การใช้สาหร่ายทะเลเป็นตัวชี้วัดปริมาณโลหะหนักในน้ำทะเลบริเวณนิคมอุตสาหกรรมแหลมฉบังและศูนย์วิจัยและพัฒนาประมงทะเลอ่าวไทยฝั่งตะวันออก (ระยอง)

นายโสภณ บุญมีวิเศษ

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THE USE OF MACROALGAE AS INDICATORS OF HEAVY METALS IN SEAWATER AT LAEM CHABANG INDUSTRIAL ESTATE AREA AND THE EASTERN MARINE FISHERIES RESEARCH AND DEVELOPMENT CENTER

Mr. Sophon Boonmewisate

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Environmental Biology

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Suranaree University of Technology has approved the thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

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ในการศึกษาสาหร่ายจากทะเลสองแห่ง คือทะเลบริเวณนิคมอุตสาหกรรมแหลมฉบัง จังหวัดชลบุรี และทะเลบริเวณศูนย์วิจัยและพัฒนาประมงทะเลอ่าวไทยฝั่งตะวันออก จังหวัดระยอง เพื่อใช้เป็นดัชนีวัดปริมาณโลหะหนัก 5 ชนิดในสาหร่ายไส้ไก่ (*Enteromorpha clathrata*), สาหร่ายเห็ดหูหนูทะเล (*Padina japonica*) และสาหร่ายทุ่น (*Sargassum polycystum*) และวัดปริมาณโลหะหนักในน้ำทะเลและในดินระหว่างเดือนกุมภาพันธ์ 2543 ถึงมกราคม 2544 พบว่าปริมาณโลหะหนักทั้ง 5 ชนิดในน้ำจากทะเลทั้งสองแห่งมีค่าต่ำกว่าค่ามาตรฐานคุณภาพน้ำทะเลชายฝั่งตามประกาศของคณะกรรมการสิ่งแวดล้อมแห่งชาติของประเทศไทย ส่วนปริมาณโลหะหนักในดินและในสาหร่ายทุกชนิดมีปริมาณมากกว่า 50 และ 100 เท่าของโลหะในน้ำทะเล แสดงว่าสาหร่ายทั้ง 3 ชนิดสามารถใช้เป็นตัวชี้วัดปริมาณโลหะหนักในน้ำได้ ปริมาณคลอโรฟิลล์เอและคลอโรฟิลล์ซีในใบของสาหร่ายทุ่นอยู่ระหว่าง 0.54 – 1.54 และ 0.16 – 0.85 ไมโครกรัมต่อลูกบาศก์เซนติเมตร ปริมาณโปรตีนอยู่ระหว่างร้อยละ 0.17 – 0.55 ต่อกรัมน้ำหนักแห้ง ปริมาณไขมันอยู่ระหว่าง 0.03 – 0.83 กรัมต่อกรัมน้ำหนักแห้ง กรดไขมันที่พบมากที่สุดคือ palmitic acid ซึ่งมีปริมาณระหว่างร้อยละ 31.24 – 37.95 มีปริมาณกรดไขมันอิ่มตัวและกรดไขมันไม่อิ่มตัวเฉลี่ยร้อยละ 58.83 และ 46.65 ตามลำดับ และพบว่าโลหะหนักชนิดเดียวหรือสองชนิดรวมกันมีผลต่อการเปลี่ยนแปลงปริมาณสารประกอบดังกล่าวต่างกัน ขึ้นอยู่กับชนิดของโลหะและความเข้มข้นที่สาหร่ายได้รับ นอกจากนี้โลหะหนักยังยับยั้งและทำลาย paraphyses และออร์แกเนลล์ด้วย

สาขาวิชา ชีววิทยา
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SOPHON BOONMEWISATE: THE USE OF MACROALGAE AS INDICATORS OF HEAVY METALS IN THE SEAWATER AT LAEM CHABANG INDUSTRAIL ESTATE AREA AND THE EASTERN MARINE FISHERIES RESEARCH AND DEVELOPMENT CENTER. THESIS ADVISOR: PAUL J. GROTE, Ph.D., 205 PP. ISBN 974-533-329-8

HEAVY METAL/ *ENTEROMORPHA*/ *PADINA*/ *SARGASSUM*/FATTY ACID

The concentrations of cadmium, chromium, copper, lead and zinc in *Enteromorpha clathrata*, *Padina japonica* and *Sargassum polycystum*, the seawater and the sediments collected from near Laem Chabang Industrial Estate Area, Chonburi Province, and the Eastern Marine Fisheries Research and Development Center, Rayong Province, were determined during February, 2000, to January, 2001. All heavy metal concentrations in the water were within the standard for Thailand's coastal seawater. The metal concentrations in the sediments and in the seaweeds were 50 and 100 times higher than those of the metal concentrations in the water. The seaweeds can be used for heavy metal monitoring. Concentrations of chlorophyll *a* and *c* in the blade of *S. polycystum* ranged from 0.54-1.54 μg.cm^{-3} and 0.16-0.85 μg.cm^{-3}, respectively. Protein content ranged from 0.17-0.55%. The lipid fraction ranged from 0.03-0.83 g/g dw. The most common fatty acid was palmitic acid. It ranged from 31.24-37.95%. Total saturated and unsaturated fatty acids were 58.83 and 46.65%, respectively. The amounts of these compounds were changed depending on concentrations of the single or combined heavy metals applied. In addition, paraphyses and organelles were inhibited and destroyed.

School of Biology
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Sophon Boonmewisate

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CHAPTER I

INTRODUCTION

Clean water is an essence for life. Nowadays, clean water is limited by contamination with pollutants. Untreated water effluent from industries and communities is released into reservoirs or rivers. Heavy metals naturally occurring in the environment are the major pollutants that outflow from the factories. It is important to distinguish between anthropogenic contamination and background or natural levels and to enable accurate evaluation of the degree of contamination in an area. Heavy metals accumulate not only in water and sediments, but also in aquatic organisms. Submerged plants are especially the first organisms that come into contact with the hazardous metals. They absorb all metals through their holdfast or root-like structures. In marine habitats, both seaweeds and phytoplanktons play the key role of producers. They act as shelters and nurseries for young animals. At the primary production level, macrophytes as well as macroalgae, anchored in the enriched sediments, tend to accumulate higher concentrations of heavy metals than the sediment. This is because there is uptake not just from the sediment but also from the water itself. Therefore, algae contain higher concentrations of heavy metals than the surrounding water. Having accumulated in the algal tissues, these metals can be transferred to higher trophic levels and finally transferred to human beings. In addition, we do not exactly know the level of metal contamination in specific areas. Although researchers have been using many animals as biomonitors for heavy metals,

metal contamination in the ecosystems should be studied. To fill this gap of study, macroalgae can be used for heavy metal monitoring. The rationale for using seaweeds as indicators of metal contamination has five main bases. First, metal concentrations in solution often are near the limits of analytical detection and may be variable with time. Second, empirical methods for distinguishing the biologically available fraction of the total concentration of a dissolved metal have not been developed for natural systems. By definition, seaweeds will accumulate only those metals that are biologically available (assuming the degree of adsorption is slight). Third, since plants do not ingest particulate-bound metals (as animals do), plants will accumulate metals only from solution (Luoma, 1983). Fourth, algae, of the microorganisms studied, are gaining attention due to the fact that algae, particularly macroalgae, are a rich resource in the oceanic environment, relatively cheap to process and able to accumulate high metal content (Zhou, Huang and Lin, 1998). Finally, their sedentary nature is another reason why algae species are well fitted as monitoring organisms in heavy metal monitoring (Topcuoğlu, Güven, Balkis, and Kirbaşoğlu, 2003). In polluted areas, a mixture of toxic agents is usually present. Therefore, the combined effects of metals on physiological changes in the plants should be studied.

1.1 Research objectives

The objectives of this study can be classified into 7 objectives as follows:

1.1.1 To measure the amounts of Cd, Cr, Cu, Pb, and Zn in macroalgae found in both the critical and non-critical areas, especially *Enteromorpha clathrata* Greville, as well as to compare the amount of the same heavy metals in seawater and sediments collected from near macroalgae in the critical and non-critical localities.

1.1.2 To investigate the amount of metals in different parts of *Sargassum polycystum* C. Agardh.

1.1.3 To study the effects of the metal pollutants, cadmium chloride, chromium chloride, cupric sulfate, lead acetate and zinc sulfate, alone and in different combined ratios, on changes in chlorophyll *a* and chlorophyll *c* concentrations, total nitrogen content, lipid fraction and fatty acid in *S. polycystum*.

1.1.4 To determine the bioaccumulation of metals in macroalgae and in the ambient water.

1.1.5 To indicate the synergistic and antagonistic effects of combined metals on seaweeds in cultures.

1.1.6 To select the kinds of macroalgae to be utilized for removing metals from wastewater.

1.1.7 To determine the effects of heavy metals on macroalgae using Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM).

1. 2 Research hypothesis

Increased population and also industries cause higher amounts of heavy metal contamination in the environment. Laem Chabang industrial estate may outflow untreated water into the sea. Most heavy metals have a long retention and bioavailability, so they are able to accumulate in both water and sediments as well as in living organisms. We can use many organisms for heavy metal monitoring, especially algae (microalgae and macroalgae), the first organisms in trophic levels of the marine food web. Therefore, the greater the amounts of heavy metals contaminating the marine environment, the higher the amounts of heavy metals which

would be accumulated in macroalgae. To compare with the critical sites, the amounts of heavy metals in the non-polluted area, the Eastern Marine Fisheries Research and Development Center, using the same species of macroalgae will be used as controls. The amounts of heavy metals in the critical area should be higher than those of the non-polluted locality. Besides, the increase of heavy metals should be more effective in causing physiological changes than the lower heavy metal concentrations, and also the combined effect of heavy metals on physiological changes should be greater than the effects of heavy metals alone. In addition to transplant experiments, this thesis will test the hypothesis that as soon as the metal concentration in polluted seawater exceeds the metal concentration in natural seawater, disturbance of the nutrient uptake will occur, partly being visible as discoloration.

In this study, *Enteromorpha clathrata*, *Sargassum polycystum*, and *Padina japonica* Yamada will be used for biomonitoring as they have some relevant uses, i.e., as human food or animal feed. The concentrations in sediments and the water body will also be examined and compared with that in macroalgae. The content of metals is likely to vary by season. Therefore, in this study, it is determined to examine the amount of metals every month. It is highly hoped that the outcome from this study would provide required and useful information for public health concerns for the Office of the National Environmental Board, Ministry of Science, Technology and Environment, to warn and prohibit all industries that outflow untreated water into the sea.

It is known that there are many metals contaminating the marine environment. Thus, for this research it has been decided to study combination effects of heavy metals on some physiological changes in seaweeds. Some physiological changes

which are easy to experiment and observe are the concentrations of chlorophyll *a* and chlorophyll *c*, crude lipid and fatty acid as well as crude protein. Because a brown alga, *Sargassum polycystum* is the most common alga in the study site, it is, therefore, selected for study as a representative of macroalgae.

1.3 Definitions of terms

Bioavailability: the degree to which a contaminant in a potential source is free for uptake (movement into or onto an organism). Some definitions of bioavailability further imply that the contaminant must affect the organisms.

Bioaccumulation: the removal of heavy metals by the active binding to living organisms from an aqueous solution.

Bioconcentration: any xenobiotic, such as heavy metals, pesticides, accumulated in living organisms, usually too higher concentration in organisms than in the environment.

Biomonitoring: use of organisms for monitoring compounds of xenobiotics.

Biosorption: the sequestration of heavy metals by the passive binding to non-living biomass from an aqueous solution.

Heavy metal: generally used to describe those metals having atomic numbers higher than that of iron (59), or having densities greater than 5 g/ml (Lobban and Harrison, 1997).

Ligand: a molecule or an anion that can bind to a metal ion to form a complex.

Seaweed: macroalgae or large algae found in marine habitats; mostly algae in divisions Chlorophyta, Phaeophyta and Rhodophyta.

Xenobiotics: Foreign substances taken up or occurring in organisms.

CHAPTER II

REVIEWS OF LITERATURE

2.1 Metals in oceanic environments

Metals in oceanic environments can be separated into metals in sediments, in the seawater as well as in living organisms. Concentrations of heavy metals in sediments usually exceed those of the water by between three and five times. With such high concentrations, the bioavailability of even a minute fraction of the total sediment metal assumes considerable importance, particularly in some filter-feeding and borrowing organisms. In addition, most metals, including mercury and lead, may be transformed in sediments to organic compounds, which have higher bioavailability and toxicity. There are various routes by which sediment metals reach the biota. For instance, in deposit-feeding bivalves, uptake may occur following the ingestion of particles, or by pinocytosis of particles at the body surface. Furthermore, desorption followed by uptake may take place during direct contact between particles and surface tissues. Uptake from solution is also important and concentrations of bioavailable metals in the seawater may be controlled by equilibrium between dissolved metals and those adsorbed by sediment particles (Bryan and Langston, 1992).

2.1.1 Cadmium (Cd)

Concentrations of cadmium in oceanic environment ranges from 0.03 – 0.3 μg/l (Crompton, 1997). However, in some areas, which are related to anthropogenic

activities, dissolved concentrations exceeding 50 μg/l have been observed. The common dissolved forms of Cd are chloride complexes, but the most bioavailable form is the free ion Cd^{2+}, the proportion of which increases with decreasing salinity (Bryan and Langston, 1992).

In UK estuaries, concentrations of cadmium in sediments range from about 0.2 μg/g dry weight in non-polluted areas to more than 10 μg/g at highly polluted areas (Bryan and Langston, 1992). There are several reports of relationships between cadmium levels in sediments and living organisms, such as in polychaete, amphipod and so forth (Bryan and Langston, 1992).

Little information of the metal in vascular plants and macroalgae is reported. In eelgrass tissue, *Zostera marina,* concentrations of cadmium reflected levels in the water column rather than those of the sediment surrounding the root and rhizome. The metal is absorbed by the leaves and translocated to the root-rhizome. Thus, providing pathway of the metal is from water to sediment (Lyngby and Brix, 1982). In marsh grasses, *Spartina alterniflora*, there was a significant relationship between tissue concentrations of cadmium and sediments (Bryan and Langston, 1992). In brown seaweed, *Ascophyllum nodosum*, the metal concentrations range from 1.2 to 4.3 μg/g (Haug, Melsom and Omang, 1974).

2.1.2 Chromium (Cr)

Concentrations of total chromium in oceanic environments, range from 0.06 – 1.26 μg/l; trivalent chromium (CrIII) range between 0.005 – 0.52 μg/l and 0.03 – 0.96 μg/l for hexavalent chromium (CrVI), as well as 0.07 – 0.32 μg/l for organic chromium (Crompton, 1997). The stable form of dissolved chromium is hexavalent

chromium (CrVI), but a significant part of Cr(III) is usually present in an organically-bound component. In inorganic forms, trivalent chromium is far less biologically available and toxic than CrVI. The trivalent form tends to be precipitated in seawater and is rapidly scavenged by particles, such as oxides of Fe, or by surfaces. Thus, in the crab, *Xantho hydrophilus*, uptake of labeled CrIII was largely confined to the body surface, whereas the hexavalent form was absorbed by the tissues where it appeared to be reduced to CrIII and organically complexed (Peternac and Legovic, 1986 qouted in Bryan and Langston, 1992).

In UK estuarine sediments, average total chromium content (HNO_3 digest) range from about 30 to more than 200 μg/g, the highest levels (800 μg/g) being found in the Loughor Estuary (tinplate production) in South Wales. Treatment of the fresh sediment with 1*N* HCl releases 90% of the total chromium from the most contaminated sediment compared with 5% from relatively pristine sediments and appears to give a good indication of the presence of anthropogenic chromium (Bryan and Langston, 1992).

Researchers have reported the chromium content in oceanic environments, in sediments from different areas, and also in invertebrates (Crompton, 1997; Oshida and Word, 1982). However, there is not such a report studied in aquatic plants or macroalgae.

2.1.3 Copper (Cu)

Concentrations of copper in oceanic waters range from 0.0063 (Baltic Sea) – 2.8 (Pacific Ocean) μg/l (Crompton, 1997). In UK estuaries, the metal levels range from 2 – 3 μg/l in the water from the Bristol Channel-Severn Estuary (Bryan and

Langston, 1992), and range from 3 – 176 μg/l in the more saline parts of Restronguet Creek (Bryan and Langston, 1992). The metal content in sediment from UK estuaries ranges from about 10 μg/g in clean areas to over 2000 μg/g in contaminated areas (Bryan and Langston, 1992). In brown seaweed, *Ascophyllum nodosum*, Haug, Melsom and Omang (1974) reported that the concentrations of Cu range from 4.5 – 111 μg/g.

There are some observation of copper concentration in vascular plants and macroalgae. The metal concentrations in the leaves and root-rhizome of eelgrass *Zostera marina* were related to those of the sediment come into the plants (Lyngby and Brix, 1987). Meanwhile, Luoma, Bryan and Langston (1982) found a very significant relationship between copper levels in the seaweed *Fucus vesiculosus* and those in sediments, it was concluded that the plant was able to desorb and accumulate copper adsorbed on particles of suspended sediment.

2.1.4 Lead (Pb)

Concentrations of lead in oceanic environments range from 0.001 – 0.014 μg/l(Bryan and Langston, 1992). Along the east coast of Britain, dissolved concentrations range from 0.015 – 0.135 μg/l and in the Humber Estuary from 0.010 – 0.055 μg/l (Bryan and Langston, 1992). Most of the lead in these waters is associated with particles. The principal dissolved forms of lead are thought to be $PbCO_3^0$ and $PbOH^+$. However, the most bioavailable inorganic form is probably the free ion Pb^+ (Freedman, Cunningham, Schindler, and Zimmeman, 1980).

In UK estuarine sediments, concentrations of inorganic lead range from about 25 μg/g in relatively non-polluted areas to more than 2,700 μg/g in the Gannel estuary

which receives waste from old lead mines (Bryan and Langston, 1992). Low levels of organolead compounds have also been found in sediments.

Very little information about lead concentration in vascular plants and seaweeds is reported. Haug, Melsom and Omang (1974) studying on brown seaweed, *Ascophyllum nodosum*, reported that the metal concentrations range from 3 – 81 μg/g.

2.1.5 Zinc (Zn)

Concentrations of zinc in oceanic environments are less than 1 μg/l (Bryan and Langston, 1992). On the other hand, the metal contents in coastal areas and estuaries are much higher. For example, dissolved zinc in a North Sea is 0.3 – 70 μg/l. The most bioavailable form of zinc is the free ion Zn^{2+}, and it is often the most abundant of the dissolved forms of zinc (Bryan and Langston, 1992).

In sediment from UK estuaries, concentrations of zinc range from less than 100 – 3,000 μg/g in Restronguet Creek. Concentrations of the metal in the seawater from the Restronguet Creek sediments were very high and ranged from 262 – 396 μg/g near the sediment surface to 67 – 216 μg/g at a depth of 10 cm (Bryan and Langston, 1992).

Little information for the bioavailability of zinc in sediments comes from research on vascular plants and macroalgae. In brown seaweed, *Ascophyllum nodosum*, Haug, Melsom and Omang (1974) reported that the concentrations of zinc range from 315 – 3,220 μg/g. Experiments with yellow water lily, *Nuphar variegatum* Campbell *et al*. (1985) found that the metal levels in the plant are mainly come from the water. Meanwhile, Lyngby and Brix (1987) studying on eelgrass, *Zostera marina*, observed very significant relationships between the metal levels in sediments and

those in the root-rhizome and leaves. Translocation of the metal between these tissues was very low. They suggested that the metal level in the sediment controlled the availability of the metal to the leaves.

Study on the availability of sediment-bound labeled zinc in the clam *Macoma balthica* showed that uptake occurred mainly from the ingestion of particles. It was found that zinc levels in *M. balthica* were more closely related to concentrations in *Fucus vesiculosus* than to concentration in 1*N* HCl extracts of sediments. It is concluded that extractable sediment zinc, dissolved zinc in the lower water and zinc in the surface water are all significant sources of bioavailable zinc in benthic organisms (Bryan and Langston, 1992).

2.2 Metals in seaweeds

The presence of metal pollutants in marine environments has been reported in a number of studies by the analysis of brown algae, Phaeophyceae, and occasionally by the analysis of other algal species. Data from experimental studies have shown the accumulation and retention of metals from solution indicating that metals are concentrated during pollution situations (Bryan and Langston, 1992).

In Norway, contamination of fjords by industrial effluents from metal mining and processing factories and pulp and paper mills was reflected in the metal concentrations in the brown seaweed, *Ascophyllum nodosum* (Haug, Melson and Omang, 1974). Although the levels for zinc and copper were increase in the Trondheimsfjord compared with average background concentrations, very high levels of zinc and cadmium were measured in samples from the Hardangerfjord (Table 1).

Table 1 Range of average concentrations (conc.) and maximum concentrations (μg/g) in *Ascophyllum nodosum* from Hardangerfjord, Norway, compared with average background concentration [1]

Metals	Range of average conc.	Maximum conc.	Average background conc.
Zn	315 – 3,220	3,700	75
Cu	4.5 - 111	160	5.5
Pb	<3 - 81	95	<3
Cd	1.2 - 14.3	20	0.7

[1] Adapted from Haug et al. (1974)

There was no evident seasonal variation in concentrations of zinc. The higher concentrations of copper and zinc were detected in older portions of the shoots. They did not attempt to quantify the relationship between metals in algae and water but concluded that analysis of *A. nodosum* provided a rapid, qualitative means of determining metal contamination fairly inexpensively (Haug, Melson and Omang, 1974).

Some studies found that there was a significant correlation between metal concentrations in *A. nodosum* frond tips, less than 2 years old, and distance from effluent discharges into the Hardangerfjord. Linear correlations of Cu, Zn and Cd with concentrations in the sea water samples collected at the same site were found. (Haug, Melson and Omang, 1974).

A study by Myklestad, Eide and Melsom (1978) involved transplantation of *A. nodosum* attached to small pieces of rock from a metal contaminated habitat to one with low metal levels for five months. Some loss of metal from the lower portions of the fronds occurred by transfer to the water rather than by translocation, since in the newly produced tips and receptacle levels were similar to those of the indigenous plants. Concentrations of Zn, Cu, Pb, Cd and Hg were monitored at intervals at each

site and some differences in behavior between metals were noted. Though the overall effect of transplants was similar, analysis of the tips of the shoots, in particular, gave some indication of changes of concentration in the waters over periods of time. Some of the data reported by Myklestad *et al*. (1978) is shown in Table 2 for two parts of the shoots in the July sampling, two months after transplantation.

Further evidence of the reversibility of metal concentrations in *A. nodosum* was presented by Myklestad, Eide and Melsom (1978) from studies of transplants from low to high levels of Zn pollution. Monitoring was continued for 12 months and the data indicated that changes in the metal concentrations in the algae reflected those in the water and a record of pollution for three years could be estimated by analyzing successive segments. Metal concentrations in transplants attained those of the native algae, though some seasonal differences appeared to affect the accumulation of Zn and Cd, which is partially linked to metabolism, more than the accumulation of Pb, which occurs mostly by ion exchange. Analysis of whole fronds gave a qualitative indication of metal pollution but some assessments on a time basis were possible if the fronds were subdivided into age classes.

Table 2 Metal concentrations (μg/g) in *Ascophyllum nodosum* transplanted from a contaminated to an uncontaminated habitat [1]

Plant parts	Areas	Zn	Cd	Pb
Tips	contaminated	900	2	8
	uncontaminated	70	<1	<3
	transplant	130	1	<3
Internode 3	contaminated	3,390	9	20
	uncontaminated	240	<1	<3
	transplant	1,950	5	9

[1] From Myklestad *et al*., (1978)

In eastern Canada, the analysis of tips of *A. nodosum* fronds collected from an estuary suggested the presence of local inputs of Zn and Cd, though the range of Zn concentrations, from 26.9 to 60.9 μg/g, was much less than those reported in the Norwegian surveys. Very variable results were obtained for concentration factors which the authors suggested could have been due to exposure of the algae to tidal flow, which would affect the supply of nutrients and other elements, and the comparison of accumulation of up to two to three years' growth with the concentration in water samples at one time. The use of *A. nodosum* as a qualitative indicator was noted (Bryan and Langston, 1992).

2.3 Seaweeds and heavy metals

Algae are groups of autotrophic organisms in unicellular or multicellular forms. They are quite similar to terrestrial plants because they have chlorophyll *a* (in all groups), but chlorophyll *b*, chlorophyll *c*, and chlorophyll *d* can also be found in some groups. Multicellular forms or macroalgae can be seen with the naked eyes. Although they are large, their cells are attached quite loosely. They have no organ systems (no roots, no stems, and no leaves). The typical term for non-organ forms is 'thallus', meaning a structure that cannot be classified into roots, stems, and leaves. Some macroalgae have organs that look like organ systems, called rhizoids, stipes, and blades, respectively, because they have no vascular tissue. Finally, macroalgae are characterized by having very simple reproductive organs, lacking protective tissue around gametic cells, and display highly varied life histories. Analyses of ribosomal RNA and chromosomal DNA sequences indicate that red algae and green plants are closely related. With the exception of the more protozoanlike divisions

(Euglenophyta, Pyrrhophyta) and some classes of Chrysophyta, macroalgae and related species may be better aligned within the Kingdom Plantae (Dawes, 1998).

The groups of algae are classified according to pigments, cell wall component, reserve food in the cells, and flagella. They are classified into 11 main divisions (adapted from Bold and Wynne, 1985; Chapman and Chapman, 1981): Cyanophyta, Rhodophyta, Chlorophyta, Charophyta, Euglenophyta, Xanthophyta, Bacillariophyta, Chrysophyta, Phaeophyta, Pyrrhophyta, and Cryptophyta. Common marine macroalgae distributed in tropical regions are *Enteromorpha*, *Caulerpa*, *Halimeda*, *Neomeris* (Chlorophyta), *Sargassum*, *Dictyota*, *Padina* (Phaeophyta), *Laurencia*, *Porphyra*, *Gracilaria* (Rhodophyta).

Brown seaweeds are one of the most abundant seaweed groups with economic importance. Within this group, nearly 400 species belong to the genus *Sargassum* and they are widely distributed in the intertidal and shallow subtidal rocky substrata of the tropical and subtropical coastal waters. The annual world production of *Sargassum* in 1994/1995 was about 7,949 ton dry weight, and it is mainly produced in India, the Philippines and Vietnam. Some of the *Sargassum* species have been used as human food, animal feed, and medicine. Besides, they have been collected and used as fertilizer and raw material for the alginate processing industry in Mainland China, the Philippines, India, Vietnam and the United States. In Hong Kong, *Sargassum* species are underutilized and only used as fertilizers by the coastal communities (Wong and Cheung, 2001a).

The importance of macroalgae can be seen not only in the production of organic materials, but also in their various ecological roles, serving as nurseries and

habitats, and as direct sources of marine animal feed as well as human food (Dawes, 1998).

Seaweeds absorb metals directly from seawater. The pattern of heavy metal (Cu, Mn, Ni, Pb and Zn) accumulation in seaweeds is rapid initially. The uptake occurs within the first four hours of exposure, then gradually increases or decreases, depending on metal and seaweed species (Vasconcelos and Leal, 2001b, Murugadas, Phang, and Tong, 1995; Tropin and Zolotukhina, 1994). The rapid uptake corresponds to passive uptake involving cell surface adsorption and simple diffusion into cells or intercellular spaces (Knauer, Behra, and Sigg, 1997; Murugadas, Phang, and Tong 1995), identified as the first phase process. An initial rapid accumulation is followed by a slower uptake, identified as a second phase process or in some cases by a continuous or non-continuous excretion (regulatory measures) in the seaweeds. Regulatory mechanisms of heavy metal uptake have been reported in microalgae (Murugadas, Phang, and Tong, 1995).

Lobban and Harrison (1997) reported that algae take metals up both passively and actively. Some, such as Pb and Sr, may be passively adsorbed by charged polysaccharides in the cell wall and intercellular matrix. Other metals (e.g., Zn, Cd) are taken up actively against large intracellular concentration gradients.

Studies of metal uptake in algae are currently performed in the research laboratory to interpret the complex process of surrounding metal-organism interaction. Most of these experiments have pointed out that free aqueous ions and some organic complexes with certain ligands, such as amino acids and citrate, are the soluble, available and also most common forms of the metals able to pass across

biological membranes (Campbell, 1995; Newman and Jagoe, 1994; Simkiss and Taylor, 1989).

In the model proposed by Morel (1983) to explain metal uptake, the physiological effect on an aquatic organism is proportional to the free-ion activity rather than to the total dissolved metal concentration. The model assumes that a fraction of the metal (reactive species) is able to bind to critical sites on the plasma membrane and rapidly exchange with ligand sites on the surface of the living cells.

Some other models developed to explain transport routes across biological membranes for trace metals have been discussed by Tessier, Buffle, and Campbell (1994); Luoma (1983). It is accepted that most trace metals cross the cytoplasmic membrane via carrier-mediated transport. After absorption in the cell wall and through the plasma membrane, metals diffuse to the inner regions (e.g. cytoplasm and organelles) of the cells, being transported as complex species with selective proteins of the lipid layer of the membrane, and released as labile hydrophilic complexes in the cytoplasm. Another route for uptake discussed by Simkiss and Taylor (1989) is that metal ions permeate within protein channels extended through the membrane, and an interaction takes place between metals and the hydrophilic surfaces lining the pore.

Factors that influence the absorption of heavy metals are types of plants, age and position of thallus, ambient pH, salinity, temperature, interactions between metals and other elements, season of the year, as well as time of the day. The absorption processes may be due to interactions between two kinds or all kinds of factors.

Each seaweed, in general, is different intrinsically in metal absorption. Villares, Puente and Carballeira (2002) reported that *Enteromorpha* sp. could accumulate more heavy metals than *Ulva* species. Topcuoğlu, Güven, Balkis and

Kirbaşoğlu (2003) studied the use of marine algae in heavy metal monitoring at the Turkish coast of the Black Sea. They reported that the highest accumulations of different metals in the algal species were Cd in *Ceramium rubrum*, Co, Cu, Mn and Ni in *Phyllophora nervosa*, Cr, Fe and Pb in *Enteromorpha linza* and Zn in *Pterocladia capillacea*. Many researchers found that biomass of *Sargassum* species is suitable to use as a biosorbent for heavy metals (Davis, Volesky, and Vieira, 2000; Seki and Suzuki, 1998; Volesky and Holan, 1995).

The age of the fronds has an important bearing on the concentrations of Fe, Mn, Zn, and Cd accumulated, with older parts being more retentive. Position of an alga on the shore (i.e., the length of time that the seaweed is immersed in seawater during the tidal cycle) effects the accumulation of heavy metals (Lobban and Harrison, 1997). In addition, the concentrations of certain metals in an organism during periods of maximum growth may be diluted (Villares *et al*., 2002). Furthermore, metal concentrations decrease in macroalgae during periods of growth and increase during the dormant period in winter (Fuge and James, 1974). Riget, Johansen and Asmund (1995) observed seasonal variation in metal concentrations in *Fucus vesiculosus*. The study was carried out in Greenland in an area far removed from anthropogenic sources of metals and thus the fluctuations found should reflect natural variations. The seasonal variation may have been due to growth, as concentrations were highest in winter when growth is minimal and decreased in summer when growth is maximal; however, not all of the variation could be explained. In *Ulva lactuca*, on the other hand, higher concentrations of metals have been found during growth periods due to higher rate of photosynthesis and respiration during the summer, which would favor the assimilation of metals. Furthermore, the

mobilization of metals from the sediment in areas covered by large amounts of macroalgae could contribute to the accumulation (Villares *et al*., 2002). Similar to other researchers, Vasconcelos and Leal (2001b) studied the Cu uptake in *Porphyra* sp. and *Enteromorpha* sp. They found that Cu absorption is higher in summer (August) than in winter (January). Wright and Mason (1999) found higher concentrations of metal in winter in *Enteromorpha* species and *Pelvetia canaliculata*, and also noted a similar pattern in metal levels in sediments and in various invertebrates, indicating that the seasonal patterns appear to be more influenced by environmental factors, such as discharges to the ocean, pH, etc., than by factors intrinsic to the organisms themselves, such as metabolism or reproduction.

The pH of the environment, particularly in solution, is important in the solubility of metals. The solubility of metals increases as pH decreases. Therefore, higher pH values usually result in higher metal cation uptakes due to lowered metal solubility. Microprecipitation of insoluble metal species may occur close to neutral pH values. This may complicate efforts to elucidate the binding mechanism since typically complexation is dominant. In addition, an environment with little or no buffering capacity tends to have higher concentrations of metals such as Al, Cu, Fe, Pb and Zn (Davis *et al*., 2000; Horne and Dunson, 1995; Xue, Stumm, and Sigg, 1988).

Salinity is one of the factors that have effects on toxicity and accumulation of heavy metals in seaweeds. Researchers reported that metal uptake increases as the salinity decreases. Because of seasonal variations in estuaries, the biota will be exposed to various levels of salinity which will have a direct effect on metal uptake

and therefore on their tissue concentrations (Amado Filho, Karez, Andrade, Yoneshigue-Valentin and Pfeiffer, 1997; Hance, 1988).

The effect of temperature on metal uptake is less consistent. In general, increases in temperature cause an increase in the rate of accumulation of metals (Phillips, 1977).

Season of the year is related to the concentrations of metals in any environment, depending on dilution of water. High precipitation in rainy season can dilute the concentrations of metals. In addition, the concentrations of different elements in an organism can vary with the season, independently of environmental concentrations; for example, the contents of certain elements may be diluted during periods of maximum growth in an organism. Villares *et al*. (2002) showed that the concentration of heavy metals differs significantly with time in some seaweeds. However, in some plants there are no significant differences. There may be different reasons for the seasonal differences found, including environmental factors, such as variations in metal concentrations in solution, interactions between metals and other elements, salinity, pH, etc.; metabolic factors, such as dilution of metal contents due to growth; or they may be due to interactions between various kinds of factors.

Tropin and Zolotukhina (1994) studied the uptake of heavy metals by seaweeds. They found that the metal content in the thallus depended not only on exposure time, but also, to a certain extent, on the time of day. The accumulation of most heavy metals, as a rule, had a maximum rate in the daytime and a minimum at night. The illumination intensity positively affects their accumulation, especially of physiologically important elements (Cu, Zn). The level of accumulation depends on plant species. *Padina gymnospora* presented the highest accumulation level of Zn. It

is indicated that both *Padina gymnospora* and *Sargassum filipendula* can be used for biomonitoring heavy metals in coastal tropical waters (Amado Filho *et al*., 1997). Moreover, biomass of these species can be used for binding and removing heavy metals from industrial wastewater (Volesky and Holan, 1995).

In laboratory studies, Strömgren (1979) cultured the apices of five species of Fucales and studied the effect of Zn on the increase in length. He found, during a period of ten days, that there were no significant growth responses at the concentration 0.1 mg/l of Zn. However, there was a signification reduction of growth during the first two to three days of exposure to 1.4 mg/l of Zn. Strömgren (1980b) studied the effect of Pb, Cd and Hg on the increase in length of five species of Fucales and found that the concentration of metal for which there had been significant reduction of growth rate was ≥ 810, ≥ 450 and ≥ 10 μg/l of Pb, Cd and Hg, respectively. Amado Filho *et al*. (1997) studied the effects of Zn on growth and accumulation in six seaweed species. They reported that all species died at 5,000 μg/l of Zn, two species (*Ulva lactuca* and *Enteromorpha flexuosa*) died at 1,000 μg/l, and one, *Hypnea musciformis*, died at 100 μg/l. The lowest concentration of Zn that presented growth inhibition in these seaweeds was 20 μg/l. They showed, in addition, a low percentage association of Zn and Cd with the polyphenolic fraction of *Sargassum* sp. and suggested that the high Zn and Cd found in whole algae from polluted areas might be greater than the capability of polyphenols to bind these metals.

It is known that there are many metals effecting the growth rate of biota in any oceanic environment. On the other hand, very little information about the effects of multiple metals on phytoplankton and macrophytes has been reported. Ince, Dirilgen,

Apikyan, Tezcanli and Üstün (1999) studied toxic interactions of heavy metals in duckweed, *Lemna minor*. They reported that Cu+Zn, Cu+Cr and Cr+Co showed antagonistic effects in any concentration. Zn+Cr showed antagonistic effects in most cases, except at 2 ml/l of Zn and 2 ml/l of Cr, in which an additive effect was observed. Co+Cu showed antagonistic effects in most cases, except 1 ml/l of Co and 1 ml/l of Cu, which had an additive effect. Zn+Co showed both additive and antagonistic effects, depending on the concentrations of the metals.

In *Chlamydomonas* sp., high concentrations of Cu and Zn inhibited cellular Mn uptake, whilst high Cu concentrations inhibited Zn uptake rates (Sunda and Huntsman, 1998). Experimenting with microalgae (*Emiliania huxleyi*), Vasconcelos and Leal (2001a) found that both Pb and Cd were slightly antagonistic to Cu uptake. Strömgren (1980a) studied combined effects of Cu, Zn and Hg on the increase in length by culturing the apices of *Ascophyllum nodosum*. When treating the algae with each metal, he found that Cu and Hg were far more toxic than Zn. When applied with multiple metals, it was noted that all combined metals (Cu+Zn, Hg+Zn and Cu+Hg) showed an antagonistic effect. The degree of antagonism was Cu+Zn > Hg+Zn > Cu+Hg, since the significant antagonistic effects occurred in 3 days, 4 days and 7 days, respectively.

Although heavy metals are toxic to aquatic organisms, they are significantly accumulated by many different freshwater and marine species. Benthic marine algae have long been known to concentrate metals several thousand times higher than their concentration in seawater (Bryan and Langston, 1992; Melhuus, Seip, Seip, and Myklestad, 1978; Seeliger and Edwards, 1977; Foster, 1976). This ability makes algae

useful in marine pollution assessment and biomonitoring studies (Phillips, 1995; Barnett and Aschcroft, 1985; Fuge and James, 1974).

Some researchers have determined heavy metals in aquatic environments, macroalgae, sediments and benthic animals (Topcuoğlu, Kirbaşoğlu and Güngör, 2002; Storelli, Storelli and Marcotrigiano, 2001; Wright and Mason, 1999; Sfriso, Marcomini and Zanette, 1995). The degree of contamination is varied in time, places, seawater, sediments, as well as biota.

Topcuoğlu, Kirbaşoğlu and Güngör (2002) studied heavy metals in organisms and sediments from the Turkish coast of the Black Sea and found that the patterns of heavy metals occurrences in macroalgae in order of decreasing contents were Zn > Cu > Cr ≥ Pb ≥ Cd whilst heavy metals in sediments were Zn > Cr > Cu > Cd > Pb. In addition, they found that the metal levels in macroalgae did not follow the same pattern as concentrations in sediments at the same station.

Few researchers have measured the concentrations of heavy metals in tropical marine environments: Murugadas, Phang, and Tong (1995) in Malaysian waters; Rajendran *et al*. (1993) in Indian waters; Ganesan *et al*. (1991) in the Gulf of Mannar, Bay of Bengal; Kureishy (1991) in the coast of Qatar; and Ho (1987) in Hong Kong waters. Very little information in Thailand was reported. Kan-Atireklap, Suwanagosoom, Buntivivutkul, and Sanguansin (1998) and Suwanagosoom, Sanguansin, Kan-Atireklap, and Buntivivutkul (1998) monitored the levels of Hg in the water column along the eastern coast of the Gulf of Thailand. However, there is not enough information about heavy metals contaminating Thailand's marine environment, including an accumulation in macroalgae.

Determination of heavy metal levels in marine organisms is usually preferred over the measuring of the metal concentrations in seawater and sediment samples. Metal concentrations in seawater are very low and show wide fluctuations. Heavy metal concentrations in sediment samples can be changed by organic matter (ligands) content, grain size composition, pH, oxidation-reduction potential, etc. (Topcuoğlu, Güven, Balkis and Kirbaşoğlu, 2003). At the same time, the metal levels in sediments are relatively invariant with time. On the other hand, marine organisms can be used as monitors to give information on concentrations of heavy metal or changes in metal availabilities in the surrounding environment.

The amount of metal concentration accumulated in seaweeds can vary in different species and for different isotopes as well as for the same isotope, since different species of seaweed have different affinities for different heavy metals (Sawidis, Brown, Zachariadis and Sratis, 2001). Many researchers, thus, used different plants and locations to find out the accumulation properties. Common macroalgae used, *in situ*, for heavy metal monitoring are showed in Table 3.

Table 3 Common macroalgae used for heavy metal monitoring

Seaweeds	Location sites	References
Division Chlorophyta		
Enteromorpha sp.	Oporto coast, Portugal	Leal, *et al*., 1997
E. sp.	Galicia, Spain	Carral, *et al*, 1995
E. prolifera	Southern Adriatic Sea, Italy	Storelli *et al*., 2001
	Gulf San Jorge, Argentina	Muse, *et al*., 1999
E. compressa	Aegean Sea, Greece	Sawidis, *et al*., 2001
	Indian Ocean, India	Rajendran *et al*, 1993
	Gulf of Mannar, Bay of Bengal	Ganesan *et al*., 1991
	Sea of Hong Kong	Ho, 1987

Table 3 Common macroalgae used for heavy metal monitoring (*cont*.)

Seaweeds	Location sites	References
Division Chlorophyta (*cont*.)		
Enteromorpha flexuosa	Turkish Coast of the Black Sea	Topcuoğlu *et al*., 2003
	Sea of Hong Kong	Ho, 1987
Ulva lactuca	Turkish Coast of the Black Sea	Topcuoğlu *et al*., 2003
	Southern Adriatic Sea, Italy	Storelli *et al*., 2001
	Aegean Sea, Greece	Sawidis, *et al*., 2001
	Gulf San Jorge, Argentina	Muse, *et al.*, 1999
	Gulf of Mannar, Bay of Bengal	Ganesan *et al.*, 1991
	Sea of Hong Kong	Ho, 1987
U. rigida	Turkish Coast of the Black Sea	Topcuoğlu *et al*., 2003
	The lagoon of Venice, Italy	Favero *et al*., 1996
	The lagoon of Venice, Italy	Sfriso *et al*., 1995
U. reticulata	Gulf of Mannar, Bay of Bengal	Ganesan *et al*., 1991
Division Phaeophyta		
Fucus ceranoides	Galicia, Spain	Carral, *et al*, 1995
F. vesiculosus	Archipelago, Stockholm	Forsberg, *et al*., 1988
	Humber estuary, England	Barnett and Aschcroft, 1985
	Bristol Channel, England	Fuge and James, 1974
Padina gymnospora	Sepetiba Bay, Rio de Janeiro	Amado Filho, *et al*., 1999
	Gulf of Mannar, Bay of Bengal	Ganesan *et al*., 1991
P. tetrastomatica	Malaysia	Murugadas, *et al*., 1995
	Gulf of Mannar, Bay of Bengal	Ganesan *et al*., 1991
P. pavonica	Aegean Sea, Greece	Sawidis, *et al*., 2001
	The Crimea, Black Sea	Zolotukhina and Radzhinskaya, 1995
P. arboresceus	Sea of Hong Kong	Ho, 1987
Sargassum sp.	Aegean Sea, Greece	Sawidis, *et al*., 2001
S. stenophyllum	Sepetiba Bay, Rio de Janeiro	Amado Filho, *et al*., 1999
S. siliquosum	Malaysia	Murugadas, *et al*., 1995
S. baccularia	Malaysia	Murugadas, *et al*., 1995
S. pellidum	the Bays of Popov Island	Tropin and Zolotukhina, 1994
S. johnstonii	Gulf of Mannar, Bay of Bengal	Ganesan *et al*., 1991
S. wightii	Gulf of Mannar, Bay of Bengal	Ganesan *et al*., 1991

Table 3 Common macroalgae used for heavy metal monitoring (*cont*.)

Seaweeds	Location sites	References
Division Phaeophyta (*cont*.)		
Sargassum binderi	the coast of Qatar	Kureishy, 1991
S. heteromorphum	the coast of Qatar	Kureishy, 1991
S. boveanum	the coast of Qatar	Kureishy, 1991
Division Rhodophyta		
Gracillaria verrucosa	Aegean Sea, Greece	Sawidis, *et al*., 2001
G. changii	Malaysia	Murugadas, *et al*., 1995
G. edulis	Malaysia	Murugadas, *et al*., 1995
G. salicornia	Malaysia	Murugadas, *et al*., 1995
G. tikvahiae	Atlantic Ocean, North Carolina	Burdin and Bird, 1994
G. filicina	Indian Ocean, India	Rajendran *et al*., 1993
G. corticata	Gulf of Mannar, Bay of Bengal	Ganesan *et al*., 1991
Porphyra sp.	Oporto coast, Portugal	Leal, *et al*., 1997
P. columbina	Gulf San Jorge, Argentina	Muse, *et al*., 1999
P. suborbiculata	Sea of Hong Kong	Ho, 1987

Ho (1987) found that the cosmopolitan green alga *Ulva lactuca* was a good bioindicator for Cu, Zn, and Pb pollution associated with sewage in Hong Kong waters because of its high accumulation capacity. For similar reasons, Forsberg, Söderlund, Frank, Petersson and Pedersén (1988) found *Fucus vesiculosus* to be a good detector of metal pollution and recommended using *Enteromorpha* as a monitor for heavy metals in estuaries. The amounts of heavy metals varied in studied sites and kinds of algae, although several researchers have reported concentrations of the metals in several macroalgae and areas. On the other hand, there has been no such report done using any seaweed in Thailand.

It is not possible to compare concentrations reported from other marine environments because of the wide variations of metal concentrations observed due to seasonal changes and to the chemical-physical characteristic of the sampling sites (Favero *et al*., 1996). Moreover, the influence of the systematic position and chemical composition of algae species on the uptake of metals has been investigated by many authors.

Topcuoğlu, Kirbaşoğlu, and Güngör (2002) reported heavy metals in the near shore sediments in the eastern Black Sea that might be related to agricultural and industrial activities of the region, especially from mining practices. At the same time, elevated concentrations of some heavy metals can be related to the geology of the sediment source. On the other hand, the heavy metal concentrations in the western Black Sea region are influenced by the rivers and atmospheric precipitation. They indicated that heavy metal levels in macroalgae did not follow the same patterns as concentrations in sediment at the same station. Furthermore, concentrations of Cd, Co, Cr, Zn, Fe, Mn and Cu in sea snail, mussel and fish samples related to sediment results in the examined stations. In addition, the concentrations of trace elements in fish tissue are also related to concentrations of elements in the sediment. Most notably, the concentrations of contaminants in sediments or water do not necessarily reflect their bioavailabiliy. In addition, the metal levels in macroalgae do not follow the same pattern as concentrations in environments.

In passive binding processes named biosorption, brown algal biomass, particularly, *Sargassum* sp., have proven to be highly effective as well as reliable and predictable in the removal of heavy metals, especially Pb^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+}, from

aqueous solutions (Hashim and Chu, 2004; Davis, Volesky and Mucci, 2003; Davis, Volesky and Vieira, 2000; Zhou, Huang and Lin, 1998).

Typical algal cell walls of brown algae and red algae are composed of a fibrillar skeleton and an amorphous embedding matrix. The most common fibrillar skeleton material is cellulose (Fig. 1). It can be replaced by xylan in red algae. The Phaeophyta algal embedding matrix is predominately alginic acid or alginate with a smaller amount of sulfated polysaccharide (fucoidan). Both the Phaeophyta and Rhodophyta divisions contain the largest amounts of amorphous embedding matrix polysaccharides. This characteristic, combined with their well-known ability to bind metals, makes them potentially excellent heavy metal biosorbents (Davis, Volesky and Mucci, 2003).

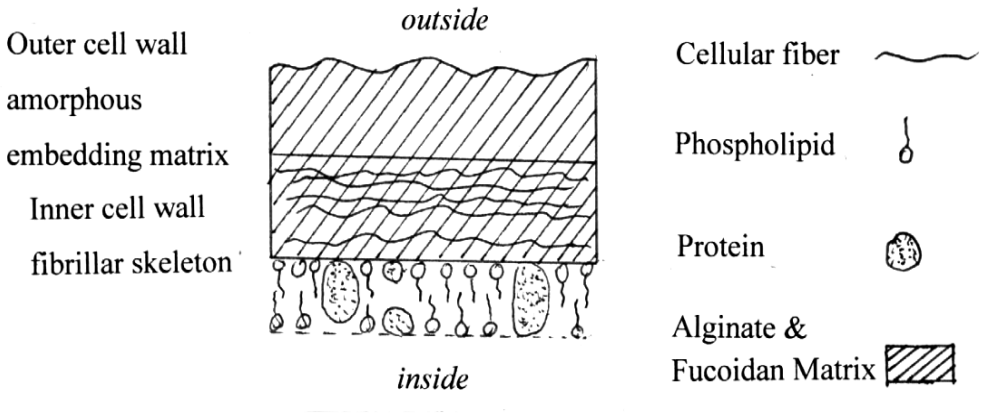

Fig. 1 Cell wall structure in the brown algae (After Davis *et al*., 2003)

Researchers have reported the effects of heavy metals on physiological changes in macroalgae. Amando Filho *et al*. (1996) submitted *Padina gymnospora* to high Zn concentrations under laboratory experimental conditions. Analytical electron microscopy of this seaweed showed the presence of Zn in dense granules along the cell walls indicating that these walls could play the main role in the Zn accumulation.

Strömgren (1980b) studied the toxicity of five metals on five seaweed species in Fucales. He found that Cu is more toxic than Hg, and that they both are far more toxic than Zn, Pb, and Cd. The amount of metal that reduces growth rate of these algae by 50% is indicated in Table 6. In brown algae, Cu is accumulated in the form of large osmophilic compounds in vacuoles (Brinkhuis and Chung, 1986). In some species, the vacuoles are small colorless vesicles, named physodes and contain reducing phenolic compounds (Davis, Volesky and Mucci, 2003). Meanwhile, Silverberg, Stokes and Ferstenberg (1976) reported that *Scenedesmus*, a green alga, deposits Cu in intracellular inclusions, a detoxification mechanism present only in metal tolerant strains.

Few researchers have reported the amount of fatty acid in different *Sargassum* species and various locations (Table 4) (Li, Fan, Han and Lou, 2002; Zhukova and Svetashev, 1999; Heiba, El-Elsa and Rizk, 1997; Vaskovsky, Khotimchenko, Xia and Hefang, 1996; Khotimchenko, 1991; Arao and Yamada, 1989). They found that the crude lipids from four *Sargassum* species range from 0.02 - 0.04 g/g dry weight. The fatty acids ranged from C12:0 – C24:0. The C14:0, C16:0, C18:1, C18:2, C18:3, C20:2 and C20:4 fatty acids were dominant. These fatty acids accounted for more than 60 – 80% of the total fatty acids reported. Palmitic acid (C16:0) was the major fatty acid present, comprising 10 – 49% of the total fatty acid content. The degree of unsaturation was : saturated > monoenes > dienes > trienes > tetraenes > pentaenes > hexaenes. The most abundant saturated fatty acid was C16:0 followed by C14:0, and far more than C18:0 or C17:0, respectively. The percentage of saturated fatty acids ranges from 14.0 – 66%. The (n-3) polyunsaturated fatty acids (PUFAs) quite vary. They range from 4.0 – 36.3%. Similarily to (n-3) PUFAs, (n-6) PUFAs range from

6.2 – 61.8%; the C_{18} PUFAs and C_{20} PUFAs range from 12.2 – 43.5% and 15.4 – 30.8%, respectively (Table 5).

Table 4 Locations, time of sampling and *Sargassum* species for which the fatty acid component was studied.

No.	Species	Location of Sampling	Time of Sampling	References
1.	*S. kjellmanianum*	Bohai Sea, Weihai,	China August, 2001	Li *et al*., 2002
2.	*S. thunbergii*	Bohai Sea, Weihai,	China August, 2001	Li *et al*., 2002
3.	*S. pallidum*	Great bay, Sea of Japan	October, 1995, 96,97	Zhukova, Svetashev, 1999
4.	*S. binderi*	Qatari coast, Qatar	March and April, 1998	Heiba *et al*., 1997
5.	*S. boveanum*	Qatari coast, Qatar	March and April, 1998	Heiba *et al*., 1997
6.	*S. denticulatum*	Qatari coast, Qatar	March and April, 1998	Heiba *et al*., 1997
7.	*S. heteromorphum*	Qatari coast, Qatar	March and April, 1998	Heiba *et al*., 1997
8.	*S. miyabei*	Yellow sea, Qingdao	Nov. 1992 – Jan. 1993	Vaskovsky *et al*., 1996
9.	*S. thunbergii*	Yellow sea, Qingdao	Nov. 1992 – Jan. 1993	Vaskovsky *et al*., 1996
10.	*S. miyabei*	Great bay, Sea of Japan	May, 1988	Khotimchenko, 1991
11.	*S. pallidum*	Great bay, Sea of Japan	August, 1989	Khotimchenko, 1991
12.	*S. herklotsii*	Vietnam, South-China Sea	April, 1987	Khotimchenko, 1991
13.	*S. baccularia*	Vietnam, South-China Sea	April, 1987	Khotimchenko, 1991
14.	*S. microcystum*	Seychelles Isds, Indian	Oceon March, 1989	Khotimchenko, 1991
15.	*S. turbinariodes*	Seychelles Isds, Indian Ocean	March, 1989	Khotimchenko, 1991
16.	*S. cristaefolum*	Seychelles Isds, Indian Ocean	March, 1989	Khotimchenko, 1991
17.	*S. ringgoldianum*	Shizuoka, Prefecture, Japan	April, 1986	Arao, Yamada, 1989

The *Sargassum* from different locations have different amounts of fatty acids. Khotimchenko (1991) studying fatty acid compositions of seven *Sargassum* species from three different areas, found that palmitic acid (C16:0) was the major fatty acid in all species (22 – 37% of total fatty acids), followed by C20:4(n-6) (12 – 18%) and C18:1(n-9) (7 – 11%), respectively. However, the content of palmitic acid was lowest

in algae from the Sea of Japan (22 – 23%) and highest in algae from the South-China Sea (33.37%). In general, researchers reported that Phaeophyta typically have high concentrations of C_{18} and C_{20}PUFAs (Li *et al*., 2002; Zhukova and Svetashev, 1999; Wahbeh, 1997; Khotimchenko, 1991; Voskovsky *et al*., 1996; Arao and Yamada, 1989). Khotimchenko (1991) found that the percentage of polyunsaturated fatty acids ranges from 17 – 26 and 17 – 28, respectively. In *Sargassum pallidum* collected from Vitiaz Bay, Sea of Japan, the percentage of polyunsaturated, monounsaturated and unsaturated fatty acids is 73 – 74, 11 and 14, respectively (Zhukova and Svetashev, 1999). Some researchers suggested that the fatty acid composition of algae from different areas changes in response to environmental factors due, probably, to changes in lipid metabolism that would lead to a better adaptation (Zhukova and Svetashev, 1999; Wahbeh, 1997). In addition, the adaptation was not the same for different species (Vaskovsky *et al*., 1996). It is known that algae accumulated polyunsaturated fatty acids (PUFAs) when there was a decrease in the environmental temperature (Khotimchenko, 1991). Season of the year can cause a change in the amount of fatty acids. In *Fucus serratus* from Roscoff in France, the amount of total lipids, triacylglycerols and fatty acids is highest in summer and lowest in winter, since the thalli have good growth and productivity as well as the synthesis of lipids being activated during the summer (Kim, Dubacq, Thomas and Giraud, 1996). On the other hand, Zhukova and Svetashev (1999) studied fatty acid component from *Sargassum pallidum* from Peter the Great Bay, Sea of Japan, in different seasons during three years. They reported that seasons and places were not related to fatty acid compositions since this plant had similar fatty acid components.

Table 5 Major fatty acid components (%) found in various *Sargassum* species

Fatty acids	*Sargassum* species								
	1 *	2	3	4	5	6	7	8	9
14:0**	4. 0	4.0	2.8	5.6	5.0	6.9	5.9	1.6	1.7
16:0	21.1	22	10.4	41.8	49.5	48.6	48.0	21.5	19.5
16:1(n-7)	2.5	3.1	3.8	-	-	-	-	2.6	6.8
17:0	0.6	0.5	-	1.6	1.1	1.5	2.0	-	-
18:0	1.3	0.8	0.3	1.2	-	1.9	1.7	1.4	1.3
18:1(n-9)	7.9	6.2	5.8	11.7	8.9	6.1	7.4	7.4	6.1
18:2(n-6)	15.0	5.0	26.5	3.4	3.5	4.0	4.1	4.3	5.4
18:3(n-6)	1.2	0.7	3.1	2.0	1.4	0.2	2.2	1.0	0.7
18:3(n-3)	4.6	9.8	4.5	-	-	-	-	9.3	8.1
18:4(n-3)	4.4	11.6	3.8	-	1.0	-	1.1	12.9	6.9
20:3(n-6)	3.2	0.6	15.5	1.6	1.3	0.5	1.8	0.9	0.5
20:4(n-6)	21.0	10.2	16.2	4.2	2.7	0.9	2.4	13.1	12.7
20:4(n-3)	1.0	0.9	0.7	-	-	-	-	0.8	0.6
20:5(n-3)	5.3	17.5	2.1	2.2	3.4	4.2	2.7	13.3	9.1
(n-3)PUFA	15.4	40.0	11.2	4.6	4.5	4.2	4.1	36.3	25.9
(n-6)PUFA	40.4	16.5	61.8	12.4	10.2	6.2	11.0	20.0	20.1
C_{18}FUFA	25.2	27.1	43.5	18.3	14.7	12.2	16.5	39.4	27.2
C_{20}FUFA	30.8	29.2	36.2	16.8	18.8	15.4	16.3	30.7	25
Saturated	27.4	28.5	14.0	54.3	60.6	66.4	63.2	26.8	25.8

* Numbers designate *Sargassum* species given in Table 4

** Some fatty acids are not shown.

Table 5 Major fatty acid components (%) found in various *Sargassum* species (*cont.*)

Fatty acids	*Sargassum* species							
	10	11	12	13	14	15	16	17
14:0	4.0	3.6	5.2	4.6	3.5	4.2	3.3	6.7
16:0	23.5	22.4	33.5	37.7	28.8	27.2	26.9	26.1
17:0	-	-	-	-	-	-	-	-
16:1(n-7)	4.0	6.1	5.4	6.3	3.7	3.3	3.4	4.0
18:0	0.7	0.8	0.9	0.9	0.9	1.2	1.0	-
18:1(n-9)	8.3	7.2	8.9	10.2	9.9	11.1	10.6	8.3
18:2(n-6)	4.8	9.8	7.2	4.9	5.6	5.4	4.6	4.3
18:3(n-6)	0.5	1.3	1.1	0.6	0.6	0.7	0.5	-
18:3(n-3)	7.0	7.2	6.2	6.3	7.9	6.7	8.9	15.2
18:4(n-3)	7.4	7.3	7.5	5.1	6.5	5.3	7.5	10.1
20:3(n-6)	0.6	3.6	0.7	0.9	1.0	1.0	1.0	-
20:4(n-6)	12.4	18.0	13.5	12.2	15.2	14.4	14.2	15.6
20:4(n-3)	0.7	1.1	0.9	0.9	1.1	1.1	1.1	1.2
20:5(n-3)	14.4	3.8	3.8	3.2	4.6	4.3	4.5	6.7
(n-3)PUFA	29.6	19.7	18.4	15.5	20.4	18.9	23.2	33.2
(n-6)PUFA	18.6	33.0	22.6	18.8	22.6	21.8	21.1	19.9
C_{18}FUFA	19.7	25.9	22.0	16.9	20.8	18.1	21.5	37.9
C_{20}FUFA	28.5	27.1	19.0	17.4	22.4	22.6	22.8	23.5
Saturated	29.0	27.8	41.3	44.8	33.9	33.7	32.0	32.8

* Numbers designate *Sargassum* species given in Table 4

** Some fatty acids are not shown.

It is known that seaweeds are traditionally used in human and animal nutrition. On the other hand, little information is available on the nutritional value of algal proteins. Their protein contents differ according to the species and seasonal conditions. Higher protein levels were observed during the end of the winter period

and spring whereas lower amounts were recorded during the summer months (Fleurence, 1999). For example, an annual monitoring of protein level from *Palmaria palmata* collected on the French Atlantic coast showed that the protein content of this alga can vary from 9 – 25% dry weight. In general, the protein fraction of brown seaweeds is low (3 – 15% dry weight) compared with that of the green or red seaweeds (10 – 47% dry weight), except for the species *Undaria pinnatifida* which has a protein level between 11 and 24% dry weight. Most brown seaweeds industrially exploited, for example, *Laminaria digitata*, *Ascophyllum nodosum*, *Fucus vesiculosus* and *Himanthalia elongata* have a protein content lower than 15% dry weight. In some green seaweeds, such as the species belonging to the genus *Ulva*, the protein content can represent between 10 and 26% dry weight of the plants. For instance, the species *Ulva pertusa*, which is frequently consumed under the name of ao-nori by the Japanese people, has a high protein level between 20 and 26% (dry product). Higher protein levels were recorded for the red seaweeds such as *Porphyra tenera* (47% dry mass) or *Palmaria palmata* (35% dry mass). These algae, known under the names of nori and dulse, respectively, have protein levels higher than those found in high-protein pulses such as soybean (Fleurence, 1999). The protein content (% dry weight) from some seaweeds was 13.6%, 17.4%, 17.6% and 24.5% from *Enteromorpha compressa*, *Padina pavonica*, *Ulva lactuca* and *Laurencia obtusa*, respectively (Wahbeb, 1997).

Wong and Cheung (2001a, 2001b) found that methods of drying samples (oven-dried and freeze-dried) showed no significant differences of crude protein content from three *Sargassum* species. In addition, they showed that oven drying was found to be more suitable for protein extraction of brown seaweeds. There were, on

the other hand, significant differences of crude protein content from different species. Crude protein content was of the order of 5.03 – 5.33, 7.56 – 8.20 and 11.3 – 11.9 g/100 g dry weight in *S. hemiphyllum*, *S. patens* and *S. henslowianum*, respectively. According to Fleurence (1999), variations in the protein content of seaweeds can be due to species and seasonal period.

Laem Chabang industrial estate, an industrial area, located in Sriracha District, Chonburi Province, may outflows untreated water into the Gulf of Thailand. Chromium, copper, lead, cadmium and zinc are heavy metals contaminating this area. The amount of these toxic metals must be measured and if the concentrations exceed standard accepted maximum levels, an urgent action for safety must be made. As macroalgae are producers, accumulated heavy metals can be transferred to other organisms of higher trophic levels. Unlike animals, the algae are not able to escape from any unsuitable place, therefore, they absorb all heavy metals from the habitat where they grow. This should lead to a conclusion that the more heavy metals in macroalgae, the more they are also concentrated in other organisms.

In Thailand, the Office of the National Environmental Board, Ministry of Science, Technology and Environment (ประกาศราชกิจจานุเบกษา, 2537), allows the amount of heavy metals in marine seawater shown in Table 6.

The Eastern Marine Fisheries Research and Development Center, Subdistrict Phe, Muang District, Rayong Province, about 80 km. from Laem Chabang industrial estate is near a fishery bridge, which is effected by anthropogenic activities, and Ko Samet (Samet Island), a great sightseeing area (Fig. 2). Since the water has good

quality, there are some seaweed communities. Certain common algae are *Padina* species, *Sargassum* species, *Enteromorpha* species, and so forth.

Table 6 The maximum allowable amounts of metals in seawater by the Office of the National Environmental Board, Ministry of Science, Technology and Environment of Thailand, and the amounts of metals that reduce growth rate of these algae by 50%

Metals	Maximum amount (μg/l)	The amounts of metals that reduced 50% growth rate in Fucales (μg/l, Strömgren, 1980b)
Cr	≤100	-
Zn	≤100	5,000-10,000
Cu	≤ 50	60-80
Pb	≤ 50	2,600
Cd	≤ 5	2,600

CHAPTER III

RESEARCH PROCEDURES

3.1 Geographic scope and duration of study

The researches were conducted both in the field and in the laboratory. In the field, the common benthic macroalga, *Enteromorpha clathrata* Greville, was collected from two locations. As a non-polluted site, the seaweed was collected from Phe, the sea near the Eastern Marine Fisheries Research and Development Center, Muang District, Rayong Province, Eastern Thailand. As a polluted site, the plant was collected from the sea near Laem Chabang, Sriracha District, Chonburi Province, Eastern Thailand, about 80 Km apart from the first location (Fig. 2). Besides, *Sargassum polycystum* C. Agardh, and *Padina japonica* Yamada, found in clean water areas were collected from Phe. In addition, sediments and the seawater nearby the seaweeds were collected. All samples were collected between February, 2000 and January, 2001.

3.2 Measurements for metals

After collecting the plants, time of sampling, pH, temperature, as well as salinity was measured. However, the collected plants needed to be the same species, and similar stages of each species were chosen. The sediments were collected from the depth of about 5 cm from the bottom of the sea. The water was collected at about 5 cm from the surface. All samples were brought to the laboratory under refrigeration

(Favero, *et al*., 1996). The laboratories in the F1 and F2 buildings of the Center for the Scientific and Technological Equipment (CSTE), Suranaree University of Technology, Muang District, Nakhon Ratchasima Province, and the Botany laboratory, Biological Department, as well as the Food Science laboratory, Food Science Department, Faculty of Science, Ramkhamhaeng University, Bangkok were used.

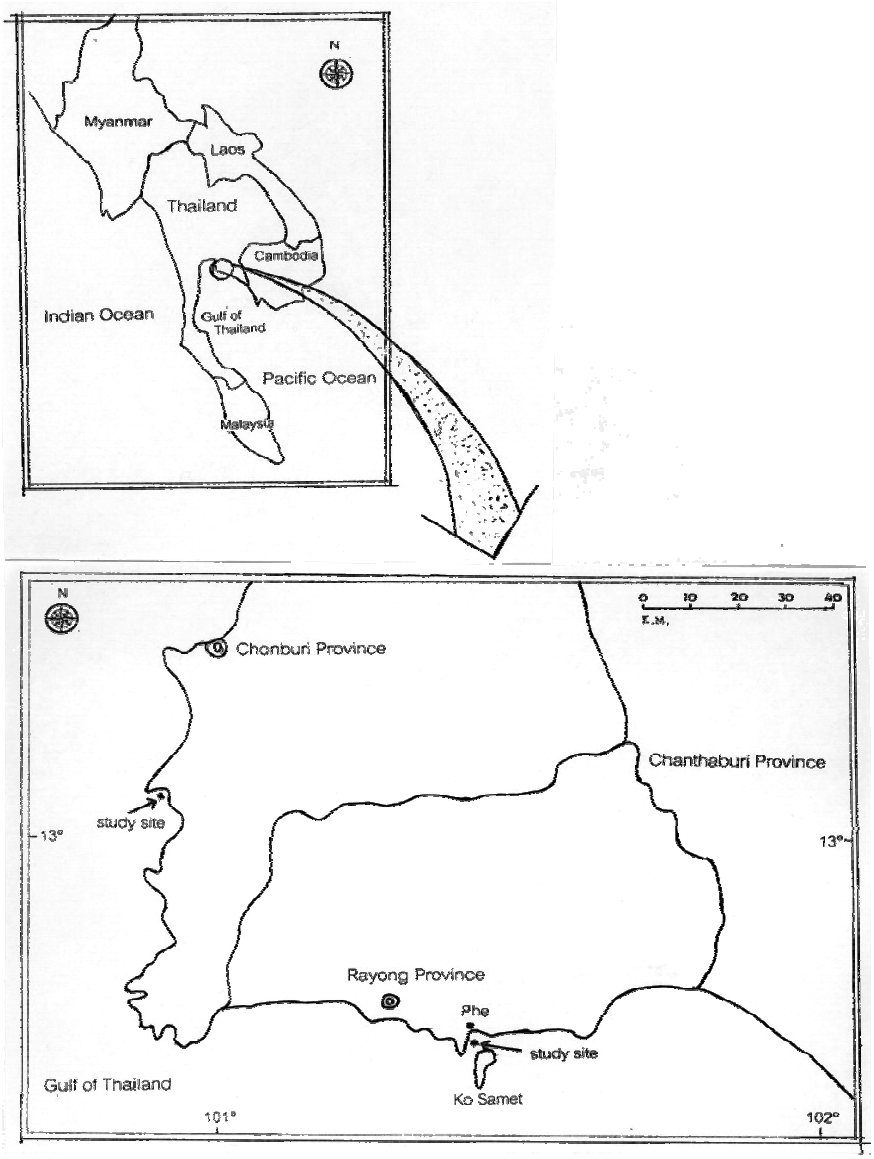

Fig. 2 Locations of study

3.2.1 Laboratory methods

Plant materials

Sufficient material to give at least 10 g dry weight of seaweed were cleaned thoroughly with distilled water to wash off associated epiphytes and debris, then washed with distilled-deionized water 2 times and dried at 50 °C until they reached a constant dry weight. For *Enteromorpha clathrata*, the whole plants were used; for *Padina japonica*, the whole plants of both gametophyte and sporophyte plant were used; for *Sargassum polycystum*, the holdfast, stipe, blade and air bladder were separated and analyzed. After homogenization, the samples were placed in polyethylene containers and stored in a desiccator at room temperature. A portion of the homogenized samples was taken with plastic spatulas. The samples were digested with HNO_3. The solution was evaporated and suspended in 0.1 *N* HCl. Metal concentrations were measured by an air-acetylene flame atomic absorption spectrophotometry (AAS) using a Perkin - Elmer, Analyst 100. Each sample was repeated three times (Amado Filho, *et al*., 1997). All metal concentrations were measured in units of μg/g.

Sediment samples

The sediment samples were dried at 50 °C, homogenized by grinding, and then sieved through a 2 mm mesh size. An accurately weighed portion of powdered and dried sediment sub-sample (about 0.5 – 1 g dry weight) was placed in an open quartz tube and 8 ml of concentrated HNO_3 were added. Then, after the carbon dioxide bubbling had ceased, 2 ml of $HClO_4$ was added. The mixture was left for cold digestion for 3 hr at room temperature, and then the quartz tube was heated at 130 °C for four more hours. After cooling, the mixture were filtered through Whatman No. 4

filter paper, and the filtrate was diluted to 100 ml with double deionized water (Sawidis, *et al*., 1995). These solutions were measured for metal concentrations as done in the plant samples and recorded in units of μg/g.

Seawater

At the laboratory, the seawater was filtered through 0.45 μm millipore membrane filters. Before filtration, the membranes were soaked overnight with 10% nitric acid and then washed with double deionized water. The filtrates were analyzed for metal concentrations, as done in the sediments and the plant samples (adapted from Muse *et al*., 1999 and Sawidis, *et al*., 1995). All metal concentrations were recorded in units of μg/l.

3.3 Effects of metals on seaweeds

3.3.1 Preliminary study and effects of metals on seaweed

For study the effects of metals on biosynthesis, *Sargassum polycystum* was grown in a 1,000-litre tank under aeration and natural condition in order to decrease some effects from culturing. After 10 days, the healthy plants were selected and grown in a 30-liter aquarium in seawater under aeration under 50% light. The water was pumped from the sea at the same area as the plant growth and filtered with No. 140 mesh size (106 μm). Individual metal (Cd, Cr, Cu, Pb and Zn) at 0, 10, 20, 50 and 100 times of the metal concentration in the seawater was added into each aquarium. After 0, 2, 4, 6 and 8 days of experimentation, the concentrations of chlorophyll *a* and chlorophyll *c*, total protein content, lipid content and fatty acid profile were analyzed in the blades of the plant.

3.3.2 Study of the effects of combined metals

These experiments were conducted at the Eastern Marine Fisheries Research and Development Center, Phe Subdistrict, Muang District, Rayong Province, between September, 2002 to February, 2003.

Sargassum polycystum were transplanted as in the previous study (3.3.1). Based on the content of metals in the water, the concentration of metals was adjusted. Various combined metals, Cd and Cr, Cd and Cu, Cd and Pb, Cd and Zn, Cr and Cu, Cr and Pb, Cr and Zn, Cu and Pb, Cu and Zn, as well as Pb and Zn were added into the media, prepared by keeping the concentration of one metal constant, while varying the other. After 0, 2, 4, 6 and 8 days, the blades were collected and carried under cool condition to the laboratory at the Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok. The blades were prepared in order to determine concentration of chlorophyll *a* and chlorophyll *c*, crude protein, lipid fraction as well as fatty acid profile, as done in the single metal experiments. The details of each combined metals are shown as follow (adapted from Jana and Choudhuri, 1994):

Combined metals 1 = Cd : Cr

Aquarium 0 = no metal added

Aquarium 1 = Cd : Cr = 0.003 : 0.0006 mg/l

Aquarium 2 = Cd : Cr = 0.003 : 0.003 mg/l

Aquarium 3 = Cd : Cr = 0.003 : 0.009 mg/l

Aquarium 4 = Cd : Cr = 0.03 : 0.003 mg/l

Aquarium 5 = Cd : Cr = 0.09 : 0.009 mg/l

Combined metals 2 = Cd : Cu

Aquarium 0 = no metal added

Aquarium 1 = Cd : Cu = 0.003 : 0.003 mg/l

Aquarium 2 = Cd : Cu = 0.003 : 0.015 mg/l

Aquarium 3 = Cd : Cu = 0.003 : 0.045 mg/l

Aquarium 4 = Cd : Cu = 0.03 : 0.015 mg/l

Aquarium 5 = Cd : Cu = 0.09 : 0.045 mg/l

Combined metals 3 = Cd : Pb

Aquarium 0 = no metal added

Aquarium 1 = Cd : Pb = 0.003 : 0.009 mg/l

Aquarium 2 = Cd : Pb = 0.003 : 0.045 mg/l

Aquarium 3 = Cd : Pb = 0.003 : 0.135 mg/l

Aquarium 4 = Cd : Pb = 0.03 : 0.045 mg/l

Aquarium 5 = Cd : Pb = 0.09 : 0.135 mg/l

Combined metals 4 = Cd : Zn

Aquarium 0 = no metal added

Aquarium 1 = Cd : Zn = 0.003 : 0.0044 mg/l

Aquarium 2 = Cd : Zn = 0.003 : 0.022 mg/l

Aquarium 3 = Cd : Zn = 0.003 : 0.066 mg/l

Aquarium 4 = Cd : Zn = 0.03 : 0.022 mg/l

Aquarium 5 = Cd : Zn = 0.09 : 0.066 mg/l

Combined metals 5 = Cr : Cu

Aquarium 0 = no metal added

Aquarium 1 = Cr : Cu = 0.0006 : 0.003 mg/l

Aquarium 2 = Cr : Cu = 0.0006 : 0.015 mg/l

Aquarium 3 = Cr : Cu = 0.0006 : 0.045 mg/l

Aquarium 4 = Cr : Cu = 0.003 : 0.015 mg/l

Aquarium 5 = Cr : Cu = 0.009 : 0.045 mg/l

Combined metals 6 = Cr : Pb

Aquarium 0 = no metal added

Aquarium 1 = Cr : Pb = 0.0006 : 0.009 mg/l

Aquarium 2 = Cr : Pb = 0.0006 : 0.045 mg/l

Aquarium 3 = Cr : Pb = 0.0006 : 0.135 mg/l

Aquarium 4 = Cr : Pb = 0.003 : 0.045 mg/l

Aquarium 5 =: Cr : Pb = 0.009 : 0.135 mg/l

Combined metals 7 = Cr : Zn

Aquarium 0 = no metal added

Aquarium 1 = Cr : Zn = 0.0006 : 0.0044 mg/l

Aquarium 2 = Cr : Zn = 0.0006 : 0.022 mg/l

Aquarium 3 = Cr : Zn = 0.0006 : 0.066 mg/l

Aquarium 4 = Cr : Zn = 0.003 : 0.022 mg/l

Aquarium 5 = Cr : Zn = 0.009 : 0.066 mg/l

Combined metals 8 = Cu : Pb

Aquarium 0 = no metal added

Aquarium 1 = Cu : Pb = 0.003 : 0.009 mg/l

Aquarium 2 = Cu : Pb = 0.003 : 0.045 mg/l

Aquarium 3 = Cu : Pb = 0.003 : 0.135 mg/l

Aquarium 4 = Cu : Pb = 0.015 : 0.045 mg/l

Aquarium 5 = Cu : Pb = 0.045 : 0.135 mg/l

Combined metals 9 = Cu : Zn

Aquarium 0 = no metal added

Aquarium 1 = Cu : Zn = 0.003 : 0.0044 mg/l

Aquarium 2 = Cu : Zn = 0.003 : 0.022 mg/l

Aquarium 3 = Cu : Zn = 0.003 : 0.066 mg/l

Aquarium 4 = Cu : Zn = 0.015 : 0.022 mg/l

Aquarium 5 = Cu : Zn = 0.045 : 0.066 mg/l

Combined metals 10 = Pb : Zn

Aquarium 0 = no metal added

Aquarium 1 = Pb : Zn = 0.009 : 0.0042 mg/l

Aquarium 2 = Pb : Zn = 0.009 : 0.022 mg/l

Aquarium 3 = Pb : Zn = 0.009 : 0.066 mg/l

Aquarium 4 = Pb : Zn = 0.045 : 0.022 mg/l

Aquarium 5 = Pb : Zn = 0.13509 : 0.066 mg/l

3.3.3 Study of the concentrations of chlorophyll *a* and chlorophyll *c*

The blades (1 g wet weight) of *Sargassum polycystum* were extracted in 90% acetone and the absorbance was measured at wavelength 630 and 664 nm. The concentrations of chlorophyll *a* and chlorophyll *c* were calculated using Jeffrey and Jumphrey's equations for 90% acetone extracts. Chlorophyll $a = 11.47\ D_{664} - 0.4\ D_{630}$, and chlorophyll $c = -3.73\ D_{664} + 24.36\ D_{630}$, where D is the absorbance in a 1 cm pathlength cell (Geider and Osborne, 1992). Concentrations of both chlorophylls are given in units of $\mu g.cm^{-3}$

3.3.4 Study of the total nitrogen content

The blades of fresh specimens were washed with fresh water followed by distilled water, then dried in an oven at 50 °C until their weight no longer changed. Two grams of dried samples were added to a mixture containing H_2SO_4 (ml): Na_2SO_4 (g): $CuSO_4$ (g) = 1000: 100: 1, then digested at 350 – 400 °C until the solution was clear. Cool solution was titrated with H_3BO_3, using bromocresol green and methyl red in ethanol as an indicator. Protein content was calculated using the equation of percent total nitrogen = ((A-B) C x 1.4)/D, using 6.25 as coefficient factor, where A = ml of acid titrated with sample, B = ml of acid titrated with blank, C = concentration of acid, and D = g of sample). Total protein contents were recorded in % total nitrogen per 2 g dry weight.

3.3.5 Study of the fatty acid content

The blades of fresh specimens were washed with fresh water followed by distilled water, then dried in an oven at 50 °C until their weight no longer changed and finally ground in a mill. Samples, 2 g, were extracted twice by percolation at room temperature in $CHCl_3$ – MeOH (1:1 v/v). After evaporation of the extract media, the lipid fraction was weighed and recorded in units of g/g dry weight. Then, this lipid fraction was saponified with 0.5 *N* methanolic NaOH solution. The fatty acids were methylated with BF_3/MeOH and the resulting esters were analyzed, in duplicate, and reported as an average (Khotimchenko, 1991), using a HP 6890 gas chromatograph (GC), analyzed by FAME in octane, using helium as a carrier. Major fatty acids were recorded in percentage of total fatty acid.

3.3.6 Study of the ultrastructure

The blades of fresh specimens are fixed and brought to the laboratory in order to study the ultrastructure. The blade of *Sargassum polycystum* treated with both each single and combined metal were fixed with 2.5% glutaraldehyde in 0.05 mol/l sodium phosphate buffer, pH 7.2, washed in buffer, then dehydrated in an ethanol series to 95%. Samples were embedded in JB-4 methacrylate resin, and sections were stained with 2% Uranyl acetate in 70% ethanol and counter stain with lead citrate. The samples were examined with TEM.

Samples for SEM, tissues were fixed as TEM's preparation and postfixed with 1% OsO_4 in buffer overnight at 4 °C. The tissues were dehydrated with an ethanol series, critical point dried in CO_2, sputter-coated with gold-palladium, and examined with SEM.

3.4 Data analysis

To test the amount of heavy metals in water, sediments, seaweeds, and the effects of heavy metals on physiological changes in seaweed, the experiments were designed in Completely Randomized Design (CRD). An analysis of variance (ANOVA) was used to analyze these data. The Duncan's method was used in order to test the difference between treatments with level of significance $\alpha = 0.05$.

3.5 Instrumentation

- AAS (Perkin-Elmer, Analyst 100)
- Light microscope and stereomicroscope with camera
- JEOL JEM-2010 TEM

- JEOL JSM-6400 SEM
- HP 6890 Gas Chromatograph
- Jenway 6400 spectrophotometer
- Glass aquarium
- Refrigerator and freezer
- Others

3.6 Chemicals

All chemicals used were analytical reagent grade. The important chemicals and their details are reported as follow:

- Acetone, CH_3COCH_3 , Merck
- Cadmium chloride, $CdCl_2$. 2½ H_2O, Carlo Erba
- Chloroform, $CHCl_3$, BDH
- Chromium III sulfate, $Cr_2(SO_4)_3.2H_2O$, Univar
- Copper sulfate anhydrous, $CuSO_4$, Carlo Erba
- Ethanol absolute, C_2H_5OH, Merck
- Lead (II) acetate 3 hydrate, $(CH_3COO)_2Pb.3H_2O$
- Methanol, CH_3OH, Merck
- Nitric acid 65%, HNO_3, Lab-Scan
- Perchloric acid 65%, $HClO_4$, Carlo Erba
- Sodium hydroxide, NaOH, Lab-Guard
- Sodium sulfate anhydrous, Na_2SO_4, Univar
- Sulfuric acid 95-97%, H_2SO_4, Merck
- Zinc Chloride, $ZnCl_2$, BDH and others

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Metals in seaweeds, water and sediments

Seaweeds, *Enteromorpha clathrata* Greville, *Padina japonica* Yamada, and *Sargassum polycystum* C. Agardh, as well as sediments and water from near the seaweeds were collected from Phe, Muang District, Rayong Province, and treated as described in chapter III. For *E. clathrata*, samples were collected from 2 sites, Phe and Laem Chabang, Sriracha District, Chonburi Province (Fig. 2). The amount of metals accumulated in all samples is shown in both table and graph forms.

4.1.1 Metals in *Enteromorpha clathrata*

Enteromorpha clathrata is classified into the division Chlorophyta, class Chlorophyceae, order Ulvales, and family Ulvaceae. It has a diplohaplontic life history with isomorphic diploid and haploid plants.

In Table 7, the mean cadmium content in the seaweed, sediments and the water from Laem Chabang is higher than in samples from Phe (6.5962, 3.4033, 0.1089 and 3.4910, 2.5800, 0.0491 μg/g, respectively). Cd content in the samples from Phe ranges from 1.4625 (May) to 5.1610 μg/g (January), from 1.3483 (September) to 5.0625 μg/g (February), and from 0.0051 (January) to 0.1245 μg/l (March), in the seaweed, sediment and water, respectively. The metal content in the samples from Laem Chabang ranges from 0.50 (April) to 19.1375 μg/g **(December),**

from 0.7750 (October) to 9.0225 μg/g (December), and from 0.011 (January) to 0.3273 μg/l (December) in the plants, sediments and water, respectively. However, Cd content is very low (less than 0.0001 μg/l) in the water from Laem Chabang in June. Therefore, it could not detect the metal concentrations. Although the seaweed used in this experiment is the same species, there are no samples in October through December since the seaweed is in the spore stage and environments, e.g., high wave and strong current, are not suitable for germination of spores. Therefore, there is no data in all samples from Phe during these months.

In Table 7, the mean data show that the metal is very highly accumulated in the seaweed, followed by in the sediment and in the water, respectively. The concentration levels of Cd in the plant and in the sediment from both sites are 71, 53 and 61, 31 times higher than in the water. The data show that the higher the metal concentration in the water and sediments, the higher the metal accumulated in the seaweeds. It is clear that *E. clathrata* can be used for Cd monitoring in ocean environment.

In the same month, the concentrations of Cd in water from both locations are not significantly different (at the 5% level). The Cd content in the plants and sediments from Phe is significantly different, except in March and May. The samples from Laem Chabang are not significantly different at the 5% level, except in September (different letter in the same month, Table 7).

Comparing the metal concentrations in the plants in the same month, from both locations, only in March there is not a significantly difference at the 5% level. In sediments from both sites, there are mostly significant differences at the 5% level, except in March and April. On the other hand, comparing different samples (the

seaweeds and the sediments) from both localities, the metal content is mostly significantly different at the 5% level, except between the seaweed from Phe and sediments from Laem Chabang in February; the seaweeds and sediments from Phe and the plant from Laem Chabang in March (different letter in the same month, Table 7).

Table 7 Cd concentrations (μg/g) in *E. clathrata*, water and sediment from Phe and Laem Chabang in the same and different months

month	*Ent*.- Phe	Wat.-Phe	Sed.-Phe	*Ent*.-Cha.	Wat.-Cha.	Sed.-Cha.
Feb-00	2.3000 [1a] ±0.074	0.0198 [1b] ±0.000	5.0625 [1c] ±0.012	1.6250 [1d2] ±0.041	0.0248 [1b2] ±0.000	2.2625 [1a] ±0.024
Mar-00	2.4750 [1ac] ±0.515	0.1245 [2b] ±0.004	2.0875 [2ac] ±0.041	3.0500 [3a4] ±0.651	0.1253 [5b] ±0.003	1.4313 [2c] ±0.017
Apr-00	1.6500 [2a] ±0.045	0.0950 [3b] ±0.002	4.7163 [3c] ±0.148	0.5000 [1d] ± 0.159	0.1158 [5b] ±0.002	4.8075 [3c] ±0.041
May-00	1.4625 [2a] ±0.027	0.0461 [3b] ±0.002	1.4818 [4a] ±0.009	2.64 [2c3] ±0.037	0.0454 [3b] ±0.001	1.2581 [2d] ±0.020
Jun-00	5.0525 [3a] ± 0.021	0.0432 [4b] ± 0.001	1.4219 [4c] ±0.041	1.4813 [1c2] ±0.045	nd	0.8375 [6d] ±0.011
Jul-00	4.9925 [3a] ± 0.014	0.0327 [5b] ± 0.001	1.6318 [5c] ± 0.011	4.9925 [4d] ±0.037	0.0824 [4b] ±0.015	1.9781 [1e4] ±0.009
Aug-00	4.8285 [3a] ±0.011	0.0492 ±0.002	1.3903 [4c] ±0.007	2.0488 [2d3] ±0.104	0.0883 [4b] ±0.001	1.8463 [4e] ±0.008
Sep-00	3.4971 [4a] ±0.062	0.0260 [1b] ±0.000	1.3483 [4c] ±0.001	1.8150 [2d] ± 0.026	0.0317 [2b3] ± 0.000	1.8138 [4d] ±0.040
Oct-00	ns	ns	ns	12.413 [5a] ± 0.345	0.0415 [3b] ± 0.000	0.7750 [6c] ±0.0329
Nov-00	ns	ns	ns	13.315 [5a] ± 0.204	0.3040 [6b] ± 0.003	7.3269 [5c] ±0.114
Dec-00	ns	ns	ns	19.1375 [6a] ±0.204	0.3273 [7b] ±0.004	9.0225 [7c] ±0.113
Jan-01	5.1610 [3a] ±0.001	0.0051 [6b] ±0.001	4.0799 [6a] ±0.023	16.770 [7c] ±0.007	0.0110 [1b] ±0.002	7.4798 [5d] ±0.011
mean	**3.4910** ±0.517	**0.0491** ±0.013	**2.5800** ±0.522	**6.5962** ±1.961	**0.1089** ±0.033	**3.4033** ±0.852

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

Cha. = Laem Chabang *Ent*. = *Enteromorpha clathrata*

nd = cannot detect (less than 0.0001 μg/l) ns = no sample

Sed. = sediment Wat. = water

Comparing the Cd concentrations in the same area, the average data from both sites show that Cd has the highest accumulation in seaweed, followed by sediment and water, respectively.

In Figure 3, Cd concentrations in *E. clathrata*, water and sediment, collected from Phe and Laem Chabang is shown. The graph shows that the metal is most highly accumulated in the seaweed from Laem Chabang in October (in the plants), and November through January (both in the plant and in the sediment). On the other hand, there are no plant samples from Phe in October, November and December since there are no plant samples during these months. In other months, the graph shows fluctuating Cd, except in the water from both sites, which is quite stable and low. The data of salinity (‰), temperature (°C) and pH of water collected from Phe and Laem Chabang are shown in Figure 4. All environmental factors are usually stable, except salinity from Laem Chabang. The salinity is quite low in June and September because the seawater is diluted by precipitation, which drains into the Gulf, especially in June. The sample was collected one hour after raining.

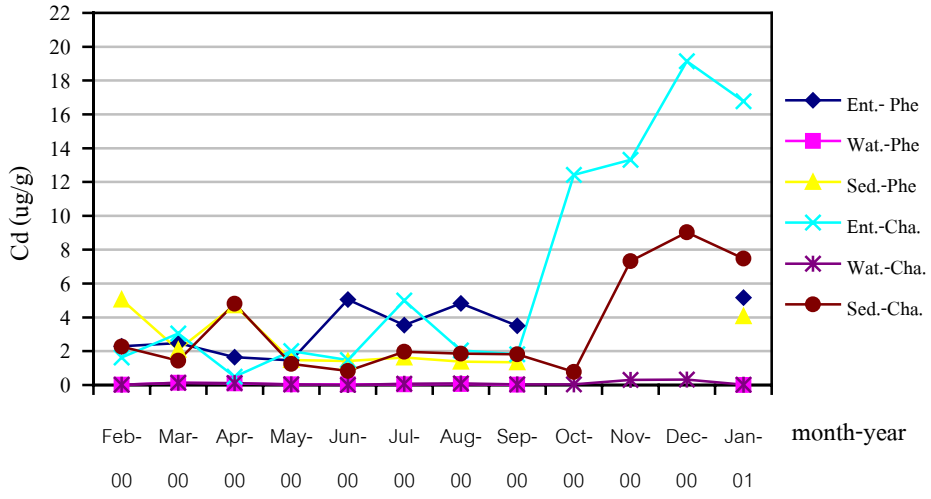

Fig. 3 Cd concentrations (µg/g) in *E. clathrata*, water and sediment from Phe and Laem Chabang

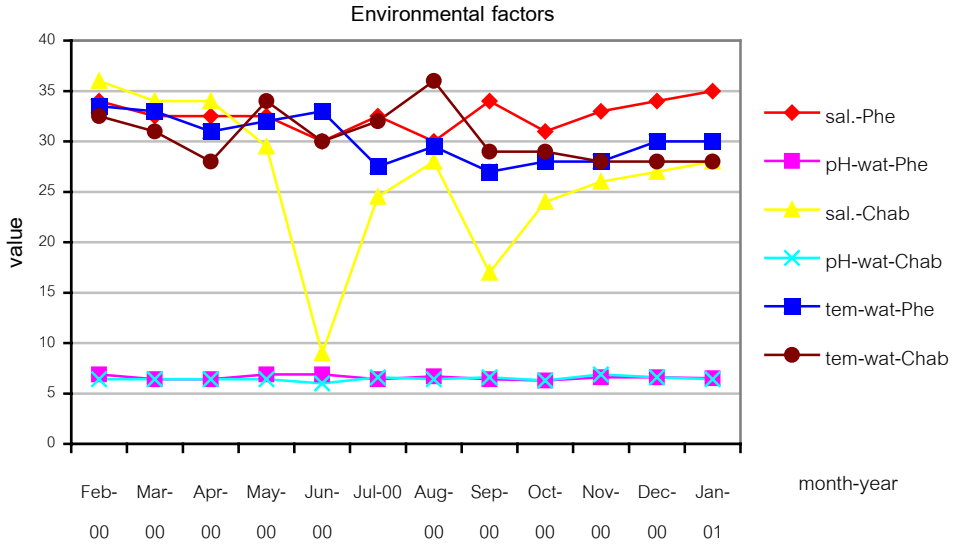

Fig. 4 Line graph of salinity (‰), temperature (°C) and pH of the water from Phe and Laem Chabang

Average total chromium concentrations from different months in *E. clathrata*, sediment and water from Phe and Laem Chabang are 46.9621, 18.6658, 0.0604, 21.2012, 7.0544 and 0.3265 µg/g, respectively. The mean data show that the higher the metal level in the sediment, the higher the metal level in the seaweed. The mean amount of Cr in the plants from Phe ranges from 4.650 (February) to 170 µg/g (June). It is of the order of 0.0052 (September) to 0.2192 µg/l (March) in the water and ranges from 5.4194 (January) to 38.8630 µg/g (June) in the sediments, respectively (Table 8). Meanwhile, the Cr concentration in all samples from Laem Chabang ranges from 3.1042 (February) to 85.925 µg/g (June); from 0.0118 (December) to 2.070 µg/l (August); and from 0.5938 (July) to 34.0060 µg/g (July) in the plants, the water and the sediments, respectively. The concentration levels of Cr in the plant and in the sediment from both sites are 778, 309 and 64, 21 times higher than in the water. On the other hand, the concentration of Cr could not be detected in the water from Phe in May and June; in the water from Laem Chabang in May, June, October, and

November; and in the sediment from Laem Chabang in February, May, and November (Table 8). In addition, *E. clathrata* was not present at Phe in October through December, since the seaweed is in the spore stage of the life cycle as described above. Therefore, there are no data of Cr in these months.

In the same month, Cr concentration in the water from both Phe and Laem Chabang is not significant different at the 5% level (the same letter in the same row, Table 8). The Cr content in *E. clathrata* and sediments from Phe and Laem Chabang are mostly significant different at the 5% level. On the other hand, comparing the same samples from both localities, the average content of Cr in the plants and sediments from Phe and in the seaweeds and sediments from Laem Chabang are mostly significant different at the 5% level (different letter in the same month).

In Table 8, Cr content in all samples shows flutuation in each month. On the other hand, the mean content of the metal is the highest in the seaweed and the sediment from Phe, except in the water, the metal is higher in the water from Laem Chabang than that of the metal from Phe. In fact, the metal should be high in the samples from Laem Chabang, the critical area. At Phe, anthropogenic activities, such as jetty activity and community effluent, may increase the metal in the marine environment. Furthermore, Laem Chabang Industrial Estate has the biggest wastewater pond in Southeast Asia. They treated the wastewater before outflow into the sea. The high metal content in this area may come from household of human activities.

Table 8 Cr concentrations (μg/g) in *E. clathrata*, water and sediment from Phe and Laem Chabang in the same and different months

month	*Ent.*- Phe	Wat.-Phe	Sed.-Phe	*Ent.*-Cha.	Wat.-Cha.	Sed.-Cha.
Feb-00	4.6500 [1a] ±0.071	0.0823 [1b3] ±0.002	7.5125 [1c] ±0.105	3.1042 [1d] ±0.052	0.0710 [1b2] ±0.001	nd
Mar-00	15.6500 [1a] ±3.558	0.2195 [4b] ±0.021	15.5630 [2a] ±0.155	3.2750 [1b] ±1.335	0.2228 [3b] ±0.030	11.2380 [3a] ±0.231
Apr-00	15.0130 [1a] ±2.520	0.0978 [3b] ±0.034	20.660 [3c4] ±1.458	25.9680 [4d5] ±2.178	0.0499 [1b2] ±0.016	17.4140 [4ac] ±1.504
May-00	31.4880 [2a] ±1.016	nd	7.2026 [1b] ±1.536	28.8550 [5a] ±2.236	nd	nd
Jun-00	170.9380 [3a] ±10.070	nd	38.8630 [5b] ±3.304	85.9250 [6c] ±2.615	nd	34.0060 [5b] ±2.209
Jul-00	87.794 [4a] ±0.141	0.0516 [1b23] ±0.001	33.562 [6c] ±0.426	14.9750 [2d] ±0.562	0.1075 [2b] ±0.022	0.5938 [1b] ±0.274
Aug-00	58.7980 [5a] ±1.181	0.0261 [1b2] ±0.001	22.9960 [4c] ±0.971	5.3088 [1d] ±0.462	2.0700 [4bd] ±0.063	5.9500 [2e] ±1.021
Sep-00	32.6970 [2a] ±0.982	0.0052 [2b] ±0.000	16.2140 [2c3] ±0.515	16.1500 [2c] ±0.276	0.0238 [1b2] ±0.001	8.6375 [2d3] ±0.048
Oct-00	ns	ns	ns	12.9560 [2a] ±0.279	nd	1.4744 [1b] ±0.059
Nov-00	ns	ns	ns	15.460 [2a] ± 0.282	nd	nd
Dec-00	ns	ns	ns	19.8680 [3a] ±0.181	0.0118 [1b] ±0.002	2.4478 [1c] ±0.076
Jan-01	5.6313 [1a] ±0.029	0.0611 [1b23] ±0.001	5.4194 [1c] ±0.033	22.5690 [3d4] ±0.072	0.0553 [1b2] ±0.002	2.8913 [1e] ±0.034
mean	**46.9621** ±0.142	**0.0604** ±0.000	**18.6658** ±3.302	**21.2012** ±6.361	**0.3265** ±0.250	**7.0544** ±3.568

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

Cha. = Laem Chabang

Ent. = *Enteromorpha clathrata*

nd = cannot detect (less than 0.0001 μg/l)

ns = no sample

Sed. = sediment

Wat. = water

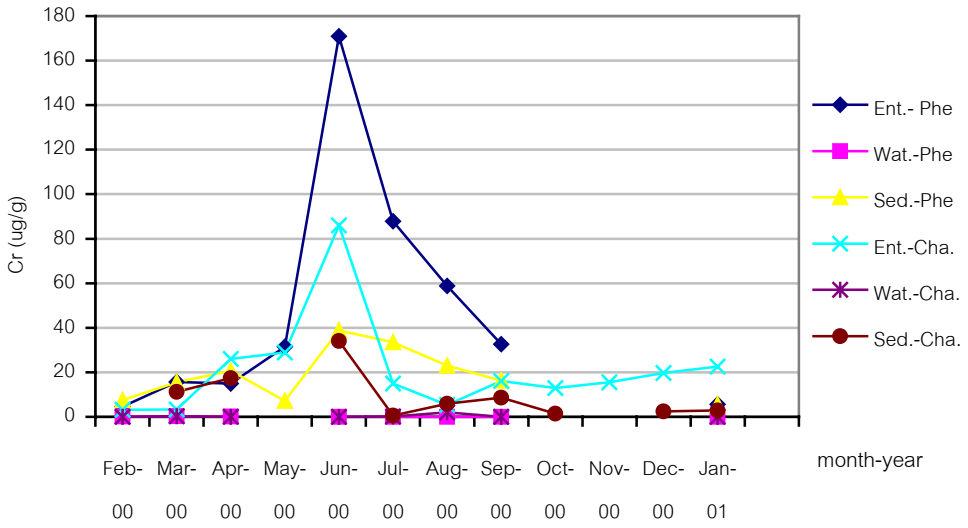

Fig. 5 Cr concentrations (µg/g) in *E. clathrata*, water and sediment from Phe and Laem Chabang

In Figure 5, a graph of Cr content in all samples from both localities is presented. The graph shows Cr content is the highest in June and slightly decreases during the rest of the year. In June, the first month of rainy season, the higher level of the metal may come from precipitation that leaches of the metal from the land, surrounded by industries, into the sea. The lowest concentration of the metal occurred in January. Meanwhile, the data of environmental factors such as salinity (‰), temperature (°C) and pH of water collected from Phe and Laem Chabang are shown in Figure 4.

The average copper concentration is 90.7974, 41.2521, 0.0522, 41.8842, 5.8556 and 0.0399 µg/g, in *E. clathrata*, the sediments and the water collected from Phe and Laem Chabang, respectively (Table 9). Cu concentrations in the plant from Phe are of the order of 11.015 (May) to 285.0 µg/g (June), and those of samples from Laem Chabang range from 20.709 (January) to 72.188 µg/g (May). Likewise, Cu content in sediments from Phe and Laem Chabang is of the order of 4.6750

(February) to 132.728 (June) and 0.6888 (November) to 10.338 μg/g (February), respectively. It ranges from 0.0204 (January) to 0.1031 μg/l (April) and from 0.0107 (January) to 0.1152 μg/l (April) in the water from Phe and Laem Chabang, respectively. The concentration levels of Cu in the plant and in the sediment from both sites are 1,739, 790 and 1,050, 147 times higher than in the water.

It is noted that Cu concentrations in the seaweed and in the sediment are very high in June, July, August and September. The higher level of the metal may come from agricultural activities, particulary shrimp farm, using $CuSO_4$ as algicide. The data show that the average Cu content in the seaweed and sediment from Phe is higher than that of the sample from Laem Chabang. The Cu content, on the other hand, in water in the same month from both localities is not significantly different at the 5% level (the same letter in the same row, Table 9). However, the metal could not be detected in the water collected from Phe in March; and from Laem Chabang in March and November. There are no samples from Phe in October through December, since there is no seaweed during these months as described above.

Like Cr content, concentrations of Cu from Laem Chabang (the critical site) are lower than that of the metal concentrations from Phe. Higher Cu content accumulated in the plant and sediment may come from other factors including human activities, especially agricultural activities, and the features of the area.

When comparing Cu concentrations in all samples in the same and different month, most Cu concentrations are significantly different at the 5% level in both the same and different month, except Cu concentration in the water from Phe and Laem Chabang in the same month (Table 9).

Table 9 Cu concentrations (μg/g) in *E. clathrata*, water and sediment from Phe and Laem Chabang in the same and different months

month	*Ent.*- Phe	Wat.-Phe	Sed.-Phe	*Ent.*-Cha.	Wat.-Cha.	Sed.-Cha.
Feb-00	22.2000 [2a] ±0.178	0.0470 [2b] ±0.000	4.6750 [1c] ±0.149	20.7090 [1d] ±0.087	0.0618 [4b5] ±0.006	10.3380 [10e] ±0.131
Mar-00	66.6000 [3a] ±0.551	nd	7.5875 [1b] ±0.013	44.5500 [6c] ±0.614	nd	10.000 [8d9] ±0.035
Apr-00	22.0980 [2a] ±0.220	0.1031 [4b] ±0.007	6.5925 [1c] ±0.317	69.975 [8d] ±0.304	0.1152 [6b] ±0.004	9.7688 [8e] ±0.174
May-00	11.0150 [1a] ±0.583	0.0612 [2b3] ±0.000	5.1613 [1c] ±0.023	72.1880 [9d] ±0.253	0.0597 [4b] ±0.000	5.5213 [6c] ±0.099
Jun-00	285.000 [7a] ±1.548	0.0563 [2b3] ±0.003	132.7280 [6c] ±1.408	38.9480 [5d] ±0.056	0.0163 [1b2] ±0.002	5.3031 [6e] ±0.063
Jul-00	129.1880 [5a] ±1.100	0.0676 [3b] ±0.001	75.9570 [5c] ±0.011	53.988 [7d] ±0.090	0.0680 [5b] ±0.001	4.2675 [5b] ±0.022
Aug-00	152.9950 [6a] ±1.056	0.0624 [2b3] ±0.001	58.8283 [4c] ±0.012	35.5088 [3d] ± 0.282	0.0513 [3b] ± 0.001	3.6650 [4b] ± 0.043
Sep-00	105.8960 [4a] ±0.580	0.0514 [2b3] ±0.002	43.1560 [3c] ±0.003	36.5130 [4d] ±0.031	0.0222 [2b] ±0.000	10.2070 [9e10] ±0.005
Oct-00	ns	ns	ns	39.1610 [5a] ±0.020	0.0511 [3b] ±0.001	6.7669 [7c] ±0.022
Nov-00	ns	ns	ns	38.7800 [5a] ±0.225	nd	0.6888 [1b] ±0.025
Dec-00	ns	ns	ns	31.4360 [2a] ±0.160	0.0219 [2b] ±0.003	2.5094 [3c] ±0.094
Jan-01	22.1850 [2a] ±0.120	0.0204 [1b] ±0.030	36.5830 [2c] ±0.006	20.8530 [1d] ±0.200	0.0107 [1b] ±0.000	1.2313 [2b] ±0.013
mean	**90.7974** ±29.300	**0.0522** ±0.008	**41.2521** ±14.377	**41.8842** ±4.732	**0.0399** ±0.010	**5.8556** ±1.028

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

Cha. = Laem Chabang

Ent. = *Enteromorpha clathrata*

nd = cannot detect (less than 0.0001 μg/l)

ns = no sample

Sed. = sediment

Wat. = water

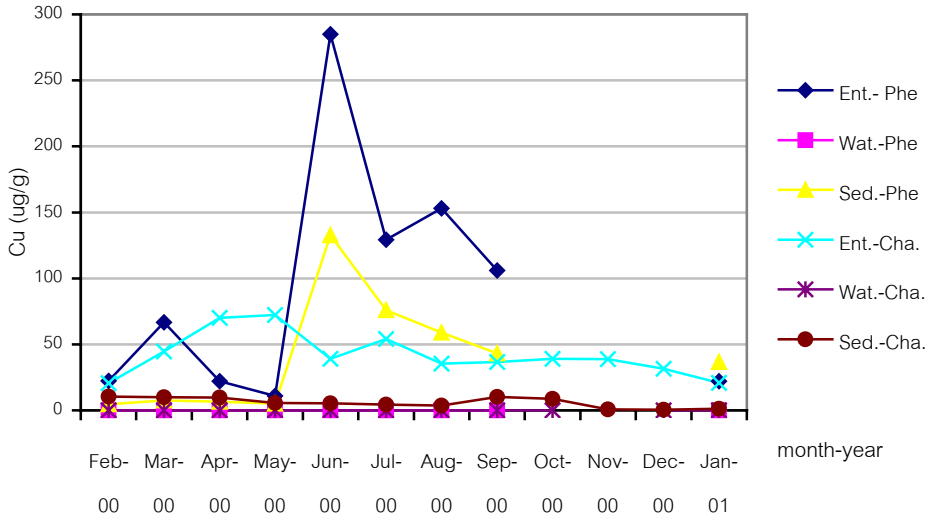

Fig. 6 Cu concentrations (µg/g) in *E. clathrata*, water and sediment from Phe and Laem Chabang

In Figure 6, the line graph shows Cu concentration in *E. clathrata*, sediment and water from both locations. The metal content is quite high in the seaweed collected from Phe from June, July, August and September, followed by the sediment and the seaweed from Phe, respectively (Table 9 and Fig. 6). The data show that Cu is more highly accumulated in the plant than in the sediment, especially in the seaweed from Phe. It is clear that the plants can be used for Cu monitoring in the oceanic environment.

The data of environmental factors such as salinity (‰), temperature (°C) and pH of water collected from Phe and Laem Chabang are shown in Figure 4.

Lead concentrations in *E. clathrata* from Phe are of the order of 13.250 (March) to 65.8270 µg/g (July) and those of the plants from Laem Chabang range from 7.6225 (June) to 64.0250 µg/g (May). Similarly, the Pb concentrations in the sediment and in the water from Phe range from 1.1588 (April) to 20.3130 (February); and from 0.3358 (March) to 0.4829 µg/l (September), respectively, and range from

0.0232 (January, 01) to 17.422 μg/g (July), and from 0.1501 (August) to 0.7667 μg/l (December) in the sediment and in the water from Laem Chabang, respectively. However, the Pb content in the sediment from Laem Chabang in Febuary could not be detected. Furthermore, there are no samples from Phe in October through December as described above.

The average Pb concentration in the plant, in the sediment and in the water from Phe and Laem Chabang is 39.6466, 12.7423, 0.4241, 34.9605, 9.2317 and 0.3191 μg/g, respectively. The mean data show that Pb concentration in both the plant and the sediment from Phe is slightly higher than that of the metal concentrations in the samples from Laem Chabang. In addition, the metal concentration is far higher than that of the metal in the water from both localities. Furthermore, the data show that most samples in both the same and different months are significantly different at the 5% level (different letter in the same row and different number in the same column, Table 10). The concentration levels of Pb in the plant and the sediment from both sites are 93, 30 and 110, 29 times higher than in the water. It is quite clear that Pb is highly accumulated in the plant. Therefore, this seaweed is suitable for useing in Pb monitoring. On the other hand, the metal content in sediment from Laem Chabang in February could not detected.

In Figure 7, the Pb concentrations are far higher in the seaweed collected from Phe, especially in June, followed by the plant from Laem Chabang, and the sediment from both localities, respectively. Meanwhile, the Pb in the water from both sites is very low.

The data of environmental factors such as salinity (‰), temperature (°C) and pH of water collected from Phe and Laem Chabang are shown in Figure 4.

Table 10 Pb concentrations (μg/g) in *E. clathrata*, water and sediment from Phe and Laem Chabang in the same and different months

month	*Ent.*- Phe	Wat.-Phe	Sed.-Phe	*Ent.*-Cha.	Wat.-Cha.	Sed.-Cha.
Feb-00	31.9500 [1a3] ±0.281	0.4280 [1b3] ±0.006	20.3130 [7c] ±0.276	28.2500 [1c3] ±0.468	0.3380 [2b3] ±0.008	nd
Mar-00	13.2500 [2a] ±0.484	0.3358 [2b] ±0.011	5.2500 [2c] ±0.527	35.2000 [4d] ±1.093	0.2188 [1b] ±0.005	0.6667 [1b] ±0.017
Apr-00	28.2930 [1a] ±2.095	0.4568 [3b] ±0.019	1.1588 [1b] ±0.325	45.443 [5c] ±1.507	0.4000 [3b4] ±0.033	6.6750 [2d] ±0.675
May-00	36.9840 [3a] ±6.450	0.3564 [1b2] ±0.069	15.4010 [5c] ±0.831	64.0250 [6d] ±2.917	0.4353 [5b] ±0.057	13.1550 [4c] ±1.346
Jun-00	51.0900 [4a] ±0.860	0.4265 [1b3] ±0.006	13.6010 [4c] ±0.401	7.6225 [2d] ±0.541	0.1884 [1b] ±0.012	10.2740 [3e] ±0.636
Jul-00	65.8720 [6a] ±0.846	0.4412 [3b] ±0.002	17.7670 [6c] ±0.016	46.2410 [5d] ±0.504	0.2131 [1b] ±0.006	17.422 [5c] ±0.133
Aug-00	55.4870 [5a] ±0.626	0.4577 [3b] ±0.005	13.5850 [4c] ±0.016	38.7850 [4d] ±1.827	0.1501 [1b] ±0.017	9.2081 [3e] ±0.172
Sep-00	44.6110 [4a5] ±0.106	0.4829 [3b] ±0.001	7.3692 [3c] ±0.002	26.6090 [1d3] ±0.243	0.1615 [1b] ±0.007	14.2020 [4e] ±0.232
Oct-00	ns	ns	ns	46.5530 [5a] ± 0.257	0.3195 [2b] ±0.004	17.1000 [5c] ±0.142
Nov-00	ns	ns	ns	24.2700 [1a] ±0.597	0.3118 [2b] ±0.007	7.3394 [2c] ±0.263
Dec-00	ns	ns	ns	28.8810 [3a] ±2.305	0.7667 [6b] ±0.050	14.7150 [4c] ±0.858
Jan-01	29.2820 [1a] ±0.116	0.4318 [1b3] ±0.002	20.2360 [7c] ±0.100	27.6460 [1d3] ±1.006	0.3261 [2b3] ±0.002	0.0232 [1b] ±0.000
mean	39.6466 ±5.393	0.4241 ±0.016	12.7423 ±2.256	34.9605 ±4.172	0.3191 ±0.049	9.2317 ±1.811

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

Cha. = Laem Chabang

Ent. = *Enteromorpha clathrata*

nd = cannot detect (less than 0.0001 μg/l)

ns = no sample

Sed. = sediment

Wat. = water

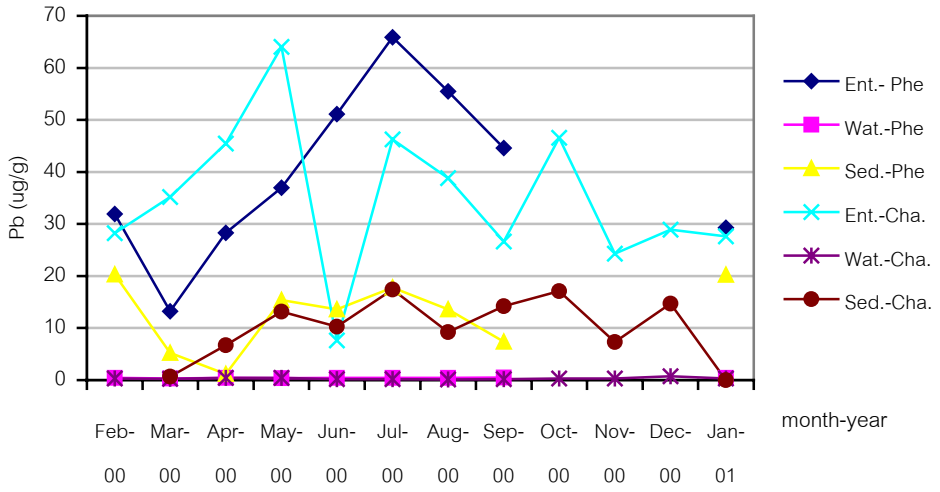

Fig. 7 Pb concentrations (µg/g) in *E. clathrata*, water and sediment from Phe and Laem Chabang

The mean concentrations of zinc in *E. clathrata*, in the sediment and in the water from Phe and Laem Chabang are 98.0383, 25.9130, 0.0547, 168.2576, 32.8275 and 0.2512 µg/g, respectively. The metal content is of the order of 32.209 (January) to 396.3750 µg/g (March), and from 10.7940 (September) to 45.30 µg/g (March), and from 0.010 (February) to 0.0929 µg/l (September) in the plant, the sediment and the water from Phe, respectively. Meanwhile, the Zn content in the seaweed, the sediment and the water from Laem Chabang ranges from 68.8130 (February) to 435.0750 µg/g (March), from 17.8410 (September) to 94.4750 µg/g (March) and from 0.0178 (February) to 0.7073 µg/g (May), respectively. However, there are no samples from Phe in October through December. The data show that the metal contents in the plant and sediment from Phe are lower than those in the samples from Laem Chabang (Table 11). The concentration levels of Zn in the plant and in the sediment from both sites are 1,792, 474 and 670, 131 times, respectively, higher than in the water.

Table 11 Zn concentrations (μg/g) in *E. clathrata*, water and sediments from Phe and Laem Chabang in the same and different months

month	*Ent.*- Phe	Wat.-Phe	Sed.-Phe	*Ent.*-Cha.	Wat.-Cha.	Sed.-Cha.
Feb-00	40.6500 [1a] ±0.000	0.0100 [1b] ±0.001	38.0000 [1c] ±0.572	68.8130 [1d] ±0.071	0.0178 [1b] ±0.001	30.7500 [1e] ±0.074
Mar-00	396.3750 [8a] ± 1.054	0.0368 [2b] ± 0.005	45.3000 [7c] ±0.061	435.0750 [11d] ±0.940	0.0430 [2b] ±0.003	94.475 [2e] ±0.153
Apr-00	46.9950 [3a] ± 0.131	0.0491 [3b] ± 0.000	23.9630 [2c] ± 0.101	174.075 [8d] ±0.155	0.0529 [2b3] ±0.001	34.0290 [3e] ±0.067
May-00	61.8100 [4a] ±0.116	0.0497 [3b] ±0.000	39.6250 [1c] ±0.062	251.6250 [10d] ±0.262	0.7073 [10e] ±0.002	42.300 [4f] ±0.053
Jun-00	78.0250 [6a] ±0.111	0.0828 [5b] ±0.001	19.7990 [6c] ±0.089	128.113 [2d] ± 0.156	0.0578 [3b] ± 0.000	29.119 [5e] ± 0.070
Jul-00	85.1750 [7a] ±0.111	0.0736 [4b] ±0.000	24.2830 [2c] ±0.081	241.6500 [9d] ±3.335	0.5242 [7b] ±0.005	31.5310 [6e] ±0.095
Aug-00	76.020 [6a] ±0.112	0.0869 [5b] ±0.001	14.3730 [4c] ±0.001	132.2880 [5d] ±0.509	0.1227 [4b] ±0.002	21.7680 [7e] ±0.042
Sep-00	65.0860 [5a] ±0.281	0.0929 [6b] ±0.011	10.7940 [3c] ±0.009	115.0880 [3d] ±4.298	0.3321 [7b] ±0.000	17.8410 [8e] ±0.114
Oct-00	ns	ns	ns	105.5380 [2a] ±0.195	0.1745 [6b] ±0.000	23.1170 [9c] ±0.036
Nov-00	ns	ns	ns	149.7630 [6a] ±0.090	0.6727 [9b] ±0.006	22.4950 [10c] ±0.061
Dec-00	ns	ns	ns	124.7630 [4a] ±0.345	0.1446 [5b] ±0.011	21.1920 [11c] ±0.053
Jan-01	32.2090 [2a] ±0.289	0.0102 [1b] ±0.003	17.0830 [5c] ±0.080	164.3000 [7d] ±0.4036	0.1649 [6b] ±0.0056	25.3130 [12e] ±0.039
mean	98.0383 ±37.765	0.0547 ±0.011	25.9133 ±4.072	168.2576 ±28.195	0.2512 ±0.072	**32.8275** ±5.936

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

Cha. = Laem Chabang *Ent.* = *Enteromorpha clathrata*

nd = cannot detect (less than 0.0001 μg/l) ns = no sample

Sed. = sediment Wat. = water

In Table 11, the zinc concentrations in all samples are significantly different at the 5% level in both the same and different months (different letter in the same row

and different number in the same column). In contrast, the metal content in the water in the same month from both sites is not significantly different at the 5% level, except in May (the same letter in the same row).

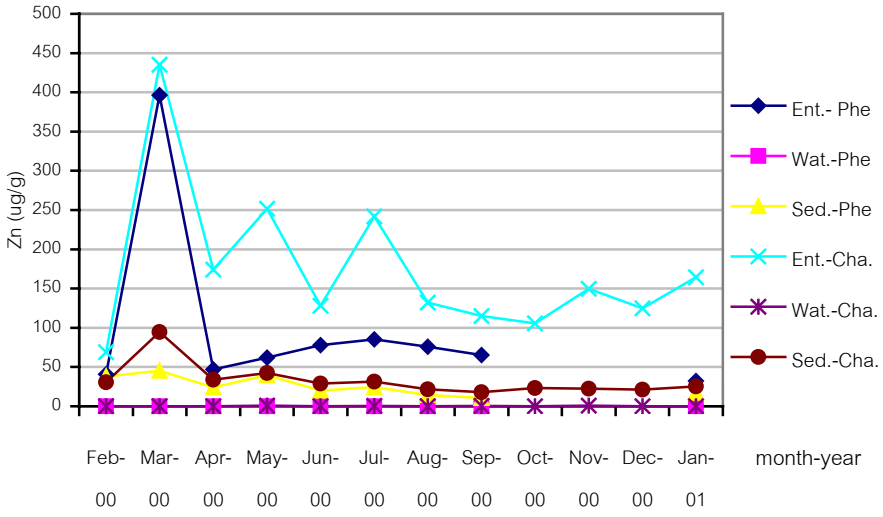

Fig. 8 Zn concentrations (µg/g) in *E. clathrata*, water and sediment from Phe and Laem Chabang

In Figure 8, the zinc contents in *E. clathrata* collected from both sites are very high, especially in March. In general, the metal concentrations in the plant from Laem Chabang are higher than that of the other area. Furthermore, the metal concentration in the plant is far higher than that of the metal in the sediment and in the water from both sites, respectively. It is clear that *E. clathrata* can be used for Zn monitoring in the marine environment.

In conclusion, all metal contents in the water from both localities in each month are very low when compared the metal in the plant and the sediment. As in Table 6, the Office of National Environment Board, Ministry of Science and Technology, Thailand, allowed Cr, Zn, Cu, Pb and Cd contamination in seawater less than 100, 100, 50, 50 and 5 µg/g, respectively. Therefore, all metal concentrations are

within Thailand's legal limits. In contrast, the metal contents in the plant and sediments are mostly higher than the limitations in Thailand's regulations. However, there are no standard levels of the metals in these samples.

In this study, bioconcentration levels of Cd, Cr, Cu, Pb and Zn in *E. clathrata* from Phe and Laem Chabang are 71 and 61; 778 and 64; 1,739 and 1,050; 93 and 110; 1,792 and 670 times higher than in the water, respectively. Therefore, the seaweed can be used for metal monitoring in oceanic environments since all five metals are highly accumulated in the plant and in far higher concentrations than in the seawater.

Concentrations of Cd, Cr and Zn are higher in the samples from Laem Chabang than the samples from Phe since these metals are related to industrial activities, especially battery and electronic productions. On the other hand, concentrations of Cu and Pb are higher in the samples from Phe since these metals are related to agricultural activities, particulary shrimp farm, using as algicide. In addition, Phe has more human communities than that of Laem Chabang.

Vasconcelos and Leal (2000b) studing *Enteromorpha* sp. found that the Cu was highly accumulated in the growing season. Similarly to this study, Cr, Cu and Pb are highly accumulated by *E. clathrata* in growing season (June, July and August). In addition, the salinity of the seawater is dilluted during these months. Therefore, absorptions of the metals increase as the salinity decreases as reported by Amado Filho, *et al*., 1997).

Ho (1987) reported that *Ulva lactuca* is suitable to use in Cu, Zn and Pb monitoring. Similarly, Forsberg, Söderlund, Frank, Petersson and Pedersen (1988) recommented that *Fucus vesiculosus* is a good detector for metal pollution. On the other hand, the latter seaweed can be grown only in temperate region. Therefore,

Enteromorpha should be a good bioindicator for heavy metals in the tropical zone, especially in estuary areas, since *Enteromorpha* is better distribution in a wide range of salinity than other seaweeds. In addition, the spore of this seaweed can germinate in low to moderate wave condition.

4.1.2 Metals in *Padina japonica*

Padina is classified into the division Phaeophyta, class Isogeneratae, order Dictyotales, and family Dictyotaceae (Bold and Wynne, 1985). The life cycle of the plants is diplo-haplontic, but they show isomorphic alternation of generations, which is the unique in the class. Most plants, on the other hand, are found in the sporophyte stage. Gametophytes can occur both as dioecious and monoecious plants. Therefore, metals in *Padina japonica* can be separated as being taken up by sporophyte and gametophyte plants.

Padina japonica can better distribute in low to moderate wave and rocky shore. One location that can be found this seaweed is the sea at Phe Subdistrict, Muang District, Rayong Province (Fig. 2). However, the plant can not occur at Laem Chabang, Chonburi Province that is sandy shore. In this study, therefore, the seaweed can be collected from one location and the samples were collected between February, 00 to January, 01.

The average concentrations of cadmium in the sporophyte stage are 7.2681 μg/g. It ranges from 1.1750 in April to 22.8338 μg/g in December. Meanwhile, the mean metal content in gametophytes is 7.2430 μg/g and ranges from 0.2125 to 23.215 μg/g in May and December, respectively. The mean data show that the metal content in both generations (7.2681 and 7.2430 μg/g) is almost the same level but far higher

than the metal content in the water and in the sediment, which is 0.1092 and 4.2716 μg/g, respectively. It ranges from 0.0051 (January) to 0.30 μg/l (November) and 1.3200 (June) to 12.2083 μg/g (January) in water and sediment, respectively. The concentration levels of Cd in sporophytes, gametophytes, and sediment are 67, 66 and 39 times higher than in the water. Thus, the degree of contamination of the metal in order of decreasing contents is sporophytes ≥ gametophytes > sediment >> water. On the other hand, there were no gametophytes in June through August (Table 12), since these are monsoon months, during which there are strong waves, so it is not suitable for germination of spores.

Table 12 Cd concentrations (μg/g) in *P. japonica*, water and sediment in the same and different months

month	sporophyte	gametophyte	water	sediment
Feb-00	2.7625 [1a] ± 0.138	2.4635 [1b3] ± 0.032	0.0223 [2c] ± 0.002	3.2125 [5d] ± 0.024
Mar-00	3.8250 [3a] ± 0.102	3.6750 [3a] ± 0.002	0.2073 [7c] ± 0.040	1.4088 [1b] ± 0.026
Apr-00	1.1750 [2a] ± 0.075	1.4800 [1b] ± 0.062	0.0971 [6c] ± 0.001	4.4338 [6d] ± 0.022
May-00	2.1538 [1a] ± 0.065	0.2125 [2b] ± 0.073	0.0496 [3c4] ± 0.002	1.8350 [2d] ± 0.010
Jun-00	8.2950 [7a] ± 0.023	ns	0.0432 [3b] ± 0.001	1.3200 [1c] ± 0.037
Jul-00	5.7875 [6a] ± 0.018	ns	0.0736 [5b] ± 0.001	3.2735 [3c4] ±0.020
Aug-	4.8525 [4a] ± 0.060	ns	0.1074 [6b] ± 0.001	2.6519 [4c] ± 0.005
Sep-00	4.2775 [3a4] ± 0.027	2.1619 [1b] ± 0.015	0.0730 [5c] ± 0.001	2.1619 [2b3] ±0.015
Oct-00	2.3525 [1a] ± 0.019	2.1988 [1b] ± 0.014	0.0582 [4c] ± 0.001	1.3281 [1d] ± 0.027
Nov-00	13.0740 [8a] ± 0.294	12.898 [4a] ± 0.163	0.3000 [8c] ± 0.002	7.0056 [7b] ± 0.086
Dec-00	22.8338 [10a] ± 0.210	23.2150 [5b] ± 0.111	0.2737 [9c] ± 0.002	10.4203 [8d] ±0.036
Jan-01	15.8275 [9a] ±	16.8823 [6a] ±	0.0051 [1c] ± 0.002	12.2083 [9b] ±0.156
mean	7.2681 ± 1.921	7.2430 ± 2.763	0.1092 ± 0.028	4.2716 ± 1.063

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

ns = no sample

Comparing the metal concentrations in all samples in the same month, they are not significantly different at the 5% level in May, November and January. However, there are no gametophyte stage in June, July and August as described above. Therefore, the seaweed can be used for Cd monitoring since the plants grow under the same conditions, such as the same time, temperature, pH and others.

In different months, the Cd concentrations in the sporophyte plant is mostly significantly different, except in February, May and October and the gametophyte form, the metal concentration is significantly different, except in February, April, September and October. Similarly, the metal concentration in the water and sediment is not significantly different at the 5% level in May and June, May and October, April and August, and July and September, respectively (Table 12).

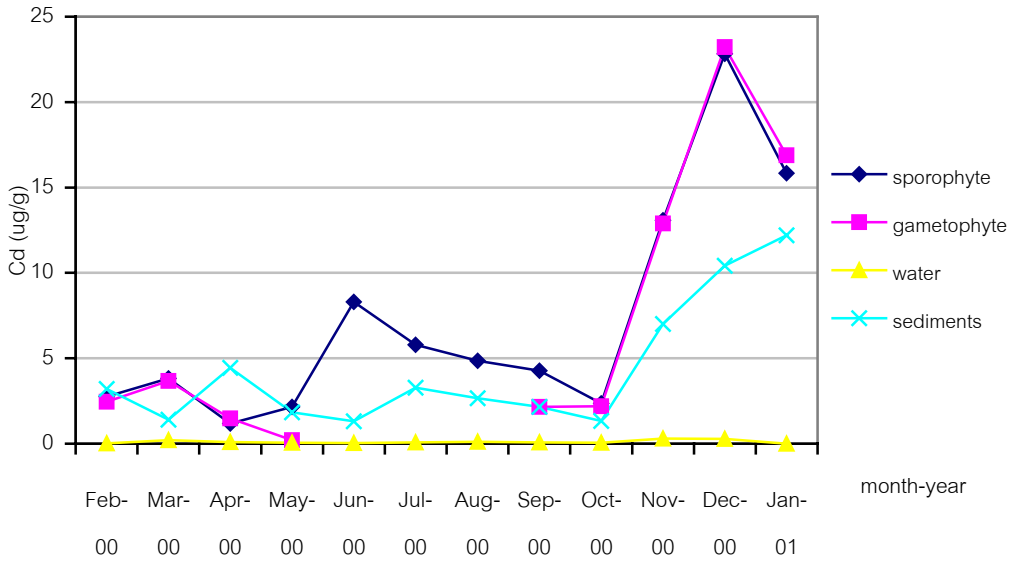

Fig. 9 Cd concentrations (μg/g) in *P. japonica*, water and sediment

In Figure 9, the amount of cadmium in both gametophyte and sporophyte plants

of *P. japonica* is quite high and has the same content, followed by the Cd in sediment and the water, respectively. All environmental factors such as salinity (‰), pH and temperature (°C) of water are quite stable year round as shown in Figure 10.

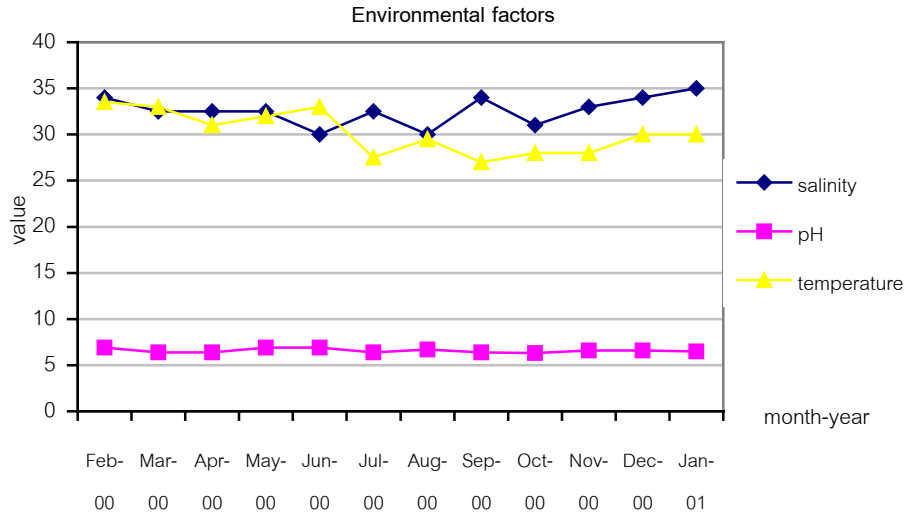

Fig. 10 Line graph of salinity (‰), pH and temperature (°C) of the water from Phe

The mean total concentrations of Cr in sporophyte and gametophyte generation are 21.2121 and 8.3261 µg/g, respectively. It ranges from 3.3275 (October) to 144.750 µg/g (June) in the sporophytes and from 2.50 (February) to 21.50 µg/g (March) in the gametophytes. In water and sediment, the mean Cr content is 0.4027 and 10.2768 µg/g, respectively. It ranges from 0.0150 to 2.7735 µg/l and 0.1781 to 25.3688 µg/g in water and sediment, respectively. The concentration levels of Cr in sporophyte, gametophyte and sediment are 53, 21 and 26 times higher than in the water. Therefore, the degree of contamination of the metal in order of decreasing contents is sporophytes > sediment > gametophytes >> water. It is clear that the seaweed can be used for the metal monitoring. However, the metal concentrations in some samples could not be detected. In addition, there were no gametophyte samples

in June throught and September, as described above (Table 13).

Table 13 Cr concentrations (μg/g) in *P. japonica*, water and sediment in the same and different months

month	sporophyte	gametophyte	water	sediment
Feb-00	4.1750 [1a]±	2.5000 [1b]±	0.0910 [1c]±	4.8875 [1d3]±
Mar-00	12.2750 [4a]±	21.500 [3b]±	0.2073 [4c]±	11.3125
Apr-00	3.6225 [1ab]±	11.4475	0.0804 [1c]±	7.6988 [4ab]±
May-00	11.8530 [4a]±	3.5688 [1b]±	nd	19.3430 [6c]±
Jun-00	144.750 [6a]±	ns	nd	25.3688
Jul-00	8.7050	ns	0.3023 [5b]±	10.9440 [5c]±
Aug-00	44.7390 [5a]±	ns	2.7735 [6b]±	22.3700 [7c]±
Sep-00	11.013 [3a4]±	ns	0.0700 [1b]±	5.1813 [1c3]±
Oct-00	3.3275 [1a]±	4.5500 [1b2]±	0.0150 [3c]±	3.3275 [1a]±
Nov-00	nd	nd	nd	0.1781 [2a]±
Dec-00	3.6050 [1a]±	8.9113 [1b2]±	0.0241 [2c3]±	6.4425 [3d4]±
Jan-01	6.4800 [1a2]±	5.8050 [1b2]±	0.0611 [1c2]±	6.2675 [3d4]±
mean	21.2121 ± 12.667	8.3261 ± 2.492	0.4027 ± 0.298	10.2768 ±2.304

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

nd = cannot detect (less than 0.0001 μg/l) ns = no sample

Comparing the Cr concentrations in all samples in the same month, they are mostly not significantly different at the 5% level, except between the sporophyte form and the sediment in March, April and October (the same letter in the same row, Table

13). All samples were collected from the same location and the same time (the same months) and the same environmental factors.

There are not significant differences in sporophytes in the following sets of months: February, April, October, December and January; July and September; March, May and September, and February, May, October, December and January in gametophytes (the same number in the same column). However, Cr concentrations could not be detected in the seaweed in November and in the water in May, June and November. Furthermore, there were no gametophytes in June, July and September (Table 13).

Comparing between two generations of the plants, the mean Cr is more accumulated in sporophyte than gametophyte stage. It is because of the metal is related to some activities of the plants. In addition, sporophyte generation have longer generation than the other generation. In contrast, the metal level in the plant and in the water is less than 0.0001 µg/l in September. This may be error in the samples or at the machine, or other reasons which I cannot give more information.

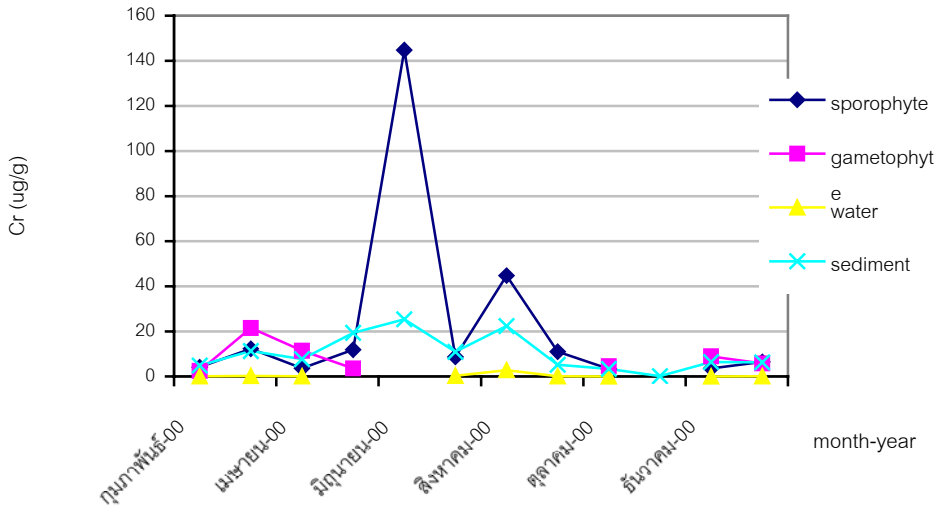

Fig. 11 Cr concentrations (μg/g) in *P. japonica*, water and sediment

In Figure 11, the graph shows the total Cr concentration in all samples. The highest level of the metal content occurred in the sporophyte stage in June. However, the degree of contamination between two generations cannot be compared since there were no gametophytes in these months.

Environmental factors such as salinity (‰), pH and temperature (°C) of water are quite stable year round as shown in Figure 10. All factors are quite stable year round, therefore the factors should have less effects on accumulation of the metal.

The mean concentrations of copper in sporophytes are 11.6134 μg/g. It ranges from 1.0165 in January to 30.4380 μg/g in February. Meanwhile, Cu content in gametophytes averages 15.8848 μg/g and ranges from 0.3263 to 60.625 μg/g in November and October. In the water and the sediment, the mean Cu content is 0.0550 and 4.6743 μg/g, respectively. It ranges from 0.0029 (January) to 0.1149 μg/l (April) and 0.0097 (December) to 12.2880 μg/g (February), respectively. On the other hand, Cu content in water could not be detected in March and November (Table 14).

Comparing the Cu content in all samples in the same month, the metal concentration is mostly significantly different at the 5% level, except in sporophytes and sediment in November; in gametophyte and sediment in May; and in water and sediment in December and January (the same letter in the same row, Table 14). The mean data show that the concentration levels of Cu in sporophytes, gametophytes and sediment are 211, 289 and 85 times higher than in the water. Therefore, the degree of contamination in order of decreasing is gametophyte > sporophyte >> sediment >> water. It is clear that the seaweed can be used for metal monitoring.

Comparing the Cu concentrations betweein two generations, the metal is high accumulated in gametophytes. It is known that Cu is one of micronutrients, therefore

the metal is essential in some stage gamete production. However, there are no gametophytes in June through September, since the duration of gametophyte generation is shorter than the others.

Table 14 Cu concentrations (μg/g) in *P. japonica*, water and sediment in the same and different months

month	sporophyte	gametophyte	water	sediment
Feb-00	30.4380 [9a]±	14.7130 [4b]±	0.0393 [3c]± 0.001	12.2880 [9d]±
Mar-00	2.9250 [2a]± 0.394	27.7750 [6b]±	nd	5.3750 [6c]± 0.032
Apr-00	13.7380 [6a]±	15.9280 [5b]±	0.1149 [7c]± 0.003	5.9400 [7d]± 0.040
May-00	12.7430 [5a]±	4.9263 [3b]± 0.111	0.0584 [4c]± 0.001	5.0800 [5b]± 0.070
Jun-00	29.0800 [7a]±	ns	0.0563 [4b]± 0.003	3.5913 [3c]± 0.031
Jul-00	13.9110 [6a]±	ns	0.0883 [6b]± 0.001	4.8681 [4c]± 0.052
Aug-00	20.0300 [7a]±	ns	0.0567 [4b]± 0.001	4.9788 [4c5]± 0.066
Sep-00	6.6900 [4a]± 0.007	ns	0.0556 [4b]± 0.001	9.0506 [8c]± 0.011
Oct-00	6.1800 [3a]± 0.036	60.6250 [7b]±	0.0683 [5c]± 0.001	3.5856 [3d]± 0.018
Nov-00	1.4638 [1a]± 0.027	0.3263 [1b]± 0.110	nd	1.2675 [2a]± 0.067
Dec-00	1.14550 [1a]±	1.6688 [2b]± 0.231	0.0097 [2c]± 0.003	0.0097 [1c]± 0.003
Jan-01	1.0165 [1a]± 0.001	1.1158 [2b]± 0.204	0.0029 [1c]± 0.001	0.0565 [1c]± 0.001
mean	**11.6134** ±3.009	**15.8848** ± 7.229	**0.0550** ± 0.011	**4.6743** ± 1.017

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

nd = cannot detect (less than 0.0001 μg/l) ns = no sample

In different month, there are not significant differences in some samples and some months, such as in sporophytes in November, December and January; and in June and August; in gametophytes in December and January; and so forth (the same number in the same column, Table 14).

In Figure 12, the graph shows fluctuation of the Cu concentrations in both the seaweed and sediment. The amount of Cu is highly accumulated in the sporophyte and gametophyte generations, especially in October, followed by in the sediment. The lowest concentration of the metal occurs in the water.

Environmental factors such as salinity (‰), pH and temperature (°C) of water are quite stable year round as shown in Figure 10.

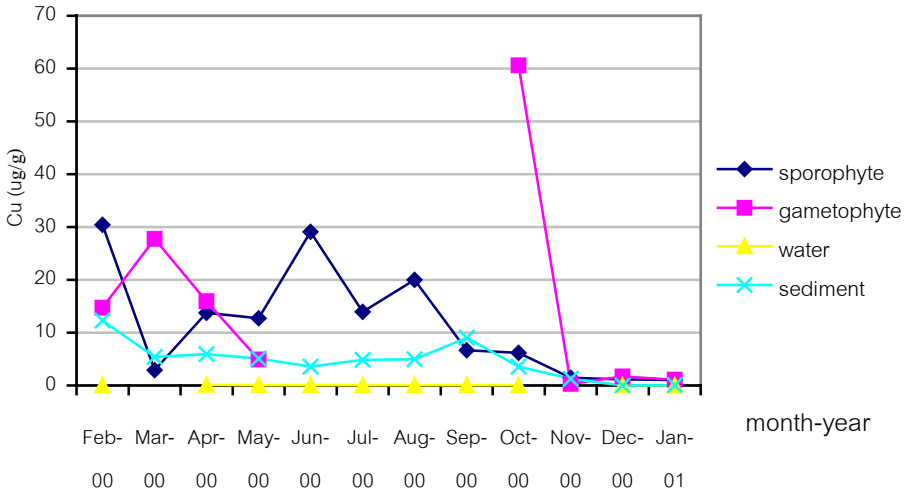

Fig. 12 Cu concentrations (µg/g) in *P. japonica*, water and sediment

The average concentrations of lead in sporophytes are 39.3204 µg/g. It ranges from 12.6450 (April) to 100.20 µg/g (December). Meanwhile, the metal content in gametophytes averages 23.5809 µg/g and ranges from 8.3125 (April) to 33.0588 µg/g (December). In water and sediment, the mean metal content is 0.3156 and 12.8924 µg/g, respectively. It ranges from 0.0536 (January) to 0.470 µg/l (October) and from 0.910 (April) to 34.3145 µg/g (July), respectively. The concentration levels of Pb in sporophyte, gametophyte and sediment are 125, 75 and 41 times higher than in the water. Therefore, the degree of contamination in order of decreasing is sporophyte >> gametophyte > sediment >> water. It is clear that the seaweed can be used for the

metal monitoring. On the other hand, there were no gametophytes in June through September (Table 15).

Table 15 Pb concentrations (μg/g) in *P. japonica*, water and sediment in the same and different months

month	sporophyte	gametophyte	water	sediment
Feb-00	21.8000 [2a3] ±	29.838 [2b3] ±0.324	0.4060 [5c67] ± 0.010	8.700 [1d23] ± 0.859
Mar-00	19.3000 [2a] ± 1.463	26.000 [2b] ± 1.482	0.2425 [3c] ± 0.008	2.3000 [1c] ± 0.576
Apr-00	12.6450 [1a] ± 1.119	8.3125 [1a] ± 3.679	0.3784 [5b6] ± 0.024	0.9100 [1b2] ±
May-00	18.3010 [2a] ± 2.812	13.676 [1a] ± 3.917	0.3692 [5b6] ± 0.033	16.473 [2a3] ± 1.449
Jun-00	94.8500 [8a] ± 0.704	ns	0.4265 [6b7] ± 0.006	9.1671 [1c23] ±1.807
Jul-00	30.3775 [5a] ±0.457	ns	0.3344 [4b5] ± 0.006	34.3145 [4a] ± 14.89
Aug-00	23.2575 [3a4] ±0.753	ns	0.2688 [3b4] ± 0.015	12.887 [b23] ±
Sep-00	26.4813 [4a] ± 0.384	ns	0.4339 [6b7] ± 0.005	15.262 [1ab23] ±1.05
Oct-00	38.4830 [6a] ± 0.199	27.573 [2b3] ±	0.4700 [7c] ± 0.012	21.574 [3d4] ±
Nov-00	33.4550 [5a] ± 0.001	23.326 [2b] ± 0.001	0.1470 [2c] ± 0.004	5.2869 [1d2] ±
Dec-00	100.2000 [9a] ±	33.059 [3b] ± 4.428	0.2569 [3c4] ± 0.131	13.244
Jan-01	52.6940 [7a] ± 0.501	26.863 [2b3] ±	0.0536 [1c] ± 0.001	14.591 [1d23] ±0.02
mean	39.3204 ± 8.4243	23.5809 ±2.9666	0.3156 ± 0.0363	12.8924 ±2.6123

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

ns = no sample

In the same month, there are not significant differences at the 5% level between sporophyte and gametophyte plants in April and May; sporophytes and sediment in May, July and September; and water and sediment in March and April as well as in August and September (the same letter in the same row, Table 15).

In different months, there are not significant differences at the 5% level in sporophyte plants in February, March and May; in gametophyte plants in April and May; in February, March, October, November and January; and in February, April, October, December and January. In the water, there are not significantly different at the 5% level in March, August and December; July, August and December; February, April, May and July. In sediment, most of the Pb is not significant differences, except in July (the same number in the same column, Table 15).

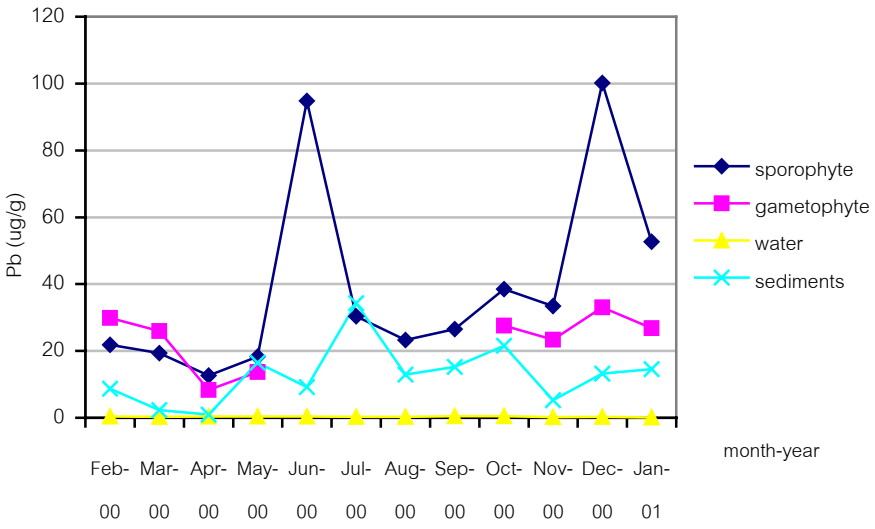

Fig. 13 Pb concentrations (μg/g) in *P. japonica*, water and sediment

In Figure 13, the graph shows that the Pb content is highly accumulated in sporophytes, especially in June and December, followed by in sediment and in gametophytes and the lowest Pb concentrations occur in the water.

The average concentrations of zinc in the sporophyte stage are 62.3403 μg/g . It is of the order of 28.416 (December) to 185.550 μg/g (March). Meanwhile, the mean Zn content in gametophyte plants is 84.1869 μg/g, and it ranges from 31.5690 (October) to 275.6750 μg/g (March). In seawater and sediment, the mean Zn content

is 0.2495 and 28.6218 μg/g, respectively. It ranges from 0.0055 (February) to 0.6602 μg/l (August) and from 13.0270 (August) to 55.9810 μg/g (May) in water and sediment, respectively. On the other hand, there were no gametophytes in June through September (Table 16). The mean data show that the concentration levels of Zn in sporophyte, gametophyte and sediment are 250, 325 and 115 times higher than in the water. Therefore, the degree of accumulation in order of decreasing contents is gametophyte > sporophyte > sediment >> water. It is clear that the plants can be used as bioindicators for metal contamination.

Table 16 Zn concentrations (μg/g) in *P. japonica*, water and sediment in the same and different months

month	sporophyte	gametophyte	water	sediment
Feb-00	88.4380 ^{10a}± 133	90.0980 ^{4b}± 0.054	0.0055 ^{1c}± 0.002	49.850 ^{8d}± 0.374
Mar-00	185.5500 ^{11a}±0.978	275.6750 ^{6b}± 0.189	0.0363 ^{2c}± 0.002	18.575 ^{3d}± 0.032
Apr-00	64.4030 ^{8a}± 0.006	37.8200 ^{2b}± 0.112	0.0529 ^{3c}± 0.001	32.573 ^{7d}± 0.138
May-00	54.4880 ^{7a}± 0.007	109.2250 ^{5b}± 0.009	0.5654 ^{8c}± 0.002	55.981 ^{9d}± 0.019
Jun-00	49.2800 ^{6a}± 0.221	ns	0.0828 ^{4b}± 0.001	32.869 ^{7c}± 0.061
Jul-00	84.9250 ^{9a}± 0.431	ns	0.1706 ^{5b}± 0.003	31.506 ^{6c}± 0.128
Aug-00	32.2860 ^{2a}± 0.471	ns	0.6602 ^{10b}± 0.001	13.027 ^{1c}± 0.391
Sep-00	42.3400 ^{5a}± 0.039	ns	0.6312 ^{9b}± 0.002	17.560 ^{2c}± 0.043
Oct-00	35.6360 ^{3a}± 0.203	31.5690 ^{1b}± 0.071	0.2960 ^{6c}± 0.001	20.298 ^{4d}± 0.046
Nov-00	43.2440 ^{5a}± 0.548	42.3100 ^{3a}± 1.081	0.3123 ^{7b}± 0.001	17.166 ^{2c}± 0.031
Dec-00	28.4160 ^{1a}± 0.260	43.3480 ^{3b}± 2.125	0.1708 ^{5c}± 0.003	21.593 ^{5d}± 0.882
Jan-01	39.0780 ^{4a}± 0.105	43.4500 ^{3b}± 0.407	0.0102 ^{1c}± 0.003	32.463 ^{7d}± 0.044
mean	62.3403 ± 12.517	84.1869 ± 29.072	0.2495 ± 0.071	28.6218 ±3.866

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

ns = no sample

Comparing the Zn content in the same month in all samples, there are significant differences at the 5% level in sporophyte and gametophyte plants, except in November (the same letter in the same row, Table 16).

In different months, there are not significant differences at the 5% level in sporophytes in September and November; in gametophytes in November, December and January; in the water in February and January, and in July and December; and in the sediment in September and November; and in April, June and January (the same number in the same column, Table 16).

In Figure 14, the Zn is highly accumulated in both sporophyte and gametophyte plants in all months, especially in March, then slightly decreases and fluctuates in the rest of the year. Meanwhile, Zn contents in the sediment are quite stable in all months. The metal content found in the water is also the lowest as observed in other metals.

Environmental factors such as salinity (‰), pH and temperature (°C) of the water are quite stable year round as shown in Figure 10.

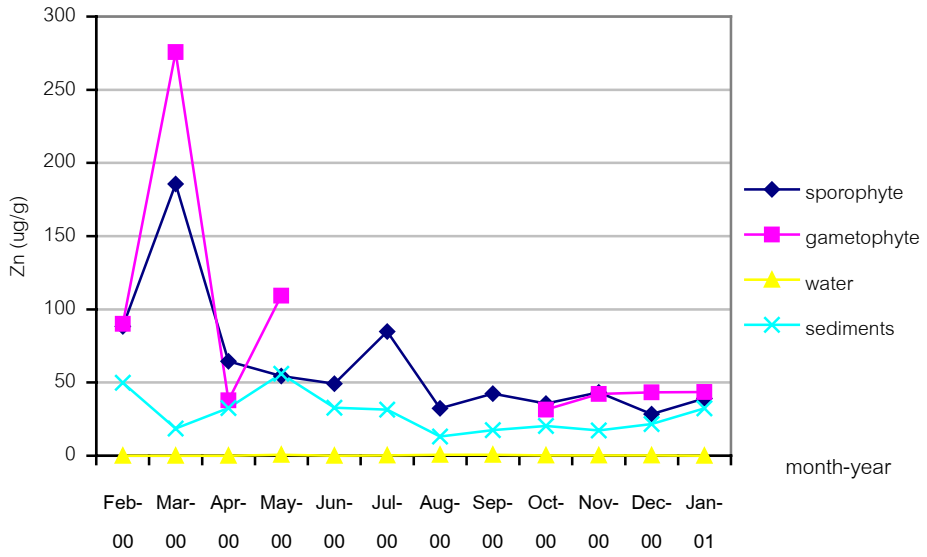

Fig. 14 Zn concentrations (μg/g) in *P. japonica*, water and sediment

In this study, bioconcentration levels of Cd, Cr, Cu, Pb and Zn in the sporophyte and gametophyte generatios are 67 and 66; 53 and 21; 211 and 289; 125 and 75; 250 and 325 times higher than in the water, respectively. Therefore, the seaweed can be used for metal monitoring in oceanic environments since all five metals are highly accumulated in the plant and in far higher concentrations than in the seawater.

Comparing all metal level between two generations, Cd concentrations in both generations are at the same level. The absorption of the metal from the water into the seaweed is very low since Cd is not a micronutrient. Therefore, the bioavailability of the metal is low, too. Cr and Pb concentrations show high levels in the sporophyte generation. Although Pb is not a micronutrient, the absorption of this metal in the sporophyte generation is very high since there may be other factors relating to absorption processes such as many ligands in the cell wall, specific structure of organelles and so forth.

The degree of contamination in order of decreasing concentrations is the

seaweed (sporophyte ≈ gametophyte) > sediment >> water, except for Cr which is accumulated at the same concentration between the gametophyte generation and sediment.

Comparing the two generations, Cu and Zn are micronutrients, these metals may be related to gamete production. Therefore, the metals are highly accumulated in gametophyte generations. However, the sporophyte generation has longer life span than the other generation. Thus, Cr and Pb are more highly accumulated in the sporophytes. Furthermore, all metals dissolved in the seawater are at a far lower concentration than in the sediment and the plants. Because *Padina japonica* is distributed in tropical areas, there is little knowledge of this seaweed as a bioindicator. In this study, it can be concluded that the plant is suitable for use in heavy metal monitoring in oceanic environments, since Cd, Cr, Cu, Pb and Zn accumulated in the plant are many times higher in concentration than in the sediment and in the water. Although the gametophyte generation can not be found in some months, the sporophyte form can be used as a bioindicator. However, there is a disadvantage to study with this seaweed since the degree of contamination between the two generations cannot be compared year round.

It is noted that the degree of contamination, in different months, in all samples is variable because there are many factors effecting accumulation. For example, metal species, age of the plant, precipitation, pH, salinity, light, temperature and so on. Therefore, the samples should be collected and determined at the same time, under the same conditions.

4.1.3 Metals in *Sargassum polycystum*

Sargassum polycystum is classified into the division Phaeophyta, class Phaeophyceae, order Fucales, and family Sargassaceae. The plants in the Fucales have a diplontic life history with the sporophyte producing eggs and sperm, thus they can be found only in the sporophyte generation (Dawes, 1998).

Sargassum polycystum cannot be found in Laem Chabang's sea. Therefore, the metals in the plants can be determined only the samples collected from Phe, Muang District, Rayong Province. The metals can be determined in the holdfast, stipe (stem), blade (leaf), and air bladder, as well as in water and in sediment collected from near the seaweed. However, there are no plant samples in March, April and May since the plants disappear during the life history of the years.

The average cadmium concentration in the seaweed is 6.0855, 5.7682, 5.7025, and 5.6617 μg/g dry weight in the holdfast, stem, blade and air bladder, respectively. It ranges from 2.0539 (June) to 22.7213 μg/g (December); from 1.4836 (June) to 23.180 μg/g (November); from 1.7275 (October) to 21.9238 μg/g (December); and from 1.8572 (June) to 22.1425 μg/g (December), in the holdfast, stem, blade and air bladder, respectively (Table 17).

In the water and sediment collected from near the seaweed, the mean metal content is 0.1145 and 4.0081 μg/g dry weight, and it ranges from 0.0051 (January) to 0.3727 μg/l (December); and from 1.6963 (October) to 10.4203 μg/g (December), respectively (Table 17).

Comparing the metal content in the plant in the same month, the metal content in each part of the seaweed is significantly different at the 5% level, except in February and January (the same letter in the same row, Table 17). In the rest of the

year, the data show both significant at the 5% level and non-significant differences (the same and different letter in the same row, Table 17).

Contamination levels of Cd in holdfast, stem, blade, air bladder, and sediment are 53, 50, 50, 49 and 25 times higher than in the water. Therefore, the degree of contamination in each part of the plant in order of decreasing is holdfast ≥ stem ≥ blade ≥ air bladder ≥ sediment >> water. However, from the mean data in Table 17, it can be concluded that the metal is absorbed from the seawater through all parts of the plant and higher degree of absorption from the sediment.

Table 17 Cd concentrations (μg/g) in *S. polycystum*, water and sediment in the same and different months

month	holdfast	stem	blade	air bladder	water	sediment
Feb-00	4.8539 [3a] ±0.000	4.8837 [4a] ±0.095	4.7018 [5a] ±0.000	4.8539 [3a] ±0.060	0.0215 [4c] ±0.001	4.3250 [5b] ±0.032
Mar-00	ns	ns	ns	ns	ns	ns
Apri-00	ns	ns	ns	ns	ns	ns
May-00	ns	ns	ns	ns	ns	ns
June-00	2.0539 [1a] ± 0.000	1.4836 [1c] ± 0.000	2.0277 [2a] ± 0.001	1.8572 [1b] ± 0.010	0.0658 [1d] ± 0.040	1.8198 [2b] ± 0.020
July-00	2.1775 [1a2] ± 0.002	1.9656 [2c] ± 0.010	2.0918 [2b] ± 0.002	1.9495 [1c2] ± 0.006	0.0653 [2e] ± 0.002	1.8490 [2d] ± 0.054
Aug-00	2.4542 [1a2] ± 0.004	2.2568 [3c] ± 0.001	2.3677 [3b] ± 0.002	2.1415 [1d2] ± 0.001	0.0647 [3f] ± 0.006	1.8200 [3e] ± 0.031
Sep-00	2.4150 [1a2b]	2.2588 [b3c] ± 0.039	2.5150 [3a] ± 0.125	2.1538 [1c2] ± 0.013	0.0765 [3e] ± 0.000	1.9263 [3d] ± 0.017
Oct-00	2.5975 [1a2] ± 0.027	1.9125 [2b] ± 0.024	1.7275 [1c] ± 0.011	1.9850 [1b2] ± 0.069	0.0590 [2e] ± 0.001	1.6963 [1d] ± 0.013
Nov-00	12.4475 [4a] ± 0.282	11.5988 [5b] ± 0.213	10.6775 [6c] ± 0.150	10.6475 [4c] ± 0.093	0.3001 [5e] ± 0.002	7.0056 [6d] ± 0.086
Dec-00	22.7213 [5a] ± 0.168	23.180 [6b] ± 0.083	21.9238 [7c] ± 0.168	22.1425 [5c] ± 0.181	0.3727 [6e] ± 0.002	10.4203 [7d] ± 0.036
Jan-01	3.0489 [2a] ±0.004	2.3741 [3a] ±0.000	3.2899 [4a] ± 0.007	3.3727 [2a] ± 0.005	0.0051 [3c] ± 0.001	5.7104 [4b] ± 0.001
mean	**6.0855** ± 2.352	**5.7682** ± 2.420	**5.7025** ± 2.231	**5.6617** ± 2.264	**0.1145** ± 0.043	**4.0081** ± 1.051

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

ns = no sample

In Figure 15, the line graph shows that the metal contamination in all parts of the plant and the sediment are approximately at the same level. After dying and disappearing in March, April and May, the plant grows and blooms. The metal is less absorbed in the growing season (June, July, August and September). In addition, there is the rainy season during these months, so the metal may diluted because of precipitation. So, the metal concentration is slightly low. In the maturation stage, the metal is more highly accumulated. The values are increased, especially in December, and then slightly decreased in the deterioration stage. Furthermore, the graph shows the metal content in all parts of the plant to be higher than that of the metal in the sediment. In addition, the metal contamination in seawater is far lower than that of the plant and sediment. It can be concluded that the plant is suitable for use in Cd monitoring.

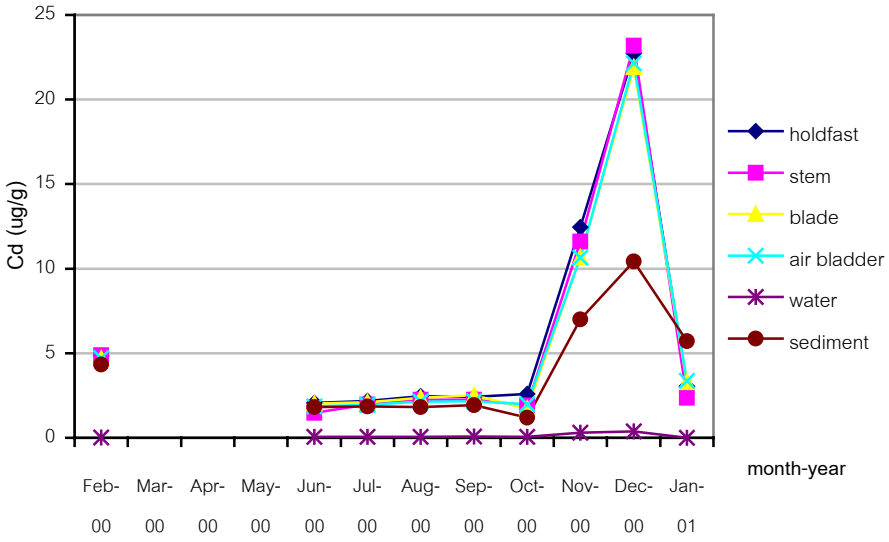

Fig. 15 Cd concentrations (μg/g) in *S. polycystum*, water and sediment

In Figure 16, the line graph shows environmental factors such as salinity (‰), temperature (ºC), and pH of the water collected from near the seaweed. All factors are quite stable in all months.

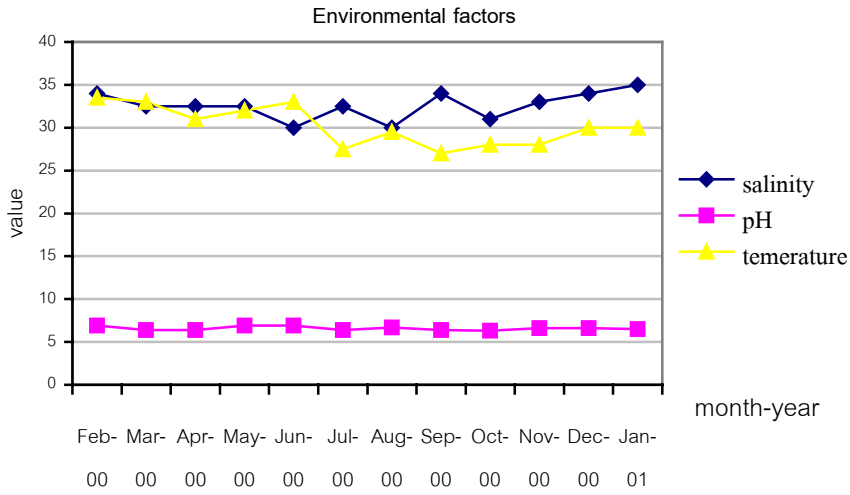

Fig. 16 Line graph of salinity (‰), pH and temperature (°C) of the water from Phe

The mean total chromium concentration in *S. polycystum* is 3.6172, 2.5925, 2.3304 and 3.5034 μg/g dry weight in the holdfast, stem, blade and air bladder,

respectively. It ranges from 0.6475 (December) to 7.3938 μg/g (January); from 0.660 (December) to 5.6750 μg/g (February); from 0.4013 (December) to 6.8950 μg/g (January); and from 0.2275 (October) to 7.9632 μg/g (February), respectively. The average data show that the metal is most highly accumulated in the holdfast, followed by air bladder, stem and bladder, respectively. In the water and the sediment, the average Cr concentration is 0.0651 μg/l and 4.1784 μg/g, and it ranges from 0.0122 (November) to 0.2663 μg/l (October) and from 0.2375 (November) to 6.4425 μg/l (December), respectively (Table 18).

Contamination levels of Cr in holdfast, stem, blade, air bladder, and sediment are 56, 40, 36, 54 and 64 times higher than in the water. Therefore, the degree of contamination in all samples in order of decreasing is sediment > holdfast > air bladder > stem > blade >> water. However, the mean data and Figure 17 show that the metal is absorbed through all parts of the plant at the same rate, except slightly high via the holdfast than the others.

Table 18 Cr concentrations (μg/g) in *S. polycystum*, water and sediment in the same and different months

month	holdfast	stem	blade	air	water	sediment
Feb-00	5.7104 [4a] ± 0.021	5.6750 [3a] ± 0.051	6.5887 [5b] ± 0.061	7.9632 [5c] ± 0.041	0.1160 [3d] ± 0.001	5.5500 [5a] ± 0.204
Mar-00	ns	ns	ns	ns	ns	ns
Apri-00	ns	ns	ns	ns	ns	ns
May-00	ns	ns	ns	ns	ns	ns
June-00	3.6474 [2a3] ± 0.010	2.3030 [2b] ± 0.030	1.8817 [4b] ± 0.009	3.3799 [2c] ± 0.014	0.0169 [1d] ± 0.024	3.5035 [3c] ± 0.051
July-00	3.5461 [2a] ± 0.002	2.2395 [2b] ± 0.008	1.0689 [1c23] ± 0.051	2.4113 [2d] ± 0.031	0.0161 [1e] ± 0.110	3.6186 [3a] ± 0.061
Aug-00	3.2049 [2a] ± 0.050	2.3206 [2b] ± 0.030	1.2293 [2c34] ± 0.071	3.1742 [2a] ± 0.014	0.0272 [1d2] ± 0.031	4.4386 [4e] ± 0.041
Sep-00	3.2211 [2a] ± 0.000	2.2050 [2ab] ± 1.070	0.7500 [1b2] ± 0.216	2.3333 [2ab] ± 0.706	0.0460 [1c2] ± 0.008	5.2250 [5d] ± 0.135
Oct-00	4.3474 [3a] ± 0.001	2.0329 [2b] ± 0.012	1.6327 [3b4] ± 0.013	0.2275 [1c] ± 0.005	0.2663 [4c] ± 0.030	2.3225 [2b] ± 0.172
Nov-00	0.8359 [1a] ± 0.042	0.7655 [1a] ± 0.004	0.5260 [1ac2] ± 0.020	4.7913 [4b] ± 0.120	0.0122 [1c2] ± 0.210	0.2375 [1ac] ± 0.251
Dec-00	0.6475 [1a] ± 0.168	0.6600 [1a] ± 0.260	0.4013 [1a] ± 0.240	1.9700 [2b] ± 0.436	0.0241 [1a] ± 0.004	6.4425 [6c] ± 0.200
Jan-01	7.3938 [5a] ± 0.045	5.1313 [3b] ± 0.038	6.8950 [5c] ± 0.178	5.2800 [4b] ± 0.018	0.0611 [2d] ± 0.001	6.2675 [6e] ± 0.021
mean	3.6172 ± 0.708	2.5925 ± 0.575	2.3304 ± 0.849	3.5034 ± 0.750	0.0651 ± 0.027	4.1784 ± 0.670

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

ns = no sample

The ability of Cr accumulation according to part of the seaweed in the same month is mostly significantly different at the 5% level, except in July. However, there are not significant differences in some parts of the plants, such as: holdfast and stem in February; stem and blade in June; holdfast and air bladder in August and so forth (the same letter in the same row, Table 18).

In different months, the metal concentrations are not significantly different at the 5% level in all parts of the seaweed, such as in the holdfast in June, July, August and September; in June and October; and in November and December; in the stem in June, July, August, September and October; in November and December; and in February and January; in the blade in July, September, October, November and December; in July, August and September; and in July and October; in the air bladder in June, July, August, September and December; and in November and January (the same number in the same column, Table 18).

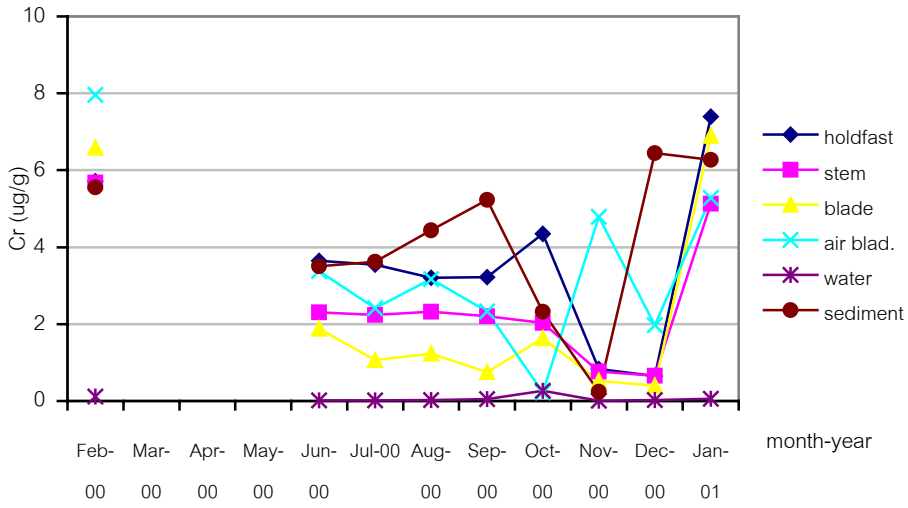

Fig. 17 Cr concentrations (μg/g) in *S. polycystum*, water and sediment

In Figure 17, the graph shows that the metal contamination is the lowest in the seawater and quite high in the sediment. It is clear that the whole plant can be used for the metal monitoring. In the seaweed, the accumulation pattern is fluctuating in all parts during the year. The contamination level, in contrast, is lower in the growing stage than in mature plants.

Environmental factors such as salinity (‰), temperature (ºC), and pH of the

water collected from near the seaweed are shown in Figure 16.

The mean concentration of copper in *S. polycystum* is 6.3086, 6.9918, 13.5690 and 8.0889 μg/g dry weight in the holdfast, stem, blade and air bladder, respectively. It ranges from 2.7813 (October) to 9.0216 μg/g (June); from 2.6825 (October) to 11.8238 μg/g (June); from 8.9763 (September) to 21.5163 μg/g (October); and from 1.3888 (December) to 19.4120 μg/g (February), in the holdfast, stipe, blade and air bladder, respectively. The average data show that the metal is the most accumulated in the blade, followed by air bladder, stem and holdfast, respectively. In the water and the sediment, an average Cu concentration is 0.0480 μg/l and 2.9873 μg/g, respectively (Table 19). Contamination levels of Cu in holdfast, stem, blade, air bladder, and sediment are 1,114, 1,160, 1,676, 751 and 1 times higher than in the water. Therefore, the degree of contamination in order of decreasing is blade > stem ≥ holdfast > air bladder >> sediment = water. This indicates that Cu is absorbed the most into the plant through the blade, stem or holdfast.

The ability of Cu accumulation in all samples in the same month is mostly significantly different at the 5% level. However, there are not significant differences at the 5% level between holdfast and sediment in February; between holdfast and stipe in July, August, and September; between holdfast, stipe, air bladder and sediment in October; between holdfast and air bladder in November; between holdfast and stipe in January, as well as between the water and the sediment in January (the same number in the same column, Table 19).

Table 19 Cu concentrations (μg/g) in *S. polycystum*, water and sediment in the same and different months

month	holdfast	stem	blade	air	water	sediment
Feb-00	8.0829 [3a4] ± 0.001	8.1961 [6b] ± 0.150	16.5789 [4c] ± 0.012	19.4120 [7d] ± 0.104	0.0480 [2e] ± 0.001	7.6625 [5a] ± 0.066
Mar-00	ns	ns	ns	ns	ns	ns
Apri-00	ns	ns	ns	ns	ns	ns
May-00	ns	ns	ns	ns	ns	ns
June-00	9.0216 [5a] ± 0.084	11.8238 [7b] ± 0.000	15.1716 [3c4] ± 0.064	11.0788 [6b] ± 0.123	0.0619 [2d3] ± 0.046	4.1208 [4e] ± 0.054
July-00	8.6039 [4a5] ± 0.025	8.4998 [5a6] ± 0.001	12.3214 [2b] ± 0.052	10.7349 [5c6] ± 0.024	0.0574 [2d3] ± 0.012	3.4777 [3e4] ± 0.032
Aug-00	7.7404 [3a] ± 0.014	8.1576 [5a] ± 0.013	9.3955 [1b] ± 0.002	9.5867 [4b] ± 0.032	0.0569 [2c3] ± 0.001	2.9544 [3d] ± 0.074
Sep-00	8.2190 [3a45] ± 0.094	8.3213 [5a] ± 0.023	8.9763 [1b] ± 0.123	10.4675 [5c] ± 0.048	0.0568 [2d3] ± 0.000	3.6919 [3e4] ± 0.011
Oct-00	2.7813 [1a] ± 0.049	2.6825 [1a] ± 0.019	21.5163 [5b] ± 1.232	3.0438 [2a] ± 0.070	0.0685 [3c] ± 0.001	3.6950 [3a4] ± 0.006
Nov-00	3.2671 [1a] ± 0.000	5.0414 [3b] ± 0.004	15.1843 [3c4] ± 0.085	3.0352 [2a] ± 0.042	0.0670 [3d] ± 0.006	1.2675 [2e] ± 0.068
Dec-00	3.0433 [1a] ± 0.091	3.9383 [2b] ± 0.009	13.9244 [2c3] ± 0.067	1.3888 [1d] ± 0.004	0.0097 [1e] ± 0.003	0.0097 [1e] ± 0.003
Jan-01	6.0177 [2a] ± 0.075	6.2653 [4a] ± 0.096	9.0522 [1b] ± 0.072	4.0528 [3c] ± 0.034	0.0054 [1d] ± 0.000	0.0060 [1d] ± 0.000
mean	**6.3086** ± 0.865	**6.9918** ± 0.931	**13.5690** ± 1.384	**8.0889** ± 1.916	**0.0480** ± 0.008	**2.9873** ± 0.791

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

ns = no sample

In Figure 18, the graph shows the metal is more highly accumulated in the growing stage than in the mature plant. It is clear that Cu, in low concentration, is necessary in growing processes. So, it is more highly accumulated in the maturation

stage, especially in the blade of the mature plant. As in the concentration of Cd and Cr, the Cu concentration is very low in the water and quite high in the sediment. It is clear that *S. polycystum* can be used for Cu monitoring.

Environmental factors such as salinity (‰), temperature (ºC), and pH of the water collected nearby the seaweed are shown in Figure 16.

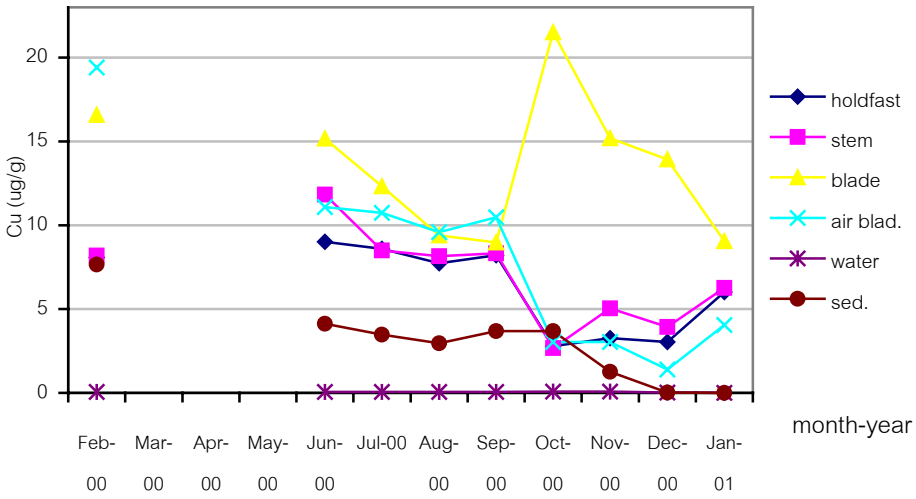

Fig. 18 Cu concentrations (µg/g) *S. polycystum*, water and sediment

The mean concentrations of lead in *S. polycystum* are 40.6196, 49.9247, 42.0553 and 33.3579 µg/g dry weight in the holdfast, stem, blade and air bladder, respectively. It ranges from 19.4975 (October) to 67.5955 µg/g (January); from 3.3838 (September) to 113.013 µg/g (December); from 6.5263 (September) to 145.548 µg/g (January); and from 2.6934 (August) to 112.275 µg/g (October), in the holdfast, stem, blade and air bladder, respectively. The average data show that the metal is accumulated most in the stem, followed by blade, holdfast, and air bladder, respectively. In seawater and sediment, the average metal concentration is 0.2668 µg/l and 12.3599 µg/g,

respectively (Table 20). However, the metal in the blade could not be detected in November.

Concentration levels of Pb in holdfast, stem, blade, air bladder, and sediment are 152, 187, 158, 125 and 45 times higher than in the water. Therefore, the degree of contamination in order of decreasing is stem > blade ≥ holdfast > air bladder >> sediment >> water.

Comparing the Cu content in the same month, the metal contamination in all samples is mostly significantly different at the 5% level, except between blade and sediment in June; stem and air bladder in September; holdfast and sediment, stem and sediment, stem and blade, and blade and air bladder in October; water and sediment in November; stem, blade and air bladder in December; and water and sediment in December and January (the same letter in the same row, Table 20).

In different months, the Pb content is not significantly different at the 5% level in July, September and October; and in July, August and September; in February and December; and in February and November in the holdfast; in June, July, August and September in the stem; in July, August and September in the blade; in June, July, August and September; and in October and November in the air bladder. Likewise in the water and the sediment, there are some non significant differences at the 5% level such as June, July, August; and December; February and June; February and September in the water; and August, September and December in the sediment, and so forth (the same number in the same column, Table 20). It is noted that the metal content in all parts of the seaweed in June, July, August and September is not significantly different at the 5% level.

Table 20 Pb concentrations (μg/g) in *S. polycystum*, water and sediment in the same and different months

month	holdfast	stem	blade	air	water	sediment
Feb-00	58.5100 [4a5]	70.6978 [3b] ± 1.155	54.0241 [4c] ± 0.986	48.6768 [3d] ± 0.884	0.3495 [4e5] ± 0.006	14.5375 [4f5] ± 2.048
Mar-00	ns	ns	ns	ns	ns	ns
Apri-00	ns	ns	ns	ns	ns	ns
May-00	ns	ns	ns	ns	ns	ns
June-00	32.5440 [3a] ± 1.041	8.8876 [1b] ± 0.041	14.7491 [2c] ± 0.046	5.7660 [1d] ± 0.471	0.2904 [3e4] ± 0.001	14.9793 [4c5] ± 1.045
July-00	24.1656 [1a2] ± 0.874	4.1076 [1b] ± 0.084	6.8428 [1c] ± 0.010	2.7370 [1d] ± 0.074	0.2382 [3e] ± 0.001	15.9808 [5f] ± 0.961
Aug-00	25.7204 [2a] ± 0.086	4.6616 [1b] ± 0.002	7.4104 [1c] ± 0.075	2.6934 [1d] ± 0.004	0.2442 [3e] ± 0.005	11.3925 [3f] ± 0.043
Sep-00	21.9588 [1a2] ± 1.056	3.3838 [1b] ± 0.282	6.5263 [1c] ± 0.420	2.9363 [1b] ± 0.636	0.3813 [5d6] ± 0.028	13.471 [3e45] ± 0.296
Oct-00	19.4975 [1a] ± 0.811	21.5463 [2bc] ± 0.628	22.3088 [3cd] ± 0.225	23.740 [2d] ± 0.538	0.4384 [6e] ± 0.003	20.3803 [6ab] ± 0.198
Nov-00	60.700 [5a] ± 224	94.2625 [4b] ± 6.019	nd	22.500 [2c] ± 2.637	0.1470 [2d] ± 0.041	5.2869 [2d] ± 0.524
Dec-00	54.885 [4a] ± 3.213	113.013 [5b] ± 8.377	121.088 [5b] ± 4.585	112.275 [5b] ± 8.559	0.2569 [3c] ± 0.131	13.244 [3c4] ± 0.882
Jan-01	67.5955 [6a] ± 2.015	128.762 [6b] ± 3.154	145.548 [6c] ± 6.985	78.8963 [4d] ± 1.151	0.0554 [1e] ± 0.001	1.9672 [1e] ± 0.004
mean	40.6196 ± 6.462	49.9247 ± 17.239	42.0553 ± 19.693	33.3579 ± 13.076	0.2668 ± 0.039	12.3599 ± 1.864

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

nd = cannot detect (less than 0.0001 μg/l) ns = no sample

In Figure 19, the line graph shows the metal level in all parts of the plant in each month. The metal is less absorbed in the growing season which is the rainy season, during the months of June, July, August and September. The ambient metal

concentration in the seawater is slightly low because it is diluted by precipitation. In the maturation stage, the metal is more highly accumulated. The values are increased and then slightly decreased in the deterioration stage. It is pointed out that metal accumulation is dependent on the age of the biota. Furthermore, the graph shows that the metal concentration in all parts of the plant is higher than that of the metal in the sediment. In addition, the metal contamination in seawater is far lower than that of the plant and sediment. It is noted that the plant can be used for the metal monitoring.

Environmental factors such as temperature (ºC), salinity (‰), and pH of the water collected from near the seaweed are shown in Figure 16.

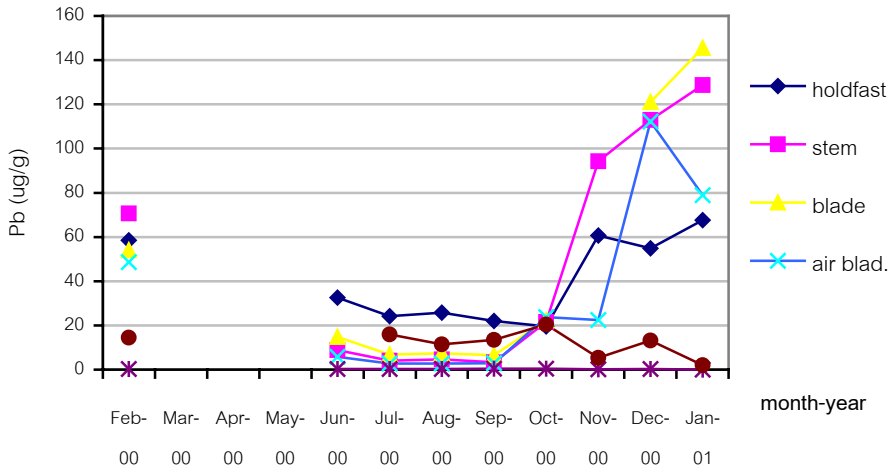

Fig. 19 Pb concentrations (μg/g) in *S. polycystum*, water and sediment

The mean concentrations of zinc in *S. polycystum* are 27.3127, 51.5066, 30.9453 and 40.2806 μg/g dry weight in the holdfast, stem, blade and air bladder, respectively. It ranges from 0.5713 (November) to 40.1829 μg/g (June); from 16.6055 (November) to 101.6740 μg/g (July); from 0.5375 (November) to 49.5746 μg/g (June); and from 0.2588 (November) to 108.930 μg/g (February), in the holdfast, stipe, blade and air bladder, respectively. In the water and the sediment, the average

metal content is 0.3641 μg/l and 22.4410 μg/g, respectively (Table 21). The average data show that contamination levels of Zn in holdfast, stem, blade, air bladder, and sediment are 750, 1,599, 850, 1,106 and 616 times higher than in the water. Therefore, the degree of contamination in order of decreasing is stem >> air bladder > blade ≥ holdfast > sediment >> water. This showed that the metal is more absorbed into the plant from seawater than from the sediment. In addition, it is clear that the plant can be used for the metal monitoring.

Table 21 Zn concentrations (μg/g) in *S. polycystum*, water and sediment in the same and different months

month	holdfast	stem	blade	air bladder	water	sediment
Feb-00	31.8093 [5a] ± 1.014	98.140 [6b] ± 3.652	38.7688 [5c] ± 0.785	108.93 [8d] ± 4.633	0.0163 [1e] ± 0.001	38.2375 [7c] ± 0.632
Mar-00	ns	ns	ns	ns	ns	ns
Apri-00	ns	ns	ns	ns	ns	ns
May-00	ns	ns	ns	ns	ns	ns
June-00	40.1829 [8a] ± 0.854	97.9860 [6a] ± 4.465	49.5746 [7b] ± 1.466	56.272 [7c] ± 1.452	0.5093 [4d5] ± 0.123	27.7460 [6e] ± 0.867
July-00	37.6123 [7a] ± 1.455	101.674 [6b] ± 4.464	48.550 [7c] ± 0.465	48.3233 [6c] ± 0.985	0.6727 [7d] ± 0.758	20.1676 [4e] ± 0.959
Aug-00	28.1925 [4a] ± 0.647	82.2483 [5b] ± 2.314	42.5453 [6c] ± 1.974	41.829 [5c] ± 1.336	0.5905 [6d] ± 0.004	15.2785 [2e] ± 0.859
Sep-00	31.7538 [5a] ± 0.591	60.0875 [4b] ± 0.277	37.475 [5c] ± 0.046	36.8588 [4c] ± 0.057	0.5369 [5d] ± 0.002	13.5395 [1e] ± 0.035
Oct-00	24.8663 [3a] ± 0.090	19.4213 [1b2] ± 0.041	22.2275 [3c] ± 0.048	26.5888 [3d] ± 0.153	0.4576 [4e] ± 0.002	15.3328 [2f] ± 0.034
Nov-00	0.5713 [1a] ± 0.078	16.6055 [1b] ± 2.482	0.5375 [1a] ± 0.033	0.2588 [1a] ± 0.024	0.3123 [3a] ± 0.001	17.6113 [3b] ± 0.031
Dec-00	16.8588 [2a] ± 1.454	22.4663 [2b3] ± 0.869	8.7413 [2c] ± 0.572	8.0938 [2c] ± 0.616	0.1708 [2d] ± 0.003	21.5933 [5b] ± 0.882
Jan-01	33.9675 [a6c] ± 0.485	25.3038 [3b] ± 0.577	30.0875 [4a] ± 3.188	35.3713 [4c] ± 0.974	0.0102 [1d] ± 0.025	32.4625 [a2c] ± 0.044
mean	27.3127 ± 4.061	58.2147 ± 12.496	30.9453 ± 5.761	40.2806 ± 10.473	0.3641 ± 0.083	22.4410 ± 2.859

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

[1] Values in vertical column followed by the same number are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 4 replications.

ns = no sample

Comparing the Zn concentration in all samples in the same month, there are mostly significant differences at the 5% level, except between blade and sediment in February; holdfast and stem in June; blade and air bladder in July, August and September; holdfast, blade, air bladder and water, stem and sediment in November, and so forth (the same letter in the same row, Table 21).

In different months, the metal in all samples is mostly significanly different at the 5% level, except in some samples and some months, such as in February and September in the holdfast; in October and November, in December and January; in February, June and July in the stem; in February and September, and in June and July in the blade; in September and January in the air bladder, and so forth (the same number in the same column, Table 21).

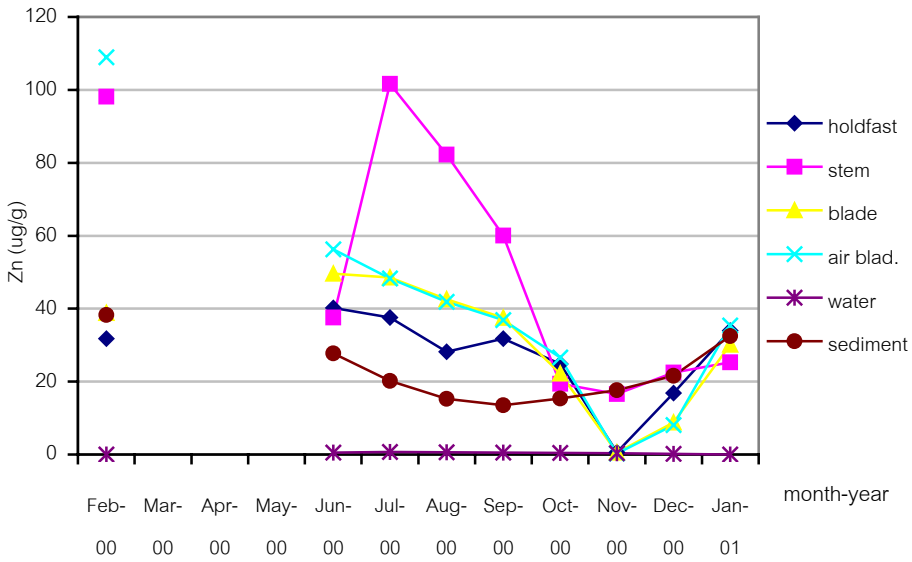

Fig. 20 Zn concentrations (μg/g) in *S. polycystum*, water and sediment

In Figure 20, the graph shows the the metal is most highly accumulated in stem, especially in February and July, and fluctuates in the other parts. After dying and disappearing in March, April and May, the plant grows and highly absorbs the metal. Thus, the graph shows the metal is more highly accumulated in the growing stage than the mature plant. It is clear that zinc is necessary in growing processes. As in the other metals, zinc concentration is very low in the water and quite high in the sediment. It is clear that *S. polycystum* can be used for copper monitoring.

In this study, bioconcentration levels of Cd, Cr, Cu, Pb and Zn in each part of *S. polycystum* range from 49 – 53, from 36 – 56, from 751 – 1676, from 125 – 189, and from 750 - 850 times higher than in the water, respectively. In addition, the seaweed is the best for Cu monitoring, followed by Zn, Pb, Cd and Cr, respectively. Therefore, the seaweed can be used for metal monitoring in oceanic environments since all metals are highly accumulated in each part of the plant and in far higher concentrations than in the seawater.

In this study, *Sargassum polycystum*, as a whole plant, shows higher accumulation than the sediment and far higher than the water collected from near the plant. Comparing each part of the plant, Cd is absorbed into each organ at the same rate. Meanwhile, Cr is highly absorbed by the holdfast, followed by the air bladder, stem and blade, respectively. Pb shows the highest accumulation in the stem, followed by the blade, holdfast and air bladder, respectively. Cu is the most absorbed by the blade, followed by the stem, holdfast and air bladder, respectively. Finally, Zn is the most absorbed via the stem, followed by the air bladder, blade and holdfast, respectively.

Concerning the age of the plant, cadmium, chromium and lead are slowly

absorbed in the growing stage and then highly accumulated in mature plants. Copper and zinc, in contrast, are rapidly absorbed into the plant in the growing season, so they show high accumulation in the plant organs and then are stable or decreased in the mature plant. Tropin and Zolotukhina (1994) reported that both Cu and Zn are important elements in the growth of seaweeds. However, Myklestad, *et al*. (1978) found that Zn, Cd and Pb absorption and accumulatin in *Ascophyllum nodosum* is higher in old parts than the tips of the plant collected from both contaminated and uncontaminated areas. In this study, it cannot be concluded which parts of the plant accumulate the metals more than the others. It depends on the plants and the metals.

Environmental factors should not have a strong effect on absorption since all factors are quite stable in each month, especially pH.

Cadmium concentrations, in this study, in seawater from Phe and Laem Chabang are of the order of 0.0051 – 0.3727 μg/l and 0.0110 – 0.3273 μg/l, respectively. According to Crompton (1997), the metal levels in the oceanic environment ranges from 0.03 – 0.30 μg/l and may exceed 50 μg/l in contaminated areas. It means that the metal level in the water from both sites is not so high. Comparing Cd levels in Table 6, the metal concentration in the seawater is lower than Thailand's standard level (5 μg/l). However, the metal in the water column from Laem Chabang, which should be the critical site, is slightly lower than that of the metal from Phe.

Laem Chabang is the area which has many industries and surrounding human communities. A lot of wastewater is produced and released into the sea. Thus, the water in this area should be full of contamination. Cd, however, from this site is slightly lower than that of Phe since the effluent is maybe treated before leaking into

the sea. In addition, there are many factors effecting the metals dissolved in the water such as pH, chelators, as well as salinity. The salinity at Laem Chabang is lower than that of Phe. Therefore, the metal is more highly dissolved in low salinity (Bryan and Langston, 1992).

Cadmium concentrations in sediments from an UK estuary range from 0.2 μg/g (clean areas) to more than 10 μg/g (contaminated sites, Bryan and Langston, 1992). In this study, the metal level from both localities is of the order of 0.770 to 9.0225 μg/g (Laem Chabang) and 1.7963 to 12.2083 μg/g (Phe). According to Bryan and Langston (1992), it means that the cadmium content from both the study sites reaches the level of contaminated areas. In Thailand, however, there are no standard levels of cadmium concentration in marine sediments.

Some researchers reported that Cd content in seaweed, *Ascophyllum nodosum*, is of the order of 1.20 – 14.3 μg/g (Haug *et al*., 1974). In this study, the metal content in *Enteromorpha clathrata* ranges from 1.4625 – 5.1610 μg/g (Phe) and from 0.5 – 19.1375 μg/g (Laem Chabang). In *P. japonica* and *S. polycystum*, it ranges from 0.2125 – 16.8823 μg/g and from 1.4836 – 23.180 μg/g, respectively. It is noted that the metal levels in each plant cannot be compared with each other. Similarly to the metal content in sediments, there are no standard levels of Cd content in any seaweed.

Total chromium concentrations in the oceanic environment range from 0.06 – 1.26 μg/g (Crompton, 1997). In UK estuarine sediments, the metal levels range from 30 – more than 200 μg/g and may be as high as about 800 μg/g in the area of tinplate industries. The metal contamination mainly comes from anthropogenic activities (Bryan and Langston, 1992). Although some researchers reported the metal concentration in animals such as barnacles, *Balanus* species (Van Weerelt *et al*.,

1984); and polychaetes, *Neanthes arenaceodentata* (Ochida *et al*., 1981), there are, in contrast, no such reports on any seaweed in Thailand.

In this study, Cr concentrations in the seawater from Phe range from 0.0052 – 2.7735 μg/l, and that of the metal from Laem Chabang is of the order of 0.0118 – 2.0700 μg/l. In sediments from Phe and Laem Chabang, it ranges from 0.1781 – 25.3688 μg/g and from 0.5938 – 34.0060 μg/g, respectively. In *E. clathrata* from Phe and Laem Chabang, it ranges from 4.650 – 170.938 μg/g and 3.1042 – 85.9250 μg/g, respectively. It is of the order of 2.50 – 44.7390 in *P. japonica* and 0.2275 – 7.9632 μg/g in *S. polycystum*, respectively. Comparing with Cr levels in Table 6, the metal in seawater is far lower than Thailand's standard level (100 μg/l). However, the metal in the water column from Laem Chabang, which should be critical site, is slightly lower than that of the metal from Phe. Although the metal content in *E. clathrata* from Phe is so high, there are no standard levels of Cr content in marine sediments as well as in the biota. Among the plants, *E. clathrata* shows far more accumulation of the metal than the others, especially the sample from Phe. Thus, it is noted that *E. clathrata* is the best seaweed to use for Cr biomonitoring.

Factors affecting Cr biological availability are temperature, bioavailable form, sediment size as well as living organisms. Sediments are far more important environments than interstitial water and the others. Thus, some researchers suggested that the organisms with sediment dwellings are more contaminated than the others (Aislabie and Loutit, 1986; Bremer and Loutit, 1986). In this study, it is clear that the metal level in the holdfast of *S. polycystum* is higher than that of the other parts and as high as the metal level in the sediments (Table 18).

Total concentrations of copper in oceanic environments range from 0.0063

(Baltic Sea) – 2.80 (Pacific Ocean) μg/l (Crompton, 1997); range from 0.2 – 2.6 μg/l (North Sea, Duinker and Nolting, 1982); range from 2.0 – 3.0 μg/l (Bristol Channel-Severn Estuary), and range from 3.0 – 176 μg/l (Restronguet Creek). In UK estuarine sediments, the metal levels are of the order of 10 (clean areas) – more than 2,000 (contaminated areas) μg/g (Bryan and Langston, 1992). The metal concentrations in brown seaweed, *Ascophyllum nodosum*, range from 4.5 – 111.0 μg/g (Haug *et al*., 1974).

In this study, Cu concentration in the water column from Phe and Laem Chabang is of the order of 0.0029 – 0.1149 μg/l and 0.0107 – 0.1152 μg/l, respectively. It is noted that the metal level is in the range of pristine water. In addition, it is under Thailand's legal standard level (50 μg/l).

The Cu concentrations in *E. clathrata* from Phe and Laem Chabang range from 11.0150 – 285.0 μg/g and from 20.7090 – 72.1880 μg/g, respectively. Meanwhile, the metal levels in the sediments from Phe and Laem Chabang are of the order of 0.0060 – 132.7280 μg/g and 0.6888 – 10.3380 μg/g, respectively. The metal level in the seaweed and sediment from Phe is widely ranging and higher than that of the metal from Laem Chabang. In *P. japonica*, the metal content ranges from 0.3263 – 60.625 μg/g, and it is of the order of 1.3888 – 19.4120 μg/g in *S. polycystum*. Among all seaweeds collected from Phe, the metal is most highly accumulated in *E. clathrata*. Thus, this seaweed can be used for the metal monitoring, whilst, Ho (1987) reported that *Ulva lactuca* is suitable to use for Cu, Zn and Pb monitoring.

There are many factors affecting Cu levels in the environment. Industrial effluent as well as anthropogenic activities can cause increasing in cupric ions (Cu^{2+}) in any habitat. Other factors, including the reductionof the metal ions by the presence

of natural organic chelators and the increase in bioavailability of Cu with decreasing salinity (Wright and Zamuda, 1987). In addition, Luoma *et al*. (1982) suggested that factors relating to accumulation of the metal are oxides of iron and organic components such as humics. In *Fucus vesiculosus*, particles of suspended sediment can effect desorption and accumulation of the metal.

In *S. polycystum*, the Cu is mostly accumulated in the blade, followed by the air bladder, stem and holdfast, respectively (Table 19). The data show that the metal is more highly absorbed from the water column than from the sediments. However, the behavior of absorption and desorption cannot be compared between two plants.

Concentrations of lead in oceanic environments are of the order of 0.001 – 0.014 μg/l. The metal level, however, is varied in each location. Bryan and Langston (1992) reported that the metal content along the East Coast of Britain and in the Humber Estuary ranges from 0.015 – 0.135 μg/l and from 0.010 – 0.055 μg/l, respectively.

In this study, concentrations of Pb from Phe and Laem Chabang are of the order of 0.0536 – 0.4829 μg/l and 0.1501 – 0.7667 μg/l, respectively. Meanwhile, the Office of the National Environmental Board, Ministry of Science, Technology and Environment, allows the metal concentration in marine seawater to be no higher than 50 μg/l (Table 6). This means that the water from both localities is far lower than Thailand's standard level.

In UK estuarine sediments, concentration of Pb ranges from 25 μg/g (pristine areas) to more than 2,700 μg/g (contaminated areas). In this study, the metal level ranges from 0.910 – 34.3145 μg/g and from 0.0232 – 14.715 μg/g in the sediments from Phe and Laem Chabang, respectively. The metal from Phe, which receives waste

from human communities, is higher than the metal from Laem Chabang, receiving wastewater from industrial activities. This means that anthropogenic activities result in higher waste production and drainage into the sea than industrial activities. However, industrial waste has to be treated before release into the marine environment.

In plant samples, Haug *et al*. (1974) reported that lead concentrations in brown seaweed, *Ascophyllum nodosum*, range from 3.0 – 81 μg/g. In this study, the metal contamination in *E*. *clathrata* from Phe and Laem Chabang is of the order of 13.250 – 65.8720 μg/g and 7.6225 – 64.025 μg/g, respectively. In *P*. *japonica*, it ranges from 8.3125 – 100.20 μg/g, and from 2.6934 – 145.5480 μg/g in *S*. *polycystum*, respectively. The study shows that all the seaweeds tested can be used in biomonitoring.

Concentrations of zinc in oceanic environments are less than 1.0 μg/l but may be higher in coastal areas. In some locations, dissolved metal ranges from 0.3 – 70 μg/l in the North Sea, 0.3 – 70.0 μg/l in Bristol Channel-Severn Estuary, and 20 – 2,046 μg/l in the Fal Estuary receiving waste zinc from Restronguet Creek (Bryan and Langston, 1992). The metal contamination in seawater collected from Phe and Laem Chabang is of the order of 0.0055 – 0.6727 μg/l and 0.0178 – 0.6727 μg/l, respectively. According to the Office of the National Environmental Board, Ministry of Science, Technology and Environment, concentrations of the metal allowed to contaminate coastal seawaters are less than 100 μg/l (Table 6). Thus, the metal content in both localities is under Thailand's legal limit.

In sediments collected from UK estuaries, concentrations of Zn range from 100 – 3,000 μg/g in Restronguet. In some areas, such as the sediments in Greece, the

concentrations are of the order of 262 – 396 μg/g near the sediment surface and 67 – 216 μg/g at a depth of 10 cm (Bryan and Langston, 1992). In this study, the metal in the sediment from Phe and Laem Chabang ranges from 10.9740 – 55.981 μg/g and from 13.5395 – 94.475 μg/g, respectively. Lyngby and Brix (1987), working on the vascular plants, *Zostera marina*, found that the bioavailability of the metal comes from the sediments. The metal is far more highly accumulated in root and rhizome, and translocation of the metal between tissues was quite low. In the brown seaweed, *S. polycystum*, the metal concentration in order of decreasing is stem > air bladder > blade > holdfast.

In the brown seaweed, *Ascophyllum nodosum*, concentrations of Zn are of the order of 315 – 3,220 μg/g. In this study, the accumulation of the metal in *E. clathrata* from Phe and Laem Chabang ranges from 32.209 – 396.375 μg/g and from 68.813 – 435.075 μg/g, respectively. It ranges from 28.416 – 185.550 μg/g in *P. japonica*, and ranges from 0.2588 – 108.930 μg/g in *S. polycystum*, respectively. The study shows that all the seaweeds are suitable for use in biomonitoring, especially *E. clathrata*.

4.2 Effects of metals on seaweed

4.2.1 Preliminary study and effects of metals on seaweed

For preliminary study, *Sargassum polycystum* the common macroalgae in the sea of Phe, Muang District, Rayong Province, was removed from natural habitats to grow in a big tank (1000 l) under natural condition and aeration for 10 days. Meanwhile, the water from the tank, pumped from the sea of Phe, was determined for Cd, Cr, Cu, Pb and Zn levels. The healthy plants were selected and grown in 30 l aquaria in the seawater under aeration, 50% light filtration and other natural

conditions. Based on the concentration of each metal in the big tank, the single metal was added into the aquarium at 5 different concentrations. After 0, 2, 4, 6 and 8 days, the blades were collected and carried to the laboratory under refrigeration in order to determine the amount of chlorophyll *a* and chlorophyll *c*, protein content, lipid fraction and fatty acid profile. The experiments were conducted at the Eastern Marine Fisheries Research and Development Center, Phe Subdistrict, Muang District, Rayong Province, between July, 2002 to September, 2002.

Effect of cadmium

Cadmium concentration in the water was determined to be 0.003 mg/l. Therefore, the metal added into each aquarium was 0.006, 0.03, 0.09, 0.15 and 0.3 mg/l, respectively (2, 10, 30, 50 and 100 times, adapted from Jana and Choudhuri, 1994).

Chlorophyll *a* and chlorophyll *c* concentrations

After treatment with cadmium at various concentrations, chlorophyll *a* and chlorophyll *c* concentrations were detemined based on Jeffrey and Jumphrey's equations (Geider and Osborne, 1992) for 90% acetone extracts. All chlorophyll concentrations are given in units of $\mu g.cm^{-3}$ and shown in Table 22.

Table 22 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Cd

Cd conc. (mg/l)	Chlorophyll *a* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Cd0 =	1.2212	0.9745	1.2872	1.3170	1.3203^{fg} ±0.0
Cd1 =	1.2212	1.2688	1.3999^{ijk} ±0.0	1.4336^{jklm} ±0.	1.2315^{bcd} ±0.
Cd2 = 0.03	1.2212	1.2095^{bi} ±0.0	1.3911	1.4039	1.4072^{ijkl} ±0.
Cd3 = 0.09	1.2212	1.4038^{ijkl} ±0.0	1.3265	1.2652^{cde} ±0.	1.3099^{fg} ±0.0
Cd4 = 0.15	1.2212	1.3459^{gh} ±0.0	1.4387^{klm} ±0.	1.4443^{lm} ±0.0	1.4973
Cd5 = 0.3	1.2212	1.4950	1.3737^{ijk} ±0.0	1.396^{hi} ±0.07	1.4612^{mn} ±0.
Cd conc. (mg/l)	Chlorophyll *c* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Cd0 =	0.4723^{bc} ±0.0	0.3991	0.4343^{ab} ±0.0	0.589^{fg} ±0.07	0.4865
Cd1 =	0.4723^{bc} ±0.0	0.4683^{b} ±0.0	0.5799^{efg} ±0.	0.7267^{jkv} ±0.0	0.4192
Cd2 = 0.03	0.4723^{bc} ±0.0	0.4390^{ab} ±0.0	0.5701	0.6210^{ghi} ±0.	0.6310^{hi} ±0.0
Cd3 = 0.09	0.4723^{bc} ±0.0	0.5926	0.4991^{cd} ±0.0	0.5032^{cd} ±0.0	0.5081^{cd} ±0.0
Cd4 = 0.15	0.4723^{bc} ±0.0	0.5388^{de} ±0.0	0.6545^{i} ±0.00	0.6982^{j} ±0.00	0.8191^{m} ±0.0
Cd5 = 0.3	0.4723^{bc} ±0.0	0.7689^{lm} ±0.0	0.5512	0.7531	0.7460^{kl} ±0.0

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications.

The letters are arranged in ascendant order as in the values.

Cd_0 = control

In Table 22, concentrations of chlorophyll *a* and chlorophyll *c* range from 0.9745 (Cd_0, day 2) to 1.4973 $\mu g.cm^{-3}$ (Cd 4, day 8) and from 0.3991 (Cd_0, day 2) to 0.8191 $\mu g.cm^{-3}$ (Cd 4, day 8), respectively. Chlorophyll *a* and chlorophyll *c* concentrations in each

treatment are mostly significantly different at the 5% level. The data show that both chlorophyll *a* and chlorophyll *c* concentrations are increased with increased time and with increase in the metal concentration. It is clear that cadmium can activate *S. polycystum* to produce chlorophyll *a* and chlorophyll *c* in its cells.

Protein content and lipid fraction

After treatment with cadmium at various concentrations, the blades of the plant were collected and dried in an oven at 50 °C until their weights no longer changed. After grilling with a mill, samples were collected in a dessicated condition. Some samples (1 g) were digested and analysed by Kjeldahl's method. Total protein content was determined in percentage of total nitrogen as described in chapter III.

Some samples (1 g) were extracted twice by $CHCl_3$ – MeOH (1:1 v/v). After evaporation of the extraction media, the lipid fraction was recorded in g/g dry weight. The lipid fractions were saponified and methylated and then analyzed for major fatty acids. Each fatty acid was determined and recorded in percentage as described in chapter III.

Total nitrogen content is of the order of 0.0657 (Cd2, day 6) to 0.2604% per gram dry weight (Cd2, day 4). The data show that protein content is decreased as the time and cadmium concentration increase (Table 23). It is clear that protein content is decreased by cadmium concentration.

The lipid fraction ranges from 0.0258 (Cd5, day 8) to 0.1176 g/g dw (Cd 0, day 6). In Table 23, the data show that cadmium concentrations have no effect on lipid synthesis in the plants. Unfortunately, there were not sufficient samples. Thus, the fatty acid profiles was not determined.

Table 23 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Cd

Cd conc. (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Cd0 = 0.003	0.2301 ^{hi} ±0.022	0.2302 ^{hi} ±0.039	0.1662 ^{efg} ±0.011	0.1401 ^{bcdef} ±0.013	0.1391 ^{bcdef} ±0.011
Cd1 = 0.006	0.2301 ^{hi} ±0.022	0.1732 ^{efg} ±0.022	0.1322 ^{bcdef} ±0.022	0.1041 ^{abc} ±0.011	0.1341 ^{bcdef} ±0.000
Cd2 = 0.03	0.2301 ^{hi} ±0.022	0.1512 ^{cdefg} ±0.031	0.2604 ^{i} ±0.018	0.0657 ^{a} ±0.000	0.1561 ^{defg} ±0.048
Cd3 = 0.09	0.2301 ^{hi} ±0.022	0.1932 ^{gh} ±0.009	0.1922 ^{gh} ±0.009	0.1091 ^{abcd} ±0.035	0.1201 ^{bcd} ±0.004
Cd4 = 0.15	0.2301 ^{hi} ±0.022	0.1762 ^{fg} ±0.028	0.2332 ^{hi} ±0.042	0.0991 ^{ab} ±0.016	0.1261 ^{bcde} ±0.002
Cd5 = 0.3	0.2301 ^{hi} ±0.022	0.1792 ^{fg} ±0.000	0.1545 ^{defg} ±0.123	0.1681 ^{bcd} ±0.000	0.1121 ^{abcd} ±0.004
Cd conc. (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Cd0 = 0.003	0.0718 ^{l} ±0.000	0.0560 ^{g} ±0.001	0.0836 ^{n} ±0.000	0.1175 ^{q} ±0.001	0.0920 ^{o} ±0.001
Cd1 = 0.006	0.0718 ^{l} ±0.000	0.0808 ^{m} ±0.000	0.1027 ^{p} ±0.001	0.0635 ^{i} ±0.001	0.0304 ^{b} ±0.000
Cd2 = 0.03	0.0718 ^{l} ±0.000	0.0624 ^{hi} ±0.000	0.0303 ^{b} ±0.000	0.0523 ^{f} ±0.000	0.0694 ^{k} ±0.000
Cd3 = 0.09	0.0718 ^{l} ±0.000	0.0663 ^{j} ±0.000	0.0328 ^{c} ±0.000	0.0625 ^{hi} ±0.001	0.0516 ^{ef} ±0.000
Cd4 = 0.15	0.0718 ^{l} ±0.000	0.0507 ^{e} ±0.001	0.0673 ^{j} ±0.000	0.0825 ^{n} ±0.000	0.0614 ^{h} ±0.000
Cd5 = 0.3	0.0718 ^{l} ±0.000	0.0407 ^{d} ±0.000	0.0558 ^{g} ±0.000	0.0692 ^{k} ±0.000	0.0258 ^{a} ±0.000

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values. Cd_0 = control.

Effect of chromium

Chromium concentration in the water was 0.0003 mg/l. Therefore, the metal added into each aquarium was 0.0006, 0.003, 0.009, 0.015 and 0.03 mg/l, respectively (2, 10, 30, 50 and 100 times, adapted from Jana and Choudhuri, 1994).

Chlorophyll *a* and chlorophyll *c* concentrations

Chlorophyll *a* and chlorophyll *c* concentrations in the blade of *S. polycystum* treated with Cr are shown in Table 24.

Table 24 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Cr

Cr conc. (mg/l)	Chlorophyll *a* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Cr0 =	1.5000	1.3383	1.5136	1.4925	1.5358
Cr1 =	1.5000	1.5036	1.3892	1.3567	1.2879
Cr2 = 0.003	1.5000	1.5790	1.4913	1.4324	1.3734
Cr3 = 0.009	1.5000	1.4113	1.3992	1.4827^{m}±0.0	1.5799
Cr4 = 0.015	1.5000	1.5434	1.1093	1.2309	1.5062
Cr5 = 0.03	1.5000	1.6236	1.5552	1.3437	1.2607
Cr conc. (mg/l)	Chlorophyll *c* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Cr0 =	0.8303	0.5182	0.8503	0.8735	0.8824
Cr1 =	0.8303	0.8388	0.6497	0. 5580	0.5579
Cr2 = 0.003	0.8303	1.5601	0.8336	0.6955	0.5573
Cr3 = 0.009	0.8303	0.6229	0.6462	0.7631^{m}±0.0	0.9650
Cr4 = 0.015	0.8303	1.0871	0.4279	0.6019	0.5831
Cr5 = 0.03	0.8303	1.2958	0.9631	0.8075	0.5272^{c}

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values. Cr_0 = control.

In Table 24, chlorophyll concentrations are of the order of 1.1093 (Cr_4, day 4) to 1.6236 $\mu g.cm^{-3}$ (Cr_5, day 2) and 0.4279 (Cr_4, day 4) to 1.5601 $\mu g.cm^{-3}$ (Cr_2, day 2) in chlorophyll *a* and chlorophyll *c*, respectively. The data show that both chlorophylls are increased

with increases time as in Cr0 but the chlorophylls are decreased with increased time and increased the metal concentrations.

Protein content and lipid fraction

Protein content and lipid fraction in the blade of the plant treated with Cr are shown in Table 25.

Total nitrogen content ranges from 0.1532 (Cr3, day 6) to 0.4091% per gram dry weight (Cr0, day 8). The data show that time and chromium concentrations has minor effect on protein synthesis in *S. polycystum*, since protein content in each treatment is mostly not significantly different at the 5% level (Table 25).

In Table 25, the lipid fraction ranges from 0.0260 (Cr5, day 8) to 0.1179 g/g dw (Cr2, day 2). Lipid in all treatments is significantly different at the 5% level. The data show that lipid content in the plant cells increase as the time increases (Cr0), but it is decreased when treatement with chromium. The metal concentration corelated with the highest level of lipid synthesis is approximately 0.003 mg/l at day 2 (Cr0, day 2).

Fatty acid profile

In Table 26, the major fatty acid found is C16:0, and far followed by C20:4(n-6) and C18:1(n-9)c, respectively. The data show that the amount of palmitic acid will be decreased if the time and chromium concentration is increased. The highest palmitic acid proportion is 39.0368% occurring in Cr0, day 4 with palmitic acid slightly fluctuating in the rest of the experiments.

Common fatty acids found in this study are C14:0 (myristic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:1(n-9)c (oleic acid), C18:2(n-6)c (linoleic acid), C18:3(n-3) (linolenic acid), C20:2 (cis-11, 14-eicosadienoic acid), and

C20:4(n-6) (Arachidonic acid).

Table 25 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Cr

Cr conc. (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Cr0 = 0.0003	0.2494 abcdef ±0.004	0.4047 g ±0.046	0.3806 fg ±0.022	0.3391 cdefg ±0.002	0.4091 g ±0.002
Cr1 = 0.0006	0.2494 abcdef ±0.004	0.3238 bcdefg ±0.066	0.2035 abc ±0.028	0.2866 abcdefg	0.3566 defg ±0.152
Cr2 = 0.003	0.2494 abcdef ±0.004	0.3719 efg ±0.000	0.2363 abcdef ±0.004	0.3325 cdefg ±0.022	0.4040 g ±0.022
Cr3 = 0.009	0.2494 abcdef ±0.004	0.2013 abc ±0.035	0.2275 abcde ±0.004	0.1532 a ±0.000	0.2232 abcde ±0.000
Cr4 = 0.015	0.2494 abcdef ±0.004	0.2844 abcdefg	0.1728 ab ±0.002	0.2983 abcdefg	0.3653 efg ±0.011
Cr5 = 0.03	0.2494 abcdef ±0.004	0.2713 abcefg ±0.008	0.2078 abcd ±0.028	0.1789 ab ±0.004	0.2494 abcdef ±0.004
Cr conc. (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Cr0 = 0.0003	0.0719 j ±0.00	0.0581 f ±0.00	0.0640 h ±0.00	ns	0.0825 l
Cr1 = 0.0006	0.0719 j ±0.00	0.0841 m	0.0308 b ±0.00	ns	0.0614 g ±0.00
Cr2 = 0.003	0.0719 j ±0.00	0.1179 p ±0.00	0.0620 g ±0.00	ns	0.0407 c ±0.00
Cr3 = 0.009	0.0719 j ±0.00	0.0923 n ±0.00	0.0306 b ±0.00	ns	0.0558 e
Cr4 = 0.015	0.0719 j ±0.00	0.0802 k ±0.00	0.0526 d ±0.00	ns	0.0692 i
Cr5 = 0.03	0.0719 j ±0.00	0.1032 o ±0.00	0.0697 j ±0.00	ns	0.0260 a ±0.00

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

Cr_0 = control ns = no samples

Table 26 Major fatty acids (%) found in the blade of *S. polycystum* treated with Cr

Cr no./day	C14:0	C16:0	C16:1	C18:1(n-9)c	C18:2(n-6)c	C18:3(n-3)	C20:2	C20:4(n-6)	Sum-Sat.	Sum-Unsat.	Others	C20:5(n-3)
0 - 5. 0 day	4.8922	33.8334	4.1746	13.6428	5.7856	6.0164	6.5121	15.3286	38.7257	51.4601	9.8143	
0 - 2 day	4.7009	31.8793	4.8599	13.3738	5.8968	6.6440	7.5199	15.1134	36.5802	53.4077	10.0121	
1 - 2 day	4.0163	26.9020	4.0922	11.3640	7.6296	7.3947	10.4559	17.0591	30.9183	57.9955	11.0862	5.0586
2 - 2 day	4.5953	32.4560	4.1024	13.0773	6.2120	6.4130	7.6834	15.4131	37.0513	52.9011	10.0476	
3 - 2 day	4.2910	30.5220	3.5904	12.6060	6.6616	6.6367	7.3244	17.5707	34.8131	54.3897	10.7972	
4 - 2 day	4.3820	29.8643	3.7553	13.3637	5.7265	6.5414	8.8980	17.1638	34.2463	55.4488	10.3048	4.1076
5 - 2 day	4.3320	28.8409	4.0732	11.8530	6.2933	7.4263	8.7938	17.3940	33.1729	55.8335	10.9936	4.2696
0 - 4 day	5.2997	39.0368	5.6693	0.0000	7.0967	6.5214	6.4961	18.9887	44.3365	44.7723	10.8913	
1 - 4 day	4.3923	33.9399	4.4002	13.8398	6.9279	6.1636	6.7922	18.4142	38.3322	56.5378	5.1299	
2 - 4 day	4.3691	33.7697	4.5585	13.2615	6.3344	5.8298	6.4391	16.5812	38.1388	53.0044	8.8568	
3 - 4 day	4.4705	31.2698	3.8580	12.0458	7.0085	6.5729	7.4229	16.6612	35.7403	53.5694	10.6903	
4 - 4 day	4.2225	29.9654	4.0118	12.3526	6.0832	7.0171	7.4014	18.7430	34.1879	55.6092	10.2029	
5 - 4 day	4.0086	29.5131	4.3708	11.5136	5.7673	6.5233	9.5066	16.7592	33.5217	54.4409	12.0374	5.4618
0 - 6 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
1 - 6 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
2 - 6 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
3 - 6 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
4 - 6 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
5 - 6 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
0 - 8 day	5.2148	37.1959	4.7239	14.9255	4.8876	4.8203	4.3414	15.1314	42.4106	48.8301	8.7592	
1 - 8 day	4.0909	32.6354	3.8286	14.1548	7.1373	4.9204	5.6899	17.5027	36.7263	53.2338	10.0399	
2 - 8 day	4.4986	33.6757	4.2007	12.7947	5.3683	6.0981	6.2324	16.0063	38.1743	50.7005	11.1252	
3 - 8 day	4.1235	35.7095	4.6985	14.2184	5.4238	4.8910	4.6853	16.7142	39.8330	50.6313	9.5357	
4 - 8 day	4.4067	34.7667	4.1770	15.0554	5.4887	5.6519	5.4387	15.3158	39.1734	51.1276	9.6990	
5 - 8 day	4.1462	34.6514	4.8247	14.1963	6.2074	4.7279	5.2868	17.6080	38.7976	52.8510	8.3514	

Effect of copper

Copper concentration in the water was 0.0015 mg/l. Thus, the metal added into each aquarium was 0.003, 0.015, 0.045, 0.075 and 0.15 mg/l, respectively (2, 10, 30, 50 and 100 times, adapted from Jana and Choudhuri, 1994).

Chlorophyll *a* and chlorophyll *c* concentrations

Concentrations of chlorophyll *a* and chlorophyll *c* in *S. polycystum* treated with copper at various concentrations are shown in Table 27.

Table 27 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Cu

Cu conc. (mg/l)	Chlorophyll *a* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Cu0 = 0.0015	0.5405 [a]±0.00	1.1937 [f]±0.00	1.2073 [h]±0.00	1.2128 [i]±0.00	1.1319 [d]±0.00
Cu1 = 0.003	0.5405 [a]±0.00	1.3629 [q]±0.00	1.2696 [l]±0.00	1.2434 [j]±0.00	1.3368 [p]±0.00
Cu2 = 0.015	0.5405 [a]±0.00	1.1915 [e]±0.00	1.2629 [k]±0.00	1.4454 [r]±0.00	1.3188 [o]±0.00
Cu3 = 0.045	0.5405 [a]±0.00	1.2066 [g]±0.00	1.2769 [n]±0.00	dead	dead
Cu4 = 0.075	0.5405 [a]±0.00	1.2752[m]±0.00	1.4543 [s]±0.00	dead	dead
Cu5 = 0.15	0.5405 [a]±0.00	0.7589 [b]±0.00	0.9849 [c]±0.00	dead	dead
Cu conc. (mg/l)	Chlorophyll *c* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Cu0 = 0.0015	0.1586 [a]±0.00	0.4320 [c]±0.00	0.4489 [d]±0.00	0.5198 [g]±0.00	0.4824 [e]±0.00
Cu1 = 0.003	0.1586 [a]±0.00	0.7441 [r]±0.00	0.5660 [k]±0.00	0.5304 [h]±0.00	0.6435 [o]±0.00
Cu2 = 0.015	0.1586 [a]±0.00	0.5471 [j]±0.00	0.4870 [f]±0.00	0.7623 [s]±0.00	0.5956
Cu3 = 0.045	0.1586 [a]±0.00	0.4278 [b]±0.00	0.6303 [n]±0.00	dead	dead
Cu4 = 0.075	0.1586 [a]±0.00	0.5438 [i]±0.00	0.5726 [i]±0.00	dead	dead
Cu5 = 0.15	0.1586 [a]±0.00	0.6970 [q]±0.00	0.6726 [p]±0.00	dead	dead

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values. Cu_0 = control

In Table 27, concentrations of chlorophyll *a* and chlorophyll *c* range from 0.5405 (Cu0) to 1.4543 $\mu g.cm^{-3}$ (Cu4, day 4) and 0.1586 (Cu0) to 0.7623 $\mu g.cm^{-3}$ (Cu2, day 6), respectively. The data show that time and copper concentrations affect both chlorophyll *a* and chlorophyll *c* synthesis. The metal concentration at approximately 0.003 to 0.015 mg/l and the 6th days of experiment is associated with the most activated chlorophyll synthesis in the plant cells. However, the plants were killed if the metal concentrations are higher than 0.045 mg/l in the 6th and the 8th days of experiments.

Protein content and lipid fraction

Crude protein content and lipid fraction in the blade of the plant treated with Cu are shown in Table 28.

In Table 28, total nitrogen content ranges from 0.1422 (Cu4, day 4) to 0.3260% per gram dry weight (Cu0). The data show that protein content is mostly not significantly different at the 5% level. Crude protein is decreased in the plant cells when treated with copper. The cells were killed within the 6th day when the metal concentration is higher than 0.045 mg/l.

The lipid fraction ranges from 0.0411 (Cu5, day 4) to 0.0910 g/g dw (Cu1, day 8). The data show that the lipid fraction in all treatments is mostly not significantly different at the 5% level. It is clear that copper concentrations do not have minor effect on lipid synthesis, though the plants were killed at the 6th day of experiment when the metal concentration is higher than 0.045 mg/l (Table 28).

Table 28 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Cu

Cu conc. (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Cu0 = 0.0015	0.3259 ^{e} ±0.006	0.3259 ^{e} ±0.015	0.3238 ^{e} ±0.009	0.2975 ^{cde} ±0.004	0.2735 ^{bcde} ±0.011
Cu1 = 0.003	0.3259 ^{e} ±0.006	0.22970 ^{bc} ±0.035	0.2319 ^{bcd} ±0.021	0.2560 ^{bcde} ±0.022	0.2341 ^{bcd} ±0.006
Cu2 = 0.015	0.3259 ^{e} ±0.006	0.2472 ^{bcde} ±0.024	0.2035 ^{ab} ±0.024	0.3150 ^{de} ±0.044	0.2210 ^{abc} ±0.012
Cu3 = 0.045	0.3259 ^{e} ±0.006	0.2385 ^{bcd} ±0.011	0.3260 ^{e} ±0.101	dead	dead
Cu4 = 0.075	0.3259 ^{e} ±0.006	0.2472 ^{bcde} ±0.004	0.1422 ^{a} ±0.000	dead	dead
Cu5 = 0.15	0.3259 ^{e} ±0.006	0.1925 ^{ab} ±0.000	0.2122 ^{ab} ±0.011	dead	dead
Cu conc. (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Cu0 = 0.0015	0.044 ^{a} ±0.000	0.0481 ^{ab} ±0.004	0.0725 ^{ab} ±0.015	0.0530 ^{ab} ±0.000	0.0729 ^{ab} ±0.000
Cu1 = 0.003	0.044 ^{a} ±0.000	0.0538 ^{ab} ±0.013	0.0729 ^{ab} ±0.026	0.0714 ^{ab} ±0.015	0.0910 ^{b} ±0.170
Cu2 = 0.015	0.044 ^{a} ±0.000	0.0515 ^{ab} ±0.006	0.0732 ^{ab} ±0.015	0.0642 ^{ab} ±0.003	0.0624 ^{ab} ±0.005
Cu3 = 0.045	0.044 ^{a} ±0.000	0.0583 ^{ab} ±0.005	0.0831 ^{ab} ±0.002	dead	dead
Cu4 = 0.075	0.044 ^{a} ±0.000	0.0453 ^{a} ±0.000	0.0884 ^{b} ±0.000	dead	dead
Cu5 = 0.15	0.044 ^{a} ±0.000	0.0694 ^{ab} ±0.008	0.0411 ^{a} ±0.000	dead	dead

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

Cu_0 = control.

Effect of lead

The lead content in the seawater was 0.0045 mg/l. Thus, the metal added into each aquarium was 0.009, 0.045, 0.135, 0.225 and 0.45 mg/l, respectively (2, 10, 30, 50 and 100 times, adapted from Jana and Choudhuri, 1994).

Chlorophyll *a* and chlorophyll *c* concentrations

Chlorophyll *a* and chlorophyll *c* concentrations when treated with Pb at various concentrations are shown in Table 29.

Table 29 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Pb

Pb conc. (mg/l)	Chlorophyll *a* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Pb0 = 0.0045	1.5006	1.3692 ^{h}±0.00	1.4437 ^{q}±0.00	1.3751 ^{j}±0.00	1.2670 ^{a}±0.00
Pb1 = 0.009	1.5006	1.2923 ^{b}±0.00	1.3703 ^{i}±0.00	1.3549 ^{f}±0.00	1.3328 ^{d}±0.00
Pb2 = 0.045	1.5006	1.4503 ^{s}±0.00	1.3456 ^{l}±0.00	1.1386^{hi}±0.00	1.3615 ^{g}±0.00
Pb3 = 0.135	1.5006	1.4354 ^{o}±0.00	1.4481 ^{r}±0.00	1.4891 ^{v}±0.00	1.5105 ^{x}±0.00
Pb4 = 0.225	1.5006	1.4050 ^{n}±0.00	1.4375 ^{p}±0.00	1.4049^{m}±0.00	1.3853 ^{k}±0.00
Pb5 = 0.45	1.5006	1.3104 ^{c}±0.00	1.4681 ^{t}±0.00	1.4711 ^{u}±0.00	1.5255 ^{y}±0.00
Pb conc. (mg/l)	Chlorophyll *c* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Pb0 = 0.0045	0.8011 ^{i}±0.00	0.5457 ^{g}±0.00	0.6669^{cd}±0.00	0.5680^{de}±0.00	0.5397^{bcd}±0.0
Pb1 = 0.009	0.8011 ^{i}±0.00	0.4678 ^{a}±0.00	0.5488^{cd}±0.00	0.5504^{cd}±0.00	0.5002^{ab}±0.00
Pb2 = 0.045	0.8011 ^{i}±0.00	0.6640 ^{g}±0.00	0.5231^{bc}±0.00	0.5528	0.5267^{bcd}±0.0
Pb3 = 0.135	0.8011 ^{i}±0.00	0.6473 ^{g}±0.00	0.6752 ^{g}±0.00	0.7478 ^{h}±0.00	0.7515 ^{h}±0.00
Pb4 = 0.225	0.8011 ^{i}±0.00	0.6040^{ef}±0.00	0.7342^{h}±0.08	0.6323 ^{fg}±0.00	0.6762^{g}±0.00
Pb5 = 0.45	0.8011 ^{i}±0.00	0.4982^{ab}±0.00	0.7653^{hi}±0.00	0.7343 ^{h}±0.00	0.6742^{g}±0.00

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values. Pb_0 = control.

In Table 29, the chlorophyll concentrations are of the order of 1.2670 (Pb0, day 8) to 1.5255 μg.cm^{-3} (Pb5, day 8) and 0.4678 (Pb1, day 2) to 0.8011 μg.cm^{-3} (Pb0) in chlorophyll *a* and chlorophyll *c*, respectively. The chlorophyll concentration is mostly significantly different at the 5% level. The data show that synthesis of both chlorophyll *a* and chlorophyll *c* can be reduced by the lead concentrations.

Protein content and lipid fraction

Total nitrogen content and lipid fraction in the blade of the plant treated with Pb are shown in Table 30.

In Table 30, total nitrogen content ranges from 0.1313 (Pb3, Pb4, day 4) to 0.2866% per gram dry weight (Pb2, day 8). Total nitrogen content is mostly not significantly different at the 5% level. The data show that synthesis of protein in the seaweed cells can be reduced by the time and the metal concentrations.

The lipid fraction is of the order of 0.0311 (Pb4, day 6) to 0.0915 g/g dw (Pb4, day 6). The data show that the lipid fraction in each treatment is significantly different at the 5% level. In addition, lead concentrations from 0.0045 to 0.135 mg/l can increase lipid synthesis in the plant cells. Synthesis of the lipid in the cells can be reduced if the metal concentrations are more than 0.225 mg/l (Table 30).

Fatty acid profile

After treatment with lead at various concentrations, the blades of *S. polycystum* were collected and determined for fatty acid profile. Major fatty acids are shown in Table 31.

In Table 31, an outstanding fatty acid found is C16:0, and far followed by C20:4(n-6) and C18:1(n-9)c, respectively. The data show that palmitic acid is slightly

increased with increased time and the metal concentrations. The highest palmitic acid proportion is 40.6494% in Pb4, day 6. However, there were not sufficient samples in 8th day of experiments.

Table 30 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Pb

Pb conc. (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Pb0 = 0.0045	0.2800 fg ±0.018	0.2713 efg ±0.008	0.2385 bcdefg ±0.007	0.2035 bc de	0.2385 bcdefg ±0.007
Pb1 = 0.009	0.2800 fg ±0.018	0.2144 bcdef ±0.018	0.1969 abcd ±0.018	0.245 bcde fg	0.2756 fg ±0.004
Pb2 = 0.045	0.2800 fg ±0.018	0.1925 abcd ±0.017	0.1903 abc ±0.008	0.252 cdef g	0.2866 g ±0.020
Pb3 = 0.135	0.2800 fg ±0.018	0.1969 abcd ±0.020	0.1313 a ±0.018	0.1794 ab ±0.044	0.2144 bcdef ±0.030
Pb4 = 0.225	0.2800 fg ±0.018	0.1969 abcd ±0.018	0.1313 a ±0.045	0.1794 ab ±0.030	0.2144 bcdef ±0.030
Pb5 = 0.45	0.2800 fg ±0.018	0.1969 abcd ±0.018	0.1969 abcd ±0.004	0.225 bcde fg ±0.002	0.2633 defg ±0.002
Pb conc. (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Pb0 =	0.0444	0.0442	0.0614 i ±0.00	0.0581	ns
Pb1 = 0.009	0.0444	0.0393	0.1122	0.0732	ns
Pb2 = 0.045	0.0444	0.0513 f ±0.00	0.0669 j ±0.00	0.0532	ns
Pb3 = 0.135	0.0444	0.0584	0.0677 j ±0.00	0.0734	ns
Pb4 = 0.225	0.0444	0.0439	0.0915	0.0311	ns
Pb5 = 0.45	0.0444	0.0431	0.0366	0.0314	ns

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

ns = no sample Pb0 = control

Table 31 Major fatty acids (%) found in the blade of *S. polycystum* treated with Pb

Pb no./day	C14:0	C16:0	C16:1	C18:1(n-9)c	C18:2(n-6)c	C18:3(n-3)	C20:2	C20:4(n-6)	Sum-Sat.	Sum-Unsat.	Others	remark
0 - 5. 0 day	4.5785	35.4201	5.5146	14.9010	7.8859	5.0926	5.5264	13.3251	39.9986	52.2456	7.7558	
0 - 2 day	4.6274	37.9199	5.2675	12.4485	7.4466	4.8076	3.9947	14.4737	42.5473	48.4386	9.0141	
1 - 2 day	5.3511	37.4249	5.0086	13.9181	7.5531	4.7336	4.7093	13.6840	42.7760	49.6066	7.6174	
2 - 2 day	5.3725	37.3864	5.4164	13.4070	6.7975	4.0620	4.5123	13.6531	42.7589	47.8484	9.3927	
3 - 2 day	5.1365	34.6152	4.8563	13.3894	6.6815	5.0571	5.0130	15.2250	39.7517	50.2222	10.0260	
4 - 2 day	6.7231	45.5726	4.6419	13.1977	4.6977	3.1846	2.7915	9.9238	52.2957	38.4372	9.2671	
5 - 2 day	5.8824	39.0661	5.0510	14.3116	5.9070	4.0877	3.8765	12.5363	44.9485	45.7702	9.2813	
0 - 4 day	4.8473	35.9492	5.1668	13.3669	6.0304	5.4260	4.9064	14.3374	40.7965	49.2339	9.9695	
1 - 4 day	4.8327	36.3806	5.2063	14.2023	6.5838	4.2531	4.5184	14.0872	41.2133	48.8511	9.9357	
2 - 4 day	4.9777	37.6563	5.3939	14.0936	6.4456	4.4394	4.0700	13.6806	42.6340	48.1231	9.2429	
3 - 4 day	4.7598	33.8419	5.0856	12.6809	6.2674	5.0004	5.7652	16.3168	38.6017	51.1163	10.2820	
4 - 4 day	4.7566	35.6576	4.8552	13.8205	6.7846	4.0357	4.4516	15.4553	40.4142	49.4028	10.1830	
5 - 4 day	4.8207	39.0235	5.2095	14.4784	6.6895	3.8562	3.6906	14.6227	43.8442	48.5468	7.6090	
0 - 6 day	4.8505	39.0501	5.6898	14.9789	6.2304	4.3981	3.7070	13.1815	43.9006	48.1856	7.9138	
1 - 6 day	4.7982	36.7196	4.7418	14.4846	8.9353	4.3680	4.0285	12.7107	41.5179	49.2688	9.2133	
2 - 6 day	5.1256	37.0443	6.4691	14.6221	6.4144	3.9590	3.7674	13.4183	42.1699	48.6503	9.1798	
3 - 6 day	4.7975	37.0099	5.0306	14.2928	6.8328	4.6841	4.1884	13.9445	41.8074	48.9733	9.2193	
4 - 6 day	4.8712	40.6494	6.9288	14.7625	6.8157	4.2387	3.6576	14.9068	45.5206	51.3101	3.1693	
5 - 6 day	4.2923	39.1657	6.0965	14.6710	6.1234	3.8137	3.1584	14.8158	43.4580	48.6790	7.8630	
0 - 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
1 - 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
2 - 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
3 - 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
4 - 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
5 - 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	

Effect of zinc

The zinc concentration in the water was 0.0022 mg/l. Thus, the metal added into each aquarium was 0.0044, 0.0220, 0.0660, 0.110 and 0.220 mg/l, respectively. (2, 10, 30, 50 and 100 times, adapted from Jana and Choudhuri, 1994).

Chlorophyll *a* and chlorophyll *c* concentrations

Concentrations of chlorophyll *a* and chlorophyll *c* when treated with zinc at various concentrations are shown in Table 32.

Table 32 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Zn

Zn conc. (mg/l)	Chlorophyll *a* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Zn0 =	1.3586 ^{j}	1.4004	1.3910	1.3813	1.5226^{w} ±0.0
Zn1 =	1.3586 ^{j}	1.2683	1.2925	1.4352	1.5233
Zn2 =	1.3586 ^{j}	1.3523	1.4086	1.3660	1.3910
Zn3 =	1.3586 ^{j}	1.2178	1.4023	1.4028	1.4196
Zn4 =	1.3586 ^{j}	1.4922	1.4386	1.2530	1.1560
Zn5 =	1.3869 ^{j}	1.1208	1.3826^{m} ±0.0	1.2448	1.2341^{d}
Zn conc. (mg/l)	Chlorophyll *c* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Zn0 =	0.7590	0.5913	0.6114	0.6060	0.8969
Zn1 =	0.7590	0.4732	0.5259	0.6386	0.8894
Zn2 =	0.7590	0.5367	0.6468	0.6025	0.6356^{m} ±0.0
Zn3 =	0.7590	0.4872	0.6380	0.6463	0.6711
Zn4 =	0.7590	0.7793	0.6959	0.5217	0.4699
Zn5 =	0.7590	1.5113	0.6359	0.5198	0.4915

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values. Zn0 = control.

In Table 32, concentrations of chlorophyll *a* and chlorophyll *c* are of the order of 1.1208 (Zn5, day 2) to 1.5233 μg.cm^{-3} (Zn1, day 8) and 0.4699 (Zn4, day 8) to 1.5113 μg.cm^{-3} (Zn5, day 2), respectively. Effects of zinc on chlorophyll *a* concentrations are slight fluctuations. In Zn0 (0.0022 mg/l), chlorophyll *a* concentration increases with increased time, but in Zn1, Zn2 and Zn3 (0.0044, 0.0220 and 0.0660 mg/l) chlorophyll *a* concentration are reduced at the 2nd day of experiment, then increased through the 8th day of experiment. Compared with the control, chlorophyll *a* concentration is decreased in Zn4 (0.110 mg/l). The data show that the plant cells are activated to produce chlorophyll *a* within the 4th day of experiment when the metal concentration is less than 0.0660 mg/l. Unlike concentration of chlorophyll *a*, chlorophyll *c* concentration is reduced by the metal when compared with the start of the experiment.

Protein content and lipid fraction

Total nitrogen protein content and lipid fraction in the blade of the plant treated with zinc are shown in Table 33.

In Table 33, crude protein content ranges from 0.1641 (Zn5, day 2) to 0.3632% per gram dry weight (Zn0). The data show that synthesis of protein in *S. polycystum* is inhibited by zinc.

The lipid fraction is of the order of 0.0199 (Zn3, day 8) to 0.1168 g/g dry weight (Zn1, day 8). Comparing with the start, synthesis of the lipid is reduced by zinc concentration except at 0.0044 mg/l at the 6th and the 8th day of the experiment (Table 33).

Table 33 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Zn

Zn conc. (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Zn0 = 0.0022	0.3631 ^{l} ±0.00	0.3237 ^{kl} ±0.00	0.2647 ^{ghi} ±0.00	0.2712 ^{ghij} ±0.00	0.3172 ^{jkl} ±0.00
Zn1 = 0.0044	0.3631 ^{l} ±0.00	0.2887 ^{ijk} ±0.00	0.2100 ^{abcdef} ±0.00	0.2887 ^{ijk} ±0.00	0.2713 ^{ghij} ±0.00
Zn2 = 0.0220	0.3631 ^{l} ±0.00	0.2581 ^{ghi} ±0.00	0.2057 ^{abcde} ±0.00	0.2865 ^{hijk} ±0.00	0.2297 ^{cdefg} ±0.00
Zn3 = 0.0660	0.3631 ^{l} ±0.00	0.2406 ^{defgh} ±0.00	0.1794 ^{ab} ±0.00	0.2559 ^{fghi} ±0.00	0.2341 ^{cdefg} ±0.00
Zn4 = 0.1100	0.3631 ^{l} ±0.00	0.1969 ^{abcd} ±0.00	0.1815 ^{ab} ±0.00	0.2515 ^{efghi} ±0.00	0.2275 ^{bcdefg} ±0.00
Zn5 = 0.2200	0.3631 ^{l} ±0.00	0.1461 ^{a} ±0.00	0.1882 ^{abc} ±0.00	0.3194 ^{kl} ±0.00	0.2494 ^{efghi} ±0.00
Zn conc. (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Zn0 =	0.1068^{u}±0.0	0.0496^{h}±0.0	0.0739^{p}±0.0	0.0971	0.0915
Zn1 =	0.1068^{u}±0.0	0.0457	0.0836	0.1258^{w}±0.0	0.1168^{v}±0.0
Zn2 =	0.1068^{u}±0.0	0.0412^{c}±0.0	0.0625	0.0763^{q}±0.0	0.0706^{o}±0.0
Zn3 =	0.1068^{u}±0.0	0.0292	0.0630^{m}±0.0	0.0601^{k}±0.0	0.0199^{a}±0.0
Zn4 =	0.1068^{u}±0.0	0.0463^{g}±0.0	0.0494^{h}±0.0	0.0536	0.070 ^{n}±0.00
Zn5 =	0.1068^{u}±0.0	0.0543	0.0431^{d}±0.0	0.5200	0.0436^{e}±0.0

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

Zn_0 = control.

Fatty acid profile

After treatment with zinc at various concentrations, the blades of *S. polycystum* were collected and determined. Major fatty acids are shown in Table 34.

In Table 34, the most common fatty acid is C16:0 and far followed by C18:1(n-9)c and C20:4(n-6), respectively. Palmitic acid is very high at the start of the experiment, and then slightly decreases with increased time and increased the metal concentration, except in Zn3, day 6 in which is found the highest proportion of palmitic acid (74.1291%).

Table 34 Major fatty acids (%) found in the blade of *S. polycystum* treated with Zn

Zn no./day	C14:0	C16:0	C16:1	C18:1(n-9)c	C18:2(n-6)c	C18:3(n-3)	C20:2	C20:4(n-6)	Sum-Sat.	Sum-Unsat.	Others	C4:0
0 - 5. 0 day	5.1223	37.3063	5.5558	14.4029	5.2107	4.3031	4.7117	12.4650	42.4286	46.6492	10.9221	
0 - 2 day	4.8074	31.1285	5.0071	13.9468	5.9988	4.8779	6.8976	14.3861	35.9359	51.1144	12.9497	
1 - 2 day	4.2613	32.2869	5.6011	19.1415	5.2968	4.2507	5.1492	12.7049	36.5482	52.1441	11.3077	
2 - 2 day	4.8299	37.1120	5.9216	16.0684	5.2013	3.7947	3.7934	12.8032	41.9419	47.5826	10.4755	
3 - 2 day	4.7238	35.4242	4.7461	15.2625	5.9076	3.9385	3.3464	14.8985	40.1480	48.0995	11.7525	
4 - 2 day	4.5960	31.5425	5.1405	13.8526	7.6010	3.5323	4.0678	15.3529	36.1385	49.5471	14.3144	
5 - 2 day	5.2718	36.2745	4.8685	15.1896	5.7135	4.0907	3.3347	11.9028	41.5463	45.0998	13.3538	
0 - 4 day	5.1746	36.7629	5.3219	13.6624	6.2077	3.9583	4.3058	14.4420	41.9375	47.8981	10.1645	
1 - 4 day	4.2847	35.0380	5.6544	16.1715	5.8536	4.0739	4.7734	12.8032	39.3226	49.3301	11.3473	
2 - 4 day	4.8895	35.3280	5.1768	13.7330	5.3618	3.8805	3.4233	12.4380	40.2175	44.0134	15.7691	
3 - 4 day	5.0114	36.5318	5.0687	17.8183	5.1909	3.8315	3.0971	12.5199	41.5432	47.5264	10.9304	
4 - 4 day	0.0000	37.4258	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	37.4258	0.0000	62.5742	40.486
5 - 4 day	4.9125	34.9173	4.7584	14.5000	6.6240	4.0308	4.0610	13.6203	39.8298	47.5946	12.5756	
0 - 6 day	4.3062	33.1426	4.8219	15.3917	6.9915	4.1787	4.3882	15.9675	37.4488	51.7395	10.8116	
1 - 6 day	3.0141	34.3993	3.9734	10.4341	5.2342	3.2166	2.4676	6.6104	37.4134	31.9363	30.6503	
2 - 6 day	4.1229	35.1274	5.5002	14.9587	10.2080	3.7759	3.4342	11.8424	39.2503	49.7194	11.0303	
3 - 6 day	0.0000	74.1291	0.0000	25.9000	0.0000	0.0000	0.0000	0.0000	74.1291	25.8709	0.0000	
4 - 6 day	0.0000	11.2397	5.2929	2.4877	4.1171	0.0000	3.2722	0.0000	11.2397	15.1698	73.5904	49.824
5 - 6 day	4.5115	35.7041	4.6418	16.8350	7.5831	3.7995	3.2946	12.7960	40.2156	48.9501	10.8344	
0 - 8 day	0.0000	34.7513	0.0000	10.0359	11.6990	0.0000	11.5634	0.0000	34.7513	33.2984	31.9503	
1 - 8 day	3.8581	34.0359	4.2706	14.6133	6.1417	3.8891	0.3031	19.7101	37.8940	48.9278	13.1782	
2 - 8 day	4.4761	35.4842	5.2237	16.6742	5.6055	3.7150	3.7691	13.2007	39.9602	48.1883	11.8514	
3 - 8 day	2.6367	23.4286	6.3861	10.1835	1.3484	0.0000	5.9839	5.1034	26.0653	29.0053	44.9293	
4 - 8 day	0.0000	4.7078	5.3791	5.6666	10.3114	0.0000	3.9177	0.0000	4.7078	25.2749	70.0173	41.168
5 - 8 day	4.7216	36.0958	4.9662	15.8955	5.3742	3.7192	3.9809	12.8316	40.8174	46.7676	12.4150	

In conclusion, effects of individual metals on concentrations of chlorophyll *a* and chlorophyll *c*, crude protein, lipid fraction and fatty acid profiles are shown in Table 35.

Table 35 Effects of metal on physiological changes in *S. polycystum*

Metal	Chlorophyll *a*	Chlorophyll *c*	Protein	Lipid	% Palmitic acid
Cd	increase	increase	decrease	no effect	ns
Cr	increase	increase	no effect	increase	decrease
Cu	increase (low)	increase (low)	decrease	no effect	ns
Pb	decrease	decrease	decrease	decrease	increase
Zn	increase	decrease	decrease	decrease	decrease

ns = no samples

In Table 35, chlorophyll *a* and chlorophyll *c* concentrations are increased as the time increases and increase with cadmium, chromium and copper. However, the plants died within 6th day of the experiment if the copper concentrations were higher than 0.045 mg/l. On the other hand, both chlorophyll concentrations are decreased with increased time and treatment with lead and zinc. Meanwhile, there is slight fluctuation when treated with chromium. It can be concluded that cadmium and copper at low concentrations activate production of both chlorophylls. Yet, both lead and zinc inhibit chlorophyll synthesis in the seaweed.

Protein concentration is decreased with increased time and treatment with all metals except chromium, for which there is variation in protein content in the seaweed. It can be concluded that all metals, except chromium, inhibit synthesis of

protein in the plant cells. Chromium concentration had no apparent effect on protein synthesis in the seaweed.

The lipid content is decreased when treated with lead and zinc, but there are slightly increases when chromium is applied. In addition, there are no effects on lipid fraction when treated with cadmium and copper. It can be concluded that both lead and zinc inhibit synthesis of lipid in the seaweed cells.

The percentage of palmitic acid is slightly decreased when treated with chromium, and it is obviously decreased when zinc is applied. Meanwhile, it is increased when treated with lead. However, there are not sufficient samples when cadmium and copper are applied.

It can be concluded that each metal has different effects on physiological changes. For example, cadmium shows activated effect on both chlorophyll syntheses, and shows inhibit effect on protein synthesis. However, there is no effect on lipid synthesis. As a micronutrient, copper shows promoted effect on chlorophyll synthesis at low concentration, and shows reduction effect on protein synthesis. Lead shows activated effect on all physiological changes, except for an inhibited effect on palmitic acid synthesis. Meanwhile, zinc shows all reduction effects on all physiological changes, except shows promoted effects on chlorophyll *a* synthesis (Table 35). Therefore, Zn is known as one of a micronutrient.

Strömgren (1998b) studying five species of Fucales, reported that Cu is more toxic than Zn, Pb and Cd. In this study, in contrast, Cu is the most toxic which is known that this metal are used as an algicide in shrimp farm and other ponds. Yet, this study treated the metals in different concentrations, depending on the metal concentration found in the water column.

4.2.2 Effects of combined metals

After 0, 2, 4, 6 and 8 days, the blades were prepared in order to determine concentrations of chlorophyll *a* and chlorophyll *c*, crude protein, lipid fraction as well as fatty acid profiles.

Effect of Cd+Cr on chlorophyll *a* and chlorophyll *c* concentrations

Concentrations of chlorophyll *a* and chlorophyll *c* treated with mixtures of Cd and Cr at various concentration are shown in Table 36.

In Table 36, concentration of chlorophyll *a* and chlorophyll *c* ranges from 1.1146 (aquarium 2, Cd:Cr = 0.006:0.003, day 6) to 1.5007 $\mu g.cm^{-3}$ (aquarium 4, Cd:Cr = 0.03:0.003, day 8) and 0.3536 (aquarium 2, day 6) to 0.8059 $\mu g.cm^{-3}$ (aquarium 4, day 8), respectively. Effects of Cd+Cr combination on chlorophyll *a* and chlorophyll *c* concentrations are slight fluctuation. In control (aquarium 0), aquarium 3, 4 and 5, chlorophyll *a* concentration increases with increased time. On the other hand, in aquarium 1 and 2 chlorophyll *a* concentration is decreased with increased time. Meanwhile, chlorophyll *c* concentration is increased with increased time in all treatments, except in aquarium 1 and 2.

Effect of Cd+Cr on total protein content and lipid fraction

Crude protein and lipid fraction in the blade of *S. polycystum* treated with mixtures of Cd+Cr at various concentrations are shown in Table 37.

In Table 37, total nitrogen content ranges from 0.1160 (aquarium 2, day 4) to 0.2275% per gram dry weight (aquarium 4, day 6). The data show that there are slight fluctuations during the experiment. It can be concluded that synthesis of protein in the plant cells is minor effect when treated with mixtures Cd+Cr.

The lipid fraction is of the order of 0.0451 (aquarium 1, day 4) to 0.1405 g/g dry weight (aquarium 5, day 8). Comparing the control, the lipid fraction is decreased with increased time and increases the mixtures of both metals (Table 37). It can be concluded that synthesis of lipid in the seaweed is inhibited by mixtures of Cd+Cr.

Table 36 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Cd+Cr

Combined metal 1 Cd : Cr (mg/l)	Chlorophyll *a* ($\mu g.cm^{-3}$) time (days)				
	0	2	4	6	8
Aqua0 = control	1.3395 ^{h} ±0.000	1.3408 ^{h} ±0.000	1.4296 ^{o} ±0.001	1.4369 ^{q} ±0.000	1.4345 ^{p} ±0.000
Aqua1=0.006 :	1.3395 ^{h} ±0.000	1.3542 ^{j} ±0.000	1.3425 ^{i} ±0.001	1.2559 ^{d} ±0.000	1.3280 ^{g} ±0.001
Aqua2=0.006 : 0.003	1.3395 ^{h} ±0.000	1.4144 ^{m} ±0.000	1.3254 ^{f} ±0.000	1.1146 ^{a} ±0.002	1.2354 ^{c} ±0.000
Aqua3=0.006 : 0.009	1.3395 ^{h} ±0.000	1.3928 ^{l} ±0.000	1.4681 ^{t} ±0.000	1.4495 ^{r} ±0.001	1.4307 ^{o} ±0.000
Aqua4=0.03 : 0.003	1.3395 ^{h} ±0.000	1.2136 ^{b} ±0.001	1.4235 ^{n} ±0.000	1.4553 ^{s} ±0.000	1.5007 ^{u} ±0.000
Aqua5=0.09 : 0.009	1.3395 ^{h} ±0.000	1.2995 ^{e} ±0.001	1.3941 ^{l} ±0.001	1.3852 ^{k} ±0.000	1.4221 ^{n} ±0.000
Combined metal 1 Cd : Cr (mg/l)	Chlorophyll *c* ($\mu g.cm^{-3}$) time (days)				
	0	2	4	6	8
Aqua0 = control	0.5794 ^{k} ±0.000	0.5084 ^{f} ±0.001	0.6243 ^{n} ±0.001	0.6618 ^{p} ±0.001	0.6723 ^{q} ±0.001
Aqua1=0.006 :	0.5794 ^{k} ±0.000	0.5228 ^{g} ±0.001	0.5229 ^{g} ±0.001	0.4675 ^{b} ±0.001	0.5374 ^{h} ±0.001
Aqua2=0.006 :	0.5794 ^{k} ±0.000	0.6104 ^{m} ±0.001	0.5419 ^{i} ±0.002	0.3536 ^{a} ±0.001	0.5009 ^{e} ±0.001
Aqua3=0.006 :	0.5794 ^{k} ±0.000	0.5696 ^{j} ±0.002	0.7080 ^{t} ±0.001	0.6953 ^{r} ±0.001	0.6941 ^{r} ±0.001
Aqua4=0.03 : 0.003	0.5794 ^{k} ±0.000	0.4716 ^{c} ±0.001	0.6444 ^{o} ±0.001	0.7225 ^{u} ±0.001	0.8059 ^{v} ±0.000
Aqua5=0.09 : 0.009	0.5794 ^{k} ±0.000	0.4824 ^{d} ±0.001	0.5946 ^{l} ±0.003	0.6132 ^{m} ±0.004	0.6988 ^{s} ±0.005

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values. Control = no metals added.

Table 37 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Cd+Cr

Combined metal 1 Cd : Cr (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.1750 cdefg h	0.2188 hi ±0.018	0.2100 ghi ±0.009	0.1554 abcde	0.2210 l ±0.011
Aqua1=0.006 : 0.0006	0.1750 cdefg h	0.1750 cdefg h	0.1947 efghi	0.1379 abc ±0.013	0.1925 efghi
Aqua2=0.006 : 0.003	0.1750 cdefg h	0.1575 abcd e	0.1160 a ±0.006	0.1882 defghi	0.1575 abcde
Aqua3=0.006 : 0.009	0.1750 cdefg h	0.1444 abcd ±0.013	0.1269 ab ±0.000	0.2057 fghi ±0.005	0.1269 ab ±0.022
Aqua4=0.03 : 0.003	0.1750 cdefg h	0.1641 bcdef g	0.1422 abc ±0.002	0.2275 l ±0.008	0.1663 bcdefg
Aqua5=0.09 : 0.009	0.1750 cdefg h	0.1685 bcdef g	0.1663 bcdef g	0.1904 efghi ±0.028	0.1422 abc ±0.011
Combined metal 1 Cd : Cr (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.0893 s ±0.000	0.0572 g ±0.001	0.0697 o ±0.000	0.0620 j ±0.001	0.0634 k ±0.002
Aqua1=0.006 : 0.0006	0.0893 s ±0.000	0.0676 n ±0.003	0.0451 a ±0.000	0.0550 e ±0.002	0.0724 p ±0.000
Aqua2=0.006 : 0.003	0.0893 s ±0.000	0.0601 i ±0.002	0.0770 q ±0.001	0.0507 c ±0.000	0.0569 g ±0.001
Aqua3=0.006 : 0.009	0.0893 s ±0.000	0.0558 f ±0.003	0.0474 b ±0.001	0.0588 h ±0.000	0.0967 t ±0.000
Aqua4=0.03 : 0.003	0.0893 s ±0.000	0.0664 m ±0.001	0.0644 l ±0.000	0.0523 d ±0.001	0.1067 u ±0.001
Aqua5=0.09 : 0.009	0.0893 s ±0.000	0.0556 f ±0.000	0.0635 k ±0.001	0.0876 r ±0.000	0.1405 v ±0.001

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

Control = no metals added.

Effect of Cd+Cr on fatty acid profile

The most fatty acid proportions found in *S. polycystum* is C16:0 (Table 38), and far followed by C18:1(n-9)c and C20:4(n-6), respectively. The most palmitic acid is 40.4421% occurring in control, day 6. The data show that palmitic acid is increased with increased time in control, aquarium 1, 2 and 3 but the fatty acid is decreased in aquarium 4 and 5. It can be concluded that synthesis of palmitic acid is inhibited at high concentrations of chromium.

Table 38 Major fatty acids (%) found in the blade of *S. polycystum* treated with Cd+Cr

Aqua.no./day	C14:0	C16:0	C16:1	C18:1(n-9)c	C18:2(n-6)c	C18:3(n-3)	C20:2	C20:4(n-6)	Sum-Sat.	Sum-Unsat.	Others	remark
0 - 5. 0 day	4.0291	36.6584	4.6101	14.5733	6.2612	4.5505	5.0086	13.9118	40.6874	48.9155	10.3971	
0. 2 day	4.4172	36.1658	5.1162	14.2516	5.2914	5.3804	5.3732	14.0700	40.5830	49.4828	9.9342	
1. 2 day	3.9018	33.9648	4.6020	14.2856	7.2019	5.2777	5.9822	13.9228	37.8666	51.2722	10.8612	
2. 2 day	3.8661	34.2533	4.6287	14.5465	5.3160	4.4698	5.8748	16.2919	38.1194	51.1277	10.7529	
3. 2 day	4.3138	35.5576	4.5080	14.3419	5.7826	5.6144	5.3230	15.1184	39.8714	50.6884	9.4402	
4. 2 day	4.3115	35.2654	4.4550	14.1309	5.4540	5.2296	5.3808	15.5237	39.5769	50.1740	10.2491	
5. 2 day	4.6000	35.6923	4.7231	13.8454	6.2001	5.0329	4.9364	15.0009	40.2923	49.7388	9.9689	
0. 4 day	4.2403	38.7992	5.1357	14.4366	6.5555	3.8672	4.1822	13.1077	43.0395	47.2848	9.6757	
1. 4 day	4.1305	37.9429	4.9237	14.7960	5.7390	3.5887	3.6543	13.5530	42.0734	46.2547	11.6719	
2. 4 day	3.7523	37.8566	4.6939	13.9149	6.9823	4.0977	3.8046	14.3375	41.6089	47.8310	10.5601	
3. 4 day	4.4489	38.1564	4.3764	13.4753	6.4200	4.6849	4.4996	13.8499	42.6053	47.3061	10.0886	
4. 4 day	4.4434	36.7502	4.5260	14.5506	6.1877	5.3273	5.0828	14.0375	41.1937	49.7120	9.0943	
5. 4 day	4.6959	37.9407	4.3155	14.8538	5.6624	4.7707	4.3031	14.6455	42.6366	48.5510	8.8124	
0. 6 day	4.4683	40.4421	5.1981	14.6154	5.5745	3.6756	3.4861	13.3491	44.9104	45.8986	9.1910	
1. 6 day	4.2326	39.2394	5.1868	14.9361	5.8578	3.9786	3.3267	13.4095	43.4720	46.6955	9.8325	
2. 6 day	4.0180	39.5792	4.9468	15.5775	5.9401	3.7704	3.4122	13.3205	43.5972	46.9675	9.4353	
3. 6 day	4.5672	36.2508	4.5439	15.3295	5.8589	4.7588	4.6944	14.5247	40.8181	49.7102	9.4717	
4. 6 day	4.6089	36.6401	4.6591	14.5307	6.8119	4.3472	4.0030	12.4890	41.2490	46.8408	11.9101	
5. 6 day	4.6431	37.2624	4.6352	14.1313	5.7620	4.6212	4.7111	13.7998	41.9055	47.6605	10.4340	
0. 8 day	4.1743	38.5775	5.4197	13.5608	6.8039	4.0745	4.0060	14.0740	42.7518	47.9389	9.3092	
1. 8 day	4.3514	38.1361	5.5716	14.2579	6.6333	4.3272	4.1451	13.9368	42.4875	48.8719	8.6407	
2. 8 day	4.7330	38.8743	5.4882	13.7603	6.5298	3.8886	3.6366	13.9885	43.6073	47.2919	9.1007	
3. 8 day	4.4019	36.9662	4.5517	14.1306	5.9044	4.8010	4.5903	14.1997	41.3681	48.1776	10.4543	
4. 8 day	4.5308	36.2937	4.6926	13.6323	5.8689	4.9151	5.0732	14.2257	40.8245	48.4078	10.7677	
5. 8 day	4.4641	35.4316	4.8335	13.3301	5.4414	5.1129	5.1443	14.4627	39.8957	48.3249	11.7794	

Effect of Cd+Cu on chlorophyll *a* and chlorophyll *c* concentrations

Concentrations of chlorophyll *a* and chlorophyll *c* treated with mixtures of Cd+Cu at various concentrations are shown in Table 39.

Table 39 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Cd+Cu

Combined metal 2 Cd : Cu (mg/l)	Chlorophyll *a* (μg.cm^{-3})				
	time (days)				
	0	2	4	6	8
Aqua0 = control	1.4720 ^{g} ±0.000	1.4407 ^{e} ±0.000	1.5004 ^{j} ±0.000	1.5682 ^{o} ±0.000	1.5877 ^{r} ±0.001
Aqua1=0.006 : 0.003	1.4720 ^{g} ±0.000	1.5507 ^{l} ±0.000	1.5581 ^{m} ±0.000	1.5826 ^{q} ±0.002	1.4915 ^{i} ±0.001
Aqua2=0.006 : 0.015	1.4720 ^{g} ±0.000	1.4330 ^{d} ±0.001	1.5775 ^{p} ±0.001	1.4878 ^{h} ±0.000	dead
Aqua3=0.006 : 0.045	1.4720 ^{g} ±0.000	1.4590 ^{f} ±0.000	1.5344 ^{k} ±0.001	dead	dead
Aqua4=0.03 : 0.015	1.4720 ^{g} ±0.000	1.5635 ^{n} ±0.000	1.4085 ^{c} ±0.000	1.3969 ^{a} ±0.000	1.4028 ^{b} ±0.001
Aqua5=0.09 : 0.045	1.4720 ^{g} ±0.000	1.4568 ^{f} ±0.000	dead	dead	dead
Combined metal 2 Cd : Cu (mg/l)	Chlorophyll *c* (μg.cm^{-3})				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.7596 ^{f} ±0.000	0.6424 ^{c} ±0.000	0.8254 ^{i} ±0.006	0.9881 ^{n} ±0.001	1.1404 ^{r} ±0.003
Aqua1=0.006 : 0.003	0.7596 ^{f} ±0.000	0.9629 ^{l} ±0.001	1.0283 ^{p} ±0.001	0.9428 ^{k} ±0.002	0.8501 ^{j} ±0.001
Aqua2=0.006 : 0.015	0.7596 ^{f} ±0.000	0.6898 ^{d} ±0.001	1.0474 ^{q} ±0.001	0.7780 ^{g} ±0.001	dead
Aqua3=0.006 : 0.045	0.7596 ^{f} ±0.000	0.8134 ^{h} ±0.001	0.9778 ^{m} ±0.003	dead	dead
Aqua4=0.03 : 0.015	0.7596 ^{f} ±0.000	1.0132 ^{o} ±0.001	0.6905 ^{d} ±0.002	0.6004 ^{b} ±0.001	0.3251 ^{a} ±0.003
Aqua5=0.09 : 0.045	0.7596 ^{f} ±0.000	0.6990 ^{e} ±0.001	dead	dead	dead

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications.

The letters are arranged in ascendant order as in the values. Control = no metals added.

In Table 39, concentrations of chlorophyll *a* and chlorophyll *c* range from 1.3969 (aquarium 4, day 6) to 1.5876 (control, day 8) and 0.3251 (aquarium4, day 8) to 1.1404 $\mu g.cm^{-3}$ (control, day 8), respectively. The data show that both chlorophyll *a* and chlorophyll *c* concentrations are increased with increased in control and the ratio of metals is Cd:Cu = 0.006:0.003 mg/l. At the ratio of Cd:Cu = 0.006:0.015 and Cd:Cu = 0.006:0.045 mg/l, both chlorophyll concentrations are variations and the plant died in the 6^{th} or 8^{th} day of experiment. Yet, at the ratio of metals = 0.03:0.045 and 0.09:0.0453 mg/l, both chlorophyll concentrations are decreased with increased time. In addition, the seaweed died at the 4^{th} day of experiment. It can be concluded that synthesis of both chlorophylls in the plant cells is inhibited by mixtures of Cd+Cu.

Effect of Cd+Cu on protein content and lipid fraction

Total nitrogen content and lipid fraction in the blade of *S. polycystum* treated with mixtures of Cd+Cu at various concentrations are shown in Table 40.

In Table 40, total nitrogen content is of the order of 0.1685 (aquarium 2, day 4, aquarium 4, day 6) to 0.2975% per gram dry weight (aquarium 1, day 8). The data show that protein content in all treatments is mostly not significantly different at the 5% level (Table 40). It can be concluded that mixtures of Cd+Cu have minor effect on protein synthesis in the seaweed. However, the seaweed was killed in the 8^{th} day in aquarium 2, in the 6^{th} and 8^{th} day in aquarium 3 and in the 4^{th} day through the 8^{th} day of experiment in aquarium 5.

The lipid fraction ranges from 0.0437 (aquarium 2, day 2) to 0.1071 g/g dry weight at the start. Comparing the control, the data show that lipid fraction is

decreased with increased time and increases the ratio of both metals. It can be concluded that synthesis of lipid in the seaweed cells is inhibited by mixtures of both metals (Table 40).

Table 40 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Cd+Cu

Combined metal 2 Cd : Cu (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.2363 ^{cde} ±0.004	0.2363 ^{cde} ±0.013	0.2232 ^{bcd} ±0.026	0.2417 ^{cdef} ±0.044	0.2800 ^{efg} ±0.008
Aqua1=0.006 : 0.003	0.2363 ^{cde} ±0.004	0.2888 ^{gh} ±0.013	0.2735 ^{efg} ±0.002	0.2516 ^{cdefg}	0.2975 ^{h} ±0.000
Aqua2=0.006 : 0.015	0.2363 ^{cde} ±0.004	0.2057 ^{abc} ±0.000	0.1685 ^{a} ±0.020	0.2210 ^{bcd} ±0.011	dead
Aqua3=0.006 : 0.045	0.2363 ^{cde} ±0.004	0.1729 ^{a} ±0.002	0.2669 ^{defg} ±0.000	dead	dead
Aqua4=0.03 : 0.015	0.2363 ^{cde} ±0.004	0.2604 ^{defg} ±0.000	0.1838 ^{ab} ±0.000	0.1685 ^{a} ±0.000	0.2733 ^{efg} ±0.000
Aqua5=0.09 : 0.045	0.2363 ^{cde} ±0.004	0.2079 ^{abc} ±0.002	dead	dead	dead
Combined metal 1 Cd : Cu (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.1071 ^{o} ±0.002	0.0721 ^{k} ±0.001	0.0868 ^{n} ±0.002	0.0724 ^{k} ±0.000	0.0746 ^{l} ±0.002
Aqua1=0.006 : 0.003	0.1071 ^{o} ±0.002	0.0722 ^{k} ±0.002	0.0716 ^{j} ±0.002	0.0713 ^{i} ±0.000	0.0445 ^{b} ±0.002
Aqua2=0.006 : 0.015	0.1071 ^{o} ±0.002	0.0437 ^{a} ±0.001	0.0621 ^{f} ±0.001	0.0717 ^{j} ±0.000	dead
Aqua3=0.006 : 0.045	0.1071 ^{o} ±0.002	0.0469 ^{d} ±0.000	0.0686 ^{h} ±0.000	0.0645 ^{g} ±0.000	dead
Aqua4=0.03 : 0.015	0.1071 ^{o} ±0.002	0.0452 ^{c} ±0.001	0.0788 ^{m} ±0.001	0.0517 ^{e} ±0.000	ns
Aqua5=0.09 : 0.045	0.1071 ^{o} ±0.002	ns	dead	dead	dead

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

Control = no metals added. ns = no sample.

Effect of Cd+Cu on fatty acid profile

The most fatty acid proportions found is $C_{16:0}$ (palmitic acid) and far followed by $C_{18:1(n-9)c}$ and $C_{20:4(n-6)}$, respectively. The most palmitic acid is 39.6738% occurring in aquarium 1, day 4. Comparing the start, the data show that palmitic acid is decreased with increased time and mixtures of Cd+Cu increase. It can be concluded that synthesis of palmitic acid is inhibited by mixtures of both metals, especially at high concentrations of copper (Table 41).

Table 41 Major fatty acids (%) found in the blade of *S. polycystum* treated with Cd+Cu

Aqua.no./day	C14:0	C16:0	C16:1	C18:1(n-9)c	C18:2(n-6)c	C18:3(n-3)	C20:2	C20:4(n-6)	Sum-Sat.	Sum-Unsat.	Others	C18:2(n-6)t
0 - 5. 0 day	4.8808	36.1034	4.3687	12.2785	5.5330	5.6246	5.8969	14.5523	40.9842	48.2540	10.7618	
0. 2 day	4.0066	33.4242	4.9826	12.3067	11.6178	5.3540	5.1443	13.4625	37.4307	52.8678	9.7015	
1. 2 day	4.5838	35.9887	5.7731	13.6035	9.1104	4.9498	5.0284	13.1369	40.5726	51.6019	7.8255	
2. 2 day	4.6112	37.5636	5.3936	16.5387	8.1685	2.9095	2.5595	13.3793	42.1748	48.9490	8.8762	
3. 2 day	3.8680	31.5349	5.1143	12.2096	8.3111	5.2357	6.8293	13.6780	35.4028	51.3780	13.2192	
4. 2 day	5.4010	34.7360	5.4327	14.8989	6.3350	5.3201	4.2767	15.0112	40.1370	51.2746	8.5884	
5. 2 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
0. 4 day	4.5431	34.1167	5.1418	14.1652	7.6458	5.1061	5.7252	14.2217	38.6598	52.0058	9.3344	
1. 4 day	4.8109	39.6738	5.4698	14.5920	6.0040	3.8970	4.3625	12.6612	44.4846	46.9864	8.5289	
2. 4 day	4.7388	37.1083	5.2639	15.4889	6.9260	3.9575	4.3275	14.1734	41.8471	50.1371	8.0158	
3. 4 day	4.3774	35.4610	5.2685	14.4473	7.2314	4.8528	5.6432	14.2999	39.8385	51.7430	8.4185	
4. 4 day	4.4864	35.5947	5.1550	13.9064	5.5476	4.6634	5.3370	13.7842	40.0811	48.3936	11.5253	
5. 4 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
0. 6 day	3.5835	31.2212	4.7017	14.1921	6.8005	3.5313	4.3625	14.0669	34.8046	47.6549	17.5405	8.2793
1. 6 day	4.2757	33.2254	5.7595	14.6383	6.0665	5.2974	6.4064	14.3153	37.5011	52.4834	10.0155	
2. 6 day	4.6676	34.4110	5.6631	14.4753	5.7380	5.0691	5.4814	13.4525	39.0786	49.8794	11.0420	1.70139
3. 6 day	4.5137	34.1265	5.8631	15.0426	7.3212	5.1672	5.0831	13.8720	38.6401	52.3492	9.0106	
4. 6 day	4.8476	35.3056	5.4014	14.9906	8.8155	4.2866	3.8186	14.0271	40.1532	51.3397	8.5071	
5. 6 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
0. 8 day	4.7141	34.7615	5.6346	15.2067	6.1539	5.3694	5.4373	13.6746	39.4756	51.4764	9.0480	
1. 8 day	4.6014	33.2163	5.3985	14.1945	7.2028	5.2149	5.9148	14.9409	37.8176	52.8664	9.3159	
2. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
3. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
4. 8 day	4.2876	34.6437	5.3718	14.8054	5.7575	4.5148	4.1361	11.5437	38.9313	46.1291	14.9396	7.0476
5. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	

Effect of Cd+Pb on chlorophyll *a* and chlorophyll *c* concentrations

Concentrations of chlorophyll *a* and chlorophyll *c* treated with mixtures of Cd and Pb at various concentrations are shown in Table 42.

Table 42 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Cd+Pb

Combined metal 3 Cd : Pb (mg/l)	Chlorophyll *a* (μg.cm^{-3}) time (days)				
	0	2	4	6	8
Aqua0 = control	1.3598 ^{ab} ±0.000	1.4865 ^{h} ±0.002	1.3814 ^{bc} ±0.000	1.4856 ^{h} ±0.000	1.5409 ^{ij} ±0.000
Aqua1=0.006 : 0.009	1.3598 ^{ab} ±0.000	1.3817 ^{bc} ±0.001	1.4757 ^{gh} ±0.001	1.4243 ^{de} ±0.000	1.4263 ^{def} ±0.000
Aqua2=0.006 : 0.045	1.3598 ^{ab} ±0.000	1.4616 ^{gh} ±0.000	1.4268 ^{def} ±0.001	1.4483 ^{efg} ±0.001	1.3930 ^{c} ±0.001
Aqua3=0.006 : 0.135	1.3598 ^{ab} ±0.000	1.5201 ^{ij} ±0.051	1.4697 ^{gh} ±0.000	1.5704 ^{k} ±0.001	1.5150 ^{i} ±0.001
Aqua4=0.03 : 0.045	1.3598 ^{ab} ±0.000	1.3367 ^{a} ±0.001	1.5227 ^{ij} ±0.000	1.5217 ^{ij} ±0.001	1.4663 ^{gh} ±0.001
Aqua5=0.09 : 0.135	1.3598 ^{ab} ±0.000	1.5496 ^{jk} ±0.000	1.5251 ^{ij} ±0.001	1.4541 ^{fg} ±0.000	1.3987 ^{cd} ±0.000
Combined metal 3 Cd : Pb (mg/l)	Chlorophyll *c* (μg.cm^{-3}) time (days)				
	0	2	4	6	8
Aqua0 = control	0.5936 ^{d} ±0.000	0.7659 ^{n} ±0.001	0.5854 ^{b} ±0.001	0.7890 ^{o} ±0.001	0.8921 ^{t} ±0.001
Aqua1=0.006 : 0.009	0.5936 ^{d} ±0.000	0.5877 ^{bc} ±0.001	0.7499 ^{m} ±0.001	0.7017 ^{i} ±0.002	0.5799 ^{a} ±0.002
Aqua2=0.006 : 0.045	0.5936 ^{d} ±0.000	0.7216 ^{k} ±0.001	0.6894 ^{g} ±0.001	0.7139 ^{j} ±0.001	0.6107 ^{f} ±0.001
Aqua3=0.006 : 0.135	0.5936 ^{d} ±0.000	0.9440 ^{v} ±0.001	0.7481 ^{m} ±0.001	0.9338 ^{u} ±0.001	0.8206 ^{p} ±0.001
Aqua4=0.03 : 0.045	0.5936 ^{d} ±0.000	0.5999 ^{e} ±0.002	0.8557 ^{r} ±0.002	0.8311 ^{q} ±0.001	0.7280 ^{l} ±0.001
Aqua5=0.09 : 0.135	0.5936 ^{d} ±0.000	0.9208 ^{u} ±0.001	0.8658 ^{s} ±0.001	0.6938 ^{h} ±0.001	0.5907 ^{cd} ±0.011

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications.

The letters are arranged in ascendant order as in the values. Control = no metals added.

In Table 42, concentrations of chlorophyll *a* and chlorophyll *c* range from 1.3367 (aquarium 4, day 2) to 1.5704 μg.cm^{-3} (aquarium 3, day 6) and 0.5799 (aquarium 1, day 8) to 0.9440 μg.cm^{-3} (aquarium 3, day 2), respectively. Comparing the start, the data show that synthesis of both chlorophyll *a* and chlorophyll *c* in the plant cells is activated by mixtures of Cd+Pb.

Effect of Cd+Pb on protein content and lipid fraction

Protein content and lipid fraction in the blade of *S. polycystum* treated with mixtures of Cd+Pb at various concentrations are shown in table 43.

In Table 43, protein content in the plant cells ranges from 0.1007 (aquarium 1, day 8) to 0.2407% per gram dry weight (aquarium 0, day 4). The data show that all treatments are mostly not significantly different at the 5% level. Comparing the start, protein concentrations are decreased with increased time and increase the ratio of both metals. On the other hand, in aquarium 0 (control) and aquarium 5, protein content is increased with increased time. It can be concluded that synthesis of protein in the cells is inhibited by mixtures of Cd+Pb (Table 43).

The lipid fraction is of the order of 0.0436 (aquarium 4, day 4) to 0.0756 g/g dry weight (control, day 0). Comparing the start, lipid fractions are decreased with increased time and increases ratio of Cd+Pb concentrations. However, there are not sufficient samples in the 8th of experiment (Table 43). It can be concluded that synthesis of lipid in the cells is inhibited by mixtures of both the metals.

Table 43 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Cd+Pb

Combined metal 3 Cd : Pb (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.1738 ^{cdef} ±0.011	0.1444 ^{abcd}	0.2407 ^{h} ±0.022	0.2100 ^{efgh} ±0.008	0.1794 ^{defg} ±0.013
Aqua1=0.006 : 0.009	0.1738 ^{cdef} ±0.011	0.1488 ^{abcd}	0.1247 ^{abc} ±0.005	0.1422 ^{abcd}	0.1007 ^{a} ±0.008
Aqua2=0.006 : 0.045	0.1738 ^{cdef} ±0.011	0.1226 ^{abc} ±0.004	0.1444 ^{abcd}	0.1641 ^{bcde}	0.1204 ^{ab} ±0.011
Aqua3=0.006 : 0.135	0.1738 ^{cdef} ±0.011	0.1422 ^{abcd}	0.1520 ^{bcd} ±0.006	0.1663 ^{bcdef}	0.1225 ^{abc} ±0.000
Aqua4=0.03 : 0.045	0.1738 ^{cdef} ±0.011	0.2276 ^{gh} ±0.004	0.1882 ^{defg} ±0.013	0.1816 ^{defg} ±0.006	0.1379 ^{abcd} ±0.006
Aqua5=0.09 : 0.135	0.1738 ^{cdef} ±0.011	0.2122 ^{defg} ±0.060	0.2144 ^{gh} ±0.004	0.2122 ^{defg} ±0.006	0.1685 ^{bcdef}
Combined metal 3 Cd : Pb (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.0756 ^{o} ±0.001	0.0755 ^{o} ±0.001	0.0711 ^{n} ±0.001	0.0611 ^{j} ±0.000	ns
Aqua1=0.006 : 0.009	0.0756 ^{o} ±0.001	0.0693 ^{m} ±0.000	0.0462 ^{c} ±0.000	0.0559 ^{g} ±0.000	ns
Aqua2=0.006 : 0.045	0.0756 ^{o} ±0.001	0.0535 ^{f} ±0.000	0.0484 ^{d} ±0.000	0.0660 ^{l} ±0.000	ns
Aqua3=0.006 : 0.135	0.0756 ^{o} ±0.001	0.0569 ^{h} ±0.000	0.0484 ^{d} ±0.000	0.0498 ^{e} ±0.000	ns
Aqua4=0.03 : 0.045	0.0756 ^{o} ±0.001	0.0601 ^{i} ±0.000	0.0436 ^{a} ±0.000	0.0452 ^{b} ±0.000	ns
Aqua5=0.09 : 0.135	0.0756 ^{o} ±0.001	0.0615 ^{k} ±0.000	0.0561 ^{g} ±0.000	0.0452 ^{b} ±0.000	ns

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

Control = no metals added

ns = no sample.

Effect of Cd+Pb on fatty acid profile

Like other treatments, the most fatty acid proportions found in the blade of *S. polycystum* is palmitic acid (C_{16}:0) and far followed by C18:2(n-6)c and C20:4(n-6), respectively. The most palmitic acid is 37.9677% occurring in aquarium 1, day 6. Comparing the start, palmitic acid is increased with increased time, except in Cd:Pb = 0.006:0.135, 0.03:0.045 and 0.09:0.135 mg/l, the fatty acid is decreased in the 6th day of experiment. However, there are not sufficient samples in the 8th day of experiment (Table 44). It can be concluded that mixtures of the metals activate synthesis of palmitic acid within the 6th day of experiment.

Table 44 Major fatty acids (%) found in the blade of *S. polycystum* treated with Cd+Pb

Aqua.no./day	C14:0	C16:0	C16:1	C18:1(n-9)c	C18:2(n-6)c	C18:3(n-3)	C20:2	C20:4(n-6)	Sum-Sat.	Sum-Unsat.	Others	C18:2(n-6)t
0 - 5. 0 day	3.8672	35.3311	5.0763	15.5312	5.6193	4.2421	4.1372	12.9744	39.1983	47.5804	13.2213	
0. 2 day	4.2335	35.0469	4.8679	15.3911	9.6922	4.3301	3.7420	13.8922	39.2804	51.9155	8.8041	
1. 2 day	4.6372	36.3727	5.2114	14.1630	6.9639	4.1758	3.2893	12.3545	41.0099	46.1580	12.8321	5.75405
2. 2 day	3.6988	34.1624	5.0733	13.7125	6.3674	3.5794	3.1956	11.9707	37.8612	43.8989	18.2398	18.2398
3. 2 day	4.3681	36.6341	5.0710	16.1724	6.5750	4.4096	3.6852	14.6938	41.0021	50.6070	8.3909	
4. 2 day	4.2895	36.6158	5.4237	15.9573	9.6169	3.8866	4.0313	12.8527	40.9053	51.7685	7.3261	
5. 2 day	4.4227	35.625	5.9132	14.9483	7.1647	4.4824	4.2973	14.8932	40.0476	51.6992	8.2532	
0. 4 day	4.3057	37.0737	4.9596	15.3658	7.5726	4.2562	4.2760	13.5329	41.3794	49.9632	8.6574	
1. 4 day	4.1382	35.6947	5.6274	14.3679	7.9684	4.7293	4.2047	13.9216	39.8329	50.8193	9.3478	
2. 4 day	3.6636	36.0300	5.5561	15.8940	5.8696	4.2208	4.3655	14.8547	39.6936	50.7607	9.5457	
3. 4 day	3.9723	37.7546	5.2374	15.0076	7.9134	3.7709	3.4198	13.8560	41.7269	49.2052	9.0680	
4. 4 day	3.9992	35.7486	5.5892	15.5329	8.5105	3.7260	3.9067	13.7000	39.7478	50.9654	9.2868	
5. 4 day	4.2576	37.2487	5.2691	15.6788	7.4249	4.1602	3.7336	13.7953	41.5063	50.0618	8.4319	
0. 6 day	4.0514	37.1958	4.8705	16.1257	7.4967	4.0998	3.9787	13.5678	41.2472	50.1393	8.6134	
1. 6 day	4.1021	37.9677	5.8756	14.6589	6.4251	4.6453	4.0763	13.7915	42.0697	49.4727	8.4576	
2. 6 day	4.3887	36.5165	5.4379	15.6719	7.9209	3.9855	3.5627	14.1723	40.9053	50.7512	8.3435	
3. 6 day	4.6666	33.8849	5.4058	15.9361	5.9849	5.1332	4.1731	14.7791	38.5515	51.4122	10.0364	
4. 6 day	4.5523	33.7576	5.3646	15.8957	5.8765	5.0452	4.1688	14.6532	38.3100	51.0039	10.6861	
5. 6 day	4.5510	33.5896	5.3564	15.7896	5.7561	5.0012	4.1466	14.6249	38.1406	50.6748	11.1846	
0. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
1. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
2. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
3. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
4. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
5. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	

Effect of Cd+Zn on chlorophyll *a* and chlorophyll *c* concentrations

Concentrations of chlorophyll *a* and chlorophyll *c* treated with mixtures of Cd and Zn at various concentrations are shown in Table 45.

Table 45 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Cd+Zn

Combined metal 4 Cd : Zn (mg/l)	Chlorophyll *a* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	1.3907 ^{d} ±0.000	1.4402 ^{gh} ±0.000	1.4651 ^{j} ±0.001	1.4318 ^{f} ±0.000	1.3846 ^{c} ±0.000
Aqua1=0.006 : 0.009	1.3907 ^{d} ±0.000	1.4419 ^{h} ±0.001	1.5399 ^{p} ±0.000	1.5313 ^{o} ±0.000	1.4812 ^{l} ±0.000
Aqua2=0.006 : 0.045	1.3907 ^{d} ±0.000	1.4312 ^{f} ±0.000	1.4394 ^{gh} ±0.001	1.4397 ^{gh} ±0.000	1.4619 ^{i} ±0.001
Aqua3=0.006 : 0.135	1.3907 ^{d} ±0.000	1.4751 ^{k} ±0.000	1.5797 ^{q} ±0.001	1.3831 ^{c} ±0.004	1.5416 ^{p} ±0.000
Aqua4=0.03 : 0.045	1.3907 ^{d} ±0.000	1.4944 ^{n} ±0.001	1.3341 ^{a} ±0.000	1.4846 ^{m} ±0.001	1.4082 ^{e} ±0.000
Aqua5=0.09 : 0.135	1.3907 ^{d} ±0.000	1.6092 ^{r} ±0.002	1.5318 ^{o} ±0.001	1.4382 ^{g} ±0.000	1.3701 ^{b} ±0.001
Combined metal 4 Cd : Zn (mg/l)	Chlorophyll *c* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.6153 ^{c} ±0.000	0.6705 ^{h} ±0.001	0.7175 ^{l} ±0.002	0.6974 ^{j} ±0.002	0.5844 ^{b} ±0.001
Aqua1=0.006 : 0.009	0.6153 ^{c} ±0.000	0.6687 ^{gh} ±0.001	0.9900 ^{s} ±0.001	0.8868 ^{r} ±0.001	0.7407 ^{n} ±0.001
Aqua2=0.006 : 0.045	0.6153 ^{c} ±0.000	0.6625 ^{f} ±0.001	0.6647 ^{fg} ±0.001	0.7009 ^{j} ±0.002	0.7065 ^{k} ±0.008
Aqua3=0.006 : 0.135	0.6153 ^{c} ±0.000	0.7336 ^{m} ±0.001	1.0346 ^{t} ±0.001	0.6376 ^{d} ±0.000	0.8883 ^{r} ±0.001
Aqua4=0.03 : 0.045	0.6153 ^{c} ±0.000	0.7897 ^{p} ±0.003	0.5705 ^{a} ±0.001	0.7833 ^{o} ±0.002	0.6409 ^{d} ±0.003
Aqua5=0.09 : 0.135	0.6153 ^{c} ±0.000	1.0947 ^{u} ±0.002	0.8612 ^{q} ±0.001	0.6917 ^{i} ±0.001	0.6523 ^{e} ±0.000

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications.

The letters are arranged in ascendant order as in the values. Control = no metals added.

In Table 45, concentrations of chlorophyll *a* and chlorophyll *c* range from 1.3341 (aquarium 4, day 4) to 1.6092 $\mu g.cm^{-3}$ (aquarium 5, day 2) and 0.5705 (aquarium 4, day 4) to 1.0947 $\mu g.cm^{-3}$ (aquarium 5, day 2), respectively. Comparing the start, both chlorophyll *a* and chlorophyll *c* concentrations are increased with increased time in all treatments. It can be concluded that mixtures of Cd+Zn promote synthesis of both chlorophylls.

Effect of Cd+Zn on protein content and lipid fraction

Protein content and lipid fraction in the blade of *S. polycystum* treated with mixtures of Cd+Zn at various concentrations are shown in Table 46.

In Table 46, total nitrogen content in the plant cells is of the order of 0.1094 (aquarium 3, day 8 and aquarium 4, day 8) to 0.2954% per gram dry weight (start). Comparing the start, the data show that protein content is slightly decreased with increased time in all treatments, especially in the mixtures of Cd:Zn = 0.006:0.022, 0.006:0.066, 0.03:0.022 and 0.09:0.066 (Table 46). It is clear that mixtures of the metals inhibit protein synthesis in the plant cells.

The lipid fraction ranges from 0.0466 (aquarium 3, day 4) to 0.1196 (aquarium 5, day 8) g/g dry weight. The data show that lipid fraction is nearly the same amount. Comparing the start, however, lipid content is slightly decreased with increased time in all treatments (Table 46). It can be concluded that mixtures of Cd+Zn inhibit lipid synthesis in the cells. Unfortunately, there are not sufficient samples for studing fatty acids profile.

Table 46 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Cd+Zn

Combined metal 4 Cd : Zn (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.2954 ^{k} ±0.011	0.2691 ^{jk} ±0.015	0.2538 ^{ij} ±0.004	0.2144 ^{fgh} ±0.004	0.2538 ^{ij} ±0.000
Aqua1=0.006 : 0.0044	0.2954 ^{k} ±0.011	0.2057 ^{efg} ±0.004	0.1400 ^{ab} ±0.008	0.1926 ^{defg} ±0.004	0.1619 ^{bcd} ±0.004
Aqua2=0.006 : 0.022	0.2954 ^{k} ±0.011	0.2954 ^{k} ±0.011	0.2232 ^{hgi} ±0.015	0.1794 ^{cde} ±0.004	0.1619 ^{bcd} ±0.004
Aqua3=0.006 : 0.066	0.2954 ^{k} ±0.011	0.2407 ^{hij} ±0.026	0.1597 ^{bcd} ±0.006	0.1554 ^{bc} ±0.006	0.1094 ^{a} ±0.007
Aqua4=0.03 : 0.022	0.2954 ^{k} ±0.011	0.2407 ^{hij} ±0.000	0.1597 ^{bcd} ±0.021	0.1554 ^{bc} ±0.006	0.1094 ^{a} ±0.006
Aqua5=0.09 : 0.066	0.2954 ^{k} ±0.011	0.2166 ^{fgh} ±0.006	0.1838 ^{cdef} ±0.000	0.2144 ^{fgh} ±0.004	0.1422 ^{ab} ±0.011
Combined metal 4 Cd : Zn (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.0870 ^{fg} ±0.002	0.0556 ^{abcd}	0.0686 ^{de} ±0.001	0.0631 ^{bcde}	0.0629 ^{bcde} ±0.001
Aqua1=0.006 : 0.0044	0.0870 ^{fg} ±0.002	0.0664 ^{cde} ±0.001	0.0453 ^{a} ±0.000	0.0570 ^{abcd}	0.0717 ^{e} ±0.002
Aqua2=0.006 : 0.022	0.0870 ^{fg} ±0.002	0.0649 ^{bcde}	0.0762 ^{ef} ±0.000	0.0515 ^{ab} ±0.000	0.0557 ^{abcd}
Aqua3=0.006 : 0.066	0.0870 ^{fg} ±0.002	0.0547 ^{abcd} ±0.000	0.0466 ^{a} ±0.000	0.0567 ^{abcd}	0.0945 ^{gh} ±0.003
Aqua4=0.03 : 0.022	0.0870 ^{fg} ±0.002	0.0642 ^{bcde}	0.0633 ^{bcde} ±0.001	0.0526 ^{abc} ±0.000	0.1027 ^{h} ±0.012
Aqua5=0.09 : 0.066	0.0870 ^{fg} ±0.002	0.0549 ^{abcd}	0.0644 ^{bcde}	0.0861 ^{fg} ±0.002	0.1196 ^{i} ±0.021

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

Control = no metals added.

Effect of Cr+Cu on chlorophyll *a* and chlorophyll *c* concentrations

Concentrations of chlorophyll *a* and chlorophyll *c* treated with mixtures of Cr and Cu at various concentrations are shown in Table 47.

Table 47 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Cr+Cu

Combined metal 5 Cr : Cu (mg/l)	Chlorophyll *a* (μg.cm^{-3}) time (days)				
	0	2	4	6	8
Aqua0 = control	1.4774 ^{c} ±0.000	1.5198 ^{i} ±0.001	1.5425 ^{k} ±0.000	1.5529 ^{l} ±0.001	1.5732 ^{o} ±0.001
Aqua1=0.006 : 0.003	1.4774 ^{c} ±0.000	1.4823 ^{d} ±0.000	1.4953 ^{f} ±0.000	1.5302 ^{j} ±0.001	1.5673 ^{m} ±0.001
Aqua2=0.006 : 0.015	1.4774 ^{c} ±0.000	1.5019 ^{g} ±0.000	1.5163 ^{h} ±0.000	1.5410 ^{k} ±0.001	1.5692 ^{n} ±0.001
Aqua3=0.006 : 0.045	1.4774 ^{c} ±0.000	1.4935 ^{e} ±0.000	dead	dead	dead
Aqua4=0.03 : 0.015	1.4774 ^{c} ±0.000	1.4662 ^{b} ±0.000	1.4178 ^{a} ±0.001	dead	dead
Aqua5=0.09 : 0.045	1.4774 ^{c} ±0.000	dead	dead	dead	dead
Combined metal 5 Cr : Cu (mg/l)	Chlorophyll *c* (μg.cm^{-3}) time (days)				
	0	2	4	6	8
Aqua0 = control	0.7498 ^{d} ±0.000	0.8596 ^{g} ±0.003	0.9007 ^{j} ±0.003	0.9288 ^{l} ±0.001	1.0076 ^{o} ±0.001
Aqua1=0.006 : 0.003	0.7498 ^{d} ±0.000	0.7440 ^{c} ±0.001	0.7901 ^{e} ±0.001	0.8884 ^{i} ±0.001	0.9902 ^{n} ±0.001
Aqua2=0.006 : 0.015	0.7498 ^{d} ±0.000	0.8031 ^{f} ±0.002	0.8644 ^{h} ±0.001	0.9430 ^{m} ±0.003	1.0307 ^{p} ±0.003
Aqua3=0.006 : 0.045	0.7498 ^{d} ±0.000	0.9173 ^{k} ±0.001	dead	dead	dead
Aqua4=0.03 : 0.015	0.7498 ^{d} ±0.000	0.7057 ^{b} ±0.002	0.6432 ^{a} ±0.001	dead	dead
Aqua5=0.09 : 0.045	0.7498 ^{d} ±0.000	dead	dead	dead	dead

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications.

The letters are arranged in ascendant order as in the values. Control = no metals added.

In Table 47, concentrations of chlorophyll *a* and chlorophyll *c* range from 1.4178 (aquarium 4, day 4) to 1.5732 μg.cm^{-3} (control, day 8) and 0.6432 (aquarium 4, day 6) to 1.0307 μg.cm^{-3} (aquarium 2, day 8), respectively. Comparing the start, the data show that both chlorophyll *a* and chlorophyll *c* concentrations are increased with increased time in most treatments, except in aquarium 3, 4 and 5 (Cr:Cu = 0.006:0.045, 0.003:0.015 and 0.009:0.045), the chlorophylls are reduced with increased time and finally the plant died (Table 47). It is clear that both chlorophylls are activated to synthesis by low concentrations of copper. However, the cells are killed by high concentrations of the metal.

Effect of Cr+Cu on protein content and lipid fraction

Total nitrogen content and lipid fraction in the blade of *S. polycystum* treated with mixtures of Cr+Cu at various concentrations are shown in Table 48.

Total nitrogen content in the seaweed ranges from 0.1663 (aquarium 2, day 8) to 0.3238% per gram dry weight (aquarium 1, day 2). The data show that protein content is reduced with increased time and increases copper concentrations. The plants were killed if copper concentrations are higher than 0.045 mg/l, such as in aquarium 4 (Cr:Cu = 0.003:0.015 mg/l, Table 48). It is clear that synthesis of protein is inhibited by mixtures of the metals.

The lipid fraction ranges from 0.0453 (aquarium 2, day 4) to 0.0601 g/g dry weight (aquarium1, day 6). Similarly to the concentrations of protein, the lipid is activated to synthesis at low concentrations of the metals but the lipid is inhibited and finally the plants were killed at high concentrations of both metals (Table 48).

Table 48 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Cr+Cu

Combined metal 5 Cr : Cu (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.2866 ^{de} ±0.024	0.2647 ^{cde} ±0.050	0.2691 ^{cde} ±0.002	0.2341 ^{abcd}	0.2079 ^{abcd}
Aqua1=0.006 : 0.003	0.2866 ^{de} ±0.024	0.3238 ^{e} ±0.009	0.2035 ^{abc} ±0.015	0.1838 ^{ab} ±0.009	0.1729 ^{ab} ±0.002
Aqua2=0.006 : 0.015	0.2866 ^{de} ±0.024	0.2494 ^{bcde}	0.1969 ^{abc} ±0.092	0.2100 ^{abcd}	0.1663 ^{a} ±0.009
Aqua3=0.006 : 0.045	0.2866 ^{de} ±0.024	0.2188 ^{abcd}	dead	dead	dead
Aqua4=0.03 : 0.015	0.2866 ^{de} ±0.024	0.3172 ^{e} ±0.011	0.2210 ^{abcd} ±0.002	dead	dead
Aqua5=0.09 : 0.045	0.2866 ^{de} ±0.024	dead	dead	dead	dead
Combined metal 5 Cr : Cu (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.0453 ^{f} ±0.000	0.0506 ^{k} ±0.000	0.0479 ^{i} ±0.000	0.0460 ^{g} ±0.000	0.0538 ^{n} ±0.000
Aqua1=0.006 : 0.003	0.0453 ^{f} ±0.000	0.0534 ^{m} ±0.001	0.0431 ^{e} ±0.000	0.0601 ^{o} ±0.001	0.0308 ^{b} ±0.000
Aqua2=0.006 : 0.015	0.0453 ^{f} ±0.000	0.0471 ^{h} ±0.000	0.0453 ^{a} ±0.000	0.0398 ^{d} ±0.000	0.0325 ^{c} ±0.000
Aqua3=0.006 : 0.045	0.0453 ^{f} ±0.000	0.0496 ^{j} ±0.000	dead	dead	dead
Aqua4=0.03 : 0.015	0.0453 ^{f} ±0.000	0.0510 ^{l} ±0.000	0.0236 ^{a} ±0.000	dead	dead
Aqua5=0.09 : 0.045	0.0453 ^{f} ±0.000	dead	dead	dead	dead

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

Control = no metals added.

Effect of Cr+Cu on fatty acid profile

The most fatty acid proportions found in the blade is palmitic acid (C16:0), and far followed by C18:1(n-9)c and C20:4(n-6), respectively. The most palmitic acid is 42.5331% occurring in aquarium 2, day 4 (Table 49). Comparing the start, the fatty acid is increased in the 4th day of experiment in all treatments, and then it is reduced. The seaweed was killed at high ratio of the metals.

Table 49 Major fatty acids (%) found in the blade of *S. polycystum* treated with Cr+Cu

Aqua.no./day	C14:0	C16:0	C16:1	C18:1(n-9)c	C18:2(n-6)c	C18:3(n-3)	C20:2	C20:4(n-6)	Sum-Sat.	Sum-Unsat.	Others	remark
0 - 5. 0 day	4.7384	35.4736	6.3971	14.7688	6.4209	4.4769	5.4221	13.8723	40.2120	51.3581	8.4299	
0. 2 day	4.8973	36.3503	6.4022	14.1221	7.5313	3.9208	4.8489	13.3594	41.2476	50.1847	8.5677	
1. 2 day	4.9373	36.2402	5.5812	15.0731	8.2834	2.9850	3.2335	14.0801	41.1776	49.2363	9.5861	
2. 2 day	5.4154	39.9701	6.8550	16.7958	5.3360	3.2802	2.6302	12.2927	45.3855	47.1898	7.4247	
3. 2 day	4.8586	38.7477	7.0932	14.8084	6.4021	4.4038	4.1839	11.7016	43.6063	48.5930	7.8007	
4. 2 day	4.5352	35.7335	6.8698	14.9961	6.2818	3.6459	4.2975	13.8984	40.2687	49.9895	9.7418	
5. 2 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
0. 4 day	4.9894	36.7487	6.3182	15.0883	6.4276	3.9753	4.1558	13.3711	41.7381	49.3364	8.9256	
1. 4 day	4.7468	36.0540	5.8980	15.8082	7.5120	3.9253	3.9538	13.2006	40.8008	50.2979	8.9014	
2. 4 day	5.0491	42.5331	6.8734	16.5654	5.4965	2.3875	2.2720	11.0775	47.5822	44.6724	7.7454	
3. 4 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
4. 4 day	4.7229	36.7506	6.7608	16.7404	6.4074	3.1040	3.7955	13.0699	41.4735	49.8780	8.6485	
5. 4 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
0. 6 day	4.7626	34.8262	6.4230	15.0057	7.8542	4.5735	4.6999	13.9626	39.5887	52.5190	7.8922	
1. 6 day	4.6585	34.1584	6.3688	15.4681	8.1102	4.2735	4.3943	13.9967	38.8169	52.6116	8.5715	
2. 6 day	5.4861	40.6953	6.6747	18.5967	5.5473	2.6120	2.2880	11.0898	46.1814	46.8087	7.0098	
3. 6 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
4. 6 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
5. 6 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
0. 8 day	4.5573	35.2626	6.4860	13.9684	7.6523	4.1804	4.6852	13.9075	39.8199	50.8798	9.3002	
1. 8 day	4.2835	35.0998	6.0764	14.7103	8.1103	3.2098	3.4654	14.3634	39.3833	49.9356	10.6811	
2. 8 day	5.3965	40.4702	6.1732	17.5889	5.4123	2.5419	2.3932	11.2150	45.8668	45.3246	8.8086	
3. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
4. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
5. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	

Effect of Cr+Pb on chlorophyll *a* and chlorophyll *c* concentrations

Concentrations of chlorophyll *a* and chlorophyll *c* treated with mixtures of Cr and Pb at various concentrations are shown in Table 50.

Table 50 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Cr+Pb

Combined metal 6 Cr : Pb (mg/l)	Chlorophyll *a* (μg.cm^{-3})				
	time (days)				
	0	2	4	6	8
Aqua0 = control	1.4684 ^{a} ±0.001	1.5535 ^{efghi} ±0.000	1.5857 ^{k} ±0.000	1.5333 ^{bcde}	1.5386 ^{cdef} ±0.000
Aqua1=0.006 : 0.009	1.4684 ^{a} ±0.001	1.5777 ^{jk} ±0.001	1.5720 ^{hijk} ±0.001	1.5437 ^{def} ±0.000	1.5424 ^{def} ±0.000
Aqua2=0.006 : 0.045	1.4684 ^{a} ±0.001	1.5481 ^{defg} ±0.000	1.5178 ^{b} ±0.000	1.5517 ^{efgh} ±0.000	1.5190 ^{bc} ±0.000
Aqua3=0.006 : 0.135	1.4684 ^{a} ±0.001	1.5707 ^{hijk} ±0.000	1.5668 ^{ghijk} ±0.001	1.5340 ^{bcde}	1.5165 ^{b} ±0.000
Aqua4=0.03 : 0.045	1.4684 ^{a} ±0.001	1.5525 ^{efghi} ±0.070	1.5658 ^{ghijk} ±0.001	1.5734 ^{ijk} ±0.002	1.5284 ^{bcd} ±0.001
Aqua5=0.09 : 0.135	1.4684 ^{a} ±0.001	1.5804 ^{k} ±0.001	1.4660 ^{a} ±0.001	1.5579 ^{fghij} ±0.003	1.5723 ^{hijk} ±0.001
Combined metal 6 Cr : Pb (mg/l)	Chlorophyll *c* (μg.cm^{-3})				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.7032 ^{a} ±0.000	1.0310 ^{fgh} ±0.001	1.1228 ^{ij} ±0.002	0.9074 ^{bcd} ±0.001	0.9668 ^{def} ±0.001
Aqua1=0.006 : 0.009	0.7032 ^{a} ±0.000	1.0485 ^{gh} ±0.001	1.1364 ^{ij} ±0.001	0.9676 ^{def} ±0.001	1.0091 ^{efgh} ±0.001
Aqua2=0.006 : 0.045	0.7032 ^{a} ±0.000	0.9407 ^{cde} ±0.001	0.8451 ^{b} ±0.001	0.9989 ^{efg} ±0.001	0.8726 ^{bc} ±0.001
Aqua3=0.006 : 0.135	0.7032 ^{a} ±0.000	1.1835 ^{j} ±0.001	1.1260 ^{ij} ±0.001	0.8980 ^{bcd} ±0.002	0.8516 ^{b} ±0.001
Aqua4=0.03 : 0.045	0.7032 ^{a} ±0.000	1.0782 ^{hi} ±0.125	1.0137 ^{fgh} ±0.001	1.0681 ^{ghi} ±0.001	0.9265 ^{cd} ±0.002
Aqua5=0.09 : 0.135	0.7032 ^{a} ±0.000	1.0259 ^{fgh} ±0.002	0.7638 ^{a} ±0.001	0.9048 ^{bcd} ±0.002	1.1739 ^{j} ±0.001

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications.

The letters are arranged in ascendant order as in the values. Control = no metals added.

In Table 50, concentrations of chlorophyll *a* and chlorophyll *c* range from 1.4660 (aquarium 5, day 4) to 1.5857 $\mu g.cm^{-3}$ (aquarium 0, day 4) and 0.7032 (start) to 1.1835 $\mu g.cm^{-3}$ (aquarium3, day 2), respectively. Comparing the start, the data show that both chlorophyll *a* and chlorophyll *c* concentrations are increased with increased time and increases mixtures of the metals. It is clear that chlorophylls are promoted to synthesis by mixtures of Cr+Pb.

Effect of Cr+Pb on total protein content and lipid fraction

Total nitrogen content and lipid fraction in the blade of *S. polycystum* treated with mixtures of Cr+Pb at various concentrations are shown in Table 51.

In Table 51, total nitrogen content ranges from 0.1685 (aquarium 1, day 4) to 0.3610% per gram dry weight (aquarium 2, day 4). The data show that crude protein content in all treatments is nearly not significantly different at the 5% level. It is clear that mixtures of Cr+Pb have minor effect on protein synthesis in the seaweed cells.

The lipid fraction is of the order of 0.0195 (aquarium 5, day 2) to 0.3052 g/g dry weight (aquarium 3, day 2). Comparing the start, lipid content is decreased when the time and mixtures of the metals are increased. This means that synthesis of lipid is inhibited by mixtures of the metals (Table 51).

Effect of Cr+Pb on fatty acid profile

Like other experiments, the most fatty acid proportions found in the blade is C16:0, and far followed by C18:1(n-9)c and C20:4(n-6), respectively. The most palmitic acid is 39.8458% occurring in aquarium 2, day 4 (Cr:Pb = 0.006:0.045 mg/l,

Table 52). Comparing the start, in all treatments, the fatty acid will be reduced when the time and the mixtures of metals are increased.

Table 51 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Cr+Pb

Combined metal 6 Cr : Pb (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.2363 ^{abc} ±0.035	0.2407 ^{abc} ±0.004	0.1991 ^{ab} ±0.006	0.2920 ^{cde} ±0.001	0.1947 ^{ab} ±0.001
Aqua1=0.006 : 0.009	0.2363 ^{abc} ±0.035	0.2057 ^{ab} ±0.021	0.1685 ^{a} ±0.001	0.2713 ^{bcd} ±0.023	0.2144 ^{abc} ±0.004
Aqua2=0.006 : 0.045	0.2363 ^{abc} ±0.035	0.2429 ^{abc} ±0.023	0.3610 ^{e} ±0.037	0.2079 ^{ab} ±0.006	0.2319 ^{abc} ±0.004
Aqua3=0.006 : 0.135	0.2363 ^{abc} ±0.035	0.2450 ^{abc} ±0.008	0.3457 ^{de} ±0.066	0.2757 ^{bcd} ±0.013	0.2947 ^{ab} ±0.011
Aqua4=0.03 : 0.045	0.2363 ^{abc} ±0.035	0.2407 ^{abc} ±0.013	0.3544 ^{e} ±0.004	0.2341 ^{abc} ±0.024	0.2013 ^{ab} ±0.008
Aqua5=0.09 : 0.135	0.2363 ^{abc} ±0.035	0.1969 ^{ab} ±0.004	0.3260 ^{de} ±0.002	0.1772 ^{a} ±0.024	0.2450 ^{abc} ±0.008
Combined metal 6 Cr : Pb (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.1175 ^{w} ±0.000	0.0807 ^{r} ±0.000	0.0535 ^{h} ±0.000	0.0663 ^{p} ±0.000	0.0620 ^{n} ±0.000
Aqua1=0.006 : 0.009	0.1175 ^{w} ±0.000	0.0305 ^{b} ±0.000	0.0365 ^{c} ±0.000	0.0687 ^{q} ±0.000	0.0813 ^{s} ±0.000
Aqua2=0.006 : 0.045	0.1175 ^{w} ±0.000	0.0599 ^{l} ±0.000	0.0602 ^{m} ±0.000	0.0559 ^{j} ±0.000	0.1144 ^{v} ±0.000
Aqua3=0.006 : 0.135	0.1175 ^{w} ±0.000	0.3052 ^{x} ±0.000	0.0540 ^{i} ±0.000	0.0520 ^{g} ±0.000	0.0868 ^{t} ±0.000
Aqua4=0.03 : 0.045	0.1175 ^{w} ±0.000	0.0387 ^{d} ±0.000	0.0640 ^{o} ±0.000	0.0500 ^{f} ±0.000	0.0459 ^{e} ±0.000
Aqua5=0.09 : 0.135	0.1175 ^{w} ±0.000	0.0195 ^{a} ±0.000	0.0578 ^{k} ±0.000	0.1023 ^{u} ±0.000	0.0602 ^{m} ±0.000

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

Control = no metals added.

Table 52 Major fatty acids (%) found in the blade of *S. polycystum* treated with Cr+Pb

Aqua.no./day	C14:0	C16:0	C16:1	C18:1(n-9)c	C18:2(n-6)c	C18:3(n-3)	C20:2	C20:4(n-6)	Sum-Sat.	Sum-Unsat.	Others	remark
0 - 5. 0 day	4.8131	37.9497	5.1833	13.3288	7.4298	6.1196	7.2901	15.1562	42.7629	54.5077	2.7294	
0. 2 day	4.4540	35.1037	5.0506	13.4251	7.2897	5.3942	6.2792	13.8763	39.5577	51.3151	9.1272	
1. 2 day	3.9700	35.2433	5.7000	15.1691	6.7132	4.6954	5.1778	14.3874	39.2134	51.8430	8.9437	
2. 2 day	4.0882	34.1128	5.9173	13.2134	6.8642	5.8719	5.8876	15.2193	38.2010	52.9736	8.8254	
3. 2 day	4.8219	35.6573	6.1466	12.9070	7.0908	4.5391	4.4795	15.2851	40.4792	50.4480	9.0728	
4. 2 day	4.6101	36.9922	5.7525	13.4078	6.1516	5.0834	5.4930	13.9737	41.6024	49.8620	8.5356	
5. 2 day	4.2927	37.4374	6.189	13.7509	7.2089	5.3633	6.4166	16.6911	41.7301	55.6198	2.6501	
0. 4 day	4.7758	36.8833	5.1489	14.2143	6.7369	4.7160	4.9683	14.6259	41.6591	50.4103	7.9306	
1. 4 day	4.3409	34.9515	5.7299	14.8545	6.7551	4.1870	6.0091	14.2819	39.2924	51.8175	8.8901	
2. 4 day	5.0428	39.8458	6.1945	14.5543	6.8830	3.2153	3.0251	12.9568	44.8886	46.8290	8.2825	
3. 4 day	4.8652	37.0055	5.8130	14.2621	6.2212	4.8446	5.2457	13.4943	41.8707	49.8809	8.2485	
4. 4 day	4.3181	35.3561	5.2511	13.8076	6.4114	4.5901	5.5146	14.1883	39.6742	49.7630	10.5629	
5. 4 day	4.1993	36.8112	5.4788	14.4413	6.0887	4.7251	5.245	15.0218	41.0104	51.0008	7.9888	
0. 6 day	4.7923	35.9986	5.6327	15.0867	8.4532	5.1144	5.1906	14.6287	40.7910	54.1063	5.1027	
1. 6 day	4.3675	35.0295	5.8953	14.9247	6.4137	5.2439	5.8787	14.0393	39.3970	52.3956	8.2074	
2. 6 day	4.3863	35.2998	5.0756	13.9830	8.7321	4.6419	5.0611	14.6305	39.6861	52.1242	8.1897	
3. 6 day	4.7571	38.0288	6.9662	15.0138	6.4994	4.1806	3.9276	13.6846	42.7859	50.2722	6.9419	
4. 6 day	4.2587	35.1990	6.2208	13.9596	7.4844	4.9684	5.1764	14.1357	39.4577	51.9453	8.5970	
5. 6 day	4.3336	35.9391	5.9551	13.9163	7.2633	4.6489	5.1458	13.8129	40.2727	50.7422	8.9851	
0. 8 day	4.4726	35.7666	6.1523	13.1549	7.2636	4.6321	4.9689	14.7505	40.2392	50.9223	8.8385	
1. 8 day	4.3341	35.8514	6.3608	14.2301	7.2008	4.3578	4.7865	14.1295	40.1855	51.0656	8.7489	
2. 8 day	4.6028	35.5123	5.9489	13.9819	7.9110	4.6381	4.7714	15.1841	40.1151	52.4354	7.4496	
3. 8 day	4.1492	37.1251	6.2926	13.8740	7.3174	4.3330	4.6503	14.0040	41.2743	50.4714	8.2543	
4. 8 day	4.0071	34.7230	5.7550	14.1007	7.4549	5.3899	5.4918	14.6574	38.7300	52.8497	8.4202	
5. 8 day	4.1459	34.9653	5.7095	13.6154	6.9198	4.8719	5.6607	15.1339	39.1111	51.9112	8.9776	

Effect of Cr+Zn on chlorophyll *a* and chlorophyll *c* concentrations

Concentrations of chlorophyll *a* and chlorophyll *c* treated with mixtures of Cr and Zn at various concentrations are shown in Table 53.

Table 53 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Cr+Zn

Combined metal 7 Cr : Zn (mg/l)	Chlorophyll *a* (μg.cm^{-3}) time (days)				
	0	2	4	6	8
Aqua0 = control	1.4096 ^{c} ±0.000	1.4890 ^{i} ±0.000	1.4381 ^{e} ±0.002	1.5000 ^{k} ±0.000	1.4650 ^{f} ±0.000
Aqua1=0.006 : 0.0044	1.4096 ^{c} ±0.000	1.4748 ^{g} ±0.001	1.4933 ^{j} ±0.000	1.4395 ^{e} ±0.001	1.4873 ^{i} ±0.001
Aqua2=0.006 : 0.022	1.4096 ^{c} ±0.000	1.5187 ^{o} ±0.000	1.5080 ^{m} ±0.001	1.5371 ^{q} ±0.001	1.5043 ^{l} ±0.001
Aqua3=0.006 : 0.066	1.4096 ^{c} ±0.000	1.5163 ^{n} ±0.000	1.4115 ^{cd} ±0.000	1.5323 ^{p} ±0.000	1.4125 ^{d} ±0.000
Aqua4=0.03 : 0.022	1.4096 ^{c} ±0.000	1.4388 ^{e} ±0.000	1.3906 ^{b} ±0.000	1.4805 ^{h} ±0.000	1.5362 ^{q} ±0.000
Aqua5=0.09 : 0.066	1.4096 ^{c} ±0.000	1.4383 ^{e} ±0.000	1.3827 ^{a} ±0.000	1.4947 ^{j} ±0.000	1.4878 ^{i} ±0.000
Combined metal 7 Cr : Zn (mg/l)	Chlorophyll *c* (μg.cm^{-3}) time (days)				
	0	2	4	6	8
Aqua0 = control	0.6180 ^{b} ±0.000	0.7879 ^{n} ±0.001	0.6615 ^{f} ±0.003	0.8134 ^{p} ±0.001	0.6897 ^{j} ±0.001
Aqua1=0.006 : 0.0044	0.6180 ^{b} ±0.000	0.7513 ^{k} ±0.001	0.7918 ^{o} ±0.001	0.6755 ^{g} ±0.001	0.7569 ^{l} ±0.002
Aqua2=0.006 : 0.022	0.6180 ^{b} ±0.000	0.8958 ^{s} ±0.002	0.8132 ^{p} ±0.001	0.9188 ^{u} ±0.001	0.7793 ^{m} ±0.001
Aqua3=0.006 : 0.066	0.6180 ^{b} ±0.000	0.8820 ^{r} ±0.001	0.6434 ^{d} ±0.001	0.8998 ^{t} ±0.001	0.6577 ^{e} ±0.002
Aqua4=0.03 : 0.022	0.6180 ^{b} ±0.000	0.6855 ^{i} ±0.001	0.6345 ^{c} ±0.002	0.7882 ^{n} ±0.001	0.8682 ^{q} ±0.001
Aqua5=0.09 : 0.066	0.6180 ^{b} ±0.000	0.6796 ^{h} ±0.001	0.5928 ^{a} ±0.001	0.7921 ^{p} ±0.001	0.7574 ^{l} ±0.001

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications.

The letters are arranged in ascendant order as in the values. Control = no metals added.

In Table 53, concentrations of chlorophyll *a* and chlorophyll *c* range from 1.3827 (aquarium 5, day 4) to 1.5362 $\mu g.cm^{-3}$ (aquarium 4, day 8) and 0.5928 (aquarium 5, day 4) to 0.8682 $\mu g.cm^{-3}$ (aquarium 4, day 8), respectively. Comparing the control, the data show that concentrations of both chlorophyll *a* and chlorophyll *c* are increased with increased time. The maximum concentration is occurred in the ratio of Cr:Zn = 0.006:0.022 mg/l. Meanwhile, in other treatment, both chlorophyll concentrations are slightly increased with increased time. In addition, some treatments are fluctuation such as the lowest chlorophyll concentrations is found in Cr:Zn = 0.009:0.066 mg/l (aquarium 5 , day 4). It can be concluded that both chlorophylls are promoted to synthesis at the maximum ratio of Cr and Zn.

Effect of Cr+Zn on total protein content and lipid fraction

Protein and lipid fraction in the blade of *S. polycystum* treated with mixtures of Cr+Zn at various concentrations are shown in Table 54.

Total nitrogen content ranges from 0.1116 (aquarium 1, day 4) to 0.2407% per gram dry weight (aquarium 0, day 6, aquarium 2, day 6 and aquarium 3, day 6). Comparing the control, the data show that nitrogen content is mostly not significantly different at the 5% level (Table 54). It can be concluded that mixtures of Cr+Zn have minor effect on protein synthesis in the seaweed cells.

In Table 54, the lipid fraction is of the order of 0.0198 (aquarium 3, day 8) to 0.0819 g/g dry weight (start). Comparing the start, the data show that lipid fraction is decreased in all treatments. However, it is very low reduction in the control and high decreased in other treatments, especially in mixtures of Cr:Zn = 0.006:0.066 and

0.009:0.066 mg/l. It can be concluded that lipid synthesis is limited by high concentrations of Zn (Table 54).

Table 54 nitrogen and lipid fraction in the blade of *S. polycystum* treated with Cr+Zn

Combined metal 7 Cr : Zn (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.2319 ef ±0.004	0.1554 abcde	0.1554 abcde	0.2407 f ±0.061	0.2078 cdef ±0.010
Aqua1=0.006 : 0.0044	0.2319 ef ±0.004	0.1247 ab ±0.015	0.1116 a ±0.037	0.2166 cdef ±0.019	0.2232 def ±0.000
Aqua2=0.006 : 0.022	0.2319 ef ±0.004	0.1794 abcdef	0.1532 abcde	0.2407 f ±0.022	0.1575 abcde
Aqua3=0.006 : 0.066	0.2319 ef ±0.004	0.1204 a ±0.002	0.1488 abcd	0.2407 f ±0.031	0.1729 abcdef
Aqua4=0.03 : 0.022	0.2319 ef ±0.004	0.2013 bcdef	0.1138 a ±0.026	0.1794 abcdef	0.1816 abcdef
Aqua5=0.09 : 0.066	0.2319 ef ±0.004	0.1794 abcdef	0.1401 abc ±0.013	0.2035 cdef ±0.006	0.2188 cdef ±0.030
Combined metal 7 Cr : Zn (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.0819 v ±0.000	0.0322 f ±0.000	0.0409 l ±0.000	0.0398 k ±0.000	0.0713 t ±0.000
Aqua1=0.006 : 0.0044	0.0819 v ±0.000	0.0415 m ±0.000	0.0617 r ±0.000	0.0382 j ±0.000	0.0666 s ±0.000
Aqua2=0.006 : 0.022	0.0819 v ±0.000	0.0537 p ±0.000	0.0550 q ±0.000	0.0300 e ±0.000	0.0438 n ±0.000
Aqua3=0.006 : 0.066	0.0819 v ±0.000	0.0515 o ±0.000	0.0377 i ±0.000	0.0202 b ±0.000	0.0198 a ±0.000
Aqua4=0.03 : 0.022	0.0819 v ±0.000	0.0408 l ±0.000	0.0367 g ±0.000	0.0379 ij ±0.000	0.0283 d ±0.000
Aqua5=0.09 : 0.066	0.0819 v ±0.000	0.0800 u ±0.000	0.0371 h ±0.000	0.0410 l ±0.000	0.0241 c ±0.000

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

Control = no metals added.

Effect of Cr+Zn on fatty acid profile

Like other experiments, the most fatty acid proportions found after treatment with mixtures of Cr+Zn is $C_{16:0}$ and far followed by $C_{18:1(n-9)c}$ and $C_{20:4(n-6)}$, respectively. The most palmitic acid is 38.6653% (aquarium 3, day 8). The data show that palmitic acid in all treatments is mostly not significantly different at the 5% level. It can be concluded that mixtures of both metals have low effect on the fatty acid synthesis (Table 55).

Table 55 Major fatty acids (%) found in the blade of *S. polycystum* treated with Cr+Zn

Aqua.no./day	C14:0	C16:0	C16:1	C18:1(n-9)c	C18:2(n-6)c	C18:3(n-3)	C20:2	C20:4(n-6)	Sum-Sat.	Sum-Unsat.	Others	remark
0 - 5. 0 day	4.2967	35.2989	4.7677	14.3848	6.1534	4.7326	5.2401	15.5711	39.5956	50.8497	9.5547	
0. 2 day	4.1964	35.7818	5.0787	13.1010	6.7295	4.7123	5.9251	15.2561	39.9782	50.8027	9.2192	
1. 2 day	3.5866	36.2494	5.9656	13.7945	5.8628	4.5691	5.8864	15.1257	39.8361	51.2042	8.9597	
2. 2 day	4.9130	35.0616	5.5616	15.9783	4.9951	4.6423	4.5675	15.2975	39.9747	51.0424	8.9830	
3. 2 day	4.5833	35.5771	4.9538	15.9146	5.3137	4.3778	4.7178	14.9062	40.1604	50.1839	9.6557	
4. 2 day	4.5788	36.5244	5.6426	14.8137	4.8853	4.9816	4.8066	14.5761	41.1032	49.7058	9.1910	
5. 2 day	4.4200	37.3462	5.8701	14.5587	4.8477	4.6463	4.2128	14.7102	41.7662	48.8458	9.3881	
0. 4 day	4.0780	34.3478	4.9597	13.9528	7.9109	4.1662	5.4696	15.1801	38.4258	51.6393	9.9350	
1. 4 day	3.7286	32.8201	4.7215	15.1598	6.2357	5.3436	5.1729	17.6596	36.5487	54.2931	9.1581	
2. 4 day	4.1046	33.6479	5.5256	16.6643	5.2187	5.4197	5.3726	14.4046	37.7525	52.6054	9.6420	
3. 4 day	4.2882	35.0398	5.4114	16.3168	5.4152	4.4242	4.1528	15.0453	39.3280	50.7657	9.9063	
4. 4 day	4.0011	36.4523	4.9455	15.5398	5.4825	4.6601	5.0782	14.3616	40.4534	50.0677	9.4789	
5. 4 day	4.1543	37.5477	5.0305	16.9754	4.2514	3.9295	4.2797	14.5497	41.7020	49.0162	9.2819	
0. 6 day	4.3517	35.1612	5.4491	15.0670	5.7163	5.3420	5.6743	13.9727	39.5129	51.2214	9.2657	
1. 6 day	3.9191	34.2866	5.1878	15.3810	7.8445	4.6129	5.4649	14.8085	38.2058	53.2996	8.4947	
2. 6 day	4.0896	35.2892	4.9512	17.2324	5.6474	4.3316	4.3514	14.8253	39.3788	51.3393	9.2819	
3. 6 day	4.2451	33.7740	5.5545	18.3573	6.7116	4.2843	4.8095	13.4662	38.0192	53.1835	8.7973	
4. 6 day	4.0485	34.4176	5.1555	17.6439	5.8578	3.9753	4.1909	15.2137	38.4662	52.0370	9.4968	
5. 6 day	4.0628	35.6182	5.0819	18.7228	4.7089	4.4498	4.6768	12.7338	39.6810	50.3740	9.9450	
0. 8 day	3.8548	36.4364	5.9817	14.5874	6.2544	5.7383	5.0122	14.0849	40.2912	51.6589	8.0499	
1. 8 day	3.8075	33.2868	5.4779	15.6027	6.6924	5.5912	5.4509	15.1592	37.0943	53.9741	8.9316	
2. 8 day	3.6986	37.2648	6.0539	14.5065	5.5235	5.3746	5.4088	13.2066	40.9634	50.0739	8.9627	
3. 8 day	4.3528	38.6653	7.2508	16.7121	5.0830	3.9959	4.0984	12.0997	43.0182	49.2398	7.7420	
4. 8 day	3.9992	32.4126	5.2123	16.0810	6.7591	5.6557	5.3060	15.5653	36.4118	54.5793	9.0088	
5. 8 day	4.4043	35.2434	5.7268	18.9568	5.5279	4.8698	3.9527	12.6678	39.6477	51.7018	8.6505	

Effect of Cu+Pb on chlorophyll *a* and chlorophyll *c* concentrations

Chlorophyll *a* and chlorophyll *c* concentrations treated with mixtures of Cu and Pb at various concentrations are shown in Table 56.

Table 56 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Cu+Pb

Combined metal 8 Cu : Pb (mg/l)	Chlorophyll *a* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	1.5054 ^{fg} ±0.000	1.5231 ^{i} ±0.000	1.5227 ^{i} ±0.000	1.5273 ^{j} ±0.001	ns
Aqua1=0.003 : 0.009	1.5054 ^{fg} ±0.000	1.5416 ^{k} ±0.001	1.4936 ^{e} ±0.000	1.4827 ^{d} ±0.000	ns
Aqua2=0.003 : 0.045	1.5054 ^{fg} ±0.000	1.5512 ^{l} ±0.001	1.4581 ^{a} ±0.000	1.5063 ^{gh} ±0.001	ns
Aqua3=0.003 : 0.135	1.5054 ^{fg} ±0.000	1.5044 ^{f} ±0.001	1.5073 ^{hi} ±0.001	1.4794 ^{c} ±0.001	ns
Aqua4=0.015 : 0.045	1.5054 ^{fg} ±0.000	1.4615 ^{b} ±0.000	1.4627 ^{b} ±0.001	dead	dead
Aqua5=0.045 : 0.135	1.5054 ^{fg} ±0.000	dead	dead	dead	dead
Combined metal 8 Cu : Pb (mg/l)	Chlorophyll *c* ($\mu g.cm^{-3}$)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.7901 ^{e} ±0.000	0.8313 ^{g} ±0.001	0.8732 ^{i} ±0.001	0.8881 ^{j} ±0.001	ns
Aqua1=0.003 : 0.009	0.7901 ^{e} ±0.000	0.8550 ^{h} ±0.001	0.7537 ^{cd} ±0.001	0.7578 ^{d} ±0.001	ns
Aqua2=0.003 : 0.045	0.7901 ^{e} ±0.000	0.8943 ^{j} ±0.009	0.6889 ^{a} ±0.001	0.7974 ^{f} ±0.001	ns
Aqua3=0.003 : 0.135	0.7901 ^{e} ±0.000	0.7562 ^{d} ±0.001	0.7856 ^{e} ±0.003	0.7486 ^{c} ±0.001	ns
Aqua4=0.015 : 0.045	0.7901 ^{e} ±0.000	0.7259 ^{b} ±0.001	0.7250 ^{b} ±0.001	dead	dead
Aqua5=0.045 : 0.135	0.7901 ^{e} ±0.000	dead	dead	dead	dead

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

Control = no metals added ns = no sample

In Table 56, concentration of chlorophyll *a* and chlorophyll *c* ranges from 1.4581 (aquarium2, day 4) to 1.5416 μg.cm^{-3} (aquarium 1, day 2) and 0.6889 (aquarium 2, day 4) to 0.8881 μg.cm^{-3} (aquarium 0, day 6), respectively. However, there are no plant samples in the 8th day of experiment since there were not sufficient samples and the seaweed died in Cu:Pb = 0.015:0.045 mg/l, from the 6th day and Cu:Pb = 0.045:0.135 from the 2nd day to the end of experiments. Comparing the start, the data show that both chlorophyll *a* and chlorophyll *c* concentrations are higher at low concentration of both metals. It can be concluded that both chlorophylls are activated to produce at low concentration of copper. If copper concentration is higher than 0.015 mg/l, the plants were killed at the 6th day of experiment.

Effect of Cu+Pb on protein content and lipid fraction

Protein content and lipid fraction in the blade of *S. polycystum* treated with mixtures of Cu+Pb at various concentrations are shown in Table 57.

In Table 57, total nitrogen content is of the order of 0.0788 (aquarium 3, day 6) to 0.3172% per gram dry weight (aquarium 0, day 4). However, there are not sufficient samples and the plants died in aquarium 4, the 6th day and 8th day and in aquarium 5, the 2nd day through the end of the experiment. (Table 57). Comparing the start and control, the data show that total nitrogen is almost in the same content. On the other hand, nitrogen content is reduced with increased time and increases concentrations of the metals.

Table 57 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Cu+Pb

Combined metal 8 Cu : Pb (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Aquarium0 = control	0.2997 [f] ±0.019	0.3129 [f] ±0.006	0.3172 [f] ±0.006	0.2210 [de] ±0.015	ns
Aqua1=0.006 : 0.0044	0.2997 [f] ±0.019	0.2888 [f] ±0.008	0.1925 [cd] ±0.008	0.1313 [ab] ±0.013	ns
Aqua2=0.006 : 0.022	0.2997 [f] ±0.019	0.2220 [de] ±0.032	0.1882 [bcd] ±0.004	0.1532 [bc] ±0.004	ns
Aqua3=0.006 : 0.066	0.2997 [f] ±0.019	0.1816 [bcd] ±0.002	0.1838 [bcd] ±0.008	0.0788 [a] ±0.079	ns
Aqua4=0.03 : 0.022	0.2997 [f] ±0.019	0.2735 [ef] ±0.002	0.2276 [de] ±0.013	dead	dead
Aqua5=0.09 : 0.066	0.2997 [f] ±0.019	dead	dead	dead	dead
Combined metal 8 Cu : Pb (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Aquarium0 = control	0.0309 [g] ±0.000	0.0300 [f] ±0.000	0.0242 [c] ±0.000	0.0328 [i] ±0.000	ns
Aqua1=0.006 : 0.0044	0.0309 [g] ±0.000	0.0290 [e] ±0.000	0.0300 [f] ±0.000	0.0203 [a] ±0.000	ns
Aqua2=0.006 : 0.022	0.0309 [g] ±0.000	0.0370 [j] ±0.000	0.0320 [h] ±0.000	0.0309 [g] ±0.000	ns
Aqua3=0.006 : 0.066	0.0309 [g] ±0.000	0.0246 [c] ±0.000	0.0236 [b] ±0.000	0.0276 [d] ±0.000	ns
Aqua4=0.03 : 0.022	0.0309 [g] ±0.000	ns	ns	dead	dead
Aqua5=0.09 : 0.066	0.0309 [g] ±0.000	dead	dead	dead	dead

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values.

Control = no metals added. ns = no sample.

In Table 57, the lipid fraction ranges from 0.0203 (aquarium 1, day 6) to 0.0370 g/g dry weight (aquarium 2, day 2). Comparing the control, lipid fraction is nearly in the same concentrations in all treatments. However, it is slowly decreased with increased time and mixtures of both metals increase. In addition, there are not sufficient samples and the plants died in aquarium 4 (the 6^{th} and the 8^{th} day) in aquarium 5 (the 2^{nd} day through the end of experiment, Table 57). It can be concluded that mixtures of the metals are minor effect on lipid synthesis in the seaweed.

Effect of Cu+Pb on fatty acid profile

The most fatty acid proportions found is C16:0 (39.1737%, aquarium 2, day 6), and far followed by C18:1(n-9)c and C20:4(n-6), respectively (Table 58). Comparing control, palmitic acid is slowly decreased with increased time and mixtures of the metals increase. It can be concluded that synthesis of palmitic acid is inhibited by mixtures of both the metals.

Table 58 Major fatty acids (%) found in the blade of *S. polycystum* treated with Cu+Pb

Aqua.no./day	C14:0	C16:0	C16:1	C18:1(n-9)c	C18:2(n-6)c	C18:3(n-3)	C20:2	C20:4(n-6)	Sum-Sat.	Sum-Unsat.	Others	remark
0 - 5. 0 day	3.6352	37.5352	6.0598	21.0163	8.4937	4.8650	5.9827	12.4120	41.1704	58.8296		
0. 2 day	3.5352	37.3352	6.0098	20.0163	7.4937	4.8650	5.2827	11.4120	40.8704	55.0796	4.0500	
1. 2 day	4.3408	35.6014	6.5700	15.6248	6.7395	4.9166	5.3882	13.4019	39.9422	52.6409	7.4169	
2. 2 day	4.8135	32.2247	6.5762	13.7624	9.7449	4.2525	5.8644	15.4951	37.0382	55.6955	7.2663	
3. 2 day	4.8155	38.3887	7.2496	14.3654	7.5644	3.7807	5.5942	12.0857	43.2042	50.6400	6.1558	
4. 2 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
5. 2 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
0. 4 day	4.9503	33.2723	6.5502	14.7892	7.0968	5.2044	6.5242	13.6982	38.2225	53.8630	7.9144	
1. 4 day	4.3122	35.2039	5.5395	16.2256	5.6552	4.1267	4.7537	12.8007	39.5161	49.1014	11.3825	
2. 4 day	4.2903	35.0863	7.5380	14.3313	7.0989	4.4027	5.3552	13.3566	39.3766	52.0827	8.5407	
3. 4 day	4.4730	34.4673	6.8053	13.9793	6.9740	4.8942	5.4182	4.1682	38.9403	42.2392	18.8205	
4. 4 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
5. 4 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
0. 6 day	4.8142	36.1862	6.6130	14.5133	7.9282	4.6221	4.9625	13.5966	41.0004	52.2356	6.7640	
1. 6 day	5.3347	35.7684	7.3214	14.5584	9.6468	4.3055	5.8631	12.2178	41.1031	53.9130	4.9839	
2. 6 day	4.7177	39.1737	6.9378	16.7736	7.9188	3.8959	4.6621	13.8024	43.8914	53.9906	2.1180	
3. 6 day	4.8649	37.1188	6.8057	14.1125	7.2048	4.1112	4.5892	13.7486	41.9837	50.5721	7.4442	
4. 6 day	5.3153	36.2932	6.4810	15.2623	7.3031	4.7156	4.2417	13.3824	41.6085	51.3860	7.0054	
5. 6 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
0. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
1. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
2. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
3. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
4. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
5. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	

Effect of Cu+Zn on chlorophyll *a* and chlorophyll *c* concentrations

Chlorophyll *a* and chlorophyll *c* concentrations treated with mixtures of Cu and Zn at various concentrations are shown in Table 59.

Table 59 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Cu+Zn

Combined Metal 9 Cu : Zn (mg/l)	Chlorophyll *a* (μg.cm^{-3})				
	time (days)				
	0	2	4	6	8
Aqua0 = control	1.5089 ^{d} ±0.001	1.5338 ^{fg} ±0.000	1.5464 ^{ij} ±0.000	1.5390 ^{gh} ±0.000	1.5470 ^{ij} ±0.000
Aqua1=0.003 : 0.0044	1.5089 ^{d} ±0.001	1.5309 ^{ef} ±0.001	1.5676 ^{l} ±0.012	1.5542 ^{k} ±0.002	1.5690 ^{l} ±0.001
Aqua2=0.003 : 0.022	1.5089 ^{d} ±0.001	1.5264 ^{e} ±0.001	1.4815 ^{c} ±0.000	1.5324 ^{efg} ±0.000	1.5120 ^{d} ±0.000
Aqua3=0.003 : 0.066	1.5089 ^{d} ±0.001	1.5509 ^{jk} ±0.000	1.5428 ^{hi} ±0.000	1.3776 ^{a} ±0.001	1.5306 ^{ef} ±0.001
Aqua4=0.015 : 0.022	1.5089 ^{d} ±0.001	1.5268 ^{ef} ±0.000	1.5383 ^{gh} ±0.000	1.5106 ^{d} ±0.000	1.4649 ^{b} ±0.000
Aqua5=0.045 : 0.066	1.5089 ^{d} ±0.001	1.5632 ^{l} ±0.000	dead	dead	dead
Combined Metal 9 Cu : Zn (mg/l)	Chlorophyll *c* (μg.cm^{-3})				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.8468 ^{e} ±0.002	0.9647 ^{n} ±0.000	1.0824 ^{q} ±0.001	0.9400 ^{k} ±0.002	1.0604 ^{p} ±0.001
Aqua1=0.003 : 0.0044	0.8468 ^{e} ±0.002	0.9123 ^{i} ±0.001	1.0095 ^{o} ±0.001	0.8836 ^{h} ±0.004	0.9569 ^{m} ±0.002
Aqua2=0.003 : 0.022	0.8468 ^{e} ±0.002	0.9126 ^{i} ±0.001	0.7600 ^{c} ±0.001	0.8761 ^{g} ±0.001	0.8131 ^{d} ±0.002
Aqua3=0.003 : 0.066	0.8468 ^{e} ±0.002	0.9483 ^{l} ±0.001	0.9170 ^{j} ±0.002	0.6279 ^{a} ±0.001	0.8665 ^{f} ±0.001
Aqua4=0.015 : 0.022	0.8468 ^{e} ±0.002	0.9191 ^{j} ±0.001	0.9166 ^{j} ±0.002	0.8663 ^{f} ±0.001	0.7297 ^{b} ±0.001
Aqua5=0.045 : 0.066	0.8468 ^{e} ±0.002	1.3083 ^{r} ±0.001	dead	dead	dead

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications.

The letters are arranged in ascendant order as in the values. Control = no metals added.

In Table 59, concentrations of chlorophyll *a* and chlorophyll *c* range from 1.3776 (aquarium 3, day 6) to 1.5690 μg.cm^{-3} (aquarium 1, day 8) and 0.6279 (aquarium 3, day 6) to 1.3083 μg.cm^{-3} (aquarium 5, day 2), respectively. Comparing the start, the data show that both chlorophyll *a* and chlorophyll *c* concentrations are increased with increased time and increases in the control and low concentrations of Cu:Zn = 0.003:0.0044 mg/l. At high concentration of both metals, both chlorophylls are increased and then decreased at the 6th day of experiment and the seaweed was killed in aquarium 5 at the 4th day through the 8th day of experiments. It can be concluded that synthesis of both chlorophylls is activated by mixtures of Cu+Zn in a short period of time.

Effect of Cu+Zn on protein content and lipid fraction

Protein content and lipid fraction in the blade of *S. polycystum* treated with mixtures of Cu+Zn at various concentrations are shown in Table 60.

Total nitrogen content ranges from 0.1444 (aquarium 3, day 6) to 0.3719% per gram dry weight (control, day 8). The data show that crude protein in all treatments is nearly not significantly different at the 5% level. However, the plants were killed in aquarium 5 (Cu:Zn = 0.045:0.066 mg/l) at the 4th day through the 8th day of experiment. Similarly to other treatment with Cu, the seaweed was killed when the metal concentrations are higher than 0.045 mg/l. It can be concluded that mixtures of Cu:Zn are minor effect on protein synthesis in the plant cells (Table 60).

In Table 60, the lipid fraction is of the order of 0.0406 (aquarium 3, day 6) to 0.1444% g/g dry weight (aquarium 2, day 4). Comparing the control, lipid fraction is

decreased with increased time and mixtures of the metals increase. However, the plants were killed in aquarium 5 (Cu:Zn = 0.045:0.066 mg/l) as described above. It can be concluded that mixtures of the metals inhibit lipid synthesis in the plants (Table 60).

Table 60 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Cu+Zn

Combined metal 9 Cu : Zn (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.2035 ^{bcd} ±0.011	0.3326 ^{h} ±0.004	0.2079 ^{bcd} ±0.028	0.2057 ^{bcd} ±0.008	0.3719 ^{i} ±0.000
Aqua1=0.003 : 0.0044	0.2035 ^{bcd} ±0.011	0.2888 ^{g} ±0.004	0.2407 ^{def} ±0.008	0.1882 ^{b} ±0.004	0.2319 ^{cdef} ±0.022
Aqua2=0.003 : 0.022	0.2035 ^{bcd} ±0.011	0.2232 ^{bcde}	0.1925 ^{bc} ±0.017	0.1882 ^{b} ±0.004	0.2406 ^{def} ±0.013
Aqua3=0.003 : 0.066	0.2035 ^{bcd} ±0.011	0.2013 ^{bcd} ±0.008	0.2560 ^{efg} ±0.024	0.1444 ^{a} ±0.000	0.2188 ^{bcde}
Aqua4=0.015 : 0.022	0.2035 ^{bcd} ±0.011	0.2035 ^{bcd} ±0.002	0.2341 ^{cdef} ±0.017	0.2166 ^{bcde} ±0.011	0.2713 ^{fg} ±0.013
Aqua5=0.045 : 0.066	0.2035 ^{bcd} ±0.011	0.1882 ^{b} ±0.000	dead	dead	dead
Combined metal 9 Cu : Zn (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.1344 ^{o} ±0.000	0.0809 ^{gh} ±0.000	0.1325 ^{o} ±0.000	0.1130 ^{n} ±0.000	0.0793 ^{g} ±0.000
Aqua1=0.003 : 0.0044	0.1344 ^{o} ±0.000	0.0495 ^{c} ±0.000	0.0956 ^{k} ±0.000	0.1008 ^{l} ±0.000	0.0774 ^{f} ±0.000
Aqua2=0.003 : 0.022	0.1344 ^{o} ±0.000	0.0885 ^{j} ±0.000	0.1444 ^{p} ±0.000	0.0852 ^{i} ±0.000	0.0961 ^{k} ±0.001
Aqua3=0.003 : 0.066	0.1344 ^{o} ±0.000	0.0801 ^{g} ±0.000	0.0828 ^{h} ±0.000	0.0406 ^{a} ±0.000	0.0448 ^{b} ±0.005
Aqua4=0.015 : 0.022	0.1344 ^{o} ±0.000	0.0603 ^{d} ±0.000	0.0646 ^{e} ±0.000	0.0663 ^{e} ±0.000	0.0592 ^{d} ±0.000
Aqua5=0.045 : 0.066	0.1344 ^{o} ±0.000	0.1081 ^{m} ±0.000	dead	dead	dead

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values. Control = no metals added.

Effect of Cu+Zn on fatty acid profile

The most fatty acid proportions found is $C_{16:0}$ (40.2284%, aquarium 4, day 4), and far followed by C18:1(n-9)c and C20:4(n-6), respectively. Comparing the control, palmitic acid is increased with time and mixtures of the metals increased. However, there are no plant samples in some treatments and the plants were killed when copper concentrations are higher than 0.045 mg/l (Table 61). It can be concluded that mixtures of both metals promote synthesis of palmitic acid.

Table 61 Major fatty acids (%) found in the blade of *S. polycystum* treated with Cu+Zn

Aqua.no./day	C14:0	C16:0	C16:1	C18:1(n-9)c	C18:2(n-6)c	C18:3(n-3)	C20:2	C20:4(n-6)	Sum-Sat.	Sum-Unsat.	Others	remark
0 - 5. 0 day	4.0821	32.3928	5.1094	14.1436	5.4511	5.4920	6.4766	17.7447	36.4749	54.4175	9.1076	
0. 2 day	4.4941	32.1325	5.3187	13.4947	8.1574	4.1292	4.9827	16.0063	36.6266	52.0888	11.2846	
1. 2 day	3.7761	31.9790	5.3055	13.2184	7.3468	4.6585	6.4771	18.1528	35.7551	55.1591	9.0858	
2. 2 day	3.8663	31.7810	5.1243	14.0355	5.8296	4.7933	5.6243	20.1826	35.6473	55.5896	8.7631	
3. 2 day	3.7139	31.8245	4.8237	16.8790	7.1292	4.6721	5.7958	17.3741	35.5384	56.6740	7.7876	
4. 2 day	4.3547	35.4180	6.4679	15.6345	6.5898	4.7489	4.1380	14.3189	39.7726	51.8981	8.3293	
5. 2 day	4.8888	35.8815	6.286	14.5313	6.0884	3.4119	4.8349	14.915	40.7703	50.0676	9.1621	
0. 4 day	4.4238	33.4404	5.2016	14.3853	7.9054	4.1123	4.6072	14.7098	37.8642	50.9216	11.2142	
1. 4 day	4.5126	34.9920	6.0379	15.1080	6.5827	5.0827	4.5303	14.1740	39.5046	51.5155	8.9799	
2. 4 day	3.7477	34.9116	5.2223	16.8667	5.6996	4.1644	3.9802	15.0751	38.6593	51.0083	10.3323	
3. 4 day	4.0948	35.2224	5.5451	15.9303	6.9544	5.2712	4.4947	12.5735	39.3171	50.7692	9.9137	
4. 4 day	4.6035	40.2284	6.9039	17.3504	5.9414	3.3332	2.7573	11.7488	44.8320	48.0350	7.1330	
5. 4 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
0. 6 day	4.3593	37.3544	5.7121	14.0545	7.0519	3.9649	4.1177	13.1107	41.7138	48.0117	10.2745	
1. 6 day	4.8162	35.0202	6.5383	15.4329	6.8364	4.3852	3.7233	13.8972	39.8363	50.8132	9.3505	
2. 6 day	4.0584	36.7471	4.8615	18.5748	6.2218	3.8177	3.8469	13.0801	40.8054	50.4028	8.7918	
3. 6 day	4.3340	38.8746	6.8019	17.0406	6.2109	3.8301	2.7340	12.1940	43.2086	48.8115	7.9799	
4. 6 day	4.8186	39.5950	6.2905	18.5853	6.2262	2.9919	2.5365	11.2123	44.4136	47.8427	7.7437	
5. 6 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
0. 8 day	5.4291	39.4008	6.7762	15.3772	6.5607	3.1433	2.4460	12.8175	44.8299	47.1209	8.0492	
1. 8 day	3.8134	36.5469	6.2250	14.3853	7.2327	4.9761	4.4792	14.2149	40.3603	51.5132	8.1265	
2. 8 day	3.9527	36.3968	5.2486	14.4673	5.8059	3.9820	4.3150	15.7357	40.3494	49.5545	10.0961	
3. 8 day	4.0447	35.8947	6.2105	16.9973	7.3193	3.7134	3.4818	13.2391	39.9393	50.9613	9.0994	
4. 8 day	4.1464	39.5146	6.6380	16.3730	7.0049	3.1902	2.5000	12.3279	43.6610	48.0339	8.3051	
5. 8 day	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	

Effect of Pb+Zn on chlorophyll *a* and chlorophyll *c* concentrations

Chlorophyll *a* and chlorophyll *c* concentrations treated with mixtures of Pb and Zn at various concentrations are shown in Table 62.

Table 62 Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* treated with Pb+Zn

Combined metal 10 Pb : Zn (mg/l)	Chlorophyll *a* ($\mu g.cm^{-3}$) time (days)				
	0	2	4	6	8
Aqua0 = control	1.4683 ^{k} ±0.007	1.4501 ^{ij} ±0.002	1.4605 ^{jk} ±0.001	1.4849 ^{l} ±0.005	1.4437 ^{hi} ±0.000
Aqua1=0.009 : 0.0044	1.4683 ^{k} ±0.007	1.5207 ^{o} ±0.005	1.4172 ^{e} ±0.000	1.4202 ^{ef} ±0.001	1.5060 ^{n} ±0.001
Aqua2=0.009 : 0.022	1.4683 ^{k} ±0.007	1.4595 ^{jk} ±0.001	1.4995 ^{mn} ±0.002	1.5183 ^{o} ±0.001	1.3696 ^{c} ±0.001
Aqua3=0.009 : 0.066	1.4683 ^{k} ±0.007	1.3675 ^{c} ±0.004	1.4270 ^{ef} ±0.001	1.4384 ^{gh} ±0.001	1.3955 ^{d} ±0.000
Aqua4=0.045 : 0.022	1.4683 ^{k} ±0.007	1.4002 ^{d} ±0.000	1.3384 ^{b} ±0.001	1.4451 ^{hi} ±0.002	1.2390 ^{a} ±0.001
Aqua5=0.135 : 0.066	1.4683 ^{k} ±0.007	1.4943 ^{lm} ±0.001	1.3379 ^{b} ±0.000	1.4286 ^{fg} ±0.002	1.4636 ^{k} ±0.001
Combined metal 10 Pb : Zn (mg/l)	Chlorophyll *c* ($\mu g.cm^{-3}$) time (days)				
	0	2	4	6	8
Aqua0 = control	0.7304 ^{ij} ±0.020	0.6927 ^{gh} ±0.002	0.7208 ^{hij} ±0.001	0.7876 ^{k} ±0.000	0.7033 ^{hi} ±0.002
Aqua1=0.009 : 0.0044	0.7304 ^{ij} ±0.020	0.8406 ^{lm} ±0.005	0.6477 ^{ef} ±0.002	0.6352 ^{de} ±0.001	0.8151 ^{l} ±0.001
Aqua2=0.009 : 0.022	0.7304 ^{ij} ±0.020	0.6920 ^{gh} ±0.003	0.8136 ^{l} ±0.001	0.8474 ^{m} ±0.003	0.5941 ^{bc} ±0.001
Aqua3=0.009 : 0.066	0.7304 ^{ij} ±0.020	0.5851 ^{b} ±0.002	0.6421 ^{ef} ±0.001	0.6390 ^{de} ±0.003	0.6487 ^{ef} ±0.001
Aqua4=0.045 : 0.022	0.7304 ^{ij} ±0.020	0.6386 ^{de} ±0.001	0.6200 ^{cde} ±0.001	0.7198 ^{hij} ±0.002	0.5542 ^{a} ±0.004
Aqua5=0.135 : 0.066	0.7304 ^{ij} ±0.020	0.7437 ^{j} ±0.001	0.6686 ^{fg} ±0.001	0.6113 ^{bcd} ±0.002	0.7051 ^{hi} ±0.001

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values. Control = no metals added.

In Table 62, concentrations of chlorophyll *a* and chlorophyll *c* range from 1.2390 (aquarium 4, day 8) to 1.5207 μg.cm^{-3} (aquarium 1, day 2) and 0.5542 (aquarium 4, day 8) to 0.8474 μg.cm^{-3} (aquarium 2, day 6), respectively. Comparing the start, the data show that concentrations of both chlorophyll *a* and chlorophyll *c* are decreased with increased time and mixtures of the metals increase. It can be concluded that synthesis of both chlorophylls is limited by mixtures of Pb+Zn.

Effect of Pb+Zn on protein content and lipid fraction

Protein content and lipid fraction in the blade of *S. polycystum* treated with mixtures of Pb+Zn at various concentrations are shown in Table 63. In Table 63, concentrations of protein range from 0.3063 (aquarium 5, day 8) to 0.5557% per 2 grams dry weight (start). The data show that crude protein in some treatments is nearly not significant differences at the 5% level. However, nitrogen content is decreased with increased time and mixtures of the metals increase (Table 63). It is clear that synthesis protein in the plant cells is inhibited by mixtures of Pb:Zn.

The lipid fraction is of the order of 0.0178 (aquarium 1, day 4) to 0.0523 g/g dry weight (control, day 8). Comparing the control, lipid fraction in all treatments, except control, is mostly not significant differences at the 5% level (Table 63). It can be concluded that mixtures of the metals are less effect on lipid synthesis in the seaweed.

Effect of Pb+Zn on fatty acid profile

The most fatty acid proportions found is C16:0 (39.2111%, aquarium 3, day 4), and far followed by C18:1(n-9)c and C20:4(n-6), respectively. Comparing control, palmitic acid in all treatments is increased with increased time and mixtures of the

metals increase (Table 64). It can be concluded that synthesis of palmitic acid is activated by mixtures of both metals.

Table 63 Nitrogen and lipid fraction in the blade of *S. polycystum* treated with Pb+Zn

Combined metal 10 Pb : Zn (mg/l)	% total nitrogen (g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.2779 ^{o} ±0.002	0.2713 ^{no} ±0.004	0.2582 ^{lmno} ±0.004	0.2538 ^{klmn} ±0.004	0.2472 ^{klm} ±0.002
Aqua1=0.003 : 0.0044	0.2779 ^{o} ±0.002	0.2621 ^{no} ±0.004	0.2232 ^{ij} ±0.004	0.2166 ^{hi} ±0.002	0.2101 ^{ghi} ±0.004
Aqua2=0.003 : 0.022	0.2779 ^{o} ±0.002	0.2604 ^{mno} ±0.006	0.2035 ^{efgh} ±0.015	0.2013 ^{defgh}	0.1969 ^{defgh}
Aqua3=0.003 : 0.066	0.2779 ^{o} ±0.002	0.2625 ^{no} ±0.000	0.2057 ^{fghi} ±0.008	0.1926 ^{cdefg}	0.1816 ^{cd} ±0.002
Aqua4=0.015 : 0.022	0.2779 ^{o} ±0.002	0.2407 ^{jkl} ±0.013	0.1904 ^{cdefg}	0.1838 ^{cde} ±0.004	0.1619 ^{ab} ±0.002
Aqua5=0.045 : 0.066	0.2779 ^{o} ±0.002	0.2363 ^{jk} ±0.013	0.1882 ^{cdef} ±0.008	0.1729 ^{bc} ±0.008	0.1532 ^{a} ±0.004
Combined metal 10 Pb : Zn (mg/l)	Lipid fraction (g/g dw)				
	time (days)				
	0	2	4	6	8
Aqua0 = control	0.0341 ^{fg} ±0.000	0.0318 ^{f} ±0.000	0.0231 ^{bc} ±0.000	0.0372 ^{h} ±0.000	0.0523 ^{l} ±0.000
Aqua1=0.003 : 0.0044	0.0341 ^{fg} ±0.000	0.0361 ^{gh} ±0.000	0.0178 ^{a} ±0.000	0.0363 ^{gh} ±0.000	0.0462 ^{j} ±0.000
Aqua2=0.003 : 0.022	0.0341 ^{fg} ±0.000	0.0274 ^{de} ±0.000	0.0508 ^{kl} ±0.005	0.0408 ^{i} ±0.000	0.0289 ^{e} ±0.000
Aqua3=0.003 : 0.066	0.0341 ^{fg} ±0.000	0.0276 ^{de} ±0.000	0.0483 ^{jk} ±0.000	0.0286 ^{e} ±0.000	0.0322 ^{f} ±0.000
Aqua4=0.015 : 0.022	0.0341 ^{fg} ±0.000	0.0256 ^{cd} ±0.000	0.0218 ^{b} ±0.000	0.0381 ^{h} ±0.000	0.0355 ^{gh} ±0.000
Aqua5=0.045 : 0.066	0.0341 ^{fg} ±0.000	0.0268 ^{de} ±0.000	0.0269 ^{de} ±0.000	0.0333 ^{fg} ±0.000	0.0290 ^{e} ±0.000

[a] Values in horizontal row followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range. Each value is the mean of 2 replications. The letters are arranged in ascendant order as in the values. Control = no metals added.

Table 64 Major fatty acids (%) found in the blade of *S. polycystum* treated with Pb+Zn

Aqua.no./day	C14:0	C16:0	C16:1	C18:1(n-9)c	C18:2(n-6)c	C18:3(n-3)	C20:2	C20:4(n-6)	Sum-Sat.	Sum-Unsat.	Others	C20:5(n-3)
0 - 5, 0 day	4.1453	31.2374	5.6634	12.1735	5.7900	4.4825	6.8127	17.2252	35.3827	52.1473	12.4700	4.1133
0, 2 day	3.4036	28.1534	4.8389	11.3801	7.4277	4.1485	7.0122	16.0553	31.5569	50.8627	17.5804	
1, 2 day	3.5476	30.8228	4.7915	13.1386	6.5732	4.6847	7.5232	17.4704	34.3704	54.1816	11.4480	
2, 2 day	3.7735	29.9984	4.9027	14.1381	6.8079	3.5920	6.0839	16.8866	33.7719	52.4113	13.8168	3.1412
3, 2 day	3.7297	32.5811	7.3124	12.0047	5.5327	4.3309	6.5269	15.6178	36.3108	51.3254	12.3638	3.5674
4, 2 day	5.4698	33.4257	7.2666	12.0006	7.1750	6.0430	5.3402	13.1507	38.8955	50.9761	10.1284	2.9572
5, 2 day	3.6347	33.8814	7.1231	11.471	5.5627	4.1948	5.8479	15.3932	37.5162	49.5927	12.8911	3.7097
0, 4 day	4.4440	37.9298	5.4775	16.3342	6.5205	4.6192	4.6841	15.1999	42.3737	52.8355	4.7908	
1, 4 day	4.0466	34.8569	7.7984	14.2812	6.4244	4.3587	5.6126	15.5918	38.9035	54.0671	7.0294	3.757
2, 4 day	5.7726	33.7259	10.2310	11.6808	3.9246	4.4205	6.6853	8.8449	39.4985	45.7871	14.7144	
3, 4 day	3.7119	39.2111	7.4575	15.0290	6.5000	3.6140	3.6284	12.3237	42.9230	48.5526	8.5244	
4, 4 day	4.3333	33.0683	5.7624	13.8664	6.2533	4.4212	6.2615	16.5215	37.4015	53.0863	9.5121	
5, 4 day	4.3258	34.7136	5.6969	15.5006	6.7568	4.2446	5.2067	15.6447	39.0393	53.0502	7.9104	
0, 6 day	3.9894	36.5480	6.5464	13.9859	7.7752	4.7908	6.4230	15.6870	40.5374	55.2083	4.2543	
1, 6 day	3.5539	33.3018	6.3290	13.1393	16.7202	3.6570	7.1557	11.7914	36.8557	58.7926	4.3516	
2, 6 day	4.5000	35.9176	5.5636	13.2640	6.9334	4.5849	4.5835	15.0774	40.4176	50.0068	9.5756	
3, 6 day	3.5616	34.4606	6.2167	13.1294	7.1109	3.7804	5.9007	13.7808	38.0222	49.9188	12.0589	3.4118
4, 6 day	3.7982	37.4879	6.3715	15.5436	7.3744	3.2272	3.7904	14.1916	41.2862	50.4988	8.2151	
5, 6 day	3.5596	33.4871	5.245	16.0331	6.6803	3.4759	5.5052	14.39	37.0467	51.3294	11.6239	
0, 8 day	3.8950	33.4893	5.2593	14.8130	6.1466	5.2375	5.9992	15.6550	37.3843	53.1107	9.5050	3.208
1, 8 day	4.7486	37.7536	5.6843	16.9919	6.8098	5.5257	4.9935	14.7785	42.5022	54.7837	2.7141	
2, 8 day	4.3292	33.7354	5.6462	15.4259	6.9471	4.2241	5.5073	15.8824	38.0646	53.6330	8.3024	
3, 8 day	3.5077	36.5537	6.0761	15.2971	6.2433	3.7069	4.7426	13.7605	40.0614	49.8265	10.1121	
4, 8 day	3.8262	37.9626	6.3580	15.6258	6.9086	3.6470	4.4051	14.5491	41.7888	51.4936	6.7176	
5, 8 day	4.0453	34.5569	5.858	14.299	6.95	4.8906	5.6876	14.3777	38.6022	52.063	9.3349	

In this study (in the control), concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *Sargassum polycystum* are of the order of 0.5405 – 1.5405 $\mu g.cm^{-3}$ and 0.1586 – 0.8468 $\mu g.cm^{-3}$, respectively. It is noted that the concentrations show the same correlation. Factors affecting of both chlorophyll concentrations are sunlight which related to season of the year, turbidity of the water, as well as a depth of the samples collected. After treatment with single or combined metals, in contrast, the concentrations will change depending on the metals (Tables 35, 65).

Little information on seaweed protein is available. Fleurence (1999) found that higher protein levels occurred during the end of the winter and spring whereas lower amounts are recorded during the summer. Protein contents in some seaweeds are reported. For example, *Palmaria palmata* (from the French Atlantic coast) ranges from 9 – 25 % (dry weight), while *Undaria pinnatifida* ranges from 11 – 14 % dry weight. In *Enteromorpha compressa*, *Padina pavonica*, *Ulva lactuca* and *Laurencia obtusa*, the protein levels are 13.6 %, 17.4 %, 17.6% and 24.5 %, respectively. In general, concentrations of protein in brown algae are far lower than in green and red algae (Wahbeb, 1977). Wong and Cheung (2001a, 2001b) reported that the protein content in *Sargassum hemiphyllum*, *S. patens*, *S. henslowianum* is of the order of 5.03 – 5.33, 7.56 – 8.20 and 11.3 – 100 g/100 g dry weight, respectively.

In this study (in the control), concentration of protein in the blade of *Sargassum polycystum* ranges from 0.3457 – 0.9188% per gram dry weight. It is noted that the protein content in other *Sargassum* species is of the same content. However, concentration of protein should vary depending on photosynthesis of the

plants. After treatment with both single and combined metals, protein content changed depending on the metals (Tables 35, 65).

There is little information about the lipid fraction in any seaweed, especially *Sargassum* species. Heiba *et al*. (1997) found that the crude lipids from four *Sargassum* species range from 0.02 – 0.04 g/g dry weight. In this study (in the control), the lipid fraction is of the order of 0.0341 – 0.8303 g/g dry weight. Similarly to other secondary products, the lipid fraction should vary depending on species of seaweeds, location, season of the year and activities of the cells (Li *et al*. 2002, Heiba *et al*. 1997). In addition, after treatment with both single and combined metals, the lipid fraction changed depending on the metals (Tables 35, 65).

Fatty acids found in *Sargassum* species range from C12:0 to C24:0. The C14:0, C16:0, C18:1, C18:2, C20:2 and C20:1 fatty acids were dominant. The most common fatty acid is C16:0 or palmitic acid. It is of the order of 10.4 to 49.5% (Li, *et al*., 2002; Zhukova and Svetashev, 1999; Heiba, *et al*., 1997; Vaskovsky, *et al*., 1996; Khotimchenko, 1991; and Arao and Yamada, 1989). Total saturated fatty acids (unbranched) are of the order of 14.0 – 66.4%, depending on species, location and season of the year (Tables 4,5).

In this study (in the control), palmitic acid ranges from 31.2374 to 37.9497% and may be decreased or increased after treatment with both single or combined metals. Other prominent fatty acids in order of decreasing are C20:4(n-6) ≥ C18:1(n-9)c >> C18:2(n-6)c ≥ C16:1, whilst, total unsaturated (branched) and saturated (unbranched) fatty acids are of the order of 46.6492 – 58.8296%, respectively. They are increased or decreased depending on the effect of both single and combined metals.

The effects of all combined metals on chlorophyll *a* and chlorophyll *c* concentration, crude protein, lipid fraction and fatty acid profile can be concluded as show in Table 65.

Table 65 Effects of combined metals on physiological changes in *S. polycystum*

Metals	Chlorophyll *a*	Chlorophyll *c*	Protein	Lipid	Palmitic acid
Cd + Cr	no effect	no effect	no effect	decrease	decrease
Cd + Cu	decrease	decrease	no effect	decrease	decrease
Cd + Pb	increase	increase	decrease	decrease	increase
Cd + Zn	increase	increase	decrease	increase	ns
Cr + Cu	increase (low)	increase (low)	decrease	increase (l.)	increase (low)
Cr + Pb	increase	increase	no effect	increase	decrease
Cr + Zn	increase	increase	no effect	decrease	no effect
Cu + Pb	increase (low)	increase (low)	decrease	no effect	decrease
Cu + Zn	increase (low)	increase (low)	no effect	decrease	increase
Pb + Zn	decrease	decrease	decrease	no effect	increase

ns = no samples

In Table 65, concentrations of both chlorophylls show a response to combined metals in the same direction. The chlorophyll concentrations are promoted (synthesis) when treated with Cd+Pb, Cd+Zn, Cr+Pb and Cr+Zn in all metal ratios. In addition, synthesis of both chlorophylls is activated at low concentrations of Cr+Cu, Cu+Pb and Cu+Zn, but it is inhibited at high concentrations of these combined metals. On the other hand, mixtures of Cd+Cu and Pb+Zn inhibit synthesis of both chlorophylls. However, mixtures of Cd+Cr showed no effect on chlorophyll synthesis.

According to Strömgren (1980a), studying *Ascophyllum nodosum*, it was found that mixtures of Cu and Zn showed an antagonistic effect on growth of the seaweed and had far more toxic effects than Hg+Zn and Cu+Hg. In addition, high concentrations of Pb and Cd inhibited Cu uptake.

In table 35 and 65, it can be concluded that Cd+Cr and Cd+Cu showed antagonist effects on all physiological changes except no data on palmitic acid synthesis. In other combined metals, they showed various effects on physiological changes. For example, Cd+Zn showed synergistic effect on protein synthesis but showed antagonistic effect on lipid synthesis. Cr+Pb showed antagonistic effect on protein synthesis. Cr+Zn showed antagonistic effects on protein and palmitic acid synthesis. Cu+Zn showed antagonistic effect on protein synthesis, and Pb+Zn showed antagonistic effect on lipid synthesis.

4.2.3 Ultrastructural study

In internal structure, it is known that seaweeds do not have a vascular system. They only have thalli composted of holdfast, stipe and blade instead of root, stem and blade, respectively. Plants in the order Fucales, especially the family Sargassaceae, are submerged and the blade's surface is unifacial. Therefore, both upper and lower epidermises are similar, and there are no stomates on either surface. The epidermal cell surface is slightly convex (Figs. 21, 22, 23). In the mature blade, the plants produce hollow chambers called cryptostoma embedded in the blade's surface on both sides (Figs. 21, 22), small spots if looking with naked eyes. Because the cryptostoma are sterile conceptacles, they are full of paraphyses (Fig. 23).

After treatment with different metals and different concentrations, the outstanding changes of the blade's surface, especially at the cryptostoma, are observed.

When treated with Cd, the blade's surface is not significantly changed (Fig. 24 compared with Figs. 21, 22). However, the epidermal cells are slightly flattened caused by the metal treatment at low concentration, 0.3 mg/l, 100 times above the metal in the water column, and for a short period of time (8 days).

Similarly to the Cd experiments, the blade's surface, treated with Cr, 0.03 mg/l, 100 times that of the water column and on the 7^{th} day of the experiment, did not show significantly changes. However, the cells surounding the cryptostoma seemed to be inhibited in growth (Fig. 25).

After treatment with 0.15 mg/l of Cu, 100 times that of the water column, and on the 4^{th} day of the experiment, cryptostoma were completely inhibited in development and most paraphyses were destroyed (Fig. 26).

Like treatement with Cu, when treated with 0.2250 mg/l of Zn, 100 times that of the seawater, cryptostoma were inhibited in growth and finally destroyed (Fig. 27).

In can be concluded that most metals, in low concentrations, do not obviously cause changes in paraphyses and cryptostoma. When treated with high concentrations of the metals, both paraphyses and cryptostoma are inhibited in growth and may be completely destroyed, especially when treated with Cu and Zn.

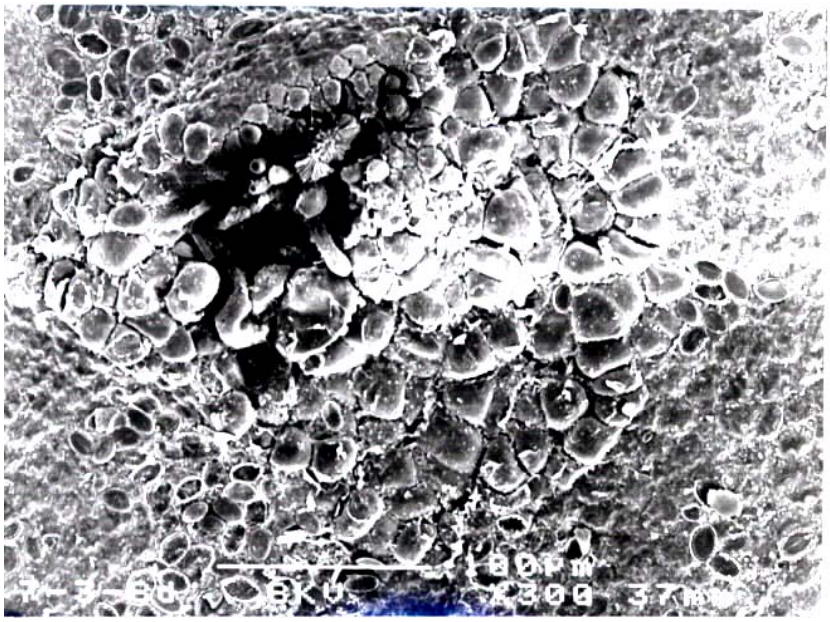

Fig. 21 SEM picture shows cryptostoma on the blade's surface (300X)

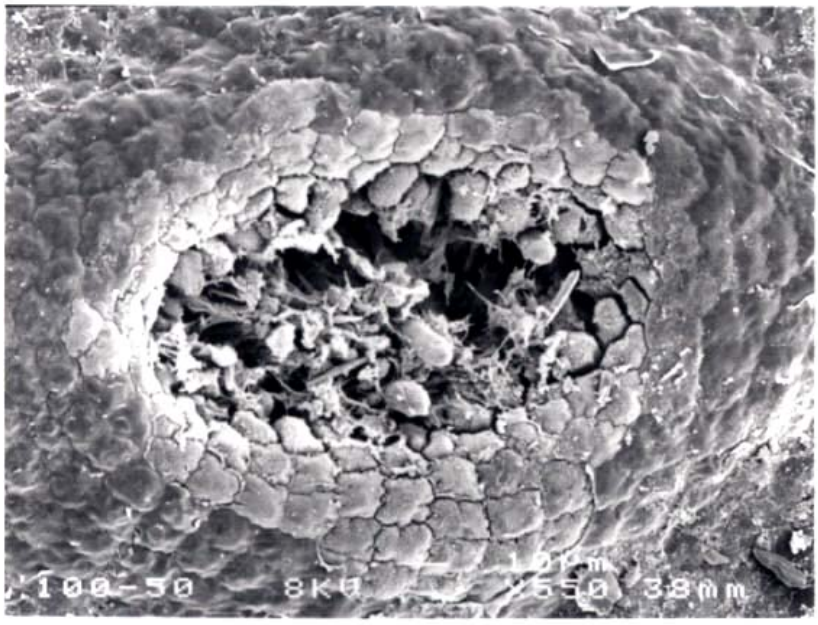

Fig. 22 SEM picture shows cryptostoma on the blade's surface (550X)

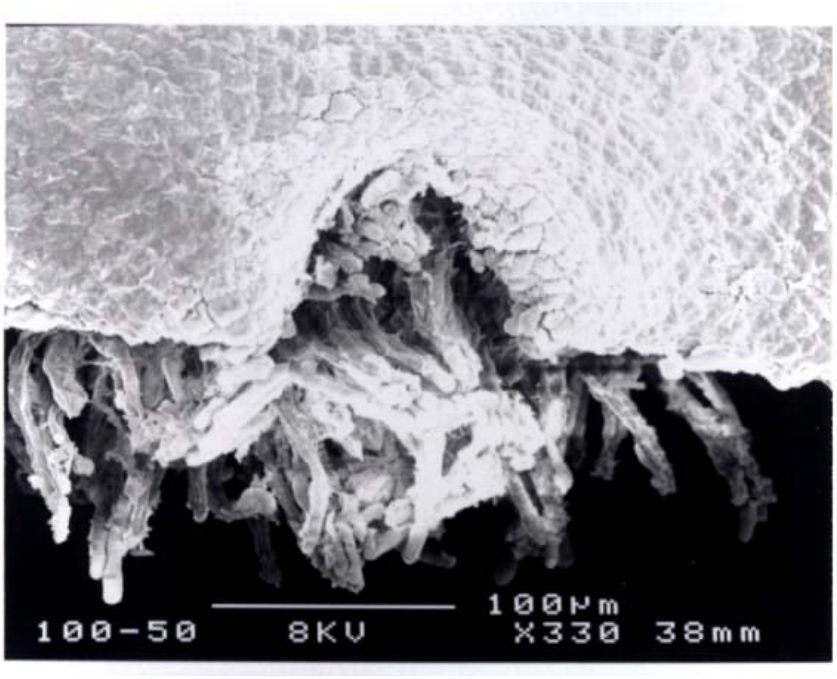

Fig. 23 SEM picture shows paraphyses in cryptostoma (330X)

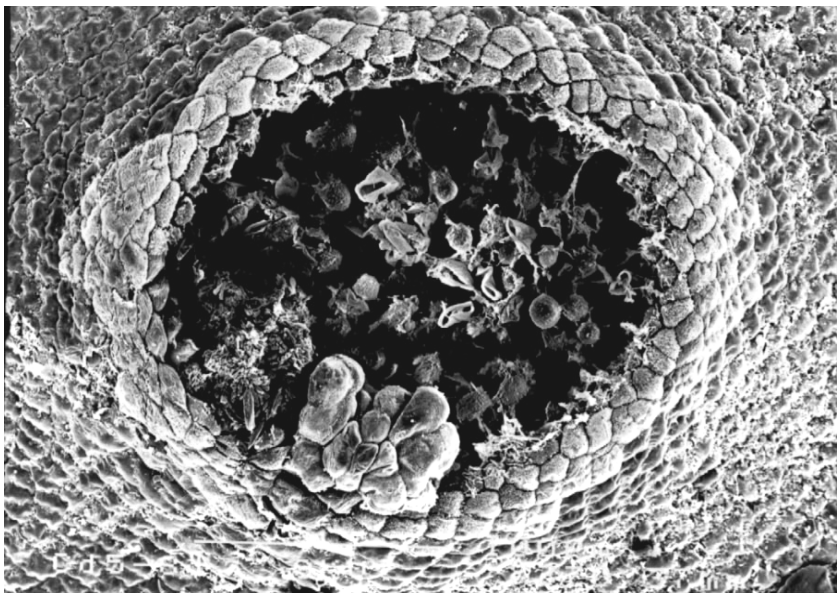

Fig. 24 SEM picture shows cryptostoma on the blade's surface treated with Cd 0.3 mg/l , 8th day (370X)

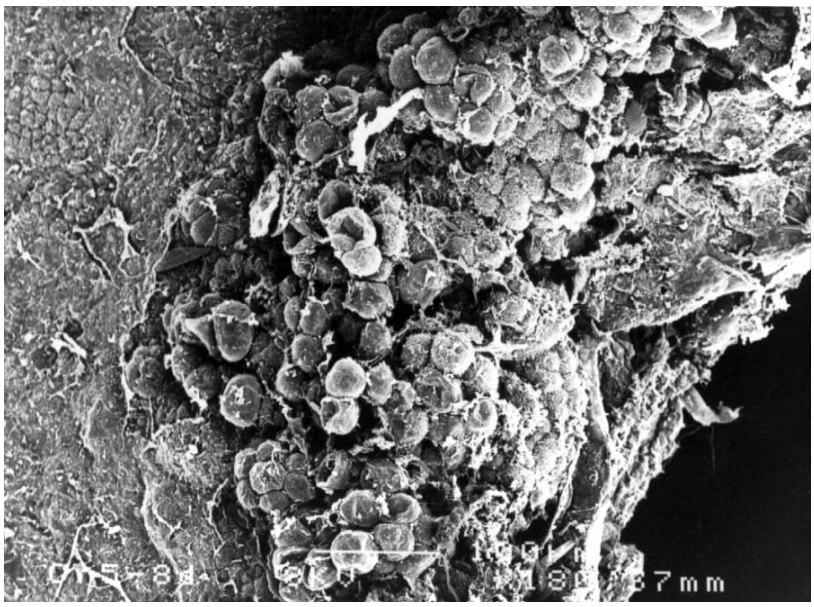

Fig. 25 SEM picture shows cryptostoma on the blade's surface treated with Cr 0.03 mg/l, 8th day (180X)

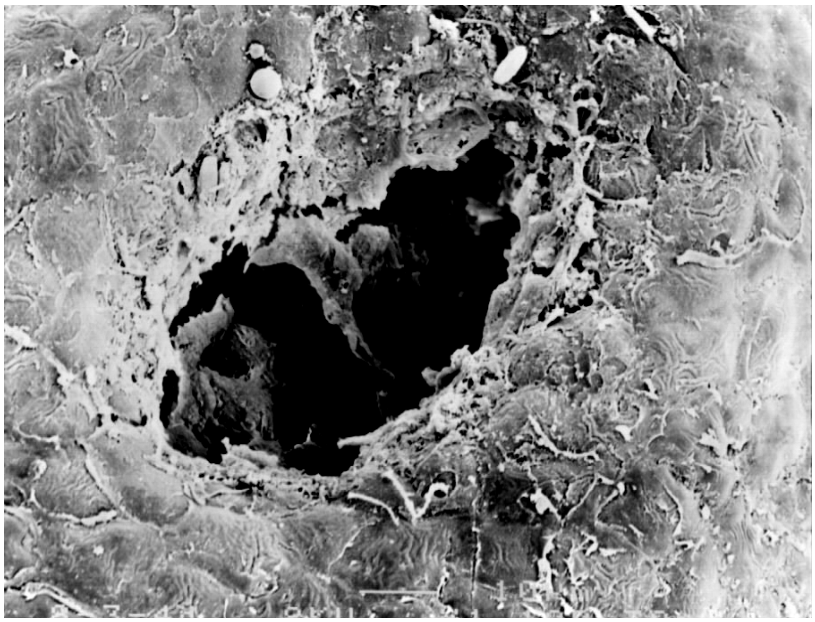

Fig. 26 SEM picture shows destroyed paraphyses in the cryptostoma treated with Cu 0.15 mg/l, 4th day (100X)

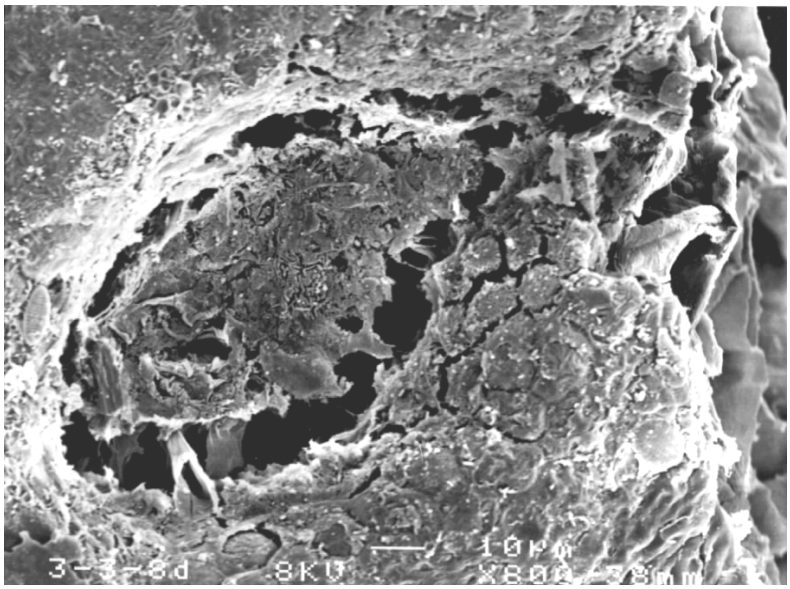

Fig. 27 SEM picture shows destroyed paraphyses in the cryptostoma treated with Zn 0.225 mg/l, 8th day (800X)

In internal structure, cells of *Sargassum polycystum*, similarly to the land plant cells, consists of a cell wall and organelles. The nuclei, chloroplasts and other organelles are enclosed in membranes. Chloroplast endoplasmic reticulum was not found. The chloroplasts are slightly round, oval or eliptical, consisting of longitudinal bands of thylakoids or discs. Membrane free areas are found between the chloroplast envelope (Brinkhuis and Chung, 1986) and thylakoids and also found among the discs (Figs. 30, 31). Other organelles are vacuoles, inclusions and so forth. However, normal nuclei are not observed. Some organelles such as Golgi apparatus, mitochondria are not found.

The most dramatic ultrastructural changes in the cells of this seaweed treated with different heavy metals and different concentrations were found in the cell wall, chloroplasts, and nuclei. However, the large osmophylic compounds (physode) in the vacuole are not obviously found.

There are some changes in the cell wall arrangement. The middle lamella are degenerated and then the wall slightly separated (Figs. 30, 31, 33). Loose arrangement of fibrils in the inner cell wall region and somewhat larger gaps are found in the cell wall (Figs. 30, 31, 33), which are different from the original intercellular space because of the remnant of fibrils in that area.

At the chloroplasts, slight swelling and detachment of the thylakoid membrane seemed to be the first symptom of toxicity, and the rupture and diffusion of the thylakoid membrane resulted in the regional swelling which caused the disruption of the parallel thylakoid band feature. The parallel pattern began to undulate (Fig. 30) and then was completely degenerated or destroyed (Figs. 31, 32, 33, 35). However, for some metals at low concentrations, such as Cu 0.03 mg/l, 4th day of experiments, the chloroplasts are not destroyed (Fig. 34).

High concentrations of metals are also effected on the nuclei and other organelles. The nuclei are destroyed (Fig. 33) and as are other organelles (Figs. 31, 32, 33, 35).

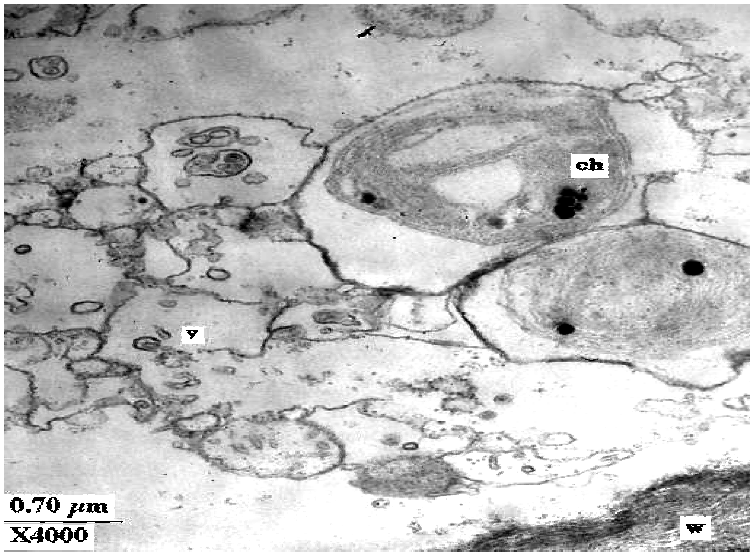

Fig. 28 TEM picture shows organelles in the cell; ch = chloroplast, w = cell wall and v = vesicles (4000X)

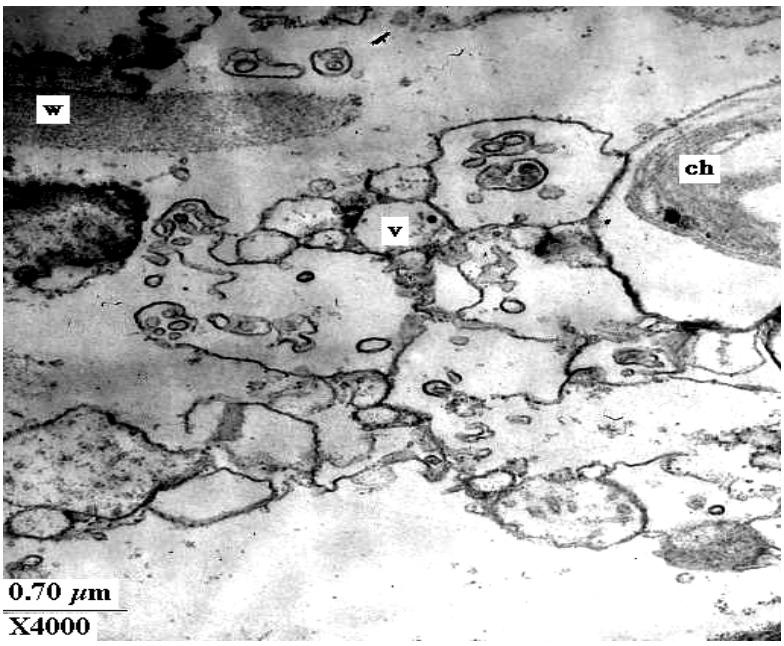

Fig. 29 TEM picture shows organelles in the cell; ch = chloroplast, w = cell wall and v = vesicles (4000X)

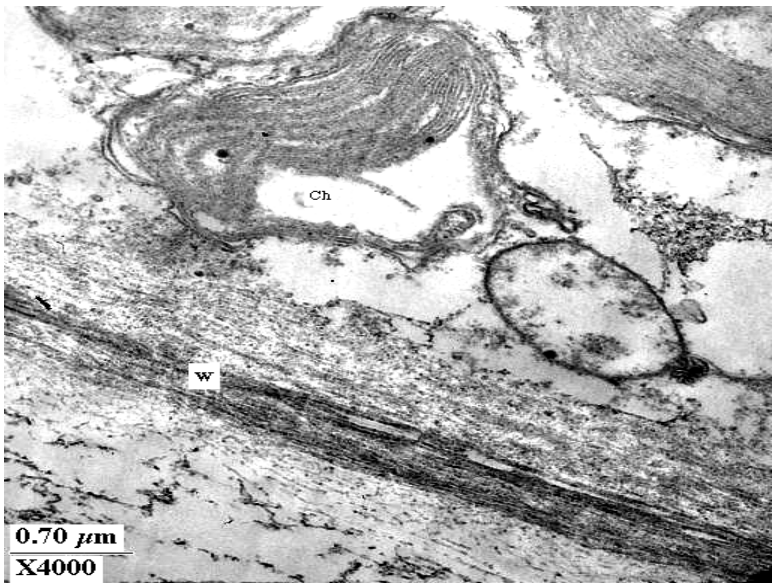

Fig. 30 TEM picture shows separated chloroplast (ch), cell wall (w) treated with Cd 0.3 mg/l, 8th day (4000X)

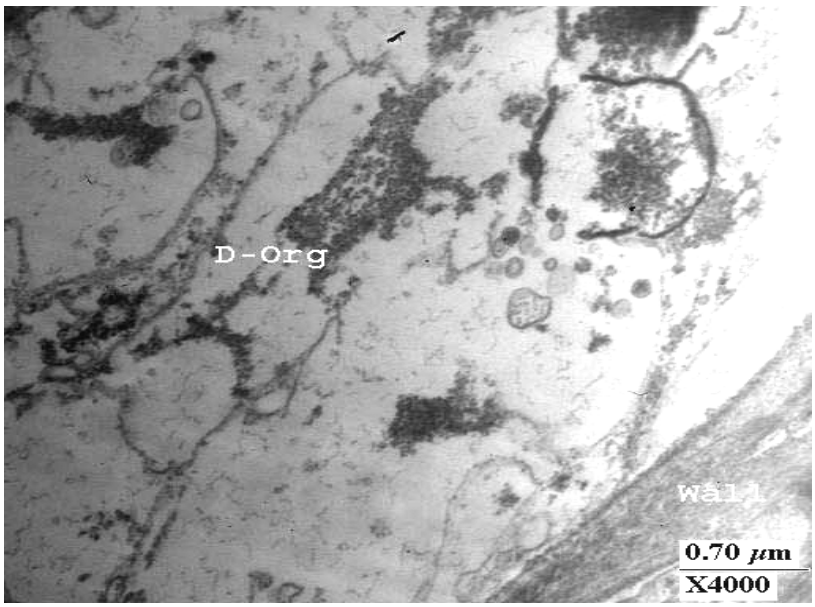

Fig. 31 TEM picture shows destroyed organelles (D-Org) treated with Cr 0.03 mg/l, 8th day (4000X)

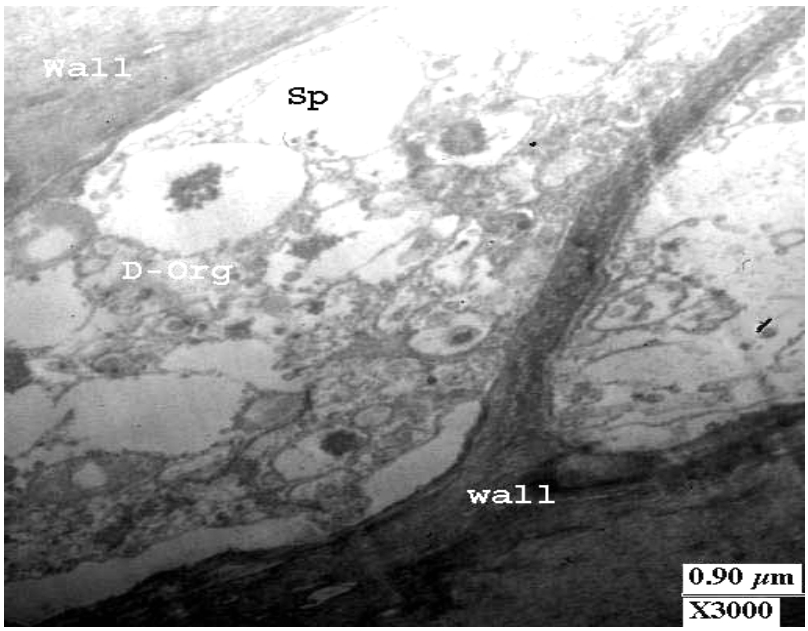

Fig. 32 TEM picture shows destroyed organelles (D-Org) treated with Cr 0.03 mg/l, 8th day (3000X)

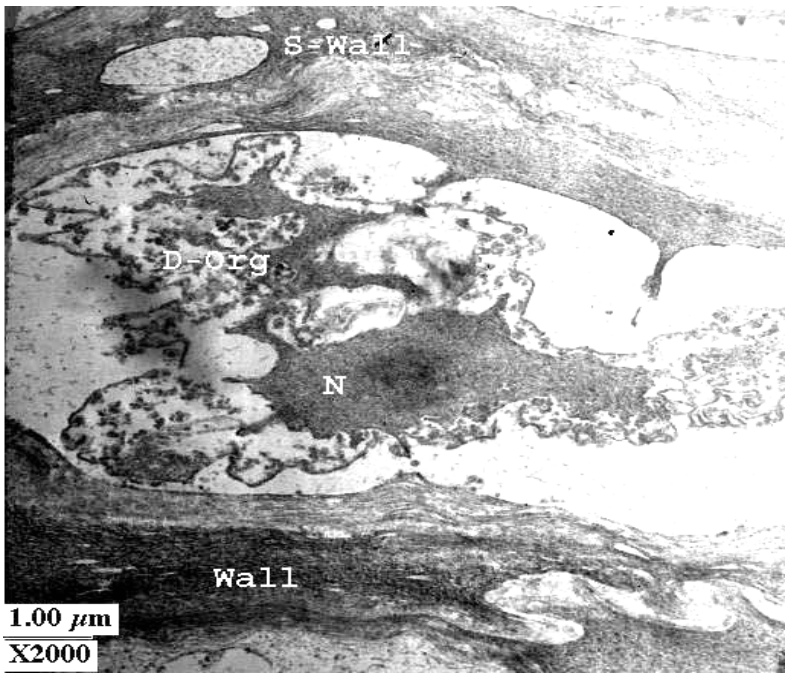

Fig. 33 TEM picture shows destroyed organelles (D-Org), destroyed nucleus (N), separated wall (S-Wall) treated with Cu 0.15 mg/l, 4th day (2000X)

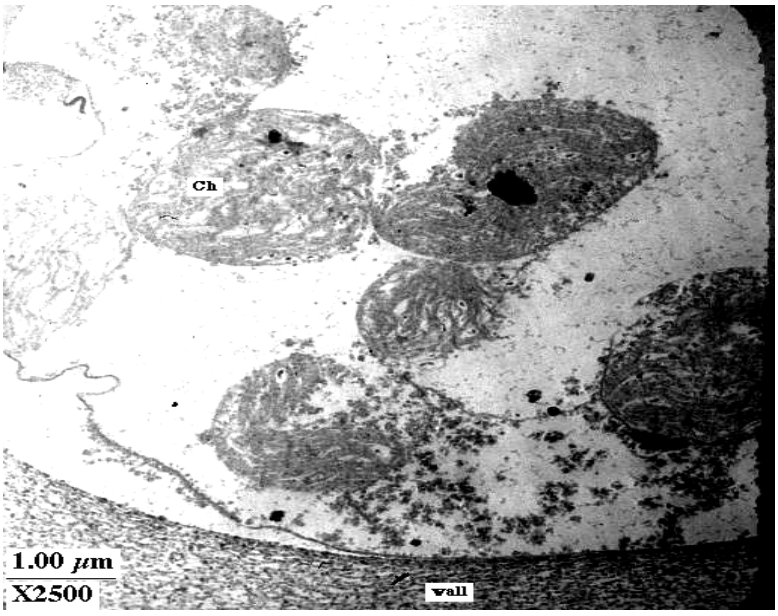

Fig. 34 TEM picture shows functional chloroplasts in such a cell treated with Cu 0.03 mg/l, 4th day (2500X)

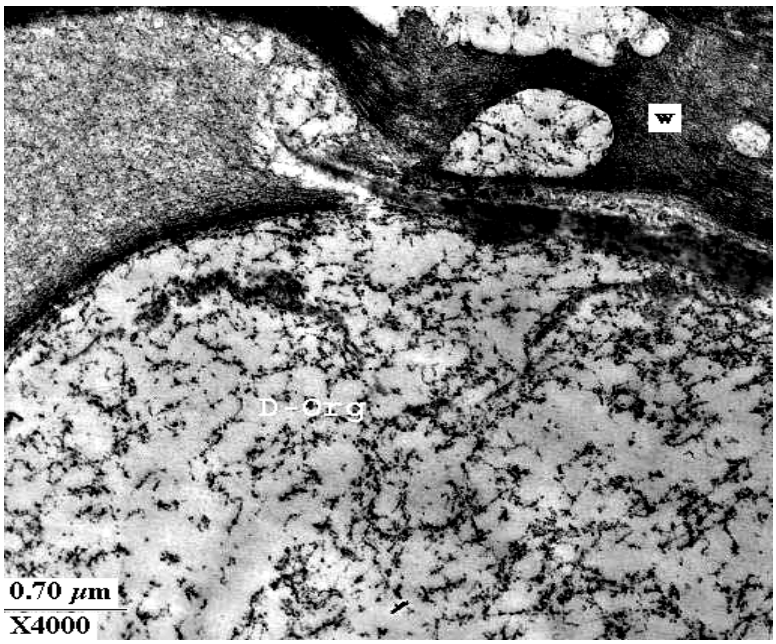

Fig. 35 TEM picture shows destroyed organelles (D-Org), large gaps in the cell wall (w) treated with Zn 0.225 mg/l, 8th day (4000X)

In this study, both treated and control specimens were prepared usign the same processes. Therefore, separated chloroplasts and destroyed organelles, such as in Figs. 31, 33 and 35 (treated) are not found in Figs. 28 and 29 (control). It is suggested that organelles were not separated by artifact but they are destroyed by heavy metals applied.

In conclusion, the blades of *Sargassum polycystum* are unifacial and have paraphyses in the cryptostoma. Growth of the paraphyses is inhibited and they may be destroyed at high concentrations of metal, especially when treated with Cu and Zn. The walls, in addition, are separated and organelles, including the nucleus, are destroyed when treated with high concentrations of metals.

CHAPTER V

CONCLUSIONS

The average concentrations of cadmium in the seawater collected from Phe and Laem Chabang are 0.0491 and 0.0998 μg/l, respectively. In the sediments, the average Cd concentrations collected form Phe and Laem Chabang are 2.5800 and 3.4033 μg/g. In *Enteromorpha clathrata* collected from Phe and Laem Chabang, the metal concentrations are 3.4910 and 6.5962 μg/g, respectively.

In the water collected from near *Padina japonica*, distributed only at Phe, the average Cd concentrations are 0.1092 μg/l. Meanwhile, the metal content in the sediments is 4.2716 μg/g. In the seaweed, the metal concentrations are 7.2681 and 7.2460 μg/g, in sporophytes and gametophytes, respectively.

In the water collected from near *Sargassum polycystum*, distributed only at Phe, the mean Cd concentrations are 0.1145 μg/l. In the sediment, the metal concentrations are 4.0081 μg/g. In the seaweed, the metal concentrations are 6.0855, 5.7682, 5.7025 and 5.6617 μg/g in the holdfast, stipe (stem), blade and air bladder, respectively.

The average total chromium concentrations in the seawater collected from Phe and Laem Chabang are 0.0604 and 0.2177 μg/l, respectively. In the sediments, the metal concentrations collected form Phe and Laem Chabang are 18.6658 and 7.0544

μg/g. The metal concentrations are 46.9621 and 21.2012 μg/g in *E. clathrata* collected from Phe and Laem Chabang, respectively.

In the water collected from near *P. japonica*, the average total Cr concentrations are 0.3021 μg/l. Meanwhile, the metal content in the sediments is 10.2768 μg/g. In the seaweed, the metal concentrations are 21.2121 and 7.2853 μg/g, in sporophytes and gametophytes, respectively.

In the water collected from near *S. polycystum*, the Cr concentrations are 0.0651 μg/l. In the sediment, the metal concentrations are 4.1784 μg/g. In the seaweed, the metal concentrations are 3.6172, 2.5925, 2.3304 and 3.5034 μg/g in the holdfast, stipe, blade and air bladder, respectively.

The mean copper concentrations in the seawater collected from Phe and Laem Chabang are 0.0522 and 0.0406 μg/l, respectively. In the sediments, the metal concentrations collected from Phe and Laem Chabang are 41.2521 and 5.8556 μg/g. In *E. clathrata* collected from Phe and Laem Chabang, the metal concentrations are 90.7974 and 41.8842 μg/g, respectively.

In the water collected from near *P. japonica*, the Cu concentrations are 0.0459 μg/l. Meanwhile, the metal content in the sediments is 4.6743 μg/g. In the seaweed, the metal concentrations are 11.6134 and 15.8848 μg/g, in sporophytes and gametophytes, respectively.

In the water collected from near *S. polycystum*, the Cu concentrations are 0.0480 μg/l. In the sediment, the metal concentrations are 2.9873 μg/g. In the seaweed, the metal concentrations are 6.3086, 6.9918, 13.5690 and 8.0889 μg/g in the holdfast, stipe, blade and air bladder, respectively.

The average lead concentrations in the seawater collected from Phe and Laem Chabang are 0.4241 and 0.3191 μg/l, respectively. In the sediments, the metal concentrations collected from Phe and Laem Chabang are 12.7423 and 9.2317 μg/g. In *E. clathrata* collected from Phe and Laem Chabang, the metal concentrations are 39.6466 and 34.9605 μg/g, respectively.

In the water collected from near *P. japonica*, distributed only at Phe, the average Pb concentrations are 0.3156 μg/l. Meanwhile, the metal concentrations in the sediments are 12.8924 μg/g. In the seaweed, the metal concentrations are 39.3204 and 23.5890 μg/g in sporophytes and gametophytes, respectively.

In the water collected from near *S. polycystum*, distributed only at Phe, the Pb concentrations are 0.2668 μg/l. In the sediment, the metal concentrations are 12.3599 μg/g. In the seaweed, the metal concentrations are 40.6169, 49.9247, 42.0553 and 33.3579 μg/g in the holdfast, stipe, blade and air bladder, respectively.

The mean zinc concentrations in the seawater collected from Phe and Laem Chabang are 0.0547 and 0.2512 μg/l, respectively. In the sediments, the metal concentrations collected from Phe and Laem Chabang are 25.9133 and 32.8275 μg/g. In *E. clathrata* collected from Phe and Laem Chabang, the metal concentrations are 98.0383 and 168.2576 μg/g, respectively.

In the water collected from near *P. japonica*, distributed only at Phe, the average Zn concentrations are 0.2495 μg/l. Meanwhile, the metal content in the sediments is 28.6218 μg/g. In the seaweed, the metal concentrations are 62.3403 and 84.1869 μg/g in sporophytes and gametophytes, respectively.

In the water collected from near *S. polycystum*, distributed only at Phe, the Zn concentrations are 0.3641 μg/l. In the sediment, the metal concentrations are 22.4410 μg/g. In the seaweed, the metal concentrations are 27.3127, 51.5066, 30.9453 and 40.2806 μg/g in the holdfast, stipe, blade and air bladder, respectively.

In the metals related to *E. clathrata* which are collected from both locations, Cd, Cr and Zn concentrations in the water from Laem Chabang are higher than that of the metal concentrations from Phe. However, Cu and Pb concentrations from Laem Chabang are slightly lower than that of the metals from Phe.

In the sediments and the seaweeds, all metal concentrations are many times higher than those of the metal concentration in the water column. The Cr, Cu and Pb concentrations show higher concentration in the samples from Phe than in the samples from Laem Chabang, but Cd and Zn concentrations show the opposite concentrations.

It is noted that all metal concentrations in the water column are within Thailand's legal limits (Cr, Zn, Cu, Pb and Cd contamination in seawater less than 100, 100, 50, 50 and 5 μg/l, respectively). However, the metal concentrations in the sediments and in the seaweeds are higher than the limitations in Thailand's regulations. In addition, it reaches the level of contaminated area as described by other researchers. However, there are no standard levels of the metals in these samples.

Focusing on the bioconcentration levels between seaweeds and water, *E. clathrata* from Phe and Laem Chabang accumulates Cd, Cr, Cu, Pb, and Zn at 71 and 61; 778 and 64; 1,739 and 1,050; 93 and 110; and 1,792 and 670 times higher than in the water, respectively. In *P. japonica*, sporophyte and gametophyte generation accumulates Cd, Cr, Cu, Pb, and Zn at 67 and 66; 53 and 21; 211 and 289; 125 and

75; and 250 and 325 times higher than in the water, respectively. In *S. polycystum*, holdfast, stem, blade, and air bladder accumulates Cd, Cr, Cu, Pb, and Zn at 53, 50, 50, and 49; 56, 40 36, and 54; 1,114, 1,160, 1,676, and 751; 152, 187, 158, and 125; 750, 1,599, 850 and 1,106 times higher than in water, respectively.

All the seaweeds can be used for metal monitoring because they accumulate the metals many times higher than those of the metal concentrations in water column. *E. clathrata* is the most suitable in the tropical areas. In addition, it can distribute in a wide variety of environmental factors, especially in very low salinity. However, there is a disadvantage for the seaweeds. The samples cannot be collected year round since they have life cycle, which disappear in some months.

Laem Chabang is the area that has many industries and surrounding human communities. A lot of wastewater is produced and released into the sea. Thus, the water in this area should be full of contamination. However, the metal concentrations from this site are slightly lower than that of Phe since the effluent is maybe treated before leaking into the sea. In addition, there are many factors effecting the metals dissolved in the water, such as pH, chelators, as well as salinity.

The bioconcentration levels of heavy metals, in this study, can give more information about the level of xenobiotics in the seaweeds. It is known that macroalgae act as primary producer in oceanic environments. Therefore, the higher the bioconcentration levels in the seaweeds, the higher the bioconcentration levels in other consumers, including human beings.

Some biochemical products, for example, concentrations of chlorophyll *a* and chlorophyll *c*, protein content, lipid fraction and fatty acid profile, were studied in the blade (leaf) of *S. polycystum*.

Concentrations of chlorophyll *a* and chlorophyll *c* in the blade of *S. polycystum* are of the order of 0.5405 – 1.5405 μg.cm^{-3} and 0.1586 – 0.8468 μg.cm^{-3}, respectively. Protein concentration in the blade of *S. polycystum* ranges from 0.1729 – 0.4594% per gram dry weight. The lipid fraction is of the order of 0.0341 – 0.8303 g/g dry weight. The most common fatty acid found in the seaweed is C16:0 (palmitic acid). It ranges from 31.2374 to 37.9497%. Other prominent fatty acids in order of decreasing are C20:4(n-6) ≥ C18:1(n-9)c >> C18:2(n-6)c ≥ C16:1, whilst, total unsaturated (branched) and saturated (unbranched) fatty acids are 46.6492 and 58.8296%, respectively.

Effects of individual single metal on physiological changes in *S. polycystrum* were studied. Concentrations of chlorophyll *a* and chlorophyll *c* are increased as the time increases and increase with Cd, Cr and Cu. However, the plants died within the 6th day of the experiment if the Cu concentrations were higher than 0.045 mg/l. On the other hand, both chlorophyll concentrations are decreased with increased time and treatment with Pb and Zn. Meanwhile, there is slight fluctuation when treated with Cr. It can be concluded that synthesis of both chlorophylls is activated by Cd and Cu, in low concentrations. Yet, both Pb and Zn inhibit chlorophyll synthesis in the seaweed.

Protein concentration is decreased with increased time and treatement with all metals except Cr, for which there is variation in protein content in the seaweed. It can be concluded that all metals, except Cr inhibit protein synthesis in the seaweed cells. Chromium concentration had no apparent effect on protein synthesis in the seaweed.

Lipid content is decreased when treated with Pb and Zn but there are slightly increases when Cr is applied. In addition, there are no effects on lipid fraction when

Cd and Cu are applied. It can be concluded that both Pb and Zn inhibit lipid synthesis in the plant cells.

The percentage of palmitic acid is slightly decreased when treated with Cr, and it is obviously decreased when Zn is applied. Meanwhile, it is increased when treated with Pb. However, there are not sufficient samples when Cd and Cu are applied.

When treatment with combined metals, concentrations of both chlorophyll show a response to combined metals in the same direction. The chlorophyll concentrations are promoted to synthesis when treated with Cd+Pb, Cd+Zn, Cr+Pb and Cr+Zn in all metal ratios. In addition, in low concentrations of Cr+Cu, Cu+Pb and Cu+Zn, synthesis of both chlorophylls are activated. In contrast, the chlorophylls are inhibited to produce at high concentrations of these combined metals. In addition, synthesis of both chlorophyll is inhibited by mixtures of Cd+Cu and Pb+Zn. However, mixtures of Cd+Cr show no effect on chlorophyll synthesis.

It is noted that mixtures of Cd+Cr and Cd+Cu showed antagonistic effect on all biochemical products. Meanwhile, the other combined metal showed various effects on physiological changes. For example, Cd+Zn showed synergistic effect on protein synthesis but showed antagonistic effects on lipid, Cr+Pb showed antagonistic effect on protein synthesis, Cr+Zn showed antagonistic effects on protein and palmitic acid synthesis and so forth.

The blades of *S. polycystum* are unifacial and have sterile paraphyses in the cryptostoma. Thus, the blades are not swollen during in sporogenesis stage. The growth of the paraphyses is inhibited and they may be destroyed at high concentrations of metals, especially when treated with Cu and Zn. The cell walls, in

addition, are separated and organelles, including the nucleus, are destroyed when treated with high concentrations of metals.

In further study, heavy metals concentrations need to be measured regularly in the seawater, including in the sediment, of Thailand since human activities change every year. The results of this study will be beware of the metal contamination and warn for public health and related offices to make regulations for safe marine environment. In addition, heavy metals in marine biota, especially, macroalgae can give a picture of the quality of our surrounding environment. Heavy metal levels in algal species are dependent both on environmental parameters and on the structural differences among the algal species. It is not possible to compare the heavy metal concentrations in macroalgae reported from other marine environment with present results due to wide variations of the environmental parameters and systematic position of the algae. Furthermore, very little information of heavy metals in Thailand was reported.

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