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Toxicity Bioassays of Cadmium on Selected Freshwater Invertebrates and the Interaction of Cadmium and Zinc on the Freshwater Shrimp, *Paratya tasmaniensis* Riek

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Abstract

In acute toxicity bioassays with cadmium sulphate at 15°C in soft water (total hardness 10 mg/l as calcium carbonate), the concentrations fatal to 50% of the test animals were determined for five freshwater invertebrate species. The 96 hr median lethal concentration (LC50) of cadmium was 0·04 mg/l for the amphipod *Austrochiltonia subtenuis* Sayce, 0·06 mg/l for the shrimp *Paratya tasmaniensis* Riek, 0·84 mg/l for the ephemeropteran nymph *Atalophlebia australis* Walker, 250 mg/l for the zygopteran nymph *Ischnura heterosticta* (Burmeister) and well over 2000 mg/l for a trichopteran larva of the Leptoceridae. The bioassays on *Paratya* indicated that there may be seasonal differences in sensitivity to cadmium. The 96 hr LC50 for zinc for *Paratya* was 1·21 mg/l. Zinc and cadmium appeared to interact less than additively at concentrations below 1 toxic unit. Above this concentration, their interaction was strictly additive.

Introduction

The South Esk River has been polluted by effluents from tin and tungsten mines since the late nineteenth century. The chief pollutants are sulphuric acid, cadmium, zinc, copper, lead, iron and manganese (Tyler and Buckney 1973), with cadmium and zinc being the major pollutants. A survey of the South Esk River indicated that both the abundance and the distribution of the macroinvertebrate fauna have been markedly affected (Thorp and Lake 1973).

The aims of this study were to evaluate the distribution of five invertebrate species in the South Esk River in relation to their sensitivity to cadmium in laboratory bioassays and also to investigate the interaction between cadmium and zinc on the shrimp *Paratya tasmaniensis*.

Materials and Methods

The bioassay procedures were based on those in 'Standard Methods' (APHA 1965) with modifications similar to those of LaRoche *et al.* (1970) and Eisler (1971).

Collection and Storage of Experimental Animals

All experimental animals were collected from Pawleena Dam near Sorell or from the Coal River near Richmond. *Paratya tasmaniensis* and the ephemeropteran nymphs were collected from Pawleena, while the zygopteran nymphs, the trichopteran larvae and the amphipods were collected from the Coal River. All of the animals were maintained for approximately 14 days at 12–13°C in aerated water collected

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from an unpolluted creek (Dunn's Creek at Ferntree), before acclimation to the experimental conditions. The aquatic macrophytes, *Myriophyllum elatinoides* and *Scirpus fluitans*, and some detritus were placed in the holding containers and small amounts of powdered fish food were added every few days.

General Bioassay Procedure

Cadmium. Cadmium stock solutions were made from cadmium sulphate (3 $CdSO_4.8H_2O$) (Univar grade, Ajax Chemicals Ltd., Sydney) in distilled water at 0·1, 1·0 and 10 mg/ml. Aliquots of these stock solutions were added to the bioassay containers about 60 min after the bioassay animals had been added. The stock solutions were added to achieve the desired cadmium concentrations.

The stock solutions used were never more than 2 weeks old in order to minimize loss of cadmium, which may be up to 5% after this period in aerated containers without organisms (Eisler 1971).

Animals were acclimatized in a room at $15 \pm 1°C$ in creek water in 5 gal glass aquariums for 7 days prior to any bioassay. All of the bioassays were conducted at $15 \pm 1°C$ under continuous subdued fluorescent light. The animals were not fed 48 hr prior to the experiments.

The test containers were flat-bottomed, half-gallon plastic containers. One litre of creek water (total hardness about 10 mg/l as calcium carbonate) was added to each container and then the test organisms added. The weights of animals and number used per container are shown in Table 1.

It was necessary to use a minimum of eight concentrations of cadmium, two replicates per concentration and at least 10 animals per concentration. The number of mortalities was recorded at 24 hr intervals over a 96 hr period, with other observations in between.

The criteria for determining death were lack of respiratory movements and no response to prodding over a period of 2–3 min. The concentrations of cadmium tolerated by 50% of the individuals of each test species were determined by probit analysis (Finney 1947).

Cadmium bioassays were performed on *Paratya tasmaniensis* Riek (Decapoda: Atyidae), *Austrochiltonio subtenuis* Sayce (Amphipoda: Ceinidae), *Atalophlebia australis* Walker (Ephemeroptera: Leptophlebiidae), *Ischnura heterosticta* (Burmeister) (Odonata: Coenagrionidae) and an unidentified species of the Leptoceridae (Trichoptera).

Difficulties experienced with some of the experimental species. Paratya proved to be the most satisfactory species. The trichopterans were extremely resistant to cadmium and their responses were irregular. The zygopteran nymphs interacted aggressively, pulling off gills and occasionally killing one another. Bioassays with ephemeropteran nymphs were disrupted by the emergence of several subimagos. The bioassays with the amphipod *Austrochiltonia subtenuis* were complicated by the presence of *A. australis*. These closely related species occur together at the collection site and can only be separated by microscopical examination after experimentation. Further bioassays were conducted only with *Paratya*.

Zinc. The bioassay procedures were similar to those performed with cadmium. Zinc stock solutions were made from zinc sulphate ($ZnSO_4.7H_2O$) (Univar grade, Ajax Chemicals Ltd., Sydney) in distilled water. Three replicates with six animals per container were used at each of eight concentrations of zinc.

Zinc and cadmium. The interaction of cadmium and zinc was investigated using a method based on that of Herbert and Shurben (1964). These authors express the threshold concentrations of two poisons P and Q as P_+ and Q_+, and the concentrations to which animals are exposed as P_s and Q_s. With one pair of poisons, it has been found that the threshold concentrations of a mixture is approximately that for which $P_s/P_+ + Q_s/Q_+$ equals one (Lloyd 1961; Herbert 1962). This threshold concentration was called one toxic unit (TU) by Sprague and Ramsay (1965).

Table 1. Weights and lengths of animals

Organism[A]	Mean length (mm)	Wt (g)
Paratya tasmaniensis	22·4	0·08 (mean of 40)
Atalophlebia australis	13·6	0·007–0·01
Austrochiltonia subtenuis		0·002–0·003
Ischnura heterosticta		0·04–0·06
Leptoceridae sp.		0·005–0·007

[A] 5–6 used per container.

In our experiments, the value of one toxic unit was assigned to the 96 hr LC50 values determined for cadmium in July and for zinc in August. Tests were made with the concentration ratios of cadmium and zinc as $Cd_S/Cd_{T50} : Zn_S/Zn_{T50} = 1 : 1 \cdot 63$, where Cd_{T50} and Zn_{T50} are the 96 hr LC50 values for cadmium and zinc respectively. A range of concentrations from 0·1 toxic units to 10 toxic units was used. The data from the experiments with cadmium alone, zinc alone and with the cadmium–zinc mixtures were then analysed jointly with a sequence of models:

(1) With separate probit lines in the three experiments;

(2) with parallel probit lines in the three experiments;

(3) with a common probit line.

This permitted (1) a joint test of fit for the probit models, (2) a test whether the lines could be regarded as parallel, and (3) if (2) were accepted, a test whether the probit lines were coincident. In particular, this amounts to a test whether the LC50's are equal.

A second procedure for testing whether zinc and cadmium interact in *Paratya* was based on the method of Warren (1971). Solutions of 0·06 mg/l cadmium (96 hr LC50 in July) and 1·8 mg/l zinc were combined in the following ratios of cadmium to zinc: 100 : 0, 75 : 25, 50 : 50, 25 : 75, 0 : 100. Percentage survival at 96 hr was plotted against each of these concentrations. The bioassay procedure was the same as that for zinc.

Results

The trichopteran larvae were most resistant, with no deaths occurring in 96 hr in concentrations up to 2000 mg/l, even when deprived of their cases. Table 2 shows that the crustaceans were most sensitive, with LC50 values for 96 hr exposure of 0·06 mg/l. with 95% confidence limits from 0·03 to 0·10 mg/l for *Paratya tasmaniensis* (July) and 0·04 mg/l with 95% confidence limits from 0·03 to 0·05 mg/l for *Austrochiltonia subtenuis* (August).

Table 2. Concentrations of cadmium and zinc allowing survival of 50% of individuals of selected invertebrate species at 15°C, in soft water, at 24, 48, 72 and 96 hr

Experiment	Time (hr)	LC50 (and 95% confidence limits)	χ^2	DF	Probability	a	b (and 95% confidence limits)
Ischnura	48	1654 (599, 0·68)[A]	2·72	3	NS	6·300	−1·957 (−4·403, 0·488)
heterosticta	72	626 (279, ∞)	4·08		NS	3·263	−1·665 (−2·290, −0·044)
Cd 29.vi.72	96	233	7·91		$0·05 > P > 0·02$	2·231	−0·942 (−1·961, −0·077)
Atalophlebia	48	269 (0, 65)	21·39	6	$0·01 > P > 0·001$	1·094	−0·450 (−1·062, 0·161)
australis	72	4·3 (1·6, 116)	14·79		$0·05 > P > 0·02$	0·581	−0·924 (−1·500, −0·347)
Cd 25.ix.72	96	0·84 (0·44, 2·25)	5·09		NS	−0·077	−1·004 (−1·503, −0·505)
Austrochiltonia	24	0·195 (0·126, 0·411)	3·86	5	NS	−1·099	−1·548 (−2·176, −0·921)
subtenuis	48	0·09 (0·07, 0·13)	9·01		NS	−2·091	−2·014 (−2·657, −0·137)
Cd 2.viii.72	72	0·05 (0·04, 0·07)	15·14		$0·01 > P > 0·001$	−2·652	−2·074 (−2·717, −1·430)
	96	0·04 (0·03, 0·05)	10·72		NS	−3·348	−2·309 (−3·045, −1·573)
Paratya	24	0·62 (0·38, 1·58)	2·85	5	NS	−0·380	−1·831 (−2·772, −0·889)
tasmaniensis	48	0·47 (0·29, 1·05)	7·31		NS	−0·559	−1·721 (−2·551, −0·891)
Cd 9.vii.72	72	0·16 (0·09, 0·29)	1·36		NS	−1·161	−1·477 (−2·163, −0·790)
	96	0·06 (0·03, 0·10)	1·95		NS	−2·201	−1·828 (−2·681, −0·974)
Paratya	24	4·5 (1·3, 78327)	1·48	5	NS	0·731	−1·118 (−2·001, −0·234)
Cd 14.x.72	48	1·2 (0·56, 14·1)	2·72		NS	0·094	−1·040 (−1·651, −0·429)
	72	0·46 (0·28, 1·16)	7·60		NS	−0·406	−1·208 (−1·776, 0·640)
	96	0·18 (0·12, 0·28)	9·41		NS	−0·146	−1·538 (−21176, −0·9593)
Paratya	24	14·5 (8·2, 63)	5·68	6	NS	1·924	−1·656 (−2·499, −0·814)
Zn	48	8·3 (5·3, 18)	6·94		NS	1·488	−1·624 (−2·277, −0·971)
1.viii.72	72	3·3 (2·4, 4·8)	2·39		NS	0·959	−1·861 (−2·426, −1·296)
	96	1·1 (0·77, 1·7)	10·68		NS	0·086	−1·444 (−1·890, −0·999)
Paratya	24	13·2 (7·4, 58)	5·69	6	NS	1·853	−1·653 (−2·494, −0·812)
Zn	48	7·5 (4·8, 17)	6·90		NS	1·420	−1·622 (−2·275, −0·970)
Toxic unit	72	3·0 (2·2, 414)	2·36		NS	0·880	−1·859 (−2·424, −1·295)
	96	1·04 (0·70, 1·5)	10·60		NS	0·025	−1·443 (−1·888, −0·998)
Paratya	24	10 (6·4, 26)	2·85	5	NS	1·856	−1·831 (−2·773, −0·889)
Cd	48	7·9 (4·9, 17)	7·3		NS	1·344	−1·721 (−2·551, −0·891)
Toxic units	72	2·7 (1·5, 4·8)	1·35		NS	0·643	−1·477 (−2·163, −0·791)
	96	1·04 (0·49, 1·6)	1·94		NS	0·031	−1·827 (−2·680, −0·9744)
Paratya	24	19 (11, 102)	1·32	5	NS	2·347	−1·838 (−2·910, −0·765)
Cd+Zn	48	5·6 (4·2, 715)	6·52		NS	2·408	−3·219 (−4·389, −2050)
Toxic units	72	3·8 (2·9, 4·9)	2·40		NS	1·997	−3·467 (−4·634, −2·299)
	96	1·8 (1·4, 2·5)	36·95		$P < 0·001$	0·673	−2·577 (−3·347, −1·806)

[A] Exclusive confidence limits.

The ephemeropteran nymph *Atalophlebia australis* was intermediate in sensitivity with a 96 hr LC50 of 0·34 mg/l with confidence limits from 0·44 to 2·25 mg/l (September). The zygopteran nymph *Ischnura heterosticta* was extremely resistant, having a 96 hr LC50 of 233 mg/l cadmium.

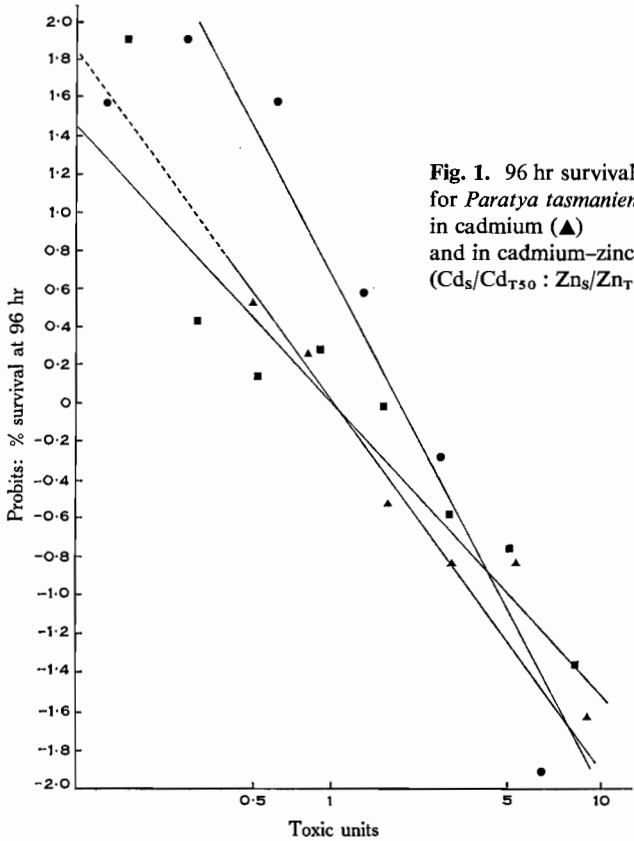

Fig. 1. 96 hr survival curves for *Paratya tasmaniensis* in zinc (■), in cadmium (▲) and in cadmium–zinc mixtures (●) ($Cd_S/Cd_{T50} : Zn_S/Zn_{T50} = 1 : 1·63$).

The bioassays on *Paratya* indicated that there may be some differences in sensitivity to cadmium at different times of the year. Of two groups of animals subject to similar acclimation and bioassay regimes, one had in early July a 96 hr LC50 of 0·06 mg/l with 95% confidence limits from 0·03 to 0·10 mg/l, while the other in mid-October had a 96 hr LC50 value of 0·18 mg/l with 95% confidence limits from 0·12 to 0·28 mg/l. *Paratya* proved to be more resistant to zinc (96 hr LC50 of 1·10 mg/l with 95% confidence limits from 0·77 to 1·7 mg/l) than to cadmium (96 hr LC50 of 0·06 mg/l) in winter bioassays (Table 2).

The results of both experiments with mixtures of cadmium and zinc suggest that the interaction may not be exactly additive (Figs. 1, 2; Table 3). The interpretation of the results is complicated by the fact that the toxicities of the supposedly equivalent solutions of zinc and cadmium were not equal. The zinc concentration of 1·8 mg/l was based on the 96 hr LC50 which had been determined by plotting percentage survival against zinc concentration on arithmetic scales. However, subsequent probit analysis gave an 96 hr LC50 value of 1·1 mg/l. In the first experiment, based

on Herbert and Shurben's (1964) method, the toxic units were recalculated to be based on 96 hr LC50 for zinc of 1·1 mg/l (see Fig. 1).

Figure 1 indicates that perhaps the combined effect of the cadmium–zinc mixtures is less toxic than the aggregated effect of cadmium and zinc separately at concentrations less than about one toxic unit, whereas above this level the combined effect is nearly additive. The 96 hr survival curves for cadmium and zinc alone are rather similar. This suggests that in *Paratya* they may have similar toxic actions.

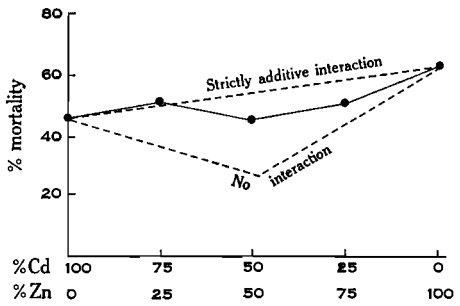

Fig. 2. Interaction of cadmium and zinc in *Paratya tasmaniensis*. Relative toxicity of combined solutions.

Discussion

The acute toxicity bioassays revealed a wide range of sensitivity to cadmium among the five invertebrate species tested. The bioassay results largely agree with the data obtained from a survey of the distribution and abundance of the macroinvertebrate fauna of the South Esk River (Thorp and Lake 1973).

Table 3. Probit analysis of zinc and cadmium mixtures ($Cd_S/Cd_{T50} : Zn_S/Zn_{T50} = 1 : 1·63$)

Time	Component	χ^2	DF	Probability
24 hr	Adequacy of probit model	9·86	16	NS
	Parallelism of lines	0·30	2	NS
	Coincidence of lines assuming parallel lines	4·02	2	NS
48 hr	Adequacy of probit model	20·72	16	NS
	Parallelism of lines	11·60	2	$0·01 > P > 0·001$
72 hr	Adequacy of probit model	6·10	16	NS
		8·61	2	$0·02 > P > 0·01$
96 hr	Adequacy of probit model	49·50	16	$P < 0·001$

For the 24 hr case where the data could be regarded as coming from a common probit model, the estimated common probit line is:

$$y = 1·971 + 1·719 \log x.$$

This compares with the three separate lines:

$$y = 1·856 + 1·831 \log x \text{ (Cd alone)}$$

$$y = 1·853 + 1·653 \log x \text{ (Zn alone)}$$

$$y = 2·347 + 1·838 \log x \text{ (Zn+Cd mixture).}$$

Relatively few heavy metal tolerance experiments have been performed on aquatic invertebrates, particularly in relation to their distribution in polluted rivers. The few studies of cadmium toxicity have been largely performed on fish (Pickering and Henderson 1966; Ball 1967; Eisler 1971; Pickering and Gast 1972). Eisler (1971) also carried out acute toxicity bioassays on various marine invertebrates. He also

found that of the animals tested, crustaceans were the most sensitive to cadmium. The 96 hr LC50 of the most sensitive species, the shrimp *Crangon septemspinosa* and the hermit crab *Pagurus longicarpus*, was 0·32 mg/l cadmium. Molluscs were more resistant, with corresponding values of between 2·2 and 25·0 mg/l cadmium.

It appears from several studies (Doudoroff and Katz 1953; Ball 1967; Shuster and Pringle 1969; Eisler 1971; Pickering and Gast 1972) that 96 hr may not be sufficient duration to adequately evaluate the lethal toxicity to cadmium to aquatic species. In Pickering and Gast's (1972) long-term study on the fathead minnow *Pimephales promelas*, the maximum acceptable concentration of cadmium was between 0·057 and 0·037 mg/l whereas the mean of five 96 hr LC50 values was 7·2 mg/l cadmium. In this study, the 96 hr LC50's for cadmium and zinc were determined in static bioassays. In static bioassays the test animals may alter their environment through decreasing oxygen, increasing carbon dioxide and increasing ammonia concentrations (Lincer *et al.* 1970). More ecologically meaningful results could have been obtained by using continuous flow bioassays.

Although the interaction between zinc and cadmium appears to be slightly less additive in less toxic solutions (less than one toxic unit), the toxicity of a mixture of zinc and cadmium could be approximately determined by simple addition of the predicted toxicitities. In most studies of mixtures of two poisons (Lloyd 1961; Herbert 1962; Herbert and Shurben 1964; Herbert and Vandyke 1964; Sprague *et al.* 1965), the toxicity of the separate components in the mixture has been additive. However, it is possible that at concentrations and combinations of cadmium and zinc different from those tested in this study, the behaviour of the solutions could deviate from the predicted behaviour because of antagonism or synergism. Brown *et al.* (1969) found that a mixture of three poisons in which zinc contributed 75% to the overall toxicity was significantly less toxic than expected. The interaction between copper and zinc is strictly additive at threshold concentrations for rainbow trout (Lloyd 1961) and for Atlantic salmon (Sprague and Ramsay 1965), but survival times in strong mixtures were much shorter for each of these salmonids than would be expected. Preliminary tests with rainbow trout at the Water Pollution Research Laboratory, Stevenage, U.K. (Ministry of Technology 1970) indicated that there was no interaction between solutions of zinc and cadmium sulphates in hard water. This was thought to be due to the differences in speed at which each of these metals act on rainbow trout. However, in the case of *Paratya*, the reason cadmium and zinc interact is probably because the speeds at which they act appear to be similar (Fig. 1).

Cadmium pollution is a particularly great problem because cadmium is not only highly toxic, as demonstrated in this study, but its toxicity is also cumulative, at least in fish (Mount and Stephen 1967; Pickering and Gast 1972) and in mammals (Flick *et al.* 1971).

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