FLUX OF CADMIUM THROUGH A LABORATORY FOOD CHAIN (MEDIA - ALGAE - MUSSEL) AND ITS EFFECTS

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ABSTRACT

The increasing pollution of the aquatic environment by cadmium is a potentially severe problem and techniques are needed to document the effect of the metal. To investigate the flux of this metal through a laboratory food chain, algae were grown in various cadmium concentrations for subsequent use as contaminated food for mussels. The results showed that in order to make valid deductions, more information about chemical mechanisms and background ecophysiological data is needed, otherwise accumulation reports may become misleading. It was found that the best growth and accumulation results were achieved by harvesting algae from a zinc deficient media containing 7 μmole dm^{-3} cadmium and at a particular life cycle phase. Two uptake mechanisms are proposed.

These "contaminated" algae were fed to mussels under different accumulation regimes. The metal gain and loss were determined and compared to a "base-line" dry body weight which had been calculated from a shell length-body weight relationship. Cadmium accumulation took place in the mussels and after some initial delay, could be correlated to weight loss. Such a weight loss was due to pathological and biochemical changes in the mussels. It was shown that the toxic effect of cadmium could be determined much earlier by the presence of special proteins.

The elutant profiles of the gel chromatography study showed the production of metal binding protein as well as a spill over of cadmium into the enzyme pool, caused by a higher uptake than elimination rate. Cadmium on metal binding protein and in the enzyme pool could be related to the poisoning effect of the metal and a pollution history for the mussels identified. The characteristics of the metal binding protein were found to be very

similar to those reported for metallothionein and had an approximate molecular weight of 10 600 daltons.

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1. INTRODUCTION

The problems of pollution in the marine environment have been given much attention in recent years. In South Africa, in particular, pollution studies have been done by Oliff et al., (1967 a & b), Oliff (1976), Eagle et al., (1977), McLachlan (1977a, b, c & d); McLachlan (1978), Eagle et al., (1979); Orren et al., (1979), Fricke et al., (1979), Hennig and Fricke (1980); Hennig et al., (in preparation) and others. Many of these studies have concentrated on meiofauna and a few on metal concentration of water and sediment.

Trace metal concentration of organisms as potential monitors have been determined by Darracott and Watling (1975); Oliff (1976); Watling and Watling (1976a & b) and Watling (1978). The last work, in particular, deals with metal accumulation in bivalve molluscs in South African coastal marine environments. However, the few reports on the accumulation ability of molluscs deal with the total metal body burden and little is known about uptake mechanisms, subsequent metabolism or detoxification.

1.1 Cadmium pollution

The "heavy metal" cadmium appears to be significant in relation to marine pollution for several reasons.

Cadmium is very toxic and believed by many to be a more dangerous threat to human health than mercury (Fowler and Benayoun, 1974). In highly specific metalloenzymes, a metal (e.g. iron, copper or zinc) is firmly associated with a protein and often constitutes the active centre of the living cell, catalyzing only one specific reaction or type of reaction. Removal of the metal results in a loss of enzyme activity which cannot readily be restored. Substitution by another metal may activate or inhibit the natural enzyme (Wittman and Förstner, 1977). This explains the extreme toxicity of cadmium as it substitutes for other metals and forms strongly bonded linkages with soft bases containing thiol groups - SH (Pearson, 1968a & b). In consequence, biological systems containing enzymes carrying thiol groups are irreversibly inactivated by Cd^{2+}. This destructive effect is due to the biological foreign nature of the metal. Cadmium is not, as far as known, an essential biological trace element. Any addition or contamination will not be lost by following a natural pathway to an enzyme pool and become a body reserve like, for instance, zinc or copper. Hence the flux of cadmium can be followed through a food chain without natural losses or gains. This was also a reason for choosing cadmium to investigate metal flux.

In South Africa the contribution of cadmium to the sea via rivers and estuaries is, at this stage, very small, but with increasing industrialization more and more cadmium will be released into the coastal marine environment. The metal originates from mining and smelting of raw base metal ores, particularly gold, lead and zinc ores (see Wittman and Förstner, 1977). It is used in some metallurgical and electrolytical processes, for example in electroplating; in the manufacture of cadmium batteries and as a catalyst. The sources, together with effluent from pigment and plastic industries as well as from sewage treatment transfer annually about 120 tonnes (see Appendix A) of cadmium into the sea. There it is readily accumulated by marine organisms, such as algae, plankton, shellfish and fish, and may be passed from there into a human food cycle.

1.1.1. Cadmium pollution in humans

During 1947 an unusual and painful disease of a "rheumatic nature" was recorded in the case of 44 patients from villages on the banks of the Jintsu River, Japan. During subsequent years, it became known as the "itai-itai" disease (meaning "ouch-ouch") in accordance with the patient's shrieks resulting from painful skeletal deformities (Kobayashi, 1978). By the end of 1965 over 100 deaths had occurred from the disease which turned out to be chronic cadmium intoxication. The most dangerous and distressing effect of cadmium poisoning in humans is that the incubation period is between five and ten years (in some cases up to 30 years). Since the human body excretes cadmium only very slowly, the metal accumulates in teeth and bones as well as interfering with the calcium metabolism, hence the skeletal deformation.

Further effects of the disease and its historical development as well as some legislation are to be found in Kobayashi (1978), Förstner and Wittman (1979) and IER.,(1980).

After the first alarming discovery and correct diagnosis of the "itai-itai" disease, global contamination of the aquatic ecosystem - similar to mercury pollution - could no longer be ruled out. Numerous investigations have however, led to the conclusion that cadmium pollution in the aquatic environment is not as serious as global mercury pollution (Förstner and Wittman, 1979). Nevertheless, a local enrichment of cadmium, as in the Jintsu River area, can occur elsewhere in other rivers or coastal waters.

1.1.2. Cadmium: Normal and polluted levels in the sea and organisms

Several sources (Krauskopf, 1956; Chester and Stoner, 1974; Riley and Skirrow, 1975; Gerlach, 1976; GESAMP, 1976; Förstner and Wittman, 1979) give the cadmium abundance of nearshore surface water ranging from 0.36 - 37.4 nmole dm^{-3} with an average of 0.80 nmole dm^{-3}. The open ocean surface waters vary between 0.18 and 1.51 nmole dm^{-3}, with an average of 0.62 nmole dm^{-3}.

The regional differences in the distribution of cadmium are summarized in Table 1. It is apparent that nearshore areas of the world oceans show a metal enrichment with relatively high levels being found in certain South African and British coastal waters. This, together with the very high cadmium concentrations reported by Cuthbert et al., (1976a & b) in Bullia digitalis from the South African West coast, is another reason for focussing attention on the flux of cadmium in a South African coastal food chain.

The organisms which are first affected by pollution in the marine environment are algae and filter-feeders (mainly bivalves). Some cadmium levels of green algae and mussels relevant to this study are given in Table 2 and 3 respectively. As can be expected, little is known about cadmium concentration of green algae from natural waters because of the collecting and separation difficulties.

Very much more is known about mussels, especially Mytilus edulis (L.), due to the 'International Mussel Watch' project, (1980). In Table 3 only known dry weight values were selected, because calculations based on wet weights are only estimates. Cadmium levels in Choromytilis meridionalis

TABLE 1: Average concentrations of cadmium in surface seawater from regions (nmole dm^{-3})

NEAR-SHORE WATER	RANGE	MEAN	REFERENCE
North Atlantic	0.62 - 6.32		Riley and Taylor, 1972
Northeastern Atlantic	0.36 - 2.67	0.71	Chester and Stoner, 1974
Britain coastal	0.09 - 37.37	2.67	Abdullah et al., 1972
South African coast 1	1.07 - 1.42	1.25	Chester and Stoner, 1974
South African coast 11	0.36 - 0.53	0.44	Chester and Stoner, 1974
Berg River, 1976	<0.18 - 1.51	0.71	NRIO, 1979
Arniston, 1977	1.33 - 5.52	2.49	" "
Jeffreys Bay, 1977	0.89 - 2.49	1.33	" "
Keurboomstrand, 1977	1.69 - 7.65	3.65	" "
Breede River, 1979	<0.27	<0.27	" "
AECI, 1979	<0.27	<0.27	" "
Camps Bay	<0.27	<0.27	" "
Hout Bay	<0.44 - 0.71	0.44	" "
Inland Sea (Japan)	0.62 - 1.25	0.98	Chester and Stoner, 1974
South Japan Coast		1.16	" "
Java Sea	0.44 - 0.53	0.53	" "
Malacca Straits	0.71 - 0.98	0.89	" "
Sea of Japan	0.80 - 1.07	0.98	" "
China Sea	0.44 - 1.07	0.71	" "
OPEN-OCEAN WATERS			
South Atlantic	0.36 - 1.51	0.62	Chester and Stoner, 1974
S.A. Offshore		0.09	Orren (pers. comm.)
Indian Ocean	0.18 - 1.25	0.62	Chester and Stoner, 1974

TABLE 2: Cadmium concentration in green algae (µg g^{-1} dry weight)

ORGANISM	CADMIUM	LOCATION	REFERENCE
Codium spp.	0.9	Irish Sea	Mullin & Riley, 1956
Chlorphyta	5	Puerto Rico	Lowman *et al.*, 1966
Halimeda gracilis	0.3	Indian Ocean	Mullin & Riley, 1956
Ulva lactuca	0.5-2.0	Atlantic	Stenner & Nickless, 1975
Ulva spp.	0.14^{x}	R.S.A.	Fourie, 1976b

^{x}wet weight

TABLE 3: Cadmium concentration in mussels (only data reported as dry body weight has been included)

ORGANISM	CADMIUM $\mu g\ g^{-1}$	LOCATION	REFERENCE
Mytilus edulis	4.3 - 38 (18.6)[x]	Tasmania	Bloom & Ayling, 1977
" "	10	New Zealand	Brooks & Rumsey, 1965
" "	1.12 - 3.20	Canada	Cossa & Bourget, 1980
" "	1 - 5 (2)	Norway	Lande, 1977
" "	4 - 60 (18)	Bristol	Nickless *et al.*, 1972
" "	1	Belgium	Noël-Lambot, 1976
" "	5.1	Irish Sea	Segar *et al.*, 1971
" "	1.7 - 3.6	Spain	Stenner & Nickless, 1975
" "	5.3 - 63 (24.4)	Port Phillip	Talbot *et al.*, 1976
" "	65.4 ± 13.1	Dorset, U.K.	75-13-5[xx] Boyden, 1975
" "	3.7 ± 0.5	Dorset, U.K.	"
" "	3.2	Port Evin, U.K.	75-D-4 Darracott & Watlin 1975
" "	3.4 ± 1.8	Port Phillip	76-F-4 Fabris *et al.*, 197
" "	7 ± 0.6	" "	"
" "	12.7 - 127 (32)	Corio Bay	"
" "	0.11 - 4.4 (1)	Westernport Bay	"
" "	5.7 ± 0.5	California	71-G-1, Grahams, 1971
" "	3.0 - 3.86 (3.5)	Humber, U.K.	75-J-1, Jones, 1975
" "	2.5 ± 0.042	England	74-L-1, Leatherland & Burton, 1974
" "	2.6 ± 0.73	Scandinavia	77-P-1, Phillips, 1977
" "	20.74	Norway	74-S-1, Stenner & Nickless, 1974
C. meridionalis	1.53 - 2.27	R.S.A.	Fourie, 1976b
"	0.9	R.S.A.	Watling & Watling, 1976b
"	0.22 - 0.43 (0.3)	R.S.A.	Orren *et al.*, 1980
"	0.37 - 3.65 (1.03)	R.S.A.	Orren *et al.*, 1980
"	0.31 - 1.16 (0.71)	R.S.A.	Present study

[x] Mean values in brackets

[xx] Computer numbers to references compiled and obtainable under the Mussel Watch program, Kidder (1977).

(Kr.) are included and a more detailed analysis is given later (see Results).

1.2 Cadmium accumulation

The ability of marine biota to accumulate cadmium is well documented (see reviews by Bryan, 1979; Coombs, 1979; Cunningham, 1979; Eisler, 1979; Eagle, 1980). The accumulation is affected by many extrinsic factors, such as temperature, salinity, chemical form of the metal, interaction, concentration and duration of exposure, position in the water column and season. These extrinsic factors in turn have different effects during different stages in the life-cycle of an animal. This makes comparison of many of the reported results extremely difficult, but even more important several different criteria (determination of LC_{50}, measurement of shell growth and oxygen consumption) have been employed in assessing the toxicity of metals to marine animals. Thus, determining the accumulation effects of sublethal doses becomes virtually impossible by traditional methods because these only monitor the organism's change into the irreversible phase leading to death.

Similarly, while many studies have traditionally been concerned with the total metal concentration, little is known about uptake mechanisms, the subsequent metabolism of metals or their detoxification.

Due to the similarity of biochemical pathways in different organisms, there is a likelihood of more generality being observed during the life-cycle within a species and across animal groups. Exposure of aquatic organisms to cadmium induces the biosynthesis of metallothionein (a heavy metal binding protein first determined by Piscator, (1964)). Metals are stored as metalloproteins, with the protein moiety showing some specificity of metal binding.

1.3 Cadmium binding protein (Metallothionein)

Metallothioneins have been shown to be present in marine vertebrates, including fish (Olafson and Thompson, 1974; Overnell and Coombs, 1979) and similar heavy metal binding proteins have been isolated from crabs, mussels, clams and limpets (Howard and Nickless, 1975; Noël-Lambot, 1976; Talbot and Magee, 1978; Jennings et al., 1979). In fact Olafson and Thompson (1974) made the statement "that metallothionein proteins may be ubiquitous in the living world".

It has been suggested (Davies et al., 1980) that intracellular toxic effects of cadmium will not become evident until the metal occurs in the enzyme pool where it may replace copper or zinc in metallo-enzymes (see also Hennig, 1981). This would only happen when the binding capacity of the metallothionein is exceeded and cadmium then spills over into the metallo-enzyme pool. For this reason the measurement of total cadmium concentrations in tissues will not always be related to toxicity, because they may be below the spillover level, as diagramatically represented in Fig. 1.

Stress due to abnormal metal levels in the environment could in principle be detected much earlier by the presence of metallothionein than by many

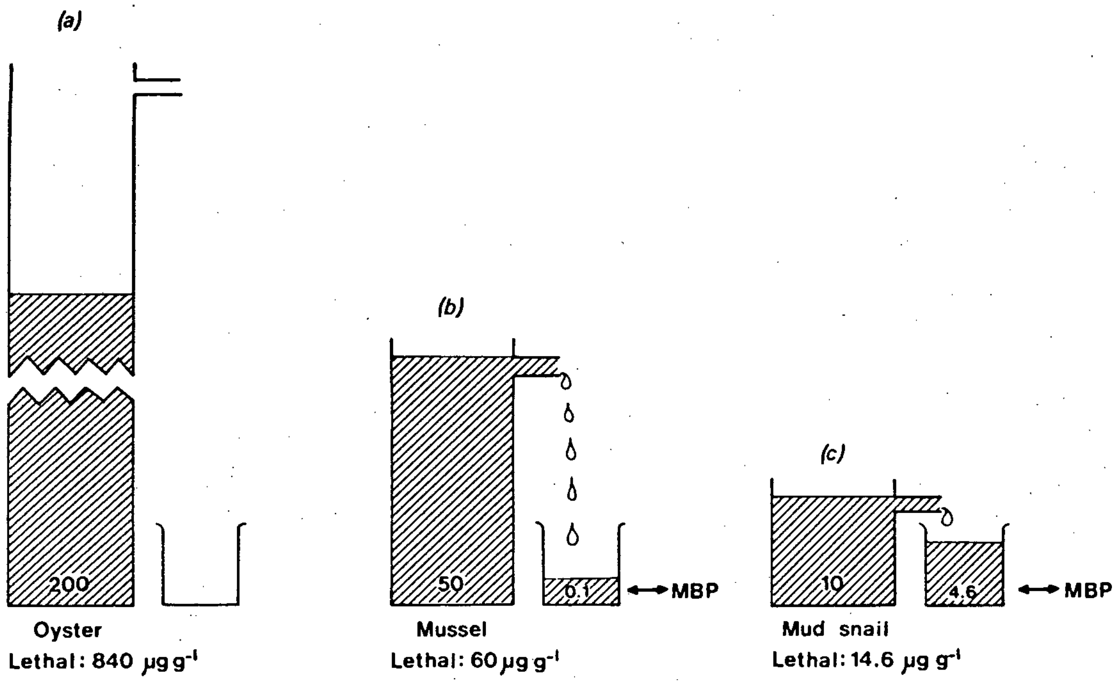

Fig. 1. Cadmium spillover into enzyme pool. Production of metallothionein (MBP), a proposed mechanism.

For example, Oysters (a) can have a cadmium body burden of 200 µg g^{-1} without showing toxic side effects. In mussels (b) metallothionein is produced as cadmium spills over into the enzyme pool. The animal is poisoned because its enzymes are inactivated. If cadmium is absorbed faster than the metal binding protein can take it away, the concentration rises in the enzyme pool and the animal dies (c).

other pathological symptoms, and if, as expected, it exists in all organisms, pollution legislation could be based on it in that isolation of metallothionein from any test animal, irrespective of metal body burden, size, age, sex and stage in the life-cycle (e.g. egg, larva, adult) would strongly suggest a polluted environment.

1.4 Objectives of present study

The primary objective of the present investigation was to induce and isolate a cadmium-binding protein from the black mussel *Choromytilus meridionalis*. As far as is known no attempts have been made so far to use the production of metallothionein for cadmium pollution monitoring.

An environmental situation was simulated whereby algae were grown in cadmium enriched seawater and these then fed to mussels in similar contaminated environments. So far, much laboratory work on metals has been done under very artificial conditions such as spiking animals or environments with extremely high concentrations of metals unlikely to occur in the real environment. No, or non-contaminated feeding has been allowed, so that metal accumulation occurred only from the surrounding water, a very unlikely situation as found by Benayoun *et al.*, (1974). Phillips (1976) has suggested that metal uptake from food is the most important route for uptake of total body trace metal concentration. By feeding the mussels in this study with contaminated algae it was hoped to make the metal available to the test animal in a more natural form.

As the investigation progressed, many gaps in current monitoring programmes studying biological effects became obvious and were investigated.

1. Absorption of metal to culture vessels in the case of algae and loss

of metal from solution in case of metal accumulation experiments with mussels, was investigated. This was to determine any possible discrepancy between spiked metal and available metal concentration.

2. Although the algae Tetraselmis suecica has frequently been used as a food source (Watling, 1978; Buxton, 1980; Griffiths, pers. comm.) no growth, weight and decay or harvesting data were available. Surprisingly, Watling (1978) found that cadmium was not accumulated in Tetraselmis chui.
3. Many metal uptake investigations do not mention exophysiological parameters such as size and life-cycle stage of food organisms, ratio of toxicant to biomass in food and test animals, size and life-cycle stage of test animals, metal body burden per unit mass, so that comparisons are often made very difficult, if not impossible.
4. Constant feeding of mussels was found to be a very difficult problem and attempts have been made to find a solution.

1.5 Background to algae: Tetraselmis suecica (Kylin) Butch

The use of artificially grown phytoplankton to maintain bivalves for long periods is well documented (Walne, 1966; Loosanoff and Murray, 1974; Winter, 1975; Helm, 1977; Griffiths, 1980; Buxton, 1980). T. suecica [x] was selected because this algae has been used for feeding (Bayne & Thompson, 1970; Griffiths, 1980; and Buxton, 1980) and is maintained on a regular basis within the algae collection of the University of Cape Town. The original culture was obtained from the Oyster Culture Unit, Knysna, where it is used as a feeding media.

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[x] According to Riley and Roth (1971 T. suecica belongs to the class: Prasinophyceae; order: Pyraminonadales and suborder: Tetraselmidaceae. Its size is approximately 6-10 µm.

Since T. suecica was used for feeding, the culturing methods (Griffiths, pers. comm.) of the algal unit were applied. Later it became apparent that these methods and media were appropriate for the general maintenance of a wide variety of feeding cultures and did not represent the optimal conditions for T. suecica.

Previous studies have shown that marine algae are capable of accumulating heavy metals to a degree many times over that present in the surrounding medium (Riley and Roth, 1971; Jensen and Rystad, 1974; Berland et al., 1977; Bentley-Mowat and Reid, 1977; Canterford et al., 1978; Jennings, 1979). Hence the possibility exists that metals are concentrated at each trophic level in the aquatic food chain (Stewart and Schulz-Baldes, 1976). This is illustrated by the well-documented occurrence of 'Minamata' disease in Japan in the 1950's (Kobayashi, 1978). So far no cadmium accumulation work has been reported for T. suecica.

1.6 Background to black mussel - Chromytilus meridionalis (Krauss)

The biology and ecophysiology of C. meriodionalis has been extensively studied by Griffiths (1980). Without such considerable background information, interpretation of the physiological and biochemical responses to cadmium would be impossible.

C. meridionalis has been the subject of research as a potential indicator for cadmium pollution by Watling (1978). She concluded that the black mussel could, strictly speaking, not be used to monitor cadmium pollution quantitatively due to the many environmental variables, but that in practice a qualitative indication can be achieved.

Other pollution effects on C. meridionalis have been investigated with regard to chlorine (Curry and Cook, 1975), low level chlorination (Stuart, 1978) and ammonium nitrate (Currie et al., 1974). None of the above investigations considered pathological effects and no simple statement regarding toxicity was made.

2. METHODS

2.1 Adsorbtion of cadmium

All glass flasks and pipettes were soaked overnight at room temperature in 70% nitric acid and washed in double distilled water. A 889.7 µmole dm^{-3} cadmium stock solution was made up each time by dissolving cadmium chloride (Merck AR) in double distilled water. Experimental solutions were prepared by dispensing predetermined volumes of stock solutions into filtered (0.45 µm) seawater (final volume 250 cm^{3}) in 250 cm^{3} Pyrex Erlenmeyer flasks. The concentrations were approximately 1.8; 4.4; 8.9; 17.8; 26.7; µmole dm^{-3} cadmium. The flasks were stored at 16 - 18° C.

All glass aquarium tanks were rinsed in 10% nitric acid and washed with seawater from Oudekraal (Salinity = 34.5^{o}/oo). The tank dimensions were 28 cm x 22 cm and 22 cm high and contained 10 dm^{3} of filtered (0.45 µm) seawater. Experimental solutions were prepared by dispensing predetermined volumes of the above stock solutions into the aquaria. The concentration was about 8.9 µmole dm^{-3} cadmium. The tanks were kept at 16 - 18°C. During the duration of the experiment a five cm^{3} acidified sample was taken every day.

Metal analysis was undertaken using a Varian Techtron AA-6 atomic absorption spectrophotometer. The metal was determined using standard analytical conditions (Appendix B1). A calibration curve was prepared using standard solutions and the metal concentrations in the samples calculated by reference to it. The instrument was standardised prior to each determination.

2.2 Growth of algae

Stock cultures

Stock were maintained in autoclaved 250 cm^3 Pyrex conical flasks sealed by a double layer of aluminium foil. The cultures were grown in autoclaved, filtered (0.45 µm) sea water enriched with a culture medium described by Walne (1966), (Appendix B2). Cultures were maintained in the algae culture room at 15°C and subjected to a 16 hour light - 8 hour darkness cycle. The light source consisted of two Crompton 20 watt 'cool white' fluorescent tubes each producing 1150 lux.

Growth study of culture

T. suecica was grown in acid washed, autoclaved 250 cm^3 Pyrex Erlenmeyer flasks. The inocula were taken from stock cultures in their logarithmic growth period. The flasks were filled with 50 cm^3 of algal inoculum and 200 cm^3 of Walne's medium in filtered autoclaved seawater containing the desired cadmium concentrations. Four replicate flasks were prepared for each concentration. The flasks were placed in single rows in an incubator rack and subjected to a continuous light cycle at 7600 lux. The temperature of the incubator rack was 16.5 $\pm$ 0.9°C.

Each day 50 cm^3 of algal culture were harvested by fitting a syringe via a plastic sleeve onto the permanent glass air vent. A similar volume of growth media/seawater/cadmium solution was replaced in the same way.

From the harvested culture four cm^3 were used for absorption measurements at 660 nm in a Beckman DB spectrophotometer (slit: 0.5 mm, path length: 10 mm). Replicate counts of algal numbers were made from each of these samples using a model TA 11 Coulter Counter with 70 µm aperture. Only particles falling into the known size range of *T. suecica* (channels 9 - 11) were counted and baseline counts for these channels (filtered seawater only) were deducted from each count.

The method for growth curve analysis proposed by Sorokin (1973) was used, allowing cell doubling times to be read directly from the growth plots. This method was modified to allow for the constant harvesting: A factor of 1.25 was multiplied by the various growth phases (OD_t) and these $\log_2$ ($1.25\ OD_t/OD_o$) values were used in the growth plots. Non-linear curve fitting was done according to Perry (1954).

The remaining algal solution (45 cm^3) was centrifuged at 2000 *g* for five minutes at room temperature in a MSE Centrifuge with a WW 495 head in teflon tubes. The cells were washed twice with filtered (0.45 µm) seawater and again centrifuged twice for five minutes each. Seawater was used as a wash liquid, because the algal cells burst immediately in distilled water due to osmotic pressure. The washed algae pellet was desalted with ammonium formate solution (3.5% in distilled water) and dried at 45°C for 24 h. Oven drying was preferred to lyophilizing, because 18% of total and spiked amounts of were cadmium found here to be lost during freeze-drying. Similar results were reported by Fourie (1976a) and Watling (1978). The dry algae weight was determined and the dry pellet was prepared for atomic absorption analysis (Appendix B3). A cell number count was done by Coulter Counter on the wash liquid to determine algal loss during washing.

The energy value of six lyophilized algal samples was measured with a

Phillipson microbomb calorimeter. The ash remaining after firing (inorganic constituency) was weighed and the energy value of the ash free dry weight of the sample calculated. All weighings were done on a Mettler electronic microbalance (Model ME30, readability 1 µg).

2.3 Accumulation of cadmium in mussels

Mussels were collected at low water spring tide (L.W.S.) level from the rocky shore at Bloubergstrand, Atlantic Ocean (21 km north of Cape Town). They were housed in a recirculating aquarium (3500 dm^3 capacity) at ambient sea temperature for 72 hours for depuration. Mussels were cleaned of epibiotic growth and the byssus threads remained intact unless discarded by the animal. The animals were placed on a shallow acid-cleaned petri-dish to which they re-attached within 10 hours. This method obviated the necessity of cutting the byssal threads, a procedure known to induce stress (Theede, 1963) and also provided a means by which mussels could be relocated and maintained in a normal upright posture.

After purging randomly selected mussels were placed in plastic bags and stored frozen (-10°C) until prepared for cadmium analysis (Orren et al., 1980). (Appendix B4).

The bulk of the mussels (16 petri-dishes with 20 mussels each) were transferred to four glass tanks containing 10 dm^3 of spiked seawater. Hence 80 juvenile mussels (size range 15.0 - 32.4 mm) were available for each experimental regime (accumulation of cadmium in 15 days, 30 days and 15 days polluted/15 day clean environment) as well as 80 animals as control.

Experimental media were prepared as in 2.1 above, to give an approximate final concentration of 7 ± 1 µmole dm^3.

After accumulation, the animals were purged for 24 hours and frozen (-10°C) in plastic bags.

2.4 Isolation of metal binding proteins

Ten partially thawed mussels were scraped from their shells and an appropriate amount of 25 mM phosphate buffer (pH 7.0) (Appendix B5) was added to give a 50% (w/v) homogenate. The homogenate was prepared by blending the mixture twice for one minute in a Du Pont Omni-mixer (Model 17106) at full speed in ice. The resulting homogenate was centrifuged at 4°C for three hours at 30 000 g in a Sorval RC 5 Superspeed refrigerated centrifuge with eight tube rotor SS 34. Supernatant material was decanted and applied to a Sephadex G-75 column kept at 4°C. Material not immediately applied was kept at 4°C. Concentration of the supernatant with Diaflo Ultrafiltration membranes (Amicon UM 2/43 mm) with a cut off point of 1000 daltons was tried, but was discontinued when cadmium was detected in the effluent.

Supernatant material (10 - 15 cm^3) was applied to a 2.6 x 100 cm fine Sephadex G-75 column and protein fractions were eluted with 20 mM Tris-HCl buffer (pH 8.6) (Appendix B5). The column was standardized using the following molecular weight markers: Bovine albumin (68000 daltons), ribonuclease (13700 daltons), cytochrome C (12500 daltons) and tryptophan (204 daltons). Approximate molecular weights of metal binding proteins were calculated according to Andrews (1964). Concentrations of cadmium were monitored in the resulting fractions using the method in Appendix B1., while absorbance at 280 nm was monitored with a rapid sampling I.S.C.O. Absorbance Monitor (Model 226) and absorbance at 250 (OD_{250}) and 280 (OD_{280}) nm was measured using a Beckman spectrophotometer (Model 25), with slit width 0.5 mm and path length 10 mm.

The mussel pellets were analysed for cadmium by standard methods (Appendix B.3).

Protein determination of the various fractions was done by the method of Lowry *et al*., (1951) (Appendix B.6).

3. RESULTS

3.1 Adsorption of cadmium to glass walls

Figure 2 summarises the adsorption of different cadmium concentrations onto the walls of Erlenmeyer flasks. Repeated filtration through a 0.45 µm membrane filter showed that adsorption onto small filterable particles was negligible. About 37% of the metal was adsorbed to walls at a concentration of 1.8 µmol dm^{-3}. At higher cadmium concentration (26.7 µmol dm^{-3}) only 18% was adsorbed, this probably is due to the fact that sites were sterically hindered (Gardiner, 1974). The extent of adsorption depends on many variables including the metal involved, the past history of the vessel, type of vessel, pH of solution, temperature and concentration of material in solution (Wyatt, 1959; Findley, 1965 and Canterford et al., 1978). For instance, Hennig and Greenwood (1981) showed that adsorption of cadmium was slightly greater in Pyrex flasks than in plastic dishes.

All these variables are unique for particular vessels, concentrations and experiments, which explains the variability of the results obtained by other workers (Table 4).

In view of these results, precontaminated tanks were used for the cadmium accumulation experiments (Hennig and Greenwood, 1981) and the concentration level was monitored daily as shown in Figs. 17 a-n for the 15 day accumulation experiment and Figs. 18 a-c for the 30 day accumulation experiment.

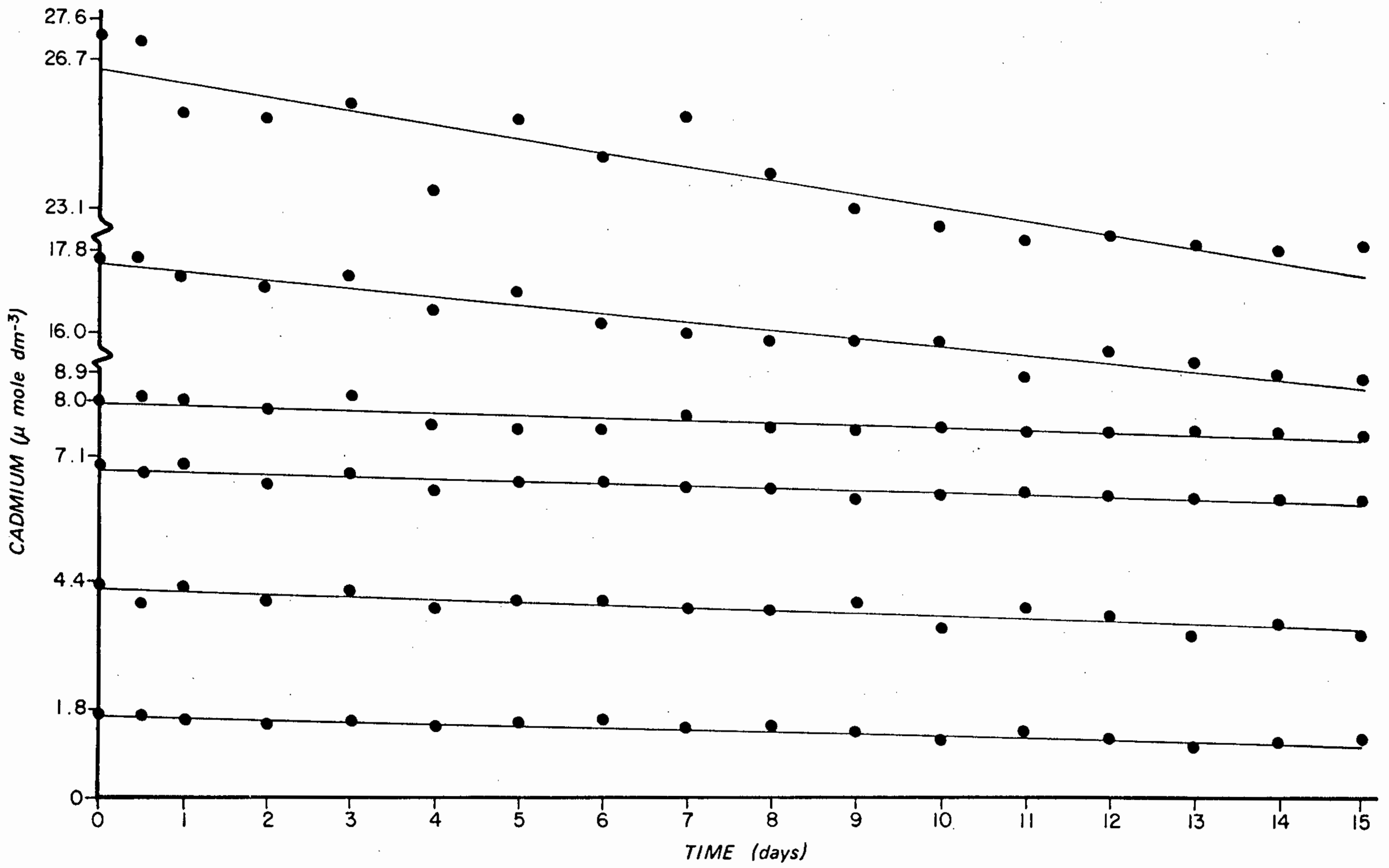


Fig. 2. Cadmium absorption to glass flasks. The 95% confidence limit of the mean (2 x S.E.) is so small that it is included within the dots.

TABLE 4: The adsorption of cadmium in seawater onto various container surfaces

Cadmium Concentration μmole dm^{-3}	Type of Vessel	Volume of solution	Temperature	Adsorbance Time	% Loss	Reference
1.8 - 26.7	Glass	200 cm^3	16 - 18°C	15 days	10 - 37	Present study
0.9 - 8.9	Glass	5 dm^3	20 - 22°C	28 days	10	Klöckner, 1979
2.58 - 1779.4	Glass	3 dm^3	20°C	11 days	5	Eisler, 1971
0 - 5.3	Glass	200 cm^3	14°C	14 days	negligible	Canterford et al., 1978
240.2	Glass	20 dm^3	20 - 25°C	4 days	0	Jackim et al., 1970
44.5	Plastic	7 dm^3	11°C	14 days	27	Cuthbert, 1975
44.5	Plastic	7 dm^3	11°C	4 days	18	Cuthbert, et al., 1976a
0.9 - 26.7	Plastic	10 cm^3	16 - 18°C	3 days	1 - 7	Hennig & Greenwood, 1981

3.2 Characteristics of algal growth in T. suecica

Due to the difficulties arising from the use of the Coulter counter (for instance: infrequent calibrations, different users need different media and apertures, as well as being time consuming) other methods to determine algae cell concentrations were investigated. A reliable and rapid method used for the 840 samples was the determination of absorbance of the sample cell solutions spectrophotometrically at 660 nm. The algal spectrum (Fig. 3) shows a peak at that point. [x]

It was found that for T. suecica the cell number (x) was closely related to cell absorbance (y) as shown in Fig. 4. As expected the relationship was non-linear. The curve that fitted the data best is described by equation

$$y = 1.041\ (0.109^{0.609^{x}})$$

---

[x] Absorbance results are given in Section 9, but due to the bulk of the data only the means of four replicates and their standard deviations are presented. Data of algal numbers, weight and algal cadmium concentration are presented in similar manner. None of these results were corrected for cell losses occurring during harvesting and washing because an average of only 0.05% cells were lost (Table 5).

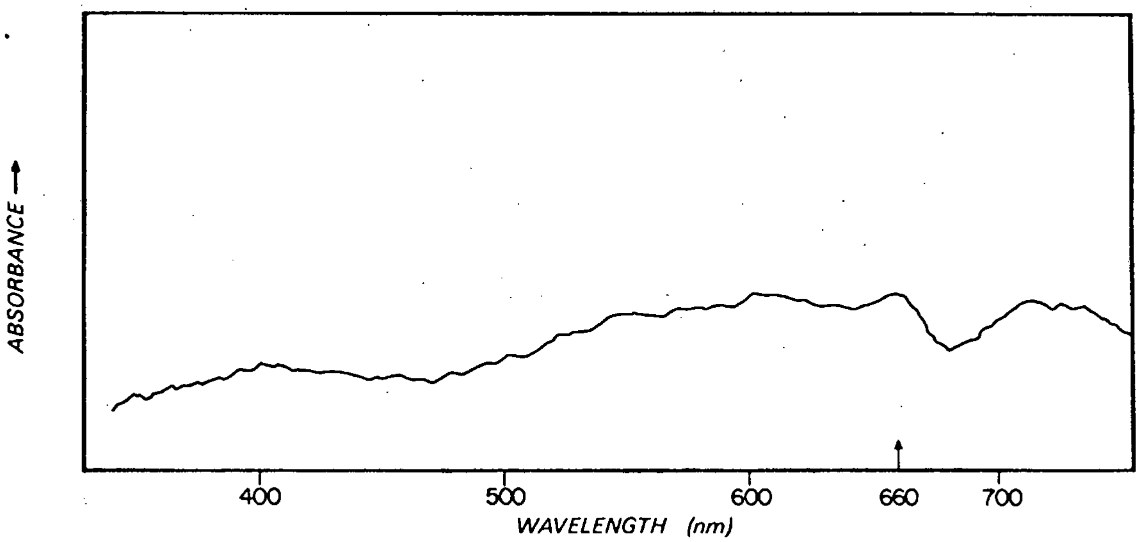


Fig. 3. Spectral properties of T. suecica cells in seawater.

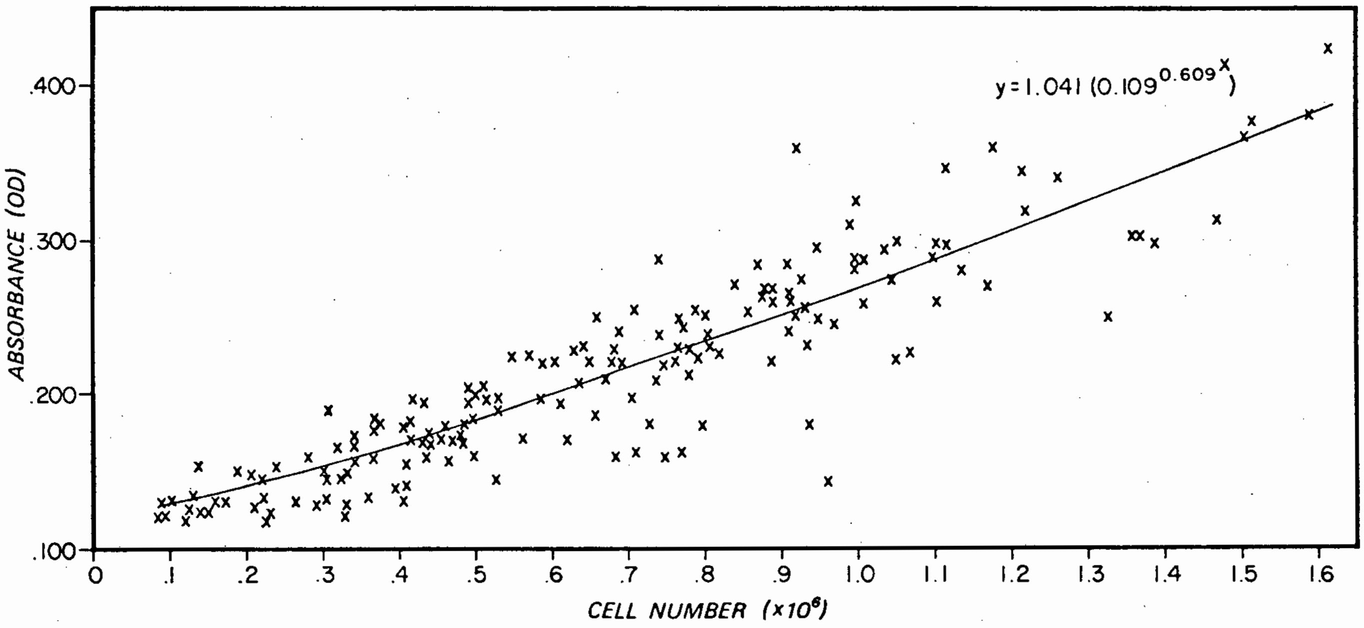


Fig. 4. Relationship between cell number and cell absorbance of <u>T. suecica</u>. ($n = 165$, $r^2 = 0.9597$, $p < 0.01$).

TABLE 5: Cells lost during centrifugation and washing

DAY	% LOSS
1	0,04
2	0,05
3	0,06
4	0,08
5	0,11
6	0,06
7	0,06
8	0,05
9	0,03
10	0,03
11	0,03
12	0,02
13	0,03
14	0,02
15	0,03
Mean	0,05
S.D.	0,02

If the characteristics of algal growth were represented by cell absorbance alone (Figs. 5 a-o, a characteristic representation is given in Fig. 5 f; the other figures are in section 8.1), recognition of trends and phase would be difficult.

Hence the method of growth curve analysis proposed by Sorokin (1973) and Fogg (1975) was used with modifications to correct for the harvesting effect, as well as transforming the data into logarithms to the base 2. This ensured that the reciprocal of the relative growth rate was equal to the doubling time.

The results are presented in Fig. 6. Now four algal growth patterns could be recognized:

1) A lag or induction phase, in which no increase in cells was apparent, lasting for four days. Period: Day 1 to 5.
2) An exponential phase, in which cell multiplication is rapid and numbers increase rapidly, lasting seven days.
   Period: Day 5 to 12.
3) A phase of declining relative growth, lasting for about one day.
   Period: Day 11 or 12.
4) A phase in which cell numbers remain more or less stable, which during the harvesting experiment could be prolonged for 30 days. (Fig. 8).
   Period: Day 12 to 30.

Due to the striking physical variability of young and old cultures and to ensure repeatability of cell numbers, weights and cadmium concentrations during the mussel feeding experiments, it was considered important to understand growth patterns and thus be able to select the most suitable

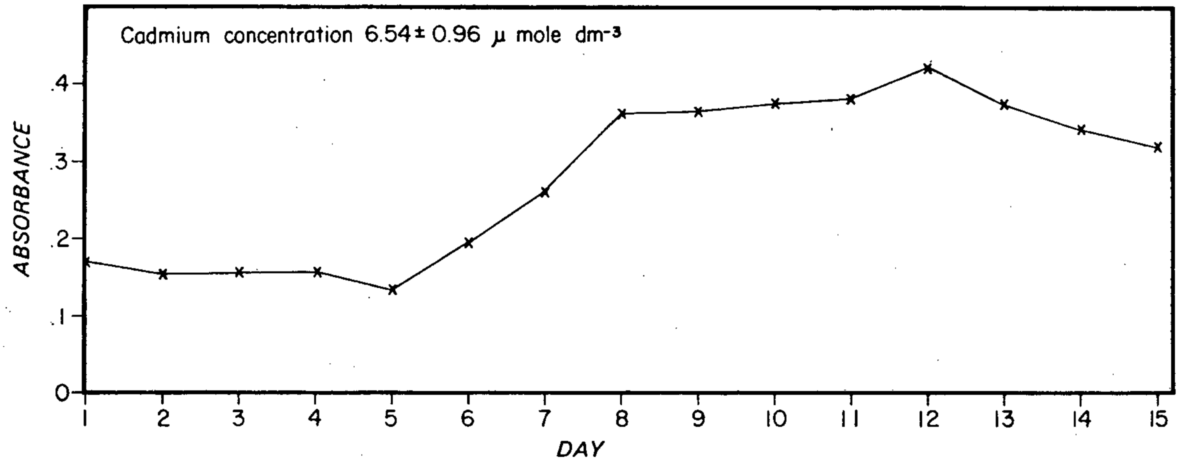


Fig. 5 f. Growth characteristics of <u>T. suecica</u> during 15 day exposure to various concentrations of cadmium.

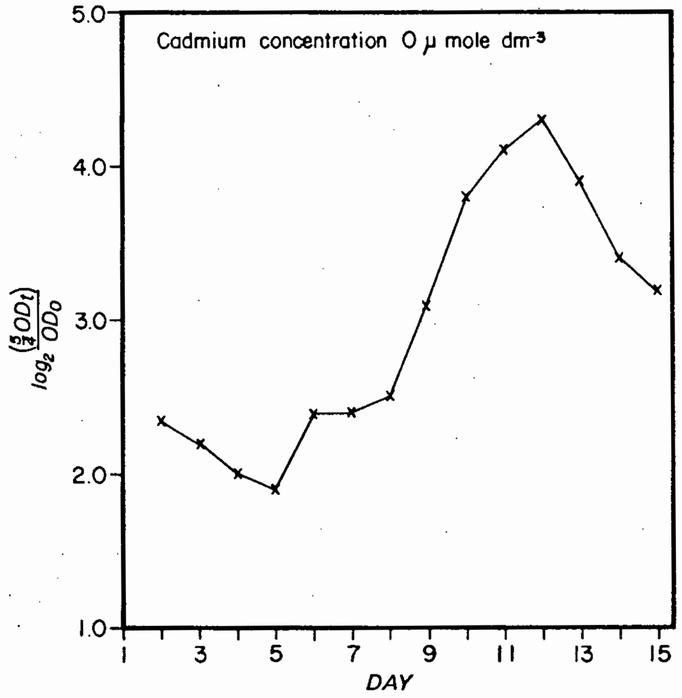


Fig. 6. Growth pattern of T. suecica during 15 days of harvesting.

combination of cell growth and metal accumulation.

A lag phase was exhibited every time the algae were subcultured. This decrease in cell numbers was due to settling-out of post-ripened cells onto the vessel's walls and base. Settling is a pre-requisite in <u>T. suecica's</u> life-cycle, and must occur before division into daughter cells can commence. (Fogg, 1975).

During the exponential phase, reproduction resulted in the increase of cells, which themselves are capable of growth, so that the actual rate of growth accelerated continuously. This can be represented by the expression

$$W = W_o e^{kt}, \qquad \text{--------- (1)}$$

where W_o is the total amount of all material in the culture at time zero, W the amount after time t, e the base of natural logarithms and k the relative growth constant measuring the efficiency of growth. In the experimental flasks the populations were large, hence cell division was not synchronized and increase in cell numbers (N) per unit volume took place smoothly, which can be represented by the corresponding expression:

$$N = N_o e^{kt} \qquad \text{--------- (2)}$$

therefore

$$k = \frac{\log N - \log N_o}{t} \qquad \text{--------- (3)}$$

The relative growth constant for the whole exponential phase of <u>T. suecica</u> is given in Table 6 and compared to various planktonic algae growth

TABLE 6: Relative growth constant (k´) for *T. suecica* in exponential growth phase

DAY	k´
7	0.041
8	0.059
9	0.064
10	0.068
11	0.062
12	0.056
Mean	0.058

constants in Table 7.

From equation (3) the mean doubling time G (which is equal to the mean generation time in T. suecica as the cells divide in two) was

$$G = \frac{0.301}{k'} \qquad \text{--------} \ (4)$$

Eventually exponential growth must come to a standstill and a phase of declining relative growth is reached. The nature of these limiting factors were not determined, but nutrient exhaustion could not be a reason as summarized in Fig. 7. A culture (four flasks) was inoculated with twice (2 cm^3) the normal used amount of nutrients. These cultures kept up a relative constant cell material concentration after the 11th day, similar to all other cultures. Hence it is suggested, that the physical size of the flasks could be a limiting factor.

A stable phase was reached at the 12th day after inoculation. Due to continuous harvesting this stage could be maintained for at least 30 days (Fig. 8) and only contamination should bring about an algal collapse.

3.3 Effects of cadmium on growth patterns of T. suecica

The growth of the algae at various cadmium concentrations is plotted against time in Figs. 9 a-n and the similarities of growth patterns compared to the control (Fig. 6) are striking. It was not possible to maintain T. suecica in a cadmium concentration above 20 μmole dm^{-3}.

TABLE 7: Relative growth constants, k′ in Log_{10} day units and mean doubling time G in hours of various algae

Species	k′	G	Temp °C	Reference
Chlorophyceae				
<u>Dunaliella tertiolecta</u>	0.30	24	16	McLachlan, 1960
<u>Tetraselmis suecica</u>	0.058	124.6	16	Present study (control)
" "	0.177	40.8	16	Present study (maximum)
Xanthophyceae				
<u>Monodus subterraneus</u>	0.074	97.7	15	Fogg <u>et al</u>., 1959
" "	0.169	42.7	30	" "
Chrysophyceae				
<u>Monochrysis lutheri</u>	0.48	15.3	20-25	Antia and Kalmakoff, 1965
Bacillariophyceae				
<u>Phaeodactylum tricornutum</u>	0.72	10.0	25	Spencer, 1954
<u>Skeletonema costatum</u>	0.55	13.1	18	Parson <u>et al</u>., 1961
<u>Chaetoceros sp.</u>	1.81	4.0	29	Thomas, 1966

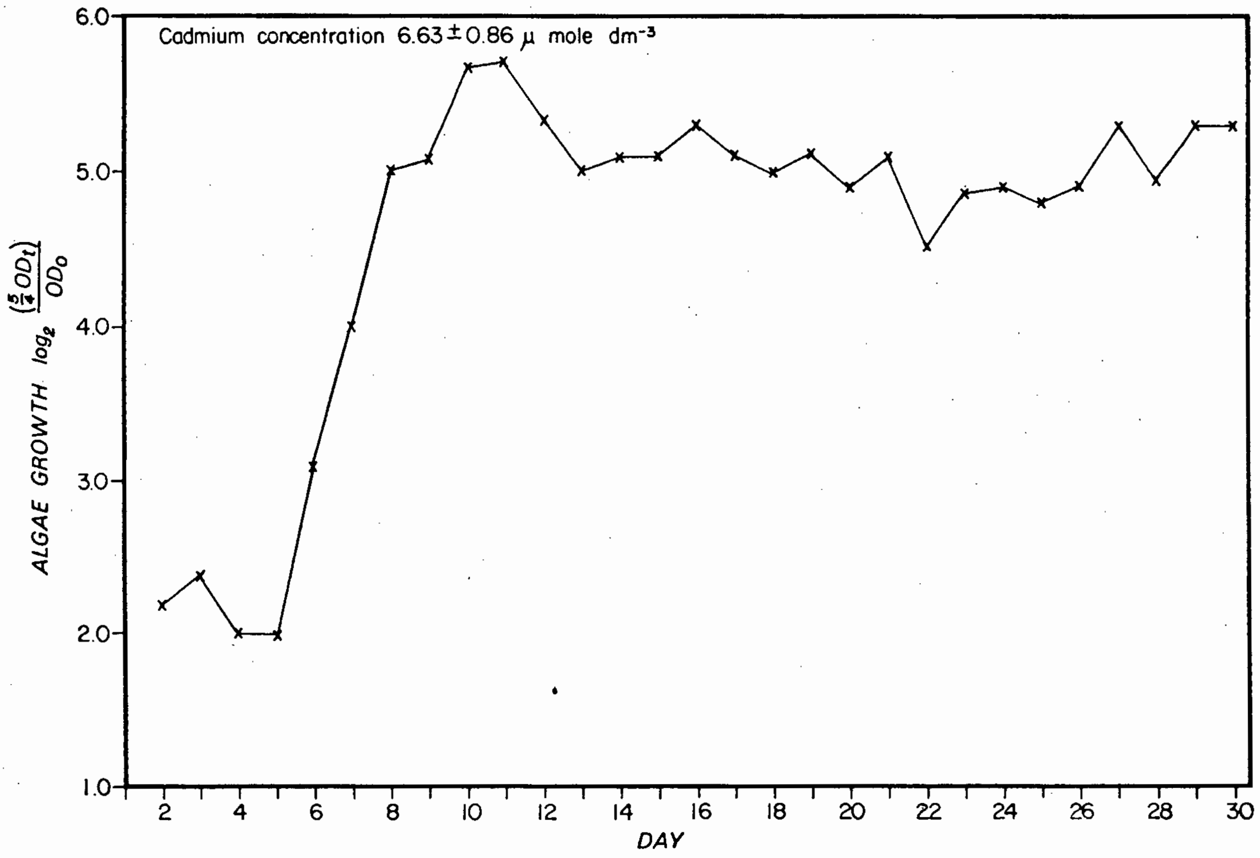


Fig. 7. Growth characteristics of T. suecica with twice the amounts of nutrients.

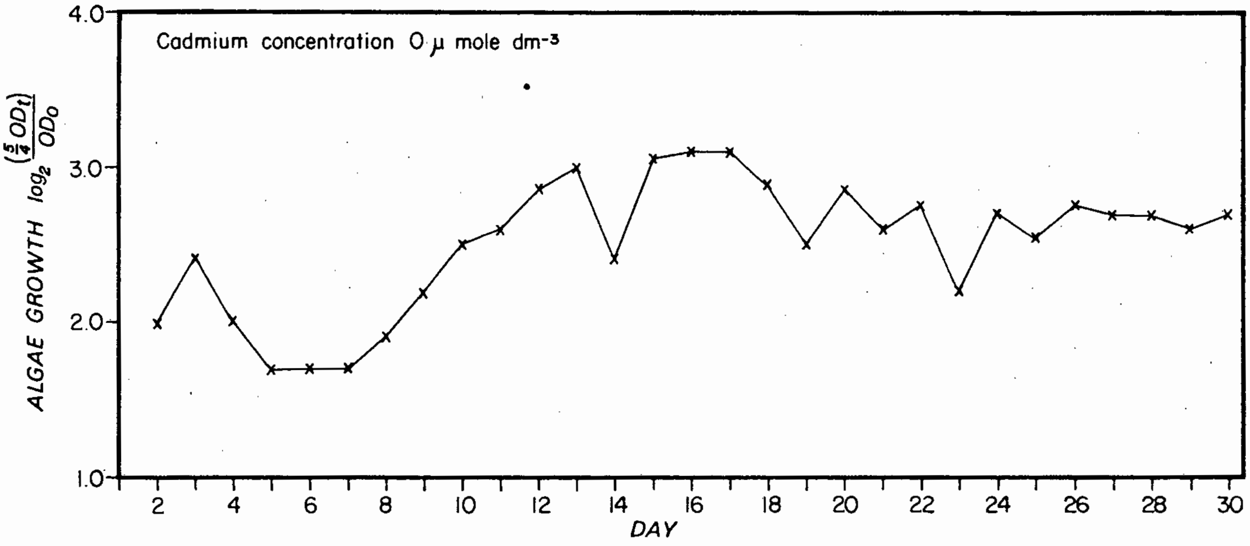


Fig. 8. Growth pattern of T. suecica during 30 days of harvesting.

3.3.1. Lag phase

The cadmium contaminated cultures showed no increase in lag phase (5 days). In each case this phase was governed only by the mean doubling time of the stock culture which was 5,1 days (Table 7). The fluctuations in the lag phase (Figs. 9c, e, g, 1, k) are due to unsynchronized cell division of the inoculate. An increased lag phase (6 days) was observed in the 19.75 μmole dm^{-3} cadmium contaminated culture, but these algae were in any case on the borderline between maintenance and death.

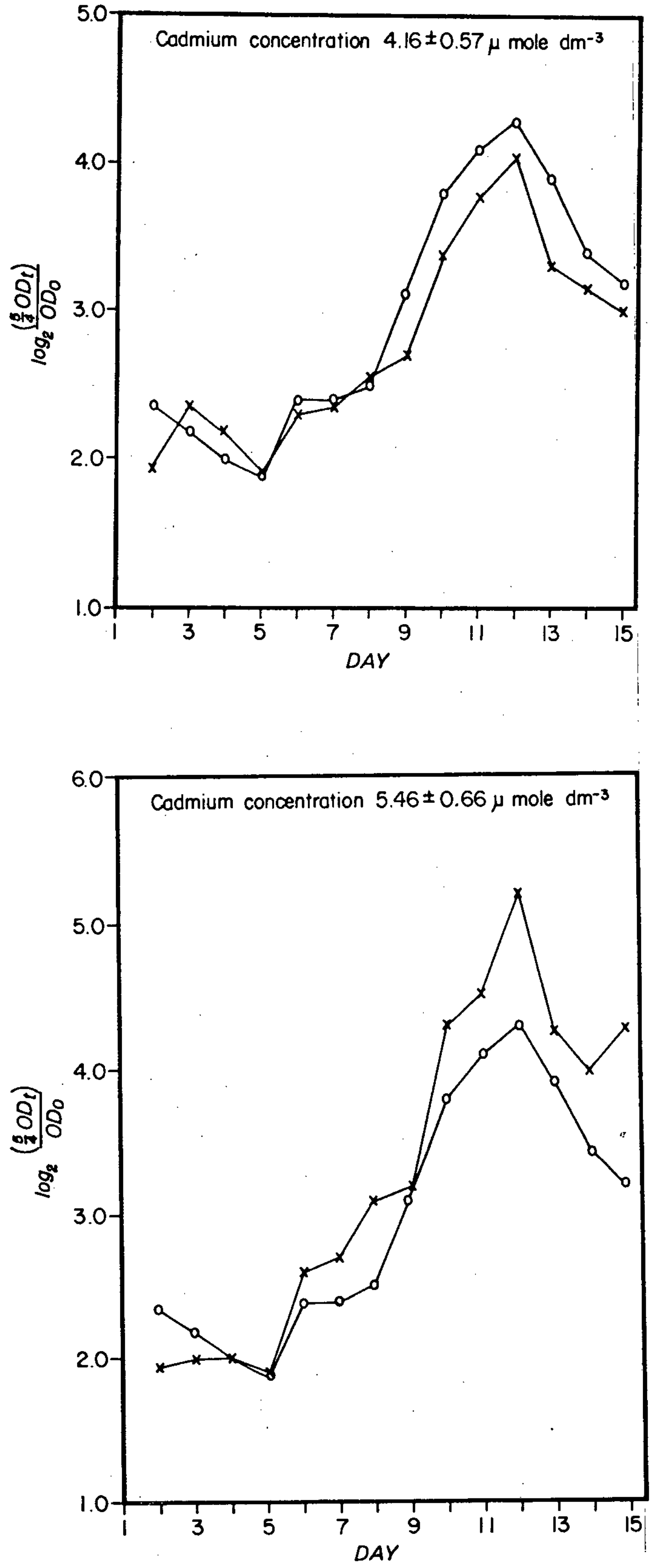
3.3.2. Exponential phase

The duration of the exponential phase was also not prolonged in contaminated cultures, possibly because identical size flasks were used. Algae numbers did increase with increasing metal concentration and the highest absorbance was measured at a cadmium concentration of 6.54 ± 0.96 μmole dm^{-3}, at higher metal concentrations, algal growth was retarded. This normal type curve behaviour is illustrated in Fig. 10. From this it also became clear, that no relative growth was achieved beyond a cadmium concentration of 17 μmole dm^{-3} as indicated by Figs. 9k, 1, m.

Since the mean generation time was inversly proportional to the relative growth (equation 4), the algae at around 7 μmole dm^{-3} cadmium doubled about every 1.85 days (Table 7), but growth was slowed down under less favourable conditions.

3.3.3. Stable phase

From Figs. 9 a-n it was difficult to extrapolate the growth trend


Cadmium concentration 4.16±0.57 μ mole dm⁻³

Cadmium concentration 5.46±0.66 μ mole dm⁻³

Figs. 9c, d. Growth pattern of T. suecica in various cadmium solutions during 15 days of harvesting (Reference growth pattern, open circles, is included for comparison).

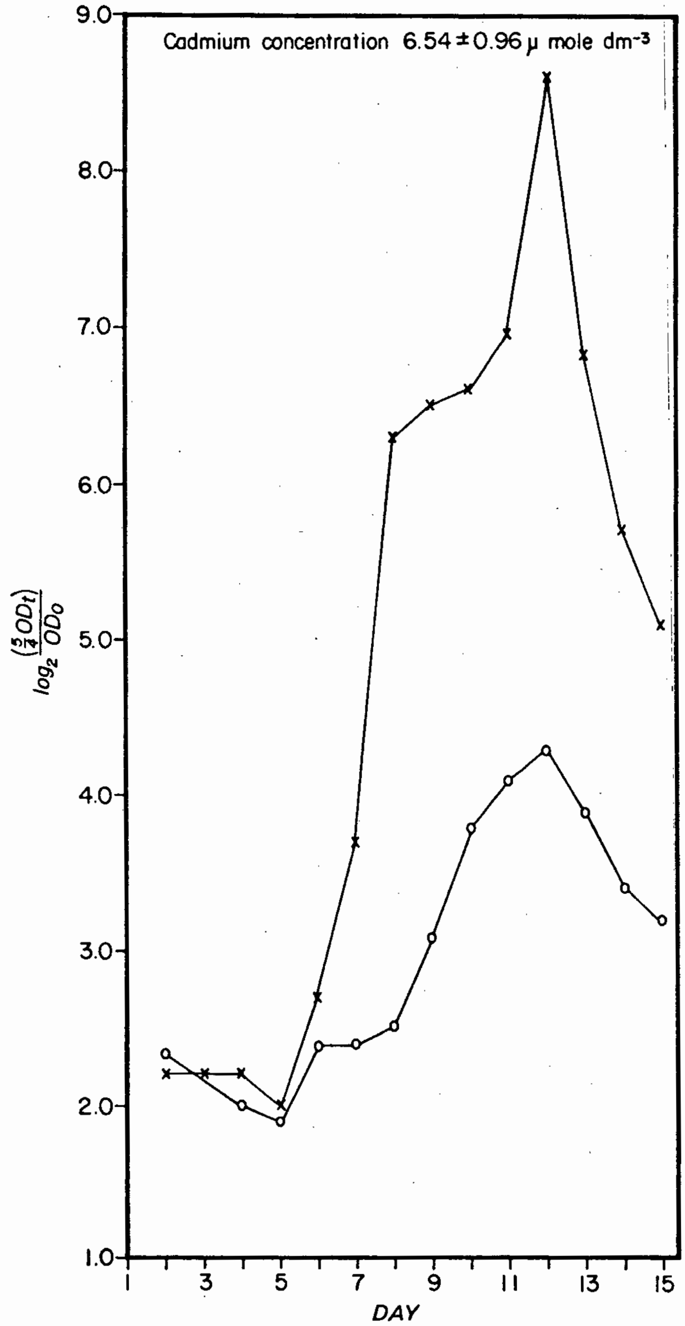
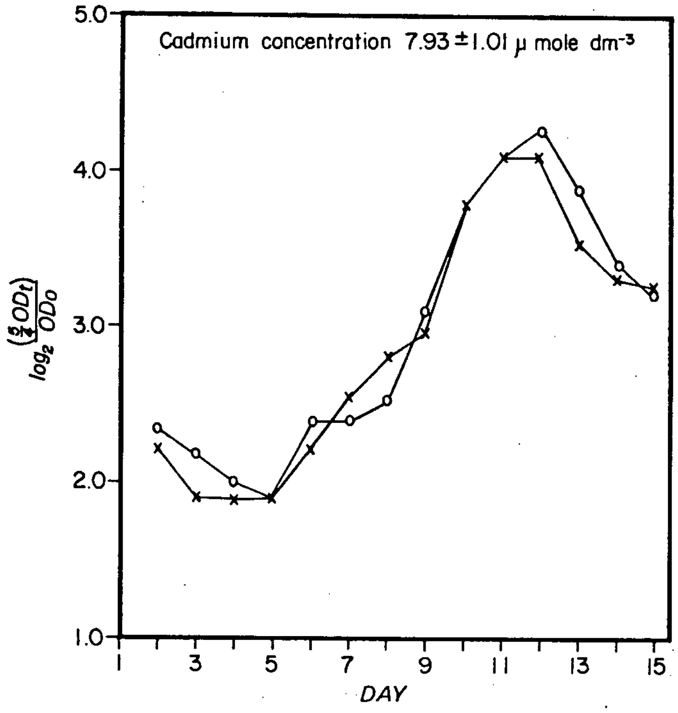
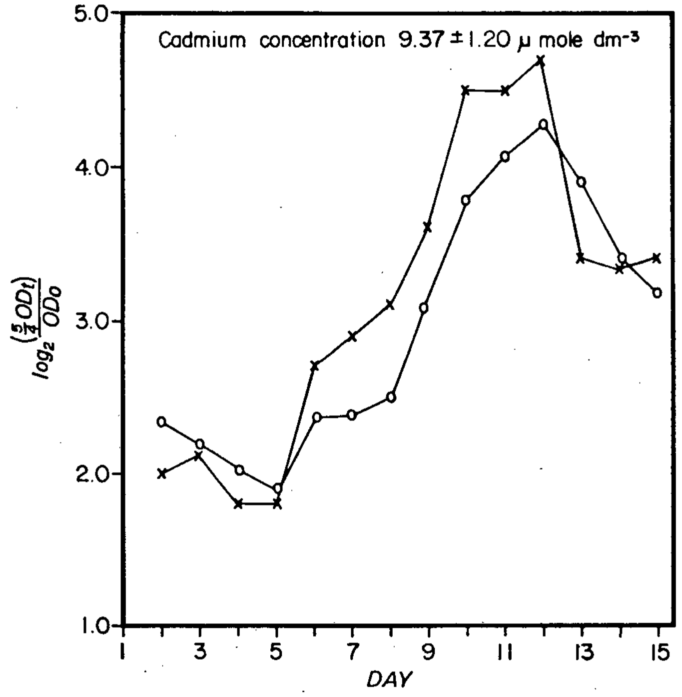
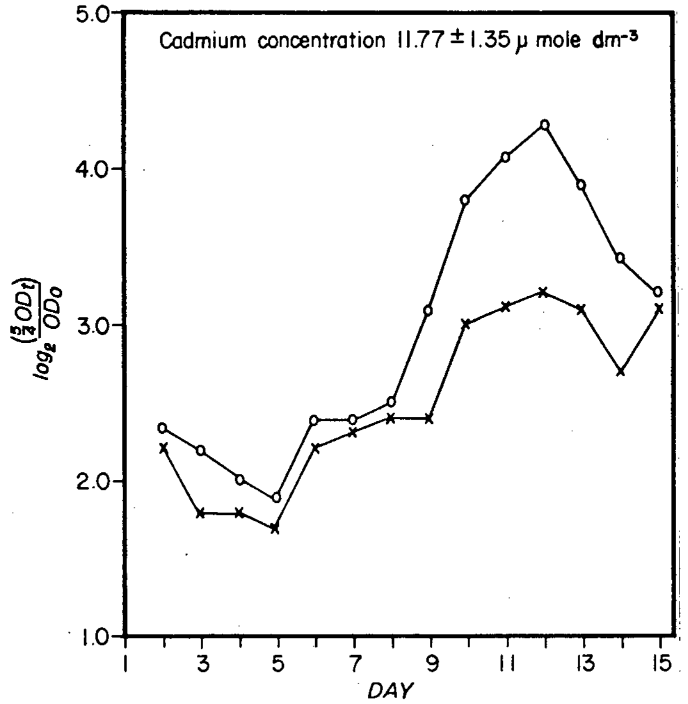


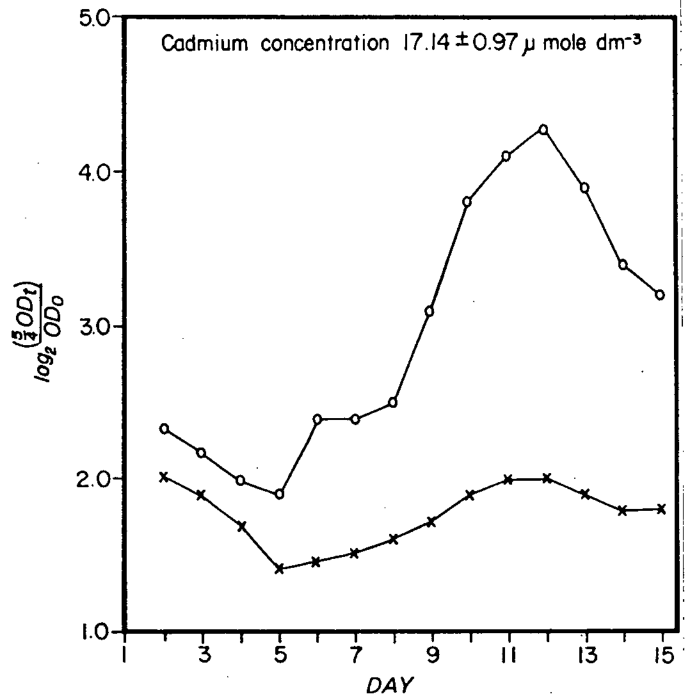
Fig. 9e. Growth pattern of <u>T. suecica</u> in various cadmium solutions during 15 days of harvesting (Reference growth pattern, open circles, is included for comparison).



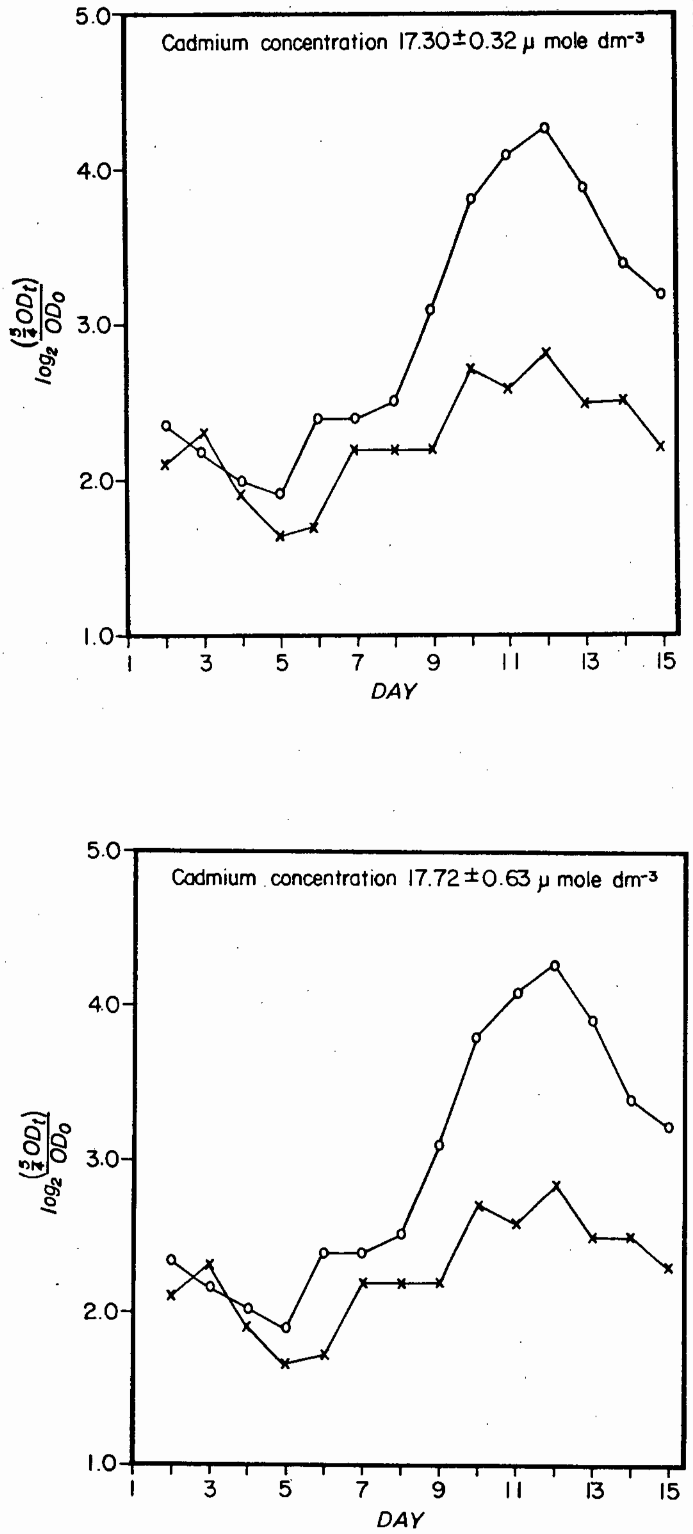


Figs. 9f, g. Growth pattern of <u>T. suecica</u> in various cadmium solutions during 15 days of harvesting (Reference growth pattern, open circles, is included for comparison).





Figs. 9j, k. Growth pattern of T. suecica in various cadmium solutions during 15 days of harvesting (Reference growth pattern, open circles, is included for comparison).



Figs. 91, m. Growth pattern of T. suecica in varius cadmium solutions during 15 days of harvesting (Reference growth pattern, open circles, is included for comparison).

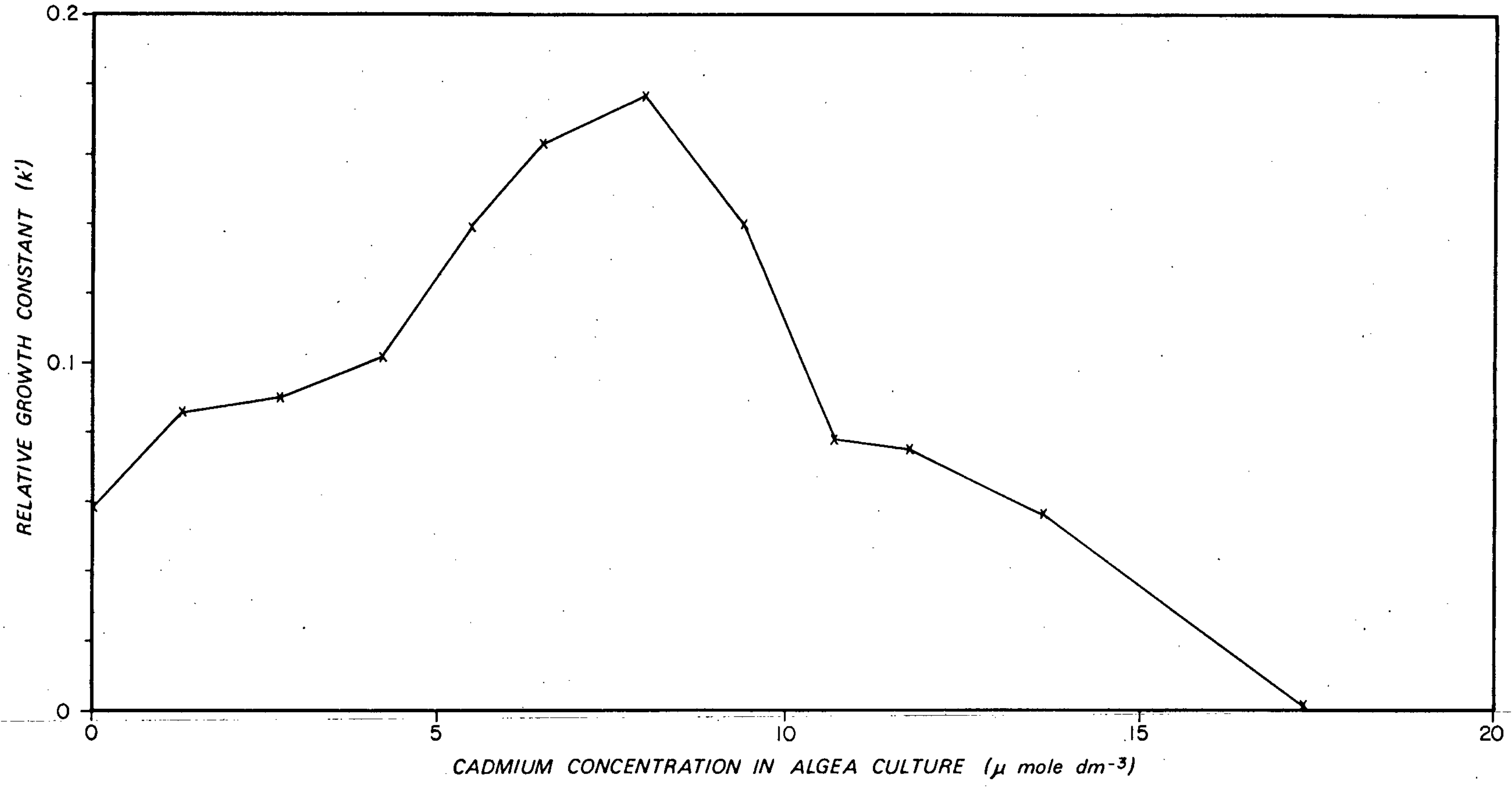


Fig. 10. Effect of cadmium on the relative growth constant (k') of *T. suecica.*

of the cultures. It was feared that the algae would immediately enter a death phase and hence be useless as a feeding media. So a harvesting experiment of 30 day duration was conducted for some selected cadmium concentrations (2.83 ± 0.28; 6.58 ± 0.93; 12.13 ± 1.17 µmole dm^{-3}). A stable growth phase (Fig. 11 a-c) was confirmed over the 30 day test. The fluctuation in algae numbers during this stable growth phase corresponded more or less to the mean generation time of 2 to 3 days calculated for these particular cadmium concentrations (Table 8). Cell growth was generally poor during the 30 day test.

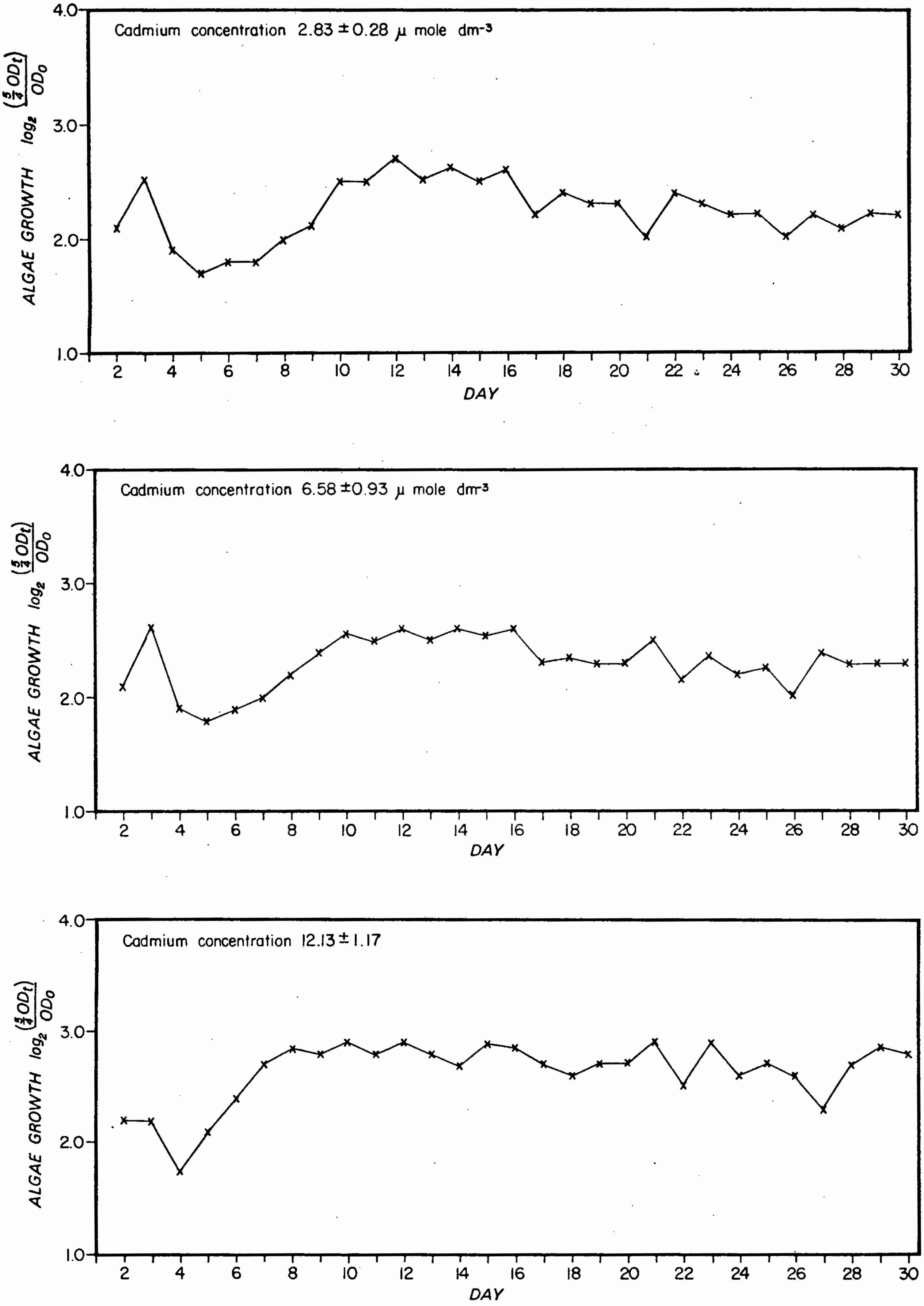
3.4 Growth pattern of *T. suecica* in zinc and zinc/cadmium solutions

The increasing growth rate and constant (k') with increasing cadmium concentration (Fig. 10) was a surprising result. It may be interpreted to mean that cadmium enhances growth and hence might be considered an essential metal. In view of this result, *T. suecica* was grown (4 flasks) in 10 times the zinc concentration (9.65 µmole dm^{-3}) of that of the culture media (Walne's media: 0.97 µmole dm^{-3} zinc) as a control. At the same time algae were added to a mixture of zinc (9.65 µmole dm^{-3}) and cadmium (6.68 µmole dm^{-3}) seeded culture media. The results are given in Fig. 12, which indicated that cadmium inhibits algae growth in the presence of sufficient zinc in the culture media.

3.5 Algal mass during accumulation of cadmium

3.5.1. Relationship of algal number and algal weight

Another important aspect of food media is the weight of the culture. It would be expected that algae number (x) is linearly related to algal mass (y), but it was found that the situation was more complicated (Fig. 13).



Figs. 11a-c. Growth of T. suecica in various cadmium solutions during 30 days of harvesting.

TABLE 8: Mean generation time (G) for *T. suecica* during the exponential growth phase cultured in various cadmium concentrations.

CADMIUM CONCENTRATION µmole dm^{-3}	G DAYS
0	5.19
1.28 ± 0.18	3.46
2.67 ± 0.34	3.34
4.16 ± 0.57	2.92
5.46 ± 0.66	2.17
6.54 ± 0.96	1.85
7.93 ± 1.01	1.70
9.37 ± 1.20	2.15
10.73 ± 1.42	3.86
11.77 ± 1.35	3.86
13.62 ± 2.01	5.28

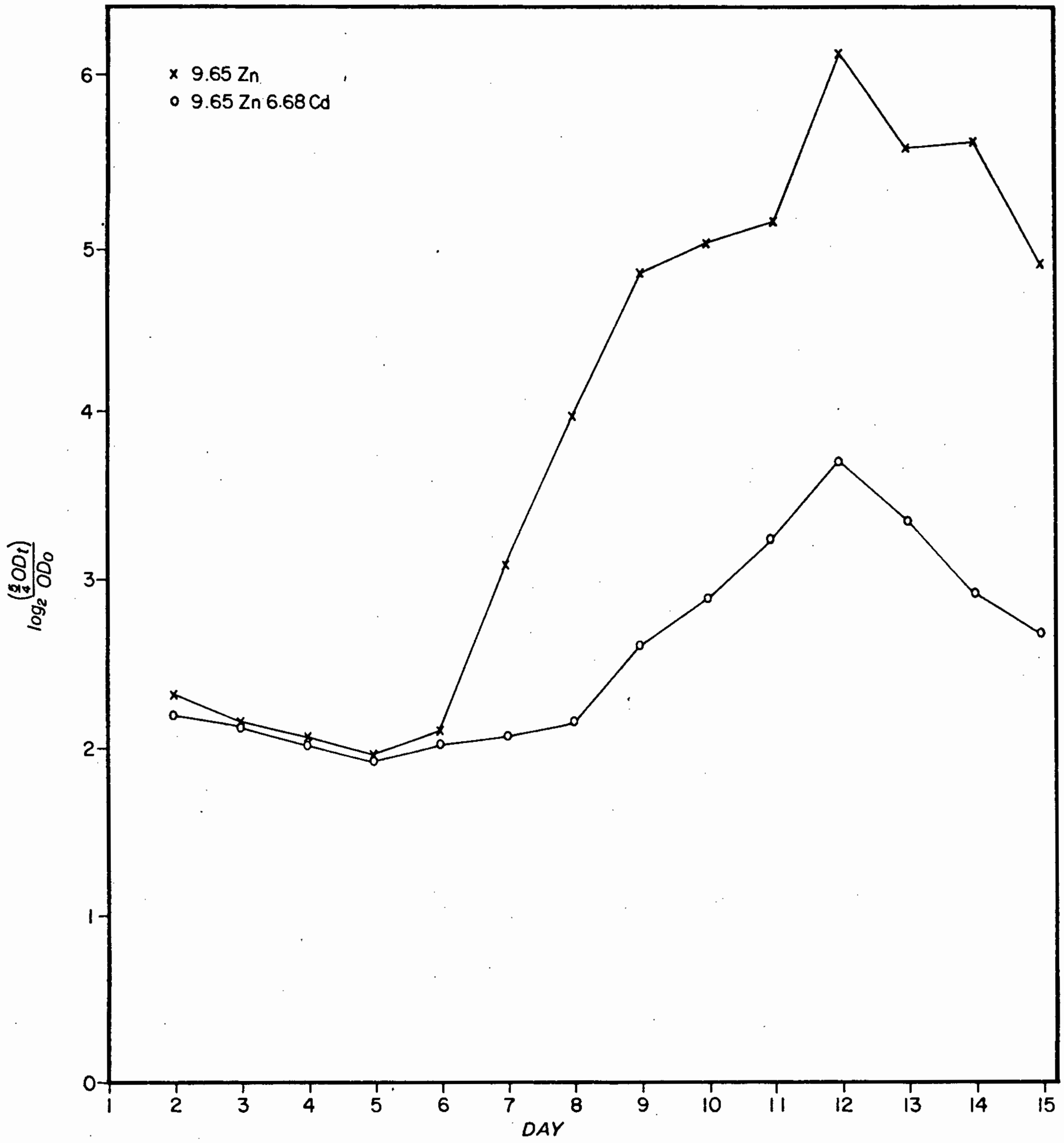


Fig. 12. Growth pattern of T. suecica in zinc (μmole dm^{-3}) and zinc/cadmium (μmole dm^{-3}) media.

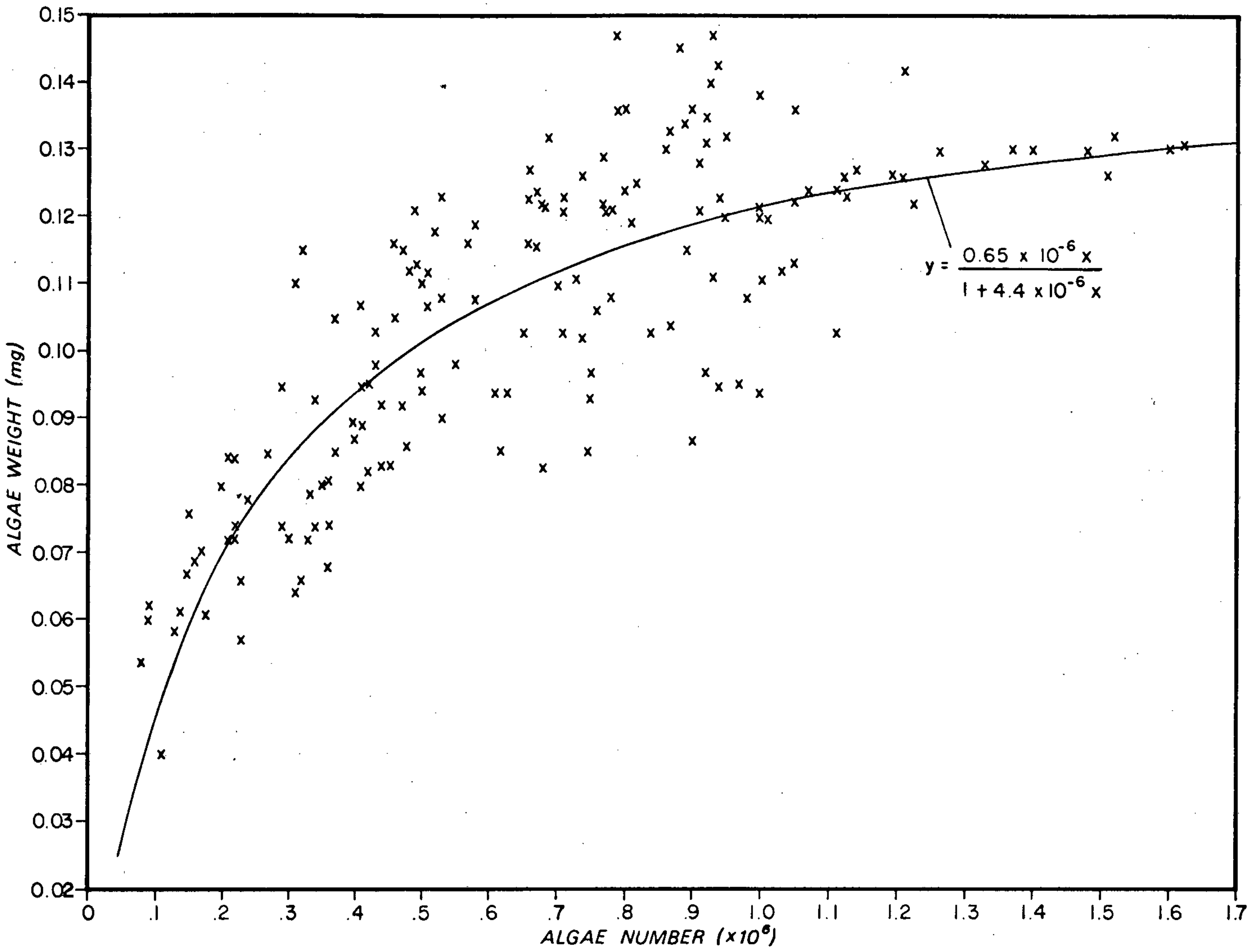


Fig. 13. Relationship between number and weight for T. suecica (n = 225, $r^2 = 0.8955$, $p < 0.01$)

The relationship was non-linear and the curve that fitted the data best is described by

$$y = \frac{0.65 \times 10^{-6} x}{1 + 4.4 \times 10^{-6} x}$$

The non-linear behaviour of this relationship (eg. algae number to mass) only became apparent after analysing the algal weight per unit number of cells (in this case 1×10^6 cells) as shown in Fig. 14. The weight of algae increased from inoculation up to day five, which from Fig. 6 was recognized as the beginning of the logarithmic growth phase. At this point the culture was synchronized and produced a population of cells which was uniform with respect to the stage reached in the cell division cycle. Cell multiplication will only happen once the algal cell is large enough to divide, as shown in figs. 4 and 6. Once the life-cycle has started and progressed into the exponential phase, cell division becomes increasingly unsynchronized and the average weight of the unit cells decreases as shown in Fig. 14. In an unsynchronized life-cycle, a harvested sample will consist of young, light daughter cells as well as old, mature cells.

Hence the non-linear behaviour of the relationship between algae number and algal mass (Fig. 13) became clear: it resulted from different proportion of light, young cells to heavy, mature cells, For instance, a harvested sample from the peak growth phase (1.5×10^6 cell cm^{-3}) would contain an increasing amount of young cells and hence the average algal mass would be low as shown in Fig. 13.

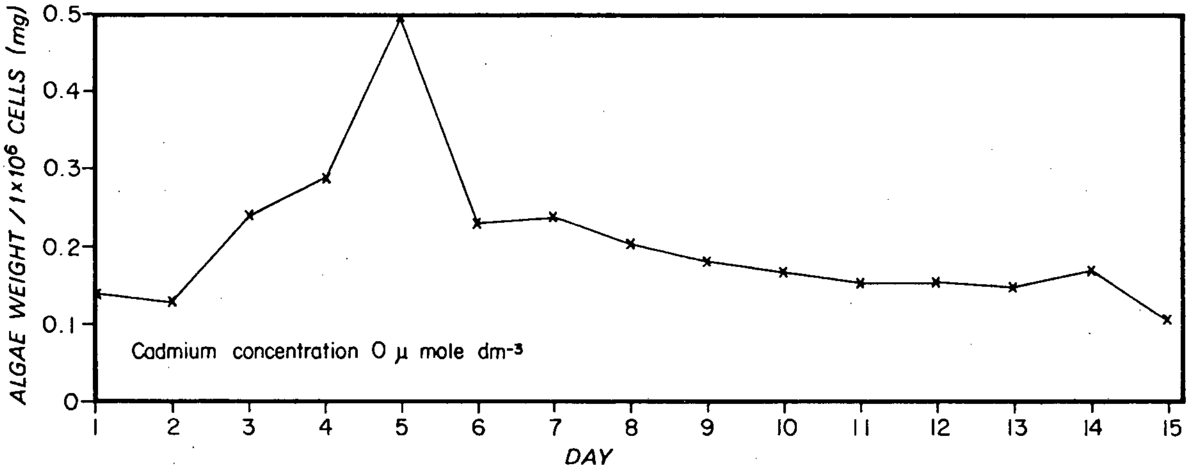


Fig. 14. Algal weight per unit number of cells during 15 days of harvesting.

3.5.2. Algal weight during 15 days of cadmium accumulation

Similar weight patterns were observed when algae were grown in low cadmium contaminated solutions (Figs. 15a-g, a typical relationship is shown in Fig. 15e, the others are in section 8.3). At medium and high cadmium contaminated levels, growth of algal cells became increasingly retarded with increasing metal concentrations and although synchronisation was still achieved more or less on the 5th day (due to the inherent mean generation time), the unit cell mass did not conform to the normal growth pattern (Figs. 15h-n, an example is shown in Fig. 15j).

3.5.3. Algal weight during 30 days of cadmium accumulation

On prolonging the harvesting to 30 days, the different cadmium contaminated cultures (2.67 ± 0.34, 6.54 ± 0.96 and 11.77 ± 1.35 μmole dm^{-3} cadmium) all reached a stable phase with respect to weight per unit cells (Figs. 16a-c). There appeared to be an increase in average cell weight as time progressed (Fig. 16a and b) in the light contaminated cultures, while with heavy cadmium contamination the algae appeared to be dying at a slow rate (Fig. 16c). This was not apparent when the growth was monitored by absorbance (Fig. 11c).

3.6 Cadmium accumulation of *T. suecica*

Since *T. suecica* was used as a food medium, the accumulation of cadmium by the cells was very important. The cadmium concentration of algal control cultures could not be detected by flame atomic absorption spectroscopy. *T. suecica* does accumulate cadmium from spiked solutions, but it seemed to

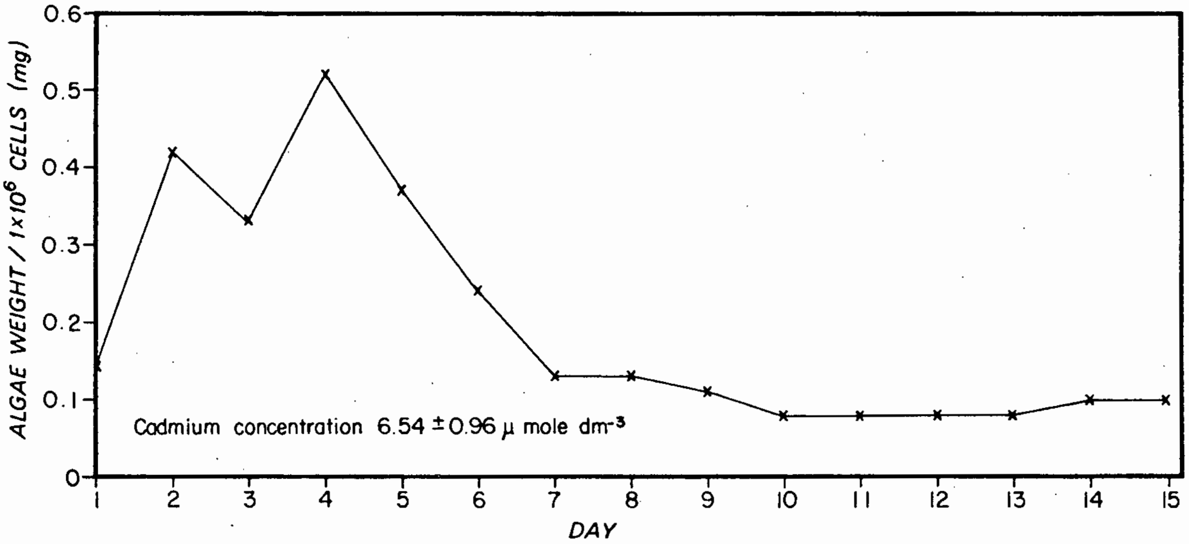


Fig. 15e. Algal mass per unit number of cells during 15 days of cadmium accumulation.

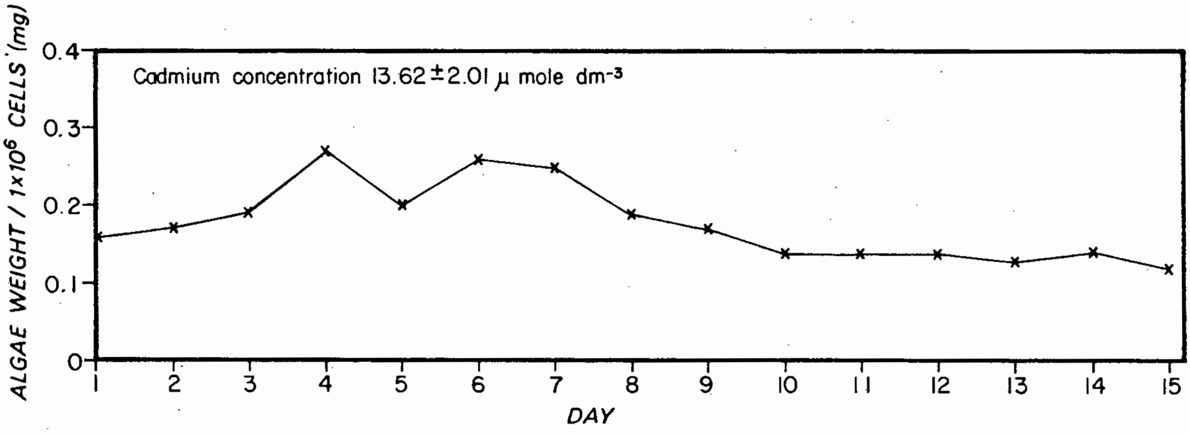
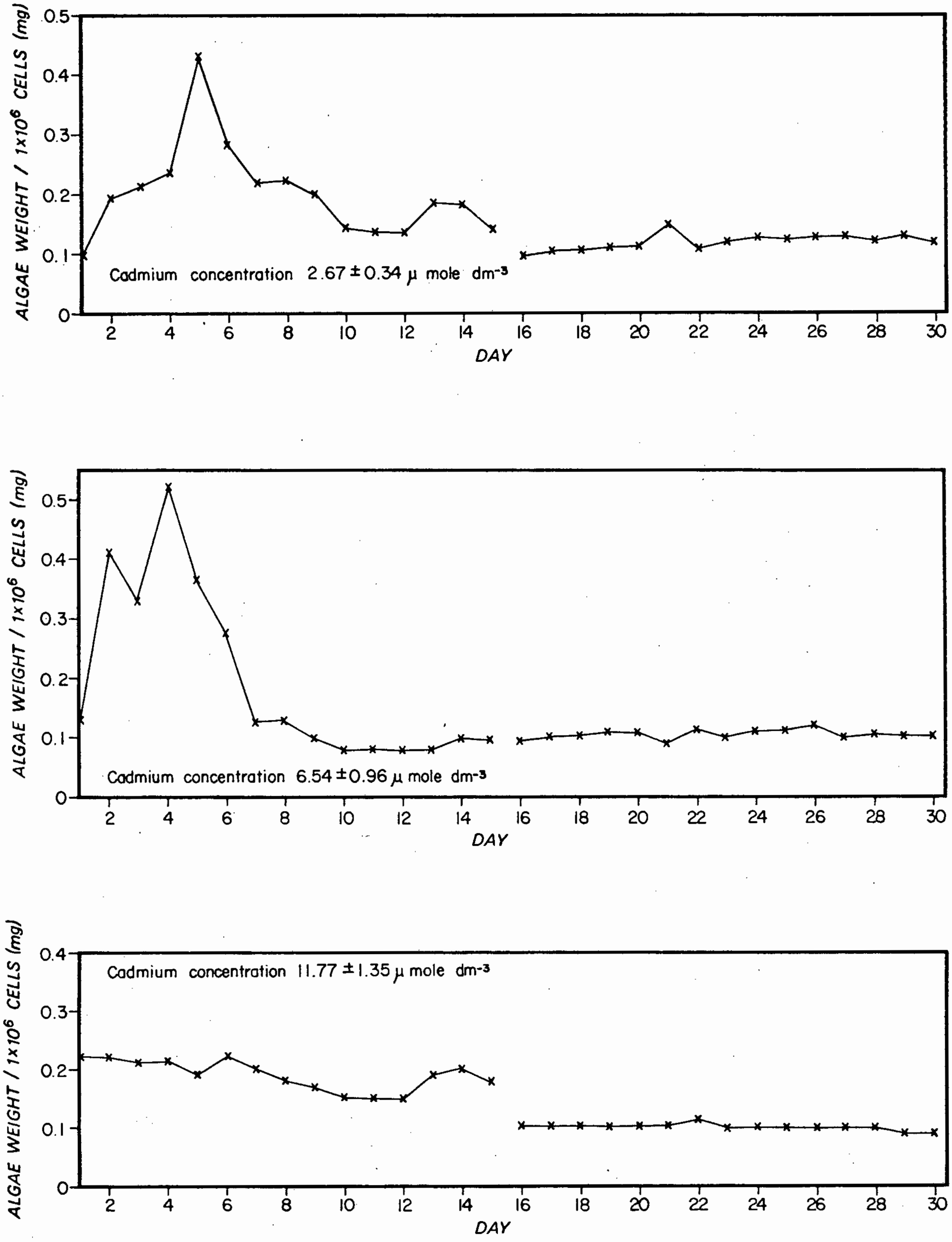


Fig. 15j. Algal mass per unit number of cells during 15 days of cadmium accumulation.



Figs. 16a-c. Algal mass per unit number of cells during 30 days of cadmium accumulation.

react differently to various concentrations as shown in Figs. 17a-n (see also section 8.4). It was found that cadmium accumulation increased with increasing number of cells and time but such a representation obscured any serious analysis, hence the data was presented on a unit weight basis. In all cases cadmium concentration was expressed per 1 mg of algal mass. Similar algal accumulation behaviour at different cadmium concentrations have been grouped together.

3.6.1. Low cadmium contamination

The accumulation of cadmium by the algae are summarized in Figs. 17a-c (Fig. 17c is a typical example, the remaining figures appear in section 8.4). Algae growing in cadmium concentrations ranging from 1.28 ± 0.18 to 4.16 ± 0.57 μmole dm^{-3} accumulated the metal at a smooth, steady rate.

3.6.2. Medium cadmium contamination

The accumulation rate from 5.46 ± 0.64 to 13.67 ± 2.01 μmole dm^{-3} cadmium containing media was not smooth and a more step-wise rate could be recognized (Fig. 17h is typical, the other figures are in section 8.4.) The most prominent feature was the high metal accumulation peak on day five. This feature commenced at a cadmium concentration of 5.46 ± 0.66 μmole dm^{-3} and persisted right throughout all concentrations tested (Figs. 17d-m, Fig. 17e is typical). This increased accumulation coincided with the synchronisation and subsequent increase in size and weight. These observations indicated adsorption rather than accumulation of cadmium ions at this particular life stage of <u>T. suecica,</u> some evidence for this is discussed in section 4.1.

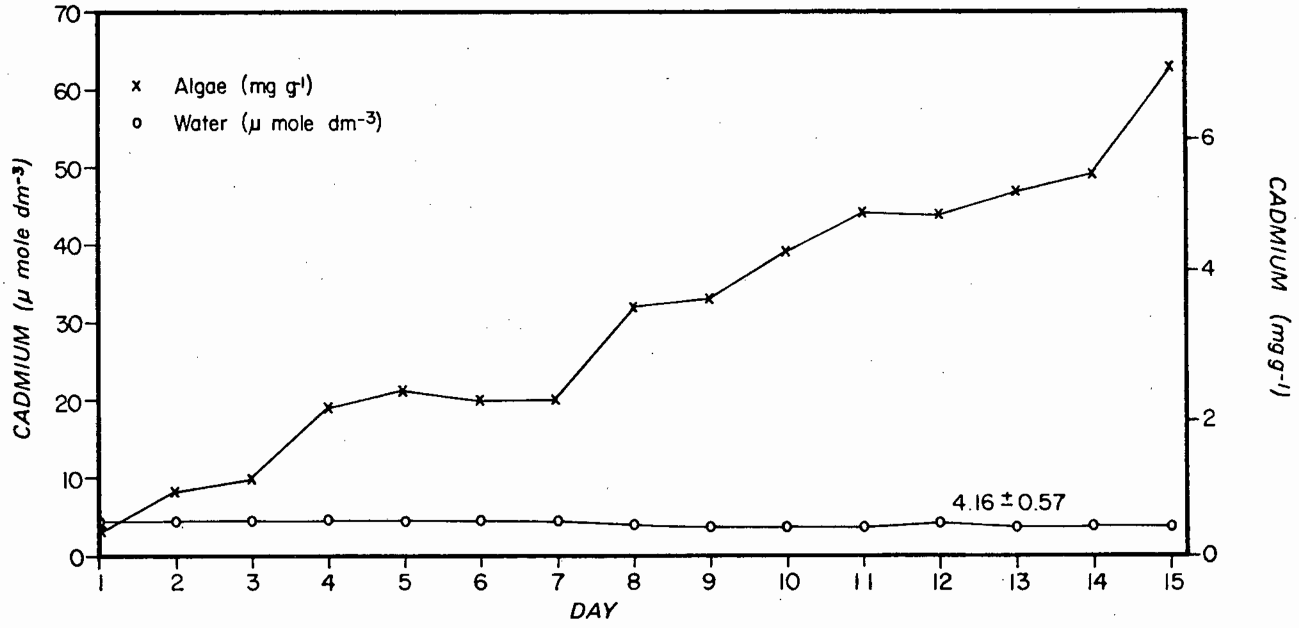


Fig. 17c. Accumulation of cadmium in T. suecica during 15 days of harvesting.

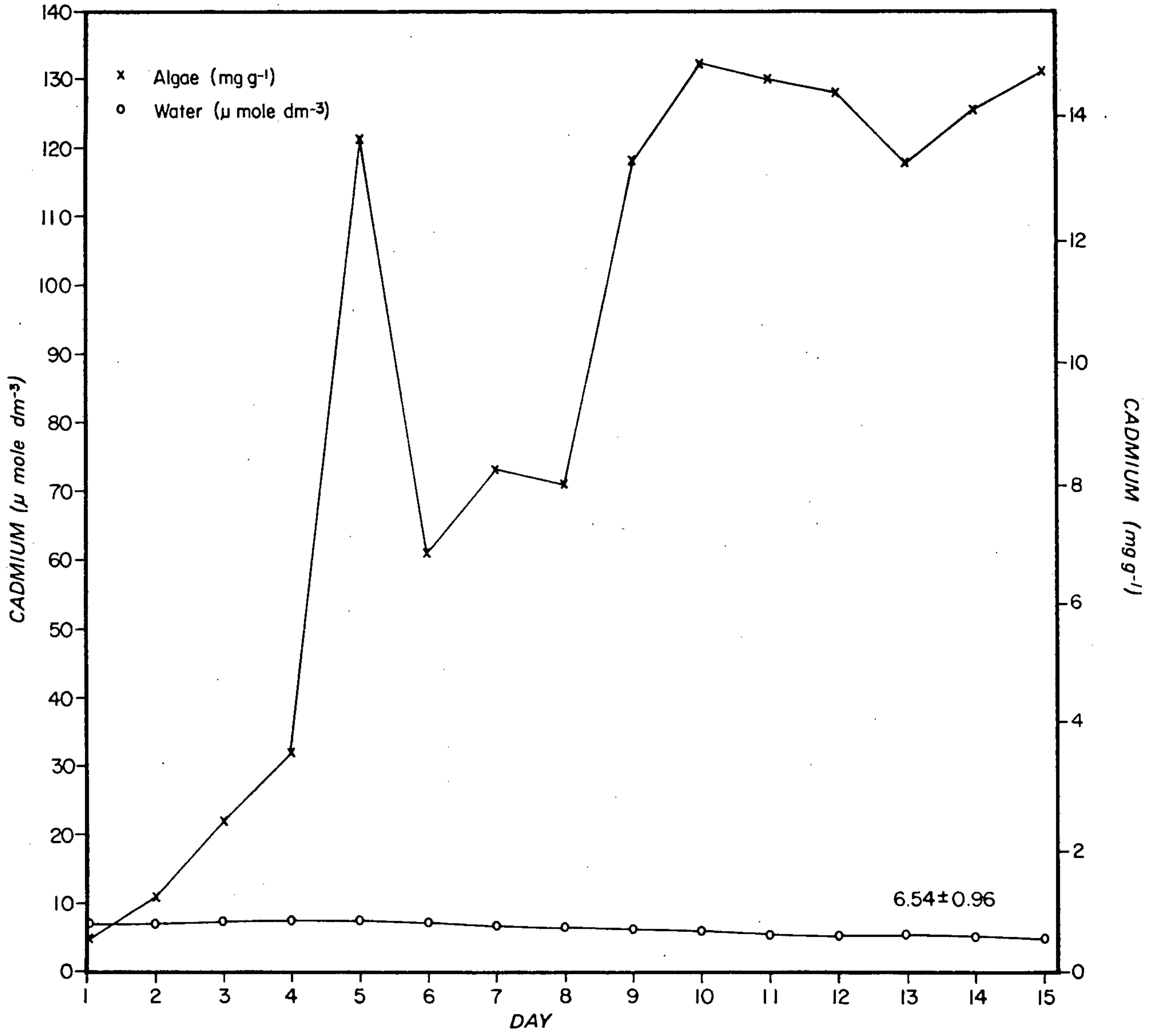


Fig. 17e. Accumulation of cadmium in T. suecica during 15 days of harvesting.

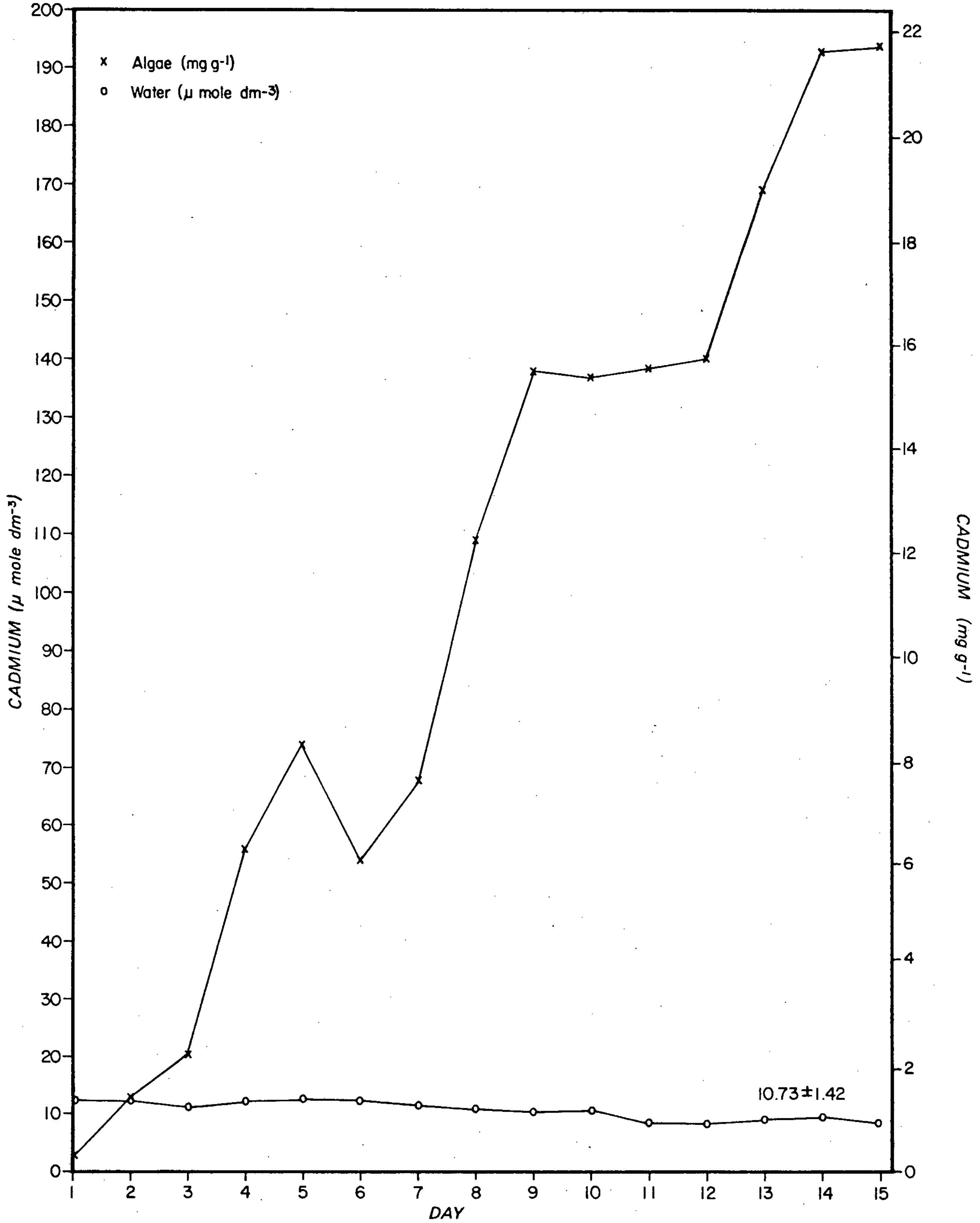


Fig. 17h. Accumulation of cadmium in T. suecica during 15 days of harvesting.

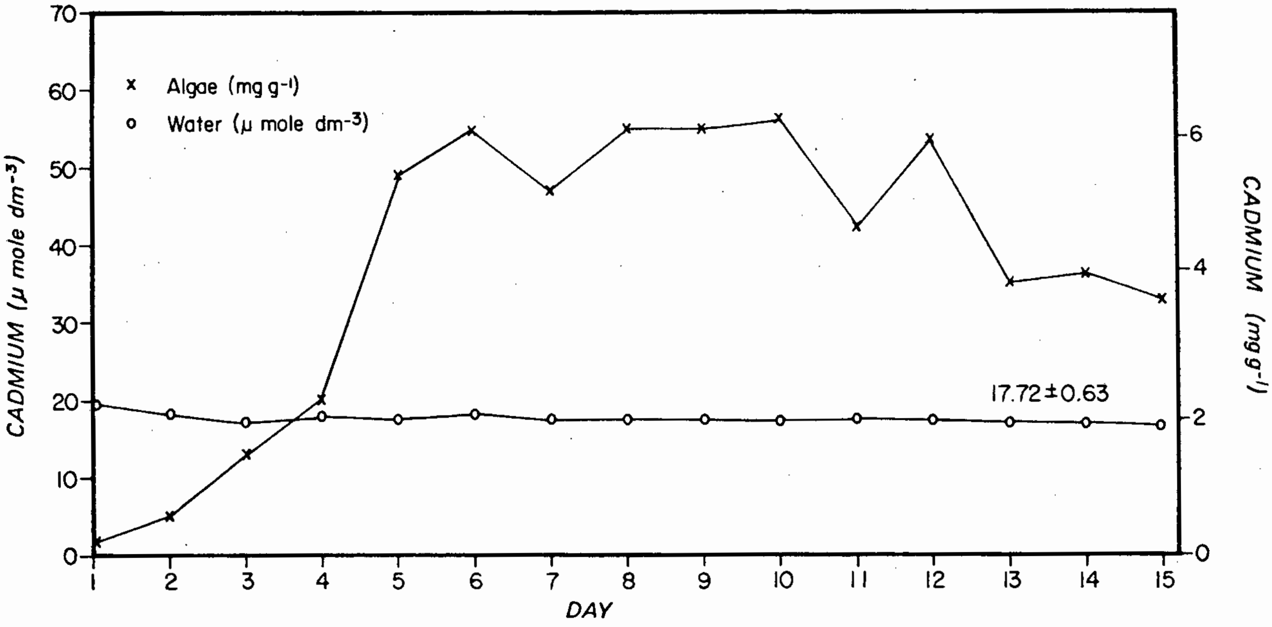


Fig. 17m. Accumulation of cadmium in T. suecica during 15 days of harvesting.

3.6.3. Heavy cadmium contamination

Comparatively little cadmium was found in algae grown in cadmium media ranging from 17.14 ± 0.97 to 19.75 ± 1.07 µmole dm^{-3} (Figs. 17j-n, Fig. 17m is typical). It has been shown that algal growth is poor at these concentrations and hence little active accumulation would have taken place as many cells died off. Adsorption and little accumulation would account for the small metal content found.

3.6.4. Cadmium accumulation of *T. suecica* during 30 days of harvesting

Algae have been grown in selected spiked cadmium media for 30 days and their metal accumulation is given in Fig. 18a-c (Fig. 18b is typical, others at section 8.5). The accumulation of cadmium in *T. suecica* followed very closely the growth pattern when the cell numbers stabilized after 15 days, the accumulated cadmium reached a plateau and then fluctuated according to the division cycle of the algae for the remainder of the experiment.

The cadmium concentrations in the media were monitored each day as shown in Figs. 17 and 18, and decreased continually over the 30 day period. With decreasing cadmium concentration in the media, the accumulated heavy metal also seemed to be decreasing (Figs. 18a and b). Thus the accumulation behaviour of *T. suecica* was similar during the long term (30 days) to that of the shorter (15 days) period. This emphasises again the need for monitoring possible metal losses or other changes during accumulation experiments.

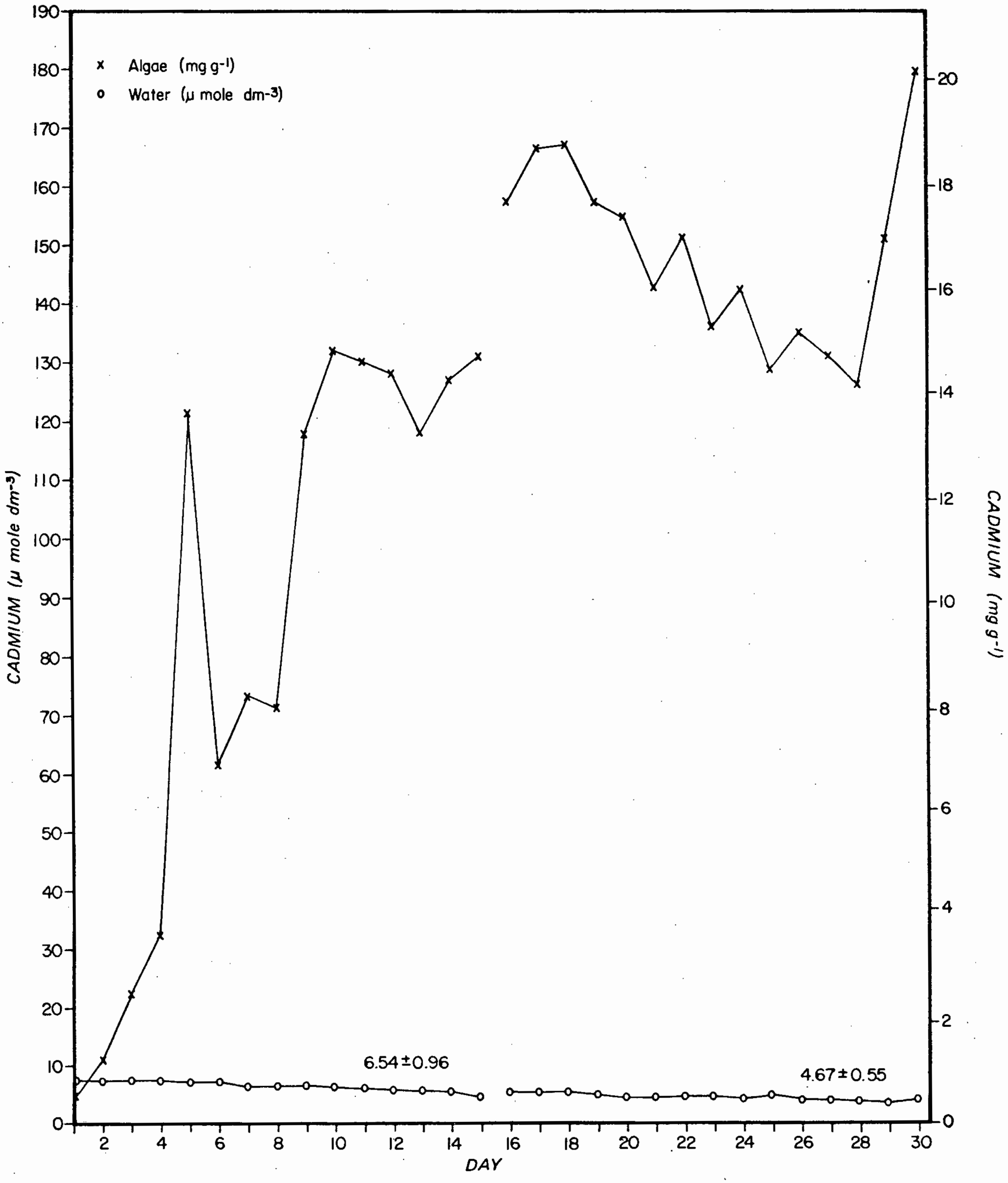


Fig. 18b. Accumulation of cadmium in T. suecica during 30 days of harvesting.

3.7 Feeding of Choromytilus meridionalis

3.7.1. Energy value of T. suecica

Rations (2 x 50 cm^3 of 1.6 x 10^6 cell cm^{-3} with weight 12.96 mg) of T. suecica in a "day-11" life-cycle phase were used as food for C. meridionalis during the accumulation experiments. The energy value of the algae in the "day-11" cycle phase is shown in Table 9. Each value represents the mean of two readings on one sample. The energy available from T. suecica averaged 13,62 kJg^{-1} AFDW (ash free dry weight) and inorganic material constituted 34.18% of the samples. Comparison with energy values of another green alga , Dunaliella primolecta and organic matter available to mussels (<100 µm particles) in seawater from Bailey's Cottage, False Bay, are given in Table 10. In its natural habitat C. meridionalis (20 mm shell length) consumes about 98 J d^{-1} (conversions: 20 mm mussel filters 0.632 dm^3h^{-1} at 1 dm^3 = 16.2 J with an assimilation efficiency of 40%, Griffiths (1980)). During the accumulation experiments the laboratory mussels, which were fed on a monoculture of T. suecica, received each 9.7 J d^{-1}, which is about one of magnitude smaller than the ration of mussels in a natural habitat (Griffiths, 1980).

3.7.2. Continuous feeding

Feeding of mussels cannot be improved by greater bulk rations, due to the decrease in assimilation efficiency at high concentration of cells (Griffiths, 1980). Continuous feeding at low concentration of algae has been shown to be desirable (Winter, 1973; Griffiths and King, 1979; Griffiths, 1980). An apparatus maintaining a certain concentration of algae has been developed by Labotron Messtechnik, Germany. This maintaining apparatus, unfortunately could only be used for short

TABLE 9: Energy values of *T. suecica* in the "Day 11" life cycle phase.

	kJg^{-1} Dry Weight	kJg^{-1} AFDW	% inorganic
	8.92	13.88	35.73
	8.97	13.47	33.36
	8.99	13.50	33.44
Mean	8.96	13.62	34.18
S.D.	0.04	0.23	1.35

TABLE 10: Energy values of algae and organic matter of seawater at Bailey's Cottage.

Source	kJg^{-1} dry weight	kJg^{-1} AFDW	% inorganic	Reference
Tetraselmus suecica	8.96	13.62	34.2	Present study
Dunaliella primolecta	9.08	14.95	30.4	Griffiths and King 1979
Organic matter of seawater (<100 μm particles)	2.76	5.74 ^{x}	53.3	Griffiths, 1980

^{x} AFDW corrected value = 6.1 kJg^{-1} AFDW experimental feeding runs lasting about 24 hours (pers. comm. Dr. D. Gaerdes, Inst. für Meeresforschung, Bremerhaven).

Considerable effort was put into an attempt to design a continuous feeding apparatus for C. meridionalis. The problems encountered with this project were numerous. The variation in main power supply and instrument light bulb intensity were corrected. Similarly, instrument conditions effected by turbulence of aeration currents, mechanical position shift and the light conditions of the experiment could be controlled. Unfortunately, all light sensitive receivers and photo-cells tested were voltage dependent and their thermal characteristics proved to be the final stumbling block and the project had to be postponed. Hence the mussels were not fed continuously throughout the accumulation experiments.

3.8 Characteristics of C. meridionalis from natural habitats and laboratory

Physical indices of test animals, which are easy to measure should be used to compare and interpret any response in natural as well as cultivated populations. For C. meridionalis, shell length (mm) was chosen as such an index against which the dry body weight of mussels was measured (Fig. 19). Data from different authors (Watling, 1978; Griffiths, 1980; Orren et al., 1980) has been included. The curve that fitted the present study data best is described by

$$(\text{g dry flesh weight}) = 2.64 \times 10^{-5} (\text{shell length (mm)})^{2.65}$$

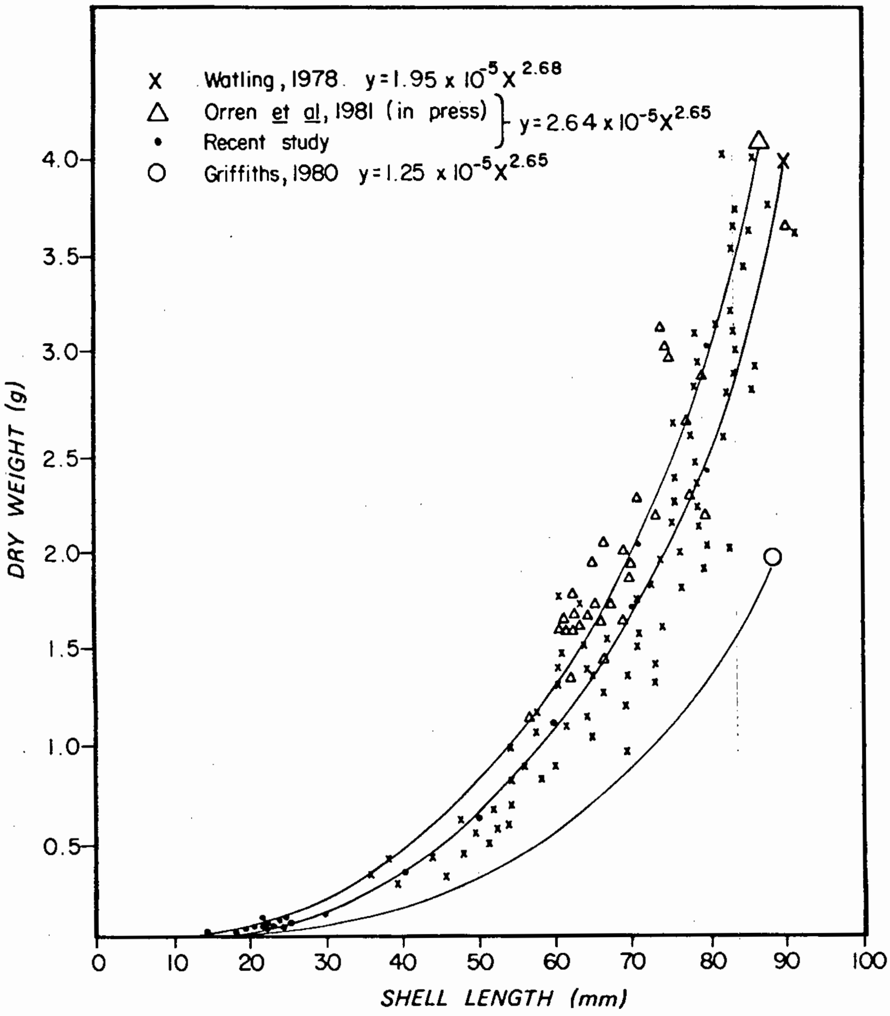


Fig. 19 Relationship of shell length and body weight in <u>C. meridionalis</u> (n = 258, $r^2 = 0.9137$, $p < 0.01$).

The mussels sampled in 1980 appeared to be similar to those of Watling (1978), but were heavier than those of Griffiths (1980). These differences are due to different habitats. Griffiths (1980) collected her mussels at Bailey's Cottage (False Bay), while the animals of both the other workers originated from the West Coast (Bloubergstrand). The West Coast mussel populations have generally larger gonads and hence are heavier (Griffiths, pers comm.).

Since Griffiths (1977) had shown that 50 - 60% of C. meridionalis body weight can be lost during spawning, this study concentrated on immature mussels with a shell length around 20 mm.

Although the algal ration fed to the laboratory mussels was very low in energy value per individual, no significant body weight loss was observed in mussels maintained for either 15 days (Fig. 20) or 30 days (Fig. 21). The statistical analysis is given in Table 11 (1,2). Actually, diet seemed to have little influence on the shell length/body weight relationship when mussels were kept for short periods. This became evident when body weights of mussels starved for 6 days (Fig. 22) were compared to weights of mussels fed for 15 days (Fig. 23), as no significant difference was found (Table 11; 1,2,6 and 1,6).

A significant difference, however, was found when all feeding regimes were compared (Table 11; 1,2,3,4,5). A more detailed analysis showed that no weight loss could be detected in mussels maintained for 15 days (Fig. 24) in a contaminated environment (Table 11; 1,3) but after 30 days (Fig. 25) in either contaminated and contaminated/clean environments (Fig. 26) a statistical highly significant weight loss was found. (Table 11; 3,4 and 3,5). The weight of the latter two in turn did

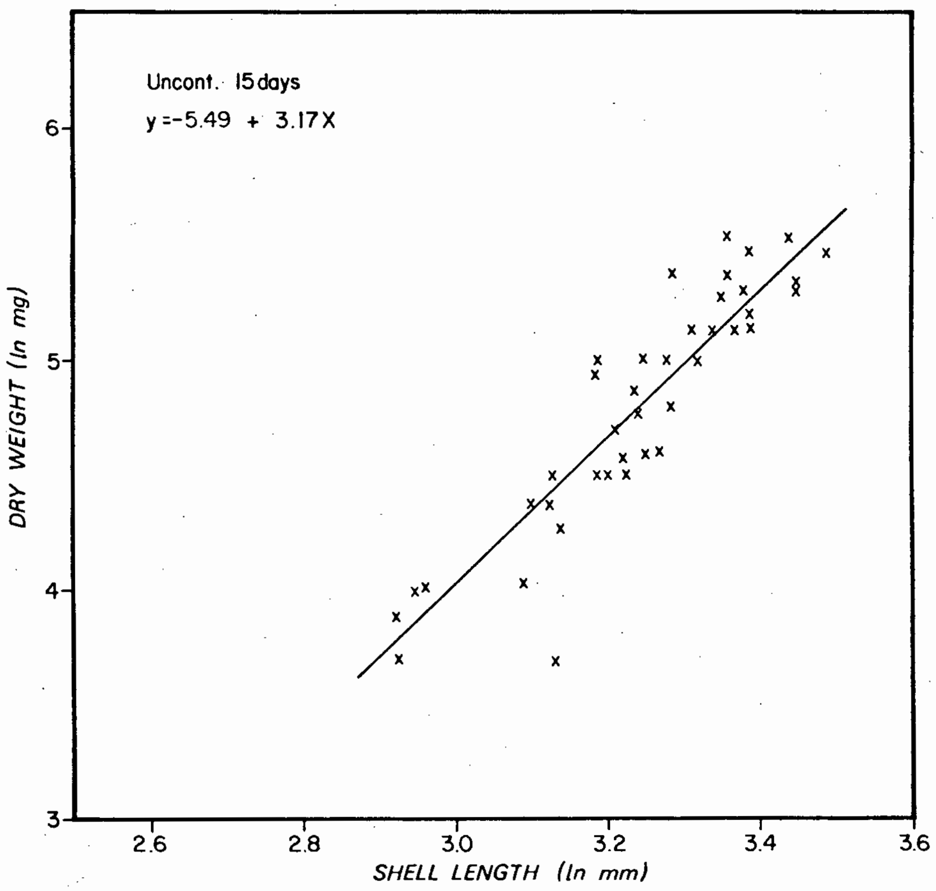


Fig. 20 Relationship of dry body mass and shell length of <u>C. meridionalis</u> maintained for 15 days on rations of <u>T. suecica</u> (n = 40, r^2 = 0.828, p <0.01).

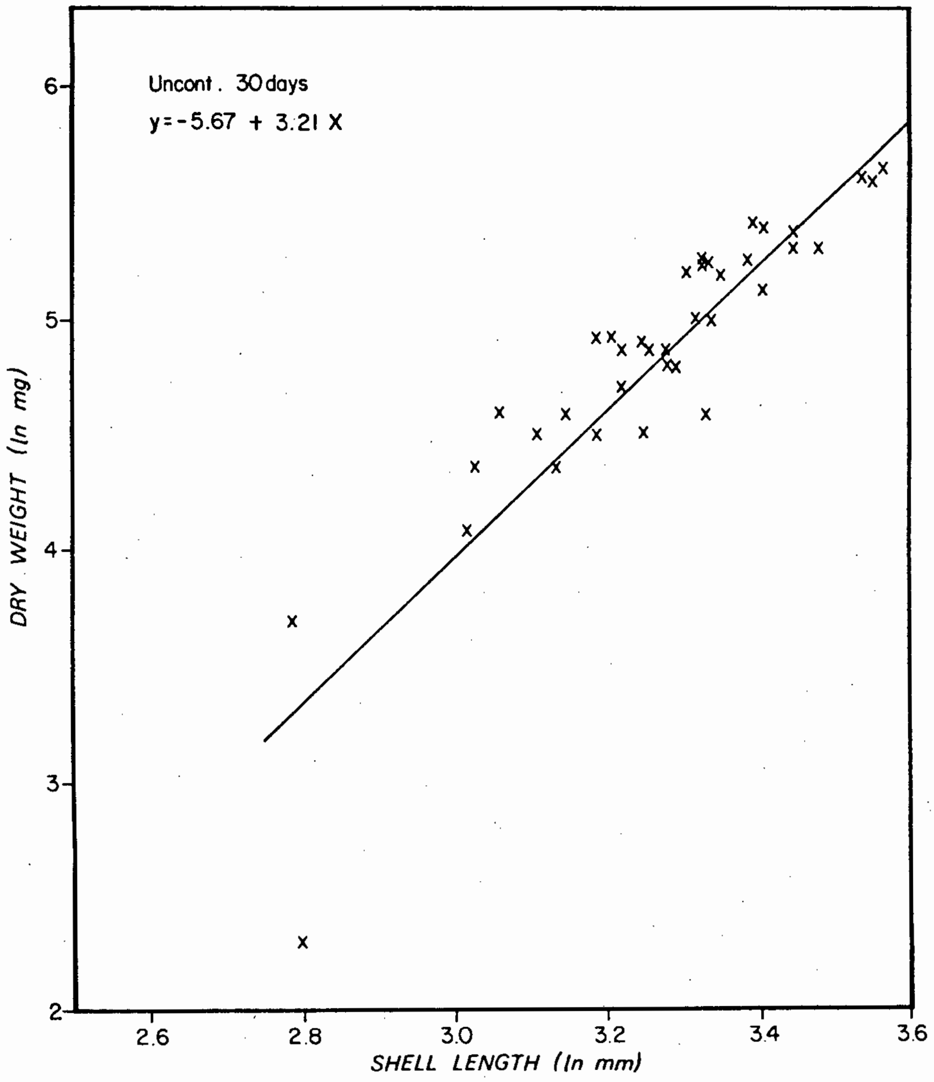


Fig. 21 Relationship of dry body mass and shell length of <u>C. meridionalis</u> maintained for 30 days on rations of <u>T. suecica</u> (n = 38, r^2 + 0,8464, $p < 0.01$).

TABLE 11: Results of analysis of variance of different feeding and starvation regimes on length-weight relationship of *C. meridionalis*.

Regimes compared	1,2,6	1,2	1,6	1,2,3,4,5	1,3	3,4	3,5	4,5
χ^2	4.63	0.44	2.98	6.55	2.69	0.86	1.69	0.17
Degrees of freedom	2	1	1	4	1	1	1	1
Significance	+	+	+	+	+	+	+	+
F_1 (slopes)	0.068	0.015	0.086	1.06	2.84	0.43	0.31	0.17
Degrees of freedom	2; 89	1; 74	1; 53	4; 287	1; 106	1; 146	1; 135	1; 145
Significance	+	+	+	+	+	+	+	+
F_2 (elevation interc.)	0.51	0.74	0.71	3.57	1.83	14.62	9.02	0.39
Degrees of freedom	2; 91	1; 75	1; 54	4; 291	1; 107	1; 147	1; 136	1; 146
Significance	+	+	+	xx	+	xx	xx	+

+	Not significant
x	Significant ($P < 0.05$)
xx	Highly significant ($P < 0.01$)
χ^2	Bartlett's test for homogeneity of variance
F1,F2	Comparison of regression lines (F-test)
1	Mussels fed uncontaminated algae for 15 days in clean media
2	Mussels fed uncontaminated algae for 30 days in clean media
3	Mussels fed "uncontaminated" algae for 15 days in Cd media
4	Mussels fed "uncontaminated" algae/Cd media for 15 days, then the same mussels were fed uncontaminated algae for 15 days in clean media
5	Mussels fed "contaminated" algae for 30 days in Cd media
6	Mussels were starved for 6 days in clean media

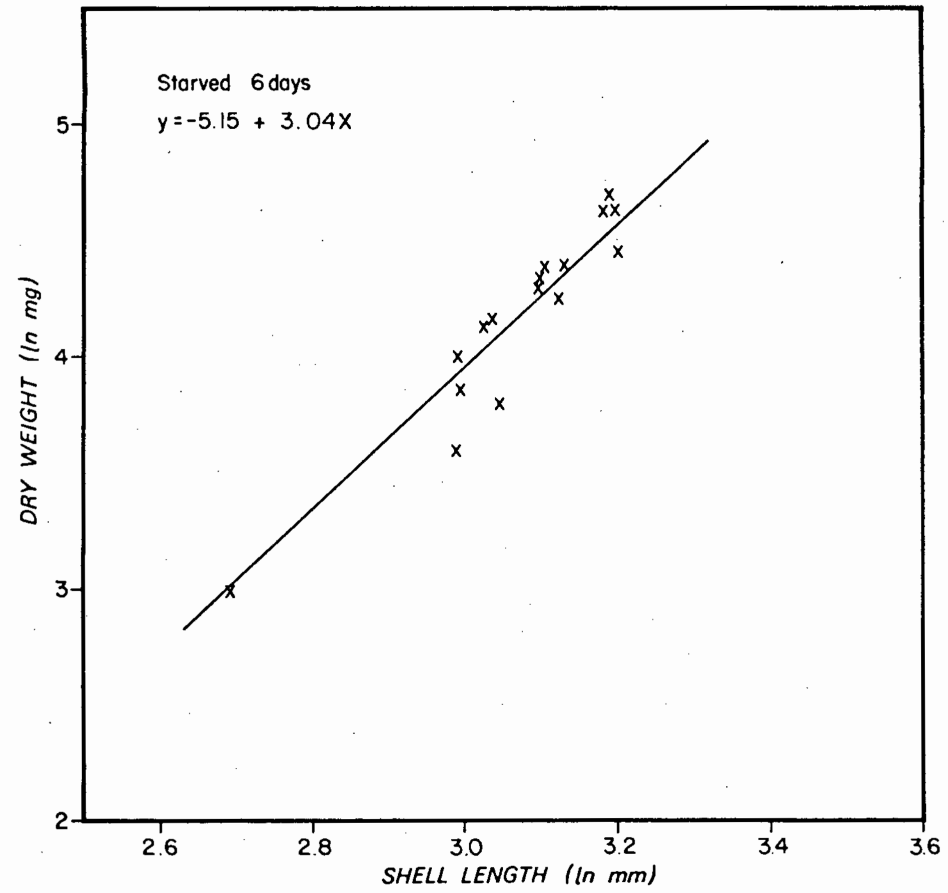


Fig. 22 Relationship of dry body mass and shell length of <u>C. meridionalis</u> starved for 6 days ($n = 17$, $r^2 = 0,8753$, $p < 0.01$)

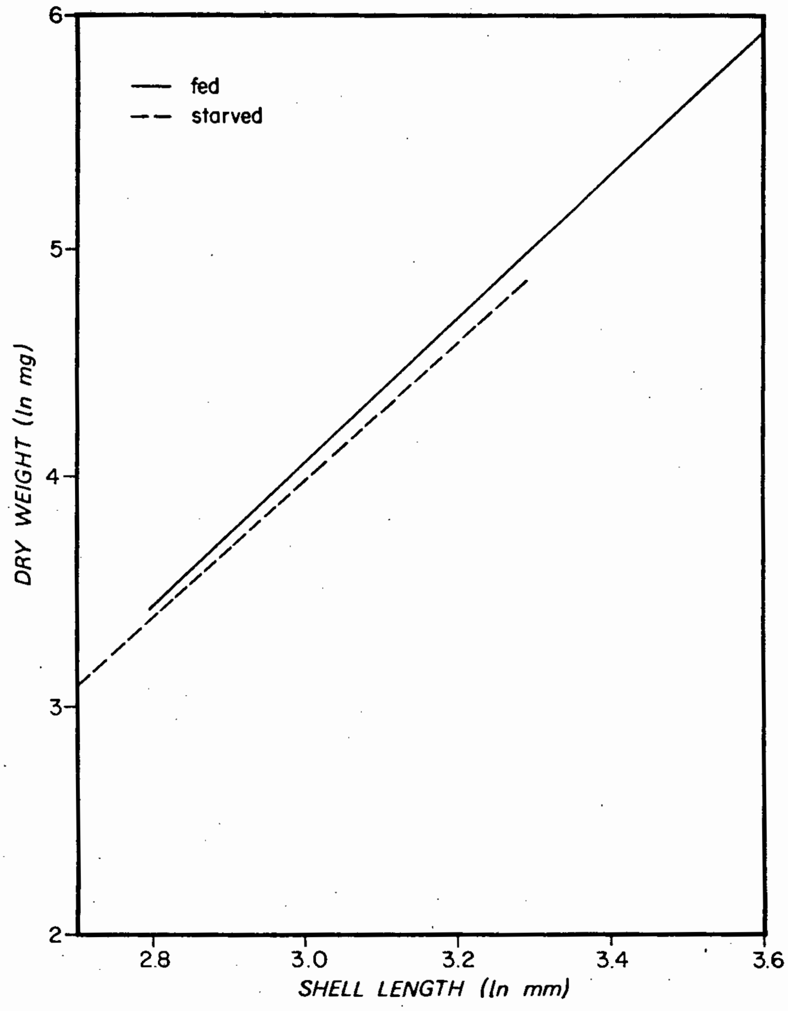


Fig. 23. Comparison between weight-lenth relationship of starved and fed C. meridionalis.

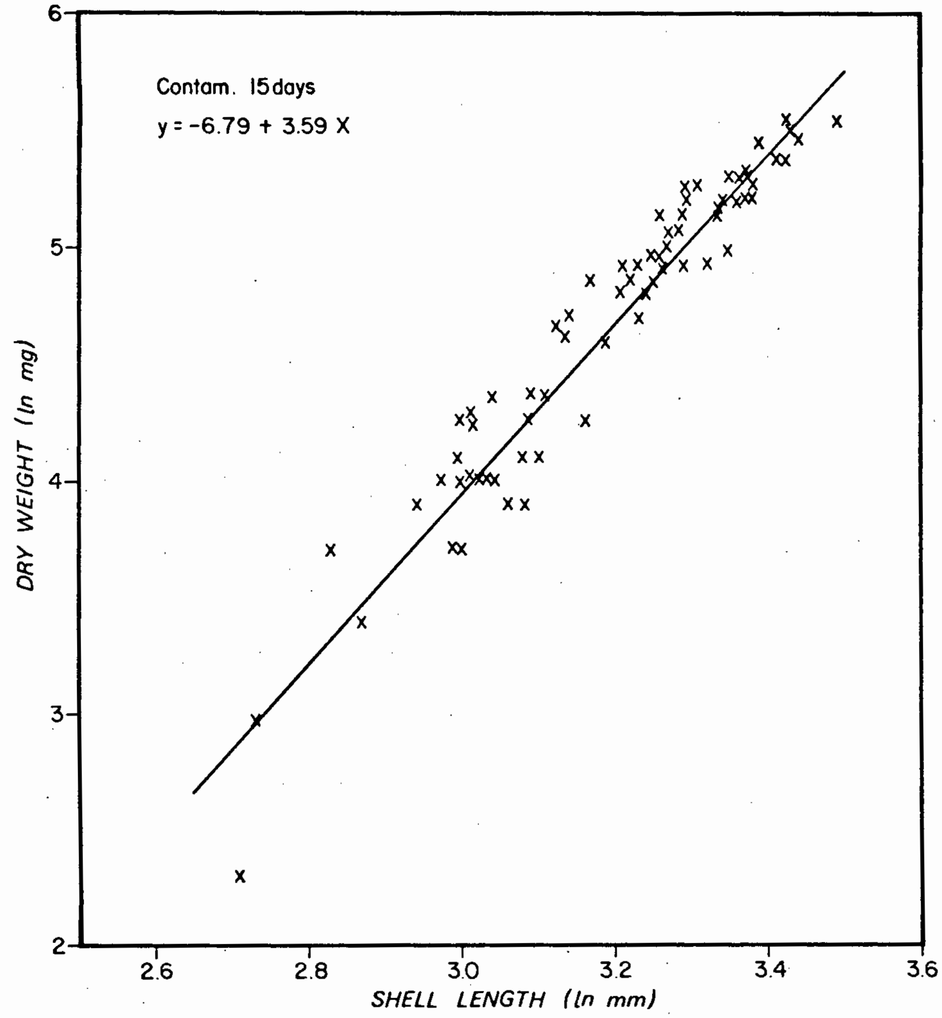


Fig. 24. Relationship of dry body mass and shell length of C. meridionalis during 15 days of cadmium accumulation (n = 70, r^2 = 0,934, p < 0,01).

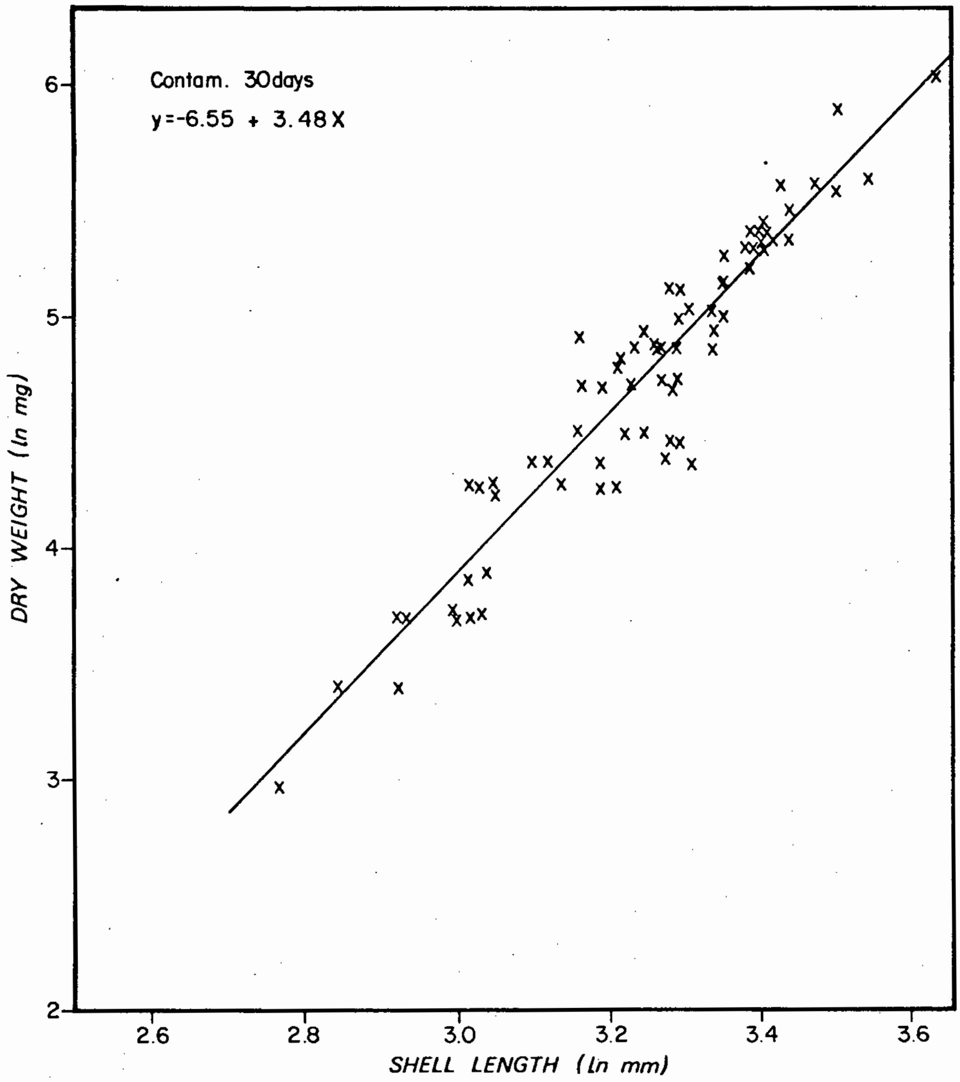


Fig. 25. Relationship of dry body mass and shell length of C. meridionalis during 30 days of cadmium accumulation ($n = 69$, $r^2 = 0.6225$, $p < 0.01$).

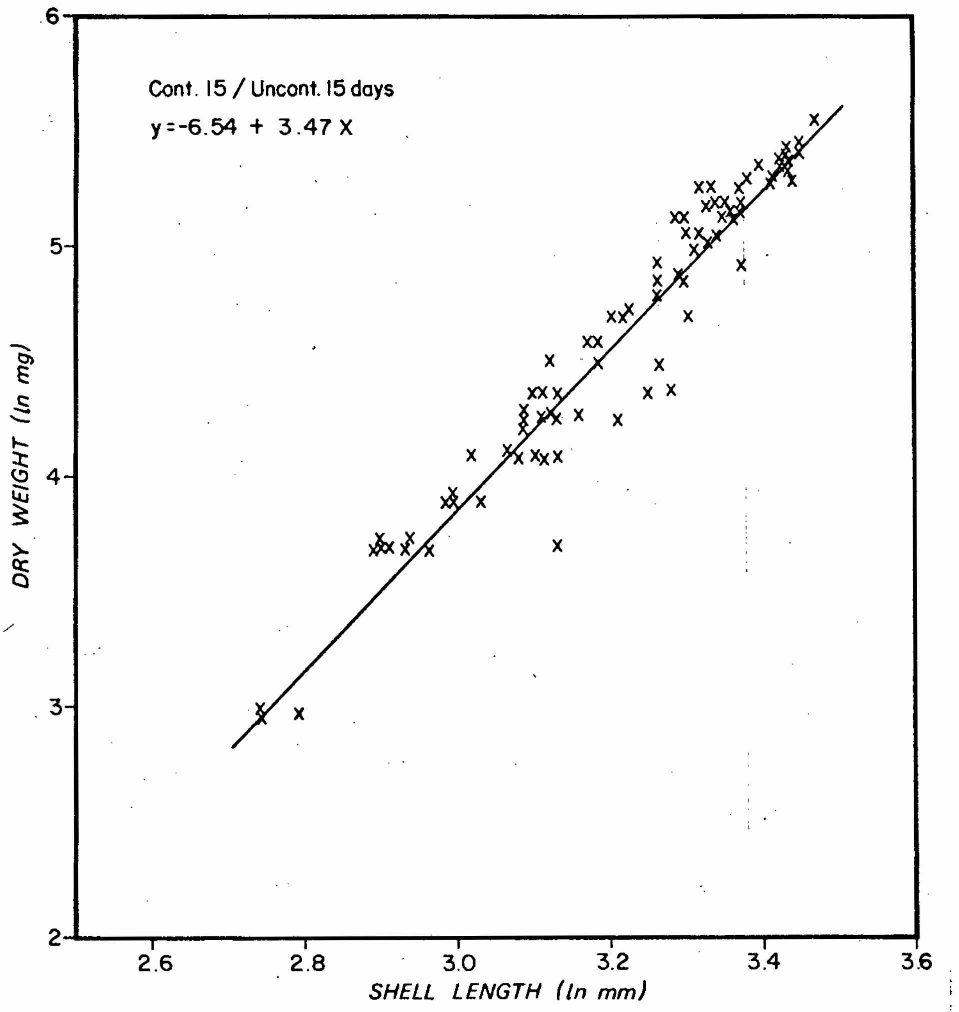


Fig. 26. Relationship of dry body mass and shell length of <u>C. meridionalis</u> during 15 days of cadmium accumulation followed by 15 days clean environment ($n = 80$, $r^2 = 0.9150$, $p<0.01$)

not differ from each other (Table 11; 4,5). In other words, the effect of cadmium on the shell length-weight relationships could not be detected after 15 days maintenance in a contaminated environment, but the damage had been done. Deterioration in mussel condition had started, which could only be detected after a 30 day period, irrespective of transfer to an uncontaminated habitat or continued contamination.

3.8.1. Cadmium content of C. meridionalis

The cadmium content of mature mussels from natural habitats is summarized in Fig. 27 and the metal content of immature mussels is given in Fig. 28. Immature, faster-growing individuals were accumulating cadmium at a greater rate than mature mussels. It is evident, however, that a detoxification mechanism exists, which allows mature mussels to eliminate cadmium. This explains why the average metal content of adult mussels is less than 0.2 $\mu g\ g^{-1}$ compared to the average metal content of 0.71 ± 0.24 $\mu g\ g^{-1}$ found in immature mussels.

In cadmium enriched environments C. meridionalis readily accumulated metal as summarised in Table 12. These results were obtained by feeding twice 50 cm^3 of 1.6 x 10^6 cell cm^{-3} of T. suecica in a "day-11" life-cycle phase. The algae were grown in either clean sea water medium or 7 ± 1 $\mu mole\ dm^{-3}$ cadmium enriched sea water medium. The latter was selected, because that was the best cell growth and cadmium accumulation condition (Fig. 10). Immature mussels (80 per tank) were maintained in:

a) clean seawater with clean food for 15 days (15 day control)
b) clean seawater with clean food for 30 days (30 day control)

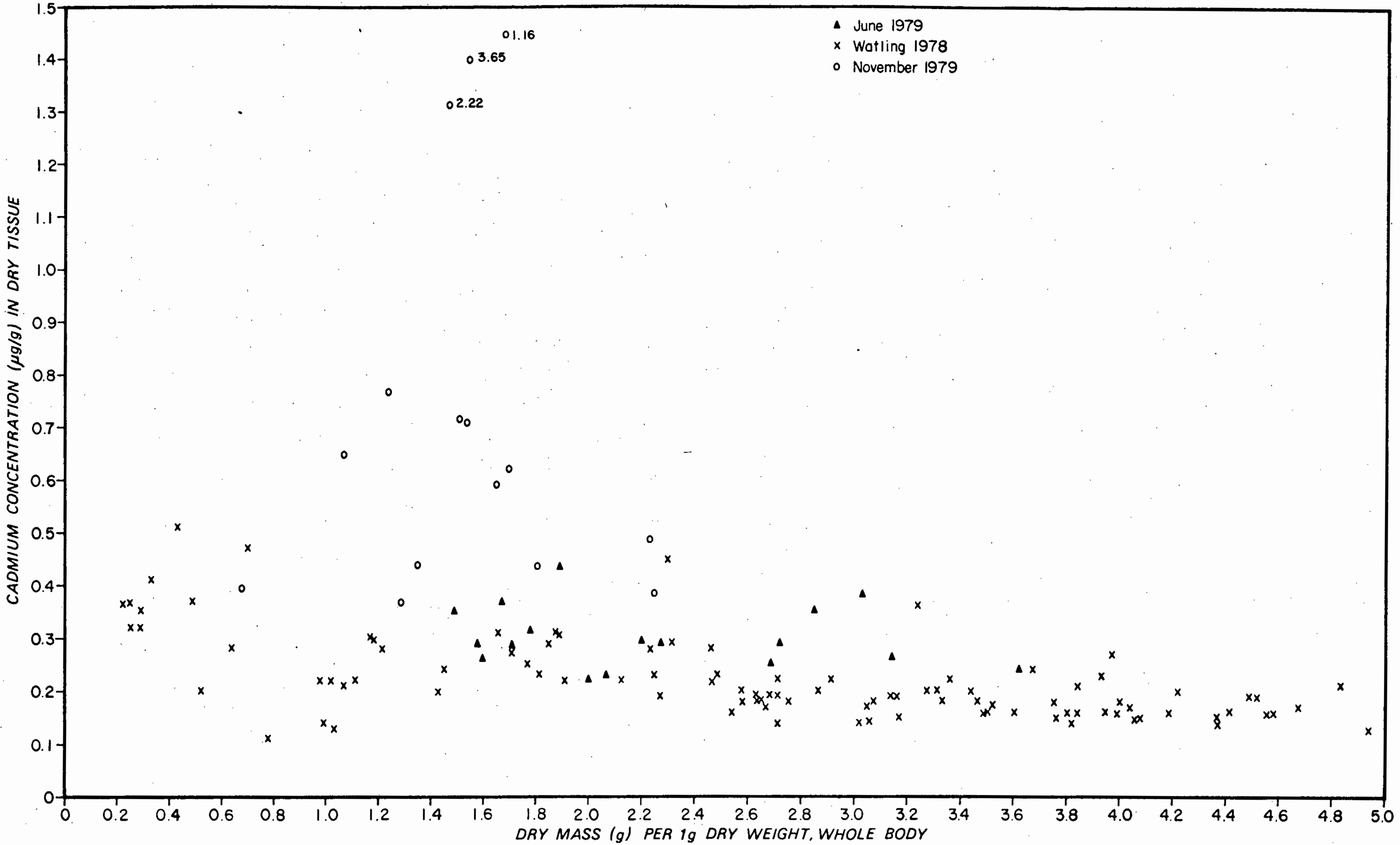


Fig. 27. Cadmium content of mature C. meridionalis from natural habitats.

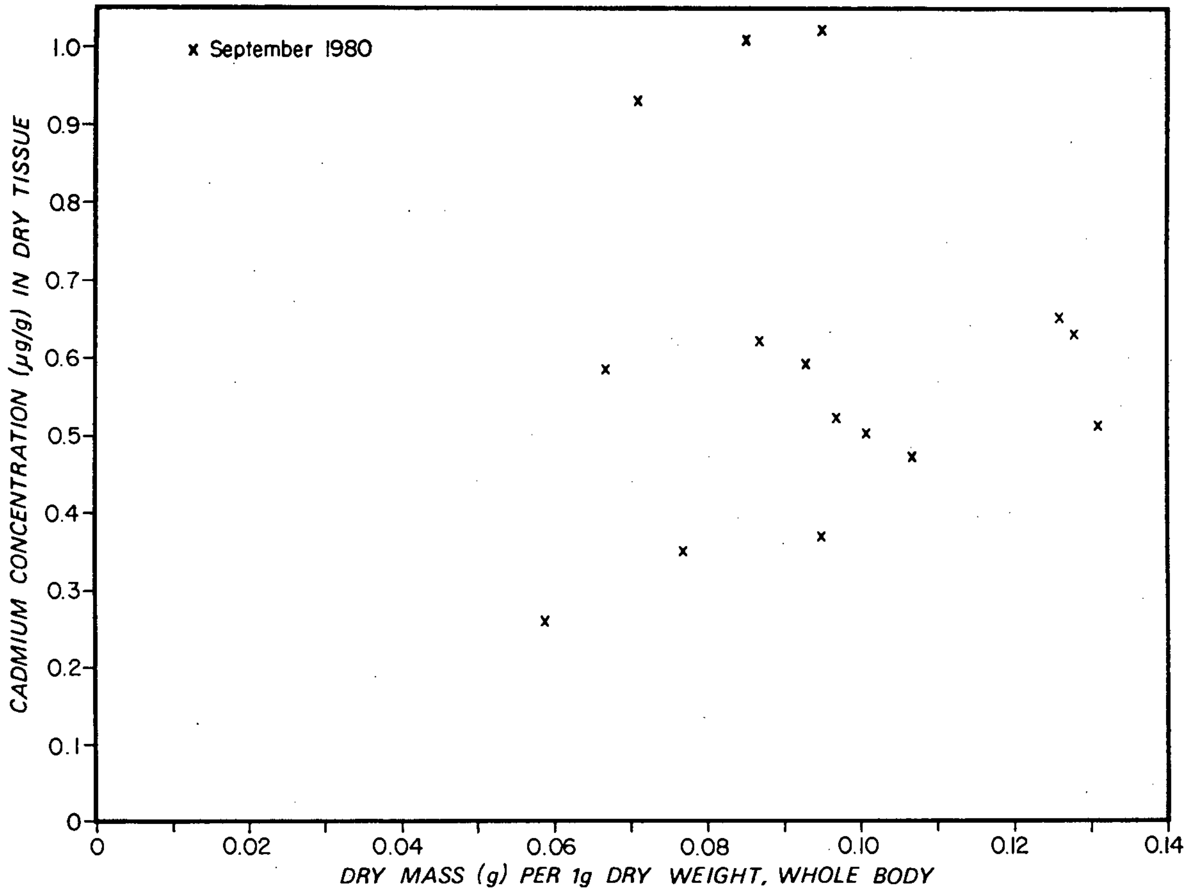


Fig. 28. Cadmium content of immature C. meridionalis from natural habitats.

TABLE 12: Cadmium content of C. meridionalis after different feeding accumulation regimes (Average value of 10 mussels all with shell length of about 20 mm)

	Flesh fraction g g^{-1}	Buffer fraction g g^{-1}	g Cadmium mg^{-1} Protein	Total Body g g^{-1}
15 day control	1.3 ± 0.1	x	x	1.3 ± 0.1
30 day control	1.4 ± 0.1	x	x	1.4 ± 0.1
15 day accumulation	49.7 ± 3.2	785.0 ± 59.1	0.62	834.7 ± 62.3
15/15 day accumulation	37.4 ± 2.1	611.4 ± 74.3	0.58	648.8 ± 76.4
30 day accumulation	61.3 ± 5.2	1082.5 ± 114.4	1.32	1143.8 ± 119.6

x not detectable

c) 7 ± 1 μmole dm^{-3} cadmium enriched tank with "contaminated" food for 15 days (15 day accumulation)

d) 7 ± 1 μmole dm^{-3} cadmium enriched seawater with "contaminated" food for 30 days (30 day accumulation)

e) 7 ± 1μmole dm^{-3} cadmium enriched seawater with "contaminated" food for 15 days and maintained for another 15 days in clean seawater fed clean food (15/15 day accumulation).

The cadmium uptake by the mussels was found to be non-linear. During the first 15 days there was a 642-fold increase, while the cadmium content increased by 37% from day 15 to day 30 (Table 12). After cadmium accumulation of 15 days, some mussels were maintained in a clean environment for another 15 days, during this time they eliminated 22% cadmium of their total body burden.

3.9 Isolation of metal binding protein (MBP)

The buffer soluble material of batches of 10 mussels from control and accumulation regimes was applied to the Sephadex G - 75 column and eluted at a rate of 22 cm^3h^{-1} at 4.02 cm^3 per fraction. Table 12 shows the rate of cadmium uptake into buffer soluble supernatant (58% of total) associated with protein. No cadmium was found in the buffer soluble fraction of the 15 day and 30 day control (Fig. 29). Two protein peaks occurred naturally in the supernatant of both controls, one at elution volume of 117 cm^3 (OD_{250}) or 161 cm^3 (OD_{280}) while the other was eluted at fraction volume 402 cm^3 (OD_{250}) or 374 cm^3 (OD_{280}). In each case the absorbance peaks at OD_{250} and OD_{280} did not quite coincide with each other.

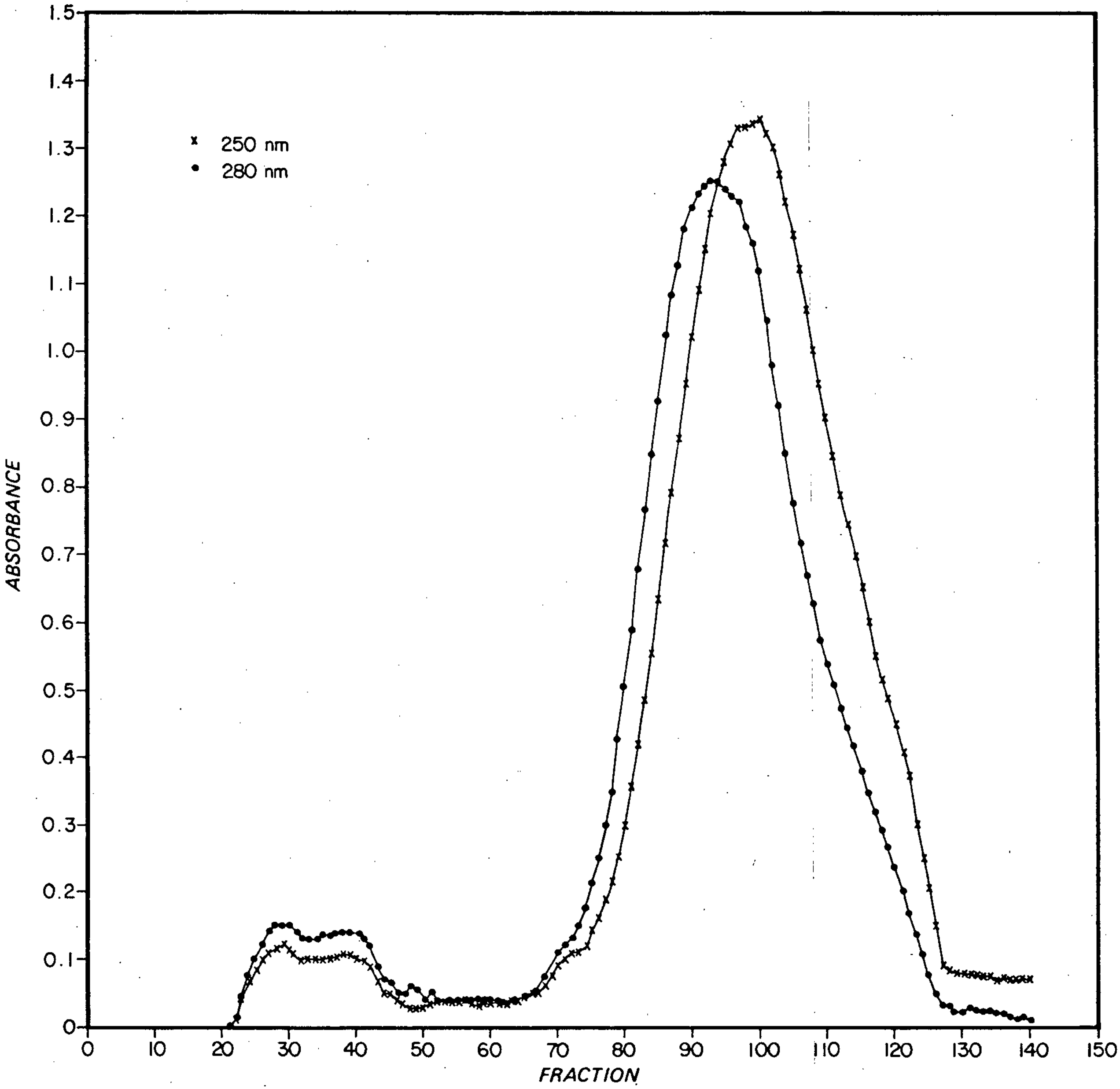


Fig. 29. Elution profile for uncontaminated <u>C. meridionalis</u>.

The results of the chromatography study for mussels exposed to 15 days accumulation of cadmium are given in Fig. 30. The absorbance peaks are only slightly offset and with the 120 cm^3 eluting volume peak a high molecular weight metal binding protein (Peak 1) was eluted. A peak consisting of low molecular weight material was eluted after 249 cm^3 (Peak 11) which was not associated with any OD_{250} or OD_{280} protein peak. The third peak of either OD_{250} or OD_{280} at 362 cm^3 again was associated with metal binding material (Peak 111). It should be noted that the cadmium concentration of Peak 1 is slightly higher than that of Peak 11. The latter was found to have 0,62 μg cadmium per mg protein (Table 12), but from Fig. 30 it can be seen that this cadmium peak (Peak 11) was not associated with the main protein peaks.

As more cadmium was accumulated during the 30 day regime the elution pattern remained very similar to that of 15 day regime (Fig. 31). Protein peaks OD_{250} and OD_{280} coincide with high molecular weight cadmium material (Peak 1) at 125 cm^3 and even the magnitudes of those cadmium peaks were proportional. The position of cadmium Peak 11 was also not changed (253 cm^3), but it was double in magnitude. Protein peaks OD_{250} and OD_{280} were again recorded at 362 cm^3 but cadmium Peak 111 had degenerated.

The case where mussels were allowed to eliminate cadmium is shown in Fig. 32. Cadmium Peak 1 was similar in magnitude and position, but cadmium Peak 11 had decreased, while Peak 111 was again similar in position and magnitude to that of the 15 day accumulation regime Peak 111. Thus the 22% loss or 37% accumulation of total cadmium body burden could mainly be related to the magnitude of cadmium Peak 11.

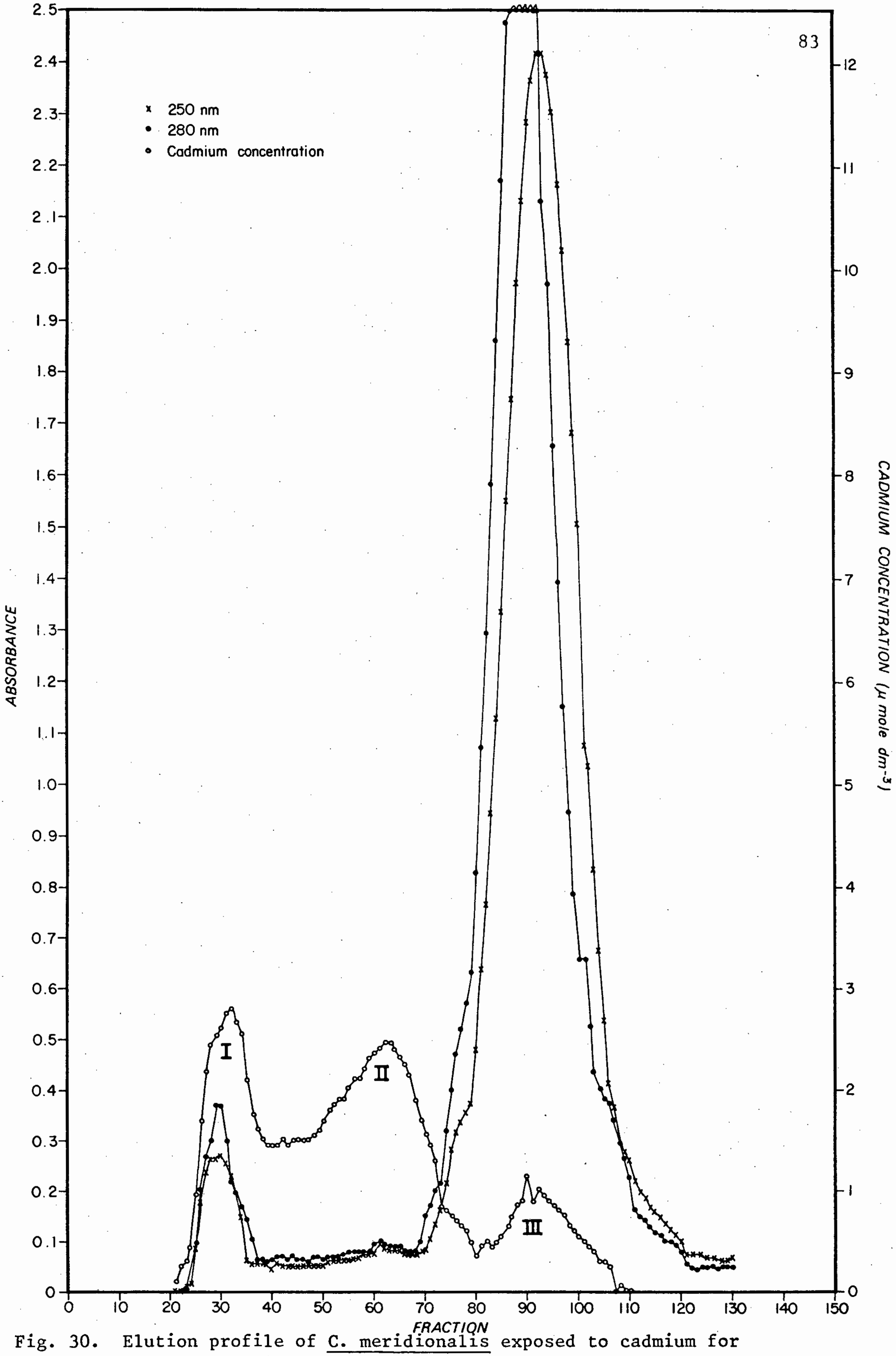


Fig. 30. Elution profile of C. meridionalis exposed to cadmium for 15 days.

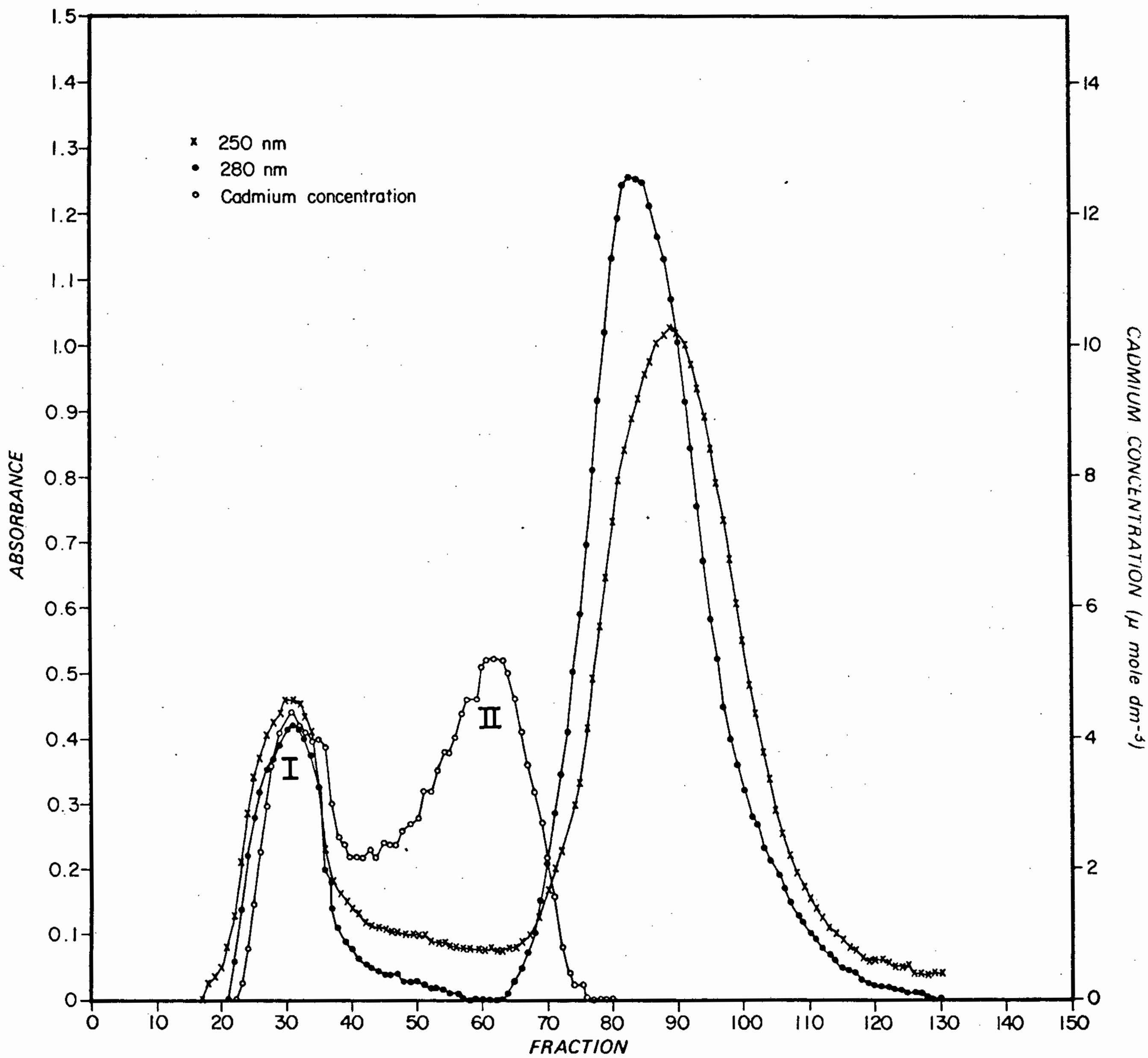


Fig. 31. Elution profile of <u>C. meridionalis</u> exposed to cadmium for 30 days.

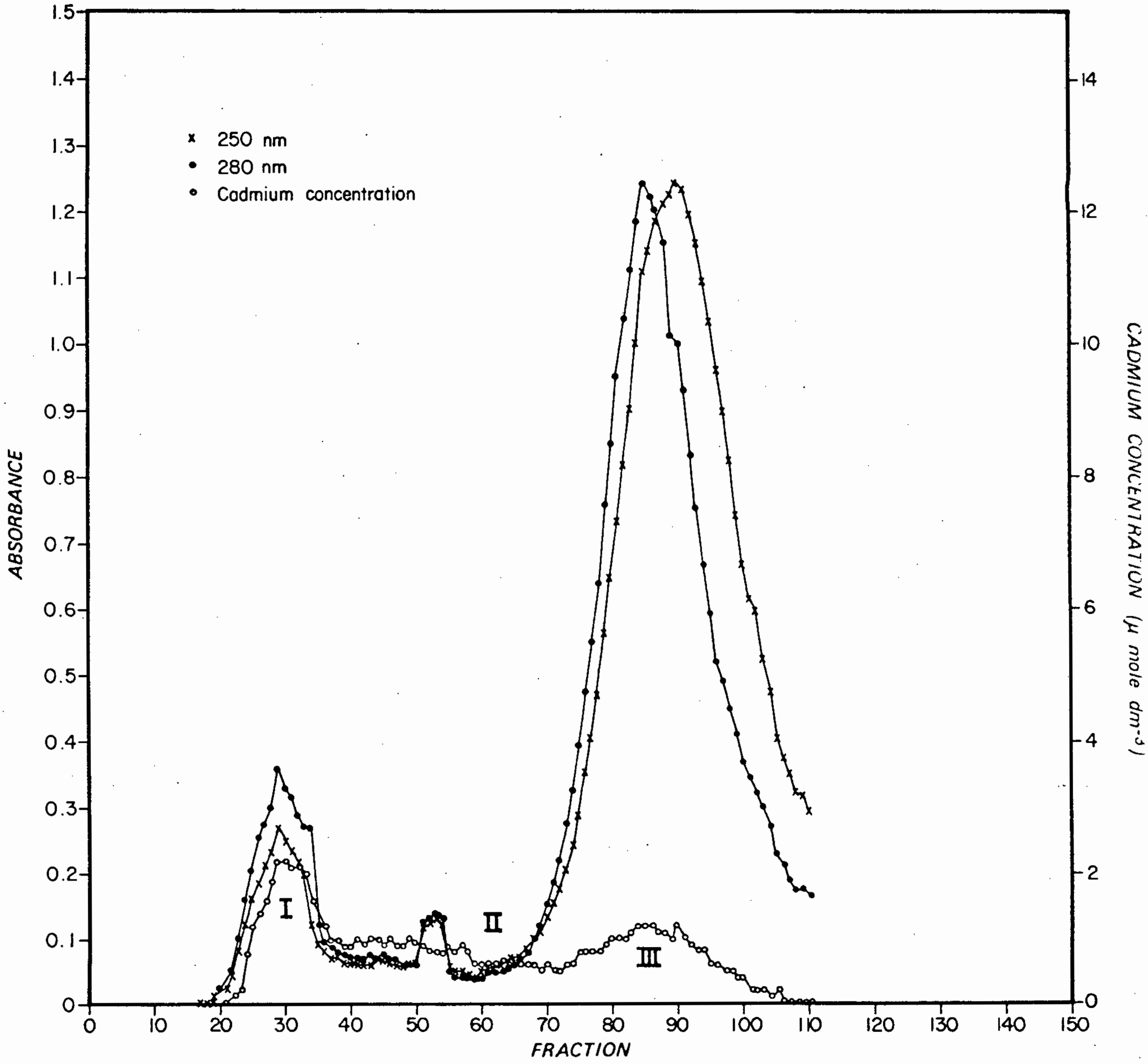


Fig. 32. Elution profile of C. meridionalis exposed to cadmium for 15 days followed by 15 days clean environment.

3.9.1. Characterisation of metal binding protein peaks

The ultraviolet absorption spectra of MBP - Peak 1 and MBP - Peak 11 are shown in Fig. 33. The characteristic spectrum is due to aromatic substances which in MBP Peak 1 are proteins containing aromatic amino acids. The gel filtration chromotographic profiles (Fig. 30, 31 and 32) showed that approximately 5% of the cadmium present in the soluble fraction was eluted with the void volume protein peak (Peak 1).

The absorption spectrum of MBP - Peak 11 was very similar to that published for cadmium metallothionein (Weser et al., 1973; George et al., 1979; Olafson et al., 1979; Overnell and Trewhella, 1979 and Ridlington and Fowler, 1979) with a high OD_{250}/OD_{280} ratio (9.6) which indicates the virtual absence of aromatic amino acid residues. There was a slight expressed peak shoulder of MBP Peak 11 at 250 nm (Fig. 33), which can be attributed to cadmium mercaptide bonding (George et al., 1979). MBP Peak 11 gave an elution volume (V_e) of 249 cm^3 and on this basis together with molecular weight markers (Table 13) and approximate molecular weight of 10 600 daltons was calculated for the metallothionein of *C. meridionalis*. The void volume (V_o) was determined with Blue Dextran, a polysaccharide with an average molecular weight of 2×10^6 (Pulido et al., 1966) and found to be 127 cm^3. The "total volume" of the packed column bed (V_t) was 381 cm^3. Hence the partition coefficient (K_{av}) was evaluated from the relationship

$$K_{av} = \frac{V_e - V_o}{V_t - V_o}$$

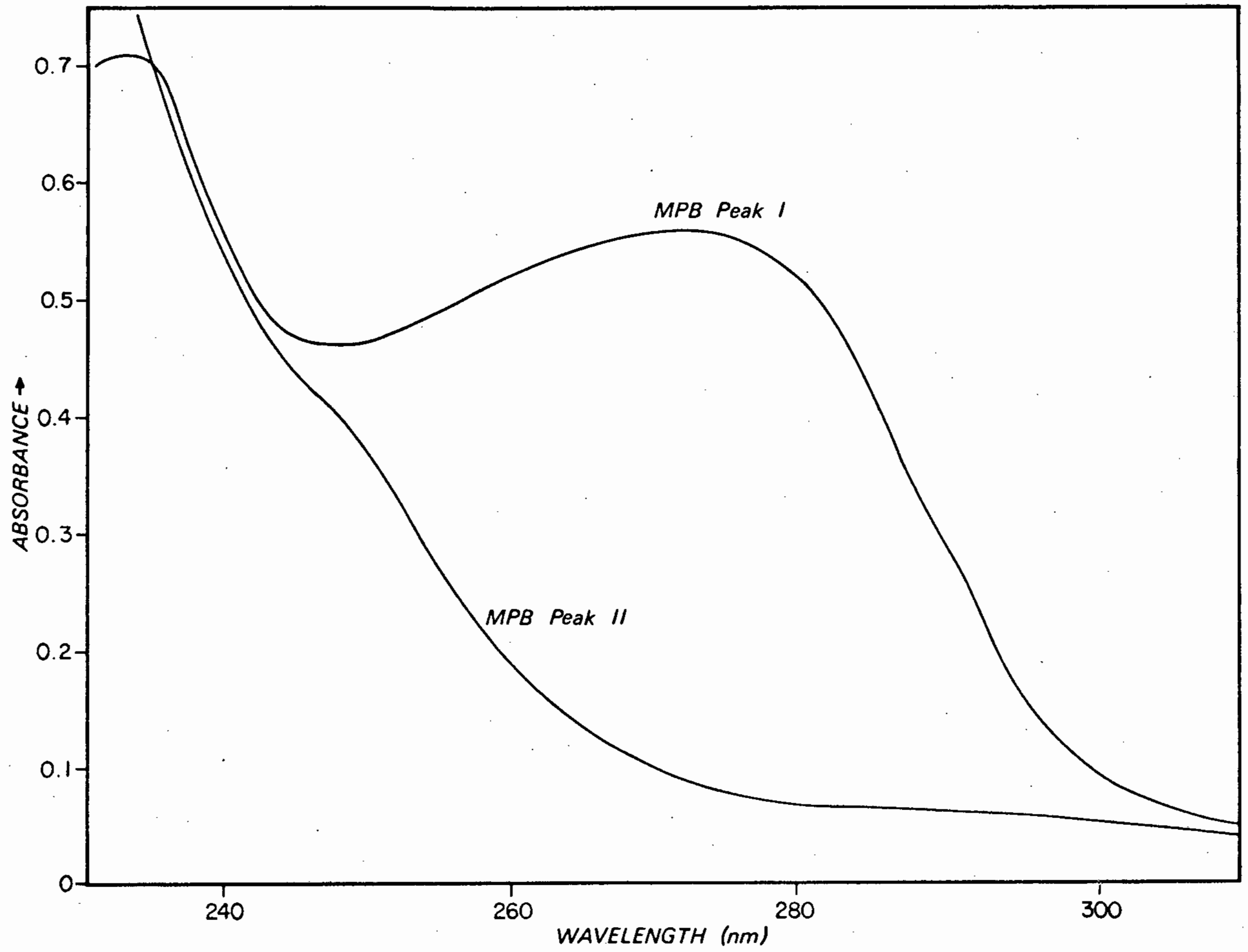


Fig. 33. Spectral properties of metal binding Peak 1 and 11 obtained from C. meridionalis.

TABLE 13: Estimation of Molecular weights of marker proteins and MBP-peak 11 of *C. meridionalis*

Protein	Molecular weight	V_e/V_o
Tryptophan	204	3.65
MBP Peak 11	10 600	1.96
Cytochrome C	12 500	1.94
Ribonuclease	13 700	1.83
Bovine albumin	68 000	1.15

and found to be 0.48. Stokes radii of marker proteins were evaluated from Kägi et al., (1974) which for MBP Peak 11 was found to be 1.27 nm while the retention coefficient (R) was 0.51.

MBP Peak 111 was associated with substantial protein peaks and Noël-Lambot (1976) found this to have the same elution volume as free cadmium. It is suggested that MBP Peak 111 is either due to interaction of MBP Peak 1 and 11 or experimental artifacts.

4. DISCUSSION

4.1 Cadmium in media and T. suecica

In section one, the increasing pollution of the aquatic environment by cadmium has been documented. There is a definite cadmium enrichment in coastal waters around the world, which stems from point sources like coastal industries, rivers and discharge pipelines of industrial and residential effluent. A few examples illustrate this: At Richards Bay (South Africa) gypsum effluent will be pumped into the sea, enriching the bay with an estimated five kilograms of cadmium per day. West German, Dutch, French and Swiss industry dump 200 tonnes of cadmium into the Rhine every year (IER., 1980). Data from industrial and domestic effluent of New York are available and are given in Table A1. There are some surprising cadmium sources: laundry, icecream industry and car washing effluents. In proportion similar cadmium concentrations can be expected in South African waste waters. The technological utilization of cadmium has led to increasing levels of this metal in the environment and in the human body. For example, the concentration of cadmium in wheat taken from the same location in Sweden are reported to have doubled between 1916 and 1972 (Jacobson and Turner, 1980).

With increasing industrialization in South Africa, cadmium pollution must increase, because so far no substitutes have been found for this metal. It is used as a hardener and pigment in plastics, as well as an essential element in the manufacture of ceramics, porcelain, electrical appliances, batteries, paints, glass and it is a waste product from gold mining, electro-plating and galvanising processes.

Cadmium and its compounds are on the "black list" contained in several

European community laws and directives concerning toxic substances, as well as the discharge of such substances into the "aquatic environment" (IER., 1980). Unfurtunately no such laws exist in South Africa and directives of most municipalities only specify that not more than "50 mg/l of heavy metals" are to be discharged into their drainage network (Basset, pers. comm.) To improve these directives and quantitative toxic levels, environmental monitoring and experimental work has to be carried out. With regard to environmental monitoring in South Africa, Watling (1978) has established the naturally occurring metal body burdern of some bivalves, while this report concentrates mainly on experimental work. It demonstrates the flux and effect of cadmium on black mussels, *C. meridionalis*, maintained in the laboratory.

For the correct interpretation of any metal accumulation work there are two important considerations to be kept in mind:

1) Information about the chemical processes and biological availability of pollutant and toxic metals, in particular, are needed.
2) A vast amount of background information is required before meaningful interpretation of the physiological responses of food organisms and test organisms to pollutants can be made.

The first point is illustrated by the adsorption of cadmium to the vessel walls. In only very few cases is the added metal concentration equal to the available metal concentration in solution. This means that figures, calculations and deductions should under no circumstances be based on spiked amounts of metal. This statement may appear to be self-evident, but Hennig and Greenwood (1981) have shown that many papers (62% in 1979) dealing with metal pollution experiments had these defects. To overcome this problem, ideally an automatically monitored open system should be used, where a predetermined amount of pollutant is injected into constantly replaced water.

If a closed system is used, the metal concentration has to be monitored daily and with each change of media. Thus, if the concentrations are not readjusted to a desired level, at least losses are reported. For instance, the metal concentration of the media used in the algal harvesting experiments are reported as mean values together with their range. A predetermined medium concentration was tried to be maintained during the mussel accumulation experiments, but due to adsorption and water changes this varied through the range values indicated.

With regard to the second above mentioned point, little background information was available on the food organism _T. suecica_. This was particularly surprising since _Tetraselmis spp_. were used as food by Winter (1969), Thompson and Bayne (1974), Gabbot and Jones (1975), Fogg (1975), Sournia (1978), Buxton (1980) and are mentioned in connection with successful metal accumulation by Riley and Roth (1971), Bentley-Mowat and Reid (1977) as well as unsuccessful metal accumulation by Watling (1978). Hence growth patterns and physiological responses to cadmium had to be investigated first.

Since such growth patterns involved the monitoring of numerous samples, a rapid counting method had to be established. It was found that cell density (above 10 000 cell cm^{-3}) was reliably determined by measuring algal absorbance. This has also been established in other green algae (Bottino _et al_., 1978).

It was soon realized that the algae went through different life-cycle stages. These had to be determined separately, because Wilson (1978) in maintaining oysters, and Sick and Baptist (1979) in feeding marine copepods, have shown that growth rates and survival varied, when algae of different life-cycle stages were used as food. The analysis of the four different life-cycle stages also provided "base-line" data against which algal response to cadmium could be measured. The duration of these life-cycle stages was rigidly fixed and did not even vary with the addition of cadmium. It appeared to be an inherent characteristic of *T. suecica* and depended entirely on the mean generation time of the inoculate.

Some of the life-cycle stages were unsuitable, in particular the lag phase (first five days) if the algae was to be used as a food organism. The best life-cycle stage to harvest *T. suecica* was at the end of the exponential growth phase (day 11) and the highest relative growth (k') was achieved at a cadmium concentration of 6 μmole dm^{-3}. From a cadmium enriched food source point of view, it was very useful that the addition of small amounts of cadmium stimulated algae growth. At high metal concentration, growth was inhibited and at metal concentration beyond 14 μmole dm^{-3} no real growth was recorded.

It was remarkable that cadmium stimulated algal growth. It suggested that cadmium was after all a biologically essential trace element, as postulated by Zingaro (1979) and Schwarz and Spallholz (in Deagen *et al.*, 1980). Recent reports appeared to be confirming this: stimulated growth in marine phytoplankton (including *Tetraselmis spp.*) and mycelium by Bentley-Mowat and Reid (1977), Duddridge and Wainwright (1980), Uthe and Chou (1981) as well as increased body weight and condition of rats (Deagen *et al.*, 1980).

Yet, when zinc was added to the growth media of T. suecica, a similar increase in algal density was achieved as with 7 µmole dm^{-3} cadmium, confirming the results of Riley and Roth (1971), Jensen and Rystad (1974), Müller and Payer (1979) and Wong and Beaver (1980), that a relatively high zinc concentration is needed for maximum algal growth.

Furthermore, when algae were growing in a zinc and cadmium medium, which on their own had a stimulating effect, algal growth was now inhibited. It is possible, that the combined effect of zinc and cadmium had an inhibiting effect on algae density, but a more likely explanation would be that the stimulation effect was due to a substitution of cadmium for zinc in a zinc deficient system. This explanation is also put forward by Deagan et al., (1980), who reported that low levels of cadmium improved the condition of rats,which were maintained on a zinc deficient diet. In light of the present data, it would be interesting to know how much zinc was in media used by workers who had found the stimulating effect of cadmium. Such a response may not have been obtained if the zinc levels had been raised in the media. This illustrates again that much background information is needed before the effect of metals on organisms can be assessed.

It should be mentioned that if such a cadmium/zinc substitution holds true in man, the implications could indeed be serious: Spoelstra and Sellschop (1980) have shown that 87% of all Kwashiorkor patients are zinc deficient. Often Kwashiorkor patients come from areas where rivers provide the only water source for drinking, cooking and farming. If such a river was cadmium enriched by mining or smelting, chronic cadmium poisioning from drinking water and contaminated vegetables in those farming communities would only be recognised at a very advanced stage; the first stage being masked by the cadmium substitution.

High levels of cadmium had an inhibitory effect on algal growth, even in a zinc deficient system and at concentrations above 17.4 μmole dm^{-3} the algae were not reproducing, but slowly dying, confirming results obtained by Bentley-Mowat and Reid (1977) and Eide et al., (1979). Yet no attempt has been made to calculate at LC_{50} value for T. suecica. It was thought to be rather meaningless, especially in view of the results obtained concerning the different life-cycle phase of the algae.

Algal cultures could be maintained for at least 30 days at a stable phase, which had a slightly lower cell density than the exponential phase. Predictable numbers of cells, however, could not be obtained with any certainty, due to the cyclic fluctuations, which corresponded to the mean generation time of a specific culture or a certain flask size. Such an uncertainty in harvesting results is undesirable in accumulation experiments, thus harvesting was restricted to the "day-11" growth phase when predictable results could be obtained.

A striking variability of growth phases and hence immature and mature cultures became apparent when the relationship of algal numbers and their dry weight was analyzed. Quite unexpectedly this relationship was non-linear. Closer examination showed that during inoculation only one life-cycle phase was introduced into the subculture. The majority of these synchronized cells grew uniformly to sizes where cell division was possible, hence the following sequence took place:

a) Uniform gain of weight per unit number of maturing cells (x 10^6 cells).
b) Settling of most of the mature algae, resulting in a reduction of numbers harvested.
c) Division and liberation of daughter cells, resulting in loss of weight

per unit number of cells harvested.

d) Repeated growth, maturing and division after settling out at a much increased rate.

Since none of the cells was in complete synchronization, these events soon became indistinguishable from each other and the weight of a harvested sample was now determined by the proportion of immature and mature cells. This explains the differences in algal weight and energy values reported for the same species by various workers (Thompson and Bayne, 1974; Griffiths and King, 1979; Buxton, 1980). Thus without stating the life-cycle of the food organism at harvesting, comparison of accumulation or feeding rates of test animals may become difficult. It is interesting to note, that the average harvest sample increased slightly in time. This may be due to metabolic changes within the mature, stable phase algal cell (Fogg, 1975).

The algal life-cycle phase is not only important with respect to weight, but also in relation to cadmium absorption; a well known phenomenon in marine algae (Riley and Roth, 1971; Saraiva and Fraizier, 1975; a review: Davies, 1978; Jennings and Rainbow, 1979b; Jennings, 1979). Hence it was very surprising that Watling (1980) found no metal accumulation in T. chui.

At low cadmium levels in T. suecica the metal uptake proceeded smoothly, similar to uptakes reported by Saraiva and Fraizier (1975); Canterford et al., (1978); Jennings (1979). This behaviour may be species independent and very well be due to a fast passive adsorption rate to the outer cell membrane of the algae and a slow cellular uptake.

At medium cadmium levels, increased metal accumulation took place, but a distinct feature, a prominent high concentration peak, was noticed on day five. Such a peak has also been reported by Kremling et al., (1978) in plankton during an ecosystem enclosure experiment. In view of the results obtained regarding the adsorbance of metal to vessel walls, two processes are proposed for the accumulation of cadmium in algae:

1) Adsorption of cadmium to the outside of the algal cell wall, followed by
2) Rapid uptake of cadmium by the growing and maturing cell, which incorporates minerals and metals together into the cell matrix for the media.

Both mechanisms would be indistinguisable at low cadmium concentrations and at higher concentration for a limited period only until active uptake exceeds adsorption. At medium metal concentrations the latter stage is just reached before cell division. The maturing cell increasingly adsorbed cadmium to its expanding outer membrane surface, together with active metal uptake. On division, the incorporated metal content remains the same and so does the adsorbed amount, but the outer cell membrane has been increased quadratically to cover both daughter cells. Thus in terms of per unit cells,a temporary drop of cadmium concentration has taken place. As the daughter cells grow steadily, adsorption does take place again, but due to the higher cellular uptake and possibly because of unsynchronized cell growth, further adsorbance peaks and apparent losses are masked. Evidence for such a dual mechanism is provided by Eisler (1971), showing the adsorbance of metal to biological material, but more important by von Westernhagen et al., (1979). They reported that herring eggs accumulated cadmium rapidly for the first few days (2-5 days) resulting in a high total metal burden, while newly hatched larvae contained very little cadmium. Later with longer exposure times the larval metal content increased.

Initially cadmium was mainly adsorbed to the outer egg membrane, which is shed on hatching resulting in a great metal loss. The newly hatched larvae had little intercellular and no adsorbed metal. This obviously changed with further exposure to the metal. Adsorption has also been shown to play a major role in zinc accumulation from water by certain marine crustaceans (Fowler and Benayoun, 1974) and hence, for a closely related metal such as cadmium, a similar mechanism is quite likely.

To summarise:

Since no substitute for cadmium has yet been found, there will be an increase of cadmium pollution throughout the world and the aquatic environment in particular. Hence more metal accumulation experiments have to be performed to establish guide-lines for permissable effluent discharge. The experiments should be carefully planned, both from a chemical and biological point of view, for instance, metal adsorbance to vessel walls and variation in organism life-cycles must be considered.

Most accumulation experiments are performed in the laboratory and it must be born in mind that established methods for maintaining organisms in the laboratory may differ greatly from nature or optimal conditions for these species. This was illustrated by the zinc deficient media used for <u>T. suecica</u>. Such a medium is recommended if high cadmium accumulation rates are to be achieved, but it should be remembered that these results cannot be used to show that cadmium is a biologically essential element.

Another oversight in this respect is often made (eg. Braek <u>et al</u>., 1980) by assuming that a food organism is one consistent, uniform entity: food. For marine animals food often consists of organisms with life-cycles, variations in weight, age and maturity. If response variations in test animals are due to variations in food and not due to the effects of test conditions, then correct interpretation of results becomes very difficult. The growth pattern phases also play an important role in accumulation and total body burden of the organism. Maximum metal concentration coincided with highest growth rate. Knowledge of growth behaviour also furthered the understanding of the metal uptake mechanisms in algae.

For the above reasons an extensive study of the food organism and its metal accumulation was made, to establish one result: as a cadmium accumulating food source, *T. suecica*, should be harvested in a "day-11" growth phase from a zinc deficient media containing 7 μmole dm^{-3} of "available" cadmium. This results in the highest cell density with an average dry weight of 0.081 mg/10^{6} cells, containing 14.64 mg g^{-1} dry weight cadmium.

4.2 Flux of cadmium through *C. meridionalis*

Contaminated food may or may not be important in accumulation experiments. Possibly this could be species dependent: Jennings and Rainbow (1979 a) found that in crabs, food contributed insignificant amounts of cadmium to the total body burden (it should be mentioned, that the "food" was only allowed to accumulate metal for four days). On the other hand, in oysters (Kerfoot and Jacobs, 1976) and in *Mytilus edulis* (review: Phillips, 1977; Janssen and Scholtz, 1979; Phillips (1979)) contaminated food made a major contribution to the total body load.

The manner of feeding could also be important, because Griffiths (1980) has shown that C. meridionalis feeds continuously. Yet, Langton and McKay (1974) showed that in oysters, discontinuous feeding was more beneficial for growth. Due to the problems encountered with the continuous feeding apparatus, the mussels were batch-fed twice daily. The calorific value of the algae food batches appeared to be enough for "scope of growth", as shown by Griffiths (1980), since no relative decrease in body weight with regard to shell length could be detected in mussels maintained on these rations for 15 days and 30 days during a control experiment. It was this relationship of body weight to shell length that was used to measure the effect of stress on the condition of C. meridionalis. Physiological responses to abnormal environments (stress) in mussels has been the subject of several investigations (Bayne and Thompson, 1970; Lynch 1974; Martin et al., 1975). These consisted of comparisons of serum constituents, carbohydrate and protein loss, oxygen consumption, nitrogen excretion and byssal thread production. The problem in measuring the effects of stress is, that knowledge of the normal seasonal variations in physiological indices is needed, to serve as a "base-line",with which induced changes in conditions can be compared. Unfortunately, most of the reports deal with Mytilus edulis and few physiological indices have been investigated in C. meridionalis. On the other hand, the relationship of body weight to shell length of C. meridionalis has received considerable attention by Watling (1978), Griffiths (1980) and Orren et al., (1980), who found some variations in body weight due to habitat but little difference within a population of one habitat at a particular time.

The body weight - shell length relationship was not very sensitive with regard to food, because no statistical difference could be found between starved mussels and mussels maintained on a very low ration for 15 to 30 days in the control experiments, although it should be remembered that weight loss or gain is usually a gradual process and has a reaction time or lag phase, before any changes are measurable. Nevertheless, the cadmium accumulation regimes must have placed the animals under stress, because loss of weight resulted. These accumulation regimes of 7 μmole dm^{-3} of contaminated food and environment were especially selected so as to minimise stress: George and Coombs (1977) and Briggs (1979) found that 7 μmole dm^{-3} cadmium saturated the detoxication system of M. edulis and this coincided with the first visible signs of cadmium toxicity. Due to the lag phase, a decrease in weight could not be detected immediately; only after 30 days could a statistical difference in body weights be found. This happened in spite of returning some mussels to a clean environment. A possible explanation could be found in the model proposed in Fig. 1: cadmium had spilled over the enzyme pool and interfered with vital enzyme processes. This was due to a continual net gain in cadmium by the mussel, because uptake (gain: 37%) exceeded detoxification (loss: 22%) after 15 days of metal accumulation (Table 12) and this poisoned the animals.

It is interesting to note that the elimination of cadmium during the recovery period was smaller than the metal uptake by mussels maintained in a contaminated environment for 30 days. Possibly the turn-over of protein in relation to the production of metal binding proteins is a rate determining step. This would also explain why immature mussels from natural environments have a higher cadmium body burden than mature animals;

an observation made by many workers (Watling and Watling, 1976 a & b; Boyden, 1977; Theede _et al_., 1979; Cossa _et al_., 1979; Cossa _et al_., 1980). It has been postulated (Williamson, 1979) that in molluscs, total cadmium body burden rises year by year: "as a cummulative total of food and absorbance from the environment increases, whilst size-related changes in metabolic rates result in a greater intake of cadmium per unit body weight in smaller individuals, giving higher tissue concentrations". This postulated mechanism is somewhat incomplete: it can be assumed that changes in metabolic rates which increase metal uptake would also increase the elimination process (eg. more food results in more faeces).

If, on the other hand, an age dependent accumulation/elimination protein ratio is assumed, the following mechanism emerges: in immature mussels, the turnover of protein is high in relation to the production of detoxification proteins because the organism is growing rapidly. In mature animals, growth is slowed down and therefore a higher proportion of detoxification proteins are available, thus elimination of more metal per unit intake is possible. The protein demands made on a mature mussel during reproduction need not be considered because Griffiths (1980) has shown that gonads are readily reabsorbed, if the need arises as in the case of stress. Such a mechanism would hold true for both accelerated or constant detoxification rates in immature and older mussels. The assumption of a constant elimination rate in both appears to be more reasonable, since in _Mytilus_ a constant half-time, regardless of lead tissue concentration has been reported by Schulz-Baldes (1974).

The results of the bio-elimination study showed that a detoxification mechanism exists: in 15 days approximately 22% of cadmium was lost by the mussels, hence the biological half lifetime ($t_{\frac{1}{2}}$) of cadmium in *C. meridionalis* was 41.3 days. Comparative data on turnover time of cadmium in mussels is scarce. Fowler and Benayoun (1974) quote a "slow but reliable release" of cadmium from *Mytilus galloprovincialis* and calculated a half life value of 307 and 1254 days for field and laboratory conditions, respectively. Schulz-Baldes (1974) found that lead in *M. edulis* had a $t_{\frac{1}{2}}$ of 45 - 57.8 days for different metal concentrations. Cunningham and Tripp (1975) showed that mercury in *Crassostrea virginica* had a $t_{\frac{1}{2}}$ value of 35.4 and 19.9 days for two metal concentrations. The biological half time of antimony in *Mytilus* tissue was 17.8 days (Walz, 1979).

Yet the accumulation potential of *C. meridionalis* must be quite substantial considering that 37% cadmium was taken up, despite the continual detoxification of 22%. Food must have played a major role in the metal uptake considering that 2335 µg cadmium were ingested per gram mussel (dry weight) during 15 days and 4670 µg during 30 days. It is premature to speculate how much of the total body burden was due to the ingested food, because not all the food was assimilated. Griffiths (1980) showed that for very small rations, like those used in the present experiments, an assimilation level of 80% can be assumed. Secondly, it is not known in which form (i.e. ions, chelate or complexes) the cadmium was taken up from either food or media.

It is evident from this study that cadmium flux between *C. meridionalis* and its environment is a relatively slow process. The value of the biological half life time of 41.3 days in immature animals should be viewed with caution because after 30 days the mussels were still accumulating cadmium at a very fast rate and equilibrium or steady state conditions had not been reached. Metal equilibrium, characterised by equal rate of uptake and loss, is commonly used for the determination of half life values. This must be much higher than 1143.8 $\mu g\ g^{-1}$ cadmium (dry weight) and will take much longer than 30 days.

Further studies are now needed on mussels and other organisms to distinguish between the influence of age and size on the accumulation of metals with long biological half lives. Until then, the sampling of marine molluscs for global monitoring of metal pollution could be invalidated by geographical variation in growth rates and age structure, even if comparisons are limited to individuals of similar size, which are sampled at the same time of year (Williamson, 1979). A more promising approach would be to use a different criterion, like the presence of metal binding proteins or metallothioneins, to define metal pollution and thus move away from the ambiguous total body burden comparisons.

To summarise:

Although the food rations for the mussels were small and they were fed in doses, the uptake of cadmium from contaminated algae was considered important.

Measurement of stress only becomes meaningful if it can be shown to deviate from normal or natural values, hence the relationship of shell length to dry body weight was determined. This served as a "base-line" against which any effect of cadmium in food or media could be measured. It was found that the influence of cadmium was delayed and a loss of body weight was only detected after 30 days. Such a decrease of body weight was brought about by cadmium, irrespective of continual contamination or change to a clean environment. It is proposed that the poisoning took place during the first 15 days of accumulation and was due to cadmium spill-over into the enzyme pool.

This was brought about by a total net gain of metal body burden, as the elimination rate could not match the metal uptake rate. The elimination mechanism was examined in relation to the high body concentration of immature mussels and the low body concentration of older animals. This was considered to be due to an increased protein requirement for growth in immature mussels, an explanation also favoured by Rice and Chien (1979), thus creating a disproportion of growth protein to detoxification proteins and not so much because of increased feeding.

The flux of cadmium between mussels and the environment is a slow process. The reported biological half-life time is very likely underestimated because no steady state and no cadmium plateau had been reached by the mussels during to 30 day accumulation experiment. It is furthermore suggested that metal pollution should rather be defined by the presence of metal binding protein than by total body burden.

4.3 Metal binding proteins in *C. meridionalis*

Studies of metal tissue concentration in biological systems have progressed along two major lines:

1) The human nutritional and health point of view, that is to find the lethal metal concentration in as many animals as possible, to identify potentially hazardous levels for man. Medical research has provided

clinicians with tests for diagnosing heavy metal disease (Kench <u>et al</u>., 1962; Kench and Sutherland, 1966; Kench, 1968; Lucis <u>et al</u>., 1970 and others, see review Jacobson and Turner, 1980). The column separation method used, was developed mainly for medical problems (Kägi and Vallee, 1961; Piscator, 1964).

2) The pollution point of view, that is to compare concentrations within suitable species on a geographical basis as a framework for an environmental monitoring programme (see: Kidder, 1977 and Int. Mussel Watch, 1980). This is dependent on many parameters such as temperature, salinity, seasonality, body size and age (reviews: Coombs, 1979 and Cunningham, 1979). It is very difficult to consider all these factors in different species around the world without a common denominator to which these parameters can be ralated.

It is well known that metallothioneins are a specific response to "heavy" metals and its isolation has been used in various mammals to monitor or investigate poisoning associated with these metals. (Shaikh and Lucis, 1971; Weser <u>et al</u>., 1973; Olafson and Thompson, 1974; Bryan and Hidalgo, 1976; Shaikh and Smith, 1977; review: Coombs 1979). The role of these metallothioneins in the detoxification of cadmium and other metals has been shown (Cherian and Shaikh, 1975) as well as the very slow rate of elimination (Stonard and Webb, 1976). All this work has been done on rats, where the metal solution can be injected directly into the animal. This study has shown that problems become rather complex when dealing with marine animals and the available techniques cannot be readily adopted without further experimentation. Metallothioneins have been isolated from 11 species of marine invertebrates (review: Coombs, 1979), but so far no attempt has been made to correlate the metallothionein production with the toxic effects in the organism.

In *C. meridionalis* metal binding proteins (MBP) have been isolated from the various accumulation regimes, as shown in Figs. 30, 31 and 32. No cadmium or MBP could be detected in mussels maintained as a control for 15 and 30 days, but small amounts of enzyme containing pool material (Peak 1, fraction 29 at OD_{250} max.) and large amounts of the low molecular-weight cytoplasmic pool material (Peak 111, fraction 93 OD_{250} max.) were eluted. Comparative data is not easy to find, but similar profiles have been reported from *Sebastodes caurims* (copper rock fish) by Olafson and Thompson (1974), Shaikh and Smith (1976) in rats, while Noël-Lambot (1976) found in untreated *Mytilus edulis* (common mussel) a large amount of enzyme-containing pool material. These untreated mussels came from the Belgian coast and some cadmium was detected in the supernatant. Ridlington and Fowler (1979) showed only the high molecular fraction of their supernatant oyster material *(Crassostrea virginica)*.

After 15 days of accumulation, the eluted protein profile (OD_{250}, OD_{280}) was similar to that of the untreated material. In addition three cadmium containing peaks were eluted. The first MBP peak (Peak 1) was associated with the protein material of the enzyme containing pool (Int. Mussel Vatch, 1980) and contained 34% of the bound cadmium. The predominant portion (57%) of the cadmium extracted from the buffer-soluble extract was found bound to a protein of low molecular weight (approximately 10 600 daltons).

MBP Peak 111, associated with the cytoplasmic pool, contained some cadmium but it was eluted below the working range of the Sephadex G-75 column. The presence of this peak has been attributed to free Cd^{2+} ions by Noël-Lambot (1976); and to the decompositon of MBP Peak 11, yielding low molecular weight amino acids containing cadmium by Talbot and Magee (1978).

Thus, the 15 day accumulation regime had initiated the production of MBP, but the detoxification system of C. meridionalis was exceeded and cadmium spilled over into the enzyme pool. This substantiates the mechanism proposed to explain the poisoning effect mentioned in connection with weight losses in mussel reported earlier on. Enzymes are either activated or inhibited, depending on enzyme and concentrations (Diamond et al., 1973; Jackim, 1974) thus poisoning the animal. This could not be determined from the total body metal concentration, nor from the shell length-weight relationship.

After 30 days of accumulation the mussels had taken up more metal, but proportionally the metal content of MBP - Peak 1 and MBP - Peak 11 remained similar, 34% and 59% respectively. Similar increases and proportional amounts are reported by Engel and Fowler (1979 a & b), Jennings et al., (1979). Although the amount of cadmium found in the elutant were higher, due to a reaction lag period, no increased effect on the weight of the mussels could be detected. Such a lag period has also been reported in rats (Stonard and Webb, 1976). Thus, the chromatographical study showed a 60% increase of toxic matter and poisoning could be recognized earlier than with other indicators often used to record stress.

This confirms the model (Fig. 1) proposed earlier and can be explained by the different mechanisms and functions of the two MBP - Peaks. There is an upper limit beyond which the mussel (or any organism for that matter) cannot sequester and therefore detoxify the accumulated cadmium. Mussels exposed to the 15 day accumulation regime were stimulated to produce metal binding protein (MBP - Peak 11), but uptake exceeded elimination and detoxification. Cadmium spilled over into the higher molecular weight fraction, the enzyme pool (MBP - Peak 1) poisoning the animal which in turn resulted in a weight loss. Further uptake and spill-over will result in increasing destruction of the mussel's general condition.

Removal of the MBP stimulus (eg. transfer to a clean environment) slowed down the detoxification mechanism. Only 35% of the total bound cadmium was associated with MBP - Peak 11 fraction, but a high percentage (52%) was still remaining in the enzyme pool fraction. Possibly further interferences with enzyme reactions will take place. So, by disregarding MBP - Peak 111 (which possibly is an artifact), during the 15/15 days accumulation/detoxification regime, the total cadmium body burden loss was 51%, but from the damaging enzyme pool only 23% was lost.

This also explains the long biological half life times of cadmium. The detoxification mechanism (MBP - Peak 11) may not be stimulated and thus slowing down the removal of metal from the enzyme pool. This underlines the dangerous character of cadmium.

It also illustrated that this technique can be used to determine the pollution exposure history of mussels. The elution of MBP - Peak 11 will show that the organism is disturbed by too high concentrations of metal in media and food. The elution of MBP Peak 1 and Peak 11 indicates an increasing deterioration of the environment and definite signs of poisoning, while elution of mainly MBP - Peak 1 material shows the removal of the pertubation stimulus, but the organism will remain poisoned for a long period. It would be interesting to test this at lower metal concentrations and with different animals.

Although it has not proved possible to isolate an homogenous preparation of mussel protein MBP - Peak 11, many properties of the protein are similar to those of mammalian and other invertebrate metallothionein. The purest source of protein came from the 30 day accumulation regime. This protein exhibits an unusual ultraviolet spectrum with low absorption at 280 nm and high absorbance at 250 nm which is consistent with the absence of tyrosine and tryptophan residues (Howard and Nickless, 1977). MBP - Peak 11 also shows the characteristic adsorption at 250 nm of the cadmium-mercaptide chromophore similar to the metallothionein isolated from rats and chickens (Weser et al., 1973), limpets (Howard and Nickless, 1977), mussels (George et al., 1979), crabs (Overnell and Trewhella, 1979), crabs, shrimps and chiton (Olafson et al., 1979), mussels (Frankenne et al., 1980), rat kidneys (Żelazowski and Szymanska, 1980), limpets (Noël-Lambot et al., 1980). All of the workers report an apparent molecular weight of approximately 10 000 - 11 000 daltons which agrees well with the weight of 10 600 daltons found in this study. The other constants determined (eg. Stokes radius, retention coefficient) also fit in well with the known literature values. No amino acid analysis was done, but it can be assumed that MBP - Peak 11 is very similar and may in fact be, metallothionein.

5. CONCLUSIONS

The purpose of this investigation was to induce and isolate a cadmium binding protein from black mussels and to correlate the presence of these proteins with toxic effects in the animals. This, as far as known, is the second attempt (Davies et al., 1980) and the first report regarding mussels, which used this method for monitoring and obtained a correlation with other indicators of contamination.

Thus, it has been shown that in C. meridionalis the levels of metals on metal-binding protein increased with increasing exposure level and toxic effects do occur when the binding capacity of the metal-binding protein is surpassed and metal spilled over into the enzyme-containing pool. Since this method quantifies the levels of metal on metal-binding protein and in the enzyme-containing pool, it is recommended that these procedures be implemented into the current marine pollution monitoring programme.

It was found that the characteristics of the isolated metal-binding protein are very similar to those described for metallothionein obtained from other animals. From the results, some proposals could be made about the possible mechanism of metal retention and accumulation in immature and mature mussels. The ration of protein used for growth to that of the metal-binding protein was thought to determine the elimination rate.

The investigation into flux of cadmium through a laboratory food chain, made it necessary to grow algae (T. suecica) in various cadmium concentrations for use as "cadmium contaminated"food for C. meridionalis. It is apparent from the present study, that without careful chemical monitoring and background ecophysiological data such an accumulation report may become misleading. When growing algae and mussels in spiked media, it was shown, that the spike of cadmium added is not the concentration available to the animals, due to cadmium adsorption to the vessel walls. Furthermore, although the growth pattern of contaminated algae apparently did not change, it is demonstrated that vast differences in number, weight, weight behaviour in time and accumulation exist at various life-cycle stages in *T. suecica*. Only when such ecophysiological data are available can algal stages (in growth and time) be selected as a food supply in acumulation experiments. The best growth and accumulation results in *T. suecica* were achieved in a medium containing $7\mu\text{mole dm}^{-3}$ cadmium. This medium was subsequently shown to be zinc deficient and hence some implications are discussed. For instance, due to the substitution reaction of cadmium, an improvement of an organism's condition need not imply that this metal is biologically essential. Furthermore such a substitution could have serious consequences in men. This highlights the dangerous nature of cadmium and together with the ever increasing cadmium load of industrial and residential effluent should be a point of grave concern. It is hoped that South Africa will soon follow international directions about defining the release of cadmium into waste water systems which ultimately reach the ocean.

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APPENDIX A

The South African industrial effluent standard for total heavy metals is a maximum of "50 mg l^{-1}" (Basset, pers. comm), but a more realistic estimated effluent concentration of cadmium would be 9 µmole dm^{-3} (Basset and Orren, pers. comm.). About 120 x $10^6 m^3 a^{-1}$ of industrial effluent are discharged along the South African Coast (Cloete, 1979), but the cadmium concentration of this is unknown. There are inherent difficulties in attempting to trace the source of metal pollution to the diffuse sources of origin. Nonetheless, a comprehensive investigation conducted by the New York City Waste Water Authorities (Klein *et al.*, (1974) quoted in Förstner and Wittman, 1979) has revealed many sources of cadmium pollution resulting from industries (Table 1a) which hitherto had received little attention.

TABLE A1: Cadmium in industrial wastewaters of New York (after Förstner and Wittman, 1979)

INDUSTRY	AVERAGE CADMIUM CONC. (µmole dm^{-3})
Meat processing	0.10
Fat rendering	0.05
Fish processing	0.13
Bakery	0.02
Miscellaneous foods	0.05
Brewery	0.04
Soft drinks and flavourings	0.03
Ice cream	0.28
Textile dyeing	0.27
Fur dressing and dyeing	1.02
Miscellaneous chemicals	0.24
Laundry	1.19
Car wash	0.16
Total	3.58

Although cadmium concentrations of South African wastewaters are not known, proportional results from New York's wastewaters could apply to South Africa. The effluent of industries from Table 1a would account for half of the estimated 9 µmole dm^{-3}. The rest is made up from electroplating industries (1.47 µmole dm^{-3}), residential waste (2.19 µmole dm^{-3}) and mining wastes: gold (0.85 µmole dm^{-3}), other (0.89 µmole dm^{-3}). Cadmium is also released from galvanised equipment: -

it is present as ~ 1% impurity in the molten zinc used in the galvanizing baths (Orren, pers. comm.).

APPENDIX B1

1. Analytical conditions for metal determination.

A Varian Techtron AA-6 with simultaneous background corrector was used for all measurements, following the manufacturers recommended conditions. Single-element hollow cathode lamps were used and the resonance lines used are listed in Table B1.

TABLE B1: Resonance line and flame used for metal determination.

Metal	Spectral line (nm)	Lamp Current mA	Flame	Slit width mm
Cd	228.7	3	air - C_2H_2	0.5
Zn	213.9	5	air - C_2H_2	0.5

APPENDIX B2

Preparation of Walne's culture medium (Walne, 1966)

Make up the following up to two dm^3 with distilled water in the order given:

Mn $Cl_2 \cdot 4H_2O$	0.72 g
Fe $Cl_3 \cdot 6H_2O$	2.60 g
$H_3\ BO_3$	67.20 g
E.D.T.A. (Na salt)	90.00 g
$Na_2\ H_2PO_4 \cdot 2H_2O$	40.00 g
Na NO_3	200.00 g
Trace Metal soln (1)	2.00 cm^3
Vitamin soln (2)	0.2 cm^3

Add one cm^3 of Walne's medium to each dm^3 of seawater for algal cultures.

1. The trace metal solution has the following composition:

Zn Cl_2	2.1 g
Co $Cl_2 \cdot 6H_2O$	2.0 g
$(NH_4)_6 \cdot Mo_7O_{24}\ 4H_2O$	0.9 g
Cu $SO_4 \cdot 5H_2O$	2.0 g

Make up to 100 cm^3 with distilled water. (It may be necessary to acidify this solution with a little HCl to obtain a clear liquid).

2. The vitamin solution has the following composion:

B_1 (Thiamine)	100 mg
B_{12}	5 mg

Make up to 100 cm^3 with distilled water.

APPENDIX B3

Preparation of biological samples for atomic absorption analysis.

Most biological materials are readily ashed by wet oxidation procedures, the purpose being the removal of the organic fraction of the sample without loss of the inorganic constituents. Nitric acid is the most widely used primary oxidizing agent for the destruction of organic matter of marine origin (Orren, 1979). Concentrated nitric acid (25 cm^3) was added to the wet biological samples (algae as well as mussels) the mixture was then allowed to stand at room temperature overnight. This is shown to improve breakdown of material. Blank determinations were run concurrently. Samples were heated to dryness at a low heat, giving black residues. Samples were then treated with a further 25 cm^3 of concentrated nitric acid and heated to dryness. At this stage the residues were grey to white. If residues are still completely black, digestion was repeated until a white residue was obtained.

25 cm^3 4:1 (v/v) mixture of nitric/perchloric acids was added and the mixture was again heated to dryness, (for precautions see Orren et al., 1980). The residues were dissolved in 10 per cent nitric acid (5 cm^3) and analysed according to Appendix B1.

APPENDIX B4

Sample preparation of mussels.

Samples of mussels should be rapidly frozen fast ($-10^{\circ}C$) in order to avoid the formation of large ice crystals within the tissue cells of the sample. Large ice crystals will rupture the cell walls and upon thawing excessive amounts of cellular liquids ("drips") will form. Since this "drip" will contain part of the elements and substances to be analysed, "drip" formation was minimized by fast freezing. All "drip" must be considered as part of the sample and wet weight (Barnard, 1976).

After thawing, the whole mussels were removed from the shell, using a glass knife to avoid metal contamination, and weighed in acid washed glass vials. The byssus threads were eliminated but the pallial fluid was included in the wet weight. Special effort was made to avoid contaminating the soft parts by contact with the outer part of the shell.

The length of one shell half was then determined with sliding calipers. Of the animals collected, six from each group were dried to constant mass in an over at $100^{\circ}C$, to determine water content while the rest were analysed individually for cadmium according to Appendix B3.

APPENDIX B5

Column chromatography preparations.

Buffer solutions

25 mM phosphate buffer pH 7.0

413 cm^3 KH_2 PO_4 (25 mM = 3.4024 g dm^{-3} added to

587 cm^3 Na_2 HPO_4 (25 mM = 4.4513 g dm^{-3}) gives the desired pH

20 mM Tris- HCl buffer pH 8.6

250 cm^3 Tris (hydroxymethyl) aminomethane (20 mM = 2.423 dm^{-3}) added to

130 cm^3 HCl (10 mM) gives the desired pH (Diem and Lentner, 1972).

Sodium azide (0.02%) was used as an anti-microbial agent in this buffer.

Gel filtration

The fine dry powdered Sephadex was allowed to swell for 48 hours in distilled water. A magnetic stirrer at very low speed was used. The swollen Sephadex was allowed to settle for two hours and the fine or broken particles were removed by decantation.

The suspension of gel was adjusted to a fairly thick slurry and de-aired for 30 minutes on a vacuum pump. The column was mounted vertically in a cool room (4°C) and the gel was poured using a glass rod. All the gel required was poured in a single operation. The airvents were closed and buffer flow started immediately after filling the column, to ensure even settling.

The operating pressure and safety loop arrangements are shown in Fig. B5. 1. The determination of void volume (V_o) was done with 5 cm^3 Blue Dextran (3 mg cm^{-3}). All buffers and vessels were precooled to 4°C. The sample was applied under the elutant with a syringe and a piece of fine capillary tubing into a sample applicator. This allowed the sample to be introduced as a layer below the element without disturbing the chromatographic bed.

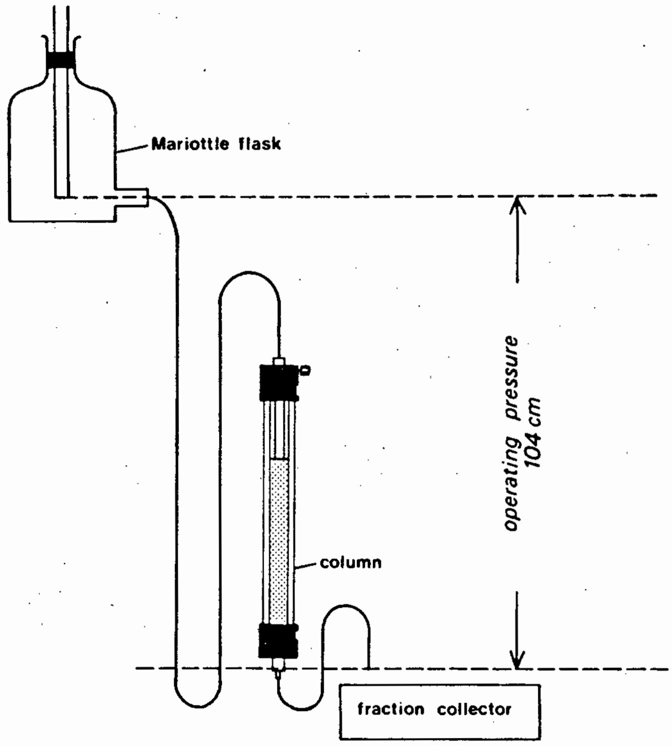


Fig. B 5.1. Operating pressure of column and safety loop arrangements.

The safety loop is placed before the column with the column outlet tubing in any position above the lower loop on the inlet side. The flow stops when the element in the inlet reaches the level of the outlet tubing.

APPENDIX B6

Protein determination of fractions.

Add one cm^3 reagent A (see below) to sample (50 mm^3) and let stand for 10 minutes at room temperature. Then add 100 mm^3 reagent B (see below). Mix immediately. Allow the mixture to stand for 30 minutes at room temperature then add one cm^3 distilled water. Read in spectrophotometer at 750 nm in one cm curvettes.

Reagent A:

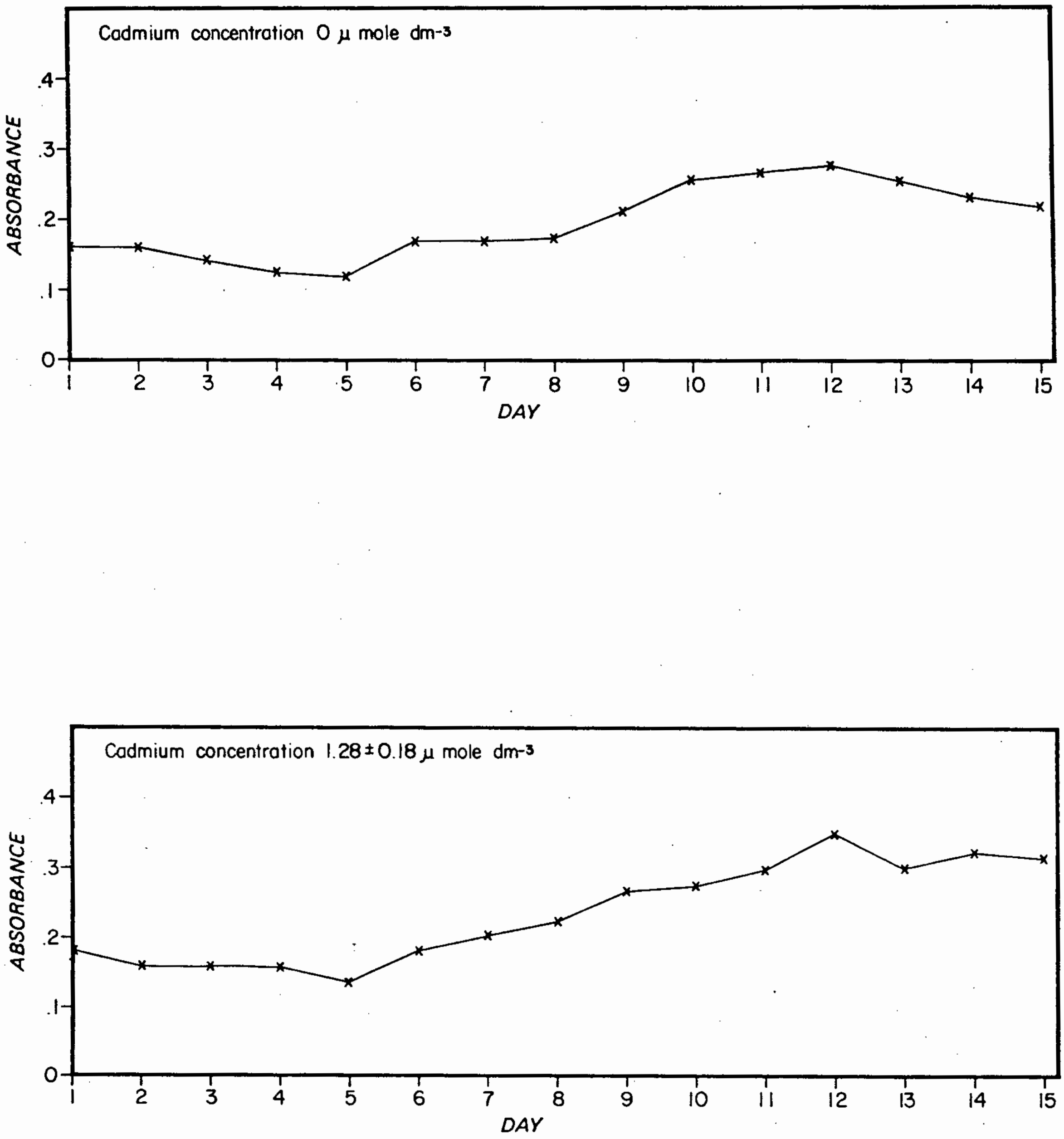
Mix 50 cm^3 $Na_2\ CO_3$ (2%) in 0.1 M NaOH with one cm^3 of $Cu\ SO_4$ (0.5%) in sodium citrate (1%) just before use.

Reagent B:

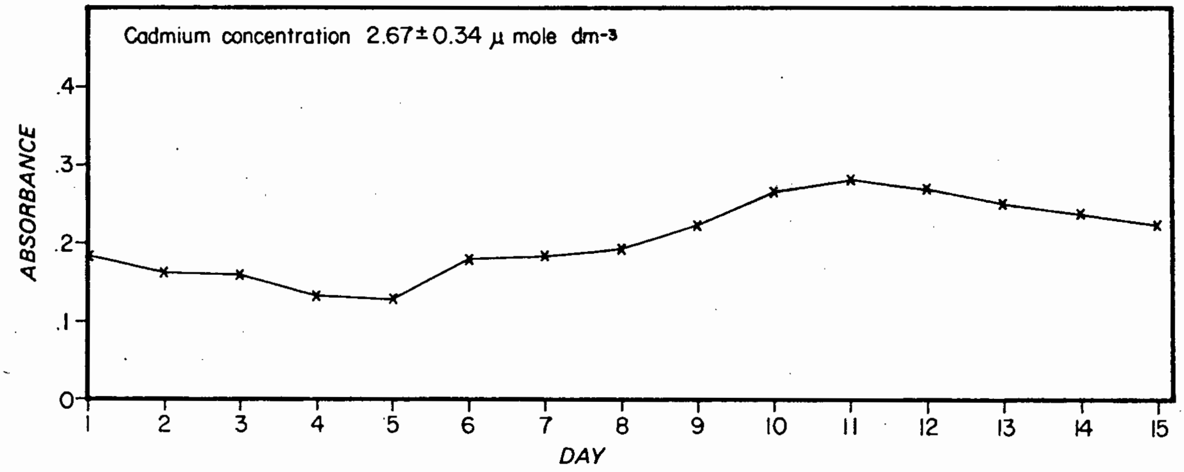
Dilute B.D.H. Folin-Ciocalteau reagent with distilled water in the ratio 1: 1.3 respectively.

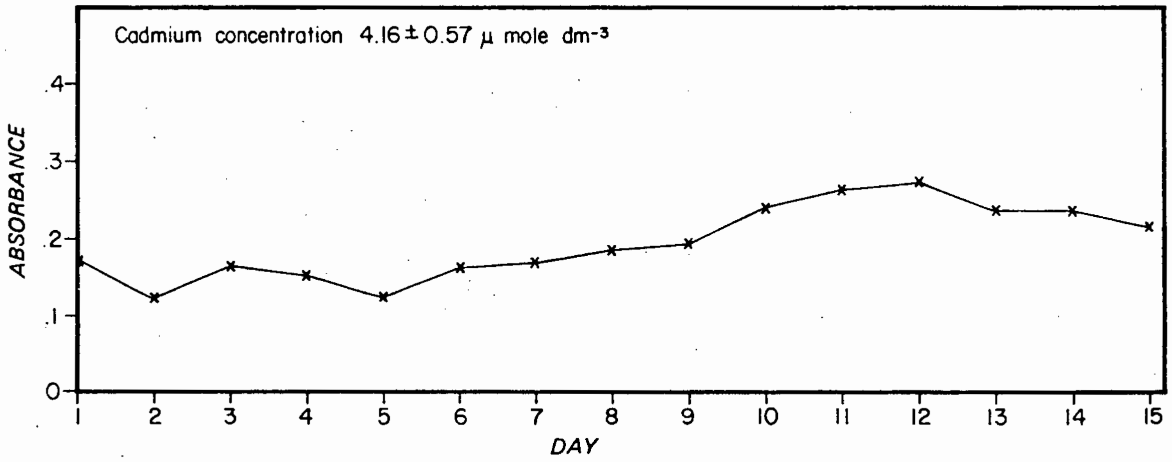
Reference: Lowry *et al.*, 1951.

SECTION 8.1

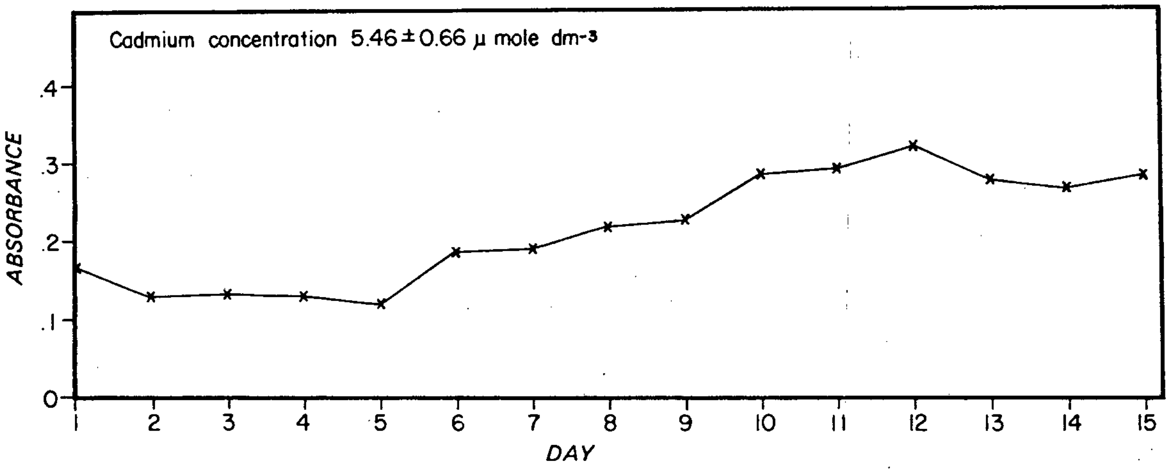


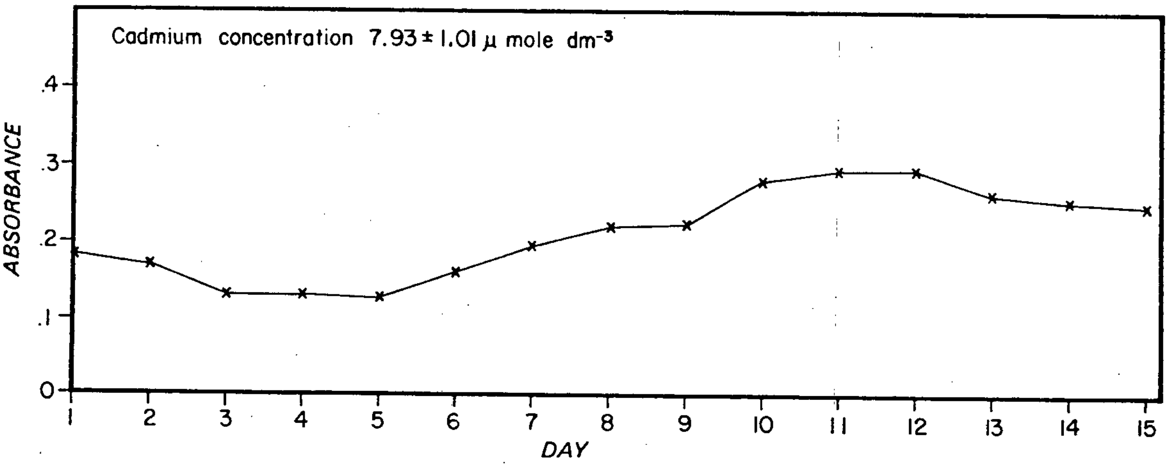
Figs. 5a,b. Growth characteristics of T. suecica during 15 day exposure to various concentrations of cadmium.



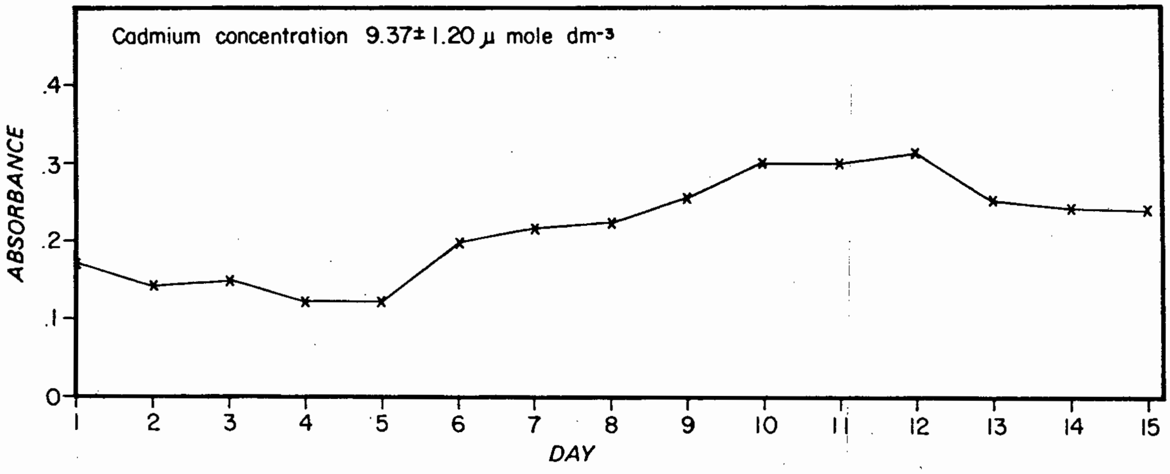


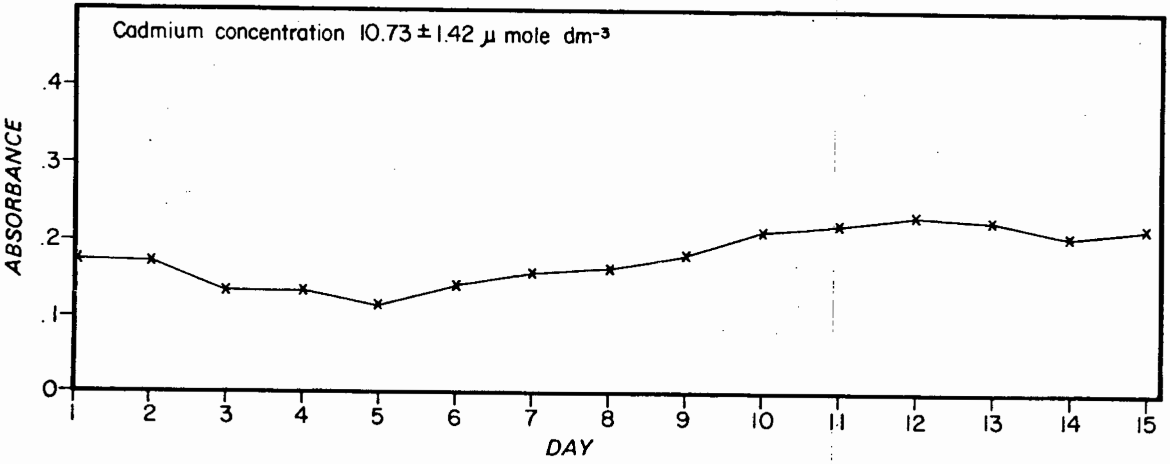
Figs. 5c,d. Growth characteristics of T. suecica during 15 day exposure to various concentrations of cadmium.



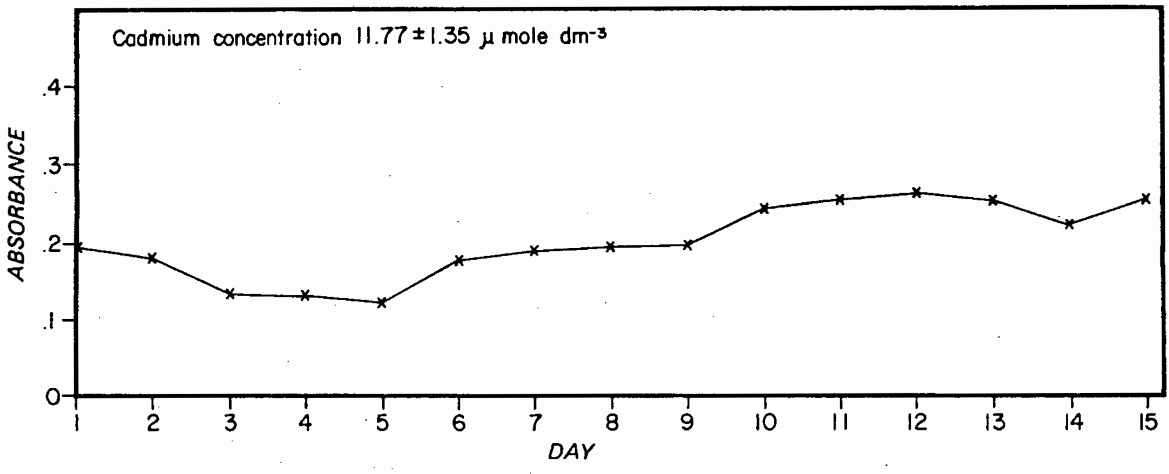


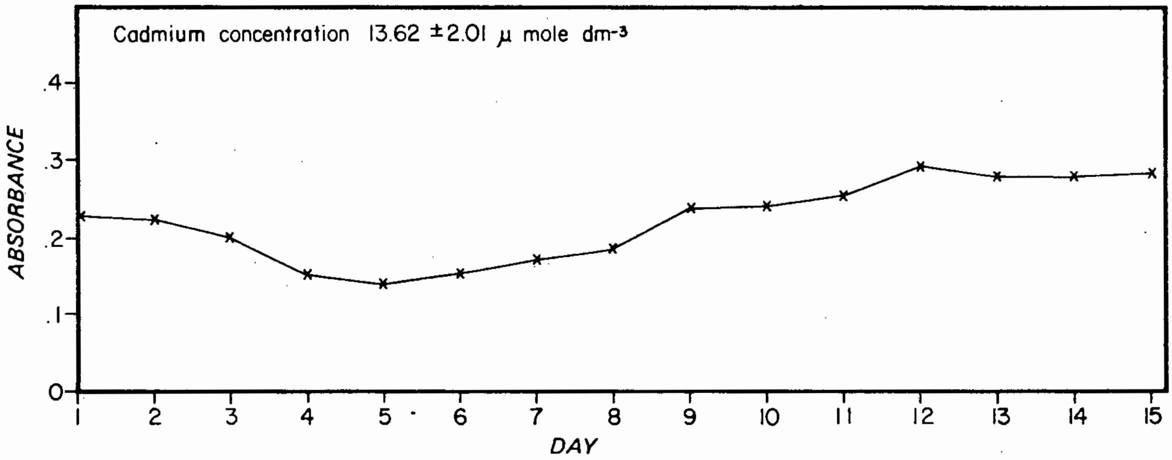
Figs. 5e,g. Growth characteristics of T. suecica during 15 day exposure to various concentrations of cadmium.



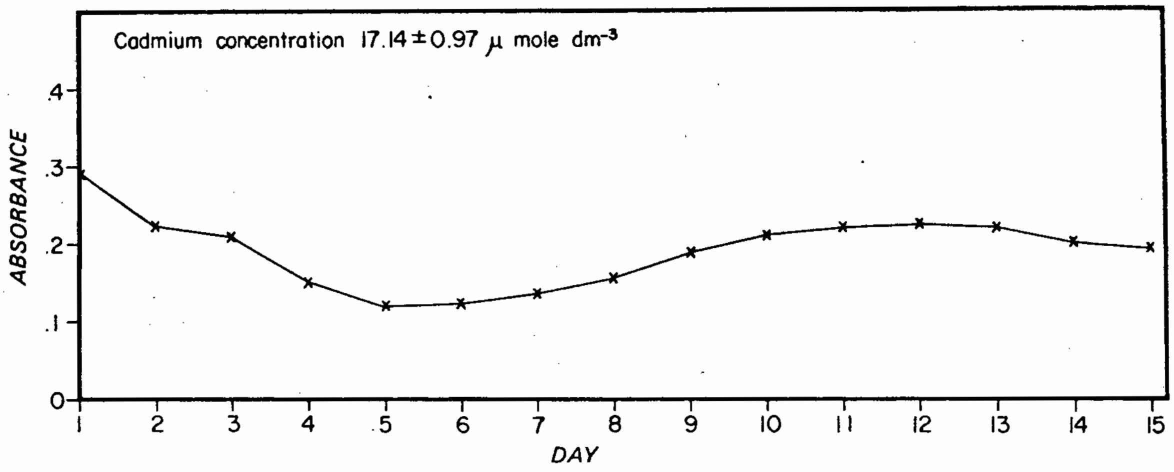


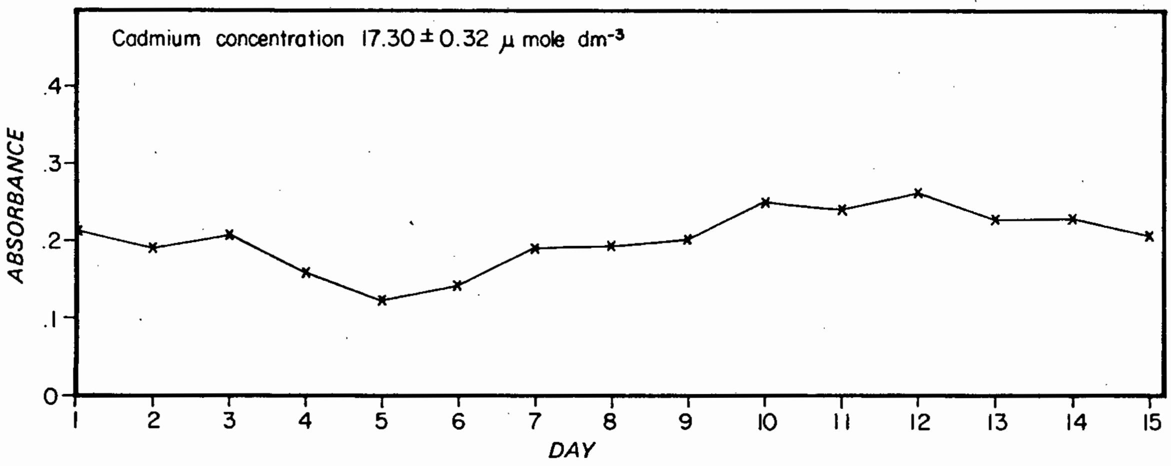
Figs. 5h,i. Growth characteristics of T. suecica during 15 day exposure to various concentrations of cadmium.



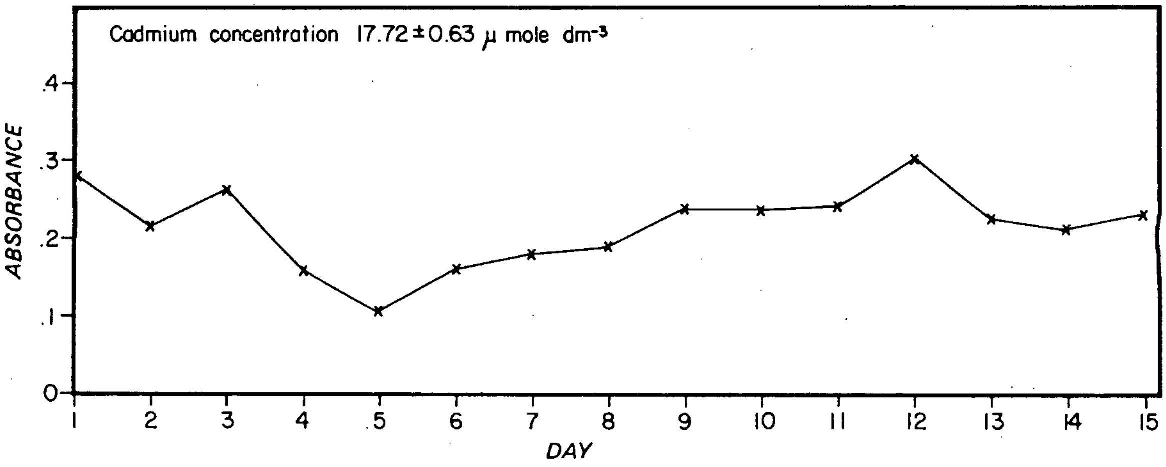


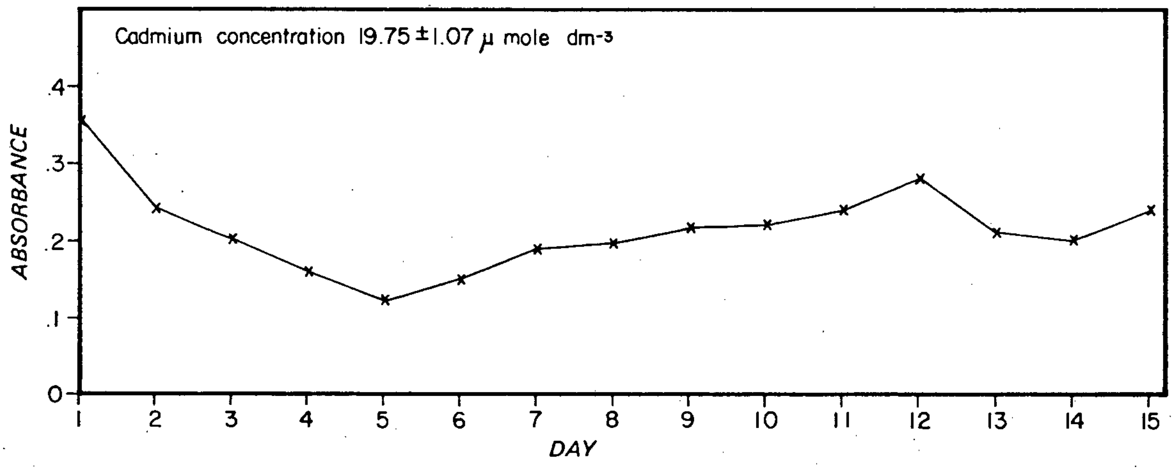
Figs. 5j,k. Growth characteristics of T. suecica during 15 day exposure to various concentrations of cadmium.





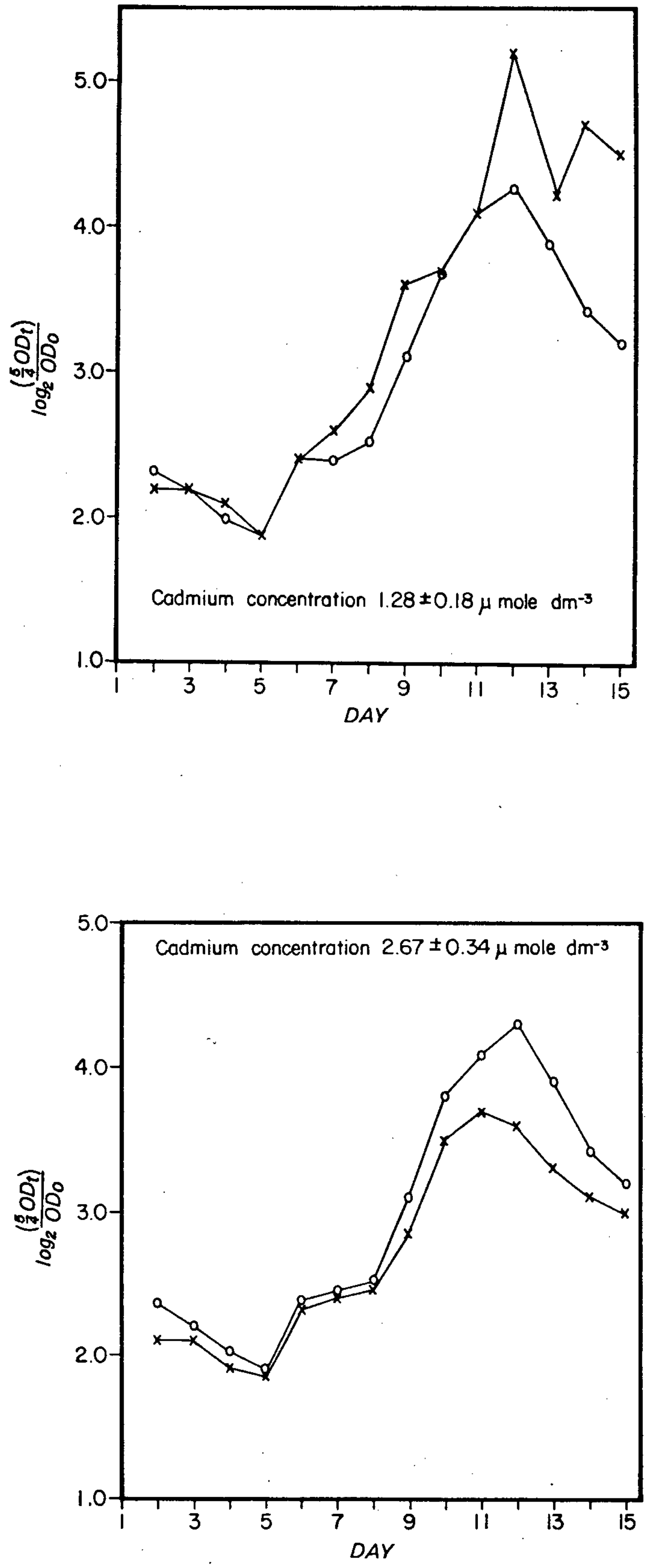
Figs. 51,m. Growth characteristics of T. suecica during 15 day exposure to various concentrations of cadmium.





Figs. 5n,o. Growth characteristics of <u>T. suecica</u> during 15 day exposure to various concentrations of cadmium.

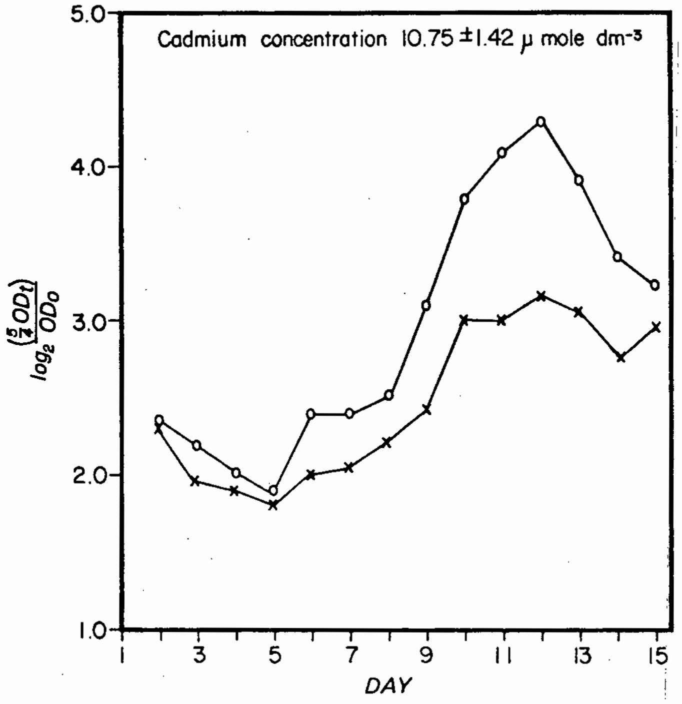
SECTION 8.2

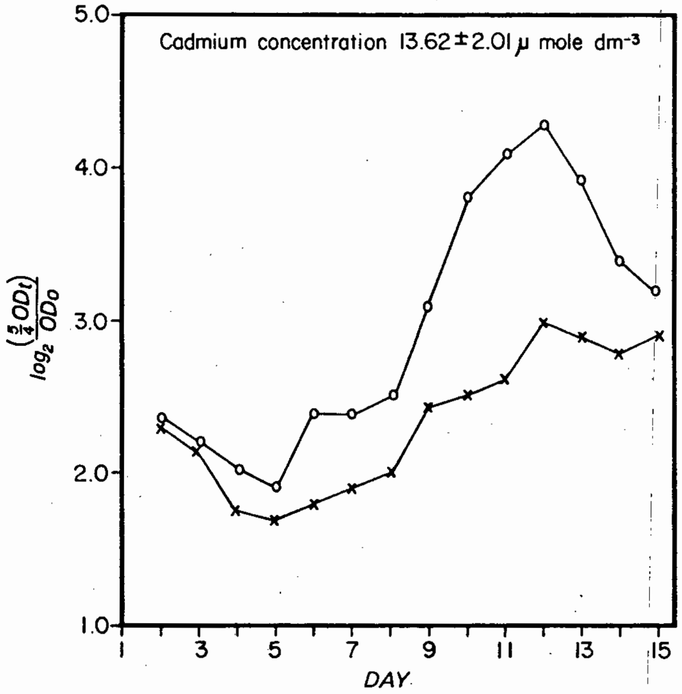


Cadmium concentration 1.28 ± 0.18 μ mole dm⁻³

Cadmium concentration 2.67 ± 0.34 μ mole dm⁻³

Figs. 9a,b. Growth pattern of T. suecica in various cadmium solutions during 15 days of harvesting (Reference growth pattern, open circles, is included for comparison).





Figs. 9h,i. Growth pattern of T. suecica in various cadmium solutions during 15 days of harvesting (Reference growth pattern, open circles, is included for comparison).

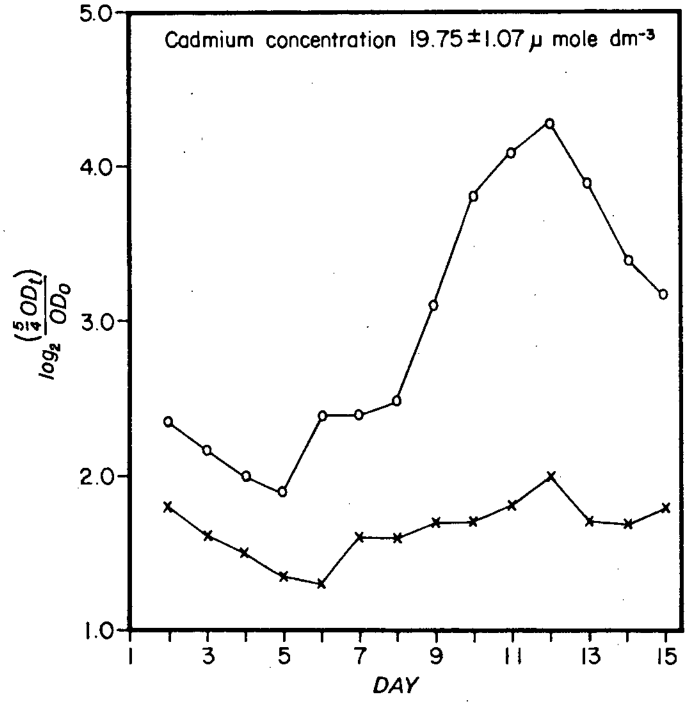
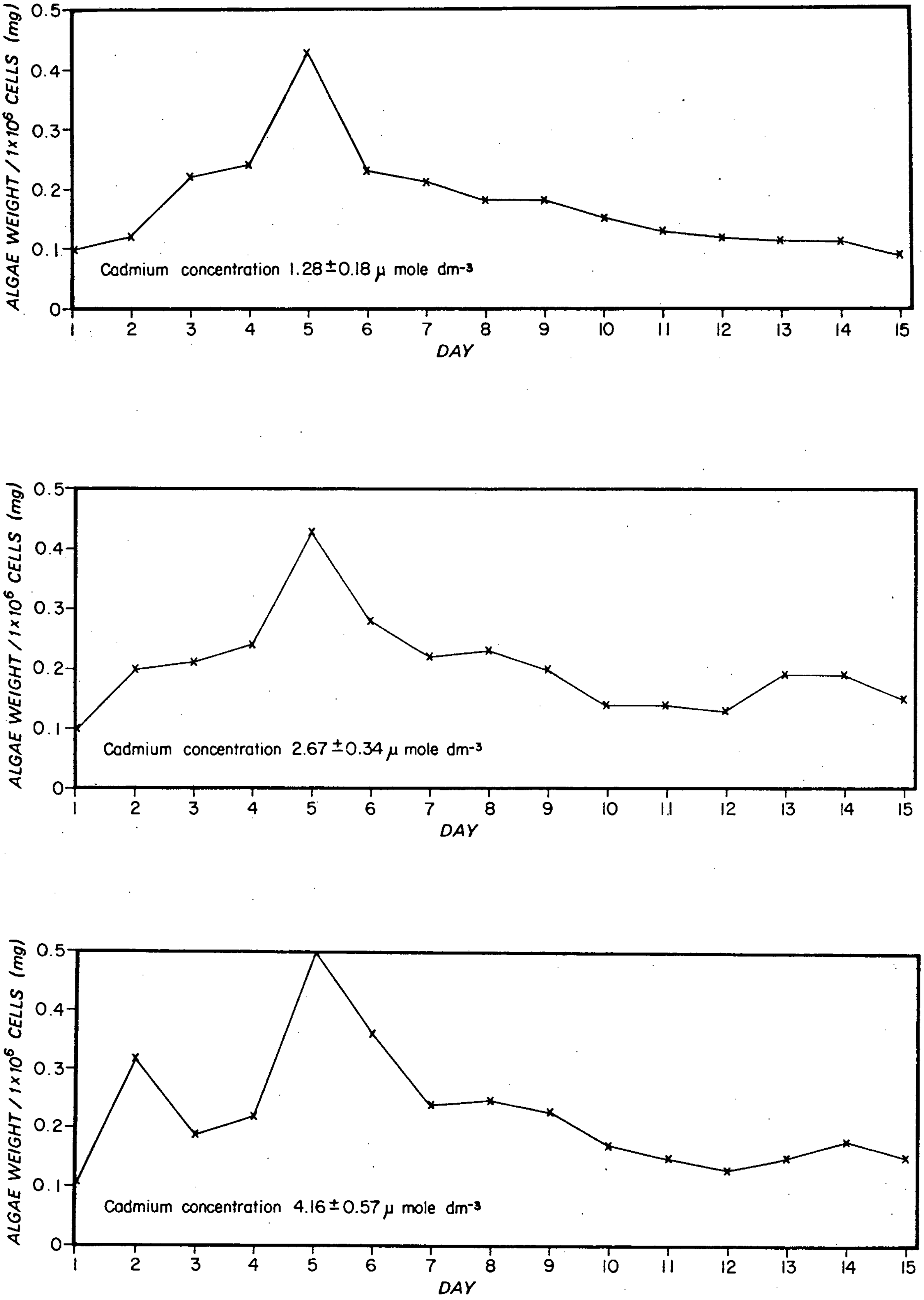
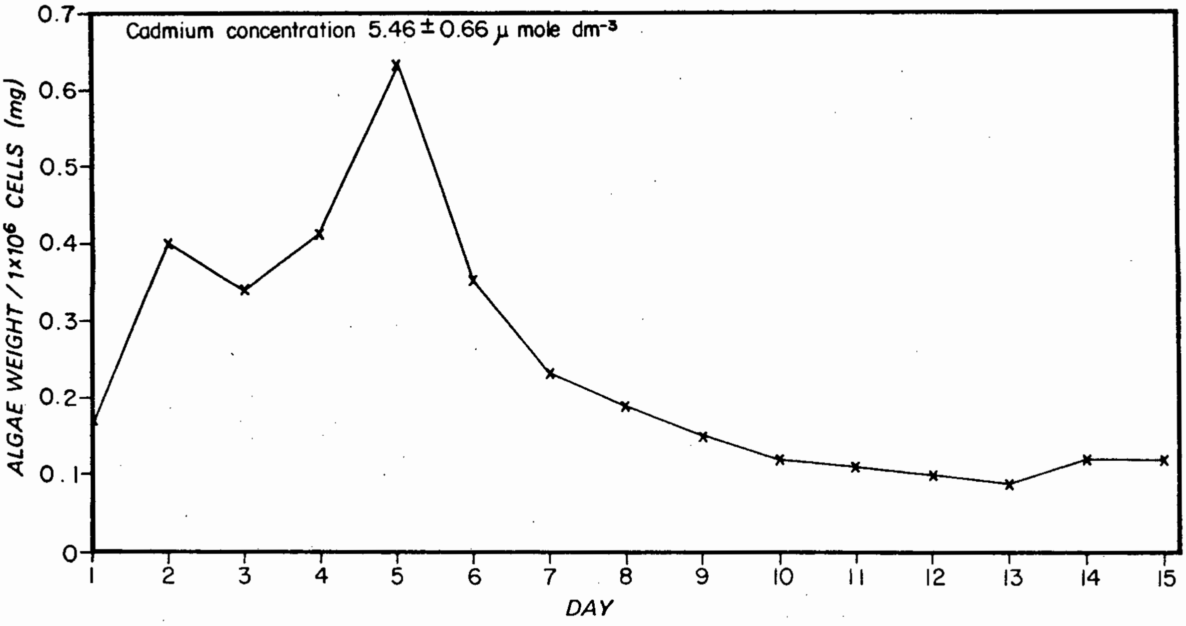


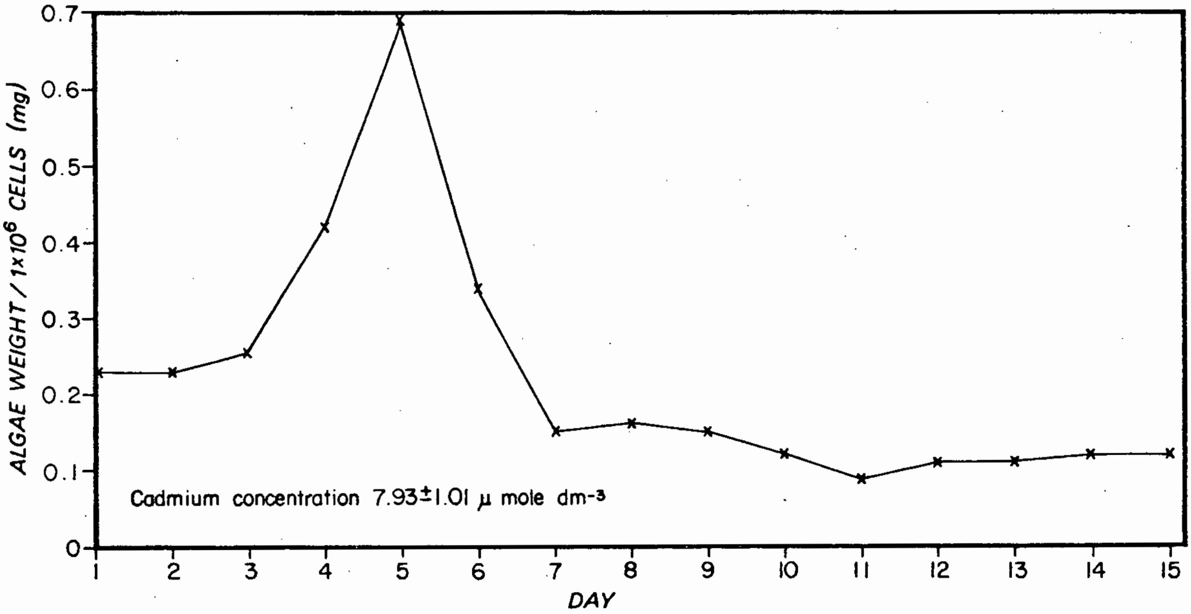
Fig. 9n. Growth pattern of T. suecica in various cadmium solutions during 15 days of harvesting (Reference growth pattern, open circles, is included for comparison).

SECTION 8.3

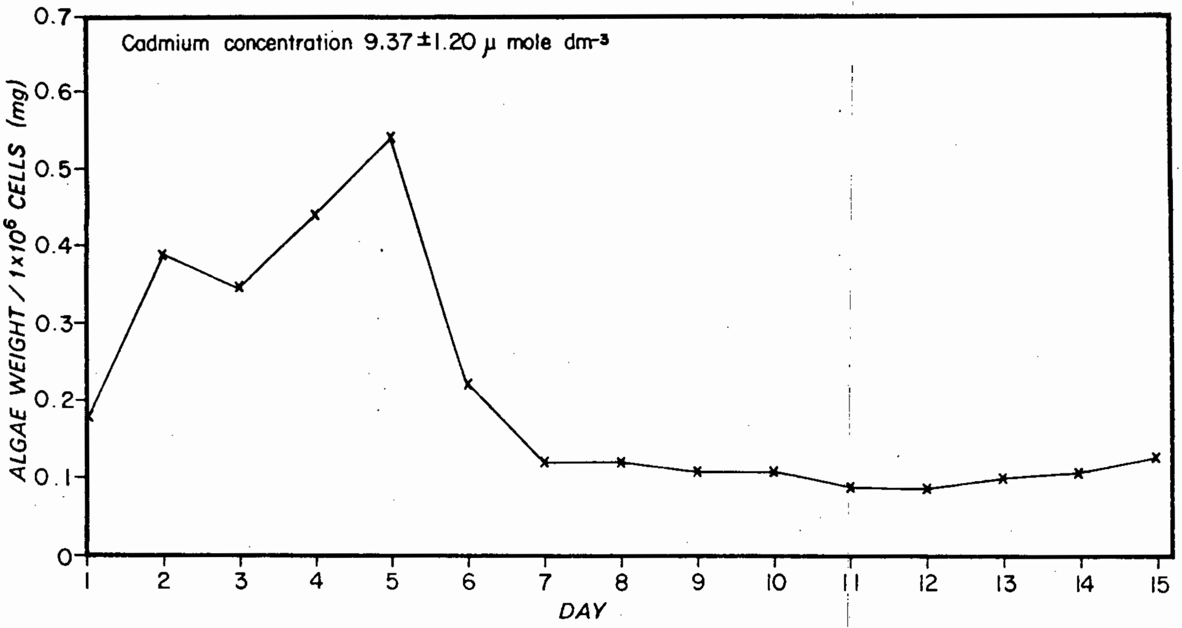


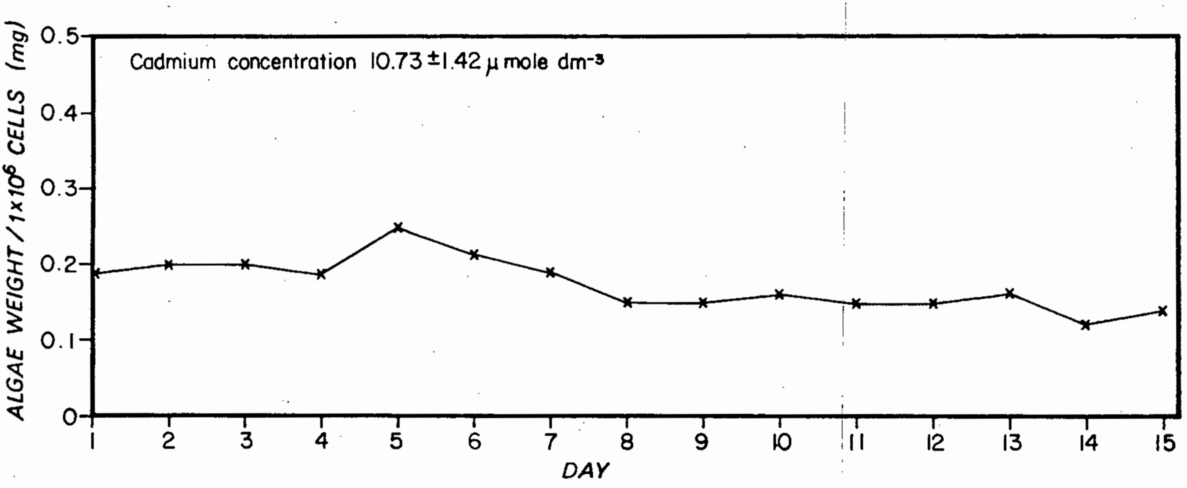
Figs. 15a-c. Algal mass per unit number of cells during 15 days of cadmium accumulation.



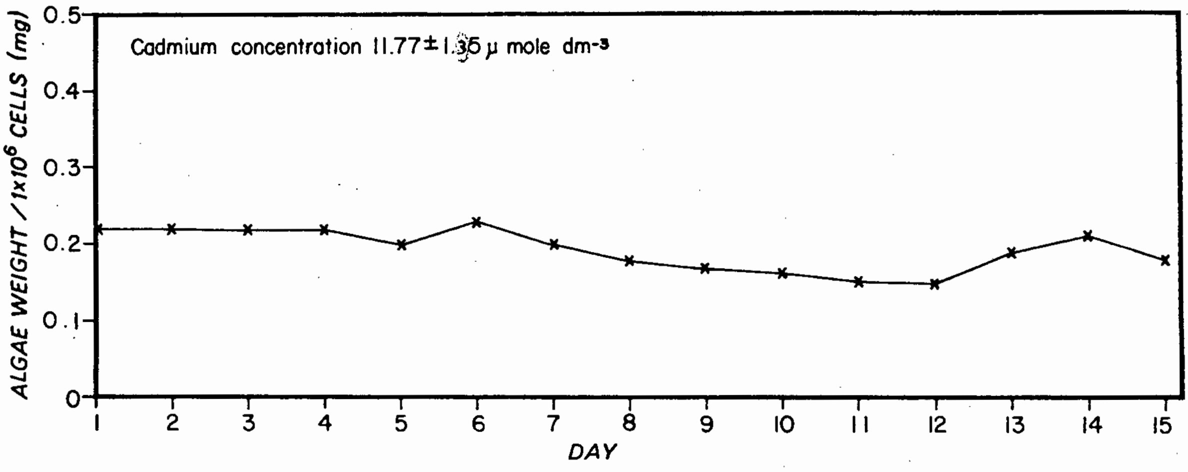


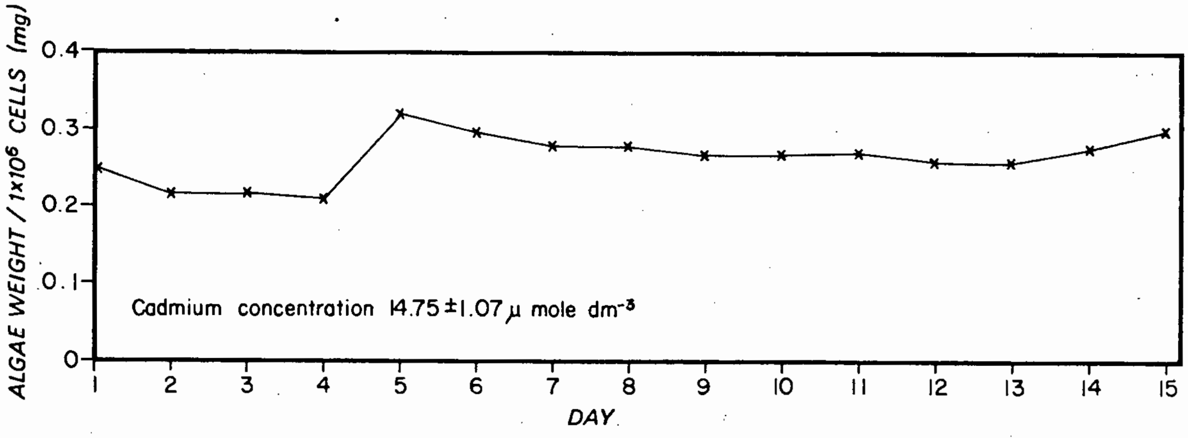
Figs. 15d,f. Algal mass per unit number of cells during 15 days of cadmium accumulation.

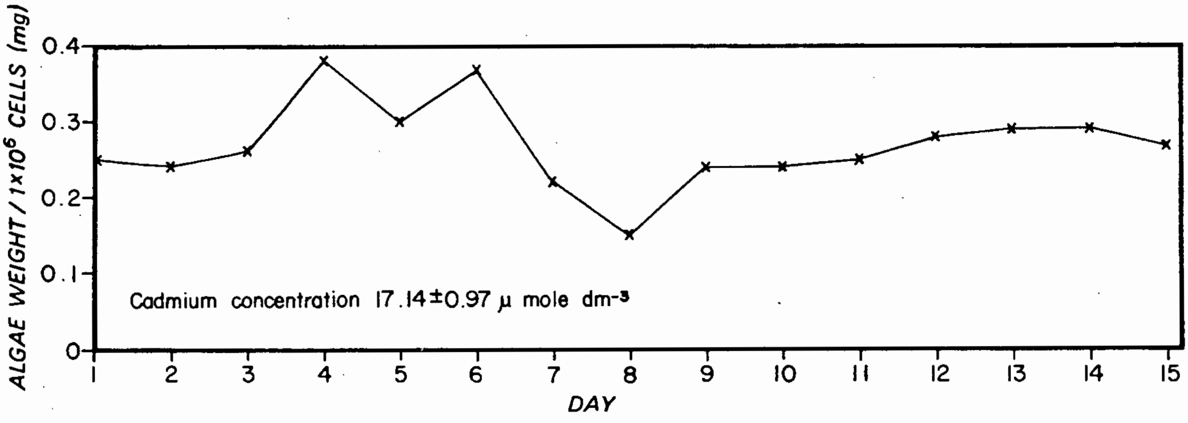




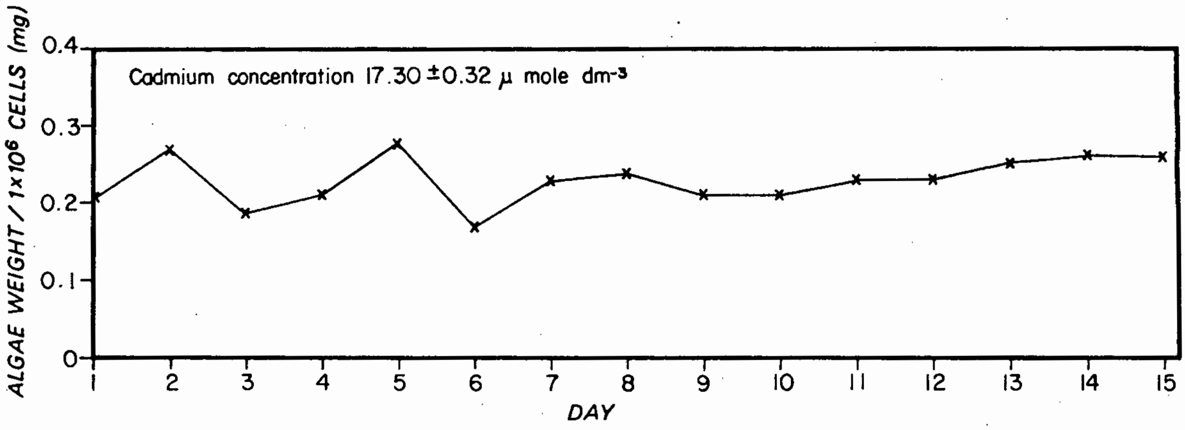
Figs. 15g,h. Algal mass per unit number of cells during 15 days of cadmium accumulation.

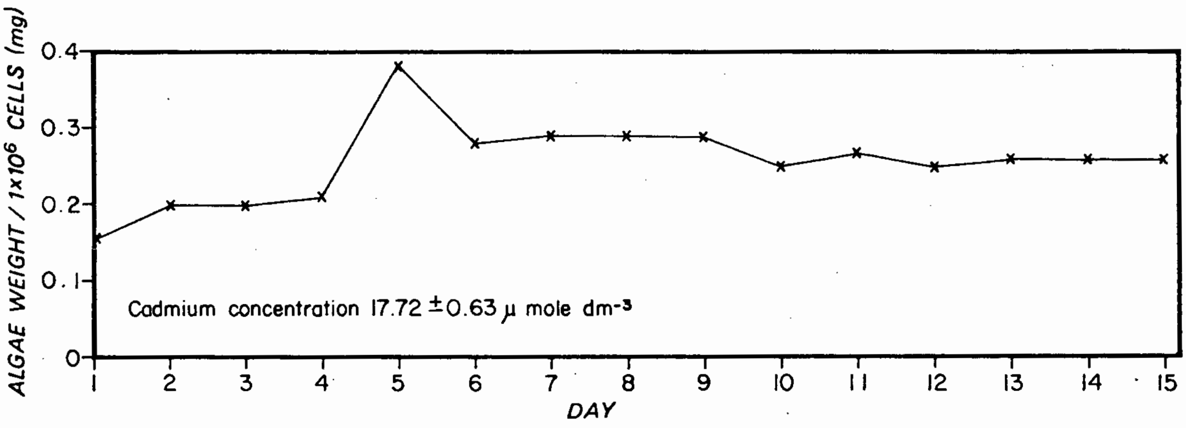






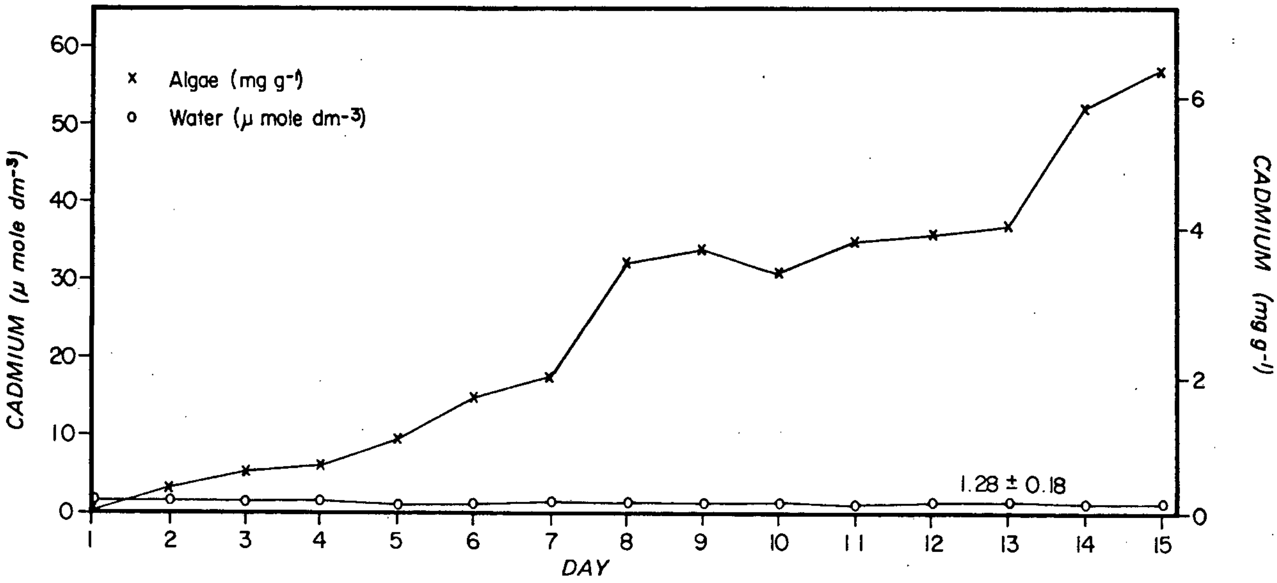
Figs. 15i,k,l. Algal mass per unit number of cells during 15 days of cadmium accumulation.

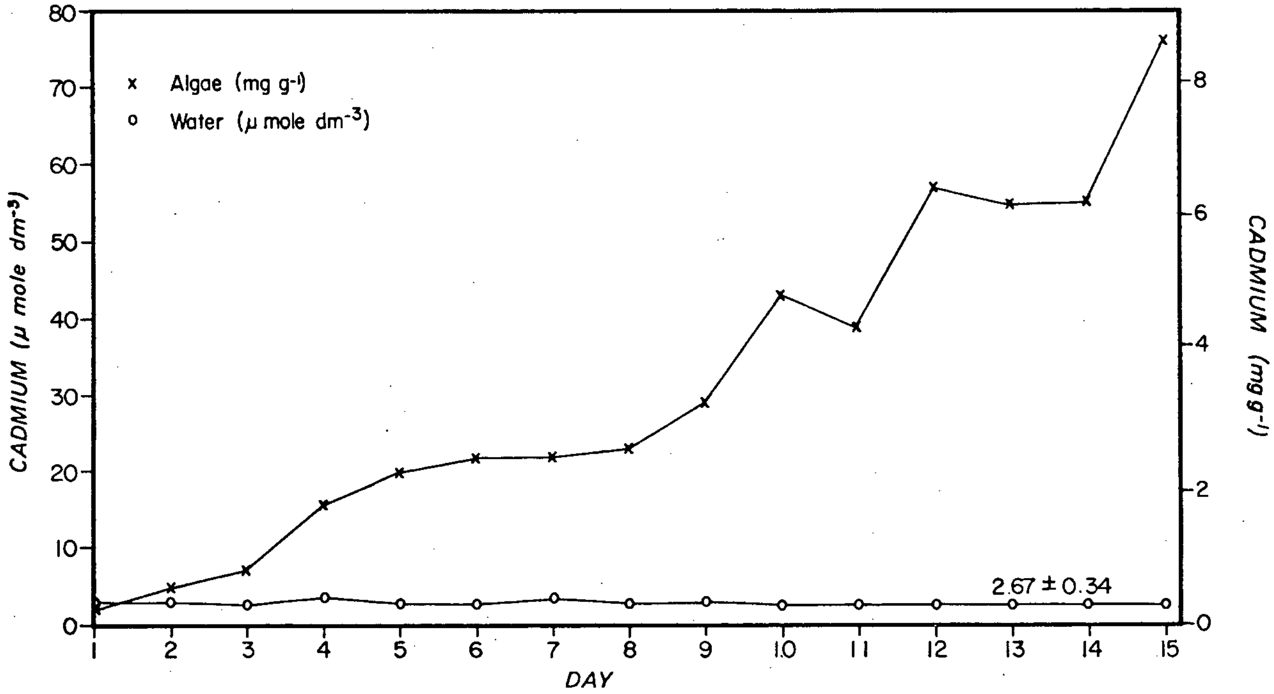




Figs. 15m,n. Algal mass per unit number of cells during 15 days of cadmium accumulation.

SECTION 8.4





Figs. 17a,b. Accumulation of cadmium in T. suecica during 15 days of harvesting.

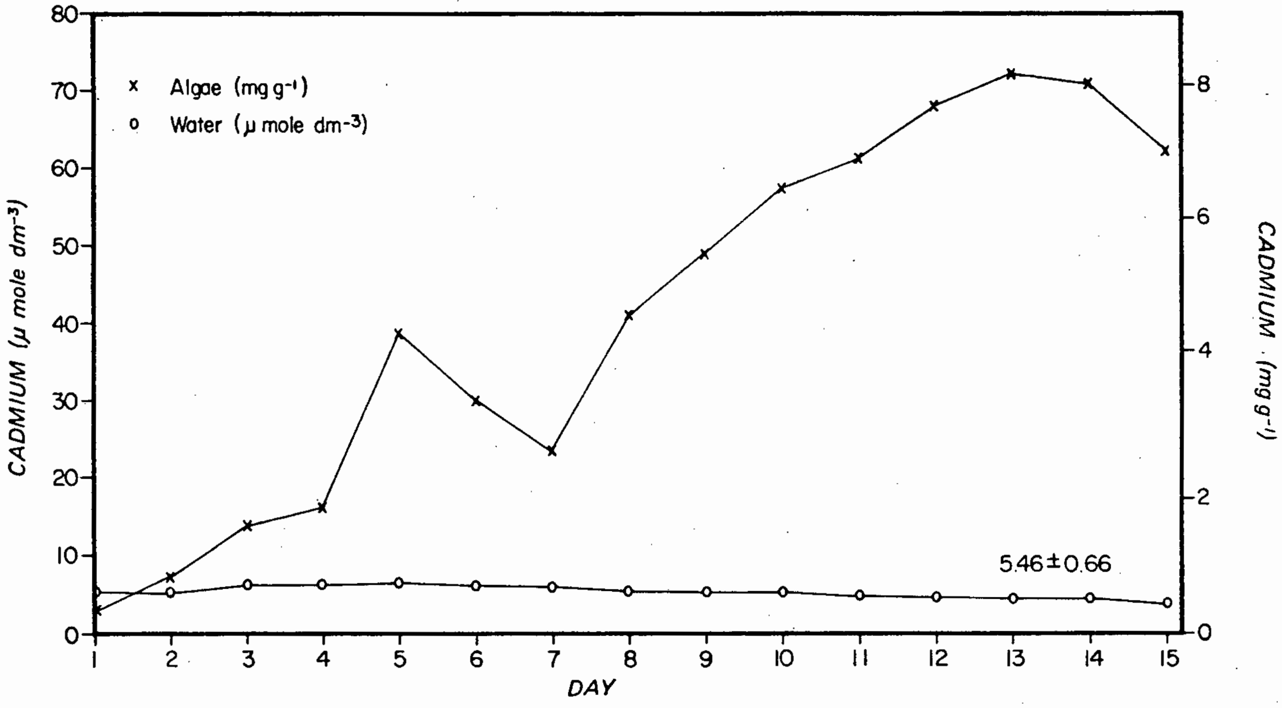


Fig. 17d. Accumulation of cadmium in T. suecica during 15 days of harvesting.

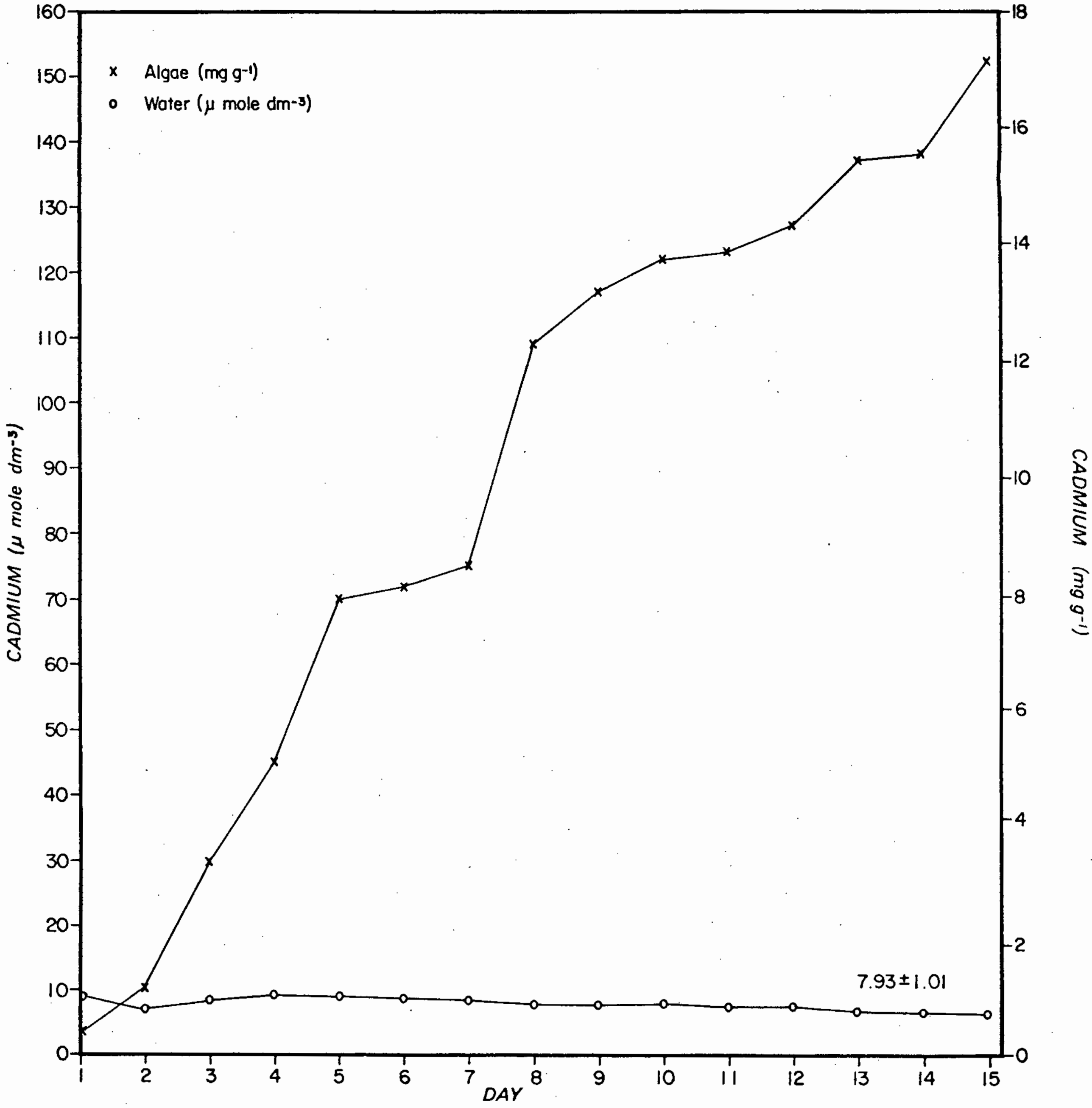


Fig. 17f. Accumulation of cadmium in <u>T. suecica</u> during 15 days of harvesting.

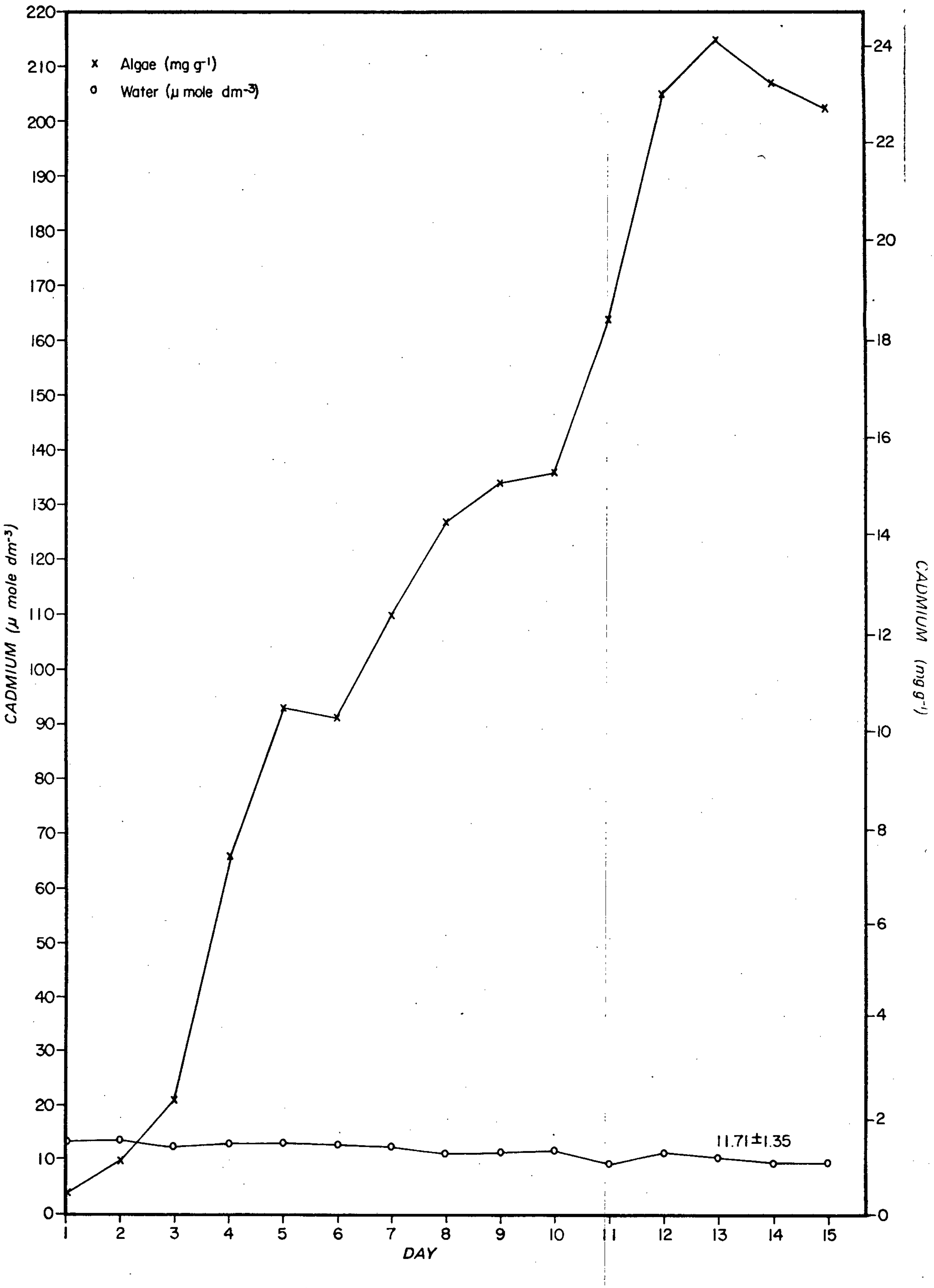


Fig. 17i. Accumulation of cadmium in T. suecica during 15 days of harvesting..

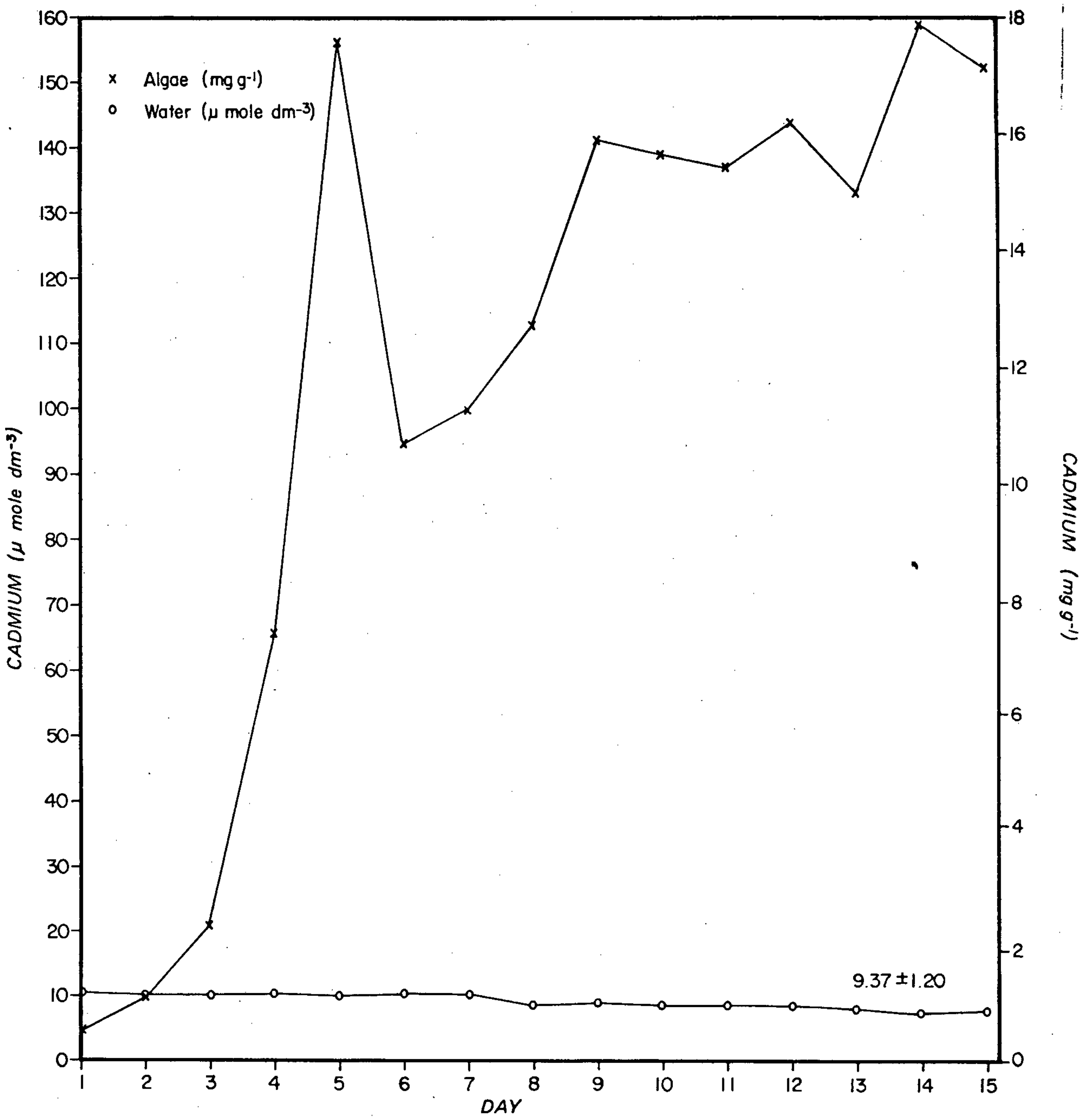


Fig. 17g. Accumulation of cadmium in T. suecica during 15 days of harvesting.

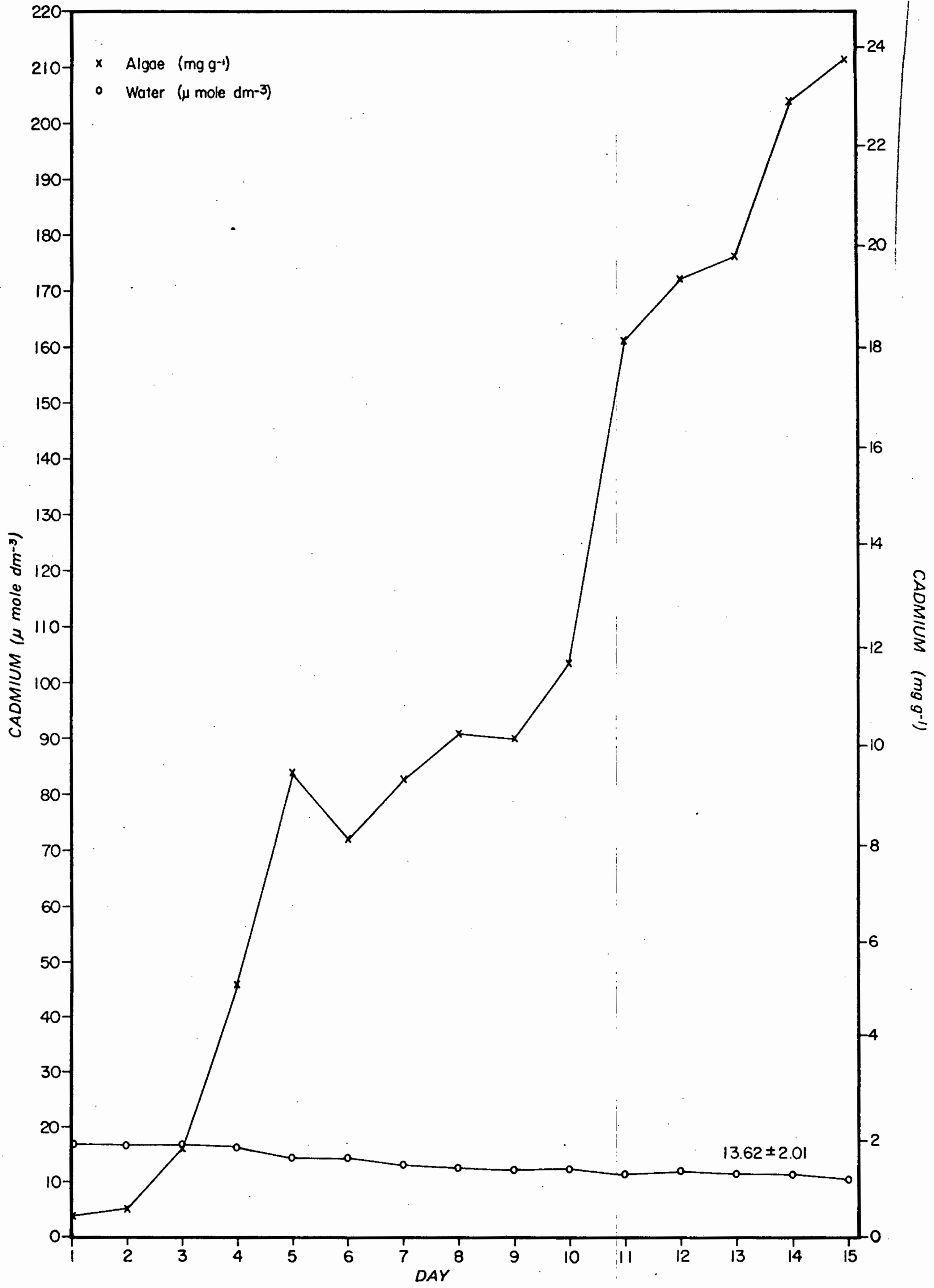
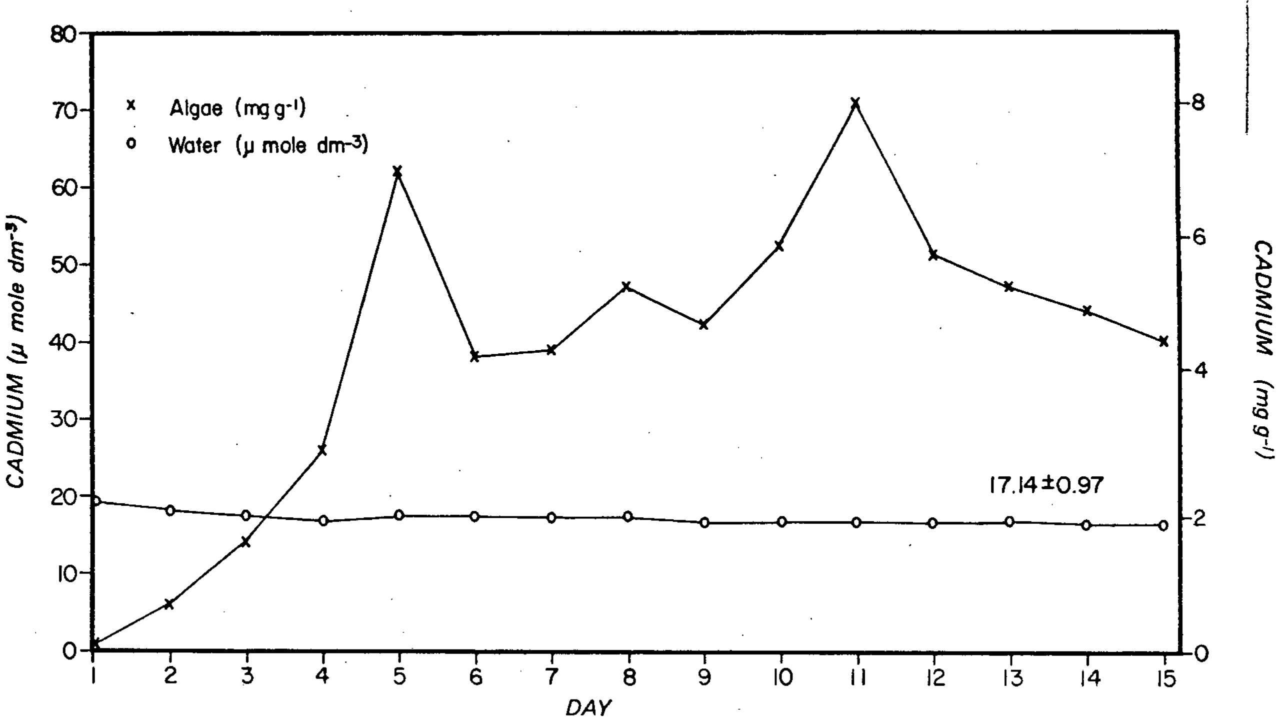
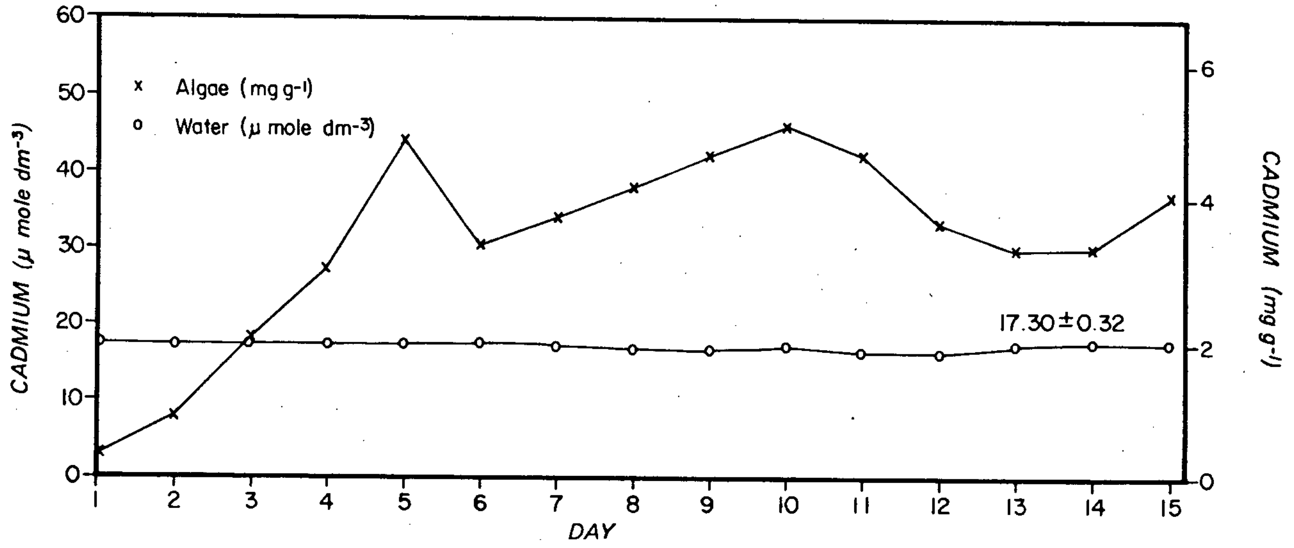


Fig. 17j. Accumulation of cadmium in <u>T. suecica</u> during 15 days of harvesting .





Figs. 17k,l. Accumulation of cadmium in T. suecica during 15 days of harvesting.

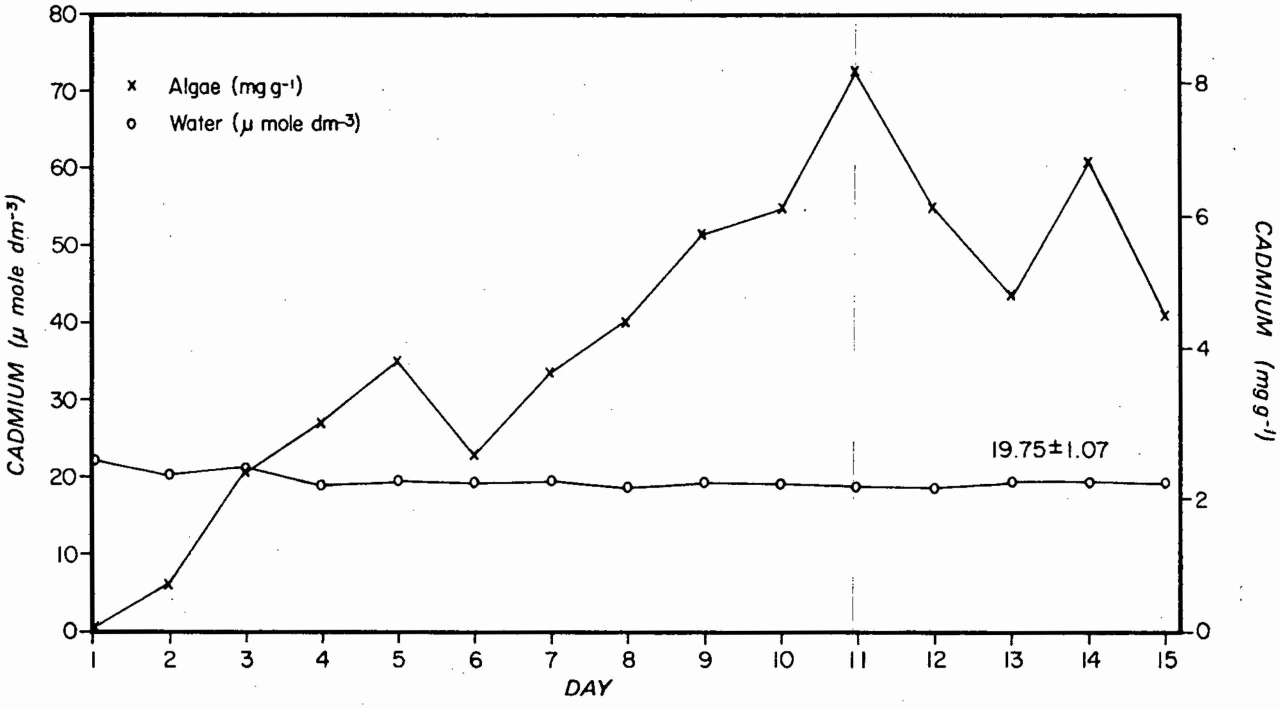


Fig. 17n. Accumulation of cadmium in T. suecica during 15 days of harvesting.

SECTION 8.5

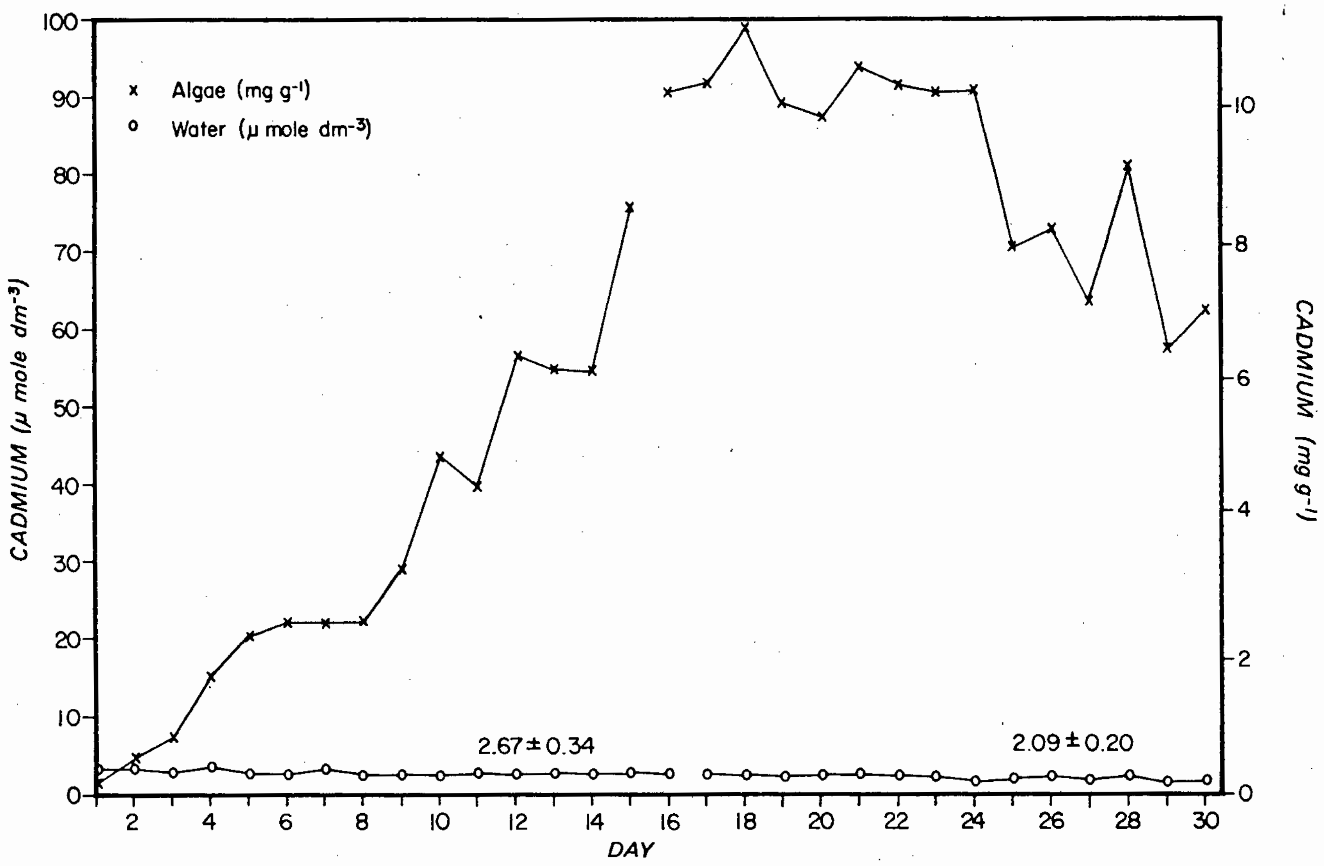


Fig. 18a. Accumulation of cadmium in T. suecica during 30 days of harvesting.

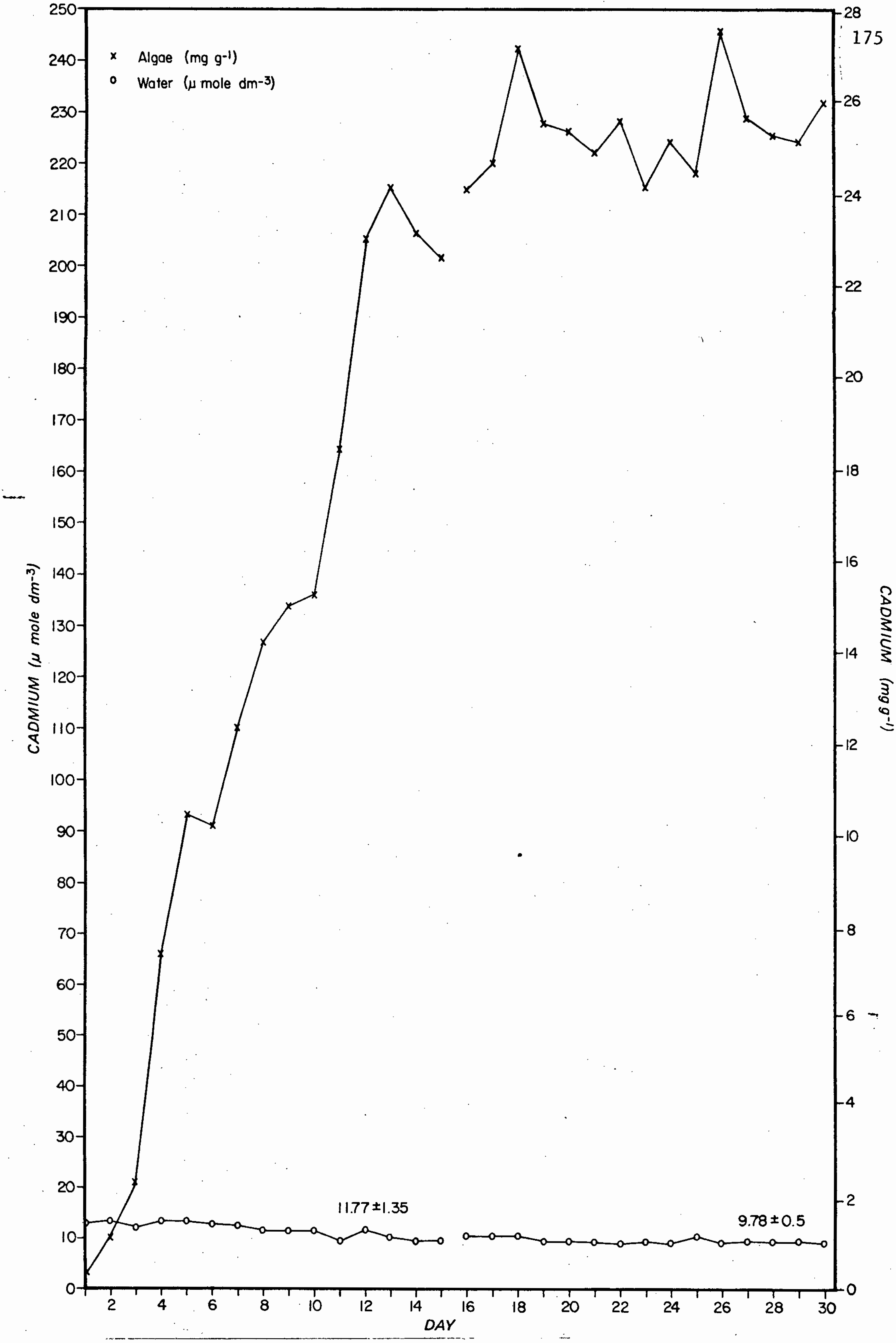


Fig. 18c. Accumulation of cadmium in T. suecica during 30 days of harvesting.

SECTION 9

RAW DATA

Algae $OD_{Abs.}$ ($\bar{x}$) Slit medium SD = Lower no.
For SD → n = 4 Wavelength = 660 nm D = time in days
Date 14/5 to 28/5 Path = 1 cm C = Cd conc. in µmole dm^{-3}
15 day experiment 4 ml of Algae taken

D C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	,164 ,017	,162 ,006	,149 ,020	,128 ,017	,120 ,012	,168 ,008	,169 ,006	,173 ,005	,212 ,015	,252 ,029	,269 ,011	,277 ,022	,257 ,022	,233 ,018	,221 ,011
1,28	,182 ,004	,162 ,007	,162 ,005	,159 ,004	,138 ,023	,182 ,014	,203 ,009	,222 ,008	,271 ,012	,272 ,024	,297 ,022	,348 ,008	,300 ,018	,325 ,018	,314 ,015
2,67	,184 ,019	,162 ,034	,162 ,018	,134 ,010	,126 ,017	,180 ,015	,185 ,017	,191 ,029	,223 ,019	,266 ,029	,280 ,018	,273 ,021	,253 ,018	,242 ,026	,232 ,026
4,16	,172 ,021	,133 ,017	,170 ,016	,158 ,010	,127 ,007	,167 ,025	,171 ,010	,186 ,013	,199 ,014	,244 ,033	,267 ,038	,278 ,013	,239 ,018	,230 ,026	,219 ,037
5,46	,170 ,016	,132 ,014	,135 ,010	,133 ,013	,123 ,006	,190 ,016	,196 ,019	,220 ,062	,231 ,013	,288 ,023	,297 ,023	,324 ,015	,280 ,016	,271 ,023	,285 ,034
6,54	,171 ,015	,153 ,022	,157 ,031	,155 ,015	,134 ,021	,195 ,017	,260 ,032	,364 ,018	,369 ,028	,374 ,014	,383 ,038	,425 ,029	,379 ,032	,343 ,016	,323 ,017
7,93	,183 ,021	,171 ,008	,137 ,010	,134 ,014	,132 ,007	,164 ,015	,198 ,010	,221 ,034	,229 ,034	,282 ,025	,299 ,023	,298 ,021	,267 ,039	,255 ,020	,260 ,031
9,37	,176 ,026	,145 ,025	,150 ,019	,124 ,015	,124 ,020	,200 ,023	,218 ,009	,229 ,016	,260 ,016	,304 ,008	,307 ,018	,315 ,011	,251 ,019	,246 ,014	,249 ,011
10,73	,179 ,004	,174 ,010	,138 ,013	,135 ,014	,118 ,005	,142 ,013	,160 ,022	,166 ,014	,183 ,024	,215 ,017	,226 ,032	,238 ,038	,231 ,023	,210 ,020	,224 ,012
13,62	,197 ,016	,181 ,008	,136 ,031	,131 ,036	,126 ,010	,180 ,008	,190 ,011	,198 ,009	,199 ,030	,248 ,017	,257 ,037	,264 ,010	,256 ,026	,227 ,037	,257 ,012
11,77	,232 ,028	,225 ,014	,206 ,022	,152 ,023	,145 ,007	,157 ,015	,177 ,016	,188 ,018	,240 ,054	,243 ,018	,254 ,015	,296 ,011	,287 ,023	,280 ,011	,284 ,012
17,30	,219	,192	,210	,160	,127	,141	,197	,198	,202	,254	,242	,266	,233	,233	,210
17,72	,283	,220	,262	,160	,110	,164	,181	,191	,240	,241	,245	,302	,231	,216	,233
17,14	,290	,232	,211	,156	,121	,125	,140	,160	,192	,216	,227	,230	,222	,202	,199
19,75	,356	,246	,204	,162	,127	,152	,193	,200	,220	,226	,246	,281	,212	,209	,241

RAW DATA

Algae Number (raw data x 200) per ml

15 day experiment

D = time in days

C = Cd conc. in µmole dm^{-3}

D C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	777 400	752 600	306 000	230 200	118 400	431 000	474 200	565 000	672 800	802 600	881 400	933 400	928 600	789 000	750 800
1,28	900 400	684 000	361 200	330 600	134 600	407 400	513 000	655 000	683 800	898 800	1035 800	1219 000	1054 200	1005 400	996 400
2,67	938 400	465 800	436 200	301 800	140 200	370 200	495 800	518 600	582 200	918 200	1147 000	1117 800	789 800	692 200	633 000
4,16	969 200	265 800	444 200	342 600	124 200	319 600	481 000	492 600	534 000	776 200	877 400	1054 200	803 200	682 200	633 600
5,46	531 000	209 200	220 200	171 400	85 600	308 600	494 000	607 800	809 000	1012 400	1129 800	1205 600	1110 800	839 800	1002 800
6,54	622 200	198 400	235 400	145 800	108 600	430 600	890 200	944 600	1187 200	1506 200	1599 200	1619 600	1519 600	1264 600	1220 200
7,93	370 200	345 600	294 000	164 400	93 800	281 400	612 200	695 400	783 800	1000 000	1399 400	1116 000	1028 200	926 000	923 600
9,37	483 800	216 200	208 000	152 600	93 600	509 800	1052 200	069 400	1100 800	1137 200	1367 400	1478 800	1334 000	983 000	951 400
10,73	445 400	419 400	408 200	364 600	227 800	413 600	500 200	709 800	728 600	777 000	816 200	944 600	778 200	746 200	761 800
13,62	424 200	401 000	398 600	334 200	334 200	457 800	531 400	588 200	708 600	774 000	856 800	888 800	664 600	579 400	708 600
11,77	647 800	551 000	498 000	337 600	313 600	406 000	461 000	657 400	740 600	910 600	919 400	948 200	910 400	744 200	871 600
17,30	571 800	387 000	402 000	367 000	148 400	368 000	383 000	385 000	393 600	469 400	455 000	495 200	470 200	462 800	398 800
17,72	599 000	437 600	547 200	377 800	239 400	384 800	400 200	406 800	485 000	490 000	486 200	505 200	430 600	417 000	433 800
17,14	492 200	463 600	408 400	209 200	145 000	229 800	408 200	629 200	455 600	458 000	476 800	448 600	384 600	372 200	389 800
19,75	529 800	371 800	346 400	337 000	204 600	346 400	377 000	377 200	395 600	410 600	425 600	445 600	408 800	377 600	391 400

RAW DATA

ALGAE WEIGHT ($\bar{x}$) in mg

For SD → n = 4

Experiment 15 days

SD = Lower no.

D = time in days

C = Cd conc. in µmole dm^{-3}

D C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	,109 ,011	,097 ,010	,074 ,034	,066 ,013	,061 ,014	,098 ,028	,115 ,023	,116 ,010	,124 ,018	,136 ,004	,145 ,010	,147 ,020	,139 ,015	,136 ,013	,086 ,014
1,28	,087 ,017	,083 ,012	,081 ,020	,080 ,018	,058 ,023	,095 ,021	,107 ,010	,116 ,009	,122 ,019	,136 ,019	,138 ,017	,142 ,019	,113 ,018	,111 ,016	,094 ,014
2,67	,095 ,025	,092 ,019	,092 ,029	,072 ,036	,061 ,010	,105 ,013	,110 ,009	,119 ,017	,119 ,028	,131 ,008	,161 ,010	,151 ,012	,147 ,009	,130 ,008	,094 ,011
4,16	,095 ,036	,085 ,023	,083 ,020	,074 ,019	,072 ,015	,115 ,028	,117 ,009	,121 ,009	,123 ,012	,129 ,006	,133 ,008	,136 ,006	,124 ,007	,122 ,007	,094 ,009
5,46	,091 ,018	,084 ,011	,074 ,006	,070 ,008	,054 ,009	,110 ,017	,113 ,022	,116 ,013	,119 ,028	,120 ,014	,123 ,013	,126 ,019	,103 ,017	,104 ,016	,122 ,014
6,54	,085 ,008	,081 ,008	,078 ,021	,076 ,017	,040 ,012	,103 ,010	,115 ,015	,123 ,011	,126 ,006	,126 ,009	,130 ,009	,131 ,019	,130 ,012	,130 ,012	,122 ,012
7,93	,085 ,018	,080 ,017	,075 ,007	,069 ,010	,065 ,011	,095 ,011	,095 ,013	,110 ,016	,121 ,017	,125 ,016	,130 ,016	,126 ,010	,112 ,010	,111 ,011	,107 ,011
9,37	,086 ,012	,084 ,008	,072 ,009	,067 ,009	,051 ,007	,112 ,008	,122 ,008	,124 ,006	,124 ,009	,127 ,009	,130 ,009	,130 ,015	,128 ,009	,108 ,009	,120 ,008
10,73	,083 ,015	,082 ,010	,080 ,016	,068 ,013	,057 ,014	,089 ,020	,097 ,010	,103 ,017	,111 ,014	,121 ,013	,125 ,012	,143 ,013	,121 ,013	,093 ,010	,106 ,010
13,62	,095 ,012	,089 ,014	,087 ,008	,072 ,010	,066 ,011	,105 ,010	,108 ,014	,108 ,009	,122 ,007	,122 ,018	,131 ,016	,134 ,038	,127 ,013	,120 ,013	,128 ,013
11,77	,103 ,010	,098 ,007	,094 ,008	,093 ,020	,064 ,019	,107 ,015	,116 ,014	,123 ,028	,126 ,005	,128 ,015	,131 ,014	,132 ,005	,121 ,005	,102 ,012	,104 ,010
17,30	,090	,077	,080	,076	,058	,102	,111	,113	,114	,119	,122	,125	,122	,122	,102
17,72	,124	,119	,105	,079	,066	,065	,093	,097	,100	,105	,134	,117	,109	,107	,114
17,14	,125	,112	,108	,081	,043	,085	,088	,093	,110	,111	,117	,124	,110	,109	,106
19,75	,137	,082	,076	,070	,065	,102	,105	,107	,108	,110	,112	,117	,106	,105	,116

RAW DATA

ALGAE Cd CONC in µmol dm^{-3} ($\bar{x}$)

Experiment 15 days

For SD → n = 4

SD = Lower no.

D = time in days

C = Cd conc. in µmole dm^{-3}

D C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
1,28	- -	0,2669 -	0,4448 -	0,5338 0,0890	0,5338 0,0890	1,4235 0,1779	1,8683 0,1779	3,7367 0,5338	4,1815 0,7117	4,2705 0,6228	4,8043 0,5338	5,0712 0,3559	4,1815 0,4448	5,7829 0,6228	5,4270 0,3559
2,67	0,1779 0,0890	0,4448 0,0890	0,7117 -	1,1566 0,1779	1,2456 0,0890	2,4021 0,1779	2,4911 0,3559	3,4698 0,2669	4,6263 0,3559	5,6940 1,3345	6,4057 0,7117	8,6299 0,9786	8,0961 0,8007	7,2064 0,9786	7,2064 1,0676
4,16	0,2669 0,0890	0,7117 -	0,8897 0,0890	1,4235 0,1779	1,5725 0,2669	2,3132 0,1779	2,3132 0,2669	3,9146 0,3559	4,0925 0,2669	4,9822 0,3559	5,8719 0,8007	6,0498 0,4448	5,8719 0,6228	6,0498 0,5338	5,9609 0,4448
5,46	0,2669 -	0,6228 0,0890	1,0676 0,3559	1,1566 0,0890	2,1352 0,2669	3,2918 0,3559	3,9146 0,3559	4,8043 0,5338	5,8719 0,2669	6,9395 0,6228	7,4733 0,8007	8,6299 0,8897	7,4733 1,0676	7,3843 0,4448	7,5623 0,8897
6,54	0,4448 0,0890	0,8897 0,0890	1,7794 0,1779	2,4911 0,1779	4,8932 0,3559	6,3167 0,2669	8,4520 0,2669	8,8078 0,2669	14,9466 0,4448	16,7260 0,6228	16,9039 1,6904	16,8149 1,0676	15,3915 1,5125	16,4591 1,1566	16,0142 0,9786
7,93	0,3559 -	0,8007 0,0890	2,2242 0,1779	3,1139 0,2669	4,5374 0,4448	6,8505 0,7117	7,1174 0,8897	12,0996 0,8007	14,2349 1,6014	15,2135 0,6228	16,1032 1,6014	15,9253 2,3132	15,3915 1,4235	15,3915 2,1352	16,3701 2,2242
9,37	0,4448 0,0890	0,8897 0,0890	1,5125 0,1779	4,4484 0,6228	7,9181 1,3345	10,5872 1,6014	12,2776 1,1566	14,0569 0,7117	17,6157 1,6904	17,7046 1,3345	17,8826 0,0890	18,7722 1,9573	16,9929 2,2242	17,2598 1,6014	18,3274 1,6904
10,73	0,2669 -	1,0676 0,890	1,6014 0,1779	3,8256 0,1779	4,2705 0,0890	4,8043 0,3559	6,5836 1,1566	11,2989 0,7117	15,3025 2,0463	16,5480 1,8683	17,2598 1,5125	19,9288 2,4021	20,5516 1,9573	17,9715 1,1566	20,5516 2,1352
13,62	0,3559 0,0890	0,8897 0,1779	1,8683 0,0890	4,8043 1,0676	6,1388 0,9786	9,6085 1,2456	11,9217 1,4235	13,7900 1,6014	16,3701 0,4448	16,6370 1,6014	21,5302 1,6904	27,4911 2,8470	27,4021 2,9359	24,7331 3,2918	25,8007 3,1139
11,77	0,4448 0,1779	0,5338 0,0890	1,5125 0,3559	4,2705 0,4448	5,3381 0,7117	7,7402 0,4448	9,6085 1,2456	11,2100 0,8007	11,3879 1,5125	13,3452 0,9786	21,0854 2,0463	11,6868 3,8256	21,2633 2,9359	20,9075 2,8470	22,0641 2,7580
17,30	0,2669	0,6228	1,4235	2,0463	2,5801	3,1139	3,8256	4,3594	4,8043	5,4270	5,1601	4,1815	3,6477	3,6477	3,8256
17,72	0,1779	0,6228	1,5125	1,6014	3,2918	3,5587	4,3594	5,3381	5,5160	5,9609	4,8043	6,3167	3,8256	3,9146	3,8256
17,14	0,1779	0,7117	1,5125	1,8683	2,6690	3,2918	3,4678	4,3594	4,6263	5,7829	8,3630	6,3167	5,2491	4,8043	4,2705
19,75	0,0890	0,5338	1,6014	1,8683	2,3132	2,4021	3,5587	4,3594	5,6050	6,0498	8,1851	6,4057	4,6263	6,4947	4,7156

RAW DATA

Cd. conc. of water in µmol dm^{-3} ($\bar{x}$)

Experiment 15 days

For SD → N = 4 17,30} 19,75} No replica

Slit 0.5

Wavelength 228,7

Background corrector

15/5 to 28/5

SD = Lower no.

D = Time in days

C = Cd conc. in µmole dm^{-3}

D C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
1,28	1,5125 0,0890	1,4235 0,0890	1,4236 -	1,5125 0,0890	1,2456 -	1,3345 0,0890	1,5125 -	1,4235 -	1,1566 -	1,1566 -	1,0676 -	1,1566 -	1,0676 -	1,0676 -	1,0676 -
2,67	3,0249 -	3,0249 0,0890	2,8470 0,0890	3,1139 0,0890	2,9359 0,0890	2,9359 -	3,0249 -	2,7580 -	2,4911 -	2,4911 -	2,2242 -	2,3132 -	2,2242 -	2,3132 -	2,3132 -
4,16	4,7153 0,0890	4,8043 0,0890	4,6263 0,0890	4,8932 -	4,6263 -	4,5374 0,0890	4,5374 -	4,1815 -	3,9146 -	3,8256 -	3,8256 -	4,0036 0,3559	3,4698 -	3,2998 -	3,1139 0,3559
5,46	5,9609 0,1779	5,8719 0,0890	6,1388 0,0890	6,0498 0,0890	6,1388 0,0890	6,1388 0,0890	6,0498 -	5,6050 0,0890	5,3381 0,0890	5,1601 0,0890	4,8932 -	4,8932 -	4,7153 0,0890	4,7153 -	4,1815 -
6,54	7,7402 -	7,3847 -	7,2954 0,0890	7,9181 0,1779	7,4733 -	7,3843 0,1779	6,9395 -	6,4057 0,0890	6,1388 0,0890	6,0498 0,0890	5,7829 0,0890	5,7829 0,0890	5,4270 -	5,4270 0,0890	4,9822 -
7,93	9,4306 0,1779	7,6512 0,1779	8,4520 0,4448	9,3416 -	9,0747 0,1779	8,8968 0,2669	8,7189 0,0890	7,9181 0,0890	7,8292 0,0890	7,5623 -	7,1174 -	7,2064 -	6,8505 0,0890	6,5836 0,0890	6,3167 -
9,37	10,8541 0,0890	10,5872 0,1779	10,5872 -	10,7651 0,0890	10,0534 0,0890	10,4982 0,0890	10,2313 0,0890	8,8078 0,2669	9,1637 0,0890	8,8968 0,7117	8,2740 0,0890	8,4520 -	8,0961 0,0890	7,7402 0,0890	7,4733 0,0890
10,73	11,9217 0,1779	12,6335 0,1779	11,7438 -	12,0107 0,1779	12,2778 0,6228	12,0996 0,3559	11,4769 -	10,4982 0,0890	10,5872 0,1779	10,3203 0,0890	8,9858 0,0890	8,4520 -	9,1637 0,0890	9,9858 0,0890	8,7189 0,0890
13,62	12,9004 0,5338	13,4342 0,5336	12,2776 0,3559	13,3452 0,5338	13,3452 0,4448	12,8114 0,3559	12,5445 -	11,5658 0,1779	11,6548 0,1779	11,6548 0,0890	9,9644 0,1779	11,2989 0,3559	10,1423 0,0890	9,9644 0,0890	9,5196 0,0890
11,77	16,1922 0,1779	16,6370 1,3345	16,1032 0,8897	16,1032 0,0890	14,8577 0,0890	14,7687 0,3559	13,7011 0,0890	12,8020 0,0890	12,9893 0,1779	12,5445 0,0890	11,5658 0,0890	12,0107 0,0890	11,8327 0,0890	11,3879 0,0890	10,7651 0,0890
17,30	17,7046	17,2598	17,6157	17,6157	17,3488	17,2598	17,3488	17,0819	16,9929	17,6157	16,8149	16,6370	17,6157	17,4377	17,0819
17,72	19,0391	18,3274	17,0819	18,2384	17,9715	18,5053	17,5267	17,3488	17,8826	17,7936	17,5267	17,6157	17,0819	16,9929	16,8149
17,14	19,3060	18,8612	17,4377	16,9039	17,3488	17,6157	17,7936	17,2598	16,3701	16,7260	16,2811	16,1922	16,1922	16,1922	16,3701
19,75	22,7758	20,3737	21,1744	19,8399	19,5730	19,1281	19,2171	18,4164	19,4840	19,3060	18,9502	18,8612	19,8399	19,8399	19,3950

RESULT

Algal growth $\log_2 \left(\frac{\frac{5}{4} OD_t}{OD_o}\right)$

D = Time in days

C = Cd conc. in µmole dm^{-3}

Date 14/5 to 28/5

15 day experiment

D C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0		2,3534	2,1972	1,9665	1,8851	2,4292	2,4421	2,4942	3,0649	3,7862	4,1419	4,3207	3,8875	3,4246	3,2142
1,28		2,1624	2,1624	2,1317	1,9289	2,3784	2,6285	2,8773	3,6333	3,6506	4,1120	5,2420	4,1711	4,6983	4,4586
2,67		2,1444	2,1444	1,8795	1,8100	2,3340	2,3896	2,4581	2,8579	3,4993	3,7378	3,6166	3,2915	3,1254	2,9816
4,16		1,9542	2,3546	2,2165	1,8960	2,3193	2,3665	2,5522	2,7249	3,4182	3,8381	4,0568	3,3332	3,1855	3,0138
5,46		1,9596	1,9898	1,9697	1,8718	2,6336	2,7154	3,0687	3,2457	4,3399	4,5436	5,2139	4,1665	3,9797	4,2740
6,54		2,1711	2,2155	2,1932	1,9718	2,6860	3,7336	6,3239	6,4862	6,6526	6,9630	8,6142	6,8233	5,6856	5,1377
7,93		2,2471	1,9129	1,8660	1,8682	2,1738	2,5535	2,8472	2,9572	3,8066	4,1192	4,0997	3,5401	3,3445	3,2663
9,37		2,0418	2,0927	1,8412	1,8412	2,6767	2,9247	3,0875	3,5965	4,4663	4,5328	4,7149	3,4406	3,3570	3,4069
10,73		2,3215	1,9503	1,9222	1,7703	1,9884	2,0669	2,2334	2,4249	2,9860	2,9860	3,1646	3,0591	2,7635	2,9572
13,62		2,2168	1,8187	1,7792	1,7405	2,2071	2,3063	2,3889	2,3994	2,9765	3,0967	3,1935	3,0831	2,7139	3,0967
11,77		2,3170	2,1583	1,7641	1,7186	1,7974	1,9368	2,0180	2,4505	2,4782	2,5821	3,0206	2,9207	2,8454	2,8882
17,30		2,1374	2,2952	1,8833	1,6528	1,7469	2,1802	2,1888	2,2237	2,7316	2,6050	2,8645	2,5139	2,5139	2,2952
17,72		3,0881	2,3878	2,8195	1,8833	1,5453	1,9133	2,0464	2,1290	2,5845	2,6361	3,3029	2,4941	2,3564	2,5139
17,14		2,0000	1,8784	1,5937	1,4355	1,4528	1,5193	1,6129	1,7747	1,9066	1,9703	1,9881	1,9411	1,8285	1,8122
19,75		1,8198	1,6430	1,4833	1,3622	1,4476	1,5996	1,6270	1,7082	1,7333	1,8198	1,9816	1,6753	1,6631	1,7978

RESULT

Algae No X$10^{6}_{\text{vs wt}}$ mg/g

15 day experiment

D = Time in days

C = Cd conc. in μmole dm^{-3}

C \ D	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0,140	0,129	0,242	0,288	0,511	0,228	0,243	0,205	0,184	0,170	0,165	0,157	0,150	0,173	0,114
1,28	0,108	0,122	0,223	0,239	0,432	0,232	0,208	0,176	0,179	0,153	0,133	0,116	0,108	0,110	0,095
2,67	0,101	0,200	0,211	0,239	0,435	0,285	0,222	0,230	0,205	0,142	0,140	0,135	0,187	0,188	0,149
4,16	0,098	0,318	0,188	0,217	0,582	0,360	0,243	0,246	0,231	0,166	0,152	0,129	0,155	0,179	0,148
5,46	0,172	0,403	0,336	0,408	0,634	0,355	0,229	0,190	0,147	0,119	0,108	0,105	0,092	0,123	0,121
6,54	0,136	0,419	0,332	0,524	0,370	0,238	0,129	0,130	0,106	0,084	0,081	0,081	0,086	0,103	0,100
7,93	0,231	0,231	0,254	0,421	0,689	0,339	0,155	0,159	0,155	0,125	0,093	0,113	0,109	0,120	0,116
9,37	0,178	0,387	0,348	0,437	0,542	0,219	0,116	0,116	0,113	0,112	0,095	0,088	0,096	0,110	0,126
10,73	0,187	0,196	0,196	0,187	0,250	0,215	0,194	0,145	0,152	0,155	0,153	0,151	0,156	0,125	0,139
13,62	0,224	0,223	0,218	0,216	0,197	0,230	0,203	0,184	0,172	0,158	0,153	0,150	0,191	0,206	0,180
11,77	0,160	0,177	0,189	0,274	0,203	0,263	0,251	0,188	0,170	0,141	0,142	0,139	0,133	0,138	0,120
17,30	0,157	0,198	0,199	0,206	0,392	0,278	0,289	0,293	0,291	0,253	0,268	0,252	0,259	0,263	0,256
17,72	0,207	0,271	0,192	0,209	0,277	0,168	0,232	0,238	0,206	0,215	0,234	0,231	0,252	0,255	0,262
17,14	0,254	0,241	0,264	0,385	0,297	0,372	0,217	0,148	0,242	0,242	0,245	0,276	0,287	0,292	0,272
19,75	0,259	0,221	0,219	0,207	0,320	0,295	0,279	0,285	0,273	0,269	0,263	0,259	0,258	0,279	0,296

RESULT

$$k' = \frac{\log_{10} N - \log_{10} N_o}{t}$$

k' is relative growth constant

15 day experiment

Log day starts on day 6

D = time in days

C = Cd conc. in µmole dm^{-3}

D / C	1	2	3	4	5	6	7	8	9	10	11	12	$\bar{X}$		
0							0,041	0,059	0,064	0,068	0,062	0,056	0,058		
1,28							0,100	0,103	0,075	0,086	0,081	0,079	0,087		
2,67							0,127	0,073	0,066	0,099	0,098	0,080	0,090		
4,16							0,178	0,094	0,074	0,096	0,088	0,086	0,103		
5,46							0,204	0,147	0,140	0,129	0,113	0,099	0,139		
6,54							0,315	0,171	0,147	0,136	0,114	0,096	0,163		
7,93							0,338	0,196	0,148	0,138	0,139	0,100	0,177		
9,37							0,315	0,161	0,111	0,087	0,086	0,077	0,140		
10,73							0,083	0,117	0,078	0,068	0,059	0,060	0,078		
13,62							0,065	0,054	0,063	0,057	0,054	0,048	0,057		
11,77							0,055	0,105	0,087	0,088	0,071	0,061	0,078		
17,30							0,017	0,010	0,010	0,026	0,018	0,021	0,017		
17,72															
17,14															
19,75															

RESULT

$\frac{\text{Cd con in } \mu g}{\text{Algae wt in mg}} = mg\ g^{-1}$

D = time in days

C = Cd conc. in µmole dm^{-3}

15 day experiment

C \ D	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1,28	-	0,36	0,62	0,76	1,03	1,69	1,97	3,64	4,07	3,53	3,92	4,03	4,14	5,86	6,46
2,67	0,21	0,54	0,87	1,81	2,30	2,56	2,55	3,28	4,37	4,90	4,49	6,45	6,18	6,22	8,60
4,16	0,32	0,94	1,20	2,15	2,35	2,26	2,22	3,62	3,73	4,35	4,97	5,00	5,31	5,58	7,13
5,46	0,33 0,45	0,83	1,62	1,86	4,42	3,38	3,89	4,68	5,56	6,49	6,86	7,70	8,19	8,02	6,99
6,54	0,59 1,20	1,24	2,56	3,66	13,68	6,93	8,27	8,04	13,31	14,89	14,64	14,43	13,29	14,25	14,75
7,93	0,47	1,28	3,35	5,06	7,89	8,08	8,44	12,33	13,20	13,73	13,88	14,25	15,41	15,53	17,16
9,37	0,58	1,19	2,35	7,50	17,55	10,65	11,29	12,75	15,92	15,67	15,51	16,27	14,97	17,91	17,14
10,73	0,36	1,46	2,26	6,30	8,42	6,07	7,61	12,33	15,51	15,44	15,54	15,71	19,03	21,74	21,79
13,62	0,42	1,21	2,41	7,47	10,49	10,27	12,41	14,30	15,11	15,33	18,52	23,11	24,23	23,24	22,73
11,77	0,48	0,62	1,80	5,19	9,43	8,15	9,33	10,22	10,16	11,68	18,13	19,32	19,77	22,97	23,75
17,30	0,33	0,92	2,00	3,04	4,99	3,42	3,89	4,35	4,72	5,14	4,77	3,77	3,37	3,37	4,21
17,72	0,16	0,59	1,62 2	2,28	5,58	6,20	5,27	6,19	6,22	6,36	4,74	6,09	3,96	4,13	3,78
17,14	0,16	0,72	1,58	2,61	6,98	4,33	4,41	5,26	4,72	5,86	8,05	5,74	5,34	4,97	4,53
19,75	0,07	0,73	2,37	3,00	3,98	2,64	3,81	4,56	5,83	6,16	8,24	6,18	4,93	6,93	4,58

RESULT

$G = \frac{0,301}{k'}$

15 day experiment

G equal the mean generation time if the cell divide into two
= mean doubling time.

Log phase starts on day 6

D = time in days

C = Cd conc. in µmole dm^{-3}

Calc. from k'

D / C	1	2	3	4	5	6	7	8	9	10	11	12	$\bar{X}$	$\bar{X}$	
0							7,26	5,12	4,67	4,46	4,84	5,38	5,29	5,19	
1,28							3,007	2,919	4,015	3,504	3,714	3,794	3,492	3,46	
2,67							2,373	4,112	4,592	3,052	3,064	3,763	3,493	3,34	
4,16							1,695	3,204	4,051	3,124	3,431	3,484	3,165	2,92	
5,46							1,473	2,045	2,157	2,334	2,670	3,052	2,289	2,17	
6,54							0,954	1,764	2,050	2,214	2,641	3,139	2,127	1,85	
7,93							0,892	1,532	2,030	2,186	2,160	3,018	1,970	1,70	
9,37							0,956	1,871	2,701	3,455	3,512	3,905	2,733	2,15	
10,73							3,646	2,567	3,850	4,397	5,098	5,035	4,099	3,86	
13,62							4,649	5,531	4,760	5,279	5,529	6,268	5,336	5,28	
11,77							5,455	2,876	3,459	3,432	4,240	4,899	4,060	3,86	
17,30							17,348	30,694	30,917	11,391	16,330	14,007	20,114		
17,72															
17,14															
19,75															

RAW DATA

Algae OD$_{Abs}$ ($\bar{x}$)
30 day experiment
For SD → n = 4

Slit medium
wavelength 660 nm
Path 1 cm
4 ml of Algae taken

Date 2/8 to 31/8
D = time in days
C = Cd conc. in μmole dm^{-3}
SD = lower no.

D / C	1	2	3	4	5	6	7	9	10	11	12	13	14	15
0	,306 ,052	,241 ,033	,312 ,039	,241 ,063	,185 ,066	,186 ,026	,227 ,049	,322 ,027	,335 ,053	,372 ,024	,388 ,070	,406 ,048	,395 ,060	,400 ,043
2,09	,348 ,018	,302 ,034	,367 ,034	,263 ,066	,216 ,019	,232 ,033	,241 ,032	,318 ,019	,367 ,033	,370 ,023	,405 ,047	,367 ,032	,390 ,053	,368 ,024
4,67	,372 ,057	,318 ,044	,412 ,055	,276 ,051	,251 ,048	,286 ,077	,305 ,091	,376 ,042	,403 ,027	,399 ,040	,418 ,035	,405 ,034	,412 ,029	,404 ,034
9,78	,327 ,063	,297 ,017	,296 ,068	,213 ,028	,277 ,039	,337 ,004	,371 ,036	,386 ,040	,402 ,015	,389 ,037	,407 ,019	,391 ,015	,371 ,027	,399 ,037
D / C	16	17	18	19	20	21	22	24	25	26	27	28	29	30
0	,405 ,040	,381 ,033	,405 ,045	,321 ,045	,372 ,045	,337 ,056	,358 ,046	,354 ,057	,333 ,029	,360 ,031	,350 ,025	,356 ,047	,337 ,021	,355 ,035
2,09	,389 ,026	,331 ,034	,357 ,060	,332 ,022	,336 ,056	,277 ,025	,352 ,010	,310 ,076	,323 ,027	,286 ,044	,321 ,017	,307 ,047	,320 ,014	,317 ,038
4,67	,411 ,026	,358 ,042	,367 ,040	,357 ,040	,362 ,036	,395 ,027	,330 ,048	,341 ,056	,350 ,024	,297 ,059	,376 ,039	,360 ,049	,356 ,058	,355 ,050
9,78	,396 ,020	,380 ,029	,367 ,024	,373 ,012	,378 ,028	,393 ,014	,349 ,014	,363 ,022	,379 ,029	,367 ,035	,386 ,050	,377 ,018	,397 ,034	,392 ,018
D / C	1	2	3	4	5	6	7	9	10	11	12	13	14	15
6,63Cd 2ml Gro	,176 ,011	,158 ,046	,177 ,032	,144 ,005	,139 ,019	,228 ,010	,283 ,027	,329 ,018	,352 ,021	,354 ,031	,339 ,015	,326 ,026	,331 ,011	,332 ,020
D / C	16	17	18	19	20	21	22	24	25	26	27	28	29	30
6,65Cd 2ml Gro	,338 ,014	,330 ,008	,328 ,028	,332 ,002	,324 ,012	,330 ,024	,307 ,006	,323 ,015	,319 ,012	,322 ,028	,340 ,030	,325 ,022	,340 ,030	,339 ,026

RAW DATA

Algae No — Channels 8–11 — D = time in days

30 day experiment — n = 4 — C = Cd conc in µmole dm^{-3}

D / C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	1195200	835800	1227400	835800	500600	506900	745900	1015400	1280900	1350100	1545700	1630000	1724800	1666800	1693200
2,09	1419000	1173600	1519300	959900	690200	784000	835 800	1015400	1259600	1519300	1535200	1719600	1519300	1640500	1524600
4,67	1545700	1259600	1756500	1032000	829600	10868	1189800	1419000	1566800	1709000	1687900	1788200	1719600	1756500	1714300
9,78	1307600	1146600	1141200	672300	1037500	1360800	1540400	1677400	1619400	1703700	1635200	1730100	1645800	1540400	1687900
D / C	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
0	1719600	1593100	1719600	1275600	1545700	1360800	1471900	1070400	1450700	1339500	1482400	1429600	1461300	1360800	1456000
2,09	1635200	1328400	1466600	1334200	1355400	1037500	1440200	1360800	1216700	1286300	1086800	1275600	1200500	1270200	1254200
4,67	1751200	1471900	1519300	1466600	1493000	1666800	1323600	1519300	1382000	1429600	1146600	1566800	1482400	1461300	1456000
9,78	1672100	1587800	1519300	1551000	1577300	1656300	1424300	1640500	1498200	1582600	1519300	1619400	1572000	1677400	1651000
D / C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
6,63 2ml Gr	443200	324300	449700	191700	760700	1070400	1307600	1307600	1318200	1440200	1450700	1371400	1302300	1328900	1334200
D / C	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6,63 2ml Gro	1366100	1323600	1312900	1334200	1291600	1323600	1200500	1275600	1286300	1264900	1280900	1376700	1296900	1376700	1371400

RAW DATA

Algae weight ($\bar{x}$) in mg
For SD → n = 4
Experiment 30 days

SD = Lower no.
D = time in days
C = Cd conc. in µmole dm^{-3}

D C	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2,09	,155 ,004	,142 ,003	,153 ,015	,148 ,014	,159 ,003	,159 ,010	,159 ,011	,166 ,012	,159 ,005	,163 ,004	,142 ,006	,168 ,007	,148 ,003	,167 ,003	,151 ,004
4,67	,164 ,005	,155 ,010	,158 ,006	,161 ,005	,165 ,006	,165 ,007	,155 ,008	,165 ,008	,154 ,010	,167 ,005	,147 ,009	,166 ,011	,160 ,012	,159 ,007	,155 ,006
9,78	,173 ,015	,169 ,012	,163 ,007	,164 ,009	,166 ,013	,168 ,013	,160 ,010	,168 ,004	,153 ,005	,158 ,010	,161 ,007	,163 ,004	,157 ,004	,166 ,005	,161 ,008

RAW DATA

Cd conc. in Algae in µmol dm^{-3} ($\bar{x}$)

For SD → n = 4

Experiment 30 days

SD = Lower no.

D = time in days

C = Cd conc. in µmole dm^{-3}

C \ D	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2,09	14,1459 1,9573	13,1673 9,7865	15,3025 1,1566	13,3452 0,6228	14,0569 0,9786	14,9466 0,4448	14,6797 0,6228	15,2135 0,5338	14,5907 0,5338	11,5658 0,7117	10,4093 0,2669	10,8541 0,4448	12,0996 1,7794	9,6975 0,5338	9,6085 0,3559
4,67	25,8897 1,6014	25,9786 1,1566	26,6014 1,8683	25,4480 0,8007	25,6228 0,9786	23,6655 0,2669	23,5765 0,7117	22,5979 0,8897	22,0641 0,8007	21,6192 0,0623	19,8399 1,6014	21,8861 0,7117	20,1959 1,6014	24,1103 2,0463	26,5125 1,6014
9,78	37,1886 1,3345	37,3665 1,4235	39,6797 1,3345	37,4555 1,5125	37,7224 1,1566	37,5445 0,8897	36,5658 1,6014	36,2100 0,8007	34,4306 1,1566	34,6975 0,0623	39,6797 1,5125	37,3665 1,6014	35,4982 1,5125	37,3665 0,8897	37,5445 1,6014

RAW DATA

C = Cd conc of water in μmol dm^{-3} D = time in days

Experiment 30 days

D C	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2,09	2,51	2,31	2,01	2,21	2,47	2,17	2,00	1,90	1,98	2,01	1,91	1,95	2,10	1,97	1,89
4,67	5,42	5,31	5,71	5,01	4,98	4,61	4,57	4,81	4,04	4,84	4,21	4,37	4,00	3,95	4,27
9,78	10,78	10,58	10,31	9,58	9,96	9,26	9,01	9,73	9,52	10,12	9,91	9,38	9,57	9,31	9.71

RAW DATA

Algae OD_{Abs} $(\bar{x})$

For SD → n = 4

15 day experiment

SD = Lower no.

Slit medium
Wavelength 60 nm
Path 1cm
4 ml of Algae taken

Date 11/8 to 25/8

Zn + Cd

SD = Lower no.

D = time in days

C = Cd conc. in µmole dm^{-3}

C \ D	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
9,65 Zn	,201 ,016	,195 ,022	,180 ,016	,168 ,010	,157 ,017	,173 ,015	,261 ,017	,321 ,013	,365 ,019	,373 ,029	,379 ,018	,421 ,021	,398 ,018	,401 ,023	,368 ,016
9,65 Zn 6,68 Cd	,198 ,019	,181 ,017	,179 ,016	,165 ,013	,156 ,015	,161 ,017	,170 ,023	,177 ,019	,221 ,024	,243 ,038	,269 ,031	,299 ,016	,278 ,023	,244 ,026	,227 ,034
	ALGAE GROWTH $\mathrm{LOG}_2 \frac{5}{(4} \frac{\mathrm{OD}_t}{\mathrm{OD}_o}$)														
9,65 Zn		2,318	2,173	2,063	1,968	2,108	3,080	3,990	4,823	4,992	5,123	6,140	5,560	5,633	4,886
9,65 Zn 6,68 Cd		2,208	2,189	2,059	1,979	2,023	2,104	2,170	2,630	2,896	3,245	3,700	3,375	2,909	2,700

RESULT

Algae Growth $\log_2 \left(\frac{5}{4}\frac{OD_t}{OD_o}\right)$

D = time in days

C = Cd conc. in µmole dm^{-3}

Date 2/8 to 31/8

30 day experiment

D C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0		1,9786	2,4192	1,9786	1,6885	1,6933	1,9017	2,1662	2,4886	2,5820	2,8671	3,0000	2,3784	3,0601	3,1037
2,09		2,1210	2,4936	1,9248	1,7122	1,7818	1,8222	1,9733	2,2072	2,4936	2,5123	2,7411	2,4936	2,6406	2,4998
4,67		2,0973	2,6106	1,9019	1,7943	1,9467	2,0348	2,2491	2,4007	2,5565	2,5328	2,6474	2,5684	2,6106	2,5625
9,78		2,1967	2,1909	1,7583	2,0833	2,4423	2,6725	2,8631	2,7809	2,9013	2,8031	2,9400	2,8180	2,6725	2,8783
D C	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
0	3,1479	2,9411	3,1479	2,4816	2,8671	2,5966	2,7557	2,2285	2,7247	2,5674	2,7713	2,6940	2,7401	2,5966	2,7324
2,09	2,6340	2,2798	2,4320	2,2855	2,3084	1,9930	2,4022	2,3142	2,1637	2,2349	2,0382	2,2238	2,1476	2,2183	2,2017
4,67	2,6046	2,3021	2,3509	2,2968	2,3237	2,5093	2,1568	2,3509	2,2127	2,2596	1,9972	2,4007	2,3129	2,2914	2,2861
9,78	2,8555	2,7370	2,6443	2,6867	2,7225	2,9329	2,5212	2,9105	2,6166	2,7299	2,6443	2,7809	2,7153	2,8631	2,8254
D C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
6,63 Cd 2ml Gro		2,1767	2,3902	2,0318	1,9824	3,0723	4,0277	5,0018	5,0513	5,6569	5,7128	5,3062	4,9772	5,1013	5,1264
D C	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6,63 Cd 2ml Gro	5,2801	5,0762	5,0265	5,1264	4,9285	5,0762	4,5328	4,8562	4,9043	4,8086	4,8802	5,3324	4,9528	5,3324	5,3062

RESULT

Algae No x 10^6 vs wt

30 day experiment

D = time in days

C + Cd conc. in µmole dm^{-3}

D C	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2,09	0,0949	0,1075	0,1047	0,1112	0,1177	0,1533	0,1110	0,1222	0,1314	0,1267	0,1314	0,1323	0,1234	0,1318	0,1210
4,67	0,0938	0,1058	0,1045	0,1100	0,1106	0,0994	0,1175	0,1091	0,1120	0,1168	0,1282	0,1064	0,1081	0,1093	0,1066
9,78	0,1035	0,1069	0,1077	0,1059	0,1056	0,1019	0,1126	0,1027	0,1024	0,1003	0,1060	0,1007	0,1001	0,0991	0,0981

RESULT

$\frac{Cd}{wt}$

Cd in Algae mg g^{-1}

Experiment 30 days

D = time in days

C = Cd conc. in µmole dm^{-3}

D / C	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2,09	10,24	10,36	11,20	10,11	9,91	10,56	10,32	10,28	10,26	7,98	8,19	7,23	9,18	6,51	7,11
4,67	17,71	18,74	18,83	17,73	17,38	16,05	17,04	15,33	16,02	14,55	15,17	14,17	14.16	16,97	19,20
9,78	24,16	24,75	27,26	25,62	25,47	25,01	25,62	24,15	25,23	24,56	27,68	25,77	25,37	25,26	26,07

RESULT

Cd conc of water mean

in µmole dm^{-3}

SD = Lower no.

C	15 days	30 days													
	- -														
	1,28 0,18														
	2,67 0,34	2,09 0,20													
	4,16 0,57														
	5,46 0,66														
	6,54 0,96	4,67 0,55													
	7,93 1,01														
	9,37 1,20														
	10,73 1,42														
	11,77 1,35	9,78 0,50													
	13,62 2,01														
	17,30 0,32														
	17,72 0,63														
	17,14 0,97														
	19,75 1,07														

RAW DATA

MUSSELS

6 days

Starved

Shell (mm)	Dry wt (mg)	Cadmium $\mu g\ g^{-1}$
22,2	73	1,16
20,6	63	1,16
21,0	45	0,77
25,0	107	0,74
24,7	85	0,59
22,8	71	0,72
24,1	105	0,66
20,9	65	0,77
22,6	79	0,63
24,2	107	0,61
24,5	95	0,63
14,6	20	0,31
19,9	55	0,52
18,2	49	1,11
22,0	73	0,51
22,0	75	0,66
19,6	37	0,48
		$\bar{x}$ = 0,71
		s = 0,24

RAW DATA

MUSSELS

15 days

Control

Shell Length (mm)	Dry weight (mg)
29,7	170
26,6	150
29,9	240
25,1	100
31,6	210
27,9	160
31,2	250
24,5	90
32,9	270
25,5	130
24,6	90
29,6	200
22,2	80
24,9	110
25,6	120
19,2	60
29,2	170
31,4	200
29,9	180
28,7	190
26,9	120

Shell Length (mm)	Dry weight (mg)
28,5	170
18,6	40
25,4	150
24,5	140
27,0	210
28,9	210
27,4	170
28,8	250
25,1	90
25,8	150
23,3	70
26,5	100
22,6	80
23,0	40
22,0	60
25,9	100
19,2	60
22,9	90
18,7	50

RAW DATA

MUSSELS

30 days

Clean water and clean food

Shell length (mm)	Dry weight (mg)
20,9	80
28,6	180
24,5	90
34,6	280
32,7	200
26,7	120
28,6	180
26,7	170
25,0	130
24,5	100
30,1	170
21,4	70
26,0	130
22,6	90
16,4	40
27,9	150
16,6	10
29,9	190
24,9	110
20,5	60

Shell length (mm)	Dry weight (mg)
25,9	130
26,9	120
28,1	190
27,9	190
25,8	90
23,3	100
25,0	140
28,3	150
31,4	200
23,1	80
35,6	290
30,4	220
35,3	270
28,2	100
31,5	210
27,5	180
29,8	210
30,2	220

RAW DATA

MUSSELS

15 days

Cd sea water Cd food (100 cm^3/d)

Shell length (mm)	Dry weight (mg)
25,1	110
27,8	170
29,2	210
24,0	130
20,5	60
21,0	80
25,9	130
17,8	30
29,4	190
24,4	100
33,1	260
27,2	190
25,2	130
20,9	60
18,8	50
28,5	180
31,0	240
26,4	160
19,6	60
20,2	70

Shell length (mm)	Dry weight (mg)
21,0	60
20,0	50
26,9	180
21,8	50
20,2	70
25,3	140
19,9	60
15,4	20
25,0	140
26,8	190
15,0	10
20,3	60
29,0	180
30,8	220
29,1	200
23,2	110
28,6	200
29,7	230
22,0	70
27,7	140
27,1	170
26,3	160
21,8	60
22,0	80
21,4	50
22,5	80

Shell length (mm)	Dry weight (mg)
20,2	40
23,7	70
19,9	40
25,4	120
26,1	140
26,9	140
28,0	170
22,7	90
30,9	240
29,0	180
26,2	140
30,6	210
30,9	250
26,6	160
23,1	100
29,6	200
26,3	170
26,3	140
20,4	70
28,7	160
29,4	180
24,7	120
22,3	60
17,0	40

RAW DATA

MUSSELS

30 days

Cd seawater Shell length (mm)	Cd food (100 cm^3/d) Dry weight (mg)
30,7	270
24,5	70
27,0	130
30,0	200
32,4	270
26,3	130
23,7	90
30,9	210
26,9	150
30,3	210
21,0	50
26,6	110
23,0	80
27,4	220
21,3	70
26,7	120
33,2	360
26,8	170
29,4	180
20,5	40
20,6	40

Shell length (mm)	Dry weight (mg)
20,8	70
21,3	70
16,0	20
20,1	40
23,9	110
22,3	80
18,9	40
24,3	80
33,4	250
26,8	120
25,1	90
23,6	140
28,5	150
26,5	80
29,4	210
31,3	240
26,1	130
34,5	270
28,6	170
17,3	30
27,7	130
26,9	120
28,3	140
28,6	150
20,2	110

Shell length (mm)	Dry weight (mg)
27,6	160
27,1	170
23,2	70
24,3	110
20,8	70
25,9	140
25,0	120
18,9	30
20,6	50
24,6	70
25,0	120
18,8	40
25,6	130
30,0	220
29,5	190
30,1	210
29,7	200
29,9	200
26,0	90
37,8	430
26,7	110
28,6	190
25,4	110

Shell length (mm)	Dry weight (mg)
28,5	180
30,2	210
24,4	100
18,4	40
27,0	170
18,2	30
18,2	40
22,5	80
29,1	180
28,0	180
25,3	110
20,6	60
20,0	50
15,6	20
28,2	190
31,7	240
31,3	200
27,5	190
22,7	90
30,3	200
22,9	80
22,0	70
22,5	70
28,8	180
27,3	160
22,3	80

Shell length (mm)	Dry weight (mg)
16,3	20
18,1	40
22,3	60
30,6	200
22,9	70
22,3	60
24,2	90
15,5	20
18,9	30
20,0	50
24,6	110
27,0	170
22,0	70
24,8	70
26,3	90
18,9	40
22,0	70
31,0	210
21,9	60
26,7	80
28,0	160
21,6	60
22,8	70
31,1	220
29,3	140
23,0	40

Shell length (mm)	Dry weight (mg)
27,1	130
23,8	70
30,8	230
27,2	110
27,9	160
25,8	80
23,1	60

Control

Fraction = 4,02 cm^3

F = Fraction No

On column 10 cm^3 at 22 cm^3/n

Column

Sephadex G-75

F	Abs 250	Abs 280	Conc Cd
1	-	-	
	-	-	
	-	-	
	-	-	
5	-	-	
	-	-	
	-	-	
	-	-	
	-	-	
10	-	-	
	-	-	
	-	-	
	-	-	
	-	-	
15	-	-	
	-	-	
	-	-	
	-	-	
	-	-	
20	-	-	
	,012	,010	
	,024	,023	
	,041	,046	
	,066	,077	
25	,086	,104	
	,102	,124	
	,113	,140	
	,117	,149	
	,119	,154	
30	,117	,150	
	,108	,141	
	,100	,132	

F	Abs 250	Abs 280	Conc Cd
	,99	,130	
	,98	,130	
35	,100	,135	
	,103	,136	
	,105	,140	
	,107	,140	
	,107	,143	
40	,104	,143	
	,100	,132	
	,088	,119	
	,067	,094	
	,053	,074	
45	,050	,068	
	,038	,053	
	,037	,049	
	,033	,061	
	,034	,056	
50	,033	,043	
	,037	,051	
	,042	,042	
	,038	,042	
	,035	,039	
55	,036	,040	
	,039	,039	
	,033	,038	
	,034	,039	
	,036	,038	
60	,037	,038	
	,035	,037	
	,036	,036	
	,039	,038	
	,040	,039	

F	Abs 250	Abs 280	Conc Cd
65	,045	,044	
	,050	,048	
	,051	,051	
	,061	,075	
	,077	,080	
70	,096	,108	
	,104	,118	
	,109	,131	
	,113	,151	
	,124	,179	
75	,145	,216	
	,164	,253	
	,188	,300	
	,216	,360	
	,251	,431	
80	,304	,505	
	,364	,588	
	,422	,683	
	,486	,767	
	,555	,849	
85	,636	,934	
	,725	1,025	
	,794	1,086	
	,872	1,134	
	,950	1,181	
90	1,020	1,212	
	1,092	1,228	
	1,148	1,245	
	1,203	1,250	
	1,249	1,244	
95	1,283	1,243	
	1,307	1,234	

F	Abs 250	Abs 280	Conc Cd
	1,329	1,224	
	1,333	1,187	
	1,336	1,164	
100	1,340	1,121	
	1,321	1,047	
	1,294	,978	
	1,262	,923	
	1,222	,851	
105	1,168	,776	
	1,119	,716	
	1,058	,670	
	1,003	,631	
	,952	,577	
110	,898	,543	
	,846	,512	
	,786	,477	
	,746	,447	
	,697	,418	
115	,650	,383	
	,600	,352	
	,554	,323	
	,517	,295	
	,488	,268	
120	,450	,238	
	,412	,207	
	,375	,174	
	,301	,141	
	,251	,111	
125	,207	,081	
	,148	,051	
	,088	,035	
	,085	,028	
	,081	,026	

F	Abs 250	Abs 280	Conc Cd
130	,079	,025	
	,078	,028	
	,078	,027	
	,076	,025	
	,077	,026	
135	,073	,021	
	,075	,019	
	,072	,015	
	,071	,014	
	,070	,015	
140	,068	,013	

15 day Cd + Cd food
Fraction : 4.02 cm^3
F = Fraction no.
On column 12 cm^3 at 22 cm^3/n

Column
Sephadex G-75

F	Abs 250	Abs 280	Conc Cd µmole dm^{-3}
1	-	-	-
	-	-	-
	-	-	-
	-	-	-
5	-	-	-
	-	-	-
	-	-	-
	-	-	-
	-	-	-
10	-	-	-
	-	-	-
	-	-	-
	-	-	-
	-	-	-
15	-	-	-
	-	-	-
	-	-	-
	-	-	-
	-	-	-
20	-	-	-
	,003	-	0,12
	,007	,004	0,26
	,009	,009	0,30
	,014	,015	0,47
25	,085	,099	0,99
	,179	,205	1,72
	,236	,274	2,21
	,263	,320	2,45
	,265	,368	2,55
30	,269	,371	2,61
	,256	,298	2,75
	,229	,222	2,77

F	Abs 250	Abs 280	Conc Cd μmole dm^{-3}
	,196	,197	2,65
	,148	,167	2,55
35	,062	,145	2,12
	,058	,105	1,76
	,053	,067	1,64
	,052	,065	1,54
	,052	,063	1,46
40	,046	,066	1,46
	,052	,070	1,46
	,050	,068	1,48
	,050	,067	1,46
	,052	,070	1,48
45	,051	,065	1,52
	,053	,066	1,54
	,052	,064	1,52
	,052	,068	1,56
	,050	,069	1,60
50	,050	,065	1,70
	,060	,070	1,82
	,060	,071	1,86
	,062	,074	1,90
	,063	,077	1,94
55	,065	,079	2,04
	,066	,079	2,08
	,068	,080	2,14
	,072	,081	2,23
	,072	,081	2,29
60	,079	,097	2,37
	,093	,101	2,41
	,084	,095	2,47
	,083	,093	2,45
	,081	,091	2,41

F	Abs 250	Abs 280	Conc Cd µmole dm^{-3}
65	,080	,089	2,33
	,075	,084	2,27
	,075	,080	2,16
	,076	,081	1,94
	,082	,099	1,72
70	,083	,150	1,56
	,101	,170	1,38
	,134	,198	1,29
	,167	,215	0,87
	,217	,320	0,79
75	,281	,398	0,75
	,314	,470	0,71
	,337	,520	0,65
	,356	,570	0,59
	,370	,631	0,49
80	,488	,825	0,36
	,635	1,074	0,45
	,769	1,292	0,49
	,944	1,582	0,47
	1,128	1,861	0,49
85	1,333	2,170	0,57
	1,555	2,476	0,65
	1,746	2,710	0,75
	1,970	2,878	0,87
	2,130	2,961	0,91
90	2,282	2,880	1,15
	2,362	2,728	0,91
	2,416	2,530	1,03
	2,416	2,133	0,95
	2,375	1,968	0,91
95	2,309	1,657	0,85
	2,164	1,388	0,79

F	Abs 250	Abs 280	Conc Cd µmole dm^{-3}
	2,032	1,152	0,75
	1,858	,945	0,67
	1,680	,786	0,63
100	1,505	,658	0,57
	1,074	,656	0,53
	1,033	,528	0,47
	,832	,437	0,42
	,671	,401	0,32
105	,536	,383	0,30
	,415	,375	0,26
	,364	,343	0,14
	,305	,297	0,16
	,274	,263	0,10
110	,264	,228	0,02
	,214	,166	0,08
	,197	,159	0,06
	,189	,147	0,04
	,165	,130	0,06
115	,158	,124	0,12
	,146	,112	0,06
	,135	,102	0,04
	,121	,099	0,04
	,108	,094	0,04
120	,099	,081	0,06
	,073	,057	0,06
	,072	,051	0,02
	,074	,048	0,08
	,069	.050	0,04
125	,067	,051	0,06
	,065	,053	0,04
	,060	,048	0,02
	,061	,051	0,08
	,063	,052	0,06
130	,062	,049	0,02

30 day Cd + Cd Food
Fractions = 4,02 cm^3
On column 6 cm^3 conc. at $22cm^3/n$
F = Fraction no.

Column
Sephadex G-75

F	Abs 250	Abs 280	Conc Cd µmole dm^{-3}
1	-	-	-
	-	-	-
	-	-	-
	-	-	-
5	-	-	-
	-	-	-
	-	-	-
	-	-	-
	-	-	-
10	-	-	-
	-	-	-
	-	-	-
	-	-	-
	-	-	-
15	-	-	-
	-	-	-
	,012	-	-
	,025	-	-
	,037	-	-
20	,053	-	-
	,082	,014	-
	,128	,061	0,03
	,210	,139	0,17
	,286	,222	0,40
25	,342	,280	0,75
	,374	,320	1,16
	,407	,356	1,53
	,425	,374	1,79
	,440	,393	2,06
30	,462	,417	2,11
	,463	,422	2,22
	,455	,415	2,13

F	Abs 250	Abs 280	Conc Cd µmole dm^{-3}
	,436	,402	2,07
	,396	,376	2,04
35	,348	,332	2,00
	,224	,204	1,97
	,178	,143	1,53
	,158	,113	1,26
	,149	,094	1,19
40	,144	,080	1,11
	,131	,065	1,09
	,122	,057	1,12
	,116	,051	1,16
	,112	,046	1,14
45	,107	,041	1,22
	,106	,040	1,21
	,105	,039	1,19
	,103	,033	1,30
	,100	,030	1,35
50	,101	,028	1,39
	,099	,025	1,58
	,093	,021	1,60
	,092	,021	1,76
	,088	,016	1,88
55	,086	,014	1,93
	,084	,011	2,04
	,083	,007	2,18
	,081	,007	2,30
	,080	,003	2,32
60	,076	,001	2,55
	,081	,002	2,62
	,076	,002	2,64
	,077	,008	2,62
	,078	,014	2,48

F	Abs 250	Abs 280	Conc Cd µmole dm^{-3}
65	,084	,029	2,34
	,092	,049	2,07
	,102	,077	1,83
	,118	,113	1,62
	,139	,156	1,35
70	,165	,208	1,09
	,206	,281	0,84
	,227	,345	0,40
	,225	,413	0,22
	,301	,498	0,12
75	,355	,592	0,12
	,417	,696	-
	,493	,808	-
	,570	,917	-
	,647	1,018	-
80	,730	1,131	-
	,797	1,188	-
	,842	1,243	-
	,892	1,256	-
	,921	1,248	-
85	,955	1,247	-
	,977	1,210	-
	1,000	1,165	-
	1,017	1,130	-
	1,026	1,068	-
90	1,019	1,005	-
	1,002	,913	-
	,972	,840	-
	,937	,752	-
	,894	,668	-
95	,844	,581	-
	,789	,520	-

F	Abs 250	Abs 280	Conc Cd µmole dm^{-3}
	,730	,453	-
	,671	,404	-
	,607	,358	-
100	,548	,321	-
	,481	,284	-
	,442	,271	-
	,380	,236	-
	,338	,216	-
105	,293	,192	-
	,255	,170	-
	,224	,153	-
	,197	,133	-
	,175	,118	-
110	,156	,104	-
	,143	,094	-
	,125	,080	-
	,112	,022	-
	,102	,061	-
115	,090	,052	-
	,082	,047	-
	,076	,040	-
	,063	,028	-
	,062	,026	-
120	,060	,024	-
	,058	,022	-
	,055	,018	-
	,051	,017	-
	,049	,016	-
125	,048	,013	-
	,044	,010	-

F	Abs 250	Abs 280	Conc Cd μmole dm^{-3}
	,044	,010	-
	,044	,006	-
	,044	,004	-
130	,042	,004	-

15 day Cd + Cd Food and 15 day clean H_2O + clean Food

Fractions = 4,02 cm^3 Column

On column 6 cm^3 conc at 22 cm^3/n Sephadex G-75

F = Fraction no.

F	Abs 250	Abs 280	Conc Cd µmole dm^{-3}
1	-	-	-
	-	-	-
	-	-	-
	-	-	-
5	-	-	-
	-	-	-
	-	-	-
	-	-	-
	-	-	-
10	-	-	-
	-	-	-
	-	-	-
	-	-	-
	-	-	-
15	-	-	-
	-	-	-
	,004	-	-
	,007	,003	-
	,013	,010	-
20	,020	,025	-
	,025	,027	0,08
	,047	,054	0,13
	,087	,102	0,24
	,124	,161	0,43
25	,158	,206	0,58
	,184	,257	0,69
	,210	,277	0,84
	,230	,304	0,96
	,269	,363	1,10
30	,248	,334	1,08
	,230	,315	1,07
	,215	,294	1,05

F	Abs 250	Abs 280	Conc Cd µmole dm^{-3}
	,196	,272	1,00
	,120	,169	0,81
35	,089	,117	0,63
	,078	,096	0,57
	,068	,087	0,53
	,067	,083	0,48
	,063	,075	0,46
40	,062	,072	0,45
	,062	,072	0,48
	,060	,068	0,46
	,064	,075	0,48
	,065	,070	0,50
45	,065	,075	0,45
	,060	,070	0,48
	,060	,071	0,45
	,057	,065	0,46
	,060	,062	0,48
50	,062	,064	0,45
	,117	,125	0,46
	,126	,132	0,43
	,133	,141	0,41
	,124	,129	0,43
55	,051	,051	0,45
	,052	,047	0,43
	,053	,050	0,45
	,046	,041	0,41
	,044	,040	0,31
60	,050	,044	0,31
	,053	,047	0,31
	,057	,048	0,29
	,059	,051	0,31
	,065	,057	0,31

F	Abs 250	Abs 280	Conc Cd µmole dm^{-3}
65	,070	,064	0,27
	,075	,069	0,27
	,085	,082	0,29
	,098	,099	0,26
	,113	,123	0,26
70	,136	,154	0,29
	,155	,187	0,26
	,175	,220	0,26
	,206	,275	0,32
	,243	,329	0,32
75	,285	,395	0,39
	,349	,475	0,38
	,407	,554	0,39
	,474	,639	0,43
	,564	,756	0,45
80	,647	,854	0,48
	,731	,949	0,48
	,817	1,037	0,51
	,898	1,112	0,57
	,,995	1.183	0,60
85	1,105	1,243	0,58
	1,114	1,221	0,58
	1,183	1,202	0,57
	1,209	1,150	0,57
	1,225	1,098	0,53
90	1,242	1,022	0,60
	1,233	,934	0,48
	1,197	,837	0,46
	1,152	,752	0,41
	1,092	,667	0,43
95	1,031	,591	0,31
	,963	,552	0,29

F	Abs 250	Abs 280	Conc Cd μmole dm^{-3}
	,897	,493	0,27
	,820	,449	0,27
	,741	,410	0,24
100	,669	,378	0,22
	,610	,348	0,13
	,597	,320	0,08
	,523	,298	0,10
	,473	,274	0,05
105	,399	,228	0,07
	,370	,209	0,03
	,354	,194	0,02
	,320	,179	0,01
	,315	,175	0,03
110	,297	,169	0,02