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A study of zinc resistance and accumulation of zinc in scapania undulata (L) Dum

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A STUDY OF ZINC RESISTANCE

AND ACCUMULATION OF ZINC IN

<u>Scapania</u> <u>undulata</u> (L) Dum.

by

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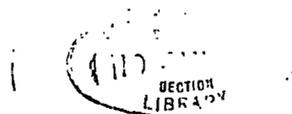
ABSTRACT

Scapania undulata (L.) Dum. is a bryophyte which is
common in the upland streams of the North East of England.
The zinc resistance of S. undulata from zinc enriched and
from low zinc sites was investigated by means of laboratory
toxicity tests and field transplants. The results obtained
do not suggest that the populations of S. undulata at the
zinc enriched sites studied are genetically adapted, zinc
resistant ecotypes of the species. The enrichment ratio of
zinc in S. undulata at a number of sites was investigated
and considerable variation between sites was found. The
effects of the concentration of zinc in the medium, light
and temperature on the uptake of zinc by S. undulata under
laboratory conditions were also investigated. The rate of
uptake of zinc was found to increase as the concentration
of zinc in the medium increased, up to a concentration of
60 mg 1^{-1}. The saturation point was found to be approximately
the same for a two day period as for the initial half hour,
with some indication of a small increase in the rate of uptake
of zinc at concentrations greater than 60 mg 1^{-1} over the two
day period. Material incubated in medium containing 1 mg 1^{-1}
zinc in the light for a period of four days was found to
contain approximately 15% more zinc than material incubated
in darkness. The rate of uptake of zinc by dead material at
$32^{O}C$ from medium containing 2 mg 1^{-1} zinc was found to be
greater than that of live material at $14^{O}C$ and there was
some indication of a greater rate of uptake by live material

at $24^{\circ}C$ than at $14^{\circ}C$. The results of these experiments are discussed in terms of the relative importances of active and passive mechanisms of uptake of zinc in S. undulata and the validity of using this species for monitoring the levels of zinc in stream waters.

ACKNOWLEDGEMENTS

CONTENTS

1. INTRODUCTION

1.1. INTRODUCTION TO THE STUDY

Many species of plant accumulate heavy metals from the medium which surrounds them. This phenomenon is exploited by geochemists who use plant analyses as a means of prospecting for minerals and by ecologists who have developed a means of monitoring atmospheric pollution which involves analysing samples of moss which are hung in net bags from buildings and trees in the area (Goodman and Roberts, 1971). Recently, it has been suggested that the heavy metal content of aquatic plants might be a useful measure of the average levels of heavy metals in stream waters (Dietz, 1973). In order to assess the validity of this proposed method of monitoring stream waters, it will be necessary to determine the effects of environmental factors on accumulation and loss of heavy metals in particular species and also to determine whether or not populations of these species at heavy metal polluted sites have become genetically adapted so as to reduce the degree to which they accumulate the element concerned. Many of the terrestial higher plants and aquatic algae which colonise heavy metal polluted sites have been found to be heavy metal resistant ecotypes of their species (Antonovics et al. 1971; Whitton and Harding in press; Say et al. in press) however no instance of genetic adaptation in a bryophyte has been reported although mosses and liverworts are commonly found at heavy metal polluted sites (Jones, 1940).

The present study concerns Scapania undulata (L.) Dum., a leafy liverwort common in fast flowing mountain streams in

northern and western parts of Britain. S. undulata has
been found to be. associated with lead and zinc pollution
in North Wales (McLean and Jones, 1974) and in the North
East of England (B.A. Whitton pers. comm). In this study
the zinc resistance of S. undulata from zinc enriched sites
and from sites with much lower concentrations of zinc has
been investigated by means of transplants between sites
with high and low levels of zinc and also by means of
toxicity tests carried out in the laboratory, the intention
being to attempt to determine whether or not populations of
S. undulata at zinc enriched sites are genetically adapted
ecotypes of the species with a greater degree of zinc
resistance than populations of the species from low zinc
sites. In addition, the enrichment ratio of zinc in
S. undulata at a number of sites has been determined and
the effect of the concentration of zinc in the medium,
light and temperature on uptake of zinc by the species
investigated.

1.2. HISTORY OF MINING IN THE NORTH EAST OF ENGLAND

Mining has been going on in the Northern Pennines since
the 12th century and was at one time one of the principal
occupations of the smallholders in the fell and dale country
of the region. In the past a form of hydraulic mining was
practised whereby streams were dammed and diverted such
that they flowed over or along the course of a vein.
Thereafter, the water was periodically allowed to rush
along the channel making in the course of years, a great

excavation or "hush". Pieces of ore dislodged by the
force of the water were collected downstream where they
were deposited on the stream bed as the rate of flow
decreased. As a result of this practice, many streams in
the area flow over mineral deposits and are enriched with
heavy metals. Mining activities intensified greatly
during the 18th and 19th centuries and the majority of
the underground shafts belong to this period. Drainage
from heaps of excavated mine waste and from underground
tunnels via drainage shafts known as adits contributes
to the heavy metal enrichment of streams in the area,
though the majority of the mines closed down in the early
years of this century (Dunham, 1949).

1.3. CHEMISTRY OF ZINC IN WATER

Zinc is one of the heavy metals, having a density
greater than five, and is recognised to be an essential
micro element for the growth and development of micro-
organisms (Myers, 1951), plants (Arnon, 1958) and
animals (Underwood, 1956). All aquatic habitats which
support a biota must therefore contain trace amounts of
zinc, though the level may be as low as 0.001 mg l^{-1}
(Whitton and Say in press). Conversely, in heavily mined
areas, the zinc levels in streams may be as high as 20 mg l^{-1}
and this pollution results in the production of an environ-
ment with reduced species diversity, as concentrations this
high are toxic to many organisms (Carpenter, 1924; Jones,
1958). Zinc pollution may also occur in water courses

receiving waste from factories engaged in zinc plating, galvanising and rayon production (Bradshaw, 1970).

Streams which have raised levels of zinc are also generally polluted to an extent with lead derived from the mineral Galena (PbS) which occurs together with the zinc ores, with cadmium which is associated with the mineral sphalerite (ZnS) and with fluoride derived from fluorite (CaF), an abundant matrix material in the area.

Zinc occurs in all three phases of the stream environment; in the sediment, the water and the biota. In the sediment and seston zinc may form an inorganic precipitate, occupy a space in the crystal lattice structure of clays or be held as an exchangeable ion on the surface of organic or inorganic solids. Inorganic zinc precipitates include zinc hydroxide ($Zn(OH)_2$), zinc carbonate ($ZnCO_3$), zinc silicate ($ZnSiO_3$) and zinc sulphide (ZnS). In waters with a pH value greater than 9, most of the zinc will be precipitated as zinc hydroxide, at pH values below 6.5, where dissolved CO_2 is abundant, a precipitate of zinc carbonate will be formed and between pH 7.5 and 10.0 zinc silicate is likely to be formed. Under reducing conditions such as may be found in the sediments of pools, zinc sulphide will be formed. Zinc may also adsorb on or coprecipitate with compounds such as calcium carbonate and ferric oxide, where these are present (Bachmann, 1963; Hem, 1972). In the liquid phase of the stream environment zinc may occur as a hydrated ion, a complexed ion or associated with soluble organic compounds

and in stream organisms, zinc may be adsorbed on to the cell surface, complexed with metabolically inactive substances in the cell wall or cutoplasm, or may be a functional component of the cell's metabolic system. Zinc is a component of enzymes such as carbonic anhydrase and alcohol dehydrogenase which are important in the regulation of cell metabolism and the biological half life of Zn^{65} has been used as a measure of metabolic rate in energy flow studies (Odum and Golley, 1961).

1.4. TOXICITY OF ZINC

Laboratory studies of the toxicity of zinc to various organisms have indicated that there is a great variation in the levels of zinc which different organisms can withstand. The alga Cladophora glomerata is reported to be particularly sensitive, being killed by 0.1 mg 1^{-1}. zinc under laboratory conditions whereas Stigeocolonium tenue is found at sites with concentrations of 20 mg 1^{-1} (Whitton and Say in press). Most of the zinc levels reported to result in 50% survival in fish lie in the range 0.5-4.0 mg 1^{-1} whereas the recommended levels for drinking water range from 2-15 mg 1^{-1}, despite recent studies which suggest a link between trace elements and some types of cancer (Stock and Davies, 1964).

The toxicity of any given level of zinc is affected by various environmental factors. Organic matter has ion exchange and complexing properties and its presence in stream waters may reduce the toxicity of zinc in two ways. Organic matter in suspension will adsorb zinc and cause it to be deposited as sediment on the stream bed or in pools (McLerran et al. 1974; Pita et al. 1974). Soluble organic

compounds will tend to complex with the zinc and may bind it so tightly that it is unavailable to the stream organisms. In general experiments in which organic matter has been added to or removed from stream waters have indicated that it has an ameliorating effect on the toxicity of heavy metals to a variety of organisms (Whitton and Say, 1975).

The toxicity of zinc is also affected by the acidity of the stream waters. Bachmann (1963) and Kuelder (1975) found that hydrogen ions antagonised the uptake of Zn^{65} by algae and Patrick (1974) found that the enrichment ratios of species living in a stream at pH 2.6 tended to be lower than those of species in a stream at pH 6.6. The cations Ca^{2+} and Mg^{2+} and the anion PO_4^{3-} have similarly been found to reduce the toxicity of zinc (Herbert, 1965; Rana and Kumar, 1974; Whitton and Harding in press; Say and Whitton in press), whereas Ni^{2+} and Cu^{2+} ions have been found to have a synergistic effect on the toxicity of zinc (Whitton and Say, 1975).

Genetic adaptation in the populations of plants which colonise old mine workings is well documented (Antonovics et al, 1971). Zinc tolerance in Agrostis tenuis and lead tolerance in Festuca ovina has been found to be dominant and polygenic (Gartside and McNeilly, 1974; Wilkins, 1960). Recent work has indicated that the populations of Stigeocolonium tenue and some Hormidium species found at zinc enriched sites are also genetically adapted ecotypes of their species (Whitton and Harding in press; Say et al in press). However,

zinc tolerance in _Typha latifolia_ apparently does not
involve the evolution of a tolerant race (McNaughton
et al, 1974) and lead resistance in _Grimmia doniana_, a
species of terrestial moss has similarly been found to
occur without any genetic adaptation (Brown and Bateson,
1972). Zinc resistant _A. tenuis_ accumulates large
quantities of zinc and resistance is thought to be
achieved by an increased ability to bind zinc to pectates
in the cell wall (Turner, 1970; Turner and Marshall, 1971).
Zinc resistant _Silene cucubalus_, _Rumex acetosa_ and
Philonotis fontana have been found to produce increased
amounts of malate and it has been suggested that the malate
complexes with zinc rendering it non-toxic or that it may
function as a carrier transferring zinc into the vacuole
and thus maintaining the cytoplasmic concentration at a
non-toxic level (Mathys, 1975).

1.5. ACCUMULATION OF ZINC

Uptake of heavy metals to produce an internal
concentration greater than in the external environment
is a widespread phenomenon in both terrestial and aquatic
organisms. Geochemists make use of "accumulator" species
in biogeochemical prospecting; analysis of the heavy metal
content of plants being used as an indication of
mineralisation and as mentioned previously, it has been
suggested that aquatic plants be used as monitoring
organisms for calculating the average heavy metal content
of stream waters (Dietz, 1973). It has generally been
found that bryophytes accumulate metals to a greater extent

than do angiosperms (Leeder, 1972; Tyler, 1972; Dietz,
1973; Patrick, 1974). Bryophytes have no roots and must
therefore be able to accumulate minerals from the very
dilute supply available in the atmosphere or water which
surrounds them. The ability of bryophytes to accumulate
minerals more efficiently than angiosperms is thought to
be due to their lack of cuticle and epidermis which results
in an increased ability to take up minerals passively by
ion exchange. This property makes them sitable as
indicators of atmospheric pollution (Goodman and Roberts,
1971; Tyler, 1972).

If bryophytes are to be used for monitoring the heavy
metal content of stream waters, then it is necessary that
accumulation of heavy metals be independent of environmental
factors other than the concentration of these metals in the
water. Dietz (1973) found that the enrichment factors
(level of element in plant (wet weight) level of element
in the water) of individual metals in a number of plants
were relatively constant. However, Whitehead and Brooks
(1972) who investigated the feasibility of using aquatic
bryophytes as indicators of uranium levels obtained results
which suggest that uranium ions may be antagonistic to the
accumulation of radium. The effect of environmental factors
on accumulation of heavy metals will obviously depend on
the mechanism whereby accumulation occurs.

Accumulation of ions against a concentration gradient
may be accomplished passively by means of ion exchange
mechanisms or through the establishment of Donnan equilibria.

The former mechanism occurs in both living and non-living systems and involves the attachment of cations to negatively charged sites on the surface of the exchange material. The proportion of exchange sites occupied by a given species of ion is dependent on the energy with which the ion is bound to the exchange material and the relative concentration of that ion in solution. In cell walls, the presence of a large number of immobile carboxyl groups ($RCOO^-$) associated with pectins and other compounds results in a high ion exchange capacity and as a result, living and dead organic material has a tendency to adsorb trace elements (Sutcliffe and Baker, 1974).

Accumulation of ions against a concentration through the establishment of Donnan equilibria involves accumulation as a result of an electrical potential difference generated by the presence of fixed or immobile charges in a solid phase adjacent to an aqueous phase. In the cell walls of plants, the presence of fixed organic cations such as amines within the cell wall matrix results in the anion concentration in the free space of the cell wall being greater than in the surrounding medium; this being necessary to balance the electrical charges. Although the movement of ions in this situation occurs passively in response to an electrical potential difference, the initial establishment of the gradient requires energy and this mechanism of accumulation therefore only occurs in living systems.

Active accumulation of ions against a concentration gradient is a well documented phenomenon in plants,

accumulation being inhibited by low temperatures, low oxygen tension and metabolic inhibitors, etc. (Devlin 69). One hypothesis as to the mechanism of active transport is that a protein "carrier" molecule sited in the membrane specifically binds certain molecules and ferries them across the membrane. Another possibility is that ions cross the membrane by means of micro-pinocytosis (Sutcliffe and Baker, 1974).

There have been few investigations into the mechanisms of accumulation in individual plants. Gutknecht (1961; 1963) has investigated mechanisms of zinc uptake in marine benthic algae. The pattern of uptake in relation to light, temperature and metabolic inhibitors suggested the operation of a metabolic accumulation process, accumulation being proportional to the rate of photosynthesis and suppressed in the dark as previously reported (Bachmann and Odum, 1960). However, further investigations indicated that killed algae absorbed more zinc than live algae and it was therefore hypothesised that physical processes of adsorption and cation exchange were responsible for zinc uptake and that the increased uptake in actively photo-synthesising and dead cells is a result of an increase in intracellular pH. These studies illustrate the problems involved in distinguishing between active and passive accumulation of ions.

Investigations of the accumulation of Zn^{65} by Candida utilis indicated that in this organism, there is an initial, rapid, temperature independent accumulation which probably

represents binding to the cell surface and a pH and temperature dependent accumulation system which is inhibited by metabolic inhibitors and depends on the presence of intact membranes and probably therefore represents active uptake (Failla, 1976).

Pickering and Puia (1969) investigated zinc uptake in _Fontinalis_ _antipyretica_ a species of aquatic moss. They found that darkness, low temperature and metabolic inhibitors had no effect on zinc uptake in short term experiments (6h), but that in the long term (48h), these factors suppressed zinc uptake. However, dead material took up more zinc than live material in both long term and short term experiments. From an analysis of the time course of uptake, they suggest that three successive processes are involved, the first two being passive adsorption processes and the third a long term metabolic accumulation process. Gullvag et al. (1974) have also studied uptake of heavy metals by bryophytes. They made an ultrastructural study of lead accumulation in two species of moss and found that uptake of lead into the cytoplasm of leaf cells of _Rhytidiadelphus_ _squarrosus_ treated with lead salt solutions, occurred by pinocytosis but that there was no uptake of lead into the cytoplasm of leaf cells of the moss _Hylocomium_ _splendens_, which has a particularly thick cell wall. Brown and Bateson (1972) have studied uptake of lead by the terrestial moss _Grimmia_ _doniana_ and have concluded that in this species also there is no uptake of lead into the cytoplasm.

RELATIONSHIP OF THIS STUDY TO PREVIOUS RESEARCH

This study concerns the nature of zinc resistance in
S. undulata and the mechanism whereby this species
accumulates zinc.

The existance of genetically adapted ecotypes able to
withstand the toxic effects of heavy metals has been
demonstrated in both higher plants and algae (section
1.4). As yet, however, no instance of genetic adaptation
in a bryophyte species has been reported although these
plants are commonly found in heavy metal polluted waters
(Jones, 1940). Because of this lack of information
regarding bryophytes, it was decided to investigate the
nature of zinc resistance in S. undulata, a species of
liverwort which is widespread in the zinc polluted streams
and rivers of the Northern Pennine area which is easily
accessible from Durham. The occurrence of heavy metal
resistant ecotypes of bryophytes would be undesirable from
the point of view of their being used for monitoring the
levels of heavy metals in stream waters (section 1.5).
Another factor which must be taken into account in assessing
the validity of this method of monitoring stream waters,
is the mechanism of accumulation of heavy metals in
bryophytes. For the purposes of monitoring, accumulation
must be as far as possible independent of environmental
factors other than the concentration of heavy metals in
the medium. At present, the evidence regarding mechanisms
of accumulation in bryophytes is conflicting (section 1.5),
therefore it was decided to investigate the mechanism of
accumulation of zinc in the liverwort S. undulata.

2. METHODS AND MATERIALS

2.1. TOXICITY TESTS

(a) OUTLINE OF EXPERIMENTAL METHOD

Scapania undulata was collected from a zinc enriched
site (2) and a low zinc site (6) and prepared as described
in section 2.1 (b). Media with a range of zinc
concentrations were prepared as described in section
2.1 (c) and eight 100 ml. flasks containing 40 ml. of
medium prepared for each concentration. 10 prepared
shoots from the zinc enriched site were then transferred
to each of four of the flasks at each concentration and
prepared material from the low zinc site transferred to
the remaining flasks. The flasks were then placed in a
shaker tank (Gallenkamp model 4A-2522-SP) at 18°C and
6000 lx (cool white fluorescent light) and incubated for
a period of six days, at the end of which time the ratio
of chlorophyll $\underline{a}$ to pheophytin $\underline{a}$ of the material at each
concentration was determined as described in sections
2.1 (d) and 2.1 (e).

(b) COLLECTION AND PREPARATION OF MATERIAL

$\underline{S}$. undulata forms dense mats, attached by rhizoids to
stable areas of substratum which may be either submerged
or in the splash zone. Collections were made from as
small an area as was practicable at each site and where
possible only completely submerged material was selected.
The material was washed in the field to remove particles
of silt trapped in the basal mat of rhizoids and dead
shoots. On return to the laboratory, 3.0 cm. shoot tips

were removed from the collected material and washed
thoroughly to remove diatoms and algae which adhered
to the leaves and would otherwise grow in the culture
flasks. In much of the material only the terminal
0.5 cm. or less of the shoot was green and apparently
photosynthetically active and in initial trials
attempts were made to culture 0.5 cm. shoot tips.
However, much of this material died within two days,
therefore in subsequent experiments 3.0 cm. shoot tips
were used. The washed shoot tips were transferred to
100 ml. flasks containing 50 ml. of zinc free culture
medium (section 2.1 (c)) and placed in the shaker tank
at $18^{\circ}C$ and 6000 lx. to equilibrate for a period of two
days before the commencement of the experiment.

(c) CULTURE MEDIUM

The medium used for all laboratory culturing was
based on the No. 10 formula of Chu (1942), modified by
lowering the pH to 6.3 reducing the level of phosphate
and using a different trace element stock. Phosphate
has been shown to reduce the toxicity of zinc to some
organisms (Rana and Kumar, 1974; Say and Whitton in
press; Harding and Whitton in press). The basal medium
was as follows:-

KH_2PO_4	8 mg l^{-1}
$MgSO_4.7 H_2O$	25 mg l^{-1}
$CaNO_3$	40 mg l^{-1}
$NaHCO_3$	8 mg l^{-1}

Na_2SiO_3 40 mg 1^{-1}

Fe (as ferric iron
 ethylenediaminetetra -
 acetate chelate)   0.5 mg 1^{-1}

'C' microelements of
Kratz and Myers (1955)
omitting zinc   0.25 mg 1^{-}

The pH of the medium was adjusted to 6.3 by addition

of 0.1 N H_2SO_4. This was done to avoid precipitation of

zinc in the course of the experiments. Zinc was added to

the basal medium from a stock solution of $ZnSO_4.7H_2O$.

(d) CHLOROPHYLL EXTRACTION

Attempts were initially made to extract the chlorophyll

from Scapania undulata using 90% acetone, this being the

solvent used most commonly for extractions of chlorophyll

and that used to extract chlorophyll from S. undulata in

a study by McLean and Jones (1974). This method was found

to be unsuitable, extraction being incomplete despite

grinding of the material and a period of 45 min. in a

water bath maintained at 70°C. In all subsequent

extractions, methanol, a much more powerful extracting

agent, was used. Methanol, like acetone, is miscible

with water hence extraction from fresh material is possible,

unfortunately, however, the spectral properties of pigment

solutions in methanol are less well known. Methanol

containing 5% water was used in preference to the anhydrous

solvent as previous workers have found that extraction is

more difficult in anhydrous solvent (Marker, 1972).

The material to be extracted was removed from the

culture flasks and the green sections of the shoots ground up in a mortar and pestle together with a small amount of 95% methanol. 20 shoot tips were used in each extraction, this being the minimum amount of material necessary to produce a suitable concentration of extract. The ground material was transferred to a 30 ml. McCartney bottle, made up to approximately 10 ml. with 95% methanol and placed in a water bath maintained at 70°C for a period of 20 min. A cover hood was used to ensure that extractions were carried out in the dark as solutions of chlorophyll in methanol are unstable in the light. The extracts were then filtered through 24 mm. G/FC filter discs under reduced pressure and stored in a refrigerator until required. All extracts were read within six hours of extraction; experiments carried out at Durham University have shown that less than 3% of the chlorophyll extracted from blue-green algae by this method is broken down within 24 h. of extraction (M. Potts, pers. comm.)

(e) DETERMINATION OF RATIO OF CHLOROPHYLL a
     TO PHEOPHYTIN a

Pheophytin a is one of a group of chlorophyll a derivatives which retain an intact porphyrin ring and therefore absorb in the same regions of the spectrum as chlorophyll a. The presence of these derivatives in a chlorophyll extract interferes with established methods of chlorophyll analysis and various methods have therefore been devised which correct for the presence of pheophytin a which is generally considered to be the most important

of the derivatives (Moss, 1967; Lorenzen, 1967; Marker, 1972).

The basis of these methods is that treatment of extracts of chlorophyll with dilute acid leads to a very rapid conversion of chlorophyll a to pheophytin a as each chlorophyll a molecule loses its bound magnesium atom. Moss's method is based on the marked differences between the spectra of chlorophyll a and pheophytin a in acetone between 430 nm. and 410 nm. This method is not readily applicable to solutions of pigments in methanol as the spectrum of pheophytin a in this region is pH sensitive (Livingstone et al, 1953; Marker, 1972). Lorenzen's method is based on the marked differences between the specific absorption coefficients of chlorophyll a and pheophytin a at 665 nm. Marker (1972) found that the spectrum of pheophytin a in methanol in this region of the spectrum also was pH sensitive and he therefore recommended neutralisation of the acidified extract with $MgCO_3$. However, other workers have found that acidification simply displaces the pheophytin peak to a slightly shorter wavelength, there being no change in peak height and they have therefore applied Lorenzen's method to extracts of chlorophyll in methanol (M. Potts pers. comm.). In this study, Lorenzen's method has been applied to solutions of pigments in 95% methanol, using a modified form of the equations derived by Marker (1972).

The absorbance of each extract was read initially at 665 nm. The extracts were then acidified by addition of one

drop of 1.0 M HCl, mixed thoroughly, allowed to stand
for 15 s. and the absorbance around 665 nm. re-read.
All absorption spectra were obtained using a Perkin
Elmer model 402 scanning spectrophotometer and 1 ml.
cuvettes. The formula used to derive the ratio of
chlorophyll $\underline{a}$ to pheophytin $\underline{a}$ from the two readings of
absorbance is as follows:-

$$\text{chlorophyll } \underline{a}/_{\text{pheophytin } \underline{a}} = 2.43(A_b-A_a)/_{A_b-243(A_b-A_a).1.46}$$

A_b = absorbance before acidification

A_a = absorbance after acidification

This formula is a modified form of the equations
derived by Marker for calculation of the absolute amounts
of chlorophyll $\underline{a}$ and pheophytin $\underline{a}$. The factor of 2.43
should be calculated from the "acid factor" of pure
chlorophyll $\underline{a}$ (I.B.P. Handbook No. 12, 1969), the "acid
factor" being the ratio of the absorbance before
acidification to the absorbance after acidification.
The factor which Marker used in his equations was derived
from the average acid factor of extracts of healthy
cultures of algae (Marker, 1972, p. 383). Marker's
equations were applied to results obtained from extracts
of $\underline{S}$. undulata and the calculated proportion of pheophytin
$\underline{a}$ was in some cases found to be negative, indicating that
the extracts concerned had a larger acid factor than the
extracts of fresh material used by Marker to estimate the
acid factor of pure chlorophyll $\underline{a}$. Marker's fresh extracts
must therefore have contained more pheophytin $\underline{a}$ or more
chlorophyll $\underline{b}$ than the extracts of $\underline{S}$. undulata.

Chlorophyll b contributes to the absorbance of extracts
at 665 nm. but is unaffected by acidification. The
presence of chlorophyll b in an extract therefore reduces
the acid factor.

In order to obtain a suitable factor for use in the
equations, material was collected from healthy populations
of S. undulata kept on ice during the return trip to
Durham and extracted immediately upon arrival. The
average acid factor of this fresh material was found to
be 1.7 from which value the factor of 2.43 is derived
(see Marker, 1972, p. 383, for method of derivation).
No values of the acid factor of pure chlorophyll a have
apparently been reported in the literature therefore it
is not possible to obtain an accurate value for the factor.

2.2. TRANSPLANT EXPERIMENTS

(a) SITE DESCRIPTIONS

For the purposes of the transplant experiments, sites
located on three zinc enriched and three low zinc streams
were selected. All six sites are located on small streams
in the region of the villages of Nenthead and Allenheads
on the border between County Durham and Northumberland
(Figures 1-6). Five of the six sites have previously been
studied by members of the Botany Department of Durham
University who kindly provided water chemistry data (Table 13).

ZINC ENRICHED SITES

Site
Number

(1) Brown Gill Burn (Map ref. NY 763423; Durham ref.

stream 0103, reach 10).

This stream rises in a recently afforested area
approximately 800 m. upstream of the site location.
The stream receives drainage from an old mine
working near its source and there is evidence of
hushing immediately upstream of the site. The
volume of flow was very small throughout the period
of the study therefore the site chosen for the
transplanted material was a shallow pool at the foot
of a small waterfall approximately 1 m. in height
Figure 1). S. undulata grows profusely both on the
waterfall and on large stones in the pool.
Unfortunately this stream dried up in the course
of the study.

(2). Gillgill Burn (Map ref. NY 791439; Durham ref.
stream 0093, reach 29).

This stream rises in a very heavily mined area
approximately 400 m. upstream of the site location.
The gradient of the stream at the site is very
steep and the substrate is mainly composed of flat
stones which are piled on top of each other to form
a series of small falls and pools. S. undulata grows
as a thick mat over much of the substrate at this
site (Figure 2). The volume of flow at this site
also was very small and the transplanted material
was placed in small pools such that water flowed
over it though it was not completely submerged.
Unfortunately this stream also dried up during the
study.

(3)   Gudham Gill (Map ref. NY 778448; Durham ref.
      stream 0092, reach 10).

      This stream rises in an area of moorland
      approximately 1.5 km. upstream of the site
      location. Hushes occur upstream and downstream
      of the site location and there is probably also
      some drainage from an adit immediately upstream
      of the site location. This is the largest of the
      six streams (Figure 3) and it was possible to
      submerge all the transplanted material. The
      volume of flow at this site remained adequate
      throughout the period of the study.

LOW ZINC SITES

(4)   Coalcleugh Moor Sike (Map ref. 799448)

      This stream has not previously been studied by
      members of the Botany Department at Durham
      University and as the stream dried up shortly
      after the commencement of the study, water
      chemistry data was not obtained for the site.
      The stream rises in an area of moorland
      approximately 400 m. upstream of the site
      location. The substrate consists mainly of
      large stones on which S. undulata grows profusely.
      At the time the transplants were performed, the
      depth of water at the site was sufficient to
      submerge all the transplanted material, however,
      within two weeks the stream had dried up
      completely (Figure 4).

(5) <u>East End Burn</u> (Map ref. NY 867453; Durham ref. stream 0082, reach 10).

This stream rises approximately 800 m. upstream of the site which is located at the boundary of a hay field. The site is heavily shaded by vegetation on the stream banks. The transplanted material was placed in a pool at the foot of a small waterfall where <u>S</u>. <u>undulata</u> grew naturally on the large stones which formed the substrate. During the course of the study, this stream ceased to flow, but the pool itself did not dry up (Figure 5).

(6) North Grain Quarry Sike (Map ref. NY 883449; Durham ref. stream 0116).

This stream rises in area of high moorland approximately 200 m. upstream of the site. The stream banks are composed of peat and the water contains large amounts of peaty material in suspension (Figure 6). The depth of water at the site was initially sufficient to submerge the transplanted material, however as the volume of flow became reduced, it became necessary to transfer the transplants to a ledge at the side of a deep pool downstream of the site. <u>S. undulata</u> is widespread in this stream but is largely confined to regions in which there is a steep gradient. The material transferred to the pool rapidly became covered in a deposit of peaty material.

(b) METHOD OF TRANSPLANTATION

Five stones with firmly attached mats of $\underline{S}$. undulata were selected at each site. As far as possible, the stones selected were of such a size that they could be completely submerged at the sites to which they were to be transferred but would not be washed away under conditions of high flow should these arise. The stones selected at each site were numbered to enable identification and one stone transferred to each of the other five sites. The stones to be transplanted were placed in buckets of stream water to prevent drying out and several trips were made to and from each site in order that each transplant should spend as short a time as possible in transit. The sites to which the transplants were transferred were all sites at which $\underline{S}$. undulata was to be found growing naturally. It was found necessary to place the transplants in situations with a rapid rate of water flow as they otherwise became overgrown with algae.

(c) TRANSPLANT MONITORING

The ratio of chlorophyll $\underline{a}$ to pheophytin $\underline{a}$ of the transplanted material was determined at weekly intervals. This method of monitoring the condition of transplanted $\underline{S}$. undulata has been used in a previous study by McLean and Jones (1974). At weekly intervals a small amount of material (approximately 20 shoots) was removed from each transplant. This material was kept on ice during the return trip to Durham and extracted immediately upon arrival as described in section 2.1 (d). The ratio of

chlorophyll a to pheophytin a in the extracted pigments
was then determined as described in section 2.1 (e).

(d) METHOD OF DETERMINATION OF THE ZINC CONTENT
OF THE TRANSPLANTS

Eight weeks after transplantation, material was
collected from the surviving transplants and analysed
for zinc. A small amount of material only remained on
each transplant stone therefore analysis was carried out
on 2.0 cm. shoot tips instead of 1.0 cm. shoot tips as
in the remainder of the study.

Four replicates of approximately 25 two cm. shoot
tips were collected from each transplant washed briefly
with distilled water and placed on filter paper to air
dry for a period of 10 min. before being placed in acid
washed Pyrex sample tubes for the return trip to Durham.
The samples were then dried for 24 h. in a forced draught
oven at 105°C, ground in a mortar and pestle, dried for a
further 2 h. and allowed to cool in a dessicator before
being weighed. The weighed samples were transferred
directly into acid washed kjeldal flasks, 5 ml. of 10 N
HNO_3 (Analar Grade) was added and the samples boiled for
20 min. by which time no solid material remained. The
small volume of digested material was then transferred
to a 25 ml. volumetric flask together with washings from
the kjeldal flask and made up to volume with double
distilled water. The samples were stored in acid washed
Pyrex sample tubes and the zinc concentration determined

on a Perkin Elmer model 403 atomic absorption spectrophotometer. This method of acid digestion has been used to release zinc from plant material in a previous study by Dietz (1973).

2.3. INVESTIGATION OF THE CONSTANCY OF THE ENRICHMENT RATIO OF ZINC IN S. UNDULATA.

(a) SITE DESCRIPTIONS

The sites involved in this section of the project include four of the six transplant sites. Site (1) and Site (4) were not included as they had dried up by the time this part of the study was undertaken. Two additional sites were included:

Site
Number

(7) <u>Gill Gill Burn (Lower)</u> (Map ref. NY 784438; Durham ref. stream 0093, reach 74).

This site is downstream of site (2) and located in the village of Nenthead. The site is a waterfall, over which S. undulata grows profusely.

(8) <u>Sikehead Stream</u> (Map ref. NY 948466; Durham ref. stream 0067, reach 99).

This site is on a small upland stream which receives drainage from disused mineworkings near its source.

Water chemistry data for these sites were provided by members of the Botany Department at Durham University and are presented in Table 12b.

(b) EXPERIMENTAL METHOD

$\underline{S}$. undulata was collected from a number of different areas of substate at each site and 10 replicates of 1.0 cm. shoot tips were removed and analysed for zinc as described in section 2.2 (d). Water samples were collected from five of the six sites at the time of sampling of the plant material and in the two preceeding weeks. As access to site (8) was restricted plant material was collected and current water chemistry data provided by P. Harding of the Botany Department at Durham. Water samples collected for zinc analysis were stored in acid washed Pyrex sample bottles to which one drop of 10 N HC_1 (Aristar Grade) was added. Analysis was carried out on a Perkin Elmer model 403 atomic absorption spectrophotometer.

The enrichment ratio was calculated from the following formula: enrichment ratio = level of zinc in plant (dry weight)/level of zinc in water.

2.4. LABORATORY ACCUMULATION EXPERIMENTS

(a) INVESTIGATION OF THE EFFECT OF CONCENTRATION ON RATE OF UPTAKE OF ZINC BY $\underline{S}$. UNDULATA

The material used in all the laboratory accumulation experiments was collected from site (6). Material was collected and prepared as described in section 2.1 (a). Media with a range of zinc concentrations between 2 and 100 mg 1^{-1} were prepared as described in section 2.1(c) and 40 ml. placed in each of three 100 ml. flasks. 10 equilibrated shoots were transferred to two of the flasks and 20 shoots transferred to the remaining flask at each

concentration. The flasks containing 20 shoots were placed in a constant temperature room maintained at $12^{o}C$ and the material in each flask removed after 30 min. and the 1.0 cm. shoot tips analysed for zinc as described in section 2.2(d). The flasks containing 10 shoots each were placed in the shaker tank at $18^{o}C$ and 6000 lx. and the material removed and analysed for zinc after two days. The amount of material in each flask was reduced in the second part of the experiment in order that the plant material should not exhaust the amount of zinc available in the medium. Material from the two flasks at each concentration was combined in order to obtain sufficient material for analysis.

(b) EFFECT OF LIGHT ON ZINC ACCUMULATION

Material was collected, fractionated and washed as described previously and maintained in zinc free culture medium in the dark for a period of four days before the start of the experiment. Light was excluded by wrapping the flasks in black polythene. Medium containing 1 mg 1^{-1} was prepared as described in section 2.1(c) and 180 ml. added to each of 10 250 ml. conical flasks. Light was excluded from five of the flasks by enclosing them in aluminium foil around which was wrapped black polythene. 40 shoots were added to each of the flasks which were then placed in the shaker tank at $18^{o}C$ and 6000 lx. In order to monitor accumulation of zinc by the plant material, samples of 0.5 ml. of the medium were removed and analysed for zinc at regular intervals. Four days after the start

of the experiment, the material in each flask was removed and analysed for zinc as described in section 2.2(d).

(c) EFFECT OF TEMPERATURE ON ZINC ACCUMULATION

Material was collected, fractionated and washed as described in section 2.1(a). Samples of the prepared material were equilibrated in zinc free culture medium in each of three constant temperature rooms maintained at 14 C, 24 C and 32 C respectively. Throughout the experiment, the flasks were shaken by hand twice a day. Medium containing 2 mg l^{-1} zinc was prepared as described in section 2.1(c) and 40 ml. placed in each of 96 100 ml. flasks and 160 ml. placed in each of three 250 ml. flasks. 32 of the 100 ml. flasks and one 250 ml. flask were placed in each of the constant temperature rooms to equilibrate with the temperature of the room for a period of four hours. 10 shoots were then transferred to each of the 100 ml. flasks at each temperature and 40 shoots to each of the 250 ml. flasks. At intervals of 6 h, 12 h, 24 h and 48 h after the start of the experiment, material was removed from eight of the 100 ml. flasks at each temperature and the 1.0 cm. shoot tips analysed for zinc as described in section 2.2(d). Material from two of the flasks was combined for each analysis. The zinc content of the medium in each of the 250 ml. flasks was monitored at regular intervals and the material in these flasks was removed and analysed for zinc seven days after the start of the experiment.

Figure 1 Site (1)

Figure 2 Site (2)

Figure 3 Site (3)

Figure 4 Site (4)

Figure 5 Site (5)

Figure 6 Site (6)

3. RESULTS

3.1. TOXICITY TEST RESULTS

The results of the toxicity tests are presented in Tables 1 and 2 and Figures 7 and 8. The results are ratios and although the distribution of many ratios approximates to the normal (Simpson et al. 1960) insufficient data was obtained to determine whether or not this was the case in the present study. For this reason, it was not possible to derive accurate confidence limits for the. mean values of the ratio at each concentration. However, it can be seen by inspection of Figures 7 and 8 that the ratio of chlorophyll a to pheophytin a in Scapania undulata shows no trend with increasing concentration of zinc in the medium over the six day period.

Initial tests carried out on S. undulata indicated that no appreciable growth occurred over a two week period under laboratory conditions. A small amount of material was maintained in flasks in the shaker tanks for a much longer period of time and it was found that S. undulata maintained in medium containing 100 mg l⁻ zinc died within three weeks, the photosynthetic pigments having been completely broken down to colourless products. Material maintained in medium containing 10 mg l⁻ zinc showed appreciable growth within a month and S. undulata maintained in medium containing 20 mg l⁻ zinc survived for up to ten weeks but showed no appreciable growth. No difference was apparent in the zinc resistance of material from site (2) and material from site (6).

Table 1. Levels of pheophytin $\underline{a}$ (expressed as $^{ph}\underline{a}$/chl $\underline{a}$ + ph $\underline{a}$) in $\underline{S}$. undulata from site (2) and site (6) in medium containing 0 mg 1^{-1} - 20 mg 1^{-1} zinc for six days (mean $\pm$ 1 S.E. ; n = 4).

Zn concentration (mg 1^{-1})	Level of ph $\underline{a}$ in site (2) material	Level of ph $\underline{a}$ in site (6) material
0.0	0.0892 $\pm$ 0.0157	0.1079 $\pm$ 0.0087
1.0	0.1001 $\pm$ 0.0083	0.0757 $\pm$ 0.0078
2.0	0.0739 $\pm$ 0.0116	0.0844 $\pm$ 0.0120
5.0	0.1024 $\pm$ 0.0133	0.1129 $\pm$ 0.0074
10.0	0.1073 $\pm$ 0.0148	0.1098 $\pm$ 0.0174
20.0	0.0711 $\pm$ 0.0290	0.1580 $\pm$ 0.0146

Table 2. Levels of pheophytin $\underline{a}$ (expressed as $^{ph}\underline{a}$/chl $\underline{a}$ + ph $\underline{a}$) in $\underline{S}$. undulata from site (2) and site (6) in medium containing 0 mg 1^{-1} - 80 mg 1^{-1} zinc for six days (mean $\pm$ 1 S.E. ; n = 4)

Zn concentration (mg 1^{-1})	Level of ph $\underline{a}$ in site (2) material	Level of ph $\underline{a}$ in site (6) material
0.0	0.0898 $\pm$ 0.0099	0.0979 $\pm$ 0.0183
10.0	0.0705 $\pm$ 0.0195	0.0918 $\pm$ 0.0061
20.0	0.0852 $\pm$ 0.0132	0.0956 $\pm$ 0.0140
40.0	0.1065 $\pm$ 0.0093	0.0818 $\pm$ 0.0148
60.0	0.0695 $\pm$ 0.0104	0.0763 $\pm$ 0.0120
80.0	0.0788 $\pm$ 0.0106	0.0803 $\pm$ 0.0097

Figure 7 Levels of pheophytin $\underline{a}$ (expressed as $^{ph}\underline{a}$/chl $\underline{a}$ + ph $\underline{a}$)
in $\underline{S}$. undulata from site (2) and site (6) as a function
of the concentration of zinc in the medium.
(a) 0.0 mg l^{-1}- 20.0 mg l^{-1}zinc
(b) 0.0 mg l^{-1}- 80.0 mg l^{-1} zinc
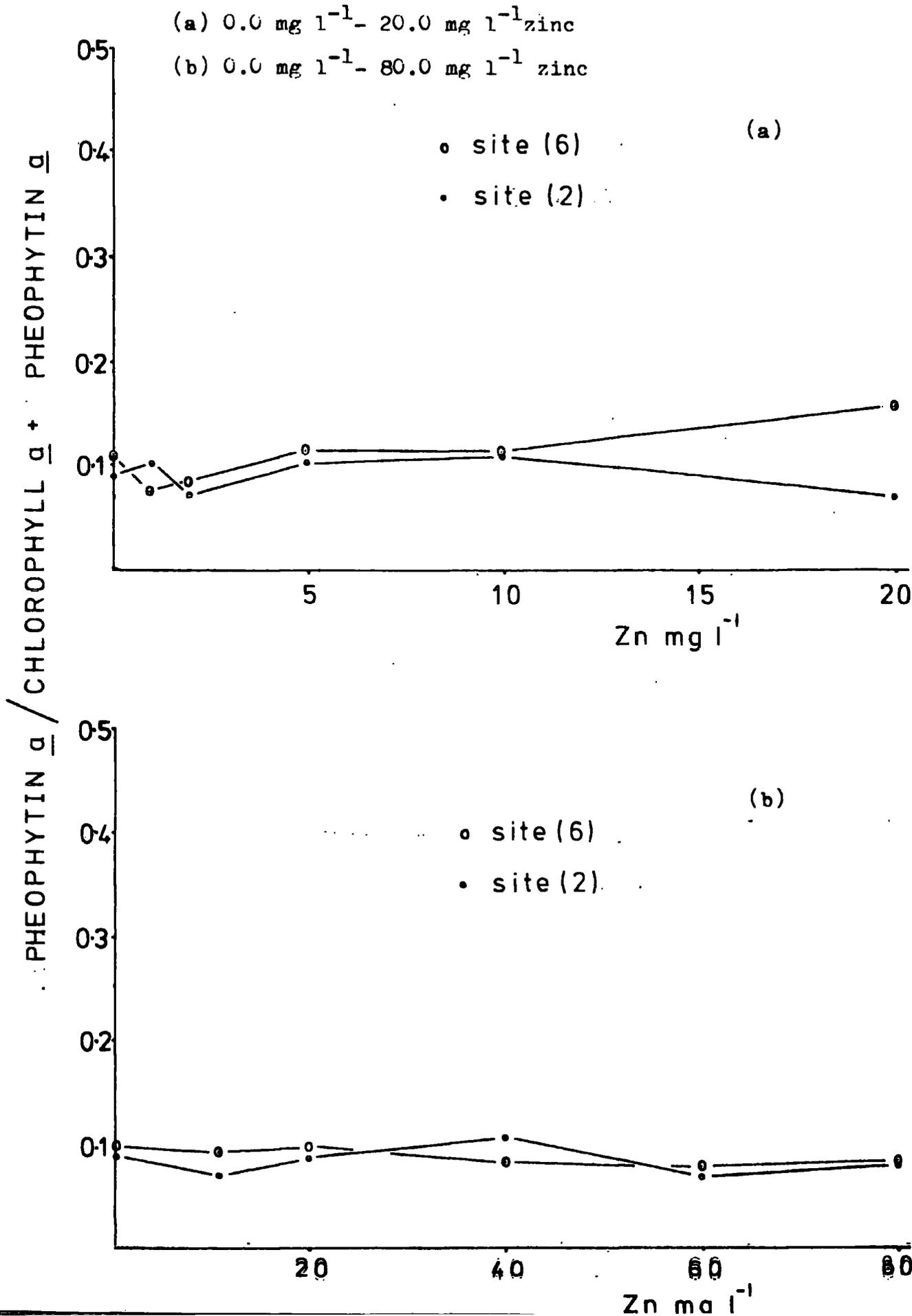
3.2. TRANSPLANT EXPERIMENT RESULTS

Unfortunately, four of the six sites selected for the transplant experiments dried up in the course of the study. Site (3) continued to flow throughout the period of the study and site (5) ceased to flow three weeks after the transplants were performed but remained as a stagnant pool for the remainder of the study period.

The results of the transplant monitoring are presented in Tables 3 - 8 and the values for the transplants at sites (3) and (5) are plotted in Figures 8 - 13. The only apparent trend in the data is a reduction in the levels of pheophytin a in the transplants at site (5) from the fourth week onwards. Monitoring of the levels of pheophytin a in the transplants was discontinued after the sixth week but the transplants at site (3) were observed for a further four weeks during which time they all remained apparently healthy. The water level at site (5) became reduced to the extent that the transplants were no longer submerged and observations at this site were therefore discontinued after the sixth week.

Eight weeks after transplantation, zinc analysis was carried out on replicates of 25 2.0 cm. shoot tips from each of the transplants at site (3). The results of this analysis are presented in table (9) and appropriate t values and probability levels in table (10). It can be seen from table 10 that _Scapania undulata_ transplanted from site (1) had a significantly greater zinc content than the material transplanted from the other four sites.

Table 3. Levels of pheophytin a (expressed as ^{ph} a/ph a + chl a) in S. undulata transplanted to site (1)

Origin of transplant	Weeks after transplantation						
	0	1	2	3	4	5	6
site (2)	0.1075	0.0896	0.0722	0.0867			
site (3)	0.1391	0.0865	0.0920				
site (4)	0.1440	0.0823	0.0753	0.0671			
site (5)	0.1497	0.0720	0.0768	0.0890			
site (6)	0.1418	0.1123	0.0920	0.1880			

Table 4. Levels of pheophytin a (expressed as ^{ph} a/chl a + ph a) in S. undulata transplanted to site (2)

Origin of transplant	Weeks after transplantation						
	0	1	2	3	4	5	6
site (1)	0.1357	0.0563	0.0560	0.1050			
site (3)	0.1391	0.1050	0.0834				
site (4)	0.1440	0.0642	0.0932	0.0840		0.0657	
site (5)	0.1497	0.1050	0.1230	0.2924			
site (6)	0.1418	0.0650	0.0750	0.0808			

Table 5. Levels of pheophytin $\underline{a}$ (expressed as $^{ph}\underline{a}$/chl $\underline{a}$ + ph $\underline{a}$) in S. undulata transplanted to site (3)

Origin of transplant	Weeks after transplantation						
	0	1	2	3	4	5	6
site (1)	0.1357	0.0899	0.0642	0.0911	0.0875	0.0541	0.0112
site (2)	0.1075	0.0652	0.0730	0.0939	0.2924	0.0874	0.1392
site (4)	0.1440	0.0890	0.0634	0.0452	0.0900	0.0535	0.0918
site (5)	0.1497	0.0653	0.0710	–	0.0781	0.1200	0.0554
site (6)	0.1418	0.0695	0.0825	0.0546	0.0649	0.0282	0.0567

Table 6. Levels of pheophytin $\underline{a}$ (expressed as $^{ph}\underline{a}$/chl $\underline{a}$ + ph $\underline{a}$) in S. undulata transplanted to site (4)

Origin of transplant	Weeks after transplantation						
	0	1	2	3	4	5	6
site (1)	0.1357	0.0630	0.0820				
site (2)	0.1075	0.1140	0.0930				
site (3)	0.1391	0.1256	0.0640				
site (5)	0.1497	0.0580	0.1250				
site (6)	0.1418	0.0350	0.0600				

Table 7. Levels of pheophytin a (expressed as ^{ph} a/chl a + ph a) in S. undulata transplanted to site (5)

Origin of transplant	Weeks after transplantation						
	0	1	2	3	4	5	6
site (1)	0.1357	0.0985	0.0489	0.1076	0.0413	0.0000	0.0229
site (2)	0.1075	0.0937	0.0920	0.0867	0.1057	0.0590	
site (3)	0.1391	0.1034	0.1410	0.0960	0.1621	0.0125	0.0000
site (4)	0.1440	0.1oo5	0.0549	0.0373	0.0206	0.0369	0.0132
site (6)	0.1418	0.0725	0.0810	0.0676	0.0340	0.0000	0.0414

Table 8. Levels of pheophytin a (expressed as ^{ph} a/chl a + ph a) in S. undulata transplanted to site (6)

Origin of transplant	Weeks after transplantation						
	0	1	2	3	4	5	6
site (1)	0.1357	0.0534	0.0562	0.0725			
site (2)	0.1075	0.0940	0.0680	0.0725			
site (3)	0.1391	0.0730	0.0950	0.3279			
site (4)	0.1440	0.0795	0.0464	0.0385			
site (5)	0.1497	0.0810	0.0836	0.1060			

Table 9. Zinc content of S. undulata transplanted to site (3) and
of S. undulata native to site (3) eight weeks after
transplantation (mean ± 1 S.E. ; n = 4 for sites (1) (3)
(4) (5) and (6), n = 2 for site (2))

Origin of transplant	Zinc content (μg / g dry wt.)
site (1)	8746 ± 227
site (2)	6342
site (3)	7347 ± 435
site (4)	7185 ± 140
site (5)	6742 ± 161
site (6)	7302 ± 493

Table 10. Values of t and probability levels for comparisons
between the values of mean zinc content tabulated in
Table 9. ($p < 0.05$ = * ; $p < 0.01$ = ** ; $p < 0.001$ = ***)

Site	(1)	(2)	(3)	(4)	(5)	(6)
(1)		4.272*	2.859*	5.859**	7.206***	2.661*
(2)			0.932	1.703	0.786	1.110
(3)				0.242	0.898	0.055
(4)					2.076	0.228
(5)						1.080
(6)						

Figure 8 Levels of pheophytin $\underline{a}$ (expressed as $^{ph}\underline{a}/chl\ \underline{a} + ph\ \underline{a}$)

in $\underline{S}$. undulata transplanted to site (3)

(a) transplant from site (1)

(b) transplant from site (2)
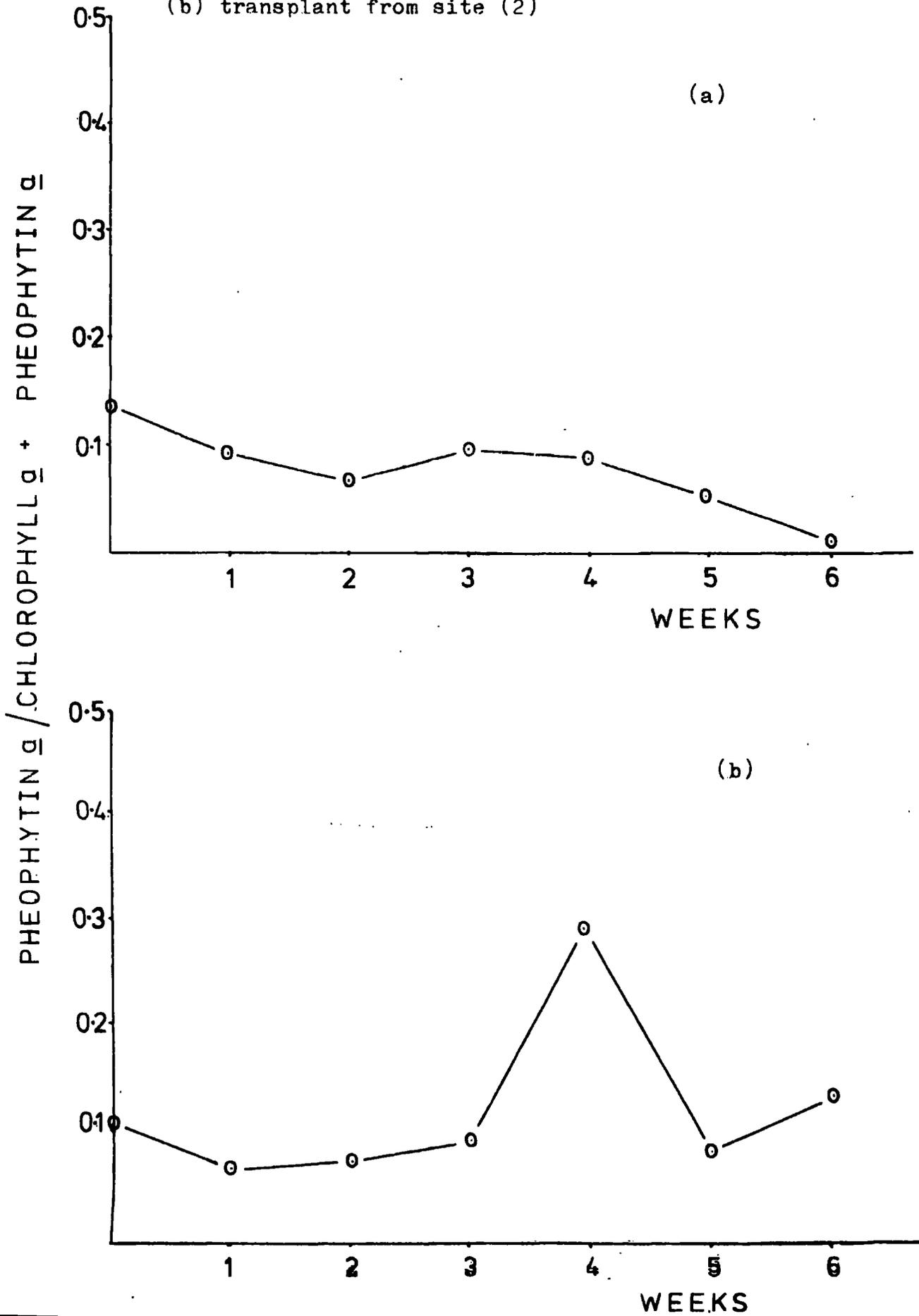
Figure 9 Levels of pheophytin $\underline{a}$ (expressed as $^{ph}\underline{a}$/chl $\underline{a}$ + ph $\underline{a}$ in $\underline{S}$. undulata transplanted to site (3)
(a) transplant from site (4)
(b) transplant from site (5)
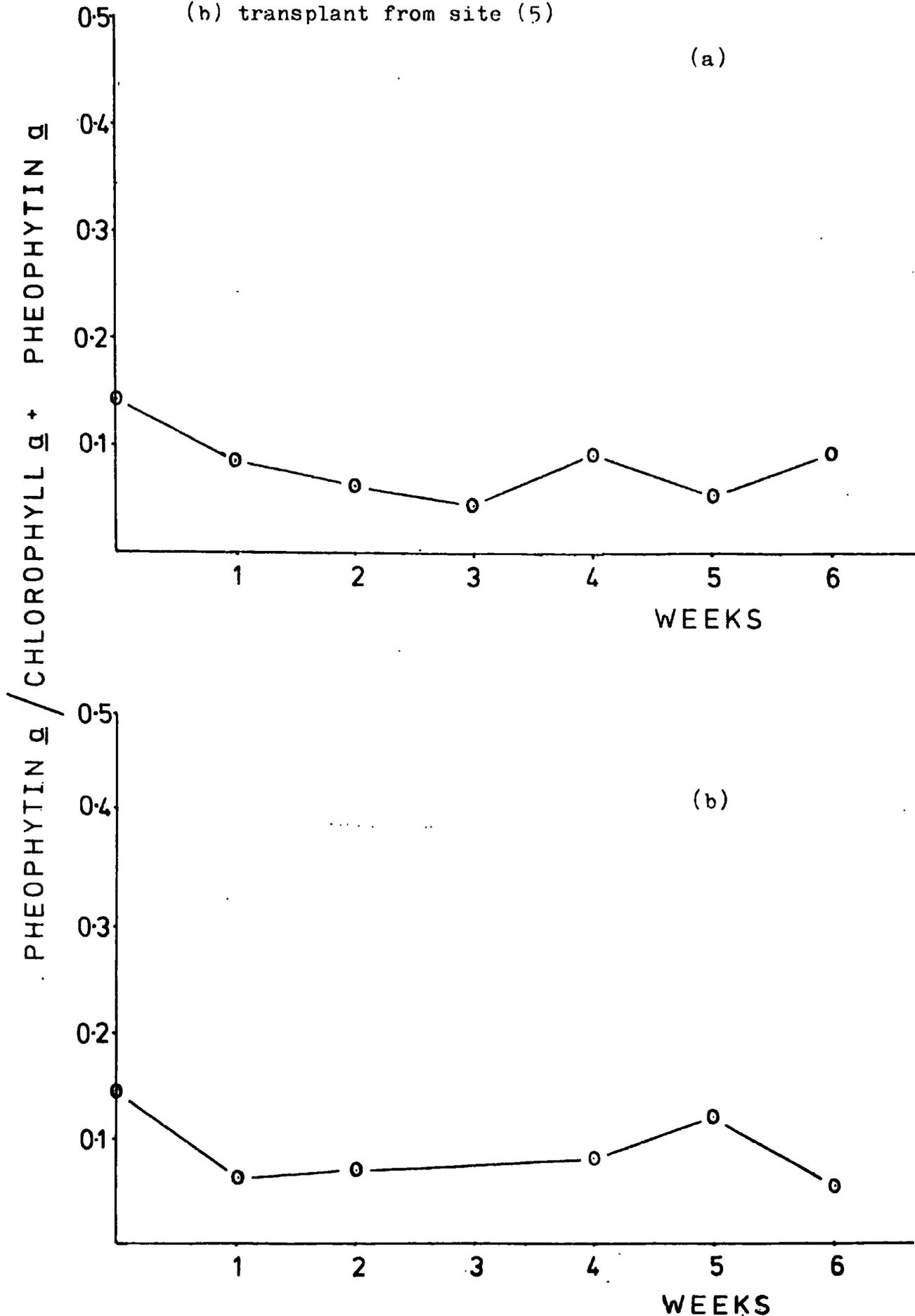
Figure 10   Levels of pheophytin $\underline{a}$ (expressed as $^{ph}\underline{a}$/chl $\underline{a}$ + ph $\underline{a}$

in $\underline{S}$. undulata transplanted to site (3) from site (6).
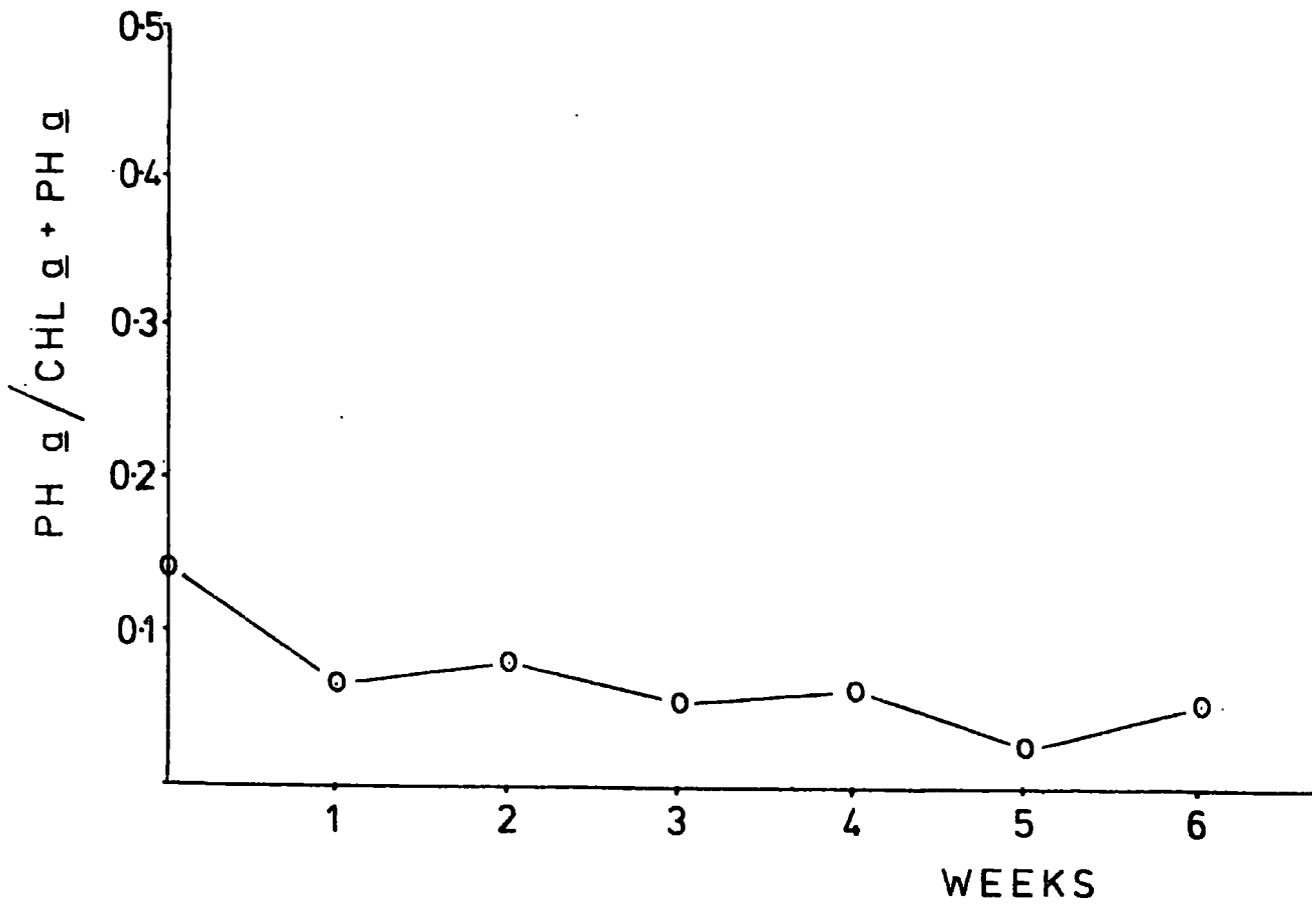
Figure 11   Levels of pheophytin <u>a</u> (expressed as ^{ph} <u>a</u>/chl <u>a</u> + ph <u>a</u>

                in <u>S</u>. <u>undulata</u> transplanted to site (5)

                (a) transplant from site (1)

                (b) transplant from site (2)
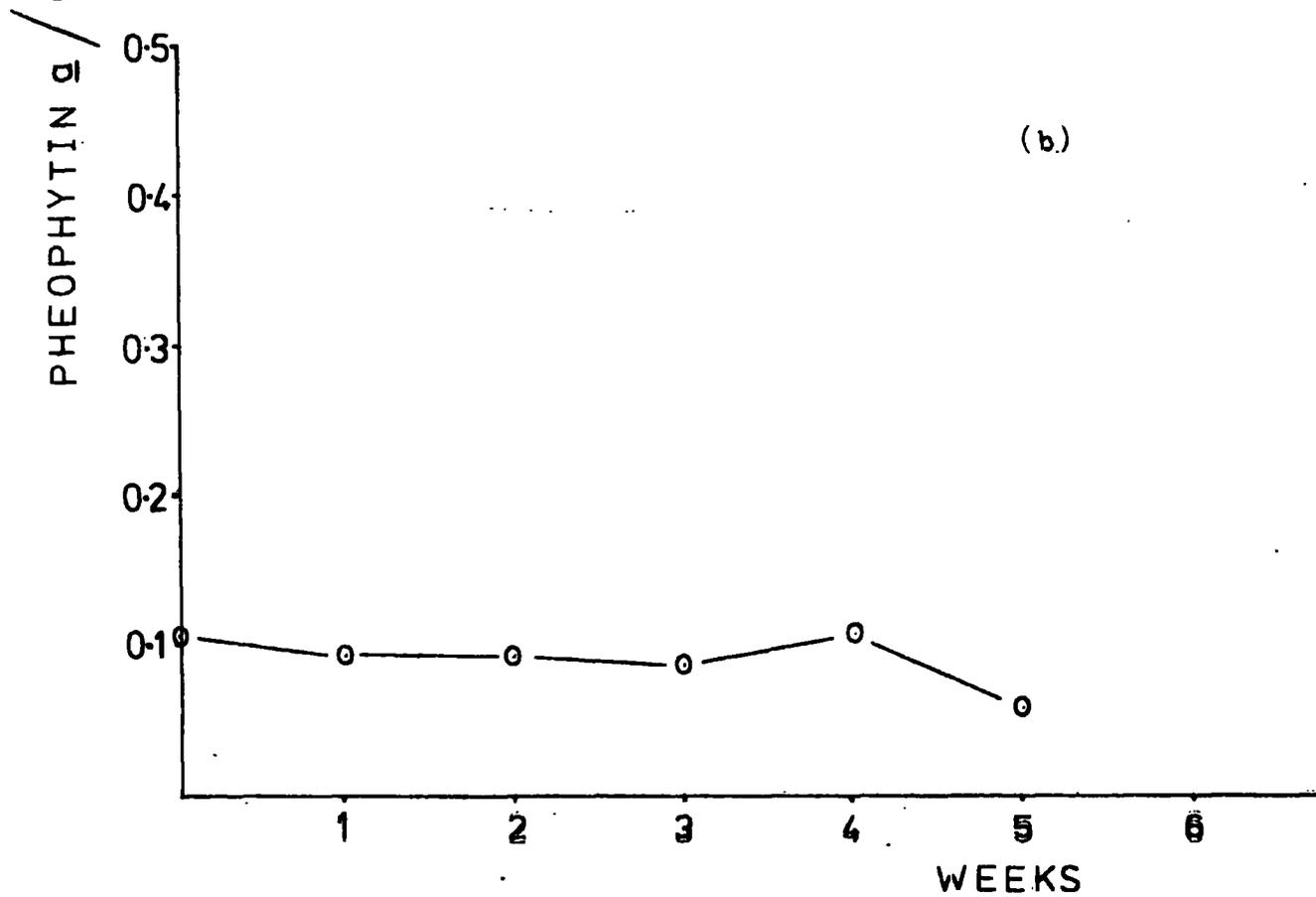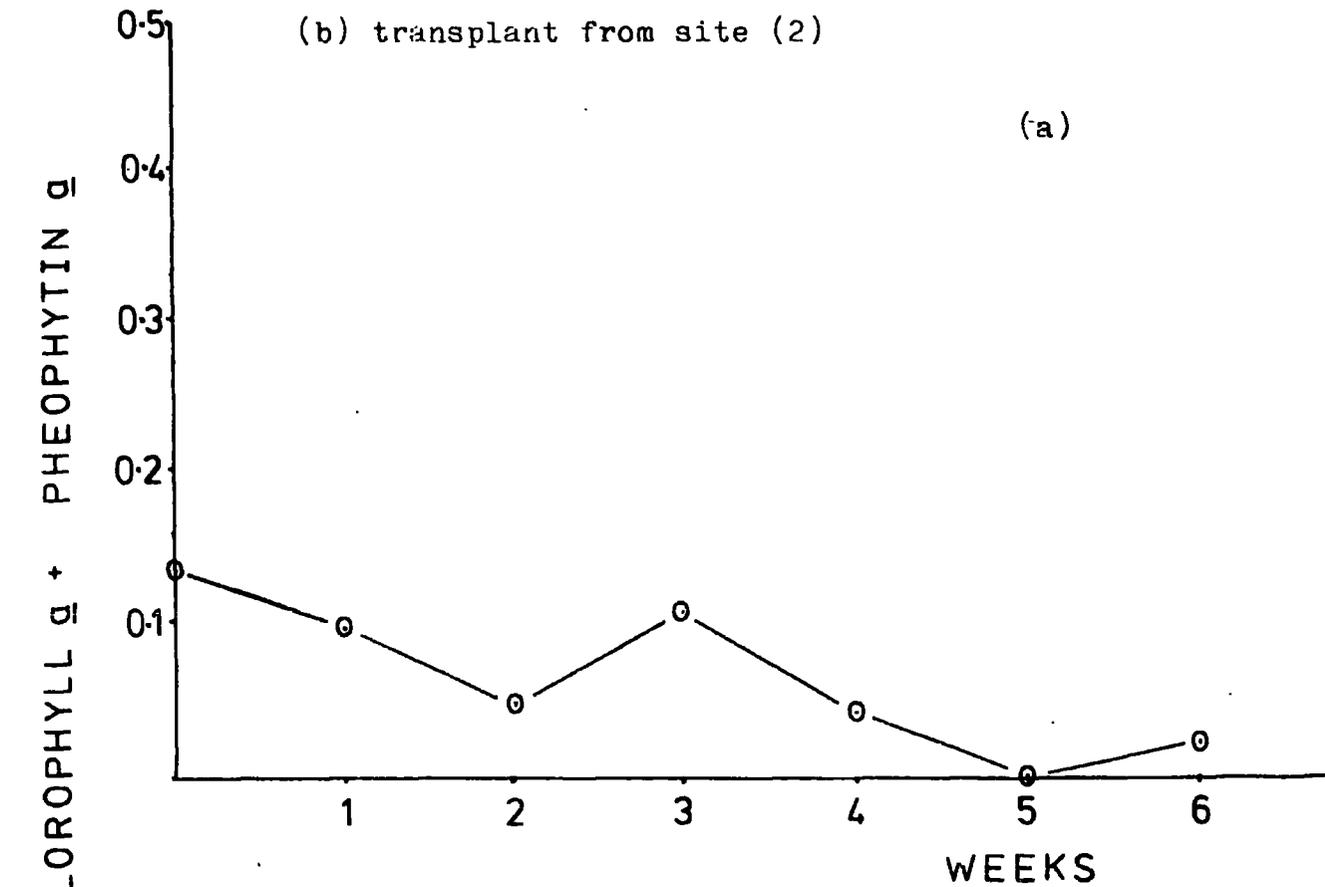
Figure 12　Levels of pheophytin $\underline{a}$ (expressed as $^{ph}\underline{a}$/chl $\underline{a}$ + ph $\underline{a}$

in $\underline{S}$. undulata transplanted to site (5)

(a) transplant from site (3)

(b) transplant from site (4)
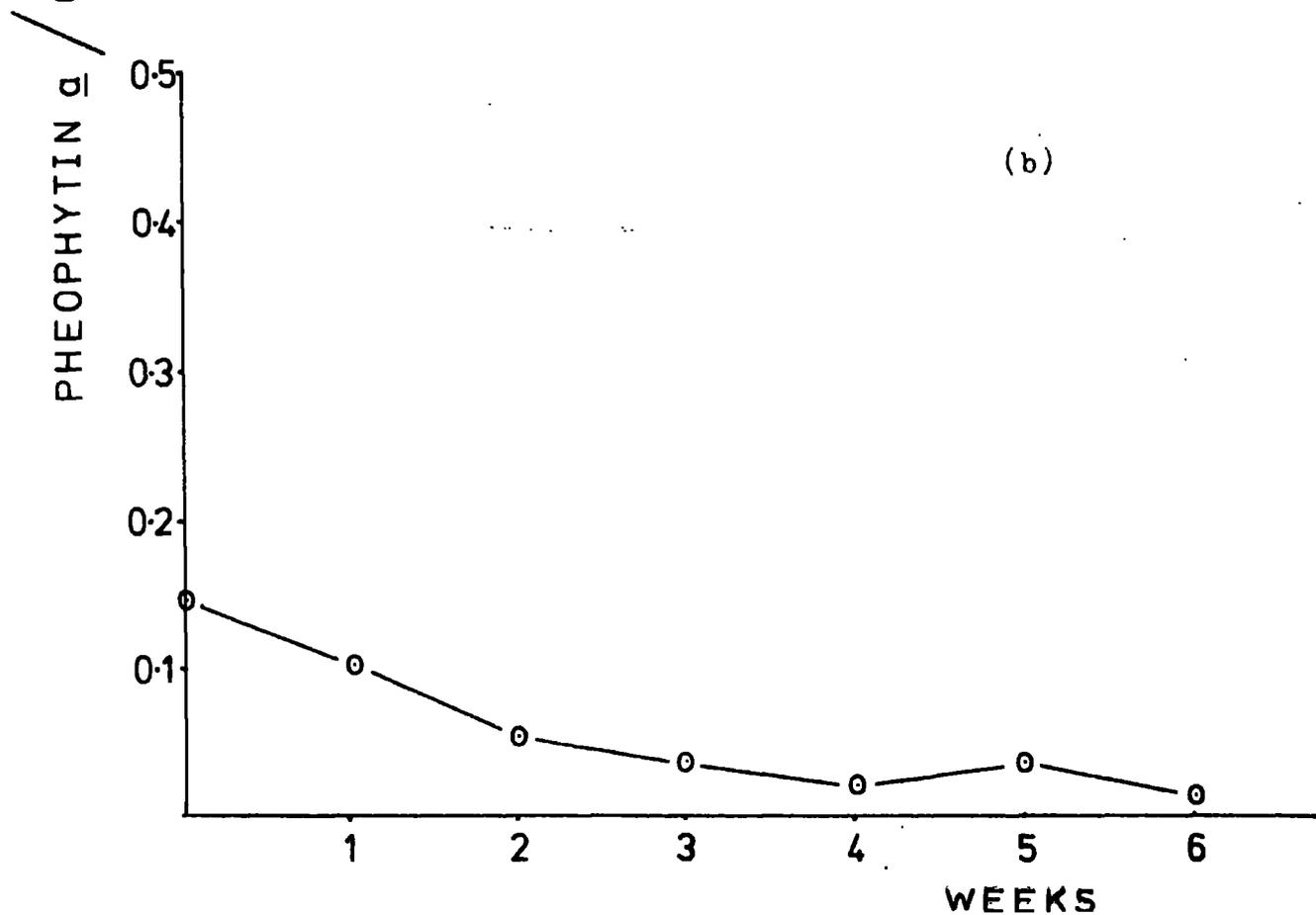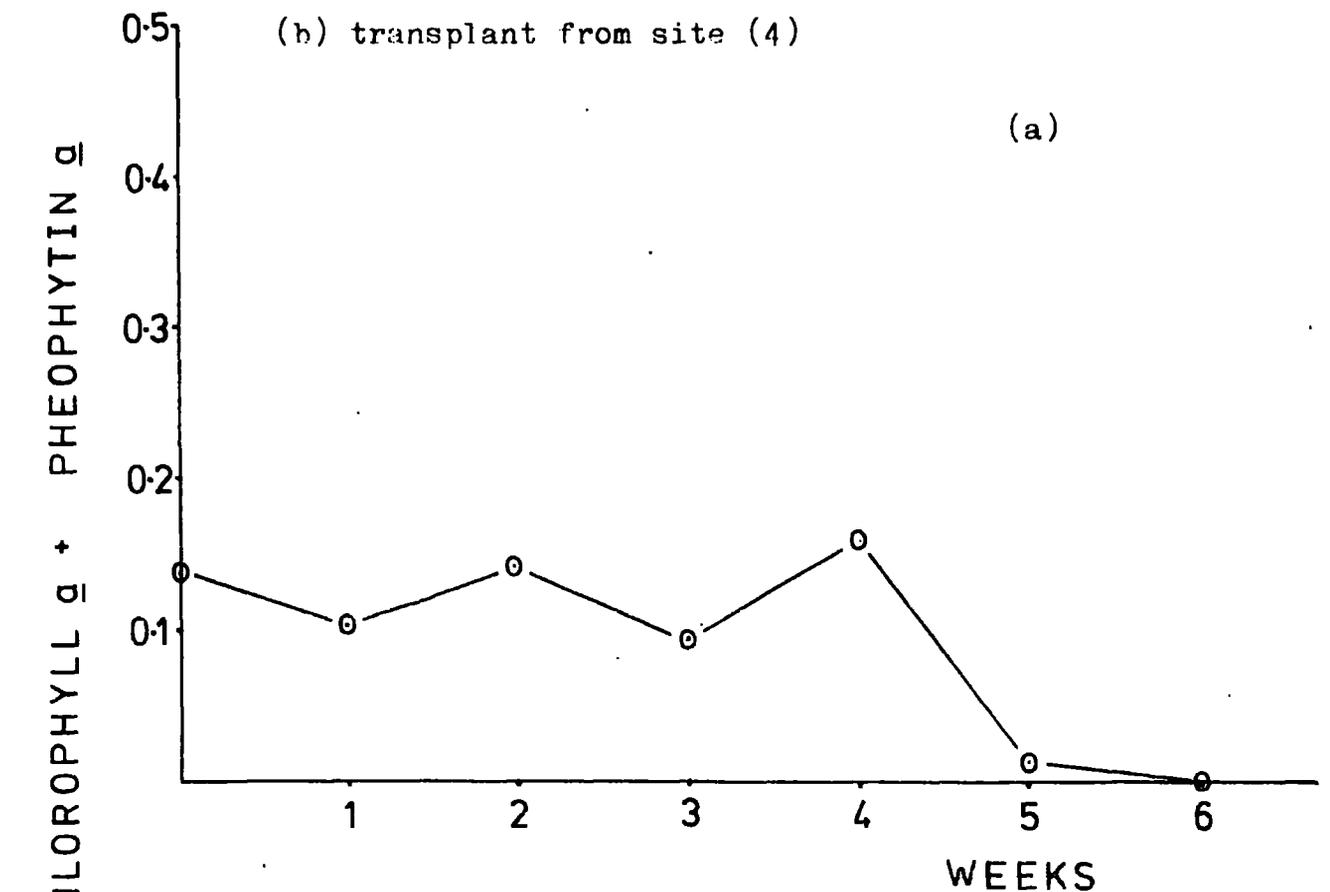
Figure 13   Levels of pheophytin a (expressed as ph a/chl a + ph a

in S. undulata transplanted to site (5) from site (6).
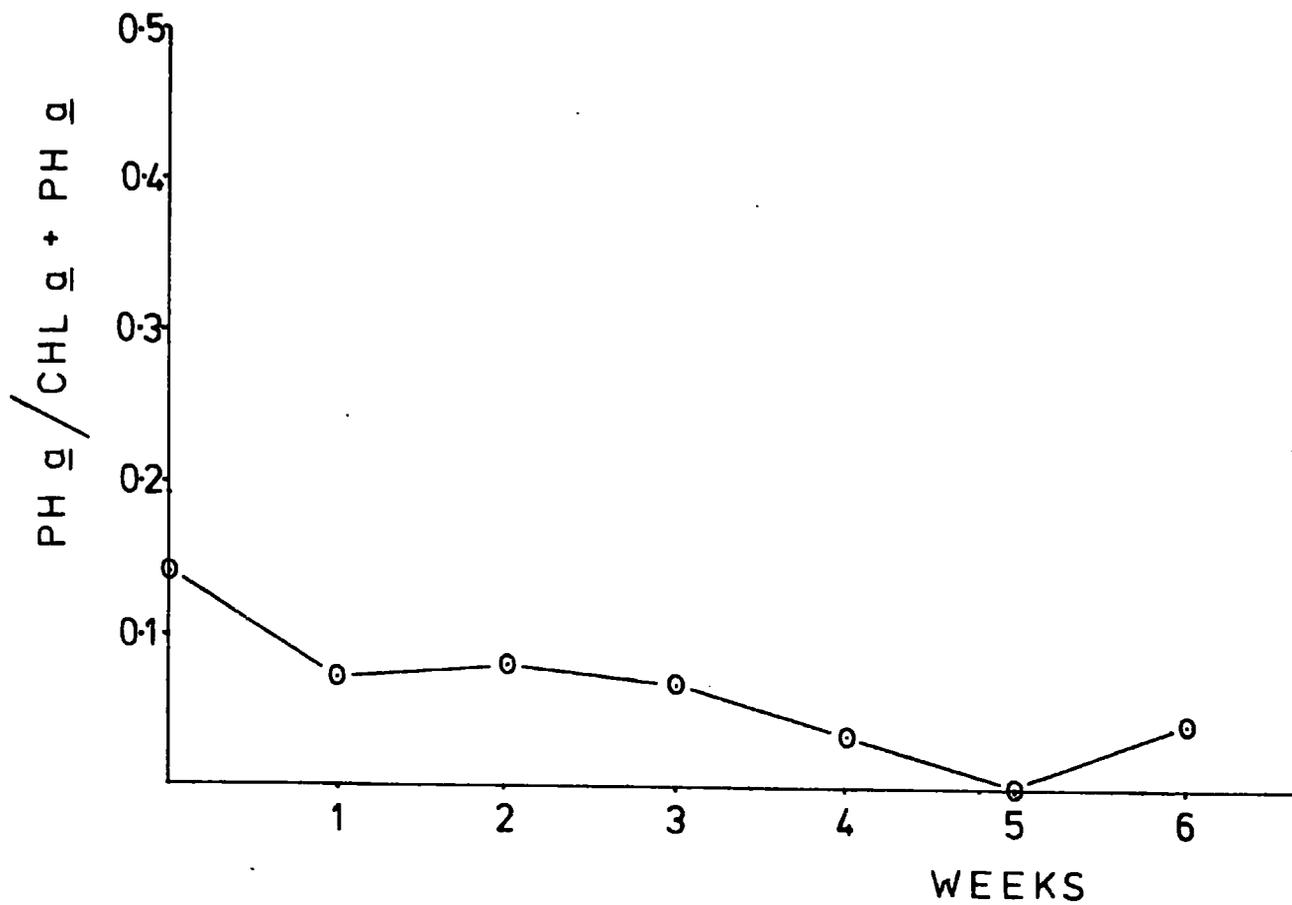
3.3. VARIATION IN THE ENRICHMENT RATIO OF ZINC IN S. UNDULATA

Values for the enrichment ratio of zinc in Scapania undulata at the six sites are presented in Table 11. The values for the concentration of zinc in the water at the six sites were obtained by averaging the values supplied by members of the Botany Department at Durham with the values obtained at the time of sampling of the plant material and in the immediately preceeding weeks. Site (8) was not sampled by the author but current water chemistry data was supplied by J. P. C. Harding of the Botany Department at Durham. Sampling was carried out during a period of exceptionally low flow at all sites and the concentration of zinc in the water at some of the sites had changed considerably from its normal level (table 12b). As the mechanism of accumulation of zinc by S. undulata is unknown, it was not known whether or not the material would have equilibrated with the altered zinc concentration of the water and for this reason an average value for the zinc content of the water was used in calculating the enrichment ratios. Because the exchangeability of accumulated zinc is not known, the accuracy of the calculated enrichment ratios is doubtful, however, the results suggest that there is considerable variation between the sites in the enrichment ratio of zinc in S. undulata.

Table 11. Zinc content of $\underline{S}$. __undulata__ and enrichment ratio of zinc in $\underline{S}$. __undulata__ at sites (2) (3) (5) (6) (7) and (8) (mean $\pm$ 1 S.E. ; n = 10).

Site	Zinc content of water (mg l^{-1})	Zinc content of $\underline{S}$. __undulata__ (μg / g dry wt.)	Enrichment ratio
(2)	2.408	2714 $\pm$ 123	1127
(3)	0.971	3184 $\pm$ 188	3280
(5)	0.126	452 $\pm$ 191	3587
(6)	0.031	129 $\pm$ 7	4215
(7)	0.965	7976 $\pm$ 291	8265
(8)	0.761	919 $\pm$ 27	1208

Table 12. Water chemistry data for sites (1)-(7).

(a)

Site	pH	Concentration of calcium (mg l^{-1})	Concentration of magnesium (mg l^{-1})	Concentration of phosphate (mg l^{-1})
(2)	6.3	19.00	5.50	0.005
(3)	6.4	10.21	2.83	0.014
(5)	5.2	3.08	1.28	0.014
(6)	4.1	2.44	0.96	0.008
(7)	7.5	20.60	4.30	0.010
(8)	6.9	12.50	2.95	0.007

Table 12(b) . Levels of zinc at the study sites (mg 1^{-1})

Site	Values obtained in the present study			Values supplied by members of the Botany Department
(1)	2.453	2.221		
(2)	1.015	0.940	1.030	6.65
(3)	0.980	1.010	0.941	0.953
(4)	0.029			
(5)	0.054	0.119	0.258	0.072
(6)	0.018	0.030	0.036	0.031
(7)	0.970	0.960	0.910	1.020
(8)				0.761

3.4. RESULTS OF LABORATORY ACCUMULATION EXPERIMENTS.

(a) EFFECT OF CONCENTRATION OF ZINC IN THE MEDIUM ON RATE OF UPTAKE OF ZINC BY S. UNDULATA

The results of this experiment are presented in Table 13 and Figure 14. The results indicate that approximately three times more zinc is taken up in 2 d. than in 30 min. at each concentration. The rate of uptake of zinc increases with increasing concentration of zinc in the medium up to a concentration of approximately 60 mg l⁻ zinc, beyond which level no further increase in the rate of uptake occurs. The saturation point is approximately the same for both the 30 min. and the 2 d. experiment, with some indication of a small increase in the rate of uptake of zinc at concentrations greater than 60 mg l⁻ zinc in the 2 d. experiment.

Table 13. Zinc content of S. undulata in media containing
2 mg l^{-1} - 100 mg l^{-1} zinc, after 30 minutes and
after two days.

Zinc content of medium (mg l^{-1})	Zinc content of S. undulata after 30 min (µg / g dry wt.)	Zinc content of S. undulata after 2 d (µg / g dry wt.)
2.0	371	1491
4.0	435	3317
6.0	864	4188
8.0	952	5029
10.0	1063	5220
15.0	1469	6477
20.0	2179	7166
25.0	1846	7592
30.0	2526	8500
35.0	3076	9106
40.0	3039	9733
60.0	4377	8967
80.0	4375	10617
100.0	4625	11397

Figure 14 Uptake of zinc by S. undulata as a function of the concentration of zinc in the medium (a) over a 30 min period (b) over a two day period.
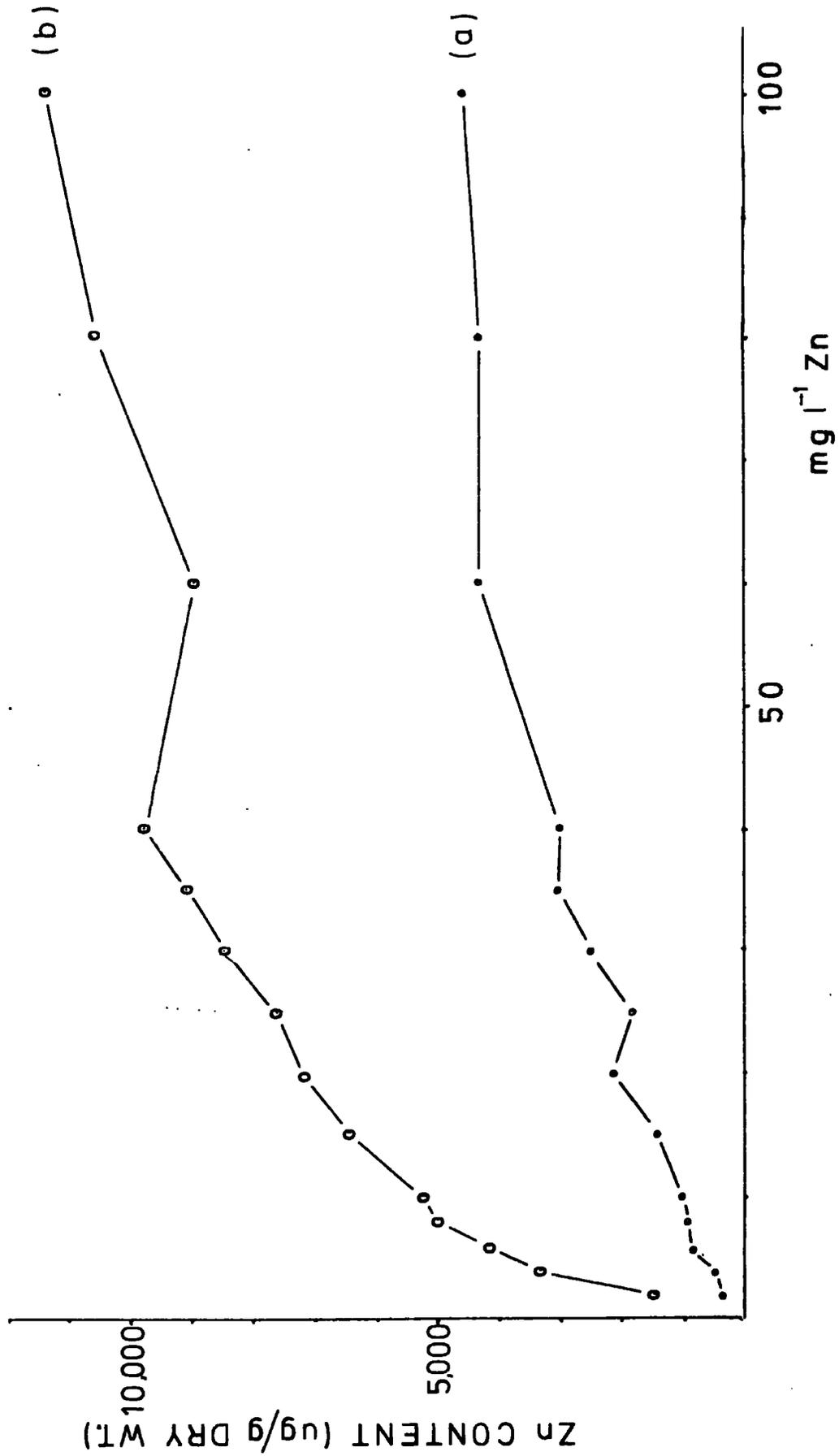
(b) EFFECT OF LIGHT ON UPTAKE OF ZINC BY S. UNDULATA

The results of this experiment are presented in Tables 14 and 15 and Figures 15 and 16. The results obtained from monitoring the concentration of zinc in the medium in each of the flasks suggest that there is no difference in the pattern of uptake of zinc by Scapania undulata in the light and in the dark (Figures 17 and 18). However, at the end of the experiment, the material in each flask was analysed for zinc and the mean zinc content of the material which had been incubated in the light was found to be significantly greater than that of material which had been incubated in the dark (p 0.01). The method which was used to monitor the uptake of zinc by S. undulata in this experiment was evidently not sufficiently accurate to detect the difference which is approximately 15% of the total amount of zinc accumulated (Table 14).

Table 14. Uptake of zinc by $\underline{S}$. undulata in the light and in the

dark from medium containing 1.0 mg l^{-1} zinc, as

estimated from the concentration of zinc in the

medium (mean $\pm$ 1 S.E. ; n = 5).

Time (h)	Uptake of zinc by $\underline{S}$. undulata in the light ($\mu g/g$ dry wt.)	Uptake of zinc by $\underline{S}$. undulata in the dark ($\mu g/g$ dry wt.)
0.5	888 $\pm$ 82	1056 $\pm$ 83
1.0	962 $\pm$ 90	1045 $\pm$ 93
2.0	1121 $\pm$ 45	1190 $\pm$ 45
3.0	1221 $\pm$ 19	1273 $\pm$ 68
4.0	1339 $\pm$ 70	1430 $\pm$ 62
5.0	1423 $\pm$ 58	1530 $\pm$ 61
6.0	1523 $\pm$ 97	1801 $\pm$ 65
8.0	1635 $\pm$ 36	1711 $\pm$ 54
16.0	1747 $\pm$ 43	1890 $\pm$ 51
22.0	1703 $\pm$ 32	1882 $\pm$ 77
28.0	1630 $\pm$ 69	1899 $\pm$ 101
40.0	1438 $\pm$ 39	1772 $\pm$ 87
52.0	1462 $\pm$ 42	1568 $\pm$ 40
64.0	1478 $\pm$ 20	1554 $\pm$ 48
76.0	1559 $\pm$ 26	1612 $\pm$ 37
89.0	1500 $\pm$ 20	1582 $\pm$ 33

Table 15. Zinc content of S. undulata after 96 hours in medium
containing 1.0 mg l^{-1} zinc, in the light and in the
dark (mean $\pm$ 1 S.E. ; n = 5).

Zinc content of S. undulata in the light (μg / g dry wt.)	Zinc content of S. undulata in the dark (μg / g dry wt.)
1203 $\pm$ 42	1038 $\pm$ 25

(t value = 3.361 ; p < 0.01)

Figure 15 Uptake of zinc by S. undulata in the dark from medium containing 1.0 mg l^{-1} zinc, as a function of time. Uptake estimated from the change in concentration of the medium (mean ± 2 S.E. ; n = 5).
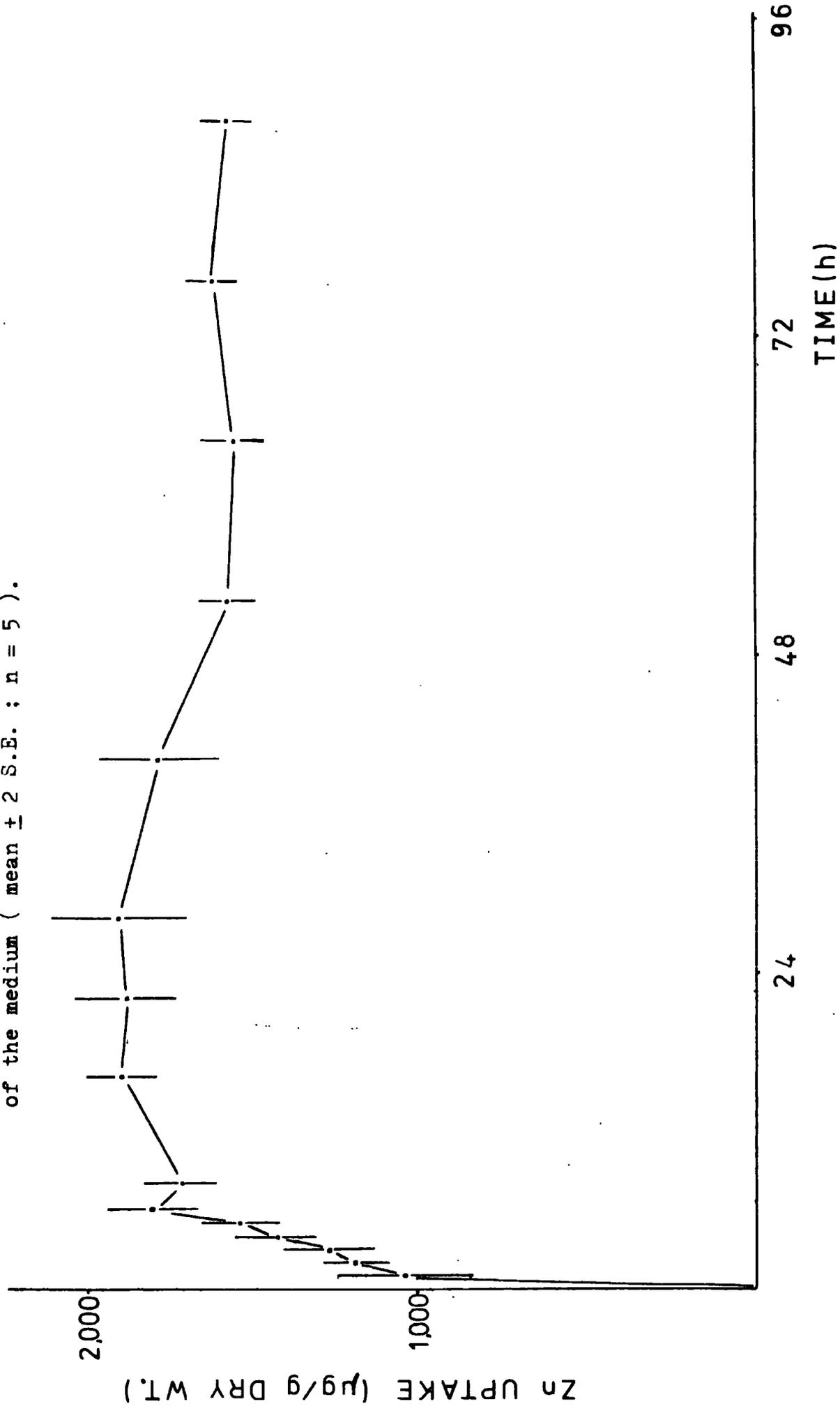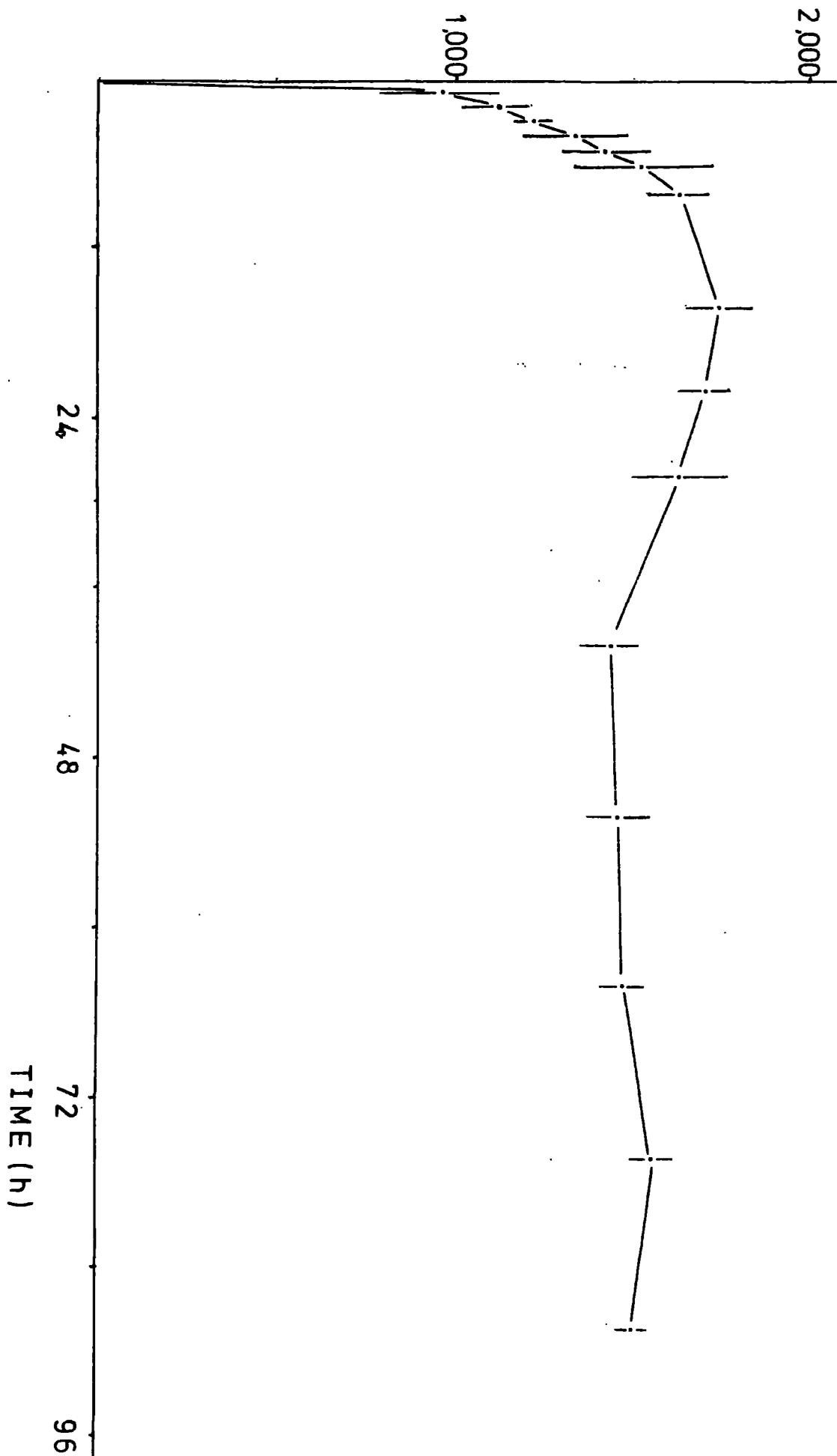
Figure 16 Uptake of zinc by S. undulata in the light from medium containing 1.0 mg l^{-1} zinc, as a function of time. Uptake estimated from the change in concentration of the medium (mean $\pm$ 2 S.E. ; n = 5).

(c) EFFECT OF TEMPERATURE ON UPTAKE OF ZINC
BY S. UNDULATA

The mean zinc content of the material at each of the
three temperatures 6 h, 12 h, 24 h and 48 h after the
start of the experiment are plotted in Figures 17 - 19.
Appropriate $\underline{t}$ values and probability levels for
comparisons between the mean values are listed in Table
17. Unfortunately, a second variable was introduced
into the experiment as the material at $32^{\circ}C$ survived for
a short period only and was apparently dead by the start
of the experiment, the chlorophyll having been completely
broken down to colourless degradation products. The
results indicate that the dead material at $32^{\circ}C$ took up
zinc significantly more rapidly than the live material
at $14^{\circ}C$. 48 h. after the commencement of the experiment,
however, there was no significant difference between the
zinc content of the material at $32^{\circ}C$ and that of the
material at $14^{\circ}C$.

One 250 ml. flask of material was maintained at each
of the temperatures and the zinc content of the medium
monitored over a seven day period. The zinc content of
the material in each flask has been calculated by
difference and the results are plotted in Figure 20.
The results indicate that accumulation of zinc at each
temperature ceases approximately four days after the start
of the experiment, the zinc content of the material at
each temperature remaining constant thereafter. The
pattern of uptake of zinc at each of the three temperatures

is similar with some indication of a greater initial
rate of uptake of zinc by the dead material at 32°C
as compared to the live material at 14°C and 24°C.
At the end of the seven day period, the material in
each of the 250 ml. flasks was analysed for zinc.
The results are presented in Table 19 and suggest a
similar equilibrium zinc content at each temperature.

Table 16. Zinc content (µg / g dry wt.) of S. undulata at 14°C 24°C and 32°C in medium containing 2.0 mg l^{-1} zinc (mean ± 1 S.E. ; n = 4).

Time (h)	Temperature 14°C	24°C	32°C
6	1320 ± 39	1557 ± 110	1773 ± 27
12	1834 ± 29	2068 ± 110	2254 ± 178
24	2072 ± 36	2605 ± 209	2694 ± 63
48	2566 ± 138	2470 ± 90	2790 ± 114

Table 17. Values of t and probability levels for comparisons between the values of mean zinc content tabulated in Table 16 ($p < 0.05$ = * ; $p < 0.01$ = ** ; $p < 0.001$ = ***).

6 h	14°C	24°C	32°C
14°C		2.026	8.599***
24°C			1.620
32°C			

12 h	14°C	24°C	32°C
14°C		2.057	2.703*
24°C			0.988
32°C			

24 h	14°C	24°C	32°C
14°C		2.508	8.539***
24°C			0.407
32°C			

48 h	14°C	24°C	32°C
14°C		0.582	1.253
24°C			2.203
32°C			

Table 18. Uptake of zinc (μg / g dry wt.) by $\underline{S}$. $\underline{undulata}$ at $14^{\circ}C$, $24^{\circ}C$ and $32^{\circ}C$ from medium containing 2.0 mg l^{-1} zinc, as estimated from the concentration of zinc in the medium.

Time (h)	Temperature		
	$14^{\circ}C$	$24^{\circ}C$	$32^{\circ}C$
2	1095	1278	1112
4	1772	1490	1983
6	1822	1956	2168
8	2570	2209	2640
10	2720	2159	2711
12	2794	3050	3556
24	3054	2862	3114
28	3017	3050	3217
32	3238	3175	3373
48	3308	3424	3817
53	3503	3668	3878
56	3821	4048	4438
72	3953	4789	4499
76	4040	4711	4744
86	4040	4715	4836
96	3989	4856	4642
100	4098	4943	4690
104	4116	5106	4615
120	4098	5227	4561
125	4116	5061	4654
144	4418	5139	4415
168	4220	4984	4686

Table 19. Zinc content of $\underline{S}$. <u>undulata</u> at $14^{\circ}C$, $24^{\circ}C$ and $32^{\circ}C$ after seven days in medium containing 2.0 mg l^{-1} zinc.

Temperature	Zinc content (µg / g dry wt.)
$14^{\circ}C$	3470
$24^{\circ}C$	3215
$32^{\circ}C$	3518

Figure 17   Uptake of zinc by live $\underline{S}$. $\underline{undulata}$ at $14^{O}C$ from
medium containing 2.0 mg l^{-1} zinc, as a function
of time (mean $\pm$ 2 S.E. ; n = 4).
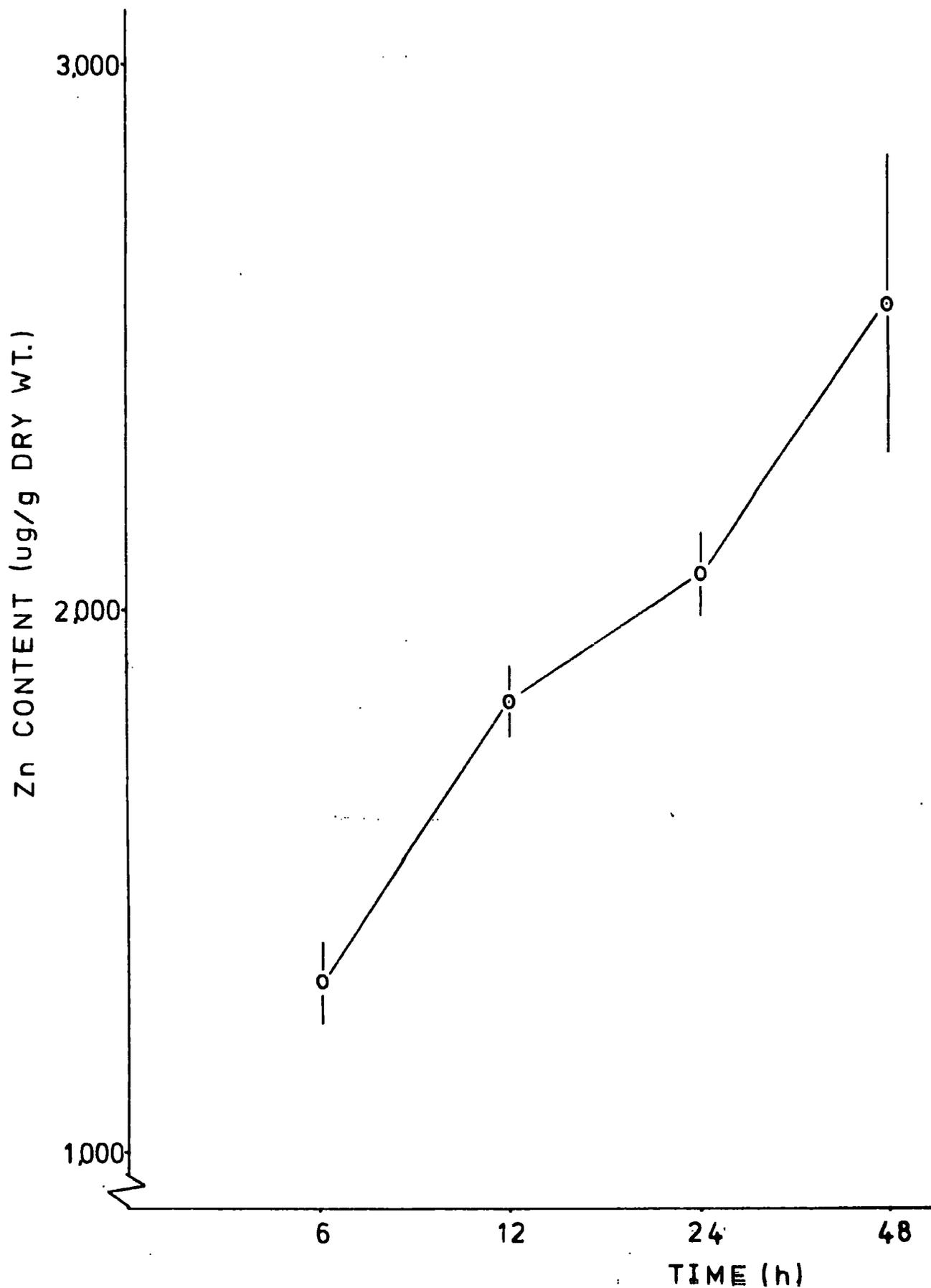
Figure 18   Uptake of zinc by live _S. undulata_ at $24^{o}C$ from medium containing 2.0 mg l^{-1} zinc, as a function of time (mean $\pm$ 2 S.E. ; n = 4).
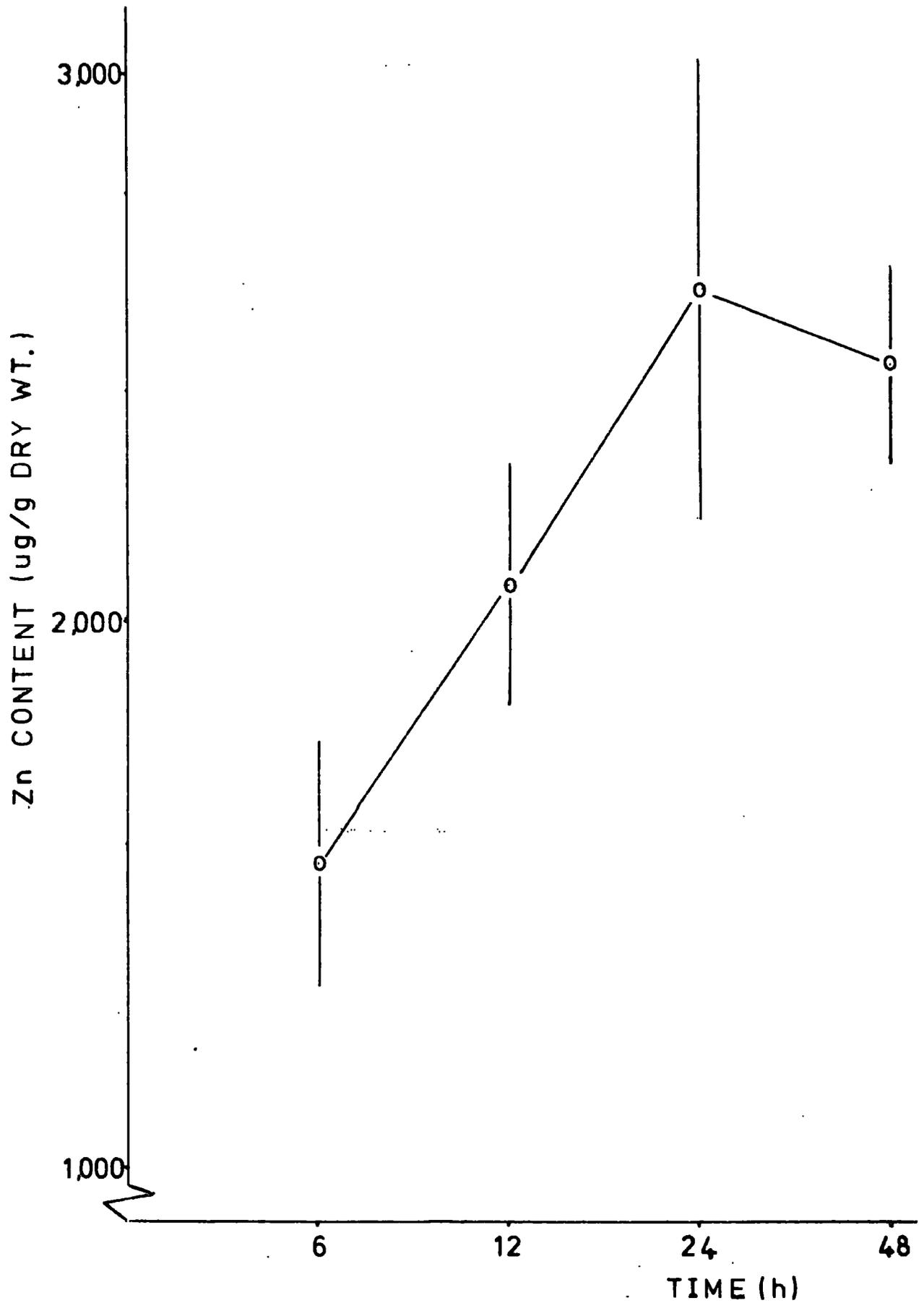
Figure 19. Uptake of zinc by dead $\underline{S}$. undulata at $32^{\circ}C$ from medium containing 2.0 mg 1^{-1} zinc, as a function of time (mean $\pm$ 2 S.E. ; n = 4).
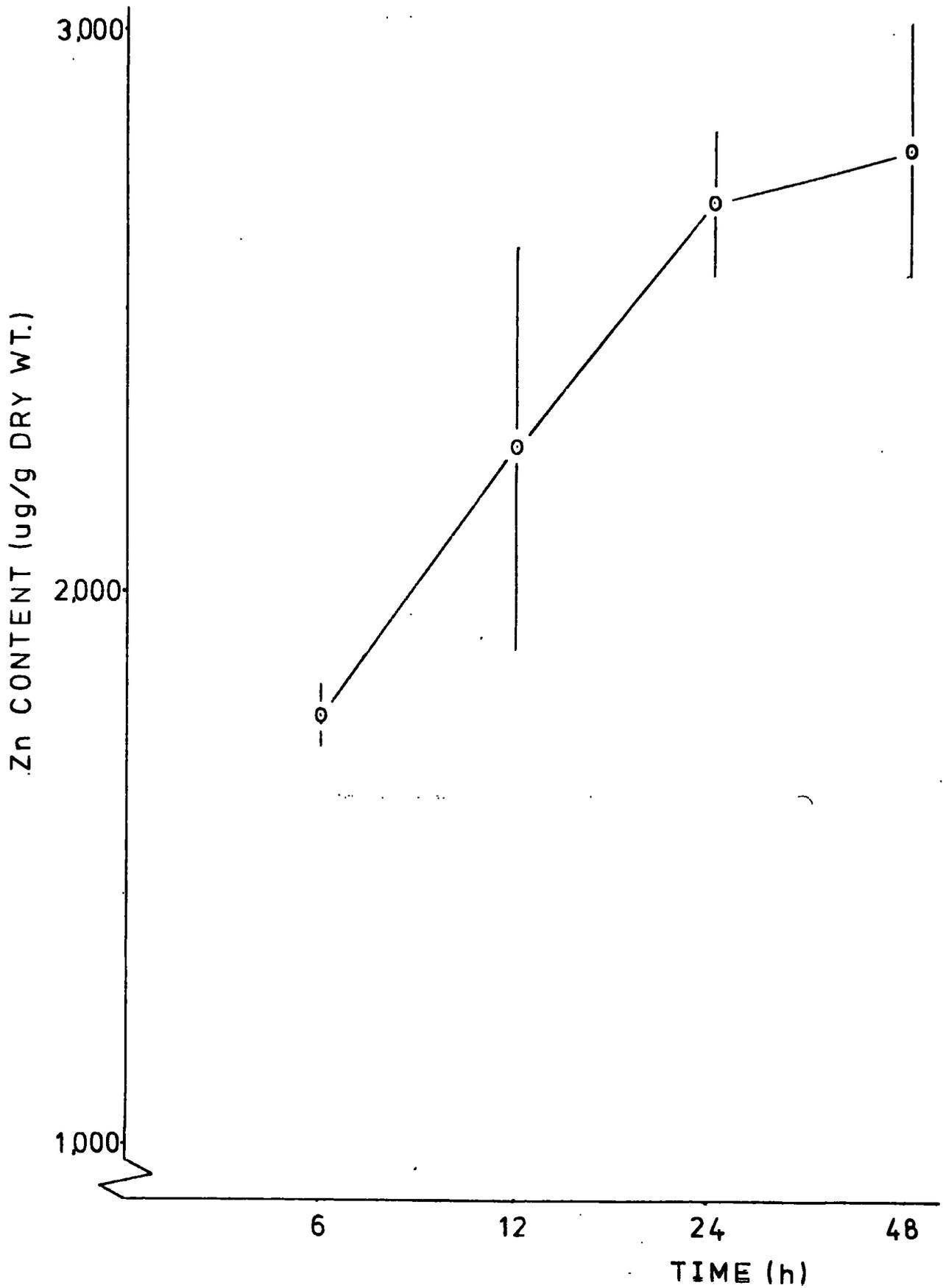
Figure 20. Uptake of zinc by live S. undulata at 14°C and 24°C and dead S. undulata at 32°C as a function of time. Uptake estimated from the change in concentration of the medium, initial concentration 2 mg l^{-1} zinc.
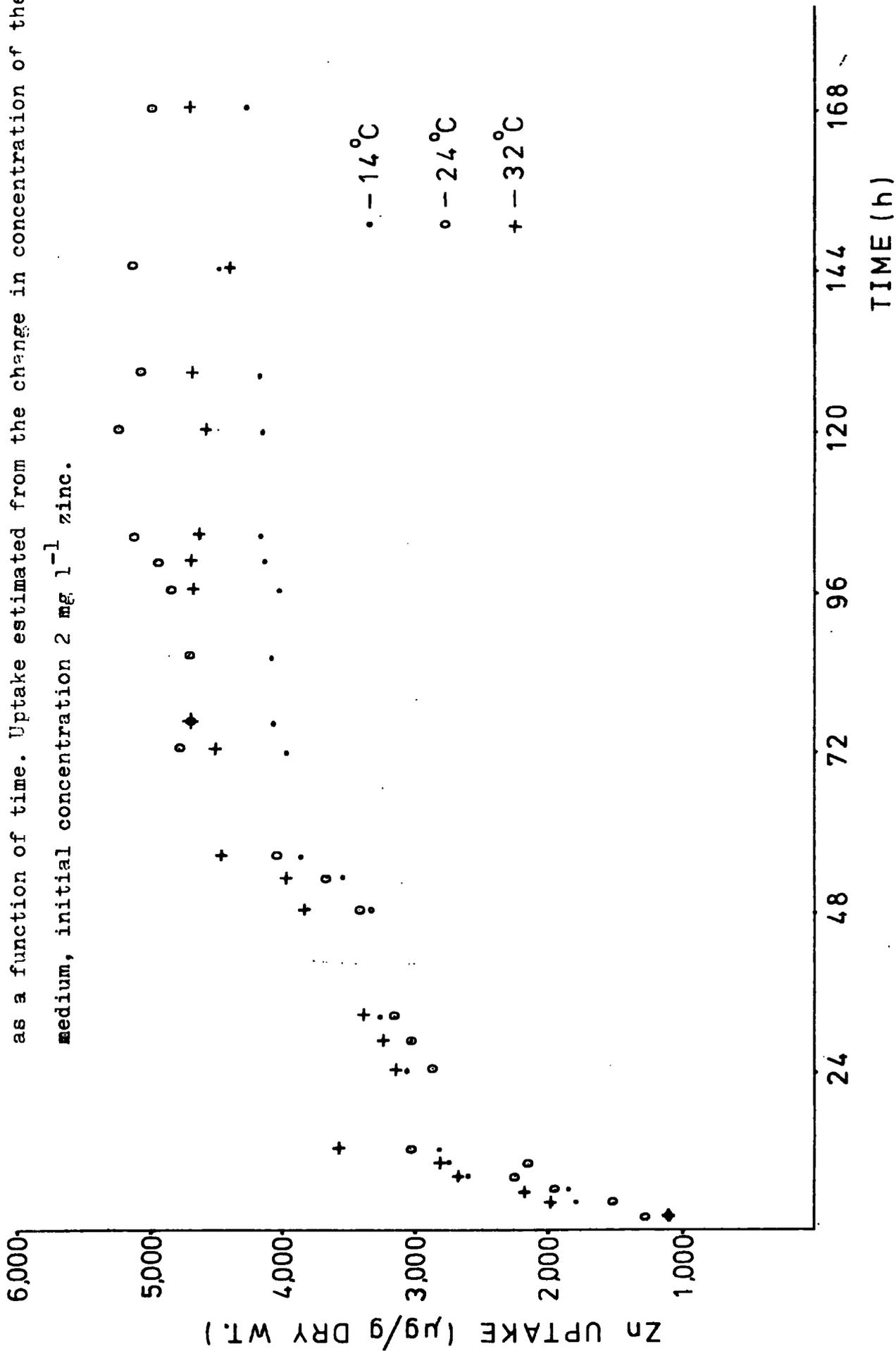
4. DISCUSSION

The purpose of the toxicity tests and the transplant experiments was to determine whether or not _Scapania undulata_ from zinc enriched sites possesses a greater degree of zinc resistance than _S. undulata_ from sites with much lower concentrations of zinc in the water. If this is the case, then it would suggest that the populations of _S. undulata_ from zinc enriched sites are zinc resistant ecotypes of the species.

The condition of the plant material in the toxicity tests and the transplant experiments was monitored by determining the ratio of chlorophyll _a_ to pheophytin _a_ in samples of the material. This method has been used to monitor the condition of transplants of _S. undulata_ in a study by McLean and Jones (1974). For reasons which will be discussed it is now considered that this ratio is not a good index of the condition of plant material.

Pheophytin _a_ is generally considered to be an intermediate in the breakdown of chlorophyll _a_ (Moss, 1968) thus:-

chlorophyll _a_ ——→ pheophytin _a_ ——→ pheophorbide _a_ ——→ colourless products
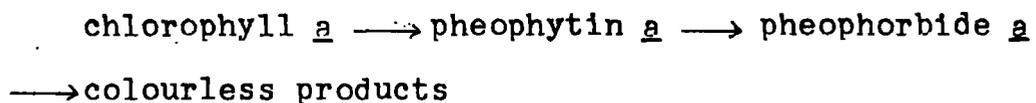
Pheophytin _a_ and pheophorbide _a_ retain an intact porphyrin ring and are therefore pigments which differ from chlorophyll _a_ only in the composition of the side chain functional group. Degradation to colourless products involves rupture of the porphyrin ring. As pheophytin _a_ is an intermediate not an end product of

the breakdown process, it will not necessarily
accumulate during chlorophyll degradation. Daley
and Brown (1972) have found that pheophytin a and
pheophorbide a do not accumulate during breakdown of
chlorophyll in senescent algal cultures except after
a prolonged exposure to darkness in some species and
they have suggested that chlorophyll a may be broken
down directly to colourless products without the
formation of pheophytin a as an intermediate, there
being no chemical reason why alteration of the side
chain functional group should occur before rupture of
the porphyrin ring. The following schematic diagram of
chlorophyll transformations has therefore been proposed
(Daley and Brown, 1972):

$$\text{chlorophyll a} \longrightarrow \text{pheophytin a} \longrightarrow \text{pheophorbide a}$$
$$\searrow \qquad \downarrow \qquad \swarrow$$
$$\text{colourless products}$$
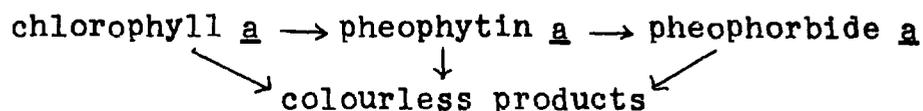
If degradation of chlorophyll a to form colourless
products may occur without the formation of pheophytin a
as an intermediate, then there is no basis for using the
ratio of chlorophyll a to pheophytin a as an index of the
condition of plant material. Furthermore, even if
pheophytin a is a necessary intermediate in the breakdown
of chlorophyll a, it will not necessarily accumulate and
the amount accumulated would, in any case, be a function
of both its rate of formation and its rate of breakdown
and would therefore bear no simple relationship to the
rate of breakdown of chlorophyll a. It is therefore

suggested that the ratio of chlorophyll a to pheophytin a is not a suitable index of the condition of plant material. This suggestion is borne out by the results obtained from monitoring the transplants at site (5) (Figures 11 - 13). Site (5) became a stagnant pool three weeks after the start of the experiment (Figure 5) and in the succeeding weeks an algal scum developed on the surface of the water. During this period, the transplanted material deteriorated and died probably as a result of the reduced light intensity and oxygen tension in the water, however the ratio of chlorophyll a to pheophytin a in the transplants increased during this period and the levels of pheophytin a were undetectable on some occasions. These results clearly indicate that pheophytin a does not necessarily accumulate during breakdown of chlorophyll a and that the ratio of chlorophyll a to pheophytin a is therefore not a measure of the amount or rate of chlorophyll degradation.

For reasons which have been discussed in the preceeding paragraph the method used to monitor the condition of $\underline{S}$. undulata in the toxicity tests and the transplant experiments is of doubtful validity therefore no conclusions can be drawn from the results. However, during the toxicity tests there was no visually apparent change in any of the material which suggests that under the experimental conditions (Section 2.1), $\underline{S}$. undulata from both site (2) and site (6) can survive concentrations of zinc in the range 2 mg l^{-1} 20 mg l^{-1} for a period of

six days. There was no apparent difference in the zinc
resistance of the material from the two sites. A long
term toxicity experiment was carried out on a very
small scale (Section 3.1) and it was found that
S. undulata from both sites could survive and grow in
medium containing 10 mg l^- zinc. In medium containing
20 mg l^- zinc, material from both sites survived for up
to 10 weeks but no appreciable growth was apparent and
at 100 mg l^- material from both sites died. Again these
results suggest that there is no difference in the zinc
resistance of the material from the two sites. The
results of the long term experiment indicate that it
would be feasible to investigate the toxicity of zinc to
S. undulata from zinc enriched and low zinc sites by
growing the material in media with a range of zinc
concentrations and using the upper limit of the range
in which growth can occur as a measure of the zinc
resistance of the material. A similar method has been
used to detect genetic adaptation in algae (Say et al.
in press; Whitton and Harding in press). In this way
further information regarding the nature of zinc resistance
in S. undulata could be obtained.

As a result of exceptionally dry weather conditions,
five of the six sites selected for the transplant
experiments dried up shortly after the start of the
study. The one site at which water continued to flow
in sufficient volume to submerge the transplants, was
site (3) a zinc enriched site with a concentration of

approximately 1.0 mg l^{-1} zinc in the water. Monitoring
of the ratio of chlorophyll a to pheophytin a in the
transplanted material at site (3) was discontinued
after the sixth week but the transplants were observed
for a further four weeks, during which time they all
remained apparently healthy. These observations suggest
that there is no difference in the zinc resistance of
the material transplanted from the two zinc enriched
sites as compared to the material transplanted from the
three low zinc sites. Ca^{2+} and Mg^{2+} and phosphate
have been found to reduce the toxicity of zinc to stream
organisms (Section 1.3) and it is therefore possible that
the transplants might have been protected from the toxic
effects of zinc by high concentrations of one or more of
these ions. The levels of these ions in the water at
site (3) were, however, determined and found to be low
(Table 12). The results of both the toxicity tests and
the transplant experiments therefore suggest that
S. undulata has not evolved zinc tolerant ecotypes.

Eight weeks after transplantation, the zinc content
of each of the transplants at site (3) was determined
(Tables 9 and 10). If the transplants are all genetically
similar as suggested, then they would be expected to have
a similar zinc content. If, however, some of the transplants
are genetically adapted, zinc resistant ecotypes then they
may have a lower zinc content, having become adapted to
reduce the degree to which they accumulate zinc. It was
found that the transplants from sites (2) (4) (5) and (6)

all contained approximately the same amount of zinc as
material native to site (3) whereas material transplanted
from site (1) had a significantly greater zinc content.
The zinc content of S. undulata at site (1) was
unfortunately not determined before the site dried up.
As this site had the highest level of zinc in the water,
however, it is probable that the transplant from site (1)
had the highest initial zinc content. Analysis of
S. undulata from site (2), the other zinc enriched site
from which material was transplanted, indicated that it
contained less zinc than the material at site (3),
therefore the transplant from site (1) was probably the
only transplant which had an initial zinc content greater
than that of material native to site (3). The fact that
the material transplanted from site (1) had apparently
not equilibrated with the lower zinc content of the water
at site (3), ten weeks after transplantation, suggests that
accumulated zinc is not readily exchangeable in S. undulata.
It is possible that the physical and chemical conditions in
the situation in which the transplant from site (1) was
placed were in some way different to the conditions which
the other transplants were subject to and that this resulted
in there being a higher equilibrium zinc content for the
site (1) transplant, however as the transplants were all
placed in the same pool, this seems unlikely. The
observation that the other four transplants all had a
similar zinc content suggests that they were genetically
similar. From the point of view of using S. undulata as

a monitoring organism, it is important to know how
rapidly zinc is accumulated and lost. If accumulated
zinc is lost only gradually when the concentration of
zinc in the water becomes reduced, as the results suggest,
then short term reductions in the zinc content of stream
waters could not be detected by monitoring the zinc
content of S. undulata. Results obtained from transplants
of Lemanea fluviatilis by J. P. C. Harding (pers. comm.)
indicate that in this species also a proportion of the
zinc is not readily exchangeable.

Investigation of the enrichment ratio of zinc in
S. undulata at six sites indicated that there was
considerable variation between sites (Table 11). It was
intended to determine enrichment ratios at a greater
number of sites, however as the majority of streams in
the area dried up, it was not possible to extend the study.
Unfortunately, as the zinc content of the water at some of
the sites had become reduced as the volume of flow became
reduced and it is not known whether or not the material at
these sites had equilibrated with the altered zinc content
of the water, the significance of the observed differences
between sites cannot be assessed. The values obtained
range between 1100 and 8200 and are rather lower than those
which have been obtained for bryophytes by other workers.
Patrick (1974) obtained values of the enrichment ratio of
zinc in S. undulata which range between 2071 and 19281 and
Leeder (1972) obtained values of the enrichment ratio of
zinc in Hygrohypnum ochraceum which vary between 45,000

and 120,000. One reason for this difference is that analysis was carried out on 1.0 cm. shoot tips whereas other workers have used entire stems; Patrick (1974) found that the zinc content of $\underline{S}$. undulata increased withthe age of the material. The enrichment ratio of zinc in aquatic plants has been found to be affected by pH, being much lower in a stream at pH 2.6 than in a stream at pH 6.7 (Patrick, 1974). The concentrations of Ca^{2+}, Mg^{2+} and PO_4^- ions as well as that of hydrogen ions have been found to affect the toxicity of zinc to algae and to affect uptake of zinc by algae (Section 1.3) and it therefore seems possible that these ions may antagonise uptake of zinc by $\underline{S}$. undulata also. No relationship between the concentrations of these ions and the enrichment ratio of zinc in $\underline{S}$. undulata is however apparent in the data obtained for the six sites in this study (Tables 11 and 12). In field studies of this type the sites vary with respect to many parameters and it is therefore difficult to isolate the factors which are responsible for variation in the data. Experiments carried out under standard conditions in the laboratory are probably a more efficient way of investigating the accumulation of heavy metals by plant species.

The initial laboratory experiment was designed to determine whether or not accumulation of zinc occurs under laboratory conditions and to obtain information regarding the duration of the uptake process and the effect of concentration on rate of uptake (Section 3.4(a)). The

results indicate that zinc is accumulated to high levels
under laboratory conditions and that uptake occurs over
a period longer than 30 min. In the initial 30 min, the
level of uptake was dependent on the concentration of
zinc in the medium up to a concentration of 60 mg l^{-1},
beyond which level, no increase in the level of uptake
occurred (Figure 11). Over the two day period, increase
in the level of uptake was greatly reduced beyond a
concentration of 60 mg l^{-1} zinc but some increase was
still evident up to the maximum of 100 mg l^{-1} zinc.
This result suggests that the mechanism of accumulation
which operates during the initial 30 min is also
responsible for the majority of zinc accumulated over
the two day period, but that a secondary mechanism is
additionally involved. A similar saturation level has
been found by Pickering and Puia (1969) for the aquatic
moss Fontinalis antipyretica in an experiment of two hours
duration which suggests that a similar mechanism of
accumulation is in operation in this species as in
S. undulata. From their results, Pickering and Puia
have suggested that a rapid passive accumulation occurs
during the initial 90 min of zinc uptake in F. antipyretica
and that thereafter active accumulation continues slowly for
a period of several days. If this model of uptake of zinc
by F. antipyretica is correct then uptake over a two hour
period is largely a result of passive accumulation and the
implication is that a similar passive mechanism of
accumulation of zinc occurs in S. undulata with a secondary

mechanism, possibly active accumulation, being additionally involved over a two day period.

McLean and Jones (1974) have investigated the effect of the concentration of zinc[65] in the medium on the uptake of zinc[65] by S. undulata. Their results indicate that saturation occurs at a concentration of approximately 10 mg l^{-1} zinc with a secondary increase in the uptake of zinc at concentrations above 25 mg l^{-1} zinc, i.e. quite different results to those obtained in the present study. The difference may be an effect of the media used; algal culture medium enriched with zinc sulphate in the present study and zinc sulphate solution in the study by McLean and Jones. Alternatively, the difference may result from the effect of accumulated radioactive zinc on the plant material in the study by McLean and Jones.

The nature and importance of active accumulation has been further investigated by a comparison of the uptake of zinc by S. undulata in the light and in darkness (Section 3.4(b)). It was found that the zinc content of material incubated in the light for a period of four days was approximately 15% greater than that of material incubated in darkness (Table 14). The suppression of accumulation of zinc by S. undulata under conditions of darkness suggests that an active mechanism is involved, however as active accumulation may not have been entirely suppressed under these conditions, 15% may be an underestimate of the proportion of zinc which is accumulated by active mechanisms.

Pickering and Puia (1969) found that uptake of zinc by the aquatic moss $\underline{F}$. antipyretica was reduced by approximately 50% in conditions of darkness after an incubation period of two days, therefore it is probable that active uptake accounts for a greater proportion of the total amount of zinc accumulated in $\underline{F}$. antipyretica than in $\underline{S}$. undulata. Unfortunately, the results of experiments of this type are not entirely unambiguous. Gutknecht (1963) has suggested that increase in the intracellular pH of photosynthesising cells may result in an increase of the Donnan potential of the protoplasm with a resultant increase in the level of zinc accumulated passively. Pickering and Puia (1969) have, however, found that uptake of zinc by $\underline{F}$. antipyretica is inhibited by DNP, a decoupling agent for adenosine triphosphate, which suggests that an active mechanism of accumulation is involved in this species. If the original interpretation of the results is accepted then the results agree with those obtained in the previous experiment and suggest that an active mechanism of accumulation accounts for a small proportion of the total amount of zinc accumulated.

The experiment described in Section 2.4(d) was designed to determine the effect of temperature on accumulation of zinc by $\underline{S}$. undulata. Most biological processes have a Q_{10} of approximately two, therefore an increase in temperature would be expected to result in an increase of the rate of active accumulation. Unfortunately, the material at 32° C died during the equilibration period therefore a second variable was introduced into the experiment. It was found

that the dead material at 32°C took up zinc more rapidly than the live material at 14°C though material at all three temperatures reached a similar equilibrium zinc content two days after the start of the experiment (Figures 16 - 19). Previous workers who have found that dead material accumulates heavy metals to an equivalent or greater extent than live material have concluded that accumulation is an entirely passive process (Gutknecht, 1963; Brown and Bateson, 1972), however there is an alternative explanation of the results. Gutknecht (1963) studied uptake of zinc[65] by three species of marine algae and found that dead algal material absorbed more zinc than live material, which indicates that the dead material has a greater ability to accumulate zinc passively than the live material. Gutknecht suggests that this may be a result of cation exchange taking place on previously inaccessible sites in the dead material or may result from an increase in the intracellular pH of the dead material which would cause a higher degree of dissociation of internal weak multibasic acids and thus an increase in the number of sites available for cation absorbtion. If the ability of S. undulata to accumulate heavy metals passively is similarly increased on the death of the plant, then it may be that in this experiment, an increase in the level of passive accumulation in the dead material at 32°C compensated for the active component of uptake in the live material. The increased rate of uptake in the dead material probably results from removal of the membrane barrier with

consequent ease of access to internal sites for cation

exchange. Further support for the suggestion that

active accumulation of zinc does take place in S. undulata,

derives from the observation that rate of uptake at $24^{\circ}C$

is greater than at $14^{\circ}C$ (Figures (16) and (17)), although

the difference is not statistically significant.

Several tentative conclusions can be drawn from the

results obtained in this study. The results of the

toxicity tests and transplant experiments suggest that

the populations of S. undulata at the zinc enriched sites

studied are not genetically adapted zinc resistant

ecotypes of the species. Brown and Bateson (1972) have

studied the lead uptake capacity of samples of the

terrestial moss Grimmia doniana from lead polluted and

unpolluted sites and have suggested that in this species

of bryophyte also, there is no differentiation into heavy

metal resistant races. This apparent lack of genetic

adaptation in populations of bryophytes growing at heavy

metal polluted sites, contrasts with the results which

have been obtained for the majority of the higher plants

and algae which have been studied (Antonovics et al. 1971;

Say et al. in press; Harding and Whitton in press). The

results of the accumulation experiments suggest that

uptake of zinc by S. undulata occurs largely by passive

mechanisms of accumulation but that an active mechanism

of accumulation does account for a small proportion of

the total amount of zinc accumulated. Similar evidence

for active accumulation of zinc by the aquatic moss

Fontinalis antipyretica has been obtained by Pickering
and Puia (1969) and other workers have demonstrated the
presence of lead inclusions in the nuclei of cells of
the moss Rhytidiadelphus squarrosus exposed to
environmental pollution and uptake of lead into the
cells by pinocytosis under experimental conditions
(Skaar et al, 1973; Gullvag et al. 1974). It seems
probable therefore that accumulation of heavy metals is
not an entirely passive process in all species of
bryophyte as has been suggested in the past (Ruhling and
Tyler, 1970; Tyler, 1972; Brown and Bateson, 1973).

From the point of view of using S. undulata as a
monitoring organism, the results of these experiments
indicate that environmental factors do affect the level
of zinc accumulated and that the enrichment ratio of
zinc is not therefore a constant property of the species.
The observation that dead material accumulates as much
zinc as live material indicates that it may be feasible
to use dead material suspended in the stream water as a
means of monitoring the levels of heavy metals in the water.
A similar method has been devised for monitoring levels of
atmospheric pollution (Goodman and Roberts, 1971). As there
is no active accumulation in dead material, the enrichment
ratios in dead material would be less variable than in live
material.

The long term toxicity test carried out in this study
was a pilot study only and further experiments of this type
are necessary to confirm or disprove the suggestion that

S. undulata has not evolved heavy metal tolerant races.
Investigation of the effect of metabolic inhibitors and
low temperature on accumulation would yield further
information on the extent of metabolic accumulation of
zinc in S. undulata. If zinc is accumulated by active
mechanisms, as suggested, then there must be a means of
maintaining cytoplasmic levels of zinc at a non-toxic
level. This may involve selective transport of zinc
into the vacuole as suggested by Mathys (1975) or may
involve immobilisation of zinc in the nucleus as
demonstrated for Rhytidiadelphus squarrosus by Gullvag
et al. (1974). Autoradiographic methods could be used to
determine the intracellular location of zinc in S. undulata
and in this way the mechanism of zinc resistance in this
species could be investigated.

SUMMARY

From a consideration of recent evidence regarding
the mechanism of breakdown of chlorophyll a, it is
suggested that the ratio of chlorophyll a to pheophytin
a is not a suitable index of the condition of plant
material.

A small amount only of useful data were obtained
from the toxicity tests and transplant experiments;
however the results obtained suggest that the populations
of _Scapania_ _undulata_ at the zinc enriched sites studied
are not genetically adapted, zinc resistant ecotypes of
the species.

The results of experiments in which the effect of
environmental factors in accumulation of zinc by
_S_. _undulata_ were investigated suggest that under the
stated experimental conditions active mechanisms of
uptake account for a small proportion of the total amount
of zinc accumulation.

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