ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLV, nr 4
1976

Influence of lead tetraethyl on the growth of *Funaria hygrometrica* L. and *Marchantia polymorpha* L.

IRENA KRUPIŃSKA

Institute of Botany, Warsaw University, Warsaw

(Received: March 18, 1976)

Abstract

The influence of various lead tetraethyl concentrations (mixture added to petrol as antiknock agent) on the growth and development of *Funaria hygrometrica* L. spores and of *Marchantia polymorpha* L. gemmulae was investigated. An inhibitory effect of the concentration applied was noted on germination and growth of spores and gemmulae development. Lead tetraethyl produces disturbances in the development of these plants, gradual degeneration of chloroplasts occurs and inhibition of growth leading to profusely branched "dwarf" forms. The disturbances are largely reversible.

INTRODUCTION

The main source of air pollution with lead compounds is the industrial emission from noniron metallurgy centres. Trees in the environs of New Haven (U.S.A.) where lead deposits are exploited exhibit an increased content of this compound in their tissues (Smith, 1972). Another source are exhaust gases of vehicles driven with petrol containing lead compounds. Numerous investigations along motorroutes and in large urban centres demonstrated that in plants exposed to motocar exhaust gases the concentration of this element markedly increases. Grasses and crop plants growing along motorways in the U.S.A. were found to contain lead tetraethyl (Cannon and Bowles, 1962). The permissible maximal lead concentration in plants established by the European Common Market Department is 10 ppm in dry mass.

Lead salts precipitated from exhaust gases were found to have a toxic effect on such plants as *Phaseolus vulgaris* or *Lycopersicum esculentum*, causing severe leaf necrosis (Steenken Folkhard, 1973, 1973a). A reduced photosynthesis and respiration rate in plants was observed

with increase of lead concentration by Bazzac, Rolfe and Windle (1974). Heavy metals from polluted air are particularly accumulated by mosses (Czarnowska, Rejment-Grochowska, 1974; Coombes and Lepp, 1974; Briggs, 1972), therefore these plants have been utilized as indices of invironment pollution. In view of the considerable pollution of the atmosphere with exhaust gases, particularly in urban centres it seemed of interest to investigate the influence of lead tetraethyl on *Bryophytes*.

MATERIAL AND METHODS

The investigations were carried out on mature *Funaria hygrometrica* L. spores and gemmulae of *Marchantia polymorpha* L. These species were chosen for the experiments because of their common occurrence, easy growth and relatively quickly obtainable results. For cultures agar was used (10 g agar, 20 ml of Knop's medium in 1000 ml distilled water). Agar with the nutrient medium in 30-ml portions was poured onto Petri dishes 9 cm in diameter and lead tetraethyl was added.

The compound was not used pure for the experiment but in the mixture which is added to petrol as antiknock agent. The composition of the mixture was as follows:

leadtetraethyl $Pb(C_2H_5)_4$ * — 61.49 per cent by weight,
dibromoethane — 17.86 per cent by weight,
dichloroethane — 18.8 per cent by weight,
and other soluble hydrocarbons.

Specific weight of lead tetraethyl is 1.6, thus in 1 ml of the mixture the content of this compound is 0.982 g. Lead content is 39.39 per cent by weight.

Lead tetraethyl is soluble in petrol (in 100%) and in all hydrocarbons from C_5 to C_{15}, therefore petrol was used for preparing the successive dilutions. Petrol had to be used also as control II since it served as solvent.

Lead tetraethyl concentration in the successive samples was:

control I — pure agar with medium
control II — pure agar with medium + 1 ml petrol
sample III — 0.7 ppm lead tetraethyl
sample IV — 13 ppm „ „
sample V — 26 ppm „ „
sample VI — 66 ppm „ „
sample VII — 133 ppm „ „

* This mixture is generally known as lead tetraethyl. For the sake of simplicity this term is also used here to denote the whole mixture. This also concerns the conclusion and abstract.

Lead tetraethyl dissolved in petrol was mixed with hot agar and poured onto the Petri dishes. The petrol added to the hot medium quickly evaporated. After cooling of the dishes spores of *Funaria hygrometrica* and *Marchantia polymorpha* gemmulae were seeded. The *Funaria* sporophores were placed for 1 h in a chloramine solution (after V a l a n n e, 1966), dried and the spores were prepared out. *Marchantia* gemmulae were isolated from the gemmiferous cups on the thallus into a vessel with distilled water and then filtered off on filter paper and transferred with a brush onto the dishes with medium. The entire experiment jointly with spore and gemmulae isolation and seeding was carried out in sterile conditions. The germination rate and growth were compared with that of the control material with the use for measurements of calibrated oculars with a scale. The cultures were run in a lumistat under stable light and temperature conditions (7920 lux for 12 h in 24 h at 22°C) during the entire experiment.

RESULTS

A. *Marchantia polymorpha*

Three replications of the experiments demonstrated the toxic action of lead tetraethyl on *Marchantia* gemmulae. Since this compound was dissolved in petrol, the growth rate of the gemmulae was compared with the controls on pure agar (control I) and agar with petrol (control II). No major differences were observed in the elongation of the gemmulae in both control media. Neither were morphological and anatomical differences noted in the structure of their thallus (Fig. 1). With increase of lead tetraethyl concentration the elongation of the gemmulae was more and more inhibited. After 11 days of culture the mean length of the control gemmulae was 5.4 mm, whereas those growing on the contamined medium (66 ppm lead tetraethyl) were only 1.2 mm long. A concentration of 0.7 ppm only slightly inhibited growth, whereas 133 ppm was for most gemmulae a lethal dose, only in some few thalli was a very slow growth observed (Fig. 1). When the lowest concentration was used (0.7 ppm) no significant morphological changes appeared as compared with the control. Higher concentrations (13 and 26 ppm) retard rhizoid formation. The thallus cells contain a much smaller number of chloroplasts and much paler-coloured ones as compared with those in the control. Gemmulae growing on the contaminated medium form on the thallus meristematic cell agglomerations. From these cell there grow in time vertically upwards cylindrical branchings at the top of which flat patch-like fragments of thallus form. They are frequently circular and indented or smooth. Most of them produce numerous rhizoids (Plate I: 1—7, Fig. 2). After a longer period of culture the thalli extend

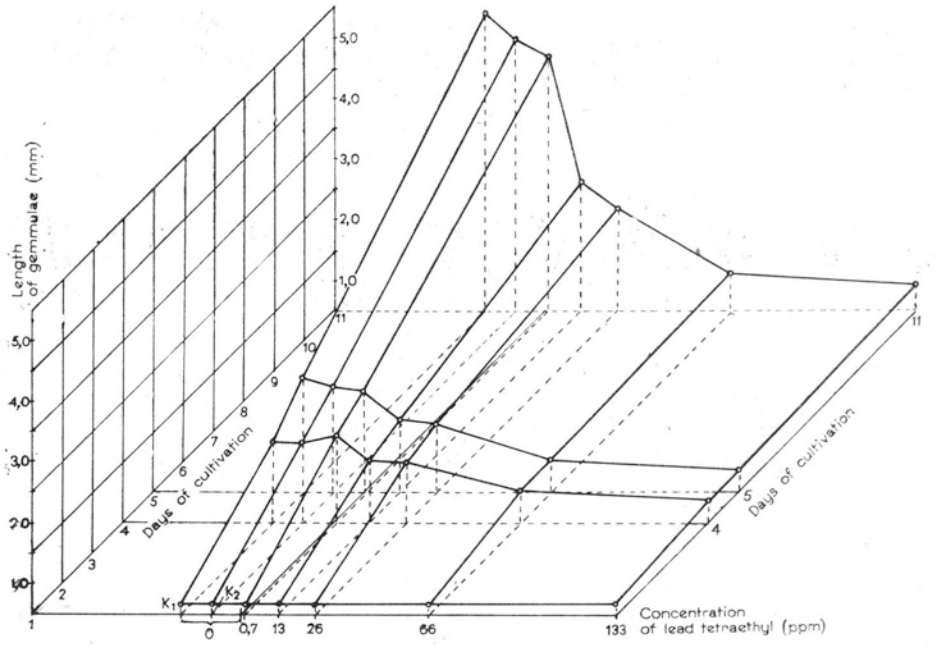


Fig. 1. Influence of various lead tetraethyl concentrations on elongation of *M. polymorpha* gemmulae. Each value is a mean of 20 measurements

changing to a more elongated shape. At a lead tetraethyl concentration of 66 ppm the above described disturbances are more pronounced. Formation on the gemmulae of meristematic cell agglomerations and their slow growth were observed as late as after 10 days of culture. About 70 per cent of gemmulae were destroyed as the result of gradual chloroplast degeneration and thallus cells break-down. At a tetraethyl concentration of 133 ppm only on few gemmulae (ca. 10%) could after 30 days of culture meristematic cell agglomerations be found. This concentration is so toxic that it may be considered as lethal.

After one month of culture on agar medium the *Marchantia polymorpha* thalli were transferred onto soil not contaminated with lead. The gemmulae continued to grow and the degeneration processes evoked by the action of lead tetraethyl receded (Plate II). The disturbances in *Marchantia polymorpha* due to this compound are, therefore, reversible to a large extent.

B. *Funaria hygrometrica*

Experiments with *Funaria hygrometrica* spores were performed analogously to those with *Marchantia polymorpha* gemmulae, with the use of the same lead tetraethyl concentrations. The experiments demonstrated

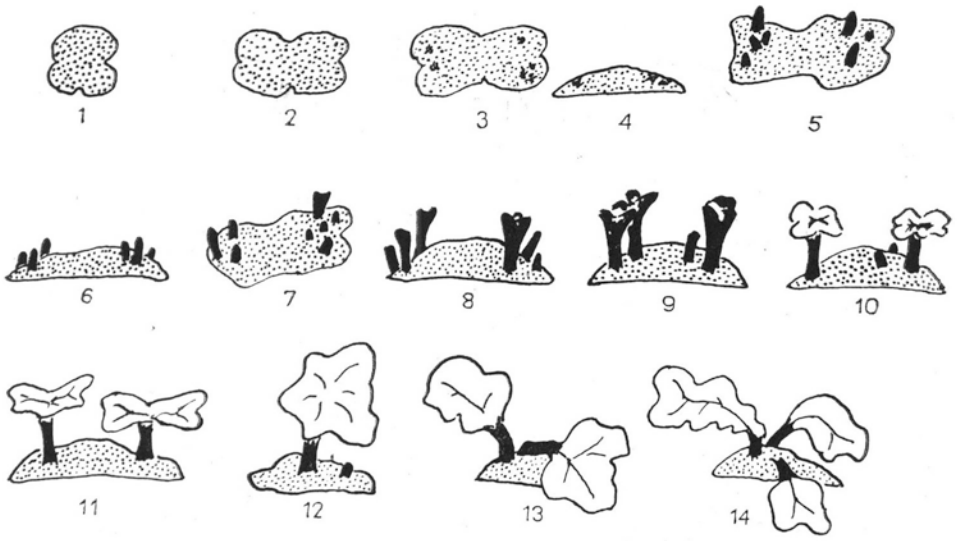


Fig. 2. Scheme illustrating the successive steps of development of *M. polymorpha* gemmulae growing on medium contaminated with lead tetraethyl (1, 2, 3, 5, 7 — view from above, the remaining are lateral views)

a retardation of spore germination on the contaminated medium. With increase in concentration of the toxic agent the germination time of the spores was protracted. Spores seeded on medium with 13 ppm of lead tetraethyl germinated with a 6-day delay as compared with the controls. At a 66 ppm concentration this delay was 10 days (Fig. 3).

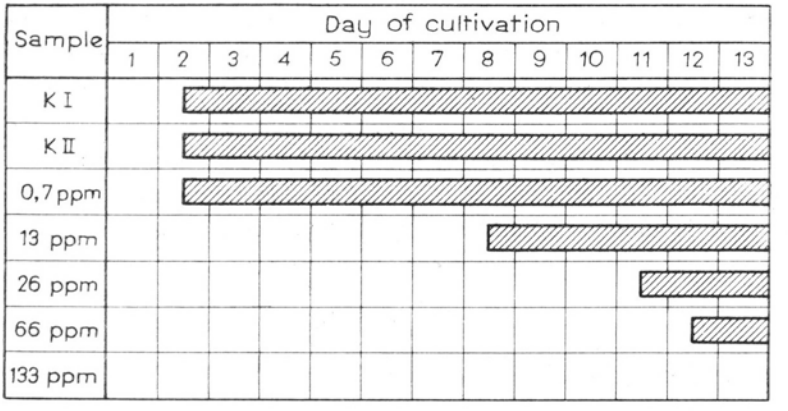


Fig. 3. Influence of various lead tetraethyl concentrations on germination rate of *F. hygrometrica* spores

The influence of various concentrations of lead tetraethyl on elongation of the spore protonema is illustrated in Fig. 4 and Plate III: 1—7. At a concentration of 133 ppm only a small number of spores (ca. 10%)

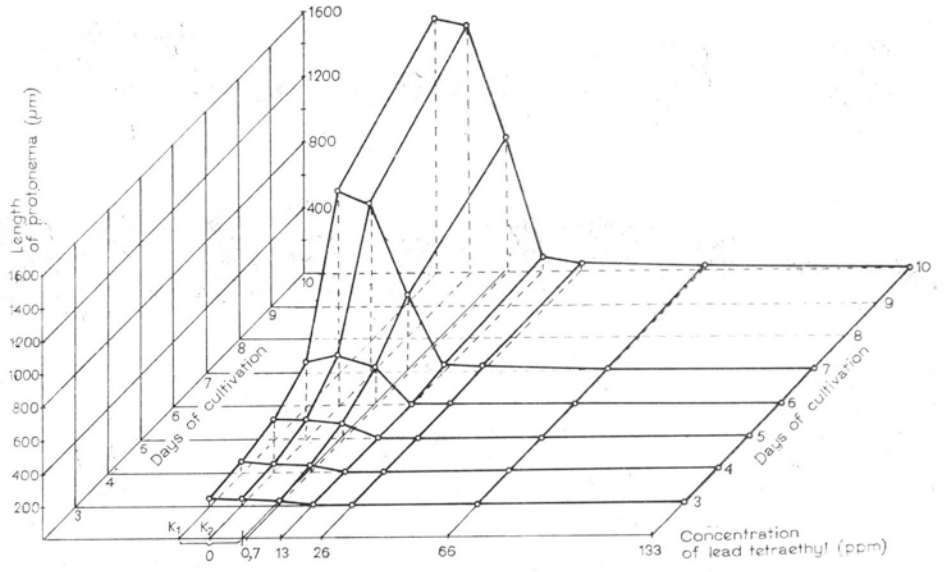


Fig. 4. Influence of various lead tetraethyl concentrations on elongation of *F. hygrometrica* protonemas. Each value represents a mean of 20 measurements

swelled and formed short protonemas, but the development process did not progress in the subsequent days of culture. In the remaining spores degeneration of chloroplasts was found and severe contraction of the protoplast (Plate IV: 1). At concentrations of 15, 26 and 66 ppm disturbances were observed in the protonema, becoming more intensive with the increase in concentration of lead tetraethyl. The protonema of spores growing on control medium are filamentously elongated and contain a high number of chloroplasts. When the medium is contaminated the protonema shows numerous bendings. It is profusely branched, bushy and the cells contain less chloroplasts. Very often 2—3 branches of protonema grow out simultaneously owing to early division of the protoplast still inside the spore. The growth of the branching protonemas is much slower than on unpolluted medium (Plate IV: 2—10).

DISCUSSION

It results from the above described experiments that lead tetraethyl has a significant effect on the course of the spore germination process in *Funaria hygrometrica* and on the development of *Marchantia polymorpha* gemmulae. Petrol in which lead tetraethyl was dissolved has no influence on the observed disturbances. This is connected with the way of medium preparation. Tetraethyl dissolved in petrol was added to hot agar. Owing to its high volatility petrol (particularly at elevated temperatures) evaporated almost completely.

Numerous investigators studied the influence of lead and other heavy metals on mosses. The effect of zinc and lead on the growth of *Marchantia polymorpha* and *Funaria hygrometrica* was tested by Coombes and Lepp (1974). *Marchantia* gemmulae tolerate a zinc concentration up to 100 ppm, while a copper level above 0.5 ppm inhibits their development. The growth of the gemmulae was also tested on aggar medium containing lead nitrate. The gemmulae tolerated this compound in a concentration as high as 400 ppm (Briggs, 1972). It results from the experimental results that lead tetraethyl in a 133 ppm concentration should be considered as a lethal dose for *Marchantia* gemmulae. Authors studying the influence of zinc and copper on *Funaria hygrometrica* and *Marchantia polymorpha* found that the presence of these metals in the medium causes reduction of chlorophyll content and inhibition of rhizoid formation. The results obtained with lead tetraethyl agree with the above mentioned observations. Steenken Folkhard (1973) when acting with lead salts precipitated from exhaust gases noticed that young leaves of the experimental plants showed changes in their shape (folds in leaf blade), and their apical parts were decolorized. Similar deformations were found in the case of *Marchantia* thalli growing on contaminated medium. The thalli did not branch dichotomically and had indented wavy edges.

When considering the influence of lead tetraethyl on *Funaria hygrometrica*, the deformation of protonema should be stressed with formation of profusely branched bent protonemas inhibited in growth. The disturbances are probably due to the action of lead tetraethyl on mitotic cell division (this will be the subject of a forthcoming paper). Ahbberg and Ramel (1972) carrying out experiments on organolead compounds and their influence on living organisms (*Allium cepa* among others) noted disturbances of the karyokinetic spindle and of cell division.

REFERENCES

Ahbberg J., Ramel C., Wachtmeister C. A., 1972. Organolead compounds shown to be genetically active. Ambio 1/1: 29—31.

Bazzac F. A., Rolfe G., Windle P., 1974. Differing sensitivity of corn and soybean photosynthesis and transpiration. J. Environ. Qual. 3/2: 156—158.

Briggs D., 1972. Population differentiation in *Marchantia polymorpha* L. in various lead pollution levels. Nature 238/5360: 166—167.

Cannon A. L., Bowles J. M., 1962. Contamination of vegetation by tetraethyl lead. Science 137: 765—766.

Coombes A. J., Lepp N. W., 1974. The effect of Cu and Zn on the growth of *Marchantia polymorpha* and *Funaria hygrometrica*. The Bryologist 77/3: 447—452.

Czarnowska K., Rejment-Grochowska I., 1974. Concentration of heavy metals — iron, manganese, zinc and copper in mosses. Acta Soc. Bot. Pol. 43(1): 39—44,

Smith W. H., 1972. Lead and mercury burden of urban woody plants. Science 176: 1237—1238.
Steenken Folkhard, 1973. Benebulungsversuche von Nutzpflanzen mit Bleisalzen aus Autoabgasen. Z. Pfl.-Krankheit. 80/6: 346—358.
Steenken Folkhard, 1973a. Gegasungen von Nutzpflanzen mit bleihaltigen und bleifriien Autoabgasen. Z. Pfl.-Krankheit. 80/9—10: 513—527.
Valanne N., 1966. The germination phases of moss spores and their control by light. Ann. Bot. Fennici 3/1: 1—42.

Author's address:

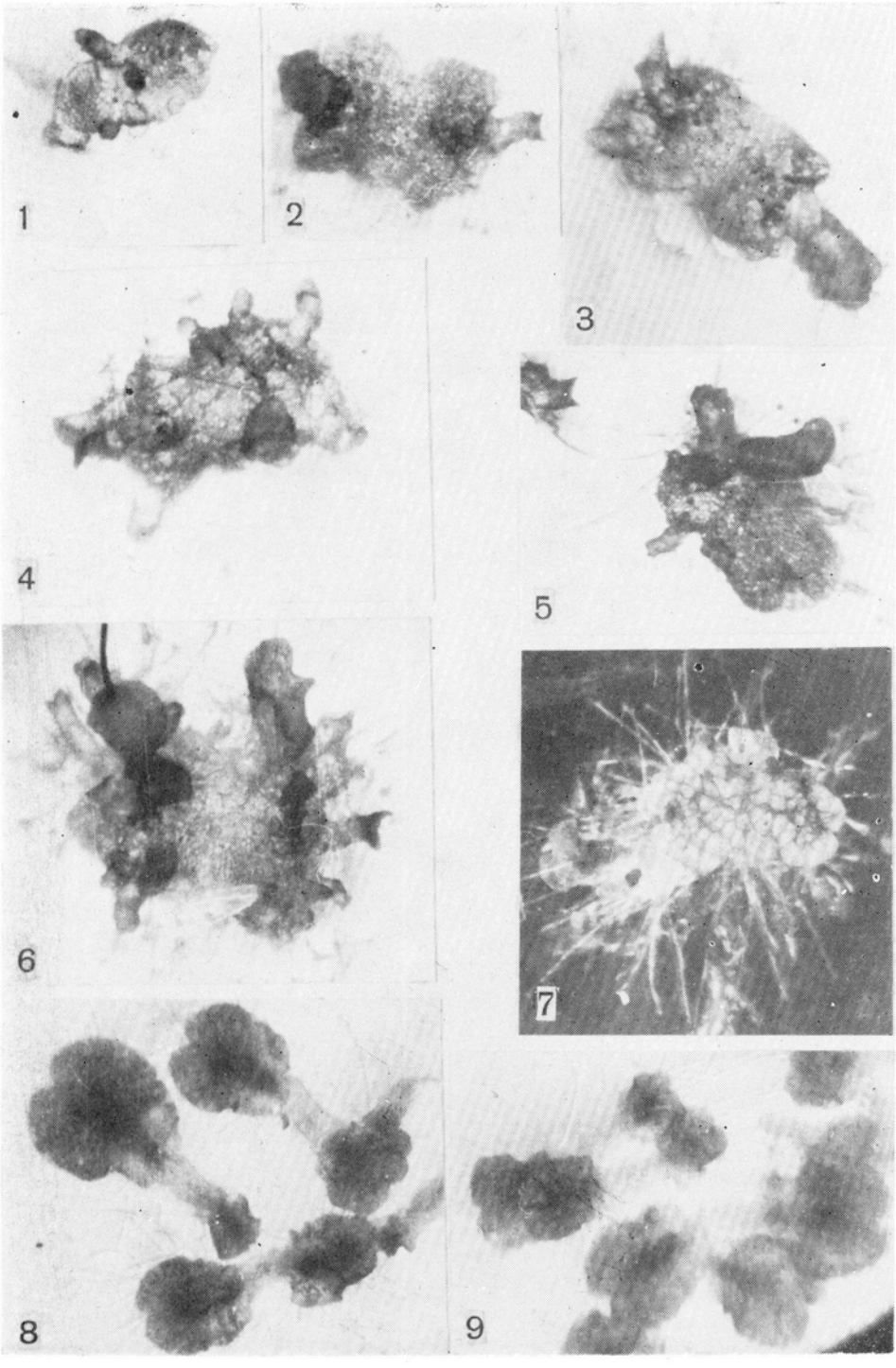
mgr Irena Krupińska
Botanical Institute of Warsaw,
Al. Ujazdowskie 4; 00-478 Warszawa; Poland

Wpływ czteroetylku ołowiu na wzrost Funaria hygrometrica L. i Marchantia polymorpha L.

Streszczenie

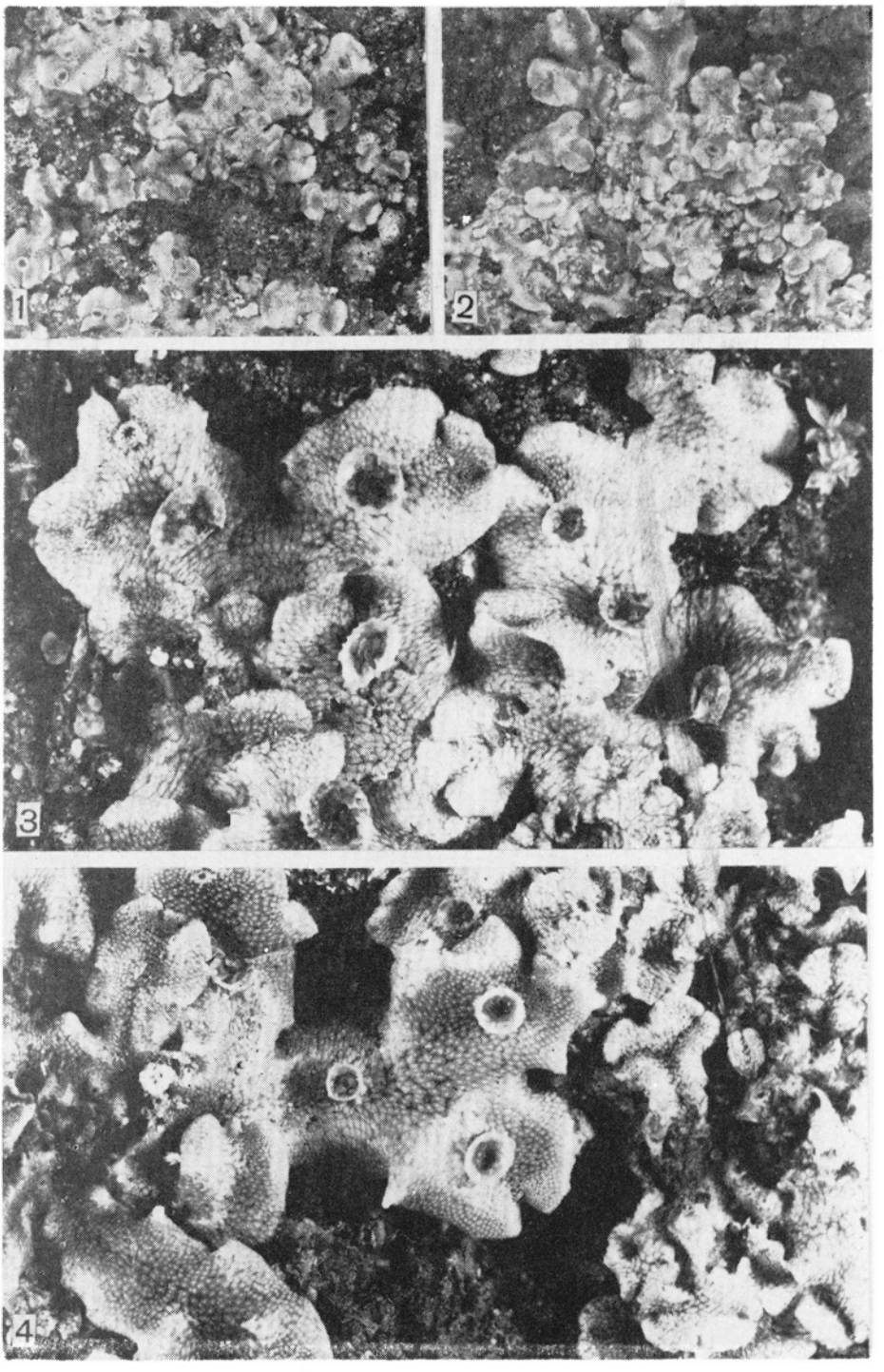
Zbadano wpływ różnych koncentracji czteroetylku ołowiu na wzrost i rozwój zarodników *Funaria hygrometrica* L. i rozmnóżek *Marchantia polymorpha* L. Hodowle prowadzono na pożywce agarowej. Analizowano następujące stężenia czteroetylku: 0,7 ppm, 13 ppm, 26 ppm, 66 ppm, 133 ppm.

Stwierdzono hamujący wpływ stosowanych koncentracji na kiełkowanie i wzrost zarodników oraz rozwój rozmnóżek. Wraz ze zwiększaniem stosowanego stężenia czteroetylku wydłuża się czas kiełkowania zarodników, hamowany jest wzrost i dalszy ich rozwój. Stężenie graniczne, przy którym rozwój rozmnóżek i kiełkowanie zarodników zostaje zahamowane jest dla obu badanych obiektów takie samo i wynosi 133 ppm. Niższe stężenia $Pb(C_2H_5)_4$ powodują występowanie szeregu zaburzeń w rozwoju tych roślin. Wraz ze wzrostem stężenia zaburzenia nasilają się. U obu badanych obiektów obserwowano degenerację chloroplastów oraz zahamowanie wzrostu prowadzące do wytworzenia mocno rozgałęzionych, „karłowatych" form. Na degenerujących rozmnóżkach *M. polymorpha* tworzą się skupienia komórek merystematycznych, które w trakcie dalszego rozwoju tworzą wyniesione ku górze, wielokrotnie powcinane lub gładkie plechy. Protonemy *F. hygrometrica* są również mocno rozgałęzione, krzaczaste i o wyraźnie zahamowanym wzroście. Zaburzenia te spowodowane są prawdopodobnie wpływem czteroetylku na podziały mitotyczne komórek. Sugestie te potwierdzają badania prowadzone na związkach organicznych ołowiu i ich wpływem na zaburzenia wrzeciona kariokinetycznego i podziały mitotyczne komórek (Ahbberg, Ramel, 1972). Zaburzenia *M. polymorpha* wywołane działaniem czteroetylku są w znacznym stopniu odwracalne.

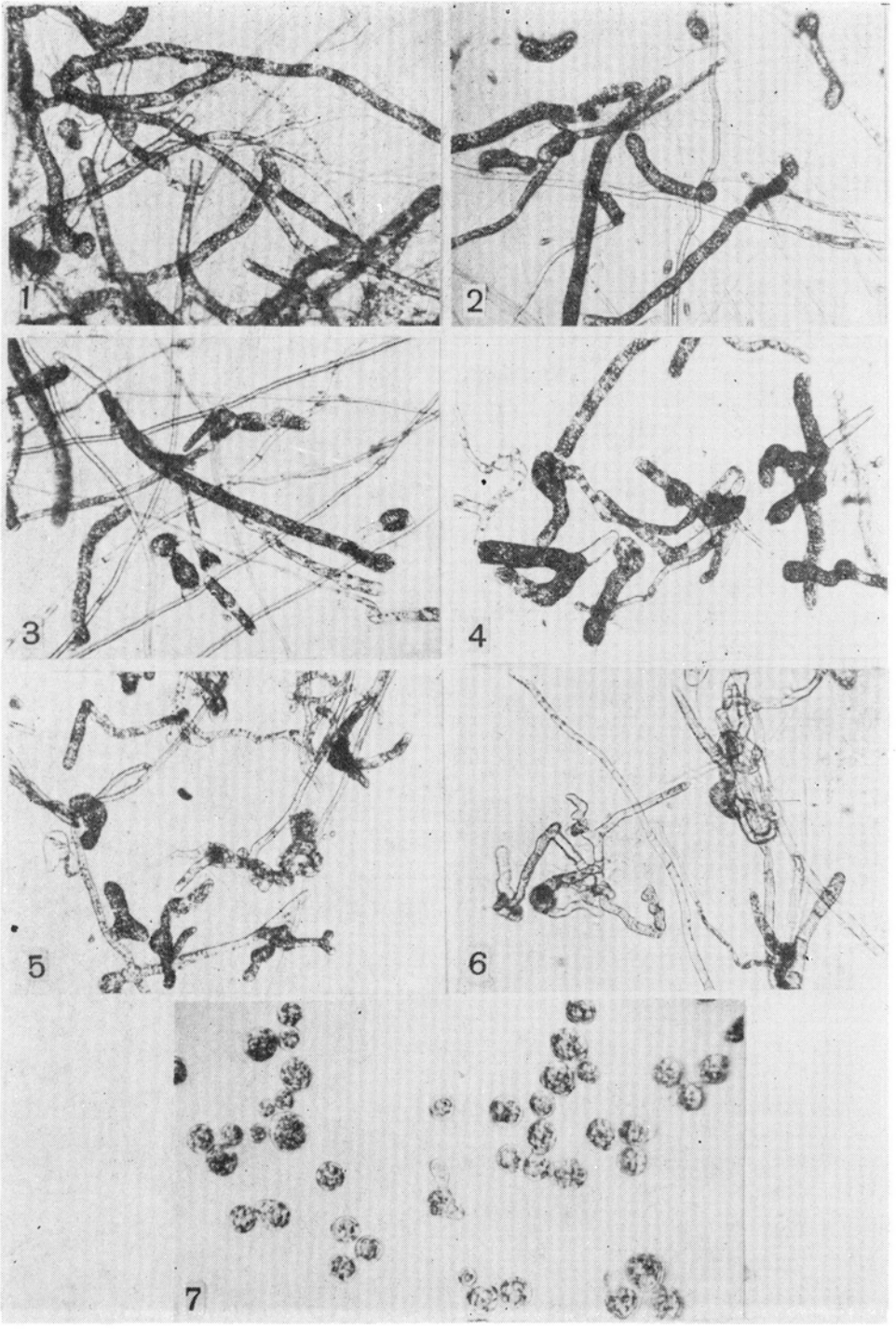


Photos 1—7. Disturbances caused by the action of lead tetraethyl on *M. Polymorpha* gemmulae. ×80. Photo 8. Control I. ×6. Photo 9. Control II. ×6 duration of culture 20 days

Plate II

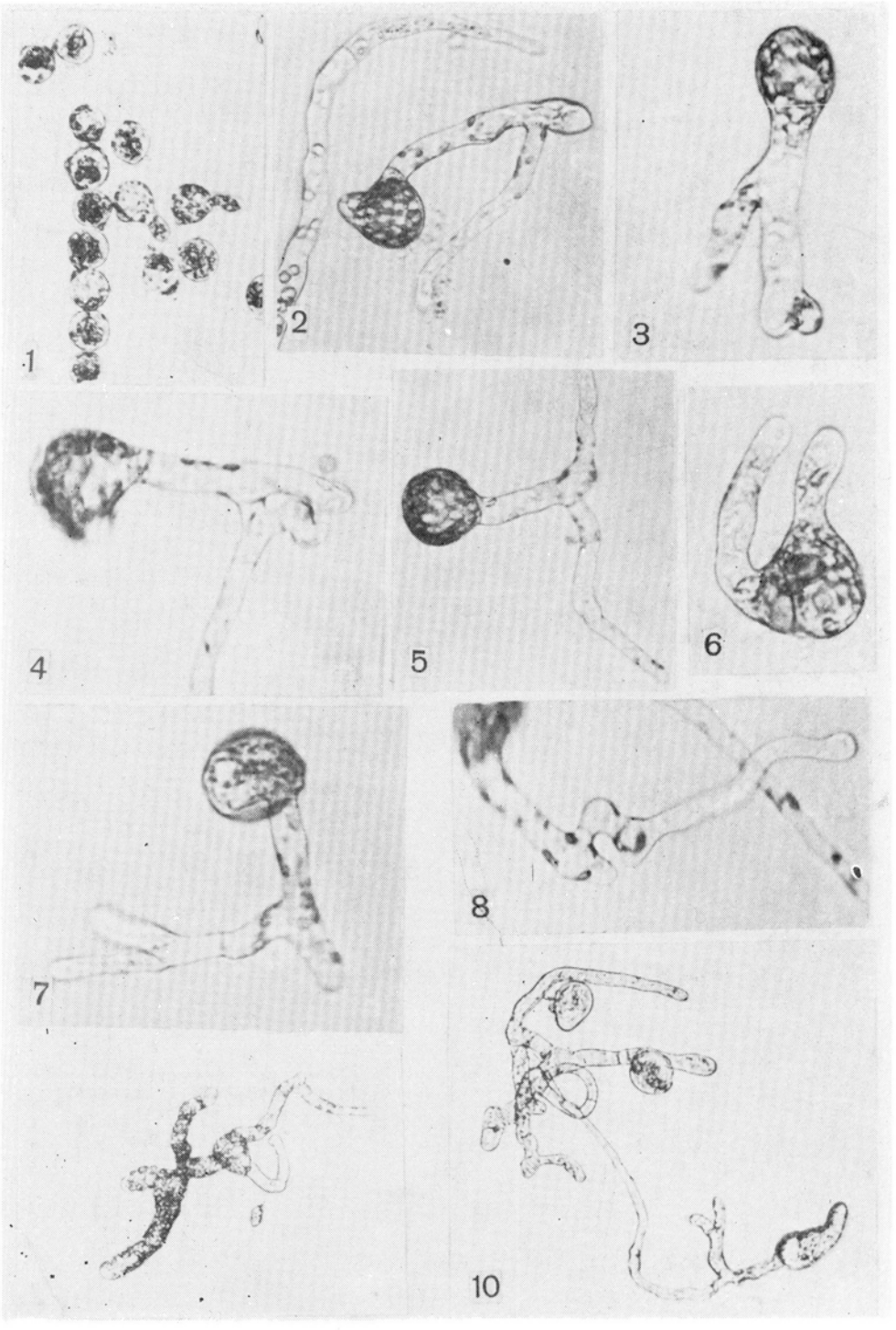


Photos 1, 2. *M. polymorpha* gemmulae (control) transplanted on soil not contaminated with lead, after 30 days of culture on agar. Photos 2, 4. *M. polymorpha* gemmulae transplanted to soil not contamined with lead, after 30 days of culture on agar with lead tetraethyl. Photos 1—2 natural size, photo 3 ×4. Culture duration 3 months



Influence of various lead tetraethyl concentrations on development of *F. hygrometrica* spores. Photo 1. control I, photo 2. control II, photo 3 — 0.7 ppm lead tetraethyl, photo 4 — 13 ppm, photo 5 — 26 ppm, photo 6 — 66 ppm, photo 7 — 133 ppm. Culture duration 30 days ×200

Plate IV



Disturbances caused by action of lead tetraethyl on *F. hygrometrica* spores. Photo 1. 133 ppm, photos 2—4. 26 ppm, photos 5—10. 66 ppm lead tetraethyl. Photos 1, 9, 10 ×200, photos 2—8 ×600