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STUDY OF HEAVY METALS AND OTHER ELEMENTS IN MACROPHYTE ALGAE USING ENERGY-DISPERSIVE X-RAY FLUORESCENCE

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Abstract

Fucus vesiculosus L. seaweeds from three estuarine stations were analysed by X-ray fluorescence, providing results for the concentration of total K, Ca, Ti, Mn, Fe, Co, Ni, Cu, Zn, As, Br, Sr and Pb. In order to study the differential accumulation of heavy metals by different parts of *Fucus*, four different structures of the algae, base, stipe reproductive organs and growing tips, were analysed.

Some elements (e.g. Cu and Fe) are preferentially accumulated in the base of the algae, whereas others (e.g. As) exhibit higher concentrations in the reproductive organs and growing tips. The pattern of accumulation in different structures is similar for Cu, Zn and Pb, but for other metals there is considerable variability between parts of the plant. This is important in determining which structures of the plant are used for biomonitoring. For samples collected at stations subject to differing metal loads, the relative elemental composition is approximately constant, notwithstanding significant variation in absolute values. The proportion of metals in *Fucus* is similar to that found in other estuaries, where metal concentrations are significantly lower.

Energy dispersive X-ray fluorescence has been shown to be a suitable technique for multi-element analysis in this type of sample. No chemical pre-treatment is required, minimising sample contamination. The small amount of sample required, and the wide range of elements which may simultaneously be detected, make it a valuable tool for pollution studies.

Keywords - Seaweeds Metals XRF Bioindicators Estuary

Introduction

Seaweeds are known to be good indicators of heavy metal contamination in marine ecosystems since the first studies of Black & Mitchell [1] on brown algae. Subsequently, the brown seaweed *Fucus vesiculosus* L. has been used as a biological indicator by many authors, including Bryan & Hummerstone [2] for Cu, Zn, Mn, Pb and Fe in British estuaries, Fuge & James [3] for eight heavy metals, Cullinane et al. [4] for zinc in Cork harbour, Ireland, and Ferreira [5] for mercury in the Tagus estuary, Portugal.

There is a general consensus in the literature regarding the dependence of algal concentrations on the metal levels in the water column ([1], [6], [2], [7], [8]), which is linked to the relative inability of seaweeds to regulate the concentration of heavy metals in the tissues, and to the tolerance of these organisms to high external levels of these pollutants.

Fucus vesiculosus and other algal species exhibit seasonal variations associated with the productivity cycle and physiological factors ([9], [10]) - these fluctuations are not necessarily correlated with variation in the water concentration of heavy metals. *Fucus vesiculosus* may thus be considered a suitable indicator organism of the annual variability in pollutant concentrations, rather than for seasonal variations ([2], [11], [12], [5]).

Not only is seasonal variability important in altering metal concentration in the tissues of the plant, but different parts of the plant also vary in their accumulation of metals. The distribution of metals in different seaweed organs was studied for *Fucus vesiculosus* by Bryan & Hummerstone [2], who found that concentrations of Cu, Zn, Fe, Pb and Al, but not Mn, increased from the growing tips to the older parts of the thallus. Seasonal changes, coupled to the heterogeneity of metal concentrations within individual plants, may explain why some authors have not found a clear relationship between metal concentrations in algae and seawater (e.g. [13]).

In the present work, energy-dispersive X-Ray fluorescence (EDXRF) is used to determine the element composition of samples of *Fucus vesiculosus*.

X-ray fluorescence analysis is a powerful technique for elemental analysis of environmental samples ([14], [15]). It is a fast, non-destructive analytical method, requiring little or no sample preparation, which allows the simultaneous determination of all elements heavier than phosphorus.

Work on trace-element content for this and other species was reported by Black & Mitchell [1] for 17 elements; subsequent literature generally focuses on 5-6 elements (e.g. [3], [16], [7], [8]).

This paper presents results for 13 different elements in the seaweed *Fucus vesiculosus.* using samples collected at different stations over a period of one year. Element composition for the older part of the thallus, stipe, growing tips and

reproductive organs is reported, and the relative proportions of different elements, observed trends and distribution of metals along the plant are discussed.

The objectives of this work are:

To describe the composition of *Fucus vesiculosus* for a range of elements, taking advantage of the features of EDXRF;

To try to identify patterns in the relative concentrations of different metals in the plants;

To examine the distribution of individual metals in different parts of a plant, and establish generalisations where possible.

Material and Methods

Study area and sampling

This work was carried out on samples from the Tagus Estuary, Portugal (Fig. 1). The estuary occupies an area of 320 km^2, of which about 130 km^2 (~ 40%) is intertidal. The tidal prism is 600 X 10^6 m^3 for a mean tide, about a third of the mean volume of 1900 X 10^6 m^3. For a modal freshwater flow of 400 m^3s^{-1}, this corresponds to an estuary number of <1%, indicating that the system should in general be vertically well-mixed, which is verified in the field, even though the circulation is not transversely homogeneous. The detailed characteristics of the estuary and its macrophyte communities have been discussed elsewhere [17]. Levels of heavy metals in other biotic and abiotic compartments of the Tagus (sediment, water, invertebrates and fish) have also been reported in the literature ([5], [18], [19]).

Fig. 1 near here

Samples of the algal species *Fucus vesiculosus* L. were collected monthly, over a yearly period, at three intertidal sites of differing metal contamination, one near the northern industrial belt (station 1), one in the centre of the estuary (station 2), and one in the southern part of the estuary (station 3). Although station 3 is not located near point-source discharges, the area is affected by the ship-building and smelting industries further downstream, due to net residual upstream flow in the southern half of the estuary. The station locations and general features of the estuary are given in Fig. 1.

Algae were washed, cleaned and stored deep-frozen (-20°C), and the material for EDXRF analysis was selected from randomly taken samples from different months for each of the three sites.

No effort was made to compare samples from identical months or to select time-series, since the objectives of this work were essentially to compare seaweed composition and metal distribution in different parts of the plants in a series of distinct conditions. Comparisons of contamination at the different stations and a study of seasonal variation have been published elsewhere [20]

Pre-treatment and analysis

Samples were analysed by Energy dispersive X Ray fluorescence (EDXRF) without any chemical pre-treatment. The algal samples were thawed, and the plants carefully washed in distilled water and separated into four parts: base, stipe, growing tips and reproductive structures. For four samples, each part was divided into two aliquots: these were dried by different methods: One in an oven (50°C) for 48 hours, and the other at room temperature, to monitor potential loss of volatile elements.

After drying, each sample was ground using a freezer-mill. The samples were prepared by mixing and pressing the powder into tablets. Each tablet was glued onto a Mylar film. To be sure that the sample holder was not going to introduce analytical errors, blanks were previously checked. A total of 180 tablets were analysed, corresponding to a minimum of three replicates per sample, to reduce the risk of analytical error.

Reference materials were used in order to assess the accuracy of the determinations (BCR CRM 60 - *Lagarosiphon major*, BCR CRM 279 - *Ulva lactuca*, and NBS 1571 - Orchard leaves). Analytical precision for sample replicates was always better than 90%.

The spectrometer used in this work is based on a three axial geometry which reduces the background by polarisation of the radiation, as has been pointed out by Standzenieks et al. [21]. The primary beam from the X-ray tube impinges on a secondary target, which emits almost monochromatic X-ray radiation. This monochromatic beam is then used to excite characteristic radiation from the atoms of the sample.

In this method the ionisation cross-section for an atomic level is greatest when the exciting X-ray energy just exceeds the binding energy of the electron in that level, and falls off drastically with an increasing difference between the excitation energy and the electron binding energy. In order to cover a broad range of elements we usually use a W X-ray tube and a Mo secondary target. Under these conditions, atoms are ionised in K shell for $15<Z<38$ and in L shell for heavier elements; characteristic K and L X-ray radiation is emitted.

The characteristic radiation emitted by the elements present in the sample is detected by a Si(Li) detector with $50mm^2$ active area, 8μm beryllium window, and 150eV resolution at 6.4keV. Data are stored in a PC computer with a Nucleus PCA card which acts as a multichannel pulse height analyser with 8192 channels. The spectra are evaluated using the fundamental parameters method. The computer code is based on a concept described by Rindby [22], which employs the information inherent to the inelastically scattered radiation to estimate the self-absorption of the samples.

Results and discussion

The tables below present the data obtained for the samples analysed in this work. A preliminary comparison of pre-treatments indicated that air-dried samples do not differ significantly from oven-dried ones. Correlation coefficients between results obtained with the 2 pre-treatments were 1.00 for station 1, 0.98 for station 2, 0.93 for station 3A and 1.00 for station 3B. Theses values are also significant at $P<0.01$.

Therefore, the results in Table 1, showing the mean values (i.e. concentrations for the base, stipe, growing tips and reproductive organs combined) for 13 elements, aggregate the different pre-treatments for identical samples.

Table 1 near here

The results presented in Table 1 also include mean values for all 13 elements at each station, and show that the highest values occur for iron in station 1, but that for most metals, station 3 has the highest values. This is probably because metals are transported upstream by bottom advective flow along the southern half of the estuary. However, for Cu, Sr, As and Pb, the mean values are similar in stations 1 and 3, and in both cases significantly above those observed at station 2. This is in keeping with observations regarding metal concentrations in the water column carried out by other authors (e.g. [23]). Lower values observed for some metals at station 2 may be justified by its location in the main flood and ebb channel of the estuary. This station is subject to much more intense mixing than the other sampling points, and is also much further away from industrial discharges both on the northern and southern shores of the estuary.

A comparison of the values obtained in this study with those of other authors has been carried out using data from Black & Mitchell [1], which, although not recent, is the only reference which contains concentrations for a wide range of elements for *Fucus vesiculosus*. The data collected by these authors refer to samples collected in different estuaries in the U.K. with a broad range of industrial point-sources. This has

similarities with the type of industrial loading to the present study site. Table 2 presents the correlation coefficients from a linear regression analysis and the F-values from ANOVAs carried out on the data.

The correlation between the proportions of elements in different samples was highly significant both for the present data (mean values at each station were compared - data in Table 1) and for data from two samples from Black & Mitchell [1], as can be observed in Table 2. These analyses were performed with all elements for both studies (i.e. 13 for the former and 15 for the latter).

Table 2 near here

The combined analysis was however carried out with less elements (9) for which values exist in both studies. It is clear that there is a significant relation between the proportions of metals in both studies, although in terms of absolute values, the levels in the Tagus estuary are generally 2-3 times greater, due to the higher concentration of metals in the water column. This may be partly because the matrix in the water for many industrialised estuaries is similar enough to ensure that the relevant metals are available for uptake, and partly because the algae regulate the concentration of different metals in their tissues to some degree.

Fig. 2 near here

The regression between the relative proportions of metals found in the two studies is illustrated in Fig. 2, with the observed and predicted values for the present study plotted against the original data of Black & Mitchell [1]. The implication of these results is that if metal ratios are similar under differing pollution conditions, some type of "Redfield" ratio may be used to estimate the concentration of one from another. Further work is clearly necessary to establish whether such an approach may be applicable.

Table 3 near here

Table 3 presents the mean concentration of different elements in the basal part, stipe, reproductive organs and growing tips of *Fucus vesiculosus*. Generally, the base of the plants contains higher concentrations of trace metals than the growing tips. An ANOVA of the data shows that the difference in concentration is significant for Ti, Fe, Cu and Pb ($P<0.10$). These results agree with the findings of Bryan & Hummerstone [2] for Cu, Zn and Pb, but not for Mn, which for our samples is higher both in the base and at the growing tips than in the stipe. The reproductive organs contain greater concentrations of potassium and calcium than other parts of the algae, reflecting their specialised function. Arsenic is also higher in these organs, possibly due to biological substitution for phosphorus.

Table 4 shows the mean ratios between 4 different trace metals in relation to lead. Lead was chosen as the base because it varies relatively little in the different parts of the algae. Individual ratios were worked out for each sample, and means subsequently calculated. The ratio between the concentration of the five metals is similar for the

base, stipe and reproductive organs, although the actual values differ in different parts of the plant (Figs. 3 and 4). An ANOVA performed on the data for these three parts indicates that the element ratios are statistically indistinguishable from each other ($F=0.002$ for an $F_{crit}=4.26$). Notable differences are a higher ratio for arsenic in the reproductive organs, and for the growing tips, a marked reduction in iron. This reduction can be seen in Fig. 3b, where iron in the growing tips remains approximately constant in all samples.

Table 4 near here

The graphs shown in Figs. 3 and 4 show the concentration of Cu, Fe, Zn, As, and Pb in the four different parts of the plants for the samples analysed. The graphs have been plotted showing the samples in ascending order of basal concentration.

Some metals (Cu, Zn and Pb) show very similar patterns of increase in concentration in the four different plant structures analysed, whereas others (Fe and As) show great variability between parts of the alga. In particular, iron shows no variation at the tips, which suggests that it is bound essentially in the base and stipe of the seaweeds. Since iron is an essential element for the algae, its presence in certain parts of the plant is determined by physiological aspects. In the case of xenobiotics such as lead, there appears to be no discrimination, and accumulation is uniform throughout the plant.

Arsenic, although also a toxic element, does not conform to this pattern. It exhibits consistently higher values at the reproductive organs and growing tips than at the base of the plant. If the increased presence of arsenic in the reproductive structures is due

to biological substitution of phosphates for arsenates, there may be potential consequences in the reproductive success of algae subject to this pollutant, which although not apparent in growth will depress recruitment.

Fig. 3 near here

Figs. 3 and 4c show that higher values for Cu, Fe and Pb concentration are found in the basal part and stipe of the algae, over a range of concentrations. In the case of iron, however, the concentrations found in different parts of the algae do not follow each other well, with an almost opposite pattern for the basal part and stipe (mid-frond). Values in the stipe and reproductive organs show a peak in sample 4, and high values for the stipe coincide with low values at the base for sample 1, with the opposite occurring at samples 7 and 8.

Fig. 4 near here

For these three metals, the basal part of the plant, and to a lesser extent the stipe, clearly accumulate more than other parts. This agrees with the results of Bryan & Hummerstone [2], although in the case of zinc (Fig. 4a), our results show a much more uniform increase of concentration in all parts of the plants, and the concentration of zinc in the growing tips is consistently higher than in the stipe.

The distribution observed for zinc also shows a number of samples where the maximum values observed were at the tips of the plant, contrary to previous work, and to the pattern observed for Cu, Fe and Pb.

Arsenic shows a clearly different variation to that observed for the other elements. The concentrations found in the basal part of the seaweeds are systematically lower than those at tips, and particularly those in the reproductive structures. In the case of arsenic, the basal part of the plant does not therefore appear to be the best indicator of contamination.

Conclusions

This study illustrates the use of the EDXRF technique in rapid assessment of metal contamination in biological material. The distribution of heavy metals in different parts of *Fucus vesiculosus* has been studied by this method, and the results obtained agree well with those of other authors.

The base of the plants shows higher concentrations for most elements than other parts, except in the case of arsenic, where the reproductive organs appear to accumulate more than other algal structures. The concentrations of Cu, Zn and Pb show a similar evolution in the different plant structures analysed, whereas Fe and As are much more variable. Thus, for the first three metals, it appears that a non-selective sampling strategy for different parts of the algae would be sufficient, whilst in other cases (e.g. Fe and As) careful selection of the plant structure for analysis may be critical for its correct application as an indicator.

With the exception of the growing tips, the ratio of the metals Fe:Zn:Cu:As:Pb remains approximately constant irrespective of absolute concentration. This pattern may be changed in systems where predominantly one element is discharged. Further work is required to establish whether it is possible to use this ratio predictively.

The EDXRF technique is well suited for multi-element determinations in environmental samples. In particular, the samples do not need any chemical pre-treatment and hence any possibility of contamination is avoided. The samples are analysed non-destructively, being retained for re-use, re-evaluation or for further studies. The small sample size required, coupled to its other features make it a valuable tool for pollution studies.

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Tables

Table 1 - Mean concentration of different elements in the tissues of the seaweed *Fucus vesiculosus* (all values in μg g^{-1} dry weight of seaweed). Values shown for all samples and also means at each station. Maximum values are highlighted.

Element	Sample name*							Station mean values**		
	1A*	2B	2C	2D*	3E	3F*	3G*	Station 1	Station 2	Station 3
K	8519	4835	12504	**13610**	9059	9773	7284	8519	**11140**	8635
Ca	11058	**16560**	11138	12743	11526	14762	14138	11058	13296	**13865**
Ti	82	93	**130**	86.5	72	97.5	74.5	82	**99**	83.2
Mn	208	155	163	**271**	170	173	219	208	**215**	190
Fe	1206	1096	**1391**	904	858	1223	967	**1206**	1074	1047
Co	13	11	12	**20**	12	12	13	13	**14.3**	12.4
Ni	6	8	8	**10**	5	8	9	6	**8.7**	7.8
Cu	34.5	18	18	16.5	35	**41**	38.5	34.5	17.3	**38.8**
Zn	463	532	323	683.5	**839**	712	654.5	463	555.5	**714.4**
As	30	21	35	25	28	41.5	**43**	30	26.5	**39.4**
Br	379	306	246	270	314	**543**	403	379	273	**441**
Sr	1283	797	852	892	1139	**1361**	1309	1283	858	**1296**
Pb	17.5	10	12	10	13	**20**	19	17.5	10.5	**18.2**

* Sample nomenclature is given by the station number and a following letter, indicating a particular sample (i.e. a month). Thus there is one sample from station 1, 3 from station 2, and 3 from station 3. Aggregated pre-treatments are indicated with an asterisk;

** Mean values were calculated on the total number of samples, prior to aggregation of pre-treatments.

Table 2 - Regression analysis and ANOVA of data from this study and from Black & Mitchell (1952), comparing the proportions of Ti, Mn, Fe, Co, Ni, Cu, Zn, Sr and Pb (9 data points were used in all cases).

Comparison between	**r**	**F**	**Source**
Mean values at stations 1 and 2	1.00	2384*	This study
Mean values at Stations 1 and 3	0.99	846*	This study
Mean values at Stations 2 and 3	0.99	422*	This study
Fucus vesiculosus (Table III) and *Fucus vesiculosus* (Table IV)	0.86	38*	Black & Mitchell
Mean for stations 1,2,3, and mean for samples in Black & Mitchell	0.94	49*	Both studies combined

* $P < 0.01$

Table 3 - Mean concentration of elements in different parts of *Fucus vesiculosus* for aggregated samples (all values in μg g^{-1} DW of seaweed) - Maximum values are highlighted

Element	Base	Stipe	Reproductive organs	Growing tips
K	8656	8862	**11883**	8823
Ca	13610	11959	**15078**	11883
Ti	**139**	128	78	48
Mn	**211**	190	183	206
Fe	**1582**	1494	1022	651
Co	**14**	13	10	<1.2
Ni	**8**	7	7	<1.5
Cu	**37**	31	28	28
Zn	**675**	579	568	627
As	31	33	**36**	33
Br	**595**	476	284	227
Sr	980	1134	**1313**	1130
Pb	**18**	17	14	14

Values reported correspond to 20-30 determinations for each part of the plant.

Table 4 - Ratios between 4 metals and Pb (Pb is given a value of 1) in the seaweed *Fucus vesiculosus* - Maximum values are indicated in bold and minimum values are shown in italic. Number of samples is indicated in brackets.

	Fe	**Zn**	**Cu**	**As**
Base (n=8)	93.6	*38.4*	2.0	*1.8*
Stipe (n=9)	**98.1**	39.9	1.9	2.1
Reproductive organs (n=9)	84.6	45.0	2.0	**2.8**
Growing tips (n=11)	*50.4*	**51.8**	2.0	2.6

Each sample consisted of at least 3 replicates.

Figure legends

Fig. 1 - The Tagus Estuary, showing seaweed sampling stations, intertidal areas, and main industrial pollution point-source areas

Fig. 2 - Regression of concentration of different metals in *Fucus vesiculosus* from ecosystems with different pollution loads.

Fig. 3 - Concentration of (a) copper and (b) iron (μg g^{-1}) in different parts of *Fucus vesiculosus*

Fig. 4 - Concentration of (a) zinc, (b) arsenic and (c) lead (μg g^{-1}) in different parts of *Fucus vesiculosus*

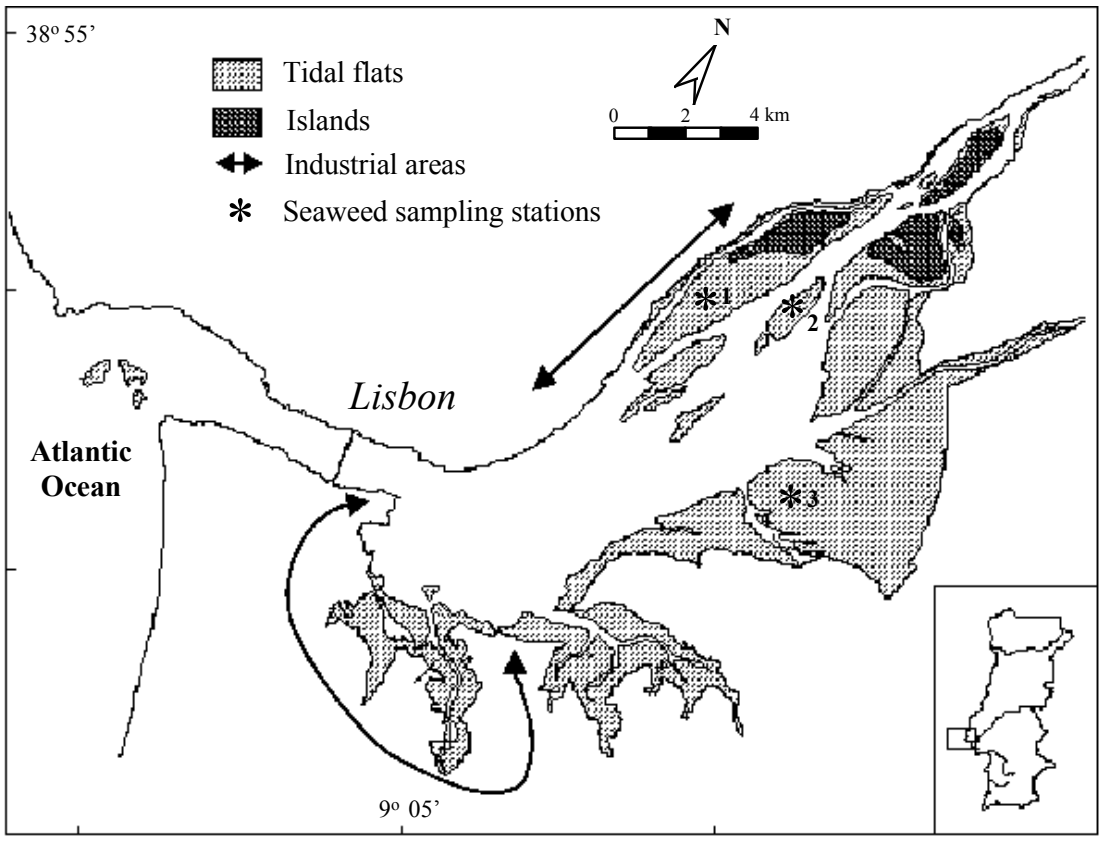

Fig. 1 - The Tagus Estuary, showing seaweed sampling stations, intertidal areas, and main industrial pollution point-source areas

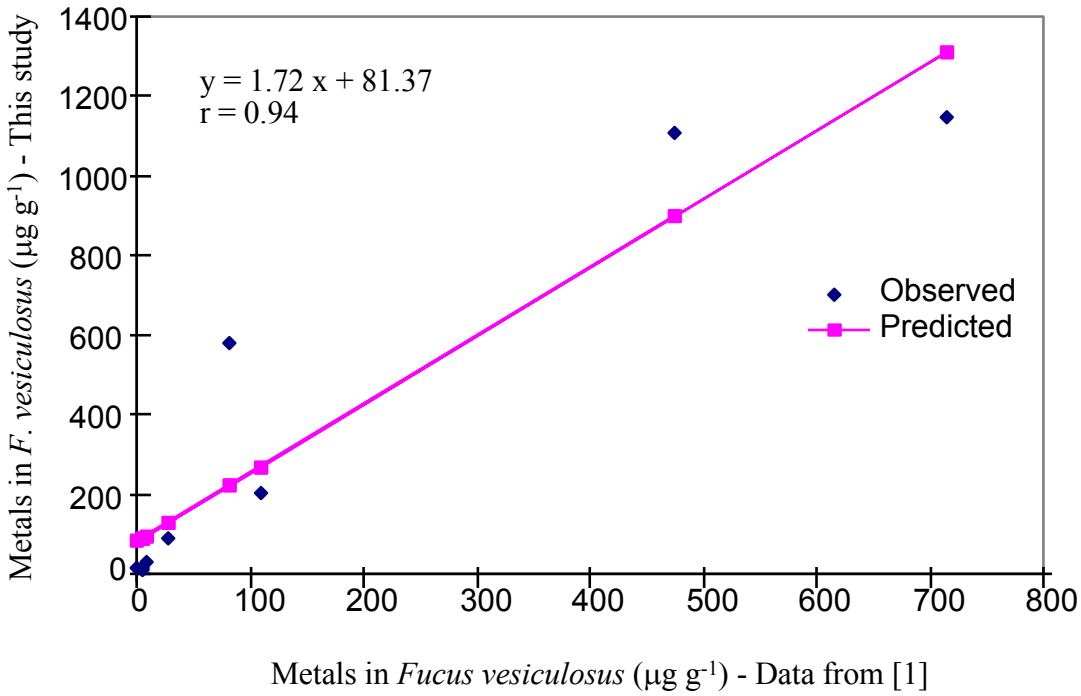

Fig. 2 - Regression of concentration of Ti, Mn, Fe, Co, Ni Cu, Zn, Sr and Pb in *Fucus vesiculosus* from ecosystems with different pollution loads.

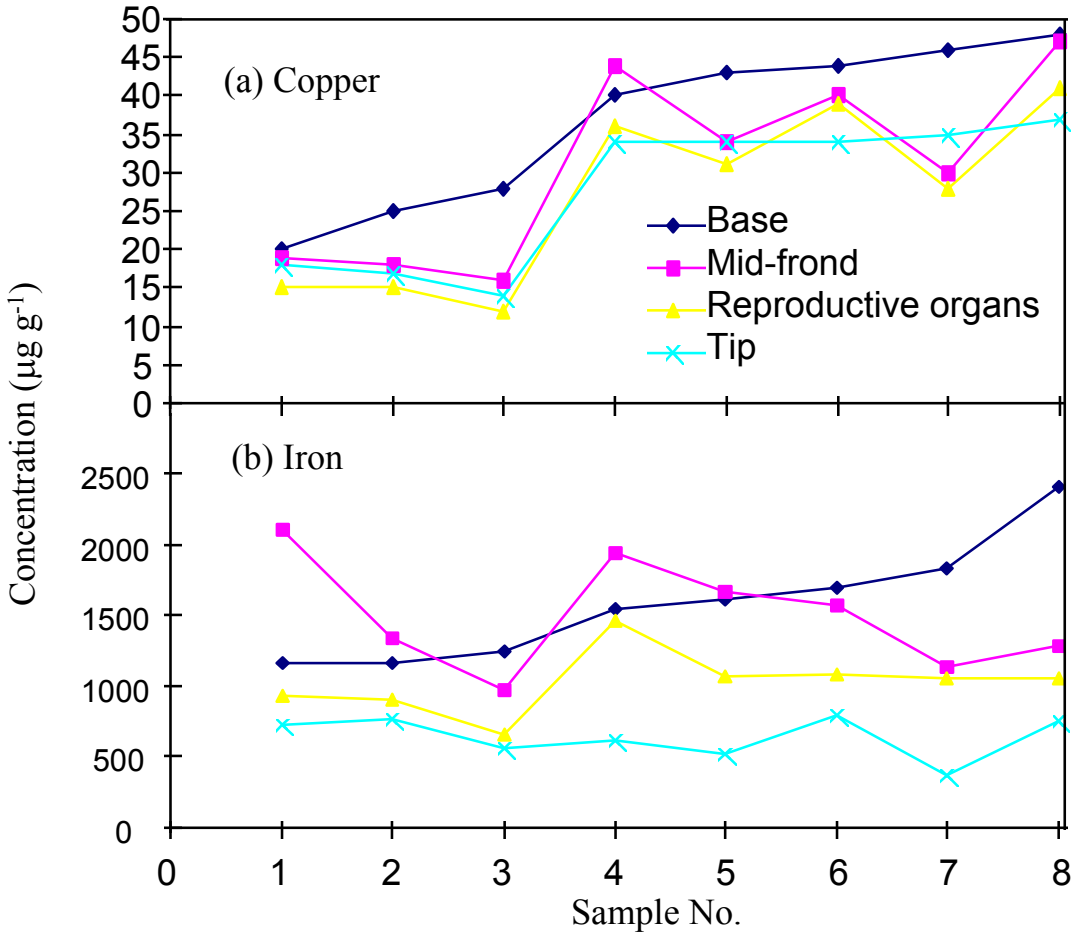

Fig. 3 - Concentration of copper and iron (µg g^{-1}) in different parts of *Fucus vesiculosus*

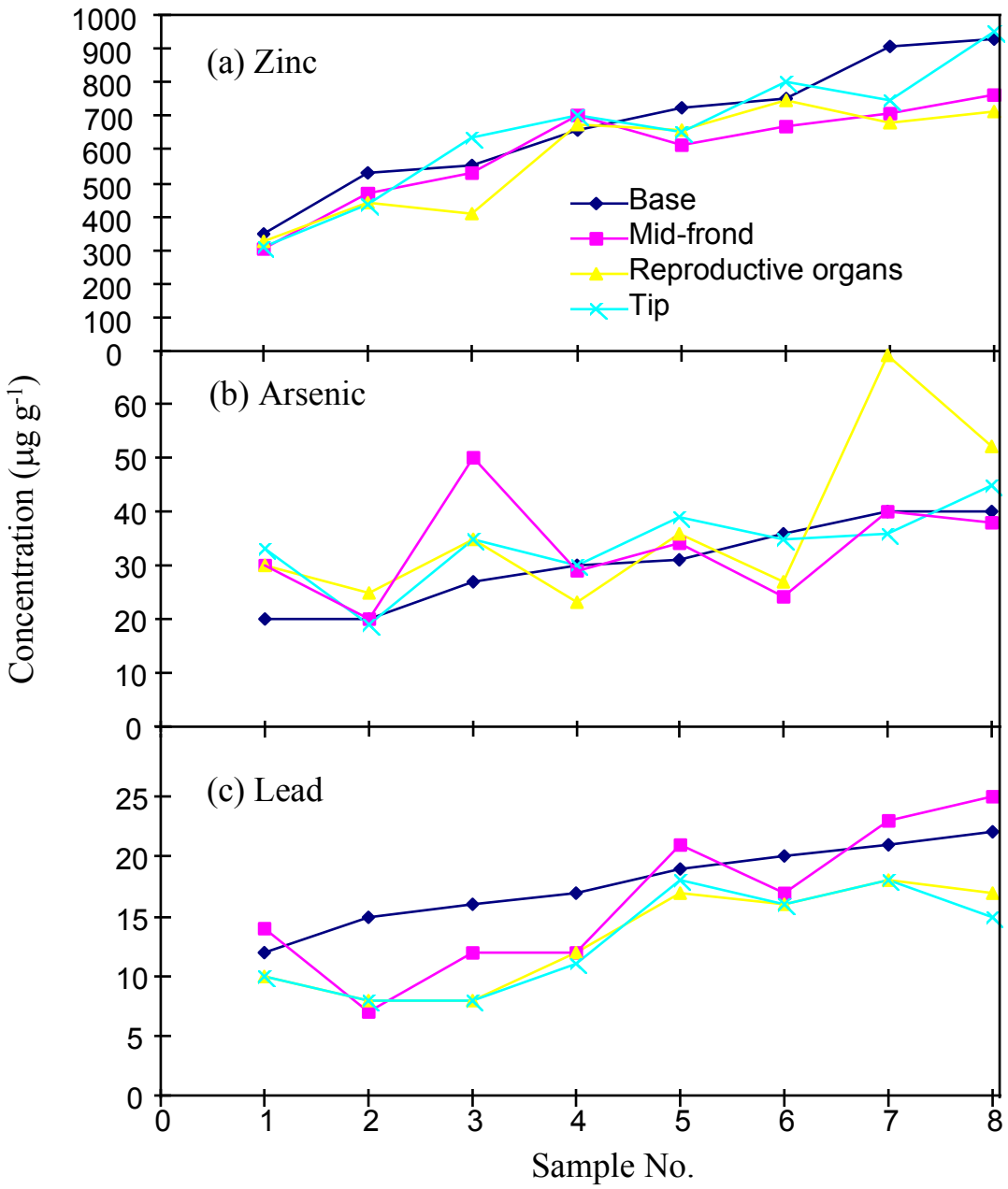

Fig. 4 - Concentration of zinc, arsenic and lead (µg g^{-1}) in different parts of *Fucus vesiculosus*