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# Spectrophotometric Determination of Nitrate and Phosphate in Selected Water Sources of Tansen

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## Abstract

*Direct human activities, such as washing and bathing in water sources, can contribute to nutrient enrichment in surface waters. However, limited information is available on the contribution of local washing and bathing practices to nitrate and phosphate enrichment in spring water sources of Tansen, necessitating systematic monitoring. Elevated nitrate and phosphate levels in water sources may pose risks to human health and contribute to eutrophication of nearby aquatic systems. Therefore, this study aimed to assess nitrate and phosphate concentrations in some water sources in and around the urban area of Tansen, Palpa, Nepal, before and after such activities using spectrophotometric analysis. The results revealed a clear increase in nutrient levels, with nitrate concentrations rising from 10.54 ± 0.83 to 108.04 ± 1.67 $mg\,L^{-1}$ and phosphate concentrations increasing from 0.248 ± 0.007 to 1.085 ± 0.033 $mg\,L^{-1}$ across all sites. Phosphate levels exceeded the typical range for unpolluted waters (0.01–0.03 $mg\,L^{-1}$), indicating potential eutrophication risks. These elevated nutrient concentrations are attributed to detergent and organic-matter residues introduced directly into the water during washing and bathing, highlighting their role as diffuse, non-point sources of pollution in small urban water bodies. While this study is limited to selected water sources and a single sampling period, the results emphasize the importance of long-term and seasonal monitoring, regulation of phosphate-containing detergents, and public awareness initiatives to reduce nutrient loading and protect local water quality.*

***Keywords:*** *Nitrate, Phosphate, Surface water quality, Diffuse nutrient pollution, Anthropogenic*

## Introduction

The deterioration of water quality due to nutrient enrichment is a growing concern worldwide (Akinnawo, 2023). Among these nutrients, nitrate ($NO_3^-$) and phosphate ($PO_4^{3-}$) are particularly important. Elevated levels of nitrogen and phosphorus in aquatic systems can trigger eutrophication, promote algal blooms, and deplete dissolved oxygen, all of which harm biodiversity and threaten human health (Ngatia et al., 2019; Yang et al., 2008). Drinking water with high nitrate concentrations can cause methemoglobinemia, commonly known as "blue baby syndrome", and may also be linked to an increased risk of certain cancers (Knobeloch et al., 2000; Ward et al., 2018). Phosphate enrichment accelerates the degradation of aquatic ecosystems (Akinnawo, 2023). Studies in Nepal have highlighted the negative impact of elevated nitrate and phosphate levels on rivers and groundwater, with concentrations often exceeding recommended limits in densely populated areas such as the Kathmandu Valley (Singh, 2004).

Bathing, washing clothes, and other routine washing activities carried out directly at or near water sources can serve as important yet often overlooked contributors to nutrient enrichment in aquatic environments (Dhar, 2023). These everyday practices introduce a variety of chemicals into water bodies, particularly phosphates and nitrates originating from soaps, detergents, and personal care products (Tolls et al., 2010; Tsiouli & Fytianos, 2023). Detergents are organic compounds containing both polar and non-polar components and consist of various constituents, including surfactants, builders, fillers, bleaches, optical brighteners, enzymes, fragrances, and colourants. Phosphate-based builders, commonly added to enhance cleaning efficiency, directly increase phosphate concentrations in water. In addition, nitrogen-containing compounds present in detergents and organic residues from human activities can undergo microbial transformation, contributing to nitrate formation. Once discharged into nearby water systems, along with additives such as sodium sulphate and common salt, these substances can elevate nutrient levels and deteriorate overall water quality (Pedrazzani et al., 2012).

When these chemicals are repeatedly released at washing sites, they can accumulate over time and disrupt the chemical balance of local water systems. Previous studies have measured phosphate levels in commercially available detergents in Tansen, Palpa, highlighting potential sources of nutrient input from domestic use (Thapa & Khadka, 2023). Similarly, Ghimire et al. (2023) reported nitrate concentrations in groundwater in Lalitpur Metropolitan City ranging from 4 to 29.6 mg/L, while phosphate levels varied between 0.104 and 3.65 mg/L. Although most values remained within WHO-recommended limits, some samples exceeded the permissible phosphate concentration of 1 mg/L, indicating localized contamination from domestic wastewater, manure, and fertilizers (Ghimire et al., 2023). However, limited research has directly examined how washing and bathing activities contribute to nitrate and phosphate enrichment in local water sources. Therefore, evaluating the impact of daily household activities on local water quality is crucial for sustainable water resource management.

Spectrophotometric analysis is one of the most widely used techniques for measuring nitrate and phosphate concentrations in water, owing to its simplicity, sensitivity, and cost-effectiveness (García-Robledo et al., 2014; Shyla et al., 2011). This method allows rapid quantification of nutrient levels, making it particularly useful for comparing pollution inputs across different sites. However, the presence of chemically similar anions like arsenate can complicate accurate phosphate measurement. Recent studies have addressed this issue by developing interference-control procedures that enable selective phosphate determination even in arsenate-contaminated waters (Subedi et al., 2022). At the same time, research on nutrient pollution mitigation has shown that biomass-based adsorbents, such as Al(III)-loaded mango waste, can effectively remove trace concentrations of phosphate from contaminated water (Poudel et al., 2020). These studies highlight both the analytical considerations and the environmental significance of monitoring phosphate in water systems. Nevertheless, localized studies that evaluate water quality before and after household activities like washing and bathing remain scarce, especially in semi-urban areas of Nepal.

Domestic activities, agricultural runoff, and improper waste disposal influence water quality in many parts of Nepal. Studies in Palpa District have reported variable physicochemical characteristics in local drinking water sources (Khadka & Khanal, 2023), highlighting the need to assess nutrient pollutants such as nitrate and phosphate associated with washing and bathing activities. Tansen Municipality in Palpa District, western Nepal, is a hilly town where community washing and bathing practices are often carried out directly at public taps and springs. These points act as both water sources and discharge sites, thereby making them potential hotspots for nutrient contamination. While Thapa and Khadka (2023) reported phosphate levels in detergents available in Tansen, no systematic study has yet examined how washing and bathing activities directly influence nutrient levels in the associated water sources (Thapa & Khadka, 2023). Hence, the present study aims to quantify and compare nitrate and phosphate concentrations in selected water sources of Tansen before and after anthropogenic washing and bathing activities using spectrophotometric methods. This investigation will provide baseline data to understand the extent of nutrient enrichment from daily domestic practices and support the formulation of effective local water quality management strategies.

## Materials and Methods

### Study area and sample collection

Water samples were collected in March 2023 from water sources in and around the Tansen urban area, Palpa, Nepal, during the dry season. The sampling sites fall within 27.86°-27.87° N and 83.54°-83.55° E at elevations of 1200-1300 m and were coded as Tindhara (TIN), Holangdi (HOL), Sindhure (SIN), Narayansthan (NAR), Dhobidhara (DHO), and Kajipauwa (KAJ). At locations with washing and bathing activities, samples were taken before (pre-activity) and after (post-activity) to assess their impact on water quality. All samples were collected in pre-cleaned 1000 mL polyethene bottles, transported in an icebox, and stored at 4 °C to prevent microbial degradation and chemical alteration. To minimize algal growth and potential interference during spectrophotometric determination of nitrate and phosphate, bottles were kept away from direct light during transport and storage.

### Chemicals and reagents

All chemicals used were of analytical reagent grade. Distilled water was freshly prepared and used for all solution preparations and dilutions. Sulphanilic acid ($C_6H_7NO_3S$, 98%) and brucine sulphate heptahydrate , $(C_{23}H_{26}N_2O_4)_2{\cdot}H_2SO_4{\cdot}7H_2O$, 98%, (Himedia, India), sodium chloride ($NaCl$, 99%), sodium nitrate ($NaNO_3$, 99%), hydrochloric acid ($HCl$, sp. gr. 1.18) (Thermo Fisher Scientific India Pvt. Ltd., India), potassium dihydrogen phosphate ($KH_2PO_4$, 99–

100%, Himedia, India), ammonium molybdate tetrahydrate $(NH_4)_6Mo_7O_{24}\cdot4H_2O$, 99.3%, Loba Cheme Pvt. Ltd., India, ascorbic acid ($C_6H_8O_6$, 98%, Triveni Interchem Pvt. Ltd., India), and concentrated sulphuric acid ($H_2SO_4$, 98%, Thermo Fisher Scientific India Pvt. Ltd., India) were used for the preparation of standard and working solutions.

## Determination of nitrate

Nitrate concentrations were determined spectrophotometrically using the brucine-sulphanilic acid method following procedures reported in the literature with minor modification(Ramalingam & Subramania, 2021). A 1000 ppm sodium nitrate stock solution was prepared in distilled water and diluted to obtain working standards (10-150 ppm). Brucine-sulphanilic acid reagent was prepared by dissolving 1 g brucine sulphate and 0.1 g sulphanilic acid in 70 mL hot distilled water, followed by the addition of 3 mL concentrated HCl, cooling, and dilution to 100 mL. A 5% NaCl solution was also prepared for use in the assay. For colour development, 10 mL of each standard or sample solution was mixed with 2 mL of 5% NaCl, 10 mL of concentrated $H_2SO_4$, and 0.5 mL of brucine-sulphanilic acid reagent. The mixture was heated at 100°C for 20 min and then diluted to 100 mL with distilled water. Absorbance was measured at the appropriate wavelength, and nitrate concentrations were determined using a calibration curve constructed from the working standards.

## Determination of phosphate

Phosphate concentrations were determined spectrophotometrically using the molybdenum blue method, using a method described in the literature(Pradhan & Pokhrel, 2013). A 1000 ppm stock solution of potassium dihydrogen phosphate ($KH_2PO_4$) was prepared and diluted to obtain working standards in the range of 0.1-1.5 ppm. Ammonium molybdate (2.5% w/v) was prepared by dissolving 2.5 g in 100 mL of distilled water, and ascorbic acid (10% w/v) was prepared by dissolving 1.76 g in 100 mL of distilled water. These solutions were used to prepare the molybdenum blue reagent for colour development. For colour development, 2 mL of each standard or sample solution was mixed with 2 mL ammonium molybdate solution, 0.5 mL concentrated sulphuric acid, and 1 mL ascorbic acid solution. The mixture was allowed to react at room temperature, forming a blue-coloured complex, and absorbance was measured at the appropriate wavelength. Phosphate concentrations were determined using a calibration curve constructed from the working standards, following the same procedure as for nitrate.

## Spectrophotometric measurement and data analysis

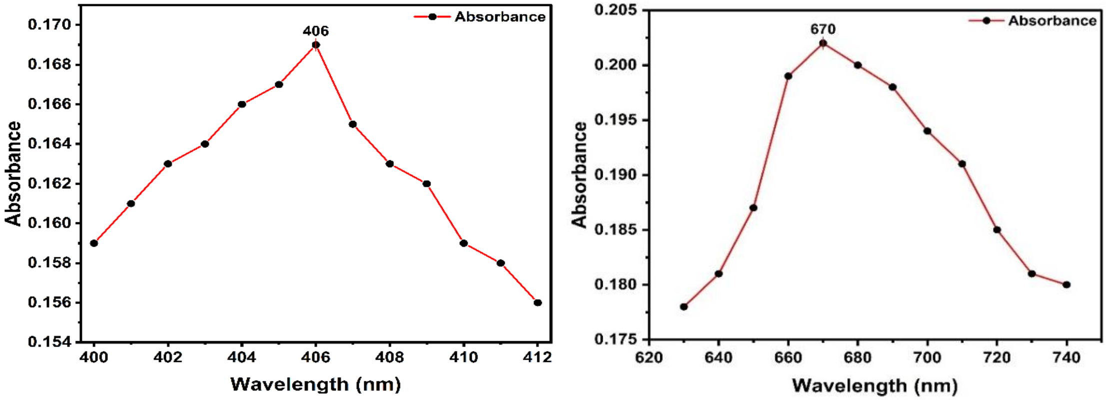
Absorbance of all standard and sample solutions was measured using a scanning visible spectrophotometer (EI-2306) under identical conditions. All measurements were performed in triplicate, and reagent blanks were used to correct for background absorbance. Concentrations of nitrate and phosphate in water samples were obtained by interpolating the measured absorbance values from the respective calibration curves.

## Results and Discussion

### Wavelength of maximum absorbance (λmax)

The absorption spectra of standard nitrate and phosphate solutions were recorded in the wavelength range of 400-750 nm using a visible scanning spectrophotometer (EI 2306). The absorbance increased steadily up to a particular wavelength and then decreased, indicating the wavelength corresponding to maximum absorbance (λmax). The λmax values were found to be 406 nm for nitrate and 670 nm for phosphate (**Figure 1**). These wavelengths were used for subsequent quantitative analyses because they provide the highest sensitivity and minimal spectral interference. The λmax values obtained are in close agreement with those reported for spectrophotometric determination of nitrate and phosphate in freshwater samples, confirming the reliability of the selected wavelengths (Isiuku & Enyoh, 2020).

### Calibration curve for nitrate and phosphate determination



(a) (b)

***Figure 1****. Absorption spectra of standard solutions showing λmax values for (a) nitrate at 406 nm and (b) phosphate at 670 nm.*

Calibration curves were prepared by plotting absorbance versus known concentrations of standard nitrate and phosphate solutions (**Figures 2a** and **2b**). A strong linear relationship was obtained in both cases, confirming adherence to the Beer-Lambert law within the studied concentration range. The linear regression equations were obtained as, for nitrate: $y = 0.01671x + 0.0052$ ($R^2 = 0.9938$) and for phosphate: $y = 0.2952x + 0.0738$ ($R^2 = 0.9830$). The high coefficients of determination ($R^2 > 0.98$) indicate excellent linearity and reproducibility of the calibration plots, validating the accuracy of the method for quantitative analysis. The low intercept values close to zero suggest negligible instrumental bias or background interference.

The calibration curves were subsequently used to determine the nitrate and phosphate concentrations in the collected water samples. The strong linearity and sensitivity of these calibration models ensure reliable quantification, even at low concentration levels, which is essential for environmental monitoring applications.

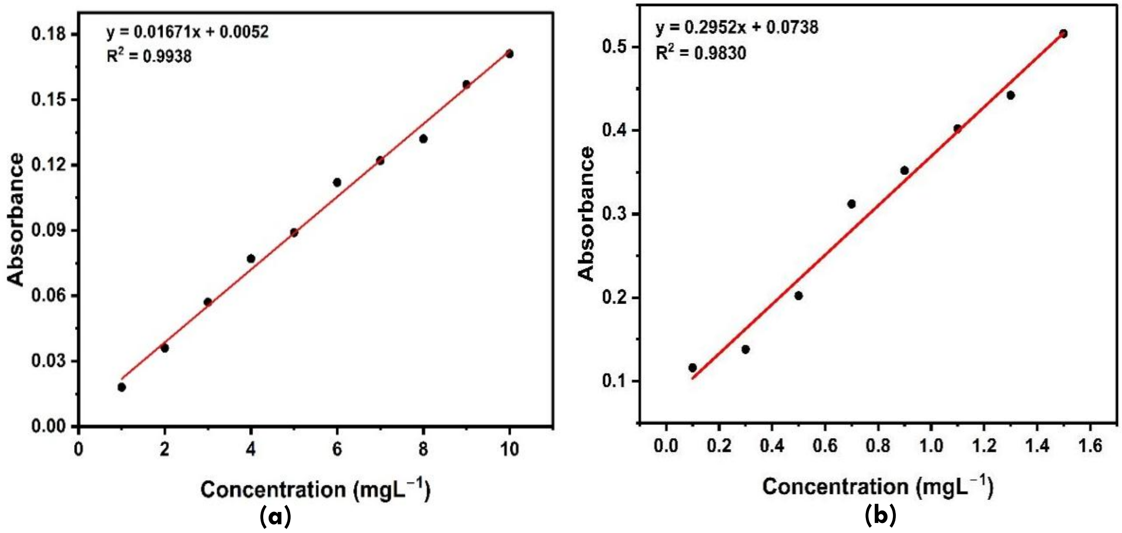


***Figure 2.*** *Calibration curves for (a) nitrate and (b) phosphate showing linear relationships between absorbance and concentration.*

The concentrations of nitrate and phosphate in water samples collected from six sites within Tansen Municipality are presented in **Tables 1, 2** and **Figure 3.** Nitrate concentrations ranged from 10.54 ± 0.83 to 45.13 ± 1.25 $mgL^{-1}$ before washing/ bathing activities and from 24.29 ± 1.25 to 108.04 ± 1.67 $mgL^{-1}$ after post activity. Similarly, phosphate concentrations ranged from 0.248 ± 0.007 to 0.605 ± 0.018 $mgL^{-1}$ before washing and increased to 0.348 ± 0.010 to 1.085 ± 0.033 $mgL^{-1}$ afterwards.

***Table 1:***

*Nitrate concentrations (mean ± SD, n = 3) in selected water sources of Tansen before (pre-activity) and after (post-activity) washing and bathing activities.*

| S.N. | Sampling Site | Absorbance | | Nitrate Concentration ($mgL^{-1}$) | |
|---|---|---|---|---|---|
| | | Pre-activity | Post-activity | Pre-activity | Post-activity |
| 1 | TIN | 0.051 ± 0.002 | 0.084 ± 0.003 | 10.54 ± 0.83 | 24.29 ± 1.25 |
| 2 | HOL | 0.134 ± 0.003 | 0.221 ± 0.004 | 45.13 ± 1.25 | 81.38 ± 1.67 |
| 3 | SIN | 0.113 ± 0.002 | 0.189 ± 0.003 | 36.38 ± 0.83 | 68.04 ± 1.25 |
| 4 | NAR | 0.100 ±0.002 | 0.146 ± 0.002 | 30.96 ± 0.83 | 50.13 ± 0.83 |
| 5 | DHO | 0.092 ± 0.002 | 0.104 ± 0.002 | 27.63 ± 0.83 | 32.63 ± 0.83 |
| 6 | KAJ | 0.108 ± 0.003 | 0.285 ± 0.004 | 34.29 ± 1.25 | 108.04 ± 1.67 |

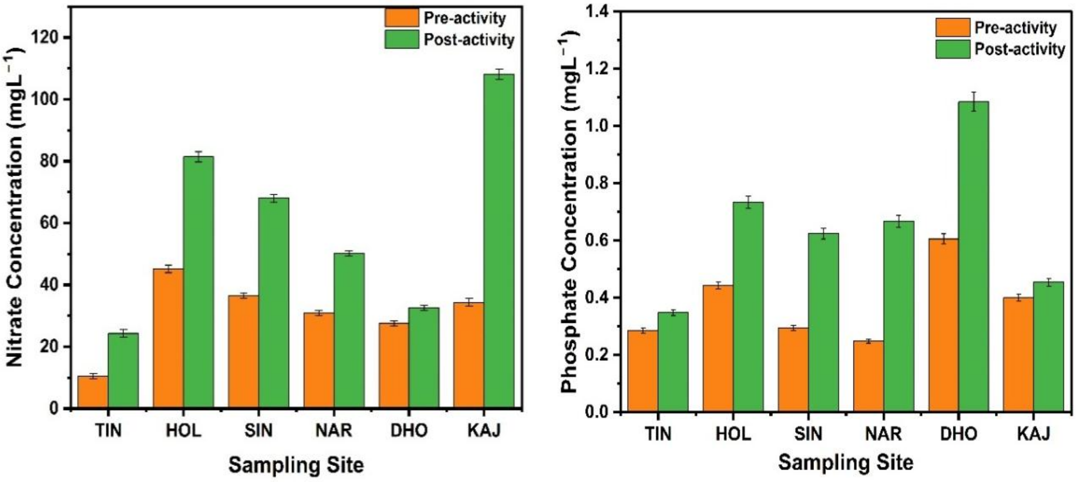
***Table 2:***

*Phosphate concentrations (mean ± SD, n = 3) in selected water sources of Tansen before (pre-activity) and after (post-activity) washing and bathing activities.*

| S.N. | Sampling Site | Absorbance | | Phosphate Concentration ($mgL^{-1}$) | |
|---|---|---|---|---|---|
| | | Pre-activity | Post-activity | Pre-activity | Post-activity |
| 1 | TIN | 0.157 ± 0.003 | 0.177 ± 0.003 | 0.286 ± 0.009 | 0.348 ± 0.010 |
| 2 | HOL | 0.207 ± 0.004 | 0.288 ± 0.006 | 0.443 ± 0.013 | 0.734± 0.022 |
| 3 | SIN | 0.160 ± 0.003 | 0.261 ± 0.006 | 0.294 ± 0.009 | 0.624 ± 0.019 |
| 4 | NAR | 0.147 ± 0.002 | 0.272 ± 0.006 | 0.248 ± 0.007 | 0.667 ± 0.020 |
| 5 | DHO | 0.250 ± 0.005 | 0.393 ± 0.010 | 0.605 ± 0.018 | 1.085 ± 0.033 |
| 6 | KAJ | 0.191 ± 0.004 | 0.204 ± 0.004 | 0.400 ± 0.012 | 0.454 ± 0.014 |

A general increase in both nitrate and phosphate levels was observed in post-activity samples across all sites, indicating that washing and bathing practices significantly contributed to nutrient enrichment. The elevated phosphate levels after washing are likely attributed to phosphate-containing detergents, while the rise in nitrate may result from organic residues and runoff. Similar patterns were reported in studies of household wastewater-affected streams in Nepal (Acharya & Pant, 2021; Singh, 2004).

The nitrate concentrations in all pre-activity samples and in two post-activity sites (TIN and DHO) were below the WHO permissible limit of 50 mg $L^{-1}$ (Mamun & Sharif, 2024), indicating minimal health concern in these locations. In contrast, the remaining post-activity sites exhibited concentrations exceeding this guideline, suggesting that washing and bathing activities may substantially elevate nitrate levels. Elevated values in these sites may pose potential health risks, particularly with continued exposure. However, the phosphate concentrations observed in the present study were substantially higher than those typically reported for unpolluted surface waters (0.01–0.03 mg $L^{-1}$), indicating a potential risk of eutrophication (Habib et al., 2015). The sites, frequently used for washing and bathing (NAR, DHO and HOL), exhibited the highest phosphate enrichment, confirming direct anthropogenic influence.



**(a)** **(b)**

***Figure 3.*** *(a) Mean nitrate and (b) mean phosphate concentrations before (pre-activity) and after (post-activity) washing and bathing activities (mean ± SD, n = 3).*

The observed increases in nitrate and phosphate concentrations suggest that anthropogenic activities, particularly washing and bathing, are contributing to nutrient enrichment in local water sources. These activities act as non-point sources of pollution, introducing nutrients indirectly through surface runoff and wastewater infiltration rather than from a single discharge point. Higher increases observed in some sources may reflect greater intensity of such activities, higher pollutant input, or site-specific factors such as reduced dilution or limited water exchange. Conversely, sources showing minimal changes might possess better natural buffering capacity, lower disturbance levels, or more effective dispersion of contaminants.

## Conclusion

This study demonstrates that washing and bathing activities significantly increase nitrate and phosphate concentrations in the surface waters of Tansen. Nitrate levels increased from 10.54-45.13 mg $L^{-1}$ before activity to 24.29-108.04 mg $L^{-1}$ after activity, while phosphate concentrations rose from 0.248-0.605 mg $L^{-1}$ to 0.348-1.085 mg $L^{-1}$, indicating clear nutrient enrichment associated with these activities. These findings confirm that routine washing and bathing act as important non-point sources of nutrient pollution, with potential implications for eutrophication and water quality deterioration. Regular monitoring, regulation of phosphate-containing detergents, and promotion of environmentally benign cleaning agents, together with community awareness and improved wastewater management, are essential to protect local water resources.

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## Conflict of Interest

The authors declare that they have no conflicts of interest related to this work.

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