THE USE OF BIOLOGICAL INDICATOR ORGANISMS TO MONITOR TRACE METAL POLLUTION IN MARINE AND ESTUARINE ENVIRONMENTS—A REVIEW

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ABSTRACT

The elucidation of the comparative pollution of aquatic areas by trace metals is theoretically possible by the analysis of water, sediments, or a member of the indigenous biota. The use of water analysis is expensive and laborious; multiple sampling must be undertaken to eliminate variations in metal concentration with time, season, freshwater run-off, currents, tides and other factors. The use of sediments is also subject to some error, according to local variations in sedimentation rates of particulate material and in the amounts of organic material present; in addition it gives little direct information on the amounts of metal entering the biomass of a given area.

The use of biological indicator organisms to define areas of trace metal pollution appears most attractive, as these organisms not only concentrate metals from water, allowing inexpensive and relatively simple analysis, but they may also represent a moving time-averaged value for the relative biological availability of metals at each site studied. However, the use of indicator organisms introduces biological variables which are not present in physico-chemical studies of water or sediments. These variables merit consideration inasmuch as they may affect the results of indicator surveys for trace metals. In addition, different indicator organisms measure different portions of the total trace metal load on an ecosystem.

The present state of knowledge on the use of indicator organisms to study trace metal pollution is reviewed, with particular reference to the use of macroalgae, bivalve molluscs and teleosts. It is suggested that the macroalgae and bivalve molluscs are the most efficient and reliable indicators developed to the present time. It is further suggested that the effects of sampling and environmental variables have been largely overlooked, and that further study in the field and in the laboratory is necessary before the results of surveys using biological indicator organisms can be relied upon.

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1. INTRODUCTION

The elucidation of the comparative trace metal pollution of coastal areas throughout the world must be attempted in order to allow the realistic planning of future industries and to minimise the impact of man on the environment. A knowledge of the relative abundance of trace metals in different areas would also allow comparative laboratory studies of metal toxicities on the biota of these areas, leading to the prediction of the ecological effects of metal pollution and affording a chance to relate laboratory studies to the environment.

The pollution of coastal areas by trace metals can be studied by the analysis of trace metals in water, sediments, or some member of the indigenous biota common to all regions. Because of the inadequate knowledge of trace metal cycling in the ecosphere at the present time, any one of these approaches may be criticised. The choice of study method must also take into account the applicability of the final method to other areas, expense, instrumentation and personnel needed, and so on.

This paper reviews the rapidly-expanding literature on the identification of pollution by trace metals in an attempt to define the most profitable methods of study.

2. SURVEYS OF TRACE METAL ABUNDANCE USING WATER, SEDIMENTS, OR BIOLOGICAL INDICATOR ORGANISMS

A. Water

Many authors have reported data specifically concerning the concentrations of trace metals in water from open ocean areas, nearshore or coastal areas or estuaries. Some data on the concentrations of metals in water are also found in studies concerned with the uptake of metals by biota. Some representative values for total or soluble concentrations of metals in water are shown in Table 1.

Trace metals in water exist 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 assign to either soluble or particulate fractions. The assignment of metals in water to soluble and particulate fractions is in any case somewhat arbitrary, being based on whether the metal passes through, or is retained by, a filter of known pore size. Pore sizes of the filters used differ from author to author, making the comparison of results difficult; however, a pore diameter of 0·45μ is most frequent. The proportions and absolute amounts of metal in each fraction vary with the metal considered, the particulate content and its nature and the time and location of sampling (Table 1: see also Spencer & Sachs, 1970; Raymont, 1972; Skei *et al.*, 1973; Price & Skei, 1975). In general, concentrations of metals are high in both soluble and particulate fractions of polluted freshwater outfalls. As this freshwater mixes with the receiving salt water at the estuary, metals

may be lost from the soluble fraction to the sediments by precipitation, or to phytoplankton by adsorption. On further mixing in the estuary, metals adsorbed to inorganic particulate material may be lost to sediments by settling in the less turbulent areas. Thus the net result of estuarine freshwater–saltwater mixing is generally a decrease in metal concentrations in the soluble fraction and a shift of high metal concentrations in the particulate fraction from mostly inorganic-associated in freshwater to mostly organic-associated (phytoplankton) in saltwater.

Superimposed on this general pattern is an increase of total metal concentrations in all phases of water with decreasing sampling distance from land-based sources of metals (Table 1). For example, Chester & Stoner (1974) found small increases in concentrations of metals in samples taken from waters closer than 400 km to a land mass over those in samples from areas more than 400 km distant from a land mass. Preston *et al.* (1972) reported rather higher values for metal concentrations in seas bordering the British Isles, and Abdullah & Royle (1974) reported even greater increases in total metal concentrations in water with distance up the Bristol Channel in England, towards the known sources of metal pollution.

The identification of polluted areas by the analysis of metal concentrations in water has certain disadvantages, however. Analysis of the low metal concentrations found in most water samples (Table 1) requires the pre-concentration of large volumes of water, either by organic extraction (Brooks *et al.*, 1967) or by resin chelation techniques (Riley & Taylor, 1968). This is expensive, laborious, and the number of steps involved increases the possibility of either positive or negative contamination. In addition, although a knowledge of the total (or soluble) concentrations of metals in water may allow the prediction of possible toxicological effects on biota, this is not always the case. The presence of humic acid or other complexing or chelating agents may render some of the metal unavailable to the biota, thus causing over-estimation of metal toxicities based on consideration of the total metal concentrations in water (Butler & Tibbits, 1972; Pagenkopf *et al.*, 1974). The toxicity of particulate-adsorbed metals is also difficult to assess (e.g. compare Lloyd, 1960 and Herbert & Wakeford, 1964, with Sprague, 1964). The form of the metal in water, i.e. the ion or ions present, may also affect the availability of the metal for uptake by biota, or may affect its toxicity in some other way. As most studies of metal pollution are orientated towards the prediction of ecosystem or human health effects, this inability to predict the metal availability to biota would appear to be a great disadvantage to the analysis of water for trace metals.

Perhaps however, the greatest disadvantage of water analyses as a means of comparing locations for their degree of metal pollution, is the large variation in concentrations of metals encountered in water with differences in season, time of day, the extent of freshwater run-off, depth of sampling, the intermittent flow of industrial effluent and hydrological factors such as tides and currents. The interacting effects of these variables may lead to as much as a 10-fold variation in the concentrations of any one metal encountered at any one location; this variation is

TABLE 1

SELECTED VALUES FOR THE CONCENTRATIONS OF CERTAIN TRACE METALS IN OPEN OCEANS, NEARSHORE WATERS, AND ESTUARINE AREAS. ALL CONCENTRATIONS IN μG/LITRE (PARTS PER BILLION). CONCENTRATIONS GIVEN ARE TOTAL (SOLUBLE PLUS PARTICULATE) OR SOLUBLE ONLY, WHERE THE LATTER IS QUOTED

Authors	*Locations*		*Zn*	*Cd*	*Pb*	*Cu*	*Fe*	*Mn*	*Ni*	*Co*	*Cr*	*Hg*	*Ag*
A. OPEN OCEANS													
Goldberg (1965)	'Average seawater'		10	0·11	0·03	3							
Pytkowicz & Kester (1971)	Review of literature	Range:	1–50	0·02–0·25	0·02–0·4	0·2–27	0·03–62	0·2–8·6	0·13–43	0·005–4·1	0·04–0·043		0·055–1·5
		Mean:	6	0·11	0·05	1	3	1·2	3·5	0·1	0·15	0·03	0·15
Goldberg (1972)	'Baseline'		3	0·02		2		0·3	2				
Leatherland *et al.* (1973)	NE Atlantic											0·03–0·09	
Preston *et al.* (1972)	NE Atlantic ('area 4a')		1·4–7·0	0·01–0·41		0·05–0·80		0·03–0·09	0·29–0·66				
Chester & Stoner (1974)	World Oceans (nearshore)	Range:	0·6–12·6	0·04–0·30		0·3–3·8	0·2–4·8	0·12–2·0	0·5–5·2				
		Mean:	2·4	0·09		0·9	1·2	0·37	1·8				
	(open ocean)	Range:	0·3–3·0	0·02–0·17		0·1–3·9	0·5–4·1	0·07–0·37	0·3–3·4				
		Mean:	1·4	0·07		0·8	1·4	0·22	1·2				
Gardner (1975)	World Oceans	Overall range:										0·005–0·08	
		Mean, N. Hemisphere:										0·033	
		Mean, S. Hemisphere:										0·011	
B. NEARSHORE WATERS													
Mullin & Riley (1956)	Irish Sea	Range:		0·03–0·25		8·7–17·3							
		Mean:		0·11		13·7							
	English Channel	Range:		0·02–0·26									
		Mean:		0·14									
Loveridge *et al.* (1960)					1·0								
Soyer (1963)													0·15
Brooks *et al.* (1967)	S. California, USA		1·1–14·1		0·6–5·2	1·6–3·0	1·9–14·6		1·0–1·6	0·1–1·2			
Fukai & Vas (1967)											0·4		
			4	0·06		5			4	0·1			
Spencer & Brewer (1969)	Gulf of Maine, USA		3·1–6·9			1·2–4·0			1·3–2·0				
Butler & Tibbits (1972)	English Channel						<2–>5						
Preston *et al.* (1972)	5 areas, UK coast		0·8–20·0	0·01–0·62	0·05–1·2	0·18–3·7	0·03–2·3	0·02–14·6	0·16–22·9				
Bryan (1973)	English Channel		2·0	0·06	0·17	0·46	0·3	0·32	0·38				
Dutton *et al.* (1973)	Straits of Dover		12	1·6		2							
	North Sea		1·6	6·2		2·8	18						
Knauer & Martin (1973)	Monterey Bay, California, USA		6·5	0·15	0·9	1·3		0·6					
	Hawaii-California transect, USA		2·0	0·02		0·8		0·2					
Fukai & Huynh-Ngoc (1976)	NW Mediterranean		0·1–11·2	0·01–0·8		0·1–22·4							

TABLE 1—*contd.*

Authors	*Locations*	*Zn*	*Cd*	*Pb*	*Cu*	*Fe*	*Mn*	*Ni*	*Co*	*Cr*	*Hg*	*Ag*
C. ESTUARINE AREAS												
Chipman *et al.* (1958)	Beaufort, N.C., USA	2·8–14·6										
Bryan (1969)	Coast of UK	1·2-26			0·4–5·9		1–23·8					
Elderfield *et al.* (1971)	Conway Estuary, UK	9	0·8		3·0							
Abdullah *et al.* (1972)	Liverpool Bay, UK	11·9	0·3	1·7	1·5							
	Cardigan Bay, UK	7·5	1·1	2·2	1·7							
	Bristol Channel, UK	10·0	1·1	1·2	2·1							
Butterworth *et al.* (1972)	Severn Estuary, UK	12–52	0·3–5·8	0·4–2·5	0·4–2·5							
Preston *et al.* (1972)	Irish Sea shoreline	3·8–49·1	0·03–1·43	0·6–2·9	0·9–3·1	2·5–24·7	0·7–25·5	0·9–9·8				0·2–0·24
Andersen *et al.* (1973)	Inner Oslofjord, Norway	40·6–72·5	0·67–0·92	1·0–2·8	8·4–10·4							
Bryan & Hummerstone (1973*a*)	Tamar Estuary, UK (Geometric mean)	4·1			2·7	2·7	21					
	Restronguet Creek, UK (Geometric mean)	113			11	0·9	28					
Halerow *et al.* (1973)	Clyde Estuary, UK	6–25	0·2–1·2	0·5–19·0	0·5–5·0	5·3–24·0	0·5–11·0	0·8–5·0				
Ireland (1973)	Cardigan Bay, UK	40–88										
Abdullah & Royle (1974)	Bristol Channel, UK	3–44	0·4–9·4	0·3–13·0	0·6–5·4	1·5–10·5	0·4–5·9	0·2–3·0				
Boyden & Romeril (1974)	Bristol Channel, UK	199–320	1·8–7·5	60–237	16·5–43	2410–9000	215–855					
Chan *et al.* (1974)	Hong Kong	30–810	10–100	40–1410	20–160	170–8900						
Hall & Valente (1974)	Lourenço Marques Bay				1–5	1–34						
Holmes *et al.* (1974)	Texas coast, USA	< 50– > 400	< 5– > 50									
Morris (1974)	Menai Straits, UK	5–50			0·5–3·0		1·–16	0·8–2·5				
Stenner & Nickless (1974*a*)	Hardangerfjord, Norway	27–3560	0·01–85	2·9–12·7	3·7–58		0–6·2	1·2–3·7				
	Skjerstadfjord, Norway	14–109	0·01–0·3	1·6–27·4	5·2–77		0·6–16·9	1·2–5·6				
Boyden (1975)	Poole Harbour, UK	2–68	0·1–7·4		1–18	2–47	5–34	3–30				
Darracott & Watling (1975)	Poole Estuary, UK	26	0·8		6							
Stenner & Nickless (1975)	Atlantic coast of Spain and Portugal	17–525	0·08–6·0	2·3–38·0	1·9–107		< 0·9–108	1·3–10·8				
Saenko *et al.* (1976)	Vostok Bay, Sea of Japan	66·6			2·5–2·7	119–375	6·4–16·6		5–7·2	0·002		

particularly evident in estuarine areas (Spencer & Brewer, 1969; Brewer *et al.*, 1972; Abdullah & Royle, 1974; Morris, 1974; Boyden, 1975). As time-integrated water sampling equipment is not readily available, the sample number at each site must be increased greatly to allow the estimation of average metal concentrations in water.

It may be possible to avoid these disadvantages by the use of biological indicator organisms. Not only is biologically-available metal then measured directly, but time-integrated estimates of available metal concentrations in water are also possible using some organisms.

B. Sediments

The use of sediments to define areas of trace metal pollution has been reported in several studies (Table 2, Fig. 1). Some of these studies are concerned with the effects of the disposal of sewage sludge (Fig. 1); others, however, are aimed principally at defining areas of industrially-derived metal pollution.

Variation of the trace metal concentrations found in sediments occurs according to the rate of trace metal deposition, the rate of particle sedimentation, the particle size and nature and the presence or absence of organic material. Most studies employ methods designed to digest the sediment samples completely, and results

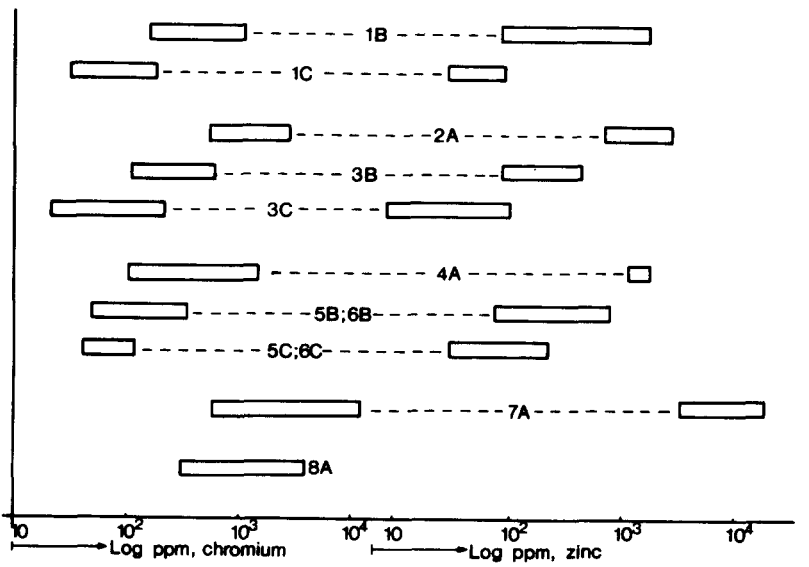

Fig. 1. Concentrations of chromium and zinc (μg/g dry weight) in sewage sludge (A), sediments from sludge disposal areas (B), and sediments from reference areas (C). Authors cited: 1. Papakostidis *et al.* (1975); 2. Gross (1971); 3. Gross (1972); 4. Topping & McIntyre (1972); 5. Steele *et al.* (1973); 6. MacKay *et al.* (1972); 7. Van Loon *et al.* (1973); 8. Weaver *et al.* (1974). From Papakostidis *et al.* (1975).

TABLE 2

CONCENTRATIONS OF SELECTED TRACE METALS IN SEDIMENTS. ALL FIGURES REFER TO μG/G (PARTS PER MILLION) IN DRY SEDIMENT, EXCEPT Fe WHICH IS WT %. SEE ALSO FIG. 1.

Authors	*Locations*	*Zn*	*Cd*	*Pb*	*Cu*	*Fe*	*Mn*	*Ni*	*Co*	*Cr*	*Hg*	*Ag*
Mullin & Riley (1956)	Irish Sea		0·36–0·41									
	N. Atlantic		0·27–0·28									
	S. Atlantic		0·13–0·98									
	N. Pacific		0·25									
	S. Pacific		0·45									
Wedepohl (1960)	Average nearshore Atlantic sediments	95		20	48	6·0	850	55	13	100		
Manheim (1961)	Baltic Sea	110		25	78		4030	43	22	90		
Hirst (1962)	Gulf of Paria			20	19		2000	31	12	100		
Brooks & Rumsby (1965)	Tasman Bay, New Zealand	< 100	< 20	< 5	102	7·3	693	219		307		
Gross (1967)	Soanich Inlet, British Colombia	80–88		20	38–45		370–400	26–33	8–9	35–86		
Bloxam *et al.* (1972)	Swansea Bay, UK	128		126	81							
Butterworth *et al.* (1972)	Severn Estuary, UK	470		163								
Mackay *et al.* (1972)	Firth of Clyde, UK (204μm)	165		86	37		1118	50	34	64		
Raymont (1972)	The Solent, UK										0·19–5·68	
Skei *et al.* (1972)	Sörfjord (Hardangerfjord), Norway	20016		11367	2424							
Bryan & Hummerstone (1973*b*, *c*)	27 estuaries in England, UK	97–2980	< 0·2–9·3				140–780					
Halcrow *et al.* (1972)	Firth of Clyde, UK											
	Sludge disposal area (204μm)	437–681	4–8	269–403	250–300	3·0–6·8	300–430	58–87		87–175	2·2	
	Firth of Clyde, UK Control area (204μm)	60–130	1–3	24–67	9–20	1·5–6·1	283–432	14–50		10–65	0·04–0·15	
	Solway Firth, UK Control area (204μm)	36–105	< 1	12–66	5–16		240–700			15–62		
Perkins *et al.* (1973)	Solway Firth, UK (204μm)				10		360	38	16	35		
Leatherland & Burton (1974)	The Solent, UK	115–800	0·55–4·2								0·26–5·60	
Stenner & Nickless (1974*a*)	Hardangerfjord, Norway	27–64	0·3–0·8	7·7–13	3–10							
	Skjerstadford, Norway	24–58	0·7–1·5	5·0–66	2–18							
Boyden (1975)	Poole Harbour, UK	3–217	< 1–7	5–190	1–60	1·0–3·1	15–160	2–67	< 1–20			
Chester & Stoner (1975)	Severn Estuary, UK	280		119	38	4·5	1820	36	7	71		
Papakostidis *et al.* (1975)	Saronikis Gulf, Athens, Greece	45–1800								35–1000	0·38–3·1	0·04–2·7
Stenner & Nickless (1975)	Atlantic coast of Spain and Portugal	6–3100	0·9–4·1	6–1600	2–1400							
Talbot *et al.* (1976*a*)	Port Phillip Bay, Australia	3·3–278	0·15–9·9	4·6–183	2·2–87	0·05–4·6	6·9–252					

therefore include any natural variation in the trace metal content of the sediments, as well as metals derived from the activities of man. Some authors report a natural concentration derived from studies of reference areas or deep sediments, however. In addition, some authors (e.g. Chester & Stoner, 1975) report the use of a certain particle-size fraction only, in an attempt to eliminate the variation in trace metal content due to variation in sediment character (sand, mud, silt, etc.) at different locations.

Three major problems exist in the interpretation of data concerning the concentrations of trace metals in sediments. Firstly, 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 to sediment deposited over a given period of time. Thus if the total quantity of inorganic particulates sedimented in an outfall of constant trace metal load increases, the metal concentration in the sediments will decrease, i.e. the metal content per standard particle will be lower, although the total quantity of metal deposited will remain the same. Unless the total quantities of particles sedimented are similar per unit area of the sea bed at all locations studied, a spurious result concerning the total input of metals at these locations will be produced. Other parameters such as particle nature, form and size may also affect the final concentrations of metals found in sediments, since charge, the presence of certain ionic groups and the surface area:volume ratio of particulates must be important in the process of metal adsorption prior to particle settling. In addition, differences in mobilisation rates (biological or physical) may lead to spurious conclusions concerning the rate of metal input.

Secondly, the concentration of a metal found in sediments is dependent on the organic content of the sediment; in general, metal concentrations are found to increase in an approximately linear fashion with increased organic content, measured as total carbon (Halcrow *et al.*, 1973). An adjustment should also be made for the presence of coal (Buchanan & Longbottom, 1970). An attempt has been made to eliminate this factor by considering metal concentrations in sediments only in relation to the percentage of total carbon present. In this case, polluted sediments are considered to be those in which metal levels fall above the natural limits of the organic matter–metal concentration zone on the concentration axis (Fig. 2). Other authors merely take this factor into account when sediment samples differ widely in organic content (J. M. Skei, pers. comm.).

Thirdly, even a complete and accurate analysis of the concentrations of metals in sediments furnishes little or no data on the amount of metal available to the biota, with the possible exception of the sediment in-fauna. It is conceivable that even in a situation where metal concentrations in sediments are very high, little metal is available to the biota in the overlying water column. Indeed, in many instances this must be the case, as the existence of polluted sediments implies the predominance of the removal of metals from the water column by precipitation or particle

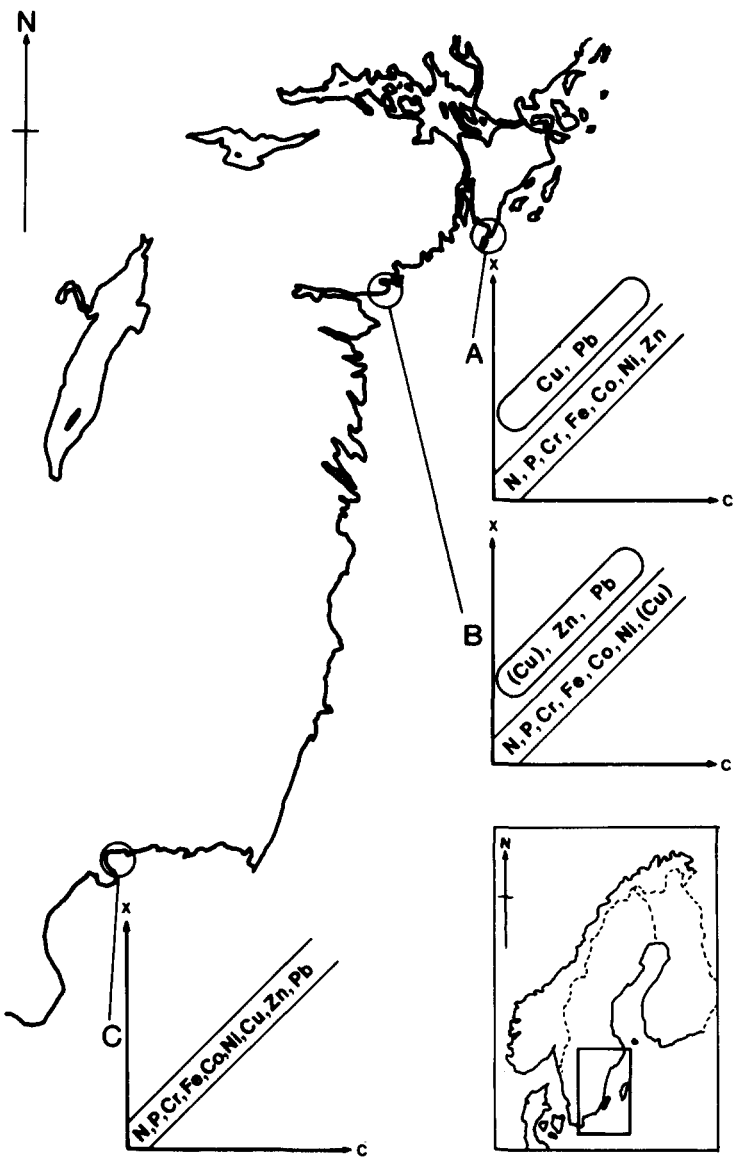

Fig. 2. Sediments from the East Coast of Sweden: (A) Nynashamn; (B) Tunaberg; (C) Pukaviken. Metal concentration (x) is plotted against % total carbon (c) in sediments. Samples are considered polluted if the metal content lies above the natural metal concentration-total carbon zone (zone through the origin) on the concentration axis, e.g. A and B are both polluted by lead; C is unpolluted. From Grimås & Ehlin (1975).

sedimentation, and also implies a low rate of mobilisation of metals from sediments. This would explain the general inability of many workers to correlate metal concentrations in water or biota with those in sediments (Bryan & Hummerstone, 1973*b*, 1973*c*; Halcrow *et al.*, 1973; Stenner & Nickless, 1974*a*, 1975; Boyden, 1975). As discussed above, the availability of metals to the biomass of a polluted region must be the prime concern, both in terms of the prediction of the effects of metal pollution on an ecosystem and in terms of possible human health risks.

C. Biological indicator organisms

In studies of the abundance of toxic pollutants, a biological indicator can be generally defined as an organism which may be used to quantify relative levels of pollution by the measurement of the toxicant concentration in its tissues. Either the entire organism, or a part of it, or a single tissue (which may sequester metals from the rest of the organism) may be used. The ideal characteristics of such an indicator were reviewed by Butler *et al.*, (1971) and are summarised as follows:

1. The organism should accumulate the pollutant without being killed by the levels encountered.
2. The organism should be sedentary in order to be representative of the area of collection.
3. The organism should be abundant in the study region.
4. The organism should be sufficiently long-lived to allow the sampling of more than one year-class, if desired.
5. The organism should be of reasonable size, giving adequate tissue for analysis.
6. The organism should be easy to sample and hardy enough to survive in the laboratory, allowing defecation before analysis (if desired) and laboratory studies of the uptake of metals.

To these requirements, Haug *et al.*, (1974) added the following:

7. The organism should tolerate brackish water.
8. The organism should exhibit a high concentration factor for metals, allowing direct analysis without pre-concentration.
9. A simple correlation should exist between the metal content of the organism and the average metal concentration in the surrounding water.

However, even these stringent requirements are not sufficient to ensure meaningful results from an indicator survey. The major requirement which must be met to avoid spurious conclusions is:

10. That *all* organisms in a survey exhibit *the same* correlation between their metal contents and those in the surrounding water, *at all locations studied, under all conditions.*

It must, therefore, be ensured that local environmental variables do not affect the

trace metal content of an organism before samples from different environments can be compared. For example, in a survey of trace metal abundance in an estuarine region, an increase in concentrations of metals in organisms from sites up-river may be either an effect of salinity or a true reflection of the relative availability and abundance of metals. Unless the effects of salinity on the net uptake of trace metals by the organism used are known, no conclusions can be made. In addition to hydrological variables such as salinity and water temperature, other possible interfering effects such as season, organism growth rate, weight and size, and the sampling position of the organism, should be considered.

The use of a biological indicator organism provides, ideally, an estimate of metal availabilities to the biomass of different regions. The choice of organism is important, as this defines the particular trace metal load measured in a survey. Metals can be derived by three possible routes: from solution, from the ingestion of food, and from the ingestion of particulate material containing metals. Not all indicator types reflect all three trace metal loads. In addition, the choice of indicator must take into account the organism suitability, i.e. the above ten requirements should be satisfied. In many cases these ten requirements have not been demonstrably satisfied prior to the use of an organism as an indicator, i.e. indicator ability has been assumed. This is patently not sufficient; for example, the mere existence of a high concentration factor for metals in some organisms is no guarantee of indicator ability. Bryan (1964, 1966, 1967, 1968) has shown a concentration factor for zinc and copper in decapod crustacea of about 10^4; both metals are nevertheless regulated to definite levels regardless of their external concentrations.

Different types of biological indicator organisms used by various authors are considered below in the light of present knowledge concerning the effects of physiological or sampling variables on their trace metal content.

3. MACROALGAE AS INDICATORS OF TRACE METAL POLLUTION

Several authors have studied the concentrations of trace metals in macroalgae, some with a view to the use of these organisms as indicators of pollution by trace metals. A selection of published values for the concentrations of several of the metals studied is shown in Table 3. The most commonly used species are *Fucus vesiculosus*, *Fucus serratus* and *Laminaria digitata*, but *Fucus spiralis*, *Ascophyllum nodosum*, *Ulva lactuca* and others have also been used.

Algae are responsive to the soluble trace metal content of their ambient surroundings; they do not, therefore, reflect total metal loads, as they do not respond (or respond only weakly and indirectly) to metal associated with organic or inorganic particulate matter. The time-integration of ambient soluble concentrations of metals in water appears to be high in the macroalgae; this is due to the extremely long biological half-lives of bound metals. For example, Zn^{65} is

TABLE 3
CONCENTRATIONS OF SELECTED TRACE METALS IN MACROALGAE. ALL FIGURES REFER TO

Authors	*Locations*	*Species*	*Zn*	*Cd*
Mullin & Riley (1956)	Atlantic coast	*Chlorophyceae*		0·31–0·86
		Rhodophyceae		0·36–0·86
		Phaeophyceae		0·13–2·08
Bryan (1969)	9 locations in England, UK	*Laminaria digitata*	4·0–10·0	
Preston *et al.* (1972)	Coasts of Britain	*Fucus vesiculosus*	32–962	0·05–21·0
		Porphyra umbilicalis	35–177	0·05–0·97
Bryan & Hummerstone (1973*a*)	4 estuaries, England, UK	*Fucus vesiculosus*	149–1240	
Ireland (1973)	Cardigan Bay, UK	*Fucus vesiculosus*	216–507	
Fuge & James (1974)	Bristol Channel, UK	*Fucus vesiculosus*	72–330	3·8–25·6
	Caernarvon Bay, UK	*Fucus vesiculosus*	39–113	0·9–4·3
Haug *et al.* (1974)	Trondheimsfjord, Norway	*Ascophyllum nodosum*	66–640	<0·7–1·0
	Hardangerfjord, Norway	*Ascophyllum nodosum*	110–3700	0·7–16·0
	6 coastal areas, Norway	*Ascophyllum nodosum*	42–370	
Leatherland & Burton (1974)	The Solent, UK	*Rhodophyceae*		
		Phaeophycae		0·15–0·4.
Stenner & Nickless (1974*a*)	Hardangerfjord, Norway	*Fucus vesiculosus*	620–2310	4·7–12·5
	Skjerstadfjord, Norway	*Fucus vesiculosus*	75–680	1·8–2·8
	Hardangerfjord, Norway	*Fucus serratus*	220–3550	3·1–13·0
	Skjerstadfjord, Norway	*Fucus serratus*	90–295	2·3–3·2
	Hardangerfjord, Norway	*Laminaria digitata*	260–290	2·3–2·5
	Skjerstadfjord, Norway	*Laminaria digitata*	170	4·3
	Hardangerfjord, Norway	*Ascophyllum nodosum*	160–1990	1·5–11·5
	Skjerstadfjord, Norway	*Ascophyllum nodosum*	65–720	1·0–1·5
	Hardangerfjord, Norway	*Enteromorpha* species	120–1820	2·7–13·0
	Skjerstadfjord, Norway	*Enteromorpha* species	35–325	0·7–3·2
Bohn (1975)	West Greenland	*Fucus vesiculosus*		
Boyden (1975)	Poole Harbour, UK	*Ulva lactuca*	28–136	1·0–4·8
Stenner & Nickless (1975)	Atlantic coast of Spain and Portugal	*Fucus* species	110–345	1·7–3·2
		Ulva lactuca	59–160	0·5–2·0
		Enteromorpha species	75–130	0·8–7·4
Foster (1976)†	Menai Straits, UK	*Fucus vesiculosus*	98–138	2·1
	Dulas Bay, UK	*Fucus vesiculosus*	228–398	1·8
	Menai Straits, UK	*Ascophyllum nodosum*	82–236	1·8
	Dulas Bay, UK	*Ascophyllum nodosum*	130–278	1·5
Saenko *et al.* (1976)	Vostok Bay, Sea of Japan	*Chlorophyceae* (4 species)	2·2–36·2	
		Rhodophyceae (4 species)	15–32	
		Phaeophyceae (8 species)	1·6–13·1	

known to be lost extremely slowly from *Fucus vesiculosus* (Gutknecht, 1965), *Laminaria digitata* (Bryan, 1969) and *Fucus serratus* (Young, 1975), although the loss of Fe^{59} from *F. serratus* was found to be measurable, with a biological half-life of about 180 days (Young, 1975).

This ability of macroalgae to reflect the soluble concentrations of metals in their surroundings with a high degree of time-integration is of importance when considering their suitability as indicators of trace metal pollution. Trace metals vary with respect to their presence in the soluble or particulate fractions of water in a polluted area. For example, zinc and cadmium are generally found mainly in the soluble fraction, whereas iron and lead exist mostly associated with particulate material (Preston *et al.*, 1972; Boyden & Romeril, 1974). Boyden & Romeril (1974) have shown that, in some circumstances, metal concentrations may decrease in the soluble fraction of water from the Severn Estuary under conditions of increased total

TABLE 3—*contd.*
μG G (PARTS PER MILLION) IN DRY ALGAE, EXCEPT FOR BRYAN (1969), WHICH IS IN μG/G WET WEIGHT

Pb	*Cu*	*Fe*	*Mn*	*Ni*	*Co*	*Cr*	*Hg*	*Ag*	*As*
	1·3–4·0	3·7–12·0	0·22–0·55						
0·5–11·3	1·6–36·0	45–1517	33–301	1·2–22·2				0·07–1·50	
0·8–10·5	2·8–23·3	104–3800	13–93	0·2–9·7				0·01–0·30	
6–31	9–301	506–1920	128–392						
	2·8–14·3	35–204	39–89	10·5–29·6	5·5–11·3				
	1·7–6·6								
<3–5	4–240						0·05–0·18		
<3·95	3–160						0·05–20·0		
							0·07–0·14		11–39
							0·16–0·22		26–47
10–202	7–36								
3–8	4–118								
5–54	6–26								
4–8	5–18								
15–37	6–12								
8	36								
0·7–62	4–44								
2–12	4–85								
27–1200	22–65								
6–24	19–110								
									35·2–35·8
16–66	11–34	3220–8890	40–1630	8–33	4–40				
5–13	9–31								
10–18	5·5–26								
4–22	6–22								
3·2	7·4–10	146–360	89–130	7·1–8·9		4·5			
2·3	49–97	41–168	52–97	4·6–7·0		3·8			
2·6	6–18	54–120	10–35	4·5–6·3		2·8			
2·2	46–96	15–40	9–25	3·9–5·2		2·2			
	1·2–21	87–1669	119–453	0·11–3·27	0·3–9·8				
	4·8–12·9	193–2809	149–1138	0·16–2·65	1·1–5·4				
	0·9–4·3	27–1077	11–554	0·19–1·35	0·6–2·7				

metal load. This decrease in soluble metal (particularly marked for cadmium and zinc) was achieved in periods of high run-off by an increase in the percentage and total amount of metals adsorbed to particulate matter, which itself increased dramatically in conditions of high run-off. In contrast, other metals, e.g. iron and manganese, increased in concentration in the soluble fraction of this estuary during periods of high run-off. In these circumstances an alga would reflect the increased total loads of iron and manganese, but would not reflect those of zinc and cadmium.

Several sources of variation in metal concentrations of macroalgae have been identified; these parameters may affect the results of an indicator survey using macroalgae by differentially affecting some of the samples taken. Variation of metal concentration with species has long been recognised (Black & Mitchell, 1952). In addition, increased concentrations of metals are found in individual plants with distance from the growth point. Bryan (1969, 1971) and Bryan & Hummerstone

(1973*a*) have shown that concentrations of zinc in *L. digitata* and those of zinc, lead, copper, iron and aluminium in *F. vesiculosus* are higher in distal (older) parts of the lamina. This was suggested to be due to the slow net accumulation of metals with age, and to the higher dry weight (and hence higher numbers of metal binding sites) of older parts of the plant. A similar effect was reported by Fuge & James (1973, 1974) for several metals in *F. vesiculosus* and by Haug *et al.* (1974) for zinc and copper in *A. nodosum*.

Seasonal variation in the concentrations of trace metals in macroalgae has also been studied. Bryan & Hummerstone (1973*a*) found little variation in copper concentration with season in *F. vesiculosus*, but concentrations of zinc, iron, aluminium and manganese all showed seasonal fluctuations. Manganese concentrations in this species also showed location-dependent seasonal variation, i.e. seasonal profiles of manganese concentration were different according to sampling position in the Tamar Estuary; this was ascribed to differences in the amount of available soluble manganese with season at these locations. Concentrations of most metals in these studies were maximal in winter. Fuge & James (1973, 1974) studied the seasonal dependence of concentrations of zinc, cadmium, copper, iron and cobalt in *F. serratus* and *F. vesiculosus*; in general, maximum concentrations were found in spring and minimum concentrations in autumn. They suggested that this profile may be due to the effects of plant growth on metal concentrations in the plant. It is probable that concentrations of metals in macroalgae increase in the winter months of low growth (and high run-off usually) and are then progressively diluted by the new growth of the plant in spring and summer. Young (1975) has reported results which agree with this concept, at least for Zn^{65} uptake in *F. serratus*. This effect of the seasonally dependent growth rate of macroalgae on their metal concentrations was suggested (Fuge & James, 1974) to account for the differences in the results of three surveys of metals in *F. vesiculosus* from the Severn Estuary and Bristol Channel in England (compare Butterworth *et al.*, 1972; Nickless *et al.*, 1972; Fuge & James, 1974). It is interesting to note that Haug *et al.* (1974) found little seasonal variation in concentrations of zinc in *A. nodosum* from Trondheimsfjord in Norway. This example serves to emphasise the differences between metals and between species which are so important in attempts to use organisms as biological indicators of trace metal pollution.

Nickless *et al.* (1972) reported variation in the concentrations of zinc and cadmium in *F. vesiculosus* according to the position of sampling of the plant on the shoreline. Both metals were present at highest concentration in plants from the mid-range of the vertical shoreline distribution. Fuge & James (1973, 1974) also observed this variation, and Bryan & Hummerstone (1973*a*) suggested that it was due to differences in metal availability to the plants, according to tidal exposure, salinity and metal stratification in waters of the estuary, and other factors. However, Bryan & Hummerstone (1973*a*) also found that metals differed appreciably in their variation with shoreline position of the alga; concentrations of zinc and copper were

unaffected in plants from the Tamar Estuary, but those of lead, iron, aluminium and manganese were higher in *F. vesiculosus* taken from the lower part of the shore. The exact correlation of this finding to the hypothesis of availability differences is obscure, particularly as the reported data were seasonally-averaged. The effect of shoreline position of sampling on the concentrations of metals found in macroalgae is probably more marked in estuaries with a high degree of water stratification, however, and samples from open coasts with better vertical mixing of water masses might be expected to show less such variation.

Morris & Bale (1975) have attempted to correlate the concentrations of zinc, cadmium, copper and manganese in waters of the Bristol Channel with those of the same metals in *Fucus vesiculosus*, as reported by Fuge & James (1974). This method of establishing indicator ability in an organism is rather suspect, as the mean concentrations of metals in water are difficult to predict accurately (Section 2A); in addition, the water analyses in this study were performed on samples from mid-Channel stations taken some two or more years after the shoreline survey of *F. vesiculosus*. No statistical tests could be applied to the data, but a general agreement between the concentrations of zinc, cadmium and copper in soluble form in water and those of the same metals in *F. vesiculosus* was apparent. By contrast, the concentrations of manganese in water showed poor agreement with those of this metal in the alga, and the uptake of manganese by this species appeared to be partly regulated. Concentration factors derived from these studies for all four metals in *F. vesiculosus* (Morris & Bale, 1975) were quite markedly different from those of previous authors for the same species (Preston *et al.*, 1972; Bryan & Hummerstone, 1973*a*). The practice of inferring mean values for the concentrations of soluble metals in water from the concentrations of metals in algae (e.g. see Preston, 1973) must be considered extremely dangerous, as the extraneous effects of sampling and environmental variables are rarely considered.

Very little information is available on the effects of environmental variables such as salinity and water temperature on the net uptake of metals by macroalgae. Although Bryan & Hummerstone (1973*a*) considered the effects of salinity inasmuch as this parameter affects the ratio of soluble to particulate metals present in the water column, no studies are known on the possible direct effects of salinity on metal binding by macroalgae. Variations in water temperature may have similar direct effects on metal binding efficiencies, and may also lead to variations in the growth rates of plants at different sites, causing lower final concentrations of metals in faster-growing weed.

The effects on growth of the presence of metals may also be important. Bryan (1969, 1971) has shown inhibition of the growth of *L. digitata* by zinc, lead and copper. Although the concentrations of lead affecting growth (0·1–0·5 mg/litre) were rather higher than those expected in most waters, the concentrations of zinc (0·10 mg/litre or less) and copper (0·011 mg/litre or less) were within the range found in severely polluted waters (see Stenner & Nickless, 1974*a*; Chan *et al.*, 1974, in Table

1). In these cases the inhibition of plant growth would cause the overaccentuation of differences between the locations for the inhibiting metals, and would also cause a spurious increase in the concentrations of other metals found in the slower-growing samples. In fact it can be seen that any parameter which differentially affects the growth rate of the samples taken will cause such effects, and this must include nutrient availability, light intensity, turbidity of the water and other factors. These factors have not been critically considered by any author to date, but it may be safe to assume that the over-estimation of pollution by some trace metals has occurred in some studies of severely polluted areas where macroalgae have been used as indicators.

The effect of the simultaneous presence of several metals on the uptake of any one metal by macroalgae has also received little attention to date. If the uptake of metals in these organisms is a true ion-exchange type of phenomenon, as suggested by some authors (Haug, 1961; Haug & Smidsrød, 1967), competition between metals for binding sites and even ion displacement may occur under some conditions. This would cause the under-estimation of pollution by some metals in areas where several metals coexist in the water column; such effects have been noted in both laboratory and field studies of the uptake of metals by macroalgae. Bryan (1969) reported that the presence of 20μg/litre of cadmium, copper or manganese decreased the net uptake of Zn^{65} by *L. digitata*; at higher concentrations of these metals the uptake of Zn^{65} could be stopped completely. Cadmium and manganese were suggested to act by competition with Zn^{65} for uptake sites, but the effects of copper were rather different and appeared to be due to a more direct toxic action of this element. Concentration factors for copper in *L. digitata* were found to increase with increased external exposure concentration of copper, which is most unusual. The toxic effects of copper were suggested to have possibly promoted its own entry into the plant. A similar increase of concentration factor for copper with increased external concentration was found by Phillips (1976*a*: see below) in studies of the mussel *Mytilus edulis*. These two reports represent almost identical findings, and it should be noted that copper is probably the most toxic of all elements to both *L. digitata* and *M. edulis*. This may suggest that a threshold of toxicity exists, beyond which an indicator organism will give spurious results due to the toxic effects of the element being measured. Whether this type of effect also causes any abnormal uptake of other metals seems to depend on the species and metals involved, as although Bryan (1969) noted such effects, Phillips (1976*a*) found no interference of copper with the uptake of other metals in the mussel. Competition between metals for binding sites (or possibly for uptake sites) has been reported recently by Foster (1976) in field studies of metals in *F. vesiculosus* and *A. nodosum*. Concentrations of zinc, cadmium, lead, copper, manganese, nickel, iron and chromium in both species of alga and in seawater from two areas in North Wales were compared. Samples of either species of alga from the heavily-polluted Dulas Bay showed lower levels of all metals except zinc and copper than did samples from the lightly-polluted Menai

Straits. The ambient levels of these metals in water were known with some certainty, after several years of study. It was suggested that zinc and copper monopolised the available binding sites on the algae, thus leading to the spurious result concerning the relative abundance of the other metals in the two areas. Although this example is from an unusually highly polluted area, such effects undoubtedly occur to a lesser extent at lower ambient levels of trace metals; this may be the greatest problem concerning the use of macroalgae as indicators of trace metal pollution, as it cannot be overcome by any simple method, e.g. a change in sampling techniques.

4. BIVALVE MOLLUSCS AS INDICATORS OF TRACE METAL POLLUTION

Many different species of bivalve molluscs have been used in attempts to monitor the concentrations of metals in the world oceans. Much of the early work concerned the indicator ability of these organisms for radioactive metals (Folsom *et al.*, 1963; Mauchline *et al.*, 1964; Folsom & Young, 1965; Bryan *et al.*, 1966; Seymour, 1966; Templeton & Preston, 1966; Young & Folsom, 1967). A number of laboratory studies on the uptake of radionuclides by bivalves have also been reported (e.g. Fretter, 1953; Jones, 1960; Keckes *et al.*, 1967*a*, *b*, 1968, 1969; Kameda *et al.*, 1968; Shimizu *et al.*, 1971; Romeril, 1971*a*; Pentreath, 1973*a*).

Bivalves accumulate both radioactive and non-radioactive trace metals with concentration factors of 10^3 to more than 10^6, depending on the species and the metal involved; tissue analyses are therefore possible with relatively unsophisticated equipment. These organisms are filter-feeders and thus obtain trace metals not only from food and from solution, but also from the ingestion of inorganic particulate material (Moore, 1971). The exact proportions of the total body trace metal content derived from each of the three routes in bivalves are uncertain. However, concentration factors attained by mussels (*Mytilus edulis*) for the uptake of both radionuclides and stable metals from solution alone in the laboratory are rather lower than those found in mussels from the environment (Pentreath, 1973*a*; Phillips, 1976*a*), suggesting uptake from food to be the most important route. Schulz-Baldes (1974) found that lead uptake by the same species from solution alone or from food alone occurred at a similar rate when exposure concentrations were the same. However, the uptake of lead from food was suggested to be the more important route in the environment, as concentrations of lead in seawater are generally very low (Table 1). Preston (1971) found that the uptake of Cr^{51} from solution alone by the oyster *Crassostrea virginica* was faster than that from food alone at the same exposure concentration, but also concluded that the food route would be more important in the environment due to the high concentration factors for Cr^{51} exhibited by phytoplankton. Several authors have shown that the uptake of metal by the ingestion of inorganic particulate matter by bivalves is also significant (Raymont, 1972; Preston *et al.*, 1972; Boyden & Romeril, 1974). Boyden & Romeril (1974) kept oysters (*Crassostrea gigas*) in waters of different particulate content and found that

the relative importance of metal uptake from solution and from inorganic particulate matter depended on the metal concerned; however, uptake from both fractions occurred for all metals. The ability of bivalves to respond to each of the three possible absorption routes of metals may be an advantage over, for example, macroalgae, in terms of their suitability as indicators of total metal pollution, particularly in estuarine areas where variable amounts of inorganic and organic particulate material are present in the water column.

Many authors have reported results on the concentration of non-radioacive trace metals in bivalves (Table 4). Several laboratory studies of the uptake of stable trace metals by bivalves have also been published (e.g. Pringle *et al.*, 1968; Shuster & Pringle, 1969; Eisler *et al.*, 1972; Scott & Major, 1972; Cunningham & Tripp, 1973, 1975*a*, *b*; Schulz-Baldes, 1974; Delhaye & Cornet, 1975; Phillips, 1976*a*).

Despite this large volume of work, the variables affecting the results of indicator surveys using bivalves remain partly unknown, particularly in terms of their quantitative effects. However, some data are available on the effects of organism age, weight, size and sex, season, position of sampling, salinity, water temperature and the coexistence of several metals, on the net uptake of metals by bivalves, or on the final concentrations of metals found in bivalves.

The effects of the age, weight or size of the organism sampled have been studied by some authors; these effects are probably all related. Romeril (1971*b*) reported an increase in the concentrations of zinc, copper and iron with increased age of *Mercenaria mercenaria*. De Wolf (1975) observed higher concentrations of mercury in larger individuals of *Mytilus edulis* collected at one location, but mussels from two other locations showed no such consistent trends. Boyden (1974) reported relationships between organism size, metal concentration and total metal content for several species of bivalves. These differences found in animals from the environment have also been noted in some laboratory studies. For example, Cunningham & Tripp (1975*a*) found a faster uptake of mercury in smaller individuals of *Crassostrea virginica* than in larger individuals. Schulz-Baldes (1974) found a similar effect for the uptake of lead by *Mytilus edulis*; again, uptake was faster in smaller animals than in larger ones. Phillips (1976*a*, 1976*b*,) has observed a weight-dependent variation in concentrations of metals in *Mytilus edulis*, both in the laboratory and in the environment. In the laboratory the uptake of zinc, cadmium, lead and copper was faster in lighter individuals than in heavier ones in some studies. In the environment, smaller and lighter individuals were found to contain significantly higher concentrations of these same metals than did larger, heavier individuals. However, this was by no means always the case; the incidence of significant relationships between tissue weight and metal concentration varied with season and between metals. Fewest such relationships were evident in late winter and early spring, at the maximum in the seasonal variation profile for metal concentrations in the mussel. Watling & Watling (1976*b*) have found

differences in the concentrations of several metals in *Choromytilus meridionalis* in relation to individual tissue weights. They also reported differences between the sexes in metal concentrations of the same species; concentrations of zinc, copper and manganese were higher in females than in males, whereas those of lead and bismuth were higher in males than in females. No such difference between the sexes has been observed in *Mytilus edulis* (Phillips, unpublished data).

The influence of season on the concentrations of trace metals found in bivalves has also been partly elucidated. Pentreath (1973*a*) reported seasonal differences in total concentrations and in tissue distributions of non-radioactive zinc, iron and manganese in *Mytilus edulis*. Pringle *et al.* (1968) found seasonal changes in the concentrations of five metals in the oyster *Crassostrea gigas* from the coast of Washington, USA; Seymour (1966) had previously reported seasonal effects on the uptake of Zn^{65} by the same species. Bryan (1973) gave more detailed seasonal profiles for the concentrations of zinc, lead, copper, cobalt, iron, manganese and nickel in tissues of the scallops *Pecten maximus* and *Chlamys opercularis* from the English Channel. In general, the concentrations of metals in these scallops were greatest in autumn and winter, and it was suggested that metal concentrations were inversely related to phytoplankton productivity. However, Fowler & Oregioni (1976), in studies of the variation in the concentrations of ten metals in *Mytilus galloprovincialis*, suggested that the seasonal maximum seen in samples collected in March (spring) was due to the reproductive state of the animals and to the high winter run-off increasing the amount of available metals. Phillips (1976*a*) reached very similar conclusions concerning the seasonal variation of concentrations of zinc, cadmium, lead and copper in *Mytilus edulis*. The seasonal fluctuations of trace metal concentrations were reciprocal to the seasonal changes in tissue weights of individual animals; thus the total metal content of each individual changed little throughout the year. Weight changes were in turn related to the sexual cycle, with a minimum in late winter or early spring (see also Dare & Edwards, 1975), coincident with the seasonal maximum of trace metal concentrations. In addition, this author noted that the seasonal fluctuation of metal concentrations in the mussel varied from site to site according to the proximity of sites to a polluted freshwater inflow. This location-dependent effect on the seasonal variation is similar to that described by Bryan & Hummerstone (1973*a*) for manganese in *Fucus vesiculosus* from the Tamar Estuary (see above). Fowler & Oregioni (1976) also found differences between each metal in terms of the amount of their seasonal fluctuation in *Mytilus galloprovincialis*. The ratio between the seasonal maximum and the seasonal minimum concentrations was greatest for chromium (factor of 8·8) and least for zinc (factor of 2·0). These differences must be related to differences in the biological half-lives of metals in mussels; in general it appears that metals have shorter half-lives in bivalves than in macroalgae, and the time-integration capacity of bivalves is, therefore, less than that of macroalgae.

TABLE 4

SELECTED VALUES FOR THE CONCENTRATIONS OF CERTAIN TRACE METALS IN WHOLE SOFT PARTS

Authors	*Locations*	*Species*	*Wet or dry weight*	*Zn*
Mullin & Riley (1956)	Atlantic coast	*Mytilus edulis*	DRY	
Brooks & Rumsby (1965)	Tasman Bay, New Zealand	*Mytilus edulis aoteanus*	DRY	91
		Ostrea sinuata	DRY	1103
		Pecten novaezelandiae	DRY	283
Pringle *et al.* (1968)	Average, Atlantic coast of USA	*Crassostrea virginica*	WET	1428
		Mya arenaria	WET	17
		Mercenaria mercenaria	WET	21
Roosenburg (1969)	Patuxent Estuary, USA	*Crassostrea virginica*	DRY	
Segar *et al.* (1971)	Irish Sea	*Pecten maximus*	DRY	230
		Modiolus modiolus	DRY	530
		Glycymeris glycymeris	DRY	120
	The Solent, UK	*Mytilus edulis*	DRY	91
		Mercenaria mercenaria	DRY	94
Raymont (1972)	The Solent, UK	*Mytilus edulis*	DRY	
		Mercenaria mercenaria	DRY	
Bryan (1973)	10 km off Plymouth, UK	*Chlamys opercularis*	DRY	462
		Pecten maximus	DRY	273
Ireland (1973)	Cardigan Bay, UK	*Mytilus edulis*	DRY	253–779
Schulz-Baldes (1973)	Weser Estuary and German Bight	*Mytilus edulis*	DRY	
Boyden & Romeril (1974)	Severn Estuary, UK	*Crassostrea gigas*	DRY	9860–99220
Leatherland & Burton (1974)	The Solent, UK	*Mytilus edulis*	DRY	269
		Mercenaria mercenaria	DRY	
Stenner & Nickless (1974*a*)	Hardangerfjord, Norway	*Mytilus edulis*	DRY	170–2370
	Skjerstadfjord, Norway	*Mytilus edulis*	DRY	105–280
Bohn (1975)	West Greenland	*Mytilus edulis*	DRY	
Boyden (1975)	Poole Harbour, UK	*Mytilus edulis*	DRY	94–162
		Ostrea edulis	DRY	1966–3397
		Crassostrea gigas	DRY	1766
Darracott & Watling (1975)	Dorset coast, UK	*Crassostrea gigas*	DRY	
De Wolf (1975)	Coasts of Europe	*Mytilus edulis*	WET	
Nielsen & Nathan (1975)	Coasts of New Zealand	*Mytilus edulis*	WET	3·8–26·0
		Perna canaliculus	WET	0·5–28·0
		Pecten novaezelandiae	WET	18–31
Stenner & Nickless (1975)	Atlantic coast of Spain and Portugal	*Mytilus edulis*	DRY	190–370
		Crassostrea angulata	DRY	310–920
Alexander & Young (1976)	S. California, USA	*Mytilus californianus* (*DG)	DRY	46–110
Fowler & Oregioni (1976)	NW Mediterranean Sea	*Mytilus galloprovincialis*	DRY	97–644
Phillips (1976*b*)	Port Phillip Bay, Australia	*Mytilus edulis*	WET	16–97
Talbot *et al.* (1976*b*)	Port Phillip Bay, Australia	*Mytilus edulis*	DRY	
		Ostrea angasi	DRY	
Watling & Watling (1976*a*)	Kynsna Estuary, South Africa	*Crassostrea gigas*	DRY	396
		Crassostrea margaritacea	DRY	886
		Ostrea edulis	DRY	660
Watling & Watling (1976*b*)	Saldanha Bay, South Africa	*Choromytilus meridionalis*	DRY	73–113
		Crassostrea gigas	DRY	424

*DG: Digestive gland only

TABLE 4—*contd.*

OF BIVALVE MOLLUSCS. ALL FIGURES REFER TO μG/G (PARTS PER MILLION) FOR WET OR DRY TISSUE

Cd	*Pb*	*Cu*	*Fe*	*Mn*	*Ni*	*Co*	*Cr*	*Hg*	*Ag*	*As*
3·21										
< 10	12	9	1960	27	7		6			
35	10	41	682	8	2		3			
249	16	9	2915	111	6		10			
3·1	0·5	91·5	67	4·3	0·19	0·10	0·40			
0·3	0·7	5·8	405	6·7	0·27	0·10	0·52			
0·2	0·5	2·6	30	5·8	0·24	0·20	0·31			
		250–1250								
13	8·3	3·3	170	140	49	8·5				
7·1	42	10	350	47	133	5·5				
3·3	3·5	5·7	68	34	1·4	0·9				
5·1	9·1	9·6	1700	3·5	3·7	1·6	1·5		0·03	
2·1	18	25	5400	18	11	4·3	0·8		1·30	
								0·43–1·86		9–15
								0·18–0·57		3–7
5·5	12·0	15·4	113	158	1·56	0·33	2·2		10·4	
32·5	2·0	8·9	196	107	0·73	0·25	1·3		2·7	
	1·3–9·7									
17–43	15–17	1760–6480	214–422	16–46	3·8–6·5	2·2–3·5				
2·5								0·42–1·9		9–15
0·3–0·6								0·16–1·5		2–12
4·8–140	15–3100	3–22								
1·9–4·7	2–6	15–130								
										14–17
3·7–65·4	7–19	7–11	87–154	3–5	5–12					
5·9–53·7	5–8	86–451	172–394	6–9	2–5					
4·6	6	200	228	18	3					
1·6–26·1										
								0·053–0·83		
0·3–1·6	0·1–2·0	1·7–18·0	8·3–49					0·02–0·48		
0·1–1·0	0·1–7·8	0·2–28·0	26–280					0·04–0·19		
0·1–0·3	0·4–3·5	0·9–2·5	9·1–698					0·03–0·06		
1·7–3·6	2–15	6·5–14								
2·9–3·5	4–11	120–435								
	2–38	14–69			3·3–20		0·8–61		0·3–46	
0·4–5·9	2·7–117	2·4–154	149–2220	3·3–70	0·9–14	0·5–7·4	0·5–29		0·1–1·9	
0·48–18·2	0·71–10·0	0·40–1·45								
4·2–83										
35·5–174										
3·7		32	128	16	1·6				1·9	
2·5		17	57	2	1·6				2·6	
3·1		38	167	6	1·7				6·4	
1–8	2–5	7–14		9–11	2–3	2–3				
9	1	33		12	1	1				

Variation in the concentrations of metals in bivalves with depth of sampling has also been reported. Nielsen (1974) found that concentrations of zinc, cadmium, lead and iron in the cultured mussel *Perna canaliculus* varied with sampling depth at one location in New Zealand waters. This was suggested to be due possibly to differences in available food or in soluble:particulate metal ratios in the water column with depth, or to the presence of hydrogen sulphide from sediment-living bacteria affecting metal solubilities in the water column. Concentrations of the same metals in mussels from a second location, of greater water circulation, showed no vertical gradients of this kind. De Wolf (1975) reported higher concentrations of mercury in individuals of *Mytilus edulis* from the intertidal zone than in those sampled subtidally, at five different locations and at various times of the year. Phillips (1976*a*) reported vertical gradients in concentrations of zinc, cadmium and lead in *M. edulis* from a location situated approximately 1 km distant from a polluted freshwater outfall. These gradients were well-defined in late winter, but were absent or much-reduced in late summer. It was suggested that the gradients seen were produced by the exposure of mussels at the top of the water column to a metal-rich freshwater layer in winter conditions of high run-off from the adjacent catchment; in summer no such vertical stratification of the water column was present.

The use of bivalves to identify sources of trace metal pollution necessitates their study in estuarine areas, as most trace metals are introduced into the marine environment in freshwater outfalls (Bryan, 1971). The effects of salinity and water temperature are, therefore, of no small importance when considering the ability of these organisms as indicators. Wolfe & Coburn (1970) reported that concentration factors for Cs^{137} in the clam *Rangia cuneata* decreased with increasing salinity but increased with increasing temperature; this was suggested to be due to the chemical similarity of caesium^{137} to potassium. Phillips (1976*a*) reported significant effects of salinity and temperature on the uptake of cadmium, lead and copper from solution by *Mytilus edulis;* the uptake of zinc, however, was unaffected by either parameter. Decreased salinities were found to increase the net uptake of cadmium and copper by this organism, but to decrease that of lead. Decreased temperatures had no effect on lead uptake by the mussel at either high or low salinity, but the net uptake of both cadmium and copper was generally decreased by this variable; this decrease was more marked at low salinities. The net uptake of copper throughout these studies was rather erratic, however, and the behaviour of copper in bivalves is discussed further below.

The effects of the coexistence of several metal pollutants on the uptake of any single metal by an organism are extremely difficult to study because of the large number of metal combinations and biological responses possible. However, Romeril (1971*a*) reported that both iron and cobalt depress the rate of Zn^{65} uptake by the oyster *Ostrea edulis*. The effects were different for soft parts and for the shell; although the presence of either iron or cobalt (at a concentration of 0·001 mg/litre) decreased the rate of net uptake of Zn^{65} into the soft parts, the equilibrium

concentration of Zn^{65} reached appeared little-changed. In contrast, the equilibrium concentration for Zn^{65} in the shell was more than halved in the copresence of iron and cobalt, and the rate of Zn^{65} uptake was also affected by these ions. Ion competition at uptake or absorption was suggested as the possible interfering mode of action. Phillips (1976*a*) has shown that the net uptake of copper by *M. edulis* is affected by the presence and change in concentration of zinc, cadmium and lead. In contrast, the net uptake of either zinc, cadmium or lead appeared indifferent to the copresence of the other metals. Few studies on this problem are known for any possible indicator organism (see Bryan, 1969, above and Eisler & Gardner, 1973, below). It is, nevertheless, an extremely important variable, as it cannot be eliminated by the use of any particular sampling regime, as can many other variables. The existence of this effect in *M. edulis* has led to the suggestion (Phillips, 1976*a*, *b*) that this organism should not be used as an indicator of copper pollution. This contention is supported by previous survey results (Stenner & Nickless, 1974*a*; Phillips, 1967*b*) where no obvious correlation between the copper concentrations in the mussel and areas of known copper pollution from industry has been found. In addition, the uptake kinetics of copper in the mussel are very different from those of other metals (e.g. compare Scott & Major, 1972, with Pentreath, 1973*a* and Phillips, 1977). It therefore appears that copper is handled atypically by the mussel, which may be a reflection of its extreme toxicity (see Section 3 above). Other bivalves may be different in this respect (Roosenburg, 1969), and certainly accumulate copper to a much greater extent than does *M. edulis* (Table 4). It may therefore be possible to use one of these species as an indicator of copper pollution, and this would seem advantageous compared to the use of either macroalgae or *Mytilus edulis*.

5. TELEOSTS AS INDICATORS OF TRACE METAL POLLUTION

Teleosts assimilate metals from both food and solution. The uptake of metals from solution may occur across the body surface (particularly the gills) or across the gastro-intestinal wall after drinking. The relative amounts of metal contributed by each route are uncertain, although the uptake of metal from food probably predominates (Hoss, 1964; Baptist & Lewis, 1969; Pentreath, 1973*b*, *c*, *d*). However, other authors have reached the opposite conclusion (Townsley *et al.*, 1960; Polikarpov, 1966), and the situation may vary with the species of fish and with the metal considered. For example, estuarine species may be exposed to higher concentrations of metals in solution than is normal in a marine situation. Variation with metal must occur because of the variation in concentration factors for metals in phytoplankton and other fish foods (Lowman *et al.*, 1971), and because of the varying ratios of metals in the soluble and particulate phases of water (see Section 2A). Teleosts probably do not respond directly to metals associated with inorganic

particulate material, but they may respond indirectly to this fraction if preying substantially on filter-feeding organisms which do derive metals from this fraction.

Published data on the concentrations of mercury in teleosts suggest that certain species are capable of acting as efficient indicators of pollution by this element (e.g. Johnels *et al.*, 1967; Ackefors *et al.*, 1970; Jernelov & Lann, 1971; Keckes & Miettinen, 1972; Grimstone, 1972). Swedish studies have pioneered the use of pike (*Esox lucius*), and this fish has several advantages as an indicator of mercury in fresh- or brackish-water ecosystems. As a high-ranking predator the pike exhibits high concentrations of mercury in axial muscle tissues, present mostly as methylmercury. These fish are also considerably territorial, and are, therefore, representative of the environment in which they are sampled. Differences in mercury concentrations arising from differences in sample ages or weights can be eliminated by the weight-normalisation methods described by Johnels *et al.* (1967), and mercury pollution of different regions is then compared on the basis of the 'standard 1 kg pike'. More recently, Dix *et al.* (1976) suggested the use of the flathead (*Platycephalus bassensis*) as a marine indicator of mercury pollution. Although seasonal and size-dependent variations were seen in these studies, they are easily eliminated. The flathead is a demersal fish and is non-migratory, and therefore also appears to be fairly representative of its collection area. However, it should be noted that factors such as salinity and water temperature have not been considered to date as possible interferences in such surveys; at least for the rainbow trout (*Salmo gairdneri*) the uptake and excretion of mercury, phenylmercuric acetate and methylmercury is temperature-dependent (Macleod & Pessah, 1973; Reinert *et al.*, 1974; Ruohtula & Miettinen, 1975). In addition, Scott (1974) has suggested that the concentration of mercury present in fish may possibly be correlated to fish condition; if this is a cause-and-effect relationship, the numbers of factors affecting the uptake of mercury by fish must be enormous. Thus it seems that even the use of teleosts as indicators of mercury pollution could be improved by the consideration of the effects of some environmental variables.

The use of teleosts as indicators of metals other than mercury is open to more criticism. However, several studies of metals in both freshwater and marine species of fish have been reported (e.g. Vinogradov, 1953; Goldberg, 1962; Uthe & Bligh, 1971; Lovett *et al.*, 1972; Vink, 1972; Cross *et al.*, 1973; Taylor, 1973; Eustace, 1974; see also Table 5).

The use of muscle tissue from teleosts as an indicator of pollution by trace metals other than mercury has been advised against in several studies (Halcrow *et al.*, 1973; Topping, 1973; Windom *et al.*, 1973; Eustace, 1974; Stenner & Nickless, 1974*a*). No consistent differences were found in these studies between concentrations of metals in the muscles of fish from polluted and non-polluted areas, for any of a number of fish species. Results from the exposure of teleosts to cadmium and copper in laboratory and field toxicity experiments have also shown no significant increase of concentrations of these metals in muscle (Eisler, 1974; Saward *et al.*, 1975). However, concentrations of metals in teleost muscle tissue do appear to vary with

TABLE 5

SELECTED VALUES FOR THE CONCENTRATIONS OF CERTAIN TRACE METALS IN FISH MUSCLE. ALL FIGURES REFER TO μG G (PARTS PER MILLION) FOR WET OR DRY TISSUE

Authors	*Location and species*	*Wet or dry weight*	*Zn*	*Cd*	*Pb*	*Cu*	*Fe*	*Mn*	*Ni*	*Cr*	*Hg*	*As*
Ackefors *et al.* (1970)	The Sound, Sweden 7 species	WET									0·05–4·16	
Portmann (1972)	Coasts of Britain	WET	4·35–6·60	<0·05 0·18	<0·5 0·99	<0·5 1·80				<0·5–0·58	0·05–0·49	
Andersen *et al.* (1973)	Oslofjord, Norway											
	(Whole body, less viscera) Sprat	DRY	85 210	<0·2 0·7	<0·3–21·8	2·6–8·5						
	Herring	DRY	95–140	<0·2 0·2	3·2 12·2	3·4 7·2						
Halcrow *et al.* (1973)	Firth of Clyde, UK 8 demersal species	DRY	17 36	0·6–1·0	5·8 15·0	2·2–3·0		20–48	2·1 3·5	0·9 1·7		
Havre *et al.* (1973)	Southern Norway	DRY		<0·005 0·13							0·25–7·27	
Leatherland *et al.* (1973)	NE Atlantic 4 species	DRY	44	0·05–0·98							0·12–0·25	2·5–5·4
Preston (1973)	England and Wales <40 km offshore	WET	5·52	0·11	0·66	0·95					0·29	
	Irish Sea >40 km offshore	WET	5·70	0·07	0·50	0·80					0·21	
	North Sea >40 km offshore	WET	4·83	0·12	<0·50	<0·50					0·10	
	Iceland; Barents Sea; Norway coast	WET	6·10	<0·05	<0·50	1·80					0·06	
Topping (1973)	Scotland, UK 4 species	WET	1·55 23·5	<0·03 0·12	<0·2 1·2	0·08–4·28						
Windom *et al.* (1973)	North Atlantic 26 species											
	(77 individuals)	DRY	7–41	<0·1 1·3		<0·3 9·5					0·1 4·5	<1·0–6·4
Brooks & Rumsey (1974)	North Island, New Zealand											
	8 species (Geometric means)	WET	2·8 21·0	0·002 0·016	0·16–0·87	0·11 0·59	2·2–9·2	0·08 1·15	0·02–0·07	0·01–0·03		
Leatherland & Burton (1974)	The Solent, UK 3 species	DRY	15	0·03							0·15 1·8	1·7–8·7
Stenner & Nickless (1974*a*)	Hardangerfjord and Skjerstadfjord											
	Norway Cod	DRY	1 33	0·1 0·35	1 2	1 3					0·1 0·4	
Stevens & Brown (1974)	Coast of Britain 5 species	DRY	23–123	<0·05–0·9	<0·2	3·0 6·6						
Bohn (1975)	West Greenland 5 species	DRY										14·7 307
Dix *et al.* (1976)	Tasmania, Australia Sand flathead	WET									0·03–1·06	
McKay *et al.* (1975)	NE Australia Black marlin	WET	5·8 14·6	0·05–0·40	0·1–0·9	0·3–1·2					0·5–16·5	0·1 1·65
Stenner & Nickless (1975)	Atlantic coast of Spain and Portugal											
	10 species	DRY	19 120	0·08–3·2	1·2 7·0	0·6 8·0					0·5–0·79	
Wright (1976)	NE Britain 9 species	WET	1·9 119	0·11–1·44		0·5–4·6			0·5 7·2			

factors other than the relative abundance of metals, for example with age, size or weight of the fish (Lovett *et al.*, 1972; Cross *et al.*, 1973; Brooks & Rumsey, 1974; McKay *et al.*, 1975), feeding habits (Topping, 1973; Windom *et al.*, 1973; Stevens & Brown, 1974), species (e.g. Windom *et al.*, 1973; Brooks & Rumsey, 1974; Eustace, 1974), and sex (mercury in some fish: T. Walker, pers. comm.). Concentrations of metals in whole bodies of the flounder *Platichthyes flesus* are also known to vary with season and with length or age of the fish (Hardisty *et al.*, 1974*b*), and possibly with the diet of the fish also Hardisty *et al.*, 1974*a*).

Data on the tissue distributions of metals in teleosts have been reported by several of the above authors. In general, the conclusions reached have been similar to those of the extensive work of Brooks & Rumsey (1974), although some authors (e.g. Halcrow *et al.*, 1973) have reported rather different distributions. Brooks & Rumsey (1974) found that in general, zinc, cadmium, copper and iron are concentrated in soft organs of teleosts such as the liver, kidney, spleen, heart and gonads, whereas lead and manganese are concentrated in bony organs such as the gill, backbone and tail. Chromium and nickel showed little evidence of concentrating preferentially in any organ, although Wright (1976) found slightly higher concentrations of nickel in skin, liver and kidneys of teleosts than in other organs.

The possibility therefore arises that some organ other than muscle in teleosts may be capable of acting as an indicator of the environmental abundance of trace metals, although it must be stressed that the preferential concentration of a metal in any particular organ does not necessarily imply the presence of indicator ability in that organ. No author has yet succeeded in correlating the concentration of metal in any teleost organ to the ambient average concentration of that metal in the surrounding water, with the exception of studies on mercury. However, Eisler (1974) and Saward *et al.*, (1975) found that the concentrations of cadmium and copper increased in some tissues of experimental fish used in toxicity tests for these metals. Eisler (1974) found increased cadmium concentrations in gill, liver and gastro-intestinal tract of the mummichog *Fundulus heteroclitus* on exposure to cadmium in solution. It should be noted, however, that Eisler & Gardner (1973) showed decreased whole body uptake of zinc in the same organism in the copresence of cadmium; this effect was similar to those described above concerning the effects of the copresence of ions on the uptake of any one ion; it may again be indicative of ion competition at the uptake site. Saward *et al.*, (1975) studied the effect of low concentrations of copper on an experimental food chain composed of phytoplankton, the bivalve *Tellina tenuis*, and small '0'-group plaice. Decreased growth rates were found in all components of the food chain on exposure to 10–100 μg/litre added copper concentrations, and increased uptake of copper was found in the bivalve and in plaice viscera (but not in plaice muscle).

Although increased concentrations of metals may therefore be expected in some organs of teleosts exposed to high concentrations of metals in solution or in food, a clear correlation of metals in these organs to those in the ambient surroundings

remains to be established. In addition, the use of pelagic fish can be criticised on the grounds of movement; the concentrations of metals found would represent (at best) an average or composite of the past exposure of the fish to metals in different areas. Although this may allow the study of gross differences between very widely separated areas, it would seem that demersal fish might be more representative of given areas of collection, and may, therefore, be more useful to delineate polluted areas of a given ecosystem. Studies on the flathead (*Platycephalus bassensis*) in Port Phillip Bay, Australia (Phillips, unpublished data) suggested that the use of liver to measure pollution by cadmium might be justified. Concentrations of cadmium found in the livers of 32 fish taken from three sampling points correlated broadly with the pattern of cadmium pollution in this Bay, which had been previously elucidated by use of the mussel *Mytilus edulis* (Phillips, 1976*b*) and the scallop *Pecten alba* (T. Walker, details to be published). Some limited agreement was also found with data on metals in sediments (Talbot *et al.*, 1976*a*). However, concentrations of zinc and copper in muscles, livers and spleens of the same fish revealed no consistent differences between fish from polluted and non-polluted areas. It is interesting in this connection that cadmium exhibits a higher ratio of concentration in liver to concentration in muscle than many other metals (Eisler, 1974; McKay *et al.*, 1975; Wright, 1976).

In summary, the use of teleosts, or their tissues, as indicators of pollution by trace metals does not at this time seem justifiable, with the exception of studies on mercury. Although metals are known to concentrate preferentially in certain organs, the relationship of organ concentration of metals to the ambient environmental concentration of metals is uncertain and may be affected by several variables. Variables of particular importance are species, season, age, size or weight of the fish, feeding habits, salinity and water temperature, and the copresence of several metals. In general the effects of these parameters have been ignored by many authors and are even more poorly-understood for teleosts than for macroalgae or bivalve molluscs.

6. OTHER ORGANISMS AS INDICATORS OF TRACE METAL POLLUTION

A certain amount of published data exists which proposes the use of indicator organisms other than macroalgae, bivalve molluscs, or teleosts. For example, Ireland (1973, 1974) and Walker *et al.* (1975*a*) have suggested the use of the barnacle *Balanus balanoides*. This organism accumulates high concentrations of metals in its tissues, particularly in tissues associated with the gut. Ireland (1974) reported seasonal profiles for zinc, lead, copper and manganese in *B. balanoides* from two sites close to rivers draining a mining catchment in South Wales. Much of the seasonal variation in metal concentrations appeared to be due to seasonal weight fluctuations connected with reproductive state of the animals. No variables other than season and tissue weight were investigated and no correlation of tissue

concentrations of metals to the concentrations in water was proven, although such a correlation was assumed. Walker *et al.* (1975*a*) considered zinc uptake only in the same species and preferred gut tissues to whole soft parts as an indicator; the metal was found to accumulate in gut parenchyma in granular form, the granules being mostly zinc phosphate (Walker *et al.*, 1975*b*; cf. Bryan, 1973). An attempt was made to correlate concentrations of zinc in the gut tissues of various barnacle samples to zinc concentrations in seawater reported by other authors, although the concentrations of zinc in water were suggested to be unreliable due to temporal variation. However, a loose relationship was presented between these two parameters. No studies on the effects of variables, e.g. sampling position, salinity, water temperature, etc. were reported.

Other authors (Peden *et al.*, 1973; Leatherland & Burton, 1974; Navrot *et al.*, 1974; Stenner & Nickless, 1974*a*, *b*, 1975) have reported data for the concentrations of metals in the limpets *Patella vulgata* and *Patella vulgaris*; Stenner & Nickless also transplanted some limpets from one area to another in the Bristol Channel. Nothing is known concerning the variation of metal concentrations in limpets with any environmental or sampling variable.

Bryan (1966, 1967, 1968) has reported data on the concentrations and tissue distributions of metals in various decapod crustacea. These organisms appear to regulate their total body levels of zinc and copper to within definite limits, exhibiting concentration factors of about 10^4 for both metals. Temporary storage of metals by the blood and hepato-pancreas occurs during conditions of excess metal uptake; the excretion routes for metals vary between species. Martin (1974) and Wright (1976) have also reported data on the concentrations of metals in decapod crustacea; however, because of the rapid turnover of metals in these organisms, they do not appear to be useful indicators of trace metal pollution.

Bryan & Hummerstone (1973*b*, 1973*c*) have studied trace metals in the polychaete *Nereis diversicolor*. This organism also regulates its tissue concentrations of zinc, but concentrations of cadmium and copper are not regulated and are roughly proportional to those in the surrounding sediment. The uptake of manganese in this organism appears to be affected by salinity, it being higher at lower salinities of the interstitial water in sediments. Although *N. diversicolor* may be a useful indicator of the biological availabilities of some metals in sediments, this does not necessarily reflect the availability of metals in the overlying water column (see Section 2B), and the use of this organism as an indicator does not seem justified.

7. CONCLUSIONS ON THE USE OF BIOLOGICAL INDICATORS OF METAL POLLUTION

Although published information on metal concentrations in organisms collected from the environment is growing rapidly, the lack of attention to sampling techniques may invalidate many of the conclusions concerning the relative

abundance of metals in different areas. Much attention is correctly paid to interlaboratory comparisons of analytical techniques in order to maximise the analytical accuracy of the results, but unfortunately no such attention is given to biological variables affecting the organism at the site of collection (see Folsom *et al.*, 1972).

Where variables such as season, age, weight and size of the individuals studied, sampling position of biota, salinity, water temperature, and the coexistence of several metals have been studied, significant effects on metal uptake and/or the metal concentrations found in organisms from the environment have been frequently noted. That such variables as salinity and water temperature affect the net uptake of trace metals by marine organisms is inferred from much of the available literature on the toxicities of metals in the laboratory. For example, increased toxicities of metals with increasing temperatures have been reported for zinc and *Salmo gairdneri* (Lloyd, 1960), cadmium and *Fundulus heteroclitus* (Eisler, 1971), copper and *Homarus americanus* (McLeese, 1974), and zinc, cadmium and lead and estuarine isopods (Jones, 1975). Decreased salinities were also correlated to increased toxicities by this last author. Bryan (1971) suggested that variables affecting the toxicity of metals may be acting directly by causing an increased net uptake of metals; if this is so, the effects of hydrological variables on surveys using biological indicators must be considerable. The results of MacLeod & Pessah (1973) and O'Hara (1973*a*, 1973*b*) support this contention. MacLeod & Pessah (1973) showed that the increased toxicity of mercury to *Salmo gairdneri* at higher temperatures was correlated to an increased uptake of mercury. O'Hara (1973*a*, 1973*b*) reported a similar parallelism of toxicity and net uptake of cadmium in studies of the fiddler crab *Uca pugilator*; both the uptake and toxicity of cadmium were maximal at high temperature and low salinity, and minimal at low temperature and high salinity. Although it is known that metal toxicities can be affected by factors other than those acting directly through an increased uptake of metal, some at least of the above effects must be caused by direct effects on the uptake of metals.

The effects of these variables must be considered in any indicator survey, as such variables can differentially affect some samples to produce a totally spurious conclusion regarding the relative abundance of trace metals in the study area. It is possible to eliminate several of these variables (e.g. season, age, size and weight of individuals, sampling position of biota) by the application of rigorously-controlled sampling procedures (see Phillips, 1976*a*). Other variables such as salinity or water temperature might be eliminated by the use of organisms from control or reference areas, although this is frequently difficult. In these cases, laboratory studies should be undertaken to elucidate at least qualitatively, if not quantitatively, the effects of such variables. The results of surveys using biological indicator organisms cannot be unambiguously interpreted unless the effects of all such variables are known.

The best-studied indicator types to date are undoubtedly the bivalve molluscs and the macroalgae. Amongst the former group, *Mytilus edulis* may be the best

candidate because of its extensively-studied physiology, its world-wide distribution in temperate waters (Hutchins, 1947) and the amount of accumulated knowledge concerning its uptake of metals and its metal content in various waters (Section 4 and Table 4). Both macroalgae and bivalves are certainly capable indicators of trace metal pollution in some conditions. In addition, their use together would be most beneficial, as not only might this eliminate some spurious conclusions due to the effects of environmental variables, but these indicator types reflect different portions of the total trace metal load on an ecosystem, and would, therefore, give more information than is available from a survey using one species alone.

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