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RESEARCH ARTICLE

THE USE OF FOUR MOLLUSCAN BIVALVES TO ASSESS THE PRESENCE OF CADMIUM AND LEAD AND THEIR DISTRIBUTION IN KHNIFISS LAGOON

R. CHFIRI[1], S. BENBRAHIM[1], S. SOUABI[2], H. BAÂLI[1], M. BAUDU[3]

1. Institut National de Recherche Halieutique, Bd Sidi Abderrahmane 2 Ain Diab, Casablanca, Morocco

2. Faculté des sciences et Techniques, Route de Rabat, Mohammedia, Morocco

3. Université de Limoges, Groupement de Recherche Eau Sol Environnement (GRESE), Faculté des Sciences & Techniques, 123 avenue Albert Thomas, 87060 Limoges (France)

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Corresponding Author

R. Chfiri

Abstract

The aim of this study was to investigate the presence and distribution of two potentially toxic trace metals cadmium (Cd) and lead (Pb) in a prominent ecosystem for aquaculture which is Khnifiss lagoon in southern coast of Morocco.

Concentrations of Cadmium (Cd) and Lead (Pb) were examined in four species of bivalves; two transplanted species: Oysters (Crassostrea Gigas), mussels (Mytilus Galloprovincialis) and two native species: Clams (Ruditapes Decussates) and Cockles (Cardum Edule) collected from 5 stations covering the entire Khnifiss Lagoon from June 2006 and July 2007, in order to assess the presence, distribution and seasonal variation of both cadmium and lead. The sampling was conducted monthly to take into account variations into season and between seasons.

The significant value of cadmium concentration was recorded in oysters near the lagoon inlet (station S1)1.30±0.41 mg/kg w.wt. The highest significant value of lead, of1.36±0.30 mg/kg w.wt, was recorded at station S3 in the middle of the lagoon. Oysters and mussels accumulate much more cadmium than clams and cockles (oyster>mussel>>clam>cockle), but for lead the order was reversed (clam>cockle>>mussel>oyster).

A statistical analysis indicated that the cadmium level is affected by both "station" and "species" factors, while lead is affected only by the "species" factor.

INTRODUCTION:

Estuarine and coastal ecosystems are among the most widely used and threatened natural systems in the world (Halpern et al, 2008; Lotze et al, 2006; Worm et al, 2006). Their degradation due to human activities is significant and growing (Waycott et al, 2009; FAO 2007; Orth et al, 2006; UNEP, 2006; MEA 2005; Valiela et al, 2001). The impact of anthropogenic disturbance is most strongly witnessed in estuarine and coastal environments adjacent to urban areas (Nouri et al., 2008). As a matter of fact, coastal areas, including estuaries, bays, shorelines, lagoons and continental shelves, are used intensively and receive the by-products of inland human activities. The human impact on these productive and economically important environments has become a major concern (Cearetta et al., 2000).

Coastal ecosystems are subjected to various entropic pressures given the concentration of human population in the vicinity and the introduction of both household and industrial effluents into the aquatic system (Miranda, 2002). Among all classes of contaminants released into the environment by human activities, trace metals can potentially be toxic; in consideration of their bioaccumulation and biomagnification capacities, the study of trace metals has been assigned priority (Braga, 2002).

The main threats to human health from metallic elements are associated with exposure to lead, cadmium, mercury and arsenic (arsenic is a metalloid but usually classified as a metal). The accumulation and distribution of these metals in soil and aquatic environments are increasing at alarming rates and thereby adversely affecting marine life (Mohiuddin et al, 2010; Okafor and Opuene, 2007; Koukal et al, 2004).

To assess trace metal contamination in the marine environment, different types of organisms may be used, such as seaweed and filter-feeding molluscs (Rainbow, 2002). Benthic molluscs are the organisms most often used in the biomonitoring of metal contamination (Silva et al, 2006; Shulkin et al, 2003). Bivalve molluscs are able to accumulate trace metals, up to several orders of magnitude higher than levels found in their environment (Rainbow, 2002). These organisms (i.e. filter feeders) accumulate most contaminants at much higher levels than those found in the water column and, as such, are representative of the pollution in a given area. For this reason, they serve to monitor the quality of coastal waters (Rainbow, 2002). Beyond their widespread distribution over coastal areas, these species possess additional characteristics of ideal biomonitors, namely they are sedentary and easy to sample (Szefer et al, 2002).

The Khnifiss Lagoon, located on Morocco's southern Atlantic coast far from any anthropogenic activity, is designated as a biological and ecological reserve; it is a potential site for aquaculture and tourism projects provided plans address the site's ecological sensitivity. The large surface area and high biological production of the avifauna qualify it among the four sites protected under the RAMSAR convention for wetland conservation. The main objective of this work is to determine the presence, ranges and variations of two potentially toxic trace metals (Cd and Pb) in the soft tissue of four molluscan bivalve species from the Khnifiss Lagoon,in order to estimate the health risks for consumers of this seafood variety.

2. Materials and methods:

2.1 Description of the study area:

The Khnifiss Lagoon (20 km long with a 65-km^2 surface area) is located between Tantan and Tarfaya (28°02'54" N, 12°13'66" W). It is connected to the Atlantic ocean via a narrow inlet called "Foum Agouitir", about 100 m wide. The lagoon continues upstream beyond a salt marsh, called "Sebkha Tazra". It is characterised by a wide diversity of habitats and high biological productivity thanks to the influence of upwelling in ocean waters. Lying in the middle of the coastal Sahara region, it is a vital stopover for migratory birds using the East Atlantic Flyway. The large temporary pool at La'wina, bordered by cliffs and bright sand dunes, is actually the bed of the Awedri wadi (or gulley), which over a few dozen meters of its mouth is occupied by dunes that are rarely penetrated by river water.

2.2 Sampling period and study sites:

Our study concerns four molluscan species: two of these have been transplanted using a caging technique; oyster (Crassostrea Gigas) and mussel (Mytilus Galloprovincialis), while the other two are native: clam (Ruditapes Decussates) and cockle (Cardum Edule). As shown in Figure 1 and explained in Table 1, five stations were selected in the three respective zones described herein (Organization and functioning of a Moroccan ecosystem: the Khnifiss Lagoon, by Lakhdar et al, 2011. Two sampling stations were selected in each of Zone I (S1, S2) and Zone II (S3, S4), plus one station in Zone III (S5). Sampling was conducted monthly between July 2006 and June 2007.

Bivalve mollusks, such as mussels and oysters are characterized by their aptitude to concentrate both metals and organic contaminants, their immobility, their limited ability to metabolize accumulated contaminants, their abundance, their persistence, and their ease of collection They have become recognized bio-monitors of pollutants in coastal waters and have been used in different international monitoring programs such as the Mussel Watch (USA) and the RNO (France) (Yungkul et al, 2008; Rocher B et al, 2006; Stellio and Cédric, 2006)

2.3 Sampling and processing methods:

The bivalves were transferred to the laboratory into basins filled with filtered seawater from the sampling sites and allowed to flush out undigested matter for a period of 24 h, according to the method described by Sokolowski et al, 2002. The whole soft tissues of 20 individuals from each location were carefully removed by shelling the bivalves with a stainless steel knife and then rinsing them with deionised water in order to remove sediment adsorbed to the tissue surface. Next, they were freeze-dried until obtaining a constant weight (at least 72 h), ground, homogenised and stored in labelled, cleaned glass bottles until analysis.

2.4 Analytical procedures:

To analyze Cadmium and Lead, wet digestion procedure have been used, it consisted of organic matter decomposition performed as described in the AOAC Method (1999, revised in 2002): 0.20-0.30g aliquots of ground sample were digested in Teflon bombs with 5 ml of HNO_3 and 2 ml of hydrogen peroxide; they were then heated in a microwave digestion system (MARS5, CEM) for 25 min in two steps to reach 200°C. After cooling, the resultant solution was diluted to 50 ml with ultrapure water.

The reagents for cleaning treatments and wet digestion were of analytical grade. The water used to clean the glass and plastic ware, prepare standard stock solutions and dilute samples was ultrapure, i.e. produced with a Milli-Q

Water Purification System (Millipore). Glass and plastic ware used for the tissue analysis were cleaned with a 10% solution of nitric acid for 24 h and then rinsed with ultrapure water to minimise the possibility of contamination.
Cadmium and lead were analysed by graphite furnace atomic absorption spectrophotometry (Shimadzu, Model AA 6800). Quality assurance focuses on controlling blanks, and the validity of these analytical methods was verified against standard biological reference materials (Mussel tissue sample, NIST 2976, Institute for National Measurement Standards, Ottawa, ON, Canada).

3. Results and Discussion:

Seasonal cadmium and lead concentrations (in mg/kg wet weight) in soft tissue of various bivalves species from the Khnifiss Lagoon are presented in Table 3. These data are expressed as average ± standard deviation (SD) and mini-maxi values.

3.1 Cadmium:

3.1.1 Spatial variation of cadmium:

To study the spatial variation of cadmium in the lagoon, oysters (Crassostrea Gigas) present at all five stations were used. The cadmium content in oyster soft tissue at the five stations with seasonal variation are listed in Table 3 and shown in Figure 2.

These results indicate that high concentrations were observed most often in autumn, for all stations. We noticed also a clear trend of decrease in the concentration of cadmium from S1 to S5 for all seasons. Sometimes the values for the stations S1, S2 and S3 reach or exceed the standard limit set by the European Commission for consumer health protection (i.e. 1 mg/kg w.wt), while the values for S4 and S5 are below the limit.

3.1.2 Cadmium comparison between species:

The combined presence at S1 and S3 of the four species (oyster, mussel, clam and cockle) at the same time allows drawing comparisons of their accumulation power (Figure 3).
Figure 3 clearly shows that cadmium accumulation follows the order: oyster>mussel>>clam>cockle. Cadmium accumulation in oysters and mussels (transplanted species relying on a caging technique - in this case water column bioindicators) is much greater than in clam and cockle sediment bioindicators.

3.2 Lead:

3.2.1 Spatial variation of lead:

Like for cadmium, the spatial variation of lead in the lagoon has been studied using oysters (Crassostrea Gigas). The lead content in oyster soft tissue at all five stations, with seasonal variations, are recorded in Table 3 and displayed in Figure 4.
Figure 4 indicates that lead concentration values gradually increase from S1 to S5 with peaks (high values) occurring in either winter or spring.
All values for all stations lie below the standard limit set by the European Commission for consumer health protection (i.e. 1.5 mg/kg w.wt).

3.2.2 Lead comparison between species:

Like for cadmium, Figure 6 shows that lead concentrations at both S1 and S3 are comparable for all four bivalve species (oyster, mussel, clam and cockle).

Results reveal a lead accumulation trend following the order: clam>cockle>>mussel>oyster. Lead accumulation in clams and cockles is much higher than in mussels and oysters.

3.3 Statistical analysis:

The effects of the various factors (site, species, season and combination) were studied by both one-way and two-way variance analysis tests (ANOVA). Statistical analyses were also conducted using the SPSS 16 software (the significance level was set at $p< 0.05$).

Table 5 shows that for cadmium, both the station factor ($p = 0.036<0.05$) and species factor ($p = 0.000<0.05$) have significant individual effects on this element accumulation in bivalves. The season factor and combined factors (Station * Species,Station * Season andSeason * Species) have no significant effect on cadmium accumulation in bivalves. For lead, only the species factor ($p = 0.000<0.05$) has a potential effect on accumulation in bivalves.

3.4 Discussion:

This research had focused on the spatial and temporal distribution of two toxic trace metals (cadmium and lead) in the Khnefiss lagoon which is a particular marine ecosystem with prominent potential in tourism and aquaculture activities.

Cadmium is a non-essential and a toxic metal for an aquatic environment, which is released into and distributed in by industrial sources such as mining, refining of ores, and the plating process (Vidoo et al., 2000). Elevated concentrations of cadmium often constitute a threat to marine biota. In the human body, cadmium may accumulate due to food chain magnification and induce kidney dysfunction, skeletal damage and reproductive deficiencies (Commission of the European Community, 2001).

Lead is a toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. It is a cumulative toxicant that affects multiple body systems, including the neurological, haematological, gastrointestinal, cardiovascular and renal systems. Children are particularly vulnerable to the neurotoxic effects of lead, and even relatively low levels of exposure can cause serious and, in some cases, irreversible neurological damage (Fewtrell et al, 2003; IPCS, 1995).

Lead is widely reported as a notable contaminant in the marine environment (Clark, 2001; Laws, 2000). Marine contamination by this non-essential metal is mainly due to releases from anthropogenic sources, and the atmosphere constitutes the primary transport vector towards the oceans (Laws, 2000). Lead is bioaccumulated by living organisms and, more specifically, by marine invertebrates (Neff, 2002; Temara et al, 1998).

We studied first cadmium and lead distribution via oysters (Crassostrea Gigas) that are present at all stations and the results show that Cd accumulation in oysters' changes across the various points depending on the season. The accumulated concentration during summer and autumn however reveals high values. Lead accumulation varies with both location and season. The greatest amount was detected at S5 during spring.

Comparing metal accumulation in oysters (Crassostrea Gigas), mussels (Mytilus Galloprovincialis), clams (Ruditapes Decussates) and cockles (Cardum Edule), it has been found significant interspecific differences, due to the trophic and ecological features of each species.

Indeed, for cadmium the uptake by the 4 species present in stations S1 and S3 shows that oysters have the highest concentrations compared to the other species, whereas clams display the lowest relative concentration. The respective accumulation rates among species can be classified in the following order: oyster > mussel > cockle > clam.

For lead, the concentration accumulated in clams is higher than the other species, according to the following ranking: clam > cockle > mussel > oyster. Moreover, it can be noted that the clam species accumulates the lowest Cd concentration and highest Pb concentration.

In the case of bivalves, physiological processes of the organism, as well as, environmental factors influence the rate of bioaccumulation (Shulkin et al, 2003; Okazaki and Panietz, 1981).

Living organisms exhibit a certain amount of selectivity in the accumulation of metals (Oros and Gomoiu, 2007). Oysters may bioaccumulate metals from seawater due to their high filtration rates. Cd is likely to be taken up incidentally, either in particulate form (suspended mineral and organic particles or phytoplankton) or dissolved form (simple hydrated metal ions). Levels of bioaccumulation of Cd in oysters can be influenced by environmental conditions (e.g., salinity and temperature), chemical and physical characteristics of the metal, and the growth and physiology of the oysters. Food (i.e., phytoplankton and other organic particles) is thought to be one of the most likely sources of Cd in oysters (Fisheries and Oceans Canada, 2010).

This study on the effects of environmental variables and assimilation efficiency of metals showed the importance of factors such as food quantity, composition and concentration of metals in food.

A comparative study of cadmium accumulated by oysters is given in Table 5. Our results are comparable to the concentrations observed in many places across the world.

4. Conclusion:

The result of this study reveals that oysters and mussels transplanted into the Khnifiss Lagoon accumulate the cadmium element to an extent (i.e. average of 1 ppm) that could affect and might cause adverse health impacts to consumers (meanwhile, the consumption of clams or cockles would be safe). Chemical speciation of cadmium in seawater and weaker complexation reactions with the solid phase may explain this accumulation in oysters and mussels but not in clams or cockles.

Although this lagoon is far from being exposed to industrial sewage and urbanisation, we found cadmium in high proportions, though the concentrations decrease from the inlet towards the interior of the lagoon. We are suggesting that the Atlantic Ocean could be the source of this enrichment with the influence of coastal upwelling.

The presence of lead lies below the allowed threshold, with low level amounts for oysters and mussels and a substantial presence, yet still under the limit, for clams and cockles. These two latter species are in direct contact with sediment that may contain lead.

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Tables:

Station	Samples	Coordinates	
S1	Oyster, Mussel, Clam and Cockle	28°02.361’ N	12°14.403’ W
S2	Oyster	28°01.961’ N	12°14.395’ W
S3	Oyster, Mussel, Clam and Cockle	28°01.896’ N	12°16.147’ W
S4	Oyster	28°01.601’ N	12°16.756’ W
S5	Oyster	28°00.622’ N	12°17.063’ W

Table 1: Sampling station coordinates and corresponding sampling

	Cd µg/g	Pb µg/g
Reference material certified value	0.82 ± 0.16	1.19 ± 0.18
Reference material test value	0.80 ± 0.23	1.23 ± 0.35
Recovery (%)	97.61	103.36

Table 2: Comparative results of certified reference material, NIST 2976

Metal		Cadmium				Lead			
Station	**Species**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**
S1	Oyster	1.30±0.27	1.30±0.41	1.00±0.12	1.03±0.21	0.17±0.11	0.15±0.07	0.23±0.13	0.22±0.05
		1.00-1.48	1.05-1.78	0.99-1.13	0.90-1.28	0.10-0.30	0.09-0.23	0.15-0.39	0.18-0.28
	Mussel	0.76±0.45	0.81±0.03	0.89±0.11	0.94±0.25	0.18±0.13	0.24±0.06	0.28±0.15	0.26±0.06
		0.53-1.28	0.78-0.84	0.79-1.01	0.68-1.18	0.11-0.33	0.18-0.30	0.18-0.45	0.20-0.32
	Clam	0.23±0.06	0.24±0.10	0.24±0.11	0.18±0.06	0.93±0.20	1.14±0.44	1.13±0.59	0.57±0.24
		0.17-0.29	0.11-0.33	0.12-0.34	0.13-0.25	0.78-1.15	0.69-1.48	0.50-1.66	0.40-0.84
	Cockle	0.44±0.15	0.38±0.08	0.42±0.15	0.40±0.07	0.21±0.11	0.23±0.07	0.39±0.17	0.28±0.13
		0.28-0.55	0.30-0.47	0.26-0.53	0.35-0.49	0.14-0.34	0.28-0.32	0.28-0.58	0.12-0.38
S2	Oyster	1.19±0.19	1.25±0.40	0.94±0.09	1.03±0.23	0.14±0.05	0.14±0.08	0.25±0.21	0.19±0.03
		1.01-1.18	0.88-1.68	0.84-1.02	0.79-1.24	0.09-0.14	0.06-0.23	0.13-0.50	0.16-0.22
S3	Oyster	0.94±0.44	1.03±0.23	0.88±0.11	1.06±0.20	0.31±0.17	0.15±0.06	0.16±0.03	0.25±0.12
		0.65-0.73	0.86-1.30	0.80-1.01	0.90-1.29	0.11-0.37	0.08-0.19	0.13-0.19	0.16-0.39
	Mussel	0.45±0.25	0.84±0.23	0.70±0.19	0.78±0.15	0.34±0.07	0.18±0.05	0.23±0.06	0.44±0.21
		0.34-1.27	0.59-1.05	0.58-0.92	0.68-0.95	0.27-0.41	0.13-0.24	0.19-0.29	0.22-0.63
	Clam	0.11±0.07	0.20±0.03	0.15±0.05	0.26±0.04	0.70±0.28	0.72±0.36	0.68±0.35	1.36±0.30
		0.06-0.20	0.13-0.25	0.10-0.26	0.24-0.30	0.48-1.02	0.22-0.97	0.18-0.93	1.26-1.70
	Cockle	0.31±0.04	0.32±0.11	0.39±0.04	0.47±0.05	0.28±0.10	0.26±0.20	0.50±0.14	0.83±0.12
		0.27-0.34	0.20-0.40	0.35-0.44	0.42-0.51	0.17-0.39	0.11-0.48	0.38-0.65	0.70-0.94
S4	Oyster	0.78±0.20	1.05±0.28	0.78±0.07	0.98±0.07	0.24±0.13	0.21±0.09	0.34±0.10	0.33±0.16
		0.66-1.01	0.83-1.37	0.71-0.84	0.92-1.05	0.11-0.39	0.14-0.31	0.24-0.44	0.23-0.52
S5	Oyster	0.62±0.27	0.90±0.43	0.71±0.03	0.89±0.16	0.17±0.12	0.20±0.06	0.22±0.07	0.39±0.05
		0.43-0.93	0.59-1.40	0.68-0.73	0.72-1.03	0.09-0.38	0.15-0.27	0.16-0.29	0.33-0.42

Table 3: Seasonal and spatial variations of cadmium and lead
results are expressed as averages and mini-maxi, in mg/kg wet weight

Elemnt	Variation origin	DF	F	p
Cadmium	Station	4	2.856	0.036
	Species	3	1.465	0.000
	Season	3	0.035	0.844
	Station * Species	3	0.011	0.459
	Station * Season	12	0.013	1.00
	Season * Species	9	0.015	0.708
Lead	Station	4	1.267	0.299
	Species	3	0.633	0.000
	Season	3	0.105	0.25
	Station * Species	3	0.02	0.665
	Station * Season	12	0.032	0.967
	Season * Species	9	0.02	0.761

Table 4: Results of one-way and two-way ANOVA

DF: Degrees of freedom; F: Statistic value of the Fisher;

p: Significance (when $p<0.05$, the factor has a significant effect)

Location	**Oyster cadmium levels (mg/kg wet weight)**	**Reference**
Khnifiss Lagoon	Average: 0.98 Range: 0.4 - 1.5	This study
Alaska	Average: 2.2 Range: 1.6 - 4.0	(Stupakoff et al, 2007)
BC	Range: 1.5 - 3.5	(Bendell et al, 2009)
BC	Range: 1.2 - 3.6	(Lekhi et al, 2008)
Oregon	Average: 1.3 Range: 0.7 - 2.0	(Stupakoff et al, 2007)
Washington	Average: 1.2 Range: 0.4 - 2.5	(Stupakoff et al, 2007)
California	Average: 0.6 Range: 0.4- 0.8	(Stupakoff et al, 2007)
Hong Kong	Average: 0.7	(Amiard et al, 2008)
France	Range: 0.04-0.7	(Amiard et al, 2008)
England	Average: 0.2	(Amiard et al, 2008)

Table 5: Cadmium concentration in oysters from different coasts around the world in mg/kg wet weight

Figures:

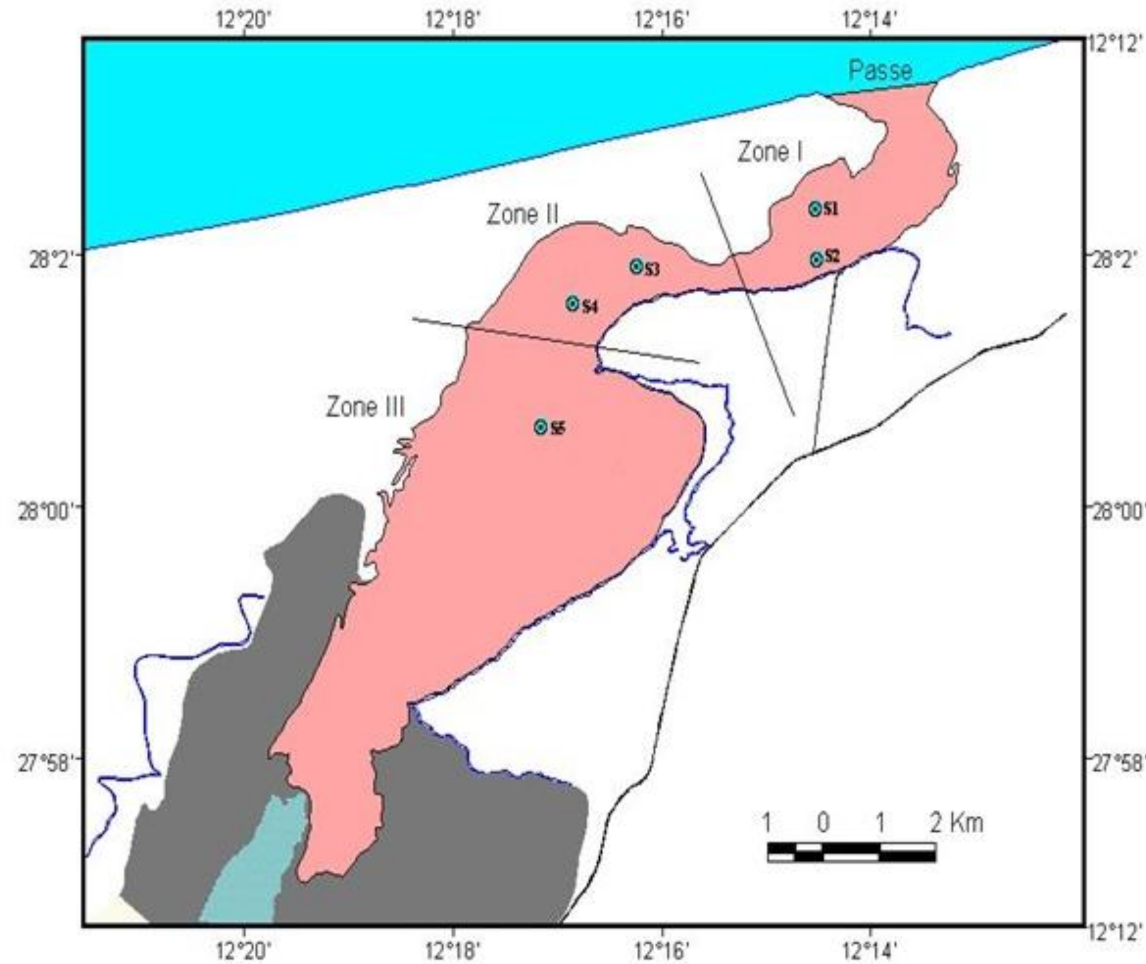

Fig 1: The location of sampling stations in the Khnifiss Lagoon and their distribution

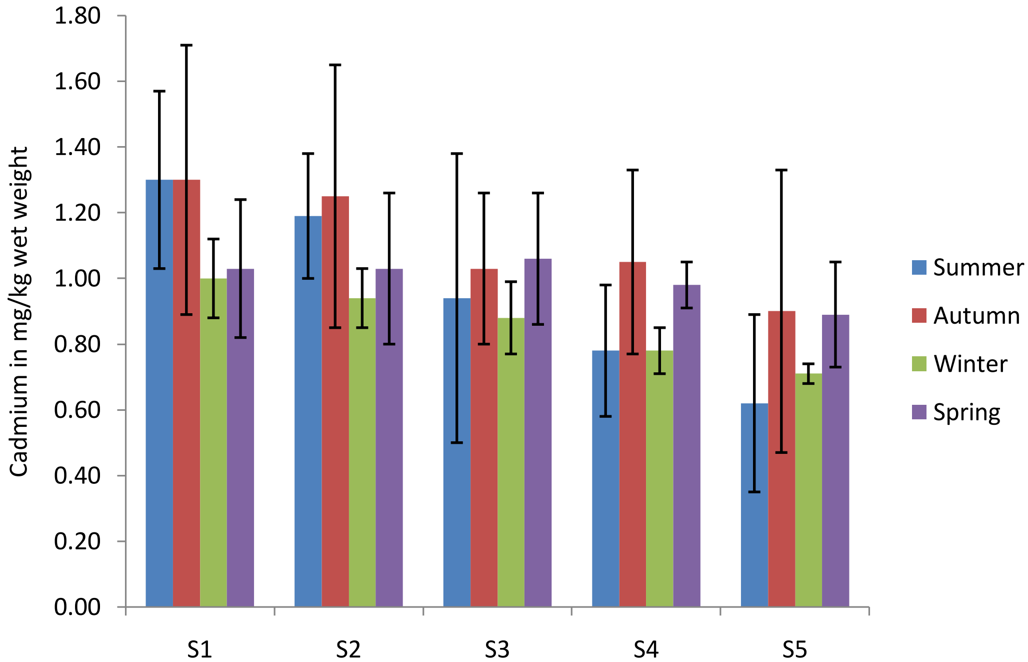

Fig 2: Seasonal and spatial variations of cadmium concentration in oyster samples - Results are expressed as a mean ± SD and mini-maxi, in mg/kg wet weight

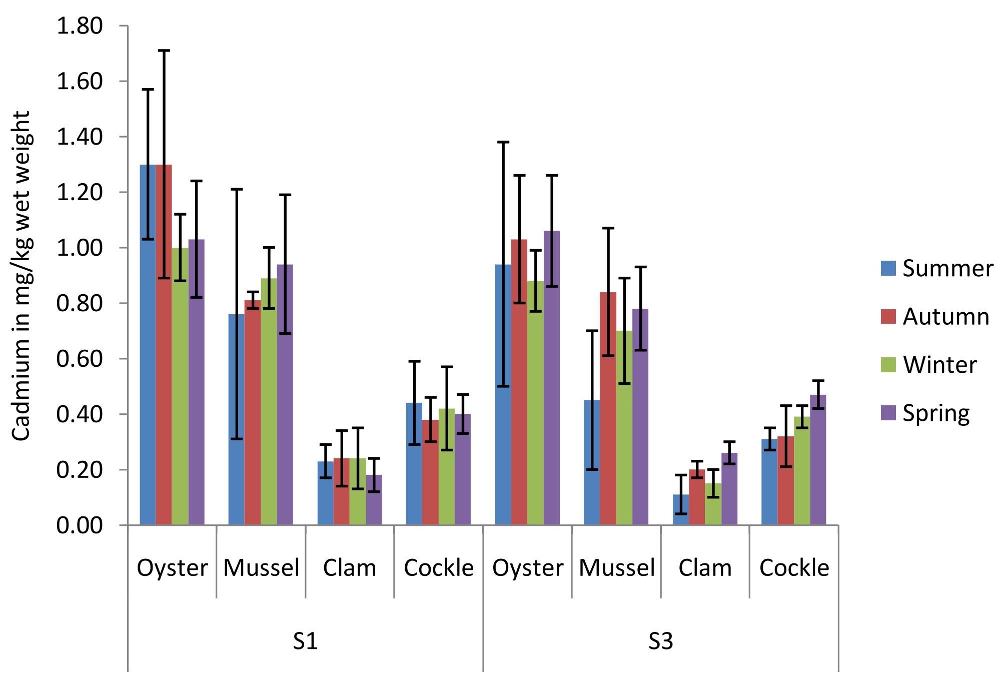

Fig 3: Comparison of cadmium levels in different bivalves at Stations 1and 3 - Results are expressed as a mean ± SD in mg/kg wet weight

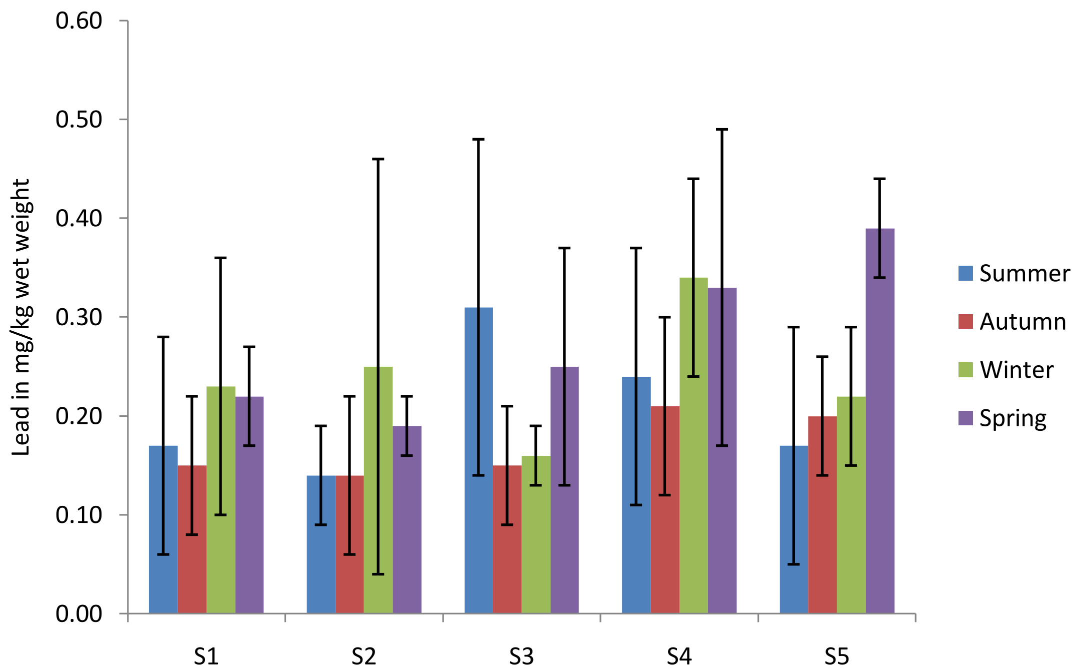

Fig 4: Seasonal and spatial variations of lead concentration in oyster samples - Results are expressed as a mean ± SD in mg/kg wet weight

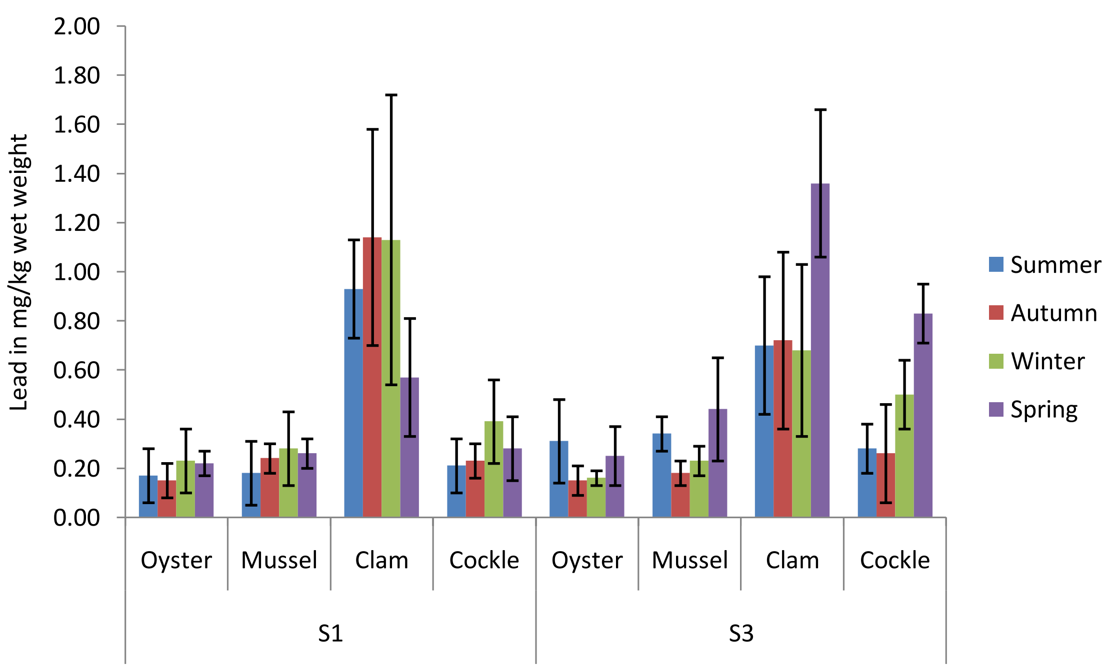

Fig 5: Comparison of lead levels in different bivalves between Stations 1 and 3 - Results are expressed as a mean ± SD in mg/kg wet weight