BASELINE SURVEYS AND METAL BINDING PROTEINS AS METAL POLLUTION INDICATORS

by

Helmke F-K O Hennig

Department of Analytical Science

University of Cape Town

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CONTENTS

ABSTRACT

The field of metal determination as a part of pollution studies, has been critically examined and metal pollution may be defined in one simple statement: The presence of metal binding proteins confirms toxic metal pollution.

It has been shown that current methods of metal determination in biological systems are of little use. This has been illustrated by both a review of metal concentration in Southern African coastal water, sediments and biotopes, and by a comparative baseline study of organisms from Gough Island and Marion Island. These showed that extrapolation of results from one geographical area to another are invalid and that this interpretation is made difficult by factors such as age, sex, size life stage of the organisms. Furthermore, it was shown that many reports on metal pollution do not even mention fundamental information such as the size or the sex of the animals.

Metal pollution could be linked to metal binding protein through an independent pollution criteria, for example, the out of season moulting of crayfish. The new definition of metal pollution has then been tested by application to five different organisms (crayfish, Jasus lalandii; hermit crab, Diogenes brevirostris; shrimp, Palaemon pacificus; black mussel, Choromytilus meridionalis and limpet, Patella granularis) kept under identical conditions and it was shown that a much more meaningful interpretation of the results could be made. The new definition was also tested with two naturally occurring metal accumulating organisms (whelk, Bullia digitalis and "kikuyu" grass) and it was shown that dramatic increases in metal may not necessarily be toxic.

It was concluded that less effort and time should be spent on metal analysis in determination of metal pollution and attention should rather be directed to the presence or absence of metal-binding proteins.

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INTRODUCTION

The studies presented here examine the role of metal determination and its relevance to pollution analysis. By using metal binding proteins as a means of determining metal pollution it is shown for the first time, that it is possible to measure a physiological parameter of stress and bioavailability of metals to biological systems.

Metal pollution is critically reviewed in this thesis and the new concept of confirming toxic metal pollution by the presence of metal binding proteins is developed. In developing this concept, individual lines of evidence (for example studies and papers) are not immediately obvious but should be read in context and in a specific sequence to develop the new idea presented here.

In the first paper, background levels and levels of metal accumulation in water, sediments and fauna are presented using a typical data set from South Africa. This was done to establish the extent and reliability of the available data and to identify any possible anomalies. A comparative study such as this, contrasts regional metal values between various areas around South Africa and also allows international comparison.

On the other hand such a study illuminates the drawbacks of pollution and "base line" work. Large variations in concentrations of metals in coastal waters have been encountered during different seasons, time of sampling, the extent of freshwater run-off, the depth of sampling, the intermittent flow of industrial effluent and hydrobiological factors such as tides and currents. Other difficulties in interpretation of metal abundance arise from analytical methodology. For instance, the low metal concentrations found in South African waters often require the preconcentration of large volumes of water by solvent extraction and errors frequently arise during these procedures. In sediments, the concentration of metals depends on the organic content as well as the particle nature, the form and effective particle size of the sediment. Errors may also arise during the

routine acid digestion method of extracting metals from sediment.

The variation of metals in fauna can be due to a large number of factors, these are dealt with separately (Paper 4).

Paper 1 is an extension of the philosophy behind the international Mussel Watch Programme and also serves as reference document for future studies. It demonstrates that in South Africa samples are usually taken from polluted areas ("hot spots") and huge gaps exist in the monitoring of coastal areas. Furthermore, the accumulation of metals by different animals does not necessarily depend on current environmental conditions. For instance, different species of limpets accumulated various metals at different rates even at the same geographical position (for example "spread of graphs" presented). Furthermore extrapolation of results from one region to another is not valid, even when working with the same species.

The study also showed that no single indicator species should be used for all metals. Accumulation of specific metals may be highly correlated in one species, while other metals are not. For example in this review bivalves show no clear accumulation trend when one is attempting to establish baseline levels for zinc. Whelk species on the other hand show less inter-organism variation.

The advantage of this type of metal determination is, however, that it can be accurate and straight forward. At hot spots, regular monitoring will show improvement or deterioration of conditions.

The data presented in this first section are also useful in establishing the order of magnitude of metal concentration in water, sediment and organisms around the South African coast.

The second paper illustrates another advantage of total metal studies. In an unpolluted environment, such as the regions around the Antarctic continent, this study shows that the relative ratios of metals in selected or representative animals may reflect their geographical origin (for example water mass). Once metal "markers" were established, the feeding grounds of predators could be determined.

The emphasis in this paper lies on "unpolluted" and "ratio of metals", because in absolute terms the metal concentration of these unpolluted Antarctic species is not necessarily low.

The third paper describes two other unpolluted environments (Marion and Gough Islands) and shows the importance of correlations which may exist between the metal content of the organisms and the existing natural metal concentration of the surrounding water and sediment.

Comparisons are drawn between animals from unpolluted Marion and Gough Islands and those from Langebaan Lagoon which can be considered as South African metal "hot spot". This highlights several important difficult interpetive problems, viz.,

a) What is pollution? High metal levels in organisms or man-induced metal levels?
b) How should pollution of an area or of an animal be defined? In terms of absolute metal values or in relation to its surroundings?
c) Are baseline studies of metal accumulation without additional environmental data, of any value?

Furthermore baseline studies appear only to be of value for a "before" and "after" study, since this paper demonstrates that extrapolation to other areas is not valid because such contradictory results are obtained.

Hence, it is argued that it is no longer sufficient to document pollution in terms of the metal concentration of contaminants.

The fourth paper criticizes the approach taken in many papers dealing with metal concentration, a problem also mentioned in Paper 1 (review of metal concentrations in Southern African coastal waters, sediments and organisms). It includes evidence as to how sex and size influences the total metal concentration of animals. It is also evident, that measures of total metal concentrations are too strongly influenced by factors such as sex and size, to be of more than limited value.

In Paper 5 and the supporting paper this idea is carried further. It is shown that crayfish (Jasus lalandii) comply to nearly all currently acceptable standards for marine monitoring organisms, that is, they are sedentary, long lived, of reasonable size, easy to sample and abundant in the sampling region, but if kept in a metal-enriched environment no accumulation of metals could be shown. This result is attributed to the short duration of the study time relative to the longevity of the study animals. The question thus arises: although the crayfish clearly seem contaminated, how can this be shown, if they do not accumulate metals?

So far little evidence can be found which links measurable physiological effects to sublethal metal concentrations. In this paper it is shown that with crayfish abnormal moulting takes place in zinc-polluted environments. However, no measurable total metal accumulation could be demonstrated. This effect was confirmed by a study on short-lived arthropods (Palaemon pacificus) held under similar conditions.

In the last paper the metal binding proteins are isolated from different animals. It is shown that this protein is very similar both in the various animals tested and to those from other studies; in fact this protein appears to be ubiquitous.

This is the first time, to the best of our belief, that metallothioneins could be linked to pollution and the bioavailability of metals. Furthermore, it is shown that studies of metal binding proteins are a much more sensitive indicatior of pollution than is the analysis of total metals. The final paper also presents evidence that the presence of metal binding protein is in fact a physiological sign of a metal stressed animal and this leads to a new concept in defining pollution.

Irrespective of factors such as size, sex, age and geographical origin, the presence of metal binding proteins in study animals indicates a metal level which affects the organism adversely. In this respect the quantity of metallothionein produced is irrelevant, the mere presence of the protein is evidence that energy is being used for detoxification, which otherwise would be channelled into growth or reproduction.

This new concept of defining pollution has been applied under field conditions and shows for the first time which metals are bioavailable to a particular organism.

REVIEW OF METAL CONCENTRATIONS IN SOUTHERN AFRICAN COASTAL WATERS, SEDIMENTS AND ORGANISMS

by

H F-K O Hennig

This report emanated from a project undertaken in the Marine Pollution Programme of the South African National Committee for Oceanographic Research (SANCOR).

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H F-K O Hennig
National Research Institute for Oceanology
P O Box 320
STELLENBOSCH
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ABSTRACT

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It is an extension of the philosophy behind the International Mussel Watch Programme and also serves as reference document for future studies. It demonstrates that in South Africa samples are usually taken from polluted areas ("hot spots") and huge gaps exist in the monitoring of coastal areas. Furthermore, the accumulation of metals by different animals does not necessarily depend on current environmental conditions. For instance, different species of limpets accumulated various metals at different rates even at the same geographical position (for example, "spread of graphs" presented). Furthermore, extrapolation of results from one region to another is not valid, even when working with the same species.

The study also showed that no single indicator species should be used for all metals. Accumulation of specific metals may be highly correlated in one species, while other metals are not. For example, in this review bivalves show no clear accumulation trend when one is attempting to establish baseline levels for zinc. Whelk species on the other hand show less inter-organism variation.

1. INTRODUCTION

How serious is chemical pollution of coastal areas? Where are the most polluted areas? Is chemical pollution increasing or decreasing? How rapidly? What are background concentrations? Where is such information to be found? These questions have been asked more and more frequently in the last five to seven years. This has led to the formation of several marine pollution monitoring groups at the major centres around the coast of South Africa, which have reported on the major impact areas. The National Programme for Environmental Sciences (Marine Pollution Section) decided in 1974 on the important monitoring areas. The different groups report to the Steering Committee of the Marine Pollution Programme, which in turn gives direction on future monitoring. Most of these recommendations were included in a Pollutant Workshop held at Plettenberg Bay in 1979 (Cloete, 1979). For the purpose of the discussion at the workshop the GESAMP definition of marine pollution was used (GESAMP, 1976) which established toxic elements as the most important pollutants.

At the Plettenberg Bay Workshop it was decided that oil was the most serious pollution problem on the southern African coast. Furthermore, based on current knowledge, the South African marine environment was thought to be still relatively unpolluted, apart from a few specific areas, and provided an excellent opportunity for baseline investigations of pollutant transfer (Cloete, 1979).

The US Mussel Watch Program which began in 1976 (Goldberg *et al.*, 1983 and Farrington, 1983) provided strategies for pollutant monitoring. Mussels and oysters from 85 different locations along the US coast were analysed. A similar project was not within the resources of South Africa. Nevertheless, it was hoped that if all available information was gathered a baseline for metals could be established for the southern coastal

environment. Unfortunately, such data were in unpublished or confidential reports and therefore were not easily accessible. Darracott and Brown (1980) included in their bibliography the titles of publications on pollution which appeared before the end of 1977, but could not include internal reports with restricted distribution or reports to committees.

In this study, data from publications, university theses, reports and minutes of internal meetings have been summarised, recalculated and redrawn. It was surprising and gratifying to realize how much information was available. When put together these, often unrelated, reports establish the distribution and relative abundance of toxic elements in the South African coastal environment. This study has also showed that even with limited resources, a pooling of all available information can establish a fairly comprehensive metal pollution profile and baseline for coastal environments. This is a lesson which is worthwhile remembering when dealing with other pollutants, for example, halogenated hydrocarbons or radioactive materials, in other resource limited areas and in third-world countries.

1.1 Methodology

The sediments, water and biological material were collected and analysed by many different investigators since 1972. This often made evaluation and direct comparison impossible, and so means ($\bar{x}$) of nearly all data sets were calculated. This introduced several errors, as was pointed out by Hennig and Orren (1983) particularly as the older references give no indication of the size or sex of the animals, nor of detailed methods. Thus, taking the mean values gave some uniformity within these studies and was thought to be the best approach.

In 1981 a manual of methods for the Marine Pollution Monitoring Programme appeared (Watling, 1981). This should have standardised the methods for the preparation and analysis of water, sediment and biological samples. Unfortunately no recommendations were given on the preparation of data. This meant that some

investigators reported their findings in terms of "wet weight", while others gave their data in terms of "dry weight". In most cases it was not possible to unify and recalculate the data to one set of conditions. Hence special attention must be given to legends of tables and figures in this report as both sets of units have been used. Evaluation of the methods of different investigations will be discussed as they appear within the framework of this study. Often an unrealistically high accuracy has been reported. This may well have been due to the use of calculators and not to the high sensitivity of the instruments. Watling (1981, in Table 1) has put the sensitivity at various wavelengths of atomic absorption spectrophotometers into a proper perspective.

Another problem which arose in the compilation of this paper was the repetition of data presented in different publications. For instance, some findings reported in internal memoranda were later published in established journals. It was decided that identical data could be represented only once and that the more accessible reference would be given. When there were differences between data sets in reports and in other publications, it was assumed the authors had additional information, and both sources of information are given here.

It is hoped that in future reports and publications a more uniform approach will be used. It is proposed that all sediment and biological data should be expressed in terms of dry mass to eliminate differences arising from different water contents in tissues, shells and carapace.

In this report, the data are divided into five different sections, arranged geographically, as follows:

I Kosi Bay to Port Shepstone - a sector including most of the Zululand and Natal coasts;

II Port Shepstone to East London - including the Transkei coast;

III East London to Cape Agulhas - a sector in which the coastal shelf broadens into the Agulhas Bank;

IV Cape Agulhas to Cape Columbine;

V Cape Columbine to the Orange River.

This division was based on South African coastal water movements as described by Harris (1978). Since the metal concentrations in sediment and biological samples are closely linked to concentrations in the associated water masses, these divisions are convenient, though perhaps an over-simplification. Each section has been divided into regions as considered suitable, depending mainly on the amount of data available. The sections are shown in Figure 1.

The coastal data originated from material collected along the beach, in the surf zone, and estuaries in which the salinity was higher than 2.5×10^{-3}. Water and sediment data from offshore stations are available from the South African Data Centre for Oceanography (SADCO), but have not been included in this report. Fish and plankton may not be strictly surf zone animals, but data on them have been included for the sake of completeness and in an attempt to establish some baseline criteria.

At several locations data from water (W), sediment (S) and biological material (B) were not all available. A more detailed location of these materials is given in Figure 2. In Table 1 the sampling points are given in order along the South African coast, starting at the eastern border and continuing clockwise around to the west coast.

Water and sediment have been treated as single components, compared with the many "components" (species) of the biological material. It was also decided that the metal concentrations of the water should be described first, since it is the more important, followed by the chemical data for the sediment and finally the distribution of metals in the various biological species.

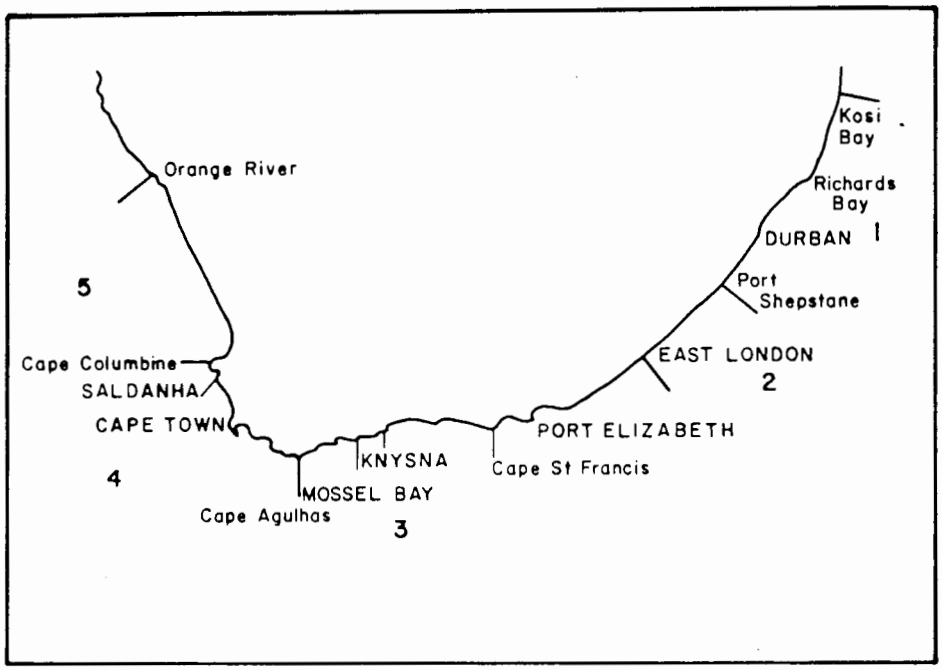


Figure 1 Location diagram of the five sectors used in the arrangement of this study

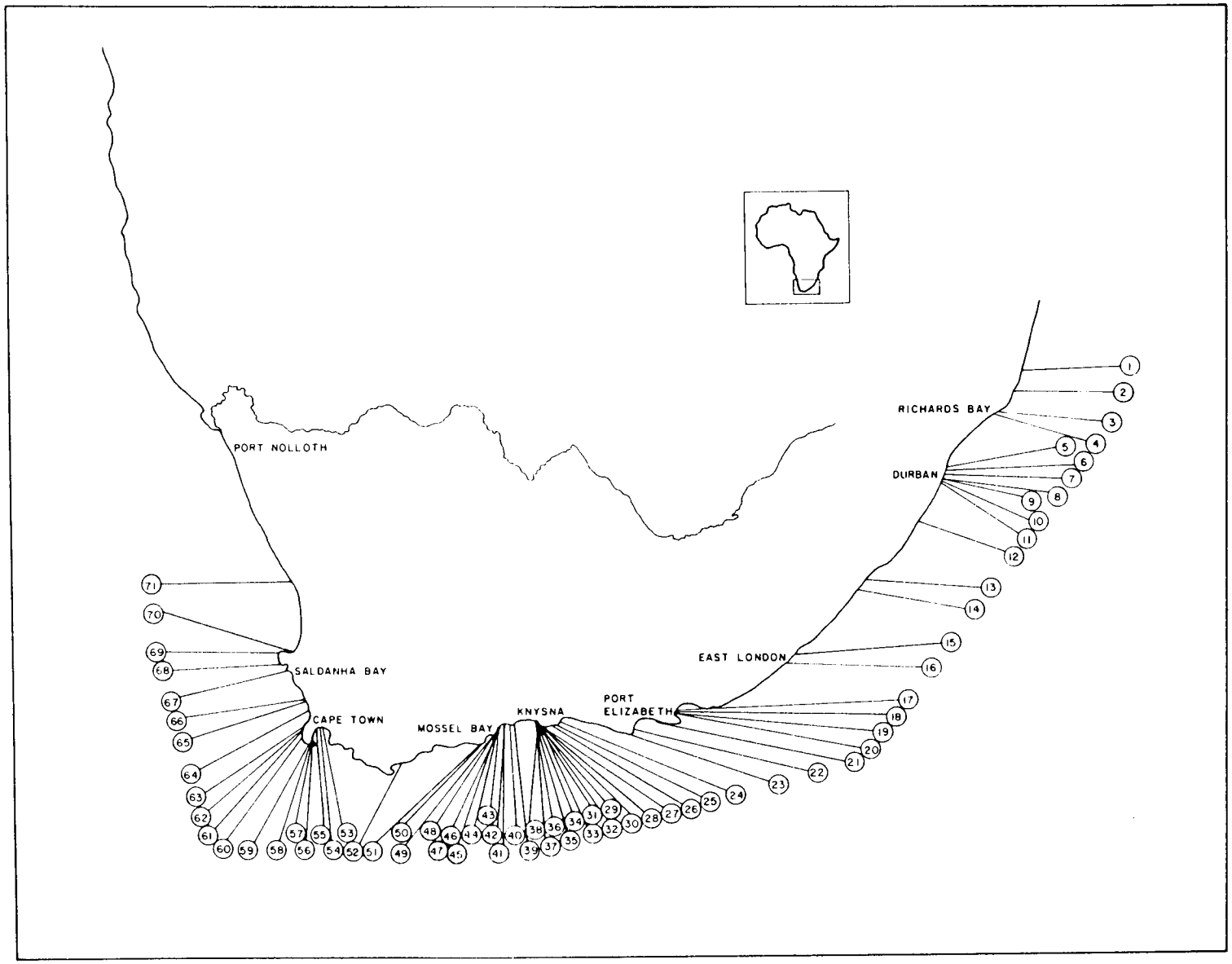


Figure 2 Location of sampling points for biological material (B), water (W) and sediment (S)

TABLE I: SAMPLE LOCATIONS AS THEY APPEAR ALONG THE SOUTH AFRICAN COAST AND IN FIGURE 1 (B = biological samples, W = water samples, S = sediment samples)

SECTION I	1.	Kosi Bay	BW
	2.	St Lucia	BWS
	3.	Richards Bay	BWS
	4.	Port Durnford Point	B
	5.	Umhlanga Rocks	B
	6.	Umgeni	B
	7.	Durban Bay	BWS
	8.	Umbogintwini	BW
	9.	Fynnlands	W
	10.	Umhlatuzana	B
	11.	Umgababa	BWS
	12.	Umzimkulu	BWS
	13.	Umzimvubu	WS
	14.	Mngazana	WS
SECTION II	15.	Bashee	BWS
	16.	Buffalo	WS
SECTION III	17.	Algoa Bay	BWS
	18.	St Croix	B
	19.	Swartkops	BWS
	20.	Port Elizabeth	BS
	21.	Maitland	B
	22.	Jeffreys Bay	WS
	23.	Cape St Francis	BWS
	24.	Keurboomstrand	BWS
	25.	Cathedral Rock	B
	26.	Noetzie	B
	27.	Knysna East Head	BW
	28.	Beacon Point	B
	29.	Knysna West Head	B
	30.	Featherbed	B
	31.	Belvedere	B
	32.	Leisure Island	B
	33.	Knysna	BS

	34.	Thesen's Point	B
	35.	Castle Rock	B
	36.	Buffalo Bay	B
	37.	Walker Point East	B
	38.	Walker Point West	B
	39.	Herold's Bay	B
	40.	Glentana	B
	41.	Tergniet	B
	42.	Little Brak River	B
	43.	Hartenbos	B
	44.	Diaz Head	B
	45.	Die Bakke	B
	46.	Mossel Bay	BWS
	47.	Dana Township	B
	48.	Cape St Blaize	B
	49.	Pinnacle Point	B
	50.	Fish Bay	B
	51.	Vlees Bay	B
	52.	Arniston	WS
SECTION IV -	53.	False Bay	B
	54.	Eerste River	S
	55.	AECI	S
	56.	Swartklip	S
	57.	Strandfontein	B
	58.	Muizenberg	B
	59.	Cape	B
	60.	Hout Bay	S
	61.	Camps Bay	S
	62.	Green Point	S
	63.	Salt River	S
	64.	Blouberg Strand	B
	65.	Melkbos	WB
	66.	Koeberg	BS
	67.	Langebaan	WB
	68.	Saldanha	WBS
	69.	Noordwesbaai	B
	70.	Berg Rivier	WS
	71.	Olifants River	WS

2. SURVEY OF TRACE METAL ABUNDANCE IN COASTAL WATERS

Treating coastal waters as a single component is an oversimplification. Trace metals exist in water partly in solution and partly in suspension, adsorbed to organic or inorganic particulate matter. In addition a certain amount of metal exists in colloids or chelates which may be difficult to categorize as either soluble or particulate fractions. This categorization is, in any case somewhat arbitrary. In coastal areas and near rivers or estuaries, the proportions and absolute amounts of metal in each fraction may vary according to the metal, the particulate content and its nature and the time and site of sampling (Phillips, 1977).

Direct comparisons of metal concentrations are complicated by the large natural variations which exist. Factors such as differences in season, time of day, the extent of freshwater runoff, depth of sampling, the intermittent flow of industrial effluent and hydrological factors such as tides and currents, all influence trace metal concentrations in both particulate and dissolved forms. Other difficulties in the determinaton of metal concentrations in coastal waters arise from the method of analysis. The low metal concentrations found in South African waters often require first the pre-concentration of large volumes of water by solvent extraction and errors may arise during these procedures.

All these limitations should be borne in mind when comparing and interpreting the metal concentrations in South African waters as summarised in Table 2.

Samples have been taken at 25 sites around South Africa over a period of nine years. The results are reported by ten different workers. Most reported results are on Cd, Co, Cu, Fe, Hg, Mn, Ni, Zn; only very limited information is available for Cr, Sb and Nb.

TABLE II: SUMMARY OF METAL CONCENTRATIONS OF COASTAL WATER AROUND SOUTH AFRICA ($\mu g\ \ell^{-1}$)

Location	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sb	Zn	Nb	Reference
Kosi River	1976	0.14	1.1		na		0.019		1.1	0.33		3.31		Oliff & Turner, 1976
Kosi Bay	1976	na 0.4	na 0.8		na 1.1		0.05 0.08		0.7 1.9	0.4 1.0		na 5.7		Cloete (ed), 1979
St Lucia E.	1976	0.187	1.534		3.329	68.81	3.949			39.15		2.312		Oliff & Turner, 1976
St Lucia E.	1978	0.03 0.25	0.08 2.4		1.2 17	460 2 000	0.03 0.13		1.2 6.1			1.4 11.7		Cloete (ed), 1979
Richards Bay	1976	0.09	2.4		0.64		0.60		2.2	1.8		1.8		Oliff & Turner, 1976
Richards Bay	1974	<0.04			4.79		0.011			10.5		12.5		Connell et al., 1975
Richards Bay	1974	<0.04			1.55		0.006			<0.4		<0.4		Connell et al., 1975
Richards Bay	1974	0.015			1.70		0.174			1.87				Connell et al., 1975
Richards Bay	1974	0.001 0.025			0.25 3.1		0.01 0.38			3,9			0.53	Cloete (ed), 1979
Richards Bay	1976	0.804 0.17	0.4 4.0		0.1 4.0		0.45 1.92		nd 13.0	nd 4.2		nd 3.8		Cloete (ed), 1979
Durban	1974	0.306			0.773	11.70	0.383			0.25		28.1		Oliff & Turner, 1976
Durban	1978	na 0.82	na 3.2		0.57 27	15 800	0.17 0.37		7 26	18 117		26 287		Cloete (ed), 1979
Umbogintwini R.	1974	1.39			31.29		0.097			0.6		43.0		Connell et al., 1975
Fynnlands	1974	1.07			12.14		0.023			1.52		20.7		Connell et al., 1975

TABLE II (continued)

Location	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sb	Zn	Nb	Reference
Umzimkulu River	1974	0.015			1.45		0.065			2.93		7.78		Connell et al., 1975
Umzimkulu River	1974	0.04 0.015			0.90 2.21		0.05 0.13			0.38 3.5		7.5 8.9		Cloete (ed), 1979
Umzimvubu River	1977	0.13 0.24	1.5 1.6		1.1 1.9	390 460	0.33 0.40			2.2 2.9		11.5 20.1		Cloete (ed), 1979
Umgababa River	1977	0.075	9.80		1.957	64.5	0.871			2.891		1.454		Oliff & Turner, 1976
Umgababa River	?	0.06	7.9		2.8	684	1.0							Cloete (ed), 1979
Mngazana River	1977	nd	nd		2.4	211	0.3			2.7		5.7		Cloete (ed), 1979
Bashee River	1975	0.159			8.730		0.11			6.009		11.429		Oliff & Turner, 1976
Bashee River	1975	0.20			8.3	120	0.14			5.71		28.6		Cloete (ed), 1979
Buffalo River	1977	na 0.035	0.7 1.5		1.5 1.7	72 122	0.16 0.23			na 0.02		0.094		Cloete (ed), 1979
Algoa Bay	1978	0.2	0.1		1.9	81	0.009	5.6	0.6	0.9		22		Watling & Watling, 1983
Swartkops River	1975	0.312			7.059		0.13			11.320		10.092		Oliff & Turner, 1976
Swartkops River	1975	0.05			2.62							3.0		Oliff & Turner, 1976
Swartkops River	1975	0.01 0.03			2.63 2.75					2.5 2.8		2.0 3.8		Cloete (ed), 1979
Swartkops River	1975	2.8 3.5			0.21 0.22		0.06			7 8.7				Cloete (ed),1979

TABLE II (continued)

Location	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sb	Zn	Nb	Reference
St Francis Bay	1979	0.3	0.3		1.3	275	0.010	4.2	0.2	0.3		1.4		Watling & Watling, 1983
Jeffreys Bay	1977	0.15			0.9	40.6		24.0	1.3	0.073		5.5		NRIO, 1981; Orren et al., 1981
Keurboomstrand	1977	0.41			1.7	15.7		11.9	0.8	0.65		3.8		NRIO, 1981; Orren et al., 1981
Knysna E	1977	0.4	0.6		1.1	192.0	0.1	3.1	0.3	1.3		1.5		Watling & Watling, 1980
Knysna E	1977	1.0	1.0		0.8	101.7	0.100	8.2	0.2	1.4		3.8		Watling & Watling, 1980
Mossel Bay	1979	0.6	0.5		1.8	45	0.037	2.1	0.16	0.3		1.7		Watling & Watling, 1983
Arniston	1977	0.27			1.0	7.9		50.5	3.2	0.68		5.9		NRIO, 1981; Orren et al., 1981
Table Bay	1980	see separate Figures												Eagle et al., 1982
Melkbos	?		0.02	0.08		1.7		0.7			0.7	1.2		Van As et al., 1975
Saldanha	1976				0.98	2.49						2.10		Henry (pers. com.)
Saldanha	1977				0.98	3.68		1.20				3.38		Henry (pers. com.)
Saldanha	1978				1.00	3.40		1.73				4.98		Henry (pers. com.)
Saldanha	1979				1.39	3.92		0.80				6.85		Henry (pers. com.)
Berg River	1976	0.08			1.3	71.3		17.2	0.9	1.5		3.7		NRIO, 1979
Olifants River	1980	0.08			0.3	170.7	0.286	11.9	0.44	0.71		6.1		NRIO, 1981

The only time series was carried out at Saldanha Bay by the Sea Fisheries Research Institute (J Henry, personal communication). This showed a substantial increase in Cu, Fe and Zn concentrations over a period of four years.

Trends in metal concentrations can be observed in Figure 3 and these are discussed below. It should be noted that the data have been log-transformed to accommodate the large ranges of metal concentration (see for example, Figure 3, Fe concentration).

2.1 Cadmium

The concentrations of cadmium ranges from "none detectable" (n.d.) to 3.5 µg ℓ^{-1} at the Swartkops River mouth. Most values are low and compare favourably with the cadmium concentration reported elsewhere, for example 0.01 to 0.62 µg ℓ^{-1} in nearshore water (Phillips, 1977) and 0.11 µg ℓ^{-1} in coastal waters (Waldichuk, 1977).

Three anomalies were observed. Higher concentrations of cadmium were found off Umbogintwini and Fynnlands. Both locations are close to pipelines carrying industrial effluent. High concentrations were also found at Port Elizabeth (Swartkops River) and at Knysna estuary. At Swartkops River the source of enrichment could be industrial effluent. Knysna on the other hand has only limited industry. Here the source could be due to geochemical factors (Watling and Watling, 1980).

2.2 Cobalt

The cobalt concentrations were higher than in comparable Californian nearshore waters (Phillips, 1977) or the value of 0,05 µg ℓ^{-1} quoted by Waldichuck (1977). Anomalies were observed at Richards Bay, Durban Bay and the Umgababa Estuary.

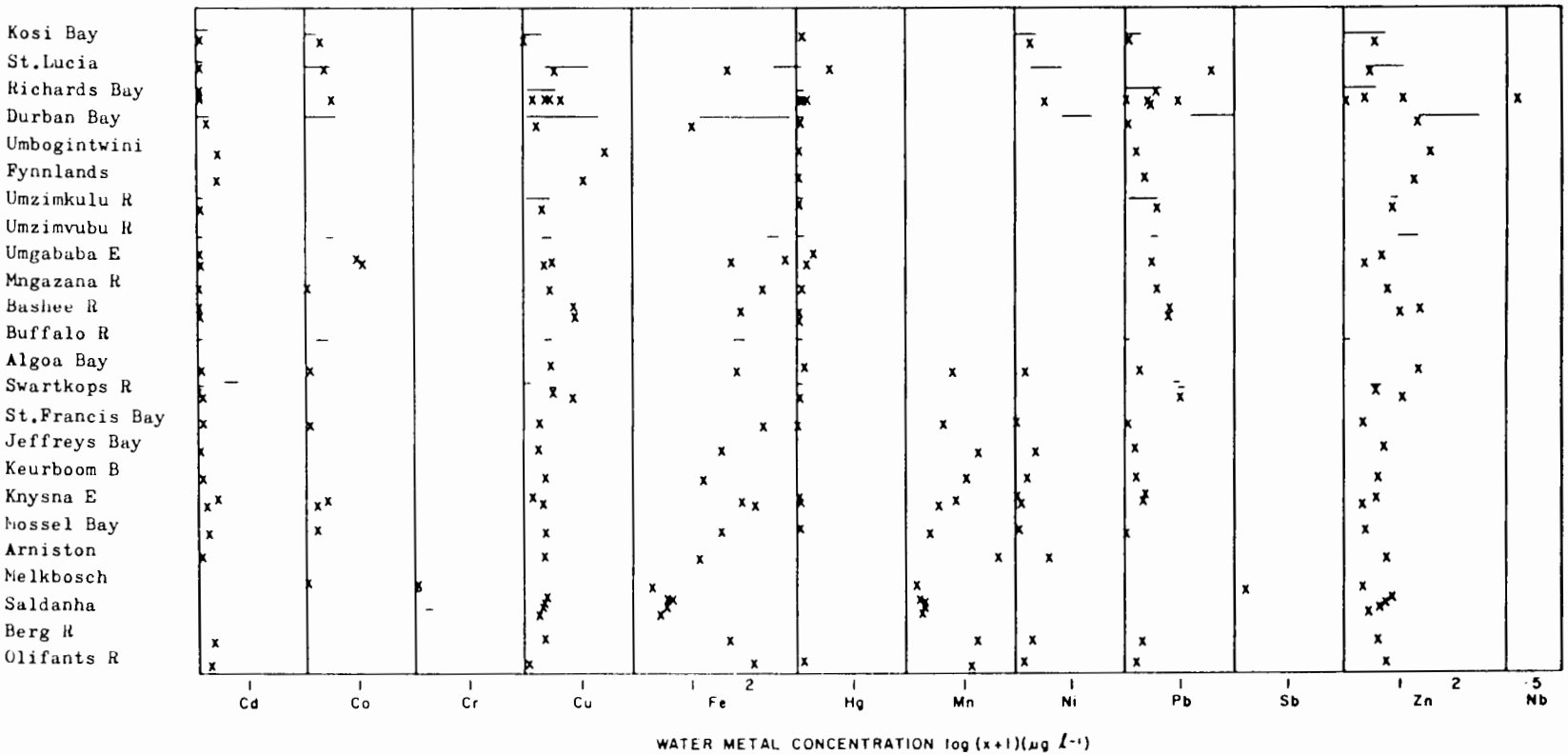


Figure 3 Metal concentrations in water at different locations along the coast of South Africa. All concentrations in µg ℓ^{-1}

2.3 Copper

The reported values in other parts of the world range from 0.18 to 4.0 µg ℓ^{-1} and were also observed at most South African locations. High values were measured at St Lucia, Durban Bay and vicinity, Bashee River and Swartkops River.

2.4 Iron

The iron concentration was very high at most of the sampling points. The only exceptions were in the vicinity of Melkbos and Saldanha Bay. Because of industrialisation at Saldanha Bay the iron concentration in the water there has been increasing but was still several orders of magnitude less than the average reported for the rest of South Africa.

2.5 Mercury

The mercury concentrations in South African coastal water were usually less than literature values, with the exception of St Lucia and Umgababa.

2.6 Manganese

This metal has been measured only in the waters of the south and west coasts. Values were high at Jeffreys Bay and Arniston, but interestingly both are unpolluted beaches used for "reference" purpose by the Marine Pollution Programme (Orren et al., 1981).

2.7 Nickel

Nickel concentrations were low compared to those from northern hemisphere locations (Phillips, 1977; Waldichuk, 1977). Anomalies were observed at St Lucia, Richards Bay, and particularly at Durban Bay and Arniston.

2.8 Lead

Lead concentrations are very difficult to measure, and values given in the literature range from 0.05 to 1.2 µg ℓ^{-1} (Phillips, 1977), while a mean of 0.03 µg ℓ^{-1} is quoted by Waldichuk (1977). Lead concentrations in the South African coastal waters were comparable, and lay in a range similar to that above, with high concentrations at St Lucia, Durban Bay, Bashee River and Swartkops River.

2.9 Zinc

The distribution of zinc in the South African coastal waters can really serve as a summary and identification of "hot spots". The elevated or polluted areas are St Lucia, Richards Bay, Durban and vicinity, Port Elizabeth (Swartkops River) and possibly Saldanha Bay. All these areas are highly industrialised.

2.10 Chromium, Niobium, Antimony

Chromium concentrations at Melkbos were low compared with those reported in the available literature (Phillips, 1977). Niobium and antimony were reported by Cloete (1979) but no other reference could be found for the two metals.

2.11 Metal concentrations in Table Bay waters, Cape Town

A detailed study has been done by Eagle et al. (1982) on the behaviour of sewage from the Green Point outfall and its effect on Table Bay. The results are given in Figures 4 to 7.

The conclusion from this study was "that the shape of the plume is dependent on the current, and hence wind, conditions prevailing at the time. At this stage there is no conclusive evidence that these metals originate from the sewage. Calculations indicate that the trace metal addition to the bay via the sewage is probably insignificant, except perhaps for zinc".

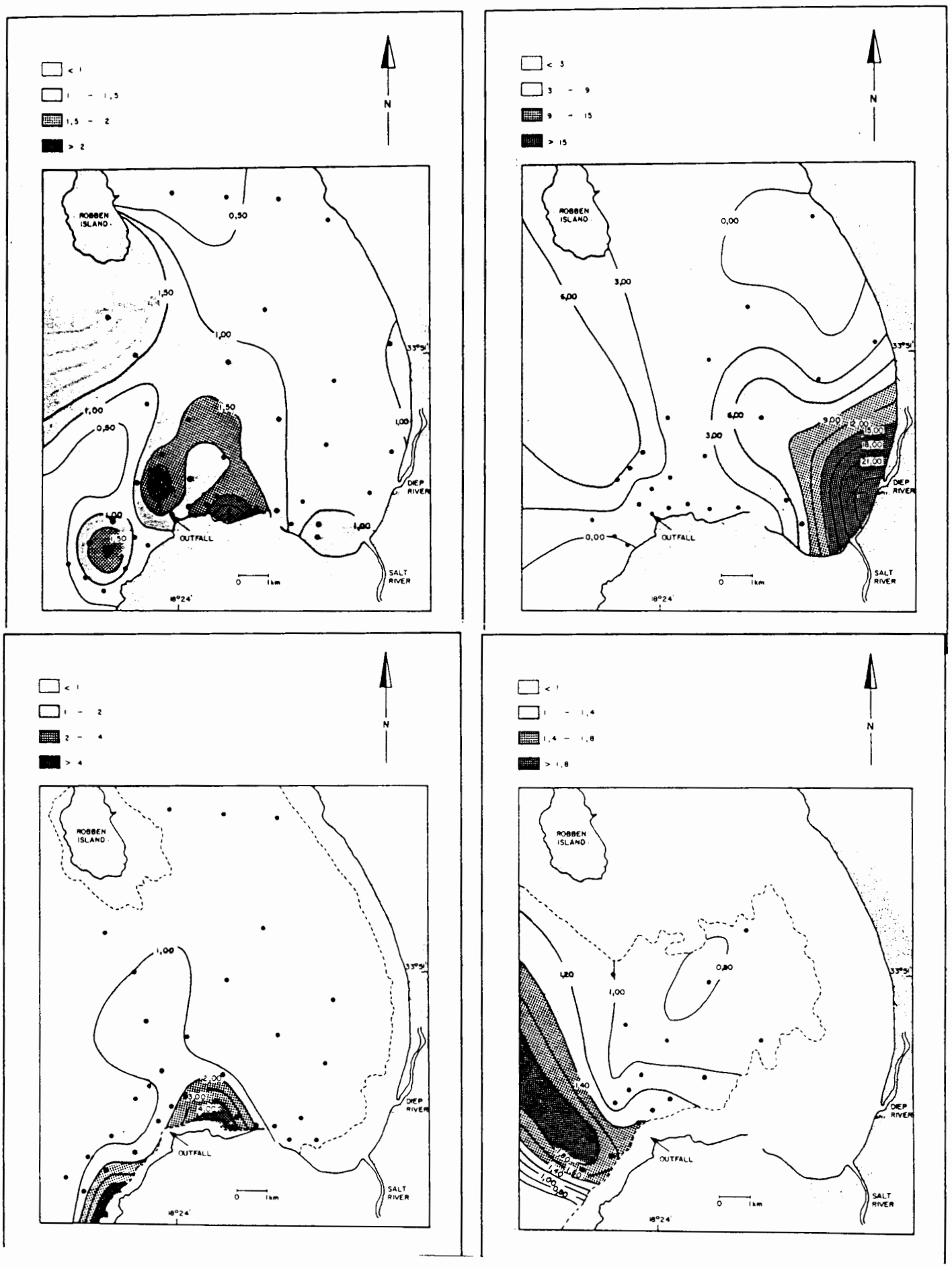


Figure 4 Metal concentrations in Table Bay waters (µg ℓ^{-1})
top left: zinc, surface - spring;
top right: iron, surface - winter;
bottom left: zinc, 10 m - spring;
bottom right: zinc, 20 m - spring

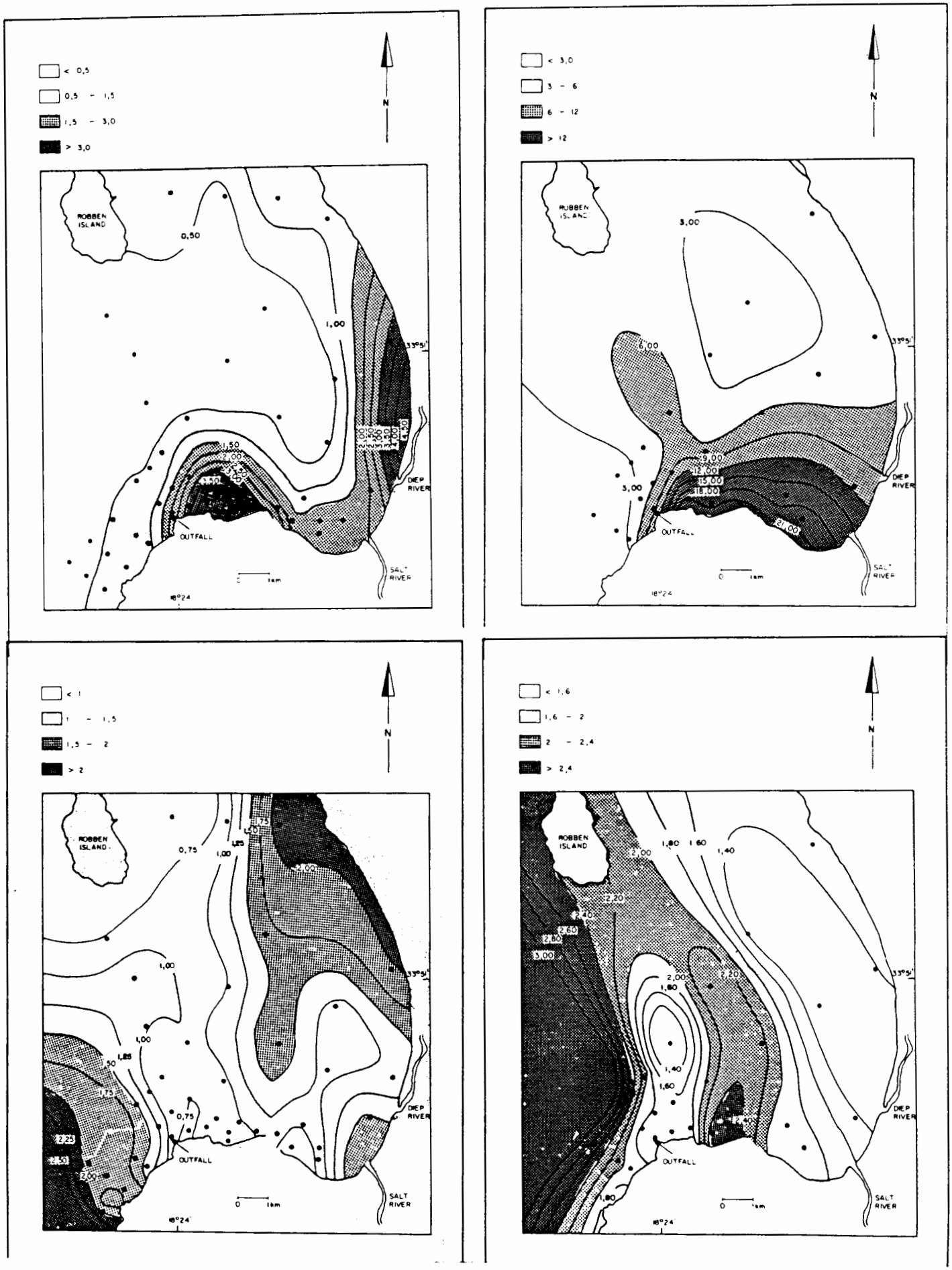


Figure 5 Metal concentrations in Table Bay waters (µg ℓ^{-1})
top left: iron, surface - spring;
top right: iron, surface - winter;
bottom left: lead, surface - spring;
bottom right: lead, surface - winter

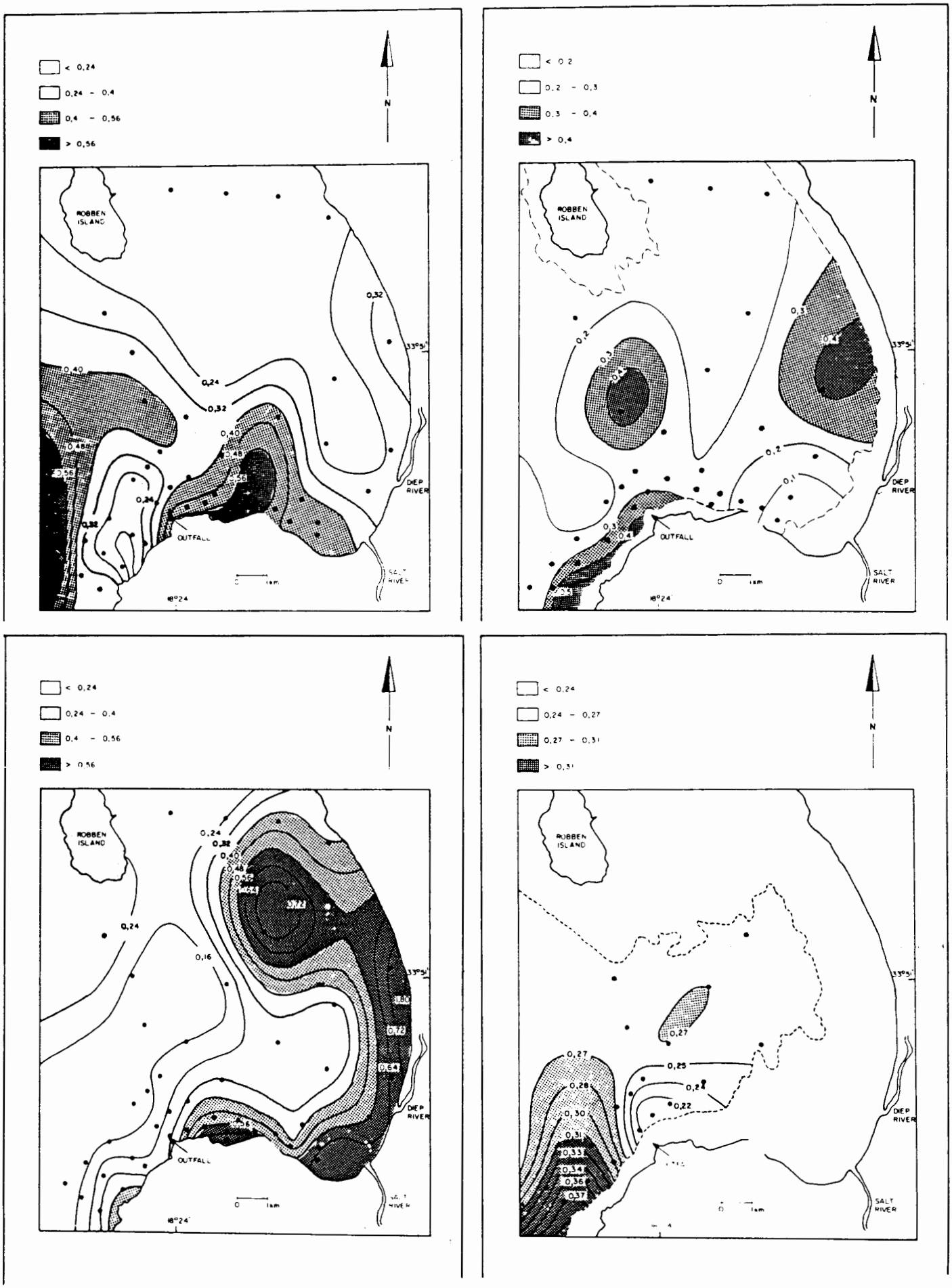


Figure 6 Metal concentrations in Table Bay waters (µg ℓ^{-1})
top left: copper, surface - spring;
top right: copper, 10 m - spring;
bottom left: manganese, surface - spring;
bottom right: manganese, 10 m - spring

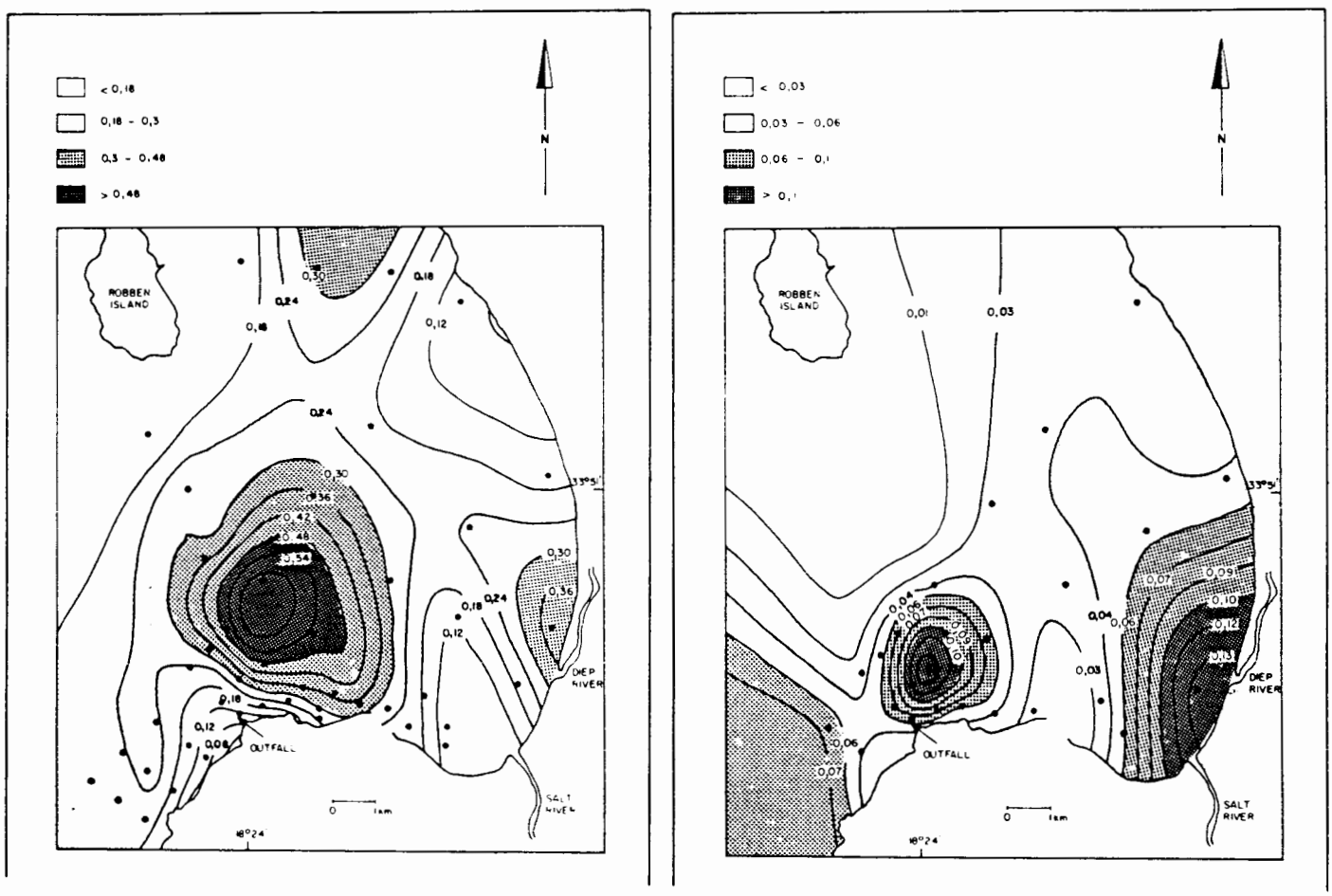


Figure 7 Metal concentrations in Table Bay waters (µg ℓ^{-1})
left: nickel, surface - spring;
right: mercury, surface - winter

2.12 Conclusions

The drawbacks and difficulties of interpreting metal concentrations in coastal waters have been pointed out. Within these limitations, the data show that the South African coastal waters are largely unpolluted. The enrichment in about four areas is due mainly to industrial effluent. It should be noted that the pollution programme has concentrated on impact areas and only very few samples were taken at non-industrial areas, away from the major cities. However, if elevated levels were to be found at these remote locations, these are likely to be of natural origin and therefore would not fit into the GESAMP definition of pollution.

Unfortunately nearly nothing is known about "normal" or "before pollution" concentrations of metals in South African coastal waters. This has produced the rather unsatisfactorily situation: there is no reference point or range of metal concentrations by which to judge any pollution or enrichment of the environment.

3. SURVEY OF TRACE METAL ABUNDANCE IN COASTAL SEDIMENTS

Three major problems exist in the interpretation of data concerning the concentrations of trace metals in sediments.

(1) The concentration of a metal in sediments is not only a function of the quantity of metal deposited, but is also a function of the ratio of metal deposited over a given period of time.

(2) The concentration of a metal found in sediments depends on the organic content of the sediment. In general, metal concentrations increase approximately linearly with increase in organic content, measured as total carbon (Halcrow *et al.*, 1973).

(3) Other variables such as particle nature, form and size may also affect the concentrations of metals in sediments. The presence of certain ionic groups and the surface area-to-volume ratio of particulates is important in the process of metal adsorption. In addition, differences in mobilisation rates (biological or physical) may lead to erronious conclusions concerning the rate of metal input.

The available data on metal concentrations in sediments are summarised in Table 3. Sediments were sampled at 29 sites over a period of nine years. The main metals reported are Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn. A small amount of information is available for Ag, Al, Ba, Bi, Br, Mo, Nb, Rb, Sr, Th, Ti, V, Y, and Zr.

Although sampling was repeated at some sites, no trend in metal level with time could be observed. The data in Table 3 have been log-transformed and graphically presented in Figure 8.

3.1 Cadmium

The cadmium concentrations in coastal sediments range from not detectable (b.d.) to 27 $\mu g\ g^{-1}$ (dry) at Port Elizabeth. Most of the reported concentrations are similar to those found in other parts of the world (Phillips, 1977; Gilmour and Kay, 1979; Cloete, 1979). High metal concentrations occurred at Richards Bay, Durban, Bashee River and Port Elizabeth.

3.2 Cobalt

Cobalt concentrations are all low and even the high values at St Lucia (27 $\mu g\ g^{-1}$ dry) cannot be considered high in comparison with other areas (Phillips, 1977). In South Africa sediment anomalies were observed at the industrial centres.

TABLE III: SUMMARY OF METAL CONCENTRATIONS OF COASTAL SEDIMENTS AROUND SOUTH AFRICA ($\mu g\ g^{-1}$ dry)

Location	Date	Al	Ag	Bi	Cd	Co	Cr	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Ti	V	Zn	Reference
St Lucia E	1978				0.08 0.4	1 27	7 150	2 61	3 000 60 000	0.007 0.04			2.9 84	0.8 19			3.4 72	Cloete (ed), 1979
Richards Bay	1974				0.57			9.9	23 640	0.022				24			98	Connell et al., 1975
Richards Bay	1974				0.46 0.76			8.3 12.0	19 000 29 000	0.017 0.029				20 32			66 175	Cloete (ed), 1979
Richards Bay	1976				0.07	16.13	74.80	24.04	5 814	0.014			27.24	17.47			87.16	Oliff & Turner, 1976
Richards Bay	1976				0.6 1.6	1.5 23.5		1.1 40.0	800 11 000	0.007 0.039			2.2 73	5.5 33.0			14 179	Cloete (ed), 1979
Durban Bay	1978				0.7 1.9	4.2 13	10 388	5.5 57	3 000 40 000	0.07 0.87	23 303		7 26	18 117			26 287	Cloete (ed), 1979
Umzimkulu E	1974				0.7			9	13 057	0.005				10			162	Connell et al., 1975
Umzimkulu E	1974				0.4 0.6			4 10	9 000 11 000	0.001 0.005				5 9			33 649	Cloete (ed), 1979
Umzimvubu R	1977				0.008 0.07	0.83 0.95	26 27	2.6 4.8	1 420 1 540	0.03 0.05			13 17	0.16 0.35			12.1 16.4	Cloete (ed), 1979
Umgababa	1976				0.28	6	15	3.7	7 016	0.014				4.6			7.8	Oliff & Turner, 1976
Umgababa	1976				0.52	12	21	9.6	12 330	0.02				9			21	Cloete (ed), 1979
Umgababa	1977				0.35		7.0	1.4	4 302	0.095			5.01	4.3			4.9	Oliff & Turner, 1976
Umgababa	1977				0.35		7.6	2.2	5 192	0.04			5.1	4.0			10.2	Cloete (ed), 1979
Mngazana R	1977				0.09	3.9	126	15	4 990	0.09			35	1.4			71	Cloete (ed), 1979

ABLE III (continued)

Location	Date	Al	Ag	Bi	Cd	Co	Cr	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Ti	V	Zn	Reference
Bashee River	1975				0.82			10.03	11 810	0.007				21.3			17.8	Oliff & Turner,1976
Bashee River	1975				0.23		5.0	2.7	4 780	0.001				5.3			8.4	Cloete (ed). 1979
Buffalo River	1977				0.04 0.06	9 11	35 40	16.2 18.6	25 000 36 000	0.05 0.16	150 470		17 21	8.8 9.5			52 69	Cloete (ed), 1979
Algoa Bay	1978				0.338	0.2	3.7	1.8	2 539	0.063	112.6		0,09	2.7			5.6	Watling & Watling, 1983
Swartkops R.	1975				1.42		44.4	5.97	5 133	0.0576				28.4			19.7	Oliff & Turner. 1976
Swartkops R.	1975				0.45		40	8.9	7 320	0.035				13.1			21	Cloete (ed), 1979
Port Elizabeth	1975				27		8	2	2 378	0.004				18			7	Oliff & Turner, 1976
St Francis Bay	1978				0.072	0.4	33	3	1 451	0.004	31		0,6	3			3	Watling & Watling, 1983
Jeffreys Bay	1977				0.05	<0.5		<0.5	2 810		18.4		<1	2.0			3.1	NRIO, 1979; Orren et al., 1981
Keurboom-strand	1977				0.002	<0.5		0.5	3 596		19.9		<1	2.0			2.8	NRIO, 1979
Keurboom-strand	1977				0.07	<0.5		<0.5	960		11.3		<1	2.0			1.5	Orren et al., 1981
Knysna E	1975	0,77	0,06	18	0.6	3	21	5	0,8%		40	4,7	7	14	370	9	17	Watling & Watling, 1977
Knysna E	1976				0.2			3.1		0.003			5,2	12			17.8	Watling & Watling, 1980
Knysna E	1977	7,76	0,06	17,84	0.72	3.43	20.5	5.16	7 551		40.40	4,79	7,41	13.94	373	8.67	16.08	Watling & Watling, 1977
Mossel Bay	1979				<0.05	<0.5		0.8	2 794		30.6		1,3	1.7			6.1	NRIO, 1980

TABLE III (continued)

Location	Date	Al	Ag	Bi	Cd	Co	Cr	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Ti	V	Zn	Reference
Mossel Bay	1978				0.021	0.4	2.7	1.9	2 574	0.001	24		0,9	4.3			4.1	Watling & Watling, 1983
Arniston	1977				0.07	<0.5		<0.5	956		11.3		<1	2.0			1.5	NRIO, 1979; Orren et al., 1981
Eerste River	1980				0.04			0.5	535		7.00		bd	1.1			1.2	NRIO, 1981
AECI	1976				0.3	<0.5		2.1	494		4.5		<1	2.4			2.8	NRIO, 1979
AECI	1979				<0.03	<0.5		1.5	706		4.5		<1	3.2			2.3	NRIO, 1980
Swartklip	1978				0.05	<0.5		<0.5	1 208		7.7		<1	2.5			1.9	NRIO, 1979; Orren et al., 1981
Hout Bay	1977				0.18	<0.8		0.4	510		8.0		<0.1	1.5			2.8	NRIO, 1979
Hout Bay	1979				0.6	<0.5		0.5	198		3.4		<1	0.4			1.1	NRIO, 1980
Hout Bay	1980				0.06			0.5	590		7.3		bd	1.0			1.5	NRIO, 1981
Camps Bay	1978				<0.1	<0.5		0.3	383		7.5		<1	1.1			1.2	NRIO, 1980
Green Point	1977				0.6	1.2		9.1	3 523		19.5		3.5	14.2			25.7	NRIO, 1979
Green Point	1980				0.1			5.4	3 055	0.014	21.5		1.3	7.4			10.9	NRIO, 1981
Green Point	1980				0.1			4.5	1 904		17.5		1.4	9.3			11.8	NRIO, 1981
Salt River	1980				bd			bd	519	bd	8.0		bd	1.3			1.4	NRIO, 1981; Orren et al., 1981b
Koeberg	1981				0.05			<0.3	268		4.35		<0.4	0.4			0.41	This study
Saldanha	1976				0.7	3		3.3			12		6	17			8.7	Watling & Watling, 1976

TABLE III (continued)

Location	Date	Ba	Br	Co	Cr	Cu	Nb	Ni	Rb	Sr	V	Y	Zn	Zr		Pb	Th	Reference
Olifants R.	1976	553 585	8 10	29 31	105 116	65 74	11 13	46 51	138 149	154 161	127 143	32	110 122	152 192				Cloete (ed), 1979
Olifants R.	1977			nd 23	15 82	3 61	nd 13	5 46	14 141	26 137	14 114	5 32	8 107	49 143		nd 28	nd 17	Cloete (ed), 1979
Berg River	1975				0.3	0.8		0.8	2 611		26		1.3	3.0			5.3	NRIO, 1979
Berg River	1975				0.3	1.6		5.3	5 152		50.9		4.6	6.0			26.9	NRIO, 1979
Berg River	?				0.67			5.23						5.99			29.6	Cloete (ed), 1979
Berg River	?				0.42			5.34						10.9			22.2	Cloete (ed), 1979
Olifants R.	19				0.21			13.2						7.9			32.7	
Olifants R.	?				0.14			23.7						7.95			44.1	Cloete (ed), 1979
Olifants R.	1980				0.7			8.9	13 435		284.3		7.67	17.8			23.73	NRIO 1981

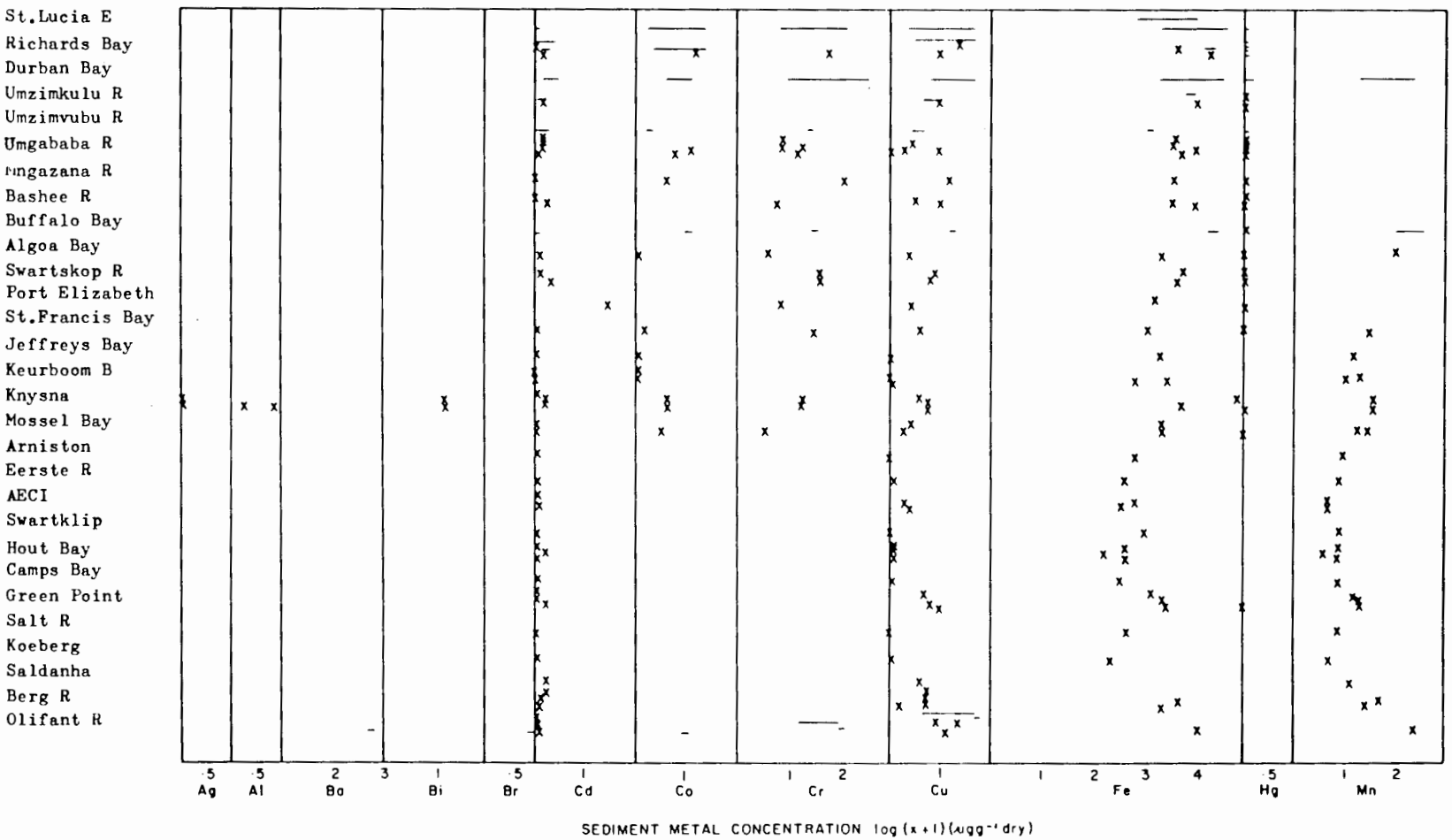
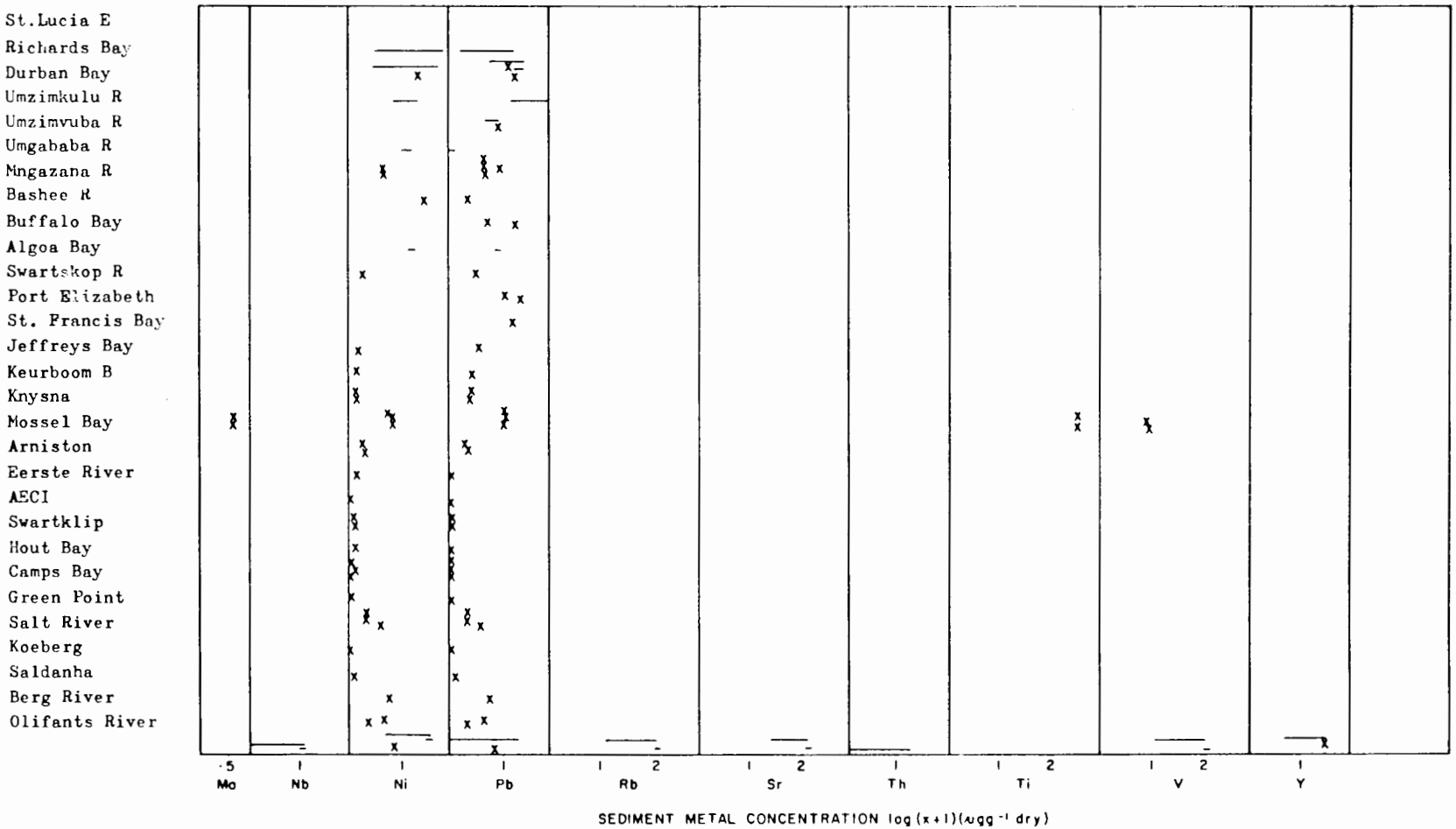
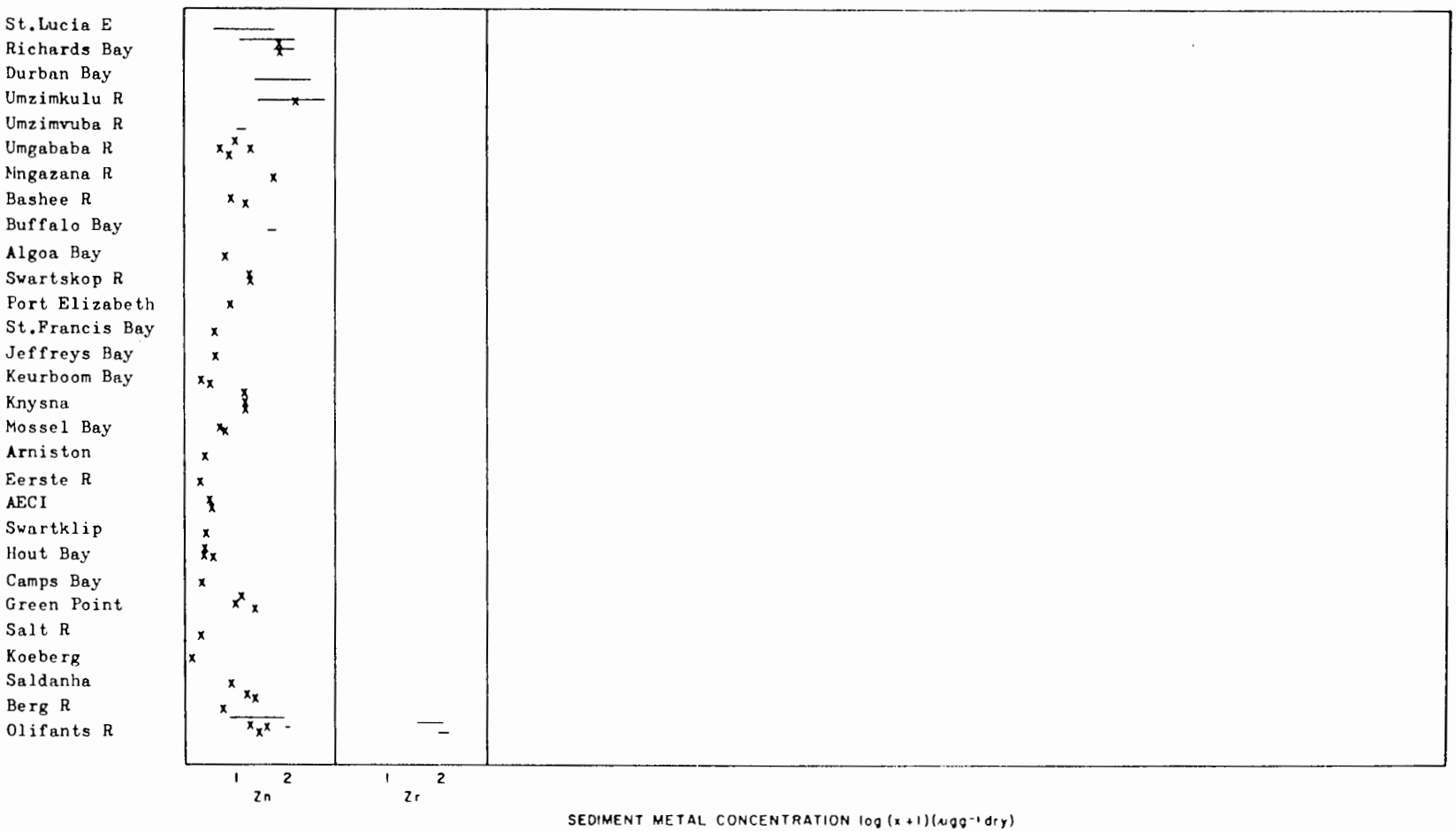


Figure 8 Metal concentrations in sediment at different locations along the coast of South Africa. All figures refer to μg g^{-1} in dry sediment


St.Lucia E
Richards Bay
Durban Bay
Umzimkulu R
Umzimvuba R
Umgababa R
Mngazana R
Bashee R
Buffalo Bay
Algoa Bay
Swartskop R
Port Elizabeth
St. Francis Bay
Jeffreys Bay
Keurboom B
Knysna
Mossel Bay
Arniston
Eerste River
AECI
Swartklip
Hout Bay
Camps Bay
Green Point
Salt River
Koeberg
Saldanha
Berg River
Olifants River
·5
1
2
Mo
Nb
Ni
Pb
Rb
Sr
Th
Ti
V
Y
SEDIMENT METAL CONCENTRATION log (x+1)(μgg⁻¹ dry)


St.Lucia E
Richards Bay
Durban Bay
Umzimkulu R
Umzimvuba R
Umgababa R
Mngazana R
Bashee R
Buffalo Bay
Algoa Bay
Swartskop R
Port Elizabeth
St.Francis Bay
Jeffreys Bay
Keurboom Bay
Knysna
Mossel Bay
Arniston
Eerste R
AECI
Swartklip
Hout Bay
Camps Bay
Green Point
Salt R
Koeberg
Saldanha
Berg R
Olifants R
1
2
Zn
1
2
Zr
SEDIMENT METAL CONCENTRATION log (x +1)(μgg⁻¹ dry)

3.3 Chromium

No trend could be observed in the distribution of chromium in the coastal sediments. Values in the range 2.6 to 388 μg g^{-1} (dry) compare well with Phillips' review (Phillips, 1977), which showed that chromium ranges from 35 to 307 μg g^{-1} (dry) in many parts of the world.

3.4 Copper

The copper levels in sediments around South Africa range from 0.5 to 74 μg g^{-1} (dry). Phillips (1977) quotes 1 to 60 μg g^{-1} (dry) for most parts of the world. It is possible that slightly higher levels are observed at industrialised areas.

3.5 Iron

In reviews, iron concentrations in sediment are usually given in percent by weight. The values are similar in range to those found in South Africa. Iron concentrations in the sediment along the east coast appear to be higher while lower levels occur along the south coast.

3.6 Mercury

There appears to be very little mercury in South African coastal sediments. A "British Control" area (see Halcrow *et al.*, 1972 in Phillips, 1977) contained 0.04 to 0.15 μg g^{-1} of mercury. In two areas in South Africa, Durban Bay and Buffalo River, mercury levels were higher.

3.7 Manganese

This metal was found in only small concentrations in South African coastal sediment. In control areas elsewhere (Phillips, 1977) concentrations usually range from 240 to 700 μg g^{-1} (dry). In South Africa higher levels were found at Durban Bay and in the Buffalo River.

3.8 Nickel

High levels of nickel in South Africa lay within the control ranges given in most published reports (Phillips, 1977). Higher nickel concentrations in South Africa occurred around Richards Bay and Durban, Knysna, Green Point and the Olifants River. The higher levels of nickel at the Olifants River were somewhat unusual but may be related to the fresh water run-off and organic matter since higher copper concentrations have also been found in sediments in this region. These minerals originate from the Namaqualand mining area and hence could have a geochemical source.

3.9 Lead

The lead concentrations in the sediment around the South African coast range from 0.4 to 117 $\mu g\ g^{-1}$ (dry). Again these values are low compared with values elsewhere in the world (Phillips, 1977). Higher concentrations have been observed at the industrial areas around the coast.

3.10 Zinc

The pattern of zinc concentrations in the coastal sediments is not very clear. This could partly be due to the relatively low concentrations compared with those in other countries. Again the industrialised areas show higher concentrations.

Concentrations of Ag, Al, Ba, Bi, Br, Mo, Nb, Rb, Sr, Th, Ti, V, Y, Zr have been reported from only two unpolluted areas: Knysna Estuary and the Olifants River. Most of these values may be the natural levels of the trace metals in the sediment of those areas, and may not be derived from the activities of man. Hence they can be assumed to be baseline values.

3.11 Metal concentration in Table Bay sediments, Cape Town

Eagle et al. (1982) have analysed selected trace metals in sediments in Table Bay (Figures 9 and 10). The conclusion drawn was that in the sediments there was a sharp increase in the concentrations of all the metals studied in a small area between the Green Point outfall and the harbour.

3.12 Conclusion

The use of sediments in the determination of metal baselines is also subject to some errors. It appears that the enrichment of sediments by metal pollutants has not yet taken place around the coast of South Africa. Comparable areas elsewhere usually contain higher metal concentrations. Against these low background values, it is possible to recognize the industrialised impact areas. Together with the water data this confirmation can be used to indicate those selected places which can be considered "polluted" or "enriched" above the baseline values found in non-industrialised regions.

In this context it is interesting to speculate whether pollution control authorities should act now and focus on enriched areas and hot spots or whether they should adopt the attitude that metal concentration in South Africa's hot spots lie well below those found in other countries and by comparison could not be called "polluted".

4. SURVEY OF TRACE METAL ABUNDANCE IN MARINE ORGANISMS

The gathering of data on metal concentrations in marine organisms was done to establish a baseline for as many organisms as possible and to arrive at approximately the "right" order of magnitude when considering any specific metal. Often baseline studies are not only of academic interest, but are used in predicting the possible effects of proposed industrialisation or sewage disposal (see Hennig et al., 1982).

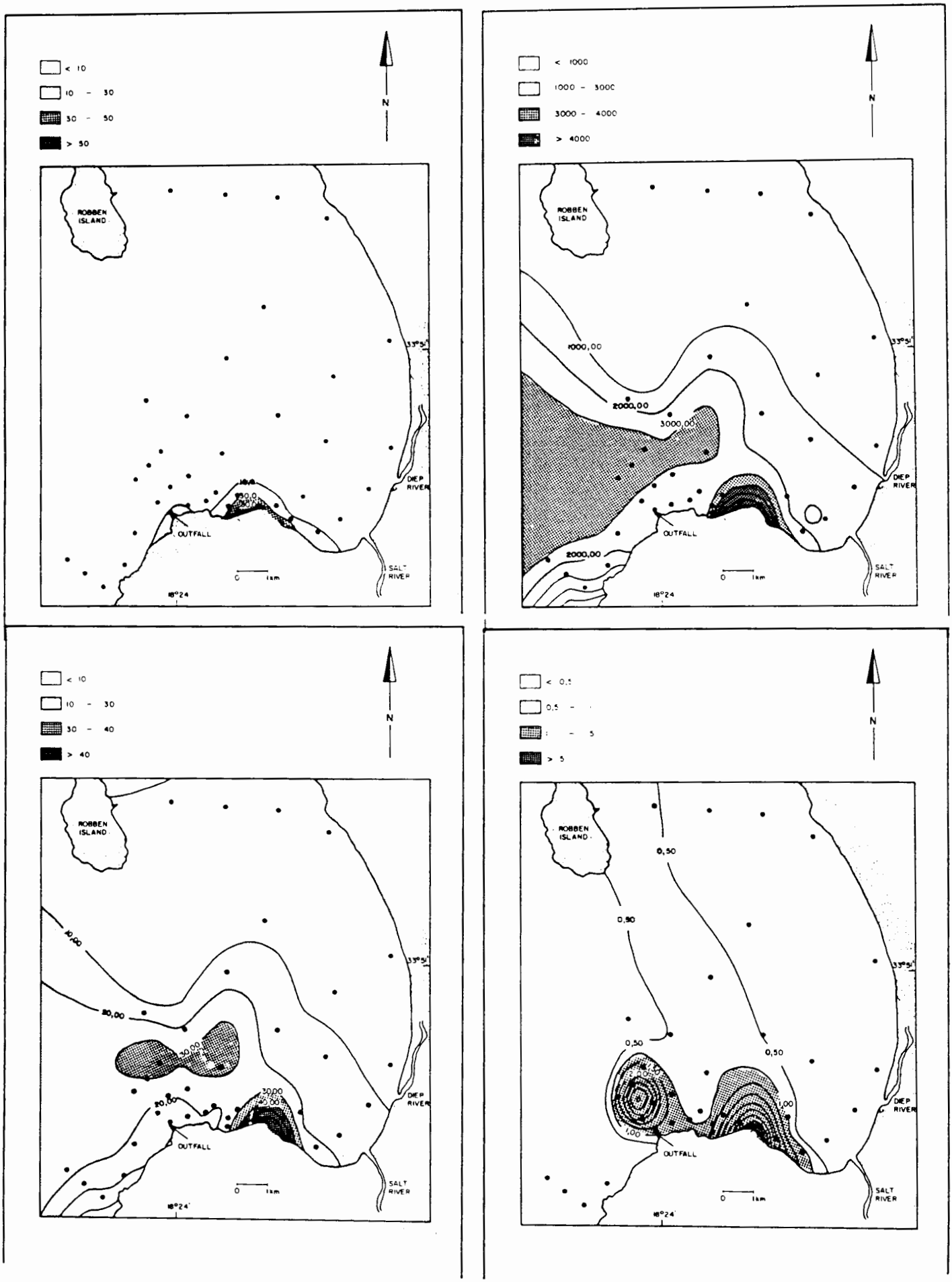


Figure 9 Metal concentrations in Table Bay sediments ($\mu g\ g^{-1}$) top left: copper; top right: iron; bottom left: manganese; bottom right: nickel

Figure 10 Metal concentrations in Table Bay sediments ($\mu g\ g^{-1}$) left: lead; right: zinc

Measurements of physical and chemical variables in sediment and water samples represent only one specific value at one specific time, season or water current pattern. Measurements of chemical variables in animals, on the other hand, represent the mean of metal concentrations integrated over the life-span of the organism, different seasons and water currents. They also represent a permanent record of the relative biological availability of metals to an individual or organism at each location studied.

Some animals accumulate metals more readily than others. These biological indicator species have been identified by Watling (1978) on the South African coast. Fortunately, enough information was available from samples of some organisms (limpets and mussels) taken from many different sections of the coast, so that a type of "Mussel Watch" study could be produced, similar to that of Goldberg et al. (1983) in the United States of America.

Therefore the biological data have been presented in two different formats: concentration of metals in the same species at different locations, and metal accumulation in different species at one point along the coast.

The data have been arranged in geographic sequence and are summarized in Table 4. This is especially useful to management and pipeline planners, since they are interested in man-made impact at a specific point.

Methods for the metal determination of biological samples are given for each author. Usually this has been done at the first described geographical area (see Table 4) and are not repeated for subsequent locations to avoid unnecessary repetition.

TABLE IV: SUMMARY OF METAL CONCENTRATION IN MARINE ORGANISMS (d = dry mass; w = wet mass; units = $\mu g\ g^{-1}$)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Cs	Sb	Al	Bi	Ag	Sr	Reference
Kosi Bay																		
d Plankton (E)	1976	12.200	20.806	17.897	884.836	6 920.274	0.152			129.516	1 096.743							Oliff and Turner, 1976
d Plankton (M)	1976	129.664	166.666	192.241	579 921.132	66 774.22	1.130			812.980	21 528.560							Oliff and Turner, 1976
d Plankton (O)	1976	7.205	13.662	6.643	806.78	3 576.571	0.057			66.784	413.393							Oliff and Turner, 1976
d Plankton (F)	1976	15.367	ND	234.240	24.415	20 461.76	3.054			84.078	1 151.88							Oliff and Turner, 1976
d *Perna perna*	1976	1.20	4.73	7.53	6.60	1 440.0				157.33	44.0							Watling, 1978
d *Cheimerius nufar* (muscle)	1976	0.519	ND	0.818	0.500	17.968	1.033			2.129	15.626							Oliff and Turner, 1976
d *Cheimerius nufar* (liver)	1976	15.812	0.830	0.490	28.716	574.713	0.941			1.985	129.581							Oliff and Turner, 1976
d *Chrysoblephus puniceus* (muscle)	1976	0.109	1.417	0.927	1.745	20.107	0.329			4.360	18.861							Oliff and Turner, 1976
d *Thunnus* sp. (liver)	1976	55.391	0.795	0.819	18.868	243.228	0.050			0.467	90.846							Oliff and Turner, 1976
d *Thunnus* sp. (muscle)	1976	0.325	1.684	1.190	1.850	33.381	0.111			0.742	53.381							Oliff and Turner, 1976
d *Perna* sp.	1976	1.186	4.70	7.51	6.62	1 441.2	0.124			157.00	43.69							Oliff and Turner, 1976
d *Bullia natalensis*	1976	12.18	11.06	6.11	5.38	226.8	0.026			31.08	50.17							Oliff and Turner, 1976
d *Crassostrea margaritacea*	1976	7.87	3.81	20.48	10.0	283.63	ND			4.30	646.9							Oliff and Turner, 1976
d *Trachinotus russellii* (muscle)	1976	0.16	1.71	3.12	1.44	35.56	0.073			6.72	20.92							Oliff and Turner, 1976
d *Lutianus argentimaculatus* (muscle)	1976	0.573	0.205	3.79	2.15	35.49	0.076			ND	ND							Oliff and Turner, 1976
d *Lutianus argentimaculatus* (liver)	1976	1.08	2.67	3.19	133.68	1 022.7	0.335			71.12	492.5							Oliff and Turner, 1976
d *Johnius hololepidotus* (muscle)	1976	0.587	1.27	34.94	6.60	118.55	0.096			3.64	7.87							Oliff and Turner, 1976
d *Johnius hololepidotus* (liver)	1976	2.72	ND	29.63	36.21	731.9	0.156			10.20	128.5							Oliff and Turner, 1976
d *Callianassa* sp.	1976	1.48	2.32	ND	290.9	514.19	0.164			6.97	50.45							Oliff and Turner, 1976
d *Panulirus homarus* (muscle)	1976	0.201	2.63	2.14	34.46	26.13	0.063			2.15	62.51							Oliff and Turner, 1976
d *Mugil cephalus* (liver)	1976	12.08	3.79	28.22	32.98	2 142.5	0.147			ND	165.24							Oliff and Turner, 1976
d *Mugil canaliculatus* (liver)	1976	1.52	0.658	62.32	22.98	693.2	0.245			6.97	117.32							Oliff and Turner, 1976
d *Mugil cephalus* (muscle)	1976	0.59	1.108	13.55	9.78	117.36	0.041			5.06	9.83							Oliff and Turner, 1976
d *Mugil canaliculatus* (muscle)	1976	1.47	1.61	20.33	3.58	62.80	ND			2.31	5.85							Oliff and Turner, 1976
d *Rhabdosargus* sp.	1976	0.17	1.03	3.82	2.56	25.85	ND			8.27	13.96							Oliff and Turner, 1976
d *Diplodus sargus* (muscle)	1976	2.06	2.70	4.04	6.64	25.06	ND			7.48	69.47							Oliff and Turner, 1976
d *Acanthopagrus berda* (liver)	1976									0.827								Oliff and Turner, 1976
d *Acanthopagrus berda* (muscle)	1976	0.40	0.63	21.44	3.85	121.4	0.108			4.74	49.45							Oliff and Turner, 1976
d *Elops machnata* (muscle)	1976	2.47	1.79	19.02	6.34	84.1	0.008			5.45	13.0							Oliff and Turner, 1976
d *Pomadasys commersonni* (liver)	1976									0.128								Oliff and Turner, 1976
d *Pomadasys commersonni* (muscle)	1976	0.382	2.75	82.56	6.93	265.99	0.181			3.34	36.19							Oliff and Turner, 1976
St Lucia																		
d *Loligo*	1976	4.543	0.516	0.848	16.311	31.239	ND			4.370	12.043							Oliff and Turner, 1976

TABLE IV (continued)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Cs	Sb	Al	Bi	Ag	V	Reference
Richards Bay																		
d Penaeus indicus	1976	0.8			38.1				ND		21.7							Oliff and Turner, 1976
d Argyrosomus hololepidotus (muscle)	1976	2.0			7.5				ND		2.4							Oliff and Turner, 1976
d Argyrosomus hololepidotus (liver)	1976	3.9			10.3				ND		82.1							Oliff and Turner, 1976
d Otolithes ruber (muscle)	1976	1.1			0				ND		32.0							Oliff and Turner, 1976
d Otolithes ruber (liver)	1976	2.7			13.4				ND		80.8							Oliff and Turner, 1976
d Rhabdosargus holubi (muscle)	1976	1.0			ND				ND		89.1							Oliff and Turner, 1976
d Rhabdosargus holubi (liver)	1976	1.8			11.6				ND		173.1							Oliff and Turner, 1976
d Mugil cephalus (muscle)	1976	0.4			ND				ND		71.8							Oliff and Turner, 1976
d Mugil cephalus (liver)	1976	0.4			169.8				ND		149.5							Oliff and Turner, 1976
d Elops machnata (muscle)	1976	0.6			ND				ND		5.0							Oliff and Turner, 1976
d Elops machnata (liver)	1976	1.0			39.5				ND		86.9							Oliff and Turner, 1976
d Pomadasys commersonni (muscle)	1976	0.6			ND				ND		72.5							Oliff and Turner, 1976
d Pomadasys commersonni (liver)	1976	1.9			39.5				ND		148.5							Oliff and Turner, 1976
Port Durnford Point																		
w Acanthopagrus berda (muscle)	1974	0.681			0.05		0.045			11.32								Connell et al., 1975
w Rhabdosargus sp. (muscle)	1974	0.066			0.3	4	0.029			0.72	56							Connell et al., 1975
w Emerita austroafricana	1974	1.040			20.43		0.008			9.07								Connell et al., 1975
w Penaeus indicus	1974	0.474			25.15		0.011			4.19	17							Connell et al., 1975
w Johnius hololepidotus (muscle)	1974	0.086			0.09	13	0.018			1.19	7							Connell et al., 1975
w Johnius hololepidotus (liver)	1974	2.011			2.47	251	0.035			0.35	24							Connell et al., 1975
w Mugil sp. (muscle)	1974	0.077			0.75	11	0.015			0.71	52							Connell et al., 1975
w Pomadasys commersonni (muscle)	1974	ND			0.25	ND	0.099			ND	ND							Connell et al., 1975
w Pomadasys commersonni (liver)	1974	0.620			58.27	657	0.018			0.15	45							Connell et al., 1975
Umgeni Estuary																		
w Mugil	1974	ND			0.387		0.17			ND	4.7							Connell et al., 1975
w Tilapia	1974	ND			0.42		0.25			ND	15.2							Connell et al., 1976
Umhlanga Rocks																		
w Perna perna	1972	0.29			14						0.95							Darracott and Watling, 1975
d Perna perna	1974	2	3	2	7	557		7	6	4	93			568		0.3	6	Watling and Watling, 1974
w Perna perna	1975	0.27	0.44	0.35	1.01	84		1.04	0.95	0.56	14.0							Watling, 1978
w Perna perna	1974						0.016											Connell et al., 1975
Durban Bay																		
w Panulirus versicolor	1974						0.079											Connell et al., 1975
w Plankton							0.004											Connell et al., 1975

TABLE IV (continued)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Cs	Sb	Al	Bi	Ag	Sr	Mo	Reference
Umbogintwini																			
w Emerita austroafricana	1974						0.237												Connell et al., 1975
w Panulirus homarus	1974						0.199												Connell et al., 1975
Fynnlands																			
w Panulirus homarus	1974						0.383												Connell et al., 1975
Umhlatuzana																			
w Mugil sp.	1974	ND			2.60		0.024			ND	23.2								Connell et al., 1975
w Mugil sp.	1974	ND			0.63		0.026			ND	4.7								Connell et al., 1975
w Therapon jarbua	1974	ND			0.56		0.041			ND	15.1								Connell et al., 1975
w Sarotheradon mossambicus	1974	ND			0.39		0.023			ND	4.3								Connell et al., 1975
Umzimkulu Estuary																			
w Mugil sp.							0.012												Connell et al., 1975
w Acanthopagrus berda	1974						0.207												Connell et al., 1975
Umgababa Estuary																			
d Panulirus homarus (muscle)	1976	0.52	2.7	1.0	41.7	87	0.481			4	81								Oliff and Turner, 1976
d Crassostrea margaritacea	1976	4.97	4.6	0.8	352.6	453	0.189			4	3 279								Oliff and Turner, 1976
d Upogebia africana	1976	2.90	12.6	0.7	145.0	1 349	0.089			26	207								Oliff and Turner, 1976
d Acanthopagrus berda (muscle)	1976	0.50	3.5	0.2	4.0	167	1.15			4	57								Oliff and Turner, 1976
d Crassostrea margaritacea	1976	1.01	0.63	0.03	81.9	46				1.35	600								Watling, 1978
Bashee Estuary																			
w Panulirus homarus	1975	0.06		0.03	4.28	1	0.0171			1.2	8.2								Oliff and Turner, 1976
w Pomadasys commersonni	1975	0.30		0.10	0.53	10	0.0449			4.6	10.2								Oliff and Turner, 1976
w Argyrosomus hololepidotus (muscle)*	1975	0.18		0.05	0.28	5	0.0454			2.5	3.5								Oliff and Turner, 1976
w Acanthopagrus berda (muscle)**	1975	0.13		0.04	0.34	5	0.4158			2.1	6.9								Oliff and Turner, 1976
w Hypacanthus amia (muscle)***	1975	0.15		0.03	0.34	5	0.2407			2.6	4.8								Oliff and Turner, 1976
w Argyrosomus (liver)*	1975	0.57		0.04	3.03	257	0.0263			5.0	20.0								Oliff and Turner, 1976
w Acanthopagrus (liver)**	1975	0.69		ND	4.05	180	0.3448			2.0	33.3								Oliff and Turner, 1976
w Hypacanthus (liver)***	1975	0.20		0.02	5.46	116	0.0646			1.2	17.4								Oliff and Turner, 1976
w Pachymetopon grande (muscle)	1975	0.32		0.40	0.60	12	0.0111			4.9	11.4								Oliff and Turner, 1976
w Pachymetopon grande (liver)	1975	21.62		0.12	18.02	808	0.0511			1.9	879.4								Oliff and Turner, 1976
w Upogebia africana	1975	0.23		0.33	15.90	208	0.0096			3.9	8.48								Oliff and Turner, 1976
w Crassostrea margaritacea	1975	0.76		0.22	9.55	38	0.0068			5.2	688.5								Oliff and Turner, 1976
w Bullia	1975	4.04		1.78	3.56	80	0.0079			17.1	23.8								Oliff and Turner, 1976
w Plankton (river)	1975	0.14		0.12	3.63	58	0.0060			1.8	10.0								Oliff and Turner, 1976
w Penaeus monodon	1975	0.37		0.22	25.46	95	0.0097			6.1	16.3								Oliff and Turner, 1976
w Perna perna	1975	0.39		0.12	1.38	54	0.0060			2.1	10.2								Oliff and Turner, 1976

TABLE IV (continued)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Cs	Sb	Al	Bi	Ag	Sr	Mo	Reference
Algoa Bay																			
w Crassostrea margaritacea	1977	0.21	0.02	0.23	7.7	39		1.18	0.06	0.15	574								Watling, 1978
w Patella longicosta		2.71	0.19	1.06	1.09	212.09		1.57	0.39	0.24	14.89								Watling and Watling, 1981a
w Perna perna		0.23	0.15	0.83	1.40	75.52		2.24	1.51	0.46	22.29								Watling and Watling, 1981a
w Crassostrea margaritacea		0.59	0.06	1.37	5.29	34.65		1.59	0.17	0.39	669.03								Watling and Watling, 1981a
w Patella oculus		1.13	0.011	1.03	0.87	463.95		1.70	0.47	0.24	13.57								Watling and Watling, 1981a
w Bullia rhodostoma		3.40	0.10	2.07	1.73	47.44		1.53	0.15	0.13	46.38								Watling and Watling, 1981a
w Crassostrea margaritacea	78/79	0.24	0.06	2.5	7.9	50		1.6	0.16	0.69	1 054								Watling and Watling, 1983
w Crassostrea margaritacea	78/79	0.99	0.07	0.04	2.7	21		1.7	0.17	0.13	249								Watling and Watling, 1983
w Perna perna	78/79	0.23	0.12	0.8	1.4	88		2.4	1.52	0.62	24.6								Watling and Watling, 1983
w Perna perna	78/79	0.22	0.21	0.9	1.3	107		2.2	1.36	0.20	17.8								Watling and Watling, 1983
w Patella granularis	78/79	3.1	0.07	3.3	2.0	403		2.4	1.15	0.23	13.9								Watling and Watling, 1983
w Patella oculus	78/79	1.2	0.13	1.4	1.2	581		2.2	0.64	0.35	15.9								Watling and Watling, 1983
w Patella oculus	78/79	1.7	0.12	1.6	1.1	574		2.0	0.99	0.04	10.7								Watling and Watling, 1983
w Patella barbara	78/79	2.5	0.10	1.7	1.0	255		2.4	0.77	0.40	267								Watling and Watling, 1983
w Patella barbara	78/79	4.0	0.24	1.5	0.9	380		1.9	0.78	0.32	24.9								Watling and Watling, 1983
w Patella longicosta	78/79	2.6	0.18	1.1	1.1	215		1.6	0.40	0.27	15.0								Watling and Watling, 1983
w Patella longicosta	78/79	2.9	0.09	1.3	1.9	268		1.6	0.68	0.36	16.2								Watling and Watling, 1983
w Patella miniata	78/79	3.7	0.04	0.6	0.8	177		0.9	0.31	0.07	9.6								Watling and Watling, 1983
w Patella miniata	78/79	3.3	0.05	1.6	1.3	675		3.0	0.87	0.12	10.3								Watling and Watling, 1983
w Patella cochlear	78/79	6.3	0.10	0.7	0.9	99		0.6	0.75	0.08	10.2								Watling and Watling, 1983
w Patella tabularis	78/79	1.40	0.08	0.4	1.1	145		1.0	0.28	0.39	17.0								Watling and Watling, 1983
w Donax serra	78/79	0.05	0.03	0.6	0.9	81		1.5	0.28	0.03	10.9								Watling and Watling, 1983
w Bullia rhodostoma	78/79	4.2	0.13	0.8	2.0	50		1.5	0.19	0.10	36.9								Watling and Watling, 1983
w Bullia rhodostoma	78/79	3.1	0.10	2.3	1.7	48		1.5	0.16	0.12	46.3								Watling and Watling, 1983
St Croix																			
d Patella cochlear	1975	16.604		3.391	6.525	403.130	0.066			40.332	36								Oliff and Turner, 1976
d Perna perna	1975	3.323		4.106	11.167	422.691	0.150			26.346	60								Oliff and Turner, 1976
w Perna perna	1975	0.50		0.62	1.68	63				3.95	9								Watling, 1978
d Oplegnathus conwayi (muscle)	1975	0.500		2.357	2.621	18.408	0.199			26.412	22								Oliff and Turner, 1976
d Oplegnathus conwayi (liver)	1975	13.650		5.868	26.555	737.211	1.034			5.648	116								Oliff and Turner, 1976
Swartkops Estuary																			
d Rhabdosargus holubi	1975	0.782		1.789	2.926	20.972	0.310			18.104	36								Oliff and Turner, 1976
d Callianassa kraussi	1975	3.468		6.526	70.128	792.228	0.571			53.514	69								Oliff and Turner, 1976
w Callianassa kraussi	1975	0.75			69.4	225.5	0.042			10.93	25.08								Oliff and Turner, 1976
w Scylla serrata	1975	0.26			28.6	6.21	0.115			3.42	40.96								Oliff and Turner, 1976

TABLE IV (continued)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Cs	Sb	Al	Bi	Ag	Sr	Mo	Reference
w Upogebia africana	1975	0.56			44.5	163.6	0.049			8.59	19.18								Oliff and Turner, 1976
w Crassostrea margaritacea	1975	0.26			79.3	27.0	0.042			3.01	333.1								Oliff and Turner, 1976
w Striliza canaliculatus	1975	0.09			0.42	4.07	0.042			1.58	6.28								Oliff and Turner, 1976
w Mugil cephalus	1975	0.06			0.28	3.89	0.017			1.32	4.43								Oliff and Turner, 1976
w Argyrosomus hololepidotus (muscle)	1975	0.04			0.15	1.05	0.152			1.02	5.04								Oliff and Turner, 1976
w Argyrosomus hololepidotus (liver)	1975	0.09			3.44	151.3	0.156			4.49	22.52								Oliff and Turner, 1976
w Hypacanthus amia	1975	0.16			0.60	2.75	0.114			1.18	7.26								Oliff and Turner, 1976
w Pomadasys commersonni (muscle)	1975	0.14			0.33	3.20	0.190			2.04	6.91								Oliff and Turner, 1976
w Pomadasys commersonni (liver)	1975	0.23			42.9	209.0	0.410			4.08	32.16								Oliff and Turner, 1976
w Rhabdosargus holubi	1975	0.13			0.35	3.36	0.146			1.93	7.84								Oliff and Turner, 1976
d Sardinops ocellata (muscle)	1979	0.405	0.2	6.958	5.560	69.160	0.018	2.414	1.539	0.2	22.320								Van der Byl, 1980
d Sardinops ocellata (liver)	1979	0.712	0.2	0.314	10.916	1 110.000	0.013	5.148	0.873	0.2	74.200								Van der Byl, 1980
d Sardinops ocellata (ovaries)	1979	0.807	0.2	6.468	4.236	117.540	0.158	6.300	0.322	0.2	224.400								Van der Byl, 1980
d Sardinops ocellata (testes	1979	0.426	0.2	2.118	4.984	111.740	ND	2.204	0.556	0.2	52.420								Van der Byl, 1980
Port Elizabeth																			
d Bullia rhodostoma	1975	12.191		9.923	33.297	257.602	0.237			86.191	198								Oliff and Turner, 1976
d Perna perna	1975	1.761		4.968	10.614	471.846	0.170			27.620	80								Oliff and Turner, 1976
d Choromytilus meridionalis	1975	3.448		4.046	18.470	376.477	10.282			26.919	102								Oliff and Turner, 1976
d Burnupena cincta	1975	16.454		8.500	102.136	236.503	0.680			19.510	1 223								Oliff and Turner, 1976
Maitland																			
w Donax serra	1975	0.04			1.6	72		1.93		0.03	28								Watling, 1978
St Francis Bay																			
w Crassostrea margaritacea	78/79	1.57	0.11	0.8	4.4	39		1.0	0.10	0.04	114								Watling and Watling, 1983
w Perna perna	78/79	0.58	0.17	0.5	1.2	94		1.5	2.02	0.10	19.0								Watling and Watling, 1983
w Patella granularis	78/79	8.9	0.06	1.6	1.3	518		1.4	1.14	0.09	12.0								Watling and Watling, 1983
w Patella oculus	78/79	3.8	0.12	0.8	0.7	466		1.0	0.51	0.06	11.2								Watling and Watling, 1983
w Patella barbara	78/79	5.9	0.10	1.4	0.8	208		1.1	0.79	0.11	14.4								Watling and Watling, 1983
w Patella longicosta	78/79	14.3	0.08	1.2	0.8	149		0.9	0.44	0.05	13.7								Watling and Watling, 1983
w Patella miniata	78/79	6.3	0.04	1.2	1.0	286		1.5	0.70	0.05	10.3								Watling and Watling, 1983
w Patella argenvillei	78/79	10.5	0.05	0.5	0.7	85		0.7	0.53	0.01	10.1								Watling and Watling, 1983
w Patella cochlear	78/79	9.6	0.06	1.6	1.4	86		0.6	0.64	0.04	9.7								Watling and Watling, 1983
w Patella tabularis	78/79	9.9	0.04	0.7	0.8	40		0.6	0.17	0.03	8.9								Watling and Watling, 1983
w Donax serra	78/79	0.04	0.07	0.8	1.0	231		2.2	0.33	0.06	13.2								Watling and Watling, 1983
w Bullia rhodostoma	78/79	5.9	0.08	1.8	1.7	39		1.6	0.18	0.10	42.4								Watling and Watling, 1983

TABLE IV (continued)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Cs	Sb	Al	Bi	Ag	Sr	Mo	Reference
Keurboom																			
w Donax serra	1975	0.14	0.04	0.16	1.18	84		1.15	0.43	0.03	15.6								Watling, 1978
w Solen capensis	1975	0.27	0.22	0.21	0.52	56		1.01	0.26	0.36	8.7								Watling, 1978
Cathedral Rock																			
w Perna perna	1975	0.37	0.04	0.46	0.70	37		0.56	0.66	0.11	7.8								Watling, 1978
w Crassostrea margaritacea	1975	2.28	0.03	1.02	4.7	36		1.30	0.79	0.37	50								Watling, 1978
Noetzie																			
w Perna perna	1975	1.07	0.24	0.16	0.84	41		0.70	1.37	0.40	12.0								Watling, 1978
w Crassostrea margaritacea	1975	2.49	0.31	0.40	4.1	23		1.20	0.54	0.69	156								Watling, 1978
Knysna East Head																			
w Patella oculus	1978	3.8	0.03	0.83	0.61	177		0.71	0.23	0.05	8.8								Watling and Watling, 1980
w Patella longicosta	1978	11.1	0.06	1.01	0.61	89		0.83	0.25	0.09	12.1								Watling and Watling, 1980
w Patella miniata	1978	5.1	0.02	1.04	0.72	230		1.21	0.30	0.04	9.0								Watling and Watling, 1980
w Patella argenvillei	1978	5.8	0.04	0.56	0.70	68		0.84	0.33	0.02	10.3								Watling and Watling, 1980
w Patella cochlear	1978	7.2	0.07	1.40	0.75	106		1.05	0.41	0.05	10.0								Watling and Watling, 1980
w Patella barbara	1978	4.2	0.06	1.26	0.94	247		1.88	0.44	0.31	13.1								Watling and Watling, 1980
w Perna perna	1975	0.61	0.17	0.35	1.4	105		1.01	1.41	0.08	18.4								Watling, 1978
w Perna perna	1978	0.55	0.07	0.76	0.94	64		0.73	1.21	0.04	12.8								Watling and Watling, 1980
Beacon Point																			
w Perna perna	1975	0.30	0.02	0.32	0.43	36		0.40	0.30	0.15	6.8								Watling, 1978
w Crassostrea margaritacea	1975	1.34	0.06	2.96	2.5	47		0.74	1.45	0.44	332								Watling, 1978
w Patella granularis	1978	3.3	0.05	0.39	1.1	377		1.3	0.74	0.14	11.9								Watling and Watling, 1980
w Patella barbara	1978	3.7	0.13	0.59	0.82	258		1.10	0.68	0.19	17.9								Watling and Watling, 1980
w Patella oculus	1978	2.39	0.09	0.62	0.71	379		1.07	0.56	0.15	10.8								Watling and Watling, 1980
w Patella longicosta	1978	7.09	0.09	0.74	0.65	121		1.07	0.34	0.15	11.2								Watling and Watling, 1980
w Patella miniata	1978	4.02	0.06	0.69	0.93	334		8.50	0.38	0.12	9.9								Watling and Watling, 1980
w Patella cochlear	1978	3.9	0.09	0.71	0.88	123		1.66	1.01	0.09	7.1								Watling and Watling, 1980
w Perna perna	1976	0.30	0.02	0.32	0.43	36		0.40	0.30	0.15	6.8								Watling and Watling, 1980
w Perna perna	1978	0.99	0.12	0.63	1.20	66		1.05	1.51	0.09	15.5								Watling and Watling, 1980
Knysna West Head																			
w Perna perna	1975	1.00	0.08	1.25	1.09	87		0.87	1.17	0.11	11.3								Watling and Watling, 1980
w Perna perna	1978	0.70	0.10	0.74	0.90	77		0.80	1.13	0.05	11.6								Watling and Watling, 1980
w Patella cochlear	1978	7.5	0.06	1.29	0.52	91		0.56	0.28	0.03	8.1								Watling and Watling, 1980
w Patella argenvillei	1978	10.8	0.12	0.28	0.95	86		1.53	0.35	0.04	10.5								Watling and Watling, 1980

TABLE IV (continued)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Cs	Sb	Al	Bi	Ag	Sr	Mo	Reference
w Patella miniata	1978	9.80	0.03	0.82	0.61	87		0.60	0.26	0.04	7.9								Watling and Watling, 1980
w Patella longicosta	1978	11.9	0.08	1.07	0.50	53		0.57	0.15	0.06	10.7								Watling and Watling, 1980
w Patella oculus	1978	4.5	0.10	0.73	0.47	143		0.80	0.18	0.07	8.6								Watling and Watling, 1980
Featherbed																			
w Crassostrea margaritacea	1975	1.73	0.32	0.34	1.1	22		0.97	0.44	0.82	95								Watling, 1978
w Perna perna	1976	0.52	0.08	0.69	1.15	99		0.78	0.98	0.13	13.1								Watling and Watling, 1980
Bellvedere																			
w Crassostrea gigas	1975	0.56	0.15	0.11	5.7	20		2.1	0.26	0.21	69								Watling, 1978
w Crassostra margaritacea	1975	0.49	0.22	0.40	3.6	11		0.46	0.38	0.33	197								Watling, 1978
w Ostrea atherstonei	1975	0.75			14.2	81		0.70		0.31	692								Watling, 1978
w Crassostrea cucullata	1978	0.67	0.06	1.18	15	46		0.63	0.33	0.10	105								Watling and Watling, 1980
w Crassostrea cucullata	1978	0.61	0.04	0.16	13	21		0.40	0.05	0.04	123								Watling and Watling, 1980
w Crassostrea margaritacea	1977	1.09	0.02	0.33	2.6	13		0.6	0.04	0.07	121								Watling and Watling, 1980
Leisure Island																			
w Perna perna	1975	0.93	0.19	1.4	1.57	93		0.91	3.2	0.17	138								Watling, 1978
w Venus verrucosa	1975	0.55	0.56	0.87	1.15	52		0.71	1.08	0.63	7.6								Watling, 1978
w Atrina squamifera	1975	2.6	0.38	0.20	0.74	45		0.54	0.59	0.13	70								Watling, 1978
w Atrina squamifera	1978	3.8	0.22	0.97	1.48	131		0.85	0.66	0.12	206								Watling and Watling, 1980
w Venus verrucosa	1975	0.47	0.21	0.23	1.13	56		0.66	0.74	0.19	7.5								Watling and Watling, 1980
w Venus verrucosa	1976	0.79			2.80	63		1.13		0.29	11.0								Watling and Watling, 1980
w Venus verrucosa	1978	0.79	0.34	0.84	1.81	123		0.98	0.66	0.20	8.9								Watling and Watling, 1980
w Mactra glabrata	1975	1.27			1.07	111		1.10		0.34	12.0								Watling and Watling, 1980
Knysna																			
w Crassostrea gigas	1972	1.3			4.4						69								Darracott and Watling, 1975
w Crassostrea margaritacea	1972	1.3			3.6														Darracott and Watling, 1975
d Ostrea edulis	1975	3.1			38	167		6	1.7	66.0	1.7								Watling and Watling, 1976a
d Crassostrea gigas	1975	3.7			32	138		16	1.6	396	1.9								Watling and Watling, 1976a
d Crassostrea margaritacea	1975	2.5			17	57		2	1.6	886	2.6								Watling and Watling, 1976a
Thesens Point																			
w Solen capensis	1975	0.58			1.09	105		1.21		0.62	16.3								Watling and Watling, 1980
w Solen capensis	1978	0.46	0.09	0.84	0.80	260		1.50	0.14	0.10	10.10								Watling and Watling, 1980
w Perna perna	1975	0.12	0.04	0.11	0.38	39		0.28	0.40	0.06	5.0								Watling, 1978

TABLE IV (continued)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Cs	Sb	Al	Bi	Ag	Sr	Mo	Reference
Castle Rock																			
w *Perna perna*	1975	0.86	0.32	0.37	1.02	44		0.97	1.98	0.50	15.6								Watling, 1978
w *Crassostrea margaritacea*	1975	2.39	0.24	0.42	3.8	15		1.35	0.46	0.85	107								Watling, 1978
Buffalo Bay																			
w *Donax serra*	1975	0.12			1.29	79		1.39		0.76	19.3								Watling, 1978
Walker Point East																			
w *Crassostrea margaritacea*	1975	1.61	0.22	0.38	3.1	11		1.50	0.34	0.73	60								Watling, 1978
Walker Point West																			
w *Perna perna*	1975	0.54	0.13	0.70	1.9	107		1.43	1.45	0.25	21.4								Watling, 1978
w *Crassostrea margaritacea*	1975	1.30	0.04	0.68	2.9	50		0.79	0.62	0.32	249								Watling, 1978
Herolds Bay																			
w *Perna perna*	197?	0.53	0.08	0.29	1.29	61		1.16	0.96	0.08	12.7								Watling and Watling, 1981b
w *Patella oculus*	197?	2.8	0.24	0.48	0.82	907		6.38	0.51	0.12	8.6								Watling and Watling, 1981b
w *Patella longicosta*	197?	13.0	0.13	0.23	0.78	143		1.37	0.30	0.09	14.2								Watling and Watling, 1981b
w *Patella barbara*	197?	4.5	0.12	0.37	0.83	353		2.03	0.38	0.22	8.8								Watling and Watling, 1981b
w *Bullia rhodostoma*	197?	4.1	0.06	2.5	1.42	63		1.31	0.17	0.11	26.5								Watling and Watling, 1981b
Glentana																			
w *Perna perna*	197?	0.95	0.13	0.33	1.27	49		1.08	1.18	0.07	16.4								Watling and Watling, 1981b
w *Crassostrea margaritacea*	197?	1.64	0.01	0.23	3.68	17		1.12	0.08	0.02	143								Watling and Watling, 1981b
w *Patella oculus*	197?	2.6	0.04	2.95	0.72	490		1.72	0.82	0.86	11								Watling and Watling, 1981b
w *Patella longicosta*	197?	10.3	0.08	5.00	1.86	121		1.43	1.10	0.17	8.8								Watling and Watling, 1981b
w *Patella barbara*	197?	3.6	0.07	0.47	0.67	320		1.79	0.30	0.14	8.4								Watling and Watling, 1981b
w *Donax serra*	197?	0.14	0.04	0.26	1.18	84		1.15	0.43	0.03	15.6								Watling and Watling, 1981b
w *Bullia rhodostoma*	197?	8.5	0.11	3.7	2.23	80		1.78	0.19	0.26	46.6								Watling and Watling, 1981b
Tergniet																			
w *Perna perna*	197?	0.46	0.06	0.86	1.20	65		1.43	0.67	0.10	13.7								Watling and Watling, 1981b
w *Patella barbara*	197?	3.8	0.13	0.96	0.83	544		2.17	0.39	0.12	9.4								Watling and Watling, 1981b
w *Patella longicosta*	197?	5.9	0.16	0.98	0.65	391		1.39	0.48	0.06	11.6								Watling and Watling, 1981b
w *Bullia rhodostoma*	197?	3.7	0.08	2.80	1.41	49		1.33	0.19	0.06	31.3								Watling and Watling, 1981b
Little Brak River																			
w *Perna perna*	197?	0.42	0.07	1.76	1.14	110		1.70	0.85	0.15	14.1								Watling and Watling, 1981b
w *Patella longicosta*	197?	4.70	0.16	1.15	1.02	347		1.24	0.42	0.11	10.17								Watling and Watling, 1981b

TABLE IV (continued)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Cs	Sb	Al	Bi	Ag	Sr	Mo	Reference
Hartenbos																			
w Perna perna	197?	0.92	0.07	0.24	1.15	83		1.65	0.66	0.10	14.7								Watling and Watling, 1981b
w Patella longicosta	197?	2.40	0.13	0.36	0.74	337		1.41	0.50	0.04	14.9								Watling and Watling, 1981b
w Bullia rhodostoma	197?	3.60	0.11	1.6	1.28	72		1.56	0.22	0.12	50.0								Watling and Watling, 1981b
Diaz Beach																			
w Bullia rhodostoma	197?	2.4	0.08	1.9	0.90	24		1.24	0.13	0.11	26.0								Watling and Watling, 1981b
w Donxa serra	197?	0.12	0.09	0.82	1.20	79		1.39	0.23	0.06	19.3								Watling and Watling, 1981b
Die Bakke																			
w Perna perna	197?	0.37	0.05	0.20	1.08	63		1.03	0.58	0.09	13.8								Watling and Watling, 1981b
w Patella longicosta	197?	4.84	0.06	0.17	0.66	96		1.03	0.21	0.08	17.5								Watling and Watling, 1981b
w Patella barbara	197?	3.9	0.13	0.34	1.00	217		1.38	0.47	0.21	18.4								Watling and Watling, 1981b
Mossel Bay																			
w Perna perna	197?	0.60	0.04	1.18	1.33	59		1.30	0.85	0.35	16.5								Watling and Watling, 1981b
w Perna perna	78/79	0.65	0.09	0.7	1.2	68		1.3	0.90	0.11	14.1								Watling and Watling, 1983
w Crassostrea margaritacea	78/79	1.72	0.01	0.3	5.4	18		0.9	0.05	0.01	178								Watling and Watling, 1983
w Patella granularis	78/79	7.6	0.04	2.2	1.2	427		2.1	0.97	0.42	13.6								Watling and Watling, 1983
w Patella oculus	197?	1.58	0.05	0.28	0.87	275		1.35	0.31	0.11	10.7								Watling and Watling, 1981b
w Patella oculus	78/79	2.8	0.11	1.0	0.8	517		2.8	0.53	0.27	9.9								Watling and Watling, 1983
w Patella barbara	197?	1.94	0.04	0.14	0.80	147		0.81	0.19	0.43	24.1								Watling and Watling, 1981b
w Patella barbara	78/79	3.9	0.17	0.5	0.8	380		2.3	0.42	0.29	13.4								Watling and Watling, 1983
w Patella longicosta	197?	0.55	0.05	0.36	1.16	174		1.24	0.30	0.31	13.5								Watling and Watling, 1981b
w Patella longicosta	78/79	9.0	0.12	1.1	1.0	213		1.3	0.46	0.13	12.6								Watling and Watling, 1983
w Patella miniata	78/79	6.6	0.10	1.0	0.7	440		2.0	0.36	0.11	7.8								Watling and Watling, 1983
w Patella argenvillei	78/79	5.7	0.16	0.6	0.8	121		1.1	0.42	0.07	14.6								Watling and Watling, 1983
w Patella cochlear	78/79	5.5	0.09	0.7	0.9	197		1.5	0.50	0.09	10.6								Watling and Watling, 1983
w Patella tabularis	78/79	1.8	0.02	0.1	0.7	107		0.5	0.11	0.27	11.5								Watling and Watling, 1983
w Donax serra	78/79	0.11	0.11	0.6	1.1	81		1.3	0.38	0.14	16.0								Watling and Watling, 1983
w Bullia rhodostoma	78/79	4.5	0.10	2.7	1.5	66		1.5	0.18	0.14	35.5								Watling and Watling, 1983
Dana Township																			
w Donax serra	197?	0.07	0.19	0.65	0.82	81		1.33	0.47	0.34	13.0								Watling and Watling, 1981b
w Bullia rhodostoma	197?	5.1	0.09	2.4	1.89	93		1.66	0.20	0.13	36.3								Watling and Watling, 1981b
Cape St Blaize																			
w Perna perna	197?	0.50	0.09	0.56	0.88	79		1.08	0.85	0.06	12.1								Watling and Watling, 1981b
w Patella oculus	197?	2.74	0.08	1.06	0.73	443		1.87	0.56	0.07	8.8								Watling and Watling, 1981b
w Patella longicosta	197?	16.2	0.10	0.86	0.58	71		0.97	0.37	0.08	11.3								Watling and Watling, 1981b
w Patella barbara	197?	2.2	0.03	0.13	0.82	130		0.49	0.16	0.38	17.4								Watling and Watling, 1981b

TABLE IV (continued)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Cs	Sb	Al	Bi	Ag	Sr	Mo	Reference
Pinnacle Point																			
w *Perna perna*	197?	1.05	0.08	0.87	0.85	58		1.04	1.05	0.06	12.7								Watling and Watling, 1981b
w *Crassostrea margaritacea*	197?	2.11	0.01	0.40	8.50	13		0.66	0.03	0.01	190								Watling and Watling, 1981b
w *Patella oculus*	197?	4.1	0.12	0.29	0.63	473		2.58	0.43	0.18	10.5								Watling and Watling, 1981b
w *Patella longicosta*	197?	16.2	0.14	0.82	0.89	189		1.25	0.38	0.14	11.2								Watling and Watling, 1981b
w *Patella barbara*	197?	5.1	0.31	0.61	0.88	703		5.10	0.76	0.33	8.3								Watling and Watling, 1981b
Fish Bay																			
w *Perna perna*	1975	1.02	0.49	0.85	1.12	52		1.20	2.08	0.64	16.7								Watling, 1978
w *Crassostrea margaritacea*	1975	1.93	0.36	1.39	3.5	28		1.56	1.39	0.92	107								Watling, 1978
w *Donax serra*	1975	0.07	0.19	0.65	0.82	81		1.33	0.47	0.34	13.0								Watling, 1978
Vlees Point																			
w *Perna perna*	197?	0.74	0.20	0.25	1.29	45		1.57	1.38	0.06	14.5								Watling and Watling, 1981b
w *Crassostrea margaritacea*	197?	1.41	0.01	0.18	4.14	25		0.89	0.03	0.01	202								Watling and Watling, 1981b
w *Patella longicosta*	197?	10.7	0.22	0.54	1.39	258		1.42	0.49	0.18	12.2								Watling and Watling, 1981b
w *Patella barbara*	197?	5.8	0.50	0.77	0.90	633		4.80	0.72	0.49	12.0								Watling and Watling, 1981b
w *Bullia rhodostoma*	197?	3.9	0.14	3.9	1.36	84		1.88	0.18	0.17	32.1								Watling and Watling, 1981b
Strandfontein																			
d *Bullia digitalis*	1981																		See diagrams
Muizenberg																			
d *Bullia digitalis*	1981																		See diagrams
d *Patella granularis*	1981	10.6			5.5	510.4		ND	ND	-	17.2						31.3		This study
Cape																			
d Penguin bones	1974	1.2-1.9								3.0-12.1									Orren, 1975
d Gannet bones	1974	0.2-0.9								1.0-1.7									Orren, 1975
d Cormorant bones	1974	1.0-6.0								0.7-1.7									Orren, 1975
Blouberg Strand																			
d *Choromytilus meridionalis*	70/73		2	2		82			8			73							Van As *et al.*, 1973
d *Donax serra*	70/73		0.2	0.7		181			3			62							Van As *et al.*, 1973
w *Choromytilus meridionalis*	1977	0.31	0.07	0.09	1.09	13		1.60	0.13	0.08	12								Watling, 1978
d *Choromytilus meridionalis* (female)	1979	0.28			5.84	21.4		8.5	0.85	0.82	75.3								Orren *et al.*, 1980
d *Choromytilus meridionalis* (male)	1979	0.32			3.21	12.8		3.87	0.71	0.95	38.7								Orren *et al.*, 1980
d *Choromytilus meridionalis* (female)	1979	1.11			7.17	51.0		9.63	1.16	2.57	90.8								Orren *et al.*, 1980
d *Choromytilus meridionalis* (male)	1979	0.94			6.29	69.9		12.1	0.95	2.87	75.7								Orren *et al.*, 1980
d *Choromytilus meridionalis*	1980	*																	See diagrams

TABLE IV (continued)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Cs	Sb	Al	Bi	Ag	Sr	Mo	Reference
w Plankton (mixed)	1971		0.089	1.726		578.5		1.47			113.2	0.003	0.664						Van As et al., 1973
w Plankton (mixed)	1972		0.004	0.07		9.26		0.18			9.3	0.001	0.027						Van As et al., 1973
w Plankton (mixed)	1973		0.076	0.43		94.4		0.80			26	0.010	ND						Van As et al., 1973
w Choromytilus meridionalis	70/73		0.038	0.10		20		2.70			16	0.007	0.20						Van As et al., 1973
w Donax serra	70/73		0.038	0.24		59		1.00			18	0.007	0.18						Van As et al., 1973
w Haliotis midae	70/73		0.025	0.50		18		0.17			12	0.001	0.08						Van As et al., 1973
w Jasus lalandii	70/73		0.004	0.08		2.7		0.27			17	0.001	0.12						Van As et al., 1973
w Parechinus	70/73		0.006	0.1		11		ND			9.4	ND	ND						Van As et al., 1973
w Pyura	70/73		0.075	ND		230		ND			12	ND	ND						Van As et al., 1973
w Seriola pappe	70/73		0.008	1.0		14		0.29			6.7	0.040	0.057						Van As et al., 1973
w Argyrozona argyrozona	70/73		0.003	0.1		3.2		0.44			3.2	0.024	0.029						Van As et al., 1973
w Johnius hololepidotus	70/73		0.002	0.72		7.2		0.14			3.8	0.031	0.018						Van As et al., 1973
w Pachymetopon grande	70/73		0.002	3.0		5.2		0.23			3.5	0.018	0.19						Van As et al., 1973
w Atractoscion pequidens	70/73		0.004	ND		7.7		0.26			5.7	0.025	0.059						Van As et al., 1973
w Chrysoblephus gibbiceps	70/73		ND	0.1		5.8		0.16			4.6	0.015	0.002						Van As et al., 1973
w Lithognathus lithognathus	70/73		0.003	0.11		5.0		0.21			4.9	0.044	0.009						Van As et al., 1973
w Merluccius capensis	70/73		0.004	0.26		4.9		0.22			3.7	0.038	0.075						Van As et al., 1973
w Xiphiurus capensis	70/73		0.003	0.92		4.4		0.21			4.8	0.028	0.037						Van As et al., 1973
w Synaptura marginata	70/73		0.003	1.3		4.5		0.17			4.0	0.006	0.14						Van As et al., 1973
w Trachurus trachurus	70/73		0.008	0.14		10		0.11			4.4	0.022	0.028						Van As et al., 1973
w Sardinops ocellata	70/73		0.033	ND		22		0.91			11	0.013	0.26						Van As et al., 1973
w Scomber japanicus	70/73		0.020	0.65		17		0.28			7.2	0.037	0.19						Van As et al., 1973
w Mugil richardsoni	70/73		ND	0.1		6.7		0.19			4.1	0.007	0.024						Van As et al., 1973
w Scombrops dubius	70/73		0.003	ND		3.5		ND			3.6	0.025	0.055						Van As et al., 1973
w Lophius piscatorius	70/73		0.006	0.1		3.0		0.12			3.7	0.014	0.066						Van As et al., 1973
w Trigla capensis	70/73		0.011	0.1		4.5		0.11			3.7	0.032	0.020						Van As et al., 1973
w Gigartina radula	70/73		0.018	0.23		40		2.0			5.6	0.023	0.24						Van As et al., 1973
w Suhria vittata	70/73		0.048	0.87		49		3.70			9.4	0.007	0.23						Van As et al., 1973
w Porphyra capensis	70/73		0.054	0.47		37		6.1			11	0.006	0.21						Van As et al., 1973
w Ulva spp.	70/73		0.038	0.45		39		4.1			5.6	0.011	0.16						Van As et al., 1973
w Ecklonia maxima	70/73		0.0064	0.13		3.3		0.25			1.2	0.010	0.082						Van As et al., 1973
Melkbos Strand						Same as Blouberg in Van As et al., 1973													Fourie, 1976
Koeberg																			
d Bullia digitalis (whole)	1975	164.9																	Cuthbert et al., 1976
d Bullia digitalis (viseral)	1975	235.5																	Cuthbert et al., 1976

TABLE IV (continued)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Sb	Al	Bi	Ag	Sr	Mo	V	Reference
d Bullia digitalis	1981	*																	See diagram
d Patella granularis	1981	9.6			2.4	282.2		3.60	10.9	ND	66								This study
d Choromytilus meridionalis	1981	3.4			4.1	81.7		1.60	3.4	3.6	124.4								This study
d Donax serra	1981	ND			0.7	18.3		0.1	0.9	ND	14.1								This study
d Semaeostomeae	1981	ND			3.0	14.9		0.05	4.8	ND	0.4								This study
Langebaan																			
w Choromytilus meridionalis	1974	0.31		1.08	1.2	14.3		2.2	0.16	0.1	15	1.0	9.4	0.44					Fourie, 1976
w Crassostrea margaritacea	1974	0.88		0.50	1.9	4.7		0.55	0.20	0.42	120	1.8	ND	0.68					Fourie, 1976
w Crassostrea gigas	1974	0.87		0.54	3.7	17		1.3	0.25	0.34	21	1.4	ND	0.49					Fourie, 1976
w Haliotis midae	1974	0.14		0.6	3.6	24		0.21	1.20	0.29	9.3	1.3	4.5	0.87					Fourie, 1976
w Jasus lalandii	1974	0.12		0.02	3.9	1.1		0.34	0.19	0.55	16	3.0	1.0	0.82					Fourie, 1976
w Mactra glabrata	1974	0.19		0.54	0.22	41		0.34	ND	ND	7.9	ND	29	ND					Fourie, 1976
w Pyura stolonifera	1974	0.13		0.30	0.52	42		1.49	0.70	0.87	9.0	4.0	61	1.7					Fourie, 1976
w Ulva sp.	1974	0.11		0.095	1.40	30		0.94	0.49	0.62	2.0	1.75	39	0.74					Fourie, 1976
d Crassostrea margaritacea	1974	7			15	45		4	1		957				2				Watling and Watling, 1976a
d Crassostrea gigas	1974	9			29	248		14	2		399				2				Watling and Watling, 1976a
d Doris verrucosa	1982	65.5			50.1	816.9		13.3	2.5	ND	373.6					625.7			This study
d Iorunna tomentosa	1982	11.8			143.5	419.5		4.1	9.0	ND	163.7					127.4			This study
d Tethya aurantia	1982	1.8			14.6	215.9		7.7	2.8		52.9					17.7			This study
d Crassostrea margaritacea	1974	7.31	0.79	0.25	10.99	47.45	118	4.06	0.91	2.62	972.3		20.98	4.33	1.09		1.06	0.08	Watling and Watling, 1974
d Crassostrea gigas	1974	5.3	0.7	1.0	9.6	176		7	0.7	1.4	217		205	1.8				0.9	Watling and Watling, 1974
d Crassostrea gigas	1974	9	1		33			12	1	1	424			4					Watling and Watling, 1976
Saldanha																			
w Jasus lalandii	74/75	0.09		0.01	5.34	1.05		0.31	0.21	0.50	14.95	2.69	1.06	0.77					Fourie, 1975
w Mactra glabrata	74/75	0.19		0.54	0.22	41.01		0.34	ND	ND	7.86	ND	29.05	ND					Fourie, 1975
w Pyura stolonifera	74/75	0.16		0.24	0.29	36.65		1.93	0.66	0.77	10.19	3.93	53.47	1.65					Fourie, 1975
w Haliotis midae	74/75	0.17		0.50	2.76	19.16		0.23	1.18	0.27	9.55	1.56	3.59	0.89					Fourie, 1975
w Crassostrea margaritacea	74/75	0.88		ND	1.85	4.65		0.55	0.20	0.42	20.9	1.84	ND	0.68					Fourie, 1975
w Crassostrea gigas	74/75	0.87		0.54	3.65	17.26		1.26	0.25	0.34	21.13	1.42	ND	0.49					Fourie, 1975
w Choromytilus meridionalis	74/75	0.26		1.09	1.13	14.57		1.82	0.26	0.15	12.53	2.28	7.99	0.78					Fourie, 1975
w Ulva sp.	74/75	0.14		0.07	1.39	25.47		0.92	0.38	0.59	1.91	1.31	31.82	0.57					Fourie, 1975
w Jasus lalandii	1974	0.03		ND	3.3	0.89		0.24	0.26	0.40	14	2.2	1.2	0.69					Fourie, 1976
w Pyura stolonifera	1974	0.12		ND	0.21	5		0.65	0.59	0.40	14	3.8	25	1.4					Fourie, 1976
w Haliotis midae	1974	0.24		0.29	1.2	11		0.26	1.2	0.67	10	2.0	17	0.92					Fourie, 1976
w Choromytilus meridionalis	1974	0.34		0.75	0.68	6.9		1.7	0.30	0.04	9.6	1.9	4.5	0.58					Fourie, 1976
w Choromytilus meridionalis	1974	0.09		1.3	1.5	23		1.3	0.41	0.35	12	3.1	9.1	0.88					Fourie, 1976

TABLE IV (continued)

Location/Species	Date	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Cs	Sb	Al	Bi	Ag	Sr	Mo	V	Reference
w Ulva spp.	1974	0.18		ND	1.5	12		0.92	0.05	0.50	1.5		1.30	10	0.70					Fourie, 1976
d Choromytilus meridionalis	1974	3.72	2.40	0.99	9.75	133.19	0.069	10.41	2.16	3.55	95.66			73.88	5.39	0.17		4.05	0.43	Watling and Watling, 1974
d Mactra glabrata (whole)	1974	6.3	2.8	1.3	5.2	376	130	1.4	2.4	2	78			130	2.9	0.17		1.3	0.9	Watling and Watling, 1974
d Mactra glabrata (heart)	1974	4.2	24.2	6.11	18.2	1 091	-	6.1	8.8	12.1	76			1 727	9.1	1.52		12.1	-	Watling and Watling, 1974
d Mactra glabrata (gonad)	1974	9.7	6.7	10.3	34.8	809	-	3.4	7.2	4.5	74			315	6.7	0.11		7.9	1.0	Watling and Watling, 1974
d Mactra glabrata (gill)	1974	34.2	14.5	8.6	21.4	513	-	4.3	12.7	6.8	87			180	7.7	6.41		5.1	0.3	Watling and Watling, 1974
d Mactra glabrata (mantle)	1974	2.6	2.3	0.9	3.0	241	-	1.0	1.5	1.5	50			66	3.8	0.11		1.9	0.6	Watling and Watling, 1974
d Mactra glabrata (adductor)	1974	3.6	1.5	0.7	1.7	93	-	0.8	0.9	2.0	70			63	3.2	0.08		1.0	0.1	Watling and Watling, 1974
d Mactra glabrata (foot)	1974	3.4	1.4	0.3	1.4	50	-	0.6	0.7	1.4	60			6	3.1	0.08		0.9	0.1	Watling and Watling, 1974
d Mactra glabrata (remainder)	1974	3.5	4.6	2.7	3.2	671	-	3.2	2.6	3.2	53			954	6.0	0.56		1.9	1.5	Watling and Watling, 1974
d Donax serra	1974	0.5	1.7	0.9	3.5	236		3.4	1.2	1.9	95			84	3.8	0.05		3.7	0.3	Watling and Watling, 1974
d Haliotis midae (gonad)	1974	5.3	2	0.7	4	798		6	4	2	64			10	3.7	0.06		2.4	0.99	Watling and Watling, 1974
d Haliotis midae (kidney)	1974	17.00	2	1.1	6	375		4	3	2	76.2			114	4.2	0.24		4.6	3.19	Watling and Watling, 1974
d Haliotis midae (gill)	1974	5.0	5	1.5	93	350		6	16	9	100			63	7.9	0.33		10.4	0.71	Watling and Watling, 1974
d Haliotis midae (heart)	1974	6.7	5	2.4	15	470		4	9	4	66			300	9.7	0.37		6.9	0.37	Watling and Watling, 1974
d Haliotis midae (mantle)	1974	2.0	3	2.5	57	197		1	33	3	105			25	4.2	0.20		2.0	0.58	Watling and Watling, 1974
d Haliotis midae (white muscle)	1974	6.00	1	0.2	3	21		1	1	1	33			1	1.9	0.2		0.20	0.01	Watling and Watling, 1974
d Gracilaria verrucosa	1974	1.4	4	1.4	2	159	0.047	7	2	3	25			172	7.1	0.08		1.2	0.13	Watling and Watling, 1974
d Ulva sp.	1974	0.80	3	10.2	5	1 330	0.094	9	8	5	24			3 647	7.0	0.07		1.5	6.65	Watling and Watling, 1974
w Choromytilus meridionalis	1972	0.43																		Darracott, 1975
w Donax serra	1975	0.09	0.30	0.17	0.64	42		0.62	0.21	0.34	17									Watling, 1978
w Crassostrea gigas	1977	1.10	0.1	0.34	9.3	32		2.2	0.1	0.1	100									Watling, 1978
d Choromytilus meridionalis (male)	197?	0.9	1.0	1.4	5.9	54		6		2.1	54				4.0	0.3				Watling and Watling, 1976b
d Choromytilus meridionalis (female)	197?	0.9	0.9	1.4	7.7	66		12		1.8	97				3.4	0.3				Watling and Watling, 1976b
w Choromytilus meridionalis	1979	0.53		0.09	1.42	41.46		1.32	0.64	0.33	26.4									John Henry (pers. com.)
w Haliotis midae	1979	0.53		0.21	0.80	29.65		0.20	0.81	0.43	12.34									John Henry (pers. com.)
w Pyura stolonifera	1979	0.14		0.10	0.72	22.0		3.25	0.53	ND	21.3									John Henry (pers. com.)
w Mactra glabrata	1979	0.69		0.32	0.67	41.78		0.72	0.49	0.22	8.26									John Henry (pers. com.)
w Jasus lalandii	1979	0.09		0.19	7.34	2.49		0.30	0.26	0.34	13.05									John Henry (pers. com.)
Noordwesbaai	1982																			See diagram
Analytical chemists																				
Homo sapiens (blood)	1974	25	25	25	649	384	9.1	25	59	125	6.2			2.5		25	132.4		12.5	Butler and Watling, 1975
Homo sapiens (blood)		7.40	0.30	26	1 070	475.00	6.50	26	38	270	6.5			0.32		24	130.0		17.0	WHO
Homo sapiens (hair)							450-635	ngg^{-1}												Butler and Watling, 1975

4.1 Section I - Kosi Bay to Port Shepstone

There were 12 sampling points (Table 1) and most of the data are for Kosi Bay. The main trace elements determined were Cd, Co, Cr, Cu, Fe, Hg, Pb and Zn.

4.1.1 Kosi Bay (Figure 11)

These data were drawn from Oliff and Turner (1976). There were no details of analytical methods for the various tissues. The data were given as $\mu g\ g^{-1}$ of dry weight, and fish length but not sex, was given. The other animals were analysed as "whole" organisms. Nearly half of the 20 species were fish. In these the liver usually had a considerably higher metal content than the muscle tissue. No trends could be observed because of the wide variety of organisms, but in each case the plankton samples showed the highest metal concentrations (Figure 11).

The only other data were from Watling (1978), who quoted Oliff and Turner (1976) and assumed that Perna sp. was in fact Perna perna. Her data were derived from wet tissue data, assuming 85 per cent water content. The accuracy is presented as in the original report and the concentrations of eight metals were determined.

4.1.2 St Lucia (Figure 12)

Only one reference (Oliff and Turner, 1976) for St Lucia was found. Loligo sp. does not seem to be affected by the enriched water and sediment of St Lucia.

4.1.3 Richards Bay (Figure 13)

Four metals were determined (Oliff and Turner, 1976). Most of the samples taken at Richards Bay were fish. These had similar metal concentrations, the highest being in the liver. Since the

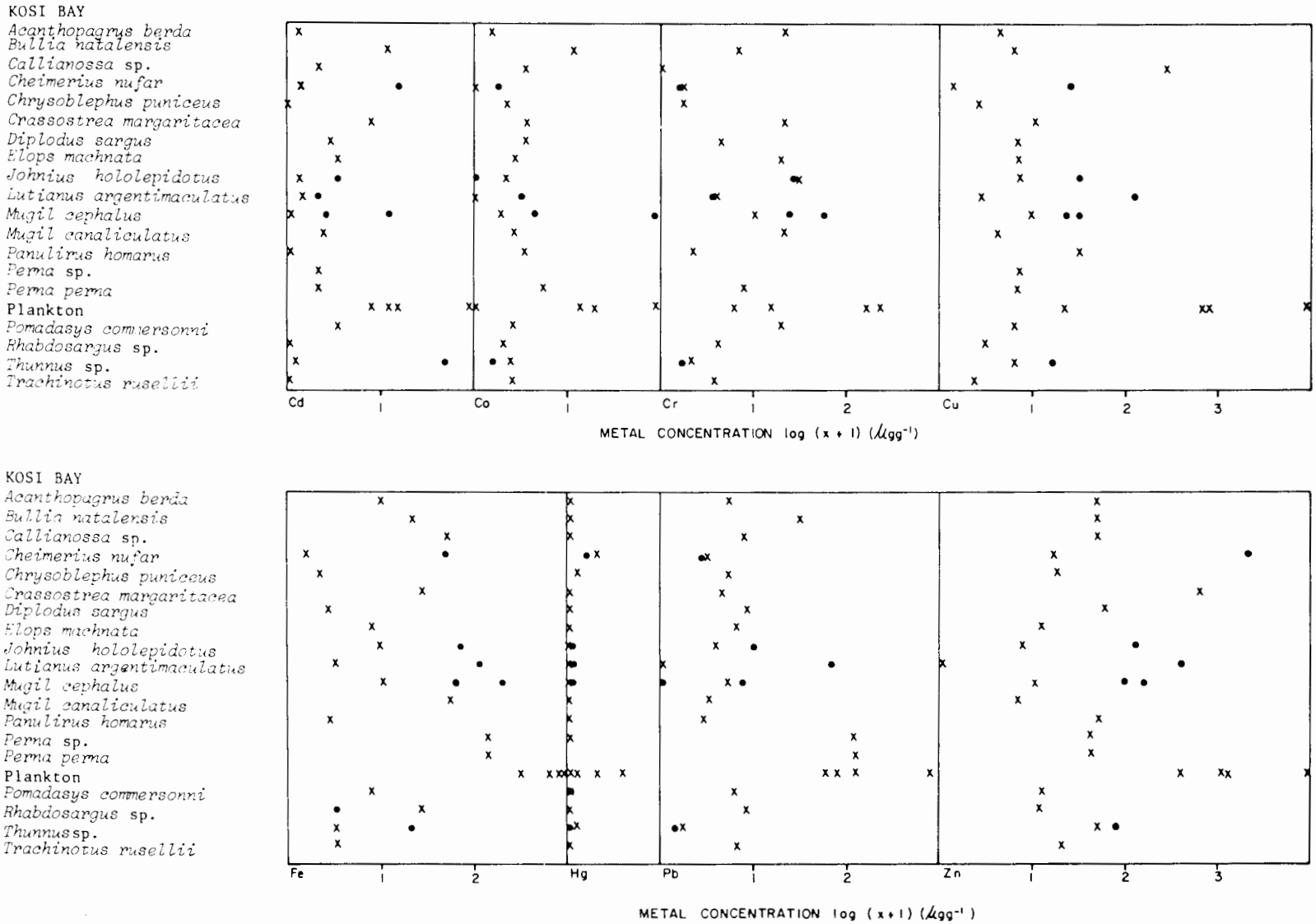


Figure 11 Metal concentrations in marine organisms from Kosi Bay (• = liver)

ST. LUCIA
Loligo

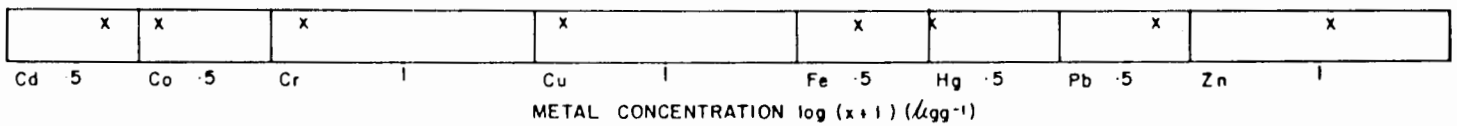


Figure 12 Metal concentrations in marine organisms from St Lucia

RICHARDS BAY
Argyrosomus hololepidotus
Elops machnata
Mugil cephalus
Otolithes ruber
Penaeus indicus
Pomadasys commersonni
Rhabdosargus holubi

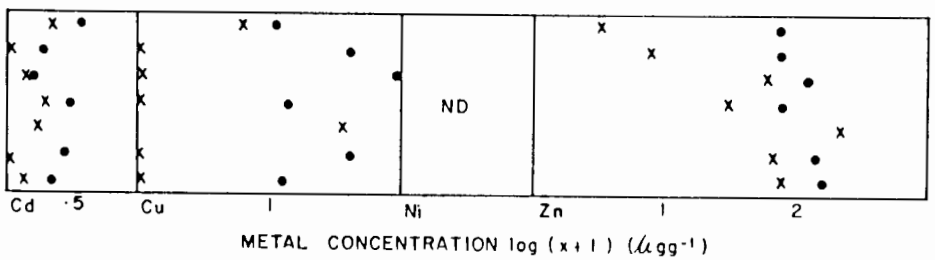


Figure 13 Metal concentrations in marine organisms from Richards Bay (• = liver)

PORT DURNFORD POINT
Acanthopagrus berda
Emerita austroafricana
Johnnius hololepidotus
Mugil sp.
Penaeus indicus
Pomadasys commersonni
Rhabdosargus holubi

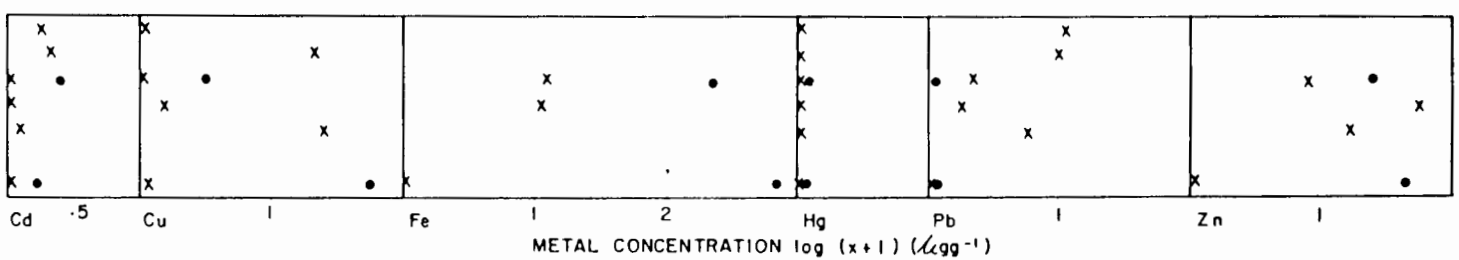


Figure 14 Metal concentrations in marine organisms from Port Durnford Point (• = liver)

data are mainly for fish they are not representative of the bio-availability of metals to the fauna at Richards Bay and no "baseline" can be established for Richards Bay. This is especially unfortunate since high metal concentrations in water and sediment were reported here.

4.1.4 Port Durnford Point (Figure 14)

These data were drawn from Connell et al. (1975). Methods of analysis were not given. The data are given as μg g^{-1} of wet weight and no mention is made of size or sex of the sample. Accuracy is as stated in the original report. In all cases, the livers had higher metal content than the muscle tissues.

4.1.5 Umgeni Estuary (Figure 15)

These data were drawn from Connell et al. (1975) and again methods were not given. Two fish species were analysed and both had lower metal concentrations than those from Richards Bay.

4.1.6 Umhlanga Rocks (Figure 16)

Darracott and Watling (1975) presented their data in terms of wet weight. No method, size or sex were quoted, the reference is "Watling, unpublished data". In Watling and Watling (1974) the reference is again "Watling, unpublished data" but more metals were presented and the results are given in terms of dry weight.

Finally Watling (1978) also quoted "Watling, unpublished data" and gave results in terms of wet weight. It is possible that all three references refer to the same 30 animals sampled at Umhlanga Rocks in June 1974.

Connell et al. (1975) measured only mercury and expressed the results in terms of wet weight. No details of method, sex, weight or size were given.

UMGENI
Mugil sp.
Tilapia sp.

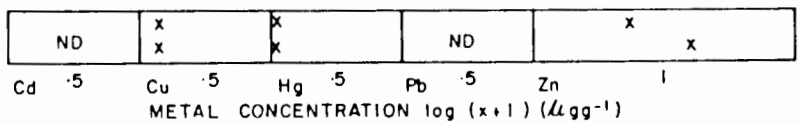
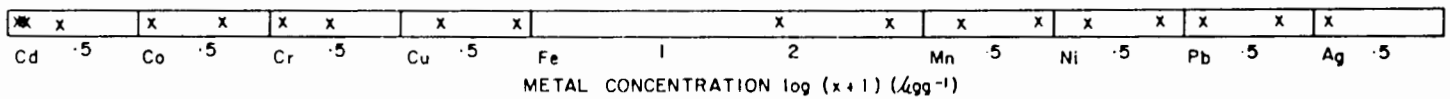


Figure 15 Metal concentrations in marine organisms from Umgeni Estuary

UMHLANGA ROCKS
Perna perna



UMHLANGA ROCKS
Perna perna

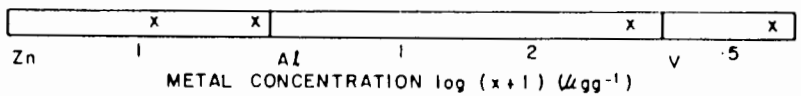


Figure 16 Metal concentrations in marine organisms from Umhlanga Rocks

The large variation in concentration range (Figure 16) arises from the different methods of reporting. Watling (1978) assumed a water content of 85 per cent in expressing her data in terms of dry weight. Although this is a very good average figure, data in this report have not been converted as no water content is known for some of the animals mentioned. It also would only have introduced errors into this study.

4.1.7 Durban Bay (Figure 17)

Only mercury levels were reported by Connell et al. (1975).

4.1.8 Umbogintwini (Figure 18)

Only mercury levels were reported by Connell et al. (1975).

4.1.9 Fynnlands (Figure 19)

It is unfortunate that only mercury levels have been recorded in these three areas (Figures 17, 18, 19) in view of the high water and sediment levels recorded. It is surprising that more data are not available for this major industrial and urban centre.

4.1.10 Umhlatuzana (Figure 20)

Connell et al. (1975) reported on three fish species. It is noticeable that copper and zinc levels vary greatly as seen in the two sets of data for Mugil sp.

4.1.11 Umzimkulu Estuary (Figure 21)

The values are reported by Connell et al. (1975).

4.1.12 Umgababa Estuary (Figure 22)

Oliff and Turner (1976) sampled the animals on 30 June 1976. No methods were reported, but sizes are given for P. homarus. Whole animals were used in the case of C. margaritacea and U. africana. The results were reported in terms of dry weight. Watling (1978) converted these data to wet tissue mass assuming 85 per cent water content.

4.1.13 Conclusion for Section I

Although high metal concentrations are reported for water and sediments, no baseline study has been done for this region. Most of the animals studied were fish, which are known to be poor metal pollution indicator species. It is possible that some of the metal concentrations referred to may originate from the same set of animals, which would reduce the metal concentrations coverage for this section even further.

4.2 Section II - Port Shepstone to East London

This section along the Transkei coast is very poorly represented. Only references to metal concentrations in the Bashee Estuary have been found.

4.2.1 Bashee Estuary (Figure 23)

Oliff and Turner (1976) reported the average sizes or masses of all their organisms. The results were expressed as wet weights. No description of the method for the determination of metal concentrations was given. Accuracy is given as reported in the original report. The fish were caught in the estuary and had low concentrations of metals. C. margaritacea could have been an old specimen (see high zinc levels).

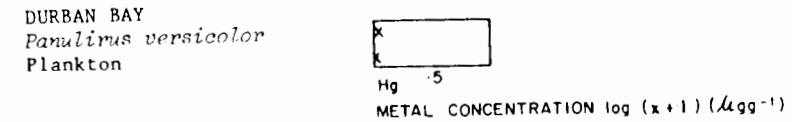


Figure 17 Metal concentrations in marine organisms from Durban Bay

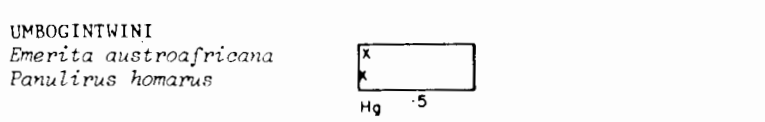


Figure 18 Metal concentrations in marine organisms from Umbogintwini

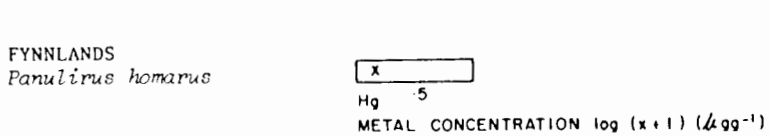


Figure 19 Metal concentrations in marine organisms from Fynnlands

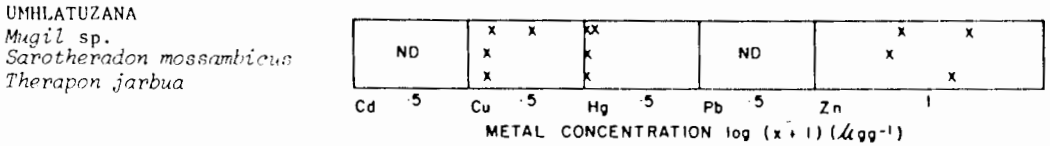


Figure 20 Metal concentrations in marine organisms from Umhlatuzana

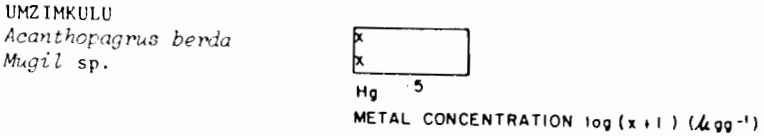


Figure 21 Metal concentrations in marine organisms from Umzimkulu Estuary

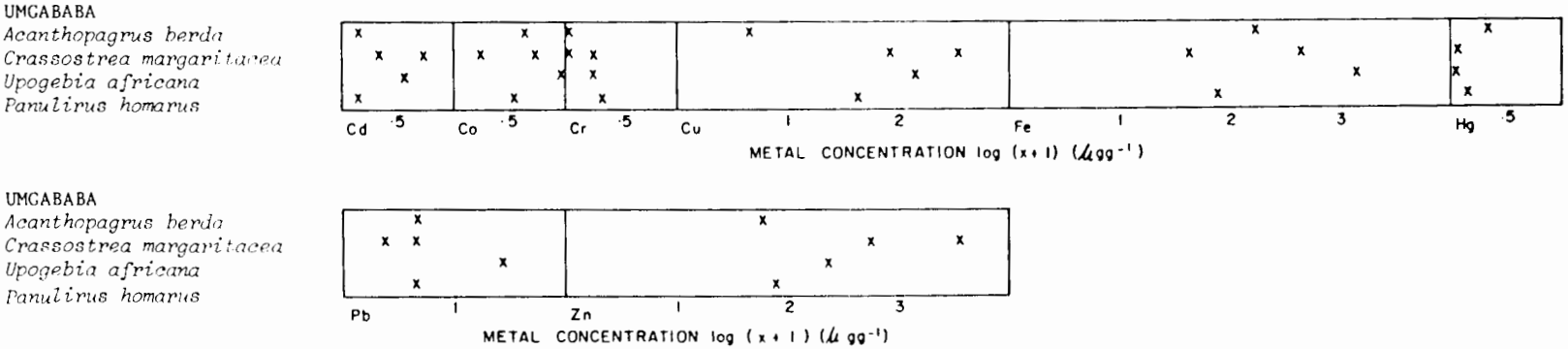


Figure 22 Metal concentrations in marine organisms from Umgababa Estuary

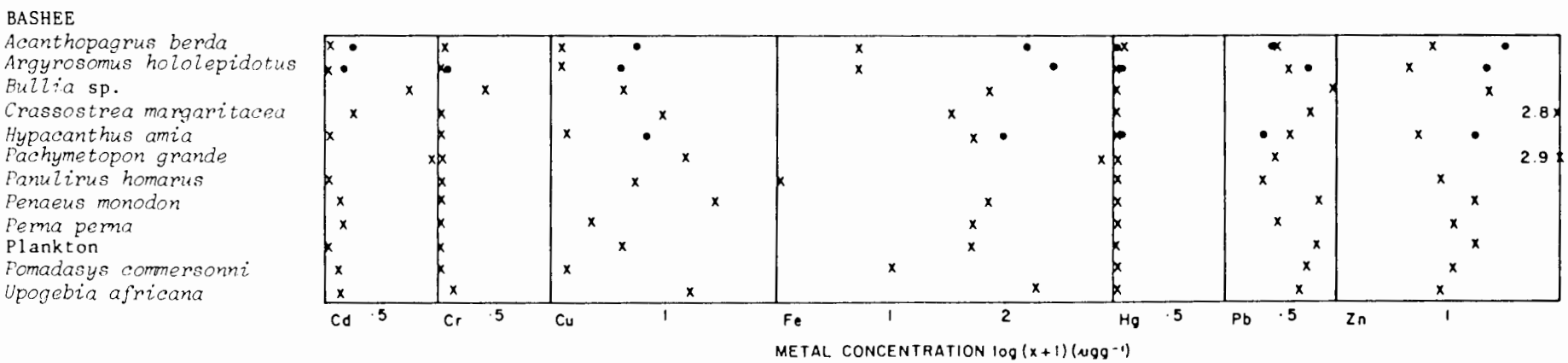


Figure 23 Metal concentrations in marine organisms from Bashee Estuary (• = liver)

4.2.2 Conclusion for Section II

Very little is known about this region in terms of metal concentrations. As it lacks heavy industry, this section could provide data which would be a valuable contribution to our metal baseline knowledge.

4.3 Section III - East London to Cape Agulhas

This is the section of the coast for which most data were available (Figure 24). Extensive studies were made around Port Elizabeth, Francis Bay, Knysna and Mossel Bay. The animals sampled were a good cross-section of the fauna commonly found in these regions. These surveys can truly be referred to as baseline studies.

4.3.1 Algoa Bay (Figure 25)

All authors report their results in terms of wet weight. Watling (1978) reported mean wet tissue masses in Algoa Bay and apart from standard deviation, no sex or size data were reported. The accuracy is as given in the original report. The method for the preparation of samples for analysis was as follows:

Living specimens were suspended in clean sea water for up to five days to allow them to purge their intestinal contents. The wet tissues were then removed from the shells and frozen. The frozen specimens were thawed, weighed into clean dry flasks and oven-dried at 90°C for 24 h. The dried samples were dissolved in 25 ml nitric acid and heated (solution temperature < 100°C) to near dryness. The residue was dissolved in 25 ml of a 4:1 nitric-perchloric acid mixture. This solution was fumed to dryness at about 140°C. The white residue was redissolved in 10 ml 10 per cent nitric acid for atomic absorption analysis.

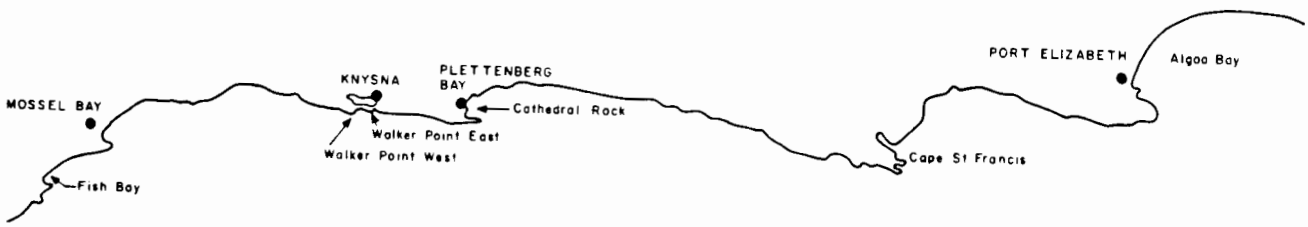


Figure 24 Location map of major sampling areas of Section III

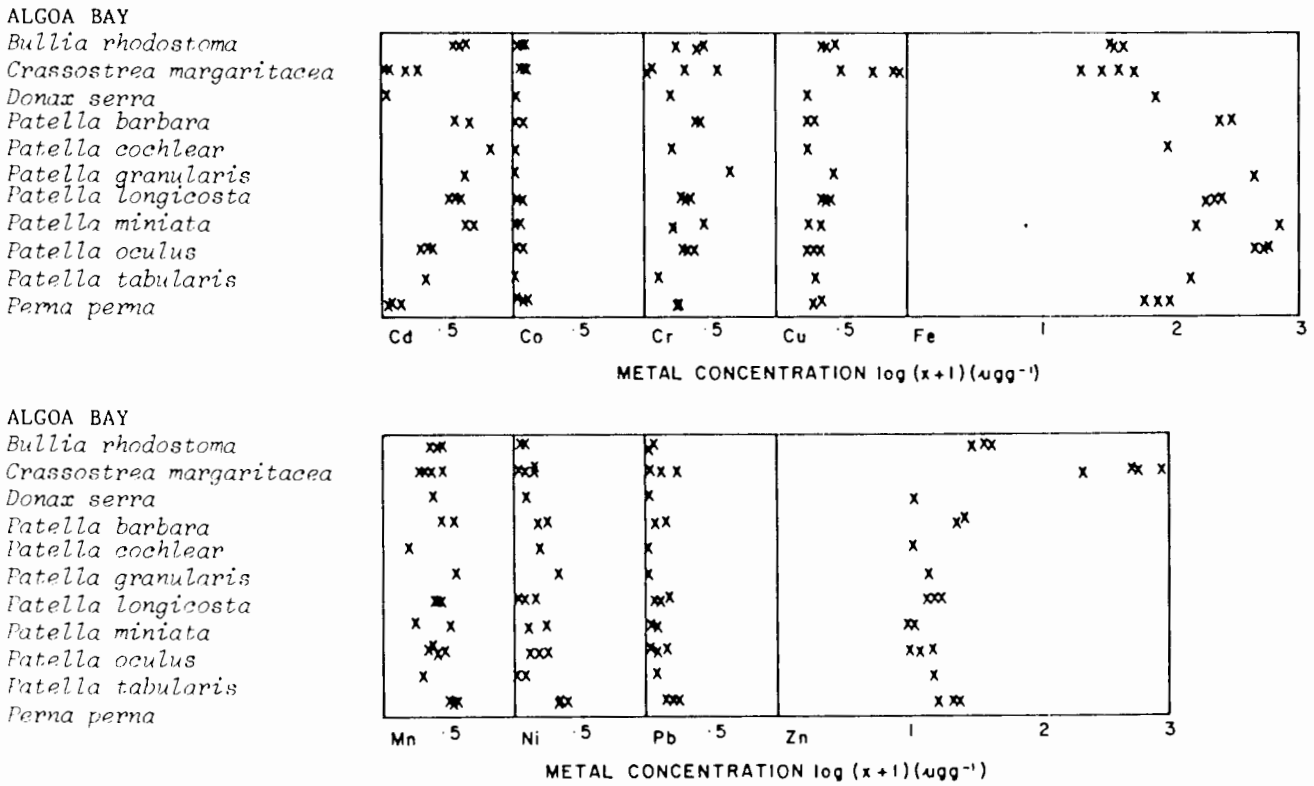


Figure 25 Metal concentrations in marine organisms from Algoa Bay

Watling and Watling (1981a) reported the number of animals used in their metal determination, also wet mass, dry mass, the mean metal concentration (as wet tissue mass) and the standard deviation. The method of preparing the animals consisted of allowing the animals to purge sediments and gut contents for 48 hours.

This was, however, either not done each time or was not reported on occasions. Whole individuals were weighed and then dried at 105°C for 48 h. The dried and weighed tissue was digested with redistilled, Analar grade nitric acid and the solution was evaporated to dryness. The residue was redissolved in a 4:1 nitric-perchloric acid mixture and the solution fumed to dryness at about 250°C. The residue was then dissolved in 10 ml of 0.1 M nitric acid.

This method was the same as that used by Watling and Watling (1983).

The interesting feature of Figure 25 was that it allows comparison of different species of Patella from the same areas. Different species accumulate different metals (see cadmium and iron). Since the limpets usually do not compete for the same niche but occupy different habitats, the different metal burden could be due to different food sources, behaviour and/or excretion mechanisms. These findings illustrate the great diversity of metal concentrations within a family of animals and serves as a warning against extrapolating metal levels from one species to another, even if they are very closely related.

It was also interesting to note that oysters concentrated copper and zinc, but that the concentrations of the other metals in oysters differed little from those in the other animals. Thus to use a single indicator species to establish metal pollution is not always appropriate.

4.3.2 St Croix (Figure 26)

All the results are those of Oliff and Turner (1976) and are reported in terms of dry mass. Watling (1978) converted Oliff's (1978) data to a wet mass basis. The method was that used for the Kosi Bay tests, reported above, again the livers of the fish contained higher metal concentrations than did the muscles.

4.3.3 Swartkops Estuary (Figure 27)

Except for the data for pilchards, all data were from Oliff and Turner (1976). For some reason a few metal concentrations are expressed in terms of dry mass, while the rest are expressed in terms of wet mass. The methods were as reported along with length and mass being reported where appropriate. Six metals were determined.

Metal concentrations in the pilchard (Van der Byl, 1980) were determined by the CSIR for 1979 fish. No detail of method, size or sex are given. The results are expressed in terms of dry mass. Except for copper, the testes contained more metal than did the ovaries.

4.3.4 Port Elizabeth (Figure 28)

Data presented are from Oliff and Turner (1976). They are expressed in terms of dry mass. Again, the metal concentrations in the various animals could not be correlated with the higher levels of metal in the water and sediment of this area.

4.3.5 Maitland (Figure 29)

Watling (1978) has reported on only one species. The results are expressed in terms of wet mass.

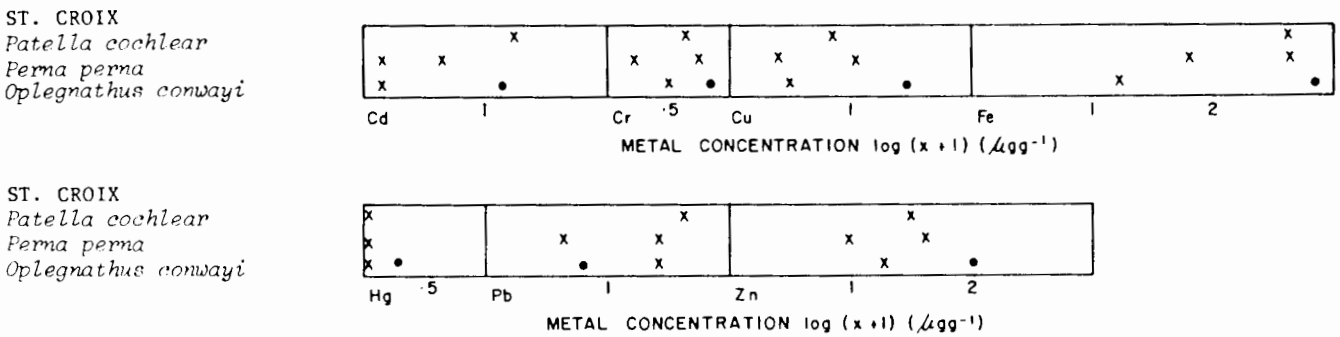


Figure 26 Metal concentrations in marine organisms from St Croix (• = liver)

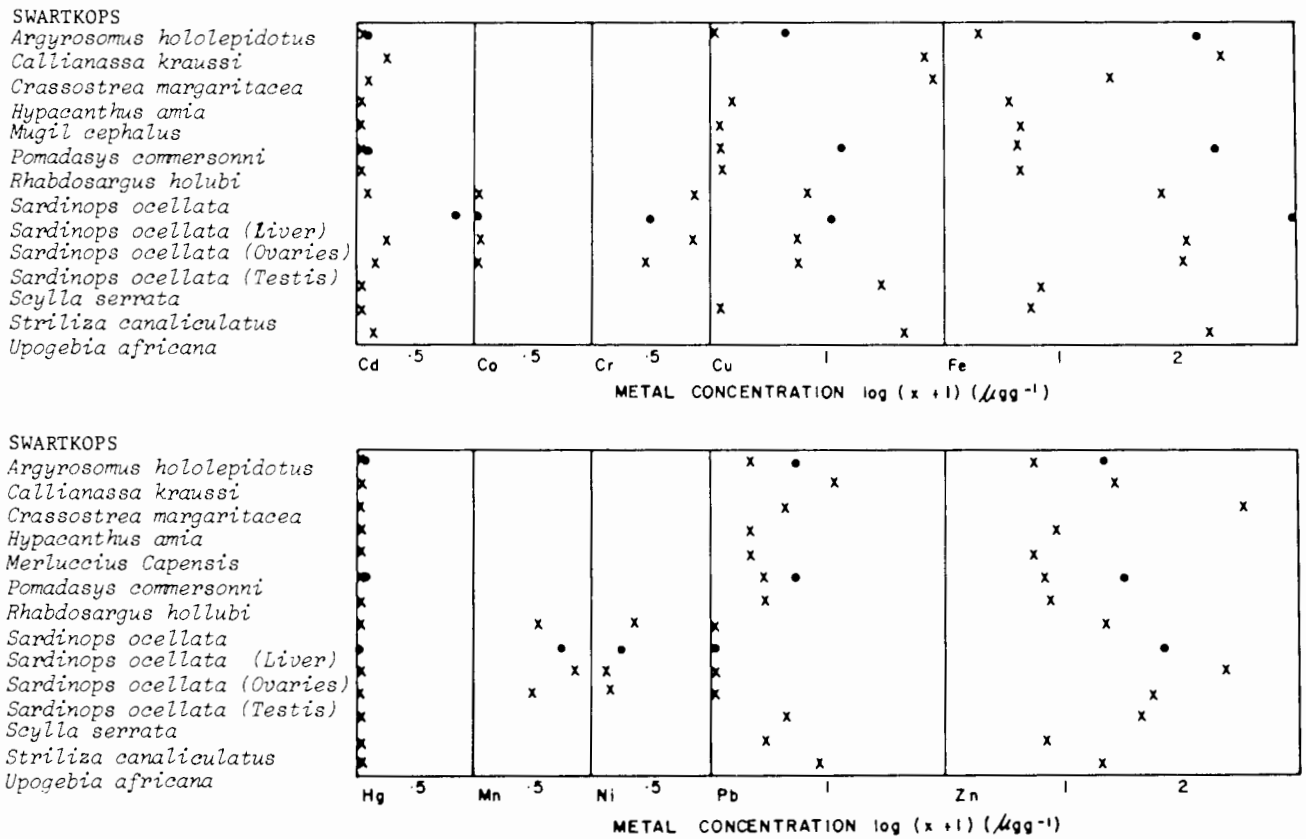


Figure 27 Metal concentrations in marine organisms from Swartkops Estuary (• = liver)

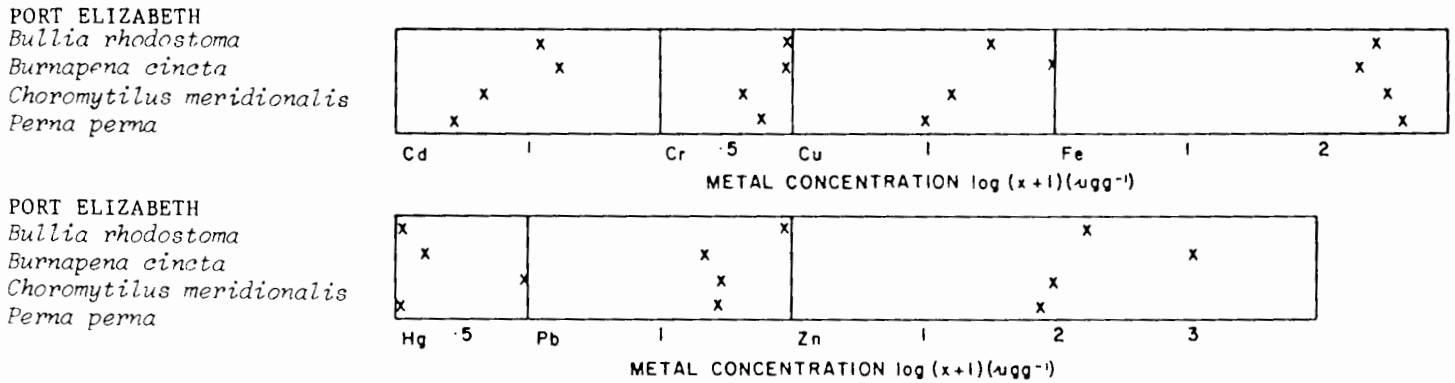


Figure 28 Metal concentrations in marine organisms from Port Elizabeth

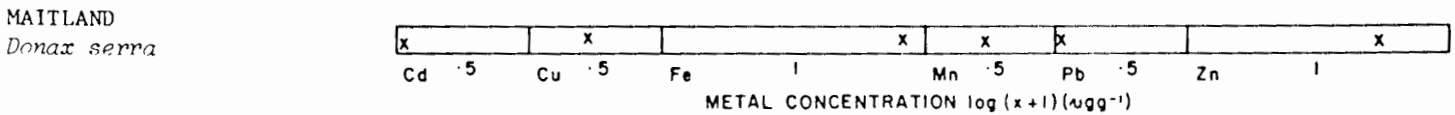


Figure 29 Metal concentrations in marine organisms from Maitland

4.3.6 St Francis Bay (Figure 30)

Watling and Watling (1983) reported data as mean metal concentration in μg g^{-1} wet tissue. The number of sampling sites and wet mass, as well as dry mass were reported.

As in other locations, the metal concentrations of the seven Patella species differed from one species to the next and seemed not to be related to metal concentrations in the water at that site. These data can serve as a baseline for future monitoring of this area.

4.3.7 Keurboomstrand (Figure 31)

Both mollusc species contained very similar metal concentrations. The results were expressed in terms of wet mass (Watling, 1978).

4.3.8 Cathedral Rock (Figure 32)

Again method and reporting were by Watling (1978). The mussel showed higher concentration of metals than did the oyster.

4.3.9 Noetzie (Figure 33)

Watling (1978) reported metal concentrations in terms of wet mass; the trends in metal levels were as observed at Cathedral Rock.

4.3.10 Knysna East Head (Figure 34)

Watling and Watling (1980) reported on the results in terms of wet mass. The animals were allowed to purge their intestinal contents for up to five days. The wet tissues were removed from shells and frozen. They were later thawed, weighed and dried at 90°C for 24 h. The dried samples were weighed, dissolved in 25 ml redistilled Analar grade nitric acid and evaporated to

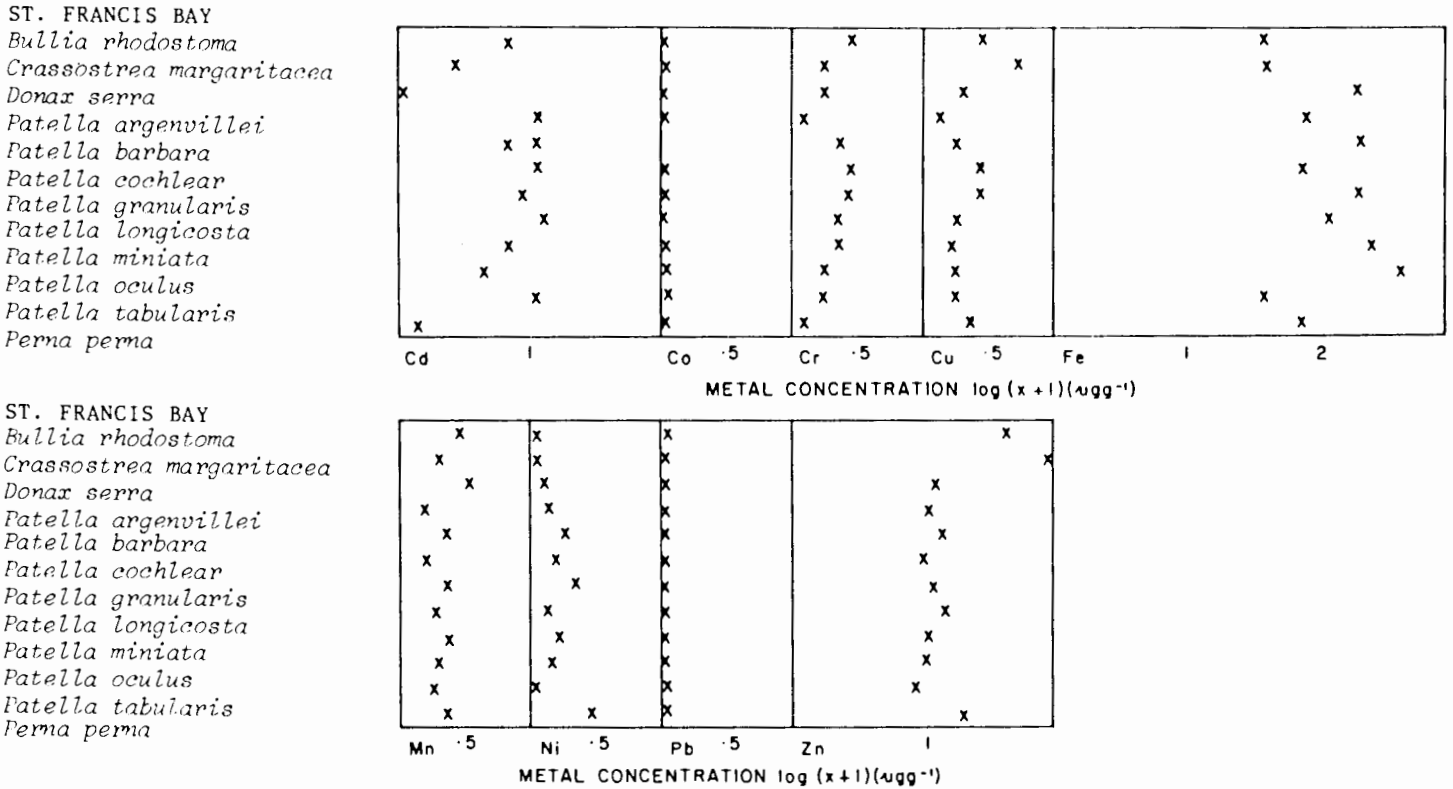


Figure 30 Metal concentrations in marine organisms from St Francis Bay

KEURBOOM
Donax serra
Solen capensis

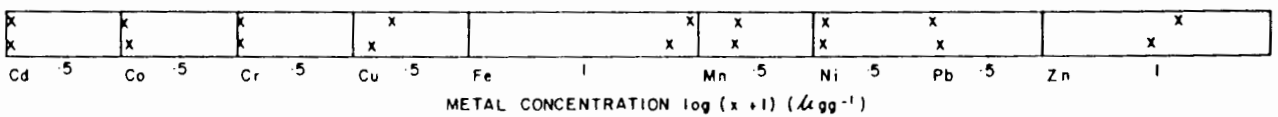


Figure 31 Metal concentrations in marine organisms from Keurboomstrand

CATHEDRAL ROCK
Crassostrea margaritacea
Perna perna

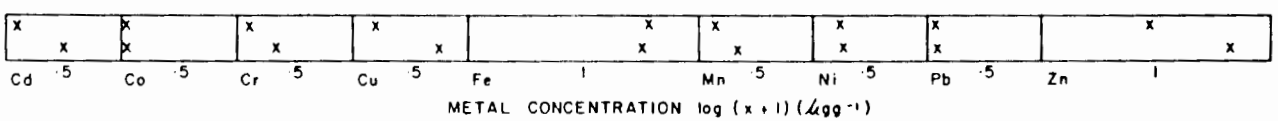


Figure 32 Metal concentrations in marine organisms from Cathedral Rock

NOETZIE
Crassostrea margaritacea
Perna perna

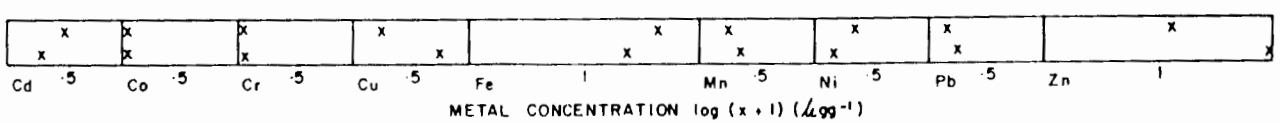


Figure 33 Metal concentrations in marine organisms from Noetzie

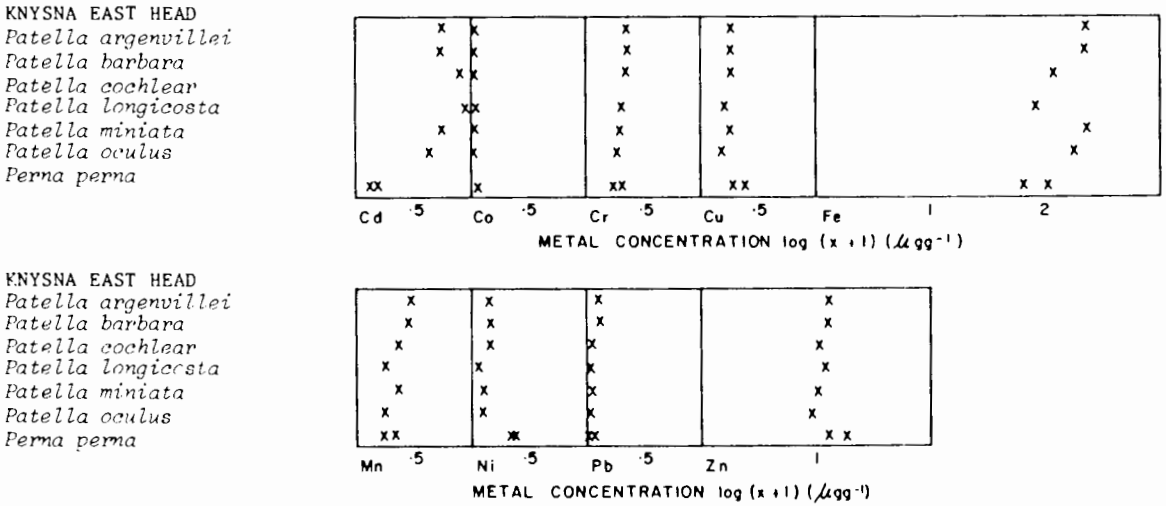


Figure 34 Metal concentrations in marine organisms from Knysna East Head

near dryness. The residue was dissolved in 25 ml of a 4:1 nitric-perchloric acid mixture and fumed to dryness at 140°C. Methods were as in Watling (1978).

The report gave concentrations for both wet mass and dry mass, the number of animals analysed, the mean metal concentration and standard deviation for concentrations of nine metals.

Watling (1978) reported on an earlier sample taken from that area. This differed slightly from the 1978 samples.

The various species of Patella differed greatly in concentrations of cadmium and iron.

4.3.11 Beacon Point (Figure 35)

The same authors, Watling (1978) and Watling and Watling (1980), have reported metal concentrations here. The same trends are observed as noticed for Knysna East Head.

4.3.12 Knysna West Head (Figure 36)

All species were reported in wet mass by Watling and Watling (1980). More cadmium was accumulated at this location.

4.3.13 Featherbed (Figure 37)

Watling and Watling (1980) reported metal levels for these two species in terms of wet tissue mass.

4.3.14 Belvedere (Figure 38)

Belvedere is the point in the Knysna lagoon which is furthest from the sea. There seems to be very little difference between metal concentrations all around the lagoon. The methods used were as in Watling and Watling (1980) and the data are expressed in terms of wet mass.

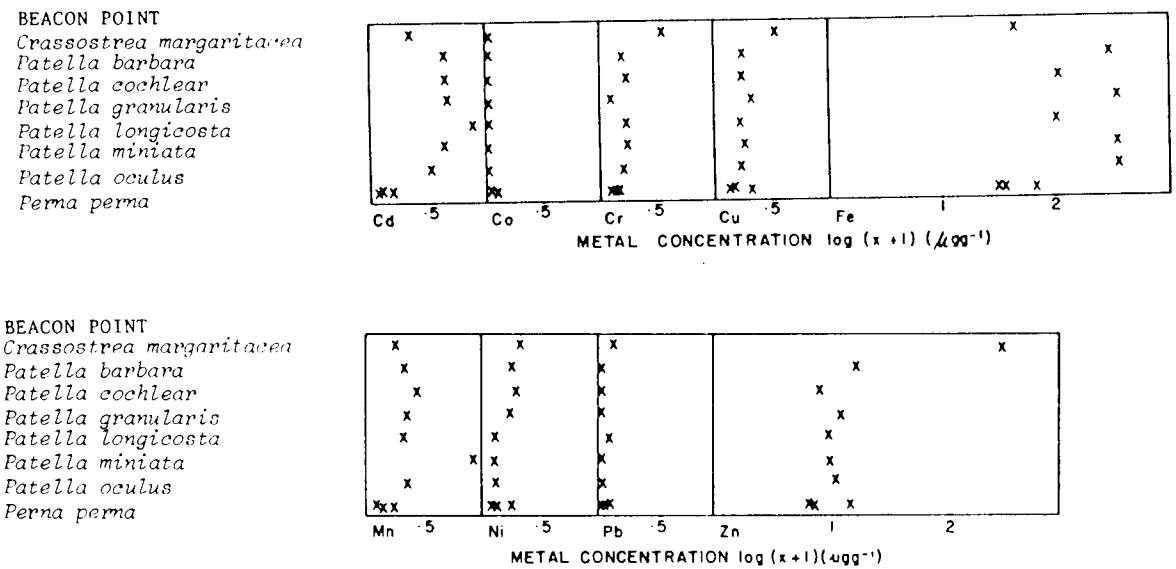


Figure 35 Metal concentrations in marine organisms from Beacon Point

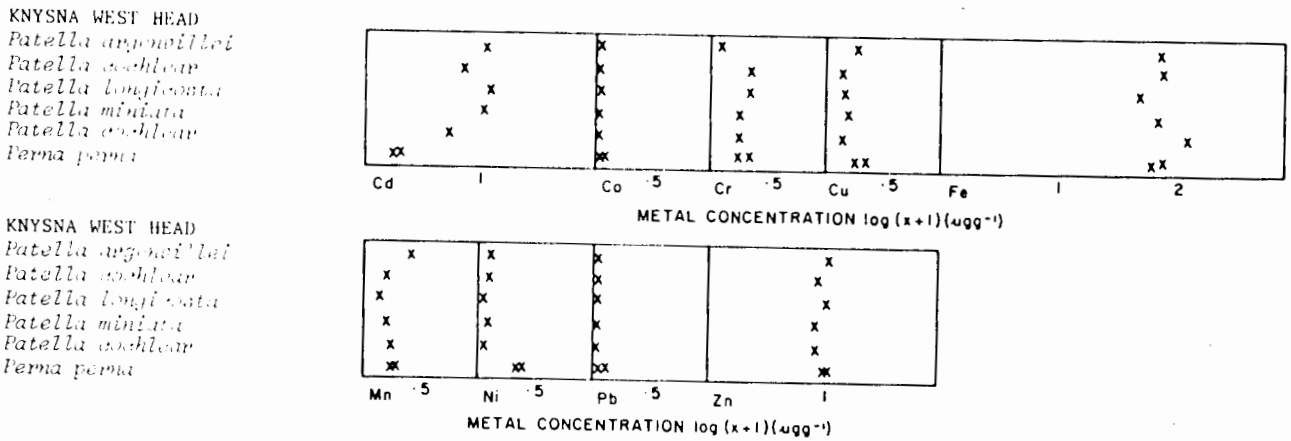


Figure 36 Metal concentrations in marine organisms from Knysna West Head

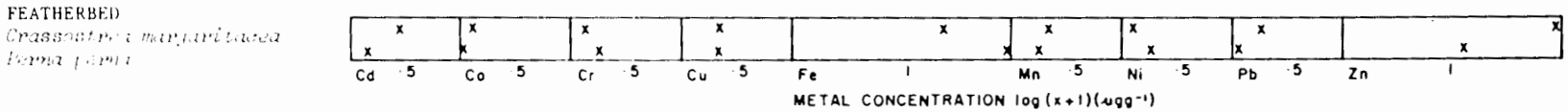


Figure 37 Metal concentrations in marine organisms from Featherbed

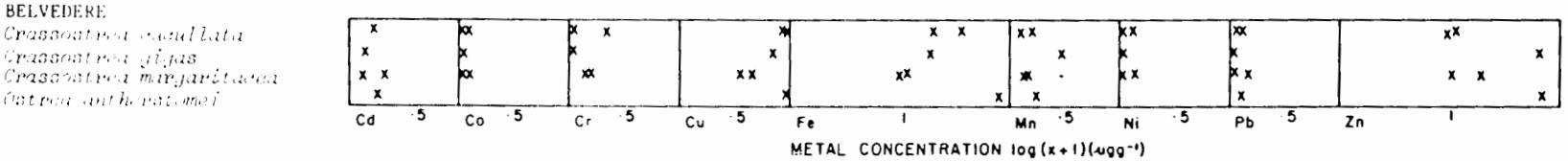


Figure 38 Metal concentrations in marine organisms from Belvedere

4.3.15 Leisure Island (Figure 39)

There seems to be little influence of the metal concentration of the water and sediment on the metal burden of various animals (see cadmium). All concentrations were expressed in terms of wet tissue mass. It is not clear if Watling (1978) and Watling and Watling (1980) were reporting on the same animal collection.

4.3.16 Knysna (Figure 40)

Seven sampling points were located in the Knysna area. Hence this geographical position may coincide with any of the others already mentioned. Darracott and Watling (1975) presented their data in terms of wet tissue mass, while Watling and Watling (1976a) report in terms of dry tissue mass. No method was given in Darracott and Watling (1975). The method for Watling and Watling (1976a) is identical to that reported in the Algoa Bay section.

4.3.17 Thesen's Point (Figure 41)

S. capensis was sampled in 1975 and 1980 with no difference observed by Watling and Watling (1980). Lower metal concentrations were found in P. perna by Watling (1978). The data are expressed in terms of wet mass.

4.3.18 Castle Rock (Figure 42)

Watling (1978) reported in terms of wet mass. These samples could be regarded as unpolluted baseline values for the animal analysed.

4.3.19 Buffalo Bay (Figure 43)

Due to the different sampling site's morphology the same species could not be sampled and a comparison was not possible. Still these metal concentrations represent background baseline data, expressed in terms of wet mass.

LEISURE ISLAND
Atrina squamifera
Mactra glabrata
Perna perna
Venus verrucosa

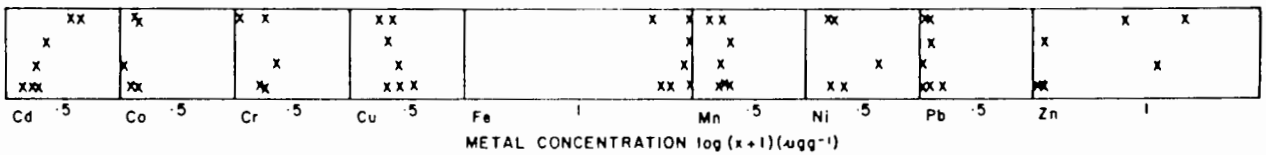


Figure 39 Metal concentrations in marine organisms from Leisure Island

KNYSNA
Crassostrea gigas
Crassostrea margaritacea
Ostrea edulis

KNYSNA
Crassostrea gigas
Crassostrea margaritacea
Ostrea edulis

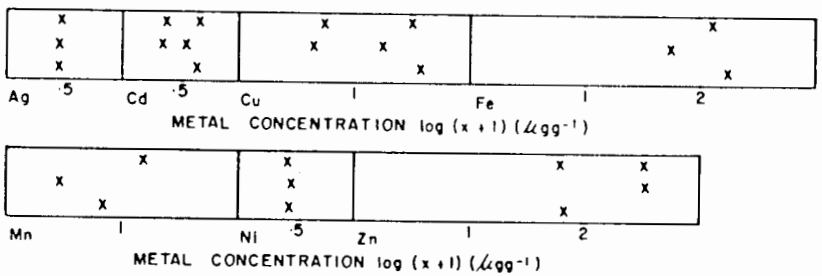


Figure 40 Metal concentrations in marine organisms from Knysna

THESENS POINT
Perna perna
Solen capensis

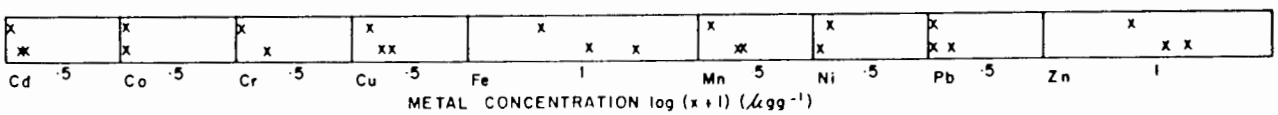


Figure 41 Metal concentrations in marine organisms from Thesen's Point

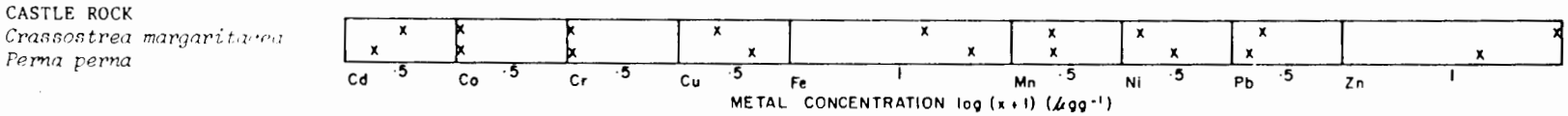


Figure 42 Metal concentrations in marine organisms from Castle Rock

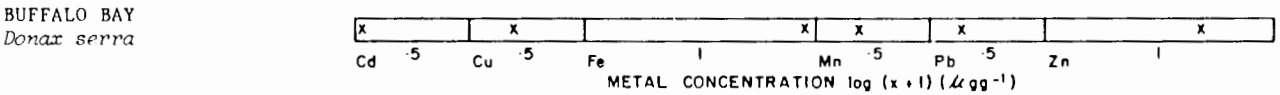


Figure 43 Metal concentrations in marine organisms from Buffalo Bay

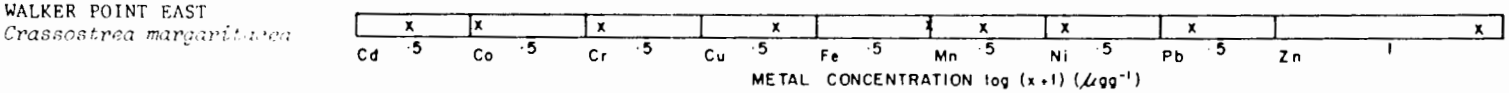


Figure 44 Metal concentrations in marine organisms from Walker Point East

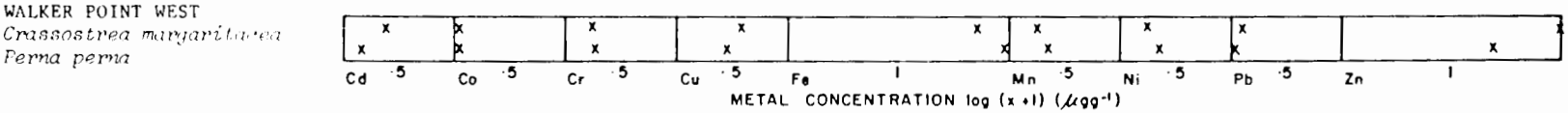


Figure 45 Metal concentrations in marine organisms from Walker Point West

4.3.20 Walker Point East (Figure 44)

The mean wet tissue mass was reported by Watling (1978) and the standard deviation of the mean metal concentration was given.

4.3.21 Walker Point West (Figure 45)

There seemed to be more iron on this side of Walker Point.

4.3.22 Herold's Bay (Figure 46)

The data from this study (Watling and Watling, 1981b) were collected during a sampling trip which covered the Mossel Bay area. The numbers of animals are given, wet mass, dry mass, mean and standard deviation expressed in terms of wet mass. It is possible that all these results were summarised by Watling and Watling (1983) so that these results have been repeated under the Mossel Bay section.

4.3.23 Glentana (Figure 47)

See Herold's Bay.

4.3.24 Tergniet (Figure 48)

See Herold's Bay.

4.3.25 Little Brak River (Figure 49)

See Herold's Bay.

4.3.26 Hartenbos (Figure 50)

See Herold's Bay.

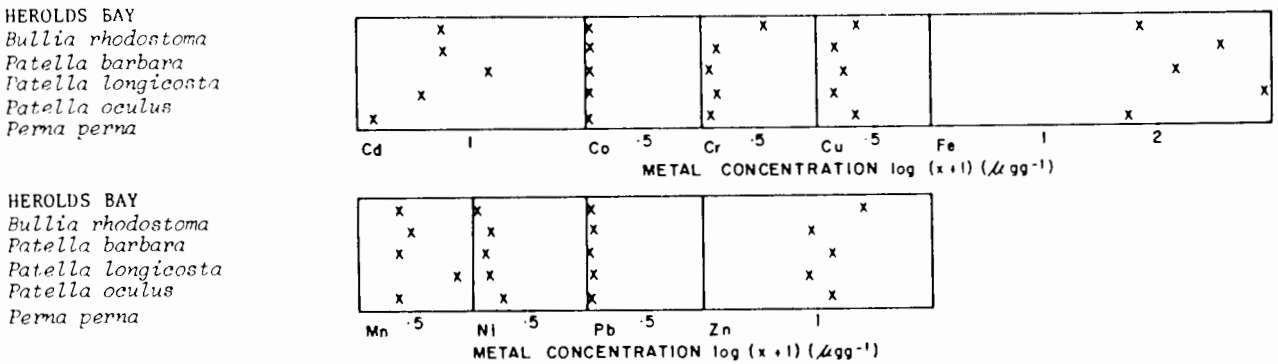


Figure 46 Metal concentrations in marine organisms from Herold's Bay

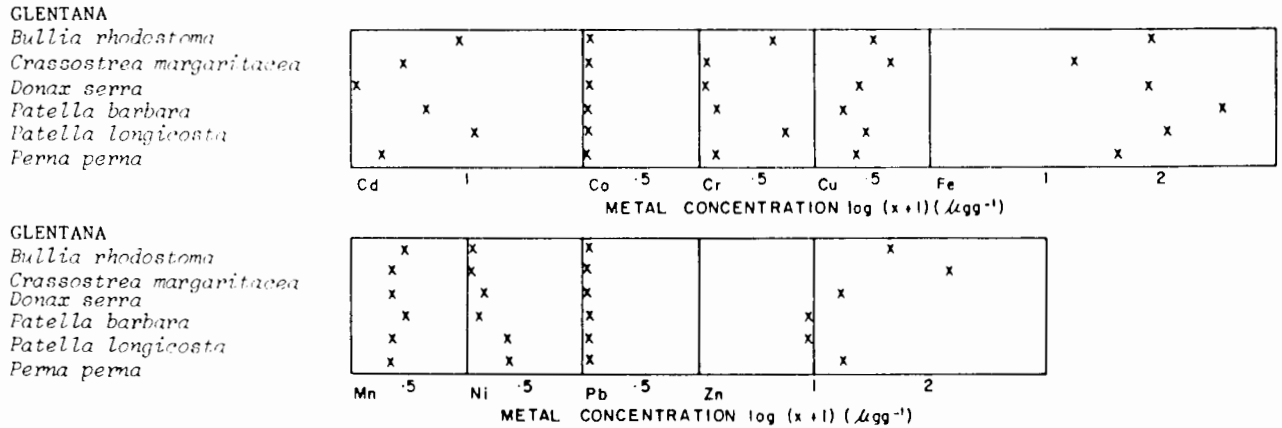


Figure 47 Metal concentrations in marine organisms from Glentana

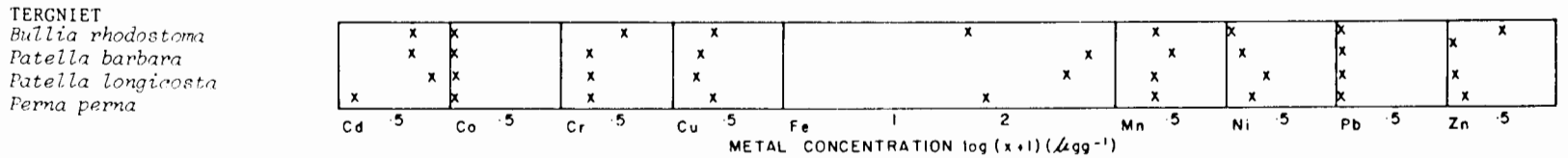


Figure 48 Metal concentrations in marine organisms from Tergniet

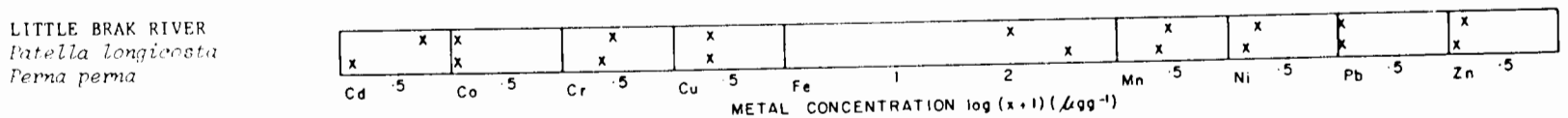


Figure 49 Metal concentrations in marine organisms from Little Brak River

HARTENBOS
Bullia rhodostoma
Patella longicosta
Perma perma

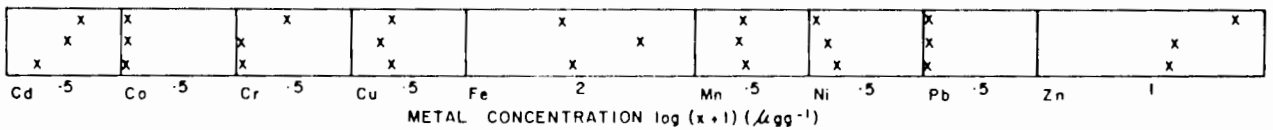


Figure 50 Metal concentrations in marine organisms from Hartenbos

DIAZ BEACH
Bullia rhodostoma
Donax serra

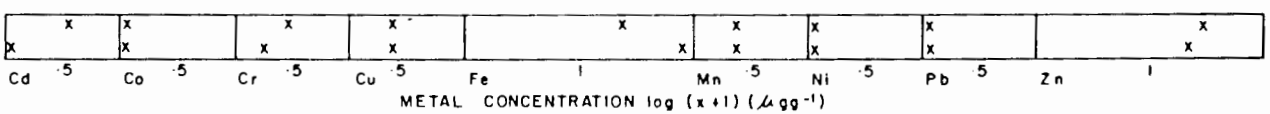


Figure 51 Metal concentrations in marine organisms from Diza Beach

DIE BAKKE
Patella barbara
Patella longicosta
Perma perma

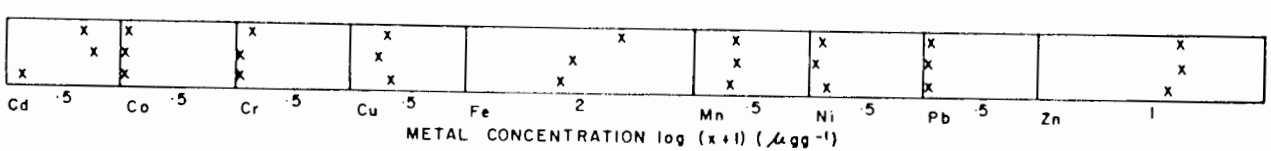


Figure 52 Metal concentrations in marine organisms from Die Bakke

4.3.27 Diza Beach (Figure 51)

See Herold's Bay. The reason for separating the data given by Watling and Watling (1981b), is that a large section of coastline had been covered by their survey. The area is being industrialised and separation of each location from the others will help to identify and pin-point any future impact areas.

4.3.28 Die Bakke (Figure 52)

See Herold's Bay.

4.3.29 Mossel Bay (Figure 53)

As mentioned under the Herolds Bay section most of these data may have been summarized by Watling and Watling (1983) and should be read in conjunction with the report by Watling and Watling (1981b). The concentrations were expressed in terms of wet mass.

4.3.30 Dana Township (Figure 54)

See Herold's Bay.

4.3.31 Cape St Blaize (Figure 55)

See Herold's Bay and Mossel Bay.

4.3.32 Pinnacle Point (Figure 56)

See Herold's Bay and Mossel Bay.

4.3.33 Fish Bay (Figure 57)

See Herold's Bay and Mossel Bay.

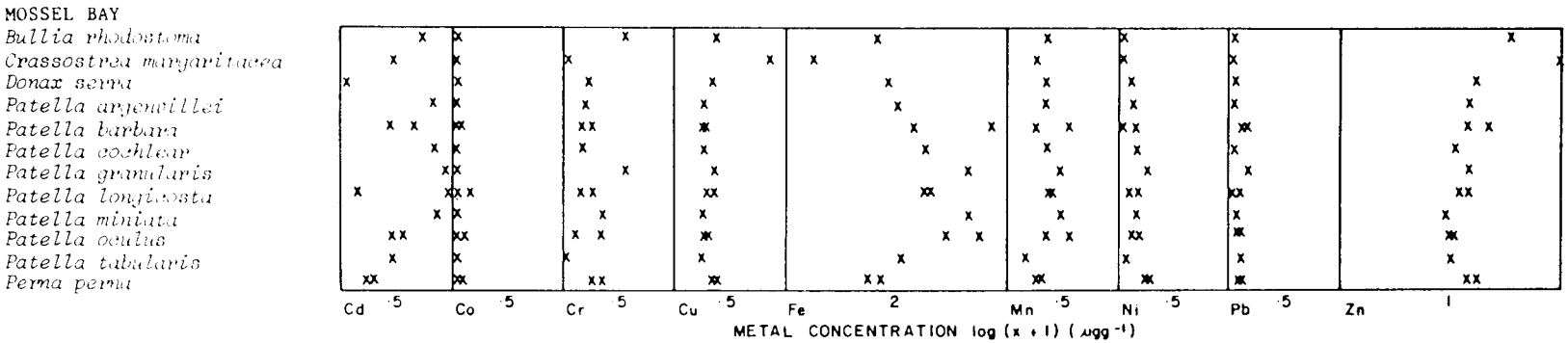


Figure 53 Metal concentrations in marine organisms from Mossel Bay

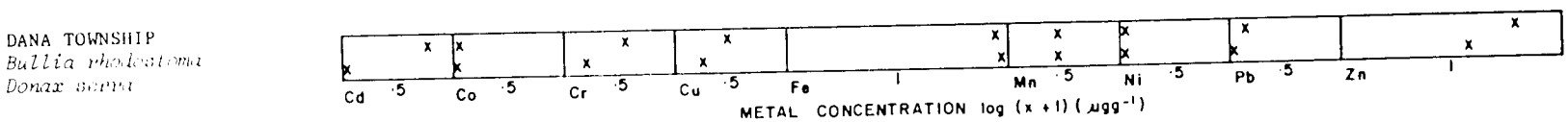


Figure 54 Metal concentrations in marine organisms from Dana Township

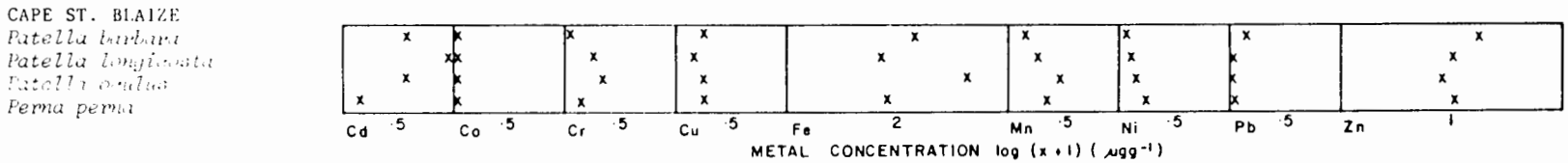


Figure 55 Metal concentrations in marine organisms from Cape St Blaize

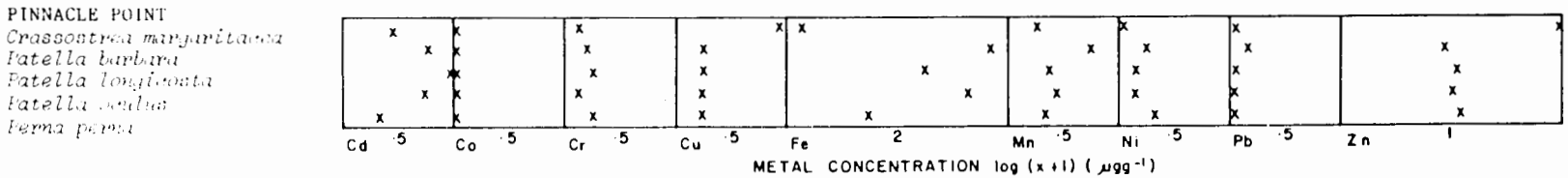


Figure 56 Metal concentrations in marine organisms from Pinnacle Point

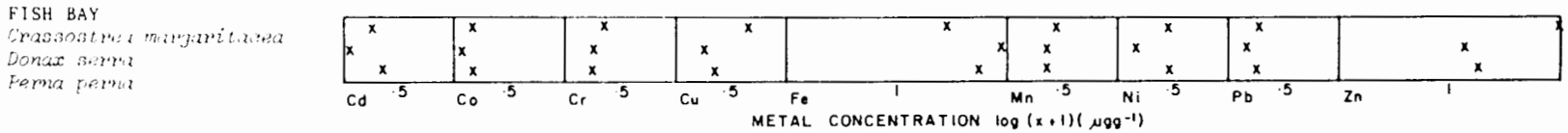


Figure 57 Metal concentrations in marine organisms from Fish Bay

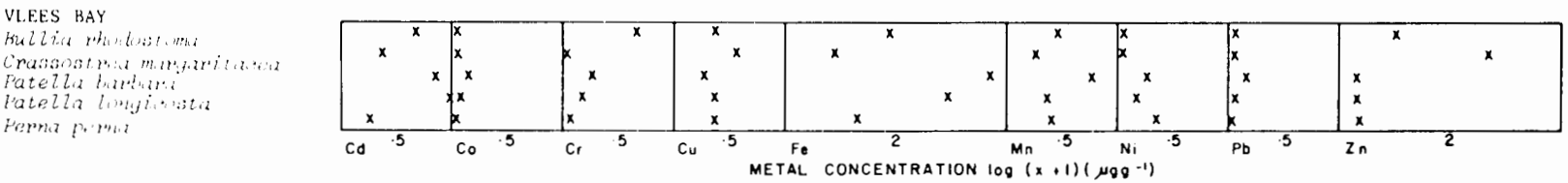


Figure 58 Metal concentrations in marine organisms from Vlees Bay

4.3.34 Vlees Bay (Figure 58)

See Herold's Bay and Mossel Bay.

4.3.35 Conclusion for Section III

This section has been well studied and samples have been taken not only from impact areas. Many samples from Knysna and along large sections of the coastline have been analysed. The reports on metal concentration are very valuable, because they give data on not only sample sites, numbers of animals analysed, but also wet and dry masses have been published. The method of preparing the organism is well documented. Again it is felt that all the available information should be published in a report such as the present one to eliminate possible duplication of data and for the sake of completeness.

4.4 Section IV - Cape Agulhas to Cape Columbine

Data have been obtained mainly from sampling sites around Cape Town and on the west coast up to Saldanha. Some of the older publications report metal concentrations before industrialisation along the west coast. There have been some very detailed studies of metal concentrations in sediment and water in Table Bay without sampling any organisms. Baseline studies and comparisons of metal levels in a wide variety of organisms have been made in this section.

4.4.1 Strandfontein (Figure 59a - e)

A detailed study and comparison of trace metals in B. digitalis at three different sites, by Hennig (unpublished), has demonstrated the size-dependence of selected metal burdens (for example, iron and lead). It also demonstrates that while there may be a linear relationship between metal concentrations in terms of wet mass and dry mass, this is not necessarily true for a dry mass/shell length relationship.

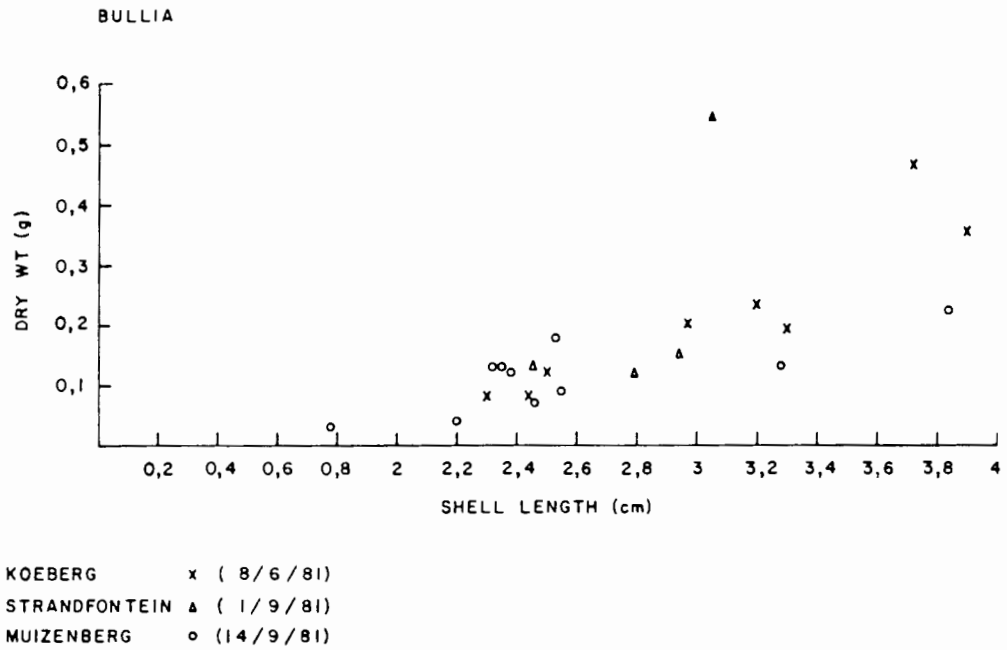


Figure 59 Comparison of metal content of Bullia digitalis from three different sites along the West Coast. Relationship of shell length and dry mass in Bullia

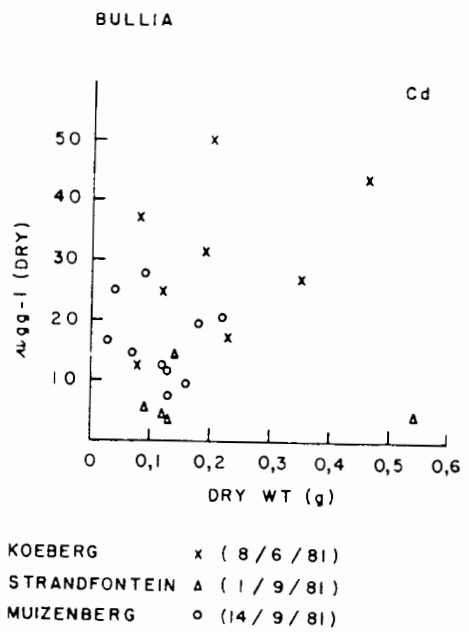
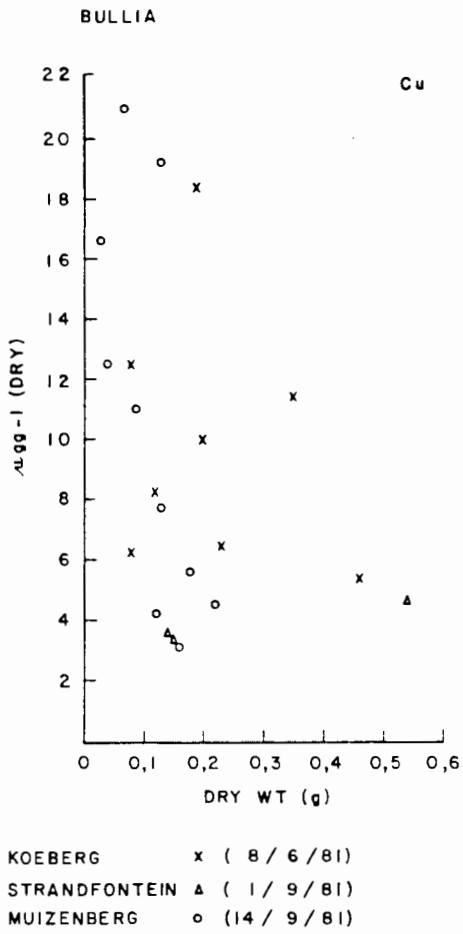


Figure 59a Cadmium concentration in Bullia



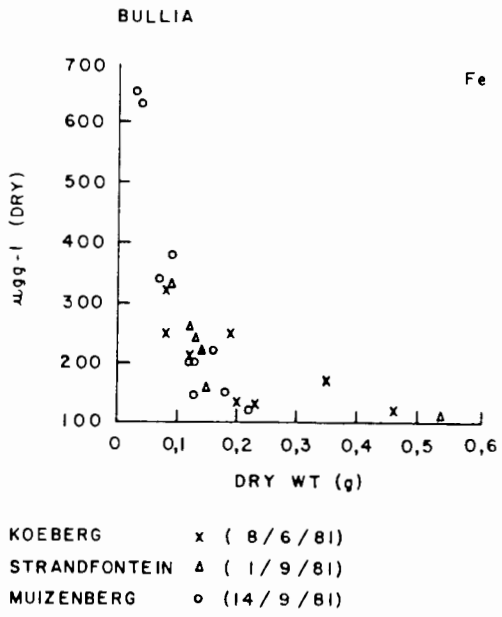


Figure 59b Copper and iron concentrations in Bullia

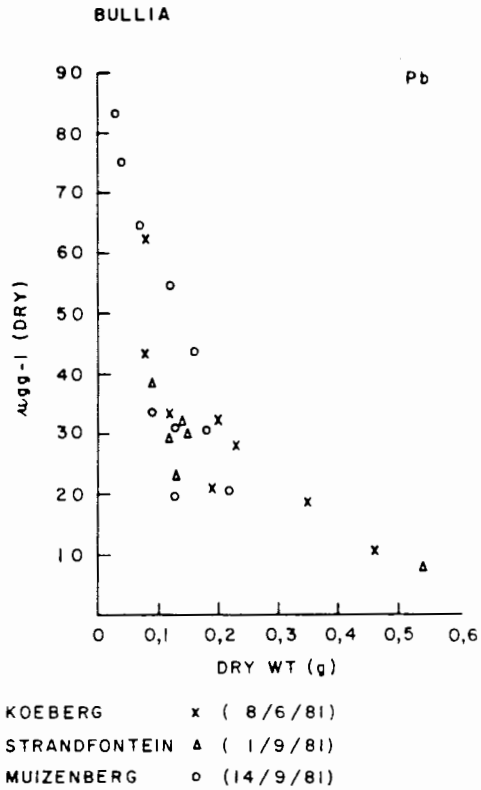


Figure 59c Lead concentrations in Bullia

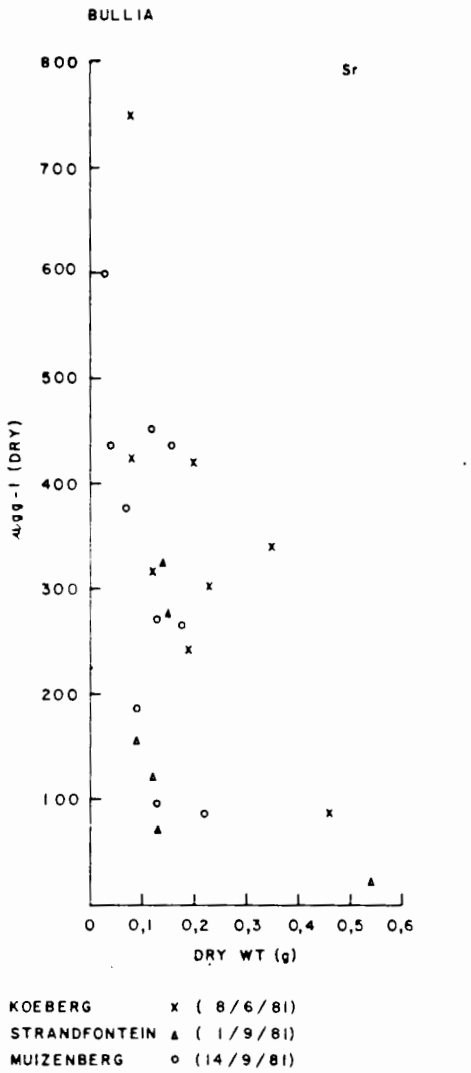


Figure 59d Strontium concentrations in Bullia

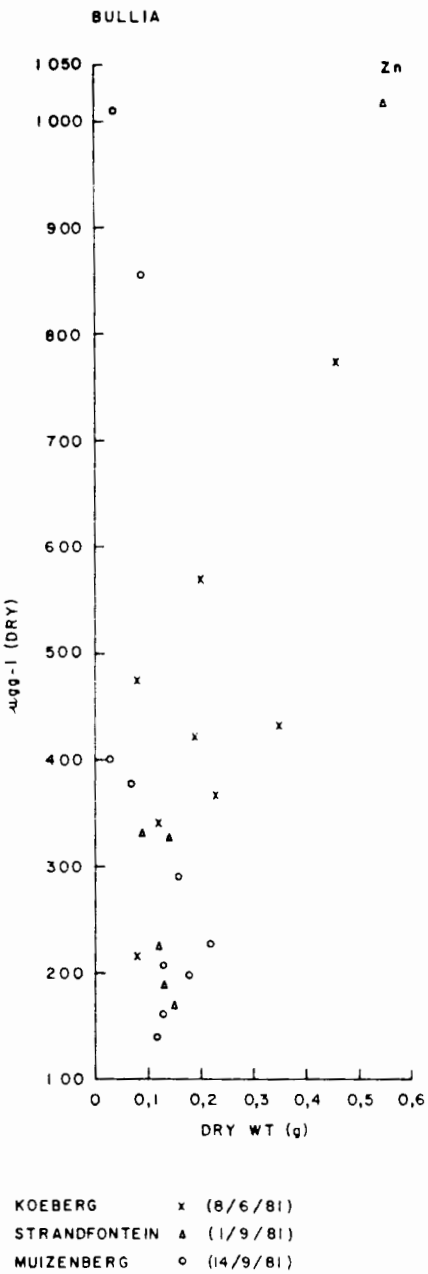


Figure 59e Zinc concentrations in Bullia

The data are expressed as µg g^{-1} dry mass. In the study, the animals were collected alive and frozen in plastic bags. After thawing, the shell was measured and the whole whelk was removed from the shell. The animals were then dried in pre-weighed, acid-cleaned glass vials to a constant mass at 60°C. Redistilled Analar grade nitric acid (25 ml) was added to the dried whelk and the mixture was allowed to stand at room temperature overnight. Blank determinations were run concurrently. Samples were heated to dryness to form a grey to white residue. A 4:1 mixture of nitric/perchloric acids (25 ml) was added and the mixture was heated again to dryness. The residue was dissolved in 10 per cent v/v nitric acid and analysed.

4.4.2 Muizenberg (Figures 59 and 60)

Metals in limpets were analyzed as in whelks (see Strandfontein), but no shell measurements were taken. The whole animal was removed from the shell with an acid washed glass knife. The rest of the method was as described in the Strandfontein section. The data were expressed in terms of dry mass.

4.4.3 Cape (Figure 61)

Some data on metal concentrations in the bones of sea birds were given by Orren (1975). No method was given; only two metals were determined. The data were expressed in terms of dry mass. Penguins seem to concentrate lead in their bones.

4.4.4 Blouberg Strand (Figure 62)

A great number of different organisms were analysed in the early 1970's by Van As et al. (1973 and 1975). The samples were collected by skin-divers, dissected and weighed. The tissues were freeze-dried and again weighed. The dried samples were then ground in an agate pestle and mortar to obtain a homogenous sample. Approximately 1 g of material was soaked in a pure quartz ampoule and irradiated with neutrons in the nuclear reactor. Samples were then analysed for Cr, Co, Cs, Fe, Sb and Zn.

MUIZENBERG
Patella granularis

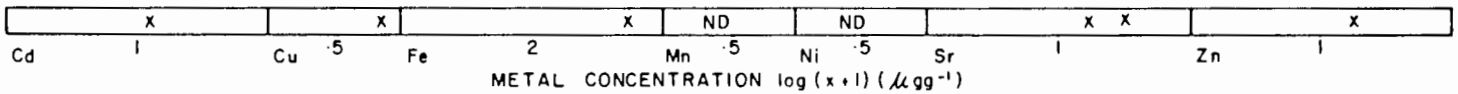


Figure 60 Metal concentrations in marine organisms from Muizenberg

CAPE
Cormorant
Gannet
Penguin

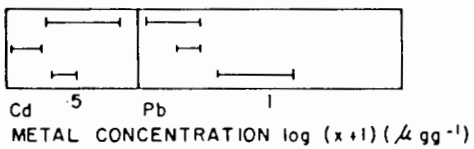


Figure 61 Metal concentrations in marine organisms from Cape

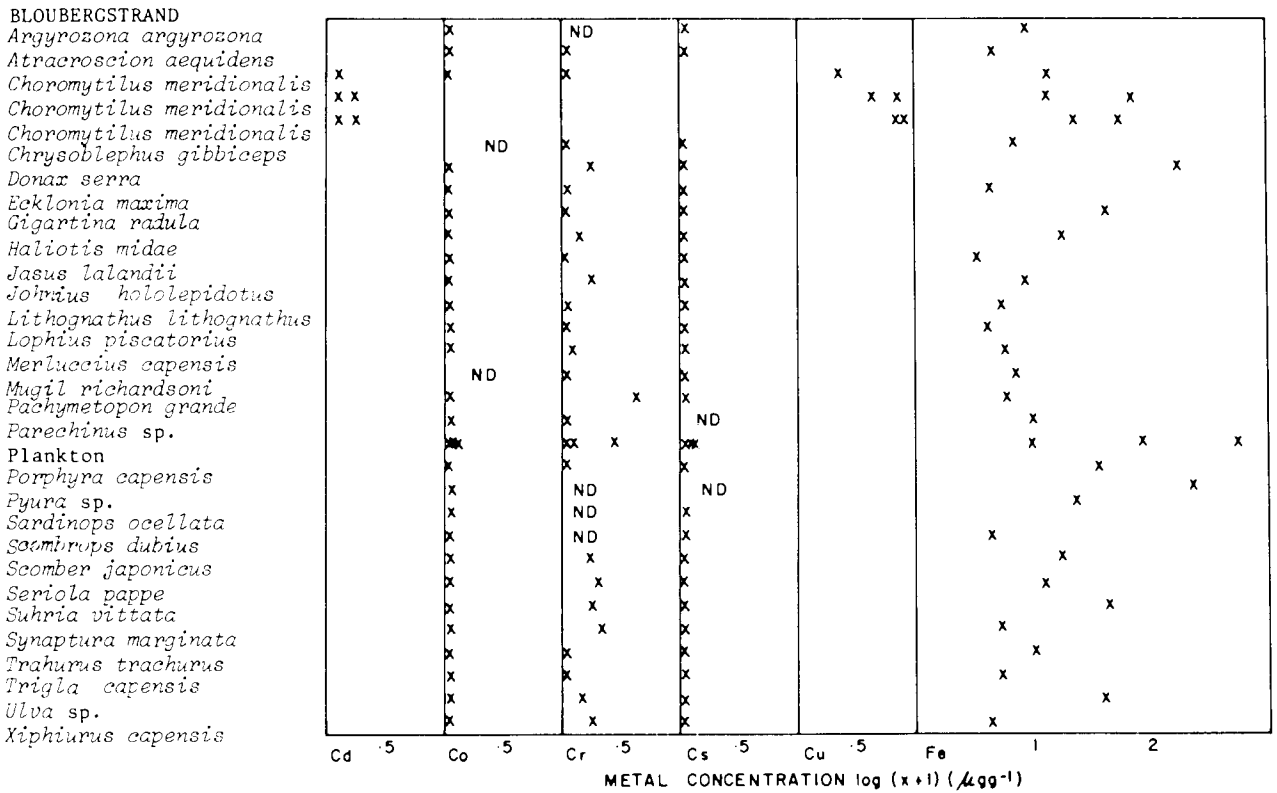
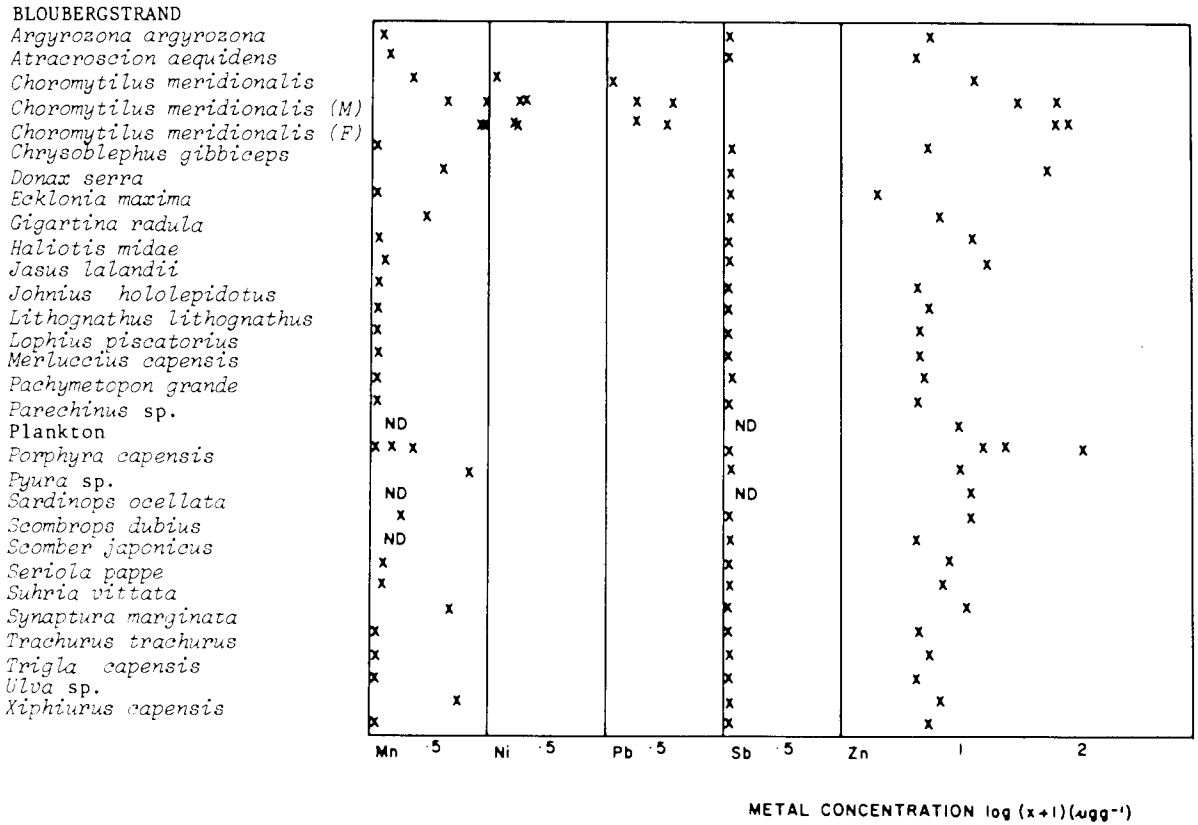


Figure 62 Metal concentration in marine organisms from Blouberg Strand


BLOUBERGSTRAND
Argyrozona argyrozona
Atractoscion aequidens
Choromytilus meridionalis
Choromytilus meridionalis (M)
Choromytilus meridionalis (F)
Chrysoblephus gibbiceps
Donax serra
Ecklonia maxima
Gigartina radula
Haliotis midae
Jasus lalandii
Johnius hololepidotus
Lithognathus lithognathus
Lophius piscatorius
Merluccius capensis
Pachymetopon grande
Parechinus sp.
Plankton
Porphyra capensis
Pyura sp.
Sardinops ocellata
Scombrops dubius
Scomber japonicus
Seriola pappe
Suhria vittata
Synaptura marginata
Trachurus trachurus
Trigla capensis
Ulva sp.
Xiphiurus capensis
ND
Mn .5
Ni .5
Pb .5
Sb .5
Zn 1
2
METAL CONCENTRATION log (x+1)(μgg⁻¹)

Analyses for Fe, Mn, Zn were carried out by atomic absorption analysis; aliquots (3 g) of the freeze-dried tissue were dissolved by refluxing in a mixture of HNO_3 and $HClO_4$. Blanks were used to determine background contribution. Data thus consist of results of atomic absorption analysis only for Mn, of neutron activation analysis for Cr, Co, Cs and Sb, and of parallel analyses for Fe and Zn. The data were expressed in terms of wet mass, mean and standard deviation were given. No numbers of organisms, size, sex or weight are given.

Watling (1978) reported results for one wet mass sample (February 1977) but gave no further information. Orren et al. (1980) reported on the metal levels in mussels collected in June and November 1979. The mussels had the byssus tracts removed and were allowed to purge for 72 hours. The dry mass was determined after oven drying at 100°C. The rest of the method was as used in the Strandfontein study.

A detailed study was made by Hennig (1981) of metal concentrations in adult (Figure 63) and immature (Figure 64 - notice the different x-axis scale) black mussels, C. meridionalis. There was a decrease of metal concentration with increase in size of animals. Such a trend is often noticeable only if sufficient animals have been sampled. For instance, 17 black mussels analysed for Cu, Fe and Zn (Figure 65), showed no such trend nor was there a difference between male and female. On the other hand, Watling (1978) found a difference between zinc levels in males and females at Knysna (Figure 66). The results have been recalculated to be expressed in terms of dry mass (Hennig and Orren, 1983). The method used by Hennig (1981) was that mentioned above by Orren, et al. (1980).

It should be noted that there are some metal values which have been determined for marine algae and kelp in this section.

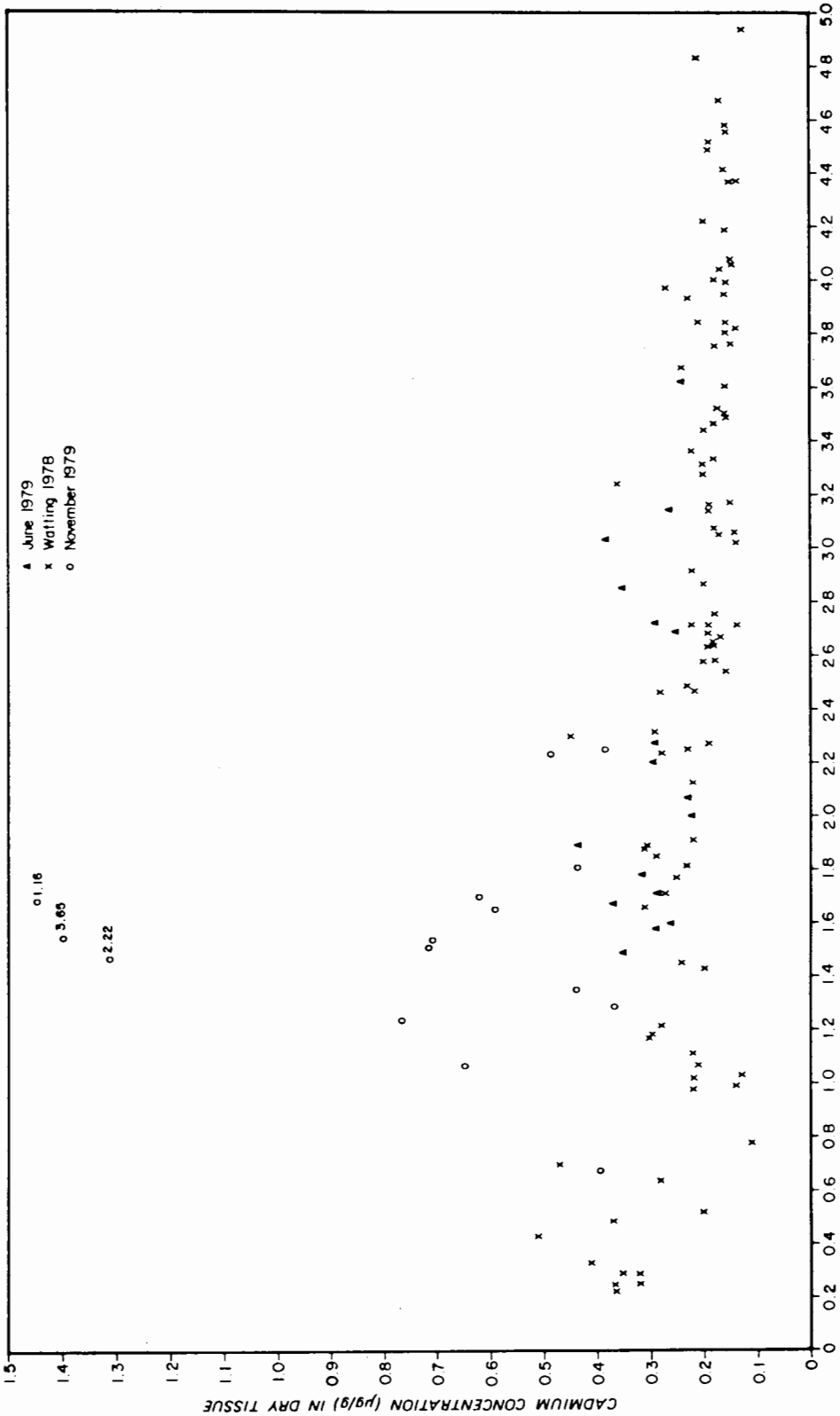


Figure 63 Cadmium content in mature C. meridionalis from Blouberg Strand

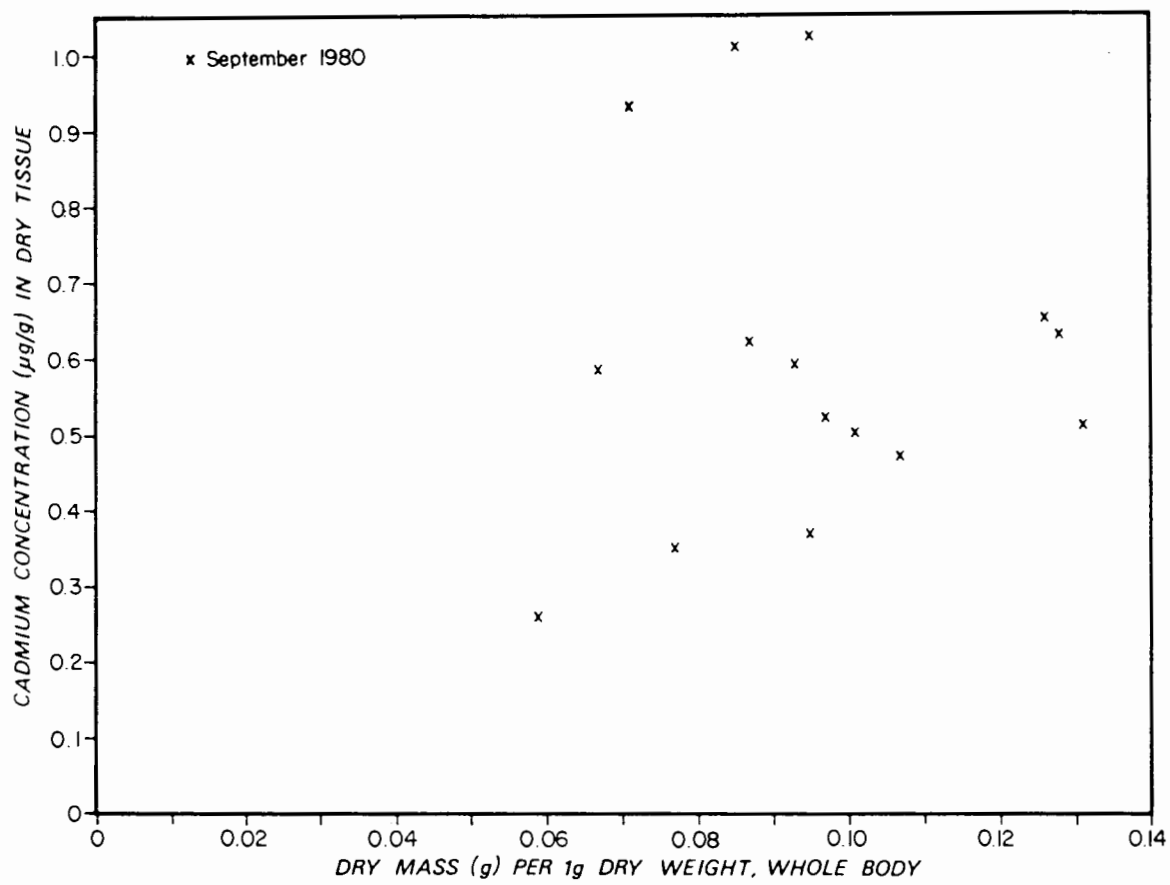


Figure 64 Cadmium content in immature C. meridionalis from Blouberg Strand

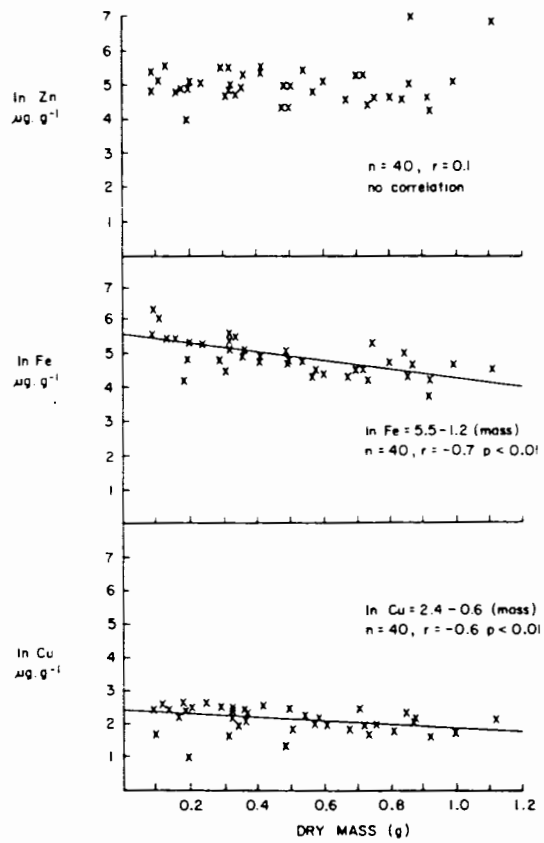


Figure 65 Metal concentrations in C. meridionalis

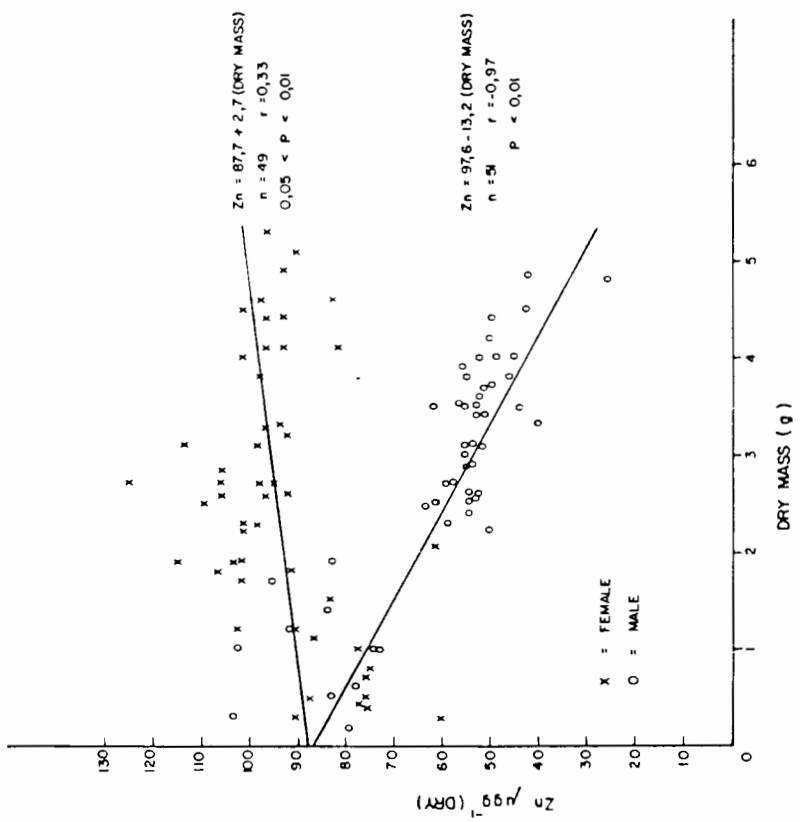


Figure 66 Zinc concentrations in black mussel (after Watling, 1978)

4.4.5 Melkbos Strand

Van As et al. (1975) conducted a survey along the littoral zone from Blouberg Strand to Bokbaai. In his report Fourie (1976) calls this Melkbos Strand.

4.4.6 Koeberg (Figure 67)

Cuthbert et al. (1976) reported B. digitalis with exceptionally high cadmium concentrations. This was followed up by later studies and comparisons (Figure 59). At the same time other animals and sediment were analysed to find the source of these high cadmium levels. The results showed a decrease in cadmium concentrations in Bullia and no high levels in sediments or other animals. The source of the high cadmium is still unknown. Cuthbert et al. (1976) did not describe the method but did state wet and dry mass of their sample animals. Wet tissue was digested and dry mass was calculated from previously established wet/dry relationships.

The present study is the first report on the metal concentrations in jellyfish. The jellyfish (2 000 ml) was heated with 10 ml redistilled Analar grade nitric acid. The sample liquified within 5 minutes. The resulting liquid was evaporated to dryness, and treated by the method used in the Strandfontein study.

When comparing these results with the results obtained for jellyfish tentacles (Cimino et al. 1983) it was found that cadmium, copper and nickel accumulated in the umbrella of the jellyfish while iron, manganese and zinc were concentrated in the tentacles. All results are expressed in terms of dry mass.

4.4.7 Langebaan (Figure 68)

Fourie (1976) presented his data on a wet-mass basis. The samples were collected by skin-divers and kept frozen at -20°C until analysed. The samples were sized, but sizes are not

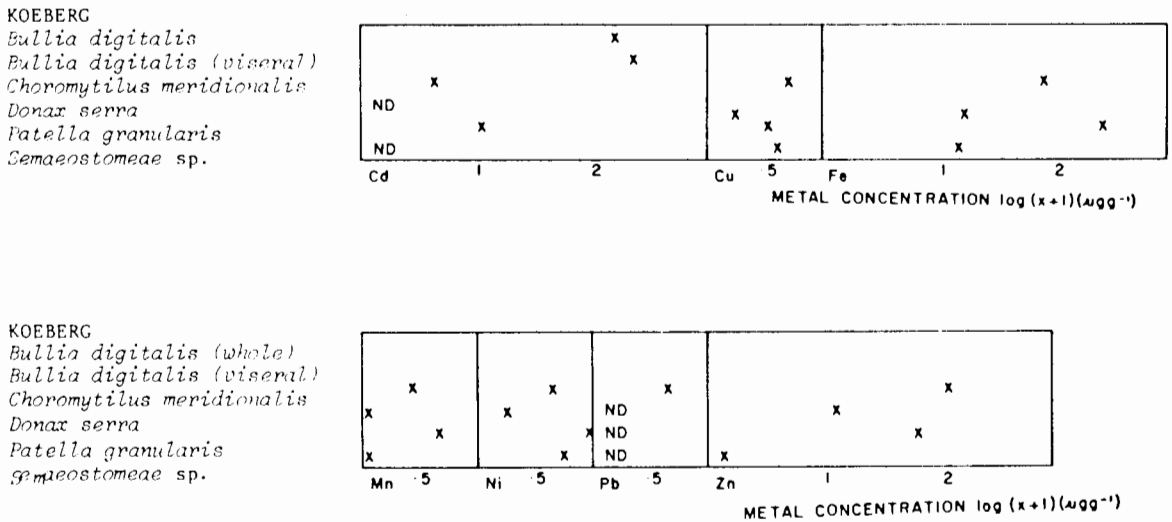


Figure 67 Metal concentrations in marine organisms from Koeberg

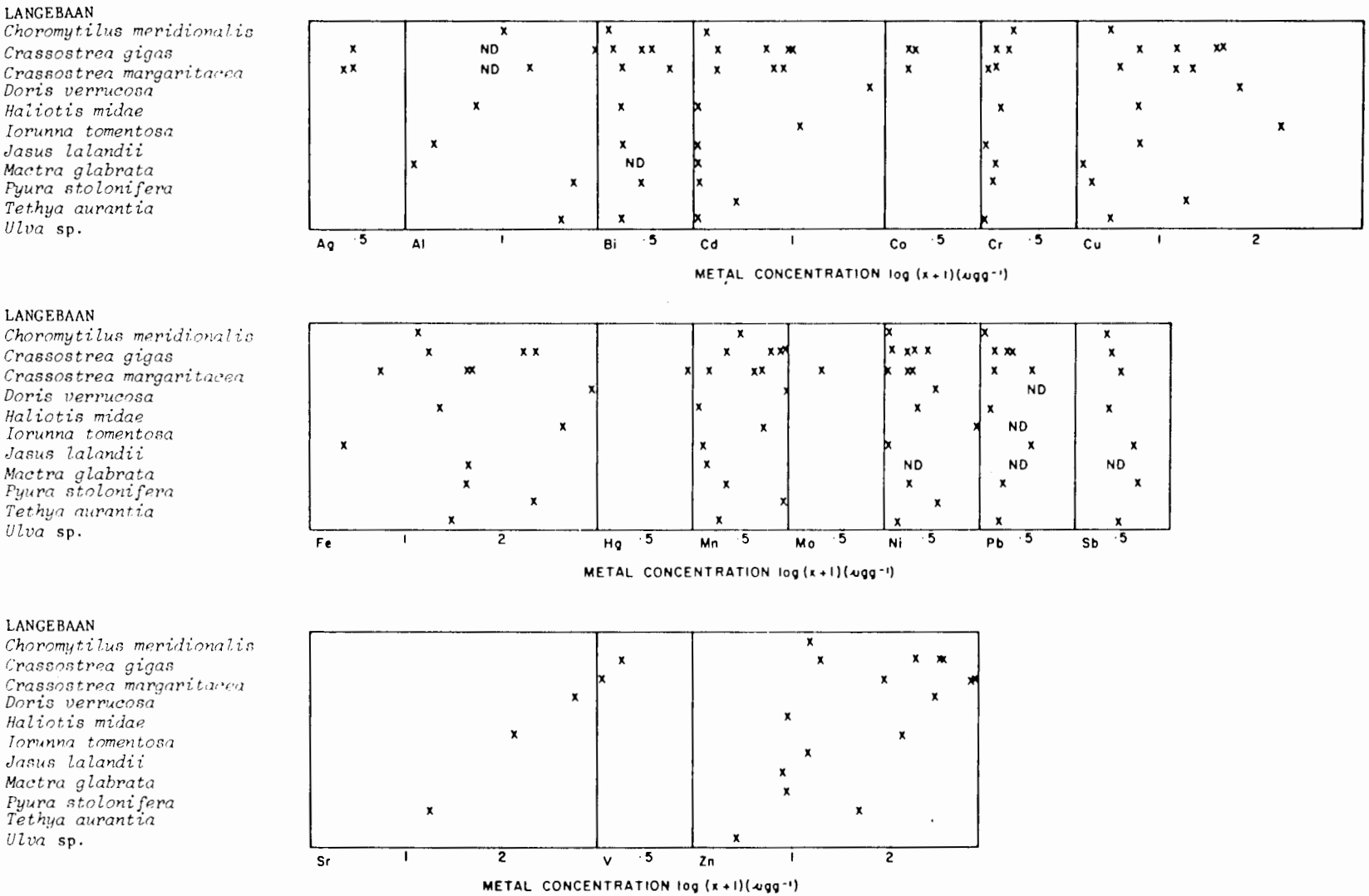


Figure 68 Metal concentrations in marine organisms from Langebaan

given. Tissues were lyophilized and wet-ashed with 30 ml atomic absorption quality (AA) nitric and 5 ml AA perchloric acids. The residues were redissolved in 1 ml concentrated hydrochloric acid and made up to 50 ml with double distilled water. About 300 organisms were sampled between October 1974 and January 1975. The data are presented per site without numbers of individuals, size, sex or mass. Metal concentrations were presented as means and sometimes standard deviation was given. Accuracy is as reported in the original paper.

Watling and Watling (1976a) quote Watling (unpublished data) on metal concentrations in various animals in terms of mean dry tissue mass.

Watling and Watling (1974) reported on the metal concentrations in some oysters which had been transplanted into the lagoon at Langebaan, and which were outside their normal habitat. The method was to dry the animals at 90°C for 48 hours and then digest them with redistilled, Analar grade nitric acid. The solutions were evaporated and the residue redissolved in 10 ml 0.1 M nitric acid. Results were calculated as µg metal per g dried tissue.

Samples for the determination of mecury were digested in redistilled, Analar grade nitric acid at 60°C under reflux. The solutions were diluted to 100 ml with double distilled water and aliquots taken for analysis. Mercury was determined by flameless atomic absorption after reduction by stannous chloride. The number of samples, dry mass, range (min, max) and mean were reported for 15 elements. This is the most complete record of elements determined.

4.4.8 Saldanha (Figure 69)

Most of the determinations were done by Watling and Watling (1974). The method was the same as that used in the Langebaan study. This again is a very comprehensive set of data.

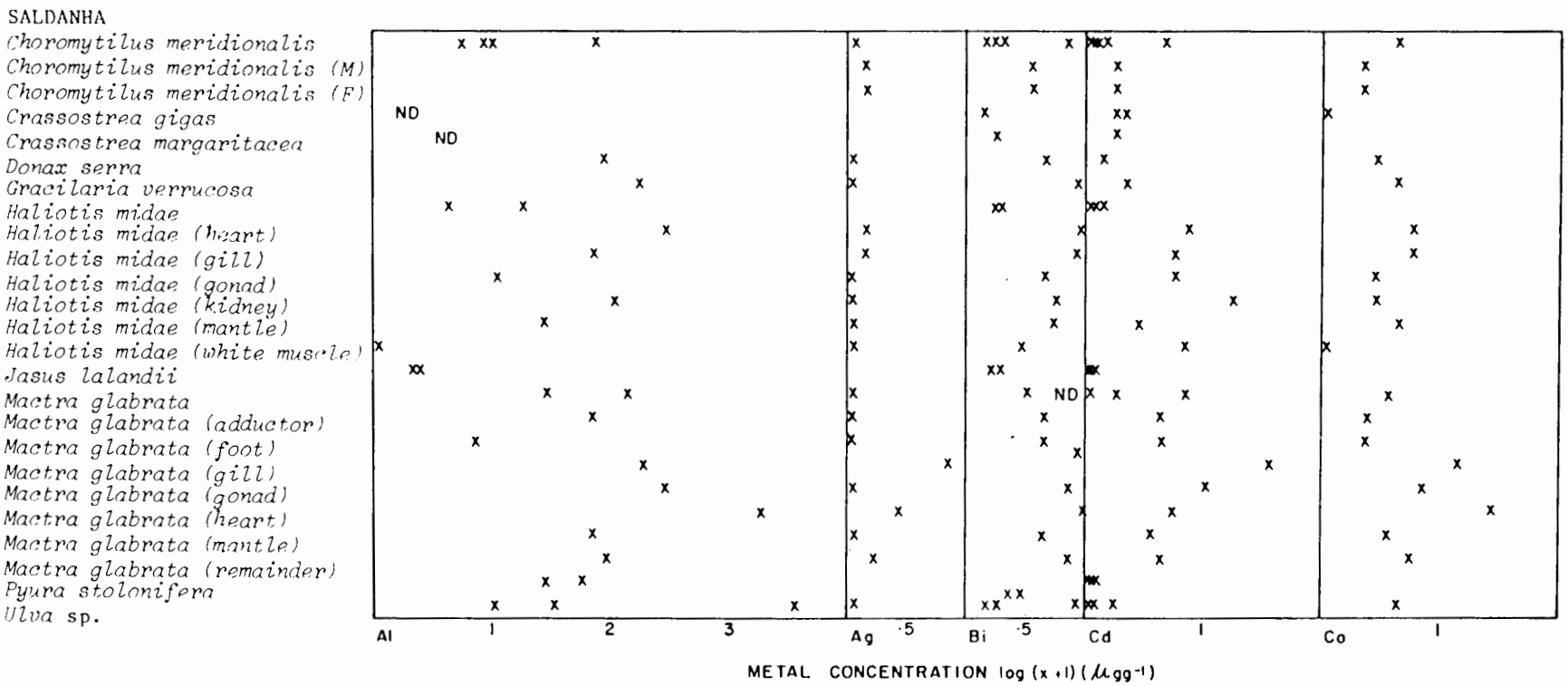
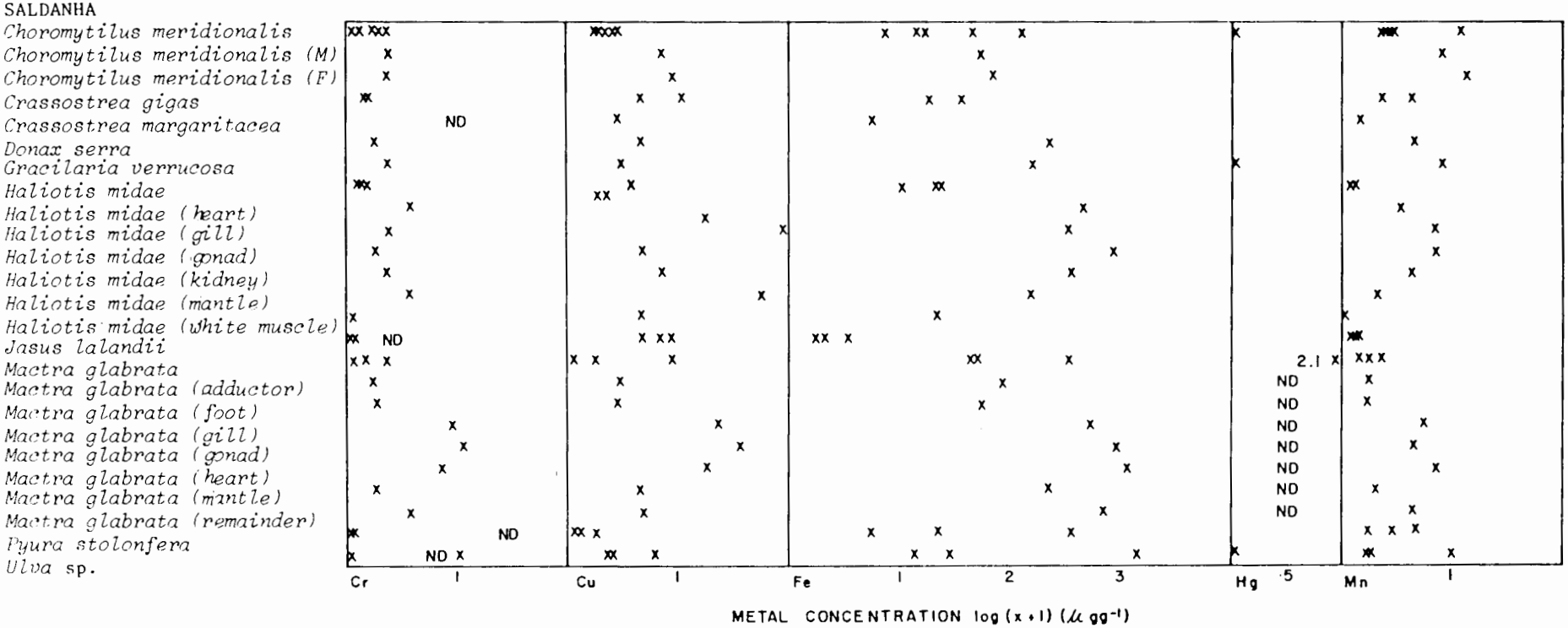
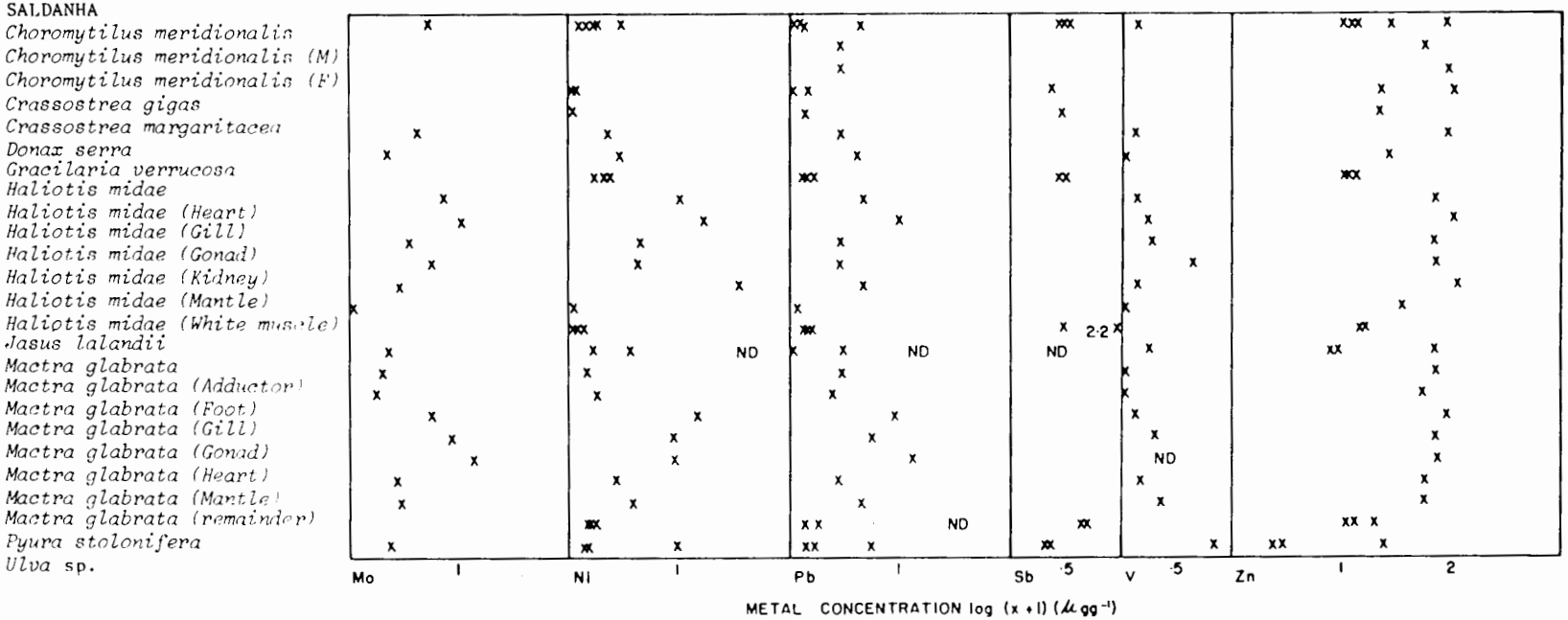


Figure 69 Metal concentrations in marine organisms from Saldanha

SALDANHA


Choromytilus meridionalis
Choromytilus meridionalis (M)
Choromytilus meridionalis (F)
Crassostrea gigas
Crassostrea margaritacea
Donax serra
Gracilaria verrucosa
Haliotis midae
Haliotis midae (heart)
Haliotis midae (gill)
Haliotis midae (gonad)
Haliotis midae (kidney)
Haliotis midae (mantle)
Haliotis midae (white muscle)
Jasus lalandii
Mactra glabrata
Mactra glabrata (adductor)
Mactra glabrata (foot)
Mactra glabrata (gill)
Mactra glabrata (gonad)
Mactra glabrata (heart)
Mactra glabrata (mantle)
Mactra glabrata (remainder)
Pyura stolonfera
Ulva sp.
ND
2.1
Cr
Cu
Fe
Hg
Mn
1
2
3
·5
METAL CONCENTRATION log (x + 1) (μ gg⁻¹)

SALDANHA


Choromytilus meridionalis
Choromytilus meridionalis (M)
Choromytilus meridionalis (F)
Crassostrea gigas
Crassostrea margaritacea
Donax serra
Gracilaria verrucosa
Haliotis midae
Haliotis midae (Heart)
Haliotis midae (Gill)
Haliotis midae (Gonad)
Haliotis midae (Kidney)
Haliotis midae (Mantle)
Haliotis midae (White muscle)
Jasus lalandii
Mactra glabrata
Mactra glabrata (Adductor)
Mactra glabrata (Foot)
Mactra glabrata (Gill)
Mactra glabrata (Gonad)
Mactra glabrata (Heart)
Mactra glabrata (Mantle)
Mactra glabrata (remainder)
Pyura stolonifera
Ulva sp.
ND
2·2
Mo 1 Ni 1 Pb 1 Sb ·5 V ·5 Zn 1 2
METAL CONCENTRATION log (x + 1) (μg g⁻¹)

Fourie (1975, 1976) may have repeated his data, but since the concentrations are different it is assumed that more information was available. His method was as that used in the Langebaan study.

John Henry (personal communication) of Sea Fisheries Research Institute, Cape Town, was kind enough to make the results from his January 1979 analyses available.

4.4.9 Noordwesbaai

The concentrations of metals in Jasus lalandii (rock lobster) tail (Figure 70a), green gland (Figure 70b) and gills (Figure 70c) are given as well as rock lobster's food mussel, Aulacomya ater (Figure 71). The results for both species were in terms of dry mass and metal data are given by Hennig, et al. (1982) but the other metals were present in such low concentrations as to show no trend.

Finally, some metal concentrations have been reported (Figure 72) in chemical analysts together with the accepted World Health Organisation's guideline values.

4.4.10 Conclusion for Section IV

The data on organisms in this section show some detail and enable some comparisons to be made. From these the shortcomings of baseline studies have emerged, but more important, the shortcomings of inadequate reporting are highlighted. Data presented here show that metal concentrations without detail of the animal size, weight, sex, dry and wet mass may give misleading baseline values.

Large sections of the coast have not been covered and there are still large gaps in our knowledge of metals in important commercial species.

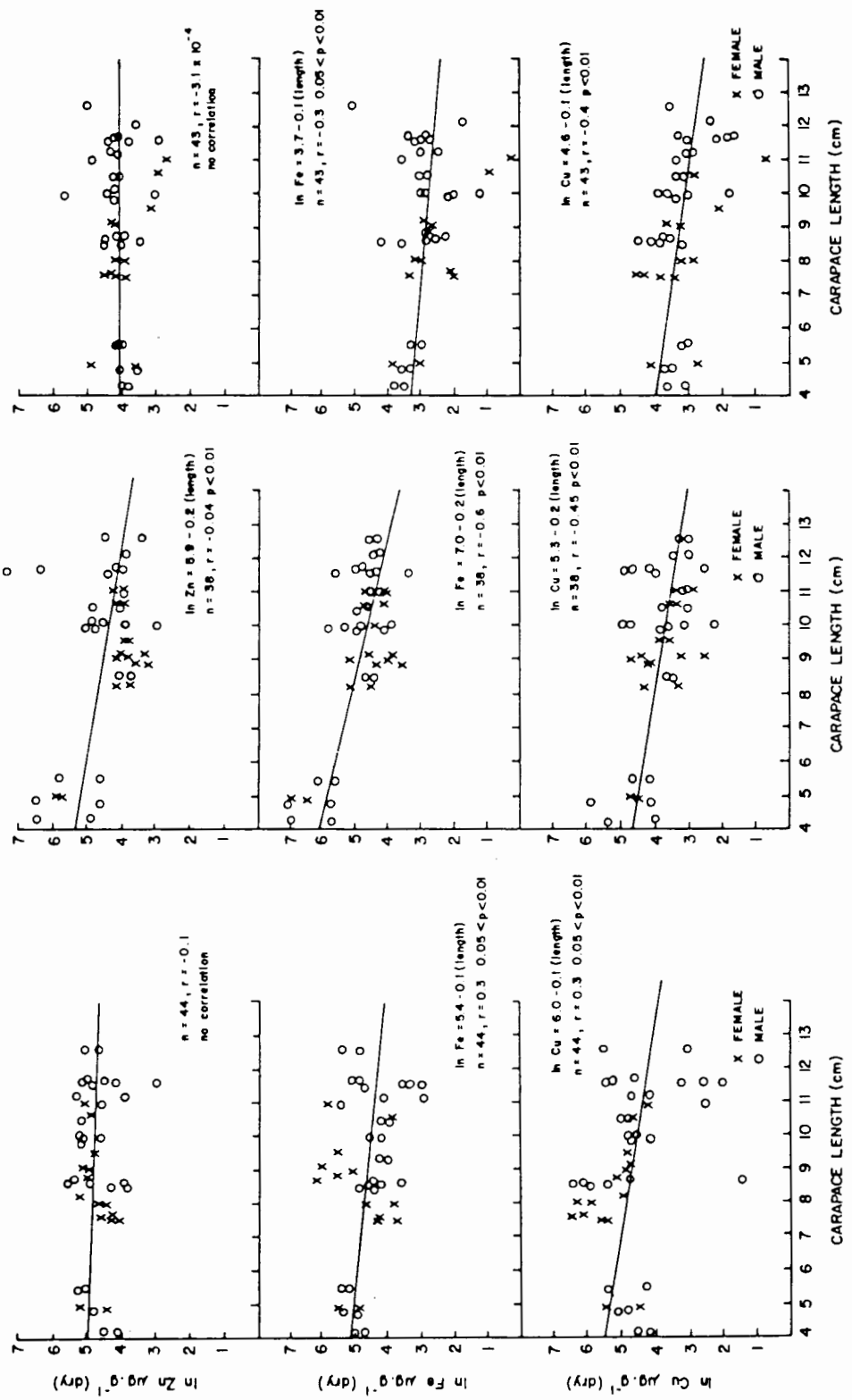


Figure 70a Metal concentrations in rock lobster tails (left)

Figure 70b Metal concentrations in rock lobster green gland (middle)

Figure 70c Metal concentrations in rock lobster gills (right)

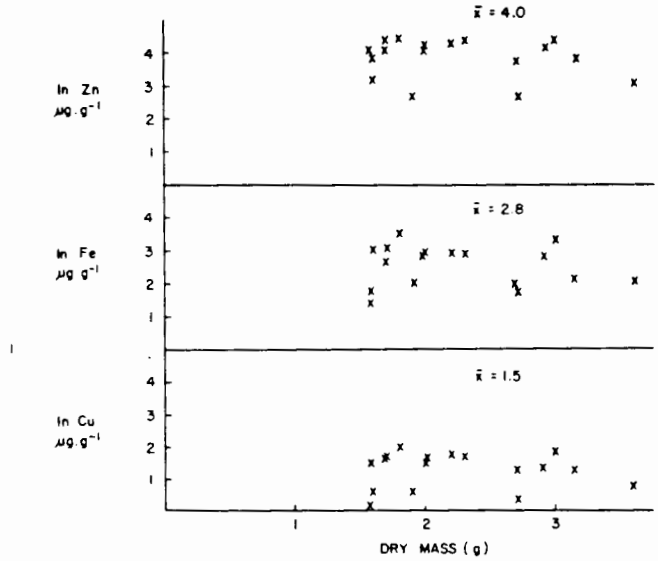


Figure 71 Metal concentrations in ribbed mussels, Aulacomya ater

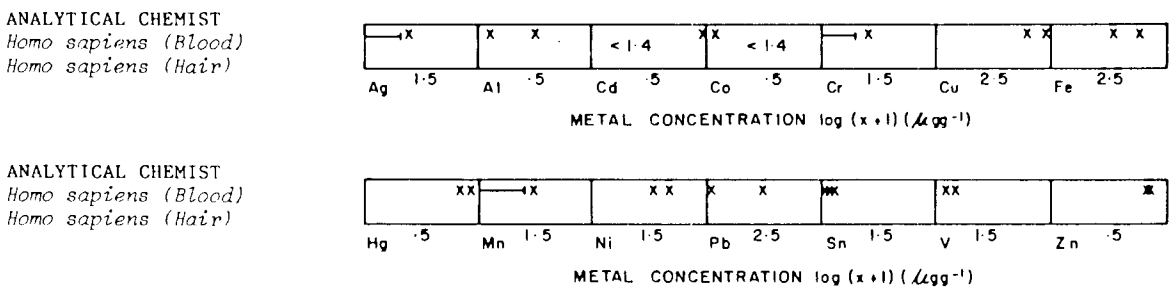


Figure 72 Metal concentrations in chemical analysts

4.5 Section V - Cape Columbine to Orange River

Although water and sediment data are available for this section, no determinations of trace metals in biological specimens have been done. Since there is very little industry along this stretch of coast, very valuable background data about the west coast could be gathered there.

5. METAL BURDENS IN DIFFERENT SPECIES

In some cases the location of an organism is not as important as the type of animal. In this section the metal concentrations in individual species are given as they vary with location.

It is hoped that it will be more useful if organisms were grouped into phyla. The system adopted is the classification system presented by Day (1974), who reported further detail on the distributions of the various animals and plants mentioned in this study.

5.1 Plankton (Figure 73)

In some cases samples were a mixture of algae and animals such as copepods. Plankton are known to have high metal concentrations (Hennig, 1981). Some of the data are expressed in terms of wet mass, some as dry mass. However it is still evident that the east coast plankton samples contain very high amounts of metals. This could be due to the metal enriched water around Kosi Bay.

5.2 Porifera (sponges) (Figure 74)

Only one sponge species Tethya aurantia, has been analysed for metal. This sponge is utilised as food by some of the nudibranchs at Langebaan. The metal concentrations were found to be low and related to the concentration of metals in the water.

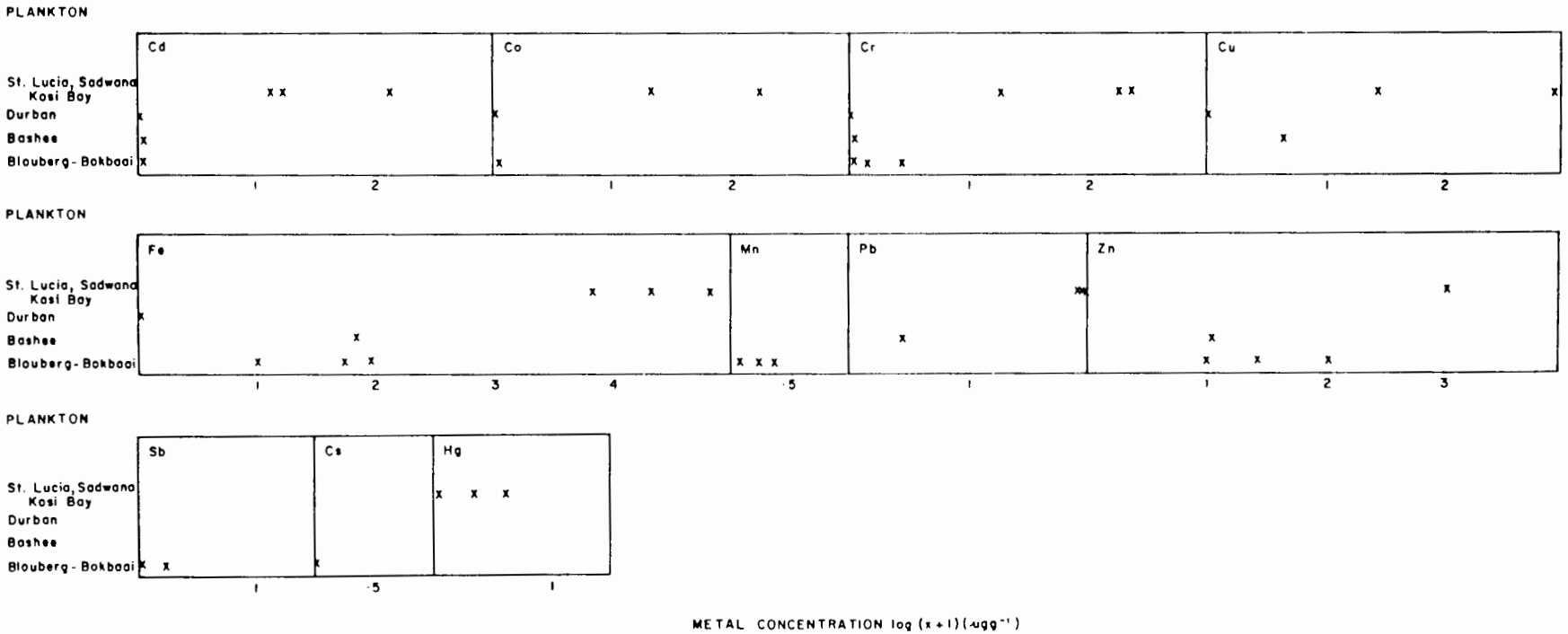


Figure 73 Metal concentrations in plankton

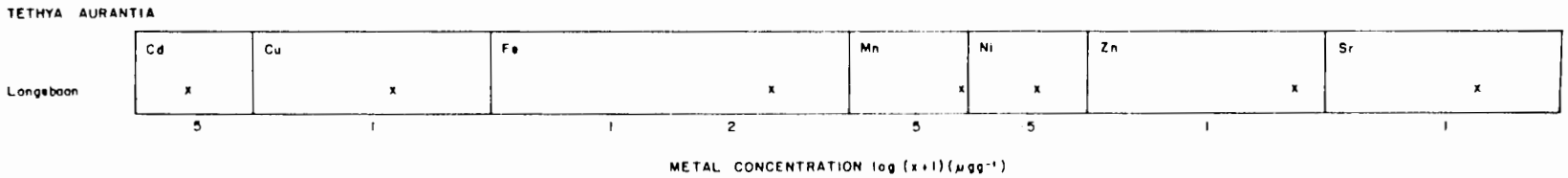


Figure 74 Metal concentrations in Porifera (sponge)

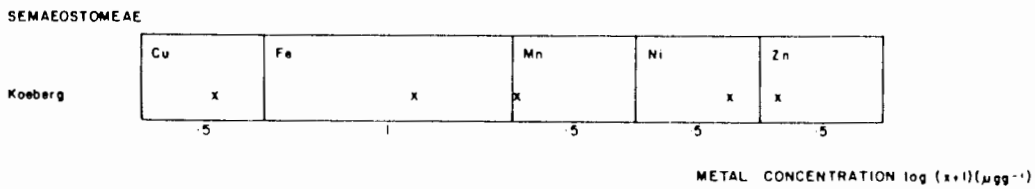


Figure 75 Metal concentrations in Cnidaria (jelly fish)

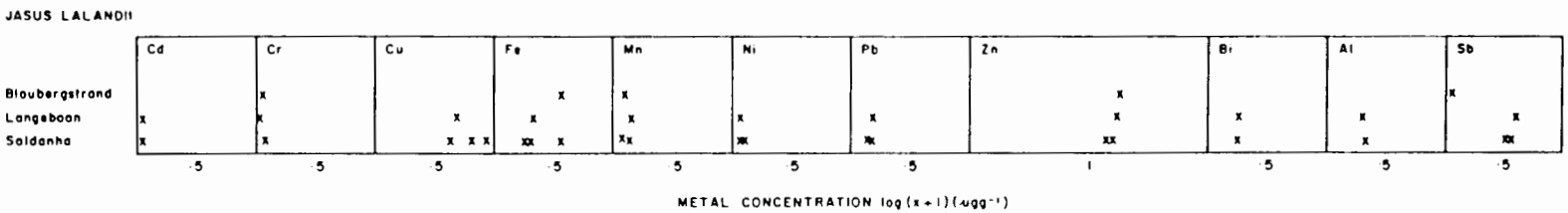


Figure 76 Metal concentrations in Jasus lalandii

5.3 **Cnidaria** (jellyfish) (Figure 75)

Only one jellyfish species Semaeostomeae sp. has been analysed. There was no metal accumulation in these jellyfish in relation to their size. More information is provided under the Koeberg location section.

5.4 **Arthropoda**

5.4.1 Macrura (lobsters, shrimps and prawns)

Jasus lalandii (Figure 76), Panulirus homarus (Figure 77), Panulirus versicolor (Figure 78), Penaeus indicus (Figure 79), Penaeus monodon (Figure 80).

There is a decreasing metal concentration from north to south, with lower metal concentrations on the west coast. Only J. lalandii has been studied in detail (see diagrams of Noordwesbaai location).

5.4.2 Anomura (hermit crabs and burrowing prawns)

Callianona sp. and Callianassa kraussi (Figure 81), Emerita austroafricana (Figure 82), Upogebia africana (Figure 83).

Animals in the industrialised areas contained higher metal burden.

5.4.3 Brachyura (crabs)

Although crabs are common all along the coast of South Africa, only one species Scylla serrata (Figure 84) has been analysed.

5.5 **Mollusca**

5.5.1 Pelecypoda (bivalves)

These include Atrina squamifera (Figure 85), Crassostrea cucullata (Figure 86), Crassostrea gigas (Figure 87), Crassostrea margaritacea (Figure 88), Choromytilus meridionalis (Figure 89),

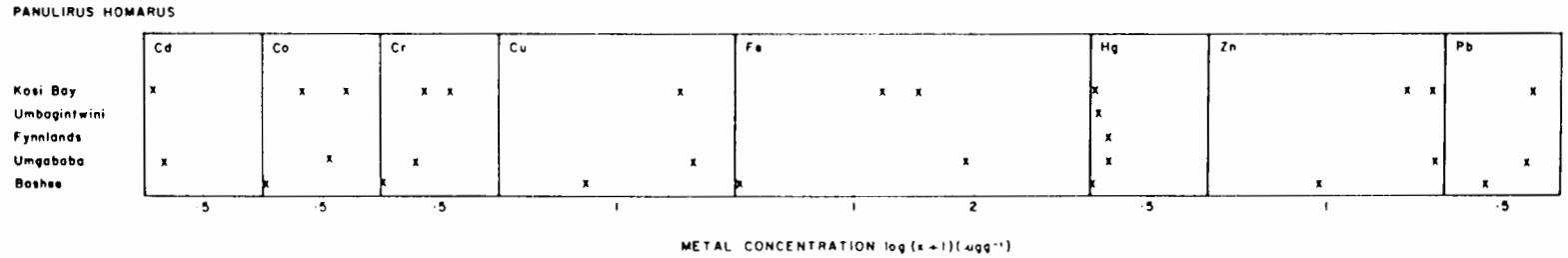


Figure 77 Metal concentrations in Panulirus homarus

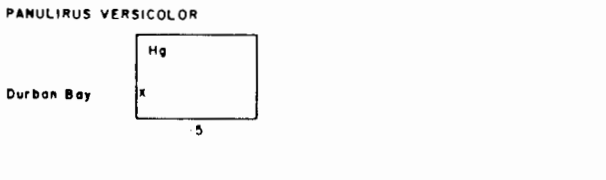


Figure 78 Metal concentrations in Panulirus versicolor

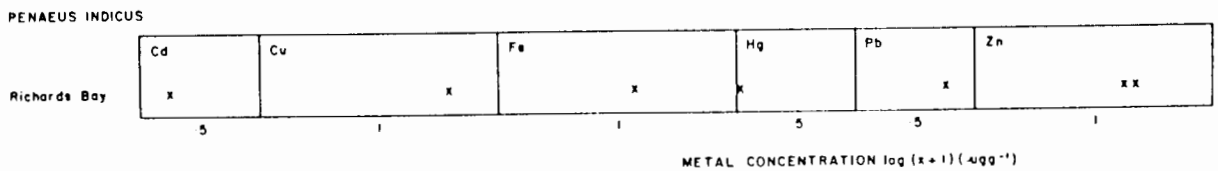


Figure 79 Metal concentrations in Penaeus indicus

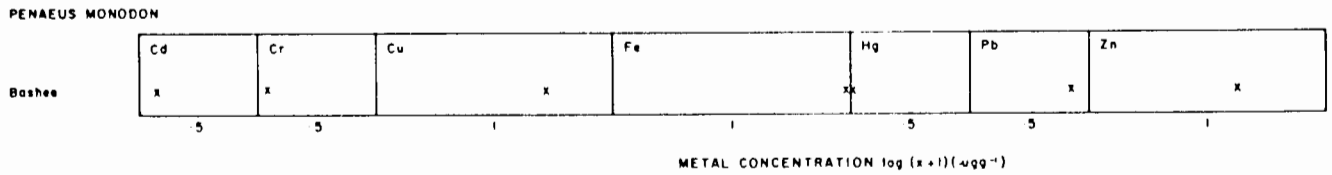


Figure 80 Metal concentrations in Penaeus monodon

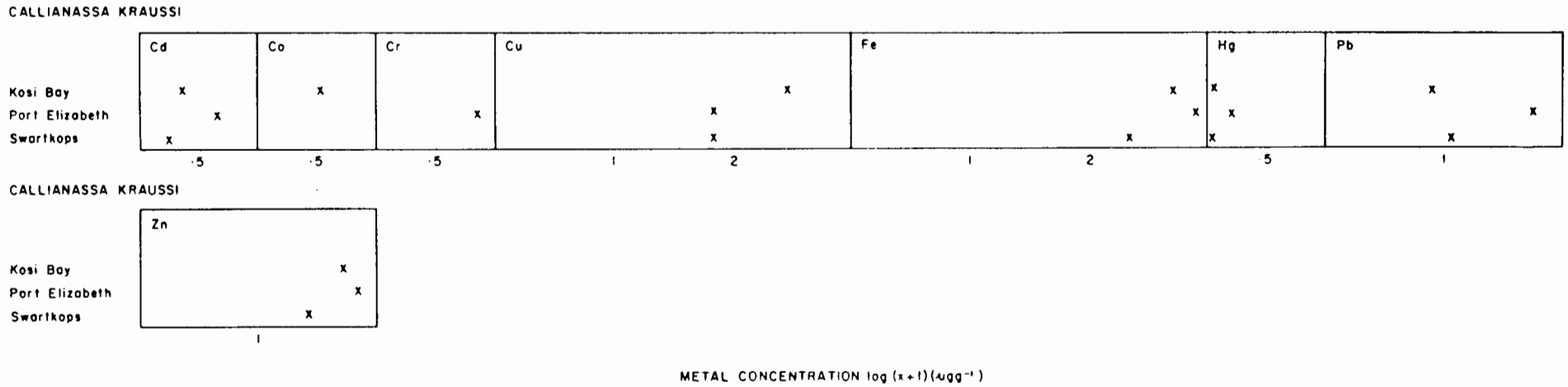


Figure 81 Metal concentrations in Callianassa kraussi

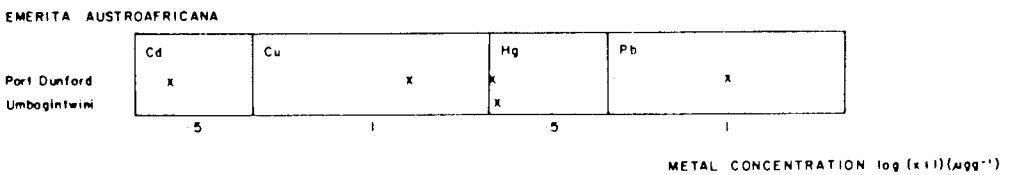


Figure 82 Metal concentrations in Emerita austroafricana

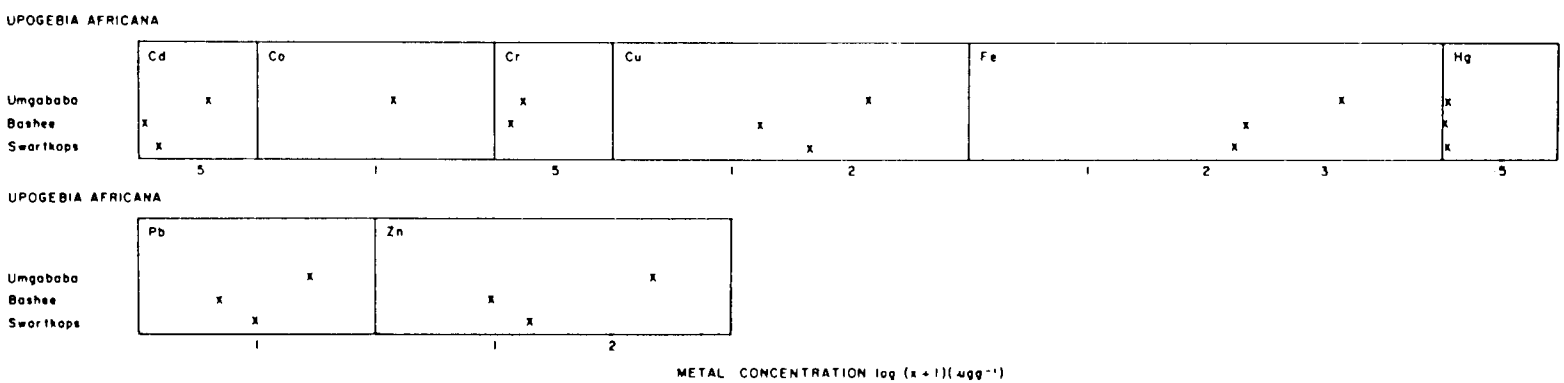


Figure 83 Metal concentrations in Upogebia africana

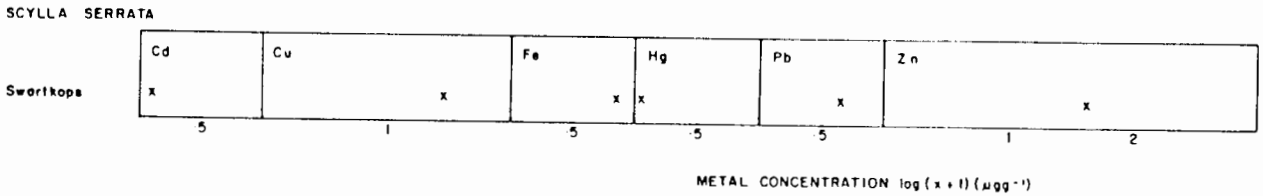


Figure 84 Metal concentrations in Scylla serrata

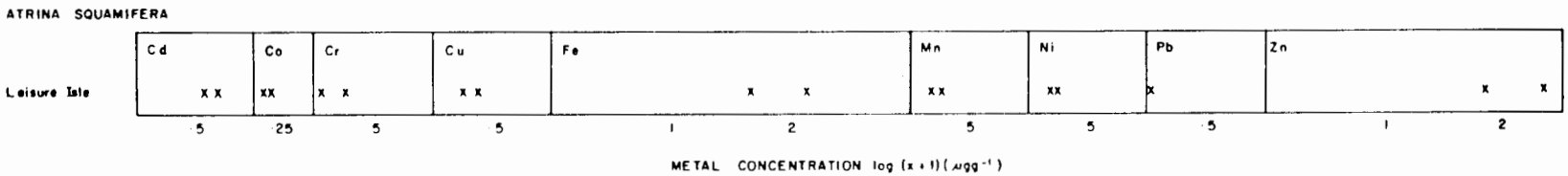


Figure 85 Metal concentrations in Atrina squamifera

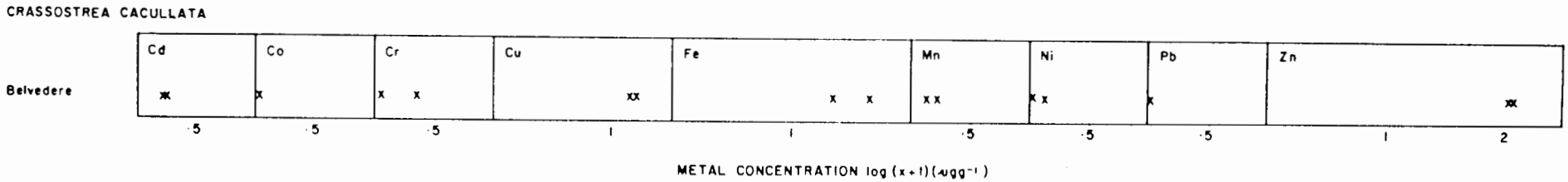


Figure 86 Metal concentrations in Crassostrea cucullata

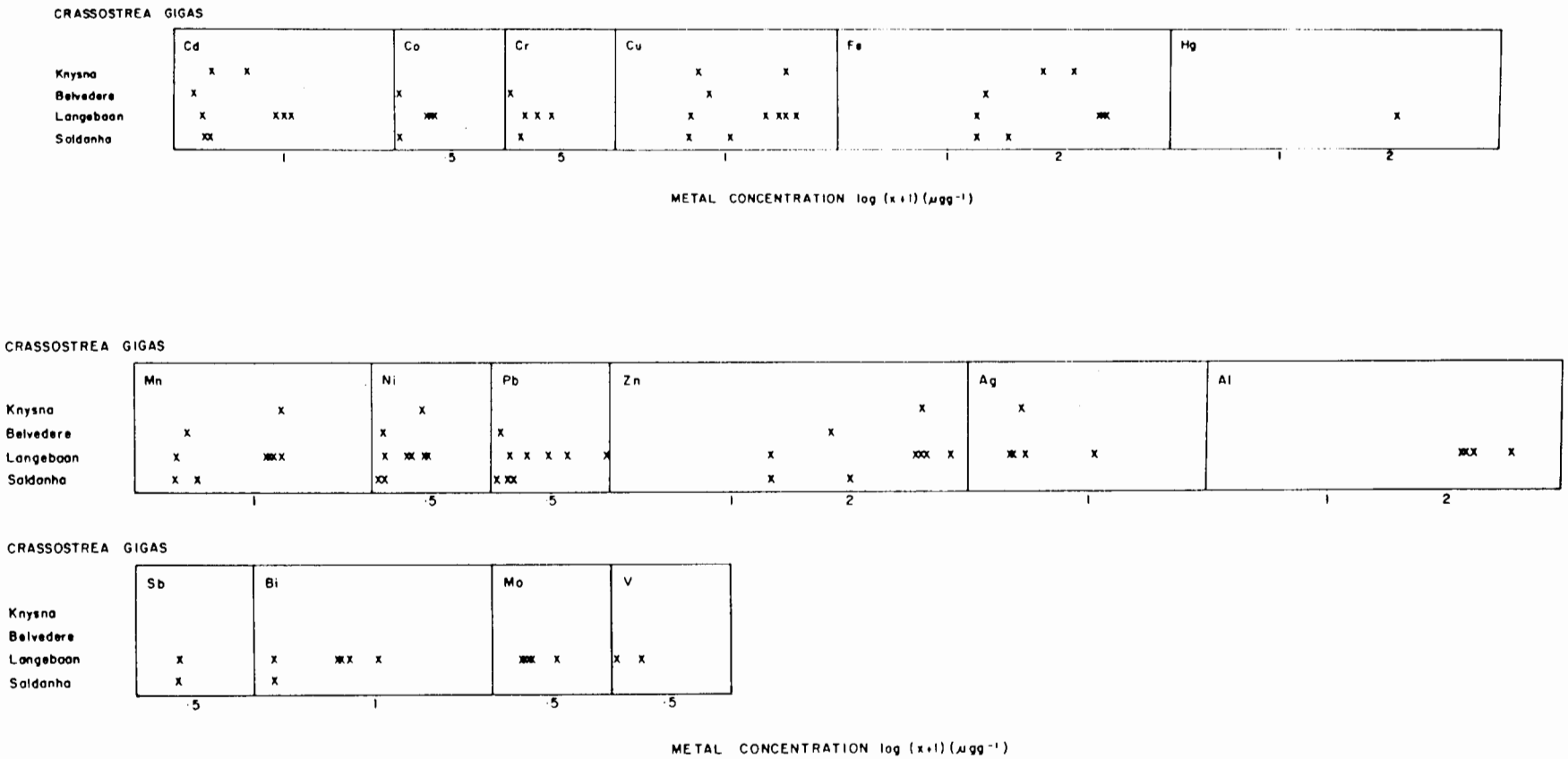


Figure 87 Metal concentrations in Crassostrea gigas

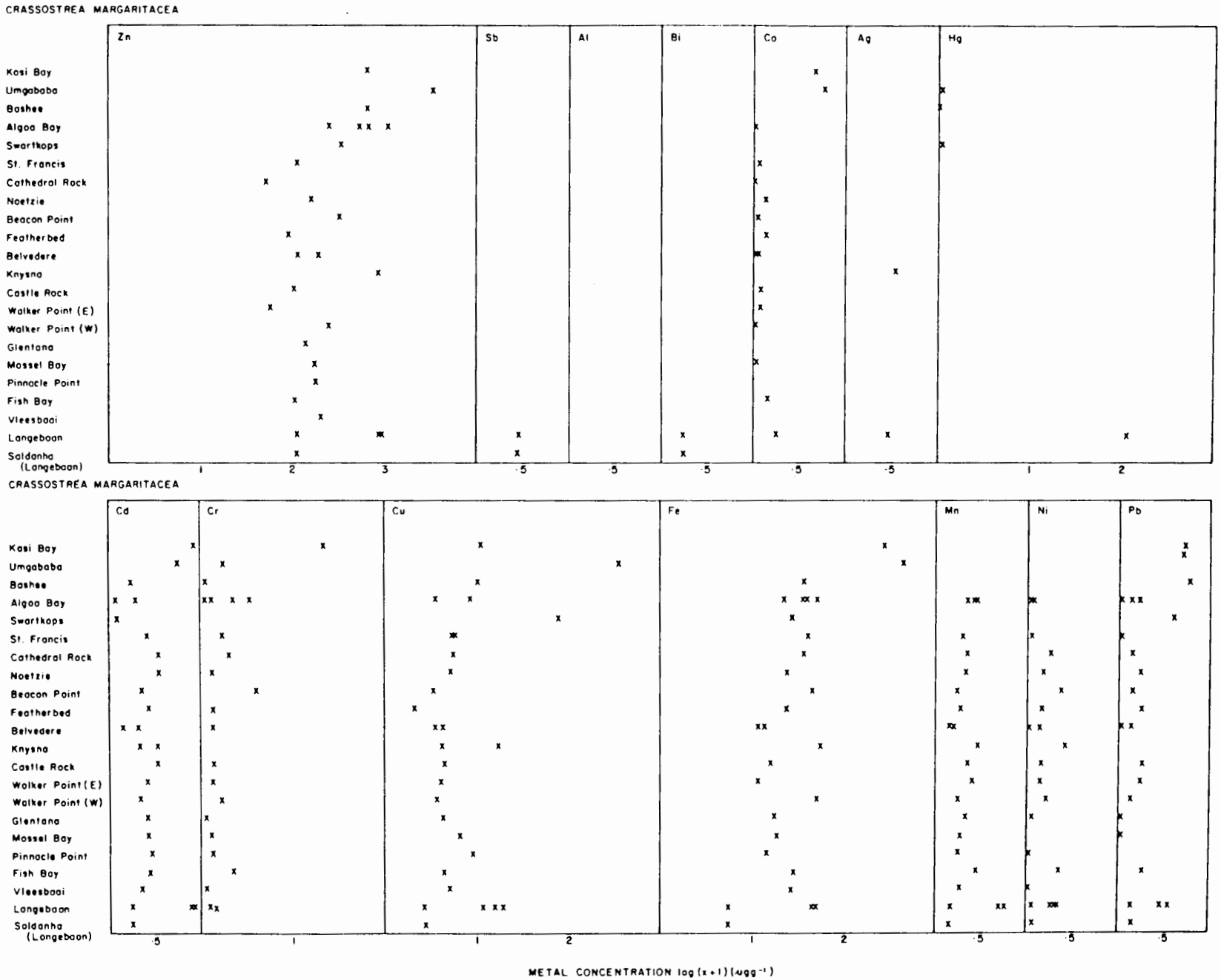


Figure 88 Metal concentrations in Crassostrea margaritacea

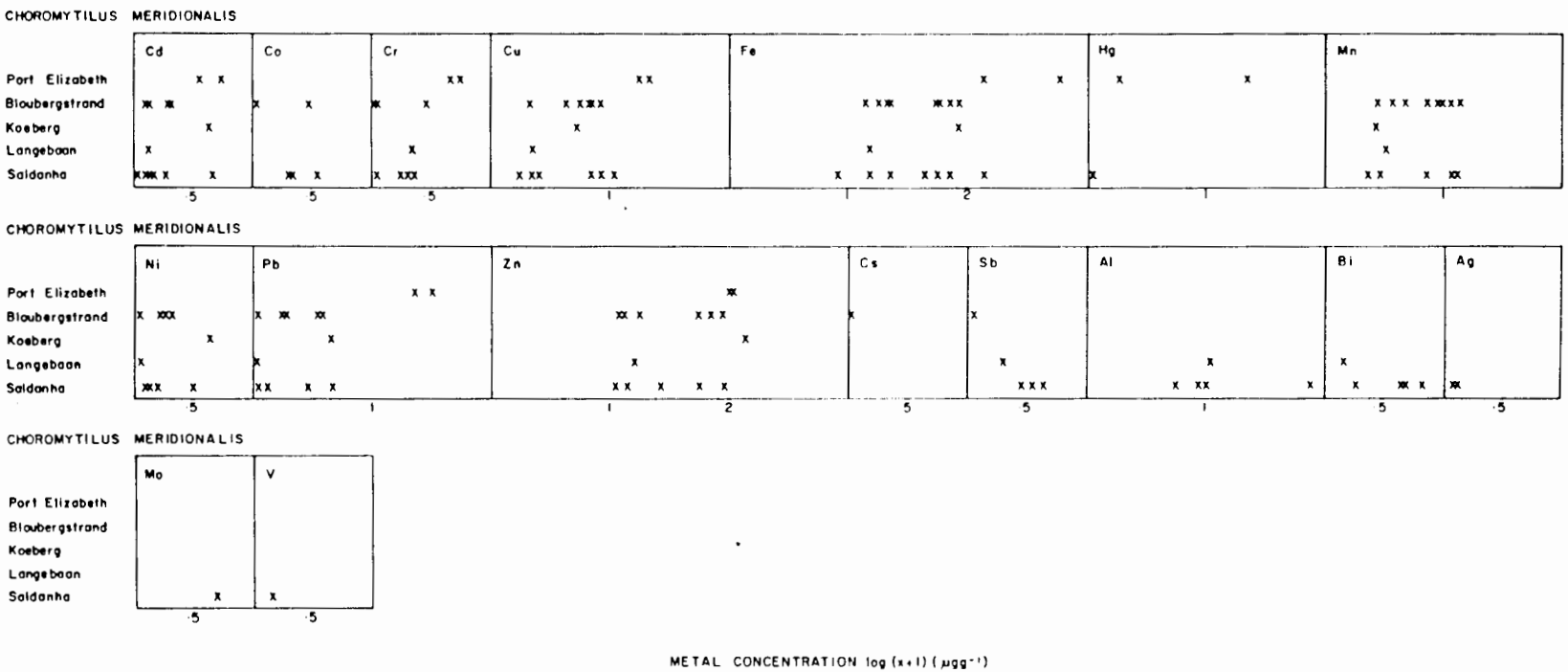


Figure 89 Metal concentrations in Choromytilus meridionalis

Donax serra (Figure 90), Mactra glabrata (Figure 91), Ostrea atherstonei (Figure 92), Ostrea edulis (Figure 93), Perna perna (Figure 94), Solen capensis (Figure 95), Venus verrucosa (Figure 96).

Bivalves exhibit several characteristics of ideal indicator species (Eisler, 1981). In general, the highest concentrations are in the gut and digestive gland, with moderate enrichment in mantle, gills and gonads, and lowest residuals in muscle.

C. margaritacea (Figure 88) gives the best sampling distribution pattern. Not all metals exhibit the same trends and it would be expected that impact areas would be easily identifiable. This is, unfortunately, not the case. There seems to be a local "hot spot" between Pinnacle Point and Fish Bay. The distribution of zinc is very varied and there appear to be factors other than environment which influence the accumulation of zinc.

C. meridionalis has been studied in detail regarding local differences in metal accumulation (see Figures 63 to 65) but little is known about metal accumulation at different locations.

D. serra was sampled at many locations. Again a more varied zinc content was found. Copper seems to decrease and lead to increase from east to west. There is little difference between concentrations of the other metals with geographical distribution.

The other well represented species is P. perna (Figure 94). At the industrial impact areas there was a greater variation in metal body burden, but the concentrations are much more consistent throughout the region when compared with the metal burdens in the oysters. Of the unpolluted areas, Fish Bay seems to induce the accumulation of more metals in molluscs.

For baseline metal concentration data, three species C. margaritacea, D. serra and P. perna provide the most complete record of metal accumulation in South Africa. When the results

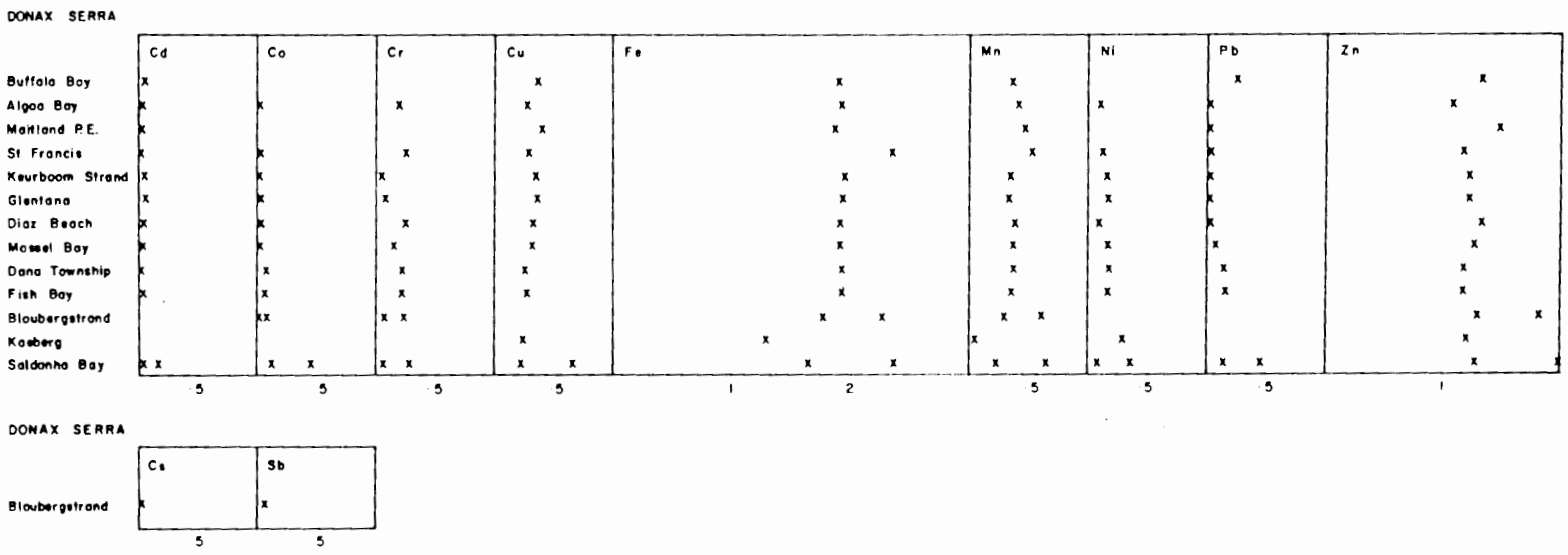


Figure 90 Metal concentrations in Donax serra

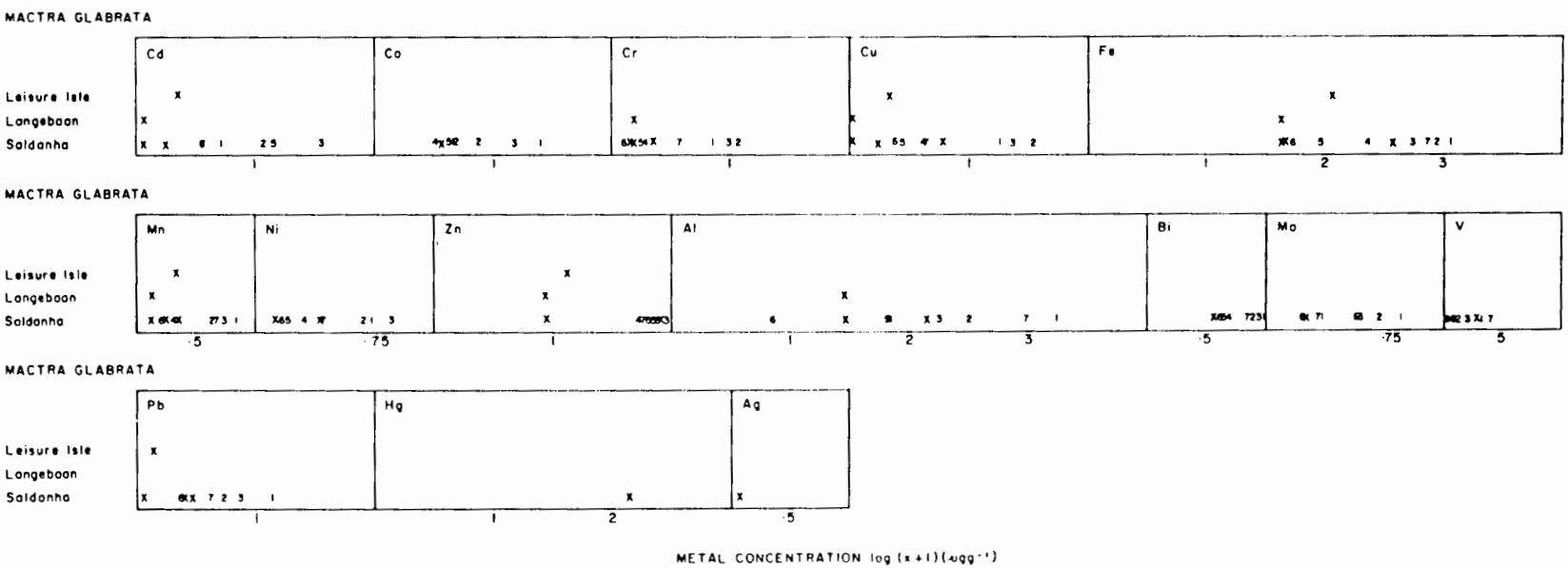


Figure 91 Metal concentrations in Mactra glabrata (1 = heart, 2 = gonad, 3 = gill, 4 = mantle, 5 = adductor muscle, 6 = foot, 7 = remainder)

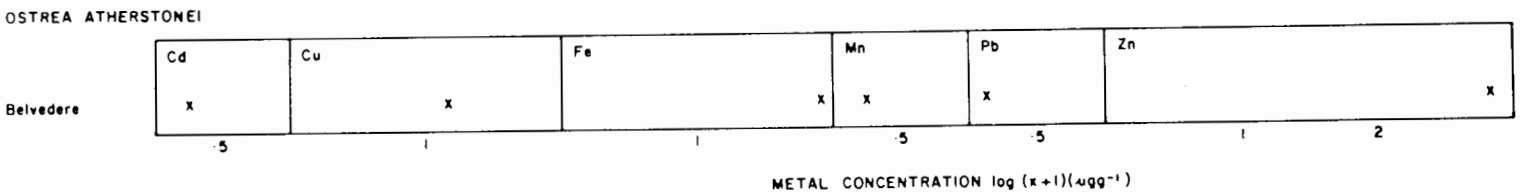


Figure 92 Metal concentrations in Ostrea atherstonei

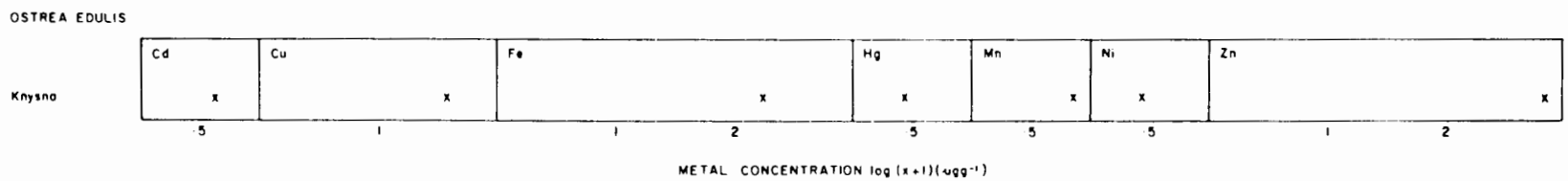


Figure 93 Metal concentrations in Ostrea edulis

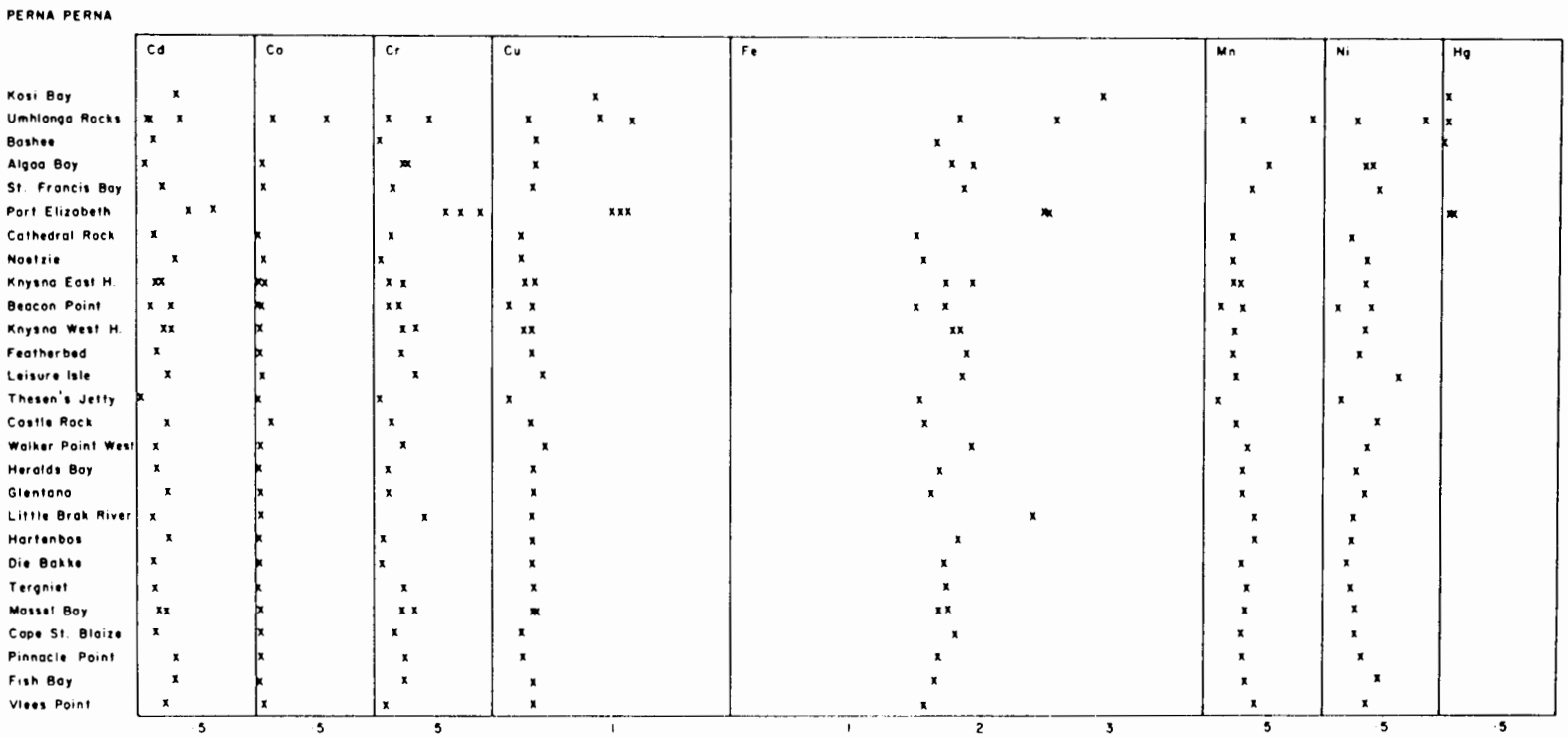
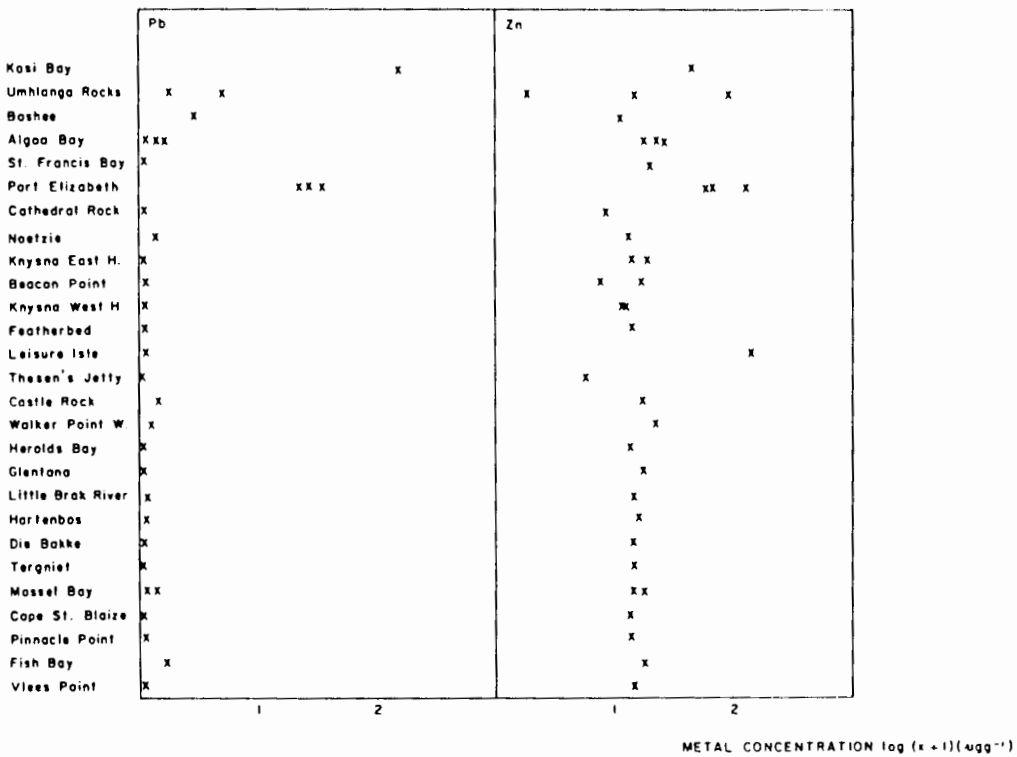


Figure 94 Metal concentrations in Perna perna

PERNA PERNA



METAL CONCENTRATION log (x + 1)(ugg^{-1})

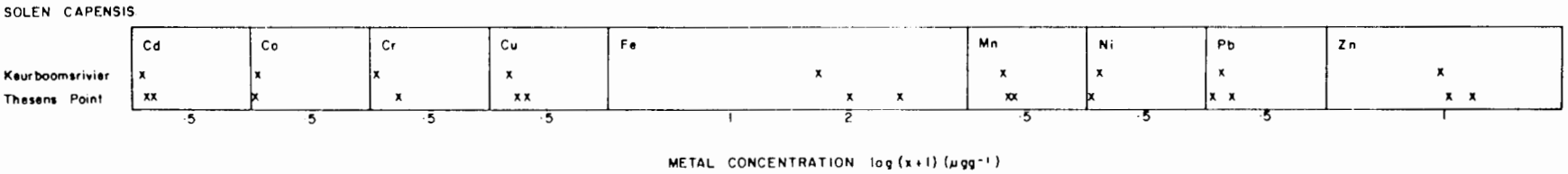


Figure 95 Metal concentrations in Solen capensis

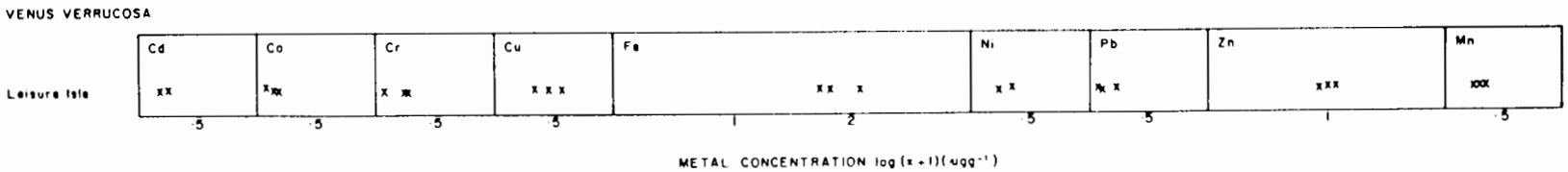


Figure 96 Metal concentrations in Venus verrucosa

for C. margaritacea and P. perna are compared with those of the Mussel Watch (Goldberg et al., 1983) it is found that cadmium, copper, nickel concentrations were lower in South Africa, while lead and zinc levels are similar, on average, in both countries.

5.5.2 Gastropoda (whelks, limpets and slugs)

5.5.2.1 Whelks

Bullia digitalis (Figure 97, see also Figure 59), Bullia natalensis (Figure 98), Bullia rhodostoma (Figure 99), Bullia sp. (Figure 100), Burnupena cincta (Figure 101), Haliotis midae (Figure 102).

5.5.2.2 Limpets

Patella argenvillei (Figure 103), P. barbara (Figure 104), P. cochlear (Figure 105), P. granularis (Figure 106), P. longicosta (Figure 107), P. miniata (Figure 108), P. oculus (Figure 109), P. tabularis (Figure 110).

5.5.3 Slugs

Doris verrucosa (Figure 111), Iorunna tomentosa (Figure 112).

Some comparisons of metal levels in Bullia have been done (Figure 59a-e) and no reason could be found for the high cadmium concentration in the animals from Koeberg, although the problem is still receiving attention. B. rhodostoma has been sampled over a wide enough region to be of value as a baseline study. The data from Port Elizabeth (Figure 99) show that Bullia makes a good indicator species for Cd, Cr, Cu, Fe, Pb and Zn. The trend lines are more uniform as in the case of mussels and oysters. This is somewhat surprising as Bullia is a scavenger.

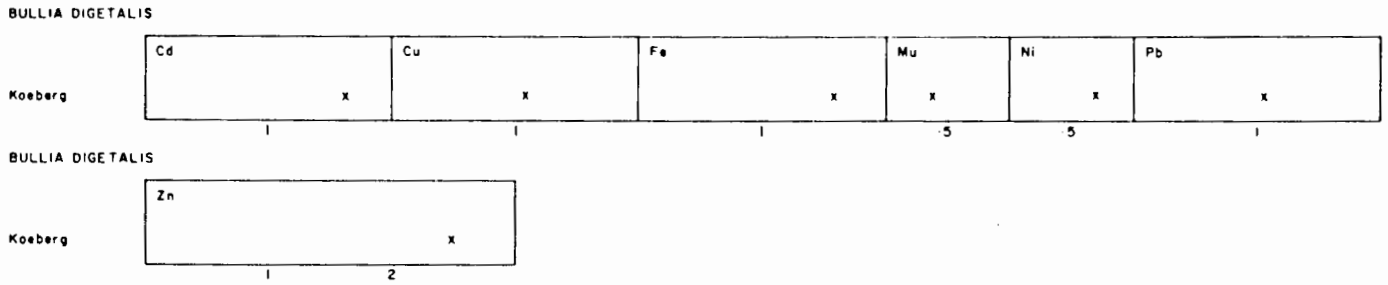


Figure 97 Metal concentrations in Bullia digitalis

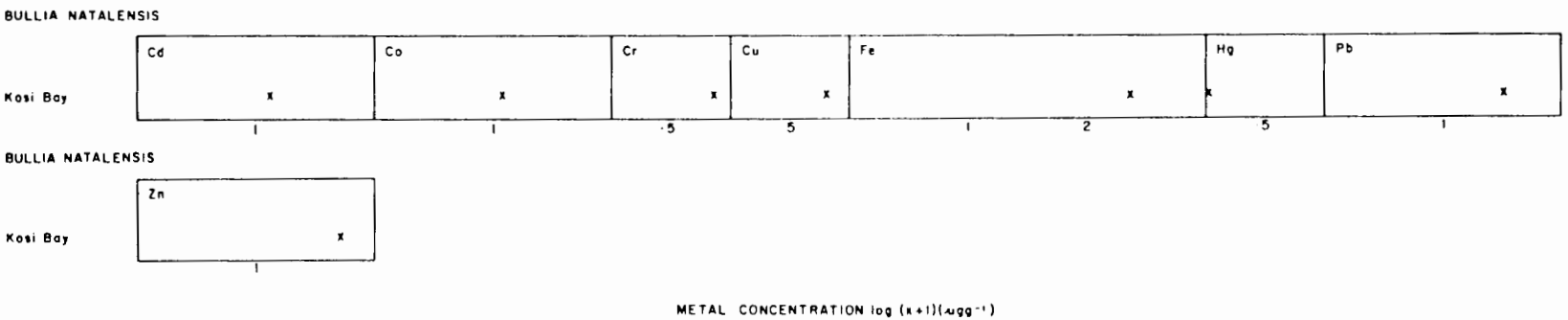


Figure 98 Metal concentrations in Bullia natalensis

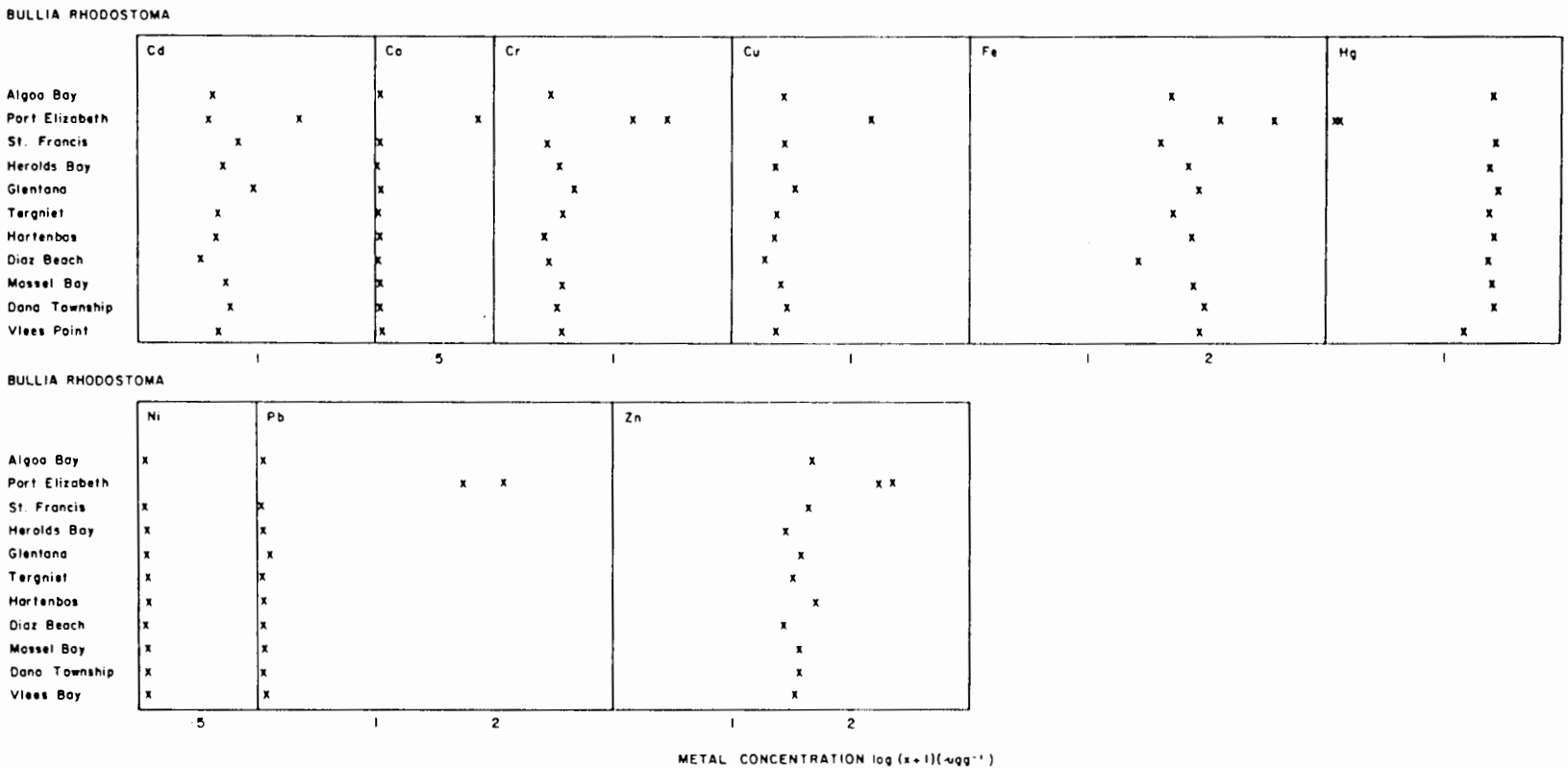


Figure 99 Metal concentrations in Bullia rhodostoma

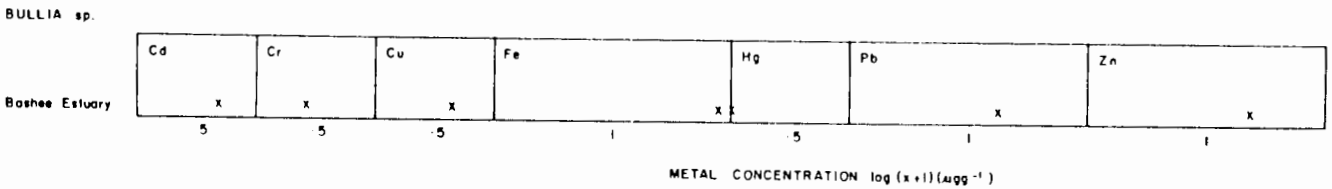


Figure 100 Metal concentrations in Bullia sp

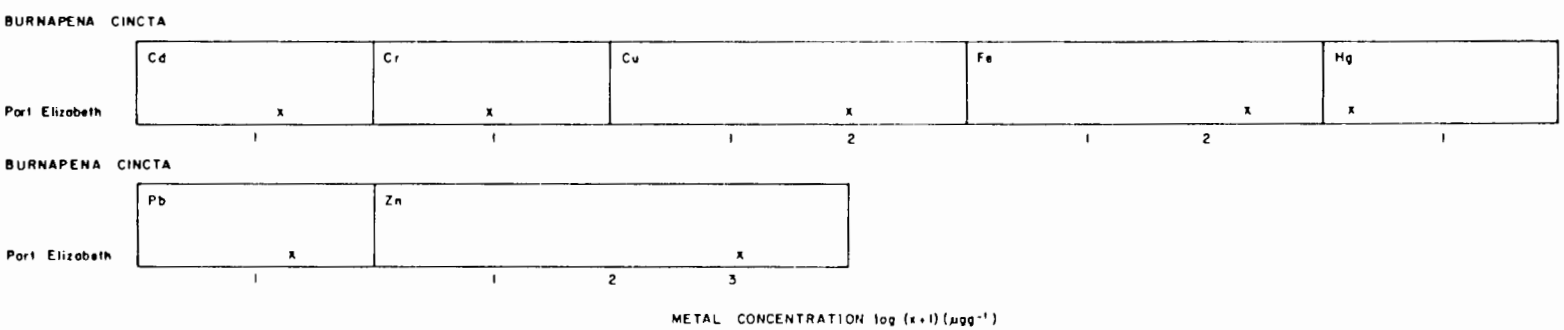


Figure 101 Metal concentrations in Burnupena cincta

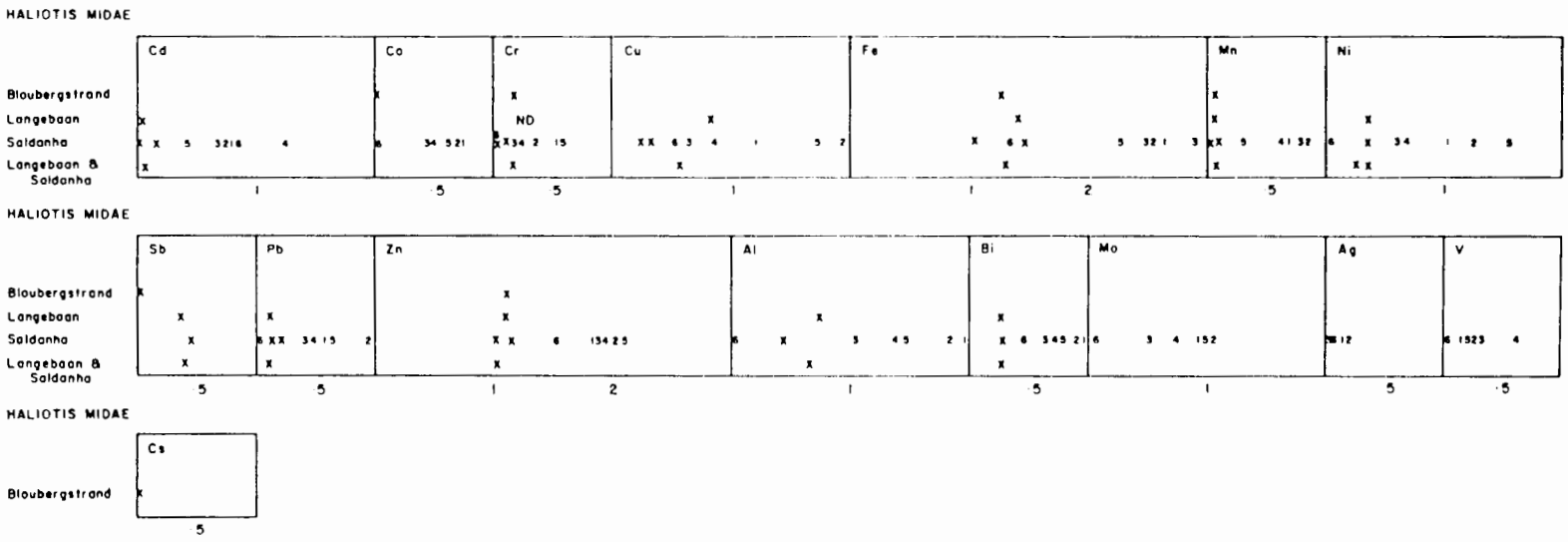


Figure 102 Metal concentrations in Haliotis midae (1 = heart, 2 = gill, 3 = gonad, 4 = kidney, 5 = mantle, 6 = white muscle)

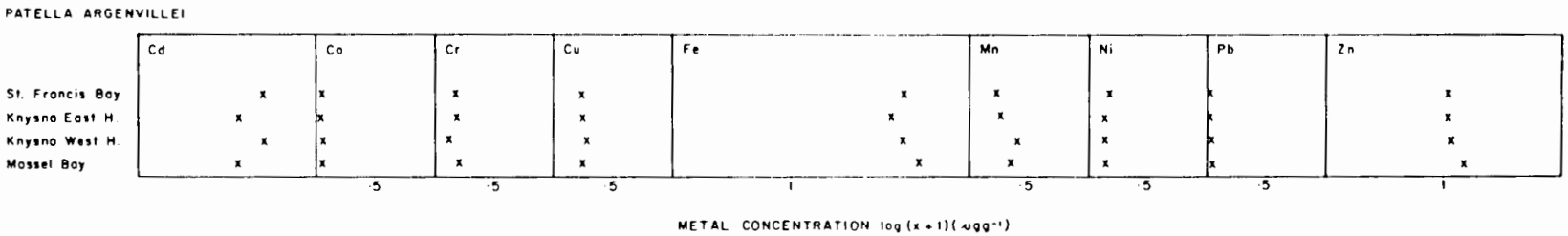


Figure 103 Metal concentrations in Patella argenvillei

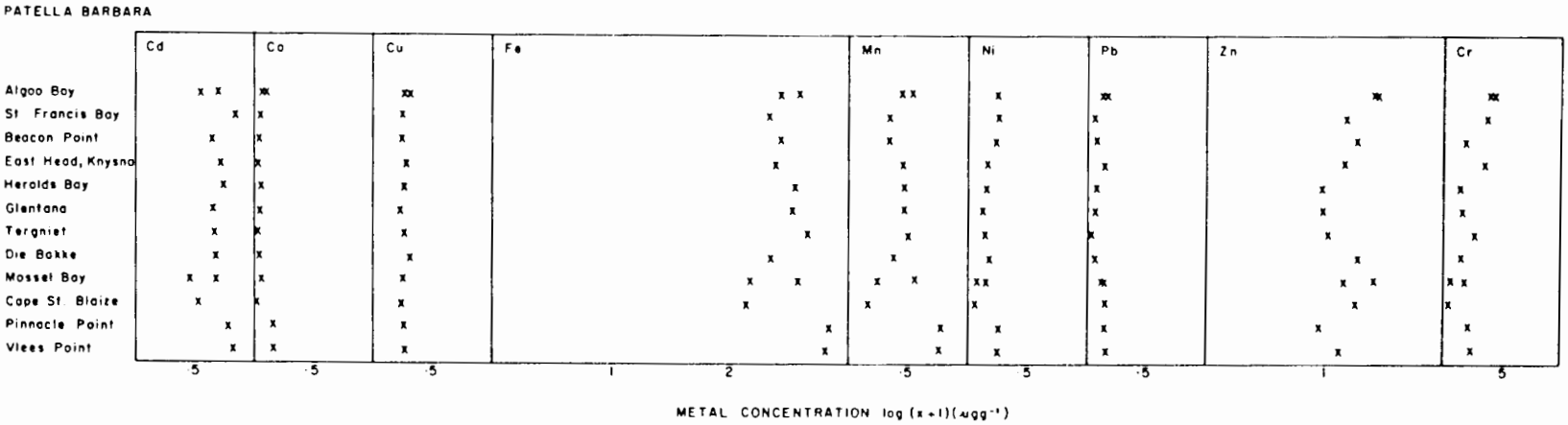


Figure 104 Metal concentrations in Patella barbara

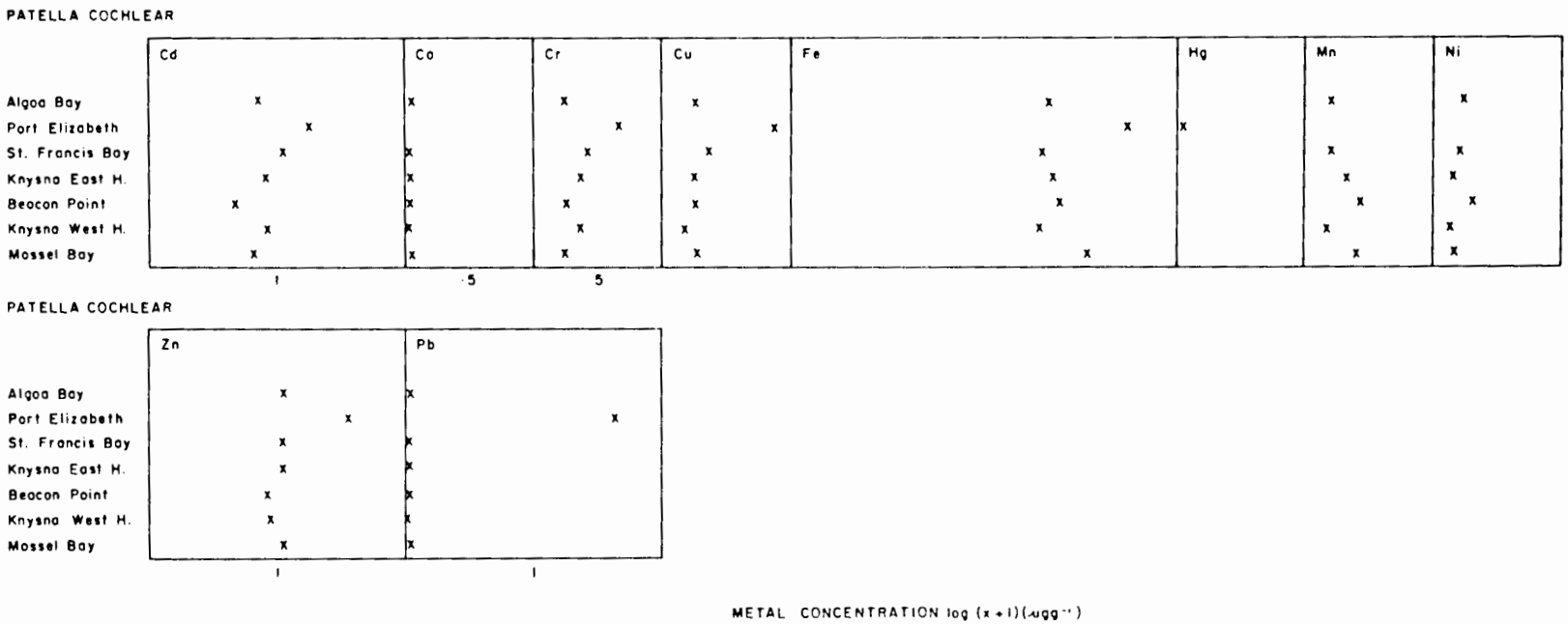


Figure 105 Metal concentrations in Patella cochlear

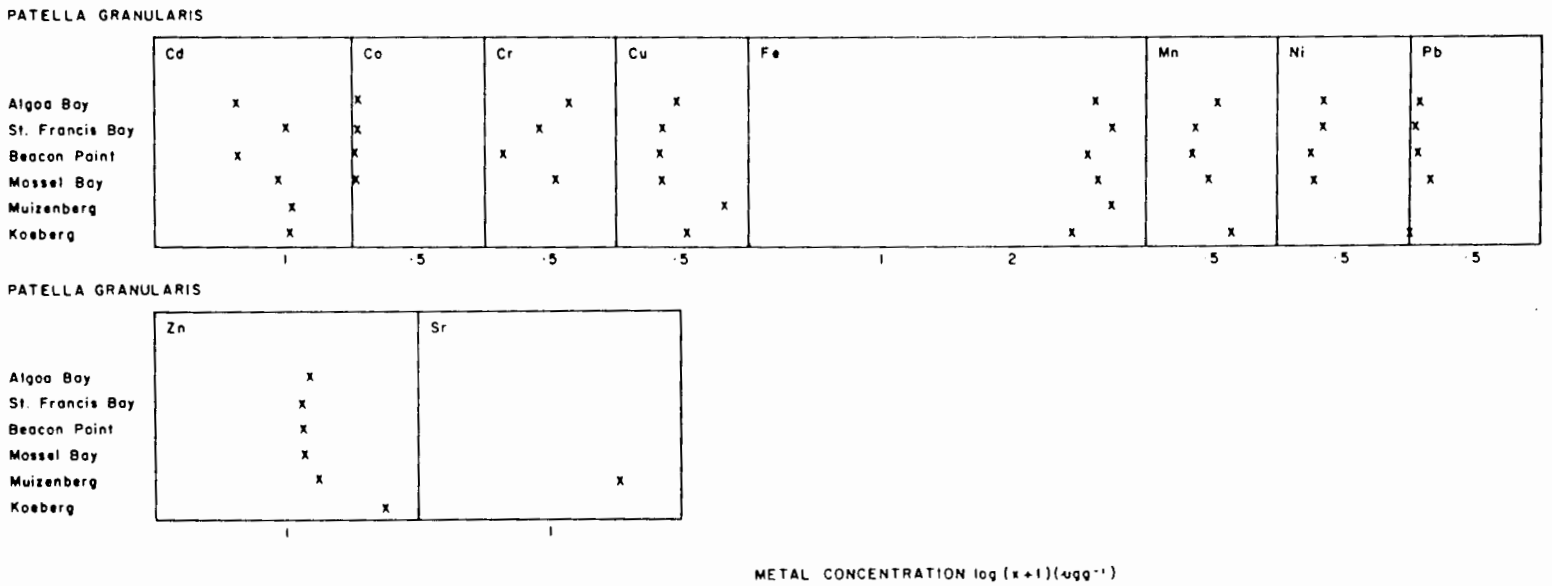


Figure 106 Metal concentrations in Patella granularis

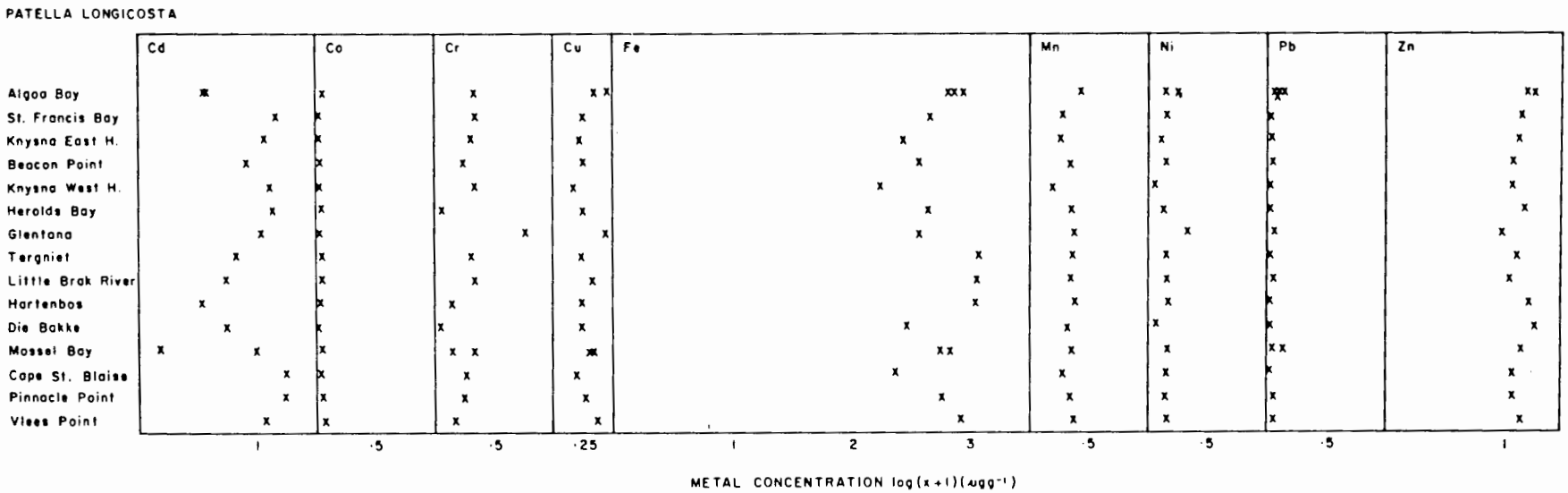


Figure 107 Metal concentrations in Patella longicosta

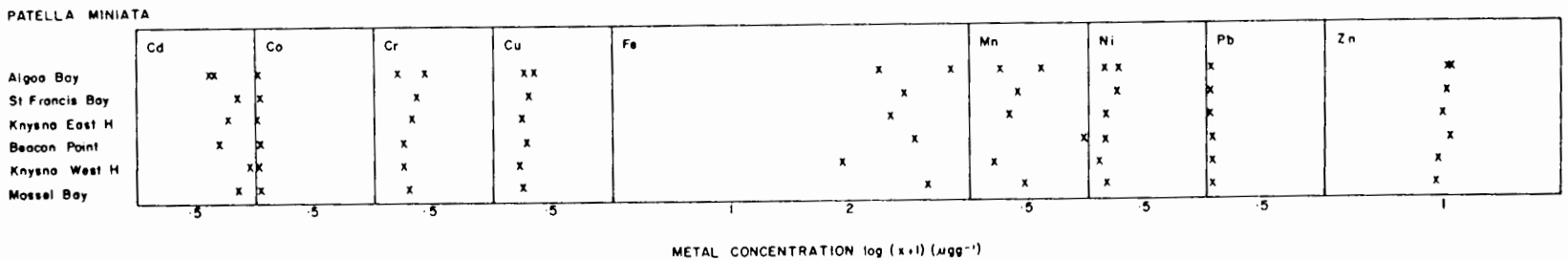


Figure 108 Metal concentrations in Patella miniata

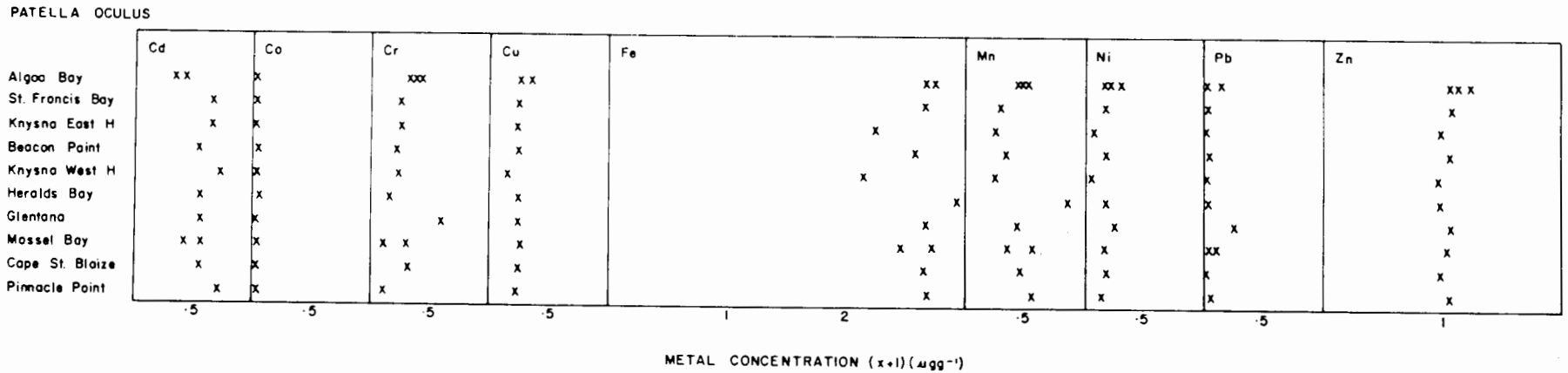


Figure 109 Metal concentrations in Patella oculus

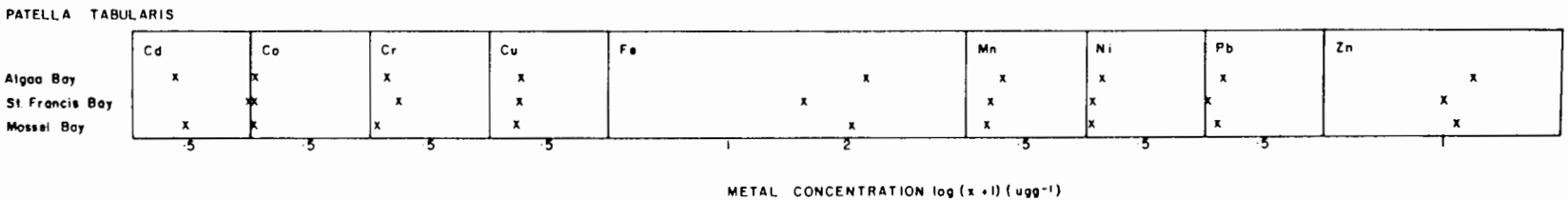


Figure 110 Metal concentrations in Patella tabularis

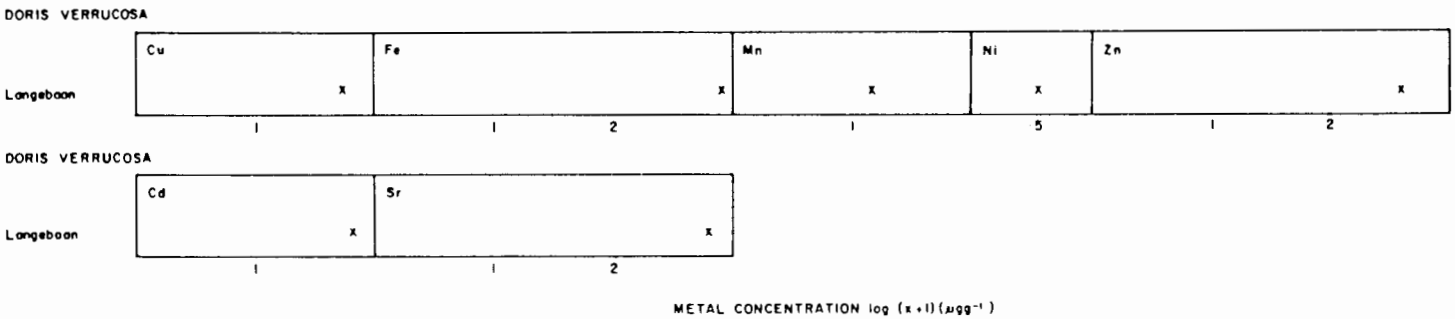


Figure 111 Metal concentrations in Doris verrucosa

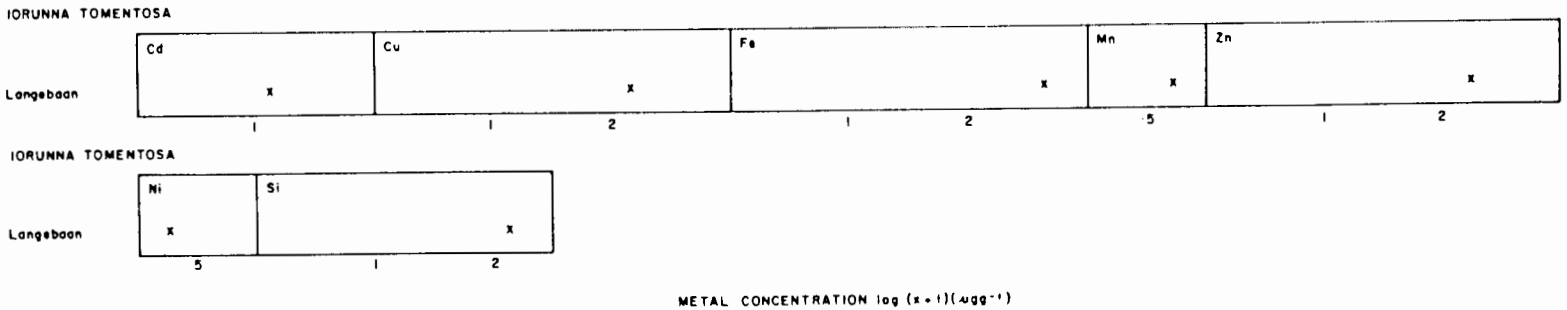


Figure 112 Metal concentrations in Iorunna tomentosa

H. midae (Figure 102) has been dissected and the different organs analysed separately; this showed that different organs accumulate different metals.

The metal burden in limpets (Figures 103 to 110) was studied in eight closely related species sampled over a wide region. Although not all figures are as complete as Figures 104 and 105, it is obvious that limpets accumulate different metals at different rates. More details are given in the Algoa Bay section and in Figure 25. Higher burdens were reported from St Francis Bay, Herold's Bay and Pinnacle Point for Cd and Cr, but Cu and Zn did not follow this trend. Data such as these should make it possible to group together those metals which display similar trends and which, therefore, may be taken up by certain animals, by the same mechanisms. The data may also be used to show that some ions (for example, Cd) may compete with others (for example, Zn).

The metal concentrations in the two nudibranchs have been used to compare the concentrations in apparently unpolluted nudibranchs from Gough Island with those in animals from coastal regions (Hennig, 1984). These appear to be background levels.

5.5.4 Cephalopoda (squids)

Loligo (Figure 113) from St Lucia is the only reference animal from this class. Since squids are an important food source, more information should perhaps be gathered.

5.6 **Echinodermata**

5.6.1 Echinoidea (sea-urchins)

Parechinus (Figure 114) is the only representative of this large phylum. Unfortunately no details of the method were given by Van As et al. (1975); hence it is not known which part of the

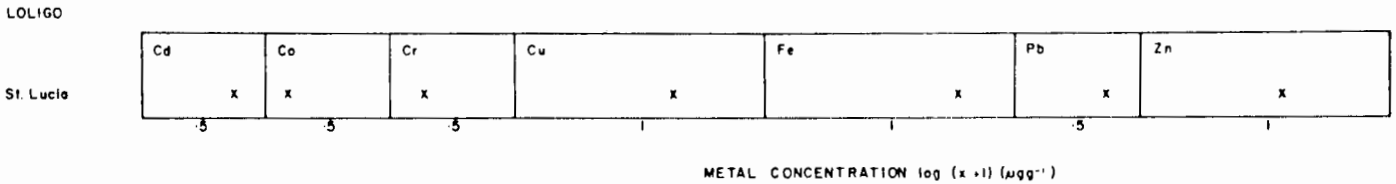


Figure 113 Metal concentrations in Loligo sp.

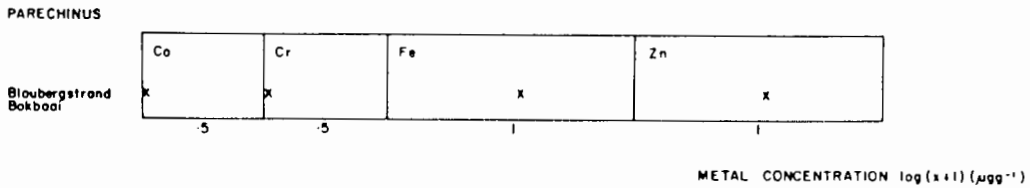


Figure 114 Metal concentrations in Parechinus sp.

sea-urchins were used. Hennig (in preparation) analysed gonads and soft parts only of sea-urchins from Gough Island and found very little metal accumulation.

5.7 **Chordata**

5.7.1 Tunicata (sea-squirts or red bait)

Pyura stolonifera (Figure 115)

Although red bait is very common on rocky shores and piers, very few metal data were available. If compared with other filter feeders the metal body-burden of red bait is very low. Unfortunately, due to lack of information it is not known if the data represent concentrations in only the fleshy part or whole animal.

5.7.2 Aves (birds)

The bones of three species of birds were analysed for cadmium and lead (Figure 116). Penguins accumulate surprisingly high levels of lead in their bones, while cormorants showed a wide range of cadmium concentrations.

5.7.3 Pisces (fish)

All the fish analysed were bony fish. They have been arranged alphabetically: Acanthopagrus berda (Figure 117), Argyrosomus hololepidotus (Figure 118), Argyrozona argyrozona (Figure 119), Atractoscion aequidens (Figure 120), Chrysoblephus gibbiceps (Figure 121), Cheimerius nufar (Figure 122), Chrysoblephus puniceus (Figure 123), Diplodus sargus (Figure 124), Elops machnata (Figure 125), Hypacanthus sp. (Figure 126), Hypacanthus amia (Figure 127), Johnius hololepidotus (Figure 128) Lithognathus lithognathus (Figure 129), Lophius piscatorius (Figure 130), Lutianus argentimaculatus (Figure 131), Merluccius capensis (Figure 132), Mugil canaliculatus (Figure 133), Mugil cephalus

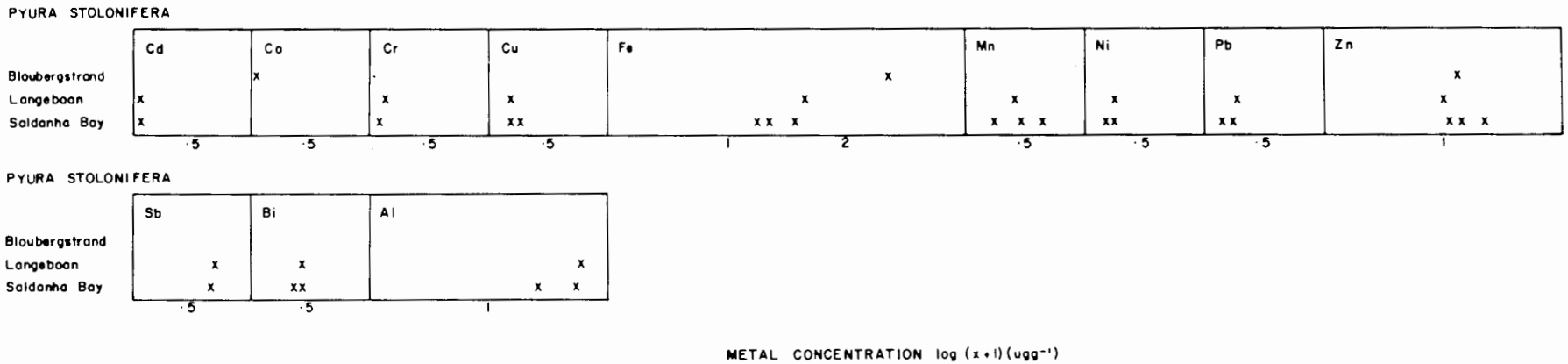


Figure 115 Metal concentrations in Pyura stolonifera

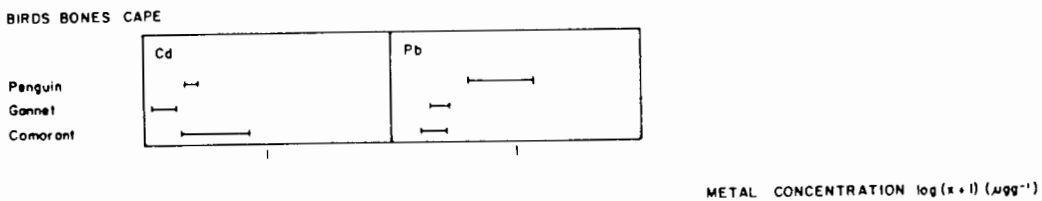


Figure 116 Metal concentrations in bird bones

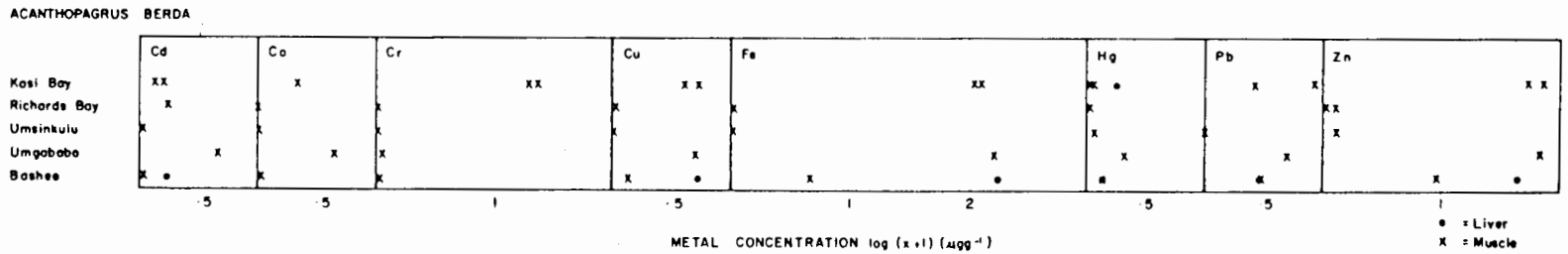


Figure 117 Metal concentrations in Acanthopagrus berda

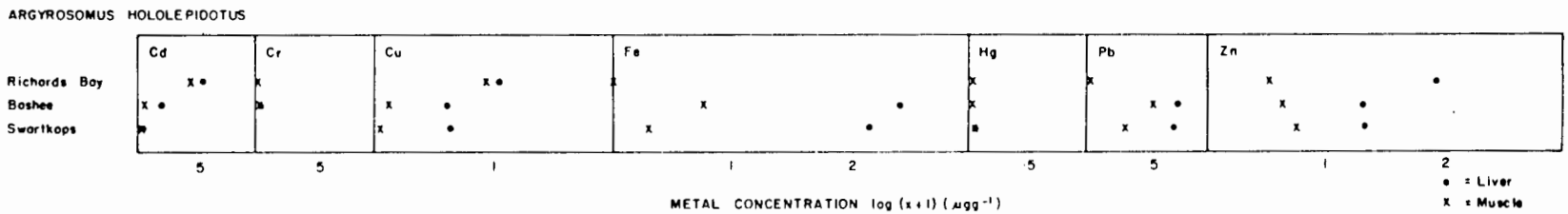


Figure 118 Metal concentrations in Argyrosomus hololepidotus

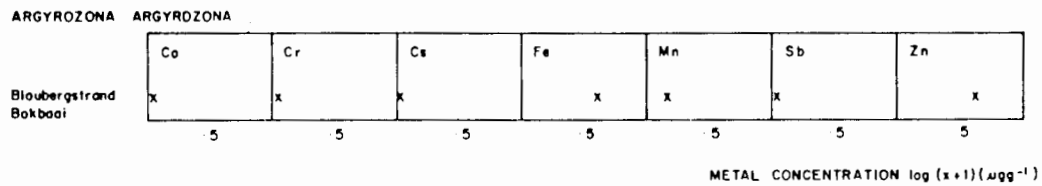


Figure 119 Metal concentrations in Argyrozona argyrozona

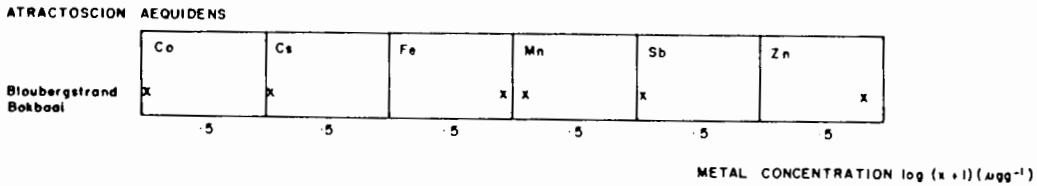


Figure 120 Metal concentrations in Atractoscion aequidens

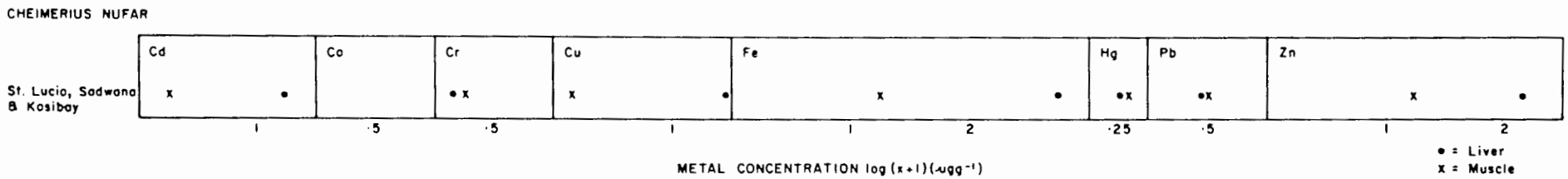


Figure 121 Metal concentrations in Cheimerius nufar

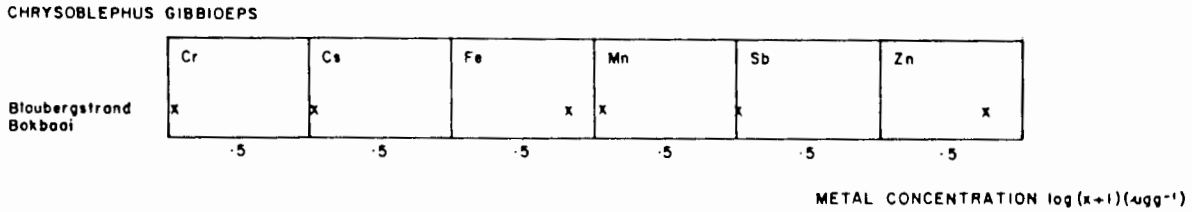


Figure 122 Metal concentrations in Chrysoblephus gibbiceps

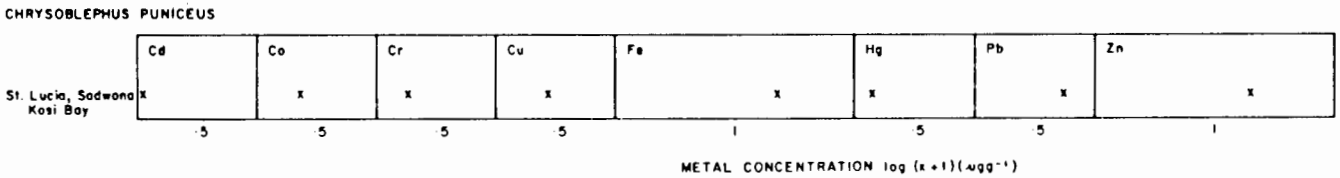


Figure 123 Metal concentrations in Chrysoblephus puniceus

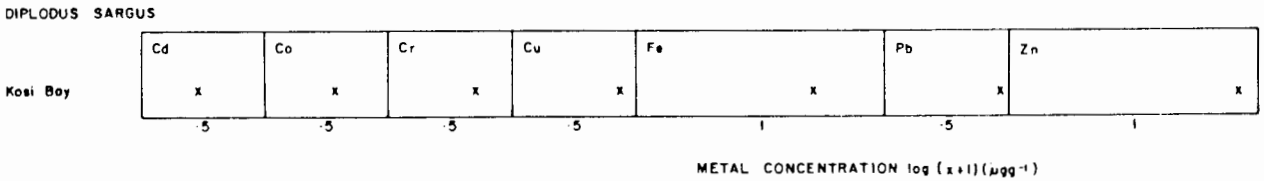


Figure 124 Metal concentrations in Diplodus sargus

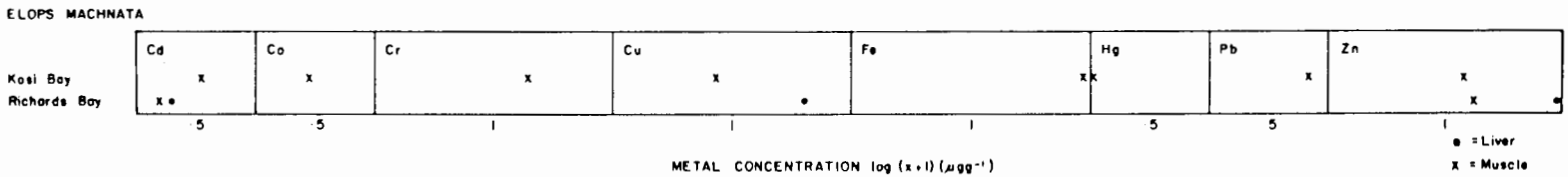


Figure 125 Metal concentrations in Elops machnata

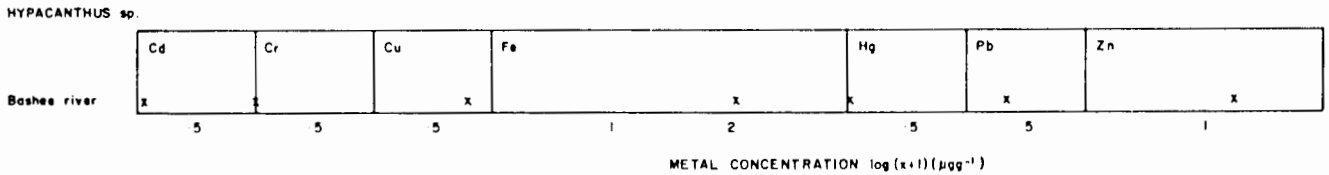


Figure 126 Metal concentrations in Hypacanthus sp.

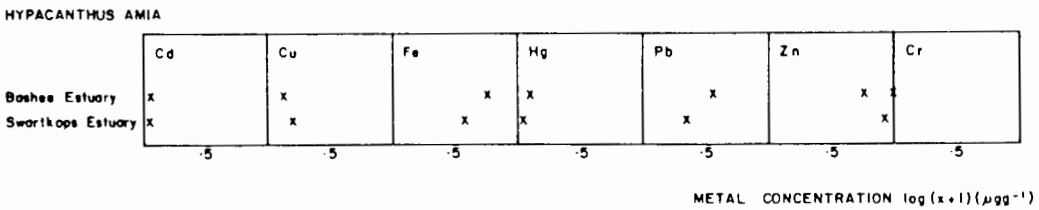


Figure 127 Metal concentrations in Hypacanthus amia

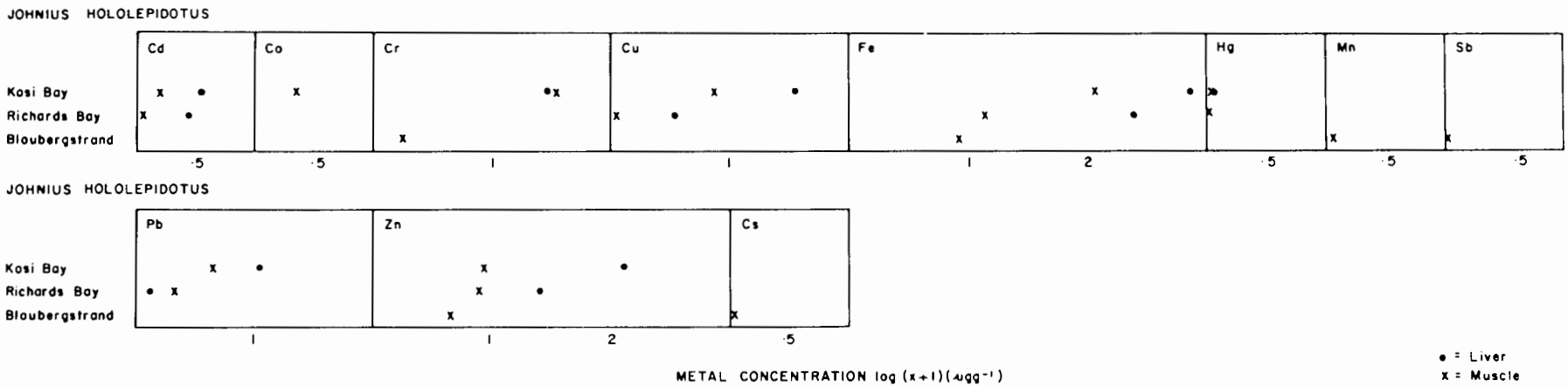


Figure 128 Metal concentrations in Johnius hololepidotus

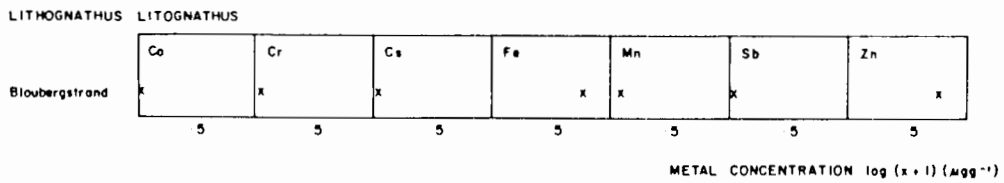


Figure 129 Metal concentrations in Lithognathus lithognathus

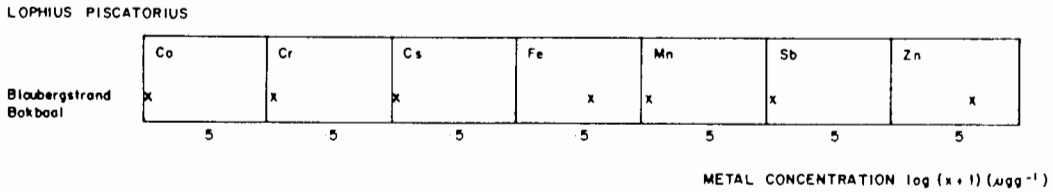


Figure 130 Metal concentrations in Lophius piscatorius

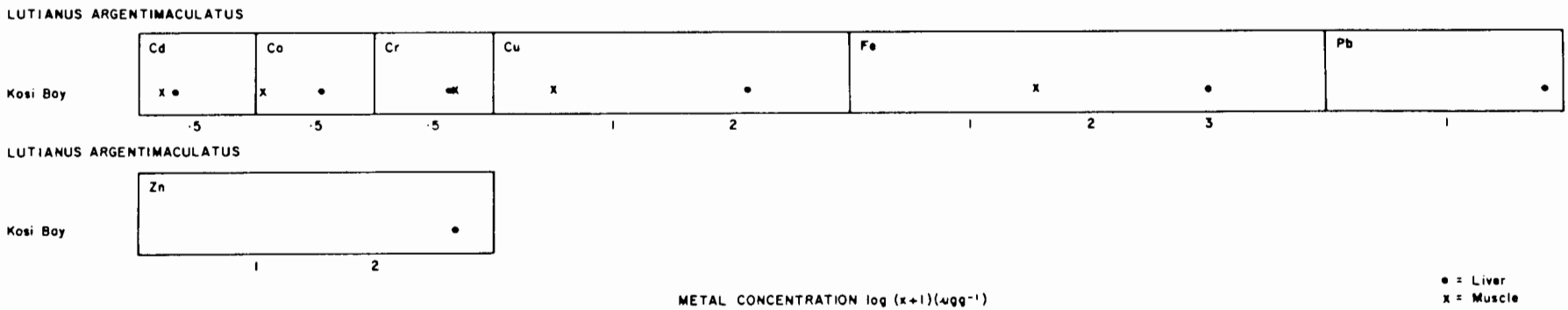


Figure 131 Metal concentrations in Lutianus argentimaculatus

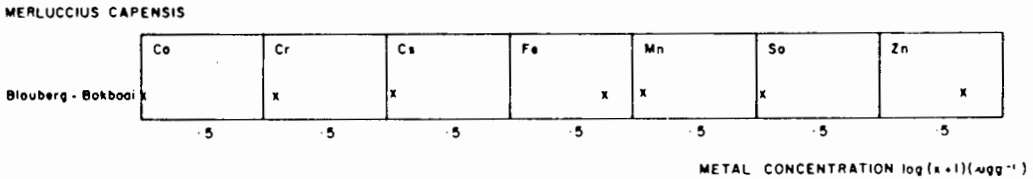


Figure 132 Metal concentrations in Merluccius capensis

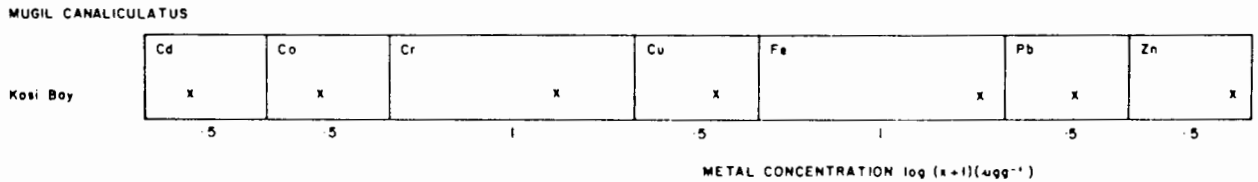


Figure 133 Metal concentrations in Mugil canaliculatus

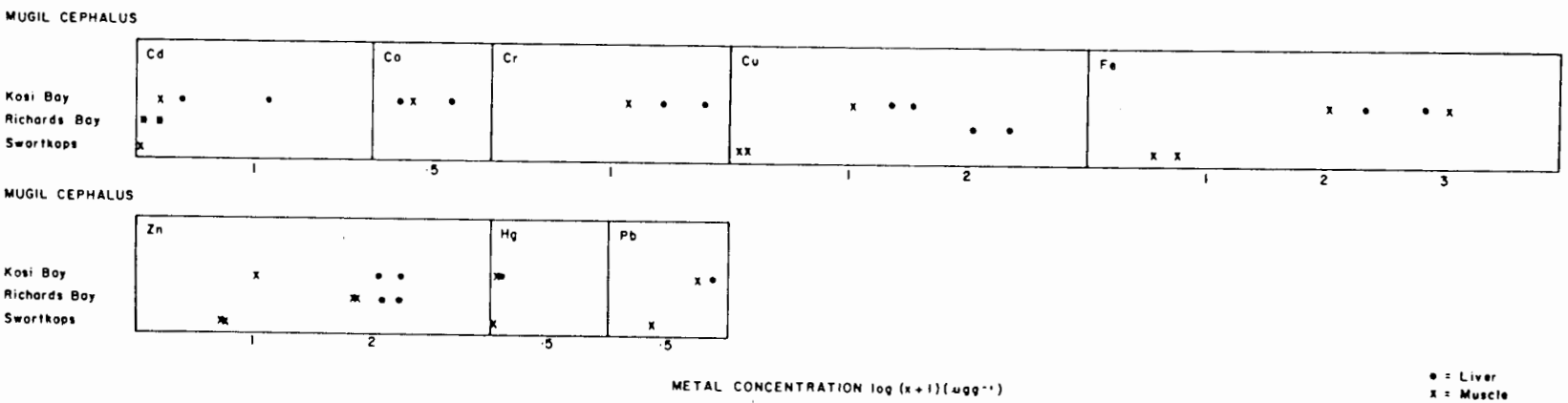


Figure 134 Metal concentrations in Mugil cephalus

(Figure 134), Mugil richardsoni (Figure 135), Mugil sp. (Figure 136), Oplegnathus conwayi (Figure 137), Otolithes ruber (Figure 138), Pomadasys commersonni (Figure 139), Pachymetopon grande (Figure 140), Rhabdosargus holubi (Figure 141), Rhabdosargus sp. (Figure 142), Sardinops ocellata (Figure 143), Sarotheradon mossambicus (Figure 144), Scomber japonicus (Figure 145), Scombrops dubius (Figure 146), Seriola pappe (Figure 147), Synaptura marginata (Figure 148), Therapon jarbua (Figure 149), Thunnus sp. (Figure 150), Tilapia sp. (Figure 151), Trachinotus russellii (Figure 152), Trachurus trachurus (Figure 153), Trigla capensis (Figure 154), Xiphiurus capensis (Figure 155).

More species of fish than of only other animals have been analysed, but these come from very different habitats and prefer different types of food. The range and accumulation were very varied and no trend can be observed. In all animals, the liver contained a higher metal concentration than did the muscle tissues. The data should not be considered as representative, although they give the correct magnitudes of metal concentration.

5.7.4 Human (Figures 156 to 157)

As a matter of interest, metal concentrations in the people who analysed trace metals are included. Data are given for blood (Figure 156) and hair (Figure 157, mercury only).

5.8 **Algae** (including seaweeds)

This is a loose collection of algae and seaweeds, so they have been arranged alphabetically.

Ecklonia maxima (Figure 158), Gigartina radula (Figure 159), Gracilaria verrucosa (Figure 160), Porphyra capensis (Figure 161), Suhria vittata (Figure 162), Ulva sp. (Figure 163).

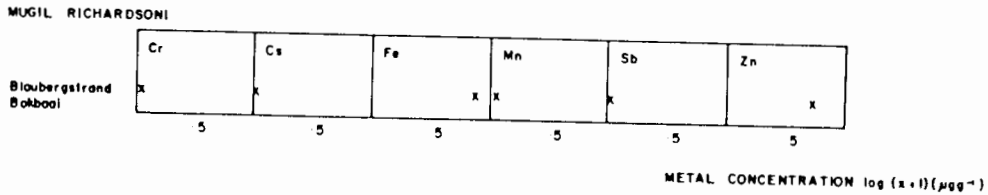


Figure 135 Metal concentrations in Mugil richardsoni

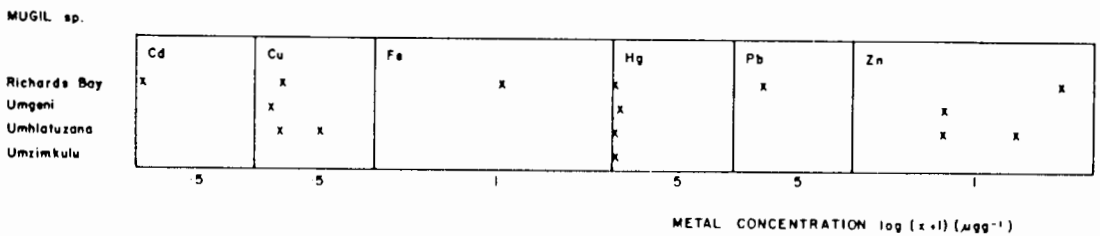


Figure 136 Metal concentrations in Mugil sp.

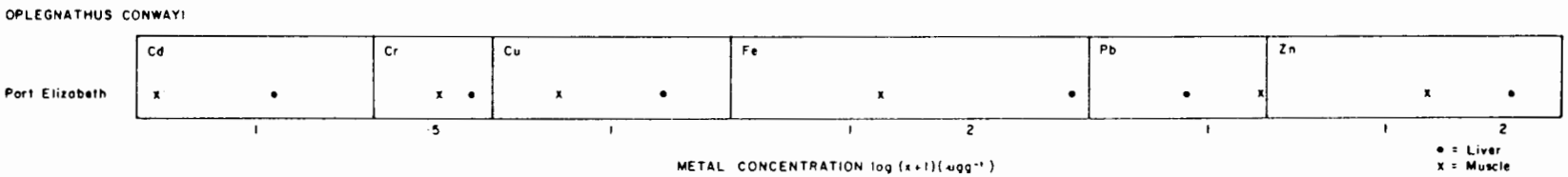


Figure 137 Metal concentrations in Oplegnathus conwayi

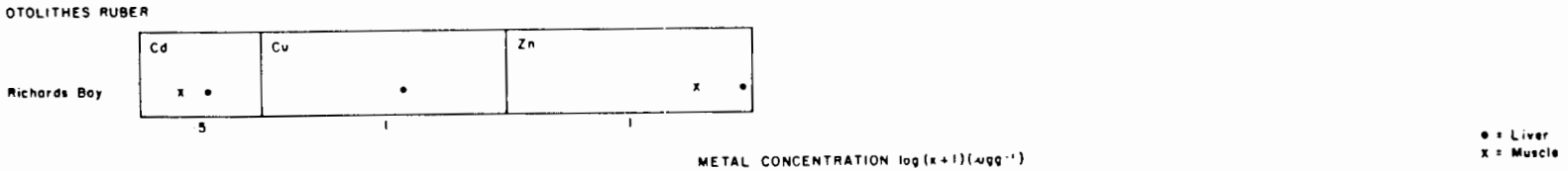


Figure 138 Metal concentrations in Otolithes ruber

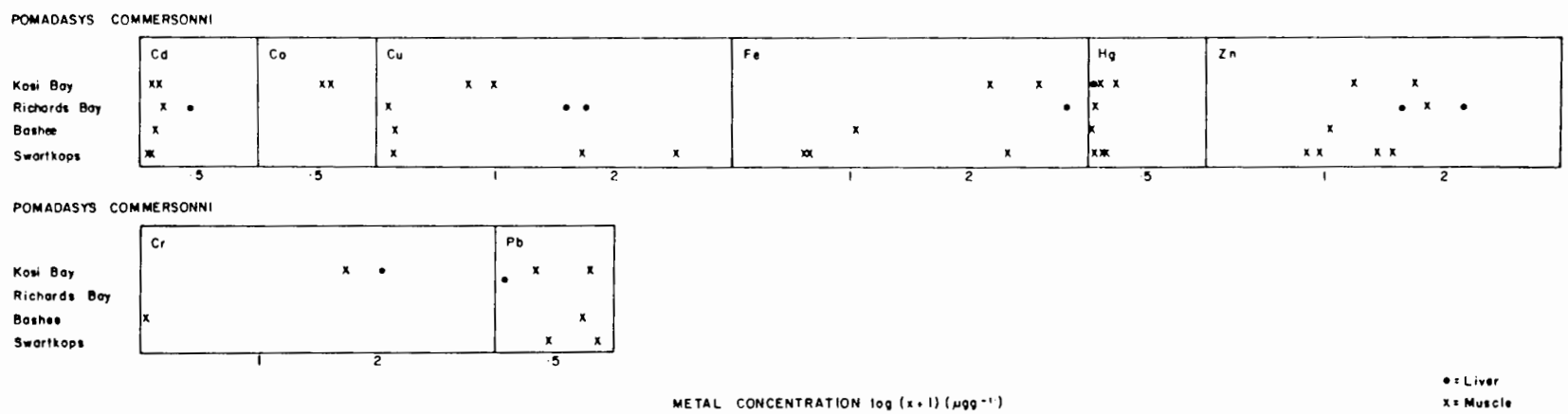


Figure 139 Metal concentrations in Pomadasys commersonni

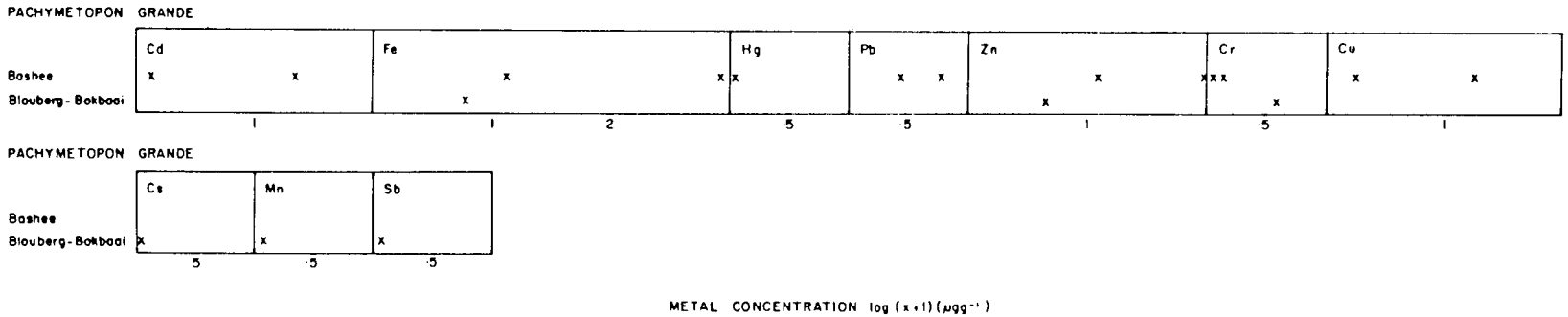


Figure 140 Metal concentrations in Pachymetopon grande

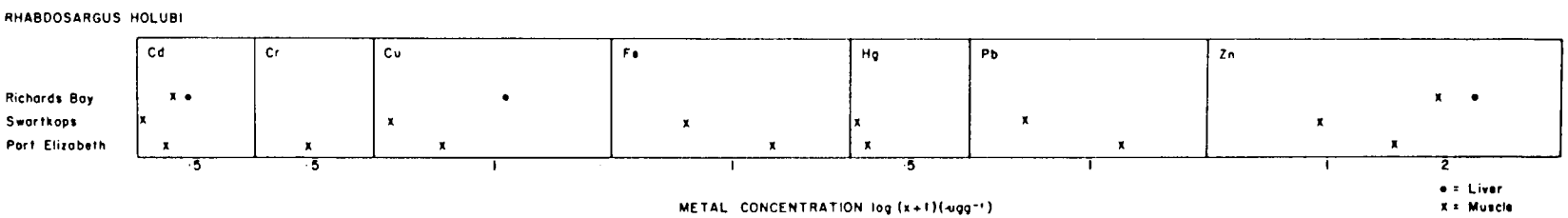


Figure 141 Metal concentrations in Rhabdosargus holubi

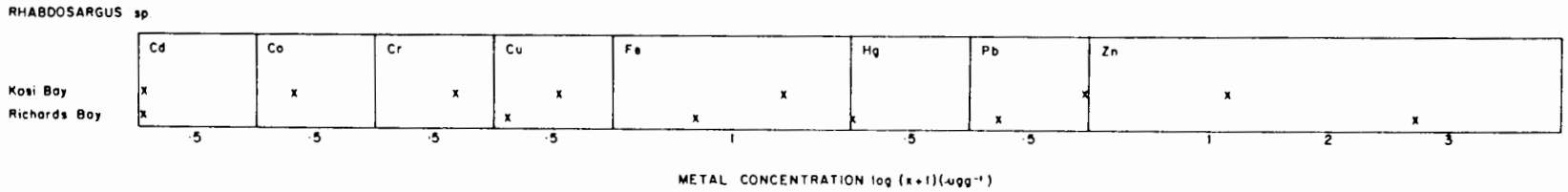


Figure 142 Metal concentrations in Rhabdosargus sp.

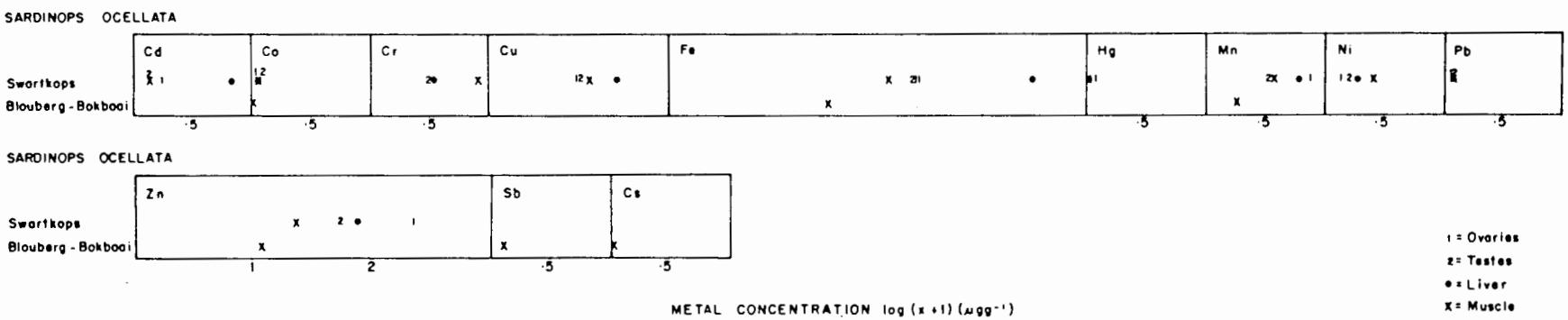


Figure 143 Metal concentrations in Sardinops ocellata

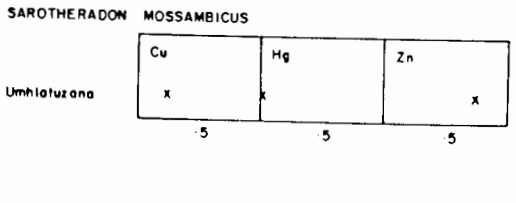


Figure 144 Metal concentrations in Sarotheradon mossambicus

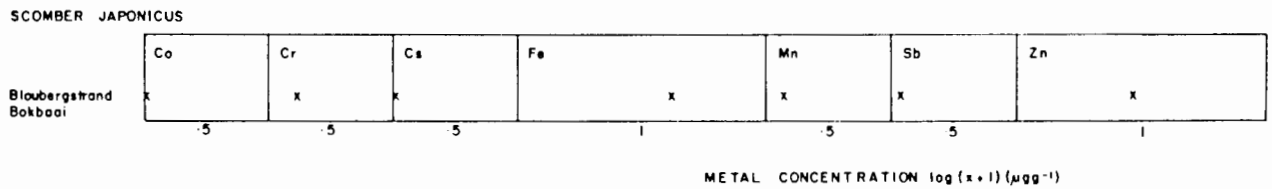


Figure 145 Metal concentrations in Scomber japonicus

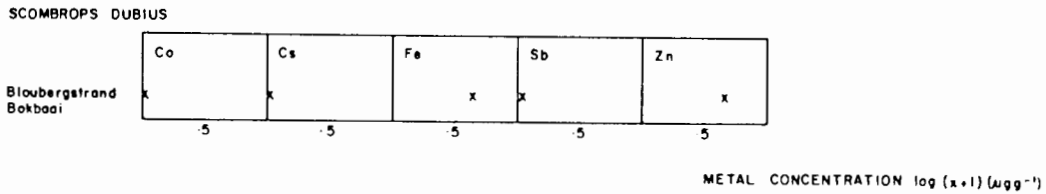


Figure 146 Metal concentrations in Scombrops dubius

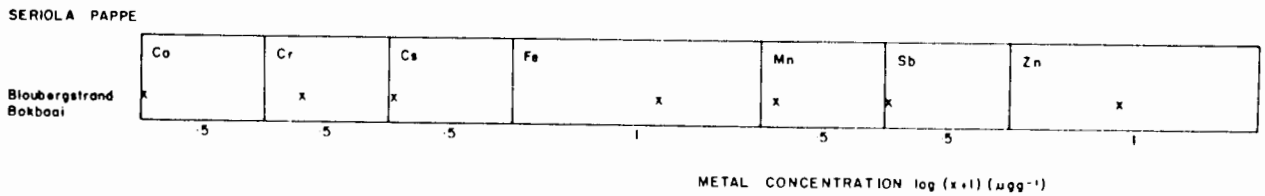


Figure 147 Metal concentrations in Seriola pappe

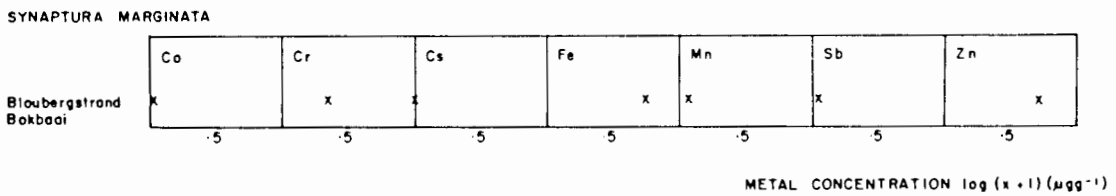


Figure 148 Metal concentrations in Synaptura marginata

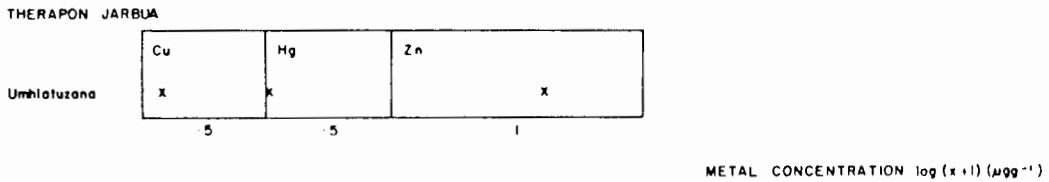


Figure 149 Metal concentrations in Therapon jarbua

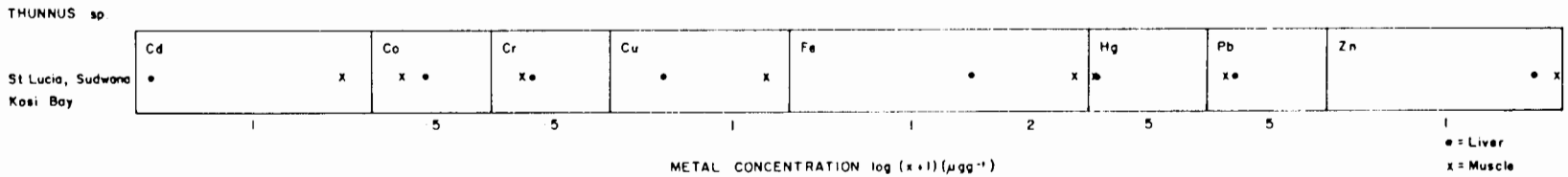


Figure 150 Metal concentrations in Thunnus sp.

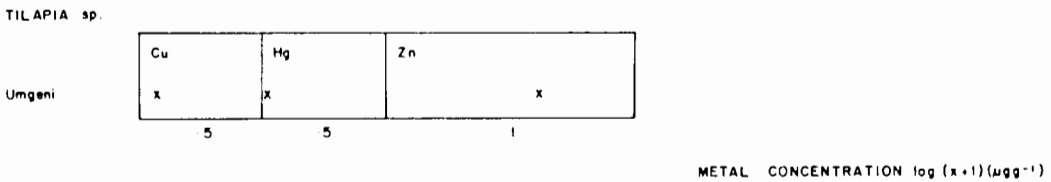


Figure 151 Metal concentrations in Tilapia sp.

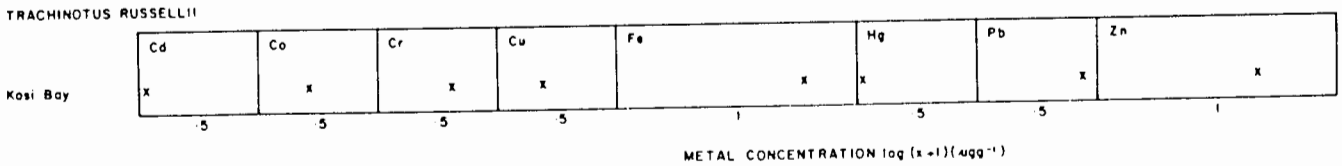


Figure 152 Metal concentrations in Trachinotus russellii

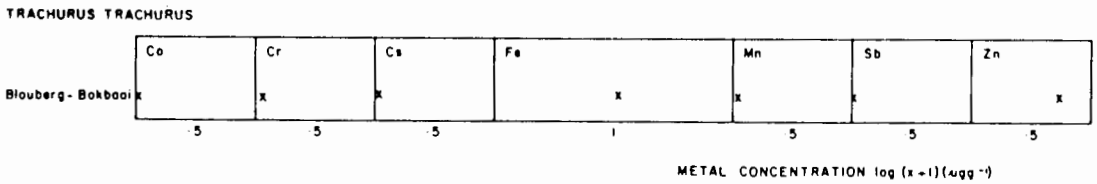


Figure 153 Metal concentrations in Trachurus trachurus

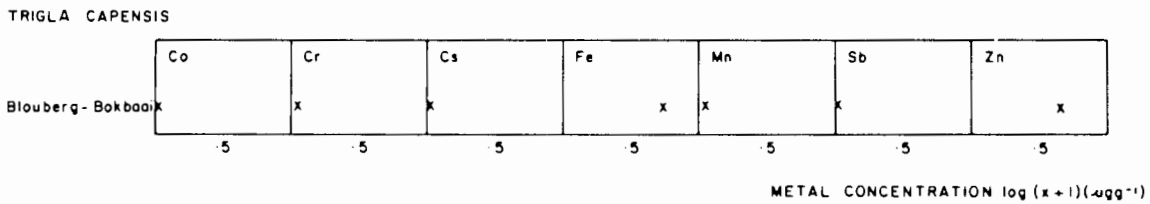


Figure 154 Metal concentrations in Trigla capensis

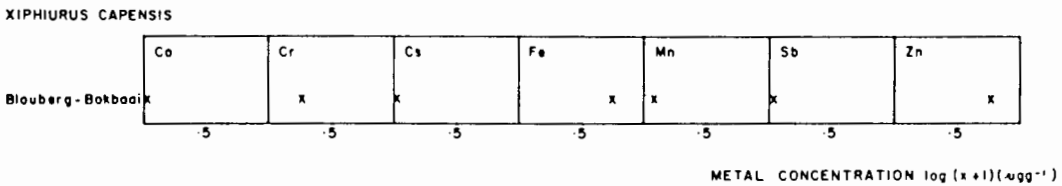


Figure 155 Metal concentrations in Xiphiurus capensis

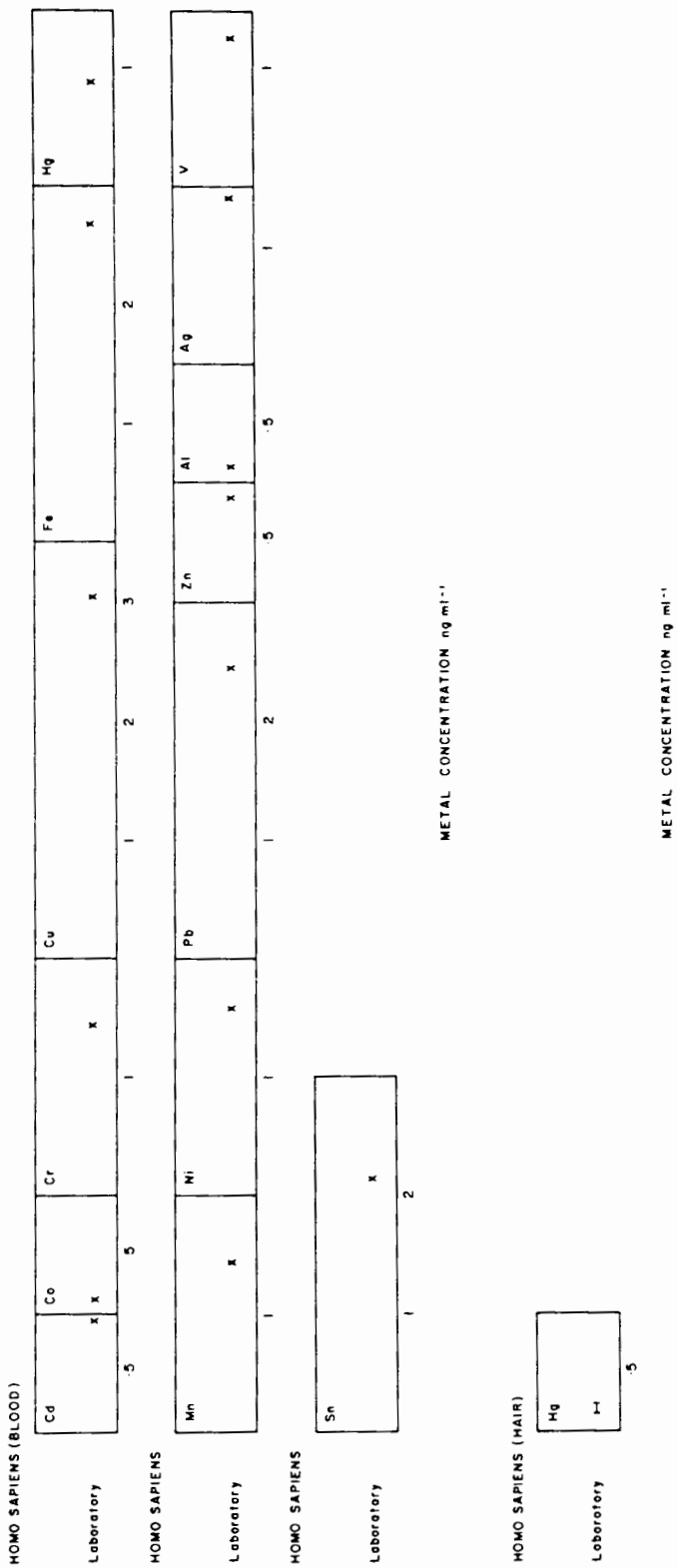


Figure 156 Metal concentrations in Homo sapiens (blood)

Figure 157 Metal concentrations in Homo sapiens (hair)

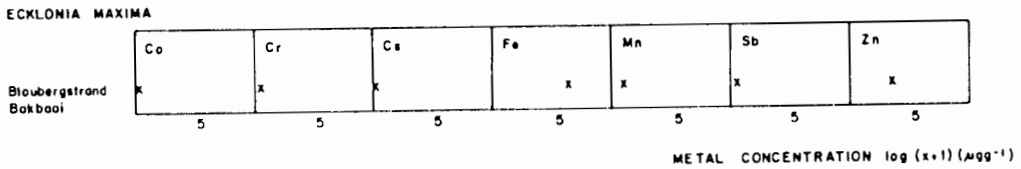


Figure 158 Metal concentrations in Ecklonia maxima

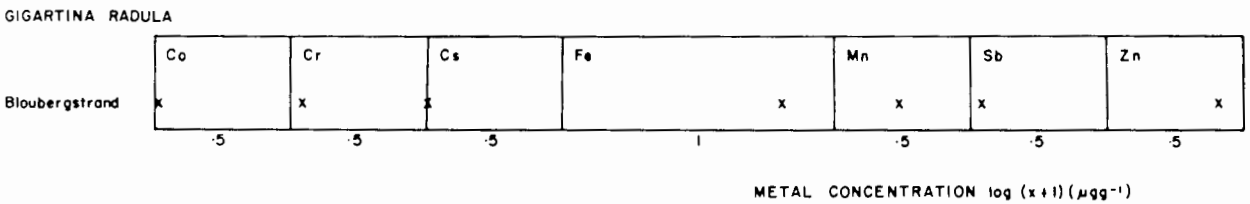
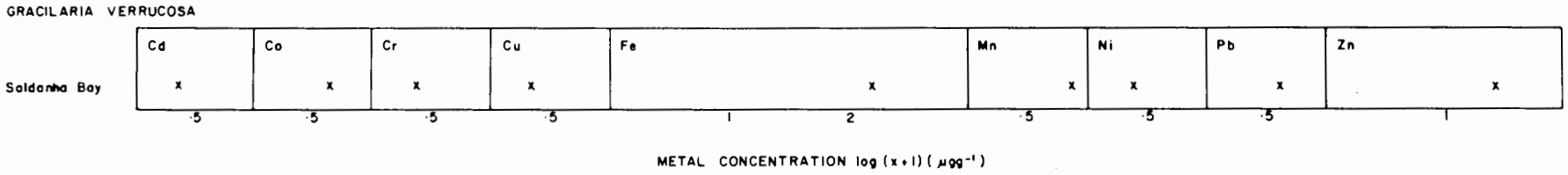


Figure 159 Metal concentrations in Gigartina radhula



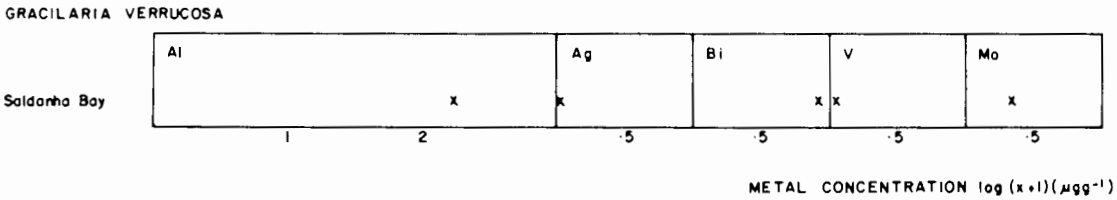


Figure 160 Metal concentrations in Gracilaria verrucosa

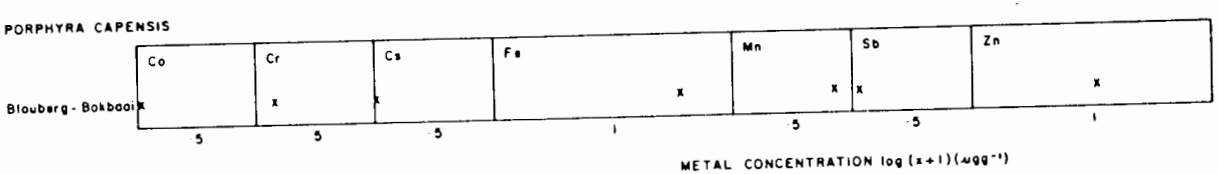


Figure 161 Metal concentrations in Porphyra capensis

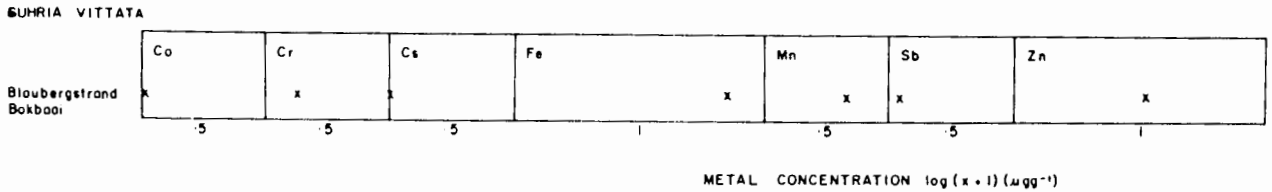


Figure 162 Metal concentrations in Suhria vittata

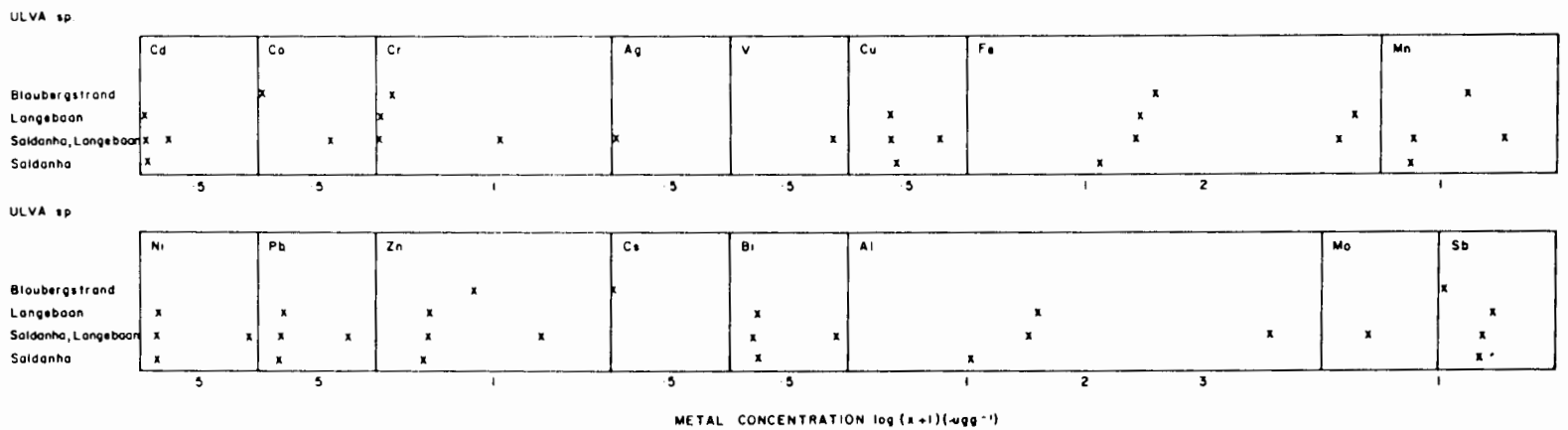


Figure 163 Metal concentrations in Ulva sp.

Most species in the section had low metal concentrations. Hence the very high levels (7 647 µg g^{-1} dry) in Ulva sp. were surprising. If these are background values and some limpets eat algae, then there is no biomagnification of metals via the food chain. This may be due to the non-bioavalibility of the metals to the grazers in the algae.

6. CONCLUSION

Metal distributions in water and sediment samples outlined impact areas and established baseline values with which future values can be compared. Although a fairly large number of samples were taken, these were unfortunately concentrated around impact areas and it is suggested that more baseline studies should be done on unpolluted reference beaches in Section I (Natal).

The value of trace metal reports would be enhanced if water, sediment and biological samples were taken simultaneously. In several locations only water or sediment samples were taken. This diminished the value of the data with respect to monitoring strategies for metals, because it is more important to know how much metal is bioavailable to animals than to know merely the total amount.

A huge gap in our knowledge still exists in data for Section IV (north part of west coast). Nearly every data point from this area would be a baseline data point.

With regard to metals in biological samples it is surprising how much data is available. For Bullia (Figure 99), C. margaritacea (Figure 88), P. barbara (Figure 104), P. longicosta (Figure 107) and P. perna (Figure 94) there were enough data to establish national and regional baselines for metal concentrations in organisms in unpolluted waters. The data emphasized that certain metal anomalies could have been identified only by analysis of the data for different locations in a review such as this. This became apparent from the consistently high concentrations in organisms from Pinnacle Point.

This review has identified "hot spots" and areas which should be watched. It has shown which organisms accumulate particular metals and that data for one particular location or organism cannot always be used for comparison of metal concentrations in other locations or other animals, even if closely related.

For instance, the question why Patella accumulate metals differently at the same location, could be investigated. As a conjecture this may be due to food differences. It may also enable us to speculate on the mechanisms of uptake and the extent to which metals are similar or dissimilar in bioavailability (see section on gastropoda).

Finally, it makes it possible to answer questions on metal concentrations asked by planners and coastal management about a vast variety of species and to be able to supply an answer which is correct to the right order of magnitude.

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Metal Concentrations in Antarctic Zooplankton Species

H.F-K.O. Hennig, G.A. Eagle, C.D. McQuaid[1] and L.H. Rickett[2]

National Research Institute for Oceanology, P.O. Box 320, Stellenbosch, 7600, South Africa

[1]Zoology Department, University of Cape Town, Rondebosch, 7700, South Africa

[2]Applied Mathematic Department, University of Cape Town, Rondebosch, 7700, South Africa

Running head : Metals in Antarctic Zooplankton

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Summary

Trace metal analyses were carried out on 15 species of zooplankton, collected between New Zealand and McMurdo Sound. Three species, Themisto gauchaudii, Eukrohnia hamata and Euphausia triacantha, were widely distributed. An attempt is made to relate the concentrations of copper, iron and zinc in these 3 species to different water-masses. Such a relationship could be used to identify feeding grounds of predators which eat zooplankton.

Introduction

It is widely known that certain well-defined oceanic phenomena, such as fronts, convergences and divergences, are permanent features of the Southern Ocean. These features separate distinct water-masses which are usually characterized with the aid of temperature-salinity (T-S) diagrams. However, apart from variations in temperature and salinity, dramatic changes in chemical properties of the water can also occur across frontal zones. For example, there is normally a characteristic sharp change in silicate concentration across the Antarctic Polar Front (Lutjeharms et al. 1983), and significant differences in the trace metal concentrations of water-masses have also been found (e.g. Burton and Young 1980; Boyle et al. 1981). Boyle et al. (op. cit.) suggested that metal concentrations were higher in cool nutrient-rich eastern boundary currents, and found that copper was distinctly higher in coastal water over the continental shelf. This was reported to be due to a nutrient trapping of diagenetically remobilized copper from mildly reducing terrigenous sediments.

A number of water masses have been identified in the Southern Ocean south of New Zealand (Burling 1961; Houtman 1967; Craig et al. 1981; Edwards and Emery 1982). In this region the Circumpolar Current is deflected rather abruptly to the south, as a result of bottom topography possibly, or the southward curve of the Antarctic land-mass. The Antarctic Polar Frontal Zone is fairly wide (approx. 500 km) and incorporates meso-scale features (Edwards and Emercy 1982). There is also evidence that individual plankters will remain within a particular water-body, and phyto-plankton assemblages have been used to identify water-masses. We suggest, therefore, that zooplankters may accumulate trace metals in their bodies, to levels which are characteristic of particular water-masses. Thus, variations in trace metal content of zooplankton may be an indication of the water-mass from which the sample was derived.

An opportunity to make preliminary studies on the trace metal concentrations in zooplankton in this area presented itself in the austral summer of 1980-81, during the Transglobe Expedition led by Sir Ranulph Fiennes. Here we report on the possibility of using trace metal concentrations in zooplankton, as indicators of the water-masses from which they were derived. This could be useful in identifying the feeding grounds or feeding depths of animals which feed on zooplankton.

Materials and Methods

Sampling was carried out between Christchurch, New Zealand, and McMurdo Sound in Antarctica (Fig. 1). Altogether there were 23 stations, although samples specifically dealt with here were collected from 17 stations (8 on the outward

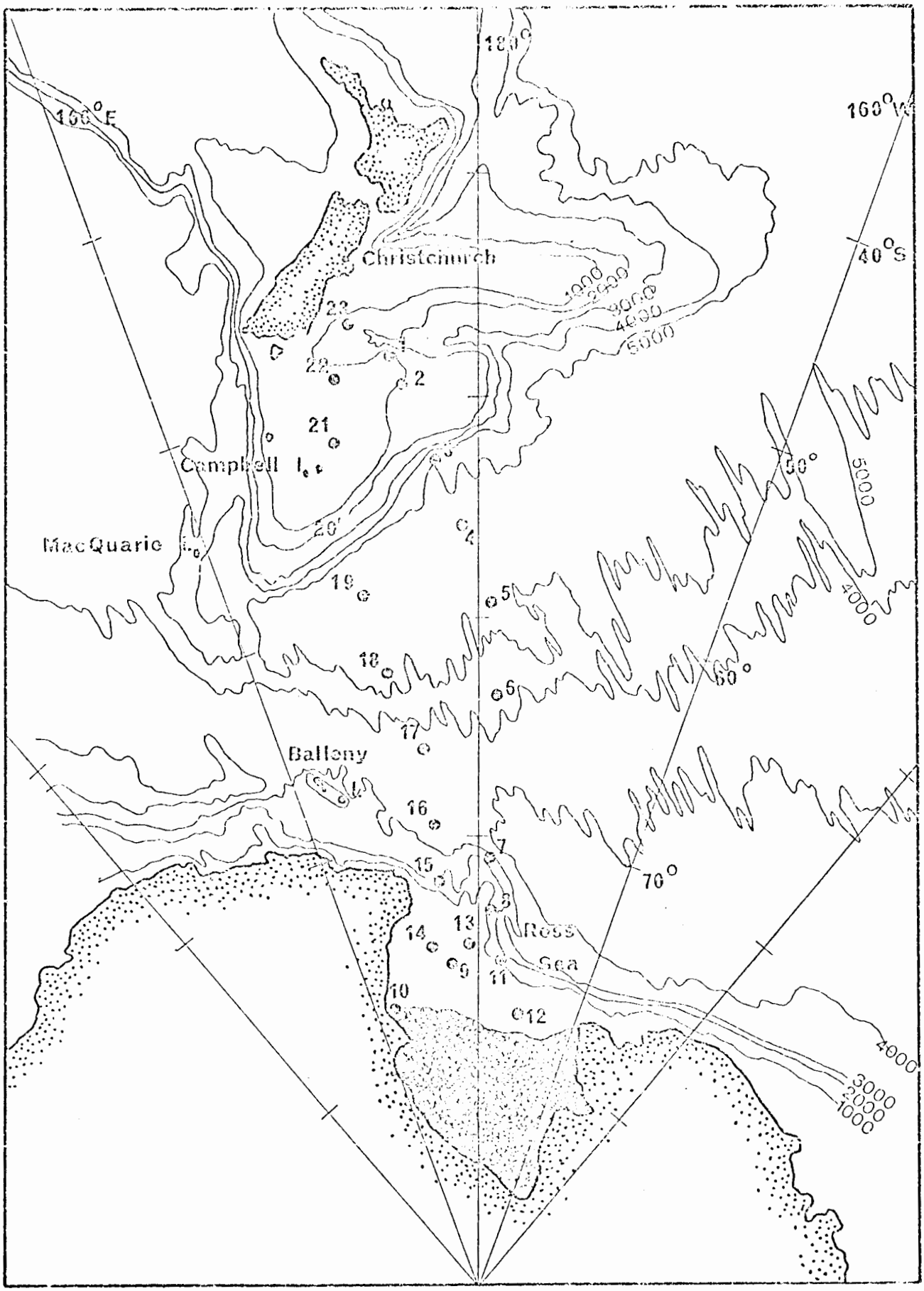

Fig. 1. Sampling positions of collected zooplankton between Christchurch, New Zealand and McMurdo Sound, Antarctica.

journey and 9 on the return leg). These stations were reasonably close to those occupied during the GEOSECS cruise (Craig et al. 1981). Surface macroplankton was collected from the M.V. Benjamin Bowring, using an N100 Discovery pattern plankton net of 1 m diam. Samples were taken 30 min after sunset on each day, until the ship moved into a zone of no sunset when samples were taken at 22h00 local time. The net was towed just below the surface for 10 min at a speed of 7.5 km h^{-1}, during which a total of approximately 4 000 m^3 of water was filtered. Start and finish positions for each trawl were taken from the ship's satellite navigation system.

Specimens were preserved in analar grade formalin and subsequently sorted and identified to species level, using pre-cleaned plastic instruments. Each species was then counted and dried for 48 h at 60°C. Samples were stored in acid-washed glass vials for analysis later in the laboratory.

Salinity samples were collected from the surface, using a temperature bucket, and stored in sealed glass bottles at room temperature for later analysis at the University of British Columbia. Surface-water temperature was measured, using a mercury bulb thermometer.

In the laboratory, zooplankters were weighed (individually for large specimens, and grouped together for smaller ones) and digested in quartz-redistilled nitric acid, and then in a 4:1 mixture of nitric-perchloric acids. The residues were dissolved in 10% nitric acid and analysed for Cd, Cu, Fe, Mn, Ni, Pb, Sr and Zn by flame atomic-absorption, using standard conditions with background correction (Watling and Watling 1976). This method of analysis of biological material is standard in our laboratory and, in a recent inter-calibration exercise, we obtained results which were within one standard deviation of the'true'values for all metals.

Results

Surface temperature decreased steadily from north to south (Fig. 2), and the Antarctic Polar Front was found from continuous surface temperature recordings to occur at approximately 62° 20'S on the southbound journey (13 January) and 62° 40'S on the northbound leg (20 February). The surface temperatures are given in more detail in Fig. 3.

Salinity also decreased towards the south, dropping from 34.77°/oo near the New Zealand coast to 33.66°/oo near the ice edge at St.7 and 16 (Fig. 2) where the summer ice-melt had already begun. The decrease in salinity was, however, less regular than that of surface temperature and values were higher in February than at the same latitudes in January (Fig. 2). If these data are plotted as a temperature-salinity diagram (Fig. 4), and the station positions considered, samples may be grouped into 4 sets representing different water bodies as follows:

1. New Zealand continental shelf (St.1, 2, 20, 21, 22, 23)
2. Deep Oceanic basin (St.3, 4, 5, 6, 18, 19)
3. Antarctic continental shelf (St. 17)
4. Ross Sea (St. 7, 8, 9, 10, 11, 12, 13, 14, 15, 16).

Fifteen of the zooplankton species collected were analysed for concentrations of 8 metals (Tables 1 - 2). Many of the species were, however, found in only a few samples. Thus, our account here is limited to concentrations of Cu, Fe, Zn and Sr in 3 species which were found to be widely distributed and which occurred in a large number of the samples.

TABLE 1 TRACE METAL CONCENTRATIONS IN THREE SPECIES OF ZOOPLANKTON (µg/g dry mass)

Species	Stations	Cu		Fe		Zn		Sr		Cd		Mn		Ni		Pb	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
Themisto gaudichaudii	1,2	875	462	283	54	1183	1081	n.d.		n.d.		n.d.		n.d.		n.d.	
	3,4,5	45	11	448	250	1006	1221	3	3	6	5	3	1	3		n.d.	
	6,7	49	26	362	147	1348	565	n.d.		4	4	n.d.		n.d.		n.d.	
	17	20		160		282		n.d.		n.d.		1		n.d.		n.d.	
	18	52		331		697		12		175		15		n.d.		n.d.	
	19	15		135		361		120		61		4		n.d.		n.d.	
	20,21,22,23	38	24	318	263	587	560	97	92	29	23	15	20	n.d.		n.d.	
Eukrohnia hamata	2	115		96		402		39		n.d.		3		n.d.		n.d.	
	4	118		85		172		19		8		3		1		n.d.	
	18	62		284		1097		16		29		9		n.d.		n.d.	
	19	6		57		89		22		5		5		n.d.		n.d.	
	20,22,23	36	18	186	52	485	65	18	17	14	9	2	2	1	1	n.d.	
Euphausia triacantha	4	168		121		310		2		11		3		n.d.		n.d.	
	16	23		250		694		n.d.		7		3		n.d.		n.d.	
	19	17		190		343		178		50		4		n.d.		n.d.	
	20,21,22,23	31	28	252	208	402	110	58	45	9	6	10	12	n.d.		n.d.	

Notes.

1. n.d. = not detected
2. Where stations have been grouped, metal concentrations given are the mean of all samples.

BLE 2 : Trace metal concentrations in Antarctic zooplankton (µg/g dry mass) (Species which were found at only a few stations)

Species	Station No.	Cd		Cu		Fe		Mn		Ni		Pb		Zn		Sr	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
prid larvae	1,2		n.d.	109	94	59	19		n.d.		n.d.		n.d.	1459	390		n.d.
	3,19	7	10	44	30	175	58		n.d.		n.d.		n.d.	416	115		n.d.
	14		n.d.	73		1812			n.d.		n.d.		n.d.	1739			n.d.
gitta gazellae	2		n.d.	175		66			n.d.		n.d.		n.d.	801		14	
	10		n.d.		n.d.	611			n.d.		n.d.		n.d.	96			n.d.
periella dilatata	3,4,5,6,18, 19	231	416	140	107	610	836	15	38		n.d.		n.d.	1657	2796	188	496
imno sp.	4,5,18,19	31	29	38	35	194	121	4	4		n.d.		n.d.	687	589	49	84
sh larvae	20	11	1	30	4	295	122	16	2		n.d.		n.d.	230	20	19	7
	4,5,19	27	33	38	47	145	109	2	2		n.d.		n.d.	466	380	28	33
ione limacina ntarctica	4,5	2	2	133	94	993	725	8	11		n.d.		n.d.	898	349		n.d.
	10,14		n.d.	103	17	528	245	3	0		n.d.		n.d.	813	514		n.d.
lpa thompsoni	21,22,23	3	2	27	31	137	80	2	1		n.d.		n.d.	250	339	56	16
	6	2		4		78		2			n.d.		n.d.	33		22	
	17	0,2		2		18		1			n.d.		n.d.	20		9	
llopus magellanicus	21,22,23	18	4	26	8	167	28	1	2		n.d.		n.d.	427	195	1	2
	6	7		35		119			n.d.		n.d.		n.d.	527			n.d.
	17		n.d.	135		139			n.d.		n.d.		n.d.	1495			n.d.
ilia antarctica	21,22,23		n.d.	74	57	365	131		n.d.		n.d.		n.d.	753	210		n.d.
	6		n.d.	12		201			n.d.		n.d.		n.d.	181			n.d.
	17		n.d.	85		175			n.d.		n.d.		n.d.	1121			n.d.
homene plebs	12,13		n.d.	76	52	577	542		n.d.		n.d.		n.d.	306	48	59	83
irus antarcticus	12		n.d.		n.d.	2008			n.d.		n.d.		n.d.	236			n.d.
pa maxima	22	3		34		170		1			n.d.		n.d.	431		41	

otes

1. n.d. = not detected
2. Where stations have been grouped, metal concentrations given are the mean of all samples.
3. s.d. = sample standard deviation (where no standard deviation is given, only one sample was obtained)

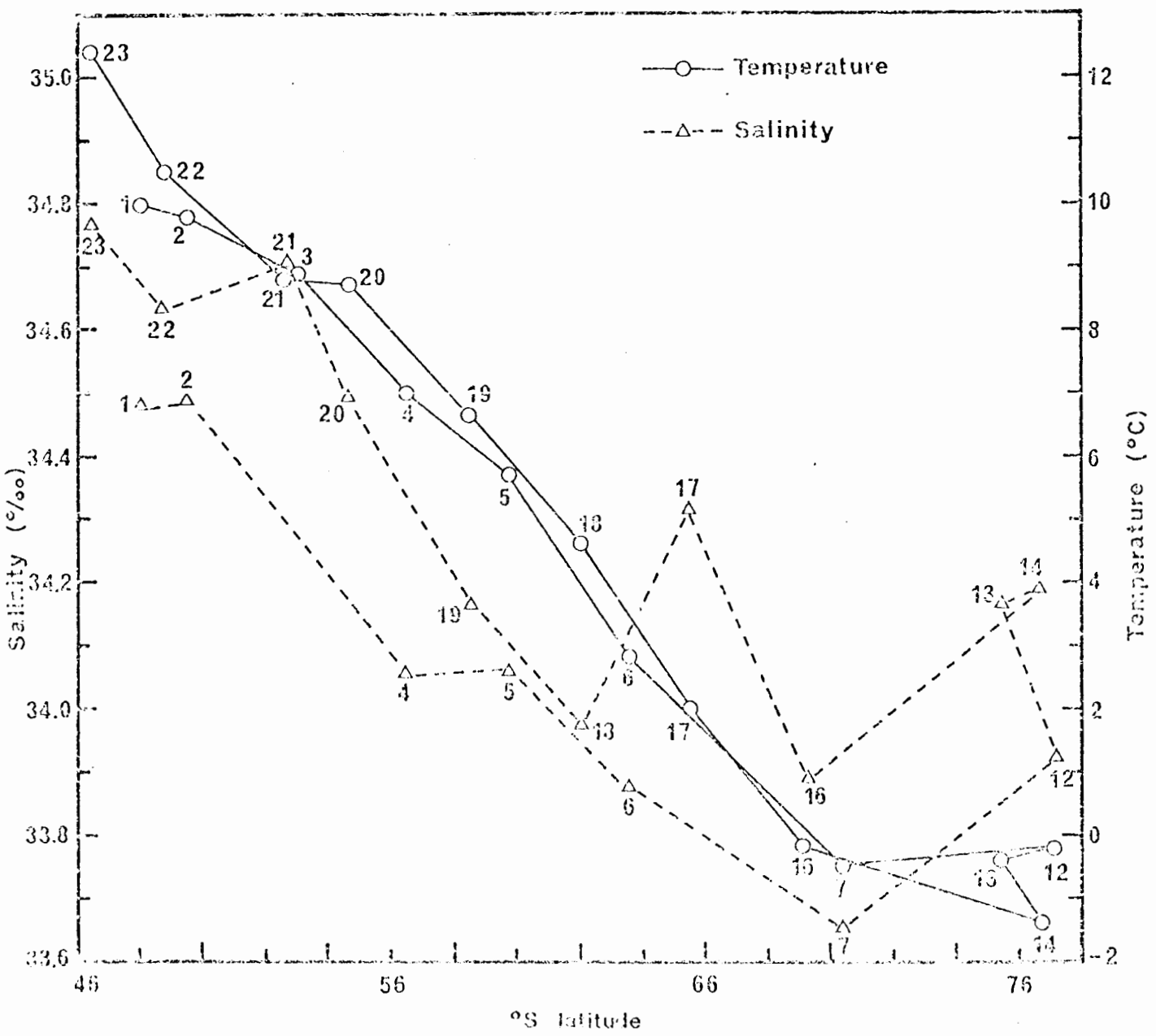

Fig. 2. Temperature and salinity between Christchurch and McMurdo Sound.

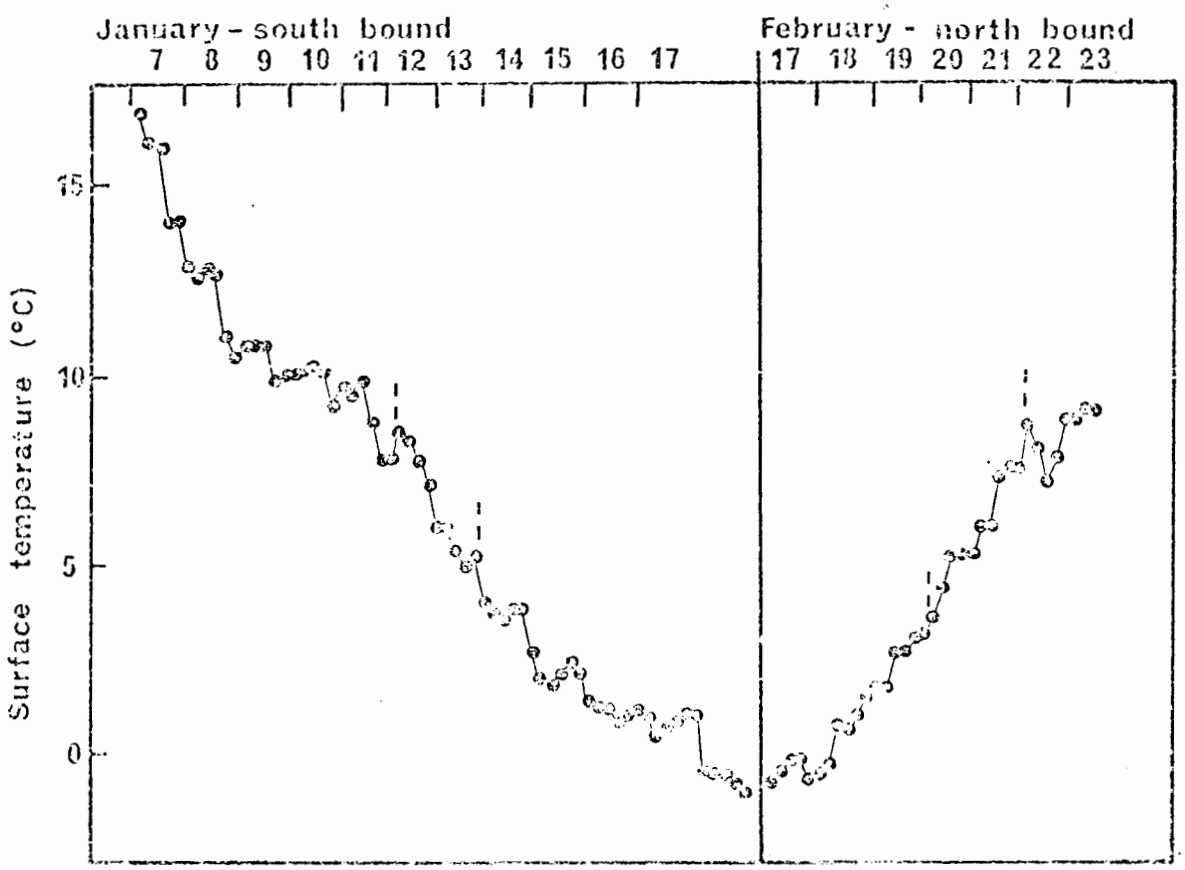

Fig. 3. Continuous surface temperature recording between Christchurch and McMurdo Sound from the M.V. Benjamin Bowring cruise.

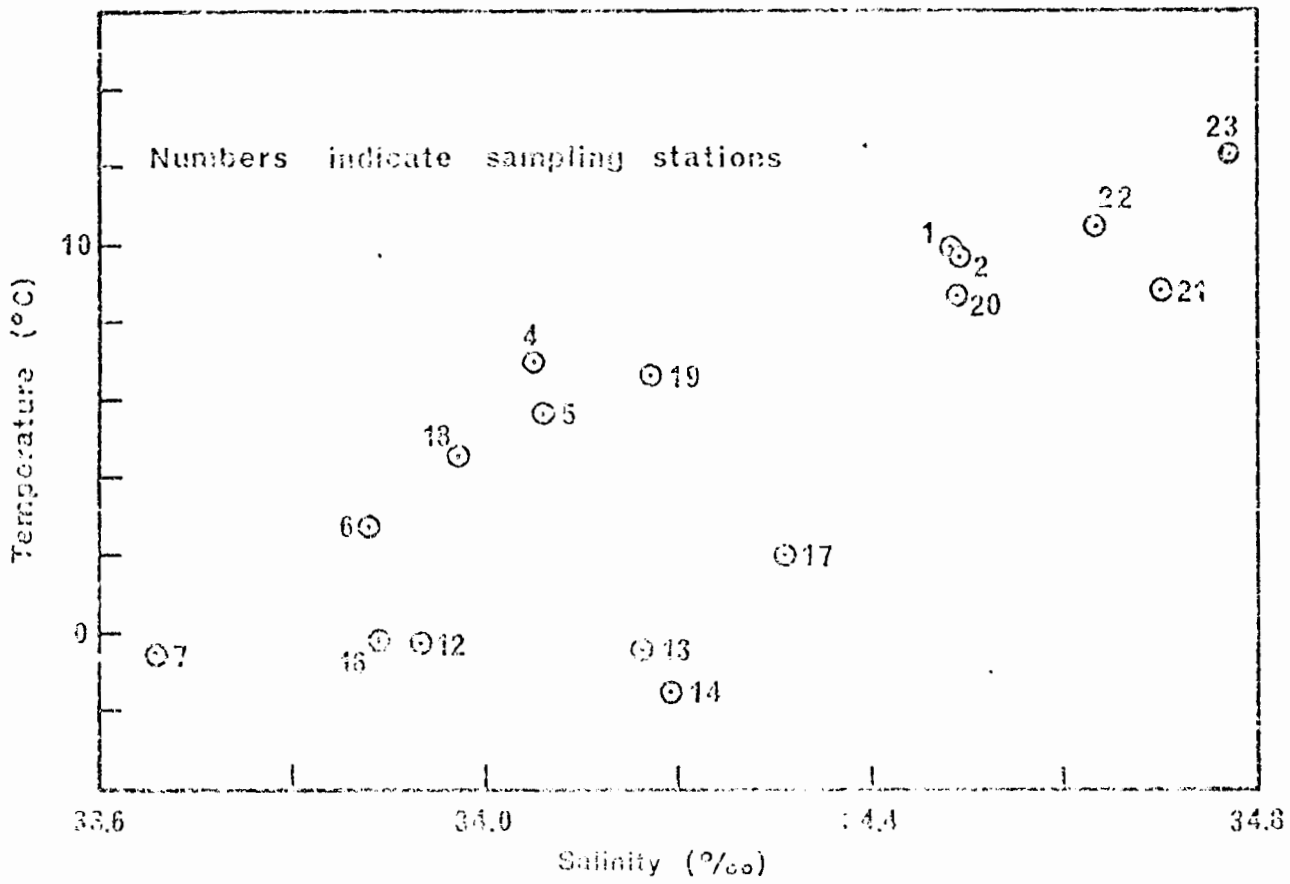

Fig. 4. Temperature-salinity diagram of stations with relevant zooplankton between Christchurch and McMurdo Sound.

There were the amphipod Themisto gaudichaudii*, the chaetognath Eukrohnia hamata and the euphausiid Euphausia triacantha.

Considerable interspecific differences in metal concentrations occurred although each species exhibited a particular pattern of metal content (Table 1), T. gaudichaudii generally contained greater concentrations than E. hamata or E. triacantha. There were striking differences between the stations representing different water-masses. Metal concentrations were generally highest at the New Zealand shelf stations and low above the oceanic basin. This pattern was clearest in the case of Cu, concentrations of which decreased from north to south with a marked drop between stations over the shelf and those over the basin (Table 1). The Cu concentrations in the shelf zooplankton were approximately 2 x those found farther south. The highest Fe concentrations occurred in animals from the Ross Sea. Zinc concentrations were also higher in animals obtained farther south, while Cd and Sr were only found in samples collected over the New Zealand shelf and in the oceanic basin.

There were also large differences in metal concentrations between

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* Some confusion has arisen regarding the nomenclature of this species which is known by most zoologists as Parathemisto gaudichaudii. In fact, Themisto is a senior synonym previously considered to be invalid as it was a junior homonym of the nudibranch Themisto, Oken (1815). Oken's classification was, however, placed on the official list of Rejected Works by Opinion 417 of the International Commission of Zoological Nomeclature (1956) leaving Themisto vacant as the valid name for the amphipod genus. This is discussed more fully by Bowman et al. (1982)

samples taken at the same latitudes in early January and in late February. Salinities were higher at the stations sampled in February (Fig. 2) however, these stations were sampled about 2 months apart. The differences were particularly noticeable in comparisons of St. 1 and 2 with 20 - 23 (i.e. over the New Zealand shelf in January and in February) and between St. 4 and 19 (i.e. over the oceanic basin). All 3 species followed a broadly similar pattern of change in these comparisons. Zinc and especially Cu concentrations decreased, sometimes quite dramatically, in all 3 species, both over the shelf region and over the oceanic basin, whereas the Sr concentration generally increased. Values for Fe generally rose between January and February over the shelf, but decreased in the same period over deeper waters.

The salinity at St. 17 was much higher than at Sts. 16 and 18 (south and north of St. 17, respectively), and the change clearly represents an intrusion between water-masses of the Ross Sea and the deep oceanic basin (Fig. 2). Similar dense water was reported by Craig et al. (1981), and Pickard and Emery (1982) found that this region has the densest water in the Southern Ocean. Of the 3 species considered, only T. gaudichaudii was found at St. 17, where it exhibited particularly low concentrations of all metals (Table 1).

Discussion

Very little chemical oceanographic information exists for this area of the Southern Ocean, the most comprehensive being that collected during the GEOSECS cruise (Craig et al. 1981). Available information on trace metals in zooplankton has been summarized in a review by Davies (1978).

Almost without exception these data were obtained in the northern hemisphere, and there is no information of which we are aware on the trace metal content of marine zooplankton from the Southern Ocean. Therefore, although our data are relatively meagre, we nevertheless feel that some discussion of their significance is warranted.

Accumulation of metals appears to be associated with the nutrient cycle (Boyle et al. 1981). For example, Cu concentrations in surface waters correlate well with nitrate concentrations (Boyle and Edmond 1975). Metals are accumulated by zooplankton in 3 ways : by adsorption onto the animals' body surfaces; by absorption from water passing over the gills and other body tissues; and by assimilation from food and detrital particles. Elimination may occur by excretion, moulting and egg-laying. Thus,the metal concentrations of any particular animal depends upon the degree of imbalance between the uptake and loss processes. Since certain of the loss processes are discontinuous (e.g. moulting), large variations in trace metal concentrations between individual animals can be expected (Cavies 1978). This was found to be the case in our study, particularly with Zn which is known to be very closely associated with the moulting cycle in certain crustaceans (Eagle et al. 1983).

Our most striking results is the difference in metal concentrations associated with samples from different groups of stations (i.e. different water-masses). This was particularly noticeable for Cu, which has been described as having a high concentration in continental shelf water (Boyle et al. 1981). Direct comparison with other published information is difficult because of differences in species and region. Nevertheless,

Martin and Knauer (1973) reported mean concentrations of 15.1 µg g Cu, 108 µg g Fe, 164 µg g Sr and 71 µg g Zn in euphausiids from Monterey Bay, California. These concentrations are lower than ours for Cu, Fe and Zn, whereas the Californian euphausiids contained more Sr. Additional data for euphausiids include, Cu and Zn concentrations between 8.5 and 42.2 µg g and between 50 and 131 µg g, respectively, in Euphausia pacifica from the north-eastern Pacific (Cutshall and Holton 1972) and mean Cu and Zn concentrations of 32.9 and 108 µg g, respectively, in euphausiids from the Mediterranean (Fowler et al. 1976). Fujita (1972) reported Zn concentrations of 166 and 456 µg g, respectively, in Themisto oblivia and Themisto japonica from the Sea of Japan. These data are of the same order of magnitude as ours for the north-bound leg of the cruise. However, certain samples collected at the beginning of the voyage, closer to New Zealand, had higher metal concentrations. For example, the Cu concentrations in Themisto gaudichaudii were particularly high at Sts. 1 and 2, and for the other 2 species high Cu concentrations were found at St. 4, as well.

It is also clear from this limited data set, that meaningful comparisons can only be made between different samples of the same species. There were large differences in metal concentrations between different species, no doubt associated with their particular mode of uptake. These would mask any other trends.

Although metal concentrations in the water were not measured, the differences in the concentrations found in the zooplankton must, at least in part, be due to differences in the concentrations in the water. In describing the water-masses and fronts south of New Zealand, Houtman (1967) stated that this is one of the principal meandering regions of the Antarctic Polar Front,

where apparent rates of change of over 4 km d^{-1} have been recorded. The rapidity and magnitude of change of trace metal concentrations (e.g. the change in Cu concentration in T. gaudichaudii between stations on the New Zealand continental shelf and the Deep Oceanic basin) leads us to speculate that the zooplankton samples came from populations occurring in different water masses. Thus, it appears that zooplankters accumulate metals to a degree which is dependent on the water-mass in which they live. There seems to be little mixing of animal populations, each staying within its own water-mass, and we suggest that metal concentrations could be used to differentiate between zooplankton populations from different water-masses. This might be used to identify feeding areas of predators which eat zooplankton. Metal concentrations of zooplankton in the stomachs of krill-eating animals, for example, might indicate their feeding grounds.

However, it must not be overlooked that seasonal variations could play an important part, as indicated by the differences in metal concentrations between the two legs of the cruise which were separated by two months. These seasonal variations should be investigated in future studies.

Finally, our data show that trace metal concentrations in zooplankton from the Antarctic are not necessarily low. This has important implications for the use of metal levels in samples from remote areas, in establishing base-line levels for pollution studies. Natural enrichment of trace metals in unpolluted water can, and does, lead to relatively high levels of trace metals in animals which have not been subjected to pollution loads.

Acknowledgements

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BASELINE STUDIES AND METAL CONTAMINATION : GOUGH ISLAND AND MARION ISLAND, SOUTHERN OCEAN

by

H F-K O Hennig

NATIONAL RESEARCH INSTITUTE FOR OCEANOLOGY
COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH
c/o Dept. of Oceanography, University of Cape Town,
Rondebosch 7700, South Africa

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ABSTRACT

The metal body burden of five marine species from Gough Island and six species from Marion Island are reported. In each case some comparison with coastal animals is made. It was found that baseline studies from remote areas sometimes show lower metal concentration depending on the environment. The size and similarity of species can play a role, but in general metal data is so inconclusive that the question is raised as to whether baseline studies are of any value. It was concluded that bioassays and not metal concentrations, would be the central feature of any marine baseline or pollution study.

BASELINE STUDIES AND METAL CONTAMINATION : GOUGH ISLAND AND MARION ISLAND, SOUTHERN OCEAN

1. INTRODUCTION

Gough Island (40° 18'S, 9° 52'W, Figure 1) is one of the areas of the world last affected by man and was in 1976 declared a wildlife reserve (Wace and Holdgate, 1976). This meant that all forms of environmental disturbance, other than those essential to the functioning of the South African weather station at Transvaal Bay, are avoided and no cropping of wildlife is permitted.

Gough's position just south of the sub-tropical convergence makes it very isolated. Even plastic pellets have only very recently been found in birds on the island (Bourne and Timber, 1982). In the 380 years since its discovery very few expeditions have visited Gough Island. The only comprehensive scientific expedition was in 1955-56 (Holdgate, 1958). In 1981 a biological expedition investigated the subtidal fringe of the island. The biological metal baseline results presented here are of the work of that expedition.

Marion Island (46° 55'S, 37° 45'E, Figure 1) is as remote as Gough Island but it has been studied more comprehensively. In 1965-66 a biological and geological expedition to Marion and Prince Edward Islands undertook general marine biological studies, mainly on the geology, algae and birds. During 1972-1973, De Villiers (1976) made a comprehensive study of the littoral ecology. In 1976 some marine biological studies were carried out (Grindley, 1978) together with zooplankton studies around the island (Grindley and Lane, 1979). In 1982 further aspects of the biology of Marion were investigated. The results of these studies were presented and published in the Proceedings of the 4th Symposium on Antarctic Biology, Wilderness (in press). The

results of the metal concentrations in specimens and in kelp are presented here.

The data from Gough and Marion Island have been analysed together for several reasons : both are remote islands. They consist of older and younger basalt and are roughly at the same latitude in the Southern Ocean. The animal population should not be contaminated from coastal water masses and they should be free from man-made pollution.

It was hoped that the concentrations of metals in marine animals from these remote islands would produce baseline data against which coastal metal enrichment of the continents could be measured and judged.

2. MATERIAL AND METHODS

Gough Island

The samples were collected by divers at water depths between about 20 metres and the shoreline. All specimens are from Repetto Bay which is on the west side of Gough Island.

Marion Island

The samples came from shallow water not deeper than 12 metres and were collected at Transvaal Cove.

Both animal collections are housed in the South African Museum.

Specimens for metal analysis were placed individually into plastic bags and kept frozen ($-20^{\circ}C$) until laboratory analysis. There, the animals were identified, weighed wet, dried at 60 C for 12 hours and weighed again. Trace metal preparation and determination was done according to Orren et al. (1980).

Animals for comparison were collected around Cape Town (Figure 1), South Africa.

3. RESULTS

Gough Island

Although there is a dense concentration of kelp and algae, the fauna associated with these were surprisingly sparse. Five different species were collected : Argobuccinum tristanensis; large sea urchins Arbacia dufresnii and a smaller sea urchin Pseudechinus novaamsterdamae; chitons Dinoplax sp. and sponge-eating nudibranchs Neodoris evinacea.

The metal concentrations in the whelks are given in Figure 2. There seems to be no correlation between size (dry mass) and metal concentration, with the possible exception of lead which may decrease with increasing size of the whelk. The zinc concentration was exceedingly high; comparable whelks from Cape Town contain two orders of magnitude less zinc as body burden. Other metal concentrations were similar to those in coastal whelks.

There was very little protein matter in the sea urchins and the shells were not included in the analysis. Generally the metal concentrations were higher in P. novaamsterdamae (Figure 3) than in A. dufresnii (Figure 4). No real trend in concentration and size could be detected.

The chiton appeared to be able to reduce its metal body burden considerably with size (Figure 5). In each case the large animals had the lowest metal concentrations per unit body mass. This might indicate that the Gough Island chitons have a well-established detoxification mechanism. The strontium and iron concentrations were generally high.

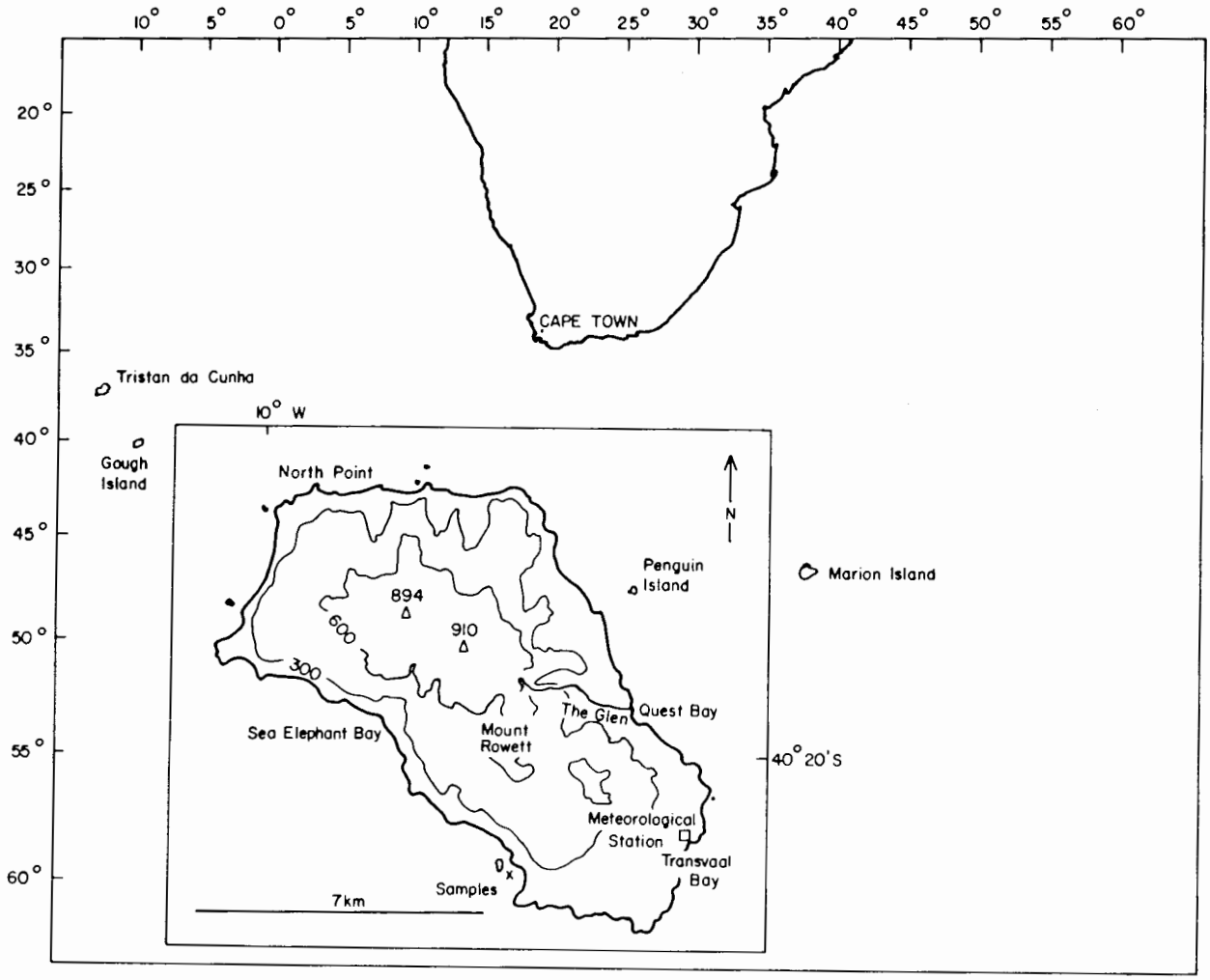

Figure 1

Position of Gough and Marion Islands in the Southern Ocean. Sampling position of Marion Island and Cape Town, South Africa.

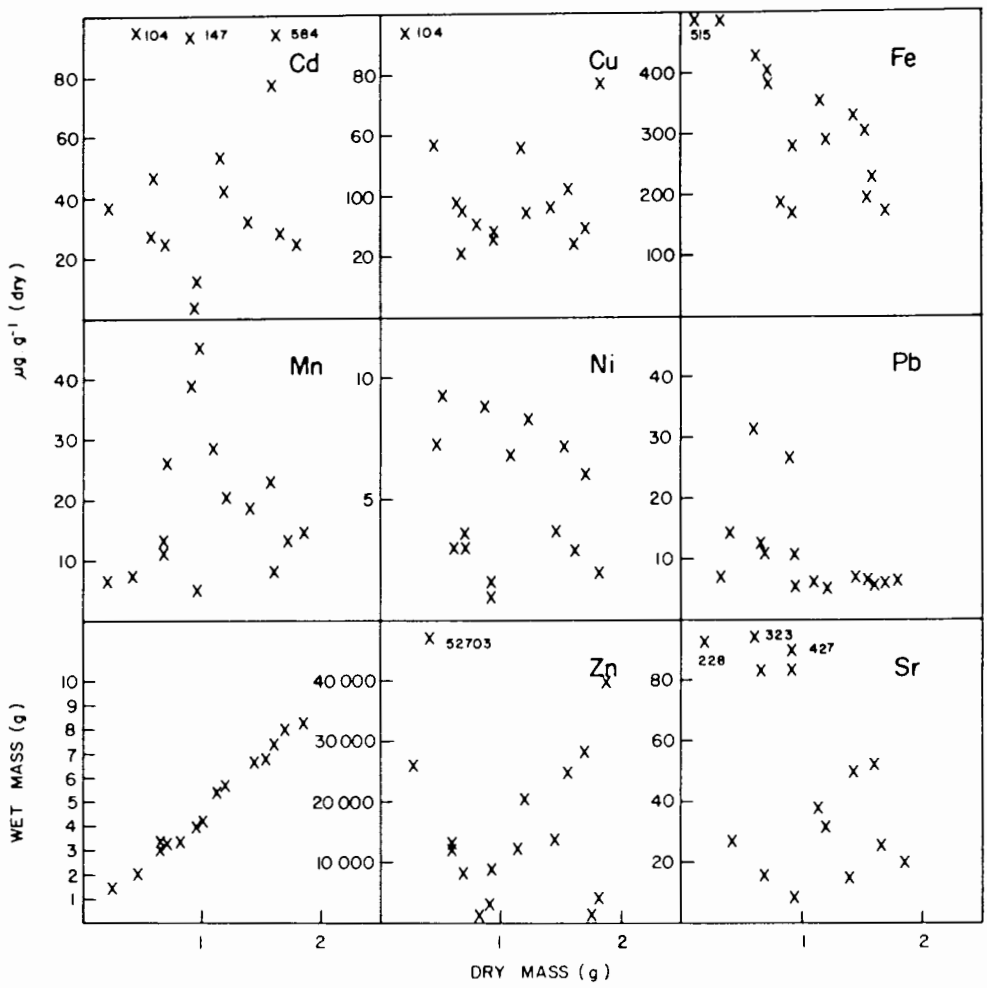

Figure 2

Metal concentration of whelks Argobuccinum tristaneusis from Gough Island.

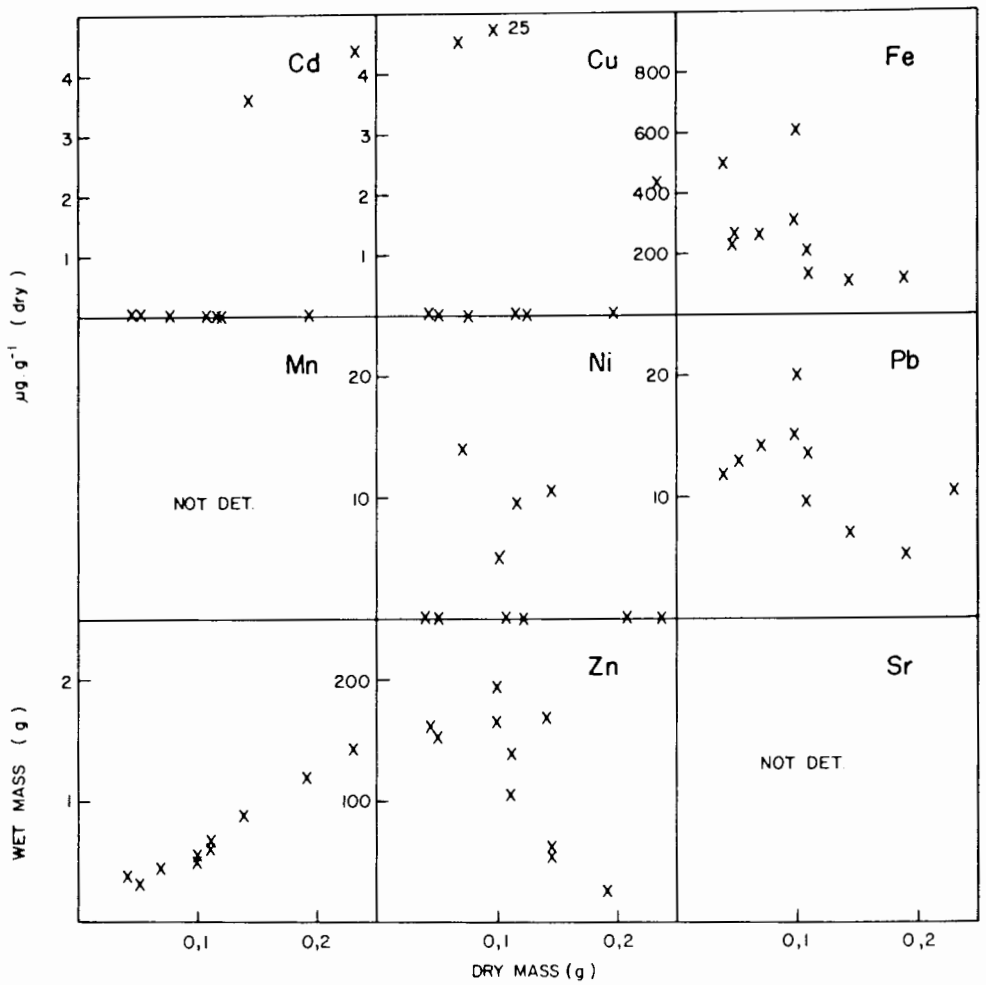

Figure 3

Metal concentration of small sea urchin Pseudochinus novaamsterdamae from Gough Island.

(NOT DET. = Not detectable, Sr concentration < 0.3 µg g^{-1})

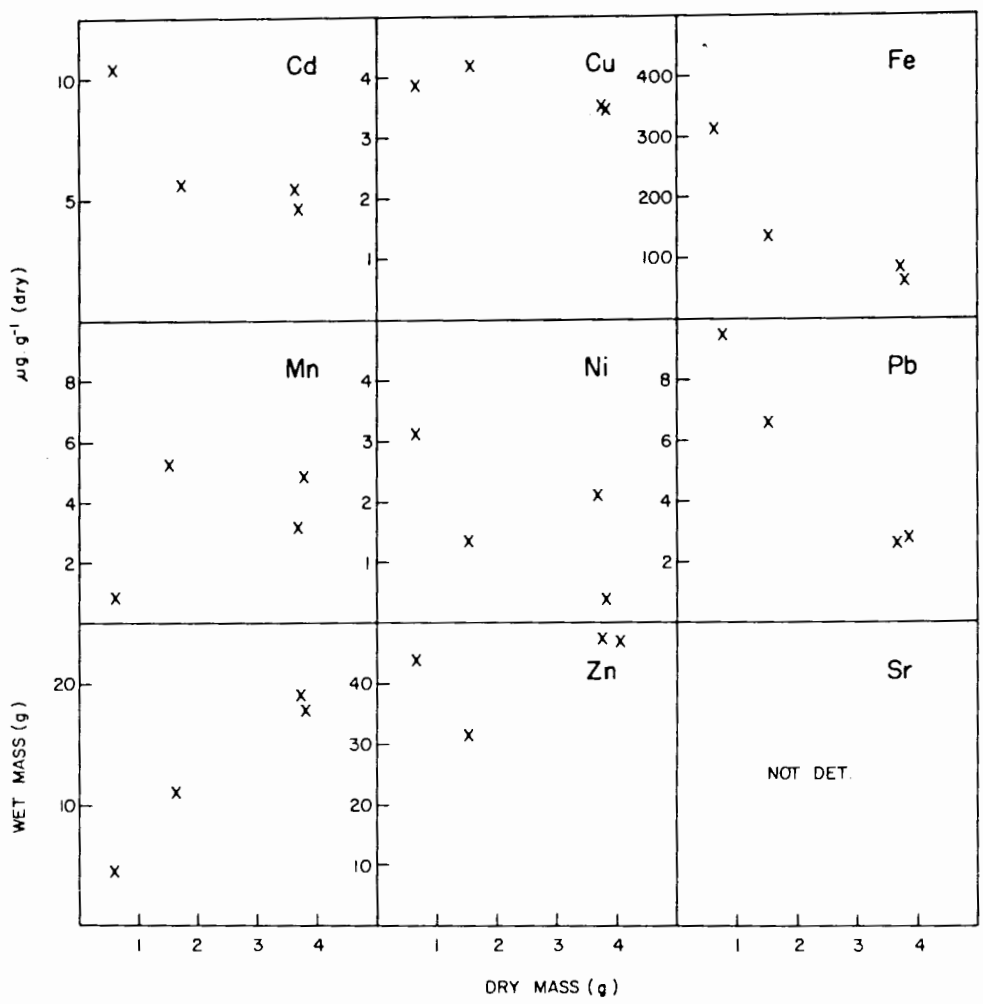

Figure 4

Metal concentration of large sea urchin Arbacia dufresnii from Gough Island.

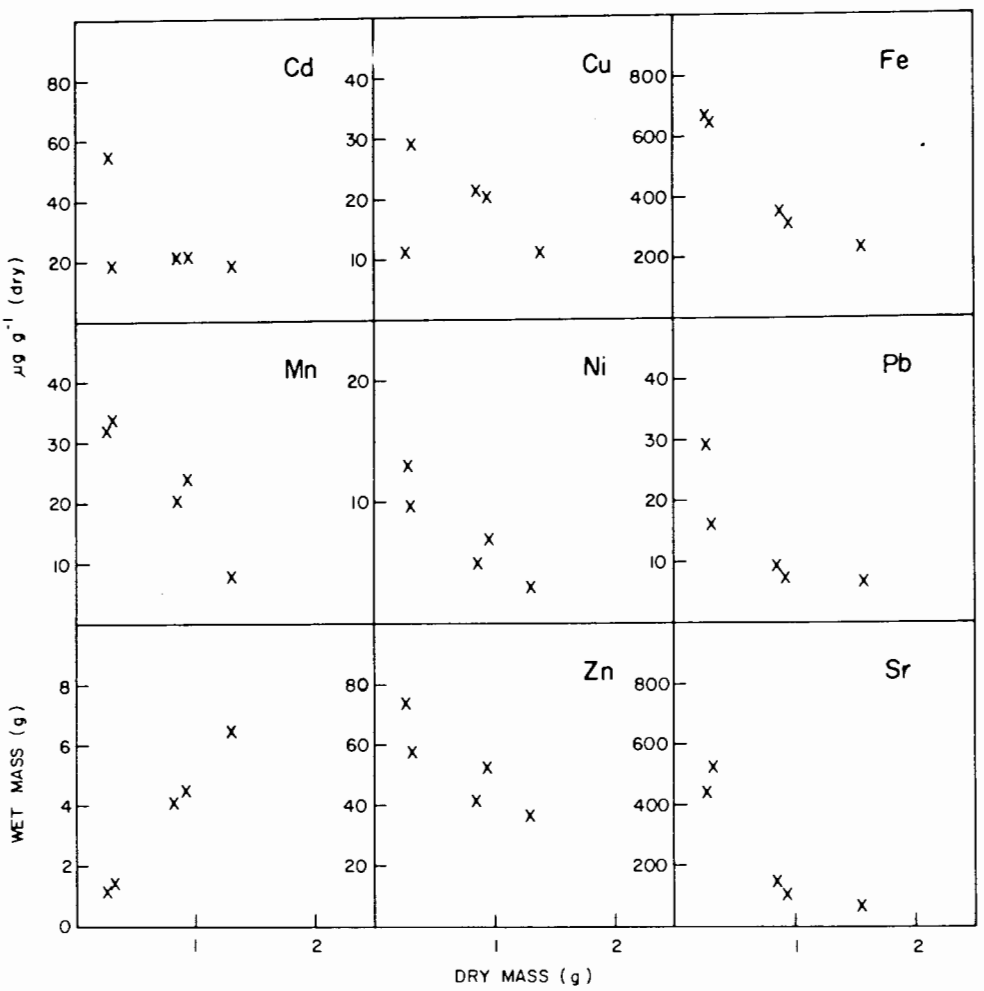

Figure 5

Metal concentrations in chiton Dinoplax sp. from Gough Island.

The metal concentrations of the nudibranchs (Figure 6) were very interesting. It was possible to obtain two species from Cape Town, Doris verrucosa, a sponge-eating nudibranch including the foodsponge and Iorunna tometosa (Figure 6). The sponge eating nudibranchs generally showed higher body metal concentrations than I. tometosa did. The Gough Island animals contained substancially higher cadmium, manganese, nickel and lead concentrations, while the Cape Town nudibranchs showed higher body burden in copper and iron. Zinc concentrations varied and strontium may be lower in D. verrucosa, if one assumed that there is a decrease of metal concentration with size.

It was surprising that there was no consistent relationship between the metal concentrations in animals from Cape Town and the very high body burden of the Gough Island specimens.

Marion Island

Six different species were collected from metal analysis : crabs (Hymenosoma sp.) isopods (Limnoria antarctica) amphipods (Hyale grandicornis) together with kelp (Durvillaea antarctia) that shelters the amphipods; starfish Anasterias rupicola and limpets Nacella delesserti. The kelp plants were divided into hold fast and stipe.

The metal concentrations in the crabs are given in Figure 7. The concentrations of copper, iron, manganese and zinc may decrease with increase in size of the animals, although more data are needed to substantiate this. There appears to be no correlation between size and the metals cadmium and strontium.

The body burden in the few isopods (Figure 7) analysed was similar in magnitude to that in the crabs. Size-metal correlation may exist for iron and zinc.

The metal concentration in the amphipods (Figure 7) were lower than those in the crabs and isopods.

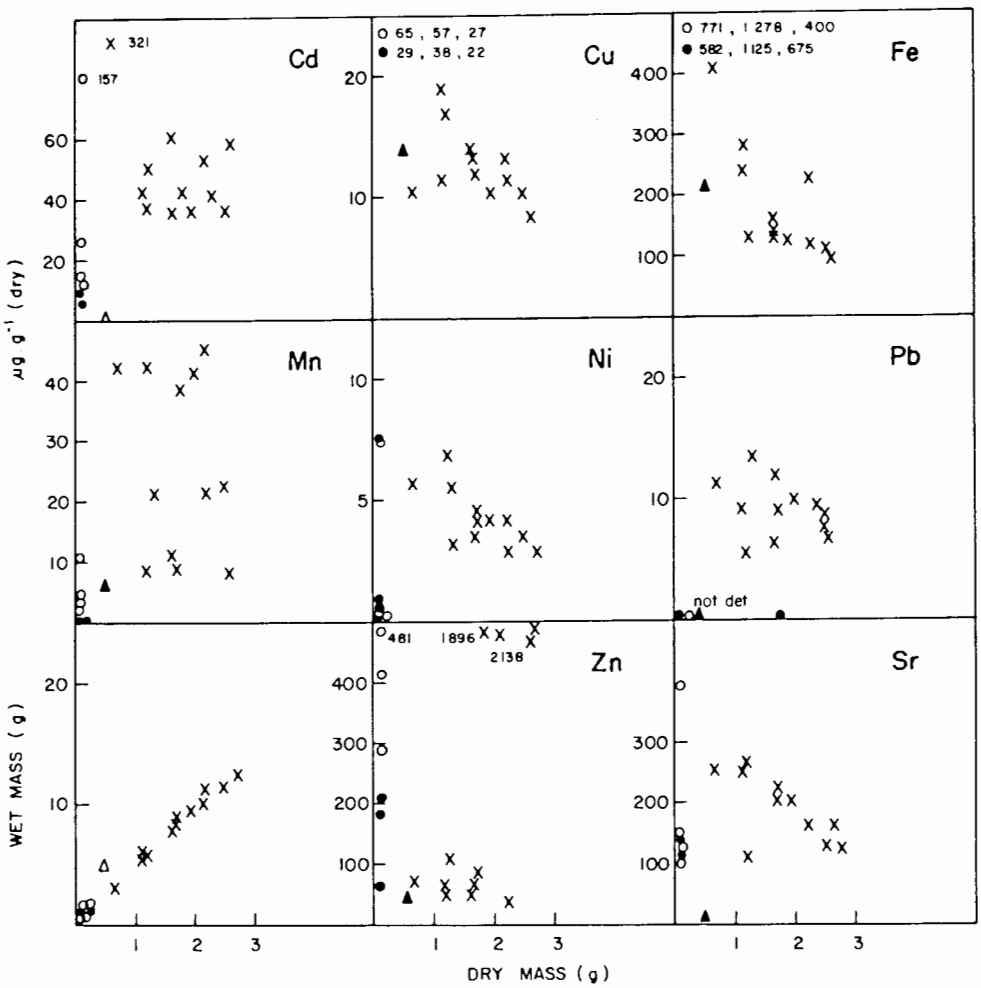

Figure 6

Metal concentration of nudibranchs Neodoris evinacea (Gough Island); Doris verrucosa and Iorunna tometosa both from Cape Town including the food sponge.

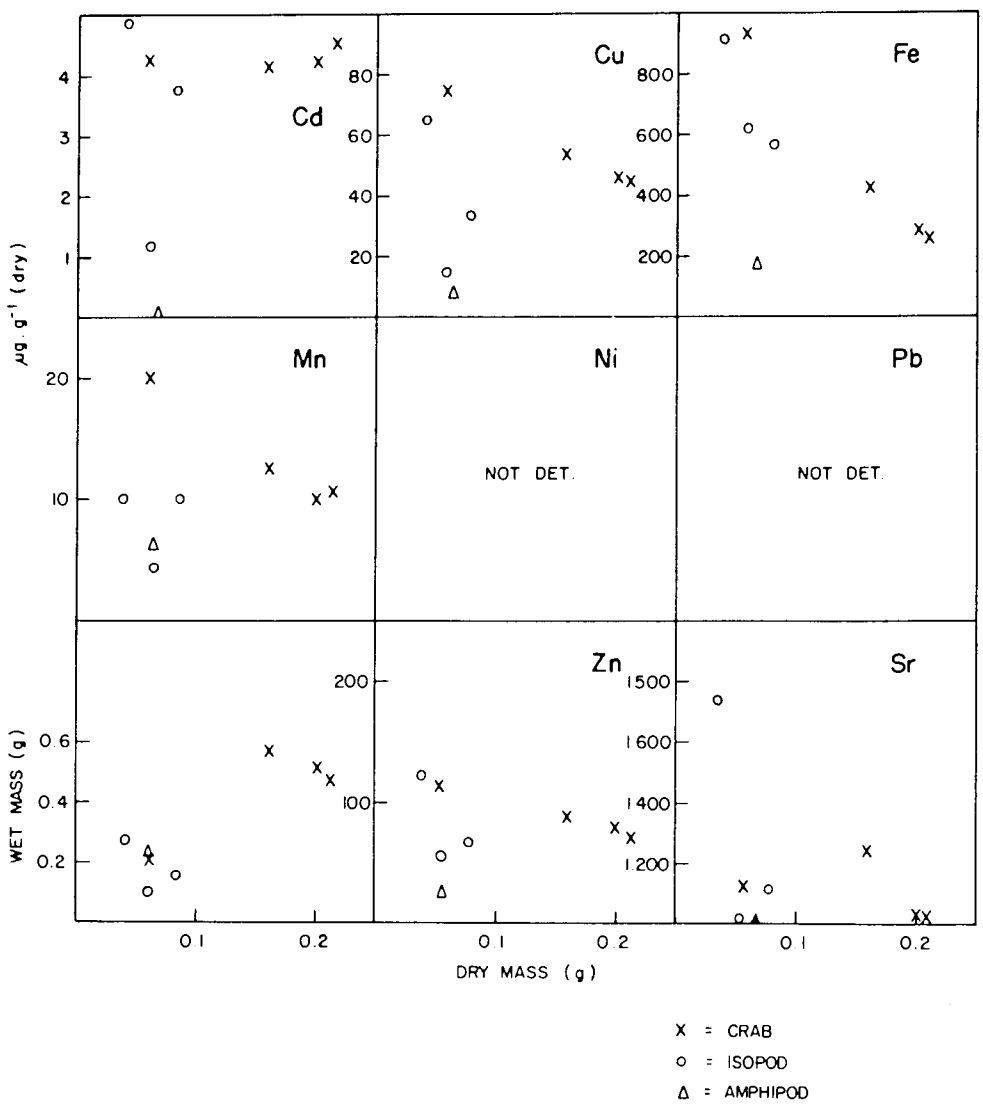

Figure 7

Metal concentration in crab, isopod and amphipod from Marion Island.

(NOT DET. = Not detectable, Ni concentration < 0.4 µg g^{-1}; Pb concentration < 0.2 µg g^{-1})

There seemed to be a relationship in the starfish (Figure 8) between body size and the metal concentration of copper, iron, zinc and strontium.

The data for the sea cucumber (Figure 8) were inclusive but the size range was not large enough for any correlation. In general, metal concentrations were very low in these animals excpt for those of zinc and strontium which were similar to those in the starfish.

The body burdens of the Marion Island limpets (Figure 9) were compared with those of Patella granularis collected at False Bay, Cape Town (Figure 9). There seemed to be very little difference between the metal concentrations in limpets from the two locations. Only manganese, zinc and strontium levels were elevated in the specimens from Marion Island and only iron, manganese and strontium seemed to diminish with increasing size of the limpets.

4. DISCUSSION

It was hoped that animals from remote islands such as Gough Island and Marion Island would show very low metal concentrations which could be used as baseline values for the study of similar species from elsewhere. The results obtained showed that these values were not low as illustrated in Figures 6 and 9. In some instances the metal concentrations in the island species were rather high especially sinc and strontium. It can be argued that the reason for these high metal concentrations could perhaps arise from the island environment. This was found to be correct; Table 1 shows the acid leached metal concentration in the dominant lava basalts of Marion and Gough Islands collected on the beach (Le Roex, in press) and of the sediment collected from sample sites near Cape Town.

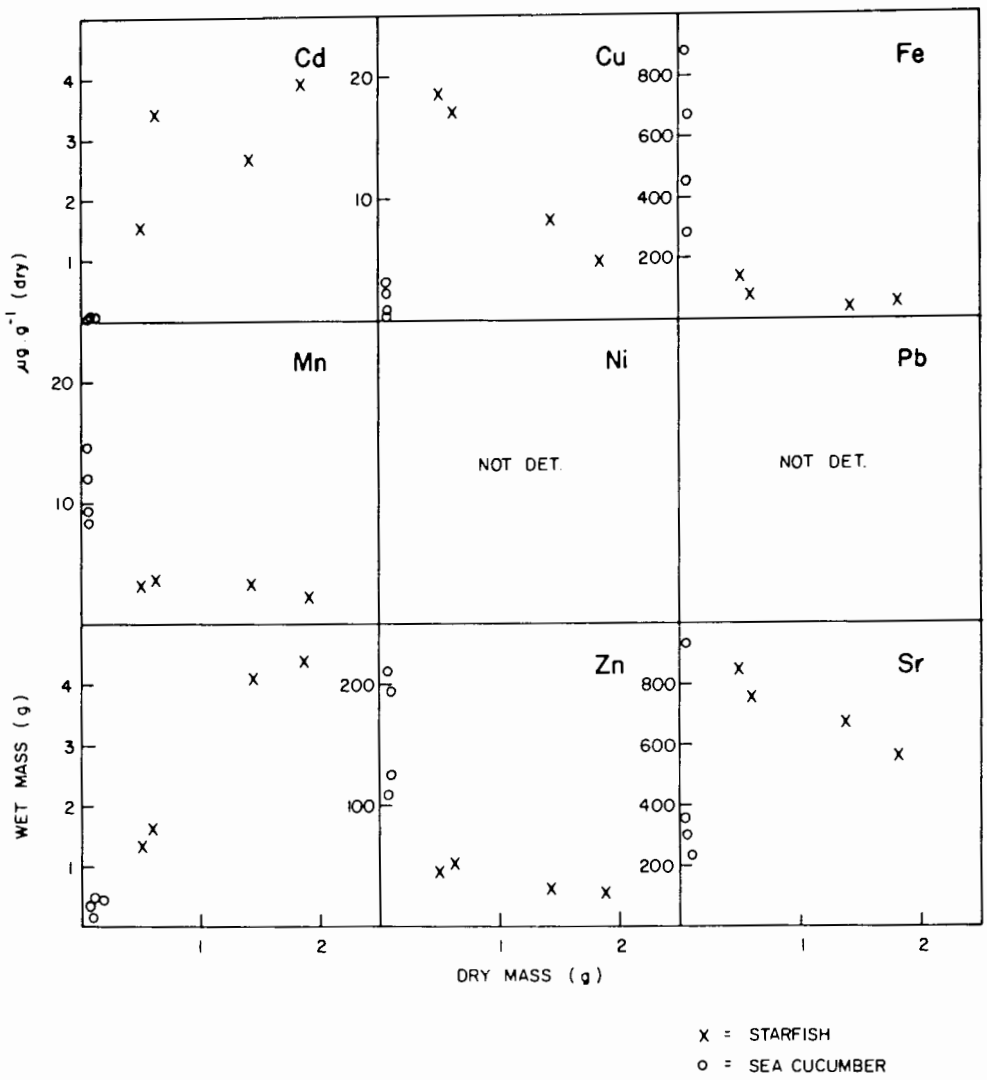

Figure 8

Metal concentration of starfish Anasterias rupicola and sea cucumber as well as different parts of kelp from Marion Island. (NOT DET. = Not detectable, Ni concentration < 0.4 µg g^{-1}; Pb concentration < 0.2 µg g^{-1})

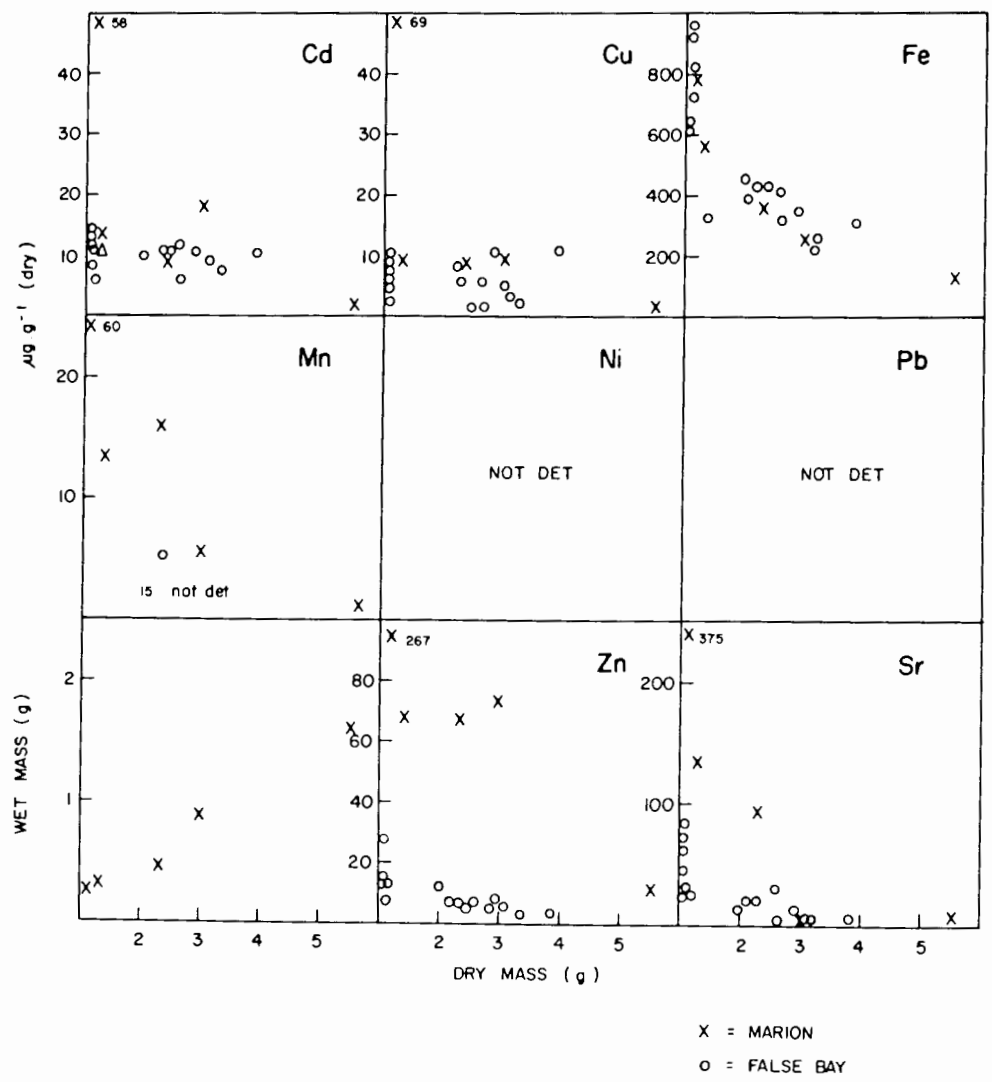

Figure 9

Metal concentration of limpets Nacella delesserti (Marion Island) and Patella granularis (Cape Town).
(NOT DET. = Not detectable, Ni concentration < 0.4 µg g^{-1}; Pb concentration < 0.2 µg g^{-1}).

The data in Table 1 explain the high body burden found of strontium and manganese in the island species, but do not explain the higher zinc and copper concentrations in nudibranchs collected in Langebaan. Nor does it explain the similar (limpet) and even lower iron concentrations (nudibranchs) in the island species which are exposed to extremely high concentration of environmental iron.

The data from this study permit the following contradictory conclusions to be drawn:

1. a. Baseline studies from remote areas show lower metal concentrations than coastal animals (copper, Figure 6).
   b. Baseline studies from remote areas do not show lower metal concentrations (cadmium, Figure 6).
2. a. Environmental metal concentrations must be taken into consideration (nickel, Figure 6; manganese, Figure 9).
   b. Environmental metal concentrations are of no importance (iron, Figure 9).
3. a. The size of the baseline animal is relevant (strontium, Figure 6).
   b. The size of the baseline animal does not play any significant role (cadmium, Figure 9).
4. a. Similar species have similar metal body burdens (copper, Figure 9).
   b Similar species hve very different metal body burdens (iron, Figure 6).
5. a. Comparisons of metal concentrations in animals from different areas show meaningful correlations (iron, Figure 9).
   b. Animals from different areas cannot be compared at all (copper, iron, Figure 6).

TABLE 1 Metal concentration ($\mu g\ g^{-1}$ dry) of rocks from Gough and Marion Island and the sediment collected from Langebaan and Cape Town (mean of 35 samples).

Location	Cd	Cu	Fe	Mn	Ni	Pb	Z	Sr
Gough/Marion	-	13.3	76000	1007	155	-	108	919
Langebaan	0.23	0.97	1678	9.88	0.96	1.52	7.98	-
Cape Town	0.1	0.3	385	2.7	0.6	1.9	1.2	-

The question now arises : Are baseline studies of any value?

1. Baseline studies can identify concentrations of various metals in marine animals from defined localities which may be identified in terms of natural origin, or those effected by pollutants (not spots)(Hamilton, 1983). It is thought that these answers are rather academic and not of much use.
2. In some cases baseline studies will show the extent to which the seas have become metal-enriched as a result of man's activities. However, the coastal animals used in this study will require considerable amounts of metal contaminants to reach level, similar to those collected from Gough and Marion Island.
3. It can define the possible dispersion of pollutants in time and space, for example effects of dilution from outfalls during different seasons. If a baseline study can be done, further surveys could indicate changes of metal concentration with time and position from the source.
4. The study can show up some biological responses to metals. It is felt that this is an important point (Hennig and Orren, 1983), but the question remains whether baseline studies are required of a large number of different localities to recognise this?

In metal baseline and pollution studies there is the tendency to make easy measurements. Samples are obtained with minimum effort, dried, pulverised to a powder and analysed. Unfortunately these results are then often used by management such as pipeline planners in a futile attempt to predict pollution effects by different metals on different animals in different areas.

It is felt that it is no longer sufficient to document pollution in terms of metal concentrations of contaminants. Determinations of metal concentrations <u>per se</u> provide no information on the availability of the compounds to the resident animals (bioavailability) or on their potential to have adverse effects (Chapman and Long, 1983).

Today, bioassays, and not investigation of total metal concentrations, should comprise the central feature of any marine pollution assessment program. A basic recommendation would be to attempt to use specific metabolic pathways that involve essential elements and which are likely to lead to the accumulation of pollutant metals. These could improve the significance of the results and may eliminate species, size, site and seasonal differences (Simkiss and Taylor, 1981). A promising approach to the study of metal accumulation would be via metal binding proteins and metallothioneins (Carpene et al., 1983).

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Suggestion to 'Baseline' —A Record of Contamination Levels

The editorial of *Marine Pollution Bulletin* (Hamilton, 1982) introduced a new section called 'Baseline'. There is a real need for such a section, especially in metal pollution work. It will not only reduce research time spent on reviews and facilitate comparison with new data but could also show up some biological response to metals. Thus we propose that some additional information should be included in the suggested format. For baseline data of biological materials it is important that size and/or weight (wet or dry) as well as sex should be included.

To illustrate these suggestions metal concentrations of the tail meat in the South African crayfish (*Jasus lalandii*) versus carapace length appear in Fig. 1. No difference between males and females was found. For zinc it would be sufficient to report a mean and standard deviation of 72.3 ± 48.1 $\mu g\ g^{-1}$ (dry). In the case of iron and copper, metal concentration decreased with length; hence, without the size data, a mean would not represent a true picture. Furthermore this decrease suggests that the animal is coping with its environmental metal intake and has some mechanism to reduce its overall metal load.

In Fig. 2 the zinc concentration of *Choromytilus meridionalis*, the South African black mussel, has been plotted against total dry body weight. An arithmetic mean of these data would be of little value. Large males have very much less zinc per gram body weight than smaller and younger animals. Females, on the other hand, either accumulate zinc with increasing size or at least are apparently not able to purge it as efficiently as the males.

We feel that with a few more details in reporting size and sex of biological material a much more meaningful 'baseline' type report can be achieved; for instance Witkowski & Frazier (1982) unfortunately do not mention the length, size or sex of their analysed adult cheloniid turtles nor the weight of the barnacle.

Crayfish

Jasus lalandii were collected at Noordwesbaai, Cape, South Africa (32° 52′ S, 17° 53′ E) by divers from a water depth of 5 m. The animals were frozen (−8°C) for two days, then defrosted to separate carapace and tail. Duplicate samples were cut with a cleaned glass knife from the meat protruding from the tail. The samples were oven-dried at 60°C to constant weight in acid-washed glass vials. Concentrated nitric acid (25 ml) was added and samples allowed to stand at room temperature overnight, before

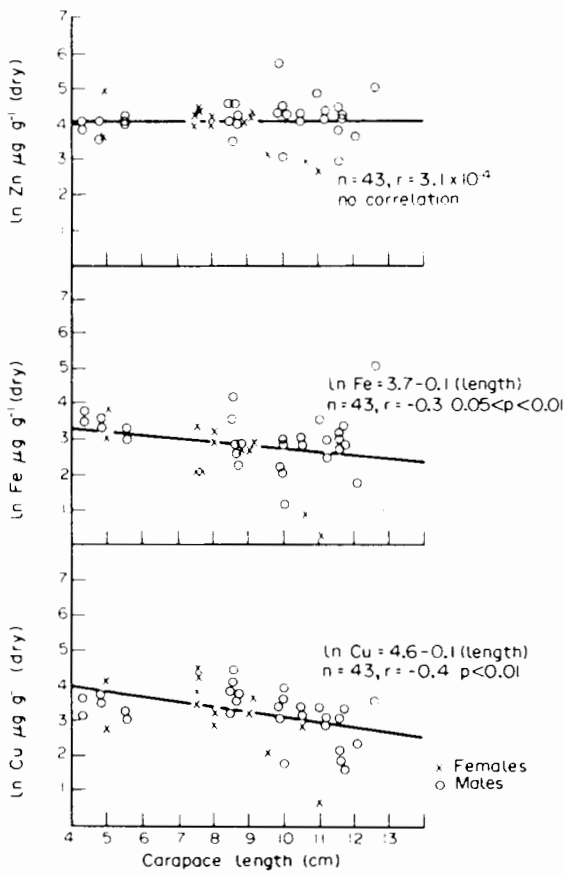

Fig. 1 Metal concentrations (ln scale) in rock lobster tail meat.

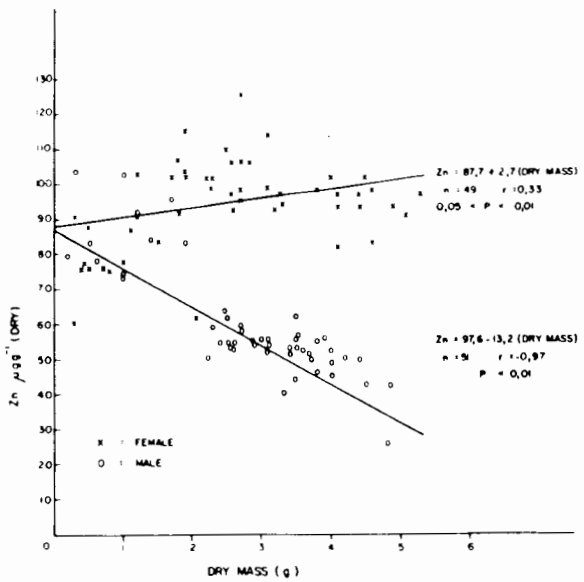

Fig. 2 Metal concentration in black mussel (after Watling, 1978).

heating to dryness at low heat. This procedure was repeated until a white or grey residue was obtained. A 4:1 mixture of nitric/perchloric acids (25 ml) was then added and samples again taken to dryness. Residues were dissolved in 10% nitric acid (5 ml) and analysed for metals using flame atomic absorption with background correction for those trace elements with resonance lines less than 280 mm. Mixed standards were prepared from commercial standard solutions, blank determinations were run concurrently while precautions and further details were as given by Orren *et al.* (1980).

Black Mussel

Choromytilus meridionalis were grown for 8 months at Featherbed, Knysna, South Africa (34° 05′ S, 22° 59′ E). The animals were allowed to purge their intestinal content in clean water for up to five days. The wet tissues were then removed and frozen (no temperature specified). Thawed specimens were oven-dried at 90°C for 24 h. Further analysis was as for crayfish (above), but details appear in Watling (1978).

H. F-K. O. HENNIG
NRIO, c/o Department of Oceanography,
University of Cape Town,
Rondebosch, 7700,
South Africa

M. J. ORREN
Department of Analytical Science,
University of Cape Town,
Rondebosch, 7700,
South Africa

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INFLUENCE OF ZINC ON THE INTERMOULT PERIODS OF TWO MARINE CRUSTACEANS: ARE METAL ACCUMULATION EXPERIMENTS OF ANY VALUE?

by

H F-K O Hennig

NATIONAL RESEARCH INSTITUTE FOR OCEANOLOGY
C/o DEPARTMENT OF OCEANOGRAPHY, UNIVERSITY OF CAPE TOWN
RONDEBOSCH, 7700, SOUTH AFRICA

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INFLUENCE OF ZINC ON THE INTERMOULT PERIODS OF TWO MARINE CRUSTACEANS: ARE METAL ACCUMULATION EXPERIMENTS OF ANY VALUE?

In accumulation experiments it has been found that zinc at a concentration of 61 μM dm^{-3} increases the moulting frequency of both prawn, Palaemon elegans and crayfish, Jasus lalandii. The exuvia could be used as a mechanism to regulate the metal burden of crustaceans. Zinc could also be responsible for the difference between moulting frequency of laboratory and field animals.

No elevated metal concentration could be found in crayfish after the accumulation experiment. This, together with the increase in moulting frequency, has been used to question the validity of unrealistic accumulation experiments and the reporting of total organ metal concentration. It is suggested that biochemical indices, especially metal binding proteins/metallothioneins should be used instead.

Key words: metal accumulation; pollution indicators; intermoult periods; zinc.

INTRODUCTION

In any marine pollution study it is important that concentrations of pollutants measured chemically should be related quantitatively to biological effects. Unfortunately most studies of acute metal or chronic effects of metals are carried out with unrealistically high metal levels. Most of these studies are of little more than academic interest since they are not likely to be met in the field. In most biological systems there is a lag phase, depending to a great extent on the generation time of the animals under investigation. Furthermore, young animals often have a larger metal burden than older animals. Thus it is often difficult to evaluate the effect of pollutants on long-lived animals either in the field or laboratory studies. This was shown by Hennig et al. (1982) in experiments with the South African crayfish, Jasus lalandii (H Milne Edwards), which can reach an age of about 40 years (Pollock, 1978). No accumulation of metals was observed in a laboratory experiment conducted over four weeks, but this was attributed to the short time scale of the experiment, in comparison with the life span of the animals (Hennig et al., 1982). It was, however, noticed that the moulting period of these study animals had been altered.

Little is known about abnormal moulting periods in crustaceans. Paterson (1969) found differences in moulting frequencies in captive adult crayfish, but she had no obvious explanation for this. She thought that certain periodic behavioural changes may have been the reason. On the other hand, Chen (1981) has reported that increased metal concentrations decreased the intermoult periods in the prawn, Palaemon elegans.

Cook and Achituv (1983) found that the moulting frequency in P. pacificus increased with increase in temperature.

This investigation examines the effect of heavy metals, especially zinc, on the moulting frequncy in P. pacificus and J.

lalandii. The different exuvia were examined for their metal concentration and zinc appeared to play a prominent role in the moulting process.

MATERIALS AND METHODS

The "base-line" metal concentration of the prawn P. pacificus was determined from animals collected from Langebaan, South Africa (Figure 1). The animals were dried and the whole bodies analysed for metals.

The experimental animals were kept in aerated 10 dm^3 all glass aquaria at 15 ± 1°C. Usually 12 animals were kept in each tank and they were fed with pieces of A. ater. This is similar to the experiments described for the crayfish below. An open system was used containing one-week old sea water from Kalk Bay, Cape Town (Figure 1). This was dispensed at a rate of 0,2 dm^3 per hour in discrete portions, by means of a modified Mount and Brungs diluter (1967). The modifications consisted of adding a suction pump to the last overflow reservoir to ensure that all partitions were emptied. The resulting mixture from the diluter was monitored daily and contained 15 $\mu M\ dm^{-3}$ zinc. Furthermore, a glass mixing spiral was added for the effective mixing of effluent and seawater (Figure 2).

The background metal concentrations in J. lalandii were determined for animals collected from Noordwesbaai, Saldanha, South Africa (Figure 1). There were 29 male and 14 female animals. The crayfish were sized, sexed and dissected. The tails, green glands and gills were analysed separately for metals.

The crayfish used in the experiment were collected from the same area. They were kept at 15 ± 1°C in aerated 10 dm^3 all glass aquaria, usually two animals per tank and they were fed daily each with a 200 mm ribbed mussel Aulacomya ater. Metal effluent

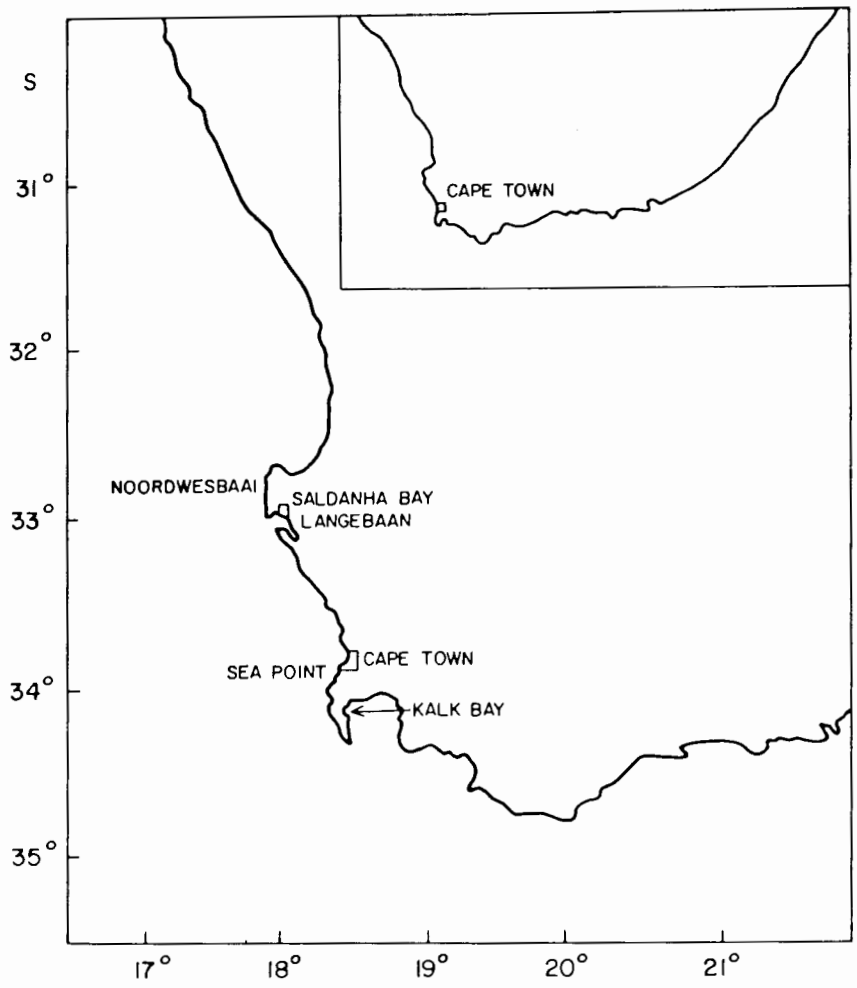

Figure 1 Location map of collection ares of different experimental animals.

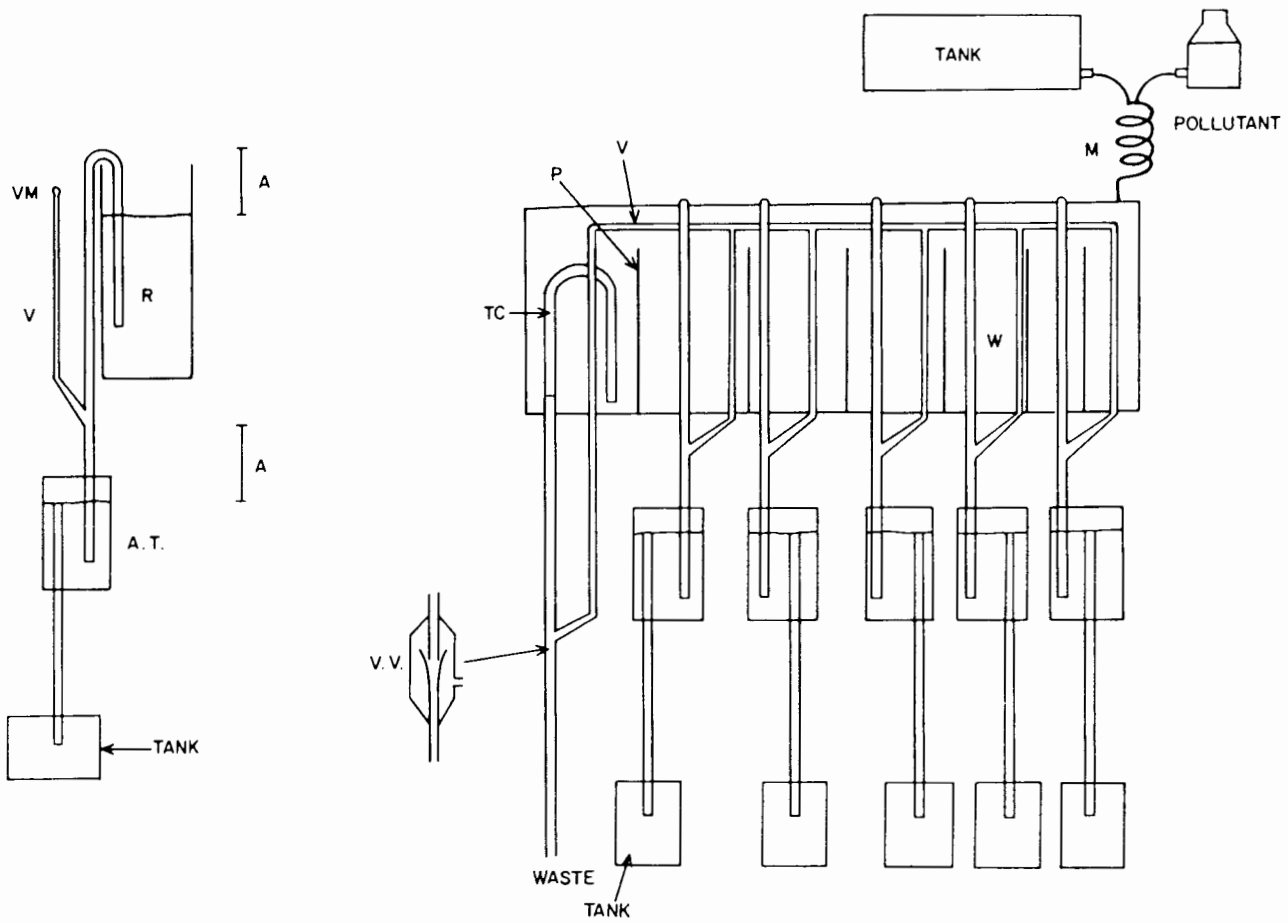

Figure 2 Modified Mount and Brungs diluter, front view and cross-section. AT = air trap; M = mixing of seawater from overhead tank and pollutant reservoir; P = partitions of each dilution cell; R = diluter cell reservoir; TC = "tantalus cup" overflow; V = ventur; VM = vacuum manifold; VV = vacuum ventur glass pump; W = water in dilution cell.

was added in predetermined volumes and the water of each tank was renewed daily. The metal concentrations in the water was monitored daily.

During some unrelated experiments 12 crayfish were kept in 50 dm^3 glass-fibre tanks, uaually three animals per tank, in a large recirculating system. These crayfish were collected from Sea Point, South Africa (Figure 1). Because of unforeseen problems the whole recirculation system was contaminated and so these crayfish were kept for over two months in sea water with a zinc concentration of 61,2 $\mu M\ dm^{-3}$. These animals were fed with Choromytilus meridionalis (Kr.).

A food mussel A. ater had been collected from Noordwesbaai (Figure 1). Forty mussels were analysed for trace metals in the same way as was done for the experimental animals. The C. meridionalis which were used as food for the Sea Point crayfish were also analysed. The whole soft part of each mussel was removed with a glass knife and analysed as described by Orren et al. (1980).

After dissection all biological samples, whole or dissected, were oven dried to constant weight at 60°C. Digestion methods used were standard: these have been described elsewhere (Orren et al., 1980). After digestion of the animals the remaining solutions were analysed by flame atomic absorption spectroscopy using standard conditions. Background correction was used for those elements for which the wave-length of the analytical line was 280 nm.

RESULTS

Prawns

The concentrations of Cu, Fe, Sr and Zn in uncontaminated whole prawns are illustrated in Figure 3. In the case of copper,

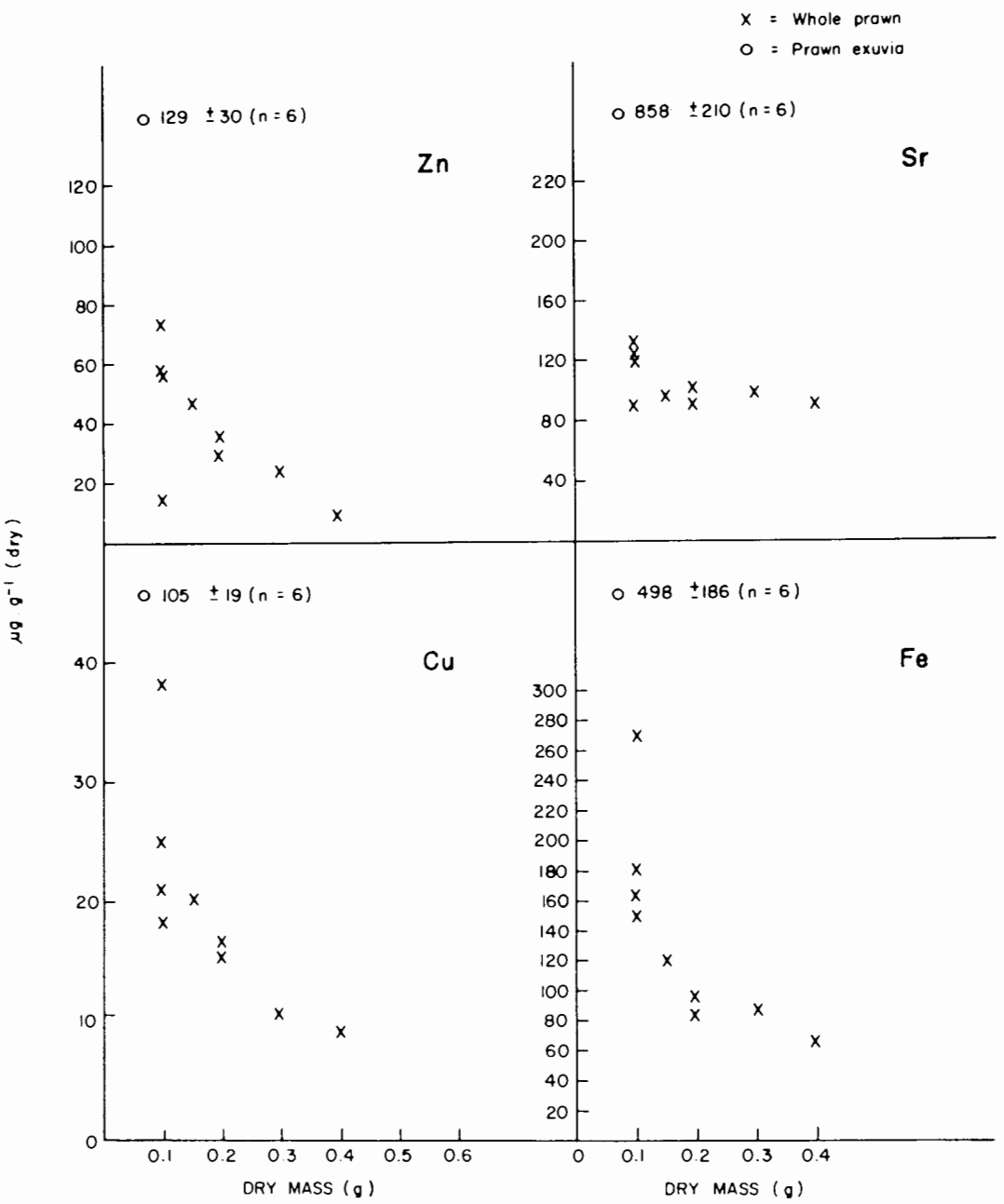

Figure 3 Metal concentration of total body mass and exuvia of prawn P. pacificus.

iron, and zinc, the metal concentrations decrease with increasing animal size. The strontium levels appeared to be constant for all sizes of P. pacificus. The main source of this metal is very likely to be the cuticle of the exoskeleton, as seen from the high Sr concentrations found in the exuvia (Figure 3).

The concentrations of Cu, Fe and Sr in the exuvia (Figure 3) were substantially higher than those in the whole animals. Zinc, on the other hand, was only slightly elevated in the exuvia. There was no correlation between metal concentration and weight of the exuvia.

Temperature has a marked influence on the moulting frequency of P. pacificus (Cook and Achituv, 1983). Their data together with additional data are presented in Figure 4. This shows that the average moulting time of P. pacificus kept in seawater with a zinc concentration of 15 $\mu M\ dm^{-3}$ was 12,4 ± 1,2 days. The animals moulted more often than in normal circumstances and the survival rate was very poor. The animals appeared to be too weak to shed their old shell properly and often died soon afterwards. The control animals had no such difficulties and none were lost.

Crayfish

The metal concentration of the different body parts of uncontaminated crayfish are illustrated in Figure 5. There were no differences between concentrations in male and female animals. It should be noted that the zinc concentration in the tails and gills showed no correlation with size, while iron and copper concentrations decreased with size. In the green gland, however, the zinc concentration did decrease with animal length. Previously animals contaminated with a metal effluent showed no measurable accumulation over a test period of 60 days (Hennig et al., 1982). Four crayfish were kept in the highest metal concentration (100 cm^3 effluent per 10 dm^3 tank), of these one

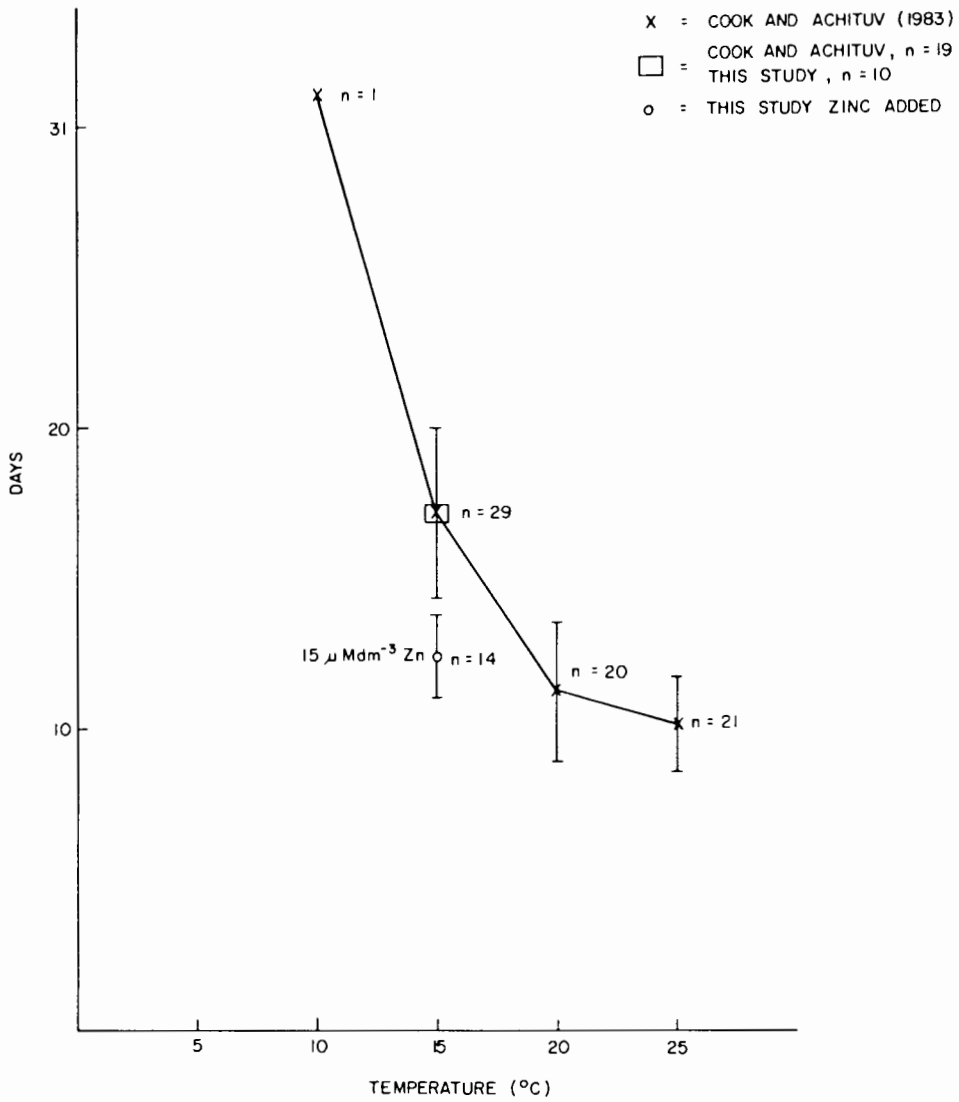

Figure 4 Moulting frequency of P. pacificus in days with different temperature and high zinc concentration.

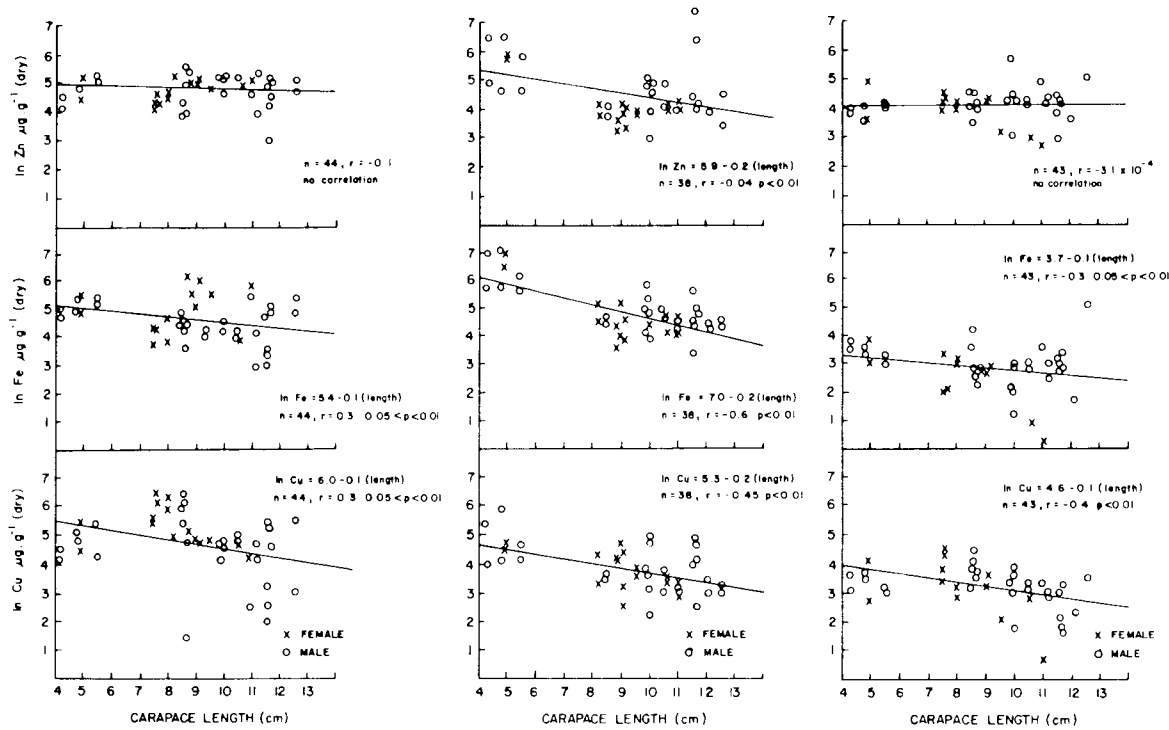

Figure 5 Metal concentration of different body parts of J. lalandii.

female moulted after 41 days (23.1.82). The metal concentrations of the exuvia are given in Table I.

Copper and zinc concentrations in the exuvia were lower than those in the whole gills while iron concentrations in the moult were higher. In the case of the experimental animals most of the copper was in the whole gills while iron and zinc concentrations were substantially higher in the exuvia. The concentrations in other body parts are difficult to compare but it was surprising to find such a high zinc concentration in the shell membrane. These levels were even higher than those in the excretory organs, the green glands. It should also be noted that the exuvia contained high concentrations of lead.

Of the 12 mature crayfish (eight males and four females) which were accidentally exposed to 61 μM of zinc, eight moulted within one week (12 to 18 June, 1982) (five males and three females).

Paterson (1969) and Pollock (1978) have shown that there is only one ecclysis per year in mature J. lalandii. Males usually moult in December/January and females in May/June.

Food

The metal concentrations in the food animals are given in Figures 6 and 7, respectively. No correlation between mussel size and zinc concentration was found for C. meridionalis. In A. ater there was a decrease in Fe and Cu concentrations with size of animal while Zn concentrations were not correlated with size. In general metal concentrations in A. ater were higher than those in the C. meridionalis.

TABLE I: METAL CONCENTRATION ($\mu g\ g^{-1}$ dry) OF CRAYFISH EXUVIA

Organ	Sex	Size (cm)	% Dry	Cd	Cu	Fe	Mn	Ni	Pb	Sr	Zn	Remarks
Gills	M	5,25	18	ND	21,3 ± 0,5	584 ± 37	ND	ND	42,7 ± 0,7	88,8 ± 0,5	17,5 ± 1,2	Control
Gills	F	11,95	18	2,9 ± 0,1	47,7 ± 0,8	629 ± 41	0,5 ± 0,1	10,8 ± 1,4	55,3 ± 0,8	121,2 ± 0,8	335,3 ± 29,1	Effluent exp.
Gills	M	7,78	18	ND	22,2 ± 0,4	593 ± 38	ND	2,1 ± 0,6	47,4 ± 0,9	95,4 ± 0,7	217,1 ± 21,3	Zinc poll.
Stomach	M	5,25	4	ND	15,0	740,0	ND	ND	31,7	659,0	80,0	Control
Stomach	F	11,95	10	4,2	37,5	162,5	33,3	4,2	33,3	533,3	550,0	Effluent exp.
Stomach	M	7,78	8	ND	19,7	587,4	ND	ND	32,8	587,4	338,9	Zinc poll.
Carapace membrane	M	5,25	14	ND	140,0	1 096,7	ND	ND	39,2	773,3	96,7	Control
Carapace membrane	F	11,95	20	2,5	90,0	310,0	3,8	43,8	36,3	216,3	2 778,4	Effluent exp.
Carapace membrane	M	7,78	5	ND	120,5	619,3	ND	ND	31,2	571,9	624,1	Zinc poll.
Carapace cuticle	M	5,25	48	ND	8,0	195,7	ND	ND	-	1 039,0	13,4	Control
Carapace cuticle	F	11,95	51	1,2	14,0	219,5	ND	ND	-	987,5	15,8	Effluent
Carapce cuticle	M	7,78	44	ND	11,0	235,4	ND	ND	-	1 13,3	11,0	Zinc pollut.

ND = not detectable

- = not determined

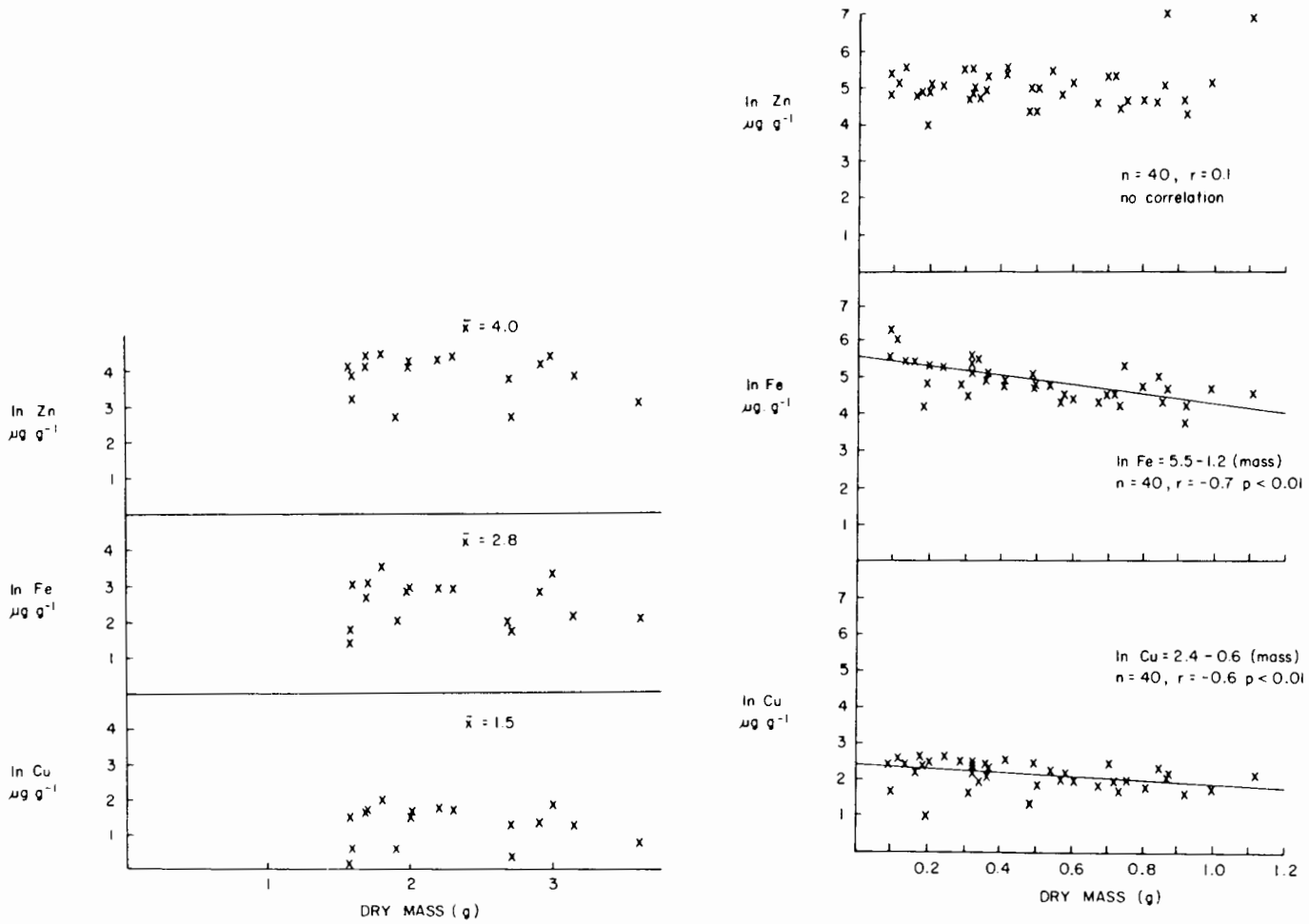

Figure 6 Metal concentration of food mussels, A. ater (left) and C. meridionalis (right).

DISCUSSION

The acute toxicity of metals to a wide variety of marine species has been determined by a number of researchers. Often the reports on the results were compiled for environmental protection agencies to assist in laying down criteria for ambient water quality (see APHA, 1971 and NAS/NAE, 1973). Some investigators (Eisler, 1971; Thorp and Lake, 1974; Ahsanullah et al., 1982) have already suggested that for many toxicants the 96 h tests are too short to enable the acute toxic effects to be determined adequately.

This study shows that even when unrealistically high metal concentrations were used no accumulation could be detected by the whole organ digestion method in longlived animals such as crayfish. For instance McLeese et al. (1981) found no excretion in lobsters (Homarus americanus) during eight months live storage in uncontaminated water. In our case, the period allowed for accumulation period was only about 0,6 per cent of the normal life span of the crayfish, whereas for the lobster it was 3,2 per cent.

Hennig and Orren (1983) have shown that young animals often have a higher metal burden per gram body mass than the older members of the same species do. However, most population studies are restricted to only one particular life stage because of lack of time and space (Gentile et al., 1982). This invalidates results of many studies relating the effects of metals on an entire population. The results of such investigations are unsatisfactory, after all an outfall to sea will affect adults as well as juveniles and eggs. After all these studies are primarily done to predict the metal effect on the whole population and not only on members of a population.

Metal concentration of food and the bio-availability of metals is usually not investigated or reported. In our study, crayfish

were fed with different mussels containing different metal concentrations. Yet the effect could not be measured by the whole organ digestion method, for example, total metal concentration.

This study has shown that zinc promotes moulting in two crustaceans and that this could be one mechanism of regulating internal concentration of heavy metals. The exuvia of the crayfish also had a surprisingly high lead content. These were comparable only to the metal levels found in the green glands. Similarly the metal concentrations in the prawn exuvia were well above those in the whole animal. This is, again, an indicator of a possible regulation mechanism and of a subject worth further investigation.

The results could also explain the difference between the moulting frequency of crayfish of both sexes in laboratory and in the field. Paterson (1969) reported too frequent moulting, under laboratory conditions, about every 4 to 6 months in J. lalandii. This may well have been due to the increase of zinc in closed circulation systems which use galvanised fittings and pipes as found in older aquarium set-ups.

Unfortunately from the point of view of prediction of pollution effects even these results are not very helpful. Although zinc caused the crayfish to moult more frequently the question of accumulation of the metal in the animal is still open. The prawns, on the other hand, are acutely affected. This was possibly not so much due to the toxicity of the metal but rather to the fact that they are unable to survive the frequent moulting and its consequent heavy energy demand.

It is not possible to come to either of these conclusions from the determination of total metal concentration in the animals. For that type of analysis in a laboratory investigation there are far too many variables, such as species, mass, sex, longevity, moulting, life cycle, temperature, food and many more. It is therefore suggested that metal pollution studies be directed

more towards bioassays which are not affected by so many variables. Those which come to mind are studies of depressed or activated enzyme reactions or presence of metal binding proteins (metallothioneins). The latter seems to be a particularly good approach; metallothionein is found in cyanobacteria, plants, lower and higher animals, different life stages and can be detected much earlier than can increased metal concentrations.

It is thought that more time should be spent on developing biochemical and cellular indices of effect than on carrying out unrealistic accumulation experiments. These conclusions were also drawn at a recent symposium (Stegeman, 1983).

ACKNOWLEDGEMENTS

Financial support from the National Programme for Environmental Sciences is gratefully acknowledged.

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METAL BINDING PROTEINS, REDEFINING THE CRITERIA OF METAL POLLUTION

BY

H F-K O Hennig

In: Environmental Health Perspectives. Conference on high affinity metal-binding proteins in non-mammalian species. September 19-21, 1984 Research Triangle Park, North Carolina, USA.

INTRODUCTION

Metal binding proteins as metallothioneins (MT) were originally isolated and characterised from equine kidney by Margoshes and Vallee (1957) and have extensively been studied in mammals (for a literature review, see Webb, 1975).

Very few information are available about the other zoological groups and particularly about the invertebrates (for review see Roesijadi, 1980/81). The information available consists mainly of descriptions on isolation of these proteins and so far very little use has been made of these unique relatively low molecular-compounds. Rugstad and Norseth (1975) suggested that metal binding proteins could be used for the study of cadmium resistance cells but only recently (Brinster et al, 1982, Palmiter et al, 1982 and overview see, Williams, 1982) has MT been used as a promoter to control and induce gene activity.

The fact that metal binding proteins are a consequence of elevated metal concentration in organisms has beem well known for many years. What has been overlooked is the unique opportunity of the presence of MT to reformulate the criteria of metal pollution. The detoxification effect of MT in animals from polluted areas has been mentioned by Olafson and Thompson (1974); Noël-Lambot (1976); Viarengo et al (1980) and Viarengo et al (1981). The only studies relating metal binding proteins to pollution are by Brown et al (1977); Hennig (1981) and Roch et al (1982). This lack is partly due to the design of most experiments which were aimed at isolation of proteins and hence of too short duration to allow for correlation to adverse physiological effect by the organism. In humans, Mills (1979) found changes in toxic elements in blood or whole blood sufficiently different to be of diagnostic significance. Sanders et al (1983) presented an equation of shifts in copper metabolism which correlated with adverse effects on growth of crab larvae.

In this study metal binding proteins have been isolated from different marine animals kept under identical enriched conditions, hence eliminating differences in method and seasons. The animals used differed in many aspects e.g. different phyla, size, mass, age, behaviour, food and life stages. As expected they also accumulated metals at different rates (see Hennig, paper 1 and 3). Furthermore a physiological effect, e.g. moulting in crustacea had been demonstrated without metal accumulation but in presence of metal binding proteins.

These findings were then related to field conditions e.g. the whelk Bullia digitalis and metal enriched grass; and a new concept of pollution was defined.

MATERIAL AND METHODS

Accumulation of Metals in Organisms.

(a) Jasus lalandii (rock lobster) have been used in two separate experiments. Adults (2) have been kept in each of twelve 10 dm^3 aquaria at 15 ± 1°C and dosed with industrial effuent (for details, see Hennig et al (1982)).

(b) Juvenile rock lobsters, hermit crabs (Diogenes brevirostris); sandshrimp (Palaemon pacificus); black mussels (Choromytilus meridionalis) and limpets (Patella granularis) were kept in an open system using one-week old seawater and solutions of cupric sulphate and zinc chloride. This was dispersed at a rate of 0,2 dm^3 per hour in discrete portions, by means of a modified Mount and Brungs diluter (for details see Hennig, paper 5). The resulting mixture from the diluter was monitored daily and contained 15 $\mu M\ dm^{-3}$ zinc and 16$\mu M\ dm^{-7}$ copper.

Background metal concentrations were determined for these animals and are presented by Hennig (in preparation, this volume).

The field studies concentrated on the whelk Bullia digitalis which was collected from Koeberg. These whelks have an unexplained high cadmium concentration for details see Cuthbert et al (1976) and Hennig (paper 1). Furthermore "Kikuyu" grass was obtained from CSIR Water Research Bellville. This grass was grown with 320 t/ha dried sewage sludge and came from site $A5_4$.

The metal determination of organisms was done by digestion and were standard; these have been described elsewhere (Orren et al., 1980). After digestion of the organisms the remaining

solutions were analysed by flame atomic absorption spectroscopy using standard conditions. Background correction was used for those elements with resonance lines of shorter wavelength than 280 nm. After accumulation, the organisms were frozen (-10°C) in plastic bags.

Isolation of Metal Binding Proteins

The partially thawed organisms were dissected or scraped from their shells and an appropriate amount of 25 mM phosphate buffer (pH 7.0) was added to give a 50 per cent (w/v) homogenate. This was prepared by blending the mixture twice for one minute in a Du Pont Omni-mixer at full speed in ice. The resulting homogenate was centrifuged at 4°C for three hours at 30 000 g in a Sorval RC5 Superspeed refrigerated centrifuge with eight tube rotor SS 34. Supernatant material (10 cm^3) was decanted and applied to a Sephadex G-75 column (2.6 × 100 cm) kept of 12 ± 2°C and protein fractions (5 mℓ) were eluted with 20 m M Tris-HCl buffer (pH8.6) (for detail see Hennig, 1981). The column was standardised using the following molecular weight markers: Bovine albumin (68 000 daltons), ribonuclease (13 700 daltons), cytochrome C (12 500 daltons) and tryptophan (204 daltons). Concentrations of metals were monitored in the resulting fractions by direct aspiration into the flame (detection limits: Cd = 0.006 µg $mℓ^{-1}$; Cu = 0.050 µg $mℓ^{-1}$; Z n = 0,009 µg $mℓ^{-1}$). Fraction absorbance at 280 nm was monitored with a rapid sampling I.S.C.O. Absorbance Monitor and absorbance at 250 nm and 280 nm was measured using a Beckman spectrophotometer with slit width 0,5 mm and path length 10 mm.

Fractions emerging at the elution volume of 10 000-12 000 daltons were pooled (Peak II) and freeze-dried.

Protein Purification

The freeze-dried material was dissolved in 1 mℓ of 20 mM and applied to a DEAE Sephadex G25 column (0,8 × 14 cm). The column was washed with 100 mℓ equilibration buffer and the

freeze-dried material was applied. This was then eluted with a linear gradient of Tris buffer, 250 mℓ each of 20 mM and 600 mM, pH 8.6. Fractions (5 mℓ) were monitored for absorbance at 280 nm and for cadmium, copper and zinc by atomic absorption spectrometry. The fractions from the metal peaks were pooled separately, scanned from 320 nm to 220 nm and freeze-dried.

The amino acid analyses were carried out in Beckman Automatic Amino Acid Analyser (Model 119) on samples carboxymethylated and hydrolysed in 5.7 N HCl for 24 hours at 110°C in tubes which had been evacuated and then sealed under nitrogen.

RESULTS

Metal Accumulation

No difference in metal concentrations could be detected by the digestion method either in the digestive gland (Figure 1) or tail meat (not shown) of crayfish.

The determination of metal in control and accumulation experiment of hermit crabs are shown in Figure 2. Both zinc and copper levels are elevated. Zinc concentration were found to be particularly high.

The metal concentration of the sandshrimps are shown in Figure 3. Both copper and zinc concentrations were found to be higher in the dosed animals compared to the control animals.

The dosed mussels (Figure 4) seem to show only an elevated zinc level, all other measured metals were found to be well within the background levels.

Limpets (Figure 5) kept in an enriched media accumulated more copper and zinc than background animals. It should, however, be noted that zinc levels from Koeberg are very much higher. The zinc enriched limpets from the experiment had just started to accumulate metals during the study time.

The digestion method of metal determination in animals kept under identical conditions thus showed

(a) no accumulation of metals in crayfish;

(b) very much elevated zinc and raised copper levels in hermit crabs;

(c) raised zinc and copper levels in shrimps and limpets;

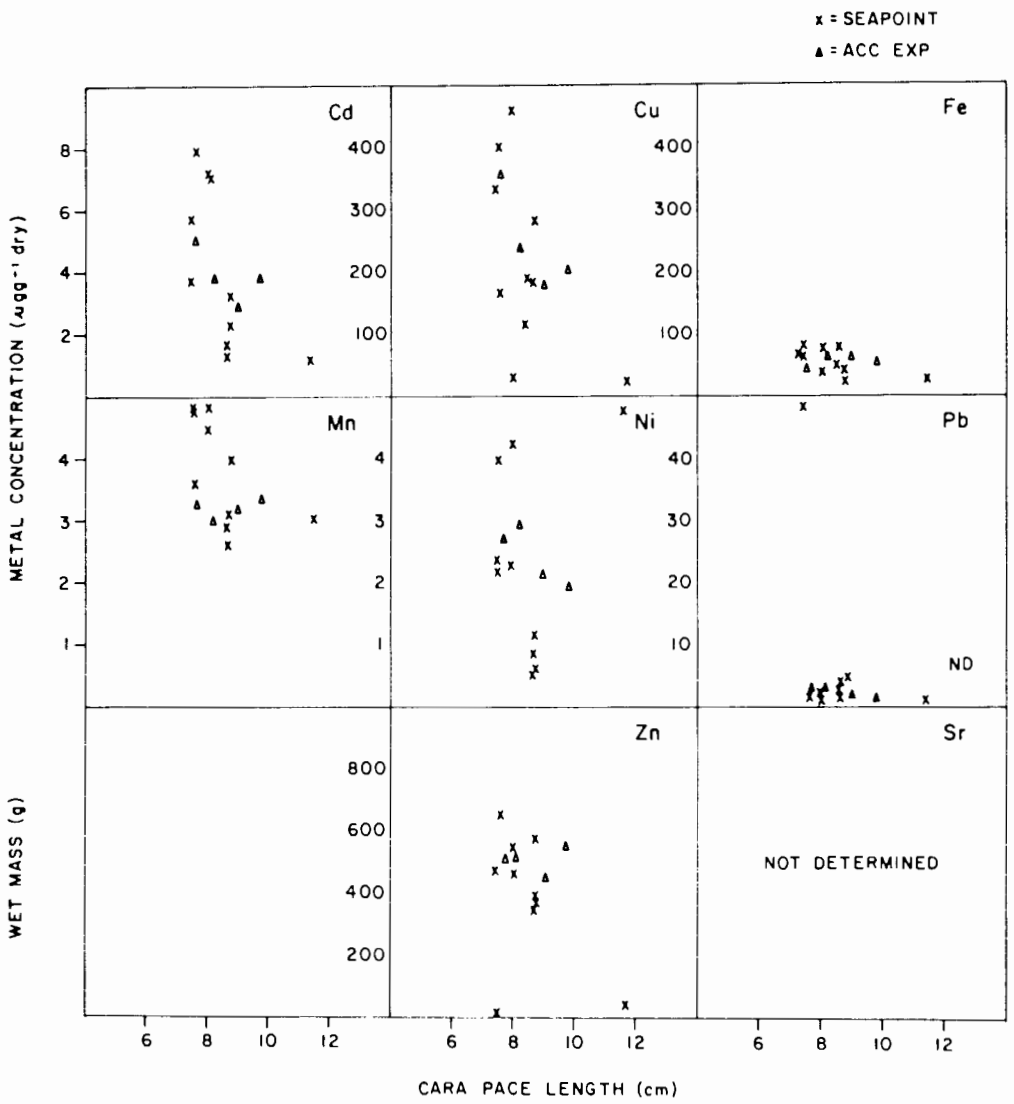


Figure 1 : Metal concentration of crayfish (Jasus lalandii) digestive gland. Background and accumulation experiment data.

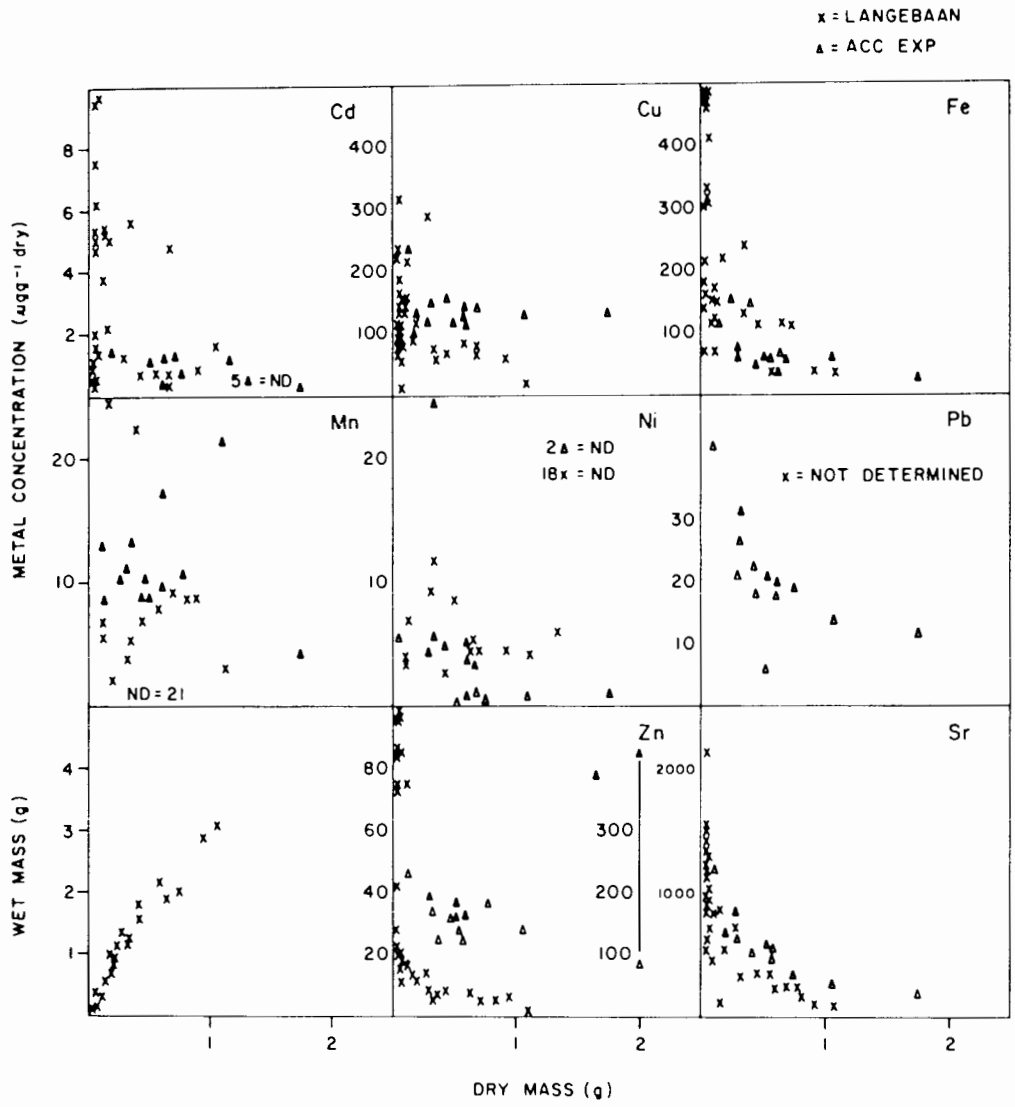


Figure 2 : Metal concentration of hermit crab (Diogenes brevirostris). Background and accumulation experiment data. The new scale for the experimental data on zinc has been inserted.

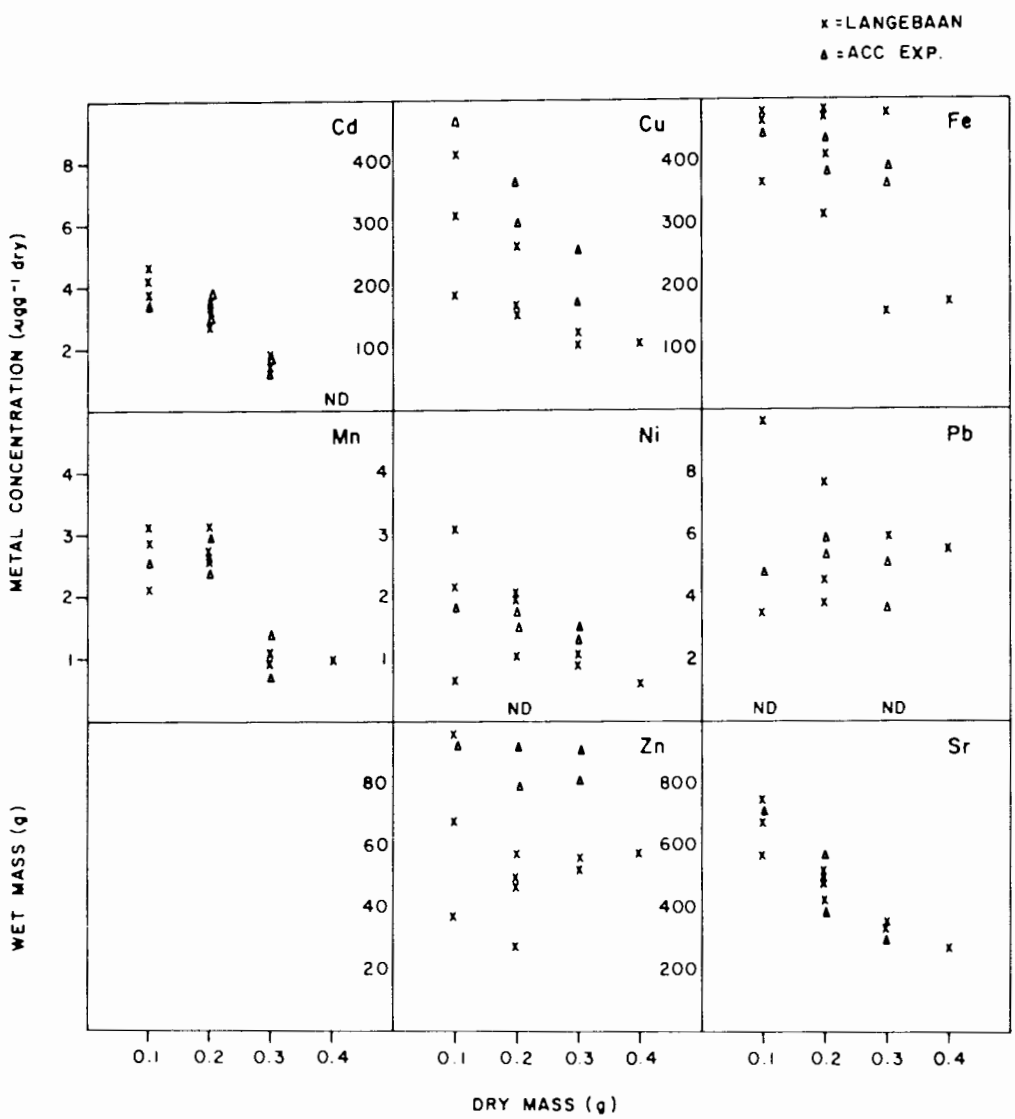


Figure 3 : Metal concentration of sand shrimps (Palaemon pacificus). Background and accumulation experiment data.

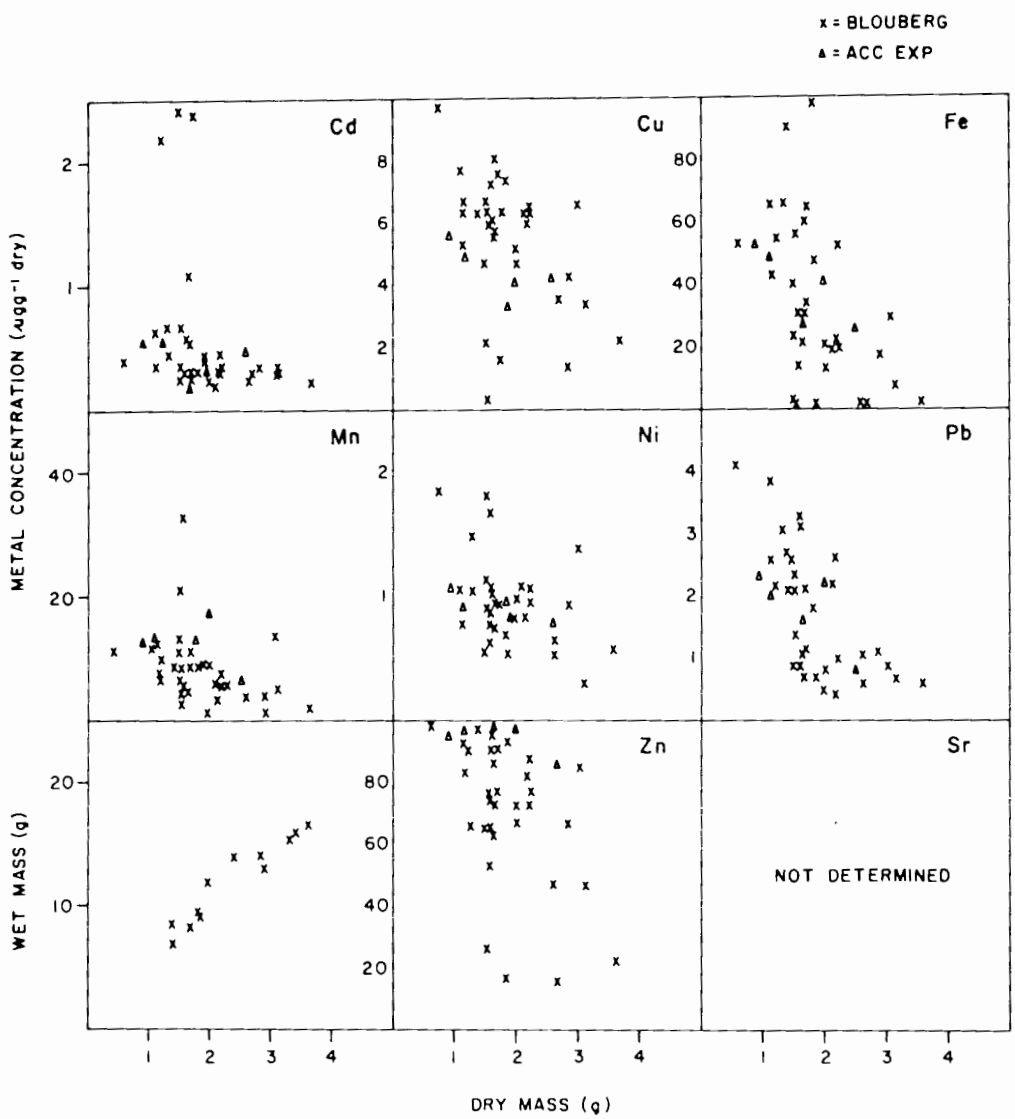


Figure 4 : Metal concentration of mussel (Choromytilus meridionalis). Background and accumulation experiment data.

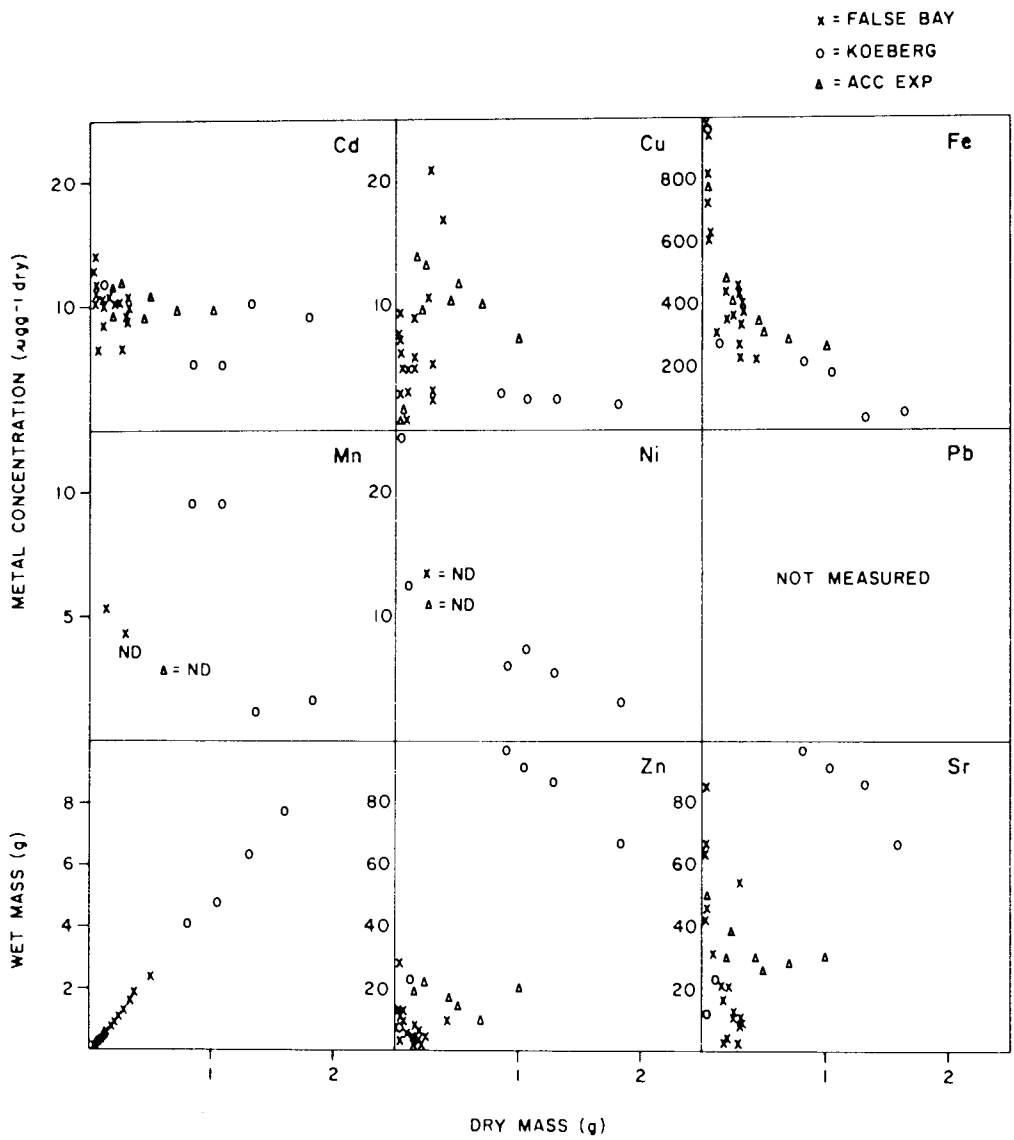


Figure 5 : Metal concentration of limpet (Patella granularis). Background and accumulation experiment data.

(d) only zinc levels were elevated in mussels.

The metal concentrations of the field samples are given in Figures 6a to 6e for Bullia digitals. The relationship between the shell length of larger whelks and their mass varied greatly hence the metal data was related to dry mass in the other Figures (Figures 6b to 6e). Bullia from Koeberg were compared to whelks from two other regions and were found to have elevated cadmium and zinc levels. Copper and strontium concentrations could also be higher in the Koeberg animals.

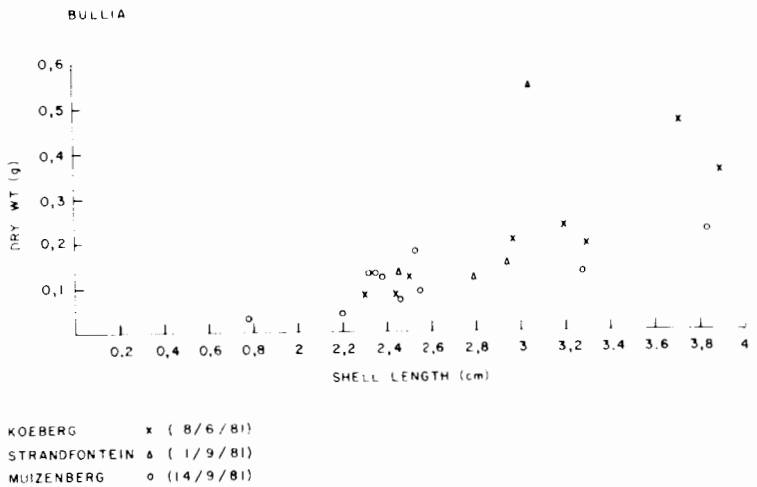
Grass was grown for eight months and harvested every four months, with the relevant metal concentrations shown in Figure 7. Elevated levels were found for the following met-als: cadmium (control = 1 ± 0 $\mu g\ g^{-1}$ dry), copper (control = 8 ± 0 $\mu g\ g^{-1}$ dry) and zinc (control = 91 ± 8 $\mu g\ g^{-1}$ dry) while iron (control = 238 ± 23 $\mu g\ g^{-1}$ dry) showed no deviation from untreated grass.

Column Chromatography

Typical G-75 Sephadex elution profiles obtained with supernatant material are shown in Figure 8 for crayfish digestive gland. Two metal peaks could be identified. The high molecular weight protein peak (I) contains both copper and zinc, while the metal binding peak (low molecular protein) contains mainly copper.

The elution profiles obtained from crayfish tail meat are shown in Figure 9. Only a metal peak associated with high molecular material (Peak I) was found.

In Figure 10 the elution profile obtained from hermit crabs are shown and high molecular material peaks were found (Peak I) and a metal binding protein peak (Peak II) containing copper and zinc was eluted.



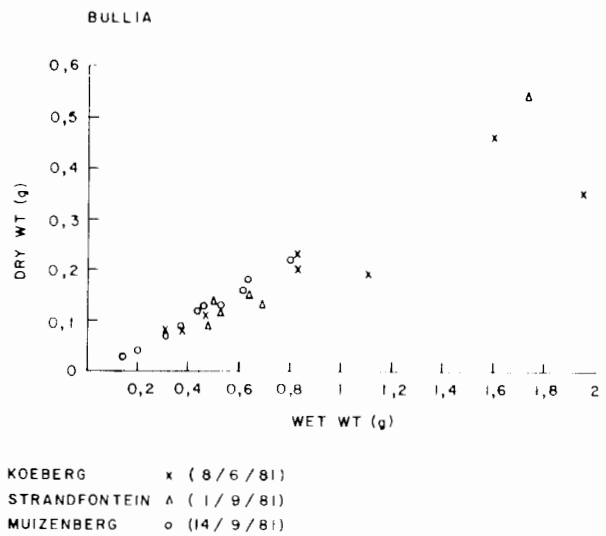
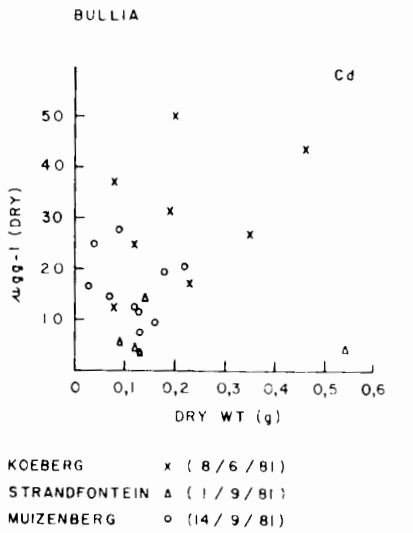


Figure 6a: Relation of shell length and dry mass as well as the relationship of wet mass to dry mass of Bullia digitalis.



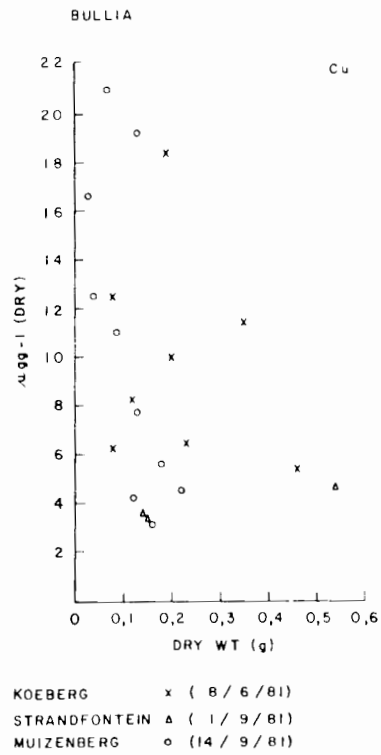
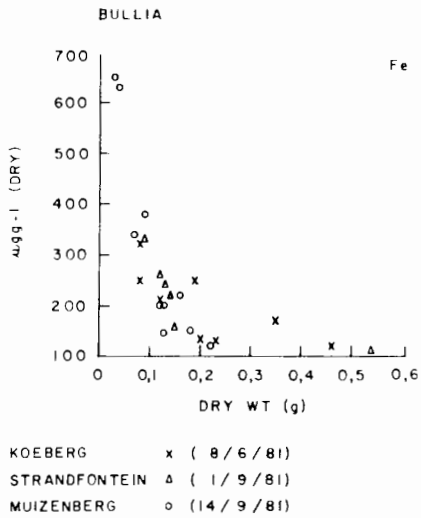


Figure 6b: Metal concentration (Cd and Cu) of Bullia digitalis.



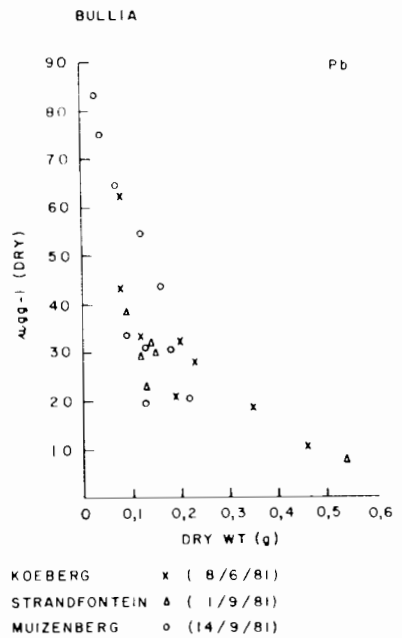


Figure 6c: Metal concentration (Fe and Pb) of Bullia digitalis.

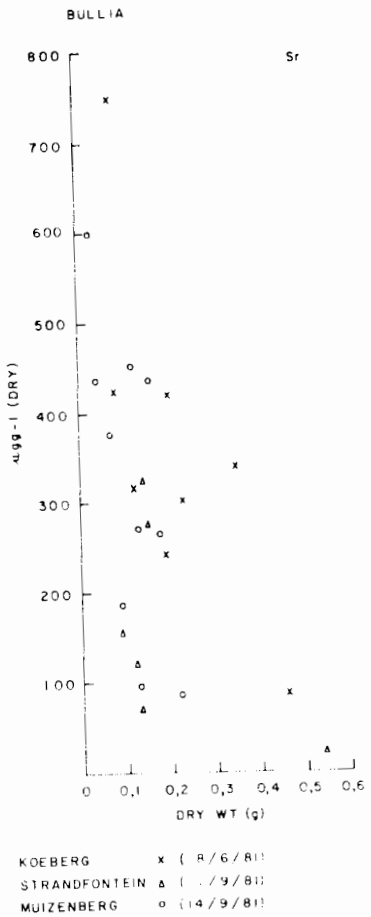


Figure 6d: Metal concentration (Sr) of Bullia digitalis.

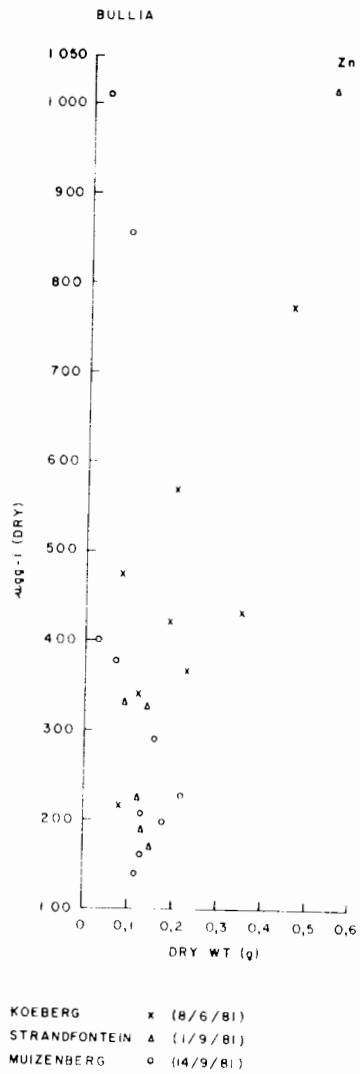


Figure 6e: Metal concentration (Zn) of Bullia digitalis.

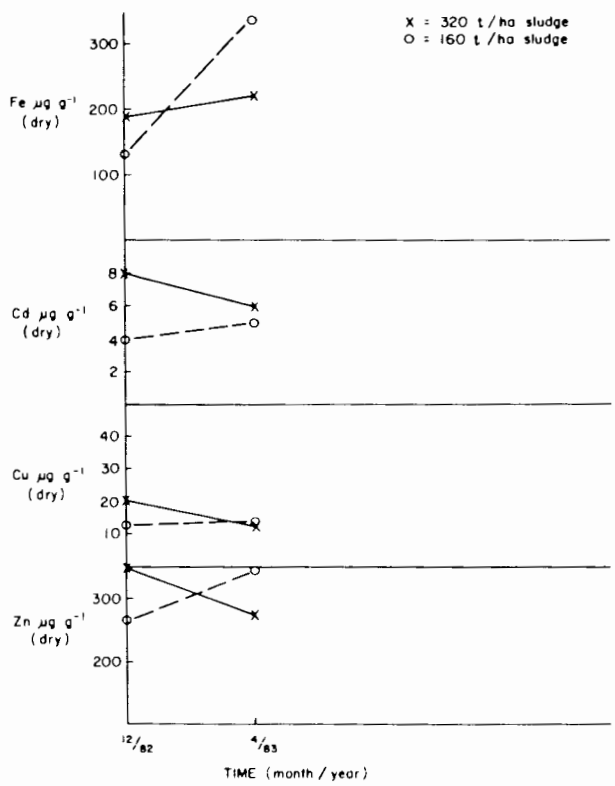


Figure 7 : Metal concentration of Kikuyu grass.

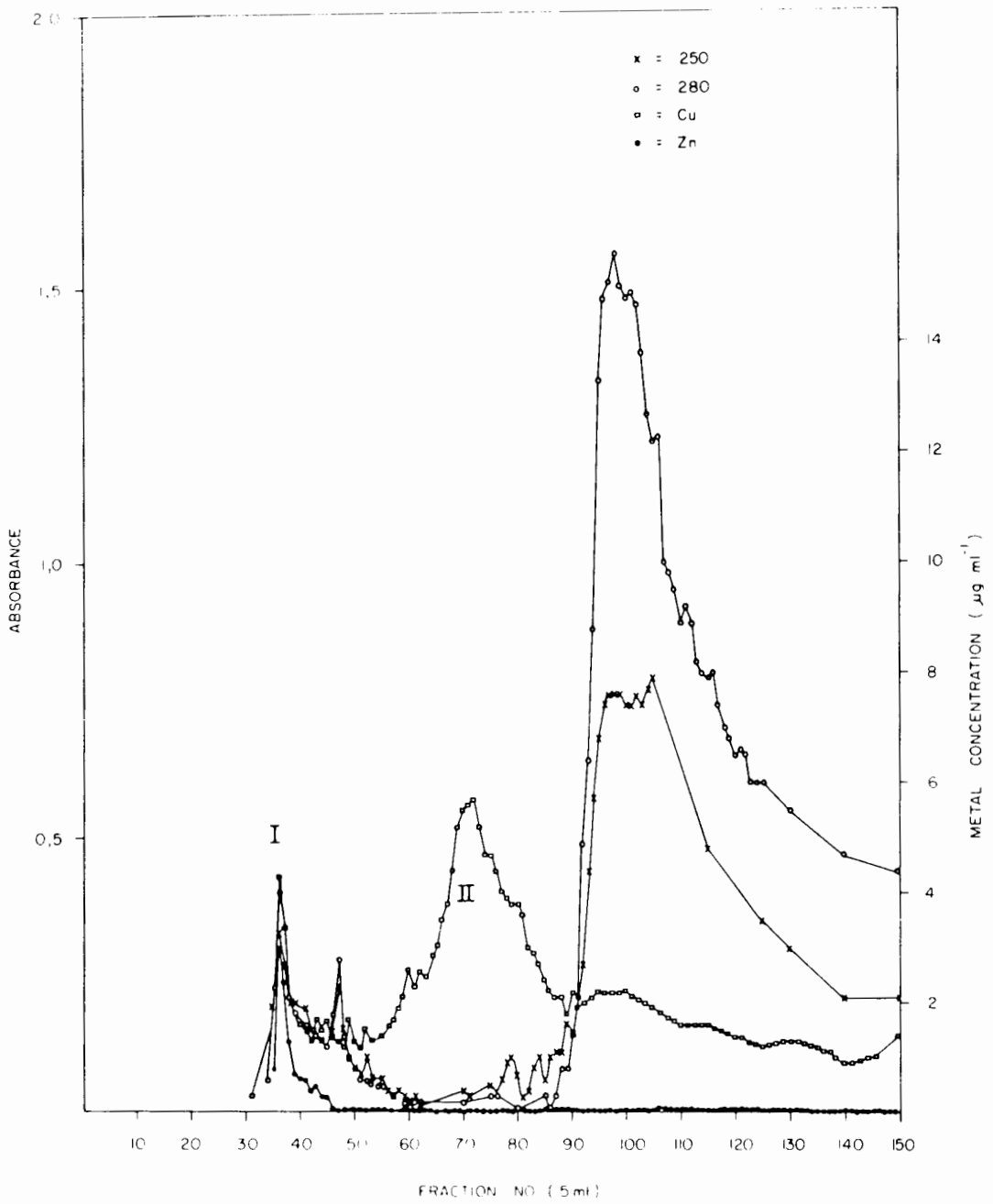


Figure 8 : Elution profiles from G-75 Sephadex column chromatography of supernatant fractions from crayfish (Jasus lalandii) digestive gland. 250 = Absorbance at 250 nm. 280 = Absorbance at 280 nm.

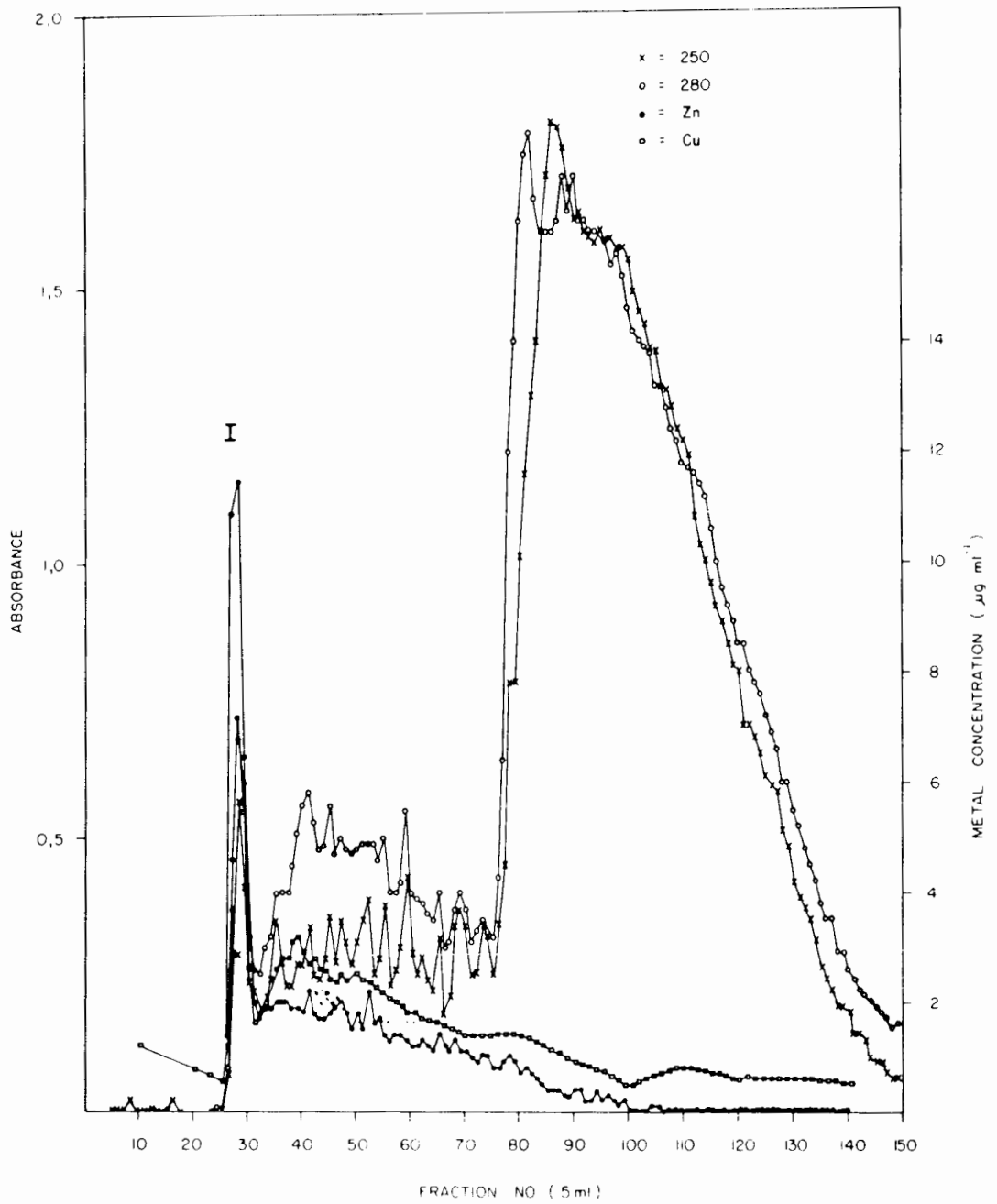


Figure 9 : Elution profiles from G-75 Sephadex column chromatography of supernatant fractions from crayfish (Jasus lalandii) tail meat. Absorbance as in Figure 8.

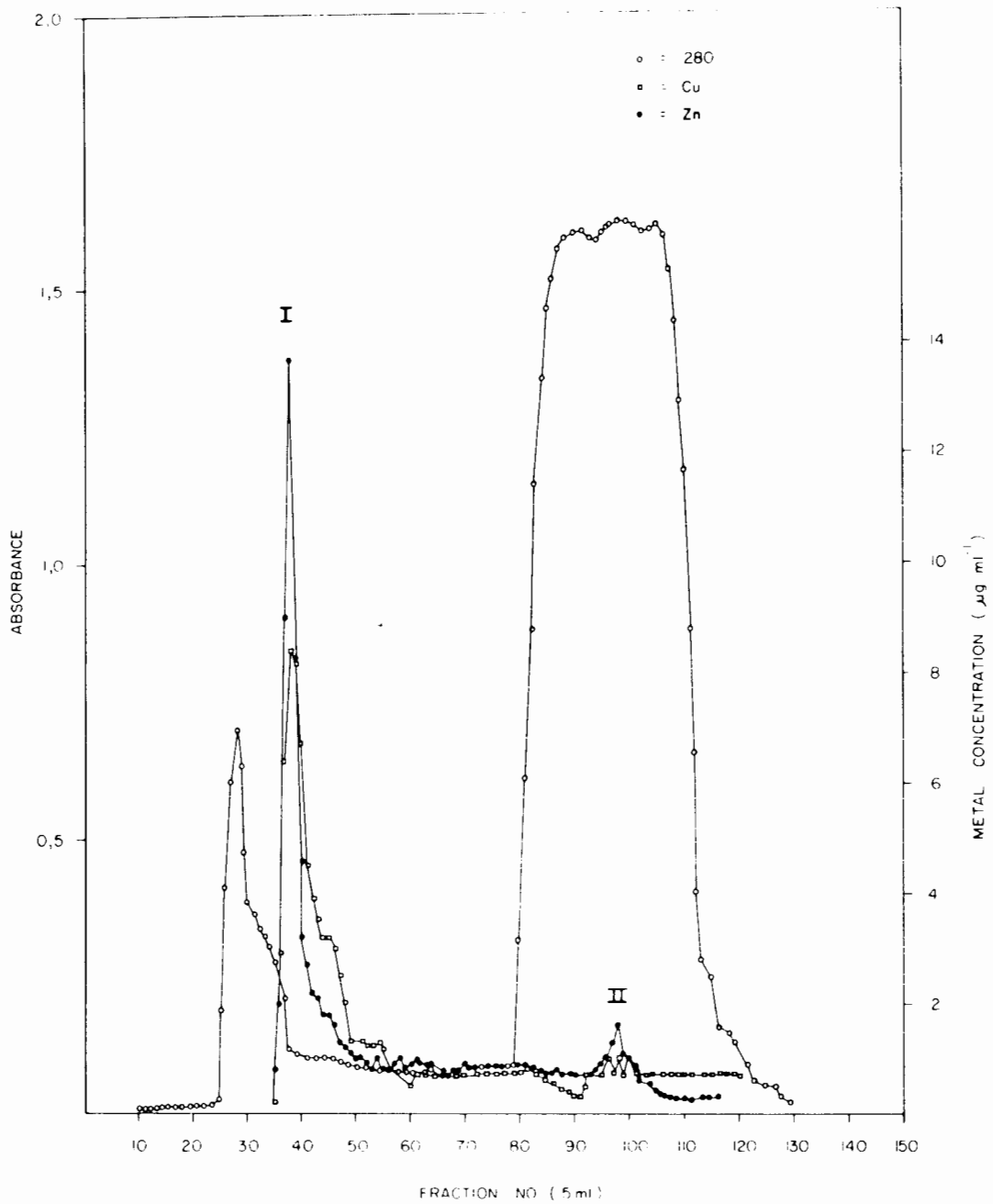


Figure 10 : Elution profiles from G-75 Sephadex column chromatography of supernatant fractions from hermit crab (Diogenes brevirostris). Absorbance as in Figure 8.

The supernatant fractions of ten shrimps each were eluted. A typical profile is shown in Figure 11. The absorbance is a function of the protein concentration and in this case not much material was available hence the absorbance at 280 nm is small. One high molecular peak (Peak I) and two low molecular peaks (Peak II) were observed.

The elution profiles obtained from black mussels are shown in Figure 12. Three peaks were obtained Peak I (high molecular material); Peak II (metal binding low molecular material) and Peak III of very small molecular fractions. Only zinc was found to be bound to any protein.

The supernatant material of limpets eluted is shown in Figure 13. Two peaks containing zinc were found, the high molecular material (Peak I) also contain copper.

The material from the field studies are shown in Figure 14 for Bullia and Figure 15 for grass.

In the case of Bullia a high (Peak I) and very small (Peak III) protein peak was observed. The metal binding protein peak (Peak II) containing all the cadmium and some copper.

Grass had all the copper associated with the higher protein fraction (Peak I) and a seperate low molecular zinc peaks (Peaks II and III).

Isolation of Metallothioneins and Metal Binding Proteins

Metal fraction peaks (Peak II around fraction numbers 89 to 100) had been pooled, freeze-dried and desalted for 3 to 12 hours, depending on the volume of the fractions.

This material was dissolved in 1 mℓ of 20 m M Tris buffer and applied to a DEAE-G25 Sephadex column. The proteins were eluted by a linear gradient and patterns are shown in Figure 16 for crayfish digestive gland.

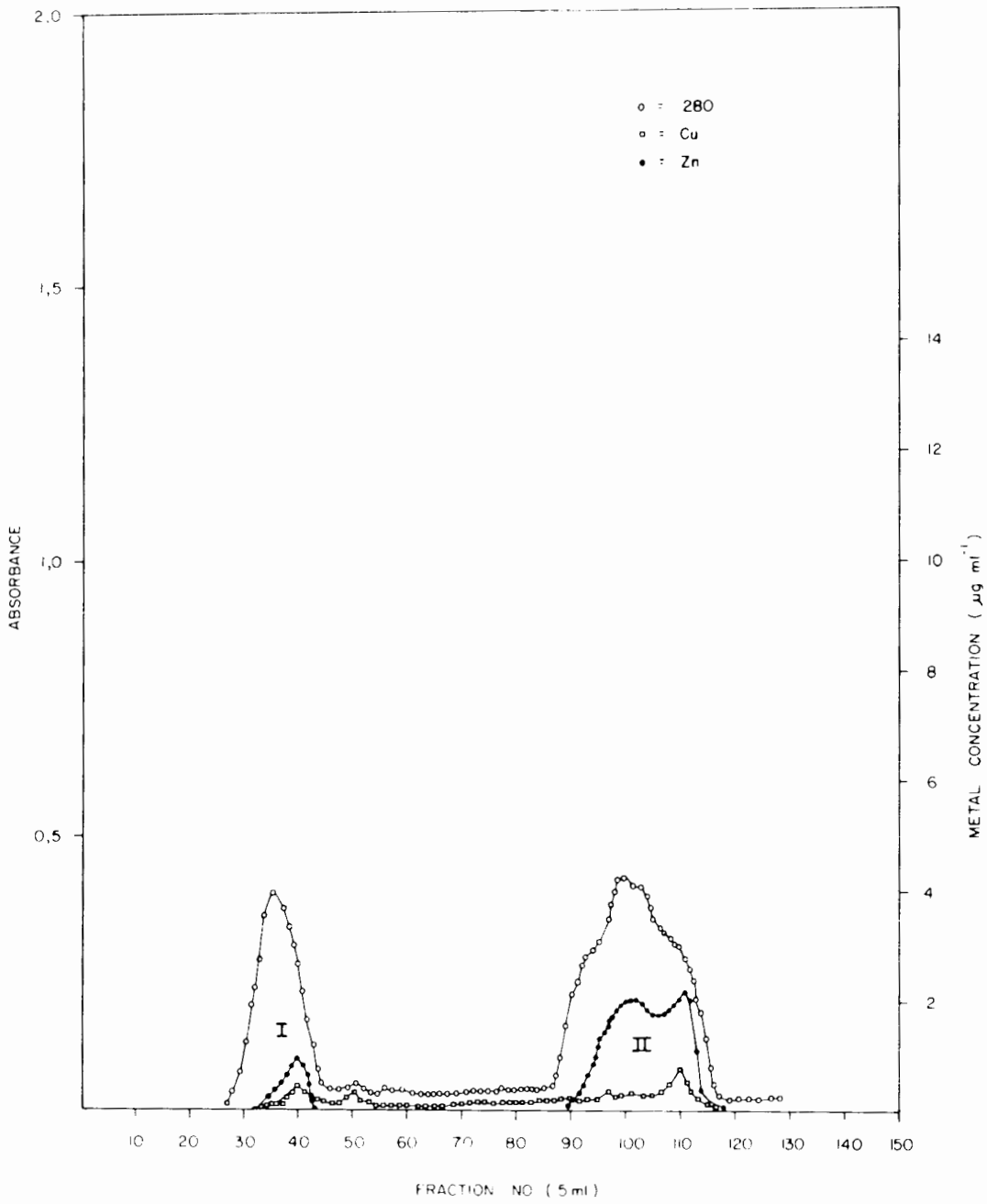


Figure 11 : Elution profiles from G-75 Sephadex column chromatography of supernatant fraction from sandshrimp (Palaemon pacificus). Absorbance as in Figure 8, also see text.

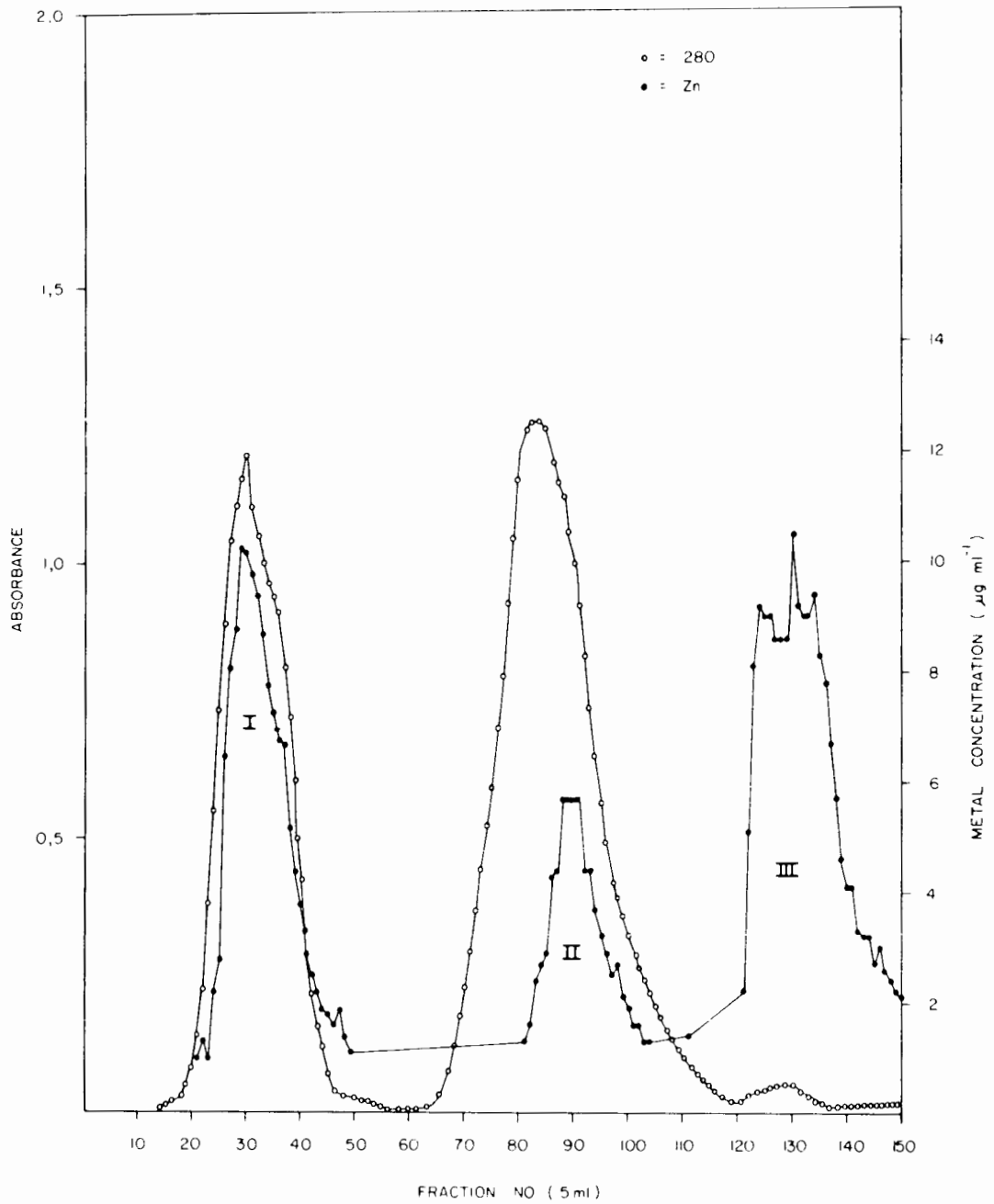


Figure 12 : Elution profiles from G-75 Sephadex column chromatography of supernatant fractions from black mussel (Choromytilus meridionalis). Absorbance as in Figure 8.

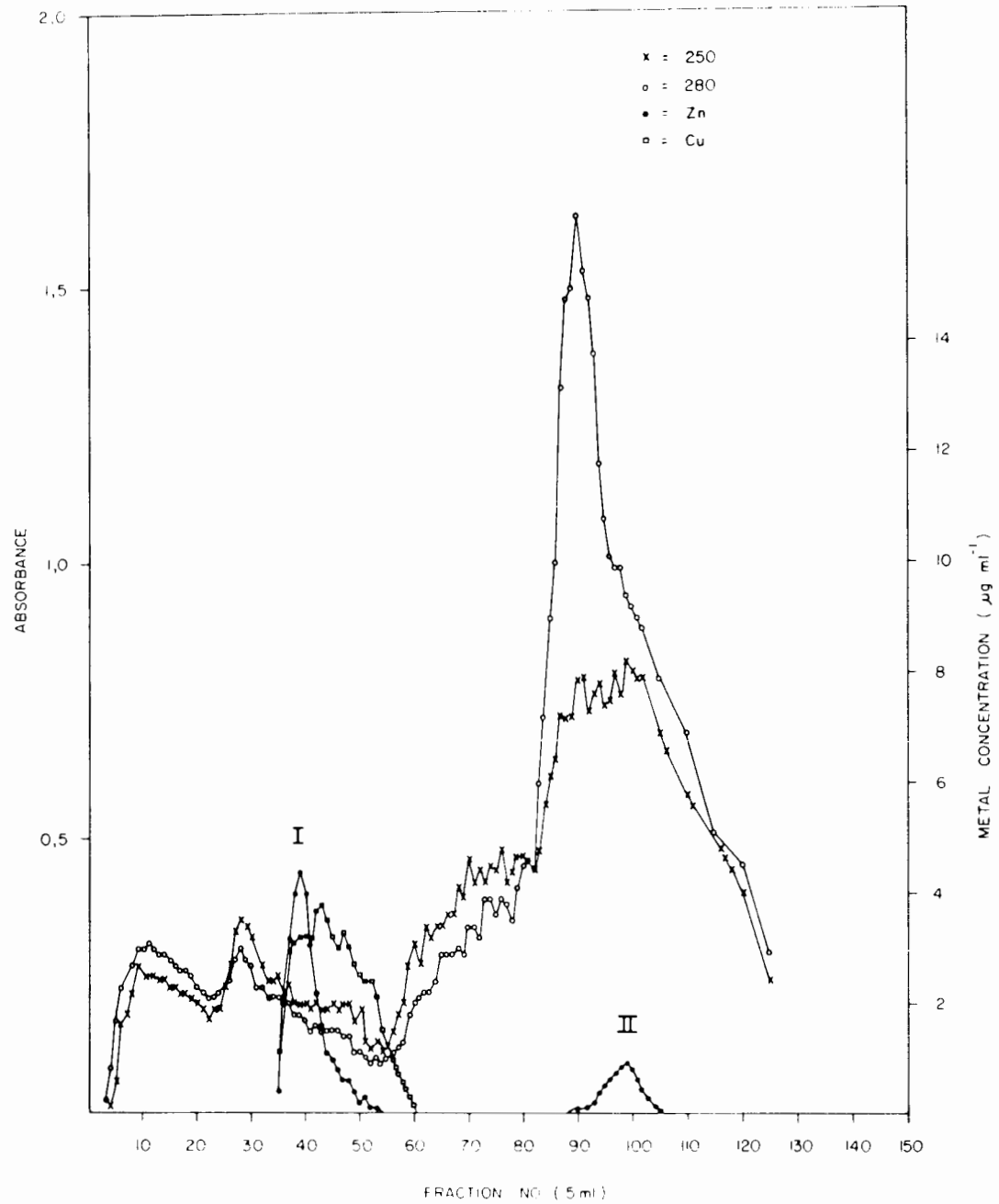


Figure 13 : Elution profiles from G-75 Sephadex column chromatography of supernatant fractions from limpets (Patella granularis). Absorbance as in Figure 8.

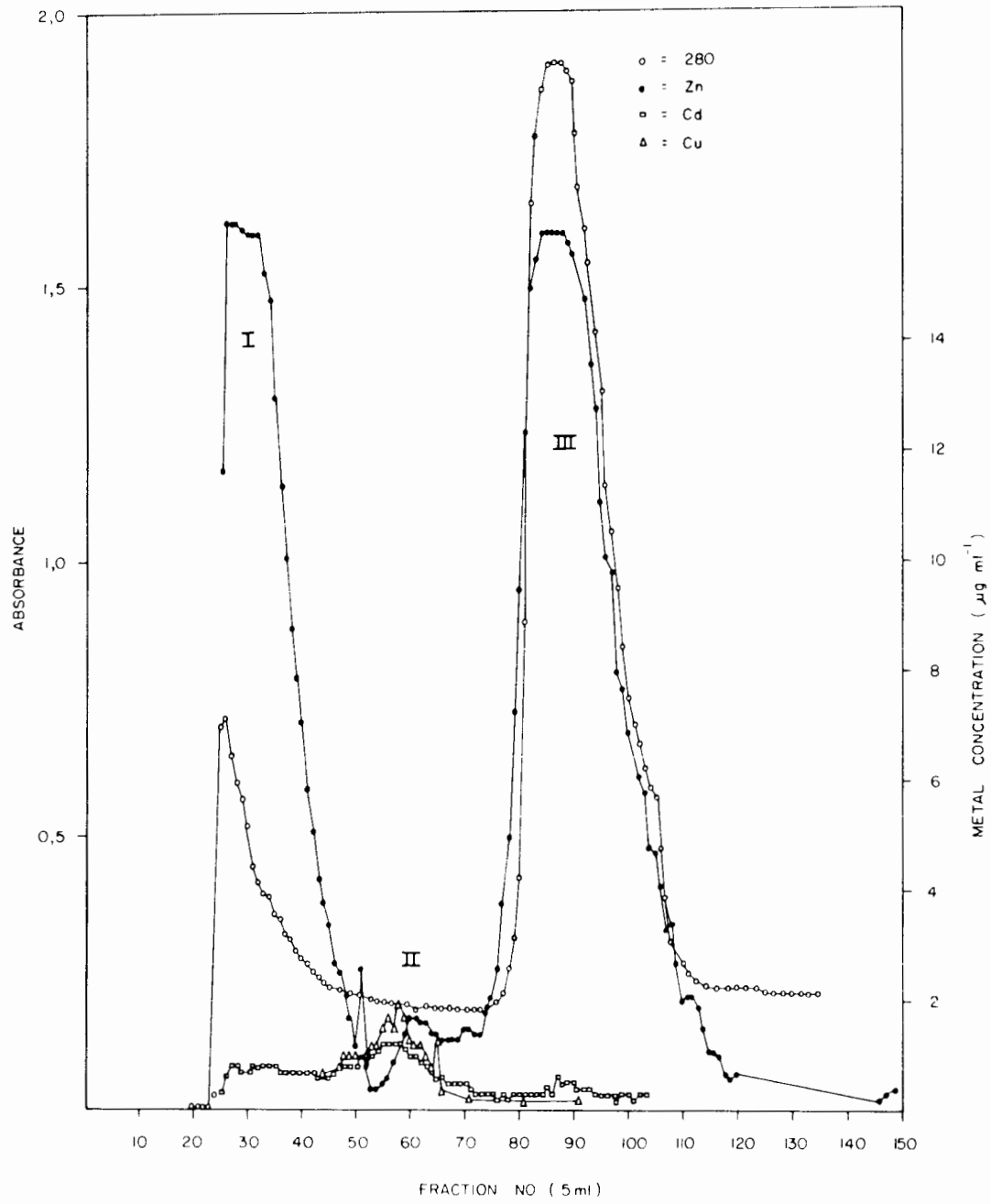


Figure 14 : Elution profiles from G-75 Sephadex column chromatography of supernatant fractions from whelks (Bullia digitalis) collected from Koeberg. Absorbance as in Figure 8.

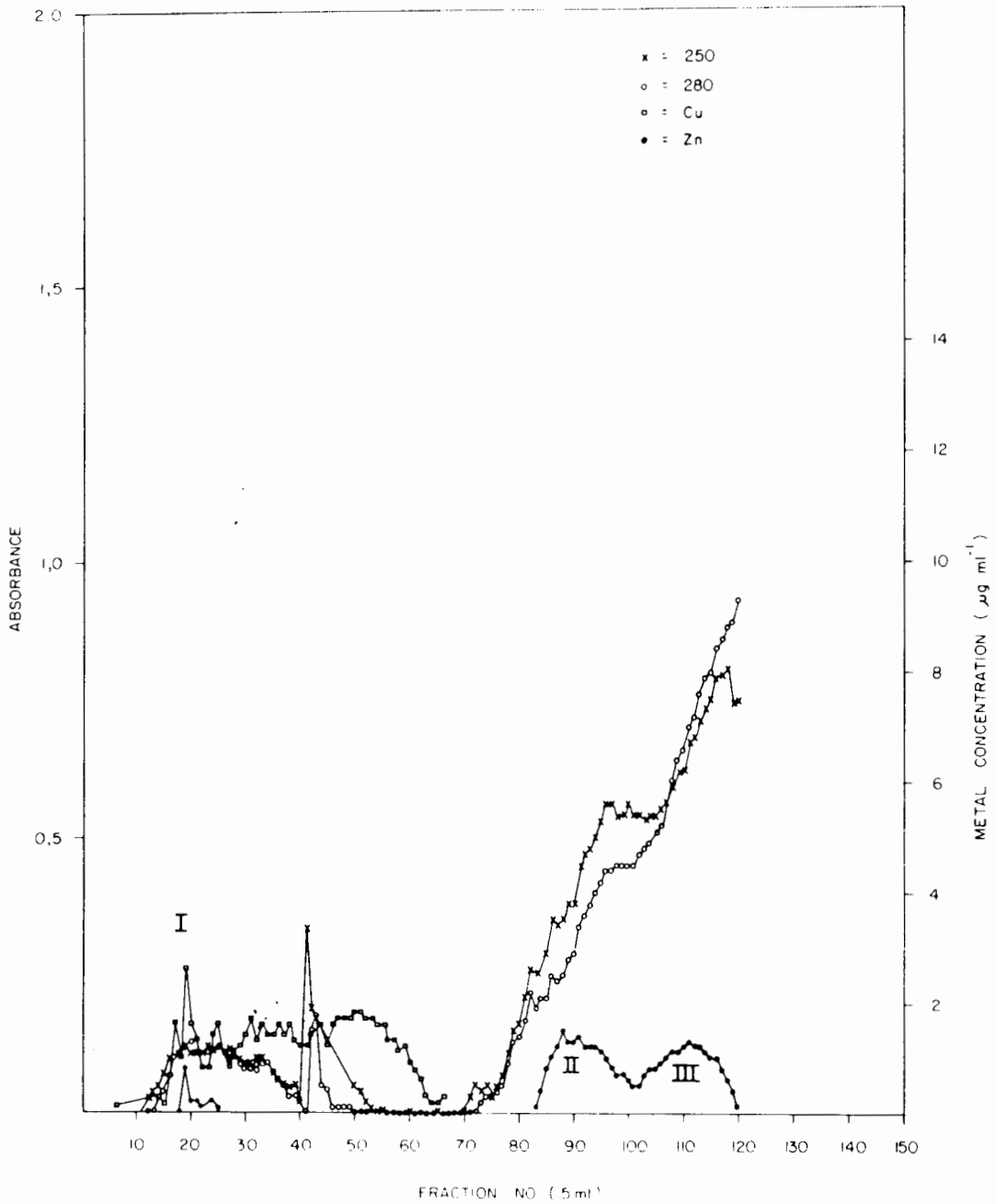


Figure 15 : Elution profiles from G-75 Sephadex column chromatography of supernatant fractions from grass grown with 320 t/ha sludge.

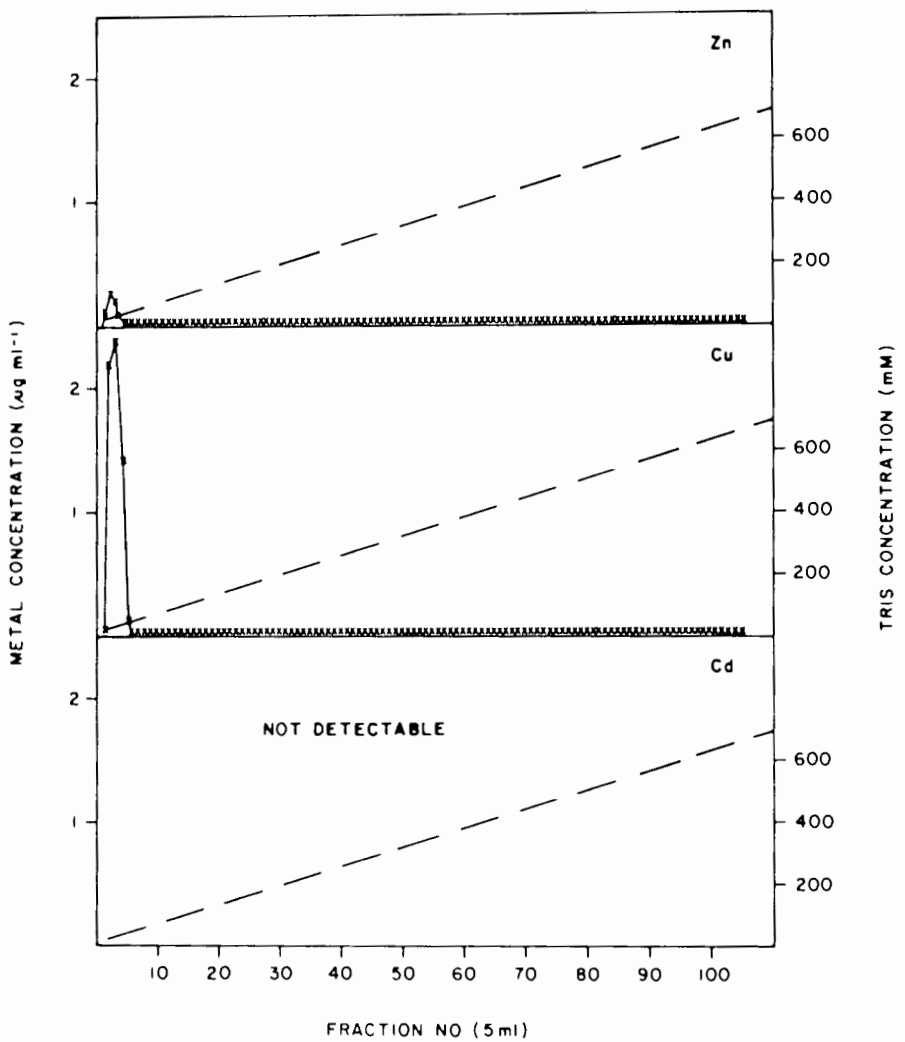


Figure 16 : Separation on DEAE Sephadex A-25 of metallothionein fraction obtained by gel filtration on Sephadex G-75 from crayfish digestive gland. Fraction = 3 mℓ, initial buffer 20 mM Tris-HCl, final 600 mM Tris-HCl pH8.6 at 15°C.

Similarly the DEAE-G25 Sephadex column elution profile are shown in Figure 17 for hermit crab G-75 Peak II; Figure 18 for shrimp G-75 Peak II; Figure 19 for mussels G-75 Peak II; Figure 20 for limpet G-75 Peak II.

The DEAE-G25 fraction from the field animals Bullia are shown in Figure 21 for the cadmium G-75 Peak II and for G-75 Peak III in Figure 22.

The grass G-75 Peak II and Peak III were applied to a DEAE-G25 Sephadex column and the elution profile are shown in Figures 23 and 24 respectively. In each case the metals were eluted at V_O of the DEAE-column.

The elution fractions corresponding to the metal binding peaks detected on Figures 16 to 24 were pooled, lyophilised and redissolved in distilled water. The ultraviolet absorption spectrum of these solutions is shown in Figure 25 for crayfish digestive gland. The absorbance of the material is dependant on the concentration of the protein and hence is relative. Ultraviolet absorption of the other material is shown: hermit crab (Figure 26); sandshrimp (Figure 27); black mussel (Figure 28); limpet (Figure 29).

The isolation of the metal peaks from crayfish digestive gland dosed with industrial effluent (see (a) under Material and Methods) is presented in Figure 30 and the absorption spectrum of the different metal fractions are given in Figure 31 for fraction numbers 91 and 92 (Zn fraction); Figure 32 for fraction numbers 96 and 97 (Cd plus Zn fraction) and Figure 33 for fractions 99 and 100 (Cd fraction).

The absorption characteristics of the field samples are shown in Figure 34 for the cadmium peak (Peak II) of Bullia and Figure 35 for the zinc peak (Peak III) of Bullia, while Figure 36 shows the grass peak (Peak III) fraction numbers 2 and 3 (V_O of column).

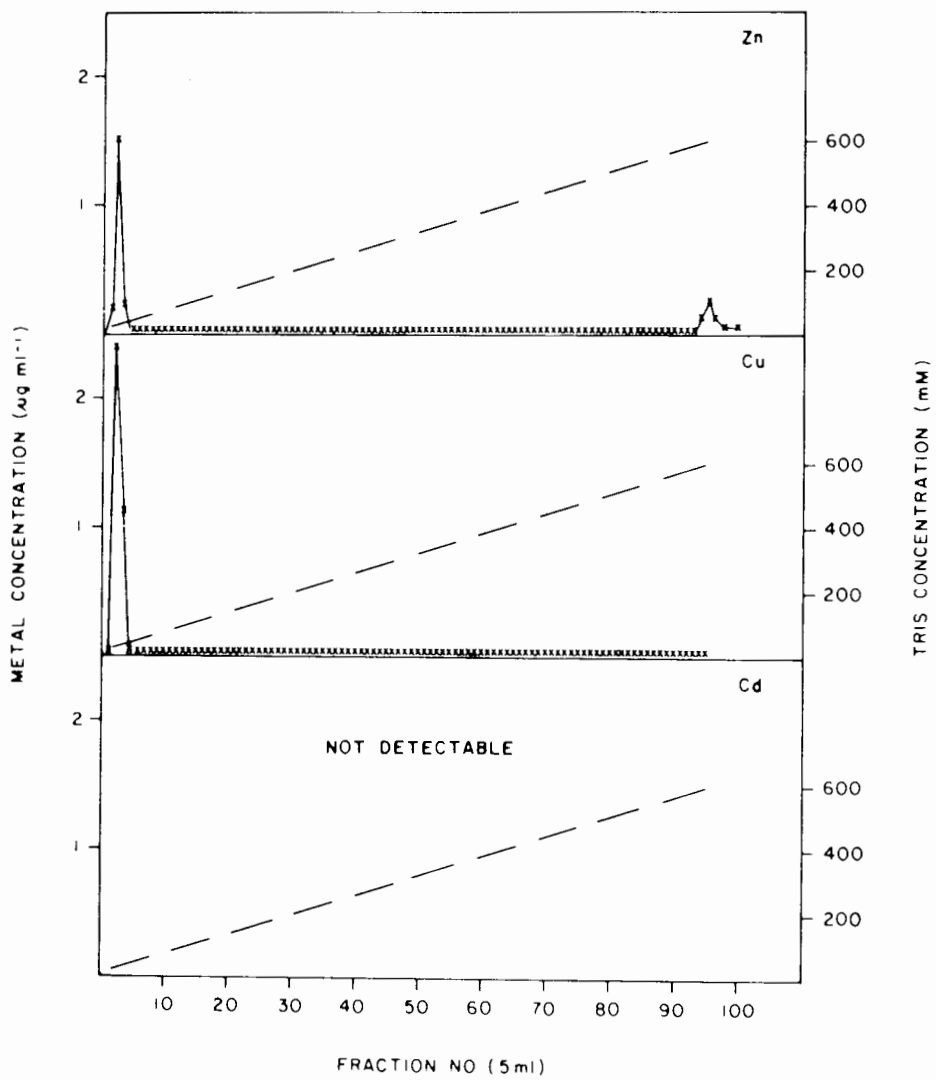


Figure 17 : Separation on DEAE Sephadex A-25 of metallothionein fraction obtained by gel filtration on Sephadex G-75 from hermit crab. Conditions as for Figure 16.

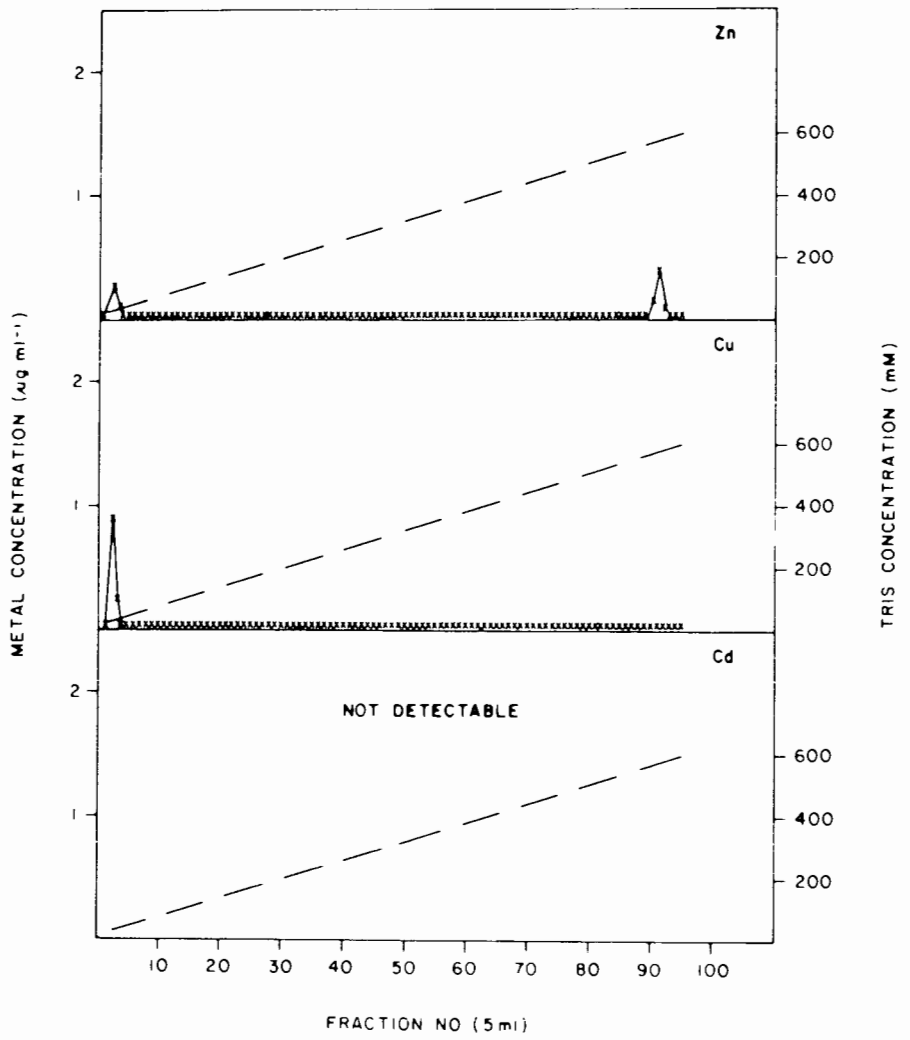


Figure 18 : Separation on DEAE Sephadex A-25 of metallothionein fraction obtained by gel filtration on Sephadex G-25 from shrimp. Conditions as for Figure 16.

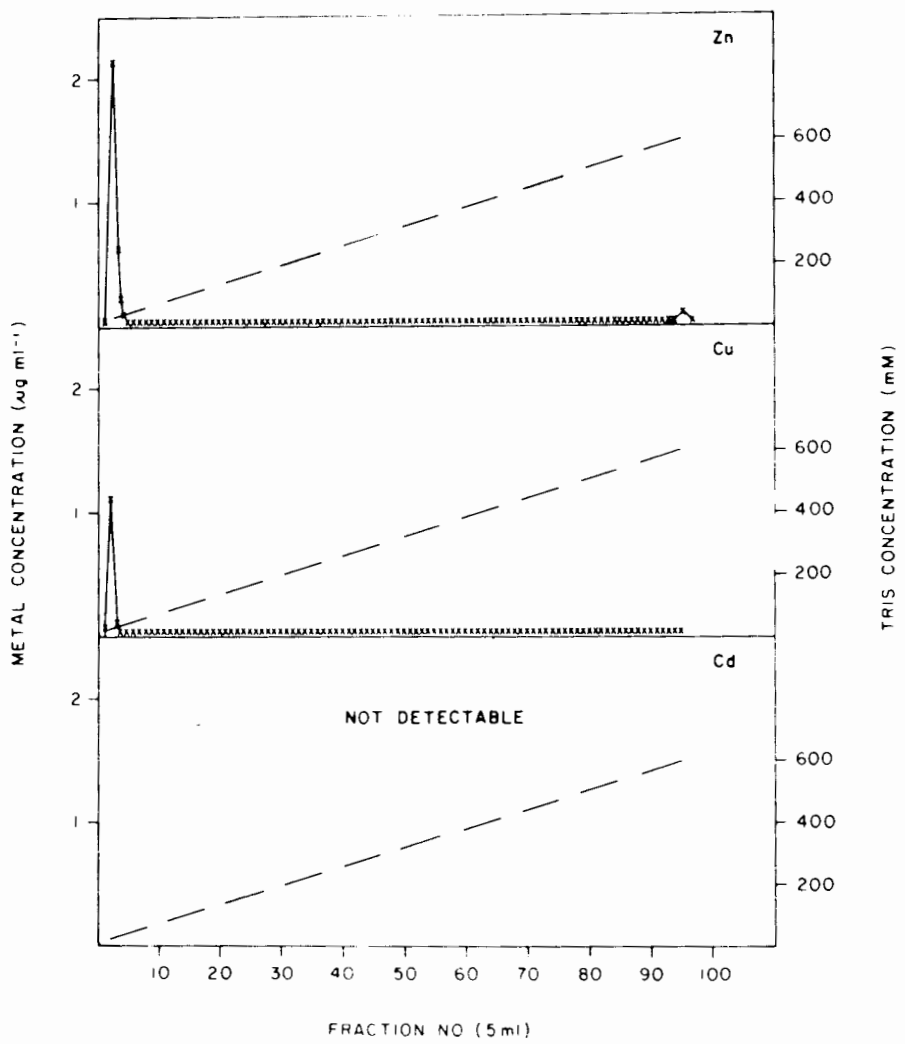


Figure 19 : Separation on DEAE Sephadex A-25 of metallothionein fraction obtained by gel filtration on Sephadex G-75 from mussel. Conditions as for Figure 16.

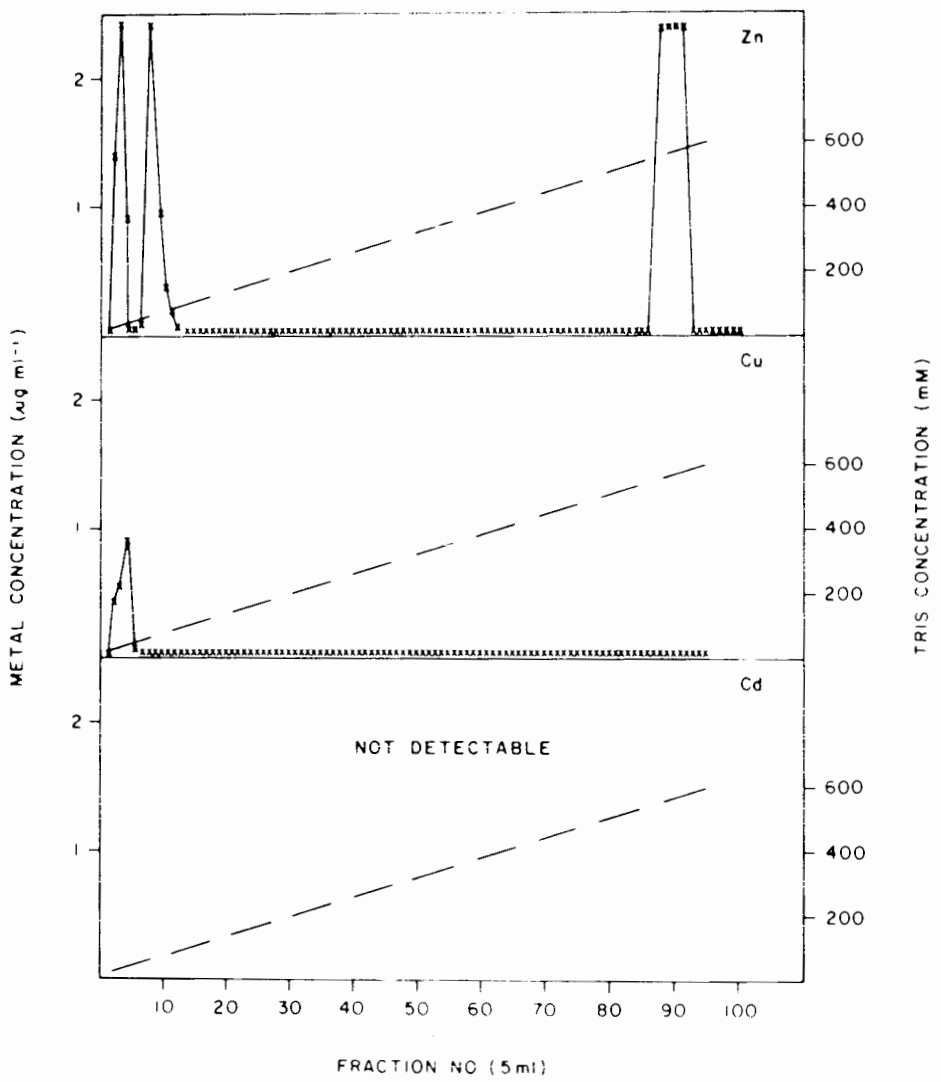


Figure 20 : Separation on DEAE Sephadex A-25 of metallothionein fraction obtained by gel filtration Sephadex G-75 from Limpet. Conditions as for Figure 16.

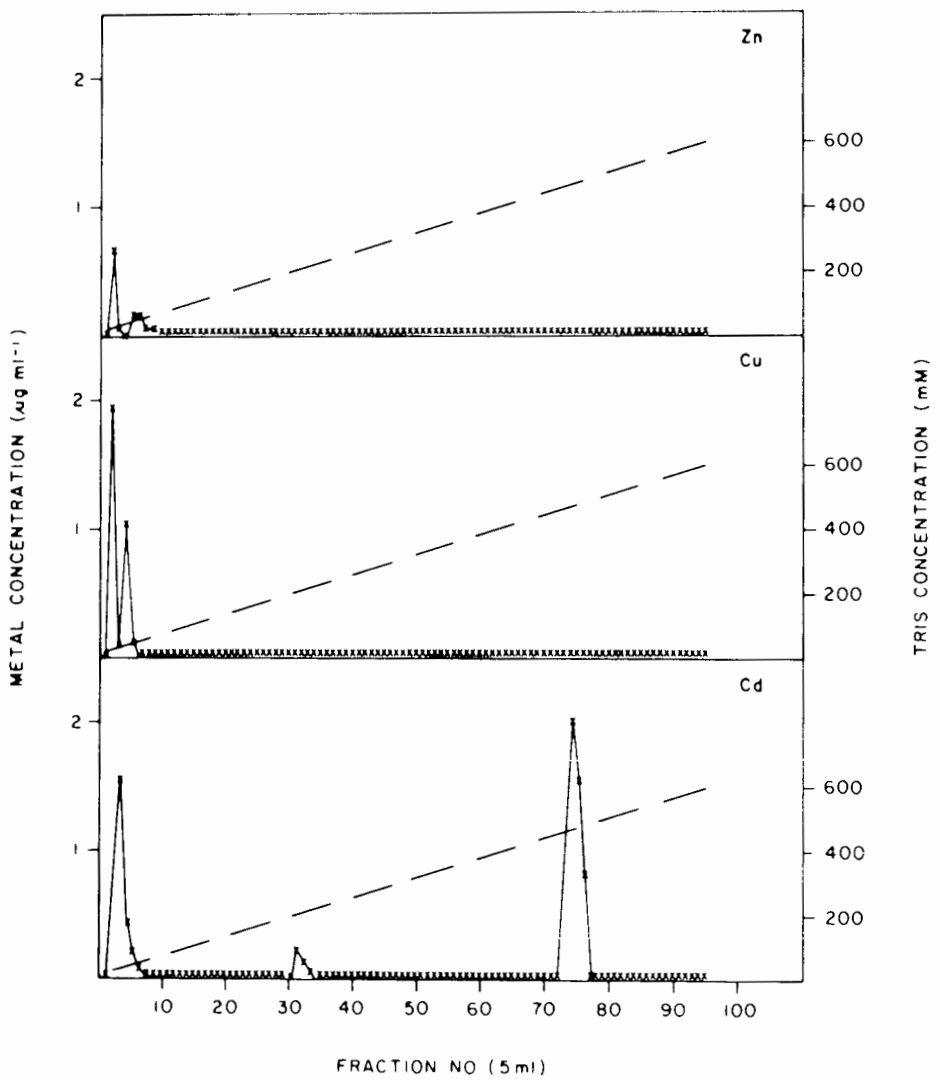


Figure 21 : Separation on DEAE Sephadex A-25 of metallothionein fraction obtained by gel filtration Sephadex G-75 from Peak II of Bullia. Conditions as for Figure 16.

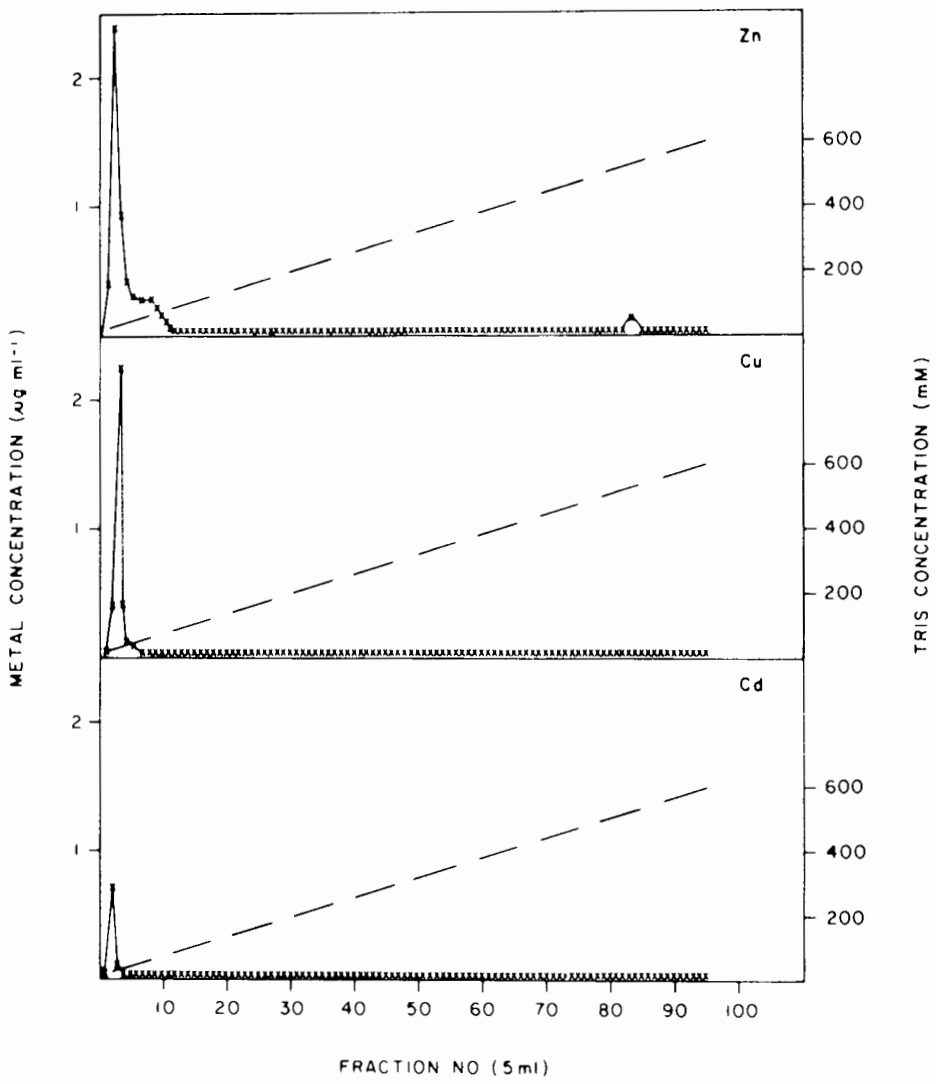


Figure 22 : Separation on DEAE Sephadex A-25 of metallothionein fraction obtained by gel filtration on Sephadex G-75 from Peak III of Bullia. Conditions as for Figure 16.

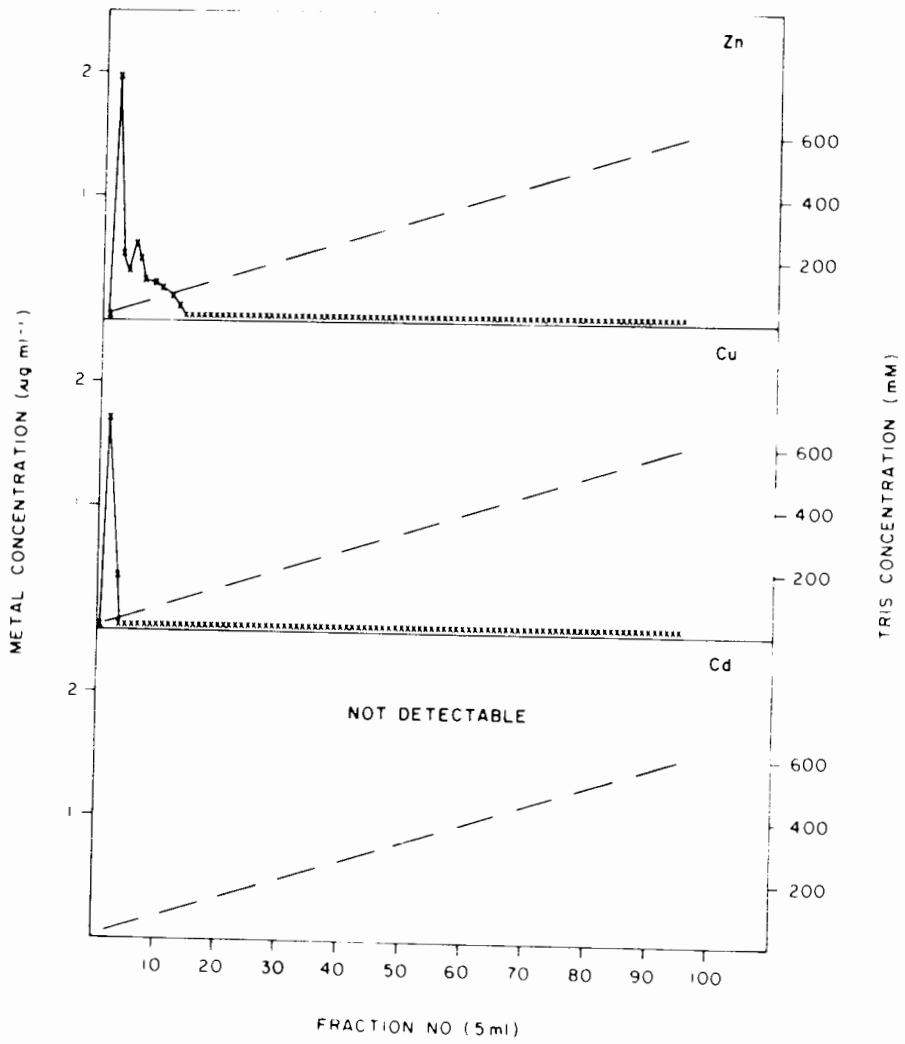


Figure 23 : Separation on DEAE Sephadex A-25 of metal binding protein fraction obtained by gel filtration on Sephadex G-75 from Peak II of Grass.

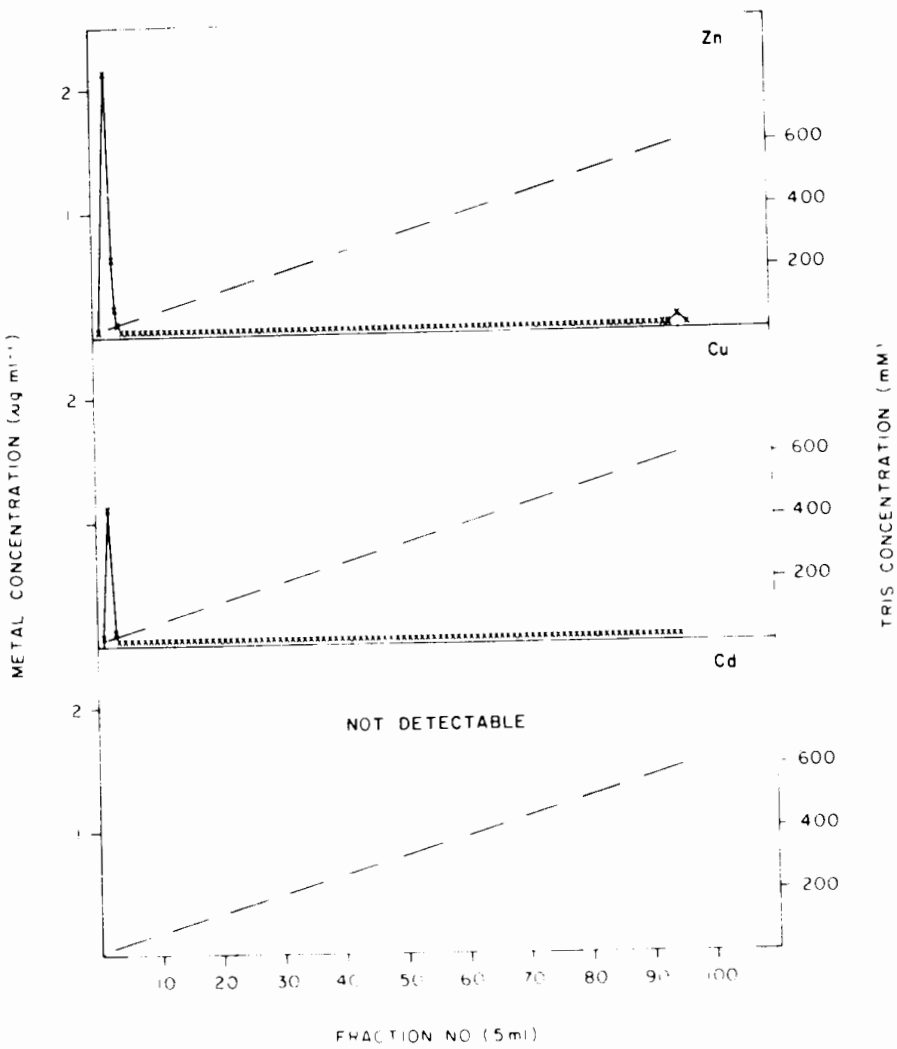


Figure 24 : Separation on DEAE Sephadex A-25 of metal binding protein fraction obtained by gel filtration on Sephadex G-75 from Peak III of Grass.

CRAYFISH HEPATIC

Figure 25 : Ultraviolet absorption spectrum of the low molecular binding protein from crayfish (4.121 mg) pH 8.6.

Figure 26 : Ultraviolet absorption spectrum of the low molecular binding protein from hermit crab (9.490 mg) pH 8.6

Figure 27 : Ultraviolet absorption spectrum of the low molecular binding protein from sandshrimp (8.097 mg) pH 8.6.

Figure 28 : Ultraviolet absorption spectrum of the low molecular binding protein from black mussel (5.924 mg) pH 8.6.

Figure 29 : Ultraviolet absorption spectrum of the low molecular binding protein from limpet (13.805 mg) pH8.6.

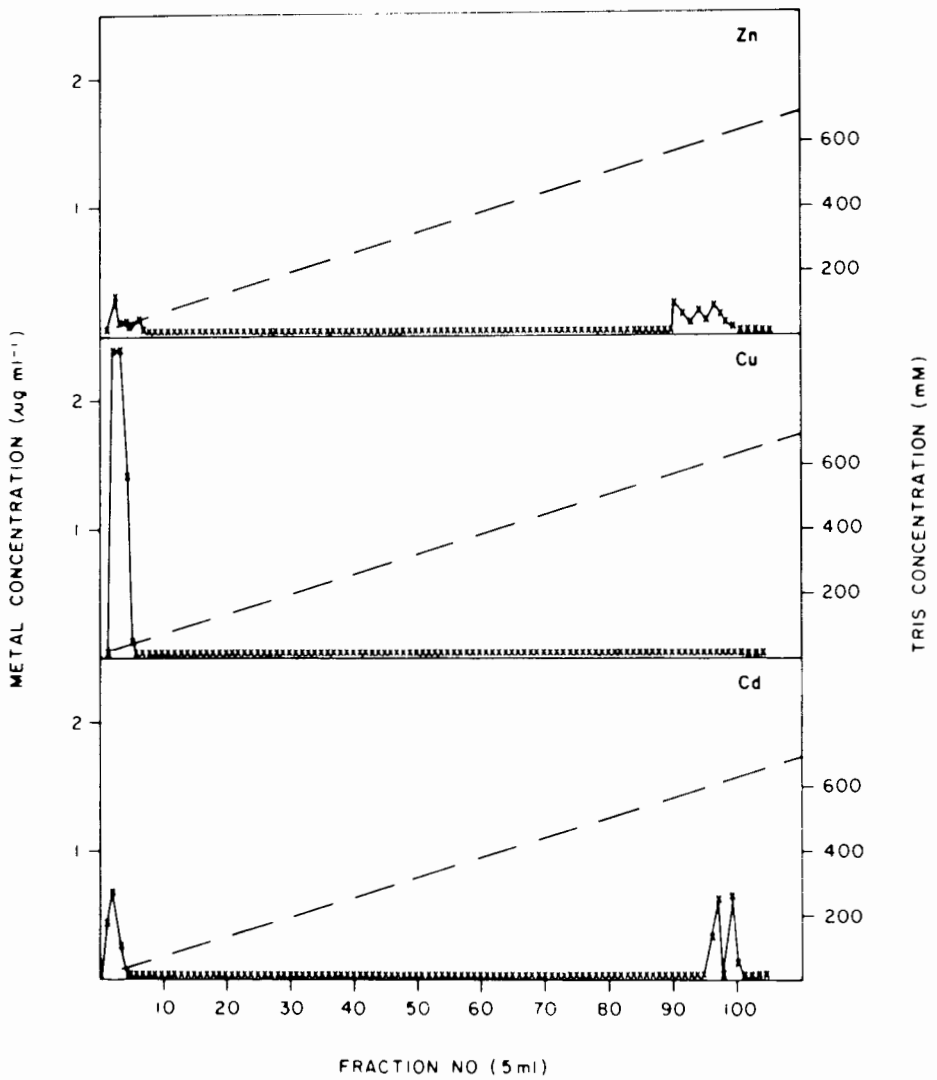


Figure 30 : Separation on DEAE Sephadex A-25 of metal binding protein fraction obtained by gel filtration on Sephadex G-75 from Peak II of crayfish digestive gland dosed with industrial effluent.

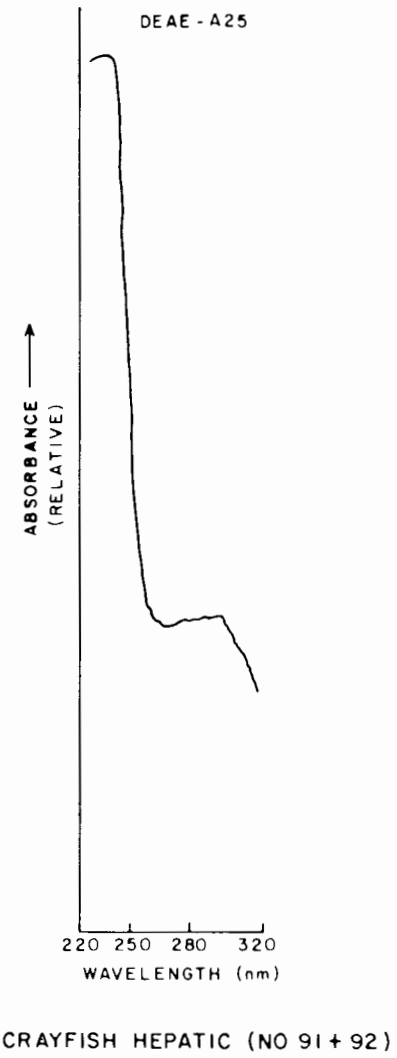
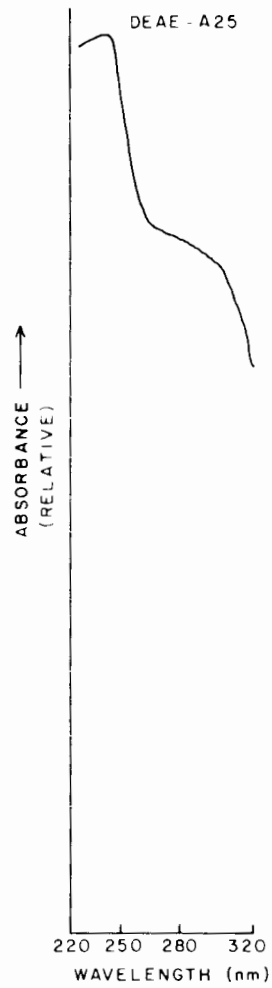


Figure 31 : Ultraviolet absorption spectrum of the low molecular weight binding protein from crayfish digestive gland (3.392 mg protein) fraction nos. 91 and 92, pH 8.6, dosed with industrial effluent.

Figure 32 : Ultraviolet absorption spectrum of the low molecular weight binding protein from crayfish digestive gland (4.008 mg protein) fraction nos. 96 and 97, pH 8.6, dosed with industrial effluent.

Figure 33 : Ultraviolet absorption spectrum of the low molecular weight binding protein from crayfish digestive gland (3.606 mg protein) fraction nos. 99 and 100 pH 8.6, dosed with industrial effluent.



BULLIA (Cd PEAK)

Figure 34 : Ultraviolet absorption spectrum of the low molecular binding protein from Bullia (Peak II-Cd) (10.481 mg) pH 8.6

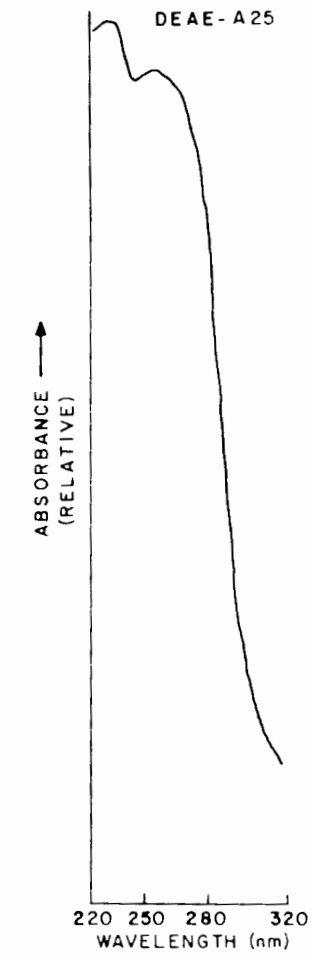


Figure 35 : Ultraviolet absorption spectrum of the low molecular binding protein from Bullia (Peak III-Zn) (13.137 mg) pH 8.6

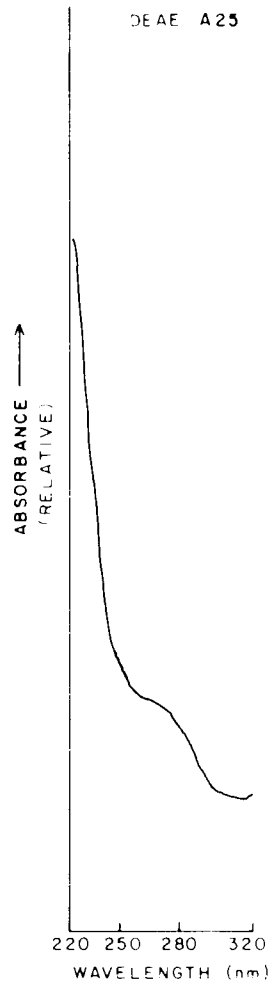


Figure 36 : Ultraviolet absorption spectrum of the low molecular binding protein from Grass Peak II (19.838 mg) pH 8.6.

The results presented here of two different experiments ((a) and (b) in Methods) five different animals and two field organisms has been simplified in Table 1, which makes comparison somewhat easier. For instance in crayfish digestive gland no metal accumulation could be determined but metal binding proteins were found. In hermit crabs the digestive method suggested very high levels of Zn but only zinc metal binding protein of a very low level were found. In shrimps the digestive method showed slightly elevated copper and zinc concentrations. But very much higher zinc metal binding protein was found.

In mussel the raised zinc levels were confirmed by the isolation of metal binding proteins.

In limpets the digestion method showed raised metal levels of copper and zinc but metal binding proteins of only zinc were isolated.

In Bullia the high levels of cadmium determined by digestion method could not be isolated in those quantities but copper and zinc binding proteins were isolated.

In the grass grown with sludge, the following results were obtained by digestion method:

(a) Cadmium in sludge grass was five times that of control;

(b) copper in sludge grass was twice that of control;

(c) zinc in sludge grass was three times that of control,

but only zinc binding proteins were found.

TABLE I: DIAGRAMATIC PRESENTATION OF RESULTS OBTAINED FROM DIFFERENT EXPERIMENTS AND FIELD ORGANISMS WITH RESPECT TO DIFFERENT METHODS OF DETERMINATION AND ISOLATION

Organism (effluent)	Metal of determination		Isolation
	Total digestion	Metal binding proteins	
Crayfish (industrial)	Not detectable	Peak II Cd + Cu + Zn	Three fractions Cd + Cu + Zn
Crayfish (Cu + Zn)	Not detectable	Peak II Cu	Cu fraction (V_o)
Hermit crabs (Cu + Zn)	≫Zn >Cu	Peak II Zn, Cu	Zn fraction (Cu)
Shrimp (Cu + Zn)	>Cu >Zn	Peak II ≫Zn >Cu	Zn fraction
Mussel (Cu + Zn)	>Zn	Peak II Zn	Zn fraction
Limpet (Cu + Zn)	>Cu >Zn	Peak II Zn	Zn fraction (Cu)
Bullia (field)	≫Cd >Zn	Peak II Cd Zn Cu	Two fractions Cd + Zn (Cu)
Grass (sludge)	≫Zn ≫Cd >Cu	Peak III Zn	Cu, Zn (V_o) fraction

≫ = High metal concentration.

> = Lower metal concentration.

V_o = Elution at V_o of DEAE-G-25 Sephadex column. (Metal) = Peak (V_o) isolated, but not further analysed.

DISCUSSIONS AND CONCLUSIONS

Up till now metal determination in biological systems has been based on the digestion method. This method has been employed in the "Mussel Water Programme" (Goldberg et al., 1983) and in any other baseline study (see Hennig, in preparation, this volume). It has been shown that this approach has many drawbacks (Eisler 1981, Phillips 1977 and Hennig, in preparation, this volume) e.g. different phyla, size, mass, age, food and life stages have usually been ignored. Some of these flaws can be overcome by sampling metal indicator species or organisms. This could be acceptable for baseline studies although extrapolating should be avoided (Hennig in preparation, this volume). For a pollution study the digestion method of metal determination is totally inadequate. For such a study the following questions are of major importance:

(1) No accumulation of metals could be determined, hence is the organism polluted or not?

(2) Accumulation of metals could be determined but does that influence the normal physiology or behaviour of the organism in any way?

To answer the first question, a better, more sensitive assay for metal is required.

The second question needs some definition on the "influence on the normal." From laboratory studies (see review Webb, 1975 and Roesijadi, 1980/81) and field studies (Olafson and Thompson, 1974; Brown et al, 1977; Noël-Lambot et al, 1980) metal accumulation in organism takes place as follows as the total metal concentration of the organism increases.

(a) Metal concentration in the body fluids increases.

(b) Metal concentration in specific organs increases.

(c) Specific detoxification or metal binding protein, and MT are produced to chelate the excess metal from the body fluids.

(d) Metal concentration exceeds the production of chelates and metals "spill over" into the enzyme pool.

(e) Obvious toxic effects are observed and the organism is visibly poisoned.

(f) The condition of (e) continues until the death of the organisms.

The first two steps (a) and (b) could be due to variations within the same species, hence "deviation from the normal" start with the third step (c). It is proposed that the production of a protein not specifically geared to growth or reproduction as is the case of metal binding protein, constitues a significant "deviation from the normal" and is therefore a powerful indicator of pollution.

So far it has been difficult to link pollution and the presence of metal binding proteins or MT. At low pollution levels some physiological change or effect of pollution had to be found.

In this study it has been shown that no metal accumulation in crayfish could be detected by digestion method but Hennig (in preparation, this volume) has shown that these crayfish moulted out of season and furthermore, metal binding proteins were found in these animals (see Method (a)). This established the link between metal binding protein in an organism without detectable metal accumulation but showing obvious pollution symptoms.

If the above argument holds metal pollution may be redefined as: "The presence of metal binding proteins confirms toxic

metal pollution." If this is then applied to the other organisms in this study, the following conclusion can be drawn for identical environmental conditions (16 $\mu M\ dm^{-3}$ Cu, 15 $\mu M\ dm^{-3}$ Zn).

In crayfish copper had reached pollution levels while the animals could detoxify zinc at this level. These conclusions could not have been drawn from the determination of metals (digestion method).

In hermit crabs the determination of metals suggested toxic levels for zinc. This cannot be confirmed by the relative amounts of metal binding proteins.

Shrimp showed considerable higher toxic zinc levels by metal binding protein than was to be expected by the digestion method.

In mussels both methods confirmed toxic pollution by zinc.

Limpets accumulated copper and zinc but only the zinc was shown to be toxic.

In the whelk Bullia the metal binding protein analysis showed toxic levels for both cadmium and zinc. This result was unexpected but it suggests the possibility that intracellular zinc accumulation may involve an active transport mechanism. It appeared that the cadmium exposure may have stimulated zinc transport into the soft tissues of the whelk. Similar results have been obtained by Frasier (1979) and these results suggest that unique mechanisms of metal uptake may exist in some marine organisms.

So far, very few reports have dealt with naturally occurring metal binding protein (Talbot and Magee, 1978; Osborn, 1978; Noël-Lambot et al., 1978; Engel and Brouwer, 1982) but it shows that this method can very well be applied to the environment.

A very convincing argument for the proposed new definition of pollution can be found in the study of grass grown with sludge.

Cadmium levels had increased drastically and the absolute levels of copper were very high, yet no ill effect or pollution could be found. The grass was obviously able to cope with these high levels. Zinc on the other hand had an toxic effect.

From the evidence presented here it is argued that metal binding protein determination should replace metal analysis in pollution work. It circumvents and largely eliminates differences due to animal size, mass, age, behaviour, food or life stage.

These metallothioneins have been isolated from humans, horses, rats, earthworms, tobacco (Wagner and Trotter, 1982) and grass and therefore can be assumed to be produced in all animals in some form or other.

Since metallothioeins are ubiqutous, this new definition of pollution should also hold for all toxic metals as well as all animals.

The absorption spectra of extracts from 7 organisms are presented here (Figures 25 to 29 and Figures 31 to 36). These together with the composition of amino acids shown in Table 2 suggest that they all belong to the family of metallothioneins reported in the literature (Table 2).

Considering toxic metalsas a whole, so far only metal binding proteins for cadmium, copper, mercury and zinc (Table 2) have been reported but that does not mean they do not exist for other metals.

In conclusion it is hoped that the great interest and enormous effort devoted to metal determination in biological systems be redirected to a more meaningful analysis of metal-binding protein.

TABLE II: PRELIMINARY AMONI ACID COMPOSITIUN OF THE ISOLATED CADMIUM, COPPER AND ZINC BINDING PROTEINS OF THE STUDY ORGANISMS TOGETHER WITH REFERENCE VALUES OBTAINED FROM THE LITERATURE

Species	Units	Lysine	Histi-dine	Argi-nine	Cysteic acid	Aspartic acid	Threo-nine	Serine	Glutamic acid	Pro-line	Gly-cine	Ala-nine	Va-line	Methio-nine	Isolen-cine	Len-cine	Tyro-sine	Phenyla-lanie	Metal	Reference
Human prenatal liver	mol %	7.9			14.1	3.1	2.4	7.6	3.5	2.6	6.1	6.2	1.8	0.8	0.9	0.6			Cu	Rydén and Deutsch, 1978
Human adult liver		8			18	3	2	8	3	2	5	6	2	1	1	1				Reydén and Deutsch, 1978
Human		8			20	4	2	8	2	2	5	7	1	1	1	-	-	-		Olafson et al, 1979
Horse liver	res %	10.41		2.22	32.62	5.00	3.85	11.55	4.47	5.10	10.08	9.43	2.57	1.50	0.63	0.60			Zn	Kägi et al, 1974
Horse kidney	res %	11.06		2.29	33.58	5.22	3.11	11.01	4.27	4.29	10.01	9.68	3.11	1.68	0.17	0.55			Zn Cd	Kägi et al, 1974
Rabbit MT-I		7	-	-	15	5	3	7	1	5	4	8	-	1	1	-	-	-		Olafson et al, 1979
Chicken	res/ mol	11.7	1.9	5.3	36.4	11.1	1.5	10.5	4.4	10.0	7.8	10.9	2.0	1.8	0.4	0.5	0	0	Cd	Weser et al, 1973
Avian		6	1	3	19	6	1	6	2	5	4	6	1	1	-	-	-	-		Olafson et al, 1979
Rat liver	res/ mol	15.5	3.6	3.4	31.6	7.3	4.7	8.6	7.1	8.3	9.6	7.5	4.7	0.5	2.9	3.4	0.8	< 1.0	Cd	Weser et al, 1973
Rat liver B	res %	13.2	1.7	2.5	7.50	10.2	7.9	7.3	12.6	4.0	10.1	4.9	4.3	3.2	5.0	5.9	2.0	3.7	Cu	Riordan and Gower, 1975
Rat A	res %	6.3		-	30.5	6.9	5.4	12.3	4.8	5.0	10.4	5.5	5.4	2.3	2.7	1.5	-	-	Zn	Richards and Cousins, 1977
Rat B	res %	8.1		-	28.4	8.1	4.0	10.6	7.5	6.8	8.1	9.5	3.6	0.9	2.4	1.2	-	-	Zn	Richards and Cousins, 1977
Rat liver Cu-LP	res %	11.4	0.9	1.8	6.8	11.8	7.8	6.4	13.6	3.4	9.4	8.0	6.1	0.4	2.0	6.5	0.5	3.5	Cu	Irons and Smith, 1977
Rat liver Cu-chelatin	res %	13.1	1.1	2.7	14.6	9.8	5.1	4.2	11.1	3.6	8.0	6.0	5.2	1.6	4.3	6.3	1.3	2.1	Cu	Irons and Smith, 1977
Rat liver Cd-BP	res %	12.8	0	0	30.7	8.0	7.2	11.3	4.8	3.8	9.8	5.7	2.8	1.0	1.8	1.0	0	0	Cd	Irons and Smith, 1977
Rat liver Cu-thionein (III-Zn)	res %	12.0	-	-	28.9	9.2	5.6	15.8	4.5	-	9.6	6.8	3.2	3.2	0.8	1.8	-	0.5	Cu	Irons and Smith, 1977
Rat kidney (Cd, Cu)-BP1	mol %	11.9	-	-	28.0	7.7	6.8	16.2	3.7	2.6	9.4	5.4	3.6	-	0.7	1.1	-	0.3	Cd Cu	Zelazowski and Szymanska, 1980
Rat kidney (Cd, Cu)-BP2	mol %	11.9	-	0.1	32.7	7.7	5.2	16.4	3.6	5.0	8.0	5.5	2.3	-	0.7	0.6	-	0.3	Cd Cu	Zelazowski and Szymanska, 1980
Rat kidney (Cd, Cu)-BP3	mol %	12.8	0.4	0.8	25.8	8.1	4.2	18.7	6.4	3.4	7.0	7.7	2.7	-	1.7	1.7	0.2	0.9	Cd Cu	Zelazowski and Szymanska, 1980
Rat liver (Cd, Zn)-MT1	mol %	16.9	-	0.1	31.7	7.4	4.6	8.0	5.6	6.4	6.4	8.0	1.9	0.6	0.7	0.9	-	-	Cd Zn	Zelazowski and Szymanska, 1980
Rat liver (Cd, Zn)-MT2	mol %	11.5	-	-	34.2	6.5	5.5	16.4	3.3	4.7	9.2	4.9	3.1	0.2	0.3	0.4	-	-	Cd Zn	Zelazowski and Szymanska, 1980
Rat liver (Cd, Zn)-MT2 + Cu	mol %	11.7	-	-	33.2	6.6	5.5	16.6	3.3	4.8	9.4	4.9	3.1	0.3	0.3	0.4	-	-	Cd Zn Cu	Zelazowski and Szymanska, 1980

TABLE II: PRELIMINARY AMONI ACID COMPOSITION OF THE ISOLATED CADMIUM, COPPER AND ZINC BINDING PROTEINS OF THE STUDY ORGANISMS TOGETHER WITH REFERENCE VALUES OBTAINED FROM THE LITERATURE (continued)

Species	Units	Lysine	Histidine	Arginine	Cysteic acid	Aspartic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	Alanine	Valine	Methionine	Isolencine	Lencine	Tyrosine	Phenylalanie	Metal	Reference
Rat liver I	mol %	12.0	0.1	-	34.5	6.8	6.7	15.2	2.1	3.2	9.1	5.1	3.3	1.4	0.2	0.3	-	-	Ag Zn	Whanger and Deagen, 1982
Rat liver II	mol %	11.9	0.2	-	32.0	6.8	2.8	18.5	4.6	3.9	6.3	7.6	1.6	1.7	1.4	0.4	0.3	-	Ag Zn	Whanger and Deagen, 1982
Rat liver I	mol %	16.4	-	-	28.5	9.0	2.5	8.1	12.0	5.4	3.1	10.0	1.9	1.7	0.8	0.3	0.3	-	Cd	Whanger and Deagen, 1982
Rat liver II	mol %	18.0	-	-	28.2	8.0	2.5	8.1	12.0	5.4	3.1	9.0	1.9	1.7	1.6	0.3	0.2	-	Cd	Whanger and Deagen, 1982
Rat pup liver	mol %	9.6		ND	30.8	7.5	3.6	14.3	6.6	4.4	9.0	8.2	2.0	1.5	1.6	0.9	-	-	Zn	Ridlington et al, 1983
Rat adult liver	mol %	18.7	ND	ND	30.5	9.2	4.3	8.7	7.5	3.9	4.5	6.6	2.8	2.1	0.5	0.8	-	-	Zn	Ridlington et al, 1983
Eel liver MT-1	res/ mol	10.5	0.4	0.8	24.4	8.3	8.8	8.9	6.1	3.9	9.6	6.3	4.2	1.6	1.3	2.2	0	1.1	Cd	Noël-Lambot et al, 1978
Eel liver MT-2	res/ mol	9.6	0.8	0.7	20.0	9.4	8.4	8.1	7.7	5.3	9.3	6.3	4.8	1.6	1.9	3.2	0	1.3	Cd	Noël-Lambot et al, 1978
Carp MT-1	mol %	11.37	-	-	34.24	8.47	7.10	11.23	3.11	4.89	10.64	5.82	1.98	1.01	0.11	-	-	-	Cd	Kito et al, 1982
Carp MT-2	mol %	10.09	-	-	31.90	9.10	8.47	10.13	3.50	4.16	11.13	5.59	2.35	1.68	0.99	-	-	-	Cd	Kito et al, 1982
Plaice	mol %	10.24	0	tr.	31.12	9.74	9.37	9.10	5.02	6.44	10.85	2.73	1.84	2.80	tr.	0.76	0	-	Cd	Kito et al, 1982
Cancer pagurus	mol %	17.4		0	29.9	9.6	7.3	9.1	5.6	8.5	7.3	4.0	tr.	1.3		0			Cd Cu	Overnell and Trewhella, 1979
Jasus lalandii	mol %	23.2	-	0	-	4.2	3.2	7.7	9.8	3.1	16.1	5.5	1.2	-	2.5	1.7	-	0.2	Cu Zn	This study
Scylla serrata MT1	mol %	13.8		1.8	26.2	6.1	5.9	12.0	10.9	7.2	9.4	4.1	1.8	0		0.6			Cd	Olafson et al, 1979
Scylla serrata MT2	mol %	13.0		3.7	28.3	11.4	4.8	7.9	9.7	11.3	6.4	2.5	0.3	0.2		0.2			Cd	Olafson et al, 1979
Scylla serrata MT1	res %	7.5		1.0	17.1	3.2	3.0	6.7	5.8	4.2	5.0	2.2	1.0						Cd	Lerch et al, 1982
Scylla serrata MT2	res/ mol	7.7		2.0	17.4	5.8	3.2	4.9	5.1	6.1	3.4	1.2							Cd	Lerch et al, 1982
Injected crab	mol %	16.5		0	33.1	8.8	7.6	9.5	4.2	8.2	6.7	3.5	0.9	0	0.6	0.5	0.1	0.1	Cd	Overnell, 1982a
Orkney crab (field) MT I	mol %	15.9		0	31.7	9.7	8.0	8.8	5.5	7.3	7.0	3.9	0.9	-	0.7	0.6	tr.	tr.	Cd	Overnell, 1982a
Orkney crab (field) MT II	mol %	13.2		0	27.8	10.1	8.0	8.6	7.3	8.4	7.4	4.4	1.6	-	1.4	1.8	tr.	tr.	Cd	Overnell, 1982a
Orkney crab (field) MT III	mol %	14.5		0	31.6	9.5	9.6	8.9	5.1	7.0	6.2	3.4	1.4	-	0.9	0.9	0.6	0.5	Cd	Overnell, 1982a
Injected crab, Cd-Cu	mol %	16.8		0	35.8	8.1	7.7	9.8	4.4	6.8	6.3	3.5	0.2	0	0.3	0.3			Cd Cu	Overnell, 1982b

TABLE II: PRELIMINARY AMONI ACID COMPOSITION OF THE ISOLATED CADMIUM, COPPER AND ZINC BINDING PROTEINS OF THE STUDY ORGANISMS TOGETHER WITH REFERENCE VALUES OBTAINED FROM THE LITERATURE (continued)

Species	Units	Lysine	Histi-dine	Argi-nine	Cysteic acid	Aspartic acid	Threo-nine	Serine	Glutamic acid	Pro-line	Gly-cine	Ala-nine	Va-line	Methio-nine	Isolen-cine	Len-cine	Tyro-sine	Phenyla-lanie	Metal	Reference
Diogenes brevirostris	mol %	27.7	-	0	-	5.5	1.7	8.5	9.8	-	26.5	9.7	1.2	-	2.5	4.0	0.6	-	Zn	This study
Palaemon pacificus	mol %	25.5	-	1.6	-	9.6	4.1	9.5	10.4	6.2	21.0	8.4	1.1	-	2.6	4.9	0.9	2.1	Zn	This study
Mytilus edulis MT I	mol %	9.5	1.5	2.4	11.2	10.8	8.0	7.6	8.9	4.9	11.7	5.9	4.3	1.4	3.7	4.7	1.7	2.3	Cd	George et al, 1979
Mytilus edulis MT II	mol %	10.1	1.2	2.3	17.6	9.5	9.5	7.5	7.6	4.4	12.2	5.9	3.9	tr.	2.6	4.3	1.2	2.1	Cd	George et al, 1979
Mytilus edulis MT II	mol %	11.5	-	1.2	20	9.1	6.3	6.5	5.8	3.8	18.6	4.7	4.2	-	3.5	3.0	-	-	Cd	George et al, 1979
Mytilus edulis MT III	mol %	9.8	3.4	1.6	6.3	12.3	8.1	8.2	7.8	5.2	10.8	5.0	3.5	tr.	2.7	2.8	0.6	1.0	Cd	George et al, 1979
Mytilus edulis	mol %	6.5	0.8	2.5	7.8	12.8	7.0	7.3	12.2	5.6	9.4	5.8	5.4	1.2	4.7	5.7	2.3	2.8	Hg	Roesijadi and Hall, 1981
Choromytilus meridionalis	mol %	27.4	-	0	-	8.7	2.8	9.9	13.6	-	20.4	7.3	0	-	3.6	5.7	-	0.9	Zn	This study
Crassostrea virginica	mol %	8.9	1.2	2.9	7.6	12.9	5.8	7.3	9.7	4.4	9.5	6.6	6.1	1.5	3.9	4.5	2.6	3.5	Cd	Ridlington and Fowler, 1979
Patella vulgata MTa	res %	8.8	1.0	0.8	21.0	11.2	7.2	8.8	7.7	3.8	11.0	8.7	3.2	< 0.5	1.8	2.6	1.8	1.0	Cd	Noël-Lambot et al, 1980
Patella vulgata MTb	res %	8.6	1.4	0.9	20.0	12.0	6.8	8.5	8.5	4.1	10.6	8.9	2.7	< 0.5	1.8	2.7	1.8	1.0	Cd	Noël-Lambot et al, 1980
Patella granularis	mol %	41.7	-	0	-	3.4	1.9	6.7	6.8	-	14.8	5.3	1.5	-	1.6	1.3	0.4	0.4	Zn	This study
Bullia digitalis	mol %	17.3	-	4.1	-	6.4	6.8	7.5	8.5	5.8	14.0	10.7	9.1	-	5.2	8.5	0.9	4.1	Zn	This study
Bullia digitalis	mol %	18.0	-	0.6	-	8.4	2.4	6.3	10.4	5.4	22.2	7.3	4.6	-	5.0	7.4	0.2	0.2	Cd	This study
Eisenia foetida	mol %	8.6	-	3.0	15.4	9.8	5.7	6.1	8.5	4.0	17.0	10.0	4.6	-	2.2	5.1	-	tr.	Cd	Yamamura et al, 1981
Jasus lalandii fraction 90-91	mol %	39.5	8.8	0	-	3.2	3.2	11.8	4.3	1.7	16.6	7.3	1.2	-	1.6	-	-	0.3	Cd	This study
Jasus lalandii fraction 96-97	mol %	23.2	-	1.0	-	4.2	2.4	11.5	9.8	3.1	26.4	7.7	3.8	-	2.5	4.0	0.3	0.2	Cd Zn	This study
Jasus lalandii fraction 99-100	mol %	28.0	-	1.2	-	3.0	1.8	7.7	7.0	6.2	16.1	5.5	0	-	1.5	1.7	0.2	0.3	Zn	This study

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CONCLUSION

Linking a physiological parameter such as production of metal binding proteins, to increased frequency of moulting out of season, in arthropods is the central theme of this thesis. For the first time, metal stress in organisms can be measured and evaluated without evidence of measurable metal enrichment. This finding is then used to re-define the criteria of toxic metal pollution.

The need for such a new measure became apparent when compiling background levels of metal concentrations in water, sediments and fauna in the South African environment. This review (Paper 1) is a typical data set, similar to work done in nearly every country, and it illuminated many of the shortcomings and drawbacks of "base line" and pollution studies.

It is now clear that all three parameters; water, sediment and organisms, have to be sampled at the same time from the same location for any meaningful interpretation (see the section on Durban harbour).

The analytical problems and errors in reporting water and sediment results have been mentioned, but these are minor in comparison to the problems arising from determinations of metals in biological samples. Here the data were often so incomplete that they can only be interpreted as possible guidelines or general trends.

The major omissions are:

a) Reporting of mean metal values without reference to size or mass. It is shown in crayfish (J. lalandii, Figure 80) and mussels (C. meridionalis, Figure 93; and P. perna, Figure 98) that a mean metal value without size may be quite meaningless.

3) The same species accumulate various metals at different rates at closely related sites (Figure 98). Thus extrapolation of results from one region to another is not valid, even when working with the same or closely related species.

Major advantages, of metal determinations are, however, the ease and accuracy of analyses, and water as well as sediments can be monitored regularly to show an improvement or a deterioration in specific polluted areas.

Furthermore, in unpolluted regions like the Antarctic (Paper 2), metal analysis of organisms could be correlated with specific areas (water masses) and thereby may identify feeding grounds of predators. Such a correlation must however be used with caution, since there appear to be seasonal variations and natural enrichments of water masses. It must be stressed that it is the differences between metal concentrations which are important, and not so much the absolute values found. Also absolute metal concentrations are not necessarily low in an unpolluted environment.

This last point was stressed in Paper 3. It was shown more emphatically than in Paper 1, that baseline studies are dependent on natural concentrations of elements. It was also pointed out that organisms in unpolluted regions can have very high metal concentrations, much higher concentrations in fact than found in anthropogenically polluted "hot spots" such as Langebaan.

In a more obvious manner than in Paper 1, it was shown that extrapolation of results from one region to another may not be valid and could produce contradictory results.

In Paper 4, the omissions mentioned in Paper 1 have been emphasized and some suggestions have been made to improve the quality

of reporting metal concentrations in base line studies. It also points out the inherent difficulties of pollution studies: metal accumulation is too strongly influenced by factors such as size and sex.

Up to this point, the papers presented here illustrate and stress the same difficulties that are encountered in metal pollution work. Each, however, discussed a different aspect of these difficulties and thus highlights the complexity of the problem.

From the arguments set out so far, it is clear that interpretation of results from pollution studies needs some parameter that would circumvent all the above mentioned limitations regarding size and sex. A new measure was needed which could be used to extrapolate both from one species to another and between different geographical regions.

The low molecular weight, sulphydryl-rich, metal binding protein, metallothionein, has proved to be such a parameter. What was however missing was the link between metal binding protein and some other symptom of toxic metal concentration in the environment of an organism, preferably observed at metal concentrations which could not be detected otherwise (see supporting paper).

Such a link was found (Paper 5) to be the abnormal and increased moulting in crayfish (J. lalandii), which was further substantiated with work done on sandshrimps (P. pacificus).

In the final paper, it was shown that these moulting organisms had indeed produced metal binding proteins and together with results from other species of different phyla and from different regions, a new definition of metal pollution was proposed: **The presence of metal binding proteins confirms toxic metal pollution.**

Furthermore it was shown that the proteins isolated were very similar one to another, an amino acid analysis of the different metal binding proteins (cadium, copper, zinc) agreed well with results reported from other organisms, ranging very widely from man to earthworm and plants. Thus the above new definition can apply to several toxic metals and it appears to be produced by all organisms as a response to metals.

The study was concluded by testing the new toxic metal criteria in two field situations and, in each case, a much more meaningful assessment of the effects of specific metals on the organism could be made.

An investigation similar to that submitted as a supporting paper was supposably done during the Southern California Coastal Water Research Project. Unfortunately the only freely available reference and citation (Jenkins et al., 1982, Mar. Poll. Bull., 13, 413-421) report on intracellular metal-binding protein of sea urchin gonads. There it is suggested that spillover takes place from the High-Molecular-Weight pool to the metallathioein-like protein pool. Precisely the opposite has been reported by all other workers who work on this mechanism.

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SCOPE

In March 1980 the then Department of Environmental Planning and Energy (now the Department of Environment Affairs, but formerly the Department of Water Affairs, Forestry and Environmental Conservation) commissioned NRIO to proceed with ocean outfall studies at Saldanha. The purpose of these studies was to investigate a favoured location for the route of an outfall to sea, should this be necessary for disposal of domestic and industrial effluent from the Saldanha-Vredenburg area in the future.

At a Steering Committee meeting in April 1981, the first results of chemical and biological surveys carried out at Noordwesbaai were presented (CSIR Report C/SEA 8112). That report contained recommendations for further survey work, but also suggested that some more details of the nature of any proposed effluent should be obtained. The toxicity of this suggested effluent to marine organisms should then be tested.

This report contains the results of four toxicity experiments which have been carried out. Two of the experiments were done by Messrs A H Fricke and H F-K O Hennig of NRIO, while the other two were kindly done by Dr A D Connell and Mr D D Airey, NIWR, Durban, at our request.

Signed

F P ANDERSON
DIRECTOR

Stellenbosch
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CONTENTS

1 INTRODUCTION

The results of preliminary chemical and biological surveys of Noordwesbaai carried out by the Marine Chemistry and Marine Biology Divisions of NRIO have been presented in two reports (CSIR Report C/SEA 8112, and CSIR Report C/SEA 8221). In the first of these, some recommendations were made for further work to be carried out. In this report we present the results of four experiments which were done in accordance with two of the recommendations, namely:

1 levels of biologically critical trace metals must be determined on biota, especially commercial species; and
2 reliable estimates as to the chemical nature of the proposed effluent must be obtained in order to assess the resulting level of pollution and the hazard it poses for man, the fishing industry and the ecology of the region.

The toxicity experiments were carried out using a test solution mixed according to the best available information regarding the possible nature of future effluent to be discharged. In addition base line levels of some trace metals were determined in the most important commercial species, *Jasus lalandii*, as well as its main food source, the ribbed mussel, *Aulacomya ater*.

2 THE EFFLUENT TESTED

It is appropriate, to begin with, to view the proposed effluent against the background of the situation at Saldanha Bay. It was suggested that the major components of any effluent discharged at Noordwesbaai would be derived from zinc and copper refineries as well as domestic sewage.

In any zinc refinery, for every tonne of concentrate, about one and a half tonnes of zinc and sulphuric acid are produced. Thus, the production of zinc depends to a great extent on the market for sulphuric acid. At present all acid produced in this way, mainly in the Vaal Triangle, is absorbed by industry in the nearby vicinity. Acid produced on the west coast could either be exported, although there is a very small international market for it, or it could be used within the Republic. This would, however, mean expensive transport of hazardous, low-value freight to the industrial areas.

A similar situation would arise with a copper refinery. This would also have to be coupled to other manufacturing plants to recover by-products, so that large transport costs would not be incurred. Obviously the by-products would depend on the chemical composition of the concentrate.

The effluents from refineries are very acid and high in toxic metals. Some sort of thickener would, therefore, have to be incorporated into any proposed refinery and neutralizing material such as calcium carbonate would have to be railed in, unless it was available locally. Removal of trace metals would also be required since the effluent would also be likely to contain far higher concentrations of heavy metals than is normally permitted to be discharged.

It must also be borne in mind that changes in both zinc and copper refining technology are occurring all the time. These could make the refining processes look very different in a few years' time, and they might be producing a completely different type of effluent. A prediction study should also take into account the source of the raw material. This in turn would depend on the geographical/geological situations of the mines supplying ore to the refineries.

With this as a background, it will readily be appreciated that unless some very specific details of the refining and other industrial processes and of the effluents are available, any toxicity testing which is done, becomes very largely an academic exercise.

Much time and effort were therefore spent in trying to get an effluent which would be as close as possible in composition to a real effluent which would be likely to be discharged through the pipeline, should this be built. The most detailed information which we were able to obtain was that from the then Department of Environmental Planning and Energy. This is set out in Table 1. In spite of numerous phone calls and letters to various government departments and local authorities, we were unable to get any information which was considered to be more accurate than this.

There was therefore no option but to use these figures, even though it was realized at the time that they were probably grossly unrealistic. It was decided, however, that to make the composition of the test solution as realistic as possible, we should use real effluents rather than laboratory solutions. We therefore obtained samples of domestic sewage, copper refinery and zinc refinery effluents and mixed these in appropriate proportions, and used this mixture as the test solution.

In addition we visited copper and zinc refineries to view their processes first-hand. From these visits we were able to ascertain from the refineries the likely content of effluents and how this would have to be treated before discharge. This information was taken into account when deciding on the final composition of our test effluent.

Thus four experiments were completed using the effluent which was prepared as shown below. In this report this mixture is referred to as "the effluent". The mixture was based on the projections for the year 2000 (See Table 1), as follows:

Raw sewage	1 840 000 $m^3.a^{-1}$	
Cu refinery effluent	120 000 $m^3.a^{-1}$	
Zn refinery effluent	687 000 $m^3.a^{-1}$	(assuming 20 per cent daily release of total process water)

The copper refinery effluent which we obtained was high in most trace metals. This type of effluent would not normally be permitted into any sewer or pipeline system. Therefore before mixing this into our test effluent we diluted it twenty times, to bring the metal concentrations into the range which would be permitted by the authorities.

The effluent was therefore made up as follows:

Sewage	72,66%
Cu refinery effluent	0,24%
Zn refinery effluent	27,10%

This effluent was analysed for trace metals. It contained the following concentrations of metals tested ($mg.dm^{-3}$, numerically equal to ppm):

Cd	0,01
Co	0,09
Cr	0,10
Cu	0,50
Fe	1,18
Mn	0,05
Ni	0,41
Pb	0,20
Zn	3,34

3 THE USE OF BIOASSAYS IN TOXICITY EXPERIMENTS

In any pollution investigation it is important that concentrations of pollutants measured chemically should, wherever possible, be related quantitatively to biological effects. Chemical measurements on their own, while being very useful in pinpointing the source of pollution, do not give an indication of the ecological significance of such pollution.

However, in most natural systems there is a large amount of biological "noise" - natural variations which occur as a result of changes in natural conditions. Thus one must try to avoid this variability and cut down the number of variables to a manageable number. To do this, one must resort to laboratory bioassays to study the biological effects of pollution, since it is normally just not feasible to do this in the natural environment. Nonetheless, one must be aware of the difficulties and dangers of extrapolating the results of laboratory experiments to natural environments.

The value of bioassays lies in the fact that organisms integrate environmental effects over a period of time and under different conditions as opposed to chemical measurements which give a picture of a situation at any one time.

The selection of the organism for the bioassay experiments is very important. Some of the requirements are the following:

(i) the biology of the organism should be well known;
(ii) the animal should be sensitive, yet robust towards laboratory handling;
(iii) the animal should be common and easily maintained.

4 RATIONALE AND METHODS

As a result of the recommendations mentioned earlier, an attempt was made to establish base line levels of metals in commercially important animals. In addition, four toxicity experiments were carried out. Each was designed to test a different aspect of toxicity of the effluent. The rationale behind each experiment and the methods used are detailed below.

4.1 Baseline levels of trace metals in biota

For this determination the most important commercial species is the rock lobster *Jasus lalandii*. Since its main food source in Noordwesbaai is the ribbed mussel *Aulacomya ater*, a number of these animals were analysed as well.

Twenty-nine male and 14 female lobsters were analysed altogether. These ranged in size from 5 to 13 cm (carapace length). However it should be borne in mind that females are much smaller than males of the same age. For example, a female may be up to 20 years older than a male of the same size (Pollock, 1978). The lobsters were dissected and the tails, green glands and gills were analysed separately for eight metals. In this report we will discuss only the copper, zinc and iron results, since these are the metals most relevant to the present study.

Mussels are well known to be efficient accumulators of trace metals. In addition they form the main food source of rock lobsters. Therefore 40 mussels were analysed for the same range of trace metals. In this case the whole soft part of each mussel was removed from the shell and analysed.

After dissection of the mussels, samples were oven dried at 60^{o}C to constant weight. Digestion methods used for all samples were standard and have been described elsewhere (Orren *et al.*, 1980). After digestion of the samples, solutions were analysed by flame atomic absorption spectroscopy using standard conditions and background correction.

4.2 Toxicity study using sea urchin eggs and larvae.

Marine organisms depend for development and survival on narrowly defined conditions, especially during their delicate juvenile stages. This is particularly relevant to the Noordwesbaai outfall site, since it is known that the main migration route of pilchard and anchovy larvae and juveniles passes within a few kilometres of the coast at Noordwesbaai (Crawford, 1980). It was not possible to do toxicity experiments with eggs of either of these species. Fortunately, however, the results of a recent detailed study using eggs and larvae of the common sea urchin *Parechinus angulosus* were available as was the expertise gained in the experiment. This animal was chosen for a number of reasons. The species is geographically widespread, extending from Lüderitz to Zululand. Although it has a bi-modal reproduction (Fricke, 1980), fertile gamete material can be obtained throughout the year. The urchins are amenable to aquarium conditions over long periods and a wealth of literature concerning them is available.

The experiments represent a short-term (80 h) bio-assay with the post-hatch stage. During this stage most animals are very sensitive to pollution and even though a pollutant may not be acutely toxic to the larvae, a decrease in the rate of development may be an equally significant ecological effect.

Experimental details of the method of sperm and egg collection and of the apparatus used are described elsewhere (Greenwood, 1980). Uniformity of genetic material in an experimental organism is essential for consistent results. Urchins were, therefore, collected from one site only, namely, a rock pool at Bloubergstrand. The urchins were maintained in an aquarium at 12^{o}C. However, they were used soon after collection, thereby avoiding the need to feed them and reducing the chance of contaminating the gamete material. In this experiment six replicates of each of five dilutions (10, 20, 100, 1000 and 5000 times) of the effluent described in Section 2 were used. Filtered seawater was used as diluent. Fertilized urchin eggs were cultured in these media in leached, conditioned petri-dishes under controlled conditions. Measurements on all subsamples of the developing larvae were commenced after 20 hours and then made at 10-hourly intervals over an 80-hour period. This was done by removing 0,2 cm^{3} of the liquid containing larvae. This volume contained sufficient larvae for the count without significantly reducing the remaining portion. The ten larvae considered to be the most developed were measured.

4.3 Toxicity study using fish eggs

Another experiment was carried out using eggs of the coral reef fish *Dascyllus trimaculatus*. The rationale for this experiment was the same as that for the experiment with the sea urchin eggs. Although this fish is an east coast species, its biology is well known and it has been used in toxicity tests by NIWR, Durban. The fish are very amenable to laboratory handling and the eggs provide a convenient bioassay method for toxicity studies.

Dascyllus eggs were obtained from a breeding stock of fish which are maintained in the laboratory. Eggs were removed from the aquarium walls using a scalpel and suction tube. The eggs were then separated from one another under a dissecting microscope and placed, in batches of about 100, into 400 cm^3 beakers containing natural seawater (salinity $35^{o}/oo$). Effluent was then added to the beakers to make up 3 replicates each of dilutions ranging from 1 in 25 to 1 in 150, plus controls. The beakers were aerated and kept at ambient temperature. Development of the eggs was followed until all had either hatched or died (approximately 6 days).

4.4 Population effects using estuarine amphipods

In an ecological context, for an effect to be relevant, it should manifest itself at a population level. It has recently been stated that "the chronic life-cycle test - whether it be on fish, invertebrates or other organisms - is, and must be, the mainstay of our profession for some time to come" (Mount, 1977). Many freshwater species, but few estuarine or marine animals are available to the biologist for these life cycle bioassays. There are various reasons for this (see also Section 3 above). One of these is that for the tests to be practical, a short-lived animal must be used. At the NIWR, Durban, this has led to the development of a rich laboratory culture of the burrowing amphipods *Grandidierella lutosa* and *G. lignorum* (Connell and Airey, 1979). The cultures are easily maintained and the full life-cycle from an egg to an adult female carrying her first eggs is less than 30 days.

Although these animals do not occur on the west coast, the considerable experience and expertise of the group in Durban made these experiments attractive. The results of the experiments give a very good idea of the toxicity of the test effluent relative to the toxicities of a large number of other effluents which have been tested by NIWR.

Aquaria were prepared using natural seawater (35^{o}/oo) from the ORI well point. A 1 cm layer of natural mud from the Karridene estuary on the Natal south coast was placed in each 10 dm^3 aquarium and the temperature was maintained at $25 \pm 0,2^{o}C$. The aquaria were connected to a Mount and Brungs (1967) proportional doser which gave dilutions of effluent between 1 in 258 and 1 in 3033. Each tank was spiked with 20 gravid female and 10 male *Grandidierella* from laboratory cultures. After one day the doser was switched on and the experiment was run for 60 days (2-3 generations of animals). The cultures were fed daily on freely available commercial tropical fish-flake food. At the completion of the experiment, the animals were separated and counted.

4.5 Accumulation experiments using the rock lobster *Jasus lalandii*

In addition to the base line determinations described in 4.1 above, experiments were done to test the accumulation rate of trace metals in rock lobsters. With hindsight it must be pointed out that the results of these experiments are of very limited value. The reason for this is that the experiments were continued for 60 days, which was the time available. When it is realised that the rock lobster is a very long-lived animal, (up to 40 years (Pollock, 1978)), the limitations of running an accumulation experiment for the period mentioned can be immediately recognised. Nonetheless, it is worth while to include the experimental details here.

Two lobsters (all above commercial catch size and collected from Noordwesbaai) were placed in each of twelve 10 dm^3 aquarium tanks, kept at $15 \pm 1^{o}C$. The tanks were aerated and the animals were given some time to acclimatise. Some trouble was experienced in getting two animals in each tank which were compatible with each other, many of the animals fighting and injuring each other after being placed together.

After 7 days the experiment was begun. Tanks were dosed with effluent as follows: 2, 10 and 100 cm^3 effluent per 10 dm^3 tank, i.e. to give dilutions of 5 000, 1 000 and 100 times, respectively. Three tanks at each dilution were prepared in this way, in addition to three control tanks. The water and effluent were completely changed daily for the duration of the experiment to ensure more or less constant concentrations of effluent and to avoid a build-up of waste products in the tanks. The lobsters were fed daily with live ribbed mussels, *Aulacomya*, collected from the same area.

After 30, 45 and 60 days, one tank from each effluent concentration and one control tank were removed from the experiment. Immediately after removal from the tanks, the animals were dissected and samples of tails, green glands and gills were removed, dried at 60°C and digested for analysis. Two animals had to be removed prematurely (after 3 and 19 days) because of injuries which they sustained while fighting. After digestion, the samples were analysed for the usual range of trace metals.

One of the control animals moulted during the experiment. The moult was separated into gills, stomach and shell lining and these were also analysed.

5 RESULTS AND DISCUSSION

5.1 Levels of trace metals in biota

The results of the trace metal analyses of the tails, green glands and gills of the rock lobsters are illustrated in Figs. 1, 2 and 3 (results for copper, zinc and iron shown only). Individual results are also given in Appendix 1. These figures clearly illustrate a few important points:

(i) there is no difference between metal concentrations in male and female for any of the organs analysed;.
(ii) concentrations of the three metals were of the same order, with zinc slightly higher than the others in the tails and iron the highest in the green glands;
(iii) there is a slight, but significant, decrease in metal concentrations with increasing size of animals; and
(iv) metals were the least concentrated in the tails, with concentrations in the green glands and gills very similar.

The fact that metal concentrations decrease with size (and age) indicates that the animals have some mechanism whereby they are able to regulate the metal levels in their bodies. The results of the analysis of the moult are interesting in this respect and give some hint as to this mechanism. The concentration of zinc in the shell lining of the moult was approximately 25 times higher than the concentration in the bodies of animals of similar size. On the basis of this one result, it appears that the rock lobster gets rid of some of the excess zinc by concentrating this in the shell, which is then lost through moulting. The concentration of iron was slightly higher in the moult than in other samples, while copper concentrations in the moult were not greater.

The concentrations of copper, zinc and iron in mussels are illustrated in Figure 4. These figures also indicate a slight but significant decrease in concentrations of copper and iron with increasing size. However, zinc concentrations in the mussels appeared to increase with size of animal. This is similar to the results obtained by Watling (1978), who used *Choromytilus meridionalis* and *Perna perna*.

Since the Noordwesbaai area has been shown to have very low concentrations of metals in water and sediments (CSIR Reports 8112 and 8221), it can be accepted that these are base line levels of the metals analysed in these animals from Noordwesbaai. These levels are not significantly different from those obtained by the Sea Fisheries Institute in *Jasus lalandii* collected from Saldanha Bay (J. Henry, personal communication).

5.2 Sea urchin larvae

Fertilization success, even at the highest pollutant concentration (1 in 10) was virtually complete. However, at this concentration severe deformation and developmental retardation was obvious after 17 hours. Larvae in higher dilutions appeared progressively less disturbed. At the other end of the scale, no difference between the control and the experiment with the highest dilution (1 in 5 000) could be detected. The three intermediate dilutions were therefore of most interest and the experiment was repeated using concentrations of 1 in 20, 1 in 100 and 1 in 1 000 of the effluent.

Six replicates of each dilution were made. At 10-hourly intervals, measurements of total lengths of 10 plutei were taken and the means calculated. Results are plotted in Figure 5.

A significant ($P < 0,05$) difference existed between the control and the 20-times dilution experiment (Figure 5). The mean size reached by the larvae in the latter was smaller and numerous morphological abnormalities were evident in all developmental stages. The differences between the control and the 100 and 1 000 times dilution experiments were clear, but not significant at the same confidence level as in the 20-times dilution experiment. This is not surprising since the effect of the effluent became visible only some 40 hours after fertilization, and four further measurements were taken before termination of the experiment. It could be expected that differences would become more marked with time.

Some important differences between these results and those obtained by Greenwood (1980) in earlier experiments can be highlighted. Greenwood detected significant effects due to zinc, applied as a pure solution. In contrast, similar concentrations of zinc in these experiments (0,05 - 0,1 $mg.dm^{-3}$)

appeared to be less toxic, in spite of the fact that other metals were also present and a synergistic effect may have been expected. This reduction in toxicity must therefore be attributed to a complexing effect of the organic matter in the sewage.

The effective copper concentration in the effluent after 20-times dilution was 0,025 $mg.dm^{-3}$. Greenwood did not detect a harmful effect at twice this concentration of copper, using a pure copper salt. It is therefore concluded that it was not the copper which was accountable for the effects observed in the 20-times dilution experiment. Similar reasoning applies in the case of nickel, the third major metal present.

It must therefore be concluded that the toxic effects of the effluent were caused, at least in part, by the zinc. This agrees with earlier results obtained by Greenwood (1980), who identified zinc as being extremely toxic to post-hatch larvae.

5.3 *Dascyllus* eggs

The results of the static experiments run with *Dascyllus* eggs are given in Table 2. The figures show a clear decrease in the success rate of hatching with increasing concentration of effluent. This is illustrated in Figure 6, in which the hatching success rate is expressed as a percentage of that in the controls. This figure shows clearly that in this experiment, no decrease in hatching success could be detected in effluent dilutions of 1 in 125 and greater. However, in dilutions of 1 in 100 and less, a very marked decline in hatch success could be observed, and this correlated positively with increase in effluent concentration.

Unfortunately in this experiment the concentrations of specific elements in the pollutant could not be monitored. Because of the uncertainty of whether specific elements plate out on the walls of the beaker or are complexed by organic matter, use cannot be made of calculated concentrations in this case. Results can therefore be based only on the dilution of the effluent used.

5.4 *Grandidierella* species

The results of the 60-day flowthrough bioassay on *Grandidierella lutosa* and *G. lignorum* are given in Table 3. This shows that, at the dilutions used, the effluent had little effect on the *Grandidierella* populations (or, in fact, on the other species of animals which were present in the mud at the start of the experiments). However, in this experiment the highest concentration of effluent was in a 1 in 258 dilution. At this concentration, no effects were observed in the other experiments either. Nonetheless, in similar experiments with some other effluents, significant effects have been noted with effluent dilutions of around 300 (Connell, personel communication). *Grandidierella* was also shown to be particularly sensitive to fluoride (Connell and Airey, 1979). From this result we must therefore conclude that this effluent was not as toxic as many others from Natal and other areas, which have been tested in a similar manner.

5.5 Accumulation experiments with *Jasus*

The concentrations of copper, zinc and iron in the tails, green glands and gills are given in Table 4. These figures indicate that there was no significant increase with time in any of the metals in any of the organs. Although the concentration of some metals may have shown a slight increase, almost all the concentrations of metals fell within the standard deviation of the mean concentrations in untreated animals. No difference could be detected either in any of the three effluent dilutions. As mentioned earlier, this is probably due to the relatively short time scale of the experiment in comparison with the life-span of the animals. At this stage, therefore, any differences in metal concentrations in treated animals must be attributed to natural variability.

This result emphasizes an important point, i.e. in order to be able to detect reasonable accumulation of metals in animals, when the concentrations of metals in the medium are near to environmental levels, the experiment must run for at least a significant portion of the animal's life. In order for such experiments to be practicable therefore, an animal with a relatively

short life-span should be used. This points to the unsuitability of *Jasus lalandii* as a test animal in this type of experiment.

However, it can be pointed out that differences were found in the concentrations of metals in different organs, as follows:

green gland:	Cu	*** <	Zn	*** <	Fe
tail	Fe	*** <	Cu	* <	Zn
gills	Fe	** =	Zn	* <	Cu

* < 90% significant
** 95% significant
*** > 99% significant

6 RECOMMENDATIONS

One particular observation comes very strongly out of the results of these experiments. Work of this nature involves an enormous amount of time and effort and, therefore, of funds. Because of this, the experiments are very difficult or impossible to repeat and must therefore supply the required results the first time. Since results with one effluent do not necessarily apply to other effluents as well, the correct choice of effluent is of the utmost importance.

In this case, in spite of the very great effort which was made to make this effluent as realistic as possible, it is still felt that this was probably some way from any effluent which may, in time, be discharged from Noordwesbaai. This detracts very much from the practical application of these results, although the experiments were valid in the academic sense. It is therefore strongly recommended that before any experiments of this nature are repeated very much more exact knowledge of any likely effluent must be obtained.

Another observation can also be made at this stage. From the results obtained it appears that experiments with eggs and larvae were more sensitive than those with adult animals. (This observation can unfortunately not be extended to the *Grandidierella* experiments where larger dilutions were used). In addition, the experiments with eggs and larvae were much shorter and therefore the logistics of running them are very much easier - less seawater and effluent are required, animals do not have to be fed and require far less space. Thus it is recommended that experiments of this nature be pursued in future, unless some easier means of measuring stress in large animals, e.g. increased heart rate, can be perfected. The use of *Grandidierella* is, however, to be encouraged since this animal is able to reveal ecological effects at a population level in a relatively short time.

7 ACKNOWLEDGEMENTS

The assistance of Dr A D Connell and Mr D D Airey of NIWR Durban in running the experiments with *Grandidierella* and *Dasycllus* is gratefully acknowledged. Although it was agreed that all sources of effluent would remain confidential, we would nevertheless like to thank a number of people in various organisations for their assistance and friendliness in supplying us with effluent.

The financial support of the Department of Environmental Affairs is acknowledged. We would also like to thank the Heads of Departments and staff of the Geochemistry, Zoology and Oceanography Departments, UCT, for their help and the use of various facilities.

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TABLE 1

Possible contributors to Saldanha Bay effluent (figures in m^3 per year).

Year	Domestic sewage	Copper refinery	Zinc refinery
1984	1 300 000	120 000	-
2000	1 840 000	120 000	3 428 000
2020	2 400 000	120 000	3 428 000
2040	2 910 000	120 000	3 428 000

TABLE 2

Hatching success of *Dascyllus* eggs

Effluent dilution	Percent hatched			Number hatched (% of control)
		$\bar{x}$	Sx	
Control	58,8			
Control	28,0	48,7	18,0	-
Control	59,4			
1 in 150	48,1			
1 in 150	50,0	50,2	2,2	103
1 in 150	52,5			
1 in 125	41,2	50,7	13,4	104
1 in 125	60,2			
1 in 100	30,1			
1 in 100	31,8	31,8	1,8	65,3
1 in 100	33,6			
1 in 75	42,0			
1 in 75	33,6	35,4	5,9	72,7
1 in 75	30,6			
1 in 50	53,3			
1 in 50	8,8	25,0	24,6	51,3
1 in 50	12,8			
1 in 36	15,7	15,7	-	32,2
1 in 25	1,0			
1 in 25	6,7	6,6	5,5	13,6
1 in 25	12,0			

TABLE 3

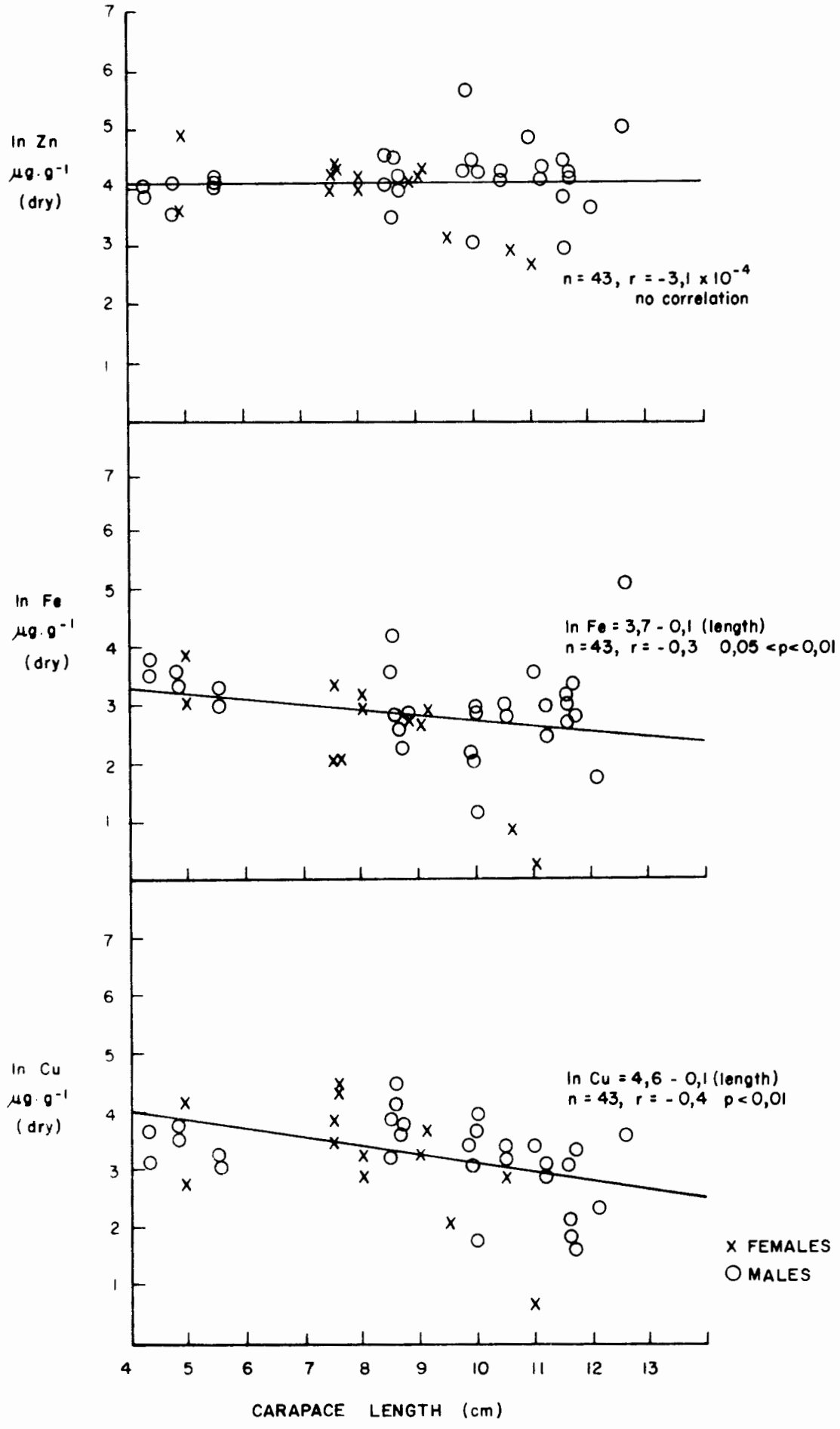
Populations of *Grandidierella* at conclusion of experiment

Effluent dilution	Controls		3033	2195	929	848	561	500	428	363	267	258
	$\bar{x}$	Sx										
Grandidierella females	95	29	125	128	121	55	123	137	173	113	118	58
males	61	15	90	84	131	38	134	89	157	115	174	95
juveniles	288	168	101	25	144	50	104	75	167	59	62	42
eggs	453	288	201	90	158	80	143	132	136	130	153	126
no. eggs per female	4,0	2,0	190	85	155	175	138	115	95	138	155	260
Ceratonereis costae adults	40	13	120	100	83	63	163	90	138	165	128	120
juveniles	451	445	168	40	36	45	18	36	95	95	92	39
Apseudes digitalis	18	19	261	33	78	178	294	0	400	239	72	67
Copepoda	480	139	84	80	94	248	129	111	114	134	52	180

TABLE 4

Accumulation of copper, iron and zinc in the rock lobster *Jasus lalandii*

Duration of accumulation (days)	Dilution	Tail $\mu g.g^{-1}$			Green gland $\mu g.g^{-1}$			Gill $\mu g.g^{-1}$		
		Cu	Fe	Zn	Cu	Fe	Zn	Cu	Fe	Zn
3	100	34,5	14,6	61,7	75,0	466,7	133,3	170,5	180,6	217,5
19	2	11,4	1,4	34,3	50,0	318,8	75,0	138,9	92,6	116,7
19	10	10,0	1,8	28,2	62,5	96,9	46,9	270,2	163,8	137,2
30	2	10,3	6,4	26,9	23,9	28,9	21,6	227,3	89,4	130,4
30	10	10,8	2,5	20,0	28,2	113,2	28,8	141,5	202,4	135,4
30	100	41,5	24,5	56,6	23,7	61,8	29,3	219,6	40,2	113,0
45	2	18,9	19,7	51,7	63,1	331,7	79,4	202,4	78,3	115,1
45	10	38,5	13,2	45,2	97,9	375,0	79,4	272,1	93,0	156,3
45	100	21,6	9,9	42,9	81,1	218,9	129,1	270,1	79,7	164,8
60	2	64,8	25,9	61,7	99,5	135,3	78,8	246,0	75,2	137,8
60	10	50,3	20,4	60,6	123,0	118,5	72,0	326,8	76,2	139,9
60	100	46,5	29,6	56,6	134,0	700,7	243,1	214,4	81,6	147,6
Baseline levels	$\bar{x}$	34,6	24,2	72,3	60,6	202,4	160,8	184,2	130,0	130,8
	S_e	6,7	10,0	13,6	19,3	82,1	87,1	48,3	28,9	16,7



TRACED L de J CHECKED RAG DATE 22/10 REF	METAL CONCENTRATIONS IN ROCK LOBSTER TAILS	FIGURE 1

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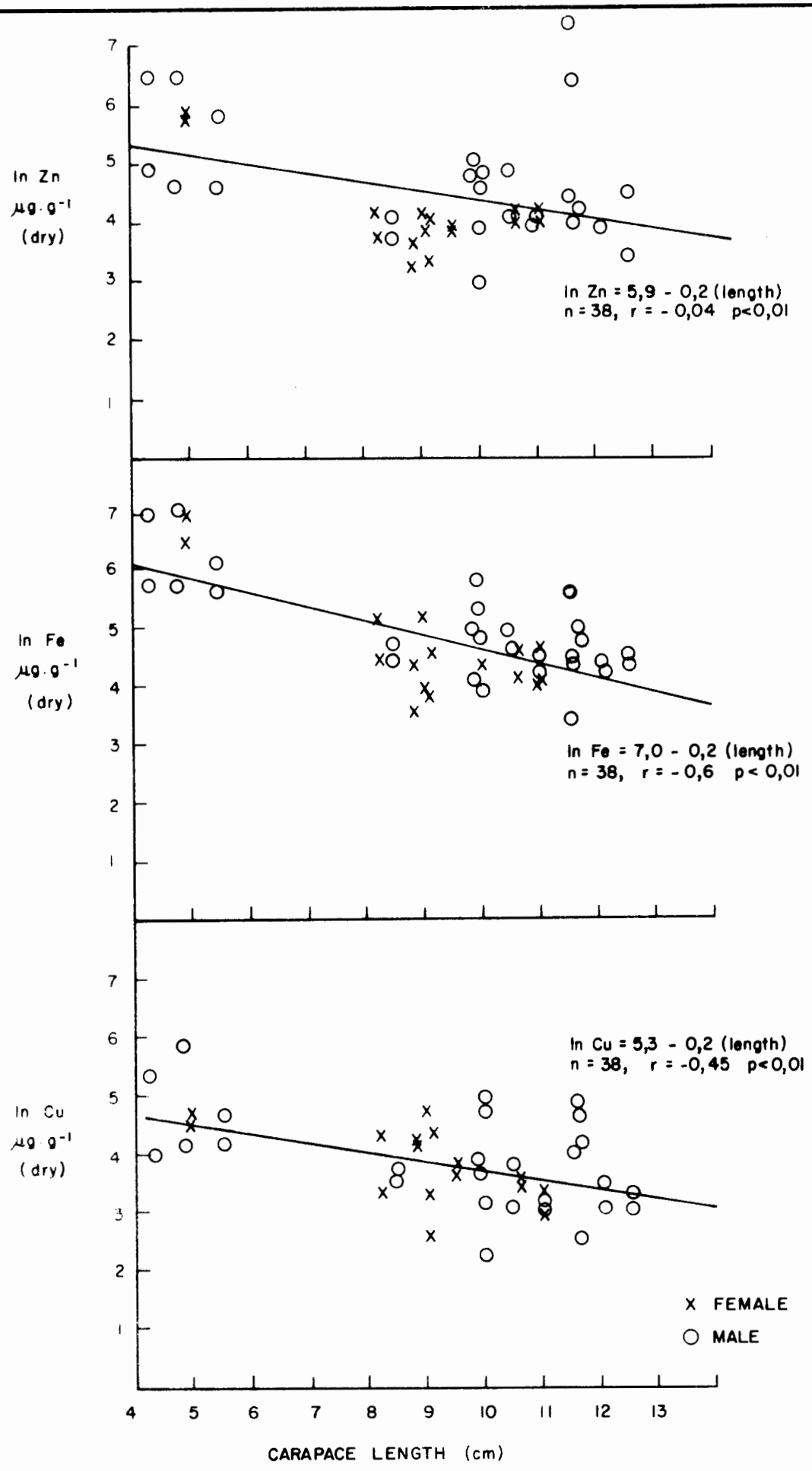
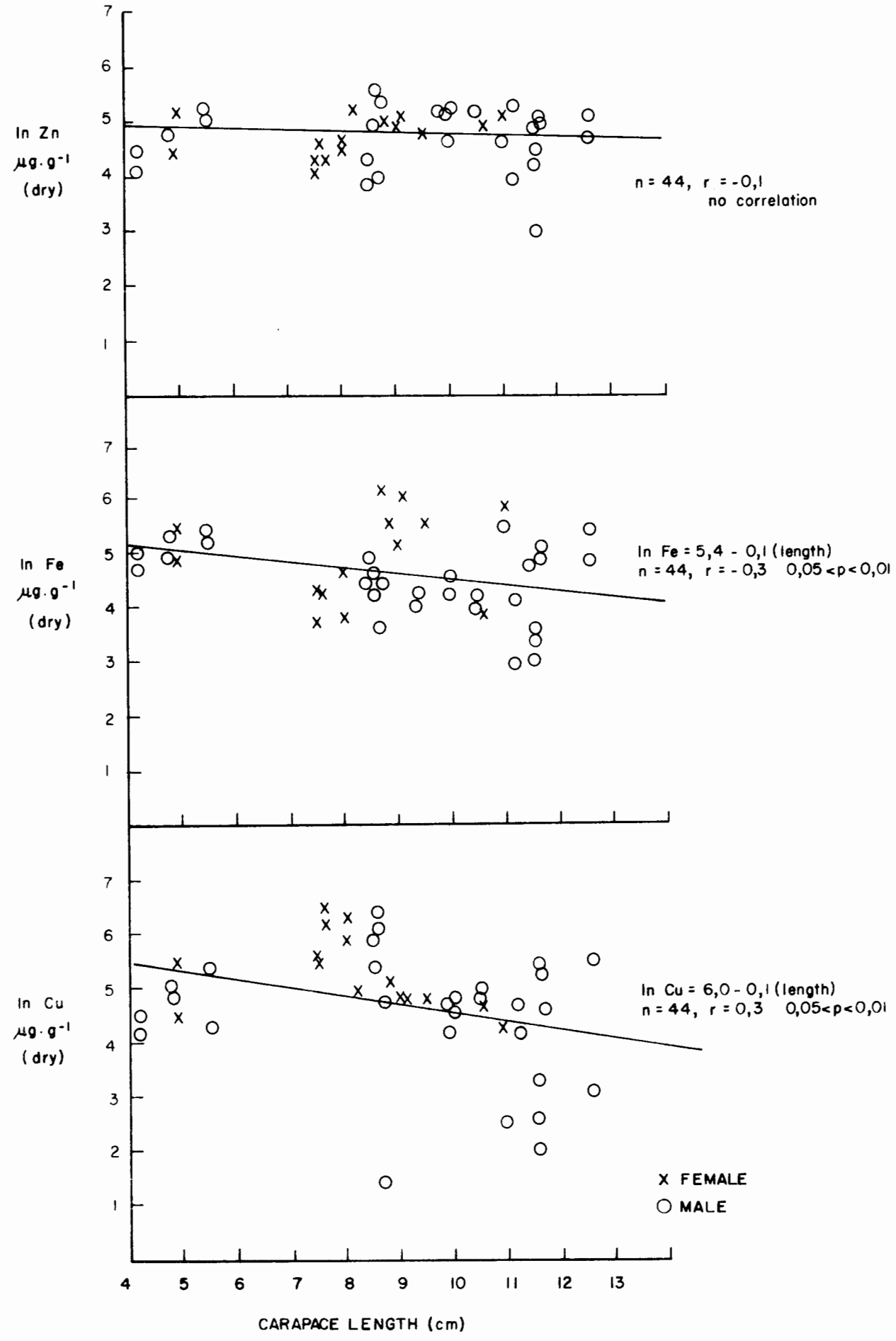


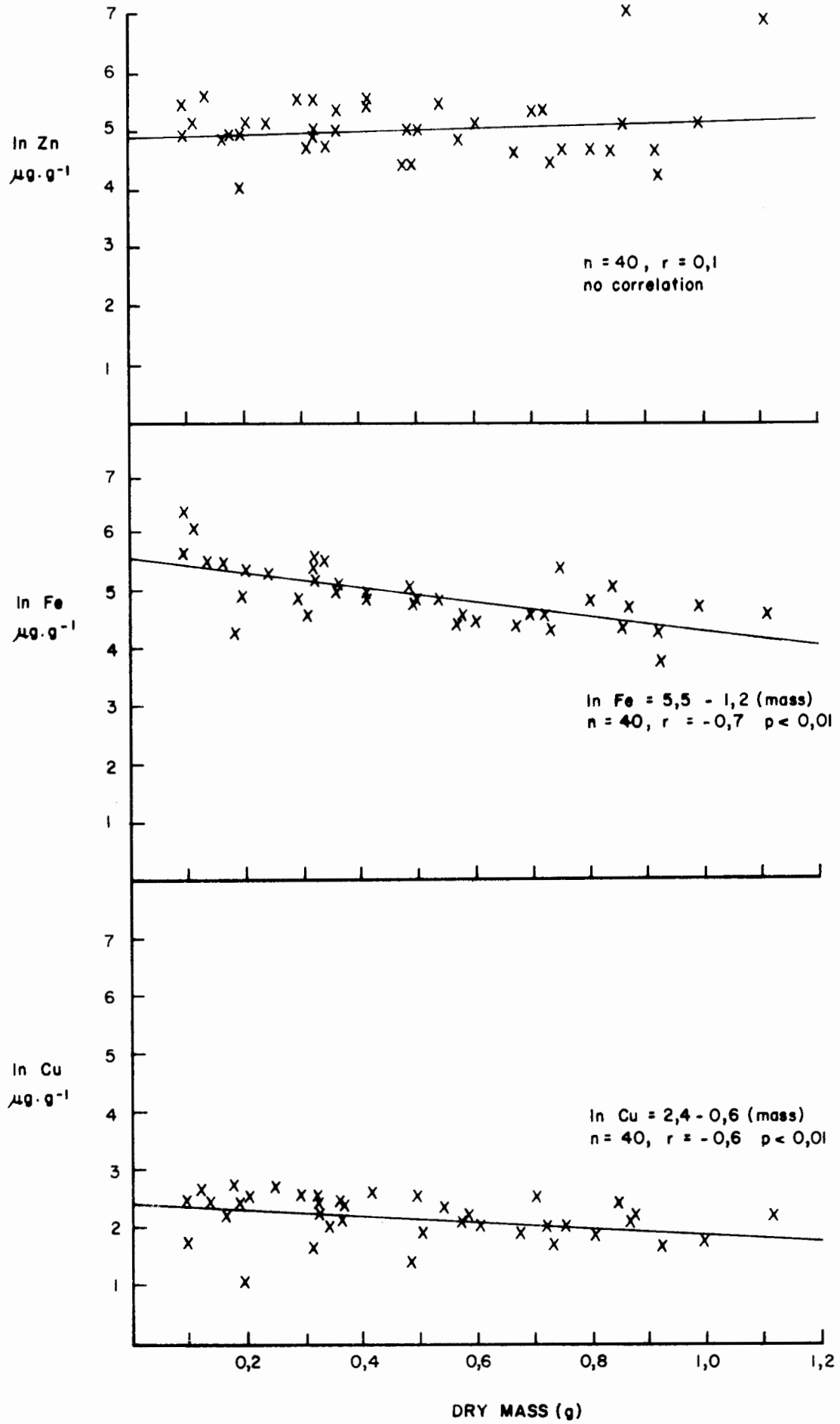
TRACED L de J CHECKED DATE 22/10 REF	**METAL CONCENTRATIONS IN ROCK LOBSTER GREEN GLAND**	FIGURE 2

NATIONAL RESEARCH INSTITUTE FOR OCEANOLOGY



TRACED : L de J CHECKED : CAG DATE 22/10 REF	METAL CONCENTRATIONS IN ROCK LOBSTER - GILLS	FIGURE 3

NATIONAL RESEARCH INSTITUTE FOR OCEANOLOGY

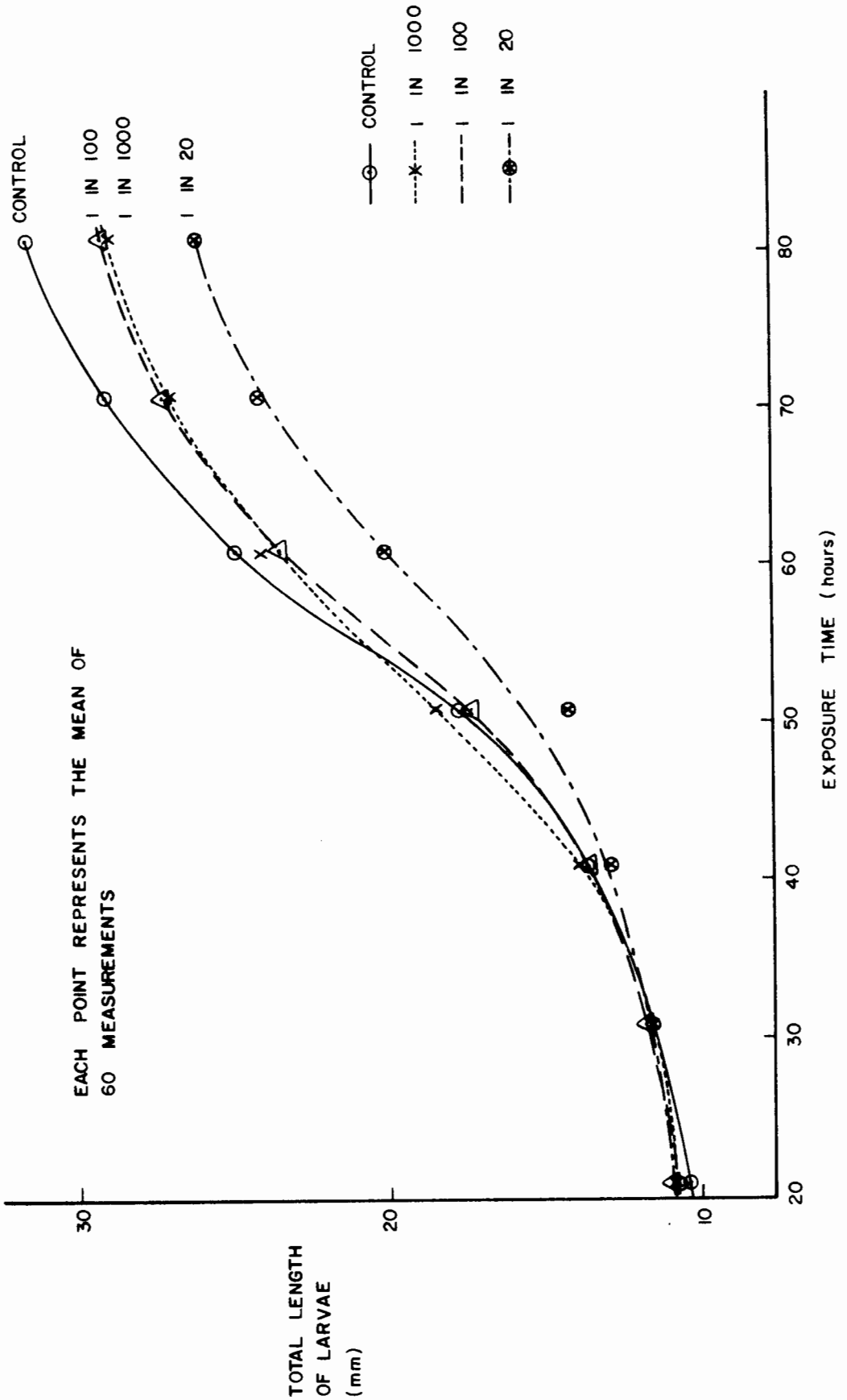


TRACED : L de J
CHECKED : [illegible]
DATE : 22/10
REF. :

METAL CONCENTRATIONS IN RIBBED MUSSELS

FIGURE 4

NATIONAL RESEARCH INSTITUTE FOR OCEANOLOGY

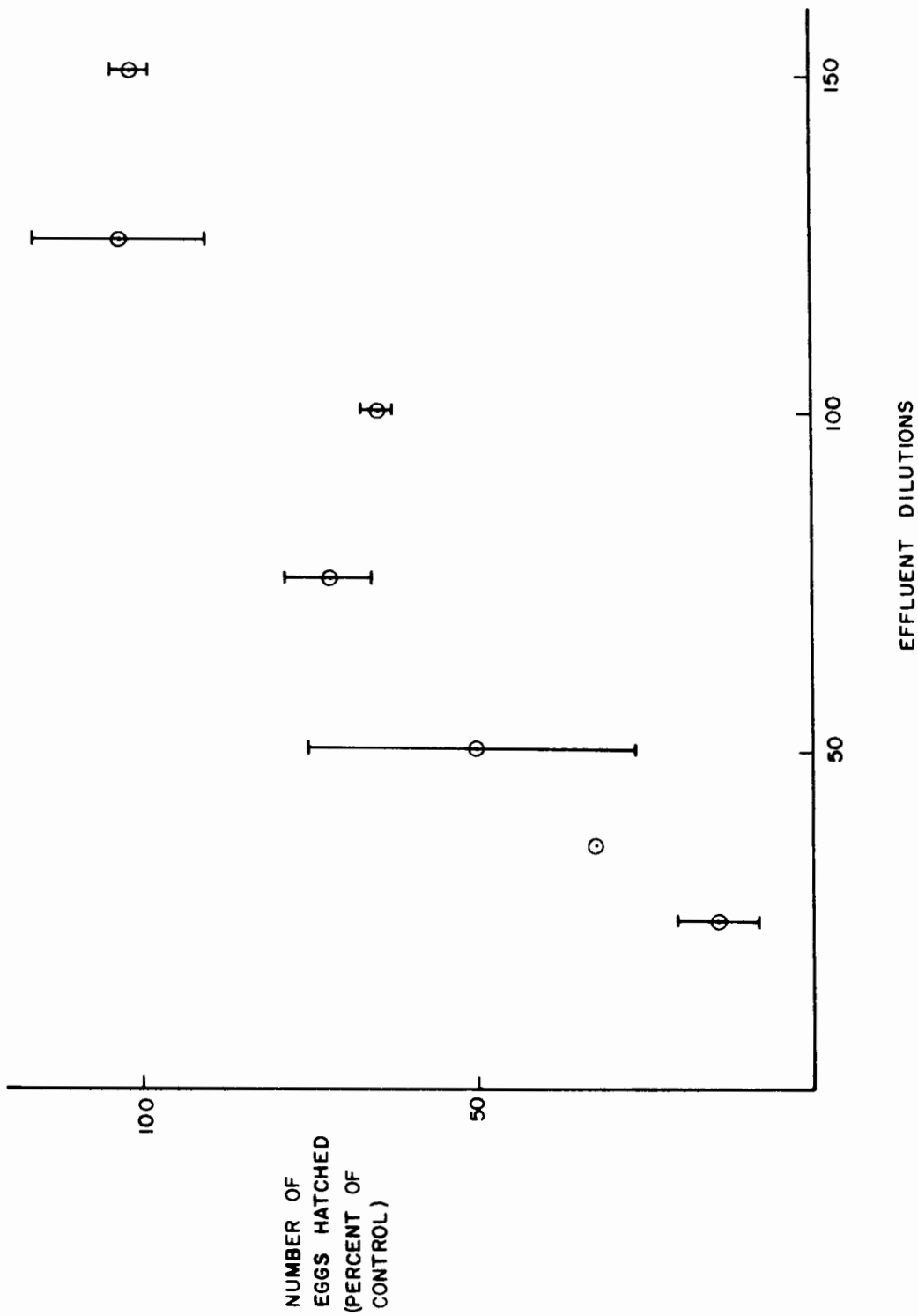


TRACED: L de J
CHECKED: RG
DATE: 22/10
REF:

GROWTH RATE OF *PARECHINUS* LARVAE

FIGURE
5

NATIONAL RESEARCH INSTITUTE FOR OCEANOLOGY



TRACED L de J CHECKED CB DATE 22/10 REF :	HATCHING SUCCESS OF *DASCYLLUS* EGGS	FIGURE 6

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APPENDIX 1

Concentrations of trace metals in rock lobsters from Noordwesbaai, ($\mu g.g^{-1}$ dry mass). (Each sample was analysed in duplicate, all readings are given).

Animal No.	Sex	Carapace length (cm)	Organ	Cd	Cu	Fe	Mn	Ni	Pb	Zn
1	F	8,03	Tail	n.d.	26,4	25,0	n.d.	1,4	3,5	64,6
				n.d.	18,1	19,3	n.d.	1,2	2,4	53,6
			Gills	22,2	531,9	44,2	1,4	2,8	8,3	101,4
				18,3	384,1	104,9	1,2	4,9	7,3	89,0
			Hepatic	7,0	38,9	80,3	4,5	2,3	1,6	468,0
				7,1	474,1	43,1	5,0	4,3	2,8	573,4
2	F	7,54	Tail	n.d.	50,0	30,0	n.d.	5,8	5,0	70,8
				n.d.	35,3	8,3	0,5	1,5	3,9	54,9
			Gills	15,9	238,6	75,0	n.d.	6,8	11,4	77,0
				11,5	265,4	40,4	n.d.	3,8	9,6	61,5
			Hepatic	3,8	166,2	91,9	4,8	2,4	115,2	4,3
3	F	7,66	Tail	n.d.	81,6	8,0	0,5	0,9	3,8	78,9
				n.d.	93,4	8,5	0,4	0,8	3,9	73,6
			Gills	17,5	700,0	67,5	n.d.	5,0	10,0	97,5
				13,2	505,9	70,6	n.d.	4,4	5,9	76,5
			Hepatic	8,0	405,4	79,8	4,9	4,0	n.d.	651,6
				5,9	308,4	65,5	3,6	2,1	1,7	480,0
4	M	11,23	Tail	0,2	17,6	11,9	1,1	n.d.	4,0	61,9
				0,3	22,8	20,4	1,4	2,2	5,2	81,8
			Gills	15,2	70,5	61,4	9,8	1,5	15,2	201,5
				6,1	112,1	18,2	n.d.	1,5	6,1	53,0
			Hepatic	1,2	38,6	33,1	3,0	4,8	2,4	30,1
5	M	8,74	Tail	0,3	45,3	9,9	n.d.	0,6	4,1	69,3
				n.d.	43,2	17,9	n.d.	0,7	4,6	62,5
			Gills	5,9	122,5	58,2	n.d.	2,0	7,8	57,8
				4,2	4,2	84,7	n.d.	4,2	18,1	236,1
			Hepatic	2,1	197,9	38,2	2,7	0,5	3,5	390,1
				3,2	283,8	44,6	4,0	0,7	3,2	530,9

Animal No.	Sex	Carapace length (cm)	Organ	Cd	Cu	Fe	Mn	Ni	Pb	Zn
6	M	8,62	Tail	n.d.	70,3	15,4	0,3	0,7	3,8	101,4
				3,0	95,5	18,7	n.d.	1,1	1,1	32,1
			Gills	14,1	471,7	67,4	2,2	5,4	7,6	142,4
				9,8	591,5	106,1	4,9	4,9	15,9	286,0
			Hepatic	1,4	184,7	55,1	3,1	0,9	2,7	387,5
				1,5	129,3	71,9	2,8	1,1	2,4	371,6
7	M	8,52	Tail	0,8	50,0	39,1	0,8	n.d.	5,5	97,7
				0,5	27,5	65,0	1,0	2,0	3,5	60,5
			Gills	20,0	384,4	131,11	5,6	2,2	7,8	73,3
				14,7	215,7	83,3	2,0	2,0	4,9	50,0
			Green gland	n.d.	34,4	112,5	43,8	3,1	31,3	62,5
				n.d.	35,9	79,4	n.d.	n.d.	8,8	41,2
8	M	10,01	Tail	1,3	40,3	20,8	n.d.	0,6	4,5	76,0
				2,3	55,5	18,0	n.d.	0,8	5,5	90,6
			Gills	14,5	102,7	67,3	n.d.	1,8	105,5	110,0
				21,9	126,6	87,5	n.d.	3,1	32,8	181,3
			Green gland	n.d.	111,1	205,6	n.d.	5,6	16,7	111,1
				n.d.	140,0	320,0	n.d.	20,0	10,0	130,0
9	M	10,54	Tail	0,7	28,4	18,9	0,7	0,7	3,4	70,9
				1,1	30,1	20,4	1,1	n.d.	4,3	66,1
			Gills	19,5	147,6	53,7	19,5	1,2	14,6	191,5
				21,4	128,6	71,4	8,9	1,8	14,3	180,4
			Green gland	n.d.	20,8	95,8	29,2	n.d.	33,3	62,5
				6,3	43,8	143,8	37,5	n.d.	25,0	137,5
10	F	4,89	Tail	n.d.	17,2	22,4	n.d.	n.d.	n.d.	37,9
				n.d.	67,1	51,2	1,2	1,2	6,1	140,2
			Gills	9,1	90,9	122,7	n.d.	n.d.	45,5	90,9
				16,7	241,7	233,3	n.d.	n.d.	50,0	175,0
			Green gland	n.d.	100,0	600,0	n.d.	25,0	125,0	325,0
				n.d.	99,1	1000,0	n.d.	50,0	25,0	325,0
11	M	5,49	Tail	n.d.	27,4	25,0	n.d.	n.d.	7,3	70,2
				n.d.	24,3	22,9	n.d.	0,7	4,4	62,9
			Gills	7,7	73,1	184,6	n.d.	n.d.	23,1	173,1
				9,1	231,9	236,4	n.d.	n.d.	36,4	209,1
			Green gland	n.d.	62,5	262,5	n.d.	n.d.	37,5	100,0
				n.d.	100,0	450,0	n.d.	25,0	n.d.	350,0

Animal No.	Sex	Carapace length (cm)	Organ	Cd	Cu	Fe	Mn	Ni	Pb	Zn
12	M	4,29	Tail	n.d.	24,1	36,2	n.d.	1,7	n.d.	51,7
				1,8	42,9	44,6	n.d.	n.d.	10,7	64,3
			Gills	5,6	88,9	161,1	n.d.	n.d.	22,2	88,9
				5,0	70,0	110,0	n.d.	n.d.	n.d.	60,0
			Green gland	n.d.	50,0	300,0	n.d.	n.d.	n.d.	133,3
				n.d.	200,0	1000,0	n.d.	n.d.	150,0	700,0
13	M	4,82	Tail	n.d.	50,0	30,7	n.d.	n.d.	3,4	61,4
				n.d.	46,8	35,5	n.d.	n.d.	1,6	35,5
			Gills	5,0	140,0	140,0	n.d.	n.d.	5,0	110,0
				8,3	158,3	200,0	n.d.	n.d.	8,3	125,0
			Green gland	n.d.	62,5	300,0	n.d.	50,0	n.d.	100,0
				n.d.	350,0	1150,0	n.d.	150,0	n.d.	700,0
14	M	9,91	Tail	4,1	33,2	9,3	1,1	1,5	4,1	74,6
				1,5	21,5	8,1	0,8	0,6	2,5	315,4
			Gills	21,4	68,6	61,4	8,6	n.d.	8,6	174,3
				26,7	121,1	64,4	8,9	3,3	7,8	187,8
			Green gland	5,3	36,8	57,9	13,2	7,9	n.d.	121,1
				7,7	46,2	138,5	19,2	11,5	n.d.	157,7
128	M	11,58	Tail	n.d.	21,4	25,6	n.d.	n.d.	6,0	91,1
				0,6	9,4	22,4	n.d.	n.d.	2,4	48,2
			Gills	0,8	13,6	32,2	n.d.	n.d.	3,4	69,5
				12,1	225,0	126,6	4,0	0,8	9,7	123,4
			Green gland	7,1	114,3	271,4	n.d.	n.d.	28,6	585,7
				9,1	122,7	81,8	4,5	n.d.	4,5	1581,8
129	M	11,74	Tail	0,5	5,8	17,4	n.d.	1,1	3,7	56,3
				0,7	31,4	31,6	n.d.	0,7	4,4	68,4
			Gills	53,1	101,6	156,3	3,1	9,4	20,3	178,1
				69,6	196,4	141,1	8,9	5,4	23,2	153,6
			Green gland	4,2	12,5	141,7	29,2	n.d.	12,5	66,7
				2,9	58,8	135,3	35,3	5,9	29,4	61,8
130	M	11,58	Tail	0,6	6,7	15,7	n.d.	n.d.	3,4	21,3
			Gills	0,9	7,5	31,1	8,5	0,9	n.d.	87,7
			Green gland	3,8	53,8	73,1	57,7	3,8	34,6	80,8
				n.d.	n.d.	119,2	n.d.	11,5	3,8	30,8

Animal No.	Sex	Carapace length (cm)	Organ	Cd	Cu	Fe	Mn	Ni	Pb	Zn
131	M	12,58	Tail	4,8	36,9	165,5	1,2	2,4	11,9	158,3
			Gills	5,0	27,7	20,3	0,8	0,2	3,3	20,8
			Green gland	5,3	21,1	86,8	2,6	n.d.	18,4	31,6
				6,3	28,1	87,5	34,4	n.d.	50,0	90,6
132	M	10,06	Tail	0,4	6,2	3,5	n.d.	0,4	3,1	22,1
			Gills	35,9	235,9	223,1	6,4	2,6	29,5	165,4
			Green gland	1,2	9,3	51,2	5,8	1,2	7,0	19,8
				3,1	21,9	118,8	15,6	3,1	12,5	50,0
133	M	12,09	Tail	0,7	11,1	6,3	n.d.	0,7	5,6	39,6
			Gills	32,5	22,2	110,4	3,5	0,7	9,7	116,7
			Green gland	1,9	20,4	75,9	14,8	1,9	18,5	53,7
				1,9	30,8	69,2	15,4	n.d.	19,2	53,8
134	M	11,03	Tail	n.d.	31,5	38,9	0,9	0,9	7,4	136,1
			Gills	0,9	13,0	225,0	n.d.	6,5	22,3	109,3
			Green gland	1,9	24,1	79,6	29,6	n.d.	14,8	57,4
				1,9	21,2	65,4	25,0	n.d.	13,5	59,6
136	F	9,15	Tail	0,7	39,7	17,8	3,4	1,4	2,1	75,3
			Gills	61,4	125,0	400,0	4,5	11,4	27,3	163,6
			Green gland	3,8	73,1	46,2	7,7	11,5	3,8	26,9
				4,2	108,3	91,7	8,3	8,3	20,8	54,2
137	F	9,52	Tail	0,8	7,9	n.d.	n.d.	1,6	4,8	22,2
			Gills	19,2	125,0	238,5	3,8	5,8	23,1	126,9
			Green gland	4,5	36,4	77,3	9,1	4,5	31,8	45,5
				6,3	43,8	137,5	12,5	18,8	31,3	50,0
138	F	8,79	Tail	0,8	45,8	16,9	n.d.	2,5	5,9	61,0
			Gills	26,4	168,1	245,8	2,8	6,9	23,6	154,2
			Green gland	5,0	60,0	80,0	5,0	5,0	25,0	40,0
				3,8	61,5	34,6	3,8	3,8	19,2	38,5
139	F	8,20	Gills	18,0	142,0	450,0	2,0	8,0	34,0	180,0
			Green gland	7,1	28,6	85,7	n.d.	14,3	21,4	42,9
				8,3	75,0	158,3	n.d.	25,0	41,7	58,3

Animal No.	Sex	Carapace length (cm)	Organ	Cd	Cu	Fe	Mn	Ni	Pb	Zn
140	F	11,01	Tail	0,7	2,1	1,4	n.d.	0,7	7,6	14,6
			Gills	24,4	78,0	322,0	2,4	3,7	44,2	172,0
			Green gland	3,8	19,2	53,8	19,2	3,8	34,6	53,8
				5,6	27,8	100,0	33,3	5,6	55,6	66,7
141	F	10,66	Tail	1,3	18,1	2,5	n.d.	0,6	5,6	47,5
			Gills	12,8	112,8	46,2	n.d.	2,6	14,1	134,6
			Green gland	4,2	33,3	62,5	41,7	12,5	33,3	58,3
				4,2	27,2	87,5	41,7	4,2	25,0	58,3
142	F	9,03	Tail	n.d.	26,9	15,7	1,9	1,9	6,5	64,8
			Gills	16,7	122,7	166,7	4,5	4,5	19,7	137,9
			Green gland	n.d.	12,5	168,8	18,8	12,5	12,5	43,8
				n.d.	25,0	50,0	16,7	8,3	33,3	58,3
135 (moulted)	F	11,95	Gills	2,9	47,1	600,0	n.d.	11,8	55,9	314,7
				2,8	48,3	657,4	0,9	9,8	54,7	355,8
			Stomach	4,2	47,5	162,5	33,3	4,2	33,3	550,0
			Shell lining	2,5	90,0	314,0	3,8	43,8	36,3	277,8

APPENDIX 2

Concentrations of trace metals in ribbed mussels from Noordwesbaai, ($\mu g.g^{-1}$ dry mass)

Animal No.	Dry mass (g)	Cd	Cu	Fe	Mn	Ni	Pb	Zn
64	0,80	8,1	6,3	111,3	3,8	3,1	8,8	100,6
65	0,70	7,9	12,1	100,0	7,9	2,9	5,7	202,9
66	0,73	10,3	5,5	84,2	1,4	2,7	8,2	81,5
67	0,36	6,9	11,1	130,6	12,5	4,2	5,6	201,4
68	0,41	7,3	13,4	124,4	7,3	3,7	8,5	250,0
69	0,32	9,4	12,5	203,1	9,4	6,3	7,8	256,3
70	0,84	5,3	11,1	141,8	1,0	1,4	5,3	97,2
71	0,75	6,0	7,3	201,3	17,3	5,3	10,7	94,7
72	0,60	8,3	7,5	77,5	5,0	1,7	8,3	156,7
73	0,50	14,0	7,0	117,0	1,0	4,0	12,0	144,0
74	0,09	16,7	5,6	561,1	n.d.	22,2	33,3	133,3
75	0,57	9,6	7,9	84,2	1,8	2,6	14,0	120,2
76	0,29	5,2	12,1	127,6	3,4	5,2	17,2	236,2
77	0,67	6,0	6,7	71,6	2,2	3,0	11,2	94,8
78	0,92	5,4	1,1	41,8	n.d.	0,5	5,4	96,2
103	0,17	11,8	14,7	208,8	2,9	n.d.	14,7	135,3
104	0,11	18,2	13,6	400,0	n.d.	n.d.	22,7	159,1
105	0,36	15,3	11,1	129,2	4,2	1,4	11,1	230,6
106	0,87	15,5	8,6	100,6	2,3	1,1	6,9	1086,2
107	0,72	18,1	7,6	94,4	6,3	0,7	6,9	205,6
108	0,31	11,3	4,8	88,7	n.d.	n.d.	6,5	114,5
109	0,34	5,9	7,4	214,7	2,9	n.d.	11,8	111,8
110	0,48	5,2	4,2	152,1	2,1	n.d.	4,2	79,2
111	0,99	22,7	6,1	97,0	3,5	n.d.	7,6	158,1
112	0,92	4,9	5,4	70,1	2,2	0,5	6,5	66,8
113	0,86	16,9	8,1	84,3	2,9	0,6	8,1	164,5
114	1,11	26,6	9,0	91,0	5,4	0,9	5,9	955,9
115	0,13	26,9	11,5	211,5	n.d.	n.d.	11,5	273,1
116	0,54	19,4	10,2	125,0	1,9	2,8	9,3	220,4
117	0,24	8,3	14,6	189,6	4,2	2,1	16,7	166,7
118	0,49	10,2	12,2	105,1	5,1	2,0	8,2	81,6
119	0,58	11,2	8,6	92,2	1,7	n.d.	9,5	149,1

Animal No.	Dry mass (g)	Cd	Cu	Fe	Mn	Ni	Pb	Zn
120	0,36	33,3	8,3	154,2	2,8	4,2	8,3	150,0
121	0,32	17,2	10,9	165,6	3,1	6,3	10,9	153,1
122	0,20	25,0	12,5	200,0	n.d.	2,5	12,5	162,5
123	0,16	12,5	9,4	215,6	n.d.	6,3	12,5	121,9
124	0,09	16,7	11,1	283,3	n.d.	n.d.	22,2	216,7
125	0,32	17,2	9,4	232,8	1,6	4,7	10,9	135,9
126	0,19	5,3	2,6	126,3	n.d.	2,6	7,9	55,3
127	0,18	8,3	11,1	66,7	n.d.	5,6	13,9	136,1

APPENDIX 3

Concentrations of trace metals in rock lobsters treated with effluent.

Volume effluent added to 10 dm^3 aquarium (cm^3)	Time of exposure (days)	Sex	Carapace length (cm)	Organ	Cd	Cu	Fe	Mn	Ni	Pb	Zn
2	19	M	7,86	Tail	n.d.	11,4	1,4	1,4	1,4	11,4	34,3
				Gills	9,3	138,9	92,6	11,1	5,6	18,5	116,7
				Green gland	n.d.	75,0	375,0	125,0	50,0	125,0	100,0
					n.d.	25,0	262,5	62,5	25,0	50,0	50,0
	30	F	7,48	Tail	n.d.	10,3	6,4	n.d.	n.d.	6,4	26,9
				Gills	10,6	227,3	89,4	10,6	3,0	13,6	130,3
				Green gland	n.d.	22,7	22,7	13,6	n.d.	13,6	18,2
					n.d.	25,0	35,0	15,0	n.d.	15,0	25,0
	45	M	8,13	Tail	n.d.	10,0	17,1	n.d.	1,4	10,0	57,1
				Gills	8,0	188,0	64,0	6,0	6,0	20,0	106,0
				Green gland	n.d.	63,6	54,5	22,7	9,1	18,2	45,5
					n.d.	55,6	155,6	27,8	16,7	33,3	72,2
		M	6,58	Tail	n.d.	27,8	22,2	n.d.	1,9	14,8	46,3
				Gills	5,6	21,67	92,6	5,6	5,6	16,7	124,1
				Green gland	n.d.	100,0	900,0	50,0	150,0	200,0	150,0
					n.d.	33,3	216,7	16,7	50,0	66,7	50,0

Volume effluent added to 10 dm^3 aquarium (cm^3)	Time of exposure (days)	Sex	Carapace length (cm)	Organ	Cd	Cu	Fe	Mn	Ni	Pb	Zn
2	60	F	8,14	Tail	n.d.	45,7	26,1	2,2	3,3	5,4	59,8
				Gills	31,0	275,9	91,4	5,2	5,2	10,3	136,2
				Green gland	n.d.	60,0	85,0	5,0	5,0	15,0	35,0
					n.d.	61,1	127,8	11,1	11,1	22,2	66,7
		F	7,87	Tail	n.d.	83,8	25,7	1,4	4,1	4,1	63,5
				Gills	30,4	216,1	58,9	3,6	5,4	14,3	139,3
				Green gland	n.d.	116,7	158,3	16,7	8,3	41,7	83,3
					n.d.	160,0	170,0	20,0	10,0	50,0	130,0
10	19	F	7,96	Tail	n.d.	10,0	1,8	n.d.	0,9	5,5	28,2
				Gills	21,3	270,2	163,8	11,7	7,4	7,4	137,2
				Green gland	n.d.	50,0	56,3	25,0	n.d.	25,0	37,5
					6,3	75,0	137,5	31,3	12,5	25,0	56,3
	30	F	8,22	Tail	0,8	10,8	2,5	n.d.	n.d.	5,8	20,0
				Gills	31,7	141,5	202,4	12,2	4,9	15,9	135,4
				Green gland	n.d.	25,0	70,0	10,0	5,0	15,0	20,0
					n.d.	31,3	156,3	12,5	18,8	18,8	37,5
	45	F	8,04	Tail	1,1	60,7	8,7	1,1	2,2	7,6	56,5
				Gills	25,0	260,0	67,5	6,3	8,8	13,8	138,8
				Green gland	n.d.	91,7	175,0	16,7	16,7	50,0	66,7
					n.d.	87,5	200,0	25,0	25,0	50,0	100,0

Volume effluent added to 10 dm^3 aquarium (cm^3)	Time of exposure (days)	Sex	Carapace length (cm)	Organ	Cd	Cu	Fe	Mn	Ni	Pb	Zn
10	45	F	7,26	Tail	1,6	16,1	17,7	n.d.	4,8	9,7	33,9
				Gills	21,1	284,2	118,4	5,3	13,2	18,4	173,7
				Green gland	n.d.	150,0	900,0	50,0	150,0	100,0	100,0
					n.d.	62,5	225,0	12,5	37,5	37,5	50,0
	60	F	8,71	Tail	n.d.	20,7	13,0	n.d.	1,1	5,4	44,6
				Gills	34,9	252,3	72,1	5,8	5,8	11,6	144,2
				Green gland	n.d.	158,3	150,0	16,7	8,3	33,3	91,7
					n.d.	94,4	66,7	11,1	11,1	38,9	50,0
			8,39	Tail	n.d.	79,8	27,7	2,1	3,2	8,5	76,6
				Gills	17,1	401,3	80,3	6,6	3,9	10,5	135,5
				Green gland	n.d.	125,0	150,0	8,3	8,3	50,0	75,0
					n.d.	114,3	107,1	14,3	7,1	42,9	71,4
100	3	F	7,21	Tail	1,6	37,9	12,1	0,8	n.d.	2,3	60,5
					1,0	31,0	17,1	1,0	n.d.	2,4	62,9
				Gills	31,3	218,8	175,0	3,1	12,5	9,4	240,6
					25,0	122,2	186,1	2,8	16,7	8,3	194,4
				Green gland	n.d.	66,7	433,3	n.d.	66,7	n.d.	133,3
					16,7	83,3	500,0	n.d.	66,7	n.d.	133,3

Volume effluent added to 10 dm^3 aquarium (cm^3)	Time of exposure (days)	Sex	Carapace length (cm)	Organ	Cd	Cu	Fe	Mn	Ni	Pb	Zn
100	30	M	9,71	Tail	n.d.	41,5	24,5	1,9	2,8	3,8	56,6
				Gills	13,0	219,6	40,2	5,4	9,8	7,6	113,0
				Green gland	n.d.	27,3	63,6	13,6	9,1	9,1	31,8
					n.d.	20,0	60,0	10,0	3,3	13,3	26,7
	45	M	6,91	Tail	n.d.	21,2	13,5	n.d.	n.d.	9,6	46,2
				Gills	10,0	276,0	100,0	12,0	20,0	18,0	188,0
				Green gland	n.d.	100,0	266,7	83,3	16,7	50,0	83,3
					n.d.	100,0	500,0	125,0	75,0	75,0	325,0
		F	7,99	Tail	1,0	21,7	6,3	2,1	2,1	5,2	39,6
				Gills	26,4	264,2	59,4	12,3	15,1	11,3	141,5
				Green gland	7,1	92,9	71,4	42,9	14,3	35,7	64,3
					n.d.	31,3	37,5	25,0	12,5	31,3	43,8
	60	M	8,54	Tail	n.d.	28,6	9,2	2,0	2,0	8,2	41,8
				Gills	8,9	169,6	58,9	8,9	10,7	14,3	117,9
				Green gland	n.d.	41,7	141,7	33,3	8,3	50,0	66,7
					n.d.	44,4	61,1	27,8	11,1	33,3	55,6
		M	6,04	Tail	n.d.	64,3	50,0	4,8	4,8	7,1	71,4
				Gills	4,5	259,1	104,5	6,8	15,7	18,2	177,3
				Green gland	n.d.	200,0	1600,0	100,0	100,0	200,0	350,0
					n.d.	250,0	1000,0	50,0	150,0	200,0	500,0