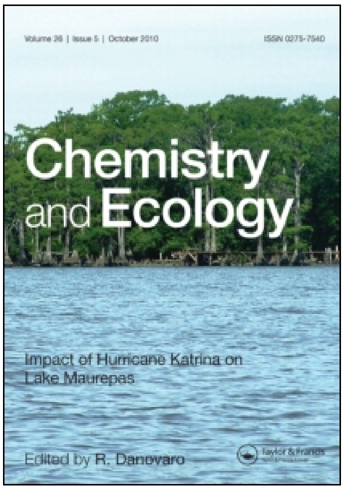

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RESEARCH ARTICLE

Metal concentrations in sediment and *Nereis diversicolor* in two Moroccan lagoons: Khnifiss and Oualidia

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Metal concentrations were measured monthly during an annual cycle (December 2004 to January 2006) in the tissues of *Nereis diversicolor* ragworms and sediment from two Moroccan Atlantic coastal lagoons: Oualidia in the north, situated in a highly industrialised urban area and Khnifiss in the south, far from anthropogenic influences. Relatively high levels of Zn, Ag and Cd were found in the sediment of Oualidia lagoon, while Ni concentrations were shown to be high at Khnifiss. The significant metal levels at Oualidia are a result of industrial and urban discharges, coupled with discharges from adjacent phosphate ore processing plants. Trace metal concentrations in worms vary according to season; they are usually high in winter and spring, in relation to the animal's reproductive cycle. Comparing the two lagoons, trace metal levels appear to be higher in worms from Khnifiss lagoon, suggesting a higher bioavailability of these elements in a relatively pristine area.

Keywords: metals; *Nereis diversicolor*; sediments; coastal lagoons; Morocco

1. Introduction

The Moroccan coast is characterised by highly diverse ecosystems and plays a key role in the national economy. However, it is subject to numerous disruptions caused by human activities. Several recent studies on metal accumulation in mussels, focusing on Moroccan beaches and estuaries [1–4], revealed contamination hotspots in particular in the Safi region. Conversely, little research has been done on lagoons, despite the fact that these areas are prone to contamination. A large, ongoing multi-disciplinary program (LagMar/REMER) focusing on Moroccan lagoon ecosystems was launched in 2004, in the aim of building a database and creating a model available to the authorities in charge of managing socio-economic aspects of these fragile areas. The study presented here was carried out in the framework of the LagMar/REMER program. It examines chemical contamination in two widely differing Atlantic coastal lagoons: Oualidia, situated between El Jadida and Safi in a highly industrialised urban area and Khnifiss in the south, situated far from anthropogenic activity and ranked as a biological and ecological reserve. Although

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various authors [5–8] have provided recent data on chemical contaminants in sediment and some bivalves in the Oualidia lagoon, information is lacking on metal contamination at Khnifiss.

This study compares metal contamination in the surface sediment of two Moroccan lagoons and examines the associated response of living organisms in terms of bioaccumulation. Previous studies [4] have already revealed high levels of Cd, Cu, Zn and Pb in mussels in the Safi region. We were keen to find out if this contamination also extended to Oualidia, and to what extent it impacted local marine life in comparison to the Khnifiss lagoon. Nickel, silver and chromium were additionally analysed in both the lagoons to complete the study. We selected the *Nereis diversicolor* ragworm as our study organism; the wide distribution and particular life cycle [9] of this species make it of particularly interest. Several studies have qualified *Nereis diversicolor* as an efficient indicator of pollution in estuarine sediment [10–14] and various authors [10,15] have demonstrated the existence of a close relationship between heavy metal concentrations in sediment and in *N. diversicolor*.

2. Materials and methods

Polychaetes *N. diversicolor* and sediment were collected monthly between December 2004 and January 2006 from the intertidal zones of Oualidia and Khnifiss lagoons at low tide (Figure 1). Fifty *Nereis* individuals were cleaned in the laboratory, purged for 48 hours in filtered seawater, dried at 70 °C until constant weight was achieved, then ground and homogenised. Aliquots of dried samples (200 mg) were digested with nitric acid [3]. Sediments collected with a pre-cleaned spatula at a maximum depth of 1 cm were placed in plastic vials, then dried at 70 °C and sieved at 63 μm. Aliquots of dried samples (200 mg) were digested with a mixture of HCl, HNO_3 and HF [16].

Cd, Pb, Cu, Mn, Ni, Zn, and Ag were analysed by ICP-MS (Thermo Electron, X series). Cr was analysed by graphic furnace atomic absorption spectrometry (VARIAN, model AA800). Fe, Al and Mn were analysed by flame atomic absorption spectrometry (VARIAN, model AA600).

Data quality was checked by comparison with analyses of standard reference material (mussel tissue from the BCR, ref. CRM 278R; oyster tissue from the NIST, ref. SRM 1566b; sediment from the NRCC, ref. BCSS-1, MESS-2 and MESS-3). All quality control results are shown in Tables 1 and 2.

Data were expressed as mean ± standard error (SD). A comparison of mean values according to sampling site (for the same season) and season (for the same site) was done by LSD testing. Differences between sites were assessed using the Tukey multiple-comparison test. Linear regression was also used to test the relationship between bivalve metal concentrations and surrounding sediment levels. Statistical analyses were performed using Statistica version 6. Significance level was $p < 0.05$.

3. Results and discussion

3.1. *Metals in sediment*

Al, Fe and Mn were measured with the aim of characterising sediment (Figure 2) with the following results: (1) Khnifiss lagoon sediment was shown to be richer in Al than Oualidia sediment, indicating that the clay fraction (fine fraction) is more abundant in Khnifiss sediment, and (2) unexpected seasonal variations in Al concentrations were observed at Oualidia throughout the sampling period, versus high stability at Khnifiss. These variations are probably due to the relative heterogeneity of surface sediment at Oualidia, (3) Fe and Mn concentrations were broadly similar

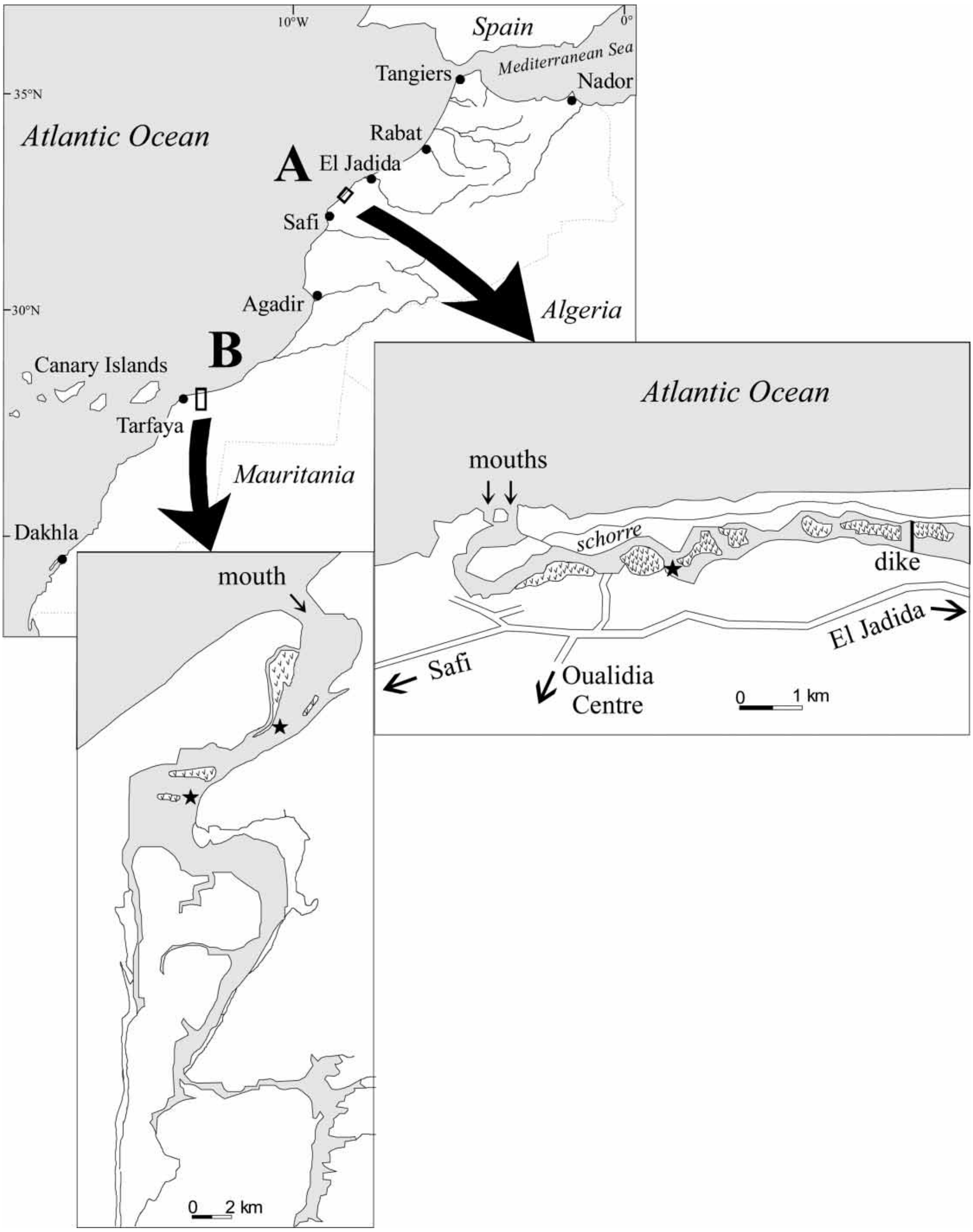

Figure 1. Study site. A, Oualidia lagoon; B, Khnifiss lagoon; ⋆, location of sampling sites.

in both lagoons, suggesting comparable redox conditions. Both parameters also proved to be highly stable throughout the sampling period at each site. This phenomenon is easily explained for Oualidia, where a sedimentation rate of less than one centimetre per year was estimated in a sediment core study [7]. However, the consistent Fe levels found in the Oualidia region did not coincide with the Al heterogeneity described above. As Fe concentrations are generally highly correlated with Al in sediment, we concluded that this anomaly was due to granulometry. Sediment characteristics at both sites were hence relatively comparable and did not require standardisation

Table 1. Sediment quality control results.

	Detection limit	BCSS-1 Analysed	BCSS-1 Certified	MESS-3 Analysed	MESS-3 Certified
Ag ($\mu g\ g^{-1}$)	0.04	0.15 ± 0.01	0.11 ± 0.03	0.23 ± 0.01	0.18 ± 0.02
Al (%)	0.4	6.4 ± 0.3	6.26 ± 0.22	8.8 ± 0.4	8.59 ± 0.23
Cd ($\mu g\ g^{-1}$)	0.01	0.29 ± 0.02	0.25 ± 0.04	0.27 ± 0.02	0.24 ± 0.01
Cr ($\mu g\ g^{-1}$)	0.15	108 ± 5	123 ± 14	95 ± 5	105 ± 4
Cu ($\mu g\ g^{-1}$)	0.4	20 ± 1	18.5 ± 2.7	34 ± 2	33.9 ± 1.6
Fe (%)	0.01	3.2 ± 0.2	3.29 ± 0.10	4.2 ± 0.2	4.34 ± 0.11
Mn ($\mu g\ g^{-1}$)	7	214 ± 10	229 ± 15	297 ± 15	324 ± 12
Ni ($\mu g\ g^{-1}$)	0.2	53 ± 3	55.3 ± 3.6	43 ± 2	46.9 ± 2.2
Pb ($\mu g\ g^{-1}$)	0.4	22 ± 1	22.7 ± 3.4	22 ± 1	21.1 ± 0.7
Zn ($\mu g\ g^{-1}$)	6	105 ± 5	119 ± 12	143 ± 7	159 ± 8

Table 2. Biota quality control results.

	Detection limit	CRM 278R Analysed	CRM 278R Certified	SRM 1566b Analysed	SRM 1566b Certified
Ag ($\mu g\ g^{-1}$)	0.02	0.15 ± 0.01	–	0.60 ± 0.03	0.666 ± 0.009
Cd ($\mu g\ g^{-1}$)	0.02	0.36 ± 0.02	0.348 ± 0.007	2.6 ± 0.1	2.48 ± 0.08
Cr ($\mu g\ g^{-1}$)	0.06	0.52 ± 0.03	0.78 ± 0.06	0.18 ± 0.01	–
Cu ($\mu g\ g^{-1}$)	0.3	9.4 ± 0.5	9.45 ± 0.13	68 ± 3	71.6 ± 1.6
Ni ($\mu g\ g^{-1}$)	0.09	0.89 ± 0.05	–	0.99 ± 0.05	1.04 ± 0.09
Pb ($\mu g\ g^{-1}$)	0.2	2.0 ± 0.1	2.00 ± 0.04	0.31 ± 0.02	0.308 ± 0.009
Zn ($\mu g\ g^{-1}$)	3	83 ± 4	83.1 ± 1.7	1596 ± 80	1424 ± 46

using a tracer. In view of the comparable quality of sediment at both lagoons and their stability over time, we opted for an annual assessment of trace metal mean values in sediment (Figure 3).

Cr, Pb and Cu concentrations were respectively in the same ranges in both lagoons ($p > 0.05$; Table 3). Zn, Ag and Cd concentrations were significantly higher at Oualidia than at Khnifiss ($p < 0.001$), reaching, in particular, a factor of two for Ag. Conversely, Khnifiss sediment appeared to contain far more Ni ($p < 0.01$) than Oualidia sediment. When we compared our figures with data recorded in other environments (Table 4), both lagoons were shown to be uncontaminated by these seven metals.

As expected, the location of sediment in either lagoon and the associated differences in biotic and abiotic conditions had a significant impact on the concentrations of each metal. First and foremost, Cd and Ag concentrations were found to be relatively high at Oualidia. Similar major Ag enrichment has already been recorded in coastal waters along the US-Mexico border, mainly ascribed to wastewater discharges [17,18]. The levels recorded in our study can be partly explained by the lagoon's poor water quality, directly associated with sewage discharge via underground seepage waters. A lack of proper water treatment facilities, coupled with a dramatic influx of visitors during the summer months, combine to intensify this phenomenon: the Oualidia catchment area enjoys a thriving tourism industry at the cost of the lagoon's health. The Oualidia area is home to approximately 6000 residents throughout the year, but this figure swells to 30,000 in the tourist season. In turn, seasonal increases in sewage discharges raise metal inputs and fluxes in the lagoon. Moreover, the neighbouring agricultural area impacts an extensive portion of the lagoon and could be a source of Cd found in fine sediment. However, the lagoon's proximity to the phosphate processing plants based at Jorf Lasfar, approximately 25 km to the northeast, is almost certainly its biggest factor of pollution. Industrial effluents from this complex are thought to contaminate the entire coastline with Cd and other metal by-products of phosphate ore processing [3,6,8]. As

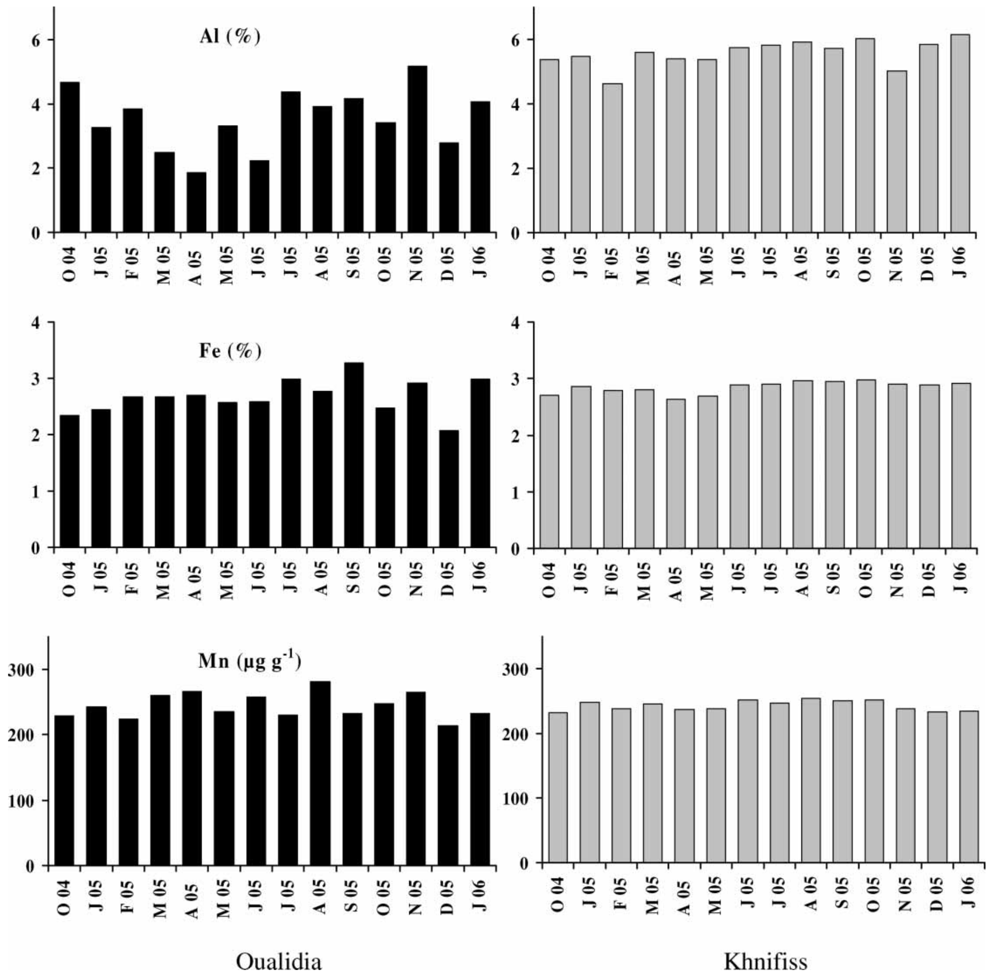

Figure 2. Temporal variations in major elements found in surface sediment from Oualidia and Khnifiss lagoons.

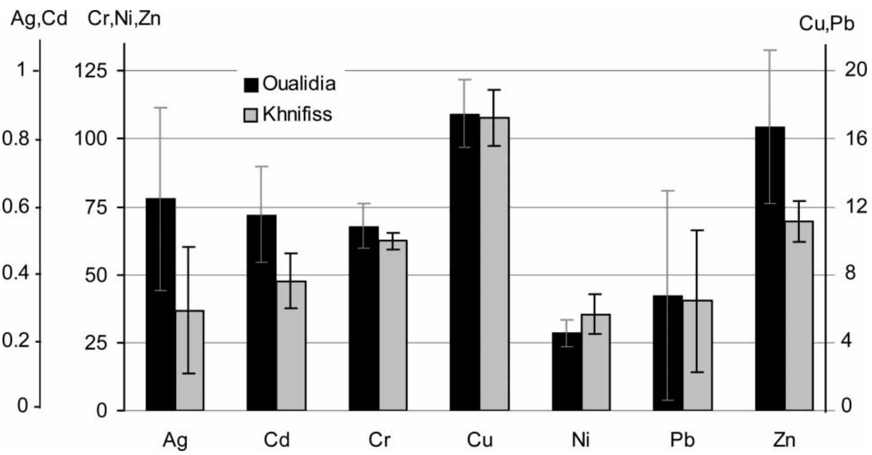

Figure 3. Respective annual mean concentrations (based on monthly measurements) of trace metals in sediment from Oualidia and Khnifiss lagoons.

Table 3. T-test results for independent samples intended to compare mean metal content in worms and sediment from lagoons.

	Ag	Cd	Cr	Cu	Ni	Pb	Zn	Fe	Mn	Al
Worms Oualidia vs. worms Khnifiss	***	***	ns	ns	***	***	ns	–	–	–
Sediment Oualidia vs. sediment Khnifiss	***	***	*	ns	**	ns	***	ns	ns	***

ns, not significant; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

these waste products are transported southeastwards by currents [19], a portion of the associated metals may reach the lagoon. A study on Ag concentrations in these effluents would certainly help explain the high levels found in the lagoon. Similar enrichment has been described in the Sidi Moussa lagoon, located to the north of Oualidia [6]. Finally, recent urban developments such as the highway skirting the lagoon, the expansion of intensive agriculture using heavy machinery, and an increase in the number of motorised fishing boats, appear to have a very limited effect on metal contaminant levels, in particular Pb and Zn, despite the heavy traffic adjacent to the site [20]. Contrary to other trace elements, we observed the highest levels of Ni at Khnifiss: this can logically be explained by the lagoon's substrate lithology, characterised by predominant schist rocks rich in Ni [21]. Finally, tannery waste products discharged directly into the water without preliminary treatment do not effect chromium concentrations in the lagoons.

3.2. Heavy metals *in Nereis diversicolor*

Seasonal variations in metal concentrations in *N. diversicolor* from the two lagoons are shown in Figure 4. With the exception of Zn, all metals at the two sites showed significant seasonal variations characterised by high values in winter and lower values in summer. These are classical natural variations, due to the reproductive cycle and variations in body weight. For example, a study conducted at the Bou Regrag estuary (Morocco) [11] revealed a fall in metal levels throughout the mating period in spring and autumn and a rise during the gametogenic phase in winter and summer. In our study, with the exception of Cd, we observed similar seasonal variations to those previously reported [22], i.e. the highest metal concentrations in *Nereis* tissues were generally found during periods of high gametogenic activity (autumn and winter).

The comparison of annual mean concentrations of various trace metals in worms (Figure 5) showed that Cu and Cr were not significantly different in either region ($p > 0.05$). Similarly, Zn levels which were almost systematically higher at Khnifiss than at Oualidia throughout the year (Figure 4), but annual mean values were not statistically different ($p > 0.05$). Ni, Cd and Pb values were higher at Khnifiss than at Oualidia (Table 3) and Cd levels were more than 10 times higher at Khnifiss than at Oualidia. Conversely, Ag was significantly higher at Oualidia than Khnifiss ($p < 0.001$). Metal concentrations found in the tissues of *N. diversicolor* collected from Oualidia and Khnifiss lagoons could be considered as moderate in comparison to the published data reported in Table 5. All metal concentrations obtained in our study were lower than those found in highly contaminated areas [11,15] and higher than those found at reference sites [23].

The differences in trace metal concentrations in worms from the two lagoons (Figure 5) were not always consistent with observations made on sediment (Figure 3). At Khnifiss, for example, Cd was higher in worms than in sediment; the same type of discrepancy was visible for Pb and Cu, and to a lesser extent for Ni. No clear differences were observed for Cr, and only Ag and Zn varied similarly at both locations and in both matrices.

The biota sediment accumulation factor (BSAF) was evaluated for each metal and each lagoon. Assuming that each lagoon is characterised by its sediment metal concentrations, we divided metal concentrations in molluscs by the concentrations found in sediment in their surrounding

Table 4. Average metal concentrations found in sediment from various regions of the world (variation coefficient in brackets).

	Ag μg g^{-1}	Al %	Cd μg g^{-1}	Cr μg g^{-1}	Cu μg g^{-1}	Fe %	Mn μg g^{-1}	Pb μg g^{-1}	Ni μg g^{-1}	Zn μg g^{-1}	References
Oualidia lagoon	0.6 (0.3)	3.5 (1)	0.6 (0.1)	68 (8)	17 (2)	2.7 (0.3)	244 (19.3)	7 (6)	29 (5)	104 (28)	This study
Khnifiss lagoon	0.3 (0.2)	5.6 (0.4)	0.37 (0.08)	62 (3)	17 (2)	2.8 (0.1)	242 (7.8)	6 (4)	35 (7)	70 (7)	
Sidi Moussa Lagoon	–	6.8 (3)	–	97 (33)	30 (6)	2.8 (1.2)	–	–	–	50 (17)	[36]
Oualidia lagoon	–	10.9 (2.5)	–	52 (6.1)	36 (23)	6.9 (1)	–	55 (17)	–	228 (16)	[7]
Iran	0.07 (0.03)	–	0.16 (0.03)	85.2 (15.3)	35 (12)	3.5 (0.6)	815 (190)	18 (4.2)	51.6 (12)	85 (18)	[37]
Bothnian bay	–	5.6 (0.8)	0.94 (0.5)	73 (8)	52 (17)	6.2 (1.6)	9 (5)	79 (32)	48 (10)	212 ()	[38]
Bidasoa estuary	1.3 (0.8)	–	1.1 (0.5)	56 (21)	100 (84)	–	309 (38)	150 (105)	35 (10)	410 (226)	[39]
Belfast lough	–	4.3–4.9	–	83–117	18–26	2.3	–	–	38	90–146	[40]

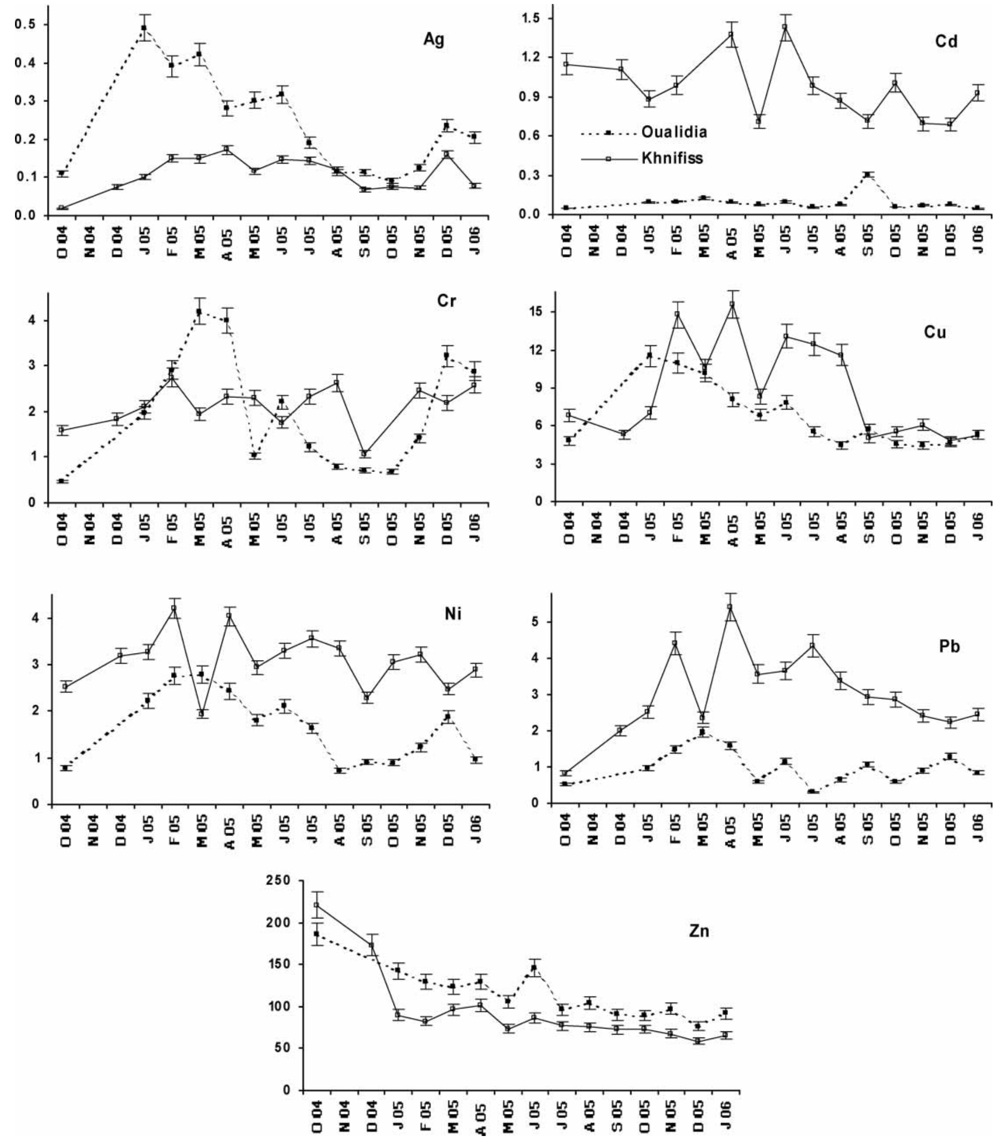

Figure 4. Monthly variations in trace metal concentrations (µg g^{-1} d.w.) in *Nereis diversicolor.*

environment: the resulting factor indicates mollusc bioaccumulation for a given metal. However, we should keep in mind that the relative accumulation of trace elements in sediment may vary between metals and sites, especially in view of the fact that sediment type varies between sites. This was our best approximation for characterising the environment with respect to metals. The bioaccumulation factor of all the studied metals was calculated for *N. diversicolor* (Table 6) and generally showed higher values at Khnifiss lagoon (Cd and Pb especially). At Oualidia, with the exception of Zn, the BSAF for all elements was less than one unit. Similar results were reported in the Bou Regrag estuary [11], where the BSAF for Zn varied from 1.7 to 3.8 between sites. A high BSAF for the same metal was reported in United Kingdom estuaries [15,24]. Trace metals can be divided into two groups according to BCF [25]. Zn and Cd are compromised in one group due to their ability to accumulate in biological tissue, and both groups showed higher BCFs at

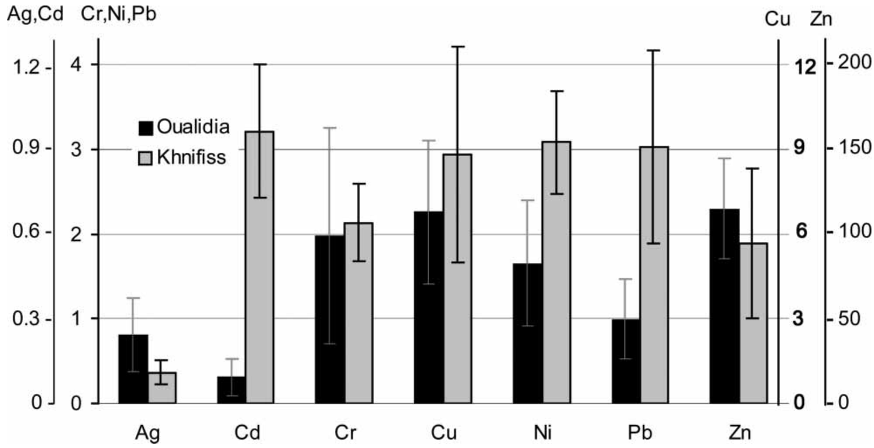

Figure 5. Respective annual mean concentrations (based on monthly measurements) of trace metals in *Nereis diversicolor* from Oualidia and Khnifiss lagoons.

Table 5. Average metal concentrations found in *Nereis diversicolor* from various regions of the world (variation coefficient in brackets).

	Ag μg g^{-1}	Cd μg g^{-1}	Cr μg g^{-1}	Cu μg g^{-1}	Ni μg g^{-1}	Pb μg g^{-1}	Zn μg g^{-1}	References
Oualidia lagoon	0.24 (0.13)	0.09 (0.06)	2.0 (1.3)	6.8 (2.5)	1.7 (0.7)	1.0 (0.5)	115 (30)	This study
Khnifiss lagoon	0.11 (0.04)	1.0 (0.2)	2.1 (0.5)	8.8 (3.8)	3.1 (0.6)	3.0 (1.1)	94 (44)	
UK estuaries	–	–	–	28–1124	–	5.9	155–199	[30]
UK estuaries		0.1–3.6					130–350	[24]
UK estuaries	–	0.03–10	0.1–10	10–1430	0.6–15	0–1190	91–510	[15]
Bou Regrag estuary	–	–	46-83	53	–	–	555–654	[11]
Nirbioi estuary	–	0.1–0.2	–	12–25	1.5	0.1	100–200	[23]
Bidasoa estuary	1.5 (0.6)	0.1 (0.2)	0.4 (0.3)	21 (13)	5.4 (2.2)	2 (1.1)	172 (14)	[34]
Plentzia estuary	0.1 (0.2)	0.1 (0.1)	-	12 (3.2)	1.3 (1.2)	0.3 (0.5)	136 (19)	[23]
Urdaibai estuary	–	0.1–1.7	0.1–5	6.3–39	1.3–7	0–10	25–300	[22]

Table 6. Biota-sediment accumulation factors (BSAF = concentration in tissues/concentration in sediment) at Oualidia and Khnifiss lagoons.

	Ag	Cd	Cr	Cu	Ni	Pb	Zn
Oualidia	0.4 ± 0.4	0.2 ± 0.2	0.03 ± 0.02	0.4 ± 0.2	0.06 ± 0.04	0.1 ± 0.2	1.1 ± 0.6
Khnifiss	0.4 ± 0.4	2.5 ± 1.2	0.03 ± 0.01	0.5 ± 0.3	0.09 ± 0.04	0.5 ± 0.5	1.4 ± 0.8

clean sites versus contaminated sites. Our study equated tissue enrichment with valency, which leads these metals to form complex ligands mainly sequestered by soluble low-molecular-weight proteins in the cytosol. This was particularly relevant for Cd, where bioaccumulation in worms from the Khnifiss lagoon in the absence of any known anthropogenic inputs was unexpected, and could be related to the permanent upwellings in this region of the Atlantic Ocean, as suggested by various authors [3,26,27].

The results of this study showed no significant correlations between total metal concentrations in sediment and in *Nereis diversicolor* ragworms (Table 7) and hence corroborate previous statements. The absence of additional metal bioaccumulation in the presence of very high metal concentrations in sediment has been observed elsewhere [23,28]. Although *Nereis diversicolor* is recognised as a good indicator of Ag, Cd, Zn Cr, Cu and Pb contamination, reflecting the

Table 7. Pearson correlation coefficients between sediment and worm heavy metal concentrations in the Oualidia and Khnifiss lagoons.

	Sediment *vs* worms			
	Oualidia lagoon		Khnifiss lagoon	
	p	r	p	r
Ag	0.25	−0.34	0.29	−0.30
Cd	0.6	0.15	0.57	−0.16
Cr	0.87	−0.05	0.79	0.08
Cu	0.19	0.37	0.33	−0.28
Ni	0.84	−0.06	0.12	−0.43
Pb	0.72	0.1	0.39	−0.25
Zn	0.36	−0.26	0.28	−0.31

bioavailability of metals in sediments [10,29], Ag and Cd concentrations are, in some cases, more accurately correlated with those of overlying waters [29]. Moreover, polychaete has been shown to regulate Zn concentrations [30].

The low bioaccumulation of metal in *N. diversicolor* in both lagoons could be related to the capacity of polychaetes to control metal incorporation from contaminated sediment. The same result was found by [31], who suggested that the presence of mucus-lined burrows and the feeding habits (superficial deposit feeder) of the nereid *Neanthes arenaceodentata* may reduce actual exposure of this species to contaminants in the sediment compared to other infaunal species with different food habits. *Nereis diversicolor* is a hemisessile polychaete living in galleries aerated by water fluxes generated through body movement. During burrowing, mucous secretions are released from glands covering the entire surface of the epidermis and are pushed against the walls of the burrow, thus consolidating it [32]. *Nereis diversicolor* creates a burrowing environment that may be buffered against surrounding sediment pollutants by the mucous layer, as previously shown. The worm may also benefit from its high ventilation rate, which increases its general tolerance to extreme environmental conditions [33,34].

Apparently, *N. diversicolor* is affected by concentrations of Ag, Cd [29] and even Cu [35] in overlying waters as well as in sediment. In fact, parapodia, where most gas exchanges take place, appear to be the favourite sites of metal uptake for this species. The potential threat of exposure to polluted sediment particles via ingestion can be circumvented using a dominant mucus-bag mode of feeding in which *N. diversicolor* is able to strain food from the respiratory current of its burrows. These galleries are indeed sites for active and rapid decomposition of organic material, since micro organisms tend to concentrate around the redox chemocline.

4. Conclusion

The joint use of sediment and *Nereis diversicolor* to study trace metal contamination in coastal lagoons demonstrated the complementarity of both indicators. Monthly sampling enabled a comprehensive assessment of changes in seasonal metal levels in *N. diversicolor* in the two lagoons. Oualidia lagoon sediment was shown to be slightly contaminated by two trace metals (Cd, Zn) and more highly contaminated by Ag, as a result of industrial and urban discharges, plus discharges from nearby phosphate ore processing plants. Conversely, higher levels of Ni were observed at Khnifiss, probably due to the natural composition of its rocky substrate. A difference in metal bioavailability was observed for *Nereis*, whose relatively high Cd, Pb and Ni levels in the Khnifiss lagoon may either be related to the vicinity of an oceanic upwelling (as for Cd) or to the predominance of schistous rocks.

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