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ZINC TOXICITY TO FRESHWATER ALGAE

by

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ABSTRACT

Studies with natural phytoplankton from Lake Ontario as well as two green and one diatom algal species revealed that the International Joint Commission water quality objective of 30 µg Zn/L was toxic to primary productivity and cell multiplication. The toxicity of Zn was dependent upon its anionic forms with nitrate being the most toxic, followed by chloride, sulphate and acetate. Studies with radioactive Zn indicated that Zn was rapidly taken up by the algae and incorporated mainly into polysaccharide and nucleic acid fractions. These results show that an objective for Zn of 30 µg/L is too high to protect algae in the Great Lakes. Based on our results and other published data, a new objective of 10 µg Zn/L has been recommended to the International Joint Commission.

RÉSUMÉ

Les études effectuées sur du phytoplancton naturel provenant du lac Ontario ainsi que sur deux algues vertes et une algue diatomée ont révélé que l'objectif de qualité de l'eau de 30 ug Zn/L fixé par la Commission mixte internationale était toxique pour la production primaire et la multiplication cellulaire. La toxicité de Zn se manifeste dans les formes anioniques, le nitrate étant la forme la plus toxique, suivi du chlorure, du sulfate et de l'acétate. Des études effectuées avec Zn radioactif ont révélé que le zinc était rapidement absorbé par les algues et incorporé avant tout dans les fractions de polysaccharide et d'acide nucléique. Ces résultats révèlent que l'objectif de 30 ug/L pour Zn est trop élevé pour protéger les algues des Grands Lacs. En se basant sur ces résultats et sur d'autres données publiées, un nouvel objectif de 10 ug Zn/L a été recommandé à la Commission mixte internationale.

EXECUTIVE SUMMARY

Studies with natural phytoplankton from Lake Ontario as well as two green and one diatom algal species revealed that Zn at concentration of 30 µg/L was toxic to the primary productivity and cell multiplication. Toxicity of zinc compound was dependent upon its anionic part with nitrate being the most toxic, followed by chloride, sulphate and acetate. This order of toxicity approximated the relative solubility of these chemicals in water with zinc nitrate being the most soluble. Studies with radioactive ^{65}Zn indicated that Zn was rapidly taken up by the algae and incorporated mainly into polysaccharide and nucleic acid fractions. These and other data show that Zn at 30 µg/L is too permissive for protecting algae in the Great Lakes.

Data obtained in this study will be extremely useful for water quality management and for setting up water quality criteria.

PERSPECTIVE DE GESTION

Des études effectuées sur du phytoplancton naturel provenant du lac Ontario ainsi que sur deux algues vertes et une algue diatomée ont révélé que Zn était toxique à une concentration de 30 ug/L pour la production primaire et la multiplication cellulaire. La toxicité des composés des zinc est apparue dépendante de sa partie anionique, le nitrate étant le plus toxique, suivi du chlorure, du sulfate et de l'acétate. Cet ordre de toxicité coïncidait approximativement avec la solubilité relative de ces produits chimiques dans l'eau, le nitrate de zinc étant la substances la plus soluble. Des études sur ^{65}Zn radioactif ont révélé que Zn était rapidement absorbé par les algues et incorporé avant tout dans les fractions de polysaccharide et d'acide nucléique. Ces données et quelques autres montrent que la norme de 30 ug/L pour Zn est trop permissive pour protéger les algues dans les Grands Lacs.

Les données recueillies dans le cadre de cette étude seront extrêmement utiles pour la gestion de la qualité de l'eau et pour l'établissement de critères de qualité de l'eau.

INTRODUCTION

Zinc is a common element and ranks as the 24th most abundant element found in the earth's crust. It has many industrial uses such as coatings for iron and steel, alloys for diecast parts and in batteries and paint production (Taylor et al., 1982). It is found in various Great Lakes water samples. The mean concentrations of Zn in the offshore waters were generally below 1 ug/L while much higher concentrations were found in the nearshore waters (Rossmann, 1984). Much of the input to the system is from anthropogenic sources and arrives via atmospheric transport and deposition (Taylor et al., 1982).

Toxicity data before 1976 considered that fishes were more sensitive to Zn than other aquatic organisms (IJC, 1976). The sensitivity of fishes to Zn varied considerably with fish species, life stages, and quality of water such as hardness, pH and alkalinity (Bradley and Sprague, 1985). The short-term acute toxicity values (generally 96 hr LC50) ranged from around 90 ug/L to almost 200 mg/L (Hart, 1983). Chronic toxicity generally began at a Zn concentration of about 70 ug/L. In view of the sensitivity of fish to Zn, a water quality objective for Zn at 30 ug/L was adopted for the Great Lakes by the IJC (1976) to protect aquatic organisms particularly fish in the Great Lakes.

However, a number of studies after 1976 indicated that Zn toxicity to algae could occur at concentrations near or below 30 ug/L. For example, Chiaudani and Vighi (1978) measured growth of Selenastrum capricornutum in water from 22 Italian lakes. Addition of Zn at 20 ug/L to the lake water

reduced algal growth by about 50% in half of the lakes. Other algae such as Anabaena spiroides (Kostyaev, 1981), Schroederella schroederi (Kayser, 1977) and Thalassiosira aesteralis (Hollibaugh et al., 1980) were affected by Zn at levels less than 30 ug/L. In this report, we demonstrate that Zn at 30 ug/L is also toxic to three freshwater algae as well as natural phytoplankton from the Great Lakes.

MATERIALS AND METHODS

Three unialgal cultures were used: Scenedesmus quadricauda (Culture collection #11, Dr. P. Healey, Dept. of Fisheries & Oceans, Freshwater Institute, Winnipeg, Manitoba), Ankistrodesmus falcatus var. acicularis (Ontario Ministry of Environment, Rexdale, Ontario), Navicula pelliculosa (Dr. C. Nalewajko, Scarborough College, University of Toronto, Ontario). The cultures were maintained in a modified CHU-10 medium, containing no complexing agents, at pH 8 (Wong et al., 1978). The inoculum for toxicity bioassays was prepared by growing the culture in 100 mL of medium at 20 C on a rotary shaker (100 rpm) under conditions of 18 hr of light (5000 lux) and 6 hr of darkness. When the cells reached the logarithmic phase of growth (about 1 week), they were used as inoculum.

Primary production was measured by the amount of ^{14}-C-sodium bicarbonate taken up by the algae over a period of 4 hr. One mL of the inoculum (about 2 x 10^5 cells/mL) was added to 13.9 mL of modified CHU-10 medium with or without the addition of various anionic forms of the zinc compounds. After a 24 hr incubation at 20 C under conditions described above, a 0.1 mL aliquot

of 2 uC/mL ^{14}-C-sodium bicarbonate (Amersham/Searle, 58.8 mC/m mole) was added to each 25-mL Erlenmeyer flask. The flasks were tightly capped with rubber stoppers wrapped in aluminum foil. A similar set of flasks was incubated in the dark. After a 4-hr incubation, the cells were filtered through a 0.45 um membrane filter and rinsed rapidly with 10 mL fresh medium to remove extracellular ^{14}-C-sodium bicarbonate. Filters containing radioactive labelled cells were dissolved in 10 mL PCS scintillation counting fluor (Amersham/Searle). Radioactivity was measured by a liquid scintillation counter (Beckman model LS8100) with automatic quenching factor and 10,000 dpm upper limit. Radioactivity taken up by algae in the dark (<5% of total) was subtracted from the total radioactive counts. Radioactivity in the algae without exposure to Zn (control) was taken as 100%. Toxicity of Zn to a natural phytoplankton was tested with water from a sampling site in Lake Ontario near Hamilton, Ontario. To compensate for the smaller number of algae, the volume of lake water was increased from 15 mL with the pure culture to 100 mL. The ^{14}C-sodium bicarbonate was also increased to 0.15 mL and 10 uC/mL. The EC_{50} value (effective concentration of the metal that causes a 50% reduction in primary productivity or cell multiplication) was estimated according to the Standard Methods for the Examination of Water and Wastewater (1980).

The effects of Zn on algal cell multiplication (growth) was measured spectrophotometrically with a Klett-Summerson Photoelectric Colorimeter (filter #66). One mL of the inoculum (A. falcatus) was added to 49 mL of the medium containing Zn concentrations from 0 to 500 ug/L in a 300-mL Nephelo culture flask with a side-arm (Bellco Co., Vineland, New Jersey). The algal growth was measured at various time intervals by tilting the medium into the

side-arm which was then inserted into the colorimeter. Since no medium was required to be withdrawn from the flask for determination, this technique was thus more convenient and less likely to be subjected to contamination (Wong and Couture, 1986). The readings in Klett units (K.U.) could be converted to cell number, using a previously constructed standard curve relating K.U. to cell number. One K.U. was equivalent to 2.85 x 10^4 cells/mL.

To determine the time course of Zn uptake, radioactive Zn ($^{65}ZnCl_2$, Amersham Corp.) was used as a tracer. Fifty mL of A. falcatus in a 250-mL Erlenmeyer flask was spiked with 0.5 mL of $^{65}ZnCl_2$ (10 uC of ^{65}Zn and 1.5 ug Zn/mL of stock solution). The flask was thoroughly mixed in a shaker. Five mL of the cell suspension was withdrawn at various time intervals, filtered through 0.45-u filter, rinsed rapidly with 10 mL of fresh medium to remove extracellular radioactivity. To compensate for radioactivity adhering to the filter, an equal volume of medium (without the cells) and $^{65}ZnCl_2$ was filtered and washed. The radioactivity representing less than 5% of the amount taken up by the cells was subtracted.

The time-course of ^{65}Zn uptake in "live" and "dead" cells was determined by dividing 200 mL of A. falcatus into four equal parts of 50 mL each. Two parts were placed in hot water (90 C) for 10 minutes and no viable cell was found when a small aliquot was plated onto a CHU-10 agar plate. These parts were designated as "dead" cells. The other two parts were left untreated and labelled as "live" cells. A small volume of concentrated sodium ethylenediaminetetraacetate (EDTA, pH adjusted to 8) was added to give a final concentration of 2 uM to one part of "live" or "dead" cells to determine the effects of EDTA on Zn uptake. At various time intervals, 5 mL

of the cells was withdrawn and filtered as described above. The Zn radioactivity was measured with an automatic well-type gamma scintillation counter (Nuclear Chicago) with 30% counting efficiency for ^{65}Zn. The concentration factor was the ratio between the concentrations of ^{65}Zn in the cells and in the medium. Cell dry weight was obtained by filtering and drying the algal cells at 80 C until constant weight.

Incorporation of Zn in cellular components was investigated with 100-mL samples of washed cell suspensions of A. falcatus (approximately 2 mg cell dry weight) incubated with labelled and unlabelled Zn for 24 hr at 20 C under light. At the end of incubation, the cells were filtered and washed with fresh medium. The cells were then fractionated with chloroform-methanol and trichloroacetic acid according to the procedures of Li et al. (1980).

RESULTS AND DISCUSSION

Primary productivity bioassays revealed that Zn was indeed toxic to the algae at 30 ug/L level (Table I). The amounts of radioactivity as an indicator of the primary productivity in two green algae, Ankistrodesmus, and Scenedesmus, one diatom, Navicula and natural phytoplankton from Lake Ontario water were reduced by 59, 55, 49 and 52% respectively when the algae were exposed to $ZnCl_2$ at 30 ug Zn/L. The pure algal cultures and the natural phytoplankton were almost equally sensitive to the metal toxicity. Even 20 ug/L was quite toxic and 10 ug Zn/L had a slight inhibitory effect. In addition to the spiked levels of Zn, the CHU-10 medium and the lake water were found to contain about 3-5 ug Zn/L.

Since the primary production involved only a short incubation time of 24

hr, the effect of Zn on a longer incubation time such as reproduction was determined over 2 weeks (Fig. 1). The growth rates and total amount of growth of A. falcatus were greatly reduced when the organism was exposed to Zn from 10 to 500 ug/L. Low concentration of Zn (5 ug/L) slightly stimulated algal growth. A number of other studies also suggest that Zn toxicity to algae can occur at concentrations near or below 30 ug/L (Table II). For example, Marshall et al. (1983) incubated natural water containing indigenous phytoplankton from Lake Michigan, in-situ for two weeks in 18 L carboys. They observed a significant drop (about two-thirds, $P < 0.01$) in both chlorophyll-a and primary production with Zn concentrations as low as 17 ug/L.

At low concentrations, Zn is an essential nutrient and is a constituent of many metalloenzymes (Bowen, 1966). However under conditions of elevated concentrations, Zn has been shown to be toxic to fish (Bradley and Sprague, 1985), invertebrates (Mount and Norberg, 1984), algae (Starodub et al., 1987) and bacteria (Babich and Stotzky, 1978). The toxic mechanism of Zn in the case of algae as proposed by Kostyaev (1981) was in the alteration of the permeability of the cell membrane which lead to a sharp reduction of potassium and sodium content of the cell, followed by inhibition of photosynthesis, then nitrogen fixation, and finally cell multiplication.

Zinc concentrations spanning three orders of magnitude from 0.015 to 30 mg/L have been reported to elicit toxic effects in algae (Spear, 1981). The variability is due to the differences in the growth media, pH, test parameters, genetic stability and tolerance of individual strains of test organisms as well as background contamination of medium or metal stocks. The common procedures of conducting algal toxicity tests with high density

monocultures grown in nutrient-rich media under near optimum conditions often result in unrealistically high Zn toxic values (Wong et al., 1979). In contrast, our experiments with low cell density and low nutrient medium would better mimic the conditions in the Great Lakes water.

The Zn toxicity was dependent upon its anionic forms with nitrate being the most toxic, followed by chloride, sulphate and acetate (Table III). This order of toxicity approximated the relative solubility of these chemicals in water with zinc nitrate being the most soluble (Merck Index, 1968). In addition to the solubility, the acetate form of Zn is also a carbon and energy source and would counter the Zn toxicity to the algae.

The accumulation of Zn by algae was tested with the "live" (nontreated) and "dead" (heat-killed) cells of A. falcatus. The cells were exposed to the medium containing radioactive Zn for various periods of time, the "dead" cells had a much faster rate of uptake and a higher level of steady state of Zn accumulation than the "live" cells (Fig. II). At the steady state of 80 minutes of incubation, the "dead" cells had a concentration factor of Zn of approximately 1000. The Zn steady state in the "live" cells was not reached even after 240 minutes of incubation. The heat treatment of the cells is known to increase the negative charge of the cells and increase the membrane permeability for metal to bind the intracellular organelles. For examples, the uptake of Sn and Cd by algae were also higher in the dead cells than the live cells (Wong et al., 1984; Sakaguchi et al., 1979). The ability of the cells to accumulate Zn is important since algae are a major vector for transferring the metal from the abiotic environment into food webs. No accumulation of Zn was observed by both types of cells when 2 uM EDTA were added to the medium, suggesting that binding of Zn-EDTA was too strong for

the cells to compete for the Zn. Similar observations of other metal-EDTA complexes have also been reported (Wong et al., 1984).

The binding of Zn in the cellular components was studied by exposing A. falcatus to Zn for 24 hr and the cells were fractionated into several components. The results show that about 80-85% of the Zn in the cells was in the polysaccharide and nucleic acid fractions, while 11-15% was in the protein fraction (Table IV). Lipid and small molecular weight metabolites contained a negligible amound of Zn. Other studies indicated that the majority of tin and vanadium taken up by algae was also found in the polysaccharide fraction (Wong et al., 1984; Lee, 1983). The binding of the metals to polysaccharide was believed to be a detoxification mechanism by the cells (Jensen et al., 1982).

Our results demonstrate that the Zn objective of 30 ug/L is too high to protect algae in the Great Lakes. Based on these results and other published results in the literatures, a new objective of 10 ug/L has been recommended by the Aquatic Ecosystem Objective Committee to the International Joint Commission (IJC, 1987).

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REFERENCES

Babich, H., and G. Stotzky. 1978. Toxicity of zinc to fungi, bacteria, and coliphages: influence of chloride ions. Appl. Environ. Microbiol. 36:906-914.

Bowen, H.J.M. 1966. Trace elements in biochemistry. Acad. Press, London, 241 pp.

Bradley, R.W., and J.B. Sprague. 1985. The influence of pH, water hardness, and alkalinity on the acute lethality of zinc to rainbow trout (Salmo gairdneri). Can. J. Fish. Aquat. Sci. 42:731-736.

Chiaudani, G., and M. Vighi. 1978. The use of Selenastrum capricornutum batch cultures in toxicity studies. Mitt. Internat. Verein. Limnol. 21:316-329.

Glooschenko, W.A., and J.E. Moore. 1973. The effect of citrate on phytoplankton in Lake Ontario. In Bioassay Techniques and Environmental Chemistry. G.E. Glass (Ed.) Ch. 16, pp. 321-334, Ann Arbor Sci. Publish., Ann Arbor, Michigan.

Greene, J.C., W.E. Miller, T. Shiroyama, R.A. Solters, and K. Putnam. 1978. Use of laboratory cultures of Selenastrum, Anabaena and the indigenous isolate Sphaerocystis to predict effects of nutrient and zinc interactions upon phytoplankton growth in Long Lake, Washington. Mitt. Int. Ver. Limnol. 21:372-384.

Hart, C.J. 1983. A study on the effects of heavy metal combinations on Lake Ontario phytoplankton communities. M.Sc. thesis, 195 pp., University of Waterloo, Waterloo, Ontario, Canada.

Hollibaugh, J.T., D.L.R. Seibert, and W.H. Thomas. 1980. A comparison of the acute toxicities of ten heavy metals to phytoplankton from Saanich Inlet, B.C., Canada. Estuarine and Coastal Marine Sci. 10:93-105.

IJC. 1976. Great Lakes Water Quality. Fourth Annual Report. Appendix A. International Joint Commission, pp. 77-81, Windsor, Ontario, Canada.

IJC. 1987. Zinc. Report of the Aquatic Ecosystem Objectives Committee. International Joint Commission, pp. 16-33, Windsor, Ontario, Canada.

Jensen, T.E., M. Baxter, J.W. Rachlin, and V. Jani. 1982. Uptake of heavy metals by Plectonema boryanum (Cyanophyceae) into cellular components, especially polyphosphate bodies: an x-ray energy dispersive study. Environ. Pollut. 27:119-127.

Kayser, H. 1977. Effect of zinc sulphate on the growth of mono- and multispecies cultures of some marine algae. Helgol. Wiss. Meersunters 30:682-696.

Kostyaev, V.Y. 1981. Effect of some heavy metals on Cyanobacteria. Microbiol. 49:665-667.

Lee, K. 1983. Vanadium in the aquatic ecosystem. In Aquatic Toxicology. J.O Nriagu (Ed.), Ch. 6, pp. 155-187. John Wiley and Sons, New York.

Li, W.K.W., H.E. Glover, and I. Morris. 1980. Physiology of carbon photoassimilation by Oscillatoria thiebautii in the Caribbean Sea. Limnol. Oceanogr. 25:447-456.

Marshall, J.S., J.I. Parker, D.L. Mellinger, and C. Lei. 1983. Bioaccumulation and effects of cadmium and zinc in a Lake Michigan plankton community. Can. J. Fish. Aquat. Sci. 40:1469-1479.

Merck Index. 1968. P.G. Stecher, M. Windholz, and D.S. Leaky (Eds.). Merck & Co. Inc. Rahway, N.J.

Mount, D.I., and T.J. Norberg. 1984. A seven-day life-cycle cladoceran toxicity test. Environ. Toxicol. Chem. 3:425-434.

Rossmann, R. 1984. Trace metal concentrations in the offshore waters of Lake

Erie and Michigan. United States Environ. Protection Agency Special Report No. 108, pp. 1-107.

Sakaguchi, T., T. Tsuji, A. Nakajima, and T. Hosikoshi. 1979. Accumulation of cadmium by green microalgae. European J. Appl. Microbiol. 8:207-215.

Spear, P.A. 1981. Zinc in the aquatic environment:chemistry, distribution, and toxicology. National Research Council of Canada, No. 17589, Ottawa, Canada, pp. 1-145.

Standard Methods for the Examination of Water and Wastewater. 1980. American Public Health Association, American Water Works Association and Water Pollution Control Federation, 15th edition, New York, pp. 641-645.

Starodub, M.E., P.T.S. Wong, and C.I. Mayfield. 1987. Short term and long term studies on individual and combined toxicities of copper, zinc and lead to Scenedesmus quadricauda. The Science of the Total Environment 63:101-110.

Taylor, M.C., A. Demayo, and K.W. Taylor. 1982. Effects of zinc on humans, laboratory and farm animals, terrestrial plants, and freshwater aquatic life. CRC Critical Rev. in Environ. Control 12:113-181.

Wong, M.H., S.H. Kwan, and F.Y. Tam. 1979. Comparative toxicity of manganese and zinc on Chlorella pyrenoidosa, Chlorella salina, and Scenedesmus quadricauda. Microbios Letters 12:37-46.

Wong, P.T.S., Y.K. Chau, and P.L. Luxon. 1978. Toxicity of a mixture of metals on freshwater algae. J. Fish. Res. Bd. Canada 35:479-481.

Wong, P.T.S., R.J. Maguire, Y.K. Chau, and O. Kramar. 1984. Uptake and accumulation of inorganic tin by a freshwater alga, Ankistrodesmus falcatus. Can. J. Fish. Aquat. Sci. 41:1570-1574.

TABLE I

The toxicity of $ZnCl_2$ to primary productivity of pure algae and natural phytoplankton from Lake Ontario water

Medium	% of primary productivity (S.D.)*			
	Ankistrodesmus	Scenedesmus	Navicula	Phytoplankton
CHU-10 (control)	100	100	100	100
CHU-10 + 5 ug Zn/L	102 (7)	98 (8)	96 (3)	104 (3)
CHU-10 + 10 ug Zn/L	95 (4)	93 (6)	91 (6)	92 (7)
CHU-10 + 20 ug Zn/L	78 (5)	80 (4)	73 (8)	72 (5)
CHU-10 + 30 ug Zn/L	41 (5)	45 (7)	51 (4)	48 (8)

* Standard deviation of 5 samples

TABLE II

Examples of Zn toxicity to freshwater algae at levels below 30 ug Zn/L

Algal species	Zn conc. (ug/L)	Toxic effect	Reference
Anabaena spiroides	3	80% reduction in nitrogen fixation	Kostyaev (1981)
Selenastrum capricornutum	30	50% growth inhibition	Greene et al.(1978)
Lake Ontario phytoplankton	10	22% reduction in primary production	Glooschenko & Moore (1973)
Lake Michigan phytoplankton	17	37% reduction in primary production	Marshall et al.(1983)
Lake Ontario phytoplankton	30	39% reduction in primary production 30% reduction in biomass	Hart (1983)

TABLE III

The effect of anionic forms of Zn on primary productivity of *A. falcatus*

Compound	EC_{50} (ug/L)*
$Zn(NO_3)_2$	16
$ZnCl_2$	24
$ZnSO_4$	32
$Zn(OAc)_2$	120

* EC_{50} = concentration of Zn causing a 50% reduction in primary productivity after a 24-hr incubation

TABLE IV

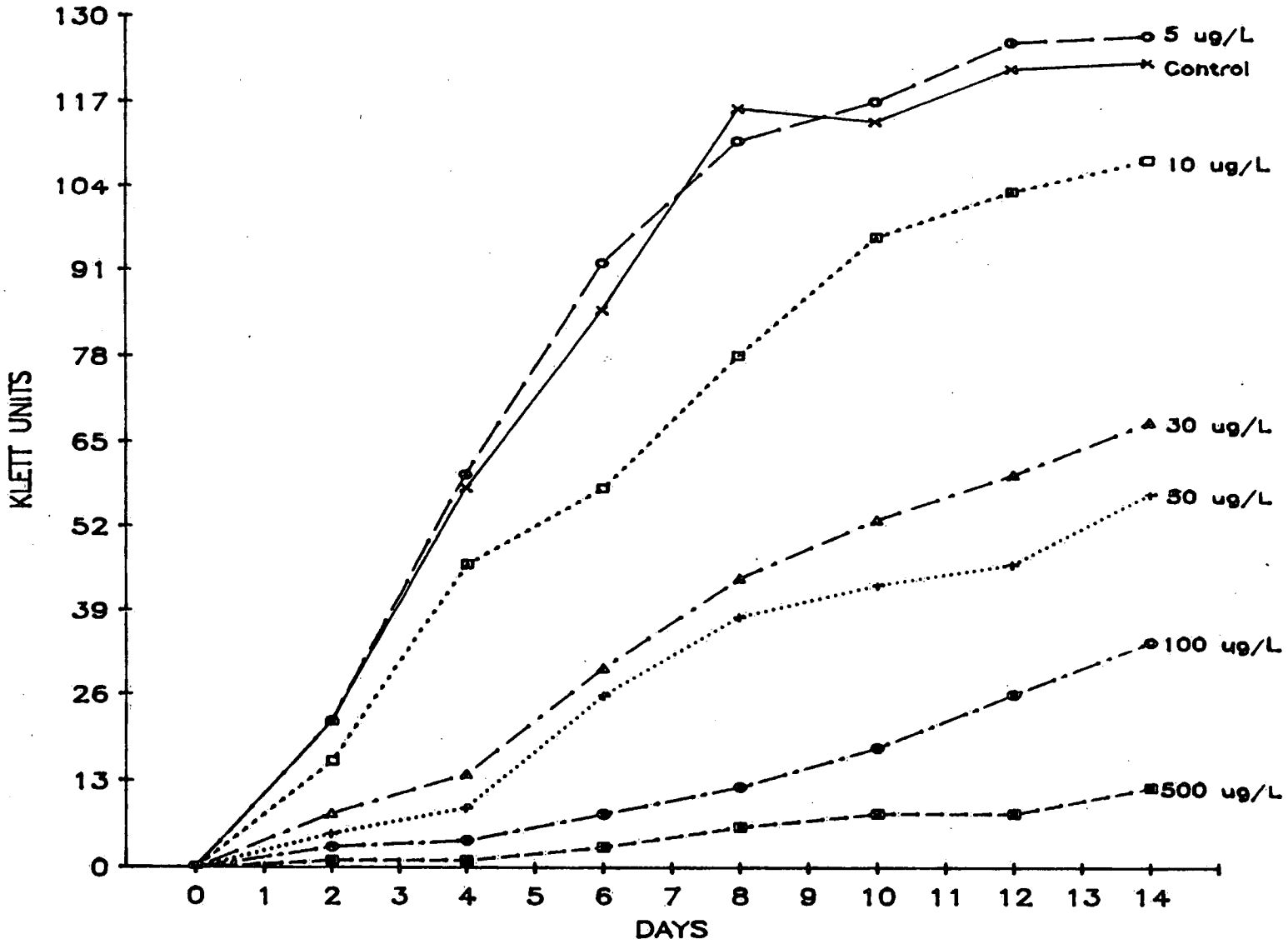
Distribution of $^{65}ZnCl_2$ in cellular components of *A. falcatus* after 24-hr exposure

Cellular component	"Live" cells	"Dead" cells
	% of distribution*	
Lipid	0.10	6.53
Small molecular weight metabolites	0.38	3.05
Protein	14.89	10.99
Polysaccharide and nucleic acid	84.62	79.42

* 100% of radioactivity representing 3.9×10^5 and 3.2×10^5 dpm in "live" and "dead" cells respectively.

Fig. I. The effects of various concentrations of $ZnCl_2$ on the growth of *A. falcatus*.

Fig. II. The effects of EDTA on the rate of Zn uptake and accumulation by "live" and "dead" cells of *A. falcatus*.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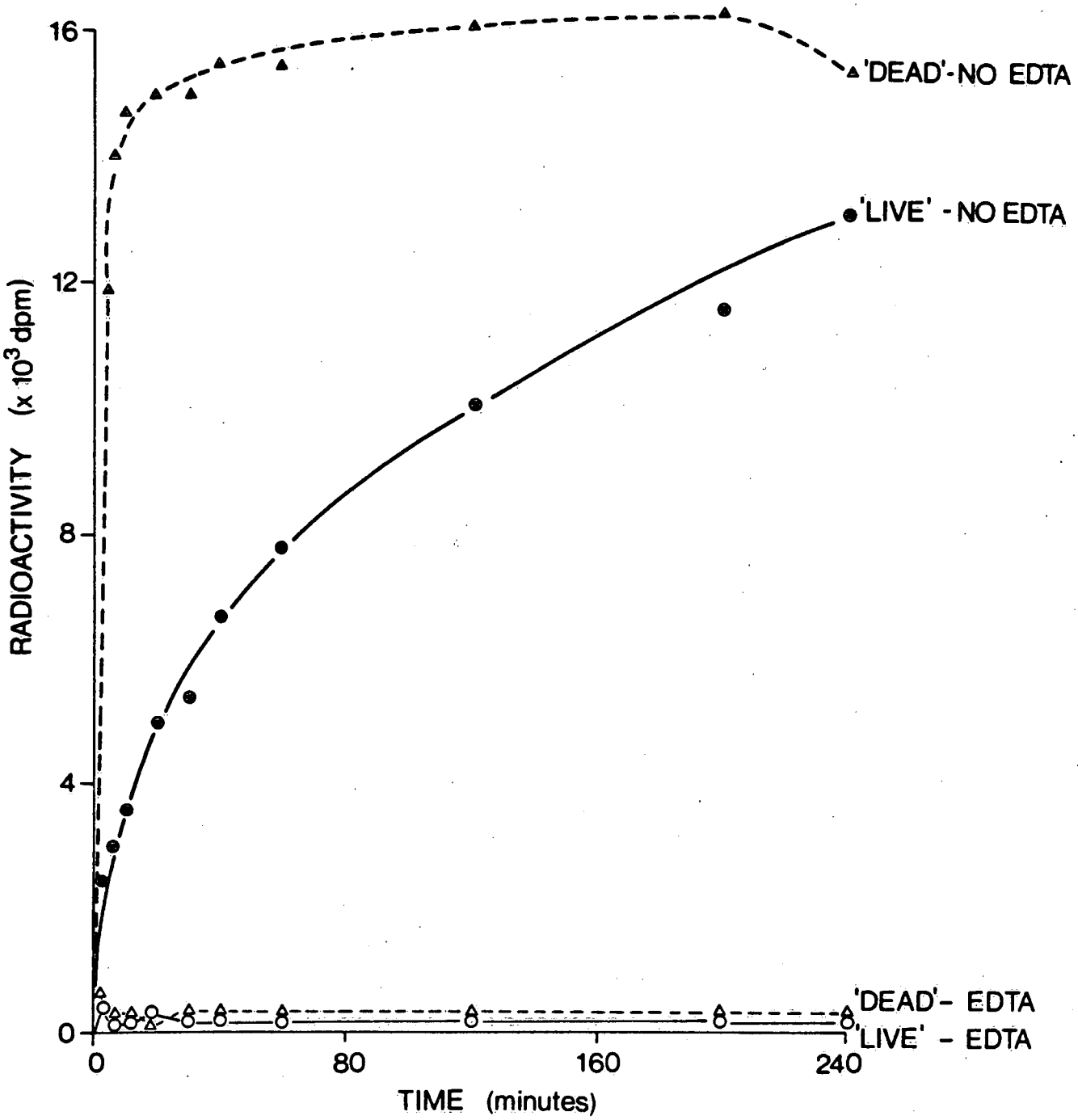
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'DEAD'- EDTA
'LIVE' - EDTA