Chapter 2

# Decomposition of Hudson Estuary Macrophytes: Photosynthetic Pigment Transformations and Decay Constants

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**ABSTRACT**: *Plant pigment decay constants were determined for four macrophytes collected from the Hudson Estuary. Typha angustifolia and Scirpus fluviatilis were used as representatives of emergent aquatic vegetation (EAV), and Potamogeton sp. and Vallisneria americana were used to represent submerged aquatic vegetation (SAV). Litter bags were maintained in an environmental chamber in the dark for a period of 104 days. The fastest rate of total mass loss was in the SAV V. americana and slowest in the EAV T. angustifolia. Changes in carotenoid and chloropigment concentration resulting from microbial and meiofaunal heterotrophy in each of the macrophytes were quantified using reverse-phase high-performance liquid chromatography (RP-HPLC) techniques. Chlorophyll c and the carotenoid, fucoxanthin, provided useful bio-markers in determining the presence of epiphytic diatom growth which only occurred on the SAV's. The highest concentrations of phaeophorbide a, commonly used as an indication of metazoan grazing, were found in the SAV V. americana. Low concentrations of phaeophorbide a in the SAV Potamogeton sp. indicate inefficient use of this SAV by meiofaunal grazers. Lutein decayed slower than all other carotenoids in both EAV's and SAV's. Microcosm studies such as this are necessary to further understand the mechanisms and kinetics of photosynthetic pigment transformations in natural systems.*

## Introduction

The importance of vascular plant materials to the energy flow of estuarine and riverine ecosystems has received considerable attention (Mann 1982; Brinson et al. 1981). The diverse array of allochthonous and autochthonous vascular plant sources found in these systems makes it difficult to identify and determine the relative importance of different sources of organic matter. Pigments characteristic of different sources can be used as bio-markers to determine mechanisms of resource utilization in food chain dynamics and to understand processes that control biogeochemical cycling of organic matter in natural systems (Boyd 1970; Daley 1973; Watts et al. 1977; Liaaen-Jensen 1985; Carpenter et al. 1988).

Many studies have described the distribution and composition of plant pigments in marine and freshwater systems (Hallegraeff and Jeffrey 1985; Welschmeyer and Lorenzen 1985; Bidigare et al. 1986; Plante et al. 1986; Klein and Sournia 1987; Vernet and Lorenzen 1987; Carpenter et al. 1988; Ridout and Morris 1988). Despite this extensive data set on composition and distribution, the kinetics and mechanisms of pigment degradation are not well understood. The three dominant chlorophyll degradation products in these systems are phaeophytin, phaeophorbide, and chlorophyllide. These tetrapyrrole derivatives are formed during bacterial, viral, or autolytic cell lysis, and by grazing processes (Sanger and Gorham 1970; Daley 1973; Jeffrey 1974; Shuman and Lorenzen 1975; SooHoo and Kiefer 1982a,b; Welschmeyer and Lorenzen 1985; Bianchi et al. 1988). The impact of grazing (Jeffrey 1974;

Hallegraeff 1981; Kleppel and Pieper 1984; Klein et al. 1986; Leavitt and Carpenter, in press) and diagenesis (Watts et al. 1977; Ridout et al. 1984; Repeta and Gagosian 1987) on the degradation of carotenoids have also received attention. There have been numerous studies that have used laboratory experiments to examine photosynthetic pigment degradation in known sources of phytoplankton (Healey et al. 1967; Hallegraeff and Jeffrey 1985) and microphytobenthos (Klein 1988). These studies have proven useful in interpreting pigment transformations and distributions in marine and freshwater environments.

We do not have this critical information for interpreting pigment dynamics in systems where macrophyte material is likely to be important because similar studies using macrophytes have not been performed. A greater understanding of the inherent differences between the decay kinetics and mechanisms of transformation - for specific pigments in known source materials - will provide valuable information for interpreting pigment chemistry in natural systems. In this study we examined pigment decay, using reverse-phase high-performance liquid chromatography, in four macrophytes from the Hudson Estuary. Using litter bags, we determined dominant pigment bio-markers for each source material and decay constants for each of the pigments during aphotic decay in controlled laboratory incubations. This experimental design allowed us for the first time to investigate the kinetics of pigment decay directly in macrophyte source materials. Our intent was to determine differences among pigments and plant types under controlled laboratory conditions. Therefore, these data are a necessary prelude for the interpretation of pigment distributions observed in the field.

# Methods

Site description and plant materials

The four macrophytes used in this study were collected from the mid-Hudson Estuary, located near Kingston, New York. This portion of the Hudson Estuary is broad and shallow, having a mean surface/volume ratio of 0.125 (Gladden et al. 1988). The tidal range is about 1 m, and the salt front (0.1 g Cl/L) only intrudes this far north during extreme drought. Beds of submerged macrophytes are common in areas less than 2 m deep, and two of the larger tidal freshwater wetlands (the Tivoli Bays) are also located in this area.

Two source materials used in this study represent the predominant forms of submerged aquatic vegetation (SAV) in the mid-Hudson Estuary (*Vallisneria americana*, *Potamogeton* sp.). The remaining two sources are forms of emergent aquatic vegetation (EAV) (*Typha angustifolia*, *Scirpus fluviatilis*). What we considered to be healthy leaves of all four macrophytes were collected in August 1988; all EAV material was collected above the water-line from intertidal areas.

Litter bag experiments

Two polyethylene trays (53 x 39 x 18 cm) were filled with unfiltered Hudson Estuary water collected off Kingston, New York. After a brief rinse, 10 g (wet weight) of each of the 4 macrophytes were added to each of three replicate litter bags (27 x 13 cm, 3 mm mesh size), resulting in a total of 12 bags. Bags containing SAV's or EAV's were submersed into one of the two trays; based on preliminary analyses of macrophyte

material, we decided to keep SAV's and EAV's in separate trays to reduce the chances of cross contamination between SAV's and EAV's. Both trays were placed in an environmental chamber and maintained in aerated Hudson Estuary water in the dark at 20°C for 104 days. River water in the trays was changed once a week. Subsamples of each macrophyte were taken from each of the three replicate litter bags at the following time intervals: 0, 14, 30, 60, 104 days. Subsamples from the three bags were pooled and stored frozen for pigment and total carbon and nitrogen analyses. Thus, for each macrophyte three litter bags were sampled repeatedly over time and these subsamples pooled. Although non-ideal from an experimental design point of view, the chromatographic run time required for individual samples precluded greater replication. To estimate variability among replicates, we performed pigment analyses (chlorophylls a, b and lutein) on each of the three bags separately at the end of the experiment. The coefficient of variation ranged from 0.3 to 7.7% of the mean across all pigment and plant species. In a separate two-week decay experiment, bags were sampled individually at time zero, one week and two weeks. Coefficients of variation ranged from 0.3 to 7.7. Carbon and nitrogen measurements were performed on initial plant material using a Carlo Erba NA 1500 nitrogen/carbon analyzer.

Decay constants for mass loss from each of the macrophytes were calculated from wet weight changes (corrected for the mass of sample removed for pigment analyses) in each of the litter bags at three times (0, 60, 85 days), assuming first-order decay kinetics. Thus, for each macrophyte there were three observations at each of three sampling times. First-order decay constants of pigments were calculated from the relationship:

$$P_t = P_i e^{-kt},$$

where $P_t$ = absolute mass of pigment at time t; i.e., pigment concentration (nmol/g dry biomass) x macrophyte biomass (g dry wt), $P_i$ = initial pigment mass, t = time (days), and k = decay constant ($day^{-1}$). Thus, the dependent variable is the absolute mass of a particular pigment (nmoles), rather than the concentration (nmol/g dry biomass). Using the absolute mass rather than the concentration makes the pigment decay dynamics independent of changes in total macrophyte mass. Use of concentrations could be misleading in a case where a pigment is degrading at, for example, 1% $d^{-1}$, while the macrophyte mass is declining at 5% $d^{-1}$. The concentration of the pigment would increase over time, when in reality, the pigment is being degraded. In this example, one might conclude erroneously that the pigment was being produced during macrophyte decomposition.

Because measurement of macrophyte biomass and pigment concentration were not always concurrent, we estimated macrophyte biomass for each of the five sampling dates (0, 14, 30, 60, 104 days), using the macrophyte decay constants in Table 1. Estimated macrophyte wet weights for each of the five sampling periods were converted to dry weights using dry wt/wet wt ratios obtained for each of the macrophytes (at time zero). These ratios were assumed to be constant over the duration of the experiment. Wet weight/dry weight ratios for the macrophytes Typha and Nuphar had low coefficients of variation (0.5%, n = 45 and 0.6%, n = 23, respectively), over the course

of a field decomposition study (Findlay, unpublished data). Decay constants were then calculated, using a least squares regression on log-transformed pigment masses.

Pigment Analyses

Pigments were extracted from pulverized macrophyte material using 100% acetone to reduce the artifactual production of chlorophyllide (Jeffrey and Hallegraeff 1987). Pigment extracts were sonicated for 5 min and allowed to stand overnight in the dark at 4°C, prior to centrifugation at 3000 rpm for 3 min in 15 ml polypropylene centrifuge tubes. The tubes were capped and stored frozen for only two days prior to pigment analysis to avoid the formation of any end-products which may form during storage. Extraction efficiencies for fresh EAV and SAV plant material were around 90%, as determined by performing three serial extractions on the same material. Pigments were determined by ion-pairing, reverse-phase HPLC (Mantoura and Llewellyn 1983). The ion-pairing (1.5 g of tetrabutylammonium acetate and 7.7 g of ammonium acetate, made up to 100 ml with water) allows for greater resolution of the dephytolated acidic chloropigments (chlorophyll c, chlorophyllide a and phaeophorbide a) (Mantoura and Llewellyn 1983).

The equipment employed consisted of a gradient pumping system (Varian-Vista 5500) controlled by a Vista 402 dual-channel system. Dual-channel detection was achieved with a Varian-Vista UV monitor set at 440 nm for absorbance and a Kratos Spectroflow 980 fluorescence detector, with excitation at 440 nm and emission at >600 nm using a bandpass filter. An auto-injector equipped with a Rheodyne model 7126 valve was connected via a precolumn to a reverse-phase $C_{18}$ Adsorbosphere column (5

μm particle size; 250 mm x 4.6 mm i.d.). After injection (100 μl sample), a gradient program that ramped (1 ml/min) from 100% mobile-phase A (80:10:10 ml, methanol: water: ion-pairing reagent) to 100% mobile-phase B (70:30 ml, methanol: acetone) in 30 min with a hold for 20 min provided sufficient resolution of all pigments of interest except for β-carotene and phaeophytin a (Fig. 1). This procedure proves to be the best technique for detrital plant pigments (SCOR, in prep.). The column was re-equilibrated between samples by linear ramping to a 100% mobile-phase A for 4 min. Example absorbance and fluorescence chromatograms of V. americana are shown in Figure 1.

Identification of all pigments was confirmed by comparing absorption spectra obtained with a Waters 990 photo diode-array detector with that of published values (Davies 1976; Braumann and Grimme 1981; Mantoura and Llewellyn 1983; Wright and Jeffrey 1987). Calibration standards for chlorophylls a and b were obtained from Sigma Chemical Co. Concentrations of pigment standards for all pigments identified in Fig. 1, except neoxanthin and violaxanthin, were determined spectrophotometrically (Perkin-Elmer 552) in 1 cm cuvettes, using extinction coefficients obtained from an inter-calibration study (SCOR in prep.). Known quantities of different pigments were injected and the peak areas were used to calculate response factors. Pigment concentrations of macrophyte extracts, corrected for sample dilution and extraction efficiency, were then calculated with these response factors. In the two cases (neoxanthin and violaxanthin) where we did not have pigment standards, concentrations of these two pigments were determined on the basis of molar extinction coefficients relative to chlorophyll a,

corrected for the absorbance spectra meaasured by diode array at 440 nm (SCOR, in prep.). Based on diode-array analyses, we did not find any α-carotene or phaeophytin a' using this run time. Thus, after ß-carotene and phaeophytin a peaks were identified using the diode-array, we calculated concentrations of these two pigments, using peak area differences between absorbance and fluorescence chromatograms.

Chlorophyll a and b derivatives were produced from chlorophyll extracts (Sigma Chemical Co.), dissolved in 100% acetone, of Anacystis nidulans (chlorophyll a) and spinach (chlorophyll b). Chlorophyllide a, phaeophytins a and b and total phaeophorbides were produced using methods described by Bianchi et al. (1988). Although a number of phaeophorbide a -like components have been reported in the literature (Mantoura and Llewellyn 1983; Hawkins et al. 1986; Rioux-Gobin et al. 1987), phaeophorbide a produced by this procedure appears as a single dominant peak.

The non-ideal experimental design requires careful application of inferential statistics. In the case of total mass loss, we calculated a decay constant for each litter bag separately and used these constants as dependent variables in an ANOVA with plant type as the main effect. In the case of pigment dynamics, we have one observation of pigment concentration for each plant type at each time point. Individual analyses of the three replicate litter bags conducted at the end of the experiment and a separate two week decay experiment showed coefficients of variation for all major pigments ranged from 0.3-7.7%. Because these sequential samples from the same litter bags are not independent, we calculated the difference in concentration between each pair of the sampling points ($t_{n+1}$ - $t_n$) to generate a set of observations of changes in pigment

concentration for each plant species. These data were used in a two-way ANOVA without replication (Sokal and Rohlf 1981), with plant species and time interval as the main effects. A one-way ANOVA was used to contrast pigment decay constants in submerged plants (Vallisneria and Potamogeton) with emergent plants (Typha and Scirpus).

## Results

### Macrophyte Mass Loss

Macrophyte decay constants were significantly ($P < 0.05$) different among the four plants in laboratory litter bags. Mass loss from V. americana was faster than mass loss from T. angustifolia, but decay constants for S. fluviatilis and Potamogeton sp. were not different (Table 1). The molar C/N ratios of the SAV's were lower than those measured for the EAV's (ANOVA, $P < 0.05$).

### Chloropigment Transformation

The chlorophyll a and b decay constants in the SAV's were significantly faster than in the EAV's (ANOVA, $P < 0.05$) (Table 2). Over the initial 14 days there appeared to be an increase in the chlorophyll a and b concentrations of certain macrophytes (Fig. 2). This may be caused by variations in extraction efficiency and/or preferential utilization of non-pigmented material (Leavitt and Brown 1988). Repeated extractions showed no difference in extraction efficiency among plant types, so it is likely

that differences in decay of non-pigmented material were responsible for changes in observed concentrations. Chlorophyll a and b concentrations of the SAV's were significantly higher ($P<0.05$) than the EAV'S (Fig. 2). The initial chlorophyll a/b ratios of the SAV's were significantly lower ($P<0.05$) than the EAV's. After an increase up to 14 days, phaeophytin a concentrations decreased significantly with time, while there were concurrent increases in phaeophorbide a (Fig. 3). There were significantly ($P<0.05$) higher concentrations of chlorophyllide a in the SAV's than in the EAV's (Fig. 4).

Carotenoid Transformation

Decay of lutein was significantly slower (ANOVA, $P<0.05$) than ß-carotene and violaxanthin in all macrophytes (Table 2). Lutein concentrations were significantly ($P<0.05$) higher in the EAV's than in the SAV's (Fig. 5). Fucoxanthin was only found in the two SAV's (Fig. 4). Decay of fucoxanthin was significantly faster than lutein (ANOVA, $P<0.05$).

## Discussion

Pigment Decay Kinetics

Chloropigment and carotenoid decay constants are likely to be dependent upon both specific pigment structure and the composition and decay properties of non-pigmented compounds in the plant. For example, pigment decay constants were generally faster in the SAV's than in EAV's, presumably due to lower concentrations of structural compounds such as lignins and surface waxes (Webster and Benfield 1986). High concentrations of these structural compounds can slow down heterotrophic activity

on macrophyte detritus (Harrison and Mann 1975; Rice and Tenore 1981), which may delay the rate at which certain photosynthetic pigments begin to decompose.

There are certain structural differences between groups of pigments (i.e., carotenoids) that can explain differences in the kinetics of their decay. For example, fucoxanthin, peridinin, and diadinoxanthin are all rapidly decaying carotenoids because of the presence of a 5,6-epoxide (Repeta and Gagosian 1987). Transformation reactions of carotenoid pigments usually occur by ester hydrolysis, dehydration, and epoxide opening (Repeta and Gagosian 1982). Although a lutein epoxide was formed during this experiment by certain bacteria (Fig. 1), parent lutein lacks an epoxide unlike the more rapidly decaying carotenoids (Repeta 1989). As an example of the utility of data from this study, the knowledge that lutein is relatively stable helps interpret pigment distributions in the field. In sediment samples collected from V. americana beds in the mid-Hudson, lutein occurs in the highest concentrations of the carotenoids and occurs to depths of 5 cm (unpubl. data). Our lab study indicates that these patterns are likely due to the stability of lutein to decomposition. In a similar study, it was concluded that ß-carotene degraded less rapidly than other carotenoids and could be used as a marker in sedimentation and paleolimnological studies (Leavitt and Carpenter, in press). Inter-site differences in pigment decay rates must be considered before selecting pigment biomarkers to be used as stable tracers.

In chloropigments, the epimers chlorophyll a' and b' are more likely to lose their Mg atom to form phaeophytin than are the parent chlorophyll molecules (Hynninen

1973). These primed compounds are formed by an epimeric inversion at the C-10 position (Vernon and Seely 1966). Although there does not appear to be any differences in decay constants between epimers and their parent molecules, chlorophyll a' did decay faster than the more polar chlorophyll a allomer (Table 2).

Mechanisms of Pigment Transformation

Pigment transformations can be used to infer availability of different macrophytes to consumers. Metazoans in particular have been shown to convert chlorophyll a to phaeophorbide a by removing the Mg atom and the phytol chain from the tetrapyrrole (Shuman and Lorenzen 1975; Carpenter and Bergquist 1985; Hawkins et al. 1986; Bianchi et al. 1988; Leavitt and Carpenter, in press). The fastest macrophyte decay constant and highest net production of phaeophorbide a were in V. americana; this may indicate preferential utilization of this substrate by detritivores. Examples of some of the consumers found on the decomposing macrophytes in this study were: cyclopoid copepods, ostracods, naidid oligochaetes, bdelloid rotifers, stalked ciliates, chydorid cladocerans, and nematodes. Potamogeton sp. had the second fastest macrophyte decay constant, but net production of phaeophorbide a was very low (Fig. 3). This pattern suggests that bacteria and fungi were responsible for most of the decay of this macrophyte. Invertebrate detritivore utilization of Potamogeton spp. is very low due to high concentrations of phenolics in this genus (Kerfoot 1988; 1989). Secondary metabolites such as phenolic acids are strong grazing inhibitors (Valiela et al. 1979; Harrison 1982).

Pigment abundances and transformations in macrophytes are affected by differences in the abundance of epiphytes. The occurrence of fucoxanthin and small amounts of chlorophyll c in both SAV's (and not the EAV's) indicates past epiphytic growth (probably diatoms) on submerged macrophytes. The xanthophyll fucoxanthin is considered a bio-marker for chrysophytes, prymnesiophytes and raphidophytes (Wright and Jeffrey 1987). Recent work shows that submerged macrophytes in the mid-Hudson Estuary have dense accumulations of epiphytic growth (R. Garritt, pers. comm.). Higher chlorophyllide a concentrations in the SAV's are due to an abundance of epiphytic diatoms (Fig. 4). Elevated concentrations of chlorophyllide a (phytol chain removed from tetrapyrrole) are found in the presence of chlorophyllase activity, which is usually associated with diatom cell senescence (Barrett and Jeffrey 1971; Jeffrey 1974) although chlorophyllide a is also produced as an intermediate in chlorophyll a synthesis. The combination of evidence from fucoxanthin and chlorophyllide suggests that these differences between SAV's and EAV's are likely to be from epiphytic diatom senescence. Surficial sediments collected from macrophyte beds (V. americana) in the mid-Hudson Estuary show extremely high concentrations of chlorophyllide a, probably due to high inputs of epiphyte detritus (unpubl. data). Epifluorescence microscopy also reveals large amounts of diatom detritus in these surficial sediments.

The composition and decay constants of plant pigments in our experimental treatments can be related to a variety of physical and chemical factors that occur in natural systems. For example, the lower chlorophyll a/b ratios in the SAV's are likely to

be attributed to the low light levels found below the surface of the Hudson Estuary (light extinction coefficient of $2m^{-1}$; Cole et al. in press). Chlorophyll b is an important light-harvesting accessory pigment found in chlorophytes (Wood 1979). In the Hudson, submerged macrophytes have significantly lower chlorophyll a/b ratios than the EAV's (Bianchi and Findlay, 1990). The higher chlorophyll b concentrations may also be due to the differential effects of light scattering and consequent changes in spectral irradiance on pigment concentration (Bidigare et al. 1987, 1989; Smith et al. 1987). The increase in phaeophorbide a concentrations, which occurred in both EAV's and SAV's, supports the idea that detrital forms of chlorophyll a, available to sedimentary diagensis, should contain a dominance of phaeophorbide a (Louda and Baker 1986).

The decay constants that we report here can only provide a general basis for comparison with decay constants in other systems. Decay constants of pigments are strongly influenced by a variety of environmental factors including the presence or absence of oxygen (Louda and Baker 1986; Furlong and Carpenter 1988). The usefulness of pigment bio-markers in natural systems is dependent upon how much we know about their decay kinetics, as well as the mechanisms of transformation. Additional studies that use controlled field and laboratory microcosm experiments are needed to further understand the biogeochemical cycling of photosynthetic pigments in natural environments.

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## Figure Captions

Figure 1. Absorbance and fluorescence chromatograms of pigments in Vallisneria americana (on day 14), analyzed by ion-pairing reversed-phase HPLC. Peaks are numbered as follows: 1, chlorophyllide a; 2, phaeophorbide a; 3, fucoxanthin; 4, neoxanthin; 5, violaxanthin; 6, lutein 5,6 epoxide; 7, antheraxanthin; 8, lutein; 9, lutein isomer; 10, chlorophyll b ; 11, chlorophyll b'; 12, chlorophyll a allomer; 13, chlorophyll a; 14, chlorophyll a'; 15, phaeophytin b; 16, phaeophytin b'; 17, ß-carotene; 18, phaeophytin a; 19, phaeophytin a'.

Figure 2. Concentrations of chlorophyll a and b (nmol g dry $wt^{-1}$) in decomposing macrophytes from experimental litter bags. Note that in all cases, these pigment concentrations were converted to absolute pigment masses in order to calculate decay constants.

Figure 3. Concentrations of phaeophytin a and phaeophorbide a (nmol g dry $wt^{-1}$) in decomposing macrophytes from experimental litter bags.

Figure 4. Concentrations of chlorophyllide a and fucoxanthin (nmol g dry $wt^{-1}$) in decomposing macrophytes from experimental litter bags.

Figure 5. Concentrations of lutein (nmol g dry $wt^{-1}$) in decomposing macrophytes from experimental litter bags.

*Vallisneria americana*

RELATIVE ABSORBANCE

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

5 10 15 20 25 30 35 40 45 50

TIME (min)

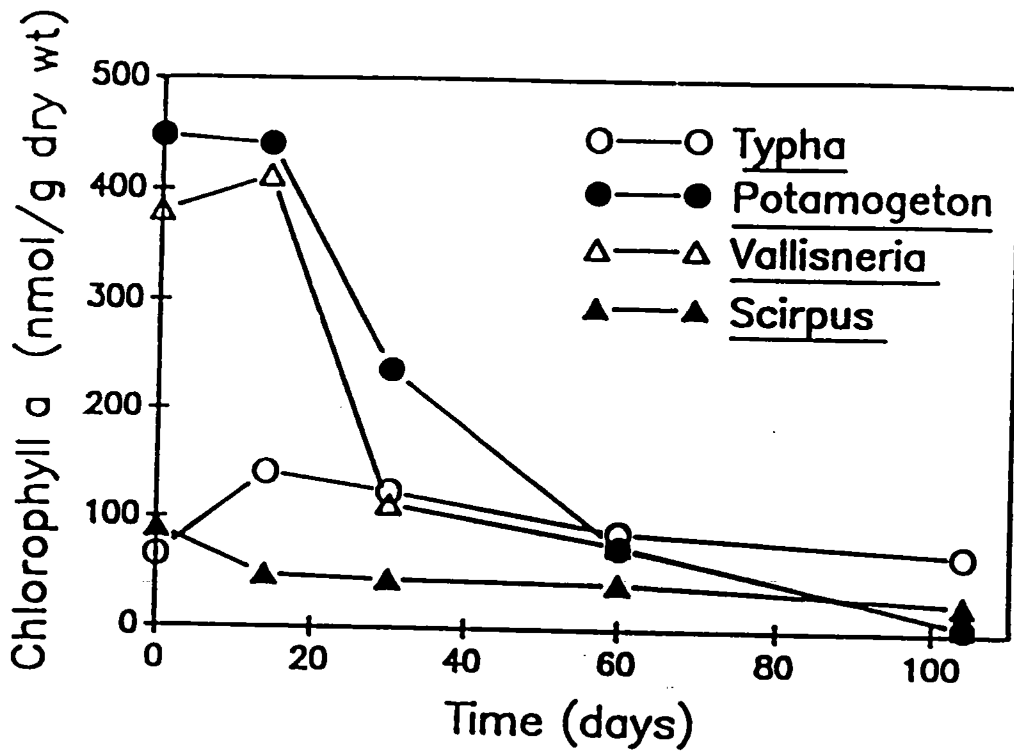
RELATIVE FLUORESCENCE

1 2 10 11 12 13 14 15 16 18 19

5 10 15 20 25 30 35 40 45 50

TIME (min)

Fig 1.



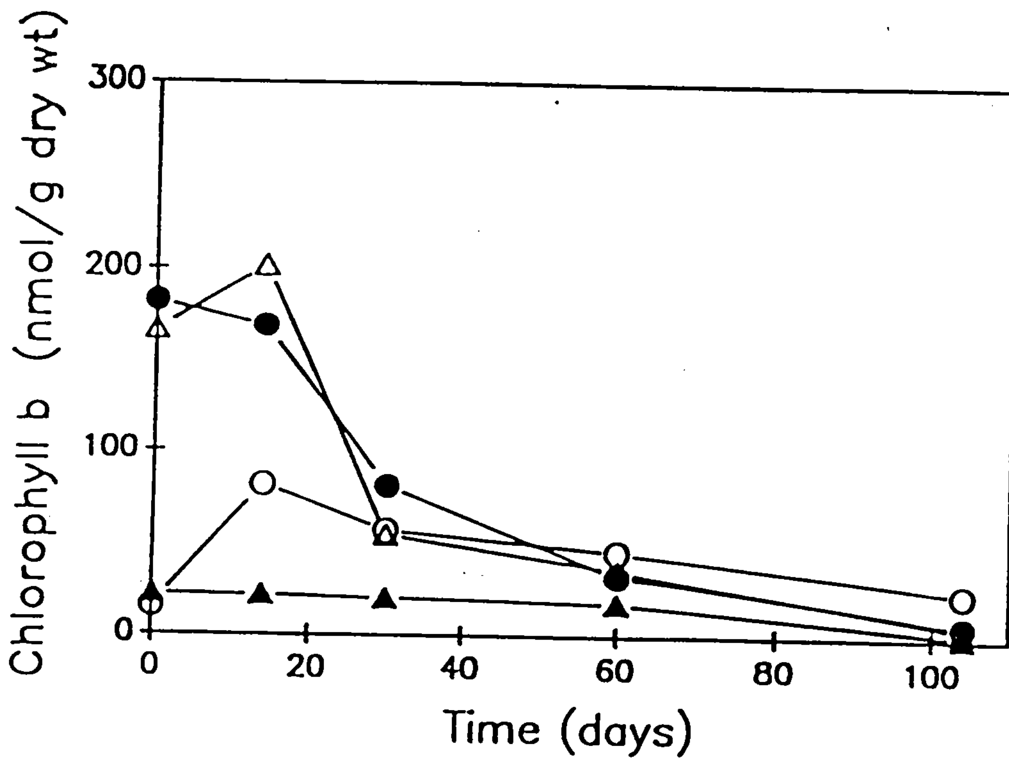
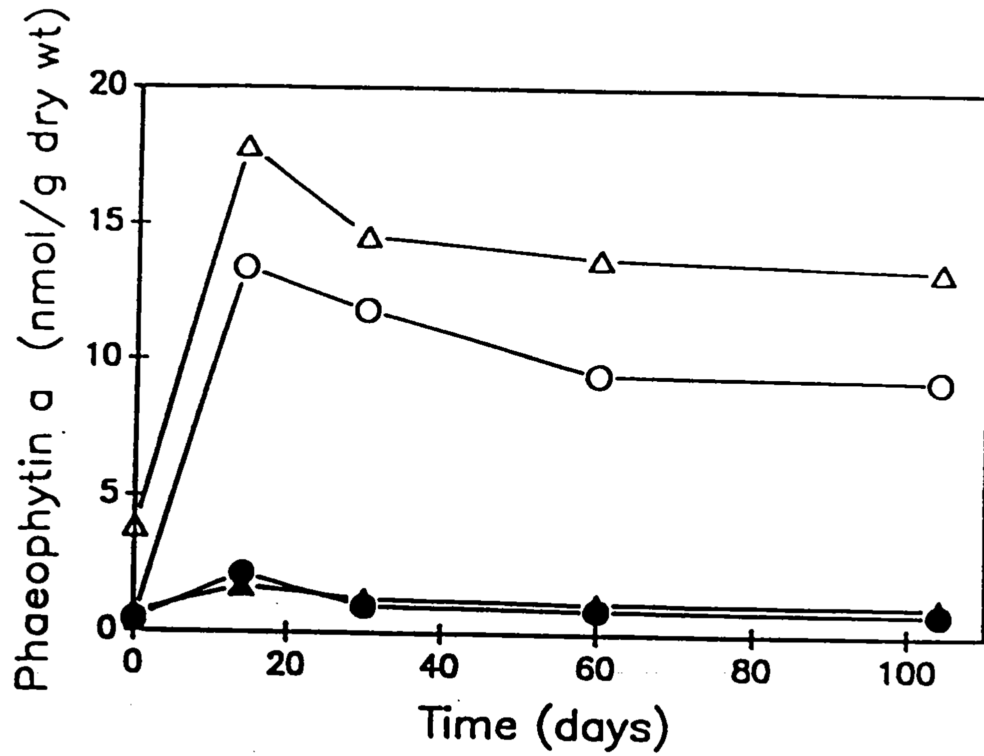




Fig 2.



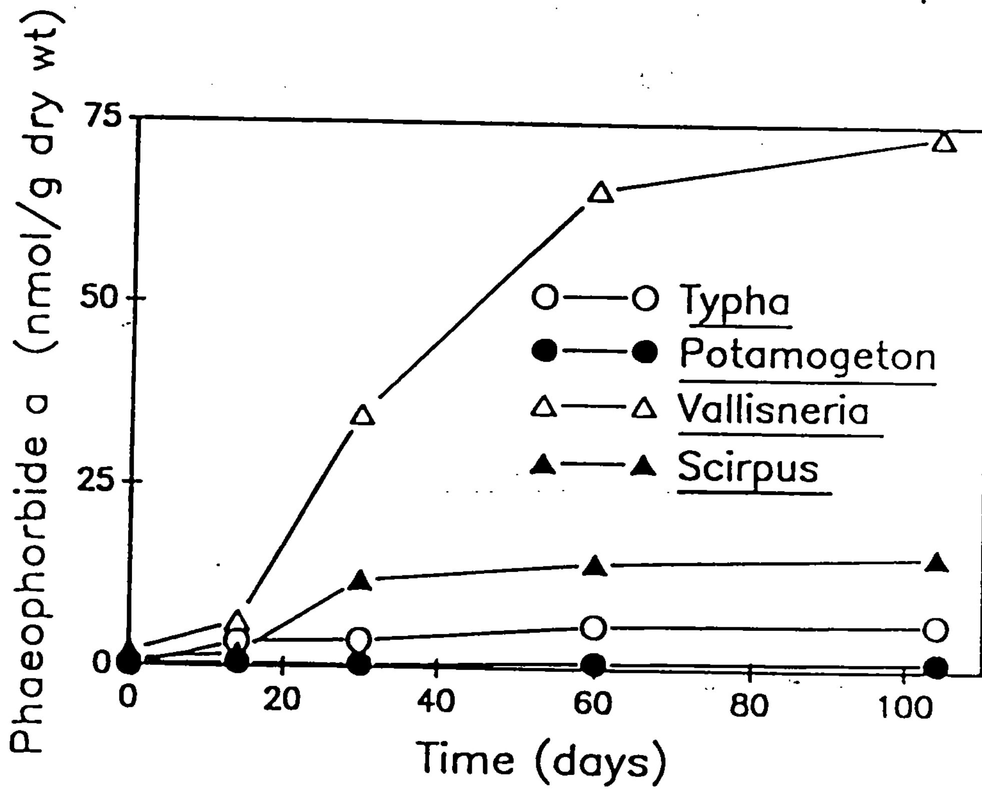
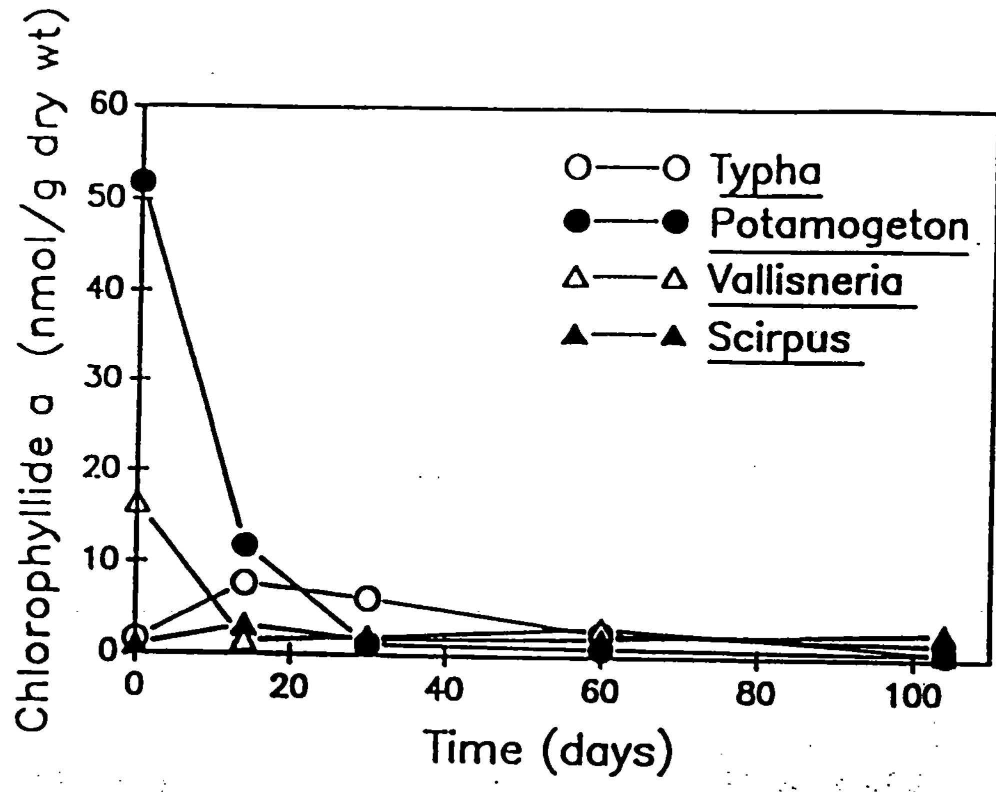




Fig 3.



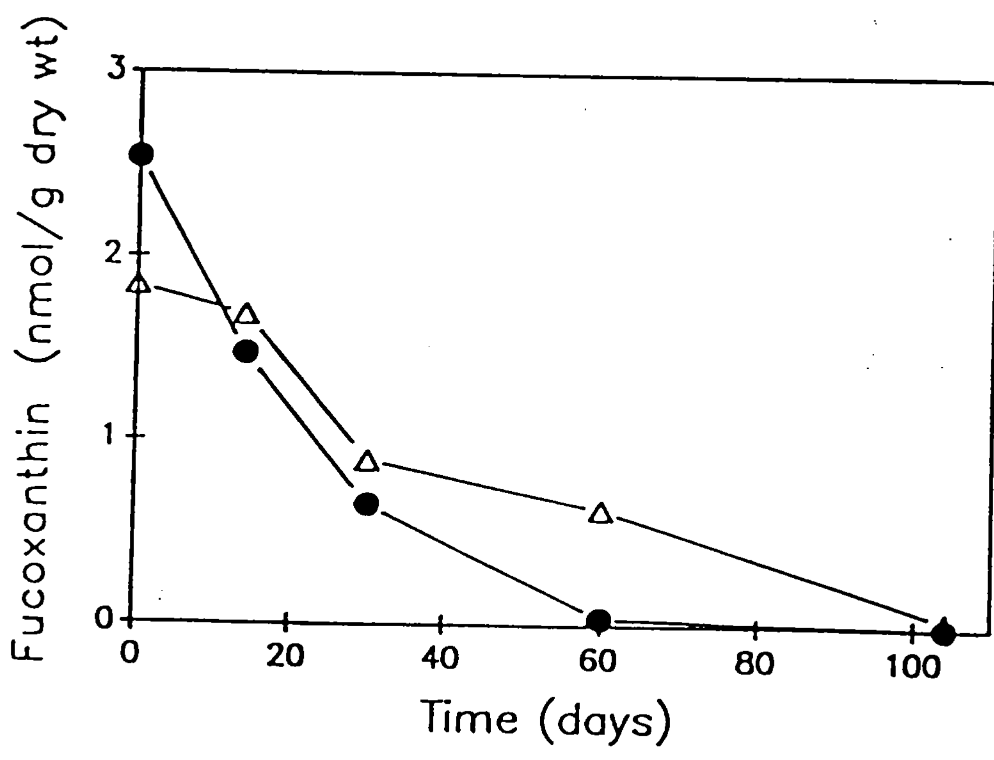




Fig 4

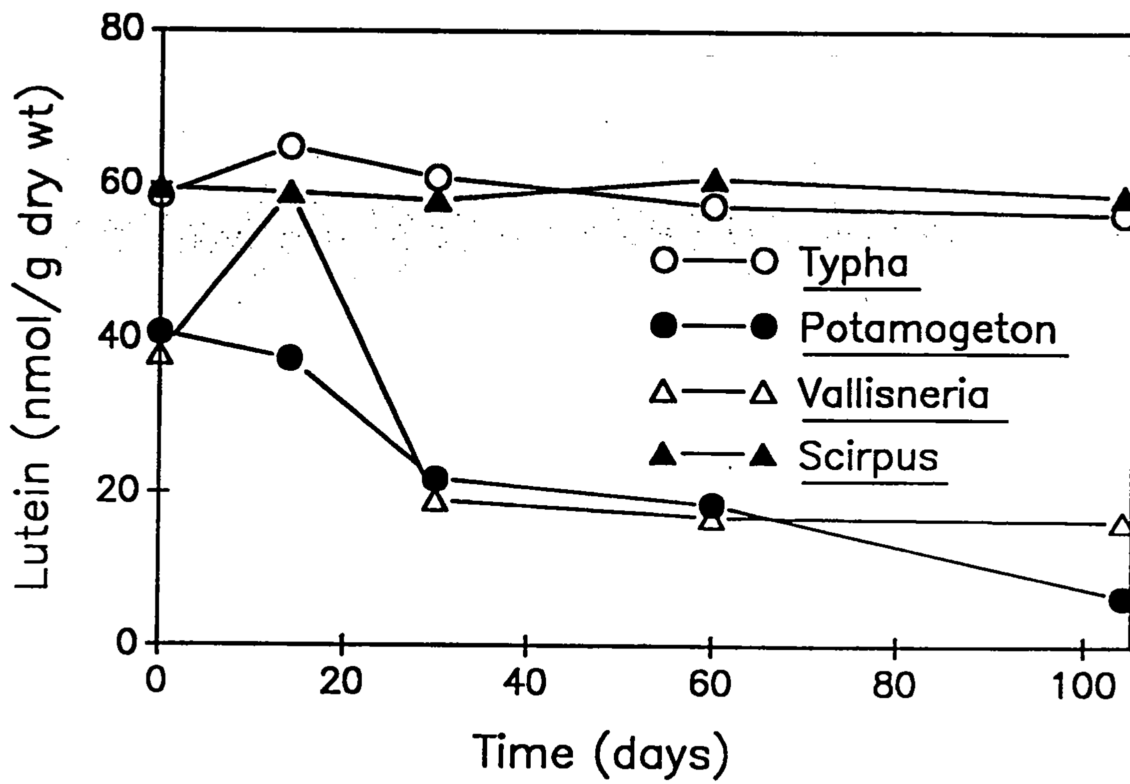



Fig 5.