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Stable Isotopic Signatures of NO_3 in Waste Water Effluent and Los Angeles River

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Abstract

A metropolitan city such as Los Angeles (LA) is an ideal study site with a very high population density, and it houses at least 3 treatment plants where sewage is treated preliminarily and then progressing to tertiary treatment before discharging into the LA River. We will gain a better understanding of the water quality in the LA River and the nitrate load in the watershed system by examining the influence of waste water treatment plants (WWTPs). The goal of this study is to pinpoint the exact source of nitrate in the LA River using the isotope signatures. We have selected sampling locations both upstream and downstream of the WWTP. This serves to monitor nitrate levels, aiding in the assessment of treatment plant effectiveness, pinpointing nitrate pollution sources, and ensuring compliance with environmental regulations. The research explores the isotopic composition of NO_3 in relation to atmospheric nitrogen and Vienna Standard Mean Ocean Water, shedding light on the contributions from various sources such as manure, sewage, soil organic nitrogen, and nitrogen fertilizers. Specifically, there is a change in the δ15NAir value between the dry and wet seasons. The isotope values in the Tillman WWTP sample changed between dry and wet seasons. Notably, the presence of nitrate originating from manure and sewage is consistent across seasons, emphasizing the significant impact of anthropogenic and agricultural activities on water quality. This investigation contributes to the broader understanding of nitrogen cycling in urban water bodies, particularly in the context of wastewater effluent discharge. The findings hold implications for water quality management and highlight the need for targeted interventions to mitigate the impact of nitrogen-containing compounds on aquatic ecosystems. Overall, the study provides a valuable framework for future research and environmental stewardship efforts aimed at preserving the health and sustainability of urban water resources. This data informs decisions regarding additional treatment or mitigation actions to safeguard downstream water quality and ecosystem health.

Keywords

Metropolitan City, Los Angeles, Treatment Plants, Sewage Treatment, Nitrate Source, Isotope Signatures, Water Quality

1. Introduction

Nitrate (NO_3), a nutrient originating safely from natural sources and dangerously from human activities, is a concern in the Los Angeles region. Contamination levels mainly arise from runoff, garden fertilizer, septic tanks, and sewage [1]. Additionally, these high levels contribute to increased aquatic plant growth, exacerbating nitrate concentrations. Nitrate negatively affects chlorine disinfection, reduces oxygen in water bodies, fosters algae growth, and triggers eutrophication, potentially elevating water toxicity due to ammonia [2]. The study by Rezaie-Boroon and Coo (2016) [2] and Takagi (2018) [3] investigated nitrate concentrations in both dry and wet periods during the past decade. Nitrate concentrations were generally higher in the dry period, particularly at close proximity to treated wastewater discharge. Nitrate levels were lowest in uncontaminated headwater areas either preceding or far downstream of wastewater treatment plants, as elevated nitrate levels were observed in samples near the Sepulveda Basin and Tillman Waste Water Treatment Plant. The authors noted nitrate sources included wastewater treatment plants, fertilizers, street runoff, and nitrifying organisms. Also, previous research by Stein and Ackerman (2005) [4] noted high nitrate levels near populous city areas. Comparison with LA River reference data (nitrate = 1 ppm) showed significantly higher concentrations (wet: 5.91 ppm, dry: 10.12 ppm) in this study, exceeding the EPA's drinking water standard of 10 ppm [5].

Nitrate (NO_3) is clean and healthy when at naturally occurring levels in groundwater and is the main benefactor to the demand of nitrogen in plants. However, this natural concentration of nitrate is tampered with by a factor of two increase due to the production and use of nitrogen fertilizers, the constant combustion of fossil fuels, and the replacement of natural vegetation with nitrogen-fixing crops such as soybeans [6]. The dominant anthropogenic input of nitrate is the utilization of nitrogen fertilizers, specifically synthetic fertilizers which have seen widespread use in the agriculture sector since the 1980s [7]. One of the well-known consequences that nitrate has on drinking water manifests itself in "blue baby syndrome", or infant methemoglobinemia, wherein bacteria that live inside infants' digestive system change nitrate into toxic nitrite (NO_2), which reacts with hemoglobin (responsible for carrying oxygen in the blood to vital tissues of the body) to form methemoglobin, which does not carry oxygen [8]. In the United States, the maximum contaminant level for nitrate in public drinking water is 10 mg/L nitrate as nitrogen (NO_3-N), which is comparable to the maximum acceptable concentration set by the World Health Organization at 11.3 mg/L. This maximum contaminant level is based on protecting the imbiber from suffering infant methemoglobinemia, which is insufficient for also protecting against other adverse side effects such as cancer and reproductive health hazards [5]. The U.S. national background level of nitrate in groundwater is 1 mg/L, this figure is roughly tripled in water nearing agricultural land use areas [9]. Since the state of California is among the highest agricultural product exporters, as well as in severe demand for water resources, it is imperative that nitrate levels are kept in check and dealt with accordingly. While the harmful effects of high nitrate concentrations are established, it remains uncertain if wastewater treatment yields nitrate-safe water for the Los Angeles River. The issue arises because there's limited research on nitrate content in wastewater effluent. Therefore, comprehending nitrate behavior in this process is crucial. Elevated nitrate levels resulting from wastewater treatment effluent could potentially harm the ecosystem. So, it is established that nitrate is harmful in high concentrations, however, it is uncertain whether wastewater treatment methods discharge a water that is of healthy nitrate concentration into the Los Angeles River. The significance lies within the fact that the amount of investigation into the nitrate signatures of wastewater effluent has been little to none, so we must try to understand more of what happens to nitrate during this process. In this case through wastewater treatment effluent, the resulting levels may show a negative impact on an ecosystem. Furthermore, given the substantial variations in nitrate concentrations observed across dry and wet periods, along with the documented presence of elevated nitrate levels downstream of the Tillman Waste Water Treatment Plant and in densely populated areas, it becomes evident that a comprehensive understanding of nitrate sources and dynamics is crucial. To better discern the contribution of wastewater treatment plants and other potential sources, conducting isotope analysis both upstream and downstream of these plants in dry and wet seasons is imperative. Such an analysis would provide valuable insights into the specific origins of nitrate contamination and help develop targeted mitigation strategies, ultimately contributing to the preservation of water quality and ecosystem health.

The primary objective of this study is to provide isotopic signatures derived from water samples collected both upstream and downstream of multiple wastewater treatment plants situated along the Los Angeles River. By accurately analyzing the water samples from various locations in dry and wet seasons for isotopic signatures, the study aims to explain the distinct compositional characteristics of nitrate sources in the river's vicinity. This effort will yield a comprehensive understanding of the extent to which wastewater treatment plants contribute to nitrate contamination, thus facilitating the formulation of targeted measures for effective water quality management and sustainable environmental conservation.

The presence of excessive nitrate levels in local rivers and coastal areas poses a multifaceted set of challenges that demand comprehensive attention and effective solutions. These issues encompass environmental, public health, and ecological concerns, requiring concerted efforts to address each aspect such as water contamination, eutrophication, and algae bloom [2]. Over the past century, stable nitrogen isotopic tracing has emerged as a prominent method for pinpointing pollution sources from various options available. Kraus, *et al*., (2017) [10] have used paired in situ high frequency nitrate measurements to better understand controls on nitrate concentrations and to estimate nitrification rate in as wastewater impacted Sacramento River. They also found out that changes in (NO_3^-) concentration were strongly related to water temperature. The estimation of the relative contribution of nitrate sources was accomplished through δ15N and δ18O ratio values of collected water samples upstream and downstream of WWTPs. The attempt to obtain isotopic signatures from water samples upstream and downstream of various wastewater treatment plants along the Los Angeles River holds paramount significance for local authorities. This initiative can provide them with a precise and data-driven understanding of the origins and distribution of nitrate contamination within the water bodies. By identifying the specific sources of nitrate pollution, whether from wastewater treatment plants or other contributors, local authorities can tailor their strategies to address the root causes of contamination more effectively. This knowledge empowers them to implement targeted measures to mitigate nitrate levels, safeguarding the health of aquatic ecosystems, ensuring the safety of drinking water supplies, and upholding the overall environmental well-being of the region. Furthermore, the insights gathered from this study can inform policy decisions and resource allocation, enabling local authorities to make informed choices that align with their mandate of preserving water quality and promoting sustainable development.

2. Materials and Methods

2.1. Water Sampling Locations

The study focuses on assessing nitrate levels in water at various locations around the Tillman Wastewater Treatment Plant and downstream where multiple wastewater treatment plants converge. This environmental monitoring and water quality assessment project covers the following key locations: 1) Upstream of Tillman Wastewater Treatment Plant: This serves as a baseline to measure natural nitrate levels in the LA River before any treatment plant influence, providing essential background data. 2) Inside Tillman Wastewater Treatment Plant: We sampled here to evaluate the initial nitrate load and assess the efficiency of nitrate removal during the treatment processes. 3) Sepulveda Basin Downstream of Tillman Wastewater Treatment Plant: This location examines the impact of treated effluent on nitrate levels, gauging the effectiveness of the Tillman WWTP in reducing nitrate concentrations and meeting regulatory standards. 4) Glendale Narrow: At this point, the Tillman WWTP effluent combines with that from the Burbank and Glendale Wastewater Treatment Plant, allowing assessment of the cumulative nitrate contributions from both plants and their combined impact on water quality. 5) Further Downstream at Arroyo Seco Confluence: In this area, effluent waters from the Tillman WWTP, Burbank WWTP, and Glendale WWTP merge, as well as mixing with untreated input from the Arroyo Seco sub-catchment. It provides a comprehensive understanding of the total nitrate loading from multiple treatment facilities at the downstream end of the study area. Notably, there were some limitations associated with accessing the chosen sampling locations during the wet season when LA River discharge was very high.

The rationale behind these specific sampling locations is to track nitrate levels at various stages of the wastewater treatment process and assess their impact on the receiving water body. It helps in evaluating the effectiveness of individual treatment plants, identifying potential sources of nitrate pollution, and ensuring compliance with environmental regulations. Additionally, data collected at these points can inform decision-makers about the need for further treatment or mitigation measures to protect water quality and ecosystem health downstream.

2.2. Study Area

The Los Angeles River Watershed is classified among the largest of the watersheds in the region at 2134 square kilometers (824 mi^2) with the L.A. River measuring a length of 88 kilometers (55 mi) (Hahn, 2018) [11]. A metropolitan is an ideal study site city such as Los Angeles with a population density of 3275 people per square kilometer, (8482 per mi^2) which is one of the most densely populated major cities in America [12]. The L.A. River watershed is supplied by runoff of the Santa Susana Mountains, the San Fernando Valley, and the San Gabriel Mountains located in the north, and drains into the L.A. River which then carries the flow to the Pacific Ocean. Originally, the L.A. River played a crucial role in the founding of the City of Los Angeles for its bountiful supply of water resources, however, today there is no reach of the river that has not been manipulated and engineered by anthropogenic interference [2]. The occurrence of numerous catastrophic floods along the L.A. River posed a problem to be solved by the U.S. Army Corps of Engineers through the construction of extensive concrete flood walls which now line nearly the entire length of the river through the city of Los Angeles, except for small stretches such as an eleven-kilometer (7 miles) soft-bottomed segment between Glendale Narrows/Burbank to north of the Arroyo Seco confluence [13]. The area of study focuses around the Tillman Wastewater Treatment plant, encompassing the upstream as well as downstream and confluence of effluents from both the Burbank and the Glendale Wastewater Treatment plant into the L.A. River (**Figure 1**). The Donald C. Tillman Water Reclamation Plant began its operations in 1985, designed to treat 40 million gallons of wastewater per day and to provide recycled water to the San Fernando Valley. The treatment process used by the Tillman plant is classified as Tertiary Treatment Nitrification/Denitrification, disinfection, and dichlorination. As the name suggests, this is a third-stage of water treatment and considered to be one of the most crucial for the treatment of toxic effluent.

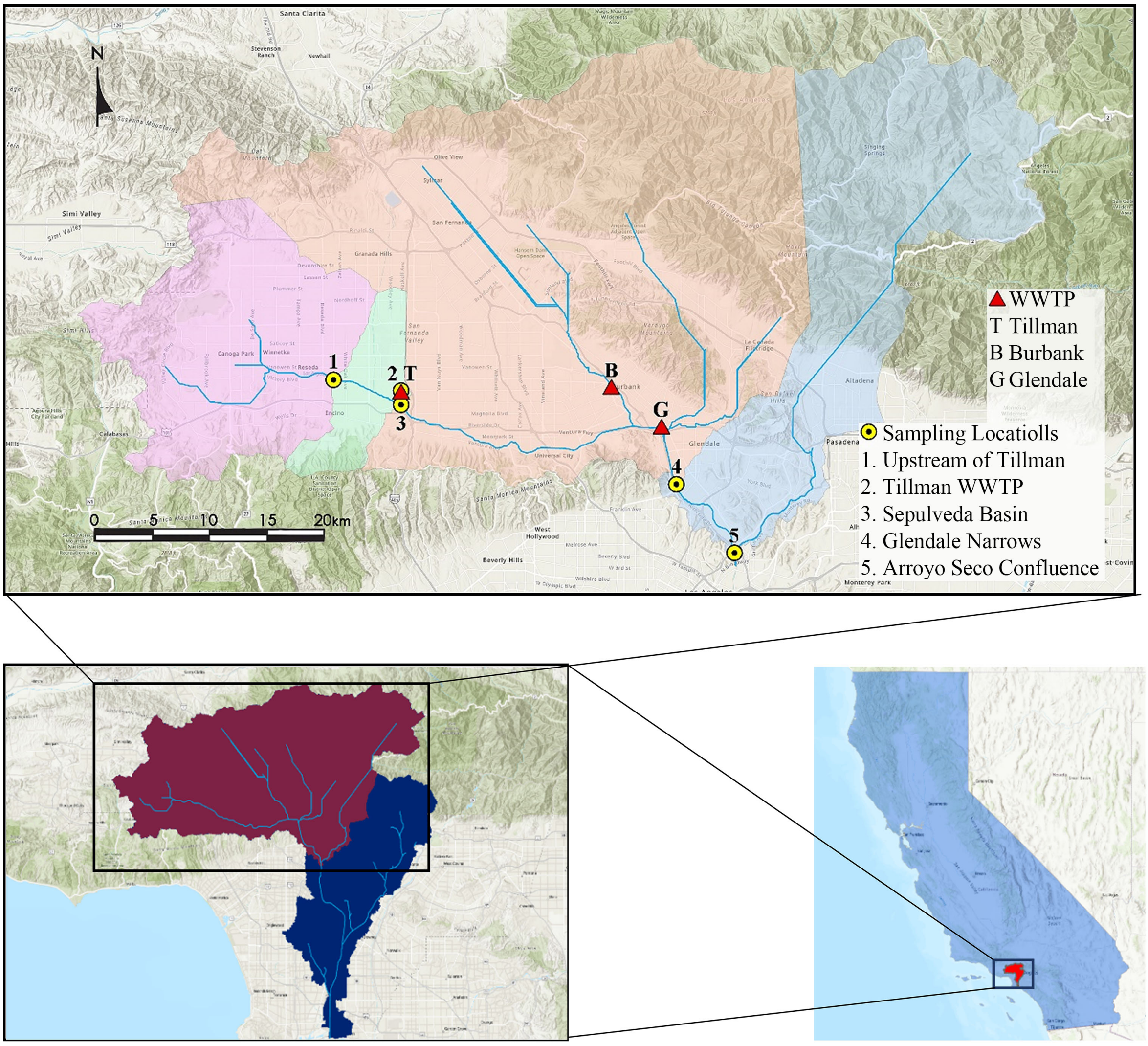

Figure 1. Map of the northern LA River watershed and the locations of the 3 Wastewater Treatment plants (WWTPs) as well as sampling sites with their associated sub catchments.

Many treatment plants focus on the first and second stages of treatment which provides a water quality that is suitable for discharge into the environment, while tertiary treatments are further in-depth and aims to provide water that is safe to drink. Nitrification is the process of breaking down ammonia contaminants into nitrite though the use of ammonia-oxidizing bacteria and then a further step is taken to break down the nitrite into nitrate through the use of nitrite-oxidizing bacteria. Next, Denitrification further lengthens this process by adding a step that reduces nitrate into nitrogen gas. Disinfection is a later stage tertiary treatment where water pathogens are purified through the use of chlorine, which then leads to the requirement of dichlorination as the final step before wastewater is then discharged into the environment [14].

2.3. Field Methods

Sampling took place over the course of a single day, at times ranging from 8:50

AM earliest to 1:07 PM latest. Samples were taken manually at the locations in 250 ml containers and were transferred immediately to a container (ice chest) kept at temperatures of 4˚C or below to avoid biological degradation processes of the samples during the sampling process and while transferring to a lab. Additionally, 50 ml samples for isotope analysis were taken and kept frozen until filtration through a 20-micron filter and shipment to an isotope lab. On site, physicochemical analysis of river water (pH, temperature, salinity) and wastewater effluent were conducted using the Accumet AP71 pH meter, HANNA Instruments HI98319 Salinity Tester, and subsequently the YSI 556 MPS meter was used in the wet season which allowed for more parameters such as DO to be recorded, these measurement devices were calibrated the day before field measurements were taken. Samples of each location were driven to Pat-Chem lab in the LA area for Nitrate as Nitrogen analysis to be carried out within 48 hours in order to avoid degradation, while the three isotope samples from Tillman, Upstream, and Downstream were frozen and shipped to UC Davis Stable Isotope Facility for isotope analysis after filtration.

2.4. Laboratory Methods

2.4.1. Isotope Analysis

The isotope analysis (δ15N-NOx, δ18O-NOx.) of collected samples was conducted at the University of California, Davis. The principle of the method is the measurement of the isotopic composition of nitrous oxide (N_2O) following the conversion of nitrate and nitrite (NOx) to N_2O by bacteria that lack N_2O reductase. It's essential to consider the measurement precision and potential sources of error when interpreting the differences in isotope ratios, especially when dealing with small variations. To satisfy this goal, we will measure the stable isotope analysis of δ15N. This will serve as a tracer for the source of N in the environment [15]. Nitrogen in the environment could rapidly be diluted, transported by waves and current, the source of δ15N values is preserved in primary sources. These values could be defined as isotope baseline for the marine food web [16].

According to Kendal *et al.* (2010) [15] each source of nitrogen has a unique δ15N signature associated with it. For example, river water and treated waste water have their own different δN15 composition therefore different signatures. According to Fry (2006) [15] [17] while atmospheric deposition has δN15 signatures (composition) of −15 to +3‰ the treated wastewater is estimated to be between 10 and 20‰ [18] [19]. Other specific examples of nitrogen sources, which have unique δ15N values are synthetic fertilizer (~3‰), combustion (~1‰), and precipitation (~−7‰) [17].

The presence of duplicates was analyzed for the purpose of assessing measurement reproducibility. It's important to evaluate whether the observed variations fall within an acceptable range for the research objectives. Statistical analysis could be applied to determine if the observed variations are statistically significant and not due to random fluctuations or measurement errors. Detailed metadata, including the conditions under which the samples were collected and the specific methods used for analysis, would greatly aid in the accurate interpretation of these isotope ratio measurements.

2.4.2. Calibration and Reporting of Stable Isotope Ratios

The water samples were measured at Stable Isotope Facility, University of California, Davis. They used quality control procedure for analyzing samples for stable isotope signatures. The isotope analysis of collected samples involved determining δ15NNOx and δ18ONOx for all water samples using the bacterial denitrifier method [20] [21]. The method's principle revolves around measuring the isotopic composition of nitrous oxide (N_2O) subsequent to the conversion of nitrate and nitrite (NOx) to N_2O through bacteria lacking N_2O reductase. Quality control and assurance mixtures are composed of pure nitrates that have been calibrated separately by EA-IRMS using certified reference materials (e.g., USGS32, USGS43, and IAEA-NO_3) distributed by USGS, NIST, and the IAEA. All are directly traceable to the primary isotopic reference material for each element (*i.e.*, Air for δ15N and VSMOW for δ18O). Calibration procedures for dissolved nitrates are applied identically across reference and sample materials. First, a pure N_2O reference gas is used to calculate provisional isotopic values of the sample peaks. Next, isotopic values are adjusted for changes in linearity and instrumental drift using IAEA-NO_3 and an in-house dissolved potassium nitrate reference material, Acros. Finally, measurements are scale-normalized to the primary reference materials using dissolved certified standard reference materials, the nitrates USGS32, USGS34, USGS35, and IAEA-NO_3. Final quality assessment is based on the accuracy and precision of the unbiased quality control materials, dissolved δ15N- and δ18O-calibrated nitrates (*i.e.*, Strem, Fisher, and NewAcros). They used quality assurance reference materials including USGS32, USGS34, USGS35, IAEA-NO_3, Acros Quality control reference materials: Strem, Fisher, NewAcros.

3. Results

3.1. Stable Isotope Analysis

We analyzed isotopic signatures of water samples in various locations upstream and downstream of WWTPs in the dry and wet seasons (**Figure 1**). For the Tillman sample, there was a change in the δ15NAir value from 13.92 to 14.03‰. This variation suggests a possible difference in nitrogen isotope composition between these measurements. Similarly, the Tillman sample shows a change in the δ18OVSMOW value from 3.48 to 3.69‰. This variability might indicate fluctuations in oxygen isotope ratios. The range of δ15NAir values (13.92 to 15.09‰) across the different samples highlights differences in nitrogen isotopic compositions. Similarly, the δ18OVSMOW values (2.70 to 9.49‰) show variability in oxygen isotopic compositions. **Table 1** shows the variations in isotope ratios (δ15N O and δ18O) for water samples at different locations observed during both the Dry and Wet Seasons of 2022-2023. This highlights alterations in the nitrogen and oxygen isotope compositions within the water samples.

Table 1. Isotope Ratios (δ15N O and δ18O) for water samples at various distances during the Dry and Wet Seasons (2022-2023), Illustrating changes in nitrogen and oxygen isotope composition in water samples. N/A: Not applicable. ‰ = Part per thousands.

		2022 Dry Season		2023 Wet Season	
	Distance km (miles)	**δ15NAir (‰)**	**δ18OVSMOW (‰)**	**δ15NAir (‰)**	**δ18OVSMOW (‰)**
Upstream	0	14.72	9.49	9.74	5.11
Tillman	5.07 (3.2)	13.92	3.48	9.79	−2.49
Tillman (lab duplicate)	N/A	14.03	3.69	9.7	−3.01
Sepulveda Basin	6.25 (3.9)	15.09	2.7	9.86	2.84

3.2. Nitrate and Nitrite Analysis

We measured the collected samples at Pat Chem Environmental Laboratory withing 48 hrs of collecting water samples. The results of water testing for various Nitrate (NO_3) and Nitrate as N inorganic constituents, (specifically nitrate and nitrite), collected on January 18, 2023. The testing method used is EPA 353.2 AA32004. The breakdown of the data and statistical analysis are shown in Table 2.

Based on the analysis, it's clear that nitrate concentrations vary significantly among the samples, with concentrations ranging from very low (2.56 mg/l) to relatively high (6.17 mg/l). Nitrite concentrations, on the other hand, are mostly below the reporting limit but show minor fluctuations around 0.08 mg/l to 0.09 mg/l in some samples.

It's important to note that these results provide a snapshot of the water's inorganic constituent concentrations at the time of testing. Interpretation of these results would involve comparing them to relevant water quality standards or guidelines to determine whether the concentrations are within acceptable limits for the intended use of the water (e.g., drinking water, agricultural use, etc.). Additionally, any trends or patterns in the data over time or across different sampling locations would be important to consider.

NO_3 chemical analysis Data (Table 3). This table presents nitrate-nitrogen (NO_3-N) concentrations in milligrams per liter at various distances from the source across different years (2012-2023).

A gradual increase in nitrate concentrations is observed from 2022 to 2023 at sampling locations, suggesting a potential change in water quality over time. However, there is no data for the 2012, 2013, 2017, and 2018 time periods when water samples were not taken at the upstream location and at Tillman WWTP.

Sepulveda Basin consistently shows moderate nitrate concentrations across the years, with a peak in 2022, and a sudden drop in 2023, likely attributed to the location being right at the discharge from the Tillman WWTP and the anomalous heavy storm event in 2023.

Glendale Narrows exhibits varying nitrate levels, much higher during dry seasons, with a notable decrease in 2013 and an increase in 2022. Arroyo Seco Confluence displays fluctuating nitrate concentrations, with a significant decrease in 2018 and a subsequent increase in 2022. The average nitrate concentration across all locations increases from 4.27 mg/l in 2012 to 3.07 mg/l in 2023.

Standard deviation (STDEV) values indicate the degree of variability in nitrate concentrations, with a decrease from 2018 to 2023.

Table 2. Nitrate-N concentrations (mg/l) at various distances across different years alternating Dry (2012 [2], 2017 [3], 2022) and Wet (2013 [2], 2018 [3], 2023) seasons, highlighting temporal trends and spatial variations in water quality in the study area (2012-2023). ND: Not determined.

		2012 Dry Season	2013 Wet Season	2017 Dry Season	2018 Wet Season	2022 Dry Season	2023 Wet Season
Sample Location	**Distance km (miles)**	**NO_3-N (mg/l)**	**NO_3-N (mg/l)**	**NO_3-N (mg/l)**	**NO_3-N (mg/l)**	**NO_3-N (mg/l)**	**NO_3-N (mg/l)**
Upstream	0	ND	ND	ND	ND	4.36	2.56
Tillman WWTP	5.07 (3.2)	ND	ND	ND	ND	4.54	3.55
Sepulveda Basin	6.25 (3.9)	3.8	3.86	4.04	4.2	6.17	3.3
Glendale Narrows	35.77 (22.2)	4.86	1.63	4.2	2.78	4.34	3.2
Arroyo Seco Confluence	44.52 (27.7)	4.16	2.53	3.68	1.3	4.77	2.75
	Average	4.27	2.67	3.98	2.76	4.84	3.07
	STDEV	0.54	1.12	0.27	1.45	0.77	0.41
	MIN	3.8	1.63	3.68	1.3	4.34	2.56
	MEDIAN	4.16	2.53	4.04	2.78	4.54	3.2
	MAX	4.86	3.86	4.2	4.2	6.17	3.55

Table 3. This table shows the Water Quality Parameters at different locations during Dry and Wet seasons (2022-2023) with variability in pH, Temperature, Salinity, and Dissolved Oxygen Levels. The average values for each parameter are calculated, providing a summary of central tendency. Standard deviations (STDEV) indicate the variability or dispersion of data around the mean. Medians, representing the middle values, are close to averages. ND = not determine. ‰ = Part per thousand.

		Dry	Season	2022		Wet	Season	2023	
Sample Location	**Distance km (miles)**	**pH**	**Temp (˚C)**	**Salinity (‰)**	**DO (ppm)**	**pH**	**Temp (˚C)**	**Salinity (‰)**	**DO (ppm)**
Upstream	0	7.07	21.7	1.1	ND	6.95	8.9	0.87	2.58
Tillman WWTP	5.07 (3.2)	6.54	24.5	0.5	ND	6.65	16.4	0.46	4.68
Sepulveda Basin	6.25 (3.9)	5.72	22.6	0.5	ND	6.58	11	0.7	4.66
Glendale Narrows	35.77 (22.2)	6.7	24.8	0.5	ND	7.05	15.2	0.6	3.25
Arroyo Seco Confluence	44.52 (27.7)	8.1	23.7	0.4	ND	8.74	15.2	0.41	1.4
	Average	6.83	23.46	0.6		7.19	13.34	0.608	3.314
	STDEV	0.87	1.3	0.28		0.89	3.22	0.19	1.4
	MIN	5.72	21.7	0.4		6.58	8.9	0.41	1.4
	MEDIAN	6.7	23.7	0.5		6.95	15.2	0.6	3.25
	MAX	8.1	24.8	1.1		8.74	16.4	0.87	4.68

Minimum (MIN), median (MEDIAN), and maximum (MAX) values provide additional insights into the range of nitrate levels observed at each location. The data suggests potential changes in nitrate levels over time and spatial variability among different sample locations. Monitoring and understanding these trends are crucial for assessing water quality, identifying sources of contamination, and implementing effective mitigation measures.

3.3. Physio-Chemical Properties

This table provides data on water quality parameters at different sample locations during the dry season of 2022 and the wet season of 2023. The parameters measured include pH, temperature (˚C), salinity (‰), and dissolved oxygen (DO, ppm). Overall, there are variations in water quality parameters between sample locations and seasons. The data can be used to identify trends, assess the impact of the Tillman WWTP, and understand seasonal variations in water quality.

The pH values generally range from 5.72 to 8.1. The highest pH is observed at the Arroyo Seco Confluence during the wet season (8.74), indicating a more alkaline condition. The lowest pH is at Sepulveda Basin during the dry season (5.72).

Temperature values range from 21.7˚C to 24.8˚C. The highest temperature is recorded at Glendale Narrows during the wet season (24.8˚C). The lowest temperature is at Upstream during the dry season (21.7˚C). Salinity remains relatively constant at 0.4 to 1.1‰. The highest salinity is observed at Upstream during the dry season (1.1‰). The lowest salinity is consistently at Arroyo Seco Confluence. Dissolved oxygen levels range from 1.4 to 4.68 ppm. The highest dissolved oxygen concentration is recorded at Tillman WWTP during the wet season (4.68 ppm). The lowest dissolved oxygen concentration is at Arroyo Seco Confluence during the wet season (1.4 ppm) (**Table 3**).

4. Discussion

4.1. Nitrate

Moreover, a U.S. nationwide study has shown that nitrate concentrations exceeding 1 mg/l are indicative of human activities adversely affecting water quality [9]. From this it is apparent that nitrate concentrations in the L.A. River are well above levels indicative of contamination being on an upward trend from 2012 to 2023, while concentrations had only been seen to drop during the wet season of 2018.

Figure 2 compares the isotopic composition of nitrogen (δ15N) and oxygen (δ18O) in nitrate from various sources. Considering δ15N vs. Air axis likely represents the nitrogen isotope composition (δ15N) of nitrate relative to atmospheric nitrogen (N2 in the air). Different sources of nitrate, such as manure, sewage, soil organic nitrogen, and nitrate from reduced nitrogen fertilizers, are compared. The δ15N values for each source indicate the extent to which the nitrogen in

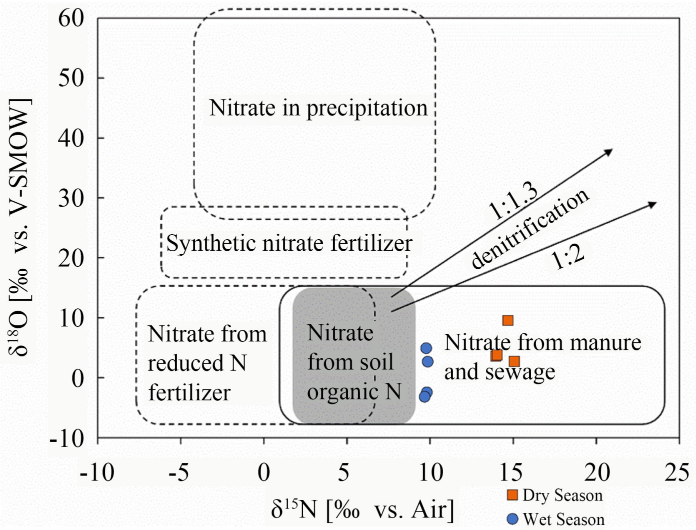

Figure 2. Expected signatures of δ15N and δ18O [15].

the nitrate has undergone various processes (e.g., denitrification, nitrification) that can alter its isotopic signature. The results further reveal spatial differences, with higher δ15NAir values downstream at the Tillman WWTP and Sepulveda Basin locations compared to upstream, suggesting potential influences from anthropogenic sources. Similar to nitrogen isotopes, δ18OVSMOW values showed variability among samples, indicating differences in oxygen isotopic compositions.

As for δ18O vs. V-SMOW axis, it represents the oxygen isotope composition (δ18O) of nitrate relative to Vienna Standard Mean Ocean Water (V-SMOW). Similar to δ15N, different nitrate sources are compared based on their δ18O values. The δ18O values provide insights into the origin and history of the nitrate, considering processes like denitrification, precipitation, and atmospheric interactions. Variations in δ15N and δ18O values among different nitrate sources can indicate distinct biogeochemical pathways and sources of nitrogen and oxygen in the nitrate. For example, different δ15N values might suggest varying contributions from natural processes (e.g., organic matter decomposition, microbial activity) or anthropogenic activities (e.g., fertilizer use). Changes in δ18O values may reflect the influence of environmental conditions and processes like microbial denitrification.

By analyzing the trends and patterns in the scatter plot or graph, one can identify relationships between the isotopic compositions of nitrate from different sources [15]. The plot clearly shows that water samples from both the dry and wet seasons in the study area have nitrate originating from manure and sewage. Understanding these isotopic signatures is valuable for tracing the origin of nitrate, identifying pollution sources, and studying nitrogen cycling in ecosystems.

As the results indicate, the δ15NAir values exhibit a notable range (13.92 to 15.09 ‰) across different samples, indicating significant differences in nitrogen isotopic compositions. Within a specific sample (Tillman), there is a change in the δ15NAir value from 13.92 to 9.79‰ between the dry and wet seasons. This suggests a potential seasonal shift in nitrogen isotope composition. The results further reveal spatial differences, with higher δ15NAir values downstream at the Tillman and Sepulveda Basin locations compared to upstream, suggesting potential influences from anthropogenic sources. Similar to nitrogen isotopes, δ18OVSMOW values showed variability (2.70 to 9.49‰) among samples, indicating differences in oxygen isotopic compositions. The δ18OVSMOW values in the Tillman sample changed from 3.48 to −3.01‰ between dry and wet seasons. The spatial variation also indicated differences in oxygen isotopes at different locations, emphasizing the complexity of the system.

The diverse δ15NAir values suggest multiple nitrogen sources, including potential anthropogenic inputs such as wastewater discharge [22]. The seasonal changes and spatial variations may be indicative of shifts in nitrogen sources or microbial processes. Fluctuations in δ18OVSMOW values reflect changes in oxygen dynamics, potentially influenced by biological activities, precipitation, or other environmental factors. Understanding these variations is crucial for assessing the overall health and dynamics of the aquatic ecosystem. The presence of significant changes in isotopic compositions downstream of WWTPs suggests potential anthropogenic contributions to nitrogen and oxygen isotopes. The isotopic signatures could serve as tracers, helping identify and quantify the impact of human activities on water quality. The observed changes in isotopic values between dry and wet seasons indicate that seasonal variations play a role in influencing the nitrogen and oxygen isotopic compositions. This could be linked to factors such as rainfall, temperature, and changes in water flow patterns [23]. While the study provides valuable insights, it is essential to acknowledge potential limitations. Further research, including more extensive spatial and temporal sampling, would enhance the understanding of isotopic dynamics and their relationship with water quality parameters. Thus, the stable isotope analysis contributes valuable information for tracing nitrogen and oxygen sources, understanding anthropogenic impacts, and unraveling the complex dynamics of aquatic ecosystems. The findings have implications for water resource management, pollution assessment, and the development of targeted mitigation strategies.

4.2. pH

The ideal target for pH in river water is 7.4, whereas lower values are particularly dangerous as an increase in acidity creates an inhospitable environment to life and speeds up leaching of heavy metals [24]. The L.A. River had been consistent from 2012-2018 showing pH averages near 7.4 in the dry seasons and 8.0 in wet seasons, however, this pH average then dropped to 6.8 in dry and 7.2 during wet seasons in the years of 2022-2023. The drop in pH is concerning as levels around 6.9 are only adequately alkaline for survival of most organisms.

4.3. Salinity

The Tillman wastewater treatment plant (WWTP) employs sodium hypochlorite

(NaClO) for water disinfection following tertiary treatment, and this is subsequently dechlorinated using sodium bisulfite ($NaHSO_3$). This chemical reaction (4.3.1) results in the formation of salt (NaCl) [2].

$$NaHSO_3 + NaClO \rightarrow NaHSO_4 + NaCl \quad (4.3.1)$$

This would explain an increase in salinity levels in water from the WWTP effluent, as well as downstream in the Sepulveda Basin area, as observed in the 2012-2013 study. During this period, the Sepulveda Basin exhibited salinity readings greater than zero, measuring 3‰ in the Dry Season and 5‰ (Part Per thousand or ‰ in the Wet Season. Additionally, the Arroyo Seco confluence registered a salinity of 2‰ in 2013. However, the 2017 Dry Season data shows a consistent 0.5‰ salinity throughout the Sepulveda Basin, Glendale Narrows, and Arroyo Seco confluence locations [3].

In the current study for this paper, during the Dry Season of 2022 and the Wet Season of 2023, samples were collected both upstream of the WWTP and directly inside the WWTP. The salinity levels recorded were highest at 1.1‰ and 0.87‰, respectively. Conversely, the WWTP's immediate sampling location resulted in the lowest recorded salinity levels along the entire sampled river. Interestingly, the Sepulveda Basin location exhibited unremarkable changes in salinity compared to the Glendale Narrows location but showed a distinct drop in salinity at the Arroyo Seco confluence. As a result, it is inconclusive whether the chemical processes at the WWTP can be directly correlated with the salinity measurements in the current 2022-2023 river conditions.

4.4. Water Temperature

Average river water temperature in the United States has been recorded to increase at a rate of 0.009˚C - 0.077˚C per year, and rates of warming were most rapid in, but not confined to, urbanizing areas [25]. When comparing the data recorded from 2012-2013 in this study, we can see a drastic increase in average L.A. River water temperatures from Dry Seasons 2012 to 2017 by 2.43 degrees, and then a decrease from 2017-2022 by 0.84 degrees, bringing an overall 2012-2022 increase of 1.59 degrees. For the Wet Seasons, the average temperature increased from 2013-2018 by 4.24 degrees, then dropped 7.03 degrees in 2018-2023 for a total drop of 2.79 degrees from 2013-2023. In both Dry and Wet seasons, the average temperature is shown to have risen from the first data set of 2012/2013 and then fallen back down in the final data set in 2022/2023. The change in temperatures from before and after the 2017/2018 study are interestingly large margins which coincide with the exiting of a drought beforehand in which we see a raising temperature, and entering of a drought afterwards in which we see lowering temperature. Comparing to the average temperature change of rivers in the U.S., the L.A. River has increased in temperature over twice as much as the high endpoint of the established rate over the past 10 years when measured during the Dry Season. However, the temperature has dropped four times the high endpoint of the established rate during the Wet Season, al-though this may be attributed to the record high wet conditions during the sampling of 2023. According to Mongolo *et al.* (2017) [26] and their findings of their pilot study reveal that the Los Angeles River experiences elevated temperatures, particularly from June to October, which currently render it unsuitable for supporting native fish species. However, these warmer conditions are conducive to a diverse range of non-native fish species. A comparison between soft-bottom and concrete-lined channel segments indicates that the thermal effects of concrete result in a broader temperature range, characterized by higher maximums and lower minimums. Additionally, this concrete-induced warming persists throughout the night, leading to warmer nighttime temperatures compared to daytime in certain segments. If this observed pattern remains consistent in further investigations, it becomes a critical factor in shaping the vision of a restored Los Angeles River, particularly if the objective of future restoration projects is to reintroduce native fish species across the entire watershed [26].

LA River water, shows a positive correlation with NO_3-N and negative correlations with both water temperature and pH. Salinity and dissolved oxygen (DO) are not easily matched with the drought history due to missing data (**Figure 3** and **Figure 4**)

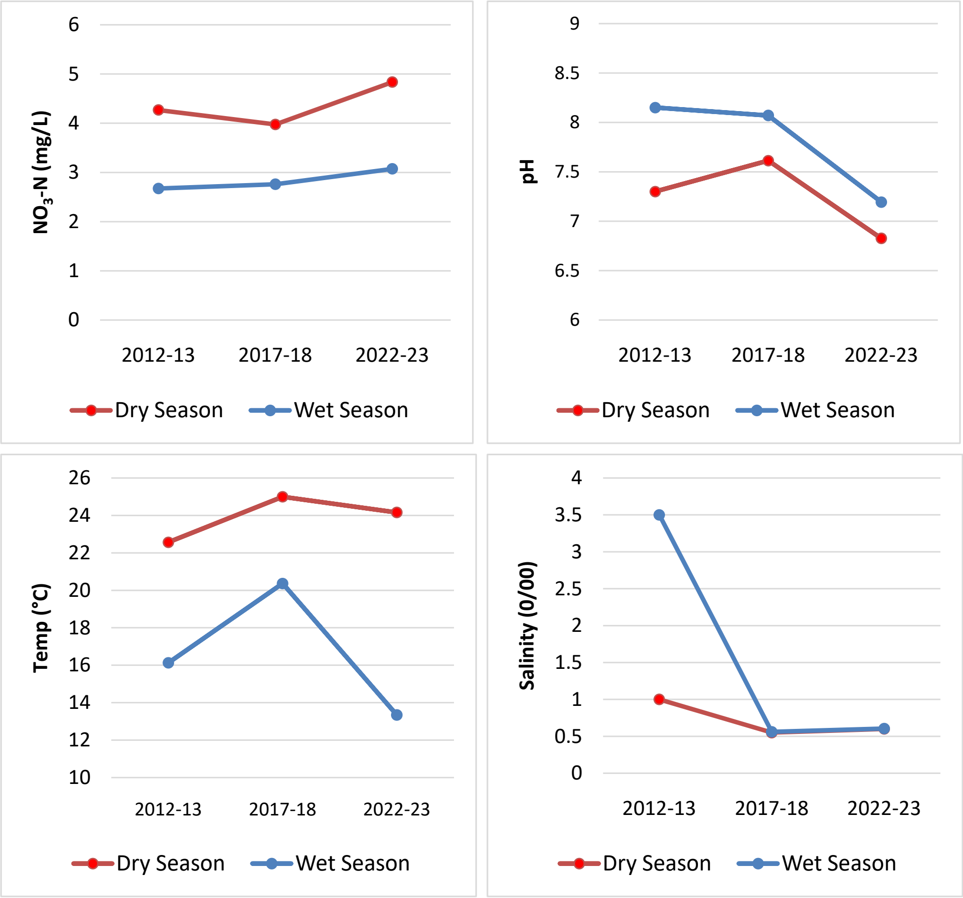

Figure 3. Physical property averages of all study locations along the L.A. River from the 2012-2023 studies [2] [3].

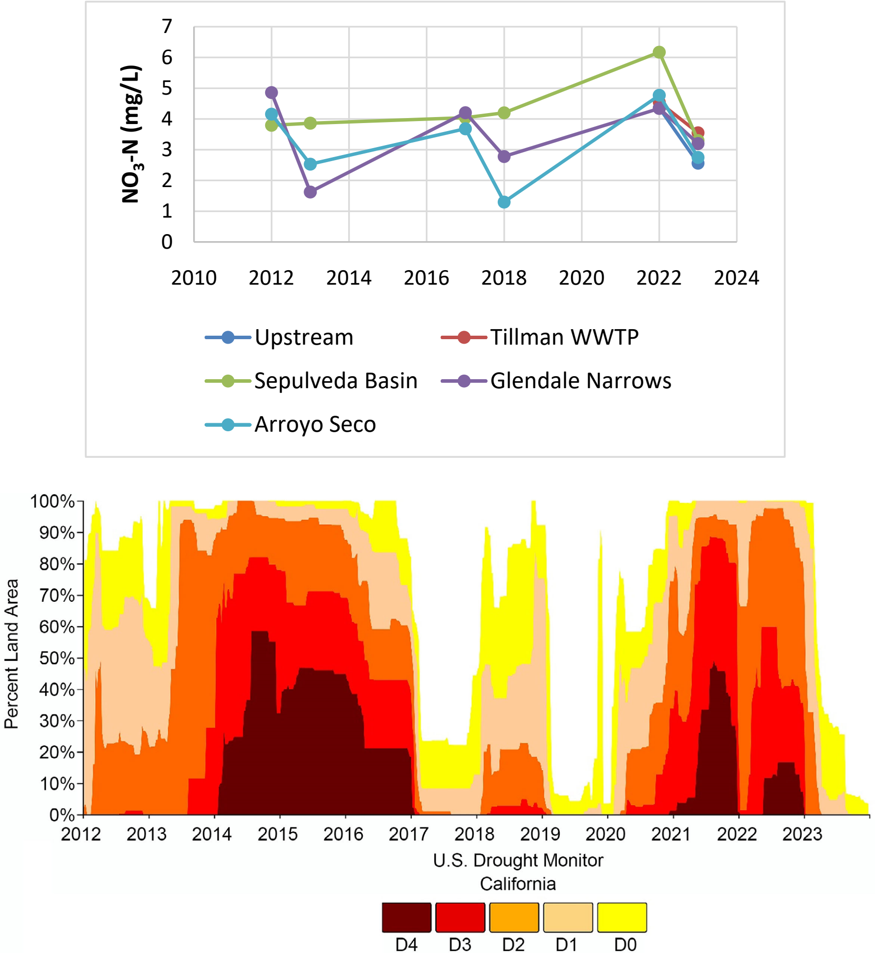

Figure 4. The graph on the left displays the change in nitrate concentration from 2010 to 2023 for each sampling location, while the figure on the right (modified from grought.gov) illustrates the historical drought recorded from 2010 to 2023 (Ranging from D_4 Exceptional Drought, to D_0 Abnormally Dry) [33].

4.5. Impacts of California State Droughts on L.A. River Water Quality

There is limited or no direct evidence regarding the impacts of drought on aquatic systems. However, there is ample evidence that certain water properties and water quality change during the dry season. The availability of monitoring studies conducted during moderate or severe droughts is limited. One observation that was consistently noted in past studies is a specific increase in some physical properties of water, such as specific conductance and temperature. This increase is attributed to two factors: decreased stream dilution capacity and an augmented influence of base flow during drought periods [2] [4] [27]. Conversely, forested watersheds and monitoring of water during the wet season have shown a decrease in specific conductance and other properties attributed to the absorption of higher-conductance subsurface flow by vegetation [28]. Additionally, in certain studies focusing on basins primarily influenced by agricultural nonpoint sources, nitrogen and phosphorus concentrations were observed to decrease during drought conditions [29]. During the years 2012-2016, California experienced its most severe drought in history, prompting a state of emergency declared by Governor Jerry Brown [30]. The drought was officially declared over in 2016-2017, following the recharging of reservoirs due to the El Niño storm event and record highs in statewide snowpack and precipitation. Governor Jerry Brown lifted the emergency status during this period [30]. California remained drought-free until Governor Gavin Newsom declared a drought emergency for the state in 2021 [31]. This drought persisted until the spring of 2023, but a record-high precipitation and snowmelt reduced the affected area to 14.6% of the state being in drought conditions [32]. This drought history aligns with the data presented in this study: 2012-2013 data coincides with a drought period, 2017-2018 data reflects a period without drought, and 2022-2023 data indicates a return to drought conditions with significant wet conditions during the wet season, marking the end of the drought. This drought trend appears to be directly correlated with nearly every dataset measured in the L.A.

Figure 4 presents two graphs depicting the dynamics over the years. On the left, the chart showcases the variation in nitrate concentration across different sampling locations from 2010 to 2023. Meanwhile, the graph on the right, adapted from drought.gov, illustrates the historical drought conditions spanning the same period, ranging from D_4 Exceptional Drought to D_0 Abnormally Dry [33].

5. Conclusions

In conclusion, the analysis of water quality parameters in the L.A. River uncovers trends and variations, showcasing the intricate interplay between natural processes and human activities. Nitrate concentrations exhibit higher contamination level, with only a brief decrease noted during the wet seasons of 2013, 2018, and 2023. The isotopic composition of nitrogen and oxygen in nitrate provides invaluable insights into pollution sources, emphasizing the significance of understanding biogeochemical pathways.

The analysis of nitrate concentrations in the L.A. River paints a concerning picture, indicating contamination levels generally not surpassing thresholds associated with adverse effects on water quality. The upward trend from 2012 to 2023, with only a temporary drop in 2018, highlights the urgent need for nitrate level monitoring in the LA river water and maybe there is a need for attention to mitigate the impact on the river ecosystem. The isotopic analysis of nitrogen (δ15N) and oxygen (δ18O) in nitrate provides valuable insights into sources and processes. The significant presence of nitrate from manure and sewage underscores the role of anthropogenic activities in water contamination. The scatter plot analysis reveals clear relationships between isotopic compositions, emphasizing the importance of understanding these signatures for tracing nitrate origins and identifying pollution sources. This information is crucial for developing targeted strategies to address specific contributors, such as organic matter decomposition, microbial activity, or fertilizer use. Thus, implementing measures to reduce anthropogenic inputs, especially from manure and sewage, is crucial. Ongoing monitoring of isotopic compositions can aid in tracking the effectiveness of intervention measures and assessing changes in nitrogen cycling within the ecosystem. Furthermore, addressing elevated nitrate concentrations in the L.A. River requires a multifaceted approach, integrating both source control and monitoring efforts. Understanding isotopic signatures provides a powerful tool for targeted intervention and long-term management, ensuring the sustainability of the river ecosystem.

In summary, this study underscores the dynamic nature of the L.A. River's water quality, shaped by a combination of anthropogenic activities and climatic factors. The findings highlight the importance of ongoing monitoring and management efforts to mitigate the impact of changing environmental conditions on the river's ecosystem.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] California Water Boards: Barstow Groundwater Nitrate Pollution (2012). https://www.waterboards.ca.gov/

[2] Rezaie Boroon, M.H. and Coo, C.B.V.L. (2015) Water Quality Assessment of the Los Angeles River Watershed, California, USA in Wet and Dry Weather Periods. *Journal of Geography, Environment and Earth Science International*, **3**, 1-17. https://doi.org/10.9734/JGEESI/2015/20717

[3] Takagi, D.S. (2018) Water Quality of the Los Angeles River in the Wet and Dry Seasons. BS Degree Project. Honor Project Report.

[4] Ackerman, D., Stein, E.D. and Schiff, K.C. (2005) Dry-Season Water Quality in the San Gabriel River Watershed. *Bulletin*, *Southern California Academy of Sciences*, **104**, 125-145. https://doi.org/10.3160/0038-3872(2005)104[125:DWQITS]2.0.CO;2

[5] Office of Water, U.S. Environmental Protection Agency (2012) U.S. Environmental Protection Agency (USEPA) Edition of the Drinking Water Standards and Health Advisories. EPA 822-S-12-001, Washington DC.

[6] Davidson, E.A., David, M.B., Galloway, J.N., Goodale, C.L., Haeuber, R., Harrison, J.A., Howarth, R.W., Jaynes, D.B., Lowrance, R.R., Nolan, B.T., *et al.* (2012) Excess Nitrogen in the U.S. Environment: Trends, Risks, and Solutions. Issues in Ecology, Ecological Society of America, Washington DC. https://doi.org/10.1016/j.hal.2008.08.015

[7] Howarth, R.W. (2008) Coastal Nitrogen Pollution: A Review of Sources and Trends Globally and Regionally. *Harmful Algae*, **8**, 14-20.

[8] Sallenave, R. (2017) Nitrate in Drinking Water. https://pubs.nmsu.edu/_m/M114.pdf

[9] Dubrovsky, N.M., Burow, K.R., Clark, G.M., Gronberg, J.M., Hamilton P.A., Hitt, K.J., Mueller, D.K., Munn, M.D., Nolan, B.T. Puckett, L.J., Rupert, M.G., Short, T.M., Spahr, N.E., Sprague, L.A., and Wilber, W.G. (2010) The Quality of Our Nation's Waters—Nutrients in the Nation's Streams and Groundwater, 1992-2004. U.S. Geological Survey Circular 135. https://doi.org/10.3133/cir1350

[10] Kraus, T.E.C., O'Donnell, K., Downing, B.D., Burau, J.R. and Bergamaschi, B.A. (2017) Using Paired *in Situ* High Frequency Nitrate Measurements to Better Understand Controls on Nitrate Concentrations and Estimate Nitrification Rates in a Wastewater-Impacted River. *Water Resources Research*, **53**, 8423-8442. https://doi.org/10.1002/2017WR020670

[11] Hahn, J. (2018) Efforts to Restore the Los Angeles River Collide with a Gentrifying City. Sierra Club. https://www.sierraclub.org/sierra/2018-4-july-august/feature/efforts-restore-los-angeles-river-California-collide-gentrifying-city

[12] U.S. Census Bureau (2023) Los Angeles Population. https://www.usapopulation.org/los-angeles-population/

[13] Los Angeles Regional Water Quality Control Board (2006) Trash Total Maximum Daily Loads for the Los Angeles River Watershed. Basin Plan Amendments.

[14] Environmental Protection Agency (EPA) (2002) Onsite Wastewater Treatment Systems Manual. https://www.epa.gov/sites/default/files/2015-06/documents/2004_07_07_septics_septic_2002_osdm_all.pdf

[15] Kendall, C., Young, M.B. and Silva, S.R. (2010) Applications of Stable Isotopes for Regional to National-Scale Water Quality and Environmental Monitoring Programs. In: West, J., Bowen, G., Dawson, T. and Tu, K., Eds., *Isoscapes*, Springer, Dordrecht, 89-111. https://doi.org/10.1007/978-90-481-3354-3_5

[16] Archana, A., Thibodeau, B., Geeraert, N., Xu, M.N., Kao, S.J. and Baker, D. (2016) Nitrogen Sources and Cycling Revealed by Dual Isotopes of Nitrate in a Complex Urbanized Environment. *Water Research*, **142**, 459-470. https://doi.org/10.1016/j.watres.2018.06.004

[17] Fry, B. (2006) Stable Isotope Ecology. Springer, New York. https://doi.org/10.1007/0-387-33745-8

[18] McClelland, J.W. and Valiela, I. (2003) Linking Nitrogen in Estuarine Producers to

Land-Derived Sources. *Limnology and Oceanography*, **43**, 577-585. https://doi.org/10.4319/lo.1998.43.4.0577

[19] Koszelnik, P. (2014) Investigating the Source of Nitrate in Poland's San River Water by Analyzing the Nitrogen and Oxygen Isotopic Ratio. *Carpathian Journal of Earth and Environmental Sciences*, **9**, 85-96.

[20] Sigman, D.M., Casciotti, K.L., Andreani, M., Barford, C., Galanter, M. and Böhlke, J.K. (2001) A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater. *Analytical Chemistry*, **73**, 4145-4153. https://doi.org/10.1021/ac010088e

[21] Casciotti, K.L., Sigman, D.M., Galanter Hastings, M., Böhlke, J.K. and Hilkert, A. (2002) Measurement of the Oxygen Isotopic Composition of Nitrate in Seawater and Freshwater Using the Denitrifier Method. *Analytical Chemistry*, **74**, 4905-4912. https://doi.org/10.1021/ac020113w

[22] Caschetto, M., Paola Galassi, D.M., Petitta, M. and Aravena, R. (2017) Evaluation of the Sources of Nitrogen Compounds and Their Influence on the Biological Communities in the Hyporheic Zone of the Sagittario River, Italy: An Isotopic and Biological Approach. *Italian Journal of Geosciences*, **136**, 145-156. https://doi.org/10.3301/IJG.2016.07

[23] Ekberg, M. and Bedaso, Z.K. (2019) Dual Isotope Tracing of Nitrate Contaminant Source in Surface and Groundwater in the Great Miami River Watershed, Southwestern Ohio. https://www.researchgate.net/publication/344810679_Dual_Isotope_Tracing_of_Nitrate_Contaminant_Source_in_Surface_and_Groundwater_in_the_Great_Miami_River_Watershed_Southwestern_Ohio

[24] U.S. Geological Survey (2019) pH and Water. https://www.usgs.gov/

[25] Kaushal, S.S., Likens, G.E., Jaworski, N.A., Pace, M.L., Sides, A.M., Seekell, D., Wingate, R.L., *et al.* (2010) Rising Stream and River Temperatures in the United States. *Frontiers in Ecology and the Environment*, **8**, 461-466. https://doi.org/10.1890/090037

[26] Mongolo, J., Trusso, N., Dagit, R., Aguilar, A. and Drill, S.L. (2017) A Longitudinal Temperature Profile of the Los Ageless River from June through October 2016. *Bulletin, Southern California Academy of Sciences*, **116**, 174-192. https://doi.org/10.3160/soca-116-03-174-192.1

[27] Caruso, B.S. (2002) Temporal and Spatial Patterns of Extreme Low Flows and Effects on Stream Ecosystems in Otago, New Zealand. *Journal of Hydrology*, **257**, 115-133. https://doi.org/10.1016/S0022-1694(01)00546-7

[28] Kobayashi, D., Suzuki, K. and Nomura, M. (1990) Diurnal Fluctuation in Stream Flow and in Specific Electrical Conductance during Drought Periods. *Journal of Hydrology*, **115**, 105-114. https://doi.org/10.1016/0022-1694(90)90200-H

[29] Sprague, L.A. (2005) Drought Effects on Water Quality in the South Platte River Basin, Colorado. *Journal of the American Water Resources Association*, **41**, 11-24. https://doi.org/10.1111/j.1752-1688.2005.tb03713.x

[30] Boxall, B. (2017) Gov. Brown Declares California Drought Emergency Is over. Los Angeles Times. https://www.latimes.com/local/lanow/la-me-brown-drought-20170407-story.html

[31] Becker, R. (2021) Newsom Declares Drought Emergency across California. Cal Matters. https://calmatters.org/environment/2021/10/california-drought-newsom-emergency/

[32] Sheffield, A. and Kalansky, J. (2023) California-Nevada Drought Status Update.

https://www.drought.gov/drought-status-updates/california-nevada-drought-status-update-2023-04-20

[33] Drought.gov. (2023) Drought in the California-Nevada Region. https://www.drought.gov/states/california