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FEEDING AND GROWTH RESPONSES TO COPPER, ZINC, MERCURY AND LEAD IN THE TERRESTRIAL GASTROPOD *ARION ATER* (LINNE)

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ABSTRACT

Food consumption and growth variations related to several dosages of heavy metals (Cu, Zn, Hg and Pb) in diet were studied in the terrestrial slug *Arion ater* for 27 days of experimental treatment.

The work reported here is part of a larger project to investigate the histological effects of metal exposure in pulmonate gastropods.

Graphics on food consumption and growth *vs.* time for each metal, and regression for each behaviour observed are presented.

INTRODUCTION

Widdows (1982) reports that field measurements of the biological impact of pollutants at community and population levels can provide an assessment of immediate impact and recovery after an acute pollution incident, or the dramatic long term consequences of high levels of chronic pollution.

Metal pollution is very important in a technologically advanced society. At present, iron, copper, cadmium, zinc, lead and mercury levels are ten times higher in specific sites than their normal occurrence in nature (Simkiss, 1984). These cations accumulate in the body of a variety of invertebrates until they reach concentrations several thousand times higher than those in the environment (Coughtrey & Martin, 1977; Ireland, 1979).

Particularly, molluscs can accumulate higher concentrations of metal ions than other groups of invertebrates (Beeby & Eaves, 1983). It must be remarked that despite the bioaccumulation of such toxicants and despite minor levels of metals lethal for other animals, authors (Schoettli & Seiler, 1970; Calabrese, Thurberg & Gould, 1977; Russell, DeHaven & Botts, 1981) have not usually found effects on mortality except in the extremely high exposure that only occurs in certain sites (sewage sludges—Russell *et al.*, 1981; areas close to smelting factories—Burkitt, Lester & Nickless, 1972; intense traffic highways—Motto, Daines, Chilko & Motto, 1970). However, the incidence of abnormal environmental concentrations of metal affects numerous phenomena involved in the development and maintenance of molluscan populations, such as feeding, growth, reproduction, general physiological activity and maturity (Bonelly De Calventi, 1965; Calabrese *et al.*, 1977; Russell *et al.*, 1981).

Although a large amount of information exists relating physiological and behavioural responses of marine molluscs to environmental stresses (Scott & Major, 1972; Calabrese *et al.*, 1977; Bayne *et al.*, 1979; Manley, 1983), information concerning this kind of response in terrestrial molluscs is poor.

This paper describes variations in feeding activity and growth in experimental populations of the slug *A. ater* (Linné) treated for 27 days with seven different dosages of four frequent metals: copper, zinc, mercury and lead. Fertilizers are the main source of copper as well as zinc and mercury pollution (Simkiss, 1984). These three cations are also discharged with industrial wastes and from pesticides and herbicides. Lead pollution occurs as a consequence of combustion of petrol additives in automobiles and reaches soil by means of atmospheric precipitation (Motto *et al.*, 1970). As copper and zinc have a metabolic role while lead and mercury do not, we chose four of them to provide a meaningful comparative study.

MATERIALS AND METHODS

Four toxicity bioassays were conducted, one for each metal. Two simultaneous sets of animals were treated with different metal concentration in diet (0, 10, 25, 50, 100, 300, and 1000 ppm) for 27 days. 198 individual

A. ater were collected from a field near the University Campus: (a) for mercury bioassay on 9 July; (b) for copper bioassay on 31 July; (c) for zinc bioassay on 7 August; and (d) for lead bioassay on 23 August. It was not possible to conduct simultaneous bioassays in view of the fact that behaviour and growth analysis was only part of a larger study which included a large volume of histochemical and morphometrical analysis. Initial planning included one week separation between samplings, but frequently taking the number of slugs required weekly was not possible; animals were then returned to the field and the collection postponed until the following week.

The slugs, selected in relation to size (4-5 cm length) and weight (5-7 g), were taken to the laboratory after each collection and distributed into 14 plastic boxes (20 × 18 × 10 cm). Box lids were replaced by nylon screens to provide efficient aeration. Animals were starved for 6 days prior to treatment in order to minimize physiological differences and provide acclimation to laboratory conditions (Akerlund, 1969).

Metal treatment was started with 12 animals per box (24 per treatment). Mean temperature during assays was 20°C, and relative humidity was maintained at saturation level using Petri dishes containing water. Daily, animals and boxes were cleaned, and water in Petri dishes changed. *Natural diet* (equiproportional triturate of lettuce, apple, carrot and pumpkin with a 1.5% agar aqueous solution) was applied to slugs (Zubiaga, 1982) mixed with metal salts ($CuSO_4$, $ZnCl_2$, $HgCl_2$, $PbNO_3$) in the proper concentration for each group. Every 3 days an individual was removed from each box for histological analysis (Marigómez, Angulo & Moya, *in press*). Food was changed daily and unconsumed food was weighed. Since slugs were weighed every 3 days, food uptake data were treated as three-day groups.

The number of individuals per box decreased systematically (from 24 slugs per treatment to 8 on the 27th bioassay day) as a consequence of periodical removal for histological analysis, so that we have used relative variables to describe feeding activity and growth: the former as grams food consumed per 100 grams slug and the latter as percentage of variation in weight related to slug weight reached. The values of weight variation plotted in graphs are the mean of the variable values for both series of each treatment, which were obtained from weight of slugs remaining every 3 days/number of slugs weighed. Thus we calculated the hypothetical weight of a 'pattern-slug' for each group of treatment. These values were compared with the mean weight of initial 'pattern slugs' to calculate the growth percentage plotted on graphs. Initial experimental populations were closely homogeneous in weight in each series (weight differences <10% total weight) and animals were randomly selected when removed for histological study. Thus, any existent variation in population densities, as well as slug/slug interactions affecting growth rates, would both have been kept at a minimum. In fact, we did not observe a significant difference between the two series for each group of exposure and consequently we decided not to plot standard deviations on graphs since they are already too complex themselves. Linear regression (on feeding activity and growth against treatment time) and correlation were calculated following the methods described by Sokal and Rohlf (1979).

RESULTS

Mortality

No effect on mortality was observed in Cu and Zn treated slugs since there was no clear relationship between metal dosage and number of dead slugs. Three, one, one, one and two individuals died in the 0, 10, 50, 100 and 1000 ppm Zn exposure respectively; and one each in the 0, 10 and 1000 ppm Cu treatment.

On the other hand, Pb and Hg treated slugs showed a major mortality when exposed to the highest dosages: one, one, one, three and two animals died in the 10, 25, 50, 300 and 1000 ppm Pb exposure respectively; and three, one, one, two and five in the 0, 25, 50, 300 and 1000 ppm Hg treatment. As the three deaths in the 0 ppm Pb exposure occurred suddenly the same day (5th) and they belonged to only one of the two control series we think that it was a chance feature different from the mortality dynamics in 1000 ppm Hg treatment where slugs died from the 16th day on.

Feeding activity

Accumulated food consumption by Cu, Zn, Hg and Pb treated and control *A. ater* is shown in Figs 1-4 to describe feeding behaviour of slugs during assays. We observed that normal linear changes turned into an exponential response; therefore, we considered linear regression and logarithmic transformation of 'Y' values (food consumption) enough to characterize groups of behavioural response (Table 1).

Growth

Percentage growth for Cu, Zn, Hg and Pb treated and control *A. ater* is shown in Figs 5-8. Normal animals and those treated with low dosages did not reduce in weight until the 15-21st bioassay days, when an intense weight reduction occurred. On the contrary, slugs treated with high metal dosages showed a linear decrease in weight from the beginning of the assays similar to that during the last days of unaffected slugs. In order to describe the growth responses to experimental treatment we decided to adjust data to linear regression. As we observed a break in the treated animals behaviour between the 15-21st days we tried to make adjustments as two separate intervals, with the break in the 15th, 18th and 21st days. We attained the best statistical significance in double regression for unaffected slugs when intervals were 'up to the 18th day', 'for the 18th day on' in Cu and Zn exposures, and 'up to the 15th', 'for the 15th day on' in Pb exposure. However, in the case of Hg exposure simple linear regression was statistically more significant. This is probably due to the incidence of another source of stress that shall be further discussed.

DISCUSSION

Mortality

A. ater is tolerant of high levels of Zn and Cu, and presents a slight intolerance to extremely high dosages of Pb and Hg in food. We can conclude that normal environmental levels of such metals would not cause significant mortality in *A. ater* populations. Similar results were attained by Russell *et al.* (1981) in cadmium-treated *Helix aspersa* Müller.

Feeding activity

Three different types of behaviour can be reported as a result of copper bioassay. One, normal, in which a continuity of feeding behaviour is observed during treatment (Fig. 1). Control slugs and those exposed to 10-25 ppm Cu are included in this group, characterized by the slope for linear regression, $b = 13.90 \pm 0.57$. A second group of strongly affected slugs (100-1000 ppm Cu fed) shows an exponential change in feeding (Table 1). We argue that initial refusal of food, which could even be due to disagreeable organoleptic characteristics, ends when animals feed on whatever is eatable because of hunger. An intermediate third response is the one observed in 50 ppm Cu treated *A. ater* ($b = 7.478$). This last group might indicate that the critical Cu concentration affecting food consumption approaches 50 ppm Cu, since slugs start in such a case with activity similar to that of unaffected slugs, but which afterwards becomes similar to that observed in strongly affected *A. ater*.

A similar response is detected in the Zn bioassay (Fig. 2), but in this case the strongly affected group includes only the series with 1000 ppm Zn exposure, and the critical Zn concentration seems to be between 300 and 1000 pm Zn, with no intermediate behaviour as in the previously mentioned assay. Linear regression correlation is significant for control and 10-300 ppm Zn treated slugs (Table 1) being characterized by a regression coefficient $b = 14.3 \pm 1.4$. As a consequence of exponential change linear regression for 1000 ppm Zn series is appropriate only after logarithmic transformation regression of food consumption ($r^2 = 0.934$; $P < 0.01$).

We conclude that low dosages of metabolically available metals, such as Cu and Zn, do not vary feeding activity of *A. ater*, but that higher doses decrease food uptake until it reaches, by acclimation, a feeding rate similar to that of unaffected series. That may be understood on the basis of the results obtained by Schoettli & Seiler (1970) and Coughtrey and Martin (1977) who found no evidence of damage or disease in *A. ater* fed on Zn and in *H. aspersa* exposed to Cu, respectively. The former authors reported that slugs have mechanisms to avoid Zn toxicity and the latter indicated that Cu does not cause apparent damage in snails since copper is a normally occurring cation in molluscan haemocyanin.

The response to Hg treatment observed in *A. ater* corresponds to the one described by Russell *et al.* (1981) in *H. aspersa* exposed to cadmium. These authors concluded that slope indicating total food consumption (30 days) on ln Cd dosage is significantly negative. We have shown that total food consumption on ln Hg dosage is characterized by the function:

$$\text{Food Consumed (27 days)} = 462.65 - 41.77 \ln [\text{Hg}]$$

(Food consumption (g/100 g slug); $r = 0.932$; $P < 0.01$).

On the basis of such a response three groups may be detected in Fig. 3: (a) control and 10 ppm Hg series with behaviour considered normal ($b = 15.489 \pm 0.2$); (b) 25-100 ppg Hg fed slugs with intermediate food consumption rate ($b = 11.423 \pm 0.4$); both of these show continuous linear activity (Table 1); and (c) 300 and 1000 ppm Hg exposures in which extremely low initial ingestion followed by a greater, but not normal, feeding activity produces an exponential dynamics pattern (Table 1).

On the contrary, Pb does not affect food consumption at all in *A. ater* ($b = 17.2 \pm 1.23$) as can be observed in Fig. 4.

Differences in regression coefficients of control *A. ater* may be attributed to the lack of synchrony of assays and we may relate them to the annual life cycle of this species. During the

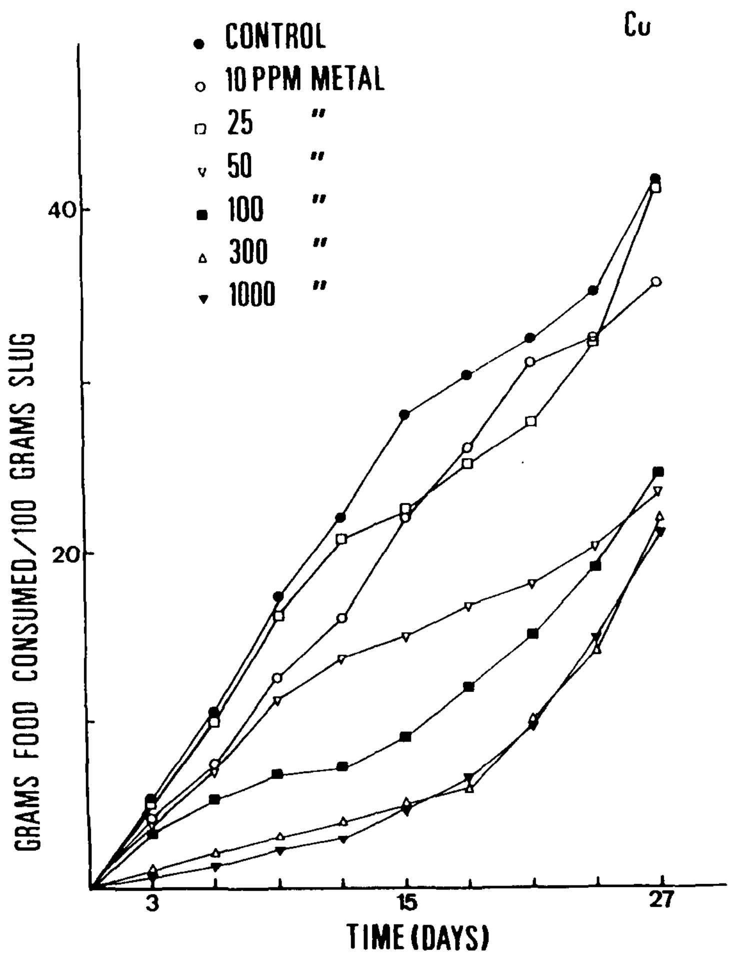

Fig. 1. Accumulated food consumption by Cu-treated and control *A. ater* (6 August–2 September).

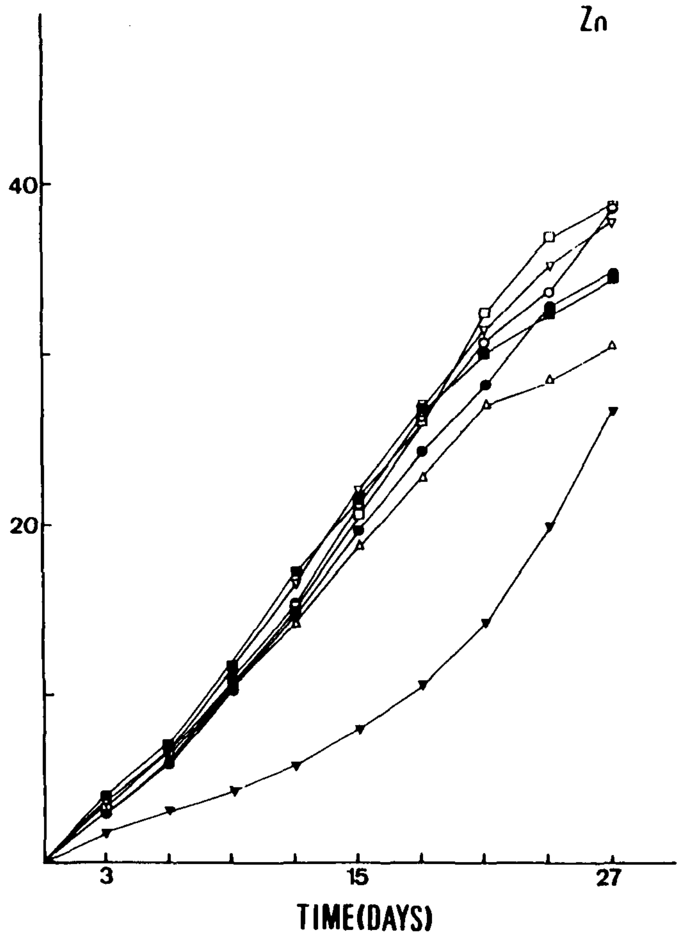

Fig. 2. Accumulated food consumption by Zn-treated and control *A. ater* (13 August–9 September).

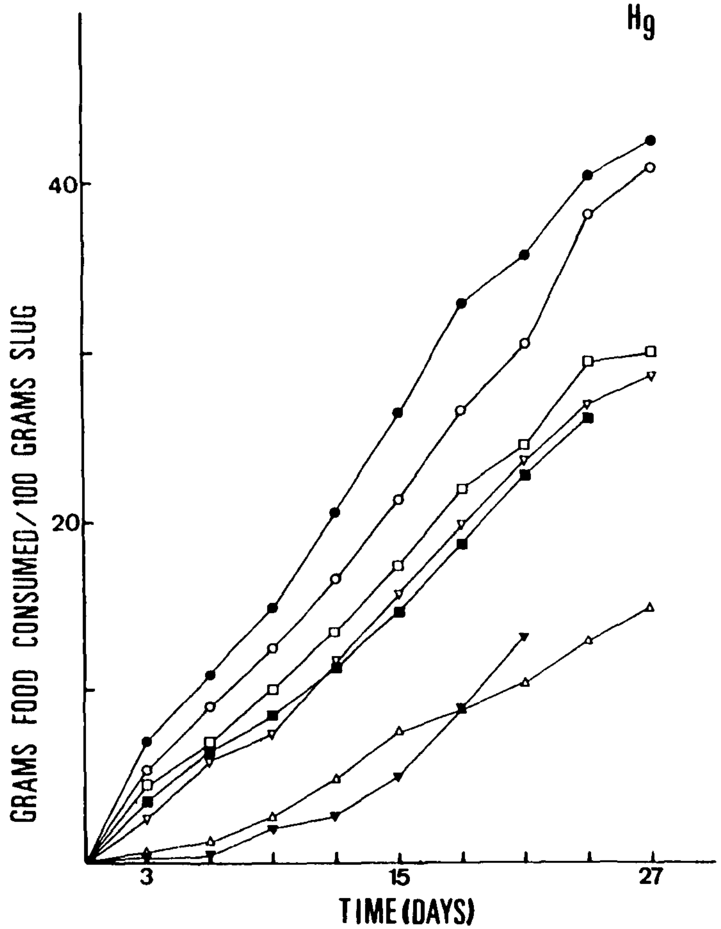

Fig. 3. Accumulated food consumption by Hg-treated and control *A. ater* (9 July–2 August).

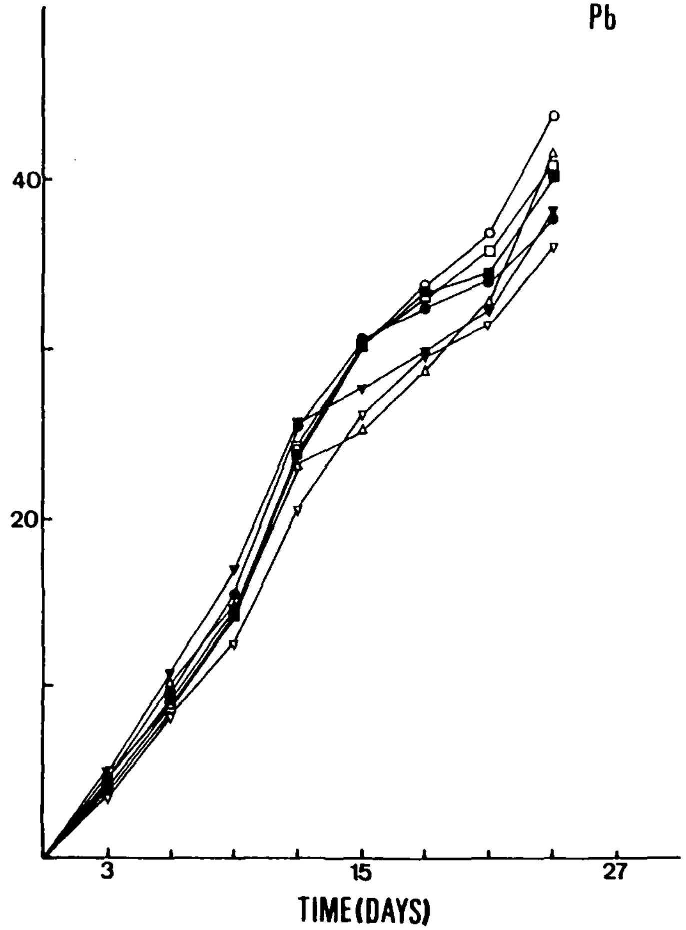

Fig. 4. Accumulated food consumption by Pb-treated and control *A. ater* (30 August–26 September).

Table 1. Correlation coefficients (r^2) for feeding activity and their significance (P) both for control and metal treated *Arion ater*. L.R.: linear regression; L.T.: linear regression after logarithmic transformation of dependent variable.

Metal	Concentration (PPM)	L.R.		L.T.	
		r^2	P	r^2	P
Hg	0	0.979	≤0.01		
	10	0.992	<0.01		
	25	0.992	<0.01		
	50	0.994	<0.01		
	100	0.990	<0.01		
	300	—	—	0.993	<0.01
	1000	—	—	0.945	<0.01
Cu	0	0.976	<0.01		
	10	0.991	<0.01		
	25	0.963	<0.01		
	50	0.971	<0.01		
	100	—	—	0.902	<0.01
	300	—	—	0.921	<0.01
	1000	—	—	0.934	<0.01
Zn	0	0.996	<0.01		
	10	0.994	<0.01		
	25	0.989	<0.01		
	50	0.997	<0.01		
	100	0.988	<0.01		
	300	0.989	<0.01		
	1000	—	—	0.954	<0.01
Pb	0	0.963	<0.01		
	10	0.982	<0.01		
	25	0.971	<0.01		
	50	0.973	<0.01		
	100	0.974	<0.01		
	300	0.979	<0.01		
	1000	0.959	<0.01		

mid summer Cu and Zn bioassays, slopes of regression lines on food consumption for control *A. ater* are lower than those of early July and late September when the Hg and Pb assays were respectively conducted. The normal depression in metabolic rate due to the increase in size that occurs during the annual life cycle (Salazar, 1980) could provide the observed decrease in feeding activity which was also seen by Laucirica (1980) in *Arion empiricorum* Fér. (since we have used a relative value related to total weight of slugs). Afterwards, the increase in metabolic rate due to maturation of the genital apparatus would demand a major food uptake. As a result of both, early summer depression and late summer increase, an inflexion point appears in mid summer when the minimum food uptake occurs.

Growth dynamics

Normal behaviour of control series is characterized by no (or little) variation in weight during the first 18 days of each bioassay. In this initial period isolated wide oscillations in weight occur; both oscillatory change and the almost null slope indicated strongly no significant linear regression ($P < 0.01$) except for Hg assay slugs (Table 2). In this case weight reduction occurs linearly from the beginning of experimental treatment: the final male phase in *A. ater* may be strongly affected by laboratory enclosure since this is a critical stage in which sexual maturity is developed. With this consideration we concluded that normal growth depression in July for control slugs was properly measured by linear regression ($P < 0.01$, Table 2). On the other hand, oscillatory results in initial growth responses to confinement may relate to variations in water content, on the basis of the continuous flux of water through slugs in tegument reported by Lyth (1983). As we knew the studies of Howes & Wells (1956) prevented incidence of day-variation in weight by weighing food and slugs always between 5.00 and 7.00 p.m. Such a prevention was probably not necessary since day-variation in weight is not significant (Lyth, 1983).

We may conclude that there is almost no variation in weight in control and unaffected series of *A. ater* during the first 18 days of assay except for normal oscillation due to changes in water content.

Series treated with low Cu dosages (10 ppm) showed a benefit in growth (Fig. 5, Table 2) until reaching an oscillatory behaviour with almost null scope from the 18th day on. Slugs treated with 25 and 50 ppm Cu behave like the control series but present a lower depression during the final period of treatment. Both degrees of apparent benefit may be due to Cu ions involved in normal metabolism (Simkiss, 1979). Simkiss, Taylor & Mason (1982) affirmed that at low concentrations most metals exert beneficial effects but at higher concentrations these become inhibitory and eventually toxic. Such beneficial low concentrations of Cu, Zn and Hg in relation to growth seem to reach 50, 300 and 25 ppm respectively; however, values under 10 ppm must be concluded for Pb treatment.

Growth responses to high Cu dosages consist of continuous weight depression until reaching 40-30% initial weight of slugs. Such a percentage could be critical and below this value slugs may be considered non-viable (inactive or moribund). The occurrence of a critical weight, however, causes no significant separation between the final weight of control and metal treated *A. ater*.

Zn assay results offer a similar perspective but include a wider range of series not affected in growth dynamics (10-300 ppm). In this case

Table 2. Correlation coefficients (r^2) for growth and their significance (P) both for metal treated and control *Arion ater*. L.R.1: linear regression for the first 18 (15 in the Pb exposure) bioassay days; L.R.2: lineal regression for the last 9 (12 in the Pb exposure) bioassay days.

	L.R.		L.R.1		L.R.2	
Treatment	r^2	P	r^2	P	r^2	P
0 ppm Hg	0.923	<0.01				
10 ppm Hg	0.771	<0.01				
25 ppm Hg	0.615	<0.05				
50 ppm Hg	0.889	<0.01				
100 ppm Hg	0.936	<0.01				
300 ppm Hg	?					
1000 ppm Hg	0.988	<0.01				
0 ppm Cu	—	—	0.087	>0.05	0.993	<0.01
10 ppm Cu	—	—	0.818	<0.05	0.155	>0.05
25 ppm Cu	—	—	0.804	<0.05	0.840	*0.05
50 ppm Cu	—	—	0.000	>0.05	0.733	>0.05
100 ppm Cu	0.955	<0.01				
300 ppm Cu	0.966	<0.01				
1000 ppm Cu	0.988	<0.01				
0 ppm Zn	—	—	0.648	*0.05	0.909	<0.05
10 ppm Zn	—	—	0.497	>0.05	0.893	*0.05
25 ppm Zn	—	—	0.774	<0.05	0.769	>0.05
50 ppm Zn	—	—	0.690	<0.05	0.958	<0.05
100 ppm Zn	—	—	0.646	*0.05	0.977	<0.05
300 ppm Zn	—	—	0.974	<0.01	0.940	<0.05
1000 ppm Zn	0.930	<0.01				
0 ppm Pb	—	—	0.236	*0.05	0.510	>0.05
10 ppm Pb	—	—	0.000	>0.05	0.773	>0.05
25 ppm Pb	—	—	0.260	>0.05	0.954	<0.05
50 ppm Pb	—	—	0.781	<0.05	0.993	<0.05
100 ppm Pb	0.928	<0.01				
300 ppm Pb	0.933	<0.01				
1000 ppm Pb	0.943	<0.01				

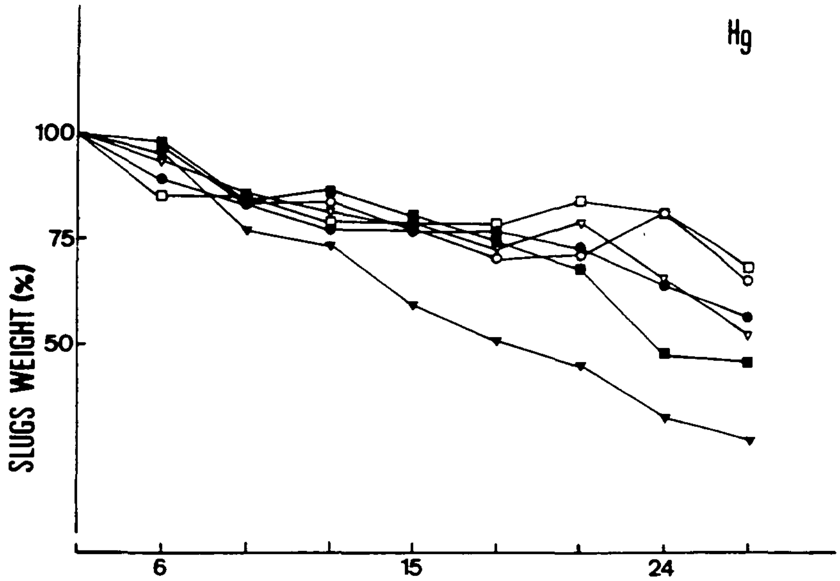

Fig. 5. Growth percentage for Cu-treated and control *A. ater*.

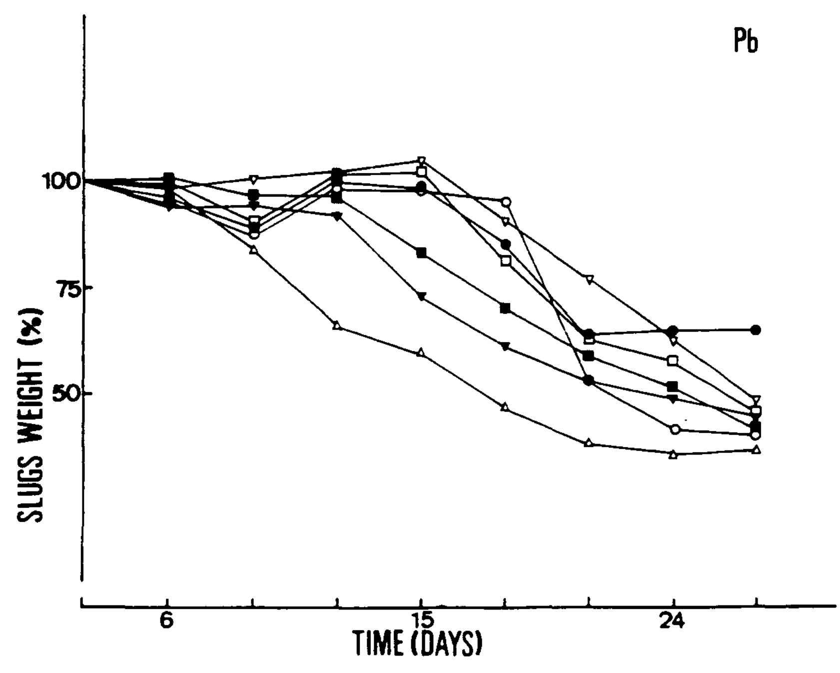

Fig. 6. Growth percentage for Zn-treated and control *A. ater*.

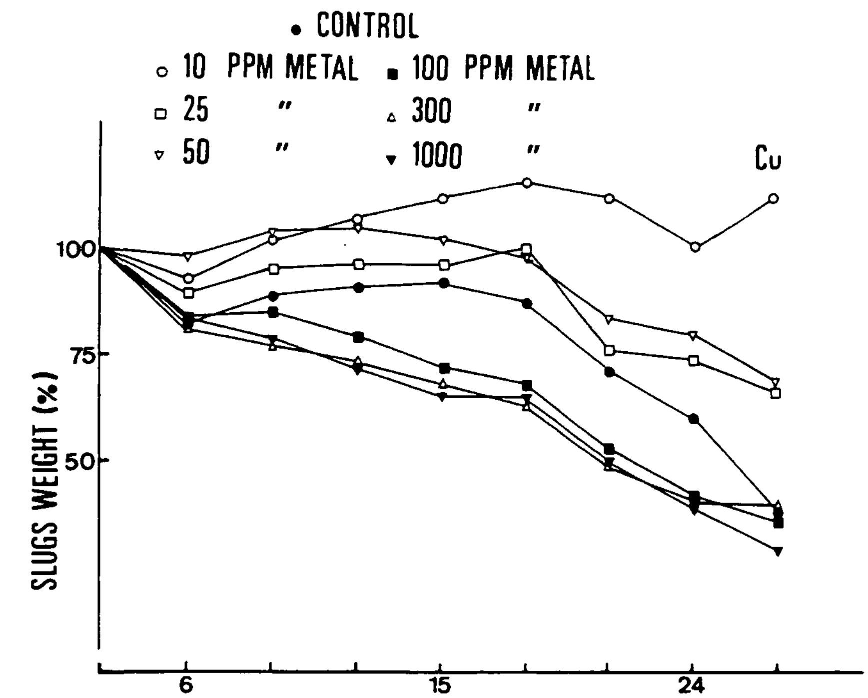

Fig. 7. Growth percentage for Hg-treated and control *A. ater*.

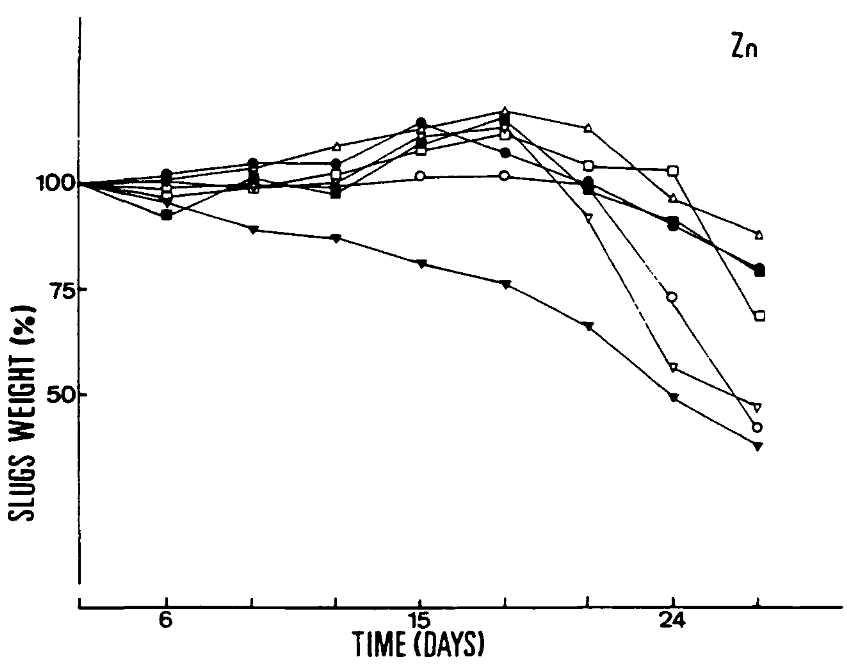

Fig. 8. Growth percentage for Pb-treated and control *A. ater*.

benefits are less noticeable and could be better defined as an innocuous action of these Zn dosages rather than a beneficial one.

A. ater treated severely with Hg show a decrease in weight with respect to the control series but such a depression is clearly significant only after exposure to 1000 ppm Hg (Fig. 7).

Series of Pb low dosages (10-50 ppm) offer a similar perspective to those in Cu and Zn assays; however, a tendency to continuous depression is observed in 50 ppm Pb treated slugs: minor initial decrease ($b = -1.899$) and subsequent major one ($b = -3.257$). Series of Pb high dosages (100-1000) grow like all the series under high metal exposure (Table 2). However the marked separation between the total weight reduction in control and that in Pb treated *A. ater* should be reported.

We can infer the existence of a variety of growth responses in *A. ater* fed with metals: (a) weight maintenance followed by depression in laboratory conditions for control and low exposures series; (b) beneficial or innocuous effect of low Cu, Zn and Hg exposure; and (c) continuous growth depression in slugs exposed to high dosages.

SUMMARY

1. Although a major mortality seems to occur in *A. ater* treated with high dosages of Hg and Pb, normal environmental levels of the metals studied would not cause a significant mortality in *A. ater* populations.

2. Feeding activity of Cu and Zn treated slugs depends on metal dosage: the linear dynamics of control and unaffected *A. ater* became exponential in strongly affected slugs. Such exponential responses may be due to acclimation to diet after the minor consumption occurring during the first 15-18 days.

3. Feeding activity of Hg treated *A. ater* is closely related to dosage: food consumption decreases on increasing Hg dosage.

4. Pb treatment does not affect feeding activity of *A. ater* at the levels of toxicant studied.

5. *A. ater* growth decreases as a consequence of stress situations. No (or slight) weight variation is described in control and unaffected slugs during the first 18 (15 in the Pb exposure) days of treatment. However, weight decreases in the generality of cases thereafter, with a similar slope to that observed in hardly affected slugs during the whole treatment.

6. Such a typical growth decrease does not occur on *A. ater* treated with low Cu, Hg and Zn dosages; these metal concentrations seem to have a positive effect on slug maintenance under laboratory conditions.

7. There is no wide separation of total weight reduction values once the slope of highly affected *A. ater* becomes reduced as the slugs' weight approaches 30% of the initial weight.

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REFERENCES

AKERLUND, D.G. 1969. Oxygen consumption of the ampullariid snail *Marisa cornuaretis* L. in relation to body weight and temperature. *Oikos*, **20**, 529–533.

BAYNE, B.L., MOORE, M.N., WIDDOWS, J., LIVINGSTONE, D.R. & SALKED, P. 1979. Measurements of the responses of individuals to environmental stress and pollution: studies with bivalve molluscs. *Philosophical Transactions of the Royal Society of London* B, **286**, 563–581.

BEEBY, A. & EAVES, S.L. 1983. Short term changes in Ca, Pb, Zn and Cd concentrations of the garden snail *Helix aspersa* Müller from a Central London car park. *Environmental Pollution* (Series A), **30**, 233-244.

BONELLY DE CALVENTI, I. 1965. Copper poisoning in the snail *Helix pomatia* and its effects on mucous secretion. *Annals of the New York Academy of Sciences*, **118**, 1015–1020.

BURKITT, A., LESTER, P. & NICKLESS, G. 1982. Distribution of heavy metals in the vicinity of an industrial complex. *Nature*, **238**, 327–328.

CALABRESE, A., THURBERG, F.P. & GOULD, E. 1977. Effects of cadmium, mercury and silver on marine animals. *Marine Fisheries Review*, **39**, 5-11.

COUGHTREY, P.J. & MARTIN, M.H. 1977. The uptake of lead, zinc, cadmium and copper within the pulmonate mollusc *Helix aspersa* Müller, and its relevance to the monitoring of heavy metal contamination of the environment. *Oecologia*, **27**, 65-74.

HOWES, N.H. & WELLS, G.P. 1934. The water relations of snails and slugs II. Weight rhythms in *Arion ater* L. and *Limax flavus* L. *Journal of Experimental Biology*, **11**, 344–351.

IRELAND, M.P. 1979. Distribution of essential and toxic metals in the terrestrial gastropod *Arion ater*. *Environmental Pollution*, **20**, 271–278.

LAUCIRICA, I. 1980. *Variaciones estacionales en el metabolismo del limaco común* Arion empiricorum Fér. *sometido a diferentes dietas sintéticas. Estudios* *sobre su balance energético.* M. Sc. Thesis. Universidad del País Vasco.

LYTH, M. 1983. Water contents of slugs (Gastropoda, Polmonata) in natural habitats, and the influence of culture conditions on water content stability in *Arion ater* (Linné). *The Journal of Molluscan Studies*, **49**, 179-184.

MANLEY, A.R. 1983. The effects of copper on the behaviour, respiration, filtration and ventilation activity of *Mytilus edulis*. *Journal of the Marine Biological Association of the U.K.*, **63**, 205-222.

MARIGOMEZ, J.M., ANGULO, E. & MOYA, J. *in press.* Copper treatment of the digestive gland of the slug, *Arion ater* L. 2. Morphometrics and histophysiology. *The Bulletin of Environmental Contamination and Toxicology.*

MOTTO, H.L., DAINES, R.H., CHILKO, D.M. & MOTTO, C.K. 1970. Lead in soils and plants: its relationship to traffic volume and proximity to highways. *Environmental Sciences Technology*, **4**, 231-237.

RUSSELL, L.K., DEHAVEN, J.I. & BOTTS, R.P. 1981. Toxic effects of cadmiun on the garden snail (*Helix aspersa*). *The Bulletin of Environmental Contamination and Toxicology*, **26**, 634–640.

SALAZAR, A. 1980. *Variaciones estacionales en el metabolismo del limaco común* Arion empiricorum *Fér. sometido a diferentes dietas sintéticas. Estudios sobre su actividad respiratoria.* M. Sc. Thesis. Universidad del País Vasco.

SCHOETTLI, G. & SEILER, H.G. 1970. Uptake and localization of radioactive zinc in the visceral complex of the land pulmonate *Arion rufus*. *Experientia*, **26**, 1212-1213.

SCOTT, D.M. & MAJOR, C.W. 1972. The effect of copper (II) on survival, respiration and heart rate in the common blue mussel, *Mytilus edulis*. *Biological Bulletin*, **143**, 679-688.

SIMKISS, K. 1979. Metal ions in cells. *Endeavour*, **3**, 2-6.

SIMKISS, K. 1984. Invertebrados que neutralizan metales tóxicos. *Mundo científico*, **39**, 864-866.

SIMKISS, K., TAYLOR, M. & MASON, A.Z. 1982. Metal detoxification and bioaccumulation in molluscs. *Marine Biology Letters*, **3**, 187-201.

SOKAL, R.R. & ROHLF, F.J. 1979. *Biometría.* Madrid, Blume.

WIDDOWS, J. 1982. Field measurements of the biological impacts of pollutants. Assimilative Capacity of the Oceans for Man's wastes, SCOPE/ICSU Academia Sinica, Taipei, Republic of China, 111-129.

ZUBIAGA, A.M. 1982. *Variaciones estacionales en la histología e histoquímica de la glándula digestiva del limaco* Arion ater *L. sometido a dieta natural.* M. Sc. Thesis. Universidad del País Vasco.