INFLUENCE OF THE ENDOCRINE DISRUPTOR 17α-ETHINYLESTRADIOL ON PHYTOPLANKTON DIVERSITY IN AQUATIC MICROCOSMS

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Introduction

17α-ethinylestradiol (EE) is a xenoestrogenic chemical, used in drugs e.g. in contraceptives. As it reveals a certain degree of persistence, it is found in natural waters, e.g. near sewage works (1, 2). Due to its known endocrine activity and its similarity with the natural hormone estradiol it can be used as a positive reference for other chemicals tested for their endocrine activity, e.g. nonylphenol (3, 4). As for a comprehensive estimation of the effect of a toxitant on an ecosystem all parts of it have to be considered, the (indirect) effects on phytoplankton were presented here. To meet the conditions in natural systems the microcosms were continuously exposed to different concentrations EE by controlled release, via diffusion through a semi-permeable tube.

Methods and Material

Cylindrical microcosms (80 cm high, 60 cm wide) made of stainless steel were filled with 230 water and a 10 cm layer of sediment derived from an oligo-mesotrophic littoral area of Lake Ammersee (Bavaria, Germany). After a five weeks pre-application period, different concentrations of EE (Sigma Aldrich) disperged in triolein were applied in four microcosms (EE-A, EE-C, EE-E, EE-F) by controlled release through semi-permeable LPDE-tubes for six weeks, then the tubes were removed. Phytoplankton samples were taken regularly before, during and for six weeks after the removal of the tubes to investigate the recovery. Four microcosms served as controls. The samples were fixed with approximately 12 drops of Lugol per 100 mL and sedimented in a plankton chamber according to the Utermoehl method. The algae cells were identified and counted in an inverse microscope. Number of taxa and diversity index H' according to Shannon/Weaver were calculated. Furthermore, various physical and chemical parameters were measured.

Results and Discussion

The time courses of the EE concentrations unveiled peaks in the first week of dosing, followed by a decline and reaching a relative constant level after the second week. To achieve higher concentrations, additional EE-filled LDPE-tubes were placed into the microcosms, leading to a second peak. Maximum concentrations reached were 50 (EE-A), 450 (EE-C), 550 (EE-E) and 720 ng/L (EE-F). Fig. 1 shows the toxodose, i.e. the integral of the concentration over time. Total loads were 300, 5300, 10800 and 12800 d*ng/L.

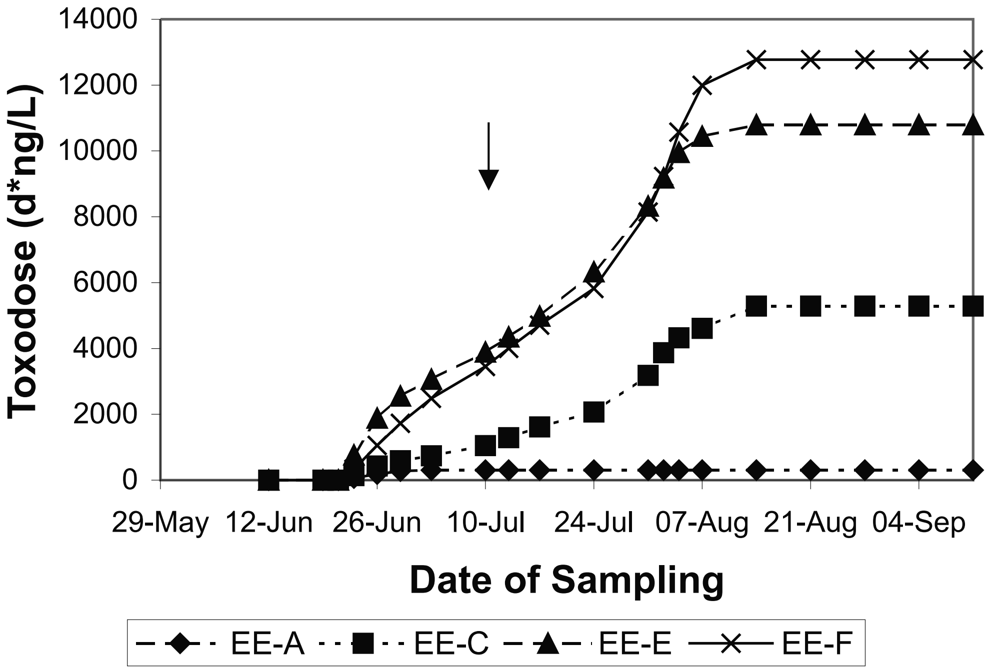

Figure 1. Toxodose of EE; arrow: LDPE-tubes added

No correlation between the investigated physical and chemical parameters and the EE concentration could be detected.

A total of 133 identified phytoplankton taxa were included into the analysis, most of them Chloro- und Bacillariophyceae (22 each), followed by Cyanophyceae (18) and Chrysophyceae (17). The number of taxa per microcosm was rather constant over time in the controls, with a mean of 21 to 25. During the post-application period, the mean as well as the variance slightly increased, both becoming smaller again at the end of the study. Thus, the phytoplankton community seemed to remain stable during the experiment, indicating a good suitability of the microcosm design for ecotoxicity testing. During the pre-application and the first half of the application period, the numbers of taxa in the treated microcosms were in the range of the controls (with the exception of some samples of EE-F). However, during the following time number of taxa increased in the three highest dosed microcosms compared to the controls. Maximum of this deviation was reached three weeks after the end of dosing. The deviations of the three highest dosed microcosms were significant (Whitney-Mann test, $p<0.05$) at August 07 and 21 and almost significant at August 14 and 28 ($p = 0.053$). During the observation period a full recovery did not occur. The diversity index H’ (not shown here) supported this results.

A former study with EE unveiled no clear effects on biocenosis including phytoplankton (5). However, due a flood in the Lake Ammersee, causing a murky water with a possibly disturbed plankton community at the time the water was taken for the microcosms, changes may have been obscured. In the study presented here, there were clear EE correlated shifts concerning phytoplankton. The finding,

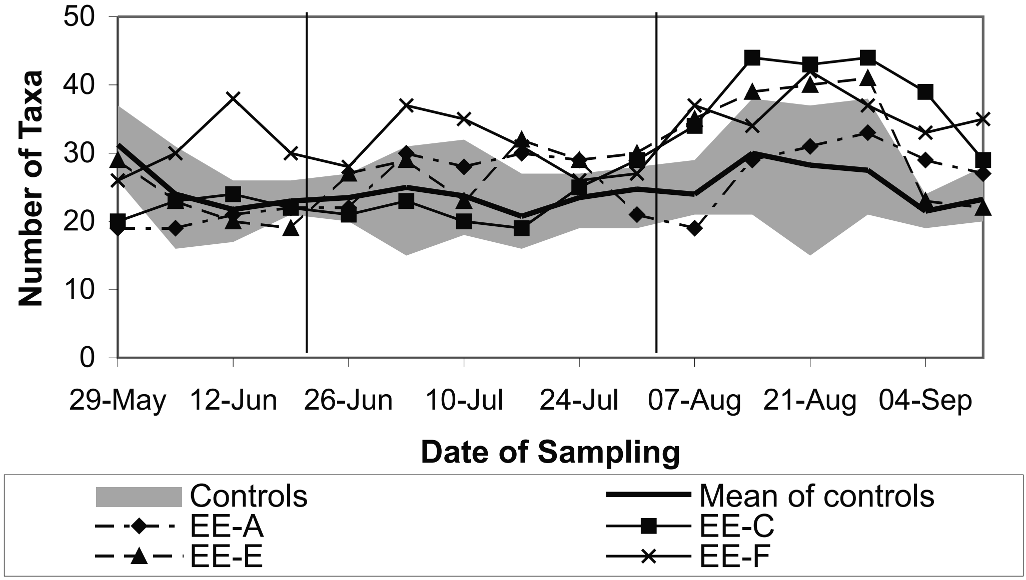

Figure 2. Number of phytoplankton taxa; vertical lines: begin and end of dosing

that the diversity increased in the treated microcosms instead of decreasing, as might be expected as a result of a stressor, indicates a indirect mode of action. Whereas the effects on phytoplankton become visible in the second half of the application period, zooplankton reacts earlier (Jaser, unpublished), supporting this assumption. An EC50 of 840µg/L was found for the phytoplanktic alga *Scenedesmus suspicatus* (growth inhibition) (6), a value were far beyond those reached in this study. Other algal species might be more sensitive, however, the large discrepance between the EC50 and the EE-concentrations influencing the phytoplankton in the microcosms also supports an indirect affectation. For a final interpretation of the results a detailed consideration connected with the development of zooplankton will be performed.

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