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Adsorption of Metals in Seawater to Limpet *(Patella vulgata)* Pedal Mucus

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Pedal mucus is deposited as a trail behind most mobile gastropods (for review see Davies and Hawkins, 1998) and by virtue of its sticky properties may act as a trap for microalgal particles that some marine molluscan grazers ingest (Davies *et al.*, 1992). Further, given mucus production and degradation rates, and the density and movement patterns of grazing gastropods, much of the benthos is covered for much of the time with a layer of mucus (Davies and Hawkins, 1998). If this mucus is capable of adhering microscopic particles, can it also be capable of adsorbing materials on a molecular scale?

Mucuses from other groups have metal-adsorbing properties. Mucus exopolymers from various marine (Loaëc et al., 1997) and freshwater (Friedman and Dugan, 1968; Míttelman and Geesey, 1985) bacteria have been shown to adsorb various metals (for a review of earlier work see Brown and Lester, 1979) and at least for one species *(Klebsiella aerogenes)* mucus serves to reduce the toxicity of a water-borne metal (Bitton and Freihofer, 1978). Mucus in fish can serve to protect the animal from its environment by accumulating metals and retarding metal transport (by over 50 %) from solution to the epithelial surface (Playle and Wood, 1991; Wilkinson and Campbell, 1993). The metal-adsorbing properties of mucus have even prompted the suggestion that it be used in water treatment and in the biodetoxification of polluted seas (Loaëc *et al.*, 1997). We therefore postulate that the mucus deposited by gastropods may also adsorb metals from solution and that subsequent ingestion of the mucus may constitute a significant route of uptake into the littoral food web.

Here we present data on the ability of the mucus of the littoral gastropod mollusc *Patella vulgata* L. (common limpet) to adsorb and thus concentrate Zn and Cu from seawater. *P. vulgata* was chosen as it is extremely common on north European shores, where it is often the dominant grazer and further because its pedal mucus production system has been well investigated (see Davies and Hawkins, 1998). Zn and Cu were chosen as toxicants as these have been shown to affect mucus production rates *in P. vulgata* (Davies, 1992) and are common coastal pollutants, entering via freshwater influx (Bryan, 1984).

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MATERIALS AND METHODS

Common limpets *(Patella vulgata)* were collected from the coast of north-east England at Whitburn (National Grid Reference: NZ 415645) and used within 36 h of collection. Each animal was used only once. All glassware was acid-washed using 2 M HCl prior to use and no metal object came in contact with the limpets or their pedal mucus. All procedures were performed at 15 ± 1°C.

The feet of -25 limpets were gently scraped free of mucus, faeces and other debris using a plastic spatula and the limpets were placed on a glass plate (200 x 200 mm) in air for 30 min. During this period the limpets did not move. Davies (1993) reports that the production of adhesive pedal mucus will have stopped by this time. The limpets were then removed and discarded. The plate was placed into a tank containing filtered (0.2 µm) fresh seawater which contained a single metal at a specific concentration. After a specified period, the plate was removed from the tank and rinsed twice by briefly dipping in de-ionised water. The mucus adhering to the plate was then collected using a plastic spatula and dried at 70 °C to constant weight. A balanced design used analytical grade Zn [as Zn $(N0_3)_2.6H_2O$] and Cu [as $Cu(NO_3)2½H_2O$], each added at 0 (control), 0.1, 1.0 and 10 mg 1^{-1} (ppm) and periods of exposure to the metal of 6 and 24 h. A 6-h exposure represents the period of immersion on a single tide by pedal mucus produced by limpets at mid-shore. A 24-h exposure represents immersion by four tides. Each treatment was replicated three times, in a random sequence of treatments.

Levels of metals in the samples of pedal mucus were measured in a Varian Spectraa 10 Plus spectrophotometer following 24 h acid digestion in HNO_3. Samples were heated gently to ensure complete digestion. Detection limits were 0.01 µg g^{-1} for Zn and 0.03 µg g^{-1} for Cu. Accuracy and precision were checked using spiked samples. Standard Reference Material was Montana Soil 2711 (National Institute of Standards and Technology, USA) which gave repeat readings precise to < 0.01 µg g^{-1}. Mucus samples from the cleaned feet of 20 freshly collected limpets (no experimental treatment performed) were also analysed for their Zn and Cu contents. In order to establish baseline levels of metals, samples of filtered (0.2µm) seawater were also analysed for Zn and Cu.

RESULTS AND DISCUSSION

Baseline levels of Zn and Cu in seawater were 0.21 mg 1^{-1} ± 0.035 SE and 0.082 mg 1^{-1} ± 0.003 SE respectively, n = 3 in each case. Although it is acknowledged that measurements of metals in seawater are normally formed from integrating data over temporal and spatial scales (see for example, Bryan, 1984), it was thought important to make these measurements as 'baseline' values against which to assess the degree of adsorption. The dry mucus produced per plate weighed between 0.6 and 8.4 mg. For Zn, the adsorption of metal to mucus showed a curved response with increasing metal concentration in seawater (Fig. 1A, note

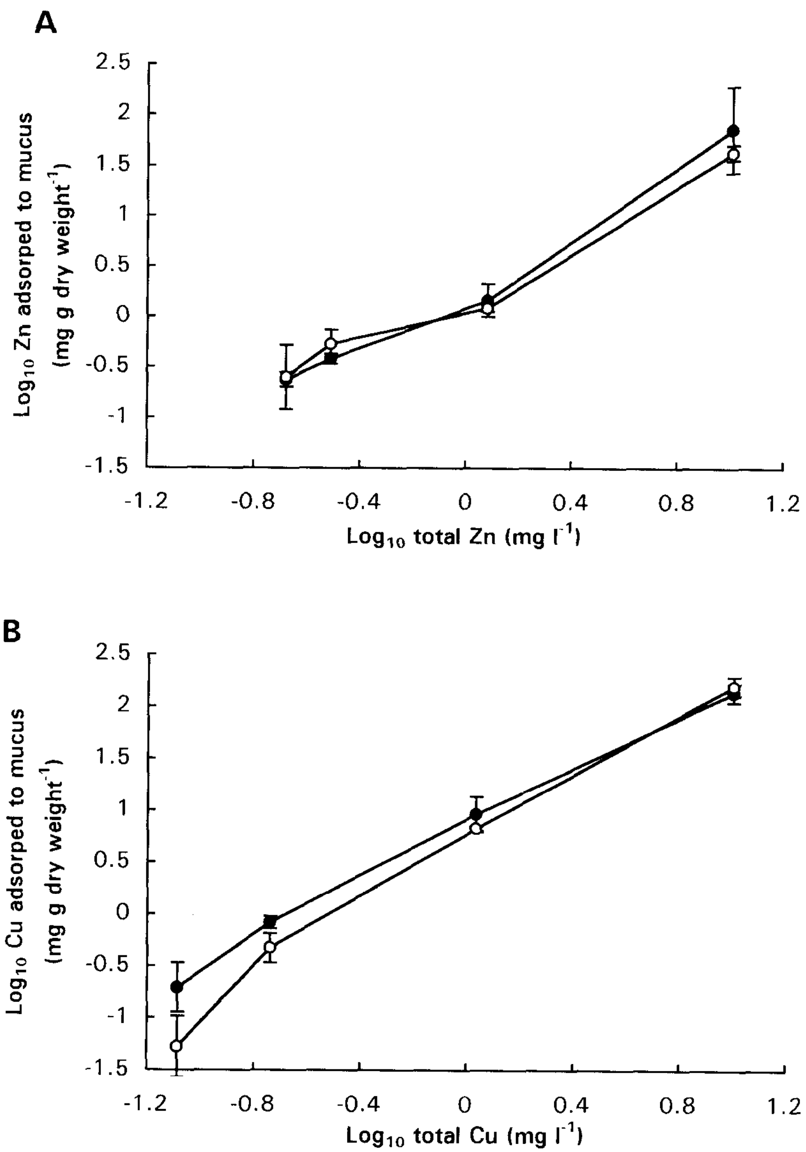

Figure 1. Mean adsorption (±SE, n = 3) of (A) Zn and (B) Cu to limpet mucus exposed at four different metal concentrations for 6 h (closed circles) and 24 h (open circles). X-data include natural levels of Zn and Cu in seawater.

logarithmic axes and inclusion of baseline values of metals in seawater). The mucus appeared to equilibrate with Zn after 6-h exposure (no more Zn was adsorbed after 24 h, Table 1). In the 10 mg 1^{-1} Zn treatment the mucus contained significantly more Zn than in the other treatments. These showed no significant difference from the control (no *added* Zn) (SNK test, Table 1). For Cu there was also a curved response between adsorbed metal and its concentration in seawater (Fig. 1B). Again the mucus was equilibrated with this metal after 6-h exposure (Table 1). Mean separation by SNK test showed that the control and 0.1 mg 1^{-1} Cu treatments did not produce significantly different adsorption, but that the 1.0 and 10 mg 1^{-1} Cu treatments produced significantly distinct levels of adsorption (Table 1). In the 10 mg 1^{-1} Cu treatment the mucus took on a green tinge after both 6 and 24 h.

Table 1. Analysis of variance summary tables for the adsorption of Zn and Cu to limpet mucus (mg g^{-1} dry wt) at 4 different metal concentrations in seawater over 2 exposure periods. In SNK tests underlined means are not significantly different at P = 0.05.

Zinc

Source	df	SS	MS	F	*P*
Concentration (1)	3	33299	11100	8.1	0.002
Time (2)	1	2798	2798	2.04	0.172
(1) x (2)	3	8366	2789	2.04	0.149
Error	16	21916	1370		
Total	23	66378			

SNK test for Concentration

0 mg l^{-1} Zn	0.1 mg l^{-1} Zn	1.0 mg l^{-1} Zn	10 mg l^{-1} Zn
0.304	0.468	1.442	86.76

Copper

Source	df	SS	MS	F	*P*
Concentration (1)	3	102287	34096	40.96	0.000
Time (2)	1	128	128	0.15	0.701
(1) x (2)	3	684	228	0.27	0.843
Error	16	13320	832		
Total	23	116418			

SNK test for Concentration

0 mg l^{-1} Cu	0.1 mg l^{-1} Cu	1.0 mg l^{-1} Cu	10 mg l^{-1} Cu
0.16	0.68	8.67	153.74

Given the baseline values of Zn and Cu in seawater, it was not surprising that adsorption showed little variation up to the 1.0 mg 1^{-1} Zn and 0.1 mg 1^{-1} Cu treatments: the baseline values approached these treatment levels. Limpet pedal mucus (n=20) contained undetectable levels of Zn and Cu prior to experimentation. For both Zn and Cu, mucus adsorbed and concentrated the metal from the seawater. To give ecologically realistic concentrating factors we have compared the levels of metal (values include baseline levels) per unit wt of seawater (assuming 1 g = 1.025 ml) with the levels of metal per unit wt of wet mucus. Wts were converted from dry to wet mucus given that the pedal mucus of *P. vulgata* is 91.75 % water (Davies *et al.*, 1990). For Zn the control treatment (0.21 mg 1^{-1}) concentrated the metal from the seawater by ~ 100 x, by 100 - 150 x for the 0.1 mg 1^{-1} treatment (actual Zn level, 0.31 mg 1^{-1}), by ~ 100 x for the 1.0 mg 1^{-1} treatment (1.21 mg 1^{-1}) and by 350 - 1000 x for the 10 mg 1^{-1} treatment (10.21 mg 1^{-1}). For Cu the control treatment (0.082 mg 1^{-1}) concentrated the metal from the seawater by 75 - 250 x, by ~ 300 x for the 0.1 mg 1^{-1} treatment (actual Cu level, 0.182 mg 1^{-1}), by 500 - 800 x for the 1.0 mg 1^{-1} treatment (1.082 mg 1^{-1}) and by ~ 1200 x for the 10 mg 1^{-1} treatment (10.082 mg 1^{-1}). Mittelman and Geesey (1985) report Cu adsorption to a bacterial mucus at levels as low as 25 µg 1^{-1}. Limpet mucus was not saturated with the metals tested, even at the 10 mg 1^{-1} treatment, though concentrations in excess of this are unlikely to occur in the sea. Loaëc *et al.* (1997) showed that a bacterial mucus saturated with Pb, Cd and Zn each at ~ 50 mg 1^{-1}.

It is clear that metals in seawater can adsorb on to the mucus deposited by intertidal gastropods and that this process may occur in waters that are not thought of as being contaminated by metals. The mechanism of adsorption of metals to, presumably, the glycosaminoglycan component of mucus is unknown, but would merit further work. Some data on the Cu-binding characteristics of bacterial exopolymers are given by Mittelman and Geesey (1985). The pedal mucus of *P. vulgata* can persist for up to 80 days in the absence of grazing (Davies *et al.,* 1992) and much of the intertidal and subtidal benthos is covered for much of the time with at least a single layer of mucus trail. The implication is that molluscs such as *P. vulgata* and the common periwinkle *Littorina littorea* that graze over the substratum in their routine foraging activities are likely to be ingesting, over time, significant amounts of mucus-borne metals, especially where the water is contaminated with such. Marine gastropods often trail-follow and can ingest material from mucus trails (Davies & Beckwith, 1999). The effects of water-borne metals on aquatic organisms are well documented, but uptake routes are often assumed as being via the food or over the general body surface through specific structures such as gills. For grazing gastropods, pedal mucus may serve as an additional uptake route. Many molluscs possess specific ionoregulatory mechanisms, including storage in granules within the tissues (e.g., Mason and Nott, 1981) and may pass on their store of metals to organisms higher in the coastal food web.

Whilst the potential for metals to accumulate up food chains from seawater via adsorption to benthic mucus is new, the ability of mucus to adsorb metals is not. Recently, the pedal mucus of the freshwater snail *Lymnaea stagnalis* has been shown to adsorb Al which was recovered following acidification (Jugdaohsingh *et al.* 1998). Also, pesticide-absorption properties of pedal mucuses of the freshwater snails *L. peregra* and *Potamopyrgus jenkinsi* have been demonstrated (Brereton *et al.*, 1999). Howell (1982) reported that the mucus produced by nematodes could adsorb Cu, Zn and Pb from seawater, but only by 10 x at 1.0 mg l^{-1}, much less than in the present system. In contrast, mucus from the hydrothermal vent polychaete *Paralvinella* sp. can absorb up to at least 29.9 mg g^{-1} Zn and 47 mg g^{-1} Cu in a metal-rich environment (Juniper *et al.,* 1996). Metal-binding activity has also been reported for the mucus of the rat small intestine (Quarterman, 1987) and the gill (Playle and Wood, 1991; Wilkinson and Campbell, 1993) and skin mucus of fish (Handy and Eddy, 1990).

Further work with this system could involve investigating the competitive interactions in a poly-metal system that perhaps better reflects natural systems.

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