RAPID MULTIELEMENT ANALYSIS OF OYSTER AND COCKLE TISSUE USING X-RAY FLUORESCENCE SPECTROMETRY, WITH APPLICATION TO MARINE POLLUTION INVESTIGATIONS

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ABSTRACT

A total of 42 elements has been analysed by the X-ray fluorescence spectrometry (XRF) method in oyster and cockle tissue. Apart from a limitation in detection of Hg in the investigated samples, this study shows that for most elements, the lower limits of detection for the XRF method are below elemental concentrations in these two bivalves from moderately polluted areas. Hence, XRF has potential to be employed for broad ranging marine pollution elemental monitoring studies using oyster and cockle tissue.

INTRODUCTION

It has been known for many years that bivalves concentrate heavy metals (Clarke and Wheeler 1922; early work reviewed by Vinogradov 1953). Brooks and Rumsby (1965) doubted on analytical grounds many results of earlier workers. Whilst Brooks and Rumsby (1965) contributed greatly to revitalising an interest in heavy metals and their investigation in marine organisms during the sixties, the analytical techniques of Margoshes and Scribner (1964) used by Brooks and Rumsby (1965) lacked sensitivity for the limited number of elements analysed. With the advent of atomic absorption spectroscopy (AAS) the number of elements determinable, as well as their lower limits of detection, improved greatly (Walsh 1955). Despite this, a recent review of heavy metals in aquatic organisms (Forstner and Wittmann 1981) showed that the range of metals regularly analysed in marine organisms is still small.

X-ray fluorescence spectrometry (XRF) has been demonstrated to be suitable for analysing a wide range of elements in various light matrix materials, such as plant (Norrish and Hutton 1977; Hutton and Norrish 1977; Murdoch and Murdoch 1977) as well as soil and plant material (Livingston 1982).

This paper investigates the use of XRF for rapid determination of 42 elements, especially with respect to marine pollution. To this end, oyster tissue from polluted and unpolluted areas in the Dampier Archipelago (20° 27' S, 116° 48' E), Western Australia, and cockle tissue from a polluted area in Albany (35° 02' S, 117° 51' E), Western Australia, were analysed. In addition, this study was undertaken to determine whether elements not normally analysed by other conventional analytical techniques may be accumulated by oysters or cockles, and hence should be included in the future marine pollution investigations of biological material using XRF.

METHODS AND MATERIALS

Specimen choice, collection and storage

Oysters were chosen for the investigation as they accumulate heavy metals (Bloom and Ayling 1977). The rock oyster Saccostrea cucullata was chosen because it is contaminated with Cu and Zn in the Dampier Archipelago (Talbot 1985).

Ninety specimens (52.5±2.5 mm length) were sampled at Dampier (20°41' S, 116°42' E), adjacent to an iron ore exporting terminal, the coolant water outfall of an electricity generating station and a sewage outfall. An additional 90 specimens were sampled in clean coastal waters off Gidley Island, 25 km north of Dampier. Each set of 90 specimens was subdivided into 3 bulk samples of 30 individuals : 30 being the minimum number which statistically constitutes a large sample (Spiegel 1972). Cockles (Katelysia Scalarina) were chosen for investigation from the western end of Princess Royal Harbour, Albany, as they are polluted with lead (Talbot 1983) and mercury (Jackson et al. 1985).

Sixty cockles (37.5±2.5 mm) were collected within 10 m of an effluent discharge pipe and 60 m from a contaminated drain mouth. The number of cockles collected was restricted by their availability.

The oysters and cockles were shucked, dried, and stored in a desiccator following the method of Talbot et al. (1985).

Analysis

Samples were dried in a Dynavac FDA/3T freeze drying unit for 48 h. Dried samples were ground in a Tema ring mill with agate elements to a fine powder in order to minimize particle size effects on analysis. Approximately 5 g of each resulting powder was mixed with 1ml of 2.5% polyvinyl alcohol aqueous solution which acts as a binding agent (K.

Norrish, unpublished work) and then pressed into 32mm diameter disks. Two separate disks were made from each material. The disks were analysed on a Philips PW1400 spectrometer using a Rh target X-ray tube operating at 60 kV and 40 mA in a vacuum path. Two measurements were made on each sample to check the measuring reproducibility. Both scintillation and flow counters were used in conjunction with a fine collimator for analyses, and the pulse height analyser was used for the measurements. All other experimental conditions are shown in Table 1.

For Ba, Bi, Ce, Cs, Dy, Er, Hg, Hf, I, La, Pb, Sb, Sc, Se, Sn, Ta, Te, Th, Tl, W and Yb, 80 s and 40 s analysing times were used for peak and background measurements respectively. For the rest of the elements, the above analysing times were halved.

The levels of the elements were measured against calibration standards which were Specpure grade SiO_2 disks (Johnson and Matthey Chemicals) impregnated with a known amount of the elements. Corrections for matrix effects were made using the intensity of the Compton scattering of Rh Kα in each sample (Bertin 1975). Spectral line interferences due to peak-overlap were corrected after the overlapping factor had been determined from samples with and without the interfering element. Table 2 lists the interfering elements for each of the spectral line overlapping corrections. In order to overcome the problem of instrumental drift of the XRF system, two synthetic monitor samples impregnated with known amounts of trace elements were analysed at both the beginning and the end of each batch of 18 samples.

Quality control

In addition to the quality control procedures used by Talbot and Chegwidden (1982) to avoid contamination of samples and apparatus, four biological reference materials (US National Bureau of Standards, NBS) SRM 1566 (oyster

Table 1. Instrumental conditions for XRF analysis of oyster tissue.

ELEMENT	LINE	XTL*	+OFFS#	-OFFS@
As	$K\alpha$	1	0.50	0.50
Ba	$L\alpha_1$	1	1.00	1.00
Bi	$L\alpha_1$	1	0.00	0.50 $
Br	$K\alpha$	1	0.00	0.50 $
Cd	$K\alpha$	2	0.70	0.50
Ce	$L\alpha_1$	1	1.00	1.00
Co	$K\alpha$	1	0.50	0.50
Cr	$K\alpha$	1	0.80	0.80
Cs	$L\alpha_1$	1	0.80	0.80
Cu	$K\alpha$	1	0.60	0.60
Dy	$L\beta_1$	1	0.60	0.60
Er	$L\beta_1$	1	0.50	0.50
Fe	$K\alpha$	1	0.50	0.50
Ga	$K\alpha$	1	0.60	0.60
Ge	$K\alpha$	1	0.50	0.50
Hf	$L\alpha_1$	2	0.50	0.50
Hg	$L\alpha_1$	1	0.50	0.50
I	$L\beta_1$	1	0.50	0.50
La	$L\alpha_1$	1	----	1.00$
Mn	$K\alpha$	1	0.50	0.50
Mo	$K\alpha$	2	0.50	0.50
Nb	$K\alpha$	2	0.50	0.50
Ni	$K\alpha$	1	0.60	0.60
Pb	$L\beta_{1,2}$	1	0.50	0.50
Rb	$K\alpha$	2	0.80	0.80
Sb	$K\alpha$	2	0.40	0.40

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Sc	$K\alpha$	1	0.80	0.80
Se	$K\alpha$	1	0.50	0.50
Sn	$K\alpha$	2	0.50	0.50
Sr	$K\alpha$	2	0.50	0.50
Ta	$L\alpha_1$	2	0.60	0.60
Te	$K\alpha$	2	0.40	0.40
Th	$L\alpha_1$	2	0.50	0.50
Ti	$K\alpha$	1	0.50	0.50
Tl	$L\beta_{1,2}$	1	----	0.50$
U	$L\alpha_1$	2	----	0.50$
V	$K\alpha$	2	----	1.00$
W	$L\alpha_1$	2	0.50	0.50
Y	$K\alpha$	1	0.50	0.50
Yb	$L\alpha_1$	1	0.80	0.00
Zn	$K\alpha$	1	0.50	0.50
Zr	$K\alpha$	2	0.50	0.50
Rh K Compton line		1	0.00	0.00

* = Analysing crystal : 1 = LiF(200), 2= LiF(220)

and @ = 2θ angular offset for background measurements.

$ = Only one background was measured

Table 2. Interfering lines used for the spectral line overlapping correction.

Element	Interferring line(s)
As	$PbL\alpha_1$
Ba	$ScK\beta$
Br	$AsK\beta$
Ce	$BaL\beta_1$
Cr	$VK\beta$
Hg	$WL\beta_2$
La	$CsL\beta_1$
Mo	$ZrK\beta$
Nb	$YK\beta$, $UL\beta_2$
Rb	$BrK\beta$
Sc	$SbL\beta_2$
Ta	$ErL\beta_2$
Th	$BiL\beta_1$
Ti	$IL\beta_2$
U	$RbK\alpha$
V	$TiK\beta$, $CsL\beta_2$
W	$YbL\beta_1$, $DyL\gamma_1$
Y	$RbK\beta$, $PbL\gamma_1$
Zr	$SrK\beta$

tissue), SRM 1571 (orchard leaves), SRM 1573 (tomato leaves) and SRM 1575 (pine needles) were used to verify the XRF method for analysing the trace elements in oyster tissue. As these standards have low concentrations of heavy elements, they were particularly useful at the lower end of the operating limits where XRF has some detection difficulties. Furthermore, these reference materials have a light elements matrix, which is similar or the same as that of oyster tissue and thus provides a control for our matrix correction method.

RESULTS AND DISCUSSION

Limits of Detection

The calculated lower limits of detection at 2σ confidence for this study are listed in Table 3. Elements in the Table have been divided into two groups. It can be seen that for those elements in Group I, the XRF method can provide rapid analyses at about 1 mg kg^{-1} level. The lower limits of detection for Group II may be improved by either using longer counting times or using a higher operating voltage for the X-ray tube.

Standard reference materials

The results for the analysis of standard reference materials are given in Table 4. They show that for the element values recommended by NBS, the authors have achieved reasonable agreement for many of the elements. With the exception of iodine, all the other discrepancies probably are due to limit of detection problems. It is possible that standards lose iodine by repeated analysis, hence giving an apparent increase in the unknown sample.

The list of NBS certified values presented in Table 4 gives a indication of the range of elements usually considered in elemental studies. The results for other elements determined in this study on the NBS standards, for which the NBS do not give values, are given in Table 5.

Table 3. Calculated lower limits of detection (L.L.D.) at 2 confidence for Rh X-ray tube operated at 60 kV and 40 mA in this study.

(1) Group I		(2) Group II	
Element	L.L.D.(mg/kg)	Element	L.L.D.(mg/kg)
As	0.5	Ba	4
Br	1	Bi	6
Cd	2	Ce	4
Co	3	Cs	3
Cr	1	Dy	9
Cu	2	Er	3
Fe	1	Hg	5
Ga	1	Hf	3
Ge	1	I	5
Mn	1	La	3
Mo	1	Pb	2
Nb	1	Sb	3
Ni	1	Sc	2
Rb	1	Se	3
Sr	1	Sn	2
Ti	2	Ta	5
U	2	Te	3
V	2	Th	2
Y	0.5	Tl	5
Zn	1	W	3
Zr	1	Yb	2

Note : (1) Analysing time : 40 s at peak, 20 s at background (s).
(2) Analysing time : 80 s at peak, 40 s at background (s).

Table 4. A comparison between the analytical results determined by the authors (mean of 3 values is given in brackets) and certified values mg/kg given by the U.S. National Bureau of Standards for SRM 1566(oyster tissue), SRM 1571 (Orchard leaves), SRM 1573 (Tomato leaves) and SRM 1575 (Pine needles). All units are mg/kg dry wt.

Element	SRM 1566	SRM 1571	SRM 1573	SRM 1575
As	13.4(9.5)	10.0 (9.5)	0.27 (1.5) x	0.21 (<0.5)
Ba	@ (<4)	44.0 (45.0)	@ (<4.0)	@ (<4.0)
Bi	@ (12.5)	0.1 (<6.0) *	@ (<6.0)	@ (<6.0)
Br	55 (55)	10.0 (9.0)	26.0 (24.5)	9.0 (8.0)
Cd	3.5 (2.0)	0.11 (<2.0) *	3.0 (2.5)	<0.5 (<2.0) *
Ce	@ (<4.0)	@ (<4.0)	1.6 (<4.0) *	0.4 (<4.0) *
Co	0.4 (<3.0)	0.2 (<3.0) *	0.6 (<3.0) *	0.1 (<3.0) *
Cr	0.69(<4.0)	2.6 (<4.0) *	4.5 (<4.0)	2.6 (<4.0)
Cs	@ (<3.0)	0.04 (<3.0)	@ (<3.0)	@ (<3.0)
Cu	63.0(64)	12.0 (15.0)	11.0 (11.0)	3.0 (3.0)
Fe	195 (195)	300.0 (280.0)	690.0 (682.0)	200.0 (218.0)
Ga	@ (6)	0.08 (<1.0) *	@ (2.5) *	@ (3.5) *
Hg	0.057(<5.0)	0.155 (<5.0)	0.1 (<5.0)	0.15 (<5.0)
I	2.8 (<5)	0.17 (8.0) x	@ (5.0) *	@ (7.0) *
La	@ (<3)	@ (8.0)	0.9 (3.0)	0.2 (3.0)
Mn	17.5(19)	91.0 (91.0)	238.0 (204.0)	675.0 (637.0)
Mo	<0.2(1.0)	0.30 (<1.0) *	@ (1.0)	@ (<1.0)
Ni	1.03(<1.0)	1.3 (<1.0)	@ (<1.0)	3.5 (4.0)
Pb	0.48(<2)	45.0 (44.5)	6.3 (6.0)	10.8 (12.5)
Rb	4.45(3.0)	12.0 (11.0)	16.5 (15.0)	11.7 (10.5)
Sb	@ (3)	2.9 (<3.0)	@ (<3.0)	0.2 (<3.0) *
Sc	@ (<2)	@ (<2.0)	0.13 (<2.0)	0.03 (<2.0)

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Se	2.1 (<3.0)	0.08 (<3.0)*	@	(<3.0)	@	(<3.0)
Sr	10.36(9.5)	37.0 (39.0)	44.9	(44.5)	4.8	(6.5)
Te	@ (<3.0)	0.01 (3.5)x	@	(4.5)	@	(<3.0)*
Th	0.1 (2)	0.064 (<2.0)*	0.17	(<2.0)	0.037	(<2.0)*
Tl	<0.005(<5.0)	@ (<5.0)	0.05	(<5.0)*	0.05	(<5.0)*
U	0.116(<2.0)	0.029 (<2.0)*	0.061	(<2.0)x	0.020	(<2.0)*
Zn	852 (848)	25.0 (27.0)	62.0	(63.5)	@	(94.0)

x Values where authors determination is 5 times greater than that of the certified value.

* Values with detection limit problems.

@ No value was given by NBS

Table 5. Elemental determinations on the U.S. National Bureau of Standards reference materials SRM 1566 (oyster tissue), SRM 1571 (Orchard leaves), SRM 1573 (Tomato leaves) and SRM 1575 (Pine needles), for which the NBS do not quote values. All units are mg/kg dry wt.

Element	SRM 1566	SRM 1571	SRM 1573	SRM 1575
Ge	<1	1	<1	3
Hf	<3	7	10	10
Ti	30	31	85	20
W	<3	3	<3	<3
Y	1	1.5	2	1
Yb	<2.0	5	2	7
Zr	1.5	<1	11	<1

Oyster tissue

Results show that in the investigated oyster tissue, the levels of elements Ba, Bi, Ce, Cs, Dy, Ga, Ge, Hg, Nb, Pb, Rb, Sb, Sc, Se, Ta, Tl, U, V and Zr are below the lower limits of detection (LLD) of the XRF system presently used. The results of other elements analysed are tabulated in Table 6. Taking into account the difference in environmental chemistry of each site, the limits of detection, and reliability of data, Table 6 shows that this oyster accumulates Cu, Fe, Ni, Ti and Zn at the Dampier site where industrial development has taken place. It is likely that the Fe, Ni and Ti emanate from the iron ore industry as samples of the ore contain 60% Fe, 0.002% Ni, and 0.04% Ti. The source of the Cu and Zn is the sewage outfall and coolant water discharges (Talbot 1985). The high concentrations of As in oysters are expected as As occurs generally in marine biota (Edmonds and Francesconi 1981 a, b; Forstner and Wittmann 1981), including bivalves (Bohn 1975).

Cockle tissue

Results show that in the investigated cockle tissue, the levels of elements Ba, Cd, Ce, Cs, Dy, Ga, Ge, Mn, Nb, Rb, Sb, Sc, Ta, Tl, U, W, and Y are below the lower limit of detection. The results of other elements analysed are tabulated in Table 7. Sixty five percent of the elements detected were present in higher concentration at the contaminated drain mouth than at the outfall. This is consistent with unpublished results of Talbot and Chang which show that the sediments are more polluted near the drain mouth. Talbot and Chang (unpublished work) have shown that the sediments near the drain mouth have higher concentrations of organic material and that this material preferentially binds a broad range of elements. The As results are consistent with the values found for oysters in the Dampier Archipelago while the Hg & Pb contamination is restricted to sediments with a high organic content : drain

Table 6. Elemental analysis of whole soft portion of the oyster Saccostrea cucullata from the Dampier Archipelago. Only those elements which were shown to be above the L.L.D. are listed. Values shown are the average of a total of six individual measurements of three samples from each site. Ranges of variation are shown in brackets. All units are mg/kg dry wt.

Elements	Dampier	Gidley Is.
As	65(56-72)	59(56-62)
Br	128(116-156)	178(168-189)
Cd	2(<2-4)	3(2-4)
Co	4(<3-6)	4(4-5)
Cr	6(3-16)	2(1-2)
Cu	224(191-266)	96(90-101)
Fe	457(298-658)	85(67-102)
Hf	7(5-11)	5(<3-10)
La	12(7-18)	6(3-10)
Mn	5(1-7)	5(4-5)
Mo	1(<1-2)	3(2-3)
Ni	78(12-179)	14(7-21)
Sn	9(5-12)	10(7-13)
Sr	61(42-81)	69(59-79)
Te	5(<3-15)	5(3-5)
Th	2(<2-5)	3(<2-5)
Ti	23(7-42)	2(<2-3)
Y	1(<0.5-2)	<0.5(0.5-1)
Yb	17(5-29)	5(4-8)
Zn	2864(2566-3173	609(571-648)

Table 7. Results of multielement analysis of Cockle Tissue (*Katalysia scalarnia*)(mg/kg dry wt) from Princess Royal Harbour, Albany, Western Australia

Element	Effluent outfall	Drain mouth
As	67(66-67)	84(84-84)
Bi	<9(<9)	10(<9-11)
Br	311(309-313)	321(320-322)
Co	7(6-7)	6(5-7)
Cr	2(2-2)	4(4-4)
Cu	18(17-18)	18(17-19)
Fe	157(154-159)	496(491-501)
Hf	6(<5-6)	6(5-6)
Hg	<9(<9)	21(20-22)
I	10(8-12)	31(30-32)
La	9(<6-9)	6(<6-7)
Mo	2(<2-2)	2(2-2)
Ni	18(18-18)	24(23-25)
Pb	4(3-4)	37(37-37)
Se	3(3-3)	7(7-7)
Sn	12(10-13)	7(6-8)
Sr	51(51-51)	68(67-68)
Te	5(<4-6)	4(<4-5)
Ti	5(4-5)	31(31-31)
V	5(4-5)	<2(<2)
Yb	6(5-6)	8(6-9)
Zn	49(49-49)	64(64-64)
Zr	<2(<2)	5(5-5)

mouth (Talbot and Williams, unpublished work).

General comments

The use of XRF in this study has been able to identify the following elements in the bivalve tissue analysed :

1) naturally occurring As;

2) Fe, Ni and Ti originating from an iron ore industry;

3) Cu and Zn from a sewage outfall; and

4) the effect of organic material on the accumulation of metals in sediments.

Advantages of X-ray fluorescence analysis

Some of the advantages of this analytical technique are that :

(1) it is rapid and can analyse a large range of elements within the same samples;

(2) the method is non-destructive; hence the one sample can be stored for future reference or can be analysed by other laboratories for quality control purposes;

(3) it is sensitive to many elements over a wide range of concentrations, and the detection range is linear for a large number of elements in the 0-500 mg kg^{-1} range. This alleviates the necessity for concentrating and diluting samples to a range suitable for analysis as is required by some techniques : such procedures are time consuming and increase error;

(4) it is particularly suitable for As analysis, as the background ranges for As in many marine biota are within the detection range and well above the lower limit of detection. It does not have the operating difficulties of the much used hydride generation method, and also is much quicker;

(5) it can analyse, relatively easily, series of elements within the same column in the periodic table. The advantage of this is that broad trends can be derived from such multielemental analysis; and

(6) it can analyse organic and inorganic materials for a wide range of elemental concentration as long as matrix corrections are made. Many other techniques only measure acid-leachable metals (unless hydrofluoric acid is used in the digest) or labile species.

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