

MELIMEX, an experimental heavy metal pollution study: Effects of increased heavy metal load on uptake of glucose by natural planktonic communities

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ABSTRACT

Glucose uptake in lake water samples has been determined with short-time ^{14}C -experiments at different additions of the single heavy metals Cu^{++} , Zn^{++} , Cd^{++} , Pb^{++} , Hg^{++} and at different additions of a combination of all metals. The degree of a metal induced inhibition of glucose uptake varied from lake to lake and from season to season, whereby the addition of legally tolerated concentrations of $10\text{ }\mu\text{g Cu/l}$ and $200\text{ }\mu\text{g Zn/l}$ inhibited glucose uptake in most samples significantly, as well as the simultaneous addition of all metals. The legally tolerated limit of $1\text{ }\mu\text{g Hg/l}$ inhibited glucose uptake in Lake Lucerne samples almost completely, showed however no effect in samples of Lake Baldegg. The equimolar toxicity sequence of heavy metals for heterotrophic microorganisms has been determined as:

$\text{Hg} > \text{Cu} > \text{Cd} > (\text{Zn}, \text{Pb})$.

Glucose uptake of plankton sampled from metal polluted limno-corrals was less inhibited than that of the control plankton, when heavy metals were added to the samples singly or in combination. It is assumed that this effect is due to the natural selection of more resistant plankton species.

1. Introduction

It is well known that heavy metals inhibit photosynthesis (see Gächter [5, 6]), that algae release organic substances by excretion and/or autolysis [2, 4, 10, 14], that organic ligands can influence the bio-availability and therefore the toxicity of heavy metals to algae [3, 9, 11, 16], invertebrates (see Lang [12] and Urech [17]) and fish [13]. Gächter [7] has shown that a raised heavy metal load leads at times to elevated amino-acid concentrations and that it also effects changes in the spectrum of copper complexing ligands in long-term experiments. Metal induced shifts in the amounts and types of organic substances can be caused by a change in photosynthesis [6] or excretion, but it can also be caused by a change of heterotrophic metabolisms of dissolved organic substances. Vaccaro et al. [19] observed in a controlled long-term ecosystem pollution experiment (CEPEX), that a single addition of $10\text{--}50\text{ }\mu\text{g Copper/l}$ led to a marked increase in the relative numbers and activity of bacterial heterotrophs. They suppose that this effect was caused by an increase of excretion and autolysis. The surviving bacteria also demonstrated an increased tolerance to copper with time.

Sugars and related compounds are the most important products of photosynthetic CO_2 -fixation. They are preferred nutrients for most heterotrophic microorganisms. Polymers are normally reduced by exoenzymes to their monomer or dimer elements, which are adsorbed by heterotrophic microorganisms and disposed in the cells for further use. It is the subject of this work to investigate whether an increase in heavy metal concentrations influences the heterotrophic utilization of a monomer substrate.

D-glucose has been chosen as a substrate because of its important role in the metabolism of autotrophic and heterotrophic organisms. Natural plankton take up D-glucose and convert it to cell substance or oxidize it to CO_2 for energy yield. Investigations on this topic have shown that both metabolisms occur in comparable rates (Bossard, unpublished). Under natural conditions more glucose is incorporated by plankton than oxidized to CO_2 . The relationship between glucose incorporation and CO_2 -production is not severely disturbed by addition of heavy metals as applied in the MELIMEX experiment [7]. Therefore the following investigations are restricted to glucose incorporation in microorganisms.

This paper shall give an answer to the following questions:

1. Do legally tolerated Cu^{++} , Zn^{++} , Cd^{++} , Pb^{++} and Hg^{++} concentrations¹⁾ inhibit the glucose incorporation of natural planktonic communities?
2. Can a natural plankton population in a long-term heavy metal pollution experiment develop an increased tolerance towards heavy metals in respect to glucose incorporation?

For the investigation of the first question water samples of a mesotrophic and a eutrophic lake have been spiked with different heavy metals. In order to answer the second question plankton has been used from the controlled ecosystem pollution experiment MELIMEX [7]. In both experiments glucose incorporation has been used as an indicator of heterotrophic activity.

2. Methods

The D-glucose incorporation was measured in short-term experiments by addition of ^{14}C -radiotracer ($0.5 \mu\text{g } ^{14}\text{C-D-glucose/l}$) to natural plankton samples, which had been incubated in brown coloured glass bottles for minutes or hours. Immediately after spiking the sample with ^{14}C -glucose, the total ^{14}C -activity was determined after mixing 7 ml of the water sample with 10 ml INSTA-GEL (universal liquid scintillation cocktail, produced by Packard Instrument Company Inc., Illinois, USA) in a TRI-CARB liquid scintillation spectrometer (Packard Instrument Company). After the incubation at room temperature, 10 ml of the sample were filtered through a cellulose nitrate filter (pore size $0.2 \mu\text{m}$). The filter was rinsed immediately afterwards with lake water to eliminate traces of dissolved ^{14}C -compounds. The moist filter was then dissolved in 7 ml of ethylene glycol monomethyl ether (Merck, Art. 859) and rigidly shaken by a vibrator. After 15 minutes the colourless solution was mixed with 10 ml INSTA-GEL.

1) Legally tolerated limits for running waters in Switzerland, fixed by the Federal Environmental Protection Agency.

The activity of dead plankton, killed with NaOH at pH 12 and incubated for 2 hours with 2×10^5 cpm ^{14}C -glucose/10 ml, amounted to less than 200 cpm/10 ml, or less than 0.1% of the total activity. Any higher activity of filter residues must therefore be the result of an active glucose uptake by living plankton.

Since glucose concentrations in the natural plankton samples were below detection limits, only relative glucose incorporation rates of subsamples originating from the same water sample have been compared with each other. The glucose incorporation rate is expressed in percentages of the total dissolved glucose after spiking.

In order to answer the first question (see introduction), the following experiments were designed:

Heavy metal nitrates have been added to lake water samples in concentrations listed in table 1.

Table 1. Disposition for spring experiments (March), heavy metal additions.

Sample series	Added heavy metal concentrations						
A 1-5	Cu ⁺⁺	0	2	5	10	20	µg/l
B 1-5	Zn ⁺⁺	0	40	100	200	400	µg/l
C 1-5	Cd ⁺⁺	0	1	2.5	5	10	µg/l
D 1-5	Pb ⁺⁺	0	10	25	50	100	µg/l
E 1-5	Hg ⁺⁺	0	0.2	0.5	1	2	µg/l
Fraction of legally tolerated limits		0	0.2	0.5	1	2	units
F 1-5	Combination of all metals for each unit						

Each series was accompanied by a reference sample, which had not been spiked with heavy metals.

The sample series A to E contained single heavy metals at four different concentrations from 0.2 to 2 units of the legally tolerated limits, while series F contained all five metals (heavy metal cocktail). After the addition of heavy metals the samples were allowed to react to the increased heavy metal concentrations for a minimum of 3 hours, before addition of ^{14}C -glucose (equilibration time).

All the bottles of one series were spiked at the same time with ^{14}C -glucose, then shaken and incubated simultaneously. The incubation was terminated by filtration of 10 ml subsamples, whereby all subsamples of one series were filtered within 1 minute, guaranteeing an absolutely equal treatment of all the samples within one series. The six series were exposed one after the other within 3 hours. The incorporation rates of the reference samples accompanying each series did not show any dependence on the equilibration time before ^{14}C -addition. This result allowed to determine an average value and the standard deviation of the reference samples, and enabled a comparison of values of different series.

In order to answer the second question (see introduction), samples were taken from the MELIMEX limno-corrals C (control) and L2 (heavy metal loaded) 1 m below surface. Immediately after filtration of the samples at a pressure difference of 0.5 atm through a Sartorius membrane filter (pore size 0.2 µm), the retentates were diluted with filtrate to reach the tenfold natural plankton concentrations.

After filtration of the lake water dark glass bottles were filled with 45 ml of C-filtrate. To each bottle heavy metals were added according to table 2. After a minimum of 3 hours equilibration each series was simultaneously spiked with 0.5 $\mu\text{g } ^{14}\text{C}$ -glucose/l and with 5 ml plankton concentrate, obtained either from limno-coral C or L2, respectively. Glucose uptake of these samples was determined as described above.

Table 2. Disposition for summer experiments (July), heavy metal additions.

Sample series	Plankton	Added heavy metal concentrations						
A 1-6	C1	Cu ⁺⁺	0	10*	50	100	250	500 $\mu\text{g/l}$
B 1-6	C1	Zn ⁺⁺	0	200*	1,000	2,000	5,000	10,000 $\mu\text{g/l}$
C 1-6	C1	Me-cocktail:						
		Cu ⁺⁺	0	5	10*	20	50	100 $\mu\text{g/l}$
		Zn ⁺⁺	0	100	200*	400	1,000	2,000 $\mu\text{g/l}$
		Cd ⁺⁺	0	2.5	5*	10	25	50 $\mu\text{g/l}$
		Pb ⁺⁺	0	25	50*	100	250	500 $\mu\text{g/l}$
D 1-6	L2	Cu ⁺⁺ : similar to A						
E 1-6	L2	Zn ⁺⁺ : similar to B						
F 1-6	L2	Me-cocktail: according to C						

* Legally tolerated limits for heavy metals in running waters.

3. Experiments

3.1 Effects of heavy metal addition on glucose uptake

Glucose uptake of natural plankton samples from Lake Lucerne and Lake Baldegg has been tested after heavy metal additions according to table 1 (March experiments) and table 2 (July experiments). Figure 1 shows the metal induced inhibition of glucose uptake, where the results of two different exposure times are presented. The mean relative glucose incorporation rate of the reference samples has been set to 100% (\pm standard deviation). The relative glucose incorporation rates of the metal-spiked samples have been calculated as fractions of the mean reference value. The metal-induced inhibition of glucose uptake is significant, if the values are below the horizontal bar, which represents the lower standard deviation ($2s_x$) of the reference values.

Copper (legally tolerated limit: 10 $\mu\text{g/l}$):

In March the addition of 2 $\mu\text{g Cu}^{++}/\text{l}$ inhibited the uptake of glucose significantly in both lakes, whereby Lake Lucerne samples reacted more sensitively than Lake Baldegg samples.

Experiments conducted in Lake Baldegg in July showed, that summer samples reacted less sensitive than spring samples. A 30% inhibition demanded in spring a copper addition of 2 $\mu\text{g/l}$, in summer one of 10 $\mu\text{g/l}$.

Zinc (legally tolerated limit: 200 $\mu\text{g/l}$):

The reaction to zinc addition was similar to copper addition: Lake Lucerne samples reacted more sensitive than Lake Baldegg samples and again spring samples in Lake

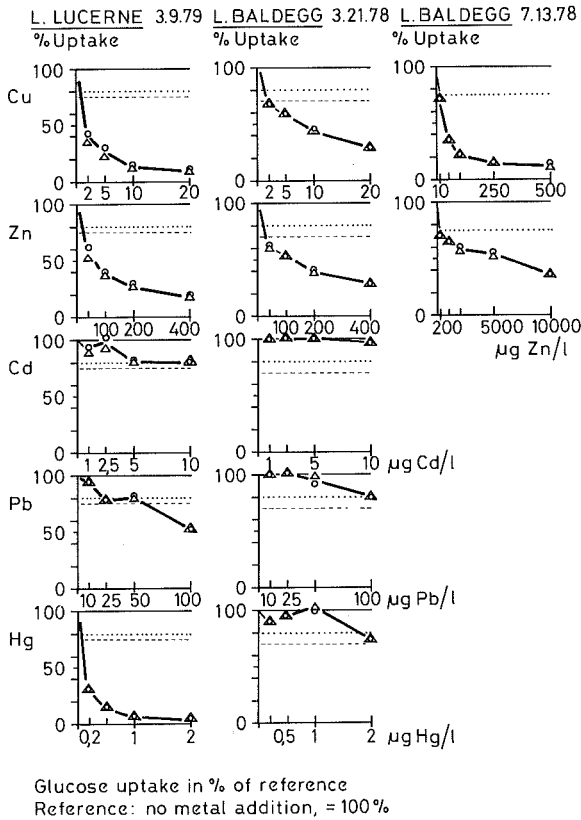


Figure 1. Inhibition of glucose uptake in lake water samples by addition of single heavy metals: Glucose uptake of the references without any heavy metal addition is set to 100%. Glucose uptake of the metal spiked values is expressed as a fraction of the references. At each heavy metal concentration two values at two different exposure times were determined: Lake Lucerne, 9.3.79: ○ 1 hr, 50 min; △ 3 hrs, 20 min, Lake Baldegg, 21.3.79: ○ 16 min; △ 32 min, Lake Baldegg, 13.7.79: ○ 32 min; △ 45 min. The standard deviation from the mean reference value ($2s_x$) is marked in the graphs by horizontal bars: for shorter exposure; - - - - - for longer exposure.

Baldegg were more sensitive than summer samples. While the addition of 40 $\mu\text{g Zn}^{++}/\text{l}$ caused in March an inhibition of 40%, the addition of 200 $\mu\text{g}/\text{l}$ inhibited glucose uptake in July by only 30%.

Cadmium (legally tolerated limit: 5 $\mu\text{g}/\text{l}$): The addition of 1–10 $\mu\text{g Cd}^{++}/\text{l}$ did not cause any significant inhibition in either of the two lakes, whereby Lake Lucerne samples showed at least a trend to inhibition.

Lead (legally tolerated limit: 50 $\mu\text{g}/\text{l}$): The addition of 10–25 $\mu\text{g Pb}^{++}/\text{l}$ to Lake Baldegg samples produced no effect and additions of 50 $\mu\text{g}/\text{l}$ and more showed a trend to inhibition.

In Lake Lucerne samples the trend to inhibition is more marked; the addition of $100 \mu\text{g Pb}^{++}/\text{l}$ caused even a significant inhibition.

Mercury (legally tolerated limit: $1 \mu\text{g}/\text{l}$):

In Lake Baldegg, additions of up to $2 \mu\text{g Hg}^{++}/\text{l}$ showed no significant effect upon glucose uptake, in contrary to Lake Lucerne, where $0.2 \mu\text{g}/\text{l}$ caused already a 70% inhibition.

Heavy metal cocktail:

Figure 2 shows the results for the simultaneous addition of several heavy metals (cocktail). The addition of $\frac{1}{5}$ of the legally tolerated limits to Lake Lucerne samples caused in March an almost complete inhibition of glucose uptake, while Lake Baldegg showed complete inhibition only by the addition of the legally tolerated limits. The same addition, however without mercury (which showed no effect in March), caused on 13 July an inhibition of only 50%, a second experiment on 24 July showed an even greater tolerance to cocktail addition.

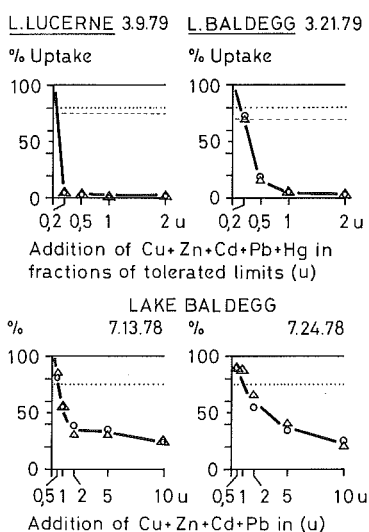


Figure 2. Inhibition of glucose uptake in lake water samples by addition of a combined heavy metal cocktail:

Addition of the heavy metal cocktail is expressed in fractions of the legally tolerated limits for the single heavy metals. The added amounts of the metals are listed in tables 1 and 2.

At each heavy metal concentration two values at two different exposure times were determined:

Lake Lucerne, 9.3.79: ○ 1 hr, 50 min; △ 3 hrs, 20 min,

Lake Baldegg, 21.3.79: ○ 16 min; △ 32 min,

Lake Baldegg, 13.7.78: ○ 32 min; △ 45 min,

Lake Baldegg, 24.7.78: ○ 20 min; △ 32 min.

The standard deviation from the mean reference value ($2s_x$) is marked in the graphs by horizontal bars:

..... for shorter exposure; ----- for longer exposure.

Toxicity sequence of heavy metals to heterotrophic microorganisms:
Table 3 shows the molar amounts of heavy metal addition in March experiments, which inhibited glucose incorporation by 20, respectively 40%.

Table 3. Toxicity sequences of heavy metals.

Metal	Lake Lucerne: metal concentration in nmole/l at an uptake inhibition of		Lake Baldegg: metal concentration in nmole/l at an uptake inhibition of	
	20%	40%	20%	40%
Cu ⁺⁺	< 30	30	< 30	80
Zn ⁺⁺	200	600	< 600	3000
Cd ⁺⁺	45	90	> 90	> 90
Pb ⁺⁺	250	500	500	> 500
Hg ⁺⁺	< 1	1	10	> 10

These results suggest the following toxicity sequence: Hg > Cu > Cd > (Zn, Pb).

3.2 *Effect of a long-term heavy metal load on the tolerance behaviour of a heterotrophic planktonic community*

In order to answer the question, whether a planktonic community at long-time metal stress reacts less sensitively to a sudden rise of heavy metal concentrations than a reference planktonic community in regard to glucose uptake, plankton, obtained from MELIMEX limno-corrals C and L2, was exposed to increased heavy metal concentrations, listed in table 2 (Cu, Zn and cocktail). The results are presented in figure 3, which shows that the metal stressed L2-plankton reacted generally with smaller inhibitions of glucose uptake than the reference

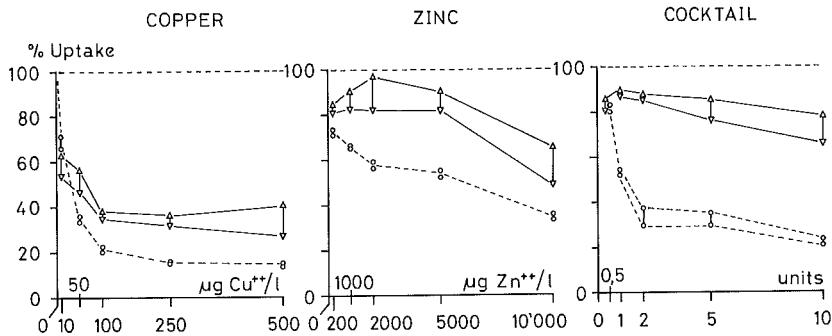


Figure 3. Inhibition of glucose uptake of heavy metal stressed and unstressed plankton in the same filtrate by addition of different heavy metals:

Glucose uptake of the references without any heavy metal addition is set to 100%. Glucose uptake of the metal spiked samples is expressed as a fraction of the references. Amounts of combined metal addition (cocktail) see table 2, being expressed in this figure as fractions of the legally tolerated limits.

Values of glucose uptake:
Δ ▽ double values for heavy metal stressed L2-plankton,
○ double values for C-plankton.

C-plankton. At concentrations higher than the legally tolerated limit the uptake inhibition of L2-plankton is significantly smaller than the one of C-plankton.

3.3 Conclusions

Based on these results, the following conclusions might be drawn:

1. The addition of heavy metal nitrates at concentrations comparable to the legally tolerated limits can inhibit glucose uptake of heterotrophic plankton.
2. The degree of inhibition at equal heavy metal additions varies from lake to lake and from season to season. This fact puts to question the practical value of generally applicable toleration limits for toxic compounds.
3. The legally tolerated limits of $10\text{ }\mu\text{g Cu/l}$ and $200\text{ }\mu\text{g Zn/l}$ are too high to enable an undisturbed glucose uptake throughout the year in the lakes of Lucerne and Baldegg. The tolerated limit of $1\text{ }\mu\text{g Hg/l}$ is by far too high for Lake Lucerne.
4. The simultaneous addition of the metals Cu, Zn, Cd and Pb inhibits glucose uptake in most cases much more than the single addition of the most toxic metal. This trivial fact illustrates the ambiguity of fixing toleration limits for single toxic compounds without testing all possible combinations of toxic substances.
5. The toxicity sequence of heavy metals (in equimolar concentrations) for heterotrophic microorganisms in the lakes of Lucerne and Baldegg is very similar to the toxicity sequence established by Gächter [5] for phytoplankton in eutrophic Lake Alpnach and mesotrophic Lake Lucerne:

heterotrophs: $\text{Hg} > \text{Cu} > \text{Cd} > (\text{Zn}, \text{Pb})$,

autotrophs [5]: $\text{Hg} > \text{Cu} > \text{Cd} > \text{Zn} > \text{Pb}$.

This comparison affirms the hypothesis, that the toxicity sequence in Swiss middle land lakes is not dependent primarily on the naturally induced variations of the composition of dissolved organic matter and planktonic communities. This statement gives rise to the hypothesis that heavy metal ions interfere primarily with metabolic mechanisms common to all planktonic microorganisms.

6. The resistance of heterotrophic planktonic communities to heavy metals can increase under long-term exposure to heavy metals. We assume that this is due to the natural selection of more resistant plankton species, as Gächter has shown for phytoplankton [7]. This observation also fits Gächter's [8] conclusions, that the uptake capacity of the plankton selected through a metal stress is generally smaller.
7. Gächter has shown in the MELIMEX experiment [7], that an increased heavy metal load changes primary production rates and the spectrum of phytoplankton species. It is assumed that this leads probably to changes in excretion and autolysis. This work has shown that the heterotrophic uptake of glucose is also affected. It was further observed [7], that an increase of heavy metal load rises the concentrations of free amino acids and induces shifts in the spectrum of copper complexing ligands. Baccini [1] found in the limno-corrals C and L seasonal variations of average complex formation constants (K_x) of dissolved organics with copper, which did not change simultaneously in limno-corrals L and C. This gives rise to the hypothesis, that K_x might also depend on plankton-specific biological mechanisms. These results suggest, that observed differences in the pool of dissolved organic ligands are not

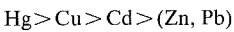
only caused by autotrophic organisms, but also by metal induced changes of heterotrophic metabolisms.

ZUSAMMENFASSUNG

Im Rahmen des MELIMEX-Experimentes [7] interessierte die Frage, ob die heterotrophe Stoffwechseltätigkeit einer natürlichen Planktongesellschaft durch eine erhöhte Schwermetallbelastung direkt beeinflusst werden kann.

Am Beispiel der heterotrophen Glucoseverwertung wurde abgeklärt, ob die Zugabe von Schwermetallen in der Grössenordnung gesetzlich erlaubter Limiten die Glucoseaufnahme hemmen kann. Zu diesem Zweck wurden Wasserproben der mesotrophen Horwer Bucht und des eutrophen Baldeggsees mit verschiedenen Konzentrationen der Schwermetalle Cu^{++} , Zn^{++} , Cd^{++} , Pb^{++} und Hg^{++} einzeln und in Kombination (siehe Tab. 1, 2) versetzt. Im ^{14}C -Kurzzeitversuch wurden die Glucoseaufnahmeraten dieser Proben mit denjenigen von unbehandelten Proben verglichen (Abb. 1, 2). Dabei zeigte sich, dass die Zugabe von $10\text{ }\mu\text{g Cu/l}$ sowie von $200\text{ }\mu\text{g Zn/l}$ die Glucoseaufnahme in den meisten Wasserproben signifikant hemmte. Der Grad der Hemmung war von See zu See und von Jahreszeit zu Jahreszeit verschieden. Die Zugabe von $1\text{ bis }10\text{ }\mu\text{g Cd}^{++}/\text{l}$ und von $10\text{ bis }100\text{ }\mu\text{g Pb}^{++}/\text{l}$ zeigte in den meisten Fällen keine Wirkung. $1\text{ }\mu\text{g Hg}^{++}/\text{l}$ hemmte die Glucoseaufnahme in der Horwer Bucht fast vollständig, zeigte jedoch im Baldeggsee keine Wirkung. Die gleichzeitige Zugabe aller Metalle verzeichnete in den meisten Fällen die grösste Hemmung. Trotz einiger Indizien konnte jedoch kein eindeutig synergistischer Effekt nachgewiesen werden.

Die Reihenfolge der Toxizität der einzelnen Schwermetalle bei äquimolaren Konzentrationen für heterotrophe Plankter lautet aufgrund der durchgeführten Versuche:



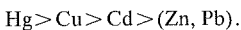
und gleicht der Toxizitätssequenz, welche Gächter [5] für Phytoplankter bestimmte.

Im weiteren wurde untersucht, ob eine natürliche Planktongesellschaft nach einer langzeitigen Schwermetallbelastung gegenüber Schwermetallen eine höhere Resistenz aufweist als eine Kontrollpopulation. Zu diesem Zweck wurde mit Schwermetall belastetes und unbelastetes Plankton aus den MELIMEX-Modellseen [7] filtriert und in unbelastetem Filtrat, welches mit verschiedenen Schwermetallkonzentrationen versetzt wurde (Tab. 2), resuspendiert. Nach Zugabe von ^{14}C -Glucose wurde die relative Hemmung der Glucoseaufnahme gemessen (Abb. 3). Aus den Resultaten wurde geschlossen, dass die Toleranz gegenüber Schwermetallen im Langzeitexperiment verbessert werden kann, was wahrscheinlich auf eine natürliche Selektion resistenterer Planktonspezies zurückzuführen ist.

RÉSUMÉ

L'absorption du glucose dans les échantillons d'eau du lac a été déterminée par des tests de courte durée au ^{14}C , en ajoutant d'une part les différents métaux lourds séparément (Cu^{++} , Zn^{++} , Cd^{++} , Pb^{++} , Hg^{++}) et en les ajoutant d'autre part combinés tous ensemble. Le degré d'inhibition de l'absorption du glucose induite par le métal varie de lac en lac et d'une saison à l'autre. L'adjonction des concentrations légales tolérées de $10\text{ }\mu\text{g Cu/l}$ et de $200\text{ }\mu\text{g Zn/l}$, aussi bien que l'adjonction simultanée de tous les métaux, inhibaient significativement l'absorption du glucose dans la plupart des échantillons. La limite légale tolérée de $1\text{ }\mu\text{g Hg/l}$ inhibait presque complètement l'absorption du glucose dans les échantillons du lac des Quatre-Cantons, mais ne montrait cependant pas d'effet dans les échantillons du lac de Baldegg.

Le degré de toxicité équimolaire des métaux lourds pour les microorganismes hétérotrophes a été déterminé comme suit:



L'absorption du glucose des échantillons de plancton, pris dans les bassins limnologiques pollués par les métaux lourds, a été moins inhibé que celle du plancton de contrôle lorsque les métaux lourds ont été ajoutés aux échantillons séparément ou en combinaison. On suppose que cet effet est dû à la sélection naturelle d'espèces de plancton plus résistantes.

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