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A STUDY OF ZINC RESISTANCE AND ACCUMULATION OF ZINC IN *Scapania undulata* (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 l^{-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 l^{-1} over the two day period. Material incubated in medium containing 1 mg l^{-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°C from medium containing 2 mg l^{-1} zinc was found to be greater than that of live material at 14°C and there was some indication of a greater rate of uptake by live material

at 24°C than at 14°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.

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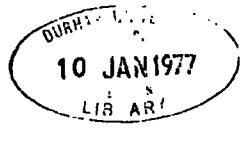
1. INTRODUCTION

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 environment 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 l^{-1}. zinc under laboratory conditions whereas Stigeocolonium tenue is found at sites with concentrations of 20 mg l^{-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 l^{-1} whereas the recommended levels for drinking water range from 2-15 mg l^{-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 suitable 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 photosynthesising 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 a to pheophytin 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

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°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 l^{-1}
Fe (as ferric iron ethylenediaminetetra - acetate chelate)	0.5 mg l^{-1}
'C' microelements of Kratz and Myers (1955) omitting zinc	0.25 mg l^{-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 *a* to pheophytin *a* from the two readings of absorbance is as follows:-

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

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 *a* and pheophytin *a*. The factor of 2.43 should be calculated from the "acid factor" of pure chlorophyll *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 *S. undulata* and the calculated proportion of pheophytin *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 *a*. Marker's fresh extracts must therefore have contained more pheophytin *a* or more chlorophyll *b* than the extracts of *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 hasnot 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) East End Burn (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 S. undulata 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. S. undulata 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 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 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 a to pheophytin a of the transplanted material was determined at weekly intervals. This method of monitoring the condition of transplanted 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) Gill Gill Burn (Lower) (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) Sikehead Stream (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

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 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 l^{-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°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°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 l^{-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°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)

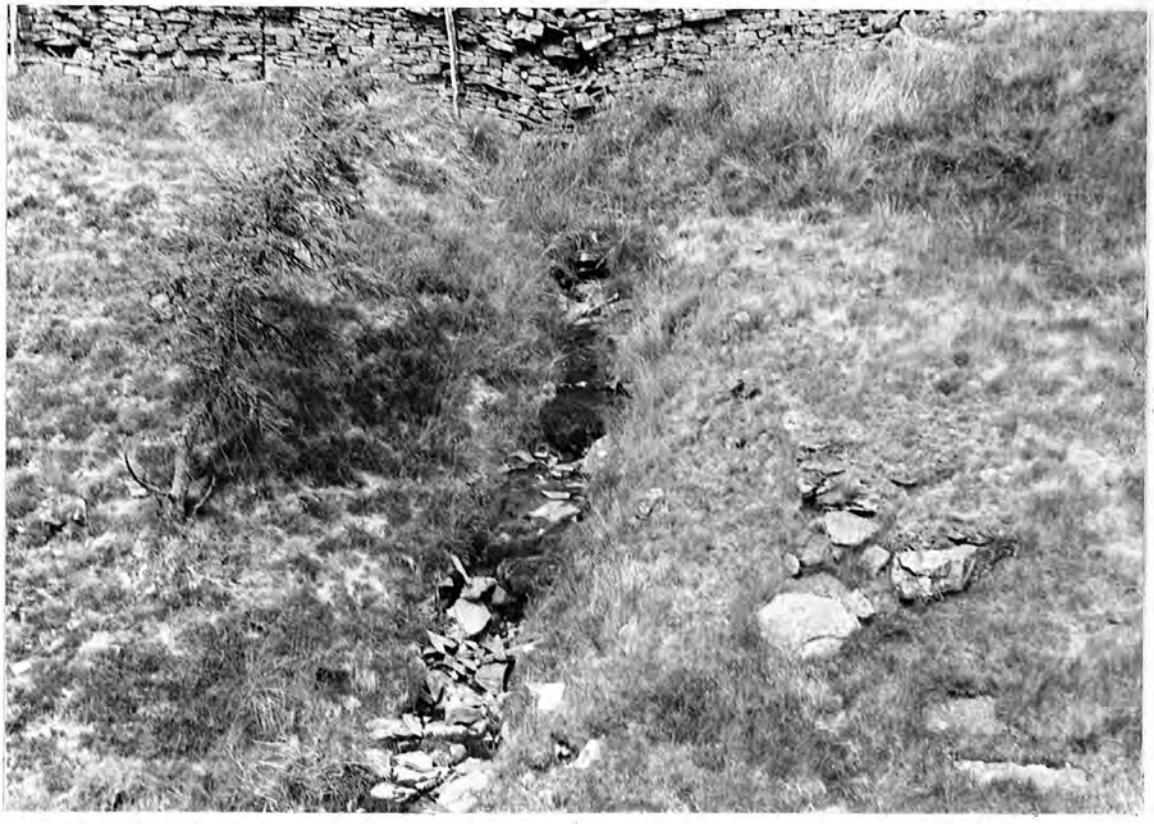


Figure 2 Site (2)

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).

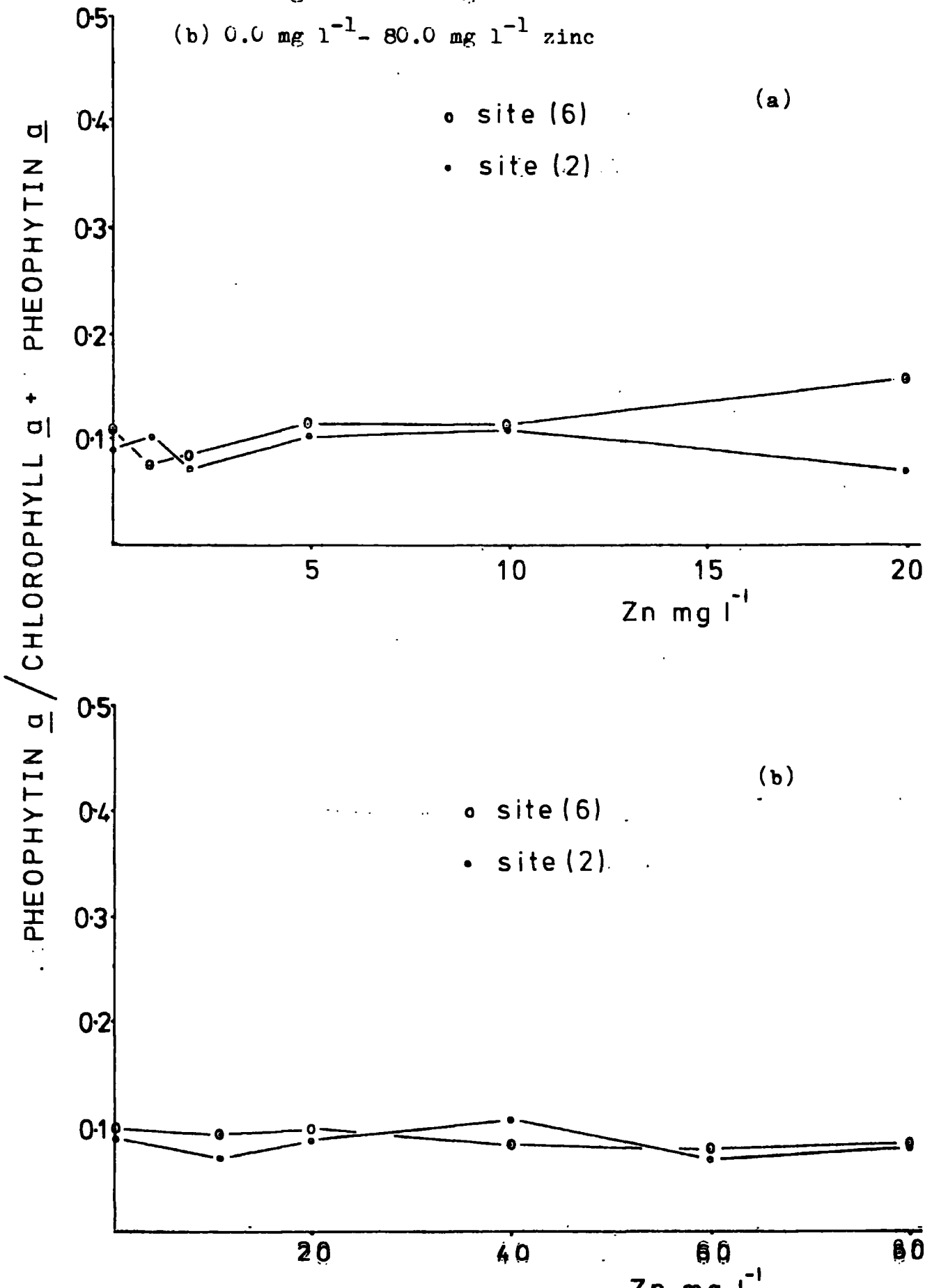
Table 1. Levels of pheophytin a (expressed as $^{ph\ a}/_{chl\ a + ph\ a}$) in S. undulata from site (2) and site (6) in medium containing 0 mg l^{-1} - 20 mg l^{-1} zinc for six days (mean ± 1 S.E. ; n = 4).

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

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

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

Figure 7 Levels of pheophytin a (expressed as $^{ph\ a}/_{chl\ a + ph\ a}$) in 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 *a* (expressed as $^{ph\ a}/_{chl\ a + ph\ a}$) 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 *a* (expressed as $^{ph\ a}/_{chl\ a + ph\ a}$) 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 a (expressed as $^{ph\ a}/_{chl\ a + ph\ a}$) in S. undulata transplanted to site (3)

(a) transplant from site (1)

(b) transplant from site (2)

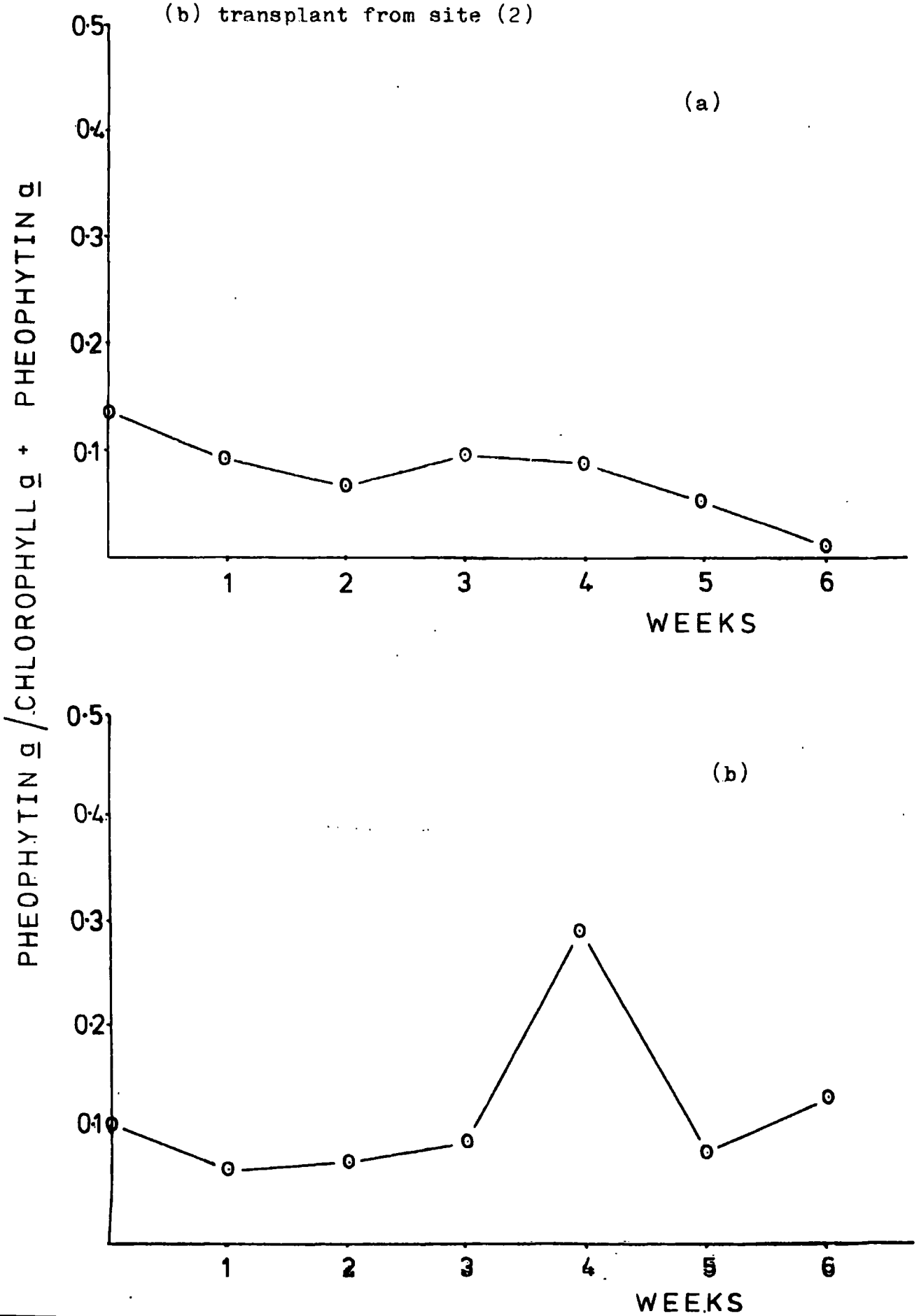


Figure 9 Levels of pheophytin a (expressed as $^{ph\ a}/_{chl\ a + ph\ a}$ in S. undulata transplanted to site (3)

(a) transplant from site (4)

(b) transplant from site (5)

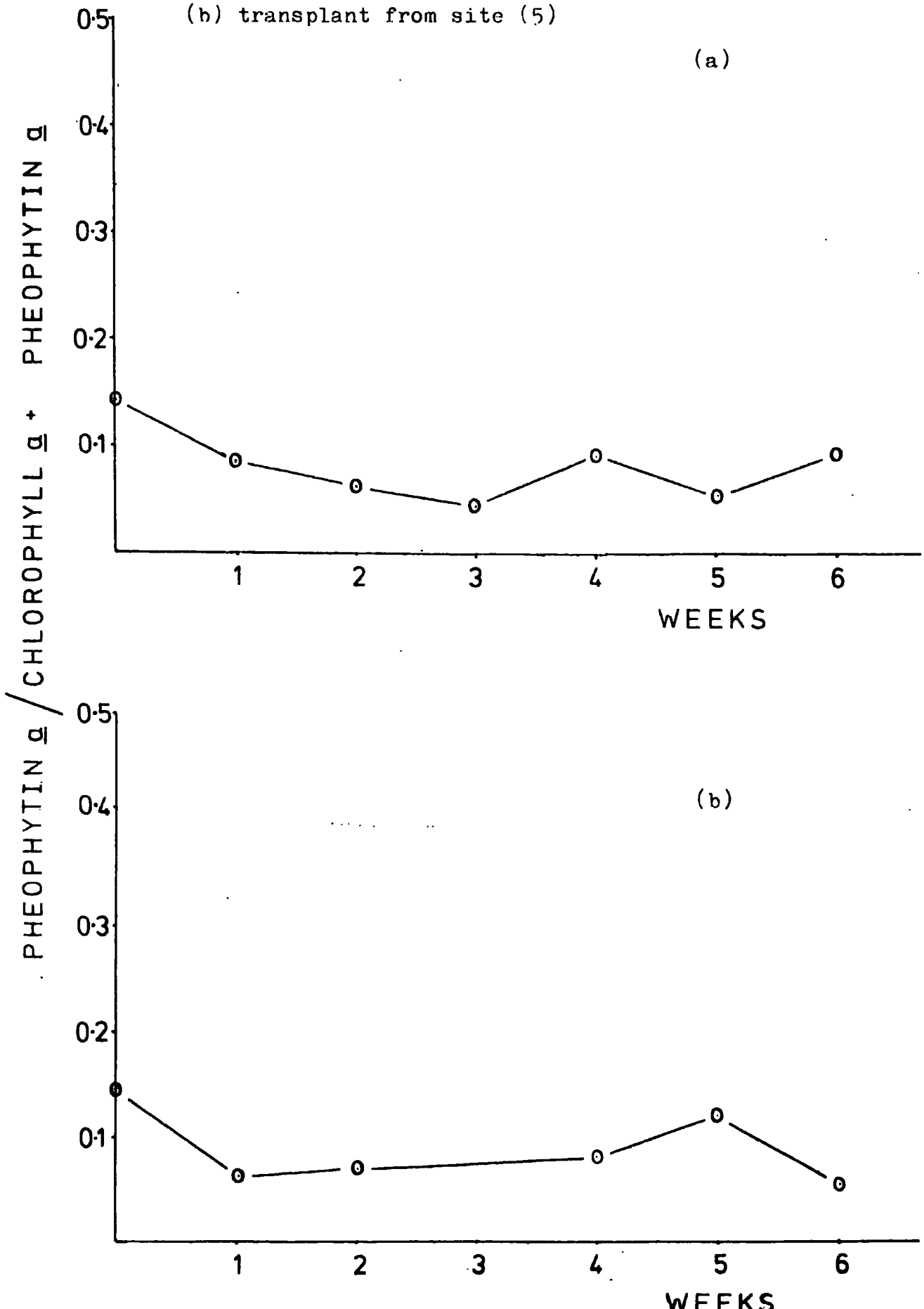


Figure 10 Levels of pheophytin a (expressed as $^{ph\ a}/_{chl\ a + ph\ a}$ in S. undulata transplanted to site (3) from site (6).

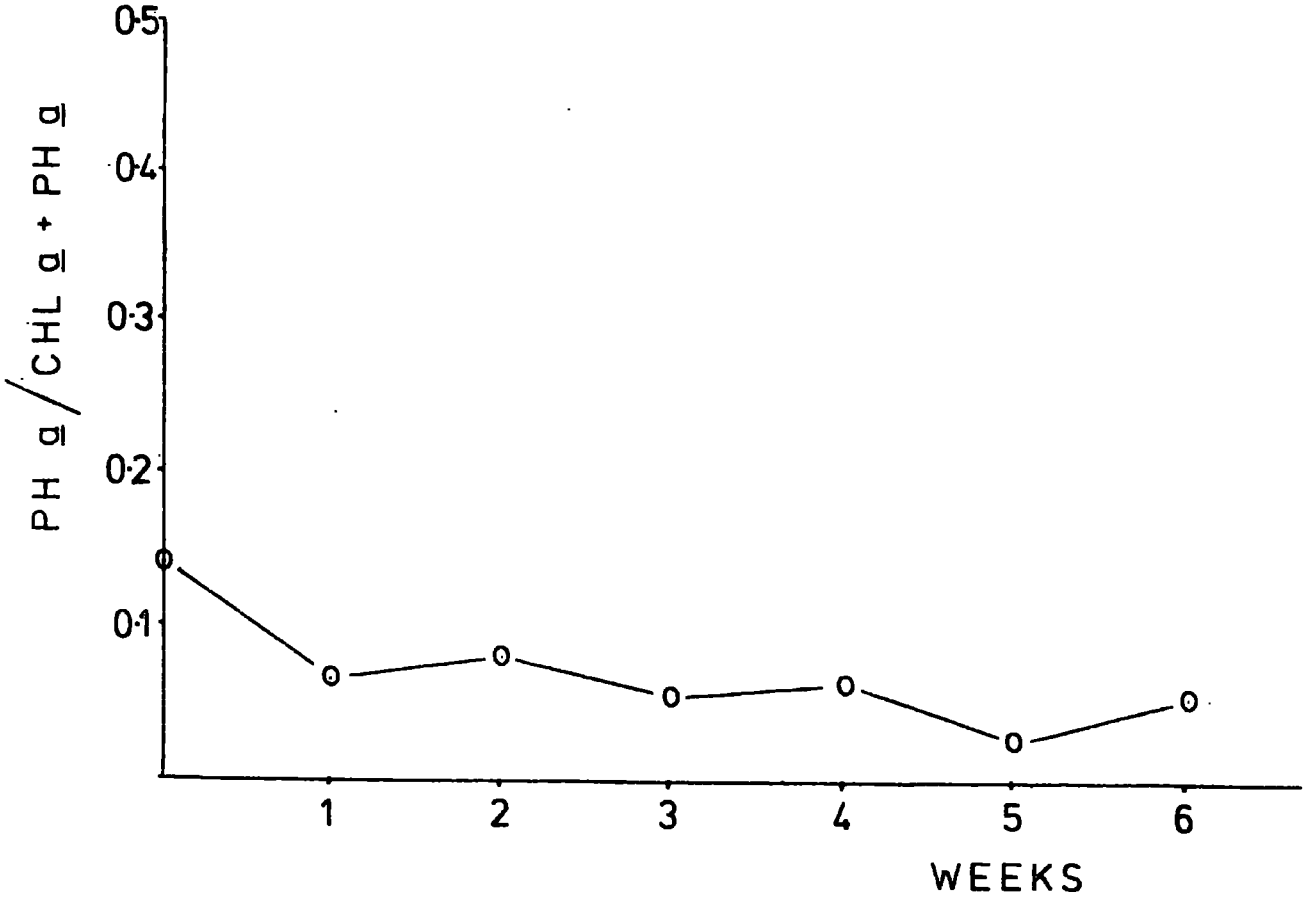


Figure 11 Levels of pheophytin a (expressed as $^{ph\ a}/_{chl\ a + ph\ a}$) in S. undulata transplanted to site (5)

(a) transplant from site (1)

(b) transplant from site (2)

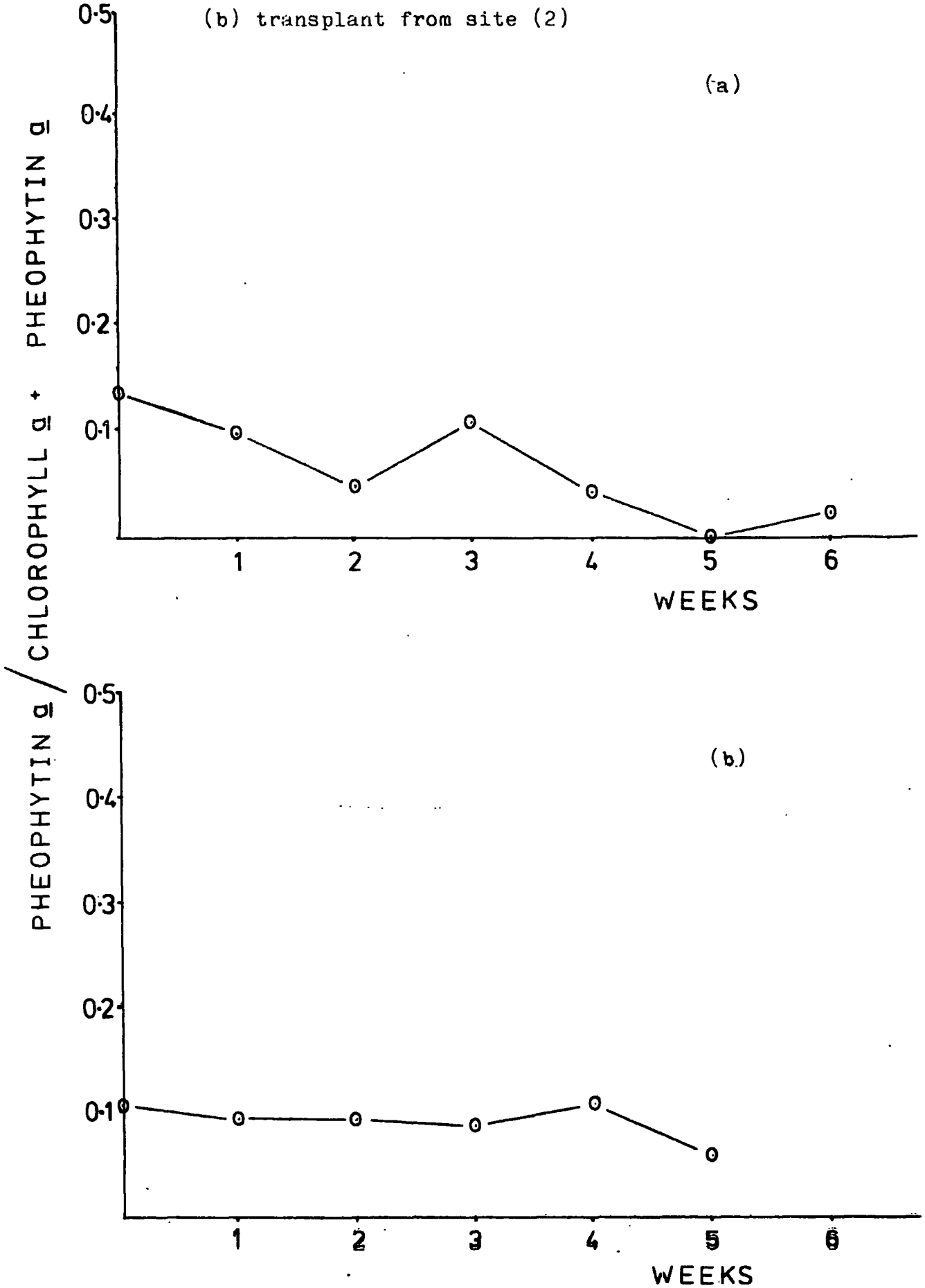


Figure 12 Levels of pheophytin a (expressed as $^{ph\ a}/_{chl\ a + ph\ a}$ in S. undulata transplanted to site (5)

(a) transplant from site (3)

(b) transplant from site (4)

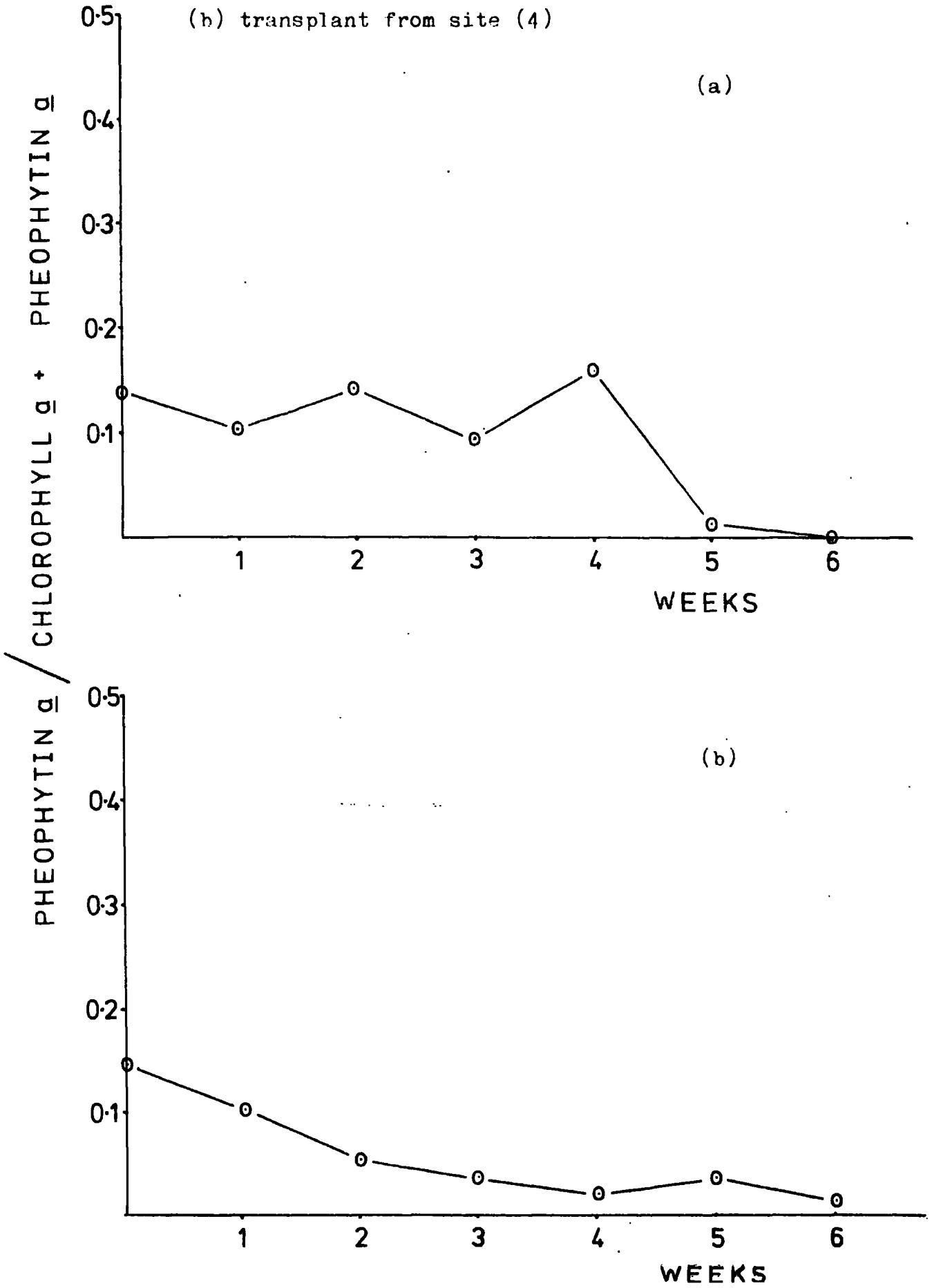
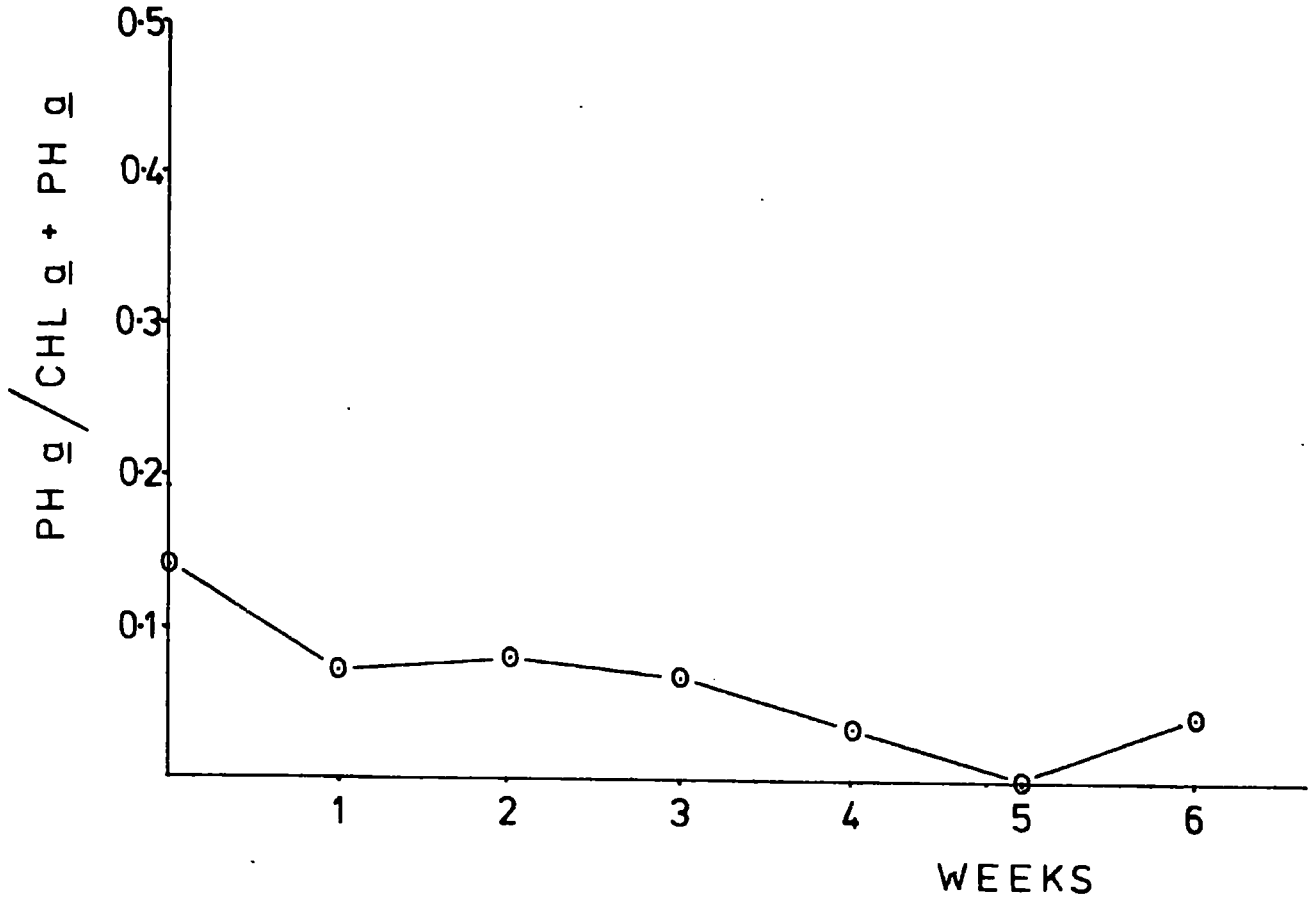


Figure 13 Levels of pheophytin a (expressed as $^{ph\ a}/_{chl\ a + ph\ a}$ in S. undulata transplanted to site (5) from site (6).



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 S. undulata and enrichment ratio of zinc in S. undulata at sites (2) (3) (5) (6) (7) and (8) (mean ± 1 S.E. ; n = 10).

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

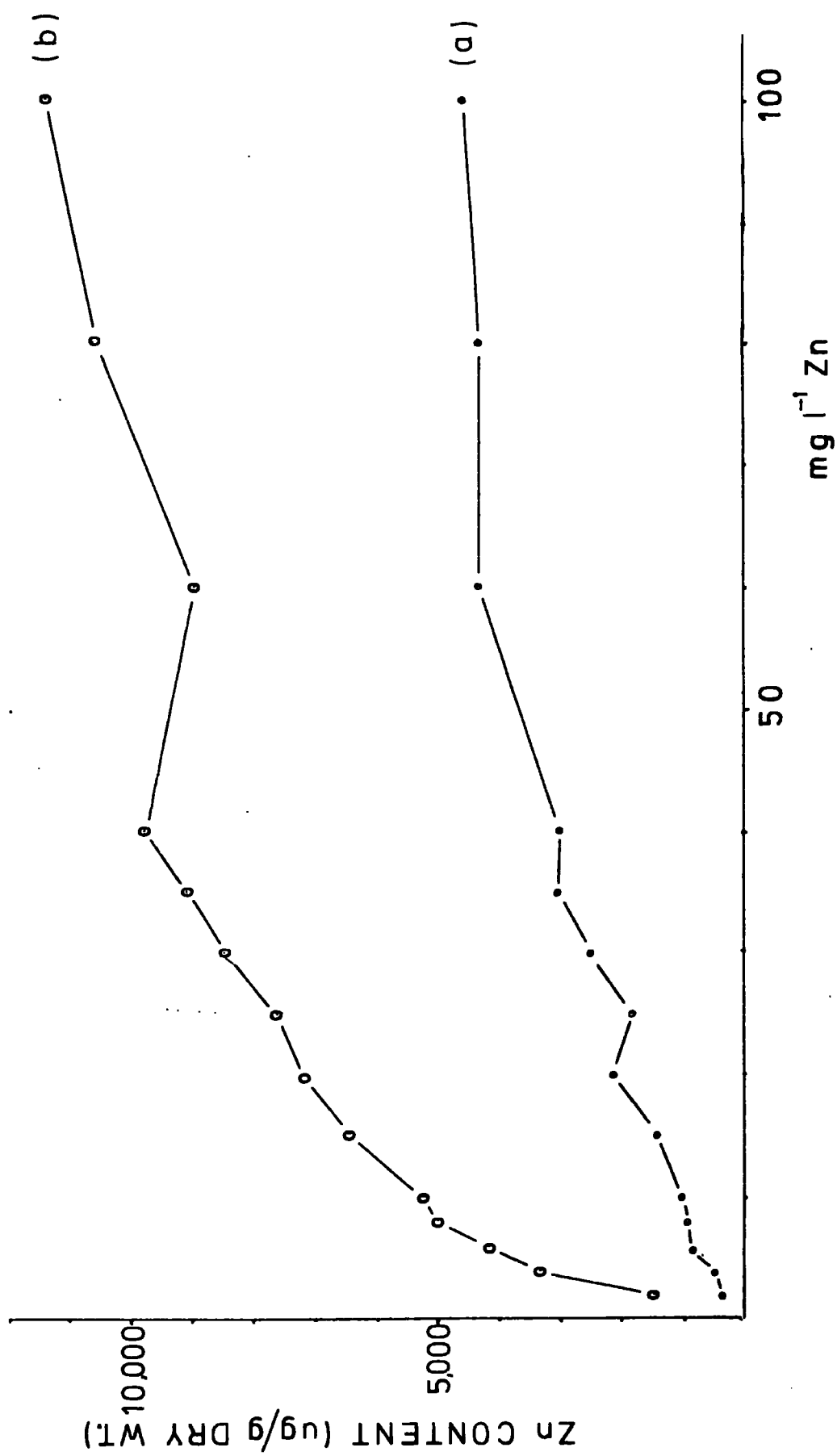


Figure 14 Uptake of zinc by S. undulata as a function of the concentration of zinc in the medium (a) over a 30 min period (b) over a two day period.

(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 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 ± 1 S.E. ; n = 5).

Time (h)	Uptake of zinc by S. undulata in the light (µg/g dry wt.)	Uptake of zinc by S. undulata in the dark (µg/g dry wt.)
0.5	888 ± 82	1056 ± 83
1.0	962 ± 90	1045 ± 93
2.0	1121 ± 45	1190 ± 45
3.0	1221 ± 19	1273 ± 68
4.0	1339 ± 70	1430 ± 62
5.0	1423 ± 58	1530 ± 61
6.0	1523 ± 97	1801 ± 65
8.0	1635 ± 36	1711 ± 54
16.0	1747 ± 43	1890 ± 51
22.0	1703 ± 32	1882 ± 77
28.0	1630 ± 69	1899 ± 101
40.0	1438 ± 39	1772 ± 87
52.0	1462 ± 42	1568 ± 40
64.0	1478 ± 20	1554 ± 48
76.0	1559 ± 26	1612 ± 37
89.0	1500 ± 20	1582 ± 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 ± 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 ± 42	1038 ± 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).

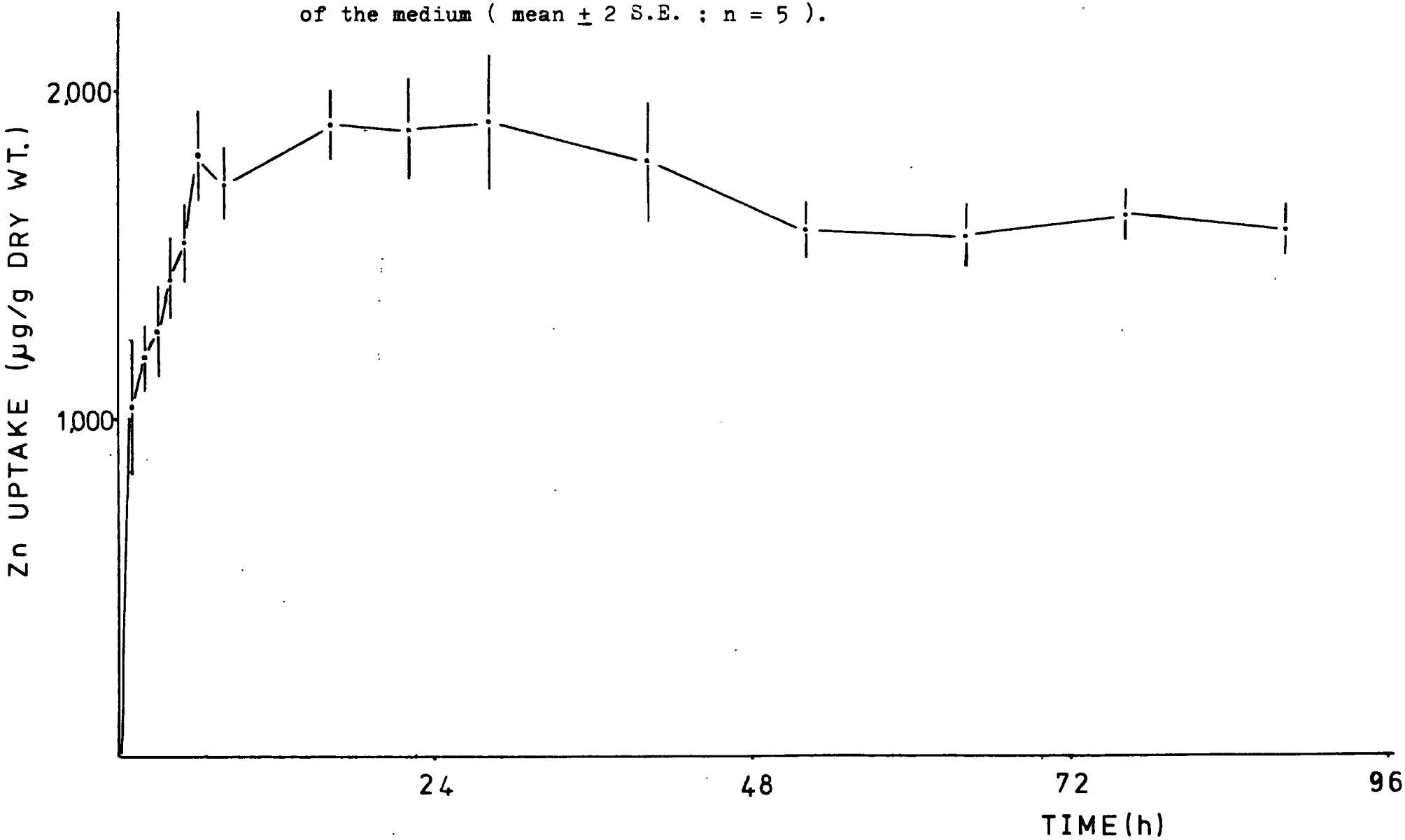
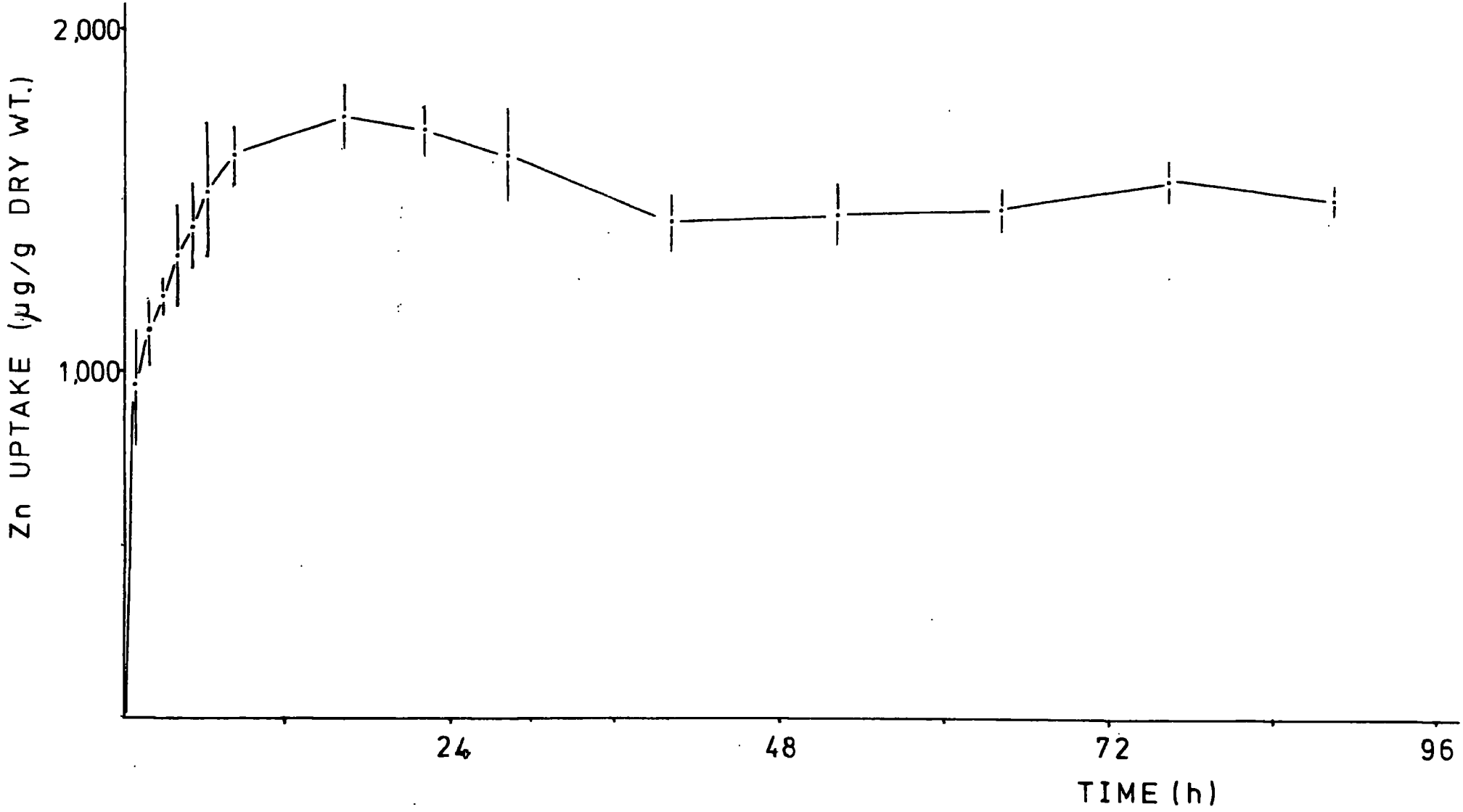


Figure 16 Uptake of zinc by S. undulata in the light from medium containing 1.0 mg l^{-1} zinc, as a function of time. Uptake estimated from the change in concentration of the medium (mean ± 2 S.E. ; n = 5).



(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 *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°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°C took up zinc significantly more rapidly than the live material at 14°C. 48 h. after the commencement of the experiment, however, there was no significant difference between the zinc content of the material at 32°C and that of the material at 14°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 S. undulata at 14°C, 24°C and 32°C from medium containing 2.0 mg l^{-1} zinc, as estimated from the concentration of zinc in the medium.

Time (h)	Temperature		
	14°C	24°C	32°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 S. undulata at 14°C, 24°C and 32°C after seven days in medium containing 2.0 mg l^{-1} zinc.

Temperature	Zinc content (µg / g dry wt.)
14°C	3470
24°C	3215
32°C	3518

Figure 17 Uptake of zinc by live S. undulata at 14°C from medium containing 2.0 mg l^{-1} zinc, as a function of time (mean ± 2 S.E. ; n = 4).

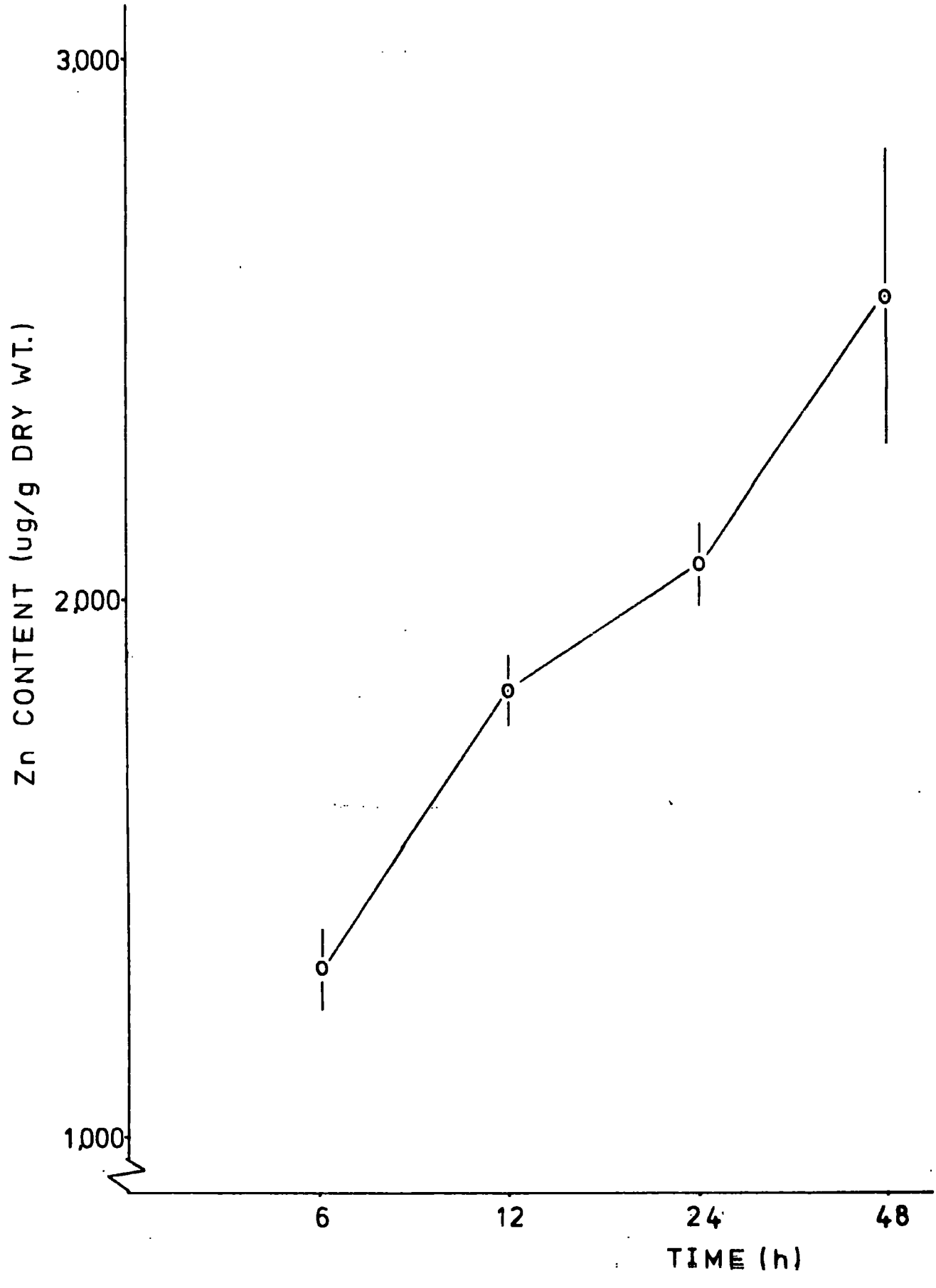


Figure 18 Uptake of zinc by live S. undulata at 24°C from medium containing 2.0 mg l^{-1} zinc, as a function of time (mean ± 2 S.E. ; n = 4).

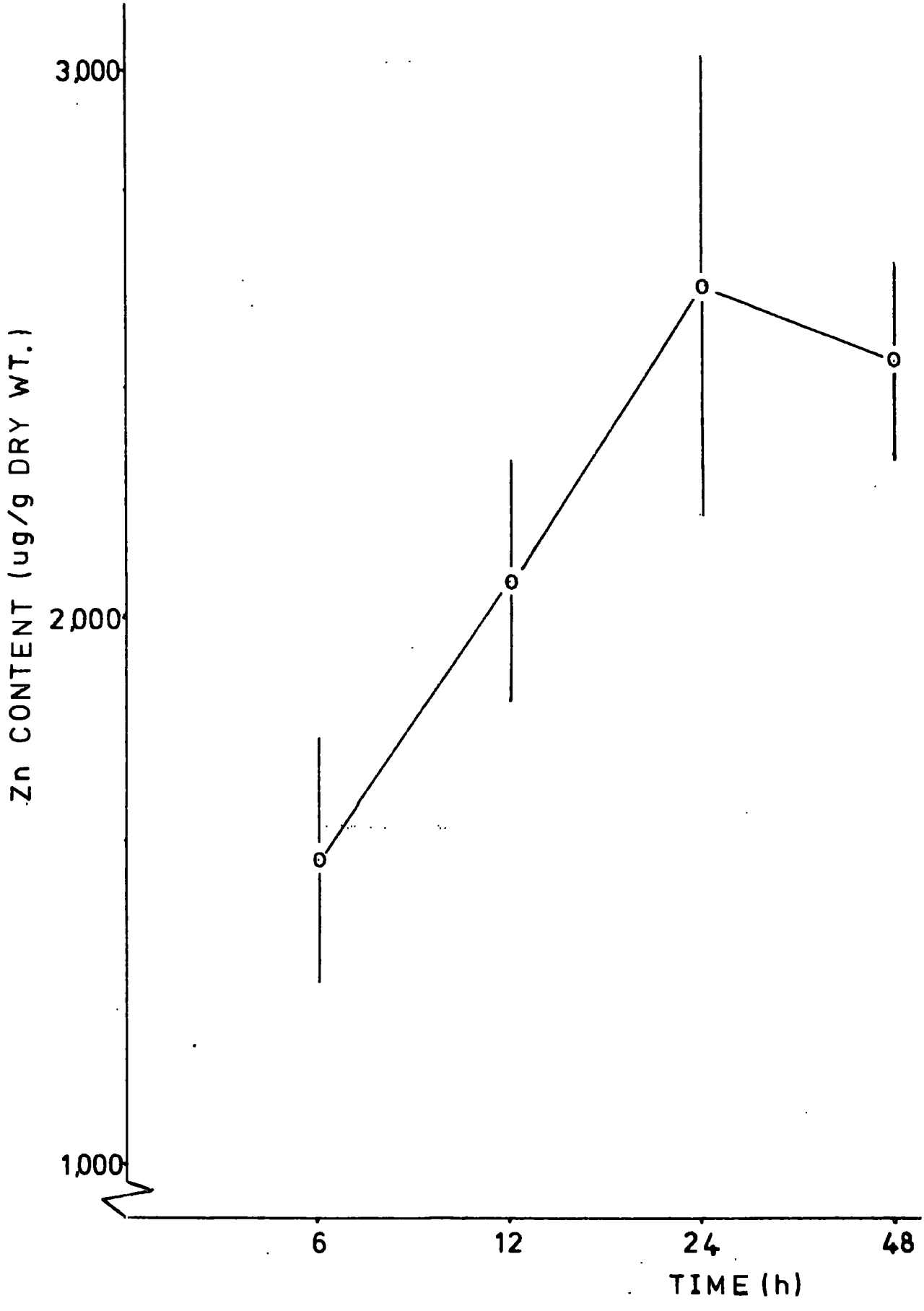
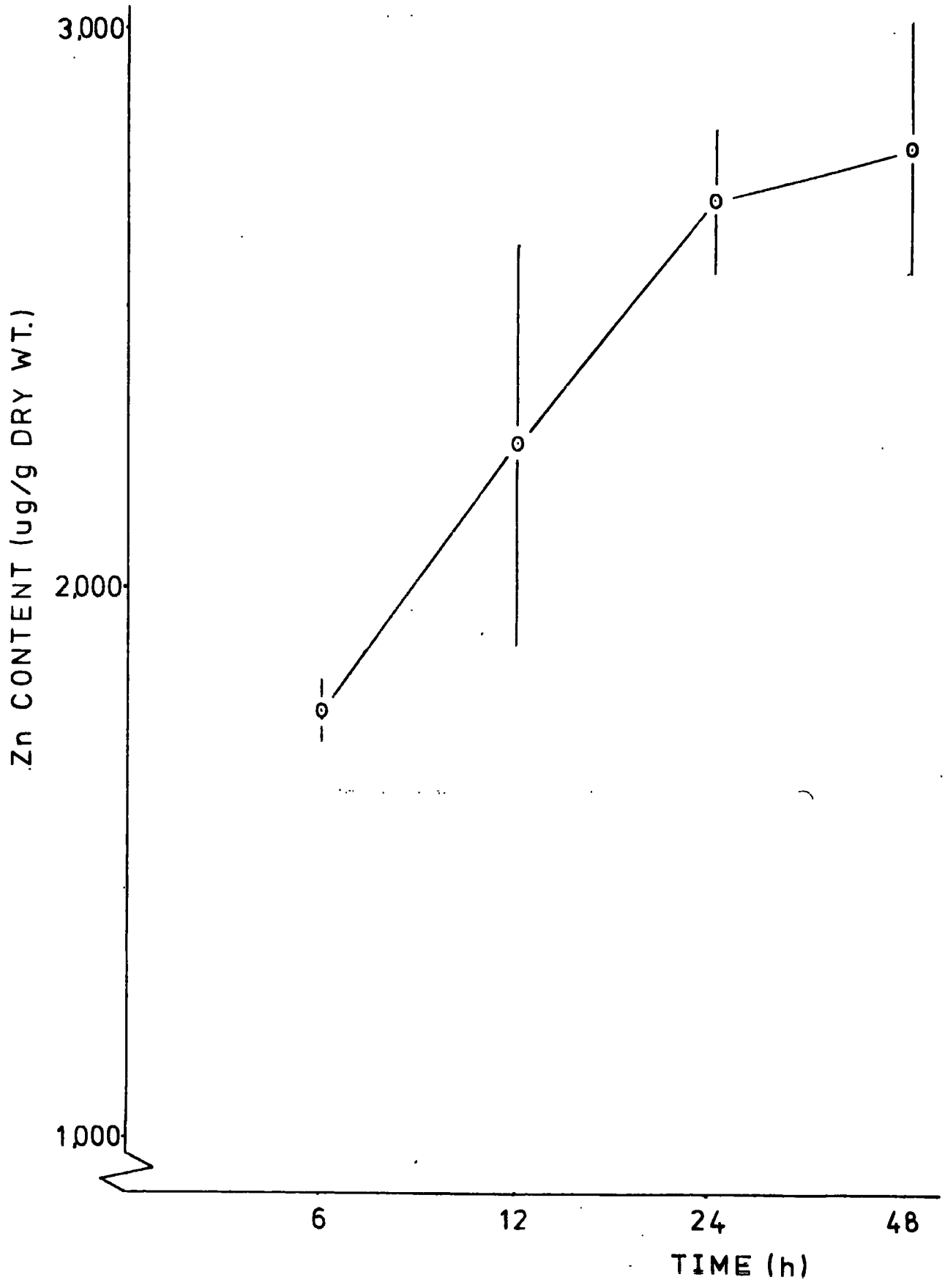


Figure 19. Uptake of zinc by dead S. undulata at 32°C from medium containing 2.0 mg l^{-1} zinc, as a function of time (mean ± 2 S.E. ; n = 4).



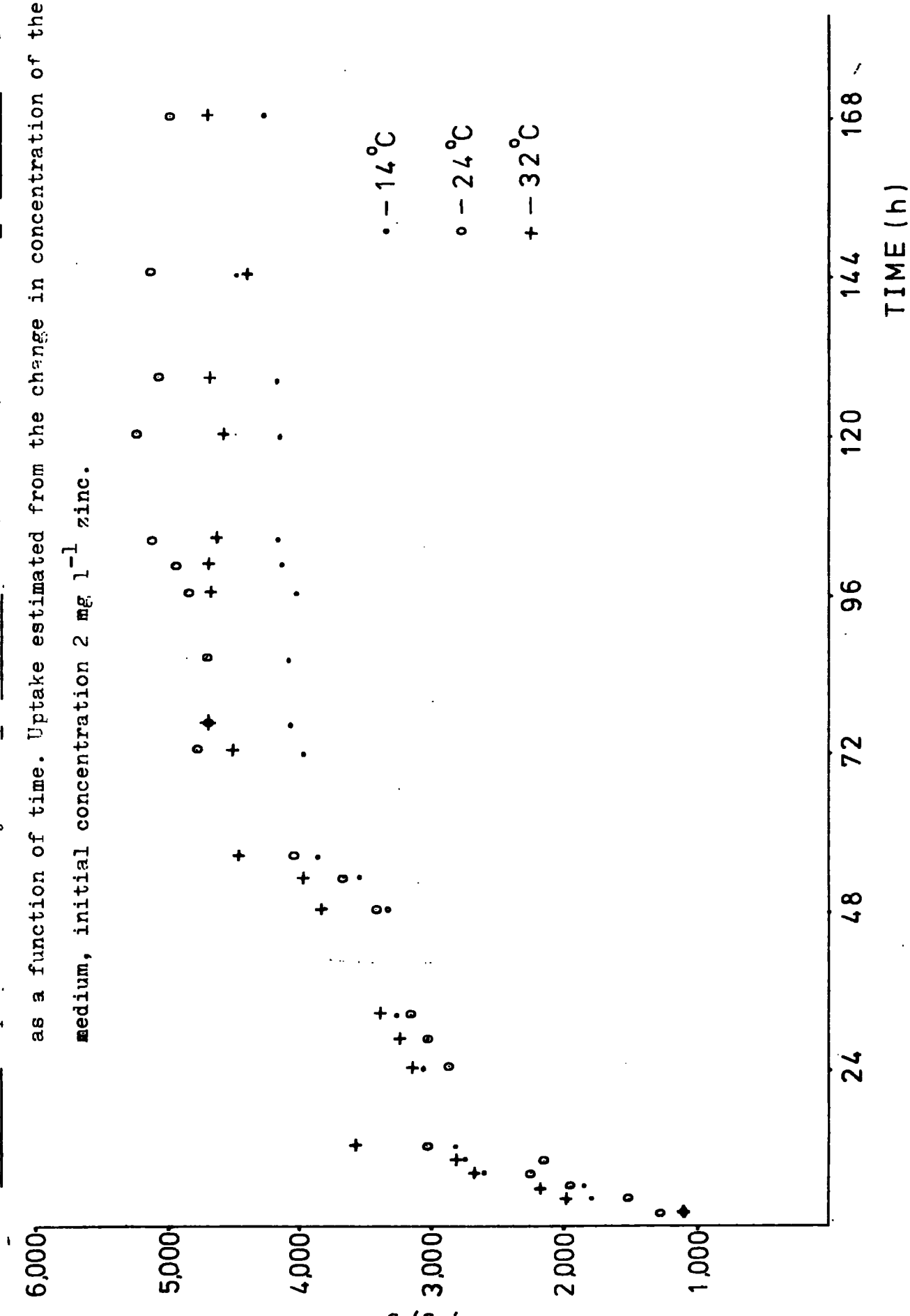


Figure 20. Uptake of zinc by live S. undulata at 14°C and 24°C and dead S. undulata at 32°C as a function of time. Uptake estimated from the change in concentration of the medium, initial concentration 2 mg l^{-1} zinc.

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.

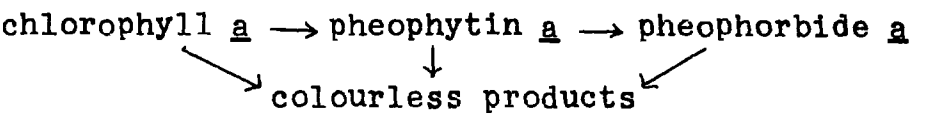
The condition of the plant material in the toxicity tests and the transplant experiments was monitored by determining the ratio of chlorophyll a to pheophytin a in samples of the material. This method has been used to monitor the condition of transplants of S. undulata in a study by McLean and Jones (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):



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 *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), *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 S. undulata increased with the 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 S. undulata also. No relationship between the concentrations of these ions and the enrichment ratio of zinc in 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 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 F. antipyretica than in 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 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 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^{o} 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°C is greater than at 14°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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