The echinoids arbacia lixula and paracentrotus lividus as indicators of heavy metal levels in the Mediterranean environment

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THE ECHINOIDS *ARBACIA LIXULA* AND *PARACENTROTUS LIVIDUS* AS INDICATORS OF HEAVY METAL LEVELS IN THE MEDITERRANEAN ENVIRONMENT

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Dedication

To Peter Dohrn, in gratefulness for those

first, disconcerning, vital kicks.

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ABSTRACT

An investigation was carried out on the potential suitability of selected marine invertebrates, especially the echinoids Arbacia lixula and Paracentrotus lividus and the gastropod Patella coerulea as indicators of the abundance of some Heavy Metals in the coastal Mediterranean environment, with particular emphasis on their use in large scale monitoring with the collaboration of unskilled personnel. Samples were collected from 32 individually described sites around the Tyrrhenian Basin, and analyzed for Heavy Metal content. A Microwave digestion technique was experimented with, but found unsuitable. The analytical results are plotted, compared with other values appearing in the literature, and subjected to statistical correlation analysis with the described environmental and demographic data collected throughout the survey. No statistically and conceptually significant correlation is found between Heavy Metal content and any of the environmental variables, though some significant intermetallic correlations are found and discussed. A significant correlation between a described composite fauno-floristic index and environmental and demographic variables is found and its implications towards an alternative monitoring technique are discussed.

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The research presented in this thesis has been carried out in the Department of Botany of the University of Durham entirely by the author, except where specifically acknowledged in the text, and has not been previously submitted for a degree in this or any other University, nor has it previously been accepted for publication.

Throughout this study, bibliographical references have been sequentially grouped in chapter endnotes.

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The Question

Reasons for interest in heavy metals

It was Samuel Butler who claimed that all progress is based upon the universal, innate desire on the part of every organism to live beyond its means. If so, it could be that the time has come for industrial man to ask himself whether or not he will be able to afford to pay the bill of progress once it is presented.

Little doubt remains that man's pressure on his environments has reached critical proportions in a number of areas, but our ability in measuring this pressure, evaluating its impact, and predicting its effects is still far from satisfactory.

Possibly due to the greater time lag between cause and apparent effect, the degradation of the marine environment has come to the public attention relatively later than that of its terrestial counterpart, and even then mainly due to such highly publicized disasters such as the Minamata mercury poisonings, nevertheless, this late entry has not diminished the seriousness of the phenomenon (1). The debate is still open whether or not life in the sea as an entity is actually under direct threat, but enough examples have shown beyond reasonable doubt that localized environmental insults, acute or chronic, can have serious to disastrous effects both on the exposed marine communities and to the human communities directly or indirectly dependent on them.

A special place in the lengthy catalogue of hazardous substances industrial societies are pouring into the environment at an increasing rate belongs to assorted toxic heavy metals, both as salts and in their metallic forms. Found in industrial waste, in waste tip leachate, and in mine waste (2), they are not as visibly offensive as raw sewage, solid waste, or spilled hydrocarbons, and are therefore less likely to be spotted or made the target for public outcry before their effects become apparent. They are, however, toxic in fairly small quantities,

usually cumulative, and highly persistent (3,4,5,6). They are prone to biological cycling and enrichment which may greatly alter their distribution and toxicity (5,7,8,9), they act mostly as metabolic poisons - often essential at trace levels - and present a highly complex toxicology, involving as it does an assortment of sublethal effects, positive and negative synergisms between elements, and different patterns of absorption - and hence biochemical activity - according to the chemical species in which they are present (5,10,11,12,13,14,15,16). To this we may add the difficulty of their detection in the environment, requiring accurate measurements of very small quantities of the metal, usually present as an organometallic complex, whose detectability might vary with surrounding conditions such as local ionic balance and adsorption on nearby surfaces (17,18,19,20).

The toxicology of heavy metals has been studied and treated fairly extensively from the clinical and biochemical aspect. Considerably less seems to be known of their behaviour in the environment before their clinical symptoms become apparent in an insulted organism.

There has been, therefore, a certain degree of pressure for developing methodologies which allow the presence of heavy metals in the environment to be measured at sublethal levels, and an early warning to be given if these seem to point out increases which might be incompatible with environmental and human health.

One path towards this goal which has been abundantly followed has been that of using specific organisms, termed accumulators, which, by absorbing and accumulating pollutants in measurable quantities, can be used for monitoring their levels in the environment, levels which may themselves be unmeasurable or measurable only with difficulty (21,22,23). Another is that in which similarly sensitive organisms are used as indicators, not so much by measuring their pollutant content, but by

measuring the manner in which they react physiologically, behaviourally, and through the structure of the communities of which they are part, to varying levels of pollutants in their environment (24,25,26,27,28,29, 30,31,32,33,34).

A combination of these two approaches has been used in the past by workers in the University of Durham and elsewhere in developing a methodology by which the kelp forest and its associated communities can and has been used for the measurement of pollution levels in inshore waters. The work of Bellamy et al (28,29,30,31), Jones (35,32,33,34) and Moore (36) has led up to the use for this purpose of the community structure of the hapteron ectofauna of the kelp Laminaria hyperborea, subsequently investigated in depth by Sheppard (37).

The use of a uniform sampling unit such as the holdfast ectofauna of a single individual of Laminaria hyperborea, a unit easily collected by a SCUBA diver not otherwise trained in scientific techniques, opens considerable possibilities for large scale surveys based on the collaboration between amateur divers distributed over a considerable area and a centralized laboratory for analysis and data evaluation. The concreteness of these possibilities was demonstrated by Bellamy (38), when a study based on the simultaneous collection of kelp holdfasts along the better part of Britain's coastline was carried out with the assistance of the British Sub-Aqua Club, and confirmed in the recently completed Underwater Conservation Year, where the usefulness of amateur divers for the coordinated collection of data and observations for universities and research establishments on a variety of subjects has been abundantly vindicated.

This study was undertaken as an attempt to investigate the possibility of transferring this concept, viz. the use of a technique aimed at the monitoring of heavy metal levels in the inshore marine

environment, through the use of indicator organisms commonly found and easily collected in view of enlisting the help of amateur divers for its widespread use, to the Mediterranean environment.

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Reason for Mediterranean Study

The Mediterranean basin, essentially a closed system with only a slow and limited exchange of water with the Atlantic Ocean, and at the same time the sink of a good portion of Europe's wastes as well as an obligate waterway for a sizeable albeit decreasing section of the world's oil traffic, is almost obviously vulnerable to man-caused contamination. Until recently, however, very little work had been done on the subject, and this mainly insofar as organic and urban pollution was concerned in view of its pathological and epidemological hazards (39,40,41). By the mid -1970's the basin's state of chronic hydrocarbon pollution had become painfully visible, and ample studies, control programs, and relevant legislative proposals have been carried out or are in progress (42,43). Around the same time works started to appear regarding heavy metal pollution in various Mediterranean areas and its monitoring (44,45,46). The importance of this problem has by now been fully accepted, and the recently presented U.N.E.P. Mediterranean Action Plan (47,48,49) includes an extensive monitoring program based on the measurement of accumulated toxic metals in three indicator organisms, the striped mullet (Mullus barbatus Linn.), the mediterranean mussel (Mytilus galloprovincialis Lamark) and the bluefin tuna (Thunnis thynnus Thynnus Linn.) (50,51).

At present, however, the available heavy metal studies tend to concentrate on estimated pollution loads (39) or metals found in seawater and sediments. Also, the greatest effort has been aimed at the behaviour of mercury, possibly as a corollary to the interest it had given rise to in the early 1970's due to its public reputation as an environmental poison.

The direct Mediterranean equivalent of the kelp forest would have been the prairies of sea grasses, Posidonia oceanica and Zostera sp., but

unfortunately their thick continuous rhyzome mats, offering shelter not only to assorted ectophytes and ectozooids, but also accumulating large amounts of sediment with its concomitant burrowing and interstitial communities and supporting a large number of grazers and higher consumers, show neither the useful separation in distinct units nor the relative ecological simplicity of the kelp holdfast ectofauna. This, on the other hand, can for the purposes of this study be considered absent from the Mediterranean, as in these waters the Laminariaceae have been described only seldom and at depths which put them beyond the reach of the vast majority of amateur divers (52,53,54,55).

It is for this reason that it was decided to concentrate this study's efforts towards the use of a direct chemical analysis of one or more indicator species as a measure of heavy metal contamination.

At the beginning of this study, even though a number of works had already appeared concerning heavy metal levels in marine organisms, both as baseline studies (56,57,58) and as possible pollution indicators (59,60,61,62,63,64), almost no data was available on the distribution of heavy metals in mediterranean organisms potentially suitable as such. Renzoni, Bacci and Falciai (44) had measured mercury levels in a number of species along the Tuscan coast, Ibragin and Patin (46) had studied the effect of heavy metal contamination on phytoplankton communities, and Majori and Petronio (22) studied the accumulation in vitro of heavy metals in mussels. More significantly for this work's purpose, Navrot (45) measured heavy metal absorption in Patella vulgata along the Israeli coast, and a pilot study by Sheppard and Bellamy (65) in the gulfs of Naples and Salerno showed promising results in measuring the total heavy metal content of the echinoids Arbacia lixula and Paracentrotus lividus, the same species together with other echinoderms being used by Papadopolou (66) to measure trace and ultratrace elements in the Saronikos gulf.

Sea urchins are just about the most obviously common and easily recognizeable organisms along the Mediterranean coast; were they suitable as pollution indicators, wide scale simultaneous collection of samples could be easily accomplished by personnel possessing only the barest of technical knowledge, making routine monitoring a distinct possibility.

The original scope of this study, therefore, can be summed up as follows:

First: to investigate the suitability of certain invertebrates, in particular the echinoids Arbacia lixula and Paracentrotus lividus and the gast ropod Patella coerulea, as heavy metal accumulators and indicators in the Mediterranean area.

Second: to investigate their suitability towards a large scale, routine monitoring system involving the use of unskilled personnel for the sampling and preparation phase.

Third: to investigate an apparently rapid method of sample preparation which had recently appeared in the literature (67). This method, described below, appeared to be ideal for use in such an extensive monitoring system, in that it would allow a rapid appraisal of pollution levels, making localized intervention a practical and meaningful proposition.

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The Sampling

Choice of sites

Ideally, a random choice of sites covering a varied section of coastline facing a homogeneous basin would have been ideal, but not surprisingly a compromise had to be reached. Sampling sites had to present reasonably easy access, the number of samples to be subsequently analysed had to be kept to a reasonable number, time and manpower limitations took their toll during the sampling campaign proper. Still, as a rule of thumb, the original plan to sample the Tyrrhenian Basin with stations at about 100km intervals was by and large adhered to. Figure 1 and table 1 show the sampling site distribution and the species collected at each site respectively. The actual choice of sampling location depended mainly on practical considerations such as ease of access and whether or not echinoids were presumed to be present, if not actually visible from above the surface, on the other hand, a conscious effort was made to avoid as far as possible direct industrial outlets and sites which might have shown a highly uncharacteristic local concentration of pollutants. It may be added that by ease of access land access is meant, except for sites 2 (Secche della Meloria), 16 (Lipari I) and 18 (Filicudi) where a boat was both necessary and available. A higher frequency of sampling was carried out in the Castellabate region, to allow for any eventual comparisons with previous workers (65).

Whilst brief individual site descriptions appear amongst the appendices, their principal environmental characteristics have been summarized in tables 2 to 7.

Table 2 refers to the physical aspects of the coast at the sampling site, table 3 to the visible human impact upon it, while table 4 contains some of the demographic data available on the site area, as summarized in the 1973 TECNECO report (39). Tables 5 and 6 summarize the general nature of the sea bottom and of the sampled echinoid communities

Figure 1

Location of sampling sites

Key in Table 1

List of sites and species there collected

Table 1

		Arbacia lixula	Paracentrotus lividus	Patella coerulea	Venerupis decussata	Tellina distorta	Mytilus edulis
1	Porto Venere	X	X	X			
2	Secche della Meloria	X	X				
3	Quercianella		X				
4	Salivoli	X		X			
5	Talamone	X	X				
6	Ansedonia	X	X				
7	Anzio			X			
8	Anzio fish market				X	X	
9	Sorrento Marina Piccola	X	X	X			
10	Sorrento fish market				X		
11	Massa Lubrense	X	X	X			
12	Agropoli	X	X	X			
13	Punta Tresino	X	X	X			
14	Punta Inferno	X	X	X			
15	Ogliastro Marina	X	X	X			
16	Lipari I	X	X				
17	Lipari II	X	X	X			
18	Filicudi	X	X	X			
19	Punta Raisi	X	X				
20	Capo Carbonara	X	X				
21	Melisenda	X	X				
22	Arbatax	X	X	X			
23	Fuile e Mare	X	X				
24	Golfo Aranci	X	X				
25	S. Teresa di Gallura	X	X	X			
26	Messina						
27	Antibes						
28	Marseilles - Cortiou						X
29	Marseilles - Mongenet			X			X
30	Malta - Fort St. Lucian		X	X			
31	Malta - Paradise Bay	X	X	X			
32	Malta - Wied iz Zurrieg	X	X				
33	Cyprus - Akrotiri	X					
34	Malta - Unspecified		X				

Coast Characteristics

Table 2

SITE	Site number
EXPOSURE	Degree of exposure of coast tract
ACCESS	Ease of access of site from land
FRESHW	Are there freshwater outlets in the vicinity?
COASTNAT	Basic nature of coast at waterline
PHISIOG	Basic physiography of coast

SITE	EXPOSURE	ACCESS	FRESHW	COASTNAT	PHYSIOG
1	SHELTERED	EASY	NO	MAINLY SAND	SLOPING
2	V.EXPOSED	V.DIFF.	NO	MIXED	FLAT
3	EXPOSED	EASY	NO	MIXED	SLOPING
4	SHELTERED	EASY	NO	MIXED	SLOPING
5	EXPOSED	EASY	NO	MAINLY SAND	CLIFFS
6	EXPOSED	DIFF.	YES	MIXED	CLIFFS
7	EXPOSED	EASY	NO	MAINLY ROCK	FLAT
9	SHELTERED	EASY	NO	MIXED	CLIFFS
11	SHELTERED	EASY	NO	MAINLY SAND	CLIFFS
12	SHELTERED	EASY	YES	MAINLY SAND	CLIFFS
13	SHELTERED	DIFF.	NO	MAINLY SAND	CLIFFS
14	EXPOSED	EASY	NO	MIXED	SLOPING
15	EXPOSED	EASY	YES	MIXED	SLOPING
16	EXPOSED	V.DIFF.	NO	MAINLY SAND	CLIFFS
17	EXPOSED	EASY	NO	MAINLY SAND	CLIFFS
18	V.EXPOSED	V.DIFF.	NO	MAINLY SAND	CLIFFS
19	EXPOSED	EASY	NO	MIXED	FLAT
20	EXPOSED	DIFF.	NO	MIXED	SLOPING
21	V.EXPOSED	V.DIFF.	NO	MAINLY SAND	SLOPING
22	EXPOSED	EASY	YES	MIXED	CLIFFS
23	EXPOSED	V.DIFF.	YES	MAINLY ROCK	FLAT
24	SHELTERED	DIFF.	NO	MIXED	SLOPING
25	EXPOSED	EASY	NO	MIXED	SLOPING

Key to table 3

HARBOUR	Are there harbours in the vicinity?
FISHPORT	Are there fishing harbours in the vicinity?
OILTERM	Are there oil terminals or refineries in the vicinity?
INDEV	Are there industrial developments in the vicinity?
INURB	How much of the coast has been built up?
QUARRY	Are there quarries in the vicinity with waste disposal at sea?
SEWAGE	Are there significant urban sewage discharges in the vicinity?
WASTE	Are there significant discharges of industrial wastes in the vicinity?

Human Impact onto Coast, by visual inspection

SITE	HARBOUR	FISHPORT	OILTERM	INDEV	INURB	QUARRY	SEWAGE	WASTE
1	PRESENT	PRESENT	-	ABUNDANT	>2/3	N.A.	N.A.	PRESENT
2	PRESENT	-	-	ABUNDANT	>2/3	-	-	PRESENT
3	-	-	PRESENT	-	<1/3	-	-	-
4	PRESENT	PRESENT	-	ABUNDANT	>2/3	PRESENT	PRESENT	PRESENT
5	-	PRESENT	-	-	>1/3<2/3	-	-	-
6	-	-	-	-	<1/3	-	PRESENT	-
7	-	PRESENT	PRESENT	-	<1/3	PRESENT	PRESENT	-
9	PRESENT	PRESENT	PRESENT	-	>2/3	-	PRESENT	-
11	-	-	-	-	>1/3<2/3	-	PRESENT	-
12	-	PRESENT	-	-	>2/3	PRESENT	PRESENT	-
13	-	-	-	-	<1/3	-	-	-
14	-	PRESENT	-	-	>2/3	-	PRESENT	-
15	-	-	-	-	>1/3<2/3	-	-	-
16	-	-	-	-	<1/3	-	-	-
17	-	PRESENT	-	-	>1/3<2/3	-	-	-
18	-	-	-	-	<1/3	-	-	-
19	-	PRESENT	PRESENT	PRESENT	<1/3	-	-	PRESENT
20	-	-	-	-	>2/3	PRESENT	-	-
21	-	-	-	-	<1/3	-	-	-
22	PRESENT	PRESENT	PRESENT	PRESENT	>2/3	PRESENT	-	PRESENT
23	-	-	-	-	<1/3	-	-	-
24	PRESENT	PRESENT	PRESENT	PRESENT	<1/3	-	PRESENT	PRESENT
25	-	PRESENT	PRESENT	PRESENT	<1/3	-	-	-

Table 3

Table 4

TECNECO demographic information (Tecneco 1973) (39)

POP Population in inhabitants per square kilometer

ESTPOLL Estimated pollution load in equivalent inhabitants per square kilometer

INLEV Tecneco Industrialization index:

5 Commune with over 100 inhabitants per square kilometer, over 1000 employed in industry, and with an average of over 10 employees per industry.

4 As above, but with an average of less than 10 employees per industry.

3 Surrounding Comuni, not qualifying under (5) or (4), but with over 15% of the population employed in industry.

2 As (3), but with between 10 and 15% of the population employed in industry.

1 Not qualifying under any of the above.

SITE	POP	ESTPOLL	INLEV
1	>1000	501-1000	5
2	>1000	501-1000	5
3	151-250	501-1000	5
4	251-350	501-1000	5
5	51-150	<200	1
6	51-150	<200	1
7	501-750	>2500	2
9	>1000	>2500	1
11	>1000	12500	1
12	251-350	201-500	1
13	51-150	201-500	1
14	51-150	201-500	1
15	51-150	201-500	1
16	51-150	<200	1
17	51-150	<200	1
18	51-150	<200	1
19	351-500	201-500	1
20	>50	<200	1
21	>50	<200	1
22	151-250	<200	5
23	>50	<200	1
24	51-150	<200	1
25	>50	<200	1

Nature of bottom, by visual inspection

SITE	CONCRETE	ROCK	SAND	MUD	PEBBLE	POSIDONIA
1	-	PRESENT	-	ABUNDANT	-	-
2	-	-	PRESENT	-	-	ABUNDANT
3	-	ABUNDANT	-	-	PRESENT	-
4	PRESENT	PRESENT	ABUNDANT	-	-	-
5	-	ABUNDANT	-	-	PRESENT	-
6	-	ABUNDANT	ABUNDANT	-	PRESENT	-
7	PRESENT	ABUNDANT	ABUNDANT	-	-	-
9	PRESENT	ABUNDANT	ABUNDANT	-	-	-
11	ABUNDANT	ABUNDANT	ABUNDANT	-	-	PRESENT
12	-	ABUNDANT	ABUNDANT	-	-	-
13	-	ABUNDANT	ABUNDANT	-	-	ABUNDANT
14	PRESENT	ABUNDANT	PRESENT	-	-	-
15	-	ABUNDANT	ABUNDANT	-	-	ABUNDANT
16	-	ABUNDANT	-	-	-	-
17	-	ABUNDANT	-	-	-	-
18	-	ABUNDANT	PRESENT	-	-	-
19	-	ABUNDANT	ABUNDANT	-	-	-
20	-	ABUNDANT	ABUNDANT	-	-	-
21	-	ABUNDANT	PRESENT	-	-	PRESENT
22	-	ABUNDANT	-	-	-	-
23	-	ABUNDANT	ABUNDANT	-	-	ABUNDANT
24	-	ABUNDANT	ABUNDANT	-	-	ABUNDANT
25	-	ABUNDANT	ABUNDANT	-	-	PRESENT

Table 5

Echinoid community data — Table 6

TECHFR — Total frequency of echinoids, visual estimate

RECHFR — Relative echinoid frequency (A : *Arbacia lixula*, P : *Paracentrotus lividus*) - Visual estimate

ECHCOM — General appearance of two echinoid communities, visual estimate

SITE	TECHFR	RECHFR	ECHCOM
1	V.ABUND.	A < P	SEPARATED
2	V.ABUND.	A = P	SEPARATED
3	V.ABUND.	P ONLY	N.A.
4	ABUND.	P ONLY	N.A.
5	PRESENT	A = P	SEPARATED
6	PRESENT	A > P	MIXED
9	ABUND.	A >> P	MIXED
11	V.ABUND.	A >> P	MIXED
12	ABUND.	A < P	CLUSTERED
13	PRESENT	A = P	MIXED
14	V.ABUND.	A = P	MIXED
15	ABUND.	A < P	MIXED
17	ABUND.	A = P	SEPARATED
18	V.ABUND.	A < P	MIXED
19	ABUND.	A = P	MIXED
20	RARE	A < P	MIXED
21	RARE	A < P	MIXED
22	ABUND.	A > P	SEPARATED
23	V.ABUND.	A << P	SEPARATED
24	PRESENT	A < P	SEPARATED
25	ABUND.	A = P	SEPARATED

Table 7

Facies distribution on sites according to the modified Ehrhardt classification described in the text (68), as well as the composite index described in appendix 5.

A = Abundant

P = Present

Absent and Scarce values omitted

Site	1	2	3	4	5	6	7	8	9	10	11	12	FACTOT
1	P	-	-	-	-	-	-	-	A	-	-	-	38
2	-	A	-	-	-	-	-	-	-	-	-	A	6
3	-	-	A	-	-	-	-	-	-	-	-	-	9
4	P	-	-	-	-	-	-	A	P	-	-	-	56
5	-	-	-	-	-	A	-	-	-	-	-	-	21
9	-	-	-	-	-	A	A	-	-	-	-	-	48
11	P	-	P	-	-	A	-	-	-	-	-	P	29
12	-	-	P	-	-	P	-	-	-	-	-	-	20
13	P	A	-	P	-	-	-	-	-	-	-	A	16
14	-	-	P	P	-	P	P	-	-	-	-	-	46
15	-	-	-	P	-	A	-	-	-	-	-	A	29
17	P	P	P	P	-	P	-	-	-	-	-	-	34
18	-	-	P	-	-	-	-	-	-	-	-	-	6
19	-	-	A	P	-	P	-	-	-	-	-	-	31
20	P	-	-	-	-	A	-	-	-	-	-	-	23
21	A	-	-	-	-	-	-	-	-	-	-	P	3
22	-	-	A	-	-	-	A	-	-	-	-	-	36
24	P	-	-	P	-	-	A	-	-	-	-	P	37
25	A	-	-	P	-	P	-	-	-	-	-	P	25

respectively, and table 7 shows the distribution, in order of increasing environmental insult - except for facies 12 - of the indicator communities used by Ehrhardt (68) to estimate organic pollution. A full description of these appears in appendices 2 and 5.

References

65) Sheppard, C.R.C. and Bellamy, D.J. (1974) Pollution of the Mediterranean around Naples Mar. Poll. Bull. 5 (3) 42-44.

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Sampling method

In view of the envisaged use of the method by amateur divers, as simple and standardized method of sampling as possible had to be used. Unfortunately, this ruled out the possibility of developing a uniform random sampling procedure *a priori*, as too many unknown variables would have been present at each site, such as ease of access, nature of bottom, and echinoid frequency.

Sheppard and Bellamy (65) had used up to 40 individual organisms per site, each constituting a sample. In this case the greater number of sites and species to be investigated made such a large sample size unrealistic in terms of time and effort required for their analysis. For this reason, an arbitrary number of ten samples per species per site was settled upon.

In the first sites visited, single individual organisms were collected as samples. It soon became apparent, however, that while an average individual of *Paracentrotus lividus* would furnish more than enough material for analysis, the same could not be said for *Patella coerulea*. So as to avoid problems during the analytical stage of the work due to insufficient material, it was arbitrarily decided to consider a valid sample a minimum of 10ml of wet tissue irrespective of how many individuals had to be bulked to reach such a volume.

On reaching a site, a sweep was carried out using basic diving equipment, covering as much bottom area as necessary to collect the required ten samples per species. The area covered varied widely, never exceeding 500 m^2, being generally a lot smaller, and at times only a few square meters of bottom would yield sufficient material.

The samples were opened immediately after collection using a stainless steel knife, and the soft parts extracted using a stainless steel scoop. These were stored at ambient temperature in snap top

polistyrene tubes, previously acid soaked in diluted HCl, together with 10ml of ethanol (Merck Pro Analysis grade, description in appendix) added as a preserving agent.

No attempt was made to separate individual organs or to eliminate the gut content, as this would have required, especially for Arbacia lixula, a lengthy dissection, probably beyond a layman's capacity.

References

65) Sheppard, C.R.C., Bellamy, D.J. (1974) Pollution of the Mediterranean around Naples Mar. Poll. Bull. 5 (3) 42-44.

The Analysis

Sample preparation

As mentioned in the introduction, it had been amongst the original intentions to try to apply a novel analytical technique (67) so as to reduce the time lag between sampling and availability of results.

The identification and measurement of heavy metals in biological tissues is certainly not a new problem, but doubts are still being raised about the methods currently in use, both as to their accuracy and as to the practical value of the results they produce. Without listing the assorted available techniques, adequately described elsewhere (69,70,71,72), suffice it to say that for the purposes of this particular study Flame Atomic Absorption Spectrometry was the method of choice due to the availability of appropriate instrumentation, namely a Perkins-Elmer 403 atomic absorption spectophotometer. Most previous work in Durham had been carried out on this instrument, and it was felt that regardless of the recognized uncertainties of the method (69,73), the existing experience would have allowed the comparison of eventual results with these past works. The one aspect of the analytical procedure which was open for experimenting upon was the sample preparation stage.

The transformation of a specimen of organic tissue into a substance suitable for trace element analysis without intervening losses has been recognized as a serious problem (74), admirably investigated by Gorsuch (75,76), and until very recently has remained an unavoidable one for all methods of optical absorption analysis. The technique used in Durham in the past had been a modification of the one described by Ulrich (77) involving wet flame sequential digestion with nitric and perchloric acid.

The use of a method involving the handling of perchloric acid at high temperatures had been for some time felt to be unsuitable in a

standard procedure which would probably have to be carried out routinely by personnel of varying degrees of skill. Perchloric acid is a highly 'temperamental' substance which, though used abundantly in this context, has occasionally been the cause of accidents of quite spectacular destructiveness (76,78,79). Unfortunately, no digestion mixture lacking it seems to give as good a recovery, particularly as far as lead is concerned. Already in the past attempts had been made in these laboratories to use less potentially hazardous techniques, such as dry ashing as described by Ulrich (77,80, Waughman, J., Personal Communication), without however obtaining satisfactory results given the existing facilities. A low temperature / medium pressure system involving small quantities of perchloric acid was described by Adrian (81,82) and used by Genakos (83), but for reasons to be mentioned below is still felt to be somewhat suspect. At the outset of this study, a method published by Abu-Samra et al (67) had struck the author as having a considerable potential in this direction, as it allowed the use of perchloric acid in quantities and under conditions which offered considerable safety and yet allowed a greatly increased throughput compared to the methods currently in use, as well as requiring only very small amounts of sample material. At the same time, the application of the Safety at Work Act to University laboratories made the search for a safe alternative technique a matter of some urgency.

This technique consisted in wet ashing the sample material in a 4:1 nitric acid / perchloric acid mixture, using a modified commercial microwave oven fitted with a fume extractor.

The equipment was reproduced using a Tappan 56-0455 microwave oven, fitted with a perspex liner connected to a fume scrubbing system and an extractor fan. Considerable technical difficulties were met both with the oven, which was found to emit a high level of stray microwaves

due to the outer casing resonating with the magnetron, and with the liner and scrubber unit which, apart from being inadequate for the amount of fumes produced, was soon corroded beyond repair. Apart from this, a series of test runs using glucose and homogenized cod muscle tissue gave results, cited in appendix 3, which seem to cast strong doubts on the chemical basis of the method.

Heavy metal values, measured on the cod tissue and compared to those obtained using the traditional method, appeared low, widely erratic, and roughly proportional only to the actual sample weight with a large mass of crystals appearing in the reaction vessels on cooling. Similar crystals appeared in even greater quantities in the digested glucose samples, which would have been expected to be totally dissociated in an efficient digestion technique.

The matter was usefully discussed with Dr. D.G. Othen, then of Durham University Chemistry Department, and on analysis the residual crystals were shown to be oxalic acid dihydrate. From this it was deduced that the use of a commercial microwave oven emitting at 915 MHz and at 2.45 GHz meant that a massive drop in energy absorption took place once the majority of the water present was lost, and before oxidation by the perchloric acid could set in. Moreover, since the technique seemed to give incomplete digestion at a temperature presumably not much higher than the boiling point of water, the Adrian method (81,82), relying on a pressure increase of a few atmospheres and the temperature of boiling water or less, also came under suspicion, though no detailed comparison was carried out.

In view of these difficulties and the very considerable amount of time taken up by them, it was decided to carry out the project using the traditional modified Ulrich (77) method, after altering the procedure and the facilities so as to comply with the more severe safety standards

(76,78,79).

The samples in etanol had been dried out at 45°C as soon as they had been conveyed to the laboratory, and stored in a silica gel dessicator. The dry samples were ground in a mortar, homogenized, and aliquots were weighed out in the reaction vessels. An analytical sample weight of 0.5g was aimed at, but quite frequently the amount of material available did not allow this, and at times bulking of samples became necessary to obtain an analyzeable quantity. Whenever possible, which was in over two thirds of the cases, samples were analyzed in duplicate.

The reaction vessels were spouted wide-necked 125ml Pyrex conical flasks, which had originally been prepared for the microwave digestion technique, but which proved themselves more efficient to the purpose than the normally used conical beakers, possibly due to the increased reflux effect of the narrower neck.

The samples were drenched with 10ml of fuming nitric acid, BDH AnalaR grade, and left to predigest for at least 12 hours. Subsequently, 2ml of concentrated perchloric acid, BDH AnalaR grade, were added and the samples, in batches of 40, were heated to boiling on a sand bath inside a suitable fume cupboard. The temperature was controlled so as to keep the samples just simmering.

The process had to be closely watched, and the operator had to be ready to quench the reaction with distilled water at the moment in which the perchloric acid oxidation began, noticeably at the sudden appearance of dense white perchlorate fumes. This procedure was repeated until the solution turned clear and changed from yellow-brown to colourless or just straw yellow. The single flasks were removed at this point and allowed to cool. The clear digest was filtered through a Whatman no. 41 filter paper so as to remove silica particles, made up to 50ml with

distilled water, and stored in polythene or polypropylene bottles which had been, as all other glassware used in the digestion, abundantly leached in hydrochloric acid so as to reduce as far as possible the effect of vessel surface contamination and adsorption.

References

67) Abu-Samra, A., Morris, J., Koirtyohann, S.R. (1975) Wet ashing of some biological samples in a microwave oven Analytical Chemistry, 47, (8) 1475.

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82) Adrian, W.J. (1973) A comparison of a wet pressure digestion method with other commonly used wet and dry-ashing methods The Analyst 98 213-216.

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Analytical Technique

The digests were analyzed for Zinc, Copper, Lead and Cadmium. Originally there had been the intention of measuring the levels of Mercury and Selenium as well. This idea was eventually abandoned because it was felt that the use of a digestion involving a lengthy period of boiling without recovery of fumes would have led to an almost complete loss of these two elements, both particularly prone to forming volatile compounds under these conditions (75, 76). Also measured were the levels of Sodium, Potassium, Calcium and Magnesium. Except for Sodium for which an EEL single beam atomic absorption spectrophotometer was used, all other elements were analysed on a Perkins-Elmer 403 double beam atomic absorption spectrophotometer. In all cases, an air/acetylene flame was used.

The instruments were zeroed on distilled water, and the obtained readings were corrected for concentration response curve, sample weight, chemical blanks, and matrix interference by writing an appropriate subset of data modification commands in the SPSS software package used for the subsequent statistical analysis of the results (84). Standard matrix interference correction factors as appearing in table 8 were used, factors previously used and found valid on the same instrument under similar conditions, and confirmed by a number of control checks throughout the analyses.

The replicate values were averaged unless, by visual inspection, one of the readings was found to differ significantly both from the other replicate and from other readings of the same nature and site, in which case it was deleted. The results were finally grouped for site and species, and group averages obtained.

Table 8

The Matrix represents the positive interference of one part of alkaline metal in terms of parts detected of heavy metal.

	Na	K	Ca	Mg
Zn	2.0×10^{-6}	3.3×10^{-6}	15.4×10^{-6}	74.0×10^{-6}
Cu	44.0×10^{-6}	3.6×10^{-6}	12.0×10^{-6}	6.9×10^{-6}
Pb	17.0×10^{-6}	20.0×10^{-6}	144.0×10^{-6}	100.0×10^{-6}
Cd	8.4×10^{-6}	2.3×10^{-6}	21.3×10^{-6}	2.9×10^{-6}

References

75) Gorsuch, T.T. (1959) Radiochemical investigation on the recovery for analysis of trace elements in organic and biological material The Analyst, 84, 135-173.

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The Results

The results obtained are shown in tables 9 to 17 and in figures 2 to 13. The tables give the number of analyzed samples per site, the average metal content in ppm, and the coefficient of variation for each value. The figures are a graphical representation of the Heavy Metal results for the three principal species studied, Arbacia lixula, Paracentrotus lividus, and Patella coerulea, the plotted values being a linear expansion of the scale shown in the upper right hand corner.

So as to make a generalized comparison between sites possible without giving any single element an undue weighing, the Total Heavy Metal Index concept as used by Sheppard (37) was applied, where the sites are ranked separately in descending order of concentration for each element measured, and an average of ranks is taken for each site, this being considered as a general ranking index giving a measure of the total heavy metal load per species per site. Tables 18 to 20 show the individual element rankings and the Total Heavy Metal Index for Arbacia lixula, Paracentrotus lividus, and Patella coerulea respectively, and figures 14 to 16 give a graphical representation of the Total Heavy Metal Index for the same species.

Result tables

Coding:	SITE	Number of site as in Table 1
	nX	Number of individual samples per site
	$\bar{x}X$	Average detected ppm of X per species per site
	cX%	Coefficient of Variation (as percentage)

Arbacia lixula

Table 9

Alkaline Metal Results

Site	nNa	x̄Na	cNa%	nK	x̄K	cK%
1	8	43014.3	35	8	11527.6	23
2	10	31964.7	27	10	7712.2	24
4	10	36728.2	25	10	12274.8	35
5	11	33275.7	18	11	12778.3	20
6	9	25297.3	21	9	10664.8	33
9	10	33589.9	16	10	11702.0	23
11	10	40717.7	27	10	10099.3	17
12	10	37191.4	23	10	11261.8	24
13	8	26656.5	44	8	6722.8	21
14	10	33258.8	41	10	13346.9	17
15	6	30283.5	23	6	7210.1	12
16	7	49758.9	14	7	14726.2	11
17	10	43753.7	12	10	16971.4	12
18	10	41932.6	24	10	11900.0	17
19	12	26851.5	40	12	7430.8	41
20	10	31406.1	34	10	8151.2	54
21	8	41135.2	24	8	12037.4	20
22	10	39356.2	18	10	15301.3	13
23	10	42865.0	12	10	12443.2	13
24	10	65776.0	17	10	14723.6	14
25	9	33987.4	12	9	12221.5	24
31	4	36755.5	10	4	19595.8	7
32	3	45964.3	1	3	21514.5	8
33	17	43128.7	18	17	7155.0	21

Site	nCa	x̄Ca	cCa%	nMg	x̄Mg	cMg%
1	8	119010.3	41	8	11019.5	25
2	10	203140.2	15	10	10402.0	16
4	10	59059.3	63	10	10916.2	33
5	11	111322.9	49	11	15028.9	36
6	9	128740.3	28	9	11177.2	19
9	10	143675.1	21	10	18115.2	16
11	10	167118.9	18	10	10809.6	10
12	10	122409.9	32	10	16232.2	20
13	8	146379.9	22	8	16620.0	16
14	10	45786.1	44	10	10373.6	24
15	6	127986.4	23	6	15969.4	22
16	6	54896.6	35	7	13745.7	15
17	10	33077.3	42	10	10561.6	18
18	10	138688.5	39	10	11656.9	31
19	8	155348.7	63	12	16642.1	32
20	1	106394.3	46	10	12141.5	34
21	8	62028.2	51	8	12006.6	15
22	10	61886.2	36	10	11068.0	16
23	10	77285.8	39	10	13122.8	19
24	10	75937.3	29	10	14277.5	22
25	9	98844.2	23	9	11026.2	13
31	4	25166.1	65	4	7535.9	15
32	3	6434.9	31	3	7659.6	1
33	17	152817.5	21	17	13433.9	17

Arbacia lixula

Table 10

Heavy Metal Results

Site	nZn	xZn	cZn%	nCu	xCu	cCu%
1	7	109.773	51	7	11.413	30
2	10	70.517	44	10	11.217	36
4	10	98.609	53	9	7.918	48
5	11	79.612	48	11	9.372	21
6	9	62.151	48	9	5.441	21
9	10	54.767	44	10	8.934	19
11	9	98.265	77	10	6.350	10
12	10	47.448	40	10	4.023	30
13	8	34.139	23	8	4.514	16
14	10	78.386	76	10	6.343	24
15	6	55.073	30	6	7.955	18
16	6	71.822	44	6	8.132	26
17	10	124.549	49	10	7.651	19
18	10	98.416	60	9	8.925	34
19	8	63.225	33	8	6.910	16
20	10	84.771	65	10	4.562	17
21	8	132.733	40	8	7.212	19
22	10	143.571	54	10	6.906	22
23	10	113.094	52	10	6.356	13
24	10	190.921	37	10	5.672	10
25	8	188.523	39	8	6.454	30
31	4	102.522	9	4	11.517	45
32	2	228.095	28	3	11.263	23
33	17	251.614	50	17	10.278	23

Site	nPb	xPb	cPb%	nCd	xCd	cCd%
1	7	51.147	46	7	10.013	36
2	10	76.701	31	10	14.366	10
4	10	25.385	36	10	2.748	90
5	11	29.880	22	11	20.126	24
6	9	10.759	24	9	9.531	32
9	10	29.754	17	10	8.129	24
11	10	34.463	13	10	15.517	15
12	10	28.481	34	10	12.071	26
13	8	41.058	16	8	11.268	23
14	10	20.510	37	10	2.130	34
15	6	43.174	29	6	16.274	40
16	6	20.115	23	6	11.707	33
17	10	24.793	36	10	12.558	17
18	10	26.616	20	10	15.542	31
19	8	33.035	44	8	18.732	15
20	10	24.384	39	10	11.068	21
21	8	24.108	25	8	10.650	19
22	10	19.532	24	10	8.739	11
23	10	24.716	30	10	12.042	30
24	10	18.843	23	10	10.322	15
25	8	19.091	30	8	9.205	22
31	4	23.995	106	4	18.802	11
32	2	7.881	1	3	17.693	22
33	17	47.028	39	17	14.832	14

Figure 2

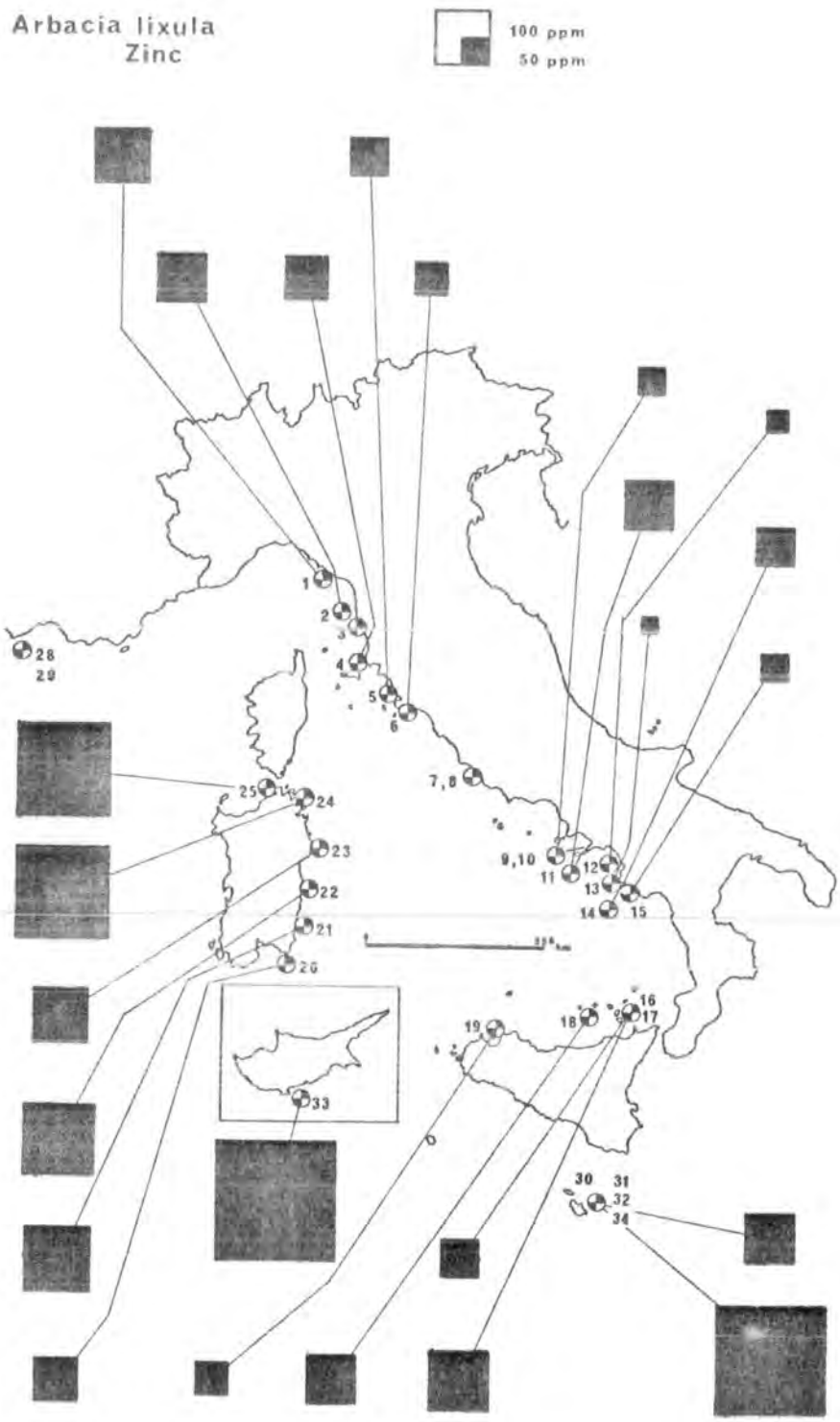
Arbacia lixula
Zinc
100 ppm
50 ppm
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Figure 3

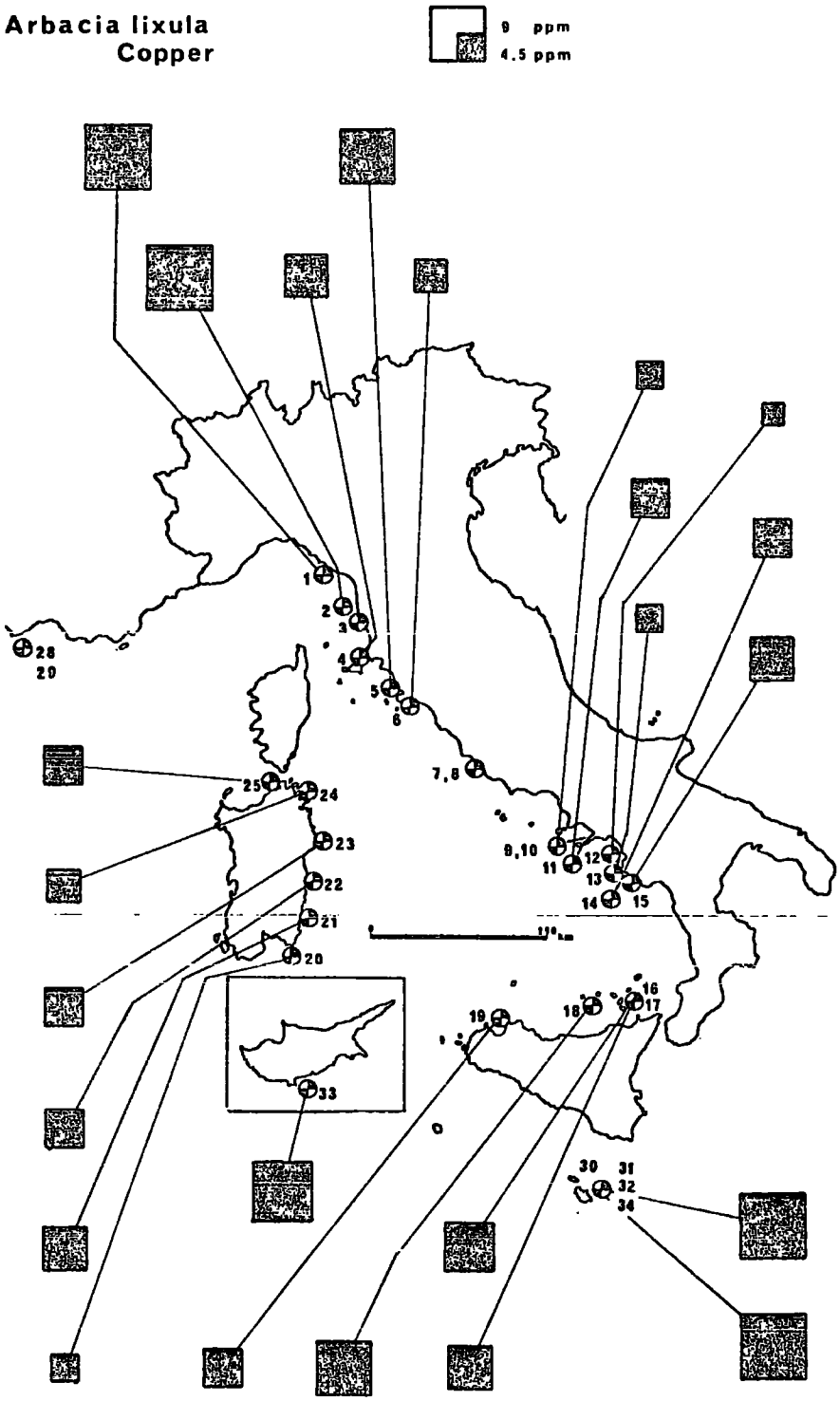
Arbacia lixula
Copper
9 ppm
4.5 ppm
1
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Figure 4

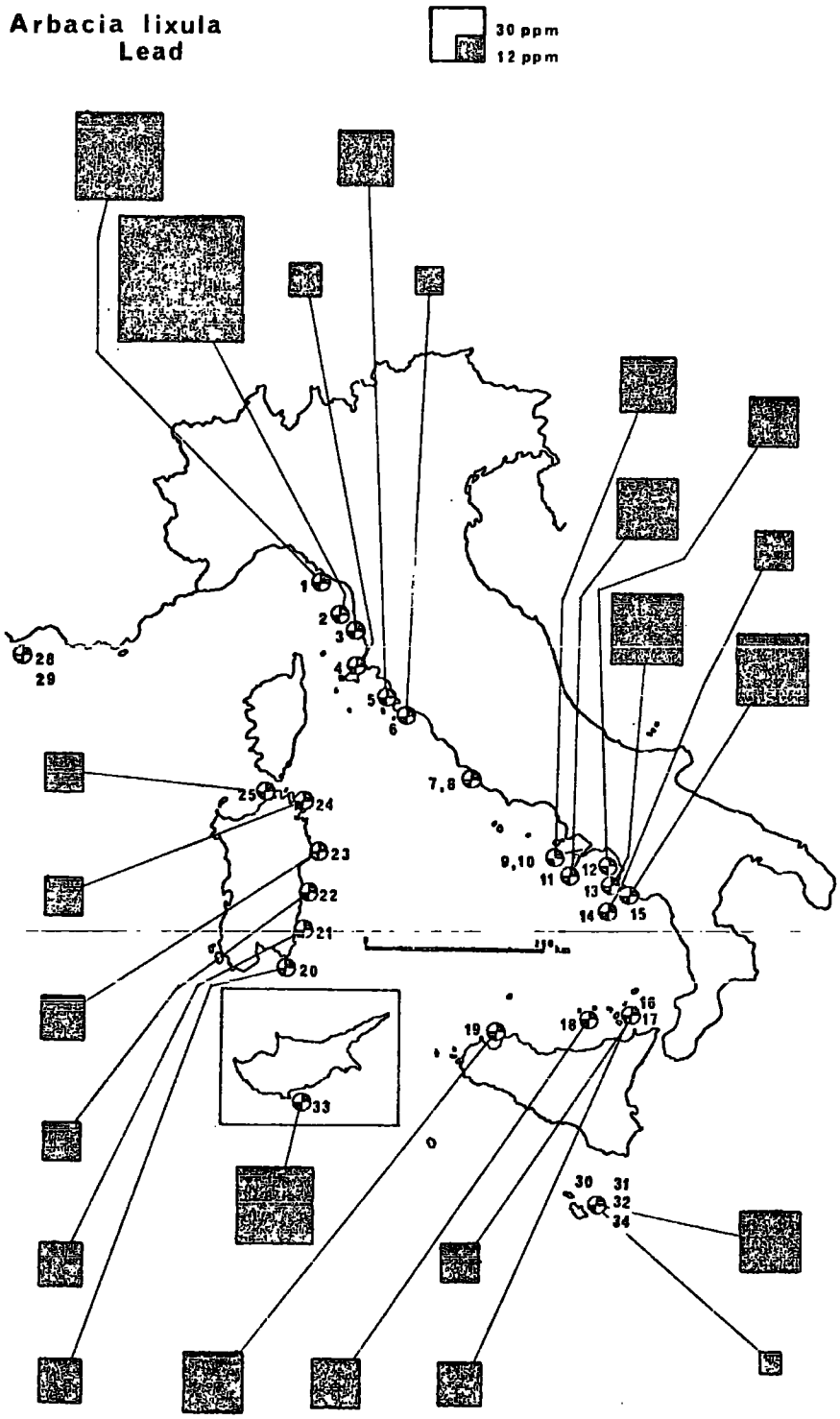
Arbacia lixula
Lead
30 ppm
12 ppm
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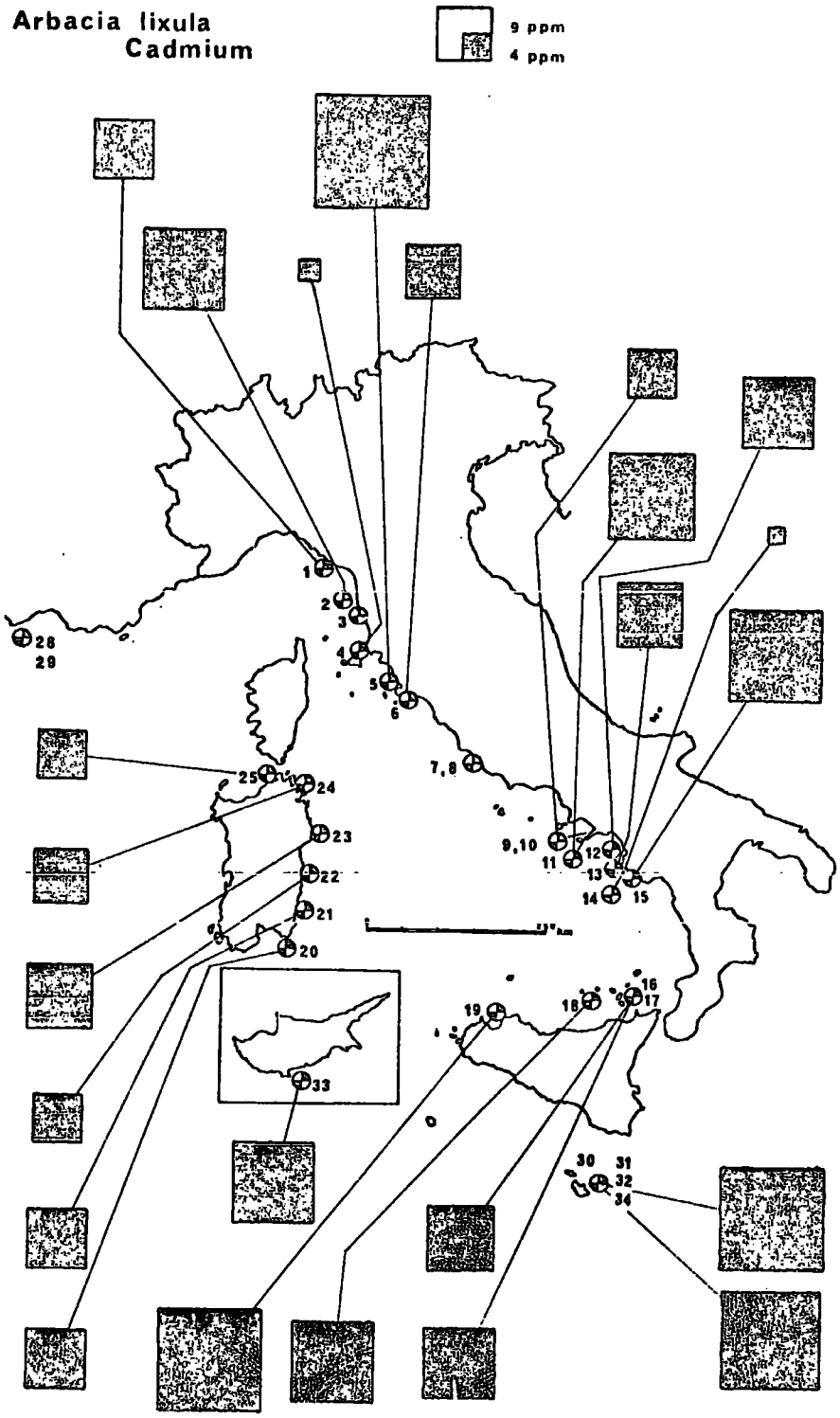
Figure 5


Arbacia lixula
Cadmium
9 ppm
4 ppm
1
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Paracentrotus lividus

Table 11

Alkaline Metal Results

Site	nNa	$\bar{x}$Na	cNa%	nK	$\bar{x}$K	cK%
1	9	61752.7	14	9	11123.9	38
2	10	71939.0	11	10	11825.9	16
3	10	57747.1	23	10	12433.5	20
5	9	57318.9	24	9	10331.0	26
6	8	42244.1	12	8	11620.1	20
9	5	44474.1	40	5	12578.4	25
11	10	54048.0	44	10	11278.2	19
12	10	31711.4	36	10	10683.1	19
13	9	35918.3	49	9	10161.1	23
14	7	40216.6	24	7	9517.7	32
15	10	64313.4	14	10	8570.3	25
16	10	48567.2	23	10	12712.3	27
17	12	58124.3	23	12	14055.4	20
18	10	68706.7	21	10	10493.8	21
19	10	74559.1	17	10	8265.3	22
20	10	63192.9	24	10	7363.7	22
21	10	66338.0	14	10	9479.8	17
22	10	58100.5	12	10	10922.3	14
23	10	60683.6	21	10	11059.9	19
24	10	61491.3	18	10	11896.8	9
25	10	60909.3	21	10	8122.0	18
30	3	25274.6	11	3	17794.0	6
32	1	31446.2	0	1	17036.8	0
34	2	29587.2	7	2	17622.9	4

Site	nCa	$\bar{x}$Ca	cCa%	nMg	$\bar{x}$Mg	cMg%
1	9	42130.1	36	9	12156.5	14
2	10	53842.7	35	10	13132.8	8
3	10	97926.9	37	10	16851.6	19
9	5	95230.0	53	5	14901.2	25
11	10	89644.2	31	10	11093.7	18
12	10	88668.8	32	10	14620.3	21
13	9	70120.1	49	9	12318.4	24
14	7	112656.6	25	7	14386.9	19
15	10	44298.4	43	10	13167.1	28
16	9	133805.3	233	10	16168.5	29
17	12	35207.0	53	12	13203.1	19
18	10	59214.1	36	10	14531.6	18
19	10	99520.2	36	10	17413.2	16
20	10	66197.9	40	10	15838.3	9
21	10	50640.5	34	10	16046.5	9
22	10	56823.1	47	10	13594.3	15
23	10	59744.7	18	10	13838.2	8
24	10	34896.2	15	10	12868.5	9
25	10	82086.4	30	10	14050.9	9
30	3	2429.4	57	3	6996.5	10
32	1	2320.3	0	1	7374.9	0
34	2	14586.6	115	2	6371.7	9

Paracentrotus lividus

Table 12

Heavy Metal Results

Site	nZn	$\bar{x}$Zn	cZn%	nCu	$\bar{x}$Cu	cCu%
1	9	226.175	75	9	20.222	23
2	10	128.282	19	10	13.112	32
3	9	103.138	53	10	15.931	28
5	9	53.016	38	9	12.982	32
6	8	98.956	35	8	13.097	20
9	5	117.469	83	5	8.969	14
11	10	58.490	49	10	5.961	35
12	10	83.971	81	10	5.111	23
13	9	116.948	92	9	6.279	44
14	7	64.525	23	7	6.778	16
15	10	106.028	63	10	7.346	31
16	9	58.748	103	9	5.554	19
17	11	67.624	58	12	7.694	22
18	10	50.058	61	10	5.727	27
19	10	58.253	42	10	5.764	15
20	10	47.445	58	10	4.543	22
21	10	75.506	96	10	8.151	30
22	10	70.613	37	10	8.823	24
23	10	51.192	40	10	6.046	9
24	10	37.825	29	10	4.354	20
25	10	64.095	55	10	5.154	19
30	3	79.297	63	3	4.287	42
32	1	183.261	0	1	9.764	0
34	2	112.589	19	2	8.962	59

Site	nPb	$\bar{x}$Pb	cPb%	nCd	$\bar{x}$Cd	cCd%
1	9	52.733	98	9	14.753	55
2	10	17.628	37	10	12.414	9
3	10	41.029	76	10	8.074	29
5	9	16.242	36	9	7.549	36
6	8	20.887	13	7	3.815	15
9	5	23.380	42	5	6.593	64
11	10	28.174	60	10	7.559	40
12	10	22.164	26	10	6.461	26
13	9	23.691	51	9	4.147	59
14	6	34.844	19	7	11.635	4
15	10	19.938	28	10	6.697	49
16	9	15.512	40	9	4.267	37
17	12	17.242	28	12	4.202	81
18	10	14.176	33	10	7.684	32
19	10	24.210	30	10	12.289	13
20	10	20.705	27	10	8.288	51
21	10	20.924	24	10	7.771	26
22	10	19.873	22	10	4.825	54
23	10	26.705	13	10	9.311	23
24	10	15.189	36	10	6.970	53
25	10	25.563	13	10	10.406	22
30	3	20.967	39	3	8.449	44
32	1	34.883	0	1	15.678	0
34	2	13.215	35	2	16.011	27

Figure 6

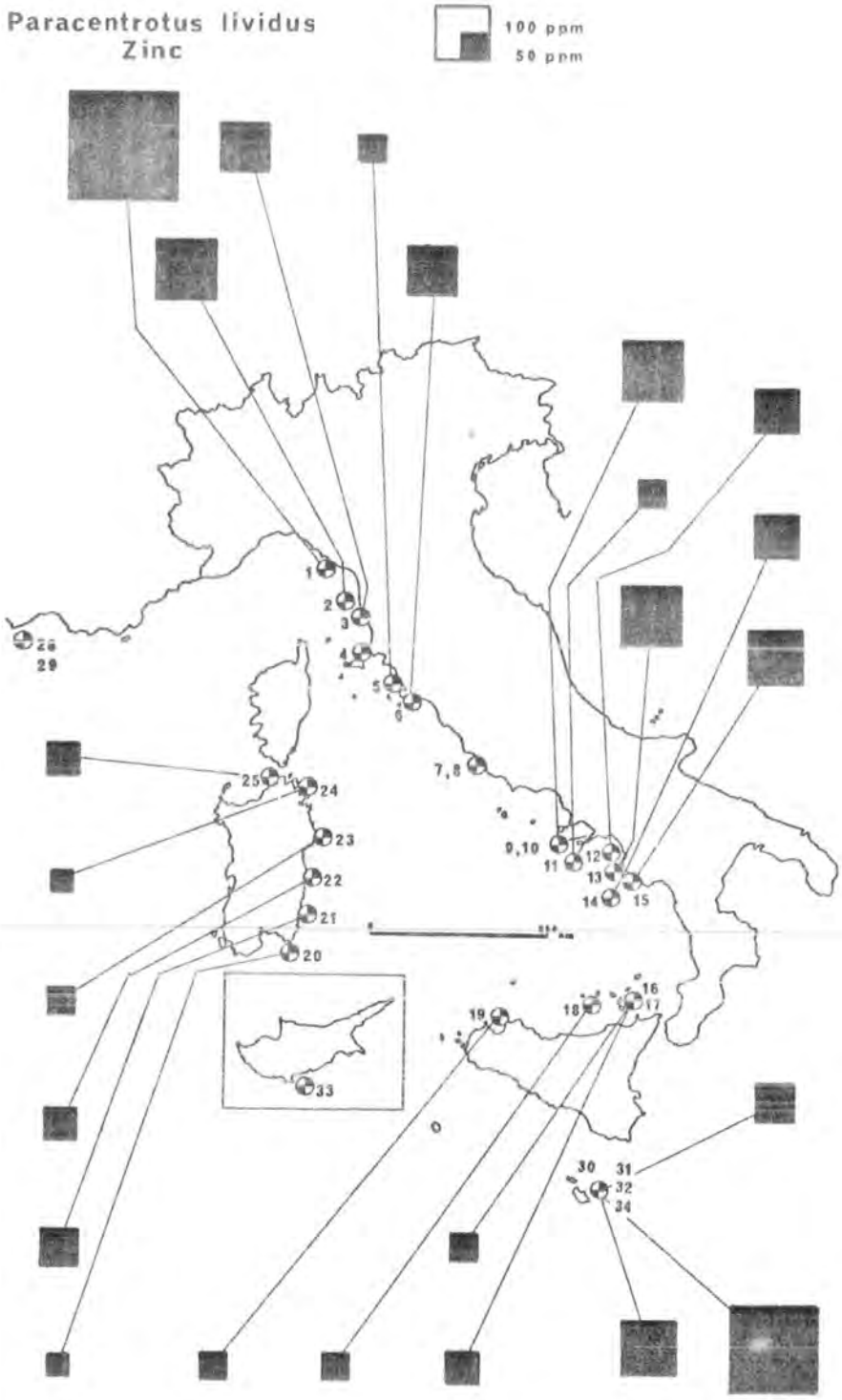
Paracentrotus lividus
Zinc
100 ppm
50 ppm
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Figure 7

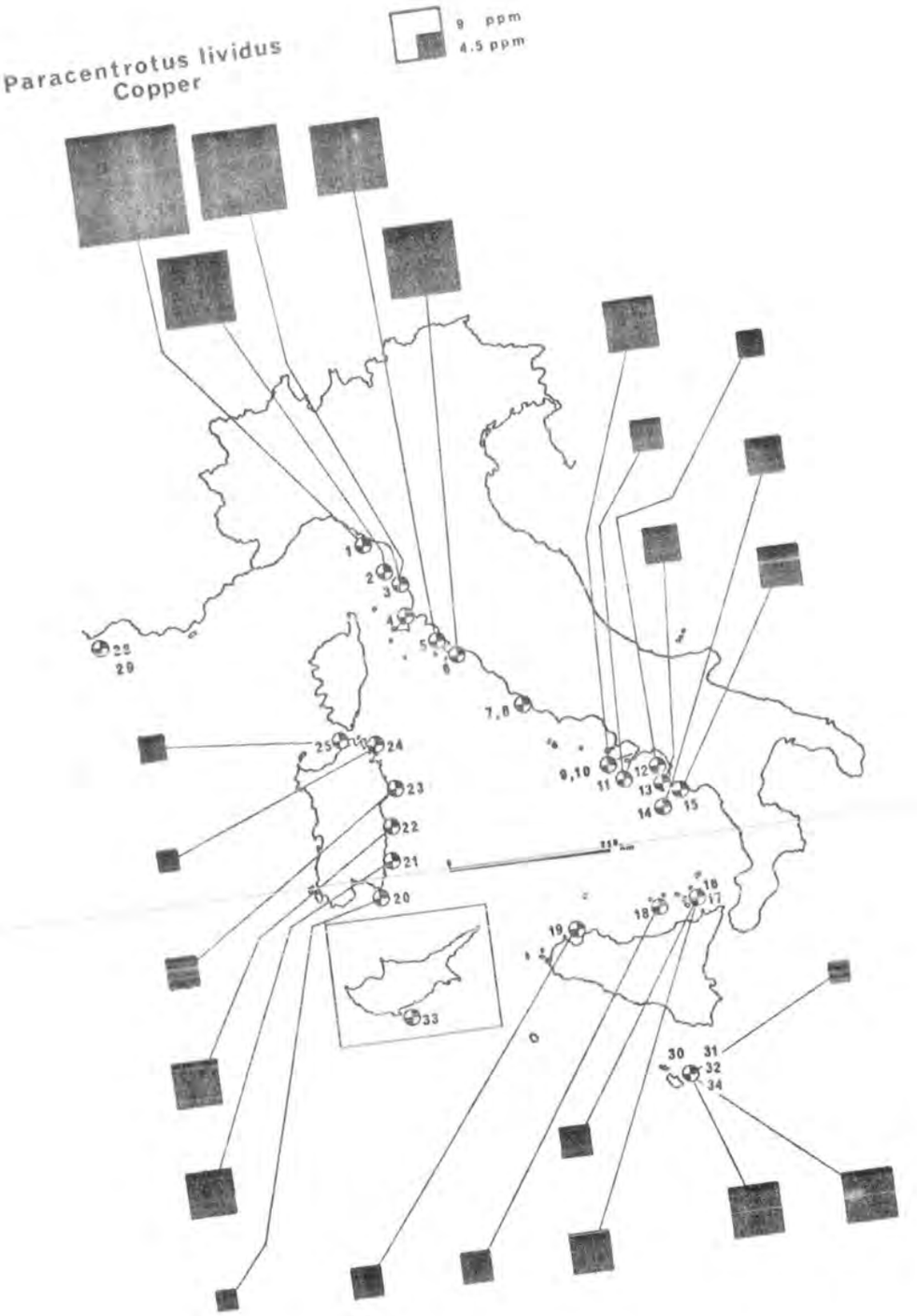
Paracentrotus lividus
Copper
9 ppm
4.5 ppm
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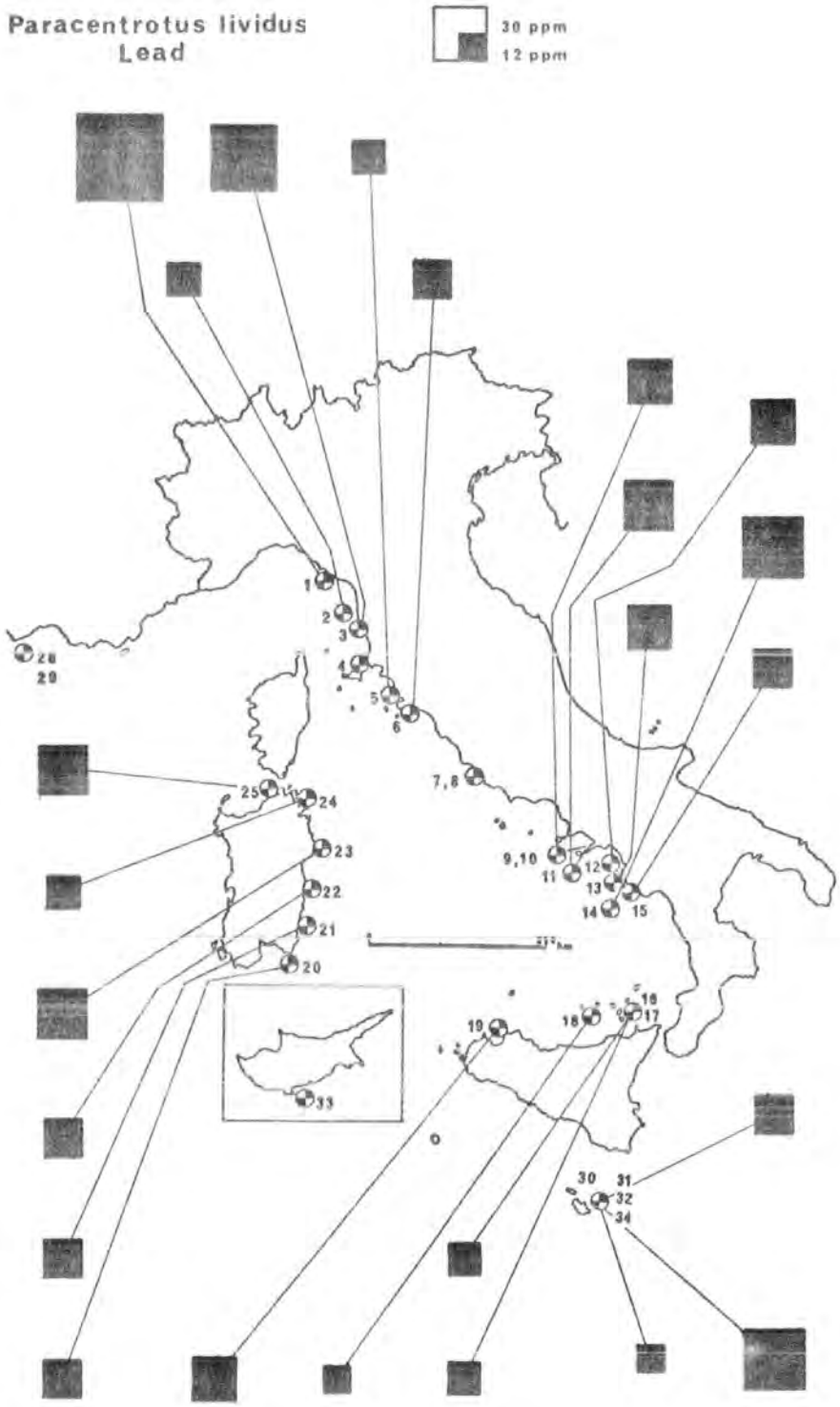
Figure 8


Paracentrotus lividus
Lead
30 ppm
12 ppm
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9,10
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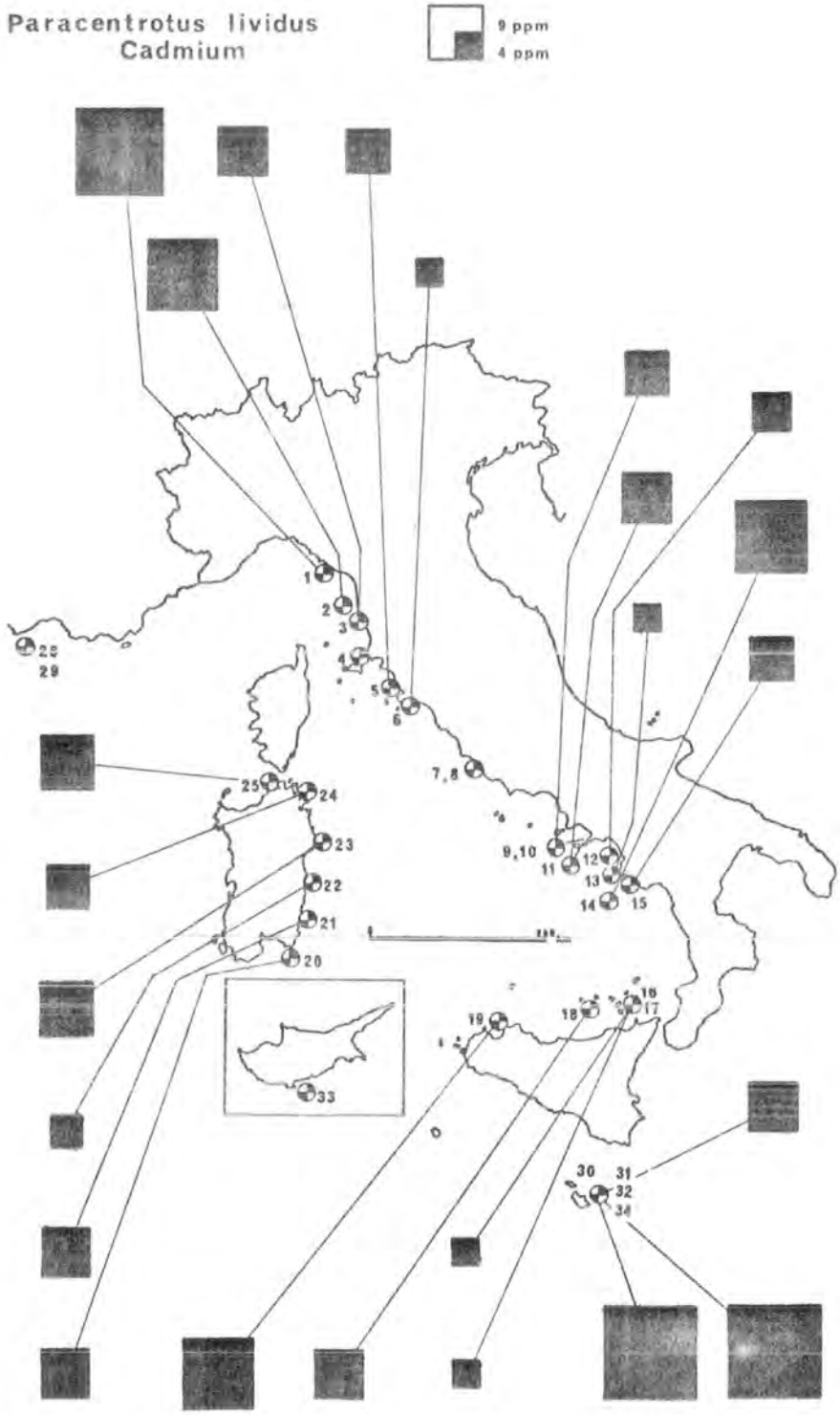
Figure 9


Paracentrotus lividus
Cadmium
9 ppm
4 ppm
1
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Patella coerulea

Table 13

Alkaline Metal Results

Site	nNa	x̄Na	cNa%	nK	x̄K	cK%
1	4	36179.3	17	4	10762.1	10
4	10	39283.0	19	10	13467.9	27
7	5	27349.6	64	5	9608.2	6
9	3	28088.0	1	3	8507.5	5
11	4	21872.9	25	4	8169.3	11
12	3	16131.7	8	3	8078.2	7
13	3	18129.5	20	3	8807.9	9
14	3	21094.0	17	3	7879.8	25
15	3	29088.0	5	3	3737.3	7
17	10	27878.5	22	10	10287.8	6
18	8	30091.8	38	8	9248.2	13
22	8	26820.0	20	8	9952.7	6
25	9	32991.1	16	10	10235.6	5
29	4	31066.3	33	4	9519.9	20
30	9	32532.7	17	9	10788.6	9
31	1	30413.2	0	1	12006.8	0

Site	nCa	x̄Ca	cCa%	nMg	x̄Mg	cMg%
1	4	18508.7	36	4	6454.9	24
4	10	26788.7	112	10	6994.8	32
7	5	16855.6	67	5	47426.9	2
9	3	19482.3	42	3	5866.3	12
11	4	32715.6	48	4	4693.8	12
12	3	35715.9	28	3	5440.1	1
13	3	6506.1	15	3	4086.6	4
14	3	13487.4	21	3	4195.7	17
15	3	8407.6	11	3	4829.7	11
17	10	10127.9	24	10	5132.9	21
18	8	10181.5	42	8	5205.9	34
22	8	6578.4	24	8	3825.0	14
25	1	11368.9	30	10	5091.3	16
29	4	35743.6	79	4	4611.2	19
30	9	51917.5	45	9	6149.3	14
31	1	11990.2	0	1	4966.8	0

Patella coerulea

Table 14

Heavy Metal Results

Site	nZn	$\bar{x}$Zn	cZn%	nCu	$\bar{x}$Cu	cCu%
1	4	90.909	15	4	14.523	39
4	10	89.826	31	10	5.100	47
7	5	55.742	6	5	9.456	8
9	3	52.092	2	3	9.666	2
11	4	60.069	10	4	5.388	6
12	3	62.201	2	3	6.361	36
13	3	47.639	1	3	4.447	17
14	3	43.085	32	3	4.494	33
15	3	61.488	19	3	7.946	22
17	10	84.768	48	10	8.720	15
18	8	54.681	16	8	7.724	30
22	8	128.035	85	8	9.440	38
25	9	99.560	54	9	5.530	33
29	4	74.004	30	4	7.086	36
30	9	191.205	40	9	18.371	15
31	1	87.682	0	1	8.618	0

Site	nPb	$\bar{x}$Pb	cPb%	nCd	$\bar{x}$Cd	cCd%
1	4	31.576	46	4	3.468	20
4	10	21.517	29	10	8.130	55
7	5	7.258	41	5	2.857	33
9	3	16.580	13	3	2.969	16
11	4	35.848	63	4	7.588	60
12	3	19.083	19	3	17.254	15
13	3	13.263	19	3	3.318	16
14	3	9.672	47	3	3.631	26
15	3	30.878	15	3	5.345	11
17	10	19.729	153	10	4.990	97
18	8	13.272	102	8	13.277	21
22	8	9.009	38	8	13.987	32
25	9	7.710	40	9	13.869	12
30	9	28.744	20	9	15.769	15
31	1	13.174	0	1	19.167	0

Figure 10

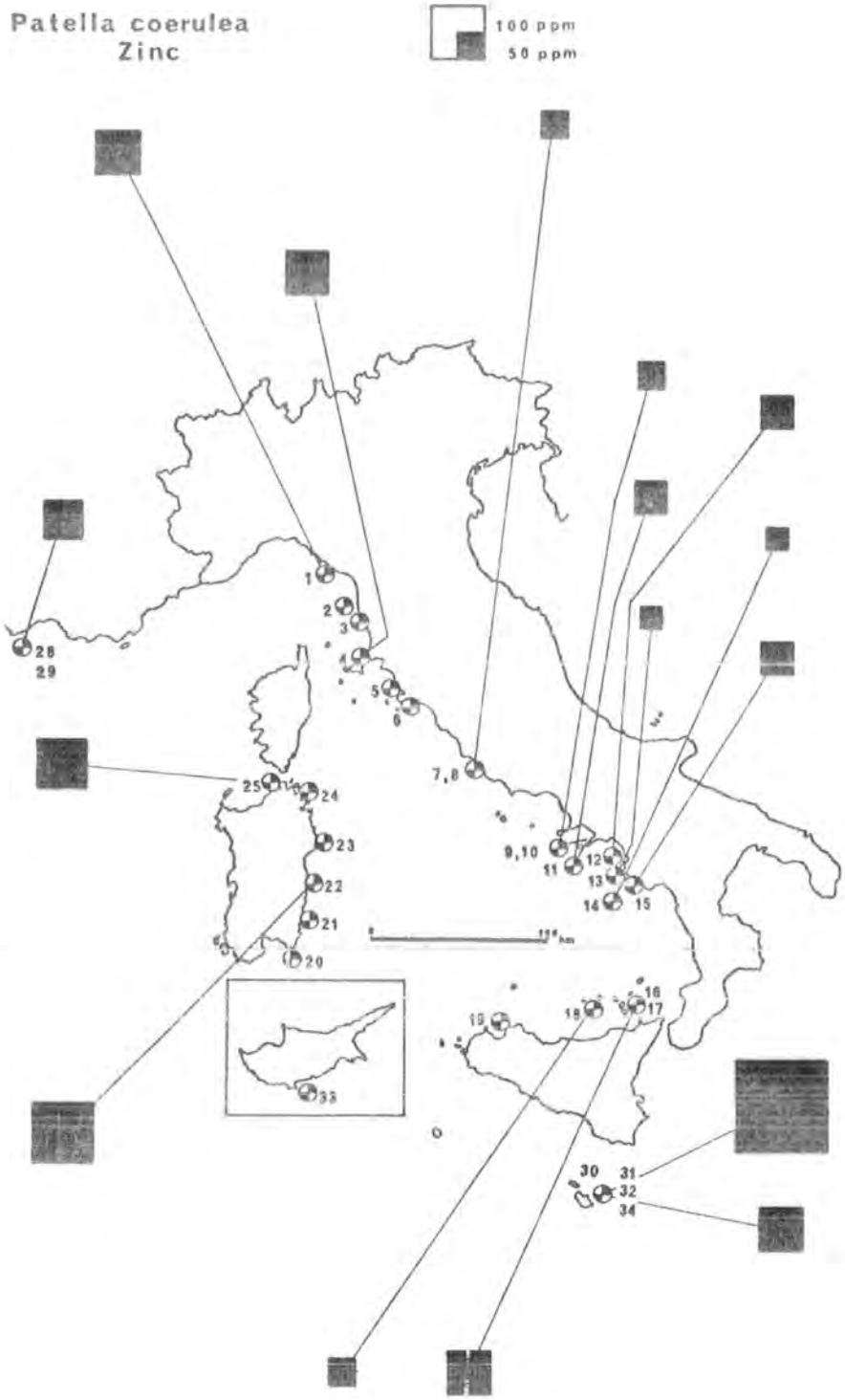
Patella coerulea
Zinc
100 ppm
50 ppm
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Figure 11

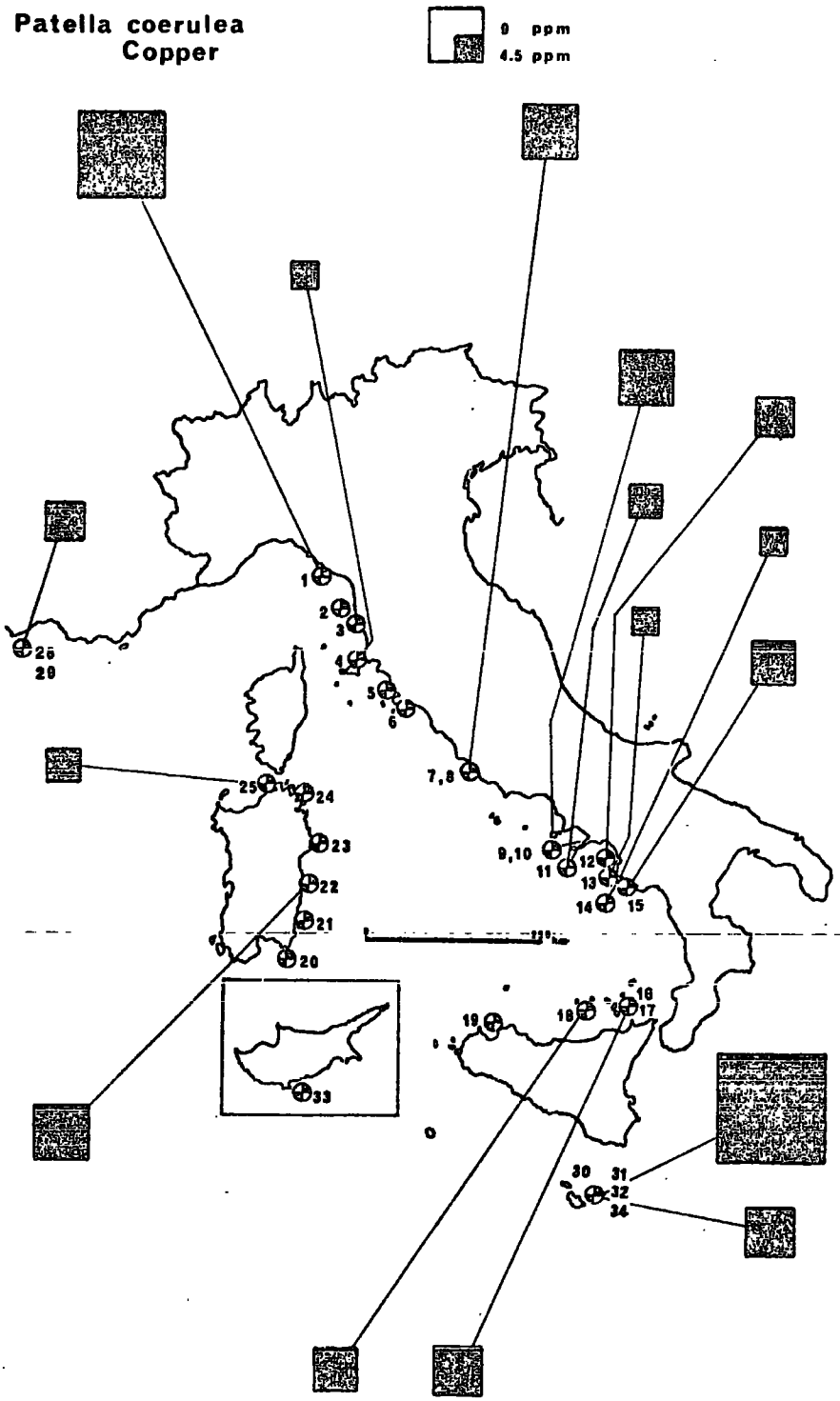
Patella coerulea
Copper
9 ppm
4.5 ppm
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Figure 12

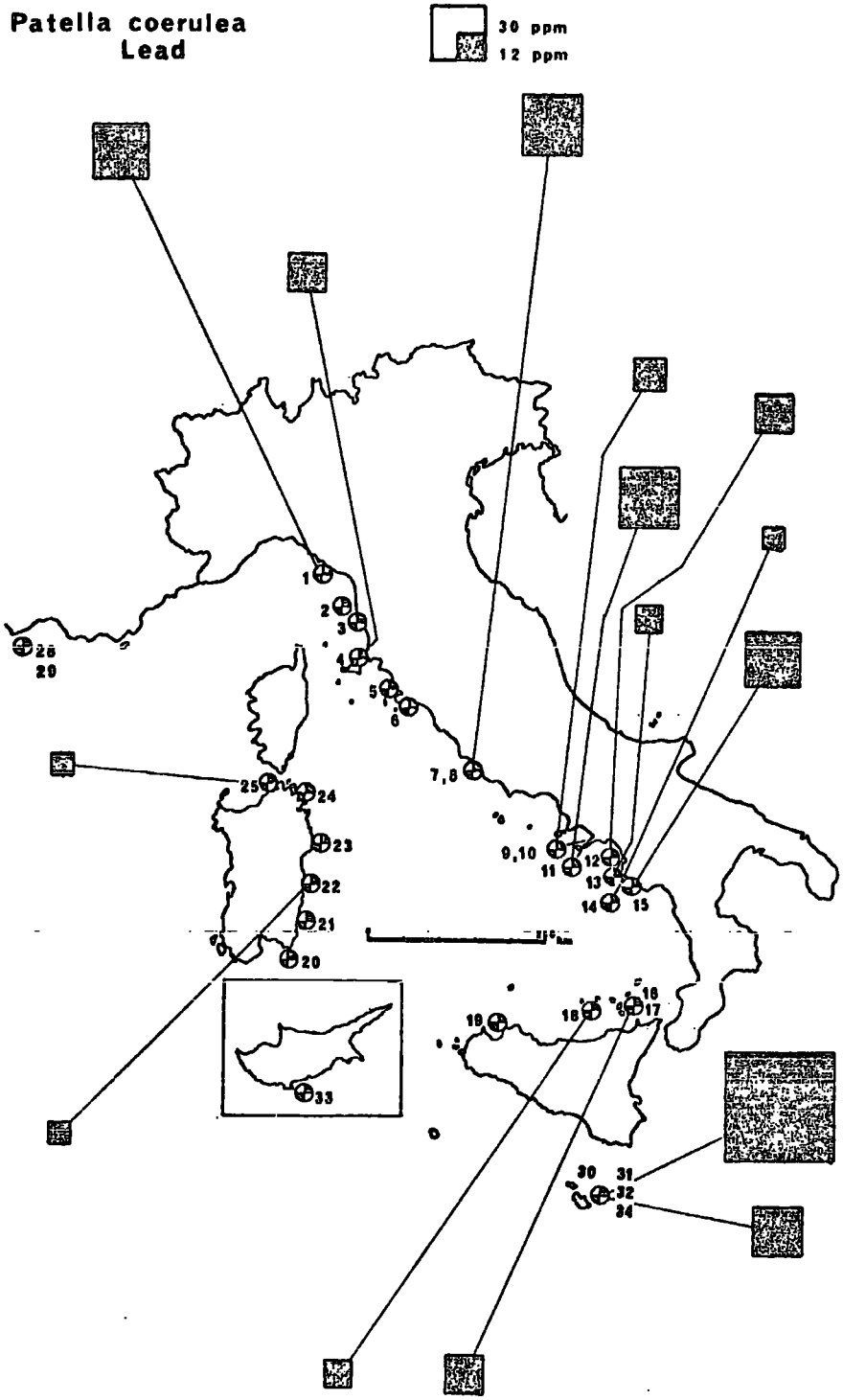
Patella coerulea
Lead
30 ppm
12 ppm
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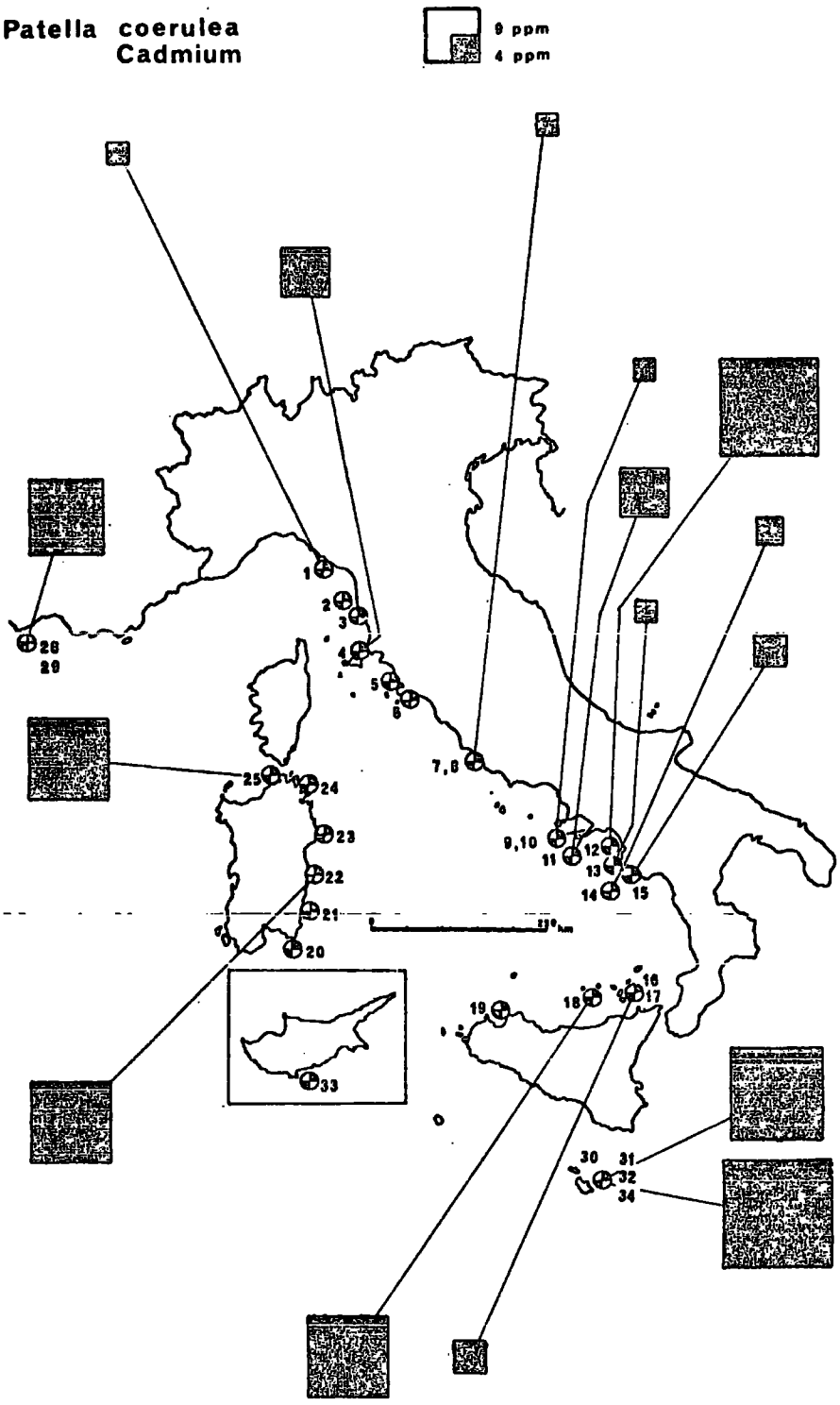
Figure 13


Patella coerulea
Cadmium
9 ppm
4 ppm
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Venerupis decussata

Table 15

Alkaline and Heavy Metal Results

Site	nNa	$\bar{x}$Na	cNa%	nK	$\bar{x}$K	cK%
8	5	29641.4	11	5	13413.4	3
10	5	25245.6	19	5	13348.1	4

Site	nCa	$\bar{x}$Ca	cCa%	nMg	$\bar{x}$Mg	cMg%
8	5	3484.6	41	5	5082.0	7
10	5	4618.8	29	5	4466.5	7

Site	nZn	$\bar{x}$Zn	cZn%	nCu	$\bar{x}$Cu	cCu%
8	5	52.449	13	5	6.638	67
10	4	55.318	3	5	8.026	8

Site	nPb	$\bar{x}$Pb	cPb%	nCd	$\bar{x}$Cd	cCd%
8	4	3.563	24	5	0.782	25
10	5	32.375	38	5	4.195	8

Tellina distorta

Table 16

Alkaline and Heavy Metal Results

Site	nNa	$\bar{x}$Na	cNa%	nK	$\bar{x}$K	cK%
8	4	22220.9	2	4	12588.6	15

Site	nCa	$\bar{x}$Ca	cCa%	nMg	$\bar{x}$Mg	cMg%
8	4	4348.3	14	4	3759.2	3

Site	nZn	$\bar{x}$Zn	cZn%	nCu	$\bar{x}$Cu	cCu%
8	4	58.182	9	4	19.843	21

Site	nPb	$\bar{x}$Pb	cPb%	nCd	$\bar{x}$Cd	cCd%
8	4	6.001	55	4	0.719	98

Mytilus edulis

Table 17

Alkaline and Heavy Metal Results

Site	nNa	$\bar{x}$Na	cNa%	nK	$\bar{x}$K	cK%
28	10	72377.1	10	10	13170.7	11
29	2	77142.2	9	2	11246.2	9

Site	nCa	$\bar{x}$Ca	cCa%	nMg	$\bar{x}$Mg	cMg%
28	10	17519.4	32	10	8036.8	9
29	2	40094.9	40	2	10868.3	0

Site	nZn	$\bar{x}$Zn	cZn%	nCu	$\bar{x}$Cu	cCu%
28	10	561.517	22	10	9.327	18
29	2	292.398	6	2	12.110	1

Site	nPb	$\bar{x}$Pb	cPb%	nCd	$\bar{x}$Cd	cCd%
28	10	115.447	37	10	8.150	52
29	2	38.398	17	2	9.318	67

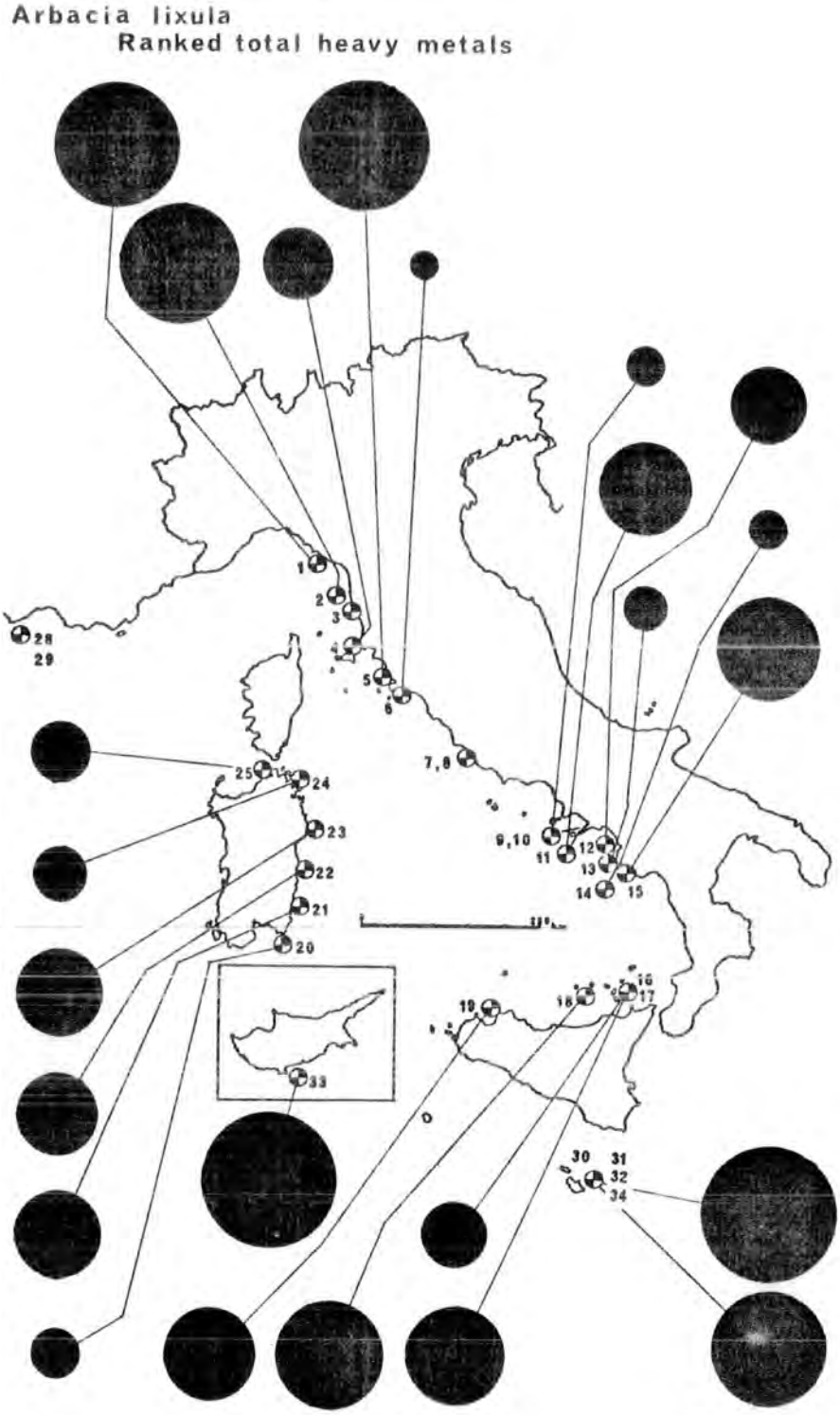
Arbacia lixula

Table 18

Individual and total heavy metal rankings

Site	Rank Zn	Rank Cu	Rank Pb	Rank Cd	Rank Total
33	1	5	3	8	4.25
31	10	1	17	2	7.5
5	15	6	8	1	7.5
1	9	2	2	18	7.75
2	18	4	1	9	8
32	2	3	24	4	8.25
18	12	8	11	6	9.25
15	22	10	4	5	10.25
17	7	12	13	10	10.5
11	13	18	6	7	11
19	20	14	7	3	11
21	6	13	16	16	12.75
23	8	17	14	12	12.75
22	5	15	20	21	14
12	24	24	10	11	14.12
4	11	11	12	23	14.25
16	17	9	19	13	14.5
25	4	16	21	20	15.25
24	3	20	22	17	15.5
20	14	22	15	15	16.5
13	25	23	5	14	16.75
9	23	7	9	22	18.12
14	16	19	18	24	19.25
6	21	21	23	19	21

Figure 14


Arbacia lixula
Ranked total heavy metals
1
2
3
4
5
6
7,8
9,10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
29
30
31
32
34
33

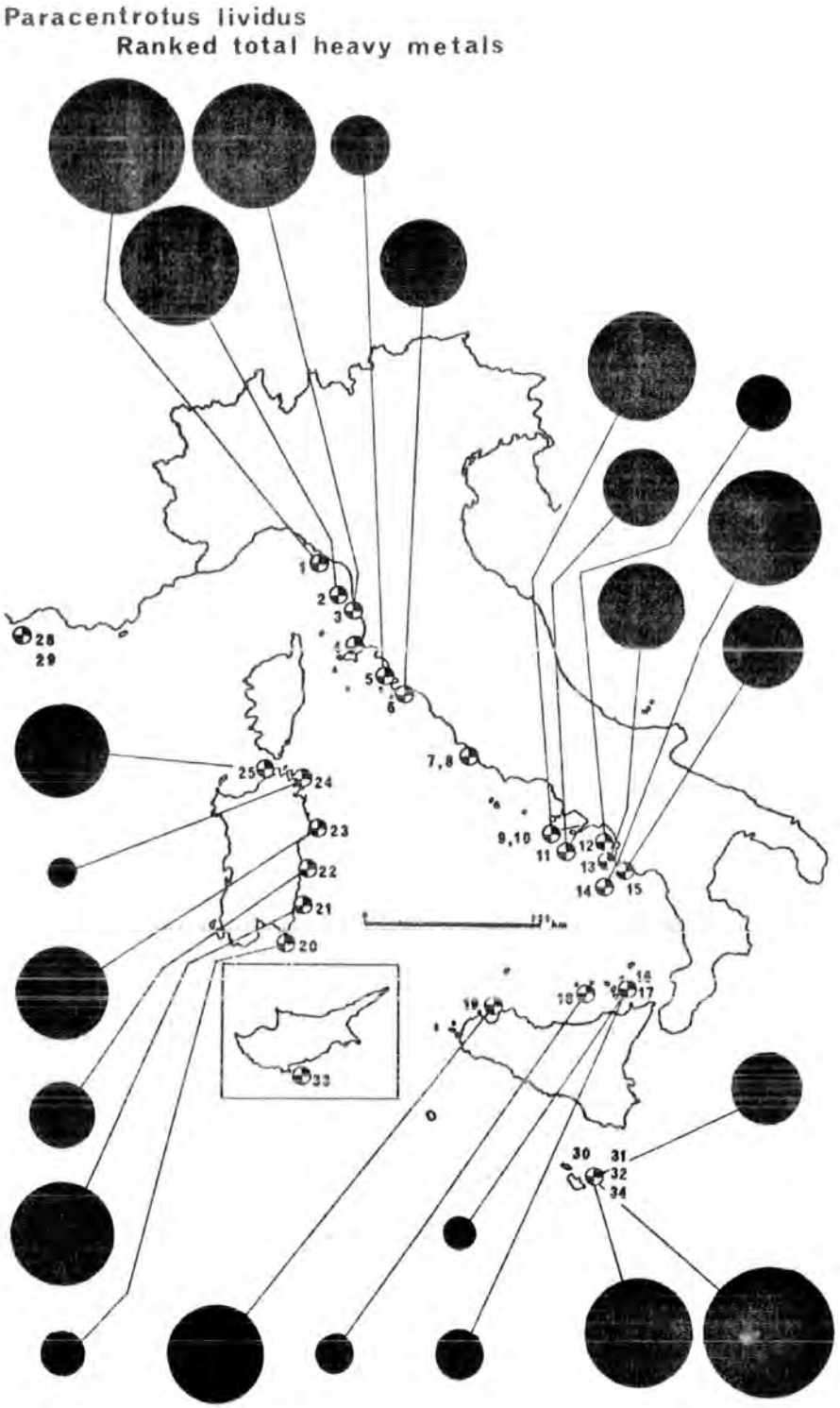
Paracentrotus lividus

Table 19

Individual and total heavy metal ranking

Site	Rank Zn	Rank Cu	Rank Pb	Rank Cd	Rank Total
1	1	1	1	3	1.5
32	2	6	3	2	3.25
3	8	2	2	11	5.75
2	3	3	18	4	7
14	15	13	4	6	9.5
9	4	7	10	18	9.75
34	6	8	24	1	9.75
21	12	10	13	12	11.75
19	19	17	8	5	12.25
23	21	15	6	8	12.5
25	16	20	7	7	12.5
6	9	4	14	24	12.75
13	5	14	9	23	12.75
15	7	12	16	17	13
11	18	16	5	14	13.4
30	11	24	12	9	14
22	13	9	17	20	14.75
5	20	5	20	15	15
12	10	21	11	19	15.25
17	14	11	19	22	16.5
20	23	22	15	10	17.5
18	22	18	23	13	19
16	17	19	21	21	19.5
24	24	23	22	16	21.25

Figure 15

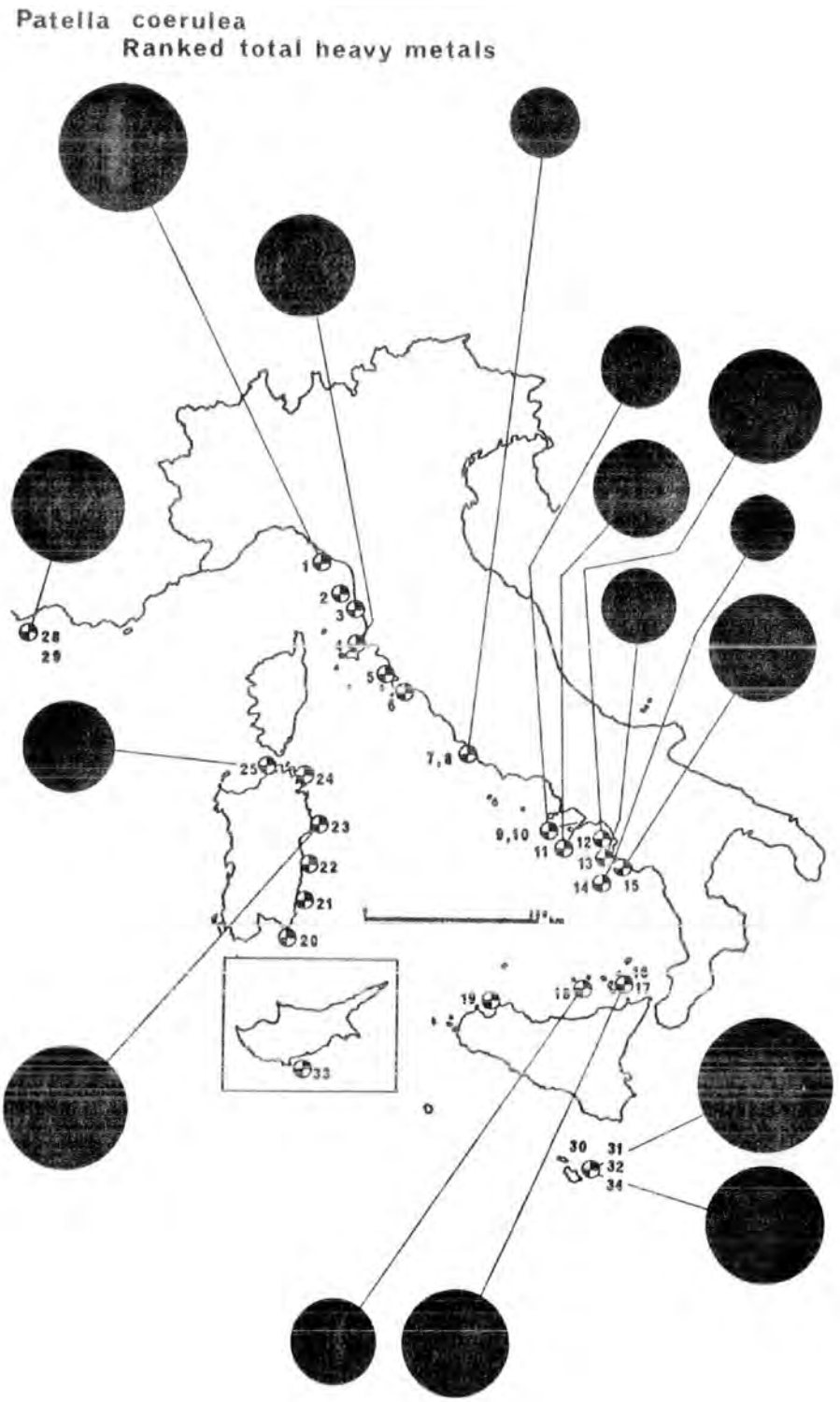

Paracentrotus lividus
Ranked total heavy metals
1
2
3
4
5
6
7,8
9,10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
29
30
31
32
34
33

Patella coerulea Table 20

Individual and total heavy metal ranking

Site	Rank Zn	Rank Cu	Rank Pb	Rank Cd	Rank Total
30	1	1	4	3	2.25
1	4	2	2	13	5.25
22	2	5	14	4	6.25
31	6	7	12	1	6.5
12	9	11	8	2	7.5
29	8	10	6	6	7.5
15	10	8	3	10	7.75
17	7	6	7	11	7.75
4	5	14	5	8	8
11	11	13	1	9	8.5
25	3	12	15	5	8.75
18	13	9	10	7	9.75
9	14	3	9	15	10.25
13	15	16	11	14	11.5
7	12	4	16	16	12
14	16	15	13	12	14

Figure 16


Patella coerulea
Ranked total heavy metals
1
2
3
4
5
6
7,8
9,10
11
12
13
14
15
16
17
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31
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33
34

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The Discussion

Points Raised

In an attempt to obtain an answer to the questions initially posed in the introduction and arisen throughout this work, the discussion of the obtained results can be broken down in a number of separate headings, treated individually in the following chapter.

1) Is there sufficient reason to believe the obtained results to be analytically accurate and suitable for interpretation in the context of this study?

Comparing the obtained results with those of other workers, in particular those who have availed themselves of the same laboratory facilities used throughout this study (37, 65, 83), they appear to be in no way less trustworthy. The spread of the individual site readings is admittedly on the large side, especially in the case of Calcium, in the echinoid samples, but this could be explained both by the possibility of occasional skeletal fragments finding their way amongst the soft tissues during dissection and by possible variation in gut contents. Interelemental correlation analysis, to be discussed below, showed no persistent correlation between the alkaline metals, Calcium in particular, and the Heavy Metal values, indicating that the matrix interference correction factors used may be considered valid. It is felt, therefore, that there is no reason not to accept the obtained values as true estimates, within the accuracy and precision of the analytical technique, of the detectable analytes within the samples.

2) Are the Heavy Metal values obtained comparable with those cited by other workers?

A summary of the obtained results together with those published by other authors is presented in tables 21 to 24. By and large the values are comparable, though usually at the higher end of the range, and with somewhat greater spread. The latter might well be caused by the use of

Table 21

Heavy Metal values cited in the literature for *Arbacia lixula* in the Mediterranean basin, in ppm dry weight. Range and averages (in parentheses if available)

Area	Ref.	Zn	Cu	Pb	Cd
Tyrrhenian Basin	Present Study	34 - 228 (107.6)	4 - 11.5 (7.72)	7.8 - 76.7 (29.4)	2.1 - 20.1 (12.2)
Naples Bay (Averages for clean and polluted sites)	(65)	76 - 120	7 - 16.6	21 - 58.6	
Saronicos Gulf	(66)	95			

Table 22

Heavy Metal values cited in the literature for Paracentrotus lividus in the Mediterranean basin, in ppm dry weight. Range and averages (in parenthesis if available)

Area	Ref.	Zn	Cu	Pb	Cd
Tyrrhenian Basin	Present Study	47.4 - 226.1 (88.1)	4.3 - 20.2 (8.4)	13.2 - 52.7 (24.1)	3.8 - 16 (8.6)
Naples Bay (Averages for clean and polluted sites)	(65)	55.6 - 122	7.6 - 13.8	20 - 42.3	
Saronicos Gulf	(66)	54			

Table 23

Heavy Metal values cited in the literature for Patella coerulea in European and Mediterranean waters. Range and averages (in parentheses if available).

Area	Ref.	Zn	Cu	Pb	Cd
Tyrrhenian Basin	Present Study	43.1 - 191.2 (80.2)	4.4 - 18.4 (8.3)	7.2 - 35.8 (18.6)	2.8 - 19.2 (9.3)
Israeli Coast	(45)	53.7 - 86.7 (66.3)	5.5 - 11.2 (8.2)		
SW Spain and Portugal	(64)	90 - 120 (106)	5 - 10 (7.75)	2 - 16 (10.25)	1.1 - 7.1 (4.2)
Irish Sea	(57)		7.7		32
Irish Sea	(85)	56 - 274 (124)	5.5 - 22 (12.1)	3.5 - 85 (7.8)	2.8 - 35 (10.7)
Bristol Channel	(60)	65 - 375 (193)		2 - 27 (8.2)	9 - 500 (127)
Dorset, U.K.	(63)				8.1
Pembs., U.K.	(63)				6
Severn Estuary	(59)				30 - 550
Loch Sunart, Western Scotland (Considered clean)	(37)	66 - 89 (77.7)	4.7 - 9.3 (6.4)	2 - 9.2 (4.5)	0.15 - 0.25 (0.19)
Shetland	(37)	76 - 145 (116)	4.7 - 8.9 (6.7)	3.2 - 7.9 (5.4)	
U.K. East Coast	(86)		14.3 - 21		
Marsden Bay U.K. NE Coast	(83)	143.1 - 227.3 (173.8)	16.5 - 19.3 (17.95)	16.7 - 20.6 (13.6)	5.6 - 7.4 (6.4)

Table 24

Heavy Metal values cited in the literature for Mytilus edulis in European waters, in ppm dry weight. Range and averages (in parentheses if available)

Area	Ref.	Zn	Cu	Pb	Cd
Marseilles Area	Present Study	292 - 561 (423)	9 - 12 (10.5)	38 - 115 (76.5)	8 - 9 (8.5)
Naples Bay	(65)	227	8	56.7	
NW Mediterranean	(87)	97 - 644 (209)	2.3 - 154 (18)	2.7 - 117 (21.5)	0.4 - 5.9 (1.9)
Rovini (+) Jugoslavia	(88)	8485			
SW Spain and Portugal	(64)	190 - 370 (265)	6.5 - 14 (10.9)	2 - 15 (6.8)	1.7 - 3.6 (2.7)
Irish Sea	(57)	91	9.6	9.1	5.1
Bristol Channel	(60)	62 - 250 (147)		1 - 30 (10.7)	4 - 60 (18)
Clyde Sea	(58)	79 - 259 (155)	5.7 - 30 (12.5)	6 - 33 (15.7)	0.6 - 3.5 (1,8)
North Sea (+) U.K.	(89)	164 - 242 (194)	10.3 - 20 (17)	15 - 27 (20)	2 - 2.7 (2.2)
France	(89)	85	15	4.8	1
Germany	(89)	127	79		
Norway	(89)	194	23	44	3
The Netherlands	(89)	109	24		1.8
The Netherlands	(62)	95			
Nieuport Belgium	(62)	29 - 57 (40)			

(+) 0.165 Dry/wet weight ratio used to convert values

a sampling area larger and more varied than most. The principal differences are in the Cadmium values for Patella coerulea (table 22), where averages more than an order of magnitude greater than the ones obtained have been published (59,60). It may be kept in mind, though, that the maximum values cited, 500 and 550 ppm, are up to twenty times the generally quoted LD_{50} values (11,72), albeit for mammals; nevertheless, such high values do not seem to have been confirmed elsewhere.

The comparison holds also for the Mytilus edulis values (table 23) even though the results are higher throughout than most others cited, especially those (87) collected from the same region. It must be said however that mussels were collected only from two sites, both claimed to present visible signs of industrial and urban pollution.

It is felt, therefore, that comparison with previously published data gives no evidence against the validity of the results.

3) Do the three principal species show generally similar detectable Heavy Metal contents?

Comparing the interspecific individual metal levels and Total Heavy Metal rank indices, no definite pattern seems to stand out. In some locations the three principal species show similar relative levels, while in others they differ markedly. The answer is therefore no.

4) Is there any obvious indication that industrialized areas, generally considered subject to gross pollution, show higher levels of detectable Heavy Metals in the species under study than more rural areas, generally considered cleaner?

Upper Tyrrhenian sites seem generally to show high concentrations, while the Sardinian sites are generally lower. On the other hand, comparison between the Gulf of Naples and the Cilento coast, where the same sites where used which Sheppard and Bellamy (65) had visited in the past, no clear gradient such as had been noticed by them was found, some of the sites along the presumably clean Cilento coast having levels

considerably higher than those of the Gulf of Naples, whose state of chronic pollution has by now become almost proverbial. Malta and Cyprus samples rank fairly highly, but the samples in these two areas were not collected by the author, and only limited information on the human impact on the surrounding coastline is available; also the Cyprus samples were not treated in the same manner as the rest, having been dried and shipped in aluminium foil packets, which might have caused some contamination. The latter results, therefore, though presented as a matter of interest, should be viewed with some suspicion. Possibly the most interesting set of results are those from the Eolian Islands (sites 16, 17 and 18), which show markedly high values and ranks for Arbacia lixula and Patella coerulea, especially Filicudi (site 18) which is the furthest one amongst all those investigated from any form of human impact, and exposed primarily to offshore W to NEE currents flowing through the Sardinian Channel without any close intervening industrial areas or conurbations. On the assumption that detectable Heavy Metal contents are directly related to the vicinity of pollution sources, this site would therefore have been expected to show the lowest levels throughout, which has not been the case. Considering these points, it is felt that there is no evidence indicating that the gross ranking of Heavy Metal levels is necessarily proportional to the localized supposed relative Heavy Metal input.

5) Does a more detailed statistical analysis of the results provide any evidence of correlation between the detectable Heavy Metal content of the organisms under study and any of the environmental factors?

Going beyond a gross ranking of the results, a detailed statistical analysis of the obtained results together with the available environmental data was carried out. Most of the site information was grouped in three composite indices as described in the appendix 5, one defining the bottom

morphology in the immediate sampling area, referred to as BOTTOM, the second combining the Ehrhardt Scale facies values, described in appendix 2, in a single weighted "Apparent Visible Pollution Index", referred to as FACTOT, and the third weighing together the shore characteristics so as to give a single "Human Impact Index", referred to as HII. Making use of the SPSS7 (84) statistical package available at the NUMAC-UDCU computing facility, the individual site/species results, the composite site indices, together with some available demographic and environmental data were subjected to partial correlation analysis.

Each site did not necessarily contain all of the three species investigated, therefore three separate matrices were produced.

Pearson's product-moment correlation coefficient r was used throughout, and first order partial correlations - that is, the correlation between x and y with the statistical influence of z removed, i.e. $r_{xy.z}$ - were extracted for all variables. Due to the large number of interactions involved, second order partial correlations - i.e. $r_{xy.zw}$ - were extracted only there where undue uncertainties persisted.

Correlations which were logically spurious or which could be accounted for by partial correlation were eliminated, and due to the somewhat limited number of cases and the possibility of inaccuracies being introduced by non-normal distribution of values, only correlations with $r > 0.4$ and $p > 0.05$ were taken into consideration. The surviving correlation coefficients with their levels of significance are summarized in tables 25, 26 and 27.

As can be seen, valid correlations between Heavy Metal contents and environmental variables were both scarce and inconsistent. No correlation was found between the Ehrhardt Scale Index FACTOT and any of the Heavy Metals, and the appearance of negative correlation coefficients between Zinc and ESTPOLL in Arbacia lixula and between Cadmium and

Arbacia lixula

Table 25

Surviving correlation matrix n = 24

	xZn	xCu	xPb	xCd
POP			+.55 (.003)	
ESTPOLL	-.49 (.008)			
INLEV				
FACTOT				
BOTTOM	-.51 (.005)	-.70 (.001)		
HII				
xNa	+.59 (.001)			
xK		Pb +.73 (.001)	-.61 (.002)	
xCa				
xMg				
xZn		Pb -.42 (.023)		
xCu	Pb -.42 (.023)		K +.74 (.001)	
xPb				
xCd				

Boxed correlations appear only when removing the influence of element in upper right corner of box.

Paracentrotus lividus

Table 26

Surviving correlation matrix n = 24

	xZn	xCu	xPb	xCd
POP				
ESTPOLL				
INLEV		+.67 (.001)		
FACTOT				
BOTTOM	-.43 (.019)	Zn +.49 (.009)	Zn +.43 (.020)	-.60 (.001)
HII				
xNa				
xK				
xCa				
xMg				
xZn		+.69 (.001)	+.62 (.001)	
xCu	+.69 (.001)			
xPb	+.62 (.001)			
xCd				

Boxed correlations appear only when removing the influence of element in upper right hand corner of box.

Patella coerulea Table 27

Surviving correlation matrix n = 16

	xZn	xCu	xPb	xCd
POP				
ESTPOLL				-.67 (.002)
INLEV				
FACTOT				
BOTTOM				
HII				
xNa				
xK				
xCa				
xMg				
xZn		+.71 (.001)		
xCu	+.71 (.001)			
xPb				
xCd				

ESTPOLL in Patella coerulea are difficult to justify in terms of environmental insult to the species, except possibly through the rather tortuous argument that an increase in pollution load in a given area might affect the resident species' excretory capacities. Such an argument, at any rate, would need far more and weightier evidence than available in its favour. In these conditions, the two positive correlations detected, Lead with POP for Arbacia lixula and Copper with INLEV for Paracentrotus lividus, remain worthy of note but can hardly be considered conclusive for the purposes of this study.

What does stand out, is the relatively persistent correlation between BOTTOM and a number of Heavy Metals. The index in question is a geomorphological rather than a geochemical one, and therefore a connection between it and the Heavy Metal content of the two echinoids is not immediately obvious. One possibility for which a sandy or silty bottom might be connected with higher values than a rocky one could be that fine particulate material, deposited on the algae on which the two species feed, may be an important vehicle for metal intake, and that therefore a greater presence of such material would correspond to a proportionally greater metal content. Though not totally implausible, this theory would also require considerably more evidence than available before being taken seriously in consideration.

In the light of these results, it is felt that insufficient evidence is provided for a correlation between Heavy Metal content and variables depending on human impact, and only inconclusive evidence for a correlation between the Heavy Metal values and the one studied variable not so dependent, bottom morphology.

6) On the same considerations as the previous heading, is there any evidence that the measured Heavy Metal levels are in any way correlated with internal, i.e. non-environmental, variables?

As can be seen in tables 25 to 27, a type of correlation which does appear in all species investigated is that between different metal contents. Persistent positive correlations between heavy and alkaline metals might be taken as indicating an insufficient correction for Atomic Absorption Spectrometry matrix interference. Sheppard (90) found generally positive correlations between heavy metals and Calcium and Magnesium, and negative correlations with Sodium and Potassium studying the same species treated here and collected in and around the Bay of Naples, and he tentatively explained them as result of the disturbance of the organism's intracellular ionic balance caused by the effect of toxic metals on membrane permeability. No such consistent results were found in the data at hand, and it can be seen from table 25, referring to Arbacia lixula, that the only persistent correlation between heavy and alkaline metals are those between Lead and Potassium, which is indeed negative, and between Sodium and Zinc, which is instead positive. Both are absent in the other two species. It can also be seen that the removal of the statistical influence of Lead makes a relatively strong positive correlation appear between Copper and Potassium. This removal also brings about a negative correlation between Zinc and Copper, while the removal of the statistical influence of Potassium brings out a relatively strong positive correlation between Lead and Copper. This seems to indicate a set of multiple cross interferences, at least between Lead, Copper, Zinc and Potassium. These may either be dependent on the analytical technique, in which case synergistic and antagonistic interference factors, both for alkaline and for Heavy Metals, would have to be allowed for as well as the normally considered Matrix Interference corrections or else might be

dependent on physiological processes in the organisms under test affecting their metal retention characteristics, and which would be unlikely to be identified with a whole body content measurement. What appears to confirm the first possibility without excluding the second is the fact that in the other two species investigated persistent correlations, albeit positive, appear between Zinc and Copper (both for Paracentrotus lividus and for Patella coerulea) and Zinc and Lead (only in Paracentrotus lividus). It is suggested, therefore, that the possibility of the existence of such multifactor interferences, involving heavy as well as alkaline metals and having a significant effect on analytical results, should be taken into consideration. Admittedly, further tests using known standards would be required to produce a conclusive confirmation.

7) On the basis of the preceding headings, is there any evidence that the measurement of total Heavy Metal content in the species under observation is a significant index of the human impact in the area and therefore, by inference, of Heavy Metal contamination of the species' immediate environment?

As described above, there seems to be no conclusive evidence towards a significant relation between total Heavy Metal content and either environmental or human impact characteristics of the sampling site, nor with states of pollution inferred from geographical and demographical considerations. Whether or not these characteristics necessarily imply an alteration of the levels of assimilable Heavy Metals in the species' environment is, of course, an unproven inference as far as this study is concerned. The detection of significant correlations might have been taken as partial evidence of it, but their absence, while leaving a verdict of not proven, does not necessarily rule it out. The indication, on the other hand, that the detectable Heavy Metal levels might depend to a large extent on non-environmental variables, both internal to the organisms or peculiar to

the analytical technique, raises considerable doubts on the usefulness of the entire procedure.

It is felt, therefore, that the method originally suggested, using total Heavy Metal content measurements in the three species investigated for the purpose of monitoring the Heavy Metal pollution of their environment, is to be considered unsatisfactory.

8) On the evidence presented, is there any indication that the technique used could be altered so as to give more valid results?

Throughout the study, the following points were recognized as weaknesses or potential causes of error:

a) Insufficient sample size.

Regardless of the large total number of samples collected, and due principally to the need to bulk the smaller individuals, the actual number of values available per site was frequently too small to give full confidence in the subsequent statistical analysis, making necessary the relatively severe limits of acceptability described under heading 5. As mentioned previously, Sheppard and Bellamy (65) used a far larger sample size, and it is possible that had this been done in this study, similar trends might have been observed. On the other hand, a greater sample size would have meant a correspondingly greater number of samples to prepare and analyze, and as already the number dealt with proved to be a considerable strain on available resources of time and facilities, the question arises whether such an increase would, in the present condition, prove itself worthy of the effort.

b) Excessive time requirements.

Admittedly more time was actually consumed in experimenting with the described microwave digestion technique (67), subsequently abandoned, than with the actual analysis, and the described preparatory technique eventually used was one known to be highly time consuming, but as one of the original

purposes of this study was to develop a method capable of producing results within a reasonably short time after the sampling, it can only be said that the technique used does not satisfy this requirement though it might be possible to accelerate the procedure using a different process for sample preparation and analysis. One such method, recently appeared in the literature, might be the combination of graphite furnace combustion of the sample, obviating the need for elaborate sample preparations, and Zeeman Effect Atomic Absorption Spectrometry, but no such facilities were available at the time of the study (91, 12, 13).

c) Uncertainties about usefulness of results.

The doubts raised concerning the exact correspondence between detected metal levels and metal content of the analyzed organism's immediate environment remain, and are likely to remain even if it were possible to analyze a sufficiently large number of samples in a sufficiently short time interval.

It is felt, therefore, that any simple alteration of the applied technique would be unlikely to significantly improve the validity of the results, a goal which would be best reached through a fairly fundamental revision of the entire experimental procedure.

9) Amongst the information obtained throughout the study, is there evidence of any other factor not involving the total Heavy Metal content of the species investigated which might be suitable for the purpose of environmental monitoring along the premises of this study?

Besides the three matrices treated so far, a fourth one was produced, involving only the site-dependent variables listed in table 28, and described in tables 4, 6,7 and appendices 2 and 5, which combined all sites from which such data was available, irrespective of the species there collected. This was subjected to the same statistical analysis as

Listing of the Environmental Variables used in the Statistical Analysis of the Results

SITE	POP	ESTPOLL	INLEV	TECHFR	RECHFR	FACTOT	BOTTOM	HII
1	8	3	5	5	5	38	26	32
2	8	3	5	5	4	6	7	27
3	3	3	5	5	7	9	45	11
4	4	3	5	4	1	56	30	37
5	2	1	1	3	4	21	45	17
6	2	1	1	3	3	0	50	13
7	6	5	2	0	0	0	35	21
9	8	5	1	4	2	48	35	40
11	8	5	1	5	2	29	35	15
12	4	2	1	4	5	20	35	28
13	2	2	1	3	4	16	35	7
14	2	2	1	5	4	46	37	23
15	2	2	1	4	5	29	35	15
16	2	1	1	0	0	0	30	7
17	2	1	1	4	4	34	30	17
18	2	1	1	5	5	6	37	7
19	5	2	1	4	4	31	35	33
20	1	1	1	2	5	23	35	17
21	1	1	1	2	5	3	37	7
22	3	1	5	4	3	36	30	47
23	1	1	1	5	6	0	35	10
24	2	1	1	3	5	37	35	43
25	1	1	1	4	4	25	35	22

Table 28

the preceding ones, and the surviving correlations appear in table 29. The persistent correlation between ESTPOLL and POP could be expected, as ESTPOLL itself is calculated by adding the industrial population-equivalent to the actual population density, POP. The persistent correlation between RECHFR and TECHFR, the echinoid frequency indices, and BOTTOM is also not surprising, as it is well known and usually noticeable that Arbacia lixula is both more common in extremely rocky environments and more tolerant of higher population densities than Paracentrotus lividus (52).

The one persistent correlation worthy of notice is the one between HII and FACTOT. Keeping in mind that the original Ehrhardt scale (68, appendices 2 and 5) was based on empirical considerations and drawn up purely in view of organic pollution, i.e. sewage, it is interesting to note that there seems to be such a relatively strong correlation between the arbitrarily structured composite index FACTOT based upon it, and the composite index HII which comprises not only human abitations, but establishments such as harbours, industry, and quarries whose discharges are generally considered under a separate heading from urban sewage.

It is suggested, therefore, that the use of such composite indices, defining in much greater detail than here both the visible environmental characteristics of an area and the community structure of its fauna and flora, could present a much more useful and reliable method of environmental monitoring than the detection and measurement of retained individual pollutants.

Site data

Table 29

Surviving correlation matrix n = 25

	POP	FACTOT	BOTTOM	TECHFR
ESTPOLL	+.89 (.001)			
HII	+.63 (.001)	+.79 (.001)		
RECHFR			+.68 (.001)	+.78 (.001)

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The Conclusions

The preceding chapter lists the positive and negative results, as well as the problems, which have cropped up throughout this study. What sort of answers, therefore, can one give to the original questions raised in the introduction, and what conclusions can be drawn from them, both in respect to the study's specific scope and to the wider aspects of environmental monitoring?

As far as the three initial technical problems are concerned, the results are almost entirely negative. The analytical technique studied, involving microwave sample digestion, proved itself totally inadequate, and the analysis had eventually to be carried out using the procedure it was meant to replace. On the other hand the use of amateur divers for the purpose of collecting samples and information was by and large vindicated, even though most of the sampling was in practice carried out by the author. When contacts were made with local divers and diving organizations, help proved itself both forthcoming and useful, and there is every reason to believe that similar or more complex work, assuming an adequate planning and experimental design, could be carried out by them with only elementary ~~scientific training and little or no scientific supervision.~~ Unfortunately, the technique used, viz. the collection and analysis of the echinoids Arbacia lixula and Paracentrotus lividus and the gastropod Patella coerulea, did not prove itself of any definite value towards the monitoring of Heavy Metal values, voiding therefore the usefulness of the sampling strategy without nevertheless touching upon the validity of the sampling method. The failure of the actual monitoring part of the study did not seem to depend so much on the inadequacy of the species investigated, as from the uncertainties surrounding the value of such an investigation as a tool for environmental monitoring. While it can be expected that the presence of contaminants in the environment will have some effect on its inhabitants,

and that the accumulation of said contaminants in the organisms in question is one of the most likely of these effects, their use as environmental indicators depends entirely on the investigator's ability to measure these effects with satisfactory accuracy and precision, and to relate the obtained measurements with either the general state of the environment, or with one or more specific factors affecting it. The assumption that these two conditions were satisfied lies at the base of most work carried out up to now using indicator organism analysis, nevertheless several workers have in recent years expressed some doubts on the matter, doubts which are shared by the author.

First, there is the question whether or not the detected metal content is necessarily proportional to that present in the environment. Boyden (94) has shown significant relationships between Heavy Metal concentrations and body weight in molluscs, and Williamson (95), working with terrestrial gastropods, found that the Cadmium body burden of the animal increased with age, but that within any year class the concentrations were lower in larger animals. Neilsen (96) found differences in Heavy Metal concentrations in cultured mussels originating at different depths within the same area, suggesting that even long-term accumulation of toxicoids could be strongly affected by such factors as transport and water column mixing. Moreover, other workers have detected and described antagonistic and synergistic effects on Heavy Metal toxicity both by other metals (97, 13) and by organic pollutants such as sewage and detergents (15). It may be added that, since most Heavy Metal capture within an organism's metabolism takes place at enzyme level (10,11), there is a distinct possibility that one pollutant's effect could be, amongst others, an alteration of another pollutant's retention. It can be seen, therefore, that there are valid reasons for doubting that the organism's metal content actually mirrors its concentration in the environment, as it may be affected by a considerable number of external

and internal variables, generally not taken into consideration.

Secondly, the question arises on whether the organism's metal content, assuming that it can be considered in a meaningful context, can be measured with satisfactory accuracy and precision. There is no shortage of analytical techniques for identifying trace quantities of metals, but the comparability of such results originating from different laboratories has frequently been queried (eg. 98), and the limited comparability of Lead and Cadmium results have been officially recognized (99). Finally, it appears that the actual chemical species in which the metal appears, is absorbed, and is retained could be as important if not more so than the total quantity present (100,20). Analytical techniques have been suggested for chemical speciation of trace metals (71,19) but their cost and complexity, together with the fact that such analyses will require standards of accuracy and repeatability even higher than those which are posing difficulties nowadays, are felt to be unlikely either to become very widespread, or to give results significantly more valid than the ones currently obtained (98).

On these counts, together with the uncertainties arisen throughout this study, the author feels that the simple measurement of Heavy Metal content of indicator organisms is unlikely to produce results commensurate with the difficulties incurred in obtaining them.

As mentioned in the introduction, the analytical pathway towards environmental monitoring had been chosen due to the absence in the Mediterranean basin of species usefully comparable to the Laminariaceae which had shown their worth in the North Sea. The community analysis pathway, not depending on any single parameter but making use of the combined response of the entire affected ecosystem, would avoid most of the problems described above though, to be sure, not without presenting difficulties of its own. Analyzing the community structure of a characteristic

sample of benthos, and even more so the very large number of samples required in this case, is neither easy nor fast, and usually involves a large amount of none too easy identifications of microflora and microfauna, generally requiring highly skilled personnel and abundant time. If, however, the use of composite diagnostic indices, as tentatively put into practice in this study, were to prove itself adequate for the purpose, such difficulties would largely disappear. The Ehrhardt scale limits itself to a dozen diagnostic facies, identifiable even by a surface observer. A scale of considerably greater detail would be well within the capacity of an average amateur diver to make use of, whose ability in identifying local macroorganisms, if only by their vernacular names, is frequently surprising. Similarly, questionnaires on environmental data, geomorphological and oceanographical information, and data referring to the human impact onto the site, can be designed so as to be within the reach of any well-informed layman. Data collected in this manner can be processed directly - even more so if prepared in machine readable form - without the intervening necessity for extensive laboratory work, and it would allow the existing but relatively scarce available scientific skills to be concentrated on the data analysis proper in the first instance, and in the subsequent investigation of and intervention in those situations where an environmental danger is apparent or suspected. The first necessary step in developing such a methodology would be a thorough investigation of the differing responses of communities to environmental insult, and the identification of useful diagnostic groupings suitable for the purpose. The second, to test in practice what sort of accuracy can be expected from such a system, as well as what kind of training, if any, should be made available to the participants.

It is the author's belief, therefore, that the diagnostic index path is the most likely process by which large scale monitoring of the marine environment, both for Heavy Metals and for other pollutants, has a chance

of being put into practice considering the present and forseeably future availability of skills, manpower, and resources.

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The Appendices

Appendix 1

Summarised descriptive notes of sampling sites.

Together with a brief site description, the site's relevant heavy metal rank values are given.

Site 1 Sampled 7/5/76

Porto Venere. Sampled off no. 35 of the lungomare, on the channel between the cape and the island of Palmaria, North of the gulf of La Spezia. Strong N to S current through the channel. Edge of waterfront boulders down to 2-3 m, then muddy detritus bottom flattening out, though channel itself is fairly deep. Boulders covered with thick algal growth, brown and green. Dominant species Ulva lactuca, Codium tormentosum, and Dictyota dichotoma. Heavy population of very small Paracentrotus on the boulders, with a few large Arbacia. Large Paracentrotus on sandy/silty bottom. Large numbers of assorted gast ropoda on the boulders. Patella abundant, very small on the waterline, larger further down, the latter covered with tufts of Jania rubens and Dictyota dichotoma. These were sampled.

Ranking:		
	Arbacia	7.75
	Paracentrotus	1.5
	Patella	5.25

Site 2 Sampled 8/5/76

Secche della Meloria. A shoal some two miles off Livorno. Marked on maps and charts, has lighthouse. Sampled over a considerable area around 1 mile west of the lower lighthouse, depth 9-12m. Posidonia bottom, flat with occasional breaks in the matted rhyzomes, leaving bare sand and some rock. Paracentrotus is abundant, and strangely enough it is almost always at an extremely high level in the Posidonia, almost at the tip of the leaves. Also very abundant was the crinoid Antedon mediterranea, also at the tip of the leaves. No Arbacia to be found, so some samples were collected in the immediate vicinity of the lighthouse, on an almost bare limestone bottom, scoured clean by an abundance of Arbacia. Here the depth was 2m at the most. No Patella was to be seen in either area.

Ranking: Arbacia 8

Paracentrotus 7

Site 3 Sampled 6/5/76

Quercianella. A residential area less than halfway between Rossignano Solvay and Livorno. Small seaway with no visible traces of sewage or other source of pollution. Water exceptionally clear, thick carpet or brown algae. Only Paracentrotus lividus was to be found, and in very large quantities up to the surface. No Arbacia lixula to be seen. Patella was exceedingly small and scarce, and not worth taking a sample of. The thick algal growth, mainly of well-developed and solid brown algae, principally Cystoseira, left little space for Patella spawn to settle. Depth 3m maximum, usually shallower.

Ranking: Paracentrotus 5.75

Site 4 Sampled 6/5/76

Salivoli. Residential suburb to the north of Piombino. As usual, the only access possible was on the 'public' beach - over the sewage outlet. Sampled along the outside of the breakwater.

First clear example of pollution facies - masses of Ulva lactuca, some Enteromorpha intestinalis. Later on, some more normal brown/red algae facies. No excessive trace of organic pollution outside the breakwater. Echinoids were frequent, but only Arbacia lixula, no Paracentrotus lividus. Patella was reasonably abundant, very small (1-1.5 cm) individuals at surface, larger (4-6cm) below 5m depth, covered by algal growth; these latter were sampled. Noteworthy was the great abundance of sponges in very shallow depths (less than 1m), usually on the North side of the rocks.

Ranking: Arbacia 14.25

Patella 8

Site 5 Sampled 5/5/76

Talamone. Sampled north of the village, beyond Capo d'Uomo, around a large protruding rock at the end of a path leading down from the tower. The rock is completely covered by algal growth, leaving no space at all for limpets. Altogether the Paracentrotus/Arbacia ratio was close to 1, but while Paracentrotus was definitely predominant on the South face of the rock, Arbacia was so on the North face. The algal growth on the two faces appeared identical.

Ranking: Arbacia 7.5

Paracentrotus 15

Site 6 Sampled 10/5/76

Ansedonia. Sampled at the N end of the beach, close to the "split". Large cliffside, sandy bottom with emerging rocks, close to large fresh-water outlet - drainage from agricultural area, maybe some sewage. Sea a mess, visibility 30cm due to surf. Collected at 4-5m depth, very at random.

Ranking: Arbacia 21

Paracentrotus 12.75

Site 7 Sampled /12/76

Anzio. Large sandy area, with a few rocky outcrops around harbour. Small fishing and turistic port, no particular industry in close vicinity. Sea conditions foul, although a few echinoids were felt more than seen, it was not possible to collect a significant sample. Patella was sampled on

the south side of the jetty (on the outside). Visibility 20cm. Maximum depth of the area 4m, the jetty is composed mainly of rock, with occasional concrete blocks.

Ranking: Patella 12

Site 8 Sampled /12/75

Anzio fish market. Sampled on same day as site 7. Bought some shell-fish from a local stand, presumably caught on the beach strip immediately south of the town. Both Tellina distorta and Venerupis decussata were alive and fresh, and presumably had been purged for some hours in the stall's tank. Venerupis acceptable, Tellina a bit too small to make one want to waste time on it. Both species of economic interest, and eaten in large quantities.

Site 9 Sampled 5/11/75

Sorrento - Marina Piccola. Sampled along jetty, towards the beach, south of the sewage outlet. Heavy organic pollution, some of which very visible, but there still was a considerable variety of organisms on the rocks and concrete boulders forming the jetty. Mainly red algae; Arbacia lixula was extremely common, but Paracentrotus was only present in small numbers and of very small size. Only 5 samples of acceptable size were found, the others were about 1-2cm in diameter, and were returned to sea. The majority of Patellae were in the 2-3cm size class, with occasional 4-5cm individuals. The 2-3cm class was sampled.

Ranking: Arbacia 18.12

Paracentrotus 9.75

Patella 10.25

Site 10 Sampled /4/76

Sorrento - fish market. Obtained some Venerupis decussata from the fish market, alive and apparently caught in the bay of Naples.

Site 11 Sampled 5/11/75

Massa Lubrense. Furthest town on the Sorrentine Peninsula facing the Gulf of Naples. Sampled the outer part of the jetty. Large cement blocks merging with a rocky bottom, degrading steeply. Sampled between 2 and 15 m for the echinoids, around 1 m for Patella. Echinoids very frequent, but Arbacia: Paracentrotus::50:1 or more. Patellae were not very frequent, with a certain variation within the 2-4cm size class.

Ranking:	Arbacia	11
	Paracentrotus	13.4
	Patella	8.5

Site 12 Sampled 7/11/75

Agropoli. Sampled on outer side of pier, close to cliff face. The pier is a recently constructed structure of limestone blocks and rubble - the concrete massing does not reach the water uncovered. The bottom was sand-covered rock, with frequent outcrops. Not very many algae, possibly because of exposed position, but this does not seem to be necessarily a hinderance. Echinoids were very abundant, but distinctly clustered. Paracentrotus lividus was the most abundant, but in some well defined areas, Arbacia was definitely predominant. Altogether, Paracentrotus: Arbacia:: 3:1. Patellae were of two size classes, abundant 2-3 cm, a few 5-7 cm. The smaller were sampled, close to the surface along the seawall.

Ranking:	Arbacia	14.12
	Paracentrotus	15.25
	Patella	7.5

Site 13 Sampled 7/11/75

Punta Tresino. Sampled at the end of the beach of Santa Maria Di Castellabate, at the southermost end of the cape. Large, sharply angled stratifications, selectively eroded and subsequently crollapsed, form a bottom of large boulders, caves, sandy stretches, and Posidonia oceanica meadows. Arbacia and Paracentrotus were equally abundant, possibly with a slight excess of Paracentrotus. Echinoids were picked mainly on vertical faces of the ridges in 2-3cm of water. Patella was sampled a bit farther north, towards the point, as close to the beach the shallows were filled with Posidonia detritus.

Ranking:	Arbacia	16.75
	Paracentrotus	12.75
	Patella	11.5

Site 14 Sampled 7/11/75

Punta Inferno. Sampled on the outer wall of the seawall in front of the small fishermen's harbour. The seawall is formed of large concrete blocks, set onto a generally bare sandy bottom. Limited flora, echinoids very abundant. Arbacia and Paracentrotus about equally abundant. Patella infrequent, in two distinct size classes 2-3cm and 5-6 cm. Assorted Muricacea were the most common inhabitants.

Ranking:	Arbacia	19.25
	Paracentrotus	9.5
	Patella	14

Site 15 Sampled 7/11/75

Ogliastro Marina. Sampled on the southermost side of the cape, at the end of the public access road. The beginning of the point's flytch scarp, emerging from the sand of the Ogliastro Marina beach, has sharply

angled stratifications, and differential erosion has created a series of ridges, parallel and continuous for over a mile, with minor ridges in between. Wherever sand has built up, Posidonia oceanica has colonized the area, from small patches to large, with well established mats over three metres thick in the middle of the bay. Echinoids were reasonably abundant, Paracentrotus being far more so than Arbacia and were sampled around 1m depth. Ratio about 10:1. Most echinoids were sampled on underside of rock ledges. Patellae were sampled along the ledges closest to shore.

Ranking: Arbacia 10.25
Paracentrotus 13
Patella 7.75

Site 16 Sampled 29/5/76

Lipari I - Pietra del Bagno. Due to injury on the way down, this series of samples were collected by Mauro Perugini without supervision. Pietra del Bagno is an isolated stack on the east coast of Lipari towards Salina. The samples were collected on the vertical wall of the stack at depth not greater than 8m. Further information missing.

Ranking: Arbacia 14.5
Paracentrotus 19.5

Site 17 Sampled 30/5/76

Lipari II. Sampled in front of the hotel Giardino sul Mare, close to the harbour, probably close to the most used sea section in the islands. Bedrock volcanic,mainly lava and some basalt boulders. Clear water with abundant flora and sessile fauna on rocky walls, but quite a few surface oil slicks. Depth sampled 0-3m, echinoids reasonably abundant, roughly

equal but Paracentrotus found on more overgrown areas, Arbacia lixula on highly grazed ones, usually shallower. Large Patella (6-7cm) around 1m depth.

Ranking:		
	Arbacia	10.5
	Paracentrotus	16.5
	Patella	7.75

Site 18 Sampled 31/5/76

Filicudi. Sampled off the East coast of the island. Completely untouched area, on opposite side of the only inhabited area, facing the open sea. As far from pollution sources as I have been able to get. Very abundant echinoids on large flat boulders at 3-6m depth. No great flora, mainly smallish clumps of Cystoseira spp. Paracentrotus: Arbacia:: 2:1. Large (5-6cm) patellae at same site.

Ranking:		
	Arbacia	9.25
	Paracentrotus	19
	Patella	9.75

Site 19 Sampled on 11/6/76

Punta Raisi. Cape a few miles west of Palermo. Site some 800m from airport perimeter, 2km from the beginning of the Palermo industrial complex. Large blocks of conglomerate sandstone, eroded by water and organisms. Massive brown algal overgrowth, mainly Cystoseira spp., and some red encrusting (Corallina spp.). Area apparently free from straightforward pollution, but a certain amount of persistent foam, a small jetty evidently serving the airport, and a drowned dog seemed to indicate otherwise. Patellae impossible to collect, as they were too tightly embedded in rock cavities, also only very small size class

present (1-2cm). Echinoids abundant, at all depths down to flat sandy bottom at 5m depth. Slight preponderance of Paracentrotus lividus over Arbacia lixula. Both species very small, bulking of 2 individuals necessary for all Arbacia, some Paracentrotus. Nearest industries: cement works, silver plating, hair treatment (wigs), secondary processing of plastics, some metalwork. Massive building along the coast.

Ranking: Arbacia 11

Paracentrotus 12.25

Site 20 Sampled 14/6/76

Capo Carbonara. Southeastermost tip of Sardinia. Pebble/boulder beach, sand bottom with outcropping boulders. Conglomerate sandstone, igneous inclusions (east of the Cagliari alluvial trench). Water clear, except for abundant Posidonia oceanica debris. Site some 1-1.5km from actual cape, on east coast. Moderate growth of brown and red encrusting algae. Echinoids relatively scarce, Paracentrotus:Arbacia::3:2. Small individuals, for Arbacia bulking was necessary. Patella was not sampleable - too small (4-8mm) and too well fitted into the rock. No visible industry in the area except a few hotels and a small quarry.

Ranking: Arbacia 16.5

Paracentrotus 17.5

Site 21 Sampled 14/6/76

Melisenda. Isolated site half-way between Capo Carbonara and Arbatax. One small hotel, some houses, a NATO radar base. Conglomerate rock, sandstone matrix and igneous inclusions, mainly granite. At sea, only granite boulders emerging from sandy bottom. Heavy brown algal growth and some Posidonia oceanica. Went offshore some 700m, scanned, and returned. Water clear, but some persistent foam along coast and abundant

Posidonia debris. Echinoids very scarce, Paracentrotus:Arbacia::3:2. Paracentrotus large, Arbacia were very variable and required bulking in some cases. Patella was very scarce and small, and therefore not sampled.

Ranking:	Arbacia	12.75
	Paracentrotus	11.75

Site 22 Sampled 15/6/76

Arbatax. Sampled along rock face outside harbour, off a large quarry. Heavily metamorphized rock, usual pinkish mother rock with thick veins of granite and basalt. Echinoids relatively abundant, but Paracentrotus: Arbacia::1:5. Paracentrotus also very deep, at least 6m. Vertical or almost vertical walls, heavily overgrown with algae, browns and greens. Patellae abundant, large, heavily overgrown with Ulva lactuca. Some bulking necessary for Arbacia, all Patella bulked. Industry present: industrial port, shipyards, paper mills.

Ranking:	Arbacia	14
	Paracentrotus	14.75
	Patella	6.25

Site 23 Sampled on 15/6/76

Fuile e Mare. Site reachable on white road leading off on Km 231 of the SS125. Small bay on gully/stream outlet. Slight water flow, probably significantly more in winter. Sand flat becoming beach: sides of gully made of igneous rock boulders, merging in the sandstone matrix further up the shore. Posidonia oceanica bottom, with frequent buildup of rhyzomes. Depth 0-4m. Sampled along left side looking seawards. Paracentrotus lividus very abundant and large, also amongst the Posidonia, Arbacia very scarce and very small. Patella present only in minute size and

quantity. Arbacia had to be bulked.

Ranking: Arbacia 12.75

Paracentrotus 12.5

Site 24 Sampled on 16/6/76

Golfo Aranci. Northeast and outermost tip of Golfo di Olbia. Olbia, large harbour, car ferry terminal, ship repair yard, some light industry. Site - end of white road off Golfo Aranci, some 2km. Bottom sandy with heavy Posidonia oceanica growth, which continues on rocks and boulders on sides. Bedrock igneous basement. Echinoids reasonably abundant, sampled between 0-2m depths. Paracentrotus:Arbacia::2:1, Arbacia mainly on exposed ridges, Paracentrotus also amongst the Posidonia. No Patella worh taking notice of. Some bulking necessary for Arbacia.

Ranking: Arbacia 15.5

Paracentrotus 21.25

Site 25 Sampled on 17/6/76

Santa Teresa di Gallura. Sampled on westerly side of cove with beach west of the town. Granite bedrock and boulders going down to sand at 2-3m. Heavy algal overgrowth, mainly brown with some red encrusting. Very sparce tufts of Posidonia oceanica, presumably increasing offshore. Echinoids reasonably frequent, particularly close to the surface. Arbacia lixula slightly more frequent than Paracentrotus, but very small. Bulking was necessary for Arbacia and Patella, which was abundant but small (2-3cm), and in particular, the animals appeared small in relation to the shell size.

Ranking: Arbacia 15.25

Paracentrotus 12.5

Patella 8.75

Site 28 Collected on 22/4/76

Marseilles - Cortiou. Collected as well as site 29 in the course of the Exercise 'Eagle Dive 2', Army Air Corps Centre Sub Acqua Club plan 42614. Sample given to me by Dr. Barry Jupp, descriptions as given: "1km from effluent behind Ilôt de la Mélette. Very dirty, no sign of Posidonia all along the coast. Would have expected some in the bay as there was some sand. Substrate mainly rock but abundant sea urchins. Cortiou bay - main sewer in northernshore - 6-10' open drain, lots of sewage pouring out. Main pollution radius 1km, with floating organic matter along coast for more than 2km. Silt, no Posidonia, Paracentrotus or Arbacia. "Mytilus edulis was collected.

Site 29 Collected on 18/4/76

Marseilles - Mongenet. Mongenet Cove (Bay of Cap Croisette). This cove is near Les Goudes, to the SW of Marseilles, and is within the polluted zone where Cystoseira stricta starts to disappear. Poor light penetration, dense suspended matter (5000-20000 coliforms/l, Bellan 1970) (101). Collected Mytilus edulis and Patella.

For sites 28 and 29, see Jupp et al 1977 (102).

Ranking: Patella 7.5 29

Site 30 Collected 14-19/8/76

Malta - Fort St. Lucian

Patella coerules 0.5m, Paracentrotus lividus 4m

Ranking: Paracentrotus 14

Patella 2.25

Site 31 Collected 13/8/76

Malta - Paradise Bay

Patella coerulea 1-3m,

Arbacia lixula 3-5m

Ranking: Arbacia 7.5

Patella 6.5

Site 32 Collected 16/8/76

Malta - Wied iz Zurrieg

Arbacia lixula and Paracentrotus lividus 11m

Ranking: Arbacia 8.25

Paracentrotus 3.25

The three above sites were collected by Dr. B. Jupp.

No further data available.

Site 33

Cyprus - Akrotiri

The material was received in 1976, dried and stored in aluminium trays.

No further data available.

Ranking: Arbacia 4.25

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Appendix 2

Ehrhardt Facies Classification

Facies description and notes on use

Ehrhardt Facies Classification

The following groupings, first described in this context by Ehrhardt in 1968 (68), do not constitute true communities, but can be considered as diagnostic "facies", not necessarily conclusive, but which if widely interpreted and considered in the light of the other site characteristics can give a reasonably precise indication on the level of organic pollution in the area, and on its chronicity. They refer only to Mediterranean areas with principally rocky substrata, and to superficial waters.

Facies 1 (Rhodophyta and/or Phaeophyta)

Characterized by the presence of Jania rubens or other large rhodophytes or phaeophytes as dominant species. Sometimes there is a sediment-accumulating undergrowth, for example of Cladophora spp.

Facies 2 (Anthozoa)

Characterized by anthozoans as dominant organisms, frequently together with other encrusting organisms.

Facies 3 (Cytoseira)

Characterized by the dominance of Cystoseira spp. in the surface waters, not to be confused with the presence of Cystoseira spp. on sandy or detritic bottoms in the immediate vicinity of marine angiosperms.

Facies 4 (Incrusting algae)

Characterized by presence of abundant incrusting rhodophytes, as for example Litophyllum incrustans.

Facies 5 (Tubiform invertebrates)

Characterized by abundant calcareous incrustations due to serpulids and /or vermatids, at times covered by a flora of incrusting algae.

Facies 6 (Corallina)

Characterized by the dominance of Corallina officinalis and Corallina mediterranea, usually associated with Ulva lactuca and

Enteromorpha spp.

Facies 7 (Ulva lactuca)

Characterized as facies 8 by an abundance of chlorophytes such as Ulva lactuca, Enteromorpha spp. and Cladophora spp., with Ulva lactuca predominating. More plants than animals.

Facies 8 (Enteromorpha)

Characterized as facies 7 by an abundance of chlorophytes such as Ulva lactuca, Enteromorpha spp. and Cladophora spp., with Enteromorpha spp. dominating. More plants than animals.

N.B. Facies 7 and 8 may appear as a temporary phase during the recolonization of an area cleared by sea action or erosion.

Facies 9 (Harbour)

Characterized by the presence of chlorophytes such as Ulva lactuca and Enteromorpha spp.,but in which the dominant organisms are filter feeders, especially Mytilus spp. and Balanus spp., together with Ciona intestinalis and sessile polichetes.

Facies 10 (Fouling)

Characterized by a scarce presence of Mytilus spp. and Balanus spp., an almost complete absence of chlorophytes, and a predominance of amphipods and sessile polichetes, usually as incrusting fouling on fixed and floating structures.

Facies 11 (Obvious pollution)

Characterized by a total absence of visible algae, an almost total absence of molluscs and lamellibranchs, and by the presence of a bottom layer of anaerobic mud, rich in nematodes and SO_2 - producing bacteria.

Facies 12 (Sea grasses)

This facies does not strictly belong in the series, and certainly cannot be placed in a fixed position on the scale, but it might well be present in sandy or detritic patches, particularly amongst facies 1 to 6, and as a rule requires relatively clean water and considerable light.

It might, therefore, be worthwhile noting separately. It is characterized by the presence of a more or less thick mat of marine angiosperms, Posidonia oceanica, Cymodocea nodosa, or Zostera spp., with their associated rich ectofauna and ectoflora, and the predators of the same.

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Appendix 3

Microwave digestion test results

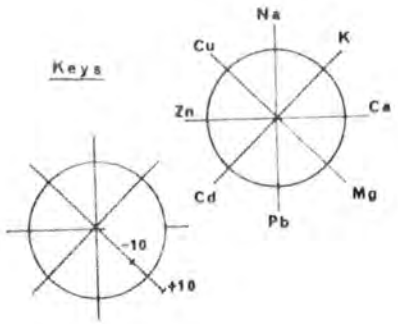
As described in the text, a series of test runs were carried out using the microwave digestion technique described by Abu Samra et.al. (67), using glucose and homogenized cod tissue, as specified in appendix 4. The runs with glucose were meant to be chemical blanks, whose purpose was to measure any level of contamination present in the system, as the glucose would have been oxidised entirely to CO_2 and water at the end of the digestion process, while the cod homogenate was meant to give an indication of the method's accuracy and repeatability. A dozen tests were carried out, using different combinations of time, sample concentration and digestion mixtures. The results, though far from uniform, gave indications consistent with the conclusions drawn in the text, and the examples shown in figures 17 and 18 can be taken as fair representatives of the lot.

Figure 17 shows the analytical results, after matrix interference correction, of a batch of homogenized cod samples digested for 35 minutes in a digestion mixture consisting of 70% HNO_3, 10% H_2SO_4, and 20% $HClO_4$. As can be seen, apart from a wide range of results, keeping in mind that the samples were uniform in nature but varying in weight, there is a definite spatial distribution pattern, the samples around the edge of the microwave chamber showing markedly higher values than the ones at the center. As non-uniform microwave density in commercial ovens is a well-known phenomenon, it appears that the results are more dependent on the available microwave density to which the samples were exposed, i.e. their degree of dissociation, than on the sample's actual metal content. On the other hand, 35 minutes in a nominal 600W microwave field for a 30ml sample could be presumed to be more than sufficient for complete dissociation, assuming that there is an efficient energy absorption. This, therefore, does not seem to be the case.

Figure 18 shows two exemplary plots of the results of the same batch

Figure 17

Deviation from mean of metal concentration in 24 individual samples, varying in weight and randomly distributed, of homogenized cod tissue digested by microwave method. The plots are arranged in the same way as the samples were laid out in the microwave oven. The circle represents the mean value, the scale on the right shows the positive or negative percentage deviation from it.



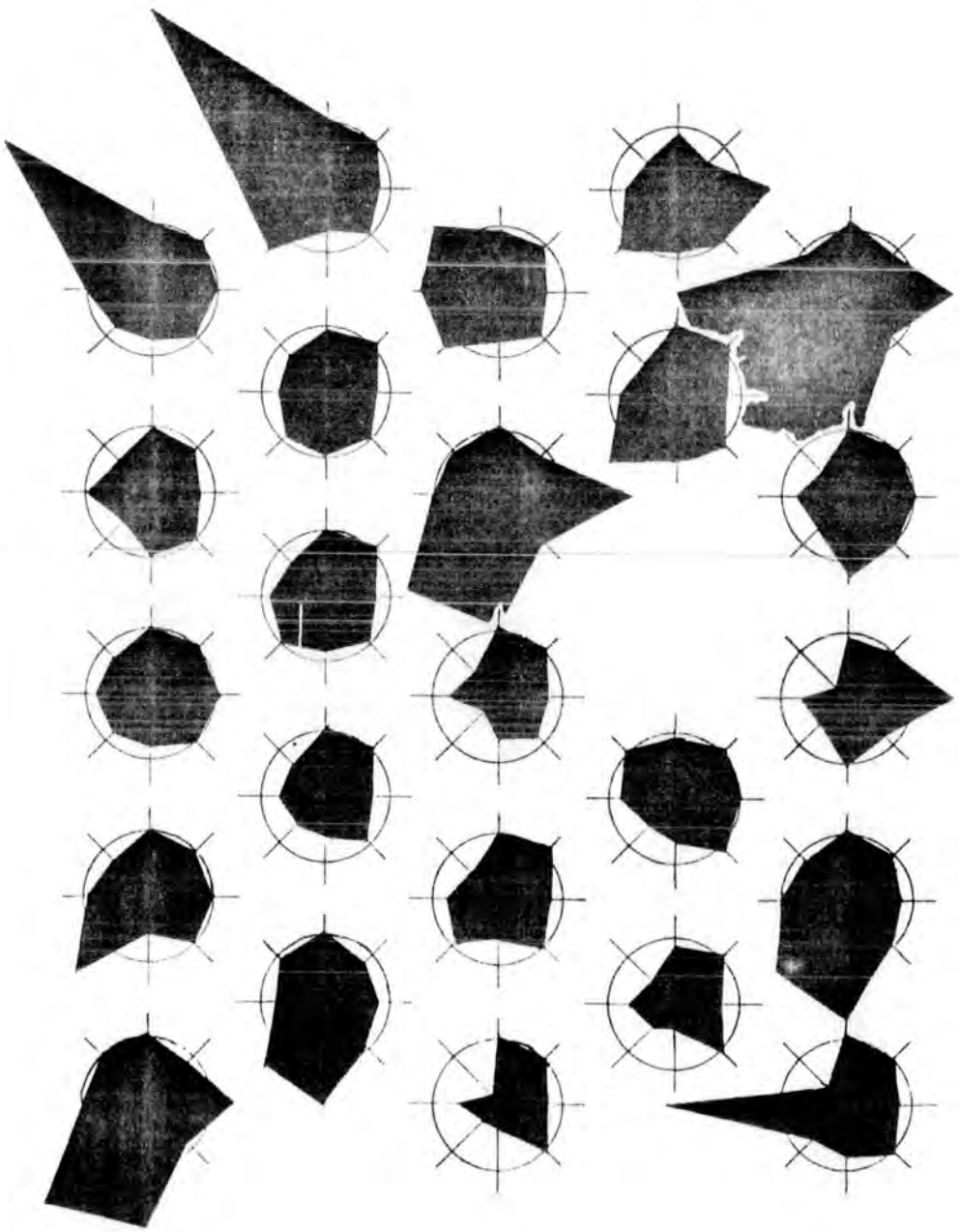
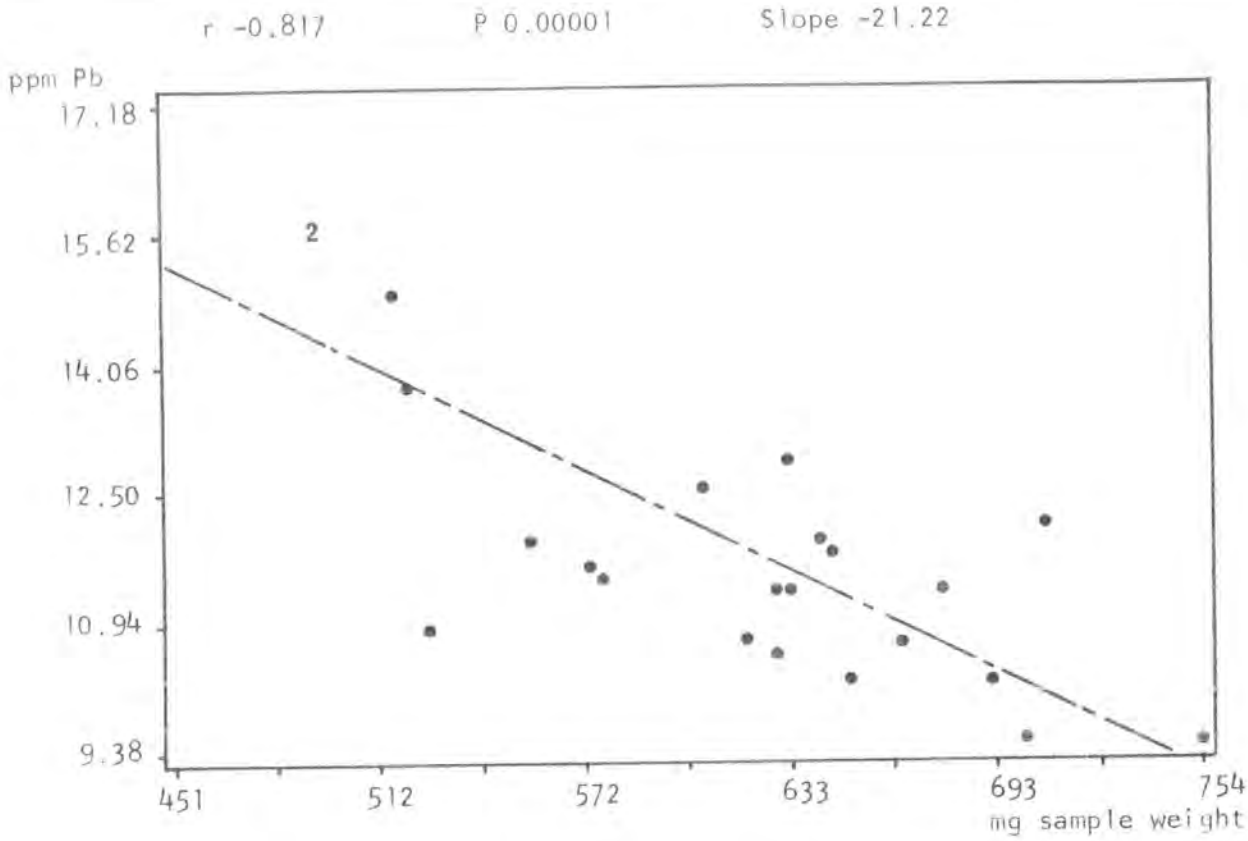


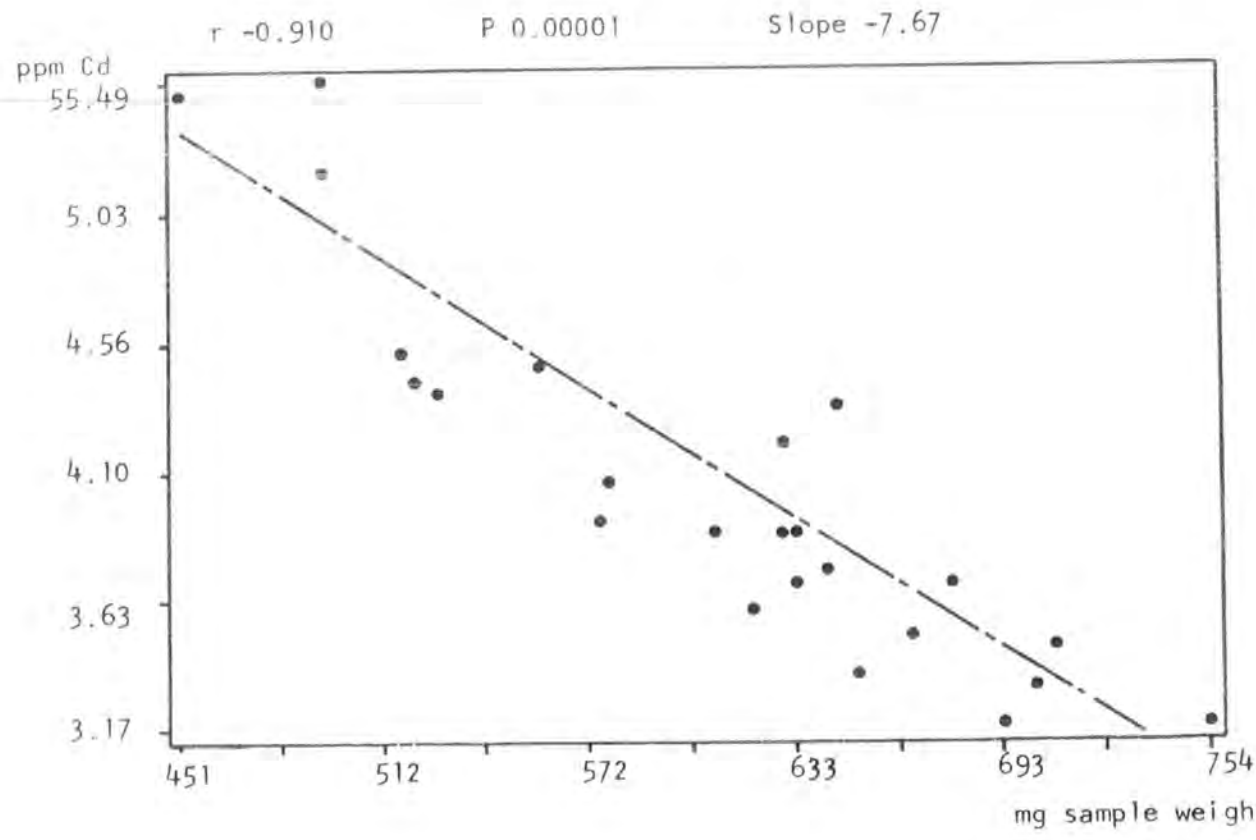
Figure 18

Microwave digestion results

Single element plots against sample weight for homogenized cod tissue.

The regression lines are plotted.





of samples. Detected values of Lead and Cadmium are plotted against the sample weight, showing a very clear relation between the two variables. Again, considering that the samples consisted of a homogeneous control substance, it would appear that the actual metal content of the sample is the least important variable affecting the detectable levels.

A full explanation of the phenomenon would probably require considerably more work, preferrably by a chemist as opposed to a biologist, but nevertheless it is felt that the reason mentioned in the text, viz. the drop in microwave absorption by the samples once all water present has been dissociated, could lie at the root of the problem.

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Appendix 4

Specifications of chemicals used throughout the analysis

Ethanol

Athanol Absolut pro analysis 983	"Merck"
GC 99.8%	
Free acid (CH_3COOH)	max 0.001%
Free alkali (NH_3)	max 0.0003%
Pb	max 0.00001%
Cu	max 0.00001%
Fe	max 0.00001%
Zn	max 0.00001%
Methanol	max 0.05%
Iso-amyl-alcohol	max 0.05%
Formaldehyde	max 0.001%
Aldehyde (CH_3CHO)	max 0.001%
Ketone or acetone	max 0.05%

D- Glucose AnalaR	BDH Chemicals (Blank Standard)
$O.(CH.OH)_4.CH.CH_2OH = 180.16$	
pH of solution	not less than 5.8
Specific rotation on $[\alpha]_D^{20^\circ}$	$+52.5^\circ$ to 53.0°
Water-insoluble matter	0.003%
Coloured impurities	Passes test
Alcohol-insoluble matter	0.005%
Sulphated ash	0.03%
Chloride (Cl)	0.0025%
Sulphate (SO_4)	0.0025%
Sulphite (SO_2)	0.0005%
Arsenic (As)	0.00002%
Iron (Fe)	0.0001%
Heavy Metals (Pb)	0.0001%
Loss on drying	0.1%

Biological Standard:

10 packets of Birdseye Frozen Cod Steaks, each 200g, giving a total of 380gd/w, dried at $105^\circ C$ and milled in a Christy and Norris 8" laboratory mill with a 1mm mesh. Mesh and hopper were cleaned before run, the fish was bought in Durham's Woolworth, and the stock prepared on 16.8.76.

Nitric Acid sp. Gr. 1.5	fuming	BDH Chemicals
95% HNO_3 = 63.01		AnalaR
Minimum assay 95.0% HNO_3		
Dilution test		Meets requirements of 'Methods of Test'
Non-volatile matter		0.001%
Chloride (Cl)		0.00005%
Phosphate (PO_4)		0.0002%
Sulphate (SO_4)		0.002%
Arsenic (As)		0.000001%
Iron (Fe)		0.0001%
Heavy metals (Pb)		0.0001%
Manganese (Mn)		0.00004%

Perchloric acid. AnalaR, BDH Chemicals Ltd., Poole, England

$HClO_4$ = 100.46 sp.gr. 1.54

Assay	60 to 62% HClO
Dilution test	Miscilbe with water, forming clear colourless solution
Non-volatile matter	max 0.003%
Chlorate (ClO_3)	max 0.0012%
Chloride (Cl)	max 0.0001%
Free Chlorine (Cl)	max 0.0005%
Nitrate (NO_3)	max 0.001%
Phosphate (PO_4)	max 0.0002%
Silicate (SiO_2)	max 0.0001%
Sulphate (SO_4)	max 0.0004%
Ammonium (NH_4)	max 0.0002%
Arsenic (As)	max 0.000005%
Copper (Cu)	max 0.00002%
Iron (Fe)	max 0.00005%
Lead (Pb)	max 0.00001%
Manganese (Mn)	max 0.00005%
Silver and Mercury (Ag+Hg)	max 0.00004%

Appendix 5

Description of composite indices

The three composite indices mentioned in the discussion were calculated as follows:

"Apparent Visible Pollution Index" - FACTOT

This is a composite statement of the obtained Ehrhardt Scale values. During the sampling, the distribution of the single facies was noted on an absent/present/abundant scale, coded 1, 2 and 3 respectively. For the present purpose, absent values and facies 12 were ignored, and the arbitrary weighing scale shown in table 30 was used.

Table 30

FACTOT weighing scale

Facies	weight	Facies	weight
1	1	7	9
2	2	8	10
3	3	9	12
4	4	10	15
5	6	11	20
6	7	12	-

The individual facies values were multiplied by their weighing, and summed to give the composite index.

"Bottom morphology" - BOTTOM

This index gives a rough indication of the "rockyness" of the sampling site, by summing the arbitrarily assigned values listed in table 31 to the recorded bottom characteristics.

Table 31

BOTTOM weighing scale

If MUD abundant	1
If MUD present	3
If SAND abundant	5
If SAND present	7
If PEBBLE present	15
If PEBBLE abundant	20
If ROCK present	25
If ROCK abundant	30

"Human Impact Index" - HII

In this case the site characteristics involving presence or absence of freshwater and waste outlets, ports and harbours, industrial sites and urban development, were ranked in order of relative injuriousness to the environment by cross estimating them on the matrix illustrated in table 32 and a composite index was created by summing the site's rank values and adding one to remove zero values.

Table 32

Human Impact Index matrix

		A	B	C	D	E	F	G	H	I	J	K
A	FRESHW	X	1	1	1	1	0	1	1	0	1	1
B	HARBOUR	0	X	0	1	-	0	0	1	0	0	1
C	FISHPORT	0	1	X	1	1	0	-	1	0	0	1
D	OILTERM	0	0	0	X	0	0	0	1	0	0	-
E	INDEV	0	-	0	1	X	0	0	1	0	1	1
F	QUARRY	1	1	1	1	1	X	1	1	0	1	1
G	SEWAGE	0	1	-	1	1	0	X	1	0	1	1
H	WASTE	0	0	0	0	0	0	0	X	0	0	1
I	INURB.1	1	1	1	1	1	1	1	1	X	1	1
J	INURB.2	0	1	1	1	0	0	0	1	0	X	1
K	INURB.3	0	0	0	-	0	0	0	0	0	0	X
	TOTAL	2	6	4	8	5	1	3	9	0	5	9
	HII	3	7	5	9	6	2	4	10	1	6	10

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