



Ecotoxicological Assessment of Potentially Toxic Elements in Sediments from Some Southern Rivers of the Caspian Sea using Single and Integrated Pollution Indices

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ABSTRACT

Aims: Potentially toxic elements are considered serious pollutants due to their high toxicity, durability in natural conditions, and bioaccumulation in the food chain.

Materials & Methods: Toxic elements (Al, As, Cr, Cd, Co, Cu, Ni, Pb, Zn, V, and Mn) in sediment samples from some coastal rivers flowing into the southern Caspian Sea (Tajan, Babolrood, and Shirood) were assessed. Single (EF, I_{geo} , Hq, PLI, and QoC) and integrated contamination indices (m-PEC-q, m-PEL-q, MERMQ, NPI, and CSI) were used to assess the ecotoxicological risk of the metals.

Findings: At all sites, the level of Cd was less than the detection limit ($<5 \text{ mg.kg}^{-1}$), indicating no significant source of pollution containing Cd. The mean concentration order of the metals in the rivers varied, suggesting that their contaminant sources significantly differed. The metal content of the Tajan River was substantially lower than that of the other rivers. EF values of Cu, Ni, and As showed partial enrichment, probably indicating their anthropogenic origin. According to the single indices of CF, I_{geo} , PLI, and Hq, the Babolrood and Shirood Rivers, sediment was significantly contaminated by As, Ni, and Zn. Based on NPI values, the Shirood River was highly polluted by As. Integrated ecotoxicological risk indices of CSI, m-ERM-Q, and m-PEL-q suggest that metals pose medium to low levels of environmental toxicity in the Babolrood and Shirood Rivers.

Discussion and Conclusion: This research demonstrated the necessity of using management and pollution control strategies such as improving wastewater treatment, promoting sustainable agriculture, and regulating industrial discharges.

Keywords: Caspian Sea Watershed; Environmental Pollution; Heavy Metal; Marine Pollution; Sediment Quality.

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Introduction

Riverine runoffs entering the estuary from upstream are rich in nutrients, so estuaries and coastal areas are among the most productive ecosystems that support marine coastal life by providing a larval nursery ground and a vital site for primary production [14, 51]. However, rapid industrialization, economic development, and construction of buildings near coastal areas and river banks [51] have caused these unique environments to be invaded by domestic and industrial pollutants, among which potentially toxic elements (PTEs) are the most hazardous contaminants. PTE pollution in riverine ecosystems originates from geogenic processes, including soil erosion, bedrock weathering, and precipitation. In addition, anthropogenic inputs, such as untreated industrial, sewage water, atmospheric deposition, and agricultural runoff, discharge remarkable toxic metals into the rivers [20, 39, 38], deteriorating water and sediment quality. Because of high toxicity, stability, non-degradability, and bioaccumulation effects, PTEs threaten the health of biotic environments and, consequently, human society [30, 57, 51, 16].

Approximately 90% of the suspended solid particulate matter in river flows, deposited in coastal and estuarine areas, is the primary carrier of trace metals [21]. Hence, sediments and suspended particles are strongly related to the burden of PTEs [6, 10]. PTE assessment of riverine sediment can capture real images of contamination in aquatic ecosystems [22] and would be a suitable solution for ecosystem health management if continually surveyed during various periods [1]. PTE contents in the surface sediments of rivers, fishing ports, and coastal areas have been studied in different countries/regions of the world, such as Hawaii [59], Taiwan [24], the Bay of Bengal [54], Iran [47], Pakistan [60], Korea [35], and Nigeria [19].

Single pollution indices evaluate the degree

of contamination for each metal and are practical tools for comparing metal contents. These indices are widely used to represent the origins of potentially toxic elements based on knowledge of the geochemical background (GB) or other reference data obtained from the literature [37]. Integrated contamination indices provide an overall assessment of sediment quality associated with a group of toxic elements and ensure future sustainability [31].

In the southern Caspian Sea (Mazandaran Strait, north of Iran), there are several rivers, three of which are the most ecologically important, namely, the Babolrood, Tajan, and Shiroom Rivers. Annually, many valuable catadromous fishes, such as kutum (*Rutilus kutum*), common carp (*Cyprinus carpio*), roach (*Rutilus rutilus*), and sturgeon, migrate to estuaries and coastal areas of these rivers for spawning [23]. Because of their high water flow, their estuary and downstream regions are of interest as nursery grounds for more than 10 million fish fingerlings artificially and semi-artificially propagated by the Iranian Fisheries Organization (IFO) and released annually into rivers for stock restoration. Based on recent statistics from 2016-2021, the annual mean numbers of kutum fingerlings released into the Babolrood, Tajan, and Shiroom Rivers were 2.5×10^6 (ind.), 8×10^6 (ind.), and 7.07×10^6 (ind.), respectively [26].

The Tajan River, located in the eastern region of the Mazandaran Strait, flows approximately 120 km long. Its several branches constitute the primary basis for agricultural production and fishery activity. In recent years, overharvesting of sand, aquaculture, and civil runoff have polluted the Tajan River. This study selected the main branch of Tajan that reaches the sea, which is likely polluted by many surrounding garden lands and industries, such as the Neka power plant, which burns mazut, as one of

the study areas.

The Babolrood River, located in the center of the Mazandaran Strait, is 109 km long and is the main water supply for agriculture, industry, drinking, and the economic hub of the people in this region. Recently, destructive changes have occurred in estuaries, river banks, and substrates of the Babolrood River due to the construction of numerous beach resorts, such as restaurants, tourism and entertainment complexes, industrial establishments, and fishing boats [25]. Moreover, rapid civil development and population growth in Babol and Babolsar cities and other surrounding villages along the Babolrood River bank and increased inputs of fertilizers and pesticides in its watershed expanded the assumption of "contaminated Babolrood River."

The Shiroom River, located in the western region of Mazandaran, is necessary for the environment and fishery. Like the other rivers mentioned above, it is surrounded by extensive agricultural farms and passes through tourist cities and villages such as Shiroom and Tonekabon; hence, it receives large amounts of urban and industrial effluents, which are expected to be polluted by wastes from rice farming lands and human activities.

However, data on PTE pollution in these rivers are scarce, fragmented, and limited to upstream areas. The purpose of the present study was to perform a comprehensive evaluation of potentially toxic element contamination in sediments from the Babolrood, Tajan, and Shiroom Rivers and to better display the sediment ecological status by single and integrated sediment quality indicators.

Materials & Methods

Surface Sediment Sampling

In this study, sampling was done at four sites in each river from June to July 2021, and

three samples were taken at each site. The sampling sites were selected according to the areas of fish spawning and fingerling release. Site 1 was inside the sea at a depth of 10 m in front of the river outlet (estuary). Site 2 was in the river outlet. Site 3 was inside the river and 1 km from the river outlet. Site 4 was selected downstream, approximately 2 km from the river outlet. The sediment samples were collected from a depth of 5 cm via an Ekman grab, packed, and then immediately transferred to the laboratory for further analysis. The geographical coordinates of the sampling sites from the Babolrood, Tajan, and Shiroom Rivers in northern Iran are shown in Figure 1.

Determination of Total Organic Matter

To determine the total organic matter (TOM) content, the sediment samples were dried at 70°C for 24 h and then heated in an oven at 550°C for four h. As described by Abrantes et al. (1999)^[2], the total organic matter content was measured as Eq.(1):

$$(\text{TOM, \%}) = \left[\frac{B - C}{B} \right] \times 100 \quad \text{Eq. (1)}$$

where B and C are the weights (g) of the dried sediment before and after combustion in the oven, respectively.

Sediment Grain Size Analysis

Grain size analysis was performed via a hydrometric method. The sediment samples were combusted in the oven at 550°C for four hours and 950°C for two hours to remove organic matter and biogenic carbonate. Then, 50 ml of Calgon solution (sodium hexametaphosphate %20 solution) was added to 50 g of dried sediment sample and mixed well via an electric mixer. Then, the solution was poured into an Isolab graduated cylinder, and the temperature was recorded. The solution was manually remixed, the hydrometer was immediately placed into the solution for 40 s, and the data were

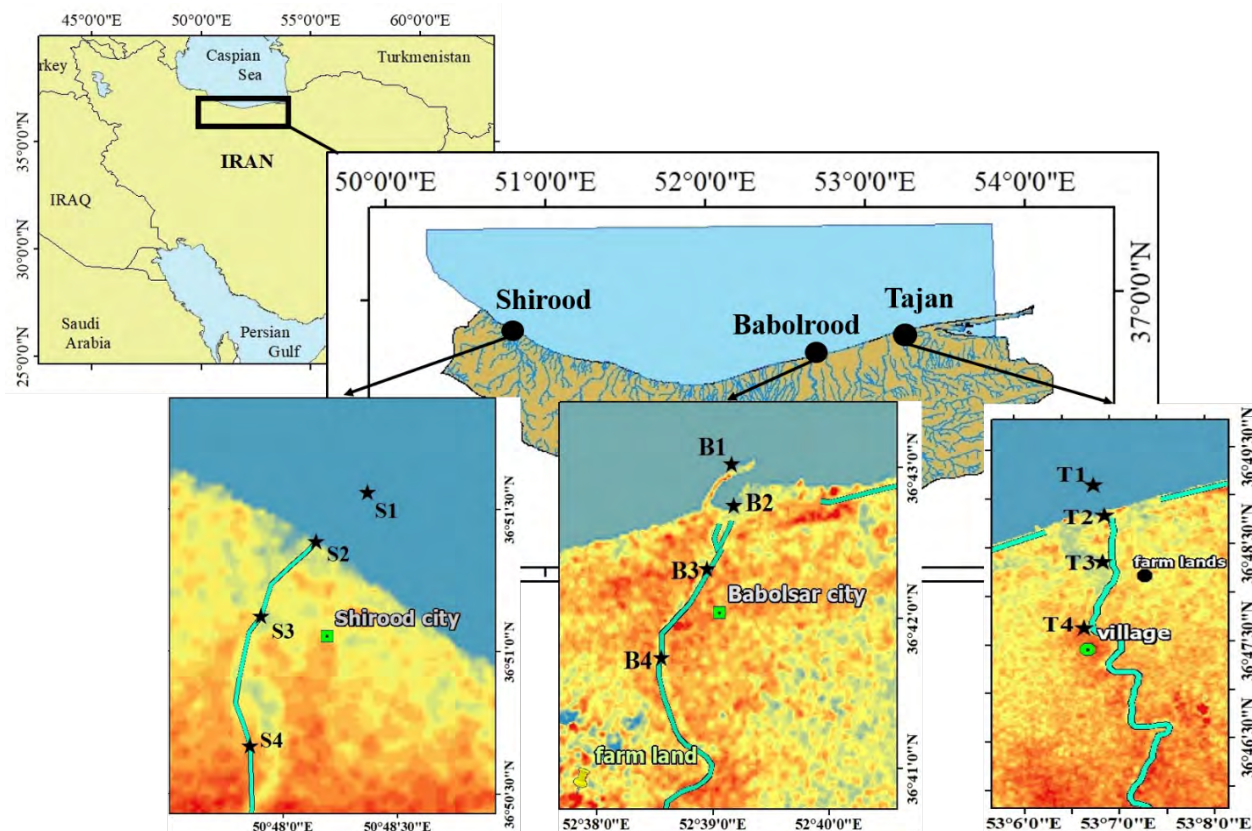


Figure 1) Map showing geographical coordinates of the sampling locations in the southern Caspian Sea, Tajan River (T1, T2, T3, T4), Babolrood River (B1, B2, B3, B4), and Shirood River (S1, S2, S3, and S4).

recorded. After 6 hours, the hydrometer was again placed into the cylinder for 60 s, and the data were recorded. The silt, sand, and clay contents were calculated as proposed by Sadeghifar and Azarmsa (2015)^[52]. All reagents applied were from Merck Company (Darmstadt, Germany).

Sediment Preparation for PTE Analysis

To measure the PTEs in the sediments, the samples were dried at room temperature and then dried at 70°C in an oven for 24 hrs. The dried samples were powdered via a mortar and then sieved through a 63 µm mesh screen to remove smaller particles. To digest the sediment samples, 0.2 g of dried sample was gently mixed with 1 ml of HNO₃:HCL and 6 ml of concentrated HF (Hydrofluoric Acid) and then kept at room temperature for an hour. The samples were subsequently heated in a sand bath at 120°C for two hours to be completely digested. Afterward, 2 ml

of deionized water was added to 2.7 g of boric acid in 50 ml polypropylene tubes and shaken well. The hot samples were kept at room temperature to be cooled, added to polypropylene tubes containing boric acid, and shaken well until completely dissolved. The solution was diluted to 50 ml and used for PTE assessment by adding deionized water. The concentrations of PTEs and metalloids (Al, As, Ni, Fe, V, Co, Cr, Mn, Cu, Pb, and Zn) were obtained via an inductively coupled plasma emission spectrometer (ICP-OES, Perkin Elmer, 8000). Arsenic was measured by atomic absorption spectrometry attached to a hydride generation device. All reagents applied were from Merck Company (Darmstadt, Germany).

Quality Assurance and Quality Control

Laboratory quality assurance and quality control methods for the metal analysis of sediment, including standard operating

procedures, analysis of reagent blanks and replicates, calibration with standards, and recovery of spiked samples, were implemented to ensure the quality of the analytical data. Standards of reference materials (SRM1646a, DS8, DS7, and MESS-3) were used for the sediment samples. With this procedure, the data recovery was between 91.08% and 102.35%. All analyses were carried out in triplicate, and the results are expressed as the means.

Sediment Pollution Indices

A list of single pollution indices for each trace element, including the contamination factor (Cf), enrichment factor (EF), geoaccumulation index (I_{geo}), hazard quotient (Hq), and quantification of contamination (QoC), and novel integrated indices, including multiple probable effect concentration quality (m-PEC-q), mean probable effects level quotient (m-PEL-q), mean effect range median quotient (MERMQ), and contamination security index (CSI), were used to assess PTEs pollution. The classification and interpretation of the values for the pollution indices are given in Table S1.

Contamination Factor of PTEs

The contamination factor of PTEs (C_f) is the degree of sediment contamination over time, which is obtained by dividing the concentration of a given toxic substance in a sediment sample by its baseline pristine reference level [4]. It is calculated as Eq. (2):

$$C_f = \frac{C_n}{C_b} \quad \text{Eq. (2)}$$

where C_f is the contamination factor of PTEs, C_n is the concentration of metal *i* in the studied sample, and C_b is the preindustrial geochemical background value of metal *i*. In this study, the background PTE concentration was referred to as the core sample (C. S) acquired from an 85 cm depth, which was aged for 1400 Cal by Bagheri et al. (2019) [8] and considered preindustrial standard

reference values (mg.kg⁻¹) (see Table S2).

Pollution Load Index

PLI defines integrated pollution of toxic elements (all the studied metals) in a polluted site. PLI in each studied site was calculated by the following formula (Eq. 3)[4]:

$$PLI_{\text{site}} = \sqrt[n]{Cf_1 \times Cf_2 \times Cf_3 \times \dots \times Cf_n} \quad \text{Eq. (3)}$$

where PLI is the pollution load index, C_f is the contamination factor of PTEs, and *n* is the number of PTEs (see Table S1).

Enrichment Factor

The enrichment factor (EF), as an effective tool for measuring the magnitude of PTE contamination, reflects the origin of the metal of interest and the contributions of natural and anthropogenic factors to surface sediment contamination. To accurately evaluate the EF values and minimize the variation effects of grain size and metal composition on the examined PTEs, Fe was applied as a reference metal so that the metal concentration could be normalized to the textural characteristics of the Earth's crust. The Eq. (4) was used to calculate the EF:

$$EF = \left(\frac{C_n}{Fe_n} \right) / \left(\frac{C_{\text{crust}}}{Fe_{\text{crust}}} \right) \quad \text{Eq. (4)}$$

where C_n and C_{crust} are the metal concentrations in sediment samples and the Earth's crust, respectively [59] (see Table S1).

Geoaccumulation Index

The geoaccumulation index (I_{geo}) is the simplest and still most frequently used measure for evaluating sediment quality in a location of interest in the present era compared with its former sediment quality for PTE pollution [48]. In this procedure, a background matrix correction factor of 1.5 is specifically applied to counteract the variations in background values driven by lithogenic effects, expressing concentrated

chemicals based on geological conditions. I_{geo} was calculated as Eq. (5)(see Table S1):

$$I_{geo} = \log_2 \frac{C_n}{1.5C_b} \quad \text{Eq. (5)}$$

Hazard Quotient

The hazard quotient (Hq) is used for health risk assessment associated with toxic metals in aquatic organisms and the environment [13]. The Hq calculation is given as Eq. (6):

$$Hq = \frac{C_n}{TEL} \quad \text{Eq. (6)}$$

where TEL is the threshold effect level of the respective metals below which adverse biological effects are not expected to occur [44] (see Tables S1 and S2).

Quantification of Contamination

The quantification of contamination (QoC) index is used to identify metals' possible source of origin in sediments [13] and is calculated via Eq. (7)(see Table S1):

$$QoC = \frac{(C_n - C_b)}{C_n} \times 100 \quad \text{Eq. (7)}$$

Contamination Security Index

The contamination security index (CSI) is an ecological risk assessment tool used to determine the toxicity limit above which adverse impacts on the sediment environment are observed [13, 49]. The CSI is calculated via Eq. (8):

$$CSI = \sum_{i=1}^n W \left(\left(\frac{C_i}{ERL} \right)^{1/2} + \left(\frac{C_i}{ERM} \right)^2 \right) \quad \text{Eq. (8)}$$

where W is the computed weight of each PTE according to Pejman et al. (2015) (see Table S2), and ERL (Effect Range Low) and ERM (Effect Range Median) are guideline values for trace metals indicating the incidence of adverse biological effects (see

Tables S1 and S2).

Mean Effect Range Median Quotient

The mean effect range median quotient (MERMQ) is used to investigate the toxicity potential of metal elements in the sediments of an aquatic ecosystem and to prioritize the management of areas based on the amount of pollution caused by these metals. Toxicity potential is only examined for the infaunal or the organisms that feed directly or indirectly on the infauna [64]. The index is calculated as Eq. (9)(see Table S1):

$$MERMQ = \frac{\sum_{i=1}^n \frac{C_i}{ERM_i}}{n} \quad \text{Eq. (9)}$$

Multiple Probable Effect Concentrations Quality

The multiple probable effect concentration quality (m-PEC-q) is calculated based on the content of the metal and the consensus-based probable effect concentration of the metal as defined by Eq. (10) [50].

$$m - PEC - q = \frac{\sum_{i=1}^n \left(\frac{C_n}{PEC_n} \right)}{n} \quad \text{Eq. (10)}$$

where PEC is the probable effective concentration of the respective metal above, adverse effects are expected to occur frequently to the aquatic biota living in or near the contaminated sediment [44] (see Tables S1 and S2).

Mean Probable Effects Level Quotients

The mean probable effects level quotient (m-PEL-q) was used to determine the possible biological effects of multiple elements in sediments as defined by Eq. (11) [13].

$$m - PEL - q = \frac{\sum_{i=1}^n (C_n / PEL_n)}{n} \quad \text{Eq. (11)}$$

where PEL is the probable effect level of

the given metal at which a large percentage of the benthic population shows a toxic response ^[56] (see Tables S1 and S2).

Nemerow Pollution Index

A Nemerow pollution index (NPI) was applied to widely assess the soil environment's quality ^[15]. NPI considers the overall level of soil pollution, taking into account the concentration of the various heavy metals under consideration ^[36, 46], and was defined as Eq. (12):

$$NPI = \sqrt{\frac{(\frac{1}{m} \sum_{i=1}^m P_i)^2 + P_{i \max}^2}{2}} \quad \text{Eq. (12)}$$

where P_i is the single pollution index of heavy metal i , $P_{i \max}$ is the maximum value of the single pollution indices of all heavy metals, and m is the count of the heavy metal species (see Table S1).

Statistical Analysis

The normality of the data was controlled via the Shapiro–Wilk test and diagrams were drawn via OriginLab Pro software (ver. 2023). Data was analyzed using one-way ANOVA, and mean differences among various rivers were determined using Duncan's multiple-range test. The Pearson correlation coefficient was used to understand the relationships among the concentrations of multiple metals, grain size, oxidation potential reduction, and total organic matter.

Findings and Discussion

Sediment Properties

Data analysis showed a normal distribution for the samples collected here. The sediment properties of the Babolrood, Tajan, and Shirood Rivers are given in Table 1. The pH values varied from 7.33 to 8.18. The oxidation-reduction potential (ORP) was negative at all the studied sites and presented the most significant variation in the Shirood River (from -33 to -75). The sand content (%) varied from 53% to 97% and was highest at site S4 of the Shirood River. The mud

amount (%) ranged from 3% to 47%, with the highest content occurring at site B2 of the Babolrood River. The total organic matter content was highest at site T4 from the Tajan River (10.118%) and lowest at site B1 from the Babolrood River (1.03%). The mean content of sediment grains was in the order of sand>mud at each river. Lithological and tectonic factors significantly affect the mean size of sediments in riverbeds ^[28], and little variation is observed among the studied sites of each river, possibly because of variations in river flow, river slopes, agricultural activities, and sediment movements that flow from adjacent lands by extreme floods.

Table 1) Physiochemical properties of the sediment-water interface and sediment characteristics at sampling sites in the southern Caspian Sea rivers (Babolrood: B1, B2, B3, and B4; Tajan: T1, T2, T3, and T4; and Shirood: S1, S2, S3, and S4).

No. sites	ORP	Sand (%)	Mud (Clay+Silt) (%)	TOM (%)
B1	-63.0	79.0	21.0	1.03
B2	-57.0	53.0	47.0	4.01
B3	-57.0	63.0	37.0	3.64
B4	-50.0	69.0	31.0	3.28
Mean±Std	-54.6	66.0	34.0	2.99
	4.04	10.8	10.8	1.34
T1	-57.0	88.0	12.0	1.27
T2	-49.0	78.0	22.0	6.24
T3	-44.0	65.0	35.0	3.64
T4	-46.0	68.0	42.0	10.1
Mean±Std	-49.0	63.9	27.7	5.32
	5.71	10.4	13.3	3.79
S1	-75.0	89.0	11.0	1.66
S2	-70.0	74.0	36.0	3.71
S3	-56.0	55.0	45.0	6.42
S4	-33.0	97.0	3.0	2.83
Mean±Std	-58.5	78.7	23.7	3.66
	18.8	18.4	19.9	2.03

Temp: Temperature; ORP: Oxidation–Reduction Potential; EC: Electrical Conductivity; TOM: Total Organic Matter.

Table 2) PTEs contents (mg.kg⁻¹ dry weight) of surface sediments collected from southern Caspian Sea rivers (Babolrood: B1, B2, B3, and B4; Tajan: T1, T2, T3, and T4; and Shirood: S1, S2, S3, and S4).

No. site	Fe*	Al*	As	Co	Cr	Cd	Cu	Ni	Pb	V	Zn	Mn
T1	3.00	3.85	8.01	14.8	70.7	<5	14.4	38.1	19.7	70.8	66.9	412
T2	3.02	3.55	5.00	9.62	63.0	<5	12.1	27.0	15.9	64.7	55.1	435
T3	3.04	3.77	6.00	10.0	59.3	<5	14.4	31.8	14.6	68.1	59.1	465
T4	3.52	4.65	5.01	16.8	69.6	<5	18.0	37.8	16.2	83.8	77.9	472
Mean ±	3.24	3.98	6.43	12.9	65.7	.	14.8	33.8	16.7	71.9	64.8	456
SD	0.35	0.58	1.63	3.60	5.55	.	2.50	5.38	2.31	8.42	10.1	27.8
B1	5.84	7.40	7.78	17.1	124	<5	32.0	58.4	22.7	134	110	610
B2	6.01	8.83	5.01	22.9	125	<5	33.9	65.9	23.1	164	122	633
B3	4.92	6.57	7.00	18.0	111	<5	39.3	52.1	21.4	123	116	536
B4	4.61	5.81	6.00	16.8	101	<5	25.4	47.8	20.6	108	112	606
Mean ±	5.44	7.25	6.98	18.8	115	.	32.7	56.1	21.9	132	115	596
SD	0.78	1.39	0.99	2.94	11.4	.	6.81	7.98	1.26	23.8	5.57	41.9
S1	6.09	6.72	14.0	26.3	108	<5	30.9	55.7	17.0	131	87.0	557
S2	5.70	6.56	6.00	20.2	110	<5	23.0	50.2	19.4	146	107	635
S3	6.01	7.98	5.00	24.7	116	<5	23.9	63.8	28.2	157	129	840
S4	5.46	5.50	6.00	18.7	104	<5	18.8	41.4	13.8	129	89.8	523
Mean ±	5.91	6.79	7.85	22.6	109	.	24.3	52.9	19.7	141	103	638
SD	0.39	1.11	4.29	3.70	4.99	.	5.12	9.51	6.27	13.4	19.1	142

*Fe and Al were expressed as %.

PTEs Content in Sediment

The concentrations of PTEs in surface sediments collected from the Babolrood, Tajan, and Shirood Rivers are presented in Table 2. The ascending order of PTEs content (mg.kg⁻¹) in the surface sediments of the Babolrood River was as: As (6.98±0.99) < Co (18.85±2.94) < Pb (21.99±1.26) < Cu (32.77±6.81) < Ni (56.16±7.98) < Zn (115.33±5.57) < Cr (115.56±11.45) < V (132.81±23.82) < Mn (596.35±41.99); in the Tajan River as: As (6.43±1.63) < Co (12.93±3.69) < Cu (14.85±2.52) < Pb (16.73±2.31) < Ni (33.82±5.38) < Zn (64.89±10.16) < Cr (65.79±5.55)

< V (71.99±8.42) < Mn (456±27.87); in the Shirood river as: As (7.85±4.29) < Pb (19.77±6.27) < Co (22.6±3.7) < Cu (24.32±5.12) < Ni (52.9±9.51) < Zn (103.55±19.81) < Cr (109.92±4.99) < V (141.205±13.4) < Mn (638.77±142.1). From the ascending order of metal contents in the rivers, it can be inferred that the order of As, Co, Pb, and Cu contents differed among the studied rivers. However, the concentration order of Ni, Zn, Cr, V, and Mn contents did not differ, indicating that the contaminant sources of some metals, including Co, Pb, Cu, and As, varied among the studied rivers. At all the river sites, the level of

Table 3) Comparison of mean PTEs content (mg.kg⁻¹ dry weight) in sediments of the south Caspian Sea rivers (Babolrood, Tajan, and Shirood) with other published river data and those for rivers sediments of the Caspian Sea basin.

Region	Country	Fe*	Al*	As	Co	Cr	Cd	Cu	Ni	Pb	V	Zn	Mn
Tajan River ^a	Iran	3.24 ^B	3.98 ^B	6.43 ^A	12.9 ^B	65.7 ^B	NE ^o	14.8 ^C	33.8 ^B	16.7 ^A	71.9 ^B	64.8 ^B	456 ^B
Babolrood River ^a	Iran	5.44 ^A	7.25 ^A	6.98 ^A	18.8 ^A	115 ^A	NE	32.7 ^A	56.1 ^A	21.9 ^A	132 ^A	115 ^A	596 ^A
Shirood River ^a	Iran	5.91 ^A	6.79 ^A	7.85 ^A	22.6 ^A	109 ^A	NE	24.3 ^B	52.9 ^A	19.7 ^A	141 ^A	103 ^A	638 ^A
Tajan River ^b	Iran	0.500	-	12.8	4.23	20.0	-	-	8.21	-	-	19.7	215
Shirood River, the estuary	Iran	2.00	-	-	-	-	-	35.0	49.0	22.0	74.0	62.0	450
Dohezar River ^d	Iran	-	-	15.5	-	64.5	-	18.3	-	25.1	-	68.2	NE
Nekarod River ^e	Iran	1.86	-	-	18.5	-	-	17.5	35.6	36.8	-	2446	434
Haraz River	Iran	-	-	3.03	-	2.55	1.94	-	-	3.69	-	-	-
Bahmanshir River ^g	Iran	-	-	-	-	113	0.220	86.5	-	28.8	-	113	425
Darreh-Morad Beyg River ^h	Iran	3.06	-	-	-	81.3	0.33	41.6	42.9	27.8	-	97.6	-
Indus River	Pakistan	2.85	-	-	66.8	90.6	1.41	71.7	128	47.3	-	-	-
Southeastern Black Sea ⁱ	Turkey	5.11	4.16	13.0	16.8	99.1	0.910	78.0	42.9	84.9	-	146	1043
Ndakotsu River ^k	Nigeria	0.62	-	-	-	-	0.050	7.61	-	10.3	-	458	176
Oder and Vistula river ^l	Poland	-	-	-	-	37.2	1.55	15.3	-	40.7	-	239	-
Jangsong tidal flat, Kangryong river estuary	PR Korea	-	-	-	12.7	15.2	-	17.0	15.5	17.9	-	68.3	-
WASV ⁿ		4.60			90.0	90.0	0.300	45.0	68.0	20.0		95.0	850

^a This study; ^b Alahabadi & Malvandi, 2018; ^c Kharat Sadeghi and Karbasi, 2006; ^d Sartipi Yarmohamadi & Ansari, 2018; ^e Abadi *et al.*, 2019; ^f Khalili *et al.*, 2021; ^g Haghazadeh *et al.*, 2021; ^h Sobhanardakani *et al.*, 2016; ⁱ Usman *et al.*, 2021; ^j Aydın *et al.*, 2023; ^k Gabi *et al.*, 2022; ^l Jaskuła, and Sojka, 2022; ^m Kim *et al.*, 2021; ⁿ World Mean Shale Value; ^o Not Evaluated in the sample; ^{*} data are expressed in %. The capital letters (A-C) show significant differences among the present studied rivers for each element (P<0.05).

Cd was less than the detection limit (<5 mg.kg⁻¹), which indicated that there was no significant source of pollution containing Cd around the studied rivers since Cd is found infrequently in the natural environment and of total Cd, more than 89% of which is derived from environmental pollution [63]. The concentrations of all the studied PTEs

in the Babolrood and Shirood Rivers were much higher than those measured in the Tajan River.

A comparison of the mean element concentrations (mg.kg⁻¹ dry weight) in the sediments of the Babolrood, Tajan, and Shirood Rivers together and with other published river data is given in Table 3.

As indicated, higher concentrations of all elements were detected in the Babolrood and Shiroad rivers than in the Tjan River, except for As and Pb, which showed no significant difference among the studied rivers. Additionally, higher contents of Fe, Co, Cr, Ni, Pb, and Zn were seen in the surface sediments of the rivers studied here than those reported in previous studies^[34, 5]. This is due to the rapid development of the surrounding industries and service units and the lack of supervision of follow-up and performance from the other side.

PTE Contamination in Sediment via Single Indices

The results of single pollution indices of PTEs, including the contamination factor (C_f), enrichment factor (EF), geoaccumulation index (I_{geo}), hazard quotient (Hq), pollution load index (PLI), and quantification of contamination (QoC), in sediment samples from the Babolrood, Tjan, and Shiroad Rivers are depicted in Figure 2. Data calculations revealed that the concentrations of all the metals considerably exceeded their background values, except for Pb, which was recorded at lower concentrations at most sites, especially in the Tjan River. The highest and lowest values of PLI were recorded in the estuary (site 3) of the Shiroad River and site 2 of the Tjan River, respectively. Based on PLI values, all studied sites in the present work were moderately polluted, but the Shiroad estuary was heavily contaminated. This increase in PTE concentration may have been caused by human activities and natural factors since many pollutant sources of human origin are found across the studied rivers. Additionally, the rivers investigated here are located in the eastern, central, and western parts of the southern Caspian Sea Basin, which includes the Gorgan metamorphic complex (Gorgan shales), containing large amounts of heavy elements due to the basic igneous nature of

an essential part of the source rock in this complex. Additionally, ultramafic rocks in this region contain large quantities of iron, chromium, nickel, cobalt, and manganese. These rocks are eroded under the hot and humid weather conditions of the area, leading to the release of the elements^[17].

Contamination Factor Assessment

As illustrated in Figure 2, all the metals had pollution coefficients between 1 and 3, indicating moderate pollution of the Babolrood, Shiroad, and Tjan Rivers, except Pb, which presented low pollution. In the Shiroad River, the C_f values of As at site S1 and Zn at site S3 (4.73 and 3.12, respectively) revealed considerable contamination. Generally, the relatively high concentration of As recorded in the Shiroad River might be attributed to using copper and sodium-based arsenical herbicides and pesticides in adjacent rice fields located downstream^[40]. These findings confirmed that the seaward and estuary areas of the Shiroad River face severe environmental degradation caused by toxic metals, which may cause toxicity to exposed organisms such as bony fishes migrating to rivers for spawning and spending nursery periods.

Enrichment Factor Assessment

EF values below 2 indicate that sediments originated entirely from crustal materials or natural weathering processes, but EF values over 2.0 represent sediment enrichment by PTE pollutants. As depicted by the heatmap in Figure 2, the EF values of the studied sites ranged from 0.27-2.16, indicating that no enrichment was partially enriched. EF values for As at site T1 (2.02) from the Tjan River, Cu, and Ni at site S1 (2.16 and 2.03, respectively) from the river Shiroad reached the maximum level. Ni is widely used in the mining, steel, metal plating, battery, and other alloy industries^[55]. Copper is naturally found in rocks, water, and air. However, its primary anthropogenic sources include

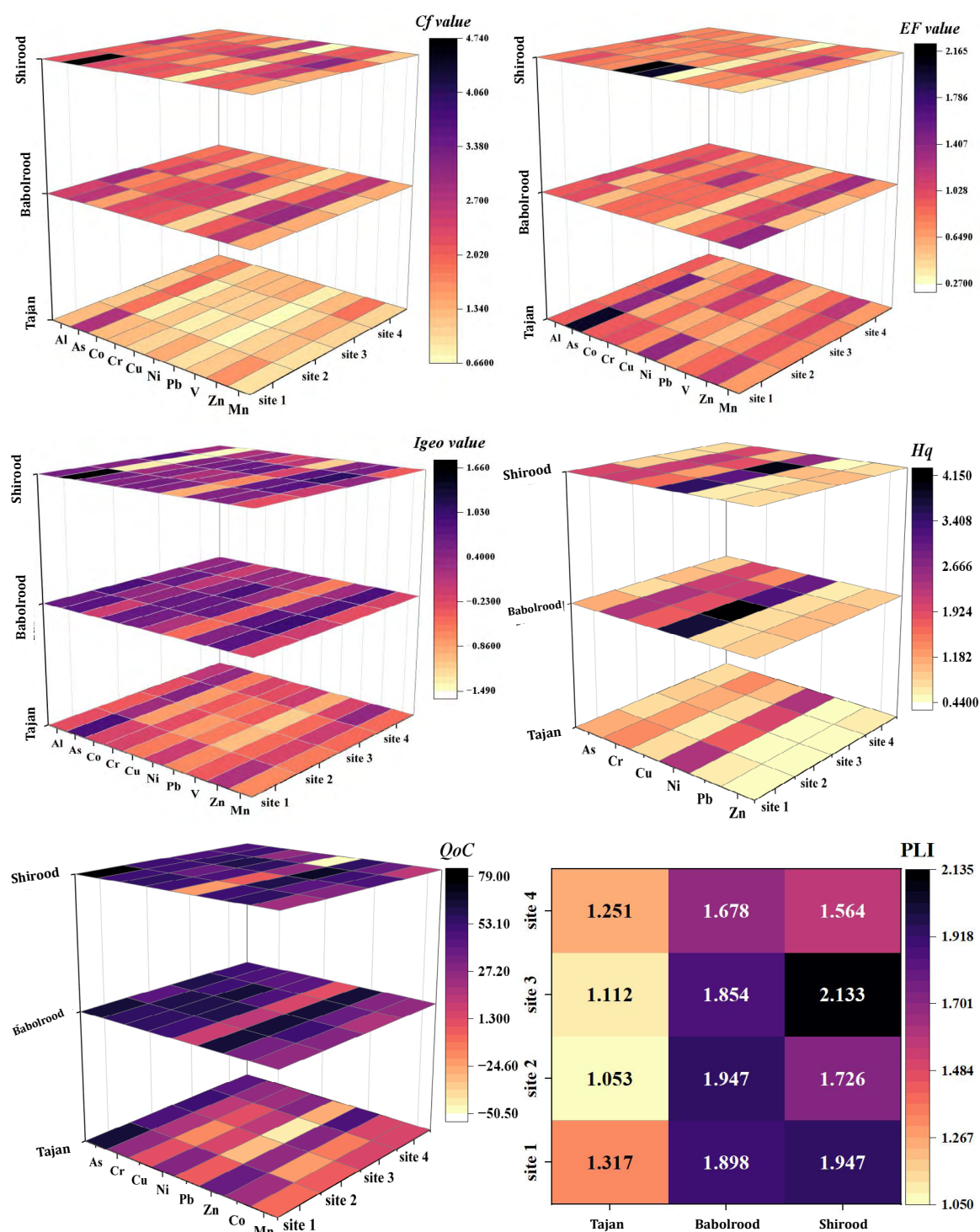


Figure 2) Spatial heatmaps of single indices for PTEs pollution in Tajan, Babolrood, and Shirood Rivers; contamination factor (C_f), enrichment factor (EF), geoaccumulation index (I_{geo}), hazard quotient (Hq), quantification of contamination (QoC), and pollution load index (PLI).

industries such as refining, metallurgy, fertilizer, fungicides, chemical manufacturing, paints, agricultural and municipal wastes, stormwater runoff, and traffic emissions [7, 41, 61]. The high contents of Ni and Cu at

the first station (inside the sea in front of the estuary) suggest that the traffic of recreational boats traveling on the beach for most of the year is the main reason for their partial enrichment in the Shirood River.

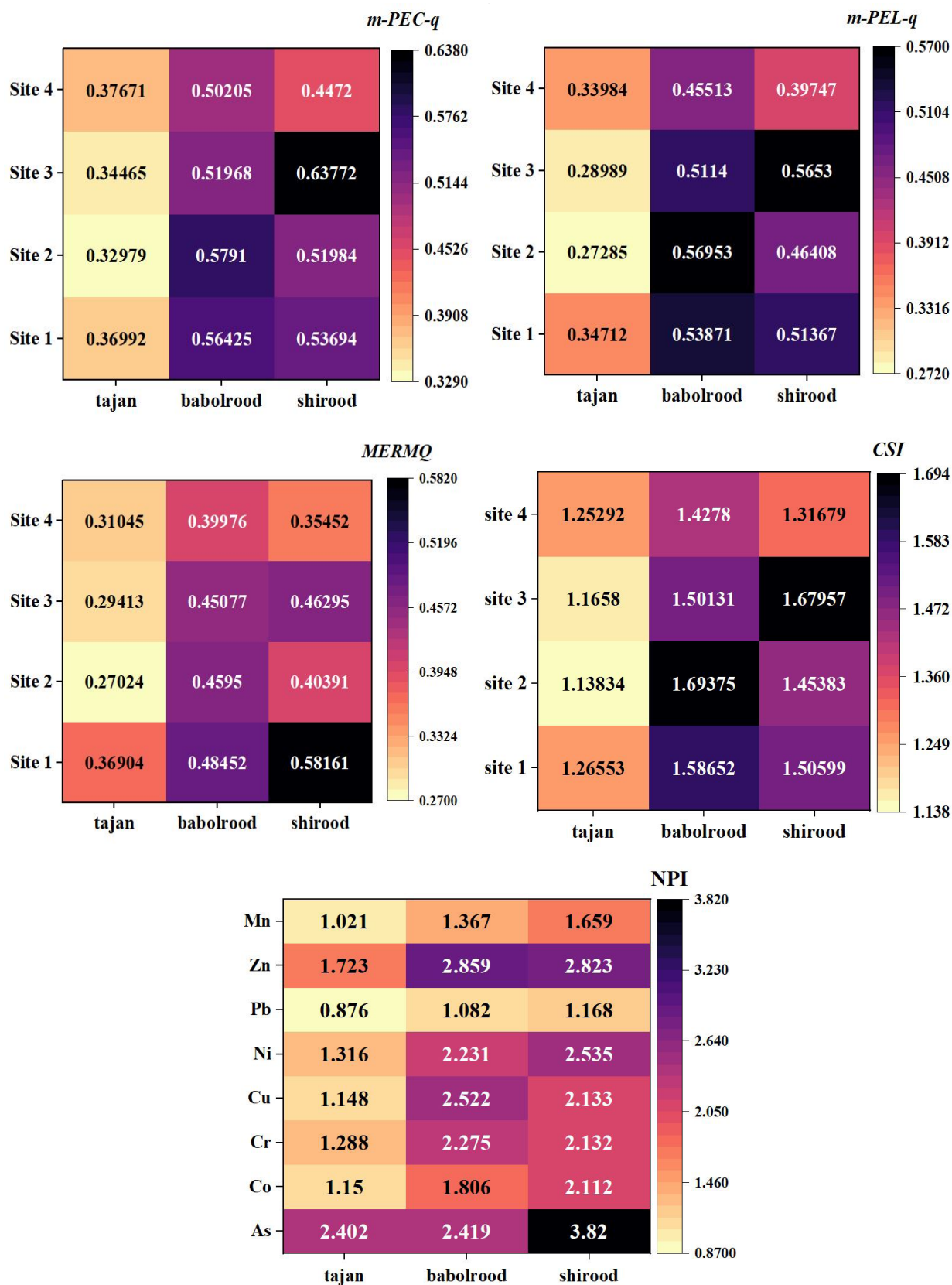


Figure 3) Spatial heatmaps of integrated indices for PTEs pollution in Tajan, Babolrood, and Shiroad Rivers; probable effect concentration quality (m-PEC-q), mean probable effects level quotient (m-PEL-q), mean effect range median quotient (MERMQ), Nemerow Pollution Index (NPI), and contamination security index (CSI).

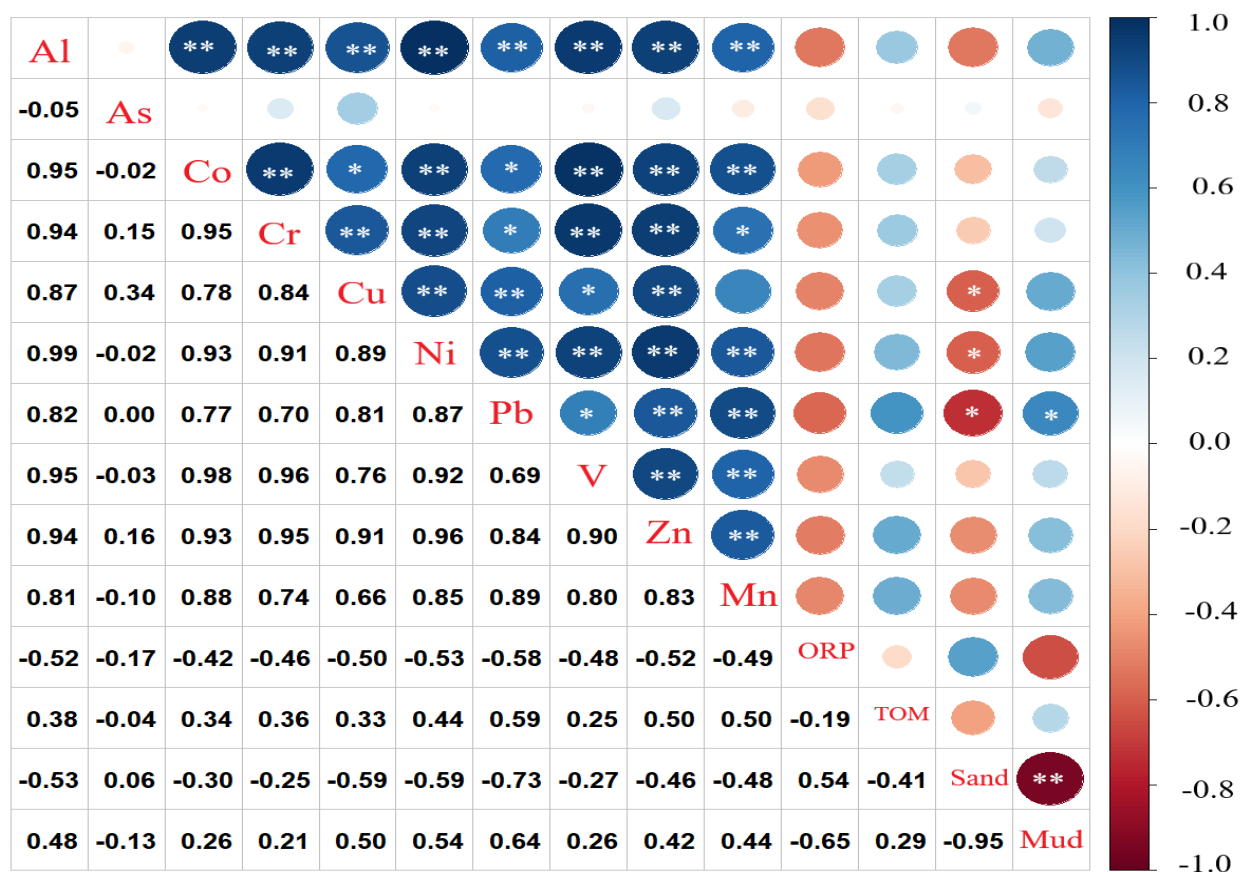


Figure 4) PCC matrix between PTEs and the general characteristics of sediments in the Babolrood, Tajan, and Shirood Rivers.

Geoaccumulation Index Assessment

The risk of geoaccumulation values for different deposited PTEs in surface sediment samples from the Tajan, Babolrood, and Shirood Rivers are illustrated in Figure 2. At all the studied sites, the I_{geo} values for the metals Al, Fe, Co, Cr, Cu, Ni, V, and Zn in the river Tajan were <1 , whereas those in the rivers Babolrood and Shirood were $0 < I_{geo} < 1$. This shows slight contamination of these metals in the Shirood and Babolrood rivers and no contamination at the studied sites in the Tajan River. Based on this index, the study sites B2, T4, and S1 were moderately polluted by As.

Quantification of Contamination Assessment

The QoC values for As, Cr, Cu, Ni, and Zn were positive at all sampling sites in the Shirood and Babolrood Rivers, probably indicating their anthropogenic sources in

nature. The QoC values for Pb, Mn, and Co were negative at several sampling sites, especially in the Tajan River, suggesting that these metals originated from natural sources. However, positive values of QoC for other metals, including As, Zn, Ni, and Cu, showed that they are of human origin in the studied rivers (Figure 2). Khalijian et al. (2022) [33] reported a level of 'no toxic risk' for Cd and 'considerable toxic risk' for As and Ni according to ecotoxic risk level (TRI) in sediments from KhazarAbad which is near the Tajan river sampling sites in the present work. They showed that the contamination levels were higher in the areas where industrial, domestic, and agricultural wastewater was discharged. According to field observations, this high level of contamination in the Babolrood River might be related to the tourism layout of Babolsar city, which is located in the

river's discharge route to the sea. The traffic of pleasure boats, leaving many traditional and worn-out fishing boats on the shore, and car traffic across the beach can discharge oil, petrol, and gasoil into the river. Moreover, the Babolrood River is also affected by domestic and industrial wastewater from Babol city, which is located before Babolsar and is known for its high population, heavy traffic, and Mansourkandeh industrial town east of the city. Recently, PTEs emitted by nonexhaust vehicles have been deposited in urban and motorway road dust and then discharged into rivers at low altitudes via rainfall-runoff [3].

Hazard Quotient Assessment

The Hq values at the studied sites ranged from 0.44–4.14, with the lowest values for Zn at all the sampling sites and the highest values for Ni, specifically in the Babolrood and Shiroad Rivers (Figure 2). The calculated Hq value ($0.1 > Hq < 10$) indicated potential to moderate hazardous triggering to aquatic organisms with the studied metals in the sediment samples of the three rivers. This finding is consistent with the results of Benson et al. (2018), who reported similar observations in sediment from the Gulf of Guinea, which could pose potential hazards to triggering moderate hazards to aquatic organisms. Hq values near or > 1 suggest that PTEs may pose a risk to the ecosystem and have toxic biological effects [13]. This result showed that the dietary intake of aquatic animals from the studied aquatic ecosystem might result in a few possible health risks, especially from Ni, Cr, and Cu poisoning in the Babolrood and Shiroad Rivers.

PTE Contamination in Sediment via Integrated Indices

Figure 3 depicts the results of the integrated pollution indices of PTEs, including the contamination security index (CSI), multiple probable effect concentration quality

(m-PEC-q), mean probable effect level quotient (m-PEL-q), probability of toxicity (m-ERM-q), and multielement contamination (MEC), in sediment samples from the Babolrood, Tajan, and Shiroad Rivers.

Contamination Security Index Assessment

The CSI values ranged from 1.13–1.26 for the Tajan River, 1.42–1.69 for the Babolrood River, and 1.31–1.67 for the Shiroad River (Figure 3). The calculated CSI values indicated that sediment samples from the Tajan River presented low contamination severity. In contrast, those from the Babolrood and Shiroad Rivers presented low to moderate contamination severity. These findings are consistent with the results of other QoC, Hq, and I_{geo} indices.

Multiple Probable Effect Concentrations Quality Assessment

In this study, for all the metals, the m-PEC-q values ranged from 0.32 - 0.63 (Figure 3) at all the sampling sites, and upper values (>0.4) were observed in the sediment samples from the Babolrood and Shiroad Rivers. The present results (m-PEC-q < 1) suggest that the sediment samples from Tajan, Babolrood, and Shiroad are nontoxic and that the incidence of toxicity is relatively low ($< 25\%$). Das et al. (2023) [18] reported a similar result in the sediment of the Ganga River, India, which presented a relatively low incidence of toxicity ($< 25\%$).

Assessment of the Means of the Probable Effects

The values of m-PEL-q at the sampling sites ranged from 0.27–0.56, and upper values (>0.4) were recorded for the Babolrood and Shiroad Rivers (Figure. 3). The values indicated that all the sampling stations had medium to low levels of contamination with all the PTEs and a 21% probability of being toxic. Similar results were reported by Benson et al. (2018) [13] in the sediment of the Guinea Gulf and Das et al. (2023) [18] in the sediment of the Ganga River, which indicated

a medium to low level of PTE contamination and a 21% probability of toxicity.

Assessment of the Mean Effect Range

In the present study, the sampling sites of the Tajan and Babolrood Rivers presented m-ERM-Q values ranging from 0.27-0.48 for all PTEs, indicating a medium to low risk associated with metal contamination, with a 21% probability of being toxic (Figure 3). In contrast, higher values ($0.5 < \text{m-ERM-Q}$) were observed in the Shiroad River (site 1=0.58), indicating a moderate to high risk, with a toxicity incidence of 49%. This implies that a considerable proportion of trace metals are delivered due to significant anthropogenic inputs into the Shiroad River and have serious adverse effects on most aquatic sediment.

Assessment of Nemerow Pollution Index

As depicted by Figure 3, all the PTEs, except for As, showed a low pollution level in the Tajan River, while the studied PTEs moderately polluted the Babolrood and Shiroad Rivers. The metal As has extremely polluted the Shiroad River, based on the NPI. Like other integrated indices, the NPI exhibited considerable pollution by PTEs in the Babolrood and Shiroad Rivers here, which implies their anthropogenic inputs, especially for As.

PTEs Content and Relationship with Sediment Properties

The results of Pearson's correlation coefficient (PCC) between the PTEs content and sediment grain size, TOM, and ORP are presented in Figure 4. All the metals were positively and significantly correlated, excluding arsenic, which was not significantly correlated ($p < 0.05$), reflecting the different geochemical characteristics compared with those of the other studied metals. This also suggests that pollutant sources of As may differ from those of other metals. The positive correlation among the studied metals might imply their same origin, mutual dependence,

and similar behavior during transportation [11, 12]. TOM was positively and significantly correlated with Pb. The sand content (%) was negatively and significantly associated with Cu, Ni, and Pb. The mud content (%) was significantly positively correlated with Pb. Although the correlation coefficients between mud content in sediment and Ni and Cu contents were insignificant, the values were remarkably high, suggesting the role of mud grains in metal movements. The results presented here were consistent with the findings of Bastami et al. (2012)^[9], who reported a significant negative relationship between sand and sedimentary metal contents but a positive correlation between mud and metals in surface sediments of Gorgan Bay, Southeast of the Caspian Sea. Generally, sediment chemical composition, grain size, and TOM content significantly affect the spatial variation in PTEs in sediment [24, 42].

Conclusion

This study provides essential information on PTE concentrations, including those of Fe, AL, As, Cu, Cr, Co, Cd, V, Ni, Pb, Zn, and Mn, in surface sediment from the Babolrood, Tajan, and Shiroad Rivers in the southern Caspian Sea. The sampling time and sites were restricted to the time and place of fish finger release in each river adjacent to the river outlet and from June to July 2021, respectively. The concentration order of PTEs varied in the studied rivers, indicating that their contaminant sources differed significantly. At all the river sites, the Cd concentration was lower than the detection limit ($< 5 \text{ mg.kg}^{-1}$), indicating no significant source of pollution containing Cd around the studied rivers. The PTE content measured in the Tajan River was significantly lower than those recorded in the Babolrood and Shiroad Rivers. The contents of all metals except Pb were higher than the background

values. EF and QoC indicate that Cu, Ni, and As are likely anthropogenic. According to the values of CF, I_{geo} , and Hq, the sediment of the Babolrood and Shirood Rivers was significantly contaminated by As, Ni, and Zn. Integrated ecological risk indices suggest that metals pose a medium to low ecological risk/toxicity level. Integrated contamination indices provide a more comprehensive view of the results than single indices. The present study revealed that the contamination level of PTEs and the studied ecological risk to the river Shirood pose serious adverse effects on most aquatics in the sediment. Hence, more accurate management strategies such as improving wastewater treatment, promoting sustainable agriculture, and regulating industrial discharge should be given to control PTE levels in sediment.

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Data availability

The data will be made available upon reasonable request.

Funding Declaration

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Competing Interest Declaration

The authors declare that they have no competing financial or nonfinancial interests as defined by Springer or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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Supplementary Data

Table S1) The contamination indices (contamination factor (C_f), enrichment factor (EF), Geo-accumulation index (I_{geo}), Pollution load index (PLI), Hazard Quotients (Hq), quantification of contamination (QoC), Mean Effect Range Median Quotient (MERMQ), Contamination Security Index (CSI), mean Probable Effects Level quotients (m-PEL-q), Nemerow Pollution index (NPI), and multiple Probable Effect Concentration quality (m-PEC-q) (data are adapted from the references of Chen et al., (2007); Cheng et al. (2007); Hakanson (1980); Adamoko et al. (2008); Kowalska et al. (2018); Malvandi (2021); Proshad et al. (2022); Benson et al. (2018); Pejman et al., 2015)).

Enrichment Factor (EF)		Geo-accumulation Index (I_{geo})		Contamination Factor	
EF Value	Contamination Level	I_{geo} Value	Contamination Level	C_f Value	Contamination Level
EF<1	Not enriched	$I_{geo} \leq 0$	Practically unpolluted	$C_f < 1$	no pollution
1<EF<3	Partially enriched	$0 < I_{geo} \leq 1$	Unpolluted to moderately-polluted	$1 \leq C_f < 3$	moderately polluted
3<EF<5	moderately enriched	$1 < I_{geo} \leq 2$	Moderately polluted	$3 \leq C_f < 6$	heavily polluted
5<EF<10	moderately to severely enriched	$2 < I_{geo} \leq 3$	Moderately to heavily polluted	$C_f \geq 6$	extremely polluted
10<EF<25	Severely enriched	$3 < I_{geo} \leq 4$	Heavily-polluted		
25<EF<50	Very severely enriched	$4 < I_{geo} \leq 5$	Heavily to extremely polluted		
EF>50	extremely high enriched	$I_{geo} \geq 5$	Extremely polluted		
Hazard Quotients (Hq)		Quantification of contamination (QoC)		Mean Effect Range Median Quotient (MERMQ)	
Hq value	Classification of effects	QoC level	Metal source identification	MERMQ value	Priority Risk and probable toxicity level
Hq<0.1	No adverse effects	QoC < 0	Natural source	≤ 0.1	Low risk with toxicity incidence of 9%
0.1<Hq<1	Potential hazards	QoC>0	Anthropogenic source	$0.1 < \text{MERMQ} \leq 0.5$	Low-moderate risk with toxicity incidence of 21%
1<Hq<10	Moderate hazard			$0.5 < \text{MERMQ} \leq 1.5$	Moderate-high risk with toxicity incidence of 49%
Hq>10	High hazard			> 1.5	High risk with toxicity incidence of 76%
Contamination Security Index (CSI)		mean Probable Effects Level quotients (m-PEL-q)		multiple Probable Effect Concentration quality (m-PEC-q)	
CSI Value	Contamination Level	m-PEL-q value	Probable Toxicity Level	m-PEC-q value	Probable toxicity level
CSI<0.5	Uncontaminated	$m\text{-PEL-q} \leq 0.1$	8%	$m\text{-PEC-q} < 1$	Nontoxic with toxicity incidence is <25%
0.5<CSI<1	Very low contamination severity	$0.11 < m\text{-PEL-q} < 1.5$	21%	$1 \leq m\text{-PEC-q} < 5$	toxicity incidence is between 25% and 75%
1<CSI<1.5	Low contamination severity	$1.51 < m\text{-PEL-q} < 2.3$	49%	$m\text{-PEC-q} \geq 5$	Toxic with toxicity incidence is >75%
1.5<CSI<2	Low to moderate contamination severity	$m\text{-PEL-q} \geq 2.3$	73%		
2<CSI<2.5	moderate contamination severity				
2.5<CSI<3	Moderate to high contamination severity	Nemerow Pollution Index (NPI)		Pollution Load Index (PLI)	
3<CSI<4	high contamination severity	NPI Value	Contamination Level	PLI Value	Contamination Level
4<CSI<5	Very high contamination severity	$PLI \leq 0.7$	No pollution	$PLI < 1$	no pollution
CSI≥5	Ultra-high contamination severity	$0.7 < PLI \leq 1$	Warning line of pollution	$1 \leq PLI < 2$	moderately polluted
		$1 < PLI \leq 2$	Low level of pollution	$2 \leq PLI < 3$	heavily polluted
		$2 < PLI \leq 3$	Moderate level of pollution	$PLI \geq 3$	extremely polluted
		$PLI > 3$	High level of pollution		

Table S2) Geochemical backgrounds, sediment quality guidelines of heavy metals given in the literature

Metal	ERL	ERM	TEL	PEC	PEL	C.S	W
Fe						22500	
Al						31300	
As	70	8.2	7.2	33	41.6	2.96	
Co						11.6	
Cr	81	370	52.3	111	160	53.01	0.134
Cu	34	270	18.7	149	108	14.33	0.075
Ni	20.9	51.6	15.9	91.3	42.8	27.43	0.215
Pb	46.7	218	30.2	129	112	20.85	0.251
V						116	
Zn	150	410	124	459	271	41.6	0.075
Mn						450	
Reference	Long et al. (1995)	Long et al. (1995)		Hongyi et al (2009)	EPA (2008)	Bagheri et al. (2019)	Pejman et al. (2015)

ERL: Effect-range-low, ERM: Effect-range-medium,
TEL: Threshold Effect Level, PEC: Probable Effect
Concentration, PEL: Probable Effect Level, C.S: Core
sample.