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Geospatial investigation on self-purification capacity of river Estuaries in the Caspian region: reducing heavy metals pollution

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In today's context, the adoption of sustainable wastewater treatment methods is crucial. River estuaries have the potential to offer an economically viable and environmentally friendly solution for wastewater treatment through the flocculation of pollutants. This study investigates the role of river estuaries flowing into the southern part of the Caspian Sea in the treatment of heavy metals. Two sets of experiments were designed for this purpose. The first set involved adjusting a series of discrete aquaria in various salinity regimes, while the second set utilized only one aquarium. The results from the first set indicate the capacity of the studied estuaries to remove heavy metals through the flocculation process in the following order: Zn (70%) > Mn (60%) > Cu (49%) > Pb (24%) > Ni (19%). However, the removal rates in the second set were reduced as follows: Zn (57%) > Mn (56%) > Cu (40%) > Pb (20%) > Ni (17%). It was observed that the flocculation process exhibits an unstable nature. Furthermore, the findings reveal that heavy metals flocculation primarily occurs upstream of the estuary. However, instability in the flocculation process occurs downstream, where water parameters undergo drastic changes. Statistical analyses indicate that an increase in pH plays a significant role in the destabilization of flocs. Conversely, the initial concentration of heavy metals, dissolved oxygen, and redox potential have a positive impact on the flocculation process.

The scarcity of viable freshwater resources and the demand for abundant amounts of water to meet humans' consumption needs have prompted a substantial number of investigations into wastewater purification approaches. In the quest to apply efficient technologies, numerous strategies have been introduced in the field of wastewater treatment; however, the majority of them are neither economically viable nor environmentally friendly[1,2]. The self-purification capacity of river estuaries in the removal of pollutants is attracting attention among different economic and environmentally friendly strategies in wastewater treatment. In fact, since fresh river water mixes with saline sea water in an estuarine zone and, as a result, a special environmental condition appears, a chemical phenomenon called flocculation occurs[3]. During this natural process, copious amounts of different pollutants, including heavy metals, are flocculated and deposited before reaching the open seas[4]. The adverse effects of heavy metals on marine environments and human health are being warned by scientists[5]. The flocculation rate of heavy metals in an estuarine zone is mainly influenced by parameters such as dissolved oxygen[6], pH[7], redox potential[8], dissolved organic carbon[9], and salinity[10].

In general, flocculation is an unstable process, and flocs might be destroyed and return to dissolved status in the water column. In other words, due to the constant change in the physical and chemical properties of the water column, the flocculation process tends to be influenced[11–14]. To our best knowledge, no study has considered the possibility of floc instability during the flocculation process in the estuaries of rivers. On the other hand, although numerous studies have been conducted regarding the flocculation of heavy metals in the river estuary, the results obtained have been contradictory. For example, while Saeedi et al. (2003) reported 100% flocculation of Mn in the Navroud estuary[15], Karbassi and Marefat (2017) reported negligible flocculation of Mn in the estuary of Sefid Rud[8]. Or, while Karbassi

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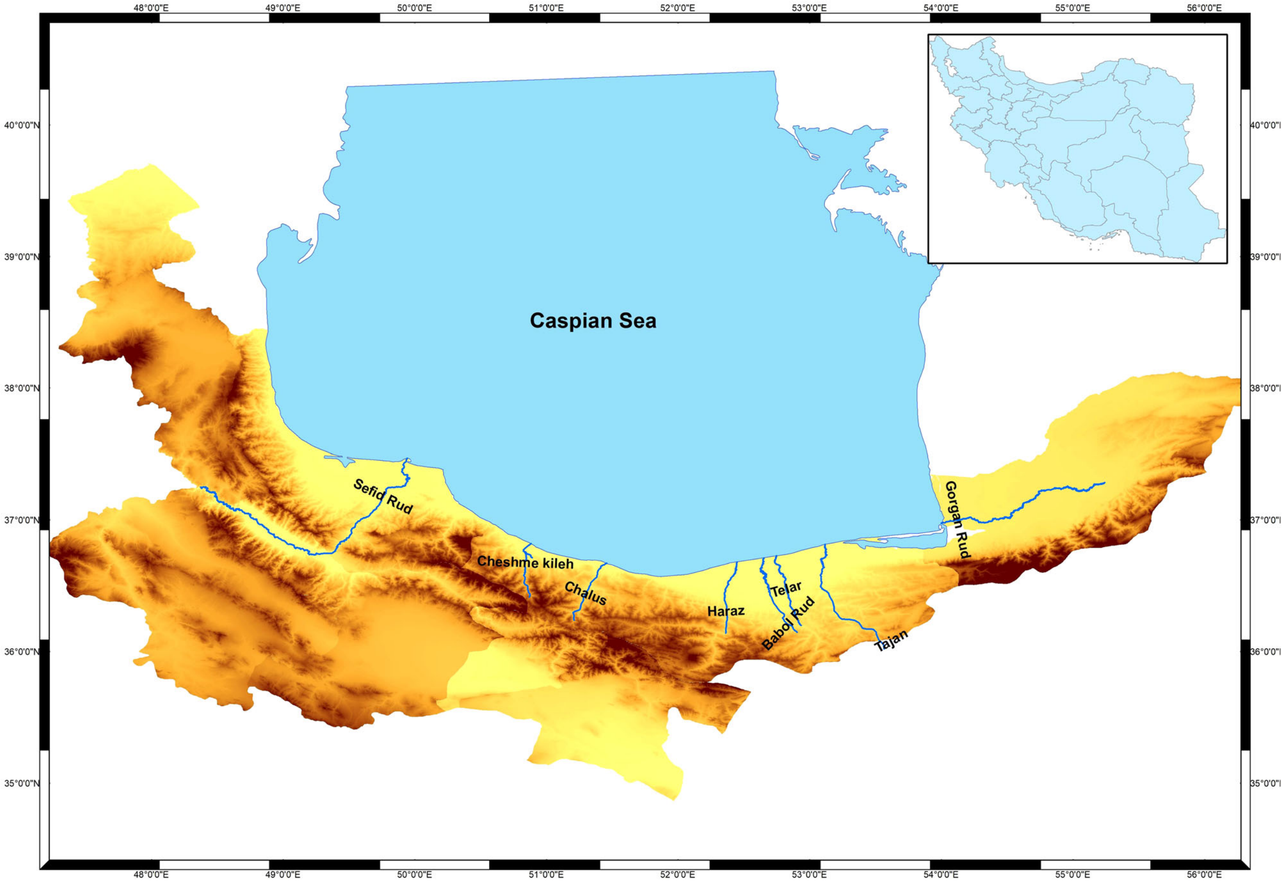

Fig. 1 | Location of rivers flowing into Caspian Sea.

and Marefat in 2017 did not observe the flocculation of Pb during estuarine mixing, Shamkhali Chenar et al. in 2012 reported the 64% flocculation of Pb in the Shafarud estuary[16]. This investigation aims to respond to this contradiction by simultaneously studying eight estuaries located in the southern part of the Caspian Sea. In addition, this study attempts to determine an instability coefficient for each metal floc formed during estuarine mixing.

Methods

Sample collection

With an area of 371,000 km^2, the Caspian Sea is the vastest lake worldwide. Figure 1 displays the eight main rivers flowing into the southern part of this lake. The average annual discharge of them is presented in Table 1. These values are based on the statistics published by the Gilan and Mazandaran Regional Water Authority. The salinity of the southern area of the Caspian Sea, where rivers reach there, is 12.5 psu[17]. Freshwater sampling stations were selected from an area where the penetration of saline water was impossible (ca. 15 km upstream). At the same time, a saline water sample was obtained from an area that was not under the effect of river freshwater (ca. 16 km from the coastline). All water samples were collected in November 2022. In addition, all sampling equipment was acid washed (by HNO_3 and HCl) and also rinsed with Milli-Q water to prevent any contamination. Subsequently, after keeping samples in a static state for 24 h, 0.22 μm Millipore HA and AP filters were used.

Table 1 | Average annual discharge and initial concentration of heavy metals in studied estuaries

River Estuary	Annual flow rate ($m^3 y^{-1}$ × 10^6)	Mn ($\mu g\,L^{-1}$)	Zn	Ni	Cu	Pb
Sefid Rud	3122	20	78	41	13	9
Tajan	7.6	22	58	40	11	6
Haraz	940	24	47	46	9	25
Talar	440	21	49	48	9	25
Babol Rud	560	28	32	48	18	27
Gorgan Rud	3	40	38	30	14	7
Cheshmeh Kileh	6.7	31	24	42	6	31
Chalus	55.3	35	30	90	15	21

Experiments

To investigate the behavior of heavy metals in the estuarine environment, two distinct sets of experiments were devised. The first set, referred to as the flocculation experiment, aimed to assess the maximum capacity of estuaries to remove heavy metals through the flocculation process. The second set, known as the instability experiment, sought to examine the reversible nature of the flocculation phenomenon, wherein flocs could lose their stability and transition into a dissolved state. For both sets of experiments, varying ratios of saline and fresh water mixtures were prepared in a series of aquaria as outlined in Table 2. Subsequently, the differences between the two

Table 2 | The ratio mixture of saline and fresh water in the aquaria

Aquarium number	Fresh water volume (L)	Saline water volume (L)	Salinity (psu)
1	5	0.4	0.5
2	5	0.6	1
3	5	0.8	1.5
4	5	1	2
5	5	1.2	2.5

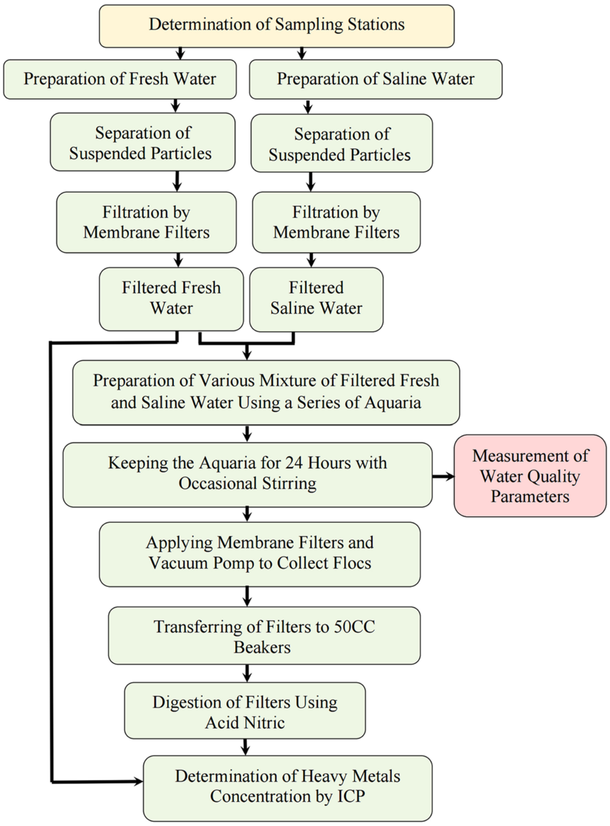

Fig. 2 | Flocculation experiment steps.

experiment sets will be explained. In the present investigation, the control salinity is considered for the fresh water, where the salinity is about 0.32 psu. It has been tried to study the flocculation of metals at salinities higher than 0.32 psu. For this purpose, the study encompasses salinity ranges between 0.5 and 2.5 psu.

Flocculation experiment

A common procedure in flocculation studies is to apply a number of separate aquaria that are prepared with various proportions of fresh and saline water. It must be mentioned that several investigators have involved this approach in their studies[6,18–20]. An important point is that, with regard to the to the oozing out of metals in lower salinity regimes, nothing of them would be left to flocculate in higher salinity regimes[21]. Thus, in this study, only lower salinity ranges would be investigated. The steps of the flocculation experiment are summarized in Fig. 2.

Instability experiment

Unlike the conventional flocculation studies applying a number of discrete aquaria to perform analysis, in the instability experiment only one aquarium would be employed in all steps. Figure 3 exhibits how the mixing process of saline and fresh water is done in the second set of experiment. In addition to this, the technique used by set 1 and 2 to measure flocs is completely different. In the flocculation experiment, first, the flocs were collected on the membrane filters with the assistance of a vacuum pump and then their amount was calculated. But, in the present innovative method, membrane filters and vacuum pump would not be applied. In fact, the floc amount would be equal to reduction in metals concentration in the solution. Therefore, this innovative method is both more economical (not applying membrane filters and vacuum pump) and more accurate (taking into account the instability of the flocs) than the conventional one. It should be noted that, in the Instability

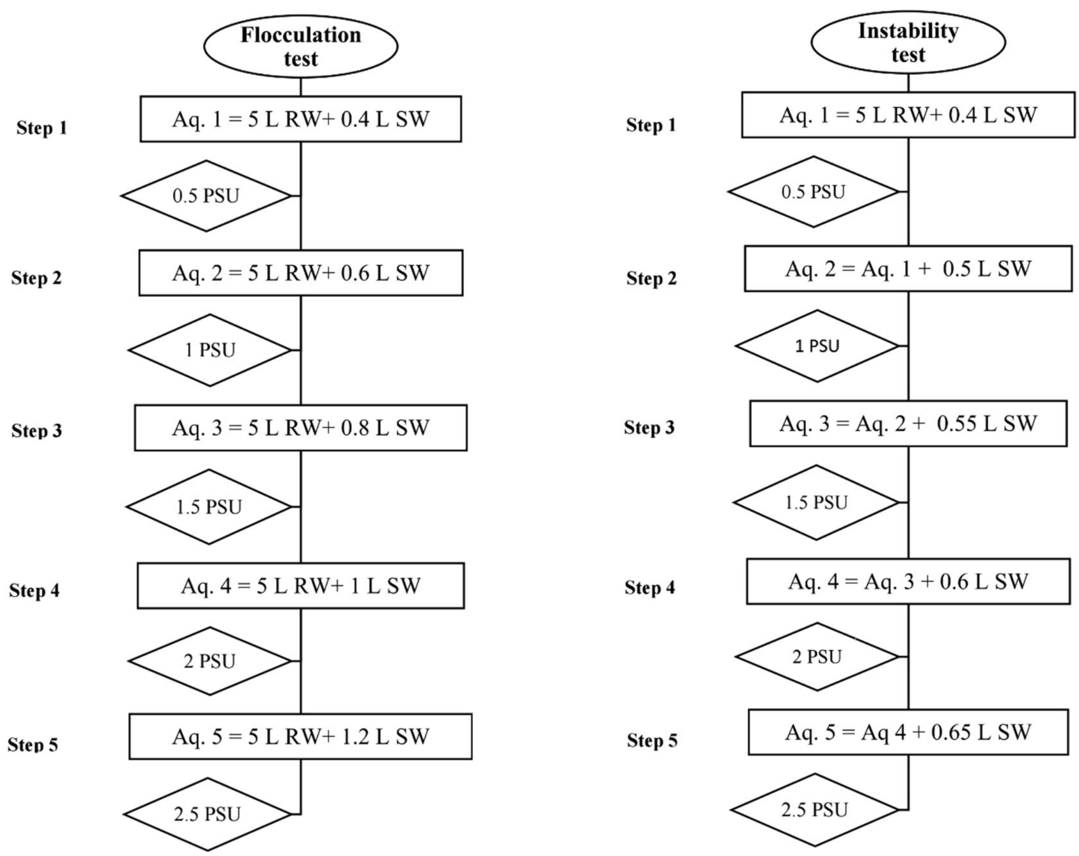

Fig. 3 | Comparison between mixing pattern in two experimental sets (RW: River Water; SW: Sea Water; Aq: Aquarium).

Table 3 | Removal percentage of (a) Mn (b) Zn (c) Ni (d) Cu in different salinity regimes according to flocculation and instability experiments (F: flocculation experiment; I: instability experiment; figures represent the flocculation percent in each salinity regime)

a) Mn	Salinity									
	0.5		1		1.5		2		2.5	
Estuary	F	I	F	I	F	I	F	I	F	I
Sefid Rud	28	28	4	4	3	3	0	−4	0	0
Tajan	36	36	12	10	1	0	0	−2	0	0
Haraz	29	29	11	11	8	8	5	3	0	0
Talar	21	21	15	15	4	3	3	1	2	0
Babol Rud	33	33	24	24	3	3	3	3	0	−1
Gorgan Rud	46	46	25	24	10	8	5	4	4	2
Cheshmeh Kileh	31	31	26	26	8	7	5	4	0	0
Chalus	37	37	19	19	14	14	4	3	1	−2

b) Zn	Salinity									
	0.5		1		1.5		2		2.5	
Estuary	F	I	F	I	F	I	F	I	F	I
Sefid Rud	56	56	31	31	11	8	2	−8	0	0
Tajan	41	41	34	34	12	5	4	0	1	0
Haraz	56	56	20	20	6	−1	4	−1	0	−1
Talar	38	38	23	23	16	15	7	0	3	−4
Babol Rud	50	50	18	18	8	3	0	−4	0	0
Gorgan Rud	47	47	21	21	9	1	1	−1	0	0
Cheshmeh Kileh	15	15	5	5	1	−10	0	−3	0	0
Chalus	14	14	7	7	4	−6	0	−3	0	−2

c) Ni	Salinity									
	0.5		1		1.5		2		2.5	
Estuary	F	I	F	I	F	I	F	I	F	I
Sefid Rud	7	7	0	−2	0	0	0	0	0	0
Tajan	10	10	0	−1	0	0	0	0	0	0
Haraz	10	10	0	−1	0	0	0	0	0	0
Talar	6	6	0	0	0	0	0	0	0	0
Babol Rud	11	11	0	−1	0	0	0	0	0	0
Gorgan Rud	52	52	9	7	5	2	0	0	0	0
Cheshmeh Kileh	20	18	0	0	0	0	0	0	0	0
Chalus	24	21	0	0	0	0	0	0	0	0

d) Cu	Salinity									
	0.5		1		1.5		2		2.5	
Estuary	F	I	F	I	F	I	F	I	F	I
Sefid Rud	41	41	11	8	3	−3	0	0	0	0
Tajan	27	26	15	10	0	−3	0	−2	0	0
Haraz	35	35	6	1	0	−4	0	0	0	0
Talar	32	32	6	−1	0	−5	0	0	0	0
Babol Rud	51	51	13	9	7	5	4	2	0	0
Gorgan Rud	39	39	9	7	7	4	0	0	0	0
Cheshmeh Kileh	22	22	2	−7	0	−1	0	0	0	0
Chalus	45	45	13	7	7	4	1	−3	0	0

Table 3 (continued) | Removal percentage of (a) Mn (b) Zn (c) Ni (d) Cu in different salinity regimes according to flocculation and instability experiments (F: flocculation experiment; I: instability experiment; figures represent the flocculation percent in each salinity regime)

e) Pb	Salinity									
	0.5		1		1.5		2		2.5	
Estuary	F	I	F	I	F	I	F	I	F	I
Sefid Rud	10	10	0	−3	0	0	0	0	0	0
Tajan	5	5	0	−2	0	0	0	0	0	0
Haraz	26	24	9	7	0	0	0	0	0	0
Talar	29	29	3	−3	0	0	0	0	0	0
Babol Rud	30	30	5	2	1	0	0	0	0	0
Gorgan Rud	8	8	0	−1	0	0	0	0	0	0
Cheshmeh Kileh	32	32	8	3	2	2	2	2	0	0
Chalus	19	19	3	0	0	0	0	0	0	0

experiment, all steps related to preparation, filtration and measurement are similar to flocculation experiment method.

Analytical techniques

To measure heavy metal concentrations (Zn, Mn, Cu, Pb, and Ni), the inductively coupled plasma mass of PerkinElmer America was used. In addition, standards from the SPEXcerprep Company were employed for calibration. After the end of each test, dissolved oxygen (DO), redox potential (Eh), pH, and salinity were analyzed using a portable multi-parameter probe (Bante 900). A DR 5000-Hach spectrophotometer was applied to monitor dissolved organic carbon (DOC). Triplicate analysis and experiments were conducted to guarantee the reliability of the results. The relative standard deviation (RSD) between replicates in the analysis was about ± 3%. It must be mentioned that only the average value of the experiments will be shown in the result section. To examine the correlation coefficient between different physicochemical parameters and heavy metal fate during estuarine mixing, the clustering approach was applied. In the present study, the weighted pair group (WPG) technique, widely utilized in environmental studies, was used for clustering[22,23]. For this end, the software MVSP (multivariate statistical package) was employed. The mean, variance, and standard deviation of the samples were calculated to determine the Z score. In general, the Z-score allows the calculation of the probability of a score occurring in a normal distribution.

Results and discussion

Flocculation process

Table 3a–e describes the flocculation process in the studied estuaries based on the results of flocculation and instability experiments. The flocculation experiment reveals that metal removal through the flocculation process mainly occurs at lower salinity regimes. In this regard, as shown in Table 3-a, the flocculation process of Mn comes to an end at salinities higher than 2.5 psu (except for Cheshmeh Kileh, Chalus, and Gorgan Rud estuaries). According to Table 3-b, the flocculation process of Zn is completely stopped at salinities higher than 2.5 psu (with the exception of Tajan and Talar estuaries). In relation to Ni, as shown in Table 3-c, the flocculation process ceases at salinities higher than 0.5 psu (except for the Gorgan Rud estuary, where the flocculation process stops at salinities higher than 1.5 psu). Table 3-d shows that the flocculation process of copper stops when the salinity is higher than 2 psu, but not in the Babol Rud and Chalus estuaries. Finally, as can be seen in Table 3-e, the flocculation process of Pb ceases at salinities higher than 1.5 psu (except for Babol Rud and Cheshmeh Kileh estuaries). Thus, it could be concluded that the maximum flocculation of metals occurs in lower salinity regimes or in upstream parts of the estuaries. Since metal oozes out at lower salinity regimes, it seems nothing will be left to flocculate at higher salinity regimes. This finding is in line with previous research, which confirms the maximum removal of metals occurs at the upstream end of estuaries. In this way, the main flocculation process was observed in low salinity regimes for Fe and Mn in Namak-abrud estuary[6], for Cr and Cd in

Table 4 | Removal rate of heavy metals during flocculation and instability experiments

River Estuary	Mn			Zn			Ni			Cu			Pb		
	F	IN	IR	F	IN	IR	F	IN	IR	F	IN	IR	F	IN	IR
Sefid Rud	35	31	4	100	87	13	7	5	2	55	46	9	10	7	3
Tajan	49	45	4	91	80	11	10	9	1	42	31	11	5	3	2
Haraz	53	51	2	86	72	14	10	9	1	41	32	9	35	33	2
Talar	45	40	5	87	72	15	6	6	0	38	26	12	32	26	6
Babol Rud	63	62	1	76	61	15	11	10	1	75	67	8	36	32	4
Gorgan Rud	90	84	6	78	68	10	66	61	5	55	50	5	8	7	1
Cheshmeh Kileh	70	68	2	21	8	13	20	18	2	24	14	10	44	39	5
Chalus	75	71	4	25	10	15	24	21	3	65	53	12	22	19	3
Average	60	56.5	**3.5**	70.5	57.2	**13.2**	19.2	17.9	**1.3**	49.3	39.9	**9.5**	24	20.7	**3.2**

F and IN represent the removal percent based on flocculation and instability experiment, respectively.
IR (instability rate) = F − IN.

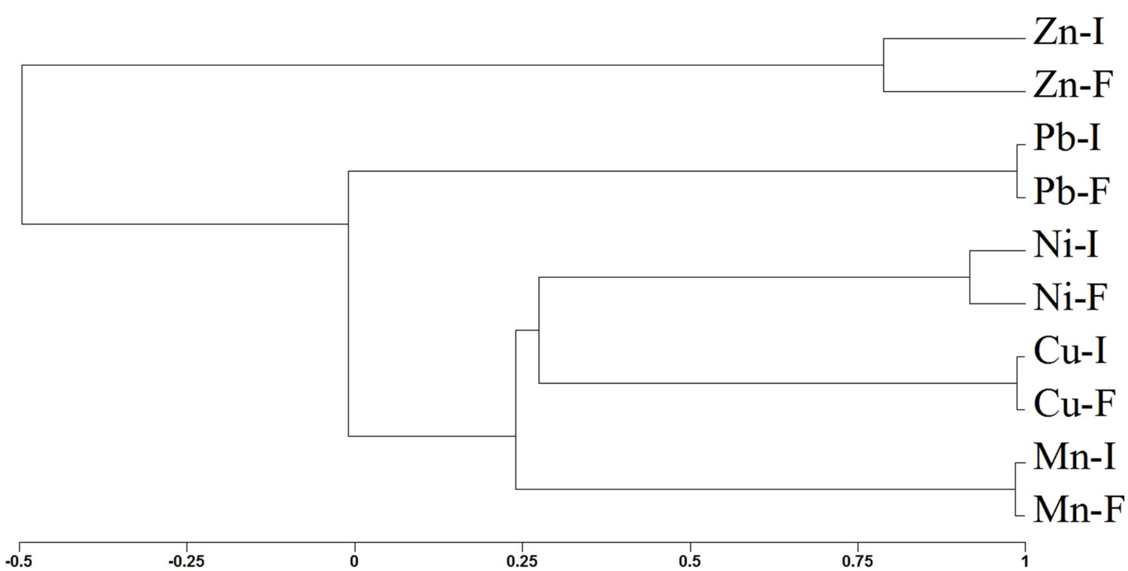

Fig. 4 | Relationship between flocculation rate and initial concentration of metal (Metal-I and Metal-F mean the initial concentration and flocculation rate of the studied metal, respectively).

Shafa Rud estuary[24], for Zn and Ni in Chalus estuary[25], and for Cu in Shalmanrood estuary[17].

On inspecting the data in Table 3(a–e), the instability experiments demonstrate that parts of the flocs, formed in lower salinity regimes, are destroyed in higher salinity regimes. The negative figures in instability experiments indicate that the fraction of flocs that occurred in the previous steps is converted to a dissolved state with increasing salinity. Accordingly, the destruction process of flocs for Zn and Mn appears at salinities of 1.5 and 2 psu, respectively. In addition, this process appears for Ni, Cu, and Pb at a salinity of 1 psu. Furthermore, as can be seen in Table 3(a–e), the instability phenomenon does not appear in very low salinity regimes (=< 0.5 psu). This might be because no significant variation in environmental conditions emerges in lower salinity ranges. In other words, when flocs experience higher salinity regimes, they are more exposed to environmental variation; hence, they are more susceptible to destruction[26–28]. A summary of results is presented in Table 4.

According to Table 4, the flocculation rates of Mn, Zn, Ni, Cu, and Pb in the estuarine zone were found to range from 35 to 90%, 21 to 100%, 6 to 66%, 38 to 75%, and 5 to 44%, respectively. Accordingly, the average amount of flocculation for Zn, Mn, Cu, Pb, and Ni was obtained at 70.5, 57.8, 49.3, 24, 19.2, and 24%, respectively. The minimum and maximum amount of flocculation belonged to the river estuaries of Sefid Rud and Gorgan Rud for Mn, Cheshmeh Kileh and Sefid Rud for Zn, Gorgan Rud and Chalus for Ni, Cheshmeh Kileh and Chalus for Cu, and Tajan and Cheshmeh Kileh for Pb, respectively. Compared to the studied metals, Zn had the highest and Ni the lowest flocculation rate. The result of this study is in line with the study done by Karbassi et al. (2015), which reported a significant amount of zinc removal during the mixing of freshwater with saline water in the Kergan Rud Estuary[29]. However, the current findings are contradictory to the study conducted on Shafa Rud estuary, which revealed that Ni had the highest flocculation rate among other metals[16]. As a consequence, the flocculation amount of metals might be completely different in two adjacent estuaries. To understand this phenomenon, as shown in Fig. 4, the relationship between the initial concentration of each metal and its flocculation rate is investigated using cluster analysis (Table 1 presents the initial concentration of the studied metals for each estuary). Figure 4 reveals that the flocculation rate has a direct relationship with the initial concentration. In other words, the higher the initial concentration, the higher the flocculation rate will be.

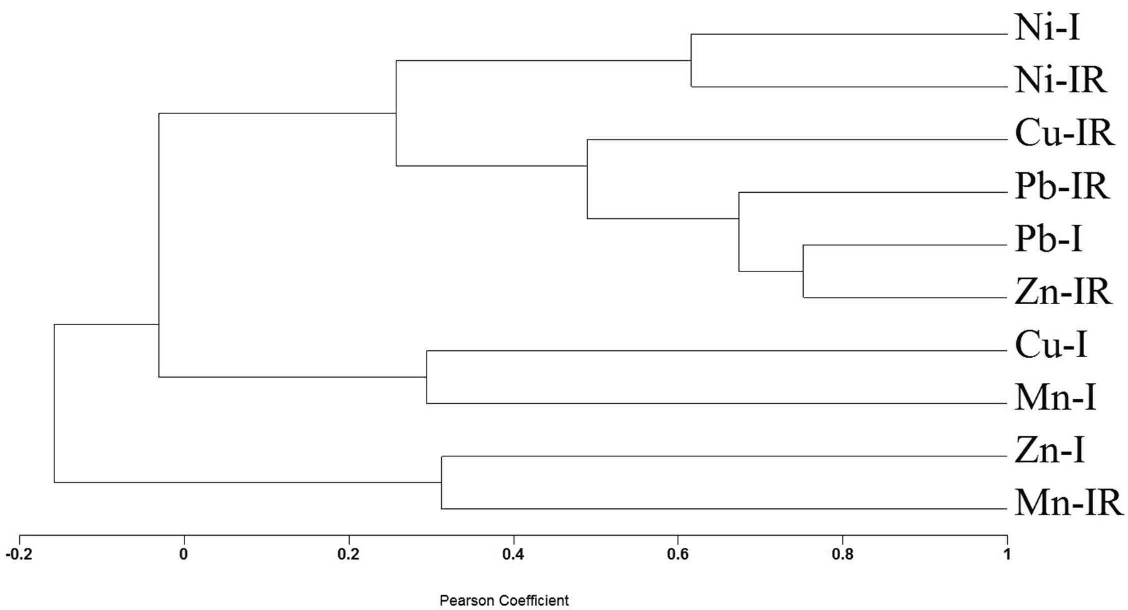

Fig. 5 | Relationship between instability rate and initial concentration of metal (Metal IR means instability rate of studied metal).

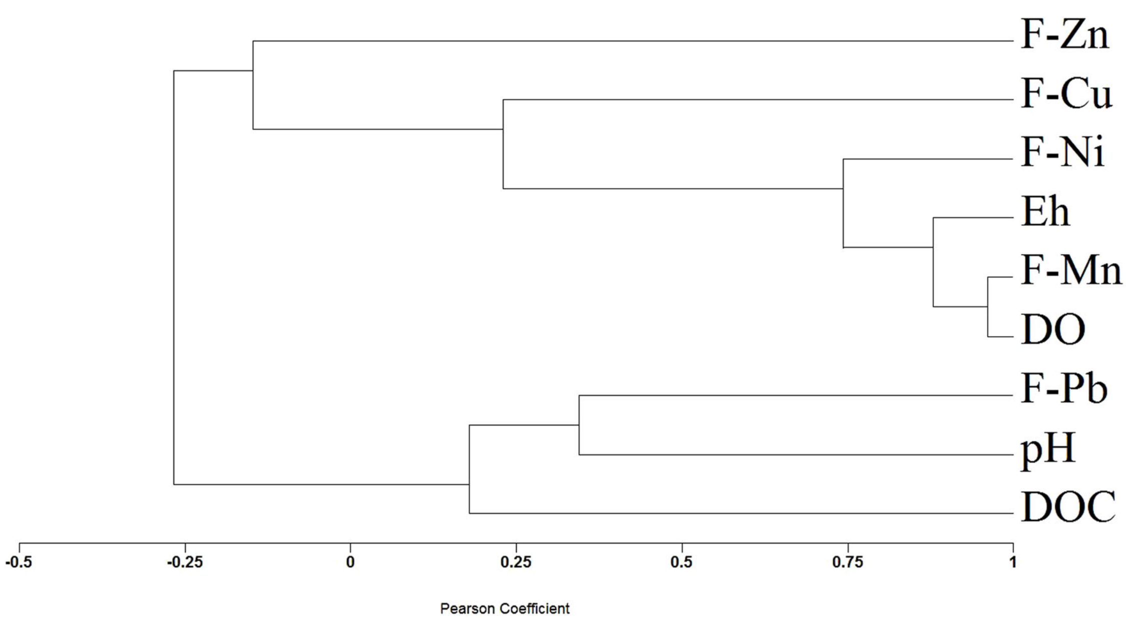

Fig. 6 | Relationship between water parameters and flocculation rate.

Based on the available literature, the initial concentration of pollutants has been observed to be one of the most important parameters influencing wastewater treatment performance[1,30–33]. Additionally, in some cases, the initial concentration of metals has been introduced as an effective factor in the flocculation process during estuarine mixing. Marefat et al. (2023) found that the reason behind the remarkable flocculation of metals in the Shafa Rud estuary is their high initial concentration in the river[24]. On the other hand, the slight flocculation of Pb in the Sefid Rud estuary was attributed to its low initial concentration[8].

As shown in Table 4, the average instability rate for flocculation of Zn, Cu, Mn, Pb, and Ni is 13.2, 9.5, 3.5, 3.2, and 1.4%, respectively. The maximum instability rate is related to the estuaries of Gorgan Rud for Mn (6%), Talar, Babol Rud, and Cheshmeh Kileh for Zn (15%), Chalus for Ni (3%), Talar and Chalus for Cu (12%), and Talar for Pb (6%). To investigate the role of metal concentration in instability experiments, cluster analysis was carried out. As shown in Fig. 5, unlike the flocculation process, there are no meaningful relationships between the instability process and the initial concentration of metals. The cluster analysis was also utilized to investigate the correlation between various parameters (Eh, pH, DO, and DOC) and processes of flocculation and instability. The lack of meaningful correlation among metals, as shown in Fig. 4, indicates that each of them follows a distinct flocculation pattern. Figure 6 demonstrates that DO and, to a lesser extent, Eh make a contribution to the flocculation of Mn. In addition, a high correlation is observed between flocculation of Ni and variations in DO and Eh levels.

Consequently, Mn and Ni are governed by dissolved oxygen during the flocculation process. This finding is in agreement with the study of Haidari (2019) and Marefat et al. (2023), which found that escalating DO led to an increase in metal flocculation in Chalus and Shafa Rud estuaries[18,24], respectively. It is worth noting that numerous scholars have confirmed that at higher dissolved oxygen, the removal of various contaminants from

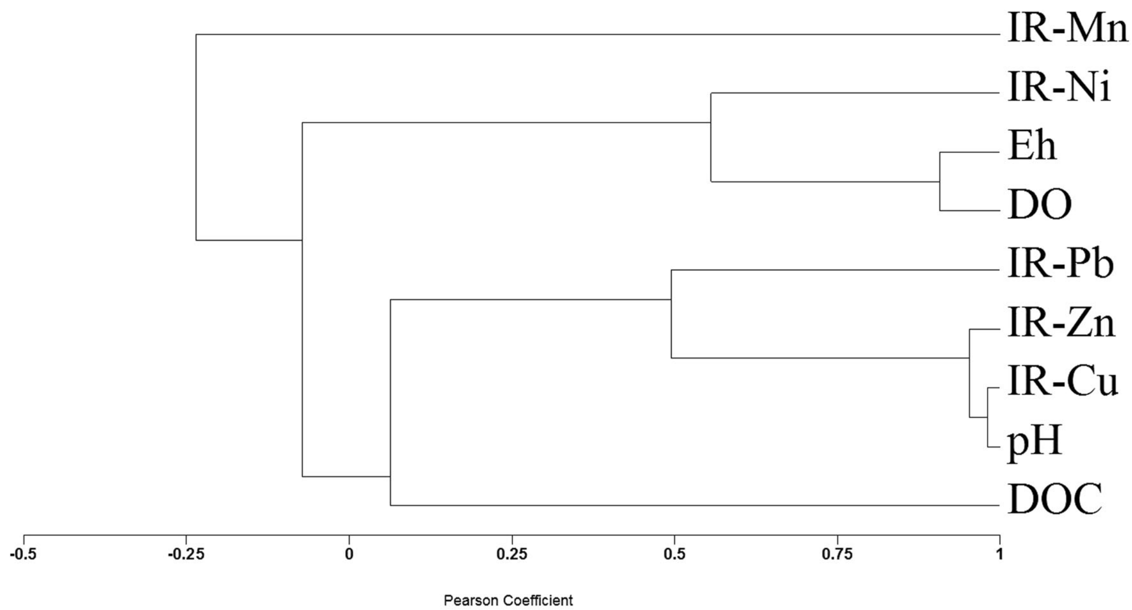

Fig. 7 | Relationship between water parameters and instability rate.

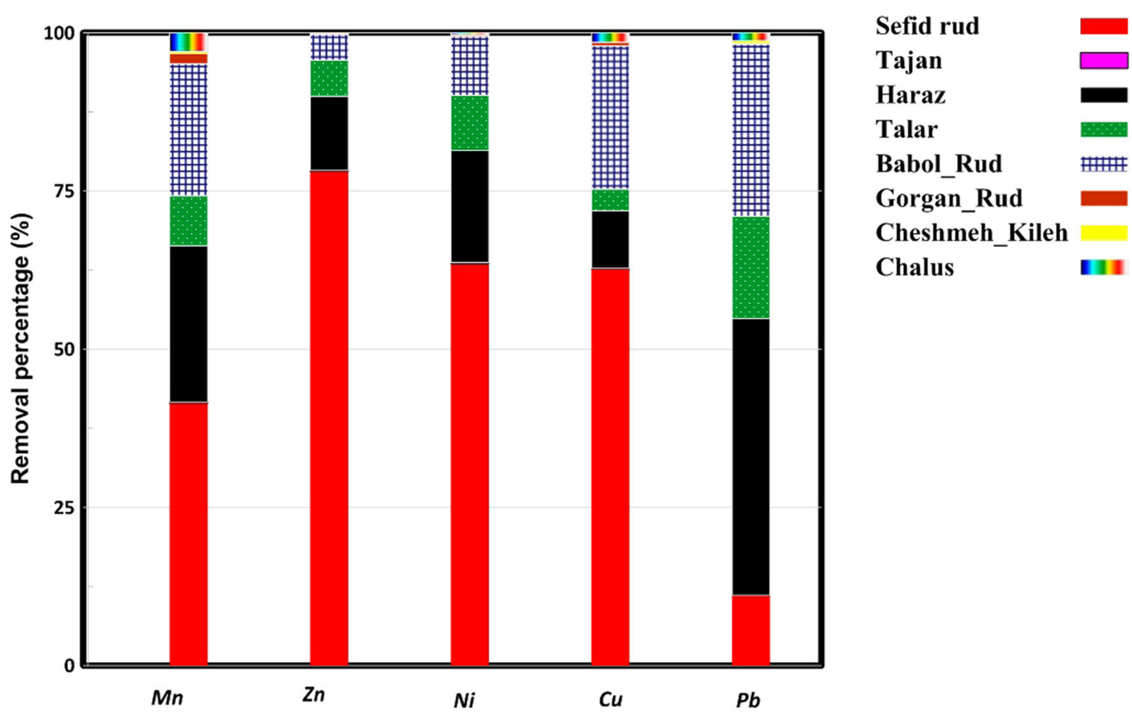

Fig. 8 | Contribution of estuaries to reducing the flow rate of metal toward the Caspian Sea.

wastewater improves[20,34,35]. The positive correlation between instability rate and pH, according to Fig. 7, implies that an increase in pH has an adverse effect on flocculation of Zn and Cu. In other words, an increase in pH destabilizes the Zn and Cu flocs. It must be mentioned that, according to the literature, pH plays an important role in the stability of colloidal forms of metals[36,37]. In addition, it could be inferred that instability occurs downstream of the estuary as water parameters experience drastic changes due to the significant mixing of seawater with fresh water. The data presented in Table 4 validated this hypothesis. Furthermore, the negative effect of pH on Ni, Co, Cr, and Cd in Shafa Rud estuary[24], Zn, Pb, and Cu in Aras estuary[38], Ni in Siyahrud estuary[7], and Pb in Cheshmeh Kileh estuary[39] was reported in previous studies.

Finally, considering the instability coefficient of the studied metals and the average discharge for each estuary, it is estimated that the studied estuaries have the capacity to reduce the average annual flow rate of Mn, Zn, Ni, Cu, and Pb from 113, 330, 225, 64, and 79 tons to 49, 141, 185, 38, and 62 tons, respectively. Figure 8 displays the contribution of each estuary to reducing the flow rate of metals toward the Caspian Sea. As shown, the estuaries of Sefid Rud, Haraz, Babol Rud, and Talar have the most contribution to make in reducing metal flow. Accordingly, the estuary of Sefid Rud plays a key role in decreasing the load of Mn, Zn, Ni, and Cu toward the Caspian Sea. However, the estuary of Haraz has made the most contribution to reducing Pb levels in the Caspian Sea.

To summarize, this study was an attempt to determine the self-purification potential of river estuaries flowing into the Caspian Sea for the removal of heavy metals. The results revealed that the studied estuaries reduced the annual discharge of Zn, Ni, Mn, Cu, and Pb by 271, 175, 46, 29, and 17 tons through the flocculation process. Moreover, analysis indicated that the flocculation process mainly occurred at lower salinity regimes in the estuarine zone. It was also found that floc destabilization occurred downstream of estuaries, so that the flocculation rates of Zn, Cu, Mn, Pb, and Ni were reduced by 13.2, 9.5, 3.5, 3.2, and 1.3% on average, respectively. As a consequence, the average annual discharge of Zn, Ni, Mn, Pb, and Cu would decrease from 330, 225, 113, 79, and 64 tons (based on the initial concentration of metal and the mean annual flow rate of each estuary) to 59, 50, 66, 61, and 34 tons, respectively. The statistical analyses revealed that the initial concentration of heavy metals, dissolved oxygen, and redox potential significantly contribute to the flocculation process. On the other hand, cluster analysis illustrated that a drastic increase in pH destabilizes the flocs of Zn and Cu.

The dynamic nature of heavy metal flocculation in estuaries, and the influence of factors like salinity and pH, which are often challenging to control, make the self-purification capacity of rivers estuary in removal of pollutants an unsuitable option for water treatment. While these complexities do pose significant challenges, it's important to note that advancements in technology and understanding may offer potential solutions. Although it may not be straightforward to compare with other management options for water treatment, exploring innovative approaches and considering the broader environmental and economic impacts could still provide valuable insights.

Data availability

All data generated during this study are included in this published article.

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Competing interests
The authors declare no competing interests.

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