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Analysis and Monitoring of the Herbicide 2,4-D in Agricultural Drainage Systems and Karun River Using HPLC Method

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ABSTRACT

The Karun River, located in Khuzestan Province in southwestern Iran, is the country's most important waterway, playing a crucial role in drinking water supply, aquaculture, industry, and agriculture. The discharge of agricultural drainage into the river has led to contamination with various pollutants, including herbicides. One of the most commonly used herbicides in the region is 2,4-dichlorophenoxyacetic acid (2,4-D), which can significantly impact river water quality. This study aims to determine the concentration of 2,4-D in agricultural drainage and the Karun River. In this study, the amount of 2,4-D herbicide was sampled and analyzed according to the standard method in summer and winter at five agricultural drainage stations and 17 Karun River stations. HPLC high-performance liquid chromatography (KNAUER) was used to measure 2,4-D. A C18 column (250 × 4.6 mm, 5 µm) was used as the stationary phase, and a 2500 ultraviolet (UV) detector made in Germany was employed for detection was used to measure 2,4-D. The results revealed that 2,4-D levels exceeded permissible limits at several stations. The highest concentration in agricultural drainage was recorded at 105.26 µg/L in summer (Station 18), while the maximum concentration in river water was 66.27 µg/L (Station 2). Additionally, 2,4-D levels decreased along the river due to processes such as adsorption to suspended solids, photodegradation (UV), and microbial biodegradation. This study provides valuable insights into the distribution of 2,4-D in the Karun River and highlights the need for effective agricultural pollution management.

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INTRODUCTION

Khuzestan Province, located in southwestern Iran, is the country's agricultural heartland, heavily reliant on the Karun River for irrigation and drinking water supply. As Iran's largest and most significant waterway, the Karun River stretches over 450 km, sustaining major downstream cities such as Ahvaz, Abadan, and Khorramshahr. However, this vital resource faces increasing environmental threats, primarily due to pollution from agricultural runoff (48%), municipal wastewater (27%), and industrial discharge (23%).

Among these sources, agricultural drainage poses the most severe risk, as it carries high volumes of dispersed pollutants, including pesticides, herbicides, and fertilizers. The reduction in river flow, combined with insufficient wastewater treatment, has intensified contamination, leading to deteriorating water quality, ecosystem disruption, and potential health hazards. Given that agriculture is the dominant water consumer in Khuzestan, monitoring and managing chemical pollutants in return flows are crucial for ensuring the river's sustainability.

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The widespread cultivation of water-intensive crops such as sugarcane and rice has further exacerbated these challenges. To maximize yields, farmers heavily rely on agrochemicals, particularly herbicides and insecticides, which subsequently infiltrate surface waters through agricultural runoff. The most commonly used agrochemicals in the region include: Atrazine, 2,4-D, Insecticides: Diazinon, Malathion, Dursban, Non-selective herbicides: Paraquat, Roundup.

Due to their persistence, solubility, and mobility in water, these chemicals significantly contribute to long-term river contamination. Among these, 2,4-Dichlorophenoxyacetic acid (2,4-D) stands out as one of the most extensively applied herbicides, particularly in sugarcane and rice farming. Its excessive use raises concerns regarding its accumulation in the Karun River, leading to ecological and health risks. Therefore, accurate measurement of 2,4-D concentrations in the Karun River is essential to understanding its distribution, persistence, and potential environmental impacts.(Jalilzadeh Yengjeh et al., 2020; Jorfi et al., 2022; Noori et al., 2007)

Phenoxyalkanoic acid herbicides, which include 2,4-D, are among the oldest and most widely used broadleaf weed control agents. Other key active ingredients in this group include 4-chloro-2-methylphenoxyacetic acid (MCPA), methylchlorophenoxypropionic acid (MCPP), and dichlorprop (DCPP). 2,4-D is extensively applied in Khuzestan for crops such as sugarcane and rice, where its usage has led to concerns about contamination through agricultural drainage entering the Karun River. Due to its high solubility and widespread application, the potential for 2,4-D contamination in surface water sources is a significant environmental concern.

Initially introduced in the 1940s, 2,4-D was the first commercial herbicide developed for broadleaf weed control. It remains one of the most commonly used herbicides worldwide due to its low cost, high efficiency, water solubility, and broad-spectrum weed control capabilities. The herbicide is rapidly absorbed through plant leaves and roots, making it highly effective.

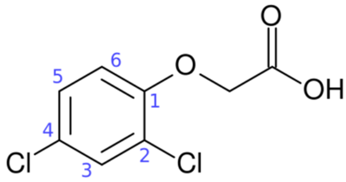
Various formulations of 2,4-D exist, with the dimethylamine salt (DMA) and 2-ethylhexyl ester (EHE) being the most commonly used, accounting for 90-95% of global usage. Over 1,500 commercial herbicide products contain 2,4-D as an active ingredient. Notably, 2,4-D was a key component of Agent Orange, the defoliant used during the Vietnam War. (Islam et al., 2018)

Phenoxy herbicides, including 2,4-D, are available in three main forms: salts (alkali, amine), esters, and acids. While all are soluble in water, salts exhibit the highest solubility. Due to their high solubility and low soil adsorption, these compounds can easily infiltrate both surface and groundwater systems, posing significant contamination risks.(Pohanish, 2014; Wafa et al., 2011)

In Canada, 2,4-D ranks among the top 10 most commonly sold herbicides, applied in agriculture, forestry, turf management, and non-food industrial sites. (Organization, 2022) Despite its widespread use, human exposure to 2,4-D is considered low, as residues in food rarely exceed trace levels, and drinking water contamination remains minimal (Organization, 2022). Commercial formulations include free acid, alkali salts, amine salts, and esters, with esters hydrolyzing rapidly to release the active acid form. While 2,4-D degrades relatively quickly in the environment, its persistence in water bodies necessitates ongoing monitoring and assessment.

Due to high water solubility and low soil adsorption, 2,4-D can easily leach through soil pores, contaminating both surface and groundwater. Commercial formulations include free acid, alkali salts, amine salts, and esters, with esters hydrolyzing rapidly to release the active acid form. While 2,4-D degrades relatively quickly in the environment, its persistence in water bodies necessitates ongoing monitoring and assessment.(López-Piñeiro et al., 2019) Despite the widespread use of 2,4-D, exposure in humans is expected to remain low. Very low levels of 2,4-D in sources of drinking water have been found in many Canadian provinces. 2,4-D does not tend to accumulate in food, and inhalation exposure is not expected to be significant. (Organization, 2022; Wafa et al., 2011)

Table 1. Physical and chemical properties of 2,4-D (Pohanish, 2014)

title	2,4-dichlorophenoxyacetic acid (2,4-D)
Chemical structure	
chemical formula	$C_8H_6Cl_2O_3$
classification	Herbicide
Molar mass ($^{gr}/_{mol}$)	221.04
Log K_{ow}	2/5 – 5/8
Melting point	140.5℃
Boiling point	160℃
Solubility of water	900 $^{mg}/_{lit}$
CAS-Number	94-75-7
EPA/OPP PC Code	030001
Business names	ACME®; AGROTECT®; AMIDOX®; AMOXONE®; KILLER®;

The types of physical and chemical properties of 2,4-D are shown in Table 1.

Accurate measurement of 2,4-Dichlorophenoxyacetic acid (2,4-D) in environmental samples is crucial for evaluating its distribution, persistence, and ecological risks. Several advanced analytical methods have been developed to detect 2,4-D in water, soil, and plant matrices. Among these methods, High-Performance Liquid Chromatography (HPLC) is widely recognized for its exceptional sensitivity, precision, and reliability. It has been the method of choice for this study due to its robust performance, ease of sample preparation, and excellent reproducibility.

Various analytical techniques are available for detecting 2,4-D residues in environmental samples. Gas Chromatography-Mass Spectrometry (GC-MS) is highly specific and offers low detection limits, making it suitable for complex matrices. Liquid Chromatography-Mass Spectrometry (LC-MS/MS) provides high sensitivity and allows for the analysis of non-volatile compounds without the need for derivatization. Spectrophotometric methods are more cost-effective but generally less sensitive than chromatographic techniques. Additionally, electrochemical sensors and biosensors are emerging technologies that enable rapid, on-site detection for real-time environmental monitoring. While each of these methods has its own advantages, HPLC is particularly favored in this research due to its superior accuracy, reproducibility, and suitability for environmental monitoring. Its widespread use in environmental science further supports its selection as the primary analytical method in this study. (Barreca et al., 2021; Carlile, 2006; Chen et al., 2018; Ismail et al., 2015; Kanu, 2021; Papadakis et al., 2018; Shin et al., 2011; Sutcharitchan et al., 2020)

Several studies worldwide have focused on the presence and levels of 2,4-D herbicide in water bodies, providing valuable insights into its environmental impact. For example, research conducted in Poland revealed that approximately 0.8% of river water samples had concentrations of 2,4-D exceeding 0.1 μg/l. (Sadowski et al., 2009) In another study, groundwater contamination by 2,4-D was found to exceed 0.1 μg/l in the analyzed area. (Buczyńska & Szadkowska-Stanńczyk, 2005)

Further investigation by Loos et al. (2009) across 27 European countries highlighted that 2,4-D was detected in 52% of the river water samples analyzed. The average concentration exceeded the European Union (EU) limit of 0.1 μg/l, with an estimated average concentration of 0.015 μg/l. These findings underscore the widespread nature of 2,4-D contamination in water systems and its potential ecological risks. The detailed results of this study are summarized in Table 2 below:

Table 2. The results of river waters monitoring for 2,4-D herbicide in European Union countries (Loos et al., 2009)

Chemical name	Limit of detection (μg L^{-1})	Frequency of detection (%)	Max concentration (μg L^{-1})	Average concentration (μg L^{-1})	90th percentile [%]
2,4-D	0.1	52	1.221	0.022	0.035

While these studies provide crucial insights into 2,4-D pollution in various regions, research on the Karun River in Iran remains notably insufficient. The study by Orooji et al. (2022) was limited to Ahvaz, focusing on water intake points for the city's treatment plants. However, this research failed to include significant sources of 2,4-D contamination such as agricultural drainage areas and upstream/downstream regions. By excluding these critical sites, the study's scope remained narrow, thus neglecting to capture the full spatial and seasonal variations of 2,4-D concentrations, which are essential for understanding its broader environmental impact. (Orooji et al., 2022)

This study aims to address these gaps by conducting comprehensive monitoring of 2,4-D levels in the Karun River and its agricultural drainage channels. Unlike previous studies, this research will extend its focus to upstream and downstream regions of the river, as well as key agricultural drainage points that significantly contribute to 2,4-D contamination. Furthermore, it will account for seasonal variations by conducting measurements in both summer and winter. The ultimate goal is to create a detailed spatial and temporal distribution map of 2,4-D pollution across multiple sampling stations, thereby providing a more holistic view of its environmental impact.

To achieve accurate and reliable results, this study employs **High-Performance Liquid Chromatography (HPLC)**, a method renowned for its high sensitivity, accuracy, and ability to separate complex components in environmental samples. HPLC is particularly effective for detecting low concentrations of herbicides such as 2,4-D, making it the ideal choice for this study. (McManus et al., 2014)

This study provides a more comprehensive view of the sources of pollution and their impact on the river system by examining samples of agricultural drainage and water from the Karun River that have not been examined in previous studies. Agricultural drains, especially in areas where sugarcane cultivation has been done, are contaminated with various agricultural pollutants. As a result, river water sampling stations have been considered near the entrance of these drains in the river. In this research, the primary goal of monitoring 2,4-D levels is to identify the main sources of pollution and understand the effect of seasonal changes in the concentration of this herbicide.

In this research, a new and advanced method of High-Performance Liquid Chromatography (HPLC) was used to measure the concentration of herbicide 2,4-D. Because of high sensitivity in low concentrations, high accuracy and high ability to separate components in complex samples, the HPLC method is the reason for choosing this method to identify types of herbicides (including 2,4-D) from polluted water.

MATERIALS & METHODS

Khuzestan province, centered in the city of Ahvaz, backed by the Karun River, is considered the agricultural hub of Iran. Karun River is considered the most important and wateriest river in Iran. This river is the only river in Iran that has access to international waters, and in addition to supplying agricultural water, it also supplies drinking water to neighboring cities. Due to the illegal transfer and extraction of water from this area to other basins, a significant decrease in the water flow of the Karun River has been observed. The increasing population has led to an increase in the number of pollutants entering the river. As a result, the pollution load of the river is increasing rapidly. Considering the extent of agricultural activities in this province, non-point sources of agricultural drains are considered the most important source of water returned to the Karun River. One of the most widely used herbicides used in agriculture in this region is 2,4-D herbicide. This herbicide is used in the cultivation of various crops such as sugarcane and rice. Seven major sugarcane development companies in Khuzestan province use this herbicide, as a result of which the water in their drains entering the Karun River is contaminated with this herbicide. In this study, 2,4-D concentrations were measured at 22 sampling stations. The locations of these stations are shown in Figure 1, where red markers indicate agricultural drainage stations, and green markers represent river stations.

After reviewing the maps and field visits, the possible points that were subject to the entry of 2,4-D herbicide into the river were identified and the stations were determined by considering them. Sampling shows the current state of the river and drinking water collection sites, while 7 major agricultural drains upstream enter the Karun and Dez rivers. Sampling was done in winter and summer in 2023. Table 3 shows the geographic location of the sampling stations of the Karun and Dez rivers.

High-performance liquid chromatography (HPLC) (KNAUER) was used to measure 2,4-D. A C18 column (250 × 4.6 mm, 5 µm) was used as the stationary phase, and detection was performed using a 2500 ultraviolet (UV) detector made in Germany. The wavelength used for the measurement of 2,4-D was 283 nm, which was previously determined using a DR5000 spectrophotometer. An isocratic mobile phase was employed with a flow rate of 0.5 mL/min. A 20 µL sample was manually injected into the system. The retention time of 2,4-D was between

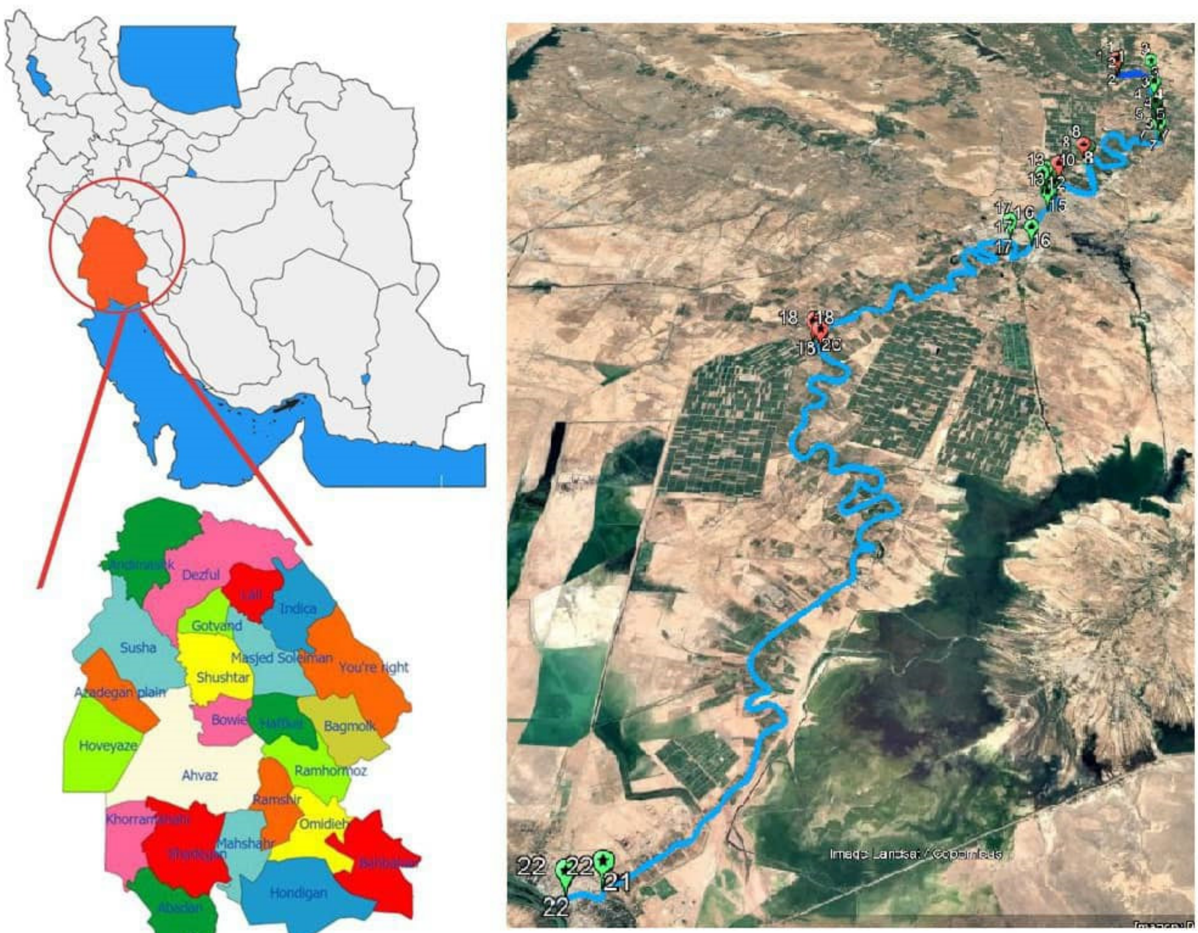


Fig. 1. Location of the study area in River Karun

Table 3. Geographical points of sampling station of Karun and Dez rivers in 2023

Geographical coordinates		**Sampling place**			
longitude	Latitude	river	drainage	Sampling Station name	number
48.8251755	31.650918		*	Imam Khomeini's agriculture and industry drainage channel	1
48.8288688	31.652345	*		Dez river, the exit point of Imam Khomeini's drain	2
48.8827489	31.646486	*		The confluence of the Karun River with the Dez River	3
48.880166	31.590521	*		Karun river in the vicinity of Malathani city	4
48.8755105	31.540470	*		Karun river in front of Hale and Dele village, below Mulathani city	5
48.881030	31.511710	*		Karun river facing Ramin power plant	6
48.872723	31.487894	*		Karun River in the vicinity of Weiss County	7
48.7506217	31.435474		*	Dehkhoda agriculture and industry drainage channel	8
48.7561678	31.430955	*		Karun River, the outlet of the Dehkhoda agricultural drain	9
48.7071300	31.3930269		*	Dehkhoda agriculture and industry drainage, in front of Beit Farsi village	10
48.700697	31.389415	*		Karun river in the vicinity of Beit Farsi village	11
48.689132	31.382486	*		Karun river near Daghaghle village	12
48.681570	31.3709926	*		Ahvaz, Shahid Modares highway, water treatment plant number 2	13
48.7002459	31.3603449	*		Ahvaz, Meli Rah Square, Water Treatment Plant No. 1	14
48.6869618	31.333886	*		Ahvaz, Abyari Street, next to the shrine of Ali Bin Mahziar, water treatment plant 3	15
48.657468	31.2614603	*		Ahvaz, Ayatollah Behbahani highway, Kot Abdullah, water treatment plant 4	16
48.629936	31.271332	*		Moinzadeh, next to the organizational houses of the police force, water treatment plant 5	17
48.3631129	31.086688		*	Amir Kabir Cultivation and Industry Drainage Channel	18
48.368593	31.083287	*		Karun River, the outlet of Amirkabir's agriculture and industry drain	19
48.373028	31.073309		*	Karun river, Shirinshahr city, next to the bridge	20
48.2086054	30.438789	*		Karun River, Khorramshahr water treatment plant	21
48.175632	30.4307089	*		The end of Karun River, not reaching Arvand River	22

3.4 and 3.7 minutes, while the total analysis time was 10 minutes.

Acetonitrile was used as the receiving phase to measure 2,4-D herbicide using HPLC.

After passing through the 0.22 head filter, the samples were prepared and placed in the sample box and sent to the laboratory. Acetonitrile with laboratory purity was obtained from Merck, Germany, and 2,4-D herbicide with 98% purity was obtained from Sigma Aldrich.

The volume of 1 ml with the efficiency of 98.69% was used as the optimal volume of the solvent.

To draw the calibration curve, concentrations of 0.01, 0.1, 0.5, 1, 2, 5 and 10 $\mu g/l$ of 2,4-D standard were prepared and the calibration curve was drawn. The calibration curve was drawn according to the area under the standard curve, and the R2 value of the calibration curve for 2,4-D was 0.9995 and the line equation was y=31901x -7320.2. After ensuring the existence of a suitable linear relationship between different standard concentrations and the area under the curve (2,4-D), the accuracy and precision of the test was evaluated. The evaluation results showed that the method has acceptable precision and accuracy. Figure 2 shows the 2,4-D regression line for device standardization.

Sampling operations were carried out in two seasons, cold and warm, in Khuzestan province, within the city of Ahvaz, from the agricultural drain and several different stations of the Karun River, from the upstream to the downstream of the river. According to the opinions

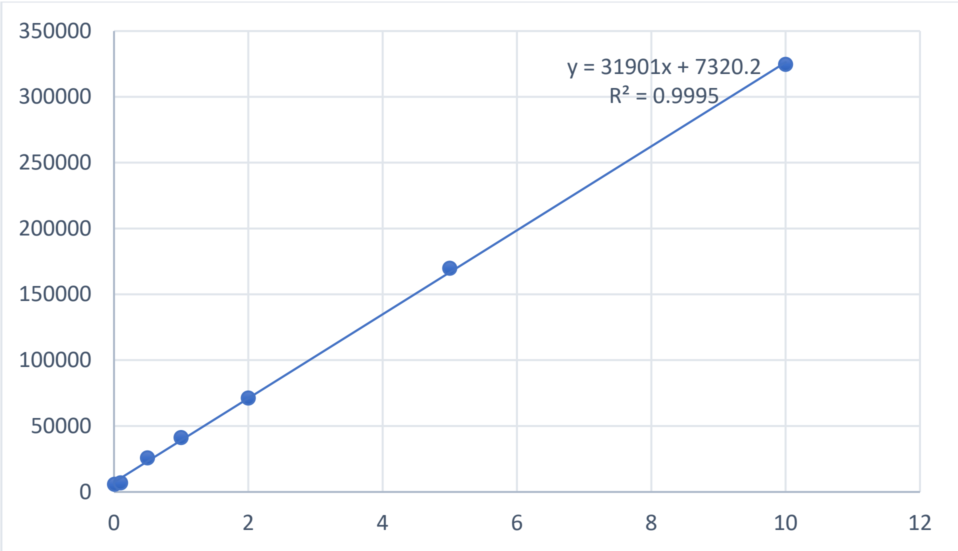


Fig. 2. 2,4-D regression line for HPLC device standardization

of environmental and agricultural experts as well as field visits, sampling during the cultivation season and the time of spraying agricultural lands, in the summer season in September as an indicator of the hot month and in the winter season in January as an indicator of the cold month Selected. The results of this research are expressed as average numbers. In each chosen month, 2 samples were taken, one at the beginning of the month and the other at the middle of the month.

At each station, samples were collected from the left bank, right bank and middle of the river to a volume of 2 lit of river water, and after mixing and preparing the samples, they were transferred to the HPLC device for measurement.

RESULTS & DISCUSSION

The Karun River serves as a crucial source of drinking water for many cities and villages in Khuzestan, as well as supporting industries such as aquaculture, agriculture, and various industrial uses. However, agricultural activities upstream, particularly those associated with Imam's cultivation, contribute significantly to the contamination of the river with the herbicide 2,4-D. This herbicide, commonly used for weed control, is highly soluble in water and poorly absorbed by soil, which results in its leaching into the river, particularly during crop cultivation.

This study was conducted to monitor and track the levels of 2,4-D in the Karun River. As shown in **Table 4**, five sampling stations from agricultural drainage channels and 17 stations along the Karun River were analyzed. The first monitoring station, located at the Imam Drainage agricultural channel, recorded 95.44 µg/l of 2,4-D in the summer, decreasing to 68.48 µg/l in the winter. Moving downstream, the concentration at Station 3 of the Karun River was measured at 36.3 µg/l in the summer and 17.7 µg/l in the winter.

The herbicide concentrations during the summer were notably higher compared to the winter season. This seasonal variation is primarily attributed to increased herbicide usage during warmer months, coupled with reduced water flow, which leads to less dilution of the herbicide in the river. For instance, a decrease in concentrations was observed from Station 4 (58.44 µg/l) to Station 5 (47.72 µg/l) during the summer season, as seen in **Fig. 3**.

Fig. 3 shows the values of 2,4-D concentrations in water samples taken from the Karun River during the summer of 2023, and Fig. 4 illustrates the same data for the winter season of 2024.

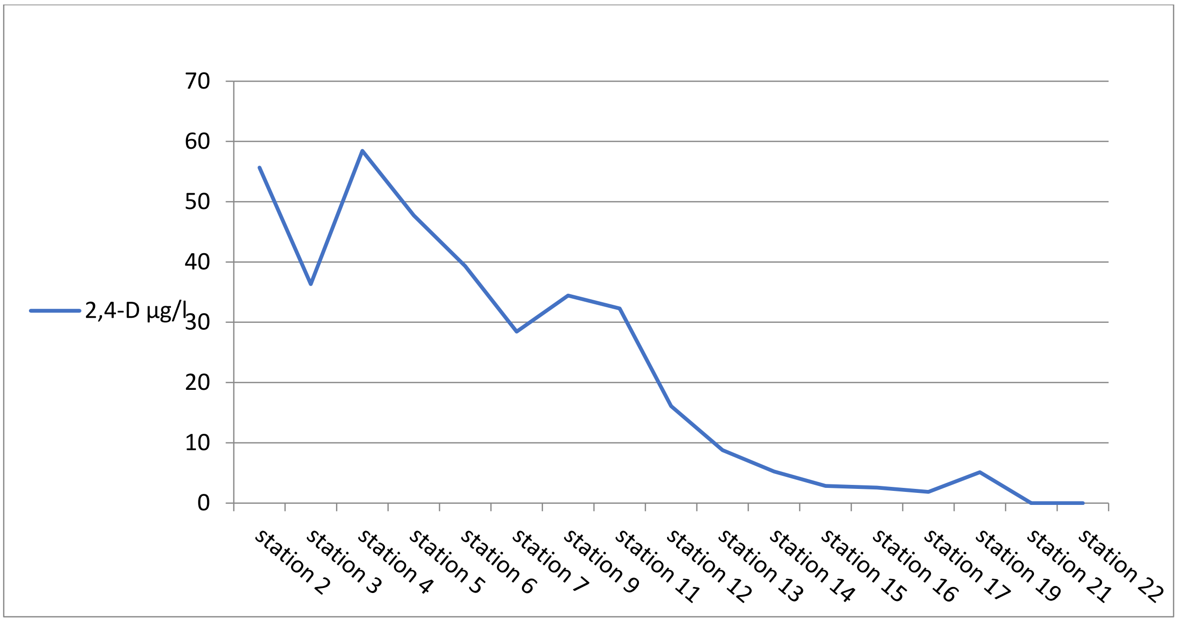


Fig. 3. The average results of measuring the amount of 2,4-D herbicide in selected stations of the Karun River in the summer season of 2023 (in $^{\mu g}/_{l}$)

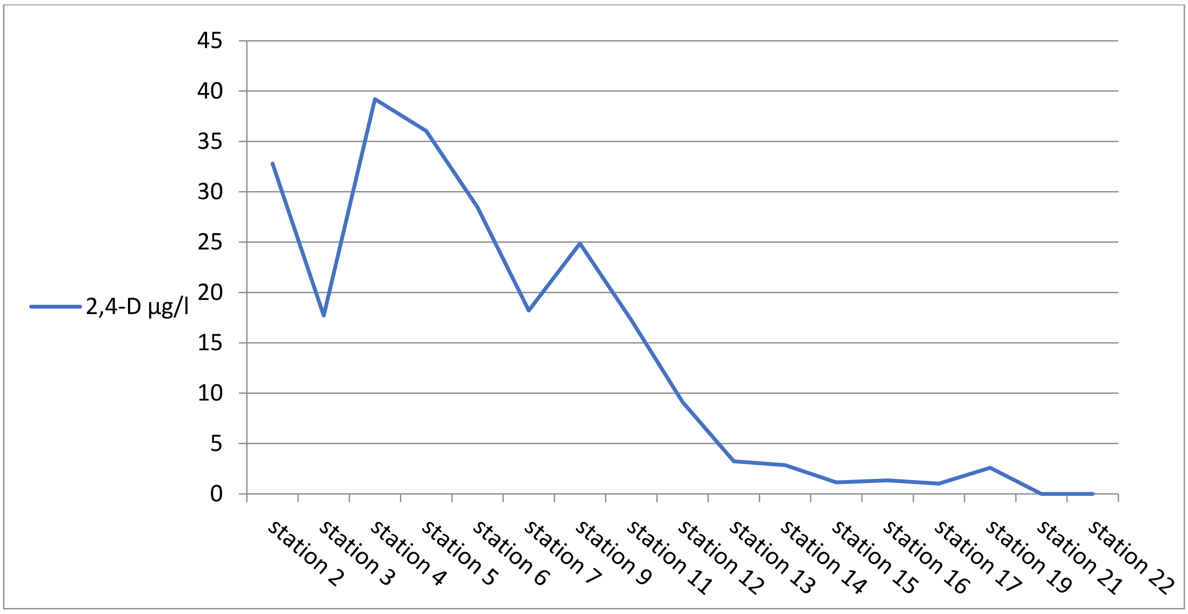


Fig. 4. The average results of measuring the amount of 2,4-D herbicide in selected stations of the Karun River in the winter season of 2024 (in $^{\mu g}/_{l}$)

These figures reveal the herbicide levels at 17 stations along a stretch of the river measuring 276 km. The highest concentration was observed at Station 4 during the summer (58.44 µg/l), while the lowest concentration in winter was recorded at Station 17 (1.02 µg/l)

In this research, monitoring and tracking of the 2,4-D herbicide in the Karun River, the most important source of drinking water for several cities and villages in Khuzestan, has been carried out. In addition to serving as a drinking water source, the Karun River is vital for aquaculture (fishery), industrial purposes, and agriculture in Iran. As seen in Table 4, 5 agricultural drainage stations and 17 sampling stations along the Karun River were studied from upstream to downstream.

Table 4. The average amount of herbicide 2,4-D in 22 sampling stations of Dez, Karun rivers and drainages entering the rivers in summer and winter seasons.

Measuring season		Sampling place			
Winter (µg/l)	**Summer (µg/l)**	**river**	**drainage**	**Sampling Station name**	**num**
68.48	95.44		*	Imam Khomeini's agriculture and industry drainage channel	1
32.79	55.65	*		Dez river, the exit point of Imam Khomeini's drain	2
17.7	36.33	*		The confluence of the Karun River with the Dez River	3
39.21	58.44	*		Karun river in the vicinity of Mulathani city	4
36.04	47.72	*		Karun river in front of Hale and Dele village, below Mulathani city	5
28.47	39.3	*		Karun river facing Ramin power plant	6
18.2	28.45	*		Karun River in the vicinity of Weiss County	7
46.32	78.69		*	Dehkhoda agriculture and industry drainage channel	8
24.87	34.44	*		Karun River, the outlet of the Dehkhoda agricultural drain	9
42.56	65.01		*	Dehkhoda agriculture and industry drainage, in front of Beit Farsi village	10
17.26	32.29	*		Karun river in the vicinity of Beit Farsi village	11
9.11	16.09	*		Karun river near Daghaghle village	12
3.23	8.77	*		Ahvaz, Shahid Modares highway, water treatment plant number 2	13
2.86	5.23	*		Ahvaz, Meli Rah Square, Water Treatment Plant No. 1	14
1.15	2.83	*		Ahvaz, Abyari Street, next to the shrine of Ali Bin Mahziar, water treatment plant 3	15
1.34	2.57	*		Ahvaz, Ayatollah Behbahani highway, Kot Abdullah, water treatment plant 4	16
1.02	1.86	*		Moinzadeh, next to the organizational houses of the police force, water treatment plant 5	17
66.27	105.26		*	Amir Kabir Cultivation and Industry Drainage Channel	18
2.59	5.11	*		Karun River, the outlet of Amirkabir's agriculture and industry drain	19
7.27	16.98		*	Karun river, Shirinshahr city, next to the bridge	20
ND	ND	*		Karun River, Khorramshahr water treatment plant	21
ND	ND	*		The end of Karun River, not reaching Arvand River	22

The first station studied was the agricultural drainage channel, Imam Drainage, where the concentration of 2,4-D was 95.44 µg/l in the summer season and 68.48 µg/l in the winter season. Moving downstream to Station 3 in the Karun River, concentrations of 36.3 µg/l in summer and 17.7 µg/l in winter were measured.

In most of the stations, the concentration of herbicide in the summer season was higher compared to the winter season. This can be attributed to the increased use of the herbicide during warmer months, along with the reduced flow rate of the Karun River in summer, leading to less dilution of the 2,4-D herbicide in the water. (The locations of the stations, according to the coordinates mentioned in Table 3, are provided).

As shown in Figures 3 and 4, the amount of 2,4-D herbicide in some stations along the river course decreased compared to previous stations. For instance, at Station 4, the concentration in the summer season was 58.44 µg/l, which decreased to 47.72 µg/l at Station 5.

Toxic organic compounds, including pesticides, can be reduced in aquatic environments through various physical, chemical, and biological mechanisms.(Melnikov, 2012) These mechanisms include surface absorption, dilution, chemical degradation, absorption by plants, microbial degradation, and photodegradation processes.(Qurratu & Reehan, 2016)

One of the physical mechanisms in the Karun River that contributes to the reduction of 2,4-D herbicide is surface adsorption of the herbicide onto suspended solids in the water and the riverbed sediments, particularly in areas where the flow velocity decreases. This leads to an accumulation of herbicide in the river sediments.(Boivin et al., 2005) Moreover, biological absorption of 2,4-D by heterotrophic bacteria in the river can also play a role in reducing its concentration(Serbent et al., 2019) Another significant process responsible for the decomposition and reduction of 2,4-D herbicide concentrations is photolysis, which occurs in the presence of dissolved oxygen and UV rays from the sun.(Su et al., 2018)

Therefore, the concentration of 2,4-D herbicide gradually decreases along the Karun River, to the point where it is not detectable in the lowest sampling points, approximately 214 km (Station 21) and 276 km (Station 22) from the upstream sampling station (Station 3).

The reduction in herbicide concentrations can also be attributed to the absence of agricultural drainage inputs near these downstream stations, which prevents the entry of 2,4-D into the river in these areas.

According to the 1993 guidelines, the permissible level of 2,4-D in drinking water was set at 0.03 mg/liter. This standard was retained in the 1998 revision, based on toxicological assessments by the JMPR in 1996. This limit remains unchanged today.(Organization, 1989, 2024)

As you move downstream along the river, the concentration of herbicide decreases. In terms of herbicide concentration, the stations in Ahvaz and Khorramshahr, located downstream, are considered safe. For example, at the Ahvaz stations (13, 14, 15, and 16), the 2,4-D concentrations measured in the summer were (8.77, 5.23, 2.83, and 2.57), and in the winter were (3.23, 2.86, 1.15, and 1.34), respectively. Station 21, located near Khorramshahr, showed no detectable levels of this pollutant.

However, in the stations closer to the upstream agricultural areas, the concentration of 2,4-D exceeded the WHO limit. This poses significant risks to water quality, public health, and local ecosystems.

Exceeding the permissible limits of herbicide concentrations can lead to severe environmental damage and harmful effects on human health. Thus, urgent measures are needed to control 2,4-D herbicide levels in the Karun River.

It is recommended to reduce the use of herbicides, better identify sources of pollution, and implement strategies to mitigate their effects on the environment and ecosystems.

CONCLUSION

The Karun River is a vital source of drinking water (both urban and rural), industrial supply, and agricultural irrigation in Khuzestan province. However, it faces significant contamination due to untreated wastewater discharge and drainage effluents, with agricultural runoff contributing to approximately 48% of the total pollution load. One of the most commonly used herbicides in the region is 2,4-D, which is applied extensively along the river, from upstream to downstream.

The results obtained from this study shown considerable seasonal and spatial variations in 2,4-D concentrations. The highest concentration in the river was observed during summer at Station 4 (58.44 μg/l), while the lowest was recorded in winter at Station 17 (1.02 μg/l). In the drainage systems, the highest concentration occurred at Station 18 (105.26 μg/l) in summer, whereas the lowest concentration was measured at Station 19 in winter (2.59 μg/l). These fluctuations underline the influence of seasonal agricultural activities, temperature changes, and hydrological conditions on herbicide distribution.

The results suggest that 2,4-D concentrations decrease along the Karun River, likely due to processes such as adsorption onto suspended solids, microbial degradation, and photolysis by UV radiation. This natural attenuation process reduces the herbicide's environmental persistence and its associated health risks. However, considering the widespread agricultural use of 2,4-D and its potential ecological impacts, continuous monitoring remains crucial. It is strongly recommended that an integrated monitoring program be established to track 2,4-D contamination in agricultural runoff, drainage effluents, and river ecosystems. Furthermore, should elevated herbicide levels persist in agricultural drainage, effective mitigation strategies, including improved irrigation management, buffer zones, and advanced water treatment technologies, should be implemented to protect the water quality of the Karun River and minimize its ecological footprint.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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