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ADAPTATION OF THE POLYCHAETE *NEREIS DIVERSICOLOR* TO ESTUARINE SEDIMENTS CONTAINING HIGH CONCENTRATIONS OF HEAVY METALS

I. GENERAL OBSERVATIONS AND ADAPTATION TO COPPER

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(Text-figs. 1–3)

The concentration of copper in *Nereis diversicolor* O. F. Müller is roughly related to the total concentration in the sediment and particularly high concentrations are found where mining pollution occurs. In contrast, the concentration of zinc in *Nereis* remains remarkably constant despite wide variations in the environment and appears to be accurately regulated.

In worms from different estuaries concentrations of copper have been related to those of the sediments, sediment extracts and interstitial water at different stations, and the influence of salinity and size of animal has also been considered. The relative importance of the absorption of copper via the gut or across the body surface is still uncertain, but uptake over the body surface appears to be important and in high-copper animals from polluted areas much of the metal is deposited in the epidermis of the body wall and in parts of the nephridia.

High-copper animals survive in polluted areas because they have developed a tolerance to the toxic effects of copper which is neither readily lost, nor readily gained by non-tolerant animals. The situation may be similar to that found on old mine dumps where populations of metal-tolerant land-plants have evolved.

INTRODUCTION

The polychaete worm *Nereis diversicolor* O. F. Müller burrows into intertidal estuarine sediments and is usually distributed from near the mouth of a river into regions which although still tidal are exposed to fresh water for some of the time. It can tolerate waters ranging from sea water with a salinity of 35‰ to fresh water (Smith, 1970), but has a clear preference for intermediate concentrations and is most abundant in the middle of the tidal reaches of an estuary. Depending on the geology of the area, as well as on the presence of wastes from mining and local industry, concentrations of trace metals may vary widely between the sediments of different estuaries. Under certain conditions some polychaetes are able to absorb metals from ingested sediment as well as from solution in the surrounding water and *Nereis* may be no exception (see, for example, Chipman, Schommers & Boyer, 1968). Concentrations of trace metals in polychaete worms in relation to those of the sediment have been studied by Cross, Duke & Willis (1970), who suggested that the concentrations of zinc, manganese and iron in the animals may be regulated. A similar suggestion was made by Bryan (1971) for *N. diversicolor*.

We have been studying the distribution of trace metals in different estuaries in South-West England and have found concentrations of copper in sediments ranging from about 20 to more than 9000 μg/g on a dry-weight basis. *N. diversicolor* was found living in or near these sediments and it was thought that a species which is successful under all these conditions merited further examination.

This paper deals with the relationship between the concentrations of copper, zinc, lead, manganese and iron in *Nereis* and in the sediments in which it lives. In the case of copper it shows how factors such as size of animal, salinity and the development of tolerance to high concentrations are involved.

MATERIALS AND METHODS

Animals

In several estuaries in South-West England *Nereis diversicolor* were collected from a series of stations which covered the range of distribution of the species in each estuary. Animals for analysis were cleaned by placing them in a layer of fine acid-washed sand covered by 50 % sea water for 7 days. This method was more effective than simply starving the animals as a way of removing the gut contents, which are an important source of analytical error if they include sediment. The sand may also remove some metals which are adsorbed on to the surface of the body because it was noticed that concentrations of manganese in animals cleaned by this method were lower than in animals starved in 50 % sea water for the same length of time. Regardless of their origin, all animals were kept in 50 % sea water because otherwise the concentrations of metals, whether calculated on a dry or a wet basis, would be influenced by the different amounts of water or salts in worms from different salinities.

From each station a sample of at least nine cleaned worms was weighed in a 100 ml. conical flask and dried at 105 °C to find the dry weight. To digest the sample, 10 ml. of redistilled nitric acid were added and the flask was covered with a glass ball and kept on a controlled hotplate overnight. When the ball had been removed the acid was evaporated off and then 2 ml. of redistilled 50 % hydrochloric acid were added and also evaporated. Finally, a known volume of hydrochloric acid was added so that when the sample was diluted with distilled water it gave a 0·1 N solution. Analyses of copper, zinc, lead, manganese and iron were made by atomic absorption using a Techtron AA4/AA5 instrument, and when low concentrations were encountered corrections for non-atomic absorption by the sample matrix were made. Concentrations have been expressed as μg/g dry weight and have been corrected for the fact that about 4 % of the dry weight is made up of sand particles which remain in the gut from the cleaning process.

Sediments

Samples of sediment adhering to the worms when they were collected were retained and near the collecting point a core was usually taken with a 6 cm diameter perspex tube to a depth of 25 cm. The extruded core was cut into 5 cm long sections and a sample from each was air-dried for analysis. Analyses were carried out using 0·2 g of finely powdered sediment which was treated in exactly the same way as the dried worm samples. When this simple digestion was compared with the method of Langmyr & Paus (1968), in which the sample was completely dissolved in hydrofluoric acid (after removing organic matter by heating to 450 °C in a platinum crucible), the analyses showed that most of the copper, zinc and lead was recovered. However, the simple digestion did not recover all the manganese and iron by comparison with the hydrofluoric acid method. In the Tamar Estuary about 93 % of the manganese and 92 % of the iron was recovered, but in Restronguet Creek the corresponding figures were 84 % and 93 % and in the Plym Estuary only 28 % and 60 %. The results quoted in this paper were all obtained using the nitric acid digestion method because if the same method is used for animals and sediments it is easier to assess the effect if contamination of the animals by sediment particles is suspected.

In addition, the selective chemical attack devised by Chester & Hughes (1967) was used, which involves extracting the dried sediment with a mixture of acetic acid and hydroxylamine hydrochloride. This method gives an estimate of metals which are adsorbed on to sediments, are incorporated in carbonate minerals or are incorporated into ferro-manganese nodules and associated iron oxides but does not extract metals which are incorporated into the clay minerals.

Interstitial water

Approximately 5 ml. samples of interstitial water were required for measuring salinities at different depths in the sediments and were obtained from sections of cores by suction using a sintered glass crucible with a fine filter-paper insert. Salinity was measured using the method for sodium analysis described by Manning (1967) and standards were diluted from sea water of known salinity.

Interstitial water for metal analyses was squeezed from about 1 kg of sediment using an enlarged version of the squeezer described by Reeburgh (1967). The water was squeezed through a 0·45 μ Millipore filter of 105 mm diameter which was supported above the three small outlet holes by a 70 mm glass filter-paper. Before use, the glass filters were heated to 450 °C to remove any organic material and both glass and membrane filters were cleaned by soaking in dilute hydrochloric acid and distilled water. Other parts of the squeezers were sometimes cleaned with a decontamination detergent but were always finally cleaned in running sea water and then soaked in sea water having a very low trace-metal content. Samples of surface sediment for squeezing were taken by skimming the oxidized surface layer from an area of about 0·5 m^2 at low tide. Deep sediment from 5–25 cm below the surface was placed directly into the squeezer from the corer. Squeezers were operated at pressures of up to 4 atm for from 8–16 h and from 100 to 250 ml. of water were obtained. Water was collected in a 500 ml. round-bottomed flask to which 10 ml. of a 3·75 pH sodium acetate–acetic buffer had been added to prevent the precipitation of iron. Analyses of the water for zinc, manganese and iron were made by direct atomic absorption and corrections for non-atomic absorption were applied. The limits of detection were about 0·01 μg/ml. for zinc and manganese and 0·05 μg/ml. for iron. Copper was extracted from 150 ml. of water in the collecting flask by adding 2 ml. of a 1 % solution of ammonium pyrollidine dithiocarbamate and then shaking for 10 min with 10 ml. of redistilled methyl isobutyl ketone. The ketone was separated and placed in a stoppered centrifuge tube and then the process was repeated except that only 5 ml. of ketone were used. The extracts were pooled, adjusted to give equal volumes and then centrifuged. Standards were prepared by extracting known amounts of copper from sea water or diluted sea water using the same methods as for the samples. Blanks were prepared by repeating the extraction in sea water from which the copper had been extracted previously and to which 10 ml. of the 3·75 pH buffer had been added. Similar extractions were used to purify the buffer before it was used in these analyses. When 150 ml. of water were extracted the limit of detection for copper using atomic absorption analysis was about 0·001 μg/ml.

Histochemical location of copper

The rubeanic acid method of Howell (1959) was applied to paraffin-wax sections of *Nereis* which had been fixed in a neutralized 5 % solution of glutaraldehyde in sea water.

RESULTS

Mean concentrations of metals in sediments and animals from different estuaries

Preliminary analyses for copper and zinc in *Nereis* and sediments from the Tamar Estuary suggested that the concentration of copper in the worm changes with that of the sediment whereas the concentration of zinc in the worm remains constant. This idea was tested by extending the analyses to six more estuaries in South-West England. These drain areas including the copper–tin mineralized zone which extends across Cornwall and into West Devon.

The mean concentrations of metals in sediments digested with nitric acid are summarized in Table 1 and show that in terms of the amount of copper in the sediment the estuaries are of three types. Less than 100 μg/g of copper are found in four estuaries but in the Tamar system about 500 μg/g are found and in Restronguet Creek several thousand μg/g are found. Mining for metals such as copper, tin and lead ceased in the Tamar Valley during the first decades of this century but the estuary drains an area in which the soils are highly mineralized and in which old mine dumps and drainage adits are still present. In Restronguet Creek, which drains into the Fal Estuary, the high concentrations of some metals result from the sedimentation of mining wastes from the Carnon River. The waters of this small river have a pH of about 3·8 and after filtration contain about 0·6 μg/ml. of copper, 4 μg/ml. of zinc, 0·02 μg/ml. of lead, 0·6 μg/ml. of manganese and 2 μg/ml. of iron.

TABLE 1. MEAN CONCENTRATIONS OF METALS IN AIR-DRIED SEDIMENTS ADHERING TO *NEREIS DIVERSICOLOR*

Estuary	Region	No. of analyses	Dry weight (μg/g) Cu	Zn	Pb	Mn	Fe
Plym	S. Devon	4	41	339	44	46	5661
Dart	S. Devon	3	44	140	154	213	29434
Avon	S. Devon	3	52	99	35	253	26814
Camel	N. Cornwall	2	73	122	21	210	13680
Tamar	S. Cornwall/Devon	16	436	518	299	366	37679
Tiddy/Tamar	S. Cornwall	2	591	532	287	372	38565
Restronguet Creek/Fal	S. Cornwall	23	3020	2237	359	425	57837

TABLE 2. MEAN CONCENTRATIONS OF METALS IN *NEREIS DIVERSICOLOR*

(Samples analysed were from different stations of the specified estuary and contained 9 or 10 animals.)

Estuary	No. of analyses	Dry weight (%)	Dry weight (μg/g) Cu	Zn	Pb	Mn	Fe	Ratio $\frac{\text{Cu animal}}{\text{Cu sediment}}$
Plym	5	15·0	28	199	5·9	7·4	350	0·68
Dart	3	15·0	22	163	4·4	9·5	366	0·50
Avon	6	15·4	33	176	3·4	9·8	391	0·63
Camel	2	15·9	31	155	0·7	7·0	404	0·42
Tamar	18	15·2	106	166	5·8	11·0	458	0·24
Tiddy/Tamar	4	14·4	257	185	4·9	9·3	562	0·43
Restronguet Creek	23	15·9	1142	194	3·5	6·0	425	0·38

Concentrations of metals in *Nereis* from all seven estuaries are summarized in Table 2 and should be compared with the figures for sediments in Table 1. Ratios of the concentrations of copper in animals and sediments are also given in Table 2 and are sufficiently similar to suggest that the two concentrations may be directly related. In contrast, the concentrations of other metals in *Nereis* are not obviously related to those of the sediment and concentrations of zinc in the worms are remarkably constant in view of the variation in the sediments. There are at least two reasons why there may be no obvious correlation between concentrations in the worms and sediments, even if the sediment is the principal source of the metal. First, the concentration of metal in the sediment which is actually available to the animal need not be related to the total concentration, and secondly, the worm may regulate the concentrations of metals such as zinc and iron in its tissues.

Bearing these points in mind, more detailed evidence concerning the factors which influence the concentrations in *Nereis* will be considered separately for each metal and in this paper will be confined to copper.

Changes in copper content of animals in different estuaries

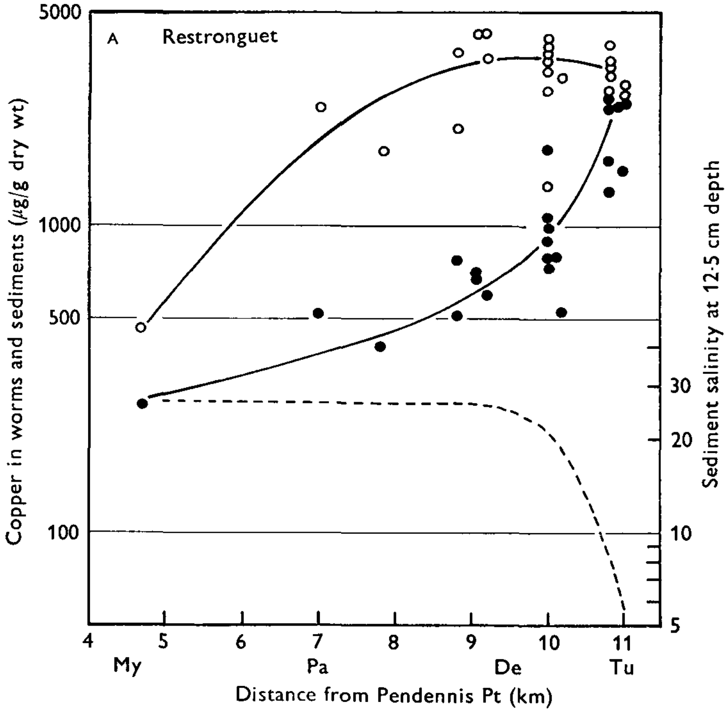
Influence of the sediment

In the estuaries of the Avon, Dart, Tamar and in Restronguet Creek the sediments and worms were analysed at different distances from the river mouth to see how concentrations change with locality or are influenced by salinity. The results are summarized in Figs. 1 A–D and relate concentrations of copper in the worms with (i) the total concentration in sediment which was adhering to the worms and came from a depth of 12–15 cm, (ii) the total concentration in the surface sediment, (iii) the concentration extracted with the acetic acid-hydroxylamine hydrochloride mixture, (iv) salinity at a depth of 12–15 cm.

Results for Restronguet Creek in Fig. 1 A show that the concentration of copper in the worm increases with distance from the mouth of the estuary although there appears to be no simple relationship between the concentrations in the sediments and animals. In this figure the results from surface sediments and sediment extracts have not been included for reasons of clarity and because they showed the same trends as the results for deeper sediments. Values for surface sediments were similar to those for deep sediments and some figures for sediment extracts are shown in Table 3. Two small rivers enter the head of Restronguet Creek, one of which, the Carnon River, is the source of pollution (see also page 848) whilst the waters of the other river are unpolluted. At low tide animals can be collected within about 1 m of the fresh water along the banks of both these rivers but, whereas at the limit of penetration of the species along the Carnon River (10 km station) the salinity of the deeper sediment exceeds 20, at the limit of penetration along the unpolluted river (11 km station) the salinity of the sediment is around 5. Over the whole area at the head of the Creek the sediments contain about 3000 μg/g of copper (9–11 km in Fig. 1 A), yet strangely much higher concentrations of copper are found in animals from the less saline parts of the unpolluted river (10·8 and 11 km stations) than in those living on the banks of the polluted Carnon River (10 km station). This apparent anomaly will be discussed again in later sections.

In the Tamar Estuary (Fig. 1 B) the concentration of copper in the worm increases sharply between the 7·5 and 11 km stations. This cannot be exactly related to changes in the sediments because the concentration in the sediment at 11 km is lower than would be expected. Similar series of analyses of *Nereis* were made on two other occasions and gave the same result. However, on these occasions a closer relationship with the deep sediment was obtained because it contained more than 450 μg/g at the 11 km station. This suggests that at this station the distribution of copper in the sediment is patchy.

Concentrations of copper in animals from the Avon and Dart estuaries tend to decrease with increasing distance from the mouth. This is again an anomalous situation because



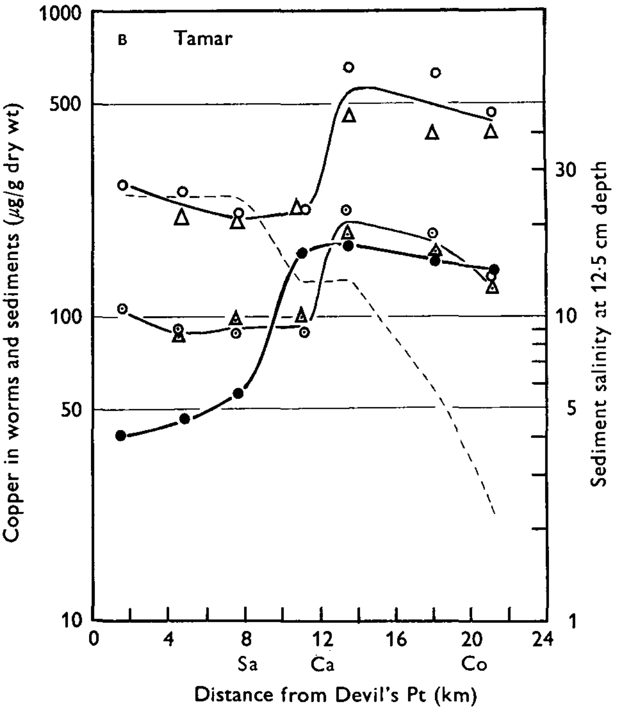


Fig. 1 A–B. For legend see opposite page.

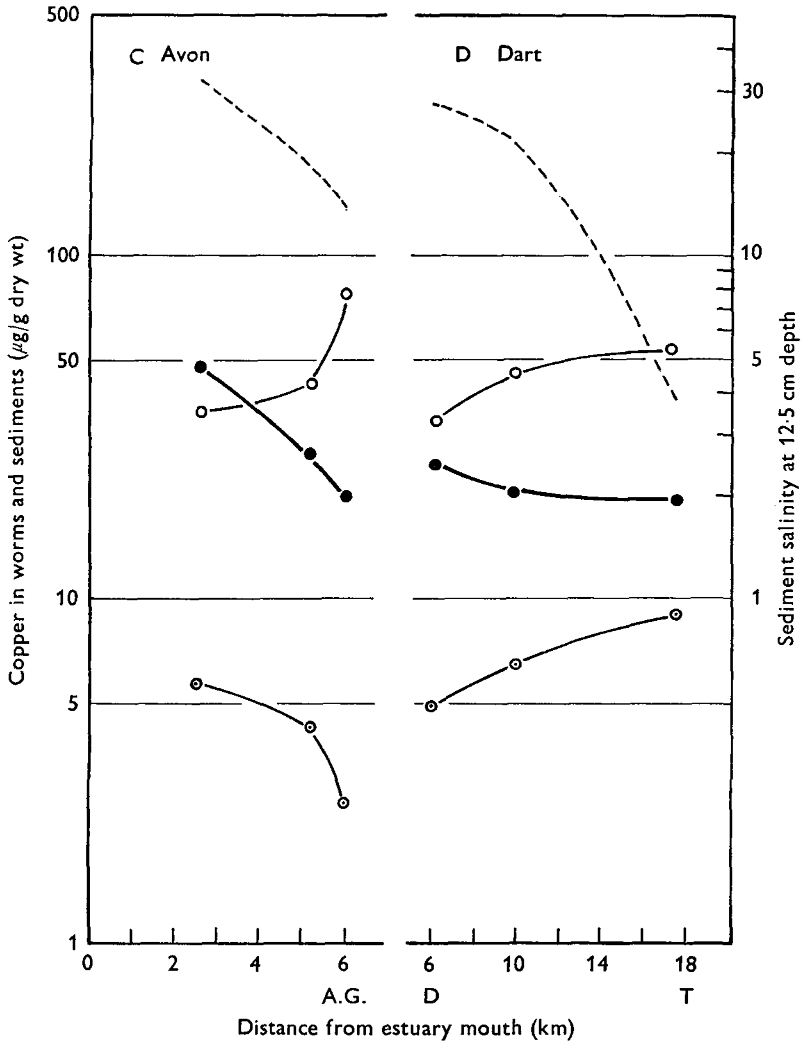


Fig. 1 A–D. Relationship between distance and the concentrations of copper in worms, sediments and sediment extracts from four estuaries. A, Restronguet Creek, Fal River; B, Tamar River; C, Avon (S. Devon); D, Dart River. ●, Copper in samples of whole worms; ○, total copper in sediment adhering to worms; △, total copper in surface sediment; ⊙, copper extracted from sediment adhering to worms; ◬, copper extracted from surface sediment; - - - - - -, salinity of sediment at depth of 12–15 cm. My, Mylor Creek; Pa, Pandora Inn; De, Devoran; Tu, Tullimaar; Sa, Saltash; Ca, Cargreen; Co, Cothele; A.G., Aveton Giffard; D, Dittisham; T, Totnes.

the total concentration of copper in the sediment increases in an upstream direction. In the Avon but not in the Dart this result can be explained by comparing the concentrations in the animals with those of the extractable copper rather than with the totals in the sediments (Figs. 1 C–D)

These results all show that the relationship between copper in *Nereis* and in sediments is not as straightforward as a comparison of the mean values from different estuaries in Tables 1 and 2 might suggest, although, as Fig. 2 shows, there is undoubtedly a general

TABLE 3. CONCENTRATIONS OF COPPER IN INTERSTITIAL WATERS OF SURFACE AND DEEP (5–25 CM) SEDIMENTS COMPARED WITH THOSE IN ANIMALS

Analyses of sediments and sediment extracts made with an acetic acid – hydroxylamine hydrochloride mixture are shown. Also shown are concentrations in water equilibrated with the sediment at 13 °C (see text).

	Distance from mouth of estuary (km)	Date	Type of sediment	Salinity of interstitial water	Interstitial water (μg/ml.)	Equilibrated water (μg/ml.)	Sediment total (μg/g)	Sediment extract (μg/g)	Copper in 10 animals (μg/g)
Restronguet Creek	8·8	28. iv. 71	Surface	> 25	0·003	0·046	3329	1386	770
			Deep	> 25	0·002	0·023	4793	1112	
	9·1	22. iii. 71	Surface	25·6	0·152	—	3557	1619	700
			Deep	29·0	0·002	—	3498	816	
		28. iv. 71	Surface	> 20	0·327	0·032	3985	1826	662
			Deep	> 20	0·003	0·053	7640	904	
	10·0	28. iv. 71	Surface	> 20	5·53	0·113	3393	2002	798
			Deep	> 20	0·017	0·050	3204	773	
	10·1	28. iv. 71	Surface	22·5	0·275	0·082	3712	2035	787
			Deep	25·6	0·008	0·085	3807	1282	
	10·8	22. iii. 71	Surface	5·2	0·036	—	3199	1754	2401
			Deep	10·6	0·005	—	2964	1182	
		28. iv. 71	Surface	< 10	0·022	0·042	3278	1729	2573
			Deep	< 10	< 0·001	0·022	2555	1109	
Tamar	7·5	28. i. 71	Surface	—	0·001	—	210	97	56
			Deep	25·4	< 0·001	—	252	84	
	11·0	28. i. 71	Surface	—	0·002	—	229	99	167
			Deep	12·9	< 0·001	—	203	60	
	18·0	28. i. 71	Surface	—	0·002	—	406	156	152
			Deep	6·5	< 0·001	—	677	266	
	21·0	28. i. 71	Surface	—	0·006	—	407	127	140
			Deep	2·3	0·003	—	453	139	
Dart	9·8	11. ii. 71	Surface	—	< 0·001	—	38	5·4	21
			Deep	21·9	< 0·001	—	46	6·4	

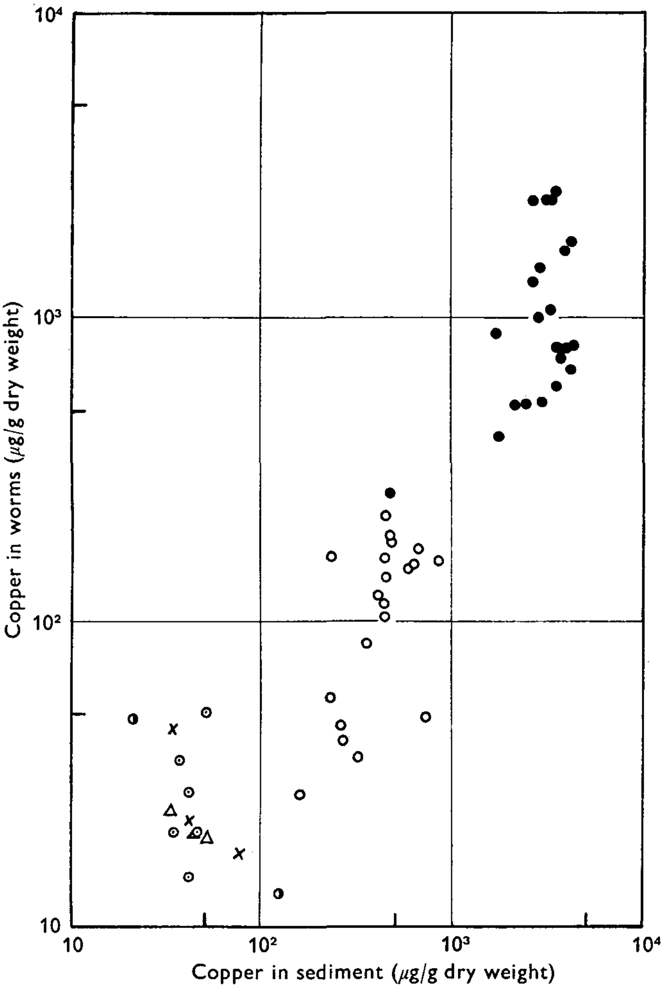


Fig. 2. Relationship between concentrations of copper in samples of whole worms and total concentrations in sediments adhering to them in different estuaries. ●, Restronguet Creek/Fal; ○, Tamar/Tiddy; ⊙, Plym; △, Dart; ×, Avon; ◑, Camel.

relationship when all the results are considered. There are obviously other factors which influence the relationship and an attempt has been made to determine them.

Influence of the interstitial water

Compared with the total concentration of copper in the sediments the concentrations in the interstitial water are extremely low. Concentrations of copper in the interstitial water, the sediments and animals from three of the estuaries are compared in Table 3. Results from Restronguet Creek show that much higher concentrations are found in water from the oxidized surface sediments than in the deeper sediments where conditions are largely reducing. In fact the figures for surface interstitial water in Restronguet Creek reflect the distance of the samples from the polluting effects of the Carnon River; hence the highest concentration at 10 km occurs on the banks of this river whereas the 10·1, 9·1 and 8·8 km stations were at a higher level on the mud banks and up to 200 m from the river at low water. At these four stations the salinity of the interstitial water exceeds 20 and the concentrations of copper in the animals are very similar (Table 3). However, at the 10·8 km station, which lies on the banks of the unpolluted river referred to on p. 849, the animals contain three times as much copper despite the fact that the concentration of copper in the interstitial water is quite low. This result confirms the apparently anomalous situation described in the previous section.

As might be expected, much lower concentrations occur in the surface interstitial water from the Tamar, and in the Dart which is a low-copper estuary the concentrations were too low to measure (Table 3).

Because the concentration of copper in surface interstitial water is higher than that in the deeper sediment it is difficult to know to what concentration the worms are exposed. The burrows permeate the deeper reduced sediment but the lining of the burrow is oxidized, and so the concentration of soluble copper to which the animals are exposed may lie somewhere between the figures for the two types of sediment. In an attempt to estimate what the concentration in the water in the burrows might be, an experiment was set up in which 250 ml. lots of surface and deep sediment from Restronguet Creek were placed in 800 ml. polypropylene beakers and covered with 550 ml. of water having a salinity similar to that of the deep sediment. Ten worms from the same station were added to each of the sediments and the water was aerated. After 24 h the water was discarded and replaced by a second lot which was aerated for a further 15 days and then filtered through a 0·45 μ Millipore filter prior to analysis. As the worm burrows were in contact with the water above the sediment for this long period it was hoped that the respiratory and feeding currents produced by the worms would equate the concentrations in the burrows with those in the water above. Results of these experiments are shown in Table 3 and, because the same sediments were used, are comparable with the figures for interstitial water. The contrast between surface and deep sediments no longer exists in these results because the deeper surface sediment tends to be reduced and the surface of the deep sediment becomes oxidized. The results of this experiment suggest that the concentration of copper in the water from the burrows is intermediate between interstitial water values for the surface and deep sediments. They do not, however, shed any light on the differences between animals from different sediments.

Influence of salinity

In Table 3 and in Fig. 1 A it can be seen that animals with the highest concentrations of copper were collected at the 10·8 and 11 km stations where the salinity of the sediment is low rather than at the 10 km station where pollution is greatest and the highest figure for copper in interstitial water is found. This situation may result from the effect of salinity but there is no certainty of this since it can be seen from Figs. 1 B–D that in other estuaries decreasing salinity does not appear to be related to higher concentrations in the worms. To solve this problem it will be necessary to study the rate of accumulation of copper under different conditions of salinity and concentration using ^{64}Cu. In addition, some unpublished work has shown that the toxicity of copper to *Nereis* increases with decreasing salinity and this also suggests a relationship between rate of accumulation and salinity.

Size of animal

At some stations in the estuaries of the Tamar, Tiddy, Avon and Restronguet Creek it was possible to collect animals of different sizes. Seven comparisons were made between samples consisting of nine small and nine large animals and in six cases higher concentrations were found in the small animals. On average, the concentration of copper in animals having an individual dry weight of 0·058 g was 15 % lower than in animals having a dry weight of 0·013 g. Samples from which the results in Figs. 1 A–D and 2 were obtained did not differ in weight by such a large factor and would only marginally be affected. However, this result does suggest that in the smaller and presumably younger animals copper equilibrium with the environment has already been achieved and there is no increase in concentration with age.

Distribution of copper in Nereis

Analyses of different parts of high-copper worms from Restronguet Creek are compared in Table 4 and show that the highest concentrations lie in the parapodia and body wall. This agrees with findings by Raymont & Shields (1964), who analysed the gut and body wall in *Nereis virens*. As the Restronguet Creek animals contain so much copper by comparison with worms from other estuaries, it was thought that the excess must be stored in some way and that it should be possible to stain it histochemically. This proved to be the case because, although no staining was seen in low-copper animals from the Plym, animals from Restronguet Creek showed the copper clearly as a green-black granular deposit in the epidermis of the body wall and parapodia and in that part of the nephridium which in transverse section lies distal to the centre line of the animal.

TABLE 4. CONCENTRATIONS OF COPPER IN DIFFERENT PARTS OF ANIMALS FROM RESTRONGUET CREEK

Sample	Copper (μg/g) Animal 1	Copper (μg/g) Animal 2
Parapodia	902	779
Body wall (including muscles)	543	484
Gut	167	198
Whole segments	638	494

This discovery suggests that the epidermis and nephridia are sites for the regulatory activity which would be expected if, as the results of the previous section suggest, the worms are in equilibirum with the environment. The fact that a large amount of copper is found in the epidermis rather than in association with the gut is not proof that copper is principally absorbed over the body surface although it would seem to be a curious place to store the metal if most of it were absorbed through the gut.

Absorption and loss of copper in different sediments

If *Nereis* is normally in equilibrium with the environment then it must excrete copper as quickly as it is absorbed. Results from the previous section suggest that the nephridia or the body surface epidermis may be sites for excretion. Unless the copper concentration is a specific characteristic of *Nereis* in different estuaries it might be expected that high-copper animals will lose copper in low-copper sediments and low-copper animals will absorb it from high-copper sediments.

Experiments were carried out in which 400 ml. batches of sediment from the Plym estuary and from Restronguet Creek were placed in 1 l. beakers and covered with 300 ml. of 50 % sea water which was aerated. After 2 days groups of ten high-copper animals from Restronguet Creek were placed in Plym sediment and low-copper Plym animals were placed in Restronguet Creek sediment. Throughout the experiment distilled water was added to correct for evaporation and the temperature was controlled at 13 °C. At intervals the groups of worms were recovered from the sediments and analysed after cleaning in sand in the usual manner.

TABLE 5. CHANGE IN COPPER CONCENTRATION IN GROUPS OF TEN LOW-COPPER PLYM ANIMALS IN HIGH-COPPER RESTRONGUET CREEK SEDIMENT

Time (days)	Copper in animals (μg/g)
0	26
12	170
18	117
23	208
35	209

Three experiments using high-copper animals and low-copper sediment were carried out. In the first experiment the initial concentration in the worms was 1020 μg/g and 5 weeks later a similar concentration was found. After 7 weeks in the second experiment a concentration of 727 μg/g was found compared with 957 μg/g originally and in the third experiment the concentration was 2683 μg/g after nearly 11 weeks compared with 2401 μg/g originally. These results show that in high-copper animals, where much of the metal seems to be stored in the epidermis of the body wall and in the nephridia, the bulk of the copper can be lost only very slowly.

Results from one of two experiments in which low-copper animals were exposed to high-copper sediments (at 13 °C) are shown in Table 5. In 28 days the concentration of copper in the worms increases by a factor of 8, showing that the availability of the metal in the high-copper sediment is much higher. A few animals appeared on the surface during this experiment and it was suspected that this might be due to the toxicity of the sediment. This implied that the low-copper Plym animals are less tolerant of the toxic affects of metals than those from Restronguet Creek and so experiments were carried out to confirm this.

Absorption of copper from solution and its toxicity to Nereis

In a preliminary experiment, animals from the Plym estuary and Restronguet Creek were exposed to 0·1, 0·25 and 0·7 μg/ml. solutions of copper (as sulphate) in 50 % sea water. From each locality two groups of nine animals were exposed to each concentration in bowls containing 250 ml. of solution. Solutions were not aerated but were changed each day and maintained at a temperature of 13 °C. Each bowl also contained pieces of glass-tubing to keep the worms separated. These concentrations of copper were used

TABLE 6. EXPERIMENTS SHOWING MEAN TIMES OF SURVIVAL IN DIFFERENT COPPER CONCENTRATIONS AND AMOUNTS OF COPPER ABSORBED BY ANIMALS

Estuary	Copper in 50 % sea water (μg/ml.)	No. of worms in experiment	Mean dry weight per worm (g)	Mean time of survival (h)	Copper in worms initially (μg/g)	Net uptake of copper by worms (μg/g)
Experiment using copper sulphate and lasting 888 h						
Plym	0·1	18	0·026	828*	35	261
Restronguet Cr.	0·1	18	0·040	888*	1295	549
Plym	0·25	18	0·035	264	35	187
Restronguet Cr.	0·25	18	0·042	888*	1295	759
Plym	0·70	18	0·031	41	35	175
Restronguet Cr.	0·70	18	0·042	247*	1295	1061
Experiment using copper sodium citrate and lasting 408 h						
Plym	0·25	10	0·030	140	26	188
Tamar	0·25	10	0·013	150	216	469
Restronguet Cr.	0·25	10	0·035	408*	2401	175
Plym	0·5	10	0·032	79	26	287
Tamar	0·5	10	0·016	50	216	289
Restronguet Cr.	0·5	10	0·032	379*	2401	353
Plym	1·0	20	0·028	29	26	250
Tamar	1·0	20	0·016	24	216	248
Restronguet Cr.	1·0	10	0·034	121	2401	1293
Plym	2·5	10	0·041	17·5	26	213
Tamar	2·5	20	0·021	16	216	279
Restronguet Cr.	2·5	10	0·046	50	2401	168

* Sample includes animals still living at the end of the experiment.

because Raymont & Shields (1964) found that in *Nereis virens* the threshold concentration for copper toxicity in sea water is 0·1 μg/ml.

When after 37 days (888 h) the experiment was ended, the animals from Restronguet Creek were still alive in the 0·1 and 0·25 μg/ml. solutions, whereas the Plym animals had died at 0·25 μg/ml. and a few deaths had occurred at 0·1 μg/ml. Animals which died were removed from the solutions and analysed together with any which survived the period of 37 days. The results are summarized in Table 6 and a comparison of the mean times for which animals survived shows clearly that the high-copper animals from Restronguet Creek are far more resistant than the low-copper animals from the Plym. The analyses show approximately how much copper was accumulated by each group and these figures suggest that the Restronguet Creek animals are more resistant not because they are less permeable to copper but because they require more copper to kill them.

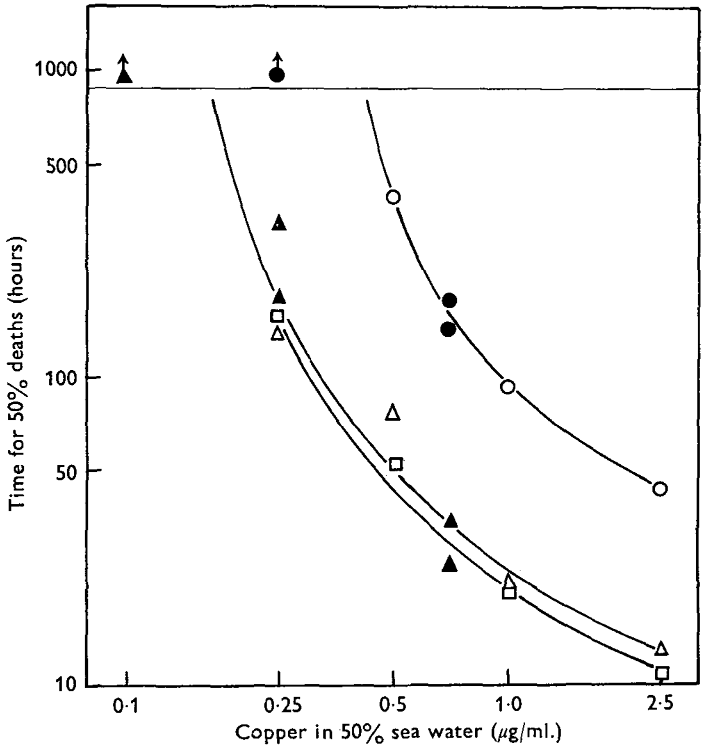


Fig. 3. Relationship between concentration of copper in 50 % sea water and the time taken to kill 50 % of animals from different estuaries. Restronguet Creek animals: ●, copper as sulphate; ○, copper as citrate. Plym animals: ▲, copper as sulphate; △, copper as citrate. Tamar animals: □, copper as citrate. Experiment not carried beyond 888 h.

A second experiment was set up in which groups of ten animals from the Plym (low copper), Tamar (medium copper) and Restronguet Creek (high copper) were exposed to concentrations of 0·25, 0·5, 1·0 and 2·5 μg/ml. of copper in 50 % sea water. This time more than 1 l. of solution was used for each group and was changed daily. Copper was used in the form of copper sodium citrate to ensure that the higher concentrations remained in solution, because Krauskopf (1956) found that sea water is saturated with copper at a concentration of 0·4–0·8 μg/ml. Raymont & Shields (1964) used copper in this form to study its toxicity to *Nereis virens* and found that the threshold concentration of 0·1 μg/ml. for this species was essentially the same for several different copper compounds. The toxic effects of copper in this second experiment are compared with the results of the first experiment in Fig. 3, where the concentration of the solution is plotted against the time taken for half the animals in each group to die. The tolerance of the Plym and Tamar animals is roughly the same although, as Table 6 shows, the Tamar animals were smaller. It was noticed that in each group of animals the smallest tended to be least resistant and it might be expected that Tamar animals of the same size as those from the Plym would be slightly more resistant.

Table 6 also shows that the amount of copper which must be accumulated in order to kill the Plym animals is roughly the same irrespective of the concentration of the toxic solution and has a mean value of about 220 μg/g. For Tamar animals the mean value is about 320 μg/g and in Restronguet Creek is 720 μg/g. However, this last figure is a mean of four values ranging from 168 to 1293 and this variability presumably results because one is trying to measure a difference between groups of animals already possessing high concentrations. We are fairly confident that the more resistant animals require more copper to kill them but cannot yet be sure whether the permeabilities of worms from different estuaries are significantly different.

For the low-copper Plym animals the toxicity threshold is a concentration of rather more than 0·1 μg/ml. in 50% sea water because very few animals died even after 37 days exposure to this concentration. This value is similar to that found by Raymont & Shields (1964) in *Nereis virens* from low-copper sediments. In a previous section it was noted that some Plym animals were affected by exposure to high-copper sediments from Restronguet Creek and this suggests that these sediments have a toxicity similar to that of 0·1 μg/ml. of copper in 50% sea water. We have already seen in Table 3 that interstitial water from the surface of these sediments sometimes contains more than 0·1 μg/ml. of copper and that water equilibrated with the sediments contains around 0·05 μg/ml. of copper. This suggests that the toxic effect of the sediment probably lies in the concentration of soluble copper in the worm burrows and also that much of the copper is absorbed across the body surface. Plym animals which were found to be affected by high-copper sediments contained more than 200 μg/g of copper and a similar amount was found in the animals from the toxicity experiment in Table 6.

This evidence, coupled with the fact that in high-copper animals much of the metal lies in the epidermis, suggests that *Nereis* may absorb an appreciable amount of copper across the body surface. However, this idea does not, at present, seem to be compatible with all the results and particularly with the finding that the highest concentrations in animals from Restronguet Creek are not found in the sediments with the highest concentrations in the interstitial water (see Table 3). Unless this anomaly depends on differences in the salinities to which the animals are exposed (see page 854), it may depend on differences in the degree of absorption from material in the gut. Experiments using radio-isotopes would seem to give the best chance of measuring the relative importance of the body surface and gut in the absorption of copper, although the short half-life of ^{64}Cu limits the length of experiments to a few days.

The possibility of changing the copper tolerance of Nereis

An important discovery from this work is that tolerance to the toxic effects of copper is very different in two populations of the same species. The difference might be genetically controlled but, on the other hand, may be developed by any animal if it is given time to do so. In land plants there is a genetic basis for the copper tolerance exhibited by populations living on old mine dumps (see, for example, Allen & Sheppard, 1971), but individual animals such as fish can increase their resistance to high metal concentrations by preliminary exposure to concentrations below the threshold value (see for example, Lloyd, 1960).

As a first step, an experiment has been carried out to see whether the tolerance of *Nereis* to copper can be changed by exposure to other types of sediment. About 30 high-copper animals from the 10·8 km station in Restronguet Creek were placed in 3–4 l. of low-copper Plym sediment which was covered with 50 % sea water and aerated. Similarly, animals from the Plym were transferred to high-copper sediment. Of these low-copper animals ten were affected by the sediment and surfaced after 22–27 days but only three more were affected and surfaced between 27–37 days. It was thought that the remaining 18 animals, which were the most resistant, might have developed some tolerance because none were affected between 37–45 days. At this point the worms were separated from the sediment and after 24 h exposed to a 1 μg/ml. solution of copper (as citrate) in 50 % sea water. Half of the animals died in 17·3 h compared with 18·3 for fresh Plym animals, which suggests that no improvement in tolerance had been achieved. However, at the end of the experiment the dead animals contained 400 μg/g of copper and had gained 374 μg/g. This is more than was required to kill Plym animals in the toxicity experiments and the results in Tables 5 and 6 suggest that perhaps half of the copper was absorbed from the high-copper sediment and half from the toxic solution. Thus, although the resistance of the worms to a 1 μg/ml. solution was not increased, more copper was necessary to kill the worms and might represent a slight increase in tolerance.

In the converse experiment high-copper animals were exposed to low-copper sediment for 76 days and were then tested in the 1 μg/ml. solution in 50 % sea water. Tests on this batch of animals prior to the experiment showed that half the worms died in 170 h and after 76 days the time was 163 h. These experiments show that copper tolerance is not a feature which is readily gained or lost and we may be dealing with a factor which is genetically controlled.

DISCUSSION

It has been shown that *Nereis diversicolor* can live successfully in estuarine sediments when the total concentration of copper ranges from about 20 μg/g in low-copper areas to more than 4000 μg/g when chronic mining pollution is encountered. The concentration of copper in the animals increases with that in the sediment and, although variation is considerable, the mean animal/sediment concentration ratio is 0·44. An exact relationship between the concentrations in animals and sediments or sediment extracts has not been found and there is evidence that factors such as salinity, size or age of animal and concentrations of copper and probably other substances in the interstitial water need to be considered. A particular problem was the finding that the highest concentrations of copper in animals from Restronguet Creek, which is heavily polluted, do not occur in those collected closest to the source of pollution but are found at a station where, although the concentration in the sediment is similar, there is less copper in the interstitial water. The explanation for this apparent anomaly may lie in the fact that the worms containing most copper were living at a low salinity, but this needs further study.

An alternative explanation for this and other anomalies may depend on the fact that *Nereis* has three methods of feeding (Harley, 1953; Goerke, 1966). The influence of the method of feeding on the concentrations of trace metals in different polychaetes has been considered by Phelps, Santiago, Luciano & Irizarry (1969) and by Cross *et al.* (1970), but their results are not in complete agreement. *Nereis* may ingest the surface layers of the sediment, it may filter-feed, or it may use its jaws to ingest larger particles. Goerke (1971) considers that it is omniverous and will take whatever food dominates in the habitat, be it animals, algae, debris or the substrate and detritus with associated micro-organisms. Obviously, if the diet is an important source of copper, variations in concentrations in the worms may occur within a limited area if the availability of food and the methods of feeding are different.

Although there is uncertainty as to how much copper *Nereis* can absorb from its food there is no doubt that the metal is readily absorbed from solution, presumably across the body surface. Appreciable amounts of copper were absorbed from solution during toxicity experiments and much of that gained by low-copper animals in high-copper sediments seemed to be absorbed in this way.

Animals from different estuaries may contain very different amounts of copper, but processes for its regulation seem to exist because at a particular station the concentration does not increase with the size or age of the worm. If copper was being accumulated continuously the highest concentrations would be expected in the oldest or largest animals unless perhaps the worm grows at such a rate that the accumulated copper is effectively diluted. In high-copper worms much of the metal is deposited in the epidermis of the body wall and in parts of the nephridia, although in what chemical form is not known. However, it seems likely that if copper is regulated these are the important regulatory organs.

The availability of copper in high-copper sediments far exceeds that in low-copper sediments because not only do worms from these sediments contain more copper but animals transferred to them from sediments containing little copper will accumulate the metal. Under normal circumstances high-copper animals almost certainly absorb and excrete much more copper than those from low-copper sediment and this ability is reflected in their greater resistance to the toxic effects of copper solutions. The time taken to kill half of the worms in a 1 μg/ml. solution of copper in 50% sea water is less than 1 day for low-copper animals but at least 4 days for high-copper animals. These more tolerant animals are able to accumulate more copper before they are killed and are clearly equipped with a better regulatory or detoxification system.

Attempts to change the tolerance of animals by exposing them to other types of sediment have shown that this is not readily achieved. By anology with the situation where land plants have become tolerant to heavy metals in mine dumps, we may be dealing with a genetically controlled difference between resistant and non-resistant populations. There has been plenty of time for a copper-tolerant population of *Nereis* to develop in Restronguet Creek since it has received mining wastes for at least 200 years. The Great County Adit which was built to drain water from the nearby copper mines into the Carnon River and then into Restronguet Creek was begun in 1748 and extended to an increasing number of mines during the remainder of the century (Barton, 1961).

Most information on heavy-metal tolerance to mining wastes comes from studies on the evolution of populations of land plants which have colonized old mine dumps (see, for example, McNeilly & Bradshaw, 1968; Turner, 1969; Allen & Sheppard, 1971). Tolerance to different metals is usually quite specific and plants tolerant to copper are not necessarily tolerant to zinc, although the two separate tolerances can exist in the same plant if the mine dumps contain high concentrations of both metals. In this context it should be remembered that in Restronguet Creek the sediments contain almost as much zinc as copper and so the worms may have developed a tolerance to zinc as well. A more detailed study of the behaviour of zinc and other metals in *Nereis* will be the subject of a second paper in this series.

Other species living in the more heavily polluted parts of Restronguet Creek may also be more tolerant of copper than normal populations and this is being examined in some of the larger organisms, including the brown seaweed *Fucus vesiculosus*, the bivalve mollusc *Scrobicularia plana*, the polychaete *Nephthys hombergi* and the amphipod *Corophium volutator*.

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