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Angewandte Physikalische Chemie

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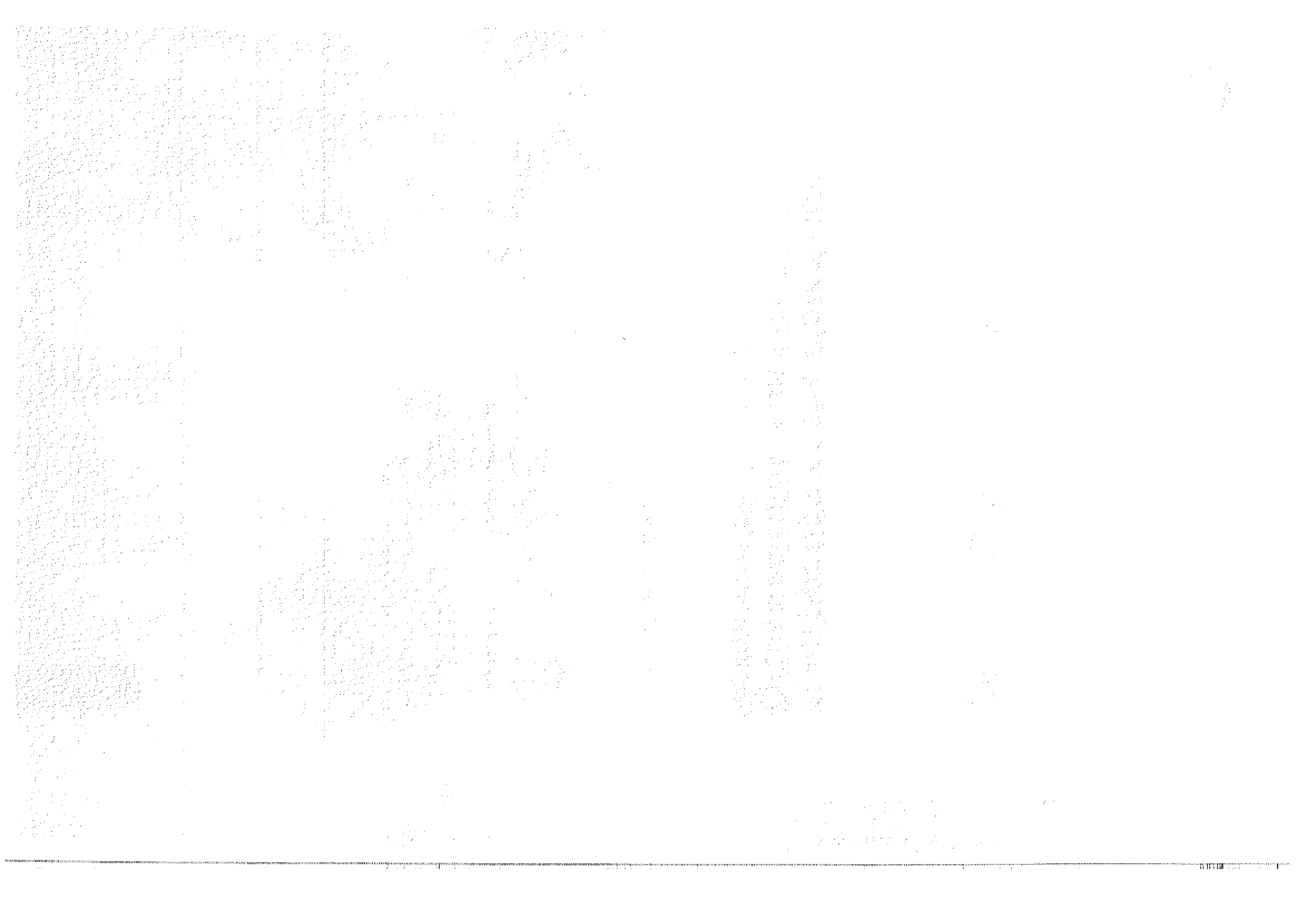

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*Abstract*

The brown alga *Fucus vesiculosus* has been suggested as an indicator of heavy metal pollution in the North Sea coastal water. A wide range of trace elements and metals was determined in *Fucus vesiculosus* using multielement techniques. The good reproducibility of Instrumental Neutron Activation Analysis (INAA) and confirmation of the obtained results with Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and Mass Spectrometry (ICP-MS) have been used as the fundamental approaches for assuring the accuracy of data presented in this work. *Fucus vesiculosus* was collected from three representative areas, Eckwarderhörne (Weser estuary), Cuxhaven (Elbe estuary), and Sylt-List (less polluted) at bimonthly intervals. After collection the algae were transferred in the gaseous phase of liquid nitrogen to the laboratory. The autecology of the sampled *Fucus vesiculosus*, with respect to maximum and minimum growth in February and August respectively, the influence of sampling time and the variation in differently aged tissues of the algae on the accumulation of heavy metals were considered. Samples collected from Eckwarderhörne were used to study the biological variability along the algae bedrock. The relative contribution ratio of the different parts to the whole plant concentration on the fresh wet basis was estimated as well as the relative variability of the element concentrations in Eckwarderhörne. Concentration of the differently aged tissues was found to affect the concentration of different elements. The *General Linear Models procedure* was applied in the course of investigating element concentration variational patterns with respect to four factors: collection site, sampling month, sampling year and combination effect adjusted for every other effect. *Stepwise selection* discrimination analysis showed that the elements Ba, Cu, and Co clearly discriminated between the three collection areas. For Eckwarderhörne and Cuxhaven the elements Co, Zn, P, Mg, and Se were considered, for Sylt-List and Cuxhaven, the elements Ba, Cu, Na, and Tb, and for Sylt-List and Eckwarderhörne the elements Cu, Cd, As, Se, and Sr. Differences in the accumulation and seasonal variations in 1993 and 1994 were studied by performing *Principal Component Analysis*

(PCA). Strong correlations between Cu, Zn, Cd, and Ag, as well as Sc,V, Cr, Fe, Pb, and Th, were found to exist in samples from the three collection sites. Fingerprinting of element concentrations in the collection areas showed different accumulation levels and revealed important information on the expanding concentration range during the two years of sampling time. Estimations of pollution load indices for each collection area, as well as, a proposed pollution load index for the North Sea coastal water were presented and compared with reported data. The variation factor of element concentrations ranged from 1.2 to 3 for most of the measured elements. In Eckwarderhörne and Cuxhaven the elements Sc, V, Cr, Fe, Ni, Cs, Hf, Pb and Th showed variation factors > 3 which might be a contribution of suspended particulate matter. P, Ag, and Hf showed variation factors > 3 in Sylt-List. It was found that the element concentrations in *Fucus vesiculosus* under certain circumstantial procedures are regional and dependent on sampling month. The study indicates the urgent need for baseline values to judge the variational behavior of the bioindicator.

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## 1. *Introduction*

During the past two decades, increasing attention has been focused on pollution of the natural environment. The threat of pollution of the human environment by heavy metals is well known by now. The distribution of heavy metals in the aquatic environment has been investigated by using bioindicator organisms, e.g. algae and mussels, because of their ability to concentrate the heavy metals, as well as other pollutants, from the surrounding waters which often have too low concentrations. Although analytical methods have been improved, indicator organisms provide a time-integrated picture of the bioavailability of pollutants rather than pollutant abundance *per se* [1]. The advantages with seaweed as bioindicators are that they are stationary, often easy to collect, and being perennials, they are also available at all times of the year. Several requirements must be fulfilled for an organism to be suitable as a bioindicator. The main requirement is that there must be a correlation between the average concentration in the surrounding water and the concentration in the bioindicator [2]. Bryan [3] found a linear relationship between zinc concentration in water and that in *Laminaria digitata*, but the concentration factors (concentration in algae : concentration in water) decreased when the concentration in the water increased. A linear relationship between cobalt concentration in water and that in *Fucus spiralis* was also observed by Van Weer [4]. Algae accumulate the dissolved fraction of the metals and radionuclides and thus the concentrations in the algae should be correlated to the water concentrations of dissolved metals or radionuclides and not to the total concentration [5]. However, various factors have been observed to affect the uptake, accumulation and release of metals and radionuclides in algae, e.g. season of the year [6, 7], age of tissue, shore position [8], and salinity [9-13], errors caused by *Epiphytes*, sea exposure, and differences depending on which part of the seaweed is analyzed [14]. These factors have been reviewed by several authors [2, 5, 15, 16]. Although various investigations have been performed, the results are unitary. Perennial algae like fucoids consist of tissues of different ages. There is a tendency for increasing concentration of heavy metals with age in algae from polluted areas, which is most consistently observed for zinc [8, 17]. There are some studies of the seasonal variations of

heavy metals in *Fucus vesiculosus* in the literature [e.g. 17, 18], and results from studies on the seasonal variations of heavy metals differ between elements and are also sometimes contradictory for the same element. The most pronounced seasonal trend, with the lowest concentrations during summer, have been observed for zinc, and this is suggested to be an effect of growth of the algae [e.g. 4, 7, 18]. However, the uptake mechanisms vary between metals, so that divalent cations may be accumulated on the basis of selective ion exchange mechanisms involving algal polysaccharides [6], while anionic arsenate accumulation in macroalgae is considered by Klumpp [19] to require energy from respiration. Trace metal uptake by seaweed is regarded as a two-stage process [6, 20, 21]. Munda and Hudnik [22] suggested that, in a rapid and reversible physico-chemical process, soluble fractions of metals are transferred from ambient water to the apparent free space (AFS) included in the cell wall and intercellular spaces. Thereafter, metals are transferred across the membranes by slower, regulated processes which require metabolic energy, and which are dependent on the concentration of metal ions in sea water and various polyanions in the cell walls and intercellular spaces of marine algae.

***Sources and distribution of heavy metals***

The term " heavy metals " is commonly thought of as pollutants, even though some of these are essential for metabolism at trace concentrations [23]. The sources and distribution of heavy metals in aquatic systems have been documented [24, 25]. Our natural waters, particularly our estuaries and fresh water systems, are not only currently being polluted to varying degrees but are also condemned to fairly long-term pollution due to metals deposited in sediments from past human activities [23, 26, 27]. The well known bioaccumulation of radionuclides of heavy metals such as Mn, Fe, Co, Zn, Ag, Pb, Po, and Pu by brown algae [e.g. 28, 29], and other edible algae, poses a pollution hazard of another kind. Table (1.1) shows the concentration in the ppb range of some elements in fresh and sea water [30].

Table 1.1 : Concentration of some elements (ppb) in fresh and sea water [30].

| | Be | Mg | Ti | V | Cr | Mn | Fe | Co | Ni | Cu | Zn | As | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fresh water | 0.1 | 4100 | 3 | 0.9 | 1 | 4 | 670 | 0.2 | 0.3 | 2 | 7 | 1.7 | |
| Sea water | 2 E-4 | 1.3E 6 | 1 | 1.9 | 0.2 | 0.03 | 1.6 | 0.002 | 0.6 | 0.25 | 0.6 | 2.6 | |
| | Se | Zr | Mo | Ag | Cd | Sn | Te | Pt | Au | Hg | Tl | Pb | Bi |
| Fresh water | 0.2 | 2.6 | 1 | 0.3 | 0.4 | 0.006 | ? | ? | 0.002 | 0.07 | 0.04 | 0.3 | 0.05 |
| Sea water | 0.09 | 0.03 | 10 | 0.002 | 0.1 | 0.0006 | ? | ? | 0.01 | 0.02 | 0.01 | 0.003 | 0.02 |

## 1.1 *Use of Fucus vesiculosus as a bioindicator*

*Fucus vesiculosus* is favorably used for monitoring programs in coastal water and estuaries. The reasons are, firstly, metal concentrations in water are normally very low and thus difficult to measure. Secondly, metal levels in this phase are transitory, varying with the level of fresh water in flow, tidal state, longshore drift and water and sediment chemistry as well as other factors such as water-sediment mixing as a consequence of wave action during storms and gales [31]. Much clearer spatial variation was evident in the heavy metal concentration determined on *Fucus vesiculosus* than in the concentration determined in the water [32]. Also, benthic intertidal algae concentrate metals by a factor of up to $10^4$ or more [33], with metal loading normally directly proportional to the concentration of soluble metals in the sea water [34]. *Fucus vesiculosus* has the indicator capabilities for showing the geographical distribution of heavy metals [35]. Metals which complex most strongly with algal tissues show the strongest correlation of algal tissues and sediment, and scavenging of these metals from particulates may be an important source of uptake by the algae [36] and/or from surface bottom sediments [37]. The binding of heavy metals to cell walls and in physodes in brown algae is likely to be a factor of importance in the relatively high tolerance of these algae for heavy metals, and hence for their suitability as indicator organisms for environmental pollution [38].

*Monitoring aspects*

Even though there are several factors which affect the uptake and accumulation of heavy metals and radionuclides in *Fucus vesiculosus* (age of tissues, growth, salinity ... etc.), *Fucus vesiculosus* can be used as a bioindicator if these factors are taken into consideration. However, algae should not be used to predict the total heavy metal concentrations in water, and the algae should be collected at the same time of the year to be comparable. Algae can be useful in tracing the source of the discharge of a metal or radionuclide as they show some relation to the concentration in water, even if it is not a quantitative relation, as well as differences between localities, and as a qualitative bioindicator for radionuclides [39]. In conclusion, Butterworth et al. [40], Nickless et al. [41], Preston et al. [33], Bryan and Hummerstone [8], and Fuge and James [7, 18] have used analyses of seaweed as a convenient and simple technique for deducing comparative environmental data on regional differences in dissolved trace metal content of estuaries and coastal water.

## 1.2 *Aim of the work*

The aim of this work was to study and test the hypothetical effect of biotic and abiotic factors on the accumulation patterns of *Fucus vesiculosus* as a bioindicator of heavy metal pollution on the North Sea coast. The major factors considered in this investigation are the influence of seasonal variation and collection sites on the element concentrations. The analytical techniques were the supporting tools in the assessment of the suitability of *Fucus vesiculosus* as a marine monitor with respect to the contribution of these factors.

Major goals were studying and comparing the differences in element concentration patterns in *Fucus vesiculosus* from each collection area, fingerprinting the influence of seasonal variation of the element concentrations with respect to the vegetation cycle of *Fucus vesiculosus* and their dependence on the sampling time of year as well as the year of sampling, and to prove that algal plants of the same species grown under identical environmental conditions exhibit marked differences in their ability to concentrate heavy metals. The elements considered here included not only heavy metals of current major environmental concern such as Pb or Cd, but also the so-called essential elements such as Cu or Co, as well as, other trace constituents of anthropogenic or geogenic origin such as As or the rare earth elements. This elemental fingerprinting approach allows conclusions with respect to different sources of element contents. The results were oriented towards the importance of standard operating procedures for long-term monitoring, design of sampling, and analysis with respect to future opportunities and applications for specimen banking.

## 2. *Multielement techniques for environmental analysis*

All possible source of metal pollution have been investigated starting with an elemental analysis of the material involved. Most elements in the environmental samples are in one sense or another more or less important either as nutrients, as elements of environmental interest, as elements of toxicological importance, or even as indicators of the origin and pathways of pollution. It is obvious that the safest approach is not to limit the scope of analysis, but to analyze for as many elements as possible, preferably with multielement techniques. Before meaningful conclusions can be drawn concerning sources, pathways, and environmental impact, large quantities of analytical data are required. Therefore, the method of analysis applied should be sensitive, accurate, and applicable to major, minor, and trace constituents, while speed and ease of analysis and automation are also highly desirable [42]. Up to now activation analysis was proved to be a very effective method for the elemental trace analysis of biological materials. The advancement in multielement analytical techniques results in analytical data that may be used in either fundamental or strategic and applied research programs.

In this work the following analytical methods were applied: Instrumental Neutron Activation Analysis (INAA), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and Mass Spectrometry (ICP-MS).

### 2.1 *Neutron activation analysis*

Neutron activation analysis (NAA) is a sensitive analytical technique useful for performing both qualitative and quantitative multielement analysis of major, minor, and trace elements in samples from almost every conceivable field of scientific or technical interest. In a recent publication, Braun and Zsindely [43] describe some current trends in the use of instrumental methods for trace metal analysis. Nuclear analytical methods have not lost, however, all of their appeal, but their application seems more and more to be restricted to solving special problems which are difficult to access by other instrumental methods.

NAA plays a very important role in the certification of reference materials (RMs) and their characterization, including homogeneity testing [44]. For some elements and applications, NAA offers sensitivities that are superior to those attainable by other methods, in the order of parts per billion or better. In addition, because of its accuracy and reliability, NAA is often recognized as the *reference method* of choice when new procedures are being developed or when other methods yield results that do not agree. The important features of NAA are its isotopic basis, the simple well understood theoretical basis, and sources of uncertainty that can be easily modeled and accurately estimated [44]. In its purely instrumental form INAA is conceptually a simple method of analysis, involving only two completely separate processes : excitation and measurement. The list of studies in which INAA has been applied as a method for multielement determinations covers archaeology, biology and life sciences, environmental sciences, nutritional science, agriculture, and medicine [45]. By neutron activation, radionuclides may be produced from all elements present in the sample, at sometimes very different production rates. This mixture of radioactivities can be analyzed in two different ways:

1. Non-destructive or instrumental neutron activation analysis (INAA). In the resulting radioactive sample, the radionuclides are determined by taking advantage of the difference in decay intervals utilizing equipment with a high energy resolution for gamma-radiation.
2. Destructive or radiochemical neutron activation analysis (RNAA). The resulting radioactive sample is chemically decomposed, and by chemical separation the total number of radionuclides is split up into many fractions with a few radionuclides each.

The fundamentals and techniques of activation analysis have been treated in depth by Erdtmann and Petri [46].

*Principles of operational instrumental neutron activation analysis*

A procedure in INAA is characterized by (i) activation via irradiation with reactor neutrons, (ii) measurement of gamma-radiation after one or more decay times, and (iii) interpretation of the resulting gamma-ray spectra in terms of elements and concentrations. In neutron activation analysis, the method is defined as the in situ production (in the sample by neutron irradiation) of a radioisotopic label (or indicator) of the analyte element, whose intensity is proportional to the concentration, and which can be calibrated with reference to an elemental standard (of the same isotopic composition).

*Theoretical introduction to INAA*

The basic essentials required to carry out analysis of samples by NAA are a source of neutrons, instrumentation suitable for detecting gamma-rays, and a detailed knowledge of the reactions that occur when neutrons interact with the target nuclei. Insight into the reactions that may take place during activation facilitates the identification of the relation between the observed radioactive nucleus, its target nucleus and associated element. Insight into the reaction rates is of importance for the quantitative analysis and for a prior estimate of the feasibility of an analysis. The most common reaction occurring in NAA is the (n,$\gamma$) reaction, but also reactions such as (n,p), (n,$\alpha$), (n,n'), and (n,2n) are important. Some nuclei, like $^{235}U$ are fissionable by neutron capture and the reaction is denoted as (n,f) yielding fission products and fast neutrons. Bode [47] reviewed the theoretical basis as follow:

The reaction rate $R$ per nucleus capturing a neutron is given by

$$R = \int_0^\infty n(v)\, v\sigma(v)\, dv \qquad (2\text{-}1)$$

where

$v$ = neutron velocity (m $s^{-1}$)

$\sigma(v)$ = neutron cross section (in $m^2$ ; 1 barn = $10^{-28}$ $m^2$ ) for neutrons with velocity $v$

$n(v)dv$ = neutron density ($m^{-3}$) of neutrons with velocities between $v$ and $v + dv$,

considered to be constant in time.

The production of radioactive nuclei is described by

$$dN / dt = RN_0 - \lambda N \qquad (2\text{-}2)$$

in which

$N_0$ = number of target nuclei

$N$ = number of radioactive nuclei

$\lambda$ = decay constant, s-1 ; $\lambda = \ln 2/t_{1/2}$ with

$t_{1/2}$ = half-life of the radionuclide, s.

The disintegration rate of the produced radionuclide at the end of the radiation time follows from

$$D(t_{ir}) = N(t_{ir})\lambda = N_0R(1 - e^{-\lambda t_{ir}}) \qquad (2\text{-}3)$$

with

$D$ = disintegration rate [Bq] of the produced radionuclide, assuming that $N = 0$ at $t = 0$ and

$N_0$ = constant.

The cross section and the neutron flux are neutron-energy-dependent. In nuclear research reactors, which are intense sources of neutrons, three types of neutrons can be distinguished;

(i) Fission or fast neutrons, their energy distribution ranges from 100 keV to 25 Mev, with maximum fraction at 2 Mev.

(ii) Epithermal neutrons, with energies between approximately 0.5 eV and 100 keV.

(iii) Thermal neutrons, their energy distribution is Maxwellian, with a most probable velocity $v_0$ of 2200 m $s^{-1}$ at 20 °C, corresponding to an energy of 0.025 eV.

The thermal neutrons have the highest flux and the cross section for thermal neutrons is often inversely proportional to the neutron velocity, and the epithermal and fast neutron fluxes strongly depend on the configuration of the reactor, particularly on the choice of moderator. Reaction of the (n,γ) and (n,f) types have the highest cross section (typically in the order of 0.1 - 100 barn) for

thermal neutrons whereas the other reactions {(n,p), (n,α), (n,n'), and (n,2n)} mainly occur with fast neutrons at cross sections 2 or 3 orders of magnitude lower.

The dependence of the activation cross section and neutron flux on the neutron energy can be taken into account in Eq. (2-1) by dividing the neutron spectrum into a thermal and an epithermal region. The division is made at $E_n$ = 0.55 eV ( the so-called cadmium cut-off energy). This approach is commonly known as the Høgdahl [48] convention.

$$R = \int_0^{v_{Cd}} n(v)\, v\sigma(v)\, dv + \int_{v_{Cd}}^{\infty} n(v)\, v\sigma(v)\, dv \qquad (2\text{-}4)$$

The first term can be integrated straightforwardly:

$$\int_0^{v_{Cd}} n(v)\, v\, dv = v_0\sigma_0 \int_0^{\infty} n(v)\, dv = n\, v_0\sigma_0 \qquad (2\text{-}5)$$

in which

$$n = \int_0^{\infty} n(v)\, dv \qquad (2\text{-}6)$$

is called the thermal neutron density, and when $\Phi_{th} = nv_0$,

$\Phi_{th}$ = *conventional* thermal neutron flux, m $^{-2}$ s $^{-1}$, for energies up to the cut-off energy of 0.55 eV.

$\sigma_0$ = thermal neutron activation cross section, m $^2$, at 0.55 eV

$v_0$ = most probable neutron velocity at 20 °C : 2200 m s $^{-1}$

The second term is reformulated in terms of neutron energy rather than neutron velocity and the infinite dilution resonance integral $I_0$, which effectively is also a cross section (m$^2$), is introduced:

$$\int_{v_{Cd}}^{\infty} n(v)\, v\, dv = \Phi_{epi} \int_{E_{Cd}}^{E_{max}} \sigma(E_n)\, dE_n /( E_n ) = \Phi_{epi}\, I_0 \qquad (2\text{-}7)$$

with

$$I_0 = \int_{E_{Cd}}^{E_{max}} \sigma(E_n)\, dE_n /( E_n ) \qquad (2\text{-}8)$$

in which

$\Phi_{epi}$ = *conventional* epithermal neutron flux per unit energy interval, at 1 eV.

From this definition of $I_0$ it can be seen that the energy dependency of the epithermal neutron flux is proportional to $1/E_n$.

In practice at nuclear reactor facilities the epithermal neutron flux $\Phi_{epi}$ does not precisely follow the inverse proportionality to the neutron energy ; the small deviation can be accounted for by introducing an epithermal flux distribution parameter $\alpha$ :

$$I_0(\alpha) = \int_{E_{Cd}}^{E_{max}} \sigma(E_n)\, dE_n / (E_n^{(1+\alpha)}) \qquad (2\text{-}9)$$

The expression of the reaction rate can thus be re-written as

$$\mathbb{R} = \Phi_{th}\sigma_0 + \Phi_{epi} I_0(\alpha) \qquad (2\text{-}10)$$

Expressing the ratio of the thermal neutron flux and the epithermal neutron flux as ;

$$f = \Phi_{th}/\Phi_{epi},$$

and the ratio of the resonance integral and the thermal activation cross section as ;

$$Q_0(\alpha) = I_0(\alpha)/\sigma_0,$$

an effective cross section can be defined as :

$$\sigma_{eff} = \sigma_0 (1 + Q_0(\alpha)/f) \qquad (2\text{-}11)$$

It simplifies the Eq (2-7) for the reaction rate to ;

$$\mathbb{R} = \Phi_{th}\ \sigma_{eff} \qquad (2\text{-}12)$$

Eq. (2-12) is valid when the self-attenuation and summation effects can be neglected. In majority of INAA procedures, thermal neutrons are used for the activation. Sometimes activation with epithermal reactor neutrons is preferred to enhance the activation of elements with a high ratio of resonance neutron cross section to thermal neutron cross section relative to the activation of elements where the corresponding ratios lower.

In principle samples can be activated in any physical state, solid, liquid, or gaseous. INAA is essentially considered to be a non-destructive method although under certain conditions some damage may occur due to ; (i) thermal heating, primarily by the absorption of both reactor and prompt gamma-rays (uranium fission), (ii) radiolysis effect, caused by the reactor gamma-rays and prompt gamma-rays, and (iii) radiation tacks mainly by fission fragments and nuclei emitting α-radiation. Radiolysis may lead to decomposition of proteins into gaseous compounds, which represent an explosion hazard because of pressure build-up.

***Principle of measurement in INAA***

The nuclear transformations yield is established by measurement of the number of nuclear decays. In the simplest cases, the number of activated nuclei $N\ (t_{ir},\ t_d)$ present at the start of the measurement is given by :

$$N\ (t_{ir},\ t_d) \quad = \quad (RN_0 / \lambda) * (1 - e^{-\lambda\, tir})\ e^{-\lambda\, td} \qquad (2\text{-}13)$$

and the number of nuclei $\Delta N$ disintegrating during the measurement is given by :

$$\Delta N\ (t_{ir},\ t_d,\ t_m) \ = \quad (RN_0 / \lambda) * (1 - e^{-\lambda\, tir})\ e^{-\lambda\, td}(\ 1 - e^{-\lambda\, tm}) \qquad (2\text{-}14)$$

in which

$t_d$ = decay time.

$t_m$ = duration of measurement

The demand for a high selectivity surpasses the demand for a high counting efficiency. Germanium semiconductor radiation detectors have the best energy resolution of the detectors available for practical application varying typically from approximately 1 keV for photons of approximately 100 keV to approximately 2 keV for photons of approximately 1.5 MeV. Replacing the number of the target $N_0$ by $(N_{Av}\ \theta\ w)/\ M$ and using Eq. (2-9) for the reaction rate, the resulting net area $A$ of a peak in the spectrum corresponding to a given photon energy is approximated by the ***activation formula*** as follows:

$$A \quad = \Delta N \gamma \varepsilon = \ \Phi_{th}\ \sigma_{eff} * \{(N_{Av}\ \theta\ w)/M\}\ (1 - e^{-\lambda\, tir})\ e^{-\lambda\, td}(\ 1 - e^{-\lambda\, tm})\ \gamma \varepsilon / \lambda \qquad (2\text{-}15)$$

where

$A$ = net peak area

$N_{Av}$ = Avogadro's number, $mol^{-1}$

$\theta$ = isotopic abundance of the target isotope $N_0$

$w$ = mass of the irradiated element, g

$M$ = atomic mass of the element, g. $mol^{-1}$

$\gamma$ = gamma-ray abundance (the probability of the disintegrating nucleus emitting a photon of this energy, photon $disintegration^{-1}$)

$\varepsilon$ = photopeak efficiency of the detector (the probability that an emitted photon of a given energy will be detected and contribute to the photopeak in the spectrum).

## 2.2 *Standardization in INAA*

An important advantage of INAA is its capacity for simultaneous multielement determinations, where symbiotic or antagonistic interelemental relationships are of interest. Standardization is the determination of the proportionality factors $F$ that relate the net peak areas in the gamma-ray spectrum to the amounts of the elements present in the sample under given experimental conditions :

$$F = A / w \qquad (2\text{-}16)$$

The terms standardization and calibration are used interchangeably to denote the determination of $F$. Both absolute and relative methods of standardization exist.

***Absolute standardization***

The physical parameters determining the proportionality factor $\theta$, $N_{Av}$, $M$, $\sigma_{eff}$, $\lambda$, $\gamma$ are taken from the literature. For many (n,$\gamma$) reactions and radionuclides the parameters $\sigma_{eff}$, $\lambda$, and $\gamma$ are not known, whilst in some cases also $\theta$ is not known accurately. Since the various parameters were often found via independent methods, their individual imprecision will add up in the calculation of

the elemental amounts, leading to large systematic errors. The other parameters A, w, $\Phi$, $\varepsilon$, $t_{ir}$, $t_d$, $t_m$, are determined, calculated or measured for the given circumstances and uncertainties can be detected.

*Relative standardization*

The unknown sample is irradiated together with a calibration sample containing a known amount of the elements of interest. The calibrated sample is measured under the same conditions as the sample (sample-to-detector distance, sample size and if possible composition). From comparison of the net peak areas in the two measured spectra the concentration of the elements of interest can be calculated :

$$X_{elem} = \{A/t_m D C w\}_{sample} / \{A/t_m D C w\}_{standard} \qquad (2\text{-}17)$$

where

$X_{elem}$ = element concentration, $g.g^{-1}$

$D$ = $e^{-\lambda td}$

$C$ = $(1 - e^{-\lambda tm})$

$w$ = mass, g.

This standardization is used in cases where the highest accuracy is required, such as the certification of reference materials. Multielement INAA on the basis of the relative standardization method is feasible when performed according to the principle of the single-comparator method. Assuming stability in the time of all relevant experimental conditions, standards for all elements are co-irradiated in turn with the chosen single comparator element. Once the sensitivity for elements relative to the comparator element has been determined (the so-called *k*-factor), only the comparator element has to be used in routine measurements instead of individual standards for each element.

*Single comparator method*

The idea of using a single comparator for multielement INAA was based on the ratio of proportionality factors of the element of interest and the comparator after correction for saturation, decay, counting, and sample weights. Girardi et al. [49] defined the *k*-factor :

$$k = \{M_a \gamma_c \varepsilon_c \theta_c \sigma_{eff,c}\}/\{M_c \gamma_a \varepsilon_a \theta_a \sigma_{eff,a}\} \quad (2\text{-}18)$$

where the subscripts a and c refer to the element of interest in the sample and comparator, respectively. Concentration then can be calculated from these *k*-factor; for an element determined via a directly produced radionuclide the concentration *X* follows from;

$$X_{elem} = \{(A/SDCw)_a / (A/SDCw)_c\}^* k \quad (2\text{-}19)$$

where, $S = (1 - e^{-\lambda tir})$

The k-factors are only valid for a specific detector, a specific counting geometry and irradiation facility, and remain valid only as long as the neutron flux parameters of the irradiation are stable. Bruin and Korthoven [50] modified the *k*-factor to a more versatile factor to be used with different detectors and counting geometries. The photopeak efficiency was removed from the factor and derived separately in the calculations from the experimentally determined efficiency curves for each detector and each counting geometry. The modified k-factor is defined by:

$$k_{IRI} = \{M_c \theta_a \sigma_{eff,a}\} \Sigma_i \gamma_{a,i} / \{M_a \gamma_c \theta_c \sigma_{eff,c}\} \quad (2\text{-}20)$$

where

$\Sigma_i \gamma_{a,i}$ = sum of the absolute intensities of the most important gamma-ray lines of the radionuclides.

The element concentration is then given by:

$$X_{elem} = \{(A/SDCw)_a / (A/SDCw)_c\}^* \{\Sigma_i \gamma_{a,i} \varepsilon_a / \gamma_a \varepsilon_c k_{IRI}\} \quad (2\text{-}21)$$

***The $K_0$-method for standardization***

Simonits et al. [51] proposed a new generalized *k*-factor which is independent of neutron flux parameters as well as of spectrometer characteristics. In the so-called $k_0$ method, the irradiation parameter $(1 + Q_0(\alpha)/f)$ and the detection parameter $\Sigma_i \gamma_{a,i}$ are removed, respectively, and altered in the expression of the *k*-factor, and the $k_0$ method is defined by :

$$k_0 = 1/k * \{(1 + Q_{0,c}(\alpha)/f)/(1 + Q_{0,a}(\alpha)/f)\}^* (\varepsilon_c/\varepsilon_a)$$

$$k_0 = M_c \theta_a \sigma_{0,a} \gamma_a / M_a \theta_c \sigma_{0,c} \gamma_c \quad (2\text{-}22)$$

By substituting for element concentration determined via a directly produced radionuclide, the element concentration is given by :

$$X_{elem} = \{(1 + Q_{0,c}(\alpha)/f)/(1 + Q_{0,a}(\alpha)/f)\}*(\varepsilon_c/\varepsilon_a)*\{(A/SDCw)_a/(A/SDCw)_c\}*1/k_0 \quad (2\text{-}23)$$

The $k_0$-factor thus has become a purely nuclear parameter for the thermal neutron spectrum. In the $k_0$-convention, Au is proposed as the comparator element. The neutron flux parameter $f$ and $\alpha$ must be measured in each irradiation facility, preferably even for each irradiation and sample [52]. At least three isotopes must be activated and measured to determine these parameters. A composite flux monitor containing adequate quantities of Au and Zr is very suitable for this purpose; in a single measurement the induced activities of $^{198}$Au, $^{95}$Zr, and $^{97}$Zr can be assessed. As such the $k_0$ method is not a single comparator but a triple comparator method.

*Synthetic multielement standards*

Another approach to the standardization problem is the preparation of synthetic multielement standards containing known concentrations of the element of interest. The availability of such primary standard materials containing appropriate and accurately known amounts of elements for a given application would be very convenient. There have been only a few reports of such synthetic standards. The US National Institute of Standards and Technology (NIST) has produced four multielement glass standards (SRMs 610, 612, 614, 616), with varying levels of trace elements. Rouchaud et al. [53] prepared multielement standards using metallic and organic polymer matrices. In the present work, synthetic multielement standards by Rossbach and Stoeppler [54] were used together with selected reference material in the irradiation of all sample sets.

*Estimation of the accuracy of analysis in INAA*

An estimate of the accuracy of INAA can be obtained by comparing the results of the analysis of certified reference materials with the data in the certificates. However, for quite few elements there are no reference materials available with certified concentrations. Therefore, as an indication of the degree of accuracy, participation in laboratory inter-comparison rounds or

proficiency testing is a must. The accuracy is expressed as a standardized difference $z$ thereby taking into account the uncertainties of the obtained results and the uncertainty in the certified value :

$$z_i = \frac{C_i - C_{ref,i}}{\sqrt{\sigma_i^2 + \sigma^2_{ref,i}}} \quad (2\text{-}24)$$

where,

$C_i$, $\sigma_i$ = observed concentration and its uncertainty respectively

$C_{ref.i}$, $\sigma_{ref.i}$ = concentration and its uncertainty in the reference respectively

In principle, INAA can provide very accurate results. In general, neutrons and γ-rays are not strongly absorbed by matter, corrections for absorption effects are therefore small and often negligible. Although in some cases interfering nuclear reactions may occur, these are easily recognized and rarely important. Other sources of error such as overlap of γ-rays lines from different radionuclides in the spectrum, dead-time losses, etc. can be assessed, overcome or corrected.

## 2.3 *INAA in comparison with other multielement techniques*

During the past decade several analytical techniques suitable for trace element analysis and in principle capable of satisfactory accuracy made their appearance or were further developed : electrothermal atomization atomic absorption spectrometry (ETA-AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS). ETA-AAS, which has excellent detection limits, is in practice a monoelement technique, so that it is difficult to compare it with a multielement technique such as INAA. ICP-AES in general has detection limits that are not satisfactory for ultratrace determination. ICP-MS is a quasi-simultaneous multielement technique with excellent detection limits. ICP-MS has clearly experienced a rapid growth during the last few years and many of its applications focus on the analysis of similar sample types (biomedical, environmental, etc.) as popular in activation analysis. Therefore, NAA will be compared to ICP-MS [55]. In ICP-MS a liquid sample is

nebulized into the ICP and the positive ions formed are extracted via an interface into a mass spectrometer, usually a quadrupole. In another variant (laser ablation ICP-MS) a laser is used to produced an aerosol directly from a solid sample. The aerosol is carried into the plasma by means of an argon stream.

*Some drawbacks in NAA and ICP-MS techniques*

Activation analysis, however, also has some drawbacks. In NAA the following disadvantages can be noted [47] :

- It is not possible to determined several elements or at least not at a low level.
- NAA is not suitable for sensitively analyzing water samples without extensive pre-treatment.
- for elements involving radionuclides with long half-lives, the turn-around time of an analysis may be in the order of 2-4 weeks.
- NAA is not available as a push-button apparatus with a complete software package to be operated on the spot .
- The need for the availability of, or access to a nuclear research reactor.

Of course, ICP-MS has also some drawbacks [55]:

- Polyatomic ions may overlap with singly charged ions of the same $m/z$. Example are ArO with $^{56}Fe$, ClO with $^{51}V$, and ArCl with $^{75}As$. These interferences occur almost exclusively up to $m/z$ = 81, corresponding to $Ar_2H$, and are a consequence of the limited resolution of a quadrupole MS. When a high-resolution double focusing magnetic sector mass spectrometer is used , instead of a quadrupole, such interferences can be resolved
- Matrix effect. For high matrix concentrations signal suppression (or enhancement) may occur. A typical figure is 35 % suppression for 9 g/l NaCl. The matrix effect can be corrected using one or several internal standards; dilution; matrix matching; standard addition; chemical separation; isotope dilution measurement.

*Simple basis of comparison between NAA and ICP-MS*

A comparison of NAA and ICP-MS for the trace element analysis of environmental materials is based on the following:

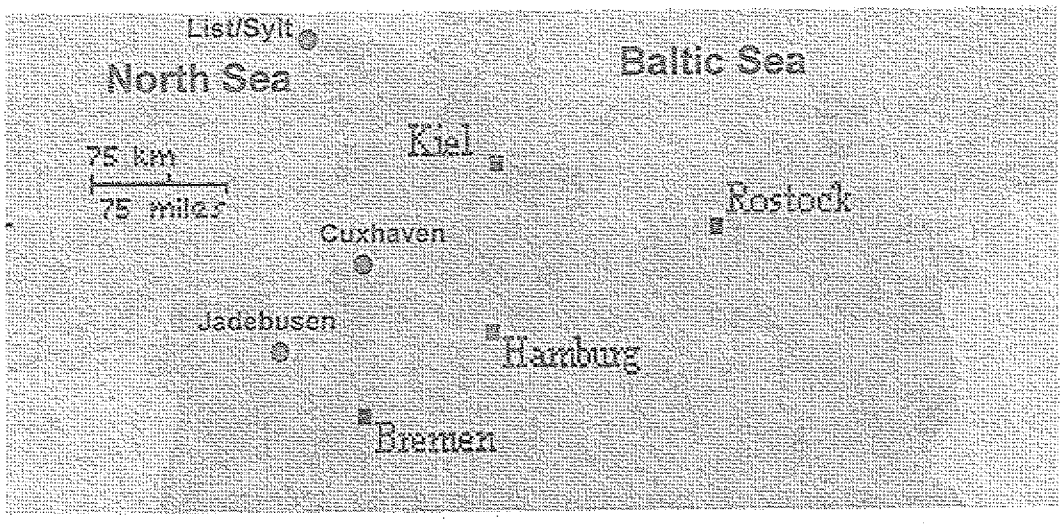
1. (Interference-free) detection limits are in general comparable, for some elements ICP-MS is better, for others NAA is.
2. For solid samples INAA has the definite advantage of not requiring sample dissolution with the attendant problems of difficult or incomplete dissolution of some matrices, possible losses of some elements such as As, Se and Hg, precipitation or adsorption losses. In addition no blank from acids or other reagents, which is generally the major source of contamination, is introduced. The later advantage also holds for RNAA. This advantage is very important for low-level material requiring considerable effort for a contamination-free dissolution, e.g. biological samples and semi-conductors.
3. The most important advantage of activation analysis is that, whenever interference occur, for instance in the analysis of biological materials where γ-radioactivity of $^{24}Na$, $^{38}Cl$, $^{42}K$, $^{80}Br$, and $^{82}Br$ and bremsstrahlung from $^{32}P$ are the main matrix activities, the radionuclide of interest can be carried through even complex radiochemical separations without any danger of contamination and with the addition of nonradioactive carrier to ease the separation. In ICP-MS, to improve the detection limits for elements interfered with by polyatomic species, a chemical separation can be carried out, but always with contamination risk and this separation must be carried out at the trace element level.
4. Another advantage of NAA is specific to the analysis of reference materials: activation analysis is based on a different principle than other analytical techniques and subject to other types of systematic errors. On the other hand, an obvious advantage of ICP-MS for analyzing reference materials is that isotope dilution ICP-MS is feasible, which allows the high precision and accuracy typical of isotope dilution methods to be achieved.

Fardy and Warner [56] concluded that NAA cannot match the superior sensitivity for a wider range of elements obtained by ICP-MS. While NAA is an inconvenient and time-consuming technique for many applications, it does not suffer from blank problems after irradiation of the sample, and becomes the preferred technique where low limits of detection are required for trace concentrations in solid samples.

## 3. *Experimental*

### 3.1 *Sample collection and preparation*

Samples of the brown alga *Fucus vesiculosus* were collected at low tide from three different well characterized sampling areas of the North Sea coast: Eckwarderhörne, Cuxhaven, and Sylt-List Algae were sampled bimonthly in 1993 and 1994 at each collection site. The collected sample size




was 2 kg. Immediately after collection the samples were cryogenically deep-frozen (-150 °C) and transported to the laboratory. Ten individuals from each site were packed in a plastic bag and stored at 4 °C for area measurements. At the collection site Eckwarderhörne, an experiment was designed in which the shore bank was divided into five spots of 1 $m^2$ each located 50 m apart. Tips, thalli, and basal parts of ten plants collected in August 1994 and February 1995 from each collection point were separated. Subsamples of plants were used for the determination of fresh weight per plant and their parts, and the maximum length of a plant. Fresh weight was determined after the plants had lain between layers of absorbent paper for 30 s. The surface area per unit fresh weight was determined by weighing fresh whole plants and the area of different parts of the whole was determined by photocopying them, cutting out the shape, and weighing it for comparison with the weight of a known area of paper. Plant surface area was assumed to be twice the area determined in this way.

*Sample preparation*

After cryogenic transportation of the 2 kg material from each site to the lab in stainless steel containers [57], a subsample of 10 g fresh weight taken from the non-homogeneous (crushed-frozen-bulk with a titanium stick) was freeze-dried. Under clean room condition, the freeze-dried samples were ground for 3 min in an agate mill to produce a coarse powder, and stored in 20 ml scintillation glass vessels, cleaned by steaming with $HNO_3$ (Merck) for 6 h and then drying before aliquoting.

Different parts of the algae were dissected using a quartz knife, and the fresh weight was determined, then freeze-dried. The dried samples were shaken in a shaking agate mill for 5 min prior to the analysis. The finely ground powder was bottled in scintillation glass vessels using a quartz spatula.

*Sample preparation for analysis*

A variety of elements with environmental importance have been analyzed in whole plants but also in different parts of the brown algae. For instrumental neutron activation analysis no chemical pre-treatment of the algae material is required. Quartz of high purity (Suprasil®) is the purest material and most suitable for container material, which provides negligible blanks (except for Si of half-life 2.6 h) and also withstands long-term irradiation of biological material with possible pressure build-up due to decomposition and production of gases. The batch of quartz tubes of 1 m length, 6 mm in diameter, and wall thickness 0.5 mm, was cut into sub-tubes of length 15 cm. Each sub-tube was directly split into two sealed-end ampoules using an oxygen- hydrogen flame. The ampoules were etched internally for 5 min with diluted 1:1 HF high-purity acid (Merck), then washed with double-distilled water and filled with conc. distilled $HNO_3$ (Merck), and boiled for 12 h. After cooling, the $HNO_3$ acid was removed and the ampoules were boiled in double-distilled water for 6 h. The acidic water was then removed and each individual ampoule was washed with double-distilled water, then dried in a semi-covered glass dish in an oven at 100 °C until they were fully dry. The dried ampoules were stored over conc. $H_2SO_4$ in a desiccator until further use.

Using a quartz spatula the freeze-dried algae samples were filled into the cleaned quartz ampoules in pairs (a and b), weighing 100 to 120 mg of material, and a pre-treated quartz wool stopper with ultra-pure distilled $HNO_3$ (Merck) was pushed along the tubes to stop the material from dispersing in the dead volume. The sealed end was then immersed in liquid nitrogen to cool before sealing the other end to prevent element losses through volatilization due to heat transfer while sealing with the oxygen-hydrogen flame. For easier identification after irradiation, the capsulated ampoules were marked with edding 3000 permanent marker.

Two aliquots of each sample together with an appropriate synthetic multielement standard, a certified reference material ( NIES No. 9 Sargasso ), and a blank ampoule from the same patch were irradiated at a thermal neutron flux density of about 5 x $10^{13}$ N $cm^{-2}$ $s^{-1}$ for 10 h. Due to the matrix activity after irradiation, the samples were cooled for approximately 6 days before handling for the clean-up. Irradiated samples were transported to the cleaning lab and identified behind a lead shield to prevent unnecessary exposure. Each irradiated ampoule was then carefully immersed in a PTFE container filled with 1:1 HF acid (Merck) for 1 min, then conc. $HNO_3$ (Merck) for 2 min, and the outside wall finally was washed in distilled water. The washed irradiated ampoules were laid between layers of absorbent paper for 5 min. Finally, the dried irradiated ampoules were transferred to a sampler holder in a ranked order for measurement.

***Sample preparation for inductively coupled plasma atomic emission spectroscopy (ICP-AES) and mass spectrometry (ICP-MS)***

For ICP-AES and ICP-MS, wet decomposition of about 200 mg freeze-dried algae material was performed at 180 °C in a closed system using a PTFE pressure bomb. The PTFE vessels were washed with double-distilled water, dried in an incubator, and then left to cool under clean bench conditions. The PTFE in batches of 8 vessels were weighed and filled with freeze-dried algae material, pre-analyzed internal reference material brown algae (IRM Algae 88), and certified reference material (NIES No. 9 Sargasso) using a quartz spatula. The quartz spatula was washed immediately with double-distilled water and then acetone (Merck) for drying after each filling. 2 ml

ultra-suprapure distilled $HNO_3$ acid was pipetted into each PTFE vessels and into two empty ones for the blank value of the $HNO_3$ acid, and all 4 PTFE vessels with relief valves were packed in a stainless steel block. The pressure ashing system was brought to the heating plates, and heated for 8 h at 180 °C. The preparation steps were repeated for the same material in different PTFE vessels as a duplicate. Thereafter, digestion solutions were pipetted into a steamed clean glass flask of 10 ml and were diluted with ultrapure water. In the case of ICP-AES prior to the determination step the samples were appropriately diluted (1:2 - 1:20) with ultrapure water and Sc was added as an internal standard element (Conc.= 10-50 µg/ml depending on the element determined), and for ICP-MS an appropriate amount of an internal standard element solution (usually Rh) was added to give concentration of 50 µg/l.

*Standards*

For INAA measurements, multielement standards were made in our lab, ***P*** for the elements (Fe, Zn, Co, Ni, Sn, Se, Cd, Ca, Na, Br, Ba, K, I, Rb, Ce, Sb, Tb, Hf, Au, As, La, Te, Nd, and Lu), ***Q*** for the elements (Cr, Fe, Sr, Zr, Yb, Cs, Sc, Sm, Ag, W, Ta, Th, Eu, Ga, Mo, In, Ge, Ru, Hg, and Na), rare earth elements (REE) standard, and a uranium (U) standard were used. The standards were used in the calculation of the element concentrations in the certified reference materials. A comparison of the obtained results with the certified and recommended values of the elements is then made. The synthetic standard used was prepared as described by Rossbach and Stoeppler [54]. The calibration standards of ICP-AES and ICP-MS for the elements Mg, S, P, Ca, Mo, Cd, V, Mn, Fe, Cu, Zn, As, and Pb were prepared from ICP-multielement standards supplied by Merck. 50 µg/l $^{103}Rh$ was used as internal standard for ICP-MS.

## 3.2 *Instrumentation*

### 3.2.1 *INAA*

The irradiated samples were counted on a GeLi detector (20 % efficiency, Canberra) constructed in the center of a steel cube of 70 x 70 x 75 cm inner dimensions, 7 cm wall thickness coated with copper sheet of 1.2 cm in thickness, surrounded by lead bricks of 5 cm thickness, and connected to a pre-amplifier, Multichannel Buffer ADCA M$^{TM}$ 918A ORTEC EG&G, an amplifier 572 ORTEC EG&G, bias supply 0-5 kV 459 ORTEC EG&G, and a computer for the analysis of the gamma spectrum collected. Another HPGe detector (25 % efficiency, ORTEC EG&G ) constructed in a steel cube of 90 x 90 x 65 cm inner dimensions, 15 cm wall thickness and coated with a sheet of copper 1.2 cm in thickness, surrounded by lead bricks of 5 cm, and connected to a pre-amplifier, Multichannel Buffer 919 Spectrum Master ORTEC EG&G, amplifier 572 ORTEC EG&G, bias supply 0-5 kV 459 ORTEC EG&G, and a computer for the analysis was used for counting the same samples counted on the GeLi detector.

*Irradiation Facilities*

The algae samples, reference material, and blank ampoules were irradiated in the FRG-2 reactor of the GKSS Research Center in Geesthacht for 10 hours with a thermal neutron flux density of $5 \times 10^{13}$ N cm$^{-2}$ sec$^{-1}$.

*Calibration*

INAA systems were calibrated with the energy lines of the $^{60}$Co, $^{137}$Cs, and $^{152}$Eu standard calibration sources of known activity, certified by the Physical-Technical Federal Institution (PTB) in Braunschweig. A computer software program for energy and efficiency calibration is integrated into the evaluation of the measured gamma spectrum. Both systems were always calibrated before measuring each irradiated set. For ICP-MS depending on the matrix and the analyte (concentration) either a standard addition calibration ($^{103}$Rh) or calibration (matrix matched) aqueous standard solution was employed. For ICP-AES aqueous acid matched standard solutions containing Sc as

internal standard element were used for calibration. The elements P and S were determined without the use of internal standard.

*Measurement*

Irradiated samples were brought into the measuring lab, counted together with the standards at the same geometrical distance from the GeLi and/or the HPGe detector. The first measurement was processed directly after sample identification. Counting time was 6000 s - 10000 s, sample-detector distance 30 cm - 60 cm, and dead time in the range of 12 to 20 %. Cooling time, dependent on the samples activities of between 6 - 10 days, then that of the second measurement was counted at a distance 15 - 20 cm and a dead time of 8 - 10 % for 20000 s - 30000 s. A cooling time of about 15 days and the third measurement was taken at distance of 0 - 2 cm, dead time 1 - 6 %, and counting time 20000 s - 50000 s. The basic set-up of the gamma ray spectrometer for use in INAA is shown in Fig. 3.1. A computer software program for the sample spectrum analysis was used (MAESTRO II EG&G ORTEC), the radioactive species were identified using their $\gamma$-lines energies reported by Erdtmann and Soyka [58] as shown in Fig. 3.2 (a-c). A Fortran program was used for calculating the concentration of the radioactive species in the sample by comparing of the net peak area of the standard to that of the sample with a correction for the decay times. Final results were compared with the certified and the recommended values of the reference material, and a correction factor was used in some cases. A correction was performed for the error resulting from the interference of gamma energies of Se and Hg, Se and Ta, and rare earth elements and U if necessary.

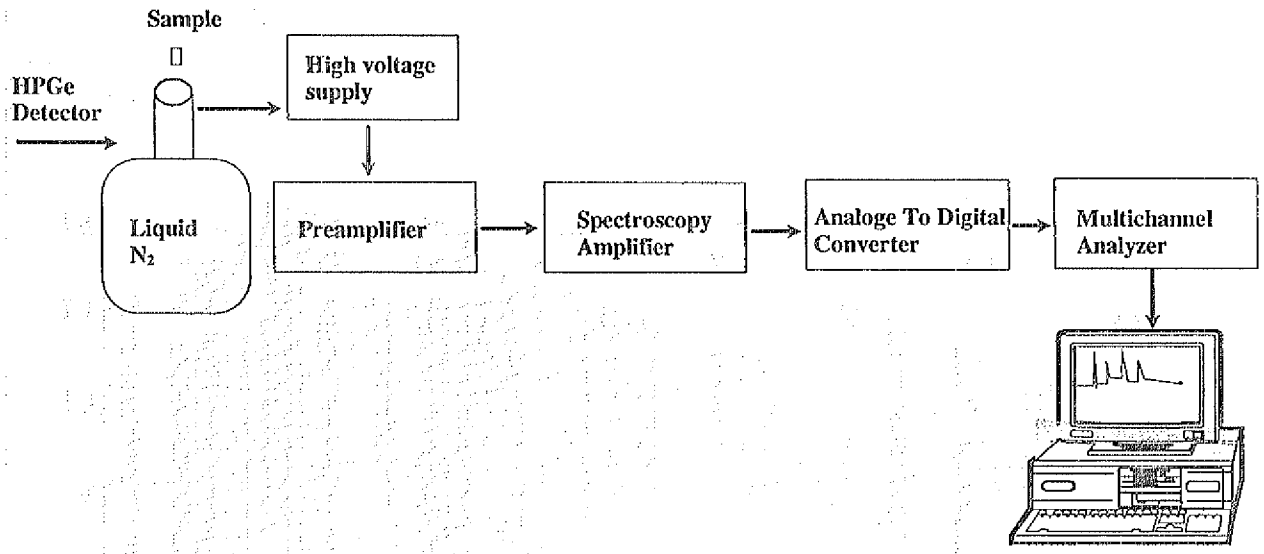



**Fig. 3.1 : Schematic set-up of gamma ray spectrometer for use in INAA**

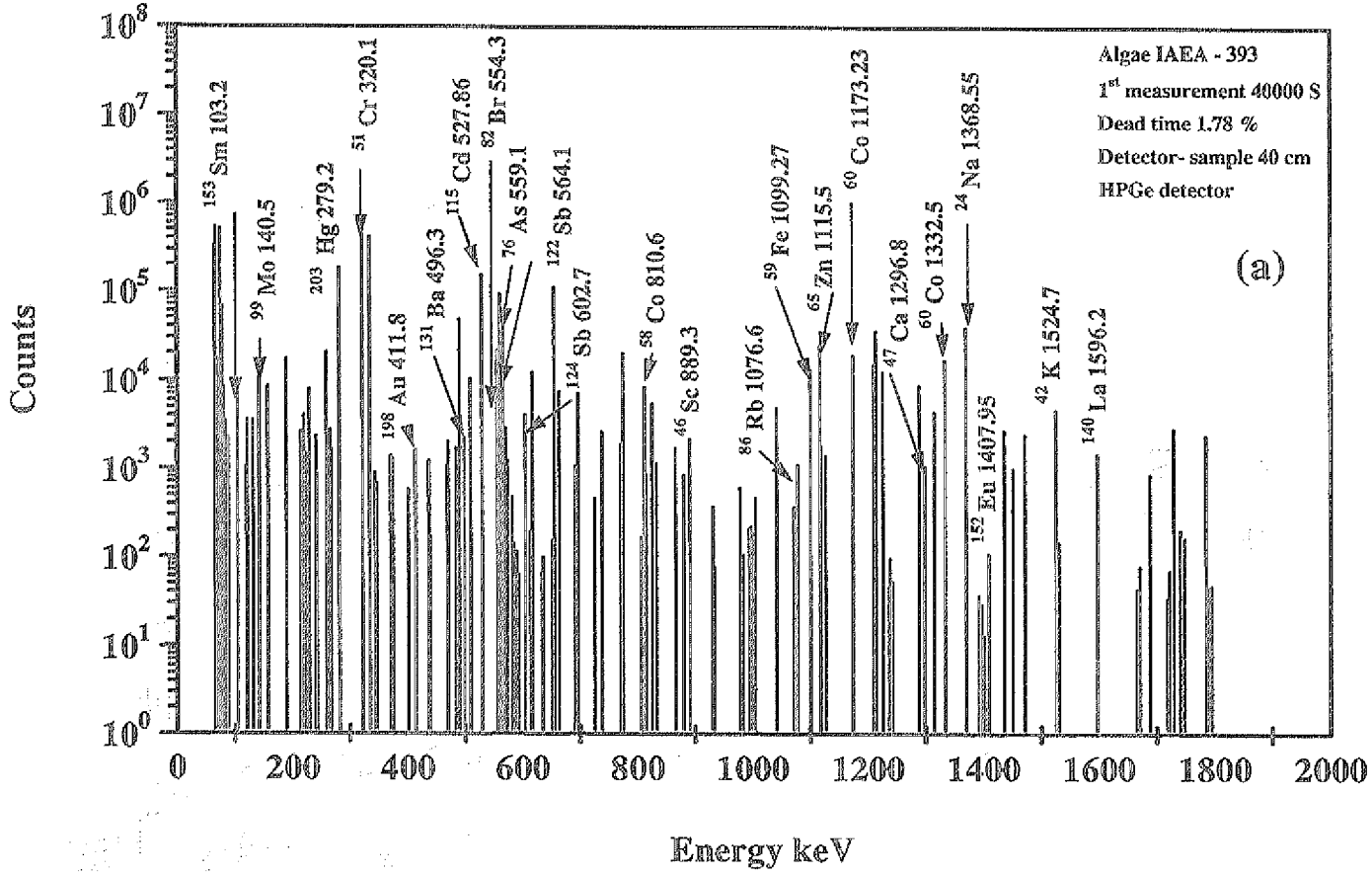
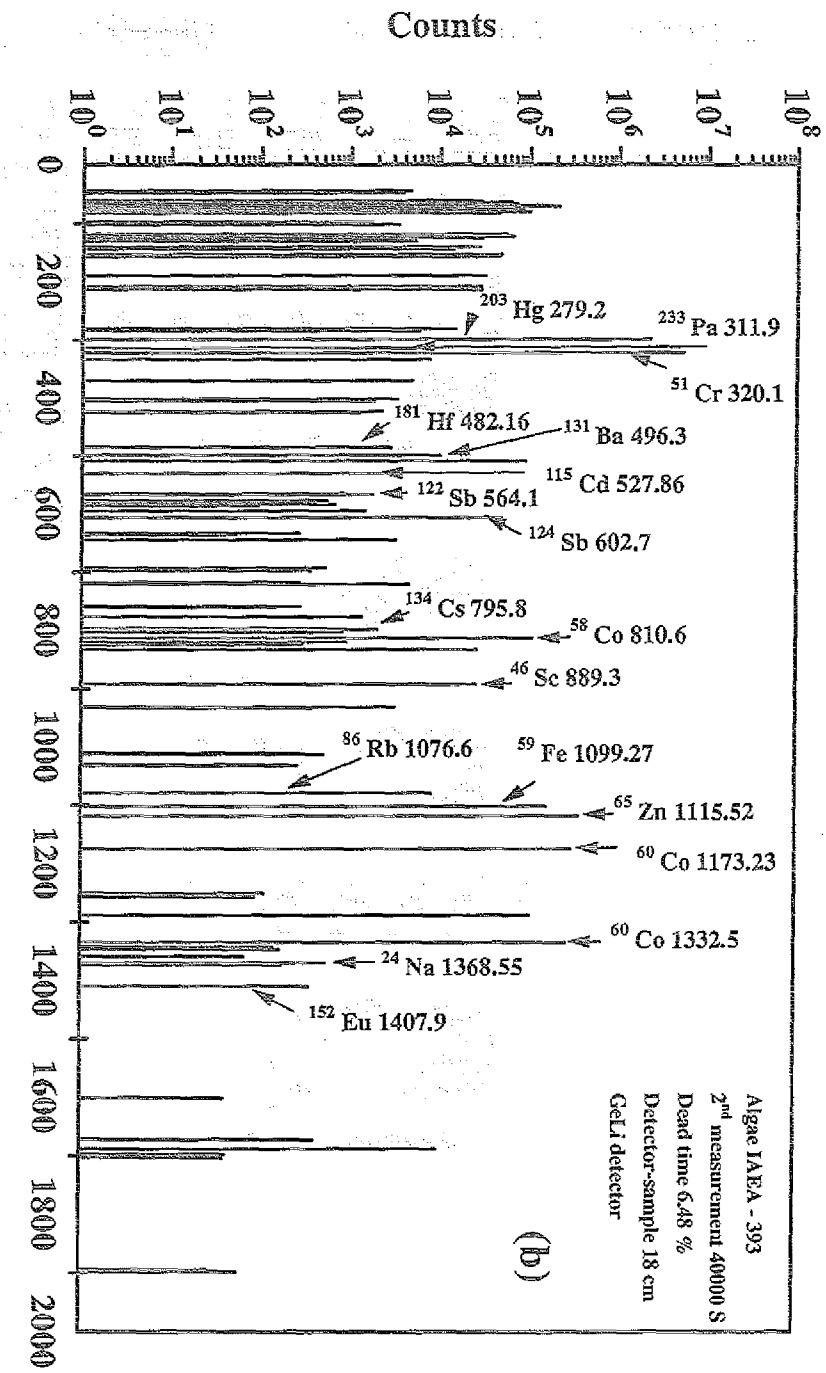
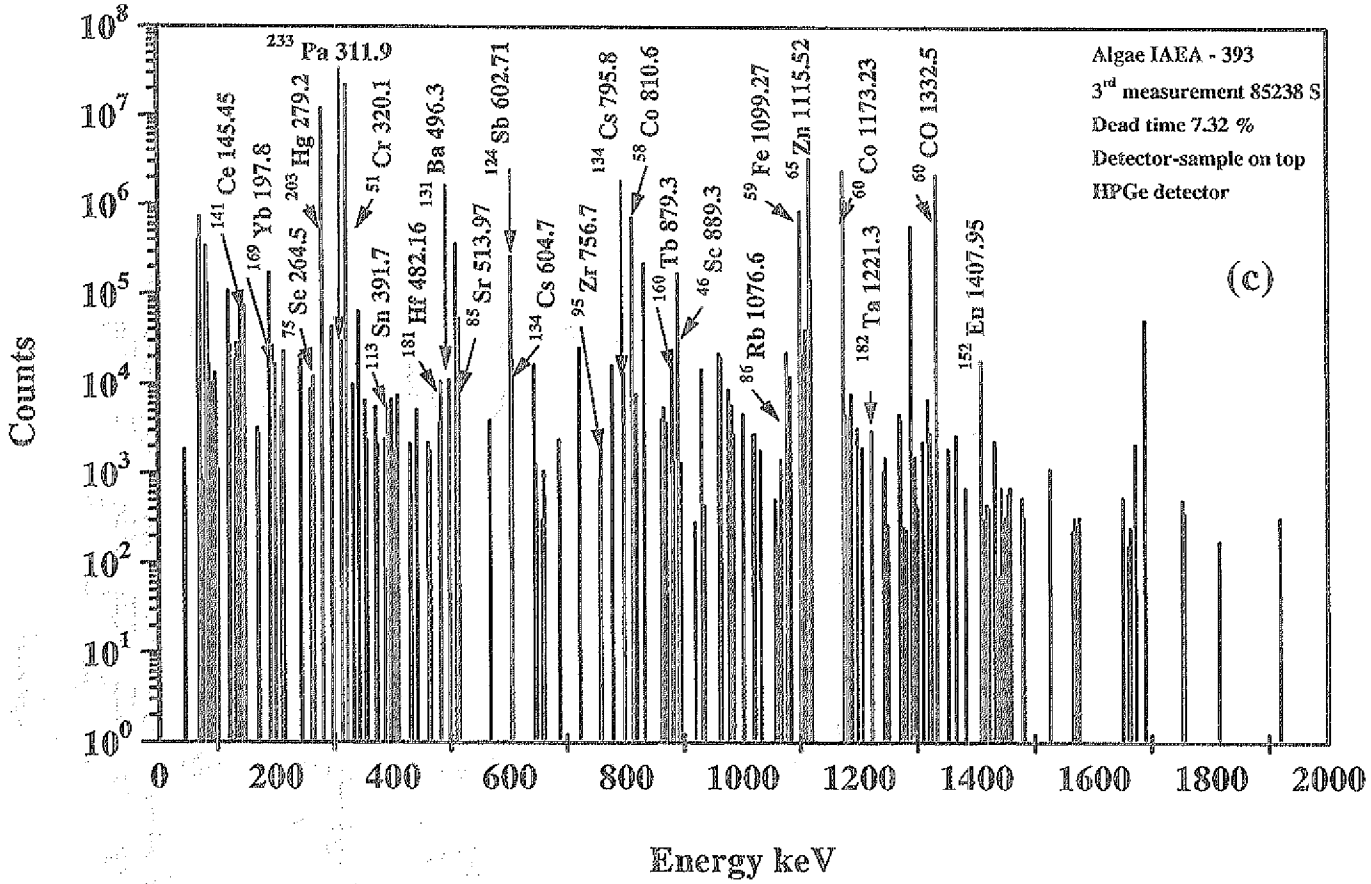



**Fig. 3.1 (a-c): The emitted gamma lines from irradiated sample with thermal neutron flux density $5x10^{13}$ N $cm^{-2}$ $s^{-1}$ for 10 hours. (a) first measurement, (b) second measurement, and (c) third measurement.**


Counts
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^7$
$10^8$
0
200
400
600
800
1000
1200
1400
1600
1800
2000
Energy keV
$^{203}$Hg 279.2
$^{233}$Pa 311.9
$^{51}$Cr 320.1
$^{181}$Hf 482.16
$^{131}$Ba 496.3
$^{115}$Cd 527.86
$^{122}$Sb 564.1
$^{124}$Sb 602.7
$^{134}$Cs 795.8
$^{58}$Co 810.6
$^{46}$Sc 889.3
$^{86}$Rb 1076.6
$^{59}$Fe 1099.27
$^{65}$Zn 1115.52
$^{60}$Co 1173.23
$^{60}$Co 1332.5
$^{24}$Na 1368.55
$^{152}$Eu 1407.9
(b)
Algae IAEA - 393
2nd measurement 40000 S
Dead time 6.48 %
Detector-sample 18 cm
GeLi detector


Counts
Energy keV
Algae IAEA - 393
3rd measurement 85238 S
Dead time 7.32 %
Detector-sample on top
HPGe detector
(c)
233 Pa 311.9
141 Ce 145.45
169 Yb 197.8
75 Se 264.5
203 Hg 279.2
51 Cr 320.1
113 Sn 391.7
181 Hf 482.16
131 Ba 496.3
85 Sr 513.97
124 Sb 602.71
134 Cs 604.7
95 Zr 756.7
134 Cs 795.8
58 Co 810.6
160 Tb 879.3
46 Sc 889.3
86 Rb 1076.6
59 Fe 1099.27
65 Zn 1115.52
60 Co 1173.23
182 Ta 1221.3
60 CO 1332.5
152 Eu 1407.95

*Detection limit of the INAA systems*

Constituents of the blank ampoules were determined in 18 quartz vials, irradiated for 10 hours with samples at a thermal neutron flux density of $5 \times 10^{13}$ N $cm^{-2}$ $s^{-1}$. Mean values with the coefficient of variation were taken as the detection limits of the measuring system and/or the limit of detection was calculated from the following formula :

$$C = 3 S_{Bv} / m \qquad (3\text{-}1)$$

where C : lower limit of detection

$S_{Bv}$ : standard deviation of the mean values of the constitutes of the blank ampoules

m : peak area per concentration in the standard.

### 3.2.2 *ICP-MS*

ICP-MS analyses were performed with an ELAN 5000 PERKIN ELMER SCIEX. The elements measuring parameters are shown in Table 3.1.

**Table 3.1 : The measuring parameters for the determined elements.**

| Element | Mass | Replicate time (ms) | Dwell time (ms) | Correction factor |
|---|---|---|---|---|
| V | 51 | 1000 | 20 | - |
| Cu | 63 | 1000 | 20 | - |
| Cu | 65 | 1000 | 20 | - |
| As | 75 | 4000 | 80 | -3.087 x mass 77 + 2.546 x mass 82 |
| As | 75 | 4000 | 80 | - |
| Se | 82 | 10000 | 200 | -1.001 x Kr 83 |
| Se | 82 | 10000 | 200 | - |
| Mo | 95 | 1000 | 20 | - |
| Mo | 98 | 1000 | 20 | -0.1095 x Ru 101 |
| Rh* | 103 | 1000 | 20 | - |
| Cd | 112 | 1000 | 20 | -0.03995 x Sn 118 |
| Cd | 114 | 1000 | 20 | -0.02747 x Sn 118 |
| Pb | 206 | 1000 | 20 | - |
| Pb | 207 | 1000 | 20 | - |
| Pb | 208 | 1000 | 20 | - |
| Pb | 208 | 1000 | 20 | +1 x Pb 207 + 1 x Pb 206 |

* Internal standard

The setting parameters were:

- plasma flow 15 l/min
- nebulizer cross flow 0.87 l/min
- auxiliary flow 0.8 l/min
- RF power 1200 W
- CEM voltage 5 kV
- sample uptake 2.1 ml/min
- 50 pbb $^{103}Rh$ was used as internal standard.

### 3.2.3 *ICP-AES*

A sequential plasma 400 atomic emission spectroscope (PERKIN ELMER, Germany) was used for ICP-AES measurements. Instrumentation and operating parameters used for ICP-AES were:

- spectrometer 400 PERKIN ELMER
- HF- generator of 40 MHz, 1kW PERKIN ELMER
- monochromator 0.408 m-Czerny-Turner PERKIN ELMER
- cross-flow nebulizer gas flow rate 0.4 l/min PERKIN ELMER
- spectral range 160 - 800 nm

***Determination of the detection limits of ICP-MS and ICP-AES***

For the determination of the detection limits of ICP-Ms and ICP-AES, 20 blank samples were analyzed. Calibration curves were dotained using a minimum of five different concentrations covering the expected concentration range. Table 3.2 (a-b) presents the wave lengths of the selected elements measured by ICP-AES, and limits of detection of ICP-AES determinations calculated based on the above data and the following equations:

detection limits $C_{NG} = 3S_{BI} / m$ (3-2)

limit of determination $C_{EG} = 6S_{BI} / m$ (3-3)

limit of quantification $C_{BG}$ = $10S_{BI} / m$ (3-4)

where;

$S_{BI}$ : standard deviation of the blank value

m : slope of the calibration curve.

**Table 3.2 (a) : Wave length of the measurements.**

| Element | λ [nm] | PMT voltage [V] | Dwell time [ms] | Element | λ [nm] | PMT voltage [V] | Dwell time [ms] |
|---|---|---|---|---|---|---|---|
| Zn | 213.856 | 600 | 300 | Ca | 422.673 | 600 | 200 |
| Mn | 257.610 | 551 | 300 | Na | 589.592* | 551 | 500 |
| Fe | 259.940 | 600 | 300 | K | 769.898 | 750 | 400 |
| V | 290.882 | 701 | 300 | P | 178.283 | 819 | 400 |
| Mg | 279.553 | 400 | 200 | S | 182.037 | 750 | 400 |
| Sc (Zn-Cu) | 255.238 | 600 | 300 | Sc (Na, K) | 567.181 | 551 | 400 |
| Sc (Mg-Ca) | 361.384 | 600 | 200 | | | | |

* in samples with a high concentration of Fe, the line 588.995 nm is used.

**Table 3.2 (b) :Limit of detection (L.D) [µg/g, d.w.] for ICP-AES determination.**

| | Zn | Mn | Fe | V | Mg | Ca | Na | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|
| L.D | 1.5 | 0.5 | 2.0 | 1.5 | 5 | 30 | 80 | 100 | 400 | 100 |
| element dilution | 1:2 | 1:2 | 1:2 | 1:2 | 1:5 | 1:5 | 1:5 | 1:5 | 1:5 | 1:5 |

# 4. *Results and Discussion*

## 4.1 *Analytical quality*

### 4.1.1 *Procedures for analytical quality assurance*

The validity and precision of the obtained data and the used analytical methods were evaluated by analyzing certified reference materials in addition to the samples. Data evaluations comprise a set of experimental and statistical procedures designed to test whether a measurement process is in a state of statistical control, and consequently whether it is capable of producing data that can be used with confidence. As accuracy is a qualitative concept, the best estimates of *true* element concentrations are obtained from information available on primary standards, or from certified element concentrations of reference materials. Fig. 4.1 shows the control chart for some elements determined by INAA in NBS SRM 1645 River Sediment, BCR CRM 146 Sewage Sludge, and IAEA-SL-1 Lake Sediment.

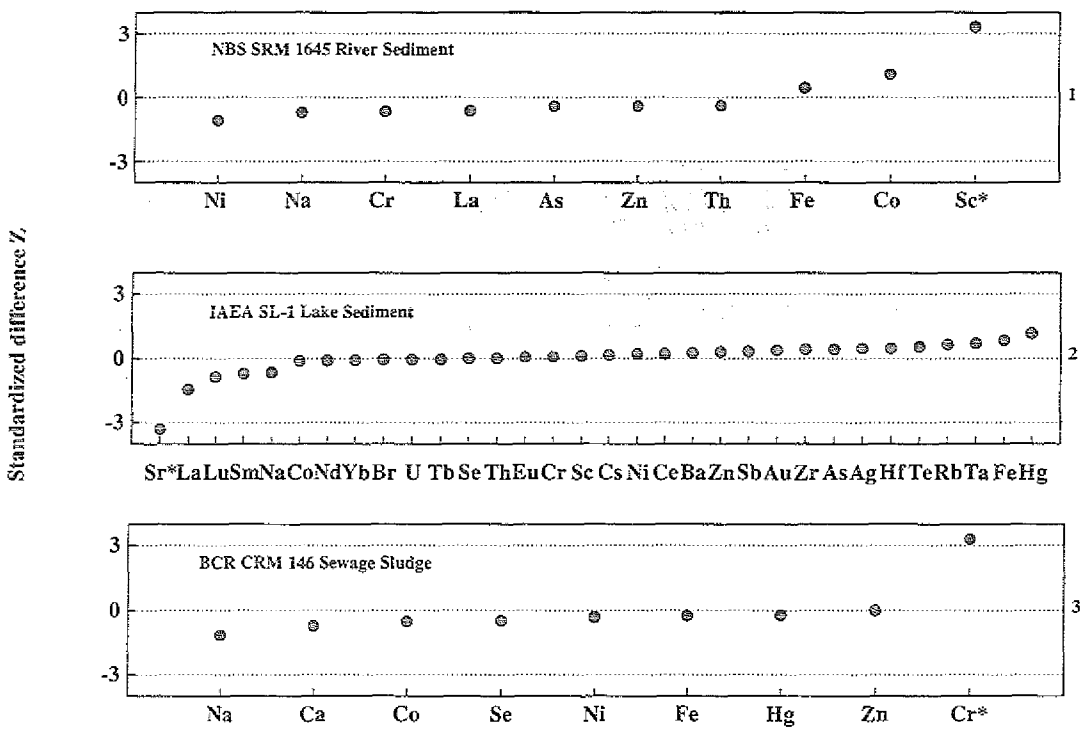



**Fig. 4.1: Control chart for comparison of results determined by INAA and certified values. Elements are sorted on Z-values (n = 3, * = out of Z-value range).**

The " closeness of agreement " is obtained by the determination of the standardized difference Z-values (see Eq. 2-24).Z-value < |3| means that the result of the analysis of reference material should be in the 99 % confidence interval of the certification value. Data presented in Fig. 4.1 demonstrate that values of Sc, Cr, and Sr are outside the confidence interval. For these elements whenever it is necessary, correction factor is applied. For all other determined elements a good agreement with the certified values was observed. Validation of INAA data and results obtained by other techniques for some selected elements were checked by the comparison with the CRM (NIES No. 9 Sargasso). Data are summarized in the control chart of Z-values as shown in Fig. 4.2. Only the phosphorus value measured by ICP-AES indicates a bias. Therefore, all phosphorus values were inspected and these measurements were corrected. Fig. 4.3 (a-b) presents a control chart for comparison of some chosen elements determined by INAA and other multielement techniques in samples from different collection sites, and different parts of algae. In all presented examples a good agreement between the methods was found.

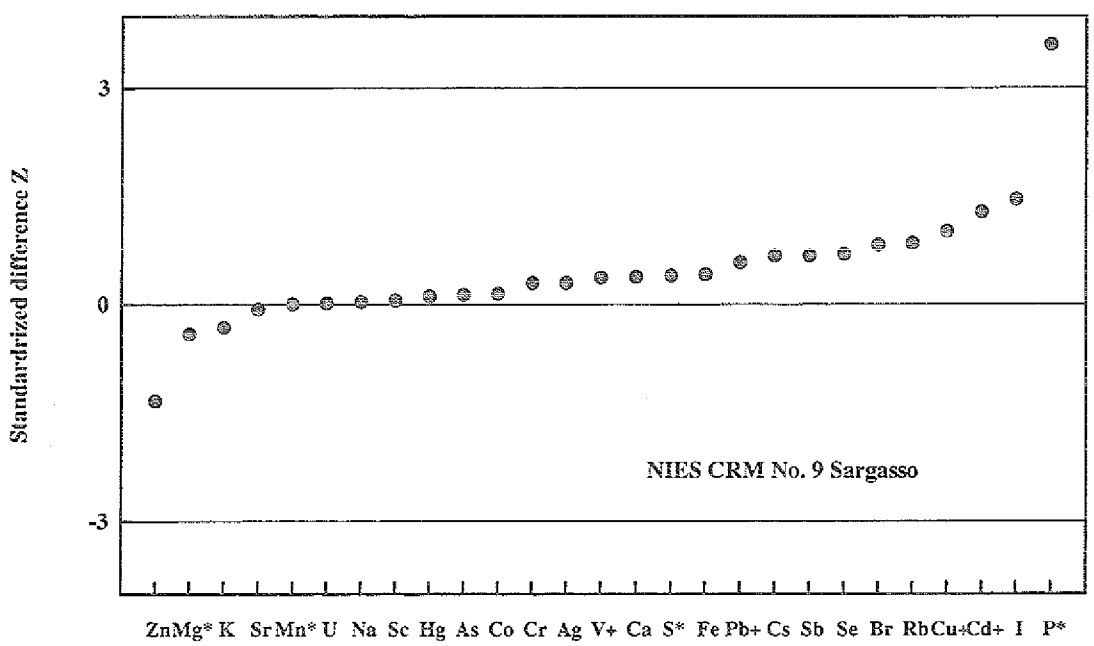



**Fig. 4.2 : Control chart for elements measured in the reference material NIES No. 9 (Sargasso) by INAA, ICP-MS (+), and ICP-AES (*). The elements are sorted on the Z-value (n = 15).**

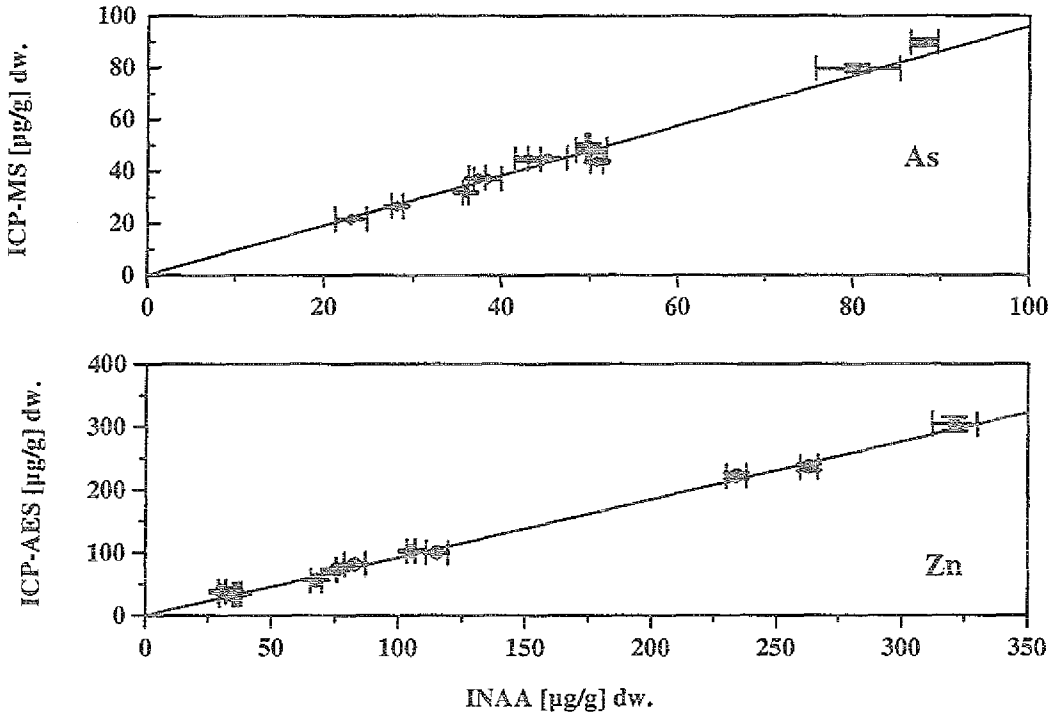



Fig. 4.3 a : Data comparison: As determined by INAA and ICP-MS, and Zn determined by INAA and ICP-AES

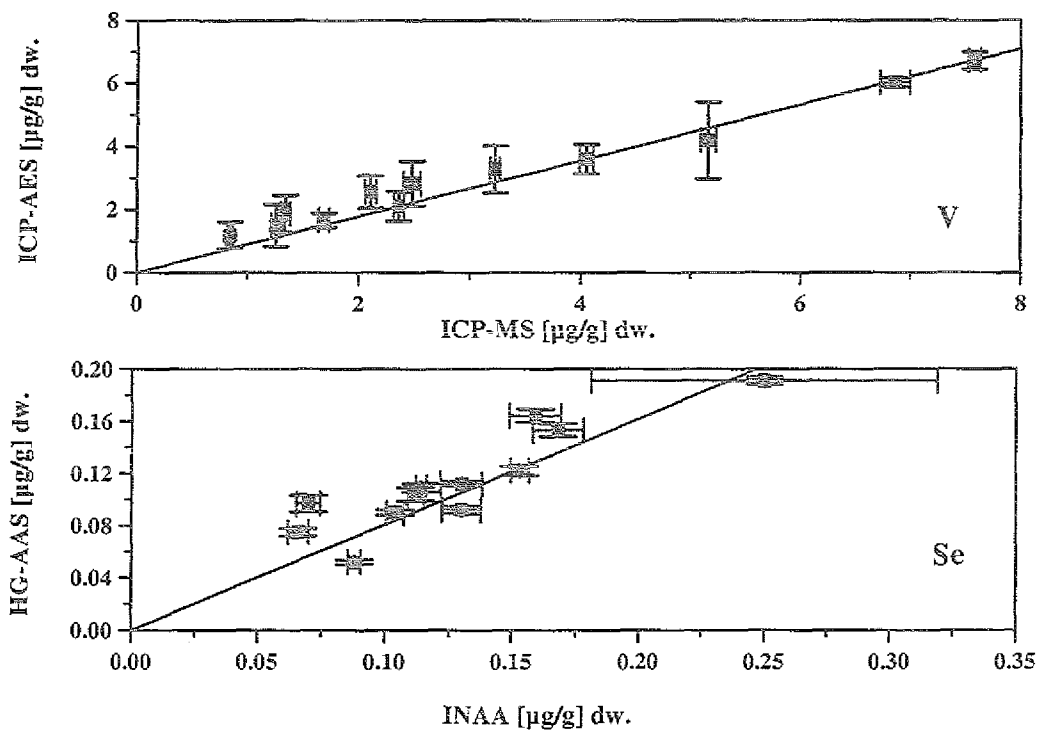



Fig. 4.3 b : Data comparison: Se determined by INAA and HG-AAS, and V determined by ICP-AES and ICP-MS.

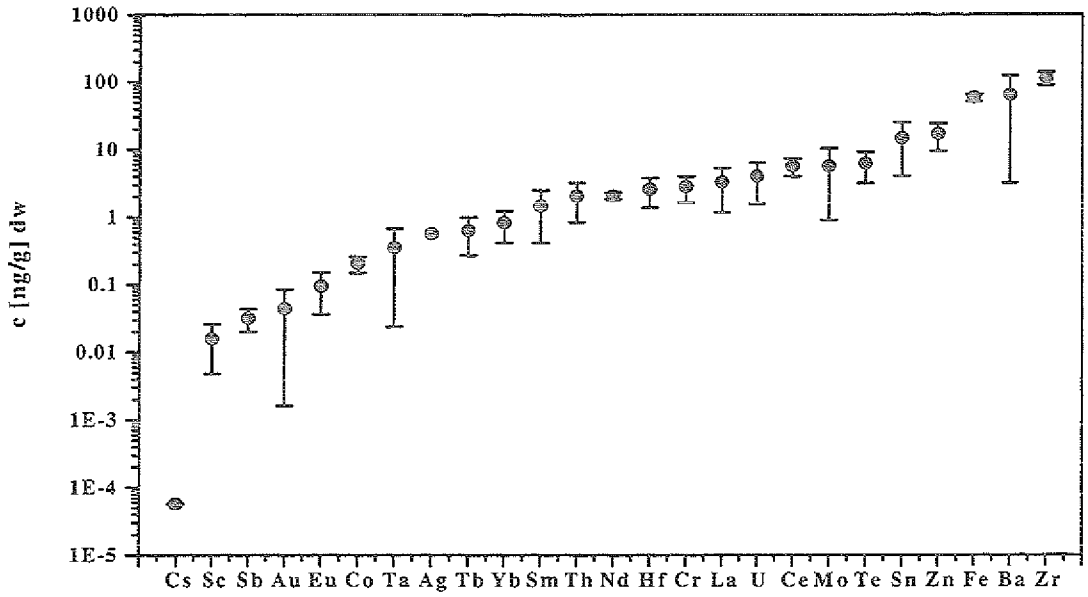



**Fig. 4.4 : Element concentration in blank quartz ampoules ( mean ± Sd, n = 15 ).**

In comparison to other techniques, INAA required no chemicals therefore the element concentrations measured in the blank quartz ampoules were considered as blank values. From Fig. 4.4 it is obvious that the lowest concentration is around $10^{-4}$ ng/g [Cs] and the highest is around 100 ng/g [Zr]. However, more than 90% of the determined elements present in the quartz ampoules are in the concentration range 0.1-1 ng/g, which is a factor of $10^3$ less than the concentrations expected in real samples.

### 4.1.2 *Detection limits and reproducibility*

Minimum detectable activity in INAA for a given peak is equal to 3 times the square root of the background. In this work the detection limit was calculated based on the determined blank value concentrations as given in Eq. (3-1). Fig. 4.5 represents the detection limits calculated from the blank values for all elements of interest. INAA is highly sensitive to Sc, Au, and Eu, but moderate sensitive to Fe, Sn, and Mo. However, for the elements determined in this work, the concentration

levels in natural samples are in a much higher range than the detection limits of INAA. Therefore, the multielement capabilities of INAA are ideally suited for the characterization of those samples.

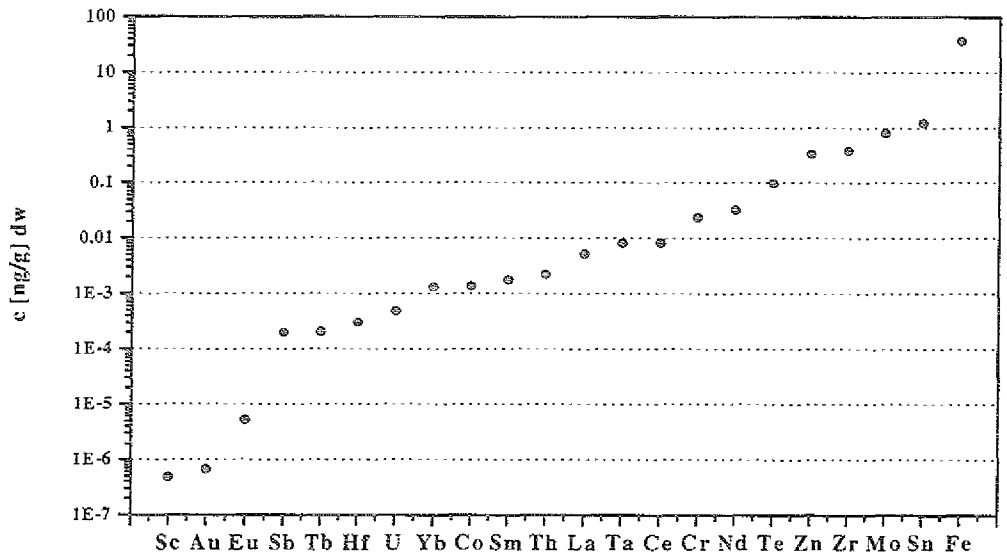



**Fig. 4.5 : INAA detection limits for elements measured in quartz ampoules irradiated for 10 h in thermal neutron flux 5 x $10^{13}$ N $cm^{-2}$ $s^{-1}$**

*Reproducibility of INAA measurements*

Fig. 4.6 represents the reproducibility of the measurements for selected elements in a set of irradiated samples from Eckwarderhörne (Sp1-Sp6), and IAEA-0392/0393 (Sp7-Sp8). In all analyzed samples the reproducibility of Fe, Co, and Zn determinations are better than 4 %. Only for Cr determinations is the reproducibility above 4 %.

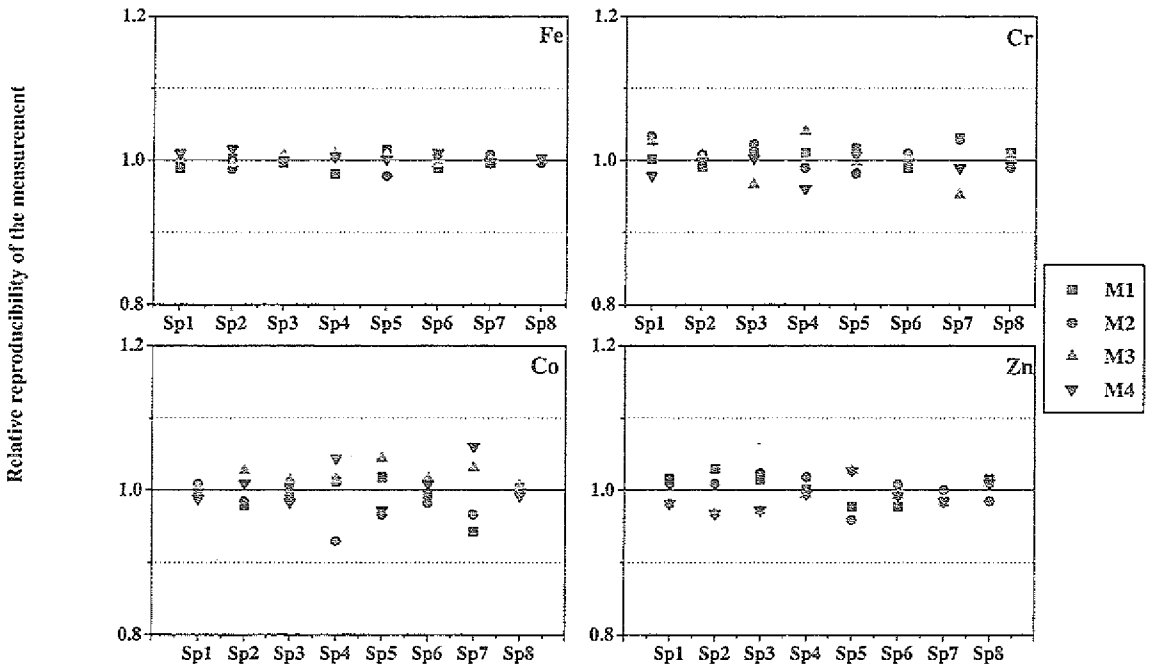



**Fig. 4.6 : Relative reproducibility for Fe, Cr, Co, and Zn determination by INAA in a set of irradiated algae samples, M = measurement (1-4)**

*Reproducibility of INAA measurements*

Fig. 4.6 represents the reproducibility of the measurements for selected elements in a set of irradiated samples from Eckwarderhörne (Sp1-Sp6), and IAEA-0392/0393 (Sp7-Sp8). In all analyzed samples the reproducibility of Fe, Co, and Zn determinations are better than 4 %. Only for Cr determinations is the reproducibility above 4 %.

*Determination of detection limits of ICP-MS, HG-AAS and ICP-AES*

The detection limits of ICP-MS, HG-AAS and ICP-AES were calculated based on Eq. (3-2). Fig. 4.7 shows the calculated detection limits for selected elements of the applied methods. ICP-AES shows less sensitivity for phosphorus and calcium and high sensitivity for Mn determination, as well as higher sensitivity for Se, Cd, and Cu (ng/g level) were obtained by HG-AAS and ICP-MS, respectively.

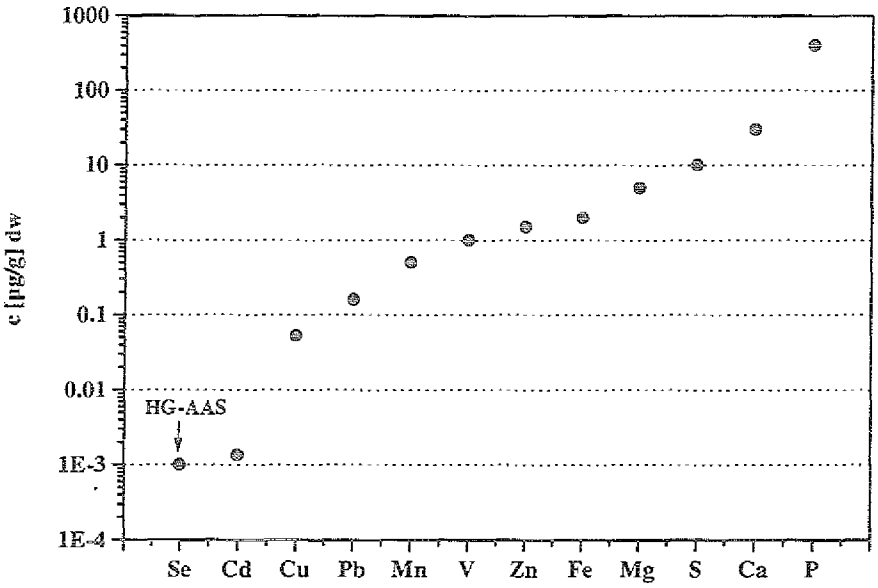



**Fig. 4.7 : Detection limits of Cd, Cu, and Pb (ICP-MS), and Mn, V, Zn, Fe, Mg, S, Ca, and P (ICP-AES) and Se (HG-AAS). n = 4**

*Reproducibility of ICP-MS and ICP-AES measurements*

The measurement reproducibility of As, Cu, Cd, and Pb were determined in a digested set of samples (Sp1-Sp6) from Eckwarderhöne, and in the candidate reference materials IAEA-0392/0393 (Sp7-Sp8). Obtained data are presented in Fig. 4.8 (a-b). Measurement reproducibility for Cu, Cd, Pb, and As determined by ICP-MS is better than 5 %. Measurement reproducibility for Zn, P and Mn, and Fe determined by ICP-AES is better than 6 %. In samples containing silica (IAEA-392/393) a higher spread of measured values of Zn, Mn, and Fe was observed. These elements are the major silicate-bound elements, and the ICP-AES analysis is based on digestion procedures which may partially decompose silicate minerals contained in the samples.

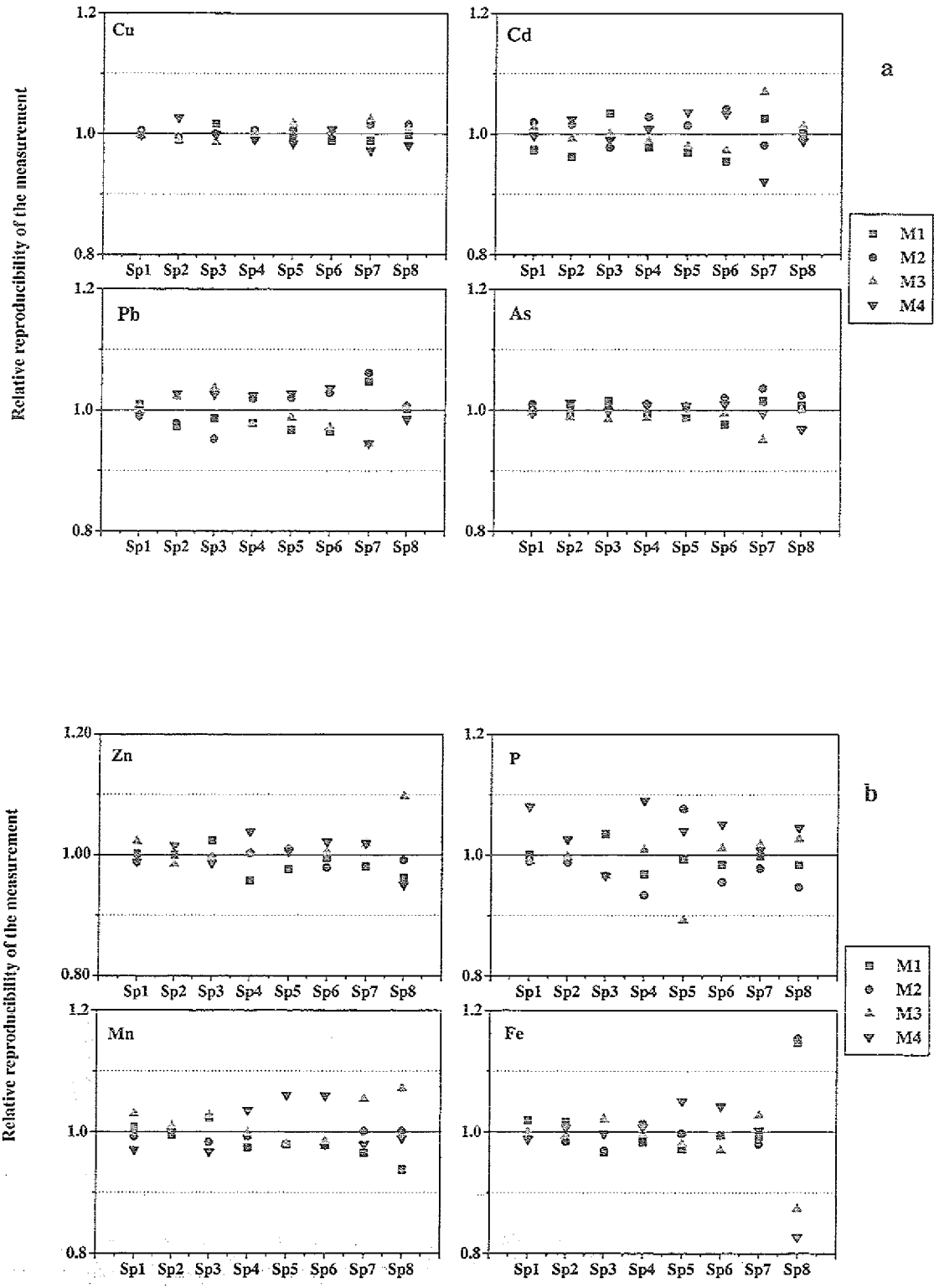



Fig. 4.8 a-b : Relative reproducibility of (a) ICP-MS for Cu, Cd, Pb, and As, and (b) ICP-AES for Zn, P, Mn, and Fe in a digested set of samples. M = measurement (1-4)

### 4.1.3 *Analytical quality control*

The analytical procedures are in statistical control when the results consistently fall within established control limits. The quality control has been done in three steps, first the results of reference materials analyzed are evaluated via the control charts (see Fig. 4.1 and 4.2). Secondly, the results of the duplicate samples have been used to assess the performance of the analytical procedure with respect to sample preparation (see Fig. 4.6 and 4.8). Thirdly, intermethod comparison of the elemental analysis, and the degree of confidence has been checked by drawing a control chart, as shown in Fig. 4.9 (a-d).

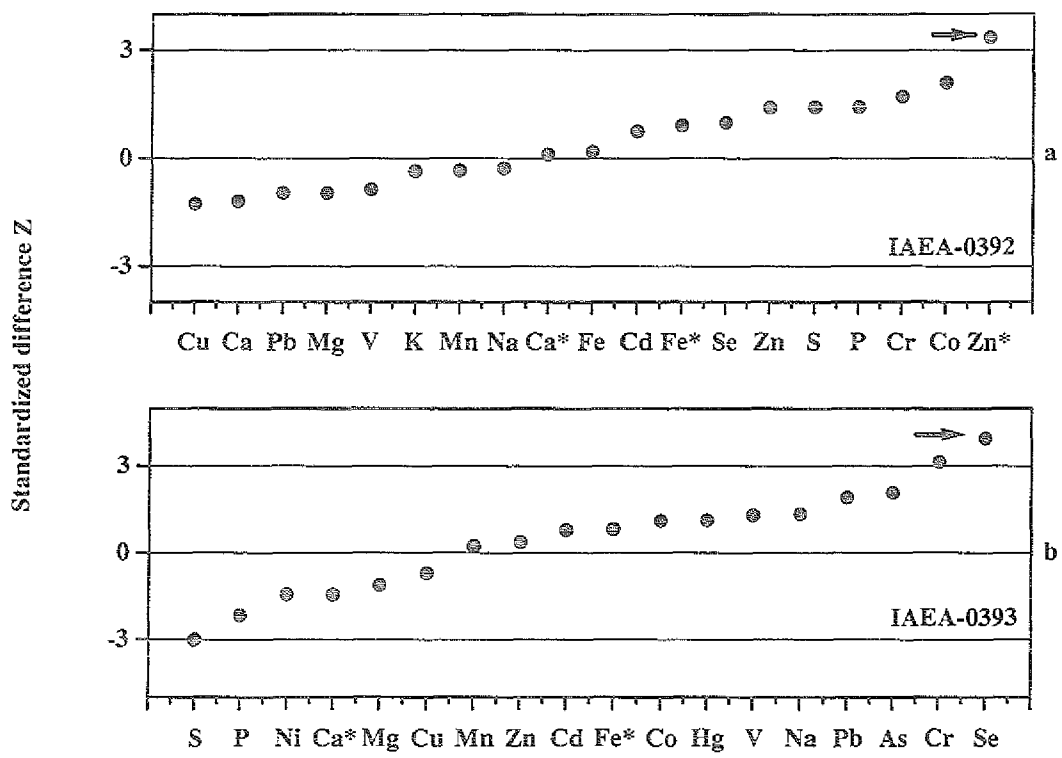



**Fig. 4.9 (a-b) : Control chart for in-house comparison for IAEA-0392/0393. Elements determined by ICP-AES, Fe*, Ca*, Zn*, Mn, Mg, P and S. Elements determined by ICP-MS, V, Cu, Cd and Pb. Elements determined by INAA, Ca, Fe, Se, Zn, Cr, Co, Hg and As.**

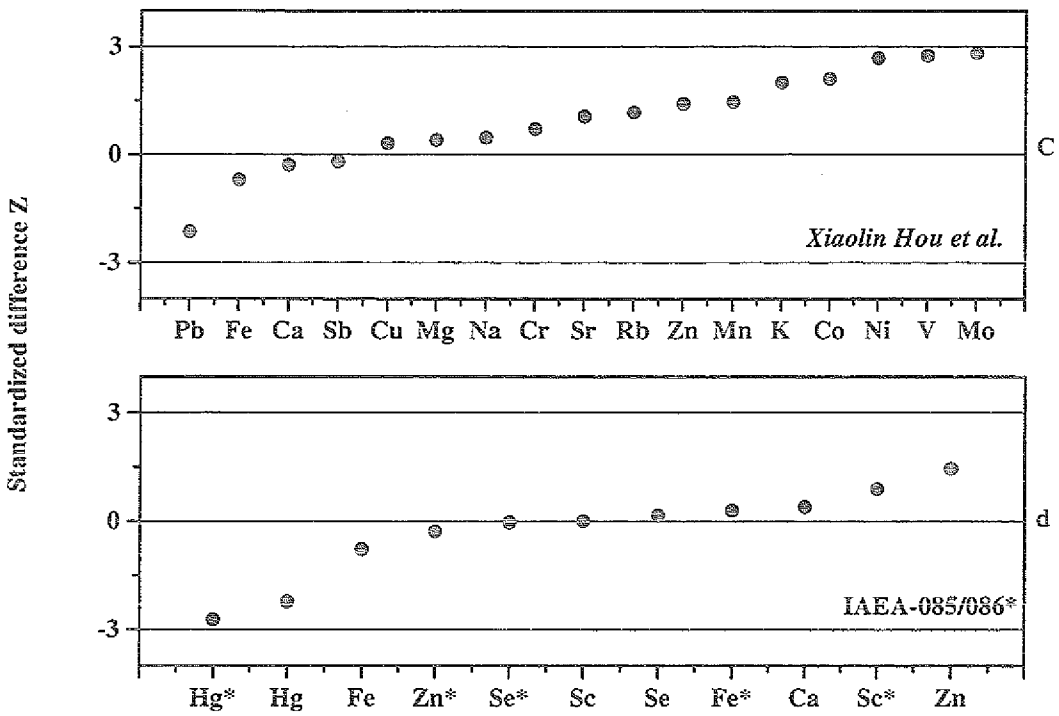



**Fig. 4.9 (c-d) : Control chart for intercomparison of results for IAEA-0392 with Xiaolin Hou et al. [59] and IAEA-085/086, (*) unspiked reference materials**

From Fig. 4.9 (a-b) it is obvious that some incidental deviations for Zn* determination in IAEA-0392 and Se determination in IAEA-0393 samples are outside the limit of the Z-value. The previous control charts assist in obtaining insight into the performance of the INAA technique of for the determination of the respective elements, at a certain concentration level, in a given matrix. For IAEA-0392/0393, the Z-value for the elements Fe*, Ca*, Zn*, Mn, Mg, P, and S correspond to that measured with ICP-AES, and the elements V, Cu, Cd, and Pb were measured by ICP-MS. In Fig. 4.9(c), the reported element concentrations were measured with INAA, Fig. 4.9(d) shows the control chart of an intercomparison of the certified and/or recommended values of the elements, Hg, Fe, Zn, Ca, Se, and Sc. With respect to the presented quality control procedures of all the implemented methods, it can be concluded that the obtained results are reliable within the value of $|Z| < 3$ and can be used for the assessment program of the analysis in this work.

### 4.1.4 *Possible sources of uncertainty*

*Sampling*

Anticipating possible sources of error, the typical sizes of collected algae samples are about 2 kg fresh mass. After cryogenically precrushing the total sample size, but before further homogenization 10 g fresh mass Subsamples are obtained and freeze-dried. To check the real representativeness of the 10 g, the German ESB analytical results of some elements of interest were compared with the results of this work (Fig. 4.10).

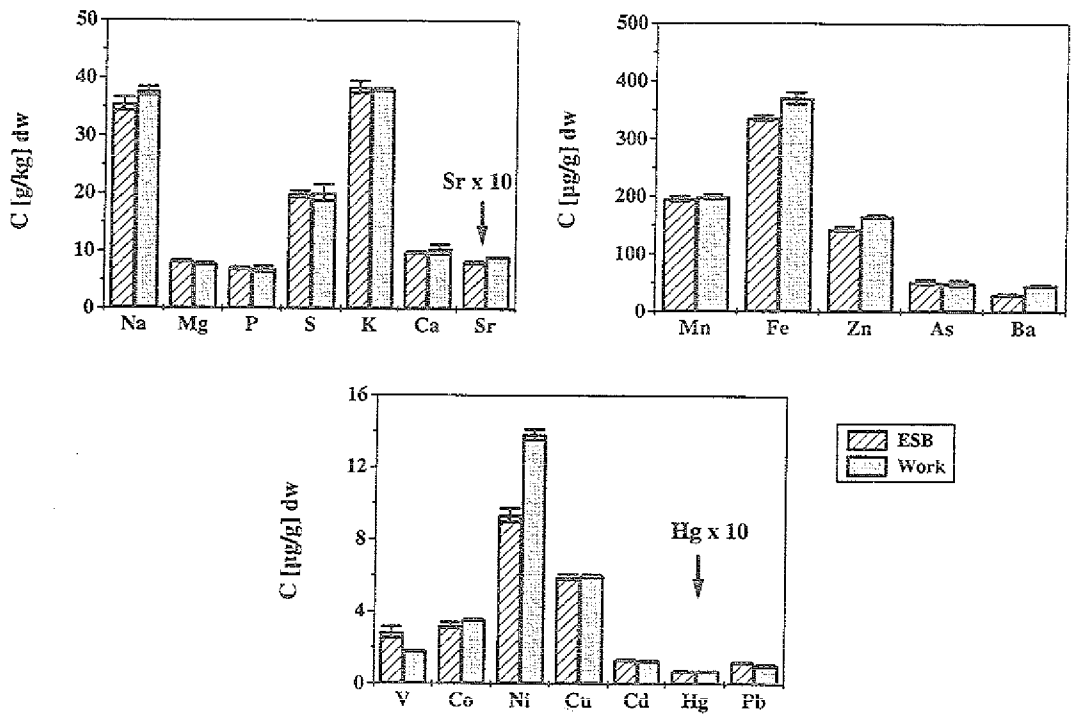



**Fig. 4.10 : Comparison of element concentrations mean value and ± SD determined in this work and in the ESB. n = 4**

Data obtained from samples collected in April 1993 from Eckwarderhörne demonstrate that only for Fe, Zn, Ba, V, Pb and Ni can differences be observed. This effect may be a result of measurements and/or the sample content of the different parts of algae. Also, the results of the German ESB annual homogenate (1993) from Eckwarderhörne were compared with the mean of the bimonthly collected samples, as shown in Fig. 4.11. This comparison demonstrates a very good agreement between the

annual homogenate (1993) of the German ESB from Eckwarderhörne and that calculated. The higher standard deviation in the results of this work represents the influence of seasonality in the environment of collected samples of *Fucus vesiculosus*.

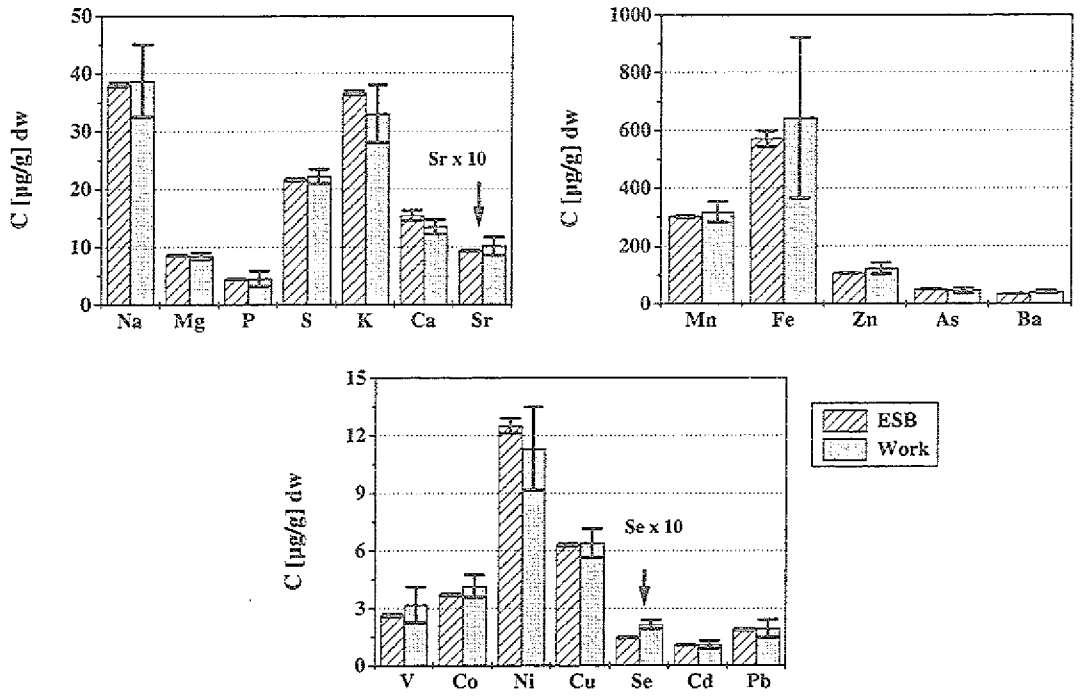



**Fig. 4.11 : Comparison of mean ± SD of the annual homogenate for German ESB from Eckwarderhörne 1993, and the mean results calculated from bimonthly collected samples with the maximum and minimum presented as ± SD (n = 4).**

*Sample processing*

Analysis of duplicate Subsamples from the same material was used as a test of analytical precision within a set of samples. Nevertheless, the uncertainty resulting from sample handling is an important factor and must be considered when monitoring aspects are under consideration. All collected algae samples were processed in an agate mill, therefore the agate material was analyzed to scan the concentration of elemental constitutes for potential contamination. Fig. 4.12 demonstrates the element concentrations found in the agate material used. Assuming that 5g freeze-dried algae material is contaminated with 0.2 g agate ≡ 60 µg Fe, which is a theoretical hypothesis, and the average concentration of Fe in the sample is 600 µg/g, this means that 2 % of the total

concentration is due to thus milling process. So that there is negligible contamination risk from the content of Ba, Ca, Na, and Fe in agate material.

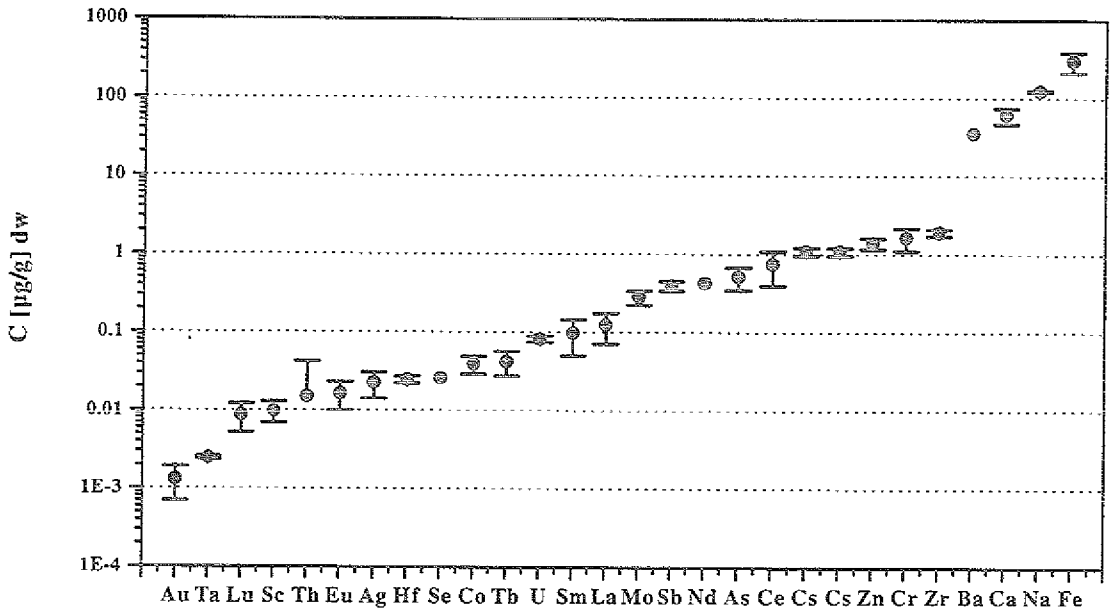
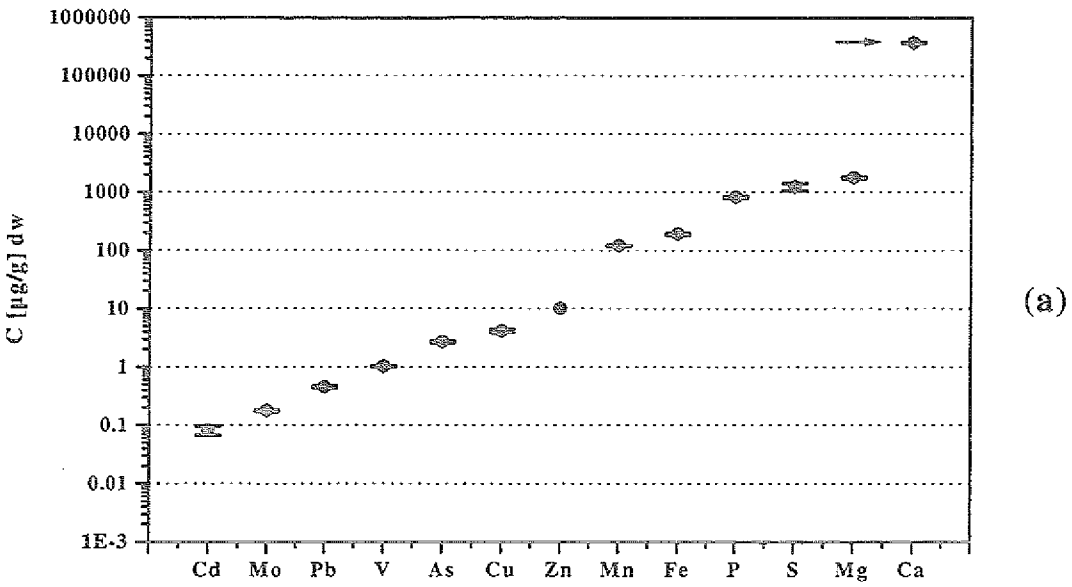



**Fig.4.12 : Concentration of elements determined by INAA in agate material**

***Contamination with other collected specimens***

In the collection of the brown algae *Fucus vesiculosus*, other specimens such as periwinkle (*Littorina littorea*), and grazing herbivorous sand shrimp (*Gammarus locusta*) are found to be present in the samples. Fig 4.13 (a-b) represents the elemental concentrations of both species. Comparing average Ca concentrations in brown algae to that of periwinkle, it was found that there is a high risk of Ca contamination where the average mass of periwinkle found per 10 g of algae sample was 0.1 g i.e. equivalent to 30 % (considering the relative contribution) and contamination also considered to be relative to the collection site. The average mass of sand shrimp is 0.09 g per 10 g of algae sample. Because of the low concentration level there is no risk of contamination from sand shrimp material to algae material.



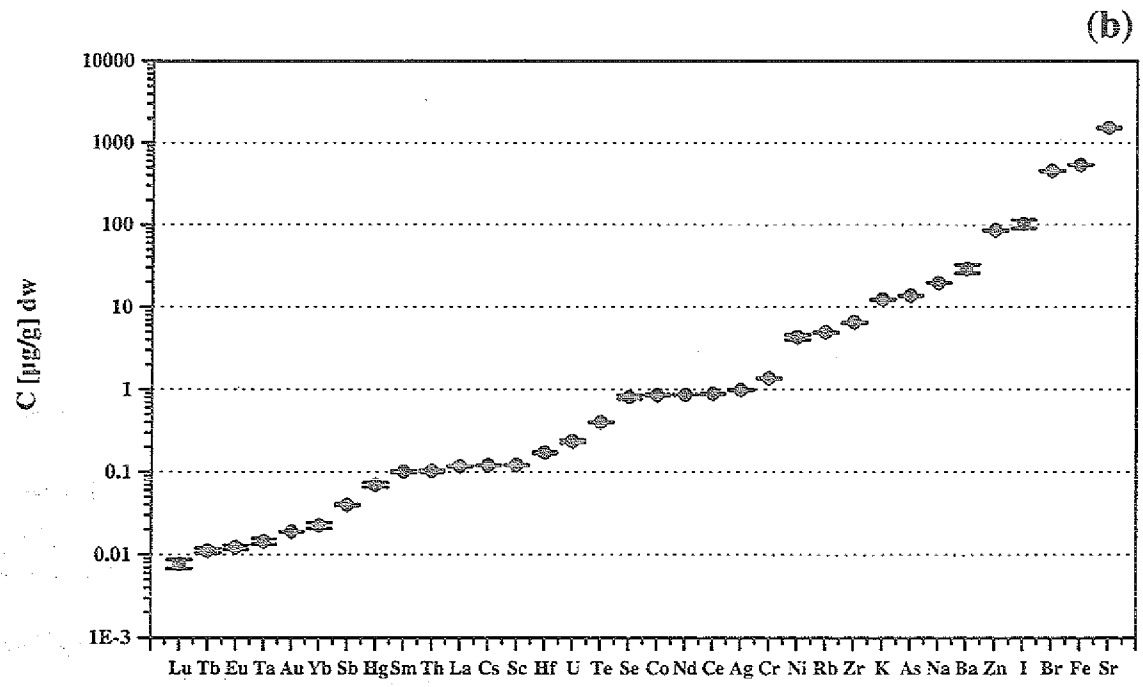




Fig. 4.13 (a-b) : Element concentrations found in (a) periwinkle (*Littorina littorea*) and (b) sand shrimp (*Gammarus locusta*)

*Contamination with particulate matter and fine sediment*

Particulate matter is a very important source of contamination. Juracic et al. [60] reported that particulate matter was found to have a large specific surface area of up to 20 $m^2g^{-1}$ in the estuarine sample. Higher levels of trace metals were usually detected in suspended matter samples, (40 ppm Ni, 200 ppm Cr, 60 ppm Pb, 100 ppm Cu, 320 ppm Zn, and 0.9 ppm Cd) than in sediment. Also, heavy metals are preferentially incorporated into suspended particles [61, 62], and the reported data indicate that more than 85 % of Pb, Cu, and Cr is associated with suspended particles. Forsberg et al. [14] reported considerably higher concentrations of Al, Fe, Cr, and V particularly in samples of *Fucus vesiculosus* with epiphytes. Therefore, careful sampling has to be performed to avoid damaged areas (receptacles) substantially affected by epiphytes, and one needs information on the contribution of particulate contamination to element concentration levels in *Fucus vesiculosus* in addition to the uncertainty of the other procedures. Otherwise, there will be no usable informative data on the algae and their respective sites. In this work, this problem was recognized throughout the course of statistic analysis, and will thus be considered in the conclusion.

## 4.2 *Parameters influencing element concentrations in brown algae*

A prerequisite in using an organism as a biological indicator is to acquire specimens that characterize the status of the sampling environment. Algae are responsive to the soluble trace element content of their ambient surroundings. They do not, therefore, reflect total element loads, as they do not respond to elements associated with organic or inorganic particulate matter. Several sources of variation in element concentrations of macroalgae have been identified [2, 14]. *Fucus vesiculosus* exhibits a vegetation cycle in growth and reproduction. It has the highest growth rate during the summer, June - August, and growth ceases in late from October to early spring.

### 4.2.1 *Seasonal variation*

Seasonal changes of metal concentrations in algae have been noted by several workers [8, 18, 23, 32, 29, 63-70]. In general, maximum concentrations of some elements (Zn, Cd, Cu, Fe, and Co) were found in spring and minimum concentrations in autumn [18]. It is suggested that this profile may be due to the effect of the algae growth on the content of element concentrations. As pointed out by Lunde [71], ash contents tend to be higher in the spring than the autumn, and this may partially be accounted for some seasonal variations of trace element concentrations. It is probable that concentrations of elements in macroalgae increase in the winter months for low growth rate and are then progressively diluted by the new growth of the plant in spring through summer. To investigate the seasonal variation of trace element concentrations in *Fucus vesiculosus*, representative samples from the North Sea coast were collected in differently characterized sites, Eckwarderhörne (Weser estuary), Cuxhaven (Elbe estuary), and Sylt-List (less polluted) at bimonthly intervals for two years (see map). From each site, three parts of 50 plants; tip, thallus, and basal were dissected.

Algal surfaces reflect dynamic equilibrium processes with the surrounding water, based in particular on ion-exchange equilibria. Therefore, they should be a good biological indicator for all elements occurring in ionic form in the aquatic environment. To study the effect of sampling time on element concentrations, all obtained data were processed with SAS/INSIGHT Software, using the *General Linear Models Procedure* for the computation process. In the model, the F-value was taken as the ratio between the mean square for the model and the mean square for error. It tests how well the model as a whole (adjusted for the mean) accounts for the element concentrations behavior with respect to the model. The higher the F-value, the stronger the evidence for the existence of element variation under the considered factors. The factors are the effect of the collection site, the sampled month, the year of sampling, and the combination effect adjusted for every other effect.

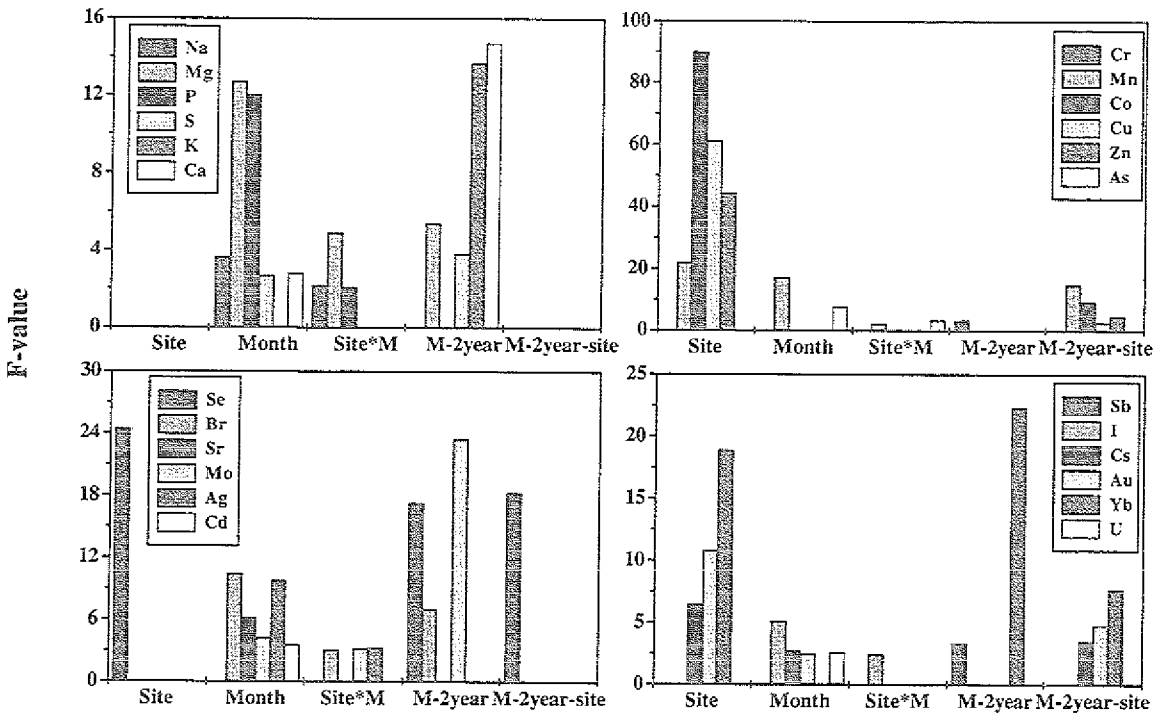


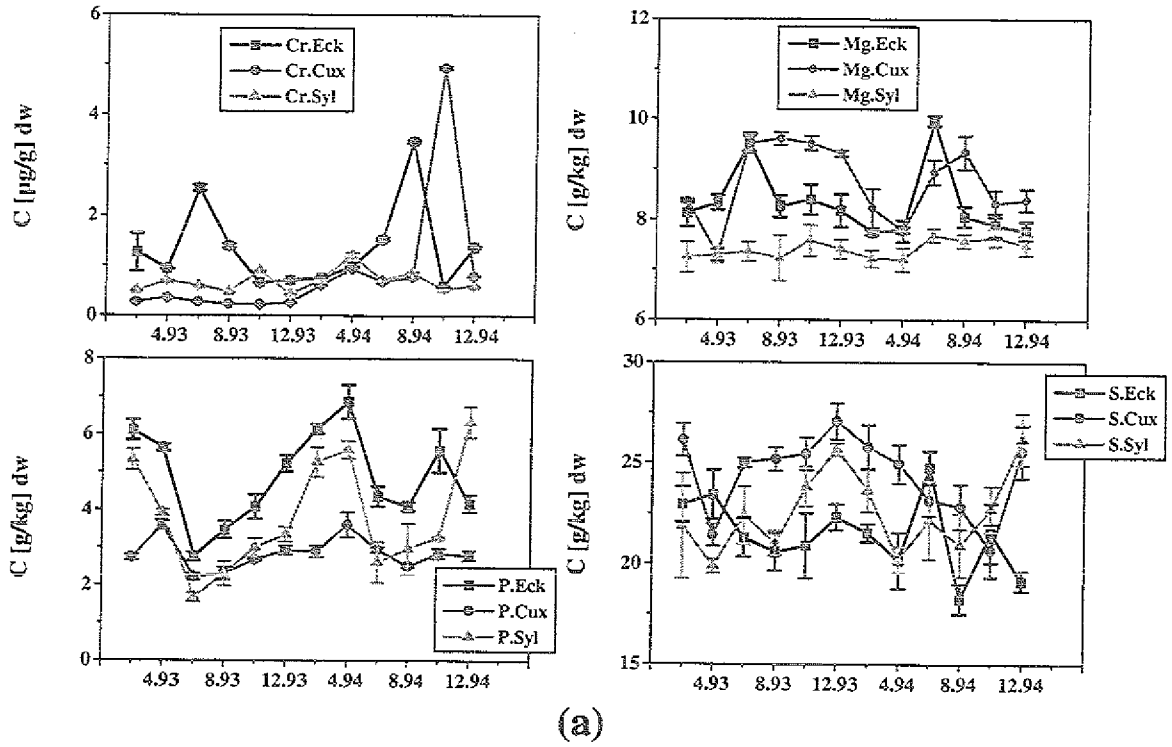

**Fig. 4.14 : The corresponding element variations of different combination effects of Site = Eckwaderhörne - Cuxhaven - Sylt-List, M = Month, and M-2year = bimonthly collection from Feb.93-Dec.94**

Results obtained by this procedure demonstrate that the sampling time and the sampling site have more influence on the element concentrations than the combination of other effects (Fig. 4.14). Concentrations of some elements have significantly different levels for the different collection time (month in one year of sampling and/or month in two years of sampling). The variation of concentrations for some selected elements as function of sampling time is presented in Fig. 4.15 (a-d). It is obvious that in the algae:

- Concentrations of the following elements are strongly dependent on the sampling time of year within the three collection sites : Mn > Mg > P > Br > Ag > As > Sr ≈ I > Mo ≈ Na > Cd > Ca ≈ S > Cs ≈ U > Au.
- Mn shows the highest seasonal variation of concentrations in a year. A similar effect was observed by Bryan & Hummerstone [8] and Carlson & Erlandsson [82], and Frazier [72]

reported that Mn and Fe dynamics are closely related. In contrast to the obtained results, Rönnberg et al. [64] reported marked seasonal variation of Mn with lower values in summer and higher values in winter, and described the uptake kinetics of Mn as possibly actively and/or metabolically regulated. Munda & Hudnik [22] suggested that Mn concentrations in algal tissues are the main distinguishing characteristic between taxonomic groups as well as in seasonal- and habitat-conditioned variations.

- Concentrations of As and Ag in algal tissues correlated significantly with concentrations in sediment [36], where seasonal variation in dissolved As inputs is reflected in As content in *Fucus vesiculosus* [63], while the removal of dissolved As is often dominated by adsorption onto Fe and to lesser extent on Mn oxyhydroxides specially in marine estuaries [73]. Also, Langston [74] and Luoma et al. [36] have reported that the correlation between As in sediment and *F.vesiculosus* may therefore be a function of the sediment's control of solute concentration rather than the direct availability of particulate As to seaweed. On the other hand, arsenate accumulation in brown algae is to some extent linked with energy-dependent metabolic processes and demonstrates the ways in which environmental parameters may affect uptake [19]. This could prove that elevated As content in algae has a different source than the dissolved As fraction in water.
- As pointed out by Munda [75], during May to July the receptacles are mature and accumulate water and $Cl^-$ and $Cl^-$-linked monovalent cations $Na^+$ and $K^+$, but this accumulation mode may be shifted depending on the vegetation cycle of *Fucus vesiculosus* in the North Sea coastal water.
- Comparisons between the samples collected in the same month but in different years have demonstrated that in this case the concentration of the following elements changes significantly; Mo > Yb > Se > Ca > K > Br > Mg > S > Cr > Sb.
- Concentration of Br, Mg, and S in *Fucus vesiculosus* depends not only on the sampling month in the year but also changes from one year to another.



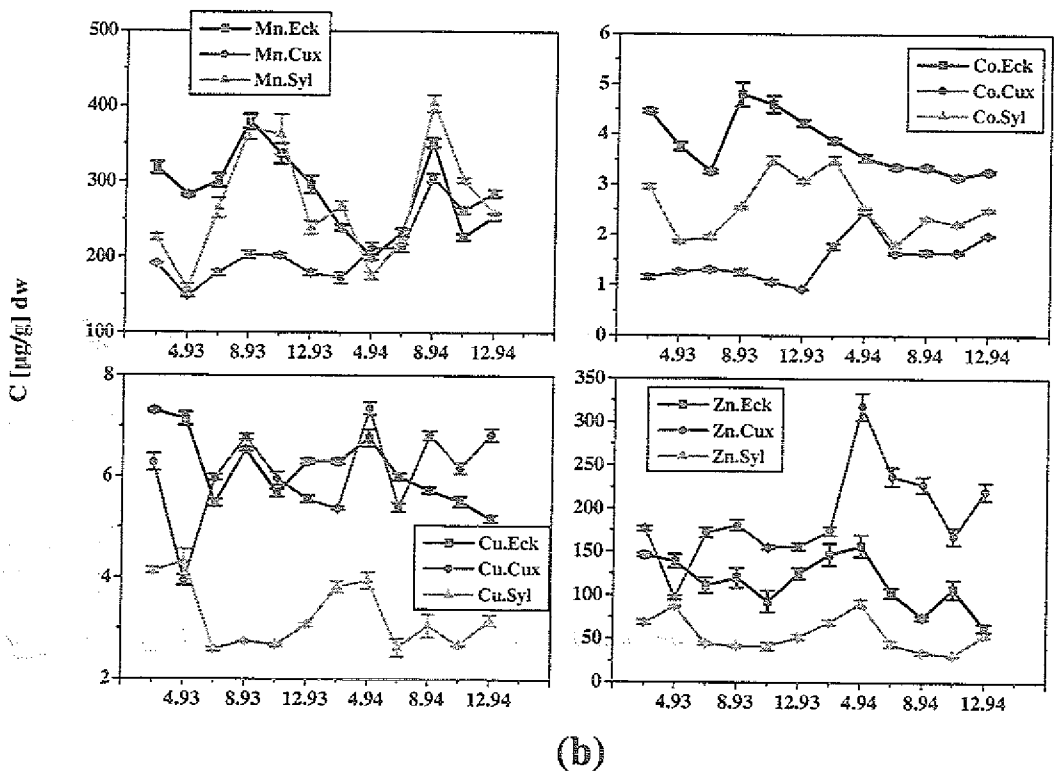
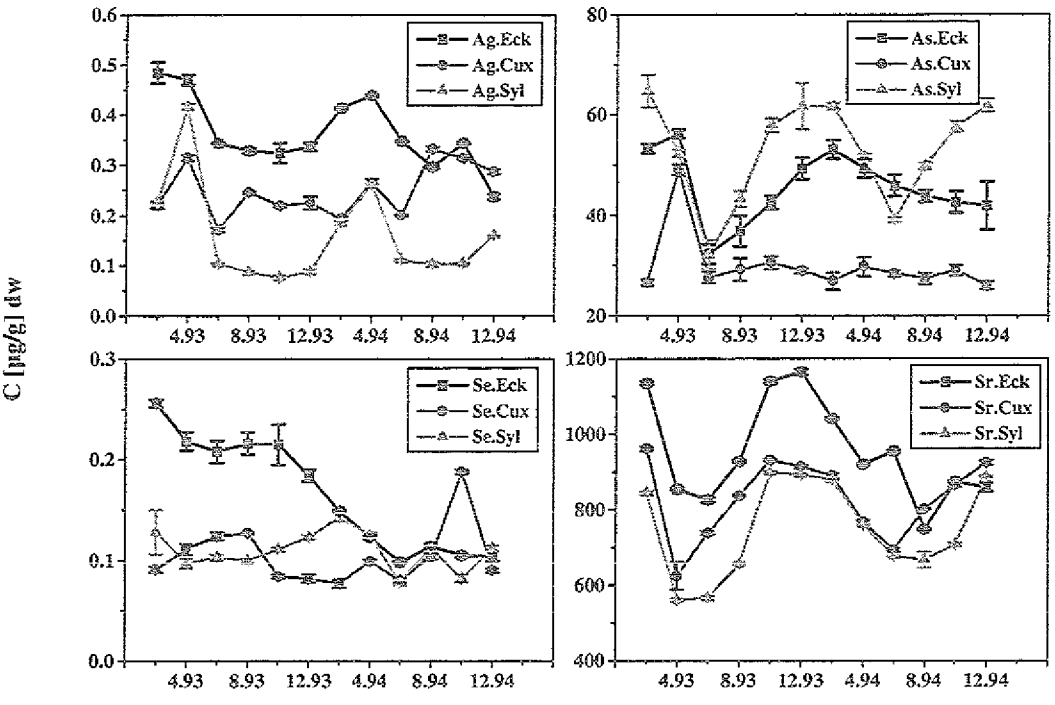
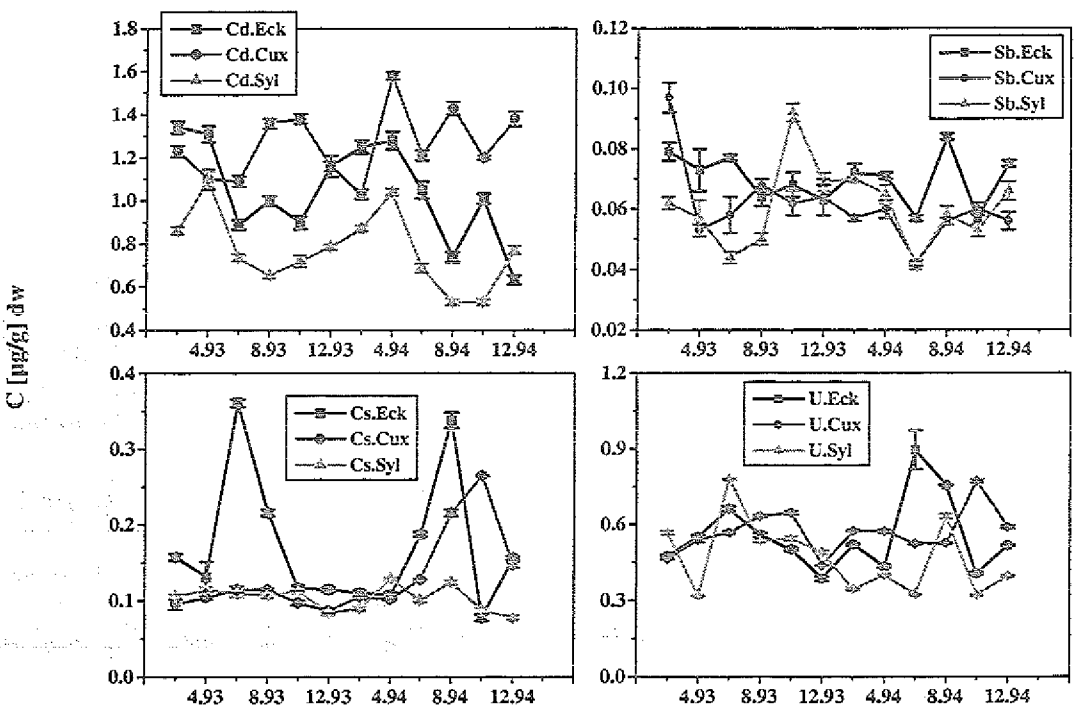




Fig. 4.15 (a-d) : Seasonal variation of some selected element concentrations in Cuxhaven, Eckwarderhörne, and Sylt-List


C [µg/g] dw
Ag.Eck
Ag.Cux
Ag.Syl
As.Eck
As.Cux
As.Syl
Se.Eck
Se.Cux
Se.Syl
Sr.Eck
Sr.Cux
Sr.Syl
4.93
8.93
12.93
4.94
8.94
12.94

(c)


C [µg/g] dw
Cd.Eck
Cd.Cux
Cd.Syl
Sb.Eck
Sb.Cux
Sb.Syl
Cs.Eck
Cs.Cux
Cs.Syl
U.Eck
U.Cux
U.Syl
4.93
8.93
12.93
4.94
8.94
12.94

(d)

- In the case of Cs, the accumulation patterns indicate summer maxima and autumn and winter minima, which is not in agreement with data reported by Carlson and Erlandsson [28]. These differences can be explained by different salinity in collection areas. They reported that the uptake of $^{137}Cs$ increased with decreasing salinity, and $^{137}Cs$ in algae and seawater from the same localities, had approximately 2.5 and 4 times higher in radioactivity concentrations at 8 % salinity relative to 15 % and 24 %, respectively. Salinity is a complex variable and does not only affect the physico-chemical characteristics of the elements but also the biological changes in the organisms, and the estuarine environments are especially complicated, e.g. great temporal and spatial variations in salinity. Therefore, element concentration levels were considered under all integrated environmental parameters with respect to the collection sites.

From the data presented in Fig. 4.15(a-d), it is obvious that the variation of element concentrations depends not only on sampling time but also on other factors. However, these fluctuations were elemental and regionally dependent.

- *Fucus vesiculosus* has its main period of P and N uptake in autumn and winter, which is used mainly in the production of reproductive tissues in spring - early summer [76]. The sharp decrease in P concentration which followed each concentration peak occurred during the periods of active growth facilitated by the availability of nutrients whose concentrations was previously low in agreement with Walsh [77] and Walsh & Hunter [66]. The similar seasonal trends of Cd and P concentration imply that Cd is directly involved in the mechanism regulating P in the algae (Fig. 4.16) with a noticeable shift in Cuxhaven. Only in the case of Mg, Mn, As, Sr and P were similar tendencies observed. The proportion of different algal parts varies as the algae grow, and this may be the reason for seasonal variation. As has been reported by Carlson [78] *Fucus vesiculosus* exhibited the highest growth rate during June and August. The mean length of vegetative tips increased almost linearly with time from May to September.

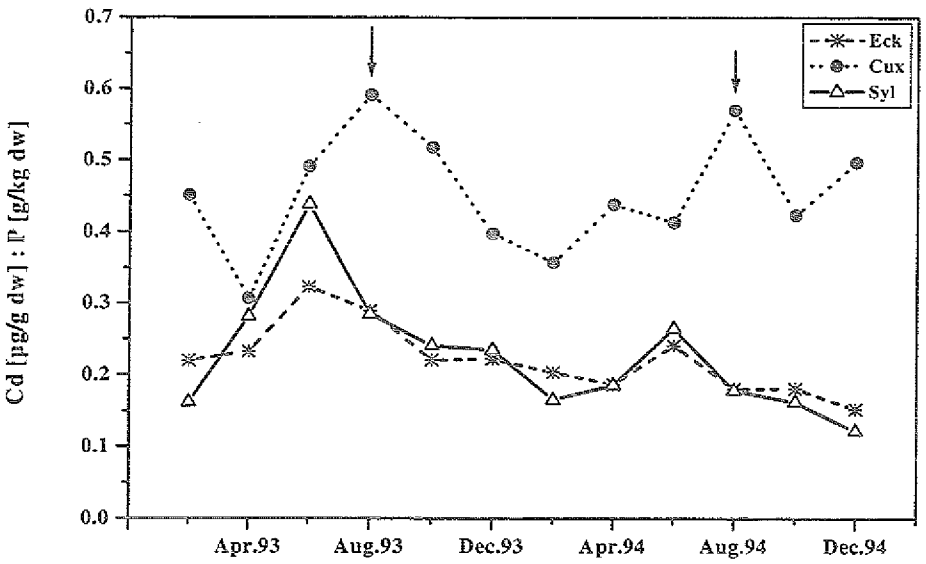



**Fig. 4.16 : Cd : P ratios of *F. vesiculosus* sampled from Eckwarderhörne, Cuxhaven, and Sylt-List.**

At the end of the year the new growth constituted over 80 % of the total plant biomass. Munda and Hudnik [22] concluded that seasonal variations of Zn were less pronounced, and a slight increase was observed from spring to autumn, in agreement with 1993 and in contrast to 1994 as shown in Fig. (4.15 b). Morris and Bale [79] concluded that Cd, Cu, and Zn in *Fucus vesiculosus* are accumulated passively, but in the case of Mn the accumulation process appears to be partially regulated. The concentration of Cu in algal tissue was about tenfold that of Cd [22], corresponding approximately to the relation of their levels in the sea water [80]. It was found that these ratios for Eckwarderhörne, Cuxhaven and Sylt-List were 4.17, 4.77, and 5.77 respectively for two years. At the three collection sites, the order of accumulation found for the concentration of Mn > Zn > Cu > Cd consisted with what has been noted by Bowen [81]. Frazier [72] reported that Zn and Cu dynamics are closely related and Foster [82] recommended *Fcucs vesiculosus* as a potential biological indicator for Cu, but it is more sensitive to increases in the mean ambient dissolved concentration of Zn. Murray and Meinke [83] demonstrated reduced adsorption for Co and Cd as well as increased adsorption of Zn in the presence of organic matter. This might be a reason for the comparability of Co and Cd levels in Eckwarderhöne and Sylt-List, and the presence of a high level

of Zn in Cuxhaven. Also, the effect of salinity interrelates with that of pH in determining metal solubility [83]. As in the case of Eckwarderhörne and Cuxhaven, Cd seasonal variation was slight. The reason is that in water with high Zn content, the Zn ions may compete with Cd, and thus inhibit Cd adsorption [84]. However, *F.vesiculosus* in water polluted by Zn contained large amounts of both Zn and Cd [85], and Price and Morel [86] suggested that in Zn- deficient waters $Cd^{2+}$ may substitute for $Zn^{2+}$ and thus become essential to growth. Also, the ion selectivity of the polysaccharides determined the nature of the accumulated ions [17]. Brown algae contain ion-binding substances with higher affinity for $Zn^{2+}$ than other cations [20]. Bryan [3] suggested that Zn in the Fucus plant is an irreversible accumulation rather than an established equilibrium between the plant and surrounding water. However, higher Zn and Mn concentration at lower salinity may probably suppress Cd uptake [9]. Also, the uptake of Co is slower than that of Mn and Zn, and decreases with increasing salinity [28]. In Cuxhaven Zn accumulation into algal tissues is enhanced in low salinity compared to Mn and Co with respect to Eckwarderhörne and Sylt-List and this disagrees (regarding Mn and Co) with Munda and Hudnik [13]. The ratio of Mn : Zn concentrations in *Fucus vesiculosus* is about 1.2 (Cuxhaven), 2.7 (Eckwarderhörne) and 5.9 (Sylt-List), indicating the domination of Mn over Zn in *Fucus vesiculosus* in agreement with Munda and Hudnik [22] who found that the Mn : Zn concentration in *Fucus virsoides* is about 3.45. It seems likely, however, that *Fucus vesiculosus* has a higher requirement for Mn than Zn, bearing in mind that the Zn, Mn, and also Cd concentration in marine algae are results of irreversible accumulation rather than an equilibrium between the plants and their surrounding water not like Cu which is apparently accumulated by different, merely physico-chemical mechanisms. Miramand and Bentley [67] reported a pronounced seasonal pattern of Zn and Cd in *Fucus serratus*. Higgins and Mackey [87] and also Manley [21] have proven that a high proportion of the *Apparent Free Space* (AFS) included bound Zn and Cd (90 - 60 %) in the cell wall and intercellular spaces, whereas it was less for Cu. The ionic selectivity of cell walls in Atlantic brown algae (Fucoids) showed that alginates have low affinity for Zn, though it is accumulated in high concentrations in brown algae [3, 18, 20]

which in disagreement with Lignell et al. [38], Pedersen [10], and Munda and Hudnik [13]. Also, the uptake kinetics for Zn was found to be active aganist intracellular concentration gradients in the brown algae *Ascophyllum nodosum* [6]. Rice and Lapointe [88] suggested a metabolic regulation of the Mn content as a result of increasing concentration with increasing growth rate in green algae *Ulva fasciata*. It is known that Zn, Mn and Cu are all essential elements, therefore the uptake kinetics may be active and/or the metals are metabolically regulated in the vegetation cycle of the algae. Eide et al. [6] realized that the uptake kinetics for Zn was found to be active to intracellular concentration gradients in the brown algae *Ascophyllum nodosum*. Data presented in Fig. 4.14 (a,b) and 4.15 allow the conclusion that for these elements the growth rate is the influencing parameter, as well as the metal concentration in water, seasonal changes, position of seaweed and particular portion of plant analyzed [8]. Bearing in mind that the data will not be particularly informative in regions where dissolved concentration levels fluctuate widely or rapidly, e.g. Cuxhaven, this confirms what has been suggested by Bryan [3]. For all other elements different parameters acted together. Rönnberg et al. [65] reported seasonal changes for the phosphorus concentration in *Fucus vesiculosus*. They found that the content of N and P in the tip showed significant seasonal variations with low levels in summer and high levels in winter. The observed concentration level for P was similar to that presented in this work. Martin et al. [70] calculated a two-monthly variation using the mean of the data for the period from 1990 to 1995 when consistent monthly samples were taken. The results obtained show significant but relatively small annual changes in the mean concentration of Cd, Cu, and Zn with minima in summer and maxima in winter. The maximum concentration for the investigated elements was observed in April regarding all sampling sites. Mg and P in Fig. 4.15(a) showed variation with respect to bimonthly sampling time and the collection sites, more effective variation caused by two years of sampling was found for S and Mg. The variation in Mn is the sum of the dependency on the sampling time and the collection sites, whether bimonthly sampling and/or in two years with maximum concentration in August and minimum in April. The variation of Co, Cu, and Zn does not only depend on the sampling months or collection sites but

also on the sampling year. Miramand and Bentley [67] showed that the concentration of Cr varied by a factor of 8 in *Fucucs serratus*, and in Eckwarderhörne, Cuxhaven, and Sylt-List were found to be 5.9, 22.2, and 2.83 respectively. Söderlund et al. [89] have suggested that Cr binds chiefly to the plant cell walls, and the potential contribution of particulate matter for Cr is about 35 %, [90]. In general, the particulate contribution for Cd and Zn is always low, whereas for Cr it is consistently high at about 63 % [91]. Also, in Fig. 4.15(c), variations in As concentrations are due to the effect of bimonthly sampling, but the response differed when the collection sites were considered as added effects. Se shows the influence of sampling time for 1993 and 1994 as well as the collection sites on concentration levels. It was found that the Se level had a decreasing tendency in Eckwarderhörne (Feb. 93 - Dec.94). Variations of Sr, Cd, and U are monthly, yearly, and collection-site dependent. The concentration of Ag varies with respect to the collection sites, but not effectually within bimonthly sampling. Correlated variation patterns were found between Cu, Zn, Cd, and Ag in Eckwarderhörne and Sylt-List, as well as Cs and Cr in Eckwarderhörne and similarity in the variation of Mn at the three collection sites. Two years' sampling time was needed to notice any observable variation for Sb.

### 4.2.2 *Influence of sampling area*

Many authors reported the variation of element concentrations in *Fucus vesiculosus* according to the sampling area [7, 8, 18, 33, 40, 41, 69, 70, 92]. From the data presented in Figures (4.14) and (4.15) it is obvious that the sampling area has an important influence on element concentration in the algae. Based on the bimonthly concentrations of some selected elements from Eckwarderhörne, Cuxhaven, and Sylt-List the mean values for two years were calculated. Concentrations of Sr, Ag, Sb, Cr are higher in Eckwarderhörne > Cuxhaven > Sylt-List (Fig. 4.16). Higher concentrations of P, Co, Se were found in Eckwarderhörne, Zn, Mg, S, Cu, and U in Cuxhaven, and As in Sylt-List. Data presented in Fig. 4.14 indicate that the order of the following element concentrations of Co > Cu > Zn > Mn > Yb > Au > Cs are strongly dependent on the sampling site if the sampling time is

not included in the calculation. If the sampling time in the year is coupled with sampling site, the elements are altered in the following order: Mg > Ag > As > Mo > Br > I > Mn ≈ Na ≈ P. If the sampling year and sampling site are coupled the concentration of the following elements change: Se > Mn > Yb > Co > Zn > Au > Cs > Cu. Only Mn is presents in all three groups of elements. In Fig. 4.17 (a-b), the central line in each box represents the median (50 %) and the edges mark the first and the third quartiles (75 % and 25 %), as well as the mean concentration value. Therefore, box plots provide a concise display of element distributions and a measure of the tendency of the deviation from the mean to be larger in one direction than the other. In some of the box plots the mean values will lie on the central line or in one of the quartiles, where some of the overall high and/or very low concentration values will also cause elongation in the extended line through the box plots to the maximum or minimum value. Such cases were found for the collection sites as follows;

**Eckwarderhörne** : **Mg** (Jun. 93 and Jun. 94), **Cr** (Aug. 94), **Cs** (Jun. 93 and Aug. 94), and **U** (Jun. 94).

**Cuxhaven** : **P** (Apr. 93 and Apr. 94), **Cr** (Aug. 94), **Zn** (Apr. 94), **As** (Apr. 93), **Se** (Oct. 94), **Sb** (Feb. 93 and Jun. 94), **Cs** (Aug. 94 and Oct. 94), and **U** (Oct. 94).

**Sylt-List** : **Ag** (Apr. 93) and **Sb** (Oct. 93).

Table 4.1 (a-c) describes the pictorial representation of the distribution of element concentrations influenced by the collection sites as well as the variation factor.

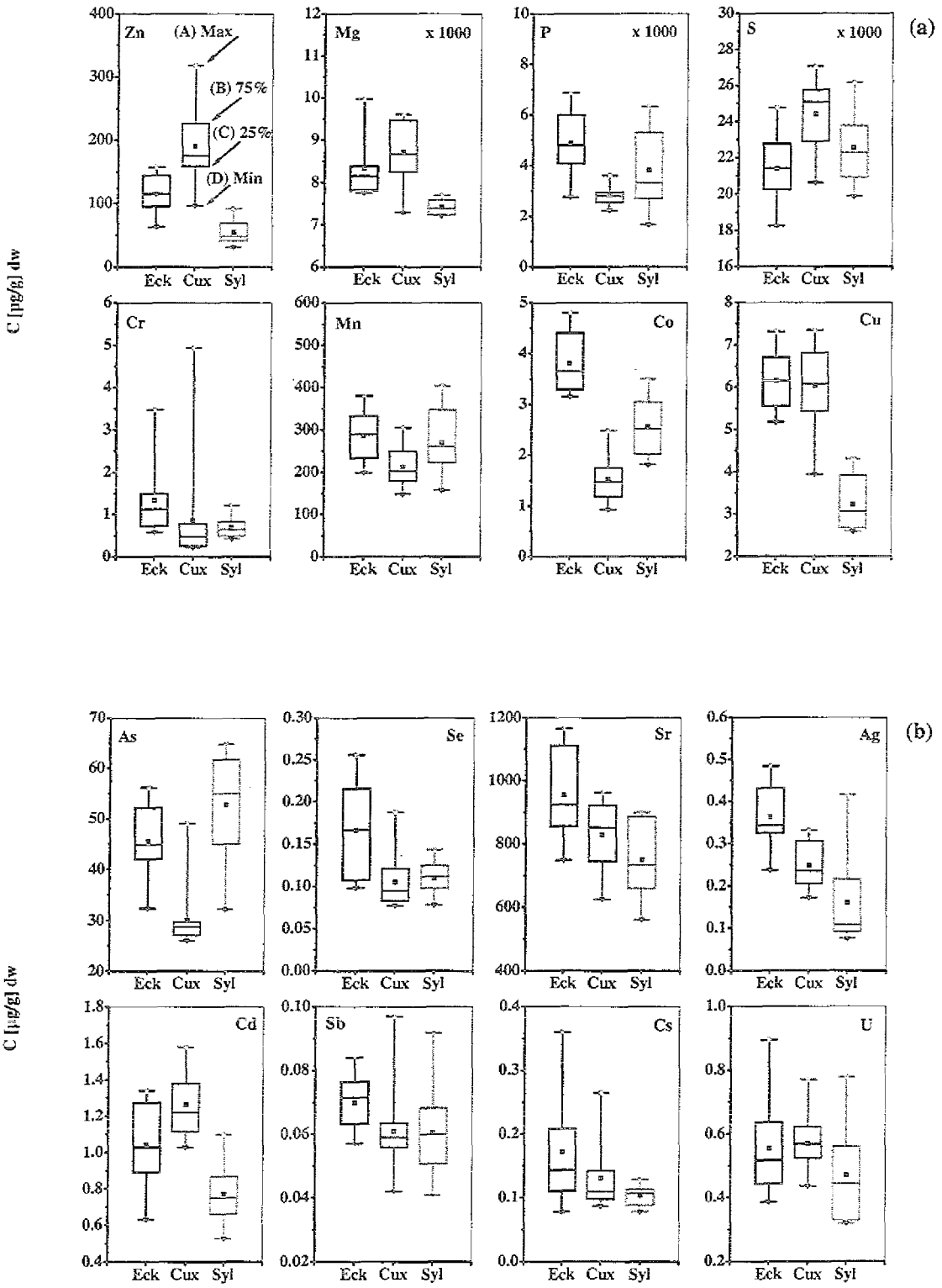



Fig. 4.17 (a-b) : Box chart of element concentrations in two years' sampling time from Eckwarderhörne, Cuxhaven, and Sylt-List.

Table 4.1 (a ) : Distribution of element concentrations in Cuxhaven for two years of sampling.

| Element | Year | February | April | June | August | October | December | Variation factor |
|---|---|---|---|---|---|---|---|---|
| Mg | 93 | ** | * | **** | **** | ***** | ** | 1.32 |
| | 94 | * | * | *** | *** | ** | *** | 1.20 |
| P | 93 | ** | **** | * | * | ** | *** | 1.62 |
| | 94 | *** | **** | *** | * | *** | **** | 1.44 |
| S | 93 | **** | * | ** | *** | *** | **** | 1.26 |
| | 94 | **** | ** | ** | * | * | **** | 1.25 |
| Cr | 93 | ** | ** | ** | * | * | * | 1.55 |
| | 94 | *** | **** | *** | *** | H | ** | 8.19 |
| Mn | 93 | ** | * | ** | *** | ** | ** | 1.37 |
| | 94 | * | *** | *** | **** | **** | ** | 1.77 |
| Co | 93 | * | ** | ** | ** | * | **** | 1.42 |
| | 94 | **** | **** | *** | *** | *** | ** | 1.51 |
| Cu | 93 | *** | * | ** | *** | ** | ** | 1.72 |
| | 94 | * | **** | * | **** | *** | *** | 1.37 |
| Zn | 93 | *** | * | ** | *** | * | *** | 1.87 |
| | 94 | ** | H | **** | **** | ** | *** | 1.89 |
| As | 93 | * | H | ** | *** | **** | *** | 1.85 |
| | 94 | * | **** | ** | ** | *** | **** | 1.15 |
| Se | 93 | ** | *** | **** | **** | ** | *** | 1.55 |
| | 94 | * | *** | * | *** | H | *** | 2.44 |
| Sr | 93 | **** | * | * | ** | **** | **** | 1.83 |
| | 94 | *** | ** | * | ** | *** | **** | 1.33 |
| Ag | 93 | ** | **** | * | *** | ** | * | 1.83 |
| | 94 | * | *** | * | **** | **** | *** | 1.71 |
| Cd | 93 | *** | * | * | *** | **** | *** | 1.27 |
| | 94 | * | **** | ** | **** | ** | *** | 1.53 |
| Sb | 93 | H | * | ** | **** | *** | **** | 1.83 |
| | 94 | ** | *** | L | ** | *** | *** | 1.43 |
| Cs | 93 | * | ** | *** | *** | * | * | 1.32 |
| | 94 | ** | ** | *** | H | H | * | 3.31 |
| Hg | 93 | **** | H | * | * | **** | *** | 2.40 |
| | 94 | ** | *** | *** | ** | ** | ** | 1.33 |
| U | 93 | * | ** | ** | **** | **** | *** | 1.48 |
| | 94 | *** | *** | * | ** | H | ** | 1.47 |

Each of the (* - intervals) represents a percentage of the maximum concentration

* min. - 25 %

** 25 - 50 %

*** 50 - 75 %

**** 75 - max.

L low

H high

Variation factor = maximum concentration divided by minimum concentration

Table 4.1 (b) : Distribution of element concentrations in Eckwarderhörne for two years of sampling.

| Element | Year | February | April | June | August | October | December | Variation factor |
|---|---|---|---|---|---|---|---|---|
| Mg | 93 | ** | *** | H | *** | **** | ** | 1.17 |
| | 94 | * | * | H | ** | ** | *** | 1.29 |
| P | 93 | **** | *** | * | ** | * | *** | 2.21 |
| | 94 | **** | **** | ** | * | *** | **** | 1.68 |
| S | 93 | **** | **** | ** | ** | ** | **** | 1.14 |
| | 94 | *** | * | **** | * | *** | **** | 1.36 |
| Cr | 93 | *** | ** | **** | *** | * | * | 3.87 |
| | 94 | ** | ** | **** | H | * | ** | 5.92 |
| Mn | 93 | *** | ** | *** | **** | **** | ** | 1.35 |
| | 94 | ** | * | * | **** | * | ** | 1.76 |
| Co | 93 | **** | *** | * | **** | **** | **** | 1.47 |
| | 94 | *** | ** | ** | ** | * | ** | 1.23 |
| Cu | 93 | **** | **** | * | *** | ** | ** | 1.33 |
| | 94 | **** | *** | ** | ** | * | *** | 1.52 |
| Zn | 93 | **** | *** | ** | *** | * | *** | 1.58 |
| | 94 | **** | **** | ** | * | ** | *** | 2.49 |
| As | 93 | **** | **** | * | * | ** | *** | 1.75 |
| | 94 | **** | *** | *** | ** | ** | **** | 1.26 |
| Se | 93 | **** | **** | *** | **** | *** | *** | 1.39 |
| | 94 | ** | ** | * | ** | * | *** | 1.52 |
| Sr | 93 | **** | * | * | *** | **** | **** | 1.40 |
| | 94 | *** | ** | *** | * | ** | **** | 1.39 |
| Ag | 93 | **** | **** | *** | ** | * | * | 1.49 |
| | 94 | *** | **** | *** | * | *** | *** | 1.85 |
| Cd | 93 | **** | **** | * | ** | ** | *** | 1.51 |
| | 94 | *** | **** | *** | * | ** | *** | 2.02 |
| Sb | 93 | **** | *** | **** | ** | ** | **** | 1.25 |
| | 94 | *** | ** | * | **** | * | *** | 1.47 |
| Cs | 93 | *** | ** | H | **** | ** | * | 2.75 |
| | 94 | * | * | *** | H | * | * | 4.33 |
| Hg | 93 | **** | **** | ** | *** | ** | *** | 1.48 |
| | 94 | H | *** | *** | * | * | ** | 2.66 |
| U | 93 | ** | *** | **** | *** | ** | *** | 1.71 |
| | 94 | *** | * | H | **** | * | ** | 2.21 |

Table 4.1 (c) : Distribution of element concentrations in Sylt-List for two years of sampling

| Element | Year | February | April | June | August | October | December | Variation factor |
|---|---|---|---|---|---|---|---|---|
| Mg | 93 | * | ** | ** | **** | * | ** | 1.09 |
| | 94 | * | * | **** | *** | **** | *** | 1.06 |
| P | 93 | **** | *** | * | * | ** | *** | 3.18 |
| | 94 | *** | **** | * | ** | ** | **** | 2.42 |
| S | 93 | ** | * | *** | ** | **** | **** | 1.29 |
| | 94 | *** | * | ** | * | *** | **** | 1.30 |
| Cr | 93 | * | *** | ** | * | **** | * | 2.10 |
| | 94 | *** | **** | *** | **** | ** | ** | 2.27 |
| Mn | 93 | ** | * | *** | **** | **** | ** | 2.36 |
| | 94 | *** | * | * | **** | *** | ** | 2.31 |
| Co | 93 | *** | * | * | *** | **** | **** | 1.87 |
| | 94 | **** | *** | * | ** | ** | ** | 1.93 |
| Cu | 93 | **** | **** | * | ** | ** | ** | 1.66 |
| | 94 | *** | **** | * | *** | * | *** | 1.50 |
| Zn | 93 | *** | **** | ** | ** | * | *** | 2.11 |
| | 94 | **** | **** | ** | * | * | *** | 2.98 |
| As | 93 | **** | ** | * | * | *** | *** | 2.02 |
| | 94 | **** | ** | * | ** | *** | **** | 1.58 |
| Se | 93 | **** | * | ** | ** | ** | *** | 1.32 |
| | 94 | **** | **** | * | *** | * | *** | 1.79 |
| Sr | 93 | *** | * | * | * | **** | **** | 1.60 |
| | 94 | *** | *** | ** | ** | ** | **** | 1.33 |
| Ag | 93 | **** | H | ** | * | * | * | 5.47 |
| | 94 | *** | **** | *** | ** | ** | *** | 2.53 |
| Cd | 93 | *** | **** | ** | * | ** | *** | 1.69 |
| | 94 | **** | **** | ** | * | * | *** | 1.96 |
| Sb | 93 | *** | ** | * | * | H | **** | 2.09 |
| | 94 | **** | *** | * | ** | ** | *** | 1.71 |
| Cs | 93 | ** | **** | *** | *** | *** | * | 1.36 |
| | 94 | ** | **** | ** | **** | * | * | 1.65 |
| Hg | 93 | *** | ** | * | * | *** | *** | 1.69 |
| | 94 | **** | **** | ** | **** | * | ** | 1.97 |
| U | 93 | **** | * | **** | *** | *** | *** | 2.44 |
| | 94 | ** | ** | * | **** | * | ** | 1.98 |

The mean Hg levels in seaweed show significant intra-specific variability between sites with different pollution as well as inter-specific variability at each site. The Hg levels found in the algae (Fig. 4.18) are heterogeneous both temporally and spatially with respect to the collection sites and this is in agreement with data of Ferreira [93]. Maxima in Hg concentration in tissues appeared in late winter-early spring, and often a moderately decrease was found in the summer. The seasonal nature of the peaks suggested that variation in Hg may be linked to physiological aspects of algal production rather than to fluctuation in dissolved Hg levels.

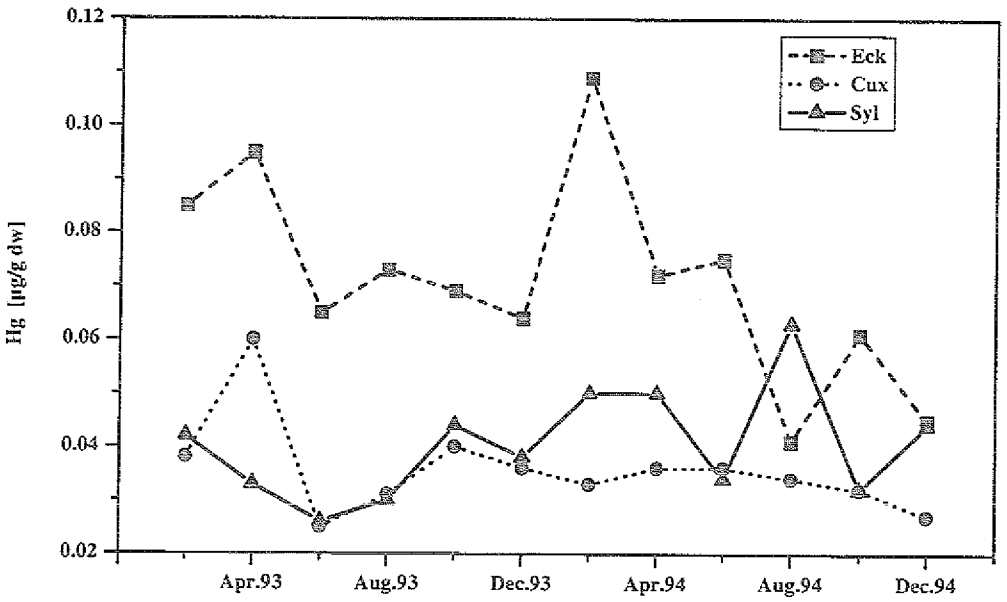



**Fig. 4.18 : Regional and seasonal variation of Hg concentration in *Fucus vesiculosus*.**

A discrimination analysis using *stepwise* selection was performed. This procedure selects a subset of quantitative variables which will be a valuable aid in discriminating between the collection sites. The significance level to enter, and to stay in stepwise discrimination analysis is 0.15. The analysis was performed on the data from the three collection sites together, Eckwarderhörne and Cuxhaven, Sylt-List and Cuxhaven, and Eckwarderhörne and Sylt-List. As a result of the analysis, Fig. 4.19 shows the effect of different sampling sites on elemental concentrations, and the possibility of differentiating between collection sites based on selected element concentrations. Table 4.2 shows the mean concentration (µg/g dw), the range (maximum - minimum), variation factor (maximum / minimum) and the coefficient of variation (SD / mean).

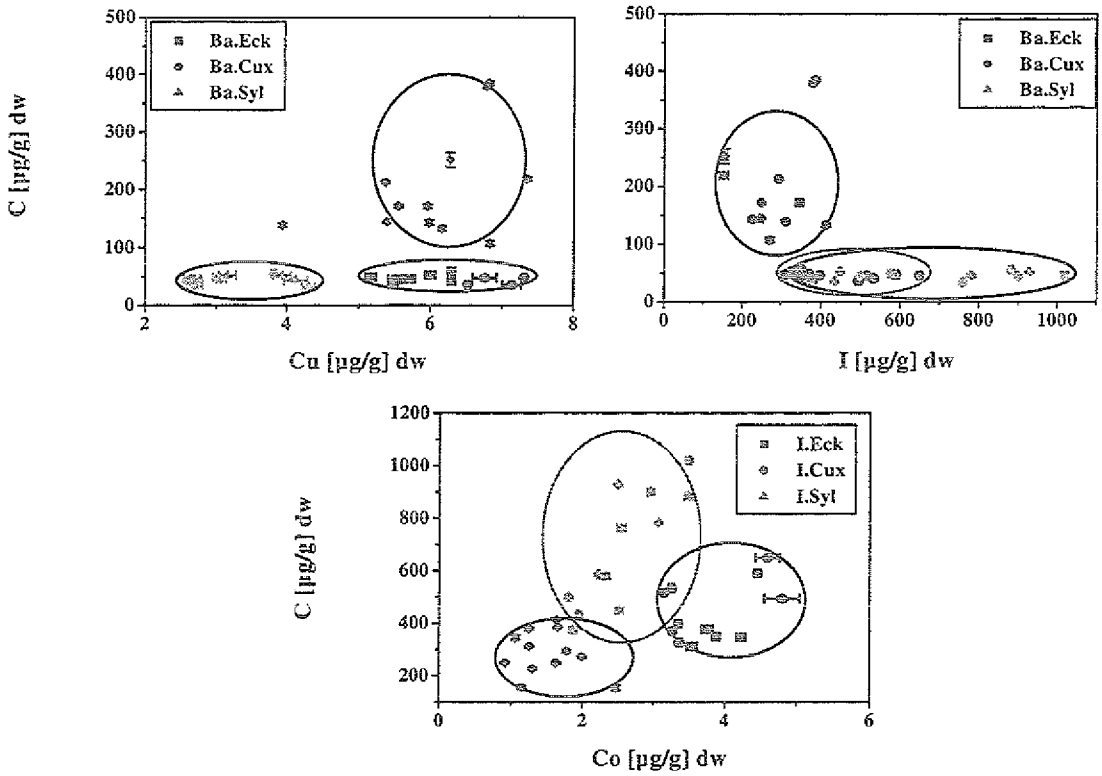



Fig. 4.19 : Discrimination between different collection sites based on element concentrations.

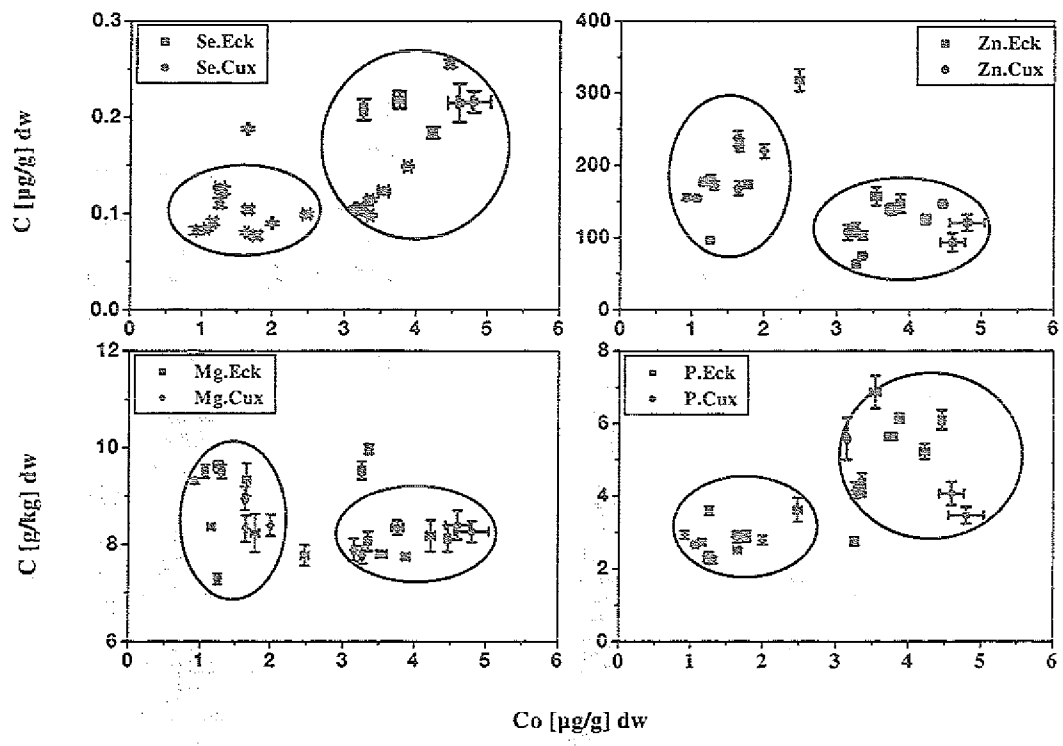



Fig. 4.20 : Discrimination between Eckwarderhörne and Cuxhaven based on element concentrations.

Table 4.2 : The mean concentrations (µg/g), ranges, variation factors (V.f), and coefficient of variations (C.v) for the elements used in the discrimination analysis.

| Element | Parameter | Eckwarderhörne | Cuxhaven | Sylt-List |
|---|---|---|---|---|
| Ba | Mean | 45.4 | 164 | 44.1 |
| | Range | 59.1 - 35.4 | 252 - 107 | 55 - 31.4 |
| | V.f | 1.67 | 2.36 | 1.77 |
| | C.v | 14.5 | 26.7 | 16.8 |
| Cu | Mean | 6.01 | 6.04 | 3.21 |
| | Range | 7.31 - 4.46 | 7.35 - 3.94 | 4.31 - 2.59 |
| | V.f | 1.64 | 1.87 | 1.66 |
| | C.v | 14 | 15 | 20.2 |
| Co | Mean | 3.81 | 1.52 | 2.56 |
| | Range | 4.8 - 3.15 | 2.48 - 0.923 | 3.49 - 1.81 |
| | V.f | 1.53 | 2.69 | 1.93 |
| | C.v | 15.3 | 29.1 | 22.7 |
| I | Mean | 440 | 286 | 683 |
| | Range | 651 - 321 | 413 - 153 | 1019 - 374 |
| | V.f | 2.03 | 2.7 | 2.72 |
| | C.v | 25.5 | 29.8 | 32.5 |
| Zn | Mean | 116 | 190 | |
| | Range | 157 - 94 | 318 - 96.8 | |
| | V.f | 2.49 | 3.29 | |
| | C.v | 25.5 | 28.9 | |
| P | Mean | 4868 | 2832 | |
| | Range | 6860 - 2755 | 3610 - 2222 | |
| | V.f | 2.9 | 1.63 | |
| | C.v | 25.4 | 15 | |
| Mg | Mean | 8337 | 8717 | |
| | Range | 9965 - 7748 | 9610 - 7290 | |
| | V.f | 1.29 | 1.32 | |
| | C.v | 8.4 | 8.8 | |
| Se | Mean | 0.166 | 0.105 | |
| | Range | 0.256 - 0.098 | 0.188 - 0.077 | |
| | V.f | 2.6 | 2.44 | |
| | C.v | 33.9 | 29.6 | |
| Na | Mean | | 30483 | 33621 |
| | Range | | 41100 - 18500 | 37500 - 28950 |
| | V.f | | 2.22 | 1.03 |
| | C.v | | 19.8 | 6.9 |
| Tb | Mean | | 0.027 | 0.014 |
| | Range | | 0.057 - 0.017 | 0.021 - 0.008 |
| | V.f | | 3.35 | 2.63 |
| | C.v | | 36.8 | 30.5 |
| As | Mean | 45.9 | | 52.9 |
| | Range | 57 - 32.5 | | 64.7 - 32 |
| | V.f | 1.75 | | 2.02 |
| | C.v | 15.7 | | 19.3 |
| Cd | Mean | 1.05 | | 0.774 |
| | Range | 1.34 - 0.633 | | 1.1 - 0.53 |
| | V.f | 2.12 | | 2.7 |
| | C.v | 22 | | 22.7 |
| Sr | Mean | 954 | | 750 |
| | Range | 1165 - 747 | | 899 - 561 |
| | V.f | 1.56 | | 1.6 |
| | C.v | 14.3 | | 17 |

The mean concentration levels of Ba, Cu, Co and I were used for the discrimination between the three collection sites. Each one of these elements represents the collection sites. The spread of the points is due to seasonal variation of two years of data and may include some extreme values. The level of I in Eckwarderhörne and Sylt-List overlaps because of the induced salinity pattern and seasonality, but the concentration levels of Cuxhaven can be distinguished from each one separately. Also, manganese dioxide in the mud would probably be reduced to manganous ion with the result that more soluble manganese would become available. McKenzie and Taylor [94] have shown that Mn oxide particles in the sediment tend to concentrate Co, therefore Co may be released into water along with Mn. Therefore also, the surface sediment contributes to the level of Co concentration in Eckwarderhörne and Sylt-List. Fig. (4.20) shows the comparison between Eckwarderhörne and Cuxhaven. The mean concentration levels of Co, Zn, Mg, Se, and P were used for the discrimination between Eckwarderhörne and Cuxhaven. These elements are characteristic not only of the collection sites, but also for the year of sampling as indicated in Table (4.1 a,b).

For the comparison between Sylt-List and Cuxhaven the elements, Ba, Cu, Na and Tb were used. As shown in Fig. 4.21 more spread in Cuxhaven values than in Sylt-List was observed for Na and this may be explained by varying levels of fresh-water inflow, tidal state, and longshore drift. The concentration levels of Cu in Cuxhaven are higher than that of Sylt-List, and the concentration levels of Na in Sylt-List are relatively higher than that of Cuxhaven. The significant higher levels of Ba, Zn, Cd, Cu, and Ni in samples from Cuxhaven could be emanated from industrial activities or be run off from the Elbe River, it could also indicate increased biological availability at Cuxhaven.

In comparison between Sylt-List and Eckwarderhörne the elements Cu, As, Sr and Cd were used. Fig. (4.22) shows relatively similar levels of As in both Eckwarderhörne and Sylt-List at different levels of Cu. The coefficient of variations of all the elements is due to the variation of the bimonthly concentration around the mean concentration of the elements.

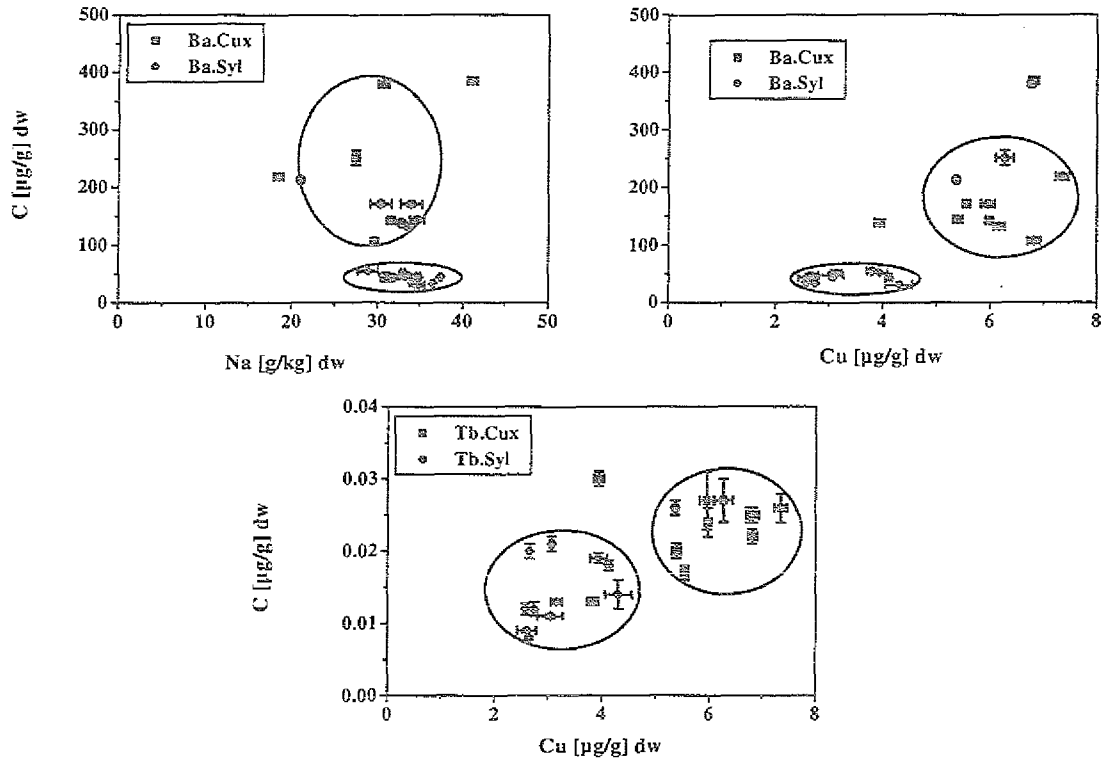



Fig. 4.21 : Discrimination between Cuxhaven and Sylt-List based on pairs of element concentrations.

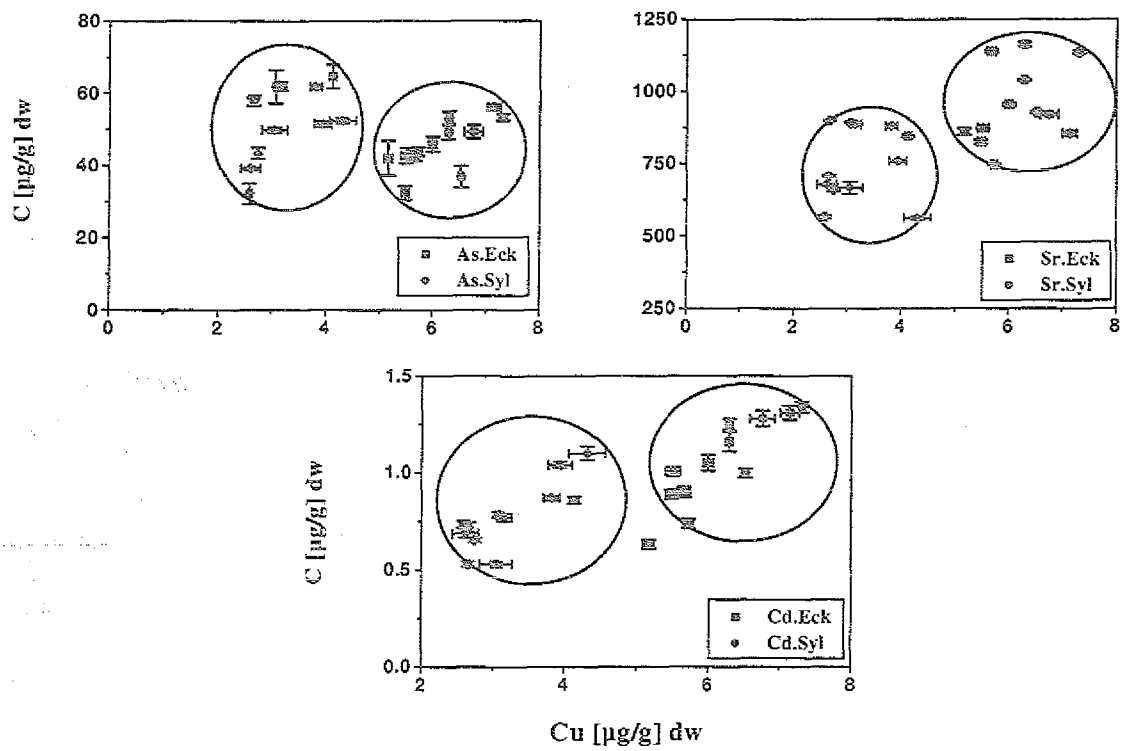



Fig. 4.22: Discrimination between Eckwarderhörne and Sylt-List based on pairs of element concentrations.

The multivariate technique of principal component analysis (PCA) using SAS/INSIGHT software, was used to examine quantitative relationships among all of the 41 determined element concentrations in the three sampling areas. PCA reduces the dimensionality of the data set while trying to preserve the structure and identifies the groups of elements which might act together in some predicted manner. The first PCA-1 has the largest variance of element concentrations in linear combination with the sampling time. A summary of the exploratory data analysis is given in Fig. 4.23, in which the first and second PCA components with the element concentrations were identified by their collection sites. In PCA the first component summarizes 28 % of the variation in the dataset. The cumulative percentage of the second component is 51 %, and three components explain 64 %. Subsequent components contribute less than 5 % each. The distribution of populations along the axis shows that the arrangement of element concentrations is fairly elongated along the first and second components. Also, it is possible to identify regional trends, and it was shown that Eckwarderhörne and Sylt-List fall into overlapping units and both almost exist in the positive loading of the second PCA-2 component. Cuxhaven exists in the positive loading of the first PCA-1 component. There are three data points outside the "normal" range: Eckwarderhörne Jun. 93 and Aug. 94, which may correspond to the fertility peak in June and the increasing biomass, as well as Cuxhaven (Oct. 94), which might be due to artificial factors such as shipping traffic at the time of sampling. They located at the extreme right with a high overall average ratio of element concentrations and bimonthly variation.

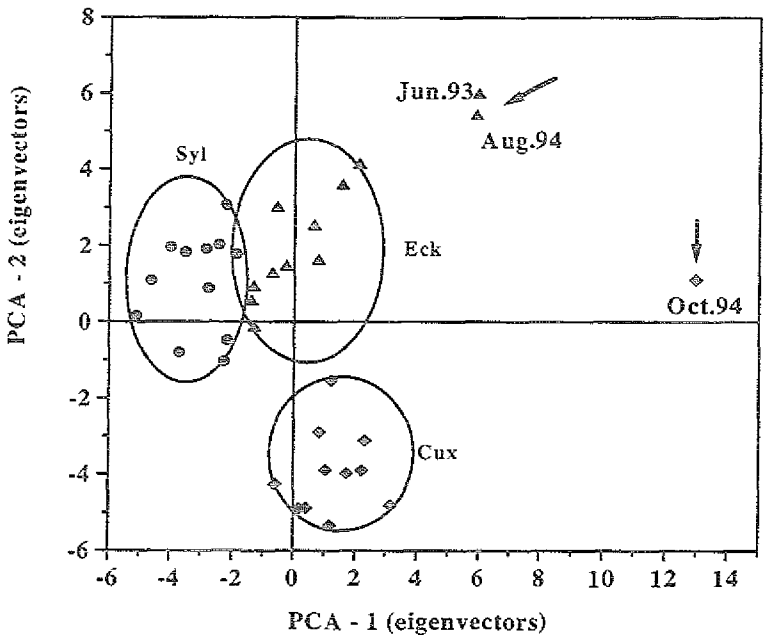



Fig. 4.23 : Range of element concentrations at collection sites, Eckwarderhörne (Eck), Cuxhaven (Cux), and Sylt-List (Syl)

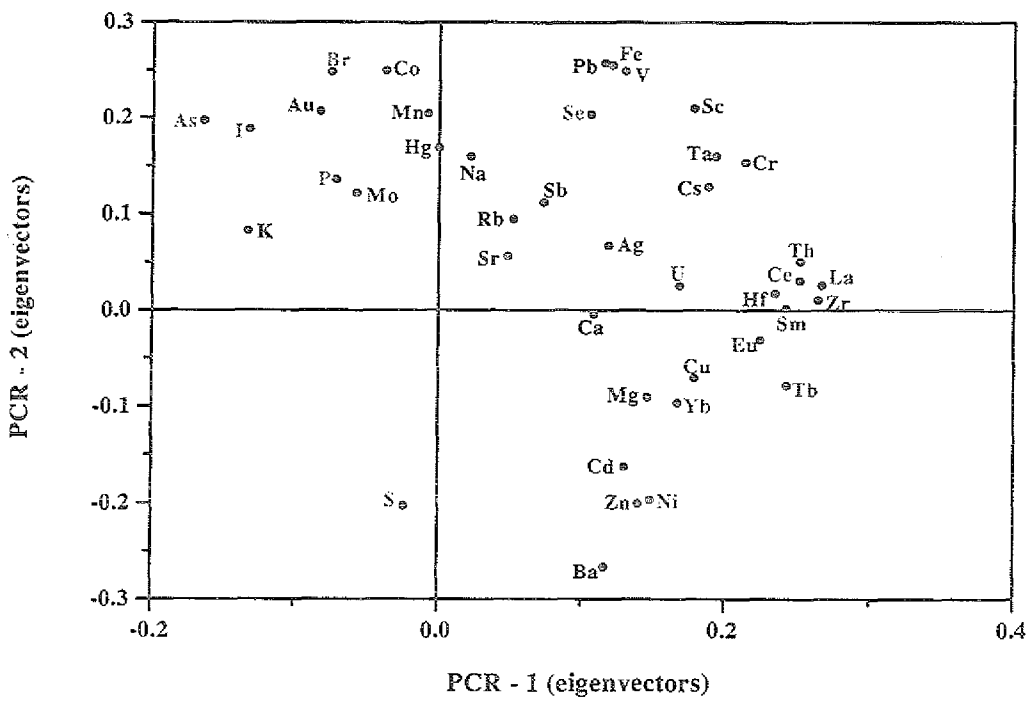



Fig. 4.24 : Scatter plot of the first PCA eigenvectors and the second PCA eigenvectors for all elements determined at the three collection sites.

Eckwarderhörne and Sylt-List were found to have a common behavior of variation patterns, in which salinities were similar and higher and less fluctuating than that of the estuary water in Cuxhaven. The eigenvectors corresponding to each of the principal components are used as the coefficient to form a linear combination of the element concentrations (principal component). Fig. 4.24 shows that the first and second PCA eigenvectors have positive load on the following elements: La, Zr, Th, Ce, Sm, Cr, Ta, Cs, Sc, U, V, Fe, Pb, Se, Sb, Rb, Sr, and Na. The elements are sorted on the basis of the highest loading with respect to the first PCA. The elements Hg, Mn, S, Co, Mo, P, Br, Au, K, I, and As have negative load with respect to the first PCA eigenvectors, and the elements Ca, Eu, Cu, Tb, Yb, Cd, Ni, Zn, S, and Ba have negative loading with respect to the second PCA eigenvectors. From Fig. 4.24 it was found that the chemical and physical properties of elements such as Sc, V, Cr, Fe, Pb, Zr, La, Hf, Ce, and Th as well as Ni, Cu, Zn, and Cd are play an important role in the characterization of the collection sites and showing site-independent correlated groups. A close look at data from each of the collection sites shows that different elemental patterns are positioned on the first and second PCA, which indicate that different environments and substrates are the major influencing factor on the accumulation of elements from their surroundings. This has been found in the stepwise discrimination analysis as well.

Fig. 4.25 shows a contour of the elements which are correlated with the PCA components is presented. The samples included in the analysis were reduced to distinct groups of elements such as Sc, V, Cr, Fe, Cs, La, Pb, Th, and rare earth elements. Also, among the distinct groups Cu, Zn, Cd, Ag, Au, and Hg, as well as P and As. In fact these elements are enriched in the surface sediment, and the algae spent 1/3 of their lifetime lying on the substrate at low tide. Mart and Nürnberg [95] concluded that the dissolved Pb in the Elbe estuary is about 9 %. The total amount of Pb is practically determined by the fraction of Pb bound to particles. Bryan and Hummerstone [8] reported that surface cleaning by additional brushing is thought to reduce Fe to 74 % , Mn to 62 %, and Cu to 89 %, respectively. Also, levels of particulate and dissolved organic matter, and their presence will influence the binding of metals on the surface of *Fucus vesiculosus* to a different

extent. Luoma et al. [36] have suggested that the concentrations of Cu, As, Pb, Zn, and Ag in algal tissues correlates significantly with concentrations in sediment. Cu and Pb, the metals with the highest selectivity coefficients for binding, showed the strongest correlation of tissues and sediment concentrations. Bryan et al. [91] reported that in general, the particulate contribution for Cd and Zn was always low, whereas for Pb and Cr it was consistently high at about 63 %. For the remaining metals particulate contributions were < 15 %, although occasionally much higher. Langston [74] and Luoma et al. [36] reported that there is a correlation between As in sediment and tissues of *F. vesiculosus*, and that the Cu concentration increased in seaweed tissues by scavenging from surface sediment. The polyphenolic proteins of *Fucus* species have a strong tendency to complex metals such as Cu, Pb, As, Zn, and Ag when they come into contact with the plant surface [96]. Autio and Kangas [32] concluded that Fe varied irregularly in algal tissues, and the mucus covering the thallus may also contain Fe, which means that not all the Fe measured was bound to the algal tissues [97]. Barreiro et al. [90] demonstrated that Fe shows a pattern similar to Cr and Al between different tissues caused merely by the quantity of fine sediment adhering to the surface, generally the finer the sediment the greater the binding capacity. Therefore, Fe has a positive load on this group, and the source is the surface sediment with which the algae came into contact during low tide. In Fig. 4.26 (a), the eigenvalues indicate that the first component accounts for 38.6 % of the total variance in the dataset of Eckwarderhörne, the cumulative percentages of the second is 57.7 %, and 70.4 % for the third. The first PCA has a positive load on Jun. 93, Aug. 93, and Aug. 94, which are the months of biomass increasing - consequently the water content of the algal tissues and the surface area - and negative load on late summer until the beginning of the fertility peak in early spring.

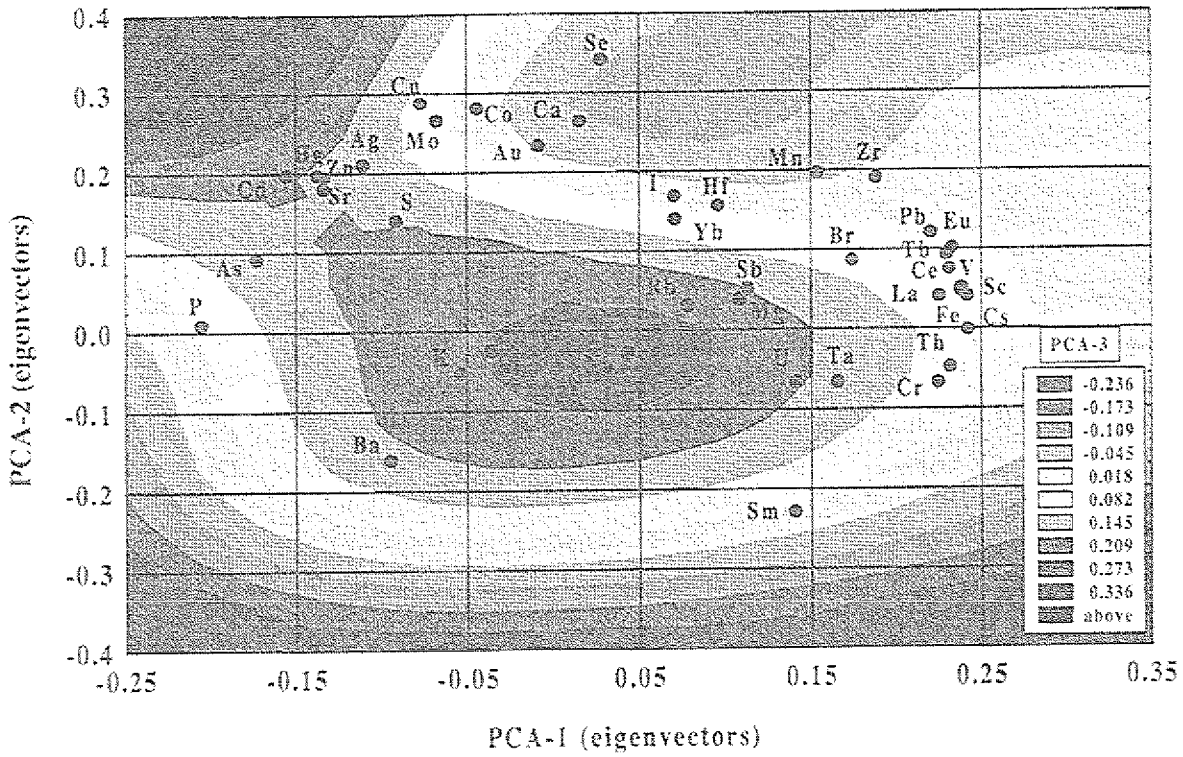



**Fig. 4.25 : Contour plot of the third against the first and the second PCA eigenvectors in Eckwarderhörne.**

Zolotukhina et al. [98] have reported that the most significant structural characteristic and the one particularly important in the rapid uptake of metals, is the specific surface area of the thallus. In fact, the surface area of the algae plays a key role in possible alternation of the accumulation factors as well as the contamination factor resulting from the suspended particulate matter and fine sediment. As an example from the data on Fe and Cs, it was found that the coefficient of variations were up to 52 % around the mean concentration. The first PCA shows positive correlation with Cr, Fe, Pb, Cs, V, Sc, and Th as well as negative correlation with P and As. The second PCA component has positive load on the year 1993, and negative on the year 1994. Also, the second PCA has positive correlation with Se, Cu, Co, Mo, Ca, Au, and Ag, as well as negative correlation with Sm. The third PCA component has positive load on February, April, and June while negative on August, October, and December, this behavior was shown for both years. The third PCA is positively correlated with Na, Rb, Mg, K, S, and U and negatively with Ca.

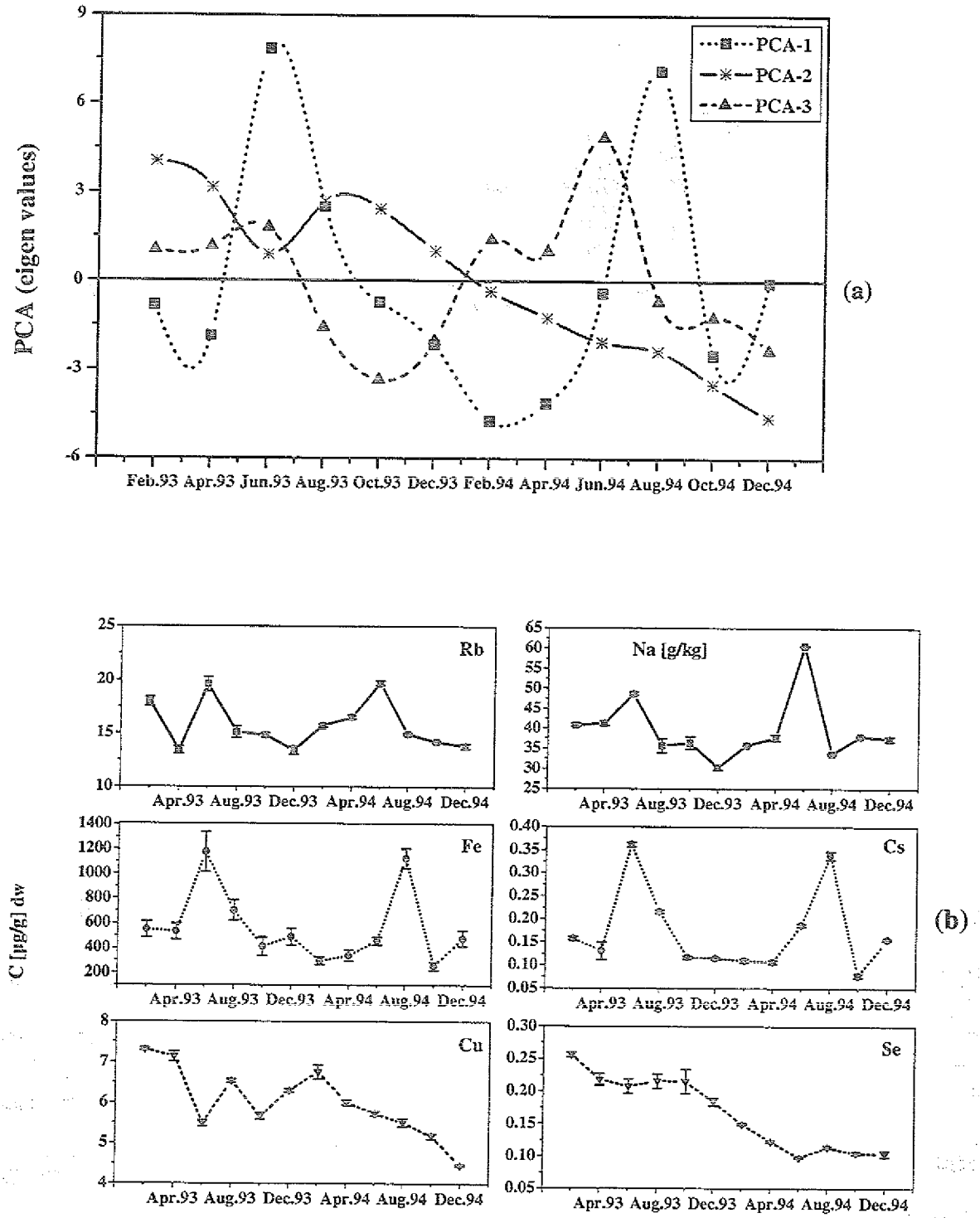



Fig. 4.26 : (a) Variation of PCA as a function sampling time in Eckwarderhörne (b) Variation of Fe and Cs (PCA-1), Cu and Se (PCA-2), and Rb and Na (PCA-3) with sampling time.

One may explains this behavior by the existence of a winter or spring maximum and a summer or autumn minimum, or biological requirement of the vegetative cycle of the algae e.g. Rb and Na as in Fig. 4.26 (b). This pattern is attributed not only to changes in the tissues due to the vegetation cycle or to bioavailability of these elements in the environment, but also to seasonal variation of temperatures. However, the interpretation of this behavior is not obvious, and it was found that for some elements such as Se and Cu concentrations may have decreased in 1994.

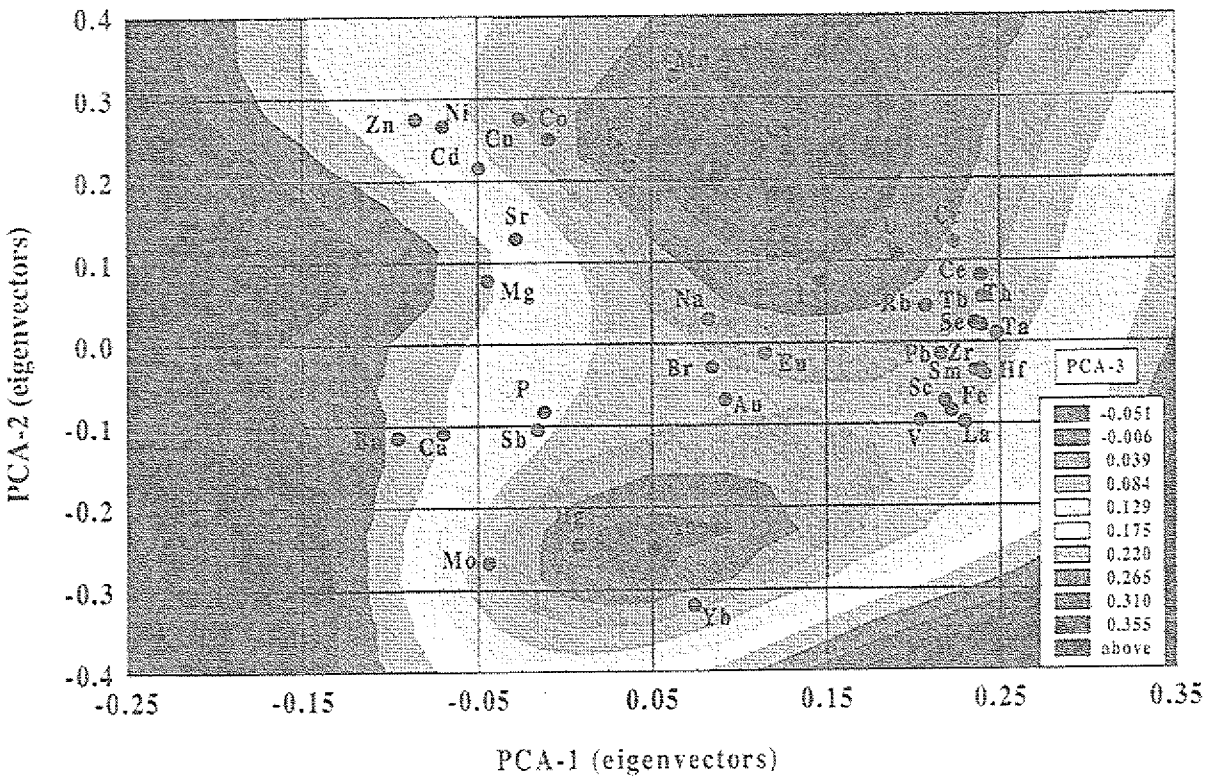



**Fig. 4.27 : Contour plot of the third against the first and the second PCA eigenvectors of all the elements determined in Cuxhaven.**

In Fig. 4.27 the estuarine water in Cuxhaven was characterized by factors such as the different mixing ratio between Elbe river water and sea water, its spectacular tidal range, high sediment load, wide salinity range, large heavy metals inputs, and consequently increased substantial upwhirling of the suspended particulate matter besides the influence of the amount of light available to the immersed algae. The positive loading of first PCA on As, Hg, V, Fe, Sc, Hf, Zr, and Pb as well as

Yb, La, Sm, Tb, Ce and Th reflects these factors when compared to those of Eckwarderhörne. The differences based on element concentrations in the same bioindicator (brown algae) collected from different collection sites for long-term monitoring is the most suitable method for studies of the potential factors affecting accumulation processes, otherwise it would not be possible to distinguish between studied sites. In the environment of the three collection sites the major effective factors influencing the elemental concentrations are salinity, wave exposure, age, plant morphology, and different inputs of pollution sources. Therefore, besides the above mentioned factors, and on the basis of the status of soluble chemical species in the ambient environment, there is a level of heterogeneity in the collection sites and within the sampling years of the same collection site. The tolerance of algae to all these factors is shown by the repetition of the accumulation patterns of the discussed element groups with respect to concentrations (Fig. 4.27).

The obvious correlated groups of elements are Sc, V, Fe, Se, and Pb, as well as Cr, Zr, Th and rare earth elements, which are correlated with Hf also, in the case of As and Hg, Cu and Ni, and Cd and Zn. It was found that Ta has the highest positive load, and shows approximately equal loading on Hf, Th, Tb, Zr, Ce, Sm, and Se. Fig. 4.28 (a) shows that in Cuxhaven the first PCA component accounts for 37.6 % of the total variance through the sampling time, and cumulative percentages of the second PCA component 55 %, and 68.8 % with the third PCA component. The first PCA component is positively correlated with Hf, Th, Zr, Se. Cr, Fe, Sc, Pb, and rare earth elements. The second PCA component is positively correlated with Mn, Cu, Zn, Ni, Co, Cs, and Cd, as well as negatively with Yb, Mo, As, and Hg. The third PCA component positively correlated with Ca, Sr, Sb, S, Ni, Mg, and Cu, as well as negatively with As, P, Hg, and Ag. Another variation mode was shown by the Cuxhaven collection site as described by the first, the second, and the third PCA components. Positive load was detected on early spring 1993 until Aug. 93. In general, by constant element contents in the body of water, the concentration should rise in slow- growing wintering plants.

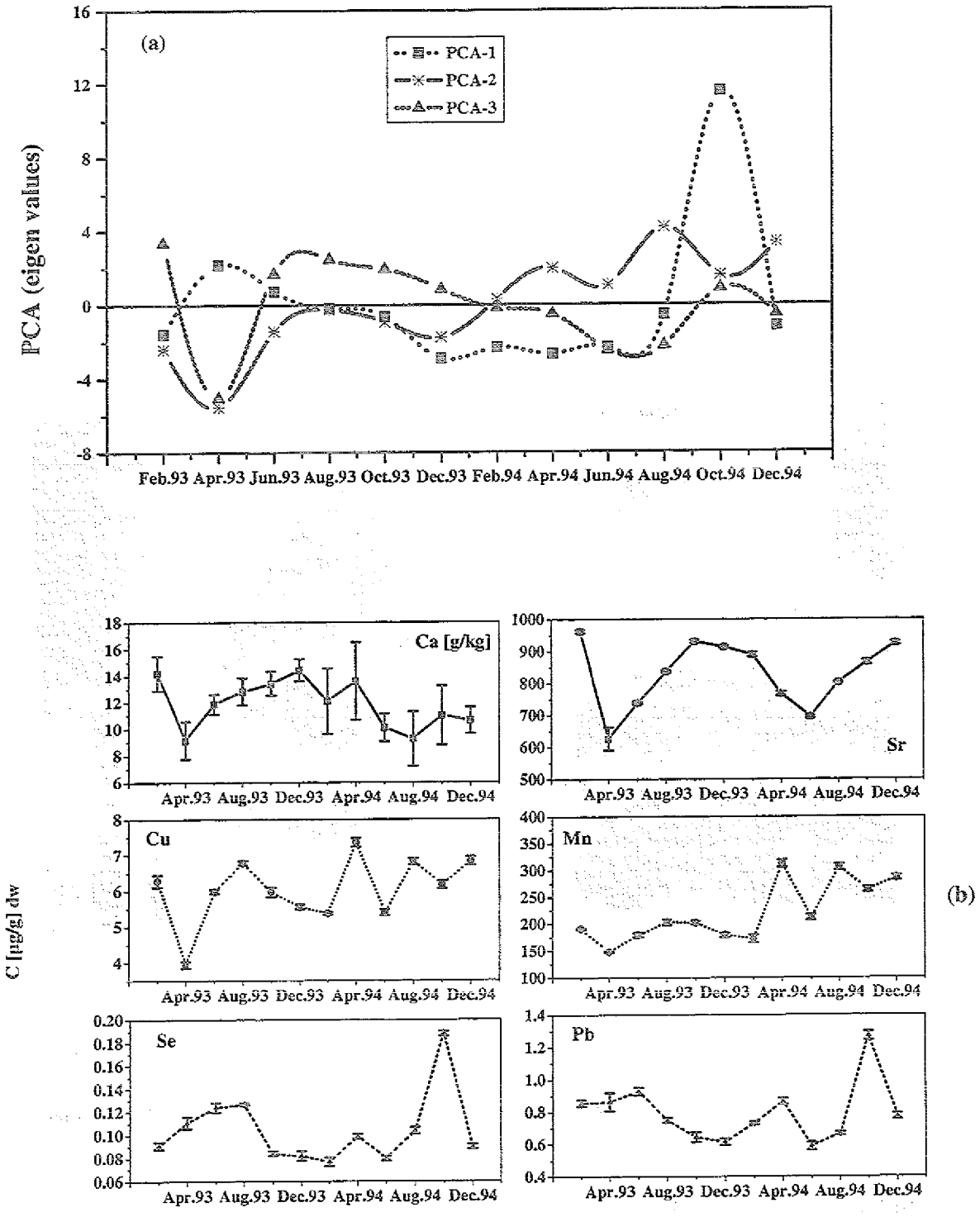



Fig. 4.28 : (a) Variation of PCA as function of sampling time in Cuxhaven (b) Variation of Se and Pb (PCA-1), Mn and Cu (PCA-2), and Ca and Sr (PCA-3) with sampling time

Thereafter concentration would decrease until the beginning of winter as a result of continuing growth. However, this does not imply that no seasonal variation exists as was reflected by the continuous negative load of the first PCA on the sampling time. In fact the overall high concentration in Oct. 94 suppresses and masks the variation mode in the summer months. Fig. 4.28 (b) indicates that Pb shows a maximum in late spring and a minimum in early winter, as well as Sc, V, Cr, and Fe. The behavior of the second cumulative PCA concludes that essential elements such as Mn is a biological-dependent element related to the physical condition of the algae.

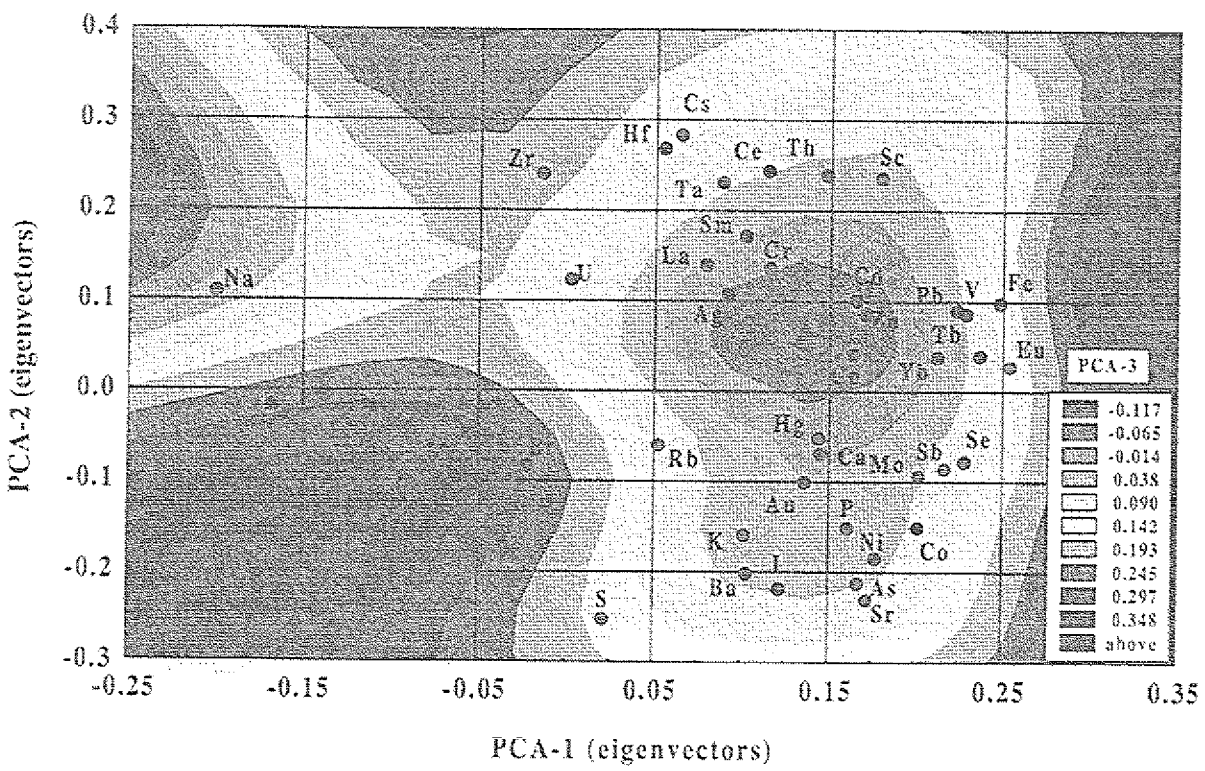



**Fig. 4.29 : Contour plot of the third against the first and the second PCA eigenvectors for all elements determined in Sylt-List.**

In Sylt-List, Fig. (4.29) shows the resultant correlation of the determined elements with that of the first, second and third PCA in two years' sampling time. The combined effect of an almost uniform degree of ambient water together with a very slow flushing rate were considered to minimize the effects of changes in metal concentrations, i.e. more or less natural behavior of seasonal variation. The element distribution patterns in Sylt-List are different from that of Cuxhaven, and have an

oblique similarity - in the biological response of the algae - with Eckwarderhörne regarding the salinity-induced pattern of accumulation with different levels of concentration in algal tissue, as well as the increase in nutrient release from the sedimenting organic matter by the metabolic activities of mussels. The first PCA is positively correlated with Fe, V, Se, Pb, Sb, Mo, Co, Eu,Tb, and Yb, and negatively with Na and Mg. The second PCA is positively correlated with Cs, Hf, Zr, Th, Sc, Ta, and Ce, and negatively with Mn, S, Sr, I, As, and Ba. The third PCA is positively correlated with Mn, U, and Mg, and negatively with Ag, Cu, Zn, Cd, and P. According to what has been discussed above, Eu, Fe, Tb, V, Se, Pb, Sb, and Yb received the highest positive loading. In Fig. 4.30 (a), the eigenvalue of the first PCA component in Sylt-List accounts for 32.7 % of the total variance of element concentration with respect to the duration of sampling time. The cumulative percentages of the second PCA component is 55.1 %, and for the third 70.5 %. The first PCA component shows positive loading on the winter and early spring, and negative loading on the summer months. This suggests that element concentrations are expected to have lower levels with increasing biomass, whereas in fact a different variation pattern was found in Eckwarderhörne and Cuxhaven taking into consideration the different environmental factors between the years. These differences could be due to the fact that Sylt-List is a relatively undisturbed natural area with respect to the other collection sites of the North Sea coast. Fig. 4.30 (b) gives an example of the variation pattern of Fe and Cs, in which it is shown that they are in correlation with the first and second PCA components.

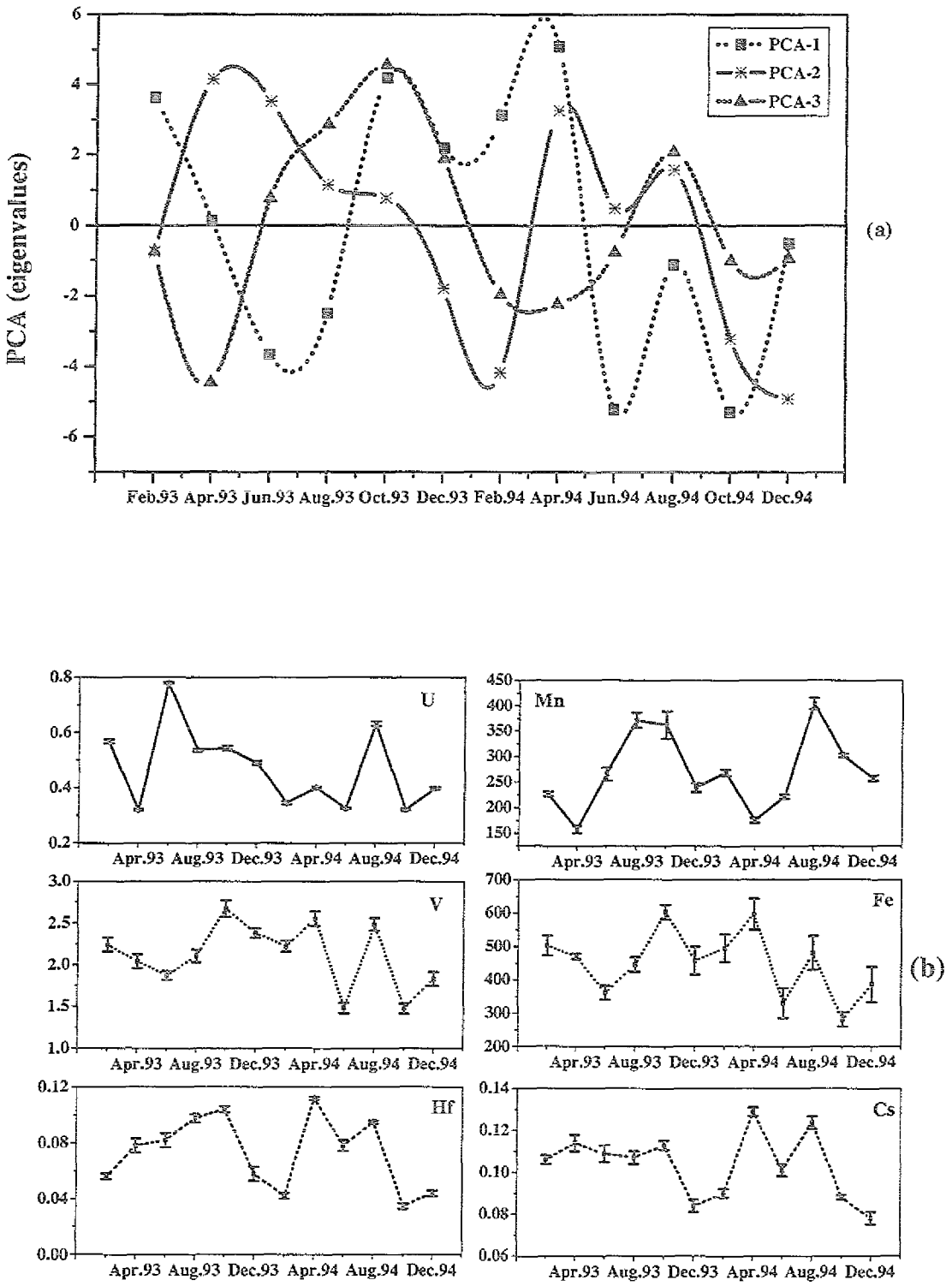



Fig. 4.30 : (a) Variation of PCA as a function of sampling time in Sylt-List (b) Variation of Fe and V (PCA-1), Cs and Hf (PCA-2), and Mn and U (PCA-3) with sampling time.

## 4.3 *Fingerprinting of elemental content*

Accumulation patterns in the collection sites of Eckwarderhörne, Cuxhaven, and Sylt-List (minimum and maximum ± SD of the seasonal variation regarding concentration values for all determined elements ) are shown in Fig. 4.31 (a-c). The characteristic feature of this matrix reveals important information on the expanding concentration range in the collection sites during the two years' sampling time. Similarities and/or differences in the environmental parameters of these localities, such as temperature, salinity, turbidity, and also nutrient content of the ambient seawater, etc. are integrated together influencing the element concentrations and their ranges. Fig. 4.32 shows the normalized variation ranges in the three collection sites as calculated from the following proposed equation:

$$\frac{C_{\max} - C_{\min}}{\sum_{i=2} C_{mean}} \qquad (4\text{-}1)$$

i = two years sampling time

$C_{max}$ = maximum concentration in two years

$C_{min}$ = minimum concentration in two years

$C_{mean}$ = mean concentration of two years

There is a comparability between the normalized variation ranges in Eckwarderhörne and Cuxhaven. This comparability may be due to grouping of elements interrelate with estuary environment than that with sea water environment as in Sylt-List. The normalized variation range of the following group of elements; Na, Mg, Sc, Fe, V, Au, Cs, Pb, Se, Hg, Hf, Rb, Sm, Eu, Tb, Cr, Th and Ce was highly pronounced in Eckwarderhörne. In Cuxhaven, Zr, Ta, Eu, Ni, Ba, La, Co, As, Cu, Zn, Sb and I showed higher normalized variation range than other elements, as well as P, K, Mo, U, Cd, S, Ca, Ag, Sr, Mn and Br in Sylt-List.

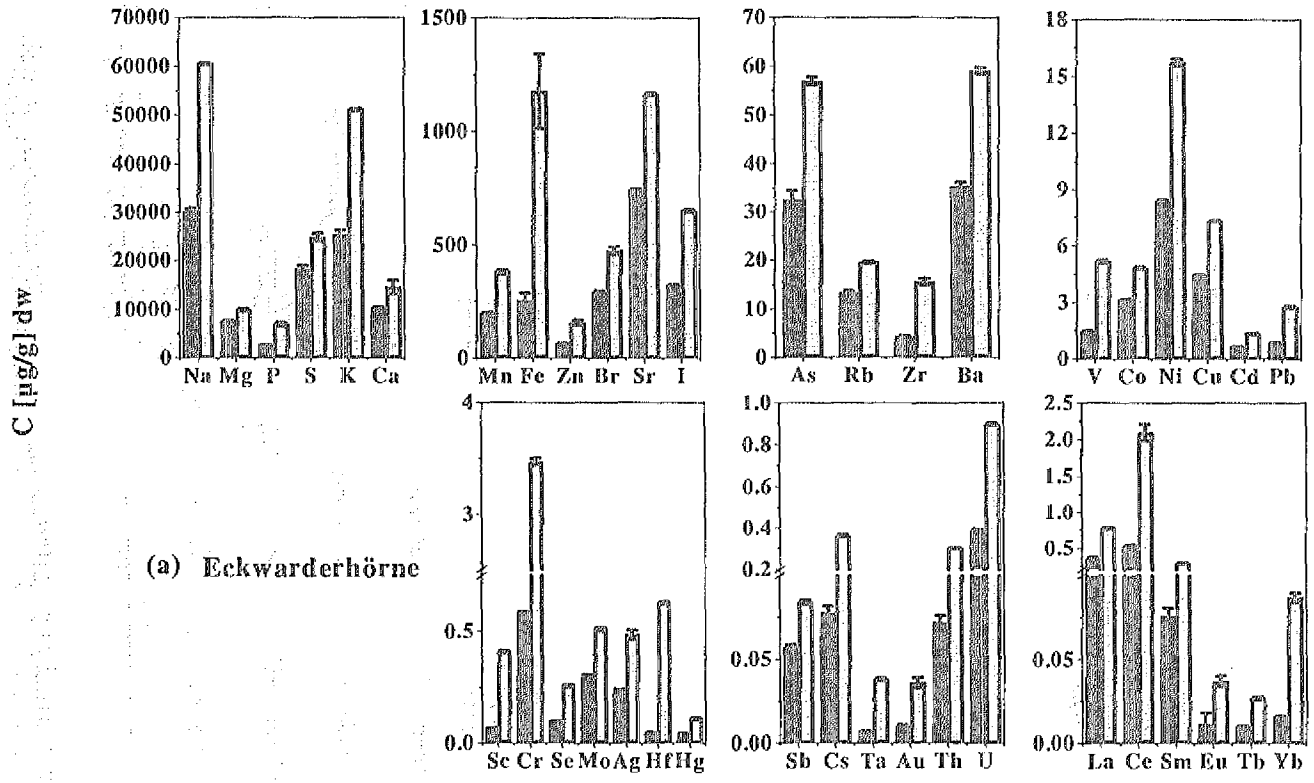
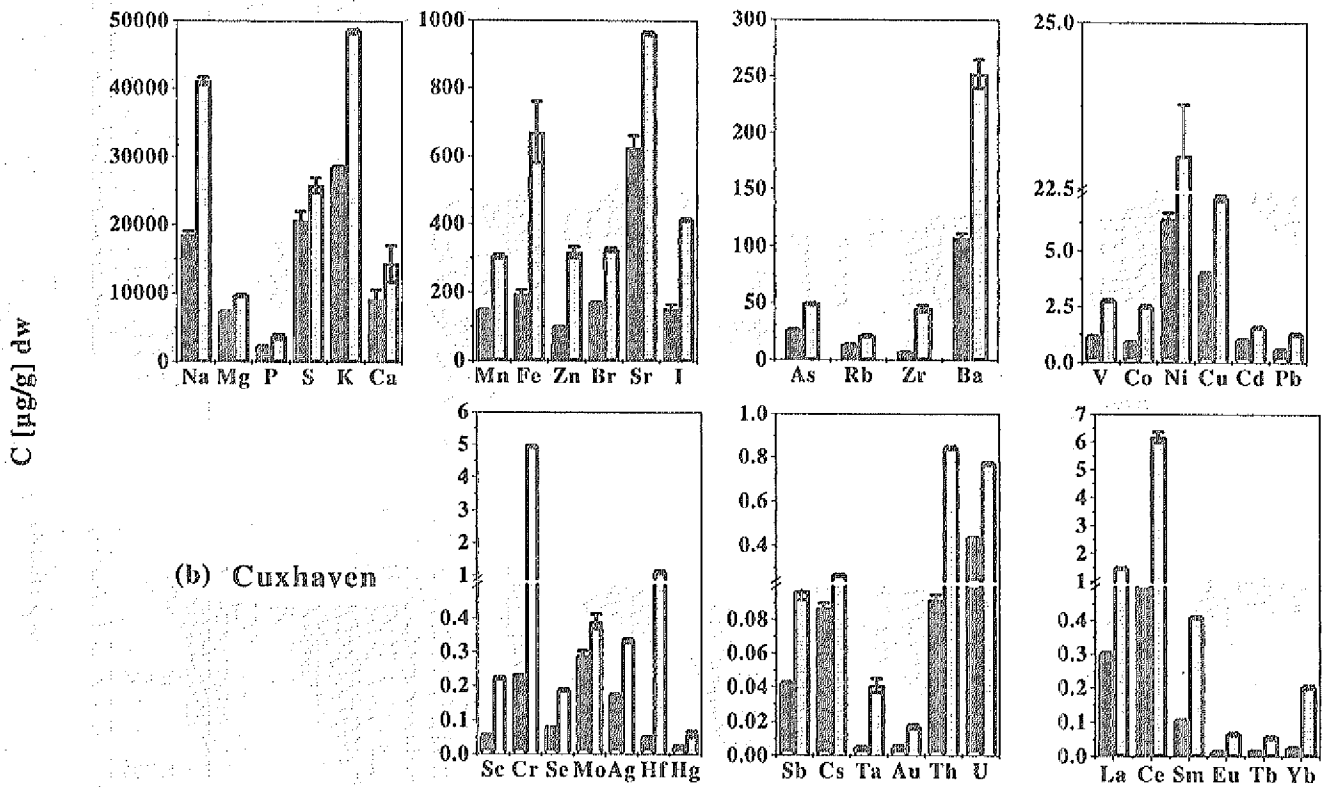
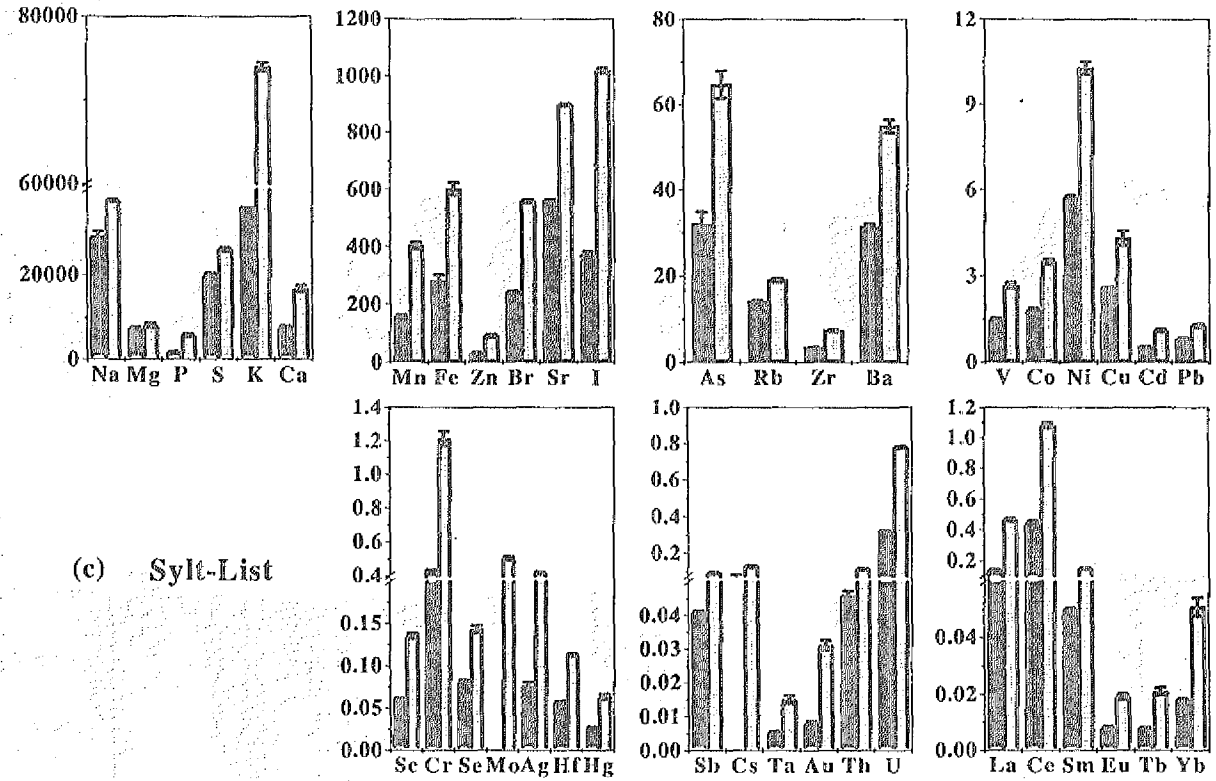



Fig. 4.31 (a-c) : Fingerprinting of the element concentration minimum and maximum values ± STD dry weight of the bioindicator Fucus vesiculosus from (a) Eckwarderhörne, (b) Cuxhaven, and (c) Sylt-List


C [µg/g] dw
(b) Cuxhaven
Na Mg P S K Ca
Mn Fe Zn Br Sr I
As Rb Zr Ba
V Co Ni Cu Cd Pb
Sc Cr Se Mo Ag Hf Hg
Sb Cs Ta Au Th U
La Ce Sm Eu Tb Yb


C [µg/g] dw
(c) Sylt-List
Na Mg P S K Ca
Mn Fe Zn Br Sr I
As Rb Zr Ba
V Co Ni Cu Cd Pb
Sc Cr Se Mo Ag Hf Hg
Sb Cs Ta Au Th U
La Ce Sm Eu Tb Yb

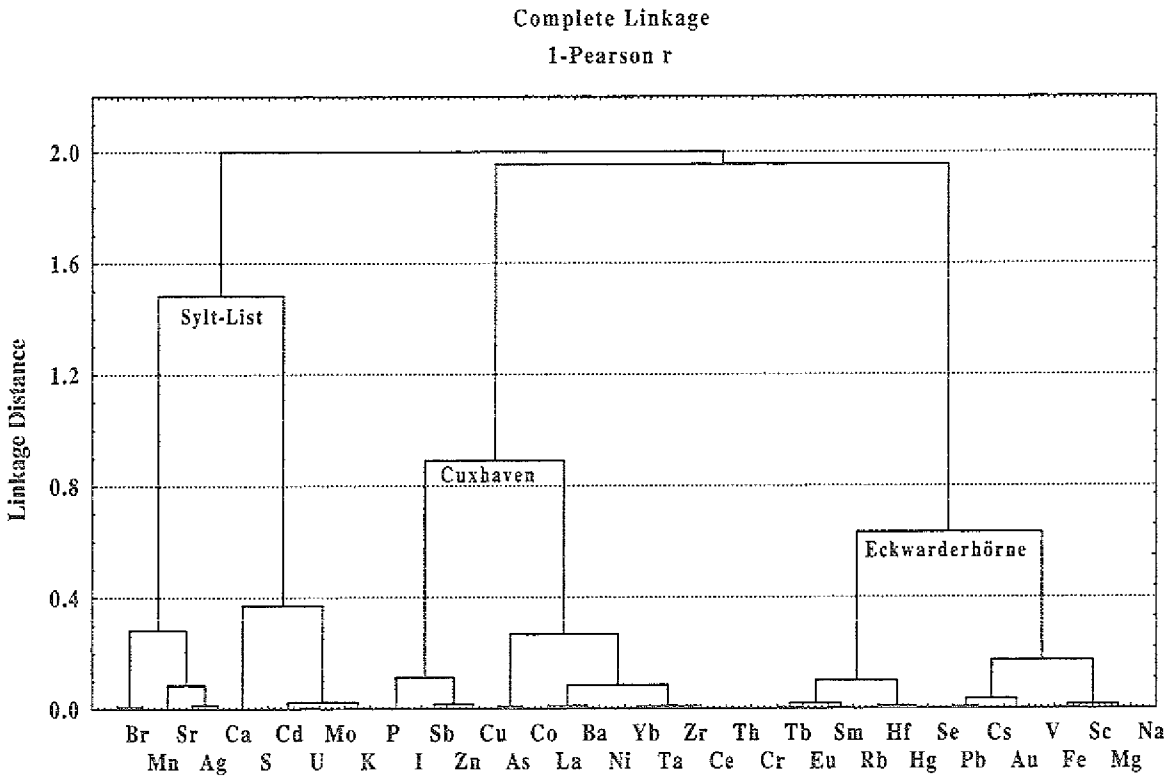



**Fig. 4.32 : Dentogram of normalized variation ranges for discriminated group of elements in the three collection sites.**

Fig. 4.33 shows selected correlations of the seasonal variation of trace metal concentrations at different locations along the North Sea coast. It demonstrates that although there are differences between sampling sites particularly because of various environmental parameters and covariables such as the specimen parameters of age, length or weight, there are similarities in the behavior of elemental groups as discussed above. The elements presented in Fig. 4.33 are randomly selected from the correlation matrix from each site as an example of the correlated group of elements. The selected correlated elements show a very good correlation factor (R = 0.82 to 0.95) with respect to two years of sampling including all the possible variabilities. These correlations are due to physico-chemical properties of the correlated elements, i.e. if there is a potential source of contamination such as in the case of mine wastes [35], the presence of elements of anthropogenic origin may raise concern about the existence of other co-correlated groups of elements which are known to be priority pollutants.

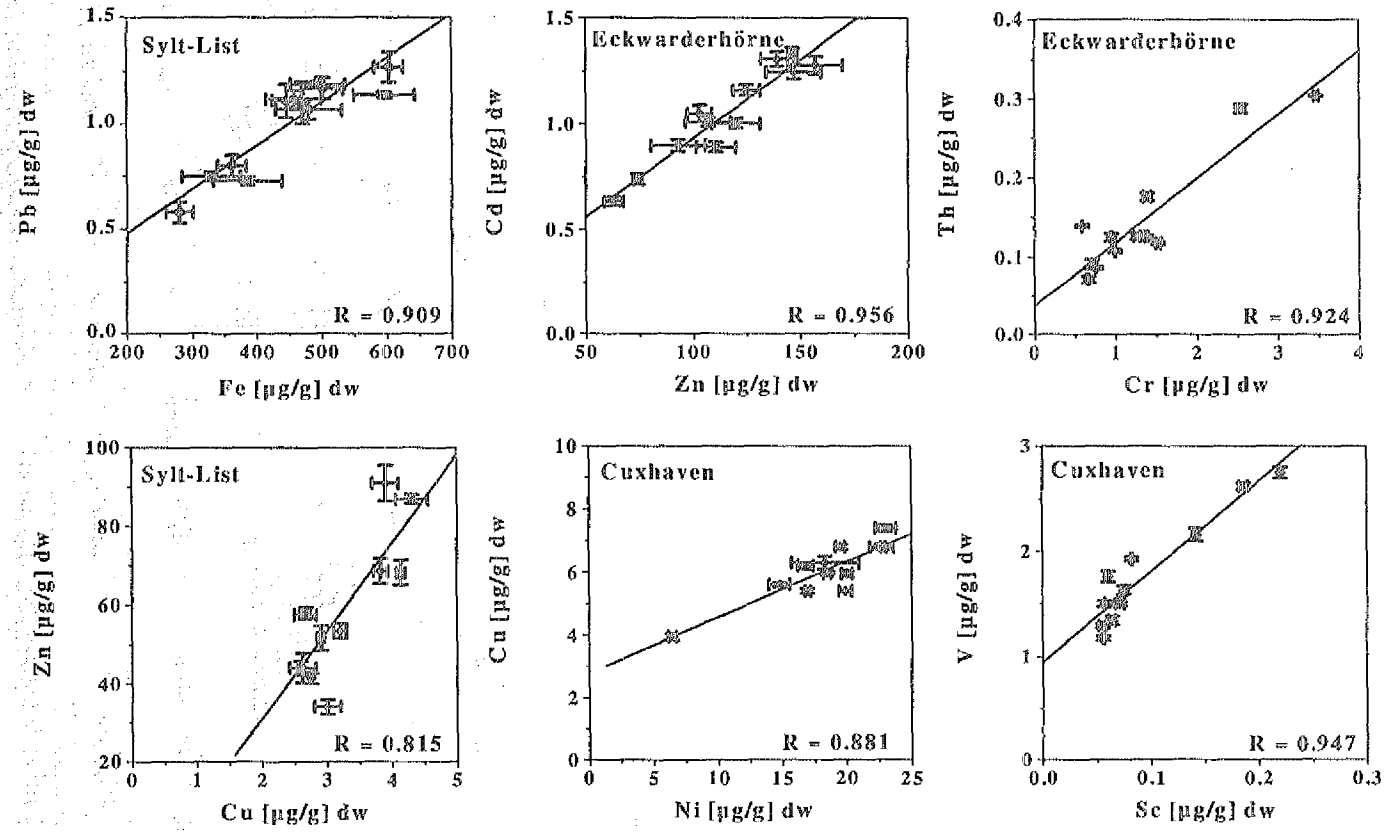



**Fig. 4.33 : Selected correlations of the seasonal variation of trace metal concentrations at different locations along the North Sea coast**

## 4.4 *Variability of trace element concentrations in different parts of Fucus vesiculosus*

Parts of algae plants of the same species grown under identical environmental conditions exhibit marked differences in their trace element concentrations. This is apparent from plant to plant of the same species, as well as from one species to another [82]. There is a tendency for increasing concentration of metals with age. Bryan [99] showed that concentrations of heavy metal tend to increase with distance from growing tips because of slow accumulation, more binding sites, and possibly some contamination of older parts with fine particles. Pedersen [10] has demonstrated a positive correlation between phenol content in *F. vesiculosus* and age of the tissues, the higher the phenol content the older the tissues. Also, Lignell et al. [38] have reported that the highest concentration of heavy metals is localized in the physodes (presumably bound to polyphenols), and in the cell walls (presumably bound to polysaccharides). Autio and Kangas [32] have concluded that samples from the top - tip and mid section - will give the accumulation in the last season and samples from the stipes give the accumulation of several years. Mathieson et al. [100] have reported that the average weight was significantly greater in spring and early summer and the reproductive periodicity of *Fucus vesiculosus* has its maximum in April. Four vegetative characters - length, wet weight, density of algal tissues, and area of different parts - were measured in Aug. 1994 and Feb. 1995. Data presented in Table 4.3 demonstrate that the sampling time has a limited influence on the length and the density (dw) of the algae. Weight, water content and area of the organisms collected in August were significantly higher than those collected in February. Depending on the element, as well as on the time of the year, the trace element concentrations differed between the algal tissues of different age. The *General Linear Models Procedure* was used to study the influence of sampling time and different parts of the algae collected from Eckwarderhörne on element concentrations. The model shows the importance of biological parameters in explaining the variation of element concentrations in different parts of the algae. In fact, conclusions made on the analytical data based only on dry weight are sometimes misleading, because the covariant factor such as different water content, age, and collection season are not included.

Table 4.3 : Vegetative characters of ten individual *Fucus vesiculosus* plants collected from five points in Eckwarderhörne, Cuxhaven, and Sylt-List.

| Site | Length (cm) | Weight (g) Wet | Water content (%)* Tip | Thallus | Basal | Density (g/cm³ dw)* Tip | Thallus | Basal | Area (m²)* Tip | Thallus | Basal |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Eck. Feb. 95* | 22.4 ± 4.5 | 8.8 ± 4.8 | 17 | 9 | 18 | 1.07 | 1.07 | 0.94 | 0.015 | 0.037 | 0.002 |
| | 24.3 ± 6.6 | 8.6 ± 5.0 | | | | | | | | | |
| | 25.2 ± 7.3 | 12.4 ± 4.8 | | | | | | | | | |
| | 25.0 ± 5.0 | 7.3 ± 3.5 | | | | | | | | | |
| | 24.1 ± 1.4 | 9.8 ± 6.1 | | | | | | | | | |
| *Aug. 94* | 26.8 ± 5.5 | 19.4 ± 12.8 | 88 | 65 | 56 | 1.02 | 1.06 | 0.98 | 0.026 | 0.056 | 0.004 |
| | 26.5 ± 9.9 | 27.8 ± 15.4 | | | | | | | | | |
| | 29.7 ± 9.9 | 27.7 ± 11.2 | | | | | | | | | |
| | 28.4 ± 5.7 | 32.5 ± 13.2 | | | | | | | | | |
| | 26.7 ± 6.9 | 26.8 ± 17.7 | | | | | | | | | |
| *Cux. Feb. 95* | 20.2 ± 4.4 | 10.6 ± 4.6 | 19 | 13 | 7 | 1.06 | 1.04 | 1.03 | 0.015 | 0.021 | 0.004 |
| | 15.6 ± 3.8 | 7.8 ± 2.8 | | | | | | | | | |
| | 18.3 ± 5.4 | 4.9 ± 1.6 | | | | | | | | | |
| | 21.4 ± 5.0 | 5.7 ± 2.2 | | | | | | | | | |
| | 20.9 ± 4.4 | 5.9 ± 1.8 | | | | | | | | | |
| *Aug. 94* | 24.8 ± 5.5 | 23.4 ± 12.5 | 89 | 68 | 63 | 1.01 | 0.97 | 0.98 | 0.022 | 0.043 | 0.006 |
| | 29.0 ± 10.2 | 35.1 ± 18.0 | | | | | | | | | |
| | 24.9 ± 7.5 | 43.2 ± 21.4 | | | | | | | | | |
| | 37.6 ± 8.3 | 54.1 ± 29.9 | | | | | | | | | |
| | 32.0 ± 7.6 | 37.4 ± 20.8 | | | | | | | | | |
| *Sylt-List Feb. 95* | 23.1 ± 2.9 | 5.2 ± 1.2 | 17 | 11 | 9 | 1.05 | 1.07 | 1.04 | 0.016 | 0.03 | 0.003 |
| | 22.5 ± 5.8 | 8.8 ± 3.2 | | | | | | | | | |
| | 21.0 ± 3.6 | 12.0 ± 4.9 | | | | | | | | | |
| | 45.0 ± 10.1 | 13.5 ± 5.5 | | | | | | | | | |
| | 32.0 ± 7.8 | 9.2 ± 4.7 | | | | | | | | | |
| *Aug. 94* | 39.2 ± 3.2 | 18.8 ± 4.5 | 83 | 67 | 59 | 1.06 | 0.96 | 1.02 | 0.022 | 0.052 | 0.007 |
| | 41.7 ± 10.7 | 30.5 ± 17.9 | | | | | | | | | |
| | 38.4 ± 6.7 | 31.1 ± 15.5 | | | | | | | | | |
| | 37.3 ± 8.4 | 26.7 ± 15.0 | | | | | | | | | |
| | 32.7 ± 9.2 | 25.5 ± 15.9 | | | | | | | | | |

◉ = is the mean value

Graphical representations based on the F-value (Fig.4.34) demonstrates that some of the elements are more influenced by the part (such as Na, Co, As, Rb, Ag, Sn, and Ba) while others (P, Sc, V, Cr, Fe, Cd, Cs, and U) are influenced by the collection season. Also, the combination effect of the parts and the sampling season strongly affects P, Ag, and Cd. However, pooling of several individual specimen into homogenates of different parts, tip, thallus, and basal parts may lead to more reliable estimates of concentration levels.

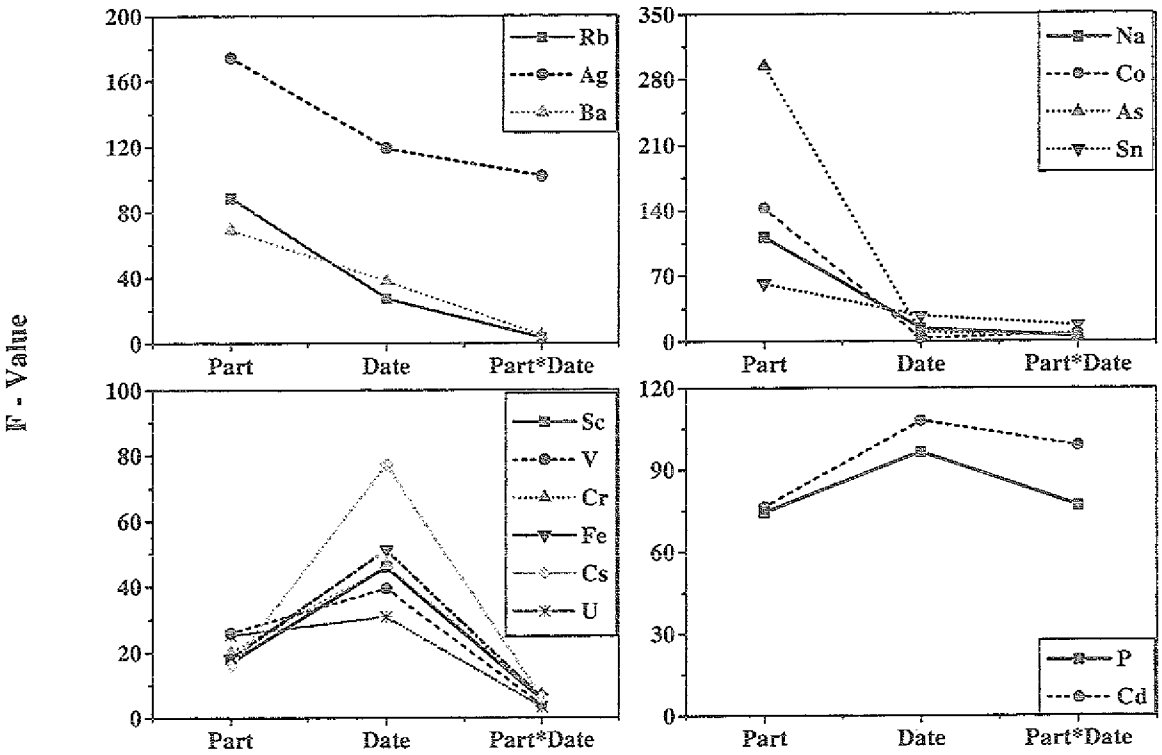



**Fig. 4.34 : Representative effects on parts of algae, collection date, and combination of both (part*date) on the element concentrations.**

### 4.4.1 *Comparison between element concentrations in parts of algae at different sampling times*

Fig. 4.35 (a-b) shows the various biological and physiological covariables such as age, sampling season, as well as the variability of the element concentrations between the individual groups from Eckwarderhörne. An increase in the variance is an indication of elevated heterogeneity in the individual group before detecting differences in the mean values between the parts of the algae. Three groups of elements are observed ;

group 1: elements showing higher concentration in samples collected in August than in February

Tip : Sc, Cr, Fe, Rb, Cs, Pb, V

Thallus : Na, Sc, V, Cr, Fe, Ag, Sn, Pb, Cs

Basal : Sc, V, Cr, Fe, Co, U, Cs, Pb

group 2: elements showing similar concentrations in both seasons

Tip : As, Na, V, U, Co, Sn

Thallus : As, P, Co, U, Rb, Cd

Basal : As, Na, P, Rb, Cd, Sn, Ba

group 3: elements showing lower concentration in samples collected in Aug. than in Feb.

Tip : P, Ag, Cd, Ba

Thallus : Ba

Basal : Ag

It is of great interest to recognize that Sc, V, Cr, Fe, Sn, Cs, Pb, and U having the same distribution of element concentration patterns. This result confirms what was found in the PCA analysis for whole plant collected from different collection sites. Also, older parts obviously contained considerably higher concentrations.

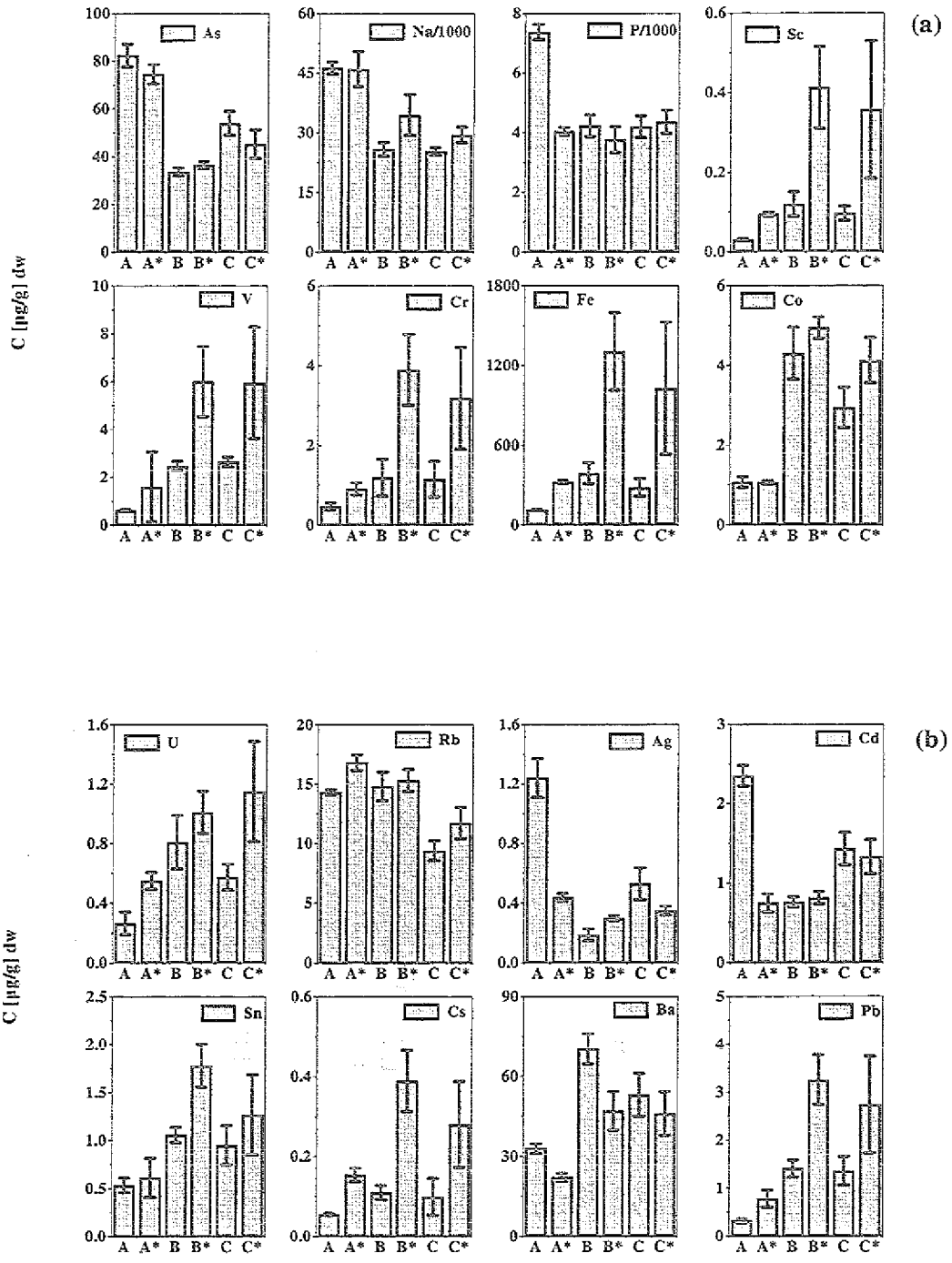



Fig. 4.35 (a-b) : Influence of calculated mean of the individual plants collected at each point and SD (representing the biological variability of the elements within the different parts).

| | | | | | |
|---|---|---|---|---|---|
| A | = | Tip Feb. 95 | A* | = | Tip Aug. 94 |
| B | = | Thallus Feb. 95 | B* | = | Thallus Aug. 94 |
| C | = | Basal Feb. 95 | C* | = | Basal Aug. 94 |

### 4.4.2 *Comparison between element concentrations in parts of algae from different sampling areas and different sampling times*

The selected element patterns in tip, thallus, and basal parts, in two different collection seasons and the three sampling areas are shown in Fig. 4.36 (a-d). There are obviously variations similar to or even greater than that found for Eckwarderhörne in both Cuxhaven and Sylt-List. It was found that the partial concentration patterns of the elements Co, Cu, Ni, Se, and Hg are almost the same with respect to the collection areas and collection seasons. It is true that the single extremes will influence the mean values, however, the biological variability may greatly exceed the analytical precision as has been shown in the different algal parts in Eckwarderhörne in comparison with Cuxhaven and Sylt-List. The comparison shows that a substantial part of the variation in concentration levels in the algae could be explained by collection season, as well as the tissue of the part. Among older parts both low and high concentrations are found whereas among tips (younger parts) low concentrations are found except for Cu, As, Ag, Zn, and Cd. This may be explained by differences in the reactivity of uptake routes in the cell walls of the surface between the different parts. The tips of the algae are fairly stationary and hence representative of the collection area and presumably homogeneous with respect to exposure to trace element concentration in the ambient water. In fact the surface area is very important if we consider the impact of suspended particulate matter. The greater the ratio of the surface area to the weight or volume of the thallus, the greater the fraction of cells involved in the uptake of ions from the environment.

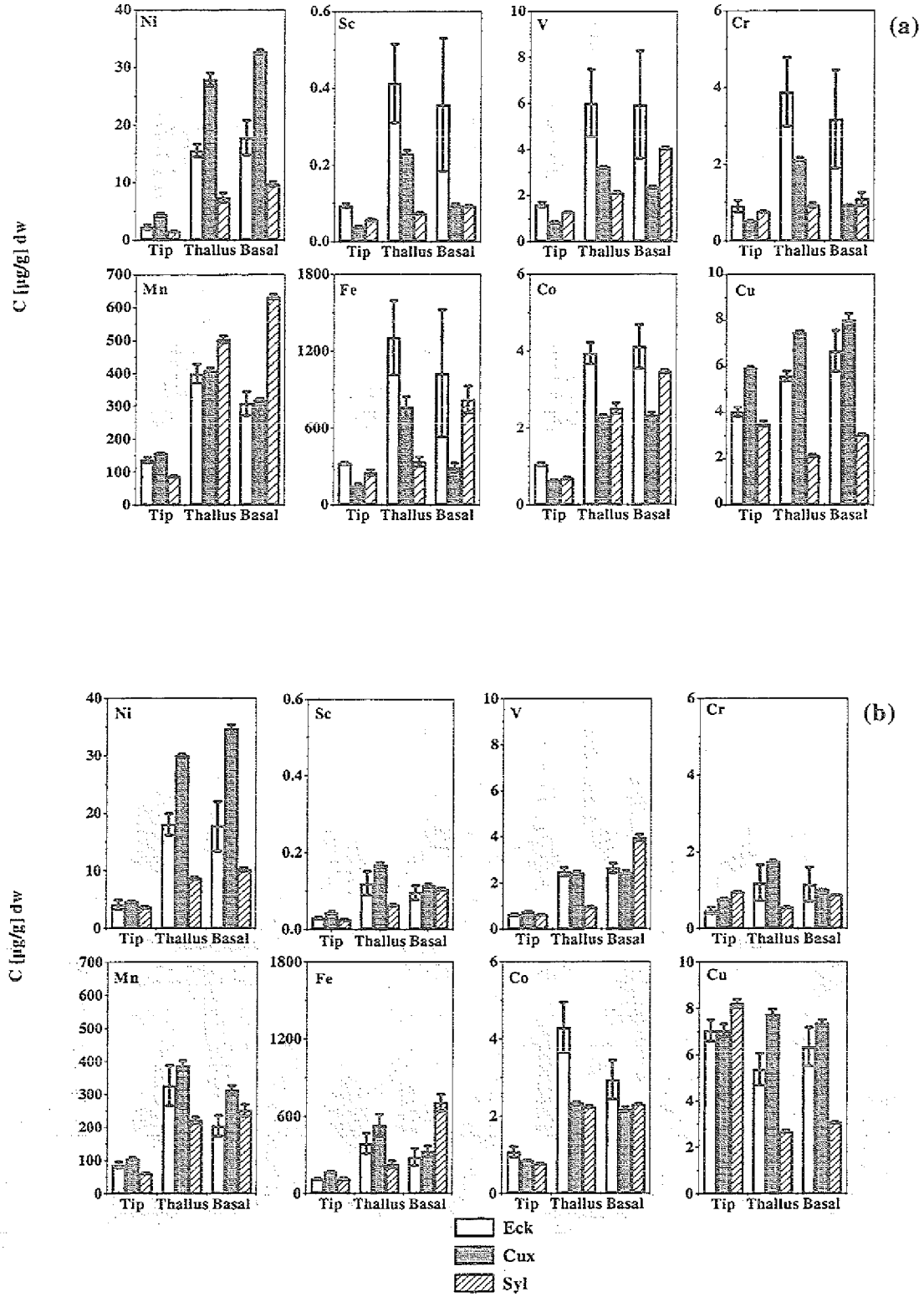
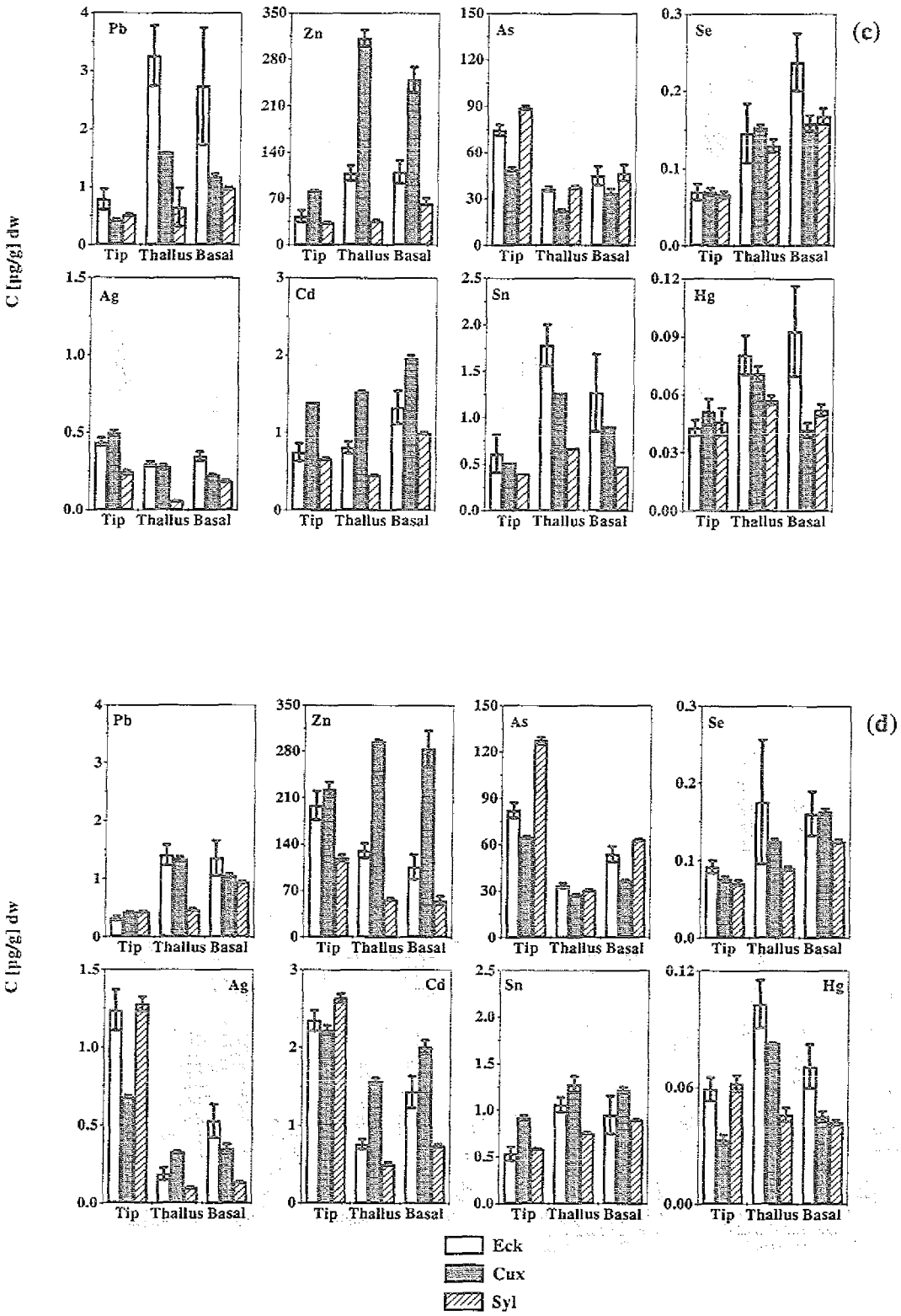



Fig. 4.36 (a-d) : Comparison between mean of the individual collection points in Eckwarderhörne (n = 5, and SD representing the biological variability of elements within different parts) and mean ± SD of concentration in different parts from Cuxhaven and Sylt-List in different collection seasons.
a and c = Aug. 94, b and d = Feb. 95




Concentrations of some selected elements from the investigated areas with respect to algal parts and collection seasons are presented in Table 4.4 a-c. Only the elements of different variation patterns in Cuxhaven and Sylt-List - from that of Eckwarderhörne were presented in Table 4.4 (b-c).

Table 4.4 (a-c) : Concentration of selected elements [µg/g dw] with respect to different parts of the algae collected in Aug. 1994 and Feb. 1995 ( *** > ** > * )

a- Eckwarderhörne

| Element | Date | Tip | Thallus | Basal |
|---|---|---|---|---|
| Sc | Aug.94 | * | *** | ** |
| | Feb.95 | * | *** | ** |
| V | Aug.94 | * | *** | ** |
| | Feb.95 | * | ** | *** |
| Cr | Aug.94 | * | *** | ** |
| | Feb.95 | * | ** | *** |
| Mn | Aug.94 | * | *** | ** |
| | Feb.95 | * | *** | ** |
| Fe | Aug.94 | * | *** | ** |
| | Feb.95 | * | *** | ** |
| Co | Aug.94 | * | ** | *** |
| | Feb.95 | * | *** | ** |
| Cu | Aug.94 | * | ** | *** |
| | Feb.95 | *** | * | ** |
| Ni | Aug.94 | * | ** | *** |
| | Feb.95 | * | *** | ** |
| Zn | Aug.94 | * | ** | *** |
| | Feb.95 | *** | ** | * |
| As | Aug.94 | *** | * | ** |
| | Feb.95 | *** | * | ** |
| Se | Aug.94 | * | ** | *** |
| | Feb.95 | * | *** | ** |
| Ag | Aug.94 | *** | * | ** |
| | Feb.95 | *** | * | ** |
| Cd | Aug.94 | * | ** | *** |
| | Feb.95 | *** | * | ** |
| Sn | Aug.94 | * | *** | ** |
| | Feb.95 | * | *** | ** |
| Cs | Aug.94 | * | *** | ** |
| | Feb.95 | * | *** | ** |
| Ba | Aug.94 | * | *** | ** |
| | Feb.95 | * | *** | ** |
| Hg | Aug.94 | * | ** | *** |
| | Feb.95 | * | *** | ** |
| Pb | Aug.94 | * | *** | ** |
| | Feb.95 | * | *** | ** |

From Table (4.4) it is clear that some elements could be efficiently detected in the tip parts. These elements are Cr, Cu, Zn, and Cd in February as well as As and Ag in February and August. Also, the concentration levels in the different parts differed from one site to another, but it was found that the difference in distribution patterns of element concentrations between parts is less pronounced in Eckwarderhörne and Cuxhaven reflecting the common estuary characteristics. However, Zn, As, Ag, and Ba show common distribution patterns with different concentration levels in Eckwarderhörne and Sylt-List, and Ni and Se in Sylt-List and Cuxhaven. Also, the high metal concentration in *F. vesiculosus* basal is due to slow and irreversible accumulation [8, 31, 101].

b- Cuxhaven

| Element | Date | Tip | Thallus | Basal |
|---|---|---|---|---|
| Cu | Aug.94 | * | ** | *** |
| | Feb.95 | * | *** | ** |
| Ni | Aug.94 | * | ** | *** |
| | Feb.95 | * | ** | *** |
| Zn | Aug.94 | * | *** | ** |
| | Feb.95 | * | *** | ** |
| Se | Aug.94 | * | ** | *** |
| | Feb.95 | * | ** | *** |
| Ag | Aug.94 | *** | ** | * |
| | Feb.95 | *** | * | ** |
| Cs | Aug.94 | ** | *** | * |
| | Feb.95 | * | *** | ** |
| Ba | Aug.94 | * | ** | *** |
| | Feb.95 | * | *** | ** |
| Hg | Aug.94 | ** | *** | * |
| | Feb.95 | * | *** | ** |

c- Sylt-List

| Element | Date | Tip | Thallus | Basal |
|---|---|---|---|---|
| Sc | Aug.94 | * | *** | ** |
| | Feb.95 | * | ** | *** |
| V | Aug.94 | * | ** | *** |
| | Feb.95 | * | ** | *** |
| Cr | Aug.94 | * | ** | *** |
| | Feb.95 | *** | * | ** |
| Mn | Aug.94 | * | ** | *** |
| | Feb.95 | * | ** | *** |
| Fe | Aug.94 | * | ** | *** |
| | Feb.95 | * | ** | *** |
| Co | Aug.94 | * | ** | *** |
| | Feb.95 | * | ** | *** |
| Cu | Aug.94 | *** | * | ** |
| | Feb.95 | *** | * | ** |
| Ni | Aug.94 | * | ** | *** |
| | Feb.95 | * | ** | *** |
| Se | Aug.94 | * | ** | *** |
| | Feb.95 | * | ** | *** |
| Cd | Aug.94 | ** | * | *** |
| | Feb.95 | *** | * | ** |
| Sn | Aug.94 | * | *** | ** |
| | Feb.95 | * | ** | *** |
| Cs | Aug.94 | *** | ** | * |
| | Feb.95 | * | ** | *** |
| Hg | Aug.94 | * | *** | ** |
| | Feb.95 | * | *** | ** |
| Pb | Aug.94 | * | ** | *** |
| | Feb.95 | * | ** | *** |

Bryan and Hummerstone [8] reported that the concentration of Cu, Zn, Mn, Fe, and Pb in older parts is much higher than in tips. Also, Tomlinson et al. [31] showed that Cu is higher in concentration in the frond than in reproductive vesicles or stipes, which is in agreement with data from Sylt-List and Cuxhaven, but only with data from Feb. 95 in Eckwarderhörne. Autio and Kangas [32] reported

consistently increasing Zn and Cu concentrations from top to the stipes of *F. vesiculosus*. Forsberg et al. [14] reported that the content of Al, Fe, Mn, Zn, Ni, and Co in older parts of the thallus significantly exceeds those of the growing tips. Cr shows a similar trend, but no tendency could be seen for Cd, Cu, and Pb. On the other hand, Carlson and Erlandsson [28] noted that the highest concentrations of $^{60}Co$ and $^{54}Mn$ were measured in the older parts of the plants in spring and summer, in agreement with the results shown in Table 4.4, while the radioactivity concentrations of $^{137}Cs$, and $^{40}K$ were highest in receptacles and new vegetative fronds, which is in agreement with data in samples from Cuxhaven and Sylt-List in case of Cs. The analysis shows that in Eckwarderhörne the following groups have the same gradient with respect to part and season: (Sc, Mn, Fe, Sn, Cs, Ba, and Pb), (As and Ag), (V and Cr), (Se and Hg), and (Cu and Cd). Johansen et al. [35] suggested that growing tips of seaweed reflect the dispersion of Pb and Zn from the sources at the mine site, and concluded that in the case of the presence of the competitive-chemically similar Zn and Pb, monitoring for Cd using biological indicators such *Fucus vesiculosus* will not be very helpful. Rönnberg et al. [102] reported that the content of Fe is 2-3 times higher in the growing tip than in basal parts of *Fucus vesiculosus*, and for Zn 1.5 times higher in the basal parts than in growing tips. This contrasts with the measured results of Fe in the different parts, but in the case of Zn it was found that the ratio between the concentration in basal to that of tip in Aug. 94 and Feb. 95 in Eckwarderhörne is (2.6 and 0.5), in Cuxhaven (3 and 1.3), and in Sylt-List (1.84 and 0.5), respectively. Forsberg et al. [14] reported significantly higher contents of Fe and Zn were found in older thallus parts than in growing tips, and a Cu concentration increased at wave-exposed sites. This is in agreement with the result found in Cuxhaven as an exposed site. In growing tips of *Fucus vesiculosus* the variation in metal levels between samples taken at different times of the year was found to be much greater than that between samples collected at the same time for Cd and Zn (Eckwarderhörne). However, for Cu the ratio between tip in Feb. 95 to that in Aug. 94 was found to be approximately twofold in contrast to that of Pb. Also, Barreiro et al. [90] proved that mature tissues accumulate more Fe, Al, Cr, Zn, Ni, Mn, and to lesser extent Co than younger tissues,

whereas, this isn't statistically significant for Cu. In Table 4.5 many of the reported results showed that the samples were washed before the drying process.

**Table 4.5 : Washing treatment of algae samples before drying**

| Type of washing | Reference |
|---|---|
| Sea water | [8, 32, 36, 66, 67, 78, 91] |
| Distilled water | [13, 19, 23, 35, 98] |
| Buffer pH 7.2 | [38] |
| Not known | [14, 64, 65, 102] |
| Tap water | [103] |
| Tap water homogenized in distilled water | [90] |

Ledent et al. [103] showed that rinsing the leaves of Mediterranean seagrass *Posidonia oceanico* (L) results in significant element leakage depending on the leaf age. In the adult leaf only Fe concentrations are reduced, but in younger leaves significant concentration reductions occur for S, Mg, Na, Zn, Pb, Cd, Fe, Cr, and Ti, while concentrations of N, P, Ca, and Cu remain unaffected. Sea water rinsing will result in estimations of total element content (including surface water and water in free space), while rinsing with distilled water will lead to an underestimation of cellular content. Of course, all these results cannot be compared and cause confusion about the reliability of the results, but on the other hand every group aims at the general informative data of the analysis. Therefore, standard operating procedures should be adopted when using the bioindicator for monitoring programs. A study using multivariate, principal component analysis (PCA) on data of both the collection seasons and the element concentrations in different algae parts in Eckwarderhörne shows that the first component PCA1 summarizes 53.3 % of the variation in the data set. The cumulative percentage of the second component PCA2 is 68.3 %. The analysis indicates that tips in both collection seasons were the lowest in variance, while the thallus and the basal parts of Aug. 94 were the highest in variance as shown in Fig. 4.37.

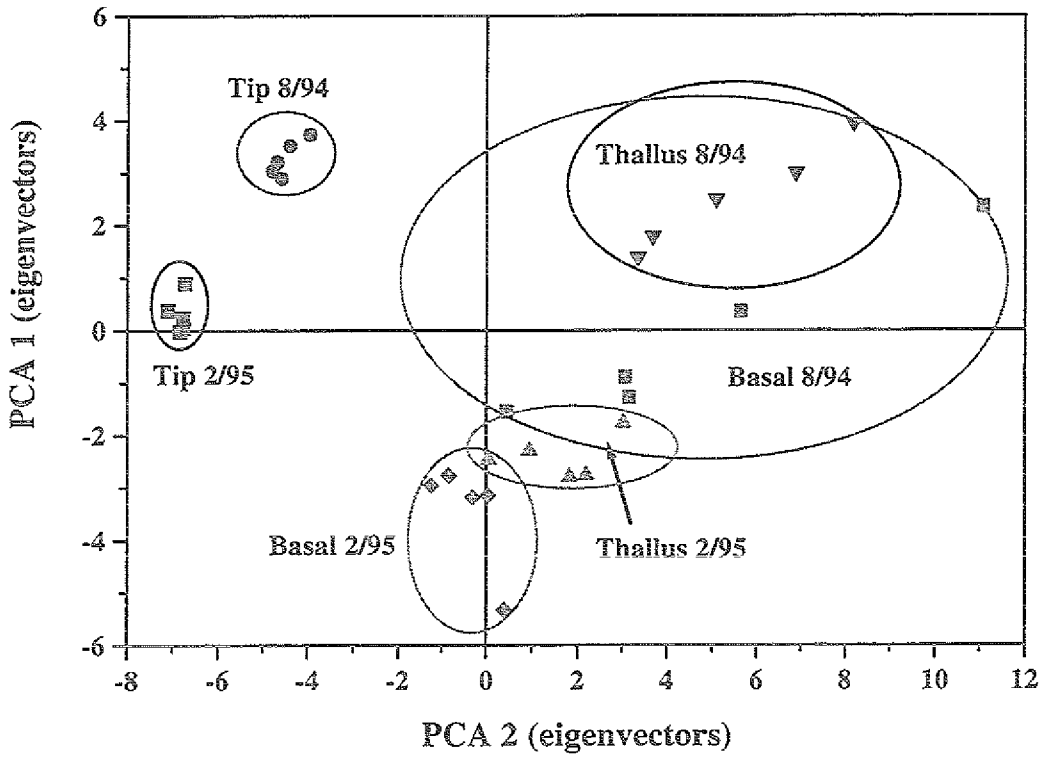



**Fig. 4.37 : Range of element concentrations in different collection seasons and different parts of algae from Eckwarderhörne.**

Older parts of the algae, e.g. thallus and basal parts, are a mixture of tissues which have been exposed for a longer and/or shorter time period making the element concentration heterogeneous with respect to the uptake, metabolism, catabolism, transport, storage of the elements, etc. Table 4.6 shows the coefficients of variation of element concentrations in different parts which represent the influence of biological variability on the element concentrations. Various explanations of this effect have been proposed [e.g. 8, 14], ranging from intrinsic characteristics of the algae (such as slow accumulation rates or a greater number of binding sites in older tissues) to factors such as higher particulate contamination or higher epiphytes present on older plant parts. Carlson and Erlandsson [28] have reported that the variation in radioactivity concentrations of $^{137}Cs$, $^{60}Co$, $^{54}Mn$, and $^{65}Zn$ in new vegetation tips of ten *Fucus vesiculosus* algae was ≥ 17 %, and the patterns of $^{54}Mn$ and $^{65}Zn$ accumulation were similar.

This study provides convincing evidence that, in the presence of biological variation, there are correlations between the element concentrations in the different parts of the algae in different seasons. Disregarding the biological variabilities in sampling seasons, correlations are indication of existence of similarities or differences in the element concentration patterns within the various group of parts. Fig. 4.38 (a-c) shows that knowledge of the biological variability within the selected matrix is a prerequisite to evaluate the usefulness of monitoring spatial and temporal changes in element concentrations. Also, the possibility of bio-magnification could be obtained by determining the concentration in representative parts of algae. One possible explanation of the increased variance in older parts is the age-dependence enrichment, in which older parts do not only represent the sampling area, but also many different seasons to which they have been exposed. On the other hand, due to physico-chemical processes, the major part of the heavy metals introduced into a water system is deposited in the sediment, where incorporation process with the organic substances and synthetic organic compounds which have been released by biodegradation take place causing enrichment by fixation of the fine sediment on the surface. The correlation of element concentrations represent the weighted arithmetic mean of its constituent in each individual parts, thus it is possibly not caused by the time of exposure but by variations in the uptake routes. However, there are distinct correlations between metal incorporation in different parts and overall weight.

Table 4.6 : Coefficient of variation (C.V. = SD/Mean) of element concentrations within different parts in Fucus vesiculosus

| Element | Date | Tip | Thallus | Basal | Element | Date | Tip | Thallus | Basal |
|---|---|---|---|---|---|---|---|---|---|
| Na | Aug.94 | 10 | 15 | 7 | Zr | Aug.94 | 21 | 22 | 32 |
| | Feb.95 | 3 | 6 | 4 | | Feb.95 | 15 | 41 | 16 |
| Mg | Aug.94 | 4 | 5 | 9 | Ag | Aug.94 | 6 | 5 | 8 |
| | Feb.95 | 3 | 2 | 2 | | Feb.95 | 11 | 21 | 20 |
| P | Aug.94 | 3 | 12 | 9 | Cd | Aug.94 | 15 | 10 | 16 |
| | Feb.95 | 4 | 9 | 9 | | Feb.95 | 6 | 8 | 14 |
| S | Aug.94 | 5 | 6 | 6 | Sn | Aug.94 | 33 | 13 | 33 |
| | Feb.95 | 2 | 5 | 5 | | Feb.95 | 14 | 7 | 21 |
| K | Aug.94 | 6 | 18 | nd | Sb | Aug.94 | 76 | 8 | 32 |
| | Feb.95 | 5 | 7 | 13 | | Feb.95 | 67 | 8 | 56 |
| Ca | Aug.94 | 3 | 15 | 13 | Te | Aug.94 | 15 | 8 | 20 |
| | Feb.95 | 3 | 3 | 12 | | Feb.95 | 23 | 23 | 24 |
| Sc | Aug.94 | 5 | 25 | 48 | Cs | Aug.94 | 11 | 20 | 39 |
| | Feb.95 | 8 | 26 | 19 | | Feb.95 | 4 | 16 | 46 |
| V | Aug.94 | 6 | 24 | 40 | Ba | Aug.94 | 7 | 15 | 18 |
| | Feb.95 | 7 | 7 | 8 | | Feb.95 | 6 | 8 | 15 |
| Cr | Aug.94 | 17 | 23 | 40 | Hf | Aug.94 | 29 | 39 | 53 |
| | Feb.95 | 18 | 39 | 39 | | Feb.95 | 26 | 61 | 38 |
| Mn | Aug.94 | 6 | 7 | 12 | Hg | Aug.94 | 10 | 13 | 25 |
| | Feb.95 | 11 | 19 | 15 | | Feb.95 | 10 | 12 | 16 |
| Fe | Aug.94 | 3 | 22 | 48 | Pb | Aug.94 | 23 | 16 | 38 |
| | Feb.95 | 3 | 21 | 24 | | Feb.95 | 13 | 13 | 22 |
| Co | Aug.94 | 4 | 7 | 14 | Ce | Aug.94 | 11 | 31 | 46 |
| | Feb.95 | 13 | 15 | 17 | | Feb.95 | 13 | 16 | 19 |
| Ni | Aug.94 | 17 | 7 | 16 | Sm | Aug.94 | 14 | 36 | 25 |
| | Feb.95 | 19 | 10 | 24 | | Feb.95 | 27 | 12 | 25 |
| Cu | Aug.94 | 5 | 4 | 13 | Eu | Aug.94 | 15 | 45 | 60 |
| | Feb.95 | 7 | 13 | 13 | | Feb.95 | 5 | 10 | 10 |
| Zn | Aug.94 | 21 | 10 | 16 | Tb | Aug.94 | 6 | 23 | 38 |
| | Feb.95 | 11 | 8 | 18 | | Feb.95 | 11 | 15 | 22 |
| As | Aug.94 | 5 | 4 | 13 | Yb | Aug.94 | 20 | 30 | 43 |
| | Feb.95 | 6 | 5 | 9 | | Feb.95 | 51 | 21 | 12 |
| Se | Aug.94 | 15 | 30 | 20 | Th | Aug.94 | 13 | 28 | 53 |
| | Feb.95 | 10 | 50 | 20 | | Feb.95 | 9 | 38 | 26 |
| Br | Aug.94 | 5 | 7 | 39 | U | Aug.94 | 10 | 14 | 29 |
| | Feb.95 | 3 | 6 | 17 | | Feb.95 | 29 | 22 | 15 |
| Rb | Aug.94 | 4 | 6 | 11 | | | | | |
| | Feb.95 | 2 | 8 | 9 | | | | | |

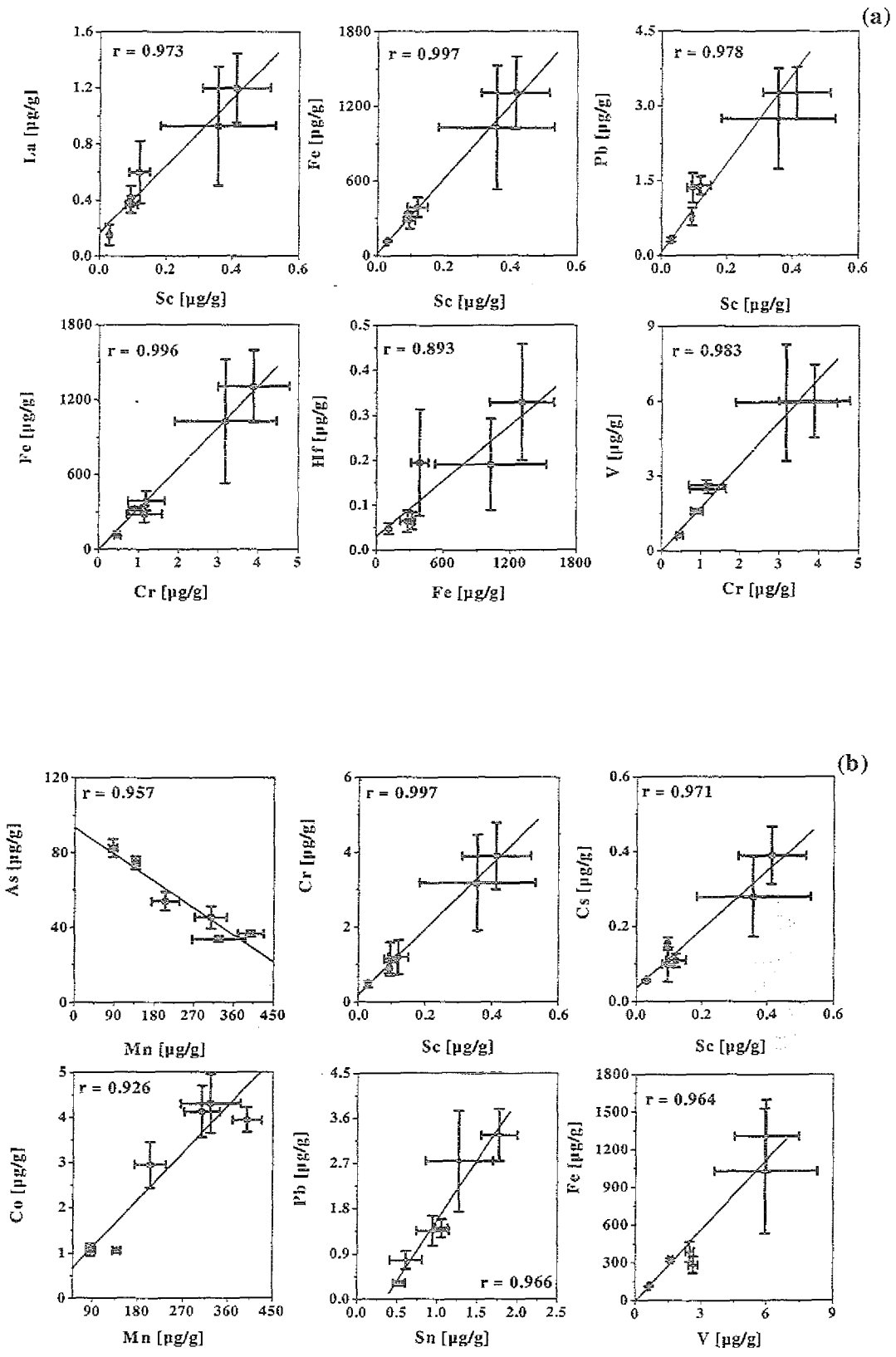
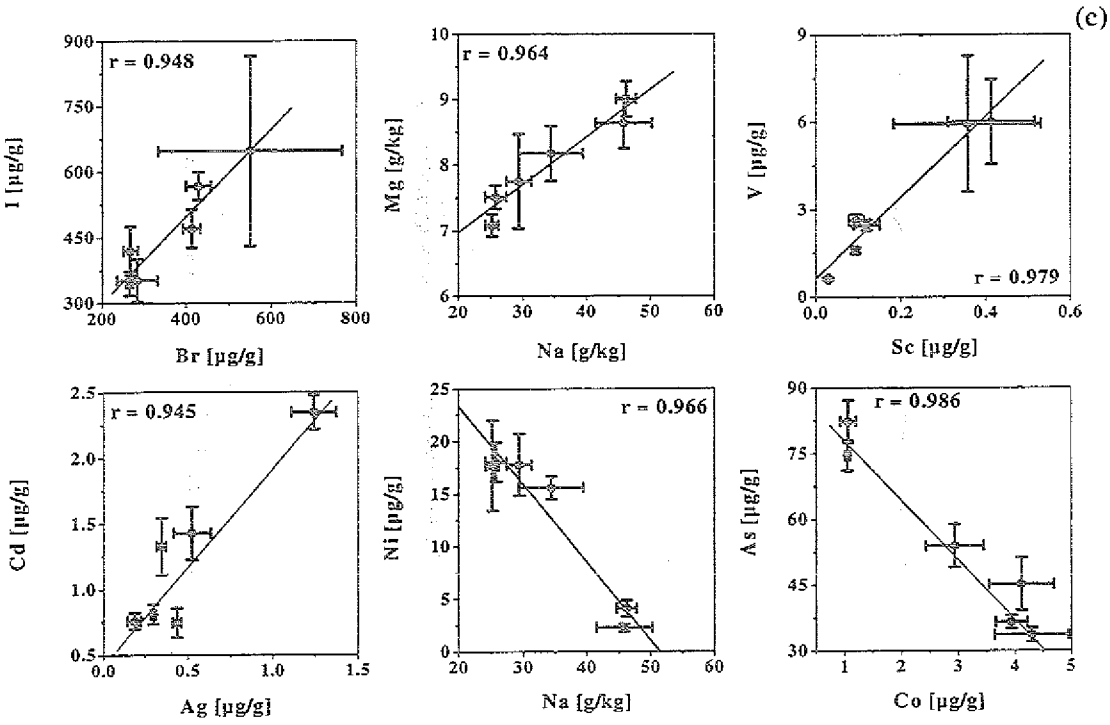



Fig. 4.38 (a-c) : Element correlations including the biological variability in different parts of the brown algae *Fucus vesiculosus* from Eckwarderhörne.




### 4.4.3 *Contribution ratio of concentrations in different parts to the total content*

In order to assess the variability in older parts, the contribution of the different parts to the total content of the element in the whole plant (in fresh weight) was calculated and the results are presented in Fig. 4.39 (a-c). It is obvious that the thallus part contributes the most to the total concentrations in both collection seasons, but for the elements Na, Mg, Cu, Zn, As, Br, Ag, Cd, and I the tips have the higher contribution in Feb. 95. Although the basal parts in (Fig. 4.32 and 4.33) indicate a higher concentration and less water content compared to the tip and thallus parts, in most of the elements they contribute on average about 8 % and 11.8 % in Aug. 94 and Feb. 95, respectively. In addition, the new growth throughout the season constitutes about 80 % of the sample element concentrations caused by increasing in vegetation. Therefore, the surface area plays key role in this contribution ratio as shown in Table (4.3). For some selected elements from Cuxhaven and Sylt-List, the contribution ratio appears to reveal a substantial decrease in the contribution of the thallus part and increase in both tip and basal parts.

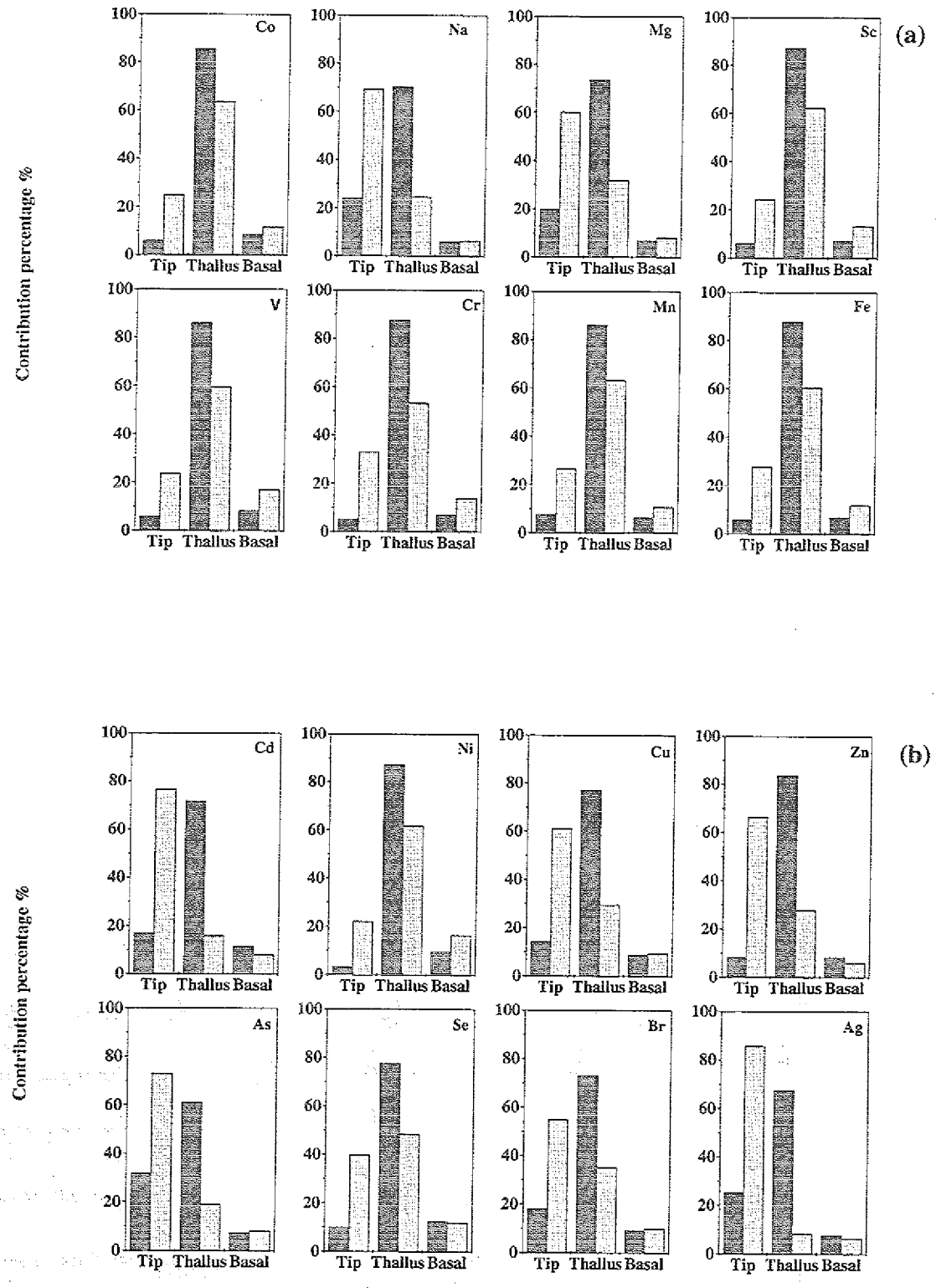
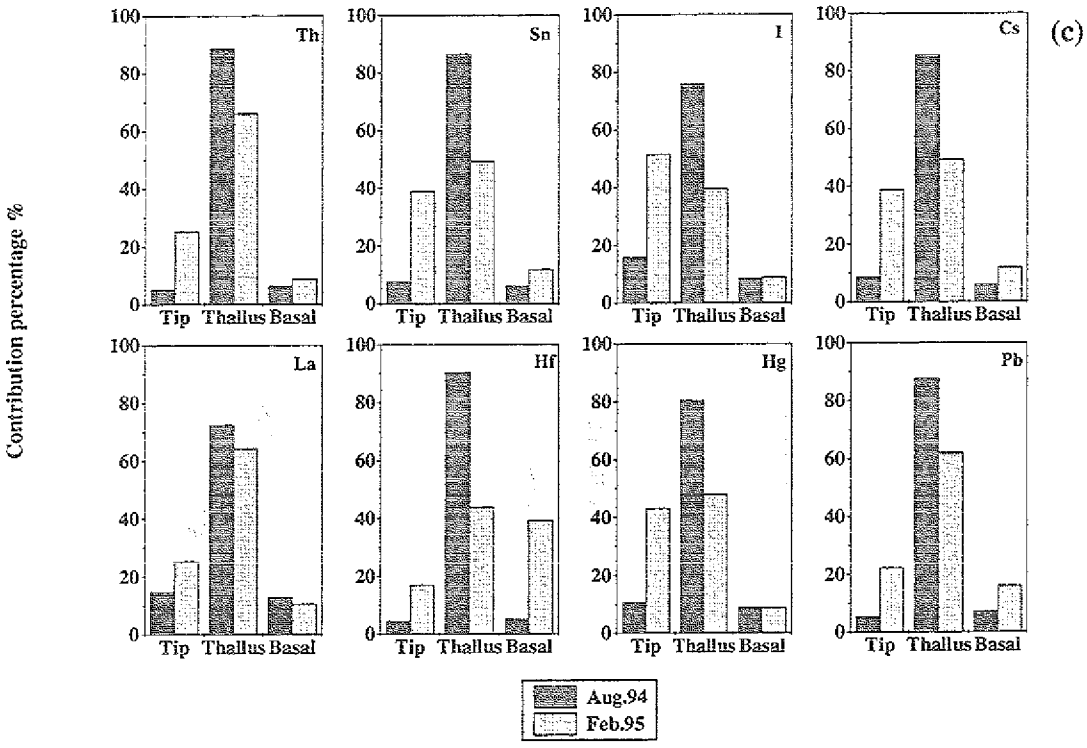



Fig. 4.39 (a-c) : The contribution of different parts to the content of whole plant of *Fucus vesiculosus* from Eckwarderhörne.




Johansen et al. [35] reported that Cd values in whole plants and growing tips do not differ significantly. For Cu values in whole plants and growing tips differ significantly, on the average 23 % lower in growing tips than the whole plant. For Pb and Zn concentration values in whole plants and growing tips differ significantly, on the average 44 % and 36 % lower in growing tips than in the whole plant respectively. In the case of Cuxhaven, the negative potential difference of the inner cell wall increases with lower salinity and this consequently increases the ion transport into the algae, on the other hand, in Sylt-List the high salinity and alters the metal bioavailability and the enrichment factor. In Fig. 4.40 (a-b), the tip indicates a higher contribution ratio for the elements Cu, As, and Cd, in Cuxhaven and Sylt-List than was found in Eckwarderhörne. Knowledge of the factors explaining the variation within and between the collection areas increases the possibilities of producing comparable or well defined specimens for monitoring programs. However, there is a pattern of morphological variation linked with plant habitat and age, and these differences reflect small-scale local factors such as wave actions. These factors are underlain by another set of factors i.e. grazing, nutrients or seasonality which probably would not be expressed morphologically but

which show themselves in differences in physiology and/or in phonology as well as the potential accumulation of trace elements. However, the heavy metal content in the different parts should by no means be regarded as constant value but rather a factor subject to the influence of varying biotic and abiotic environmental conditions.

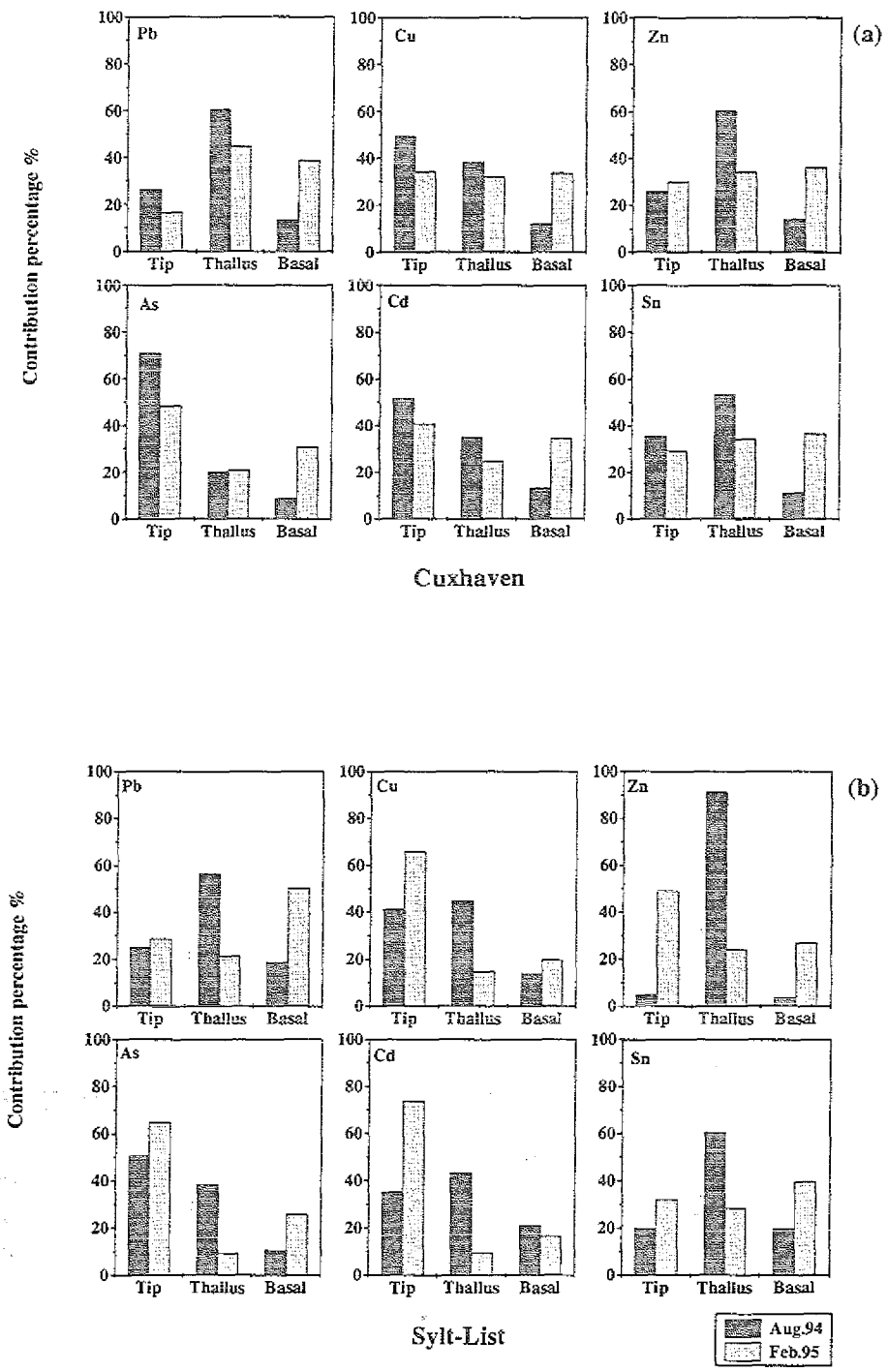



**Fig. 4.40 (a-b) : Contribution of different parts to the content of the whole *Fucus vesiculosus* plant from Cuxhaven and Sylt-List.**

### 4.5 *Estimated pollution impact of heavy metal on the North Sea coastal water*

The investigated sites Eckwarderhörne Cuxhaven, and Sylt-List, were chosen to estimate the pollution load index of the North Sea coastal water. A pollution load index [31] of each site was derived by calculating the contamination factor :

C.F. = conc. of metal in algae : base value of the metal in algae

Thereafter, the fifth root of the five highest C.Fs multiplied together is taken. Therefore,

$$\text{Pollution Load Index (PLI) for site} = \sqrt[n]{CF_1 x CF_2 \ldots\ldots x CF_n} \qquad (4\text{-}2)$$

Martin et al. [70] concluded that the use of mature thalli for the biomonitoring program would be expected to reduce any seasonal changes to a minimum, in contrast to the results in this work. However, the thallus part of *Fucus vesiculosus* collected from Sylt-List was chosen as the preliminary baseline concentration study for heavy metals, and compared with the baseline reported by Tomlinson et al. [31]. The choice of the thallus part was based on the following :

1. The thallus part is easy to identify, separate, and clean of contamination contributors.
2. The concentration levels of the elements are considered to be the integral of several seasons between 1 to 2 years.
3. The study of element concentrations in the different parts showed that the concentration in the thallus part is in the range between the tip and basal parts, except for Hg, Ba, Sn, and Sc which were higher and Cu, As, Cd, and Cr which are lower than the tip and basal parts.
4. Sylt-List is considered to be less polluted than Eckwarderhörne and Cuxhaven.

Therefore the thallus part would be a reasonable baseline for information on the pollution load indices. In Eckwarderhörne, Fig. 4.41 curve (A) shows that the highest contamination factors based on *thallus* data were found to be for Ag, Pb, Cu, Zn, and Cd in 1993, and the same elements were repeated in 1994. The data for 1995 and 1996 show other contamination factors due to V and Fe, respectively, taking into consideration that Ag was not analyzed for these years. When baseline data of Tomlinson et al. [31] were used for comparison, curve (C) showed a repeated pattern of elements except in 1995, Cd was introduced instead of Cr, and this is because Cr was not determined in those

samples of 1995. However, curve (C) clearly indicates the effect of the water column which has been influenced by the algal bedrock embedded in highly enriched surface sediment with these elements. V, Cr, Fe, Co, Ni, As, and Pb are strongly associated with particulate matter especially with precipitated iron oxyhydroxides, and effectively incorporated with sedimenting organic matter. Also, the fixation of such fine sediment is encouraged by the high surface-to-volume ratio of the filamentous algal epiphytes growing on the surface of *Fucus vesiculosus*. Therefore, the metal load of these elements driven by scavenging from particulate matter is potentially pronounced. This means that, there must be a constant factor in sampling procedures causing the appearance of these reposition patterns in each year. If the effect of the contamination factor of Fe and Mn is removed from the calculation, curve (B) shows 3 times less pollution load indices than curve (C) for each year, and is not much higher than curve (A) calculated on the basis of *thallus* data from Sylt-List.

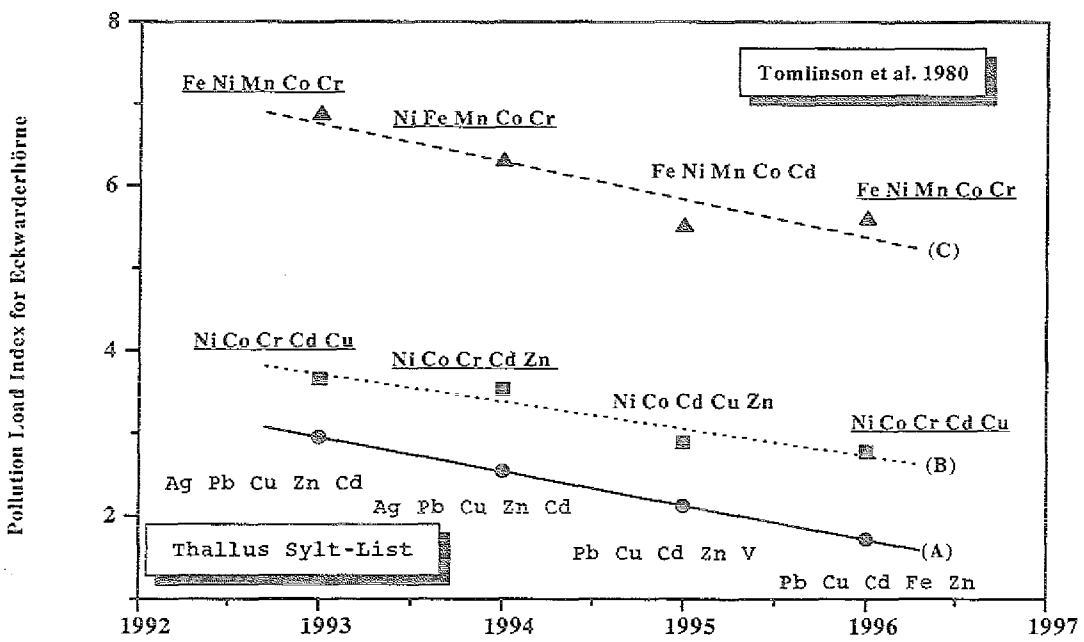



**Fig. 4.41 : Application of the pollution load index to Eckwarderhörne, (A) data based on thallus part from Sylt-List, (B) data after removal the effect of Fe and Mn, (C) data based on reported baseline by Tomlinson et al. [31]**

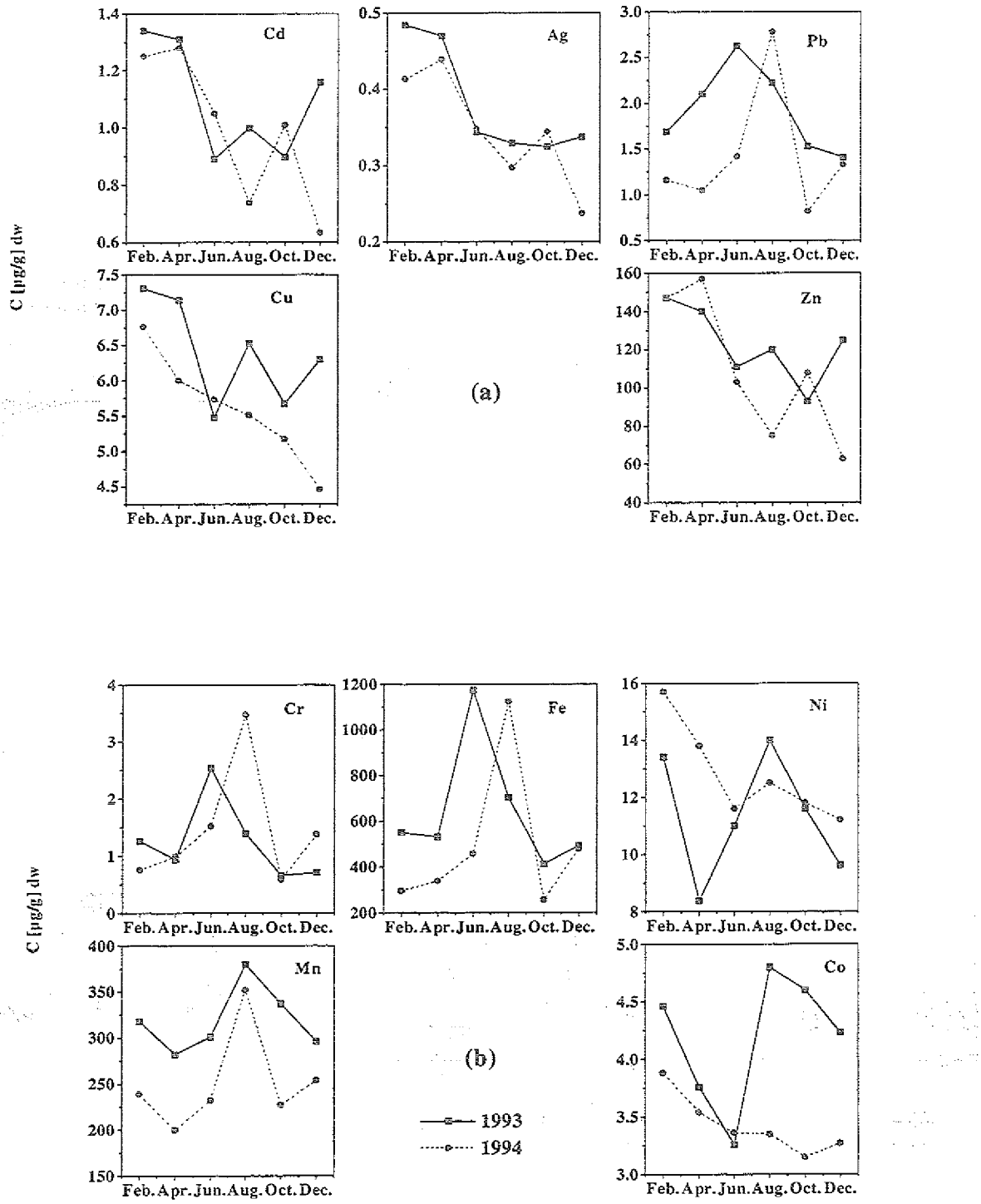



**Fig. 4.42 : Variation patterns of elements contributing the most to the pollution load index in Eckwarderhörne based on (a) data of *thallus* part from Sylt-List and (b) baseline data reported by Tomlinson et al. [31]**

The estimated pollution load indices in Eckwarderhörne indicate decreasing metals enrichment at the site in the course of the years. In Fig. 4.42 (a), it is obvious that correlated variation patterns between the elements contributing the most to the suggested pollution load index, but also some of

these variation patterns changed between the two years. These correlated variation patterns are Cd, Cu, and Zn in 1993, and Cd, Ag, and Zn in 1994. On the other hand, the Co variation pattern in 1993 (Fig. 4.42 b) correlates with Ni in 1993 but not the case in 1994. Mn shows periodical variation pattern with a lower concentration in 1994, and shows correlation with variation patterns of Cr, Fe and Pb in 1994.

**Table 4.7 : Average mean concentration values and average mean of concentration differences [µg/g dw] in 1993/1994 of the elements contribute the most to pollution load index in Eckwarderhörne**

| Element | *Variation factor in 93/94* | *Av. mean conc. value in 93/94* [a] | *Av. mean diff. in 93/94* [b] | *Mean of subgroups*[c] *to the average mean value 93/94* | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Feb. | Apr. | Jun. | Aug. | Oct. | Dec. |
| Cu | 1.64 | 6.01 | (0.883) | 17 % | 9 % (1.14) | | (1.02) | | (1.84) |
| Zn | 2.49 | 116 | (25) | 26 % | 28 % | | (45) | | (62) |
| Ag | 2.04 | 0.364 | (0.04) | 20 % (0.07) | 20 % | | | | (0.1) |
| Cd | 2.12 | 1.05 | (0.197) | 23 % | 23 % | | (0.262) | | (0.527) |
| Pb | 3.38 | 1.68 | (0.69) | | (1.05) | 20 % (1.21) | 48 % | | |
| Cr | 5.92 | 1.35 | (0.731) | | | 50 % (1.02) | 80 % (2.08) | | |
| Mn | 1.9 | 285 | (68) | (79) | (82) | | 29 % | (110) | |
| Fe | 4.59 | 568 | (293) | | | 44 % (719) | 61% (422) | | |
| Co | 1.52 | 3.81 | (0.793) | 10 % | | | 7 % (1.45) | (1.45) | (0.96) |
| Ni | 1.87 | 12.1 | (1.93) | 21 % (2.3) | (5.42) | | 10 % | | |

a - Average concentration value of the element in 1993 and 1994
b - Average of mean of the concentration difference found in 1993 and 1994
c - Subgroup = mean of each two corresponding months

As shown in Fig. 4.42 (a, b) and Table (4.7), comparing annual homogenates with the bimonthly samples, for Cu, Zn, Ag, and Cd February and April are the main contributors to the average mean concentrations in the two years. Meanwhile, for Pb, Cr, Mn, and Fe June and August, as well as February and August for Co and Ni are contribute the most to the average mean concentration in

two years. Cu and Zn show a decreasing concentration in 1994. Also, Fe and Cr coincide in the maximum concentration value, in June 1993 and August 1994, in which a shift in the accumulation pattern has occurred leading to a very high difference in concentration (range) even higher than the average mean concentration values of the two years.

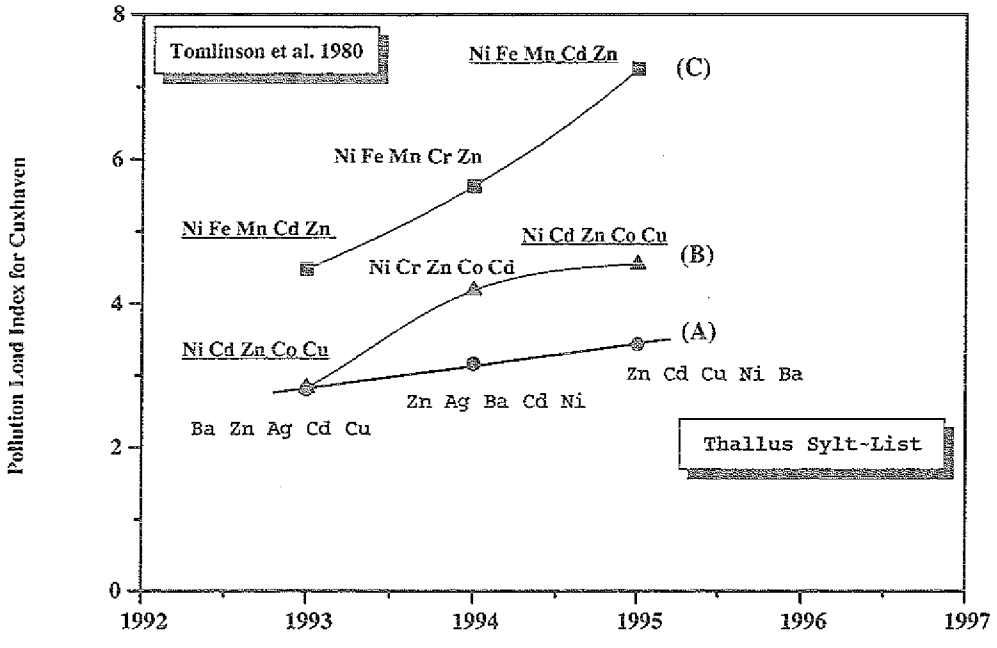



**Fig. 4.43 : Application of pollution load index to Cuxhaven**
**(A) data based on thallus part from Sylt-List**
**(B) data after removal the effect of Fe and Mn**
**(C) data based on reported baseline by Tomlinson et al. [31]**

In Cuxhaven the calculation of the pollution load index based on data of *thallus* Sylt-List is shown in Fig. 4.43 curve (A). The elements which contributing the most to the pollution status in Cuxhaven are Ba, Zn, Ag, Cd, Cu, and Ni. Within this group of elements, Cu and Zn in 1993 patterns are correlated, and Cd, Cu, and Zn in 1994 patterns. On the other hand, if baseline data from Tomlinson et al. [31] were used, elements such as Fe, Mn, and Cr were introduced as potential pollutants, and the pollution load indices raised by almost a factor of 2, curve (C).

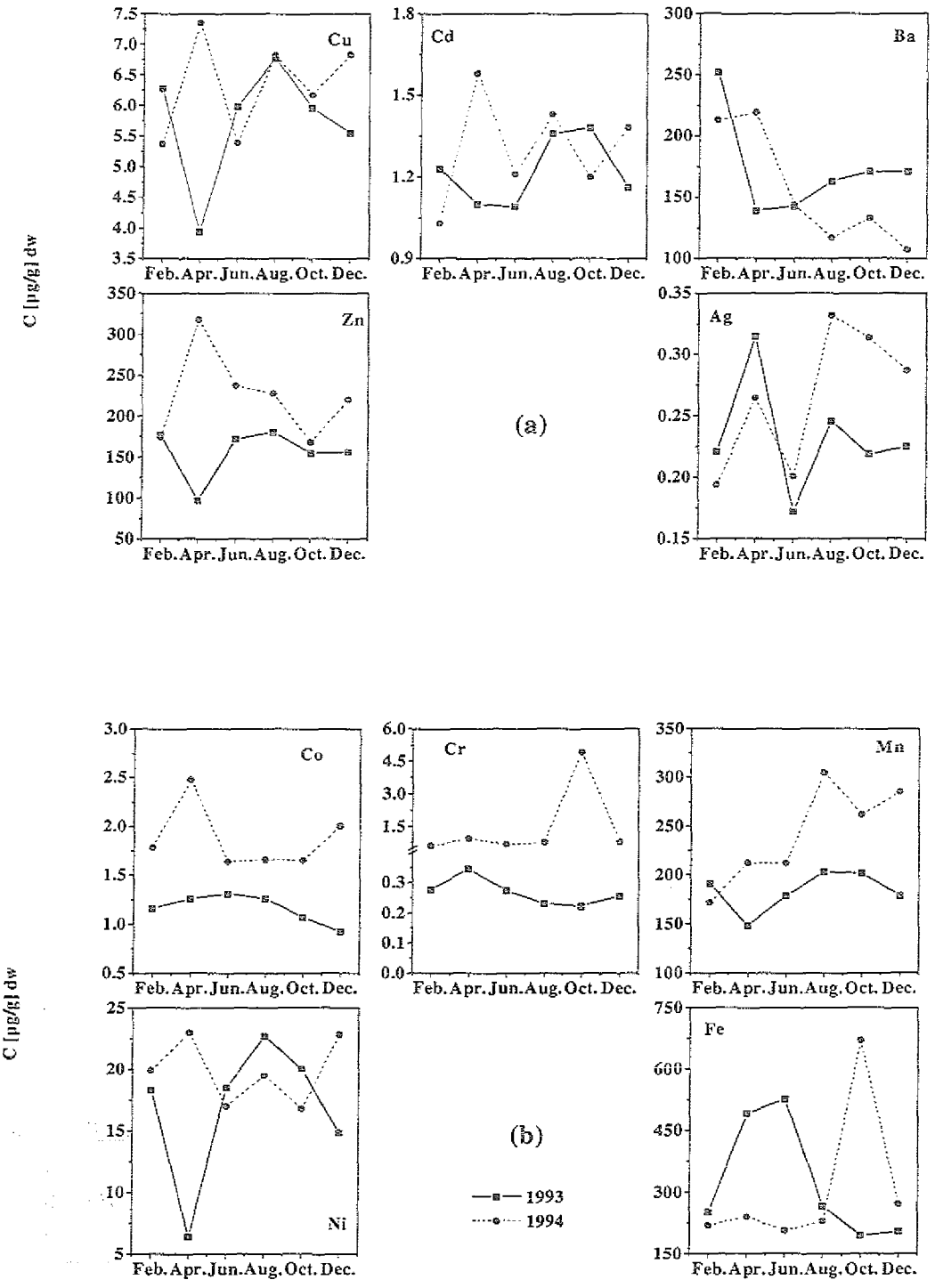



**Fig. 4.44 : Variation patterns of elements contributing the most to the pollution load index in Cuxhaven based on (a) data of *thallus* part Sylt-List and (b) data from Tomlinson et al. [31]**

However, in case when the Fe and Mn effects were removed from calculation, the resultant curve (B) introduced Co as another pollutant, with pollution load indices higher in 1994 and 1995 by

almost a factor of 1.5, also as Cr was not determined in 1995 sample. In Fig. 4.44 (a, b) and Table (4.8), It has been found that the general behavior of Cu, Cd, Zn, and Ag concentration values were higher in 1994 than 1993, but Ba was decreasing in 1994. However, the order of the elements which have the highest contamination factor changes between the years as well as the variation patterns, e.g. Cu, Cd, and Zn, beside the introduction of other elements as Ni, Ag and Ba.

**Table 4.8 : Average mean concentration values and average of concentration differences [µg/g dw] in 1993/1994 of the elements contribute the most to pollution load index in Cuxhaven**

| Element | *Variation factor in 93/94* | *Av. mean conc. value in 93/94* | *Av. mean diff. in 93/94* | *Mean of subgroups to the average mean value 93/94* | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Feb | Apr | Jun | Aug | Oct | Dec |
| Cu | 1.87 | 6.04 | (1.07) | | | | 12 % (3.41) | | 2.5 % (1.28) |
| Zn | 3.29 | 190 | (69) | | 9 % (221) | 7.5 % | 7.5 % | | |
| Ag | 1.93 | 0.249 | (0.058) | | 16 % | | 16 % (0.086) | 7 % (0.095) | 2.7 % (0.062) |
| Cd | 1.53 | 1.26 | (0.212) | | 7 % (0.48) | | 10.5 % | 2.1 % | |
| Ba | 2.36 | 164 | (45) | 42 % (80) | 9 % | | | | (64) |
| | | | | | | | | | |
| Cr | 22.2 | 0.861 | (*1.19*) | | | | | 300 % (*4.71*) | |
| Mn | 2.06 | 213 | (64) | | | | 19.5 % (103) | 9.2 % | 9.2 % (106) |
| Fe | 3.44 | 315 | (197) | | 16 % (251) | 16.6 % (320) | | 37.5 % (*476*) | |
| Co | 2.69 | 1.52 | (0.705) | | 23 % (1.22) | | | | (1.08) |
| Ni | 3.58 | 18.3 | (5.68) | 4 % | (16.6) | | 15 % | | 2.7 % (8) |

If the data of Tomlinson et al. [31] were used, other elements were introduced such as Ni, Fe, Mn, Co, and Cr. The variation patterns of Fe and Cr correlate negatively with that of Mn in 1993, and Ni positively with Cu, Zn, and Cd in 1994. In Cuxhaven, the influence of the sampling months on element concentrations as have been shown in Apr. ( Zn, Ag, Cd, Ba, Fe, and Co), Aug. (Cu, Zn, Ag, Cd, Mn, and Ni), and Oct. (Ag, Cd, Cr, Mn, and Fe), is an evidence that these months are very

important in standardization of the monitoring program, as well as Feb. especially for Ba. The general behavior for Ni, Co, Mn, and Cr was increasing concentration level in 1994 than in 1993.

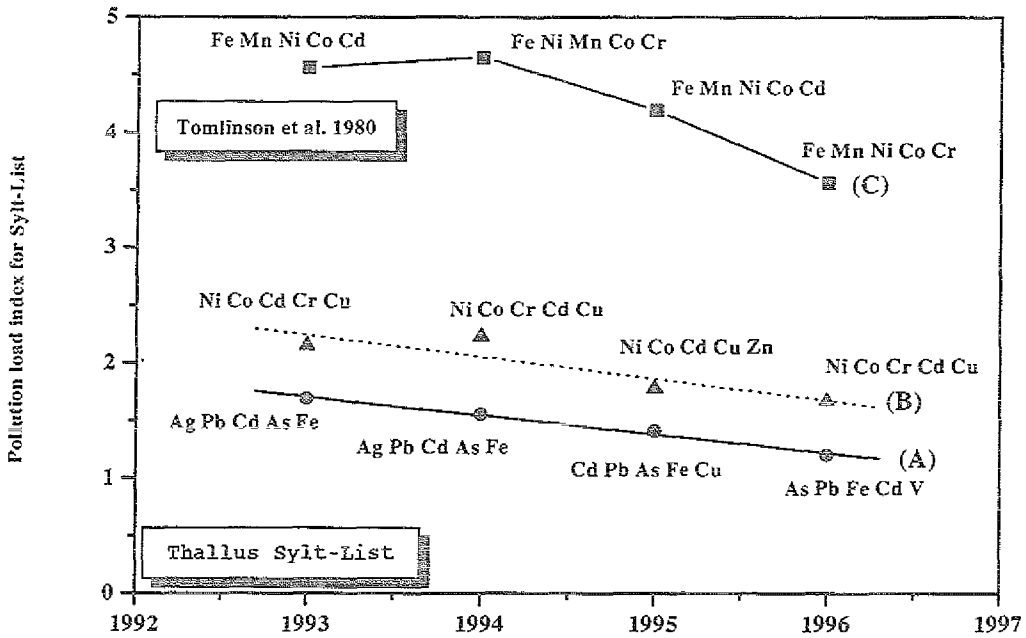



**Fig. 4.45 : Application of pollution load index to Sylt-List.**
**(A) data based on thallus part from Sylt-List**
**(B) data after removal the effect of Fe and Mn**
**(C) data based on reported baseline by Tomlinson et al. [31]**

The pollution load indices in Sylt-List based on concentration levels of thallus part as baseline are represented by curve (A) in Fig. 4.45. As a result of the use of this baseline, the elements Ag, Pb, Cd, As, and Fe appeared to have the highest contamination factors for 1993 and 1994. In 1995 and 1996, Ag was not determined in the samples, but Cu and V were introduced as contamination contributors. In case of the calculation based on data of Tomlinson et al. [31], the resultant was curve (C), which is 3 times higher in pollution load indices than curve (A). The elements which have the highest contamination factors were Fe, Mn, Ni, Co, Cd, and Cr (Cr was not determined in 1995 samples). If the effect of contamination factor of Fe and Mn was removed from calculation, the resultant is curve (B) which showed 30 % higher than curve (A).

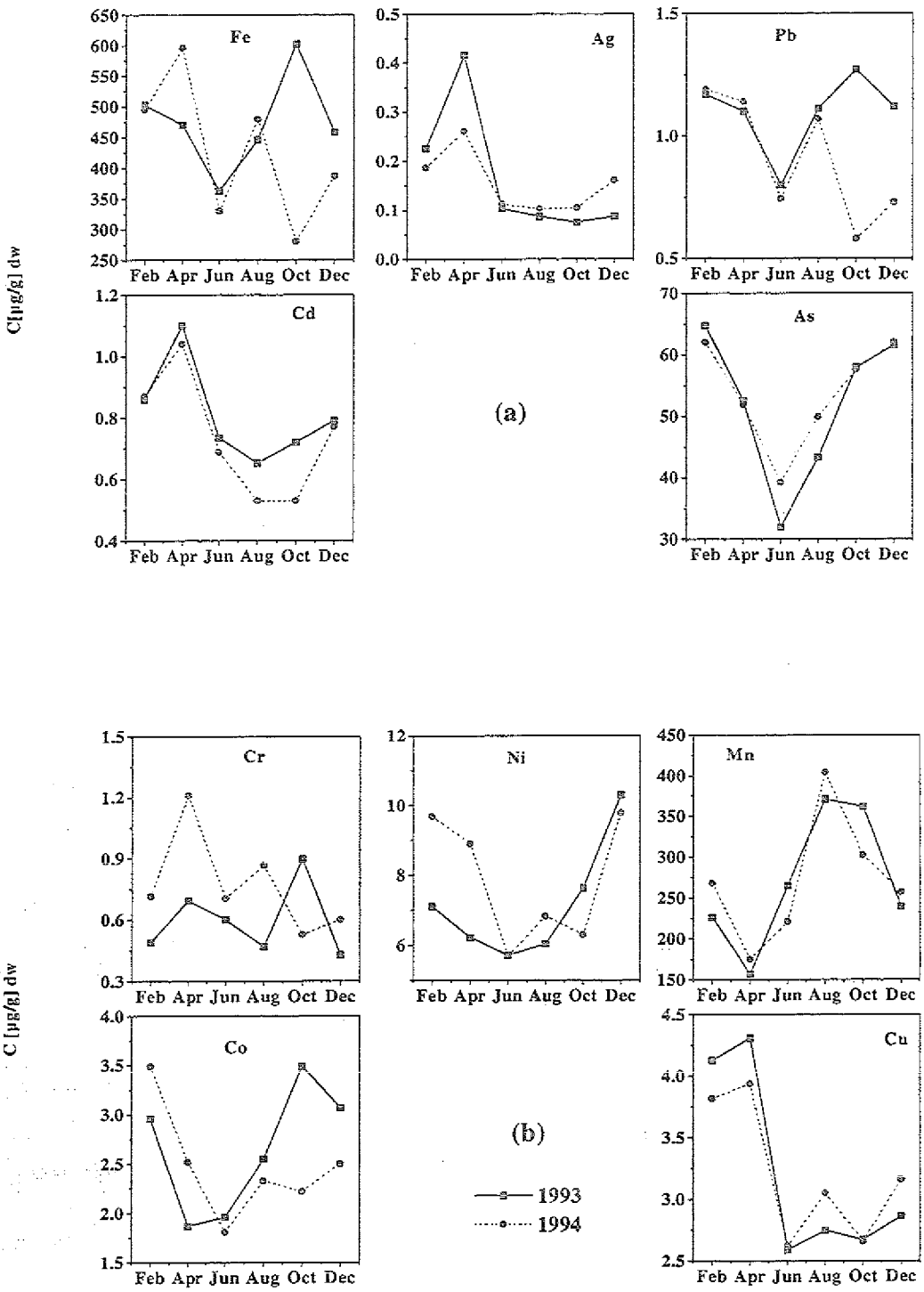



Fig. 4.46 : Elements contributing the most to the pollution load index in Sylt-List based on (a) data of *thallus* part Sylt-List and (b) data from Tomlinson et al. [31]

In this case, the elements showing higher contamination factors were Ni, Co, Cd, Cr, Cu, and Zn, which is introduced because Cr was not determined in 1995 samples. In Fig. 4.46 (a, b) and Table (4.9) the variation patterns are given of the elements contributing the most to pollution load indices in 1993 and 1994.

**Table 4.9 : Average mean concentration values and average of concentration differences [µg/g dw] in 1993/1994 of the elements contributing the most to the pollution load index in Sylt-List**

| Element | *Variation factor in 93/94* | *Av. mean conc. value in 93/94* | *Av. mean diff. in 93/94* | *Mean of subgroups to the average mean value 93/94* | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Feb. | Apr. | Jun. | Aug. | Oct. | Dec. |
| Fe | 2.15 | 451 | (100) | 10.6 % (126) | 18.1 % | | 2.7 % | (322) | |
| As | 2 | 53 | (3.02) | 19.7 % | | (7.3) | (6.6) | 9 % | 16.7 % |
| Ag | 5.47 | 0.16 | (0.054) | 28 % (0.155) | 200 % | | | | (0.073) |
| Cd | 2.08 | 0.774 | (0.075) | 11.7 % | 38.3 % | | (0.123) | (0.19) | |
| Pb | 2.19 | 1 | (0.206) | 17.7 % | 11.7 % | | 8.7 % | (0.69) | (0.39) |
| | | | | | | | | | |
| Cr | 2.83 | 0.684 | (0.297) | | 39 % (0.516) | | (0.397) | 4 % (0.371) | |
| Mn | 2.57 | 271 | (36) | (42) | | (44) | 43 % | 22.7 % (60) | |
| Co | 1.93 | 2.56 | (0.565) | | 25.8 % (0.65) | | | 11.3 % (1.27) | 8.6 % |
| Ni | 1.8 | 7.52 | (1.32) | 11.7 % (2.58) | (2.67) | | | | 33.5 % |
| Cu | 1.66 | 3.21 | (0.22) | 23.7 % (0.31) | 28.4 % (0.37) | | (0.3) | | (0.3) |

The variation patterns of Ag and Cd were repeated and correlated in 1993 and 1994. Also, the variation patterns of Fe and Pb correlated in both 1993 and 1994. For As, the variation pattern of 1993 is identical with 1994. The variation pattern of 1993 for Cu was repeated in 1994, which is correlated with Ni in 1994 as well as Fe in 1994. The variation pattern of Cr in 1994 is correlated with that of Fe in 1994, as well as the co-correlated elements. The variation pattern of Mn in 1993 was repeated in 1994, and correlated with that of Co in 1993. Also, it can be seen that February and

April are important months for Fe, Ag, Cd, Pb, Cu, Co, Ni, and As, and August for Mn, as well as December for Ni and As.

It could be concluded from the above discussion that many of the elements which have the highest contamination factors are correlated especially in the same year and/or in the two years as described above. There are interesting months with mean concentrations in which the element concentrations are higher than the average mean in two years, and this could be of help in a more detailed study of these elements.

*Derivation of the North Sea coastal index*

From information on the pollution load indices in Eckwarderhörne, Cuxhaven, and Sylt-List, it is shown clearly that Eckwarderhörne is by far the most polluted collection site with regard to heavy metals if one considers the pollution load index as defined by Fig. 4.47, but the pollution indices cease.

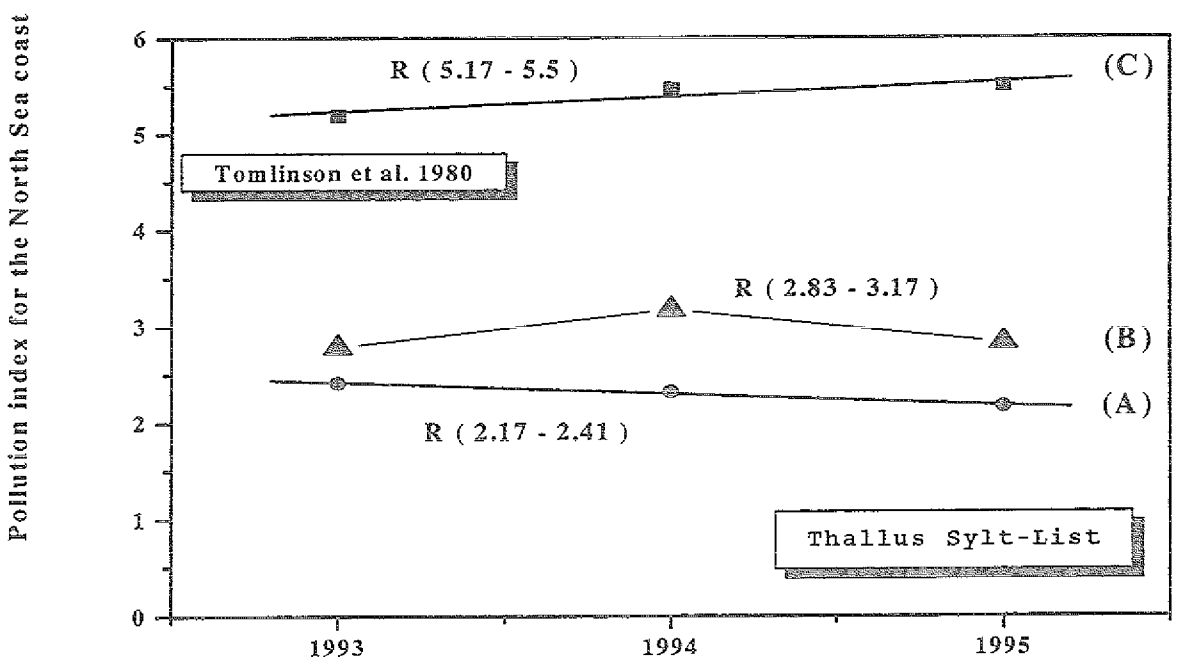



**Fig. 4.46 : Pollution indices derived from the coastal indices of Eckwarderhörne, Cuxhaven, and Sylt-List. (R) = Range . (A) data based on *thallus* part from Sylt-List, (B) data after removal the effect of Fe and Mn, and (C) data based on reported baseline by Tomlinson et al. [31]**

In Cuxhaven the pollution indices show an increasing effect, whereas Sylt-List is the least polluted site. The proposed estimation of PLI of the North Sea coastal water is given by :

$$\text{PLI of North Sea coast} = \sqrt[3]{Eckwarderhörne(PLI) * Cuxhaven(PLI) * Sylt(PLI)}$$

The ranges of the coastal indices from 1993 to 1995 are given in Fig. 4.47 based on both baselines of *thallus* Sylt-List and Tomlinson et al. [31]. A value of zero indicates perfection, a value of one means that only baseline of pollutants are present, and a value above one would indicate progressive deterioration of coastal quality. Comparing these coastal indices with that of 2.2 [31], 3.8 [7], 7.4 [105], and 29.0 (polluted) and 3.3 (unpolluted) of Melhuus et al. [85] indicates the very important need for baseline reference values, and provides some understanding of trends over time, as well as a means for quantifying the quality of the North Sea coastal water in the simplest way.

## *Conclusions*

Environmental research and policy may not only be restricted to the reduction or registration of the existing environmental pollution, but may also be based on the principle of preventive care. Comprehensive knowledge is essential about the tendency and the rate of changes in the ecosystems. Integration of ecological observations - which result from long-term monitoring programs - is an important basis for enhancing this knowledge. The analysis of the environmental bioindicator brown algae ***Fucus vesiculosus*** sampled under the aspects of the German Environmental Specimen Bank [57, 106] explores the application of the bioindicator and examines its representativeness.

Comprehensive data which were collected during part of the long-term observation program - in particular during 1993 and 1994 - and have been evaluated based on the present status of the heavy metal content at three different collection sites - Eckwarderhörne, Cuxhaven, and Sylt-List on the North Sea coast. The distribution of heavy metals with respect to space and time depends primarily on their physicochemical properties. By an analytical characterization of each sample from each collection site, and after an assessment of the data, differently recognizable ecological observations were found. An important aspect of this ecological observation was that the bioindicator shows the variational concentrations of heavy metals in the collection areas as in case of Mn, Se, Yb and Cs. *Fucus vesiculosus* as a bioindicator was not effectively environmentally relevant for all determined elements. Even if *Fucus vesiculosus* reflects major differences in elements concentration levels showing geographical differences, small time variations may not be efficiently reflected. e.g. for Cu, Cd, and Pb. At the same time, the cross-relationship between the accumulated elements like Sc, V, Cr, Fe, Pb, and Th, especially if we considered the three collection areas, demonstrates the possible contamination of the samples by fine sediment and particulate matter. Unless care is taken in inspecting the collected samples before grinding, higher values of Ca due to the existence of periwinkle (*Littorina littorea*) can be expected. Based on the entire composition of *Fucus*

*vesiculosus*, the analyzed samples were regionally representative, and there are comparable results in Eckwarderhörne and Sylt-List because of similarities of the biological communities, and in some cases between Eckwarderhörne (partially estuarine) and Cuxhaven regarding the functional structure of estuaries. The analysis defined an irregular seasonal variation of some element concentrations, but not for all the three collection area. e.g. for Cr, Fe, Ag, Cs, Hf, and Th.

The information gained from the relative variation in element concentrations in different parts of *Fucus vesiculosus* collected from Eckwarderhörne as individual groups and from Cuxhaven and Sylt-List as representative parts is suitable for defining the optimized part for the element of interest, as well as the accessibility of the adequate sample size. A reliable comparison - between the collection areas - was obtained due to the application of statistical tools, taking the time-scales into account for the trend analysis which reveals the ecological significance of the indicator function for observing the specific ecosystem.

The relevance of the fingerprinting analysis expands the choices of exclusion and/or selection of the sample for special element investigations. The spatial and temporal dimensions of element concentrations were realized using multi-element techniques [69].

The quality assurance of the data was adapted by intralaboratory comparisons, reproducibility control of the results with respect to each applied technique, accuracy control of each analytical method by repeated measurement of CRMs, and comparison of the analysis of candidate reference materials distributed by IAEA.

The presented monitoring results are discussed with respect to the position of *Fucus vesiculosus* as a bioindicator reflecting spatial and temporal trends in metal levels of its environment. The interspecific differences seen for each site highlight the effect of physical and biological factors on the accumulation process. Measuring the whole plant may reflect the accumulation of trace metals or even discharge of radionuclides from several months or years proceeding the sampling. Therefore, they can be used for long-term monitoring, whereas short-term variation can be better observed by investigating new vegetative parts of the algae. Also it is important to sample at the

same time of the year, as uptake and accumulation differ due to variation in growth rate of the algae. *Fucus vesiculosus* can be a representative bioindicator organism if the following precautions are taken :

- Samples should be collected at equal intervals at approximately the same time from monitoring sites, and sampling methodology should be developed to standardize as many factors as possible.
- Parts of *Fucus vesiculosus* of comparable age should be used when comparing different localities.
- The samples should be washed with filtered sea water and freed from epiphytes as far as possible.
- The contribution of the suspended particulate matter to the concentration of elements must be taken into consideration and appropriate corrections made.
- Comparison should be made between collection areas subject to identical environmental conditions.
- Baseline values should be made available from the same specimen.

*The results of the present study are to be considered as the further step in research aimed at quantifying and assessing the assimilative capacity of the bioindicator Fucus vesiculosus.*

*SUMMARY*

The brown alga *Fucus vesiculosus* of the North Sea coast is sensitive to changes in the environment caused by anthropogenic or other disturbances. The presented study shows that the use of *Fucus vesiculosus* as an indicator will give an integrated measure of environmental conditions at the site over longer time periods.

The study of elemental content of the brown alga *Fucus vesiculosus* sampled from Eckwarderhörne, Cuxhaven, and Sylt-List is based on the irradiation of the freeze-dried materials with a thermal neutron flux density of about 5 X $10^{13}$ N cm $^{-2}$ s $^{-1}$. In Instrumental Neutron Activation Analysis (INAA), the measured gamma-ray energies are characteristic of specific indicator radionuclides, and their intensities are proportional to the amounts of the various target nuclides in the sample. Also, the samples were processed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Atomic Emission Spectrometry (ICP-AES) to confirm the obtained results as well as the determination of some other elements of environmental interest. The precision of the analytical methods was evaluated by analyzing certified reference material alongside the samples.

The measurements were carried out for two years of sampling to provide an overview of heavy metals content of the bioindicator *Fucus vesiculosus*. Numerous factors may influence the heavy metal content of *Fucus vesiculosus*, and hence the credibility and usefulness of the bioindicator. Such factors - temperature, salinity, nutrition, particulate matter, etc. - will frequently vary seasonally, geographically, with the vegetation cycle, and as a function of other conditions that may over-or underestimate the interpretation of the observations. Therefore, collection procedures were adapted during the course of this study. The analysis of the samples proved that the data sets exhibit considerable spatial and temporal variations. As a result of the extensive information gained from the analysis, statistical analysis were applied to the data to determine which changes may have occurred spatially or temporally. The *General Linear Models Procedure*, was used to examine the

elemental variations with respect to collection site, sampling month, sampling year, and the combination effect adjusted for every other effect. Discrimination analysis using *Stepwise Selection* was used to discriminate between the collection sites based on quantitative element concentrations. Also, the multivariate technique, *Principal Component Analysis*, was applied to the investigation of the seasonality of the element concentrations. Fingerprinting of the elemental content of *Fucus vesiculosus* in each of the collection sites provided evidence of spatial variations. Correlations between element concentrations of the same variational patterns were studied.

Some vegetative characters of *Fucus vesiculosus* were measured in August 1994 and February 1995. The *General Linear Models Procedure* was used on the element concentrations of different parts collected in two different seasons from Eckwarderhörne to study the effect of the part, collection time, and combination effect of both on the level of element concentrations. A comparison of the concentration in different parts between different collection sites at different seasons was studied. The *Principal Component Analysis* of both collection seasons and element concentrations in different parts of *Fucus vesiculosus* sampled from Eckwarderhörne indicate a lower variance in tips while thallus and basal parts have the highest variance. Different parts representing the different metabolic activities were analyzed to show the variability in trace element concentrations and to study the contribution ratio of each part to the whole body burden. The results of the collected samples were used together with Environmental Specimen Bank (ESB) data of 1995 and 1996 to propose an estimate of the pollution index of the North Sea coast.

The present study concluded that the chemical analysis of *Fucus vesiculosus* validates the function of the bioindicator and *Fucus vesiculosus* can be used as a bio-indicator organism for the bioavailable forms of several metals if certain precautions are taken.

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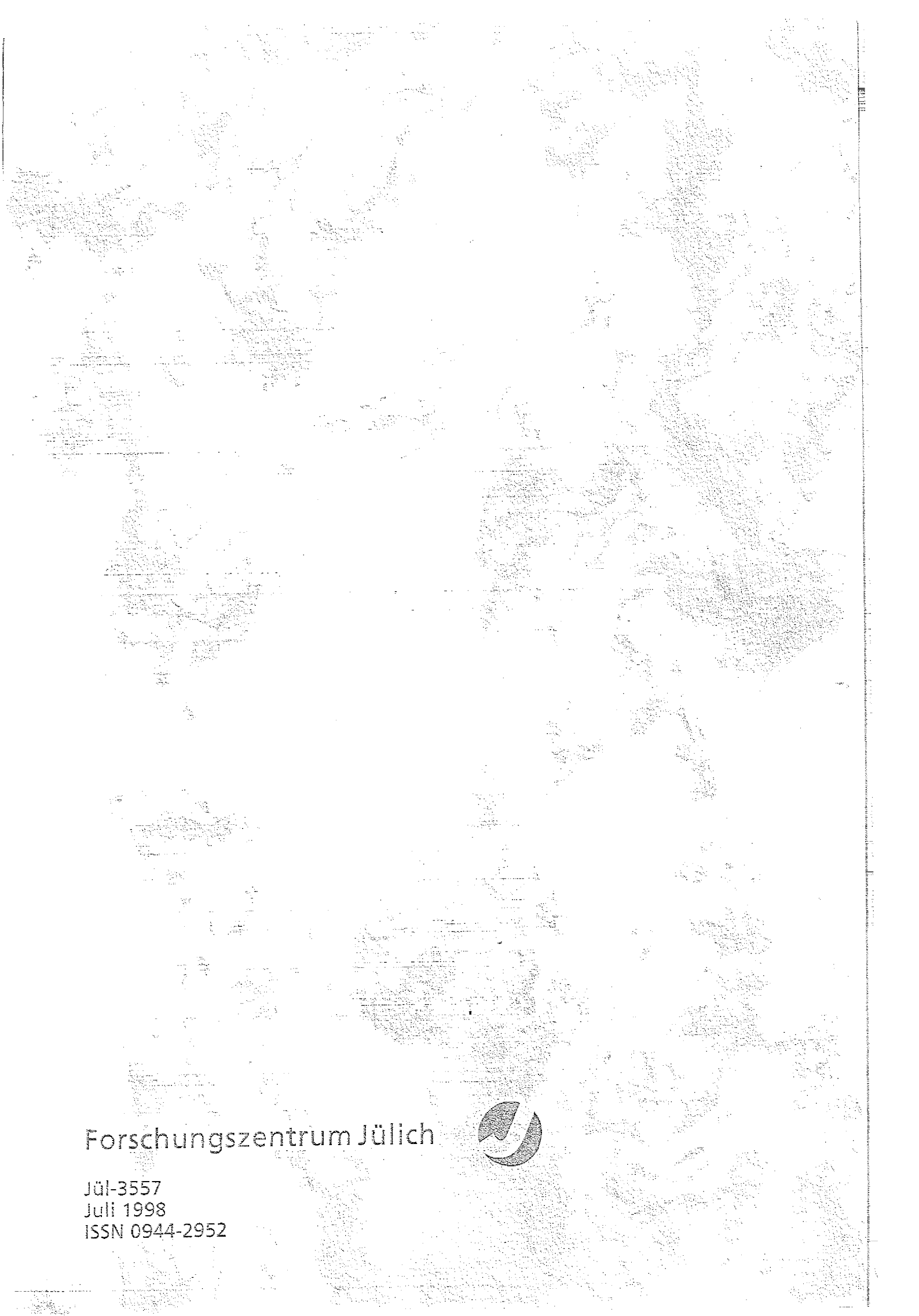

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