

Metals in Sediment, Seagrass and Gastropods Near a Nickel Smelter in Greece: Possible Interactions

ARTEMIS NICOLAIDOU*† and JAMES A. NOTT†

*Department of Zoology and Marine Biology, University of Athens, Panepistimiopolis, GR 15784 Athens, Greece

†Plymouth Marine Laboratory, Citadell Hill, Plymouth, UK

Accumulation of Cd, Co, Cr, Cu, Fe, Mn, Ni and Zn was studied in the sediments, *Cymodocea nodosa* leaves, roots and stems and in two gastropods from an area adjacent to a ferro-nickel smelting plant and a control site on the east coast of Greece. In the sediment, the metals, with the exception of Cu, have significantly higher concentrations in the polluted than in the clean site. There is a tendency for *C. nodosa* tissues in the polluted area to have higher concentrations of metals although the differences are not always statistically significant. Co, Mn, Ni and Zn are in higher concentrations in the leaves than in the other tissues, a trend more obvious in the polluted site. Concentrations of metals in the viscera of the gastropods *Cerithium vulgatum* and *Monodonta mutabilis* are higher than in the muscle (except for Cd) and significantly higher in the animals from the polluted site. It is suggested that Mn is taken up from the water by *C. nodosa* leaves, which in the form of detritus enter *C. vulgatum*. Mn concentrates in the viscera of *C. vulgatum* in the form of granules. Zn follows the same route with additional amounts being taken by *C. vulgatum* directly from the water. © 1998 Elsevier Science Ltd. All rights reserved

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The small bay of Larymna, in the Northern Evoikos Gulf, Greece, is dominated by a ferro-nickel smelting plant which extracts iron and nickel from laterite ore. The by-products of smelting, namely cooling water from the furnaces, dust and slag, enter the marine environment directly and through spillages. A preliminary survey in the littoral zone of the area (Nicolaidou and Nott, 1989) showed that gastropods had the higher concentrations of metals, among the biota. In addition, they were the most abundant animal group in the area and they became the subject of a number of studies. These include the cytology of heavy

metal accumulation (Nott and Nicolaidou, 1989a), metal metabolism and bioreduction (Nott and Nicolaidou, 1989b), the differential uptake of metals by different species of gastropods (Nicolaidou and Nott, 1990) and the effect of animal size on metal accumulation (Catsiki *et al.*, 1994). Finally, gastropods from the area were used in laboratory experiments to elucidate aspects of transfer of metals to the predators (Nott and Nicolaidou, 1990, 1994).

The present paper focuses on two other major ecosystem components: the coastal sediment and the sea grass *Cymodocea nodosa*. Metal concentrations in the sediments of the deeper parts of the bay and at a slag dumping site offshore were studied by Voutsinou-Taliadouri and Varnavas (1987) who found very high concentrations of most metals. Little information exists in the literature concerning metal accumulation in *Cymodocea* (Nienhuis, 1986). The most comprehensive work on metal accumulation by *C. nodosa*, a widespread species along the Mediterranean coast, is that of Malea and Haritonidis (1995). Other relevant studies are those of Chabert *et al.* (1983) and Malea (1993).

As the gastropods *Cerithium vulgatum* and *Monodonta mutabilis* were the numerically dominant animal species, some data from Nicolaidou and Nott (1990) are further analysed and included here. Comparison is made with material collected at the same time from Vravra, a control area in the South Evoikos Gulf.

Materials and Methods

Samples were collected from a *Cymodocea nodosa* bed at approximately 70 cm depth. The plants were washed to remove sediment. Five samples of leaves, stems and roots were analysed individually. The animals were brought alive to the laboratory where they were kept for a week in artificial seawater, periodically changed, to eliminate the gut contents. They were then dissected to separate the viscera from the remaining structures which consisted mainly of muscle tissue.

The preparation of samples for atomic absorption spectroscopy (AAS) was carried out according to the

†Corresponding author.

methods described by Bryan *et al.* (1985). The samples, including sediments for total metals, were dried at 80°C and digested with concentrated 'Aristar' (BDH) nitric acid. Following evaporation, they were redissolved in 1 N 'Aristar' (BDH) hydrochloric acid. The metals Co, Cr, Cu, Fe, Mn and Zn were analysed by flame atomic absorption in a Perkin-Elmer 603 spectrophotometer with background correction for Co and Cr; carbon furnace atomic absorption (Perkin-Elmer 76B) was used for measuring Cd and Ni. The sediments assessed for available metals were extracted in stirred 10% HCl at 80°C for 2 h. The acid was passed through a 0.45 µm Millipore filter before AAS analysis.

The statistical significance of the differences between metal concentrations was tested by one-way analysis of variance (ANOVA) and Tuckey's least significant difference test (95% confidence intervals).

Results

Sediments

The concentrations of metals at the clean site (Table 1) are within the range of average values for Aegean and coastal sediments. At the polluted site, the metals have significantly higher concentrations with the exception of Cu which has higher concentrations in the clean sediment. The bioavailable metals, as measured by extractability from the sediments with dilute hydrochloric acid, also have higher concentrations at the polluted site, again with the exception of Cu. For Cu the difference between the two sites is not statistically significant. At Larymna the metal most soluble in HCl is Mn showing 57.0% solubility, followed by Cu and Cd

(51.5 and 50.0% respectively). The lowest solubility observed is that of Ni (14.9%). Mn is also the most soluble metal (51.0%) in the clean site. The solubility of the other metals is of the same order of magnitude except for Co and Cr for which it is approximately 10 times lower than in the polluted site. This suggests that these two metals are either chemically different or are associated with different components (organic or inorganic) of the sediment.

Cymodocea nodosa

There is a tendency for plants in the polluted area to have higher concentrations of metals, although the differences are not always significant. In addition, there is marked variation in the metal content between leaves, stems and roots and this depends on both the metal and the site. The mean metal concentrations are shown in Table 1.

Cobalt. Higher concentrations of this metal occur in the leaves of *C. nodosa*. The mean concentration at the polluted site is significantly higher than in the leaves at the clean site. Both values are higher than concentrations in the roots and stems at both sites, which do not differ significantly.

Chromium. Variability in the concentration of Cr is high and the only statistically significant difference occurs between the highest value in the roots at the polluted site and the lowest in the leaves at the clean site. The general tendency is for the metal concentration to decrease from the roots to the stems to the leaves and from the polluted to the clean site.

Cadmium. High variability occurs in the concentrations of Cd and there are no obvious trends. The highest

TABLE 1
Mean concentrations of metals in sediment and biota (units of µg g⁻¹ dry wt, except for Fe, which is in mg g⁻¹ dry wt)

	Cd	Co	Cr	Cu	Fe	Mn	Ni	Zn
<i>Cymodocea nodosa</i>								
Leaves polluted	2.3±0.2	18.3±2.4	4.8±1.4	23.5±5.9	287.0±78.8	422.4±34.3	77.3±3.3	147.1±8.1
Roots polluted	2.6±0.6	1.4±2.5	10.5±8.7	22.4±6.0	3832±2071	17.4±3.6	26.4±1.6	62.4±6.3
Stems polluted	0.4±0.1	1.6±1.0	9.1±4.0	8.9±1.6	2054±418	21.9±3.2	15.0±4.0	87.1±9.0
Leaves clean	1.2±0.2	5.2±0.4	2.0±0.6	9.6±0.5	143.6±18.1	186.5±16.5	7.6±0.3	57.5±6.2
Roots clean	2.1±0.7	1.1±1.5	5.1±1.4	12.8±2.4	247.0±80.0	12.4±2.0	5.2±2.8	22.92±1.6
Stems clean	2.1±1.6	1.1±0.4	3.28±2.8	7.7±4.0	182.8±54.6	20.4±15.6	1.2±1.6	23.0±19.5
<i>Cerithium vulgatum</i>								
Viscera polluted	8.0±2.4	180.4±63.8	40.5±19.5	110.3±35.8	2675±788	3646±1234	319.4±159.1	3838±1026
Muscle polluted	8.4±1.7	-	36.3±13.7	16.9±6.7	355.6±202.8	19.7±10.1	5.7±1.9	58.0±4.4
Viscera clean	8.7±2.7	2.6±0.9	7.1±3.7	115.7±56.4	987.9±282.2	692±193.7	31.9±10.0	570.1±288.0
<i>Monodonta mutabilis</i>								
Viscera polluted	3.0±1.1	2.1±0.6	9.0±4.2	222.8±42.1	1031±280	67.1±19.7	9.3±1.5	88.6±11.7
Muscle polluted	3.6±0.5	-	-	43.15±9.6	158.9±28.1	9.4±3.7	2.2±0.5	109.0±36.7
Viscera clean	5.8±1.0	2.0±0.5	25.2±14.6	222.6±83.6	3632±1280	16.8±6.1	6.5±1.7	135.3±20.1
<i>Sediment</i>								
Polluted total	0.1±0.02	49.9±2.0	677.6±46.6	13.2±0.7	39.2±1.8	339.9±12.2	889.4±39.7	152.5±6.8
Polluted HCl	0.05±0.01	16.2±1.4	301.2±10.7	6.8±0.2	11.3±0.5	193.6±6.7	132.3±5.3	64.1±1.2
Polluted %HCl	50.0	32.5	44.5	51.5	28.8	57.0	14.9	42.0
Clean total	0.1±0.02	6.7±0.3	105.0±13.4	16.6±1.7	13.2±1.6	270.5±12.6	60.5±6.2	44.3±3.9
Clean HCl	0.02±0.01	0.2±0.1	6.0±0.6	7.1±2.0	1.2±0.3	138.0±4.1	4.6±1.0	21.4±4.6
Clean %HCl	20.0	3.0	5.7	42.8	9.1	51.0	7.6	48.3

concentration of this metal occurs in the roots of *C. nodosa* in the polluted site, but it is not significantly different from all the other values except for the stems at the polluted site, which have the lowest concentration.

Copper. Concentrations of Cu are higher in the roots and leaves of *C. nodosa* and lower in the stem, although this difference is only significant at the polluted site.

Iron. The concentration of Fe in the roots at the polluted site is significantly higher than the concentration in the stems at the same site and both are higher than the other values which do not differ significantly between them.

Manganese. There is preferential accumulation of Mn in the leaves of *C. nodosa* where concentrations are significantly higher than in the roots and stems at both sites which do not differ significantly. In addition, the concentration in leaves from the polluted site is significantly higher than in leaves from the clean site.

Zinc. As with Mn, Zn also accumulates at higher concentrations in the leaves than the stems and roots at both sites. However, at the polluted site there is also a significant difference between the other two tissues, with stems having higher concentrations than roots. All three tissues at the polluted site have higher concentrations than the same tissues in the clean site.

Nickel. Concentrations of Ni are significantly higher in all tissues at the polluted site than at the clean site. At the polluted site, concentrations decrease significantly from leaves to roots to stems, while there is no significant difference between the tissues at the clean site.

Gastropods

The mean values in Table 1 show that, in general, the concentrations of metals in *Cerithium vulgatum* are higher than in *Monodonta mutabilis* (with the exception of Cu). The concentrations are also higher in the viscera of both species than in the muscle and higher in the polluted site than in the clean (with the exception of Cd).

Cobalt, manganese, nickel and zinc. Co, Mn, Ni and Zn in the viscera of *C. vulgatum* from the polluted site have significantly higher concentrations than in all the other samples, which do not differ between them.

Cadmium. Cd is significantly higher in *C. vulgatum* than in *M. mutabilis* with the exception of the highest concentration in *M. mutabilis* viscera from the clean site which does not differ significantly from the lowest level in *C. vulgatum* viscera from the polluted site. As mentioned earlier, concentrations of Cd are generally higher at the clean site than at the polluted. Concentrations may also be subliminal as they can be two to three orders of magnitude less than other metals.

Copper. Cu concentrations in the gastropods are related to tissue type and species but not to pollution. Significantly higher concentrations occur in the viscera of *M. mutabilis* followed by the viscera of *C. vulgatum*

while the muscle has the lowest concentrations. There are no significant differences between sites.

Iron. Fe occurs at highest concentrations in the viscera of *M. mutabilis* from the clean site followed by the viscera of *C. vulgatum* from the polluted site. Both are significantly higher than all the other samples.

Chromium. Cr occurs at higher concentrations in the viscera of *C. vulgatum* from the polluted site followed by the viscera in *M. mutabilis* from the clean site. Both concentrations are higher than the same tissue of *C. vulgatum* from the clean site and *M. mutabilis* from the polluted site.

Discussion

At the site of the smelting plant, the concentrations of all metals, except Cu, are higher than the average concentrations in clean coastal sediments (Smith and Cronan, 1975; Voutsinou-Taliadouri *et al.*, 1987) but are comparable with those in the offshore dumping site and other polluted areas in Greece. Thus, in comparison with the Bay of Kavala, which is affected by oil platforms and a fertilizer factory on the shore (Voutsinou-Taliadouri, 1985), Larymna has higher concentrations of Ni and Co. The values in Larymna, however, are lower than those found in the Thermaikos Gulf (Voutsinou-Taliadouri and Leondaris, 1985) which is influenced by the sewage and industrial effluent from Thessaloniki, the second largest city in Greece. The concentrations of metals in the sediments in the control site of the present investigation fall within the range found for clean coastal sediments in Greece (Smith and Cronan, 1975; Voutsinou-Taliadouri *et al.*, 1987).

In animals from Larymna, the concentrations of metals in *M. mutabilis* match those observed in polluted sites in the Bay of Izmir in the Mediterranean (Tuncer and Uysal, 1983). They also follow the trend for the same species sampled at Lebanon although the latter were analyses of the whole animals minus shell (Shiber and Satila, 1978) and the animals were not allowed to empty their guts.

The tissues of *C. nodosa* in the polluted site have, in most cases, higher concentrations than the respective tissue in the clean site. This is true for Ni, Zn Fe (except for leaves), Cu (except for stems) and for Mn and Co in the leaves. It seems, therefore, that the plants are also contaminated in the bay of Larymna. The mean metal concentrations in the leaves of *C. nodosa* in the polluted site decrease in the order: Mn > Fe > Zn > Ni > Cu > Co > Cr > Cd. This is the same for the leaves in the clean site, only with reverse order for Ni and Cu. The same order, Mn > Fe > Zn > Cu > Cd, is given by Brix and Lyngby (1984) for the metals which they studied in *Zostera marina*. They also found more Fe in the roots than the leaves which is also the case in the present study.

Comparisons of the concentrations of each metal in the sediments and the biota can be used as a basis for

speculation on possible pathways of these metals from the environment to organisms.

The most bioavailable metal in the sediment (over 50% HCl extractable) is Mn in both clean and polluted sites. In *C. nodosa* Mn tends to concentrate in the leaves. In both clean and polluted sites Mn is in higher concentration in the leaves than in the sediment. Measurements of Mn in the seawater (Nakopoulou *et al.*, 1985) showed concentrations of 3.06 ppb at the entrance of Larymna bay as opposed to 34.42 ppb in the inner bay, 84% of which was dissolved. Probably, Mn from solution is taken up by *C. nodosa* through the leaves. Higher concentrations of Mn in the leaves than in the roots were found in *Z. marina* by Brinkhuis *et al.* (1980) and Drifmeyer *et al.* (1980), in *Posidonia spp.* by Tiller *et al.* (1989) and in other seagrasses by Pulich *et al.* (1976). There is probably no translocation from the water to the sediment through the roots and the leaves of the plant. Schroeder and Thorhaug (1980) mention that Mn and Zn dissolved in seawater are concentrated in the leaves of *Thalassia testudinum* but little translocation to below ground tissues occurs. On the contrary, Skei and Melsom (1982) suggest that Mn may be released from the sediment to the overlying water under reduced conditions (which exist in the sediment in Larymna) and accumulated in the leaves of *Zostera*. A large part of the Mn in Larymna probably enters the sediment as *C. nodosa* leaf detritus, a more bioavailable form, which is subsequently taken up by *C. vulgatum* as food. In the body of the gastropod, the excess metal concentrates in the viscera. Electron probe X-ray microanalyses showed that it is accumulated in both the mineralized phosphate granules of the basophil cells and the lysosomal residual bodies of the digestive cells (Nicolaidou and Nott, 1989). This explains the very high concentrations of Mn in the viscera of *C. vulgatum* which is 10 times higher than in the sediment.

The role of seagrasses as reservoirs and pathways of Mn, Zn and other metals has been described by Parker *et al.* (1963) for *Halodule wrightii* and *Thalassia testudinum*, and by Drifmeyer *et al.* (1980) for *Z. marina*. Zn in Larymna may follow the same route as Mn. It also has higher concentrations in the leaves than in the roots and stems which was also found for *Z. marina* (Lyngby and Brix, 1982, 1983; Brix and Lyngby, 1984), *Posidonia spp.* (Tiller *et al.*, 1989) and *Halophila stipulacea* both in the field (Malea, 1994a,b) and experimentally (Malea *et al.*, 1995). Although in *C. nodosa* more Mn is accumulated than Zn, the elevated concentrations of the two metals in *C. vulgatum* are similar. This may be partly due to the fact that dead seagrass leaves, which are the detritus source of metals, concentrate the metals differentially with respect to the living leaves. It is known that dead *Zostera* leaves absorb five times as much ^{65}Zn as live leaves (Lyngby *et al.*, 1982) and dead leaves of *Halodule wrightii* accumulate ^{54}Mn from solution at 70% of the rate of live leaves (Pulich, 1987). It is more likely, however, that

C. vulgatum receives additional Zn directly from the water. In uptake experiments using ^{65}Zn , Renfro *et al.* (1975) found that shrimps and crabs receiving ^{65}Zn from both food and water did not attain significantly higher ^{65}Zn body burdens than those individuals which accumulated the isotope from the water only. Similarly, Howard and Brown (1987) found that coral accumulated Zn from solution but not Mn.

The concentration of Cd in the leaves of *C. nodosa* was comparable to that given for *Z. marina* (Brix *et al.*, 1983; Brix and Lyngby, 1984) although much higher values are known from the literature for *Posidonia oceanica* (Malea *et al.*, 1994) and *Z. marina* (Damyanova *et al.*, 1981) from polluted environments. The concentrations in the sediments were lower than in the plants. This is in disagreement with the literature where concentrations of Cd in *Posidonia spp.* (Ward, 1987), *Z. marina* (Lyngby and Brix, 1987) and *C. nodosa* (Malea and Haritonidis, 1995) are known to reflect concentrations in the sediments. Higher concentrations in the leaves than in the other tissues were found for *Posidonia* (Tiller *et al.*, 1989) and *Z. marina* (Brix and Lyngby, 1984) and acropetal translocation was suggested for *P. oceanica* by Malea *et al.* (1994) and for *Z. marina* by Brinkhuis *et al.* (1980). Conversely, Faraday and Churchill (1979) in Cd uptake experiments found translocation from the leaves to the roots of *Z. marina* but not the opposite. In the present case, Cd seems to accumulate more in the roots, but variability is too high for statistically significant results to be obtained. Concentrations of Cd in both gastropods is higher than plants and sediments, which indicates that it may be received directly from the water.

There is little information concerning the flow of Co through seagrass ecosystems except that the leaves of *Thalassia testudinum* are very active in taking up the element (Parker, 1966). This may explain the significantly higher concentration of Co in the leaves than the roots and stems observed both in the polluted and clean sites in the present investigation.

There is a seeming discrepancy between the concentrations of Cu in sediment and seagrasses from polluted and reference sites. Although soluble Cu concentrations in the sediment are similar in the two sites, the concentrations in the leaves and roots are higher in the polluted site. However, solubility is higher in the polluted site, thus more Cu may be available in the water. It is possible that *C. nodosa* may accumulate Cu also from the water through its leaves, as suggested by Drifmeyer *et al.* (1980) for *Z. marina*. Lyngby and Brix (1987) found good positive correlation between Cu concentrations in sediments and *Z. marina* leaves and root/rhizomes. However, the concentrations of the metal in their areas of study were higher and with a greater range (82–790 $\mu\text{g g}^{-1}$) than in the present case.

The concentrations of Cr are high especially in the polluted sediment and this contrasts markedly with the much lower concentration in *C. nodosa*. Wasserman *et*

al. (1992) who studied 25 metals in sediments and *Z. noltii* in the bay of Arcachon, categorize Cr among the metals concentrating mostly in the sediment, as opposed to those which accumulate preferentially in plants or those which are indifferent. Their concentrations in the sediment ($60 \mu\text{g g}^{-1}$) were two to three times higher than in the plants, which is in agreement with the values found here for *C. nodosa* and acid-soluble Cr in the sediment of the clean site. In the polluted site, however, the concentrations in the sediment were up to 60 times higher than those in the plant tissues. Thus, concentrations of Cr in *C. nodosa* do not increase proportionally to those in the sediment, suggesting that perhaps this seagrass is not a good indicator of Cr in sediments. As opposed to *C. nodosa*, the concentrations of Cr in *C. vulgatum* from the polluted site are much higher than in the clean site. This can be taken as an indication that this element is accumulated by the gastropod directly from the sediment rather than through the plant material.

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