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ADAPTATION OF THE POLYCHAETE *NEREIS DIVERSICOLOR* TO ESTUARINE SEDIMENTS CONTAINING HIGH CONCENTRATIONS OF ZINC AND CADMIUM

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(Text-figs. 1–11)

Concentrations of zinc and cadmium in the polychaete *Nereis diversicolor* O. F. Müller have been compared with those of the sediments in the estuaries of 26 rivers which drain the old metalliferous mining areas of South-West England. Whereas in the sediments concentrations of zinc varied by a factor of 30 from about 100 to 3000 μg/g, concentrations in the worms, after correcting for size, varied by only 2·7 from 130 to 350 μg/g dry weight. Concentrations of cadmium in the sediments varied by a factor of 46 from about 0·2 to 9·3 μg/g and concentrations in the worms were roughly proportional to them and varied by a factor of 45 from 0·08 to 3·6 μg/g. These results suggest that zinc is regulated by the worm whereas cadmium is not.

Experimental work has shown that with increasing concentrations in solution the rate of absorption of cadmium by *Nereis* increases more rapidly than that of zinc and is more nearly proportional to the external concentration. This helps to explain why, in the field, concentrations of zinc in the worms vary less than those of cadmium, but another reason is that populations from high-zinc sediments are better at regulating zinc than normal populations. In toxicity experiments they are more resistant to zinc than normal worms and this adaptation is explained by a reduced permeability to zinc and probably to more effective excretion.

Some factors which influence the absorption of heavy metals are discussed and the adaptation of *Nereis* to high concentrations of metals is compared with that in other organisms.

INTRODUCTION

In the first paper of this series (Bryan & Hummerstone, 1971) it was shown that in the estuarine polychaete *Nereis diversicolor* O. F. Müller the concentration of copper is roughly proportional to that of the surrounding sediment. On the other hand, concentrations of zinc appeared to be relatively independent of those in the sediment and it was suggested that the level of zinc in the worm is regulated.

This paper deals in more detail with field and experimental observations on the behaviour of zinc and cadmium in *Nereis* from estuaries which drain the old metalliferous mining areas of Cornwall and Devon in South-West England. Because it was found that animals from sediments containing high concentrations of copper had developed a resistance to its toxic effects (Bryan & Hummerstone, 1971), the adaptation of *Nereis* to sediments containing high concentrations of zinc has also been studied.

MATERIALS AND METHODS

The techniques employed in this work have already been described by Bryan & Hummerstone (1971, 1973) and will be described only briefly. Worms for analysis were cleaned by exposing them to fine acid-washed sand covered with 50 % sea water (S ‰ = 17·5) for 6 days and to 50 % sea water for a further day. Samples containing at least 10 animals were dried in conical flasks and digested with 10 ml of redistilled nitric acid. After digestion, the sample was evaporated and the residue redissolved in a known volume, usually 10–25 ml of dilute nitric acid. Samples of 0·4 g of fine sediment were digested with 20 ml of nitric acid and, after evaporation, the residue was diluted to 10 ml with 1·0 N nitric acid. The solutions were analysed for zinc and cadmium by atomic absorption using a Perkin–Elmer 306 instrument with background correction. Samples of interstitial water which had been squeezed from the sediments were analysed for zinc by direct atomic absorption and the detection limit for the method was around 0·01 μg/ml.

Experiments using the radioisotopes ^{65}Zn and ^{115m}Cd were carried out in the same way as experiments described previously using ^{54}Mn (Bryan & Hummerstone, 1973). All experiments were carried out at 13 °C, mainly in darkness. Results for the animals expressed on a dry weight basis may be converted to a wet weight basis by dividing by 7, since about 85 % of the body weight is water.

RESULTS

Field observations

Zinc and cadmium in sediments

The estuaries from which samples were obtained are shown in Fig. 1 and listed in Table 1. In Fig. 1 the positions, within the catchment areas of the rivers, of mines where zinc production was recorded are also shown. By comparison with tin or copper or even lead, zinc was never mined on a large scale in Cornwall and Devon and only about 85000 tons of ore, produced mainly between 1850 and 1885, have been recorded (Dines, 1956). Cadmium ores do not appear to have been found in the area and the metal is mentioned by Dines only as a contaminant of zinc ores. Although not exploited on a large scale, blende, the principal ore of zinc, is widely distributed (Fig. 1) and is associated with both lead and copper lodes (Dewey, 1921; Dines, 1956). As a result, sediments contaminated with zinc usually contain high concentrations of other metals. Those from Restronguet Creek contain high concentrations of copper and arsenic and those from the Gannel contain high levels of lead.

Taylor (1964) gives the average concentration of zinc in the continental crust as 70 μg/g and that of cadmium as 0·2 μg/g. Concentrations of zinc in estuarine sediments are summarized in Table 1 and vary by a factor of 30 from around 100 μg/g in some of the estuaries in South Devon to nearly 3000 μg/g at the head of Restronguet Creek. The sediments were those adhering to the animals and came from a depth of about 10 cm. High concentrations from the Fal and Tamar systems, Helford, Hayle and Gannel Estuaries certainly reflect not only the metalliferous nature of the area but also the association of these estuaries with drainage from past mining activity. Even the lower concentrations of zinc may not be strictly natural since, except perhaps for the Kingsbridge Estuary, none of the estuaries can be dissociated from past mining activity or the working of alluvial deposits.

Concentrations of cadmium in the sediments vary by a factor of about 46 from around 0·2 μg/g in some of the South Devon estuaries to an average of 9·3 μg/g in the

Upper Plym Estuary. Although the relatively high levels in the Gannel Estuary can be associated with mining, the high level in the Plym probably comes from industrial sources and is confined to the upper layers of the sediment. Compared with Taylor's average Zn/Cd ratio of 350, 17 of the 30 values in Table 1 lie between 200 and 500, two from the Plym are below 200, and 11, including values from Restronguet Creek, exceed 500.

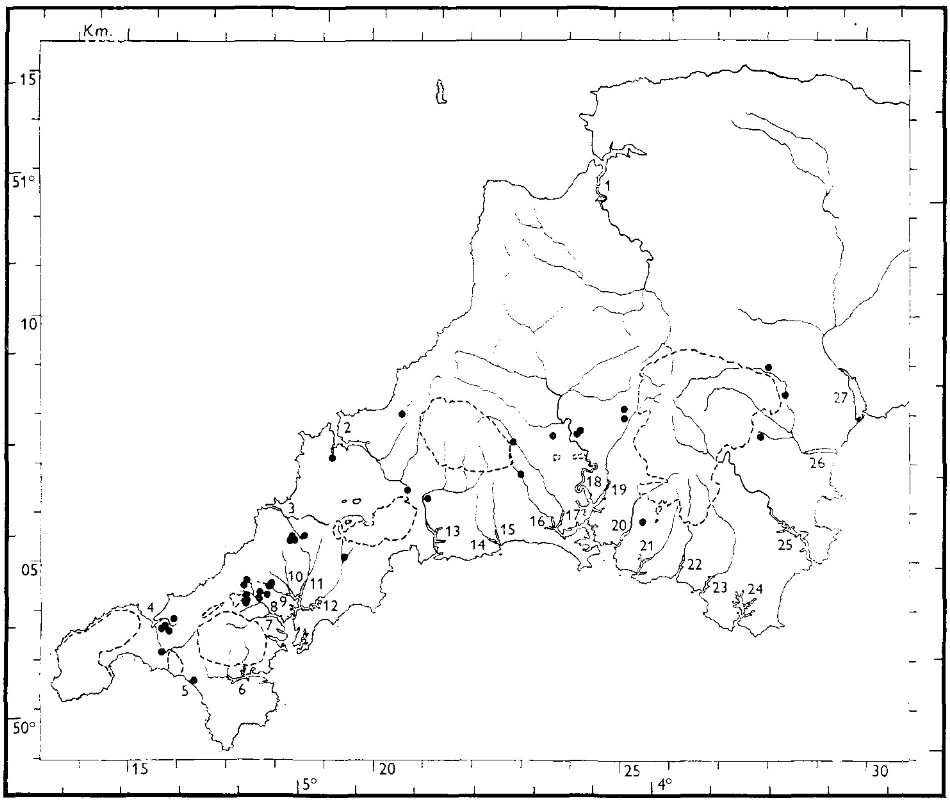

Fig. 1. South-West England. Positions of estuaries from which worms and sediments were collected are shown by numbers which correspond with names in Table 1. ●, Positions of mines associated with estuaries and known to have included zinc in their production (Dines, 1956); – –, granite areas, high ground.
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For comparison with concentrations in the sediments, the levels of zinc in the interstitial water squeezed from the sediments and in the river water entering the estuaries are also summarized in Table 1. Interstitial water from the oxidized surface layers of the sediment often contains more zinc than that from the deeper sediment, but even at the surface concentrations are usually below 0·1 μg/ml. However, in the Fal and Gannel Estuaries and Restronguet Creek, higher concentrations were found and are particularly relevant to the levels in the worms from these areas.

TABLE 1. ZINC AND CADMIUM IN *NEREIS*, SEDIMENTS AND WATER FROM ESTUARIES IN SOUTH-WEST ENGLAND

Region	No.	Estuary	Station*	*Nereis* (µg/g dry wt) Zn	*Nereis* Cd	*Nereis* Zn/Cd	Sediment† (µg/g air dry wt) Zn	Sediment† Cd	Sediment† Zn/Cd	Interstitial water‡ Zn (µg/ml) Surface	Interstitial water‡ Zn (µg/ml) Deep	Interstitial water‡ Mean salinity	River water,§ soluble Zn (µg/ml)
North Coast	1	Torridge	Several (3)	155	0·08	1938	142	0·42	338	—	—	8·7	—
	2	Camel	Wadebridge	130	0·17	765	203	0·71	286	0·016	0·040	2·4	0·018
	3	**Gannel**	**Upper**	**312**	**2·35**	**133**	**1840**	**4·20**	**438**	**0·92**	**0·029**	**12·2**	**0·15**
			Middle	222	1·68	132	1010	4·00	253	0·42	0·41	20·0	—
			Penpoll	153	—	—	254	0·90	282	0·035	0·040	29·0	—
	4	Hayle	Grigg's Quay	180	0·46	391	795	0·67	1187	0·060	0·022	20·0	0·30
Fal system	7	Restronguet Cr.	Tullimaar	215	0·33	652	2790	1·80	1550	0·021	0·012	9·0	0·024
	8	**Restronguet Cr.**	**Devoran**	**225**	**1·13**	**199**	**2980**	**3·10**	**961**	**1·14**	**0·41**	**25·5**	**3·71**
	9	Calenick	Calenick	180	0·81	222	1160	2·80	414	—	—	7·0	0·12
	10	Truro	Truro	230	0·41	561	792	1·32	600	—	—	7·7	0·009
	11	Tresillian	Tresillian	190	0·52	365	496	0·42	1181	—	—	7·7	—
	12	**Fal**	**Head of estuary**	**350**	**0·48**	**729**	**476**	**0·32**	**1488**	**0·21**	**< 0·01**	**10·5**	**0·11**
South Cornwall	5	Porthleven	Harbour	180	0·49	367	735	2·41	305	—	—	33·4	—
	6	Helford	Gweek	155	0·12	1292	606	1·75	346	—	—	22·0	0·040
	13	Fowey	Lostwithiel	145	0·41	354	205	0·72	285	—	—	3·2	0·025
	14	West Looe	Several (2)	155	0·27	574	169	0·56	302	0·018	0·018	3·1	0·004
	15	East Looe	Several (3)	165	0·15	1100	159	0·26	611	0·031	0·022	8·0	0·028
Tamar system	16	Tiddy	Several (3)	150	0·76	197	472	0·22	2145	0·01	< 0·010	4·8	0·007
	17	Lynher	Several (3)	165	0·81	204	570	0·71	803	0·025	0·012	2·5	0·040
	18	Tamar	Several (3)	160	0·85	188	673	2·27	296	0·037	0·017	14·2	0·017
	19	Tavy	Lopwell	160	0·91	176	230	0·44	523	—	—	8·9	0·048
South Devon	**20**	**Plym**	**Upper (4)**	**160**	**3·6**	**44·5**	**256**	**9·3**	**27·5**	**0·016**	**0·096**	**17·3**	**0·013**
			Lower (3)	160	1·22	131	358	2·5	143	—	—	31·5	—
	21	Yealm	Puslinch	190	0·19	1000	139	< 0·2	> 695	—	—	18·6	—
	22	Erme	Several (2)	155	0·30	517	116	0·32	363	—	—	18·2	—
	23	**Avon**	**Lower Ford**	**150**	**0·15**	**1000**	**102**	**0·22**	**464**	**0·016**	**0·020**	**13·8**	—
	24	Kingsbridge	Newquay	140	0·12	1167	97	< 0·2	> 485	—	—	33·1	—
	25	Dart	Several (3)	185	0·58	319	136	0·54	252	< 0·01	0·020	17·8	0·005
	26	Teign	Several (2)	180	0·56	321	268	1·26	213	—	—	11·0	—
	27	Exe	Topsham	160	0·29	552	274	1·17	234	—	—	3·5	—

* 'Several' shows that more than one station was used along the estuary.
† Sediment used was that adhering to the worms and came from a depth of about 10 cm.
‡ Interstitial water was squeezed from an oxidized surface layer about 1 cm thick and from a core section 5–25 cm deep.
§ Filtered through 0·45 nm millipore filter and analysed following concentration by evaporation and removal of organic material with nitric acid.

Zinc and cadmium in Nereis

In order to compare concentrations of zinc in *Nereis* from different estuaries it was necessary to correct for the size of the animals. Fig. 2 shows how concentrations in animals from different estuaries or from different stations in the Gannel Estuary change with the average dry weight. From these curves concentrations in worms having a dry weight of 0·05 g can be deduced and are summarized in Table 1. Concentrations of

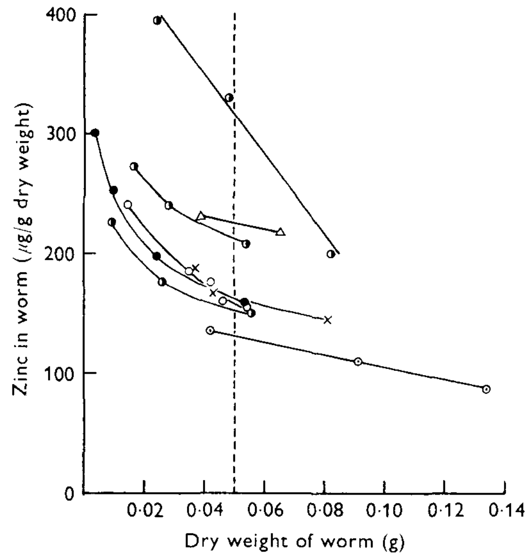

Fig. 2. Relationship between the concentration of zinc and the average dry weight per worm. ⊙, Camel Estuary; ◑, Gannel; △, Restronguet Creek; ○, West Looe; ×, East Looe; ●, Plym.

cadmium were corrected by the same factor as those for zinc because it was shown in the Plym Estuary that although size affected the concentrations it did not change the Zn/Cd ratio appreciably. The highest average concentration of cadmium, in worms from the Plym Estuary, is about 45 times the lowest concentration – a similar factor to that observed for both cadmium and zinc in the sediments. On the other hand, the highest concentration of zinc in the worms is only 2·7 times the lowest, suggesting that, compared with cadmium, zinc is effectively regulated.

In terms of Zn/Cd ratios, this last observation means that with increasing concentrations of both metals in the sediments the ratios in the worms tend to fall relative to those in the sediments. These ratios are given in Table 1 and, with three exceptions, ratios for the animals exceed those in the sediment when its concentration of zinc is less than 350 µg/g but are lower when its concentration is more than 350 µg/g.

Since the animals appear to treat zinc and cadmium differently, it seems best to consider their relationships with the sediment separately, although in the experimental section it will be shown that the concentration of zinc in solution does influence the rate of absorption of cadmium by the worms (see page 851).

The relationship between the animals and the sediments for zinc is shown in Fig. 3.

For an increase from 100 to 1000 μg/g in the concentration of zinc in the sediment the level in the majority of worms increases by no more than 100 μg/g. However, there are three outstanding results: (1) animals from the Devoran Station on Restronguet Creek contain little more zinc than the other worms although the sediment contains nearly

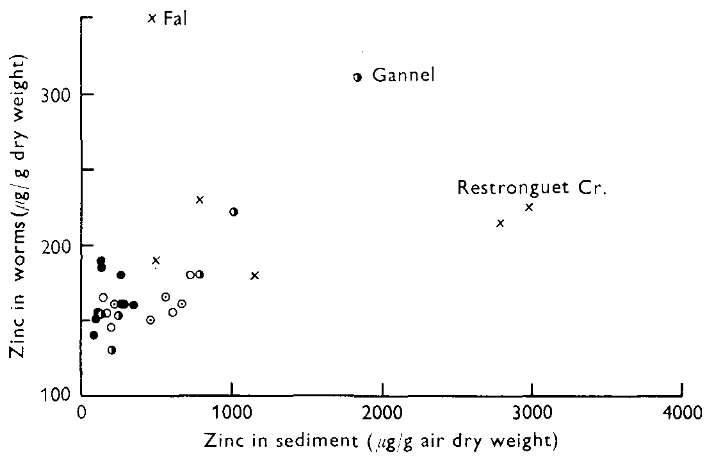

Fig. 3. Relationship between concentrations of zinc in worms and sediments. ◑, North Coast Estuaries; ×, Fal System; ○, South Cornwall; ⊙, Tamar System; ●, South Devon.

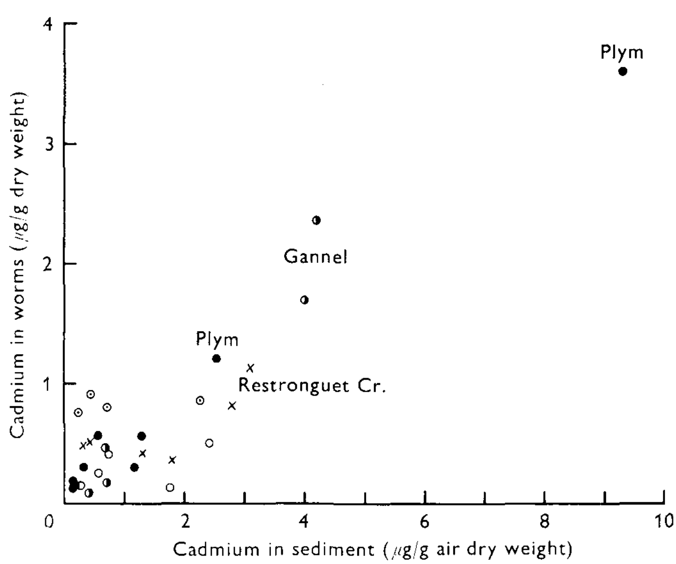

Fig. 4. Relationship between concentrations of cadmium in worms and sediments. Symbols as for Fig. 3.

3000 μg/g, the concentration in the interstitial water is high and at low tide the animals lie about 1 m from the water of the Carnon River which contains nearly 4 μg/ml of zinc (Table 1); (2) animals from the upper part of the Gannel Estuary contain more zinc than those from Restronguet Creek although levels in the environment are generally

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lower; (3) animals from the Fal Estuary contain the highest concentration of zinc but levels in the environment are lower than in the previous two examples. One possible explanation is that different populations of worms differ in their capacity to deal with high concentrations of zinc and an experimental study of this is described later.

Results for cadmium in Fig. 4 suggest that concentrations in the worms increase in direct proportion to those in the sediment. There are certainly some deviations from this at the lower levels, which are only partially explained by the fact that the limit of detection for cadmium was around 0·1 μg/g for both worms and sediments.

Absorption of zinc and cadmium from solution and their toxicities to Nereis

Bryan & Hummerstone (1971) found that worms from Restronguet Creek, where the sediments contain about 3000 μg/g of copper, are more resistant to the toxic effects of copper than populations from uncontaminated estuaries. Similar concentrations of zinc are found in these sediments and so experiments were carried out to see whether this population is also more resistant to zinc and cadmium.

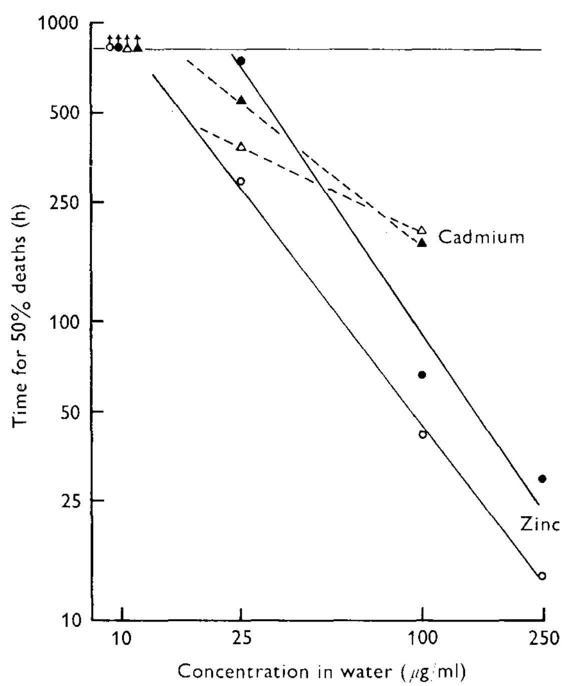

Fig. 5. Relationship between concentrations of zinc and cadmium in sea water ($S‰ = 17·5$) and the time taken to kill 50 % of the worms. ○, △, Avon animals; ●, ▲, Restronguet Creek (Tullimaar) animals. The experiment was not carried beyond 816 h.

Animals in groups of ten were exposed to a range of concentrations of zinc and cadmium in glass bowls containing 250 ml of solution and lengths of glass tubing to keep the worms separated. The solutions were aerated and changed daily, when any dead animals were removed and kept for analysis. Times taken for 50 % of the animals to die were estimated using probability paper and values obtained at a salinity of 17·5 are plotted in Fig. 5. At this salinity neither metal is very toxic. Although at a concentration of 100 μg/ml cadmium is much less toxic than zinc it is probably rather more toxic at low concentrations. It should be noted that at 100 and 250 μg/ml precipitation of zinc occurred and this could have increased its toxicity due to ingestion by the worms.

TABLE 2. TOXICITIES OF ZINC AND COPPER TO RESISTANT AND NON-RESISTANT WORMS

		96 h LC50 concentrations (μg/ml)	
	Salinity (‰)	Avon animals	Restronguet Cr. animals
Zinc (sulphate)	0·35	1·5	2·3
	3·5	11·0	14·6
	17·5	55	94
Copper (citrate)	17·5	0·54	2·3

TABLE 3. EXPERIMENTS SHOWING MEAN TIMES OF SURVIVAL IN DIFFERENT CONCENTRATIONS OF ZINC AND CADMIUM AND AMOUNTS ABSORBED BY ANIMALS

			Avon worms			Restronguet Cr. worms		
Concentration in water (μg/ml)	Salinity (‰)	pH	Mean time of survival (h)	Concentration in worms (μg/g dry weight)	Rate of net absorption (μg/g/day)	Mean time of survival (h)	Concentration in worms (μg/g dry weight)	Rate of net absorption (μg/g/day)
Zn			Experiment using zinc sulphate and lasting 816 h					
250*	17·5	7·05	24	2410	2230	30	2330	1680
100*	17·5	7·3	48	2500	1150	72	2230	672
25	17·5	7·8	382	3630	216	732	2930	89
10	17·5	7·8	816†P	2040	55	816†	868	19
0	17·5	7·8	816†	180	0	816†	223	0
Zn			Experiment using zinc sulphate and lasting 408 h					
25	3·5	7·7	52	1150	439	70	1510	442
10	3·5	7·7	128	1140	178	216	1780	173
5	3·5	7·7	349†	1010	55	394†	1010	48
0	3·5	7·8	408†	198	0	408†	229	0
Zn			Experiment using zinc sulphate and lasting 216 h					
5	0·35	7·6	46	812	310	72	1190	319
2·5	0·35	7·7	70	638	144	115	764	113
1·0	0·35	7·6	180	673	60	216†	501	31
0	0·35	7·8	216†	216	0	216†	224	0
Cd			Experiment using cadmium sulphate and lasting 816 h					
100	17·5	7·6	233	4010	413	187	1300	167
25	17·5	8·1	406	1970	116	576	1630	68
10	17·5	8·1	809†P	1860	55	816†	629	18·5
2·5	17·5	8·1	816†	471	13·9	816†	225	6·6
1·0	17·5	8·1	816†	208	6·1	816†	140	4·1

* Some zinc was precipitated.
† Sample includes animals still living at the end of the experiment.
†P Living worms but poor condition.

Concentrations of zinc killing 50% of the worms in 96 h (96 h LC 50 concentrations) for both Restronguet Creek (Tullimaar) and Avon animals are summarized in Table 2 and compared with values for copper (as citrate) obtained at the same time of year. The difference in resistance between the two populations is smaller for zinc than for copper and this may be because zinc is much less toxic than copper. In the interstitial water from Restronguet Creek concentrations of copper were found which approached the toxic threshold for unadapted worms (Bryan & Hummerstone, 1971). Concentrations of zinc exceeding 1 μg/ml have rarely been found in the interstitial water whereas Fig. 4 suggests that the threshold for zinc toxicity in the unadapted Avon animals is more than 10 μg/ml at a salinity of 17·5. On this basis no adaptation to zinc appears to be necessary. However, the intake of zinc by *Nereis* in contaminated conditions is almost certainly increased by absorption from contaminated food, its toxicity increases markedly at lower salinities (Table 2) and conditions contributing to the development of a resistant population were almost certainly worse in the past than at present, since mining was at its peak in the middle of the last century.

Animals which died during the toxicity experiments were retained and analysed together with any survivors at the end of the experiments. Assuming that the rate of net uptake is linear, the rate of net gain per day can be found by dividing the final concentration in the pooled animals (minus control value) by the mean time of survival. These results are summarized in Table 3 and show that, with one exception, the mean times of survival for the Restronguet Creek animals always exceed those for the Avon animals. In most cases this is because zinc or cadmium was absorbed more slowly by the Restronguet Creek worms, and when this was not the case the Restronguet Creek animals required more metal to kill them. At lower salinities the toxicity of zinc increases, because not only is it absorbed more rapidly but the worms are also killed by lower internal concentrations. The greater toxicity of zinc than cadmium at high concentrations when precipitated zinc was present is explained by the higher rate of absorption of zinc. At lower concentrations, where the toxicities are closer, the rates of absorption are also closer.

In order to confirm some of the points raised by the previous observations regarding rates of absorption and the adaptation of worms to high levels of zinc, experiments using radioisotopes have been carried out.

Experiments with ^{65}*Zn*

Rates of absorption in adapted and unadapted worms

Using ^{65}Zn the rate of absorption of zinc was measured at seven different concentrations of stable zinc (added as sulphate) in water having a salinity of 17·5. Simultaneous experiments were carried out with animals from Restronguet Creek (Tullimaar) and the Avon Estuary and 4–5 worms were used at each concentration. The absorption of ^{65}Zn was followed for 7 days and the average uptake curves for Restronguet Creek worms are shown in Fig. 6. Rates of absorption of stable zinc were calculated from the slopes of the curves between 30 and 120 h and it is thought that the intercept of the curves on the vertical axis may represent the adsorption of zinc on the surface of the body. Results from these experiments (on a wet weight basis) are summarized in Fig. 7 and show that

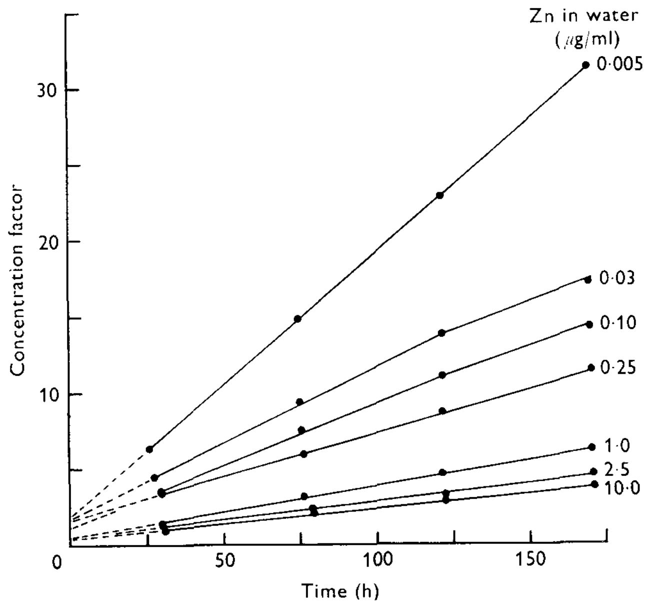

Fig. 6. Absorption of ^{65}Zn by Restronguet Creek (Tullimaar) animals from sea water (S‰ = 17·5) containing different concentrations of stable zinc. Each point is an average from 4–5 worms. Concentration factor is the ratio: ^{65}Zn per g wet weight/^{65}Zn per g of water.

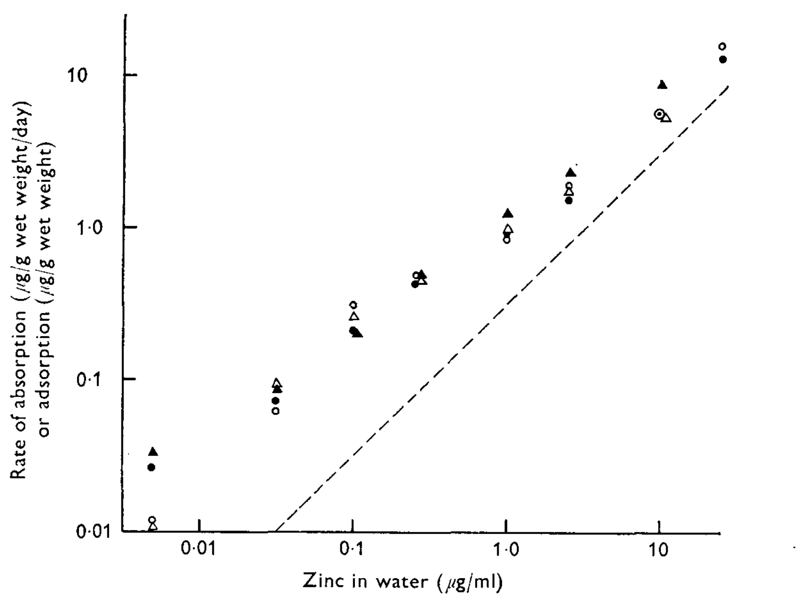

Fig. 7. Relationship between the concentration of zinc in sea water (S‰ = 17·5) and the rate of absorption and degree of adsorption of zinc in two populations of worms. ▲, Absorption by Avon animals; ●, absorption by Restronguet Creek animals; △, adsorption by Avon animals; ○, adsorption by Restronguet Creek animals. On a dry weight basis values would be about seven times higher. – –, line of direct proportionality.

zinc is absorbed more rapidly (about 30%) by the unadapted worms from the Avon Estuary. This agrees with the results from the toxicity experiments where higher concentrations of zinc were involved.

The rate at which zinc is absorbed is not proportional to the concentration in the water but, with the exception of the lowest concentration where no carrier was added, it is roughly proportional to the amount of zinc which appears to be adsorbed on the body surface (Fig. 7).

Absorption of naturally occurring zinc

Concentrations of zinc in interstitial waters from Restronguet Creek are given in Table 1 and, as they were measured by direct atomic absorption, should include any zinc which was combined with soluble organic compounds. To see whether any effects

TABLE 4. COMPOSITION OF WATER FOLLOWING CONTACT WITH SEDIMENTS FROM RESTRONGUET CREEK FOR 3 DAYS

Station	Type of sediment	S‰	μg/ml Mn	Zn
Devoran	Surface	17·5	0·55	0·49
	Deep	17·5	0·32	0·26
Tullimaar	Surface	17·5	0·48	0·093
	Deep	17·5	0·64	0·036

which might come from these compounds could be detected, the absorption of ^{65}Zn by worms from Restronguet Creek (Tullimaar) was compared in media containing either natural zinc leached from the sediment or zinc added as sulphate. Solutions containing natural zinc were prepared by placing 400 g of sediment in a beaker and covering this with 200 ml of sea water having a salinity of about 20. Eight worms were added to the system so that the aerated water was circulated through their burrows in the sediment. After 3 days the water was filtered through a 0·45 nm Millipore filter and the salinity was measured. The salinity was adjusted to 17·5 with distilled water and the concentrations of zinc and manganese in the solution were measured. Four solutions having the composition shown in Table 4 were prepared and four similar artificial solutions were also made. After labelling with carrier-free ^{65}Zn each solution was used to study the absorption of the isotope by three animals. The results are summarized in Fig. 8 and, because the volumes of solution were limited, depletion of ^{65}Zn was sufficient to cause more bending of the curves than would otherwise be expected. The isotope is absorbed at roughly the same rate from both types of solution and, because the rate of absorption of ^{65}Zn depends on the concentration of stable zinc in solution, this suggests that zinc acts in the same way in both natural and artificial solutions. If an appreciable fraction of bound zinc was present it might be expected that the rate of absorption of the isotope by the worms would be markedly affected. The results indicate that much of the zinc in the interstitial water from Restronguet Creek is available to the worms and may be in an inorganic form or only weakly bound by soluble organic compounds. Although a different type of approach was used, Bryan & Hummerstone (1971) concluded that much of the copper in the interstitial water from these sediments is available to the worms.

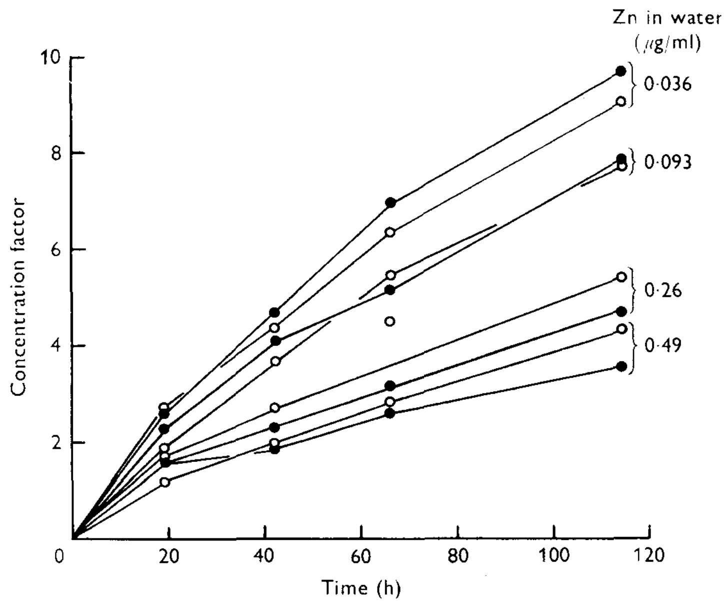

Fig. 8. Absorption of ^{65}Zn from sea water (S‰ = 17·5) containing different concentrations of zinc by animals from Restronguet Creek (Tullimaar). ●, Zinc leached from sediment; ○, zinc added as sulphate. Each point is an average from three worms.

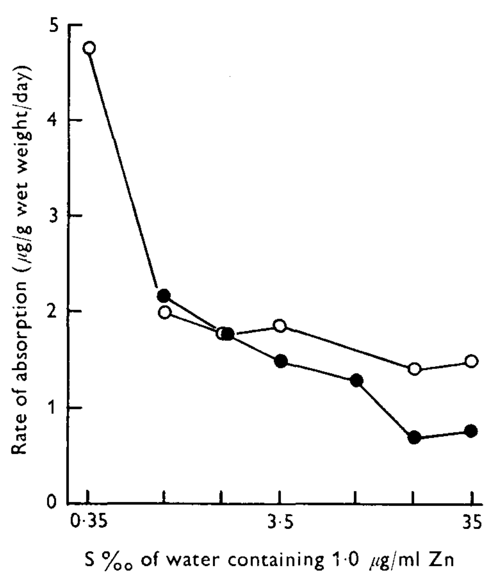

Fig. 9. Effect of salinity on the rate of absorption of zinc by two populations of worms. ○, Avon worms; ●, Restronguet Creek (Tullimaar) worms. Each point is an average from 4–5 worms.

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Influence of salinity on zinc absorption

Analyses from the toxicity experiments showed that zinc is absorbed more rapidly at low salinities and this has been confirmed using ^{65}Zn (Fig. 9). Although experiments with animals from both Restronguet Creek and the Avon Estuary were carried out, they were not done simultaneously. It was noted that whereas the pH of the more saline media was about 8, that of the lower salinities was nearer to 7. Unlike manganese (Bryan & Hummerstone, 1973), there was no evidence from the field observations that animals from places exposed to low average salinities contain more zinc than those from more saline areas. Presumably any increase in absorption is controlled by increased excretion.

Experiments with ^{115m}Cd

The effect of zinc on the absorption of cadmium

Simultaneous experiments were carried out with worms from Restronguet Creek (Tullimaar) and the Avon Estuary in which the effect of zinc on the absorption of ^{115m}Cd was studied. Rates of absorption of cadmium at different external concentrations

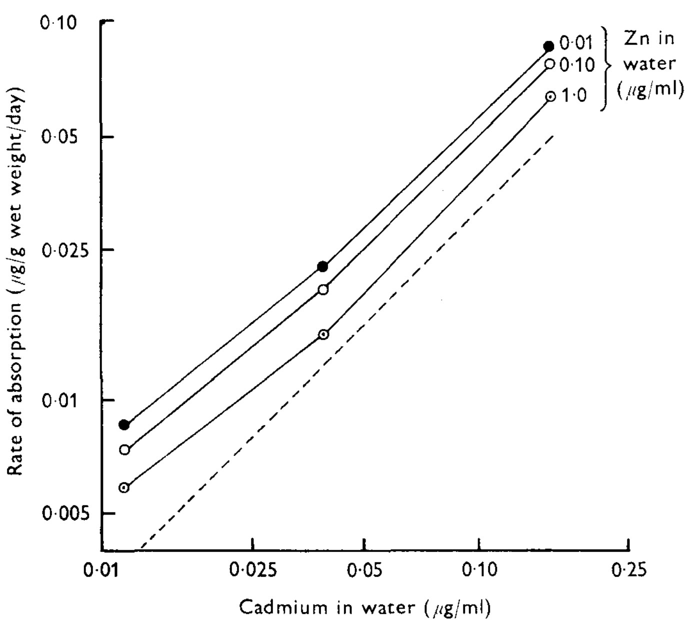

Fig. 10. Effect of zinc on the relationship between the rate of absorption of cadmium and the concentration in sea water ($S‰ = 17·5$). Animals were from Restronguet Creek (Tullimaar) and each point is an average for four worms. – –, Line of direct proportionality.

and the effect of zinc on these are shown in Fig. 10 for the Restronguet Creek worms. These worms absorbed cadmium about 15% more slowly than worms from the Avon Estuary, but otherwise the results were very similar. The three concentrations of zinc used in these experiments covered the range of values observed for interstitial water in Table 1 but the lowest level of cadmium was controlled by the amount of carrier supplied with the ^{115m}Cd. Increasing the concentration of zinc in the water from 0·01–0·1 μg/ml and from 0·01–1·0 μg/ml decreased the rates of absorption of cadmium by average values of 9 and 37% respectively. Effects of this magnitude on concentrations of cadmium in *Nereis* in the field would be difficult to detect and may explain why no obvious effect of zinc on the distribution of cadmium between the worms and the sediment was observed.

Adaptation of Nereis *to zinc-contaminated sediments*

Field observations showed that in three of the estuaries, the Fal, the Gannel and Restronguet Creek, concentrations of zinc in the interstitial water exceeded 0·1 μg/ml and increased in the order Fal < Gannel < Restronguet Creek. On the other hand concentrations in the worms were in the reverse order. Animals from the Devoran Station

TABLE 5. RATES OF ABSORPTION (μg/g/DAY) OF ZINC IN WORMS FROM THE AVON ESTUARY COMPARED WITH WORMS FROM CONTAMINATED ESTUARIES

Avon worms

		Zinc in water of 17·5 $S‰$ (μg/ml)		
		0·01	0·1	1·0
Rates of absorption	Wet weight basis	0·041	0·202	1·16
	± S.D.	0·0093	0·035	0·11
	Dry weight basis	0·297	1·36	7·97

Worms from contaminated estuaries

Estuary	Zn in control worms (μg/g dry weight)	Relative rates of absorption in animals having mean dry weight of 0·036 g			Mean
Avon	212	100	100	100	100
Fal	442	100	113	83	99
Gannel	243	78	88	62	76
Restronguet Cr.					
Tullimaar	278	63	76	57	65
Devoran	285	68	74	62	68

on Restronguet Creek did not contain much more zinc than animals from uncontaminated estuaries although the interstitial water contains as much as 1 μg/ml of zinc which experiments (p. 849) have shown is probably available to the worms. Other experiments have shown that these worms are less permeable to zinc and more resistant to toxic concentrations than normal animals. It was thought that the higher concentrations of zinc in worms from the Fal and Gannel Estuaries might be explained if it could be shown that they are more permeable and not as well adapted as the animals from Restronguet Creek. Using ^{65}Zn and animals of similar size, the rates of absorption of zinc by animals from all three estuaries and from the Avon Estuary were carried out simultaneously. The results are summarized in Table 5 and show that the permeability of the Fal animals to zinc is similar to that for animals from the uncontaminated Avon Estuary. Results for the Gannel worms are intermediate and suggest that they possess a degree of adaptation. Unfortunately, although five worms from each station were used at each concentration, the standard deviations of the results were sometimes around ±20% and this raises doubts about their significance.

When the animals from the media containing 1 μg/ml of zinc were analysed for stable zinc after 7 days it was found that, whereas those from other stations had absorbed a measurable amount, very little had been gained by the worms from Restronguet Creek – suggesting that zinc was being excreted as fast as it was absorbed. However, the differences between control and experimental analyses on which this statement is based were quite small and so to avoid this problem an experiment was carried out in which

TABLE 6. RELATIVE RATES OF NET ABSORPTION OF ZINC FROM WATER ($S‰ = 17·5$) CONTAINING ADDED ZINC

(The highest absorption rates obtained for the Avon animals are assigned the value of 100%)

			5 μg/ml Zn for 15 days		10 μg/ml Zn for 9 days		
Estuary	Dry weight of worm (g)	Zn in control worms (μg/g dry weight)	Rate (μg/g dry weight/day)	%	Rate (μg/g dry weight/day)	%	Mean (%)
Avon	0·016	204	39·6	100	62·7	100	100
	0·035	174	29·8	100	48·0	100	
Fal	0·016	470	36·0	91	62·0	99	95
Gannel	0·016	311	25·7	65	62·0	99	75
	0·035	248	14·1	47	42·2	88	
Restronguet Cr. Tullimaar	0·035	203	14·8	50	23·7	49	50
Restronguet Cr. Devoran	0·016	274	23·8	60	41·2	66	57
	0·035	254	14·6	49	25·4	53	

the net uptake of zinc was observed in solutions containing 5 and 10 μg/ml of zinc. From each station two groups of six or seven worms, one heavier than the other, were exposed to water containing 5 μg/ml for 15 days and two similar groups were kept in water with no zinc added. By plotting the analyses against the average weight of the animals in each group it was possible to compare control and experimental concentrations in worms of exactly the same weight. The difference between these values divided by the time of exposure gave the rate of net absorption of zinc. Similar experiments were carried out in water containing 10 μg/ml of zinc and the results are summarized in Table 6. When expressed as percentages of the results for the Avon animals they tend to confirm the results in Table 5. The rate of net absorption of zinc by the Restronguet Creek animals is rather more than half that of the Avon animals. This difference is greater than that in Table 5 and is greater than was found in the earlier radioisotope experiments (see for example Fig. 6) where the average difference was about 30%. Since the net rate of absorption is the total rate, as measured with ^{65}Zn, minus the rate of excretion, the greater difference in Table 6 suggests that not only are the Restronguet Creek worms less permeable to zinc than those from the Avon Estuary but they are also better at excreting zinc.

Contamination of Restronguet Creek with metals due to mining has been occurring for at least 200 years and conditions leading to the development of a metal resistant population may in the past have been worse than at present. On a reduced scale the same may be true of the Gannel Estuary, although the worms are less well adapted and, despite the apparently lesser degree of contamination, they contain more zinc than those from Restronguet Creek. The Fal animals appear to be little better adapted than the control worms from the Avon and this may explain why, although contamination is less than in the other two estuaries, the worms contain much higher levels of zinc. The Fal is silted up with china clay wastes and conditions may never have been sufficiently bad for an adapted population to evolve.

DISCUSSION

At a concentration of 25 μg/ml in the water, the rate of absorption of zinc by Restronguet Creek animals as measured with ^{65}Zn was similar on a dry weight basis to the net rate of absorption found by analysis. This occurs because at such a high concentration the rate of excretion, by which the two values differ, is comparatively small.

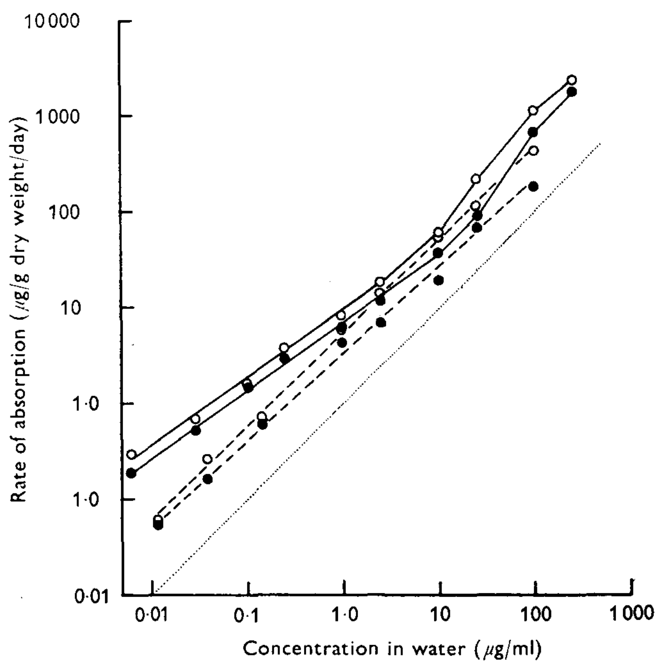

Fig. 11. Relationship between rates of absorption of zinc and cadmium by two populations of worms and the concentrations in the water (S‰ = 17·5). These graphs combine the results from the radioisotope and toxicity experiments on a dry weight basis. ○—○, Zinc in Avon animals; ●—●, zinc in Restronguet Creek animals; ○- - -○, cadmium in Avon animals; ●- - -●, cadmium in Restronguet Creek animals; . . . ., line of direct proportionality. The worms weighed about 0·05 g.

As a result, the values from the radioisotope and toxicity experiments can be combined and are summarized in Fig. 11. They show clearly that the rate of absorption of cadmium is more nearly proportional to the external concentration than that of zinc and that between concentrations of 0·01 and 10 μg/ml the rates of absorption converge. This is an important observation because it can be used to explain why, in the field, concentrations of zinc in the worms change less than concentrations of cadmium in response to changes in the sediments. This is not the only reason, since it has also been shown that worms from high-zinc sediments are better able to regulate zinc than those from uncontaminated estuaries. These experimental results tend to imply that zinc and cadmium are mainly absorbed from solution in the interstitial water, but, in addition, the worms probably absorb appreciable amounts from their food. Although *Nereis* can filter-feed and grasp larger particles in its jaws, it also feeds by ingesting the surface layers of the sediment (Harley, 1953; Goerke, 1971). Thus the metals may come from the same source whether they are absorbed from solution or from food.

Why the rates of absorption of zinc and cadmium change in different ways in relation to the external concentration is not known, but there are several possibilities based either on the chemical form of the metal in the water or on the processes of absorption.

The more abundant chemical forms of zinc and cadmium in sea water appear to be quite different. Zirino & Yamamato (1972) have predicted that at the pH of sea water (8·1) zinc exists as $Zn(OH)_2^0$ (62%), Zn^{2+} (17%) and $ZnCl^+$ (6·4%), whereas cadmium exists as $CdCl^0$ (51%), $CdCl^+$ (39%) and $CdCl_3^-$ (6%). There is, for example, a much higher proportion of divalent zinc than divalent cadmium ions in sea water and this may have a bearing on the uptake processes. Zirino and Yamamato also showed that with decreasing pH the proportion of divalent zinc ions increases markedly and this could be one of the reasons why at low salinities zinc was absorbed more rapidly by *Nereis*, since the pH was shown to be lower. However, there are other factors such as the lack of competition from calcium and magnesium at low salinities and the discovery by Fletcher (1970) that at low salinities the potential difference across the body wall (negative inside) increases appreciably. This has been discussed by Bryan & Hummerstone (1973) in relation to the increase in manganese absorption which was observed in *Nereis* at low salinities. However, unlike manganese, concentrations of zinc in animals from the field did not change appreciably with salinity. Compared with zinc, the forms of cadmium in sea water are relatively independent of pH (Zirino & Yamamato, 1972), and this could explain why in Fig. 10 the rate of absorption of zinc increases relative to cadmium at high concentrations which are sufficient to reduce the pH of the water. However, at the two highest concentrations, zinc was precipitated and may have been ingested.

The rate of absorption of zinc appears to be related to the amount which can be adsorbed or bound in some other way by the body surface. In crustaceans a similar situation exists and uptake appears to depend initially on an adsorption process (Bryan, 1971). In support of this, unpublished work using the lobster *Homarus gammarus* showed that the initial uptake of zinc was similar in both living isolated gills and in the gill surfaces which are cast when the lobster moults. Differences between the rates of absorption of zinc and cadmium may therefore depend not only on their chemical forms in the water but also on differences between the ways in which they are adsorbed and then transported inwards. Different carrier mechanisms or mechanisms having different characteristics towards the two metals may exist, especially as zinc is an essential element and cadmium is not, but there is no evidence for this. Nor is there any evidence for the active transport of these metals by marine organisms, although in other fields the active transport of metals such as nickel has been described in yeast cells and in the protozoan *Paramecium* (Fuhrmann & Rothstein, 1968; Andrivon, 1970).

An important discovery in this work is that animals from estuaries where the sediments have contained high levels of zinc for a long time, the worms are adapted to resist its toxic effects. If, as seems likely, this is genetic adaptation, the pressure to evolve probably develops in the least saline areas of an estuary. The toxicity of zinc increases at low salinities and processes such as those of ionic and osmotic regulation are probably most vulnerable under these conditions. The best adapted worms were from Restronguet Creek and had previously been shown to be resistant to copper (Bryan & Hummerstone, 1971). Although the sediments from this area contain roughly equal amounts of copper and zinc and the latter is generally more readily available in the interstitial water, the worms are better adapted to copper than zinc. This is probably because zinc is much less toxic than copper.

In the plant kingdom, metal tolerant populations have been observed in freshwater algae (Myslik & Hutchinson, 1971), marine algae (Russell & Morris, 1970) and in land plants (Bradshaw, 1970). The adaptation of land plants to old mining and smelting dumps is a genetic process and has been studied in some detail (Bradshaw, 1970). Usually, resistance to two metals only occurs in land plants if both are present in the soil and adaptation to each is developed separately. In *Nereis* the adaptations to zinc and copper have probably developed separately because they appear to be completely different processes. The adaptation to zinc involves a decrease in the permeability of the body surface and probably an improved ability to excrete the metal. These populations are also rather less permeable to cadmium and manganese so that the adaptation is not completely specific. Decreased permeability has been described as the mechanism of arsenite resistance in the bacterium *Pseudomonas* (Beppu & Arima, 1964) and arsenate resistance in yeast, where uptake is an active process (Cerbón, 1969). In the case of copper, unpublished results have shown that absorption is more rapid in the resistant than non-resistant *Nereis*. The level of copper in the worms tends to be proportional to that in the sediment and resistant worms may contain 100 times more copper than non-resistant worms (Bryan & Hummerstone, 1971). Increased resistance appears to depend on a complexing system which detoxifies the metal and stores it in the epidermis and nephridia. This system has similarities to that in land plants where zinc, for example, is much more strongly bound by the cell walls in tolerant than in non-tolerant individuals, thus perhaps protecting the other parts of the cell (Turner & Marshall, 1971).

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