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Temporal changes in nickel and vanadium concentrations and in condition index and metallothionein levels in three species of molluscs following the "*Erika*" oil spill

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Abstract – The petroleum spilt by the tanker "*Erika*" contained environmentally high concentrations of nickel (45 mg kg^{-1}) and vanadium (83 mg kg^{-1}). Our aim was to show that nickel and vanadium concentrations in marine organisms could be used as tracers of their exposure to oil deposits along the coast. Two biomarkers were determined, condition index (CI) and metallothionein levels. Samples were collected monthly from January to May 2000 from five sites along the coast of Vendée and Loire Atlantique: (1) Lérat, (2) La Govelle, (3) Saint Gildas, (4) La Bernerie and (5) La Fosse. Among benthic invertebrates, mussels *Mytilus edulis* (filter-feeders), periwinkles *Littorina littorea* (grazing-feeders) and dogwhelks *Nucella lapillus* (carnivora, bivalve predators) were selected. In addition, mussels were collected from a control site, Fier d'Ars (Ré Island). The species chosen as bioindicators have responded to the presence of oil in their environment by accumulating nickel and vanadium. The bioaccumulation of vanadium occurred early one month after oil spill whereas nickel bioaccumulation was deferred, probably as a consequence of a lower stability of vanadylporphyrins compared to nickelporphyrins which are known in particular for their role in stabilizing emulsions (film at the water/oil interface). Interspecific differences may be explained by different food habits: periwinkles grazed contaminated algae; mussels as filter-feeders retained particles and colloids from the water column; dogwhelks fed on mussels. Spatio-temporal changes of nickel and vanadium concentrations may result from (i) the arrival of new oil slicks, (ii) the action of cleaning of the coasts contributing to the re-suspension of petroleum. In all of the three species, few changes of the CI were observed from site to site. CI variations were linked to sexual ripening in mussels. Mussels originating from the control site showed MT concentrations significantly lower than those in specimens from impacted sites. The highest MT concentrations were observed in January and February, and then a consistent decrease was registered in March and May. MT concentrations in periwinkles increased very significantly in March and May. An increase in MT concentrations was also shown at this period in dogwhelks. Depending on the species, positive correlations were shown between MT and nickel and/or vanadium concentrations.

Key words: Nickel / Vanadium / Condition index / Metallothionein / Molluscs / Mussel / *Mytilus edulis* / Dogwhelk / *Nucella lapillus* / Periwinkle / *Littorina littorea*

Résumé – Bioaccumulation du nickel et du vanadium, de l'indice de condition et de la métallothionéine chez trois espèces de mollusques, suite au naufrage de l'« *Erika* ». Le pétrole de l'« *Erika* » présentait des concentrations élevées en nickel (45 mg kg^{-1}) et en vanadium (83 mg kg^{-1}). Notre objectif était de montrer que les concentrations en nickel et en vanadium dans les organismes pouvaient rendre compte de leur exposition aux dépôts de pétrole sur le littoral. Deux biomarqueurs ont été également mesurés, l'indice de condition (IC) et les concentrations en métallothionéine. Les prélèvements ont été réalisés pendant quatre mois (janvier, février, mars et mai 2000) sur cinq sites du littoral de la Vendée et de la Loire Atlantique : (1) Lérat, (2) La Govelle, (3) Saint Gildas, (4) La Bernerie et (5) La Fosse et pour *Mytilus edulis* sur un site indemne de pollution, le Fier d'Ars (Ile de Ré). Les invertébrés benthiques retenus sont les moules *Mytilus edulis* (filtreurs), les littorines *Littorina littorea* (brouteurs) et les nucelles *Nucella lapillus* (prédateurs de bivalves). Les espèces indicatrices choisies (*Mytilus edulis*, *Nucella lapillus* et *Littorina littorea*) répondent à la présence d'hydrocarbures dans leur milieu par une bioaccumulation du nickel et du vanadium. La bioaccumulation du

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vanadium est précoce, un mois après le naufrage, celle du nickel intervenant plus tardivement probablement en raison d'une stabilité moindre des vanadylporphyrines comparées aux nickelporphyrines qui, notamment, stabilisent les émulsions (film à l'interface eau/pétrole). Les différences de bioaccumulation entre les espèces peuvent s'expliquer par leur régime alimentaire propre : la littorine broute les algues souillées, la moule filtre la colonne d'eau (particules, colloïdes), la nucelle perce les moules. Les évolutions spatio-temporelles des concentrations en nickel et vanadium peuvent s'expliquer par (i) l'arrivée de nouvelles nappes et (ii) les travaux de nettoyage favorisant la remise en suspension du pétrole. Les indices de condition (IC) varient peu pour les trois espèces entre les sites. Les seules observations sont des variations temporelles liées à la maturation sexuelle chez la moule. Chez la moule, les organismes du Fier d'Ars ont des concentrations en MT significativement plus faibles que celles des autres sites impactés. Les concentrations maximales en MT sont observées en janvier et en février puis nous notons une baisse sensible des concentrations en MT en mars et mai. Les concentrations en MT des littorines augmentent de façon nette et significative en mars et mai. Les concentrations en MT des nucelles augmentent également en mars et mai. Selon les espèces des corrélations positives sont relevées entre les concentrations de nickel et/ou de vanadium et celles de la métallothionéine.

1 Introduction

Following the sinking of the tanker *Erika* at the end of 1999, intertidal benthic organisms have been exposed to numerous pollutants, especially polycyclic aromatic hydrocarbons. Oil from the *Erika* also contains relatively high concentrations of nickel (45 mg kg^{-1}) and vanadium (83 mg kg^{-1}) (Tiercelin et al. 2000). Furthermore, some authors (Bu-Olayan and Subrahmanyan 1997) noted that nickel levels had increased considerably in molluscs following the Gulf War (1994). It thus seemed possible to draw a parallel between oil exposure and nickel and vanadium bioaccumulation.

Even though a large number of these pollutants are bioavailable and thus are bioaccumulated by organisms such as mussels, it is still not known whether this bioaccumulation leads to organism damage. To determine whether this is the case, a number of biomarkers of defence or damage are available (Lagadic et al. 1997, 2000; Amiard et al. 2000; Lafontaine et al. 2000). Among the markers of defence are metallothioneins, which are induced in relation to the environmental concentration of various metals, including nickel (Barka et al. 2001). Metallothioneins (MT) are cystein-rich, heat-stable proteins, which bind several metals (Amiard and Cosson 1997; Cosson and Amiard 2000). The use of MT as a biomarker of defence has only just been validated (Mourgaud et al. 2002) and has recently been the subject of bibliographical reviews, particularly in molluscs (Cosson 2000; Isani et al. 2000). Amongst the markers of damage, the most widely used is the condition index which indicates whether the nutritional health status of an individual is generally good or bad.

Our aim was therefore to test the hypothesis that the bioaccumulation of nickel and vanadium in various mollusc species could provide information concerning the exposure of these animals to oil spilled following the sinking of the *Erika* and to check whether the organisms exposed in this manner suffer any harmful effects.

2 Material and methods

2.1 Sampling

Samples were collected monthly from January to May 2000 from five sites along the coast of Vendée and Loire Atlantique: (1) Lérat, (2) La Govelle, (3) Saint Gildas, (4) La Bernerie and (5) La Fosse (Fig. 1). In addition, mussels were collected from Fier d'Ars, a site in the Ré Island, which had not been impacted by the oil spill. Among benthic invertebrates, mussels *Mytilus edulis* (filter-feeders), periwinkles *Littorina littorea* (grazing-feeders) and dogwhelks *Nucella lapillus* (carnivora, bivalve predators) were sampled. For each species, eight individuals were collected at each date and each site. The wet weight of soft tissue is 1.05 ± 0.34 g, 0.38 ± 0.11 g and 0.67 ± 0.28 g respectively for mussels, periwinkles and dogwhelks.

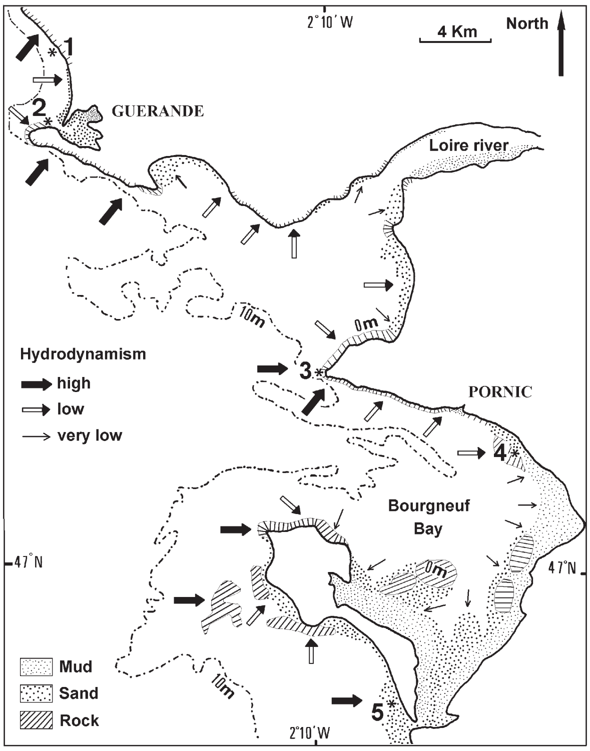

Fig. 1. Map of sampling sites on the South coast of Brittany (France) impacted by the oil spill: (1) Lérat, (2) La Govelle, (3) Saint Gildas, (4) La Bernerie and (5) La Fosse.

2.2 Determination of the condition index (CI)

The condition index (CI) was determined according to the recommendation of the French Association for Standardization (AFNOR, NF V45056, Sept. 1985):

$$\text{CI} = 100 \times \frac{\text{Drained weight of soft tissues}}{\text{Total weight}}.$$

This index is reliable (Bodoy et al. 1986) and is relevant for samples which must be kept wet for subsequent chemical and biochemical analysis.

2.3 Dissection and compartmentalization

The pre-treatment of samples devoted to metal and metallothionein analysis was carried out avoiding secondary contamination. All laboratory ware was soaked during 2 h in 10% HCl, rinsed three times with deionised water and dried in desiccators. Soft tissues were separated from the shell, using a scalpel for mussels and a hand vice for gastropods. Individuals were homogenized with an Ultra-Turrax in a buffer solution (20 mM Tris, 150 mM NaCl, 0.01 M ß-mercaptoethanol, 0.1 mM PMSF, pH 8.6). Then the homogenate was separated into two aliquots for metal analysis and metallothionein analysis.

2.4 Digestion and metal determination using flameless atomic absorption spectrophotometry (AAS)

To determine metal concentrations, it was necessary to carry out a digestion of shellfish homogenates using suprapur nitric acid (Carlo Erba) at 60 °C for 12 h minimum. The volume was then adjusted to 5 ml with deionised water. Ni and V were determined in these acid solutions by flameless AAS using Zeeman effect (Varian SpectrAA-880 GTA-100) according to the analytical method described by Amiard et al. (1987). This methodology was validated through international intercalibration exercises (Table 1). Moreover, internal quality controls were based on the analysis of the metals of interest in standard reference materials (DORM-1, NRC Canada or IAEA-407 for nickel, IAEA-140 for vanadium). Metal concentrations are expressed in mg kg^{-1} wet weight.

2.5 Determination of MT concentrations using Differential Pulse Polarography (DPP)

Soft tissues homogenates were separated between soluble (S1) and insoluble (P1) fractions by centrifugation (25 000 g for 55 min at 4 °C). The cytosolic heat-stable compounds including metallothioneins (S2) were isolated by centrifugation of the soluble fraction (12 000 g for 10 min) after heat-treatment (75 °C for 10 min). The amount of MT was determined in the heat-denaturated cytosolic fraction by differential pulse polarography, a technique based on-SH compound determination according to the Brdicka reaction (Brdicka 1933) as described by Thompson and Cosson (1984) and validated by Olafsson and Olsson (1991). The PAR Model 174 analyser, with the PAR/EG&G Model 303 static mercury drop electrode (SMDE) were used. The temperature of the cell was maintained at 5 °C. The standard addition method was used for calibration with rabbit liver MT (Sigma Chemical Co., St Louis, MO) in the absence of purified bivalve MT. MT concentrations are expressed in mg kg^{-1} wet weight.

Table 1. Results of internal and external quality controls of nickel and vanadium (Coquery et al. 2000, 2001).

Internal control	Ni (mg kg^{-1} dw)	V (mg kg^{-1} dw)
DORM-1		
Certified value	1.20 ± 0.30	
Our value	1.16 ± 0.11	
IAEA-140		
Certified value		3.67 ± 0.48
Our value		3.65 ± 0.53

External control		
IAEA 405		
Certified value	3.1 to 33.9	
Our value (Lab No. 55b)	33.7 ± 2.4	
Score Z*	0.3	

* Performance is acceptable if Z is <2.0.

2.6 Statistical analysis

Normal distribution and variance homogeneity for each group of data were checked using version 2.03 of the SigmaStat$^{\text{TM}}$ software. For data which were not normally distributed, normalising transformations were performed using the neper logarithm function. The inter-site variations of the measured variables (mean concentrations of nickel and vanadium, condition index and metallothionein concentration) were assessed by analysis of variance (ANOVA) and the critical differences between the sites were determined by the Scheffé F-test using StatView software (SE + Graphics$^{\text{tm}}$, version 1.03). Temporal variations (between two months) in mean concentrations of nickel, vanadium, metallothionein and condition index for organisms from the same site were assessed using the Student *t*-test with the SigmaStat$^{\text{TM}}$ software. Certain statistical analyses were superfluous due to vast differences between means.

3 Results

3.1 Comparison between sites

Nickel

The mean concentrations of nickel in mussels increased markedly between January and May (Fig. 2). The concentrations were particularly high at the sites located in the Bay of Bourgneuf (Pointe Saint Gildas, La Bernerie and La Fosse). Mussels from the control site (Fier d'Ars) had significantly lower concentrations of nickel than those from the sites affected by the oil from the *Erika*. Amongst the five sites impacted, the lowest concentrations of nickel were found in the organisms from the Lérat site.

Nickel concentrations also increased significantly in dogwhelks between January and May 2000 (Fig. 2). Mean concentrations were approximately 1.0 ± 0.5 mg kg^{-1} in January-February and 3.5±1.4 mg kg^{-1} in May. The most contaminated sites were La Bernerie, Lérat and Pointe Saint Gildas.

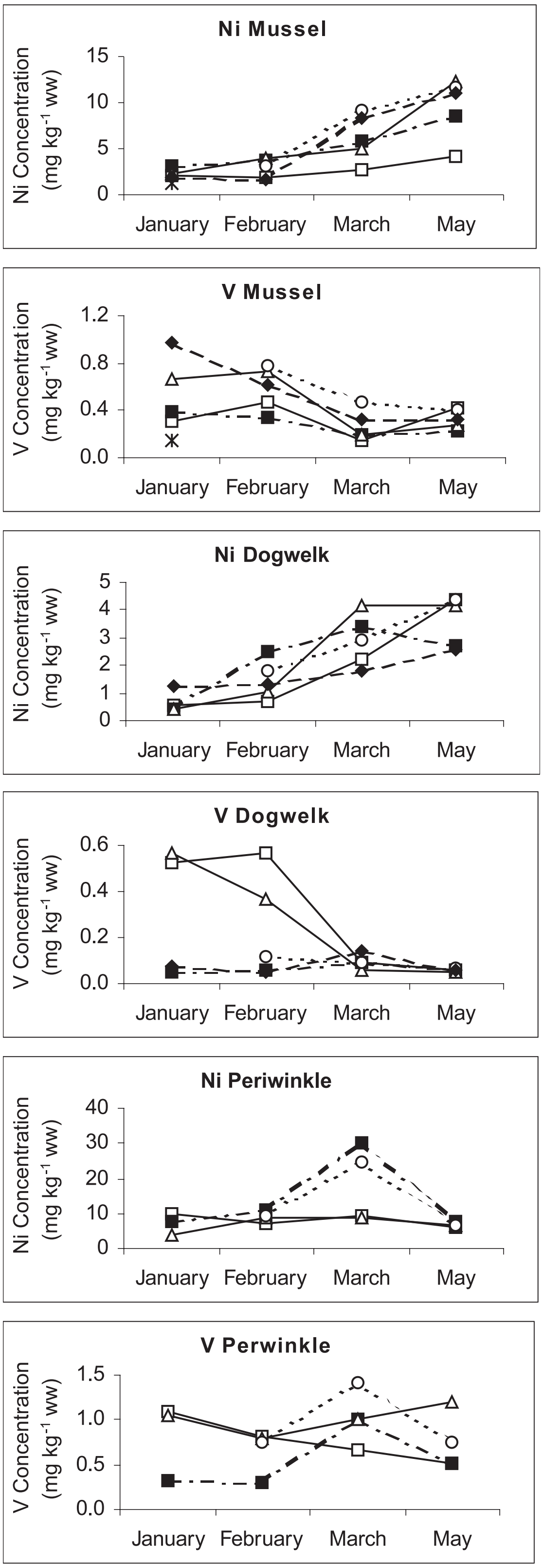

Fig. 2. Temporal variations of nickel and vanadium concentrations (means in mg kg^{-1} wet weight) in mussels (*Mytilus edulis*), dogwhelks (*Nucella lapillus*) and periwinkles (*Littorina littorea*) in various sites of Loire-Atlantique and Vendée. * Fier d'Ars, control; □— Lérat; ■- - – La Govelle; Δ— St Gildas; ○- - - La Bernerie; ♦– – La Fosse.

The periwinkles (Fig. 2) from the sites of La Govelle and La Bernerie were the most contaminated in March, with high nickel concentrations.

Vanadium

The concentrations of vanadium in mussels were very high at La Fosse and Pointe Saint Gildas in January and February and at La Bernerie in February (0.7 ± 0.3 to 1.0 ± 0.5 mg kg^{-1}, Fig. 2). These concentrations decreased between February and May albeit remaining higher than those at the control site of Fier d'Ars.

The mean vanadium concentrations in dogwhelks (Fig. 2) were maximal for organisms from the sites of Lérat and Saint Gildas in January and February (0.53 ± 0.24 and 0.57 ± 0.34 mg kg^{-1}). Then, the mean concentration in March and May was 0.07 ± 0.02 mg kg^{-1} with a maximum of 0.14 ± 0.04 mg kg^{-1} in March for organisms from La Fosse.

Vanadium concentrations in periwinkles tended to fluctuate (Fig. 2). Peak concentrations were observed in January at the Saint Gildas and La Fosse sites and in March at La Bernerie, La Govelle and la Pointe Saint Gildas, with a mean concentration of around 1.1 ± 0.2 mg kg^{-1}.

3.2 Comparison in the three studied species

The concentrations of the two elements nickel and vanadium were the highest in periwinkles (4.10 to 30.34 and 0.29 to 1.42 mg kg^{-1} respectively for Ni and V) (Fig. 2). Intermediate concentrations were detected in mussels (1.29 to 12.32 and 0.15 to 0.98 mg kg^{-1} respectively for Ni and V) whereas the lowest concentrations were found in dogwhelks (0.44 to 4.39 and 0.05 to 0.57 mg kg^{-1} respectively for Ni and V) (Fig. 2).

3.3 Variations in condition index (CI)

In mussels (*Mytilus edulis*), there was little difference between the sites in terms of mean CI values, except for CI of organisms from La Bernerie in March (14.3) which was significantly lower than those for organisms from Lérat, La Govelle and Saint Gildas (20.7 to 21.9) (Fig. 3). Temporal variations were also noted, the highest CI values were determined in March. In dogwhelks (*Nucella lapillus*), there was no difference between the mean CI values (14.7 to 20.2), except for those at La Govelle which had CI values significantly higher than the others (21.1 to 25.4). In periwinkles (*Littorina littorea*), the mean CI values were relatively constant (18.9 to 26.3), except for CI of specimens from La Bernerie which was lower in March (14.9).

3.4 Variations in metallothionein (MT) concentration

In mussels from Fier d'Ars MT concentrations were significantly lower than those from the impacted sites. Maximal MT concentrations were found in January and February and decreased considerably in March and May. There was little intersite variability, especially when the concentrations were the

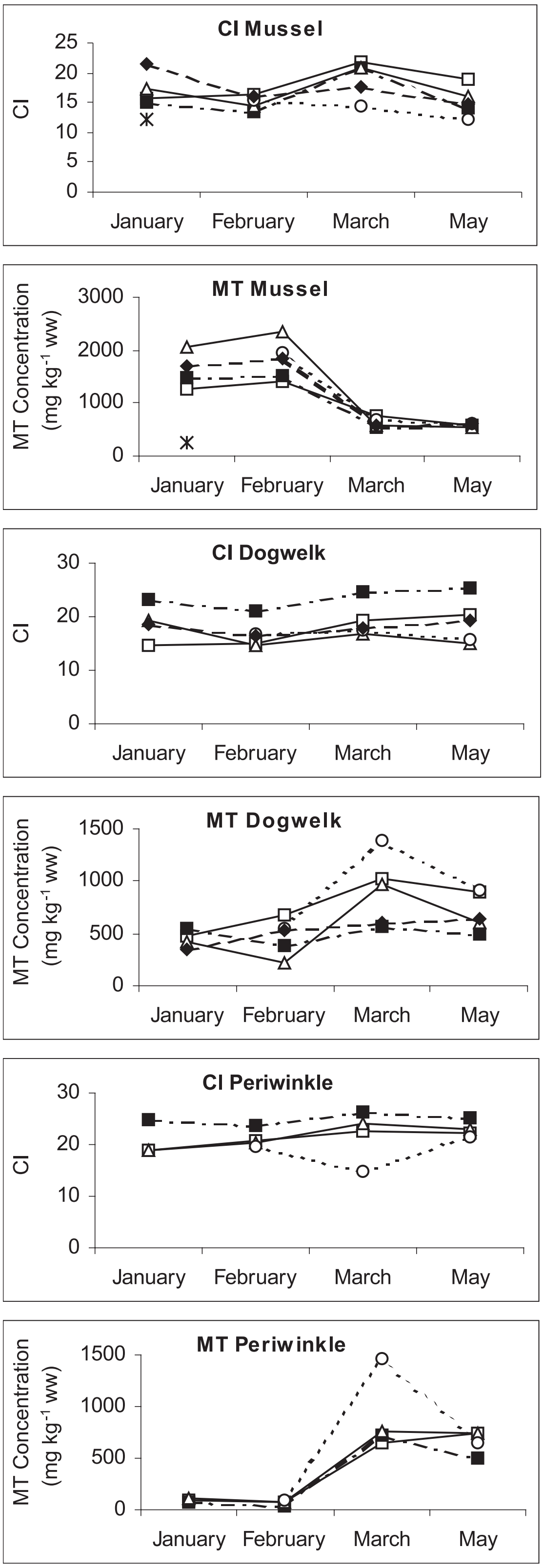

Fig. 3. Temporal variations of mean of condition index (CI) and metallothionein concentrations (means in mg kg^{-1} wet weight) in mussels (*Mytilus edulis*), dogwhelks (*Nucella lapillus*) and periwinkles (*Littorina littorea*) in various sites of Loire-Atlantique and Vendée. * Fier d'Ars, control; □— Lérat; ■– – – La Govelle; Δ— St Gildas; ○- - - La Bernerie; ♦– – La Fosse.

Table 2. Spearman rank order correlation between four parameters (nickel, vanadium, MT and condition index). n = number of samples; * significant with $p < 0.05$.

	V	MT	CI
Mussels (n = 145)			
Ni	−0.0729	**−0.351 ***	−0.101
V	1	**0.708 ***	−0.188
MT		1	−0.0733
Dogwhelk (n = 147)			
Ni	**−0.332 ***	**0.469 ***	0.00846
V	1	−0.155	**−0.242 ***
MT		1	−0.0414
Periwinkle (n = 122)			
Ni	0.136	0.0612	0.0107
V	1	**0.285 ***	**−0.204 ***
MT		1	−0.173

lowest in March and May. In dogwhelks, MT concentrations increased significantly in March and May. In periwinkles, MT concentrations increased markedly and significantly in March and May for all sites.

3.5 Correlations between the four variables studied

Correlation factors are shown in Table 2. Vanadium and nickel concentrations were not correlated in mussel and periwinkle tissues whereas, in dogwhelk, a negatively significant correlation was shown. Generally, the condition index was not correlated with either MT or metal concentrations, except with vanadium in both of the gastropod species, this correlation being negatively significant. MT concentrations correlated positively and significantly with those of vanadium in mussels and periwinkles, and those of nickel in dogwhelks. In mussels, a significant inverse correlation was observed between the levels of MT and nickel.

4 Discussion

Bioaccumulation of the two metals occurred in the following decreasing order: periwinkles (grazing-feeders) > mussels (filter-feeders) > dogwhelks (carnivore, bivalve predators). Given the fact that it was the dogwhek which represent the upper link that displayed the lowest concentrations of nickel and vanadium, there was no bioamplification.

The difference in bioaccumulation between periwinkles and mussels can be explained by their specific diets: periwinkles directly graze on algae soiled by oil, and mussels, by filtering suspended particulate matter from the water, are exposed indirectly to oil. These interspecific differences could also be explained by the mechanisms of intracellular detoxification of different metals: certain species privilege the insolubilisation of metals in the form of granules which are stored for life or regularly eliminated, others can link metals to intracellular proteins, the most common being metallothioneins (MT) (Viarengo and Nott 1993).

The similarity between mussels and dogwhelks in terms of nickel and vanadium bioaccumulation can be explained by their close trophic relationship. In fact, dogwhelks pierce holes in the shells of certain bivalves, especially mussels, in order to feed on them.

In the case of *Mytilus edulis*, nickel and vanadium bioaccumulation was found to be clearly higher in the organisms affected by the oil from the *Erika* than in those from the control site. In the case of *Littorina littorea* and *Nucella lapillus*, even though there were no corresponding controls, the temporal variations observed in these organisms suggest their contamination by vanadium and nickel present in oil. In mussels and dogwhelks, the two contaminants occurred at different time points, appearing in January–February for vanadium and in March–May for nickel. These peaks of pollution can be explained either by a rapid elimination of vanadium from the organisms or by a temporal difference in the bioavailability of each metal in the water column. The elimination of vanadium from soft tissue has not been reported in the published literature. We have noted (unpublished data) that such elimination from the soft tissues of mussels is extremely rapid (biological half-life of approximately 4 days). On the other hand, this element binds strongly to the shell and byssus of bivalves (Miramand et al. 1980; Miramand and Fowler 1998) and is then eliminated slowly. In periwinkles *(Littorina littorea)*, bioaccumulation of the two metals appears to be more temporally in concordance with the maximum values in March 2000. This is probably related to the fact that periwinkles directly graze on algae soiled by oil.

The maximum concentrations of nickel and vanadium that we measured at our sites are amongst the highest reported by various authors for sites contaminated by these metals but not necessarily due to oil pollution (Bryan et al. 1983; De Wolfe et al. 2000; Roux et al. 2001). Several hypotheses may be proposed to explain the spatiotemporal evolution of nickel and vanadium bioaccumulation: i) the arrival of new layers of oil due to persistent discharge from the wreck or to meteorological conditions; ii) the clean-up effort which results in the oil being put back into suspension and being re-deposited on the coast in a changed (mature) form.

The temporal degradation of oil from the *Erika* (fuel No. 2) is reputed to be slow but could vary considerably according to its location on the coast (the level in the intertidal area) and to the hydrodynamics of the site (sheltered or exposed). Furthermore, the clean-up effort has significantly altered the oil's structure, with a large proportion emulsifying and dispersing in the water column following the application of dispersents. The heavier constituents such as the asphatenes are known to degrade very slowly. These constituents contain porphyrines which bind very strongly to metals such as nickel and vanadium (Yen 1975). However, the finest emulsions bring these porphyrines into close contact with the sea water (Lee 1999). Vanadylporphyrines are less stable and more hydrophilic than nickelporphyrines due to the stronger polarity of the VO^{2+} ion. Moreover, nickelporphyrines are known to strongly stabilise emulsions by forming a film at the water/oil interface, which is not the case with vanadylporphyrines (Lee 1999). It can thus be surmised that vanadylporphyrines degrade more easily and that vanadium is released much more rapidly into the water column than nickel. Vanadium and then nickel, present in the seawater in their dissolved form or as fine colloids, could then bioavailable to mussels. This phenomenon also appears to apply to dogwhelks which become rapidly contaminated by vanadium and then subsequently by nickel. It can also be noted that the rapid bioaccumulation of vanadium coincides with the bioaccumulation of HAP in mussels, which is also very fast (Tronczynski et al. 2004).

The condition index (CI) displayed little variation with two exceptions. The first exception is the seasonal variation of this index in mussels which can be explained by the sexual cycle of the species under study. Indeed, sexual maturation takes place in January and February with breeding in March. Mussels then start to grow again and increase their size and weight of soft tissues. The mussels from the sites studied during the month of March must certainly have been sampled before breeding (high CI). The second exception is a low index value in periwinkles from La Bernerie in March. This can be linked to the high concentration of nickel, vanadium and MT, but probably also to breeding, which takes place in spring in this species, generally during the night of a high tide (Daguzan 1975). In dogwhelks, no index variation was noted. In this species and in these latitudes, ovulation takes place all year round with peaks in winter and spring (Fretter and Graham 1962).

In terms of the biochemical marker, we are certain that the thiol- groups measured by differential impulsion polarography in the heated cytosols of mussels and dogwhelks are indeed MT. In fact, in *Mytilus edulis*, nine isoforms of metallothionein have been sequenced (Mackay et al. 1993) and MT induction has been observed by both active and passive biomonitoring or in the laboratory in larvae and adults (Cosson and Amiard 1998; Geffard et al. 2002; Mourgaud et al. 2002). The presence of MT has also been detected in *Nucella lapillus* (Leung and Furness 2001) and in *Littorina littorea* (Bebianno et al. 1992; Leung and Furness 1999a).

The role of metallothioneins in metal detoxification in invertebrates is far from being negligible. In agreement with this role, metallothioneins were more concentrated *Mytilus edulis* taken from the impacted sites compared to those taken from a non-contaminated site. Few authors have measured MT in mussels in toto because often only a particular organ is retained (generally the gills or the digestive glands). In another study of the same species (*M. galloprovincialis* variety), transplanted into the Mediterranean at various sites, Mourgaud et al. (2002) considered that a value below 500 $mg\,kg^{-1}$ signified a clean zone. This was the case for the Fier d'Ars site (approximately 250 $mg\,kg^{-1}$). On the contrary, values above 650 $mg\,kg^{-1}$ were recorded for the polluted sites. All of our values were above this threshold for samples taken in January and February 2000 and between 500 and 650 $mg\,kg^{-1}$ in May 2000. Bebianno and Serafim (1998) measured high concentrations of metallothionein (780 to 2600 $mg\,kg^{-1}$) in the south of Portugal in a relatively polluted zone. According to Leung and Furness (1999b) normal values for *Nucella lapillus* are approximately 150 $mg\,kg^{-1}$. Our lowest values were similar to this concentration whereas our maximum values were nine times higher. In *Littorina littorea*, Bebianno et al. (1992) recorded mean values of 660 $mg\,kg^{-1}$, which is more than two fold lower than our maximum values. The high values of metallothionein concentration in the three species studied clearly indicate a metal contamination of the sites affected by the oil from the *Erika*.

In mussels and periwinkles, a relationship was observed between the concentrations of vanadium and MT. In January and February, organisms from the sites of Saint Gildas and La Bernerie displayed maximal concentrations of both MT and metals. However, given that no correlation has any significance in terms of the cause–effect relationship, one cannot conclude that vanadium induces MT. In effect, MT are known to be induced by other metals present in the environment, or by stress factors, which could in this case result from oil exposure. In *Nucella lapillus*, the concentrations of nickel appeared to be linked to MT concentrations. The opposite applies to mussels. Although the affinity between nickel and MT is known in vitro, only one experiment (Barka et al. 2001) in vivo has shown MT to be induced by nickel, in copepods (*Tigriopus brevicornis).*

This study was carried out using samples of feral organisms of three species (passive biomonitoring). The results would probably be more easily exploitable if we had performed active biomonitoring based on transplantation of organisms from a controlled population, as recommended by De Koch and Kramer (1994) or Mourgaud et al. (2002).

5 Conclusion

The condition index (CI) shows little variation for the three species between the sites and is not very informative. On the other hand, we have noted that, depending on the species, positive correlations exist between nickel and/or vanadium concentrations and those of metallothioneins.

The bioaccumulation of nickel and vanadium in mussels displays obvious temporal changes with concentrations at sites affected by the sinking of *Erika* distinctly higher than those at a control site. This indicates that the mussel constitutes a good matrix to perform biomonitoring of these two metals which are present in numerous oils. This is not surprising, given that mussels strongly bioaccumulate a number of contaminants and are used as a sentinel species in numerous monitoring programs (Mussel Watch, RNO, etc.). Dogwhelks are equally sensitive and are a species likely to be used in monitoring programs. Periwinkles on the other hand respond completely differently, probably due to their diet, and this is a disadvantage. The use of these two metallic markers and metallothioneins in dogwhelks and especially mussels thus appears to be a reliable way of monitoring the quality of the environment affected by oil from the tanker *Erika*.

Nevertheless, the results of all fieldwork observations are subject to caution and any conclusion remains hypothetical. Thus, it is necessary to carry out various laboratory experiments under controlled conditions in order to be able to confirm whether the nickel and vanadium present in oil do indeed accumulate in bivalves and are capable of inducing metallothionein synthesis.

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