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Copper and Cadmium Accumulation in Populations of *Littorina saxatilis* from the Isle of Man Having Differential Tolerance to Zinc and Lead

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Abstract: The accumulation of Cu and Cd from solution was studied in *Littorina saxatilis* from five sites around the Isle of Man with different degrees of metal contamination. The winkles were exposed to increasing concentrations of each metal (0, 0.01, 0.1, 0.5 and 1.0 mg L^{-1} added Cu and added Cd) after acclimation to laboratory conditions. The experimentation was conducted in triplicate runs and after the exposure period, tissue metal concentrations were determined by atomic absorption spectrophotometry. Different accumulation profiles were observed for Cu and Cd. Cd accumulation was monotonic over the range of exposure concentrations, giving linear regressions with high coefficients of determination. Cu accumulation on the other hand was linear up to 0.5 mg L^{-1} but polynomial (quadratic) fits described the accumulation patterns better when all concentrations are included. Tissue Zn concentrations showed a significant relationship with Cd accumulation. No significant interpopulation differences in Cu accumulation were shown by Analysis of Covariance. Significant differences in Cd accumulation were found between populations although not reflective of the tolerance profile.

Keywords: Heavy metals, bioaccumulation, gastropod mollusc, littorinid, semi-static bioassay and metal-tolerance

INTRODUCTION

Heavy metals are natural constituents of seawater derived from geochemical and volcanic processes. Rivers are a major contributor of heavy metals to the sea and the nature of input depends on the occurrence of metal and ore bearing deposits in the drainage area (Clark, 1992). The sources of anthropogenic input of heavy metals into the aquatic environment include industrial processes (Buckley, 1995; Chen, 1995; Shear, 1996), sewage sludge (Blomqvist *et al.*, 1992), power stations (Philips and Unni, 1991) and mining (Davies, 1987). The mining of heavy metals by man is increasing the mobilization of most elements over that achieved by natural geological weathering. Even when mining has stopped, drainage water from spoil heaps is a continuous source of contamination (Bryan, 1983; Hunt, 1994; Daka, 2003).

A distinction can be made between essential and non-essential heavy metals on the basis of known biological functions (Depledge, 1990). Essential elements which include zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) are vital components of enzymes and respiratory pigments (Bryan, 1976; White, 1985). For example, carbonic anhydrase, carboxy-peptidase A and B and several dehydrogenases contain zinc, pyruvate carboxylase contains manganese, haemocyanin and haemoglobin contain copper and iron respectively. Other metals (non-essential) such as mercury (Hg), cadmium (Cd) and silver (Ag) have no known biological function (Bryan, 1984).

Aquatic invertebrates accumulate heavy metals in their tissues, whether or not these metals are essential to metabolism (Rainbow, 2002). The kinetics of metal handling in a species could depend on a number of factors, including whether the metal is essential or non-essential. Prior exposure to the same or other metal(s) could also affect accumulation. Hisstorical mine-related contamination has led to different metal burdens in sediment and biota in estuaries around the Isle of Man (Daka *et al.*, 2003; Daka, 2005). Also, *Littorina saxatilis* from the most contaminated estuary in the Isle of Man has been shown exhibit tolerance to zinc and lead, with contrasting mechanisms (Daka and Hawkins, 2004). In this paper, the accumulation patterns of copper (an essential metal) and cadmium (a non-essential metal) were assayed in the gastropod mollusc *L. saxatilis* to determine how tolerance to zinc and lead could affect the accumulation of these metals.

MATERIALS AND METHODS

Test organism/sites of collection: Large, ovoviviparous littorinids identified as *Littorina saxatilis* (Reid, 1996; Daka and Hawkins, 2004) were collected from five sites around the Isle of Man (Fig. 1) with varying degrees of mine-related metal contamination and uncontaminated sites (Daka, 2003; 2005). These were at the mouths of the estuaries at Laxey (high contamination especially with Zn), Peel (contaminated), Castletown and Ramsey, and the open shore in Castletown Bay near Derbyhaven.

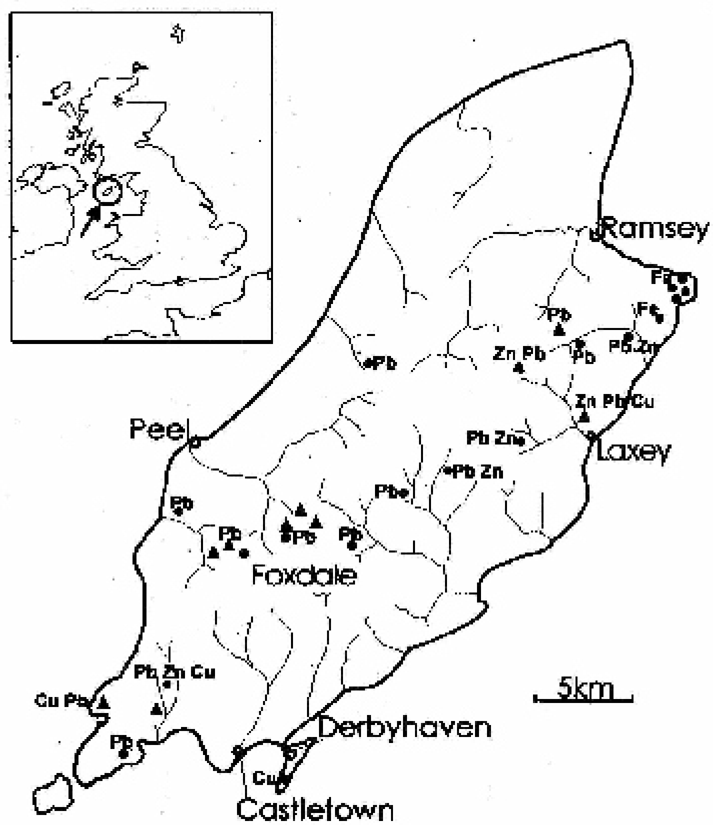


Fig 1: Locations of sampling sites around the Isle of Man (open circles) and past mining activities. Filled triangles and circles represent major and minor producing mines respectively with an indication of the order of importance of the ores produced

Animals of a similar size (~10 2 mm shell height) were used to control for the effect of site. The study was conducted at the Port Erin Marine Laboratory, Isle of Man in 1996 as part of a larger study on population differences in the toxic effects of heavy metals in *Littorina saxatilis*.

Experimentation: *Littorina saxatilis* were collected from the above sites and transported to the laboratory in moist seaweed. Animals of similar size were acclimatized to laboratory conditions (constant temperature room of 10±1ºC, salinity ~34 psu) in tanks containing aerated seawater. They were subsequently transferred to test chambers (Parrish, 1985; Mance, 1987), by suspending samples in labelled nylon mesh bags. Test chambers consisted of 2L conical flasks aerated through a hollow glass tube through the stopper.

The animals were introduced to test solutions of the appropriate metal concentration after acclimatization for six days. Metal salts used were the sulphates of copper and cadmium ($CuSO_4.5H_2O$ and $3CdSO_4.8H_2O$). The exposure concentrations were: 0.01, 0.1, 0.5 and 1.0 mg L^{-1} added Cu and added Cd. Three replicate treatments of each concentration and controls (no metal added) were used. The solutions were aerated continuously with air filtered through a glass microfibre filter (Whatman Hepa-cap). All tanks, flasks and bags were acid-washed before use. Test solutions were replaced every other day. The exposures were terminated after 6-day periods and samples of 8 pooled individuals per treatment were analysed for each metal.

Samples were oven-dried to constant weight and digested according to Harper (1989). Blanks and reference material of *Mytilus edulis* (European Communities of Reference-BCR ref. material 278) were included. The concentrations of metals in samples were blank-corrected and expressed as $\mu g\ g^{-1}$ dry weight. Measured values of the metals in the reference materials were all within 10 % of the certified values.

Data Analysis: Linear regression models were used to determine metal accumulation rates; for Cu polynomial regressions were also applied. Analysis of covariance (ANCOVA) was applied to the regression slopes to test for significant differences in accumulation between different populations. The analyses were performed using MINITAB R14.

RESULTS

At the lower concentrations (0.1 to 0.5 mg L^{-1} Cu), Cu accumulation increased with concentrations of the metal but above 0.5 mg L^{-1} a sharp decline in net uptake occurred in animals from all sites (Fig. 2). Linear curve fits for the tissue Cu concentrations over the spectrum of exposure concentrations were poor with very low coefficients of determination (Fig. 2, Table 1). The

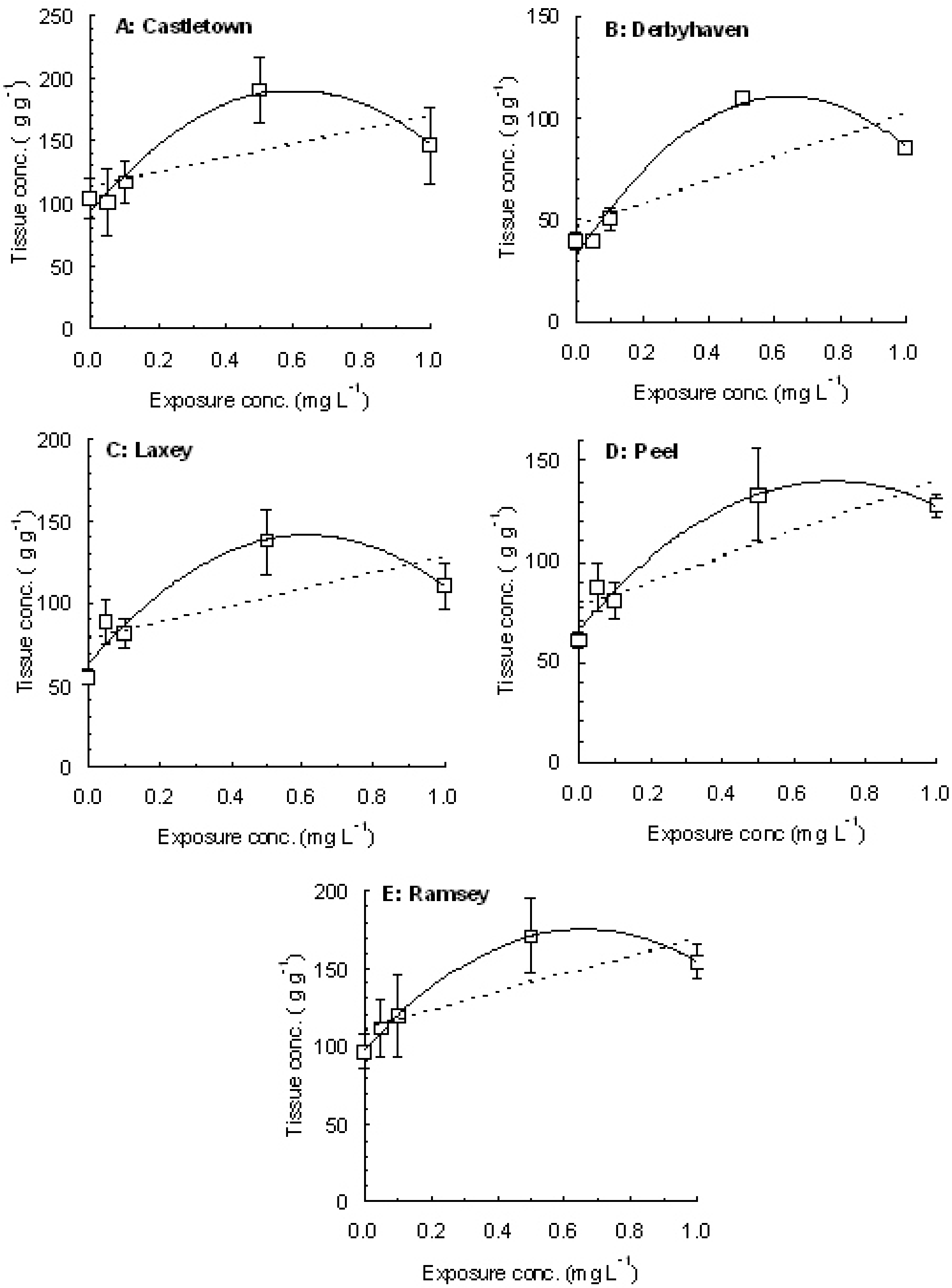


Fig 2: Regressions of tissue copper burdens (μg g^{-1} dry weight) with increasing exposure concentration (mg L^{-1} added Cu) in *Littorina saxatilis* from sites around the Isle of Man. Dotted line = linear regression; solid line = polynomial (quadratic) regression

exclusion of the values for 1.0 mg l^{-1} Cu improved the linearity of the fit lines (Table 1). However, ANCOVA on the regression coefficients with all values included or without the values for 1.0 mg L^{-1} showed no significant difference in slopes between sites (Table 1). Polynomial curve fits gave a better representation of the accumulation of Cu when all concentrations are included in the regression with coefficients of determination ranging from 93.1 % to 99.8 % (Table 2).

Regression lines for the net uptake of Cd with increasing Cd concentration (Fig. 3) show that individual regressions were significant for animals from all the five sites ($p<0.001$). The slopes varied with site and individuals from Peel had the highest, whilst animals from Derbyhaven had the least (Table 3) and the differences in slope were significant (ANCOVA). The effects of initial metal concentrations in the tissue of animals in the field on the accumulation of Cd were examined by plotting the

Table 1: Regression equations using linear models for the accumulation of copper in *L. saxatilis*. Regressions with all data and with data for the highest zinc exposure concentration (1 mg L^{-1}) excluded are presented.

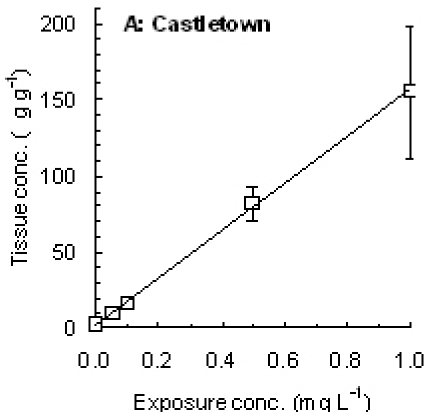
	All data		Data for 1 mg L^{-1} excluded	
Site	*Equation*	r^2	*Equation*	r^2
Castletown	$y = 56.358x + 113.14$	0.411	$y = 182x + 98.6$	0.791
Derbyhaven	$y = 55.998x + 46.302$	0.569	$y = 147x + 37.5$	0.981
Laxey	$y = 49.491x + 78.434$	0.454	$y = 143x + 67.6$	0.796
Peel	$y = 63.598x + 76.86$	0.725	$y = 130x + 69.2$	0.782
Ramsey	$y = 58.974x + 110.86$	0.644	$y = 140x + 101.0$	0.718
ANCOVA	$F_{4,75} = 0.36$, $p>0.05$		$F_{4,50} = 0.74$, $p>0.05$	

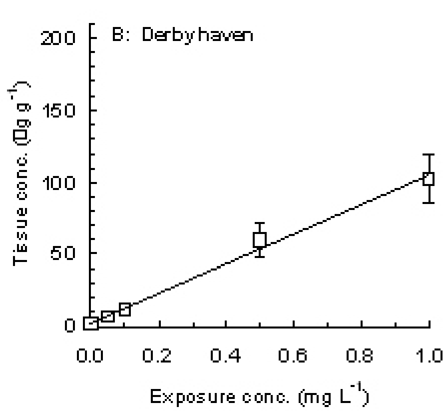
Table 2: Regression equations using polynomial (first order) models for the accumulation of copper in *L. saxatilis*.

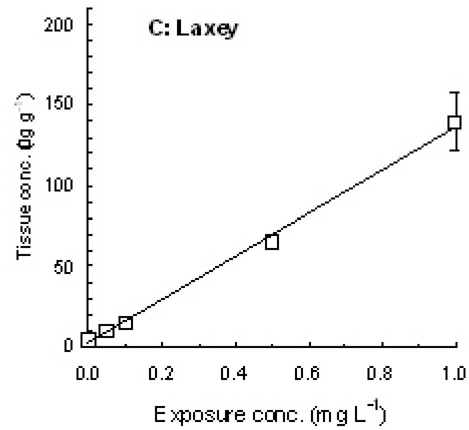
Site	*Equation*	r^2
Castletown	$y = -266.59x^2 + 319.79x + 93.519$	0.958
Derbyhaven	$y = -193.73x^2 + 247.43x + 32.044$	0.975
Laxey	$y = -208.02x^2 + 255.05x + 63.125$	0.931
Peel	$y = -146.75x^2 + 208.61x + 66.061$	0.955
Ramsey	$y = -179.2x^2 + 236.06x + 97.668$	0.998

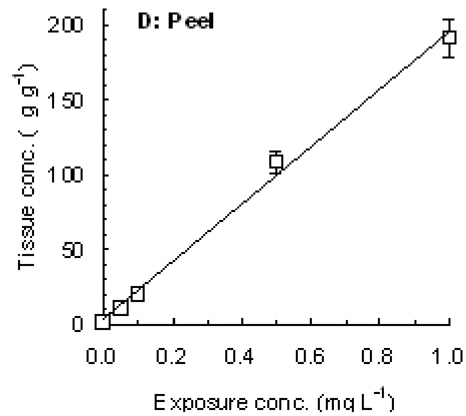
Table 3: Regression equations using linear models for the accumulation of cadmium in *L. saxatilis*.

Site	*Equation*	r^2
Castletown	$y = 154.02x + 2.3989$	0.999
Derbyhaven	$y = 102.89x + 2.5868$	0.995
Laxey	$y = 134.46x + 2.4959$	0.998
Peel	$y = 193.01x + 2.8639$	0.996
Ramsey	$y = 158.67x - 2.2849$	0.994
ANCOVA	$F_{4,65} = 17.53$, $p<0.001$	









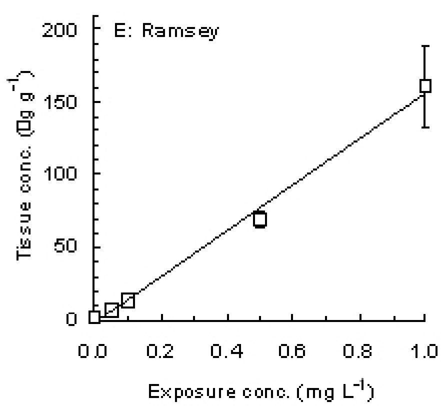


Fig 3: Regressions of tissue cadmium burdens (μg g^{-1} dry weight) with increasing exposure concentration (μg L^{-1} added Cd) in *Littorina saxatilis* from sites around the Isle of Man

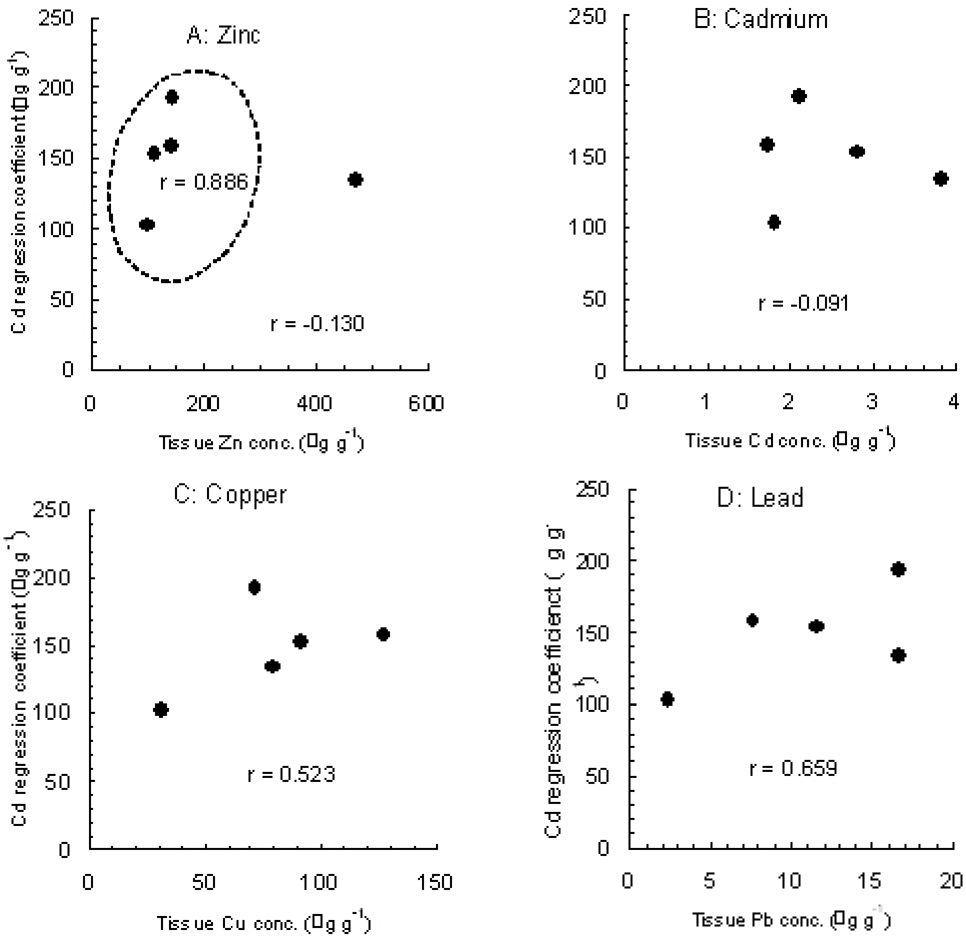


Fig 4: Relationships between cadmium accumulation rates in *L. saxatilis* and tissue concentrations of zinc, cadmium, copper and lead

relationship between Cd accumulation rate (regression coefficient) and the tissue metal concentrations (Fig. 4). There was an increase in the accumulation of Cd with increasing Zn concentration in the tissue, although at the highest Zn concentration a reversal occurred (Fig. 4A). Correlations with all data gave a non-significant negative relationship ($r = -0.130$, $p>0.05$) but exclusion of the highest Zn value produced a significant positive correlation ($r = 0.886$, $p<0.05$). The tissue concentrations of Cu, Pb and Cd in the winkles did not show any clear relationship with the accumulation of Cd and gave non-significant correlations (Fig. 4B to D).

DISCUSSION

Different profiles of accumulation were found between the essential metal – copper and the non-essential metal – cadmium in *L. saxatilis*. Cadmium accumulation was monotonic over the entire range of concentrations to which the winkles were exposed, giving a significant linear relationship whereby nearly all of the variation of the regression was accounted for by the coefficient of determination (0.994 – 0.999) On the other hand, linearity of the regression of copper accumulation was observed up to 0.5 mgl^{-1} Cu, above which it was lost and a quadratic fit gave a better coefficient of determination.

Depledge (1990) and Rainbow (2002) discuss a number of generalized models of metal accumulation in invertebrates which might elucidate the different metal accumulation profiles observed for Cu and Cd in *L. saxatilis* in this study. Accumulation patterns for essential metals include regulation of body metal concentration, accumulation without excretion, and accumulation with some excretion either from the metabolically active pool or from the detoxified store. In the littoral crustacean *Palaemon elegans*, the body concentration of Zn does not show any change over an increasing range of dissolved Zn exposures until a threshold external dissolved availability is reached. Although new Zn is entering the body in significant amounts at all exposures but an equivalent amount of Zn is excreted to match the rate of Zn uptake. When the rate of Zn uptake from solution exceeds the rate of excretion, the body concentration of Zn then rises above the regulated values. There also appears to be regulation of Cu in the same species over a wide range of

Cu availabilities until regulation breaks down. Then an increase in body Cu concentration follows any further increase in dissolved Cu exposure concentration, with a pattern reflective of accumulation with some excretion. Littorinids are known to be capable of regulating the levels of certain metals especially those essential for metabolic activities such as Cu, Zn, Mn and Fe (Bryan, 1983; Webb, 1990). The Cu exposure concentrations applied in this study were rather high and therefore presumably above the threshold of regulation for Cu (according to the above models) and accumulation did take place from 0.01 mg/l up to 0.5 mg Cu L^{-1} exposure concentration. The pattern depicted by the accumulation above 0.5 mg L^{-1} Cu defies any of the models. Given that the threshold of regulation had been exceeded, one would expect tissue concentration to be higher at 1.0 mg L^{-1} than 0.5 mg L^{-1}. Rather, a reversal occurred whereby higher mean concentrations were found in the tissue of animals exposed to the lower concentration (i.e. 0.5 mg L^{-1}). It is unlikely that vigorous Cu excretion would have taken place at 1 mg l^{-1} Cu exposure to compensate for the influx of the metal far and above that at 0.5 mg L^{-1} but the physiological basis for this is uncertain. Perhaps an interplay of physiological and behavioural mechanisms (mainly retraction and shell closure) is involved. Iintracellular activities, the complex interactions of which may determine the distribution and concentrations of metals in *Littorina littorea* have been identified (Mason, 1983), and these could be applicable to *L. saxatilis*. One type involved very specific cells, such as pore cells and connective tissue calcium cells, which occur diffusely in the connective tissue and which accumulate specific metals (i.e. Cu and Mg respectively) along precise metabolic pathways protected from the influence of other interfering metals. These cells are involved in the metabolism of the copper-containing respiratory protein haemocyanin. Webb(1990) showed that Cu may be bound to a different type of ligand (from Zn, Mn and Fe) in *L. saxatilis* since excretion of these metals occurred but no Cu excretion was observed under his experimental conditions, suggesting that behavioural responses might be important in explaining the Cu accumulation patterns observed.

Non-essential metals may be accumulated without excretion or with some excretion (Rainbow, 2002). The accumulation pattern observed in this study confirm that no regulation of Cd was apparent in its uptake from solution by *L saxatilis.* It is, however, not possible to state whether accumulation is accomplished with or without excretion as no radioactive tracers were used in the experiments. Basophil cells of the digestive gland and the nephrocytes of the kidney, occur at specific sites and apparently produce non-specific ligands capable of binding a wide variety of metals (Mason and Simkiss 1983). Much of the Cd accumulated by aquatic invertebrates is bound to metallothionein in the cytosol of the organ predominantly used for accumulated Cd storage (Langston, 1998; Rainbow, 2002). It is therefore likely that Cd accumulation occurred without much excretion.

Interpopulation differences in metal accumulation owing to differences in initial metal levels in the winkles at the site of collection were assessed by ANCOVA on regression coefficients. No significant differences were found in the slopes (using linear regressions) for Cu accumulation, whereas there were significant differences in regression coefficients for Cd accumulation. The differences did not appear to be related to initial Cd levels in the tissues of the animals. However, initial concentrations of Zn in the tissues showed a positive correlation with Cd accumulation, giving a synergistic relationship at lower concentrations (Fig. 4), but an outlier showed at very high tissue concentrations of Zn it was antagonistic to Cd accumulation. The chemical similarity between Zn and Cd implies that competitive binding to uptake sites, presumably to non-specific ligands could explain this situation. The Zn and Pb tolerant winkles from Laxey did not show any special accumulation pattern in comparison with the animals from other sites.

In conclusion, the accumulation Cu and Cd in *L. saxatilis* show different patterns of accumulation, basically reflecting the essential or non-essential nature of the metals. The Cu accumulation pattern also highlights the importance of behavioural mechanisms in the accumulation of the metal above the threshold of regulation, while Cd was accumulated without regulation. Tolerance to Zn and Pb did not reflect in the interpopulation profiles of Cu and Cd accumulation.

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REFERENCES

Blomqvist, S., U. Larsson and H. Borg, 1992. Heavy metal decrease in the sediments of a Balthic Bay following tertiary sewage treatment. Mar. Pollut. Bull. 24: 258-266.

Bryan, G.W. and Gibbs, P.E., 1983. Heavy metals in the Fal Estuary, Cornwall: A study of long term contamination by mining waste and its effects on estuarine organisms. Mar. biol. Ass. UK. Occ. Publ. Ser. 2, 112pp.

Bryan, G.W., 1976. Heavy metal contamination in the sea. In: Marine pollution, Johnston, R.(Ed.). Academic Press, London, pp: 185-302.

Bryan, G.W., 1984. Pollution due to heavy metals and its compounds. In: Marine Ecology vol. 5. Kinne, O. (Ed.), John Wiley, Chichester, pp:1289-1431.

Bryan, G.W., W.J. Langston, L.G. Hummerstone, G.R. Burt and Y.B. Ho, 1983. An assessment of the gastropod Littorina littorea as an indicator of heavy metal contamination in United Kingdom estuaries. J. Mar. Biol. Ass. UK., 63: 327-345.

Buckley, D.E., J.N. Smith and G.W. Winters, 1995. Accumulation of contaminant metals in marine sediments: Environmental factors and historical trends. Appl. Geochem., 10: 175-195.

Chen, M.H. and J.T. Wu, 1995. Copper, cadmium and lead in sediments of the Kaohsiung River and its harbour area, Taiwan. Mar. Pollut. Bull., 30: 879-884.

Clark, R.B., 1992. Marine Pollution. Oxford University Press, Oxford.

Daka, E. R., 2005. Heavy metal concentrations in *Littorina saxatilis* and *Enteromorpha intestinalis* from Manx Estuaries. Mar. Pollut. Bull., 50: 1452-1456.

Daka E. R. and S. J. Hawkins, 2004. Tolerance to heavy metals in Littorina saxatilis from a metal contaminated site in the Isle of Man. J. Mar. Biol. Assoc UK, 84:393-400.

Daka E.R., J.R. Allen and S.J. Hawkins, 2003. Heavy metal contamination in sediment and biomonitors from sites around the Isle of Man. Mar Pollut Bull, 46: 784-794.

Davies, B.E., 1987. Consequencies of environmental contamination by lead mining in Wales. Hydrobiologia, 149: 213-220.

Depledge M H and P.S. Rainbow 1990. Models of regulation and accumulation of trace metals in marine invertebrates. Comp Biochem Physiol 96C: 1-7.

Harper, D.J., C.F. Fileman, P.V. May and J.E. Portman, 1989. Methods of analysis for trace metals in marine and other samples. Aquatic Environment Protection: Analytical Methods, no3. MAFF, Dir. Fish. Res., 38pp.

Hunt, L.E. and A.G. Howard, 1994. Arsenic speciation and distribution in the Carnon Estuary following the acute discharge of contaminated water from a disused mine. Mar. Pollut. Bull., 28: 33-38.

Langston, W.J. and M. Zhou, 1987. Cadmium accumulation, distribution and metabolism in the gastropod Littorina littorea: The role of metal-binding proteins. J. Mar. Biol. Ass. UK., 67: 585-601.

Mance, G., 1987. Pollution threat of heavy metals in aquatic environments. Elsevier Applied Science, London.

Mason, A.Z. and K. Simkiss, 1983. Interactions between metals and their distribution in tissues of Littorina littorea (L) collected from clean and polluted sites. J. Mar. Biol. Ass. UK., 63: 661-672.

Parrish, P.R., 1985. Acute toxicity tests. In: Fundamentals of aquatic toxicology. Rand G.M. and S.R. Petrocelli (Eds.), Hemisphere Publishing Company, Washington DC, pp: 31-57,

Philips, S. and S. Unni, 1991. Content of metallic ions in water and sediments in reservoirs and rivers receiving ash effluents from thermal power stations. Trop. Ecol., 32: 236-244.

Rainbow P.S. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environ. Pollut. 120: 497-507.

Reid D. G., 1996. Systematics and evolution of Littorina, The Ray Society, London.

Shear, N.M., C.W. Schmidt, S.L. Huntley, D.W. Crawford and L.B. Finley, 1996. Evaluation of factors relating to combined sewer overflows with sediment contamination of the lower Passaic River. Mar. Pollut. Bull., 32: 288-304.

Webb, S.F., 1990. Intraspecific differences in the response of the littoral gastropod Littorina saxatilis (Olivi) to heavy metals. Ph.D Thesis, University of Manchester, U.K.

White, K.N. and P.S. Rainbow, 1985. On the metabolic requirements for copper and zinc in molluscs and crustaceans. Mar. Environ. Res., 16: 215-229.