Vol. 48: 295–303, 1988 | MARINE ECOLOGY – PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published October 3

Accumulation and tolerance to cadmium, copper, lead and zinc by the green mussel *Perna viridis*

H. M. Chan*

Department of Zoology, University of Hong Kong, Pokfulam Road, Hong Kong

ABSTRACT: The green mussel *Perna viridis* (Bivalvia: Mytilacea) was exposed to a range of dissolved concentrations of Cd, Cu, Pb and Zn for 21 d. Accumulation of Zn has been interpreted in terms of a regulation mechanism to maintain constant body concentrations at ca 100 µg Zn g^{-1} dry wt at external dissolved Zn levels up to a threshold concentration (between 178 and 362 µg Zn l^{-1}). Beyond this threshold, net accumulation of body Zn continued until body Zn concentration reached a plateau at 200 µg g^{-1} Cadmium, Cu and Pb were linearly accumulated by *P. viridis* in proportion to ambient metal concentrations over 7 d. Toxicity of Cd, Cu, Pb and Zn was evaluated in 96 h bioassays; LC-50 values in µg l^{-1} were 620 for Cu, 1570 for Cd, 6090 for Zn, and 8820 for Pb.

INTRODUCTION

Marine invertebrates accumulate trace metals to varying degrees, and body concentrations may reach high levels (e.g. Bryan 1979, Prosi 1979). Marine invertebrates, especially molluscs, therefore have been used widely as monitors of trace metal pollution (Phillips 1977, 1980, Bryan at al. 1980, 1985), concentrations of metals in whole or parts of these organisms being taken as measures of ambient concentration. Most of these studies assume that a simple linear relation exists between metal concentration in water and in marine organisms. This may not necessarily be true. The net accumulation of any metals taken up will depend for example on the relative rates on metal excretion and storage of metal in detoxified form.

It is, therefore, inappropriate to assume that a given species will accurately reflect the ambient concentrations of pollutants in its environment without regulating the amounts accumulated. Phillips & Segar (1986) concluded that laboratory and field studies are required to prove the indicator ability of any untried species. Consequently, the aim of the present study was to examine the pattern of accumulation of essential (Cu and Zn) and non-essential (Pb and Cd) trace metals by the green-mussel *Perna viridis* (Bivalvia: Mytilacea). Additionally, short-term toxic effects of metals on *P. viridis* have also been evaluated.

* Present address: School of Biological Sciences, Queen Mary College, University of London, Mile End Road, London E1 4NS, United Kingdom

MATERIALS AND METHODS

Perna viridis were collected by subaqua diving from a sublittoral population at Ma Liu Shui, Tolo Harbour, Hong Kong in November 1986. They were maintained in a laboratory seawater aquarium at 20 °C and 33 ppt salinity for at least 1 mo and used as stock for all subsequent experiments.

Experiment I: Accumulation and regulation. Prior to metal exposure, mussels were acclimatized in artificial seawater (HW Marinemix, Wiegandt GMBH & Co., F. R. Germany) for 5 d. Groups of 20 mussels (of 55 to 65 mm shell length) were selected in order to minimize effects caused by size differences. Artificial seawater was chosen as exposure medium since it represents a medium easier to reproduce than natural seawater, particularly with respect to dissolved organic matter which might chelate added metals. Moreover, artificial seawater prepared with freshly distilled water was confirmed to contain lower concentrations of Cd, Cu, Pb and Zn than natural seawater.

Each group of 20 *Perna viridis* was exposed for 21 d to one of a number of metal concentrations in 5 l artificial seawater of 33 ppt salinity at 20 °C ± 2 °C under a 12:12 h light:dark regime in acid-washed 10 l perspex tanks with continuous aeration. The mussels were not fed during the experiments.

Media were changed twice weekly, and dissolved metal levels were monitored periodically by chelation-solvent extraction and flame atomic absorption spectrometry (Kremling & Peterson 1974, Bone & Hibbert 1979). Analyses confirmed that under these conditions

0171-8630/88/0048/0295/$ 03.00

the concentrations of dissolved Cd, Cu, Pb and Zn remained within 10 % of the declared values. The artificial seawater used was found to contain inherent levels of 3.1, 11.0, 6.0 and 0.7 $\mu g\ l^{-1}$ Cu, Zn, Pb and Cd respectively. Additional metals were added as required from standard stock solutions of the metal (1000 $\mu g\ l^{-1}$), either as sulphate (Cu and Zn) or chloride (Pb and Cd) (Analar grade, BDH, UK). Salinity changes were minute but were nevertheless equalized in all experimental tanks by addition of similar aliquots of distilled water as required.

Four series of experiments were conducted. Groups of 20 mussels each were exposed to (a) copper: concentrations of 3.1 (control), 31.6, 56.2, 100, 178, and 316 $\mu g\ l^{-1}$; (b) zinc: 11.0 (control), 31.6, 56.2, 100, 316, 562 and 3162 $\mu g\ l^{-1}$; (c) lead: 6 (control), 100, 316, 562, 1000 and 1778 $\mu g\ l^{-1}$; (c) cadmium: 0.7 (control), 31.6, 56.2, 100, 178 and 316 $\mu g\ l^{-1}$. Dead mussels were removed daily (mussels were considered dead when the shell remained agape after tapping). After 21 d, surviving mussels were rinsed in clean artificial seawater and stored frozen. Upon thawing, the 20 mussels in each experimental tank were divided into 4 groups of 5 (3 to 4 when mortalities had occurred). Tissues were removed from the shells with stainless steel equipment, pooled and homogenized by a non-contaminating polytron homogenizer. The byssus was discarded. Aliquots of 5 g of the homogenate were used to determine metal levels, after digestion with 25 ml conc. nitric acid at 120 °C. The digest was analysed for Cd, Cu, Pb and Cd by flame atomic absorption spectrophotometry. A further 5 g aliquot of the homogenate was dried at 110 °C and the dry:wet weight ratio determined. All metal concentrations are expressed in $\mu g\ g^{-1}$ dry tissue weight.

Experiment II: Rate of accumulation. Groups of 80 *Perna viridis* (35 to 45 mm shell length) were acclimatized in artificial seawater for 5 d. All groups were exposed to various metal concentrations in 15 l artificial seawater in acid-washed 30 l tanks. Metal concentratrion regimes were: Cu: 50 and 100 $\mu g\ l^{-1}$; Zn: 100 and 200 $\mu g\ l^{-1}$; Pb: 100 and 200 $\mu g\ l^{-1}$; Cd: 100 and 200 $\mu g\ l^{-1}$. Media were changed daily and metal levels monitored. Dead mussels were removed daily over the 7 d study; 15 live mussels were randomly sampled from experimental tanks at the end of Days 1, 2, 5 and 7 and analysed for Cd, Cu, Pb and Zn.

Experiment III: Mortality. Groups of 40 *Perna viridis* (shell length 30 to 40 mm) were maintained in 5 l artificial seawater in acid-washed 10 l perspex tanks. Experimental concentrations were established on the basis of results of previous trial experiments: Cu: 3.1 (control), 100, 250, 400, 630 and 1000 $\mu g\ l^{-1}$; Zn: 11 (control), 2500, 5000, 7500, 10 000 and 12 500 $\mu g\ l^{-1}$; Pb: 6 (control), 2500, 7500, 10 000, 12 500 and 15 000 $\mu g\ l^{-1}$; Cd: 0.7 (control), 250, 750, 1000, 1500 and 2500 $\mu g\ l^{-1}$. Media were changed daily. Mussels were disturbed as little as possible and inspected twice daily; dead ones being removed. Cumulative mortalities were recorded after 96 h. Experiments were conducted between December 1986 and January 1987.

RESULTS

Experiment I: Accumulation and regulation

Table 1 gives a record of survivorship of mussels exposed to the increasing levels of Cu, Zn, Pb and Cd after 21 d. Regression analysis of accumulated concentration against dose concentration indicate that Cu, Pb and Cd are all accumulated in proportion to exposure levels at all external metal exposures (Fig. 1a, c, d). Data for the Cu control experiment (3.1 $\mu g\ l^{-1}$), however, do not fall close to the line of best fit indicating

Table 1. *Perna viridis*. Percent surviving exposure for 21 d to various concentrations of Cu, Zn, Pb, and Cd salts. Initial number per group = 20. Dash: exposure not conducted

Exposure levels ($\mu g\ l^{-1}$)	Cu	Zn	Pb	Cd
0.7	–	–	–	100 (control)
3.1	100 (control)		–	–
6.0	–	–	90 (control)	–
11.0	–	65 (control)	–	–
31.6	95	75	–	90 (control)
56.2	95	60	–	75
100	95	75	60	75
178	80	–	–	60
316	0	80	75	60
562	–	75	65	–
1000	–	–	75	–
1778	–	–	65	–
3160	–	0	–	–

that a considerable amount of Cu was present in the mussel body even at low ambient concentrations. This concentration may be equivalent to the baseline level for the metabolic requirements of the mussel (White & Rainbow 1985).

There is no significant difference ($p > 0.05$) between the mean Zn body concentrations of controls and mussels exposed to Zn levels of up to 100 μg l^{-1} after 21 d (Fig. 1b); these were significantly lower than those for higher exposure levels (316 and 562 μg l^{-1}). This implies an ability of *Perna viridis* to maintain a constant body Zn concentration at ca 100 μg g^{-1} at exposures up to 100 μg l^{-1}. At higher levels of dissolved Zn, there is an increase in body Zn concentration which then

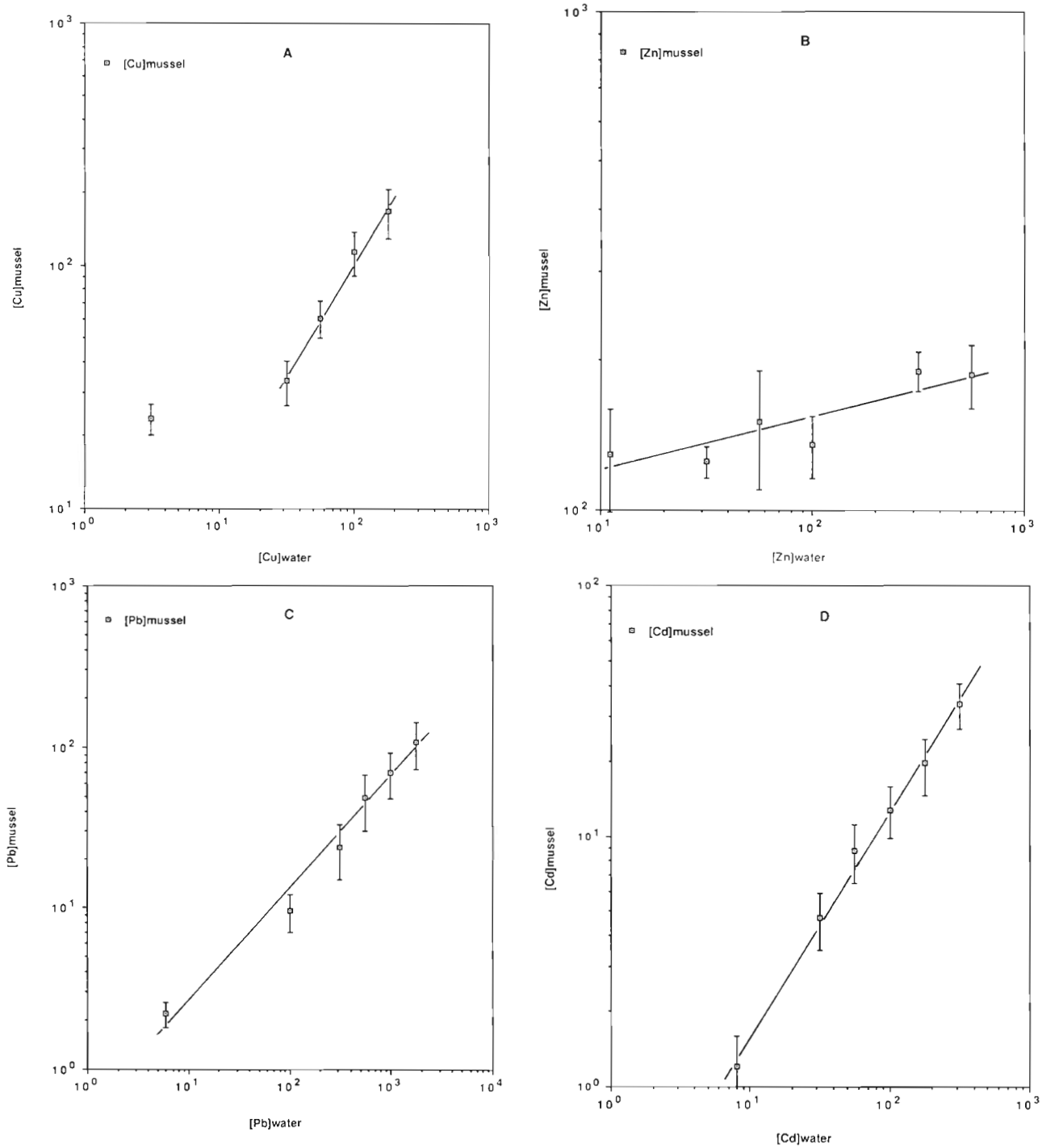

Fig. 1. *Perna viridis*. Mean body metal concentrations (μg g^{-1} dry wt ± SD) in individuals exposed to increasing concentrations of dissolved metals (μg l^{-1}) for 21 d at 20 °C (log-log plot). (A) Copper, (B) zinc, (C) lead, (D) cadmium

reaches a plateau at around 250 μg g^{-1}. The highest measured tissue concentration of dead mussels exposed to 3162 μg l^{-1} was only 250 μg g^{-1}.

Tissue metal concentration as a function of the level of contamination in experimental media can be expressed in terms of concentration factor (CF), where

$$CF = \frac{\text{conc. in contaminated organisms (μg } g^{-1} \text{ wet wt)}}{\text{conc. in contaminated media (μg } l^{-1})}$$

In the case of Cu, Pb and Cd, concentration factors showed an initial decrease with increasing metal concentrations in the experimental media, then rapidly reached constant values of 200, 10 and 25 for Cu, Pb and Cd respectively (Table 2). In these instances, the increase in the levels of Cu, Pb and Cd in the mussels therefore paralleled the increase of dissolved concentration of these metals in the experimental media. The decreasing trend did not stop for Zn, however, implying that the body Zn concentration was independent of the ambient Zn level and remained almost constant.

Experiment II: Rate of accumulation

The pattern of net accumulation was similar for Cu, Pb and Cd (Fig. 2a, b, d). These metals were continuously accumulated in the tissues of *Perna viridis* throughout exposure, and correlations of the regression lines ($p < 0.01$) indicate that accumulation is linear with time. Assuming a linear time course over the 7 d experiment, the rate of net accumulation was calculated to be 8.57 and 17.1 μg g^{-1} d^{-1} in 50 and 100 μg l^{-1} copper media respectively. Rates of uptake in 100 μg l^{-1} and 200 μg l^{-1} contaminated media were estimated to be 1.96 and 3.62 μg g^{-1} d^{-1} for Pb, and 0.62 and 1.13 μg g^{-1} d^{-1} for Cd. The Cu, Pb and Cd concentrations in the organism increased with increases in the metal concentrations of the experimental media after a given time from the start of the experiment.

The pattern of Zn accumulation was different from that of the other 3 metals (Fig. 2b). Analysis of variance has shown that there is no significant difference between the Zn concentrations of the control mussels and those exposed to 100 and 200 μg Zn l^{-1} ($F_s = 2.47$, $p > 0.05$, d.f. = 2,33). No significant correlation between body Zn concentration and time of exposure could be obtained. The Zn concentration of all mussels remained almost constant at around 100 μg g^{-1}. Evidently *Perna viridis* can maintain a constant body Zn concentration at exposures up to 200 μg l^{-1} throughout the experimental period.

Experiment III: Toxicity

Results obtained from toxicity tests are expressed as LC_{50}, i.e. the concentration of trace metals killing 50 % of the mussels during the 96 h exposure period. The LC_{50} values were estimated by probit analysis and the results are summarized in Table 3. Metal toxicity in *Perna viridis* increases in the sequence Pb < Zn < Cd < Cu. The LC_{50} 96 h for *P. viridis* in μg l^{-1} is 620 for Cu, 6090 for Zn, 8820 for Pb, and 1570 for Cd.

A visible sign of metal toxicity was an increase in mucous secretion by the treated mussels, resulting in media foaming. The frothing increased with increasing metal concentration. Another response of the mussels to metal exposure was sustained valve adduction, particularly at higher concentrations.

No deaths occured in the controls.

Table 2. *Perna viridis*. Concentration factors* of surviving after exposure to various concentrations of Cu, Zn, Pb and Cd salts for 7 d. Dash: no exeriment at this exposure level; NS = no survivors

Exposure levels (μg l^{-1})	Cu	Zn	Pb	Cd
0.7	–	–	–	198 (control)
3.1	1508 (control)	–	–	–
6.0	–	–	66 (control)	–
11.0	–	2342 (control)	–	–
31.6	214	796	–	29
56.2	220	592	–	31
100	230	286	24	26
178	186	–	–	24
316	NS	122	14	23
562	–	66	15	–
1000	–	–	12	–
1778	–	–	11	–
3160	–	NS	–	–

* $\frac{\text{Concentration in mussel soft tissues in μg } g^{-1} \text{ wet wt}}{\text{concentration in medium in μg } l^{-1}}$

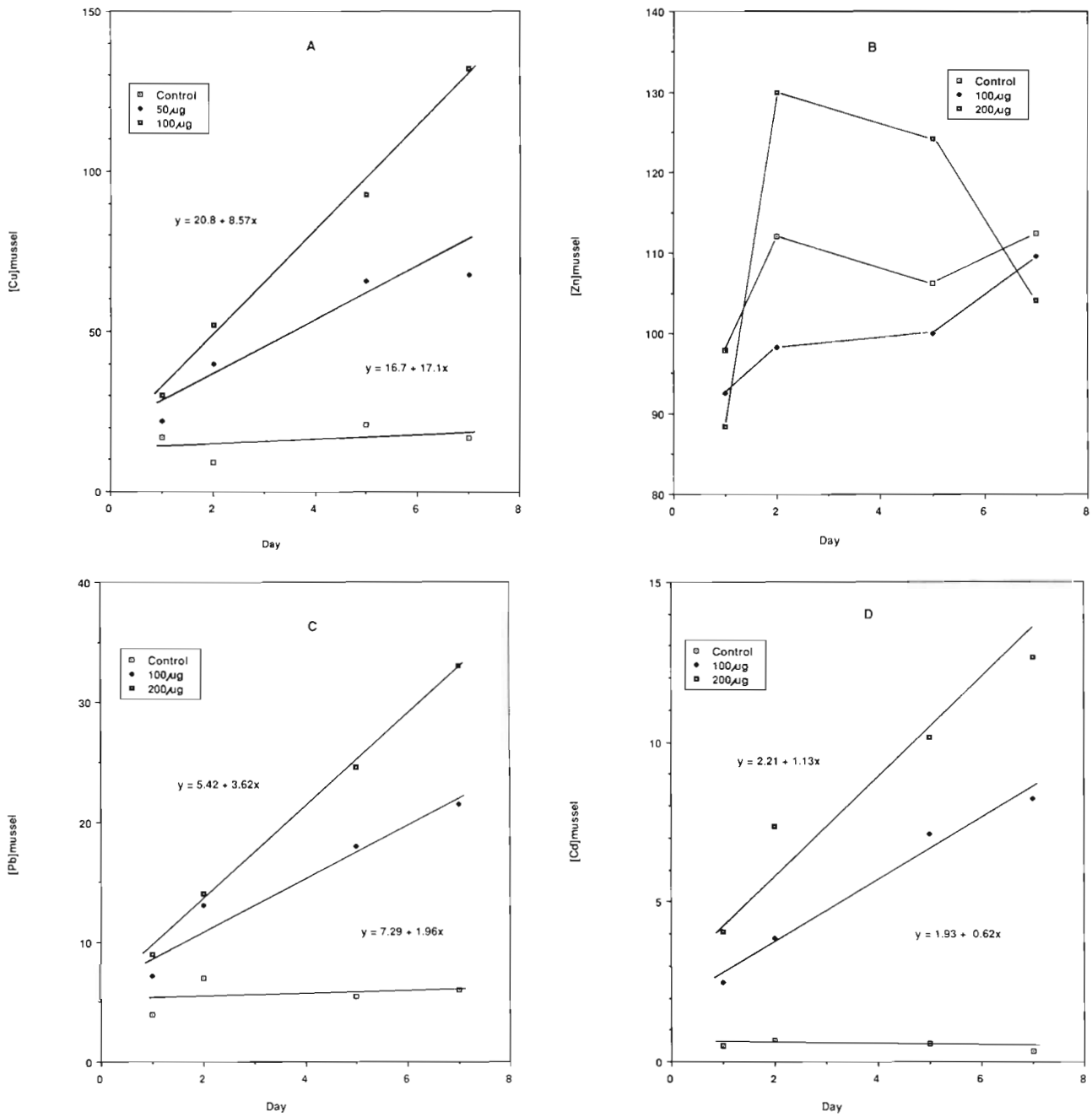

Fig. 2. *Perna viridis*. Mean metal uptake at different exposure concentrations with time. (A) Copper, (B) zinc, (C) lead, (D) cadmium

Table 3. *Perna viridis*. Probit equations and LC-50 (96 h) values for Cu, Zn, Pb, and Cd

Metal	Probit regression equation (y= probits, x = log dose)	96 h LC-50 ($\mu g\ l^{-1}$)	95 % fiducial limit	
			lower	upper
Cu	y = 2080 x − 1060	620	440	1010
Zn	y = 2840 x − 7120	6090	4320	8560
Pb	y = 2570 x − 5990	8820	4740	12610
Cd	y = 2210 x − 2070	1570	1280	1960

DISCUSSION

In the present study a linear accumulation by *Perna viridis* of Cu, Pb and Cd over a wide range of concentrations has been recorded. A similar accumulation pattern of mussels has been reported for Cu (D'Silva & Kureishy 1978, Ritz et al. 1982), Pb (Schulz-Baldes 1974, Tan & Lim 1984), and Cd (Poulsen et al. 1982, Ritz et al. 1982, Amiard-Triquet et al. 1986). The rate of accumulation of each metal is proportional to the level of contamination over 7 d. *P. viridis* concentrated Cu, Pb and Cd from experimental media up to 200, 10 and 25 times respectively. These concentration factors remained almost constant over a wide contamination range, increases in body concentration of these metals paralleling increases in ambient dissolved metal concentrations.

Martin (1979) reported that in *Mytilus edulis* the lethal accumulation level was 59.9 $\mu g\ l^{-1}$ for Cu and the lethal time was 6 d for exposure concentrations higher than 100 $\mu g\ l^{-1}$. The present study, however, has shown that *Perna viridis* can accumulate over 100 $\mu g\ g^{-1}$ Cu when exposed to 200 $\mu g\ l^{-1}$ medium for 7 d. Cu concentrations for in situ *P. viridis* can reach 300 $\mu g\ g^{-1}$ (Phillips 1985). Since experimental mussels were collected from a fairly clean site according to Phillips (1985) and had been maintained in laboratory conditions for 60 d, the argument put forward by Martin (1979) that tolerance was induced by adaptation cannot apply to *P. viridis*. Other factors such as temperature and salinity may also affect the toxicity of trace metals to mussels (McLusky et al. 1986). However, these experimental conditions are often not stated (e.g. Martin 1979). It is therefore not possible to make direct comparisons. Nevertheless, results of this study suggest that *P. viridis* can accumulate high levels of Cu as compared with other mussels (Eisler 1981). Since the maximum sublethal concentration accumulated under the experimental conditions (178 $\mu g\ g^{-1}$) is not much lower than the concentration found in dead mussels exposed to 316 $\mu g\ l^{-1}$ for 11 d (256 $\mu g\ g^{-1}$), the range between tolerable and lethal concentrations is narrow for Cu. Mortality has presumably resulted from a failure to balance the rate of incorporation of Cu into detoxification pathways to the increased rate of uptake under high Cu exposure concentrations.

An ability to accumulate and tolerate high body concentrations of Cu, Pb and Cd implies that *Perna viridis* possesses mechanisms that prevent interaction of the toxic metals with essential enzymes. Metal detoxification by marine invertebrates is reportedly achieved by employing physiological and biochemical processes (Simkiss 1981) which may, for example, include the production of physiologically inert granular deposits (Mason & Nott 1981, Brown 1982) or the induction of metal-binding proteins such as metallothioneins (Cherian & Goyer 1978, Roesijadi 1981). Viarengo et al. (1981) have demonstrated that Cu^{2+}, when present in seawater at the sublethal concentration of 0.08 $mg\ l^{-1}$, is able to induce, within 48 h, the synthesis of Cu-binding proteins having a molecular weight similar to that of the metallothioneins (12 000) in the gills and mantle of *Mytilus galloprovincialis*. It is tentatively suggested that Cu accumulated from natural concentrations is stored bound to these high molecular weight proteins and thus detoxified (see Roesijadi 1981 for a review of the significance of metallothioneins in marine invertebrates).

The mechanism of immobilization and detoxification of Pb by *Mytilus edulis* has been described by Schulz-Baldes (1977). He demonstrated that Pb is taken up in gills and viscera, distributed by the blood and finally stored as a phosphorus or sulphur-rich complex in membrane-bound vesicles (i.e. in intracellular storage sites) within the excretory cells of the kidney. He suggested that uptake into the cells occurs by pinocytosis. This immobilization of Pb in a chemically inert form outside the cytoplasm would be an internal detoxification process.

Similar detoxification mechanisms have been reported for Cd. Janssen & Scholz (1979) suggested that Cd is concentrated in the midgut gland of *Mytilus edulis*. They showed that Cd is transported via the haemolymph, and that selective discrimination and accumulation occurs at the basement lamina of the digestive tubules. Within the tubules, Cd is immobilized in membrane-bound vesicles, which are finally released into the intestine and faeces.

The above mechanism may apply to *Perna viridis*, but this has not been investigated. The deposition of metals in membrane-bound cytoplasmic bodies, however, appears to be a general biological process which occurs in a variety of phyla (Fowler et al. 1981).

Results from Experiments 1 and 2 show that *Perna viridis* can regulate total body levels of Zn up to a threshold exposure level. A similar Zn regulating ability has been recorded for decapod crustaceans (Bryan 1976, White & Rainbow 1982, Rainbow 1985, 1988). Among molluscs, *Haliotis tuberculata* and *Scrobicularia plana* are able to regulate Zn body levels (Bryan et al. 1977, Bryan 1979). Little experimental data pertaining to Zn regulation in mussels is available. Amiard-Triquet et al. (1986) have demonstrated the ability of *Mytilus edulis* to maintain a normal concentration in all groups of organs for 4 d; in the visceral mass, and the 'remainder' (mantle, muscles, gonads) for 8 d; and in the 'remainder' alone for up to 16 d. But with higher doses and increasing exposure time, the regulatory mechanism seemed to be perturbed. Tissue Zn accumulation by *M. edulis* increased linearly when exposed

Table 4. Acute toxicity of Cu, Zn, Pb, and Cd to marine mussels

Species	Metal	LC-50 in μg l^{-1} (time)	Source
Perna viridis	Cu	140 (48 h)	D'Silva & Kureishy (1978)
	Zn	2310 (48 h)	
	Pb	4460 (168 h)	Tan & Lim (1984)
	Cd	1020 (168 h)	
	Cu	620 (96 h)	Present study
	Zn	6090 (96 h)	
	Pb	8820 (96 h)	
	Cd	1570 (96 h)	
Mytilus edulis	Cu	480 (96 h)	Amiard-Triquet et al. (1986)
	Zn	> 5000 (96 h)	
	Cu	1550 (96 h)	
Mytilus edulis planulatus	Zn	2500 (96 h)	Ahsanullah (1976)
	Cd	1620 (96 h)	

to 100–200 μg Zn l^{-1} over 35 to 86 d (D'Silva & Kureishy 1978, Ritz et al. 1982). Results of D'Silva & Kureishy (1978) for *P. viridis*, however, reveal that the ratio between Zn concentration of the most contaminated mussels and the control 35 d exposure was less than 3. Field studies have shown that the levels of Zn in *M. edulis, Trichomya hirsuta* and *Septifer bilocularis* do not vary considerably between polluted and unpolluted areas (Phillips & Yim 1981, Klumpp & Burdon-Jones 1982, Lobel et al. 1982, Phillips 1985). Results of field studies on *P. viridis* (Chan in press) confirm these observations and thus corroborate the hypothesis that Zn is regulated by all mussels (Mytilacea) hitherto studied.

George & Pirie (1980) have presented a metabolism scheme for Zn in *Mytilus edulis*. The metal is taken up by both gill and mantle (possibly as soluble Zn), as well as the gut (possibly as particulates or mucus-bound). Zn from the gill and gut is transported via the haemolymph to the kidney where it is stored in membrane-bound granules which occupy about 20 % of the kidney volume. These granules, which have a long half life, are eventually excreted in the urine (George 1983, Roesijadi et al. 1984, Lobel 1986). It is possible that the half life of the Zn-bound granules is much shorter for *P. viridis* resulting in the narrow range of tissue concentrations recorded.

In the present study LC-50s for Cu, Zn and Pb are generally higher than values previously reported for marine mussels (Table 4), while data for Cd had a similar range. The reasons for these differences are not known but may be related to differences in age, size and condition of the test animal, and the test environment. Toxicity is also influenced not only by the intrinsic toxicity of the element, but also by its availability as determined by occurrence, complexation of other chemical reactions and absorption potential. Bryan (1976) has listed a series of factors influencing toxicity of heavy metals in solution. These include: the dissolved form of the metal, the presence of other metals, factors influencing the physiology and behaviour of the organism. Environmental factors such as temperature and salinity may also affect the toxicity of metals to the organism (McLusky et al. 1986). Nevertheless, when sensitivities to metals (Cu, Zn, Pb, Cd) are compared (Table 4), a common sequence is obtained. *Perna viridis* is most sensitive to Cu and least senstitive to Pb.

The present study confirms the ability of *Perna viridis* to accumulate dissolved Cu, Pb and Cd in proportion to exposure levels. *P. viridis* is also efficient in regulation Zn, but not Cu as reported for *Mytilus edulis* by Phillips (1976, 1980) and Amiard-Triquet et al. (1986). Difference between minimum and maximum values of Zn concentrations among experimentally contaminated and control mussels are of the same order of magnitude as those observed in wild populations of mussels resulting from spatial and temporal factors (Chan unpubl.). *P. viridis*, therefore, is of limited value as an indicator of Zn pollution.

Acknowledgements. I thank my supervisor Professor Brian Morton for his constructive criticism on this manuscript. I am also grateful to Dr P. S. Rainbow and Dr D. J. H. Phillips for invaluable advice. This work formed part of a thesis submitted to University of Hong Kong for the degree of Master of Philosophy.

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This article was presented by Dr G. W. Bryan; it was accepted for printing on August 1, 1988