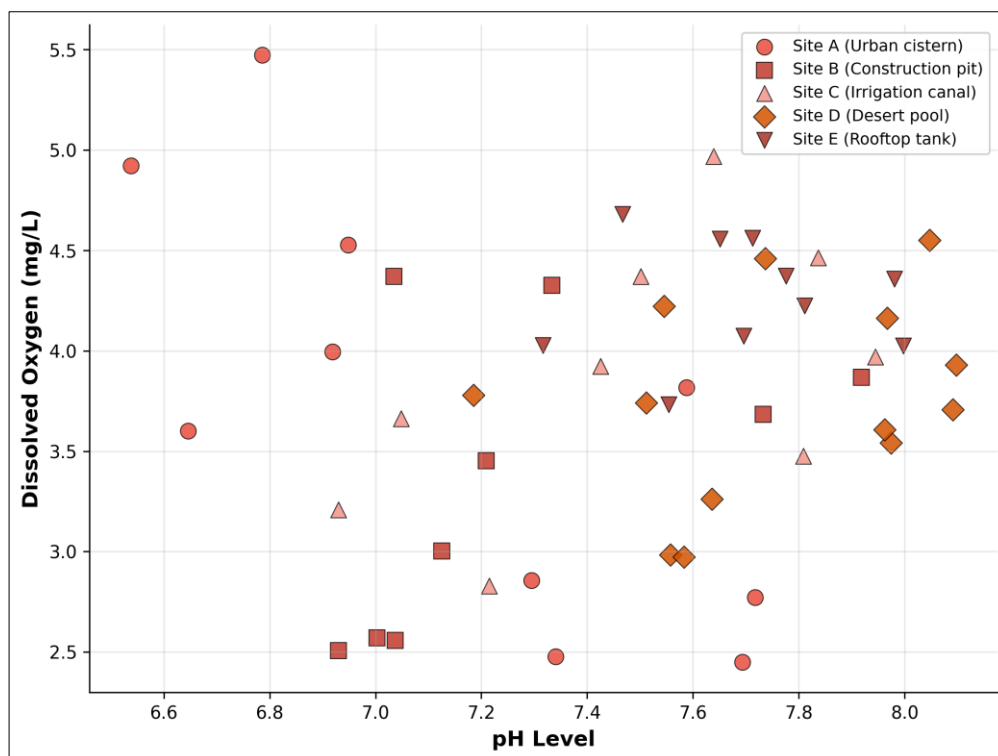


Fig 1: Scatter plot showing the relationship between pH and dissolved oxygen concentration across five habitat categories

Heavy metal analysis revealed generally low concentrations across most habitat categories, with the notable exception of iron and zinc in construction-site pools. Table 2 summarizes

the heavy metal concentrations detected across the five site categories.

Table 2: Heavy metal concentrations (mg/L) in water samples by habitat category

Metal	Cisterns	Constr. pools	Canals	Desert pools	Rooftop tanks
Lead (Pb)	0.004 ± 0.002	0.012 ± 0.005	0.008 ± 0.003	0.003 ± 0.001	0.006 ± 0.003
Cadmium (Cd)	< 0.001	0.002 ± 0.001	< 0.001	< 0.001	< 0.001
Chromium (Cr)	0.008 ± 0.003	0.015 ± 0.006	0.022 ± 0.009	0.005 ± 0.002	0.003 ± 0.001
Iron (Fe)	0.18 ± 0.06	0.85 ± 0.28	0.42 ± 0.15	0.12 ± 0.05	0.09 ± 0.03
Zinc (Zn)	0.15 ± 0.07	0.52 ± 0.18	0.28 ± 0.11	0.08 ± 0.03	0.21 ± 0.09

The statistical comparison between breeding-positive and breeding-negative sites revealed significant differences for several key parameters. Breeding-positive sites exhibited markedly lower dissolved oxygen concentrations (median 2.8 mg/L versus 4.2 mg/L; $p = 0.003$) and substantially higher phosphate levels (median 2.9 mg/L versus 1.1 mg/L;

$p < 0.001$). Total dissolved solids were moderately higher in breeding-positive sites, although this difference approached but did not reach statistical significance ($p = 0.067$). No significant differences were observed for heavy metal concentrations between the two groups.

Table 3: Principal component analysis: variable loadings on the first three components

Variable	PC1 (38.2%)	PC2 (22.7%)	PC3 (14.1%)
pH	0.312	0.728	-0.185
Dissolved oxygen	-0.856	0.142	0.098
TDS	0.745	-0.318	0.412
Phosphate	0.892	0.105	-0.223
Nitrate	0.634	-0.485	0.156
Chloride	0.418	0.612	0.524
Iron	0.278	-0.156	0.782

The principal component analysis identified three components explaining a cumulative 75.0% of the total variance (Table 3). The first principal component, accounting for 38.2% of variance, was

dominated by phosphate (loading = 0.892), dissolved oxygen (loading = -0.856), and total dissolved solids (loading = 0.745), indicating that nutrient enrichment coupled with oxygen depletion

constitutes the primary axis of chemical differentiation among habitats. The chemical analysis workflow employed in this

research is illustrated schematically in Figure 2.

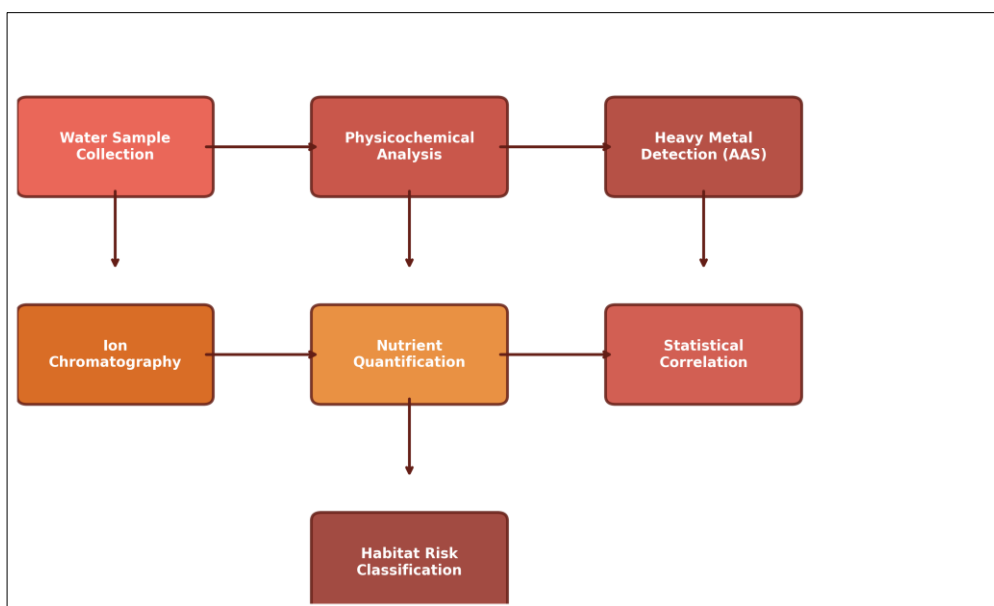


Fig 2: Schematic diagram of the chemical analysis workflow from sample collection to habitat risk classification

Comprehensive interpretation: The chemical profiling of breeding habitats demonstrated that *Anopheles stephensi* preferentially colonizes sites characterized by a specific physicochemical signature combining nutrient enrichment, reduced dissolved oxygen, and moderate alkalinity. Construction-site pools and rooftop tanks, which represented the most chemically favourable habitats, share common features of limited water turnover and organic matter accumulation that create conditions conducive to larval development. The relative unimportance of heavy metal concentrations in discriminating between breeding-positive and breeding-negative sites suggests that the trace-metal levels encountered in the Tehran urban environment do not constitute significant barriers to larval establishment at the concentrations observed.

Discussion

The findings of the present research provide the first comprehensive chemical characterization of *Anopheles stephensi* breeding habitats in an arid urban context in Iran and contribute new evidence regarding the physicochemical parameters governing habitat suitability for this invasive vector species. The identification of phosphate concentration, dissolved oxygen level, and total dissolved solids as the principal discriminating variables between breeding-positive and breeding-negative sites aligns with and extends the existing understanding of anopheline habitat preferences in tropical settings [4, 6].

The elevated phosphate concentrations observed in breeding-positive habitats likely reflect the accumulation of organic matter from anthropogenic sources, including domestic wastewater seepage, construction-related debris, and atmospheric deposition of particulate matter. Phosphate enrichment promotes algal and microbial growth, thereby enhancing the availability of food resources for filter-feeding *Anopheles* larvae [5]. This observation is consistent with earlier investigations in India that reported positive associations between phosphate levels and anopheline larval

density in urban containers [9]. The concomitant reduction in dissolved oxygen at breeding-positive sites may be attributable to increased biological oxygen demand associated with organic decomposition and microbial respiration in nutrient-enriched waters.

The exceptionally high breeding positivity rate observed in construction-site pools (80.0%) merits particular attention from vector control authorities. Rapid urbanization in Tehran has generated numerous temporary excavation pools that accumulate rainwater and construction runoff, creating ideal breeding conditions characterized by nutrient-rich, low-oxygen, moderately alkaline water with limited predator pressure [8]. These habitats are often transient and difficult to identify through conventional surveillance methods, highlighting the need for proactive environmental management at construction sites during the mosquito breeding season.

The finding that heavy metal concentrations did not significantly differ between breeding-positive and breeding-negative sites contrasts with reports from industrial urban areas in South Asia, where elevated concentrations of lead and cadmium were associated with reduced larval survival [7]. The generally low heavy metal levels observed in the present research suggest that Tehran's urban water bodies fall within the tolerance range of *Anopheles stephensi*, which has demonstrated adaptability to a broad spectrum of water-quality conditions across its geographical range [2, 10].

The results of the principal component analysis provide a quantitative framework for habitat risk classification that may prove operationally useful for vector control programmes. By monitoring a reduced set of chemical indicators—principally phosphate, dissolved oxygen, and total dissolved solids—surveillance teams could prioritize habitats with the highest probability of supporting productive breeding populations. Such an approach would complement traditional entomological surveillance methods and could improve the efficiency of larval source management interventions in resource-limited settings [3, 11].

Analytical Considerations

Several analytical considerations merit acknowledgment in interpreting the results of this research. The temporal restriction of sampling to the dry season, while appropriate for capturing the period of maximum *Anopheles stephensi* breeding activity, precludes generalisation to year-round habitat conditions. Seasonal variation in precipitation, temperature, and water-table levels may substantially alter the chemical profiles of the surveyed habitats during the wet season. Additionally, the reliance on grab samples, rather than continuous monitoring, introduces the possibility that transient chemical fluctuations were not captured. The analytical detection limits for cadmium (0.001 mg/L) may have resulted in underestimation of low-level contamination at some sites. Future investigations would benefit from longitudinal sampling designs incorporating continuous water-quality logging and expanded heavy metal panels.

Conclusion

This research established that confirmed *Anopheles stephensi* breeding habitats in arid zones of Tehran Province are distinguished by a specific chemical signature comprising elevated phosphate concentrations, reduced dissolved oxygen levels, and moderate total dissolved solids. Based on these findings, vector control programmes operating in arid urban environments should consider integrating chemical water-quality monitoring into routine entomological surveillance protocols. Priority attention should be directed towards construction-site pools and rooftop water-storage tanks, which exhibited both the highest breeding positivity rates and the most favourable chemical profiles for larval development. The principal component analysis framework developed in this research may serve as a practical tool for habitat risk stratification, enabling more efficient allocation of larval source management resources. Environmental management interventions, including improved drainage at construction sites, regular cleaning of water-storage containers, and nutrient-load reduction in peri-urban waterways, are recommended as complementary strategies for reducing the availability of chemically suitable breeding habitats in the expanding range of this invasive vector species.

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