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Third Report Concerning International Atomic Energy Agency Research Contract

No.: 37

A supplement to the application for financial aid the last year of the research project.

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FOREWORD

This is the third report to the International Atomic Energy Agency under the Research Contract No. 37.

The study at Kjeller, Norway, is an experimental investigation of the accumulation of some radioactive isotopes in some aquatic organisms, and the aspects of the distribution of these substances in the food chain of a stream.

The research programme entered upon was intended as a three year experimental investigation of some important biological problems related to the disposal of radioactive wastes in a small Norwegian river. This report covers most of the work performed during the summer 1961.

The experimental approach has been made in a specially designed model recipient. Equipment of this construction is for the first time used in research on radioecological implications of radioactive waste disposal problems.

The present account is an accompanying paper to the application for financial aid the last year of the research programme.

Blindern, November 25, 1961.

Karen Halvorsen Olav Skulberg

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1. OUTLOOK AND STATEMENT

Although inland waters are generally regarded unsuited as recipients for radioactive wastes, nevertheless, one of the important means of disposing such materials is provided by streams. The development of atomic energy is thus faced with a water pollution problem. The efforts to solve and manage a practical and safe waste disposal programme must be given serious attention (Straub et al. 1959). An understanding of the effects of introducing the wastes into the aquatic environment ought to be the background for the sanitary engineer in the attempt to solve problems of this nature.

The processes operating in the recipient, affecting the distribution of the radioactivity, have to be studied. When the significant biotic and abiotic factors are recognized, it may be possible to define permissible levels of radioactive contaminants which will still preserve the water for man's direct and indirect use without deleterious effects to man or organisms. The knowledge of these factors may also make it possible to establish a practical programme for supervision based on a few selected organisms or other environmental components.

A considerable amount of research work has been put into the field of radioecology since the importance of these problems was recognized. A list with selected references to the literature available on marine and aquatic radiobiology of this category up to May 1960 contains 422 headings (Klement et al. 1960). Since then written contributions to the topic have been steadily increasing. An examination of the literature, however, demonstrates that the ecological research on phenomena related to water pollution problems with radioactive substances is in its commencement. This situation is stressed by several authors (e.g. Dunster 1959, Foster 1959, Nelson 1960, Tsivoglou et al. 1960, Jaag 1961).

A still better indication of the state of research is perhaps represented by the ecological studies of the streams that receive radioactive wastes in North-America (e.g. the Columbia River, the Ottawa River and the Clinch River), and in smaller scale in Western-Europe (e.g. the Thames, the Rhine, the Elbe, and the Rhone). These investigations are performed to obtain primary information about the problems involved.

A statement in the paper ORNL - 2557 "Report of the joint programme of studies on the decontamination of radioactive waters", page 11, reads as follows: "Summarizing, it may be stated that data available at present on concentration of radioactive materials by natural agents are of a preliminary nature. There is much to be learned about the many complex mechanisms responsible for this concentration".

The need of extending and improving the research in radioecology is reflected in the programme for studies of this nature in the various centers for development of the use of atomic energy, as well as in universities and other scientific institutions (e.g. U.S. Atomic Energy Commission 1961).

During the Scientific Conference on Radioactive Waste Disposal, Monaco, November 16 - 21, 1959, and on the Second Panel on Coordination of Research Contracts on Selected Topics in Radiobiology, Vienna, March 28 - 30, 1961, the status of research on biological implications of reconcentration of radioactive wastes in streams were commented upon by several scientists.

The current opinions at these meetings were that basic and applied studies are needed and desirable in order to obtain further information on the dispersion, movement, distribution and biological cycling of radionuclides in a wide variety of aquatic environments.

In the present state of development concerning radioecology there is a demand for observations and experiments made in different geographical regions. The wide range of habitats, the variety of communities, the different climatic and other physiographical factors involve a great amount of research work to be done. On the other hand, data collected furnish the science with evidence that make comparison and criticism possible. This will form the base for progress in the field towards a more adequate understanding of the complex of phenomena considered.

The effectuation of the research programme concerned with the International Atomic Energy Agency Research Contract No. 37, was intended as a contribution to the international efforts to obtain information related to biological implications of stream pollution with radioactive wastes.

At the same time the results obtained may furnish a base for the evaluation of the safe use of the river Nitelv as a recipient of low level radioactive wastes. The actual problem is for the first time studied under conditions of nature prevailing in Scandinavia. The geographical position will determine the physiographical factors as well as the selection of organisms in the communities which are considered.

Most of the knowledge existing about the abiotic and biotic factors that may influence the dispersion of radionuclides disposed to stream environments represents analytical data and observations made during regional surveys of contaminated watercourses. These studies are very important contributions and have founded a base for further endeavour (e.g. Krumholz 1954, Junkins et al. 1960, Tsivoglou et al 1960, Morton et al. 1961, Sedlet 1961). A regional survey of this kind was made at the river Nitelv and provided a background for the experimental investigation involved in the International Atomic Energy Agency Research Contract No. 37 (Stedje 1960).

Another type of research consists in laboratory investigations on single problems and selected organisms. This kind of experimental investigations is reported from many research institutes (e.g. Boroughs et al. 1957, Davis 1959, Auerbach 1959, Department of Scientific and Industrial Research 1959, Polikarpov 1960). Laboratory experiments are also performed in connection with the International Atomic Energy Agency Research Contract No. 37.

A third category of investigation on these problems is represented by what may be called "controlled experiments in natural aquatic communities". This synecological approach has the advantage that the organisms are in situ on their respective biotopes during the experiment. The environmental factors and the organisms interact upon each other in the same way as in the actual recipient. Thus it is possible to describe and define the conditions for bioaccumulation and transfer of radionuclides by the components of the aquatic communities. Experimental work (to some extent) of this kind is performed by several investigators (e.g. Hutchinson et al. 1950, Krumholz 1956, Pendleton et al. 1958, Foster 1959, U.S. Atomic Energy Commission 1961 - contract AT (11-1)-655).

The model recipient wherein the experimental undertakings for the International Atomic Energy Agency Research Contract No. 37 are performed, provide experimental facilities specially designed for investigations of the third category. For a description of the build up and function of the experimental plant reference is given to previous reports (Skulberg 1960, 1961). Some of the very important advantages offered by the device are summarized in the following: (For information related to the use of channel systems for investigations of general water pollution problems see Wuhrmann 1951).

The experiments are conducted with the river water itself. The aspects of the change in water quality with meteorological conditions and the seasons, and the effect of such change on the processes studied, are given attention. The factors of the environment are either controllable (e.g. water flow and velocity, nature and degree of radioactive contamination, type and qualities of the bed of the running water, in part the chemical and physical properties of the water) or subject for measurements. The opportunity is at hand to study the organisms and their biotic interrelationships very closely. The entities of biota, with their characteristics in addition to the characteristics of individuals of species, can be considered. By this the importance of population and community relations on the actual problems is emphasized.

The diverse uses to which the model recipient may be put are many. We consider it more suitable for precise experimental investigation on radioecology of flowing water than most other experimental facilities used for studying biological problems related to radioactive waste disposal. Our experience obtained during the introductory work in the experimental plant in the autumn 1960 and from the investigations the summer 1961 support this opinion.

The experimental work during the summer 1961 have mainly progressed along two lines which we will designate as an autecological and a synecological approach. The aims and the objectives are in accordance with the written research programme of the previous applications to the International Atomic Energy Agency.

2. AUTECOLOGICAL APPROACH

Work classified under this category performed during the summer 1961 comprise experiments conducted in the well system and the recirculation channel of the experimental plant.

The study of the ability of single organisms to transfer radionuclides through the different trophic levels in the ecosystem includesinvestigations of the accumulation phenomena. It is important to get knowledge about the nature and extent of the several processes effecting maximum and minimum concentrations of elements in the various species of the aquatic communities. The purpose of the experiments here reported was to obtain information about levels of radioactive contamination that may occur in selected species of river biota as a result of exposure to radionuclides under known conditions. The variation between the different species in their capacity to concentrate the radionuclides used was considered.

Important organisms from an ecological point of view were sampled from different habitats in the river Nitelv during the early summer. The organisms were cultivated and maintained in the experimental plant, and from this collection specimens of good condition and as equal as possible were selected and transplanted in the well system and recirculation channel in due time before the experiments were started.

The objects for the experiments were the following species:

Algae

1. Chara Braunii Gmel.

   Family Characeae.

   The species is a cosmopolite, and has a wide distribution in Europe. The collecting of the species was done in the potametum sociation, where it occurs in loose population at small depths.

Vascular plants:

2. Alisma Plantago-aquatica L.

Family Alismataceae.

A borealcircumpolar plant.

In the river Nitelv this species is a constituent of magnocaricetum, eleocharetum and potametum sociations. The collecting of the species was done in the eulittoral zone.

3. Callitriche verna L.

Family Callitrichaceae.

A borealcircumpolar plant.

Found together with the forementioned species in the eleocharetum sociation of macrovegetation in the river Nitelv.

4. Scirpus acicularis L.

Family Cyperaceae.

A borealcircumpolar plant.

Growing in dense population in the eleocharetum sociation. The collecting of the species was done in the eulittoral zone.

Invertebrates:

5. Anodonta cygnea L.

Family Unionacea.

The species has a wide range on the northern hemisphere.

The sampling of the animals was done in the sublittoral zone, outside the potametum sociation.

Vertebrates:

6. Phoxinus phoxinus Linné

Family Cyprinidae.

A cyprinid fish of Europe and northern Asia.

The fish was caught with an electric fishing apparatus.

7. Salmo trutta Linné

Family Salmonidae

Fresh-water distribution in the countries of Europe north of the Mediterranean eastwards to Greece.

The fish used for the experiment were reared in a fish hatchery before the transfer to the well system.

The species number 1, 4, 6, and 7, are to our knowledge for the first time studied with the purpose of getting information about their ability to concentrate radioisotopes. The regional wide distribution of these species and their abundance on the different biotopes made them experimental objects of considerable interest.

2.1. Experiments with Phoxinus phoxinus and Salmo trutta

The osmoregulation, salt metabolism and excretion of teleosts have been the subject for investigations of zoophysiologists for a long time (Brown 1957). Although valuable and fundamental knowledge is the result of these efforts, it is obviously little known about the concentration of the species of elements in fishes under different environmental conditions. This matter of fact is in part a consequence of the analytical difficulties of measuring the concentrations of some elements being at hand in minute quantities only. The use of radioisotopes have not only created a requirement for knowledge about the topic, but also given a useful tool to provide the necessary information.

The teleosts absorb the salts which they use for the control of their osmotic concentration and other metabolic needs, partly from the diet and partly from their external environment.

An experiment was accomplished to obtain information about the ability of Phoxinus phoxinus and Salmo trutta to take up radiophosphorus directly from the surrounding water.

This experiment, which is described in the following, was started on June 21 and finished on August 10, 1961. Two tanks in the well system of the experimental plant were used for the purpose. Each tank was filled with 1000 litres of water ($\kappa_{20} = 55{,}5 \cdot 10^{-6}$, pH 7,3 and turbidity 12,8 mg SiO_2/l). 100 individuals (average length 6 cm) of Phoxinus phoxinus were transferred to one of the tanks and 50 individuals (average length 10 cm) of Salmo trutta to the other. The fish represented healthy stocks and were in advance accustomed to their special environment in the well system. On June 21 the radioisotope P^{32} was administered to each tank. The dose was 470 µc. During the whole experiment the water in the tanks were maintained in stagnant condition. The fish received no food during the time of exposure. The temperature in the water varied between 12°C and 18°C. During the last days of the experiment a few deaths occurred among the individuals of both species.

The uptake of radiophosphorus to the fish was measured 8 times during the exposure time. 10 individuals of Phoxinus phoxinus and 5 individuals of Salmo trutta were each time used for this operation. The samples of individuals of the two species were treated as a group. On the same days observations of turbidity and determinations of the radioactivity associated with the particles and water in the tanks were performed.

The data obtained from the experiment with Salmo trutta are plotted in figure 1. The highest value of concentration of radiophosphorus in the fish corresponds to $45 \cdot 10^{-6}$ µc/mg ash weight. Obviously more than thirty days are required for the P^{32} content of the fish to reach an equilibrium with that of the surrounding water. When equilibrium was reached, the concentration (µc/g wet weight) of P^{32} in Salmo trutta was approximately 10 times the concentration (µc/ml) in the milieu.

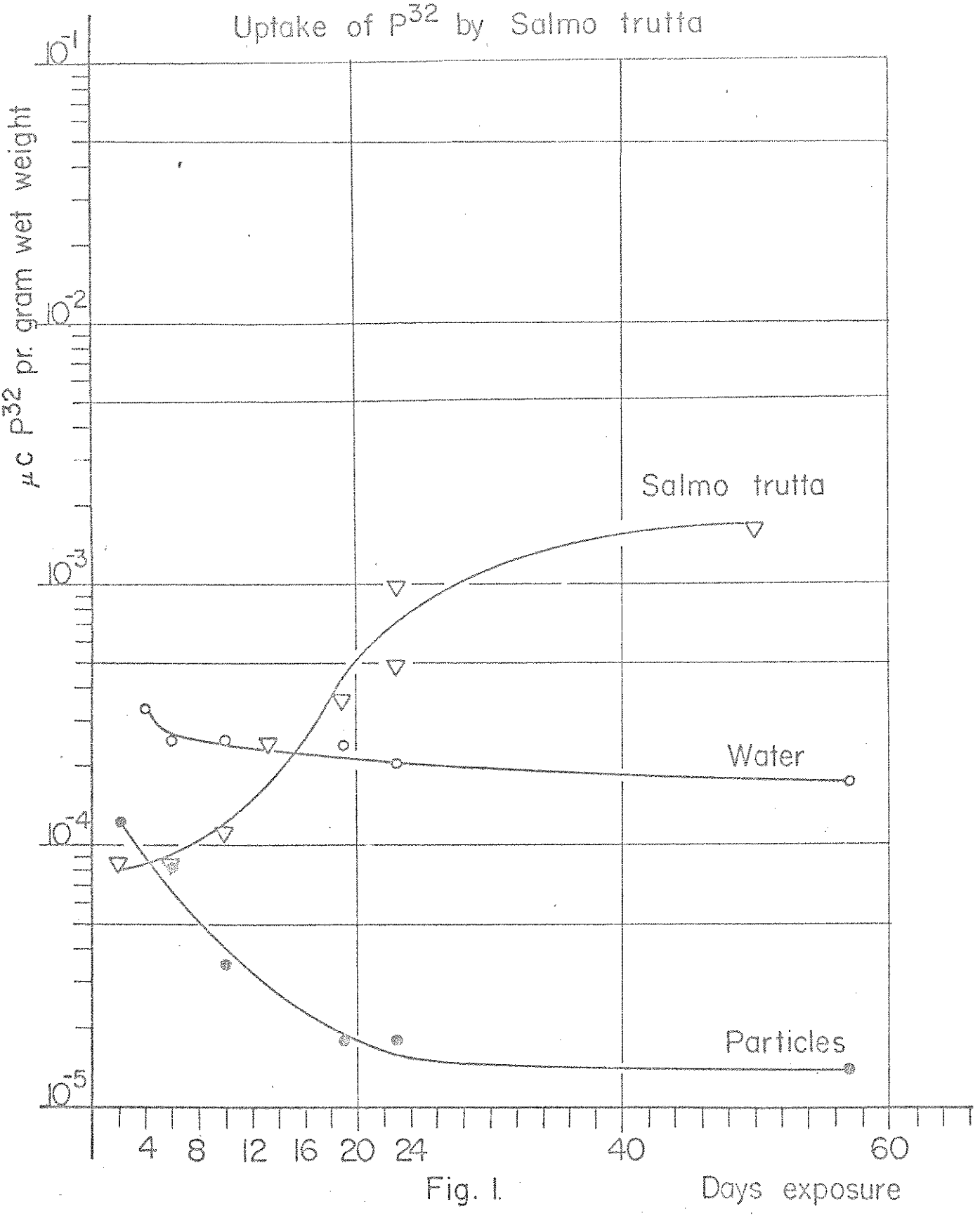

Uptake of P32 by Salmo trutta
μc P32 pr. gram wet weight
10-1
10-2
10-3
10-4
10-5
Salmo trutta
Water
Particles
4
8
12
16
20
24
40
60
Days exposure

Fig. 1.

The corresponding data obtained from the experiment with Phoxinus phoxinus are shown in figure 2. The highest value of concentration of radiophosphorus for this species measured was 11900 • 10^{-6} /uc/mg ash weight. The concentration of P^{32} in the fish approached an equilibrium with that of the environment during twenty days. At that moment the concentration (/uc/g wet weight) of P^{32} in Phoxinus phoxinus was approximately 1000 times the concentration (/uc/ml) in the milieu.

The osmotic concentration of body fluids in teleosts living in fresh water is greater than that of their surrounding water. This involve a tendency of continuous loss of salts by diffusion through surface tissues, and from feces and urine. To compensate for the salt loss, salts are actively absorbed through the gill membrane (Harris 1960), and by uptake of salts contained in ingested food. During the experiments described above with Salmo trutta and Phoxinus phoxinus, the fish were kept fasting. Thus the ability of the gills to take up ions dissolved in the surrounding medium against the diffusion gradient is of prime importance. The high level of the equilibrium between the content of phosphorus in the organism and in the water reflects the biologically essential role of this substance.

An interesting result of the experiment was the demonstration of the considerable difference between the two species with respect to their uptake of P^{32} from the external environment. To emphasize this finding the various concentration factors

$$\text{Concentration factor} = \frac{\dfrac{\text{Quantity of radioactivity (/uc)}}{\text{wet weight (g)}}}{\dfrac{\text{Quantity of radioactivity (/uc)}}{\text{Volume of water (ml)}}}$$

(Krumholz et al. 1957) during the experiments with the two species are calculated and plotted in figure 3. We are at the moment only able to indicate that the accumulation of phosphorus is regulated by some metabolic processes which have properties characterizing the species. In this connection the physiological peculiarities otherwise of these species may be recalled. Salmo trutta is a coldstenotherm, euryhalin, poly - oxybiontic and carnivor species. Phoxinus phoxinus, on the other hand, is an eurytherm, stenohaline, eury - oxybiontic and omnivor species.

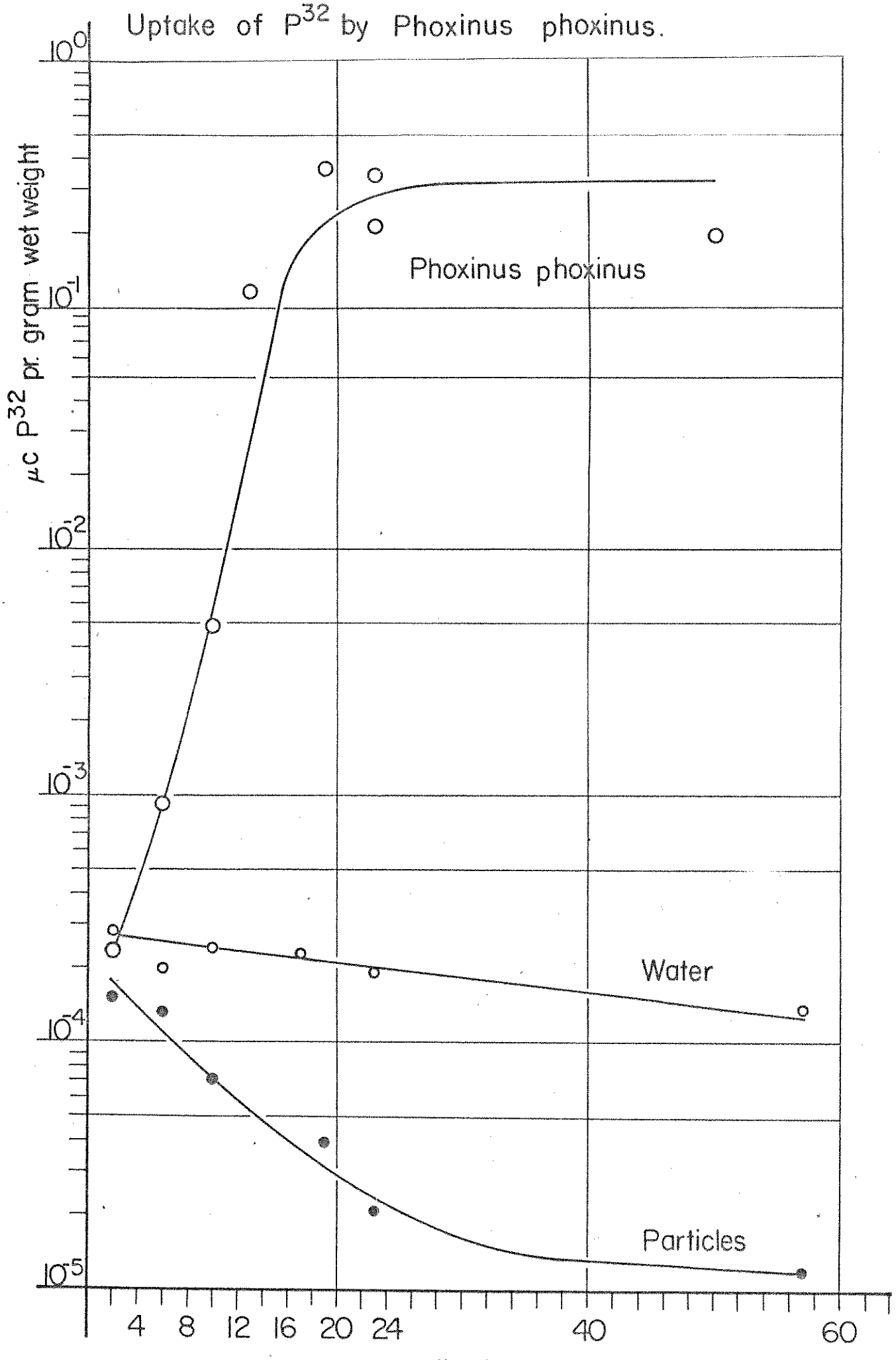

Uptake of P^{32} by Phoxinus phoxinus.
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
μc P^{32} pr. gram wet weight
Phoxinus phoxinus
Water
Particles
4
8
12
16
20
24
40
60
Days exposure

Fig. 2.

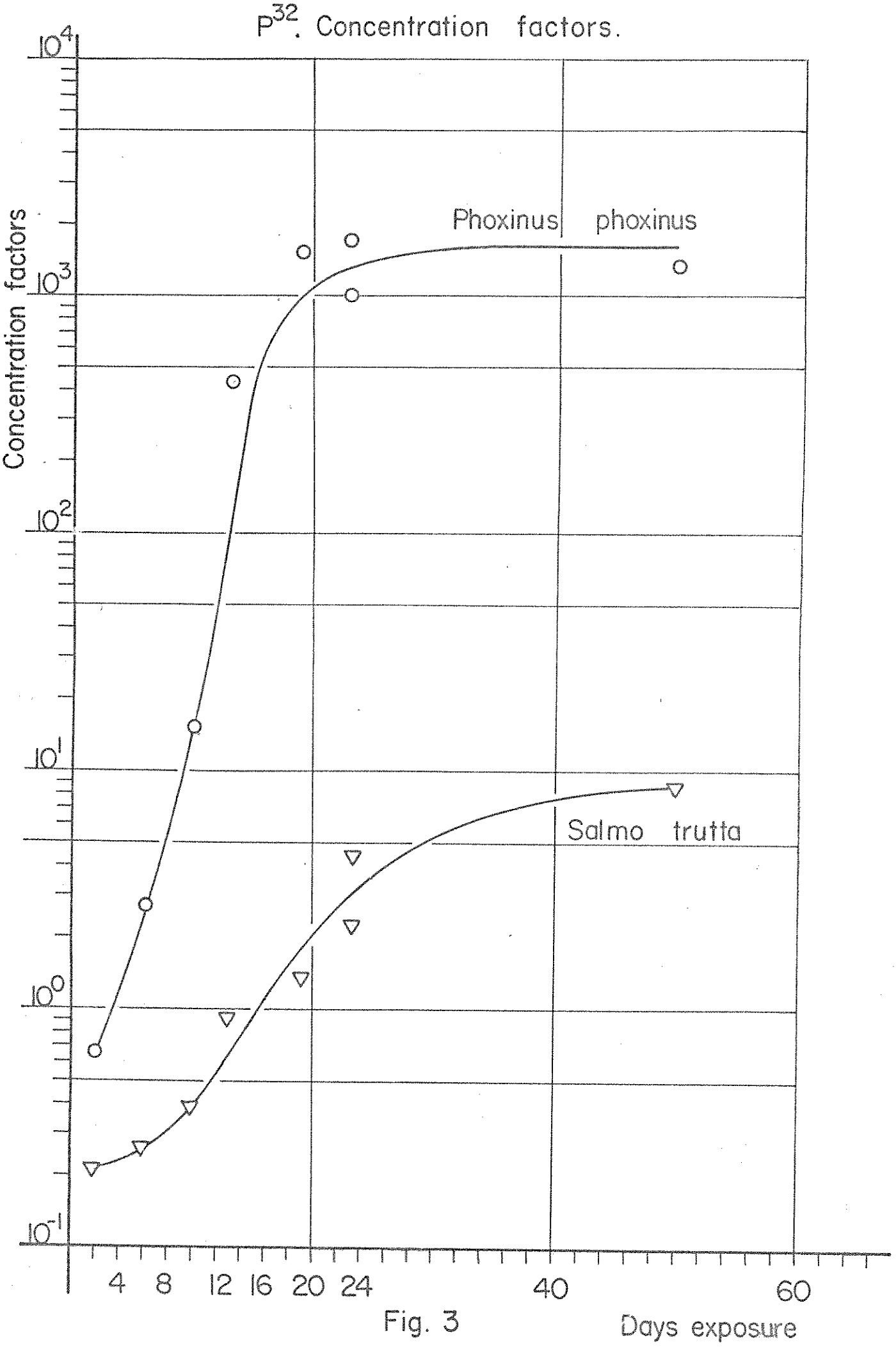

P32. Concentration factors.
Concentration factors
10^4
10^3
10^2
10^1
10^0
10^-1
Phoxinus phoxinus
Salmo trutta
4 8 12 16 20 24 40 60
Days exposure

Fig. 3

The experiment described was intended as an integrating part of the investigation concerned with uptake of radionuclides by fish of the river Nitelv. Together with experiments with administering radionuclides to the fish in a food chain mode, an understanding of these problems in the actual environment may be approached.

2.2. Uptake of radiophosphorus by some hydrophytes of macrovegetation

The macrovegetation of inland waters is in many regions a considerable fraction of the biotic mass of primary producers (Reid 1961). The ecological importance of the macrovegetation concerning the problems of radioactive contamination of inland waters is among others attached to the role the macrovegetation plays as a link between aquatic and terrestrial food chains. But also inside the aquatic environment the macrovegetation has an essential function as reservoir for chemical elements in the biogeochemical cycles. An exploration and evaluation of the processes performed by the macrovegetation in connection with the movement of chemical elements in the aquatic environment are therefore required. In handling low-level waste the aquatic environment has to be used as diluting agent. The task to establish necessary precautions against the hazards of contamination of constituents of hydrobiota will require knowledge of the reconcentration mechanism of the macrovegetation. The experiments reported here are concerned with some individual links in the transfer of radiophosphorus from water to hydrophytes of the macrovegetation.

The macrovegetation in the littoral zone of the river Nitelv is divided between a magnocaricetum, an eleocharetum and a potametum sociation (Skulberg 1960). The variation of water level in the river with the seasons and meteorological conditions involves that the sublittoral and the lower eulittoral zones are most exposed to a possible radioactive contaminant. According to this reasoning it was decided to use important species from the eleocharetum and the potametum sociation as objects for the experiment. Alisma Plantago-aquatica, Scirpus acicularis, Callitriche verna and Chara Braunii were chosen.

The experiment was started on July 5 and lasted ten days to July 14. The recirculation channel was filled to a height of 10 cm with water from the river Nitelv. This water level corresponds to a water volume of 2400 litres. The water masses were characterized by these analytical data: pH = 7,3, $\kappa_{20} = 54,4 \cdot 10^{-6}$ and turbidity = 22 mg SiO_2/l. The velocity of the water during the experiment was approximately 10 cm/sec. The hydrophytes, which had been cultivated in the well system of the experimental plant in metal baskets with plastic coating, were directly transferred to the recirculation channel. The quantity of P^{32} added to the water represented 290 /uc. At daily intervals observations and measurements of these factors were made: Radioactivity content of organisms, water and particles; turbidity and temperature in the water. The meteorological conditions during the experimental period were recorded.

The determination of radioactivity was performed on samples of whole plants minus roots, the procedure being the same as previously reported. The specimens of plants used for the experiment were in a state of vegetative growth and demonstrated the typical habitus of the species. The remaining plants from the experiment were followed in their development. They completed their vegetation cycle in the same manner as parallel plants not used for experiments.

The conditions in the recirculating water were fairly constant during the experiment. The variation in temperature was in the interval 15 - 20°C. As a consequence of sedimentation the turbidity of the water decreased from 24 mg SiO_2/l on July 5 to 17 mg SiO_2/l on July 14.

The data obtained from the radio-assay are given in figures 4 and 5. The curves of the uptake of P^{32} demonstrate a characteristic pattern of uptake for each species.

1. <u>Chara Braunii</u>

The level of radioactivity in the thallus when equilibrium between external and internal concentrations of the radionuclide is established was reached rapidly. The highest value of concentration of radiophosphorus in the plant body corresponds to 1200 . 10^{-6} /uc/mg ash weight.

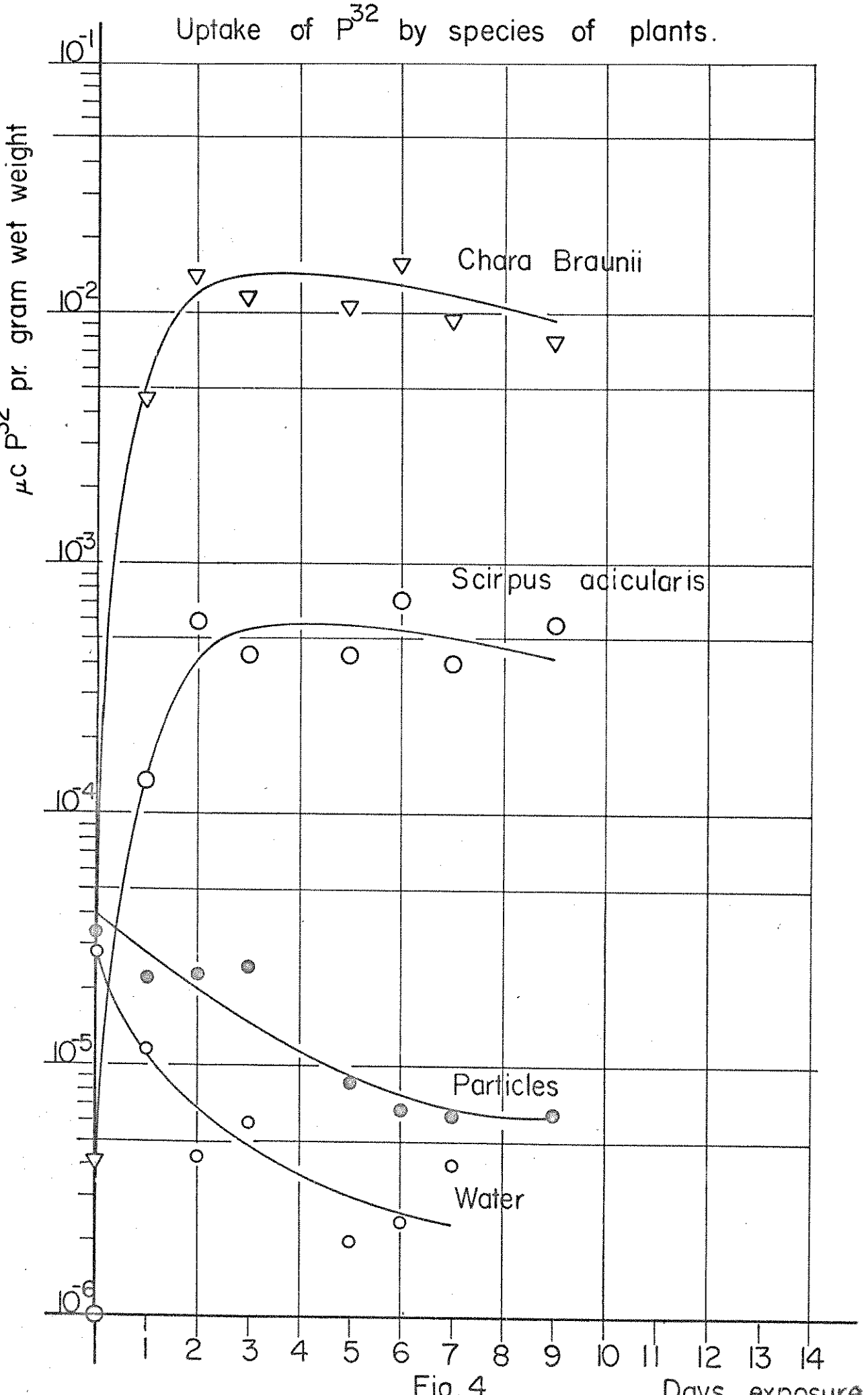

Uptake of P^{32} by species of plants.
$\mu c\ P^{32}$ pr. gram wet weight
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
Chara Braunii
Scirpus acicularis
Particles
Water
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days exposure

Fig. 4

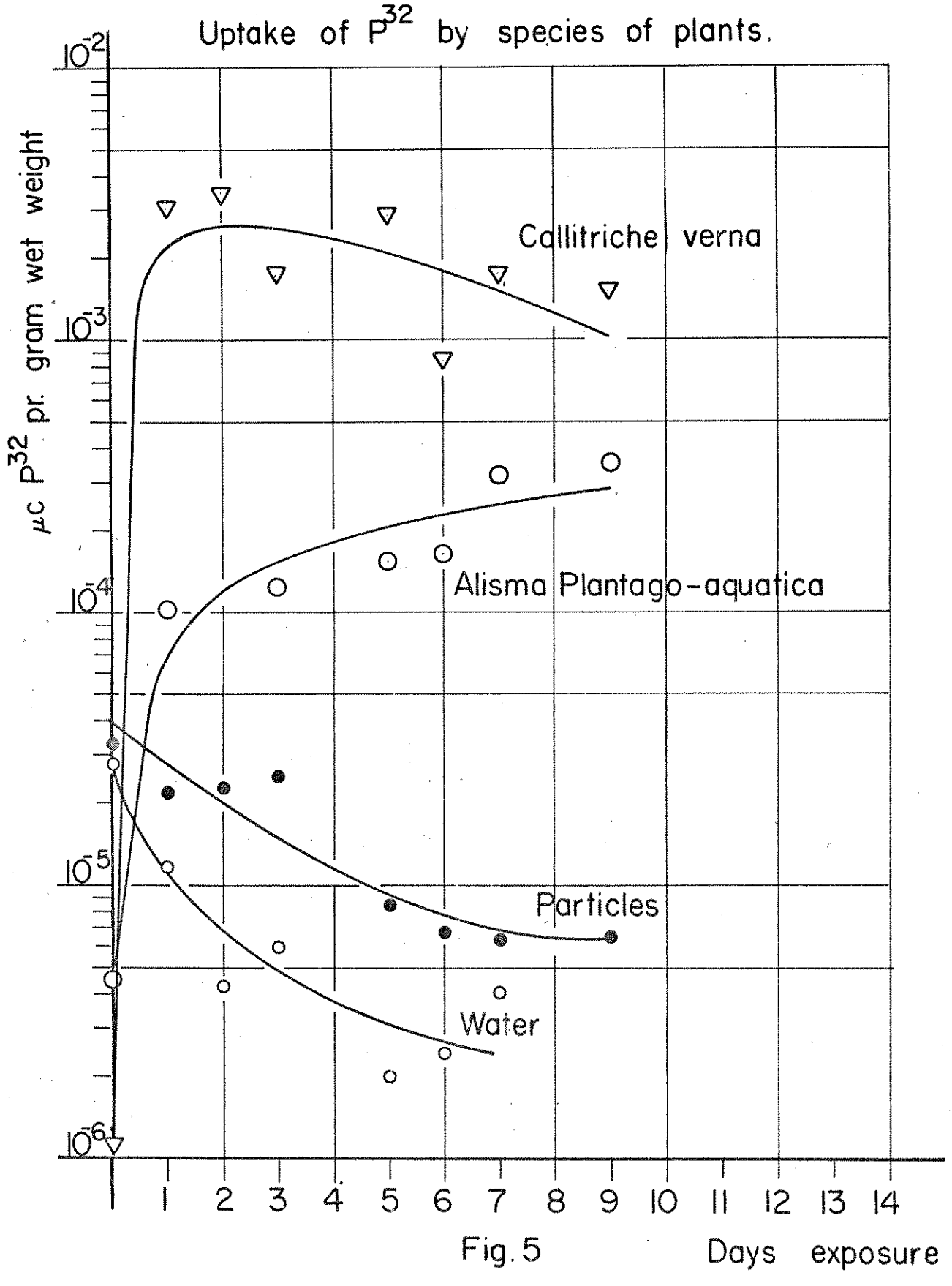

Uptake of P32 by species of plants.
μc P32 pr. gram wet weight
10-2
10-3
10-4
10-5
10-6
Callitriche verna
Alisma Plantago-aquatica
Particles
Water
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days exposure

Fig. 5

2. Scirpus acicularis.

The increase of the content of radioactivity in the plant body took place more slowly than in the preceeding species. But on the second day of observation also this species had reached a condition of equilibrium with the surrounding milieu. The highest value of concentration of radiophosphorus in the plant body corresponds to $76 \cdot 10^{-6}$ /uc/mg ash weight.

3. Callitriche verna.

The data obtained indicate a particularly rapid uptake of radiophosphorus. This plant presents some difficulties as object for experiments of this kind, and that may be the cause of the greater variance of individual measurements obtained for this species. The highest value of concentration of radiophosphorus in the plant body corresponds to $526 \cdot 10^{-6}$ /uc/mg ash weight.

4. Alisma Plantago-aquatica.

This species differ from the others in the fact that it did not reach the equilibrium condition during the experimental period. The highest value of concentration of radiophosphorus in the plant body corresponds to $33 \cdot 10^{-6}$ /uc/mg ash weight.

Phosphorus is closely related to the vital growth processes and other developmental functions of the plants. The supply of this element in forms available as nutrient is very limited in the actual water. Evidence indicates that phosphorus deficiency is a factor that must be considered in the growth of hydrophytes in the river Nitelv. The rate and amount of uptake of P^{32} during the experiment reflect the high demand for this element by each of the species. An interesting result of the experiment is the demonstrated difference between the four species in their ability to concentrate P^{32}.

A comparison of the maximum concentration factors (definition see page 15) obtained for the actual species is shown in the diagram of figure 6. Chara Braunii and Callitriche verna concentrate P^{32} to a high level, Alisma Plantago-aquatica and Scirpus acicularis to a lesser degree.

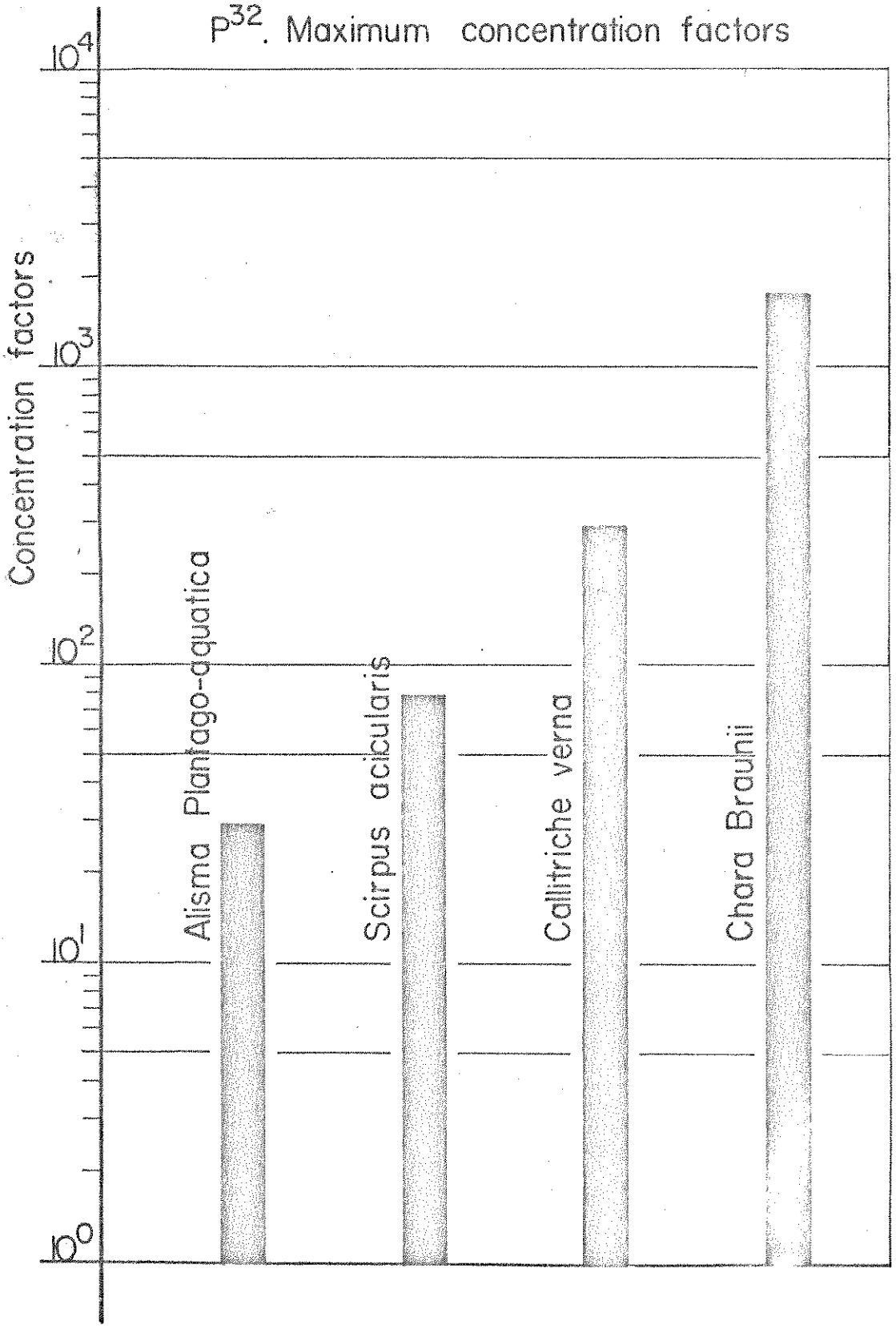

P32. Maximum concentration factors
Concentration factors
10^4
10^3
10^2
10^1
10^0
Alisma Plantago-aquatica
Scirpus aciculariś
Callitriche verna
Chara Braunii

Fig. 6.

The morphological differences between the species considered will influence the rate of uptake. Alisma Plantago-aquatica has the growth form of a typical helophyte, this may partly explain that equilibrium state of phosphorus content was not reached during the exposure time. Chara Braunii and Scirpus acicularis have the growth form of elodeids. Callitriche verna, although a nymphaeid, was represented with submerged specimens only. The three species with an elodeidan growth form reached the equilibrium state during approximately two days.

The physiological properties of the species, however, are more important than morphological differences when the variation in maximum concentration factors is discussed.

The algae (represented by Chara Braunii) are known to have a particularly high content of minerals in their thalli (Baumeister 1958). Our experiment shows that Chara Braunii in an exceptional degree has the ability to concentrate P^{32}.

The process of osmoregulation by hydrophytes is an important factor to consider when the apparently great species difference in uptake of radionuclides is to be explained. The demonstration of the dissimilarity between aquatic and terrestrial plants with respect to accumulation phenomena are of interest in this connection (Pendleton et al. 1958). Most likely great variations will be found between e.g. typical species of the eleocharetum sociation in the eulittoral zone and typical species of the potametum sociation in the sublittoral zone. The result of our experiment indicates that important progress can be made along this line of reasoning.

The experience we have gained during our work with the cultivation of organisms and the operation of the experimental facilities furnish a good basis for advance in the investigation. Important parameters to be given attention are the water velocity, the concentrations of radionuclides in the water and the duration of exposure.

2.3. Observations on Anodonta cygnea

It has been a long established fact that molluscs possess an outstanding ability to concentrate large quantities of radionuclides from very dilute solutions of the elements. Several species of molluscs have been used as objects for investigations of this phenomenon. An example of the extraordinary values of concentration factors found concerns Tridacna gigas, which is reported (Weiss 1957) to concentrate Co^{60}. Radiocobalt was measured in the animal in quantities ten thousands of times greater than its concentration in sea-water.

The accumulation of radionuclides by lamellibranchiates inhabiting inland waters have been studied in the field and in the laboratory (Polikarpov 1960). Anodonta cygnea is reported to be used as object for experiments (Timofeev - Resovskii 1961), and Unio sp. (Ravera, O., Ispra, Italy, personal communication). The use of molluscs in experiments on uptake of radioisotopes have several advantages, and interesting findings have resulted from the work (Fretter 1953).

The species Anodonta cygnea is in the river Nitelv inhabiting the sublittoral zone. The biotopes showing the highest densities in population are outside the potametum sociation of macrovegetation. The ecological niche of the species ranks it among the highly important invertebrates when food chain relations of the stream environment are considered. The structural specialization of the feeding apparatus, which make the animal dependent upon a food supply filtered from the water, involves the possibility of a concentration of radioactivity to levels which are extreme for organisms in aquatic communities. Anodonta cygnea represents an organism of major interest when assessing the amounts of radiocontaminants which may be safely discharged to inland waters.

In connection with the experiment on uptake of radiophosphorus by species of macrovegetation in the recirculation channel July 5 - July 14, a series of determinations of the uptake of this radionuclide by Anodonta cygnea was performed. The specimens used were exposed to the radioactive water masses of the recirculation channel. The animals were kept in small baskets. The preparation of the specimens collected for measurements consisted of a dissecting and a partition of the animal in body and shell.

The two fractions were applied to weight analysis (wet weight, dry weight and ash weight were determined). The ash was used for measurement of radioactivity.

The data obtained from this examination are plotted in figure 7. The marked accumulation of P^{32} is demonstrated. The level of radionuclide concentration in the body was rapidly changing during the first two days of the experimental period. Afterwards only a slow increase of radioactivity in the animal body was observed. A steady state of uptake and loss of P^{32} was not reached during the time of experiment. This pattern of uptake is fairly in agreement with what is previously reported for gastropods (Polikarpov 1960). The highest value of concentration of radiophosphorus in the animal body corresponds to $376 \cdot 10^{-4}$ /uc/mg ash weight.

The values obtained from the determinations of the radioactivity attached to the shell fraction of Anodonta cygnea are of the order 10^{-4} /uc/mg wet weight. It is reasonable to regard the movement of P^{32} from the water to the shell as an effect of sorption phenomena. Particles of detritus, microorganisms and the surface of the shell itself may have a share in this result.

During the experimental work in the throughflow channels (see page 39) it was convenient to get additional information about the uptake of radionuclides by Anodonta cygnea. Collections of animals were throughout each experiment exposed to the contaminated water at the end of the actual channel. Data obtained from this work are listed in table 1.

The conditions in the throughflow channels with respect to water velocity (approximately 4,4 cm/sec.) and length of dosing time (approximately 15 min.) were nearly identical during the experiments.

The results indicate that uptake of the various radionuclides under the prevailing conditions is of little amount. The length of contamination time is a limiting factor. An increase of the concentration of radionuclides in the water has as a consequence an intensified uptake of the actual element.

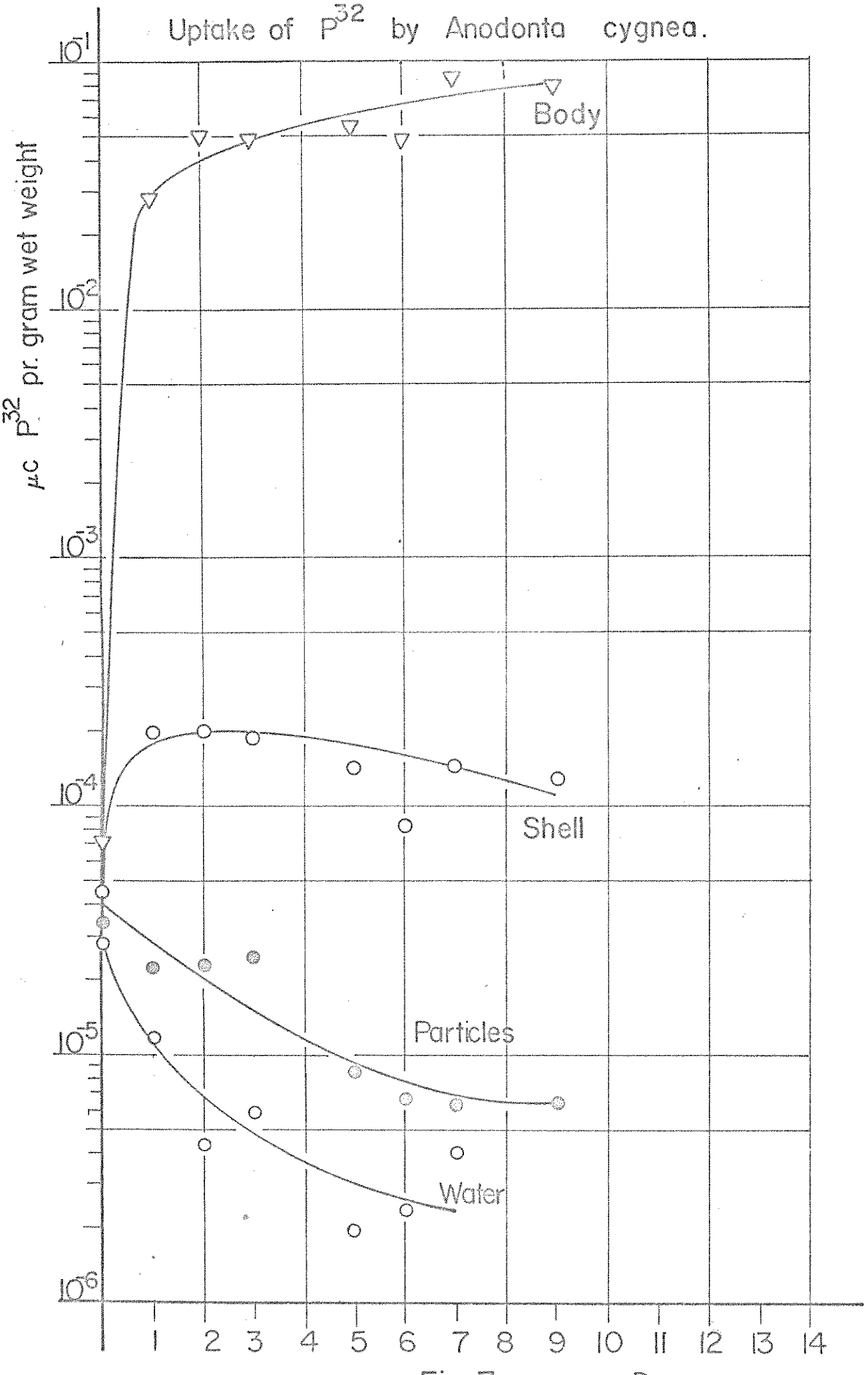

Uptake of P^{32} by Anodonta cygnea.
μc P^{32} pr. gram wet weight
10^{1}
10^{2}
10^{3}
10^{4}
10^{5}
10^{6}
Body
Shell
Particles
Water
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days exposure

Fig. 7

Table 1

Uptake of radionuclides by Anodonta cygnea

Sr^{89}	Water /uc • 10^{-5}/ml	4,0	9,2	9,2
	Body /uc • 10^{-5}/g wet weight	1,1	0,8	1,1
	Body /uc • 10^{-5}/g ash weight	59	41	39
Ru^{106}	Water /uc • 10^{-5}/ml	9,3	12,2	45
	Body /uc • 10^{-5}/g wet weight	0,4	0,8	1,3
	Body /uc • 10^{-5}/g ash weight	6	14	20
I^{131}	Water /uc • 10^{-5}/ml	12,3	23,0	46,0
	Body /uc • 10^{-5}/g wet weight	0,9	2,4	4,2
	Body /uc • 10^{-5}/g ash weight	5,3	89	184
U(nat.)	Water /uc • 10^{-5}/ml	1,5	3,0	6,0
	Body /uc • 10^{-5}/g wet weight	2,5	2,3	3,9
	Body /uc • 10^{-5}/g ash weight	156	152	352

Further experiments with Anodonta cygnea will include a study of uptake during longer time of exposure.

The parameters, water velocity and radionuclide concentration have to be considered.

3. SYNECOLOGICAL APPROACH

The current observations of the organism development and the changes of communities in the model recipient formed the base for the experimental investigation of this category. The taxonomic work in fauna and flora analysis have been given considerable attention.

The observations and measurements in the throughflow channels during the experiments took aim at the description of the distribution and uptake of radionuclides by the major species in the channel biota. It has been regarded of much importance to record the accumulation of radionuclides by the organisms in situ in their respective communities. Much of our attention has been given to the uptake of radioactive substances in the first trophic level, where the algae represent the important entrance possibilities for transfer of radionuclides into the food chain.

The algal communities have been characterized by the following species being in dominance:

1. Spirogyra cf. formosa (Transeau) Czurda.

   Family Zygnemataceae.

   Figure 8 and 9.

   The species found in the throughflow channels had vegetative cells 84 - 94 /u x 56 - 247 /u , with plane end walls; 7 - 8 chromatophores, making 1,5 - 2 turns in the cell. Conjugating filaments were not observed.

   The diagnosis is close to that of Spirogyra formosa.

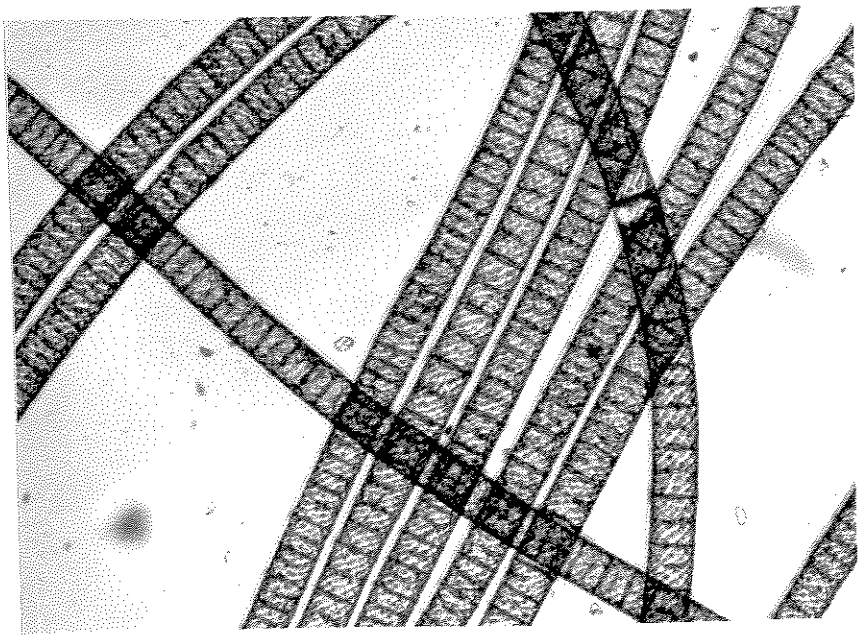


Figure 8

Spirogyra cr. formosa

Sample taken from vegetation with almost single species population

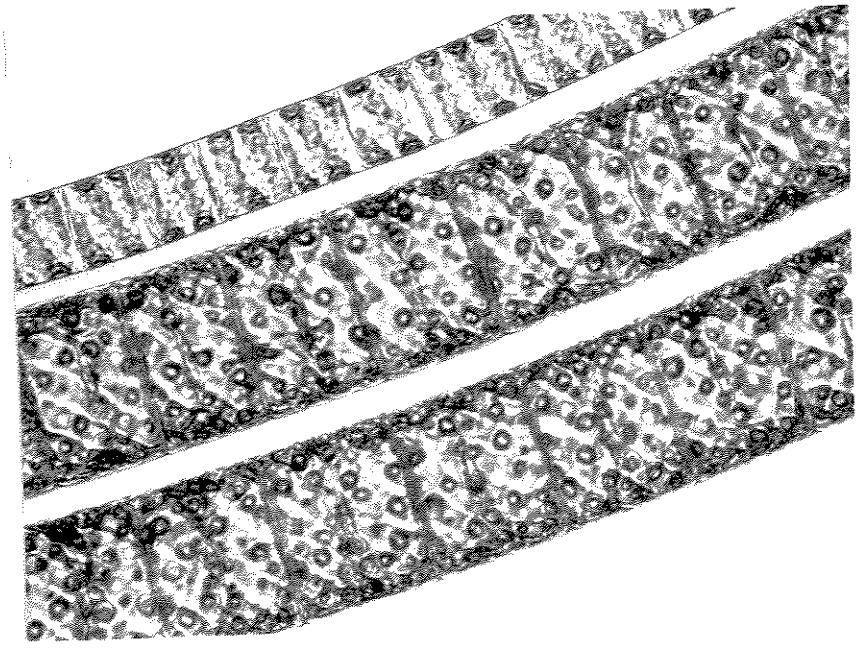


Figure 9

Spirogyra cf. formosa

Detail of thallus.

2. Spirogyra cf. porticalis (Müller) Cleve.

Family Zygnemataceae.

Figure 10 and 11.

The actual species had vegetative cells 40 - 52 /u x 48 - 134 /u , with plane end walls; 1 chromatophore, making 2 - 5 turns in the cell. No conjugating filaments were observed.

The diagnosis is close to that of Spirogyra porticalis.

3. Vaucheria De Candolle sp.

Family Vaucheriaceae.

Figure 12.

The species growing in the throughflow channel was a tubular coenocyte with fairly abundant branching. The thallus was 48 - 101 /u broad. During the autumn akinetes developed, their dimensions being approximately 160 x 240 /u .

Sexual reproduction did not take place, and no identification of the species was possible.

4. Oedogonium Link sp.

Family Oedogoniaceae.

Figure 13.

The species found in the throughflow channels had unbranched filaments of cylindrical cells with dimensions approximately 43 x 100 /u .

Only sterile specimens were found, and the identification of the species was impossible.

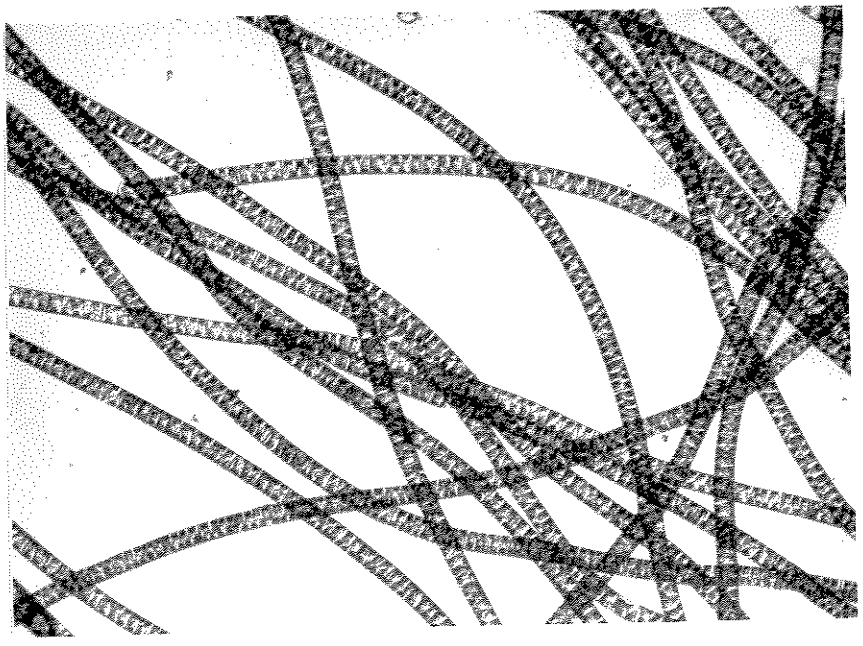


Figure 10

Spirogyra cf. porticalis

Sample taken from an algal community dominated by this species.

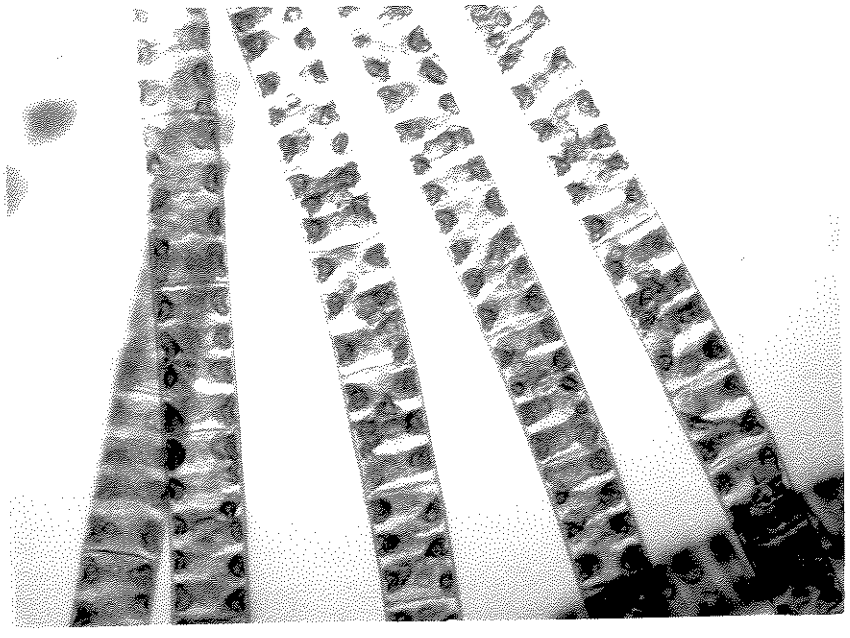


Figure 11

Spirogyra cf. porticalis

Detail of thallus.

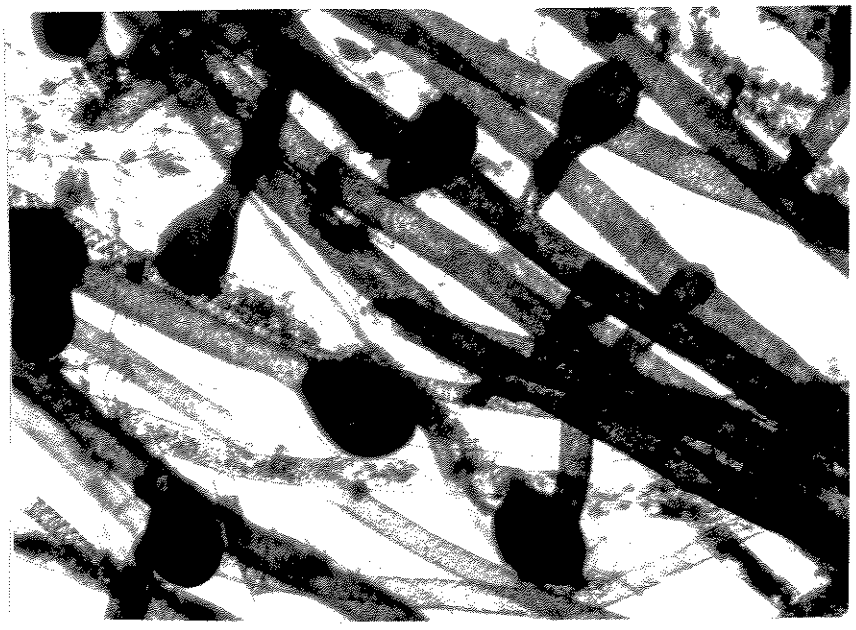


Figure 12

Vaucheria sp.

The specimens show the typical akinetes at ends of short laterals.

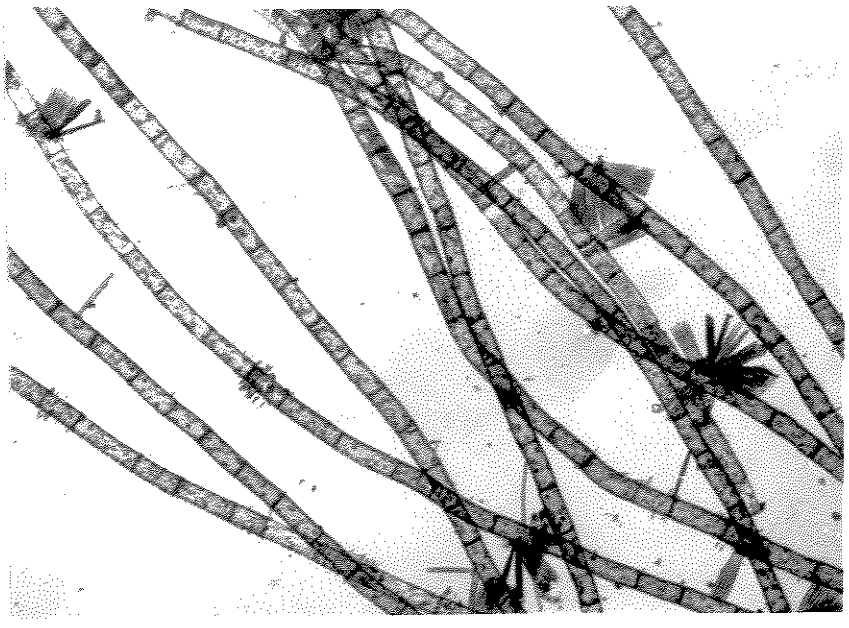


Figure 13

Oedogonium sp.

Sample taken from algal community dominated by this species.

5. Fragilaria capucina var. mesolepta (Rabh.) Grun.

Family Fragilariaceae.

Figure 14 and 15.

The species collected from the throughflow channels demonstrated the taxonomic characteristica described for Fragilaria capucina var. mesolepta. Dimensions of the frustules 2,7 - 6,7 /u x 25 - 48 /u.

The communities of river biota in the throughflow channels developed satisfactorily, no transfer of organisms to these channels was performed (Skulberg, 1961).

3.1 Observations of sorption phenomena in the mud-water interface of the bottom deposits.

The laboratory studies of sorption phenomena of radionuclides to sediments from the river Nitelv, and the experience gained in the model recipient, demonstrate that when solutions of radionuclides are mixed with the water, a significant portion of the radionuclides will be associated with the suspended particles and on the bottom sediments. The relationship between suspended particles in the water, the bottom sediments and the organisms living in the environment with respect to dispersion and concentration of radionuclides, makes out an important complex of the problems studied in the present research project. The experiments carried out during the summer 1961 in the throughflow channels in the experimental plant gave an opportunity to collect more information about this topic.

The nature of the bottom sediments in the throughflow channels is the same as in the river Nitelv. The major fraction of the deposits consists of pleistocene clay. Decaying organic residues from polluting substances in the water and from the channel biota constitute another important fraction. The water-mud interface represents a stratum with characteristic communities of organisms (bacteria, protozoans and algae are common).

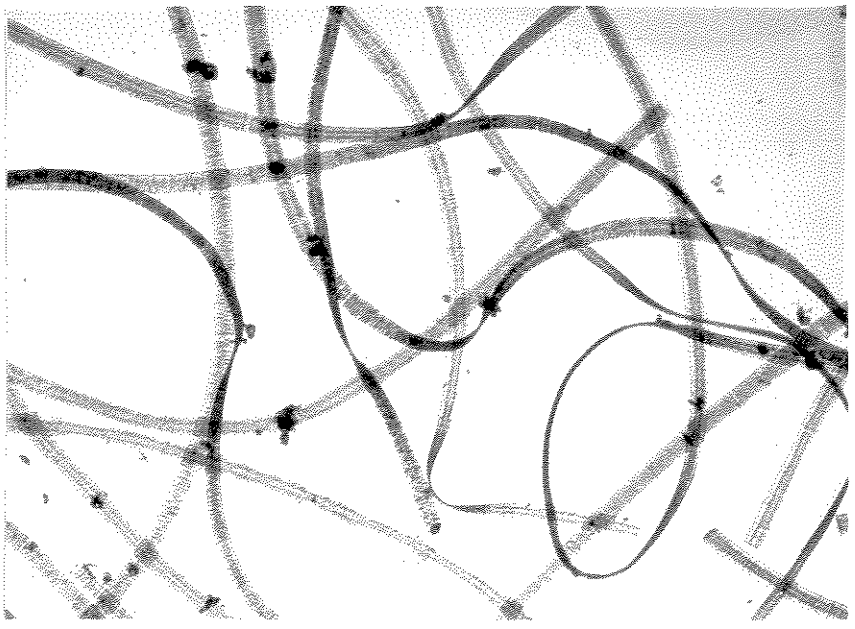


Figure 14

Fragilaria capucina var. mesolepta

Habitus in living condition.

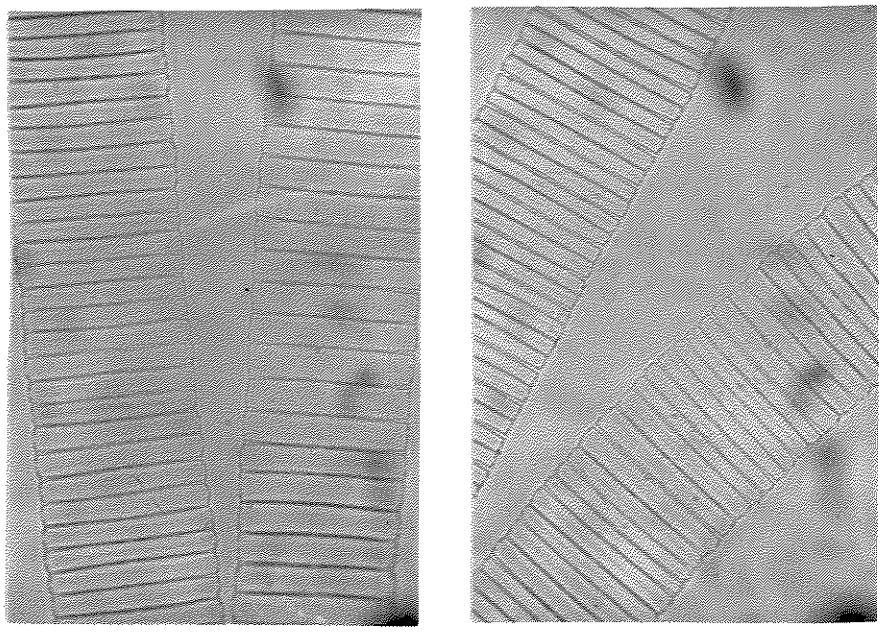


Figure 15

Fragilaria capucina var. mesolepta

Detail of frustules showing the morphological characteristics of the species. Preparated specimens.

The sampling of mud for the laboratory treatment was conducted with a giant pipette made by attaching a rubber bulb to a piece of glass tubing of suitable diameter and length. After the collecting, the samples were applied to weight analysis; wet weight, dry weight and ash weight being determined. The samples were used for measurements of radioactivity.

One example of the results obtained in the throughflow channels is plotted on the diagram in figure 16. The longitudinal variation of radionuclide uptake in the mud-water interface is demonstrated. During the experiments the water flow was 0,5 l/sec. The concentration of radionuclides in the water was in the experiment with Sr^{89} $9,2 \cdot 10^{-5}$ /uc/ml, and in the experiment with P^{32} $5,8 \cdot 10^{-5}$ /uc/ml. The dosing time represented 15 minutes. The result was interpreted in the following way. The inorganic component of the bottom deposits account for the major uptake of the radionuclides. The fact that the course of the curves is similar for both radionuclides (with their very different biological affinities) and the amount of the uptake, indicates this. The curves demonstrate that the radionuclides were sorbed to the sediments in a higher degree with increasing channel length. This reflects the importance of the time of contact for the sorption phenomenon.

Maximum concentration factors observed in the mud-water interface during the experiments in the throughflow channels are diagrammatized in figure 17. It is of interest to compare the obtained data with the results from our laboratory experiments on sorption of radionuclides to river sediments (Skulberg 1961). In table 2 some of these results are recapitulated. For better comparison the distribution coefficient K_d has been calculated. The distribution coefficient is defined as (Sorathesn et al. 1960):

$$K_d = \frac{f_c/M}{f_s/V}$$

where: f_c = fraction of radioactivity sorbed by sediments.
f_s = fraction of radioactivity left in solution.
M = weight of sediment in grams.
V = volume of solution in millilitres.

Longitudinal variation of radionuclide uptake in mud-water interface.

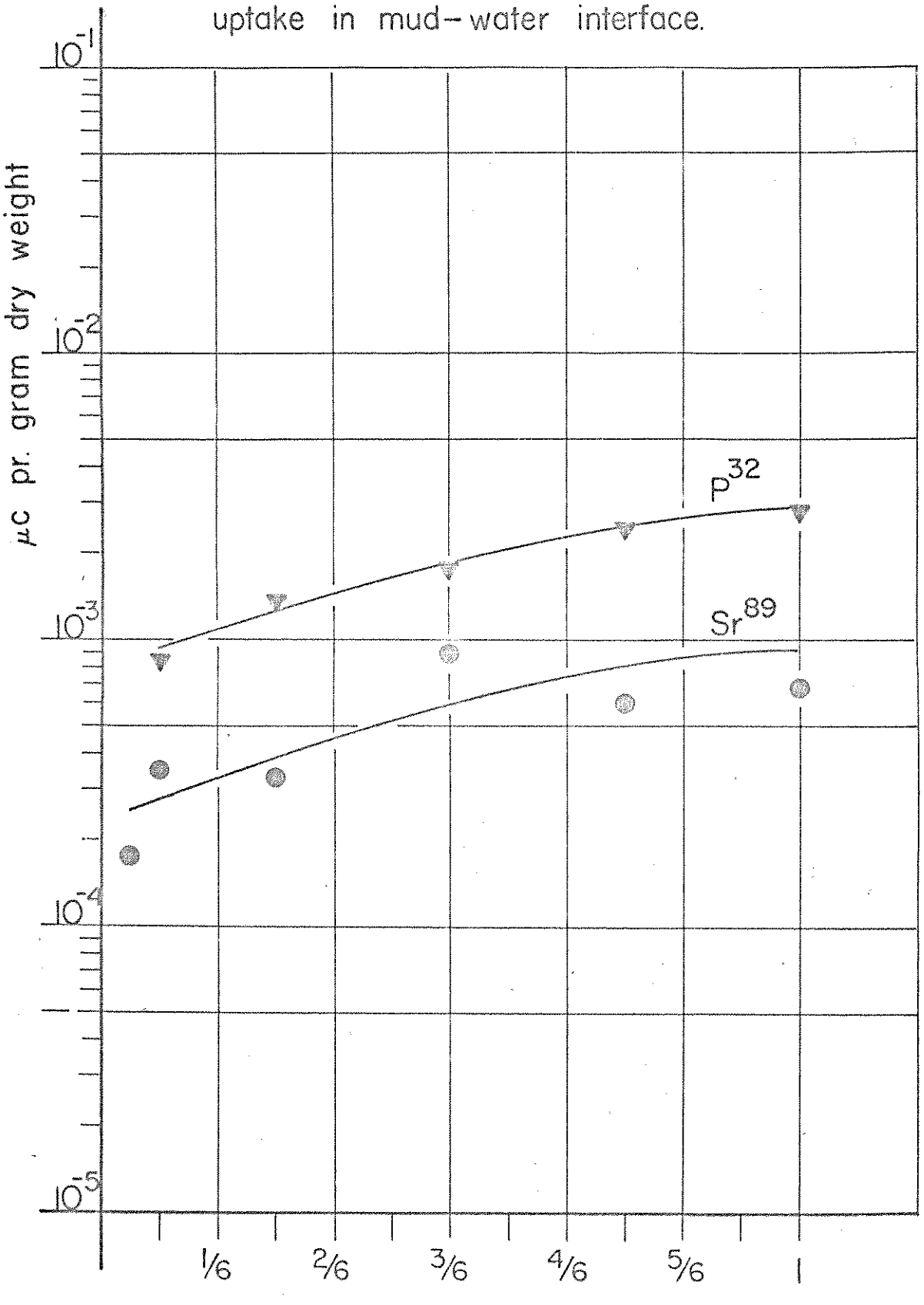


Fig. 16

Mud–water interface.

Maximum concentration factors.

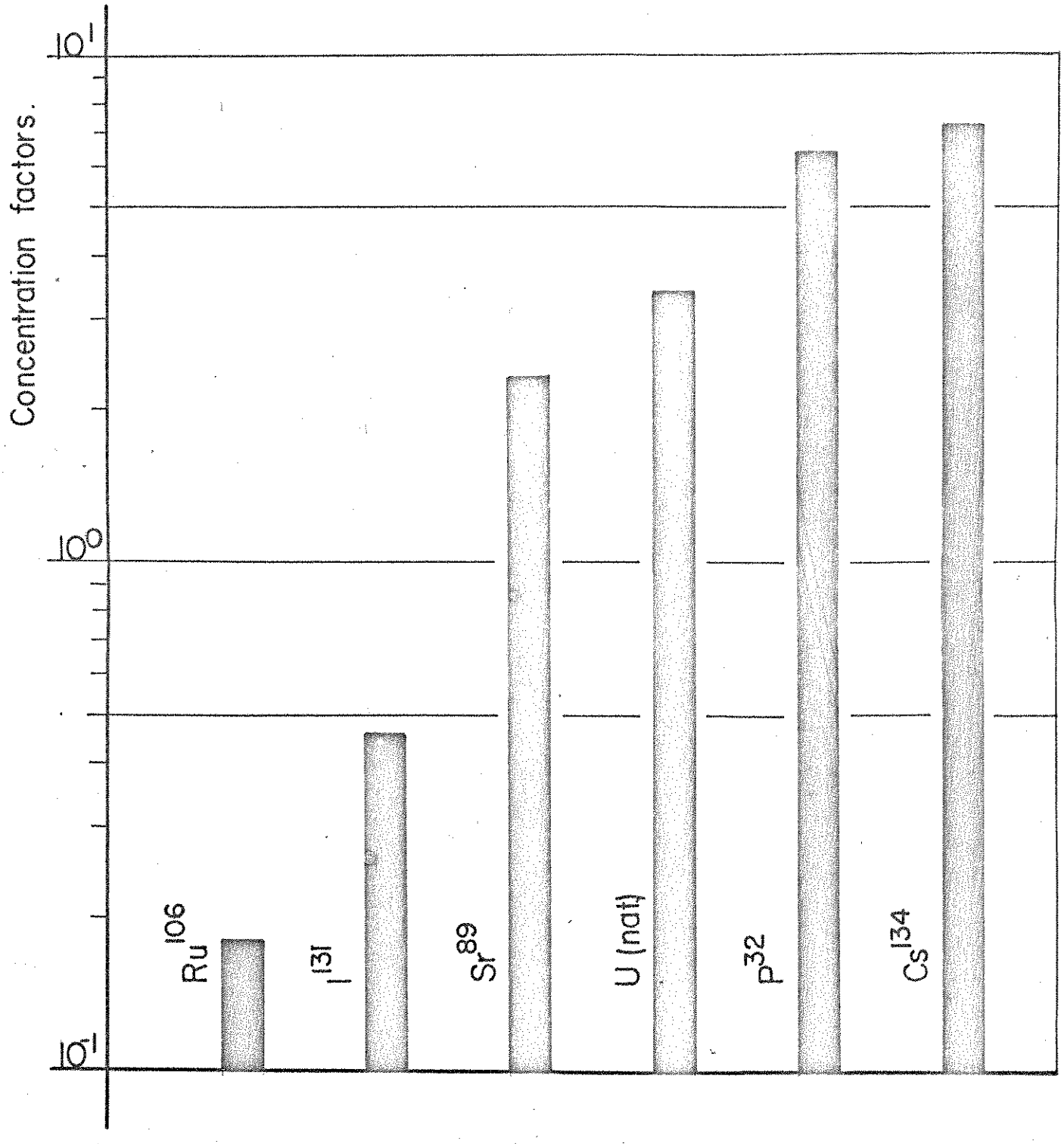


Fig. 17

Table 2.

Sorption of radionuclides to river sediments

I Sediments suspended in distilled water.

II Sediments suspended in river water.

	Radio-nuclide	Concentration	Contact time	Per cent activity sorbed	K_d
I	Sr^{89}	0,077 g/300 ml	1 day	14,5 ± 1,0	660
	Ru^{106}	0,077 g/300 ml	1 day	59,0 ± 2,6	5300
	Cs^{134}	0,077 g/300 ml	1 day	65,2 ± 1,9	7300
	P^{32}	0,077 g/300 ml	1 day	70,0 ± 2,8	9100
II	Sr^{89}	0,077 g/300 ml	1 day	14,0 ± 1,6	630
	Ru^{106}	0,077 g/300 ml	1 day	15,0 ± 1,2	680
	Cs^{134}	0,077 g/300 ml	1 day	50,0 ± 1,6	3900

From the data on sediment sorption it can be seen that the sediments have a high sorption capacity for cesium and phosphorus, whereas the sorption capacity for strontium and ruthenium is much lower. The concentration of radionuclides in the samples of the bottom deposits of the throughflow channels collected from the mud-water interface appears to be in good agreement with these findings.

3.2. Studies on uptake of some radionuclides by species of the algal communities.

The organisms near the base of the food pyramid will be the first ones affected by radionuclides disposed to the environment. A major fraction of the different radioactive contaminants will be held by the organisms which make up the primary trophic level (Davis et al 1958). The ability of the organisms which constitute the biotic mass in the first trophic level to concentrate various radionuclides is an important topic of re-

search. The different factors affecting accumulation, e.g. quantity of dissolved solids, percentage composition of different elements, pH, the oxygen-carbondioxide ratio, biological relationships etc., have to be given consideration. An approach to a more detailed knowledge about biological implications of radioactive contamination of aquatic environments include intensified studies of the problems attached to the first trophic level. The purpose of the experiments carried out in the model recipient this summer was to obtain explicit information about the result of processes involving uptake of radionuclides to major species in algal communities.

Several investigators have performed research on the problems connected with uptake of radionuclides by algal species (e.g. Boroughs 1957, Williams 1958, Steel 1954). In the surveys accomplished in the recipients of atomic energy facilities the algae represent a type of environmental media sampled for monitoring purposes (Lackey 1957). The results obtained during these surveys are an important contribution to the knowledge about the uptake of radionuclides in the first trophic level (Krumholz 1954, Foster 1959). Still there is a lack of knowledge about what certain environmental factors, biotic and abiotic, may imply with respect to potential uptake and reconcentration of radionuclides by the species of algal communities inhabiting inland waters.

The experiments in the model recipient concerned with problems of this kind were performed as short time exposure of the communities of algae to low level concentrations of radionuclides. The facilities of the experimental plant has proved suitable for the purpose. The procedure is briefly described here. The actual radionuclide was dosed to the water at the starting point of the throughflow channel used for the experiment. The movement of the radionuclide along the channel was observed with a Geiger-Müller counter of the "dip" type directly immersed in the channel water. Samples of the water during the experiments were collected for the precise determination of radioactivity. Specific materials e.g. major species in the algal communities, invertebrates (a few collections only) and sediments from the mud-water interface were sampled several places along the channel and were applied to radiochemical analyses.

The experiments were carried out in order to determine the dispersion of specific radionuclides in terms of media sampled. Information of quantitative and qualitative nature of the radionuclide uptake was obtained. From the variation of radionuclide concentration in the various media sampled, the removal of radionuclides from the water and their uptake by the organisms were studied.

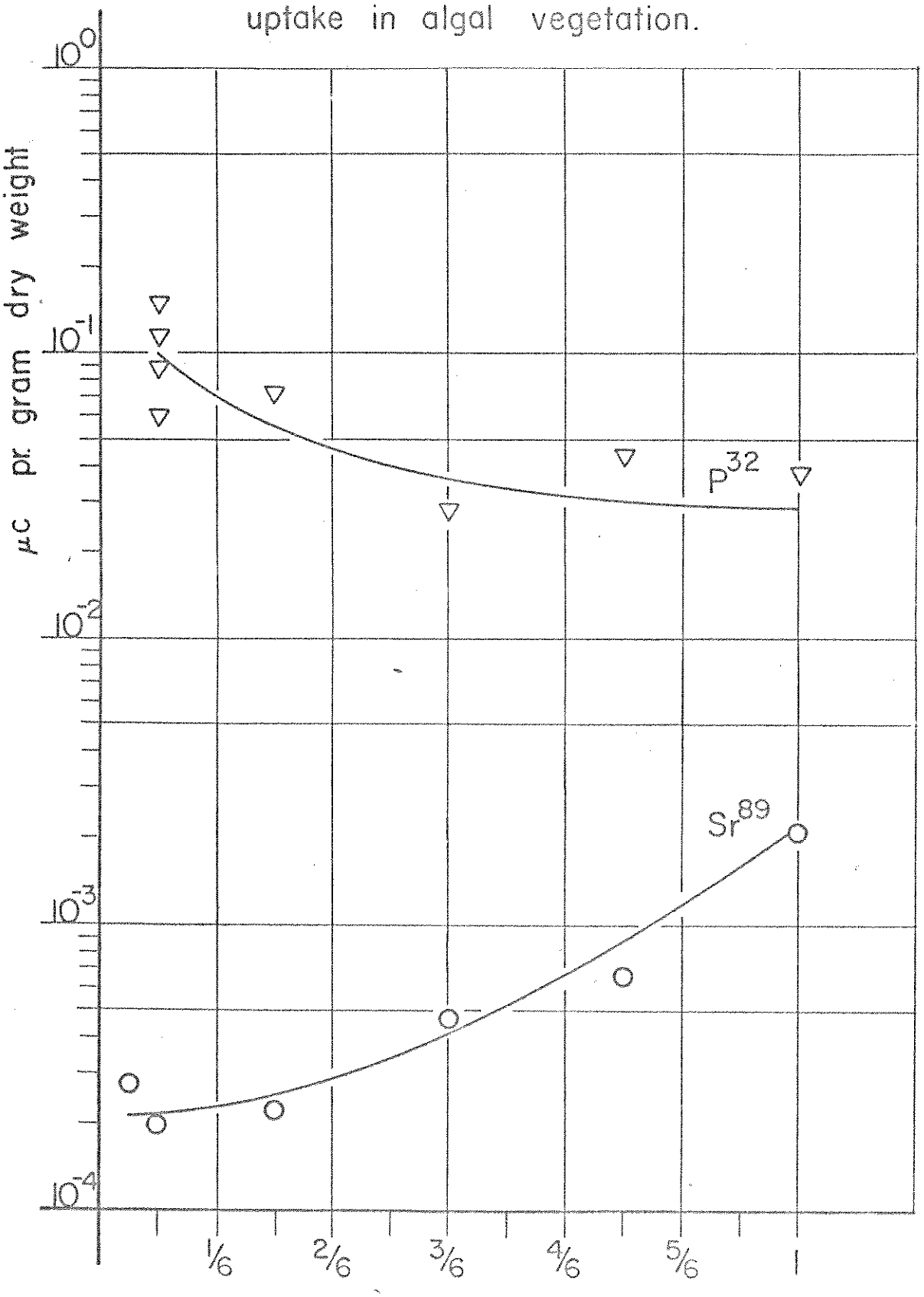
An example of the data obtained is plotted in figure 18. These particular experiments show the longitudinal variation of radionuclide uptake in algal vegetation. During the experiment the velocity of water was approximately 4,4 cm/sec. The dosing time was 15 minutes and the concentration of P^{32} was approximately $5{,}8 \cdot 10^{-5}$ µc/ml, and correspondingly for Sr^{89} approximately $9{,}2 \cdot 10^{-5}$ µc/ml. The result is interpreted in this way: The metabolic essential phosphorus is eagerly taken up by the algae, the concentration of the substance in the water is a relative more important factor than the exposure time for the amount of uptake. Strontium demonstrates quite another affinity to the algae, for this substance the exposure time determines the course of the curve.

Strong species differences in ability to concentrate various radionuclides were apparent among the algae. A summary of the results obtained is given in table 3 and on the diagrams in figure 19 and 20. (Concerning the level of radionuclide concentration in the water during the experiment in the model recipient see data noted in table 1, page 27).

It is of interest to note that considerable differences prevail between genera of the same class, as well as between species of the same genus. Very little is known about the physiological properties of filamentous algae (Geitler 1959), and a discussion of the metabolic relations the values of concentration factors may reflect, is not possible at present.

The experimental investigation in the model recipient on the problems related to uptake of radionuclides by algal communities is an important contribution of the present study. Among the various media sampled for environmental monitoring the sessile algae is recognized having many advantages (Straub et al. 1959).

Longitudinal variation of radionuclide uptake in algal vegetation.



Fraction of total length

Total channel length 120m.

Fig. 18

Table 3

Values of concentration factors determined on various species of algae and various radionuclides.

Radionuclide \ Species		Spirogyra cf. formosa	Spirogyra cf. porticalis	Fragilaria capucina var. mesolepta	Oedogonium sp.	Vaucheria sp.
P^{32}	max.	246	148	37	32	103
	min.	62	111	18	28	63
Cs^{134}	max.	-	1,6	-	5,8	-
	min.	-	1,2	-	5,1	-
Sr^{89}	max.	0,9	0,9	-	2,8	-
	min.	0,1	0,2	-	0,2	-
Ru^{106}	max.	0,8	-	-	0,4	-
	min.	0,1	-	-	0,2	-
I^{131}	max.	0,3	-	-	2,4	-
	min.	0,1	-	-	0,1	-
U(nat.)	max.	11	-	-	47	-
	min.	6	-	-	6	-

P^{32}. Maximum concentration factors.

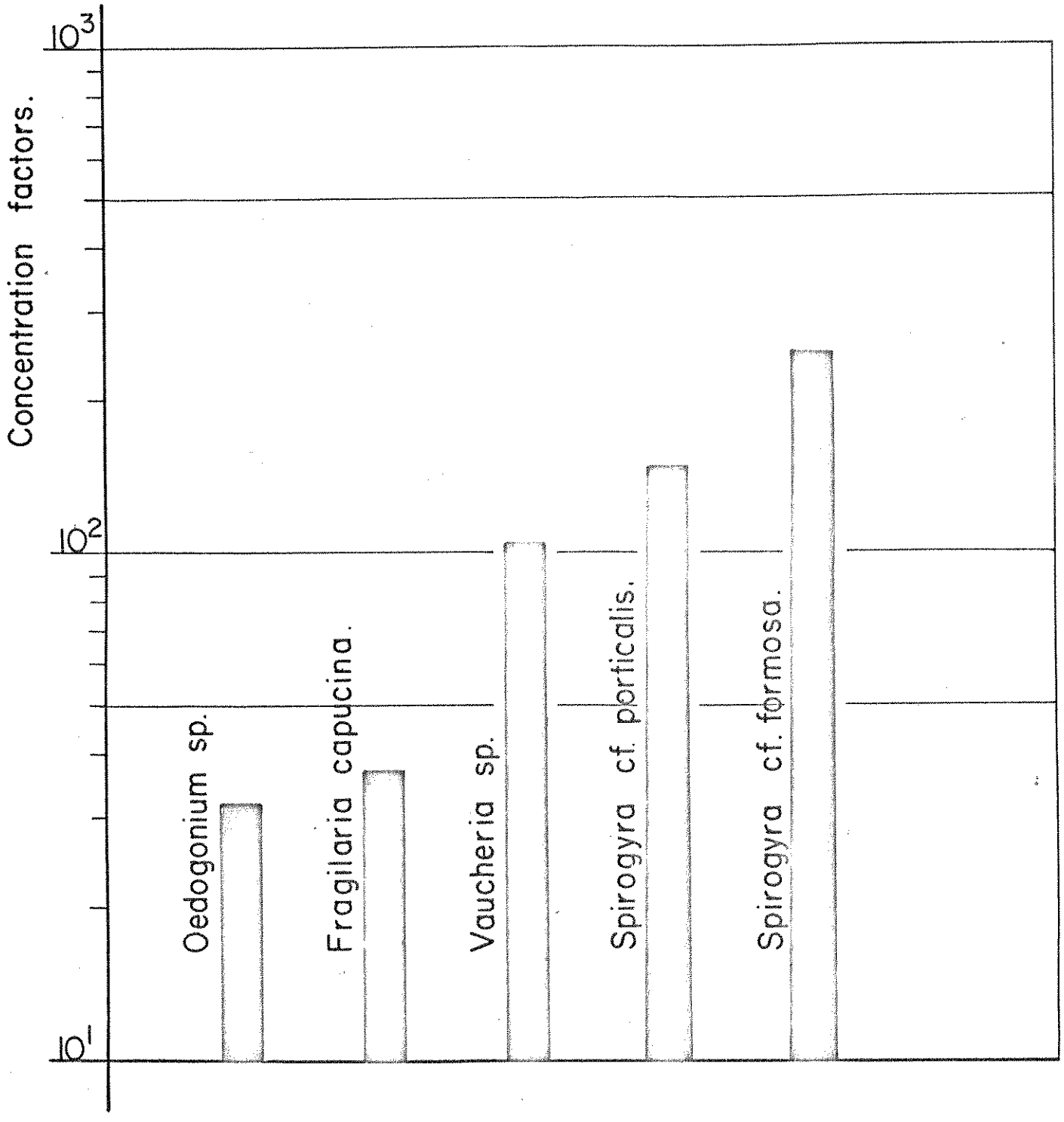


Fig. 19

Spirogyra cf. formosa.
Maximum concentration factors.

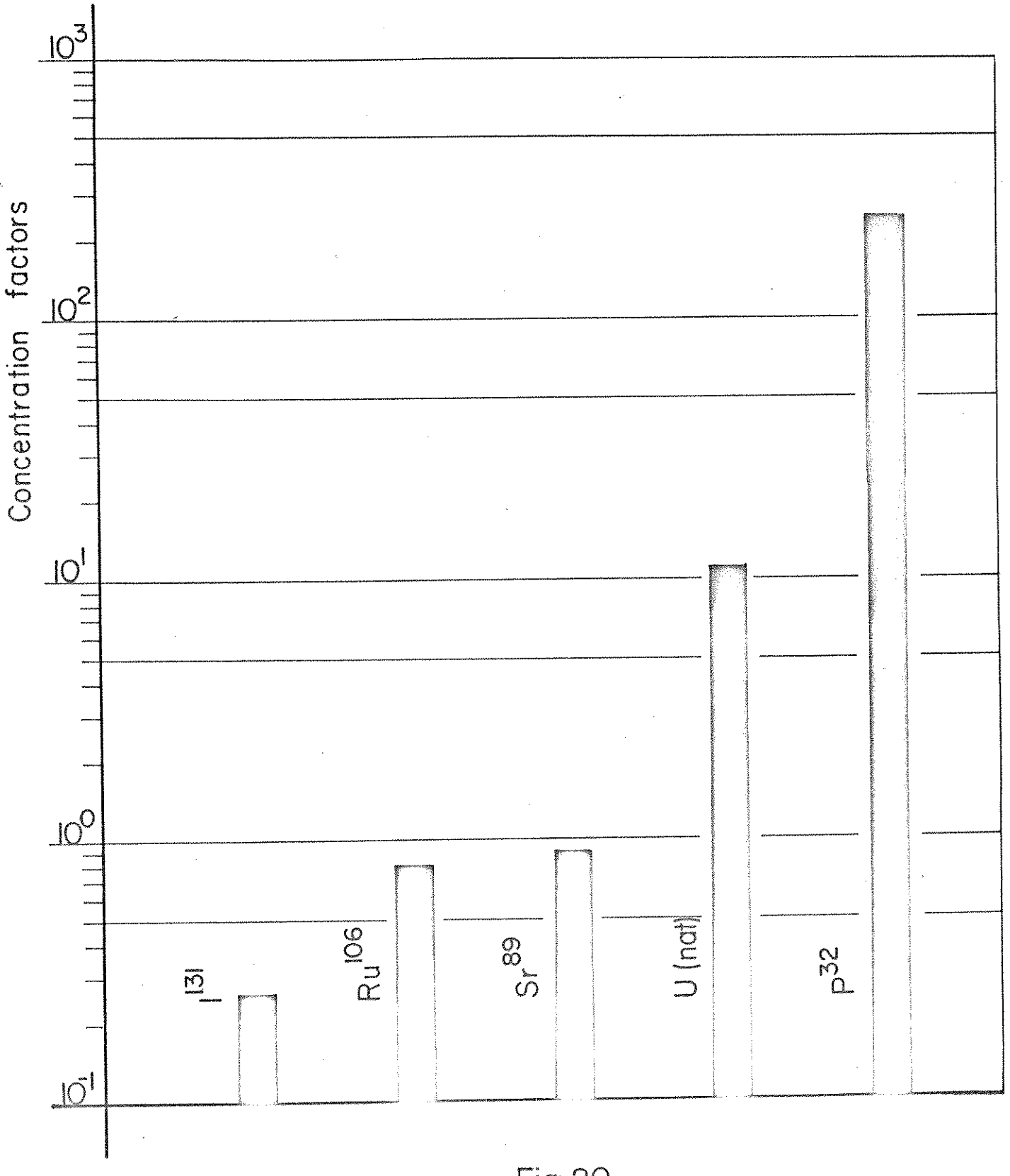


Fig. 20

The algae represent several important entrance possibilities of radionuclides into the food chain. To elucidate the order of magnitude of uptake of radionuclides by the algal constituents in communities of the first trophic level is of primary interest.

Further aspects of work along these lines under the research project involve the influence of long exposure times. The water-clay-algae interrelationship will be given attention.

4. REMARKS ON FURTHER PROGRESS OF THE RESEARCH PROJECT.

The results of the experimental work on the research project this summer have revealed a base for further approach towards the accomplishment of the present study. We have gained experience in operating the experimental plant both with respect to the cultivation and maintenance of the actual organisms and the management of the practical problems involved with experiments of this kind.

In connection with the investigations performed in the model recipient a study of the selfpurification process and how this process influence the decontamination of the water will be a fruitful advance. For this purpose it will be necessary to quantitate the results from the experiments. Parameters to be used are: Rate of elimination, the quantity of radioactive substance removed from the water per time unit. Specific elimination, the quantity of radioactive substance which is removed from the water per time unit and weight unit of the organism. Elimination capacity, the quantity of removed radioactive substance during the passage of water through the channel.

A few organisms representing the littoral and sublittoral zone possessing different physiological properties (e.g. helophytes, nymphaeids and elodeids) will be studied with respect to their ability to concentrate radionuclides. Variations in concentration of radionuclide, water velocity and exposure time will be considered.

The investigations on Anodonta cygnea will be directed toward its food chain relations. A convenient procedure represents the uptake of radionuclides to unicellular algae, and the transfer of the radionuclides to the animal. The concentration resulting from this incorporation of radionuclides from the lower trophic level, may be compared with the concentration obtained when the radionuclides are administered directly to the animal by the water.

The problems related to radioecological implications of pollution by radioactive wastes disposed to inland waters are many and of complex nature. We believe that the informations obtained through the International Atomic Energy Agency Research Contract No. 37 will be of interest to this field of science.

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