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Toxicological studies of energy flows in ecological systems

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Abstract

A short survey is given about calorimetric investigations on ecological systems. Relevant literature is sparse, and moreover often dedicated to "indirect" calorimetry. Systems under research range from very complex entities like forests, lakes or marine shore sites to simpler parts of them as soils and sediments or various types of animals living in these habitats. Two examples of toxic effects are presented in some more detail: the influence of the organic pollutant pentachlorophenol on the different components of a forest system and that of the heavy metal cadmium on an aquatic organism.

INTRODUCTION

Energy flows through a biological system are sensitive indicators of its status and of interdependences with neighbouring or overlapping units. This holds for well defined microbial cultures as well as for highly structured ecological entities like forests, lakes or smaller parts of them. On all levels of complexity, toxic effects interfere with the system, lead to changing life conditions and performances modifying their energy flows.

Ecological systems are not *per se* the sum of their inorganic and organic components. They establish complex networks due to the many mutual interactions between the various components. Although they have to be kept in mind it is unavoidable in most experiments to investigate single, isolated parts of them as a first approximation to the ecological system and to obtain basic data about it.

This paper gives a short review of calorimetric activities in this field of research and presents two experiments in some more detail.

SHORT SURVEY ABOUT ECOTOXICOLOGIC INVESTIGATIONS

Energy flows through ecological systems may be monitored not only by oxygen consumption or carbon dioxide production ("indirect" calorimetry) and by turnover rates of radioactively labelled substrates, but also by reaction and combustion calorimetry (refs. 1-3). As "direct" calorimetry is a non-specific method and detects any kind of heat dissipation, it is the only means to measure anaerobic as well as aerobic metabolism in the system under investigation. Moreover, the reaction enthalpy only depends upon the educts and products and not upon the specific pathway used under the ruling conditions. Although this direct calorimetry offers the chance to draw a global picture of the energy flow in a system and to determine toxic influences in a non-specific manner, only few examples can be found in the literature. However, some information and experimental tips may be obtained from "pure" calorimetric research without the ecological background presented in some recent reviews and monographs (refs. 4,5).

Calorimetric experiments in connection with toxic effects have been performed using:

- microorganisms in batch or chemostat cultures of special strains (refs. 6-8) or in surface attached mixed cultures obtained from activated sludges and immobilized in a biofilm (ref. 7), looking for the actions of antislime agents (ref. 6), aromatic compounds (ref. 7) or heavy metals (refs. 7,8),
- soils of various origins. With the addition of small amounts of glucose, soils exhibited a strong temperature dependence in the form of an Arrhenius plot (ref. 9) and a drastic change in their heat profiles after addition of heavy metals or aliphatic compounds (ref. 10). Zelles and coworkers (ref. 11) observed a stimulating effect on the soil bioactivity by liming as a countermeasure to acidification, while addition of nitrogen or liming had no or negative effects in Berlin soils (refs. 12,13),
- sediments. Gustafsson and Gustafsson (ref. 14) measured the "total" activity in natural aquatic sediments and found a steady decrease with depth. Moreover, it was shown that oxygen consumption accounts only for the first few millimeters of sediment activity and thus underestimates the amount of metabolized organic matter in the lower layers (ref. 15). Calorimetry offers the most correct approach to determine the energy flow and the carbon consumption in such a soft bottom community (refs. 16-18),
- sludges. Weppen and Schuller (ref. 7) developed a special chemostat culture as a simple analogon of the chemiorganotrophic microbiocoenosis of an activated sludge and tested the influences of various xenobiotics. Other authors looked for waste water treatment, digesting rates for various substrates and toxic effects of heavy metals and organic pollutants (refs. 19,20),
- various soil invertebrates. Beetles, pillbugs and worms were investigated in their response to pentachlorophenol (see below), and the cabbage looper *Trichoplusia ni* as just one further example, but under the influence of benzene vapor (ref. 21),
- aquatic animals. Gnaiger (refs. 22,23) published data on oligochaetes, zooplankton, fish eggs and larvae under anaerobic and aerobic conditions and with addition of two antibiotics. Measurements on the influence of cadmium on a freshwater snail are reported in some detail below.

The various systems were stressed by aliphatic or aromatic organic compounds (refs. 7, 19,21,24-27), antibiotic drugs such as bacteriostatic, bacteriocidic or anti-slime agents (refs. 6,24), heavy metals like mercury, copper and cadmium (refs. 8,19,27,28) or atmospheric pollutants as found in pig fattening houses and other lifestock facilities (refs. 21,29).

CALORIMETRIC EQUIPMENT

Various calorimetric systems for ecological investigations are described in the literature. They can be grouped as combustion bomb, batch and flow calorimeters (refs. 2,3). In the experiments presented below the two latter methods were applied:

- Pentachlorophenol investigations were performed by means of a batch calorimeter (type MS70, SETARAM/Lyon) with 4 vessels of 100 ml and a sensitivity of 60 mV W^{-1}. Animals, soil and litter were isolated in 45 ml plastic containers with gauze covers, thermally equilibrated outside the calorimeter and then inserted into the metallic vessels. The volume was large enough to avoid any significant drop in oxygen concentration (ref. 25).
- For cadmium/snail investigations a flow calorimeter was used (type 2107-122, LKB/Bromma). The standard flow-through sorption cell was enlarged to approximately 3 ml housing animals up to 600 mg fresh weight. The sensitivity amounted to 38 mV W^{-1}, the pump rate to 9.5 ml h^{-1}. Further details for both systems are given in refs. 2,25,26,28.

The direct calorimetric evaluations were complemented by indirect manometric and polarographic determinations. PCP uptake was screened by radioactive labelling, cadmium uptake by atomic absorption spectrometry. Of course, the most complete information is obtained by simultaneous direct and indirect calorimetry as in combination experiments of flow calorimetry and respirometry (ref. 22).

INFLUENCE OF PENTACHLOROPHENOL ON A FOREST SYSTEM

Pentachlorophenol (PCP) was the most intensively used pesticide against bacteria, fungi, algae, molluscs, insects and herbs for wood protection (ref. 30). Although nowadays forbidden in most countries, it is an ubiqitous long-term contaminant and a heavy burden in many ecological system. PCP is known to be a powerful uncoupler of the main energy producing step in living organisms, oxidative phosphorylation (ref. 31), which transfers the chemical energy of the substrate to ATP. Due to uncoupling less ATP is formed than necessary for the maintenance of the organism thus accelerating its metabolic turnover. Consequently, oxygen consumption and heat production rates increase.

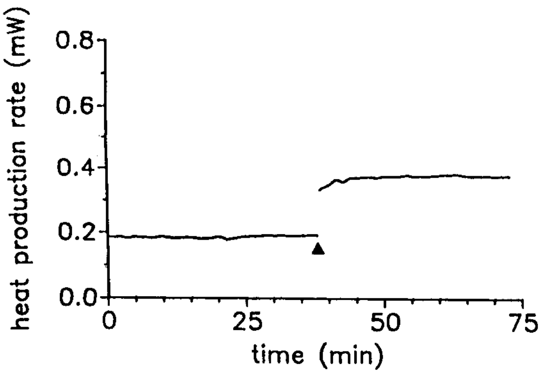

Fig. 1a Heat production rate of 5.8 g ww forest soil as function of the time before and after (triangle) addition of pentachlorophenol to a final concentration of 34.5 μg (g ww soil)$^{-1}$ (adapted from ref. 26).

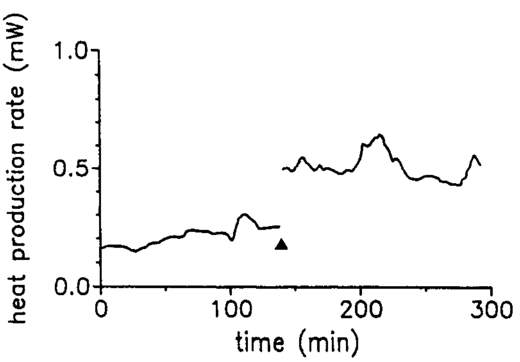

Fig. 1b Heat production rate of an earthworm (365 mg ww) before and after (triangle) the uptake of 31.8 μg PCP(g ww)$^{-1}$ (adapted from ref. 25).

Various biotic parts of a forest system as soil, litter in different degrees of degradation, and several small invertebrates of such a biotope (beetles, pillbugs, worms) were investigated calorimetrically (ref. 25). All components exhibited a significant stimulation of energy turnover under the influence of PCP. Figure 1 gives two examples of such stimulations. Figure 1a shows an approximately twofold increase in heat production of soil after the addition of PCP. The break in the calorimetric trace is due to mechanical disturbance of the system. A similar augmentation is seen in the heat output of an earthworm, *Eisenia foetida* (Fig. 1b). Moreover, the graph demonstrates by its temporal structures before and after addition of PCP that there is no significant disturbance of the animal in the sense of an increased locomotor activity.

The relative enhancement of heat production rates increases with growing PCP concentration. Fig. 2 demonstrates this dependence for forest soil (1) and for rotten litter (2) exhibiting different sensitivity to PCP. As PCP never reached or even exceeded lethal concentrations, no maximum stimulation or subsequent poisoning of the system could be observed. It becomes clear from Fig. 2 that the relative enhancement in soil is much stronger than that in litter, although its weight-specific heat production rate is smaller by a factor of 5. Slopes similar to those shown here are found with low PCP concentrations for the other components of the ecological system.

INFLUENCE OF CADMIUM ON A WATER SNAIL

Cadmium was used as a model substance for heavy metals. It is toxic to most living systems (ref. 32) due to its strong oxidative and denaturating power on proteins. Most frequently used tests for heavy metals determine the mortality in a given period of time. Sublethal effects are not as easily observed and need more time, but they are more sensitive to potential hazards by toxic

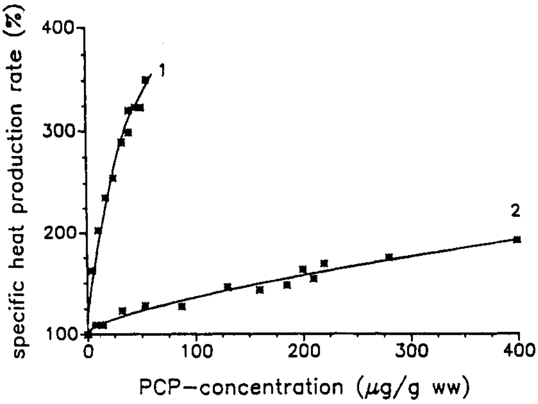

Fig. 2 Increase of the relative specific heat production rate of soil (1) and litter (2) with increasing pentachlorophenol concentrations.

substances in low concentrations. A total picture of the energy metabolism as rendered by direct calorimetry should therefore be the most suited tool for such investigations.

Sublethal cadmium concentrations from 0.01 to 1000 $\mu g\ l^{-1}$ were chosen for their action on the freshwater snail *Planorbis corneus* and exhibited drastic reductions of heat production rates after a few hours (ref. 28). *P.corneus* was taken as a test object, because it is widespread in European stagnant waters and an important member at the beginning of the food chain. The modified calorimetric vessel was large enough for snails with a mean total wet weight of 150 mg, an active tissue of 72 mg and a maximum shell diameter of 12 mm.

Calorimetric curves were not as smooth and steady as those of litter or soil (Fig. 1a) but structured owing to locomotor activity as presented in Fig. 1b. These fluctuations occur around a steady state remaining constant for the whole experimental period. It indicates constant physiological conditions for the snail in the slow water stream. This phase of establishing the experimental baseline is exhibited during the first 4.5 h of Fig. 3 with a mean weight specific rate of heat production of 453 ± 80 $\mu W\ g^{-1}$ of fresh weight. After this time the water was completely withdrawn and exchanged against the chosen cadmium solution. After less than 1 h the flow-through vessel is equilibrated again and the effect of cadmium becomes visible. A more or less pronounced lag phase of 1.5 to 2.5 h appears as a "shoulder" or as a prolongation of the pre-period depending upon the cadmium concentration. It is followed by a steady decrease of the heat production rate to a new final plateau. This level varies between 81 and 12 % of the pre-period value, strongly depending upon the cadmium concentration. The best correlation to the cadmium concentration is established by the slope of the decrease, which also appears to be the quickest response to the disturbance. Therefore, this slope can be used for monitoring poisoning effects.

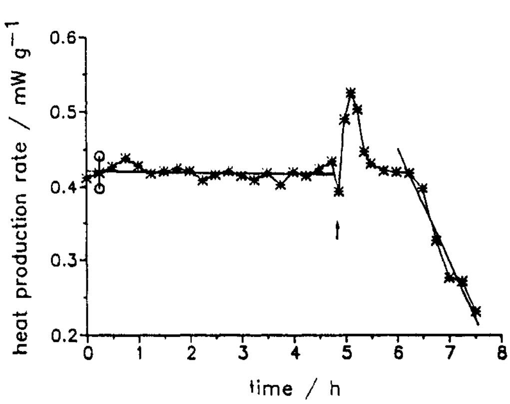

Fig. 3 Mass specific heat production rate of a freshwater snail before and after (arrow) the addition of 1 $\mu g\ l^{-1}$ cadmium chloride. The graph represents the mean of 3 individual experiments with 3 different snails. The standard deviation is indicated near to the ordinate as a vertical bar with open circles (adapted from ref. 28).

DISCUSSION

This short review demonstrates that (direct) calorimetry is not just another, appropriate tool to determine energy flows through ecological systems, but sometimes the only one to monitore metabolism under special conditions. Although modern calorimeters are more expensive, by far, than polarographic setups, they can be constructed and adapted to special tasks by the customer more cheaply (see e.g. refs. 16,17). They are not as tedious and time consuming as they are supposed to be and offer sensitivities which compete with other methods.

Under atmospheric conditions the transfer of electrons from substrate to oxygen as a terminal acceptor renders the major part of energy for an organism. Thus, heat output of the catabolic reaction may be estimated from oxygen consumption as long as the substrate is known. This close connection between respiration and heat dissipation (indirect calorimetry) is guaranteed by the fact that the reaction enthalpy is independent of the kind of carbon source when the electron transfer from the substrate to the oxidant is calculated. This "oxycaloric coefficient" amounts to approximately -110 kJ (mol electrons)$^{-1}$. But under hypoxic or anoxic conditions when fermentative reactions become necessary to cover the energy demand of the organism indirect calorimetry is no longer possible. Such conditions of reduced oxygen supply are found periodically or permanently in aquatic systems, sediments and sometimes in soil. Eutrophication of waters and the related anoxia are consequences of pollution and present a worldwide problem often connected with increased concentrations of toxic substances (ref. 3).

Biochemistry alone fails under such conditions to estimate energy flow through an ecological system, as only 60 % of the total anoxic heat production can be explained by the biochemical pathways which are known today. Thus, only direct calorimetry offers the chance to cover the whole area of ecological, physiological and toxicological questions (refs. 3,23).

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